Synthesis and Pharmacological Characterization of Novel Druglike Corticotropin-Releasing Factor 1 Antagonists

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To identify new CRF_1 receptor antagonists, an attempt to modify the bis-hetherocycle moiety present in the top region of the dihydropirrole[2,3]pyridine template was made following new pharmacophoric hypothesis on the CRF_1 receptor antagonists binding pocket. In particular, the 2-thiazole ring, present in the previous series of compounds, was replaced by more hydrophilic non aromatic hetherocycles able to make appropriate H-bond interactions with amino acid residues Thr192 and Tyr195. This exploration, followed by an accurate analysis of the substitution of the pendant aryl ring, enabled to identify in vitro potent compounds showing excellent pharmacokinetics and outstanding in vivo activity in animal models of anxiety, both in rodents and primates.

Introduction

Corticotropin releasing factor (CRF[a]) is a 41 amino acid peptide that plays a central role in mediating the behavioral, neuroendocrine, and autonomic responses to stress.[1] CRF is synthesized in the hypothalamus and liberated into the hypophyseal-portal system triggering the release of adrenocorticotrophic hormone (ACTH) from the pituitary gland. ACTH in turn, induces the synthesis and the release of glucocorticoids from the adrenal cortex. In mammals, exposure to stress increases the release of CRF in both hypothalamic and extrahypothalamic brain areas and produces the same effects observed with exogenous administration of CRF, i.e., increase of ACTH and cortisol (human) or corticosterone (rodent) blood levels, enhanced autonomic nervous system activity, and anxiety-like behavior and is responsible, at least in part, for the HPA (hypothalamic pituitary adrenal) axis hyperactivity characteristic of patients with major depression.[2−5] 7*H*-Pyrrolo(2,3-d)pyrimidin-4-amine,*N*-butyl-*N*-ethyl-2,5-dimethyl-7-(2,4,6-trimethylphenyl) (CP-154,526),[6−8] 3-[6-(dimethylamino)-4-methyl-3-pyridinyl]-2,5-dimethyl-*N*,*N*-dipropylpyrazolo[1,5-*a*]pyrimidin-7-amine (R121919, also known as NBI30775),[9] 7*H*-Pyrrolo[2,3-*d*]pyrimidin-4-amine, *N*-butyl-*N*-ethyl-2,5,6-trimethyl-7-(2,4,6-trimethylphenyl) (Antalarmin),[10] and pyrazolo[1,5-*a*]-1,3,5-triazin-4-amine, 8-(2,4-dichlorophenyl)-*N*-[2-methoxy-1-(methoxymethyl)ethyl]-2,7-dimethyl- (DMP-696)[11] are among the first CRF_1 receptor antagonists discovered, and their activity in animal models of anxiety and depression like-behavior is widely recognized.[6−9] In particular, NBI30775 and DMP696 showed an anxiolytic-like effect consistent with a displacement of CRF from its binding sites in the brain, measured by ex-vivo autoradiography.[11,12] These anxiolytic-like properties have been confirmed in a clinical study with NBI30775.[9] Notably, it was shown that CRF_1 receptor blockade does not impair the ACTH and cortisol secretory activity either at baseline or following an exogenous CRF challenge.

This large body of evidence suggests that the identification of compounds that block CRF_1 receptors in the central nervous system may provide an important therapeutic approach to anxiety and depression, with a favorable side effect profile with respect to antidepressant and anxiolytic drugs currently on the market.

As part of a wide exploration aimed toward the identification of innovative, druggable series of CRF_1 receptor antagonists, we recently reported[13] the discovery of compounds of type **1** as depicted in Figure 1, bicycle derivatives capped with appropriate bihetherocycle moieties in the top region of the molecules, specifically designed to make appropriate H-bond acceptor interaction with residues Thr192 and Tyr195 present in the putative CRF_1 receptor antagonists binding site. In particular compounds of type **2**, the dihydropyrrole[2,3]pyridine derivatives bearing an appropriate 2-thiazole moiety at the C-3 position of the pyrazole and different substituents at the ortho position of the pendant phenyl ring, were among the most in

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[a] Abbreviations: CRF, corticotropin-releasing factor; ACTH, adrenocorticotrophic hormone; HPA, hypothalamic pituitary adrenal; DMF, *N*,*N*-dimethylformamide; THF, tetrahydrofurane; NMP, *N*-methylpyrrolidinone; CHO, Chinese hamster ovary; QSAR, quantitative structure−activity relationship; ED, effective dose; cAMP, cyclic adenosine monophosphate; SPA, scintillation proximity assay; DMSO, dimethylsulfoxide; ESP, electrostatic potential; DMEM, Dulbecco's modified eagle's medium; PBS, phosphate buffered saline; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; PVT WGA, polyvinyltoluene wheat germ agglutinin; SSRI, selective serotonin reuptake inhibitor; SCX, strong cation exchange; PEG, polyethylene glycol.

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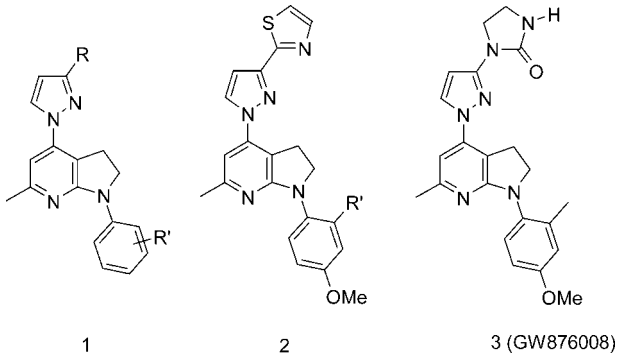


Figure 1. Exploration of the dihydropyrrole[2,3]pyridine template.

vitro potent compounds belonging to this series. Exploiting this novel pharmacophoric hypothesis, we managed to reduce further the overall lipophilicity of this series of compounds, one of the main drawbacks for the drugability of CRF_1 receptor antagonists impacting detrimentally on both the volume of distribution (V_{ss}) and the elimination half-life ($t_{1/2}$), characteristics that may be responsible for significant drug accumulation and undesired toxicological findings.[14] To this end, a wider exploration of the top region of this template was undertaken with the specific aim to replace the thiazole ring with more hydrophilic hetherocycles containing both H-bond acceptor and H-donor groups. This exploration was then followed by an appropriate selection of the best substituents of the "pendant" aryl ring. Among the different compounds synthesized, the imidazolone derivative **3** (GW876008), shown in Figure 1, exhibited similar in vitro potency as the corresponding thiazole analogues of type **2**, outstanding pharmacokinetics and in vivo activity in different animal models of anxiety.

Chemistry. Compounds **3**−**22** were prepared by the innovative and rather straightforward synthetic route shown in Scheme 1. Commercially available aniline derivatives **23** were transformed into the corresponding amides **24** by reaction with 2-chlorobutirylchloride in the presence of NaH_2PO_4. These compounds were treated with sodium hydride in dry DMF ant 0 °C to afford the γ-lactam derivatives **25** in high yield after purification by flash chromatography. The following reaction with freshly distilled $POCl_3$ and 2-aminoethylcrotonate in dry dichloroethane enabled preparation of compounds **26**, which were then dissolved in DMF and heated at 90 °C in the presence of sodium hydride. The bicycle derivatives **27** were obtained, although in limited chemical yield, after fast filtration of the crude reaction residue on silica gel. Then these intermediates were transformed in good yield into the key triflate derivatives **28**. The following reaction with KI in dry NMP at 150 °C for 15 h, enabled obtaining of the corresponding more reactive iodine intermediates **29**, which were finally coupled in good yield with a series of substituted pyrazole derivatives to give title compounds **3**−**22** shown in Table 1.

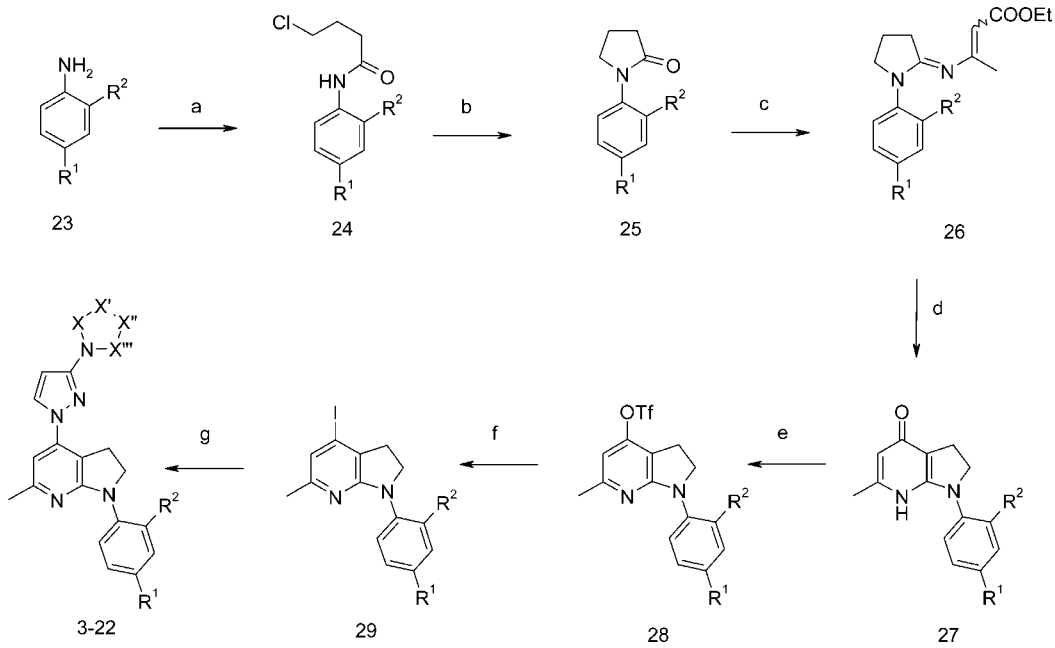
Compound **7** was prepared in three steps from intermediate **31**, as shown in Scheme 2, by sequential reaction with phenyl chloroformate at 0 °C in the presence of pyridine, followed by treatment with 2,2-bis(ethy1oxy)ethanamine in pyridine at 60 °C for 3 h to get intermediate **37** and final transformation into the title compound by acid hydrolysis.

Compounds **9** and **10** were synthesized from the *N*-acetyl derivative **30** as shown in Scheme 3. Deacetylation reaction occurred in 89% yield in basic conditions by irradiation in a microwave apparatus. Then, the alkylation reaction of the aminoderivative **31** with ethyl bromoacetate with NaH in DMF enabled obtaining of the aminoester **32**, which was reduced to the corresponding amino alcohol **33** with $LiAlH_4$. Finally, the following cyclization reaction run in the presence of (methoxycarbonylsulfamoyl)triethylammonium hydroxide in CH_2Cl_2 at reflux for 1 h gave compound **34**, which was then hydrolyzed in basic medium to give the title compound **9**. Alternatively, the amino derivative **31**, dissolved in *n*-butanol, was transformed into the sulfonic acid derivative **35** by reaction with 1,2-oxathiolane 2,2-dioxide by irradiation in a microwave apparatus. Then the following reaction with freshly distilled $POCl_3$ at reflux for 1 h in the presence of $NaHCO_3$, enabled preparation of the final compound **10**.

Results and Discussion

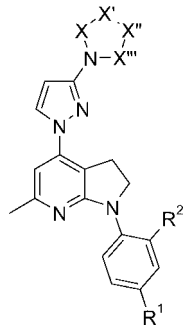
To identify innovative druglike CRF_1 receptor antagonists, an appropriate strategy was designed starting from compound of type **2**, shown in Figure 1, with a 2-fold objective: (a) clarify further the SAR associated to the bihetherocycle moiety present in the top region of the molecule, exploiting the information available in house on the nature of the putative CRF_1 receptor antagonists binding pocket,[13] (b) reduce the overall lipophilicity of the molecules, perceived as a critical physicochemical characteristic at the base of the optimization of the pharmacokinetics and, more generally, the druggability of the CRF_1 receptor antagonists. To this end, and in view of the presence within the putative CRF_1 receptor antagonists binding pocket of Thr192 and Tyr195, amino acid residues potentially acting as H-bond donors, we replaced the critical thiazole moiety by a γ-lactam ring in which the carbonyl group might function as a H-bond acceptor group, as the nitrogen atom present in the terminal thiazole ring. Once prepared as previously reported, compound **4** was tested in vitro, in terms of ability to inhibit the binding of [^{125}I] sauvagine to h-CRF_1 Chinese hamster ovary (CHO) transfected cells. As shown in Table 1, it exhibited a limited reduction of receptor binding potency with respect to the compound **2** (pIC_{50} = 6.91 and 7.27, respectively), confirming that the presence of a carbonyl group acting as H-bond acceptor was tolerated and the thiazole ring could be replaced by alternative nonaromatic hetherocycle moieties. This initial positive finding gave rise to a wider exploration of this region of the molecule. In particular, with the aim to further reduce the overall lipophilicity, aiming at increasing in the meantime the in vitro affinity, we introduced an additional H-bond donor group. To this end, the imidazolinone derivative of type **3** was designed and synthesized. Notably, as shown in Table 1, a marginal increase of the in vitro affinity of compound **3** with respect to the corresponding γ-lactam derivative **4** was observed (pIC_{50} = 7.18 and 6.91, respectively) despite the significant reduction of general lipophilicity (calculated ClogP = 4.109 and 5.182 for compounds **3** and **4**, respectively).[13] This result was an important observation as far as the optimization of this class was concerned, further confirming the working hypothesis and the SAR of compounds of type **1** and **2**. For the CRF_1 receptor antagonists in fact, the overall lipophilicity, and more specifically the presence in the top region of the molecule of lipophilic aliphatic side chains, has always been considered one the main drivers to increase the in vitro potency, however, impacting negatively on their druggability. Conversely, in our series, the appropriate decoration of the top region with appropriate hydrophilic hetherocycles, suitably oriented to make appropriate interaction with some specific amino acid residues present in the putative CRF_1 receptor antagonists binding pocket, enabled optimization of their in vitro affinity by reducing at the same time their ClogP. When compound **3** was docked in silico in this putative model, as shown in Figure 2, it was

Scheme 1. General Synthetic Route for Compounds **3**−**22**[a]



[a] (a) 4-chlorobutirrylchloride, CH_2Cl_2, K_2HPO_4, room temp 1 h; (b) NaH, DMF, 0 °C, 30 min; (c) $POCl_3$, 2-aminoethylcrotonate, 1.2-dichloroethane, T = 60 °C, 8 h; (d) NaH, DMF, T = 90−100 °C, 1.5 h; (e) Py, Tf_2O, CH_2Cl_2, from −78 °C to room temp, 1 h; (f) KI, NMP, T = 150 °C, 18 h; (g) 1-(1*H*-pyrazol-3-yl)-2-imidazolidinone, CuI, dodecane, NMP, (1R,2R)-1,2-cyclohexanediamine, K_2CO_3, T = 150 °C, 15 h.

Table 1. Top and Bottom region SAR

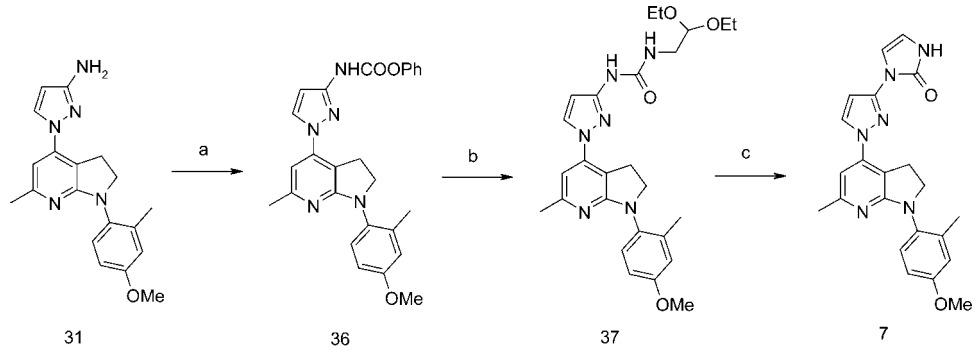


compd	R^1	R^2	X	X′	X″	X‴	pIC_{50}[a]
NBI30775							7.55 ± 0.20
DMP-696							7.40 ± 0.06
2							7.27 ± 0.18
3	OCH_3	CH_3	CH_2	CH_2	NH	CO	7.18 ± 0.18
4	OCH_3	CH_3	CH_2	CH_2	CH_2	CO	6.91 ± 0.12
5	OCH_3	CH_3	CH_2	CH_2	NCH_3	CO	6.10 ± 0.07
6	OCH_3	CH_3	CH_2	CH_2	O	CO	5.71 ± 0.06
7	OCH_3	CH_3	CH	CH	NH	CO	6.92 ± 0.08
8	OCH_3	CH_3	CH	CH_2CH_2	NH	CO	6.84 ± 0.05
9	OCH_3	CH_3	CH	CH_2	NH	SO_2	6.51 ± 0.05
10	OCH_3	CH_3	CH	CH_2	CH_2	SO_2	5.49 ± 0.26
11	OCH_2CH_3	CH_3	CH	CH_2	NH	CO	6.50 ± 0.05
12	O-*i*-C_3H_7	CH_3	CH	CH_2	NH	CO	5.91 ± 0.04
13	OCF_3	CH_3	CH	CH_2	NH	CO	7.17 ± 0.13
14	CN	CH_3	CH	CH_2	NH	CO	7.17 ± 0.19
15	1-pyrazole	Me	CH	CH_2	NH	CO	6.43 ± 0.07
16	CN	CF_3	CH	CH_2	NH	CO	7.49 ± 0.29
17	OCH_3	CHF_2	CH	CH_2	NH	CO	7.25 ± 0.14
18	CN	OCF_3	CH	CH_2	NH	CO	6.92 ± 0.23
19	CN	CH_2CH_3	CH	CH_2	NH	CO	7.30 ± 0.26
20	1-pyrazole	OCH_3	CH	CH_2	NH	CO	6.44 ± 0.00
21	Cl	Cl	CH	CH_2	NH	CO	6.80 ± 0.18
22	CF_3	CF_3	CH	CH_2	NH	CO	7.05 ± 0.19

[a] Mean pIC_{50} ± SEM, h-CRF_1.

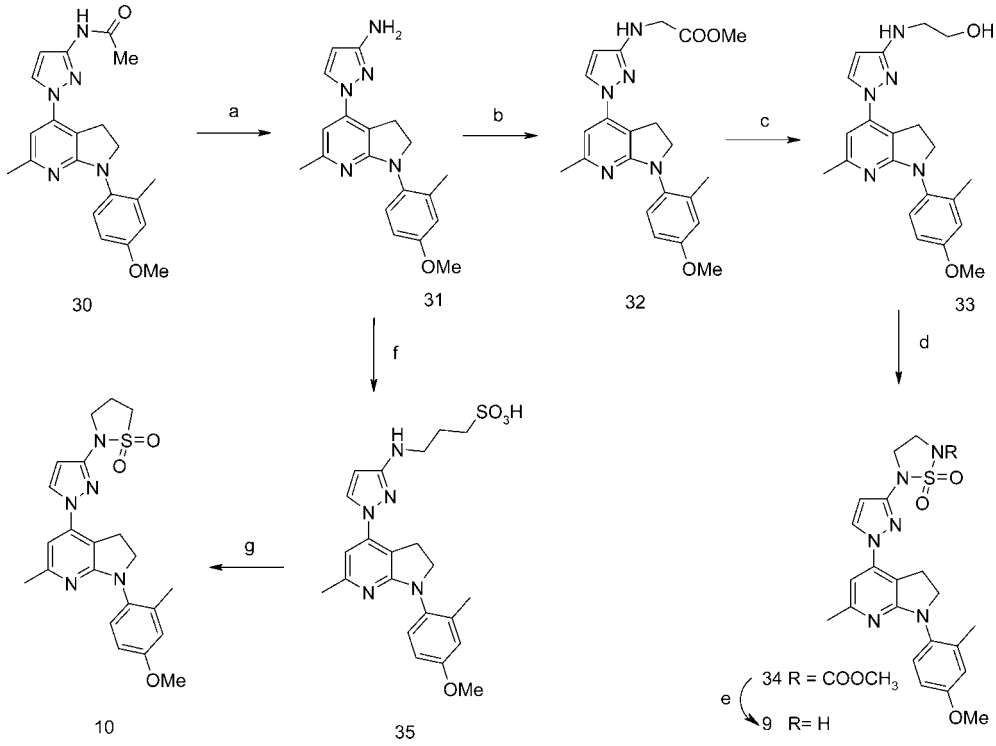
observed that the carbonyl group of the imidazolinone moiety might be responsible for an H-bond acceptor interaction with the hydroxyl group of Thr192, as in the case of the corresponding thiazole derivative **2**. On top of that, an additional H-bond donor positive interaction might occur between the NH present in the imidazolinone moiety and the carbonyl group of the Cys188 amino acid residue. The following substitution and/or modification of this five-membered imidazolinone heterocycle further endorsed this working hypothesis. In fact, the corresponding substituted imidazolinone derivative **5** and the carbamate **6** showed, in fact, a significant drop of in vitro potency with respect to compound **3** (pIC_{50} = 6.10 and 5.71, respectively), confirming the preference for the simultaneous presence of H-bond acceptor and donor groups. Further modifications of the imidazolinone ring were made. Neither the introduction of a double bond within the five-membered ring nor the corresponding six-membered ring showed improved in vitro potency (pIC_{50} = 6.92 and 6.84 for compound **7** and **8**, respectively), most likely due to the fact that the imidazolinone moiety lies into a limited-size hydrophilic subpocket. Finally, both the sulfamide derivative **9** and the corresponding γ-sultam **10** derivative **10** were found to be significantly less active than the analogue compounds **3** and **4** (pIC_{50} = 6.51 and 5.49 compared to 6.91 and 7.18, respectively). This result may be explained by the need for an appropriate spatial orientation of the carbonyl group, resulting in a more effective H-bond acceptor interaction with both Thr192 and Cys188. In addition, it is worth noting that, as in the case of the γ-lactam **4**, compound **10** was less active than **9**, most likely due to the absence of the necessary H-bond donor group. Furthering these results, an optimization of the substitution of the pendant phenyl ring was undertaken, following the indication coming from the QSAR (quantitative structure−activity relationship) analysis performed previously.[13] As expected, the concomitant presence of both a lipophilic group of limited size in the ortho position and an H-bond acceptor group in the para position, enabled maximization of the in vitro potency. In particular, compound **16** (R_1 = CN, R_2 = CF_3) was the most potent exemplar of this series (pIC_{50} = 7.49). However, when the most potent com-

Scheme 2. Synthetic Route for Compound **7**[a]



[a] (a) CH_2Cl_2, $T = 0$ °C, pyridine, phenyl chloroformate, room temp, 1 h; (b) pyridine, 2,2-bis(ethy1oxy)ethanamine, $T = 60$ °C, 3 h; (c) CH_2Cl_2, HCl 6N, room temp, 45 min.

Scheme 3. Synthetic Route for Compounds **9**, **10**, **34**, and **35**[a]



[a] (a) EtOH, room temp, NaOH, microwave irradiation, $T = 130$ °C, 20 min; (b) DMF, room temp, NaH, ethyl 2-bromoacetate, $T = 80$ °C, 7.2 h; (c) $T = -78$ °C, $LiAlH_4$ 1 N in THF, THF, 20 min, NaOH 1 N; (d) (methoxycarbonylsulfamoyl)triethylammonium hydroxide, THF, reflux, 1h; (e) MeOH, CH_2Cl_2, NaOH, room temp, 30 min; (f) *n*-BuOH, room temp, 1,2-oxathiolane 2,2-dioxide, microwave irradiation (20 + 60 + 60 min, $T = 150-180$ °C); (g) $POCl_3$, reflux, 1 h.

pounds identified were characterized in terms of in vivo pharmacokinetics in rats,[15] derivative **3** emerged as the most appropriate for progression into the in vivo animal model studies.

Having identified compound **3** as the most attractive member of this series, the mechanism of the antagonism was evaluated using Schild analysis. By increasing the concentration of compound **3**, a right shift of the sauvagine concentration−response curve was observed, which resulted in a slope of the Schild plot close to one (0.95 ± 0.04, $n = 6$). In addition, it did not affect the E_{max} of sauvagine. Its potency, measured as the concentration required doubling the sauvagine ED_{50}, was 7.14 ± 0.12, which is considered the compound pA_2 value. Moreover, at concentrations up to 10 μM, compound **3** did not affect cAMP levels in the absence of sauvagine, confirming its neutral antagonist nature with no evidence of partial agonism. As far as the receptor selectivity is concerned, in CHO cells expressing the human CRF_2 receptor, compound **3** did not measurably affect sauvagine-stimulated (0.5 nM) cAMP up to 10 μM. In addition, it produced less than 50% displacement of binding in the full Cerep battery of 83 G-protein coupled receptors, transporters, ion channels, and enzymes at 10 μM. In summary, these results indicate that **3** is a competitive and highly selective CRF_1 receptor antagonist.

The in vivo pharmacokinetic profile of compound **3** is shown in Table 2. Low plasma clearance, high oral bioavailability, limited volume of distribution, and appropriate half-life were observed. Moreover, a brain/plasma ratio of 3.7 was seen 1 h after the iv administration of the compound, while in gerbils, the brain/plasma ratio was 4.7 after oral administration. This excellent profile was confirmed also in marmosets. On the basis of these positive results, compound **3** was fully characterized in vivo in different animal models of anxiety both in rodents and in primates, namely gerbil forepaw treading,[16,17] ultrasonic

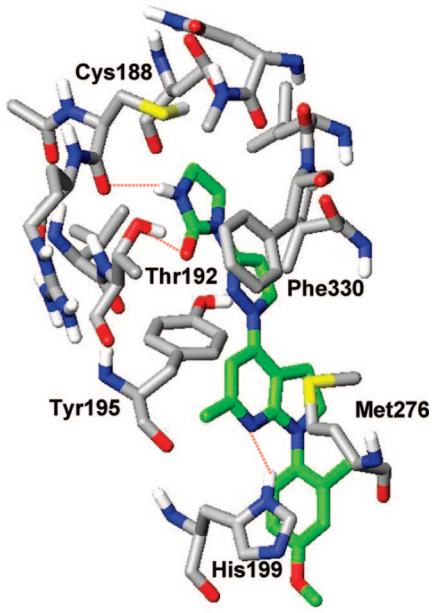


Figure 2. An energetically favorable docking solution of compound **3** in the putative site of the CRF_1 receptor antagonists.

Table 2. In Vivo Pharmacokinetic Profile of Compound **3** in Rat and Marmoset[a]

	rat	marmoset
Cl (mL/min/kg)	19 [18−21]	16 [13−19]
V_{ss} (L/kg)	2.4 [2.3−2.6]	4.7 [4.4−4.9]
$t_{1/2}$ (h)	1.6 [1.4−1.7]	3.7 [3.5−4.0]
F (%)	66 [58−73]	65 [57−75
B/P[b]	3.7 [2.9−4.4]	

[a] All data are reported as mean and range. Doses: 1 mg/kg, iv, 10 mg/kg, po. [b] Assessed 1 h after iv administration. B/P (gerbil) = 4.7 (3.1−6.2), assessed after po administration.

vocalization in rat pups,[18,19] and marmoset human threat test.[20,21] As shown in Figures 3 and 5, NBI30775, the compound that exhibited a positive effect in an open label phase IIA clinical trial in major depressive disorder, was used as reference standard on the gerbil forepaw treading and marmoset human threat test (Figure 3 and 5, respectively), whereas fluoxetine was the reference standard in the ultrasonic vocalization model in rat pups (Figure 4).

In the gerbil forepaw treading model, the intracerebroventricular (icv) injection of CRF in Mongolian gerbils induces a stereotyped behavior characterized by vertical up and down movements of one or both forepaws. This behavior, named "forepaw treading" or "piano playing", can be selectively reversed by systemic administration of CRF_1 receptor antagonists. Briefly, male gerbils (55−75 g) were injected icv with CRF at the dose of 0.6 μg/gerbil (5 μL/gerbil). Preliminary experiments showed that at this dose, piano playing behavior is present starting from 15 min after the injection and reaches its maximal effect after 45 min, remaining stable up to 90 min. The behavior has been scored as time spent in "piano playing" over 10 min starting 50 min after CRF injection. When gerbils were treated orally with compound **3** at 3, 10, and 30 mg/kg, 1 h before test, (Figure 3), a significant dose dependent reduction of time spent in "piano playing" was observed at both 10 and 30 mg/kg. Moreover, a significant long lasting effect was observed at the 30 mg/kg po dose when the compound was given at 60, 180, and 360 min prior to the test. Because this behavioral response is induced by the central administration of the agonist, the effect observed for compound **3** can be attributed to the central activity of the compound itself.

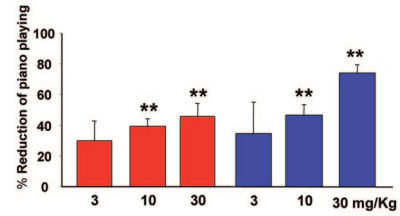


Figure 3. Effect of oral administration of compound **3** (red) and NBI30775 (blue) in piano playing model in gerbil. Data are expressed as mean ± SEM (** = <0.01 ANOVA followed by Dunnett's test).

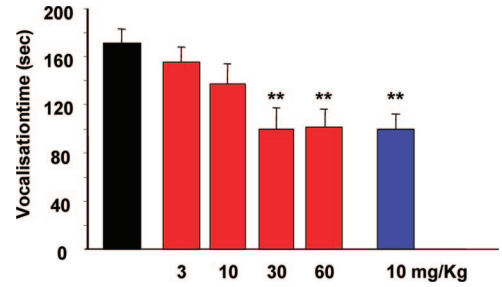


Figure 4. Effect of vehicle (black), compound 3 (red) and fluoxetine (blue) in rat pup vocalization. Data are expressed as mean ± SEM (** = <0.01 ANOVA followed by Dunnett's test).

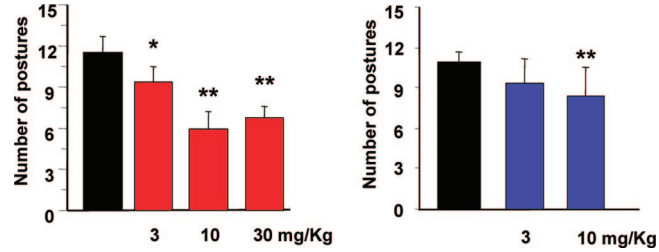


Figure 5. Effect of vehicle (black), compound **3** (red), and NBI30775 (blue) in the human threat in marmosets. Data are expressed as mean ± SEM (** = <0.01 ANOVA followed by Dunnett's test).

In the ultrasonic vocalization model, rat pups are removed from their home cages and placed in an acoustically isolated environment where vocalizations are recorded for a 5 min period. This behavior reflects a state of distress and is a sensitive test for anxiolytic and antidepressant drugs. Benzodiazepines, buspirone, and SSRIs are all effective in reducing the duration of vocalization in this paradigm. When given at 3, 10, 30, and 60 mg/kg, ip, with a pretreatment time of 30 min, compound **3** produced a dose dependent reduction of the duration of vocalization. This effect was significant at 30 and 60 mg/kg.

In the marmoset human threat test, ethological, physiological, and pharmacological evidence suggests that territorial postures performed by the marmoset in response to the presence of the tester in front of the animal's home cage (human threat) reflect the level of stress in this species. This behavioral effect is maintained on repeated exposure to the potential intruder. When animals were treated orally with compound **3** at the doses of 3, 10, and 30 mg/kg one hour before test, a significant dose dependent reduction of the number of postures was observed at all doses. Notably, this compound did not show any sedative effect, as measured in the same experiment by the number of physiological jumps made by the animals.

Conclusions

Despite the large number of in vitro potency and in vivo potent small molecules CRF_1 receptor antagonists disclosed over

the last years, the identification of druggable compounds with appropriate physicochemical and developability features is still highly desirable and remains an area of major challenge in medicinal chemistry today. In particular, most of these molecules are highly lipophilic, resulting in inappropriate pharmacokinetic and toxicological profiles.

The appropriate exploration of the top region of the dihydropyrrole[2,3]pyridine template, driven by the hypothetical presence within the CRF_1 receptor antagonists binding pocket of specific amino acid residues able to make H-bond interaction with the heterocycle moiety present in the upper region of the molecule, enabled the identification of druglike exemplars of CRF_1 receptor antagonists, exhibiting limited overall lipophilicity and high in vitro affinity. In particular, among the number of compounds prepared, derivative **3** showed appropriate in vivo pharmacokinetics and outstanding activity in vivo in an appropriate panel of animal models of anxiety both in rodents and primates, resulting as one of the most promising drug candidate recently reported in the CRF_1 receptor antagonist field.

Experimental

HPLC mass spectra (HPLC-MS) were recorded on a Hewlett-Packard 1100 LC-MSD. All target compounds gave purities >95% a/a (UV). In particular, compound **3** gave a purity of 100% (R_t = 7.06 min). Analytical chromatographic conditions: column: X Terra MS C18 5 μm, 50 mm × 4.6 mm. Mobile phase: A, H_2O + 0.1% TFA; B, CH_3CN + 0.1% TFA. Gradient: 10% (B) for 1 min, from 10% (B) to 90% (B) in 12 min. Flow rate: 1 mL/min. UV wavelenght range: 200−400 nm. Mass range: 100−900 amu. Ionization: ES+. ^{1}H NMR spectra were recorded on a Varian Mercury (300 MHz) or a Bruker DPX (300 MHz) or a Bruker Avance (400 MHz) spectrometer. ^{1}H and ^{13}C NMR for compound **3** were performed on a Varian Inova (600 MHz). Chemical shifts were expressed in parts per million downfield from $CDCl_3$ as an internal standard (δ = 7.24 ppm) and were assigned as singlets (s), doublets (d), triplets (t), quartet (q), broad singlets (bs), doublets of doublets (dd), or multiplets (m). A strategy comprising of nuclear Overhauser effect (NOE) correlation and/or ^{1}H,^{15}N long-range scalar correlations measurements has been implemented in order to allow elucidation of possible regioisomers structure of compounds of the present invention. Proposed structures were verified by measurement of the vicinity in the space of key hydrogen atoms, thus 1D nuclear Overhauser difference spectra were used to measure ^{1}H,^{1}H-dipole−dipole correlations. Mass spectra analyses were performed on a V.G. Platform (Waters, Manchester, UK) mass spectrometer operating in positive electrospray ion mode. Analytical thin layer chromatography (TLC) was performed on glass plates (Merck Kieselgel 60 F254). Visualization was accomplished by UV (ultraviolet) light (254 nm), I_2. Column chromatography was performed on silica gel (Merck Kieselgel 70−230 mesh). All reactions were carried out under anhydrous nitrogen using standard Schlenk techniques. Most chemicals and solvents were analytical grade and used without further purification. Infrared spectra were measured on a Nicolet Magna 760 FT-IR (Fourier transform infrared) instrument.

Biology. CRF_1 Receptor Antagonists Binding Assay. The CRF_1 receptor-binding assay uses the homogeneous technique of scintillation proximity (SPA). The ligand (^{125}I-Sauvagine) binds to the CRF_1 receptor present in membranes prepared from recombinant CHO cells, which in turn bind to wheat germ agglutinin coated SPA beads. The signal generated is measured as CPM and is directly proportional to the amount of ligand bound to the receptor. The [^{125}I]-Sauvagine SPA is performed in a final volume of 100 μL using Costar white, clear bottomed, 96 well plates, 5 μL drug solution in neat DMSO and 95 μL of a mixture of PVT WGA (polyvinyltoluene wheat germ agglutinin) beads (1.0 mg/well), CRF_1−CHO cell membranes (20 μg/well), and [^{125}I]-Sauvagine (0.025 nM) in 50 mM HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid)/KOH pH 7.40, 2 mM EDTA (ethylenediaminetetraacetic acid), 10 mM $MgCl_2$. All values in brackets are final assay concentrations. The nonspecific binding is measured in the presence of 1 μM unlabeled CRF. After addition of all reagents, plates were shaken gently for 15 min, incubated overnight at room temperature, and read the following day using a Perkin-Elmer Trilux 1 min counting time per well.

DMP-696 and NBI30775 affinities values were reported to be determined by filtration binding assay using different radioligands, concentrations, and cell lines (i.e., [^{125}I]Sauvagine at 200−250 pM in Ltk for NBI30775 and [^{125}I]Ovine CRF at 150 pM in 293EBNA for DMP-696, respectively). Taken all together, these methodological differences may account for the discrepancies with data reported in literature.

Functional Antagonism. CHO cells stable transfected with either the human CRF_1 or the human CRF_2 receptor were seeded into 96-well microtiter tissue culture plates and grown overnight. The culture medium was aspirated, and the cells were washed once with PBS (phosphate buffered saline). Each well received assay medium (DMEM (Dulbecco's modified eagle's medium) without phenol red supplemented with 1 mM isobutylmethylxanthine, pyruvate, HEPES, L-glutamine with or without various dilutions of compound **3** followed by sauvagine. To determine functional antagonism at the human CRF_1 receptor, seven concentrations of Sauvagine were tested (ranging from 3.2 pM to 3.2 μM) in the assay medium containing varying concentrations of compound **3** (0, 100 nM, 320 nM, 1 μM, 3.2 μM, and 10 μM). To determine functional antagonism at the human CRF_2 receptor, a fixed concentration of sauvagine (0.5 nM) was tested against varying concentrations of compound **3** (ranging from 0.32 nM to 10 μM). After 30 min incubation at 37 °C, cells were lysed and total cAMP (Cyclic adenosine monophosphate) was determined using a chemiluminescent immunoassay kit (Tropix-PE Biosystems, Bedford, MA).

Receptor Selectivity. The receptogram screen was performed by Cerep assessing the binding of 10 μM on 83 G-Protein coupled receptors, channels, enzymes, and transporters.

Ultrasonic Vocalizations in Rat Pups. The rat pup vocalization test has been validated as a test for anxiolytic/antidepressant-like activity. Rat pups between 9 and 12 days postnatal emit ultrasonic vocalizations (42 KHz) in response to separation from their mother and littermates. This test was originally proposed by Gardner[18] as a sensitive test for anxiolytic and antidepressant drugs. Benzodiazepines, buspirone, CRF_1 antagonists, and SSRIs (selective serotonin reuptake inhibitors) have all been reported to be active in this test. Rat pups were housed with their mother and littermates under standard laboratory conditions. At the age of 9−12 days, pups were screened over a 1 min session for their ability to emit ultrasonic vocalization (42 KHz) after removal from their home cage. Only subjects with duration of vocalization longer than 20 s over the 1 min training session were included in the following test session. Immediately following the training session, animals were returned to their home cages, randomly assigned to treatment groups and treated intraperitoneally (10 mL/kg) with vehicle, compound **3** at 3, 10, 30, or 60 mg/kg and Fluoxetine (10 mg/kg) 30 min before testing (n = 5−10). During the test period, vocalizations were recorded in a sound proof box using a microphone for 5 min. The signal was filtered and transformed into digital block pulse and processed by dedicated software (Ultravox system, Noldus, Netherlands).

Gerbil Forepaw Treading. The intracerebroventricular (icv) injection of CRF in mongolian gerbils induces a stereotyped behavior characterized by vertical up and down movements of one or both forepaws. This behavior has been named "forepaw treading" or "piano playing", and it can be selectively reversed by systemic administration of CRF_1 receptor antagonists.[16,17]

Animals were randomly assigned to treatment groups and treated orally (10 mL/kg) with vehicle, compound **3** at 10 or 30 mg/kg, and NBI-30775 (10 mg/kg) (n = 8−9). Ten minutes later, gerbils were injected icv with 5 μL CRF at a concentration of 0.12 mg/mL to a final CRF amount of 0.6 μg per gerbil. After treatment with CRF, gerbils were placed individually in a standard cage. Gerbil forepaw treading behavior was scored over a 10 min test

period by an observer blind to the drug treatment starting 50 min after CRF injection.

Human Threat Test in Marmosets. It has been hypothesized that the number of territorial postures elicited by marmosets, confronted with a human observer in close proximity to the home cage, may reflect the level of stress induced in the animals. Clinically effective anxiolytic drugs are able to attenuate this response, which is manifest as a decrease in the number of postures performed, in the absence of sedative effects, recorded as a decrease in the number of jumps.[20]

Both marmosets in each housing pair were involved in the test, which was carried out with the animals situated in their home cage. The behavioral response to a human observer standing in close proximity to the home cage was recorded.[21] The number of postures and jumps from the back of the cage to the cage front were measured during the 2 min test to assess potential anxiolytic-like activity and exclude sedation or locomotor stimulation. Only animals that showed a baseline response of at least 10 postures exhibited in the 2 min test period were included in the statistical analysis. Drug treatments were assigned according to a blind crossover design. One hour before the test, four couples of animals were treated orally (1 mL/kg) with vehicle or compound **3** at 3, 10, or 30 mg/kg. After a washout period of at least three days, treatments were reassigned and the study was complete when all animals had received all treatments.

All in vivo studies were conformed to GlaxoSmithKline ethical standards and were conducted in accordance with the Italian law (Legislative Decree no. 116, 27 January 1992), which acknowledges the European Directive (86/609/EEC).

Sprague−Dawley rat pups (Charles River, Italy) and male Mongolian gerbils (55−75 g Charles River, Germany) were housed under standard laboratory conditions on a 12 h light/dark cycle with lights on at 6 a.m. and food and water available ad libitum.

Human threat test studies utilized laboratory-bred male (vasectomized) and female common marmosets over 2 years of age, weighing 300−500 g. The animals were caged in couples, in a housing room maintained at 25 ± 1 °C, 60% humidity and a 12 h light/dark cycle (lights on at 6 a.m., with 30 min simulated dawn and twilight). Water was available ad libitum and food administered twice daily (7 a.m. and 14 p.m.).

Data Analysis. Data were subjected to one-way analysis of variance (ANOVA, completely randomized design), followed by Dunnett's test. Statistical significance was considered when $p < 0.05$. All data were analyzed by using GB-Stat software (version 8.0, Dynamic Microsystems Inc.).

pIC_{50} values, defined as the negative logarithm of the antagonist concentrations that cause 50% inhibition of ^{125}I-Sauvagine binding at the human recombinant CRF_1 receptor, were determined by fitting the displacement binding data using a four parameter-logistic equation.

The effect of compound **3** on the sauvagine EC_{50} was analyzed using the method of Arunlakshana and Schild[22] to obtain estimates of the compound pA_2 value (negative logarithm of the concentration of compound **3** required to double the sauvagine EC_{50}, with an unconstrained Schild slope).

Docking Experiments. Flexible docking experiments in the putative binding site of the CRF_1 receptor model were carried out with routines (MCMM) implemented in MacroModel/Batchmin. Random sampling was performed for 2K steps followed by energy minimization of the complexes sampled with AMBER united atom force field (1K steps). ESP charges (AM1 Hamiltonian, MOPAC) were utilized for the ligands while charges from force field were used for the protein.

The synthesis of compound **3** is presented as general procedure for the preparation of compounds **3**−**22**, shown in Scheme 1.

4-Chloro-*N*-[2-methyl-4-(methyloxy)phenyl]butanamide (24). To a solution of 2-methyl-4-methoxy aniline **23** (10 g, 72.9 mmol) in 100 mL of dry dichloromethane under nitrogen atmosphere at room temperature, K_2HPO_4 (25.4 g, 146 mmol) was added. 4-Chloro-butyrilchloride (8.2 mL, 72.9 mmol) was then added dropwise. The mixture was stirred at room temperature for 1 h. The mixture was then diluted with water (50 mL), extracted with EtOAc (3 × 100 mL), washed with brine, and dried over anhydrous Na_2SO_4. After removal of solvent in vacuo, a purple solid was obtained, which was triturated with acetonitrile (100 mL) and then with Et_2O (100 mL) to give the title compound (10.8 g, 61% yield) as pale-purple solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.12 (t, 2H), 2.18 (s, 3H), 2.58 (t, 2H), 3.64 (t, 2H), 3.75 (s, 3H), 6.79−6.72 (m, 2H), 7.05 (d, 1H). MS: *m*/*z* 242 $[M + H]^+$.

1-[2-Methyl-4-(methyloxy)phenyl]-2-pyrrolidinone (25). To a suspension of sodium hydride (95%, 1.24 g, 49.1 mmol) in 20 mL of dry DMF under a nitrogen atmosphere at 0 °C, a solution of intermediate **24** (10.8 g, 44.7 mmol) in 100 mL of dry DMF was added dropwise. The mixture was stirred at this temperature for 30 min. The mixture was then diluted with H_2O (100 mL) and extracted with Et_2O (3 × 100 mL) and EtOAc (100 mL). The organic extracts were collected, washed with brine (2 × 150 mL), and dried over anhydrous Na_2SO_4. Removal of solvent in vacuo gave a purple oil (12.5 g), which was purified by chromatography (Biotage, Cartridge 75M, eluant EtOAc/cyclohexane = 2/1, 4 L) to give the title compound (7.42 g, 80% yield) as pale-pink solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.12 (t, 2H), 2.18 (s, 3H), 2.55 (t, 2H), 3.65 (t, 2H), 3.75 (s, 3H), 6.79−6.72 (m, 2H), 7.05 (d, 1H). MS: *m*/*z* 206.3 $[M + H]^+$.

Ethyl(2*E*)-3-({(2*E*)-1-[2-methyl-4-(methyloxy)phenyl]-2-pyrrolidinylidene}amino)-2-butenoate (26). To a solution of intermediate **25** (870 mg, 4.24 mmol) in 10 mL of dry 1,2-dichloroethane was added freshly distilled $POCl_3$ (0.79 mL, 8.48 mmol) dropwise under a nitrogen atmosphere at room temperature, followed by β-amino-ethylcrotonate (547 mg, 4.24 mmol). The resulting mixture was heated to 60 °C for 8 h. The reaction mixture was diluted with EtOAc (20 mL) and washed with saturated aqueous $NaHCO_3$ (20 mL) until neutral pH. The organic phase was then dried over anhydrous Na_2SO_4. After filtration, the solvent was evaporated in vacuo to give the title compound (1.2 g, 90% yield) as yellow oil, which was used without any further purification in the next step. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.15−1.25 (m, 3H), 2.2 (m, 5H), 2.3 (s, 3H), 2.62 (t, 2H), 3.65 (t, 2H), 3.74 (s, 3H), 4.01 (q, 2H), 5.07 (s, 1H), 6.79−6.72 (m, 2H), 7.05 (m, 1H). MS: *m*/*z* 389 $[M + H]^+$.

6-Methyl-1-[2-methyl-4-(methyloxy)phenyl]-1,2,3,7-tetrahydro-4*H*-pyrrolo[2,3-*b*]pyridin-4-one (27). To a suspension of sodium hydride (95%, 192 mg, 7.59 mmol) in 4 mL of dry DMF under nitrogen atmosphere at room temperature, a solution of intermediate **26** (1.2 g, 3.79 mmol) in 8 mL of dry DMF was added dropwise. The resulting orange suspension was heated to 90−100 °C for 1.5 h. The reaction mixture was then diluted with H_2O (10 mL) and Et_2O (20 mL). NaOH 3N was then added until pH 8−9, the aqueous phase was extracted several times with Et_2O, and the combined extracts were washed with brine. The organic layer were collected, dried over sodium sulfate, filtered, and concentrated under reduced pressure to give 350 mg of a black oil (crude 1). The aqueous phase was again extracted with EtOAc (3 × 50 mL) and with CH_2Cl_2 (3 × 50 mL) and the combined organic phase washed with brine and dried over anhydrous Na_2SO_4. Removal of the solvent in vacuo gave a brown oil (crude 2), which was combined with crude 1, filtered over a silica gel pad, eluted with CH_2Cl_2 (200 mL), CH_2Cl_2/MeOH 5% (400 mL), CH_2Cl_2/MeOH 10% (500 mL), to give the title compound (175 mg, 18% yield) as pale-brown foam. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.8 (b, 1 H), 7.08 (d, 1 H), 6.80 (d, 1 H), 6.75 (dd, 1 H), 5.92 (s, 1 H), 3.72 (s, 3H), 3.68 (t, 2H), 2.89 (t, 2H), 2.12 (s, 3H), 2.02 (s, 3H). MS: *m*/*z* 271 $[M + H]^+$.

6-Methyl-1-[2-methyl-4-(methyloxy)phenyl]-2,3-dihydro-1*H*-pyrrolo[2,3-*b*]pyridin-4-yl-4- trifluoromethyl)benzenesulfonate (28). To a solution of intermediate **27** (120 mg, 0.44 mmol) in 5 mL of dry CH_2Cl_2 under a N_2 atmosphere at −78 °C, dry pyridine (78 μL, 0.968 mmol) was added, followed by triflic anhydride (75 μL, 0.484 mmol). The mixture was stirred for 1 h at room temperature. The reaction mixture was diluted with CH_2Cl_2 (15 mL) and washed with aqueous saturated NH_4Cl (5 mL) and brine (5 mL). The organic phase was then dried over anhydrous Na_2SO_4,

filtered, and after removal of the solvent in vacuo, the residue was filtered over a silica gel pad (CH_2Cl_2) to give the title compound (148 mg, 84% yield) as pale-yellow oil. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.17 (d, 1H), 6.85 (d, 1H), 6.77 (dd, 1H), 6.40 (s, 1H), 3.89 (t, 2H), 3.73 (s, 3H), 3.16 (t, 2H), 2.17−2.11 (bs, 6H). MS: *m/z* 403 $[M + H]^+$.

4-Iodo-6-methyl-1-[2-methyl-4-(methyloxy)phenyl]-2,3-dihydro-1*H*-pyrrolo[2,3-*b*]pyridine (29). To a solution of intermediate **28** (913 mg, 2.27 mmol) in anhydrous NMP (7 mL) was added KI (1.13 g,) and the reaction mixture was stirred at 150 °C for 18 h. It was then cooled down to room temperature and poured in water. The aqueous phase was extracted with EtOAc (3 × 30 mL), and the combined organic extracts were dried over anhydrous Na_2SO_4. The solid was filtered, and the solvent evaporated in vacuo. The crude residue was then purified by flash chromatography (cyclohexanes/EtOAc 9:l) to give the title compound as a clear oil, which solidified upon standing (681 mg, 79% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.14 (d, 1H), 6.81−6.74 (m, 2H), 6.70 (s, 1H), 3.84 (t, 2H), 3.81 (s,3H), 3.03 (t, 2H), 2.22 (s, 6H). MS: *m/z* 381 $[M + H]^+$.

1-(1-{6-Methyl-1-[2-methyl-4-(methyloxy)phenyl]-2,3-dihydro-1*H*-pyrrolo[2,3-*b*]pyridin-4-yl}-1*H*-pyrazol-3-yl)-2-imidazolidinone (3). In a sealed vial, at room temperature, under nitrogen atmosphere, compound **29** (60 mg, 0.158 mmol), CuI (6 mg, 0.032 mmol), and K_2CO_3 (4.5 mg, 0.4 mmol) were added to a solution of dodecane (14.3 μL, 0.063), (1*R*,2*R*)-1,2-cyclohexanediamine (14 μL, 0.095), and 1-(1*H*-pyrazol-3-yl)-2-imidazolidinone (intermediate **8**, 48 mg, 0.316) in anhydrous NMP (*N*-methylpyrrolidinone) (5 mL) and the reaction mixture was stirred at 130 °C for 3.5 h. It was then cooled down to room temperature and poured in EtOAc/H_2O. The organic phase was separated, and the aqueous layer was extracted with EtOAc (2 × 10 mL). The combined organic extracts were dried over anhydrous Na_2SO_4, the solids were filtered, and the solvent evaporated in vacuo. The crude compound was purified by flash chromatography (EtOAc/cyclohexanes 6:4, then 1:1, then 3:7) followed by a SCX cartridge (100% MeOH, then 2 M NH_3/MeOH), to give the title compound as a white solid (34 mg, 53%). ^{1}H NMR (600 MHz, DMSO-d_6) δ8.31 (d, J = 2.75 Hz, 1H), 7.17 (d, J = 8.79 Hz, 1H), 7.06 (s, 1H), 6.86 (d, J = 2.75 Hz, 1H), 6.68−6.83 (m, 2H), 3.87−4.06 (m, 1H), 3.83 (t, J = 8.38 Hz, 1H), 3.76 (s, 2H), 3.46 (t, J = 8.24 Hz, 2H), 3.31 (s, 3H), 2.16 (d, J = 12.36 Hz, 3H). MS: *m/z* 405 $[M + H]^+$. ^{13}C NMR (150.81 MHz, DMSO-d_6): 163.64, 158.06, 157.34, 155.81, 150.28, 141.51, 137.27, 133.84, 129.65, 127.36, 115.69, 111.87, 106.64, 101.25, 97.28, 55.15, 51.54, 43.33, 37.21, 26.66, 23.93, 18.34.

1-(1-{6-Ethyl-1-[2-methyl-4-(methyloxy)phenyl]-2,3-dihydro-1*H*-pyrrolo[2,3-*b*]pyridin-4-yl}-1*H*-pyrazol-3-yl)-2-pyrrolidinone (4). Compound obtained as a pale-yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.35 (d, 1H), 7.20 (d, IH), 6.95 (d, 1H), 6.85 (d, 1H), 6.75 (m, 2H), 3.90 (m, 4H), 3.70 (s, 3H), 3.45 (t, 2H), 2.50 (m, 2H), 2.15 (s, 3H), 2.10 (m, 2H), 2.10 (s, 3H). MS: *m/z* 404 $[M + H]^+$.

1-Methyl-3-(1-{6-methyl-1-[2-methyl-4-(methyloxy)phenyl]-2,3-dihydro-1*H*-pyrrolo[2,3-*b*]pyridin-4-yl}-1*H*-pyrazol-3-yl)-2-imidazolidinone (5). Compound obtained as a pale-yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.76 (d, 1H), 7.15 (d, 1H), 6.93 (d, 1H), 6.80−6.75 (m, 3H), 6.52 (s, 1H), 3.96 (t, 2H), 3.84 (t, 2H), 3.77 (s, 2H), 3.50 (t, 2H), 3.42 (t, 2H), 3.89 (s, 3H), 2.28 (s, 3H), 2.20 (s, 3H). MS: (*m/z*) 419 $[MH]^+$.

3-(1-{6-Methyl-1-[2-methyl-4-(methyloxy)phenyl]-2,3-dihydro-1*H*-pyrrolo[2,3-*b*]pyridin-4-yl}-1*H*-pyrazol-3-yl)-1,3-oxazolidin-2-one (6). Compound obtained as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.85 (d, 1H), 7.16 (d, 1H), 6.93 (d, 1H), 6.81 (d, 1H), 6.77 (dd, 1H), 6.55 (s, 1H), 4.54 (t, 2H), 4.2 (t, 2H), 3.87 (t, 2H), 3.8 (s, 3H), 3.44 (t, 2H), 2.32 (s, 3H), 2.24 (s, 3H). MS: *m/z* 406 $[M + H]^+$.

1-(1-{6-Methyl-1-[2-methyl-4-(methyloxy)phenyl]-2,3-dihydro-1*H*-pyrrolo[2,3-*b*]pyridin-4-yl}-1*H*-pyrazol-3-yl)tetrahydro-2(1*H*)-pyrimidinone (8). Compound as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.80 (d, 1H), 7.2 (d, 1H), 7.0 (d, 1H), 6.80 (d, 1H), 6.75 (dd, 1H), 6.60 (s, 1H), 4.95 (bs, 1H), 4.05 (dd, 2H), 3.90 (t,2H), 3.80 (s, 3H), 3.45 (t, 2H), 3.40 (bm, 2H), 2.45 (s, 3H), 2.25 (s, 3H), 2.05 (m, 2H). MS: *m/z* 419 $[M + H]^+$.

1-(1-{1-[4-(Ethyloxy)-2-methylphenyl]-6-methyl-2,3-dihydro-1*H*-pyrrolo[2,3-*b*]pyridin-4-yl}-1*H*-pyrazol-3-yl)-2-imidazolidinone] (11). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.82 (d, 1H), 7.15 (d, lH), 6.90 (d, 1H), 6.78 (d, 1H), 6.75 (dd, 1H), 6.70 (dd, 1H), 4.70 (bs, 1H), 4.2−4.0 (m, 4H), 3.80 (t, 2H), 3.60 (t, 2H), 3.40 (t, 2H), 2.30 **(s,** 3H), 2.25 (s, 2H), 1.45 (t, 3H). MS: *m/z* 419 $[M + H]^+$.

1-[1-(6-Methyl-1-{2-methyl-4-[(1-methylethyl)oxy]phenyl}-2,3-dihydro-1*H*-pyrrolo[2,3-*b*]pyridin-4-yl)-1*H*-pyrazol-3-yl]-2-imidazolidinone (12). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.90 (d, 1H), 7.10 (d, lH), 6.90 (d, 1H), 6.8−6.7 (m, 2H), 6.55 (s, 1H), 4.60 (bs, 1H), 4.50 (m, 1H), 4.10 (t, 2H), 3.90 (t, 2H), 3.60 (t, 2H), 3.45 (t, 2H), 2.30 (s, 3H), 2.20 (s, 3H), 1.26 (d, 6H). MS: *m/z* 475 $[M + H]^+$.

1-[1-(6-Methyl-1-{2-methyl-4-[(trifluoromethyl)oxy]phenyl}-2,3-dihydro-1*H*-pyrrolo[2,3-*b*]pyridin-4-yl)-1*H*-pyrazol-3-yl]-2-imidazolidinone (13). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.84 (d, 1H), 7.30 (d, 1H), 7.12 (s, 1H), 7.09 (d, 1H), 6.98 (d, 1H), 6.63 (s, 1H), 4.68 (s, 1H), 4.10 (t, 2H), 3.91 (t, 2H), 3.63 (t, 2H), 3.50 (t, 2H), 2.36 (s, 3H), 2.29 (s, 3H). MS: *m/z* 459 $[M + H]^+$.

3-Methyl-4-{6-methyl-4-[3-(2-oxo-1-imidazolidinyl)-1*H*-pyrazol-1-yl]-2,3-dihydro-1*H*-pyrrolo[2,3-*b*]pyridin-1-yl}benzonitrile (14). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.82 (d, 1H), 7.6 (bs, 1H), 7.49 (m, 1H), 7.34 (m, 1H), 6.96 (d, 1H), 6.65 (s, 1H), 4.7 (bs, 1H), 4.11 (t, 2H), 3.92 (t, 2H), 3.62 (t, 2H), 3.49 (t, 2H), 2.41 (s, 3H), 1.22 (t, 3H).). MS: *m/z* 414 $[M + H]^+$.

1-(1-{6-Methyl-1-[2-methyl-4-(1*H*-pyrazol-1-yl)phenyl]-2,3-dihydro-1*H*-pyrrolo[2,3-*b*]pyridin-4-yl}-1*H*-pyrazol-3-yl)-2-imidazolidinone (15). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.90 (d, 1H), 7.84 (d, 1H), 7.72 (d, 1H), 7.68 (m, 1H), 7.64 (m, 1H), 7.37 (d, 1H), 6.97 (d, 1H), 6.62 (s, 1H), 6.45 (t, 1H), 4.77 (s, 1H), 4.11 (t, 2H), 3.94 (t, 2H), 3.63 (t, 2H), 3.50 (t, 2H), 2.34 (s, 6H). MS: *m/z* 441 $[M + H]^+$.

4-{6-Methyl-4-[3-(2-oxo-1-imidazolidinyl)-1*H*-pyrazol-1-yl]-2,3-dihydro-1*H*-pyrrolo[2,3-b]pyridin-1-yl}-3-(trifluoromethyl)-benzonitrile (16). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.01 (d, 1H), 7.85 (d, 1H), 7.84 (dd, 1H), 7.69 (d, 1H), 7.00 (d, 1H), 6.75 (s, 1H), 4.65 (s, 1H), 4.10 (t, 2H), 3.98 (t, 2H), 3.64 (t, 2H), 3.51 (t, 2H), 2.36 (s, 3H). MS: *m/z* 454 $[M + H]^+$.

1-(1-{1-[2-(Difluoromethyl)-4-(methyloxy)phenyl]-6-methyl-2,3-dihydro-1*H*-pyrrolo[2,3-*b*]pyridin-4-yl}-1*H*-pyrazol-3-yl)-2-imidazolidinone (17). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.84 (d, 1H), 7.21 (m, 2H), 7.02 (dd, 1H), 6.87 (t, 1H), 6.97 (d, 1H), 6.64 (s, 1H), 4.84 (bs, 1H), 4.13 (t, 2H), 3.93 (t, 2H), 3.9 (s, 3H), 3.66 (t, 2H), 3.53 (t, 2H), 2.33 (s, 3H). MS: *m/z* 441 $[M + H]^+$.

4-{6-Methyl-4-[3-(2-oxo-1-imidazolidinyl)-1*H*-pyrazol-1-yl]-2,3-dihydro-1*H*-pyrrolo[2,3-*b*]pyridin-1-yl}-3-[(trifluoromethyl)-oxy]benzonitrile (18). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.05 (d, 1H), 7.84 (m, 1H), 7.54 (bs, 1H), 7.5 (m, 1H), 6.98 (m, 1H), 6.78(s, 1H), 4.63 (bs, 1H), 4.11 (m, 4H), 3.62 (t, 2H), 3.48 (t, 2H), 2.41 (s, 3H). MS: *m/z* 470 $[M + H]^+$.

3-Ethyl-4-{6-methyl-4-[3-(2-oxo-1-imidazolidinyl)-1*H*-pyrazol-1-yl]-2,3-dihydro-1*H*-pyrrolo[2,3-*b*]pyridin-1-yl}benzonitrile (19). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.82 (d, 1H), 7.6 (bs, 1H), 7.49 (m, 1H), 7.34 (m, 1H), 6.96 (d, 1H), 6.65 (s, 1H), 4.7 (bs, 1H), 4.11 (t, 2H), 3.92 (t, 2H), 3.62 (t, 2H), 3.49 (t, 2H), 2.66 (m, 2H), 2.41 (s, 3H), 1.22 (t, 3H). MS: *m/z* 414 $[M + H]^+$.

1-(1-{6-Methyl-1-[2-(methyloxy)-4-(1*H*-pyrazol-1-yl)phenyl]-2,3-dihydro-1*H*-pyrrolo[2,3-*b*]pyridin-4-yl}-1*H*-pyrazol-3-yl)-2-imidazolidinone (20). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.89 (d, 1H), 7.82 (d, 1H), 7.69 (d, 1H), 7.58 (d, 1H), 7.42 (d, 1H), 7.16 (dd, 1H), 6.94 (d, 1H), 6.62 (s, 1H), 6.44 (t, 1H), 4.68 (bs, 1H), 4.13−3.66 (t, 4H), 3.89 (s, 3H), 3.93−3.53 (t, 4H), 2.34 (s, 3H). MS: *m/z* 457 $[M + H]^+$.

1-{1-[1-(2,4-Dichlorophenyl)-6-methyl-2,3-dihydro-1*H*-pyrrolo[2,3-*b*]pyridin-4-yl]-1*H*-pyrazol-3-yl}-2-imidazolidinone (21). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.82 (s, lH), 7.78 (s, lH), 7.44 (s, lH), 7.42 (s, lH), 7.26 (s, lH), 6.95 (s, lH), 6.66 (s, lH), 4,57 (b s,

lH), 4.09 (t, 2H), 3.96 (t, 2H), 3.61 (t, 2H), 3.48 (t, 2H), 2.34 (s, 3H). MS: *m/z* 429 $[M + H]^+$.

1-(1-{1-[2,4-Bis(trifluoromethyl)phenyl]-6-methyl-2,3-dihydro-1*H*-pyrrolo[2,3-*b*]pyridin-4-yl}-1*H*-pyrazol-3-yl)-2-imidazolidinone (22). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.96 (s, 1H), 7.83 (d, 1H), 7.80 (b s, 1H), 7.61 (d, 1H), 6.97 (d, 1H), 6.70 (s, 1H), 4.66 (b s, 1H), 4.08 (t, 2H), 3.91 (t, 2H), 3.61 (t, 2H), 3.50 (t, 2H), 2.31 (s, 3H). MS: *m/z* 497 $[M + H]^+$.

Phenyl(1-{6-methyl-1-[2-methyl-4-(methyloxy)phenyl]-2,3-dihydro-1*H*-pyrrolo[2,3-*b*]pyridin-4-yl}-1*H*-pyrazol-3-yl)carbamate (36). To a suspension of **31** (391 mg, 1.17 mmol) in anhydrous CH_2Cl_2 (8 mL), at 0 °C, under nitrogen atmosphere, were added pyridine (103 μL, 1.29 mmol) and phenyl chloroformate (161 μL, 1.29 mmol). The reaction mixture was stirred at room temperature for 1 h. The solvent was evaporated in vacuo, and then brine was added (50 mL) and the solution extracted with EtOAc (3 × 15 mL). The combined organic extracts were dried over anhydrous Na_2SO_4, the solids were filtered, and the solvent evaporated in vacuo. The crude residue was purified by flash chromatography, (MeOH/NH_3 solution 0.5 M in MeOH) to give 284 mg (53%) of the title compound as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 10.8 (bs, 1 H), 8.28 (d, 1H), 7.39−7.46 (m, 4H), 7.18 (m, 4H), 6.73 (dd, 1H), 6.64 (s, 1H), 3.81 (t, 2H), 3.72 (s, 3H), 3.32 (t, 2H), 2.14 (s, 3H), 2.12 (s, 3H). MS: *m/z* 456 $[M + H]^+$.

2,2-Bis(ethyloxy)ethyl(1-{6-methyl-1-[2-methyl-4-(methyloxy)-phenyl]-2,3-dihydro-1*H*-pyrrolo[2,3-*b*]pyridin-4-yl}-1*H*-pyrazol-3-yl)carbamate (37). A mixture of **36** (284 mg, 0.62 mmol), pyridine (50 μL, 0.74 mmol), and 2,2-bis(ethy1oxy)ethanamine (108 μL, 0.74 mmol) was heated for 3 h at 60 °C. H_2O (50 mL) was then added and the solution extracted with CH_2Cl_2 (3 × 15 mL). The combined organic extracts were dried over anhydrous Na_2SO_4, the solids were filtered, and the solvent was evaporated in vacuo. The crude residue was purified by flash chromatography (cyclohexanes/EtOAc 1:l) to give 214 mg (84%) of the title compound as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.21 (s, 1H), 8.24 (d, 1H), 7.15 (d, 1H), 6.95 (bs, 1H), 6.95 (d, 1H), 6.78 (dd, 1H), 6.72 (s, 1H), 6.42 (s, 1H), 4.5 (m, 1H), 3.82 (t, 2H), 3.60 (m, 1H), 3.48 (m, 1H), 3.65 (s, 3H), 3.3 (m, 6H), 3.23 (t, 2H), 3.16 (s, 3H), 2.14 (s, 3H), 2.12 (s, 3H). MS: *m/z* 495 $[M + H]^+$.

1-(1-{6-Methyl-1-[2-methyl-4-(methyloxy)phenyl]-2,3-dihydro-1*H*-pyrrolo[2,3-*b*]pyridin-4-yl}-1*H*-pyrazol-3-yl)-1,3-dihydro-2*H*-imidazol-2-one (7). To a solution of **37** (50 mg, 0.1 mmol) in anhydrous CH_2Cl_2 (2 mL) was added HCl 6N (200 μL). The reaction mixture was stirred at room temperature for 45 min. It was then neutralized with 1 M $NaHCO_3$ (1 mL), and the solvent was evaporated in vacuo. The crude residue was purified by flash chromatography (cyclohexanes/EtOAc 1:l) to give 18 mg (45%) of the title compound as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.85 (d, 1H), 7.79 (bs, 1H), 7.12 (d, 1H), 7.03 (d, 1H), 6.96 (m, 1H), 6.76 (d, 1H), 6.72 (dd, 1H), 6.52 (s, 1H), 6.33 (m, 1H), 3.82 (t, 2H), 3.74 (s, 3H), 3.41 (t, 2H), 3.27 (s, 3H), 2.18 (s, 3H). MS: *m/z* 403 $[M + H]^+$.

***N*-(1-{6-Methyl-1-[2-methyl-4-(methyloxy)phenyl]-2,3-dihydro-1*H*-pyrrolo[2,3-*b*]pyridin-4-yl}-1*H*-pyrazol-3-yl)acetamide (30).** A mixture of intermediate **29** (500 mg, 1.32 mmol), *N*-1*H*-pyrazol-3-ylacetamide (329 mg, 2.64 mmol), CuI (50 mg, 0.264 mmol), K_2CO_3 (382 mg, 2.77 mmol), dodecane (60 μL, 0.264 mmol), and (1*R*,2*R*)-1,2-cyclohexanediamine (45 μL, 0.396) in anhydrous NMP (5 mL), in a sealed vial, was heated at 150 °C for 4 h. It was cooled down to room temperature and poured into saturated aqueous NH_4Cl. EtOAc was added, and the organic phase was separated. The aqueous layer was then extracted with EtOAc (2 × 10 mL). The combined organic extracts were dried over anhydrous Na_2SO_4, the solids were filtered, and the solvent was evaporated in vacuo. The residue was purified on SCX (strong cation-exchange) cartridge (CH_2Cl_2, then MeOH, then concentrated aqueous NH_4OH/MeOH, 25:75) and then by flash chromatography (cyclohexanes/EtOAc 7:3) to give the title compound as a white solid (358 mg, 72%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.62 (bs, 1H), 8.24 (d, 1H), 7.15 (d, 1H), 6.84 (d, 1H), 6.78−6.73 (m, 3H), 3.83 (t, 2H), 3.74 (s, 3H), 3.4 (t, 2H), 2.16 (s, 3H), 2.14 (s, 3H), 2.04 (s, 3H). MS: *m/z* 378 $[M + H]^+$.

1-{6-Methyl-1-[2-methyl-4-(methyloxy)phenyl]-2,3-dihydro-1*H*-pyrrolo[2,3-*b*]pyridin-4-yl}-1*H*-pyrazol-3-amine (31). To a dispersion of **30** (358 mg, 0.95 mmol) in EtOH (7 mL), at room temperature, was added 25% aqueous solution NaOH (2.5 mL) and the reaction mixture was irradiated in a microwave apparatus for 20 min at 130 °C. The solvent was evaporated in vacuo, and the crude residue was partitioned between EtOAc and brine. The organic phase was separated, and the aqueous layer was extracted with EtOAc (2 × 10 mL). The combined organic extracts were dried over anhydrous Na_2SO_4, the solids were filtered, and the solvent evaporated in vacuo to give the title compound (282 mg, 89%), which was used in the next step without any further purification. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.98 (d, 1H), 7.12 (d, 1H), 6.83 (d, 1H), 6.75 (dd, 1H), 6.67 (s, 1H), 5.77 (d, 1H), 5.10 (bs, 2H), 3.78 (t, 2H), 3.74 (s, 3H), 3.35 (t, 2H), 2.13 (s, 3H), 2.12 (s, 3H). MS: *m/z* 336 $[M + H]^+$.

Methyl *N*-(1-{6-Methyl-1-[2-methyl-4-(methyloxy)phenyl]-2,3-dihydro-1*H*-pyrrolo[2,3-*b*]pyridin-4-yl}-1*H*-pyrazol-3-yl)glycinate (32). To a solution of **31** (200 mg, 0.6 mmol) in anhydrous DMF (5 mL), at room temperature, under nitrogen atmopshere, was added NaH 60% in oil (26 mg, 1.1 equiv). The reaction mixture was stirred at room temperature for 20 min, ethyl 2-bromoacetate (73 μL, 1.1 equiv) was then added dropwise and the reaction mixture was heated at 80 °C. Continuous additions of the alkyl bromide were done at 80 °C over a period of 7.2 h (5 × 36 μL, 5 × 0.55 equiv). The reaction mixture was cooled down to room temperature and poured into H_2O. EtOAc was added, and the phases were separated. The aqueous layer was further extracted with EtOAc (2 × 10 mL). The combined organic extracts were dried over anhydrous Na_2SO_4, the solids were filtered, and the solvent was evaporated. The residue was purified by flash chromatography (silica gel, cyclohexanes/EtOAc 7:3) to give the title compound as a yellow oil (155 mg, 62%). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.09 (d, 1H), 7.13 (d, 1H), 6.83 (d, 1H), 6.76 (dd, 1H), 6.37 (s, 1H), 6.17 (t, 1H), 5.86 (d, 1H), 4.09 (q, 2H), 3.77 (t, 2H), 3.74 (s, 3H), 3.36 (t, 2H), 2.13 (bs, 6H), 1.17 (t, 3H). MS: *m/z* 422 $[M + H]^+$.

2-[(1-{6-Methyl-1-[2-methyl-4-(methyloxy)phenyl]-2,3-dihydro-1*H*-pyrrolo[2,3-*b*]pyridin-4-yl}-1*H*-pyrazol-3-yl)amino]ethanol (33). To a solution of 1 N $LiAlH_4$/THF (0.5 mL, 0.5 mmol), under nitrogen atmosphere, was added dropwise at −78 °C a solution of intermediate **32** (77.5 mg, 0.184 mmol) in anhydrous THF (5 mL). The reaction mixture was stirred at −78 °C for 20 min. Continuous additions of 1 N $LiAlH_4$/THF were done at this temperature over a period of 40 min (3 × 0.2 mL, 3 × 0.2 mmol). To the reaction mixture was added water (42 μL), 1 N NaOH (42 μL), water again (0.12 mL), and a precipitate was formed. The solid was filtered and washed with EtOAc and CH_2Cl_2. The combined organic extracts were concentrated in vacuo, and the residue was purified by flash chromatography (silica gel, cyclohexanes/EtOAc 7:3) to give the title compound as a yellow solid (25 mg, 36%). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.04 (d, 1H), 7.13 (d, 1H), 6.83 (d, 1H), 6.76 (dd, 1H), 6.66 (s, 1H), 5.82 (d, 1H), 5.58 (t, 1H), 4.59 (t, 1H), 3.78 (t, 2H), 3.74 (s, 3H), 3.55 (q, 2H), 3.40 (t, 2H), 3.20 (q, 2H), 2.13 (bs, 6H). MS: *m/z* 380 $[M + H]^+$.

Methyl-5-(1-{6-methyl-1-[2-methyl-4-(methyloxy)phenyl]-2,3-dihydro-1*H*-pyrrolo[2,3-*b*]pyridin-4-yl}-1*H*-pyrazol-3-yl)-1,2,5-thiadiazolidine-2-carboxylate 1,1-dioxide (34). To a vial under nitrogen atmosphere were added intermediate **33** (20 mg, 0.052 mmol), (methoxycarbonylsulfamoyl)triethylammonium hydroxide (40 mg, 0.166 mmol), and anhydrous THF (1 mL). The reaction mixture was refluxed for 1 h. Then, it was cooled down to room temperature and diluted with CH_2Cl_2. 1 N HCI was added, and the organic phase was separated. The aqueous layer was further extracted with CH_2Cl_2 (3 × 10 mL). The combined organic extracts were concentrated in vacuo. The crude residue was purified by flash chromatography (cyclohexanes/EtOAc 7:3) to give the title compound as a white solid (8.2 mg, 32%). ^{1}H NMR (300 MHz, $CDCl_3$)

δ: 7.88 (d, 1H), 7.17 (d, 1H), 6.82 (d, 1H), 6.77 (dd, 1H), 6.51 (s, 1H), 6.5 (d, 1H), 4.1 (bt, 4H), 3.95 (s, 3H), 3.87 (t, 2H), 3.81 (s, 3H), 3.46 (t, 2H), 2.32 (s, 3H), 2.24 (s, 3H). MS: *m*/*z* 499 $[M + H]^+$.

4-[3-(1,1-Dioxido-1,2,5-thiadiazolidin-2-yl)-1*H*-pyrazol-1-yl]-6-methyl-1-[2-methyl-4-methyloxy)phenyl]-2,3-dihydro-1*H*-pyrrolo[2,3-*b*]pyridine (9). Compound **34** (10 mg, 0.022 mmol) and $POCl_3$ (1 mL) were mixed in a vial under nitrogen atmosphere. The reaction mixture was refluxed for 1 h. Then saturated aqueous $NaHCO_3$ was added until neutral pH and the mixture was partitioned between water and EtOAc. The two phases were separated, and the aqueous layer was further extracted with EtOAc (3 × 10 mL). The combined organic extracts were concentrated, and the residue was purified by flash chromatography (cyclohexanes/EtOAc 1:l to EtOAc/NH_3 solution 0.5 M in MeOH 7:3) to give the title compound as a white solid (4.2 mg, 50%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.84 (d, 1 H), 7.16 (d, 1H), 6.82 (d, 1H), 6.77 (dd, 1H), 6.52 (s, 1H),6.51 (d, 1H), 3.98 (t, 2H), 3.87 (t, 2H), 3.80 (s, 3H), 3.46 (m, 2H), 3.37 (t, 2H), 2.57 (m, 2H), 2.32 (s, 3H), 2.23 (s, 3H). MS: *m*/*z* 439 $[M + H]^+$.

3-[(1-{6-Methyl-1-[2-methyl-4-(methyloxy)phenyl]-2,3-dihydro-1*H*-pyrrolo[2,3-*b*]pyridin-4-yl}-1*H*-pyrazol-3-yl)amino]-1-propanesulfonic acid (35). To a suspension of **31** (25 mg, 0.0746 mmol) in *n*-BuOH (1 mL), at room temperature, under nitrogen atmosphere, was added 1,2-oxathiolane-2,2-dioxide (30 μL, 0.224 mmol). The reaction mixture was subjected to microwave irradiation (20 + 60 + 60 min at 150 °C /180 °C). The solvent was evaporated in vacuo, and the residue was purified by flash chromatography (EtOAc to 7:3 EtOAc/NH_3 solution 0.5 M in MeOH) and SCX cartridge (MeOH and NH_3 solution 0.5 M in MeOH) to give the title compound as a yellow oil (10 mg, 30%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.54 (bs, 1H), 7.05 (d, 1H), 6.72 (m, 1H), 6.66 (m, 1H), 6.48 (bs, 1H), 5.78 (bs, 1H), 3.72 (m, 5H), 3.3 (m, 4H), 2.97 (m, 2H), 2.03−2.2 (m, 8H). MS: *m*/*z* 458 $[M + H]^+$.

4-[3-(1,1-Dioxido-2-isothiazolidinyl)-1*H*-pyrazol-1-yl]-6-methyl-1-[2-methyl-4-(methyloxy)phenyl]-2,3-dihydro-1*H*-pyrrolo[2,3-*b*]pyridine (10). Compound **35** (10 mg, 0.022 mmol) and $POCl_3$ (1 mL) were mixed in a vial under nitrogen atmosphere. The reaction mixture was refluxed for 1 h. Then salurated aqueous $NaHCO_3$ was added until neutral pH and the mixture was partitioned between water and EtOAc. The two phases were separated, and the aqueous layer was further extracted with EtOAc (3 × 10 mL). The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by flash chromatography (cyclohexanes/EtOAc 1:l to EtOAc /NH_3 solution 0.5 M in MeOH 7:3) to give the title compound as a white solid (4.2 mg, 50%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.84 (d, 1 H), 7.16 (d, 1H), 6.82 (d, 1H), 6.77 (dd, 1H), 6.52(−, 1H), 6.51 (d, 1H), 3.98 (t, 2H), 3.87 (t, 2H), 3.80 (s, 3H), 3.46 (m, 2H), 3.37 (t, 2H), 2.57 (rn, 2H), 2.32 (s, 3H), 2.23 (s, 3H). MS: *m*/*z* 439 $[M]^+$.

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Supporting Information Available: Experimental for the synthesis of compound **8** and elemental analysis data for compounds **3**−**22**. The material is available free of charge via the Internet at http://pubs.acs.org.

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