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The design, synthesis and structure–activity relationships of 1-aryl-4-aminoalkylisoquinolines: A novel series of CRF-1 receptor antagonists

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Abstract—The design, synthesis and structure–activity relationships of a novel series of CRF-1 receptor antagonist, the 1-aryl-4-alkylaminoisoquinolines, is described. The effects of substitution on the aromatic ring, the amino group and the isoquinoline core on CRF-1 receptor binding were investigated.
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Corticotropin releasing factor (CRF) is a 41-amino acid peptide that acts as the prime regulator of the hypothalamic–pituitary–adrenal (HPA) stress response.[1] A large body of evidence exists for the hypothesis that over-stimulation of the CRF system may underlie the pathology of several diverse neuropsychiatric diseases including depression, anxiety and stress-related disorders.[2] CRF exerts its actions through high-affinity binding to its receptors CRF-1 and CRF-2, both of which belong to the class B subfamily of G-protein coupled receptors.[3] They are encoded by two separate genes and display distinct pharmacology and distribution within the CNS and periphery.[4] While, the therapeutic benefits of blocking the CRF-2 receptor remain to be fully established, evidence exists that antagonism of the CRF-1 receptor produces anxiolytic and antidepressant effects. Centrally acting CRF-1 receptor antagonists are therefore expected to have utility in the treatment of anxiety, depression and stress-related disorders. The first potent bicyclic CRF-1 antagonist, CP-154,526, was reported in 1996, and in the ensuing years a large number of small molecule antagonists have been disclosed,[5] representative examples are illustrated in Figure 1.[6] Potent CRF-1 antagonists including CP-154,526,[7] R121919,[8] NBI 27914,[9] NGD 98-1,[10] the tricycle **1**,[11] and SSR125543A[12] have been widely studied in animal models of depression and

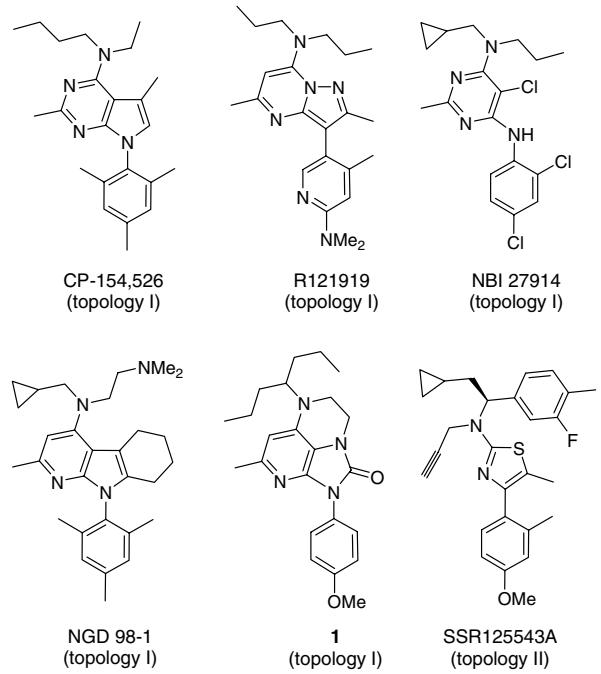

Figure 1. Structures of representative CRF-1 receptor antagonists.

Keywords: CRF-1 receptor antagonist; 1-Aryl-4-aminoalkylisoquinoline; Depression; Anxiety.
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anxiety and have shown encouraging results. Notable amongst these, R121919, demonstrated efficacy in treating depressed patients in an open label phase IIa clinical trial.[13] Clinical development of R121919 was discontinued, due to significant, albeit reversible, elevation of liver enzymes at high doses. A number of other CRF-1 receptor antagonists are reportedly undergoing clinical trials at the present time.

Structural features common to most CRF-1 antagonists include: a core central heterocycle that contains a critical hydrogen bond acceptor, an out-of-plane pendant aromatic ring and a top side-chain filling a largely hydrophobic area of the receptor. They have previously been classified into two topologies based on distinct structural features.[5b] In the first topology, which contains the majority of compounds to have reached advanced preclinical development or clinical stage, the hydrogen bond acceptor and the pendant aryl substituent are separated by two atoms (e.g., R121919), while in the second topology a single intervening atom is present (e.g., SSR125543A). We set out to examine the possibility of identifying a novel series of CRF-1 receptor antagonists from the relatively underexplored topology II template. In this paper the design, synthesis and evaluation of the first bicyclic CRF-1 receptor antagonists from topology II, the 1-aryl-4-alkylaminoisoquinolines, is described.

The design strategy was to rearrange the positioning of the key structural features of a known high-affinity topology I template to generate a novel series. In such a way repositioning of the pendant aryl group, the hydrogen bond acceptor and the 2-methyl group of the previously reported quinoline **2**[14] led us to consider the novel isoquinoline **3**, which formally belongs to the topology II class (Fig. 2).

Potential alignments of low-energy conformers of the CRF-1 antagonist **2** and the proposed template **3** were first examined to better understand comparisons between the pharmacophores of the two templates. The analyses were performed using the Maestro 7.0 software package from Schrodinger.[15] The analysis was encouraging and revealed that there was an excellent alignment between **2** and **3** with respect to all of the key pharmacophore features: the top side chain, the critical hydrogen bond acceptor and the pendant aromatic ring which

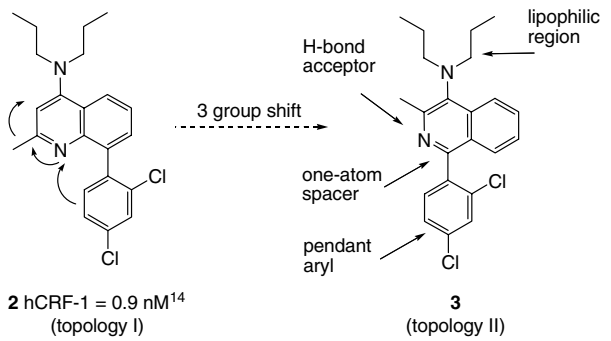


Figure 2. Design of 1-aryl-4-aminoalkylisoquinolines as potential CRF-1 receptor antagonists.

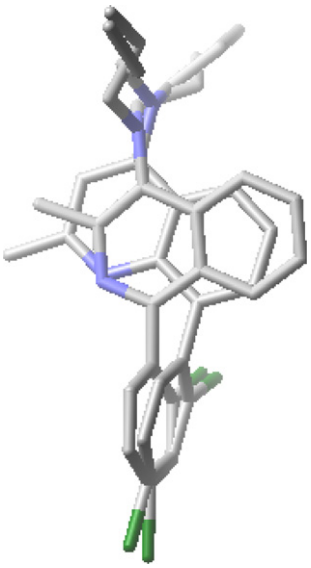
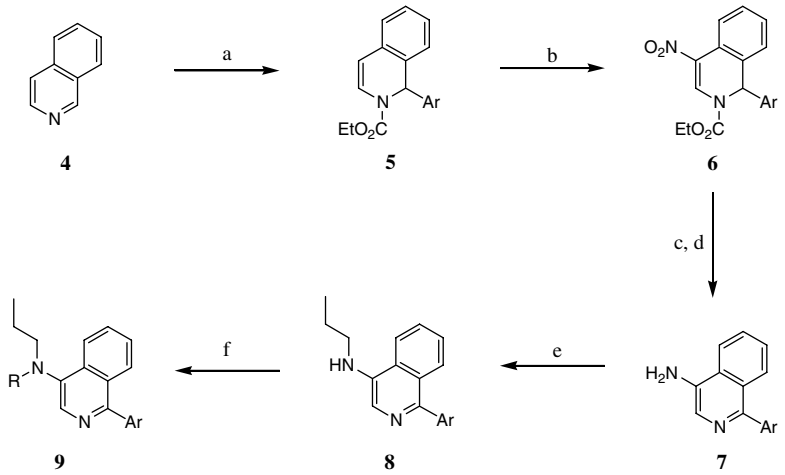


Figure 3. Alignment of the low-energy conformers of quinoline **2** (background) and isoquinoline **3** (foreground).

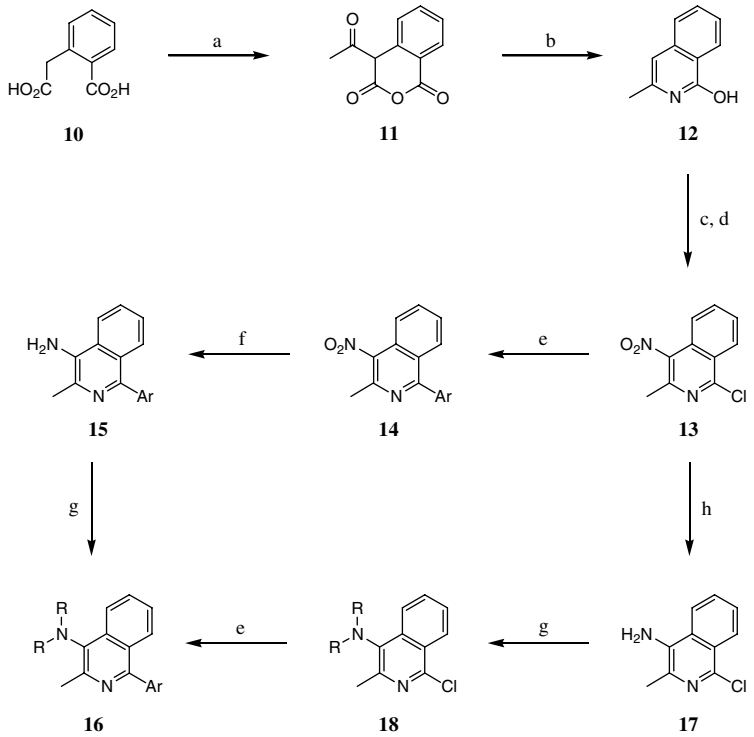
adopts a nearly orthogonal conformation in both series (Fig. 3).

There was limited literature precedent for the synthesis of 1-aryl-4-aminoisoquinolines[16] and the methods developed for the synthesis of the compounds described in this study are illustrated in Schemes 1 and 2. The synthesis of analogues unsubstituted at the 3-position of the isoquinoline is outlined in Scheme 1. Nucleophilic alkylation of isoquinoline (**4**) with an aryl Grignard in the presence of ethyl chloroformate proceeded smoothly and gave in good yield the desired 1,2-dihydroisoquinoline **5**. Nitration of **5** proceeded at the 4-position and gave the 4-nitro-isoquinoline **6** in moderate yield. Deprotection of the carbamate **6**, spontaneous air-oxidation and hydrogenation of the nitro group gave the 4-amino-isoquinoline **7** in 35% overall yield. Reductive amination of **7** gave the monoalkyl amine **8** and finally alkylation with the appropriate alkyl halide gave the dialkylamine **9**.

The synthesis of the 3-alkyl-substituted isoquinolines described in the study is depicted in Scheme 2. Cyclization of homophthalic acid (**10**) with acetic anhydride in pyridine gave 4-acetyl-1,3-isochromandione (**11**). Treatment of **11** with refluxing ammonium hydroxide gave an excellent yield of 3-methyl-isoquinolone (**12**) over the two steps. Regioselective nitration and subsequent chlorination in the presence of diethyl aniline gave the versatile intermediate 1-chloro-4-nitroisoquinoline (**13**). The bi-functional isoquinoline **13** could be used to incorporate either the 4-amino substituent or the 1-aryl substituent in the final step of the synthesis. For example, Suzuki coupling of **13** under standard conditions allowed introduction of the 1-aryl substituent first. Hydrogenation of the nitro group of **14** then gave the amine **15** and reductive amination gave the target compound **16**. The 1-aryl group could also be introduced in the final step. Dithionate reduction of the nitro group of **13** gave the amine **17** and reductive amination yielded the alkylamine **18**. Finally, Suzuki coupling with an aryl



Scheme 1. Reagents and conditions: (a) ArMgBr, THF, 0 °C then $ClCO_2Et$, (70–92%); (b) HNO_3, HOAc, 0 °C, (46%); (c) HBr, HOAc, 100 °C, air; (d) Pd/C, H_2 (50 psi), HCl, MeOH, (35% over 2 steps); (e) $CH_3CH_2CO_2H$, $NaBH_4$, 100 °C, (83%); (f) RBr or RI, ^{t}BuOK, DMSO, rt, (49–78%).



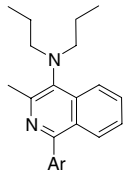
Scheme 2. Reagents and conditions: (a) Ac_2O, pyridine, rt; (b) NH_4OH, 100 °C, (75% over 2 steps); (c) HNO_3, 0 °C, (91%); (d) $OPCl_3$, $PhNEt_2$, 95 °C, (72%); (e) $ArB(OH)_2$, 2 M K_2CO_3, $Pd(PPh_3)_4$, PhMe, 85 °C, (31–72%); (f) Pd/C, H_2, MeOH, (95%); (g) aldehyde, $NaBH(OAc)_3$, HOAc, CH_2Cl_2, rt, (54–69%); (h) $Na_2S_2O_4$, NH_4OH, THF, H_2O, (72%).

boronic acid allowed introduction of a variety of aryl substituents at the 1-position of **16**. It should be noted that coupling of **18** with 2,6-disubstituted aryl boronic acids was sluggish and additional boronic acid, palladium catalyst and prolonged reaction times were necessary to drive the reaction to completion.

The compounds described in this study are listed in Tables 1–5. The affinity of these compounds for the CRF-1 receptor was determined by using a modified version of the assay described by Grigoriadis and De Souza[17] by examining their competition with ^{125}I-sauvagine at membrane preparations of CRF-1 receptors endogenously expressed in IMR-32 human neuroblastoma cells.

The effects of the substituents on the 1-aryl group were investigated first (Table 1). The 4-dipropylamine and the 3-methyl substituent were kept constant. The first example, the 2,4-dichloro analogue **3**, had a very encouraging affinity (K_i = 103 nM) which validated our original design of the isoquinolines as novel, topology II, CRF-1 receptor ligands, although the ~100-fold loss in activity relative to the quinoline **2** (K_i = 0.9 nM)[14] was somewhat disappointing. To further understand the SAR and improve the affinity of the new series, a num-

Table 1. Effects of the 1-aryl substituent on CRF-1 binding

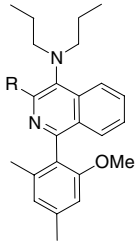


3, 26a-k

Compound	Ar	K_i (nM)
CP154526	—	2.7[7]
2		0.9[14]
3	2,4-Dichloro	103
26a	Ph	>10,000
26b	2-Chloro	1685
26c	3-Chloro	>10,000
26d	4-Chloro	962
26e	2-Methyl-4-chloro	21
26f	2-Methyl-4-methoxy	28
26g	2-Ethyl-4-methoxy	36
26h	2,4-Methoxy	42
26i	2-Methoxy-4-difluoromethoxy	17
26j	2-Methoxy-4,6-dimethyl	6
26k	2-Methoxy-4,6-ditrifluoromethyl	8
26l	2,6-Dimethyl-4-methoxy	26

K_i values are means of three independent experiments.

Table 2. Effects of the 3-methyl substituent on CRF-1 binding

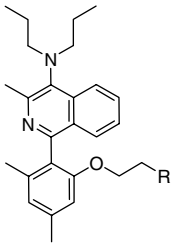


26j, 27a-b

Compound	R	K_i (nM)
26j	Me	6
27a	H	49
27b	Et	9

K_i values are means of three independent experiments.

Table 4. Effects of the 2-aryl substituent on CRF-1 binding



29a-g

Compound	R	Solubility[a] (μg/mL)	K_i (nM)
26j		<0.5	6
29a	NMe_2	112	265
29b	Pyrrolidine	105	173
29c	Piperidine	27	120
29d	*N*-Methylpiperazine	135	524
29e	Morpholine	NA	30
29f	4-Hydroxypiperidine	125	41
29g	3-Hydroxypiperidine	131	32

K_i values are means of three independent experiments.
[a] At pH 5.4.

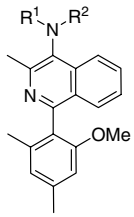
Table 5. Representative data for compounds **26j** and **26k**

Compound	CRF-1 K_i (nM)	At T_{20} K_i (nM)	Microsomal $T_{1/2}$ (min)	Solubility[a] (μg/mL)	M log *P*
26j	6	12	4	<0.5	4.6
26k	8	22	36	<0.5	5.7

[a] At pH 5.4.

ber of substituted aryl groups were subsequently prepared. For optimal CRF-1 binding, previous studies have suggested that the aryl ring lies out of the plane of the core heterocycle and the results in the isoquinoline series are in accord with that model.[18] The unsubstituted phenyl analogue **26a** and the 3-chloro analogue **26c** were inactive. However, an *ortho* substituent forces the aryl ring to be out of the plane with respect to the isoquinoline core and the 2-chloro analogue **26b** showed modest

Table 3. Effects of the 4-amino substituent on CRF-1 binding



28a-f

Compound	R^1	R^2	Solubility[a] (μg/mL)	K_i (nM)
26j	^{n}Propyl	^{n}Propyl	<0.5	6
28a	^{n}Propyl	CH_2–^{c}Propyl	<0.5	5
28b	CH_2–^{c}Propyl	CH_2–^{c}Propyl	<0.5	7
28c	H	^{n}Propyl	NA	165
28d	H	CH_2CH_2–pyrrolidine	103	>10,000
28e	^{n}Propyl	CH_2CH_2–pyrrolidine	120	215
28f	^{n}Propyl	CH_2CH_2–imidazole	107	1063

K_i values are means of three independent experiments.
[a] At pH 5.4.

affinity (K_i = 1685 nM). The 4-chloro analogue **26d** also showed weak affinity (K_i = 962 nM) which underscores the importance of *para* substitution even in the absence of an *ortho* substituent. An increase in affinity was observed by combining an *ortho* and *para* substituent (analogues **26e**–**26i**) and the addition of a second *ortho* substituent, which further hinders the rotation of the aryl group, gave another boost in affinity. The 2-methoxy-4,6-dimethyl analogue **26j** (K_i = 6 nM) was the first compound with activity below 10 nM in the series and replacing both methyl groups with a trifluoromethyl group gave an analogue of similar affinity (**26k**, K_i = 8 nM). Switching the 2-methoxy-4-methyl substituents resulted in a loss of affinity (**26l**, K_i = 26 nM).

The effects of substitution at the 3-position of the isoquinoline on CRF-1 receptor binding were examined next (Table 2). The 4-dipropyl amine and the 2-methoxy-4,6-dimethylphenyl group were kept constant to allow comparison with the 3-methyl analogue **26j**. Increasing the size of the alkyl group from methyl to ethyl produced a compound of comparable affinity to **26j** (**27b**, K_i = 9 nM), but replacing the 3-methyl substituent with hydrogen resulted in a significant loss of affinity (**27a**, K_i = 49 nM).

The next region of the isoquinoline that was investigated was the 4-amino substituent. To allow comparison to **26j** the 3-methyl substituent of the isoquinoline was fixed and the 2,4-dimethyl-6-methoxyphenyl group was retained at the 1-position (Table 3). Exchange of one or both of the ^{n}propyl groups by a ^{c}propylmethyl group gave compounds of similar affinity (**28a**, K_i = 5 nM and **28b**, K_i = 7 nM). Replacement of an ^{n}propyl group by hydrogen resulted in a large decrease in affinity (**28c**, K_i = 165 nM). In an attempt to increase the solubility of the series, several basic amines were incorporated into the side chain (**28d**–**28f**). The solubility at pH 5.4 was increased in comparison to **26j**, however, a substantial loss in affinity was also observed.

The 2-methoxy substituent of the aromatic ring offered a handle to probe the SAR at this position and a number of analogues with an *O*-ethyl linker were prepared (Table 4). The first example, the *N*,*N*-dimethyl analogue **29a**, had relatively weak affinity for the CRF-1 receptor and a number of cyclic amines were subsequently investigated. The pyrollidine was slightly more active and increasing the ring size to piperidine resulted in a further increase in affinity (**29b**, K_i = 173 nM and **29c**, K_i = 120 nM, respectively). Introduction of additional heteroatoms to the ring was also investigated. The *N*-methylpiperazine **29d** had significantly less affinity than the piperidine **29c**, but replacement by morpholine resulted in a significant increase in binding affinity (**29e**, K_i = 30 nM). Other oxygenated analogues were also tolerated moderately well. The 3- and 4-hydroxypiperidine analogues were only slightly less active (**29f**, K_i = 41 nM and **29g**, K_i = 32 nM, respectively) and both had good solubility at pH 5.4 relative to **26j**.

Compounds **26j** and **26k** were profiled further and shown to inhibit sauvagine stimulated cAMP accumulation in AtT20 cells expressing the CRF-1 receptor[19] with K_i's of 12 and 22 nM, respectively. These values tracked well with binding affinity and confirm that **26j** and **26k** are functional antagonists at the CRF-1 receptor (Table 5). The rat microsomal half-life of **26j** was very short ($T_{1/2}$ < 5 min), however, **26k** in which both of the aryl ring methyl groups have been replaced by trifluoromethyl had a much longer microsomal half-life ($T_{1/2}$ = 36 min). The pharmacokinetic properties of **26k** were examined in rat but modest oral exposure and a large volume of distribution were observed (F = 4.8% at 20 mg/kg, C_{max} = 387 ng/mL, T_{max} = 0.7 h, $T_{1/2}$ = 5.4 h, C_l = 14.5 mL/min/kg and V_d = 13 L/kg). In general, poor microsomal stability, minimal aqueous solubility and low oral bioavailability precluded the further assessment of this series.

In summary, a series of 1-aryl-4-alkylaminoisoquinolines has been described having affinity for the CRF-1 receptor. The original hypothesis of transforming a topology I template into a topology II template proved to be successful. A number of new synthetic routes were developed for the synthesis of substituted isoquinolines which resulted in the identification of several compounds with <10 nM CRF-1 binding affinity. For optimal CRF-1 binding affinity 2,4,6-trisubstitution was found to be necessary on the aryl ring of this series. At this stage however, the unacceptable physiochemical (e.g., high lipophilicity and low aqueous solubility) and poor pharmacokinetic profiles of the series rendered them unsuitable for further development. The results of our continuing efforts to resolve these issues will be reported in due course.

References and notes

1. Vale, W.; Spiess, J.; Rivier, C.; Rivier, J. *Science* **1981**, *213*, 1394.
2. (a) Holsboer, F. *J. Psychiatr. Res.* **1999**, *33*, 181; (b) Bale, T. L.; Vale, W. V. *Annu. Rev. Pharmacol. Toxicol.* **2004**, *44*, 525.
3. Spiess, J.; Dautzenberg, F. M.; Sydow, S.; Hauger, R. L.; Ruhmann, A.; Blank, T.; Radulovic, J. *Trends Endocrinol. Metab.* **1998**, *9*, 140.
4. (a) Chalmers, D. T.; Lovenberg, T. W.; Grigoriadis, D. E.; Behan, D. P.; De Souza, E. B. *Trends Pharmacol. Sci.* **1996**, *17*, 166; (b) Lovenberg, T. W.; Liaw, C. W.; Grigoriadis, D. E.; Clevenger, W.; Chalmers, D. T.; De Souza, E. B.; Oltersdorf, T. *Proc. Natl. Acad. Sci U.S.A.* **1995**, *92*, 836.
5. For recent reviews, see (a) Chen, C. *Curr. Med. Chem.* **2006**, *13*, 1261; (b) Gilligan, P. J.; Li, Y.-W. *Curr. Opin. Drug Discov. Dev.* **2004**, *7*, 487; (c) Kehne, J.; De Lombaert, S. *Curr. Drug Targets—CNS Neurol. Disord.* **2002**, *1*, 467; (d) Gilligan, P. J.; Robertson, D. W.; Zaczek, R. *J. Med. Chem.* **2000**, *43*, 1641.
6. For some recent examples, see (a) Dyck, B.; Grigoriadis, D. E.; Gross, R. S.; Guo, Z.; Haddach, M.; Marinkovic, D.; McCarthy, J. R.; Moorjani, M.; Regan, C. F.; Saunders, J.; Schwaebe, M. K.; Szabo, T.; Williams, J. P.; Zhang, X.; Bozigian, H.; Chen, T. K. *J. Med. Chem.* **2005**, *48*, 4100; (b) Lowe, R. F.; Nelson, J.; Dang, T. N.; Crowe, P. D.; Pahuja, A.; McCarthy, J. R.; Grigoriadis, D. E.; Conlon, P.; Saunders, J.; Chen, C.; Szabo, T.; Chen, T. K.; Bozigian, H. *J. Med. Chem.* **2005**, *48*, 1540;

(c) Hodgetts, K. J.; Yoon, T.; Huang, J.; Gulianello, M.; Kieltyka, A.; Primus, R.; Brodbeck, R.; De Lombaert, S.; Doller, D. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2497; (d) Yuan, J.; Gulianello, M.; De Lombaert, S.; Brodbeck, R.; Kieltyka, A.; Hodgetts, K. J. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2133.

7. (a) Chen, Y. L.; Mansbach, R. S.; Winter, S. M.; Brooks, E.; Collins, J.; Corman, M. L.; Dunaiskis, A. R.; Faraci, W. S.; Gallaschun, R. J.; Schmidt, A.; Schulz, D. W. *J. Med. Chem.* **1997**, *40*, 1749; (b) Schultz, W. D.; Mansbach, R. S.; Sprouse, J.; Braselton, J. P.; Collins, J.; Corman, M., ; Tingley, F. D., III; Winston, E. N.; Chen, Y. L.; Heym, J. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 10477.
8. Chen, C.; Wilcoxen, K. M.; Huang, C. Q.; Xie, Y. F.; McCarthy, J. R.; Webb, T. R.; Zhu, Y.-F.; Saunders, J.; Liu, X. J.; Chen, T. K.; Bozigian, H.; Grigoriadis, D. E. *J. Med. Chem.* **2004**, *47*, 4787.
9. Chen, C.; Dagnino, R., Jr.; De Souza, E. B.; Grigoriadis, D. E.; Huang, C. Q.; Kim, K. I.; Liu, Z.; Morgan, T.; Webb, T. R.; Whitten, J. P.; Xie, Y. F.; McCarthy, J. R. *J. Med. Chem.* **1996**, *39*, 4358.
10. Holsboer, F. *Curr. Opin. Invest. Drugs* **2003**, *4*, 46.
11. Guo, Z.; Tellew, J. E.; Gross, R. S.; Dyck, B.; Grey, J.; Haddach, M.; Kiankarimi, M.; Lanier, M.; Li, B. F.; Luo, Z.; McCarthy, J. R.; Moorjani, M.; Saunders, J.; Sullivan, R.; Zhang, X.; Zamani-Kord, S.; Grigoriadis, D. E.; Crowe, P. D.; Chen, T. K.; Williams, J. P. *J. Med. Chem.* **2005**, *48*, 5104.
12. Griebel, G.; Simiand, J.; Steinberg, R.; Jung, M.; Gully, D.; Roger, P.; Geslin, M.; Scatton, B.; Maffrand, J.-P.; Soubrie, P. *J. Pharmacol. Exp. Ther.* **2002**, *301*, 333.
13. (a) Zobel, A. W.; Nickel, T.; Kunzel, H. E.; Ackl, N.; Sonntag, A.; Ising, M.; Holsboer, F. *J. Psychiatr. Res.* **2000**, *34*, 171; (b) Kunzel, H. E.; Zobel, A. W.; Nickel, T.; Ackl, N.; Uhr, M.; Sonntag, A.; Ising, M.; Holsboer, F. *J. Psychiatr. Res.* **2003**, *37*, 525.
14. Huang, C. Q.; Wilcoxen, K. M.; McCarthy, J. R.; Haddach, M.; Webb, T. R.; Gu, J.; Xie, Y. F.; Grigoriadis, D.; Chen, C. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3375.
15. Maestro 7.0; See Schrodinger Ltd. Available from: http://www.Schrodinger.com.
16. For the synthesis of 4-amino-1-phenyl-isoquinoline see Sawanishi, H.; Sashida, H.; Tsuchiya, T. *Chem. Pharm. Bull.* **1985**, *33*, 4564.
17. Grigoriadis, D. E.; De Souza, E. B. *Methods Neurosci.* **1991**, *5*, 510.
18. (a) Chen, C.; Dagnino, R., Jr.; De Souza, E. B.; Grigoriadis, D. E.; Huang, C. Q.; Kim, K.-I.; Liu, Z.; Moran, T.; Webb, T. R.; Whitten, J. P.; Xie, Y. F.; McCarthy, J. R. *J. Med. Chem.* **1996**, *39*, 4358; (b) Hodge, C. N.; Aldrich, P. E.; Wasserman, Z. R.; Fernandez, C. H.; Nemeth, G. A.; Arvanitis, A.; Cheeseman, R. S.; Chorvat, R. J.; Ciganek, E.; Christos, T. E.; Gilligan, P. J.; Krenitsky, P.; Scholfield, E.; Strucely, P. *J. Med. Chem.* **1999**, *42*, 819.
19. Chaki, S.; Okuyama, S.; Nakazato, A.; Kumagai, T.; Okubo, T.; Ikeda, Y.; Oshida, Y.; Hamajima, Y.; Tomisawa, K. *Eur. J. Pharmacol.* **1999**, *371*, 205.