1-Amino-4-benzylphthalazines as Orally Bioavailable Smoothened Antagonists with Antitumor Activity

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Abnormal activation of the Hedgehog (Hh) signaling pathway has been linked to several types of human cancers, and the development of small-molecule inhibitors of this pathway represents a promising route toward novel anticancer therapeutics. A cell-based screen performed in our laboratories identified a new class of Hh pathway inhibitors, 1-amino-4-benzylphthalazines, that act via antagonism of the Smoothened receptor. A variety of analogues were synthesized and their structure−activity relationships determined. This optimization resulted in the discovery of high affinity Smoothened antagonists, one of which was further profiled in vivo. This compound displayed a good pharmacokinetic profile and also afforded tumor regression in a genetic mouse model of medulloblastoma.

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The Hedgehog (Hh[a]) signaling pathway plays a critical role in the development and maintenance of numerous organs and tissues.[1] In its resting state, the 12-pass transmembrane protein Patched (Ptch) inhibits the activity of the 7-pass transmembrane receptor protein Smoothened (Smo). Hh family protein ligands Sonic Hedgehog (Shh), Desert Hedgehog (Dhh), and Indian Hedgehog (Ihh) can all bind to Ptch, leading to relief of Smo repression. Activated Smo then initiates a signaling cascade via a cytosolic complex of proteins, resulting in activation of the Gli1 family of transcription factors. Active Gli1 signaling can cause cell proliferation and differentiation. Clear links exist between genetic activation of the Hh pathway (via mutations in Ptch, Smo, suppressor of Fused (SUFU), or Gli) and tumorigenesis in several cancers, such as basal cell carcinoma and medulloblastoma.[2−4]

Small molecules have been identified that bind Smo and act as either agonists or antagonists of the Hh pathway (Figure 1).[5] Activation of Hh signaling via introduction of a Hh−protein ligand has shown therapeutic benefit in models of Parkinson's disease and peripheral nerve damage;[6,7] hence, small-molecule agonists such as Hh-Ag1.5 (**1**) may provide interesting opportunities for drug development.[8] Smo antagonists such as natural product cyclopamine (**2**) and HhAntag691 (**3**) have been shown to induce remissions of medulloblastoma in Ptch$^{+/-}$p53$^{-/-}$ mice.[9,10] Recently, Genentech reported the first in-human, phase I clinical trial results of treatment of advanced basal cell carcinoma patients with their Smo antagonist GDC-0449 (**4**); on the basis of positive results, the compound has recently been advanced to phase II.[11] Several other reports on inhibitors of the Hh pathway have appeared in the recent literature.[12,13] Herein, we report a portion of our own efforts in this area,[13a] which resulted in the discovery of a structurally unrelated, potent class of Smo antagonists.[14]

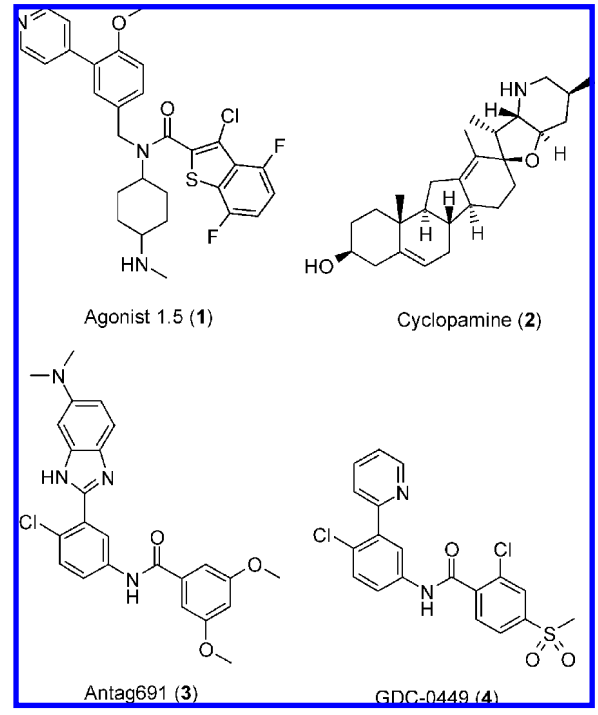

Figure 1. Structures of previously reported Smoothened agonist (**1**) and antagonists (**2**−**4**).

Cell-based screening of a portion of the Novartis compound collection identified 1-amino-4-benzylphthalazines, such as compound **5**, as micromolar inhibitors of the Hh pathway (Figure 2).[15,16] Membrane filter binding assays using mouse and human Smo were employed both to confirm interaction with the receptor and to demonstrate that such compounds showed

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[a] Abbreviations: cyp, cyclopropyl; Hh pathway, Hedgehog pathway; HPBCD, hydroxypropyl β-cyclodextrin; PEG, polyethylene glycol; Ptch, Patched; Smo, Smoothened; TPGS, D-α-tocopheryl polyethylene glycol 1000 succinate.

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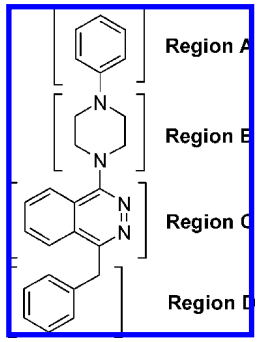


Figure 2. Structure of **5**, a Hedgehog antagonist screening hit.

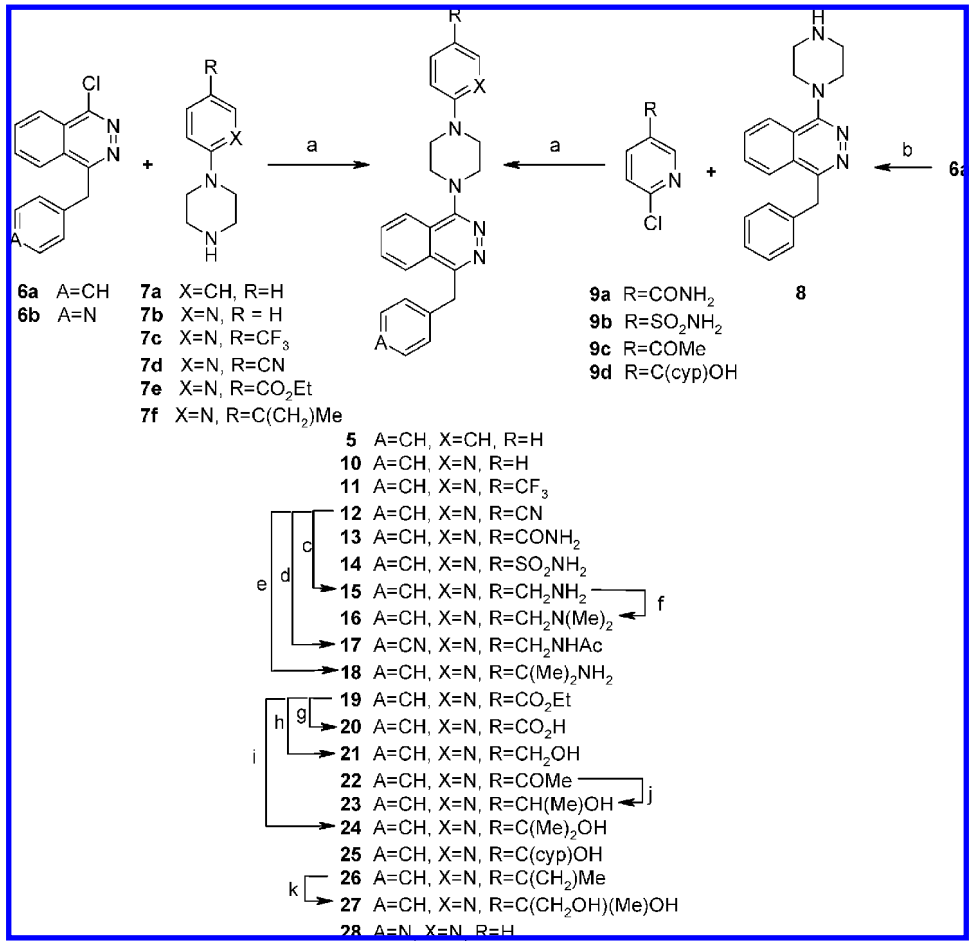
sufficient cross-reactivity with human Smo. We then initiated a chemistry effort to investigate the structure−activity relationships around each of the ring systems of **5** (regions A−D), which culminated in the identification of a potent, orally bioavailable Smo antagonist with excellent antitumor activity demonstrated in vivo.

Chemistry

Initial synthetic routes explored to analogues of **5** focused on introducing modifications to regions A and D (Figure 2). 1-Benzyl-4-chlorophthalazine **6a** served as a general intermediate to introduce different A rings via one of two routes (Scheme 1). In the first route, substituted piperazines **7a**−**f** were added to **6a** under thermal conditions to afford compounds **5**, **10**, **11**, **12**, **19**, and **26**. In the second route, **6a** was first combined with excess piperazine to generate intermediate **8**, which was then reacted with a series of 2-chloropyridines **9a**−**d** to generate compounds **13**, **14**, **22**, and **25**. Further functionalization of some of these compounds afforded additional analogues. 5-Cyanopyridine analogue **12** was converted to primary amine **15** via nickel boride reduction,[17] to amide **17** via a reduction/acetylation sequence, and to tertiary amine **18** via addition of a preformed methylcerium reagent.[18] Primary amine **15** was converted to dimethylamine **16** via reductive amination with formaldehyde. Hydrolysis of ethyl ester **19** afforded carboxylic acid **20**; alternatively, treatment of **19** with $LiAlH_4$ afforded primary alcohol **21**, while reaction with excess MeMgI provided tertiary alcohol **24**. Reduction of methyl ketone **22** with $NaBH_4$ yielded secondary alcohol **23**. Finally, osmium-catalyzed dihydroxylation[19] of olefin **26** afforded diol **27**. Reaction of 1-(4-pyridyl)-4-chlorophthalazine **6b** with **7d** afforded 4-pyridyl analogue **28**.

To further explore changes to region D, an alternative route was developed (Scheme 2). Addition of substituted piperazine **7d** to 1,4-dichlorophthalazines **29a** and **29b**[20] afforded chlorophthalazine intermediates **30** and **31a**,**b**. These were then coupled via palladium catalysis with a variety of benzylzinc reagents to provide analogues **32**−**39**.[21] Chlorophthalazines **39a**,**b** were converted to the corresponding cyano derivatives **40a**,**b** under palladium catalysis using $Zn(CN)_2$.[22]

Scheme 1[a]



[a] Reagents and conditions: (a) Et_3N, NMP, 180 °C, microwave; (b) piperazine, Et_3N, NMP, 180 °C, microwave; (c) $NiCl_2$, $NaBH_4$, EtOH; (d) (i) $NiCl_2$, $NaBH_4$, EtOH; (ii) Ac_2O; (e) $CeCl_3$, MeMgBr, THF; (f) $NaBH(OAc)_3$, H_2CO, CH_2Cl_2; (g) LiOH, EtOH, 60 °C; (h) $LiAlH_4$, THF; (i) MeMgI, THF; (j) $NaBH_4$, MeOH, 0 °C; (k) K_2OsO_4 (cat.), NMO, acetone/H_2O, cyp = cyclopropyl.

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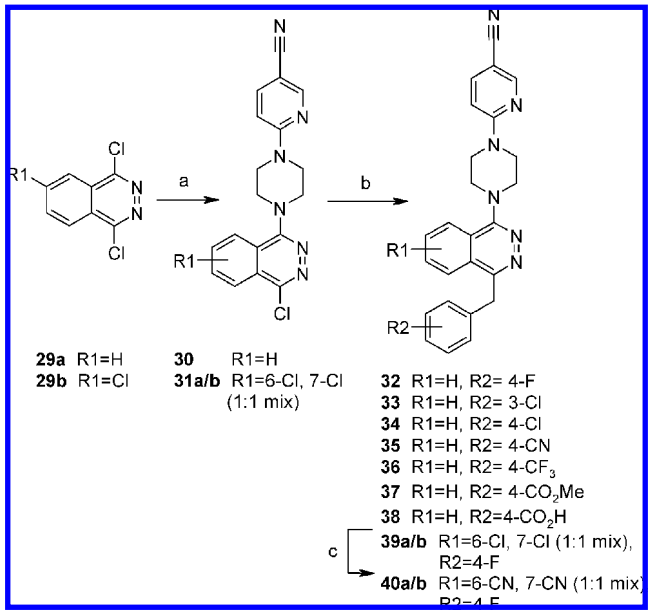
Scheme 2[a]



[a] Reagents and conditions: (a) **7d**, triethylamine, NMP, 180 °C, microwave; (b) R2 = benzylzinc chloride, $Pd(PPh_3)_4$ (cat.), THF; (c) $Zn(CN)_2$, $Pd_2(dba)_3$ (cat.), XPhos (cat.), DMF, 120 °C, microwave.

Similar chemistry was used to explore modifications to region B (Scheme 3). Thus, reaction of **6a** with homopiperazine, (*R*)-methylpiperazine, or (*S*)-methylpiperazine afforded intermediates **41a**−**c**. In the cases involving methylpiperazine, complete regioselectivity was observed in the displacements, presumably because of the steric hindrance introduced by the methyl substituent. These intermediates were then reacted with 6-chloronicotinonitrile to afford **42**−**44**. To obtain the methylpiperazine analogues of the opposite regiochemistry, substituted piperazines **45a**,**b** were prepared and then reacted with **6a** to afford **46**, **47**. Similarly, substituted piperidine **45c** afforded **48**.

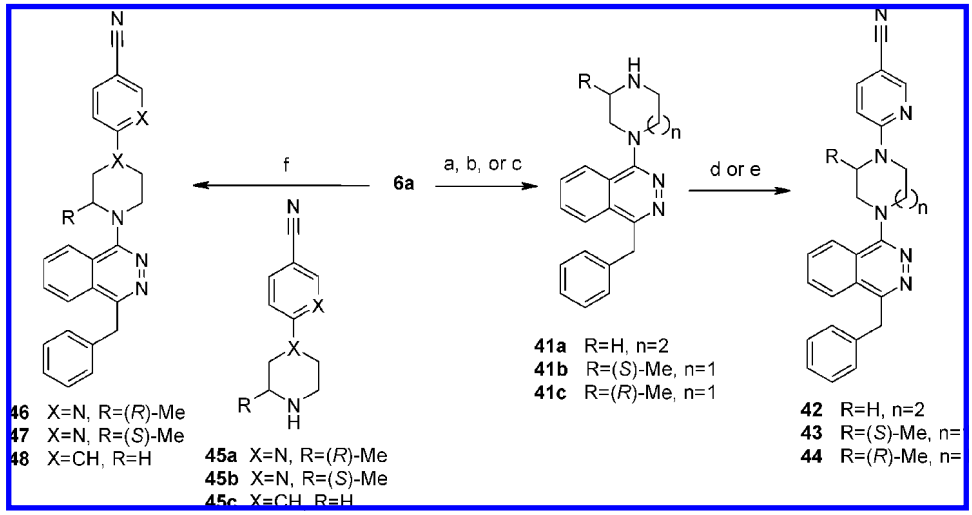
Finally, modifications were made to region C via several different routes (Schemes 4 and 5). Pd-catalyzed coupling of 4-bromo-1-chloroisoquinoline (**49**) with benzylzinc chloride afforded **50**, which was then reacted under Buchwald−Hartwig amination conditions[23] with **7d** to afford isoquinoline **51**. Alternatively, initial reaction of **49** with *n*-BuLi/$ZnBr_2$ followed by Pd-catalyzed coupling with benzyl bromide provided **52**,[24] which was then reacted with **7d** under thermal conditions to provide isoquinoline **53**. Reaction of 3-piperidinylindole **54** with 6-chloronicotinonitrile afforded intermediate **55**, and N-alkylation under phase-transfer conditions yielded **56**. 4,7-Dichlorofuro[2,3-*d*]pyridazine[25] (**57**) was first reacted with **7c** under thermal conditions and then coupled with benzylzinc chloride in the presence of $Pd(PPh_3)_4$ to afford a mixture of regioisomers **58** and **59**, which were separable by reverse-phase HPLC (Scheme 5). 4,7-Dichloro-1*H*-imidazo[4,5-*d*]pyridazine[26] (**60**) was reacted under an identical sequence of conditions to afford a single regioisomer **61**.

Results and Discussion

Structure−Activity Relationships (SAR). All compounds were first evaluated in a cell-based reporter gene assay for their ability to inhibit the Hh pathway. A mouse cell line (TM3) was stably transfected with a Hh-responsive reporter construct to afford Hh-dependent luciferase expression, and the ability of compounds to induce pathway inhibition was evaluated via competition with Smo agonist **1** at two different concentrations, 1 and 25 nM. A shift to a higher IC_{50} at the higher agonist concentration in this TM3-Gli-Luc IC_{50} shift assay ("Gli shift" assay) suggested antagonism of Smo. Membrane filter binding assays, which assessed the ability of compounds to displace radiolabeled **1** from Smo, were also employed to confirm that compounds were acting via interaction with the Smo receptor. Since our reporter gene assay was conducted in mouse cells, filter binding assays were conducted with both mouse and human Smo to ensure that the desired cross-reactivity was observed.

Structure−activity relationships resulting from modifications to regions A and D are shown in Table 1. Changing region A from a phenyl to a 2-pyridyl (compound **10**) afforded significant improvements in both Hh pathway inhibition and Smo binding affinity relative to **5**. The introduction of electron-withdrawing groups, such as $-CF_3$ and $-CN$, at the para-position of this ring (compounds **11** and **12**) offered further enhancements in activity. A primary amide (compound **13**) or sulfonamide (compound **14**) at this position was well-tolerated. Electron-

Scheme 3[a]



[a] Reagents and conditions: (a) homopiperazine, Et_3N, NMP, 180 °C, microwave; (b) (*R*)-methylpiperazine, Na_2CO_3, dioxane, 100 °C; (c) (*S*)-methylpiperazine, Na_2CO_3, dioxane, 100 °C; (d) 6-chloronicotinonitrile, Na_2CO_3, DMF/dioxane, 180 °C, microwave; (e) 6-chloronicotinonitrile, Et_3N, NMP, 180 °C, microwave; (f) **6a**, Et_3N, DMF, 180 °C, microwave.

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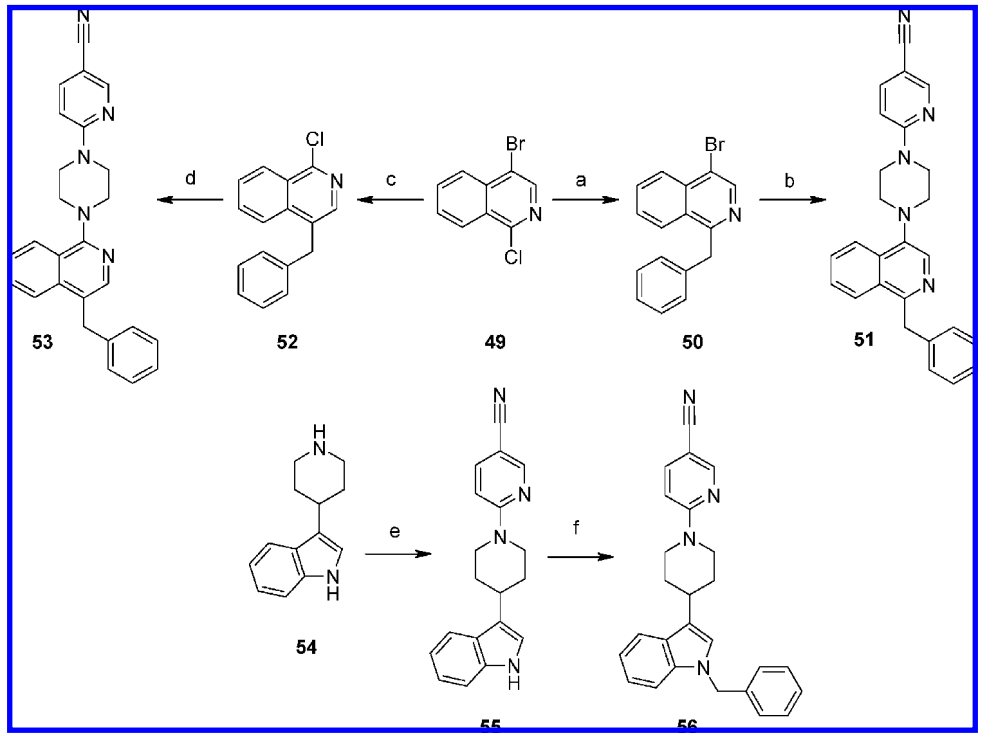
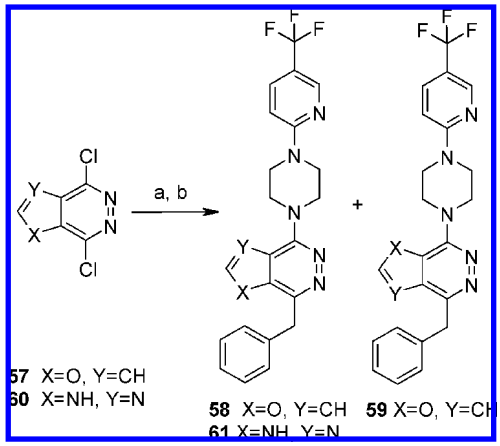
Scheme 4[a]



[a] Reagents and conditions: (a) benzylzinc chloride, $Pd(PPh_3)_4$ (cat.), THF; (b) **7d**, $Pd_2(dba)_3$, (rac)-BINAP, KO-*t*-Bu, dioxane, 80 °C; (c) (i) *n*-BuLi, $ZnBr_2$; (ii) benzyl bromide, $Pd(PPh_3)_4$ (cat.), THF; (d) **7d**, triethylamine, NMP, 180 °C, microwave; (e) 6-chloronicotinonitrile, K_2CO_3, DMF, 95 °C; (f) benzyl bromide, NaOH, $Bu_4N^+OH^-$, THF/H_2O.

Scheme 5[a]



[a] Reagents and conditions: (a) **7c**, Et_3N, NMP, 180 °C, microwave; (b) benzylzinc chloride, $Pd(PPh_3)_4$ (cat.), THF.

donating substituents at this position were also quite effective, with methylene-linked amines (e.g., compound **16**) and amides (e.g., compound **17**) offering excellent enhancements relative to **10** and with a tertiary amine (compound **18**) displaying impressive activity. Notably, a carboxylic acid at this position resulted in significant loss of activity (compound **20**). Primary (compound **21**), secondary (compound **23**), and tertiary (compound **24**) alcohols were all well-tolerated, and again, the compound with the largest steric bulk at this position (**24**) showed the highest affinity. The cyclopropyl ring of compound **25** proved significantly inferior to the dimethyl substitution found in **24**. Somewhat diminished activity was also observed upon changing from **24** to diol **27**.

In region D, replacement of phenyl by a 4-pyridyl ring resulted in significant loss of activity (compound **28** vs **10**). A *p*-fluoro or *m*-chloro substituent had little effect on activity (compounds **32** and **33** vs **12**), whereas *p*-chloro or *p*-cyano substitution showed distinct improvements (compounds **34** and **35**). Other electron-withdrawing groups at this position, such as $-CF_3$, $-CO_2Me$, and $-CO_2H$, resulted in significant losses of activity (compounds **36**−**38**).

Table 2 shows structure−activity relationships established for region B. A change from piperazine to homopiperazine was tolerated, but a ∼2- to 3-fold drop in activity was observed (compound **42** vs **12**). Of the four chiral methyl substitutions investigated on the piperazine ring, two substitutions afforded similar potency to the unsubstituted piperazine (compounds **43** and **46**), while the other two each improved potency >2 fold (compounds **44** and **47**). Replacement of piperazine by piperidine afforded a large drop in activity (compound **48**).

Finally, structure−activity relationships were established for region C (Table 3). Introduction of a chloro substituent on the phthalazine ring afforded a significant enhancement in activity (compounds **39a**,**b**), but the more strongly electron-withdrawing cyano substituent led to a loss in activity (compounds **40a**,**b**). Replacement of the phthalazine by either isoquinoline regioisomer afforded significant loss in activity (compounds **51** and **53**). Conversion to a 3-piperidin-4-ylindole core also resulted in a loss of activity (compound **56**). Significant reductions in activity were again observed upon replacement of the phthalazine by either furo[2,3-*d*]- or 1*H*-imidazo[4,5-*d*]pyridazine cores (compounds **58**, **59**, **61**).

After completion of an investigation of the SAR in four distinct regions, several conclusions could be drawn. First, excellent cross-reactivity between mouse and human Smo was observed within the series; the human Smo filter binding IC_{50} values for almost all compounds were within 2- to 3-fold of those observed with mouse Smo. In addition, consistent IC_{50} shifts were seen between the low and high agonist concentrations

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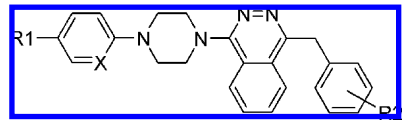
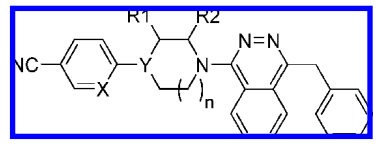
Table 1. IC_{50} Values for Select Compounds in the TM3-Gli-Luc IC_{50} Shift Assay and Mouse (Ms) and Human (Hu) Smo Membrane Filter Binding Assays[a]



				IC_{50}, nM			
compd	R1	X	R2	Gli shift 1 nM[b]	Gli Shift 25 nM[b]	MsBdg[c]	HuBdg[c]
5	H	CH	H	414 ± 4	2246 ± 1514	1095	4237
10	H	N	H	258 ± 28	772 ± 75	643	1243
11	CF_3	N	H	134 ± 30	1327 ± 35	74	95
12	CN	N	H	69 ± 22	821 ± 205	71	112
13	$CONH_2$	N	H	46 ± 10	149 ± 1	173	724
14	SO_2NH_2	N	H	104 ± 14	919 ± 49	90	306
16	$CH_2N(Me)_2$	N	H	14 ± 2	246 ± 46	287	654
17	CH_2NHAc	N	H	35 ± 13	327 ± 39	96	196
18	$C(Me)_2NH_2$	N	H	1.3 ± 0.3	42 ± 13	6	15
20	CO_2H	N	H	2665 ± 153	>5000	1644	2261
21	CH_2OH	N	H	374 ± 40	2448 ± 38	187	484
23	CH(Me)OH	N	H	38 ± 16	222 ± 30	61	67
24	$C(Me)_2OH$	N	H	2.7 ± 0.9	35 ± 3	5	8
25	C(cyp)OH[d]	N	H	55	345	10	21
27	$C(CH_2OH)(Me)OH$	N	H	17 ± 3	136 ± 10	28	70
28	H	N	4-pyridyl[e]	690 ± 98	4493 ± 30	3690	>5000
32	CN	N	4-F	112 ± 20	975 ± 19	69	103
33	CN	N	3-Cl	112 ± 18	693 ± 132	54	82
34	CN	N	4-Cl	48 ± 1	498 ± 20	20	25
35	CN	N	4-CN	20 ± 5	256 ± 2	23	34
36	CN	N	4-CF_3	366 ± 14	1239 ± 221	226	185
37	CN	N	4-CO_2Me	265 ± 151	>5000	165	2929
38	CN	N	4-CO_2H	>5000	>5000	>5000	>5000

[a] See Experimental Section for detailed descriptions of each assay. [b] Results expressed as the mean ± standard deviation of two separate IC_{50} determinations. For each determination, concentration−inhibition curves were obtained in triplicate and then averaged to afford a single IC_{50} curve with a ≥95% confidence interval. [c] Results expressed are from a single IC_{50} determination. Concentration−inhibition curves were obtained in triplicate and then averaged to afford a single IC_{50} curve with a ≥95% confidence interval. [d] cyp = cyclopropyl. [e] In this case, benzene ring is replaced by, not substituted with, a 4-pyridyl ring.

Table 2. IC_{50} Values for Select Compounds in the TM3-Gli-Luc IC_{50} Shift Assay and Mouse (Ms) and Human (Hu) Smo Membrane Filter Binding Assays[a]



						IC_{50}, nM			
compd	X	Y	R1	R2	*n*	Gli Shift, 1 nM[b]	Gli Shift, 25 nM[b]	MsBdg[c]	HuBdgc[c]
12	N	N	H	H	1	69 ± 22	821 ± 205	71	112
42	N	N	H	H	2	210 ± 12	1632 ± 361	153	391
43	N	N	(*S*)-Me	H	1	79 ± 2	735 ± 13	66	59
44	N	N	(*R*)-Me	H	1	30 ± 10	244 ± 24	17	34
46	N	N	H	(*R*)-Me	1	86 ± 30	738 ± 74	180	217
47	N	N	H	(*S*)-Me	1	25 ± 16	210 ± 91	22	17
48	CH	CH	H	H	1	3776 ± 326	>5000	>5000	>5000

[a] See Experimental Section for detailed descriptions of each assay. [b] Results expressed as the mean ± standard deviation of two separate IC_{50} determinations. For each determination, concentration−inhibition curves were obtained in triplicate and then averaged to afford a single IC_{50} curve with a ≥95% confidence interval. [c] Results expressed are from a single IC_{50} determination. Concentration−inhibition curves were obtained in triplicate and then averaged to afford a single IC_{50} curve with a ≥95% confidence interval.

in the Gli shift assay. To summarize the SAR observed, a 2-pyridyl ring in region A was optimal, with large substituents at the 5-position providing the best improvements in potency. Piperazine substitution in region B was superior to homopiperazine or piperidine, and some improvements in potency could be obtained via the introduction of a chiral methyl substituent. In region C, the phthalazine ring system afforded superior affinity. Finally, the introduction of certain electron-withdrawing groups to the benzyl ring of region D provided moderate to excellent improvements in potency. Two compounds with excellent in vitro activity in the Gli shift and filter binding assays were identified, **18** and **24**. Given the propensity for multi-ring compounds that contain a strongly basic nitrogen to act as inhibitors of hERG channel activity,[27] we selected **24**, which lacks this functionality, for further profiling in vivo.

In Vivo Studies. Pharmacokinetic studies of **24** were conducted in nude mice, with both intravenous and oral dosing (Table 4). Since medulloblastoma is a potential clinical indication for Smoothened antagonists,[4] the brain concentration of **24** was also evaluated. Following a single 1 mg/kg intravenous

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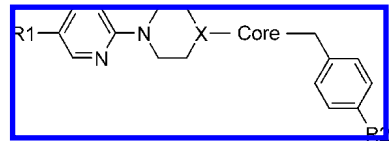
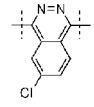
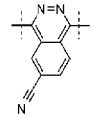
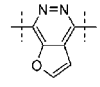
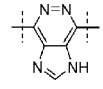
Table 3. IC_{50} Values for Select Compounds in the TM3-Gli-Luc IC_{50} Shift Assay and Mouse (Ms) and Human (Hu) Smo Membrane Filter Binding Assays[a]



Compound	R1	X	Core	R2	Gli Shift, 1 nM[b] (IC_{50}, nM)	Gli Shift, 25 nM[b] (IC_{50}, nM)	MsBdg[c] (IC_{50}, nM)	HuBdg[c] (IC_{50}, nM)
11	CF_3	N		H	134 ± 30	1327 ± 35	74	95
12	CN	N		H	69 + 22	821 + 205	71	112
39a/b	CN	N		F	34 ± 14	220 ± 32	12	13
40a/b	CN	N		F	197 ± 27	656 ± 51	499	708
51	CN	N		H	910 ± 150	2500 ± 894	>5000	>5000
53	CN	N		H	1427 ± 144	2987 ± 59	>5000	>5000
56	CN	CH		H	382 ⊥ 32	1507 ⊥ 324	386	401
58	CF_3	N		H	212 ı 25	2560 1960 =	139	230
59	CF_3	N		H	1798 ± 1278	>5000	>5000	>5000
61	CF_3	N		H	724 ± 192	1549 ± 111	>5000	>5000

[a] See Experimental Section for detailed descriptions of each assay. [b] Results expressed as the mean ± standard deviation of two separate IC_{50} determinations. For each determination, concentration−inhibition curves were obtained in triplicate and then averaged to afford a single IC_{50} curve with a ≥95% confidence interval. [c] Results expressed are from a single IC_{50} determination. Concentration−inhibition curves were obtained in triplicate and then averaged to afford a single IC_{50} curve with a ≥95% confidence interval.

dose to nude mice, **24** showed low plasma clearance relative to hepatic blood flow (90 (mL/min)/kg),[28] low volume of distribution compared to total body water (~0.7 L/kg),[28] and short half-life at 0.53 h. Following a single 5 mg/kg oral dose, a short T_{max} was observed, indicating rapid absorption, and the compound showed good oral bioavailability at 73%. The oral exposure AUC_{0-inf} brain/plasma ratio was moderate (0.33).

$Ptch^{+/-}$ mice have been reported to spontaneously develop medulloblastomas,[4c] and the deletion of p53 was found to cause earlier onset and to increase incidence of these tumors such that 95% of $Ptch^{+/-}p53^{-/-}$ mice develop medulloblastomas within 12 weeks of birth.[29] Treatment with Smo antagonists has been shown to effectively decrease the incidence of medulloblastoma in these mice, both directly in the transgenic model[4c] and in allograft models derived from the $Ptch^{+/-}p53^{-/-}$ medulloblastoma tumors.[29] The favorable PK profile observed with compound **24** along with its high affinity for Smo led us to evaluate this compound in a $Ptch^{+/-}p53^{-/-}$ mouse medulloblastoma allograft model.

After 8 days of oral administration, when the vehicle group had to be sacrificed because of excessive tumor size (tumors greater than 10% of mouse body weight), **24** demonstrated dose-

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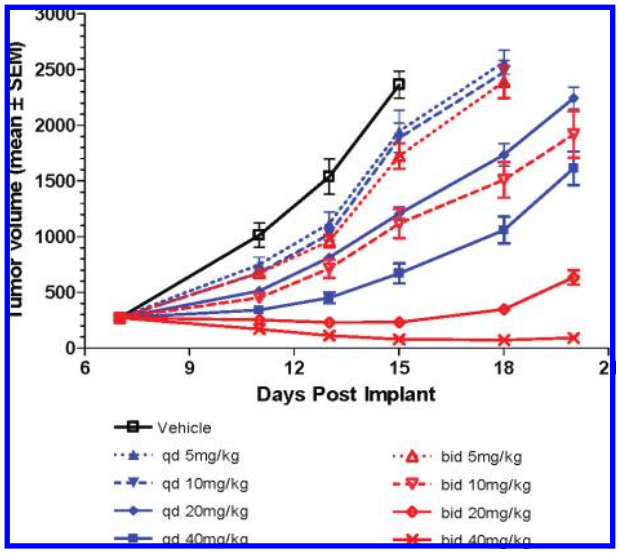

Figure 3. Antitumor activity upon treatment with **24** or vehicle in a Ptch$^{+/-}$p53$^{-/-}$ medulloblastoma allograft model in nude mice. Treatments started on day 7 after implantation (5 million cells/animal). Compound **24** was administered po at 5 mg/kg q.d. and b.i.d., 10 mg/kg q.d. and b.i.d., 20 mg/kg q.d. and b.i.d., and 40 mg/kg q.d. and b.i.d. for 12 days total. Vehicle control of **24**, 20% of 0.1 N HCl, 20% of HPBCD 40% in water, 60% of pH 7.4 buffer. Initial group size: 8 animals for all groups. Vehicle group was taken down 11 days after treatment because of excessive tumor size (greater than 10% of mouse body weight). $p < 0.05$ as determined by the ANOVA rank (Tukey) test.

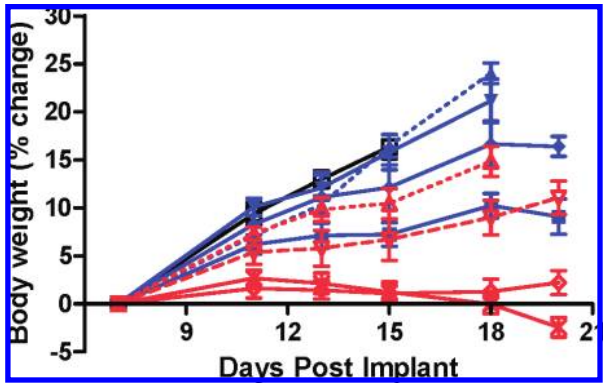

Figure 4. Body weight change upon treatment with **24** or vehicle in a Ptch$^{+/-}$p53$^{-/-}$ medulloblastoma allograft model in nude mice. See Figure 3 caption for study conditions.

related antitumor activity (Figure 3 and Table 5). When dosed at 40 mg/kg q.d., **24** showed a significant antitumor effect corresponding to a *T*/*C* value of 19% ($p < 0.05$ compared to vehicle controls). Dosing of **24** at 20 and 40 mg/kg b.i.d. resulted in 15% and 71% regression, respectively. In addition, **24** was well tolerated, affording no significant body weight loss at all doses investigated (Figure 4).

Table 4. Pharmacokinetic Profile of **24** in Nude Mouse[a]

mean PK parameter	mouse plasma PK iv (1 mg/kg)[b]	mouse plasma PK po (5 mg/kg)[c]	mouse brain PK po (5 mg/kg)[c]	mouse brain/plasma, po (5 mg/kg)[c]
AUC_{0-inf} (μM·h)	7.23	26.5	8.82	0.33
CL ((mL/min)/kg)	5.24			
V_{ss} (L/kg)	0.2			
$T_{1/2}$ (h)	0.53			
C_{max} (μM)		15.7	2.39	0.15
T_{max} (h)		0.25	0.25	
F (%)		73		

[a] The reported data are average values generated after either iv (1 mg/kg) or po (5 mg/kg) dose of compound **24** to female nude nude mice. Plasma and brain were collected ($n = 3$/time point) at scheduled times over 24 h after dosing. [b] Vehicle: 5% DMA, 15% PEG300, 30% (10% TPGS), 50% D5W. [c] Vehicle: 10% DMA, 30% PEG300, 60% (10% TPGS).

Table 5. In Vivo Antitumor Activity of **24** against Ptch$^{+/-}$p53$^{-/-}$ Medulloblastoma Allografts Implanted Subcutaneously in Nude Mice[a]

dose (mg/kg)[b]	% *T*/C	% regression
5, q.d.	80	none
10, q.d.	77	none
20, q.d.	45	none
40, q.d.	19	none
5, b.i.d.	69	none
10, b.i.d.	40	none
20, b.i.d.		15
40, b.i.d.		71

[a] See Figure 3 caption for study conditions. [b] The freebase was dosed po as a suspension in 20% of 0.1 N HCl, 20% of HPBCD, 40% in water, 60% of pH 7.4 buffer.

Inhibition of the Hedgehog pathway in tumors upon treatment with **24** was analyzed in the Ptch$^{+/-}$p53$^{-/-}$ medulloblastoma allograft model. Tumor-bearing mice were treated with a single dose of 5 or 40 mg/kg **24**, and tumors were harvested at different time points after dosing. Expression of Gli1 mRNA as a pharmacodynamic marker for Hedgehog pathway activity was analyzed by real-time PCR in tumor samples (Figure 5). Time- and dose-dependent inhibition of Gli1 mRNA was seen. Inhibiton was stronger and more prolonged at 40 mg/kg compared to 5 mg/kg. The degree of Gli1 mRNA inhibition correlated well with the tumor growth inhibition observed in the efficacy study (Table 5), indicating that the effect of **24** on tumor growth is mediated by inhibition of the Hedgehog pathway.

Conclusion

A novel series of Hh pathway inhibitors, 1-amino-4-benzylphthalazines, has been identified and confirmed to act via antagonism of the Smo receptor. Structure−activity relationships have been established around each ring system, and these efforts have resulted in the discovery of compounds with low nanomolar affinity for Smo. One of these compounds, **24**, displayed a good pharmacokinetic profile in mice, including a demonstrated ability to cross the blood−brain barrier. Treatment with

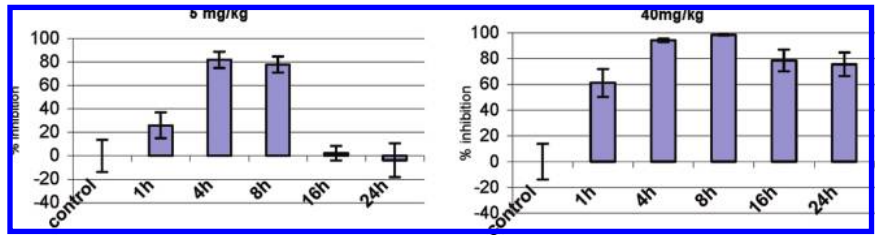

Figure 5. Inhibition of Gli1 mRNA expression upon treatment with **24**. Tumors were harvested 1, 4, 8, 16, and 24 h after a single po dose of 5 or 40 mg/kg **24**. Gli1 mRNA levels were analyzed by real-time PCR and normalized to β-actin expression. Data are shown as percent inhibition relative to vehicle-treated control tumors.

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24 in a genetic mouse model of medulloblastoma afforded dose-related reduction in tumor growth, with regression observed at the two highest doses administered. Further optimization of this series toward the selection of a clinical candidate will be reported in due course.

Experimental Section

TM3-Gli-Luc Reporter Gene Assay. Test compounds were prepared for assay by serial dilution in DMSO and then added to empty assay plates. TM3Hh12 cells (TM3 cells containing Hh-responsive reporter gene construct pTA-8xGli-Luc) were cultured in F12 Ham's/DMEM (1:1) containing 5% horse serum, 2.5% fetal bovine serum (FBS), and 15 mM HEPES, pH 7.3. Cells were harvested by trypsin treatment, resuspended in F12 Ham's/DMEM (1:1) containing 5% horse serum and 15 mM HEPES, pH 7.3, added to assay plates, and incubated with test compounds for approximately 30 min at 37 °C in 5% CO_2. Then 1 or 25 nM Ag1.5 (2)[8] was added to assay plates and incubated at 37 °C in the presence of 5% CO_2. After 48 h, either Bright-Glo (Promega E2650) or MTS reagent (Promega G258B) was added to the assay plates and luminescence or absorbance at 492 nm was determined. IC_{50} values, defined as the inflection point of the logistic curve, were determined by nonlinear regression of the Gli-driven luciferase luminescence or absorbance signal from MTS assay vs $\log_{10}$ (concentration) of test antagonist using the R statistical software package.

Membrane Filter Binding Assays. Smo membranes were prepared from CHO-K1 cells that were stably transfected with HA-tagged cDNA encoding mouse or human Smo. Test compounds were prepared for assay by serial dilution in DMSO and then added to empty assay plates. Smo membranes (10 μg of total protein) were added to these assay plates and incubated with 1.5 nM in binding buffer (50 mM Tris-HCl, 10 mM EDTA, and 5 mM magnesium chloride, pH 7.2) for 48 h at 37 °C. The 96-well Unifilter GF/B filter plates (Perkin-Elmer) were prepared by 60 min of incubation in binding buffer containing 0.5% w/v polyethyleneimine (Acros) and 0.1% (w/v) bovine serum albumin (Jackson Immuno Research) followed by three rinses with 2% β-hydroxypropylcyclodextrin (HPCD, Acros) in distilled water. Assay plates were harvested into filter plates using a Unifilter-96 cell harvester (Perkin-Elmer). Loaded filter plates were washed three times with 2% HPCD buffer to remove unbound [^{3}H]Hh-Ag 1.5, dried in a 60 °C oven for 30 min, and then cooled. The bottom of the plate was sealed, 50 μL of Microscint-O (Perkin-Elmer) was added to each well, the top of the plate was sealed, and the plate was incubated 100 min to overnight. The amount of bound [^{3}H]Hh-Ag 1.5 was determined by scintillation counting on a TopCount (Perkin-Elmer). The data were analyzed for saturation binding using global fitting with Graphpad Prizm software.

Pharmacokinetic Studies. Female nude mice ($n = 3$ per time point) were administered intravenously via tail vein at 1 mg/kg as a solution of 5% DMA, 15% PEG300, 30% (10% TPGS), 50% D5W or 5 mg/kg orally by gavage in 10% DMA, 30% PEG300, 60% (10% TPGS). At the allotted times over 24 h after dosing, the mice were sacrificed, blood was collected via cardiac puncture, and brain was removed. The plasma was separated by centrifugation. The tissue samples were homogenized in 1 mM PBS buffer (1 g of tissue plus 3 mL of buffer). The concentration of the compound was measured in plasma and tissue homogenate by the LC/MS/MS method after protein precipitation with acetonitrile. Relevant estimated pharmacokinetic parameters for plasma such as clearance and volume of distribution half-life were derived by noncompartmental analysis.

LCMS Assay. All plasma samples (25 μL), tissue homogenate (50 μL), including standards and blanks, were prepared for analysis by protein precipitation with acetonitrile containing Glyburdine as internal standard (150 μL). Then samples were centrifuged for 5 min at 4000 rpm. For LC/MS/MS analysis, 120 μL of the supernatant was transferred into 1 mL capacity 96-well plate and 10 μL aliquot of each sample was injected onto an ACE C18 (3 μm, 3.0 cm × 2.1 mm) MAC-MOD analytical HPLC column. Separation was achieved using an Agilent 1100 HPLC (Palo Alto, CA) system and 1 min linear gradient elution with mobile phase containing solvents A (99.9:0.1% water/formic acid) and B (99.9:0.1% acetonitrile/formic acid). Mobile phase was delivered at a flow rate of 0.7 mL/min. The mass spectral (MS/MS) detection was performed on a Sciex API 4000 triple quadruple mass spectrometer using multiple reaction monitoring with data acquisition in positive ion mode using electrospray ionization (ESI).

In Vivo Efficacy Study. Mouse $Ptch^{+/-}p53^{-/-}$ medulloblastoma cells ($(1.0-5.0) \times 10^6$), dissociated directly from tumor fragments, were inoculated subcutaneously into the right flank of Harlan nu/nu mice. Treatment was initiated approximately 7 days after implantation. Animals were randomized into treatment groups with similar mean tumor volumes of 271 mm^3 with individual tumor sizes ranging from approximately 200 to 340 mm^3. Tumor volumes (mm^3) and body weights (g) were recorded two or three times per week from all groups for analysis. Dose was body weight adjusted at time of dosing. Comparisons between treatment groups was performed using ANOVA (Tukey) rank sum test.

Gli1 mRNA Expression Analysis. Gli1 mRNA levels in tumors were analyzed by real-time PCR. Briefly, total RNA from approximately 100 mg of flash frozen tumor tissue was purified using the Aurum total RNA fatty and fibrous tissue kit (Biorad, Hercules CA). cDNA was synthesized by reverse transcription using qScript cDNA Supermix (Quanta Bioscience Gaithersburg, MD) using 2−5 μg of total RNA. Relative quantitation of mRNA expression was performed by real-time quantitative PCR using TaqMan gene expression reagents (Applied Biosystems, Foster City, CA). An amount of 2 μL of the cDNA reaction was used in a 25 μL reaction with 2× master mix containing Amplitaq gold DNA polymerase, Amperase UNG, dNTPs, UTP, and probes for mouse β-actin (Mm 4352341E) and Gli1 (Mm494645). The reaction was run on an ABI 7900HT fast real-time PCR system.

Chemistry. All nonaqueous reactions were carried out under a nitrogen atmosphere unless otherwise noted. All solvents employed were commercially available "anhydrous" grade, and reagents were used as received unless otherwise noted. A Biotage Initiator Sixty system was used for microwave heating. Flash column chromatography was performed on either an Analogix Intelliflash 280 using Si 50 columns (32−63 μm, 230−400 mesh, 60 Å) or on a Biotage SP1 system (32−63 μm particle size, KP-Sil, 60 Å pore size). Preparative high pressure liquid chromatography (HPLC) was performed using a Waters 2525 pump with 2487 dual wavelength detector and 2767 sample manager. Columns were Waters C18 OBD 5 μm, either 50 mm × 100 mm Xbridge or 30 mm × 100 mm Sunfire. Systems were run with either a 5−95% or 10−90% ACN/H_2O gradient with either a 0.1% TFA or 0.1% NH_3OH modifier. NMR spectra were recorded on a Bruker AV400 (Avance 400 MHz) instrument. Analytical LC−MS was conducted using an Agilent 1100 series with UV detection at 214 and 254 nm and an electrospray mode (ESI) coupled with a Waters ZQ single quad mass detector. One of two methods was used: method A, 5−95% ACN/H_2O with 5 mM ammonium formate with a 2 min run, 3 μL injection through an inertisil C8 3 cm × 5 mm × 3 μm; method B, 20−95% ACN/H_2O with 10 mM ammonium formate with a 2 min run, 3 μL injection through an inertisil C8 3 cm × 5 mm × 3 μm.

All tested compounds were ≥95% pure by HPLC unless otherwise noted. Analytical HPLC UV purity was assessed at both 254 and 214 nm using an Agilent 1100 HPLC system and one of the following methods. For method 1, an Inertsil 150 mm × 4.6 mm C18 column was used at a flow rate of 1.2 mL/min with a gradient of 10−95% acetonitrile/water with 0.1% TFA over 15 min. For method 2, an Inertsil ODS3 3 μm 3.0 mm × 100 mm C18 column was used at a flow rate of 1.5 mL/min with a gradient of 10−95% acetonitrile/water with 0.1% TFA over 15 min. For method 3, an ACE 3 μm 30 mm × 2.1 mm C18 column was used at a flow rate of 1.0 mL/min with a gradient of 5−98% acetonitrile/water with 0.1% TFA over 2.20 min. For method 4, an Inertsil ODS3 3 μm 3.0 mm × 100 mm C18 column was used at a flow

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rate of 1.0 mL/min with a gradient of 5−95% acetonitrile/water with 0.1% TFA over 7.75 min. LC/ESI-MS data were recorded using a Waters LCT Premier mass spectrometer with dual electrospray ionization source and Agilent 1100 liquid chromatograph. The resolution of the MS system was approximately 12 000 (fwhm definition). HPLC separation was performed at 1.0 mL/min flow rate with a gradient from 10% to 95% in 2.5 min. Ammonia formate (10 mM) was used as the modifier additive in the aqueous phase. Sulfadimethoxine (Sigma, protonated molecule *m*/*z* 311.0814) was used as a reference and acquired through the LockSpray channel every third scan.

General Procedure A for Addition of Amines to 1-Chlorophthalazines. The desired 1-chlorophthalazine (2 mmol, 1 equiv) and amine (2.6 mmol, 1.3 equiv) were added to a microwave vial equipped with a stir bar. NMP (3 mL) was then added followed by triethylamine (3.2 mL, 6 mmol, 3 equiv). The vial was sealed and irradiated in the microwave at 180 °C (high absorption setting) for 30 min. Water (50 mL) was then added to the reaction mixture to form a precipitate, which was isolated by filtration, washed with additional cold water, and then dried in vacuo. Products were further purified when necessary by either flash chromatography on silica gel or reverse phase HPLC.

1-Benzyl-4-(4-phenylpiperazin-1-yl)phthalazine (5). 5 was synthesized from **6a** and **7a** in 21% yield according to general procedure A. ^{1}H NMR (400 MHz, MeOH-d_4) δ 8.24 (d, J = 8.1 Hz, 1H), 8.15 (d, J = 7.6 Hz, 1H), 7.91 (t, J = 7.3 Hz, 1H), 7.85 (t, J = 7.1 Hz, 1H), 7.22−7.31 (m, 6H), 7.14−7.19 (m, 1H), 7.07 (d, J = 8.1 Hz, 2H), 6.88 (t, J = 7.3 Hz, 1H), 4.63 (s, 2H), 3.62−3.67 (m, 4H), 3.44−3.49 (m, 4H). Anal. RP-HPLC t_R = 7.01 min (method 1). HR-MS (*m*/*z*, MH$^+$): measd 381.2072, calcd 381.2079.

5′-Isopropenyl-3,4,5,6-tetrahydro-2*H*-[1,2′]bipyrazinyl (7f). Methyltriphenylphosphonium iodide (3.13 g, 7.55 mmol) was dissolved in THF (15 mL) and chilled to 5 °C. Potassium *tert*-butoxide (8.20 mL, 1 M in THF, 8.20 mmol) was added dropwise via syringe over 5 min. The mixture was stirred for 30 min, and then 1-(6-chloropyridin-3-yl)ethanone (1.00 g, 6.30 mmol) was added in one portion. The mixture was stirred an additional 30 min at 5 °C, and then the reaction was quenched via addition of saturated aqueous NH_4Cl (10 mL). THF was removed in vacuo, and the organics were extracted with EtOAc (3 × 20 mL). The combined organic layers were washed with brine and then stirred with decolorizing charcoal for 20 min. The charcoal was removed via filtration, and the filtrate was dried over $MgSO_4$ and concentrated. Diethyl ether (15 mL) was added to the residue and stirred 30 min. The resulting solids were removed via filtration and rinsed several times with additional ether. Filtrate and ether rinses were combined and concentrated, and the residue was purified by flash chromatography on silica gel (0−10% EtOAc in heptanes) to afford 2-chloro-5-isoprenylpyrazine as a clear oil (755 mg, 78% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.40 (d, J = 2.2 Hz, 1H), 7.63 (dd, J = 8.3, 2.5 Hz, 1H), 7.21 (dd, 1H), 5.34 (s, 1H), 5.13 (quin, J = 1.4 Hz, 1H), 2.07 (dd, J = 1.5, 0.8 Hz, 3H).

2-Chloro-5-isoprenylpyrazine (313 mg, 2.00 mmol), piperazine (861 mg, 10.00 mmol), and Et_3N (0.696 mL, 5.00 mmol) were combined in NMP (4 mL) in a vial and heated in the microwave (high absorption setting) at 180 °C for 30 min. The mixture was then diluted with H_2O (10 mL) and extracted with EtOAc (3 × 15 mL). The combined organic layers were dried over $MgSO_4$ and concentrated. The residue was purified by flash chromatography on silica gel (0−10% ammonium hydroxide in MeOH (10%) in DCM) to afford the title compound as an orange solid (309 mg, 76% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.32 (dd, J = 2.6, 0.7 Hz, 1H), 7.63 (dd, J = 8.9, 2.6 Hz, 1H), 6.62 (dd, J = 8.8, 0.6 Hz, 1H), 5.27 (d, J = 0.5 Hz, 1H), 4.95−4.98 (m, 1H), 3.54−3.61 (m, 4H), 2.97−3.07 (m, 4H), 2.12 (s, 3H). MS (*m*/*z*, MH$^+$): 204.1.

1-Benzyl-4-piperazin-1-ylphthalazine (8). Compound **6a** (1.06 g, 4.18 mmol) and piperazine (1.82 g, 20.9 mmol) were added to a microwave vial, followed by NMP (5 mL) and triethylamine (6.62 mL, 12.5 mmol). The vial was sealed and irradiated in the microwave at 180 °C (high absorption setting) for 30 min. CH_2Cl_2 (10 mL) was added to form a precipitate, which was washed with additional CH_2Cl_2 and dried in vacuo to afford the title compound as a white powder (745 mg, 58% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.22−8.16 (m, 1H), 8.14−8.08 (m, 1H), 7.95−7.85 (m, 2H), 7.36−7.31 (m, 2H), 7.30−7.24 (m, 2H), 7.22−7.14 (m, 1H), 4.59 (s, 2H), 3.42 (br s, 4H), 2.80 (br s, 4H). MS (*m*/*z*, MH$^+$): 305.1.

General Procedure B for Addition of Compound 8 to Heteroaryl Chlorides. Compound **8** (0.33 mmol, 1 equiv) and the desired heteroaryl chloride (0.46 mmol, 1.4 equiv) were combined in a 2 mL microwave vial. Triethylamine (0.49 mmol, 1.5 equiv) and NMP (1 mL) were then added. The vial was sealed and irradiated in the microwave (high absorption setting) at 180 °C for 30 min. Water (15 mL) was then added to the reaction mixture to form a precipitate, which was isolated by filtration, washed with additional cold water, and then dried in vacuo. The products were further purified when necessary by either flash chromatography on silica gel or reverse phase HPLC.

1-(6-Chloropyridin-3-yl)cyclopropanol (9d). A suspension of methyl 6-chloropyridine-3-carboxylate (1 g, 5.83 mmol) in anhydrous ether (17 mL) was charged with ethyl magnesium bromide (8.5 mL, 26 mmol, 3 M in Et_2O). The mixture was stirred for 1 h, and then titanium isopropoxide (1.73 mL, 5.84 mmol) was added. The mixture was stirred for 16 h and was then quenched with NH_4Cl (saturated aqueous). The aqueous phase was adjusted to pH 3 with 1 N HCl, and the mixture was partitioned between DCM and brine. The organic layer was dried over Na_2SO_4 and concentrated. The residue was purified via flash chromatography on silica gel (10−80% EtOAc/heptanes) to afford the title compound as a brown greasy solid (180 mg, 18%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.33−8.31 (m, 1H), 7.68−7.65 (m, 0.5 H), 7.57−7.54 (m, 0.5 H), 7.32−7.28 (m, 1H), 1.36−1.33 (m, 1H), 1.07−1.04 (m, 1H), 0.95−0.91 (m, 2H). MS (*m*/*z*, MH$^+$): 170.1.

1-Benzyl-4-(4-pyridin-2-yl-piperazin-1-yl)phthalazine (10). 10 was synthesized from **6a** and **7b** in 41% yield according to general procedure A. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13−8.18 (m, 1H) 8.04 (d, J = 7.6 Hz, 1H), 7.92 (d, J = 7.6 Hz, 1H), 7.61−7.72 (m, 2H) 7.41−7.48 (m, 1H), 7.26 (d, J = 8.0 Hz, 2H) 7.18 (t, J = 8.0 Hz, 2H), 7.06−7.12 (m, 1H), 6.67 (d, J = 8.6 Hz, 1H), 6.59 (dd, J = 7.7, 5.1 Hz, 1H), 4.55 (s, 2H), 3.70−3.77 (m, 4H), 3.55−3.60 (m, 4H). Anal. RP-HPLC t_R = 6.35 min (method 4). HR-MS (*m*/*z*, MH$^+$): measd 382.2036, calcd 382.2032.

1-Benzyl-4-[4-(5-trifluoromethylpyridin-2-yl)piperazin-1-yl]phthalazine (11). 11 was synthesized from **6a** and **7c** in 9% yield according to general procedure A. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.44 (s, 1H), 8.11−8.17 (d, J = 7.6 Hz, 1H), 8.02 (d, J = 7.6 Hz, 1H), 7.83−7.74 (m, 2H), 7.68 (dd, J = 8.8, 2.5 Hz, 1H), 7.35 (d, J = 7.1 Hz, 2H), 7.26 (t, J = 7.1 Hz, 2H), 7.18 (t, J = 7.1 Hz, 1H), 4.64 (s, 2H), 6.75 (d, J = 9.1 Hz, 1H), 3.94−3.90 (m, 4H), 3.63−3.67 (m, 4H). Anal. RP-HPLC t_R = 7.21 min (method 1). HR-MS (*m*/*z*, MH$^+$): measd 450.1926, calcd 450.1906.

6-[4-(4-Benzylphthalazin-1-yl)piperazin-1-yl]nicotinonitrile (12). 12 was synthesized from **6a** and **7d** in 51% yield according to general procedure A. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.45 (d, J = 2.5 Hz, 1H), 8.02−8.12 (m, 2H), 7.73−7.82 (m, 2H), 7.67 (dd, J = 9.0, 2.0 Hz, 1H), 7.33−7.36 (m, 2H), 7.27 (t, J = 7.0 Hz, 2H), 7.19 (t, J = 7.0 Hz, 1H), 6.71 (d, J = 9.0 Hz, 1H), 4.64 (s, 2H), 3.96 (m, 4H), 3.65 (m, 4H). Anal. RP-HPLC t_R = 8.98 min (method 1). HR-MS (*m*/*z*, MH$^+$): measd 407.1987, calcd 407.1984.

6-[4-(4-Benzylphthalazin-1-yl)piperazin-1-yl]nicotinamide (13). 13 was synthesized from **8** and **9a** in 15% yield according to general procedure B. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.68 (d, J = 2.4 Hz, 1H), 8.26−8.17 (m, 2H), 8.02 (dd, J = 9.0, 2.4 Hz, 1H), 7.98−7.88 (m, 2H), 7.81 (br s, 2H), 7.36−7.31 (m, 2H), 7.31−7.23 (m, 2H), 7.22−7.13 (m, 1H), 6.95 (d, J = 9.0 Hz, 1H), 4.60 (s, 2 H), 3.96−3.84 (m, 4H), 3.56−3.43 (m, 4H). RP-HPLC t_R = 5.87 min (method 1). HR-MS (*m*/*z*, MH$^+$): measd 425.2096, calcd 425.2090.

6-[4-(4-Benzylphthalazin-1-yl)piperazin-1-yl]pyridine-3-sulfonic Acid Amide (14). 14 was synthesized from **8** and **9b** in 30% yield according to general procedure B. ^{1}H NMR (400 MHz, DMSO-d_6)

δ 8.52 (d, J = 2.4 Hz, 1H), 8.26−8.18 (m, 2H), 7.99−7.87 (m, 3H), 7.37−7.32 (m, 2H), 7.32−7.25 (m, 2H), 7.23 (s, 2H), 7.22−7.15 (m, 1H), 7.06 (d, J = 9.1 Hz, 1H), 4.61 (s, 2H), 3.99−3.89 (m, 4H), 3.55−3.47 (m, 4H). RP-HPLC t_R = 7.18 min (method 1). HR-MS (m/z, MH^+): measd 461.1757, calcd 461.1760.

{6-[4-(4-Benzylphthalazin-1-yl)piperazin-1-yl]pyridin-3-yl}methylamine (15). To a 100 mL round-bottom flask equipped with a stir bar, **12** (340 mg, 0.836 mmol) was added, followed by EtOH (8 mL) and $NiCl_2$ (119 mg, 0.92 mmol). $NaBH_4$ (95 mg, 2.51 mmol) was added in portions, and the mixture was then stirred for 2 h. The resulting mixture was filtered through a Celite pad and rinsed with EtOH (50 mL). Purification by preparative HPLC (C-18 column; modifier = TFA) afforded the title compound (34 mg, 10%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16−8.10 (m, 2H), 8.00 (d, J = 7.6 Hz, 1H), 7.77−7.72 (m, 2H), 7.54−7.52 (m, 1H), 7.35−7.33 (m, 2H), 7.27−7.24 (m, 2H), 7.19−7.17 (m, 1H), 6.75 (d, J = 8.6 Hz, 1H), 4.62 (s, 2 H), 3.81−3.78 (m, 6H), 3.66−3.63 (m, 4H). Anal. RP-HPLC t_R = 5.73 min (method 1). HR-MS (m/z, MH^+): measd 411.2295, calcd 411.2297.

{6-[4-(4-Benzylphthalazin-1-yl)piperazin-1-yl]pyridin-3-ylmethyl}dimethylamine (16). To a solution of **15** (35 mg, 0.064 mmol) in CH_2Cl_2 (10 mL) was added $NaBH(OAc)_3$ (41 mg, 0.19 mmol) followed by formaldehyde (13 mg, 30% in H_2O, 0.128 mmol), The mixture was stirred at room temperature for 30 min. Saturated aqueous $NaHCO_3$ was added, and the layers were separated. The aqueous layer was extracted with CH_2Cl_2. The combined organic layers were washed with water, saturated aqueous $NaHCO_3$, and brine, then dried over Na_2SO_4, filtered, and concentrated. Purification by preparative HPLC (acetonitrile/water, 10−50%) afforded the title compound (15 mg, 57%). ^{1}H NMR (400 MHz, CD_2Cl_2) δ 8.06 (d, J = 7.5 Hz, 1H), 7.98 (s, 1H), 7.93 (d, J = 8.5 Hz, 1H), 7.70 (m, 2H), 7.43 (m, 1H), 7.24−7.10 (m, 5H), 6.67 (d, J = 9.0 Hz, 1H), 4.53 (s, 2H), 3.71 (m, 4H), 3.51 (m, 4H), 3.22 (s, 2H), 2.11 (s, 6H). RP-HPLC t_R = 5.29 min (method 1). HR-MS (m/z, MH^+): measd 439.2600, calcd 439.2610.

***N*-{6-[4-(4-Benzylphthalazin-1-yl)piperazin-1-yl]pyridin-3-ylmethyl}acetamide (17).** To a 100 mL round-bottom flask equipped with a stir bar was added **12** (150 mg, 0.362 mmol), followed by EtOH (7 mL) and $NiCl_2$ (52 mg, 0.398 mmol). $NaBH_4$ (27 mg, 0.723 mmol) was added in portions, and the mixture was then stirred for 2 h. Ac_2O (1.08 mmol) was added, and the reaction was stirred for 1 h, at which point it was filtered through a pad of Celite and rinsed with MeOH (50 mL). Purification by preparative HPLC (C-18 column; modifier = propanol) afforded the title compound (85 mg, 52%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 (m, 2H), 8.03 (d, J = 8.0 Hz, 1H), 7.77 (m, 2H), 7.55 (dd, J = 9.1, 2.5 Hz, 1H), 7.35 (d, J = 7.0 Hz, 2H), 7.27 (t, J = 7.5 Hz, 2H), 7.18 (t, J = 7.5 Hz, 1H), 6.75 (d, J = 8.6 Hz, 1H), 5.71 (s, 1H) 4.63 (s, 2H), 4.33 (d, J = 6.1 Hz, 2H), 3.82 (t, J = 5.5 Hz, 4H), 3.65 (t, J = 5.5 Hz, 4H), 2.01 (s, 3H). Anal. RP-HPLC t_R = 5.83 min (method 1). HR-MS (m/z, MH^+): measd 453.2393, calcd 453.2403.

1-{6-[4-(4-Benzylphthalazin-1-yl)piperazin-1-yl]pyridin-3-yl}1-methylethylamine (18). Cerium(III) chloride hydrate (455 mg, 1.84 mmol) was added to a 40 mL vial and heated to 150 °C under high vacuum for 2 h. The hot vial was back-filled with N_2 and cooled to room temperature prior to charging with THF (2 mL). This mixture was stirred for 2 h and then cooled to −78 °C, at which point methylmagnesium bromide (0.62 mL, 3 M in THF, 1.9 mmol) was added. After the mixture was stirred for 30 min, a solution of **12** (250 mg, 0.62 mmol) in THF (1 mL) was added. The mixture was allowed to gradually warm to room temperature and was stirred overnight. The mixture was filtered through Celite and then evaporated to afford a crude material. Purification by preparative HPLC (10−100% acetonitrile/water; modifier = 3% *n*-PrOH) afforded the title compound as a white solid (100 mg, 37%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.33−8.31 (m, 1H), 8.21−8.18 (m, 2H), 7.95−7.88 (m, 2H), 7.74 (dd, J = 8.6 Hz, 2.5 Hz, 1H), 7.34−7.32 (m, 2 H), 7.29−7.25 (m, 2H), 7.19−7.15 (m, 1H), 6.86 (d, J = 8.6 Hz, 1H), 4.59 (s, 2H), 3.73 (br, 4H), 3.48 (br, 4H), 1.46 (s, 6H). Anal. RP-HPLC t_R = 5.33 min (method 1). HR-MS (m/z, MH^+): measd 439.2618, calcd 439.2610.

6-[4-(4-Benzylphthalazin-1-yl)piperazin-1-yl]nicotinic Acid Ethyl Ester (19). 19 was synthesized from **6a** and **7e** in 76% yield according to general procedure A. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.70 (d, J = 1.9 Hz, 1H), 8.15−8.27 (m, 2H), 8.01 (dd, J = 9.0, 2.40 Hz, 1H), 7.88−7.97 (m, 2H), 7.31−7.36 (m, 2H), 7.24−7.30 (m, 2H), 7.14−7.21 (m, 1H), 6.99 (d, J = 9.1 Hz, 1H), 4.60 (s, 2H), 4.28 (q, J = 7.0 Hz, 2H), 3.86−4.04 (m, 4H), 3.39−3.61 (m, 4H), 1.31 (t, J = 7.1 Hz, 3H). RP-HPLC t_R = 8.31 min (method 1). HR-MS (m/z, MH^+): measd 454.2225, calcd 454.2243.

6-[4-(4-Benzylphthalazin-1-yl)piperazin-1-yl]nicotinic Acid (20). EtOH (4 mL) and H_2O (3 mL) were added to a 10 mL round-bottom flask containing **19** (42 mg, 0.093 mmol). Solid LiOH (39 mg, 0.93 mmol) was added, and the mixture was stirred overnight at 60 °C. The mixture was then concentrated under reduced pressure to remove EtOH and was acidified to pH ∼3 using 10% citric acid solution. The resulting precipitate was isolated by filtration and washed with additional H_2O to afford the title compound (39 mg, 99%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.27 (br s, 1H), 8.62 (s, 1H), 8.21 (m, 2H), 8.01 (d, 1H), 7.93 (m, 2H), 7.25−7.34 (m, 5H), 6.97 (d, 1H), 6.60 (s, 2H), 3.95 (m, 4H), 3.50 (m, 4H). Anal. RP-HPLC t_R = 6.56 min (method 1). HR-MS (m/z, MH^+): measd 426.1929, calcd 426.1930.

{6-[4-(4-Benzylphthalazin-1-yl)piperazin-1-yl]pyridin-3-yl}methanol (21). Compound **19** (700 mg, 1.543 mmol) was added to a 1 L round-bottom flask, followed by THF (20 mL). Lithium aluminum hydride (1.85 mL, 1 M in THF, 1.85 mmol) was then added dropwise at room temperature. The mixture was stirred at room temperature for 15 h. Aqueous saturated sodium sulfate (1 mL) was added, and the precipitated lithium salts were removed via filtration. The filtrate was concentrated in vacuo and the residue purified by flash chromatography on silica gel (0−8% MeOH/CH_2Cl_2) to afford the title compound as a pale-yellow solid (355 mg, 56%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.22−8.13 (m, 2 H), 8.08 (d, J = 2.0 Hz, 1 H), 7.94−7.84 (m, 2 H), 7.53 (dd, J = 8.7, 2.4 Hz, 1 H), 7.31 (dm, J = 7.1 Hz, 2 H), 7.25 (ddm, J = 7.6, 7.6 Hz, 2 H), 7.15 (ddm, J = 7.3, 7.3 Hz, 1 H), 6.89 (d, J = 8.7 Hz, 1 H), 4.99 (t, J = 5.6 Hz, 1 H), 4.57 (s, 2 H), 4.36 (d, J = 5.7 Hz, 2 H), 3.78−3.70 (m, 4 H), 3.51−3.43 (m, 4 H). RP-HPLC t_R = 5.63 min (method 1). HR-MS (m/z, MH^+): measd 412.2134, calcd 412.2137.

1-{6-[4-(4-Benzylphthalazin-1-yl)piperazin-1-yl]pyridin-3-yl}ethanone (22). 22 was synthesized from **8** and **9c** in 74% yield according to general procedure B. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.80 (d, J = 2.5 Hz, 1H), 8.11−8.06 (m, 2H), 8.03−8.01 (m, 1H), 7.80−7.72 (m, 2H), 7.35−7.33 (m, 2H), 7.28−7.24 (m, 2H), 7.20−7.16 (m, 2H), 4.63 (s, 2 H), 4.00−3.97 (m, 4H), 3.69−3.63 (m, 4H), 2.52 (s, 3H). Anal. RP-HPLC t_R = 1.25 min (method 2 purity 89.3%/100.0%). LRMS: 424.3 (M + H).

1-{6-[4-(4-Benzylphthalazin-1-yl)piperazin-1-yl]pyridin-3-yl}ethanol (23). To a 25 mL round-bottom flask was added **22** (60 mg, 0.139 mmol) and MeOH (4 mL). The resulting solution was cooled to 0 °C, and then sodium borohydride (11 mg, 0.277 mmol) was added portionwise. The mixture was stirred at 0 °C for 40 min, and then the reaction was quenched by the addition of aqueous saturated $NaHCO_3$. The solution was diluted with H_2O (25 mL), and the organics were extracted with EtOAc (3 × 25 mL), dried over $MgSO_4$, and concentrated. The residue was recrystallized from EtOAc/heptanes to afford the title compound as yellow needles (19 mg, 32%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (d, J = 2.3 Hz, 1H), 7.96 (dd, J = 18.6, 8.0 Hz, 2H), 7.77−7.61 (m, 3H), 7.27−7.19 (m, 2H), 7.18−7.11 (m, 2H), 7.10−7.02 (m, 1H), 6.86 (d, J = 9.1 Hz, 1H), 4.78 (q, J = 6.4 Hz, 1H), 4.55 (s, 2H), 3.89 (s, 3H), 3.69−3.47 (m, 5H), 2.08 (d, J = 2.9 Hz, 1H), 1.38 (d, J = 6.6 Hz, 3H). RP-HPLC t_R = 5.81 min (method 1). HR-MS (m/z, MH^+): measd 426.2304, calcd 426.2294.

2-{6-[4-(4-Benzylphthalazin-1-yl)piperazin-1-yl]pyridin-3-yl}propan-2-ol (24). To a solution of **19** (300 mg, 0.65 mmol) in THF (3 mL) at 23 °C was added dropwise MeMgI (0.86 mL of 3.0 M solution in Et_2O, 2.6 mmol). The mixture was stirred for 2 h, and then the reaction was quenched by addition of aqueous

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saturated NH_4Cl (3 mL). Additional water (10 mL) was added, and the organics were extracted with EtOAc (3 × 20 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated. The residue was purified by flash chromatography on silica gel (30−100% EtOAc/heptanes) to afford the title compound as a light-yellow powder (202 mg, 71%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.36 (d, J = 4.0 Hz, 1H), 8.13 (d, J = 8.0 Hz, 1H), 8.03 (d, J = 8.0 Hz, 1H), 7.83−7.74 (m, 3H), 7.37−7.18 (m, 5H), 6.82 (d, J = 8.0 Hz, 1H), 4.66 (s, 2H), 3.92−3.87 (m, 4H), 3.70−3.65 (m, 4H), 1.61 (s, 6H). RP-HPLC t_R = 6.01 min (method 1). HR-MS (*m*/*z*, MH^+): measd 440.2437, calcd 440.2450.

1-{6-[4-(4-Benzylphthalazin-1-yl)piperazin-1-yl]pyridin-3-yl}cyclopropanol (25). 25 was synthesized from **8** and **9d** in 15% yield according to general procedure B. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.78 (d, J = 2.0 Hz, 1H), 8.21−8.19 (m, 2H), 8.04 (dd, J = 9.1, 2.5 Hz, 1H), 7.93−7.90 (m, 2H), 7.33−7.31(m, 2H), 7.28−7.24 (m, 2H), 7.18−7.14 (m, 1H), 6.97 (d, J = 9.1, 1H), 4.59 (s, 2H), 3.97−3.95 (m, 4H), 3.50−3.48 (m, 4H), 2.93−2.90 (m, 2H), 1.06 (t, J = 7.4, 2H). Anal. RP-HPLC t_R = 7.44 min (method 1). HR-MS (*m*/*z*, MH^+): measd 438.2314, calcd 438.2294.

1-Benzyl-4-[4-(5-isopropenylpyridin-2-yl)piperazin-1-yl]phthalazine (26). 26 was synthesized from **6a** and **7f** in 34% yield according to general procedure A. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.27 (d, J = 2.3 Hz, 1H), 8.05 (d, J = 7.5 Hz, 1H), 7.95 (d, J = 7.7 Hz, 1H), 7.61−7.76 (m, 3H), 7.25−7.31 (m, 2H), 7.16−7.23 (m, 2H), 7.08−7.15 (m, 1H), 6.70 (d, J = 8.7 Hz, 1H), 5.24 (s, 1H), 4.95 (s, 1H), 4.57 (s, 2H), 3.82 (br s, 4H), 3.53−3.68 (m, 4H), 2.06 (s, 3H). Anal. RP-HPLC t_R = 5.01 min (method 1). MS (*m*/*z*, MH^+): 422.3.

(±)-2-{6-[4-(4-Benzylphthalazin-1-yl)piperazin-1-yl]pyridin-3-yl}propane-1,2-diol (27). To a 10 mL round-bottom flask was added **26** (68 mg, 0.158 mmol), acetone (1 mL), *tert*-butyl alcohol (0.5 mL), and H_2O (0.5 mL). To this suspension was then added potassium osmate(VI) dihydrate (536 μg, 1.58 μM) and NMO (21 mg, 0.174 mmol), and the mixture was stirred at room temperature for 3 h. Sodium sulfite (350 mg) was added to the resulting clear orange solution, and the mixture was stirred for 1 h. Additional H_2O (25 mL) was added, and the organics were extracted with EtOAc (3 × 25 mL), dried over $MgSO_4$, and concentrated. Purification of the residue by flash chromatography on silica gel (0−10% MeOH/CH_2Cl_2) afforded a clear oil which was then triturated with EtOAc to afford the title compound as a white powder (52 mg, 72%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.30−8.13 (m, 3H), 7.99−7.84 (m, 2H), 7.64 (dd, J = 8.8, 2.5 Hz, 1H), 7.38−7.23 (m, 4H), 7.22−7.14 (m, 1H), 6.87 (d, J = 8.8 Hz, 1H), 4.89−4.81 (m, 1H), 4.67 (dd, J = 5.8, 5.8 Hz, 1H), 4.60 (s, 2H), 3.81−3.69 (m, 4H), 3.54−3.45 (m, 4H), 3.43−3.34 (m, 2H), 1.39 (s, 3H). RP-HPLC t_R = 5.01 min (method 1). HR-MS (*m*/*z*, MH^+): measd 456.2426, calcd 456.2400.

1-Pyridin-4-ylmethyl-4-(4-pyridin-2-ylpiperazin-1-yl)phthalazine (28). 28 was synthesized from **6b** and **7b** in 42% yield according to general procedure A. ^{1}H NMR (400 MHz, MeOH-d_4) δ 8.81 (d, J = 6.6 Hz, 2H), 8.58−8.52 (m, 1H), 8.42−8.36 (m, 1H), 8.23−8.04 (m, 6H) 7.42 (d, J = 9.1 Hz, 1H), 7.08 (t, J = 6.6 Hz, 1H), 4.93 (br s, 2H), 4.17 (br s, 4H), 4.14−4.07 (m, 4H). Anal. RP-HPLC t_R = 4.99 min (method 4, purity 92.3%/97.9%). HR-MS (*m*/*z*, MH^+): measd 383.1995, calcd 383.1984.

6-[4-(4-Chlorophthalazin-1-yl)piperazin-1-yl]nicotinonitrile (30). To a 100 mL round-bottom flask was added **7d** (9.60 g, 50 mmol), 1,4-dichlorophthalazine (11.2 g, 55.1 mmol), Et_3N (3.5 mL, 250 mmol), and NMP (100 mL). The mixture was heated to 80 °C for 2.5 h. After cooling to room temperature, the mixture was poured into H_2O (500 mL) and the precipitate isolated by filtration, rinsing with additional H_2O. Crude material was purified by recrystallization (CH_2Cl_2/heptanes) to afford the title compound as a beige solid (8.96 g, 51%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.39 (s, 1H), 8.24−8.20 (m, 1H), 8.08−8.03 (m, 1H), 7.93−7.86 (m, 2H), 7.62 (d, J = 8.0 Hz, 1H), 6.65 (d, J = 12.0 Hz, 1H), 3.95−3.88 (m, 4H), 3.67−3.62 (m, 4H). MS (*m*/*z*, MH^+): 351.0.

6-[4-(4,7-Dichlorophthalazin-1-yl)piperazin-1-yl]nicotinonitrile and 6-[4-(4,6-Dichlorophthalazin-1-yl)piperazin-1-yl]nicotinonitrile (31a and 31b). 31a and **31b** were synthesized from **7d** and **29b** in 66% yield as a 1:1 mixture of regioisomers according to the procedure for compound **30**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.53 (d, J = 2.2 Hz, 1H), 8.30−8.17 (m, 2H), 8.16−8.06 (m, 1H), 7.91 (dd, J = 9.0, 2.3 Hz, 1H), 7.02 (dd, J = 9.1, 3.2 Hz, 1H), 4.01−3.90 (m, 4H), 3.63−3.49 (m, 4H).

General Procedure for Coupling of Benzylzinc Halides with 1-Amino-4-chlorophthalazines. The desired 1-amino-4-chlorophthalazine (0.57 mmol) was added to a sealed tube along with THF (13 mL). $Pd(PPh_3)_4$ (110 mg, 0.114 mmol) was added at room temperature, and N_2 was bubbled through the resulting solution for 30 min to degas. The desired benzylzinc chloride (3.42 mL, 0.5 M in THF, 1.71 mmol) was then added dropwise. The tube was sealed and stirred for 3−24 h. For certain substrates, heating to 80 °C for 16 h was necessary to achieve good conversion. The reaction mixture was concentrated in vacuo, and the residue was purified by flash chromatography on silica gel (0−100% EtOAc/heptane) followed by trituration with EtOAc.

6-{4-[4-(4-Fluorobenzyl)phthalazin-1-yl]piperazin-1-yl}nicotinonitrile (32). 32 was synthesized from **30** and 4-fluorobenzylzinc chloride in 50% yield according to the general coupling procedure. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.54 (d, J = 2.3 Hz, 1H), 8.26−8.18 (m, 2H), 7.99−7.87 (m, 3H), 7.37 (dd, J = 8.6, 5.7 Hz, 2H), 7.10 (t, J = 8.9 Hz, 2H), 7.04 (d, J = 9.1 Hz, 1H), 4.59 (s, 2H), 3.99−3.94 (m, 4H), 3.53−3.47 (m, 4H). Anal. RP-HPLC t_R = 8.50 min (method 1). HR-MS (*m*/*z*, MH^+): measd 425.1889, calcd 425.1890.

6-{4-[4-(3-Chlorobenzyl)phthalazin-1-yl]piperazin-1-yl}nicotinonitrile (33). 33 was synthesized from **30** and 3-chlorobenzylzinc chloride in 47% yield according to the general coupling procedure. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.55 (d, J = 2.2 Hz, 1H), 8.29−8.19 (m, 2H), 7.99−7.94 (m, 2H), 7.92 (dd, J = 9.1, 2.3 Hz, 1H), 7.44 (s, 1H), 7.35−7.22 (m, 3H), 7.04 (d, J = 9.1 Hz, 1H), 4.62 (s, 2H), 4.02−3.90 (m, 4H), 3.54−3.46 (m, 4H). Anal. RP-HPLC t_R = 9.43 min (method 1). HR-MS (*m*/*z*, MH^+): measd 441.1587, calcd 441.1594.

6-{4-[4-(4-Chlorobenzyl)phthalazin-1-yl]piperazin-1-yl}nicotinonitrile (34). 34 was synthesized from **30** and 4-chlorobenzylzinc chloride in 40% yield according to the general coupling procedure. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.55 (d, J = 2.2 Hz, 1H), 8.23−8.18 (m, 2H), 7.98−7.92 (m, 2H), 7.91 (d, 2.40 Hz, 1H), 7.39−7.29 (m, 4H), 7.04 (d, J = 9.1 Hz, 1H), 4.60 (s, 2H), 4.01−3.90 (m, 4 H), 3.54−3.46 (m, 4H). Anal. RP-HPLC t_R = 9.44 min (method 1). HR-MS (*m*/*z*, MH^+): measd 441.1609, calcd 441.1594.

6-{4-[4-(4-Cyanobenzyl)phthalazin-1-yl]piperazin-1-yl}nicotinonitrile (35). 35 was synthesized from **30** and 4-cyanobenzylzinc chloride in 16% yield according to the general coupling procedure except that the reaction was run for 66 h at 75 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.47 (d, J = 2.2 Hz, 1H), 8.16 (d, J = 2.0 Hz, 1H), 8.14 (d, J = 2.0 Hz, 1H), 7.92−7.86 (m, 2H), 7.85 (dd, J = 9.1, 2.4 Hz, 1H), 7.69 (d, J = 8.3 Hz, 1H), 7.47 (d, J = 8.2 Hz, 1H), 6.97 (d, J = 9.1 Hz, 1H), 4.64 (s, 2H), 3.97−3.80 (m, 4H), 3.53−3.34 (m, 4H). Anal. RP-HPLC t_R = 8.50 min (method 1). HR-MS (*m*/*z*, MH^+): measd 432.1917, calcd 432.1937.

6-{4-[4-(4-Trifluoromethylbenzyl)phthalazin-1-yl]piperazin-1-yl}nicotinonitrile (36). 36 was synthesized from **30** and 4-(trifluoromethyl)benzylzinc bromide in 81% yield according to the general coupling procedure except that the reaction was run at 75 °C. 4-(Trifluoromethyl)benzylzinc bromide was prepared as follows: Activated Zn powder (120 mg, 1.883 mmol) was added to a sealed tube along with THF (2 mL) and TMSCl (0.317 mL, 2.51 mmol), and the mixture was heated at 60 °C for 1 h. 4-(Trifluoromethyl)-benzyl bromide (300 mg, 1.255 mmol) was then added, and the mixture was stirred at 60 °C for 66 h, cooled to room temperature, and used immediately. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.51 (d, J = 2.0 Hz, 1H), 8.19 (dd, J = 5.9, 3.2 Hz, 2H), 7.96−7.85 (m, 3H), 7.65−7.59 (m, 2H), 7.53 (d, J = 8.1 Hz, 2H), 7.00 (d, J = 9.1 Hz, 1H), 4.68 (s, 2H), 3.97−3.87 (m, 4H), 3.51−3.41 (m, 4H).

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Anal. RP-HPLC t_R = 9.44 min (method 1). HR-MS (*m*/*z*, MH^+): measd 475.1838, calcd 475.1858.

4-{4-[4-(5-Cyanopyridin-2-yl)piperazin-1-yl]phthalazin-1-ylmethyl}benzoic Acid Methyl Ester (37). 37 was synthesized from **30** and 4-(bromomethylzinc)benzoic acid methyl ester in 50% yield according to the general coupling procedure. 4-(Bromomethylzinc)benzoic acid methyl ester was prepared as described for 4-(trifluoromethyl)benzylzinc bromide and was used immediately. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.54 (d, J = 2.4 Hz, 1H), 8.24−8.17 (m, 2H), 7.99−7.90 (m, 3H), 7.88 (d, J = 8.2 Hz, 2H), 7.48 (d, J = 8.2 Hz, 2H), 7.04 (d, J = 9.2 Hz, 1H), 4.69 (s, 2H), 4.00−3.93 (m, 4H), 3.82 (s, 3H), 3.56−3.46 (m, 4H). Anal. RP-HPLC t_R = 8.63 min (method 1, purity 92.92%/100%). HR-MS (*m*/*z*, MH^+): measd 465.2032, calcd 465.2039.

4-{4-[4-(5-Cyanopyridin-2-yl)piperazin-1-yl]phthalazin-1-ylmethyl}benzoic Acid (38). Compound **37** (60 mg, 0.129 mmol) and MeOH (2 mL) were added to a 10 mL round-bottom flask. NaOH (0.775 mL, 1 M, 0.775 mmol) was then added, and the mixture was stirred at room temperature for 24 h. The MeOH was removed in vacuo, the flask was placed in an ice bath, and the remaining solution was acidified to pH ~3.0 with acetic acid. The organics were extracted with DCM, washed with water followed by brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by trituration with EtOAc to afford the title compound as an off-white solid (36 mg, 62%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.79 (s, 1H), 8.54 (d, J = 2.4 Hz, 1H), 8.26−8.17 (m, 2H), 7.99−7.89 (m, 3H), 7.86 (d, J = 8.2 Hz, 2H), 7.45 (d, J = 8.2 Hz, 2H), 7.04 (d, J = 9.1 Hz, 1H), 4.68 (s, 2H), 4.00−3.93 (m, 4H), 3.54−3.47 (m, 4H). Anal. RP-HPLC t_R = 8.50 min (method 1). HR-MS (*m*/*z*, MH^+): measd 451.1896, calcd 451.1882.

6-{4-[7-Chloro-4-(4-fluorobenzyl)phthalazin-1-yl]piperazin-1-yl}nicotinonitrile and 6-{4-[6-Chloro-4-(4-fluorobenzyl)phthalazin-1-yl]piperazin-1-yl}nicotinonitrile (39a,b). 39a,**b** were synthesized from **31a**,**b** and 4-fluorobenzylzinc chloride in 81% yield as a 1:1 mixture of regioisomers. ^{1}H NMR (1:1 mix of **39a** and **39b**) (400 MHz, $CDCl_3$) δ 8.38 (m, 1H), 7.98 (m, 1H), 7.88 (m, 1H), 7.69−7.59 (m, 2H), 7.21 (m, 2H), 6.91 (m, 2H), 6.64 (m, 1H), 4.51 (s, 1H), 4.49 (s, 1H), 3.89 (m, 4H), 3.56 (m, 4H). Anal. RP-HPLC t_R = 6.44, 6.54 min (regioisomers elute separately) (method 1). HR-MS (*m*/*z*, MH^+): measd 459.1483, calcd 459.1500.

4-[4-(5-Cyanopyridin-2-yl)piperazin-1-yl]-1-(4-fluorobenzyl)phthalazine-6-carbonitrile and 1-[4-(5-Cyanopyridin-2-yl)piperazin-1-yl]-4-(4-fluorobenzyl)phthalazine-6-carbonitrile (40a,b). To a solution of **39a** and **39b** (46 mg, 0.1 mmol, 1:1 mix of regioisomers) in DMF (2 mL) was added $Zn(CN)_2$ (24 mg, 0.2 mmol), $Pd_2(dba)_3$ (9.2 mg, 0.1 equiv), and X-phos (6 mg, 0.125 equiv). The mixture was degassed by bubbling N_2 through the solution for 5 min and then heated in the microwave (high absorption setting) at 120 °C for 45 min. EtOAc (4 mL) was added, and the mixture was passed through a plug of silica. The filtrate was washed with water, brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified via flash chromatography on silica gel (25% heptane in EtOAc) gave the title compounds as a yellow powder (41 mg, 91%, 1:1 mix of regioisomers). ^{1}H NMR (1:1 mix of **40a** and **40b**) (400 MHz, $CDCl_3$) δ 8.37 (m, 0.5H), 8.36 (s, 1H), 8.26 (m, 0.5H), 8.14 (d, J = 8.6 Hz, 0.5H), 8.02 (d, J = 8.6 Hz, 0.5H), 7.91 (m, 0.5H), 7.87 (m, 0.5H), 7.61 (m, 1H), 7.20 (m, 2H), 6.90 (m, 2H), 6.63 (m, 1H), 4.53 (s, 2H), 3.90 (m, 4H), 3.58 (m, 4H). Anal. RP-HPLC t_R = 6.28 min (regioisomers coelute) (method 1, purity 90.3%/93.8%). HR-MS (*m*/*z*, MH^+): measd 450.1840, calcd 450.1842.

1-Benzyl-4-[1,4]diazepam-1-ylphthalazine (41a). 1-Benzyl-4-chlorophthalazine (1.11 g, 4.35 mmol) and homopiperazine (2.20 g, 21.7 mmol) were added to a microwave vial, followed by NMP (5 mL) and triethylamine (1.84 mL, 13.1 mmol). The vial was sealed and irradiated in the microwave at 180 °C (high absorption setting) for 30 min. The mixture was diluted with H_2O, and the organics were extracted with CH_2Cl_2. The combined organic fractions were dried over $MgSO_4$ and then concentrated in vacuo to afford the title compound as a yellow oil (640 mg, 42%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.47 (t, J = 9.1 Hz, 2H), 8.19 (dt, J = 8.6, 1.0 Hz, 1H), 8.11 (t, J = 7.6 Hz, 1H), 7.29−7.37 (m, 4H), 7.22−7.28 (m, 1H), 4.77 (s, 2H), 4.23−3.27 (m, 2H), 4.14 (t, J = 6.1 Hz, 2H), 3.67−3.71 (m, 2H), 3.41−3.46 (m, 2H), 2.39 (q, J = 5.6 Hz, 2H). MS (*m*/*z*, MH^+): 319.2.

1-Benzyl-4-((*S*)-3-methylpiperazin-1-yl)phthalazine (41b). Solid Na_2CO_3 (400 mg, 3.8 mmol) was added to a solution of 1-benzyl-4-chlorophthalazine (250 mg, 0.98 mmol) and (*S*)-2-methylpiperazine (400 mg, 4.0 mmol) in dioxane (5 mL). The resulting suspension was heated at 100 °C for 48 h. The reaction mixture was then concentrated, and EtOAc (50 mL) and water (15 mL) were added. The organic fraction was washed with H_2O and then brine, dried over Na_2SO_4, and concentrated in vacuo to afford the title compound as a white solid (200 mg, 64%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (d, J = 7.1 Hz, 1H), 7.89 (d, J = 8.2 Hz, 1H), 7.68−7.58 (m, 2H), 7.28−7.24 (m, 2H), 7.22−7.14 (m, 2H), 7.11−7.06 (m, 1H), 3.69 (d, J = 12.4 Hz, 2H), 3.61 (s, 2H), 3.03−3.20 (m, 4H), 2.74 (dd, J = 12.6, 10.2 Hz, 1H), 1.07 (d, J = 6.3 Hz, 3H). MS (*m*/*z*, MH^+): measd 319.1.

1-Benzyl-4-((*R*)-3-methylpiperazin-1-yl)phthalazine (41c). 41c was synthesized from **6a** and (*R*)-methylpiperazine in 58% yield according to the procedure described for **41b**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (d, J = 7.1 Hz, 1H), 8.00 (d, J = 7.7 Hz, 1H), 7.79−7.69 (m, 2H), 7.39−7.34 (m, 2H), 7.32−7.25 (m, 2H), 7.20 (d, J = 7.2 Hz, 1H), 4.65−4.61 (m, 2H), 3.82−3.76 (m, 2H), 3.30−3.13 (m, 4H), 2.85 (dd, J = 12.6, 10.2 Hz, 1H), 1.17 (d, J = 6.3 Hz, 3H). MS (*m*/*z*, MH^+): measd 319.1.

6-[4-(4-Benzylphthalazin-1-yl)[1,4]diazepan-1-yl]nicotinonitrile (42). 42 was synthesized from 6-chloronicotinonitrile and **41a** in 34% yield according to general procedure B. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.40 (d, J = 2.5 Hz, 1H), 7.97−8.03 (m, 2H), 7.68−7.75 (m, 2H), 7.60 (dd, J = 10.4, 2.0 Hz, 2H), 7.32 (d, J = 7.1 Hz, 2H), 7.25 (t, J = 7.6 Hz, 2H), 7.17 (t, J = 7.1 Hz, 1H), 6.59 (d, J = 9.1 Hz, 1H), 4.60 (s, 2 H), 4.11 (br s, 2H), 3.88−3.94 (m, 4H), 3.67 (t, J = 5.6 Hz, 2H), 2.19 (q, J = 5.6 Hz, 2H). Anal. RP-HPLC t_R = 7.21 min (method 1, purity 100.00%/92.50%). HR-MS (*m*/*z*, MH^+): measd 421.2142, calcd 421.2141.

6-[(*S*)-4-(4-Benzylphthalazin-1-yl)-2-methylpiperazin-1-yl]nicotinonitrile (43). Solid Na_2CO_3 (50 mg, 0.47 mmol) was added to a solution of 6-chloronicotinonitrile (50 mg, 0.36 mmol) and **41b** (100 mg, 0.31 mmol) in DMF (1 mL) and dioxane (2 mL) in a microwave vial. The vial was sealed and irradiated in the microwave at 180 °C (high absorption setting) for 30 min. The reaction mixture was concentrated in vacuo and then diluted with CH_2Cl_2. This solution was washed with H_2O and then brine. The organics were dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified via flash chromatography on silica gel (50−90% EtOAc/hexanes) to afford the title compound as a white solid (30 mg, 23%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.39 (d, J = 2.3 Hz, 1H), 8.09 (d, J = 8.7 Hz, 1H), 7.97 (d, J = 7.8 Hz, 1H), 7.77−7.71 (m, 1H), 7.77−7.66 (m, 1H), 7.60 (dd, J = 9.0, 2.3 Hz, 1H), 7.30−7.25 (m, 2H), 7.23−7.16 (m, 2H), 7.14−7.09 (m, 1H), 6.60 (d, J = 9.0 Hz, 1H), 4.77−4.67 (m, 1H), 4.56 (s, 2H), 4.31 (d, J = 13.1 Hz, 1H), 3.90 (d, J = 11.9 Hz, 1H), 3.75 (dt, J = 12.8, 2.1 Hz, 1H), 3.53 (ddd, J = 12.7, 3.5 Hz, 1H), 3.36 (dd, J = 12.7, 3.6 Hz, 1H), 3.20 (td, J = 12.5, 3.5 Hz, 1H), 1.42 (d, J = 6.3 Hz, 3H). Anal. RP-HPLC t_R = 9.03 min (method 1). HR-MS (*m*/*z*, MH^+): measd 421.2151, calcd 421.2141.

6-[(*R*)-4-(4-Benzylphthalazin-1-yl)-2-methylpiperazin-1-yl]nicotinonitrile (44). 44 was synthesized from 6-chloronicotinonitrile and **41c** in 42% yield according to the procedure described for **43**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.39 (d, J = 2.0 Hz, 1H), 8.09 (d, J = 7.5 Hz, 1H), 7.97 (d, J = 7.7 Hz, 1H), 7.76−7.66 (m, 2H), 7.59 (dd, J = 9.0, 2.3 Hz, 1H), 7.30−7.24 (m, 2H), 7.22−7.16 (m, 2H), 7.14−7.08 (m, 1H), 6.60 (d, J = 9.1 Hz, 1H), 4.76−4.68 (m, 1H), 4.59−4.54 (m, 2H), 4.30 (d, J = 13.0 Hz, 1H), 3.94−3.86 (m, 1H), 3.78−3.71 (m, 1H), 3.53 (td, J = 12.7, 3.4 Hz, 1H), 3.35 (dd, J = 12.8, 3.7 Hz, 1H), 3.20 (td, J = 12.5, 3.5 Hz, 1H), 1.44 (d, J = 6.7 Hz, 3H). Anal. RP-HPLC t_R = 9.01 min (method 1). HR-MS (*m*/*z*, MH^+): measd 421.2153, calcd 421.2141.

6-((*R*)-3-Methylpiperazin-1-yl)nicotinonitrile (45a). Triethylamine (5.51 g, 4 mL, 54.6 mmol) was added to a solution of

6-chloronicotinonitrile (2.76 g, 20 mmol) and (*R*)-2-methylpiperazine (2.00 g, 20 mmol) in DMF (15 mL). The resulting solution was stirred at room temperature for 36 h. A white precipitate of triethylamine hydrochloride formed during the course of the reaction. Water (15 mL) and EtOAc (100 mL) were added, and the organic layer was separated, dried over Na_2SO_4, and concentrated under reduced pressure to give a white residue. This solid was further dried under high vacuum to yield the title compound as a white solid (2.3 g, 59%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.32 (d, J = 2.4 Hz, 1H), 7.52 (dd, J = 9.1, 2.3 Hz, 1H), 6.52 (d, J = 9.0 Hz, 1H), 4.24−4.14 (m, 2H), 3.07−3.01 (m, 1H), 2.94−2.72 (m, 3H), 2.52 (dd, J = 12.8, 10.4 Hz, 1H), 1.07 (d, J = 6.3 Hz, 3H). MS (m/z, MH^+): 203.1.

6-((*S*)-3-Methylpiperazin-1-yl)nicotinonitrile (45b). 45b was prepared from 6-chloronicotinonitrile and (*S*)-2-methylpiperazine in 69% yield according to the procedure described for **45a**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.38 (s, 1H), 7.58 (d, J = 9.6 Hz, 1H), 6.59 (d, J = 9.1 Hz, 1H), 4.31−4.19 (m, 2H), 3.15−3.08 (m, 1H), 3.04−2.92 (m, 1H), 2.91−2.81 (m, 2H), 2.65−2.57 (m, 1H), 1.15 (d, J = 6.3 Hz, 3H). MS (m/z, MH^+): 203.1.

6-[(*R*)-4-(4-Benzylphthalazin-1-yl)-3-methylpiperazin-1-yl]nicotinonitrile (46). Triethylamine (300 μL, 218 mg, 2.15 mmol) was added to a solution of **6a** (250 mg, 0.98 mmol) and **45a** (200 mg, 1.0 mmol) in DMF (2 mL) in a microwave vial. The vial was sealed and irradiated in the microwave at 180 °C (high absorption setting) for 30 min. The reaction mixture was diluted with EtOAc (50 mL) and water (15 mL). The organic layer was separated, washed with water (10 mL) and brine (2 × 10 mL), and then dried over sodium sulfate. The solvent was evaporated under reduced pressure and the residue then purified by flash chromatography on silica gel (50−90% EtOAc/ hexanes) to afford the title compound as a white solid (20 mg, 5%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.35 (dd, J = 2.3, 0.6 Hz, 1H), 8.07 (dd, J = 7.3 Hz, 1H), 7.96 (d, J = 8.3 Hz, 1H), 7.76−7.75 (m, 2H), 7.57 (dd, J = 9.0, 2.3 Hz, 1H), 7.30−7.24 (m, 2H), 7.23−7.16 (m, 2H), 7.15−7.08 (m, 1H), 6.61 (d, J = 9.1 Hz, 1H), 4.57 (s, 2H), 4.17−4.08 (m, 1H), 4.01−3.92 (m, 1H), 3.84 (d, J = 4.4 Hz, 2H), 3.77 (ddd, J = 12.6, 8.8, 3.4 Hz, 1H), 3.66−3.56 (m, 1H), 3.53−3.44 (m, 1H), 1.16 (d, 3H). Anal. RP-HPLC t_R = 9.05 min (method 1, purity 96.00%/94.79%). HR-MS (m/z, MH^+): measd 421.2153, calcd 421.2141.

6-[(*S*)-4-(4-Benzylphthalazin-1-yl)-3-methylpiperazin-1-yl]nicotinonitrile (47). 47 was prepared from **6a** and **45b** in 20% yield according to the procedure described for **46**. ^{1}H NMR (400 MHz, CDCl3) δ 8.35 (d, J = 1.4 Hz, 1H), 8.07 (d, J = 8.2 Hz, 1H), 7.96 (d, J = 7.8 Hz, 1H), 7.81−7.62 (m, 2H), 7.57 (dt, J = 9.0, 1.9 Hz, 1H), 7.31−7.24 (m, 2H), 7.24−7.16 (m, 2H), 7.15−7.08 (m, 1H), 6.61 (d, J = 9.1 Hz, 1H), 4.58 (s, 2H), 4.12 (br s, 1H), 3.95 (br s, 1H), 3.83 (d, J = 2.2 Hz, 2H), 3.76 (br s, 1H), 3.67−3.56 (m, 1H), 3.51 (br s, 1H), 1.16 (d, J = 5.2 Hz, 3H). Anal. RP-HPLC t_R = 9.02 min (method 1). HR-MS (m/z, MH^+): measd 421.2134, calcd 421.2141.

4-[1-(4-Benzylphthalazin-1-yl)piperidin-4-yl]benzonitrile (48). 48 was synthesized from **6a** and **45c** in 75% yield according to general procedure A. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.09 (m, 2H), 7.82 (m, 2H), 7.74 (d, J = 8.6 Hz, 2H), 7.52 (d, J = 8.1 Hz, 2H), 7.26 (m, 2H), 7.20 (m, 2H), 7.10 (m, 1H), 4.51 (s, 2H), 3.83 (m, 2H), 3.04 (m, 2H), 2.99 (m, 1H), 2.02−1.88 (m, 4H). Anal. RP-HPLC t_R = 4.86 min (method 1). HR-MS (m/z, MH^+): measd 405.2083, calcd 405.2079.

1-Benzyl-4-bromoisoquinoline (50). To a 40 mL vial was added **49** (490 mg, 2.00 mmol), tetrakis(triphenylphosphine)palladium(0) (40 mg, 0.034 mmol), and THF (4 mL). After all solids were dissolved, benzylzinc bromide (8.0 mL, 0.5 M in THF, 4.0 mmol) was added dropwise via syringe, and the resulting reaction mixture was stirred at 25 °C for 12 h. The mixture was poured into a cold solution of saturated NH_4Cl and extracted with EtOAc. The organics were combined, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by flash chromatography on silica gel (EtOAc/ heptanes gradient) to afford the title compound (150 mg, 25% yield).

6-[4-(1-Benzylisoquinolin-4-yl)piperazin-1-yl]nicotinonitrile (51). To a 40 mL vial was added **50** (120 mg, 0.40 mmol), **7d** (160 mg, 0.84 mmol), $Pd_2(dba)_3$ (40 mg, 0.04 mmol), (±)-2,2′-bis(diphenylphosphino)-1,1′-binaphthyl (60 mg, 0.096 mmol), and dioxane (5 mL). After the vial was flushed with nitrogen for 5 min, the reaction mixture was stirred for 2 min, and then NaO-*t*-Bu (150 mg, 1.55 mmol) was added. The vial was then sealed and heated at 80 °C for 12 h. After cooling, the mixture was loaded directly onto a silica column and purified via flash chromatography. Additional purification via reversed phase HPLC (0−100% of 0.1% NH_3 in water/0.1% NH_3 in acetonitrile) afforded the title compound (40 mg, 25%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.54 (d, J = 2.3 Hz, 1H), 8.30 (d, J = 8.5 Hz, 1H), 8.21 (d, J = 8.5 Hz, 1H), 8.18 (s, 1H), 7.91 (dd, J = 9.0, 2.3 Hz, 1H), 7.78 (t, J = 7.6 Hz, 1H), 7.65 (t, J = 7.6 Hz, 1H), 7.32−7.21 (m, 4H), 7.18−7.12 (m, 1H), 7.04 (d, J = 9.1 Hz, 1H), 4.57 (s, 2H), 3.96 (br s, 4H), 3.17 (br s, 4H) Anal. RP-HPLC t_R = 1.84 min (method 3). HR-MS (m/z, MH^+): measd 406.2032, calcd 406.2032.

1-Chloro-4-benzylisoquinoline (52). In a 200 mL round-bottom flask, **49** (2 g, 8.2 mmol) was dissolved in THF (40 mL) and cooled to −78 °C. *n*-Butyllithium (3.28 mL, 2.5 M in hexanes, 8.7 mmol) was then added dropwise. The mixture was stirred for 15 min, and then zinc(II) bromide (2.26 g, 8.7 mmol) was added as a single portion, and the mixture was allowed to warm to room temperature. Tetrakis(triphenylphosphine)palladium(0) (0.30 g, 0.26 mmol) was added followed by benzyl bromide (1.03 mL, 8.7 mmol). The mixture was then warmed to 65 °C and stirred for 16 h. The crude mixture was poured into a cold solution of saturated NH_4Cl and extracted with EtOAc. The organics were combined, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by flash chromatography on silica gel (EtOAc/heptanes gradient) to afford the title compound (1.6 g, 77%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.31 (m, 2H), 8.14 (d, J = 8.5 Hz, 1H), 7.87 (t, J = 7.1 Hz, 1H), 7.80 (t, J = 8.1 Hz, 1H), 7.27 (m, 4H), 7.18 (m, 1H), 4.42 (s, 2H). MS (m/z, MH^+): 254.

6-[4-(4-Benzylisoquinolin-1-yl)piperazin-1-yl]nicotinonitrile (53). To a 6 mL microwave reaction vial was added **52** (50 mg, 0.20 mmol), **7d** (41 mg, 0.22 mmol), and NMP (1 mL). The mixture was heated in the microwave (high absorption setting) at 180 °C for 1 h. The mixture was the diluted with MeOH (1 mL) and purified via reverse-phase HPLC (10−90% acetonitrile/0.1% TFA in water). Pure fractions were pooled, basified to pH 8 with aqueous saturated $NaHCO_3$, and then extracted with EtOAc. Organics were combined, dried over Na_2SO_4, filtered, and concentrated in vacuo to afford the title compound (50 mg, 62%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.53 (d, J = 2.3 Hz, 1H), 8.22 (d, J = 8.2 Hz, 1H), 8.09 (s, 1H), 7.97 (d, J = 8.3 Hz, 1H), 3.38 (s, 4H), 7.90 (dd, J = 9.1, 2.4 Hz, 1H), 7.70 (t, J = 7.2 Hz, 1H), 7.61 (t, J = 7.4 Hz, 1H), 7.26 (d, J = 4.4 Hz, 4H), 7.02 (d, J = 9.1 Hz, 1H), 7.17 (m, 1H), 4.28 (s, 2H), 3.94 (s, 4H). Anal. RP-HPLC t_R = 5.74 min (method 1). HR-MS (m/z, MH^+): measd 406.2032, calcd 406.2032.

4-(1*H*-Indol-3-yl)-3,4,5,6-tetrahydro-2*H*-[1,2′]bipyridinyl-5′-carbonitrile (55). To a suspension of **54** (500 mg, 2.5 mmol) in DMF (10 mL) was added K_2CO_3 (3.0 g, 21.7 mmol). The mixture was heated to 60 °C for 2 min, and then a solution of 6-chloronicotinonitrile (420 mg, 3 mmol) in DMF (2 mL) was added. The temperature was increased to 95 °C, and the mixture was stirred for 2 h. The mixture was then cooled to room temperature and was concentrated in vacuo to remove volatiles. The resulting residue was redissolved in EtOAc and washed with water followed by brine. The organic layer was dried over Na_2SO_4, filtered, and concentrated in vacuo to afford the title compound, which was carried onto the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.35 (d, J = 1.9 Hz, 1H), 7.92 (br s, 1H), 7.57 (d, J = 7.8 Hz, 1H), 7.53 (dd, J = 9.1, 2.3 Hz, 1H), 7.31 (d, J = 8.1 Hz, 1H), 7.00−7.17 (m, 2H), 6.89 (d, J = 1.9 Hz, 1H), 6.59 (d, J = 9.1 Hz, 1H), 4.50 (d, J = 13.1 Hz, 2H), 2.94−3.23 (m, 3H), 2.13 (d, J = 12.8 Hz, 2H), 1.85−1.82 (m, 2H). MS (m/z, MH^+): 303.1.

4-(1-Benzyl-1*H*-indol-3-yl)-3,4,5,6-tetrahydro-2*H*-[1,2′]bipyridinyl-5′-carbonitrile (56). Compound **55** (50 mg, 0.166 mmol) was dissolved in THF (2 mL) and aqueous sodium hydroxide (50%, 2 mL). Tetrabutylammonium hydroxide (0.2 mL, 1 M in THF) was added and the mixture stirred for 2 min at ambient temperature before adding benzyl bromide (34 mg, 0.199 mmol). The mixture

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was stirred for 1.5 h. It was then diluted with EtOAc and washed with several portions of H_2O. The organics were washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The resulting residue was washed with hot heptane (2 × 10 mL) to yield the title compound (33 mg, 65%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.33 (br s, 1H), 7.57 (d, J = 7.7 Hz, 1H), 7.49 (d, J = 8.5 Hz, 1H), 7.32−6.91 (m, 8H), 6.79 (s, 1H), 6.55 (d, J = 9.1 Hz, 1H), 5.18 (s, 2H), 4.47 (d, J = 12.0 Hz, 2H), 3.19−2.93 (m, 3H), 2.10 (d, J = 12.6 Hz, 2H), 1.78−1.49 (m, 2H). Anal. RP-HPLC t_R = 2.16 min (method 3). HR-MS (m/z, MH$^+$): measd 393.2074, calcd 393.2079.

4,7-Dichlorofuro[2,3-*d*]pyridazine (57). Furan-2,3-dicarboxylic acid (1.0 g, 6.41 mmol) was dissolved in MeOH (10 mL). To this solution was added thionyl chloride (1.4 mL, 19.22 mmol). The mixture was stirred at room temperature for 16 h. H_2O (1 mL) was added, and the MeOH was removed in vacuo. Additional H_2O was added, and the organics were extracted with EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated to afford furan-2,3-dicarboxylic acid dimethyl ester (650 mg, 55%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.02 (d, J = 1.8 Hz, 1H), 6.94 (d, J = 1.9 Hz, 1H), 3.84 (s, 3H), 3.81 (s, 3H).

Furan-2,3-dicarboxylic acid dimethyl ester (1.6 g, 8.69 mmol) was added to a solution of EtOH (10 mL) and hydrazine hydrate (1.46 mL, 55% in water). The reaction was heated to reflux for 6 h, then cooled and concentrated in vacuo to form a slurry. This was diluted with H_2O, and the resulting precipitate was isolated by filtration, washing with additional H_2O. This solid was transferred to a round-bottom flask, and HCl was added (7.2 mL, 2 N in H_2O). The mixture was heated to reflux for 4 h, then cooled to room temperature. The resulting precipitate was isolated by filtration, washing several times with H_2O, to afford 5,6-dihydrofuro[2,3-*d*]pyridazine-4,7-dione (930 mg, 70%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.77 (br s, 1H), 8.21 (d, J = 1.9 Hz, 1H), 7.03 (d, J = 1.5 Hz, 1H), 3.42 (br s, 1H).

5,6-Dihydrofuro[2,3-*d*]pyridazine-4,7-dione (930 mg, 6.11 mmol) was combined with pyridine (1.8 mL) and $POCl_3$ (18 mL), and the mixture was heated to reflux for 4 h. The mixture was then cooled to room temperature and concentrated in vacuo. The resulting viscous solution was poured over ice, and organics were extracted with CH_2Cl_2. The combined organics layers were washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified via flash chromatography on silica gel (0−8% MeOH/CH_2Cl_2) to afford the title compound (577 mg, 50%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.66 (d, J = 2.2 Hz, 1H), 7.43 (d, J = 2.2 Hz, 1H).

7-Benzyl-4-[4-(5-trifluoromethylpyridin-2-yl)piperazin-1-yl]furo[2,3-*d*]pyridazine (58) and 4-Benzyl-7-[4-(5-trifluoromethylpyridin-2-yl)piperazin-1-yl]furo[2,3-*d*]pyridazine (59). Compound **57** (250 mg, 1.32 mmol), **7c** (290 mg, 1.26 mmol), and Et_3N (270 μL, 1.98 mmol) were combined in dioxane (2 mL). The reaction mixture was heated to 80 °C for 70 h, then cooled to room temperature and concentrated in vacuo. The residue was purified by flash chromatography on silica gel (EtOAc/heptanes) to afford a regioisomeric mix (60:40) of 7-chloro-4-[4-(5-trifluoromethylpyridin-2-yl)piperazin-1-yl]furo[2,3-*d*]pyridazine and 4-chloro-7-[4-(5-trifluoromethylpyridin-2-yl)piperazin-1-yl]furo[2,3-*d*]pyridazine (210 mg, 41%).

This regioisomeric mixture (210 mg, 0.547 mmol) was combined with benzylzinc bromide (6.75 mL, 0.5 M in THF, 3.28 mmol) and tetrakis(triphenylphosphine)palladium(0) (31.5 mg, 0.027 mmol) in THF (12 mL), and the mixture was heated to 80 °C for 40 h. The mixture was cooled to room temperature and diluted with H_2O. Organics were extracted with EtOAc, dried over $MgSO_4$, and concentrated. The residue was purified by flash chromatography on silica gel (EtOAc/heptanes) to afford a mix of the title compounds. Regioisomers were separated by reverse-phase HPLC (30% isocratic gradient of CH_3CN/H_2O with a formic acid modifier (0.1%)). Compound **58**: 17 mg, 7%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.45 (s, 1H), 8.26 (s, 1H), 7.84 (d, J = 9.1 Hz, 1H), 7.39−7.33 (m, 1H), 7.32−7.24 (m, 4H), 7.19 (dd, J = 6.8 Hz, 1H), 7.00 (d, J = 9.1 Hz, 1H), 4.38 (s, 2H), 3.89−3.81 (m, 8H). Anal. RP-HPLC t_R = 5.4 min (method 1). HR-MS (m/z, MH$^+$): measd 440.1683, calcd 440.1698. Compound **59**: 18 mg, 7%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.45 (s, 1H), 8.25 (s, 1H), 7.85 (d, J = 9.1 Hz, 1H), 7.36−7.30 (m, 2H), 7.27 (q, J = 7.6, 7.6 Hz, 2H), 7.18 (dd, J = 7.2, 7.2 Hz, 1H), 7.11 (s, 1H), 7.04 (d, J = 9.1 Hz, 1H), 4.37 (s, 2H), 3.94−3.88 (m, 4H), 3.88−3.81 (m, 4H). Anal. RP-HPLC t_R = 5.55 min (method 1). HR-MS (m/z, MH$^+$): measd 440.1683, calcd 440.1698.

4,7-Dichloro-1*H*-imidazo[4,5-*d*]pyridazine (60). 1*H*-Imidazole-4,5-dicarboxylic acid dimethyl ester (592 mg, 3.21 mmol) was combined with hydrazine (600 mg, 18.8 mmol) and MeOH (10 mL). The reaction mixture was heated to 115 °C for 30 min, then cooled to room temperature. The resulting precipitate was isolated by filtration, washing several times with H_2O. The precipitate was then combined with hydrazine (1.38 mL) and heated to reflux for 4 h. The reaction mixture was poured into ice−water and then brought to pH ∼2 with HCl (12 N). The new precipitate was isolated by filtration to afford 5,6-dihydro-1*H*-imidazo[4,5-*d*]pyridazine-4,7-dione (293 mg, 60%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.41 (br s, 1H), 8.27 (s, 1H), 3.37 (br s, 2H).

5,6-Dihydro-1*H*-imidazo[4,5-*d*]pyridazine-4,7-dione (1.0 g, 6.57 mmol) was combined with $POCl_3$ (28 mL) and Me_2NH (1 mL), and the reaction mixture was heated at reflux for 16 h. Volatiles were removed in vacuo, and the viscous mixture was poured slowly into ice-cold H_2O (45 mL) while maintaining an internal temperature of <5 °C. The mix was stirred for 1 h at room temperature, and the resulting precipitate was isolated by filtration, washing with additional H_2O, to afford the title compound (830 mg, 67%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 14.43 (br s, 1H), 8.87 (s, 1H).

7-Benzyl-4-[4-(5-trifluoromethylpyridin-2-yl)piperazin-1-yl]-1*H*-imidazo[4,5-*d*]pyridazine (61). Reaction of **60** and **7c** according to the procedure described for **58** and **59** afforded a single regioisomer, 7-chloro-4-[4-(5-trifluoromethyl-pyridin-2-yl)piperazin-1-yl]-1*H*-imidazo[4,5-*d*]pyridazine, in 57% yield. Reaction of this compound with benzylzinc bromide and Pd(PPh_3)$_4$ as described afforded the title compound in 7% yield. ^{1}H NMR (400 MHz, MeOD) δ 8.40−8.35 (m, 1H), 8.30−8.27 (m, 1H), 7.74 (dd, J = 9.1, 2.5 Hz, 1H), 7.34−7.29 (m, 2H), 7.28−7.22 (m, 2H), 7.21−7.14 (m, 1H), 6.94 (d, J = 9.1 Hz, 1H), 4.46 (s, 2H), 4.21−4.15 (m, 4H), 3.90−3.84 (m, 4H). Anal. RP-HPLC t_R = 1.56 min (method 3). HR-MS (m/z, MH$^+$): measd 440.1799, calcd 440.1811.

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