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Heteroaryl-Substituted 4-(1*H*-pyrazol-1-yl)-5,6-dihydro-1*H*-pyrrolo-[2,3-*d*]pyrimidine Derivatives as Potent and Selective Corticotropin-Releasing Factor Receptor-1 Antagonists

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Depression and anxiety are psychiatric disorders that still constitute a major health concern worldwide. As a consequence of this high unmet need, more efficacious anxyolitic and antidepressant agents, exhibiting improved side-effect profiles, is highly desirable.[1] Corticotropin-releasing factor[2] (CRF) is a 41 amino acid peptide that interacts with two distinct CRF receptors, known as CRF_1 and CRF_2. CRF is considered the primary regulator of the hypothalamus–pituitary–adrenal (HPA) axis and is implicated in the endocrine, behavioural, and autonomic response to stress via activation of the CRF_1 receptor.[3,4]

As part of a broad chemical programme aimed towards the discovery of drug-like classes of CRF_1 receptor antagonists, which has proven to be highly challenging, we recently disclosed the synthesis and pharmacological characterization of cyclopenta[*d*]pyrimidines and dihydropyrrolo[2,3-*d*]pyrimidines.[5] In particular, this initial effort led to the identification of compound **1** (Figure 1), a CRF_1 antagonist endowed with high in vitro affinity, a good pharmacokinetic (PK) profile in rats, and interesting activity in an in vivo animal model of anxiety. However, the progression of this compound was limited by its high clog*P* value, the strong inhibition of CYP1A2 isoform of cytochrome P450 ($IC_{50} < 0.1$ μM), and a poor PK profile in dogs. Herein we report the identification of novel potent and selective CRF_1 antagonists. A distinctive feature of this new class of dihydropyrrolo[2,3-*d*]pyrimidine compounds is the presence of a pyrazole substituted with an additional heterocycle at the 'top' region. This characteristic sets it apart from most of the other known CRF_1 antagonists, which typically require a lipophilic alkylamino chain, and should result in improved physicochemical properties and metabolic stability. To expand our knowledge on the SAR around compound **1**, we focused our attention on the substitution pattern of the pyrazole ring with diverse heterocycles. This also enabled modulation of the physicochemical parameters and, whenever possible, a decrease in the lipophilicity, thus giving compounds that exhibit better drug-like properties. To this end, we set out to elaborate a more rational approach by computational methods. Subsequently, we tied these findings with those of a broader exploration of the substitution pattern of the 'bottom' aryl/heteroaryl ring. Thus, the synthesis and SAR of templates **2** and **3** (Figure 1) are presented, as well as the in vitro and in vivo activity and the PK profile of the best compound identified.

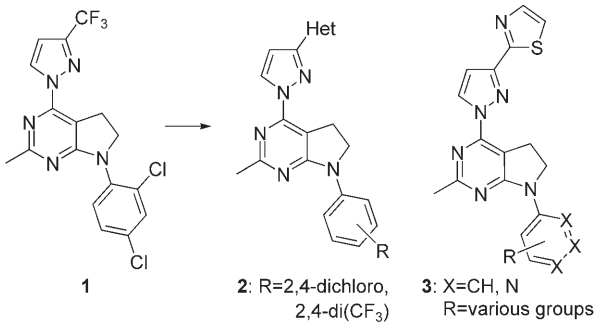

Figure 1. Conversion of template **1** into templates **2** and **3**.

We initially focused our strategy on replacement of the CF_3 group present on the pyrazole fragment. Similarity searches based on both molecular electrostatic potential (MEP) and shape were performed. This approach[6] could lead to the iden-

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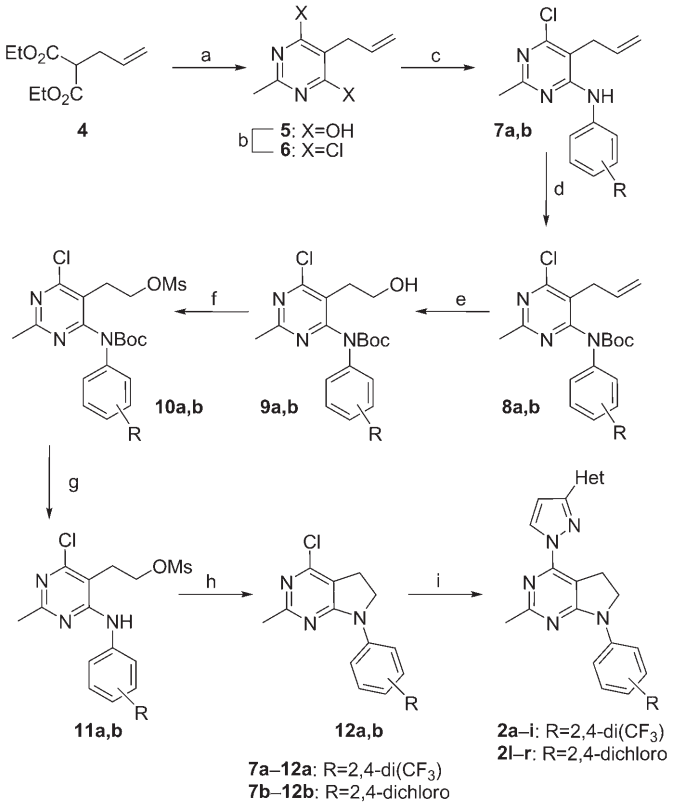
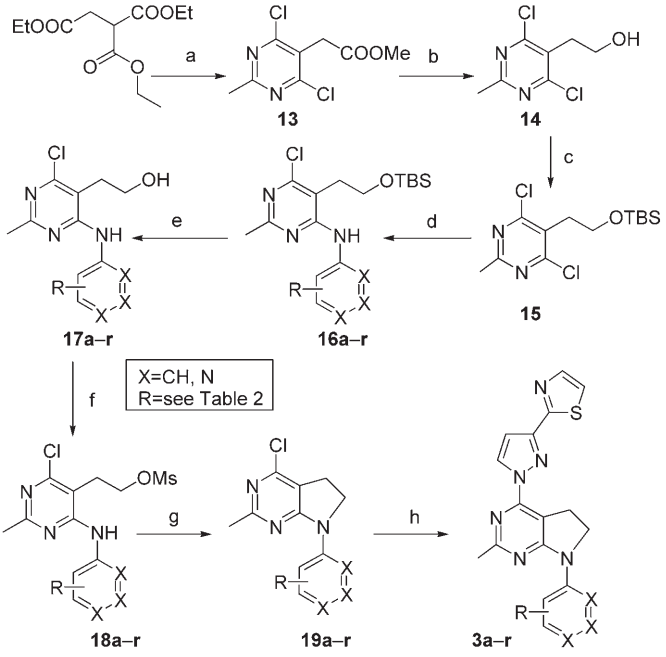


Scheme 1. Reagents and conditions: a) i) acetamidine·HCl, NaOMe, RT, 10 min, then ii) intermediate **4**, RT, 2 days, 71%; b) $POCl_3$, reflux, 3 h, 91%; c) i) (*R*)-aniline, NaH 80%/oil, THF, 0 °C, 20 min, then ii) intermediate **6**, reflux, 3 h, 27%; d) $(Boc)_2O$, 4-dimethylaminopyridine, CH_2Cl_2, RT, 18 h, 84%; e) i) O_3, CH_2Cl_2, −78 °C, 10 min, then ii) $NaBH_4$, −78 °C→RT, 3 h, 82%; f) CH_3SO_2Cl, Et_3N, CH_2Cl_2, RT, 18 h, quant.; g) 20% trifluoroacetic acid/CH_2Cl_2, RT, 2 h, 88%; h) NaH 80%/oil, THF, RT, 2 h, 60%; i) substituted pyrazole, NaH 80%/oil, DMF, 100–130 °C, 4–18 h, 92%. The yields reported are for compound **6a** (Table 1) only.

tification of structures with a high degree of novelty that might not be unearthed by similarity-searching techniques based on 2D and 3D patterns of atoms. The aim is to uncover bioisosteres for known active compounds.

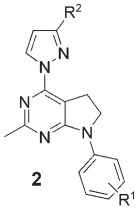
To prepare these compounds, keeping the pendant substituted phenyl ring constant (2,4-dichlorophenyl and/or the 2,4-di(trifluoromethyl)phenyl), the aryl/heteroaryl-pyrazole was introduced in the last step. This was achieved by following the synthetic pathway shown in Scheme 1 for the preparation of compounds of general structure **2**. Condensation of allylmalonate **4** in the presence of acetamidine[7,8] followed by treatment with phosphorus oxychloride gave dichloropyrimidine **6**, which was then treated under nucleophilic aromatic substitution conditions with 2,4-bistrifluoromethylaniline or 2,4-dichloroalanine to give intermediates **7a** and **7b**, respectively. Protection of the resulting secondary aniline with a *tert*-butyloxycarbonyl (Boc) group and subsequent ozonolysis of the allylic double bond followed by reductive workup, yielded the intermediate alcohols **9a** and **9b**. These were then transformed into their methanesulfonyl derivatives **10a** and **10b**, and following removal of the Boc group, closure of the five-membered ring was readily achieved through intramolecular nucleophilic substitution to form the key advanced intermediates **12a** and **12b**. Finally, aromatic nucleophilic displacements, under basic conditions, with the appropriate pyrazole derivative and **12** gave access to the target compounds **2a**–**r**.

Once the exploration of the 'top' region was complete, and the thiazole was identified as the best substituent, we sought to investigate the SAR of the 'bottom' aryl group. In order to effectively carry out this exploration, a slightly modified route, shown in Scheme 2, was set up to prepare compounds of general structure **3a**–**r**. In particular, condensation of acetamidine hydrochloride with ethoxycarbonylsuccinic acid diethyl ester followed by treatment of the resulting dihydroxypyrimidine with phosphorous oxychloride yielded the dichloropyrimidine derivative **13**, which was in turn subjected to reduction with diisobutylaluminum hydride (DIBAH) followed by protection with a *tert*-butyldimethylsilyl (TBS) group to afford intermediate **15**. At this stage the introduction of the various anilines took place via nucleophilic aromatic displacement to form intermediates **16a**–**r**. Removal of the TBS group followed by methanesulfonylation of the resulting primary alcohol led to the formation of intermediates **18a**–**r**, which were then subjected to intramolecular cylisation under basic conditions to give the cyclised chloropyrrolo[2,3-*d*]pyrimidines **19a**–**r**. Finally, nucleophilic aromatic substitution in the presence of 2-(1*H*-pyr-

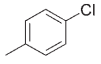
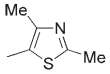


Scheme 2. Reagents and conditions: a) i) acetamidine·HCl, Na, MeOH, RT, 20 min; ii) 2-ethoxycarbonylsuccinic acid diethyl ester, RT, 48 h; iii) $POCl_3$, reflux, 3.5 h, 45%; b) 1 M DIBAH/hexanes, CH_2Cl_2, −78 °C→0 °C, 3 h, 92%; c) TBSCl, imidazole, DMF, 0 °C→RT, 3 h, 83%; d) NaH 80%/oil, (*R*)-aniline, THF, 0 °C, 15 min, reflux, 3 h, 10%; e) $Et_3N{\cdot}3HF$, DMF, RT, 2 h, quant.; f) CH_3SO_2Cl, Et_3N, CH_2Cl_2, RT, 2 h, quant.; g) THF, NaH, RT, 2 h, 65%; h) NaH 80%/oil, 2-(1*H*-pyrazol-3-yl)-1,3-thiazole, *N*-methylpyrrolidone, 0 °C, 15 min, 120 °C, 3 h, 50%. The yields reported are for compound **3b** (Table 2) only.

Table 1. In vitro characterization of template **2**: 3-pyrazolyl derivative amines.



2

Compd	R^1	R^2	pIC_{50}	Compd	R^1	R^2	pIC_{50}
2a	2,4-di(CF_3)		7.5	**2i**	2,4-di(CF_3)		6.1
2b	2,4-di(CF_3)		7.0	**2l**	2,4-dichloro		6.8
2c	2,4-di(CF_3)		7.0	**2m**	2,4-dichloro		6.6
2d	2,4-di(CF_3)		6.9	**2n**	2,4-dichloro		6.6
2e	2,4-di(CF_3)		6.9	**2o**	2,4-dichloro		6.4
2f	2,4-di(CF_3)		6.6	**2p**	2,4-dichloro		6.2
2g	2,4-di(CF_3)		6.6	**2q**	2,4-dichloro		7.4
2h	2,4-di(CF_3)		6.3	**2r**	2,4-dichloro		6.1

azol-3-yl)-1,3-thiazole led to the formation of the desired final compounds **3a**–**r**.

The in vitro potency of all the compounds prepared following the route shown in Scheme 1 is reported in Table 1[9] (all compounds reported herein were inactive on CRF_2). Compounds **2a** and **2q**, characterized by the presence of the thiazole ring, were the most potent compounds identified. The increase of the steric bulk in this region of the molecule (entries **2h** and **2p**, $pIC_{50}=6.3$ and 6.1, respectively) resulted in a significant drop of in vitro potency, which might indicate the presence of a small pocket at the receptor binding site. In addition, both the pyrazole and the thiophene were tolerated (entries **2c** and **2l**, $pIC_{50}=7.0$ and 6.8, respectively), as well as the pyridine and the pyrazine (entries **2b**, **2f**, and **2d**, $pIC_{50}=$ 7.0, 6.6, and 7.0, respectively). Notably, the morpholine maintained promising signs of activity (entry **2e**, $pIC_{50}=6.9$). Results of the exploration of the pendant aryl ring while keeping the 2-(1*H*-pyrazol-3-yl)-1,3-thiazole group constant are reported in Table 2. Different patterns of *ortho* and *para* substituents, endowed with various physicochemical properties, were tolerated (entries **3f** and **3g**, $pIC_{50}=6.9$ and 6.8, respectively). However, the only compound with in vitro potency similar to that of **2a** and **2q** was the 2,4,6-trichlorophenyl derivative **3a** ($pIC_{50}=$ 7.5), indicating that a more conformationally restricted arrangement of the pendant aryl group might be beneficial.

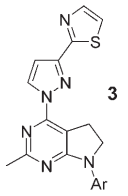
Replacement of the phenyl ring with a weakly basic pyridine as in **3b** ($pIC_{50}=7.2$) was also tolerated. However, introduction of electron-withdrawing groups on the pyridine core, as in **3q** ($pIC_{50}=5.3$), resulted in a significant drop of in vitro potency. As a result of this exploration, compound **2a** was selected for further in vitro and in vivo characterization.

The PK profile of compound **2a** is shown in Table 3. When administered to Han–Wistar rats at 1 mg kg^{-1} (po) and 0.4 mg kg^{-1} (iv) good exposure in plasma was observed along with high bioavailability ($F=52\%$), low plasma clearance ($Cl_p=$ 12 mL min^{-1} kg^{-1}), and acceptable volume of distribution ($V_d=$ 3.7 L kg^{-1}). The compound half-life was also adequate ($t_{1/2}=$ 5.1 h). In addition, the compound was endowed with high brain penetration (B/P = 2.3) and exposure in the brain (200 ng g^{-1}). As far as the inhibition of the CYP450 isoforms is concerned, a significant improvement of the profile ($IC_{50}=$ 5.3 μM for 2D6 in the worst case) with respect to compound **1** (for which $IC_{50}<0.1$ μM toward isoform 1A2, as mentioned earlier) was observed.

Based on this interesting PK profile, compound **2a** was tested in vivo in the rat pup vocalization anxiety model.[10] Significant reduction of the rat pup vocalization time by 50%, at a dose of 10 mg kg^{-1} (ip), was observed. Duration of vocalisation was 179.8 ± 19.8 s and 88.3 ± 18.6 s in vehicle- and **2a**-treated animals, respectively.[11]

In summary, the in silico rational design, synthesis, and SAR characterization of a novel series of potent and selective CRF_1 receptor antagonists belonging to the dihydropyrrolo[2,3-*d*]-pyrimidine class has been described. Replacement of the "tra-

Table 2. In vitro characterization of template **3**: exploration of the 'bottom' aryl/heteroaryl group.



3

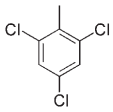
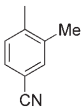
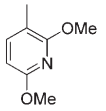
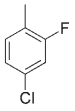
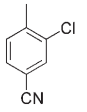
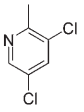
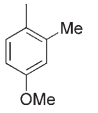
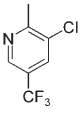
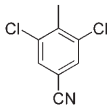
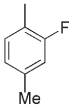
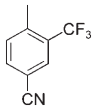
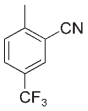
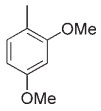
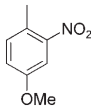
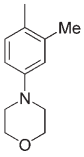
Compd	Ar	pIC_{50}	Compd	Ar	pIC_{50}
3a		7.5	**3i**		6.7
3b		7.2	**3l**		6.6
3c		7.1	**3m**		6.2
3d		6.9	**3n**		6.2
3e		6.8	**3o**		6.1
3f		6.9	**3p**		5.5
3g		6.8	**3q**		5.3
3h		6.7	**3r**		4.6

Table 3. Pharmacokinetic characterization of compound **2a** in Han–Wistar rats.

Test	Results ($n=3$)
2a dose [mg kg^{-1}]	0.4 (iv), 1 (po)
Cl_p [mL min^{-1} kg^{-1}]	12
V_d [L kg^{-1}]	3.7
$t_{1/2}$ [h]	5.1
C_{max} oral [μg mL^{-1}]	0.11
F [%]	52
B/P ratio	2.3 (1 h, iv)
C_{max} brain [(ng g^{-1})/(μg kg^{-1})]	200 (1 h, iv)

ditional" alkylamine chain with heteroaryl-substituted pyrazoles maintained good levels of potency. Compound **2a** was identified as the most potent within this sub-series and hence selected for further in vitro and in vivo characterization. The compound showed an excellent pharmacokinetic profile in rat and significant anxiolytic in vivo activity, further confirming that CRF_1 antagonists may play a role in the treatment of anxiety and depression.

Acknowledgements

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Keywords: anxiety · corticotropin releasing factor · CRF_1 antagonists · depression

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[9] All compounds were characterized in vitro by displacement of ^{125}I[CRF] from recombinant human CRF receptors, expressed in CHO cell membranes. For a detailed description of the in vitro assay, see the experimental section in Ref. [5].

[10] C. R. Gardner, *J. Pharmacol. Methods* **1985**, *14*, 181–187. Twelve rat pups between 9 and 11 days postnatal emit ultrasonic vocalization in response to separation from their mother and littermates. This behaviour can reflect a state of distress. This test has been originally proposed by Gardner as a sensitive test for anxiolytic drugs. Benzodiazepines, buspirone, CRF_1 antagonists, fluovoxamine, and tianeptine have been reported to be active in this test.

[11] All work involving animals was carried out in compliance with Italian national legislation (DL116/92), which acknowledges the European Directive 86/609, and according to internal review by the GSK Committee on Animal Research and Ethics (CARE).

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