N-Phenylphenylglycines as Novel Corticotropin Releasing Factor Receptor Antagonists

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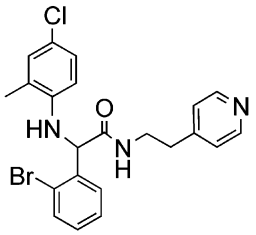
Abstract: Screening of a computationally designed synthetic library led to the discovery of the *N*-phenylphenylglycines (NPPGs) as a novel class of human corticotropin releasing factor (h-CRF_1) antagonists. Several NPPGs with greater potency than the original hit **1** were rapidly identified, and resolution of the racemate demonstrated that only the *R*-enantiomer displays activity. This structural class represents the first example of a non-peptide CRF_1 antagonist with a stereochemically distinct receptor binding affinity.

Corticotropin releasing factor (CRF; also known as corticotropin releasing hormone)[1] is the primary physiological regulator of the hypothalamic−pituitary−adrenal axis, presiding over a large number of neuronal, endocrine, and immune processes. This 41 amino acid peptide is a major modulator of the body's response to stress,[2] and regulation of its function has become an important target for drug design. Studies of CRF agonists have shown that abnormal hormonal levels may be important in neuropsychiatric disorders such as anxiety and depression,[3] substance abuse,[4] eating disorders,[5,6] premature parturition,[7] and gastrointestinal maladies.[8] Peptide agonists and antagonists have been available for many years, and they are valuable tools for the investigation of CRF-mediated physiology. Studies with peptide ligands provide support for a role for CRF in coordinating the body's response to stress. In the past 10 years, considerable progress in the identification of non-peptide modulators of CRF has provided opportunities for clinical studies on treatment for some of the disorders described above. Several classes of antagonists have already been investigated to date,[9] but there is still a need for the identification of structurally diverse CRF antagonists.

Corticotropin releasing factor receptor antagonists such as anilinopyrimidines, triazines, and bicyclic compounds have been reviewed by Gilligan.[2] In a previous paper we described that arylamidrazones are a novel class of CRF antagonists.[10] Here, we report the discovery of the *N*-phenylphenylglycines (NPPGs) as a new class of CRF antagonists utilizing computational design tools and parallel synthesis to rapidly advance from "hit to lead".



1 *K*i = 1035 nM

Figure 1. Initial NPPG hit.

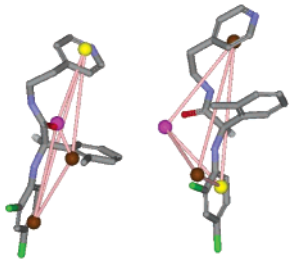


Figure 2. Compound **30A** (the *R* enantiomer) aligned to two high information content pharmacophores. Each pharmacophore contains one hydrogen-bond acceptor (magenta), an aromatic group (yellow), and two hydrophobic groups (brown).

The initial hit from the *N*-phenylphenylglycine series, **1** (Figure 1), was discovered using computational library design methods. A library design space biased toward pharmacophores contained in compounds exhibiting CRF_1 antagonist activity was generated from an initial data set that included 2973 actives (K_i < 100 nM) and 1212 inactives (K_i > 10μM). The library design space was derived using molecular signatures composed of three-dimensional pharmacophore descriptors described in the Supporting Information.

To select the subset of all pharmacophore hypotheses potentially associated with CRF_1 activity, the signatures of all compounds were systematically analyzed in the context of their associated activity data. The pharmacophores were ranked on the basis of their ability to discriminate between actives and inactives across the entire data set. The ranking criterion was "information content", a function of the active and inactive compounds (see ref 11 for the equation). To assess the significance of the information content value, the activity data were randomly permuted (10 trials) to determine the average information content level expected from random chance correlations in the data set. All 77 075 pharmacophores with information content values greater than those expected by random chance were utilized as our CRF_1 library design space. Figure 2 shows two examples of pharmacophores with high information content. The arrangement of the hydrogen bond acceptor and aromatic and hydrophobic groups in these high-ranking pharmacophores is consistent with the general CRF_1 antagonist SAR proposed by Gilligan.[2] The lower information content pharmacophores encompass a more diverse collection of feature combinations

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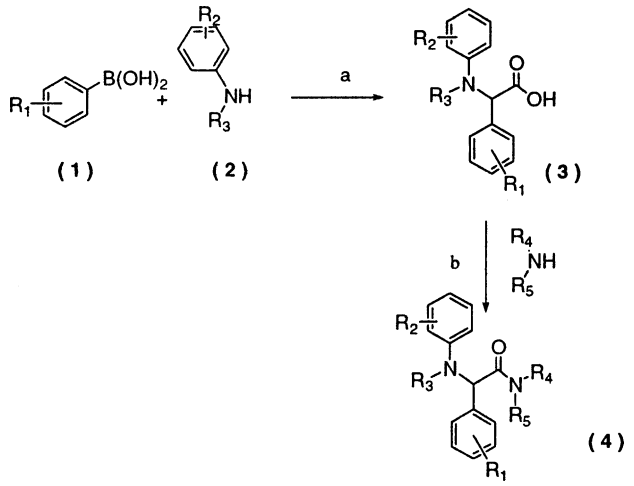
Scheme 1[a]



[a] Conditions: (a) HCOCOOH, toluene, 80 °C, 12 h; (b) HATU, DIEA, DMSO, 12 h.

and arrangements, representing alternative descriptions of activity from the known SAR.

Because our challenge was to identify novel classes of CRF_1 antagonists, we used the full 77 075 pharmacophore CRF_1 design space to select chemical scaffolds and design libraries for synthesis. New scaffolds covering a broad range of chemical diversity were evaluated for their ability to produce virtual libraries that could match pharmacophores in the CRF_1 design space. Each of the 77 075 pharmacophores was treated equally, i.e., no extra importance was placed on scaffolds that were able to cover more high than low information content pharmacophores. Also examined was whether each scaffold covered pharmacophores that were not matched by other scaffolds. Pharmacophore coverage in the CRF_1 design space, complementarity of the pharmacophores covered, and synthetic feasibility constraints were the main criteria used to select a set of chemistries for library design and synthesis. The NPPG class was one of these selections.

To design libraries that systematically explore the portion of CRF_1 pharmacophore space covered by the new chemical classes, we applied the strategy of informative design[12] by which compounds are used to "interrogate" the target receptor and to determine which chemical features are required for activity. The method constructs a library such that the maximum number of conclusions can be drawn from the new biological screening results. Informative design was implemented as a maximum Shannon entropy criterion to search a binary descriptor space (e.g., pharmacophore signatures) and systematically identify descriptors important for activity.

For the first NPPG combinatorial library, 12 amines were selected computationally from a list of 367 possible amines using the whole molecule (product) based three-dimensional pharmacophore descriptors and informative design.[12] The 77 075 bit pharmacophore signatures were generated for the final molecular products of each of the 367 amines in combination with the two sets of additional building blocks, anilines and boronic acids in the chemical reaction for the formation of NPPGs (Scheme 1).

The result of this design was a selection of compounds whose combination of on-bits (accessible pharmacophores) and off-bits (inaccessible pharmacophores) in their molecular signatures systematically investigated the CRF_1 pharmacophore space. While one would not expect to test all 77 075 hypotheses with a small combinatorial library for one chemical scaffold, the NPPG library design in combination with informative library designs for additional chemical series provided sufficient information to refine our CRF-biased pharmacophore space for subsequent rounds of design and synthesis. (In the combined library designs for multiple classes, ~99% of the 77 075 pharmacophores were matched by at least one compound and ~60% were covered by 50 or more compounds.) The final NPPG combinatorial matrix design for synthesis was composed of all products resulting from the combination of the 12 computationally selected amines with 6 anilines and 6 boronic acids preselected by medicinal chemistry intuition. The NPPG compounds included the 4-ethylaminopyridine building block and the product **1**.

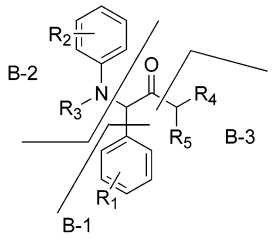


Figure 3. NPPG's synthetic strategy.

The *N*-phenylphenylglycine amides were synthesized in a two-step process involving a boronic acid Mannich reaction[13] followed by amidation (Scheme 1). Condensation of boronic acids (**1**) and amines (**2**) with glyoxylic acid afforded the intermediate acids (**3**) that were converted to the NPPGs (**4**) by amidation.

The first NPPG library utilized 6 anilines (**2**), 6 boronic acid (**1**), and 12 amines. Of 36 expected acids (**3**), 30 examples were successful and were converted to amides (**4**). Of 180 possible racemic products, 174 were isolated by LC/MS in >85% purity.[14] One mixture with modest affinity in the in vitro h-CRF_1 binding assay was identified. Racemic mixture **1** afforded a K_i of 1035 nM. Mixture **1** was also tested against CRF_2 and found to be inactive at 10 μM.

To optimize the NPPG series, each of the three branches of the molecule were evaluated independently (Figure 3). Initially, B-1 was varied while retaining B-2 and B-3 (Table 1). Substitution of the bromo at the 2-position with other substituents (13 examples) led to the 2-vinyl- and 2-methyl-substituted variants (**4**, K_i = 752 nM; **5**, K_i = 922 nM), which were slightly more potent than the original example **1**. Other substituents (CF_3, OPh, SCH_3, OCH_2CH_3, Ph) in the 2-position resulted in inactive racemates. Monosubstitution in the 3- or 4-position afforded inactive or weakly active racemic mixtures as illustrated by comparison of the three monomethyl products **5**, **9**, and **12** and the three monochloro products **2**, **8**, and **11**. Interestingly, the 2- and 3-methoxy mixtures **7** and **10**, respectively, were inactive as were the dimethoxy variants **13**−**15**. The unsubstituted phenyl derivative **16** was inactive. Racemates derived from heteroaromatic boronic acids were also inactive.

Next B-2 was varied, keeping B-1 and B-3 constant. A variety of amines were examined including aromatic,

Table 1. Structure−Activity Relationship Data[15]

compd[a]	R_1	R_2	R_4[c]	K_i (nM)
1	2-Br	2-Cl, 4-CH_3	A	1035
2	2-Cl	2-Cl, 4-CH_3	A	3346
3	2-F	2-Cl, 4-CH_3	A	>10000
4	2-$CHCH_2$	2-Cl, 4-CH_3	A	752
5	2-CH_3	2-Cl, 4-CH_3	A	922
6	2-$CH(CH_3)_2$	2-Cl, 4-CH_3	A	3295
7	2-OCH_3	2-Cl, 4-CH_3	A	>10000
8	3-Cl	2-Cl, 4-CH_3	A	>5000
9	3-CH_3	2-Cl, 4-CH_3	A	>10000
10	3-OCH_3	2-Cl, 4-CH_3	A	>10000
11	4-Cl	2-Cl, 4-CH_3	A	>10000
12	4-CH_3	2-Cl, 4-CH_3	A	>10000
13	2,5-di-OCH_3	2-Cl, 4-CH_3	A	>10000
14	2,4-di-OCH_3	2-Cl, 4-CH_3	A	>10000
15	2,6-di-OCH_3	2-Cl, 4-CH_3	A	>10000
16	H	2-Cl, 4-CH_3	A	>10000
17	2-Br	2-Cl	A	7334
18[b]	2-Br	2-Cl	A	>10000
19	2-Br	2,4-di-Cl	A	204
20	2-Br	2-Cl, 4-CH_3	B	>10000
21	2-Br	2-Cl, 4-CH_3	C	>10000
22	2-Br	2-Cl, 4-CH_3	D	>10000
23	2-Br	2-Cl, 4-CH_3	E	>10000
24	2-Br	2-Cl, 4-CH_3	F	>10000
25	2-Br	2-Cl, 4-CH_3	G	>10000
26	2-Br	2-Cl, 4-CH_3	H	1075
27	2-Br	2-Cl, 4-CH_3	I	1066
28	2-CH_2CH_3	2,4-di-Cl	A	330
29	2,6-di-CH_3	2,4-di-Cl	A	338
30	2- CH_3	2,4-di-Cl	A	370
31	2- CH_3,4-F	2,4-di-Cl	A	512
32	2-$CHCH_2$	2-Cl, 4-CH_3	A	752
33	2-CF_3	2,4-di-Cl	A	1002
34	2-CH_3	2-F, 4-CH_3	A	439
35	2-CH_3	2-CF_3, 4-Cl	A	469
36	2-CH_3	2-CF_3, 4-CH_3	A	641
37	2-CH_3	2,4-di-Cl	I	387
38	2-CH_3	2,4-di-Cl	L	>10000
39	2-CH_3	2,4-di-Cl	M	>10000
40	2-Br	2,4-di-Cl	N	514

[a] R_3 = H unless otherwise specified. [b] R_3 = Me. [c] See Figure 4.

heteroaromatic, aliphatic, primary, and secondary (74 reactions attempted). Only aniline containing racemic mixtures were found to have activity. Removal of the methyl in the 4-position (**17**) resulted in a decrease in activity, and N-methylation resulted in an inactive mixture (**18**). Ultimately 2,4-dichloro substitution resulted in the most active racemate **19** (K_i = 204 nM).

Finally, B-3 was investigated using aromatic, heteroaromatic, aliphatic, primary, and secondary nitrogen substituents (210 reactions attempted). The 4-pyridyl feature was strongly favored over other nitrogen regioisomers because 2- and 3-pyridyl variants were inactive (**1** vs **20** vs **21**). Molecules featuring a phenyl (**22**), imidazole (**23**), or a 4-fluorophenyl group (**24**) resulted in inactive mixtures. Shortening the aliphatic chain linker (**25**) resulted in a loss of activity as well. Racemic mixtures **26** and **27** derived from anilines rather than aliphatic amines showed weak binding affinities with K_i values of 1075 and 1066 nM, respectively.

Using the resulting optimized side chains, a final round of synthesis focused on small modifications of the preferred substituents (Table 1, Figure 4). The optimal R_2 was 2,4-dichloro substitution (compare **30**, **34**−**36**, and **5**). The greatest affinity was observed for racemic mixture **19** when R_1 = 2-Br (K_i = 204 nM). Substituents that also resulted in an active mixture were R_1 = 2-CH_2-CH_3 (**28**), 2,6-di-CH_3 (**29**), 2-CH_3 (**30**), 2-CH_3, and 4-F

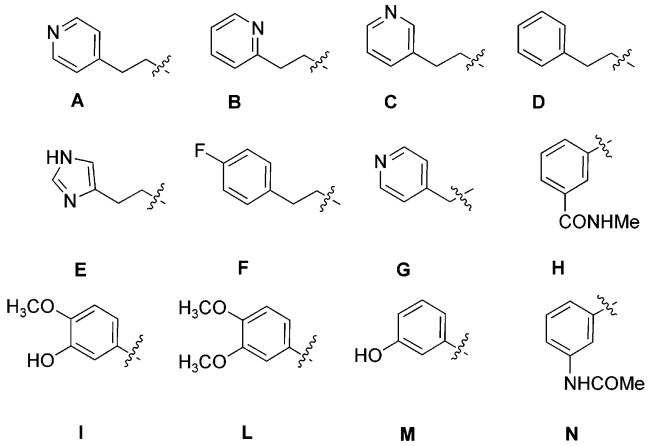


Figure 4. R_4 substitution for compounds in Table 1.

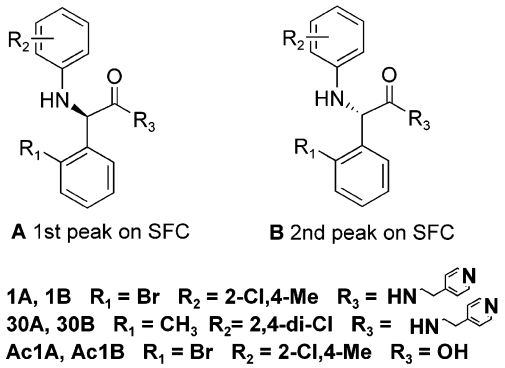


Figure 5. Resolved NPPGs.

(**31**). The free phenol **37** resulted in an active mixture but only when paired with an obligate C_4 methoxy substituent (**37** vs **38** and **39**).

Racemic mixture **1** was resolved by chiral supercritical fluid chromatography (SFC; Figure 5). The absolute configuration of the two enantiomers **1A** (*R*) and **1B** (*S*) was established by determining the configuration of the precursor acid **Ac1B**, which corresponds to the second compound **1B** eluted by SFC. The racemic mixture of the intermediate acid was resolved into the two pure enantiomers **Ac1A** and **Ac1B** (see Figure 5). Each of the two acids was coupled with 2-pyridin-4-ylethylamine, giving **1A** from **Ac1A** and **1B** from **Ac1B**. X-ray analysis of **Ac1B** confirmed the *S* configuration, allowing assignment of **1B** as *S* and **1A** as *R* (see Supporting Information). The two pure enantiomers **1A** and **1B** were tested for binding affinity to the CRF_1 receptor. Compound **1A** showed a 2-fold increase in binding affinity compared to the racemic mixture (K_i = 546 nM), whereas **1B** was inactive. This is one of the first examples of a non-peptide antagonist of the CRF_1 receptor in which chirality plays a role.

Racemic mixtures **5** and **30** were also resolved, yielding **5A** and **5B** and **30A** and **30B**, respectively. The absolute configuration of each was assigned by correlation of their retention time (chiral SFC) to those for **1A** and **1B**. Also, as in the previous example, **5A** and **30A** were more potent when compared to the racemic mixture (K_i = 244 and 154 nM, respectively). Compound **5B** was inactive, and **30B** showed a K_i of 3180 nM. To establish the selectivity of this class of compounds, **30A** was also serotonin receptors 5-HT_{2A}, 5-HT_{1A}, 5-HT_{1B},

5-HT_{1D}, 5-HT_6, and 5-HT_7 and resulted inactive at 10 μM in all cases.

To assess the pharmacokinetics of an early lead in this series, we included **30A** in a dog cassette dose study.[16] Briefly, **30A** was coadministered intravenously with seven other compounds to beagle dogs ($n = 2$) at a dose of 0.2 mg/kg for each compound. In addition, the same set of compounds was administered orally to beagle dogs ($n = 2$) at a dose of 1 mg/kg. Plasma samples were collected at 0, 0.1, 0.25, 0.5, 1, 2, 4, 6, 8, and 24 h postdose for the intravenous experiment and at 0, 0.5, 1, 2, 4, 6, 8, and 24 h postdose for the oral experiment. Drug concentrations were determined in the plasma samples using LC/MS/MS. The following pharmacokinetic parameters were derived noncompartmentally from the described study: total body clearance, 1.3 (L/h)/kg, volume of distribution at steady state, 1.8 L/kg; half-life, 1.7 h; oral bioavailability, 7%. Although the parameters are not optimal, the total body clearance estimate categorizes **30A** as a moderate clearance compound. Thus, **30A** provides a compelling starting point for further potency and bioavailability optimization.

We have described the discovery of a novel series of CRF_1 antagonists by using a computational library design strategy. Optimization of the core structure was accomplished through parallel synthesis of small, targeted libraries. In addition, resolution of the most active mixture in the series (**30**, $K_i = 370$ nM) showed that the receptor seems to have a stereochemical bias, since only the *R* enantiomer retained activity (**30A**, $K_i = 154$ nM). This structural class represents the first example in which the enantiomers of a non-peptide molecule demonstrated differences in CRF_1 binding affinity. In addition, preliminary pharmacokinetic studies on **30A** were encouraging and provide incentive for additional work in this series.

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Supporting Information Available: Experimental and computational procedures, ^{1}H NMR, LC/MS, HRMS, and crystal structure data. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Vale, W.; Spiess, J.; Rivier, C.; Rivier, J. Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and beta-endorphin. *Science* **1981**, *213*, 1394−1397.

(2) Gilligan, P. J.; Robertson, D. W.; Zaczeck, R. Corticotropin releasing factor (CRF) receptor modulators: progress and opportunities for new therapeutic agents. *J. Med. Chem.* **2000**, *43*, 1641−1660 and references therein.

(3) Aguilera, G. Corticotropin releasing hormone, receptor regulation and the stress response. *Trends Endocrinol. Metab.* **1998**, *9*, 329−336.

(4) Koob, G. F. Stress, corticotropin-releasing factor, and drug addiction. *Ann. N. Y. Acad. Sci.* **1999**, *897*, 27−45.

(5) Pelleymounter, M. A.; Joppa, M.; Carmouche, M.; Cullen, M. J.; Brown, B.; Murphy, B.; Grigoriadis, D. E.; Ling, N.; Foster, A. C. Role of corticotropin-releasing factor (CRF) receptors in the anorexic syndrome induced by CRF. *J. Pharmacol. Exp. Ther.* **2000**, *293*, 799−806.

(6) Kaye, W. H. Neuropeptide abnormalities in anorexia nervosa. *Psychiatry Res.* **1996**, *62*, 65−74.

(7) Keller, P. A.; Elfick, L.; Garner, J.; Morgan, J.; McCluskey, A. Corticotropin releasing-hormone: therapeutic implications and medicinal chemistry developments. *Bioorg. Med. Chem.* **2000**, *8*, 1213−1223.

(8) McCarthy, J. R.; Heinrichs, S. C.; Grigoriadis, D. E. Recent advances with the CRF_1 receptor: design of small molecule inhibitors, receptor subtypes, and clinical indications. *Curr. Pharm. Des.* **1999**, *5*, 289−315.

(9) Saunders, J.; Williams, J. New developments in the study of corticotropin releasing factor. *Annu. Rep. Med. Chem.* **2001**, *36*, 21−30 and references therein.

(10) Wilson, D. M.; Termin, A. P.; Mao, L.; Ramirez-Weinhouse, M. M.; Molteni, V.; Grootenhuis, P. D. J.; Miller, K.; Keim, S.; Wise, G. Arylamidrazones as novel corticotropin releasing factor receptor antagonists. *J. Med. Chem.* **2002**, *45*, 2123−2126.

(11) Srinivasan, J.; Castellino, A.; Bradley, E. K.; Eksterowicz, J. D.; Grootenhuis, P. D. J.; Putta, S.; Stanton, R. V. Evaluation of a novel shape-based computational filter for lead evolution: application to thrombin inhibitors. *J. Med. Chem.* **2002**, *45* (12), 2494−2500.

(12) Miller, J. L.; Bradley, E. K.; Teig, S. L. Luddite: an information-theoretic library design tool. *J. Chem. Inf. Comput. Sci.* **2003**, *43* 47−54.

(13) (a) Petasis, N. A.; Zavialov, I. A. A new and practical synthesis of α-amino acids from alkenyl boronic acids. *J. Am. Chem. Soc.* **1997**, *119*, 445−446. (b) Petasis, N. A.; Goodman, A.; Zavialov, I. A new synthesis of α-arylglycines from aryl boronic acids. *Tetrahedron* **1997**, *53*, 16463−16470. (c) Petasis, N. A.; Zavialov, I. Highly stereocontrolled one-step synthesis of anti-β-amino alcohols from organoboronic acids, amines, and α-hydroxy aldehydes. *J. Am. Chem. Soc.* **1998**, *120*, 11798−11799.

(14) Zeng, L.; Kassel, D. B. *Anal. Chem.* **1998**, *70*, 4380.

(15) (a) Arvanitis, A. G.; Gilligan, P. J.; Chorvat, R. J.; Cheeseman, R. S.; Christos, T. E.; Bakthavatchalam, R.; Beck, J. P.; Cocuzza, A. J.; Hobbs, F. W.; Wilde, R. G.; Arnold, C.; Chidester, D.; Curry, M.; He, L.; Hollis, A.; Klaczkiewicz, J.; Krenitsky, P. J.; Rescinito, J. P.; Scholfield, E.; Culp, S.; De Souza, E. B.; Fitzgerald, L.; Grigoriadis, D.; Tam, S. W.; Wong, Y. N.; Huang, S.; Shen, H.-S. L. Non-peptide corticotropin releasing hormone antagonists: syntheses and structure−activity relationships of 2-anilinopyrimidines and -triazines. *J. Med. Chem.* **1999**, *42*, 805−818. (b) Mean K_i values were reported when more than one determination was performed. If the deviation between initial duplicate K_i values exceeded 15%, additional duplicate studies were performed until this criterion was met and determinations were averaged. Internal control compounds were run on every plate.

(16) (a) Berman, J.; Halm, K.; Adkison, K.; Schaffer, J. Simultaneous pharmacokinetic screening of a mixture of compounds in dog using API LC/MS/MS analysis for increased throughput. *J. Med. Chem.* **1997**, *40*, 827−829. (b) He, L.; Gilligan, P. J.; Zaczek, R.; Fitzgerald, L. W.; McElroy, J.; Shen, H.-S. L.; Saye, J. A.; Kalin, N. H.; Shelton, S.; Christ, D.; Trainor, G.; Hartig, P. 4-(1,3-Dimethoxyprop-2-ylamino)-2,7-dimethyl-8-(2,4-dichlorophenyl)-pyrazolo[1,5-*a*]-1,3,5-triazine: a potent, orally bioavailable CRF_1 receptor antagonist. *J. Med. Chem.* **2000**, *43*, 449−456.

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