

Structure-Guided Design of IACS-9571, a Selective High-Affinity Dual TRIM24-BRPF1 Bromodomain Inhibitor

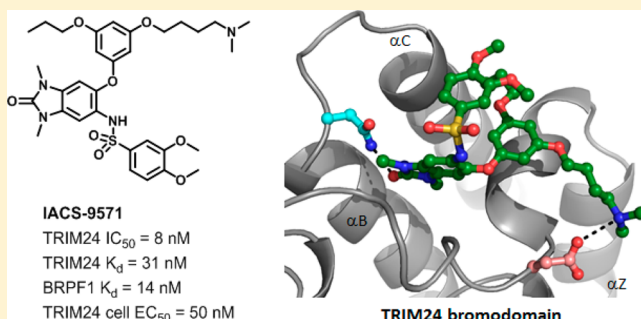
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S Supporting Information

ABSTRACT: The bromodomain containing proteins TRIM24 (tripartite motif containing protein 24) and BRPF1 (bromodomain and PHD finger containing protein 1) are involved in the epigenetic regulation of gene expression and have been implicated in human cancer. Overexpression of TRIM24 correlates with poor patient prognosis, and BRPF1 is a scaffolding protein required for the assembly of histone acetyltransferase complexes, where the gene of MOZ (monocytic leukemia zinc finger protein) was first identified as a recurrent fusion partner in leukemia patients (8p11 chromosomal rearrangements). Here, we present the structure guided development of a series of *N,N*-dimethylbenzimidazolone bromodomain inhibitors through the iterative use of X-ray cocrystal structures. A unique binding mode enabled the design of a potent and selective inhibitor **8i** (IACS-9571) with low nanomolar affinities for TRIM24 and BRPF1 (ITC K_d = 31 nM and ITC K_d = 14 nM, respectively). With its excellent cellular potency (EC_{50} = 50 nM) and favorable pharmacokinetic properties (F = 29%), **8i** is a high-quality chemical probe for the evaluation of TRIM24 and/or BRPF1 bromodomain function in vitro and in vivo.



INTRODUCTION

Bromodomains are protein interaction modules found within a diverse set of chromatin-regulator proteins and specifically recognize, or “read”, acetylated lysine (KAc) residues on the histone tails of chromatin as well as KAc residues on other proteins.^{1,2} Inhibitors of bromodomains, and in particular the BET (bromodomain and extra-terminal) subfamily, have gained much attention with the development of selective chemical probes^{3–6} and the advancement of several inhibitors into the clinic for various oncology indications.^{7–10} Additionally, targeting bromodomains is being explored for the treatment of inflammatory, neurodegenerative, and cardiovascular diseases.^{11,12} The development of this class of inhibitors has demonstrated the potential utility of treating diseases by the disruption of the protein–protein interaction network of chromatin reader modules and is enabling pharmacological studies into how bromodomain-containing proteins (BCPs) regulate gene transcription and cell signaling.^{1,13}

The functions of BCPs, other than BET bromodomains, are much less well understood; however, selective chemical

probes^{6,14–17} are now beginning to emerge, which should help unravel their biological role in normal development and human disease. Equally encouraging are inhibitors that have now been identified for more difficult to drug bromodomains,¹⁸ such as BAZ2B^{19,20} and ATAD2A.^{21–23} Unlike the BET subfamily, which contains two bromodomains that primarily drive their binding and therefore function, a large proportion of the other BCPs contain auxiliary domains, many with diverse and sometimes unknown functions. These multidomain containing BCPs can also be part of multiprotein complexes. Accordingly, the relative contribution of the bromodomain within these complexes has yet to be elucidated. For example, the bromodomain-PHD finger protein (BRPF) family acts as scaffolding proteins to assemble complexes of MYST-family histone acetyltransferases (HATs).²⁴ BRPF1 is a subunit of the

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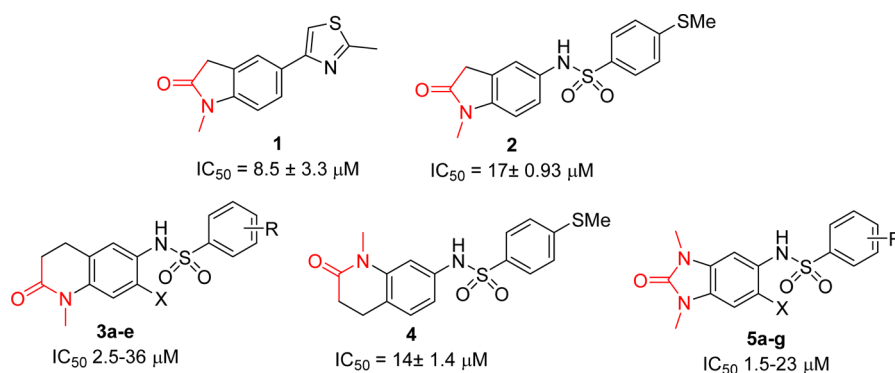


Figure 1. Chemotypes for TRIM24 identified by three different hit-finding approaches: in silico virtual screening (**1**), acetyllysine mimetic (highlighted in red) library building and SAR exploration (**2–5**), and HTS screening (**5f** and **5g**). TRIM24 $IC_{50} \pm SEM$ are from at least three determinations.

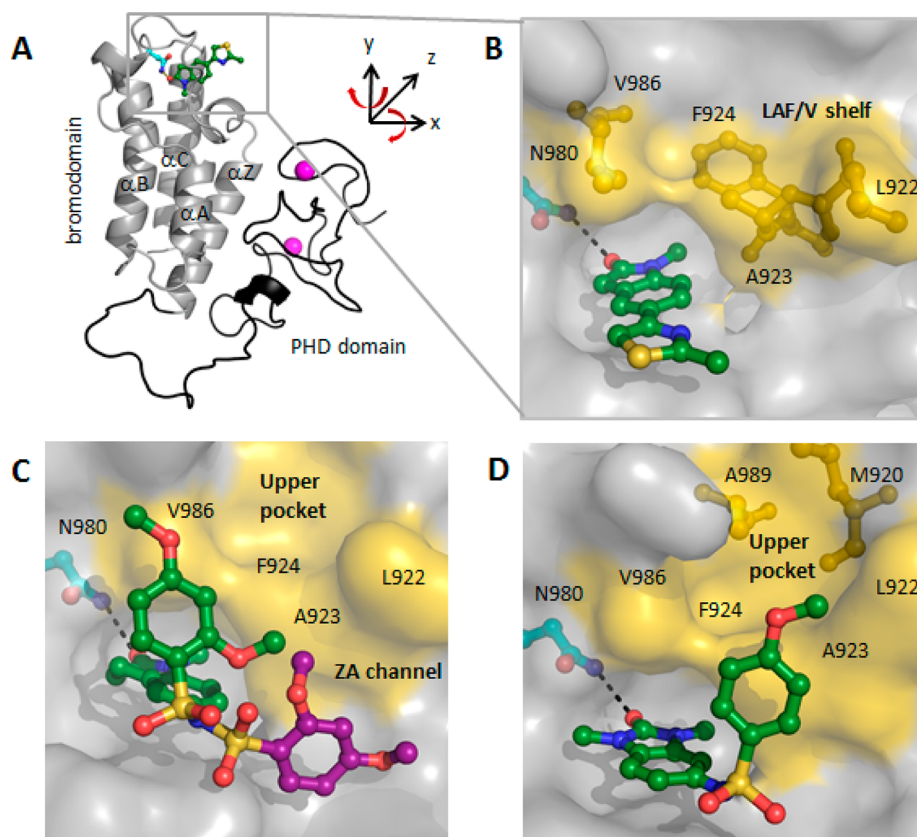


Figure 2. TRIM24 (PHD-bromo) X-ray complexes of initial virtual screening hit and lead templates. (A) Compound **1** bound to TRIM24 (1.9 Å resolution) bromodomain (gray ribbon), PHD domain (black), N980 (cyan), and zinc (magenta) is depicted (PDB code 4YAB). (B) Acetyllysine binding site with **1**, lipophilic residues L922, A923, and F924 on the ZA loop, and V986 on the BC α -helix form an "LAF/V-shelf" (yellow sticks) (PDB code 4YAB). (C) Complex of **3b** (1.7 Å resolution) shown in two poses based upon partial occupancy, second pose (purple) in ZA channel (PDB code 4YAD). (D) Complex of **5b** (2.2 Å resolution) with arylsulfonamide interacting with LAF/V-shelf (PDB code 4YAT).

monocytic leukemic zinc finger (MOZ) complex whose translocations are associated with an aggressive form of acute myeloid leukemia.²⁵ The BRPF1 protein contains multiple conserved domains involved in gene transcription, chromatin binding, and remodeling. The BRPF1 bromodomain has been demonstrated to bind to several histone peptides, including H2AK5ac, H4K12ac, H4K8ac, H4K5ac, and H3K14ac; knowledge of the importance of these observations and the biological function of the BRPF1 bromodomain is largely unknown.²⁶

The tripartite motif containing proteins (TRIMs) are a large family of proteins whose structures are characterized by a highly

conserved sequence of domains in the N-terminal region: RING domain, B-box zinc-fingers, and a coiled-coil region (also collectively known as an RBCC domain).²⁷ Many functions have been ascribed to TRIM proteins, including their ability to act as E3 ligases for ubiquitin and small-ubiquitin-like modifier (SUMO) activities.^{28,29} A subgroup of TRIM proteins (TRIM24, -28, and -33) also contain a dual C-terminal plant homeodomain and bromodomain motif (PHD-bromo), which makes these interesting targets for exploring epigenetic regulation through bromodomain inhibition.³⁰ TRIM24 was originally identified as a transcriptional intermediary factor

(TIF1 α) which acts as a co-repressor of the retinoic acid receptor that interacts with multiple nuclear receptors via an LXXLL motif.³¹ The C-terminal PHD-bromo domain of TRIM24 acts as a dual “reader” of unmodified H3K4 and acetylated H3K23 marks within the same histone tail;³² additionally, TRIM24 can also bind to non-histone KAc marks on other proteins, including p53.³³ Genetic knock-down of TRIM24 in cancer cells results in antiproliferative phenotypes, and overexpression of TRIM24 has also been linked to poor prognosis for several cancers, including breast,^{32,34} head and neck,³⁵ non-small-cell lung,³⁶ hepatocellular,³⁷ and glioblastoma.³⁸ Because of the interest in TRIM24 here at MD Anderson and the druggability of BCPs,¹⁸ we set out to develop a potent (cellular EC₅₀ < 100 nM) and selective TRIM24 chemical probe in order to interrogate the role of the bromodomain in human disease.

RESULTS AND DISCUSSION

Identification of Scaffolds. Since there were no known small-molecule TRIM24 inhibitors³⁹ at the start of our study, we explored three different hit identification approaches for finding novel scaffolds: virtual in silico high-throughput screening (HTS), construction of a focused KAc mimetic library, and a traditional small-molecule library HTS. Three different chemotypes, as shown in Figure 1, were identified from this diverse approach: an *N*-methylindolinone (**1** and **2**), the *N*-methyltetrahydroquinolin-2-one (**3** and **4**), and the *N,N*-dimethylbenzimidazolone (**5**).¹⁴

The in silico virtual screening was performed using the “Virtual Screening Workflow” implemented within Schrödinger’s Maestro modeling software suite and the reported TRIM24 X-ray crystal structures (PDB codes 3O33 and 3O34, apo and peptide substrate-bound complexes, respectively). Commercially available compounds within the ZINC library⁴⁰ that bound to the KAc binding pocket, with a constraint of making at least one hydrogen bond (H-bond) interaction with N980 side chain, were docked, scored, and ranked. After visual inspection of the top ranked poses and applying appropriate physicochemical property filters, the highest scoring hits were evaluated for binding to the bromodomain of TRIM24 using a biochemical bromodomain-H3K23Ac peptide displacement assay (AlphaScreen).⁴¹ Fragment **1** (Figure 1) was identified as a TRIM24 inhibitor with IC₅₀ = 8.5 μ M, and subsequent X-ray cocrystal structure determination of **1** with TRIM24(PHD-bromo) (Figure 2A) validated the initially proposed docking hypothesis. Compound **1** was verified to be a KAc mimetic, whereby the carbonyl of the methyloxindole moiety interacts with the conserved N980 side chain through an H-bond (Figure 2B). Although **1** was ligand efficient (LE = 0.41), the linked-thiazole group is coplanar with the oxindole template and is primarily solvent exposed, which made further modifications unlikely to interact with the protein. We sought to improve potency by interacting with the lipophilic sequence of residues 922-LAF-924 on the ZA-loop and V986 on the B-loop. This LAF/V sequence highlighted in yellow (Figure 2B) forms a lipophilic shelf, or “LAF/V-shelf”, akin to the “WPF” shelf of the BET-family of bromodomains (BRD2–4 and BRDT) whereby ligand interactions within this region have been demonstrated to be a way to improve potency for a wide variety of inhibitors.^{42,43} In order to vector off orthogonally from the template and interact with the LAF/V-shelf, we reasoned that a sulfonamide linkage to an aromatic substituent, as in PFI-1,⁵ would be a way to achieve this.

We explored a variety of template replacements for the indolinone and constructed a KAc mimetic library of 5,6- and 6,6-fused heterocycles containing a sulfonamide linkage to an aryl group. In contrast to the indolinone sulfonamide **2** which lost potency (IC₅₀ = 17 μ M), both the tetrahydroisoquinolin-2-one **3** and the *N,N*-dimethylbenzimidazol-2-one **5** scaffolds were identified as viable chemotypes with micromolar inhibition for TRIM24 (Figure 1). A summary of structure–activity relationships (SARs) for the scaffolds is shown in Table 1. Both **3a** and **5a** have comparable potencies of 4.8 and 4.9

Table 1. Structure–Activity Relationship for Sulfonamides 3a–e and 5a–g

compd	R	X	TRIM-24 IC ₅₀ (μ M) ^a
3a	4-SMe	H	4.8 \pm 2.9 (5)
3b	2,4-di-OMe	H	2.5 \pm 0.56 (3)
3c	4- <i>O</i> -isobutyl	H	3.7 \pm 3.2 (3)
3d	4-cyclohexyl	H	2.8 \pm 1.3 (6)
3e	H	OMe	36 \pm 24 (9)
5a	4-SMe	H	4.9 \pm 0.82 (5)
5b	4-OMe	H	9.3 \pm 5.1 (3)
5c	H	H	23 \pm 2.6 (3)
5d	4- <i>O</i> -isobutyl	H	4.0 \pm 1.3 (5)
5e	4-cyclohexyl	H	1.7 \pm 0.69 (5)
5f	3-CN	OMe	10 \pm 2.7 (6)
5g	H	O(4-MeO-Ph)	1.5 \pm 0.79 (11)

^aMean \pm SEM (number of measurements).

μ M, respectively, and were reasonable starting points from which to optimize. Interestingly, the regioisomeric derivative **4**, which has the same sulfonamide attachment point relative to the KAc mimetic (**red**) compared to **5a**, was less potent with an IC₅₀ = 14 μ M. Both the template and the positioning of the sulfonamide-linker appeared to be important for obtaining potency; therefore, evaluation of the relative binding modes between the two templates would be important for selecting a series to progress.

The TRIM24 cocrystal structure complexes of **3b** (Figure 2C) and **5b** (Figure 2D) informed on the importance of the relative regiochemistry of the sulfonamide-linker attachment and the role in defining how the aryl group interacts with the lipophilic LAF/V-shelf. Both templates interact with N980 and bind similarly within the KAc binding site; however, the positioning of the aryl group was remarkably different between the two compounds. The complex with **3b** also revealed the conformational mobility of the arylsulfonamide; two poses of the aryl group were observed within the same chain of the complex based upon partial occupancy. The relative atomic densities suggested an approximately 60:40 ratio between the two conformations (Figure 2C) with the aryl group (**green**) only partially interacting with V986 of the BC loop and in the less populated conformation (**purple**) directing the aryl group toward the ZA channel. In contrast, the complex of **5b** showed a single conformation of the arylsulfonamide, which makes lipophilic contacts with both A923 and F924 of the LAF/V-shelf (Figure 2D). Another attractive feature of scaffold **5** relative to **3** is the symmetrical nature of the two *N*-methyl groups, which allows the scaffold to always place a methyl group deep into the pocket. Scaffold **5** was also attractive synthetically, as it allowed for a more rapid development of this series relative to the regiochemical control necessary for the prosecution of **3**.

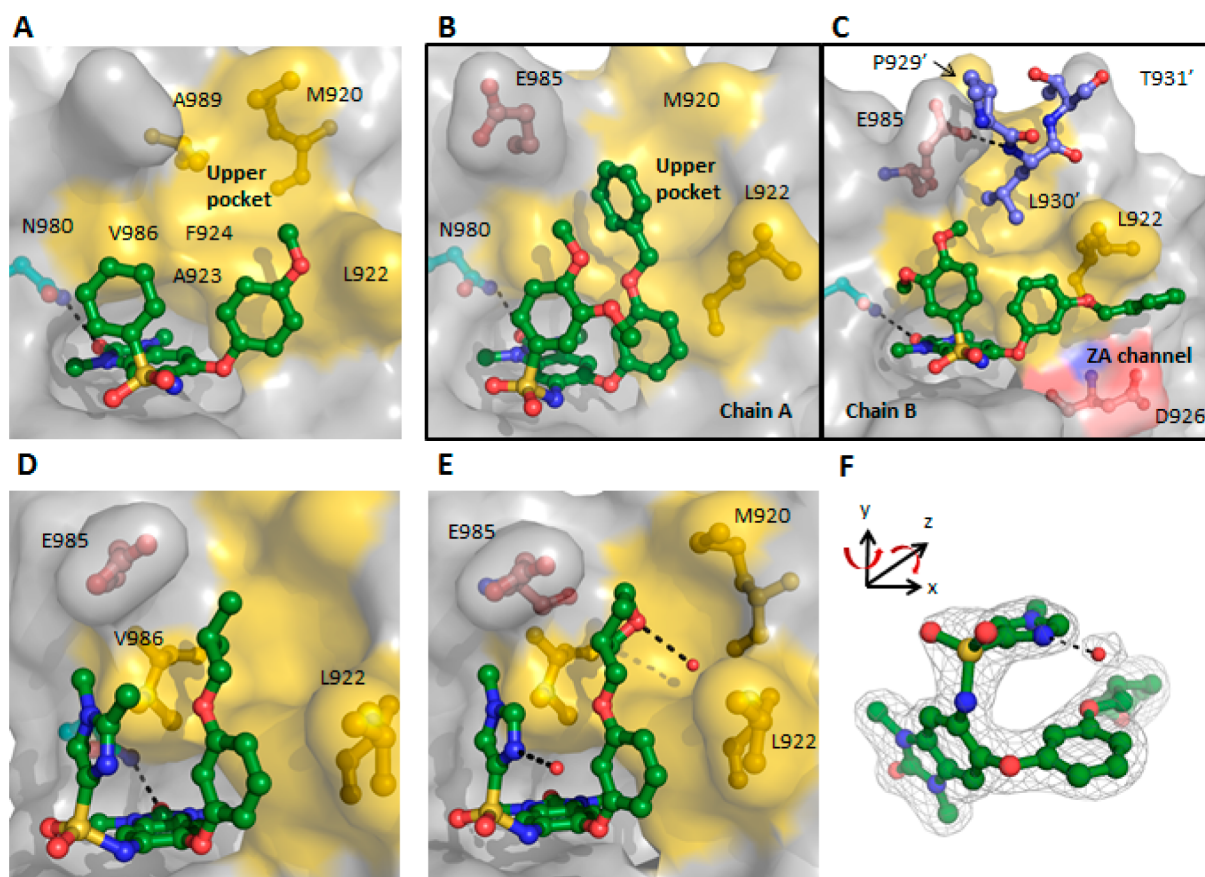


Figure 3. New unexpected binding mode enabled the design of interactions with the “upper pocket”. (A) Cocystal structure of **5g** (2.3 Å resolution) depicting a “flipped” binding mode with the aryl ether group now interacting with the LAF/V-shelf (PDB code 4YAX). (B, C) Cocystal structure of **7b** (1.5 Å resolution) depicted in two binding conformations in the asymmetric unit (PDB code 4YBM): (B) chain A, showing the benzyl group filling the “upper pocket”; (C) chain B, with the benzyl group occupying the ZA channel. Depicted in blue are three residues of chain A TRIM24 protein with L930' occupying the “upper pocket”. (D) Cocystal structure of **7g** (1.8 Å resolution) with the isobutyl ether group occupying the “upper pocket” (PDB code 4YBS). (E) Cocystal structure of **7l** (1.8 Å resolution) with the 3-tetrahydropyranyl group H-bonded to a conserved water in the “upper pocket” and the imidazole H-bonded to a water that is π -stacked with the aryl ether group (PDB code 4YBT). (F) $2F_o - F_c$ map of **7l** and the π -stacked water molecule contoured at 1.0σ (PDB code 4YBT).

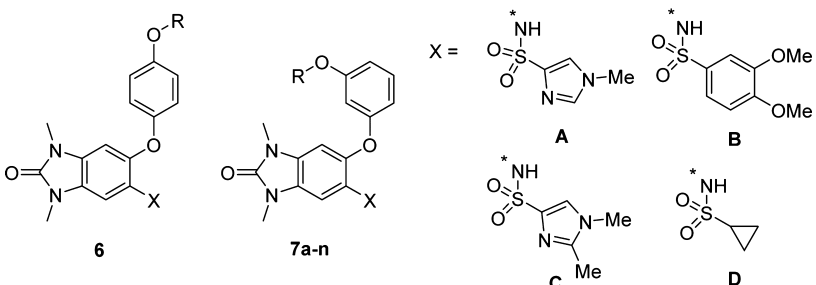
The positioning of the aryl group in the complex of **5b** looked ideal for targeting the lipophilic region just beyond the LAF/V-shelf or “upper pocket” (depicted as a yellow surface in Figure 2D). The “upper pocket” is defined by lipophilic residues A989 and M920, which we thought we could improve potency by exploiting this region; therefore, we evaluated a series of para-substituted aryl derivatives for both templates (Table 1). The SAR with substituents in the para-position showed very little improvement going from the thiomethyl group of **3a** and **5a** (IC_{50} of 4.8 and 4.9 μM , respectively) to the much larger cyclohexyl group of **3d** and **5e** (IC_{50} of 2.8 and 1.7 μM , respectively). The poor ligand binding efficiency of adding the larger cyclohexyl group (LE = 0.28 for **5e**) was discouraging. After evaluating a library of aryl sulfonamides, there did not appear to be a likelihood of achieving the desired nanomolar TRIM24 potency through this approach.

Concurrent with the lead optimization of this series, an HTS screen was performed at the Texas Screening Alliance for Cancer Therapeutics with a 61,000 compound library using the same AlphaScreen conditions we employed for our in vitro assay. Derivative **5f**, containing a 5-methoxy substituent, was identified as a hit and verified as a modest TRIM24 inhibitor (IC_{50} = 10 μM). This is in contrast to **3e** with IC_{50} = 36 μM . Knowledge of the binding mode of the unsubstituted series, as

previously discussed, made substitution at the 5-position of the dimethylbenzimidazolone a nonobvious vector from which to explore further changes. We subsequently performed a hit expansion of 4-arylsulfonamide-5-substituted compounds and identified **5g** as a promising new lead with IC_{50} = 1.5 μM .

New Binding Mode Allowed for Significant Improvements in Potency. The cocystal structure of **5g** with TRIM24 quite surprisingly revealed a new “flipped” binding mode in which the aryl ether group now interacted with the LAF/V-shelf and the arylsulfonamide group was oriented towards the conserved asparagine (Figure 3A). The aryl ether ring of **5g** makes lipophilic contacts with A923 (3.75 Å between the $C\alpha$ and the center of the aryl ring), while the phenylsulfonamide makes lipophilic contact with V986. The lone pairs of the oxygen of the methoxy group directed towards L922 would appear to be an unfavorable interaction and may influence the positioning of this residue. The meta position appeared to be more favorable, as it allows more room for additional derivatizations, directing groups towards the “upper pocket”. Although the ligand efficiency of **5g** was lower (LE = 0.26), we recognized that this unexpected binding mode offered a new opportunity to optimize interactions with the LAF/V-shelf and target the “upper pocket” in order to improve potencies.

Table 2. Structure–Activity Relationships for Improved in Vitro Cellular Potencies and Solubility



compd	R	X	TRIM-24 IC ₅₀ (μM) ^a	cell EC ₅₀ (μM) ^{a,b}	solubility ^c (μM)
6	benzyl	A	2.0 ± 0.93 (6)	>33 (4)	nt
7a	benzyl	A	0.22 ± 0.041 (5)	6.2 ± 2.0 (4)	59
7b	benzyl	B	0.14 ± 0.056 (29)	3.9 ± 0.87 (13)	0.6
7c	benzyl	C	0.27 ± 0.094 (6)	4.9 ± 1.3 (4)	nt
7d	Et	C	0.18 ± 0.045 (5)	2.3 ± 1.1 (4)	74
7e	<i>n</i> -propyl	B	0.043 ± 0.0037 (6)	0.83 ± 0.17 (7)	1.1
7f	<i>n</i> -propyl	C	0.053 ± 0.015 (9)	0.95 ± 0.31 (9)	62
7g	CH ₂ CH(Me) ₂	C	0.057 ± 0.016 (65)	1.3 ± 0.27 (53)	87
7h	<i>n</i> -butyl	C	0.16 ± 0.055 (4)	3.0 ± 0.81 (5)	nt
7i	CH ₂ CH(Me) ₂	D	0.11 ± 0.023 (7)	5.0 ± 0.53 (7)	0.78
7j	CH ₂ CH(Me) ₂	H	2.4 ± 1.6 (11)	>36 (5)	2.6
7k	(CH ₂) ₃ OMe	C	0.13 ± 0.035 (5)	2.7 ± 0.36 (6)	100
7l	CH ₂ (tetrahydrofuran-3-yl)	A	0.10 ± 0.038 (5)	1.9 ± 0.41 (5)	77
7m	(CH ₂) ₄ NMe ₂	B	0.27 ± 0.12 (4)	3.2 ± 0.44 (2)	66

^aMean ± SEM (number of measurements). ^bTRIM24 AlphaLisa assay in HeLa cells. ^cKinetic solubility in phosphate buffer, pH = 7.0. nt = not tested.

We evaluated the SAR of a series of aryl ether substitutions to improve the potency and assess the role of the sulfonamide functionality on enhancing the solubility of the molecule (Table 2). In order to develop a potent cellular molecular probe, compounds were evaluated in an AlphaLisa cellular target engagement assay, which measured the displacement of ectopically expressed TRIM24(PHD-bromo) from endogenous histone H3 in HeLa cells. The effect of substituents on the kinetic solubility of the compound at pH 7.0 was also monitored. The larger *p*-benzyl substituent of **6** (IC₅₀ = 2.0 μM) did not improve upon the potency of **5g** and had no cellular activity; however, the meta-regioisomer **7a** was 10-fold more potent than **6** with IC₅₀ = 0.22 μM and cellular EC₅₀ = 6.2 μM, thereby confirming the meta position to be the best vector for further exploration.

We found that a wide variety of aryl- and heteroaromatic sulfonamides were tolerated and had minor effects on the intrinsic potency of the molecules. The sulfonamide appears to play a structural role in positioning the aryl ether group, since few contacts with the protein are made by this functional group. In fact, this moiety could be used to modify physical properties, such as solubility and permeability, without adversely affecting the binding affinity of the molecule. For example, imidazole **7a** had an improved aqueous solubility of 59 μM compared with arylsulfonamide **7b** (0.6 μM) while maintaining similar in vitro potencies of 0.22 versus 0.14 μM, respectively. In this example, the improved solubility was accompanied by a reduction in apparent permeability (*P*_{app}), as determined by a Caco-2 permeability assay; compound flux from the apical to basolateral side was determined to be 4.0 × 10⁻⁶ cm·s⁻¹ for **7a** versus 21 × 10⁻⁶ cm·s⁻¹ for **7b**. The cellular EC₅₀ of 6.2 and 3.9 μM for **7a** and **7b**, respectively, resulted in identical 28-fold cell shifts for both compounds. For further development, we

focused on improving both the potency and the cell shift, since this would best reflect improvements in overall physical properties of the molecule.

In the presence of **7b**, TRIM24 cocrystallized with two molecules in the asymmetric unit. In the first TRIM24 protein (chain A), the 1.5 Å resolution structure confirmed that the benzyl ether targeted the “upper pocket” as we initially envisioned (Figure 3B) and that the conformation of the arylsulfonamide now undergoes an offset *π*–*π* interaction with the aryl ether ring, positioning the other aryl group to interact with the LAF/V-shelf (3.8 Å from the methoxy group of the arylsulfonamide and the center of aryl ether ring). Conversely, in the second TRIM24 protein (chain B), the complex revealed a second conformation with **7b** bound, such that the benzyl ether occupies the ZA channel (Figure 3C). Interestingly, although the ligand was not occupying the “upper pocket” in this alternative binding conformation, residue L930' from chain A involved in the crystal packing does occupy the “upper pocket”. The excellent density and high resolution of the leucine showed an ideal and tight fit with the lipophilic residues of the “upper pocket”. This observation suggested that alkyl ether substituents could be more ligand efficient replacements for the benzyl group. Therefore, a series of alkyl ether substituents were evaluated, and we found that a three-carbon atom length appeared to be optimal for potency, exemplified by the *n*-propyl (**7e,f**) and isobutyl (**7g**) analogues, which improved intrinsic potencies by 3- to 5-fold with respect to **7a**. Improved ligand efficiency was also achieved comparing **7e** (IC₅₀ = 0.043 μM, LE = 0.27) with **7b** (IC₅₀ = 0.14 μM, LE = 0.23).

The TRIM24 cocrystal complex of **7g** (Figure 3D) nicely recapitulated the leucine interactions previously observed in Figure 3C. Since the aryl ether ring appeared to be driving the

Table 3. Structure–Activity Relationship of Disubstituted Aryl Ethers

compd	Y	n	Z	TRIM-24 IC ₅₀ (μM) ^a	cell EC ₅₀ (μM) ^{a,b}	cell shift
8a	A	6	NHBoc	0.16 ± 0.026 (6)	5.5 ± 0.32 (5)	34
8b	A	6	NH ₂	0.010 ± 0.0025 (14)	0.17 ± 0.069 (11)	17
8c	A	6	OH	0.060 ± 0.017 (8)	1.8 ± 0.40 (8)	30
8d	B	6	NH ₂	0.013 ± 0.0020 (15)	0.17 ± 0.059 (18)	13
8e	A	5	NH ₂	0.011 ± 0.0021 (6)	0.11 ± 0.023 (5)	10
8f	A	4	NH ₂	0.0079 ± 0.0029 (8)	0.12 ± 0.0032 (8)	15
8g	B	4	NH ₂	0.0083 ± 0.0026 (4)	0.059 ± 0.011 (4)	7
8h	B	3	NH ₂	0.013 ± 0.0048 (4)	0.12 ± 0.0066 (4)	9
8i	B	4	NMe ₂	0.0076 ± 0.0029 (16)	0.050 ± 0.013 (14)	7

^aMean ± SEM (number of measurements). ^bTRIM24 AlphaLISA assay in HeLa cells.

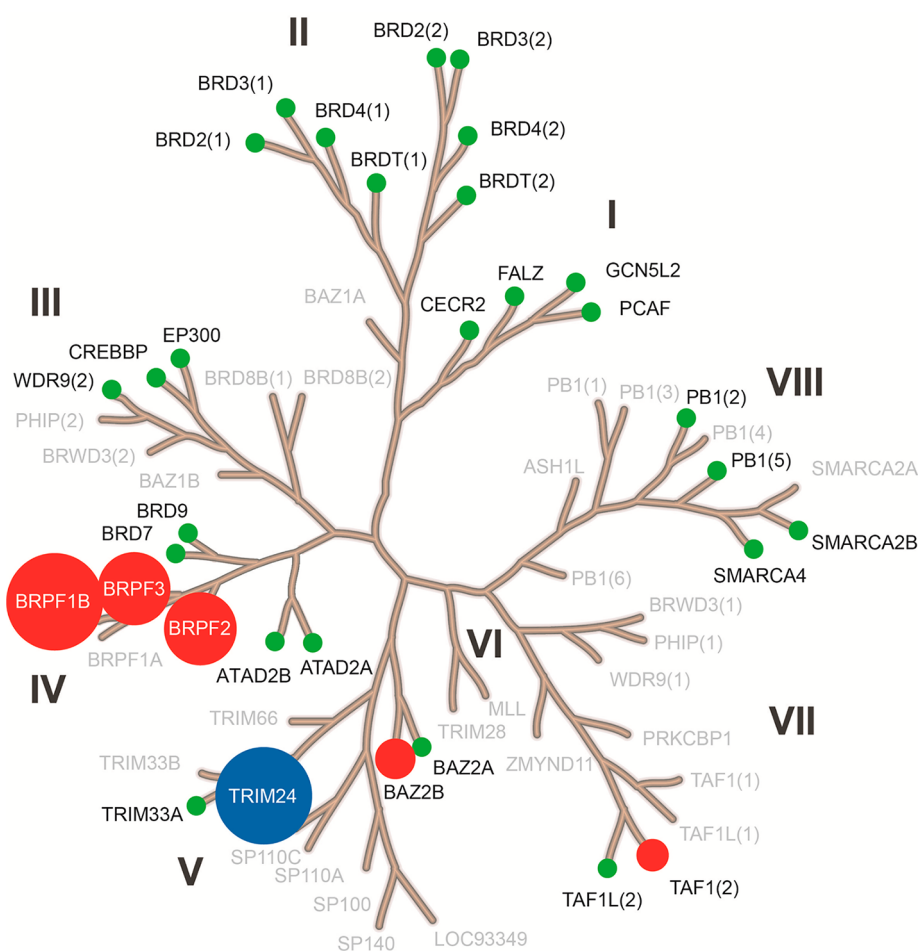


Figure 4. Selectivity profile of **8i** against a panel of 32 bromodomains (DiscoverX) at 1 μM test concentration. The larger is the circle size, the greater is the inhibition: TRIM24(PHD-bromo) in blue; BRPF1–3, BAZ2B, and TAF1(2) in red; 26 bromodomains with percent of control of >35 in green. Bromodomains in gray are not tested. See also Table S2, Supporting Information.

binding affinity and the interactions with the “upper pocket” appeared to be optimized, we sought to minimize or completely remove the sulfonamide group. However, replacement of the arylsulfonamide with a smaller cyclopropyl group as in **7i** (IC₅₀

= 0.11 μM) resulted in a 3-fold loss in cellular potency (EC₅₀ = 5.0 μM) relative to **7g** and complete removal of the sulfonamide (**7j**, IC₅₀ = 2.4 μM) resulted in a 44-fold loss in intrinsic potency and no cellular activity. Other polar functional

groups on the alkyl ether chain were also well tolerated, such as the methyl ether (**7k**, IC_{50} = 0.13 μ M), tetrahydrofuran (**7l**, IC_{50} = 0.10 μ M), and dimethylamine (**7m**, IC_{50} = 0.27 μ M) providing good solubilities; however, cellular potencies were weaker with respect to **7e**.

Although the sulfonamide group makes minimal contact with the protein, the SAR and structural data demonstrate that the arylsulfonamide is an important functional group that helps fill the much more open and wider binding pocket of TRIM24 compared to the smaller more defined pocket of the BET family bromodomains. This is also reflected by the lower druggability scores (Dscore) calculated for TRIM24 (0.67) compared to those for the BET family (0.86–0.94).¹⁸ Furthermore, the cocrystal complex of **7l** with TRIM24 (Figure 3E) also supports the notion that the sulfonamide plays an important structural role in positioning the aryl ether ring in order to direct substituents into the “upper pocket”. The basic nitrogen of the imidazole is H-bonded to a structural water molecule which is positioned directly above the aryl ether group. A different orientation of **7l** (Figure 3F) shows the folded U-shape conformation of the molecule, and the ligand density map clearly demonstrates this solvent exposed water undergoing a π -stacking interaction with the aryl ether. The importance of the sulfonamide stacking interaction with the aryl ether may also explain the observed loss of potency for **7j** which has no sulfonamide group. It is interesting to note that a similar intramolecular aromatic stacking interaction was also an important feature in the design of a potent BAZ2A/B molecular probe.²⁰

Targeting the ZA-Channel for Improved Cellular Potencies. Both **7f** and **7g** displayed the best balance between potency and solubility reflected by their submicromolar cell potencies (EC_{50} of 0.95 and 1.3 μ M, respectively); however, they exhibited large cell shifts of 18- and 23-fold, respectively. We sought to further improve cell potencies by improving the intrinsic potencies and/or lowering the cell shift. Interactions of the *n*-propyl ether group with the “upper pocket” already appeared optimal, so we sought another region of the protein to interact with, in order to further improve potencies. The ZA channel of TRIM24 offered a second site to pick up further interactions. The two different bound conformations of the benzyl group in the **7b** cocrystal (Figure 3B and Figure 3C) inspired the strategic design of a bidirectional aryl ether, whereby one vector was used to interact with D926 in the ZA channel, while the other vector maintained the interaction with the “upper pocket”.

A series of bidirectional aryl ethers were then evaluated, and their SARs are shown in Table 3. The substantial improvement in potency (16-fold) going from the Boc-protected amine **8a** (IC_{50} = 160 nM) to the basic amine analogue **8b** (IC_{50} = 10 nM), as well as the excellent cellular potency of **8b** (EC_{50} = 170 nM), suggested that the bidirectional design principles were realized. The importance of the basic amine is also further demonstrated by the alcohol analogue **8c** which is 11-fold less potent in cells compared to **8b**. Shortening of the chain length to four carbons, as in **8f**, improved both the in vitro and cellular potencies (IC_{50} of 7.9 and 120 nM, respectively) as well as the observed cell shift (15-fold). We also found that with the use of the basic amine on the bidirectional molecules, the imidazole group was no longer needed for solubility. In fact, the dimethoxyphenylsulfonamide **8g**, with a kinetic solubility of 42 μ M (at pH 7.0), displayed a lower cell shift compared to the imidazole **8f** (7-fold versus 15-fold, respectively).

The dimethylamine analogue **8i** (IACS-9571) is one of the most potent TRIM24 inhibitors within this series, with an IC_{50} = 7.6 nM in the biochemical AlphaScreen and a cellular EC_{50} = 50 nM in the AlphaLisa cellular target engagement assay. **8i** has excellent solubility (76 μ M) and a low cell shift (7-fold). Selectivity profiling of **8i** against a panel of 32 bromodomains (DiscoverX) at 1 μ M confirmed the TRIM24 interaction specificity and also revealed strong interactions with family IV bromodomains, BRPF1–3 (Figure 4 and Table S2, Supporting Information).⁴⁴ Subsequent dose–response determinations demonstrated **8i** to be a selective dual TRIM24/BRPF1 inhibitor (K_d = 1.3/2.1 nM) with 9- and 21-fold selectivity against BRPF2 and BRPF3, respectively (Table 4). Much

Table 4. Binding Affinity Data for **8i**

bromodomain	K_d (nM) ^a
TRIM24(PHD-bromo)	1.3
BRPF1	2.1
BRPF2 ^b	12
BRPF3	27
BAZ2B	400
TAF1(domain 2)	1800
BRD4(domains 1, 2)	>10000

^aMean of four determinations in bromoELECT recombinant protein binding assays performed at DiscoverX (<http://www.discoverx.com>).

^bAlso known as BRD1.

weaker affinities with BAZ2B (K_d = 400 nM) and the second domain of TAF1 (K_d = 1800 nM) were also observed. Importantly, **8i** does not interact with the BET subfamily of bromodomains, displaying greater than 7700-fold selectivity versus BRD4(1,2) relative to TRIM24. No interactions were detected against 26 other bromodomains (green circles) at 1 μ M concentration of **8i**. The dual TRIM24/BRPF1 inhibitor profile of **8i** was also confirmed by ITC resulting in K_d values of 31 and 14 nM, respectively (Figure 5). The cocrystal structure of **8i** with TRIM24(PHD-bromo) ultimately confirmed the bidirectional design principle we envisioned, with the *n*-propyl ether occupying the “upper pocket” as well as the salt-bridge interaction of the dimethylamine group with D926 in the ZA channel (Figure 6). The dual TRIM24/BRPF1 potency of **8i** is not surprising since potent BRPF1 dimethylbenzimidazolone inhibitors have been described,¹⁴ and BRPF1 also contains an acidic residue (E655) in the ZA channel which the dimethylamine could potentially interact with.

The pharmacokinetics of **8i** was studied in female CD1 mice, and after iv administration of a 1 mg/kg dose, a moderate clearance of 43 mL·min^{−1}·kg^{−1} was observed, representing approximately half the rate of hepatic blood flow. The terminal iv half-life was 0.7 h, and after oral dosing of a 10 mg/kg dose, the bioavailability of **8i** was 29%, making the inhibitor useful for in vivo studies.

CONCLUSIONS

Iterative use of structural biology and medicinal chemistry optimization allowed us to develop a series of highly potent and selective dual inhibitors of TRIM24 and BRPF1 starting from a diverse hit-finding approach that utilized in silico virtual and small-molecule HTS screens as well as KAc mimetic library design. The unique binding mode observed while investigating new substitution patterns on the benzimidazolone template enabled the design of potent and selective dual inhibitors. Also,

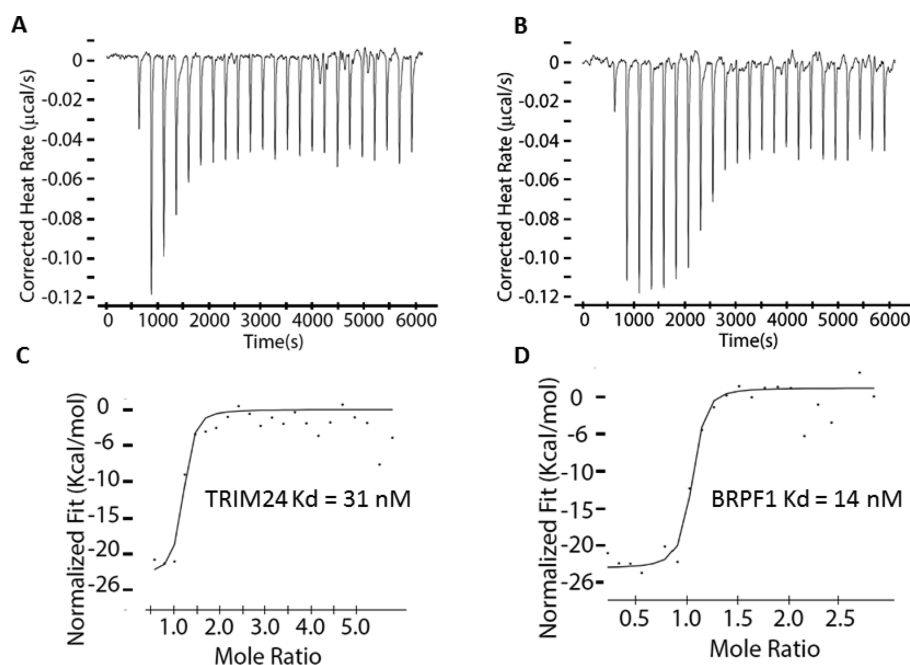


Figure 5. ITC data for **8i**: (A) release of energy for the titration with TRIM24(PHD-bromo); (B) release of energy for the titration with BRPF1B; (C) integrated binding heats for (PHD-Bromo), $K_d = 31$ nM; (D) integrated binding heats for BRPF1B, $K_d = 14$ nM.

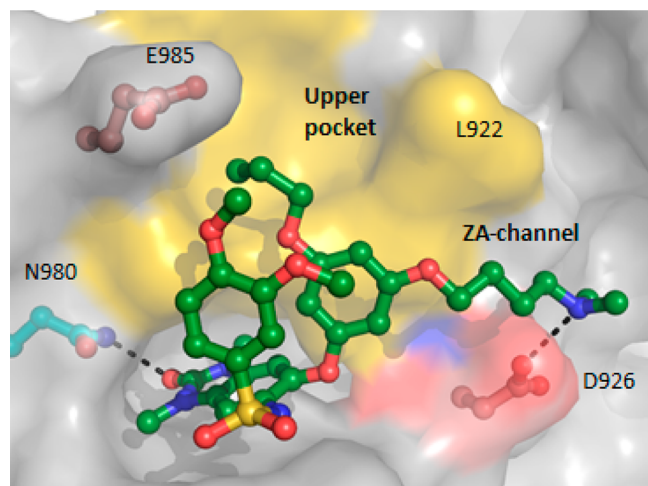


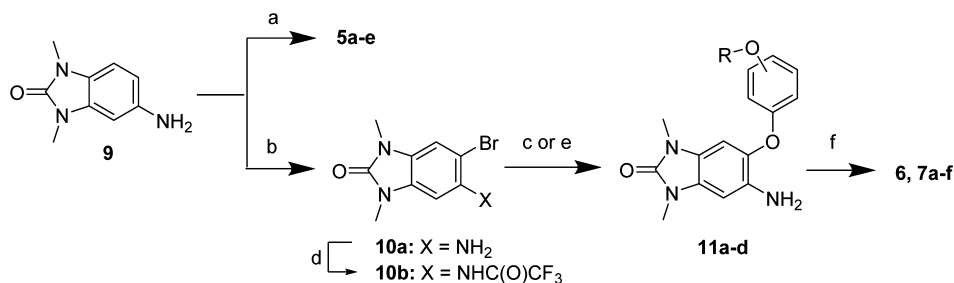
Figure 6. Cocystal structure of **8i** with TRIM24(PHD-bromo) (1.8 Å resolution) showing the *n*-propyl ether group occupying the “upper pocket” and the dimethylamino group forming a salt bridge to D926 in the ZA channel (PDB code 4YC9).

utilization of a TRIM24 cellular target engagement assay and targeting low cell shifts allowed for the optimization of compounds that displayed the best physicochemical properties. The excellent cellular potency, bromodomain selectivity, and desirable physical properties displayed by **8i** provide an ideal chemical probe to investigate the biological and pharmacological role of TRIM24 and/or BRPF1 bromodomain inhibition in vitro and in vivo. The biological activity of **8i** and related compounds will be reported in a subsequent manuscript.

CHEMISTRY

The sulfonamides **5a–e** were synthesized from aniline **9** using the corresponding sulfonyl chlorides (Scheme 1). The 5-phenylether derivatives **6** and **7a–f** were synthesized from either bromide **10a** or **10b** and a phenol, through either one of two different Ullmann coupling methods⁴⁵ to give intermediates **11a–d**. Use of the more electrophilic trifluoroacetamide **10b** and milder Ullmann coupling conditions⁴⁶ with copper iodide, *N,N*-dimethylglycine, and cesium carbonate in dioxane at 80 °C was the preferred method for generating intermediates **11a–d**. An alternative and more convergent route was utilized

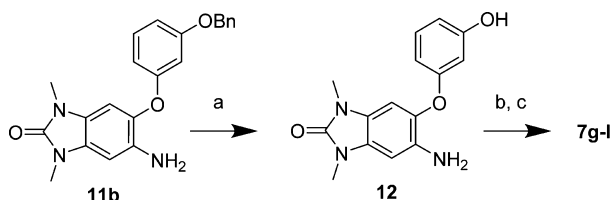
Scheme 1. Synthesis of Sulfonamides **5a–e**, **6**, **7a–f**^a



^aReagents and conditions: (a) sulfonyl chloride, pyr, DCM; (b) Br₂, AcOH, CHCl₃, 0 °C; (c) phenol intermediate, CuCl, quinolin-8-ol, potassium phosphate, diglyme, 120–130 °C; (d) TFA₂O, DMAP, Et₃N, DCM; (e) phenol intermediate, CuI, *N,N*-dimethylglycine, Cs₂CO₃, dioxane, 80 °C; (f) sulfonyl chloride, pyr, DCM.

for the synthesis of **7g–l** via alkylation of the intermediate phenol **12** (Scheme 2). The bidirectional derivatives **8a–c** were

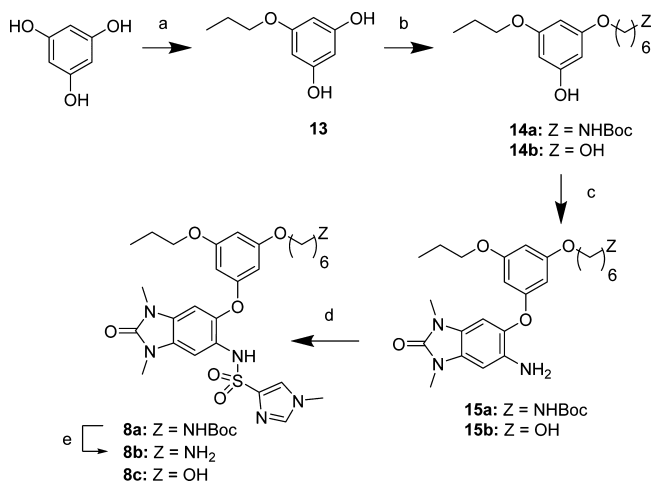
Scheme 2. Synthesis of Sulfonamides **7g–l**^a



^aReagents and conditions: (a) BBr_3 , DCM, -78°C ; (b) R-Br, K_2CO_3 , DMF; (c) sulfonyl chloride, pyr, DCM.

synthesized through iterative alkylation of phloroglucinol to give the bifunctionalized phenols **14a** and **14b**, then Ullmann coupling with **10b** to give intermediates **15a** and **15b**, followed by sulfonamide formation to give **8a** and **8c** (Scheme 3).

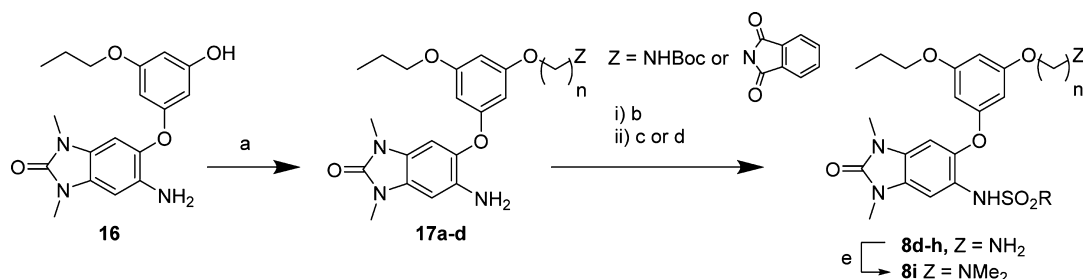
Scheme 3. Synthesis of Derivatives **8a–c**^a



^aReagents and conditions: (a) 1-bromopropane, K_2CO_3 , DMF; (b) $\text{Z}(\text{CH}_2)_6\text{Br}$, K_2CO_3 , DMF; (c) **10b**, CuI, *N,N*-dimethylglycine, Cs_2CO_3 , dioxane, 80°C ; (d) 1-methyl-1*H*-imidazole-4-sulfonyl chloride, pyr, DCM; (e) TFA, DCM.

Alternatively, the advanced phenol intermediate **16** was alkylated and transformed in a similar manner to give amines **8d–h** (Scheme 4). Reductive alkylation of **8h** using formaldehyde and sodium cyanoborohydride gave the dimethylamine **8i**.

Scheme 4. Synthesis of Amines **8d–i**^a



^aReagents and conditions: (a) $\text{Z}(\text{CH}_2)_n\text{Br}$, K_2CO_3 , DMF; (b) sulfonyl chloride, pyr, DCM; (c) TFA, DCM; (d) hydrazine hydrate, MeOH, 80°C ; (e) formaldehyde, AcOH, $\text{NaBH}(\text{OAc})_3$, Et_3N , MeOH.

EXPERIMENTAL SECTION

Synthetic Methods. Column chromatography was performed on a Biotage system using Biotage SNAP columns with Biotage KP-Sil silica or Biotage Zip Si columns with Biotage KP-Sil silica or a Teledyne ISCO system with RediSep Rf normal phase silica cartridges (unless otherwise stated). All NMR spectra were recorded on Bruker instruments operating at 300, 500, or 600 MHz. NMR spectra were obtained as CDCl_3 , CD_3OD , D_2O , $(\text{CD}_3)_2\text{SO}$, $(\text{CD}_3)_2\text{CO}$, C_6D_6 , or CD_3CN solutions (reported in ppm), using tetramethylsilane (0.00 ppm) or residual solvent (CDCl_3 , 7.26 ppm; CD_3OD , 3.31 ppm; D_2O , 4.79 ppm; $(\text{CD}_3)_2\text{SO}$, 2.50 ppm; $(\text{CD}_3)_2\text{CO}$, 2.05 ppm; C_6D_6 , 7.16 ppm; CD_3CN , 1.94 ppm) as the reference standard. When peak multiplicities are reported, the following abbreviations are used: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br-s (broadened singlet), dd (doublet of doublets), dt (doublet of triplets). Coupling constants, when reported, are reported in hertz (Hz). All temperatures are reported in $^\circ\text{C}$. The purity of all final compounds was >95% and was confirmed by LC/MS analysis. Low-resolution mass spectral data were obtained on either a Waters H class UPLC with a Waters Acquity UPLC BEH C18 1.7 μm , 2.1 mm \times 50 mm column, UV detection between 200 and 400 nm, evaporating light scattering detection, and a SQ detector mass spectrometer with ESI ionization or a Water I class UPLC with a Waters Acquity UPLC CSH C18 1.7 μm , 2.1 mm \times 50 mm column, UV detection at 254 and 290 nm, evaporating light scattering detection, and a SQ detector 2 mass spectrometer with ESI ionization. Preparative HPLC was performed using a Waters Autopurify system with a Waters Xbridge Prep C18 5 μm OBD, 19 mm \times 150 mm or 50 mm \times 100 mm column and SQ detector mass spectrometer with ESI ionization. Reagents were purchased from commercial suppliers such as Sigma-Aldrich, Alfa Aesar, TCI, or Acros and were used without further purification unless otherwise indicated. Anhydrous solvents (e.g., THF, DMF, DMA, DMSO, MeOH, DCM, toluene) were purchased from Sigma-Aldrich and used directly. The following compounds were obtained from commercial vendors: **1**, Enamine catalog no. Z118580532; **3b**, Life Chemicals catalog no. F2278-0147; **3c**, Life Chemicals catalog no. F2278-0177; **5f**, ChemDiv catalog no. G433-0348; **5g**, ChemDiv catalog no. G433-0914.

General Procedure for the Synthesis of **2, **3a**, **3d**, and **5a–e** (Method A: Sulfonamide Coupling).** The following commercially available intermediates were used: 5-amino-1-methyl-1,3-dihydro-2*H*-indol-2-one (Combi-Blocks catalog no. ST-1422 for **2**); 6-amino-1-methyl-3,4-dihydroquinolin-2(1*H*)-one (Enamine catalog no. EN300-69848 for **3a** and **3d**); 5-amino-1,3-dimethyl-1*H*-benzo[*d*]imidazol-2(3*H*)-one (Enamine catalog no. EN300-59698 for **5a–e**) (0.1 mmol) in DCM (1 mL), treated with the sulfonyl chloride (0.1–0.20 mmol) and pyridine (20 μL , 0.2 mmol). The reaction mixture was stirred for 2–24 h at ambient temp, then concentrated and purified by mass-directed preparative HPLC to give sulfonamides **2**, **3a**, **3d**, and **5a–e**. **N-(1-Methyl-2-oxo-2,3-dihydro-1*H*-indol-5-yl)-4-(methylsulfonyl)benzene-1-sulfonamide (2).** ^1H NMR (600 MHz, $\text{DMSO}-d_6$) δ 9.95 (br-s, 1H), 7.58 (dt, J = 8.6, 2.0 Hz, 2H), 7.35 (dt, J = 8.6, 2.0 Hz, 2H), 7.01 (s, 1H), 6.93 (dd, J = 8.3, 2.1 Hz,

1H), 6.82 (d, $J = 8.3$ Hz, 1H), 3.48 (s, 2H), 3.03 (s, 3H), 2.99 (s, 3H). MS (ESI) m/z 349 [M + H]⁺.

N-(1-Methyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)-4-(methylsulfanyl)benzene-1-sulfonamide (3a). 23% yield. ¹H NMR (500 MHz, DMSO-*d*₆) δ 10.01 (s, 1H), 7.95 (q, $J = 7$ Hz, 4H), 6.88 (s, 1H), 6.80 (q, $J = 5$ Hz, 1H), 6.67 (d, $J = 6$ Hz, 1H), 3.33 (s, 3H), 3.15 (s, 3H), 2.76 (t, $J = 6$ Hz, 2H), 2.37 (t, $J = 8$ Hz, 2H). MS (ESI) m/z 363 [M + H]⁺.

4-Cyclohexyl-N-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)benzene-1-sulfonamide (3d). 81% yield. ¹H NMR (600 MHz, DMSO-*d*₆) δ 10.10 (s, 1H), 7.65 (d, $J = 8.3$ Hz, 2H), 7.39 (d, $J = 8.3$ Hz, 2H), 6.99–6.91 (m, 3H), 3.15 (s, 3H), 2.75 (t, $J = 6.9$ Hz, 2H), 2.59–2.53 (m, 1H), 2.46 (t, $J = 6.9$ Hz, 2H), 1.81–1.72 (m, 4H), 1.73–1.64 (m, 1H), 1.43–1.29 (m, 4H), 1.27–1.15 (m, 1H). MS (ESI) m/z 399 [M + H]⁺.

Synthesis of N-(7-Methoxy-1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)benzenesulfonamide (3e). *Step 1.* To a solution of 7-hydroxy-3,4-dihydroquinolin-2(1H)-one (1.64 g, 10 mmol) in 30 mL of H₂SO₄ was added water (7.6 mL) dropwise with stirring. The reaction mixture was cooled to 0 °C and HNO₃ (0.76 mL) added dropwise while stirring. The reaction mixture was stirred for 15 min at 0 °C and then quenched by adding reaction mixture to a slurry of 100 mL of H₂O and ice. The resulting solution was extracted with EtOAc (3 × 10 mL) and the combined organic layers were concentrated under reduced pressure to afford 7-hydroxy-6-nitro-3,4-dihydroquinolin-2(1H)-one (1.3 g, 63%) which was used without further purification. MS (ESI) m/z 209 [M + H]⁺.

Step 2. To a solution of 7-hydroxy-6-nitro-3,4-dihydroquinolin-2(1H)-one (1.1 g, 5 mmol) in 10 mL of DMF were added iodomethane (2.5 mL, 20 mmol) and K₂CO₃ (5.47 g, 20 mmol). The suspension was stirred at rt overnight. The reaction mixture was then diluted with water (20 mL) and extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with water (10 mL), brine (10 mL) and then dried over anhydrous sodium sulfate, concentrated under reduced pressure, then the residue was purified by flash chromatography on silica (EtOAc/hexanes = 1:2) to afford 7-methoxy-1-methyl-6-nitro-3,4-dihydroquinolin-2(1H)-one as a yellow solid (950 mg, 81%). MS (ESI) m/z 237 [M + H]⁺.

Step 3. To a solution of 7-methoxy-1-methyl-6-nitro-3,4-dihydroquinolin-2(1H)-one (900 mg, 3.8 mmol) in 30 mL of EtOH and 15 mL of H₂O were added iron powder (640 mg, 11.4 mmol) and ammonium chloride (2.1 g, 38 mmol). The mixture was heated at reflux for 2 h and then cooled to rt. The resulting suspension was filtered through Celite, and the filtrate was diluted with 50 mL of water. The filtrate was extracted with EtOAc (3 × 10 mL) and washed with water (10 mL) and brine (10 mL), dried over anhydrous sodium sulfate, then the combined organic layers were concentrated under reduced pressure to afford a crude product. The residue was purified by flash chromatography (hexanes/EtOAc = 1:1) on silica to afford 6-amino-7-methoxy-1-methyl-3,4-dihydroquinolin-2(1H)-one as a white solid (700 mg, 81%). ¹H NMR (500 MHz, CDCl₃) δ : 6.54 (s, 1H), 6.48 (s, 1H), 3.87 (s, 3H), 3.33 (s, 3H), 2.77–2.74 (m, 2H), 2.61–2.58 (m, 2H). MS (ESI) m/z 207 [M + H]⁺.

Step 4. Sulfonamide formation with 6-amino-7-methoxy-1-methyl-3,4-dihydroquinolin-2(1H)-one using method A afforded 3e as a white solid (100 mg, 58%). ¹H NMR (500 MHz, DMSO) δ : 9.39 (s, 1H), 7.68 (d, $J = 7.0$ Hz, 2H), 7.59 (m, 1H), 7.53 (m, 2H), 6.99 (s, 1H), 6.79 (s, 1H), 3.31 (s, 3H), 3.27 (s, 3H), 3.26 (s, 3H). MS (ESI) m/z 347 [M + H]⁺.

N-(1-Methyl-2-oxo-1,2,3,4-tetrahydroquinolin-7-yl)-4-(methylsulfanyl)benzene-1-sulfonamide (4). *Step 1.* A mixture of 7-amino-3,4-dihydroquinolin-2(1H)-one (100 mg, 0.62 mmol) and phthalic anhydride (110 mg, 0.74 mmol) in AcOH (1 mL) was stirred at 100 °C overnight, cooled to rt, and poured into water, and the resulting suspension was filtered. Collected solids were dried to give 2-(2-oxo-1,2,3,4-tetrahydroquinolin-7-yl)isoindoline-1,3-dione as a white solid (120 mg, 67%). MS (ESI) m/z 293 [M + H]⁺.

Step 2. To a mixture of 2-(2-oxo-1,2,3,4-tetrahydroquinolin-7-yl)isoindoline-1,3-dione (120 mg, 0.41 mmol) and iodomethane (300 mg, 2.05 mmol) in DMF (2 mL) was added K₂CO₃ (113 mg, 0.82

mmol). The mixture was stirred at rt for 48 h. The reaction mixture was diluted with water and the resulting suspension was filtered and collected solids were dried to give 2-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-7-yl)isoindoline-1,3-dione as a white solid (120 mg, 100%). MS (ESI) m/z 307 [M + H]⁺.

Step 3. To a solution of 2-(1-methyl-2-oxo-1,2,3,4-tetrahydroquinolin-7-yl)isoindoline-1,3-dione (120 mg, 0.39 mmol) in MeOH (5 mL) was added hydrazine hydrate (122 mg, 1.95 mmol). The mixture was stirred at rt for 1 h and then concentrated and purified by reverse-phase HPLC to give 7-amino-1-methyl-3,4-dihydroquinolin-2(1H)-one (50 mg, 72%). MS (ESI) m/z 177 [M + H]⁺.

Step 4. Synthesized 4 by method A (28 mg, 45%). ¹H NMR (500 MHz, CDCl₃) δ 7.67 (d, $J = 8.5$ Hz, 2H), 7.23 (d, $J = 9$ Hz, 2H), 6.99 (d, $J = 7.5$ Hz, 1H), 6.99 (br-s, 1H), 6.80 (d, $J = 2.5$ Hz, 1H), 6.60 (dd, $J = 7.5$, 2.5 Hz, 1H), 3.26 (s, 3H), 2.80 (t, $J = 7.5$ Hz, 2H), 2.61 (t, $J = 7.5$ Hz, 2H), 2.49 (s, 3H). MS (ESI) m/z 363 [M + H]⁺.

N-(1,3-Dimethyl-2-oxo-2,3-dihydro-1H-1,3-benzodiazol-5-yl)-4-(methylsulfanyl)benzene-1-sulfonamide (5a). ¹H NMR (500 MHz, MeOD-*d*₄) δ 7.56 (d, $J = 8.5$ Hz, 2H), 7.26 (d, $J = 8.5$ Hz, 2H), 6.93–6.97 (m, 2H), 6.76 (dd, $J = 8.0$, 1.5 Hz, 1H), 3.35 (s, 3H), 3.34 (s, 3H), 2.47 (s, 3H). MS (ESI) m/z 364 [M + H]⁺.

N-(1,3-Dimethyl-2-oxo-2,3-dihydro-1H-1,3-benzodiazol-5-yl)-4-methoxybenzene-1-sulfonamide (5b). 39% yield; ¹H NMR (600 MHz, DMSO-*d*₆) δ 9.87 (s, 1H), 7.62 (d, $J = 8.9$ Hz, 2H), 7.02 (d, $J = 8.9$ Hz, 2H), 6.96 (d, $J = 8.2$ Hz, 1H), 6.86 (d, $J = 1.9$ Hz, 1H), 6.68 (dd, $J = 8.3$, 1.9 Hz, 1H), 3.77 (s, 3H), 3.24 (s, 3H), 3.23 (s, 3H). MS (ESI) m/z 348 [M + H]⁺.

N-(1,3-Dimethyl-2-oxo-2,3-dihydro-1H-1,3-benzodiazol-5-yl)benzenesulfonamide (5c). 53% yield; ¹H NMR (600 MHz, DMSO-*d*₆) δ 10.02 (s, 1H), 7.71–7.68 (m, 2H), 7.60–7.57 (m, 1H), 7.54–7.49 (m, 2H), 6.96 (d, $J = 8.3$ Hz, 1H), 6.86 (d, $J = 1.9$ Hz, 1H), 6.68 (dd, $J = 8.3$, 1.9 Hz, 1H), 3.24 (s, 3H), 3.23 (s, 3H). MS (ESI) m/z 318 [M + H]⁺.

N-(1,3-Dimethyl-2-oxo-2,3-dihydro-1H-1,3-benzodiazol-5-yl)-4-(2-methylpropoxy)benzene-1-sulfonamide (5d). 29% yield; ¹H NMR (600 MHz, DMSO-*d*₆) δ 9.86 (s, 1H), 7.60 (d, $J = 8.9$ Hz, 2H), 7.01 (d, $J = 8.9$ Hz, 2H), 6.96 (d, $J = 8.2$ Hz, 1H), 6.86 (d, $J = 1.9$ Hz, 1H), 6.68 (dd, $J = 8.3$, 1.9 Hz, 1H), 3.76 (d, $J = 6.5$ Hz, 2H), 3.24 (s, 3H), 3.23 (s, 3H), 2.03–1.94 (m, 1H), 0.95 (d, $J = 6.7$ Hz, 6H). MS (ESI) m/z 390 [M + H]⁺.

4-Cyclohexyl-N-(1,3-dimethyl-2-oxo-2,3-dihydro-1H-1,3-benzodiazol-5-yl)benzene-1-sulfonamide (5e). 40% yield; ¹H NMR (600 MHz, DMSO-*d*₆) δ 9.97 (s, 1H), 7.61 (d, $J = 8.3$ Hz, 2H), 7.37 (d, $J = 8.3$ Hz, 2H), 6.97 (d, $J = 8.3$ Hz, 1H), 6.84 (d, $J = 1.9$ Hz, 1H), 6.71 (dd, $J = 8.3$, 1.9 Hz, 1H), 3.24 (s, 3H), 3.22 (s, 3H), 2.57–2.50 (m, 1H), 1.81–1.65 (m, 5H), 1.41–1.28 (m, 4H), 1.25–1.15 (m, 1H). MS (ESI) m/z 400 [M + H]⁺.

5-Amino-6-bromo-1,3-dimethyl-1H-benzo[d]imidazol-2(3H)-one (10a). To a 0 °C solution of 5-amino-1,3-dimethyl-1H-benzo[d]imidazol-2(3H)-one (4.0 g, 23 mmol) in 25 mL of CHCl₃ and 25 mL of AcOH was carefully added bromine (3.5 g, 23 mmol) dropwise. The mixture was stirred at rt for 30 min, then concentrated and purified by silica gel chromatography (pet ether/EtOAc 1:1) to afford 10a as a yellow solid (3.2 g, 69%). ¹H NMR (500 MHz, DMSO-*d*₆) δ 7.18 (s, 1H), 6.59 (s, 1H), 4.96 (s, 2H), 3.22 (s, 3H), 3.21 (s, 3H). MS (ESI) m/z 257 [M + H]⁺.

N-(6-Bromo-1,3-dimethyl-2-oxo-2,3-dihydro-1H-benzo[d]imidazol-5-yl)-2,2,2-trifluoroacetamide (10b). To a 0 °C solution of 10a (1.50 g, 5.9 mmol) in DCM (45 mL) were added DMAP (72 mg, 0.59 mmol), triethylamine (1.63 mL, 11.7 mmol), and trifluoroacetic anhydride (0.91 mL, 6.4 mmol). The reaction mixture was stirred for 2 h and warmed to ambient temperature. The reaction mixture was then quenched with water and the organic phase was washed with brine, dried over sodium sulfate, filtered, and evaporated to give 10b as a yellow solid (2.20 g, 100%). ¹H NMR (600 MHz, DMSO-*d*₆) δ 11.23 (br-s, 1H), 7.57 (s, 1H), 7.31 (s, 1H), 3.34 (s, 3H), 3.32 (s, 3H). MS (ESI) m/z 353 [M + H]⁺.

Representative Procedure for the Synthesis of 6 and 7a–c (Method B: Ullmann Coupling). *Step 1.* A mixture of 3-(benzyloxy)phenol (156 mg, 0.78 mmol), quinolin-8-ol (30 mg, 0.21 mmol), copper(I) chloride (10 mg, 0.10 mmol), potassium phosphate

(200 mg, 0.94 mmol), and **10a** (200 mg, 0.78 mmol) in diglyme (10 mL) was degassed under a nitrogen atmosphere. Then the reaction mixture was heated to 130 °C for 72 h. The cooled reaction mixture was filtered through a pad of silica gel. The collected filtrate was then concentrated and purified by column chromatography (0–100% EtOAc in hexanes and then 0–40% methanol in EtOAc) to give 5-amino-6-(3-(benzyloxy)phenoxy)-1,3-dimethyl-1*H*-benzo[d]imidazol-2(3*H*)-one (**11b**) as a solid (213 mg, 73%). MS (ESI) *m/z* 376 [M + H]⁺. ¹H NMR (600 MHz, CDCl₃) δ 7.34 (m, 5H), 7.18 (t, *J* = 7.8 Hz, 1H), 6.65 (d, *J* = 2.8 Hz, 1H), 6.58 (s, 1H), 6.55 (m, 2H), 6.48 (s, 1H), 4.98 (s, 2H), 3.66 (br-s, 2H), 3.34 (s, 3H), 3.27 (s, 3H).

Step 2. Sulfonamide formation, using method A.

Representative Procedure for the Synthesis of 7d–f (Method C: Ullmann Coupling).⁴⁶ Step 1. A mixture of **10b** (50 mg, 0.14 mmol), 2-(dimethylamino)acetic acid (15 mg, 0.14 mmol), 3-ethoxyphenol (29 mg, 0.21 mmol), copper(I) iodide (8 mg, 0.04 mmol), and cesium carbonate (139 mg, 0.43 mmol) was charged in a flask with dioxane (1 mL). The reaction mixture was heated to 80 °C and stirred for 16 h. The reaction was monitored for complete deprotection of trifluoroacetamide (addition of methanol was used to facilitate this step). The cooled reaction mixture was diluted in methanol, filtered and the filtrate was evaporated and purified by column chromatography (hexanes/EtOAc 1:1) to give 5-amino-6-(3-ethoxyphenoxy)-1,3-dimethyl-1*H*-benzo[d]imidazol-2(3*H*)-one (**11c**) as an orange solid (13 mg, 29%). MS (ESI) *m/z* 314 [M + H]⁺.

Step 2. Sulfonamide formation, using method A.

N-[6-[4-(Benzyloxy)phenoxy]-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-1,3-benzodiazol-5-yl]-1-methyl-1*H*-imidazole-4-sulfonamide (6**).** 6% yield; ¹H NMR (600 MHz, DMSO-*d*₆) δ 9.32 (s, 1H), 7.57 (d, *J* = 3.3 Hz, 2H), 7.46 (d, *J* = 7.5 Hz, 2H), 7.38 (t, *J* = 7.1 Hz, 2H), 7.30 (t, *J* = 6.7 Hz, 1H), 7.08 (s, 1H), 6.95 (d, *J* = 9.5 Hz, 2H), 6.82 (d, *J* = 9.5 Hz, 2H), 6.67 (s, 1H), 5.06 (s, 2H), 3.57 (s, 3H), 3.28 (s, 3H), 3.17 (s, 3H). MS (ESI) *m/z* 520 [M + H]⁺.

N-[6-[3-(Benzyloxy)phenoxy]-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-1,3-benzodiazol-5-yl]-1-methyl-1*H*-imidazole-4-sulfonamide (7a**).** 21% yield; ¹H NMR (600 MHz, DMSO-*d*₆) δ 9.35 (s, 1H), 7.56 (d, *J* = 3.5 Hz, 2H), 7.41 (d, *J* = 7.5 Hz, 2H), 7.36 (t, *J* = 7.1 Hz, 2H), 7.31 (t, *J* = 6.7 Hz, 1H), 7.17 (t, *J* = 8.7 Hz, 1H), 7.09 (s, 1H), 6.75 (s, 1H), 6.70 (dd, *J* = 2.4, 8.3 Hz, 1H), 6.29–6.31 (m, 2H), 5.05 (s, 2H), 3.55 (s, 3H), 3.29 (s, 3H), 3.19 (s, 3H). MS (ESI) *m/z* 520 [M + H]⁺.

N-[6-[3-(Benzyloxy)phenoxy]-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-1,3-benzodiazol-5-yl]-3,4-dimethoxybenzene-1-sulfonamide (7b**).** 13% yield; ¹H NMR (600 MHz, CDCl₃) δ 7.30–7.40 (m, 5H), 7.24 (dd, *J* = 8.3, 2.0 Hz, 1H), 7.06 (m, 2H), 6.82 (s, 1H), 6.70 (d, *J* = 8.6 Hz, 1H), 6.65 (dd, *J* = 8.3, 2.0 Hz, 1H), 6.42 (s, 1H), 6.17 (t, *J* = 2.6 Hz, 1H), 6.05 (dd, *J* = 8.3, 2.0 Hz, 1H), 4.97 (s, 2H), 3.79 (s, 3H), 3.60 (s, 3H), 3.45 (s, 3H), 3.25 (s, 3H). MS (ESI) *m/z* 576 [M + H]⁺.

N-[6-[3-(Benzyloxy)phenoxy]-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-1,3-benzodiazol-5-yl]-1,2-dimethyl-1*H*-imidazole-4-sulfonamide (7c**).** 47% yield; ¹H NMR (600 MHz, DMSO-*d*₆) δ 9.31 (s, 1H), 7.48 (s, 1H), 7.42–7.34 (m, 4H), 7.33–7.29 (m, 1H), 7.18 (t, *J* = 8.3 Hz, 1H), 7.15 (s, 1H), 6.73 (s, 1H), 6.70 (dd, *J* = 8.3, 2.2 Hz, 1H), 6.31–6.26 (m, 2H), 5.04 (s, 2H), 3.42 (s, 3H), 3.29 (s, 3H), 3.19 (s, 3H), 2.07 (s, 3H). MS (ESI) *m/z* 534 [M + H]⁺.

N-[6-(3-Ethoxyphenoxy)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-1,3-benzodiazol-5-yl]-1,2-dimethyl-1*H*-imidazole-4-sulfonamide (7d**).** 36% yield; ¹H NMR (600 MHz, DMSO-*d*₆) δ 9.34 (s, 1H), 7.50 (s, 1H), 7.18–7.14 (m, 2H), 6.76 (s, 1H), 6.61 (dd, *J* = 7.7, 2.2 Hz, 1H), 6.26 (dd, *J* = 8.0, 2.2 Hz, 1H), 6.19 (t, *J* = 2.2 Hz, 1H), 3.96 (q, *J* = 6.9 Hz, 2H), 3.45 (s, 3H), 3.29 (s, 3H), 3.20 (s, 3H), 2.09 (s, 3H), 1.30 (t, *J* = 7.0 Hz, 3H). MS (ESI) *m/z* 472 [M + H]⁺.

N-[1,3-Dimethyl-2-oxo-6-[3-(propoxyphenoxy)-2,3-dihydro-1*H*-1,3-benzodiazol-5-yl]-3,4-dimethoxybenzene-1-sulfonamide (7e**).** 66% yield; ¹H NMR (600 MHz, DMSO-*d*₆) δ 9.51 (s, 1H), 7.19–7.14 (m, 2H), 7.11 (s, 1H), 7.08–7.02 (m, 1H), 6.89 (d, *J* = 9.0 Hz, 1H), 6.74 (s, 1H), 6.55 (dd, *J* = 8.2, 2.2 Hz, 1H), 6.08 (dd, *J* = 8.1, 2.0 Hz, 1H), 6.05–6.03 (m, 1H), 3.79 (t, *J* = 6.5 Hz, 2H), 3.77 (s, 3H), 3.57 (s, 3H), 3.30 (s, 3H), 3.19 (s, 3H), 1.73–1.64 (m, 2H), 0.95 (t, *J* = 7.4 Hz, 3H). MS (ESI) *m/z* 528 [M + H]⁺.

N-[1,3-Dimethyl-2-oxo-6-[3-(propoxyphenoxy)-2,3-dihydro-1*H*-1,3-benzodiazol-5-yl]-1,2-dimethyl-1*H*-imidazole-4-sulfonamide (7f**).** 93% yield; ¹H NMR (600 MHz, DMSO-*d*₆) δ 9.32 (s, 1H), 7.49 (s, 1H), 7.17–7.13 (m, 2H), 6.76 (s, 1H), 6.61 (dd, *J* = 7.7, 2.2 Hz, 1H), 6.25 (dd, *J* = 8.0, 2.2 Hz, 1H), 6.23 (t, *J* = 2.2 Hz, 1H), 3.86 (t, *J* = 6.5 Hz, 2H), 3.45 (s, 3H), 3.29 (s, 3H), 3.20 (s, 3H), 2.08 (s, 3H), 1.73–1.66 (m, 2H), 0.96 (t, *J* = 7.5 Hz, 3H). MS (ESI) *m/z* 486 [M + H]⁺.

5-Amino-6-(3-hydroxyphenoxy)-1,3-dimethyl-1*H*-benzo[d]imidazol-2(3*H*)-one (12**).** To a –78 °C solution of **11b** (400 mg, 1.07 mmol) in DCM (20 mL) was added tribromoborane (5.3 mL, 5.3 mmol). The reaction mixture was allowed to gradually warm to rt, then quenched by the dropwise addition of methanol, concentrated, and purified by column chromatography (20–100% EtOAc/hexanes and then 0–40% methanol/EtOAc) to give **12** as a solid (240 mg, 79%). ¹H NMR (600 MHz, DMSO-*d*₆) δ 9.38 (s, 1H), 7.06 (t, *J* = 8.1, 1H), 6.78 (s, 1H), 6.58 (s, 1H), 6.39 (dd, *J* = 8.0, 2.2 Hz, 1H), 6.33 (dd, *J* = 8.1, 2.3, 1H), 6.23 (t, *J* = 2.3, 1H), 4.57 (br-s, 2H), 3.24 (s, 3H), 3.20 (s, 3H). MS (ESI) *m/z* 286 [M + H]⁺.

General Procedure for the Synthesis of Sulfonamides 7g–i, 7k, 7l. To a solution of **12** (50 mg, 0.18 mmol) in anhydrous DMF (1 mL) were added potassium carbonate (0.2–0.4 mmol) and alkyl bromide (0.18–0.25 mmol). The reaction mixture was stirred at ambient temperature for 1 day, and then the reaction mixture was diluted with water and extracted with EtOAc. The separated organic layer was dried over sodium sulfate, filtered, and concentrated to give the intermediate 5-amino-6-(3-(alkyloxy)phenoxy)-1,3-dimethyl-1*H*-benzo[d]imidazol-2(3*H*)-one as a crude residue which was used without further purification in the sulfonamide formation step using method A to give **7g–i, 7k, 7l**.

N-[1,3-Dimethyl-6-[3-(2-methylpropoxy)phenoxy]-2-oxo-2,3-dihydro-1*H*-1,3-benzodiazol-5-yl]-1,2-dimethyl-1*H*-imidazole-4-sulfonamide (7g**).** 3% yield; ¹H NMR (600 MHz, DMSO-*d*₆) δ 9.27 (s, 1H), 7.47 (s, 1H), 7.17–7.14 (m, 2H), 6.77 (s, 1H), 6.63 (d, *J* = 8.9 Hz, 1H), 6.27 (s, 1H), 6.24 (d, *J* = 8.2 Hz, 1H), 3.69 (d, *J* = 6.5 Hz, 1H), 3.40 (s, 1H), 3.29 (s, 3H), 3.29 (s, 3H), 3.20 (s, 3H), 2.09 (s, 3H), 2.01–1.97 (m, 1H), 0.96 (d, *J* = 6.7 Hz, 6H). MS (ESI) *m/z* 501 [M + H]⁺.

N-[6-(3-Butoxyphenoxy)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-1,3-benzodiazol-5-yl]-1,2-dimethyl-1*H*-imidazole-4-sulfonamide (7h**).** 13% yield; ¹H NMR (600 MHz, DMSO-*d*₆) δ 9.33 (s, 1H), 7.50 (s, 1H), 7.16 (t, *J* = 7.3 Hz, 2H), 6.77 (s, 1H), 6.62 (dd, *J* = 8.3, 2.5 Hz, 1H), 6.25 (m, 2H), 3.91 (t, *J* = 6.4 Hz, 3H), 3.29 (s, 3H), 3.20 (s, 1H), 2.09 (s, 3H), 1.68–1.65 (m, 2H), 1.43–1.39 (m, 2H), 0.93 (t, *J* = 7.5 Hz, 3H). MS (ESI) *m/z* 501 [M + H]⁺.

N-[1,3-Dimethyl-6-[3-(2-methylpropoxy)phenoxy]-2-oxo-2,3-dihydro-1*H*-1,3-benzodiazol-5-yl]cyclopropane-sulfonamide (7i**).** 28% yield; ¹H NMR (600 MHz, DMSO-*d*₆) δ 9.11 (s, 1H), 7.21 (t, *J* = 8.2 Hz, 1H), 7.17 (s, 1H), 6.92 (s, 1H), 6.66–6.63 (m, 1H), 6.53–6.47 (m, 2H), 3.69 (d, *J* = 6.4 Hz, 2H), 3.33 (s, 3H), 3.25 (s, 3H), 1.97 (m, 1H), 0.95 (d, *J* = 6.7 Hz, 6H), 0.85–0.80 (m, 4H). MS (ESI) *m/z* 446 [M + H]⁺.

1,3-Dimethyl-5-[3-(2-methylpropoxy)phenoxy]-2,3-dihydro-1*H*-1,3-benzodiazol-2-one (7j**).** Synthesized from 5-bromo-1,3-dimethyl-1,3-dihydro-2*H*-benzo[d]imidazol-2-one using method C, 32% yield. ¹H NMR (500 MHz, DMSO-*d*₆) δ 7.21 (t, *J* = 8.5 Hz, 1H), 7.14 (d, *J* = 8.5 Hz, 1H), 6.98 (d, *J* = 2.2 Hz, 1H), 6.77 (dd, *J* = 8.4, 2.2 Hz, 1H), 6.65–6.60 (m, 1H), 6.48–6.44 (m, 2H), 3.69 (d, *J* = 6.5 Hz, 2H), 3.33 (s, 3H), 3.29 (s, 3H), 1.97 (m, 1H), 0.94 (d, *J* = 6.7 Hz, 6H). MS (ESI) *m/z* 327 [M + H]⁺.

N-[6-[3-(Methoxypropoxy)phenoxy]-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-1,3-benzodiazol-5-yl]-1,2-dimethyl-1*H*-imidazole-4-sulfonamide (7k**).** 13% yield; ¹H NMR (600 MHz, DMSO-*d*₆) δ 9.35 (s, 1H), 7.50 (s, 1H), 7.18–7.15 (m, 2H), 6.78 (s, 1H), 6.63 (d, *J* = 8.3 Hz, 1H), 6.25 (dd, *J* = 8.4 Hz, 2H), 3.96 (t, *J* = 6.4 Hz, 2H), 3.46–3.43 (m, 5H), 3.29 (s, 3H), 3.23 (s, 3H), 3.20 (s, 3H), 2.09 (s, 3H), 1.92 (m, 2H). MS (ESI) *m/z* 516 [M + H]⁺.

N-[1,3-Dimethyl-2-oxo-6-[3-(oxolan-3-ylmethoxy)phenoxy]-2,3-dihydro-1*H*-1,3-benzodiazol-5-yl]-1-methyl-1*H*-imidazole-4-sulfonamide (7l**).** 5% yield; ¹H NMR (600 MHz, DMSO-*d*₆) δ 9.32 (s, 1H), 7.56 (d, *J* = 3.5 Hz, 2H), 7.17 (t, *J* = 8.7 Hz, 1H), 7.09 (s,

1H), 6.78 (s, 1H), 6.63 (dd, $J = 8.3, 2.4$ Hz, 1H), 6.28 (m, 2H), 3.88 (dd, $J = 9.3, 6.4$ Hz, 1H), 3.83 (t, $J = 7.7$ Hz, 1H), 3.73 (m, 1H), 3.65 (dd, $J = 15.3, 7.4$ Hz, 1H), 3.03 (s, 3H), 3.52 (dd, $J = 8.3, 5.4$ Hz, 1H), 3.27 (s, 3H), 3.20 (s, 3H), 2.63 (m, 1H), 1.99 (m, 1H), 1.64 (m, 1H). MS (ESI) m/z 515 $[M + H]^+$.

Synthesis of *N*-(6-{3-[4-(Dimethylamino)butoxy]phenoxy}-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-1,3-benzodiazol-5-yl)-3,4-dimethoxybenzene-1-sulfonamide (7m). *Step 1.* To a solution of resorcinol (585 mg, 5.32 mmol) in DMF (15 mL) were added K_2CO_3 (735 mg, 5.32 mmol) and 2-(4-bromobutyl)isindoline-1,3-dione (500 mg, 1.772 mmol). The mixture was stirred at 50 °C overnight and then diluted with water (50 mL) and then adjusted to acidic pH with 1 N HCl. The product was extracted with EtOAc (2 × 50 mL), washed with brine (20 mL), dried over Na_2SO_4 , and concentrated. Purification was by silica gel chromatography (0% EtOAc/hexane to 100% EtOAc/hexane). Purification was by prep-HPLC using a gradient of 40–80% ACN/water containing 0.1% TFA to afford 2-(4-(3-hydroxyphenoxy)butyl)isindoline-1,3-dione (263 mg, 48% yield) as a white solid. 1H NMR (600 MHz, DMSO- d_6) δ 9.32 (s, 1H), 7.90–7.80 (m, 4H), 7.05–6.97 (m, 1H), 6.34–6.29 (m, 2H), 6.30–6.25 (m, 1H), 3.90 (t, $J = 6.1$ Hz, 2H), 3.63 (t, $J = 6.6$ Hz, 2H), 1.79–1.65 (m, 4H). MS (ESI) m/z 312 $[M + H]^+$.

Step 2. 2-(4-(3-((6-Amino-1,3-dimethyl-2-methylene-2,3-dihydro-1*H*-benzo[d]imidazol-5-yl)oxy)phenoxy)butyl)isindoline-1,3-dione was prepared from **10b** using method C to give the product as an orange liquid (118 mg, 35% yield). 1H NMR (600 MHz, DMSO- d_6) δ 7.89–7.79 (m, 4H), 7.19–7.12 (m, 1H), 6.79 (s, 1H), 6.58 (s, 1H), 6.55 (dd, $J = 2.2, 8.3$ Hz, 1H), 6.42–6.37 (m, 2H), 4.57 (br-s, 2H), 3.92 (t, $J = 5.9$ Hz, 2H), 3.61 (t, $J = 6.5$ Hz, 2H), 3.24 (s, 3H), 3.20 (s, 3H), 1.76–1.64 (m, 4H). MS (ESI) m/z 487 $[M + H]^+$.

Step 3. *N*-(6-(3-(4-(1,3-Dioxoisindolin-2-yl)butoxy)phenoxy)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[d]imidazol-5-yl)-3,4-dimethoxybenzenesulfonamide was synthesized using method A to give the product as a light-orange liquid (160 mg, 99% yield). 1H NMR (600 MHz, DMSO- d_6) δ 9.53 (s, 1H), 7.90–7.80 (m, 4H), 7.18–7.13 (m, 2H), 7.12 (s, 1H), 7.06–7.00 (m, 1H), 6.87 (d, $J = 9.0$ Hz, 1H), 6.73 (s, 1H), 6.53 (dd, $J = 8.2, 2.1$ Hz, 1H), 6.06 (dd, $J = 7.9, 2.0$ Hz, 1H), 6.06–6.02 (m, 1H), 3.86 (t, $J = 5.9$ Hz, 2H), 3.75 (s, 3H), 3.63 (t, $J = 6.5$ Hz, 2H), 3.56 (s, 3H), 3.30 (s, 3H), 3.18 (s, 3H), 1.78–1.65 (m, 4H). MS (ESI) m/z 687 $[M + H]^+$.

Step 4. To a solution of *N*-(6-(3-(4-(1,3-dioxoisindolin-2-yl)butoxy)phenoxy)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[d]imidazol-5-yl)-3,4-dimethoxybenzenesulfonamide (160 mg, 0.233 mmol) in MeOH (4 mL) was added hydrazine hydrate (29 μ L, 0.47 mmol), and the reaction mixture was heated to 80 °C for 90 min. The cooled reaction mixture was concentrated and then purified by prep-HPLC using a gradient of 20–60% ACN/water containing 0.1% TFA to afford the TFA salt of *N*-(6-(3-(4-aminobutoxy)phenoxy)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[d]imidazol-5-yl)-3,4-dimethoxybenzenesulfonamide (103 mg, 66% yield) as a white solid. 1H NMR (600 MHz, DMSO- d_6) δ 9.52 (s, 1H), 7.68 (br-s, 3H), 7.20–7.15 (m, 2H), 7.09 (s, 1H), 7.09–7.04 (m, 1H), 6.89 (d, $J = 9.0$ Hz, 1H), 6.73 (s, 1H), 6.55 (dd, $J = 8.2, 2.1$ Hz, 1H), 6.12 (dd, $J = 8.2, 2.1$ Hz, 1H), 6.08–6.04 (m, 1H), 3.85 (t, $J = 5.9$ Hz, 2H), 3.77 (s, 3H), 3.59 (s, 3H), 3.30 (s, 3H), 3.19 (s, 3H), 2.89–2.81 (m, 2H), 1.78–1.62 (m, 4H). MS (ESI) m/z 557 $[M + H]^+$.

Step 5. To a solution of *N*-(6-(3-(4-aminobutoxy)phenoxy)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[d]imidazol-5-yl)-3,4-dimethoxybenzenesulfonamide TFA salt (50 mg, 0.075 mmol) in MeOH (3 mL) was added triethylamine (10 μ L, 0.08 mmol), acetic acid (9 μ L, 0.15 mmol), formaldehyde (16 μ L, 0.60 mmol), and sodium triacetoxyborohydride (40 mg, 0.19 mmol). The reaction mixture was stirred at rt for 3 h and then was quenched with a few drops of TFA and concentrated. Purification by prep-HPLC using a gradient of 20–60% acetonitrile/water containing 0.1% TFA afforded the TFA salt of **7m** (14 mg, 27% yield) as a yellow liquid. 1H NMR (600 MHz, DMSO- d_6) δ 9.50 (s, 1H), 9.34 (br-s, 1H), 7.21–7.15 (m, 2H), 7.12–7.05 (m, 2H), 6.89 (d, $J = 9.0$ Hz, 1H), 6.73 (s, 1H), 6.56 (dd, $J = 8.2, 2.0$ Hz, 1H), 6.14 (dd, $J = 8.1, 1.9$ Hz, 1H), 6.09–6.04 (m, 1H), 3.86 (t, $J = 5.9$ Hz, 2H), 3.77 (s, 3H), 3.59 (s, 3H), 3.29 (s, 3H),

3.19 (s, 3H), 3.14–3.06 (m, 2H), 2.77 (d, $J = 4.9$ Hz, 6H), 1.79–1.64 (m, 4H). MS (ESI) m/z 585 $[M + H]^+$.

Synthesis of *tert*-Butyl 6-(3-((1,3-Dimethyl-6-(1-methyl-1*H*-imidazole-4-sulfonamido)-2-oxo-2,3-dihydro-1*H*-benzo[d]imidazol-5-yl)oxy)-5-propoxyphenoxy)hexyl)carbamate (8a). *Step 1.* 5-Propoxybenzene-1,3-diol (**13**). A solution of benzene-1,3,5-triol (1.0 g, 7.9 mmol) in DMF (10 mL) was treated with potassium carbonate powder (1.2 g, 8.7 mmol) and 1-bromopropane (0.80 mL, 8.7 mmol), and the mixture was stirred at 50 °C for 16 h. The cooled reaction mixture was diluted with water, carefully quenched with 1 N HCl until acidic, then extracted with EtOAc. The organic layer was washed with brine and dried over sodium sulfate, concentrated, and purified by column chromatography (EtOAc/hexanes 3:7) to give 5-propoxybenzene-1,3-diol (**13**) as a yellow liquid (0.49 g, 39%). 1H NMR (600 MHz, $CDCl_3$) δ 6.00 (d, $J = 2.1$ Hz, 2H), 5.96 (t, $J = 2.1$ Hz, 1H), 5.48 (br-s, 2H), 3.84 (t, $J = 6.5$ Hz, 2H), 1.80–1.72 (m, 2H), 1.00 (t, $J = 7.4$ Hz, 3H). MS (ESI) m/z 169 $[M + H]^+$.

Step 2. To a solution of **13** (344 mg, 2.05 mmol) and *tert*-butyl (6-bromohexyl)carbamate (268 mg, 0.956 mmol) in 3 mL of DMF was added potassium carbonate (326 mg, 2.36 mmol), and the resulting mixture was stirred at 50 °C for 16 h. The cooled reaction mixture was neutralized with 1 M HCl, diluted with EtOAc, and the separated organic layer was washed with sat. aq NaCl solution, dried over sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified via silica gel chromatography (1:9 to 1:1 EtOAc/hexanes) to give **14a** (174 mg, 23%) as a light-brown viscous liquid. 1H NMR (600 MHz, $CDCl_3$) δ 6.05 (m, 1H), 6.01 (m, 2H), 5.15 (br-s, 1H), 4.53 (br-s, 1H), 3.90 (m, 2H), 3.86 (t, $J = 6.5$ Hz, 2H), 3.12 (m, 2H), 1.81–1.72 (m, 4H), 1.54–1.33 (m, 6H), 1.44 (s, 9H), 1.01 (t, $J = 7.5$ Hz, 3H). MS (ESI) m/z 368 $[M + H]^+$.

Step 3. A solution of **10b** (100 mg, 0.284 mmol), **14a** (147 mg, 0.400 mmol), and 2-(dimethylamino)acetic acid (51 mg, 0.50 mmol) in diethylene glycol dimethyl ether (6 mL) was degassed with nitrogen. Copper(I) iodide (34 mg, 0.18 mmol) and Cs_2CO_3 (410 mg, 1.26 mmol) were added, and the mixture was degassed with nitrogen for an additional 2 min. The reaction mixture was heated to 80 °C for 1.5 days. The cooled reaction mixture was filtered through a pad of Celite, concentrated, then purified by flash chromatography (EtOAc/hexanes 1:9 to 1:1) to give **15a** as a solid (54 mg, 35%). 1H NMR (600 MHz, $CDCl_3$) δ 6.63 (s, 1H), 6.47 (s, 1H), 6.15 (t, $J = 2.1$ Hz, 1H), 6.08 (t, $J = 2.1$ Hz, 1H), 6.06 (t, $J = 2.1$ Hz, 1H), 4.50 (br-s, 1H), 3.87 (t, $J = 6.4$ Hz, 2H), 3.84 (t, $J = 6.6$ Hz, 2H), 3.64 (br-s, 2H), 3.37 (s, 3H), 3.31 (s, 3H), 3.11 (m, 2H), 1.80–1.70 (m, 4H), 1.54–1.34 (m, 6H), 1.44 (s, 9H), 1.00 (t, $J = 7.4$ Hz, 3H). MS (ESI) m/z 543 $[M + H]^+$.

Step 4. Method A gave **8a** as an amorphous solid (68% yield). 1H NMR (600 MHz, $CDCl_3$) δ 7.97 (br-s, 1H), 7.60 (s, 1H, overlapped with $CHCl_3$), 7.35 (s, 1H), 7.31 (s, 1H), 7.20 (s, 1H), 6.57 (s, 1H), 6.14 (s, 1H), 5.77 (br-s, 1H), 5.72 (br-s, 1H), 3.86 (t, $J = 6.4$ Hz, 2H), 3.83 (t, $J = 6.5$ Hz, 2H), 3.62 (s, 3H), 3.45 (s, 3H), 3.31 (s, 3H), 3.12 (br-t, $J = 6.3$ Hz, 2H), 1.76 (m, 4H), 1.51 (m, 2H), 1.46 (m, 2H), 1.44 (s, 9H), 1.37 (m, 2H), 1.01 (t, $J = 7.3$ Hz, 3H). MS (ESI) m/z 687 $[M + H]^+$.

***N*-(6-{3-[(6-Aminoethyl)oxy]-5-propoxyphenoxy}-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-1,3-benzodiazol-5-yl)-1-methyl-1*H*-imidazole-4-sulfonamide (8b).** To a solution of **8a** (18 mg, 0.026 mmol) in DCM (4 mL) was added TFA (500 μ L, 6.49 mmol), and the resulting mixture was stirred at 23 °C for 16 h. The reaction mixture was concentrated to give the TFA salt of **8b** as a brown-colored viscous liquid (17 mg, 93%). 1H NMR (DMSO- d_6) δ 8.52 (br-s, 1H), 7.94 (br-s, 3H), 7.48 (br-s, 1H), 7.24 (s, 1H), 7.18 (s, 1H), 6.56 (s, 1H), 6.11 (s, 1H), 5.85 (s, 1H), 5.56 (s, 1H), 3.89 (t, $J = 6.1$ Hz, 2H), 3.79 (t, $J = 6.6$ Hz, 2H), 3.61 (s, 3H), 3.41 (s, 3H), 3.28 (s, 3H), 2.97 (br-s, 2H), 1.77–1.65 (m, 6H), 1.46 (m, 2H), 1.42 (m, 2H), 0.98 (t, $J = 7.3$ Hz, 3H). MS (ESI) m/z 587 $[M + H]^+$.

Synthesis of *N*-(6-{3-[(6-Hydroxyhexyl)oxy]-5-propoxyphenoxy}-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-1,3-benzodiazol-5-yl)-1-methyl-1*H*-imidazole-4-sulfonamide (8c). *Step 1.* Preparation was in a similar manner to **14a** from **13** using 6-bromohexan-1-ol to give **14b** as a viscous brown liquid (39% yield). 1H NMR (600

MHz, CDCl₃) δ 6.06 (m, 1H), 6.03–5.98 (m, 2H), 5.25 (br-s, 1H), 3.90 (t, J = 6.5 Hz, 2H), 3.86 (t, J = 6.7 Hz, 2H), 3.67 (t, J = 6.5 Hz, 2H), 1.82–1.73 (m, 4H), 1.64–1.57 (m, 2H), 1.52–1.36 (m, 5H), 1.01 (t, J = 7.4 Hz, 3H). MS (ESI) m/z 269 [M + H]⁺.

Steps 2 and 3. Preparation was done using method C to give **15b**, used without further purification. MS (ESI) m/z 444 [M + H]⁺. **15b** was converted to **8c** using method A (38% yield). ¹H NMR (600 MHz, DMSO-*d*₆) δ 9.30 (s, 1H), 7.60 (m, 1H), 7.58 (m, 1H), 7.07 (s, 1H), 6.80 (s, 1H), 6.17 (s, 1H), 5.83 (m, 2H), 4.38 (t, J = 6.5 Hz, 1H), 3.86 (t, J = 6.5 Hz, 2H), 3.83 (t, J = 6.5 Hz, 2H), 3.37 (t, J = 6.5 Hz, 2H), 3.59 (s, 3H), 3.27 (s, 3H), 3.21 (s, 3H), 1.67 (m, 4H), 1.45–1.28 (m, 6H), 0.94 (t, J = 7.5 Hz, 3H). MS (ESI) m/z 588 [M + H]⁺.

Synthesis of N-(6-(3-((6-Aminoethyl)oxy)-5-propoxyphenoxy)-1,3-dimethyl-2-oxo-2,3-dihydro-1H-benzod[imidazol-5-yl]-3,4-dimethoxybenzenesulfonamide (8d). *Step 1.* Preparation was from **13** using method C to give **16** as a brown solid (43% yield). ¹H NMR (600 MHz, DMSO-*d*₆) δ 9.40 (s, 1H), 6.81 (s, 1H), 6.66 (br-s, 1H), 5.99 (s, 1H), 5.93 (s, 1H), 5.85 (s, 1H), 3.80 (t, J = 6.6 Hz, 2H), 3.26 (s, 3H), 3.21 (s, 3H), 1.66 (m, 2H), 0.93 (t, J = 7.5 Hz, 3H). MS (ESI) m/z 344 [M + H]⁺.

Step 2. To a solution of **16** (800 mg, 2.33 mmol) in anhydrous DMF (60 mL) were added potassium carbonate (1.29 g, 9.32 mmol) and 2-(6-bromohexyl)isoindoline-1,3-dione (2.89 g, 9.32 mmol) in a vial. The reaction mixture was stirred at 80 °C for 1 h. To the cooled reaction mixture was added water (50 mL), the aqueous phase was extracted with EtOAc (3 × 50 mL), the combined organic layers were concentrated under reduced pressure, and the residue was purified by silica gel chromatography (EtOAc/hexanes 1:4 to 100% EtOAc) to give 2-(6-(3-((6-amino-1,3-dimethyl-2-oxo-2,3-dihydro-1H-benzod[imidazol-5-yl]oxy)-5-propoxyphenoxy)hexyl)isoindoline-1,3-dione **17a** as a viscous liquid (800 mg, 60%). ¹H NMR (600 MHz, DMSO-*d*₆) δ 7.87–7.82 (m, 4H), 6.83 (s, 1H), 6.69 (br-s, 1H), 6.17 (s, 1H), 6.00 (s, 2H), 3.86 (t, J = 6.2 Hz, 2H), 3.84 (t, J = 6.5 Hz, 2H), 3.56 (t, J = 7.1 Hz, 2H), 3.26 (s, 3H), 3.21 (s, 3H), 1.70–1.56 (m, 6H), 1.39 (m, 2H), 1.31 (m, 2H), 0.93 (t, J = 7.4 Hz, 3H). MS (ESI) m/z 573 [M + H]⁺.

Step 3. To a solution of **17a** (150 mg, 0.262 mmol) in anhydrous DCM (1 mL) were added pyridine (0.042 mL, 0.524 mmol) and 3,4-dimethoxybenzene-1-sulfonyl chloride (74.4 mg, 0.314 mmol). The mixture was stirred for 1 h before quenching with MeOH (0.5 mL). The resulting mixture was treated with hydrazine hydrate (8.22 μ L, 0.262 mmol) and then concentrated and purified by mass-triggered preparative HPLC (mobile phase, A = 0.1% TFA/H₂O, B = 0.1% TFA/ACN; gradient, B = 20–60%, 12 min; column, C18) to give **8d** as a white solid, 30% yield (over 2 steps). ¹H NMR (600 MHz, DMSO-*d*₆) δ 9.44 (s, 1H), 7.61 (s, 2H), 7.19–7.18 (m, 2H), 7.07 (s, 1H), 6.90 (d, J = 8.9 Hz, 1H), 6.75 (s, 1H), 6.11 (s, 1H), 5.69 (s, 1H), 5.66 (s, 1H), 3.81 (t, J = 6.4 Hz, 2H), 3.78–3.75 (m, 5H), 3.35 (s, 3H), 3.29 (s, 3H), 3.20 (s, 3H), 2.80–2.76 (m, 2H), 1.69–1.64 (m, 4H), 1.55–1.52 (m, 2H), 1.40–1.33 (m, 4H), 0.95 (t, J = 7.5 Hz, 3H). MS (ESI) m/z 643 [M + H]⁺.

N-(6-(3-((5-Aminopentyl)oxy)-5-propoxyphenoxy)-1,3-dimethyl-2-oxo-2,3-dihydro-1H-1,3-benzodiazol-5-yl)-1-methyl-1H-imidazole-4-sulfonamide (8e). **8e** was prepared in a similar manner to **8d** except using a BOC-protected intermediate. 27% yield (3 steps). ¹H NMR (600 MHz, DMSO-*d*₆) δ 9.28 (s, 1H), 7.60 (s, 4H), 7.63 (s, 1H), 7.05 (s, 1H), 6.80 (s, 1H), 6.17 (s, 1H), 5.87 (t, J = 2.1 Hz, 2H), 3.91 (t, J = 6.1 Hz, 2H), 3.84 (t, J = 6.5 Hz, 2H), 3.61 (s, 3H), 3.26 (s, 3H), 3.22 (s, 3H), 2.85–2.82 (m, 2H), 1.68 (m, 4H), 1.56 (m, 2H), 1.42 (m, 2H), 0.95 (t, J = 7.3 Hz, 3H). MS (ESI) m/z 573 [M + H]⁺.

N-(6-(3-(4-Aminobutoxy)-5-propoxyphenoxy)-1,3-dimethyl-2-oxo-2,3-dihydro-1H-1,3-benzodiazol-5-yl)-1-methyl-1H-imidazole-4-sulfonamide (8f). **8f** was prepared in a similar manner to **8d**. 50% yield (3 steps). ¹H NMR (600 MHz, DMSO-*d*₆) δ 9.27 (s, 1H), 7.64 (s, 3H), 7.61 (s, 1H), 7.07 (s, 1H), 6.80 (s, 1H), 6.18 (s, 1H), 5.89–5.87 (m, 2H), 3.91 (t, J = 6.1 Hz, 2H), 3.84 (t, J = 6.5 Hz, 2H), 3.61 (s, 3H), 3.27 (s, 3H), 3.22 (s, 3H), 2.85–2.82 (m, 2H), 1.74–1.64 (m, 7H), 0.95 (t, J = 7.3 Hz, 3H). MS (ESI) m/z 559 [M + H]⁺.

N-(6-(3-(4-Aminobutoxy)-5-propoxyphenoxy)-1,3-dimethyl-2-oxo-2,3-dihydro-1H-1,3-benzodiazol-5-yl)-3,4-dimethoxybenzene-1-sulfonamide (8g). **8g** was prepared in a similar manner to **8d**. 26% yield (3 steps). ¹H NMR (600 MHz, DMSO-*d*₆) δ 9.47 (s, 1H), 7.59 (s, 3H), 7.25 (d, J = Hz, 1H), 7.09–7.06 (m, 2H), 7.00 (s, 1H), 6.81 (s, 1H), 6.00 (s, 1H), 5.71 (s, 1H), 5.62 (s, 1H), 3.84 (s, 3H), 3.75 (t, J = 6.5 Hz, 2H), 3.70 (s, 3H), 3.31 (s, 3H), 3.25 (s, 3H), 1.69–1.65 (m, 3H), 1.38–1.35 (m, 2H), 0.95 (t, J = 7.5 Hz, 4H). MS (ESI) m/z 615 [M + H]⁺.

N-(6-(3-(3-Aminopropoxy)-5-propoxyphenoxy)-1,3-dimethyl-2-oxo-2,3-dihydro-1H-1,3-benzodiazol-5-yl)-3,4-dimethoxybenzene-1-sulfonamide (8h). **8h** was prepared in a similar manner to **8d** except using a BOC-protected intermediate. 13% yield (3 steps). ¹H NMR (600 MHz, DMSO-*d*₆) δ 9.44 (s, 1H), 7.68 (br-s, 3H), 7.20–7.17 (m, 2H), 7.06 (s, 1H), 6.90 (d, J = 9.0 Hz, 1H), 6.73 (s, 1H), 6.13 (t, J = 2.1 Hz, 1H), 5.72 (t, J = 2.1 Hz, 1H), 5.67 (t, J = 2.1 Hz, 1H), 3.89 (t, J = 6.1 Hz, 2H), 3.775 (t, J = 6.5 Hz, 2H), 3.770 (s, 3H), 3.61 (s, 3H), 3.29 (s, 3H), 3.20 (s, 3H), 2.95–2.89 (m, 2H), 1.98–1.90 (m, 2H), 1.71–1.63 (m, 2H), 0.94 (t, J = 7.5 Hz, 3H). MS (ESI) m/z 601 [M + H]⁺.

N-(6-(3-(4-(Dimethylamino)butoxy)-5-propoxyphenoxy)-1,3-dimethyl-2-oxo-2,3-dihydro-1H-1,3-benzodiazol-5-yl)-3,4-dimethoxybenzene-1-sulfonamide (8i). To a solution of **8h** TFA salt (180 mg, 0.25 mmol) in methanol (3 mL) were added triethylamine (34 μ L, 0.25 mmol), acetic acid (28 μ L, 0.49 mmol), formaldehyde (0.054 mL, 1.98 mmol), and sodium triacetoxymethylborohydride (131 mg, 0.618 mmol). The reaction mixture was stirred at rt for 3 h and then concentrated under reduced pressure. The residue was purified by prep-HPLC using a gradient of 20–60% ACN/water containing 0.1% TFA to afford the TFA salt of **8i** (106 mg, 57%) as a white solid. ¹H NMR (600 MHz, DMSO-*d*₆) δ 9.46 (s, 1H), 9.30 (br-s, 1H), 7.19 (m, 2H), 7.07 (s, 1H), 6.90 (d, J = 9.0 Hz, 1H), 6.75 (s, 1H), 6.13 (t, J = 2.2 Hz, 1H), 5.71 (t, J = 2.0 Hz, 1H), 5.67 (t, J = 2.0 Hz, 1H), 3.84 (t, J = 5.9 Hz, 2H), 3.77 (m, 5H), 3.62 (s, 3H), 3.29 (s, 3H), 3.20 (s, 3H), 3.12–3.05 (m, 2H), 2.78 (d, J = 4.7 Hz, 6H), 1.77–1.63 (m, 6H), 0.95 (t, J = 7.3 Hz, 3H). ¹³C NMR (600 MHz, DMSO-*d*₆) δ 160.3, 160.0, 159.3, 154.1, 152.0, 148.4, 143.9, 131.8, 128.2, 126.0, 121.9, 120.5, 110.4, 109.4, 106.4, 100.6, 95.9, 95.8, 95.2, 68.9, 66.7, 56.3, 55.6, 55.4, 42.1, 27.1, 27.0, 25.6, 21.9, 20.7, 10.4. MS (ESI) m/z 644 [M + H]⁺.

■ ASSOCIATED CONTENT

Supporting Information

Assay conditions, materials and methods, crystallographic data collection and refinement statistics (Table S1), bromodomain profiling data for **8i** (Table S2), ¹H NMR spectral data for **8i**, and a csv file containing molecular formula strings. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.5b00405.

Accession Codes

The models and structure factors of TRIM24 ligand cocrystal structures have been deposited with the PDB with the following accession codes: **1**, 4YAB; **3b**, 4YAD; **5b**, 4YAT; **5g**, 4YAX; **7b**, 4YBM; **7g**, 4YBS; **7l**, 4YBT; **8i**, 4YC9.

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

ACN, acetonitrile; AcOH, acetic acid; Alpha, amplified luminescence proximity homogeneous assay; BET, bromodomain and extra-terminal; BCP, bromodomain-containing protein; BOC, *tert*-butyl carbonate; BRD4, bromodomain containing protein 4; BRPF1, bromodomain and plant homeodomain finger containing 1; DCM, dichloromethane; DMF, dimethylformamide; EtOAc, ethyl acetate; H-bond, hydrogen bond; HTS, high throughput screening; ITC, isothermal calorimetry; KAc, acetylated lysine; PHD, plant homeodomain; rt, room temperature; SAR, structure–activity relationship; SEM, standard error of the mean; SUMO, small-ubiquitin-like modifier; TBAF, tetrabutylammonium fluoride; TFA, trifluoroacetic acid; TRIM24, tripartite motif containing protein 24

REFERENCES

- (1) Filippakopoulos, P.; Knapp, S. The bromodomain interaction module. *FEBS Lett.* **2012**, *586*, 2692–2704.
- (2) Filippakopoulos, P.; Picaud, S.; Mangos, M.; Keates, T.; Lambert, J. P.; Baryte-Lovejoy, D.; Felletar, I.; Volkmer, R.; Muller, S.; Pawson, T.; Gingras, A. C.; Arrowsmith, C. H.; Knapp, S. Histone recognition and large-scale structural analysis of the human bromodomain family. *Cell* **2012**, *149*, 214–231.
- (3) Filippakopoulos, P.; Qi, J.; Picaud, S.; Shen, Y.; Smith, W. B.; Fedorov, O.; Morse, E. M.; Keates, T.; Hickman, T. T.; Felletar, I.; Philpott, M.; Munro, S.; McKeown, M. R.; Wang, Y.; Christie, A. L.; West, N.; Cameron, M. J.; Schwartz, B.; Heightman, T. D.; La Thangue, N.; French, C. A.; Wiest, O.; Kung, A. L.; Knapp, S.; Bradner, J. E. Selective inhibition of BET bromodomains. *Nature* **2010**, *468*, 1067–1073.
- (4) Picaud, S.; Da Costa, D.; Thanasopoulou, A.; Filippakopoulos, P.; Fish, P. V.; Philpott, M.; Fedorov, O.; Brennan, P.; Bunnage, M. E.; Owen, D. R.; Bradner, J. E.; Taniere, P.; O'Sullivan, B.; Muller, S.; Schwaller, J.; Stankovic, T.; Knapp, S. PFI-1, a highly selective protein interaction inhibitor, targeting BET Bromodomains. *Cancer Res.* **2013**, *73*, 3336–3346.
- (5) Fish, P. V.; Filippakopoulos, P.; Bish, G.; Brennan, P. E.; Bunnage, M. E.; Cook, A. S.; Fedorov, O.; Gerstenberger, B. S.; Jones, H.; Knapp, S.; Marsden, B.; Nock, K.; Owen, D. R.; Philpott, M.; Picaud, S.; Primiano, M. J.; Ralph, M. J.; Sciammetta, N.; Trzuppek, J. D. Identification of a chemical probe for bromo and extra C-terminal bromodomain inhibition through optimization of a fragment-derived hit. *J. Med. Chem.* **2012**, *55*, 9831–9837.
- (6) SGC. Epigenetics Probes Collection. <http://www.thesgc.org/chemical-probes/epigenetics>.
- (7) Mirguet, O.; Gosmini, R.; Toum, J.; Clement, C. A.; Barnathan, M.; Brusq, J. M.; Mordaunt, J. E.; Grimes, R. M.; Crowe, M.; Pineau, O.; Ajakane, M.; Dugan, A.; Jeffrey, P.; Cutler, L.; Haynes, A. C.; Smithers, N. N.; Chung, C. W.; Bamborough, P.; Uings, I. J.; Lewis, A.; Witherington, J.; Parr, N.; Prinjha, R. K.; Nicodeme, E. Discovery of epigenetic regulator I-BET762: lead optimization to afford a clinical candidate inhibitor of the BET bromodomains. *J. Med. Chem.* **2013**, *56*, 750 1–7515.
- (8) Filippakopoulos, P.; Knapp, S. Targeting bromodomains: epigenetic readers of lysine acetylation. *Nat. Rev. Drug Discovery* **2014**, *13*, 337–356.
- (9) Garnier, J. M.; Sharp, P. P.; Burns, C. J. BET bromodomain inhibitors: a patent review. *Expert Opin. Ther. Pat.* **2014**, *24*, 185–199.
- (10) Gallenkamp, D.; Gelato, K. A.; Haendler, B.; Weinmann, H. Bromodomains and their pharmacological inhibitors. *ChemMedChem* **2014**, *9*, 438–464.
- (11) Mirguet, O.; Lamotte, Y.; Donche, F.; Toum, J.; Gellibert, F.; Bouillot, A.; Gosmini, R.; Nguyen, V. L.; Delannee, D.; Seal, J.; Blandel, F.; Boullay, A. B.; Boursier, E.; Martin, S.; Brusq, J. M.; Krysa, G.; Riou, A.; Tellier, R.; Costaz, A.; Huet, P.; Dudit, Y.; Trotter, L.; Kirilovsky, J.; Nicodeme, E. From ApoA1 upregulation to BET family bromodomain inhibition: discovery of I-BET151. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 2963–2967.
- (12) McLure, K. G.; Gesner, E. M.; Tsujikawa, L.; Kharenko, O. A.; Attwell, S.; Campeau, E.; Wasiak, S.; Stein, A.; White, A.; Fontano, E.; Suto, R. K.; Wong, N. C.; Wagner, G. S.; Hansen, H. C.; Young, P. R. RVX-208, an inducer of ApoA-I in humans, is a BET bromodomain antagonist. *PLoS One* **2013**, *8*, e83190.
- (13) Josling, G. A.; Selvarajah, S. A.; Petter, M.; Duffy, M. F. The role of bromodomain proteins in regulating gene expression. *Genes* **2012**, *3*, 320–343.
- (14) Demont, E. H.; Bamborough, P.; Chung, C. W.; Craggs, P. D.; Fallon, D.; Gordon, L. J.; Grandi, P.; Hobbs, C. I.; Hussain, J.; Jones, E. J.; Le Gall, A.; Michon, A. M.; Mitchell, D. J.; Prinjha, R. K.; Roberts, A. D.; Sheppard, R. J.; Watson, R. J. 1,3-Dimethyl benzimidazolones are potent, selective inhibitors of the BRPF1 bromodomain. *ACS Med. Chem. Lett.* **2014**, *5*, 1190–1195.
- (15) Hay, D. A.; Fedorov, O.; Martin, S.; Singleton, D. C.; Tallant, C.; Wells, C.; Picaud, S.; Philpott, M.; Monteiro, O. P.; Rogers, C. M.; Conway, S. J.; Rooney, T. P.; Tumber, A.; Yapp, C.; Filippakopoulos, P.; Bunnage, M. E.; Muller, S.; Knapp, S.; Schofield, C. J.; Brennan, P. E. Discovery and optimization of small-molecule ligands for the CBP/p300 bromodomains. *J. Am. Chem. Soc.* **2014**, *136*, 9308–9319.
- (16) Picaud, S.; Strocchia, M.; Terracciano, S.; Lauro, G.; Mendez, J.; Daniels, D. L.; Riccio, R.; Bifulco, G.; Bruno, I.; Filippakopoulos, P. The 9H-purine scaffold reveals induced-fit pocket plasticity of the BRD9 bromodomain. *J. Med. Chem.* **2015**, *58*, 2718–2736.
- (17) Vangamudi, B.; Paul, T. A.; Shah, P. K.; Kost-Alimova, M.; Nottebaum, L.; Shi, X.; Zhan, Y.; Leo, E.; Mahadeshwar, H. S.; Protopopov, A.; Futreal, A.; Tieu, T. N.; Peoples, M.; Heffernan, T. P.; Marszalek, J. R.; Toniatti, C.; Petrocchi, A.; Verhelle, D.; Owen, D. R.; Draetta, G.; Jones, P.; Palmer, W. S.; Sharma, S.; Andersen, J. N. The SMARCA2/4 ATPase domain surpasses the bromodomain as a drug target in SWI/SNF mutant cancers: Insights from cDNA rescue and PFI-3 inhibitor studies. *Cancer Res.* **2015**, *10.1158/0008-5472.CAN-14-3798*.
- (18) Vidler, L. R.; Brown, N.; Knapp, S.; Hoelder, S. Druggability analysis and structural classification of bromodomain acetyl-lysine binding sites. *J. Med. Chem.* **2012**, *55*, 7346–7359.
- (19) Ferguson, F. M.; Fedorov, O.; Chaikuad, A.; Philpott, M.; Muniz, J. R. C.; Felletar, I.; von Delft, F.; Heightman, T.; Knapp, S.; Abell, C.; Ciulli, A. Targeting low-druggability bromodomains: fragment based screening and inhibitor design against the BAZ2B bromodomain. *J. Med. Chem.* **2013**, *56*, 10183–10187.
- (20) Drouin, L.; McGrath, S.; Vidler, L. R.; Chaikuad, A.; Monteiro, O.; Tallant, C.; Philpott, M.; Rogers, C.; Fedorov, O.; Liu, M.; Akhtar, W.; Hayes, A.; Raynaud, F.; Müller, S.; Knapp, S.; Hoelder, S. Structure enabled design of BAZ2-ICR, a chemical probe targeting the bromodomains of BAZ2A and BAZ2B. *J. Med. Chem.* **2015**, *58*, 2553–2559.

- (21) Harner, M. J.; Chauder, B. A.; Phan, J.; Fesik, S. W. Fragment-based screening of the bromodomain of ATAD2. *J. Med. Chem.* **2014**, *57*, 9687–9692.
- (22) Chaikuad, A.; Petros, A. M.; Fedorov, O.; Xu, J.; Knapp, S. Structure-based approaches towards identification of fragments for the low-druggability ATAD2 bromodomain. *MedChemComm* **2014**, *5*, 1843–1848.
- (23) Poncet-Montange, G.; Zhan, Y.; Bardenhagen, J. P.; Petrocchi, A.; Leo, E.; Shi, X.; Lee, G. R.; Leonard, P. G.; Geck Do, M. K.; Cardozo, M. G.; Andersen, J. N.; Palmer, W. S.; Jones, P.; Ladbury, J. E. Observed bromodomain flexibility reveals histone peptide- and small molecule ligand-compatible forms of ATAD2. *Biochem. J.* **2015**, *466*, 337–346.
- (24) Carlson, S.; Glass, K. C. The MOZ histone acetyltransferase in epigenetic signaling and disease. *J. Cell. Physiol.* **2014**, *229*, 1571–1574.
- (25) Brown, T.; Swansbury, J.; Taj, M. M. Prognosis of patients with t(8;16)(p11;p13) acute myeloid leukemia. *Leuk. Lymphoma* **2012**, *53*, 338–341.
- (26) Lubula, M. Y.; Eckenroth, B. E.; Carlson, S.; Poplawski, A.; Chruszcz, M.; Glass, K. C. Structural insights into recognition of acetylated histone ligands by the BRPF1 bromodomain. *FEBS Lett.* **2014**, *588*, 3844–3854.
- (27) Hatakeyama, S. TRIM proteins and cancer. *Nat. Rev. Cancer* **2011**, *11*, 792–804.
- (28) Marin, I. Origin and diversification of TRIM ubiquitin ligases. *PLoS One* **2012**, *7*, e50030.
- (29) Chu, Y.; Yang, X. SUMO E3 ligase activity of TRIM proteins. *Oncogene* **2011**, *30*, 1108–1116.
- (30) Herquel, B.; Ouararhni, K.; Davidson, I. The TIF1 α -related TRIM cofactors couple chromatin modifications to transcriptional regulation, signaling and tumor suppression. *Transcription* **2011**, *2*, 231–236.
- (31) Le Douarin, B.; Zechel, C.; Garnier, J. M.; Lutz, Y.; Tora, L.; Pierrat, P.; Heery, D.; Gronemeyer, H.; Chambon, P.; Losson, R. The N-terminal part of TIF1, a putative mediator of the ligand-dependent activation function (AF-2) of nuclear receptors, is fused to B-raf in the oncogenic protein T18. *EMBO J.* **1995**, *14*, 2020–2033.
- (32) Tsai, W. W.; Wang, Z.; Yiu, T. T.; Akdemir, K. C.; Xia, W.; Winter, S.; Tsai, C. Y.; Shi, X.; Schwarzer, D.; Plunkett, W.; Aronow, B.; Gozani, O.; Fischle, W.; Hung, M. C.; Patel, D. J.; Barton, M. C. TRIM24 links a non-canonical histone signature to breast cancer. *Nature* **2010**, *468*, 927–932.
- (33) Allton, K.; Jain, A. K.; Herz, H. M.; Tsai, W. W.; Jung, S. Y.; Qin, J.; Bergmann, A.; Johnson, R. L.; Barton, M. C. Trim24 targets endogenous p53 for degradation. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 11612–11616.
- (34) Chambon, M.; Orsetti, B.; Berthe, M. L.; Bascoul-Mollevis, C.; Rodriguez, C.; Duong, V.; Gleizes, M.; Thénot, S.; Bibeau, F.; Theillet, C.; Cavaillès, V. Prognostic significance of TRIM24/TIF-1 α gene expression in breast cancer. *Am. J. Pathol.* **2011**, *178*, 1461–1469.
- (35) Cui, Z.; Cao, W.; Li, J.; Song, X.; Mao, L.; Chen, W. TRIM24 overexpression is common in locally advanced head and neck squamous cell carcinoma and correlates with aggressive malignant phenotypes. *PLoS One* **2013**, *8*, e63887.
- (36) Li, H.; Sun, L.; Tang, Z.; Fu, L.; Xu, Y.; Li, Z.; Luo, W.; Qiu, X.; Wang, E. Overexpression of TRIM24 correlates with tumor progression in non-small cell lung cancer. *PLoS One* **2012**, *7*, e37657.
- (37) Liu, X.; Huang, Y.; Yang, D.; Li, X.; Liang, J.; Lin, L.; Zhang, M.; Zhong, K.; Liang, B.; Li, J. Overexpression of TRIM24 is associated with the onset and progress of human hepatocellular carcinoma. *PLoS One* **2014**, *9*, e85462.
- (38) Zhang, L. H.; Yin, A. A.; Cheng, J. X.; Huang, H. Y.; Li, X. M.; Zhang, Y. Q.; Han, N.; Zhang, X. TRIM24 promotes glioma progression and enhances chemoresistance through activation of the PI3K/Akt signaling pathway. *Oncogene* **2014**, *34*, 1–11.
- (39) Bennett, J. M.; Federov, O.; Tallant, C.; Monteiro, O. P.; Meier, J.; Gamble, V.; Savitski, P.; Nunez-Alonso, G. A.; Haendler, B.; Rogers, C.; Brennan, P. E.; Müller, S.; Knapp, S. Discovery of a chemical tool inhibitor targeting the bromodomains of TRIM24 and BRPF. *J. Med. Chem.* **2015**, DOI: 10.1021/acs.jmedchem.5b00458.
- (40) Irwin, J. J.; Shoichet, B. K. ZINC—a free database of commercially available compounds for virtual screening. *J. Chem. Inf. Model.* **2005**, *45*, 177–182.
- (41) Philpott, M.; Yang, J.; Tumber, T.; Federov, O.; Uttarkar, S.; Filippakopoulos, P.; Picaud, S.; Keates, T.; Felletar, I.; Ciulli, A.; Knapp, S.; Heightman, T. D. Bromodomain-peptide displacement assays for interactome mapping and inhibitor discovery. *Mol. Biosyst.* **2011**, *7*, 2899–2908.
- (42) Hewings, D. S.; Federov, O.; Filippakopoulos, P.; Martin, S.; Picaud, S.; Tumber, A.; Wells, C.; Olcina, M. M.; Freeman, K.; Gill, A.; Ritchie, A. J.; Sheppard, D. W.; Russell, A. J.; Hammond, E. M.; Knapp, S.; Brennan, P. E.; Conway, S. J. Optimization of 3,5-dimethylisoxazole derivatives as potent bromodomain ligands. *J. Med. Chem.* **2013**, *56*, 3217–3227.
- (43) Hewings, D. S.; Rooney, T. P.; Jennings, L. E.; Hay, D. A.; Schofield, C. J.; Brennan, P. E.; Knapp, S.; Conway, S. J. Progress in the development and application of small molecule inhibitors of bromodomain-acetyl-lysine interactions. *J. Med. Chem.* **2012**, *55*, 9393–9413.
- (44) Fabian, M. A.; Biggs, W. H., 3rd; Treiber, D. K.; Atteridge, C. E.; Azimioara, M. D.; Benedetti, M. G.; Carter, T. A.; Ciceri, P.; Edeen, P. T.; Floyd, M.; Ford, J. M.; Galvin, M.; Gerlach, J. L.; Grotzfeld, R. M.; Herrgard, S.; Insko, D. E.; Insko, M. A.; Lai, A. G.; Lelias, J. M.; Mehta, S. A.; Milanov, Z. V.; Velasco, A. M.; Wodicka, L. M.; Patel, H. K.; Zarrinkar, P. P.; Lockhart, D. J. A small molecule-kinase interaction map for clinical kinase inhibitors. *Nat. Biotechnol.* **2005**, *23*, 329–336.
- (45) Monnier, F.; Taillefer, M. Catalytic C-C, C-N, and C-O Ullmann-type coupling reactions. *Angew. Chem., Int. Ed.* **2009**, *48*, 6954–6971.
- (46) Cai, Q.; Zou, B.; Ma, D. Mild Ullmann-type biaryl ether formation reaction by combination of ortho-substituent and ligand effects. *Angew. Chem., Int. Ed.* **2006**, *45*, 1276–1279.