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In Vitro Intrinsic Clearance-Based Optimization of N^3-Phenylpyrazinones as Corticotropin-Releasing Factor-1 (CRF_1) Receptor Antagonists

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A series of pyrazinone-based heterocycles was identified as potent and orally active corticotropin-releasing factor-1 (CRF_1) receptor antagonists. Selected compounds proved efficacious in an anxiety model in rats; however, pharmacokinetic properties were not optimal. In this article, we describe an in vitro intrinsic clearance-based approach to the optimization of pyrazinone-based CRF_1 receptor antagonists wherein sites of metabolism were identified by incubation with human liver microsomes. It was found that the rate of metabolism could be decreased by incorporation of appropriate substituents at the primary sites of metabolism. This led to the discovery of compound **12x**, a highly potent (IC_{50} = 1.0 nM) and selective CRF_1 receptor antagonist with good oral bioavailability (F = 52%) in rats and efficacy in the defensive withdrawal anxiety test in rats.

Introduction

Novel pharmacological approaches leading to the development of improved treatments for stress-related diseases, such as anxiety and depression, are of significant interest to the pharmaceutical industry. Despite the availability of currently marketed anxiolytic and antidepressant drugs, issues with currently available therapies, such as a delayed onset of action, undesirable side effects, and a lack of efficacy in subgroups of patients, stimulate continued interest in novel treatments. One such potential novel method of treatment for stress-related disorders includes corticotropin-releasing factor-1 (CRF_1[a]) receptor antagonists.[1−6] Corticotropin-releasing factor (CRF), a 41 amino acid neuropeptide,[7] coordinates the body's response to stress via its function as the primary physiological regulator of the secretion of adrenocorticotropic hormone (ACTH) from the anterior pituitary gland.[8,9] Two receptor subtypes, CRF_1 and CRF_2, have been identified and are widely distributed throughout the central and peripheral nervous systems.[10−14] A substantial body of evidence exists supporting the hypothesis that excess levels of CRF contribute to stress-related disorders, such as depression and anxiety, and that antagonists of CRF_1 receptors may be able to treat these conditions.[15−18] In addition, small molecule CRF_1 receptor antagonists, such as **1** (DMP696),[19] were shown to be efficacious in behavioral models for anxiety and depression in preclinical studies.[1,20−22]

The outcome of a limited number of clinical trials reported to date produced mixed results. In a small open-label clinical trial with **2** (R121919, also known as NBI30775)[23] for depression, it was found that depressed patients showed reductions in depression symptoms, as rated by both patients and clinicians.[24] A placebo-controlled clinical study, designed to evaluate whether subchronic treatment with **3** (NBI-34041)[25] would decrease the stress hormone response following a psychological stressor, indicated that **3** may improve resistance to psychological stress by reducing stress hormone secretion.[26,27] It was concluded from a double-blind, placebo-controlled clinical trial with **4** (CP-316311)[28] for the treatment of major depressive disorder that this compound lacked efficacy in this study.[29] Several additional compounds, including **5** (CP-376395)[30] and **6** (BMS-562086),[1] are reported to have entered clinical trials, and the results from these trials may provide further insight into the clinical utility of CRF_1 receptor antagonists (Figure 1).

As previously described in the companion paper (DOI 10.1021/jm900301y),[31] the pyrazinone ring system[32] served as a useful scaffold based on its structural relationship to the well-established pharmacophore model of known CRF_1 receptor antagonists.[6] The structure−activity relationships (SAR) of a series of pyrazinone-based analogues were investigated, leading to highly potent and efficacious compounds such as compound **7**[33] (Figure 2). Although **7** was very potent (IC_{50} = 0.26 nM), it had high clearance in rats. A metabolite ID study was subsequently conducted.[31] Briefly, the results of this study suggested that the three major metabolites were *O*-demethylation of the upper methoxy group, *O*-demethylation of the lower methoxy group, and *O*-demethylation of both. These results provided us with a starting point from which to further optimize this class of compounds by assessing

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[a]Abbreviations: CRF, corticotropin-releasing factor; ACTH, adrenocorticotropic hormone; SAR, structure−activity relationships; cAMP, cyclic adenosine monophosphate.

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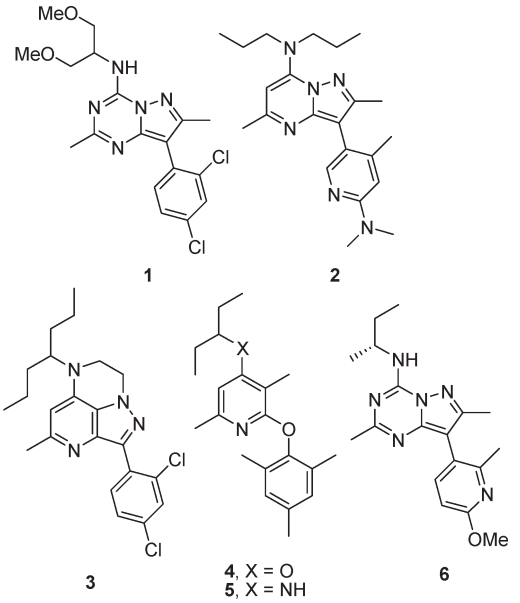


Figure 1. CRF_1 receptor antagonists.

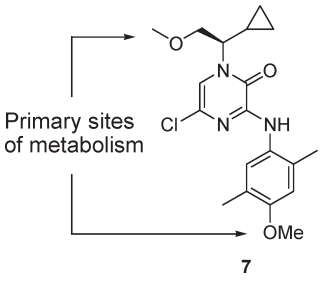


Figure 2. Summary of the metabolite ID study with compound **7**.

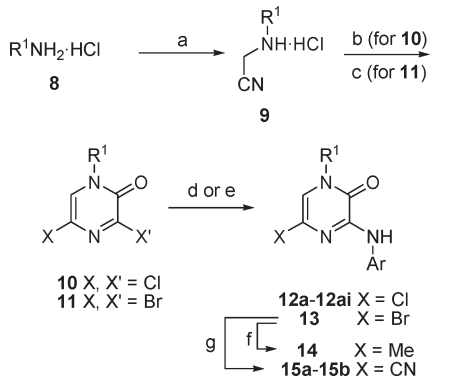
their rate of intrinsic clearance in vitro. Progress toward further optimization of the pharmacokinetic properties of pyrazinone-based compounds using an in vitro intrinsic clearance-directed approach is described herein.

Results and Discussion

Chemistry. 1-Alkyl-3-anilinopyrazin-2-ones were prepared as shown in Scheme 1. Cyanomethylamines **9** were prepared in high yield by treatment of alkylamine hydrochlorides **8**[34] with chloroacetonitrile in the presence of potassium iodide and potassium carbonate. The cyanomethylamine intermediate was condensed with either oxalyl chloride or oxalyl bromide to form the dichloro- or dibromopyrazinone products **10** or **11**, respectively,[35,36] as previously described.[31] Intermediates **10** and **11** were then coupled with a variety of anilines in the presence of a base to furnish chloro- and bromopyrazinones **12** and **13**, respectively. Synthesis of 5-methyl- and 5-cyanopyrazinone analogues was then completed in one additional step from bromopyrazinone **13** to furnish **14** (X = Me) or **15** (X = CN).

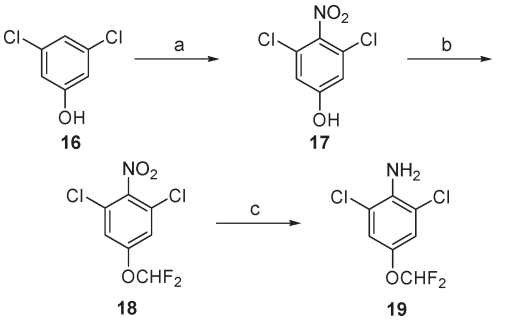
The various aniline groups were either available from commercial sources, synthesized according to cited references, or synthesized as shown in Schemes 2 and 3. Preparation of 2,6-dichloro-4-(difluoromethoxy)aniline (**19**) was carried out via a three-step synthesis whereby commercially available 3,5-dichlorophenol was nitrated, resulting in a mixture of 2- and 4-nitro regioisomeric products (Scheme 2).

Scheme 1[a]



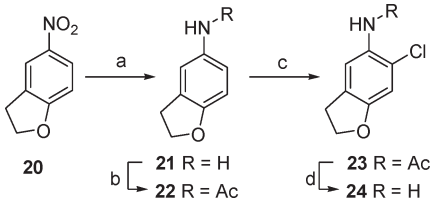
[a] Reagents and conditions: (a) chloroacetonitrile, KI, K_2CO_3, CH_3CN, 50 °C (84−96%); (b) $(COCl)_2$, toluene, 55 °C (43−71%) or $(COCl)_2$, dioxane/CH_2Cl_2, 55 °C (69−74%); (c) $(COBr)_2$, CH_2Cl_2, 45 °C (64%); (d) base, $ArNH_2$, THF; (e) $ArNH_2$, $Pd(OAc)_2$, BINAP, K_2CO_3, toluene; (f) K_2CO_3, $MeB(OH)_2$, $Pd(Pt\text{-}Bu_3)_2$, dioxane, 120 °C (52%); (g) $Zn(CN)_2$, $Pd(PPh_3)_4$, Zn dust, NMP (33−63%).

Scheme 2[a]



[a] Reagents and conditions: (a) $NaNO_2$, H_2SO_4, H_2O (25%); (b) ClF_2CCO_2Me, K_2CO_3, DMF (72%); (c) H_2, Pd/C, EtOH/HCl (47%).

Scheme 3[a]

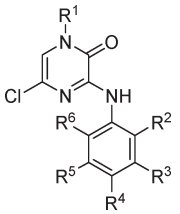


[a] Reagents and conditions: (a) $SnCl_2 \cdot 2H_2O$, EtOH (65%); (b) AcCl, CH_2Cl_2/pyridine (94%); (c) NCS, CH_3CN (74%); (d) KOH, EtOH/H_2O (81%).

After workup of the reaction mixture and isolation of the products, the mixture was treated with hexanes, resulting in selective precipitation of the desired 4-nitro intermediate **17**. Formation of the difluoromethyl ether was carried out by heating **17** with methyl 2-chloro-2,2-difluoroacetate in the presence of potassium carbonate to furnish **18** in good yield. Subsequent reduction of the nitro group by hydrogenation provided the desired product **19** in 47% yield.

6-Chloro-2,3-dihydrobenzofuran-5-amine (**24**) was synthesized by the four-step route shown in Scheme 3. 5-Nitro-2,3-dihydrobenzofuran **20** was prepared by nitration of 2,3-dihydrobenzofuran using the conditions of Bekkali et al.[37] The nitro group of **20** was subsequently reduced with $SnCl_2 \cdot 2H_2O$ in 65% yield. Acetylation followed

Table 1. CRF_1 Receptor Binding Affinity and Intrinsic Clearance Data for **12a**−**12i**



Cmpd	R^1	R^2	R^3	R^4	R^5	R^6	IC_{50} (nM)[a]	Intrinsic Clearance (human, mL/min/kg)
12a		Me	H	Me	H	Me	0.63 ± 0.14	340
12b		Cl	H	Cl	H	Me	0.53 ± 0.18	210
12c		Cl	H	CN	H	Cl	1.2 ± 0.2	160
12d		Cl	H	OMe	H	Cl	0.44 ± 0.16	400
12e		Cl	H	OCF_3	H	Cl	1.8 ± 0.8	77
12f		Cl	H	CF_3	H	Cl	2.3 ± 1.0	150
12g		Cl	H	CF_3	H	Cl	11 ± 1	32
12h		Cl	H	CN	H	Cl	1.7 ± 0.1	210
12i		Cl	H	CN	H	Cl	18 ± 3	35

[a]All values are the average of at least $n = 3 \pm$ standard deviation. The IC_{50} of *o*-CRF = 2.9 ± 1.0 nM and the IC_{50} of **1** = 1.2 ± 0.2 nM in this assay.

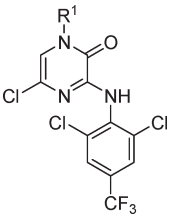
by chlorination with NCS and subsequent hydrolysis with KOH provided the target compound (**24**) in good yield.

Biology. The general screening strategy described previously was employed.[38] In addition, after determining the IC_{50} of test compounds, selected compounds were incubated with human liver microsomes to evaluate their rate of intrinsic clearance. The intrinsic clearance results were used for rank ordering purposes. On the basis of prior in-house experience in our CRF program, compounds with an intrinsic clearance rate of approximately 80 mL/min/kg or less were considered acceptable for further advancement. This number served only as a guide, however, because other properties (potency, plasma protein binding, etc.) needed to be considered as well before selecting compounds for further evaluation.

Early in our investigation, many of the most potent compounds evaluated had high rates of intrinsic clearance (e.g., compounds **12a**−**12d**, **12f**, and **12h** in Table 1) even though these compounds lacked one or both of the metabolically labile methoxy groups present in **7**. However, comparison of compounds **12f** versus **12g** and **12h** versus **12i** revealed that shortening the ethyl group in R^1 to a methyl group resulted in a significant reduction in the rate of intrinsic clearance, albeit with reduced binding affinity. A study was subsequently undertaken to identify compounds that were both highly potent and also had a low rate of intrinsic clearance.

On the basis of the low rate of intrinsic clearance of **12g**, a variety of alkyl groups at R^1 were examined with the 2,6-dichloro-4-(trifluoromethyl)aniline group as the aryl substituent (Table 2). Compounds where R^1 contained either an ethyl group (**12f** and **12j**) or a propyl group (**12k**) had higher rates of intrinsic clearance compared to analogues where

Table 2. Effect of R^1 on the Rate of Intrinsic Clearance



Cmpd	R^1	IC_{50} (nM)[a]	Intrinsic Clearance (human, mL/min/kg)
12g		11 ± 1	32
12f		2.3 ± 1.0	150
12j		1.3 ± 0.6	89
12k		3.7 ± 1.5	110
12l		4.1 ± 0.6	10
12m		1.6 ± 0.1	64
12n		8.5 ± 0.5	53

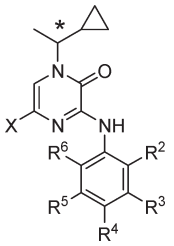
[a]All values are the average of at least $n = 3 \pm$ standard deviation.

R^1 contained a methyl group (**12g** and **12l**). Other ether containing analogues (**12m** and **12n**) had moderately higher rates of intrinsic clearance compared to **12g** and **12l**.

Although **12l** had a low rate of intrinsic clearance, it was 10-fold less potent than compounds such as **12d**[39] (Table 1). In an effort to identify compounds with good potency and metabolic stability, a broader study of the SAR on the aniline ring was carried out (Table 3). The in vitro rate of intrinsic clearance was measured for selected compounds. Compound **12o**, with a methoxy group at R^4, had an IC_{50} of 1.1 nM. Compounds with a methyl, chloro, or cyano group at R^4 (**12p**−**12t**) were similar in potency to **12o**. Analogues with a trifluoromethoxy (**12u**) or trifluoromethyl (**12l**) group were less potent than the corresponding methoxy analogue (**12o**). In contrast, replacement of the methoxy group with a difluoromethoxy group led to a slight improvement in binding affinity (compare **12w** vs **12o**). The improved potency of the difluoromethoxyphenyl analogue (**12w**) compared to the trifluoromethoxyphenyl analogue (**12u**) may arise from the hydrogen of the difluoromethoxy group acting as a hydrogen bond donor[40,41] to an amino acid residue in the CRF_1 receptor. SAR studies of the R^2 substituent indicated that Cl and Me were preferred (compare **12r** vs **12aa**; **12z** and **12ac** vs **12ab**). Comparison of compounds **12ad** vs **12af** also showed that the analogue with a methyl group at R^5 was more than 30-fold more potent than the corresponding methoxy analogue. Analogues with a fluoro or cyano group at R^5 (**12ag**[42] and **12ah**,[43] respectively) and the bicyclic analogue where R^4 and R^5 are tied together to form a 5-membered ring (**12ai**) were less potent than analogues with a methyl group at R^5 (**12ac** and **12ad**).

Analysis of the intrinsic clearance data indicated that the metabolic stability of the substituent at R^4 was critical for determining the rate of intrinsic clearance (Table 3). Compounds where R^4 was either methyl or methoxy had higher rates of intrinisic clearance. Analogues with groups at R^4, which were not readily oxidized, such as trifluoromethoxy, trifluoromethyl, difluoromethoxy, chloro, cyano, and ethoxy, generally had lower rates of intrinsic clearance. In contrast to the influence of R^4 on the rate of intrinsic clearance, the substituent at R^2 appeared to have an insignificant effect on the rate of intrinsic clearance (compare **12s**,

Table 3. Effect of Phenyl Group Substituents on CRF_1 Receptor Binding Affinity and the Rate of Intrinsic Clearance



compd	enantiomer	X	R^2	R^3	R^4	R^5	R^6	IC_{50} (nM)[a]	intrinsic clearance (human, mL/min/kg)
12l	*R*	Cl	Cl	H	CF_3	H	Cl	4.1 ± 0.6	10
12o	*R*	Cl	Cl	H	OMe	H	Cl	1.1 ± 0.1	130
12p	*R*	Cl	Me	H	Me	H	Me	1.7 ± 0.2	180
12q	*R*	Cl	Cl	H	Me	H	Me	0.98 ± 0.13	140
12r	*R*	Cl	Cl	H	Cl	H	Cl	0.91 ± 0.23	ND[b]
12s	*R*	Cl	Cl	H	CN	H	Cl	2.4 ± 1.1	58
12t	*R*	Cl	Me	H	CN	H	Cl	2.9 ± 0.8	38
12u	*R*	Cl	Cl	H	OCF_3	H	Cl	6.5 ± 2.1	30
12v	*S*	Cl	Cl	H	CF_3	H	Cl	7.9 ± 4.5[c]	ND
12w	*R*	Cl	Cl	H	$OCHF_2$	H	Cl	0.46 ± 0.14	22
12x	*S*	Cl	Cl	H	$OCHF_2$	H	Cl	1.0 ± 0.3	62
12y	*R*	Cl	Cl	H	SO_2Me	H	Cl	63 ± 13	ND
12z	*R*	Cl	Me	H	Cl	H	Cl	1.2 ± 0.1	44
12aa	*R*	Cl	CN	H	Cl	H	Cl	18 ± 0.1	ND
12ab	*R*	Cl	OMe	H	Cl	Me	H	36 ± 18	ND
12ac	*R*	Cl	Me	H	Me	Me	H	4.8 ± 0.5	ND
12ad	*R*	Cl	Me	H	OMe	Me	H	0.42 ± 0.10	61
12ae	*R*	Cl	Me	H	OEt	Me	H	1.2 ± 0.3	32
12af	*R*	Cl	Me	H	OMe	OMe	H	15 ± 5	100
12ag	*R*	Cl	Cl	H	OMe	F	H	8.2 ± 1.1	180
12ah	*R*	Cl	Cl	H	Me	CN	H	19 ± 6	160
12ai	*R*	Cl	Cl	H	OCH_2-	$-CH_2$	H	120 ± 30	ND
14	*R*	Me	Me	H	Cl	H	Me	3.1 ± 0.8	ND
15a	*R*	CN	Cl	H	CF_3	H	Cl	7.5 ± 3.9	13
15b	*R*	CN	Cl	H	$OCHF_2$	H	Cl	4.3 ± 1.6	ND

[a]All values are the average of at least $n = 3 \pm$ standard deviation unless indicated otherwise. [b] ND = not determined. [c] Value determined by two measurements.

12t, and **12z**). In summary, the data from Tables 2 and 3 indicate that the rate of intrinsic clearance of pyrazinone-based CRF_1 receptor antagonists is primarily a function of the nature of the R^1 substituent and the type of substituent at R^4.

Shown in Table 4 are the pharmacokinetic profiles of **12l**, **12x**, **14**, and **15b** after iv and oral dosing at the indicated doses in male Sprague−Dawley rats.[44] These compounds were chosen for their diversity of substituents at C5 of the pyrazinone core, aniline substituents, and chirality. Compounds **12l**, **12x**, and **15b** were low clearance compounds in vivo (based on a hepatic blood flow of 55 mL/min/kg in rats) with half-lives ranging from 7.8 to 17 h. Methylpyrazinone **14** was a moderate clearance compound, which nevertheless had a half-life of 12 h. Compound **12l** had 37% oral bioavailability when dosed as an aqueous suspension (1% polysorbate 80/0.5% methylcellulose). Compounds **12x** and **15b** each had low oral bioavailability when dosed as an aqueous suspension, likely due to the poor aqueous solubility of these compounds (<0.001 mg/mL at pH = 1.0 and 6.5 for both **12x** and **15b**). Methylpyrazinone **14** showed improved solubility (1.36 mg/mL at pH = 1 and 0.013 mg/mL at pH = 6.5) compared to chloropyrazinone **12x** and cyanopyrazinone **15b**. Nevertheless, compound **14** had low oral bioavailability, possibly due to its higher rate of clearance. Compounds **12x**, **14**, and **15b** were also dosed as a solution (in an oleoyl macrogolglycerides-based vehicle).[45] The oral bioavailability of **12x** and **15b** were each significantly enhanced, improving to 52% and 80%, respectively (Table 4). In contrast, **14** did not show much improvement in oral bioavailability, indicating that solubility was less of an issue with this compound.

On the basis of potency, protein binding results (data not presented) and the results of the intrinsic clearance assay and/or rat pharmacokinetic experiments, CRF_1 receptor occupancy was measured for a group of selected compounds bearing the 1-(1-cyclopropyl)ethyl group as the R^1 substituent. Selected compounds were also tested in the defensive withdrawal test for anxiety in rats to determine behavioral efficacy.[19,46] It was found in previous studies with **1** that the in vitro IC_{50} was similar to the plasma free concentration of **1**, corresponding to 50% receptor occupancy in the parietal cortex (in vivo IC_{50}). In turn, a minimum of 50% receptor occupancy was associated with anxiolytic efficacy in the defensive withdrawal test.[47] CRF_1 receptor occupancy was determined by ex vivo autoradiography.[47]

Compounds were initially dosed at 10 mg/kg. Exit latencies were measured 60 min after oral administration of the test compounds. Compound **12l**, which had reasonable

Table 4. Discrete Pharmacokinetic Properties of Compounds **12l**, **12x**, **14**, and **15b** in Rats

PK parameters	**12l**	**12x**	**14**	**15b**
iv dose[a] (mg/kg)	5[b]	2	2	2
Cl (mL/min/kg)	21	17[c]	30	21
V_{ss} (L/kg)	24	4.7	12	13
$t_{1/2}$ (h)	17	7.8	12	12
po dose (10 mg/kg)[d]				
AUC (nM·h)	7350	1300	950	1900
C_{max} (nM)	490	100	95	160
F%	37	5	6	10
po dose (10 mg/kg)[e]				
AUC (nM·h)	ND[f]	12000	1700	15000[c]
C_{max} (nM)	ND	1700	270	1600
F%	ND	52	10	80

[a]Vehicle: PEG/ethanol, 90:10 (v/v); $n = 3$ rats. [b]Vehicle: propylene glycol/DMAC/water, 80:10:10 (v/v); $n = 3$ rats. [c]$n = 2$ rats. [d]Vehicle: 1% polysorbate 80 in 0.5% methylcellulose; $n = 3$ rats. [e]Vehicle: oleoyl macrogolglycerides[45]/DMAC/polysorbate 80, 85:10:5; $n = 3$ rats. [f]ND = not determined.

potency (IC_{50} = 4.1 nM) and excellent metabolic stability, had a high level of receptor occupancy (81%) and caused a significant decrease in exit latency (Table 5). However, this compound was not efficacious at lower doses (data not presented). Compounds **12s** (IC_{50} = 2.4 nM) and **12t** (IC_{50} = 2.9 nM), despite being potent and having low rates of intrinsic clearance, showed low levels of CRF_1 receptor occupancy, perhaps due to poor brain penetration. Compound **15a** (IC_{50} = 7.5 nM), the cyano analogue of **12l**, had a lower level of receptor occupancy compared to **12l**. Compound **15b**, a reasonably potent compound (IC_{50} = 4.3 nM) with low in vivo clearance in rats, showed 55% CRF_1 receptor occupancy and was efficacious at 10 mg/kg. Compound **12x** showed a high level of receptor occupancy at 10 mg/kg and was also efficacious at this dose. **12x** had the combination of excellent potency (IC_{50} = 1.0 nM) and low in vivo clearance (Cl = 17 mL/min/kg). It was also determined that **12x** was a functional antagonist (IC_{50} = 4.9 ± 1.2 nM), had no agonist properties, and was not active in a CRF_2 receptor binding assay (CRF_2 IC_{50} > 10 μM), indicating that **12x** was a selective CRF_1 antagonist.[48] A dose−response experiment was conducted to determine the lowest dose required for **12x** to produce an anxiolytic effect, and the results are shown in Table 5 and Figure 3. In this study, **12x** occupied CRF_1 receptors in a dose-dependent manner (Table 5). Figure 3 shows exit latencies for each dose at 60 min after oral administration of **12x** or the positive control compound **1** (dosed at 10 mg/kg).[47] At 3 mg/kg, there was a substantial, although not quite statistically significant, decrease in the exit latency (46% compared to vehicle). At 10 mg/kg, the reduction was greater (61%) and statistically significant.

Conclusion

In conclusion, screening of compounds in an intrinsic clearance assay in human liver microsomes facilitated the identification of the substituents most susceptible to metabolism. Blocking metabolism at these sites resulted in the identification of compounds with improved pharmacokinetic properties in rats, leading to the discovery of **12x**. Compound **12x** was a high affinity CRF_1 receptor antagonist (IC_{50} = 1.0 nM) and was a potent inhibiter of CRF-stimulated cyclic adenosine monophosphate (cAMP) production in human Y-79 retinoblastoma cells (IC_{50} = 4.9 nM), indicating that it behaves as an antagonist. **12x** had low in vivo clearance (Cl = 17 mL/min/kg) in rats and good oral bioavailability (F = 52%) when dosed as a solution. In addition, **12x** was efficacious in the defensive withdrawal model of anxiety in rats. Further evaluation of **12x** will be reported in due course.

Table 5. Mean Total and Plasma Free Concentrations and CRF_1 Receptor Occupancies the Defensive Withdrawal Test for Anxiety in Rats Following Oral Doses of Selected Compounds

compd	oral dose (mg/kg)	mean total plasma conc (nM)[a,b]	mean plasma free conc (nM)[a,c]	CRF_1 receptor occupancy (%)[a,d,e]	% decrease in exit latency[b]
12l	10	2000 ± 400	6.7 ± 1.4	81 ± 10	68[g]
12s	10	120 ± 40	2.3 ± 0.8	11 ± 5	ND[f]
12t	10	250 ± 40	11 ± 2	34 ± 7	ND
15a	10	1400 ± 400	16 ± 5	68 ± 10	ND
15b	10	ND	ND	55 ± 6	61[g]
12x	10	790 ± 170	6.1 ± 1.3	83 ± 3	61[h]
12x	3	270 ± 100	2.1 ± 0.8	73 ± 8	46
12x	1	80 ± 27	0.62 ± 0.2	43 ± 6	8

[a]±SEM. [b]$n = 8$. [c]Based on an unbound fraction in plasma of 0.33%, 1.9%, 4.4%, 1.1%, 3.0%, and 0.77% for **12l**, **12s**, **12t**, **15a**, **15b**, and **12x**, respectively, as determined by equilibrium dialysis. [d]$n = 4$. [e]Receptor occupancy of **1** at 10 mg/kg = 71 ± 4. [f]ND = not determined. [g]$p < 0.01$. [h]$p \leq 0.05$ vs vehicle.

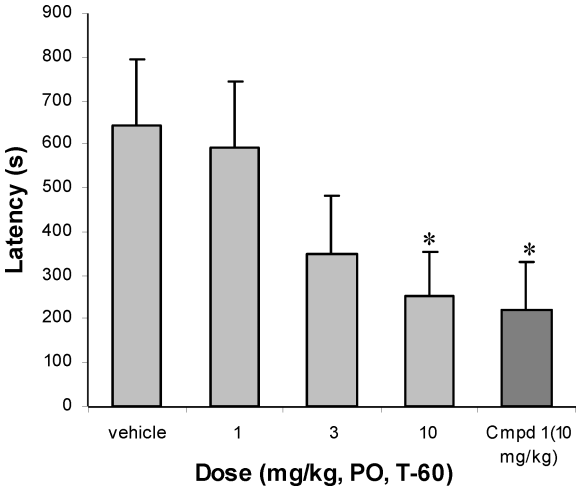


Figure 3. Anxiolytic-like effects of **12x** in the defensive withdrawal test in rats in rats at 1, 3, and 10 mg/kg with **1** as a positive control, *$p \leq 0.05$ vs vehicle.

Experimental Section

Chemistry. All procedures were carried out under a nitrogen atmosphere unless otherwise indicated using anhydrous solvents purchased from commercial sources without further purification. Reactions requiring anhydrous conditions were performed in glassware, which was flame-dried or oven-dried and placed under a nitrogen atmosphere. Column chromatography was performed on silica gel using the solvent systems indicated. Solvent systems are reported as v/v percent ratios. All reactions were monitored by TLC using EM Science, 0.25 mm, precoated silica gel plates or by LC/MS. Yields refer to chromatographically and spectroscopically pure compounds, except as otherwise indicated. Melting points were obtained on a Laboratory Devices, Inc. Mel Temp 3.0 melting point apparatus and are uncorrected. Proton NMR spectra were recorded on either a Varian (Palo Alto, CA) Inova 300, 400, or 500 MHz or Bruker 400 or 500 MHz NMR spectrometer. Chemical shifts are

reported in δ values relative to tetramethylsilane. Atmosphere pressure chemical ionization (APCI) low-resolution mass spectra were obtained on a Finnigan Navigator LC/MS single quadrupole mass spectrometer. Electrospray ionization (ESI) high-resolution mass spectra were obtained on a Finnigan MAT95S or Thermo Scientific MAT900 mass spectrometer. All final products had a purity of ≥95%. The purity of final products was determined by either combustion analysis or HPLC. Combustion analyses were performed by Quantitative Technologies, Inc., Whitehouse, NJ. HPLC purity was measured using two methods for each compound: method A, Phenominex analytical C18 column (4.6 mm × 50 mm, 5 μm); mobile phase: A = H_2O with 0.1% TFA, B = acetonitrile with 0.1% TFA, 0−1 min, 20% B, 1−7 min, 20% B → 95% B, 7−8 min, 95% B, flow rate = 3 mL/min, λ = 254 nm, run time = 8 min; method B: Phenominex analytical Synergi polar RP (phenoxy) column (4.6 mm × 50 mm, 4 μm); mobile phase: A = 90% H_2O/10% methanol with 0.1% TFA, B = 90% methanol/10% H_2O with 0.1% TFA, 0−4 min, 40% B → 100% B, 4−6 min, 100% B, flow rate = 4 mL/min, λ = 254 nm, run time = 6 min.

Procedures for the preparation of compounds **9**−**11**, **12a**, **12b**, **12c**, **12p**, **12s**, and **12ad** were previously described.[31]

General Methods for Coupling of the Dichloropyrazinones with Anilines (12). Method A: (*R*)-5-Chloro-1-(1-cyclopropylpropyl)-3-(2,6-dichloro-4-(trifluoromethoxy)phenylamino)pyrazin-2(1*H*)-one (12e). To a solution of (*R*)-3,5-dichloro-1-(1-cyclopropylpropyl) pyrazin-2(1*H*)-one (**10**) (100 mg, 0.405 mmol) and 2,6-dichloro-4-(trifluoromethoxy)aniline (100 mg, 0.405 mmol) in THF (4 mL) at 0 °C was added NaHMDS (0.850 mL, 0.850 mmol, 1 M in THF) slowly while maintaining the temperature of the reaction mixture below 10 °C. The reaction mixture was stirred at 0 °C for 2 h. The mixture was transferred to a separatory funnel containing saturated aqueous $NaHCO_3$ solution (5 mL) and the aqueous layer was extracted with ethyl acetate (3 × 10 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated. The residue was purified by column chromatography on silica gel (20 → 30% ethyl acetate in hexanes) to afford **12e** (120 mg, 65% yield) as a yellow solid; mp 134−134.5 °C; $[\alpha]^{25}_D$ −10.6 (*c* 0.422, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.79 (s, 1H), 7.33 (d, J = 0.7 Hz, 2H), 6.86 (s, 1H), 4.17−4.03 (m, 1H), 1.97−1.82 (m, 2H), 1.10−1.05 (m, 1H), 0.97 (t, J = 7.3 Hz, 3H), 0.86−0.79 (m, 1H), 0.59−0.52 (m, 2H), 0.37−0.31 (m, 1H). HRMS (ESI) *m*/*e* 456.0260 [$(M + H)^+$, calcd for $C_{17}H_{16}N_3O_2Cl_3F_3$ 456.0260]. Anal. ($C_{17}H_{15}N_3O_2Cl_3F_3$) C, H, N.

Method B: (*R*)-5-Chloro-1-(1-cyclopropylpropyl)-3-(2,6-dichloro-4-methoxyphenylamino)pyrazin-2(1*H*)-one (12d). To a solution of 2,6-dichloro-4-methoxyaniline (190 mg, 1 mmol) in DMF (2 mL) at 0 °C was added sodium hydride (60 mg, 1.5 mmol, 60% in mineral oil). After stirring for 20 min, (*R*)-3,5-dichloro-1-(1-cyclopropylpropyl)pyrazin-2(1*H*)-one (**10**) (246 mg, 1 mmol) dissolved in DMF (2 mL) was then added via cannula. The reaction mixture was warmed to room temperature and was then heated at 55 °C for 16 h. The reaction mixture was cooled to room temperature and was transferred to a separatory funnel containing ether (25 mL). The organic layer was washed with water (4 × 5 mL), brine (5 mL), dried over $MgSO_4$, filtered, and concentrated. The product was purified by column chromatography on silica gel (20% ethyl acetate in hexanes) to afford **12d** (180 mg, 45% yield) as a colorless solid: mp 171.5−172 °C; $[\alpha]^{25}_D$ −10.7 (*c* 0.294, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.64 (s, 1H), 6.98 (s, 2H), 6.81 (s, 1H), 4.10−4.06 (m, 1H), 3.83 (s, 3H), 1.96−1.83 (m, 2H), 1.11−1.04 (m, 1H), 0.97 (t, J = 7.7 Hz, 3H), 0.85−0.79 (m, 1H), 0.60−0.51 (m, 2H), 0.38−0.34 (m, 1H). HRMS (ESI) *m*/*e* 402.0553 [$(M + H)^+$, calcd for $C_{17}H_{19}N_3O_2Cl_3$ 402.0543]. Anal. ($C_{17}H_{18}N_3O_2Cl_3$) C, H, N. (Improved yields could be obtained using 2.2 equiv of NaH.)

Method C: (*S*)-5-Chloro-1-(1-cyclopropylethyl)-3-[2,6-dichloro-4-(difluoromethoxy)phenylamino]pyrazin-2(1*H*)-one (12x). To a dry 500 mL round-bottom flask under N_2 was added $Pd(OAc)_2$ (193 mg, 0.860 mmol), BINAP (536 mg, 0.860 mmol), and toluene (200 mL). After stirring for 1.5 h, (*S*)-3,5-dichloro-1-(1-cyclopropylethyl)pyrazin-2(1*H*)-one (**10**) (5.00 g, 22.0 mmol), 2,6-dichloro-4-(difluoromethoxy)aniline (**19**) (5.00 g, 21.5 mmol), and potassium carbonate (20.0 g, 145 mmol) were added. The reaction mixture was heated at reflux for 18 h. The reaction mixture was cooled to room temperature and was filtered through a pad of celite with ethyl acetate rinsing. The reaction mixture was transferred to a separatory funnel containing saturated aqueous $NaHCO_3$ solution (200 mL). The aqueous layer was extracted with ethyl acetate (3 × 200 mL). The combined organic layers were washed with brine (100 mL), dried over $MgSO_4$, filtered, and concentrated. The residue was purified by column chromatography (20% ethyl acetate in hexanes) to give 8.0 g of product, which was subsequently recrystallized from EtOH (40 mL) to furnish **12x** (7.0 g, 77% yield) as a pink solid; mp 164−165 °C; $[\alpha]^{25}_D$ +20.2 (*c* 0.353, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69 (s, 1H), 7.21 (s, 2H), 6.90 (s, 1H), 6.52 (t, J = 72.6 Hz, 1H), 4.31−4.25 (m, 1H), 1.44 (d, J = 6.7 Hz, 3H), 1.12−1.04 (m, 1H), 0.79−0.73 (m, 1H), 0.61−0.54 (m, 1H), 0.51−0.45 (m, 1H), 0.41−0.35 (m, 1H). HRMS (ESI) *m*/*e* 424.0181 [$(M + H)^+$, calcd for $C_{16}H_{15}N_3O_2Cl_3F_2$ 424.0198]. Anal. ($C_{16}H_{14}N_3O_2Cl_3F_2$) C, H, N.

(*R*)-5-Chloro-3-[2,6-dichloro-4-(trifluoromethyl)phenylamino]-1-[1-(methoxymethyl)propyl]pyrazin-2(1*H*)-one (12f). Compound **12f** was prepared according to the procedure described for the synthesis of **12d** (method B) using 4-(trifluoromethyl) aniline (115 mg, 0.500 mmol) and (*R*)-3,5-dichloro-1-(1-methoxybutan-2-yl)pyrazin-2(1*H*)-one (**10**) (125 mg, 0.500 mmol). The product was purified by column chromatography to afford **12f** (60 mg, 27% yield) as a colorless solid; mp 116−116.6 °C; $[\alpha]^{25}_D$ +44.3 (*c* 0.312, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.89 (s, 1H), 7.68 (s, 2H), 6.95 (s, 1H), 5.03−4.96 (m, 1H), 3.70 (dd, J_{AB} = 10.7, J_{AX} = 5.5 Hz, 1H), 3.61 (dd, J_{BA} = 10.7, J_{BX} = 3.3 Hz, 1H), 3.39 (s, 3H), 1.95−1.77 (m, 2H), 0.97 (t, J = 7.3 Hz, 3H). HRMS (ESI) *m*/*e* 444.0277 [$(M + H)^+$, calcd for $C_{16}H_{16}N_3O_2Cl_3F_3$ 444.0260]. HPLC method A: t_R = 5.95 min, > 99%; method B: t_R = 3.56 min, 99.3%.

(*R*)-5-Chloro-3-[2,6-dichloro-4-(trifluoromethyl)phenylamino]-1-(2-methoxy-1-methylethyl)pyrazin-2(1*H*)-one (12g). Compound **12g** was prepared according to the procedure described for the synthesis of **12d** (method B) using 4-(trifluoromethyl) aniline (230 mg, 1.00 mmol) and (*R*)-3,5-dichloro-1-(1-methoxypropan-2-yl)pyrazin-2(1*H*)-one (**10**) (236 mg, 1.00 mmol). The product was purified by column chromatography to afford **12g** (178 mg, 41% yield) as a pale-yellow solid; mp 64.5−65.5 °C; $[\alpha]^{25}_D$ +44.0 (*c* 0.314, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.89 (s, 1H), 7.68 (s, 2H), 6.95 (s, 1H), 5.24−5.18 (m, 1H), 3.64−3.61 (m, 2H), 3.39 (s, 3H), 1.46 (d, J = 7.0 Hz, 3H). HRMS (ESI) *m*/*e* 430.0088 [$(M + H)^+$, calcd for $C_{15}H_{14}N_3O_2Cl_3F_3$ 430.0104]. Anal. ($C_{15}H_{13}N_3O_2Cl_3F_3$) C, H, N.

(*R*)-3,5-Dichloro-4-{6-chloro-4-[1-(methoxymethyl)propyl]-3-*oxo*-3,4-dihydropyrazin-2-ylamino}benzonitrile (12 h). Compound **12h** was prepared according to the procedure described for the synthesis of **12d** (method B) using 4-amino-3,5-dichlorobenzonitrile (93 mg, 0.500 mmol) and (*R*)-3,5-dichloro-1-(1-methoxybutan-2-yl)pyrazin-2(1*H*)-one (**10**) (125 mg, 0.500 mmol). The product was purified by column chromatography to afford **12h** (51 mg, 26% yield) as a colorless solid; mp 182−183 °C; $[\alpha]^{25}_D$ +52.3 (*c* 0.286, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.94 (s, 1H), 7.72 (s, 2H), 6.98 (s, 1H), 5.03−4.96 (m, 1H), 3.70 (dd, J_{AB} = 10.6, J_{AX} = 5.5 Hz, 1H), 3.60 (dd, J_{BA} = 10.7, J_{BX} = 3.3 Hz, 1H), 3.38 (s, 3H), 1.95−1.60 (m, 2H), 0.97 (t, J = 7.3 Hz, 3H). HRMS (ESI) *m*/*e* 401.0333 [$(M + H)^+$, calcd for $C_{16}H_{16}N_4O_2Cl_3$ 401.0339]. Anal. ($C_{16}H_{15}N_4O_2Cl_3$) C, H, N.

(*R*)-3,5-Dichloro-4-[6-chloro-4-(2-methoxy-1-methylethyl)-3-*oxo*-3,4-dihydropyrazin-2-ylamino]benzonitrile (12i). Compound **12i** was prepared according to the procedure described for the synthesis of **12d** (method B) using 4-amino-3,5-dichlorobenzo-

nitrile (47 mg, 0.253 mmol) and (*R*)-3,5-dichloro-1-(1-methoxypropan-2-yl)pyrazin-2(1*H*)-one (**10**) (60 mg, 0.253 mmol). The product was purified by column chromatography to afford **12i** (60 mg, 61% yield) as a colorless solid. The product was purified further by recrystallization from ethyl acetate to afford **12i** as a colorless crystalline solid; mp 195−196 °C; $[\alpha]^{25}_{D}$ +47.8 (*c* 0.288, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.94 (s, 1H), 7.71 (s, 2H), 6.98 (s, 1H), 5.23−5.17 (m, 1H), 3.62 (d, J = 5.1 Hz, 2H), 3.39 (s, 3H), 1.46 (d, J = 7.0 Hz, 3H). HRMS (ESI) *m*/*e* 387.0193 $[(M+H)^+$, calcd for $C_{15}H_{14}N_4O_2Cl_3$ 387.0182]. HPLC method A: t_R = 4.71 min, 99.3%; method B: t_R = 3.19 min, > 99%.

(*R*)-5-Chloro-1-(1-cyclopropylpropyl)-3-(2,6-dichloro-4-(trifluoromethyl)phenylamino)pyrazin-2(1*H*)-one (12j). Compound **12j** was prepared according to the procedure described for the synthesis of **12d** (method B) using 2,6-dichloro-4-(trifluoromethyl)aniline (296 mg, 1.2 mmol) and (*R*)-3,5-dichloro-1-(1-cyclopropylpropyl)pyrazin-2(1*H*)-one (**10**) (246 mg, 1 mmol). The product was purified by column chromatography to afford **12j** (110 mg, 25% yield) as a colorless solid; mp 155.5−156 °C; $[\alpha]^{25}_{D}$ −10.2 (*c* 0.314, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.91 (s, 1H), 7.69 (s, 2H), 6.90 (s, 1H), 4.13−4.04 (m, 1H), 1.98−1.82 (m, 2H), 1.14−1.05 (m, 1H), 0.98 (t, J = 7.7 Hz, 3H), 0.87−0.80 (m, 1H), 0.60−0.53 (m, 2H), 0.37−0.33 (m, 1H). HRMS (ESI) *m*/*e* 440.0310 $[(M+H)^+$, calcd for $C_{17}H_{16}N_3OCl_3F_3$ 440.0311]. Anal. ($C_{17}H_{15}N_3OCl_3F_3$) C, H, N.

5-Chloro-3-[2,6-dichloro-4-(trifluoromethyl)phenylamino]-1-(1-propylbutyl)pyrazin-2(1*H*)-one (12k). Compound **12k** was prepared according to the procedure described for the synthesis of **12d** (method B) using 2,6-dichloro-4-(trifluoromethyl)aniline (230 mg, 1.00 mmol) and 3,5-dichloro-1-(heptan-4-yl)pyrazin-2(1*H*)-one (**10**) (263 mg, 1.00 mmol). The product was purified by column chromatography to afford **12k** (165 mg, 36% yield) as a colorless solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.93 (s, 1H), 7.69 (s, 2H), 6.69 (s, 1H), 5.03−4.96 (m, 1H), 1.77−1.60 (m, 4H), 1.37−1.22 (m, 4H), 0.95 (t, J = 7.3 Hz, 6H). HRMS (ESI) *m*/*e* 456.0619 $[(M+H)^+$, calcd for $C_{18}H_{20}N_3OCl_3F_3$ 456.0642]. Anal. ($C_{18}H_{19}N_3OCl_3F_3$) C, H, N; calcd C, 47.33; found C, 46.83.

(*R*)-5-Chloro-1-(1-cyclopropylethyl)-3-[2,6-dichloro-4-(trifluoromethyl)phenylamino]pyrazin-2(1*H*)-one (12l). Compound **12l** was prepared according to the procedure described for the synthesis of **12d** (method B) using 2,6-dichloro-4-(trifluoromethyl)aniline (172 mg, 0.750 mmol) and (*R*)-3,5-dichloro-1-(1-cyclopropylethyl)pyrazin-2(1*H*)-one (**10**) (174 mg, 0.750 mmol). The product was purified by column chromatography to afford **12l** (149 mg, 47% yield) as a colorless solid. The product was purified further by recrystallization from hexane/ethyl acetate to afford **12l** as a colorless crystalline solid; mp 137.5−138.8 °C; $[\alpha]^{25}_{D}$ −18.6 (*c* 0.258, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.90 (s, 1H), 7.69 (s, 2H), 6.98 (s, 1H), 4.35−4.25 (m, 1H), 1.50 (d, J = 6.9 Hz, 3H), 1.17−1.07 (m, 1H), 0.85−0.76 (m, 1H), 0.66−0.57 (m, 1H), 0.56−0.47 (m, 1H), 0.45−0.36 (m, 1H). HRMS (ESI) *m*/*e* 426.0153 $[(M+H)^+$, calcd for $C_{16}H_{14}N_3OCl_3F_3$ 426.0155]. Anal. ($C_{16}H_{13}N_3OCl_3F_3$) C, H, N.

(*R*)-5-Chloro-1-(1-cyclopropyl-2-methoxyethyl)-3-[2,6-dichloro-4-(trifluoromethyl)phenylamino]pyrazin-2(1*H*)-one (12m). Compound **12m** was prepared according to the procedure described for the synthesis of **12e** (method A) using (*R*)-3,5-dichloro-1-(1-cyclopropyl-2-methoxyethyl)pyrazin-2(1*H*)-one (**10**) (100 mg, 0.380 mmol) and 2,6-dichloro-4-(trifluoromethyl)aniline (90 mg, 0.380 mmol) with DMF (1 mL) as the solvent. The product was purified by column chromatography to afford **12m** (30 mg, 17% yield) as a fluffy colorless solid: $[\alpha]^{25}_{D}$ +58.4 (*c* 0.311, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82 (s, 1H), 7.66 (s, 2H), 7.06 (s, 1H), 4.21−4.10 (m, 1H), 3.76 (dd, J_{AB} = 10.5, J_{AX} = 5.4 Hz, 1H), 3.69 (dd, J_{BA} = 10.5, J_{BX} = 3.4 Hz, 1H), 3.37 (s, 3H), 1.37−1.31 (m, 1H), 0.87−0.76 (m, 1H), 0.67−0.60 (m, 1H), 0.55−0.49 (m, 1H), 0.41−0.34 (m, 1H). HRMS (ESI) *m*/*e* 456.0276 $[(M+H)^+$, calcd for $C_{17}H_{16}N_3O_2Cl_3F_3$ 456.0260]. HPLC method A: t_R = 6.05 min, 99.4%; method B: t_R = 3.61 min, 99.1%

5-Chloro-3-[2,6-dichloro-4-(trifluoromethyl)phenylamino]-1-[2-methoxy-1-(methoxymethyl)ethyl]pyrazin-2(1*H*)-one (12n). Compound **12n** was prepared according to the procedure described for the synthesis of **12d** (method B) using 2,6-dichloro-4-(trifluoromethyl)aniline (230 mg, 1.00 mmol) and 3,5-dichloro-1-(1,3-dimethoxypropan-2-yl)pyrazin-2(1*H*)-one (**10**) (267 mg, 1.00 mmol). The product was purified by column chromatography to afford **12n** (178 mg, 39% yield) as a colorless solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.88 (s, 1H), 7.68 (s, 2H), 7.08 (s, 1H), 5.28−5.22 (m, 1H), 3.80 (dd, J_{AB} = 10.6, J_{AX} = 6.3 Hz, 2H), 3.72 (dd, J_{BA} = 10.6, J_{BX} = 4.7 Hz, 2H), 3.40 (s, 6H). HRMS (ESI) *m*/*e* 460.0209 $[(M+H)^+$, calcd for $C_{16}H_{16}N_3O_3Cl_3F_3$ 460.0209]. HPLC method A: t_R = 5.62 min, > 99%; method B: t_R = 3.48 min, 99.3%.

(*R*)-5-Chloro-1-(1-cyclopropylethyl)-3-(2,6-dichloro-4-methoxyphenylamino)pyrazin-2(1*H*)-one (12o). Compound **12o** was prepared according to the procedure described for the synthesis of **12d** (method B) using 2,6-dichloro-4-methoxyaniline (190 mg, 1.00 mmol) and (*R*)-3,5-dichloro-1-(1-cyclopropylethyl)pyrazin-2(1*H*)-one (**10**) (233 mg, 1.00 mmol). The product was purified by column chromatography to afford **12o** (170 mg, 44% yield) as a colorless solid; mp 161.5−162 °C; $[\alpha]^{25}_{D}$ −19.8 (*c* 0.270, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.64 (s, 1H), 6.97 (s, 2H), 6.89 (s, 1H), 4.32−4.27 (m, 1H), 3.83 (s, 3H), 1.46 (d, J = 6.6 Hz, 3H), 1.17−1.05 (m, 1H), 0.81−0.75 (m, 1H), 0.64−0.58 (m, 1H), 0.53−0.45 (m, 1H), 0.43−0.39 (m, 1H). HRMS (ESI) *m*/*e* 388.0361 $[(M+H)^+$, calcd for $C_{16}H_{17}N_3O_2Cl_3$ 388.0386]. Anal. ($C_{16}H_{16}N_3O_2Cl_3$) C, H, N.

(*R*)-5-Chloro-3-(2-chloro-4,6-dimethylphenylamino)-1-(1-cyclopropylethyl)pyrazin-2(1*H*)-one (12q). Compound **12q** was prepared according to the procedure described for the synthesis of **12e** (method A) using (*R*)-3,5-dichloro-1-(1-cyclopropylethyl)pyrazin-2(1*H*)-one (**10**) (233 mg, 1.00 mmol) and 2-chloro-4,6-dimethylaniline (186 mg, 1.20 mmol). The product was purified by column chromatography to afford **12q** (188 mg, 53% yield) as a colorless solid; mp 164.5−165 °C; $[\alpha]^{25}_{D}$ −18.3 (*c* 0.258, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.77 (s, 1H), 7.14 (s, 1H), 7.01 (s, 1H), 6.85 (s, 1H), 4.35−4.25 (m, 1H), 2.33 (s, 3H), 2.27 (s, 3H), 1.46 (d, J = 9.5 Hz, 3H), 1.15−1.07 (m, 1H), 0.82−0.73 (m, 1H), 0.64−0.45 (m, 1H), 0.44−0.38 (m, 1H). HRMS (ESI) *m*/*e* 352.1006 $[(M+H)^+$, calcd for $C_{17}H_{20}N_3OCl_2$ 352.0983]. Anal. ($C_{17}H_{19}N_3OCl_2$) C, H, N.

(*R*)-5-Chloro-1-(1-cyclopropylethyl)-3-(2,4,6-trichlorophenylamino)pyrazin-2(1*H*)-one (12r). Compound **12r** was prepared according to the procedure described for the synthesis of **12e** (method A) using (*R*)-3,5-dichloro-1-(1-cyclopropylethyl)pyrazin-2(1*H*)-one (**10**) (100 mg, 0.430 mmol) and 2,4,6-trichloroaniline (85 mg, 0.430 mmol) with DMF (1 mL) as the solvent. The product was purified by column chromatography to afford **12r** (100 mg, 60% yield) as a tan solid; mp 237−237.5 °C; $[\alpha]^{25}_{D}$ −25.6 (*c* 0.164, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 (s, 1H), 7.41 (s, 2H), 6.91 (s, 1H), 4.30−4.22 (m, 1H), 1.44 (d, J = 6.6 Hz, 3H), 1.11−1.05 (m, 1H), 0.79−0.72 (m, 1H), 0.61−0.54 (m, 1H), 0.51−0.44 (m, 1H), 0.40−0.34 (m, 1H). HRMS (ESI) *m*/*e* 435.9385 $[(M+H)^+$ calcd for $C_{15}H_{14}N_3OCl_3Br$ 435.9386]. HPLC method A: t_R = 5.97 min, 98.9%; method B: t_R = 3.76 min, 99.1%.

(*R*)-3-Chloro-4-[6-chloro-4-(1-cyclopropylethyl)-3-*oxo*-3,4-dihydropyrazin-2-ylamino]-5-methylbenzonitrile (12t). Compound **12t** was prepared according to the procedure described for the synthesis of **12d** (method B) using 4-amino-3-chloro-5-methylbenzonitrile (190 mg, 1.00 mmol) and (*R*)-3,5-dichloro-1-(1-cyclopropylethyl)pyrazin-2(1*H*)-one (**10**) (233 mg, 1.00 mmol). The product was purified by recrystallization from hexanes/ethyl acetate to afford **12t** (115 mg, 32% yield) as a colorless crystalline solid; mp 207−208 °C; $[\alpha]^{25}_{D}$ −26.4 (*c* 0.246, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.90 (s, 1H), 7.56 (s, 1H), 7.45

(s, 1H), 6.89 (s, 1H), 4.25−4.20 (m, 1H), 2.30 (s, 3H), 1.40 (d, J = 5.9 Hz, 3H), 1.12−1.00 (m, 1H), 0.78−0.68 (m, 1H), 0.60−0.51 (m, 1H), 0.49−0.40 (m, 1H), 0.39−0.28 (m, 1H). HRMS (ESI) m/e 363.0778 [(M + H)$^+$, calcd for $C_{17}H_{17}N_4OCl_2$ 363.0779]. HPLC method A: t_R = 5.28 min, 99.7%; method B: t_R = 3.48 min, > 99%.

(*R*)-5-Chloro-1-(1-cyclopropylethyl)-3-[2,6-dichloro-4-(trifluoromethoxy)phenylamino]pyrazin-2(1*H*)-one (12u). Compound **12u** was prepared according to the procedure described for the synthesis of **12d** (method B) using 2,6-dichloro-4-(trifluoromethoxy)aniline (295 mg, 1.20 mmol) and (*R*)-3,5-dichloro-1-(1-cyclopropylethyl)pyrazin-2(1*H*)-one (**10**) (233 mg, 1.00 mmol). The product was purified by column chromatography to afford **12u** (182 mg, 41% yield) as a colorless solid; mp 125−125.5 °C; $[\alpha]^{25}_D$ −16.1 (c 0.304, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.76 (s, 1H), 7.33 (s, 2H), 6.95 (s, 1H), 4.34−4.24 (m, 1H), 1.47 (d, J = 6.6 Hz, 3H), 1.16−1.07 (m, 1H), 0.82−0.75 (m, 1H), 0.66−0.58 (m, 1H), 0.57−0.47 (m, 1H), 0.45−0.38 (m, 1H). HRMS (ESI) m/e 442.0104 [(M + H)$^+$, calcd for $C_{16}H_{14}N_3O_2Cl_3F_3$ 442.0104]. Anal. ($C_{16}H_{13}N_3O_2Cl_3F_3$) C, H, N.

(*S*)-5-Chloro-1-(1-cyclopropylethyl)-3-[2,6-dichloro-4-(trifluoromethyl)phenylamino]pyrazin-2(1*H*)-one (12v). Compound **12v** was prepared according to the procedure described for the synthesis of **12d** (method B) using 2,6-dichloro-4-(trifluoromethoxy)aniline (172 mg, 0.75 mmol) and (*S*)-3,5-dichloro-1-(1-cyclopropylethyl)pyrazin-2(1*H*)-one (**10**) (174 mg, 0.75 mmol). The product was purified by column chromatography to afford **12v** (149 mg, 47% yield) as a colorless solid; $[\alpha]^{25}_D$ + 18.1 (c 0.306, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.90 (s, 1H), 7.69 (s, 2H), 6.98 (s, 1H), 4.35−4.25 (m, 1H), 1.50 (d, J = 6.9 Hz, 3H), 1.17−1.07 (m, 1H), 0.85−0.76 (m, 1H), 0.66−0.57 (m, 1H), 0.56−0.48 (m, 1H), 0.45−0.37 (m, 1H). HRMS (ESI) m/e 426.0161 [(M + H)$^+$, calcd for $C_{16}H_{14}N_3OCl_3F_3$ 426.0155]. Anal. ($C_{16}H_{13}N_3OCl_3F_3$) C, H, N.

(*R*)-5-Chloro-1-(1-cyclopropylethyl)-3-[2,6-dichloro-4-(difluoromethoxy)phenylamino]pyrazin-2(1*H*)-one (12w). Compound **12w** was prepared according to the procedure described for the synthesis of **12e** (method A) using (*R*)-3,5-dichloro-1-(1-cyclopropylethyl)pyrazin-2(1*H*)-one (**10**) (124 mg, 0.535 mmol) and 2,6-dichloro-4-(difluoromethoxy)aniline (**19**) (122 mg, 0.535 mmol). The product was purified by column chromatography to afford **12w** (140 mg, 62% yield) as a pale-yellow solid; mp 163−164 °C; $[\alpha]^{25}_D$ −12.3 (c 0.290, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69 (s, 1H), 7.21 (s, 2H), 6.91 (s, 1H), 6.52 (t, J = 72.6 Hz, 1H), 4.30−4.23 (m, 1H), 1.44 (d, J = 6.9 Hz, 3H), 1.12 (m, 1H), 0.80−0.73 (m, 1H), 0.61−0.55 (m, 1H), 0.51−0.45 (m, 1H), 0.41−0.35 (m, 1H). HRMS (ESI) m/e 424.0205 [(M + H)$^+$, calcd for $C_{16}H_{15}N_3O_2Cl_3F_2$ 424.0198]. Anal. ($C_{16}H_{14}N_3O_2Cl_3F_2$) C, H, N.

(*R*)-5-Chloro-1-(1-cyclopropylethyl)-3-(2,6-dichloro-4 (methylsulfonyl)phenylamino)pyrazin-2(1*H*)-one (12y). Compound **12y** was prepared according to the procedure described for the synthesis of **12x** (method C) using (*R*)-3,5-dichloro-1-(1-cyclopropylethyl)pyrazin-2(1*H*)-one (**10**) (150 mg, 0.644 mmol) and 2,6-dichloro-4-(methylsulfonyl)aniline (155 mg, 0.644 mmol). The residue was purified by column chromatography to give **12y** (181 mg, 64% yield) as a colorless solid; $[\alpha]^{25}_D$ −25.2 (c 0.529, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99 (s, 2H), 7.28 (s, 1H), 7.02 (s, 1H), 4.41−4.22 (m, 1H), 3.15 (s, 3H), 1.49 (d, J = 6.8 Hz, 3H), 1.19−1.08 (m, 1H), 0.86−0.76 (m, 1H), 0.67−0.58 (m, 1H), 0.57−0.48 (m, 1H), 0.46−0.35 (m, 1H). HRMS (ESI) m/e 436.0052 [(M + H)$^+$, calcd for $C_{16}H_{17}N_3O_3Cl_3S$ 436.0056]. Anal. ($C_{16}H_{16}N_3O_3Cl_3S$) C, H, N.

(*R*)-5-Chloro-1-(1-cyclopropylethyl)-3-(2,4-dichloro-6-methylphenylamino)pyrazin-2(1*H*)-one (12z). Compound **12z** was prepared according to the procedure described for the synthesis of **12e** (method A) using (*R*)-3,5-dichloro-1-(1-cyclopropylethyl) pyrazin-2(1*H*)-one (**10**) (150 mg, 0.644 mmol) and 2,4-dichloro-6-methylaniline (125 mg, 0.709 mmol). The product was purified by column chromatography to afford **12z** (59 mg, 24% yield) as a colorless solid; mp 165−166 °C; $[\alpha]^{25}_D$ −20.5 (c 0.288, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.48 (s, 1H), 7.32 (d, J = 2.2 Hz, 1H), 7.19 (d, J = 1.9 Hz, 1H), 6.88 (s, 1H), 4.32−4.22 (m, 1H), 2.27 (s, 3H), 1.44 (d, J = 6.6 Hz, 3H), 1.16−1.05 (m, 1H), 0.82−0.73 (m, 1H), 0.63−0.54 (m, 1H), 0.51−0.44 (m, 1H), 0.42−0.36 (m, 1H). HRMS (ESI) m/e 372.0429 [(M + H)$^+$, calcd for $C_{16}H_{17}N_3OCl_3$ 372.0437]. HPLC method A: t_R = 5.98 min, > 99%; method B: t_R = 3.74 min, > 99%.

(*R*)-3,5-Dichloro-2-[6-chloro-4-(1-cyclopropylethyl)-3-*oxo*-3,4-dihydropyrazin-2-ylamino]benzonitrile (12aa). Compound **12aa** was prepared according to the procedure described for the synthesis of **12e** (method A) using (*R*)-3,5-dichloro-1-(1-cyclopropylethyl)pyrazin-2(1*H*)-one (**10**) (150 mg, 0.644 mmol) and 2-amino-3,5-dichlorobenzonitrile (120 mg, 0.644 mmol). The product was purified by column chromatography to afford **12aa** (60 mg, 24% yield) as a pale-yellow solid; mp 183.4−184 °C; $[\alpha]^{25}_D$ −21.5 (c 0.514, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 (s, 1H), 7.68 (d, J = 2.3 Hz, 1H), 7.61 (d, J = 2.3 Hz, 1H), 6.99 (s, 1H), 4.29−4.21 (m, 1H), 1.44 (d, J = 6.8 Hz, 3H), 1.12−1.05 (m, 1H), 0.80−0.73 (m, 1H), 0.62−0.55 (m, 1H), 0.51−0.44 (m, 1H), 0.41−0.34 (m, 1H). HRMS (ESI) m/e 383.0244 [(M + H)$^+$, calcd for $C_{16}H_{14}N_4OCl_3$ 383.0233]. Anal. ($C_{16}H_{13}N_4OCl_3$) C, H, N.

(*R*)-5-Chloro-3-(4-chloro-2-methoxy-5-methylphenylamino)-1-(1-cyclopropylethyl)pyrazin-2(1*H*)-one (12ab). Compound **12ab** was prepared according to the procedure described for the synthesis of **12e** (method A) using (*R*)-3,5-dichloro-1-(1-cyclopropylethyl)pyrazin-2(1*H*)-one (**10**) (60 mg, 0.260 mmol) and 4-chloro-2-methoxy-5-methylaniline (45 mg, 0.260 mmol) with DMF (1 mL) as the solvent. The product was purified by column chromatography to afford **12ab** (70 mg, 74% yield) as a brown solid; $[\alpha]^{25}_D$ −8.3 (c 0.400, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.89 (s, 1H), 8.52 (s, 1H), 6.86 (s, 2H), 4.24−4.20 (m, 1H), 3.88 (s, 3H), 2.36 (s, 3H), 1.42 (d, J = 6.8 Hz, 3H), 1.12−1.05 (m, 1H), 0.77−0.71 (m, 1H), 0.58−0.51 (m, 1H), 0.48−0.42 (m, 1H), 0.36−0.31 (m, 1H). HRMS (ESI) m/e 368.0935 [(M + H)$^+$, calcd for $C_{17}H_{20}N_3O_2Cl_2$ 368.0933]. HPLC method A: t_R = 7.07 min, 97.2%; method B: t_R = 4.35 min, 97.8%.

(*R*)-5-Chloro-1-(1-cyclopropylethyl)-3-(2,4,5-trimethylphenylamino)pyrazin-2(1*H*)-one (12ac). Compound **12ac** was prepared according to the procedure described for the synthesis of **12e** (method A) using (*R*)-3,5-dichloro-1-(1-cyclopropylethyl)pyrazin-2(1*H*)-one (**10**) (70 mg, 0.300 mmol) and 2,4,5-trimethylaniline (41 mg, 0.300 mmol) with DMF (1 mL) as the solvent. The product was purified by column chromatography to afford **12ac** (41 mg, 41% yield) as a light-brown solid; $[\alpha]^{25}_D$ −18.3 (c 0.371, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.16 (s, 1H), 8.02 (s, 1H), 6.95 (s, 1H), 6.82 (s, 1H), 4.27−4.19 (m, 1H), 2.27 (s, 6H), 2.20 (s, 3H), 1.42 (d, J = 6.8 Hz, 3H), 1.12−1.04 (m, 1H), 0.77−0.71 (m, 1H), 0.58−0.54 (m, 1H), 0.51−0.42 (m, 1H), 0.37−0.31 (m, 1H). HRMS (ESI) m/e 332.1529 [(M + H)$^+$, calcd for $C_{18}H_{23}N_3OCl$ 332.1530]. Anal. ($C_{18}H_{22}N_3OCl$) C, H, N.

(*R*)-5-Chloro-1-(1-cyclopropylethyl)-3-(4-ethoxy-2,5-dimethylphenylamino)pyrazin-2(1*H*)-one (12ae). Compound **12ae** was prepared according to the procedure described for the synthesis of **12e** (method A) using (*R*)-3,5-dichloro-1-(1-cyclopropylethyl)pyrazin-2(1*H*)-one (**10**) (60 mg, 0.260 mmol) and 4-ethoxy-2,5-dimethylaniline (42 mg, 0.260 mmol) with DMF (1 mL) as the solvent. The product was purified by column chromatography to afford **12ae** (60 mg, 65% yield) as a tan solid; $[\alpha]^{25}_D$ −12.7 (c 0.521, benzene). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (s, 1H), 7.85 (s, 1H), 6.80 (s, 1H), 6.65 (s, 1H), 4.27−4.20 (m, 1H), 4.00 (q, J = 7.1 Hz, 2H), 2.28 (s, 3H), 2.22 (s, 3H), 1.43 (d, J = 6.8 Hz, 3H), 1.40 (t, J = 6.9 Hz, 3H), 1.13−1.04 (m, 1H), 0.78−0.71 (m, 1H), 0.59−0.52 (m, 1H), 0.49−0.43 (m, 1H), 0.37−0.31 (m, 1H). HRMS (ESI) m/e 362.1640 [(M + H)$^+$, calcd for $C_{19}H_{25}N_3O_2Cl$ 362.1635]. Anal. ($C_{19}H_{24}N_3O_2Cl$) C, H, N.

(*R*)-5-Chloro-1-(1-cyclopropylethyl)-3-(4,5-dimethoxy-2-methylphenylamino)pyrazin-2(1*H*)-one (12af). Compound **12af** was prepared according to the procedure described for the synthesis of **12e** (method A) using (*R*)-3,5-dichloro-1-(1-cyclopropylethyl)pyrazin-2(1*H*)-one (**10**) (70 mg, 0.300 mmol) and 4,5-dimethoxy-2-methylaniline (50 mg, 0.300 mmol) with DMF (1 mL) as the solvent. The product was purified by column chromatography to afford **12af** (15 mg, 14% yield) as a pale-yellow solid; $[\alpha]^{25}_D$ −20.5 (*c* 0.271, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (s, 1H), 8.06 (s, 1H), 6.83 (s, 1H), 6.70 (s, 1H), 4.25−4.21 (m, 1H), 3.90 (s, 3H), 3.85 (s, 3H), 2.28 (s, 3H), 1.42 (d, J = 6.8 Hz, 3H), 1.11−1.05 (m, 1H), 0.76−0.71 (m, 1H), 0.57−0.51 (m, 1H), 0.48−0.43 (m, 1H), 0.36−0.31 (m, 1H). HRMS (ESI) *m*/*e* 364.1444 [(M + H)$^+$, calcd for $C_{18}H_{23}N_3O_3Cl$ 364.1428]. HPLC method A: t_R = 5.16 min, >99%; method B: t_R = 3.52 min, >99%.

(*R*)-5-Chloro-3-(2-chloro-5-fluoro-4-methoxyphenylamino)-1-(1-cyclopropylethyl)pyrazin-2(1*H*)-one (12ag). Compound **12ag** was prepared according to the procedure described for the synthesis of **12d** (method B) using 2-chloro-5-fluoro-4-methoxyaniline (87 mg, 0.500 mmol) and (*R*)-3,5-dichloro-1-(1-cyclopropylethyl)pyrazin-2(1*H*)-one (**10**) (116 mg, 0.500 mmol). The product was purified by column chromatography to afford **12ag** (63 mg, 34% yield) as an off-white solid; mp 147−147.5 °C; $[\alpha]^{25}_D$ −14.3 (*c* 0.226, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.84 (s, 1H), 8.63 (d, J = 13.6 Hz, 1H), 7.04 (d, J = 8.4 Hz, 1H), 6.94 (s, 1H), 4.28−4.23 (m, 1H), 3.90 (s, 3H), 1.46 (d, J = 6.9 Hz, 3H), 1.18−1.08 (m, 1H), 0.82−0.75 (m, 1H), 0.65−0.56 (m, 1H), 0.54−0.47 (m, 1H), 0.41−0.34 (m, 1H). HRMS (ESI) *m*/*e* 372.0672 [(M + H)$^+$, calcd for $C_{16}H_{17}N_3O_2Cl_2F$ 372.0682]. Anal. ($C_{16}H_{16}N_3O_2Cl_2F$) C, H, N.

(*R*)-4-Chloro-5-[6-chloro-4-(1-cyclopropylethyl)-3-*oxo*-3,4-dihydropyrazin-2-ylamino]-2-methylbenzonitrile (12ah). Compound **12ah** was prepared according to the procedure described for the synthesis of **12d** (method B) using 5-amino-4-chloro-2-methylbenzonitrile (**26**) (166 mg, 1.00 mmol) and (*R*)-3,5-dichloro-1-(1-cyclopropylethyl)pyrazin-2(1*H*)-one (**10**) (233 mg, 1.00 mmol). The product was purified by column chromatography followed by recrystallization from hexanes/ethyl acetate to afford **12ah** (99 mg, 27% yield) as a colorless solid; mp 168−168.5 °C; $[\alpha]^{25}_D$ −28.0 (*c* 0.298, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.04 (s, 1H), 8.90 (s, 1H), 7.40 (s, 1H), 7.00 (s, 1H), 4.31−4.21 (m, 1H), 2.53 (s, 3H), 1.47 (d, J = 6.6 Hz, 3H), 1.17−1.09 (m, 1H), 0.85−0.76 (m, 1H), 0.64−0.55 (m, 1H), 0.54−0.46 (m, 1H), 0.40−0.34 (m, 1H). HRMS (ESI) *m*/*e* 363.0775 [(M + H)$^+$, calcd for $C_{17}H_{17}N_4OCl_2$ 363.0779]. Anal. ($C_{17}H_{16}N_4OCl_2$) C, H, N.

(*R*)-5-Chloro-3-(6-chloro-2,3-dihydrobenzofuran-5-ylamino)-1-(1-cyclopropylethyl)pyrazin-2(1*H*)-one (12ai). Compound **12ai** was prepared according to the procedure described for the synthesis of **12e** (method A) using (*R*)-3,5-dichloro-1-(1-cyclopropylethyl)pyrazin-2(1*H*)-one (**10**) (55 mg, 0.237 mmol) and 6-chloro-2,3-dihydrobenzofuran-5-amine (**24**) (40 mg, 0.237 mmol) with DMF (1.2 mL) as the solvent. The product was purified by column chromatography to afford **12ai** (47 mg, 54% yield) as a colorless solid; mp 172−173 °C; $[\alpha]^{25}_D$ −8.8 (*c* 0.351, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.89 (s, 1H), 8.52 (s, 1H), 6.86 (s, 2H), 4.24−4.20 (m, 1H), 3.88 (s, 3H), 2.36 (s, 3H), 1.42 (d, J = 6.8 Hz, 3H), 1.12−1.05 (m, 1H), 0.77−0.71 (m, 1H), 0.58−0.51 (m, 1H), 0.48−0.42 (m, 1H), 0.36−0.31 (m, 1H). HRMS (ESI) *m*/*e* 366.0785 [(M + H)$^+$, calcd for $C_{17}H_{18}N_3O_2Cl_2$ 366.0776]. Anal. ($C_{17}H_{17}N_3O_2Cl_2$) C, H, N.

(*R*)-3-(4-Chloro-2,6-dimethylphenylamino)-1-(1-cyclopropylethyl)-5-methylpyrazin-2(1*H*)-one (14). Part A. To a solution of the (*R*)-3,5-dibromo-1-(1-cyclopropylethyl)pyrazin-2(1*H*)-one (**11**) (400 mg, 1.23 mmol) and 4-chloro-2,6-dimethylaniline (240 mg, 1.23 mmol) in DMF (2 mL) at 0 °C was added NaHMDS (3.80 mL, 3.80 mmol, 1 M in THF). The reaction mixture was stirred at 0 °C for 1 h. The mixture was transferred to a separatory funnel containing ether (25 mL). The organic layer was washed with water (4 × 5 mL), brine (5 mL), dried over $MgSO_4$, filtered, and concentrated. The residue was purified by column chromatography on silica gel (15% ethyl acetate in hexanes) to afford (*R*)-5-bromo-3-(4-chloro-2,6-dimethylphenylamino)-1-(1-cyclopropylethyl)pyrazin-2(1*H*)-one (**13**) (380 mg, 77% yield) as an off-white solid; $[\alpha]^{25}_D$ −18.8 (*c* 0.329, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52 (s, 1H), 7.08 (s, 2H), 6.88 (s, 1H), 4.27−4.20 (m, 1H), 2.20 (s, 6H), 1.43 (d, J = 6.8 Hz, 3H), 1.11−1.03 (m, 1H), 0.78−0.71 (m, 1H), 0.60−0.53 (m, 1H), 0.49−0.43 (m, 1H), 0.39−0.32 (m, 1H). HRMS (ESI) *m*/*e* 396.0492 [(M + H)$^+$, calcd for $C_{17}H_{20}N_3OBrCl$ 396.0478]. Anal. ($C_{17}H_{19}N_3OBrCl$) C, H, N.

Part B. (*R*)-5-Bromo-3-(4-chloro-2,6-dimethylphenylamino)-1-(1-cyclopropylethyl)pyrazin-2(1*H*)-one (**13**) (380 mg, 0.960 mmol) from above, potassium carbonate (400 mg, 2.88 mmol), methylboronic acid (120 mg, 2.01 mmol), and $Pd(P\textit{t}\text{-}Bu_3)_2$ (196 mg, 0.380 mmol) were combined in dioxane (2 mL) in a sealed vial and the reaction mixture was heated at 120 °C for 16 h. The mixture was cooled to room temperature and transferred to a separatory funnel containing saturated aqueous $NaHCO_3$ solution (10 mL). The aqueous layer was extracted with ethyl acetate (3 × 10 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated. The residue was purified by column chromatography on silica gel (15% ethyl acetate in hexanes) to afford **14** (165 mg, 52% yield) as an off-white solid. The product was purified further by recrystallization from hexanes to furnish **14** as a colorless crystalline solid; mp 145−146.6 °C; $[\alpha]^{25}_D$ +21.3 (*c* 0.307, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 (s, 1H), 7.07 (s, 2H), 6.54 (s, 1H), 4.30−4.22 (m, 1H), 2.20 (s, 6H), 2.05 (s, 3H), 1.41 (d, J = 6.8 Hz, 3H), 1.13−1.06 (m, 1H), 0.75−0.68 (m, 1H), 0.55−0.47 (m, 1H), 0.46−0.41 (m, 1H), 0.39−0.33 (m, 1H). HRMS (ESI) *m*/*e* 332.1520 [(M + H)$^+$, calcd for $C_{18}H_{23}N_3OCl$ 332.1530]. HPLC method A: t_R = 3.86 min, >99%; method B: t_R = 2.40 min, >99%.

(*R*)-4-(1-Cyclopropylethyl)-6-(2,6-dichloro-4-(trifluoromethyl)phenylamino)-5-*oxo*-4,5-dihydropyrazine-2-carbonitrile (15a). To a solution of (*R*)-5-bromo-1-(1-cyclopropylethyl)-3-(2,-6-dichloro-4-(trifluoromethyl)phenylamino)pyrazin-2(1*H*)-one (**13**) (100 mg, 0.210 mmol), prepared according to the procedure for compound **14** part A, in *N*-methylpyrrolidinone (NMP) (2 mL) was added zinc cyanide (28 mg, 0.240 mmol), zinc dust (18 mg, 0.25 mmol), and $Pd(PPh_3)_4$ (73 mg, 0.060 mmol). The reaction mixture was heated under N_2 at 110 °C for 6 h. The mixture was then cooled to room temperature and was transferred to a separatory funnel containing saturated aqueous $NaHCO_3$ solution (10 mL). The aqueous layer was extracted with ethyl acetate (3 × 10 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated. The product was purified by column chromatography on silica gel (5% → 20% ethyl acetate in hexanes) to afford **15a** (55 mg, 63% yield) as an off-white solid; mp 106.5−107.5 °C; $[\alpha]^{25}_D$ −20.5 (*c* 0.300, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 (s, 1H), 7.68 (s, 2H), 7.49 (s, 1H), 4.31−4.23 (m, 1H), 1.47 (d, J = 6.6 Hz, 3H), 1.13−1.07 (m, 1H), 0.85−0.79 (m, 1H), 0.65−0.60 (m, 1H), 0.56−0.49 (m, 1H), 0.41−0.36 (m, 1H). HRMS (ESI) *m*/*e* 415.0343 [(M + H)$^+$, calcd for $C_{17}H_{12}N_4OCl_2F_3$ 415.0340]. HPLC method A: t_R = 6.37 min, 98.4%; method B: t_R = 3.49 min, > 99%.

(*R*)-4-(1-Cyclopropylethyl)-6-[2,6-dichloro-4-(difluoromethoxy)phenylamino]-5-*oxo*-4,5-dihydropyrazine-2-carbonitrile (15b). Compound **15b** was prepared according to the procedure described for the synthesis of **15a** using (*R*)-5-bromo-1-(1-cyclopropylethyl)-3-(2,6-dichloro-4-(difluoromethoxy)phenylamino)pyrazin-2(1*H*)-one (**13**) (380 mg, 0.810 mmol). The product was purified by column chromatography to furnish **15b** (110 mg, 33% yield) as a colorless solid; $[\alpha]^{25}_D$ −29.0 (*c* 0.400, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63 (s, 1H), 7.44 (s, 1H), 7.22 (s, 2H), 6.53 (t, J = 72.6 Hz, 1H), 4.29−4.22 (m, 1H), 1.46 (d, J = 6.8 Hz, 3H), 1.13−1.04 (m, 1H), 0.84−0.78 (m, 1H), 0.65−0.58 (m, 1H), 0.54−0.48 (m, 1H), 0.39−0.33 (m, 1H). HRMS (ESI) *m*/*e*

415.0547 [(M + H)$^+$, calcd for $C_{17}H_{15}N_4O_2Cl_2F_2$ 415.0540]. Anal. ($C_{17}H_{14}N_4O_2Cl_2F_3$) C, H, N.

3,5-Dichloro-4-nitrophenol (17). To a well stirred mixture of 3,5-dichlorophenol (25.01 g, 153 mmol) and sodium nitrite (14.45 g, 276 mmol) in water (250 mL) was slowly added a solution of conc H_2SO_4 (12 mL) in water (60 mL). The reaction mixture was then heated at reflux for 2 h while monitoring by HPLC (starting material, t_R = 3.73 min; 3,5-dichloro-4-nitrophenol, t_R = 3.45 min; 3,5-dichloro-2-nitrophenol, t_R = 3.63 min; YMC C18 S5 4.6 mm × 50 mm column; mobile phase: A = 10% MeOH/90% water with 0.2% phosphoric acid, B = 10% water/90% MeOH with 0.2% phosphoric acid, 30% B → 100% B; flow rate = 4 mL/min; λ = 210 nm; run time = 6 min). Additional sodium nitrite (21.11 g, 306 mmol) was added, and the reaction mixture was heated at reflux for an additional 2 h. A third portion of sodium nitrite (42.2 g, 612 mmol) was added, and the reaction mixture was heated at reflux for an additional 2 h until the reaction was complete, resulting in the formation of 3,5-dichloro-4-nitrophenol and 3,5-dichloro-2-nitrophenol in a 1:2 ratio. The mixture was cooled to room temperature and was transferred to a separatory funnel, and the layers were separated. The thick oily layer was diluted with ethyl acetate (800 mL) and was washed with water (300 mL), brine (300 mL), dried over $MgSO_4$, filtered, and concentrated. The residue was treated with hexanes (700 mL), and the desired product precipitated out of solution. The solid was collected on a Buchner funnel and was washed with hexanes and dried under vacuum to give **17** (8.01 g, 25% yield) as a brown solid. ^{1}H NMR (400 MHz, CD_3OD) δ 6.89 (d, J = 1.6 Hz, 2H). ^{13}C NMR (100 MHz, CD_3OD) δ 161.3, 128.4, 117.1.

1,3-Dichloro-5-(difluoromethoxy)-2-nitrobenzene (18). To a solution of **17** (5.65 g, 27.2 mmol) in DMF (60 mL) at room temperature was added potassium carbonate (13.16 g, 95.2 mmol). The resulting suspension was stirred vigorously for 20 min and was then treated with methyl 2-chloro-2,2-difluoroacetate (9.83 g, 68.0 mmol). The reaction mixture was stirred at 90 °C for 2 h. The mixture was cooled to room temperature and concentrated. The residue was transferred to a separatory funnel containing ethyl acetate (500 mL), and the organic layer was washed with water (300 mL), brine (300 mL), dried over $MgSO_4$, filtered, and concentrated. The product was purified by column chromatography on silica gel (0% → 5% ethyl acetate in hexanes) to furnish **18** (5.07 g, 72% yield) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.23 (s, 2 H), 6.56 (t, J = 58.0 Hz, 1H).

2,6-Dichloro-4-(difluoromethoxy)aniline (19). Palladium (10%) on carbon (2.01 g) was added to a solution of **18** (5.07 g, 19.6 mmol) in EtOH (20 mL) and conc HCl (3 mL) in a Parr bottle. The mixture was placed on a Parr shaker under an H_2 atmosphere at 50 psi for 2 h. The reaction mixture was filtered through a pad of celite and 2 M NH_3 in MeOH (300 mL) was added to the filtrate. The filtrate was concentrated, and the residue was transferred to a separatory funnel containing ethyl acetate (500 mL). The organic layer was washed with water (100 mL), brine (100 mL), dried over $MgSO_4$, filtered, and concentrated. The product was purified by column chromatography on silica gel (0% → 2% ethyl acetate in hexanes) to furnish **19** (2.12 g, 47% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.04 (s, 2H), 6.36 (t, J = 72.0 Hz, 1H), 4.40 (s br, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 141.0, 138.5, 120.8, 119.4, 115.8 (t, J = 260.4 Hz). LRMS (ESI) m/e 225.9 [(M − H)$^-$, calcd for $C_7H_4NOCl_2F_2$ 226.0].

2,3-Dihydrobenzofuran-5-amine (21). 5-Nitro-2,3-dihydrobenzofuran (1.0 g, 6.71 mmol) was dissolved in EtOH (50 mL) (some heating was required). $SnCl_2 \cdot 2H_2O$ (12.4 g, 67.1 mmol) was added, and the mixture was heated at 75 °C for 5.5 h. The mixture was cooled to room temperature and concentrated. The residue was dissolved in ethyl acetate and slowly transferred to an Erlenmeyer flask containing saturated aqueous $NaHCO_3$ solution (100 mL). The flask was swirled, and the solution was filtered through pad of celite with ethyl acetate rinsing. The filtrate was transferred to a separatory funnel and the aqueous layer was extracted with ethyl acetate (3×). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated. The product was purified by column chromatography on silica gel (50% → 70% ethyl acetate in hexanes) to afford **21** (586 mg, 65% yield) as a colorless solid; mp 80−81 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.59−6.57 (m, 2H), 6.45 (dd, J = 8.6, 2.5 Hz, 1H), 4.48 (t, J = 8.8 Hz, 2H), 3.36 (s br, 2H), 3.11 (t, J = 8.6 Hz, 2H). LRMS (APCI) m/e 177.4 [(M + H + CH_3CN)$^+$, calcd for $C_{10}H_{13}N_2O$ 177.1].

***N*-(2,3-Dihydrobenzofuran-5-yl)acetamide (22).** To a solution of **21** (300 mg, 2.22 mmol) in CH_2Cl_2 (10 mL) and pyridine (5 mL) at 0 °C was added acetyl chloride (150 μL, 2.66 mmol) dropwise via syringe and the reaction mixture was stirred at 0 °C for 30 min. The reaction was quenched by the addition of saturated aqueous $NaHCO_3$ solution, and the mixture was transferred to a separatory funnel. The aqueous layer was extracted with ethyl acetate (3 × 20 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated. The product was purified by column chromatography on silica gel (70% → 80% ethyl acetate in hexanes) to furnish **22** (317 mg, 94% yield) as a tan solid; mp 93−95 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.46 (s, 1H), 7.22 (s, 1H), 6.99 (dd, J = 8.5, 1.8 Hz, 1H), 6.68 (d, J = 8.5 Hz, 1H), 4.54 (t, J = 8.9 Hz, 2H), 3.17 (t, J = 8.6 Hz, 2H), 2.12 (s, 3H). LRMS (APCI) m/e 178.4 [(M + H)$^+$, calcd for $C_{10}H_{12}NO_2$ 178.2].

***N*-(6-Chloro-2,3-dihydrobenzofuran-5-yl)acetamide (23).** *N*-Chlorosuccinimide (197 mg, 1.47 mmol) was added to a solution of **22** (238 mg, 1.34 mmol) in acetonitrile (7 mL), and the reaction mixture was heated at 65 °C for 1 h. The mixture was cooled to room temperature and transferred to a separatory funnel containing saturated aqueous $NaHCO_3$ solution. The aqueous layer was extracted with ethyl acetate (3 × 20 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated. The product was purified by column chromatography on silica gel (40% → 60% ethyl acetate in hexanes) to give **23** (210 mg, 74% yield) as a colorless solid; mp 155−156 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 (s, 1H), 7.34 (s, br, 1H), 6.78 (s, 1H), 4.56 (t, J = 8.8 Hz, 2H), 3.18 (t, J = 8.9 Hz, 2H), 2.19 (s, 3H). LRMS (APCI) m/e 212.3 [(M + H)$^+$, calcd for $C_{10}H_{11}NO_2Cl$ 212.7].

6-Chloro-2,3-dihydrobenzofuran-5-amine (24). A solution of **23** (179 mg, 0.848 mmol) dissolved in ethanol (6 mL) and water (1 mL) was treated with potassium hydroxide (1.40 g, 25 mmol) and was heated at 90 °C for 4 h. The mixture was cooled to room temperature and was transferred to a separatory funnel containing saturated aqueous $NaHCO_3$. The aqueous layer was extracted with ethyl acetate (3 × 20 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated. The product was purified by column chromatography on silica gel (30% ethyl acetate in hexanes) to afford **24** (116 mg, 81% yield) as a tan solid; mp 99−100 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.76 (s, 1H), 6.65 (s, 1H), 4.49 (t, J = 8.6 Hz, 2H), 3.68 (s br, 2H), 3.09 (t, J = 8.9 Hz, 2H). LRMS (APCI) m/e 211.3 [(M + H + CH_3CN)$^+$, calcd for $C_{10}H_{12}N_2OCl$ 211.1].

5-Amino-4-chloro-2-methylbenzonitrile (26). To a solution of H_2SO_4 (20 mL) at 0 °C was added 4-chloro-2-methylbenzonitrile (5.0 g, 33.0 mmol) in portions, followed by HNO_3 (3.0 mL). The reaction mixture was stirred at 0 °C for 1.5 h. The mixture was then slowly poured over ice and the product was collected by filtration. The solid was washed with cold water and dried under vacuum to afford 4-chloro-2-methyl-5-nitrobenzonitrile (6.47 g, 99% yield) as a solid, which was used directly in the next step.

To a suspension of 4-chloro-2-methyl-5-nitrobenzonitrile (6.47 g, 32.9 mmol) from above and Sn (11.7 g, 98.8 mmol) in water (82 mL) at 0 °C was added conc HCl (33 mL). The reaction mixture was heated at 55 °C for 3 h. The mixture was cooled to 0 °C and was quenched by the slow addition of solid $NaHCO_3$. The

mixture was transferred to a separatory funnel, and the aqueous layer was extracted with ethyl acetate (3×150 mL). The combined organic layers were washed with water, brine, dried over $MgSO_4$, filtered, and concentrated to give **26** (4.45 g, 81% yield) as an off-white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.18 (s, 1H), 6.94 (d, J = 2.8 Hz, 1H), 4.11 (s br, 2H), 2.38 (s, 3H). LRMS (ESI) m/e 164.9 [$(M - H)^-$, calcd for $C_8H_6N_2Cl$ 165.0].

Biology. Intrinsic Clearance Studies. Estimates of intrinsic clearance were generated using human liver microsomes. Pooled human liver microsomes were obtained from BD Gentest (Woburn, MA) in which all compounds were evaluated using the same preparation of microsomes. The stability in liver microsomes was determined by a high-throughput in-house assay using final substrate concentrations of 1 μM and 0.5 mg/mL microsomal protein in which the final organic concentration of solvent was 0.1% acetonitrile. Incubations were performed at 37 °C in sodium phosphate buffer (100 mM), pH 7.4, in the presence and absence of 1 mM NADPH and quenched after 0, 10, 20, and 30 min. Samples were analyzed by an in house high-throughput LC/MS/MS. Intrinsic clearances were calculated as described by Obach et al.[49]

Rat Pharmacokinetic Studies. Pharmacokinetic parameters were estimated in Spague−Dawley rats following intravenous (2 mg/kg; n = 3) and oral (10 mg/kg; n = 3) dosing. Intravenous doses were prepared in a vehicle consisting of PEG:ethanol, 90:10 (v/v) at a volume of 1 mL/kg. The oral doses were prepared in a vehicle consisting of 1% polysorbate 80 in 0.5% methylcellulose suspension or Labrafil M 1944CS/DMAC/polysorbate 80, 85:10:5 (v/v) at a volume of 3 mL/kg. Blood samples were collected via a jugular vein catheter at 0, 0.17, 0.25, 0.5, 1, 2, 4, 6, 8, and 24 h postdose for the intravenous experiment, and at 0, 0.25, 0.5, 1, 2, 4, 6, 8, and 24 h postdose for the oral experiment. Plasma was separated by centrifugation and stored frozen at −20 °C until analysis. Concentrations were determined by LC/MS/MS.

LC/MS/MS Conditions. Sample preparation was conducted as follows. Aliquots (typically, 50 μL) of the biological matrix from in vivo study and standard/QC samples were treated with acetonitrile (200 μL) containing an appropriate internal standard, followed by vortex mixing for 2 min. The supernatant was then separated from the precipitated proteins after a 20 min centrifugation at 3000 rpm and 200 μL was transferred to a 96-well plate. The supernatant was evaporated under nitrogen using a TurboVap, with the plate heater set at 37 °C and then reconstituted using 75 μL of 0.1% formic acid.

An aliquot (5 μL) was injected onto a Synergi Fusion-RP column (2 mm × 50 mm, 4 μm) (Phenomenex, Torrance, CA) at room temperature for LC/MS/MS-based analysis (mobile phase = 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B); flow rate = 0.4 mL/min). A combination of isocratic and linear gradients were used for peak separation. The HPLC was interfaced to a Micromass Quattro Ultima LC/MS/MS tandem mass spectrometer (Micromass, Manchester, UK) equipped with an electrospray interface operating in the positive ionization mode. Detection of each analyte was achieved through selected reaction monitoring.

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Supporting Information Available: Tables of elemental analysis data and high-resolution mass spectral data with HPLC purity data for compounds lacking elemental analysis data. This material is available free of charge via the Internet at http://pubs.acs.org.

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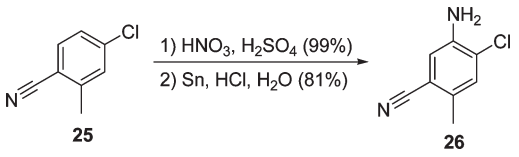

25 **26**

(44) Pharmacokinetic parameters were derived noncompartmentally.

(45) Oleoyl macrogolglycerides, also known as Labrafil M 1944CS, was purchased from Gattefossé, 36 Chemin de Genas, BP 603, 69804 Saint-Priest, France (http://www.gattefossecanada.ca/en/products/pharmaceutical/gattefosse_oral.shtml).

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(48) The antagonist properties of compound **12x** were assessed in a cell-based functional assay by measuring its ability to inhibit CRF-stimulated cAMP production in human Y-79 retinoblastoma cells. Compound **12x** produced a concentration-dependent inhibition of CRF (1 nM)-induced cyclic adenosine monophosphate (cAMP) production (IC_{50} value of 4.9 ± 1.2 nM) and completely suppressed CRF-stimulated cAMP production at higher concentrations. No agonist properties were detected at concentrations up to 10 μM. The IC_{50} of sauvagine = 0.58 ± 0.28 nM, $n = 2$ (0.77 nM, 0.38 nM) in the CRF_2 receptor binding assay.

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