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Discovery of Potent, Orally Bioavailable Phthalazinone Bradykinin B1 Receptor Antagonists

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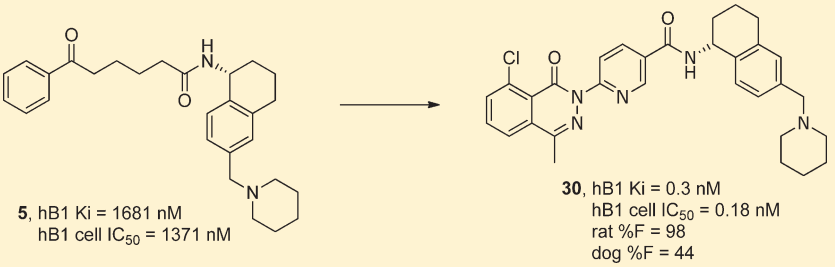
ABSTRACT:



The bradykinin B1 receptor is rapidly induced upon tissue injury and inflammation, stimulating the production of inflammatory mediators resulting in plasma extravasation, leukocyte trafficking, edema, and pain. We have previously reported on sulfonamide and sulfone-based B1 antagonists containing a privileged bicyclic amine moiety leading to potent series of 2-oxopiperazines. The suboptimal pharmacokinetics and physicochemical properties of the oxopiperazine sulfonamides led us to seek B1 antagonists with improved druglike properties. Using a pharmacophore model containing a bicyclic amine as anchor, we designed a series of amide antagonists with targeted physicochemical properties. This approach led to a novel series of potent phthalazinone B1 antagonists, where we successfully replaced a sulfonamide acceptor with a cyclic carbonyl unit. SAR studies revealed compounds with subnanomolar B1 binding affinity. These compounds demonstrate excellent cross-species PK properties with high oral bioavailability and potent activity in a rabbit biochemical challenge pharmacodynamic study.

INTRODUCTION

The kinins are a group of naturally occurring peptides, including the nonapeptide bradykinin (BK), the decapeptide kallidin (Lys-BK), and their active des-Arg$^{9/10}$ metabolites.[1] They are formed by the proteolytic action of plasma kallikrein and tissue kallikrein on precursors called high molecular weight and low molecular weight kininogens, respectively.[2] Kinins are important biological mediators in cardiovascular homeostasis, contraction and relaxation of smooth muscles, inflammation, and nociception.[3,4]

Kinins exert their biological influences by binding to and activating two cell surface G-protein-coupled receptors, the B1 and B2 receptors.[5] BK and Lys-BK activate the receptor B2 subtype, whereas des-Arg^9-BK and Lys-des-Arg^{10}-BK are inactive on this receptor. Conversely, des-Arg^9-BK and Lys-des-Arg^{10}-BK are ligands for the B1 receptor, with BK and Lys-BK being less potent. Both receptors are expressed on cells that initiate, exacerbate, and/or maintain inflammation and pain. They are linked via the G-proteins $G_{q\alpha/11}/G_{i\alpha1,2}$ to phospholipase C activation, leading to intracellular Ca^{2+} generation by inositol 1,4,5-phosphate.[6] There are, however, temporal differences in their expression, function, and regulation. B2 receptors are ubiquitously and constitutively expressed. B1 receptors, on the other hand, are absent or expressed at low levels in normal tissue but are induced/up-regulated following tissue injury and/or inflammation. After agonist stimulation, the B2 receptor is rapidly desensitized and internalized interrupting its function; this is in contrast to the B1 receptor that does not internalize upon activation.[7]

In vivo studies focusing on the role of B1 and B2 receptors in pain are consistent with their expression/regulation patterns. Preclinical pain models and knockout studies suggest that the B2 receptor plays a significant role in acute pain processing and the development of primary nociceptor sensitization and is the principal kinin receptor involved in cardiovascular and renal function. In contrast, the inducible nature of the B1 receptor suggests a limited role in acute pain processing but a prominent role in establishing and maintaining chronic pain. Evidence of relationship of the B1 receptor to chronic pain has come from model studies with synthetic antagonists. Peptide antagonists have been shown to reduce neurogenic pain induced by capsaicin as well as inflammatory pain induced by UV irradiation, carrageenan, complete Freund's adjuvant, or lipopolysaccharide.[8] The induction of functional B1 receptors and reversal of established hyperalgesia by B1 antagonists also have been demonstrated in models of neuropathic pain including streptozotocin-induced diabetes, chronic constriction injury, and partial sciatic nerve lesion.[9] These data suggest that the B1 receptor

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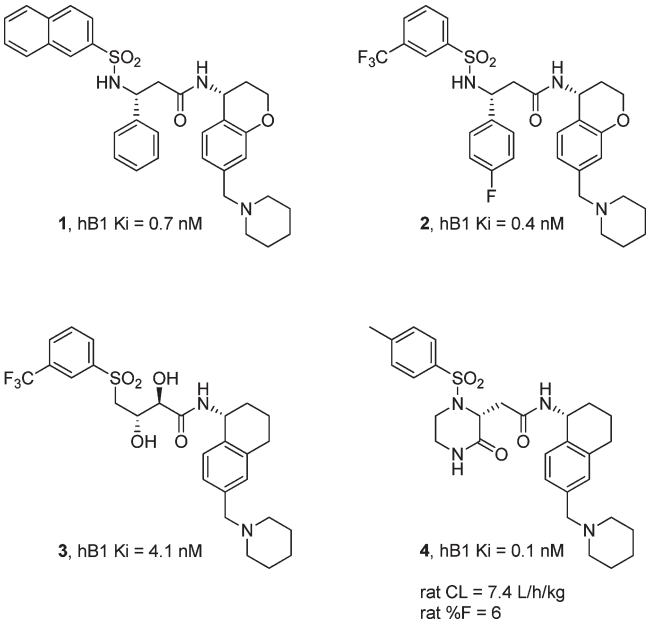


Figure 1. Amgen's first generation B1 receptor antagonists.

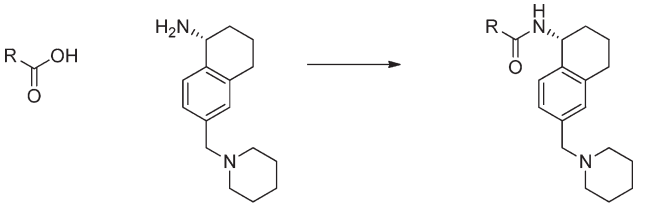
is involved in the potentiation, propagation, and maintenance of chronic pain. As such, the B1 receptor is an interesting therapeutic target for the treatment of chronic inflammatory and neuropathic pain conditions.

Along with studies with peptide antagonists, efforts toward developing small molecule antagonists of the B1 receptor by the biopharmaceutical industry have also been published. This work has been the subject of multiple recent reviews.[10] At Amgen, initial discovery of potent arylsulfonamide based antagonists containing a bicyclic amine motif (compound **1**, Figure 1)[11] was followed by SAR studies seeking to improve the PK and PD properties of the series (compounds **2** and **3**, Figure 1).[12,13] These experiments culminated in the synthesis of sulfonamides containing 2-oxopiperazine moieties as potent B1 antagonists and in the identification of compound **4** (Figure 1) as a lead compound.[14]

Further profiling of these compounds identified deficiencies in compound **4**, which were consistent across the 2-oxopiperazine class of B1 antagonists, making them undesirable for further development. This was exemplified by poor PK properties in rats, from high clearance to poor oral bioavailability (CL = 7.4 L h^{-1} kg^{-1}, F = 6% for compound **4**). We initiated a second generation discovery effort to address the identified liabilities. In particular, we sought B1 receptor antagonists that would possess improved rat PK properties (CL < 2 L h^{-1} kg^{-1}; F > 25%) while maintaining the antagonistic potency that distinguished the 2-oxopiperazine series. Since PD studies in our program have been conducted in rabbits, these new compounds would also need to display high binding affinity at the rabbit B1 receptor.[12]

To seek new antagonists of the B1 receptor with optimal PK properties, we employed a 2-fold approach of high-throughput screening and rational design. Unfortunately, a high-throughput rescreen of our corporate collection did not yield any validated hits suitable for optimization. Hence, we focused on identifying a new chemotype by design from first principles. To establish guidelines to drive the hit generation effort, we turned our attention to the physicochemical properties of compound **4** which could perhaps offer a rationale for suboptimal PK

Scheme 1. Preparation of Amide Targets[a]



[a] Reagents and conditions: EDCI/HOBt, DMF, room temp.

properties. These include high PSA (99), multiple hydrogen bond donors, and low lipophilicity (cLogD = 0.78). The relationship between physicochemical properties and in vivo PK has been the focus of elegant reviews.[15,16] We therefore sought to focus on new chemical matter that possessed druglike physicochemical properties, with PSA ≤ 80 and cLogD > 1, in the hopes of identifying B1 antagonists with improved PK properties. Examination of the first generation chemotypes suggested a pharmacophore containing a hydrophobic unit on the left and a hydrogen bond acceptor, a linker moiety, and a basic domain on the right occupied by the bicyclic amine.[17] Using this as a design template, we decided to employ the bicyclic amine in **4**, (*R*)-6-(piperidin-1-ylmethyl)-1,2,3,4-tetrahydronaphthalen-1-amine (compound **6**, Scheme 1), as an anchoring element and prepared a small 35-member amide library that contained linker units joining the amine to a hydrogen bond acceptor and a hydrophobic moiety (Figure 2a). Testing this library in human B1 binding and cellular assays revealed a hit, albeit of modest potency, compound **5** (hB1 binding K_i = 1681 nM, hB1 cellular IC_{50} = 1371 nM, Figure 2b).[18] Importantly, this compound displayed physicochemical properties that were in the range we had sought in a new hit (PSA = 49, cLogD = 3.57). With this result in hand, we embarked upon a lead identification and optimization effort that led to potent phthalazinone-based B1 receptor antagonists with excellent PK properties. This SAR effort is described herein, along with a PD study in rabbits with lead compounds.

RESULTS AND DISCUSSION

We adopted two strategies to increase the binding affinity of compound **5** for the human B1 receptor. One focused on the long aliphatic linker moiety in the middle of the molecule. We reasoned that decreasing the number of rotatable bonds in this section would attenuate the loss in entropy upon receptor binding. The other modification was incorporation of hydrophobic substituents onto the left phenyl ring, since we believed it occupied a hydrophobic pocket on the receptor. Continuing with the initial strategy of making small, focused libraries to advance the SAR studies, we obtained 40 commercial carboxylic acids that included one or both of the above structural features and coupled them to amine **6** (Scheme 1). The majority of this library was inactive in the hB1 binding assay except for the compounds shown in Figure 3. Compounds **7**−**9** shared the common feature of a phenyl ring in the central linker unit. Rigidifying the core of the molecule had led to a 3- to 5-fold increase in binding affinity (Figure 3). A carbonyl moiety, as the proposed required hydrogen bond acceptor, was also present in all three structures, similar to compound **5**.

With the success of applying the principle of reduction of rotatable bonds to improving binding affinity, we asked the

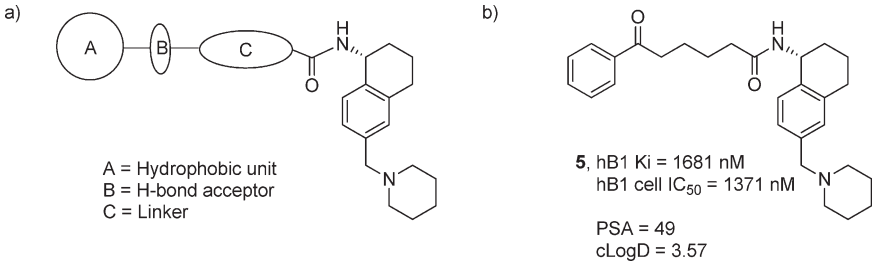


Figure 2. (a) Library design. (b) Identified hit with desirable physicochemical properties.

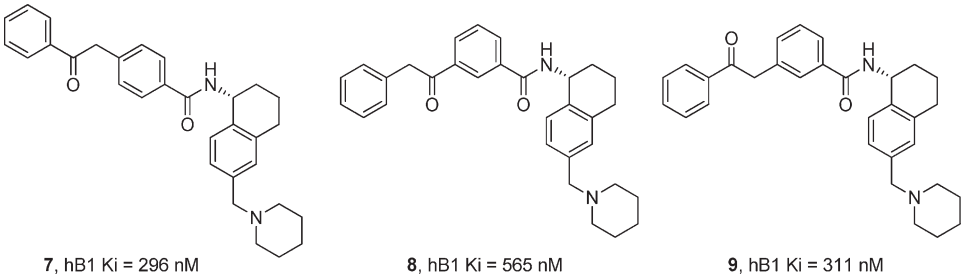


Figure 3. Compounds with measurable hB1 binding affinity from second amide library.

question if a further restriction in the degrees of freedom of the structures in Figure 3 would "lock" the conformation into a better binding mode. To address this, compounds **10**−**19** shown in Table 1 were synthesized and tested in the hB1 binding assay. These compounds possessed a 6,6-bicyclic unit on the left-hand sector of the molecule, fixing the location of the carbonyl H-bond acceptor compared to the precursor molecules in Figure 3. The amide linkages for each structure were examined in both the meta (1,3-) and the para (1,4-) orientation for each bicyclic moiety.

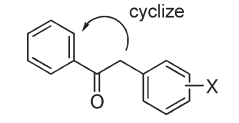
As can be seen from Table 1, this approach led to further gains in B1 receptor binding affinity. Certain structures were more preferred than the others; chromenones **10** and **11** (hB1 K_i = 64 and 183 nM, respectively), thiochromemones **14** and **15** (hB1 K_i = 159 and 18 nM, respectively), and phthalazinone **17** (hB1 K_i = 35 nM) were among the most potent compounds in this series. Other bicycles like the 1-methyl-3-phenylquinolin-4(1*H*)-ones (**12** and **13**) and 3-phenylquinazolin-4(3*H*)-ones (**18** and **19**) were less potent.

Since the key goal for this discovery effort was to identify compounds with improved PK properties, we decided to examine the more potent chemotypes from Table 1 in a rat PK study to select the best opportunities for further optimization. These data for compounds **11**, **15**, and **17** are shown in Table 2.

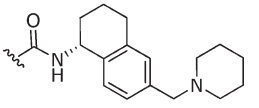
While all three compounds possessed acceptable PK properties in rat, phthalazinone **17** demonstrated the lowest clearance (0.9 L h^{-1} kg^{-1}) and the highest oral bioavailability (51%) in this group. Comparison of these data to the corresponding figures for compound **4** (CL = 7.4 L h^{-1} kg^{-1}, F = 6%) shows a marked improvement in the in vivo PK profile for this new generation of B1 antagonists. Encouraged by this result, we embarked upon further SAR studies that focused on optimizing the potency of phthalazinone **17** to identify candidates for further preclinical evaluation.

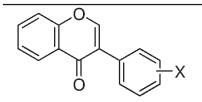
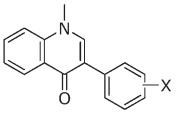
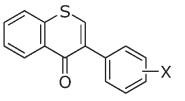
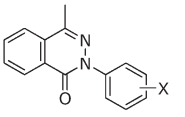
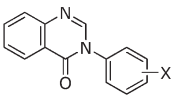
We first modified the 4-position of the phthalazinone bicycle to probe the effect of substitution and increased steric bulk at this site on receptor potency. These data are shown in Table 3. While changing the 4-methyl group from compound **17** to hydrogen

Table 1. Bicycle SAR: Cyclizing the Linker to the Hydrophobic Moiety[a]



X=



Structure	Compound	X-linkage	hB1 Ki (nM)
	**10**	*meta*	64±10
	11	*para*	183±32
	**12**	*meta*	331±263
	13	*para*	>10,000
	**14**	*meta*	159±38
	15	*para*	18±6
	**16**	*meta*	6,366±793
	17	*para*	35±8
	**18**	*meta*	>10,000
	19	*para*	614±27

[a] Values represent the average of three or more determinations ± SD.

(compound **20**, hB1 K_i = 52 nM) or 4-ethyl (compound **21**, hB1 K_i = 29 nM) had no significant effect on B1 potency, increasing

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the size of the 4-substituent led to loss in binding affinity. Isopropyl (compound **22**, hB1 K_i = 214 nM), trifluoromethyl (compound **23**, hB1 K_i = 98 nM), and phenyl (compound **24**, hB1 K_i = 418 nM) substitutions led to less potent analogues. For further explorations of the SAR, the methyl functionality was selected as the 4-subsituent.

Next, the effect of substitutions at the 5, 6, 7, or 8 position of the phthalazinone ring on receptor potency was examined in the binding assay. These data, with chlorine or fluorine atom substitution, are shown in Table 4. With the exception of the 7-position (compound **28**, hB1 K_i = 198 nM), the resulting compounds showed a marked improvement in potency compared to unsubstituted phthalazinone **17**. Of particular interest was compound **27**, with a chlorine atom at the 8-position of the phthalazinone ring. This compound possessed subnanomolar potency at the B1 receptor (hB1 K_i = 0.9 nM) and was now comparable to some of our most potent B1 antagonists listed in Figure 1. This exciting gain in binding affinity allowed us to progress into the next step in our optimization campaign of identifying lead candidates for in vivo pharmacodynamic and efficacy studies.

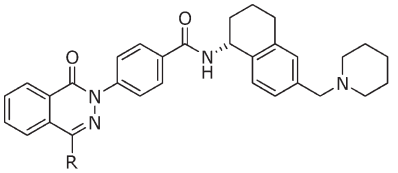
B1 receptor antagonism programs have been hampered by the lack of receptor homology across species, especially when considering preclinical studies in animal models of efficacy. The rabbit B1 receptor has a higher sequence homology to the human receptor (82%)[19] compared to the rat (71%).[20] Hence, we and others have evaluated the pharmacodynamic effects of B1 antagonists in vivo in rabbits.[12−14,21] For such studies to be feasible, the compounds under consideration have to be potent antagonists at

Table 2. In Vivo PK Parameters of Select Compounds from Table *1*

	rat			
compd	$t_{1/2}$ [a] (h)	CL [a] (L h^{-1} kg^{-1})	V_{ss} [a] (L/kg)	F [b] (%)
11	4.2	1.78	9.27	29
15	2.9	2.93	11.47	27
17	6.1	0.9	8.09	51

[a] Dosed iv at 2 mg/kg, formulated in 100% DMSO. [b] Dosed orally at 5 mg/kg, formulated in 1% Tween 80, 2% HPMC, pH 2.2; n = 3; interanimal variability was less than 20%.

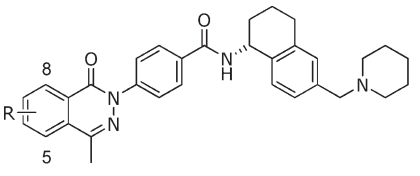
Table 3. SAR at the 4-Position of the Phthalazinones[a]



Compound	R	hB1 Ki (nM)
20	H	52±8
17	Me	35±8
21	Et	29±1
22	*i*Pr	214±35
23	CF_3	98±24
24	Ph	418±140

[a] Values represent the average of three or more determinations ± SD.

Table 4. SARs at the 5- to 8-Positions of the Phthalazinones[a]

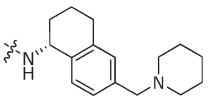


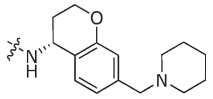
Compound	R	hB1 Ki (nM)
17	H	35±8
25	8-F	1.9±0.5
26	5,8-di-F	3.5±0.8
27	8-Cl	0.9±0.3
28	7-Cl	198±70
29	6-Cl	4.7±1.4

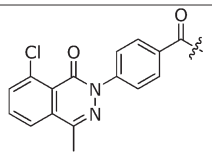
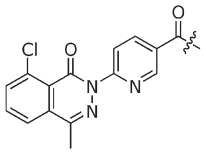
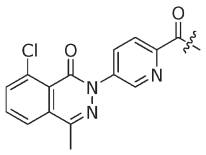
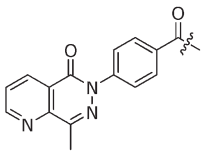
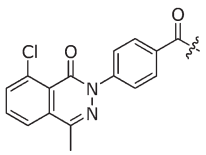
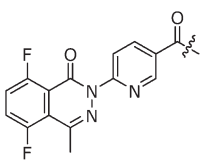
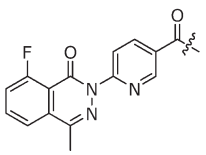
[a] Values represent the average of three or more determinations ± SD.

dx.doi.org/10.1021/jm200808v |*J. Med. Chem.* 2011, 54, 7232–7246

Table 5. Introduction of Heteroatoms and Effect on Human and Rabbit B1 Potency[a]

Amine A = 

Amine B = 

Compound	Acid fragment	Amine fragment	hB1 Ki (nM)	hB1 Cell IC_{50} (nM)	Rabbit B1 Cell IC_{50} (nM)
27		A	0.9±0.3	1.0±0.4	21.4±6.8
30		A	0.3±0.1	0.18±0.07	1.1±0.5
31		A	3.1±0.7	4.0±1.8	31.1±4.8
32		A	69.9±11.3	146.5±55.8	342.3[b]
33		B	2.5±0.8	1.1±0.1	17.5±1.2
34		A	0.6±0.4	0.3±0.1	2.2±0.7
35		A	0.6±0.4	0.09±0.02	0.6±0.3

[a] Values represent the average of three or more determinations ± SD. [b] $n = 1$.

the rabbit B1 receptor. As the next step in our SAR studies, we probed the effect of various heteroatom substitutions on potent fluoro- and chloro-substituted phthalazinones. In addition to evaluating the antagonistic activity at the human B1 receptor, we also measured the IC_{50} in a rabbit B1 cellular calcium-flux assay to find compounds that would possess optimal activity across

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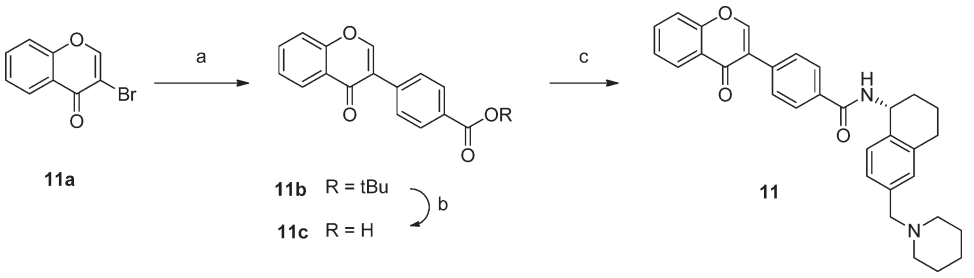
Table 6. Physicochemical and in Vivo PK Parameters of Select Compounds from Table 5

		rat PK				dog PK			
compd	PSA/cLogD	$t_{1/2}$ [a] (h)	CL [a] (L h^{-1} kg^{-1})	V_{ss} [a] (L/kg)	F [b] (%)	$t_{1/2}$ [c] (h)	CL [c] (L h^{-1} kg^{-1})	V_{ss} [c] (L/kg)	F [d] (%)
27	67/3.05	10.4	0.67	7.85	90	8.9	1.14	10.6	60
30	80/2.18	4.4	0.8	5.22	98	5.1	1.42	7.43	44
35	80/1.64	3.3	1.41	6.45	61	nd[e]	nd[e]	nd[e]	nd[e]

[a] Dosed iv at 2 mg/kg, formulated in 100% DMSO. [b] Dosed orally at 5 mg/kg, formulated in 1% Tween 80, 2% HPMC, pH 2.2. [c] Dosed iv at 1 mg/kg, formulated in 10% Captisol, pH 4.0. [d] Dosed orally at 1 mg/kg, formulated in 10% Captisol, pH 4.0. Interanimal variability was less than 20%. [e] nd = not determined.

Scheme 2. Synthesis of Chromenone 11[a]



[a] Reagents and conditions: (a) 4-(*tert*-butoxycarbonyl)phenylboronic acid, $Pd(PPh_3)_4$, 2.0 M sodium carbonate, dioxane, 140 °C (42%); (b) TFA; (c) **6**, EDCI, HOBt, DMF, room temp (31%, two steps).

species. These data are shown in Table 5. Interestingly, this set of modifications led to unexpected gains in potency at the rabbit receptor. The compounds were also tested in a functional assay in CHO cells expressing the human B1 receptor, verifying the antagonistic profile of each compound in an aequorin-based calcium flux assay. The human B1 receptor functional potency closely correlates with binding affinity SAR.

Compound **27**, which demonstrated subnanomolar potency at the human receptor, inhibited rabbit B1 receptor signaling with IC_{50} = 21 nM. Incorporation of a nitrogen atom in the core phenyl ring ortho to the phthalazinone substituent afforded the potent nicotinamide **30** (hB1 K_i = 0.3 nM, rabbit B1 cell IC_{50} = 1.1 nM). Addition of nitrogen to the other position of the core phenyl moiety did not offer any such gain with respect to rabbit potency (picolinamide **31**, hB1 K_i = 3.1 nM, rabbit B1 cell IC_{50} = 31 nM). Compound **32**, with nitrogen in the phthalazinone moiety, lost activity against both species, while replacing the tetralin amine in compound **27** with chroman in compound **33** had minimal affect on B1 antagonism. Fluorine-substituted phthalazinones when linked to the nicotinamide core (compounds **34** and **35**) gave rise to very potent compounds at both human and rabbit receptors, with compound **35** displaying subnanomolar potency at both receptors (hB1 K_i = 0.6 nM, rabbit B1 cell IC_{50} = 0.6 nM).

Select potent compounds were subsequently tested in rat and dog PK studies. As seen in Table 6, the lead phthalazinones possessed excellent oral bioavailability (61−98% in rats and 44−60% in dogs) and exhibited low clearance in both species. The physicochemical properties of the lead compounds are also included, demonstrating improvements over the 2-oxopiperazine **4** scaffold. Compounds **30** and **35** were tested against a 100-kinase panel from Ambit Biosciences, a GPCR panel at CEREP and the hERG, Kv1.5, and Nav1.5 ion channels, and showed no activity at 1 μM.

CHEMISTRY

The compounds in this report were synthesized by the coupling of (*R*)-6-(piperidin-1-ylmethyl)-1,2,3,4-tetrahydronaphthalen-1-amine (compound **6**) to various commercially available or synthesized carboxylic acids, employing EDCI/HOBt mediated amidation (Scheme 1).

Compounds **5**, **7**, **8**, and **9** were prepared from commercially available carboxylic acids as described in Scheme 1. Schemes 2 and 3 depict the syntheses of the para-substituted analogues shown in Table 1. The meta-substituted compounds were prepared in an analogous fashion, employing 3-(*tert*-butoxycarbonyl)phenylboronic acid in the Suzuki−Miyaura coupling step.

The synthesis of chromenone **11** is shown in Scheme 2. Commercially available 3-bromochromone (**11a**) was reacted with 4-(*tert*-butoxycarbonyl)phenylboronic acid in a palladium(0)-mediated Suzuki−Miyaura coupling reaction to afford the arylated ester **11b**. Deprotection of the *tert*-butyl ester with TFA and amide coupling with amine **6** afforded the final product, compound **11**.

Quinolinone **13** was prepared as shown in Scheme 3. Conjugate addition of *N*-methylaniline to acrylic acid followed by Freidel−Crafts acylation afforded the quinolinone core **13c**. Bromination, Suzuki−Miyaura coupling, and deprotection of the *tert*-butyl ester under acidic conditions gave acid **13f**, which was converted to the desired amide **13** as described previously.

Thiochromenone **15** was prepared in a sequence analogous to that for chromenone **11**, as shown in Scheme 2, beginning with the known 3-bromo-4*H*-thiochromen-4-one.[22] Phthalazinone **17** was prepared from commercially available 4-(4-methyl-1-oxophthalazin-2(1*H*)-yl)benzoic acid, and 4-oxoquinazoline **19** was synthesized from commercially available 4-(4-oxoquinazolin-3(4*H*)-yl)benzoic acid, using the amide coupling conditions in Scheme 1.

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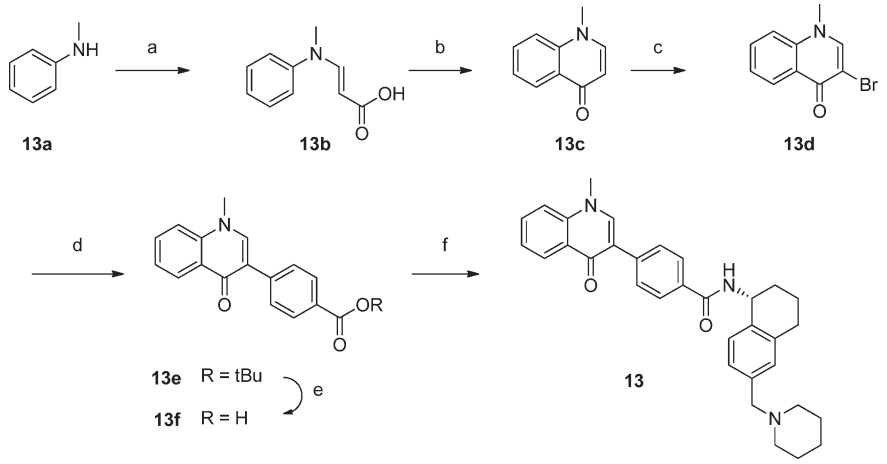
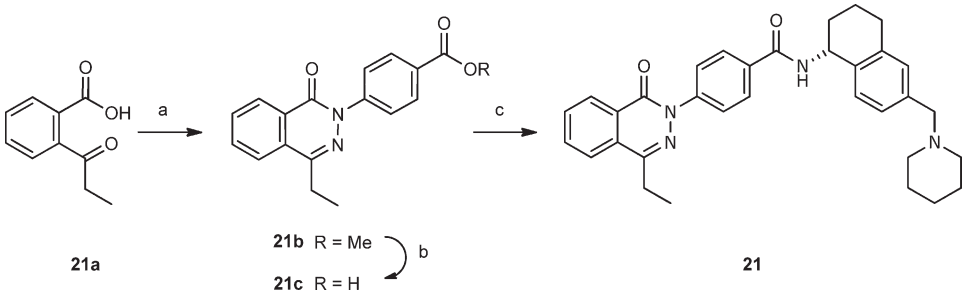
Scheme 3. Synthesis of Quinolinone 13[a]



[a] Reagents and conditions: (a) acrylic acid, toluene, heptanes, 40−80 °C (99%); (b) P_2O_5, methanesulfonic acid, 110 °C (19%); (c) bromine, HCl, dioxane, chloroform, room temp (64%); (d) 4-(*tert*-butoxycarbonyl)phenylboronic acid, $Pd(PPh_3)_4$, 2.0 M sodium carbonate, dioxane, 150 °C (10%); (e) TFA (98%); (f) **6**, EDCI, HOBt, DMF, room temp (52%).

Scheme 4. Synthesis of Phthalazinone 21[a]



[a] Reagents and conditions: (a) 4-hydrazinobenzoic acid, conc sulfuric acid, MeOH, 75 °C (57%); (b) 1 N HCl, 1,4-dioxane, reflux (99%); (c) **6**, EDCI, HOBt, DMF, room temp (79%).

For the compounds shown in Table 3, the preparation of phthalazinones **17**, **20**, and **24** followed the procedure outlined in Scheme 1, from commercially available substituted benzoic acids. Phthalazinone **21** was prepared from commercially available 2-propionylbenzoic acid **21a** as shown in Scheme 4. Acid-catalyzed condensation with 4-hydrazinobenzoic acid in methanol afforded the 4-phthalazinobenzoate **21b**, which was deprotected and coupled to amine **6**, affording the desired amide **21**. Compound **23** was synthesized by analogous route, employing 2-(2,2,2-trifluoroacetyl)benzoic acid as the starting material.

Isopropylphthalazinone **22** was prepared from methyl 2-iodobenzoate as shown in Scheme 5. The iodide was converted to the isopropyl ketone **22b** using Grignard conditions, which was then further processed to the final product as described in Scheme 4, using the same sequence of steps described previously.

Phthalazinones in Table 4 were prepared by analogous keto ester condensation with 4-hydrazinobenzoic acid. A representative example of compound **27** is shown in Scheme 6. 2-Bromo-6-chlorobenzoic acid was methylated using dimethyl sulfate to give ester **27b**, followed by Pd(0)-mediated Stille coupling with (1-ethoxyvinyl)tin. The resulting vinyl ether was hydrolyzed in situ with 6 N hydrochloric acid, affording the keto ester **27c**, which was converted to the desired product **27** using the chemistry described above.

The compounds in Table 5 were also prepared by a similar procedure, either using 4-hydrazinobenzoic acid to construct the phthalazinone core in compounds **27**, **32**, and **33** or using 6-hydrazinylnicotinic acid to synthesize the compounds **30**, **34**, and **35**. The synthesis of 5-hydrazinylpicolinate for making compound **31** is shown in Scheme 7. 5-Aminopicolinic acid was converted to the methyl ester **31b**, followed by treatment with sodium nitrite and hydrochloric acid. The resulting diazonium intermediate was reduced in situ to the hydrazine using tin(II) chloride, which was used to complete the synthesis in a similar fashion.

PHARMACOLOGY

Because of their desirable rabbit B1 potency and cross-species PK properties, the nicotinamide phthalazinones **30** and **35** were selected for evaluation in a rabbit preclinical pharmacology experiment. B1 agonists like des-Arg-kallidin (DAK) cause a drop in blood pressure in animals where receptor expression has been stimulated by administration of an inflammation source, for example, lipopolysaccharide. Treatment of these animals with B1

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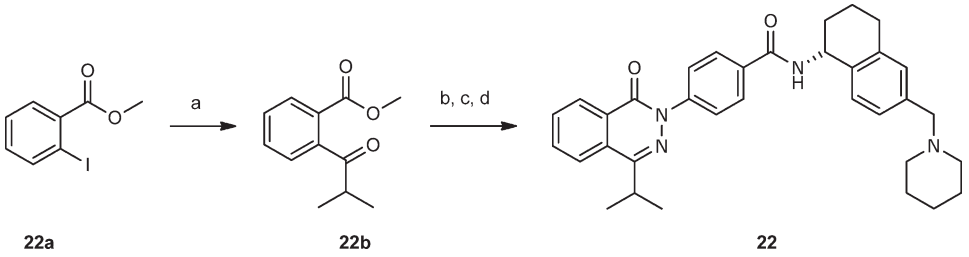
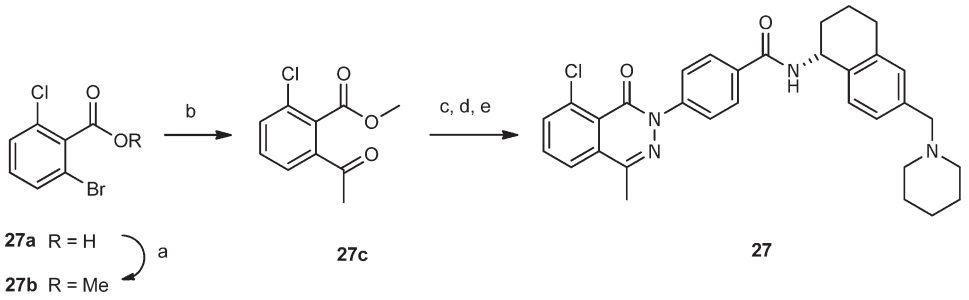
Scheme 5. Synthesis of Phthalazinone 22[a]



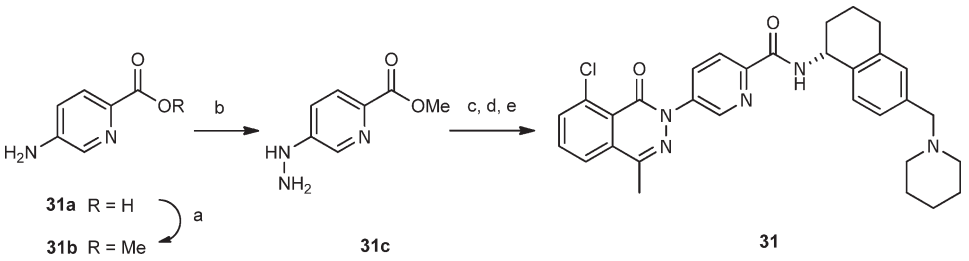
[a] Reagents and conditions: (a) ^{i}PrMgCl, THF, −78 °C, then isobutyryl chloride, −78 °C to room temp (15%); (b) 4-hydrazinobenzoic acid, conc sulfuric acid, methanol, 75 °C (22%); (c) 1 N HCl, 1,4-dioxane, reflux (99%); (d) **6**, EDCI, HOBt, DMF, room temp (40%).

Scheme 6. Synthesis of Phthalazinone 27[a]



[a] Reagents and conditions: (a) LiOH, Me_2SO_4, THF, 85 °C (97%); (b) (1-ethoxyvinyl)tin, $PdCl_2(PPh_3)_2$, 1,4-dioxane, 150 °C, then 6 N HCl, room temp (78%); (c) 4-hydrazinobenzoic acid, conc sulfuric acid, MeOH, 80 °C (71%); (d) 1 N HCl, 1,4-dioxane, reflux (99%); (e) **6**, EDCI, HOBt, DMF, room temp (94%).

Scheme 7. Synthesis of Phthalazinone 31[a]



[a] Reagents and conditions: (a) thionyl chloride, methanol, 70 °C (62%); (b) $NaNO_2$, 6 N HCl, 0 °C to room temp, then cool to 0 °C, $SnCl_2$, warm to 10 °C (67%); (c) **27c**, conc sulfuric acid, methanol, 80 °C (99%); (d) 1 N HCl, 1,4-dioxane, reflux (99%); (e) **6**, EDCI, HOBt, DMF, room temp (68%).

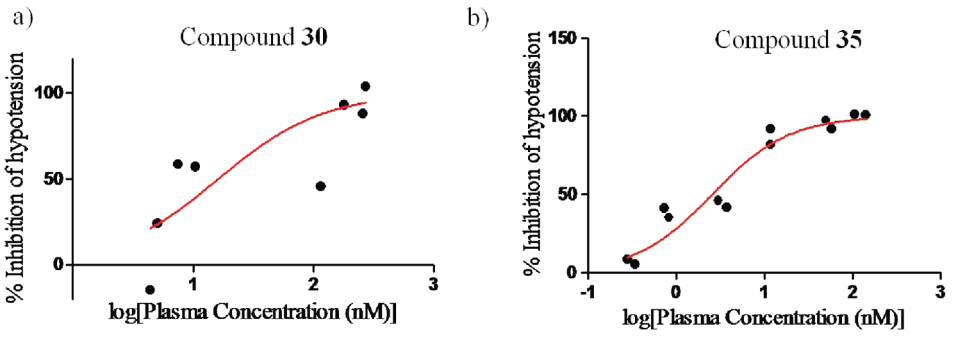


Figure 4. (a) Compound **30** and (b) compound **35** reverse DAK-induced hypotension in rabbits in plasma concentration-dependent fashion. The compounds were dosed subcutaneously. See ref 12 for protocol details and the Experimental Section for administered doses. The *x*-axis is the measured plasma concentration of each compound at the time of measurement of the PD effect.

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Table 7. Correlation of in Vitro and in Vivo Activity for Select Compounds

	rabbit			
compd	B1 cell IC_{50} (nM)	plasma F_u	cell IC_{50}/F_u (nM)	PD plasma IC_{50} [a] (nM)
30	1.1	0.09	12.0	16.2
35	0.6	0.24	2.4	2.5
3	11.2	0.21	53.3	127.0[b]
4	4.1	0.28	14.6	10.3[c]

[a] PD plasma IC_{50}: compound **30** $CI_{95\%}$ 4.25−62.14 nM, R^2 = 0.6063; compound **35** $CI_{95\%}$ 1.67−3.95 nM, R^2 = 0.9267. [b] Reference 13. [c] Reference 14.

antagonists has been shown to reverse the observed hypotension effect.[23,24] This PD assay is therefore a rapid way to establish in vivo on-mechanism activity of new B1 antagonist chemotypes. In this instance, treatment of rabbits with bacterial lipopolysaccharide, followed by administration of DAK, caused a drop in blood pressure, which was then reversed by increasing doses of phthalazinones **30** and **35**. The % inhibition of hypotensive response was plotted against the measured plasma concentration of the drug at two time points for each dose (30 and 75 min) to obtain a quantitative measure of the inhibition of hypotension (Figure 4).

We also measured the rabbit plasma protein binding of each compound. Phthalazinone **35** displayed a larger free fraction (F_u = 0.24) compared to phthalazinone **30** (F_u = 0.09). As shown in Table 7, the in vivo IC_{50} values correlate very well with rabbit cellular potency when corrected for free fraction. Comparison of these values with our previously reported B1 antagonists **3** and **4** demonstrates that phthalazinone **35** is the most potent compound identified in this PD assay in our laboratories, possessing a desirable balance of in vitro potency and plasma free fraction.[25]

CONCLUSION

Structure−activity relationship studies on Amgen's sulfonamide based B1 antagonists had identified very potent scaffolds with subnanomolar binding affinity to the target. However, suboptimal PK properties of the first generation sulfonamide lead, 2-oxopiperazine **4**, compelled us to undertake a search for novel chemotypes with improved physicochemical and PK properties suitable for preclinical optimization. Using a pharmacophore-based approach anchored by the bicyclic amine moiety, we synthesized small, focused amide libraries with restricted physicochemical properties. Starting from the initial hit compound **5**, multiple iterations of a strategy of reducing rotatable bonds afforded potent scaffolds, of which the phthalazinones series was selected for further optimization based on superior rat PK properties. It is interesting to note that a sulfonamide moiety has been successfully replaced with a cyclic carbonyl unit in this scaffold-modification exercise, and this could be of use in other medicinal chemistry programs where sulfonamides lead to undesirable properties.

Further SAR led to potent B1 antagonists like compounds **27**, **30**, and **35** with subnanomolar binding affinity to the human B1 receptor and exceptional cross-species PK properties, exemplified by oral bioavailabilities in the 44−98% range in rats and dog. On the basis of their potencies at the rabbit receptor, compounds **30** and **35** were tested in a rabbit PD study. We demonstrated that both compounds potently reversed B1-agonist induced hypotension in a proof of on-mechanism PD activity, being the most potent in this setting among the compounds discovered in our program. Further preclinical evaluation of phthalazinone B1 antagonists, including efficacy studies in pain models, will be reported shortly.

EXPERIMENTAL SECTION

Unless otherwise noted, all materials were obtained from commercial suppliers and used without further purification. Anhydrous solvents were obtained from EMD or Aldrich and used were directly. All reactions involving air- or moisture-sensitive reagents were performed under a nitrogen or argon atmosphere. Microwave-assisted reactions were conducted with a Smith synthesizer from Personal Chemistry (Uppsala, Sweden). Silica gel chromatography was performed using medium pressure liquid chromatography (MPLC) on a CombiFlash Companion (Teledyne Isco) with RediSep normal-phase silica gel (35−60 μm) columns and UV detection at 254 nm. Preparative reversed phase HPLC was performed using a Shimadzu Prominence system and Phenomenex Gemini C18 column (30 μm, 150 mm × 30 mm i.d.), eluting with a binary solvent system (A, H_2O with 0.1% TFA; B, CH_3CN with 0.1% TFA; gradient elution) with UV detection at 254 nm. All final compounds were purified to ≥95% purity as determined by Agilent 1100 series high performance liquid chromatography (HPLC) with UV detection at 254 nm using one of the following methods: Method A: Zorbax SB-C8 column (150 mm × 4.6 mm, 3.5 μm); mobile phase, A = H_2O with 0.1% TFA, B = CH_3CN with 0.1% TFA; gradient: 5−95% B (0.0−15.0 min); flow rate, 1.5 mL/min. Method B: Zorbax analytical C18 column (50 mm × 3 mm, 3.5 μm, 40 °C); mobile phase, A = H_2O with 0.1% TFA, B = CH_3CN with 0.1% TFA; gradient, 5−95% B (0.0−3.6 min); flow rate, 1.5 mL/min. Method C: YMCODS-AM (100 mm × 2.1 mm, 5 μm, 40 °C); mobile phase, A = H_2O with 0.1% HCO_2H, B = CH_3CN with 0.1% HCO_2H; gradient, 10% B (0.0−0.5 min), 10−100% B (0.5−7.0 min), 100% B (7.0−10 min); flow rate, 0.5 mL/min. Method D: Phenomenex Synergi MAX-RP (50 mm × 2.0 mm, 4.0 μm, 40 °C); mobile phase, A = H_2O with 0.1% TFA, B = CH_3CN with 0.1% TFA; gradient, 10% B (0.0−0.2 min), 10−100% B (0.2−3.0 min), 100% B (3.0−4.5 min), 100−10% B (4.5−5.0 min); flow rate, 0.8 mL/min. Low resolution mass spectrometry (MS) data were obtained using an Agilent G1956B mass spectrometer operated in electrospray ionization (ESI) mode (positive or negative). NMR spectra were obtained at ambient temperature with a Bruker Avance II spectrometer operating at 300 or 400 MHz. Chemical shifts are reported in ppm downfield of an internal standard, tetramethylsilane (δ 0.00 ppm). Data are reported as follows: chemical shift, number of protons, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, br = broad, m = multiplet), and coupling constants.

(*R*)-6-(Piperidin-1-ylmethyl)-1,2,3,4-tetrahydronaphthalen-1-amine Dihydrochloride (6). *Step 1.* (*R*)-methyl CBS-oxaborolidine (7.4 mL of a 1 M solution in toluene, 7.4 mmol) in toluene (190 mL) was cooled to −10 °C and was treated with $BH_3 \cdot SMe_2$ (17 mL, 180 mmol). A solution of methyl 5-oxo-5,6,7,8-tetrahydronaphthalene-2-carboxylate (30 g, 150 mmol)[26] in THF (200 mL) was added over 5 h using a syringe pump. After the addition was complete, the mixture was stirred for an additional 1 h and then was poured into an addition funnel. The reaction mixture was added to methanol (200 mL), and the mixture was cooled in an ice−salt bath over 30 min at such a rate that the internal temperature was kept below 0 °C. The mixture was concentrated in vacuo, diluted with diethyl ether (1 L), and washed with 1 M H_3PO_4, saturated sodium bicarbonate solution, and brine. The organic layer was dried over $MgSO_4$ and concentrated. The residue was dissolved in diethyl ether again (500 mL) and washed with 1 M H_3PO_4, saturated sodium bicarbonate solution, and brine. After the organic layer was dried over $MgSO_4$, the mixture was concentrated in vacuo to give

methyl 5-(*S*)-hydroxy-5,6,7,8-tetrahydronaphthalene-2-carboxylate as a white-yellow solid.

Step 2. A solution of methyl 5-(*S*)-hydroxy-5,6,7,8-tetrahydronaphthalene-2-carboxylate (29 g, 140 mmol) in toluene (280 mL) was cooled in an ice−salt bath. Diphenylphosphorylazide (36 mL, 170 mmol) was added. 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU, 25 mL, 170 mmol) was added over 10 min at such a rate that the internal temperature was kept below 1 °C. The ice in the bath was allowed to melt, and the mixture was stirred for 23 h. The reaction contents were poured into a 2 L separatory funnel, and the lower dark brown layer was removed. Water (500 mL) was added to the remaining top layer, and the mixture was extracted with Et_2O. The combined organic layers were washed with 1 M H_3PO_4, water, saturated $NaHCO_3$, and brine. The organic layer was dried over $MgSO_4$, filtered, and concentrated in vacuo. Purification by flash column chromatography (silica, 50% hexane/DCM) of the crude material provided methyl 5-(*R*)-azido-5,6,7,8-tetrahydronaphthalene-2-carboxylate.

Step 3. A solution of lithium aluminum hydride (470 mL of a 1 M solution in THF, 470 mmol) in THF (700 mL) was cooled in a ice−salt bath, and methyl 5-(*R*)-azido-5,6,7,8-tetrahydronaphthalene-2-carboxylate (27 g, 120 mmol) in 100 mL of THF was added over 30 min. The reaction mixture was warmed to 25 °C overnight, then cooled in an ice−salt bath the next morning. Water (18 mL) in THF (20 mL) was added to the reaction mixture over 4 h. Vigorous gas evolution occurred. Then 5 M NaOH (18 mL) was added over 30 min followed by 54 mL of water. After being stirred for an additional 1 h, the mixture was filtered, and the filtrate was concentrated in vacuo. The residue was reconstituted in MeOH and CH_3CN and concentrated in vacuo again to provide (*R*)-(5-amino-5,6,7,8-tetrahydronaphthalen-2-yl)methanol as light-brown solid.

Step 4. Methanol (4.0 L) was added to (*R*)-(5-amino-5,6,7,8-tetrahydronaphthalen-2-yl)methanol (321 g, 1.81 mol), followed by di-*tert*-butyl dicarbonate (514 g, 2.36 mol) in methanol (500 mL). The reaction mixture was stirred at 25 °C for 4 h, followed by concentration, in vacuo. The residue was dissolved in 1,2-dichloroethane (2.0 L) and concentrated again to remove residual methanol. The crude product was used in the next step.

Step 5. A solution of (*R*)-*tert*-butyl 6-(hydroxymethyl)-1,2,3,4-tetrahydronaphthalen-1-ylcarbamate (312.7 g, 1.13 mol) in dichloromethane (4.0 L) was treated with 225 mL (112.8 mmol) of a 0.5 N KBr solution (aq), followed by (2,2,6,6-tetramethylpiperidin-1-yl)oxyl or (2,2,6,6-tetramethylpiperidin-1-yl)oxidanyl (TEMPO, 1.76 g, 11.3 mmol). The reaction mixture was cooled to 0−2 °C using an ice bath, and a bleach solution (prepared from commercial bleach (1.6 L), water (2.18 L), and sodium bicarbonate (236 g)) was added in a dropwise fashion. The internal temperature was kept below 10 °C. After addition of a total of 3.4 L of bleach solution, the reaction was quenched with saturated sodium bisulfite solution. The layers were separated, and the aqueous phase was extracted with dichloromethane. The combined extracts were dried over $MgSO_4$ and concentrated to give (*R*)-*tert*-butyl 6-formyl-1,2,3,4-tetrahydronaphthalen-1-ylcarbamate as a thick brown syrup, which was used in the next step without purification.

Step 6. A solution of (*R*)-*tert*-butyl 6-formyl-1,2,3,4-tetrahydronaphthalen-1-ylcarbamate (306 g, 1.13 mol), piperidine (558 mL, 5.64 mol), and catalytic acetic acid (12 mL in 1,2-dichloroethane (5.0 L) was heated to 55 °C under nitrogen. After 30 min, $NaBH(OAc)_3$ (598 g, 2.82 mol, 2.5 equiv) was added slowly. After 1 h, the reaction mixture was cooled to 25 °C and was quenched with saturated sodium bicarbonate solution (8.0 L) slowly (caution: effervescence). The organic layer was separated, and the aqueous layer was extracted with dichloromethane). The combined organic layers were dried ($MgSO_4$) and concentrated in vacuo to afford crude (*R*)-*tert*-butyl 6-(piperidin-1-ylmethyl)-1,2,3,4-tetrahydronaphthalen-1-ylcarbamate, which was carried forward to the next step.

Step 7. (*R*)-*tert*-Butyl 6-(piperidin-1-ylmethyl)-1,2,3,4-tetrahydronaphthalen-1-ylcarbamate (388 g, 1.13 mol) was dissolved in methanol (4.0 L). A solution of hydrogen chloride (4.0 N in 1,4-dioxane, 592 mL, 2.4 mol, 2.1 equiv) was added in a dropwise fashion via an addition funnel. The reaction mixture was heated to 55 °C for 1 h, cooled to 25 °C, and concentrated in vacuo. The brown oil was dissolved in methanol and treated with diethyl ether to cause a white solid to precipitate which was collected by filtration and washed with diethyl ether. The solid was dried under high vacuum, affording the title compound **6** as the dihydrochloride salt (297 g, 84% over four steps). ^{1}H NMR (400 MHz, D_2O) δ ppm 1.34 (qt, J = 12.9, 3.9 Hz, 1H), 1.47−1.63 (m, 2H), 1.64−1.74 (m, 2H), 1.74−1.86 (m, 4H), 1.86−1.98 (m, 1H), 2.02−2.15 (m, 1H), 2.64−2.90 (m, 4H), 3.34 (bd, J = 12.4 Hz, 2H), 4.14 (s, 2H), 4.52 (t, J = 5.2 Hz, 1H), 7.23 (s, 1H), 7.24 (d, J = 8.0 Hz, 1H), 7.35 (d, J = 8.0 Hz, 1H); ^{13}C NMR (100 MHz, D_2O) δ ppm 17.7, 21.1, 22.7, 27.1, 28.0, 48.7, 52.8, 60.0, 128.9, 129.0, 129.3, 132.5, 132.9, 139.2; HRMS calcd for $C_{16}H_{24}N_2$ $[M + H]^+$ 245.2018, found 245.2017. This amine was used in peptide coupling steps as its salt or by converting to the free base before addition to the reaction mixture. Conversion to the free base was accomplished by washing it in a dichloromethane/saturated sodium bicarbonate mixture, separating the organic layer, drying over $MgSO_4$, and concentrating in vacuo.

(*R*)-3-(4-Oxo-4*H*-chromen-3-yl)-*N*-(6-(piperidin-1-ylmethyl)-1,2,3,4-tetrahydronaphthalen-1-yl)benzamide (10). **10** was prepared by a method analogous to that used for the preparation of compound **11**. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.30 (1 H, d, J = 7.5 Hz), 8.09 (1 H, s), 7.95 (1 H, s), 7.81 (1 H, d, J = 8.0 Hz), 7.65−7.78 (2 H, m), 7.51 (2 H, t, J = 7.5 Hz), 7.45 (1 H, t, J = 7.8 Hz), 7.21−7.33 (1 H, m), 7.00−7.16 (2 H, m), 6.39 (1 H, d, J = 8.5 Hz), 5.40 (1 H, q, J = 6.5 Hz), 3.42 (2 H, s), 2.70−2.95 (2 H, m), 2.37 (4 H, s), 2.09−2.21 (1 H, m), 1.80−2.01 (3 H, m), 1.50−1.65 (4 H, m), 1.42 (2 H, s); ESI MS calcd for $C_{32}H_{32}N_2O_3$ $[M + H]^+$ 493.2, found 493.2.

(*R*)-4-(4-Oxo-4*H*-chromen-3-yl)-*N*-(6-(piperidin-1-ylmethyl)-1,2,3,4-tetrahydronaphthalen-1-yl)benzamide (11). *Step 1.* A mixture of 3-bromochromone (250 mg, 1.11 mmol), 4-(*tert*-butoxycarbonyl)phenylboronic acid (296 mg, 1.33 mmol), $Pd(PPh_3)_4$ (257 mg, 0.222 mmol), and 2.0 M sodium carbonate (1.67 mL, 3.33 mmol) in 1,4-dioxane (5 mL in a sealed 10 mL tube was evacuated and backfilled with argon (three cycles). The reaction mixture was heated to 140 °C for 20 min in a microwave (Personal Chemistry, 300 W). The solution was then diluted with EtOAc (50 mL) and washed with water and brine. The organic solution was dried over magnesium sulfate, concentrated in vacuo, and purified by flash column chromatography (silica, 0−100% DCM/hexane) to give *tert*-butyl 4-(4-oxo-4*H*-chromen-3-yl)benzoate (152 mg, 42.4%).

Step 2. A solution of *tert*-butyl 4-(4-oxo-4*H*-chromen-3-yl)benzoate (135 mg, 0.419 mmol) in TFA (5 mL, 67.3 mmol) was stirred at 25 °C for 10 min. The solution was concentrated, azeotroped in DCM, and dried in vacuo to give 4-(4-oxo-4*H*-chromen-3-yl)benzoic acid (127 mg; crude material was advanced to the next step).

Step 3. 4-(4-Oxo-4*H*-chromen-3-yl)benzoic acid (100 mg, 0.376 mmol), **6** (110 mg, 0.451 mmol), HOBt (74.8 mg, 0.488 mmol), and EDCI (93.6 mg, 0.488 mmol) were combined in DMF (2 mL). The reaction mixture was stirred at 25 °C for 16 h and then quenched with 1 N sodium hydroxide solution (30 mL) and extracted with EtOAc. The organic solution was washed with water, followed by brine. It was dried over magnesium sulfate and concentrated in vacuo. Purification by flash column chromatography (silica, 5−10% MeOH/DCM) gave **11** (58.2 mg, 31.5% over two steps) as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.79 (1 H, d, J = 8.5 Hz), 8.65 (1 H, s), 8.17 (1 H, d, J = 8.0 Hz), 7.99 (2 H, d, J = 8.0 Hz), 7.86 (1 H, t, J = 7.8 Hz), 7.68−7.76 (3 H, m), 7.55 (1 H, t, J = 7.5 Hz), 7.05−7.17 (2 H, m), 7.03 (1 H, s), 5.21−5.28 (1 H, m), 3.34 (2 H, s), 2.71−2.85 (2 H, m), 2.30 (4 H, s), 1.94−2.04 (2 H, m), 1.71−1.88 (2 H, m), 1.43−1.52 (4 H, m),

1.32−1.42 (2 H, m); ESI MS calcd for $C_{32}H_{32}N_2O_3[M + H]^+$ 493.2, found 493.2.

(*R*)-3-(1-Methyl-4-oxo-1,4-dihydroquinolin-3-yl)-*N*-(6-(piperidin-1-ylmethyl)-1,2,3,4-tetrahydronaphthalen-1-yl)-benzamide (12). **12** was prepared by a method analogous to that used for the preparation of compound **13**. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 9.00 (1 H, d, J = 8.6 Hz), 8.51 (1 H, d, J = 2.3 Hz), 8.30 (1 H, s), 8.15 (1 H, dd, J = 9.0, 2.3 Hz), 7.99 (1 H, d, J = 7.6 Hz), 7.93 (1 H, dd, J = 11.6, 7.9 Hz), 7.78 (1 H, d, J = 9.0 Hz), 7.60 (1 H, t, J = 7.7 Hz), 7.17 (1 H, d, J = 7.8 Hz), 7.05−7.14 (2 H, m), 7.03 (1 H, s), 6.08 (1 H, d, J = 7.6 Hz), 5.14−5.38 (1 H, m), 3.86 (3 H, s), 3.34 (2 H, s), 2.64−2.85 (2 H, m), 2.23−2.36 (4 H, m), 1.91−2.08 (2 H, m), 1.67−1.93 (2 H, m), 1.43−1.54 (4 H, m), 1.29−1.40 (2 H, m); ESI MS calcd for $C_{33}H_{35}N_3O_2$ $[M + H]^+$ 506.2, found 506.2.

(*R*)-4-(1-Methyl-4-oxo-1,4-dihydroquinolin-3-yl)-*N*-(6-(piperidin-1-ylmethyl)-1,2,3,4-tetrahydronaphthalen-1-yl)-benzamide (13). *Step 1.* A solution of *N*-methylaniline (1.476 mL, 13.63 mmol) and acrylic acid (982 mg, 13.63 mmol) in toluene (6 mL) and heptanes (3 mL) was heated to 40 °C under nitrogen for 14 h. The temperature was increased to 80 °C, and the reaction continued for another 2 h. The reaction mixture was cooled to 23 °C, the solvents were concentrated, and the residue was dried under vacuum, affording 3-(methyl(phenyl)amino)propanoic acid (2.44 g, 99.9%) as a dark yellow oil.

Step 2. A suspension of phosphorus pentoxide (3.865 g, 27.23 mmol) and methanesulfonic acid (17.6 mL, 272.3 mmol) was stirred at 23 °C. After 2 h, the reaction mixture was quickly filtered, and the filtrate was added to 3-(methyl(phenyl)amino)propanoic acid (2.440 g, 13.62 mmol). The reaction mixture was heated to 110 °C for 3 h. It was then cooled to 23 °C, poured into ice−water, and extracted with DCM. The organic layers were combined, washed with saturated sodium bicarbonate solution and brine, dried over magnesium sulfate, and concentrated, affording 1-methyl-2,3-dihydroquinolin-4(1*H*)-one (425 mg, 19.4%) as an orange oil.

Step 3. A solution of 1-methyl-2,3-dihydroquinolin-4(1*H*)-one (153 mg, 0.949 mmol) in chloroform (8 mL) was treated with hydrogen chloride, 4.0 M in 1,4-dioxane (0.26 mL, 1.04 mmol) at 23 °C under nitrogen. After 5 min, the reaction mixture was cooled to 0 °C and was treated with a solution of bromine (50 μL, 0.95 mmol) in chloroform (2 mL) in a dropwise fashion over 20 min. The reaction mixture was warmed to 23 °C and stirred under nitrogen for 2 h. The reaction mixture was concentrated under vacuum and diluted with ice-cold water. The precipitate was collected by filtration, washed with water, and dried under vacuum, affording 3-bromo-1-methylquinolin-4(1*H*)-one (144 mg, 64%) as a dark yellow solid.

Step 4. A suspension of 3-bromo-1-methylquinolin-4(1*H*)-one (142 mg, 0.596 mmol), 4-(*tert*-butoxycarbonyl)phenylboronic acid (265 mg, 1.193 mmol), 2.0 M sodium carbonate solution (0.59 mL, 1.193 mmol), and $Pd(PPh_3)_4$ (69 mg, 60 μmol) in 1,4-dioxane (5 mL) was degassed and backfilled with argon in a sealed vial. The reaction mixture was heated to 150 °C in a microwave for 20 min. The reaction mixture was diluted with EtOAc (75 mL) and washed with saturated sodium bicarbonate solution and brine, dried over magnesium sulfate, concentrated in vacuo, and purified by flash column chromatography (silica, 0.5−10% MeOH/EtOAc), affording *tert*-butyl 4-(1-methyl-4-oxo-1,4-dihydroquinolin-3-yl)benzoate (81 mg, 10%) as a white solid.

Step 5. A solution of *tert*-butyl 4-(1-methyl-4-oxo-1,4-dihydroquinolin-3-yl)benzoate (80 mg, 0.239 mmol) in TFA (4.43 mL, 59.63 mmol) was stirred at 23 °C. After 30 min, the reaction mixture was concentrated and azeotroped with toluene (3 × 10 mL), affording 4-(1-methyl-4-oxo-1,4-dihydroquinolin-3-yl)benzoic acid trifluoroacetate salt (92 mg, 98%) as a white solid.

Step 6. A suspension of 4-(1-methyl-4-oxo-1,4-dihydroquinolin-3-yl)-benzoic acid trifluoroacetate salt (92 mg, 234 μmol) and **6** (63 mg, 257 μmol) in DMF (5 mL) was treated with TEA (39 μL, 281 μmol). The reaction mixture was stirred at 23 °C for 10 min, followed by the addition of HOBt (38 mg, 0.28 mmol) and EDCI (45 mg, 0.23 mmol). The reaction mixture was stirred at 23 °C under nitrogen. After 20 h, the reaction mixture was diluted with DCM and washed with saturated sodium bicarbonate solution and brine, dried over magnesium sulfate, concentrated in vacuo, and purified by flash column chromatography (silica, 1−9% MeOH/DCM), affording **13** (61 mg, 52%) as a light yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.83 (1 H, d, J = 8.6 Hz), 8.48 (1 H, d, J = 2.3 Hz), 8.15 (1 H, dd, J = 9.0, 2.3 Hz), 8.06 (2 H, d, J = 8.4 Hz), 8.00 (1 H, d, J = 7.6 Hz), 7.87 (2 H, d, J = 8.6 Hz), 7.79 (1 H, d, J = 9.0 Hz), 7.15 (1 H, d, J = 8.0 Hz), 7.06 (1 H, d, J = 8.0 Hz), 6.99−7.05 (1 H, m), 6.09 (1 H, d, J = 7.8 Hz), 5.11−5.34 (1 H, m), 3.87 (3 H, s), 3.34 (2 H, s), 2.56−2.92 (2 H, m), 2.29 (4 H, s), 1.90−2.10 (2 H, m), 1.54−1.95 (2 H, m), 1.47 (4 H, s), 1.29−1.41 (2 H, m); ESI MS calcd for $C_{33}H_{35}N_3O_2$ $[M + H]^+$ 506.3, found 506.2.

(*R*)-3-(4-Oxo-4*H*-thiochromen-3-yl)-*N*-(6-(piperidin-1-ylmethyl)-1,2,3,4-tetrahydronaphthalen-1-yl)benzamide (14). **14** was prepared by a method analogous to that used for the preparation of compound **15**. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 8.79 (d, J = 8.48 Hz, 1H), 8.56 (s, 1H), 8.46 (d, J = 7.16 Hz, 1H), 8.06 (s, 1H), 7.94 (dd, J = 3.87, 7.53 Hz, 2H), 7.74−7.82 (m, 1H), 7.63−7.72 (m, 2H), 7.48−7.56 (m, 1H), 6.98−7.21 (m, 3H), 5.25 (br s, 1H), 3.32 (s, 2H), 2.76 (br s, 2H), 2.30 (br s, 4H), 1.98 (br s, 2H), 1.68−1.90 (m, 2H), 1.29−1.56 (m, 6H); ESI MS calcd for $C_{32}H_{32}N_2O_2S$ $[M + H]^+$ 509.2, found 509.1.

(*R*)-4-(4-Oxo-4*H*-thiochromen-3-yl)-*N*-(6-(piperidin-1-ylmethyl)-1,2,3,4-tetrahydronaphthalen-1-yl)benzamide (15). *Step 1.* A vessel containing 3-bromo-4*H*-thiochromen-4-one (250 mg, 1.037 mmol),[21] 4-(*tert*-butoxycarbonyl)phenylboronic acid (253 mg, 1.141 mmol), potassium carbonate (430 mg, 3.111 mmol), and $Pd(PPh_3)_4$ (24 mg, 21 μmol) was purged with nitrogen. A mixture of toluene (2.72 mL)/H_2O (1.03 mL) (2.6:1) was then added, and the reaction mixture was stirred at 60 °C. After 72 h, the reaction mixture was cooled to 25 °C and diluted with DCM and saturated aqueous sodium bicarbonate. The aqueous layer was washed with DCM, and the combined organic layers were dried over sodium sulfate, concentrated in vacuo, and purified by flash column chromatography (silica, 10% EtOAc/hexanes) to give *tert*-butyl 4-(4-oxo-4*H*-thiochromen-3-yl)benzoate as a light yellow solid. This was then washed with MeOH to give the desired pure product as a white solid (282.4 mg, 80%).

Step 2. A solution of *tert*-butyl 4-(4-oxo-4*H*-thiochromen-3-yl)benzoate (282.4 mg, 0.834 mmol) and TFA (1.7 mL) was stirred at 25 °C for 10 min. The reaction mixture was concentrated in vacuo and coevaporated with DCM to give 4-(4-oxo-4*H*-thiochromen-3-yl)benzoic acid as a white solid, which was used in the next step without purification.

Step 3. To a solution of 4-(4-oxo-4*H*-thiochromen-3-yl)benzoic acid (264 mg, 0.935 mmol) and **6** (251 mg, 1.029 mmol) in DMF (9.35 mL) was added TEA (0.156 mL, 1.122 mmol). The reaction mixture was stirred at 25 °C for 10 min. EDCI (179 mg, 0.935 mμmol) and HOBt (172 mg, 1.122 mmol) were then added, and the reaction mixture was stirred at 25 °C for 16 h. The reaction mixture was then diluted with brine and extracted with DCM. The combined organic layers were washed with brine, dried over sodium sulfate, filtered, and concentrated in vacuo. The resulting mixture was purified by flash column chromatography (silica, 0.5−10% MeOH/DCM) to give the crude product as a light yellow solid. This was washed with MeOH to give **15** as a white solid (138.9 mg, 29% over two steps). ^{1}H NMR (300 MHz, $CDCl_3$) 1.44−1.58 (m, 6H), 2.0−2.15 (m, 4H), 2.38 (m, 4H), 2.83 (m, 2H), 3.43 (s, 2H), 5.39 (m, 1H), 6.45 (m, 1H), 7.09 (m, 2H), 7.27 (m, 1H), 7.63 (m, 4H), 7.84 (m, 3H), 8.63 (s, 1H); ESI MS calcd for $C_{32}H_{32}N_2O_2S$ $[M + H]^+$ 509.2, found 509.1.

(*R*)-3-(4-Methyl-1-oxophthalazin-2(1*H*)-yl)-*N*-(6-(piperidin-1-ylmethyl)-1,2,3,4-tetrahydronaphthalen-1-yl)benzamide (16). **16** was prepared by a method analogous to that used for the preparation of compound **17**. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.87 (1 H, d, J = 8.5 Hz), 8.36 (1 H, d, J = 7.5 Hz), 8.16 (1 H, t,

J = 1.5 Hz), 7.91−8.07 (4 H, m), 7.73−7.79 (1 H, m), 7.59 (1 H, t, J = 8.0 Hz), 7.15 (1 H, d, J = 8.0 Hz), 7.06 (1 H, d, J = 8.0 Hz), 7.02 (1 H, s), 5.21−5.30 (1 H, m), 3.33−3.39 (2 H, m), 2.70−2.83 (2 H, m), 2.62 (3 H, s), 2.29 (4 H, s), 1.91−2.04 (2 H, m), 1.69−1.89 (2 H, m), 1.41−1.53 (4 H, m), 1.31−1.41 (2 H, m); ESI MS calcd for $C_{32}H_{34}N_4O_2$ $[M + H]^+$ 507.2, found 507.2.

(*R*)-4-(4-Methyl-1-oxophthalazin-2(1*H*)-yl)-*N*-(6-(piperidin-1-ylmethyl)-1,2,3,4-tetrahydronaphthalen-1-yl)benzamide (17). Phthalazinone **17** was prepared from commercially available 4-(4-methyl-1-oxophthalazin-2(1*H*)-yl)benzoic acid and amine **6**. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.32 (1 H, d, J = 8.4 Hz), 8.21 (1 H, d, J = 8.4 Hz), 8.17 (1 H, s), 7.95 (1 H, d, J = 8.4 Hz), 7.04 (2 H, s), 6.99 (1 H, s), 4.85−4.94 (1 H, m), 3.58 (1 H, t, J = 8.0 Hz), 3.47−3.55 (1 H, m), 3.36−3.45 (2 H, m), 3.33 (2 H, s), 2.98−3.09 (1 H, m), 2.69 (2 H, d, J = 7.2 Hz), 2.29 (4 H, s), 2.03−2.15 (2 H, m), 1.75−1.89 (2 H, m), 1.54−1.72 (2 H, m), 1.42−1.50 (4 H, m), 1.32−1.42 (2 H, m); ESI MS calcd for $C_{32}H_{34}N_4O_2$ $[M + H]^+$ 507.3, found 507.2.

(*R*)-3-(4-Oxoquinazolin-3(4*H*)-yl)-*N*-(6-(piperidin-1-ylmethyl)-1,2,3,4-tetrahydronaphthalen-1-yl)benzamide (18). **18** was prepared by a method analogous to that used for the preparation of compound **19**. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.85 (1 H, d, J = 8.4 Hz), 8.40 (1 H, s), 8.22 (1 H, dd, J = 8.0, 1.0 Hz), 8.04−8.11 (2 H, m), 7.90 (1 H, td, J = 8.2, 7.0, 1.4 Hz), 7.71−7.79 (2 H, m), 7.66 (1 H, t, J = 7.8 Hz), 7.61 (1 H, t, J = 7.6 Hz), 7.12−7.19 (1 H, m), 7.00−7.11 (2 H, m), 5.20−5.29 (1 H, m), 3.33−3.42 (2 H, m), 2.65−2.84 (2 H, m), 2.30 (4 H, s), 1.90−2.06 (2 H, m), 1.70−1.88 (2 H, m), 1.41−1.55 (4 H, m), 1.31−1.41 (2 H, m); ESI MS calcd for $C_{31}H_{32}N_4O_2$ $[M + H]^+$ 493.2, found 493.2.

(*R*)-4-(4-Oxoquinazolin-3(4*H*)-yl)-*N*-(6-(piperidin-1-ylmethyl)-1,2,3,4-tetrahydronaphthalen-1-yl)benzamide (19). 4-Oxoquinazoline **19** was synthesized from commercially available 4-(4-oxoquinazolin-3(4*H*)-yl)benzoic acid and amine **6**. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.90 (1 H, d, J = 8.6 Hz), 8.38 (1 H, s), 8.22 (1 H, dd, J = 8.0, 1.2 Hz), 8.08 (2 H, d, J = 8.4 Hz), 7.90 (1 H, dt, J = 8.4, 7.0, 1.6 Hz), 7.74−7.79 (1 H, m), 7.65 (2 H, d, J = 8.6 Hz), 7.61 (1 H, dt, J = 8.0, 7.2, 1.0 Hz), 7.05−7.20 (2 H, m), 7.03 (1 H, s), 5.20−5.30 (1 H, m), 3.35 (2 H, s), 2.71−2.83 (2 H, m), 2.30 (4 H, s), 1.94−2.06 (2 H, m), 1.73−1.89 (2 H, m), 1.43−1.52 (4 H, m), 1.32−1.42 (2 H, m); ESI MS calcd for $C_{31}H_{32}N_4O_2$ $[M + H]^+$ 493.3, found 493.2.

(*R*)-4-(1-Oxophthalazin-2(1*H*)-yl)-*N*-(6-(piperidin-1-ylmethyl)-1,2,3,4-tetrahydronaphthalen-1-yl)benzamide (20). Phthalazinone **20** was synthesized from commercially available 4-(1-oxophthalazin-2(1*H*)-yl)benzoic acid and amine **6**. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.86 (1 H, d, J = 8.6 Hz), 8.62 (1 H, s), 8.35 (1 H, d, J = 7.8 Hz), 7.98−8.08 (4 H, m), 7.94 (1 H, td, J = 8.1, 6.4, 2.0 Hz), 7.74 (2 H, d, J = 8.6 Hz), 7.16 (1 H, d, J = 8.0 Hz), 7.01−7.10 (2 H, m), 5.21−5.30 (1 H, m), 3.34 (2 H, s), 2.69−2.86 (2 H, m), 2.30 (4 H, s), 1.92−2.08 (2 H, m), 1.70−1.90 (2 H, m), 1.42−1.54 (4 H, m), 1.32−1.41 (2 H, m); ESI MS calcd for $C_{31}H_{32}N_4O_2$ $[M + H]^+$ 493.3, found 493.2.

(*R*)-4-(4-Ethyl-1-oxophthalazin-2(1*H*)-yl)-*N*-(6-(piperidin-1-ylmethyl)-1,2,3,4-tetrahydronaphthalen-1-yl)benzamide (21). *Step 1.* A suspension of commercially available 2-propionylbenzoic acid (422 mg, 2.36 mmol) and 4-hydrazinobenzoic acid (378 mg, 2.48 mmol) in methanol (10 mL) was treated with concentrated sulfuric acid (5.26 mL, 9.473 mmol). The mixture was stirred at 75 °C in a sealed vessel. After 17 h, the mixture was cooled to 23 °C and poured into ice−water (150 mL). The precipitated solid was collected by filtration, washed with water (25 mL) and diethyl ether (10 mL), and dried under vacuum, affording methyl 4-(4-ethyl-1-oxophthalazin-2(1*H*)-yl)benzoate (417 mg, 57%) as a yellow solid.

Step 2. A suspension of methyl 4-(4-ethyl-1-oxophthalazin-2(1*H*)-yl)benzoate (208 mg, 675 μmol) and 1 N hydrochloric acid (6.74 mL, 6.74 mmol) in 1,4-dioxane (10 mL) was heated to 100 °C. After 17 h, the mixture was concentrated, azeotroped with toluene (10 mL), and dried under vacuum, affording 4-(4-ethyl-1-oxophthalazin-2(1*H*)-yl)benzoic acid (198 mg, 99%) as a brown solid.

Step 3. A solution of 4-(4-ethyl-1-oxophthalazin-2(1*H*)-yl)benzoic acid (190 mg, 646 μmol), **6** (174 mg, 710 μmol), HOBt (105 mg, 775 μmol), and EDCI (149 mg, 775 μmol) in DMF (10 mL) was stirred at 23 °C. After 17 h, the solution was diluted with EtOAc (100 mL) and washed with saturated sodium bicarbonate solution (75 mL), water (75 mL), and brine (75 mL), dried over $MgSO_4$, concentrated in vacuo, and purified by flash column chromatography (silica, 2−6% MeOH/DCM), affording **21** (264 mg, 79%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.54 (1 H, d, J = 7.8 Hz), 7.84−7.94 (6 H, m), 7.75−7.82 (1 H, m), 7.28 (1 H, d, J = 7.8 Hz), 7.13 (1 H, d, J = 8.0 Hz), 7.09 (1 H, s), 6.35 (1 H, d, J = 8.2 Hz), 5.34−5.45 (1 H, m), 3.42 (2 H, s), 3.06 (2 H, q, J = 7.4 Hz), 2.71−2.93 (2 H, m), 2.38 (4 H, s), 2.08−2.24 (1 H, m), 1.78−2.00 (3 H, m), 1.51−1.67 (4 H, m), 1.33−1.50 (5 H, m); ESI MS calcd for $C_{33}H_{36}N_4O_2$ $[M + H]^+$ 521.2, found 521.3.

(*R*)-4-(4-Isopropyl-1-oxophthalazin-2(1*H*)-yl)-*N*-(6-(piperidin-1-ylmethyl)-1,2,3,4-tetrahydronaphthalen-1-yl)benzamide (22). *Step 1.* A solution of methyl 2-iodobenzoate (0.8 mL, 5.45 mmol) in THF (20 mL) in a flame-dried round bottomed flask was cooled to −20 °C under nitrogen. Isopropylmagnesium chloride, 2.0 M solution in tetrahydrofuran (2.99 mL, 5.99 mmol), was added in a dropwise fashion over 10 min. After 3 h, isobutyryl chloride (0.85 mL, 8.16 mmol) was added in a dropwise fashion. The reaction mixture was warmed to 23 °C overnight. After 15 h, the reaction mixture was quenched with MeOH (3 mL), diluted with EtOAc, and washed with water (100 mL). The organic solution was washed with brine (100 mL), dried over magnesium sulfate, concentrated in vacuo, and purified by flash column chromatography (silica, 5−15% EtOAc/hexane), affording methyl 2-isobutyrylbenzoate (166 mg, 15%) as a light yellow oil.

Step 2. A solution of methyl 2-isobutyrylbenzoate (150 mg, 727 μmol) and 4-hydrazinobenzoic acid (116 mg, 764 μmol) in methanol (10 mL) was treated with concentrated sulfuric acid (1.61 mL, 29.09 mmol). The mixture was stirred at 75 °C in a sealed vessel. After 2 days, the mixture was cooled to 23 °C, diluted with water (50 mL), and extracted with dichloromethane (2 × 100 mL). The organic layers were combined, washed with brine (75 mL), dried over $MgSO_4$, concentrated in vacuo, and purified by flash column chromatography (silica, 5−25% EtOAc/hexane), affording methyl 4-(4-isopropyl-1-oxophthalazin-2(1*H*)-yl)benzoate (51 mg, 22%) as a yellow oil.

Step 3. A solution of methyl 4-(4-isopropyl-1-oxophthalazin-2(1*H*)-yl)benzoate (51 mg, 158 μmol) in 1,4-dioxane (8 mL) was treated with 1 N hydrochloric acid (7.91 mL, 7.91 mmol). The mixture was heated to reflux. After 14 h, the mixture was cooled to 23 °C, concentrated, azeotroped with toluene (10 mL), and dried under vacuum, affording 4-(4-isopropyl-1-oxophthalazin-2(1*H*)-yl)benzoic acid (48 mg, 99%) as a yellow solid.

Step 4. A solution of 4-(4-isopropyl-1-oxophthalazin-2(1*H*)-yl)-benzoic acid (49 mg, 159 μmol), **6** (42 mg, 173 μmol), HOBt (23 mg, 173 μmol), and EDCI (33 mg, 173 μmol) in DMF (5 mL) was stirred under nitrogen at 23 °C. After 16 h, the mixture was diluted with EtOAc (75 mL) and washed with saturated sodium bicarbonate solution (50 mL), water (50 mL), and brine (50 mL), dried over $MgSO_4$, concentrated in vacuo, and purified by flash column chromatography (silica, 2.5−8% MeOH/DCM), affording **22** (31 mg, 40%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.52−8.59 (1 H, m), 7.82−7.95 (6 H, m), 7.75−7.82 (1 H, m), 7.29 (1 H, d, J = 7.8 Hz), 7.13 (1 H, d, J = 7.8 Hz), 7.09 (1 H, s), 6.37 (1 H, d, J = 8.2 Hz), 5.34−5.44 (1 H, m), 3.50−3.61 (1 H, m), 3.43 (2 H, s), 2.74−2.88 (2 H, m), 2.38 (4 H, s), 2.09−2.21 (1 H, m), 1.84−2.00 (3 H, m), 1.52−1.63 (4 H, m), 1.42−1.49 (2 H, m), 1.40 (6 H, d, J = 6.7 Hz); ESI MS calcd for $C_{34}H_{38}N_4O_2$ $[M + H]^+$ 535.2, found 535.3.

(*R*)-4-(1-Oxo-4-(trifluoromethyl)phthalazin-2(1*H*)-yl)-*N*-(6-(piperidin-1-ylmethyl)-1,2,3,4-tetrahydronaphthalen-1-yl)benzamide (23). Phthalazinone **23** was synthesized by analogous

route to compound **21**, employing 2-(2,2,2-trifluoroacetyl)benzoic acid as the starting material. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.88 (1 H, d, J = 9.0 Hz), 8.46 (1 H, d, J = 8.0 Hz), 7.95−8.21 (5 H, m), 7.75 (2 H, d, J = 8.5 Hz), 7.17 (1 H, d, J = 8.0 Hz), 6.95−7.12 (2 H, m), 5.19−5.32 (1 H, m), 3.35−3.53 (2 H, m), 2.68−2.86 (2 H, m), 2.30 (4 H, s), 1.93−2.07 (2 H, m), 1.69−1.90 (2 H, m), 1.41−1.55 (4 H, m), 1.29−1.43 (2 H, m); ESI MS calcd for $C_{32}H_{31}F_3N_4O_2$ $[M + H]^+$ 561.2, found 561.1.

(*R*)-4-(1-Oxo-4-phenylphthalazin-2(1*H*)-yl)-*N*-(6-(piperidin-1-ylmethyl)-1,2,3,4-tetrahydronaphthalen-1-yl)benzamide (24). Phthalazinone **24** was synthesized from commercially available 4-(1-oxo-4-phenylphthalazin-2(1*H*)-yl)benzoic acid and amine **6**. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.83 (1 H, d, J = 8.4 Hz), 8.39 (1 H, d, J = 8.0 Hz), 8.02 (1 H, t, J = 7.6 Hz), 7.81−7.88 (3 H, m), 7.60 (2 H, d, J = 8.6 Hz), 7.51 (2 H, dd, J = 6.7, 2.6 Hz), 7.37−7.43 (3 H, m), 7.35 (1 H, d, J = 8.2 Hz), 6.97−7.13 (3 H, m), 5.13−5.23 (1 H, m), 3.33 (2 H, s), 2.65−2.83 (2 H, m), 2.29 (4 H, br s), 1.88−2.01 (2 H, m), 1.67−1.84 (2 H, m), 1.42−1.52 (4 H, m), 1.33−1.41 (2 H, m); ESI MS calcd for $C_{37}H_{36}N_4O_2$ $[M + H]^+$ 569.3, found 569.3.

(*R*)-4-(8-Fluoro-4-methyl-1-oxophthalazin-2(1*H*)-yl)-*N*-(6-(piperidin-1-ylmethyl)-1,2,3,4-tetrahydronaphthalen-1-yl)benzamide (25). Phthalazinone **25** was prepared from 2-bromo-6-fluorobenzoic acid using a procedure analogous to that for compound **27**. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.80−7.88 (1 H, m), 7.84 (4 H, q, J = 30.7, 8.6 Hz), 7.60 (1 H, d, J = 8.0 Hz), 7.46 (1 H, dd, J = 10.3, 8.5 Hz), 7.29 (1 H, d, J = 8.0 Hz), 7.15 (2 H, d, J = 7.8 Hz), 6.35 (1 H, d, J = 8.2 Hz), 5.35−5.45 (1 H, m), 3.49 (2 H, s), 2.72−2.92 (2 H, m), 2.64 (3 H, s), 2.45 (3 H, br s), 2.09−2.23 (1 H, m), 1.94−2.01 (1 H, m), 1.84−1.94 (2 H, m), 1.63 (5 H, br s), 1.43 (2 H, br s); ESI MS calcd for $C_{32}H_{33}FN_4O_2$ $[M + H]^+$ 525.3, found 525.2.

(*R*)-4-(5,8-Difluoro-4-methyl-1-oxophthalazin-2(1*H*)-yl)-*N*-(6-(piperidin-1-ylmethyl)-1,2,3,4-tetrahydronaphthalen-1-yl)benzamide (26). Phthalazinone **26** was prepared from 2-bromo-3,6-fluorobenzoic acid using a procedure analogous to that for compound **27**. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.84−7.96 (2 H, m), 7.72−7.80 (2 H, m), 7.46−7.57 (1 H, m), 7.35−7.46 (1 H, m), 7.21−7.29 (1 H, m), 7.12 (1 H, d, J = 7.8 Hz), 7.07 (1 H, s), 6.39 (1 H, d, J = 8.2 Hz), 5.33−5.46 (1 H, m), 3.41 (2 H, s), 2.75−2.92 (2 H, m), 2.73 (3 H, d, J = 7.6 Hz), 2.37 (4 H, br s), 2.07−2.21 (1 H, m), 1.82−2.01 (3 H, m), 1.52−1.64 (4 H, m), 1.33−1.48 (2 H, m); ESI MS calcd for $C_{32}H_{32}F_2N_4O_2$ $[M + H]^+$ 543.2, found 543.2.

(*R*)-4-(8-Chloro-4-methyl-1-oxophthalazin-2(1*H*)-yl)-*N*-(6-(piperidin-1-ylmethyl)-1,2,3,4-tetrahydronaphthalen-1-yl)benzamide (27). *Step 1.* A solution of 2-bromo-6-chlorobenzoic acid (5.60 g, 23.8 mmol) and lithium hydroxide monohydrate (1.10 g, 26.2 mmol) in THF (50 mL) was stirred at 25 °C for 1 h. Dimethyl sulfate (2.70 mL, 28.5 mmol) was added, and the mixture was stirred at 85 °C for 21 h. The solution was quenched with ammonium hydroxide/water (1:1, 50 mL), stirred for 30 min, then concentrated in vacuo. It was diluted with water (50 mL) and extracted with EtOAc. The organic solution was washed with brine (75 mL), dried over magnesium sulfate, concentrated, and dried in vacuo to give methyl 2-bromo-6-chlorobenzoate (5.745 g, 96.8%).

Step 2. A suspension of methyl 2-bromo-6-chlorobenzoate (2.846 g, 11.41 mmol), tributyl(1-ethoxyvinyl)tin (4.24 mL, 12.55 mmol), and dichlorobis(triphenylphosphine)palladium(II) (0.40 g, 0.57 mmol) in 1,4-dioxane (18 mL) in a sealed microwave tube was degassed and backfilled with argon. The reaction vessel was heated in a microwave at 150 °C for 30 min, and vinyl ether formation was confirmed by LCMS. ESI MS calcd for $C_{12}H_{13}ClO_3$ $[M + H]^+$ 241.0, found 241.0. The reaction mixture was diluted with 6 N hydrochloric acid (10 mL) and stirred at 23 °C for 3 h. The reaction mixture was diluted with EtOAc (250 mL) and washed with 10% hydrochloric acid solution (150 mL), dried over magnesium sulfate, and concentrated in vacuo. It was purified by flash column chromatography (silica, 10−25% EtOAc/hexane), affording methyl 2-acetyl-6-chlorobenzoate (1.895 g, 78.14%) as a yellow solid.

Step 3. A solution of methyl 2-acetyl-6-chlorobenzoate (1.88 g, 8.88 mmol) and 4-hydrazinobenzoic acid (1.48 g, 9.76 mmol) in methanol (75 mL) was treated slowly with concentrated sulfuric acid (17.26 mL, 621.2 mmol). The reaction mixture was heated to 80 °C in a sealed vessel for 5 h. It was then cooled to 23 °C, and the precipitate was poured into an ice−water solution (100 mL). The solid was collected by filtration and washed with water (25 mL), affording methyl 4-(8-chloro-4-methyl-1-oxophthalazin-2(1*H*)-yl)benzoate (2.084 g, 71%) as off-white crystals.

Step 4. A solution of methyl 4-(8-chloro-4-methyl-1-oxophthalazin-2(1*H*)-yl)benzoate (2.00 g, 6.08 mmol) and 5 N hydrochloric acid (100 mL, 100 mmol) in 1,4-dioxane (100 mL) was stirred at 110 °C. After 22 h, the solution was concentrated and dried in vacuo to give 4-(8-chloro-4-methyl-1-oxophthalazin-2(1*H*)-yl)benzoic acid (2.09 g; crude material was used directly in the next step).

Step 5. A suspension of 4-(8-chloro-4-methyl-1-oxophthalazin-2(1*H*)-yl)benzoic acid (1.91 g, 6.07 mmol), **6** (1.78 g, 7.28 mmol), HOBt (1.07 g, 7.89 mmol), and EDCI (1.51 g, 7.89 mmol) in DMF (30 mL) was stirred at 25 °C for 4.5 h. The solution was diluted with EtOAc (300 mL) and washed with 1 N sodium hydroxide solution and brine. It was dried over magnesium sulfate, concentrated in vacuo, then purified by flash column chromatography (silica, 2−8% MeOH/DCM) to give **27** (3.08 g, 94% over two steps) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) 7.88 (2 H, d, J = 8.6 Hz), 7.69−7.83 (5 H, m), 7.28 (1 H, d, J = 7.8 Hz), 7.07−7.20 (2 H, m), 6.33 (1 H, d, J = 8.2 Hz), 5.34−5.46 (1 H, m), 3.39−3.55 (2 H, m), 2.73−2.92 (2 H, m), 2.63 (3 H, s), 2.42 (4 H, s), 2.10−2.23 (1 H, m), 1.81−2.01 (3 H, m), 1.61 (4 H, s), 1.45 (2 H, s); ESI MS calcd for $C_{32}H_{33}ClN_4O_2$ $[M + H]^+$ 541.2, found 541.2.

(*R*)-4-(7-Chloro-4-methyl-1-oxophthalazin-2(1*H*)-yl)-*N*-(6-(piperidin-1-ylmethyl)-1,2,3,4-tetrahydronaphthalen-1-yl)benzamide (28). Phthalazinone **28** was prepared from 2-bromo-5-chlorobenzoic acid using a procedure analogous to that for compound **27**. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 8.49 (1 H, d, J = 1.8 Hz), 7.86−7.96 (2 H, m), 7.72−7.85 (4 H, m), 7.28 (1 H, d, J = 8.0 Hz), 7.14 (2 H, d, J = 8.0 Hz), 6.36 (1 H, d, J = 8.2 Hz), 5.34−5.44 (1 H, m), 3.48 (2 H, s), 2.77−2.89 (2 H, m), 2.65 (3 H, s), 2.46 (4 H, br s), 2.11−2.22 (1 H, m), 1.84−2.01 (3 H, m), 1.62 (4 H, s), 1.39−1.51 (2 H, m); ESI MS calcd for $C_{32}H_{33}ClN_4O_2$ $[M + H]^+$ 541.2, found 541.2.

(*R*)-4-(6-Chloro-4-methyl-1-oxophthalazin-2(1*H*)-yl)-*N*-(6-(piperidin-1-ylmethyl)-1,2,3,4-tetrahydronaphthalen-1-yl)benzamide (29). Phthalazinone **29** was prepared from 2-bromo-4-chlorobenzoic acid using a procedure analogous to that for compound **27**. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.46 (1 H, d, J = 8.4 Hz), 7.90 (2 H, d, J = 8.6 Hz), 7.73−7.83 (4 H, m), 7.28 (1 H, d, J = 8.2 Hz), 7.13 (1 H, d, J = 8.0 Hz), 7.09 (1 H, s), 6.35 (1 H, d, J = 8.2 Hz), 5.33−5.46 (1 H, m), 3.42 (2 H, s), 2.73−2.96 (2 H, m), 2.64 (3 H, s), 2.38 (4 H, br s), 2.10−2.21 (1 H, m), 1.80−2.01 (3 H, m), 1.50−1.64 (4 H, m), 1.34−1.48 (2 H, m); ESI MS calcd for $C_{32}H_{33}ClN_4O_2$ $[M + H]^+$ 541.2, found 541.2.

(*R*)-6-(8-Chloro-4-methyl-1-oxophthalazin-2(1*H*)-yl)-*N*-(6-(piperidin-1-ylmethyl)-1,2,3,4-tetrahydronaphthalen-1-yl)nicotinamide (30). Phthalazinone **28** was prepared from 2-bromo-6-chlorobenzoic acid and 6-hydrazinylnicotinic acid using a procedure analogous to that for compound **27**. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 9.01 (1 H, d, J = 2.2 Hz), 8.26 (1 H, dd, J = 8.4, 2.3 Hz), 7.65−7.85 (4 H, m), 7.26 (1 H, d, J = 7.6 Hz), 7.14 (1 H, d, J = 7.8 Hz), 7.10 (1 H, s), 6.36 (1 H, d, J = 8.2 Hz), 5.34−5.46 (1 H, m), 3.37−3.45 (2 H, m), 2.72−2.93 (2 H, m), 2.65 (3 H, s), 2.38 (4 H, br s), 2.11−2.24 (1 H, m), 1.82−2.00 (3 H, m), 1.52−1.63 (4 H, m), 1.36−1.47 (2 H, m); ESI MS calcd for $C_{31}H_{32}ClN_5O_2$ $[M + H]^+$ 542.2, found 542.2.

(*R*)-5-(8-Chloro-4-methyl-1-oxophthalazin-2(1*H*)-yl)-*N*-(6-(piperidin-1-ylmethyl)-1,2,3,4-tetrahydronaphthalen-1-yl)picolinamide (31). *Step 1.* A suspension of 5-aminopicolinic

acid (4.98 g, 36.07 mmol) in methanol (50 mL) was cooled to 0 °C under nitrogen, and thionyl chloride (7.89 mL, 108.2 mmol) was added in dropwise fashion over 10 min. The reaction mixture was stirred at 23 °C for a further 10 min and then heated to reflux under nitrogen for 48 h. The reaction mixture was cooled to 23 °C, concentrated in vacuo, diluted with saturated sodium bicarbonate solution (150 mL), and extracted with EtOAc. The combined organic layers were washed with brine, dried over magnesium sulfate, and concentrated in vacuo, affording methyl 5-aminopicolinate (3.390 g, 61%) as a light yellow solid.

Step 2. A solution of methyl 5-aminopicolinate (980 mg, 6.44 mmol) in 6 N hydrochloric acid (10.74 mL, 64.41 mmol) was cooled to 0 °C. A solution of sodium nitrite (667 mg, 9.66 mmol) in water (5 mL) was added in dropwise fashion from an addition funnel. The reaction mixture was stirred at 0 °C for 15 min, followed by 23 °C for 30 min, followed by cooling to 0 °C. Tin(II) chloride dihydrate (3.079 g, 13.53 mmol) was added, followed by warming to 10 °C over 2 h. The reaction mixture was quenched with 5 N sodium hydroxide solution (until the solution was basic to pH paper) and was extracted with DCM. The combined organic layers were dried over magnesium sulfate and concentrated in vacuo, affording methyl 5-hydrazinylpicolinate (718 mg, 67%) as a yellow solid.

Step 3. A solution of methyl 2-acetyl-6-chlorobenzoate (0.825 g, 3.8 mmol) and methyl 5-hydrazinylpicolinate (0.713 mg, 4.2 mmol) in methanol (15 mL) was treated with concentrated sulfuric acid (7.5 mL, 13.5 mmol). The mixture was heated to 80 °C in a sealed vessel. After 4 h, the mixture was cooled to 23 °C, and the precipitate was poured into an ice−water solution (100 mL). The solid was collected by filtration and washed with water (25 mL), affording an off-white gel-like solid, which was dissolved in dichloromethane/methanol (1:1), concentrated, and azeotroped with toluene (5 mL), affording methyl 5-(8-chloro-4-methyl-1-oxophthalazin-2(1*H*)-yl)picolinate (1.27 g, 99%) as a brown solid.

Step 4. A suspension of crude methyl 5-(8-chloro-4-methyl-1-oxophthalazin-2(1*H*)-yl)picolinate (1.2 g, 3.8 mmol) in 1,4-dioxane (20 mL) was treated with 1 N hydrochloric acid (19.3 mL, 19.3 mmol). The mixture was heated to reflux. After 15 h, the mixture was cooled to 23 °C and concentrated in vacuo. The crude product was azeotroped with toluene (2 × 5 mL) and dried under vacuum, affording 5-(8-chloro-4-methyl-1-oxophthalazin-2(1*H*)-yl)picolinic acid (1.2 g, 99%) as a yellow solid.

Step 5. A suspension of 5-(8-chloro-4-methyl-1-oxophthalazin-2(1*H*)-yl)picolinic acid (1.255 g, 3.98 mmol), TEA (1.1 mL, 7.95 mmol), **6** (1.069 g, 4.37 mmol), HOBt (0.591 g, 4.37 mmol), and EDCI (0.838 g, 4.37 mmol) in DMF (25 mL) was stirred under nitrogen at 23 °C for 15 h. The solution was diluted with EtOAc (250 mL) and washed with saturated sodium bicarbonate solution (150 mL), water (100 mL), and brine (100 mL). The organic solution was dried over magnesium sulfate, concentrated in vacuo, and purified by flash column chromatography (silica, 2.5−10% MeOH/DCM), affording **31** (1.469 g, 68%) as an off-white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.89 (1 H, d, J = 1.8 Hz), 8.36 (1 H, dd, J = 8.4, 0.6 Hz), 8.21−8.31 (2 H, m), 7.83 (1 H, dd, J = 7.2, 2.0 Hz), 7.71−7.80 (2 H, m), 7.18−7.34 (1 H, m), 7.06−7.14 (2 H, m), 5.32−5.47 (1 H, m), 3.44 (2 H, s), 2.72−2.94 (2 H, m), 2.64 (3 H, s), 2.39 (4 H, s), 2.05−2.27 (1 H, m), 1.81−2.01 (3 H, m), 1.54−1.67 (4 H, m), 1.36−1.47 (2 H, m); ESI MS calcd for $C_{31}H_{32}ClN_5O_2$ $[M + H]^+$ 542.2, found 542.2.

(*R*)-4-(8-Methyl-5-oxopyrido[2,3-*d*]pyridazin-6(5*H*)-yl)-*N*-(6-(piperidin-1-ylmethyl)-1,2,3,4-tetrahydronaphthalen-1-yl)benzamide (32). Phthalazinone **32** was prepared from methyl 2-bromonicotinate using a procedure analogous to that used for the preparation of compound **27**. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 9.12 (1 H, dd, J = 4.6, 1.9 Hz), 8.77 (1 H, dd, J = 8.1, 1.9 Hz), 7.88−7.95 (2 H, m), 7.76−7.85 (2 H, m), 7.72 (1 H, dd, J = 8.1, 4.6 Hz), 7.28 (1 H, d, J = 7.8 Hz), 7.13 (1 H, d, J = 8.0 Hz), 7.09 (1 H, s), 6.37 (1 H, d, J = 8.2 Hz), 5.34−5.45 (1 H, m), 3.43 (2 H, s), 2.78−2.95 (2 H, m), 2.76 (3 H, s), 2.38 (4 H, s), 2.09−2.21 (1 H, m), 1.81−2.06 (3 H, m), 1.52−1.63 (4 H, m), 1.31−1.49 (2 H, m); ESI MS calcd for $C_{31}H_{33}N_5O_2$ $[M + H]^+$ 508.2, found 508.2.

(*R*)-4-(8-Chloro-4-methyl-1-oxophthalazin-2(1*H*)-yl)-*N*-(7-(piperidin-1-ylmethyl)chroman-4-yl)benzamide (33). A solution of 4-(8-chloro-4-methyl-1-oxophthalazin-2(1*H*)-yl)benzoic acid (189 mg, 0.60 mmol, intermediate for compound **27**) and (*R*)-7-(piperidin-1-ylmethyl)-3,4-dihydro-2*H*-chromen-4-amine dihydrochloride (211 mg, 0.661 mmol)[10] in DMF (12 mL) was treated with TEA (0.21 mL, 1.50 mmol), and the mixture was stirred at 23 °C for 5 min. The addition of HOBt (97.4 mg, 0.721 mmol) and EDCI (138 mg, 0.721 mmol) followed, and the reaction mixture was then stirred for 5 h. The solution was diluted with EtOAc (100 mL) and washed with saturated sodium bicarbonate solution, water, and brine. It was dried over magnesium sulfate, concentrated in vacuo, and purified by flash column chromatography (silica, 2−8% MeOH/DCM), affording **33** (232 mg, 71%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.84−7.95 (2 H, m), 7.65−7.83 (5 H, m), 7.20 (1 H, d, J = 8.0 Hz), 6.90 (1 H, dd, J = 7.8, 1.4 Hz), 6.84 (1 H, d, J = 1.4 Hz), 6.39 (1 H, d, J = 7.2 Hz), 5.34 (1 H, dd, J = 12.3, 7.0 Hz), 4.27−4.37 (1 H, m), 4.14−4.26 (1 H, m), 3.41 (2 H, s), 2.63 (3 H, s), 2.28−2.47 (5 H, m), 2.12−2.25 (1 H, m), 1.51−1.67 (4 H, m), 1.31−1.48 (2 H, m); ESI MS calcd for $C_{31}H_{31}ClN_4O_3$ $[M + H]^+$ 543.2, found 543.2.

(*R*)-6-(5,8-Difluoro-4-methyl-1-oxophthalazin-2(1*H*)-yl)-*N*-(6-(piperidin-1-ylmethyl)-1,2,3,4-tetrahydronaphthalen-1-yl)nicotinamide (34). Phthalazinone **28** was prepared from 2-bromo-3,6-fluorobenzoic acid and 6-hydrazinylnicotinic acid using a procedure analogous to that for compound **27**. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 9.02 (1 H, d, J = 2.2 Hz), 8.26 (1 H, dd, J = 8.4, 2.3 Hz), 7.77 (1 H, d, J = 8.2 Hz), 7.46−7.60 (1 H, m), 7.34−7.48 (1 H, m), 7.21−7.30 (1 H, m), 7.14 (1 H, d, J = 7.8 Hz), 7.09 (1 H, s), 6.40 (1 H, d, J = 8.2 Hz), 5.34−5.46 (1 H, m), 3.42 (2 H, s), 2.77−2.92 (2 H, m), 2.75 (3 H, d, J = 7.4 Hz), 2.37 (4 H, br s), 2.10−2.24 (1 H, m), 1.78−2.01 (3 H, m), 1.49−1.68 (4 H, m), 1.33−1.50 (2 H, m); ESI MS calcd for $C_{31}H_{31}F_2N_5O_2$ $[M + H]^+$ 544.2, found 544.2.

(*R*)-6-(8-Fluoro-4-methyl-1-oxophthalazin-2(1*H*)-yl)-*N*-(6-(piperidin-1-ylmethyl)-1,2,3,4-tetrahydronaphthalen-1-yl)nicotinamide (35). A solution of 6-(8-fluoro-4-methyl-1-oxophthalazin-2(1*H*)-yl)nicotinic acid (6.9 g, 23.3 mmol, prepared from 2-bromo-6-fluorobenzoic acid in a procedure analogous to that for compound **30**), **6** (6.2 g, 25.7 mmol), HOBt (3.1 g, 23.3 mmol), and EDCI (4.4 g, 23.3 mmol) in DMF (100 mL) was stirred at 23 °C under nitrogen. After 15 h, the mixture was diluted with EtOAc (500 mL) and washed with saturated sodium bicarbonate solution (200 mL), water (200 mL), and brine (200 mL), dried over $MgSO_4$, concentrated in vacuo, and purified by flash column chromatography (silica, 1−10% MeOH/DCM), affording **35** (7.6 g, 62%) as an off-white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 9.04 (1 H, d, J = 2.5 Hz), 8.25 (1 H, dd, J = 8.3, 2.3 Hz), 7.81−7.90 (1 H, m), 7.75 (1 H, d, J = 8.5 Hz), 7.61 (1 H, d, J = 8.0 Hz), 7.39−7.50 (1 H, m), 7.21−7.29 (1 H, m), 7.13 (1 H, d, J = 7.5 Hz), 7.05 (1 H, s), 6.57 (1 H, d, J = 8.0 Hz), 5.35−5.46 (1 H, m), 3.40 (2 H, s), 2.71−2.90 (2 H, m), 2.66 (3 H, s), 2.37 (4 H, s), 2.10−2.22 (1 H, m), 1.81−2.05 (3 H, m), 1.51−1.66 (4 H, m), 1.32−1.50 (2 H, m). ESI MS calcd for $C_{31}H_{32}FN_5O_2$ $[M + H]^+$ 526.2, found 526.2.

Biological Assays. Details of the in vitro binding and cellular assays have been described previously in ref 11. The PK and PD assay methods have been previously described in detail in ref 12.

Doses of compound used in the rabbit blood pressure assay (Figure 4) were as follows: 0.03, 0.1, 1, and 3 mg/kg for compound **30** and 0.003, 0.01, 0.03, 0.1, 0.3, and 1 mg/kg for compound **35**.

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■ ABBREVIATIONS USED

BK, bradykinin; PSA, polar surface area; SAR, structure−activity relationship; PK, pharmacokinetics; PD, pharmacodynamics; DAK, des-Arg-kallidin; HPLC, high-performance liquid chromatography; ESI MS, electrospray ionization mass spectrometry; DCM, dichloromethane; DMF, *N*,*N*-dimethylformamide; EDCI, *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride; EtOAc, ethyl acetate; HOBt, 1-hydroxybenzotriazole; HPMC, hydroxypropyl methylcellulose; MeOH, methanol; $Pd(PPh_3)_4$, tetrakis(triphenylphosphine)palladium(0); TEA, triethylamine; TFA, trifluoroacetic acid; THF, tetrahydrofuran

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