CHEMMEDCHEM

DOI: 10.1002/cmdc.200600257

Cyclopenta[*d*]pyrimidines and Dihydropyrrolo[2,3-*d*]pyrimidines as Potent and Selective Corticotropin-Releasing Factor 1 Receptor Antagonists

Roberto Arban,[a] Roberto Benedetti,[a] Giorgio Bonanomi,[a] Anna-Maria Capelli,[a] Emiliano Castiglioni,[a] Stefania Contini,[a] Fabio Degiorgis,[a] Pina Di Felice,[b] Daniele Donati,[a] Elettra Fazzolari,[a] Gabriella Gentile,[a] Chiara Marchionni,[c] Carla Marchioro,[a] Flavia Messina,[c] Fabrizio Micheli,[a] Beatrice Oliosi,[a] Francesca Pavone,[a] Alessandra Pasquarello,[a] Benedetta Perini,[a] Marilisa Rinaldi,[a] Fabio M. Sabbatini,[a] Giovanni Vitulli,[d] Paola Zarantonello,[a] Romano Di Fabio,*[a] and Yves St-Denis*[a]

*Two new classes of potent and selective CRF_1 receptor antagonists are presented. Exploration of general templates **3** and **4** through modifications of the top amine and bottom phenyl substituents led to optimization of the in vitro affinity and pharmacokinetic profiles. The typical alkyl chains present in the top region of CRF_1 antagonists were replaced by substituted heteroaryl moieties, leading to a dramatic improvement of the metabolic stability. This improvement was apparent when the compounds were dosed in vivo: several compounds exhibited low plasma clearance, good oral bioavailability, and high brain penetration. As a consequence of their outstanding pharmacokinetic profiles, these CRF_1 antagonists, as exemplified by compound **4 fi** (4-(4-bromo-3-methyl-1*H*-pyrazol-1-yl)-7-(2,4-dichlorophenyl)-2-methyl-6,7-dihydro-5*H*-pyrrolo[2,3-d]pyrimidine), produced a dose-dependent "anxiolytic-like" effect when administered orally, decreasing the vocalization of rat pups.*

Introduction

Depression and anxiety are psychiatric disorders that constitute a major health concern worldwide, and new pharmacological approaches for improved efficacy and fewer side effects relative to currently marketed drugs are highly desired.[1] Corticotropin-releasing factor (CRF), a 41-amino acid peptide synthesized by specific hypothalamic nuclei in the brain, was originally isolated by Vale and colleagues in 1981 from ovine hypothalamus. CRF has been found to produce profound alterations in endocrine, nervous and immune system function, and it is believed to be the major physiological regulator of the basal and stress release of adrenocorticotropic hormone (ACTH) from the anterior pituitary.[2] In addition to its role in stimulating ACTH release, CRF is also believed to coordinate many of the endocrine, autonomic, and behavioral responses to stress, and may be involved in the pathophysiology of affective disorders. The fundamental role of CRF is to prepare the organism for a response to various stressors such as physical trauma, insults to the immune system, and social interactions, through control of the hypothalamic–pituitary–adrenal (HPA) axis.

During the last decade, several research groups have published their work in the area of small-molecule CRF_1 receptor antagonists.[3] Compounds such as CP-154,526[4] (**1**) and DMP-696[5] (**2**) were among the first to show good binding affinities coupled with interesting in vivo activities (Figure 1). A vast majority of the compounds published to date share structural features with antagonists **1** and **2**: a bicyclic heterocyclic core substituted with a highly lipophilic alkyl amine side chain and a 2,4-disubstituted or 2,4,6-trisubstituted aromatic or heteroaromatic ring. More recently, new high-affinity analogues containing monocyclic and tricyclic cores have been disclosed,[6] thus broadening the structural diversity and increasing the

[a] *Dr. R. Arban, Dr. R. Benedetti, Dr. G. Bonanomi, Dr. A.-M. Capelli, Dr. E. Castiglioni, Dr. S. Contini, Dr. F. Degiorgis, Dr. D. Donati, Dr. E. Fazzolari, Dr. G. Gentile, Dr. C. Marchioro, Dr. F. Micheli, Dr. B. Oliosi, Dr. F. Pavone, Dr. A. Pasquarello, Dr. B. Perini, Dr. M. Rinaldi, Dr. F. M. Sabbatini, Dr. P. Zarantonello, Dr. R. Di Fabio, Dr. Y. St-Denis*
GlaxoSmithKline Medicines Center, Psychiatry CEDD
Via A. Fleming 4, 37135 Verona (Italy)
Fax: (+39) 045-8218192
E-mail: romano.m.di-fabio@gsk.com
yves.a.st-denis@gsk.com

[b] *Dr. P. Di Felice*
Pfizer Italia S.r.l., Via del Commercio 25/27, 63046 Marino del Tronto (Italy)

[c] *Dr. C. Marchionni, Dr. F. Messina*
Nerviano Medical Sciences, Viale Pasteur 10, 20014 Nerviano (Italy)

[d] *Dr. G. Vitulli*
GlaxoSmithKline, Gunnels Wood Road, Stevenage, Herts, SG1 2NY (UK)

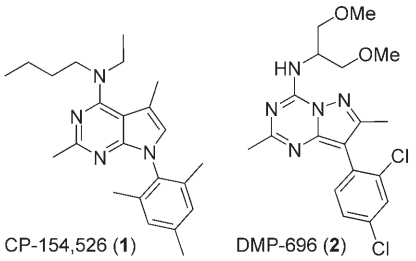


Figure 1. Known small-molecule CRF_1 antagonists.

knowledge of different scaffolds able to interact with the receptor. While significant progress has been made toward CRF regulation through administration of CRF receptor antagonists, there remains a need for effective small molecules endowed with druglike properties, as very few candidates have reached advanced preclinical development or clinical phases so far.

Herein we report our efforts towards the identification of potent and selective classes of CRF_1 receptor antagonists. Our initial approach in the identification of suitable templates was based on the use of a pharmacophore elaborated with examples of small-molecule CRF_1 antagonists. A set of representative structures were selected from the literature[7] to span the available chemical diversity. All compounds included in the study were endowed with high affinity toward the CRF_1 receptor and were assumed to bind the receptor with a common binding mode according to SAR data available in the literature. As can be observed in Figure 2, the model[8] consisted of one

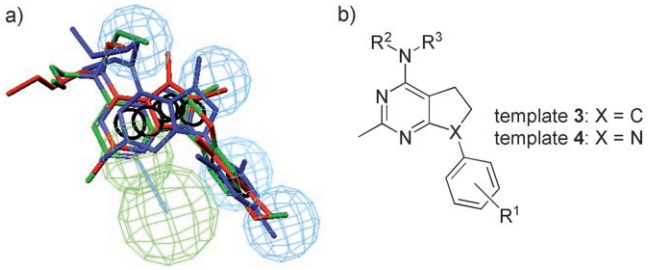


Figure 2. a) Pharmacophore model and b) templates **3** and **4**.

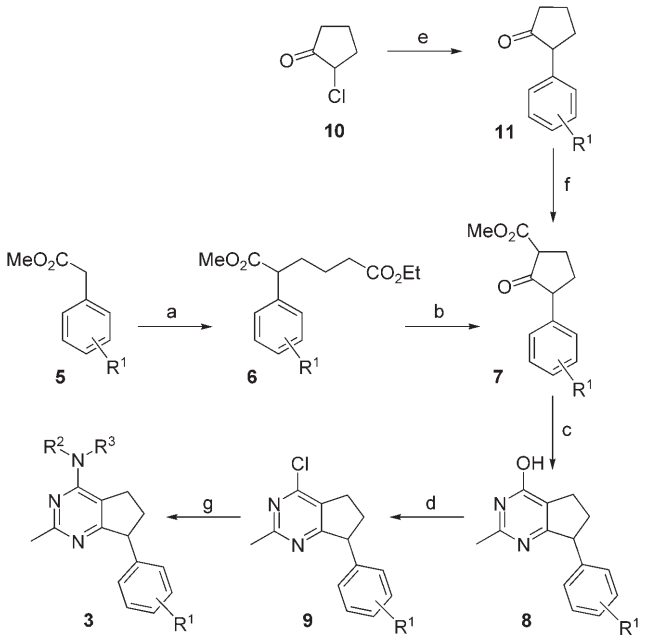
hydrogen bond acceptor and four hydrophobic features. It encoded the key structural requirements for high-affinity binding at this seven-transmembrane (7TM) receptor. These features, in particular the hydrogen bond acceptor functionality (shown in green in Figure 2), the aromatic ring with its *para* substituent, and the hydrophobic region mapped by the chains linked to the nitrogen atom (in blue) are also shared by other potent inhibitors that are structurally different from those used to derive the model. The pharmacophore thus produced was used to filter our original ideas. Templates **3** and **4** (Figure 2) were chosen for our original work, as they would allow a wide exploration of two of the important features represented by the pharmacophore: 1) the *bottom* aromatic ring and 2) the *top* hydrophobic region.

Results and Discussion

Chemistry

As our first goal was the exploration of the top region of the proposed template primarily in terms of replacing the alkyl amine region of the bicyclic templates, the planned synthetic pathways had to allow introduction of the amine moiety in the last step. This would have enabled a rapid exploration of the substituents through the production of suitable libraries of related analogues. Two different synthetic pathways were set up for the preparation of templates **3** and **4**.

Compounds of general structure **3** were prepared as outlined in Scheme 1. Substituted phenyl acetic esters **5** were de-



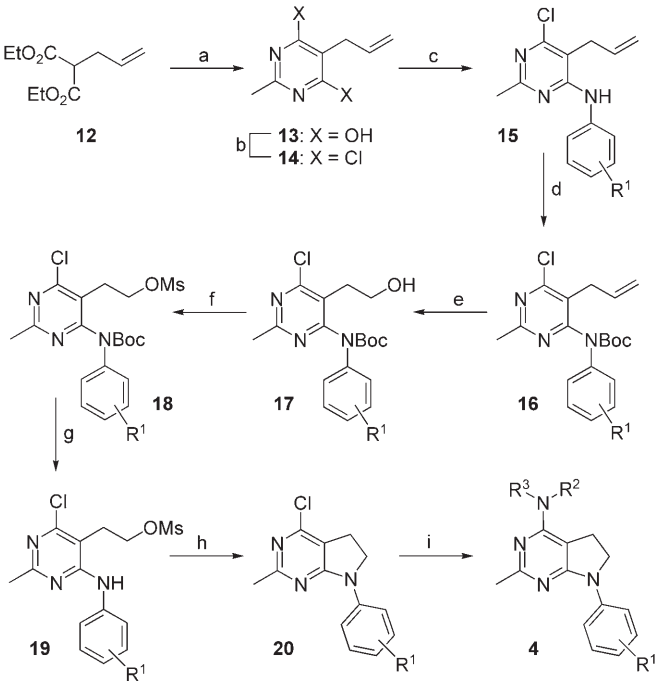
Scheme 1. Reagents and conditions: a) 1) LDA, THF, −78 °C, 45 min, then 2) ethyl-4-iodobutyrate, RT, 4 h; b) MeONa, toluene, 90 °C, 45 min; c) 1) acetamidine·HCl, MeONa, RT, 10 min, then 2) intermediates **7**, RT, 2 days; d) $POCl_3$, reflux, 2.5 h; e) 1) substituted bromobenzenes, Mg, THF, reflux, 1 h, then 2) intermediate **10**, 0 °C→reflux, 2 h; f) 1) LDA, THF, −78 °C, 15 min, then 2) $ClCO_2Me$, 15 min; g) alkyl amines (neat), 130–160 °C, 10–48 h; or HN-heterocycle, NaH/oil (80% *w/w*), DMF, 100–130 °C, 4–18 h. LDA = lithium diisopropylamide, DMF = *N*,*N*-dimethylformamide.

protonated with LDA at −78 °C in tetrahydrofuran (THF), and the anion thus formed was quenched with ethyl iodo-5-butyrate to give diesters **6**, which underwent a Dieckmann cyclization to β-ketoesters **7** upon treatment with sodium methoxide in toluene at 90 °C. Condensation of **7** with acetamidine (prepared in situ by the addition of acetamidine hydrochloride to a solution of sodium methoxide)[9] gave 4-hydroxycyclopentan[*d*]pyrimidines **8**, which readily reacted with phosphorus oxychloride to give 4-chlorocyclopenta[*d*]pyrimidines **9**.

For those phenyl acetic esters **5** which did not undergo a facile alkylation with ethyl iodo-5-butyrate (as was the case if the phenyl ring of **5** was substituted with electron-rich moiet-

ies such as methoxy groups; see compounds **3ca**, **3cb**, **3da**, **3db**, **3ea**, and **3eb**), an alternative synthetic pathway leading to cyclic β-ketoesters **7** was developed. 2-Chlorocyclopentanone **10** was thus coupled with substituted benzene Grignard reagents (prepared from the substituted bromobenzenes and metallic magnesium) in THF at reflux, yielding 2-phenylcyclopentanones **11**.[10] Deprotonation of **11** with freshly prepared LDA at −78 °C followed by acylation with methyl chloroformate resulted in the formation of cyclic β-ketoesters **7**, which were converted into intermediates **9** as described above. Nucleophilic aromatic substitution on template **9** was effected with the neat amines at high temperature, or, in the case of less nucleophilic reagents, in the presence of a strong base such as sodium hydride in an polar aprotic solvent, thus yielding final compounds **3**.

Scheme 2 illustrates the preparation of compounds of general structure **4**. Condensation of allyl malonate **13** with acetamidine (generated as for template **3**)[9,11] followed by treatment with phosphorus oxychloride yielded dichloropyrimidine **14**. Nucleophilic aromatic substitution of **14** with deprotonated disubstituted anilines afforded diaryl amines **15**, which were protected with a *tert*-butoxycarbonyl (Boc) group to give intermediates **16**. Ozonolysis of the double bond of **16** followed by reductive workup yielded alcohols **17**, which in turn were mesylated to afford intermediates **18**. Deprotection of **18** to aminomesylates **19**, followed by base-catalyzed cyclization yielded pyrrolo[2,3-*d*]pyrimidines **20**.



Scheme 2. Reagents and conditions: a) 1) acetamidine·HCl, MeONa, RT, 10 min, then 2) intermediate **12**, RT, 2 days, then b) $POCl_3$, reflux, 3 h; c) 1) R^1–anilines, NaH/oil (80% *w/w*), THF, 0 °C, 20 min, then 2) intermediate **14**, reflux, 3 h; d) $(Boc)_2O$, DMAP, CH_2Cl_2, RT, 18 h; e) 1) O_3, CH_2Cl_2, −78 °C, 10 min, then 2) $NaBH_4$, −78 °C→RT, 3 h; f) CH_3SO_2Cl, Et_3N, CH_2Cl_2, RT, 18 h; g) TFA/CH_2Cl_2 (20% *v/v*), RT, 2 h; h) NaH/oil (80% *w/w*), THF, RT, 2 h; i) alkyl amines (neat), 130–160 °C, 10–48 h; or HN-heterocycle, NaH/oil (80% *w/w*), DMF, 100–130 °C, 4–18 h. Boc = *tert*-butoxycarbonyl, DMAP = 4-dimethylaminopyridine, Ms = methanesulfonyl.

As in the case of intermediates **9** (Scheme 1), pyrrolo[2,3-*d*]pyrimidines **20** readily underwent aromatic nucleophilic displacement with the appropriate amines, yielding the target compounds **4**. In both cases, this transformation allowed a broad exploration of the top region of the two bicyclic pyrimidine templates.

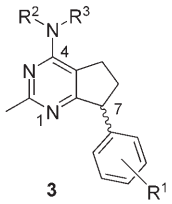
SAR, in vitro affinity, and metabolic stability

The aim of the exploration of both templates **3** and **4** was to maximize the in vitro potency while improving metabolic stability, mainly through the exploration of both the top region of the template ($-NR^2R^3$) and the proper substitution on the pendant phenyl ring ($-R^1$). To test the validity of the cyclopenta[*d*]pyrimidine and dihydropyrrolo[2,3-*d*]pyrimidine bicyclic cores designed according to the five-point pharmacophore model discussed previously, an initial set of known acyclic amines was introduced on both templates (entries **3aa**, **3ab**, **3ad**–**3eb**, and **4aa**–**4db**). As reported in Tables 1 and 2, high affinity was achieved in both cases, even though, as a rule, compounds **4** had higher binding potencies. It is interesting to note that the conformation of the stereogenic center of **3** (position 7 of the bicyclic core) had an effect on the affinity for the receptor, as can be observed with **3ad** and **3ae** (Table 1). In this case, the use of a chiral amine introduced on the top region of the template allowed chromatographic separation of both diastereomers. The higher in vitro affinity of compounds **4** shown in Tables 1 and 2 suggest that the spatial orientation of the pendant phenyl ring in this template is preferred over both diastereomers of **3**. Therefore, our attention was focused primarily on the optimization of the former template.

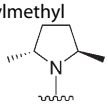
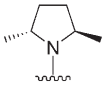
Most of the compounds prepared exhibited low in vitro metabolic stability, as expressed by the high turnover (often >80%) in incubations with rat liver microsomes (Tables 1 and 2; a high percentage means the compound is highly metabolized). The two most stable compounds of this series, **4cb** and **4cc**, both share a common structural feature: a 2,4-bis(trifluoromethyl)phenyl moiety at the bottom. The lower percent turnover of these two compounds can be partially explained by the higher stability of the phenyl ring when substituted with these two highly lipophilic electron-withdrawing groups. We thought that one way of stabilizing compounds **4** would have been to use constrained amines, as they might be metabolically more stable then their acyclic counterparts. As can be observed in Table 3, in some cases the substitution of template **4** with cyclic amines maintained the high affinities observed with the previous series of compounds (**4eb** and **4ef**, Table 3), but had no effect on metabolic stability. The only exception is compound **4eg**, for which the introduction of a polar amide moiety limited the extent of metabolic degradation. Unfortunately, the introduction of polar groups on the amine moiety was not well tolerated by the receptor.

As reported in Table 3, substitution of template **4** with cyclic amines appears to be tolerated by the receptor. We hypothesized that one way of making metabolically more stable, yet

Table 1. In vitro characterization of template **3**.[a]

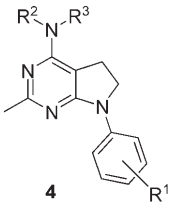


3

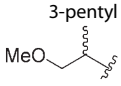
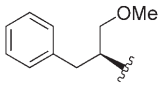
Compd	R^1	R^2	R^3	pIC_{50}[b]	Met. Stability [% Turnover]
1				8.11	nd[e]
2				7.92	11
3aa	2,4-di-Cl	cyclopropylmethyl	H	5.68	56
3ab	2,4-di-Cl	butyl	ethyl	6.67	nd
3ac	2,4-di-Cl	cyclopropylmethyl	propyl	6.76	94
3ad[c]	2,4-di-Cl			7.22	92
3ae[d]	2,4-di-Cl			6.57	88
3ba	2,4-di-F	butyl	ethyl	5.59	85
3bb	2,4-di-F	cyclopropylmethyl	propyl	5.52	85
3ca	2,4-di-OMe	butyl	ethyl	6.27	nd
3cb	2,4-di-OMe	cyclopropylmethyl	propyl	6.70	93
3da	2-Me-4-F	butyl	ethyl	5.98	93
3db	2-Me-4-F	cyclopropylmethyl	propyl	6.19	nd
3ea	2,4-di-Me	butyl	ethyl	6.02	92
3eb	2,4-di-Me	cyclopropylmethyl	propyl	6.17	91

[a] Compounds are reported as racemic mixtures except **3ad** and **3ae**. [b] All compounds reported showed no affinity ($pIC_{50} < 5.0$) for the CRF_2 receptor. [c] Isomer 1. [d] Isomer 2. [e] nd = not determined.

Table 2. In vitro characterization of template **4**: acyclic amines.



4

Compd	R^1	R^2	R^3	pIC_{50}	Met. Stability [% Turnover]
4aa	2,4-di-Cl	cyclopropylmethyl	H	5.82	93
4ab	2,4-di-Cl	3-pentyl	H	7.93	90
4ac	2,4-di-Cl		H	5.64	73
4ad	2,4-di-Cl		H	6.89	96
4ae	2,4-di-Cl	cyclopentyl	H	5.48	95
4af	2,4-di-Cl	butyl	ethyl	7.25	88
4ag	2,4-di-Cl	cyclopropylmethyl	propyl	7.77	90
4ba	2-Br-4-*i*Pr	3-pentyl	H	8.00	57
4bb	2-Br-4-*i*Pr	butyl	ethyl	7.78	65
4bc	2-Br-4-*i*Pr	cyclopropylmethyl	propyl	8.03	70
4ca	2,4-di-CF_3	4-heptyl	H	7.57	nd
4cb	2,4-di-CF_3	butyl	ethyl	7.46	33
4cc	2,4-di-CF_3	cyclopropylmethyl	propyl	7.38	48
4da	2-Cl-4-CF_3	butyl	ethyl	7.11	63
4db	2-Cl-4-CF_3	cyclopropylmethyl	propyl	7.26	87

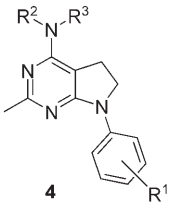
potent compounds would be through the introduction of cyclic moieties that are not easily degraded. Thus, we turned our attention to substituted *N*-heteroaryl rings. In this case, derivatives of imidazoles, triazoles, and pyrazoles were prepared. The results of this exploration are presented in Table 4. Even though the potencies of these new analogues are generally lower than those of their aliphatic counterparts, a few examples have maintained a good level of activity despite the drastic structural modification.

Pyrazoles appear to be better tolerated than imidazoles (**4fi** versus **4fn**, and **4fd** versus **4fo**; Table 4), whereas triazoles have intermediate values. There does not seem to be a clear rule with respect to the substitution pattern of the pyrazole ring, but in many cases, a substituent at the C3 position had a beneficial effect on the in vitro affinity (**4ff**, **4fh**, and **4fi**). The electronic content of the ring seems to have no significant effect on the affinity (**4fa** versus **4fe**), while lipophilicity is the apparent governing factor, as was the case with the acyclic and cyclic aliphatic substituents previously described. Interestingly, as originally hypothesized, the metabolic stability of the few examples tested was improved. All compounds tested had a turnover value $\leq 70\%$, and in some cases, the metabolic degradation was extremely limited (**4fg** and **4fh**: 3%). These interesting preliminary in vitro results prompted us to immediately characterize some examples in vivo to find a potential correlation with the in vitro results so far obtained.

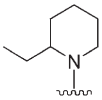
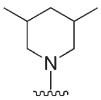
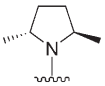
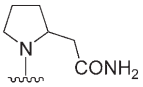
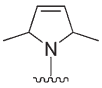
Pharmacokinetic characterization

A selection of structurally diverse compounds was chosen for in vivo DMPK studies in rat.

Table 3. In vitro characterization of template **4**: cyclic amines.

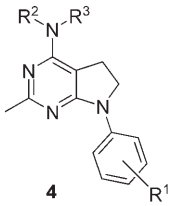


4

Compd	R^1	R^2 and R^3	pIC_{50}	Met. Stability [% Turnover]
4ea	2,4-di-Cl		6.34	nd
4eb	2,4-di-Cl		7.36	88
4ec	2,4-di-Cl		5.79	80
4ed	2,4-di-Cl		5.39	92
4ee	2,4-di-Cl		6.34	93
4ef	2,4-di-Cl		7.72	100
4eg	2,4-di-Cl		5.66	49
4eh	2,4-di-Cl		6.06	87

Some examples of both templates **3** and **4** were included, as well as examples bearing aliphatic chains, aliphatic heterocyclic moieties, and aromatic heterocycles in the top part of the molecule (Table 5). As expected from the in vitro percent turnover, compounds bearing dialkylamines or a cyclic aliphatic amine had higher in vivo plasma clearance (Cl_p) than compounds bearing aromatic moieties, as exemplified by **4ag**, **4ef**, and **4fi**. Moreover, compounds with a 2,4-bis(trifluoromethyl) substituent (R^1) on the bottom phenyl ring had higher metabolic stability than 2,4-dichloro-substituted compounds, even with R^2 and R^3 present as alkyl chains (for example, **4ag** versus **4cc**). Compounds **4fd** and **4fi**, both bearing a substituted pyrazole ring instead of the typical aliphatic side chains, showed low plasma clearance (21 and 9 mL min^{-1} kg^{-1}, respectively). Despite its good clearance, only low systemic exposure was observed for **4fd** after oral administration. This low bioavailability (%*F*) could be explained by solubility- or permeability-limited absorption from the GI tract or a transporter-mediated efflux. Compound **4fi**, on the other hand, demonstrated excellent properties, with a moderate volume of distribution and good bioavailability. Moreover, the compound was endowed with

Table 4. In vitro characterization of template **4**: heteroaryl amines.



4

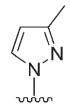
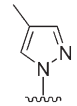
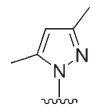
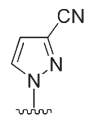
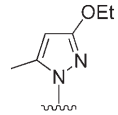
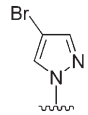
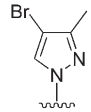
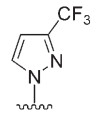
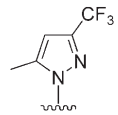
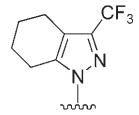
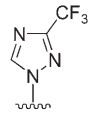
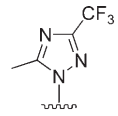
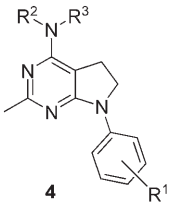
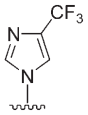
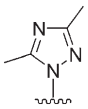
Compd	R^1	R^2 and R^3	pIC_{50}	Met. Stability [% Turnover]
4fa	2,4-di-Cl		6.57	58
4fb	2,4-di-Cl		5.76	nd
4fc	2,4-di-Cl		6.34	70
4fd	2,4-di-Cl		6.47	59
4fe	2,4-di-Cl		6.67	nd
4ff	2,4-di-Cl		7.48	nd
4fg	2,4-di-Cl		5.68	3
4fh	2,4-di-Cl		6.85	3
4fi	2,4-di-Cl		7.32	nd
4fj	2,4-di-Cl		7.03	nd
4fk	2,4-di-Cl		6.02	nd
4fl	2,4-di-Cl		6.53	nd
4fm	2,4-di-Cl		5.82	nd

Table 4. (Continued)



4

Compd	R^1	R^2 and R^3	pIC_{50}	Met. Stability [% Turnover]
4 fn	2,4-di-Cl		5.51	nd
4 fo	2,4-di-Cl		5.32	21

high brain penetration, as expressed by the brain-to-plasma ratio (2.3:1).

In vivo pharmacology

Because of its good in vitro activity and excellent pharmacokinetic profile (low plasma clearance, high %*F*, and high brain-to-plasma ratio), compound **4 fi** was chosen for further pharmacological characterization (**4 fi** was shown to behave as a functional antagonist in a CRF-stimulated cAMP formation assay performed in the same cell system used for the binding assay: CHO cells expressing human recombinant CRF_1 receptor (data not shown)).

As can be observed in Figure 3, **4 fi** was able to significantly decrease rat pup vocalization time[12] by 50% when dosed orally at 3 mg kg^{-1}, and by 70% at 10 mg kg^{-1}. A slightly lower activity was observed for DMP-696 (**2**) in the same model.

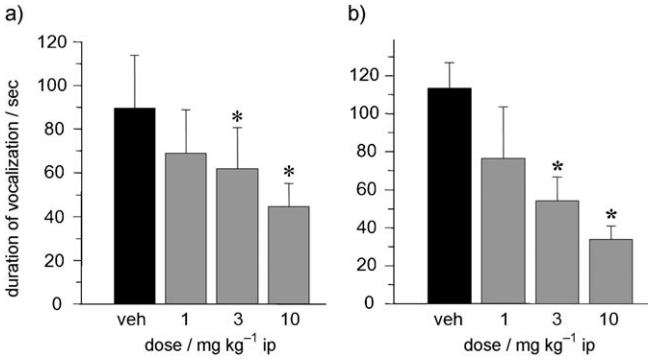
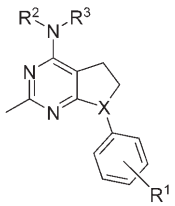
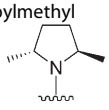
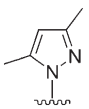
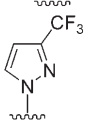


Figure 3. Compounds a) **2** (ED_{50} = 3.5 mg kg^{-1}) and b) **4 fi** (ED_{50} = 2.0 mg kg^{-1}) in the rat pup distress call model in vivo; veh = vehicle; *$p \leq 0.05$

Table 5. Pharmacokinetic characteristics of selected examples.



Compd	X	R^1	R^2	R^3	Vd [L kg^{-1}][a]	Cl_p [mL min^{-1} kg^{-1}]	$t_{1/2}$ [h]	*F* [%]	B/P[b]
3 ac	C	2,4-di-Cl	cyclopropylmethyl	propyl	5.4	41	3.1	22	0.35
4 ab	N	2,4-di-Cl	propyl	H	10	57	3.9	nd	nd
4 ag	N	2,4-di-Cl	cyclopropylmethyl	propyl	7.6	40	5.2	21	0.6
4 bc	N	2-Br-4-*i*Pr	cyclopropylmethyl	propyl	1.9	23	1.9	nd	nd
4 cb	N	2,4-di-CF_3	butyl	ethyl	4.5	18	7.1	43	1.1
4 cc	N	2,4-di-CF_3	cyclopropylmethyl	propyl	4.8	20	6.9	76	1.2
4 db	N	2-Cl-4-CF_3	cyclopropylmethyl	propyl	1.9	22	2.0	nd	nd
4 ef	N	2,4-di-Cl			7.4	44	3.4	8	1.5
4 fd	N	2,4-di-Cl			4.9	21	4.8	4	nd
4 fi	N	2,4-di-Cl			3.3	9	6	86	2.3

[a] Vd = volume of distribution. [b] Brain/plasma ratio.

Conclusions

In summary, we present herein the characterization of two classes of CRF_1 receptor ligands. Both classes presented potent and selective antagonists. Through modifications of the pyrimidine ring amine substituents, the pharmacokinetic properties of the dihydropyrrolo[2,3-*d*]pyrimidine template **4** were greatly improved. Replacement of the alkyl chains and cycloalkyl moieties with heteroaryl substituents led to metabolically stable compounds while retaining good levels of potency. From this series, compound **4 fi** exhibited outstanding pharmacokinetics in rat. Based on these positive characteristics, **4 fi** was tested in vivo in the rat pup vocalization model and proved to be active, thus confirming that CRF_1 antagonists may play a role in the treatment of anxiety and depression. Further exploration of template **4** to provide improved analogues will be reported in due course.

Experimental Section

General methods: ^{1}H NMR spectra were recorded on a Varian Inova (400 MHz) spectrometer in $CDCl_3$ or [D_6]DMSO. Chemical shifts are reported in ppm, referring to the $CHCl_3$ residual line as an internal standard ($\delta = 7.26$ ppm), or to the DMSO residual line ($\delta = 2.49$ ppm), and were assigned as singlets (s), doublets (d), triplets (t), quadruplets (q) or multiplets (m). MS analysis was performed on a VG Platform (Waters, Manchester, UK) instrument operating in positive electrospray ion mode. Analytical TLC was performed on glass plates (Merck Kieselgel 60 F_{254}). Visualization was carried out by UV light ($\lambda = 254$ nm) or 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.

Pharmacophore generation: Ligand conformational searches were carried out using the BEST routine (max 200 conformations, energy window of 10 kcal), while common-feature alignments were performed using the HipHop algorithm. Standard Catalyst pharmacophore features (H-bond acceptor, aromatic rings, and hydrophobic groups) were selected according to SAR data available in the literature. Among the different top-scoring pharmacophore solutions generated by the program, the most satisfactory models were chosen according to quality of ligand conformations, rmsd of the ligand conformations to pharmacophore features, and common volumes.

Chemistry: *Cyclopenta[*d*]pyrimidine scaffold: Method A:*

6-Ethyl-1-methyl-2-(2,4-dichlorophenyl)hexanedioate (6 a): A solution of *n*BuLi in hexanes (1.6 M, 3.42 mL, 1 equiv) was added to a solution of *i*Pr_2NH (0.921 mL, 1.2 equiv) in THF (anhyd, 22 mL) at 0 °C under N_2, and the reaction mixture was stirred at 0 °C for 10 min. It was then cooled to −78 °C, and a solution of methyl-(2,4-dichlorophenyl)acetate (1.2 g, 5.478 mmol) in THF (anhyd, 3.3 mL) was added dropwise. The mixture was stirred at −78 °C for 45 min before the addition of a solution of ethyl-4-iodobutyrate (1.72 g, 1.3 equiv) in THF (anhyd, 2 mL). The cooling bath was then removed, and the mixture was stirred at RT for 4 h. The solvents were evaporated under reduced pressure. The residue was dissolved in CH_2Cl_2, washed with water (2×25 mL) and brine (1× 25 mL), and dried over anhyd Na_2SO_4. The solids were filtered, and the solvent was evaporated. The crude product was purified by flash chromatography (silica gel, *c*Hex/EtOAc 9:1). Mixed fractions were repurified with the same eluent, thus affording **6 a** as a pale-yellow oil (1.36 g, 4.088 mmol, 75%): ^{1}H NMR (400 MHz, $CDCl_3$): $\delta =$ 7.63 (d, 1H), 7.44 (dd, 1H), 7.40 (d, 1H), 4.05 (dd, 1H), 4.01 (q, 2H), 3.59 (s, 3H), 2.28 (dt, 2H), 2.01 (m, 1H), 1.74 (m, 1H), 1.47 (m, 1H), 1.35 (m, 1H), 1.14 ppm (t, 3H); MS (*m/z*) 332 $[M]^+$, 300 $[M-CH_3OH]^+$.

Methyl-3-(2,4-dichlorophenyl)-2-oxocyclopentanecarboxylate (7 a): Sodium (376 mg, 4 equiv) was added portion-wise, under N_2, to MeOH (anhyd, 5 mL). After consumption of metallic sodium, MeOH was evaporated under nitrogen flux. Toluene (anhyd, 30 mL) was then added to the residue followed by a solution of **6 a** (1.36 g, 4.088 mmol) in toluene (anhyd, 30 mL). The mixture was heated at 90 °C for 45 min. The reaction mixture was acidified with glacial AcOH, diluted with EtOAc, washed with water (2×20 mL) and dilute aqueous $NaHCO_3$ (2×20 mL), and dried over anhyd Na_2SO_4. The solids were filtered, and the solvent was evaporated. **7 a** was obtained as a pale-yellow oil (1 g, 3.48 mmol, 85%) as a mixture of two diastereomers in a 7:3 ratio and was used in the following step without further purification: ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.38$ (m, 1H), 7.19 (m, 1H), 7.06, 7.01, (2d, 1H), 3.93–3.85, 3.78 (m+dd, 1H), 3.79, 3.76 (2 s, 3H), 3.49–3.44, 3.40 (m+dd, 1H), 2.60–1.90 ppm (m, 4H); MS (*m/z*) 286 $[M]^+$.

7-(2,4-Dichlorophenyl)-2-methyl-6,7-dihydro-5*H*-cyclopenta[*d*]-pyrimidin-4-ol (8 a): Sodium (240 mg, 3 equiv) was added portion-wise to MeOH (anhyd, 6 mL) under N_2. After consumption of metallic sodium, acetamidine hydrochloride (1.04 g, 3 equiv) was added. After 10 min of stirring the precipitated NaCl was filtered off and washed with MeOH (anhyd, 2 mL). The solution of free acetamidine was added to neat **7 a** (1 g, 3.483 mmol), and the mixture was stirred at RT for 2 days and then at 70 °C for 7 h. The solvent was evaporated, and the crude product was purified by flash chromatography (silica gel, gradient: CH_2Cl_2/MeOH 98:2→95:5). The pure **8 a** was obtained as a white solid (455 mg, 1.54 mmol, 44%): ^{1}H NMR (400 MHz, [D_6]DMSO): $\delta = 12.31$ (s, 1H), 7.60 (d, 1H), 7.34 (dd, 1H), 7.04 (d, 1H), 4.54 (t, 1H), 2.75–2.60 (2 m, 2H), 2.58–2.50 (m, 1H), 2.20 (s, 3H), 1.80–1.72 ppm (m, 1H); MS (*m/z*) 295 $[M\mathrm{H}]^+$.

4-Chloro-7-(2,4-dichlorophenyl)-2-methyl-6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidine (9 a): Compound **8 a** (453 mg, 1.535 mmol) was suspended in $POCl_3$ (16 mL), and the mixture was held at reflux for 2.5 h. The excess $POCl_3$ was then evaporated, and the residue was dissolved in CH_2Cl_2. A few drops of concd NH_4OH were added, and the mixture was diluted with water and CH_2Cl_2. The phases were separated, and the aqueous layer was extracted with CH_2Cl_2 (3×20 mL). The combined organic extracts were washed with water and brine (2×20 mL), and dried over anhyd Na_2SO_4. The solids were filtered, and the solvent was evaporated. Crude **9 a** was obtained as a brown oil (378 mg, 1.2 mmol, 79%) and was used in the next step without further purification: ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.44$ (d, 1H), 7.19 (dd, 1H), 6.82 (d, 1H), 4.83 (t, 1H), 3.05 (m, 2H), 2.79 (m, 1H), 2.68 (s, 3H), 2.00 ppm (m, 1H); MS (*m/z*) 313 $[M\mathrm{H}]^+$.

***N*-(Cyclopropylmethyl)-7-(2,4-dichlorophenyl)-2-methyl-6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidin-4-amine (3 aa):** Compound **9 a** (104 mg, 0.332 mmol) was dissolved in DMSO (anhyd, 0.75 mL). (Cyclopropylmethyl)amine (0.288 mL, 10 equiv) was added, and the mixture was stirred in a closed vial at 160 °C for 4 h. The mixture was cooled to RT, diluted with AcOEt, washed with water (3× 10 mL), and dried over anhyd Na_2SO_4. The solids were filtered, and the solvent was evaporated. The crude product was purified by flash chromatography (silica gel, *c*Hex/AcOEt 7:3), affording pure

3aa as a white solid (90 mg, 0.258 mmol, 78%): ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.38$ (d, 1H), 7.12 (dd, 1H), 6.76 (d, 1H), 4.64 (m, 1H), 4.51 (brt, 1H), 3.40 (m, 2H), 2.80 (m, 2H), 2.66 (m, 1H), 2.50 (s, 3H), 1.84 (m, 1H), 1.10 (m, 1H), 0.58 (m, 2H), 0.30 ppm (m, 2H); MS (*m/z*) 348 $[M\text{H}]^+$.

***N*-Butyl-7-(2,4-dichlorophenyl)-*N*-ethyl-2-methyl-6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidin-4-amine (3ab):** This compound was prepared by using the same procedure as described for **3aa**, except that *N*-ethyl-1-butanamine was used instead of (cyclopropylmethyl)amine: ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.39$ (d, 1H), 7.12 (dd, 1H), 6.76 (d, 1H), 4.56 (m, 1H), 3.58 (m, 4H), 3.02 (m, 2H), 2.60 (m, 1H), 2.45 (s, 3H), 1.79 (m, 1H), 1.62 (m, 2H), 1.37 (m, 2H), 1.21 (t, 3H), 0.98 ppm (t, 3H); MS (*m/z*): 378 $[M\text{H}]^+$.

***N*-(Cyclopropylmethyl)-7-(2,4-dichlorophenyl)-2-methyl-*N*-propyl-6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidin-4-amine (3ac):** This compound was prepared by using the same procedure as described for **3aa**, except that *N*-(cyclopropylmethyl)-1-propanamine was used instead of (cyclopropylmethyl)amine: ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.35$ (d, 1H), 7.10 (dd, 1H), 6.75 (d, 1H), 4.55 (dd, 1H), 3.65–3.40 (m, 4H), 3.00 (t, 2H), 2.60 (m, 1H), 2.40 (s, 3H), 1.75 (m, 1H), 1.60 (m, 2H), 1.05 (m, 1H), 0.90 (t, 3H), 0.55 (m, 2H), 0.25 ppm (m, 2H); MS (*m/z*) 390 $[M\text{H}]^+$.

(7*R*)- and (7*S*)-7-(2,4-Dichlorophenyl)-4-[(2*R*,5*R*)-2,5-dimethyl-1-pyrrolidinyl]-2-methyl-6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidine (3ad + 3ae): These compounds were prepared by using the same procedure as described for **3aa**, except that (2*R*,5*R*)-2,5-dimethylpyrrolidine was used instead of (cyclopropylmethyl)amine. The two isomers were separated by flash chromatography (silica gel, *c*Hex/EtOAc 9:1): Isomer 1: ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.39$ (d, 1H), 7.13 (dd, 1H), 6.75 (d, 1H), 4.64 (dd, 1H), 4.50 (brs, 1H), 2.99 (m, 2H), 2.65 (m, 1H), 2.47 (s, 3H), 2.22 (m, 2H), 1.78 (m, 1H), 1.65 (m, 2H), 1.15 ppm (brd, 6H); MS (*m/z*) 376 $[M\text{H}]^+$; Isomer 2: ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.39$ (d, 1H), 7.12 (dd, 1H), 6.77 (d, 1H), 4.54 (dd, 1H), 4.50 (brs, 1H), 2.95 (m, 2H), 2.63 (m, 1H), 2.48 (s, 3H), 2.23 (m, 2H), 1.81 (m, 1H), 1.66 (m, 2H), 1.14 ppm (brd, 6H); MS (*m/z*) 376 $[M\text{H}]^+$.

***N*-Butyl-7-(2,4-difluorophenyl)-*N*-ethyl-2-methyl-6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidin-4-amine (3ba):** This compound was prepared by using method A, except that methyl-(2,4-difluorophenyl)acetate was used instead of methyl-(2,4-dichlorophenyl)acetate in the first step, and *N*-ethyl-1-butanamine was used instead of (cyclopropylmethyl)amine in the last step: ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 6.88$ (m, 1H), 6.77 (m, 2H), 4.40 (t, 1H), 3.6 (m, 2H), 3.5 (m, 2H), 3.03 (m, 2H), 2.52 (m, 1H), 2.42 (s, 3H), 1.86 (m, 1H), 1.60 (m, 2H), 1.35 (m, 2H), 1.19 (t, 3H), 0.95 ppm (t, 3H); MS (*m/z*) 346 $[M\text{H}]^+$.

***N*-(Cyclopropylmethyl)-7-(2,4-difluorophenyl)-2-methyl-*N*-propyl-6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidin-4-amine (3bb):** This compound was prepared using method A, except that methyl-(2,4-difluorophenyl)acetate was used instead of methyl-(2,4-dichlorophenyl)acetate in the first step, and *N*-(cyclopropylmethyl)-1-propanamine was used instead of (cyclopropylmethyl)amine in the last step: ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 6.87$ (m, 1H), 6.77 (m, 2H), 4.40 (t, 1H), 3.58 (m, 2H), 3.45 (m, 2H), 3.04 (m, 2H), 2.55 (m, 1H), 2.43 (s, 3H), 1.86 (m, 1H), 1.65 (m, 2H), 1.07 (m, 1H), 0.92 (t, 3H), 0.58 (m, 2H), 0.27 ppm (m, 2H); MS (*m/z*) 358 $[M\text{H}]^+$.

*Cyclopenta[*d*]pyrimidine scaffold: Method B:*

2-[2,4-Bis(methyloxy)phenyl]cyclopentanone (11c): A solution of 1-bromo-2,4-dimethoxybenzene (0.34 mL, 1.2 equiv) in dry THF (0.5 mL) was added dropwise, under N_2, to a suspension of Mg turnings (64 mg, 1.3 equiv) in dry THF (0.7 mL) and in presence of a catalytic amount of I_2. The reaction mixture was stirred at reflux for 1 h and then cooled to 0 °C. A solution of 2-chlorocyclopentanone (0.2 mL, 2 mmol) in THF (anhyd, 0.5 mL) was added to this mixture dropwise, and the reaction mixture was heated at reflux for 2 h. The mixture was allowed to cool to RT, was diluted with Et_2O and slowly mixed with ice and 1 M HCl. The organic layer was then separated, washed twice with brine, and dried over anhyd Na_2SO_4. The solids were filtered, and the solvent was evaporated. The crude red oil was purified by flash chromatography (silica gel, *c*Hex/EtOAc 8:2) to give compound **11c** (297 mg, 1.35 mmol, 67%) as a yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 6.97$ (d, 1H), 6.44 (m, 2H), 3.78 (s, 3H), 3.74 (s, 3H), 3.30 (dd, 1H), 2.4–2.3 (m, 3H), 2.13–2.04 (m, 2H), 1.86 ppm (m, 1H); MS (*m/z*) 220 $[M]^+$.

Methyl-3-[2,4-bis(methyloxy)phenyl]-2-oxocyclopentanecarboxylate (12c): *n*BuLi in hexanes (1.6 M, 0.66 mL, 1.12 equiv) was added to a solution of freshly distilled diisopropylamine (0.148 mL, 1.2 equiv) in THF (anhyd, 3.5 mL) at 0 °C under N_2, and the resulting mixture was stirred for 10 min and then cooled to −78 °C. A solution of compound **11c** (195 mg, 0.88 mmol) in THF (anhyd, 1 mL) was added dropwise, and the reaction mixture was stirred for 15 min. Methyl chloroformate (0.075 mL, 1 equiv) was then added to the enolate solution, and the reaction mixture was stirred for 15 min. The cold reaction mixture was poured into HCl (0.5 N, 10 mL), and diethyl ether (10 mL). The phases were separated, and the organic layer was washed with satd aq $NaHCO_3$, satd aq NaCl, and was dried over anhyd Na_2SO_4. The solids were filtered, and the solvent was evaporated. The crude oil was purified by flash chromatography (silica gel, *c*Hex/Et_2O 7:3) to give **12c** as a yellow oil (66 mg, 27%): ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 6.97$ (d, 1H), 6.44–6.42 (m, 2H), 3.8–3.69 (m, 9H), 3.41 (m, 2H), 2.31 (m, 1H), 2.08 ppm (m, 1H); MS (*m/z*) 278 $[M]^+$.

7-[2,4-Bis(methyloxy)phenyl]-2-methyl-1,5,6,7-tetrahydro-4*H*-cyclopenta[*d*]pyrimidin-4-one (7c): Acetamidine hydrochloride (50 mg, 2.3 equiv) was added to a solution of freshly prepared MeONa (37 mg, 2.3 equiv) in MeOH (anhyd, 1 mL). The resulting suspension was filtered and added to a flask containing compound **12c** (65 mg, 0.23 mmol) in MeOH (anhyd, 1 mL). The reaction mixture was stirred for 1 day and then a second portion of the free acetamidine was prepared as described above and added to the reaction flask. After stirring for 2 days, the solution was concentrated in vacuo, and the crude oil was purified by flash chromatography (silica gel, 100% EtOAc). Compound **7c** was obtained as a white solid (35 mg, 0.12 mmol, 53%): ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 10.44$ (brs, 1H), 6.79 (d, 1H), 6.46 (d, 1H), 6.41 (dd, 1H), 4.53 (t, 1H), 3.78 (s, 6H), 2.88 (m, 1H), 2.78 (m, 1H), 2.55 (m, 1H), 2.41 (s, 3H), 1.90 ppm (m, 1H); MS (*m/z*) 287 $[M\text{H}]^+$.

7-[2,4-Bis(methyloxy)phenyl]-4-chloro-2-methyl-6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidine (9c): A solution of compound **8c** (21 mg, 0.07 mmol) in $POCl_3$ (1 mL) was heated at 100 °C for 3 h and then concentrated in vacuo. The crude oil was diluted with EtOAc and washed with concd NH_4OH. It was then dried over anhyd Na_2SO_4, the solids were filtered, and the solvent was evaporated to give compound **9c** (20.6 mg, 0.068 mmol, 90%) as an orange oil, which was used in the next step without further purification: ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 6.82$ (d, 1H), 6.40 (m, 2H), 4.6 (dd, 1H), 3.8 (s, 3H), 3.7 (s, 3H), 3.1–2.85 (m, 2H), 2.65 (s, 3H), 2.60 (m, 1H), 2.05 ppm (m, 1H).

7-[2,4-Bis(methyloxy)phenyl]-*N*-butyl-*N*-ethyl-2-methyl-6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidin-4-amine (3ca): *N*-Ethyl-1-butanamine (20 mg, 10 equiv) was added to compound **9c** (6 mg, 0.019 mmol) in a screw-cap vial under N_2, and the reaction mixture

was heated at 100 °C for 3 h. It was then diluted with CH_2Cl_2 and washed with water. The aqueous layer was extracted with CH_2Cl_2 (2×10 mL), and the combined organic extracts were dried over anhyd Na_2SO_4. The solids were filtered, and the solvent was evaporated. The crude oil obtained was purified by preparative TLC (*c*Hex/EtOAc 6:4) to give compound **3 ca** (6 mg, 0.016 mmol, 85%) as a clear oil: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.67 (d, 1H), 6.46 (d, 1H), 6.38 (dd, 1H), 4.44 (dd, 1H), 3.79 (s, 3H), 3.78 (s, 3H), 3.67–3.44 (m, 4H), 3.00 (m, 2H), 2.45 (m, 1H), 2.44 (s, 3H), 1.83 (m, 1H), 1.6 (m, 2H), 1.36 (m, 2H), 1.20 (t, 3H), 0.97 ppm (t, 3H); MS (*m/z*) 370 $[MH]^+$.

7-[2,4-Bis(methyloxy)phenyl]-*N*-(cyclopropylmethyl)-2-methyl-*N*-propyl-6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidin-4-amine (3 cb): This compound was prepared using method B, except that *N*-(cyclopropylmethyl)-1-propanamine was used instead of *N*-ethyl-1-butanamine in the last step: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.64 (d, 1H), 6.45 (d, 1H), 6.36 (dd, 1H), 4.45 (dd, 1H), 3.78 (s, 3H), 3.76 (s, 3H), 3.6–3.4 (m, 2H), 3.55–3.49 (m, 2H), 3.1–2.9 (m, 2H), 2.45 (m, 1H), 2.44 (s, 3H), 1.81 (m, 1H), 1.66 (m, 2H), 1.08 (m, 1H), 0.91 (t, 3H), 0.6–0.2 ppm (m, 4H); MS (*m/z*) 382 $[MH]^+$.

***N*-Butyl-*N*-ethyl-7-(4-fluoro-2-methylphenyl)-2-methyl-6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidin-4-amine (3 da):** This compound was prepared using method B, except that 1-bromo-4-fluoro-2-methylbenzene was used instead of 1-bromo-2,4-dimethoxybenzene in the first step: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.88 (dd, 1H), 6.75 (td, 1H), 6.64 (dd, 1H), 4.32 (m, 1H), 3.64 (m, 2H), 3.54 (m, 2H), 3.02 (m, 2H), 2.52 (m, 1H), 2.45 (s, 3H), 2.39 (s, 3H), 1.78 (m, 1H), 1.65 (m, 2H), 1.36 (m, 2H), 1.20 (t, 3H), 0.96 ppm (t, 3H); MS (*m/z*) 342 $[MH]^+$.

***N*-(Cyclopropylmethyl)-7-(4-fluoro-2-methylphenyl)-2-methyl-*N*-propyl-6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidin-4-amine (3 db):** This compound was prepared using method B, except that 1-bromo-4-fluoro-2-methylbenzene was used instead of 1-bromo-2,4-dimethoxybenzene in the first step, and *N*-(cyclopropylmethyl)-1-propanamine was used instead of *N*-ethyl-1-butanamine in the last step: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.87 (dd, 1H), 6.74 (td, 1H), 6.63 (dd, 1H), 4.31 (dd, 1H), 3.56 (m, 2H), 3.51 (dd, 2H), 3.04 (m, 2H), 2.50 (m, 1H), 2.44 (s, 3H), 2.38 (s, 3H), 1.80 (m, 1H), 1.66 (q, 2H), 0.92 (t, 3H), 1.08–0.85 (m, 1H), 0.54 (m, 2H), 0.29 ppm (m, 2H); MS (*m/z*) 353 $[M]^+$.

***N*-Butyl-7-(2,4-dimethylphenyl)-*N*-ethyl-2-methyl-6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidin-4-amine (3 ea):** This compound was prepared using method B, except that 1-bromo-2,4-dimethylbenzene was used instead of 1-bromo-2,4-dimethoxybenzene in the first step: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.98 (d, 1H), 6.86 (dd, 1H), 6.56 (d, 1H), 4.32 (m, 1H), 3.70–3.44 (m, 2H + 2H), 3.02 (m, 2H), 2.50 (m, 1H), 2.42 (s, 3H), 2.35 (s, 3H), 2.25 (s, 3H), 1.8 (m, 1H), 1.6 (m, 2H), 1.35 (m, 2H), 1.19 (t, 3H), 0.96 ppm (t, 3H); MS (*m/z*) 338 $[MH]^+$.

***N*-(Cyclopropylmethyl)-7-(2,4-dimethylphenyl)-2-methyl-*N*-propyl-6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidin-4-amine (3 eb):** This compound was prepared using method B, except that 1-bromo-2,4-dimethylbenzene was used instead of 1-bromo-2,4-dimethoxybenzene in the first step, and *N*-(cyclopropylmethyl)-1-propanamine was used instead of *N*-ethyl-1-butanamine in the last step: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.98 (d, 1H), 6.87 (dd, 1H), 6.58 (d, 1H), 4.33 (m, 1H), 3.66–3.44 (m, 2H + 2H), 3.05 (m, 2H), 2.50 (m, 1H), 2.42 (s, 3H), 2.35 (s, 3H), 2.26 (s, 3H), 1.85 (m, 1H), 1.60 (m, 2H), 1.08 (m, 1H), 0.91 (t, 3H), 0.53 (m, 2H), 0.29 ppm (m, 2H); MS (*m/z*) 350 $[MH]^+$.

Pyrrolo[2,3-d]pyrimidine scaffold: Method C:

6-Hydroxy-2-methyl-5-(2-propen-1-yl)-4(1*H*)-pyrimidinone (13): Sodium (2 g, 3 equiv) was added portion-wise to MeOH (anhyd, 100 mL) at 0 °C under N_2. After consumption of metallic sodium, acetamidine hydrochloride (8.4 g, 3 equiv) was added. After 10 min of stirring, the precipitated NaCl was filtered off. Diethyl-2-propen-1-ylpropanedioate (6 mL, 30.42 mmol) was added to the solution of free acetamidine, and the mixture was stirred at RT for 2 days. The reaction mixture was concentrated, then neutralized with concentrated hydrochloric acid and filtered to obtain compound **13** (4.25 g, 15.57 mmol, 84%) as a white solid: ^{1}H NMR (400 MHz, [D_6]DMSO): δ = 11.61 (br s, 2H), 5.75 (m, 1H), 4.92 (m, 1H), 4.84 (m, 1H), 2.94 (d, 2H), 2.19 ppm (s, 3H); MS (*m/z*) 166 $[M]^+$.

4,6-Dichloro-2-methyl-5-(2-propen-1-yl)pyrimidine (14): Compound **13** (6.0 g, 36.14 mmol) was mixed with $POCl_3$ (70 mL) and heated at reflux for 3 h. The resulting solution was cooled to RT and poured slowly into ice/water (600 mL) with vigorous stirring. The product was extracted with EtOAc (3×50 mL). The combined organic extracts were washed with $NaHCO_3$ (satd aq, 60 mL) and NaCl (satd aq, 40 mL) and dried over anhyd Na_2SO_4. The solids were filtered, and the solvent was evaporated in vacuo. The crude oil was purified by flash chromatography (silica gel, *c*Hex 100%) to give compound **14** (4.78 g, 23.54 mmol, 65%) as a light-yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.85 (m, 1H), 5.15 (dq, 1H), 5.11 (dq, 1H), 3.61 (dt, 2H), 2.67 ppm (s, 3H); MS (*m/z*) 202 $[M]^+$.

6-Chloro-*N*-(2,4-dichlorophenyl)-2-methyl-5-(2-propen-1-yl)-4-pyrimidinamine (15 a): A solution of 2,4-dichloroaniline (798 mg, 1 equiv) in THF (anhyd, 22 mL) under N_2 was treated with sodium hydride 95%/oil (393 mg, 3 equiv) at 0 °C for 15 min before compound **14** (1 g, 4.92 mmol) was added. The mixture was heated at reflux for 3 h, cooled to RT and quenched with water (20 mL). The product was extracted with EtOAc (2×20 mL) and dried over anhyd Na_2SO_4. The solids were filtered, and the solvent was evaporated in vacuo. The crude product was purified by flash chromatography (silica gel, *c*Hex/EtOAc 96:4) to give compound **15 a** (725 mg, 2.20 mmol, 45%) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.52 (d, 1H), 7.40 (d, 1H), 7.27 (dd, 1H), 7.21 (br s, 1H), 5.90 (m, 1H), 5.26 (m, 2H), 3.58 (m, 2H), 2.57 ppm (s, 3H); MS (*m/z*) 327 $[M]^+$.

1,1-Dimethylethyl-[6-chloro-2-methyl-5-(2-propen-1-yl)-4-pyrimidinyl]-(2,4-dichlorophenyl)carbamate (16 a): $(Boc)_2O$ (194 mg, 2.0 equiv) and DMAP (cat.) were added to a solution of compound **15 a** (146 mg, 0.444 mmol) in CH_2Cl_2 (anhyd, 11 mL) under N_2. The reaction mixture was stirred at RT for 18 h. The solution was diluted with water (10 mL) and extracted with EtOAc (3×15 mL). The combined organic extracts were dried over anhyd Na_2SO_4, filtered, and concentrated to dryness in vacuo. Flash chromatography of the crude product (silica gel, *c*Hex/EtOAc 95:5) gave compound **16 a** (164 mg, 0.383 mmol, 86%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.47 (d, 1H), 7.20 (dd, 1H), 7.17 (d, 1H), 5.75 (tq, 1H), 5.05(dd, 1H), 4.97 (dd, 1H), 3.52 (d, 2H), 2.58 (s, 3H), 1.44 ppm (s, 9H); MS (*m/z*) 428 $[MH]^+$.

1,1-Dimethylethyl-[6-chloro-5-(2-hydroxyethyl)-2-methyl-4-pyrimidinyl]-(2,4-dichlorophenyl)carbamate (17 a): A solution of compound **16 a** (160 mg, 0.373 mmol) in a mixture of CH_2Cl_2 (anhyd, 9 mL) and CH_3OH (anhyd, 1 mL) was ozonized (5 g h^{-1}) at −78 °C for 10 min. When all the starting material had disappeared (according to TLC), the reaction mixture was first flushed with oxygen and then with nitrogen for 20 min. $NaBH_4$ (56 mg, 4 equiv) was added to the cooled reaction mixture, and the temperature was allowed to warm to RT. The solution was stirred for 3 h at RT. It was then di-

luted with water (10 mL) and extracted with CH_2Cl_2 (3×10 mL). The combined organic extracts were dried over anhyd Na_2SO_4, filtered, and concentrated to dryness in vacuo. The crude product was purified by flash chromatography (silica gel, *c*Hex/EtOAc 85:15) to give compound **17a** (120 mg, 0.277 mmol, 74%) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.49 (d, 1H), 7.37 (d, 1H), 7.23 (dd, 1H), 3.93 (q, 2H), 3.05 (t, 2H), 2.59 (s, 3H), 1.89 (br s, 1H), 1.45 ppm (s, 9H); MS (*m/z*) 432 $[MH]^+$.

2-[4-Chloro-6-((2,4-dichlorophenyl){[(1,1-dimethylethyl)oxy]carbonyl}amino)-2-methyl-5-pyrimidinyl]ethyl methanesulfonate (18a): Et_3N (0.545 mL, 5 equiv) and CH_3SO_2Cl (0.120 mL, 2 equiv) were added to a solution of compound **17a** (337 mg, 0.779 mmol) in CH_2Cl_2 (anhyd, 15 mL) at RT under N_2. The reaction mixture was stirred at RT for 18 h. Water (15 mL) and EtOAc (15 mL) were added, the phases were separated, and the aqueous layer was extracted with additional EtOAc (2×15 mL). The combined organic extracts were washed with H_2O (20 mL), dried over anhyd Na_2SO_4, filtered and concentrated in vacuo. The crude product was purified by flash chromatography (silica gel, *c*Hex/EtOAc 75:25) to give compound **18a** (327 mg, 0.640 mmol, 82%) as a white foam: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.49 (d, 1H), 7.34 (d, 1H), 7.26 (m, 1H), 4.52 (t, 2H), 3.24 (t, 2H), 2.98 (s, 3H), 2.58 (s, 3H), 1.45 ppm (s, 9H); MS (*m/z*) 510 $[MH]^+$.

2-{4-Chloro-6-[(2,4-dichlorophenyl)amino]-2-methyl-5-pyrimidinyl}ethyl methanesulfonate (19a): A solution of compound **18a** (327 mg, 0.640 mmol) in 20% TFA/CH_2Cl_2 (10 mL) was stirred at RT for 2 h. The solvent was removed in vacuo and the residue was partitioned between EtOAc (10 mL) and $NaHCO_3$ (satd aq, 10 mL), and the layers were separated. The aqueous layer was extracted with EtOAc (3×10 mL), and the combined organic extracts were dried over anhyd Na_2SO_4, filtered, and concentrated to dryness in vacuo to deliver compound **19a** (224 mg, 0.545 mmol, 85%) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.39 (d, 1H), 7.49 (d, 1H), 7.44 (br s, 1H), 7.34 (dd, 1H), 4.56 (t, 2H), 3.28 (t, 2H), 3.03 (s, 3H), 2.61 ppm (s, 3H); MS (*m/z*) 410 $[MH]^+$.

4-Chloro-7-(2,4-dichlorophenyl)-2-methyl-6,7-dihydro-5*H*-pyrrolo[2,3-*d*]pyrimidine (20a): NaH 95%/oil (20 mg, 1.5 equiv) was added to a solution of compound **19a** (224 mg, 0.545 mmol) in THF (anhyd, 10 mL) at RT under N_2. The reaction mixture was stirred for 2 h at RT. It was then diluted with water (10 mL) and extracted with EtOAc (2×15 mL). The combined organic extracts were dried over anhyd Na_2SO_4, filtered, and concentrated to dryness in vacuo. The crude product was purified by flash chromatography (silica gel, *c*Hex/EtOAc 75:25) to give compound **20a** (158 mg, 0.502 mmol, 92%) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.51 (s, 1H), 7.33 (m, 2H), 4.04 (t, 2H), 3.21 (t, 2H), 2.44 ppm (s, 3H); MS (*m/z*) 313 $[MH]^+$.

***N*-(Cyclopropylmethyl)-7-(2,4-dichlorophenyl)-2-methyl-6,7-dihydro-5*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine (4aa):** A mixture of compound **20a** (15 mg, 0.048 mmol) and (cyclopropylmethyl)amine (0.250 mL, large excess) in a screw-cap vial was heated at 130 °C for 6 h. The excess amine was evaporated, and the residue purified by flash chromatography (silica gel, *c*Hex/EtOAc 8:2) yielding compound **4aa** (9.5 mg, 0.027 mmol, 57%) as a yellow solid: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.43 (d, 1H), 7.36 (d, 1H), 7.25 (dd, 1H), 4.38 (br s, 1H), 3.94 (t, 2H), 3.34 (t, 2H), 3.01 (t, 2H), 2.36 (s, 3H), 1.07 (m, 1H), 0.56 (q, 2H), 0.27 ppm (q, 2H); MS (*m/z*) 349 $[MH]^+$.

7-(2,4-Dichlorophenyl)-*N*-(1-ethylpropyl)-2-methyl-6,7-dihydro-5*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine (4ab): This compound was prepared using method C, except that 3-pentanamine was used instead of (cyclopropylmethyl)amine in the last step: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.43 (d, 1H), 7.38 (d, 1H), 7.25 (dd, 1H), 4.02 (s, 1H), 3.93 (m, 1H), 3.93 (t, 2H), 2.97 (t, 2H), 2.33 (s, 3H), 1.65 (m, 2H), 1.5 (m, 2H), 0.94 ppm (t, 6H); MS (*m/z*) 365 $[MH]^+$.

7-(2,4-Dichlorophenyl)-2-methyl-*N*-[1-methyl-2-(methyloxy)ethyl]-6,7-dihydro-5*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine (4ac): This compound was prepared using method C, except that 1-(methyloxy)-2-propanamine was used instead of (cyclopropylmethyl)amine in the last step: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.43 (d, 1H), 7.37 (d, 1H), 7.25 (dd, 1H), 4.43–4.32 (m, 2H), 3.94 (t, 2H), 3.44 (d, 2H), 3.39 (s, 3H), 2.94 (t, 2H), 2.35 (s, 3H), 1.26 ppm (d, 3H); MS (*m/z*) 367 $[MH]^+$.

7-(2,4-Dichlorophenyl)-2-methyl-*N*-[(1*S*)-2-(methyloxy)-1-(phenylmethyl)ethyl]-6,7-dihydro-5*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine (4ad): This compound was prepared using method C, except that (2*S*)-1-(methyloxy)-3-phenyl-2-propanamine was used instead of (cyclopropylmethyl)amine in the last step: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.44 (d, 1H), 7.37 (d, 1H), 7.35–7.20 (m, 6H), 4.53 (br m, 1H), 4.45 (br m, 1H), 3.94 (t, 2H), 3.40 (m, 5H), 3.01 (dd, 1H), 2.91 (m, 3H), 2.39 ppm (s, 3H); MS (*m/z*) 443 $[MH]^+$.

***N*-Cyclopentyl-7-(2,4-dichlorophenyl)-2-methyl-6,7-dihydro-5*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine (4ae):** This compound was prepared using method C, except that cyclopentanamine was used instead of (cyclopropylmethyl)amine in the last step: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.44 (d, 1H), 7.38 (d, 1H), 7.26 (dd, 1H), 4.34 (m, 2H), 3.94 (t, 2H), 3.03 (t, 2H), 2.35 (s, 3H), 2.05 (m, 2H), 1.8–1.4 ppm (m, 6H); MS (*m/z*) 363 $[MH]^+$.

***N*-Butyl-7-(2,4-dichlorophenyl)-*N*-ethyl-2-methyl-6,7-dihydro-5*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine (4af):** This compound was prepared using method C, except that *N*-ethyl-1-butanamine was used instead of (cyclopropylmethyl)amine in the last step: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.43 (d, 1H), 7.37 (d, 1H), 7.25 (dd, 1H), 3.84 (t, 2H), 3.56 (q, 2H), 3.48 (dd, 2H), 3.25 (t, 2H), 2.32 (s, 3H), 1.59(m, 2H), 1.36 (m, 2H), 1.19 (t, 3H), 0.97 ppm (t, 3H); MS (*m/z*) 379 $[MH]^+$.

***N*-(Cyclopropylmethyl)-7-(2,4-dichlorophenyl)-2-methyl-*N*-propyl-6,7-dihydro-5*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine (4ag):** This compound was prepared using method C, except that (cyclopropylmethyl)propylamine was used instead of (cyclopropylmethyl)amine in the last step: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.43 (d, 1H), 7.37 (d, 1H), 7.25 (dd, 1H), 3.84 (t, 2H), 3.50 (dd, 2H), 3.46 (d, 2H), 3.28 (t, 2H), 2.31 (s, 3H), 1.65 (m, 2H), 1.07 (m, 1H), 0.93 (t, 3H), 0.53 (m, 2H), 0.29 ppm (m, 2H); MS (*m/z*) 391 $[MH]^+$.

7-[2-Bromo-4-(1-methylethyl)phenyl]-*N*-(1-ethylpropyl)-2-methyl-6,7-dihydro-5*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine (4ba): This compound was prepared using method C, except that 2-bromo-4-isopropylaniline was used instead of 2,4-dichloroaniline in the third step, and 3-pentanamine was used instead of (cyclopropylmethyl)amine in the last step: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.43 (d, 1H), 7.24 (d, 1H), 7.15 (dd, 1H), 4.00 (br s, 1H), 3.90 (m, 1H), 3.89 (t, 2H), 2.94 (t, 2H), 2.84(m, 1H), 2.29 (s, 3H), 1.65–1.4 (m, 4H), 1.21 (d, 6H), 0.91 ppm (t, 6H); MS (*m/z*) 417 $[MH]^+$.

7-[2-Bromo-4-(1-methylethyl)phenyl]-*N*-butyl-*N*-ethyl-2-methyl-6,7-dihydro-5*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine (4bb): This compound was prepared using method C, except that 2-bromo-4-isopropylaniline was used instead of 2,4-dichloroaniline in the third step, and *N*-ethyl-1-butanamine was used instead of (cyclopropylmethyl)amine in the last step: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.47 (d, 1H), 7.28 (d, 1H), 7.19 (dd, 1H), 3.83 (t, 2H), 3.55 (q, 2H), 3.48 (m, 2H), 3.24 (t, 2H), 2.89 (m, 1H), 2.31 (s, 3H), 1.58 (m, 2H), 1.36

(m, 2H), 1.25 (d, 6H), 1.18 (t, 3H), 0.97 ppm (t, 3H); MS (*m/z*) 431 $[MH]^+$.

7-[2-Bromo-4-(1-methylethyl)phenyl]-*N*-(cyclopropylmethyl)-2-methyl-*N*-propyl-6,7-dihydro-5*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine (4bc): This compound was prepared using method C, except that 2-bromo-4-isopropylaniline was used instead of 2,4-dichloroaniline in the third step, and (cyclopropylmethyl)propylamine was used instead of (cyclopropylmethyl)amine in the last step: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.46 (d, 1H), 7.28 (d, 1H), 7.18 (dd, 1H), 3.84 (t, 2H), 3.51 (m, 2H), 3.46 (m, 2H), 3.27 (t, 2H), 2.89 (m, 1H), 2.31 (s, 3H), 1.66 (m, 2H), 1.25 (d, 6H), 1.09 (m, 1H), 0.93 (t, 3H), 0.53 (m, 2H), 0.28 ppm (q, 2H); MS (*m/z*) 431 $[MH]^+$.

***N*-Butyl-7-[2-chloro-4-(trifluoromethyl)phenyl]-*N*-ethyl-2-methyl-6,7-dihydro-5*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine (4da):** This compound was prepared using method C, except that 2-chloro-4-(trifluoromethyl)aniline was used instead of 2,4-dichloroaniline in the third step, and *N*-ethyl-1-butanamine was used instead of (cyclopropylmethyl)amine in the last step: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.68 (d, 1H), 7.61 (d, 1H), 7.51 (dd, 1H), 3.92 (t, 2H), 3.57 (q, 2H), 3.49 (t, 2H), 3.27 (t, 2H), 2.33 (s, 3H), 1.61 (m, 2H), 1.37 (m, 2H), 1.19 (t, 3H), 0.98 ppm (t, 3H); MS (*m/z*) 413 $[MH]^+$.

7-[2-Chloro-4-(trifluoromethyl)phenyl]-*N*-(cyclopropylmethyl)-2-methyl-*N*-propyl-6,7-dihydro-5*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine (4db): This compound was prepared using method C, except that 2-chloro-4-(trifluoromethyl)aniline was used instead of 2,4-dichloroaniline in the third step, and (cyclopropylmethyl)propylamine was used instead of (cyclopropylmethyl)amine in the last step: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.68 (d, 1H), 7.61 (d, 1H), 7.52 (dd, 1H), 3.93 (t, 2H), 3.52 (t, 2H), 3.47 (d, 2H), 3.30 (t, 2H), 2.33 (s, 3H), 1.66 (m, 2H), 1.07 (m, 1H), 0.93 (t, 3H), 0.55–0.29 ppm (m, 4H); MS (*m/z*) 425 $[MH]^+$.

7-(2,4-Dichlorophenyl)-2-methyl-4-(2-methyl-1-piperidinyl)-6,7-dihydro-5*H*-pyrrolo[2,3-*d*]pyrimidine (4ea): This compound was prepared using method C, except that 2-methylpiperidine was used instead of (cyclopropylmethyl)amine in the last step: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.42 (d, 1H), 7.35 (d, 1H), 7.23 (dd, 1H), 4.71 (m, 1H), 4.33 (br d, 1H), 3.83 (t, 2H), 3.24 (m, 2H), 3.02 (dt, 1H), 2.32 (s, 3H), 1.78–1.49 (m, 6H), 1.21 ppm (d, 3H); MS (*m/z*) 377 $[MH]^+$.

7-(2,4-Dichlorophenyl)-4-(2-ethyl-1-piperidinyl)-2-methyl-6,7-dihydro-5*H*-pyrrolo[2,3-*d*]pyrimidine (4eb): This compound was prepared using method C, except that 2-ethylpiperidine was used instead of (cyclopropylmethyl)amine in the last step: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.42 (d, 1H), 7.36 (d, 1H), 7.24 (dd, 1H), 4.44 (m, 1H), 4.38 (m,1H), 3.83 (m, 2H), 3.22 (t, 2H), 2.97 (m, 1H), 2.31 (s, 3H), 1.73 (m, 2H), 1.80–1.10 (m, 6H), 0.88 ppm (t, 3H); MS (*m/z*) 391 $[MH]^+$.

7-(2,4-Dichlorophenyl)-4-(3,5-dimethyl-1-piperidinyl)-2-methyl-6,7-dihydro-5*H*-pyrrolo[2,3-*d*]pyrimidine (4ec): This compound was prepared using method C, except that 3,5-dimethylpiperidine was used instead of (cyclopropylmethyl)amine in the last step: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.43 (d, 1H), 7.35 (d, 1H), 7.24 (dd, 1H), 4.41 (m, 2H), 3.84 (t, 2H), 3.24 (t, 2H), 2.32 (s, 3H), 2.30 (m, 2H), 1.85 (m, 1H), 1.66 (m, 2H), 0.91 (d, 6H), 0.80 ppm (m, 1H); MS (*m/z*) 391 $[MH]^+$.

7-(2,4-Dichlorophenyl)-2-methyl-4-(1-pyrrolidinyl)-6,7-dihydro-5*H*-pyrrolo[2,3-*d*]pyrimidine (4ed): This compound was prepared using method C, except that pyrrolidine was used instead of (cyclopropylmethyl)amine in the last step: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.42 (d, 1H), 7.36 (d, 1H), 7.24 (dd, 1H), 3.83 (t, 2H), 3.66 (t, 4H), 3.35 (t, 2H), 2.33 (s, 3H), 1.92 ppm (m, 4H); MS (*m/z*) 349 $[MH]^+$.

7-(2,4-Dichlorophenyl)-2-methyl-4-(2-methyl-1-pyrrolidinyl)-6,7-dihydro-5*H*-pyrrolo[2,3-*d*]pyrimidine (4ee): This compound was prepared using method C, except that 2-methylpyrrolidine was used instead of (cyclopropylmethyl)amine in the last step: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.42 (d, 1H), 7.37 (d, 1H), 7.24 (dd, 1H), 4.39 (m, 1H), 3.88 (m, 2H), 3.76 (m, 1H), 3.60 (m, 1H), 3.30 (m, 2H), 2.32 (s, 3H), 2.06 (m, 2H), 1.90 (m, 1H), 1.65 (m, 1H), 1.21 ppm (d, 3H); MS (*m/z*) 363 $[MH]^+$.

7-(2,4-Dichlorophenyl)-4-[(2*R*,5*R*)-2,5-dimethyl-1-pyrrolidinyl]-2-methyl-6,7-dihydro-5*H*-pyrrolo[2,3-*d*]pyrimidine (4ef): This compound was prepared using method C, except that (2*R*,5*R*)-2,5-dimethylpyrrolidine was used instead of (cyclopropylmethyl)amine in the last step: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.41 (d, 1H), 7.39 (d, 1H), 7.24 (dd, 1H), 4.4 (m, 2H), 3.9–3.8 (m, 2H), 3.16 (m, 2H), 2.32 (s, 3H), 2.18 (m, 2H), 1.6 (m, 2H), 1.13 ppm (d, 6H); MS (*m/z*) 377 $[MH]^+$.

2-{1-[7-(2,4-Dichlorophenyl)-2-methyl-6,7-dihydro-5*H*-pyrrolo-[2,3-*d*]pyrimidin-4-yl]-2-pyrrolidinyl}acetamide (4eg): This compound was prepared using method C, except that 2-(2-pyrrolidinyl)acetamide was used instead of (cyclopropylmethyl)amine in the last step: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.44 (d, 1H), 7.34 (d, 1H), 7.26 (dd, 1H), 7.12 (br s, 1H), 5.29 (br s, 1H), 4.62 (br m, 1H), 3.86 (t, 2H), 3.81 (m, 1H), 3.60 (m, 1H), 3.36 (m, 2H), 2.81 (dd, 1H), 2.32 (s, 3H), 2.24 (dd, 1H), 2.01 ppm (m, 4H); MS (*m/z*): 406 $[MH]^+$.

7-(2,4-Dichlorophenyl)-4-(2,5-dimethyl-2,5-dihydro-1*H*-pyrrol-1-yl)-2-methyl-6,7-dihydro-5*H*-pyrrolo[2,3-*d*]pyrimidine (4eh): This compound was prepared using method C, except that 2,5-dimethyl-2,5-dihydro-1*H*-pyrrole was used instead of (cyclopropylmethyl)amine in the last step: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.43 (d, 1H), 7.38 (d, 1H), 7.25 (dd, 1H), 5.80 (s, 2H), 4.94 (q, 2H), 3.84 (t, 2H), 3.32 (t, 2H), 2.33 (s, 3H), 1.42 ppm (d, 6H); MS (*m/z*) 375 $[MH]^+$.

7-(2,4-Dichlorophenyl)-2-methyl-4-(5-methyl-1*H*-pyrazol-1-yl)-6,7-dihydro-5*H*-pyrrolo[2,3-*d*]pyrimidine (4fa) and 7-(2,4-dichlorophenyl)-2-methyl-4-(3-methyl-1*H*-pyrazol-1-yl)-6,7-dihydro-5*H*-pyrrolo[2,3-*d*]pyrimidine (4fb): 3-Methyl-1*H*-pyrazole (16 mg, 3 equiv) was added to a suspension of NaH 80%/oil (5.5 mg, 3 equiv) in DMF (anhyd, 0.3 mL) at RT under N_2, and the reaction mixture was stirred at RT for 30 min. Compound **20a** (20 mg, 0.064 mmol) was then added, and the reaction mixture was heated at 100 °C for 6 h. The solvent was evaporated, and the residue partitioned between CH_2Cl_2/H_2O. The phases were separated, and the aqueous phase extracted with CH_2Cl_2 (2×10 mL). The combined organic extracts were dried over anhyd Na_2SO_4, the solids were filtered, and the solvent was evaporated. The residue was purified by flash chromatography (silica gel, *c*Hex/EtOAc 9:1) to obtain compound **4fa** (18 mg, 0.050 mmol, 78%) along with its regioisomer **4fb** (1.5 mg, 0.004 mmol, 7%) as white solids: **4fa** ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.49 (d, 1H), 7.50 (d, 1H), 7.39 (d, 1H), 7.31 (dd, 1H), 6.23 (d, 1H), 4.02 (t, 2H), 3.64 (t, 2H), 2.46 (s, 3H), 2.37 ppm (s, 3H); MS (*m/z*) 360 $[MH]^+$; **4fb** ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.59 (d, 1H), 7.51 (d, 1H), 7.38 (d, 1H), 7.32 (dd, 1H), 6.17 (br d, 1H), 4.01 (t, 2H), 3.55 (t, 2H), 2.70 (s, 3H), 2.46 ppm (s, 3H); MS (*m/z*) 360 $[MH]^+$.

7-(2,4-Dichlorophenyl)-2-methyl-4-(4-methyl-1*H*-pyrazol-1-yl)-6,7-dihydro-5*H*-pyrrolo[2,3-*d*]pyrimidine (4fc): This compound was prepared using 4-methyl-1*H*-pyrazole and compound **20a**, as reported for the preparation of compound **4fa**: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.38 (m, 1H), 7.58 (br s, 1H), 7.50 (d, 1H), 7.39 (d, 1H),

7.32 (dd, 1H), 4.02 (t, 2H), 3.62 (t, 2H), 2.46 (s, 2H), 2.17 ppm (s, 3H); MS (*m/z*) 360 $[MH]^+$.

7-(2,4-Dichlorophenyl)-4-(3,5-dimethyl-1*H*-pyrazol-1-yl)-2-methyl-6,7-dihydro-5*H*-pyrrolo[2,3-*d*]pyrimidine (4 fd): This compound was prepared using 3,5-dimethyl-1*H*-pyrazole and compound **20 a**, as reported for the preparation of compound **4 fa**: ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.49$ (d, 1H), 7.37 (d, 1H), 7.30 (dd, 1H), 5.96 (s, 1H), 3.99 (t, 2H), 3.54 (t, 2H), 2.64 (s, 3H), 2.44 (s, 3H), 2.27 ppm (s, 3H); MS (*m/z*) 374 $[MH]^+$.

1-[7-(2,4-Dichlorophenyl)-2-methyl-6,7-dihydro-5*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl]-1*H*-pyrazole-3-carbonitrile (4 fe): This compound was prepared using 1*H*-pyrazole-3-carbonitrile and compound **20 a**, as reported for the preparation of compound **4 fa**: ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 8.74$ (d, 1H), 7.53 (dd, 1H), 7.4–7.3 (m, 2H), 6.83 (d, 1H) 4.09 (t, 2H), 3.66 (t, 2H), 2.49 ppm (s, 3H); MS (*m/z*): 371 $[MH]^+$.

7-(2,4-Dichlorophenyl)-4-[3-(ethyloxy)-5-methyl-1*H*-pyrazol-1-yl]-2-methyl-6,7-dihydro-5*H*-pyrrolo[2,3-*d*]pyrimidine (4 ff): This compound was prepared using 3-(ethyloxy)-5-methyl-1*H*-pyrazole and compound **20 a**, as reported for the preparation of compound **4 fa**: ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.48$ (d, 1H), 7.37 (d, 1H), 7.29 (dd, 1H), 5.63 (s, 1H), 4.26 (q, 2H), 3.96 (t, 2H), 3.57 (t, 2H), 2.66 (s, 3H), 2.41 (s, 3H), 1.40 ppm (t, 3H); MS (*m/z*) 404 $[MH]^+$.

4-(4-Bromo-1*H*-pyrazol-1-yl)-7-(2,4-dichlorophenyl)-2-methyl-6,7-dihydro-5*H*-pyrrolo[2,3-*d*]pyrimidine (4 fg): This compound was prepared using 4-bromo-1*H*-pyrazole and compound **20 a**, as reported for the preparation of compound **4 fa**: ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 8.63$ (s, 1H), 7.68 (s, 1H), 7.50 (d, 1H), 7.37 (d, 1H), 7.32 (dd, 1H), 4.03 (t, 2H), 3.59 (t, 2H), 2.44 ppm (s, 3H); MS (*m/z*) 424 $[MH]^+$.

4-(4-Bromo-3-methyl-1*H*-pyrazol-1-yl)-7-(2,4-dichlorophenyl)-2-methyl-6,7-dihydro-5*H*-pyrrolo[2,3-*d*]pyrimidine (4 fh): This compound was prepared using 4-bromo-3-methyl-1*H*-pyrazole and compound **20 a**, as reported for the preparation of compound **4 fa**: ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 8.55$ (s, 1H), 7.50 (d, 1H), 7.37 (d, 1H), 7.31 (dd, 1H), 4.01 (t, 2H), 3.59 (t, 2H), 2.43 (s, 3H), 2.31 ppm (s, 3H); MS (*m/z*) 440 $[MH]^+$.

7-(2,4-Dichlorophenyl)-2-methyl-4-[3-(trifluoromethyl)-1*H*-pyrazol-1-yl]-6,7-dihydro-5*H*-pyrrolo[2,3-*d*]pyrimidine (4 fi): This compound was prepared using 3-(trifluoromethyl)-1*H*-pyrazole and compound **20 a**, as reported for the preparation of compound **4 fa**: ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 8.65$ (m, 1H), 7.51 (d, 1H), 7.38 (d, 1H), 7.33 (dd, 1H), 6.68 (m, 1H), 4.04 (t, 2H), 3.65 (t, 2H), 2.46 ppm (s, 3H); MS (*m/z*) 414 $[MH]^+$.

7-(2,4-Dichlorophenyl)-2-methyl-4-[5-methyl-3-(trifluoromethyl)-1*H*-pyrazol-1-yl]-6,7-dihydro-5*H*-pyrrolo[2,3-*d*]pyrimidine (4 fj): This compound was prepared using 5-methyl-3-(trifluoromethyl)-1*H*-pyrazole and compound **20 a**, as reported for the preparation of compound **4 fa**: ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.52$ (d, 1H), 7.37 (d, 1H), 7.33 (dd, 1H), 6.43 (s, 1H), 4.03 (t, 2H), 3.55 (t, 2H), 2.72 (s, 3H), 2.46 ppm (s, 3H); MS (*m/z*) 428 $[MH]^+$.

7-(2,4-Dichlorophenyl)-2-methyl-4-[3-(trifluoromethyl)-4,5,6,7-tetrahydro-1*H*-indazol-1-yl]-6,7-dihydro-5*H*-pyrrolo[2,3-*d*]pyrimidine (4 fk): This compound was prepared using 3-(trifluoromethyl)-4,5,6,7-tetrahydro-1*H*-indazole and compound **20 a**, as reported for the preparation of compound **4 fa**: ^{1}H NMR (400 MHz, $CDCl_3$): $\delta =$ 7.51 (d, 1H), 7.37 (d, 1H), 7.32 (dd, 1H), 4.015 (t, 2H), 3.58 (t, 2H), 3.20 (t, 2H), 2.65 (t, 2H), 2.44 (s, 3H), 1.82 ppm (m, 4H); MS (*m/z*) 468 $[MH]^+$.

7-(2,4-Dichlorophenyl)-4-(3,5-dimethyl-1*H*-1,2,4-triazol-1-yl)-2-methyl-6,7-dihydro-5*H*-pyrrolo[2,3-*d*]pyrimidine (4 fl): A solution of compound **20 a** (30 mg, 0.095 mmol) and hydrazine hydrate (5 μL, 0.095 mmol) in methanol (0.95 mL) was heated at 130 °C (screw-cap vial) for 18 h. The mixture was then evaporated to dryness, and the crude product obtained, together with 2,2,2-trifluoro-*N*-formylacetamide (43 mg, 0.3 mmol) in *N*-methylpyrrolidone (200 μL) were heated at 100 °C (screw-cap vial) for 5 h. The mixture was diluted with cold satd aq NaCl and extracted with EtOAc (3×10 mL). The combined organic extracts were dried over anhyd Na_2SO_4, the solids were filtered, and the solvent was evaporated in vacuo. The crude compound was purified by flash chromatography (silica gel, EtOAc/*c*Hex 9:1) to give compound **4 fl** as a white solid (42 mg, 0.094 mmol, 99%): ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 8.13$ (s, 1H), 7.54 (d, 1H), 7.38 (d, 1H), 7.35 (dd, 1H), 4.08 (t, 2H), 3.48 (t, 2H), 2.47 ppm (s, 3H); MS (*m/z*): 415 $[MH]^+$.

7-(2,4-Dichlorophenyl)-4-(3,5-dimethyl-1*H*-1,2,4-triazol-1-yl)-2-methyl-6,7-dihydro-5*H*-pyrrolo[2,3-*d*]pyrimidine (4 fm): This compound was prepared using *N*-acetylacetamide and compound **20 a**, as reported for the preparation of compound **4 fl**: ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.51$ (d, 1H), 7.37 (d, 1H), 7.33 (dd, 1H), 4.02 (t, 2H), 3.54 (t, 2H), 2.84 (s, 3H), 2.46 (s, 3H), 2.40 ppm (s, 3H); MS (*m/z*) 375 $[MH]^+$.

7-(2,4-Dichlorophenyl)-2-methyl-4-[4-(trifluoromethyl)-1*H*-imidazol-1-yl]-6,7-dihydro-5*H*-pyrrolo[2,3-*d*]pyrimidine (4 fn): This compound was prepared using 4-(trifluoromethyl)-1*H*-imidazole and compound **20 a**, as reported for the preparation of compound **4 fa**: ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 8.34$ (s, 1H), 8.05 (s, 1H), 7.54 (s, 1H), 7.36 (m, 2H), 4.13 (t, 2H), 3.45 (t, 2H), 2.47 ppm (s, 3H); MS (*m/z*) 415 $[MH]^+$.

7-(2,4-Dichlorophenyl)-4-(2,4-dimethyl-1*H*-imidazol-1-yl)-2-methyl-6,7-dihydro-5*H*-pyrrolo[2,3-*d*]pyrimidine (4 fo): This compound was prepared using 2,4-dimethyl-1*H*-imidazole and compound **20 a**, as reported for the preparation of compound **4 fa**: ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.53$ (d, 1H),7.38 (d, 1H), 7.34 (dd, 1H), 6.83 (s, 1H), 4.04 (t, 2H), 3.22 (t, 2H), 2.56 (s, 3H), 2.47 (s, 3H), 2.23 ppm (s, 3H); MS (*m/z*) 374 $[MH]^+$.

Biology and pharmacokinetics:

All work involving animals was carried out in compliance with Italian national legislation (DL 116/92), which acknowledges the European Directive 86/609, and according to internal review by the GSK Committee on Animal Research and Ethics (CARE).

In vitro binding assay: CRF binding affinity was determined in vitro by the compound's ability to displace [^{125}I]CRF and [^{125}I]sauvagine for CRF_1 and CRF_2, respectively, from recombinant human CRF receptors expressed in Chinese hamster ovary (CHO) cell membranes. For membrane preparation, CHO cells from confluent T-flasks were collected in SPA buffer (HEPES/KOH 50 mM, EDTA 2 mM, $MgCl_2$ 10 mM, pH 7.4) in 50-mL centrifuge tubes, homogenized with a Polytron instrument and centrifuged at 50000 *g* for 5 min at 4 °C (Beckman centrifuge with JA20 rotor). The pellet was resuspended, homogenized, and centrifuged as before. The scintillation proximity assay (SPA) was carried out in Optiplate by addition of 100 μL of the reagent mixture to 1 μL of compound (100% DMSO solution) per well. The assay mixture was prepared by mixing SPA buffer, WGA SPA beads (2.5 mg mL^{-1}), BSA (1 mg mL^{-1}), and membranes (50 and 5 μg protein mL^{-1} for CRF_1 and CRF_2, respectively) and 50 pM radioligand. The plate was incubated overnight (>18 h) at room temperature and read with a

Packard Topcount instrument with a WGA SPA ^{125}I counting protocol.

Rat pup ultrasonic vocalization test: Sprague–Dawley rat pups (*Rattus norvegicus*, Charles River, Italy) 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. During the test period, animals were maintained at $22\pm1\,^{\circ}C$, and their vocalizations were recorded in a sound-proof box using a bat detector. The signal was filtered and transformed into digital block pulse and processed by dedicated software (Ultravox system, Noldus, Netherlands). 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 and randomly assigned to treatment groups. The test session was carried out on the same day of the training session and vocalizations were recorded over a 5-min test period. Pups were treated intraperitoneally (10 mL kg^{-1}) with vehicle or **4 fi** (1, 3 and 10 mg kg^{-1}) 30 min before testing.

In vitro pharmacokinetics: Pooled rat liver microsomes from UK-SDG (diluted to 0.27 mg mL^{-1}, equivalent to a P450 content of 125 pmol mL^{-1}) were incubated in 1-mL round 96-deep-well polypropylene plates with a series of test compounds (5 μM) in a total volume of 0.2 mL. Pooled human liver microsomes supplied by XenoTech were also used (diluted to 0.324 mg mL, equivalent to a P450 content of 125 pmol mL^{-1}). Stock solutions of the test compounds were prepared in DMSO (10%) to maintain a 1% (*v*/*v*) final concentration in each incubate. Incubations were started by adding β-NADPH (25 μL, 8 mM) in Tris buffer (0.1 M, pH 7.4). Control incubations contained only microsomes in Tris buffer (0.1 M, pH 7.4). Plates were incubated at 37 °C in a heated shaker water bath for 30 min. Incubations were terminated by the addition of perchloric acid (20 μL, 60% *v*/*v*). Samples were centrifuged at 3000 *g* for 10 min, and the supernatants were analyzed by LC–MS.

Rat in vivo pharmacokinetics: The compounds were administered intravenously in cassette to Han Wistar male rats ($n=3$ animals) and were formulated at 2 mL kg^{-1} in DMSO/60% aqueous PEG 400 5:95 (*v*/*v*) as an iv bolus in the caudal vein (0.4 mg kg^{-1} for each compound, total dose of 1.6 mg kg^{-1}). In a second experiment, the compounds were administered orally in cassette to Han Wistar male rats ($n=3$ animals) and were formulated at 5 mL kg^{-1} in DMSO/0.5% HPMC 5:95 (*v*/*v*) by oral gavage (0.8 mg kg^{-1} for each compound, 3.2 mg kg^{-1} total dose). Blood samples were taken up to 24 h, and plasma was stored at −20 °C, pending assay. Plasma samples were assayed by automated protein precipitation and HPLC–MS–MS analysis.

Acknowledgements

The authors thank the Computational, Analytical and Structural Sciences group for analytical support.

Keywords: antagonists · corticotropin-releasing factor · drug design · receptors · structure–activity relationships

[1] a) J. Fawcett, R. L. Barkin, *J. Clin. Psychiatry* **1997**, *58 Suppl. 6*, 32–39; b) C. L. DeVane, *J. Clin. Psychiatry* **2000**, *61 Suppl. 11*, 4–8.
[2] W. Vale, J. Spiess, C. Rivier, J. Rivier, *Science* **1981**, *213*, 1394–1397.
[3] For some reviews and opinions on the subject, see: a) P. J. Gilligan, Y.-W. Li, *Curr. Opin. Drug Discovery Dev.* **2004**, *7*, 487–497; b) E. P. Zorrilla, G. F. Koob, *Expert Opin. Invest. Drugs* **2004**, *13*, 799–828.
[4] D. W. Schulz, R. S. Mansbach, J. Sprouse, J. P. Braselton, J. Collins, M. Corman, A. Dunaiskis, S. Faraci, A. W. Schmidt, T. Seeger, P. Seymour, F. D. Tingley III, E. N. Winston, Y. L. Chen, J. Heym, *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 10477–10482.
[5] L. He, P. J. Gilligan, R. Zaczek, L. W. Fitzgerald, J. McElroy, H. S. Shen, J. A. Saye, N. H. Kalin, S. Shelton, D. Christ, G. Trainor, P. Hartig, *J. Med. Chem.* **2000**, *43*, 449–456.
[6] J. Saunders, J. Williams, *Prog. Med. Chem.* **2003**, *41*, 195–247.
[7] a) P. J. Gilligan, D. W. Robertson, R. Zaczek, *J. Med. Chem.* **2000**, *43*, 1641–1660; b) J. R. McCarthy, S. C. Heinrichs, D. E. Grigoriadis, *Annu. Rep. Med. Chem.* **1999**, *34*, 11–20; c) J. R. McCarthy, S. C. Heinrichs, D. E. Grigoriadis, *Curr. Pharm. Des.* **1999**, *5*, 289–315.
[8] All pharmacophore modeling work was performed with Catalyst 4.5 (Accelrys Inc).
[9] N. Zanatta, M. B. Fagundes, R. Ellensohn, M. Marques, H. G. Bonacorso, M. A. Martins, *J. Heterocycl. Chem.* **1998**, *35*, 451–455.
[10] M. Hori, T. Kataoka, H. Shimizu, E. Imai, T. Iwamura, K. Maeda, *Chem. Pharm. Bull.* **1986**, *34*, 3599–3605.
[11] R. G. Melik-Ogandzhanyan, G. G. Danagulyan, S. A. Fagradyan, V. S. Mirzoyan, V. M. Okhikyan, L. G. Alaverdova, R. V. Agababyan, L. G. Akopyan, S. A. Papoyan, *Khim.-Farm. Zh.* **1983**, *17*, 299–303.
[12] C. R. Gardner, *J. Pharmacol. Methods* **1985**, *14*, 181–187.

Received: November 3, 2006
Published online on March 5, 2007

