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Bioorganic & Medicinal Chemistry Letters 15 (2005) 3713–3716
Bioorganic & Medicinal Chemistry Letters

Substituted tetraazaacenaphthylenes as potent CRF_1 receptor antagonists for the treatment of depression and anxiety

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Received 17 March 2005; revised 6 May 2005; accepted 11 May 2005
Available online 8 June 2005

Abstract—Two isomers of the hexahydro-tetraazaacenaphthylene templates (**1** and **2**) are presented as novel, potent, and selective corticotropin releasing factor-1 (CRF_1) receptor antagonists. In this paper, we report the affinity and SAR of a series of compounds, as well as pharmacokinetic characterization of a chosen set. The anxiolitic activity of a selected example (**2ba**) in the rat pup vocalization model is also presented.

Depression and anxiety are psychiatric disorders that constitute a major health concern worldwide, and new pharmacological approaches for improved efficacy and reduced side effects profiles 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 Wale and colleagues[2] in 1981 from ovine hypothalamus. It plays an important role as a neurotransmitter in the mediation of anxiety and depression related behaviors, and could represent a new opportunity for the treatment of such diseases. 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 the 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] and DMP-696[5] were among the first to show good binding affinities coupled with interesting in vivo activities. A vast majority of the compounds published to date have in common the structural features shared by the two aforementioned antagonists: a bicyclic heterocyclic core substituted with a highly lipophilic alkylamine side chain and a 2,4-disubstituted or 2,4,6-trisubstituted aromatic or heteroaromatic ring (Fig. 1). More recently, new high-affinity analogs containing monocyclic and tricyclic cores have been disclosed,[6] thus broadening the structural diversity and known SAR for the receptor. Notwithstanding these encouraging progresses, very few molecules have reached the advanced preclinical development or clinical phases to date.

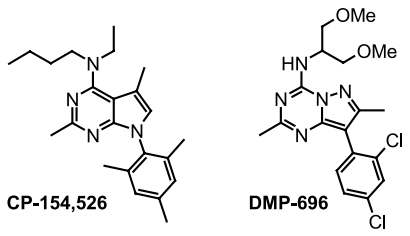

Figure 1. Known bicyclic small molecule CRF_1 receptor antagonists.

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doi:10.1016/j.bmcl.2005.05.040

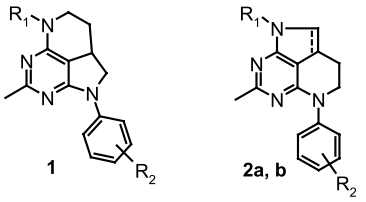
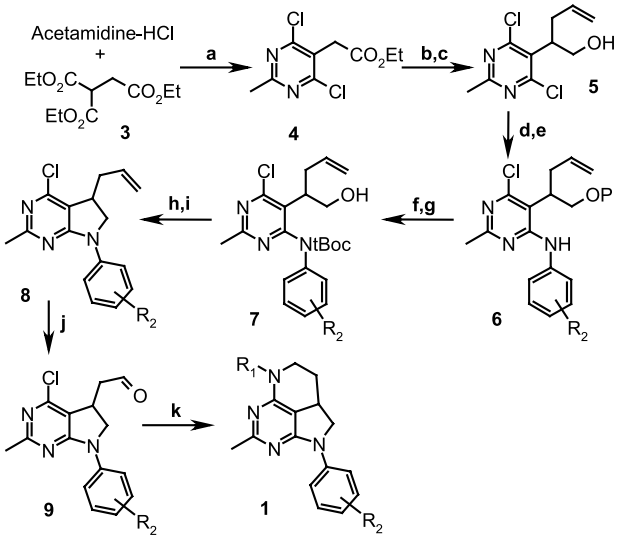


Figure 2. General structures **1** and **2**.

We wish to report here our efforts toward the identification of novel, potent, and selective CRF_1 receptor antagonists. In our search for heterocyclic templates with high receptor affinities, hexahydro- and tetrahydro-tetraazaacenaphthylenes of general structures **1** and **2** were identified (Fig. 2).[7,8]

The top amine substituents (R_1 in **1** and **2**) as well as the bottom aromatic portion (substituents R_2) were explored, leaving the central acenaphthylene core intact. It was our belief that modification of these two regions would allow for the optimization of the general physicochemical characteristics of the molecules, thus leading to a better/suitable PK profile.

The synthetic procedure for the preparation of the tetraazaacenaphthylene template **1** is described in Scheme 1. Intermediate **4** has already been described in the literature.[9,10] Allylation of **4** followed by reduction of the ethyl ester functionality yielded alcohol **5**, which was protected with a *t*-butyl-dimethylsilyl group (P, in Scheme 1).

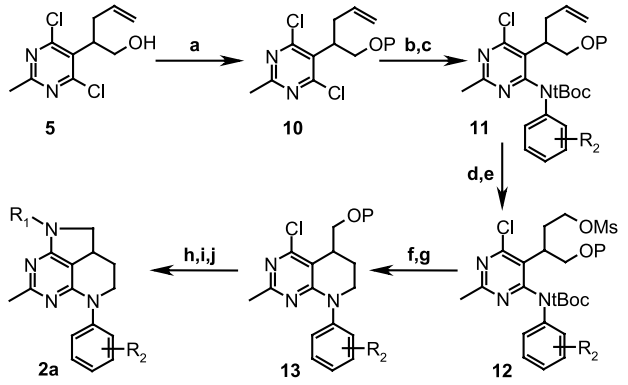


Scheme 1. Reagents and conditions: (a) (i) acetamidine hydrochloride, Na, MeOH, rt, 20 min; (ii) 2-ethoxycarbonyl–succinic acid diethyl ester, rt, 48 h; (iii) $POCl_3$, reflux, 3.5 h, 73%; (b) 1 M LiHMDS/Hex, allyl bromide, THF, 0 °C to rt, 68%; (c) 1 M DIBAl-H/Hex, CH_2Cl_2, −78 to 0 °C, 3 h, 86%; (d) *t*-BDMSi-Cl, imidazole, DMF, 0 °C to rt, 3 h, 81%; (e) NaH 80%/oil, R_2-aniline, THF, 0 °C, 15 min, then compound **5**, THF, reflux, 3 h, 72%; (f) $(t\text{-Boc})_2O$, DMAP, CH_2Cl_2, rt, 18 h, 83%; (g) $Et_3N{\cdot}3HF$, DMF, rt, 18 h, 74%; (h) CH_3SO_2Cl, Et_3N, CH_2Cl_2, rt, 18 h, 98%; (i) (i) TFA 20%/CH_2Cl_2, rt, 2 h, (ii) Et_3N, THF, rt, 1 h, quant.; (j) O_3, CH_2Cl_2, −78 °C, 30 min, then Me_2S, rt, 18 h, 27%; (k) R_1-amine, THF, rt, 90 min, then 1 M $NaBH_3CN$/THF, rt, 18 h, 7%. The yields reported are for compound **1b** (Table 1) only. P, protecting group.

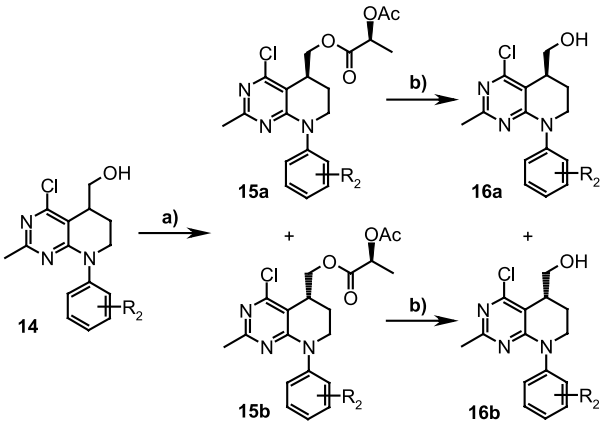
The protected alcohol was then subjected to a nucleophilic aromatic substitution with deprotonated anilines to give aminopyrimidines **6**. The amino group of **6** was protected (*t*-Boc) and the alcohol desilylated to give intermediates **7**. Mesylation of alcohols **7**, followed by cleavage of the *t*-Boc protecting group gave an aminomesylate intermediate, which cyclised to substituted pyrrolidino-pyrimidines **8** upon treatment with Et_3N in THF. Ozonolysis of **8** to give aldehydes **9** was followed by imine formation with the appropriate alkylamines, which was then reduced in situ to give the desired compound **1**.

Scheme 2 illustrates the preparation of tetraazaacenaphthylene template **2**. The alcohol group of intermediate **5** (from Scheme 1) was similarly protected using a *tert*-butyldiphenylsilyl group (P, in Scheme 2) to give better stability. Nucleophilic aromatic substitution with different anilines followed by protection of the amino group were performed as reported in Scheme 1, to give intermediate **11**. Ozonolysis of **11** followed by reductive workup gave an alcohol, which was reacted with methanesulfonyl chloride in the presence of Et_3N to give mesylates **12**. Cleavage of the *t*-Boc protecting group, followed by treatment with Et_3N gave the substituted piperidinopyrimidines **13**. Deprotection of the alcohol group of **13** was followed by mesylate formation and cyclization with the appropriate amines (neat) to yield the desired compounds **2a**.

Both templates were initially prepared as racemic mixtures. In order to verify if the stereogenic center of the different templates had an effect on the affinity for the receptor, the two enantiomers of two examples of template **2a** were prepared, as described in Scheme 3. Intermediate **14** (racemic, from Scheme 2) was acylated with



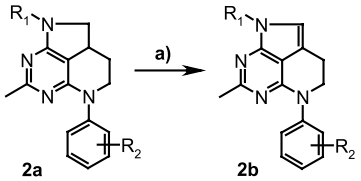
Scheme 2. Reagents and conditions: (a) *t*-BDPSi-Cl, DMAP, imidazole, DMF, 0 °C to rt, 2 h, 90%; (b) NaH 80%/oil, R_2-aniline, THF, 0 °C, 30 min, then compound **10**, THF, reflux, 5 h, 46%; (c) $(t\text{-Boc})_2O$, DMAP, CH_2Cl_2, rt, 48 h, 93%; (d) O_3, CH_2Cl_2, −78 °C, 30 min, then $NaBH_4$, rt, 3 h, 54%; (e) CH_3SO_2Cl, Et_3N, CH_2Cl_2, rt, 18 h, 95%; (f) TFA 20%/CH_2Cl_2, rt, 18 h, 100%; (g) Et_3N, THF, 0 °C to rt, 16 h, 48%; (h) $Et_3N{\cdot}3HF$, DMF, 40 °C, 4 h, 98%; (i) CH_3SO_2Cl, Et_3N, CH_2Cl_2, rt, 16 h, 93%; (j) neat R_1-amine, 120 °C, 8 h, 47%. The yields reported are for compound **2aa** (Table 2) only. P, protecting group.



Scheme 3. Reagents and conditions: (a) (*S*)-2-acetoxypropionyl chloride, DMAP, CH_2Cl_2, 0 °C, 30 min, then chiral HPLC separation, 46%; (b) LiOH, THF/H_2O, 50 min, 90%. The yields reported are for compounds **2ag** and **2ah** (Table 2) only.

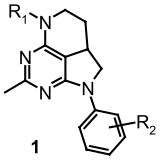
enantiopure (*S*)-2-acetoxypropionyl chloride. Initial attemps to separate diastereoisomers **15a** and **15b** using conventional flash chromatography proved to be unsuccessful, thus prompting us to use chiral HPLC.[11] The two separated enantiomerically pure compounds were hydrolyzed to give alcohols **16a** and **16b**, which were subjected to the conditions reported above in Scheme 2 (steps **i** and **j**) to complete the synthesis of the chirally pure enantiomers.

Finally, the unsaturated tetraazaacenaphthylenes **2b** were easily obtained by the DDQ oxidation of saturated templates **2a**, as shown in Scheme 4.



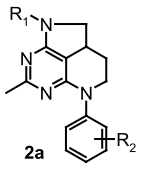
Scheme 4. Reagents and conditions: (a) DDQ, CH_2Cl_2, rt, 3 h, 65%. The yield reported is for compounds **2ba** (Table 3) only.

Table 1. Affinity results for template **1** and standards



Compound	R_1	R_2	pIC_{50}
CP-154,526			7.76
DMP-696			7.42
1a	Cyclopropylmethyl	2,4-Dichloro	5.35
1b	3-Pentyl	2,4-Dichloro	6.74
1c	4-Heptyl	2,4-Dichloro	7.52
1d	4-Heptyl	2,4-Bis-CF_3	7.39

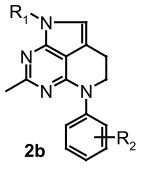
Table 2. Affinity results for template **2a**



Compounds	R_1	R_2	pIC_{50}
2aa	Pent-3-yl	2,4-Dichloro	7.16
2ab	1,3-Di-MeO-prop-2-yl	2,4-dichloro	5.90
2ac	Hept-4-yl	2,4-Dichloro	7.65
2ad	Hept-4-yl	2,4-Bis-CF_3	7.44
2ae[a]	Hept-4-yl	2,4-Dichloro	7.96
2af[a]	Hept-4-yl	2,4-Dichloro	7.89
2ag[a]	Hept-4-yl	2,4-Bis-CF_3	7.55
2ah[a]	Hept-4-yl	2,4-Bis-CF_3	7.84

[a] Chirally pure enantiomers, prepared as in Scheme 3.

Table 3. Affinity results for template **2b**



Compound	R_1	R_2	pIC_{50}
2ba	Hept-4-yl	2,4-Bis-CF_3	7.85
2bb	Hept-4-yl	2-Me-4-CN	7.35
2bc	Pent-3-yl	2,4-Bis-CF_3	7.58
2bd	Pent-3-yl	2-Me-4-CN	7.57
2be	Pent-3-yl	2-Cl-4-CN	7.21

CRF binding affinity has been determined in vitro by the compounds' ability to displace ^{125}I-oCRF from recombinant human CRF receptors expressed in Chinese Hamster Ovary (CHO) cell membranes.[7,8] Affinity results for templates **1**, **2a**, and **2b** are presented in Tables 1–3, respectively. As a general rule, affinity for the receptor seems to be controlled by the lipophilicity of the compounds (see **1a** vs **1c** in Table 1, or **2ab** vs **2aa** vs **2ae** in Table 2). The top region of the molecules (R_1) requires lipophilic chains in order to maintain high affinities (see **1a** vs **1b** vs **1c** in Table 1). Polar groups do not seem to be well tolerated in that region (see **2aa** vs **2ab** in Table 2). As far as the substituents on the bottom phenyl ring are concerned, reduction of lipophilicity was tolerated, as can be seen in Table 3, where a cyano group has replaced one of the lipophilic moieties (see **2bc** vs **2bb** or **2bd** in Table 3).

As can be seen in Table 2, the configuration of the stereogenic center in template **2b** has a limited effect on the affinity of this series for the receptor (see **2ae** vs **2af**, and **2ag** vs **2ah** in Table 2). The chirally pure enantiomers have more or less the same affinity as the racemic mixtures. The same conclusion can be reached looking at the compounds in Table 3, where the chiral

Table 4. DMPK parameters for **1c**, **2ac**, **2ad**, and **2ba**[a]

Parameters	1c	2ac	2ad	2ba
Plasma clearance (mL/min/kg)	31	32	15	10
Bioavailability (%)	31	14	36	33
Brain/Plasma	1.8	2.0	1.1	1.3

[a] Pharmacokinetic experiments were performed in Hans–Wistar rats after oral (1 mg/kg) and intravenous (0.5 mg/kg) administrations; brain/plasma ratios and brain levels were measured at 1 h after a 0.5 mg/kg iv dose.

center has been eliminated through the introduction of an insaturation. Analogs of template **2b** are almost equipotent to their equivalents of template **2a**, whether they are chirally pure or racemates (see **2ba** vs **2ad** vs **2ag**, and **2ah**).

Four compounds were selected for the in vivo PK characterization (**1c**, **2ac**, **2ad**, and **2ba**). Table 4 reports the in vivo parameters measured for these compounds: low to moderate plasma clearance, good oral bioavailability, and brain penetration were observed.

In particular, compound **2ba** exhibited a high affinity for the receptor and good PK characteristics. Based on this positive profile, it was evaluated in the rat pup vocalization model.[12] **2ba** both at 30 and 60 mg/kg was able to reduce significantly the rat pup vocalization time by 55% with respect to vehicle.

In summary, the synthesis of two novel tetraazaacenaphthylene templates was described. Compounds belonging to these series exhibited high affinities for the CRF_1 receptor. Selected examples of both templates showed good in vivo PK characteristics. Furthermore, compound **2ba** exhibited good activity in the rat pup vocalization model, thus confirming that CRF_1 antagonists may play a role in the treatment of anxiety and depression.

Acknowledgment

The authors would like to thank Dr. Carla Marchioro for analytical support.

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11. Daicel CHIRALPAK AD; mobile phase: *n*-hexane/isopropyl alcohol 9:1; flow rate: 6.5 mL/min; detector type: diode array detector (DAD) (225 nm, 292 nm).
12. Rat pups between 9 and 11 days postnatal emit ultrasonic vocalization in response to separation from their mother and littermates. This behavior can reflect a state of distress. This test has been originally proposed by Gardner (*J. Pharmacol. Methods*, **1985**, *14*, 181) as a sensitive test for anxiolytic drugs. Benzodiazepines, buspirone, CRF1 antagonists, fluovoxamine, and tianeptine have been reported to be active in this test.