Optimization of Chromone-2-carboxamide Melanin Concentrating Hormone Receptor 1 Antagonists: Assessment of Potency, Efficacy, and Cardiovascular Safety

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Evaluation of multiple structurally distinct series of melanin concentrating hormone receptor 1 antagonists in an anesthetized rat cardiovascualar assay led to the identification of a chromone-2-carboxamide series as having excellent safety against the chosen cardiovascular endpoints at high drug concentrations in the plasma and brain. Optimization of this series led to considerable improvements in affinity, functional potency, and pharmacokinetic profile. This led to the identification of a 7-fluorochromone-2-carboxamide (**22**) that was orally efficacious in a diet-induced obese mouse model, retained a favorable cardiovascular profile in rat, and demonstrated dramatic improvement in effects on mean arterial pressure in our dog cardiovascular model compared to other series reported by our group. However, this analogue also led to prolongation of the QT interval in the dog that was linked to affinity for hERG channel and unexpectedly potent functional blockade of this ion channel.

Introduction

Melanin-concentrating hormone (MCH) is a cyclic, 19-amino acid peptide that is produced predominantly in neurons in the lateral hypothalamus and zona incerta.[1] This neuropeptide is understood to have a significant role in the complex control of food intake and body weight regulation in rodents.[2] Three general lines of evidence involving experiments with the MCH peptide, the MCH receptor, and small molecule antagonists have emerged to suggest that inhibition of the interaction of MCH with its receptor in the brain would be a potential antiobesity therapy.

A single injection of the MCH peptide into the lateral hypothalamus of rodents stimulates food intake and chronic administration leads to increased body weight.[3,4] Similarly, transgenic mice overexpressing the MCH gene are susceptible to obesity and insulin resistance.[5] In contrast, mice lacking the gene encoding MCH are hypophagic, lean, and maintain elevated metabolic rates.[6]

Genetically altered animals that lack the single gene encoding the MCH receptor in rodents (MCHr1) maintain elevated metabolic rates and remain lean despite hyperphagia on a normal diet.[7,8] Finally, the observation that chronic administration of small-molecule antagonists leads to the reduction of body weight provides further validation of MCHr1 blockade as a novel target for antiobesity pharmacotherapy.[9−13]

Our group recently disclosed a moderate throughput anesthetized rat assay that was used to eliminate MCHr1 antagonists that demonstrated cardiovascular liabilities prior to lead optimization.[14] Evaluation of over 130 compounds representing multiple structurally distinct series of MCHr1 antagonists revealed one class that achieved a favorable cardiovascular profile with respect to its effect on mean arterial pressure (MAP) and heart rate (HR) with high exposures in plasma (>20 μg/mL, 40 μM) and brain (>20 μg/g). Two chromone-2-carboxamide aminopiperidines in particular, **1** and **2**, exhibited potent MCHr1 antagonism and excellent safety against the chosen cardiovascular endpoints at very high drug concentrations in plasma and brain (Table 1). Therefore, the chromone-2-carboxamide series was selected as a chemical series worthy of further optimization. Herein, we report the synthesis, structure−activity relationships, cardiovascular effects, pharmacokinetics, and in vivo efficacy of this series of MCHr1 antagonists.

Chemistry

The synthesis of 2-carboxychromones **4** was accomplished in three steps by acylation of 2′-hydroxyacetophenones **3** with diethyl oxalate, ring closure by treatment with concentrated hydrochloric acid and acetic acid, and hydrolysis (Scheme 1, steps a−c). In the course of our work, it was discovered that the initial protocol of basic hydrolysis of the ester (step c) led to ring-opened products in some cases and that an acidic hydrolysis of the ester was far superior. Optimization of the chemical route soon revealed that the ring closure and hydrolysis (steps b and c) could be conducted efficiently in one pot. In addition, converting commercially available 2-methoxybenzoic acids to the 2-hydroxyacetophenones in four steps allowed access to differentially substituted 2-carboxychromones. The 2-methoxybenzoic acids **5** were treated with oxalyl chloride followed by methoxymethylamine to produce the Weinreb amide. Treatment with a Grignard reagent gave 2-methoxyacetophenone that was demethylated with boron tribromide to give **3**. However, while this was an efficient route to several 2-carboxychromones, it suffered from limited availability of 2-hydroxyacetophenones and 2-methoxybenzoic acids.

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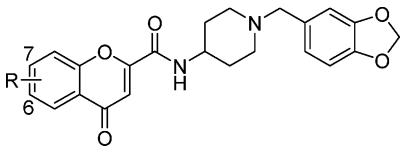
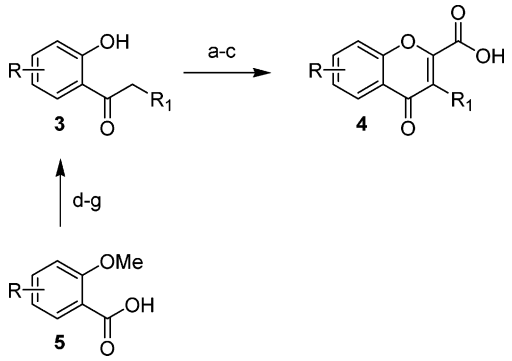
Table 1. MCHr1 Activity and Anesthetized Rat Cardiovascular Profile



		IC_{50} (nM)		end of study levels		mean arterial pressure	heart rate
compd	R	MCHr1 binding[a,c]	Ca^{2+} release[b,c]	plasma (μg/mL)[c]	brain (μg/g)[c]	effect[c]	effect[c]
1	6,7-dichloro	3 ± 2	83 ± 10	20 ± 2	63 ± 14	no effect	10% decrease[d]
2	7-OMe	11 ± 3	73 ± 16	29 ± 2	158 ± 13	no effect	no effect

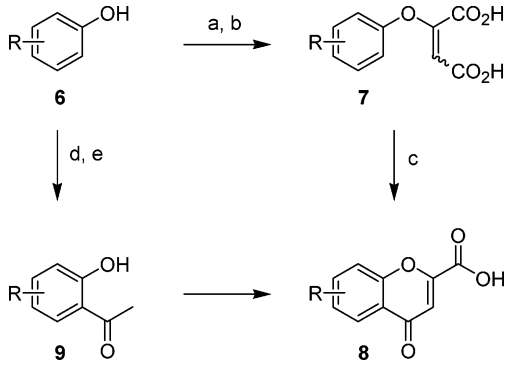
[a] Displacement of [^{125}I]MCH from MCHr1 expressed in membranes from IMR-32 (I3.4.2) cells (MCH binding K_d = 0.66 ± 0.25 nM, B_{max} = 0.40 ± 0.08 pmol/mg). [b] Inhibition of MCH-mediated Ca^{2+} release in intact IMR-32 cells (MCH EC_{50} = 62.0 ± 3.6 nM). [c] All values are mean values ± SEM and are derived from at least three independent experiments. [d] At 100 mg/kg.

Scheme 1[a]



[a] (a) EtO_2CCO_2Et, NaOEt; (b) glacial AcOH, concd HCl; (c) LiOH, H_2O or AcOH, H_2O, Δ; (d) oxalyl chloride, cat. DMF, CH_2Cl_2; (e) HN(Me)OMe; (f) R_1CH_2MgX, THF; (g) BBr_3, CH_2Cl_2.

Scheme 2[a]

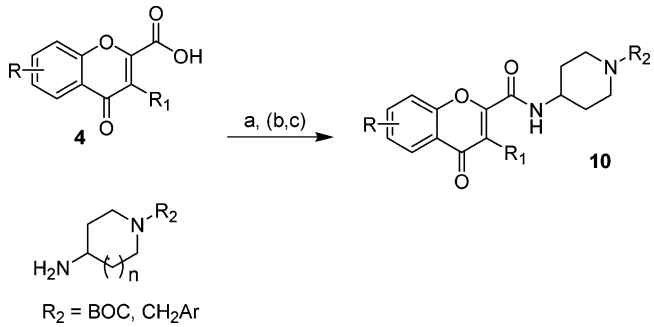


[a] (a) Dimethylacetylenedicarboxylate (DMAD); (b) NAOH; (c) P_2O_5, CH_3SO_3H, doxane, Δ; (d) AcCl, Et_3N; (e) $AlCl_3$.

A second route to the desired 2-carboxychromones that started from widely available substituted phenols was accomplished as described in Scheme 2. Treatment of a phenol **6** with dimethylacetylenedicarboxylate followed by hydrolysis gave a mixture of olefin diacids **7**. Closure of the ring from **7** was then affected by heating this mixture with Eaton's reagent in dioxane to give **8**. This route had the advantage of a large selection of differentially substituted commercially available phenols but suffered from low yields for the ring closure of **7** to **8** (only the *cis*-olefin closed to the desired product) and generation of a difficult to separate regioisomeric mixture of substituted chromone carboxylic acids when starting with 3-substituted or 3,4-disubstituted phenols.

This regioselectivity problem was solved by acetylation of the phenol **6** followed by a Fries rearrangement to give exclusively transfer of the acetyl moiety to the less hindered position to generate **9**. The 2′-hydroxyacetophenone **9** was then processed as shown in Scheme 1 to generate the desired 2-carboxychromone **8**.

Scheme 3[a]



[a] (a) EDC, HOBt, DMF; (b) TFA; (c) $NaBH(OAc)_3$, ArCHO.

Table 2. Pharmacokinetic Parameters[a] after a 10 mg/kg Oral Dose in DIO Mice

	plasma			brain			
compd	C_{max}	$t_{1/2}$	AUC	C_{max}	$t_{1/2}$	AUC	*T*/*P*
1	33	0.9	39	0	0	0	0
2	3596	3.1	18761	548	4.9	4398	0.24

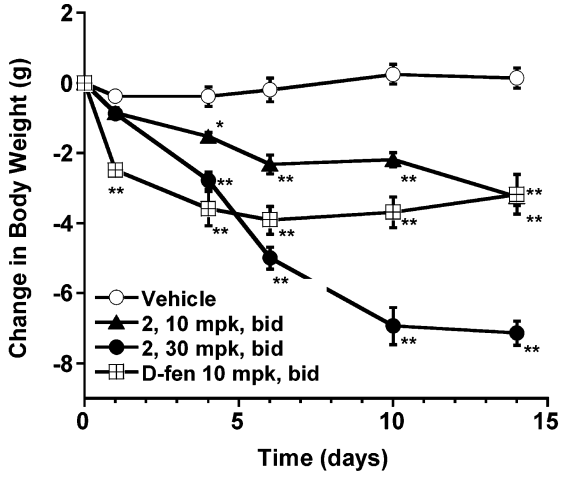
[a] C_{max} (ng/mL or ng/g); $t_{1/2}$ (h); AUC (ng·h/mL or ng·h/g), *T*/*P* (tissue (brain) to plasma AUC ratio), n = 3.

The general assembly of these chromone-amide antagonists was straightforward (Scheme 3). Acylation of a mono-*N*-protected-diamine core with the 2-carboxychromone **4** under standard amide coupling conditions was followed by deprotection and alkylation of the second nitrogen to give the requisite analogues **10**. In addition, after it was discovered that one of the best northern fragments was the piperonyl (vide infra), the intact 4-amino-1-(piperonyl)piperidine piece was coupled to the carboxychromone **4**.

Results and Discussion

Due to their favorable profiles in the anesthetized rat cardiovascular assay, compounds **1** and **2** were orally dosed at 10 mg/kg in diet-induced obese (DIO) mice for pharmacokinetic evaluation (Table 2). The 6,7-dichloro analogue **1** was poorly absorbed, showed little drug in plasma, and was undetectable in the brain, which obviously precluded further development. The 7-methoxy analogue **2** showed a significantly better pharmacokinetic profile with high levels in the plasma (AUC = 18.8 μg·h/mL) as well as good levels in the brain (AUC = 4.4 μg·h/mL). The half-life in plasma was 3.1 h and in brain 4.9 h with a reasonable but suboptimal brain to plasma AUC ratio of 0.24.

Thus, because of its good pharmacokinetic profile, **2** was selected for proof-of-concept evaluation in DIO mice that were



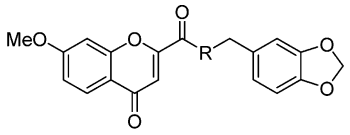
Cpd	Dose (mg/kg, po, 2x/day)	# Days	% Change Body Weight (from vehicle)	Peak Plasma Conc (μg/mL)	Peak Brain Conc (μg/g)
2	10	14	7.3	10.1 ± 1.8	4.9 ± 1.5
2	30	14	15.9	32.9 ± 2.4	27.1 ± 3.4

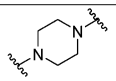
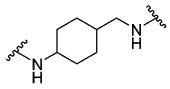
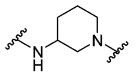
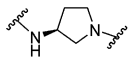
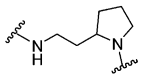
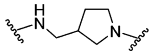
Figure 1. Effect of **2** and D-fenfluramine (D-fen, 10 mg/kg, po, twice a day) on the body weight of DIO mice. All values are mean values ± SEM for $n = 12$: (**) $p < 0.01$ for comparisons against vehicle group.

fed a high fat diet (60% fat) ad libitum and in lean mice that were fed normal chow (Figure 1). Animals were orally dosed twice a day with **2**, dexfenfluramine as reference weight loss agent (10 mg/kg), or vehicle. The vehicle-treated DIO mice had a small weight gain (1%) over the course of 2 weeks, while the dexfenfluramine-treated group lost weight rapidly then regained some by the end of the study to ultimately give a cumulative 7% loss in body weight. Treatment with **2** led to dose-dependent weight loss of 7.3% and 15.9% at 10 and 30 mg/kg, respectively, and a cumulative decrease in food intake of 16 and 37%. Consistent with earlier reports on MCHr1 antagonists from our group, the observed weight loss was due solely to the loss of fat mass, as determined by dual-energy X-ray absorptiometry (DEXA) imaging.[15] All mice appeared healthy throughout the study and there were no adverse events observed in a test of motor coordination (edge-balance test)[16] or in a systematic behavioral assay analogous to that described by Irwin (modified Irwin test).[17] End of study drug levels (1 h post dose C_{max}) for the lowest efficacious dose of 10 mg/kg were 4.9 μg/g in brain and 10.1 μg/mL (23 μM) in plasma. The exceptionally high efficacious peak plasma levels, along with results of a subsequent efficacy study that showed no weight loss at lower doses (1 and 3 mg/kg, twice a day, data not shown) left us with limited opportunity to achieve an acceptable therapeutic window with this analogue. Therefore, we focused our efforts on the optimization of the chromone amides with particular emphasis on improving potency and the brain-to-plasma ratio while maintaining a favorable cardiovascular profile.

In terms of cardiovascular safety in the rat, the chromone amide had emerged as the best heterocycle from screening a set of piperonyl-substituted 4-aminopiperidines. In order to determine if 4-aminopiperidine was the optimal core for this series, we conducted a survey of alternate diamino-linker replacements (Table 3). Contraction of the linker to a piperazine (**11**), extension to a 4-amino-1-aminomethyl linker (**12**), or conversion to 3-aminopiperidine (**13**), led to a significant loss of affinity for the MCH receptor. Pyrrolidine linkers also lost considerable affinity with 3-aminopyrrolidine **14** and 2-ethylaminopyrrolidine **15**, both having $IC_{50} > 2$ μM in our binding

Table 3. SAR of 4-Aminopiperidine Replacement Analogues[a]



Cpd	R	Binding IC_{50} (nM)	CaFlux IC_{50} (nM)
11		>2000 (N = 3)	nt
12		>2000 (N = 3)	nt
13		>2000 (N = 2)	nt
14		>2000 (N = 2)	nt
15		>2000 (N = 3)	nt
16		1140 ± 600 (N = 3)	8300 ± 1600 (N = 3)

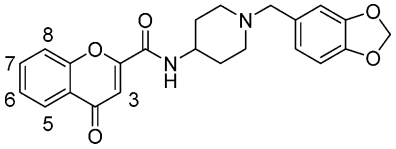
[a] nt: not tested.

assay. A 3-aminomethylpyrrolidine analogue (**16**) retained some activity (binding $IC_{50} = 1140$ nM, Ca flux $IC_{50} = 8300$ nM), albeit extremely weak. This brief study, in addition to experience on related series of MCHr1 antagonists, prompted us to move forward with 4-aminopiperidine as the most potent core for this series.

Confident that we had identified the best core, we next examined substitution on the chromone heterocycle while keeping the remainder of the molecule intact. A large number of differentially substituted chromone amide analogues were constructed that contained the 4-aminopiperidine core with piperonyl attached to the ring nitrogen (Table 4). A careful survey of substitution at the 7-position was undertaken first. Thus, while the 7-hydroxy analogue **17** had very little affinity for the receptor and no effect in the functional assay, conversion of the hydroxy to a difluoromethoxy **18** recovered much of the activity, although this analogue was still significantly weaker than methoxy analogue **2**. Electron-neutral substituents such as 7-ethyl (**19**) or 7-bromo (**20**) had moderate activity, but a shift to electron-withdrawing 7-chloro (**21**) gave a 2-fold boost to the binding affinity ($IC_{50} = 5$ nM) versus the methoxy **2** and was equipotent in the calcium flux assay. The 7-fluoro chromone (**22**) had even greater potency in binding affinity ($IC_{50} = 3$ nM) as well as an increase in functional potency ($IC_{50} = 29$ nM) to give the most potent analogue identified thus far in the series. Alternate electron withdrawing substituents at the 7-position such as cyano (**23**) or trifluoromethyl (**24**) were moderate at best.

We also explored the 6-position with less beneficial results. When compared to the 7-substituted analogues, monosubstituted analogues at the 6-position were generally significantly less potent as exemplified by analogues 6-chloro (**25**), 6-trifluoromethyl (**26**), and 6-bromo (**27**). The 6-methyl (**28**) and 6-trifluoromethoxy (**29**) also had relatively low affinity for the receptor but lack direct comparators. Clearly, the best substituent at the 6-position was fluoro (**30**), both in terms of affinity ($IC_{50} = 65$ nM) and functional potency ($IC_{50} = 196$ nM). However, compound **30** was only equipotent with the unsubstituted

Table 4. SAR of 2-Carboxychromone Amide Analogues



compd	R	IC$_{50}$ (nM) binding	Ca flux	hERG
2	7-OMe	11 ± 3	73 ± 16	11.5 (3.9
17	7-OH	1480 ± 310	>10000[b]	63.7[c]
18	7-OCHF$_2$	64 ± 20	160 ± 61	3.6 ± 1.3
19	7-Et	59 ± 18	529 ± 263	7.4 ± 3.1
20	7-Br	32 ± 11	82 ± 25	2.5 ± 0.6
21	7-Cl	5 ± 2	79 ± 17	3.6 ± 0.3
22	7-F	3 ± 1	29 ± 7	15.1 ± 4.6
23	7-CN	47 ± 11	137 ± 18	5.0 ± 1.2
24	7-CF$_3$	178 ± 50	3070 ± 250	nt
25	6-Cl	734 ± 647	2050 ± 100	nt
26	6-CF$_3$	351 ± 142	nt	nt
27	6-Br	187 ± 42	>6000[b]	nt
28	6-CH$_3$	393 ± 55	nt	nt
29	6-OCF$_3$	132 ± 2	>9000[b]	nt
30	6-F	65 ± 9	196 ± 30	4.0 ± 0.8
31	H	66 ± 14	192 ± 26	6.7 ± 1.2
32	5-Cl	24 ± 9	>10000[c]	nt
33	3-Me-7-Cl	2 ± 1	67 ± 6	nt
1	6,7-diCl	3 ± 2	83 ± 10	1.4 ± 0.2
34	6-F-7-Cl	3 ± 1	71 ± 25	10.5 ± 0.3
35	6-F-7-OMe	2 ± 1	16 ± 1	15.9 ± 2.3
36	6,7-difluoro	2 ± 1	13 ± 1	6.2 ± 1.3
37	6,8-difluoro	99 ± 33	2720 ± 550	nt
38	5,6-dichloro	75 ± 24	4750 ± 650	nt
39	6,7,8-trifluoro	91 ± 23	418 ± 93	nt

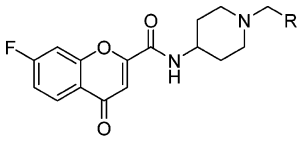
[a] All values are mean values ± SEM and are derived from at least three independent experiments. nt: not tested. [b] $n = 3$. [c] $n = 2$.

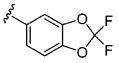
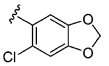
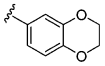
analogue **31**. A 5-chloro-substituted analogue (**32**) had reasonable affinity (IC$_{50}$ = 24 nM) but no functional potency (IC$_{50}$ >10 μM). Methyl substitution at the 3-position (**33**) was tolerated but did not have a favorable effect on potency (**33** vs **21**). Due to possible beneficial effects on pharmacokinetics, 6-fluoro substitution was combined with the best moieties at the 7-position to give 6-fluoro-7-chloro, 6-fluoro-7-methoxy, and 6,7-difluoro analogues **34**, **35**, and **36**, respectively. All three had exceptional affinity for the receptor (IC$_{50}$ = 2−3 nM), but **34** had only moderate functional potency (IC$_{50}$ = 71 nM) while **35** and **36** had exceptional functional potency as well (IC$_{50}$ = 16 and 13 nM, respectively). Additional multiply substituted analogues such as 6,8-difluoro (**37**), 5,6-dichloro (**38**), or 6,7,8-trifluoro (**39**) were significantly less potent.

The more potent analogues were also evaluated for their affinity for the hERG ion channel using a competitive, radiometric, binding assay ([^{3}H]dofetilide).[18] Fortunately, 7-fluoro and 7-methoxy substitution not only improved MCHr1 potency but also led to a 2−3-fold decrease in affinity for the hERG channel (**2** and **22** vs **31**). Fluorine at the 6-position resulted in a moderate increase in hERG activity of 1.5-fold (**30** vs **31**). Importantly, the most potent analogues from this set (**22**, **35**, and **36**) demonstrated not only a significant improvement in MCHr1 potency but also possessed a large separation between hERG affinity and MCHr1 affinity and functional potency.

Finally, the structure−activity relationship of the group attached to the piperidine nitrogen was examined. A set of 7-fluorochromone-4-aminopiperidines was constructed (Table 5) for comparison to piperonyl substituted compound **22** (binding IC$_{50}$ = 3 nM, Ca flux IC$_{50}$ = 29 nM). The unsubstituted benzyl analogue **40** was approximately 20-fold less potent in terms of affinity and functional potency. Moving the methylenedioxy moiety from the 3,4-position (as in the piperonyl **22**) to the 2,3-position resulted in a dramatic loss in binding affinity (**41**, IC$_{50}$ >700 nM). Changing the hydrogens of the 3,4-methylenedioxy to fluorine (**42**) led to an almost 20-fold drop in potency. A chlorine at the 2-position (**43**) reduced affinity (IC$_{50}$ = 22 nM) and functional potency (IC$_{50}$ = 266 nM) by about a factor of 8. Expanding the ring to the benzodioxane (**44**) had a more significant effect on affinity (>20-fold decrease) than it did on functional potency (6-fold decrease). Scission of the five-membered methylenedioxy ring to the 3,4-dimethoxy (**45**) led to a dramatic loss in affinity (IC$_{50}$ = 427 nM), but the monosubstituted 3-methoxy (**46**) and 4-methoxy (**47**) analogues had reasonable activity, with the 4-methoxy being the more potent of the two. Systematic monosubstitution of fluorine around the ring (**48**−**50**) showed that 3-fluoro (**49**) had reasonable affinity (IC$_{50}$ = 12 nM), but all three decreased significantly in functional potency (IC$_{50}$ = 789−2150 nM). The combination of 4-methoxy with fluorine revealed that 3-fluoro-4-methoxy (**52**) was a moderately potent analogue. Other moieties at the 4-position such as acetyl (**53**) or acetamide (**54**) led to significantly less potent analogues.

Table 5. SAR of Benzylpiperidine Analogues[a]

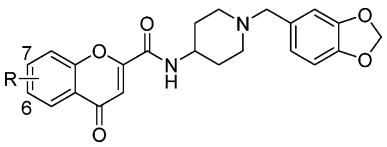


Cpd	R	Binding IC$_{50}$ (nM)	CaFlux IC$_{50}$ (nM)	hERG IC$_{50}$ (μM)
40	Ph	60 ± 28	592 ± 127	19.9 ± 2.8
41		>700 (N = 3)	nt	nt
42		57 ± 26	601 ± 111	5.6 ± 2.0
43		22 ± 2	266 ± 21	13.5 ± 0.6
44		73 ± 48	171 ± 36	12.2 ± 2.4
45	3,4-dimethoxyphenyl	427 ± 131	nt	nt
46	3-methoxyphenyl	31 ± 17	390 ± 121	10.4 ± 2.6
47	4-methoxyphenyl	13 ± 7	101 ± 23	5.4 ± 1.2
48	2-fluorophenyl	566 ± 106	2150 ± 60	nt
49	3-fluorophenyl	12 ± 6	966 ± 122	21.2 ± 1.8
50	4-fluorophenyl	159 ± 53	789 ± 101	nt
51	2-fluoro-4-methoxyphenyl	53 ± 37	411 ± 25	14.7 ± 5.1
52	3-fluoro-4-methoxyphenyl	24 ± 6	168 ± 12	8.5 ± 0.9
53	4-acetylphenyl	39 ± 21	581 ± 280	20.3 ± 6.3
54	4-(NHAc)phenyl	1690 ± 480	>10,000 (N = 2)	47.7 ± 4.5

[a] All values are mean values ± SEM and are derived from at least three independent experiments. nt: not tested.

Generally, the compounds shown in Table 5 had only moderate impact on hERG affinity. Exceptions were the difluoropiperonyl (**42**) and 4-methoxy substitution (**47**), which led to a 3-fold increase in hERG affinity while one analogue with a relatively polar acetamide at the 4-position (**54**) had a greater than 3-fold decrease in affinity. However, the MCHr1 activity with this analogue (**54**) had dropped even more dramatically, resulting in an inferior hERG/MCHr1 ratio. In the

Table 6. Anesthetized Rat Cardiovascular Profile



compd	R	IC_{50} (nM) MCHr1 binding[a,c]	IC_{50} (nM) Ca^{2+} release[b,c]	end of study levels plasma (mg/mL)[c]	end of study levels brain (mg/g)[c]	mean arterial pressure effect (infusion range)[c]	heart rate effect (infusion range)[c]
22	7-F	3 ± 1	29 ± 7	62 ± 8	126 ± 17	no effect	no effect
35	6-F-7-OMe	2 ± 1	16 ± 1	105 ± 16	149 ± 11	15.3% decrease (60 mg/kg)	<15% decrease (60 mg/kg)
36	6,7-diF	2 ± 1	13 ± 1	34 ± 6	25 ± 8	>15% decrease (60 mg/kg)	no effect

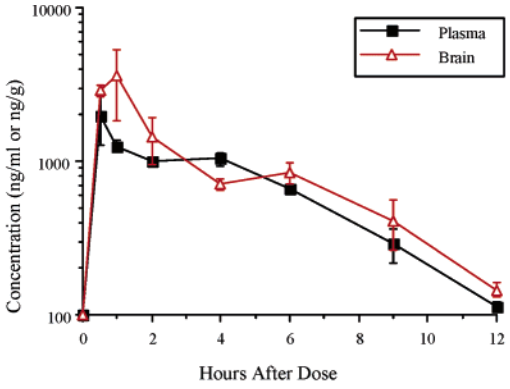
[a] Displacement of [^{125}I]MCH from MCHr1 expressed in membarnes from IMR-32 (I3.4.2) cells (MCH binding K_d = 0.66 ± 0.25 nM, B_{max} = 0.40 ± 0.08 pmol/mg). [b] Inhibition of MCH-mediated Ca^{2+} release in intact IMR-32 cells (MCH EC_{50} = 62.0 ± 3.6 nM. [c] All values are mean values ± SEM and are derived from at least three independent experiments.

end, piperonyl was found to be the optimal N-linked piperidine, and analogues **22**, **35**, and **36** were selected for further evaluation.

As described in a previous publication, recurring hemodynamic cardiovascular side effects observed for several different classes of MCHr1 antagonists led us to move an anesthetized rat cardiovascular assay earlier in our triaging process.[14] In this assay, three male Sprague−Dawley rats are anesthetized with inactin (100 mg/kg), have catheters placed in the femoral artery to monitor mean arterial pressure and heart rate, and are administered iv escalating doses in half log increments such that the complete dose is administered at the end of each 30-min infusion period. We took the three most potent analogues (**22**, **35**, **36**) into this assay. The 7-fluoro analogue **22** had no effect on mean arterial pressure (MAP) and heart rate (Table 6) and drug concentrations reached exceptionally high levels in the plasma (62 μg/mL, 146 μM) and brain (126 μg/g). The 6-fluoro-7-methoxy analogue **35** led to a moderate decrease in heart rate, showed a significant effect on MAP (15.3% decrease) at extremely high plasma concentrations (105 μg/mL, 231 μM), and all rats went into convulsions six minutes before the end of the protocol. The 6,7-difluoro analogue **36** had no effect on heart rate but did cause a significant effect on mean arterial pressure (>15% decrease), and two out of the three rats dropped below the minimum allowed mean arterial pressure limit of 70 mmHg during the 60 mg/kg dose.

On the basis of its superior cardiovascular profile in the rat, analogue **22** was orally dosed at 10 mg/kg in DIO mice for pharmacokinetic evaluation (Figure 2). This analogue had a nearly identical half-life in plasma and brain of about 2.5 h but rapidly penetrated the brain, resulting in good levels in the plasma (AUC = 9.29 μg h/mL) but higher levels in the brain (AUC = 12.7 μg h/g) and a very favorable brain-to-plasma ratio of 1.4. Thus, the analogue **22** was not only 3-fold more potent than **2** in terms of functional potency but also had a 6-fold improvement in brain to plasma AUC ratio.

Analogue **22** was further evaluated in an efficacy study in DIO mice (Figure 3). Due to its superior in vitro potency, compound **22** was dosed orally twice a day at 3 and 10 mg/kg while **2** had been dosed at 10 and 30 mg/kg. The fluorochromone **22** had no effect on body weight at 3 mg/kg but had a significant loss of body weight (7.2%) at the 10 mg/kg dose. Weight loss was due solely to the loss of fat mass as determined by DEXA imaging, and all mice appeared healthy throughout the study, displaying no signs of overt toxic effects in the edge-balance or modified Irwin tests (data not shown). Although the study with **22** was run for 22 days as opposed to 14 days for **2**, weight loss after administration of **22** had leveled out by day 14 with very little change thereafter.



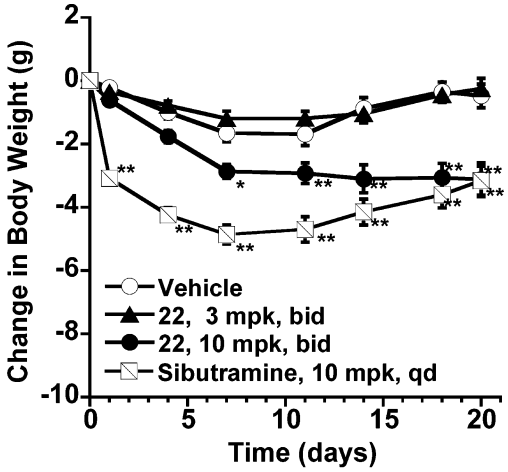
Cpd	Plasma Cmax	Plasma $t_{1/2}$	Plasma AUC	Brain Cmax	Brain $t_{1/2}$	Brain AUC	T/P
22	1779	2.5	9292	4367	2.4	12658	1.4

Cmax (ng/mL or ng/g); t1/2 (hr); AUC (ng·hr/mL or ng·hr/g), T/P (tissue to plasma AUC ratio), N = 3.

Figure 2. Pharmacokinetic parameters after a 10 mg/kg oral dose in DIO mice.

End of study drug levels for the lowest efficacious dose of 10 mg/kg showed that **22** did have a 3-fold lower peak plasma level compared to **2** (7.7 μM vs 23 μM). Interestingly, at 10 mg/kg **22** and **2** had nearly identical peak brain levels (C_{max} = 4.7 and 4.9 μg/g respectively), but **22** had dramatically lower trough levels (0.4 vs 2.2 μg/g). This was undoubtedly due to the longer brain half-life of **2** (4.9 h) versus **22** (2.4 h) and may explain why the more potent **22** showed the same efficacy as **2** at 10 mg/kg and was not efficacious at lower doses.

The therapeutic index for this project was initially defined as the ratio of the highest plasma concentration that was safe in our dog cardiovascular model[19] over the peak plasma concentration required to achieve at least a 5% weight loss in the DIO mouse model. Due to comparable efficacy at 10 mg/kg and 3-fold lower plasma C_{max} observed for **22** versus **2**, we evaluated **22** in our anesthetized dog cardiovascular model. An array of clinically relevant endpoints was assessed in the dog, including blood pressure, heart rate, cardiac contractile function, pulmonary arterial pressure, and QT interval. Compound **22** was infused (iv) in three escalating doses at 3, 10, and 30 mg/kg/30 min, which produced peak plasma concentrations of 5.9 ± 0.3, 25.8 ± 1.6, and 85.1 ± 7.9 μM at the end of each dosing period. This represented 0.8-, 3.3-, and 11.0-fold above the mouse efficacious C_{max}. By the end of the 30 mg/kg infusion period,



Cpd	Dose (mg/kg, po, 2x/day)	# Days	% Change Body Weight (from vehicle)	Peak Plasma Conc (μg/mL)	Peak Brain Conc (μg/g)
22	3	22	0	0.84 ± 0.05	1.11 ± 0.06
22	10	22	7	3.27 ± 0.34	4.67 ± 0.66

Figure 3. Effect of **22** and sibutramine (10 mg/kg, po, once a day) on the body weight of DIO mice. All values are mean values ± SEM for $n = 12$: (**) $p < 0.01$ for comparisons against vehicle group.

22 had produced no physiologically relevant change in mean arterial pressure (Figure 4C). This constituted a significant improvement on this parameter versus the best compounds from earlier publications such as the coumarin **55** and alkyl indazole **56** (Figure 4A,B).

However, **22** produced a biphasic effect on heart rate initially decreasing to −22 ± 1% at 10.94 μg/mL before trending toward baseline to end at −11 ± 3% at 36.13 ± 3.34 μg/mL (Figure 5A). Importantly, **22** also produced a pronounced and dose-dependent increase in the QT-interval corrected for heart rate (QTc; Van de Water correction formula)[20] to 9 ± 0.5%, 16 ± 0.8%, and 30 ± 1.6% above baseline at the end of each dosing period (Figure 5B).

The QT prolongation led to the closer evaluation of activity at the hERG channel. A competitive binding assay ([^{3}H]-dofetilide) had given an IC_{50} of 15.1 μM for **22**. Given the exceptional potency of this analogue at MCHr1 (IC_{50} = 29 nM Ca flux and 3 nM binding), this compound possessed a significant in vitro selectivity window versus hERG binding (500−5000-fold). However, the high plasma drug levels required to achieve efficacy (3.27 μg/mL, 7.7 μM, 265-fold above the IC_{50}) prevented this in vitro selectivity window from translating into a reasonable in vivo therapeutic index. Furthermore, the functional effect of **22** on hERG current was evaluated using HEK 293 cells stably expressing hERG. Drug effects were evaluated on the basis of tail currents measured during 4-s repolarization test pulses to −50 mV preceded by a 3-s depolarizing conditioning pulse to 0 mV. In this assay, **22** demonstrated an IC_{50} of 1.6 μM and led to 90% current block when evaluated at the efficacious C_{max} (7 μM), thus precluding the need for further development of this analogue.

Conclusion

In conclusion, a series of MCHr1 antagonists was optimized for potency, triaged for cardiovascular safety in anesthetized rat, and evaluated for pharmacokinetic profile in DIO mice. This led to the identification of two analogues (**22** and **2**) that were tested in our DIO mouse efficacy model and showed significant, dose-dependent weight loss with twice-a-day oral dosing.

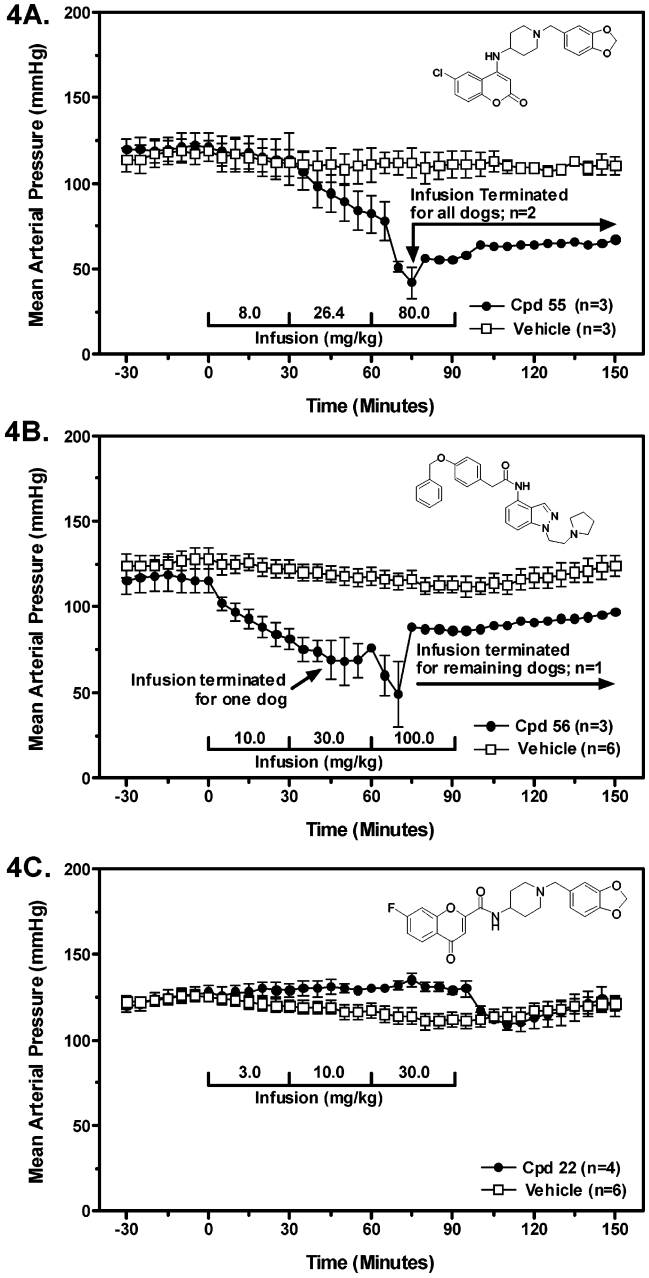


Figure 4. Effect on mean arterial pressure in anesthetized dog.

Further evaluation of **22** in an anesthetized dog cardiovascular assay demonstrated significant improvement in effects on mean arterial pressure compared to earlier disclosed series of MCHr1 antagonists. However, this analogue also had biphasic effects on heart rate and revealed considerable QT prolongation. The QT prolongation was linked to activity at the hERG channel both in terms of binding and functional blockade. While screening for changes in mean arterial pressure and heart rate in rat led to an improved cardiovascular profile, clearly significant hurdles remain. Addressing the hERG activity, along with efforts to lower efficacious peak plasma drug levels, will be the topic of future communications from our group.

Experimental Section

General. Unless otherwise specified, all solvents and reagents were obtained from commercial suppliers and used without further purification. All reactions were performed under nitrogen atmosphere unless specifically noted. Normal-phase flash chromatography was performed using Merck silica gel 60 (230−400 mesh)

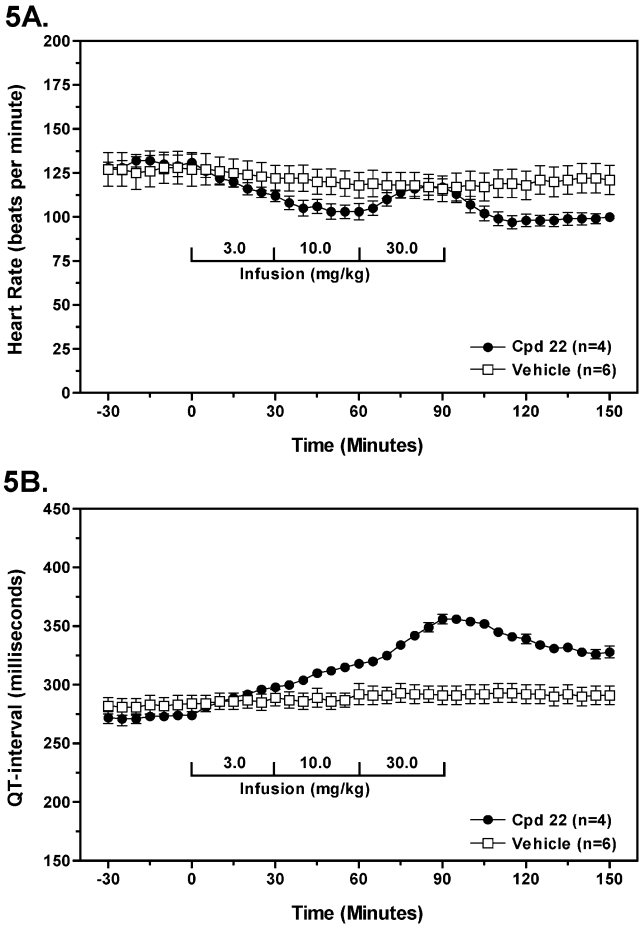


Figure 5. Effect of **22** on HR and QTcV interval in anesthetized dog.

from E. M. Science or on Biotage, Analogix or Isolute pre-packed columns. Following workup, reaction mixtures were dried over $MgSO_4$ or Na_2SO_4; filtered through a fritted glass funnel, filter paper, or a plug of cotton; and concentrated with a rotary evaporator at ca. 15 mmHg, with warming when necessary. Thin-layer chromatography systems were the same as those used for column chromatography, with R_f approximately = 0.3. Analytical LC−MS was performed on a Finnigan Navigator mass spectrometer and Agilent 1100 HPLC system running Xcalibur 1.2 and Open-Access 1.3 software. The mass spectrometer was operated under positive APCI ionization conditions. The HPLC system comprised an Agilent Quaternary pump, degasser, column compartment, autosampler, and diode-array detector with a Sedere Sedex 75 evaporative light-scattering detector. The column used was a Phenomenex Luna Combi-HTS C8(2), 5 μm, 100 Å (2.1 mm × 30 mm). A gradient of 10−100% acetonitrile (A) and 0.1% trifluoroacetic acid in water (B) was used, at a flow rate of 1.5 mL/min (0−0.1 min 10% A, 0.1−3.1 min 10−100% A, 3.1−3.9 min 100−10% A, 3.9−4.0 min 100−10% A). ^{1}H NMR spectra were recorded at 300 MHz unless specified otherwise; all values are referenced to tetramethylsilane as internal standard and are reported as shift (multiplicity, proton count, coupling constants). Mass spectral analysis was accomplished using fast atom bombardment (FAB-MS), electrospray (ESI-MS), or direct chemical ionization (DCI-MS) techniques. Elemental analyses were obtained at QTI Laboratories. Routine analytical reversed-phase chromatography was performed using a Zorbax SP-C18, 5 μM, 4.6 × 250 mm column with UV detection analyzed at 254 nM (water with 0.1% trifluoracetic acid and CH_3CN gradient, 0−100% CH_3CN over 18 min at 1.5 mL/min). Additional reversed-phase purity evaluation was performed on Phenomenex Luna C18(2), 5 μM (method A) and Phenomenex 5 μM Phenyl-Hexyl (method B) 4.6 × 250 mm columns with UV detection at 254 nM (water with 0.1% trifluoroacetic acid and CH_3CN gradient, 0−90% CH_3CN over 6 min, 90% CH_3CN 6−18 min, 90−0% CH_3CN 18−20 min). Preparative reversed-phase chromatography was performed using a Zorbax SB-C18, 7 μM, 21.2 × 250 mm column with UV detection analyzed at 220 and 254 nM (water with 0.1% trifluoracetic acid and CH_3CN with 0.1% trifluoroacetic acid gradient, 5−95% CH_3CN over 30 min at 15 mL/min).

General Procedures. Method A. Conversion of Phenols to 2′-Hydroxyacetophenones. Acetyl chloride (3.42 mL, 48.1 mmol) was added slowly to a solution containing the phenol (38.5 mmol), triethylamine (6.7 mL, 48.1 mmol), and CH_2Cl_2 (50 mL) at 0 °C. The solution was permitted to warm to room temperature as it was stirred overnight. After 15 h the solution was concentrated, and the resulting residue was filtered though a plug of silica gel (5 g), eluting with Et_2O, to give a solid after concentration of the filtrate. The solid was added to a slurry of aluminum trichloride (16 g, 118 mmol) in either CH_2Cl_2 or CS_2 (35 mL). After 1 h at room temperature, the mixture was heated between 60 and 70 °C and the solvent removed by distillation. Once the solvent was gone, the temperature was raised to 140 °C. After 1.5 h, the reaction was allowed to cool to ambient temperature and combined very carefully with 50% aqueous HCl (30 mL). The resulting solid was collected by filtration to provide the desired 2′-hydroxyacetophenone.

Method B. Conversion of 2′-Hydroxyacetophenones to 2-Carboxychromones. The 2′-hydroxyacetophenone (32.4 mmol) was dissolved in diethyl oxalate (22 mL, 162 mmol) and added to sodium ethoxide (22 wt %) in EtOH (42 mL, 130 mmol). The yellow, heterogeneous solution was heated at 40 °C for 30 min before the mixture was cooled to room temperature and filtered to collect the yellow precipitate. The yellow solid was diluted with CH_2Cl_2, washed with 10% aqueous AcOH and brine, dried ($MgSO_4$), filtered, and concentrated to a yellow solid. This solid was combined with glacial acetic acid (33 mL) and concentrated HCl (1 mL) and heated at 80 °C for 14 h before the dark solution was allowed to cool to room temperature and combined with distilled water (35 mL). The resulting precipitate, generally a mixture of the ethyl ester and carboxylic acid, was collected by filtration and dried in a vacuum oven at 50 °C overnight. Final conversion to the carboxylic acid was accomplished by heating the compound with glacial acetic acid (80 mL) and 6 M HCl (40 mL) at 80 °C for 17 h. The resultant solution was allowed to cool to ambient temperature, diluted with distilled water (120 mL), cooled in an ice bath, and the precipitate was collected by filtration. Alternatively, the initial dark solution from the ring closure step (at room temperature) was treated directly with glacial acetic acid (50 mL) and 6 M HCl (40 mL) and heated at 80 °C for 17 h followed by the same procedure for isolation of precipitate 2-carboxychromone.

Method C. Conversion of 2-Methoxybenzoic Acids to 2′-Hydroxyacetophenones. 2-Methoxybenzoic acid (15.0 mmol), DMF (0.25 mL), and CH_2Cl_2 (60 mL) were combined and cooled to 0 °C. Oxalyl chloride (7.5 mL, 15.0 mmol of a 2 M solution in CH_2Cl_2) was added carefully dropwise. Gas evolution was observed. After 0.5 h at 0 °C, the ice bath was removed. After 1 h at room temperature, the solution was cooled to 0 °C, and *N*,*O*-dimethylhydroxylamine hydrochloride (3.22 g, 33.0 mmol) was added followed by the slow addition of diisopropylethylamine (5.8 mL, 33.0 mmol). The solution was permitted to slowly warm to room temperature as it was stirred overnight. After 15 h, the solution was diluted with CH_2Cl_2; washed with 1 M HCl, 1 N NaOH, and brine; dried ($MgSO_4$); filtered; and concentrated to a solid. The crude Weinreb amide (14.3 mmol) was combined with THF (60 mL) and cooled to −78 °C. Methylmagnesium bromide (5.3 mL, 15.8 mmol of a 3 M solution in Et_2O) was added dropwise. The mixture was permitted to warm to room temperature slowly as it was stirred overnight. After 16 h, the reaction was quenched with 2 N HCl and the mixture was diluted with EtOAc; washed with distilled water, saturated $NaHCO_3$, and brine; dried ($MgSO_4$); filtered; and concentrated. Next, boron tribromide (17.4 mL, 17.4 mmol) was added slowly to the 2′-methoxyacetophenone (8.71 mmol) in CH_2Cl_2 (20 mL) at 0 °C. After 1 h, the ice−water bath was removed and the dark mixture was stirred at ambient temper-

ature for another 0.5 h before the solution was added carefully to a mixture of ice-cold 2 N aqueous HCl. The aqueous layer was extracted with CH_2Cl_2; the organic layers were combined, washed with saturated aqueous $NaHCO_3$ and brine, dried ($MgSO_4$), filtered, and concentrated. The resulting crude material was purified by column chromatography, eluting with a gradient of EtOAc in hexane, to provide the desired 2′-hydroxyacetophenone (2.29 mmol, 26%).

Method D. Conversion of Phenols to 2-Carboxychromones. Benzyltrimethylammonium hydroxide (40% weight in water, 0.5 mL, 1.47 mmol) was added to a mixture of the phenol (123 mmol), dimethylacetylenedicarboxylate (16.6 mL, 135 mmol), and dioxane (250 mL), and the solution was heated at 90 °C. After 1.5 h, the solution was allowed to cool, combined with 20% aqueous NaOH (100 mL), and then heated at 90 °C. After 1 h, the slurry was cooled in an ice bath, 2 M HCl was added until the pH was less than 6, and the resulting precipitate was isolated by filtration and washed with distilled water. The crude solid (89.5 mmol) was combined with Eaton's reagent (182 mL, 98.4 mmol) and heated at 70 °C for 16 h. The solution was cooled to ambient temperature and added to ice (1 kg). The resulting white precipitate was collected by filtration to give the 2-carboxychromone.

Method E. Conversion of Carboxylic Acids to Amides. A solution of 2-carboxychromone (0.25 mmol) and amine (0.26 mmol) in DMF (1 mL) was charged with hydroxybenzotriazole (36 mg, 0.26 mmol), Et_3N (38 uL, 0.28 mmol), and *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDCI, 53 mg, 0.28 mmol). The resultant homogeneous solution was stirred at room temperature for 18 h; diluted with EtOAc; washed with distilled water, 1 M K_2CO_3, and brine; dried (Na_2SO_4); and concentrated to give a solid. Purification by flash silica gel chromatography (0−5% MeOH in CH_2Cl_2) or reversed phase HPLC (Zorbax SB-C18, 7 μM, 21.2 × 250 mm column, CH_3CN, H_2O with 0.1% TFA) provided the desired amides. If the amine used in the reaction was a hydrochloride salt, an extra equivalent of Et_3N was added for each equivalent of HCl.

Method F. Reductive Alkylation of Amines. Sodium triacetoxyborohydride (0.200 mmol) was added to a mixture of the free amine (0.100 mmol), aldehyde (0.100 mmol), acetic acid (20 μL), and THF (1 mL). After 18 h, the reaction was quenched with methanol and/or water. The solution was diluted with EtOAc, washed with 1 M NaOH or 1 M K_2CO_3, washed with brine, dried (Na_2SO_4), filtered, and concentrated. Purification by flash silica gel chromatography (0−5% MeOH in CH_2Cl_2) or reversed phase HPLC as described above provided the desired alkylated amines. If the amine starting material was a salt, either the free amine was generated by partitioning between dichloromethane and aqueous potassium carbonate prior to submission to the reductive alkylation or 2−4 equiv of KOAc was added to the reaction for each equivalent of amine salt.

***N*-[1-(1,3-Benzodioxol-5-ylmethyl)piperidin-4-yl]-6,7-dichloro-4-oxo-4*H*-chromene-2-carboxamide (1).** 6,7-Dichloro-4-oxo-4*H*-chromene-2-carboxylic acid (**1A**) was produced from 3,4-dichlorophenol as detailed in methods A and B. Compound **1A** (65 mg, 0.25 mmol) and 1-benzo[1,3]dioxol-5-ylmethylpiperidin-4-ylamine **1B**[10,14] (62 mg, 0.26 mmol) were processed as detailed in method E to provide the title compound as a white powder (86 mg, 72%): ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.54−1.69 (m, 2 H), 1.76−1.84 (m, 2 H), 1.95−2.07 (m, 2 H), 2.83 (d, J = 11.9 Hz, 2 H), 3.37−3.41 (m, 2 H), 3.78 (d, J = 7.8 Hz, 1 H), 5.99 (s, 2 H), 6.73−6.77 (m, 1 H), 6.83−6.89 (m, 3 H), 8.13 (s, 1 H), 8.16 (s, 1 H), 8.82 (d, J = 8.1 Hz, 1 H); MS (APCI, CH_3OH/NH_4OH) m/z 475 [M + H], 473 [M − H]; Anal. ($C_{23}H_{20}Cl_2N_2O_5$) C, H, N.

***N*-[1-(1,3-Benzodioxol-5-ylmethyl)piperidin-4-yl]-7-methoxy-4-oxo-4*H*-chromene-2-carboxamide (2).** A solution of commercially available 7-hydroxy-4-oxo-4*H*-chromene-2-carboxylic acid ethyl ester (10 g, 42.7 mmol) in 90 mL of acetone was charged with K_2CO_3 (13 g, 93.8 mmol) and iodomethane (3.3 mL, 53.4 mmol) and the reaction heated at reflux. After 19 h, the reaction was allowed to cool to room temperature and 50 mL of ethyl acetate was added. The resultant solid was filtered off, rinsed with 1/1 ethyl acetate/acetone, and air-dried to give 7-methoxy-4-oxo-4*H*-chromene-2-carboxylic acid ethyl ester (**2A**) as an off-white solid (10.6 g, 100%). A solution of example **2A** (10 g, 40 mmol) in 120 mL of THF and 40 mL of distilled water was charged with of lithium hydroxide hydrate (3.4 g, 81 mmol). The resulting brown, homogeneous solution was stirred for 2 h and then quenched by slow addition of 15 mL of 3 M H_2SO_4 portionwise via pipet. The resulting solid was filtered off, washed with distilled water, and air-dried to give 7-methoxy-4-oxo-4*H*-chromene-2-carboxylic acid (**2B**) as a light yellow powder (7.7 g, 82%). A solution of **2B** (7.5 g, 34 mmol) was coupled to 4-amino-1-BOC-piperidine (6.8 g, 34 mmol) using general method E to give 13 g of a brown residue. This residue was dissolved in 90 mL of dichloromethane, cooled to 0 °C, and then charged with 45 mL of trifluoroacetic acid in three portions. After 15 min at 0 °C, the reaction was allowed to warm to room temperature, stirred for 1.5 h, and then concentrated to give a brown oil. This oil was dissolved in dichloromethane (300 mL) and washed with 1 M K_2CO_3. The aqueous layer was extracted with 95/5 CH_2Cl_2/methanol (3 × 300 mL), and the organic extracts were combined, dried (Na_2SO_4), and concentrated to give 4-[(7-methoxy-4-oxo-4*H*-chromene-2-carbonyl)amino]piperidine (**2C**) as an orange solid (7.8 g, 76%). Compound **2C** (7.5 g, 24.8 mmol) was alkylated with piperonal (3.7 g, 24.8 mmol) using general method F to give 11.1 g of an orange foam. This foam was dissolved in a minimal amount of CH_2Cl_2, placed on a 70-g Isolute silica gel column, and eluted with 0−100% ethyl acetate in hexane (0 to 20 min), 100% ethyl acetate (20−35 min), 100% CH_2Cl_2 (35−40 min), 0−5% methanol/CH_2Cl_2 (40−60 min), 5/95 methanol/CH_2Cl_2 (60−70 min) at 30 mL/min with detection at 254 nM wavelength to give the title compound **2** as an off-white, amorphous solid (5.3 g, 49%): ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.63 (m, 2 H), 1.80 (m, 2 H), 2.00 (m, 2 H), 2.85 (m, 2 H), 3.39 (bs, 2 H), 3.76 (m, 1 H), 3.93 (s, 3 H), 5.98 (s, 2 H), 6.73 (m, 2 H), 6.85 (m, 2 H), 7.10 (dd, J = 9.0, 2.5 Hz, 1 H), 7.24 (d, J = 2.4 Hz, 1 H), 7.95 (d, J = 8.8 Hz, 1 H), 8.83 (d, J = 7.8 Hz, 1 H); MS (ESI, MeOH/NH_4OH) m/z 437 [M + H], 459 [M + Na], 435 [M − H]; Anal. ($C_{24}H_{24}N_2O_6 \cdot 0.5H_2O$) C, H, N.

2-(4-Benzo[1,3]dioxol-5-ylmethylpiperazine-1-carbonyl)-7-methoxychromen-4-one (11). A solution of 7-methoxy-4-oxo-4*H*-chromene-2-carboxylic acid (**2B**, 100 mg, 0.45 mmol) and piperazine-1-carboxylic acid *tert*-butyl ester (101 mg, 0.54 mmol) were coupled in a manner similar to method E, followed by treatment with 4 N HCl in dioxane to give **7-methoxy-2-(piperazine-1-carbonyl)-chromen-4-one (11A).** Compound **11A** and benzo[1,3]dioxole-5-carbaldehyde (62 mg, 0.41 mmol) were processed in a manner similar to method F to give the title compound **11** (156 mg, 82% yield): ^{1}H NMR (DMSO-d_6) δ 2.41 (m, 4H), 3.44 (s, 2H), 3.53 (m, 2H), 3.61 (m, 2H), 5.99 (s, 2 H), 6.44 (s, 1H), 6.76 (dd, J = 7.9 Hz, 1.5 Hz, 1H), 6.85 (d, J = 7.9 Hz, 1H), 6.88 (d, J = 1.5 Hz, 1 H), 7.10 (dd, J = 8.9 Hz, 2.4 Hz, 1 H), 7.21 (d, J = 2.4 Hz, 1 H), 7.95 (d, J = 8.9 Hz, 1H); MS (ESI, MeOH/NH_4OH) m/z 422.9 [M + H], 444.9 [M + Na]; Anal. ($C_{23}H_{22}N_2O_6 \cdot 0.25H_2O$) C, H, N.

7-Methoxy-4-oxo-4*H*-chromene-2-carboxylic Acid (4-{[(Benzo-[1,3]dioxol-5-ylmethyl)amino]methyl}cyclohexyl)amide (12). A solution of **2B** (99 mg, 0.45 mmol) and 4-[[(benzo[1,3]dioxol-5-ylmethyl)amino]methyl}cyclohexylamine (118 mg, 0.45 mmol) were coupled in a manner similar to method E to give the title compound **12** (161 mg, 77% yield): ^{1}H NMR (DMSO-d_6) δ 1.46−1.70 (m, 9H), 1.99 (m, 1H), 2.46 (d, J = 6.7 Hz, 2H), 3.61 (s, 2H), 3.87−3.88 (bs, 1H), 3.93 (s, 3H), 5.97 (s, 2H), 6.75−6.83 (m, 3H), 6.92 (s, 1H), 7.11 (dd, J = 8.9 Hz, 2.4 Hz, 1H), 7.24 (d, J = 2.4 Hz, 1H), 7.95 (d, J = 8.9 Hz, 1H), 8.60 (d, J = 7.6 Hz, 1H); MS (ESI, MeOH/NH_4OH) m/z 465.2 [M + H]; Anal. ($C_{26}H_{28}N_2O_6 \cdot 0.5H_2O$) C, H, N.

7-Methoxy-4-oxo-4*H*-chromene-2-carboxylic acid (1-Benzo-[1,3]dioxol-5-ylmethylpiperidin-3-yl)amide (13). A solution of **2B** (80 mg, 0.36 mmol) and 3-amino-piperidine-1-carboxylic acid *tert*-butyl ester (87 mg, 0.43 mmol) were coupled in a manner similar to method E, followed by treatment with 4 N HCl in dioxane to give 7-methoxy-4-oxo-4*H*-chromen-2-carboxylic acid piperidin-3-ylamide (**13A**). Compound **13A** and benzo[1,3]dioxole-5-carbal-

dehyde (48 mg, 0.32 mmol) were processed in a manner similar to method F to give the title compound **13** (126 mg, 80% yield): ^{1}H NMR (DMSO-d_6) δ 1.37−1.56 (m, 2H), 1.67−1.86 (m, 2H), 1.91−2.04 (m, 2H), 2.66−2.86 (m, 2H), 3.49−3.48 (m, 2H), 3.93 (s, 3H), 3.94−3.98 (m, 1H), 5.97 (s, 2 H), 6.74−6.77 (m, 2H), 6.83−6.89 (m, 2H), 7.10 (dd, J = 8.9 Hz, 2.4 Hz, 1 H), 7.21 (d, J = 2.4 Hz, 1 H), 7.95 (d, J = 8.9 Hz, 1 H), 8.69 (d, J = 7.6 Hz, 1 H); MS (ESI, MeOH/NH_4OH) m/z 437.2 [M + H], 459.2 [M + Na]; Anal. ($C_{24}H_{24}N_2O_6$·0.5H_2O) C, H, N.

7-Methoxy-4-oxo-4*H*-chromene-2-carboxylic Acid (1-Benzo-[1,3]dioxol-5-ylmethylpyrrolidin-3-yl)amide (14). A solution of **2B** (80 mg, 0.36 mmol) and 3-aminopyrrolidine-1-carboxylic acid *tert*-butyl ester (81 mg, 0.43 mmol) were coupled in a manner similar to method E, followed by treatment with 4 N HCl in dioxane to give 7-methoxy-4-oxo-4*H*-chromen-2-carboxylic acid pyrrolidine-3-ylamide (**14A**). Compound **14A** and benzo[1,3]dioxole-5-carbaldehyde (45 mg, 0.30 mmol) were processed in a manner similar to method F to give the title compound **14** (100 mg, 66% yield): ^{1}H NMR (DMSO-d_6) δ 1.81−1.89 (m, 1H), 2.14−2.23 (m, 1H), 2.47 (m, 2H), 2.62−2.71 (m, 1H), 2.75−2.79 (m, 1H), 3.52 (s, 2H), 3.93(s, 3H), 4.35−4.43 (m, 1H), 5.98 (s, 2 H), 6.74−6.78 (m, 2H), 6.83−6.89 (m, 2H), 7.10 (dd, J = 8.9 Hz, 2.5 Hz, 1 H), 7.24 (d, J = 2.5 Hz, 1 H), 7.95 (d, J = 8.9 Hz, 1 H), 8.96 (d, J = 7.4 Hz, 1 H); MS (ESI, MeOH/NH_4OH) m/z 423.2 [M + H], 445.2 [M + Na]; Anal. ($C_{23}H_{22}N_2O_6$·0.5H_2O) C, H, N.

7-Methoxy-4-oxo-4*H*-chromene-2-carboxylic Acid [2-(1-Benzo-[1,3]dioxol-5-ylmethylpyrrolidin-2-yl)ethyl]amide (15). A solution of **2B** (80 mg, 0.36 mmol) and 2-(2-aminoethyl)pyrrolidine-1-carboxylic acid *tert*-butyl ester (92 mg, 0.43 mmol) were coupled in a manner similar to method E, followed by treatment with 4 N HCl in dioxane to give 7-methoxy-4-oxo-4*H*-chromen-2-carboxylic acid (2-pyrrolidin-2-ylethyl)amide (**15A**). Compound **15A** and benzo[1,3]dioxole-5-carbaldehyde (47 mg, 0.31 mmol) were processed in a manner similar to method F to give the title compound **15** (115 mg, 71% yield): ^{1}H NMR (DMSO-d_6) δ 1.49−1.71 (m, 4H), 1.87−1.99 (m, 2H), 2.06−2.13 (m, 1H), 2.49 (m, 1H), 2.78−2.82 (m, 1H), 3.09−3.12 (m, 1H), 3.34−3.44 (m, 2H), 3.88−3.92 (m, 4H), 5.90 (d, J = 12.0 Hz, 2 H), 6.73−6.78 (m, 3H), 6.83 (s, 1H), 7.06 (d, J = 2.5 Hz, 1 H), 7.24 (dd, J = 8.9 Hz, 2.5 Hz, 1 H), 7.95 (d, J = 8.9 Hz, 1 H), 9.18 (t, J = 5.5 Hz, 1 H); MS (ESI, MeOH/NH_4OH) m/z 451.2 [M + H], 473.2 [M + Na]; Anal. ($C_{25}H_{26}N_2O_6$·0.5H_2O) C, H, N.

***N*-[1-(1,3-Benzodioxol-5-ylmethyl)pyrrolidin-3-ylmethyl]-7-methoxy-4-oxo-4*H*-chromene-2-carboxamide (16).** 7-Methoxy-4-oxo-4*H*-chromene-2-carboxylic acid (**2B**) (110 mg, 0.500 mmol) and 3-(aminomethyl)-1-*N*-Boc-pyrrolidine (100 mg, 0.500 mmol) were processed as described in method E to give (1-*N*-Boc-pyrrolidin-3-ylmethyl)-7-methoxy-4-oxo-4*H*-chromene-2-carboxamide (**16A**) as a yellow solid (167 mg, 83%). Trifluoroacetic acid (0.5 mL) was added dropwise to compound **16A** (165 mg) and CH_2Cl_2 (1 mL) at 0 °C. After 1 h, volatiles were removed, and the resulting yellow oil was triturated with 2 N HCl in ether (1 mL). The supernatant was removed and the resulting yellow salt was combined with piperonal (65 mg, 0.46 mmol) and processed as detailed in method F to give the title compound **16** (30 mg, 17% over two steps) as a white solid: ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.43−1.54 (m, 1 H), 1.83−1.95 (m, 1 H), 2.27−2.39 (m, 2 H), 2.39−2.49 (m, 2 H), 2.51−2.57 (m, 2 H), 3.23−3.30 (m, 2 H), 3.47 (s, 2 H), 3.92 (s, 3 H), 5.96 (s, 2 H), 6.73−6.86 (m, 3 H), 7.11 (dd, J = 9.0, 2.5 Hz, 1 H), 7.17 (d, J = 2.4 Hz, 1 H), 7.95 (d, J = 8.8 Hz, 1 H), 9.10 (t, J = 5.6 Hz, 1 H); MS (ESI, MeOH/NH_4OH) m/z 437 [M + H], 459 [M + Na], 435 [M − H]; Anal. ($C_{24}H_{24}N_2O_6$·0.5H_2O) C, H, N.

***N*-[1-(1,3-Benzodioxol-5-ylmethyl)piperidin-4-yl]-7-hydroxy-4-oxo-4*H*-chromene-2-carboxamide (17).** Commercially available ethyl 7-hydroxy-4-oxo-4*H*-chromene-2-carboxylate (1.00 g, 4.27 mmol), glacial acetic acid (17 mL), and 6 N aqueous HCl (8.5 mL) were combined and heated at 80 °C for 16 h before the mixture was allowed to cool to room temperature. The resulting pale yellow solid was collected by filtration to give 7-hydroxy-4-oxo-4*H*-chromene-2-carboxylic acid (**17A**) as a pale yellow solid (820 mg, 93%). Compound **17A** (800 mg, 3.88 mmol) and 1-(1,3-benzodioxol-5-ylmethyl)piperidin-4-amine dihydrochoride **1B** (1.28 g, 4.07 mmol) were processed as detailed in method E to give the title compound **17** (909 mg, 54%) as an off-white solid: ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.64−1.74 (m, 2 H), 1.76−1.84 (m, 2 H), 2.00−2.16 (m, 2 H), 2.87 (m, 2 H), 3.45 (s, 2 H), 3.72−3.85 (m, 1 H), 6.00 (s, 2 H), 6.70 (s, 1 H), 6.74−6.80 (m, 1 H), 6.84−6.97 (m, 3 H), 7.02 (d, J = 2.0 Hz, 1 H), 7.88 (d, J = 8.8 Hz, 1 H), 8.83 (d, J = 8.1 Hz, 1 H), 10.98 (s, 1 H); MS (ESI, MeOH/NH_4OH) m/z 421 [M − H]; HPLC method A (t_R = 4.90 min, 100%), method B (t_R = 5.0 min, 98.5%).

***N*-[1-(1,3-Benzodioxol-5-ylmethyl)piperidin-4-yl]-7-(difluoromethoxy)-4-oxo-4*H*-chromene-2-carboxamide (18).** A solution of **17** (0.011 g, 0.026 mmol) in DMF (1 mL) at −78 °C was treated with liquid chlorodifluoromethane (1 mL) and K_2CO_3 (0.011 g, 0.078 mmol). The cold bath was removed, and the suspension was allowed to warm to ambient temperature and stirred for 2 h. The reaction mixture was purified by preparative reverse-phase HPLC as described in the general section to provide **18**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.80 (m, 2 H), 2.07 (m, 2 H), 3.09 (m, 2 H), 3.43 (m, 2 H), 3.99 (m, 1 H), 4.22 (m, 2 H), 6.09 (s, 2 H), 6.85 (s, 1 H), 6.96 (m, 1H), 7.02 (dd, 1 H), 7.08 (d, 1 H), 7.35 (dd, 1 H), 7.50 (m, 1 H), 7.51 (t, 1 H), 8.10 (d, 1 H), 9.08 (d, 1 H); MS (ESI) m/z 473 [M + H]$^+$.

***N*-[1-(1,3-Benzodioxol-5-ylmethyl)piperidin-4-yl]-7-ethyl-4-oxo-4*H*-chromene-2-carboxamide (19).** 3-Ethylphenol (5 g, 40.9 mmol), pyridine (8.3 mL, 102 mmol), acetic anhydride (4.3 mL, 45 mmol), and dichloromethane (10 mL) were stirred at room temperature overnight. The solution was washed with water and brine, dried (Na_2SO_4), and concentrated to dryness. All of the crude product was suspended in BF_3−OEt_2 (40 mL) and heated to reflux. After 2 h, the solution was cooled to ambient temperature, diluted with water (20 mL), and extracted with ethyl acetate (3 × 25 mL). The combined organics were washed with water and brine, dried (Na_2SO_4), and concentrated to give 1-(4-ethyl-2-hydroxyphenyl)-ethanone (**19A**). The crude **19A** was processed according to general method B to give 7-ethyl-4-oxo-4*H*-chromene-2-carboxylic acid (**19B**, 1.9 g, 21% from 3-ethylphenol). Compound **19B** (50 mg, 0.23 mmol) and 1-benzo[1,3]dioxol-5-ylmethylpiperidin-4-ylamine dihydrochloric acid salt (70 mg, 0.23 mmol) were processed according to general method E to give title compound **19** (83 mg, 84%): ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.19−1.30 (m, 3 H), 1.80−1.91 (m, 1 H), 1.99−2.13 (m, 3 H), 2.74−2.84 (m, 2 H), 3.00−3.15 (m, 2 H), 3.41 (s, 2 H), 4.01 (d, J = 11.5 Hz, 1 H), 4.21 (d, J = 4.8 Hz, 2 H), 6.07−6.11 (m, 2 H), 6.96−7.09 (m, 3 H), 7.42 (td, J = 7.2, 1.5 Hz, 1 H), 7.57−7.61 (m, 1 H), 7.97 (t, J = 7.5 Hz, 1 H), 9.08 (d, J = 7.1 Hz, 1 H), 9.46 (s, 1 H); MS (ESI) m/z 435 [M + H]$^+$, HPLC method A (t_R = 5.44 min, 100%), method B (t_R = 5.55 min, 100%).

***N*-[1-(1,3-Benzodioxol-5-ylmethyl)piperidin-4-yl]-7-bromo-4-oxo-4*H*-chromene-2-carboxamide (20).** 3-Bromophenol (5 g, 28.9 mmol), pyridine (5.8 mL, 72 mmol), acetic anhydride (3.0 mL, 31.8 mmol), and dichloromethane (6 mL) were stirred at room temperature overnight. The solution was washed with water and brine, dried (Na_2SO_4), and concentrated to dryness. All of the crude product was suspended in BF_3−OEt_2 (25 mL) and heated to reflux. After 2 h, the solution was allowed to cool to room temperature, diluted with water (20 mL), extracted with ethyl acetate (3 × 25 mL), washed with water and brine, dried (Na_2SO_4), and concentrated to dryness to give 1-(4-bromo-2-hydroxyphenyl)ethanone (**20A**, 5.5 g, 89%). The crude **20A** was processed according to general method B to give 7-bromo-4-oxo-4*H*-chromene-2-carboxylic acid (**20B**, 2.1 g, 31%). 1-Benzo[1,3]dioxol-5-ylmethyl-piperidin-4-ylamine (37 mg, 0.12 mmol) and **20B** (32 mg, 0.12 mmol) were processed according to general method E to give the title compound **20** (14 mg, 24%): ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.63 (qd, 2 H, J = 11.9, 3.7 Hz), 1.71−1.87 (m, 2 H), 1.96−2.07 (m, 2 H), 2.83 (d, 2 H, J = 11.2 Hz), 3.39 (s, 2 H), 3.67−3.86 (m, 1 H), 5.99 (s, 2 H), 6.75 (dd, 1 H, J = 7.9, 1.5 Hz), 6.82 (s, 1 H), 6.85 (d, 1 H, J = 7.7 Hz), 6.86 (s, 1 H), 7.72 (dd, 1 H, J = 8.5, 1.8 Hz), 7.96 (d,

1 H, $J = 8.4$ Hz), 8.06 (d, 1 H, $J = 1.8$ Hz), 8.80 (d, 1 H, $J = 7.9$ Hz); MS (ESI) *m*/*z* 485 $[M + H]^+$. Anal. ($C_{23}H_{21}BrN_2O_5$) C, H, N.

***N*-[1-(1,3-Benzodioxol-5-ylmethyl)piperidin-4-yl]-7-chloro-4-oxo-4*H*-chromene-2-carboxamide (21).** 4-Chloro-2-methoxybenzoic acid (5 g, 26.8 mmol) was processed as detailed in method C followed by method B to provide 7-chloro-4-oxo-4*H*-chromene-2-carboxylic acid (**21A**) as a beige solid (204 mg). Compound **21A** (202 mg, 0.899 mmol) and 4-amino-1-*N*-Boc-piperidine (189 mg, 0.944 mmol) were processed as detailed in method E to give *tert*-butyl 4-{[(7-chloro-4-oxo-4*H*-chromen-2-yl)carbonyl]amino}piperidine-1-carboxylate (**21B**) as a yellow solid (240 mg, 66%). Trifluoroacetic acid (2 mL) was added dropwise to compound **21B** and CH_2Cl_2 (4 mL) at 0 °C. After 1 h, volatiles were removed, and the residue was diluted with 1 N aqueous HCl (40 mL) and washed with Et_2O (2 × 20 mL). The aqueous layer was combined with saturated aqueous K_2CO_3 until basic and extracted with EtOAc (3 × 20 mL). Organic extracts and washes were combined, washed with brine, dried ($MgSO_4$), filtered, and concentrated to give (piperidin-4-yl)-7-chloro-4-oxo-4*H*-chromene-2-carboxamide (**21C**) as an amber solid (120 mg, 67%). Compound **21C** (1.19 g, 3.88 mmol) and piperonal (583 mg, 3.88 mmol) were processed as described in method F to provide the title compound **21** (345 mg, 20%) as a white solid: ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.56−1.69 (m, 2 H), 1.76−1.84 (m, 2 H), 1.96−2.06 (m, 2 H), 2.79−2.87 (m, 2 H), 3.39 (s, 2 H), 3.72−3.81 (m, 1 H), 5.99 (s, 2 H), 6.73−6.77 (m, 1 H), 6.82−6.87 (m, 3 H), 7.59 (dd, $J = 8.5$, 2.0 Hz, 1 H), 7.90 (d, $J = 1.7$ Hz, 1 H), 8.04 (d, $J = 8.8$ Hz, 1 H), 8.81 (d, $J = 7.8$ Hz, 1 H); MS (ESI, MeOH/NH_4OH) *m*/*z* 441 [M + H], 439 [M − H]; Anal. ($C_{23}H_{21}ClN_2O_5$) C, H, N.

***N*-[1-(1,3-Benzodioxol-5-ylmethyl)piperidin-4-yl]-7-fluoro-4-oxo-4*H*-chromene-2-carboxamide (22).** 4′-Fluoro-2′-hydroxyacetophenone (5.00 g, 32.4 mmol) was processed as described in method B to give ethyl 7-fluoro-4-oxo-4*H*-chromene-2-carboxylate (**22A**) as a white solid (4.6 g, 68%). Compound **22A** (2.00 g, 8.47 mmol) and 4-amino-1-*N*-Boc-piperidine (1.87 g, 9.32 mmol) were coupled as described in method E to provide *tert*-butyl 4-{[(7-fluoro-4-oxo-4*H*-chromen-2-yl)carbonyl]amino}piperidine-1-carboxylate (**22B**) as a pale yellow solid (2.9 g, 88%). Trifluoroacetic acid (7.4 mL) was added dropwise to compound **22B** (2.90 g, 7.43 mmol) and CH_2Cl_2 (16 mL) at 0 °C. After 1 h, volatiles were removed, and the resulting yellow residue was triturated with 2 N HCl in ether. The supernatant was removed, and the resulting yellow salt (**22C**) (450 mg, 1.55 mmol) and piperonal (244 mg, 1.63 mmol) were processed as described in method F to provide the title compound **22** as a white solid (532 mg, 81%): ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.51−1.72 (m, 2 H), 1.72−1.87 (m, 2 H), 1.92−2.10 (m, 2 H), 2.83 (d, 2 H, $J = 11.8$ Hz), 3.39 (s, 2 H), 3.70−3.85 (m, 1 H), 5.99 (s, 2 H), 6.75 (dd, 1 H, $J = 7.9$, 1.4 Hz), 6.81−6.87 (m, 3 H), 7.43 (td, 1 H, $J = 8.7$, 2.5 Hz), 7.62 (dd, 1 H, $J = 9.5$, 2.5 Hz), 8.11 (dd, 1 H, $J = 8.9$, 6.4 Hz), 8.83 (d, 1 H, $J = 8.1$ Hz); MS (APCI) *m*/*z* 425 $[M + H]^+$; Anal. ($C_{23}H_{21}FN_2O_5$) C, H, N.

***N*-[1-(1,3-Benzodioxol-5-ylmethyl)piperidin-4-yl]-7-cyano-4-oxo-4*H*-chromene-2-carboxamide (23).** *N*-[1-(1,3-Benzodioxol-5-ylmethyl)piperidin-4-yl]-7-bromo-4-oxo-4*H*-chromene-2-carboxamide (**20**, 10 mg, 0.02 mmol), zinc cyanide (3 mg, 0.025 mmol), tetrakis(triphenylphosphine) palladium(0) (10 mg, 0.009 mmol), and DMF (500 μL) were stirred at 130 °C for 16 h. The reaction was allowed to cool to room temperature, filtered through Celite, and purified by preparative reversed-phase HPLC as described in the general section to give the title compound **23** (1.5 mg, 17%): ^{1}H NMR (300 MHz, Solvent) δ 1.92−1.99 (m, 2 H), 2.24 (s, 2 H), 3.16 (d, $J = 2.4$ Hz, 1 H), 3.53−3.62 (m, 3 H), 4.21−4.26 (m, 3 H), 6.03 (s, 2 H), 6.92−6.95 (m, 1 H), 6.98−7.02 (m, 3 H), 7.04 (s, 1 H), 7.83 (dd, $J = 8.1$, 1.4 Hz, 1 H), 8.13 (s, 1 H), 8.28 (d, $J = 8.1$ Hz, 1 H); MS (ESI) *m*/*z* 432 $[M + H]^+$; HPLC method A ($t_R = 5.24$ min, 100%), method B ($t_R = 5.37$ min, 100%).

***N*-[1-(1,3-Benzodioxol-5-ylmethyl)piperidin-4-yl]-7-trifluoromethyl-4-oxo-4*H*-chromene-2-carboxamide (24).** Iodomethane (3.4 mL, 54 mmol) was added to a stirred solution of 2-hydroxy-4-(trifluoromethyl)benzoic acid (5.00 g, 24.3 mmol), potassium carbonate (8.40 g, 53.5 mmol), and DMF (50 mL), and the system was heated at 90 °C for 20 h. The resultant milky solution was allowed to cool to room temperature; diluted with Et_2O (250 mL); washed with distilled water, 1 N aqueous NaOH, 1 N aqueous HCl, and brine; dried ($MgSO_4$); filtered; and concentrated to give methyl 2-methoxy-4-(trifluoromethyl)benzoate (**24A**) as an off-white solid (4.40 g, 77%). Compound **24A** was combined with 6 M aqueous NaOH in methanol (25 mL) and heated at 80 °C for 3 h before the solution was allowed to cool to room temperature and diluted with 6 M aqueous HCl (16 mL) and distilled water. The resulting white precipitate was collected by filtration to give 2-methoxy-4-(trifluoromethyl)benzoic acid (**24B**) as white crystals (3.35 g, 81%). Compound **24B** was processed in a manner analogous to method C followed by method B to give 4-oxo-7-(trifluoromethyl)-4*H*-chromene-2-carboxylic acid (**24C**) as a white solid (290 mg). Compound **24C** (287 mg, 1.11 mmol) and 4-amino-1-*N*-Boc-piperidine (245 mg, 1.22 mmol) were processed as detailed in method E to give *tert*-butyl 4-{[(7-trifluoro-4-oxo-4*H*-chromen-2-yl)carbonyl]amino}piperidine-1-carboxylate (**24D**) as an amber solid (335 mg, 69%). Trifluoroacetic acid (1.5 mL) was added dropwise to compound **24D** (335 mg, 0.760 mmol) and CH_2Cl_2 (3 mL) at 0 °C. After 1 h, volatiles were removed, and the resulting dark residue was diluted with CH_2Cl_2 (40 mL), washed with aqueous K_2CO_3 (40 mL) and brine, dried (Na_2SO_4), filtered, and concentrated to give (piperidin-4-yl)-7-trifluoromethyl-4-oxo-4*H*-chromene-2-carboxamide (**24E**) as an amber solid (84 mg, 32%). Compound **24E** (20 mg, 59 μmol) and piperonal (9 mg, 59 μmol) were processed as detailed in method F to give the title compound **24** as a white solid (12 mg, 43%): ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.56−1.70 (m, 2 H), 1.77−1.85 (m, 2 H), 1.97−2.08 (m, 2 H), 2.80−2.88 (m, $J = 11.5$ Hz, 2 H), 3.40 (s, 2 H), 3.74−3.86 (m, 1 H), 5.99 (s, 2 H), 6.73−6.77 (m, 1 H), 6.83−6.89 (m, 3 H), 7.86 (d, $J = 8.1$ Hz, 1 H), 8.19−8.26 (m, 2 H), 8.88 (d, $J = 8.1$ Hz, 1 H); MS (ESI) *m*/*z* 475 $[M + H]^+$, 473 [M − H]; HPLC method A ($t_R = 5.61$ min, 100%), method B ($t_R = 5.68$ min, 100%).

***N*-[1-(1,3-Benzodioxol-5-ylmethyl)piperidin-4-yl]-6-chloro-4-oxo-4*H*-chromene-2-carboxamide (25).** Commercially available 6-chloro-4-oxo-4*H*-chromene-2-carboxylic acid (2.8 g, 12.5 mmol) was coupled to 4-amino-1-BOC-piperidine (2.5 g, 12.5 mmol) using general method E to provide *tert*-butyl 4-{[(6-chloro-4-oxo-4*H*-chromen-2-yl)carbonyl]amino}piperidine-1-carboxylate (**25A**). To a solution of **25A** (2.5 g, 6.1 mmol) in 16 mL of dichloromethane at 0 °C was added trifluoroacetic acid (8 mL). The mixture was allowed to slowly warm to room temperature, stirred for 2 h, and then concentrated to give a yellow oil. The oil was diluted in 95/5 dichloromethane/methanol and washed with 1 M K_2CO_3, the aqueous layer was extracted with additional dichloromethane, and the organic extracts were dried (Na_2SO_4) and concentrated to provide 6-chloro-4-oxo-*N*-piperidin-4-yl-4*H*-chromene-2-carboxamide (**25B**). Reductive alkylation of **25B** (31 mg, 0.1 mmol) with piperonal (15 mg, 0.1 mmol) was accomplished by general method F. Filtration of semipurified material through a plug of silica gel (2 g, SepPak), followed by rinsing through with 95/5 dichloromethane/methanol and concentration, provided the title compound **25**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.65 (m, 2 H), 1.77 (m, 2 H), 1.99 (m, 2 H), 2.83 (m, 2 H), 3.39 (s, 2 H), 3.78 (m, 1 H), 5.97 (s, 1 H), 5.99 (s, 2 H), 6.80 (m, 1 H), 6.90 (m, 2 H), 7.82 (d, $J = 8.8$ Hz, 1 H), 7.97 (m, 2 H), 8.89 (d, $J = 8.1$ Hz, 1 H); MS (ESI, MeOH/NH_4OH) *m*/*z* 441 [M + H], 439 [M − H]; Anal. ($C_{23}H_{21}ClN_2O_5 \cdot 0.5H_2O$) C, H, N.

4-Oxo-7-trifluoromethyl-4*H*-chromene-2-carboxylic Acid (1-Benzo[1,3]dioxol-5-ylmethyl-piperidin-4-yl)amide (26). 4-Oxo-7-trifluoromethyl-4*H*-chromene-2-carboxylic acid (10 mg, 0.039 mmol, synthesized from 4-trifluoromethylphenol according to general method D) was coupled to 1-benzo[1,3]dioxol-5-ylmethylpiperidin-4-ylamine (13 mg, 0.055 mmol, **1B**) according to general method E. Purification by preparative reverse phase chromatography as described in the general section followed by partition between dichloromethane and aqueous 1 M K_2CO_3 provided the title compound **26** as an off-white solid (15 mg, 83%): ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.59−1.72 (m, 2 H)

1.74−1.86 (m, 2 H), 1.94−2.10 (m, 2 H), 2.77−2.91 (m, 2 H), 3.27−3.30 (m, 3 H), 3.37−3.44 (m, 2 H), 3.69−3.92 (m, 1 H), 5.99 (s, 2 H), 6.71−6.80 (m, 1 H), 6.85 (d, J = 8.1 Hz, 2 H), 6.91 (s, 1 H), 7.99 (d, J = 8.5 Hz, 1 H), 8.21−8.33 (m, 2 H), 8.95 (d, J = 8.1 Hz, 1 H); MS (ESI, MeOH/NH_4OH) *m/z* 475 [M + H], 473 [M − H], Anal. ($C_{24}H_{21}F_3N_2O_5 \cdot 0.5H_2O$) C, H, N.

***N*-[1-(1,3-Benzodioxol-5-ylmethyl)piperidin-4-yl]-6-bromo-4-oxo-4*H*-chromene-2-carboxamide (27).** Commercially available 6-bromochromone-2-carboxylic acid (71 mg, 0.263 mmol) was coupled to 1-(1,3-benzodioxol-5-ylmethyl)piperidin-4-amine dihydrochoride (162 mg, 0.526 mmol) as described in method E to give the title compound **27** (70 mg, 55%) as a white solid: ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.60−1.70 (m, 2 H), 1.75−1.82 (m, 2 H), 1.96−2.05 (m, 2 H), 2.79−2.86 (m, 2 H), 3.39 (s, 2 H), 3.72−3.83 (m, 1 H), 5.99 (s, 2 H), 6.73−6.77 (m, 1 H), 6.83−6.86 (m, 3 H), 7.75 (d, J = 9.2 Hz, 1 H), 8.04−8.08 (m, 1 H), 8.11 (d, J = 2.7 Hz, 1 H), 8.89 (d, J = 7.8 Hz, 1 H); MS (APCI) *m/z* 485, 487 $[M + H]^+$, 483, 485 [M − H]; analytical HPLC method A (t_R = 5.47 min, 100%), method B (t_R = 5.57 min, 98.3%).

***N*-[1-(1,3-Benzodioxol-5-ylmethyl)piperidin-4-yl]-6-methyl-4-oxo-4*H*-chromene-2-carboxamide (28).** Commercially available 6-methylchromone-2-carboxylic acid (54 mg, 0.263 mmol) was coupled to 1-(1,3-benzodioxol-5-ylmethyl)piperidin-4-amine dihydrochoride (162 mg, 0.526 mmol) as described in method E to give the title compound **28** (56 mg, 51%) as clear, colorless crystals: ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.61−1.71 (m, 2 H), 1.75−1.82 (m, 2 H), 1.96−2.04 (m, 2 H), 2.44 (s, 3 H), 2.80−2.86 (m, 2 H), 3.39 (s, 2 H), 3.71−3.84 (m, 1 H), 5.99 (s, 2 H), 6.73−6.86 (m, 4 H), 7.65−7.73 (m, 2 H), 7.83 (br. s, 1 H), 8.85 (d, J = 7.8 Hz, 1 H); MS (APCI) *m/z* 421 $[M + H]^+$, 420 [M − H]; analytical HPLC method A (t_R = 5.26 min, 100%), method B (t_R = 5.40 min, 100%).

***N*-[1-(1,3-Benzodioxol-5-ylmethyl)piperidin-4-yl]-6-trifluoromethyl-4-oxo-4*H*-chromene-2-carboxamide (29).** 6-Trifluoromethoxychromone-2-carboxylic acid (29 mg, 0.106 mmol) and 1-(1,3-benzodioxol-5-ylmethyl)piperidin-4-amine dihydrochoride (21 mg, 0.0683 mmol) were processed as described in method E to give the title compound **29** (16 mg, 20%): ^{1}H NMR (500 MHz, pyridine-d_5) δ 2.00−2.09 (m, 2 H), 2.11−2.17 (m, 2 H), 2.30−2.39 (m, 2 H), 3.09−3.17 (m, 2 H), 3.67 (s, 2 H), 4.31−4.39 (m, 1 H), 6.00 (s, 2 H), 6.87−6.90 (m, 3 H), 7.07 (s, 1 H), 7.45−7.51 (m, 2 H), 8.16 (d, J = 1.9 Hz, 1 H), 9.80 (d, J = 7.8 Hz, 1 H); MS (APCI) *m/z* 491 $[M + H]^+$, 489 [M − H].

***N*-[1-(1,3-Benzodioxol-5-ylmethyl)piperidin-4-yl]-6-fluoro-4-oxo-4*H*-chromene-2-carboxamide (30).** Commercially available 6-fluoro-4-oxo-4*H*-chromene-2-carboxylic acid (2.8 g, 12.5 mmol) was coupled to 4-amino-1-BOC-piperidine (2.5 g, 12.5 mmol) using general method E to provide 4-[(6-fluoro-4-oxo-4*H*-chromene-2-carbonyl)amino]piperidine-1-carboxylic acid *tert*-butyl ester (**30A**). A solution of **30A** (2.5 g, 6.4 mmol) in 18 mL of dichloromethane at 0 °C was treated with 9 mL of trifluoroacetic acid, and the mixture was stirred for 2 h and then concentrated to give an orange oil. This oil was diluted with 95/5 dichloromethane/methanol and washed with 1 M K_2CO_3. The aqueous washes were back-extracted with additional dichloromethane, and the combined organic extracts were dried (Na_2SO_4), filtered, and concentrated under reduced pressure to provide 4-[(6-fluoro-4-oxo-4*H*-chromene-2-carbonyl)-amino]piperidine (**30B**, 1.4 g, 75%) Reductive alkylation of **30B** (29 mg, 0.1 mmol) with piperonal (15 mg, 0.1 mmol) according to general procedure F followed by flash silica gel chromatography eluting with dichloromethane/methanol provided the title compound **30** (33 mg, 79%): ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.64 (m, 2 H), 1.79 (m, 2 H), 1.99 (m, 2 H), 2.85 (m, 2 H), 3.39 (s, 2 H), 3.77 (m, 1 H), 5.97 (s, 1 H), 5.99 (s, 2 H), 6.75 (m, 2 H), 6.85 (m, 3 H), 7.74 (m, 1 H), 7.85 (m, 1 H), 8.88 (d, 1 H); MS (ESI, MeOH/NH_4OH) *m/z* 425 [M + H], 423 [M − H], 459 [M + Cl]; Anal. ($C_{23}H_{21}FN_2O_5 \cdot 0.25H_2O$) C, H, N.

***N*-[1-(1,3-Benzodioxol-5-ylmethyl)piperidin-4-yl]-4-oxo-4*H*-chromene-2-carboxamide (31).** Commercially available chromone-2-carboxylic acid (50 mg, 0.263 mmol) was coupled to 1-(1,3-benzodioxol-5-ylmethyl)piperidin-4-amine dihydrochoride (162 mg, 0.526 mmol) as described in method E to give the title compound **31** (82 mg, 77%) as a white solid: ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.59−1.72 (m, 2 H), 1.75−1.83 (m, 2 H), 1.96−2.07 (m, 2 H), 2.83 (d, J = 11.9 Hz, 2 H), 3.39 (s, 2 H), 3.73−3.83 (m, 1 H), 5.99 (s, 2 H), 6.73−6.77 (m, 1 H), 6.82−6.87 (m, 3 H), 7.51−7.56 (m, 1 H), 7.75−7.79 (m, 1 H), 7.90 (ddd, J = 8.7, 7.0, 1.7 Hz, 1 H), 8.05 (dd, J = 7.8, 1.4 Hz, 1 H), 8.88 (d, J = 7.8 Hz, 1 H); MS (APCI) *m/z* 407 $[M + H]^+$, 405 [M − H]; analytical HPLC method A (t_R = 5.08 min, 100%), method B (t_R = 5.24 min, 100%).

***N*-[1-(1,3-Benzodioxol-5-ylmethyl)piperidin-4-yl]-5-chloro-4-oxo-4*H*-chromene-2-carboxamide (32).** 3-Chlorophenol (10.0 g, 77.8 mmol) was processed as detailed in method D to obtain a mixture of 5-chloro-2-carboxychromone and 7-chloro-2-carboxychromone (2.36 g). This 2-carboxychromone mixture (0.224 mg, 1.13 mmol) and 4-amino-1-*N*-Boc-piperidine (340 mg, 1.70 mmol) were processed according to method E to provide the 5-chlorochromone-2-carboxamides and 7-chlorochromone-2-carboxamides (228 mg, 50%). Trifluoroacetic acid (1 mL) was added to the carboxamides (224 mg, 0.551 mmol) in CH_2Cl_2 (2 mL) at 0 °C. After 1 h, the solution was diluted with CH_2Cl_2 (40 mL) and Et_2O (15 mL) and extracted with 1 N aqueous HCl (2 × 30 mL). The aqueous extracts were combined and treated with aqueous K_2CO_3 until basic (pH 10). The aqueous layer was extracted with CH_2Cl_2 (3 × 40 mL); the organic extracts were combined, dried ($MgSO_4$), filtered, and concentrated to a fine yellow solid (83 mg, 49%). The yellow solid (80 mg, 0.261 mmol) was combined with piperonal (43 mg, 0.287 mmol) and processed according to method F to obtain a mixture of regioisomeric products. Purification by preparative reversed-phase HPLC as described in the general section provided title compound **32** (10 mg, 9%): ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.56−1.71 (m, 2 H), 1.72−1.83 (m, 2 H), 1.94−2.05 (m, 2 H), 2.78−2.87 (m, 3.39 Hz, 2 H), 3.39 (s, 2 H), 3.73−3.82 (m, 1 H), 5.99 (s, 2 H), 6.73−6.79 (m, 2 H), 6.81−6.89 (m, 2 H), 7.54 (dd, J = 7.8, 1.4 Hz, 1 H), 7.72−7.76 (m, 1 H), 7.78−7.83 (m, 1 H), 8.85 (d, J = 7.5 Hz, 1 H); MS (APCI) *m/z* 441 $[M + H]^+$; analytical HPLC method A (t_R = 5.29 min, 100%), method B (t_R = 5.41 min, 98.9%).

***N*-[1-(1,3-Benzodioxol-5-ylmethyl)piperidin-4-yl]-7-chloro-3-methyl-4-oxo-4*H*-chromene-2-carboxamide (33).** 7-Chloro-3-methyl-4-oxo-4*H*-chromene-2-carboxylic acid **33A** was prepared from 2-methoxy-4-chlorobenzoic acid as described in general method C, substituting ethylmagnesium bromide for methylmagnesium bromide, followed by method B. Compound **33A** (480 mg, 2 mmol) and 1-benzo[1,3]dioxol-5-ylmethylpiperidin-4-ylamine (680 mg, 2.2 mmol) were processed as detailed in method E to provide the title compound **33** as a white powder (630 mg, 69%): ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.54 (dq, J = 11.6, 3.3 Hz, 2 H), 1.80 (d, J = 11.6 Hz, 2 H), 2.01 (t, J = 11.6 Hz, 2 H), 2.09 (s, 3H), 2.78 (d, J = 11.6 Hz, 2 H), 3.37 (s, 2 H), 3.71−3.77 (m, 1 H), 5.97 (s, 2 H), 6.73 (dd, J = 7.9, 1.5 Hz, 1 H), 6.82−6.84 (m, 2 H), 7.54 (dd, J = 8.6, 1.8 Hz, 1 H), 7.90 (d, J = 1.8 Hz, 1 H), 8.03 (d, J = 8.6 Hz, 1 H), 8.83 (d, J = 7.6 Hz, 1 H); MS (ESI, MeOH/NH_4OH) *m/z* 455 [M + H], 453 [M − H]; analytical HPLC method A (t_R = 5.55 min, 100%), method B (t_R = 5.65 min, 100%).

***N*-[1-(1,3-Benzodioxol-5-ylmethyl)piperidin-4-yl]-7-chloro-6-fluoro-4-oxo-4*H*-chromene-2-carboxamide (34).** 3-Chloro-4-fluorophenol was processed according to general procedures A and B to give 7-chloro-6-fluoro-4-oxo-4*H*-chromene-2-carboxylic acid (**34A**). Compound **34A** (306 mg, 1.26 mmol) and 1-benzo[1,3]-dioxol-5-ylmethylpiperidin-4-ylamine dihydrochloric acid salt (407 mg, 1.32 mmol) were coupled using general method E to give the title compound **34** (400 mg, 69%): ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.60 (m, 2H), 1.80 (m, 2H), 2.00 (m, 2H), 2.80(m, 2H), 3.40 (s, 2H), 3.80 (m, 1H), 6.00 (s, 2H), 6.70−6.90 (m, 4H), 7.95 (d, 1H), 8.10 (d, 1H), 8.80 (d, 1H); MS (ESI) *m/z* 459$[M + H]^+$; analytical HPLC method A (t_R = 5.51 min, 100%), method B (t_R = 5.60 min, 92.0%).

***N*-[1-(1,3-Benzodioxol-5-ylmethyl)piperidin-4-yl]-6-fluoro-7-methoxy-4-oxo-4*H*-chromene-2-carboxamide (35).** A mixture of 1-(4,5-difluoro-2-hydroxyphenyl)ethanone (4.00 g, 23.2 mmol), K_2CO_3 (3.84 g, 27.8 mmol), and MOM-Cl (2.12 mL, 27.8 mmol) in acetone (30 mL) was stirred at room temperature for 16 h. The

reaction was then quenched by the addition of H_2O and Et_2O. The layers were separated, and the aqueous layer was extracted with additional Et_2O (3 × 20 mL). The combined organic layers were dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The residue was purified by MPLC (10% EtOAc in hexane to 35% EtOAc in hexane) to yield 1-(4,5-difluoro-2-methoxymethoxyphenyl)ethanone (**35A**) as a colorless oil (3.75 g, 75%). To a solution of compound **35A** (3.00 g, 13.9 mmol) in MeOH (30 mL) was added solid NaOMe (11.3 g, 209 mmol) in three portions. The resulting solution was heated at 40 °C for 8 h. The cooled reaction was then quenched by addition of H_2O and EtOAc. The layers were separated, and the aqueous was extracted with additional EtOAc. The combined organic layers were dried (Na_2SO_4), filtered, and concentrated under reduced pressure to yield 1-(5-fluoro-4-methoxy-2-methoxymethoxyphenyl)ethanone (**35B**) as an oil (2.97 g, 94%) that was used without further purification. To an ambient solution of compound **35B** (2.97 g, 13.03 mmol) in MeOH (11 mL) and H_2O (11 mL) was added TFA (7.28 mL). The reaction was stirred at room temperature for 16 h and was then diluted with H_2O and Et_2O. The layers were separated, and the aqueous layer was extracted with additional Et_2O (3 × 25 mL). The combined organic layers were dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The residue was purified on SiO_2 gel by MPLC (15% EtOAc in hexane to 50% EtOAc in hexane) to yield 1-(5-fluoro-4-methoxy-2-hydroxyphenyl)ethanone (**35C**) (1.61 g, 67%). Compound **35C** (1.61 g, 8.75 mmol) was processed according to method B to give 6-fluoro-7-methoxy-4-oxo-4*H*-chromene-2-carboxylic acid (**35D**) as a beige solid (1.50 g, 72%). Compound **35D** (1.0 g, 4.20 mmol) and 1-(1,3-benzodioxol-5-ylmethyl)piperidin-4-amine dihydrochloride (1.61 g, 5.25 mmol) were processed in a manner similar to method E to give the title compound **35** as a white solid (572 mg, 45%) after purification of the crude residue by recrystallization from hot CH_3CN: ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.53−1.72 (m, 2 H), 1.72−1.87 (m, 2 H), 1.92−2.10 (m, 2 H), 2.84 (d, 2 H, J = 11.2 Hz), 3.39 (s, 2 H), 3.68−3.87 (m, 1 H), 4.02 (s, 3 H), 5.99 (s, 2 H), 6.75 (dd, 1 H, J = 7.9, 1.6 Hz), 6.78 (s, 1 H), 6.83−6.87 (m, 2 H), 7.46 (d, 1 H, J = 7.0 Hz), 7.73 (d, 1 H, J = 10.9 Hz), 8.82 (d, 1 H, J = 8.0 Hz); MS (ESI) m/z 455 $[M + H]^+$; analytical HPLC method A (t_R = 5.29 min, 100%), method B (t_R = 5.40 min, 100%).

***N*-[1-(1,3-Benzodioxol-5-ylmethyl)piperidin-4-yl]-6,7-difluoro-4-oxo-4*H*-chromene-2-carboxamide (36).** Employing method A, 3,4-difluorophenol (5 g, 38.5 mmol) was converted to 4′,5′-difluoro-2′-hydroxyacetophenone (**36A**), an off-white solid (4.23 g, 64%). Compound **36A** (1.39 g, 8.07 mmol) was converted to 6,7-difluoro-2-carboxychromone (**36B**), an off-white solid (1.04 g, 57%), though method B. Compound **36B** (1.03 g, 4.55 mmol) and 4-amino-N-1-Boc-piperidine (1.00 g, 5.01 mmol) were coupled as described in method E to give *tert*-butyl 4-{[(6,7-difluoro-4-oxo-4*H*-chromen-2-yl)carbonyl]amino}piperidine-1-carboxylate (**36C**) as an off-white solid (900 mg, 48%). Trifluoroacetic acid (3.5 mL) was added dropwise to compound **36C** (0.9 g, 2.2 mmol) and CH_2Cl_2 (7 mL) at 0 °C. After 1 h, volatiles were removed and the resulting orange residue was diluted with CH_2Cl_2 (100 mL), washed with aqueous K_2CO_3 (1 × 100 mL) and with brine (1 × 100 mL), dried (Na_2SO_4), filtered, and concentrated to *N*-[1-(1,3-benzodioxol-5-ylmethyl)piperidin-4-yl]-6,7-difluoro-4-oxo-4*H*-chromene-2-carboxamide (**36D**) as an orange solid (200 mg, 29%). Compound **36D** (140 mg, 0.454 mmol) and piperonal (75 mg, 0.500 mmol) were processed as detailed in method F to provide the title compound **36** (24 mg, 12%) as a pale orange solid: ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.52−1.71 (m, 2 H), 1.73−1.87 (m, 2 H), 1.94−2.10 (m, 2 H), 2.76−2.92 (m, 2 H), 3.35−3.46 (m, 2 H), 3.67−3.82 (m, 1 H), 5.99 (s, 2 H), 6.77 (s, 1 H), 6.81−6.92 (m, 3 H), 7.91 (dd, J = 10.5, 6.4 Hz, 1 H), 7.95−8.05 (m, 1 H), 8.84 (d, J = 8.1 Hz, 1 H); MS (ESI, MeOH/NH_4OH) m/z 443 $[M + H]^+$, 441 $[M - H]^+$; analytical HPLC method A (t_R = 5.34 min, 100%), method B (t_R = 5.42 min, 97.0%).

***N*-[1-(1,3-Benzodioxol-5-ylmethyl)piperidin-4-yl]-7,8-difluoro-4-oxo-4*H*-chromene-2-carboxamide (37).** Commercially available acetic acid 2,4-difluorophenyl ester was processed according to general methods A (skip acetylation part of procedure) and B to give 6,8-difluoro-4-oxo-4*H*-chromene-2-carboxylic acid (**37A**, 0.91 g, 69%). Compound **37A** (25 mg, 0.11 mmol) was coupled to 1-(1,3-benzodioxol-5-ylmethyl)piperidin-4-amine dihydrochloride (34 mg) using general method E to give the title compound **37** (12 mg, 25%): ^{1}H NMR (300 MHz, CD_3OD) δ 1.28−1.30 (m, 1 H), 1.75 (dd, J = 11.9, 3.4 Hz, 1 H), 1.92 (d, J = 4.4 Hz, 2 H), 2.14−2.23 (m, 2 H), 2.97 (d, J = 14.2 Hz, 2 H), 3.49 (s, 2 H), 3.90 (td, J = 10.5, 5.4 Hz, 1 H), 5.93 (s, 2 H), 6.75−6.79 (m, 2 H), 6.87 (s, 1 H), 6.97 (s, 1 H), 7.59−7.69 (m, 3 H); MS (APCI) m/z 443 $[M + H]^+$; analytical HPLC method A (t_R = 5.29 min, 100%), method B (t_R = 5.40 min, 100%).

***N*-[1-(1,3-Benzodioxol-5-ylmethyl)piperidin-4-yl]-5,6-dichloro-4-oxo-4*H*-chromene-2-carboxamide (38).** 3,4-Dichlorophenol (20.0 g, 123 mmol) was processed as detailed in method D to provide a mixture of dichloro-2-carboxychromone isomers (**38A**) (16.8 g, 53%). Compound **38A** (100 mg, 0.386 mmol) and 1-(1,3-benzodioxol-5-ylmethyl)piperidin-4-amine dihydrochoride (237 mg, 0.772 mmol) were processed as described in method E. The title compound **38** (9 mg, 5%) was separated from the undesired isomer through preparative reversed-phase HPLC as described in the general section: ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.60−1.70 (m, 2 H), 1.75−1.82 (m, 2 H), 1.95−2.05 (m, 2 H), 2.79−2.86 (m, 2 H), 3.39 (s, 2 H), 3.71−3.84 (m, 1 H), 5.99 (s, 2 H), 6.73−6.77 (m, 1 H), 6.80 (s, 1 H), 6.85 (d, J = 7.8 Hz, 2 H), 7.78 (d, J = 9.2 Hz, 1 H), 8.10 (d, J = 9.2 Hz, 1 H), 8.87 (d, J = 7.8 Hz, 1 H); MS (ESI, MeOH/NH_4OH) m/z 475 $[M + H]$, 473 $[M - H]$; analytical HPLC method A (t_R = 5.59 min, 100%), method B (t_R = 5.68 min, 98.4%).

***N*-[1-(1,3-Benzodioxol-5-ylmethyl)piperidin-4-yl]-6,7,8-trifluoro-4-oxo-4*H*-chromene-2-carboxamide (39).** 6,7,8-Trifluoro-4-oxo-4*H*-chromene-2-carboxylic acid (**39A**) was prepared from commercially available 2,3,4-trifluorophenol as detailed in method D. Compound **39A** (75 mg, 0.30 mmol) and 1-benzo[1,3]dioxol-5-ylmethylpiperidin-4-ylamine (99 mg, 0.32 mmol) were coupled as detailed in method E to provide the title compound **39** as a tan powder (94 mg, 67%): ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.65 (dq, J = 11.6, 3.4 Hz, 2 H), 1.77 (d, J = 11.6 Hz, 2 H), 2.00 (t, J = 11.6 Hz, 2 H), 2.81 (d, J = 11.6 Hz, 2 H), 3.38 (s, 2 H), 3.71−3.80 (m, 1 H), 5.99 (s, 2 H), 6.74 (d, J = 7.9 Hz, 1 H), 6.83−6.85 (m, 2 H), 6.93 (s, 1H), 7.88 (t, J = 7.9, 1 H), 8.85 (d, J = 7.9 Hz, 1 H); MS (ESI, MeOH/NH_4OH) m/z 461 $[M + H]$, 459 $[M - H]$; analytical HPLC method A (t_R = 5.42 min, 100%), method B (t_R = 5.53 min, 100%).

***N*-[1-Benzylpiperidin-4-yl]-7-fluoro-4-oxo-4*H*-chromene-2-carboxamide (40).** Compound **22C** (30 mg, 0.103 mmol) and benzaldehyde (16 mg, 0.155 mmol) were processed in a manner analogous to method F to provide the title compound **40** (32 mg, 82%): ^{1}H NMR (500 MHz, pyridine-d_5) δ 1.80−1.88 (m, 2 H), 2.01−2.08 (m, 4 H), 2.81−2.86 (m, 2 H), 3.40 (s, 2 H), 4.20−4.29 (m, 1 H), 6.48 (dd, J = 9.4, 2.2 Hz, 1 H), 7.03−7.08 (m, 1 H), 7.11−7.16 (m, 2 H), 7.26−7.31 (m, 1 H), 7.44 (s, 1 H), 8.28 (dd, J = 8.9, 6.4 Hz, 1 H), 9.52 (d, J = 7.5 Hz, 1 H); MS (ESI, MeOH/NH_4OH) m/z 381 $[M + H]$, 379 $[M - H]$; analytical HPLC method A (t_R = 5.14 min, 100%), method B (t_R = 5.26 min, 100%).

***N*-[1-(1,3-Benzodioxol-4-ylmethyl)piperidin-4-yl]-7-fluoro-4-oxo-4*H*-chromene-2-carboxamide (41).** 2,3-(Methylenedioxy)-benzaldehyde (13 mg, 0.087 mmol) and compound **22C** (19 mg, 0.058 mmol) were processed in a manner analogous to method F to provide the title compound **41** as the TFA salt after purification by preparative reversed-phase HPLC as described in the general section (16 mg, 47%): ^{1}H NMR (500 MHz, pyridine-d_5) δ 2.22−2.27 (m, 2 H), 2.28−2.36 (m, 2 H), 2.52−2.60 (m, 2 H), 3.26−3.32 (m, J = 12.2 Hz, 2 H), 3.88 (s, 2 H), 4.29−4.36 (m, 1 H), 6.09 (s, 2 H), 6.91−7.00 (m, 2 H), 7.09 (d, J = 7.8 Hz, 1 H), 7.33−7.35 (m, 2 H), 7.65 (dd, J = 9.2, 2.3 Hz, 1 H), 8.31 (dd, J = 8.9, 6.4 Hz, 1 H); MS (ESI, MeOH/NH_4OH) m/z 425 $[M + H]^+$, 423 $[M - H]$; analytical HPLC method A (t_R = 5.20 min, 100%), method B (t_R = 5.33 min, 100%).

***N*-[1-[(2,2-Difluoro-1,3-benzodioxol-5-yl)methyl]piperidin-4-yl]-7-fluoro-4-oxo-4*H*-chromene-2-carboxamide (42).** Compound

22C (29 mg, 0.10 mmol) and 2,2-difluoro-1,3-(methylenedioxy)-benzaldehyde (19 mg, 0.10 mmol) were processed in a manner analogous to method F to provide the title compound **42** as a white solid: ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.58−1.71 (m, 2 H), 1.76−1.85 (m, 2 H), 1.99−2.10 (m, 2 H), 2.84 (d, J = 11.2 Hz, 2 H), 3.48−3.52 (m, 2 H), 3.74−3.82 (m, 1 H), 6.82 (s, 1 H), 7.14 (dd, J = 8.1, 1.4 Hz, 1 H), 7.32−7.37 (m, 2 H), 7.43 (td, J = 8.7, 2.7 Hz, 1 H), 7.61 (dd, J = 9.5, 2.4 Hz, 1 H), 8.11 (dd, J = 9.0, 6.3 Hz, 1 H), 8.83 (d, J = 7.8 Hz, 1 H); MS (APCI, MeOH/NH_4-OH) m/z 461 [M + H]$^+$, 459 [M − H].

***N*-{1-[(6-Chloro-1,3-benzodioxol-5-yl)methyl]piperidin-4-yl}-7-fluoro-4-oxo-4*H*-chromene-2-carboxamide** (43). Compound **22C** (19 mg, 0.065 mmol) and 6-chloropiperonal (12 mg, 0.065 mmol) were processed according to general method F to provide the title compound **43** (14.5 mg, 39%) as the trifluoroacetic acid salt after purification by preparative reversed-phase HPLC as described in the general section The TFA salt was dissolved in dichloromethane, washed with 1 M K_2CO_3, dried (Na_2SO_4), and concentrated to give the free base for elemental analysis: ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.57−1.72 (m, 2 H), 1.76−1.86 (m, 2 H), 2.07−2.19 (m, 2 H), 2.85 (d, 2 H, J = 11.5 Hz), 3.48 (s, 2 H), 3.74−3.88 (m, 1 H), 6.06 (s, 2 H), 6.83 (s, 1 H), 7.01 (s, 1 H), 7.05 (s, 1 H), 7.43 (td, 1 H, J = 8.7, 2.5 Hz), 7.61 (dd, 1 H, J = 9.5, 2.5 Hz), 8.12 (dd, 1 H, J = 8.9, 6.4 Hz), 8.83 (d, 1 H, J = 7.9 Hz); MS (ESI, MeOH/NH_4OH) m/z 459 [M + H]$^+$, 457 [M − H]$^+$; Anal. ($C_{23}H_{20}ClFN_2O_5 \cdot H_2O$) C, H, N.

***N*-[1-[(2,3-Dihydro-1,4-benzodioxin-6-yl)methyl]piperidin-4-yl]-7-fluoro-4-oxo-4*H*-chromene-2-carboxamide (44).** Compound **22C** (29 mg, 0.10 mmol) and 1,4-benzodioxan-6-carbaldehyde (17 mg, 0.10 mmol) were processed in a manner analogous to method F to provide the title compound **44** as a white solid (17 mg, 31%): ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.55−1.70 (m, 2 H), 1.75−1.83 (m, 2 H), 1.95−2.04 (m, 2 H), 2.79−2.86 (m, 2 H), 3.36 (s, 2 H), 3.72−3.82 (m, J = 8.1, 4.1 Hz, 1 H), 4.20 (s, 4 H), 6.72−6.82 (m, 4 H), 7.43 (td, J = 8.7, 2.4 Hz, 1 H), 7.61 (dd, J = 9.5, 2.4 Hz, 1 H), 8.11 (dd, J = 8.8, 6.4 Hz, 1 H), 8.82 (d, J = 7.8 Hz, 1 H); MS (APCI, MeOH/NH_4OH) m/z 439 [M + H]$^+$, 437 [M − H]; analytical HPLC method A (t_R = 5.23 min, 100%), method B (t_R = 5.32 min, 96.02%).

***N*-[1-[(3,4-Dimethoxybenzyl)methyl]piperidin-4-yl]-7-fluoro-4-oxo-4*H*-chromene-2-carboxamide (45).** Compound **22C** (22.5 mg, 0.0681 mmol) and 3,4-dimethoxybenzaldehyde (19 mg, 0.102 mmol) were processed in a manner analogous to method F to provide the title compound **45** (32 mg, 74%): ^{1}H NMR (500 MHz, pyridine-d_5) δ 1.86−1.95 (m, 2 H), 2.03−2.12 (m, 4 H), 2.92−2.98 (m, 2 H), 3.44 (s, 2 H), 3.72 (s, 3 H), 3.77−3.79 (m, 3 H), 4.29 (m, 1 H), 6.44 (dd, J = 9.3, 2.3 Hz, 1 H), 6.92−6.98 (m, 2 H), 7.15 (td, J = 8.5, 2.1 Hz, 1 H), 7.46 (s, 1 H), 8.29 (dd, J = 8.9, 6.4 Hz, 1 H), 9.65 (d, J = 7.9 Hz, 1 H); MS (ESI, MeOH/NH_4OH) m/z 441 [M + H]$^+$, 439 [M − H]; analytical HPLC method A (t_R = 5.12 min, 100%), method B (t_R = 5.21 min, 97.8%).

***N*-[1-[(3-Methoxybenzyl)methyl]piperidin-4-yl]-7-fluoro-4-oxo-4*H*-chromene-2-carboxamide (46).** Compound **22C** (30 mg, 0.103 mmol) and 3-methoxybenzaldehyde (21 mg, 0.155 mmol) were processed in a manner analogous to method F to provide the title compound **46** (35 mg, 83%): ^{1}H NMR (500 MHz, pyridine-d_5) δ 1.81−1.90 (m, 2 H), 2.02−2.09 (m, 4 H), 2.88−2.93 (m, 2 H), 3.45 (s, 2 H), 3.68 (s, 3 H), 4.21−4.29 (m, 1 H), 6.45 (dd, J = 9.4, 2.2 Hz, 1 H), 6.93 (dd, J = 8.1, 2.5 Hz, 1 H), 7.00 (d, J = 7.5 Hz, 1 H), 7.09 (s, 1 H), 7.14 (td, J = 8.6, 2.5 Hz, 1 H), 7.29 (t, J = 7.8 Hz, 1 H), 7.44 (s, 1 H), 8.28 (dd, J = 8.9, 6.4 Hz, 1 H), 9.51 (d, J = 7.5 Hz, 1 H); MS (ESI, MeOH/NH_4OH) m/z 411 [M + H]$^+$; analytical HPLC method A (t_R = 5.24 min, 100%), method B (t_R = 5.33 min, 97.1%).

***N*-[1-[(4-Methoxybenzyl)methyl]piperidin-4-yl]-7-fluoro-4-oxo-4*H*-chromene-2-carboxamide (47).** Compound **22C** and 3-methoxybenzaldehyde were processed in a manner analogous to method F to provide the title compound **47** as a solid: ^{1}H NMR (500 MHz, pyridine-d_5) δ 1.82−1.91 (m, 2 H), 2.01−2.10 (m, 4 H), 2.88−2.93 (m, 2 H), 3.41 (s, 2 H), 3.69 (s, 3 H), 4.24−4.30 (m, 1 H), 6.43 (d, J = 9.4 Hz, 1 H), 6.98 (d, J = 8.4 Hz, 2 H), 7.10−7.15 (m, 1 H), 7.31 (d, J = 8.7 Hz, 2 H), 7.44 (s, 1 H), 8.28 (dd, J = 8.7, 6.2 Hz, 1 H), 9.55 (d, J = 7.5 Hz, 1 H); MS (ESI, MeOH/NH_4OH) m/z 411 [M + H]$^+$, 409 [M − H]; analytical HPLC method A (t_R = 5.21 min, 100%), method B (t_R = 5.31 min, 98.1%).

***N*-[1-[(2-Fluorobenzyl)methyl]piperidin-4-yl]-7-fluoro-4-oxo-4*H*-chromene-2-carboxamide (48).** Compound **22C** (19 mg, 0.058 mmol) and 2-fluorobenzaldehyde (11 mg, 0.087 mmol) were processed in a manner analogous to method F to provide the title compound **48** as the TFA salt after purification by preparative reversed-phase HPLC (7 mg, 20% yield): ^{1}H NMR (500 MHz, pyridine-d_5) δ 2.23 (s, 6 H), 3.10 (s, J = 11.5 Hz, 2 H), 3.74 (s, 2 H), 4.20−4.33 (m, 1 H), 7.19−7.25 (m, 1 H), 7.27 (t, J = 7.5 Hz, 1 H), 7.33−7.37 (m, 2 H), 7.41−7.48 (m, 1 H), 7.54−7.60 (m, 1 H), 7.66 (dd, J = 9.2, 2.3 Hz, 1 H), 8.31 (dd, J = 9.1, 6.2 Hz, 1 H); MS (APCI, MeOH/NH_4OH) m/z 399 [M + H]$^+$, 397 [M − H]; analytical HPLC method A (t_R = 5.15 min, 97.3%), method B (t_R = 5.27 min, 100%).

***N*-[1-[(3-Fluorobenzyl)methyl]piperidin-4-yl]-7-fluoro-4-oxo-4*H*-chromene-2-carboxamide (49).** Compound **22C** (30 mg, 0.103 mmol) and 3-fluorobenzaldehyde (19 mg, 0.155 mmol) were processed in a manner analogous to method F to provide the title compound **49** (28 mg, 68%): ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.66 (qd, J = 11.9, 3.9 Hz, 2 H), 1.76−1.86 (m, 2 H), 2.02−2.12 (m, 2 H), 2.84 (d, J = 11.9 Hz, 2 H), 3.52 (s, 2 H), 3.73−3.85 (m, 1 H), 6.82 (s, 1 H), 7.04−7.17 (m, 3 H), 7.33−7.46 (m, 2 H), 7.61 (dd, J = 9.5, 2.4 Hz, 1 H), 8.11 (dd, J = 8.8, 6.4 Hz, 1 H), 8.83 (d, J = 7.8 Hz, 1 H); MS (ESI, MeOH/NH_4OH) m/z 399 [M + H]$^+$, 397 [M − H]; analytical HPLC method A (t_R = 5.24 min, 100%), method B (t_R = 5.34 min, 100%).

***N*-[1-[(4-Fluorobenzyl)methyl]piperidin-4-yl]-7-fluoro-4-oxo-4*H*-chromene-2-carboxamide (50).** Compound **22C** (19 mg, 0.058 mmol) and 4-fluorobenzaldehyde (11 mg, 0.087 mmol) were processed in a manner analogous to method F to provide the title compound as a TFA salt after purification by preparative reversed-phase HPLC (16 mg, 47%): ^{1}H NMR (500 MHz, solvent) δ 2.26−2.35 (m, 4 H), 2.59−2.67 (m, 2 H), 3.27−3.33 (m, J = 12.2 Hz, 2 H), 3.94 (s, 2 H), 4.35−4.42 (m, 1 H), 7.20 (t, J = 8.7 Hz, 2 H), 7.33−7.35 (m, 2 H), 7.60−7.66 (m, 3 H), 8.31 (dd, J = 8.9, 6.4 Hz, 1 H); MS (APCI, MeOH/NH_4OH) m/z 399 [M + H]$^+$, 434 [M + Cl]; analytical HPLC method A (t_R = 5.24 min, 100%), method B (t_R = 5.33 min, 100%).

***N*-[1-[(2-Fluoro-4-methoxybenzyl)methyl]piperidin-4-yl]-7-fluoro-4-oxo-4*H*-chromene-2-carboxamide (51).** Compound **22C** (29 mg, 0.10 mmol) and 2-fluoro-4-methoxybenzaldehyde (16 mg, 0.10 mmol) were processed in a manner analogous to method F to provide the title compound as a white solid (25 mg, 58%): ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.54−1.68 (m, 2 H), 1.75−1.83 (m, 2 H), 1.99−2.10 (m, 2 H), 2.79−2.87 (m, 2 H), 3.46 (s, 2 H), 3.73 (m, 1 H), 3.76 (s, 3 H), 6.74−6.84 (m, 3 H), 7.28 (t, J = 8.8 Hz, 1 H), 7.43 (td, J = 8.7, 2.4 Hz, 1 H), 7.61 (dd, J = 9.5, 2.4 Hz, 1 H), 8.11 (dd, J = 8.8, 6.4 Hz, 1 H), 8.82 (d, J = 7.8 Hz, 1 H); MS (APCI, MeOH/NH_4OH) m/z 429 [M + H]$^+$, 427 [M − H]; analytical HPLC method A (t_R = 5.29 min, 100%), method B (t_R = 5.36 min, 95.1%).

***N*-[1-[(3-Fluoro-4-methoxybenzyl)methyl]piperidin-4-yl]-7-fluoro-4-oxo-4*H*-chromene-2-carboxamide (52).** Compound **22C** (29 mg, 0.10 mmol) and 3-fluoro-4-methoxybenzaldehyde (16 mg, 0.10 mmol) were processed in a manner analogous to method F to provide the title compound as a white solid (22 mg, 51%): ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.57−1.70 (m, 2 H), 1.80 (m, 2 H), 1.98−2.07 (m, 2 H), 2.79−2.86 (m, 2 H), 3.42 (s, 2 H), 3.72−3.81 (m, 1 H), 3.82 (s, 3 H), 6.82 (s, 1 H), 7.03−7.15 (m, 3 H), 7.43 (td, J = 8.7, 2.5 Hz, 1 H), 7.61 (dd, J = 9.5, 2.4 Hz, 1 H), 8.11 (dd, J = 9.0, 6.3 Hz, 1 H), 8.82 (d, J = 8.1 Hz, 1 H); MS (APCI, MeOH/NH_4OH) m/z 429 [M + H]$^+$, 427 [M − H]; Anal. ($C_{23}H_{22}F_2N_2O4$) C, H, N.

***N*-[1-{4-[(4-Aminopiperidin-1-yl)methyl]phenyl}ethanonyl]-7-fluoro-4-oxo-4*H*-chromene-2-carboxamide (53).** Compound **22C** (29 mg, 0.10 mmol) and 4-acetobenzaldehyde (15 mg, 0.10 mmol) were processed in a manner analogous to method F to provide the title compound as a white solid (25 mg, 58%): ^{1}H

NMR (300 MHz, DMSO-d_6) δ 1.59−1.73 (m, 2 H), 1.77−1.85 (m, 2 H), 2.03−2.13 (m, 2 H), 2.57 (s, 3 H), 2.80−2.87 (m, 2 H), 3.57 (s, 2 H), 3.75−3.84 (m, 1 H), 6.82 (s, 1 H), 7.39−7.48 (m, 3 H), 7.61 (dd, J = 9.5, 2.4 Hz, 1 H), 7.93 (d, J = 8.1 Hz, 2 H), 8.12 (dd, J = 9.0, 6.3 Hz, 1 H), 8.84 (d, J = 8.1 Hz, 1 H); MS (APCI, MeOH/NH_4OH) m/z 423 $[M + H]^+$, 421 $[M - H]$; Anal. ($C_{24}H_{23}FN_2O_4$) C, H, N.

***N*-[1-*N*-[1-{4-[(4-Aminopiperidin-1-yl)methyl]phenyl}ethanon-yl]-7-fluoro-4-oxo-4*H*-chromene-2-carboxamide (54).** Compound **22C** (29 mg, 0.10 mmol) and 4-acetamidobenzaldehyde (17 mg, 0.10 mmol) were processed in a manner analogous to method F to provide the title compound as a white solid (20 mg, 45%): ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.56−1.70 (m, 2 H), 1.75−1.84 (m, 2 H), 1.95−2.01 (m, 2 H), 2.02 (s, 3 H), 2.79−2.87 (m, 2 H), 3.38−3.43 (m, 2 H), 3.72−3.81 (m, 1 H), 6.81−6.82 (m, 1 H), 7.21 (d, J = 8.5 Hz, 2 H), 7.42 (td, J = 8.7, 2.4 Hz, 1 H), 7.52 (d, J = 8.5 Hz, 2 H), 7.61 (dd, J = 9.5, 2.4 Hz, 1 H), 8.11 (dd, J = 9.0, 6.3 Hz, 1 H), 8.82 (d, J = 7.8 Hz, 1 H), 9.88 (s, 1 H); MS (APCI, MeOH/NH_4OH) m/z 438 $[M + H]^+$, 436 $[M - H]$; analytical HPLC method A (t_R = 4.91 min, 100%), method B (t_R = 5.02 min, 100%).

In Vitro Compound Evaluation. MCHR1 Binding Assay Using IMR-32 Membrane Preparations. Inhibition of binding of the melanin concentrating hormone receptor by MCH was determined using cell membranes prepared from a subcloned cell line derived from IMR-32 cells (I3.4.2) and described in our publication.[22] In 96-well plates, I3.4.2 membranes (6 μg/well) were incubated in the presence of test compound in binding buffer (25 mM HEPES pH 7.4, 1 mM $CaCl_2$, 5 mM $MgCl_2$ and 0.5% BSA) and with 0.05 nM [^{125}I]MCH (PerkinElmer; 2200 Ci/mmol) per well for 60 min at room temperature. Nonspecific binding controls consisted of I3.4.2 membranes, 0.05 nM [^{125}I]MCH, and 300 nM human MCH. Total binding controls of I3.4.2 membranes and 0.05 nM [^{125}I]MCH were also included on each plate. The plates were centrifuged for 5 min at 1380*g* in a Beckman GS-6R desktop centrifuge. The reaction buffer was carefully aspirated from each well without disturbing the pellet. Wash buffer (25 mM HEPES, pH 7.4, 1 mM $CaCl_2$, 5 mM $MgCl_2$, and 0.5 M NaCl) was added to each well and then transferred to a 0.5% polyethylenimine-treated GF/B filtration plate (Packard) using a plate Filtermate Harvester (Packard). The filter plate was washed three times with wash buffer, MicroScint 20 (PerkinElmer) was added to each well, and the plate was read using a Topcount microplate scintillation counter (PerkinElmer).

Assay for Release of Intracellular Calcium. Activation of the melanin concentrating hormone receptor 1 by MCH induces the release of Ca^{2+} from intracellular stores. This intracellular Ca^{2+} release is measured using a fluorometric imaging plate reader (FLIPR, Molecular Devices Corp.) in conjunction with the Ca^{2+}-sensitive dye reagent (Calcium Assay Reagent, Molecular Devices). Release of $Ca^{2+}$from intracellular stores causes an increase in fluorescence of the dye that is proportional to Ca^{2+} concentration. Briefly, the assays are performed as follows. IMR-32 cells expressing G_α16 G protein were plated at 100 000 cells/well in 96-well plates. After 2 days, cells are loaded with the Calcium Assay Reagent for 1 h at room temperature. Test compounds are prepared at 60 μM in 6% DMSO. The cell plate is placed in the FLIPR and 50 μL/well of test compound is delivered. The calcium signal is followed for 3 min to assay for potential agonist activity by the test compounds. Then 50 μL/well of 6 μM human MCH (in D-PBS containing 0.1% BSA) was added and the ligand-induced calcium signal was followed for an additional 3 min. Antagonist activity as determined by the test compound's ability to inhibit 50% of MCH-induced Ca^{2+} flux is reported.

In Vivo Compound Evaluation. Pharmacokinetic Analysis of Plasma and Brain Exposure in DIO Mice. Male C57Bl/6J mice are placed on a diet of 60% kcal from fat (Reseach Diets D12492) for 3−4 months, during which time they become obese (45 g vs 30 g for lean controls on normal chow). Compounds are dosed in DIO-mice orally at 10 mg/kg in a vehicle containing 1% Tween-80 and water. Plasma samples are drawn and whole brains are harvested at 0.5, 1, 2, 4, 6, 8, 12, and 24 h after the dose, and drug-concentrations are determined by mass spectroscopy analysis in comparison with a standard curve. The three mice with highest concentrations were averaged to provide the peak plasma or brain concentration (C_{max}) ± SEM; the time for these three samples was averaged to provide the time to peak plasma or brain concentration (T_{max}) ± SEM. The mean plasma concentration data were submitted to multiexponential curve fitting using WinNonlin. The area under the mean plasma concentration−time curve from 0 to t h (time of the last measurable plasma concentration) after dosing (AUC_{0-t}) was calculated using the linear trapezoidal rule for the plasma concentration−time profile. The residual area was extrapolated to infinity, determined as the final measured mean plasma concentration (C_t) divided by the terminal elimination rate constant (β), and added to AUC_{0-t} to produce the total area under the curve ($AUC_{0-\infty}$).

Compound Evaluation in Anesthetized Rat Cardiovascular Assay. Male Sprague−Dawley rats (325−375 g) are anesthetized with the long-acting barbiturate inactin (100 mg/kg). Catheters (PE-50) were placed in the femoral artery for measurement of mean arterial pressure (MAP) and heart rate (HR). Hemodynamic data was acquired using a Ponemah software platform. (Gould Instrument Systems, Valley View, Ohio). Additional catheters were placed in the femoral vein for compound administration (1−2 mL/kg) and saline infusion (10 μL/min) to maintain hydration. A large blood sample was taken after the highest dose or when MAP decreased below 70 mmHg. This minimum pressure level was implemented to ensure a functional cardiovascular system for the collection of the end-of-study (EOS) blood sample used for drug plasma level determinations. A compromised vasculature can cause venous pooling and yield unrepresentative plasma results. Escalating doses were given in half log increments, such that the complete dose was administered by the end of each 30-min infusion period. The highest doses infused were either 100 or 300 mg/kg, representing the limits of solubility achieving the highest possible plasma concentrations. These high doses required a vehicle of poly(ethylene glycol) 400 (PEG 400), which produces cardiovascular effects (increasing MAP and decreasing HR). These effects seen with PEG 400 are minimally variable and highly reproducible. All cardiovascular results are expressed as mean ± SEM, (n = 3 rats per compound). This level of quantitation was used to define screening of MCHr1 antagonists for cardiovascular effects.

Compound Evaluation in the Anesthetized Dog Cardiovascular Assay.[19] Male beagle dogs (n = 3/group) were anesthetized with pentobarbital (35.0 mg/kg, iv) and immediately placed on a constant iv infusion (6.0 mg/kg/h, 0.21 mL/min) to maintain a surgical plane of anesthesia as previously described.[14] Animals were intubated with a cuffed endotracheal tube and ventilated with room air by means of a mechanical respiration pump (Harvard Apparatus, Model 613). Expiratory CO_2 was monitored with an end-tidal CO_2 monitor (Criticare Systems; Model POET TE) and maintained at 4−5%. Briefly, polyethylene catheters were inserted into the right femoral vein and artery for infusion of test agents and collection of blood samples, respectively. A Swan-Ganz catheter (5.5 F) was advanced into the pulmonary artery via the right jugular vein for measurement of cardiac output via thermodilution utilizing a cardiac output computer (Abbott Laboratories, Oximetrix 3). Central venous pressure and pulmonary arterial pressure were measured through the distal port of the catheter. A dual-tip micromanometer catheter (Millar, Model SPC-770, 7F) was advanced into the left ventricle of the heart via the right carotid artery for measurement of left ventricular and aortic blood pressure. The primary hemodynamic variables were recorded using commercial software and a signal processing workstation (Ponemah, Gould Instrument Systems, Inc). Animals were randomly divided into one of five treatment or vehicle (PEG-400) groups. Following the completion of the surgical protocol, animals were allowed to stabilize for 1 h and baseline data were collected at 5-min intervals.

hERG Affinity Assay.[18] Membranes were prepared as described in our publication; frozen membrane aliquots were thawed and homogenized again in a glass Dounce homogenizer (approximately

10 passes). The following were added to each 200-μL well: 20 μL of assay buffer, 1 μM astemizole (for nonspecific bounds) or test compound, 50 μL of [^{3}H]dofetilide, and 130 μL of membrane homogenate (final protein concentration = 30 μg/well). The plates were incubated at 37 °C for 45 min, aspirated onto GF/B filter plates, and washed with 2 mL of cold wash buffer. The radioactivity was counted and the ligand concentration was adjusted on the basis of the calculated K_d at each K^+ concentration. The signal-to-noise ratio was calculated using the following equation: (total bounds − nonspecific bounds)/nonspecific bounds.

hERG Ionic Current Studies.[21] hERG channels were stably expressed in HEK 293 cells and maintained in Minimal Eagle Medium (MEM) supplemented with 10% fetal bovine serum, 1% penicillin−streptomycin, 2 mM L-glutamine, 0.1 mM nonessential amino acids, 1 mM sodium pyruvate and 200 mg/mL of G418. Media were changed every 48 h and cells were passaged weekly. Currents were recorded using a MultiClamp 700A and pClamp data acquisition software. Patch pipets were constructed with borosilicate glass capillary tubes (resistance 1.5−2.9 MΩ). The pipet solution contained (in mM) K-aspartate, 125; KCl, 20; EGTA, 10; $MgCl_2$, 1; HEPES, 5; MgATP, 5; pH 7.3. The bath solution contained (in mM) NaCl, 140; KCl, 5; $MgCl_2$, 1; $CaCl_2$, 2; glucose, 5; HEPES, 20; pH 7.4. Compounds were dissolved in DMSO and then diluted in the bath solution. Cells were exposed to a single concentration of drug. DMSO concentrations never exceeded 0.1% (by volume). Effects of compounds on hERG current were assessed using a voltage clamp protocol that stepped to 0 mV for 3 s, followed by a step to −50 mV for 4 s from a holding potential of −80 mV once every 15 s. Current measurements were made at 36.5−37 °C. IC_{50} values were calculated from the effect of compounds on hERG tail current at −50 mV corrected for run down/DMSO vehicle effects with a logistic fitting routine using the following equation: $y = [(A_1 - A_2)/(1 + (x/IC_{50}))^s] + A_2$, where A_1 = initial x value, A_2 = final x value, and s = slope.

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Supporting Information Available: Results from combustion analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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