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Novel substituted tetrahydrotriazaacenaphthylene derivatives as potent CRF_1 receptor antagonists

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Abstract—Corticotropin-releasing factor (CRF), a 41 amino acid peptide neurohormone synthesised by specific hypothalamic nuclei in the brain, is implicated in stress-related function. Antagonism of CRF_1 receptors is an attractive therapeutic approach for the treatment of depression and anxiety. Unsaturated tetrahydrotriazaacenaphthylenes of general structure **3** have been identified as potent and selective CRF_1 receptor antagonists with a suitable oral pharmacokinetic profile.

The health burden of stress-related diseases, including depression and anxiety disorders, is rapidly increasing, requiring the identification of more efficacious pharmacotherapies exhibiting improved tolerability with respect to current treatments.[1] Recent findings support a major role for neuropeptides in mediating the response to stress and emotional behaviors. Corticotropin-releasing factor (CRF), a 41 amino acid peptide synthesised by specific hypothalamic nuclei in the brain, was originally isolated by Wale and colleagues in 1981 from ovine hypothalamus.[2] This neuropeptide is known to be the major physiological regulator of the basal and stress-mediated release of adrenocorticotropic hormone (ACTH) from the anterior pituitary. In addition CRF has been recognised to coordinate many of the endocrine, autonomic and behavioral responses to stress via activation of CRF_1 receptors.

Therefore the identification of CRF_1 antagonists is an attractive therapeutic approach for the treatment of depression and anxiety.[3]

Despite major efforts by many pharmaceutical companies and academic groups during the last decade, the discovery of potent small molecule CRF_1 receptor antagonists has proved to be challenging.[4]

Compounds such as CP-154,526[5] and DMP-696[6] (Fig. 1) were amongst the first CRF-1 antagonists showing high affinity along with interesting signs of in vivo activity in animal models of anxiety and depression.

These molecules share common pharmacophoric features: (a) a bicyclic heterocyclic core substituted in the top region of the molecule with a lipophilic alkylamine

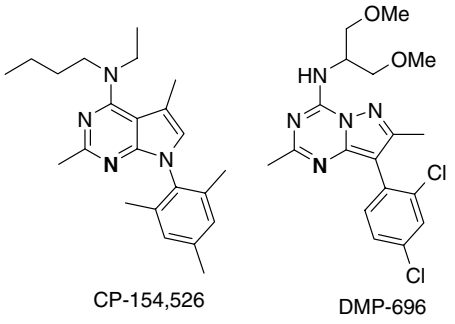

Figure 1. Bicyclic CRF_1 receptor antagonists.

Keywords: CRF_1 antagonists; Anxiety; Depression; Corticotropin-releasing factor.
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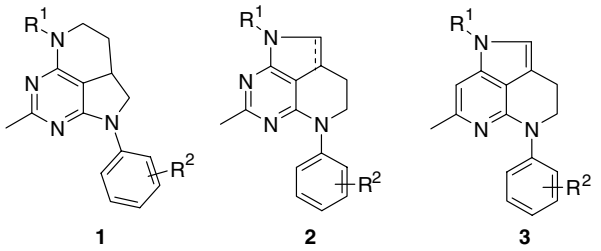


Figure 2. Tricyclic CRF_1 receptor antagonists.

side chain; (b) an aromatic nitrogen acting as H-bond acceptor[7–9]; (c) a pendant 2,4-disubstituted or 2,4,6-trisubstituted aromatic moiety or heteroaromatic ring in the bottom region of the molecule.

As part of a broad chemical strategy aimed towards the discovery of new series of CRF_1 antagonists, the tricyclic templates of general structures **1** and **2** have been designed and explored in detail[9] in our laboratories.

In this article, we report the preparation of the corresponding tetrahydrotriazaacenaphthylenes of general structure **3** (Fig. 2), suitably substituted in both the top region of the molecule (R^1) and in the bottom aromatic portion (R^2).

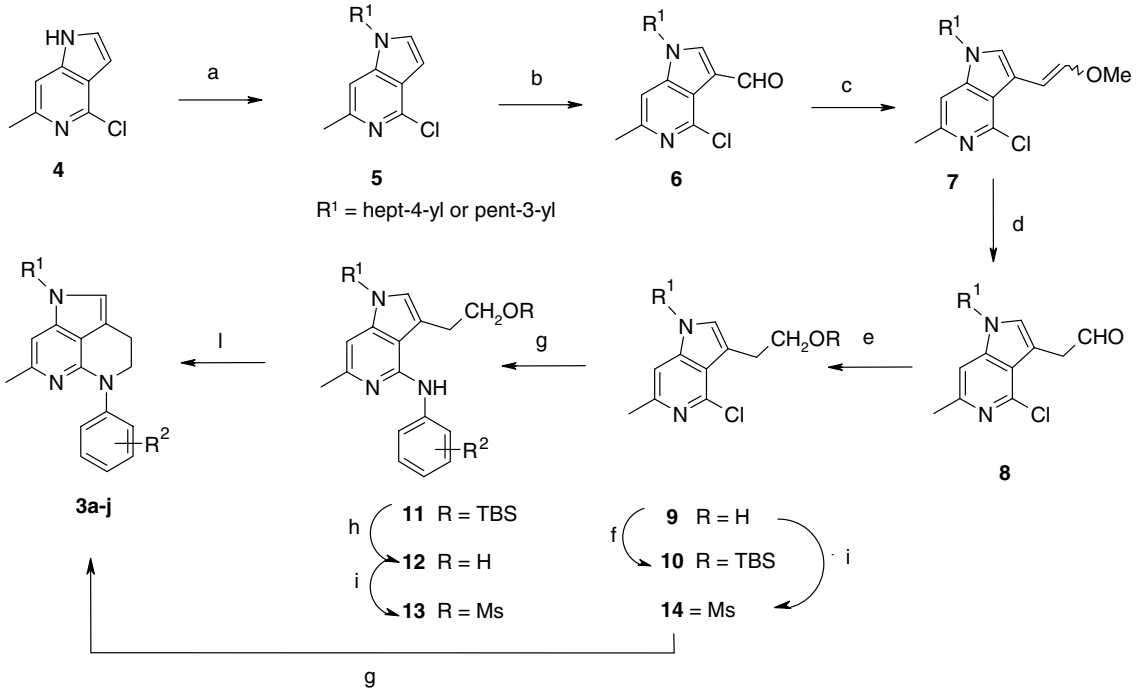
The synthetic route setup is described in Scheme 1. Intermediates **8** (R^1 = hept-4-yl or pent-3-yl) were prepared in four steps starting from the azanidole **4**.[10,11]

The alkylation of **4** with the necessary alkyl bromides occurred in high yields. Then, the formylation of the C-3 position afforded the aldehyde intermediates **6**, which were homologated by Wittig-type reaction in the presence of (methoxymethyl)triphenylphosphonium chloride and BuLi, giving the corresponding methyl enolethers **7** as mixture of the cis/trans isomers. Acid hydrolysis generated the aldehyde derivative **8** in good yield, after purification by flash chromatography. These compounds were reduced to the corresponding alcohol **9** and then protected as TBS.

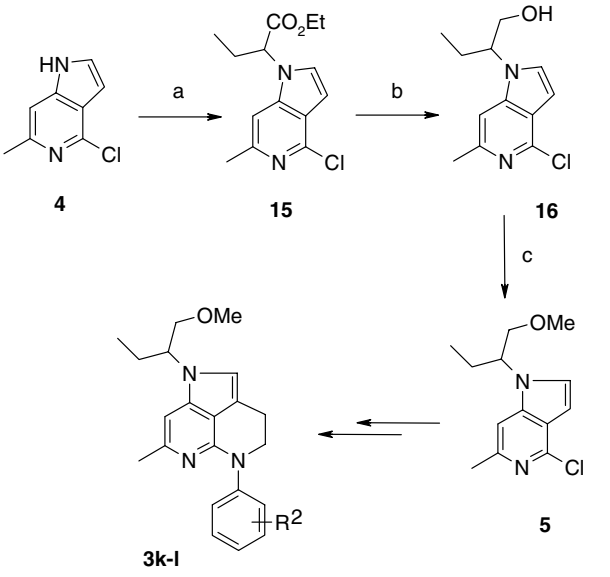
Intermediates **10** were reacted with the substituted anilines using a Buchwald amination reaction,[12] affording compounds **11**. The best conditions for this reaction used $Pd_2(dba)_3$ (10%) as a catalyst, 2-(dicyclohexylphosphino)-2′-methylbiphenyl as a ligand, DME as a solvent and K_3PO_4 as a base, under microwave irradiation (150 W, T = 100 °C, P = 60 p.s.i., cooling, t = 20 min). In the absence of microwave irradiation, reaction yields reduced and conversion times increased. Also, the use of different bases and solvents such as *t*BuOK and toluene significantly reduced yields.

After removal of the TBS, intermediates **11** afforded alcohol derivatives **12**. The final intramolecular cyclization reaction to give compounds **3a–j** was successfully carried out by refluxing the mesylates **13** in toluene in the presence of triethylamine.

Alternatively, the mesylate derivatives **14**, smoothly prepared from alcohols **9**, were transformed straight into



Scheme 1. Reagents and conditions: (a) R_1Br, NaH 80% oil, DMF, 75–81%; (b) DMF, $POCl_3$, reflux, 70–75%; (c) (methoxymethyl)-triphenylphosphonium chloride, 1.6 M BuLi/THF 0 °C 10 min, then compound **10**, THF, rt, 68–70%; (d) 2N HCl, 0 °C during the addition, then 70 °C, 1.5 h, 67–70%; (e) $NaBH_4$, MeOH, 0 °C, 1 h, 89–93%; (f) imidazole, TBSCl, DMAP, DMF, 0 °C, then rt overnight; (g) tris(dibenzylideneacetone)palladium(0), 2-(dicyclohexylphosphino)-2′-methylbiphenyl, K_3PO_4 ArNH2, DME, rt, then microwave irradiation, 100 °C, 150 W, P = 60 p.s.i., 20 min (cooling on), 84–88%; (h) $Et_3N{\cdot}3HF$, DMF, rt, overnight, 48–50%; (i) MsCl, Et_3N, CH_2Cl_2, rt; (l) toluene, Et_3N, reflux, 29–31% (two steps).



Scheme 2. Reagents and conditions: (a) ethyl 2-bromobutanoate, NaH 80% in oil, DMF, 0 °C to rt 1 h, 81%; (b) DIBAL-H, CH_2Cl_2, 0 °C, then rt, 45 min, 42%; (c) NaH 80%/oil, MeI, THF, 0 °C to rt, 30 min, 79%.

the final unsaturated tetrahydrotriazaacenaphthylenes via a Buchwald amination reaction in the presence of the appropriately substituted anilines.

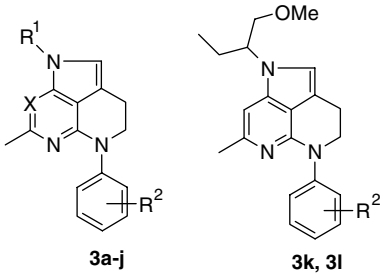
In order to prepare racemic compounds **3k** and **3l**, the modified synthetic approach described in Scheme 2 was setup. Alkylation of the nitrogen on the azaindole **4** with ethyl 2-bromobutanoate gave intermediate **15**, which was subsequently reduced to the primary alcohol **16**. This intermediate was smoothly transformed into the corresponding methyl ether **5**. Final compounds were prepared from compound **5** as described in Scheme 1.

Compounds **3a**–**l** were characterized in vitro by displacement of ^{125}I-CRF from recombinant human CRF receptors expressed in CHO-cell membranes. Results are presented in Table 1.[13]

Based on the results obtained the following conclusions can be drawn: (a) moving from tetrahydrotetrazazaacenaphthylenes (**2**) to the corresponding tetrahydrotriazaacenaphthylenes (**3**) a significant improvement of in vitro potency was observed only for compound **3b**, which can be considered as one of the most potent CRF_1 antagonists identified to date (pIC_{50} = 8.4 vs. 7.8 for **3b** and **2a**, respectively), while **3a** and **3i** were less active than the analogues **2a** and **2i** (pIC_{50} = 7.0 and 6.9 vs. 7.8 and 7.6, respectively); (b) different substituents on the *pendant* phenyl ring allow modulation of in vitro affinity; (c) replacement of one of the methyl groups with a methoxy in the top chain of the molecule reduces the affinity for CRF_1 receptors (pIC_{50} = 7.9 and 7.4 for **3e** and **3k**, respectively).

Four compounds were selected for further PK characterization[14] namely **3a**, **3b**, **3d**, and **3i**.[15] As shown in Table 2, **3b**, the most potent compound in vitro, showed

Table 1. In vitro potency of compounds **3a**–**l**



Entry	R^1	R^2	X	pIC_{50}[a]
CP-154,526				7.8
DMP-696				7.4
1	Hept-4-yl	2-Cl, 4-Cl	C	7.5
3a	Hept-4-yl	2,4-Bis-CF_3	C	7.0
2a	Hept-4-yl	2,4-Bis-CF_3	N	7.8
3b	Hept-4-yl	2-CH_3, 4-CN	C	8.4
2b	Hept-4-yl	2-CH_3, 4-CN	N	7.3
3c	Hept-4-yl	2-CH_3, 4-OCH_3	C	6.9
3d	Hept-4-yl	3-F, 4-OCH_3	C	7.4
3e	Pent-3-yl	2-CH_3, 4-CN	C	7.9
3f	Pent-3-yl	2-CH_3, 4-OCH_3	C	7.9
3g	Pent-3-yl	3-F, 4-OCH_3	C	7.3
3h	Pent-3-yl	2-CH_3, 4-OCF_3	C	7.1
3i	Pent-3-yl	2,4-Bis-CF_3	C	6.9
2i	Pent-3-yl	2,4-Bis-CF_3	N	7.6
3j	Pent-3-yl	4-OCH_3	C	7.2
3k	—	2-CH_3, 4-CN	C	7.4
3l	—	2-CH_3, 4-OCH_3	C	6.8

[a] $pIC_{50} = -\log IC_{50}$.

moderate-high plasma clearance (Cl_p = 54 mL/min/kg) and low oral bioavailability (F = <5%). Compound **3d** showed similar plasma clearance but better bioavailability (37%). Compounds **3a** and **3i** instead showed reduced plasma clearance (Cl_p = 8 and 21 mL/min/kg, for **3a** and **3i**, respectively), leading to oral bioavailability of 71 and 28%, for **3a** and **3i**, respectively. Therefore 2,4-bis-CF_3 substitution in the aromatic region of the molecule proved to be a positive modulator of the metabolic stability and was associated to increased oral exposure, thus allowing the progression of both compounds to in vivo efficacy studies. Conversely the presence of methoxy or cyano substituents in the *pendant* phenyl ring, as in **3d** and **3b**, proved to increase plasma clearance and distribution volume.

In summary, the synthesis and the characterization of a new series of unsaturated triazaacenaphthylene templates has been described. Several compounds exhibited high affinity for the CRF_1 receptor; in particular com-

Table 2. PK profile of compounds **3a**, **3b**, **3d** and **3i** in Sprague–Dawley male rats

Parameters	**3a**	**3b**	**3d**	**3i**
Cl_p (mL/min/kg)	8	54	47	21
F (%)	71	<5	37	28
Vd_{ss} (L/kg)	2.5	17	24	6.8
$T1/2$ (h)	5.3	6.7	8.6	7.6
AUC (ng h/mL)[a]	1479	<15	131	222

[a] AUC normalised by oral dose.

pound **3b** is one of the most potent CRF_1 antagonists ever reported. The SAR associated with this chemical series indicated that in vitro potency is sensitive to the nature of the substituents on the top region of the molecule, whereas modifications in the aromatic ring in the bottom region were better tolerated. From a PK point of view, a 2,4-bis-CF_3 substitution on the phenyl ring in the bottom region of the molecule contributed to increase the oral exposure by reducing plasma clearance (first pass metabolism) and the distribution volume.

Acknowledgments

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13. For a detailed description of the in vitro assay used for the characterization of the molecules prepared, see the experimental section in Ref. 9.
14. PK profile was measured in Sprague–Dawley male rats ($n = 3$), after po (1 mg/kg, dose) and iv (0.4 mg/kg dose) administration. For experimental details, see Ref. 9.
15. ^{1}H NMR, **3a**: (400 MHz, $CDCl_3$) δ ppm 8.02 (s, 1H), 7.82 (d, 1H), 7.68 (d, 1H), 6.59 (s, 1H), 6.47 (s, 1H), 4.07 (m, 1H), 3.74 (t, 2H), 2.35 (s, 2H), 1.78 (m, 4H), 1.22 (m, 4H), 0.87 (t, 6H). **3b**: (500 MHz, $CDCl_3$) δ ppm 7.57 (s, 1H), 7.53 (dd, 1H), 7.41 (d, 1H), 6.63 (s, 1H), 6.51 (s, 1H), 4.10 (m, 1H), 3.90 (t, 2H), 3.12 (t, 2H), 2.41 (s, 3H), 2.27 (s, 3H), 1.77 (m, 4H), 1.20 (m, 4H), 0.91 (t, 6H). **3d**: (500 MHz, $CDCl_3$) δ ppm 7.35 (dd, 1H), 7.20 (dd, 1H), 6.95 (t, 1H), 6.58 (s, 1H), 6.50 (s, 1H), 4.08 (m, 1H), 3.94 (t, 2H), 3.90 (s, 3H), 3.07 (t, 2H), 2.45 (s, 3H), 1.80 (m, 4H), 1.20 (m, 4H), 0.87 (t, 6H). **3i**: (300 MHz, $CDCl_3$) δ ppm 8.05 (d, 1H), 7.90 (d, 1H), 7.80 (dd, 1H), 6.57 (s, 1H), 6.49 (s, 1H), 3.89 (t, 2H), 3.87 (m, 1H), 3.08 (m, 2H), 2.43 (s, 3H), 1.85 (m, 4H), 0.81 (t, 6H).