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GNE-371, a Potent and Selective Chemical Probe for the Second Bromodomains of Human Transcription-Initiation-Factor TFIID Subunit 1 and Transcription-Initiation-Factor TFIID Subunit 1-like

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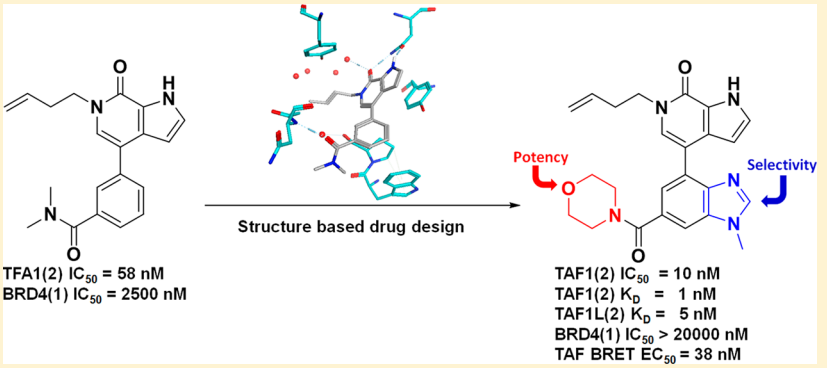
Supporting Information



ABSTRACT: The biological functions of the dual bromodomains of human transcription-initiation-factor TFIID subunit 1 (TAF1(1,2)) remain unknown, although TAF1 has been identified as a potential target for oncology research. Here, we describe the discovery of a potent and selective in vitro tool compound for TAF1(2), starting from a previously reported lead. A cocrystal structure of lead compound **2** bound to TAF1(2) enabled structure-based design and structure−activity-relationship studies that ultimately led to our in vitro tool compound, **27** (GNE-371). Compound **27** binds TAF1(2) with an IC_{50} of 10 nM while maintaining excellent selectivity over other bromodomain-family members. Compound **27** is also active in a cellular-TAF1(2) target-engagement assay (IC_{50} = 38 nM) and exhibits antiproliferative synergy with the BET inhibitor JQ1, suggesting engagement of endogenous TAF1 by **27** and further supporting the use of **27** in mechanistic and target-validation studies.

INTRODUCTION

Bromodomains are protein modules that serve as epigenetic readers. These modules, consisting of ∼110 amino acids, specifically recognize acetylated lysine residues on histone tails and play important roles in chromatin-structural changes and transcriptional regulation.[1−3] Bromodomain-containing proteins have been implicated in cancer, inflammation, and viral replication; therefore, it is important to understand the potential phenotypes produced by inhibition of these bromodomains.[4−8] Although a number of inhibitors of the bromodomain and extra terminal (BET) family and non-BET bromodomains have been disclosed recently,[9−20] a highly selective inhibitor of the second bromodomain of the human transcription-initiation-factor TFIID subunit 1 (TAF1(2)) has not been identified to date. Herein, we report our discovery of a TAF1(2)-bromodomain inhibitor, which will be a valuable tool compound for investigations into the pharmacological relevance of non-BET bromodomain proteins.

TAF1 contains both a histone acetyltransferase (HAT) domain and a tandem bromodomain module, comprising the individual bromodomains TAF1(1) and TAF1(2).[21] Although the function of TAF1 has not been fully elucidated, it has been implicated in pathways associated with cancer.[22] Recently, Sdelci et al. showed that TAF1(2) synergizes with bromodomain-containing protein 4 (BRD4) in controlling

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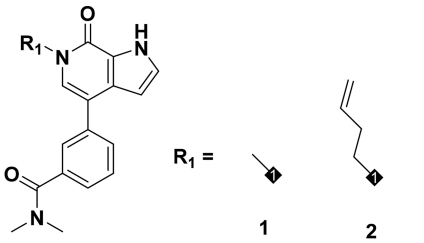
cancer-cell proliferation, making TAF1(2) a potentially promising oncology target.[23] In addition, TAF1(2) is capable of recognizing not only acetyl lysine but also butyryl and crotonyl lysines, although the biological significance of this unusual property of TAF1(2) is currently unknown.[24] A potent and selective inhibitor of TAF1(2) would help clarify the TAF1(2) bromodomain function, which would not be possible through full-protein-knockdown experiments. This inhibitor may also aid the development of novel therapeutic agents. Herein we report such an inhibitor of TAF1(2) that has favorable biochemical properties, is potent in a cellular target-engagement assay, and is suitable for use as a chemical probe for TAF1(2).

RESULTS AND DISCUSSION

Recently, we reported modulation of the selectivity of a pyrrolopyridone scaffold for various bromodomains by varying the hydrophobic substituent on the pyridone nitrogen.[25,26] Replacing the methyl on the pyrrolopyridone, **1**, with a 1-butenyl group (compound **2**) exploited the ability of TAF1(2) to bind to butyrylated and crotonylated lysine residues[24,25] and greatly improved the selectivity over a small set of bromodomains while maintaining TAF1(2) activity (Table 1). This modest change to the inhibitor rearranged and

Table 1. Comparison of *N*-Butenyl (2) and *N*-Methyl (1) Substitutions on the Pyridone Nitrogen of the Pyrrolopyridone Core



	1	2
TAF1(2) IC_{50} (μM)	0.059	0.046
TAF1(1) IC_{50} (μM)	3.6	9.9
BRD4(1) IC_{50} (μM)	0.092	2.5
BRD4(2) IC_{50} (μM)	0.065	5.5
BRD9 IC_{50} (μM)	0.23	1.4
CECR2 IC_{50} (μM)	0.24	4.8
CBP IC_{50} (μM)	0.56	>20

stabilized a conserved water network in the binding site, providing an excellent starting point for further optimization to increase both potency against TAF1(2) while also improving the bromodomain-selectivity profile, particularly against BET-family members.[25]

The *N*-butenyl group maintains potency against TAF1(2) and improved selectivity against the other tested bromodomains. All bromodomain assays were run in the TR-FRET format as described in the Supporting Information. All IC_{50} data are reported as means of values from at least two determinations.

The X-ray cocrystal structure of **2** in complex with the second bromodomain of TAF1 (PDB code 5I1Q) reveals that the pyrrolopyridone core makes a 2-point hydrogen-bond interaction with the highly conserved asparagine residue (Asn1583), which is also important for recognition of histone acetyl lysine (KAc, Figure 1A).[25] The gatekeeper residue, Tyr1589, forms a π-stacking interaction with the pyrrolopyridone; the 4-phenyl extends into a hydrophobic region called the ZA channel,[27,28] interacting with residues that form a lipophilic region known as the WPF motif,[28] which includes van der Waals contacts with Pro1527 and Phe1528 and an edge-to-face interaction with Trp1526.[26] The sterically hindered amide positions the carbonyl out of plane, resulting in a direct hydrogen bond to the backbone NH of Asn1533 of the ZA channel. The dimethyl amine occupies an open space above the WPF shelf that provides an opportunity for further hydrophobic interactions by extending deeper into the pocket (Figure 1B).

We also previously observed that the benzamide of compound **1** bound to BRD4(1) had a flipped conformation, directed away from the BRD4(1) WPF shelf and instead into the ZA channel toward the solvent (Figure 2).[25] The phenyl ring maintains hydrophobic contacts to the WPF shelf; however, the π−π edge-to-face interaction is not observed, nor is a hydrogen-bond interaction to the carbonyl. Nevertheless, the dimethyl substituents can form hydrophobic contacts with the gatekeeper Ile146 to retain potency.

Initial attempts to improve potency for TAF1(2) targeted substituents of the benzamide and the phenyl ring. From the structure of compound **2** bound to TAF1(2), we hypothesized that bulkier substitutions on the benzamide could lead to increased potency by improving the hydrophobic protein−surface interactions in that region (Figure 1B). We therefore explored a limited set of benzamide substitutions on the phenyl ring (Table 2). Replacement of the dimethyl amine with larger hydrophobic substituents, such as diethyl amine (**3**), pyrrolidine (**4**), or *N*-methylcyclohexylamine (**5**), had little effect on TAF1(2) potency; however, these substituents did result in a 3−5-fold erosion of selectivity against BRD4(1). Introducing a basic amine (compounds **6** and **7**) was unfavorable for TAF1(2) potency and selectivity over BRD4(1), BRD9, and especially CECR2. The morpholine amide (**8**) at this position slightly increased TAF1(2) potency, although BRD4(1) selectivity remained unaffected. From the cocrystal structure of **8** bound to TAF1(2), we observe that the morpholine ether does not make a direct interaction and instead hydrogen-bonds to a water molecule approaching the solvent interface. However, the ring optimally fills the accessible volume in the lipophilic-shelf region, which may account for the slight potency improvement (Figure 3A). As the benzamide of compound **2** is flipped when bound to BRD4(1), further amide substituents alone would be well-tolerated by BRD4(1) and would not result in improved selectivity for TAF1(2).

Having found an increase in potency with the morpholine amide, we next explored disubstituted phenyl analogues. We hypothesized that increased steric bulk might be well-tolerated in the TAF1(2) ZA channel (Figure 3B), while also resulting in unfavorable interactions within the smaller BRD4(1) binding pocket (Figure 2). The 5-fluoro (**9**) and 5-amino (**10**) analogues showed no improvement in potency toward TAF1(2), whereas **10** slightly lost selectivity against BRD4(1) (Table 3). Adding a larger $OCHF_2$ substituent (**11**) showed a 4-fold increase in potency compared with that of **8** but again resulted in increased affinity for BRD4(1), whereas compound **12** (5-CN) was equipotent to **8** for TAF1(2) and BRD4(1). However, both **11** and **12** were significantly more potent

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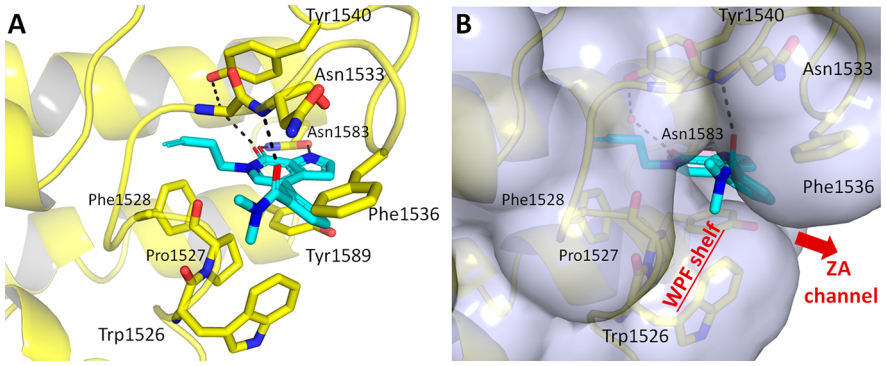

Figure 1. (A) Co-crystal structure of compound **2** (cyan) bound to TAF1(2) (PDB code 5I1Q, 1.5 Å resolution).[25] TAF1(2) residues are in yellow. The pyrrolopyridone core makes two strong hydrogen bonds with the conserved Asn1583, and the butenyl group extends into the water channel. (B) Carbonyl of the benzamide forming a hydrogen bond with the NH of Asn1533.

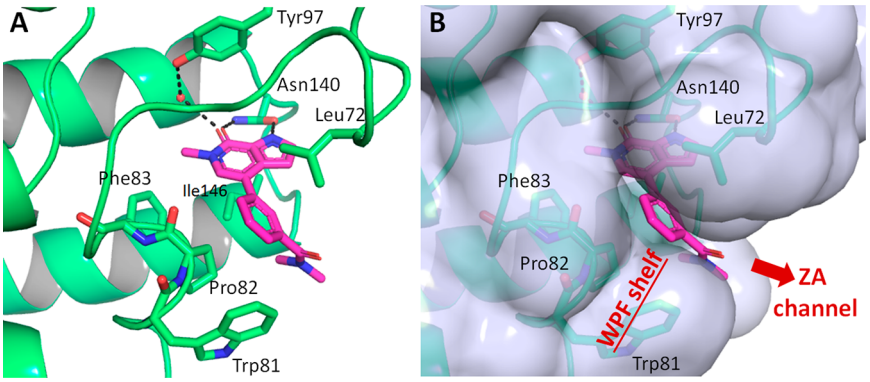

Figure 2. (A) Compound **1** (magenta) bound to BRD4(1) (residues in salmon; PDB code 5I80, 1.5 Å resolution).[25] (B) Protein surface in gray, with the accessible region near the WPF shelf.

against CECR2, having submicromolar affinity. Substitution at the 4-position with a fluorine (**13**), chlorine (**14**), methyl (**15**), or 4,5-difluoro (**16**) provided at least a 5-fold decrease in potency for BRD4(1), whereas the TAF1(2) potency was maintained. We believe the improved selectivity for TAF1(2) over BRD4(1) for compound **15** (142-fold) compared with that of **8** (43-fold) may be the result of the methyl forcing the benzamide orthogonal to the phenyl ring rather than at the 50° angle observed for compound **1** in Figure 2A, resulting in a negative interaction with the protein surface. Incorporation of a pyridine nitrogen at position 5 on the phenyl ring (compound **17**) improved TAF1(2) potency and also provided improved selectivity over BRD4(1) (200-fold) relative to that of **8** (43-fold). This is most likely due to the flipped conformation of the benzamide in BRD4(1), where the pyridine nitrogen is facing the lipophilic WPF-shelf region, resulting in a desolvation penalty. Addition of a methyl (**18**) at position 4 of the pyridine maintained potency against TAF1(2) while increasing the selectivity window over BRD4(1) (~1250-fold). Investigation at the 4-position further with methoxy (**19**) and ethoxy groups (**20**) greatly increased TAF1(2) affinity, with single-digit nanomolar potency observed. Unfortunately, these substitutions failed to further improve selectivity over the bromodomain-family members in our internal bromodomain panel.

Although compounds **19** and **20** met our initial goal of 100-fold selectivity, further efforts continued with the aim of obtaining an even greater selectivity window over BRD4 (IC_{50} > 20 μM) to ensure that any observed cellular phenotypes were not compromised by BET inhibition. To achieve this, we explored trisubstituted-phenyl-ring systems to induce steric clashes in the BRD4 binding site. First we designed a number of benzimidazole analogues to not only investigate steric effects but also explore the potential of improving the edge-to-face interaction with Trp1526. Compound **21** had similar potency for TAF1(2) compared with that of **8**, and had slightly improved selectivity over BRD4(1) (Table 4). The *N*-methyl regioisomers (**22** and **23**) had similar TAF1(2) potencies to that of **21** and resulted in better selectivity over BRD4, but their selectivity over CECR2 fell ~6-fold. Increasing the steric bulk with the ethyl compounds (**24** and **25**) did not result in appreciable selectivity gains. We hypothesized that making the benzimidazole regioisomer, **26**, might introduce a hydrogen bond with the gatekeeper Tyr1589 in TAF1(2). This could both improve potency for TAF1(2) and also selectivity as a result of modeled clashes with the sterically hindered β-branched gatekeeper residues Ile146 and Val147 in BRD4(1) and BRD4(2), respectively. Surprisingly, analogue **26** was equipotent to compound **8** in the TAF1(2) assay, but perhaps more importantly, **26** had significantly lower affinity for BRD4 and BRD9. We probed whether locking this compound in the preferred tautomer would enable a stronger interaction to the gatekeeper Try1589 and increase TAF1(2) potency. Compound **27** resulted in 3-fold-improved TAF1(2) potency compared with that of **26**, further increased BRD4 selectivity (>2000-fold), and improved selectivity for the other bromodomains screened. The cocrystal structure of **27** confirms that the benzimidazole ring is indeed making a hydrogen-bond interaction with Tyr1589 (Figure 4).

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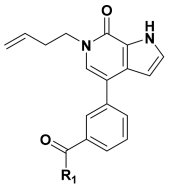
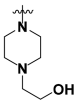
Table 2. Structure–Activity Relationships of Phenylamides



Compound	R_1	IC_{50} (μM)[a] TAF1(2)	TAF1(1)	BRD4(1)	BRD4(2)	BRD9	CECR2	CBP
2		0.046	9.9	2.5	5.5	1.4	4.8	>20
3		0.046	17	0.69	1.5	1.8	2.9	>20
4		0.035	16	0.76	1.9	1.0	4.7	>20
5		0.067	13	0.75	2.4	1.7	4.0	>20
6		0.089	9.5	1.5	5.0	0.90	0.55	>20
7		0.083	14	3.2	5.0	0.89	1.1	>20
8		0.023	11 (470x)	1.0 (43x)	6.9 (300x)	1.5 (65x)	3.3 (143x)	>20 (870x)

[a]All IC_{50} data are reported as means of values from at least two determinations.

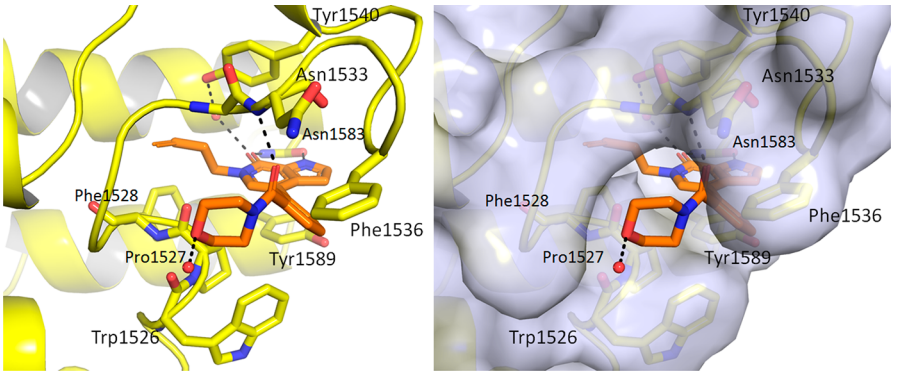


Figure 3. (A) Cocrystal structure of compound **8** (orange) bound to TAF1(2) (PDB code 6DF4, 1.3 Å resolution). The morpholine amide occupies significant volume on the WPF shelf. (B) Accessible volume extending from compound **8** off of the aryl ring.

The bromodomain selectivity of compound **27** was further assessed by screening against 40 bromodomain-family members in a BROMOscan panel (see Supporting Information Table S3 for full data profile).[29] Exquisite selectivity for **27** was observed, with only BRD4 (full length protein, K_D = 8900 nM), CECR2 (K_D = 1200 nM), and BRD9 (K_D = 3400 nM) potencies below 10 μM, whereas the potencies for TAF1(2) and its homologue transcription-initiation-factor TFIID subunit 1-like TAF1L(2) were very strong (1 and 5 nM, respectively). Compound **27** was also screened at 1 μM in a diverse panel of 35 kinases, with no inhibition >17% observed (Table S4).

To confirm cellular permeability and assess the target-engagement potential of **27**, we made use of nanoBRET assays.[30] Treatment of cells transfected with nLuc–TAF1(2) with a fluorescent, tagged ligand resulted in a BRET signal that could be diminished by competition with the test compounds. In this assay, **27** showed an IC_{50} of 0.038 μM (Figure 5A). Compound selectivity was determined by measuring displacement of a tracer compound from BRD4. BET inhibitor JQ1 demonstrated a potency of 0.3 μM, whereas **27** failed to displace tracer even at the highest test concentration (20 μM, Figure 5B), indicating a cellular selectivity of >500-fold for TAF1(2) over BRD4, consistent with the high biochemical selectivity observed.

Screening of a diverse panel of 393 cancer-cell lines for sensitivity to **27** at different doses (up to 20 μM, 8 day assay) revealed little to no effect on cell viability for any tested line (not shown). However, positive interactions between TAF1 and BRD4 in mediating transcriptional activation have been reported recently.[23] In particular, a cellular screen for small molecules capable of activating expression of a reporter gene

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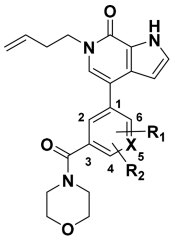
Table 3. Structure-Activity Relationships of Morpholine Amides



				IC_{50} (μM)[a]					
Compound	R_1	R_2	X	TAF1(2)	TAF1(1)	BRD4(1)	BRD4(2)	BRD9	CECR2
8	H	H	C	0.023	11	1.0	6.9	1.5	3.3
9	5-F	H	C	0.017	15	1.2	5.0	1.6	2.2
10	5-NH2	H	C	0.026	4.3	0.48	4.0	0.88	2.3
11	5-OCHF2	H	C	0.006	6.1	0.60	9.0	0.96	0.70
12	5-CN	H	C	0.015	8.1	1.8	5.0	3.0	0.35
13	4-F	H	C	0.010	9.6	5.1	11	1.4	1.8
14	4-Cl	H	C	0.006	9.7	5.0	5.0	0.86	2.0
15	4-Me	H	C	0.035	15	5.0	5.0	1.0	5.0
16	5-F	4-F	C	0.011	11	7.9	17	2.2	2.1
17	H	H	N	0.016	11	3.2	5.0	2.1	0.83
18	4-Me	H	N	0.013	9.3	16	13	2.0	1.7
19	4-OMe	H	N	0.007	6.9	8.6	9.6	1.7	1.0
20	4-OEt	H	N	0.004	5.3	6.3	7.6	1.6	0.59

[a]All IC_{50} data are reported as means of values from at least two determinations.

sensitive to BRD4 inactivation yielded not only known BET-bromodomain inhibitors as hits but also novel compounds, some of which were not BET-bromodomain binders but instead targeted TAF1(2). Synergistic loss of viability of H23 lung-cancer cells was observed when cells were treated with combinations of JQ1 and a TAF1(2) inhibitor, CeMME13, optimized for selectivity.[23] Because of the higher potency of **27** compared with that of CeMME13, we carried out a similar combination study with JQ1 and **27** to verify the earlier finding (Figure 6). Enhanced sensitivity to JQ1 can be seen for concentrations of **27** as low as ∼100 nM, consistent with the potency of **27** in the target engagement assay. The drug combination shows modest synergy in a Bliss model (overall score = 15, maximum score = 43; Figure S1). The excellent biochemical potency, selectivity, physical properties, and observed cellular activity led us to designate compound **27** as GNE-371, a chemical probe for further biological evaluation of TAF1 bromodomain 2.

Chemistry. Compounds **3**−**20** were made from the previously reported pyrrolopyridone intermediate **28**,[25] either via coupling of an aryl benzamide boronic acid or ester (Scheme 1) or by Suzuki coupling to form the benzoic acid intermediate followed by amide coupling (Scheme 2).

Preparation of compounds **21**−**25** began with an amide coupling with 2-amino-3-nitrobenzoic acid (**30**) and morpholine to form **31** (Scheme 3). After bromination to afford **32** and iron reduction of the nitro group, the diamine (**33**) was cyclized with triethoxymethane to give the benzimidazole (**34**). After SEM protection and borylation, Suzuki coupling with **28** was followed by SEM deprotection with TFA. Either tosyl deprotection or N-alkylation of the benzimidazole followed by tosyl deprotection resulted in compounds **21**−**25**.

The preparation of compounds **26** and **27** began with reduction of methyl 4-amino-3-bromo-5-nitrobenzoate (**38**) to the dianiline (**39**) with tin chloride (Scheme 4). Cyclization with triethoxymethane afforded benzimidazole (**40**), which was then converted to the boronic acid (**41**). Suzuki coupling with 4-bromo-6-(but-3-en-1-yl)-1-tosyl-1*H*-pyrrolo[2,3-*c*]-pyridin-7(6*H*)-one (**28**) yielded ester **42**. Intermediate **42** was then either saponified to the acid followed by amide coupling to yield compound **26** or alkylated with methyl iodide to yield the desired *N*-methylbenzimidazole regioisomer (**43**) as the major product. Saponification of **43** followed by amide coupling led to compound **27**.

CONCLUSION

In summary, we have identified a highly potent and selective in vitro probe (**27**) of the TAF1(2) bromodomain through a structure-based-design approach. On the basis of the potency and exquisite selectivity profile and observed activity in cellular engagement and phenotypic assays, compound **27** will be suitable in interrogating the biology of TAF1(2) without phenotypic contamination from BET inhibition.

EXPERIMENTAL PROCEDURES

General Methods. All solvents and reagents were used as obtained. NMR analysis was performed in a deuterated solvent with a Varian Avance 300 MHz or Bruker Avance 400 or 500 MHz NMR spectrometer and referenced to trimethylsilane (TMS). Chemical shifts are expressed as δ units using TMS as the external standard. The

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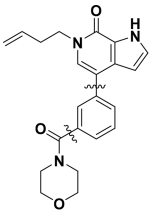
Table 4. Structure-Activity Relationships of Benzimidazoles



Compound	R	IC_{50} (μM)[a] TAF1(2)	TAF1(1)	BRD4(1)	BRD4(2)	BRD9	CECR2
8		0.023	11	1.0	6.9	1.5	3.3
21		0.016	5.9	2.3	4.4	2.2	0.82
22		0.010	7.0	3.6	4.4	1.4	0.23
23		0.016	8.4	8.2	12	0.75	0.36
24		0.016	>20	2.0	1.0	2.4	2.6
25		0.006	6.8	3.4	6.8	0.62	0.59
26		0.028	>20	>20	>20	18	1.2
27		0.010	>20 (>2000x)	>20 (>2000x)	>20 (>2000x)	9.5 (950x)	1.2 (120x)

[a]All IC_{50} data are reported as means of values from at least two determinations.

following abbreviations were used in the NMR descriptions: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; and br, broad peak. All coupling constants (*J*) are reported in hertz. Mass spectra were measured with a Finnigan SSQ710C spectrometer using an ESI source coupled to a Waters 600MS high-performance-liquid-chromatography (HPLC) system operating in reverse-phase mode with an X-bridge Phenyl column of dimensions 150 × 2.6 mm, with 5 μm sized particles. Preparatory-scale silica-gel chromatography was performed using medium-pressure liquid chromatography (MPLC) on a CombiFlash Companion (Teledyne ISCO) with RediSep normal-phase silica-gel (35−60 μm) columns and UV detection at 254 nm. Reverse-phase (HPLC) was used to purify compounds as needed by elution from a Phenomenex Gemini-NX C18 column (20.2 × 50 mm, 5 μm) stationary phase, using the mobile phase indicated and operating at a 35 mL/min flow rate on a Waters 3100 mass-directed prep instrument. Chemical purities were >95% for all final compounds as assessed by LC-MS analysis. The following analytical method was used to determine chemical purities of final compounds: an HPLC-Agilent 1200, water with 0.05% TFA, acetonitrile with 0.05% TFA (buffer B), and an Agilent SB-C18 (1.8 μM, 2.1 × 30 mm) were used at 25 °C with a gradient of 3−95% buffer B in 8.5 min and 95% in 2.5 min (400 μL/min; 220 and 254 nm; Agilent quadrupole 6140, ESI positive, 90−1300 amu).

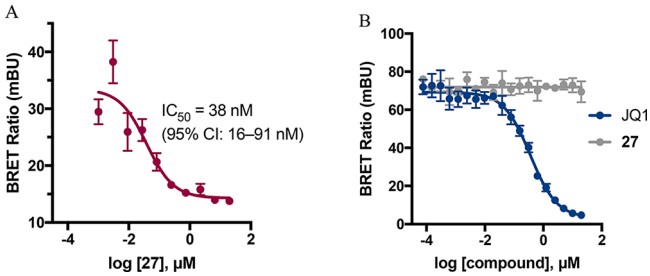


Figure 5. Cellular target-engagement assays. Each concentration point was measured in triplicate and is shown as the mean ± SD. IC_{50} values were determined by fitting to a 4-parameter binding equation in Prism 7 (GraphPad Software). (A) Compound **27** tested in a nanoBRET TAF1(2) assay. The uncertainly in the IC_{50} is indicated by the 95% confidence interval (CI) of the fit. (B) JQ1 and **27** tested in a nanoBRET BRD4 assay (Promega N2131) using the full-length nLuc−BRD4 construct supplied by the manufacturer. JQ1 IC_{50} in this experiment is 0.36 μM.

Time-Resolved-Fluorescence-Resonance-Energy-Transfer Assays. Compound potencies were evaluated in a panel of biochemical bromodomain-binding assays. Binding of biotinylated small-molecule ligands to recombinant His-tagged bromodomains was assessed by time-resolved fluorescence-resonance-energy transfer (TR-FRET) as previously described.[24,25]

3-(6-(But-3-en-1-yl)-7-oxo-6,7-dihydro-1*H*-pyrrolo[2,3-*c*]-pyridin-4-yl)-*N*,*N*-diethylbenzamide (3). To a solution of 4-bromo-6-(but-3-en-1-yl)-1,6-dihydro-7*H*-pyrrolo[2,3-*c*]pyridin-7-one **29** (45 mg, 0.17 mmol) and 3-(6-but-3-enyl-7-oxo-1*H*-pyrrolo[2,3-*c*]pyridin-4-yl)-*N*,*N*-diethyl-benzamide (45 mg, 0.25 mmol) in acetonitrile (1 mL), and 1 M potassium carbonate in water (1 mL) was added $Pd(dppf)Cl_2$ (14 mg, 0.02 mmol). The reaction was stirred

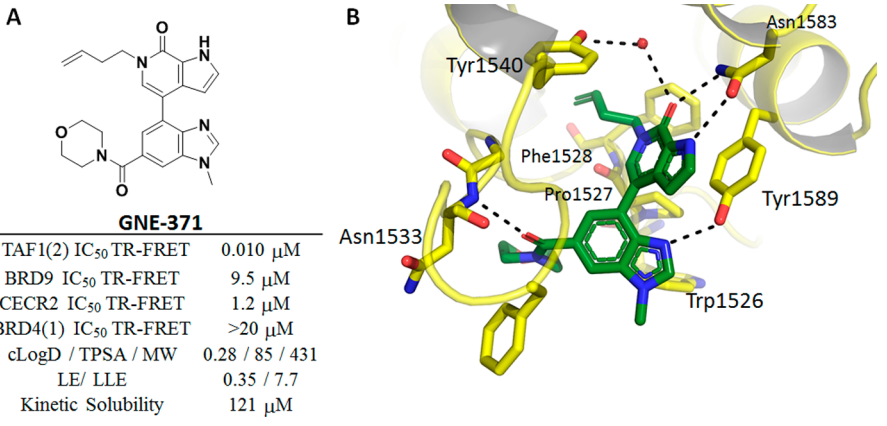


Figure 4. (A) Profile of compound **27** (GNE-371). All bromodomain assays were run in TR-FRET format. IC_{50} data are an average of at least two independent experiments. LE = $-RT \ln(IC_{50})/N_{heavy\ atoms}$. LLE = pIC_{50} − cLogP. (B) Cocrystal structure of compound **27** (green) bound to TAF1(2) (PDB code 6DF7, 2.0 Å resolution).

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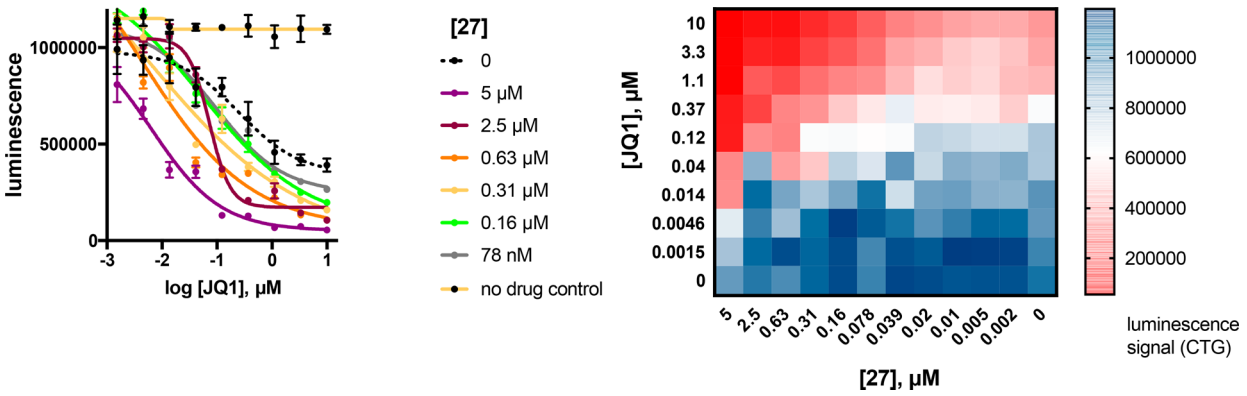
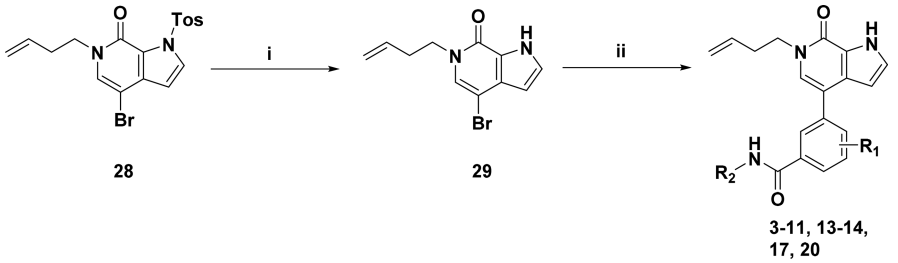


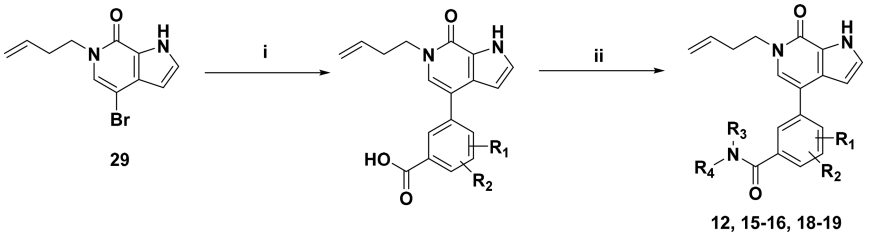
Figure 6. H23 lung-cancer cells treated with combinations of JQ1 and **27**. Viability was measured by luminescence after 48 h with CellTiter-Glo 2.0 reagent (Promega). Dose−response curves for indicated concentrations of **27** are shown on the left. An expanded set of the data from the same experiment is shown in heatmap format on the right.

Scheme 1. Synthesis of Compounds 3−11, 13, 14, 17, and 20[a]



[a]Reagents and conditions: (i) MeOH, 10 M KOH solution, 50 °C, 77%; (ii) R-B(OH)$_2$, Pd(dppf)Cl$_2$, CH$_3$CN, 1 M K$_2$CO$_3$ in water solution, 100 °C.

Scheme 2. Synthesis of Compounds 12, 15, 16, 18, and 19[a]



[a]Reagents and conditions: (i) R$_1$R$_2$-B(OH)$_2$, Pd(dppf)Cl$_2$, CH$_3$CN, 1 M K$_2$CO$_3$ in water solution, 140 °C; (ii) R$_3$R$_4$NH, HATU, DIPEA, DMF.

at 100 °C for 10 min under microwave irradiation. The mixture was filtered through a pad of Celite, using ethyl acetate (20 mL) to rinse the cake. The filtrate was washed with brine (10 mL), dried over sodium sulfate, and concentrated in vacuo. The residue was purified by preparative HPLC (25−35% ACN/0.1% NH_4OH in H_2O) to afford 3-(6-(but-3-en-1-yl)-7-oxo-6,7-dihydro-1*H*-pyrrolo[2,3-*c*]-pyridin-4-yl)-*N*,*N* diethylbenzamide (17 mg, 28% yield) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.13 (s, 1H), 7.72−7.61 (m, 1H), 7.57−7.48 (m, 2H), 7.41 (s, 1H), 7.38−7.27 (m, 2H), 6.42 (d, J = 2.8 Hz, 1H), 5.86 (ddt, J = 17.1, 10.2, 6.8 Hz, 1H), 5.13−4.97 (m, 2H), 4.12 (dd, J = 7.9, 6.5 Hz, 2H), 3.45−3.20 (m, 4H), 2.48−2.40 (m, 2H), 1.12 (s, 6H). LC-MS: m/z 364 (M+H)$^+$.

6-(But-3-en-1-yl)-4-(3-(pyrrolidine-1-carbonyl)phenyl)-1,6-dihydro-7*H*-pyrrolo[2,3-*c*]pyridin-7-one (4). Compound **4** was prepared in the same manner as shown for compound **3** except (3-(pyrrolidine-1-carbonyl)phenyl)boronic acid was used instead. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.12 (s, 1H), 7.78 (dp, J = 3.8, 1.9 Hz, 1H), 7.72−7.65 (m, 2H), 7.58−7.33 (m, 3H), 6.43 (dd, J = 2.8, 1.9 Hz, 1H), 5.86 (m, 1H), 5.13−4.98 (m, 2H), 4.12 (t, J = 7.3 Hz, 2H), 3.46 (m, 4H), 2.50−2.46 (m, 2H), 1.86 (m, 4H). LC-MS: m/z 362 (M+H)$^+$.

3-(6-(But-3-en-1-yl)-7-oxo-6,7-dihydro-1*H*-pyrrolo[2,3-*c*]-pyridin-4-yl)-*N*-cyclohexyl-*N*-methylbenzamide (5). Compound **5** was prepared in the same manner as shown for compound **3** except (3-(cyclohexyl(methyl)carbamoyl)phenyl)boronic acid was used instead. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.13 (s, 1H), 7.70−7.62 (m, 1H), 7.56−7.48 (m, 2H), 7.43−7.34 (m, 2H), 7.30 (d, J = 7.6 Hz, 1H), 6.41 (m, J = 2.9, 1.5 Hz, 1H), 5.86 (m, 1H), 5.14−4.98 (m, 2H), 4.12 (m, 2H), 3.25 (s, 3H). 2.98−2.71 (m, 4H), 2.50−2.47 (m, 2H), 1.62 (d, J = 37.3 Hz, 4H), 1.02−0.90 (m, 2H). LC-MS: m/z 404 (M+H)$^+$.

6-(But-3-en-1-yl)-4-(3-(4-methylpiperazine-1-carbonyl)-phenyl)-1,6-dihydro-7*H*-pyrrolo[2,3-*c*]pyridin-7-one (6). Compound **6** was prepared in the same manner as shown for compound **3** except (3-(4-methylpiperazine-1-carbonyl)phenyl)boronic acid was used instead. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.13 (s, 1H), 7.68 (dt, J = 7.9, 1.3 Hz, 1H), 7.57−7.51 (m, 2H), 7.41 (s, 1H), 7.37 (d, J = 2.8 Hz, 1H), 7.33 (dt, J = 7.6, 1.3 Hz, 1H), 6.42 (d, J = 2.8 Hz, 1H), 5.86 (m, 1H), 5.16−4.98 (m, 2H), 4.20−4.12 (m, 2H), 3.75−3.52 (m, 4H), 2.41−2.26 (m, 6H), 2.20 (s, 3H). LC-MS: m/z 391 (M+H)$^+$.

6-(But-3-en-1-yl)-4-(3-(4-(2-hydroxyethyl)piperazine-1-carbonyl)phenyl)-1,6-dihydro-7*H*-pyrrolo[2,3-*c*]pyridin-7-one

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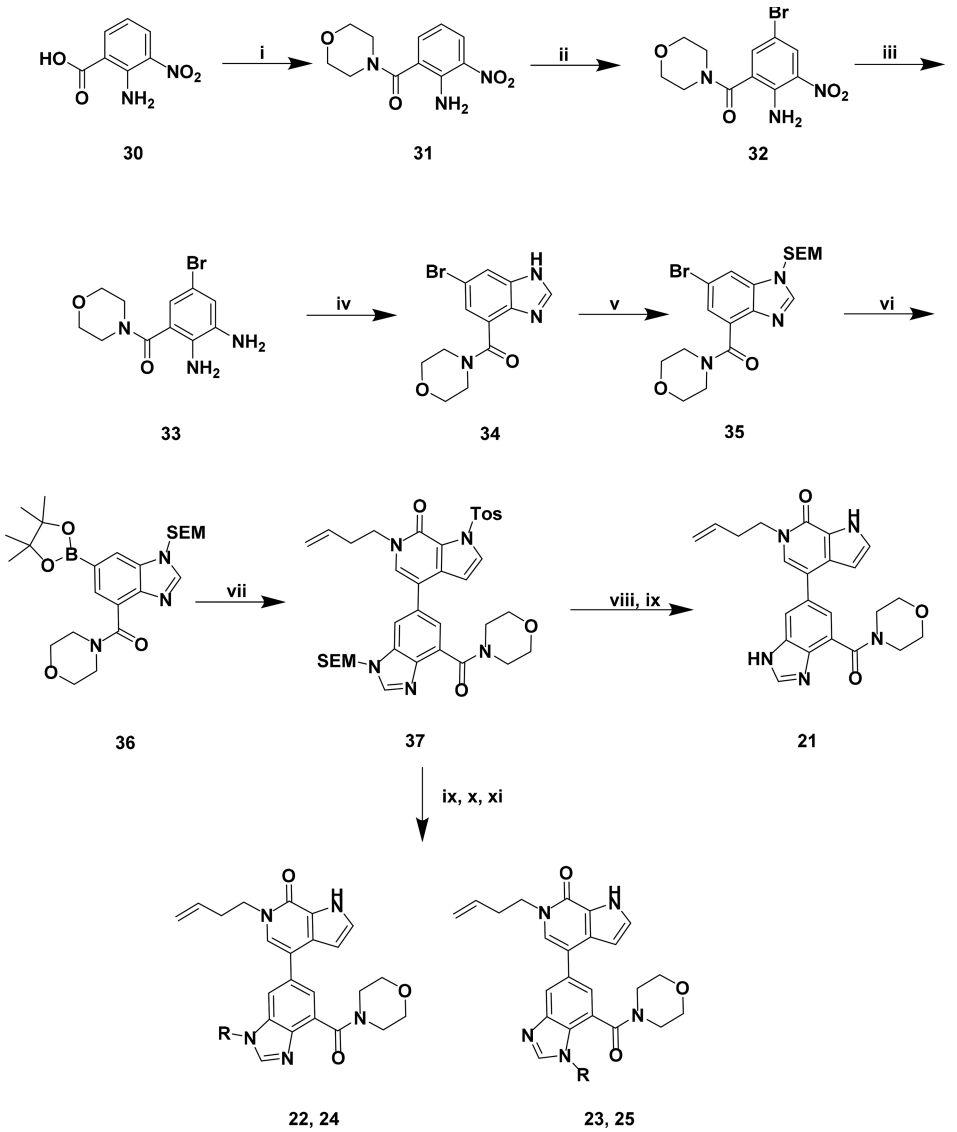
Scheme 3. Synthesis of Compounds 21−25[a]



[a]Reagents and conditions: (i) morpholine, HATU, DIPEA, DMF, 73%; (ii) bromine, acetic acid, 0 °C, 76%; (iii) Fe powder, NH_4Cl, EtOH, H_2O, reflux, 92%; (iv) triethoxymethane, TsOH, rt, 76%; (v) SEM-Cl, NaH, DMF, 0 °C, 70%; (vi) bis(pinacolato)diboron, Pd(dppf)Cl_2, KOAc, dioxane, 110 °C, 41% (crude); (vii) **28**, Pd(dppf)Cl_2, Cs_2CO_3, dioxane, H_2O, 110 °C, 77%; (viii) NaOH, dioxane, H_2O, 80 °C, 5%; (ix) TFA, DCM, rt, 98%; (x) NaH, R-I, DMF, 0 °C; (xi) NaOH, dioxane, H_2O, 80 °C.

(7). Compound **7** was prepared in the same manner as shown for compound **3** except (3-(4-(2-hydroxyethyl)piperazine-1-carbonyl)-phenyl)boronic acid was used instead. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.14 (s, 1H), 7.68 (dt, J = 7.8, 1.4 Hz, 1H), 7.61−7.49 (m, 2H), 7.46−7.30 (m, 3H), 6.42 (dd, J = 2.9, 1.6 Hz, 1H), 5.95−5.77 (m, 1H), 5.14−4.97 (m, 2H), 4.48−4.42 (m, 1H), 4.30−4.12 (t, J = 7.2 Hz, 2H), 3.70−3.62 (m, 2H), 3.45−3.39 (m, 2H), 2.45−2.38 (m, 2H). LC-MS: m/z 421 $(M+H)^+$.

6-(But-3-en-1-yl)-4-(3-(morpholine-4-carbonyl)phenyl)-1,6-dihydro-7*H*-pyrrolo[2,3-*c*]pyridin-7-one (8). Compound **8** was prepared in the same manner as shown for compound **3** except (3-(morpholine-4-carbonyl)phenyl)boronic acid was used instead. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.13 (s, 1H), 7.69 (dt, J = 7.9, 1.4 Hz, 1H), 7.61−7.50 (m, 2H), 7.41 (s, 1H), 7.40−7.31 (m, 2H), 6.42 (dd, J = 2.8, 1.9 Hz, 1H), 5.97−5.78 (m, 1H), 5.14−4.97 (m, 2H), 4.12 (t, J = 7.2 Hz, 2H), 3.62−3.20 (m, 8H), 2.50−2.46 (m, 2H). LC-MS: m/z 378 $(M+H)^+$.

6-(But-3-en-1-yl)-4-(3-fluoro-5-(morpholine-4-carbonyl)-phenyl)-1,6-dihydro-7*H*-pyrrolo[2,3-*c*]pyridin-7-one (9). Compound **9** was prepared in the same manner as shown for compound **3** except (3-fluoro-5-(morpholine-4-carbonyl)phenyl)boronic acid was used instead. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.18 (s, 1H), 7.52 (s, 1H), 7.45 (t, J = 1.5 Hz, 1H), 7.40−7.36 (m, 2H), 7.23 (m, J = 8.8, 2.5, 1.3 Hz, 1H), 6.45 (d, J = 2.8 Hz, 1H), 6.00−5.75 (m, 1H), 5.21−4.92 (m, 2H), 4.12 (t, J = 7.3 Hz, 2H), 3.62−3.20 (m, 8H), 2.49−2.45 (m, 2H). LC-MS: m/z 396 $(M+H)^+$.

4-(3-Amino-5-(morpholine-4-carbonyl)phenyl)-6-(but-3-en-1-yl)-1,6-dihydro-7*H*-pyrrolo[2,3-*c*]pyridin-7-one (10). To a solution of 4-bromo-6-but-3-enyl-1*H*-pyrrolo[2,3-*c*]pyridin-7-one (**29**, 200 mg, 0.70 mmol) and [3-(morpholine-4-carbonyl)-5-nitro-phenyl]boronic acid (300 mg, 0.90 mmol) in acetonitrile (2.5 mL) and 1 M potassium carbonate in water (2.5 mL) was added Pd(dppf)Cl_2 (30 mg, 0.04 mmol). The mixture was stirred at 100 °C for 15 min under microwave irradiation. The mixture was filtered through a pad of Celite and rinsed with ethyl acetate (20 mL). The filtrate was washed with brine (10 mL), dried over sodium sulfate, and concentrated in vacuo to yield 6-but-3-enyl-4-[3-(morpholine-4-carbonyl)-5-nitro-phenyl]-1*H*-pyrrolo[2,3-*c*]pyridin-7-one (300 mg, quant) as an off-white solid. This crude material was used in the next step without further purification. LC-MS: m/z 424 $(M+H)^+$.

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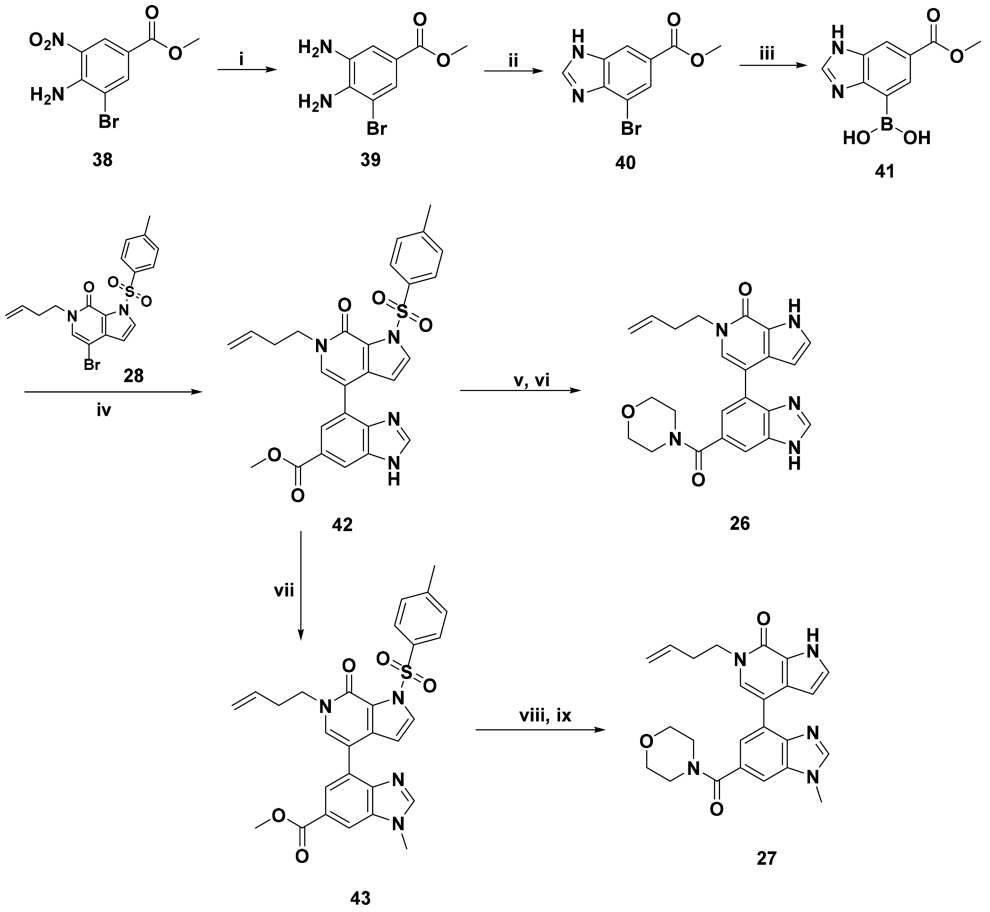
Scheme 4. Synthesis of Compound 27[a]



[a]Reagents and conditions: (i) $SnCl_2{\cdot}2H_2O$, ethyl acetate, 90 °C, 95%; (ii) triethoxymethane, $TsOH{\cdot}H_2O$, THF, rt, 93%; (iii) bis(pinanolato)diboron, $Pd(dppf)Cl_2$, KOAc, 1,4-dioxane, 120 °C, 92% (crude); (iv) $Pd(dppf)Cl_2$, Cs_2CO_3, 1,4-dioxane, water, 100 °C, 77%; (v) NaOH, methanol, water, 74%; (vi) morpholine, HATU, triethylamine, DMF, 21%; (vii) iodomethane, K_2CO_3, DMF, rt, 75%; (viii) NaOH, methanol, water, 92%; (ix) morpholine, HATU, triethylamine, DMF, 33%.

To a mixture of 6-but-3-enyl-4-[3-(morpholine-4-carbonyl)-5-nitro-phenyl]-1*H*-pyrrolo[2,3-*c*]pyridin-7-one (170 mg, 0.40 mmol) in ethanol (8 mL) and water (3 mL) were added iron (220 mg, 4.0 mmol) and ammonium chloride (43 mg, 0.80 mmol). The reaction was heated at 80 °C for 2 h. The mixture was filtered through a Celite pad, the cake washed with ethanol, and the filtrate was concentrated in vacuo. The residue was purified by preparative HPLC (25−35% ACN/0.1% NH_4OH in H_2O) to give 3-(6-(but-3-en-1-yl)-7-oxo-6,7-dihydro-1*H*-pyrrolo[2,3-*c*]pyridin-4-yl)-5-(morpholine-4-carbonyl)-benzonitrile (37 mg, 23% yield) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.06 (s, 1H), 7.35 (d, J = 2.8 Hz, 1H), 7.28 (s, 1H), 6.89 (t, J = 1.9 Hz, 1H), 6.67 (t, J = 1.5 Hz, 1H), 6.53 (t, J = 1.8 Hz, 1H), 6.42 (d, J = 2.8 Hz, 1H), 5.93−5.78 (m, 1H), 5.36 (s, 2H), 5.12−4.96 (m, 2H), 4.10 (t, J = 7.2 Hz, 2H), 3.59 (s, 8H), 2.47 (d, J = 6.9 Hz, 2H). LC-MS: m/z 394 $(M+H)^+$.

6-(But-3-en-1-yl)-4-(3-(difluoromethoxy)-5-(morpholine-4-carbonyl)phenyl)-1,6-dihydro-7*H*-pyrrolo[2,3-*c*]pyridin-7-one (11). To a solution of 3-bromo-5-hydroxybenzoic acid (2.00 g, 9.2 mmol) in DMF (20 mL) were added HATU (3.89 g, 10.2 mmol), DIPEA (1.32 g, 10.2 mmol), and morpholine (887 mg, 10.2 mmol). The reaction was stirred at ambient temperature for 10 h and then poured into water (40 mL) and extracted with ethyl acetate (3 × 50 mL). The combined organic layers were concentrated in vacuo. The residue was purified by flash chromatography (1:2 petroleum ether/ethyl acetate) to give (3-bromo-5-hydroxyphenyl)(morpholino)-methanone (900 mg, 31% yield) as a white solid. LC-MS: m/z 288.1 $(M+H)^+$.

To a solution of (3-bromo-5-hydroxyphenyl)(morpholino)-methanone (900 mg, 3.2 mmol) in DMF (20 mL)/H_2O (5 mL) were added K_2CO_3 (877 mg, 6.3 mmol) and sodium 2-chloro-2,2-difluoroacetate (966 mg, 6.3 mmol). The reaction was heated at 100 °C for 2 h. After cooling to ambient temperature, the mixture was poured into water (600 mL), and the resulting mixture was extracted with ethyl acetate (3 × 50 mL). The combined organic layers were concentrated in vacuo. The residue was purified by flash chromatography (1:1 petroleum ether/ethyl acetate) to give (3-bromo-5-(difluoromethoxy)phenyl)(morpholino)methanone (400 mg, 38% yield) as a yellow solid. LC-MS: m/z 338.1 $(M+H)^+$.

A mixture of (3-bromo-5-(difluoromethoxy)phenyl)(morpholino)-methanone (400 mg, 1.19 mmol), bis(pinanolato)diboron(455 mg, 1.79 mmol), potassium acetate (175 mg, 1.79 mmol), and $Pd(dppf)Cl_2$ (73 mg, 0.1 mmol) in dioxane (25 mL) was heated at 120 °C under N_2 for 5 h. Upon completion, the reaction mixture was concentrated in vacuo. The residue was dissolved in ethyl acetate (60 mL) and washed with water (2 × 30 mL). The separated organic layer was concentrated under reduced pressure. The crude product was purified by flash chromatography (1:1 petroleum ether/ethyl acetate) to give (3-(difluoromethoxy)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2 yl)phenyl)(morpholino)methanone (300 mg, 66% yield) as a yellow oil. LC-MS: m/z 384.2 $(M+H)^+$.

6-(But-3-en-1-yl)-4-(3-(difluoromethoxy)-5-(morpholine-4-carbonyl)phenyl)-1,6-dihydro-7*H*-pyrrolo[2,3-*c*]pyridin-7-one (**11**) was then prepared in a similar procedure as shown for compound **3** except (3-(difluoromethoxy)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl) morpholine methanone was used instead. ^{1}H NMR

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(400 MHz, DMSO-d_6) δ 12.18 (s, 1 H), 7.55−7.36 (m, 3H), 7.18−7.15 (m, 2H), 6.42 (s, 1H), 5.87−5.80 (m, 1H), 5.07−4.99 (m, 2H), 4.11−4.08 (m, 2H), 3.63−3.53 (m, 4H), 3.36−3.26 (m, 4H), 2.48−2.46 (m, 2H). LC-MS: m/z 444 $(M+H)^+$.

3-(6-(But-3-en-1-yl)-7-oxo-6,7-dihydro-1*H*-pyrrolo[2,3-*c*]-pyridin-4-yl)-5-(morpholine-4-carbonyl)benzonitrile (12). To a solution of 3,5-dibromobenzoic acid (2.79 g, 10 mmol) in DMF (30 mL) were added HATU (3.80 g, 10 mmol), DIPEA (1.32 g, 10.2 mmol), and morpholine (870 mg, 10.2 mmol). The reaction was stirred at ambient temperature for 10 h and then poured into water (40 mL) and extracted with ethyl acetate (3 × 150 mL). The combined organic layers were concentrated in vacuo. The residue was purified by flash chromatography (1:2 petroleum ether/ethyl acetate) to give (3,5-dibromophenyl)(morpholino)methanone (1.7 g, 49% yield) as a white solid. LC-MS: m/z 348.1, 350.1 $(M+H)^+$.

To a solution of (3,5-dibromophenyl)(morpholino)methanone (2.8 g, 7.3 mmol) in NMP (50 mL) was added CuCN (650 mg, 7.3 mmol). The reaction was heated at 175 °C for 2 h. After the mixture was cooled to ambient temperature, it was poured into concentrated ammonium hydroxide (200 mL), and the resulting mixture was extracted with ethyl acetate (3 × 150 mL). The combined organic layers were concentrated in vacuo. The residue was purified by flash chromatography (1:1 petroleum ether/ethyl acetate) to give 3-bromo-5-(morpholine-4-carbonyl)benzonitrile (1.0 g, 47% yield) as a yellow solid. LC-MS: m/z 295.1, 297.1 $(M+H)^+$.

A mixture of 3-bromo-5-(morpholine-4-carbonyl)benzonitrile (1.47 g, 5.0 mmol), bis(pinacolato)diboron (1.52 g, 6.0 mmol), potassium acetate (588 mg, 6.0 mmol), and Pd(dppf)Cl_2 (730 mg, 1.0 mmol) in dioxane (100 mL) was heated at 120 °C under N_2 for 3 h. Upon completion, the reaction mixture was concentrated in vacuo. The residue was purified by flash chromatography (1:1 petroleum ether/ethyl acetate) to give crude 3-(morpholine-4-carbonyl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzonitrile (600 mg, 35% yield) as a yellow oil.

A mixture of 4-bromo-6-(but-3-en-1-yl)-1,6-dihydro-7*H*-pyrrolo-[2,3-*c*]pyridin-7-one (266 mg, 1.0 mmol), methyl 2-methyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl) benzoate (342 mg, 1.0 mmol), cesium carbonate (325 mg, 1.0 mmol), and Pd(dppf)Cl_2 (50 mg, 0.06 mmol) in dioxane (20 mL) and water (2 mL) was heated at reflux temperature for 4 h under nitrogen atmosphere. The mixture was then filtered through Celite, using ethyl acetate (20 mL) to rinse the cake. The filtrate was concentrated in vacuo. The residue was purified by preparative HPLC (35−65% ACN/0.1% NH_4HCO_3 in water) to give 3-(6-(but-3-en-1-yl)-7-oxo-6,7-dihydro-1*H*-pyrrolo[2,3-*c*]pyridin-4-yl)-5-(morpholine-4-carbonyl)benzonitrile (32 mg, 8% yield) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.21 (s, 1H), 8.09 (s, 1 H), 7.90 (s, 1H), 7.83 (s, 1H), 7.58 (s, 1H), 7.36 (s, 1H), 6.46 (s, 1H), 5.87−5.70 (m, 1H), 5.08−4.98 (m, 2H), 4.11−4.06 (m, 2H), 3.63−3.53 (m, 4H), 3.36−3.26 (m, 4H), 2.48−2.46 (m, 2H). LC-MS: m/z 403.3 $(M+H)^+$.

6-(But-3-en-1-yl)-4-(4-fluoro-3-(morpholine-4-carbonyl)-phenyl)-1,6-dihydro-7*H*-pyrrolo[2,3-*c*]pyridin-7-one (13). Compound **13** was prepared in the same manner as shown for compound **3** except (4-fluoro-3-(morpholine-4-carbonyl)phenyl)-boronic acid was used instead. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.14 (s, 1H), 7.71 (m, J = 8.6, 5.1, 2.4 Hz, 1H), 7.58 (m, J = 6.4, 2.4 Hz, 1H), 7.47−7.33 (m, 3H), 6.41 (d, J = 2.9 Hz, 1H), 5.95−5.78 (m, 1H), 5.15−4.96 (m, 2H), 4.20−4.02 (m, 2H), 3.67−3.60 (m, 4H), 3.56 (m, J = 5.1 Hz, 4H), 2.49 (m, J = 4.7 Hz, 2H). LC-MS: m/z 396 $(M+H)^+$.

6-(But-3-en-1-yl)-4-(4-chloro-3-(morpholine-4-carbonyl)-phenyl)-1,6-dihydro-7*H*-pyrrolo[2,3-*c*]pyridin-7-one (14). Compound **14** was prepared in the same manner as shown for compound **3** except (4-chloro-3-(morpholine-4-carbonyl)phenyl)-boronic acid was used instead. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.92 (s, 1H), 7.65 (dd, J = 8.5, 2.2 Hz, 1H), 7.62−7.51 (m, 3H), 7.42 (s, 1H), 6.15 (dd, J = 1.9, 1.0 Hz, 1H), 5.85 (ddt, J = 17.0, 10.2, 6.7 Hz, 1H), 5.19−4.96 (m, 2H), 4.09 (t, J = 7.3 Hz, 2H), 3.82−3.21 (m, 8H), 2.47 (t, J = 6.6 Hz, 2H). LC-MS: m/z 426 $(M+H)^+$.

6-(But-3-en-1-yl)-4-(4-methyl-3-(morpholine-4-carbonyl)-phenyl)-1,6-dihydro-7*H*-pyrrolo[2,3-*c*]pyridin-7-one (15). Compound **15** was prepared in a similar manner as shown for compound **12** except methyl 2-methyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoate was used instead.

^{1}H NMR (400 MHz, DMSO-d_6) δ 12.10 (s, 1H), 7.54 (dd, J = 8.0, 1.9 Hz, 1H), 7.45−7.21 (m, 4H), 6.39 (d, J = 2.8 Hz, 1H), 5.86 (ddt, J = 17.0, 10.2, 6.7 Hz, 1H), 5.15−4.93 (m, 2H), 4.11 (t, J = 7.2 Hz, 2H), 3.59 (d, J = 50.2 Hz, 6H), 3.21 (s, 2H), 2.47 (d, J = 7.3 Hz, 2H), 2.27 (s, 3H). LC-MS: m/z 392 $(M+H)^+$.

6-(But-3-en-1-yl)-4-(3,4-difluoro-5-(morpholine-4-carbonyl)phenyl)-1,6-dihydro-7*H*-pyrrolo[2,3-*c*]pyridin-7-one (16). Compound **16** was prepared in the same manner as shown for compound **12** except (3,4-difluoro-5-methoxycarbonyl-phenyl)-boronic acid was used instead. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.18 (s, 1H), 7.72 (ddd, J = 11.7, 7.5, 2.2 Hz, 1H), 7.48 (s, 1H), 7.45−7.36 (m, 2H), 6.45 (d, J = 2.8 Hz, 1H), 5.86 (ddt, J = 17.0, 10.2, 6.7 Hz, 1H), 5.14−4.99 (m, 2H), 4.10 (t, J = 7.3 Hz, 2H), 3.68−3.50 (m, 4H), 3.57 (t, J = 4.8 Hz, 2H), 3.35 (t, J = 4.7 Hz, 2H), 2.50−2.44 (m, 2H). LC-MS: m/z 414 $(M+H)^+$.

6-(But-3-en-1-yl)-4-(5-(morpholine-4-carbonyl)pyridin-3-yl)-1,6-dihydro-7*H*-pyrrolo[2,3-*c*]pyridin-7-one (17). Compound **17** was prepared in the same manner as shown for compound **3** except [5-(morpholine-4-carbonyl)-3-pyridyl]boronic acid was used instead. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.19 (s, 1H), 8.89 (d, J = 2.3 Hz, 1H), 8.58 (d, J = 1.9 Hz, 1H), 8.01 (t, J = 2.1 Hz, 1H), 7.56 (s, 1H), 7.40 (d, J = 2.8 Hz, 1H), 6.46 (d, J = 2.8 Hz, 1H), 5.87 (ddt, J = 17.0, 10.2, 6.7 Hz, 1H), 5.18−4.95 (m, 2H), 4.13 (t, J = 7.2 Hz, 2H), 3.65 (m, 8H), 2.47−2.43 (m, 2H). LC-MS: m/z 379 $(M+H)^+$.

6-(But-3-en-1-yl)-4-(6-methyl-5-(morpholine-4-carbonyl)-pyridin-3-yl)-1,6-dihydro-7*H*-pyrrolo[2,3-*c*]pyridin-7-one (18). Compound **18** was prepared in the same manner as shown for compound **12** except ethyl 2-methyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine-3-carboxylate was used instead. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.17 (s, 1H), 8.74 (t, J = 1.6 Hz, 1H), 7.81 (d, J = 1.9 Hz, 1H), 7.58−7.25 (m, 2H), 6.52−6.27 (m, 1H), 5.98−5.71 (m, 1H), 5.23−4.93 (m, 2H), 4.11 (t, J = 7.3 Hz, 2H), 3.62 (d, J = 56.5 Hz, 6H), 3.27−3.18 (m, 2H), 2.50−2.48 (m, 2H), 2.46 (s, 3H). LC-MS: m/z 393 $(M+H)^+$.

6-(But-3-en-1-yl)-4-(6-methoxy-5-(morpholine-4-carbonyl)-pyridin-3-yl)-1,6-dihydro-7*H*-pyrrolo[2,3-*c*]pyridin-7-one (19). Compound **19** was prepared in the same manner as shown for compound **12** except methyl 2-methoxy-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)nicotinate was used instead. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.14 (s, 1H), 8.46 (d, J = 2.5 Hz, 1H), 7.89 (d, J = 2.5 Hz, 1H), 7.49−7.30 (m, 2H), 6.40 (d, J = 2.8 Hz, 1H), 5.97−5.78 (m, 1H), 5.18−4.95 (m, 2H), 4.10 (t, J = 7.3 Hz, 2H), 3.96 (s, 3H), 3.65 (s, 4H), 3.56 (t, J = 4.8 Hz, 2H), 3.23 (t, J = 4.7 Hz, 2H), 2.49−2.44 (m, 2H). LC-MS: m/z 409 $(M+H)^+$.

6-(But-3-en-1-yl)-4-(6-ethoxy-5-(morpholine-4-carbonyl)-pyridin-3-yl)-1,6-dihydro-7*H*-pyrrolo[2,3-*c*]pyridin-7-one (20). A mixture of morpholine (1.11 g, 12.69 mmol), 5-bromo-2-chloronicotinic acid (2.0 g, 8.46 mmol), 1-[bis(dimethylamino)-methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluoro-phosphate (3.37 g, 8.88 mmol), and triethylamine (2.36 mL, 16.9 mmol) in DMF (20 mL) was stirred at 18 °C for 2 h. The resulting mixture was poured into water (100 mL) and extracted with ethyl acetate (3 × 50 mL). The combined organic layers were dried over sodium sulfate and concentrated under reduced pressure to give crude (5-bromo-2-chloropyridin-3-yl) (morpholino)methanone (2.2 g, 85%) as a brown solid. LC-MS: m/z 306.9 $(M+H)^+$.

To a freshly prepared sodium ethanolate solution, in which sodium (56 mg, 1.64 mmol) was added carefully to ethanol (10 mL) and stirred until no solid remained, was added (5-bromo-2-chloropyridin-3-yl)(morpholino)methanone (500 mg, 1.64 mmol). The mixture was heated at 90 °C for 2 h and then concentrated under reduced pressure. The residue was diluted with water (20 mL) and extracted with ethyl acetate (3 × 10 mL). The combined organics were dried over sodium sulfate and concentrated under reduced pressure to give

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crude (5-bromo-2-ethoxypyridin-3-yl) (morpholino)methanone (380 mg, 74%) as a brown solid. LC-MS: m/z 314.9 $(M+H)^+$.

A mixture of (5-bromo-2-ethoxypyridin-3-yl)(morpholino)-methanone (380 mg, 1.21 mmol), bis(pinanolato)diboron (460 mg, 1.78 mmol), $Pd(dppf)Cl_2$ (38 mg), and potassium acetate (236 mg, 2.39 mmol) in dioxane (10 mL) was heated at 120 °C under microwave irradiation for 1 h. The mixture was concentrated under reduced pressure, and the residue was purified by preparative TLC (5% methanol in dichloromethane, R_f = 0.5) to afford (6-ethoxy-5-(morpholine-4-carbonyl)pyridin-3-yl)boronic acid (220 mg, 65%) as brown solid. LC-MS: m/z 280.9 $(M+H)^+$.

A mixture of 4-bromo-6-(but-3-en-1-yl)-1-tosyl-1*H*-pyrrolo[2,3-*c*]pyridin-7 (6*H*)-one (**28**, 220 mg, 0.52 mmol), (6-ethoxy-5-(morpholine-4-carbonyl)pyridin-3-yl) boronic acid (220 mg, 0.78 mmol), $Pd(dppf)Cl_2$ (10 mg), and cesium carbonate (340 mg, 1.04 mmol) in dioxane/water (6:1 mL) was heated at 110 °C for 1 h under microwave conditions. The resulting mixture was concentrated under reduced pressure, and the residue was purified by preparative TLC (50% ethyl acetate in petroleum ether, R_f = 0.2) to afford 6-(but-3-en-1-yl)-4-(6-ethoxy-5-(morpholine-4-carbonyl)pyridin-3-yl)-1-tosyl-1*H*-pyrrolo[2,3-*c*]pyridin-7(6*H*)-one (120 mg, 41%) as brown oil. LC-MS: m/z 423 $(M+H)^+$.

A mixture of 6-but-3-enyl-4-[6-ethoxy-5-(morpholine-4-carbonyl)-3-pyridyl]-1-(*p*-tolylsulfonyl)pyrrolo[2,3-*c*]pyridin-7-one (120 mg, 0.21 mmol) and 10% aqueous sodium hydroxide (0.17 mL, 0.21 mmol) in methanol (15 mL) was heated at 90 °C for 1 h and then concentrated to dryness under reduced pressure. The residue was diluted with water (20 mL). The crude solid product was collected by filtration and purified by preparative HPLC (32−52% ACN/0.1% NH_4OH in water) to give 6-(but-3-en-1-yl)-4-(6-ethoxy-5-(morpholine-4-carbonyl)pyridin-3-yl)-1,6-dihydro-7*H*-pyrrolo[2,3-*c*]pyridin-7-one (30 mg, 34%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.16 (s, 1H), 8.44 (s, 1H), 7.88 (s, 1H), 7.43−7.37 (m, 2H), 6.41 (s, 1H), 5.91−5.80 (m, 1H), 5.09−5.01 (m, 2H), 4.50−4.30 (m, 2H), 4.12−4.08 (m, 2H), 3.78−3.55 (m, 4H), 3.33−3.23 (m, 4H), 2.50−2.45 (m, 2H), 1.37−1.33 (m, 3H). LC-MS: m/z 578 $(M+H)^+$.

6-But-3-enyl-4-[7-(morpholine-4-carbonyl)-3*H*-benzimidazol-5-yl]-1*H*-pyrrolo[2,3-*c*]pyridin-7-one (21). To a solution of 6-(but-3-en-1-yl)-4-(4-(morpholine-4-carbonyl)-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-benzo[*d*]imidazol-6-yl)-1-tosyl-1*H*-pyrrolo[2,3-*c*]pyridin-7(6*H*)-one **37** (500 mg, 0.71 mmol) in dioxane/H_2O (10 mL, 1:1) was added sodium hydroxide (57 mg, 1.42 mmol). The reaction mixture was heated at 80 °C for 3 h, at which time LC-MS indicated the reaction had gone to completion. The mixture was concentrated under reduced pressure. The residue was diluted with ethyl acetate (30 mL), washed with water (2 × 20 mL), and concentrated under reduced pressure to give the crude 6-(but-3-en-1-yl)-4-(4-(morpholine-4-carbonyl)-1-((2-(trimethylsilyl)ethoxy)-methyl)-1*H*-benzo[*d*]imidazol-6-yl)-1*H*-pyrrolo[2,3-*c*]pyridin-7(6*H*)-one (350 mg, 90% yield).

To a solution of the above crude in dichloromethane (15 mL) was added trifluoroacetic acid (15 mL). After addition, the reaction mixture was stirred at ambient temperature for 3 h, at which time LC-MS indicated that the reaction had gone to completion. The reaction mixture was concentrated under reduced pressure. The crude was purified by reverse-phase chromatography (13−33% ACN/0.1% HCl in water) to give 6-but-3-enyl-4-[7-(morpholine-4-carbonyl)-3*H*-benzimidazol-5-yl]-1*H*-pyrrolo[2,3-*c*]pyridin-7-one (11 mg, 5% yield). ^{1}H NMR (400 MHz, CD_3OD) δ 9.58 (s, 1H), 8.16 (s, 1H), 7.91 (s, 1H), 7.48 (s, 2 H), 6.57 (s, 1H), 5.97−5.88 (m, 1H), 5.09 (t, J = 10.4 Hz, 2H), 4.26 (t, J = 7.2 Hz, 2 H), 4.02−3.48 (m, 8 H), 2.65−2.57 (m, 2 H). LC-MS: m/z 418 $(M+H)^+$.

6-(But-3-en-1-yl)-4-(1-methyl-4-(morpholine-4-carbonyl)-1*H*-benzo[*d*]imidazol-6-yl)-1,6-dihydro-7*H*-pyrrolo[2,3-*c*]-pyridin-7-one and 6-(But-3-en-1-yl)-4-(1-methyl-7-(morpholine-4-carbonyl)-1*H*-benzo[*d*]imidazol-5-yl)-1,6-dihydro-7*H*-pyrrolo[2,3-*c*]pyridin-7-one (22 and 23). To a solution of 6-(but-3-en-1-yl)-4-(4-(morpholine-4-carbonyl)-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-benzo[*d*]imidazol-6-yl)-1-tosyl-1*H*-pyrrolo[2,3-*c*]pyridin-7(6*H*)-one (500 mg, 0.71 mmol) in dichloromethane (10 mL) was added trifluoroacetic acid (10 mL). The mixture was stirred at ambient temperature for 3 h and then concentrated under reduced pressure to give 6-(but-3-en-1-yl)-4-(4-(morpholine-4-carbonyl)-1*H*-benzo[*d*]imidazol-6-yl)-1-tosyl-1*H*-pyrrolo[2,3-*c*]pyridin-7(6*H*)-one (400 mg, 98% yield) as a yellow oil. LC-MS: m/z 572.1 $(M+H)^+$.

To a stirred and cooled (0 °C) solution of 6-(but-3-en-1-yl)-4-(4-(morpholine-4-carbonyl)-1*H*-benzo[*d*]imidazol-6-yl)-1-tosyl-1*H*-pyrrolo[2,3-*c*]pyridin-7(6*H*)-one (500 mg, 0.87 mmol) in DMF (10 mL) was slowly added sodium hydride (60%, 42 mg, 0.92 mmol). After addition, the mixture was stirred at 0 °C for 1 h, and then iodomethane (149 mg, 0.92 mmol) was added. The resulting mixture was stirred at ambient temperature for 2 h. Upon completion, the reaction was quenched by the addition of saturated aqueous ammonium chloride (10 mL) and then extracted with ethyl acetate (3 × 20 mL). The combined organic layers were concentrated in vacuo. The residue was purified by silica-gel chromatography (1:1 hexanes/ethyl acetate) to give the mixture of the methylated 6-but-3-enyl-4-[3-methyl-7-(morpholine-4-carbonyl)benzimidazol-5-yl]-1-tosyl-1*H*-pyrrolo[2,3-*c*]pyridin-7-one and 6-but-3-enyl-4-[1-methyl-7-(morpholine-4-carbonyl)benzimidazol-5-yl]-1-tosyl-1*H*-pyrrolo[2,3-*c*]pyridin-7-one regioisomers (250 mg, 49% yield) as a brown solid.

To a solution of the above regioisomers (250 mg, 0.43 mmol) in dioxane/H_2O (10 mL, 1:1) was added sodium hydroxide (34 mg, 0.86 mmol). The reaction was heated at 80 °C for 3 h and then concentrated in vacuo and partitioned between dichloromethane (40 mL) and water (30 mL). The separated organic layer was concentrated under reduced pressure, and the residue was purified by reverse-phase chromatography (18−48% ACN/0.1% NH_4OH in water), resulting in the title compounds as white solids. Compound **22** (11 mg, 6% yield). ^{1}H NMR (400 MHz, CD_3OD) δ 9.54 (s, 1H), 8.14 (s, 1H), 7.87 (s, 1H), 7.48 (s, 2H), 6.55 (d, J = 2.8 Hz, 1H), 5.96−5.90 (m, 1H), 5.13−5.06 (m, 2H), 4.27−4.24 (m, 2H), 4.14 (s, 3H), 4.04−3.54 (m, 8H), 2.64−2.59 (m, 2H). LC-MS: m/z 432 $(M+H)^+$. Compound **23** (6.3 mg, 3% yield). ^{1}H NMR (400 MHz, CD_3OD) δ 9.59 (s, 1H), 8.26 (s, 1H), 7.95 (s, 1H), 7.53−7.49 (m, 2H), 7.62 (d, J = 2.8 Hz, 1 H), 5.98−5.89 (m, 1H), 5.13−5.06 (m, 2H), 4.29−4.26 (m, 5H), 4.02−3.46 (m, 8H), 2.66−2.61 (m, 2H). LC-MS: m/z 432 $(M+H)^+$.

6-(But-3-en-1-yl)-4-(1-ethyl-4-(morpholine-4-carbonyl)-1*H*-benzo[*d*]imidazol-6-yl)-1,6-dihydro-7*H*-pyrrolo[2,3-*c*]pyridin-7-one and 6-(But-3-en-1-yl)-4-(1-ethyl-7-(morpholine-4-carbonyl)-1*H*-benzo[*d*]imidazol-5-yl)-1,6-dihydro-7*H*-pyrrolo[2,3-*c*]pyridin-7-one (24 and 25). Compounds **24** and **25** were prepared in the same manner as shown for compounds **22** and **23** with iodoethane used instead. Compound **24** (71 mg, 12% yield). ^{1}H NMR (400 MHz, CD_3OD) δ 8.34 (s, 1H), 7.98 (s, 1 H), 7.49 (s, 1H), 7.42 (d, J = 2.8 Hz, 1H), 7.31 (s, 1H), 6.51 (d, J = 2.8 Hz, 1H), 6.97−5.88−5.79 (m, 1H), 5.12−5.04 (m, 2H), 4.41−4.39 (m, 2H), 4.24−4.20 (m, 2H), 3.92−3.81 (m, 4H), 3.69−3.31 (m, 4H), 2.62−2.56 (m, 2H), 1.49 (t, J = 7.2 Hz, 3H). LC-MS: m/z 446 $(M+H)^+$. Compound **25** (105 mg, 26% yield). ^{1}H NMR (400 MHz, CD_3OD) δ 8.32 (s, 1H), 7.87 (s, 1H), 7.54 (s, 1H), 7.40 (s, 1H), 7.35 (s, 1H), 6.58 (s, 1H), 5.93−5.87 (m, 1H), 5.10−5.02 (m, 2H), 4.43−4.38 (m, 2H), 4.21 (t, J = 7.2 Hz, 2H), 3.88−3.81 (m, 4H), 3.62−3.35 (m, 4H), 2.61−2.56 (m, 2H), 1.55 (t, J = 7.2 Hz, 3H). LC-MS: m/z 446 $(M+H)^+$.

6-But-3-enyl-4-[6-(morpholine-4-carbonyl)-1*H*-benzimidazol-4-yl]-1*H*-pyrrolo[2,3-*c*]pyridin-7-one (26). To a solution of methyl 4-(6-(but-3-en-1-yl)-7-oxo-1-tosyl-6,7-dihydro-1*H*-pyrrolo[2,3-*c*]pyridin-4-yl)-1*H*-benzo[*d*]imidazole-6-carboxylate (**42**, 400 mg, 0.77 mmol) in methanol (10 mL) was added a solution of sodium hydroxide (1 N in water, 2.0 mL, 2.0 mmol) at ambient temperature. The resulting mixture was heated at 100 °C for 2 h. After cooling to ambient temperature, the mixture was adjusted to pH 3−4 by addition of 1 N aqueous HCl. The resulting mixture was extracted with ethyl acetate (3 × 30 mL). The combined organic layers were concentrated under reduced pressure to give 4-(6-(but-3-en-1-yl)-7-oxo-6,7-dihydro-1*H*-pyrrolo[2,3-*c*]pyridin-4-yl)-1*H*-benzo-[*d*]imidazole-6-carboxylic acid (200 mg, 74% yield) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.94 (br s, 1H), 12.16 (s, 1H),

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8.84 (s, 1H), 8.19 (s, 1H), 8.06 (s, 1H), 7.67 (s, 1H), 7.45 (d, J = 8.0 Hz, 0.5 H), 7.34 (s, 1H), 7.07 (d, J = 7.6 Hz, 0.5 H), 6.24 (s, 1H), 5.87−5.80 (m, 1H), 5.08−4.97 (m, 2H), 4.13−4.09 (m, 2H), 2.50−2.48 (m, 1H), 2.25 (s, 1H). LC-MS: m/z 418 $(M+H)^+$.

A mixture of 4-(6-(but-3-en-1-yl)-7-oxo-6,7-dihydro-1*H*-pyrrolo[2,3-*c*]pyridine-4-yl)-1*H*-benzo[*d*]imidazole-6-carboxylic acid (100 mg, 0.28 mmol), morpholine (73 mg, 0.84 mmol), HATU (141 mg, 0.37 mmol), and DIPEA (72 mg, 0.56 mmol) in DMF (10 mL) was heated at 60 °C for 8 h. The reaction mixture was concentrated in vacuo, and the residue was dissolved in dichloromethane (50 mL), washed with brine (2 × 20 mL), and concentrated under reduced pressure. The crude product was purified by preparative HPLC(10−40% ACN/0.1% HCl in water) to give 6-but-3-enyl-4-[6-(morpholine-4-carbonyl)-1*H*-benzimidazol-4-yl]-1*H*-pyrrolo[2,3-*c*]pyridin-7-one as a white solid (25 mg, 21% yield). ^{1}H NMR (400 MHz, CD_3OD) δ 9.56 (s, 1H), 7.97 (d, J = 4.0 Hz, 1H), 7.78 (s, 1H), 7.58 (s, 1H), 7.45 (d, J = 2.8 Hz, 1 H), 6.28 (d, J = 2.8 Hz, 1H), 5.94−5.88 (m, 1H), 5.15−5.05 (m, 2H), 4.27 (t, J = 7.2 Hz, 2H), 3.93−3.47 (m, 8H), 2.65−2.59 (m, 2H). LC-MS: m/z 418 $(M+H)^+$.

6-But-3-enyl-4-[1-methyl-6-(morpholine-4-carbonyl)-benzimidazol-4-yl]-1*H*-pyrrolo[2,3-*c*]pyridin-7-one (27, GNE-371). To a solution of methyl 4-(6-(but-3-en-1-yl)-7-oxo-1-tosyl-6,7-dihydro-1*H*-pyrrolo [2,3-*c*]pyridin-4-yl)-1-methyl-1*H*-benzo[*d*]-imidazole-6-carboxylate (**43**, 350 mg, 0.66 mmol) in methanol/H_2O (15:3 mL) was added sodium hydroxide (130 mg, 3.3 mmol). The reaction was heated at 80 °C for 16 h and then concentrated in vacuo. The residue was diluted with H_2O (8 mL), and the solution was adjusted to pH 5−6 by the addition of 2 N aqueous HCl. The resulting precipitate was collected by filtration, washed with water and dried under reduced pressure to give 4-(6-(but-3-en-1-yl)-7-oxo-6,7-dihydro-1*H*-pyrrolo[2,3-*c*]pyridin-4-yl)-1-methyl-1*H*-benzo[*d*]-imidazole-6-carboxylic acid (220 mg, 92% yield) as a yellow solid. LC-MS: m/z 362.9 $(M+H)^+$.

To a solution of 4-(6-(but-3-en-1-yl)-7-oxo-6,7-dihydro-1*H*-pyrrolo[2,3-*c*]pyridin-4-yl)-1-methyl-1*H*-benzo[*d*]imidazole-6-carboxylic acid (220 mg, 0.61 mmol) in DMF (5 mL) were added triethylamine (120 mg, 1.2 mmol), HATU (460 mg, 1.2 mmol) and morpholine (110 mg, 1.2 mmol). The reaction was stirred at ambient temperature for 16 h and then concentrated in vacuo. The residue was purified by reverse-phase chromatography (19% ACN/0.1% NH_4OH in water) to give 6-but-3-enyl-4-[1-methyl-6-(morpholine-4-carbonyl)benzimidazol-4-yl]-1*H*-pyrrolo[2,3-*c*]pyridin-7-one (86 mg, 33% yield) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.09 (s, 1H), 8.33 (s, 1H), 7.84 (s, 1H), 7.64 (s, 1H), 7.46 (s, 1H), 7.35−7.34 (m, 1H), 6.37 (s, 1H), 5.93−5.84 (m, 1H), 5.12−5.07 (m, 2H), 4.15−4.11 (m, 2H), 4.08 (s, 3H), 3.63−3.57 (m, 8H), 2.46−2.44 (m, 2H). LC-MS: m/z 432 $(M+H)^+$. ^{13}C NMR (101 MHz, DMSO, 360 K) δ 170.30, 154.20, 146.14, 142.16, 135.61, 135.45, 130.52, 130.18, 129.45, 128.77, 127.17, 124.12, 120.22, 117.29, 111.54, 108.39, 103.39, 66.72, 47.38, 45.79, 34.01, 31.33. HRMS: calcd for $C_{24}H_{26}N_5O_3$ m/z $(M+H)^+$ 432.2030, found 432.2029.

(2-Amino-3-nitrophenyl)(morpholino)methanone (31). To a solution of morpholine (2.4 g, 27.45 mmol) in DMF (80 mL) were added 2-amino-3-nitrobenzoic acid (**30**, 5.0 g, 27.45 mmol), HATU (12.5 g, 32.94 mmol), and DIPEA (10 mL). The reaction was stirred at ambient temperature for 15 h and then concentrated in vacuo. The residue was diluted with ethyl acetate (100 mL) and washed with water (2 × 50 mL). The organic solution was concentrated under reduced pressure, and the residue was purified by silica-gel chromatography (3:1 hexanes/ethyl acetate) to give (2-amino-3-nitrophenyl)(morpholino)methanone (5.0 g, 73% yield) as a yellow solid. LC-MS: m/z 252 $(M+H)^+$.

(2-Amino-5-bromo-3-nitrophenyl)(morpholino)methanone (32). To a solution of (2-amino-3-nitrophenyl)(morpholino)-methanone (**31**, 5.0 g, 19.90 mmol) in acetic acid (50 mL) was added Br_2 (3.82 g, 23.88 mmol). The reaction was stirred at 0 °C for 30 min. The mixture was poured into ice-cold water (30 mL). The resulting precipitate was collected by filtration and dried under reduced pressure to give (2-amino-5-bromo-3-nitrophenyl)-(morpholino)methanone (5.0 g, 76% yield) as a yellow solid. LC-MS: m/z 332 $(M+H)^+$.

(2,3-Diamino-5-bromophenyl)(morpholino)methanone (33). To a solution of (2-amino-5-bromo-3-nitrophenyl)-(morpholino)methanone (**32**, 5.0 g, 15.15 mmol) in EtOH/H_2O (5:1, 100 mL) were added NH_4Cl (4.1 g, 75.73 mmol) and iron powder (4.2 g, 75.73 mmol). The reaction mixture was heated at reflux temperature for 15 h, at which time LC-MS indicated that the reaction had gone to completion. The solid was removed by filtration, and the filtrate was concentrated under reduced pressure. The residue was dissolved in ethyl acetate (100 mL) and washed with brine (2 × 40 mL). The separated organic layer was concentrated to give (2,3-diamino-5-bromophenyl)(morpholino)methanone (4.2 g, 92% yield) as a yellow oil. LC-MS: m/z 302.0 $(M+H)^+$.

(6-Bromo-1*H*-benzo[*d*]imidazol-4-yl)(morpholino)-methanone (34). To a solution of (2,3-diamino-5-bromophenyl)-(morpholino)methanone (**33**, 4.2 g, 12.72 mmol) in DMF (40 mL) were added triethoxymethane (2.8 g, 19.08 mmol) and 4-methylbenzenesulfonic acid (220 mg, 1.27 mmol). The reaction mixture was stirred at ambient temperature for 15 h, at which time LC-MS indicated that the reaction had gone to completion. The solvent was evaporated under reduced pressure, and the residue was diluted with water (40 mL). The mixture was extracted with ethyl acetate (3 × 50 mL). The combined organic extracts were concentrated under reduced pressure. The crude product was purified by silica-gel chromatography (10:1 DCM/MeOH) to give (6-bromo-1*H*-benzo[*d*]imidazol-4-yl)(morpholino)methanone (3.0 g, 76% yield) as a brown solid. LC-MS: m/z 311.9 $(M+H)^+$.

(6-Bromo-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-benzo[*d*]-imidazol-4-yl)(morpholino)methanone (35). To a solution of (6-bromo-1*H*-benzo[*d*]imidazol-4-yl)(morpholino)methanone (**34**, 3.0 g, 9.67 mmol) in DMF (50 mL) was slowly added sodium hydride (60%, 464 mg, 11.61 mmol) at 0 °C. After addition, the mixture was stirred at 0 °C for 1 h, and then (2-(chloromethoxy)ethyl)-trimethylsilane (1.9 g, 11.61 mmol) was added dropwise. The resulting mixture was stirred at ambient temperature for another 2 h, at which time LC-MS indicated that the reaction had gone to completion. The reaction was quenched by the addition of saturated aqueous ammonium chloride (50 mL) and then extracted with ethyl acetate (3 × 50 mL). The combined organic extracts were concentrated under reduced pressure. The crude product was purified by silica-gel chromatography (1:1 hexanes/ethyl acetate) to give (6-bromo-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-benzo[*d*]imidazol-4-yl)(morpholino)methanone (3.0 g, 70% yield) as a yellow solid. LC-MS: m/z 441 $(M+H)^+$.

Morpholino(6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-benzo[*d*]imidazol-4-yl)methanone (36). A mixture of (6-bromo-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-benzo[*d*]imidazol −4-yl)-(morpholino)methanone (**35**, 3.0 g, 6.81 mmol), bis(pinanolato)-diboron (3.6 g, 13.62 mmol), potassium acetate (1.7 g, 17.64 mmol), and Pd(dppf)Cl_2 (0.5 g, 0.68 mmol) in dioxane (45 mL) was heated at 110 °C under microwave irradiation for 45 min, at which time LC-MS indicated that the reaction had gone to completion. The mixture was concentrated under reduced pressure. The residue was dissolved in ethyl acetate (80 mL), washed with water (2 × 50 mL), and concentrated in vacuo to give morpholino(6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-benzo[*d*]imidazol-4-yl)methanone (1.35 g, 41% yield) as a brown oil. LC-MS: m/z 405.9 $(M+H)^+$.

6-(But-3-en-1-yl)-4-(4-(morpholine-4-carbonyl)-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-benzo[*d*]imidazol-6-yl)-1-tosyl-1*H*-pyrrolo[2,3-*c*]pyridin-7(6*H*)-one (37). A mixture of morpholino(6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-benzo[*d*]imidazol-4-yl)methanone (450 mg, 0.92 mmol), 4-bromo-6-(but-3-en-1-yl)-1-tosyl-1*H*-pyrrolo-[2,3-*c*]pyridin-7(6*H*)-one (**36**, 389 mg, 0.92 mmol), Pd(dppf)Cl_2 (66 mg, 0.09 mmol), and cesium carbonate (601 mg, 1.85 mmol) in dioxane/H_2O (10 mL, 4:1) was heated at 110 °C under microwave irradiation for 45 min, at which time LC-MS indicated the reaction

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had gone to completion. The mixture was concentrated under reduced pressure. The residue was dissolved in ethyl acetate (30 mL), washed with water (2 × 20 mL), and concentrated under reduced pressure. The residue was purified by silica-gel chromatography (2:1 hexanes/ethyl acetate) to give 6-(but-3-en-1-yl)-4-(4-(morpholine-4-carbonyl)-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-benzo[*d*]-imidazol-6-yl)-1-tosyl-1*H*-pyrrolo[2,3-*c*]pyridin-7(6*H*)-one (500 mg, 77% yield) as a yellow solid. LC-MS: m/z 703 $(M+H)^+$.

Methyl 3,4-Diamino-5-bromobenzoate (39). A mixture of methyl 4-amino-3-bromo-5-nitrobenzoate (**38**, 1.0 g, 3.6 mmol) and $SnCl_2{\cdot}2H_2O$ (1.6 g, 7.3 mmol) in ethyl acetate (30 mL) was heated at 90 °C for 18 h. Upon completion, the reaction was cooled, diluted with water (30 mL), and extracted with ethyl acetate (3 × 30 mL). The combined organic layers were concentrated under reduced pressure to give methyl 3,4-diamino-5-bromobenzoate (850 mg, 95% yield) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74 (d, J = 1.6 Hz, 1H), 7.35 (s, 1H), 4.25 (br s, 2H), 3.86 (s, 3H), 3.50 (br s, 2H).

Methyl 4-Bromo-1*H*-benzo[*d*]imidazole-6-carboxylate (40). To a solution of methyl 3,4-diamino-5-bromobenzoate (850 mg, 3.5 mmol) in THF (30 mL) were added triethoxymethane (1.04 g, 7.0 mmol) and $TsOH{\cdot}H_2O$ (66 mg, 0.35 mmol). The resulting mixture was stirred at ambient temperature for 5 h and then concentrated in vacuo. The residue was diluted with water (30 mL) and extracted with ethyl acetate (3 × 30 mL). The combined organic layers were concentrated under reduced pressure to give methyl 4-bromo-1*H*-benzo[*d*]imidazole-6-carboxylate (820 mg, 93% yield) as a pale-white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.40 (s, 1H), 8.22 (s, 2H), 3.97 (s, 3H).

(6-(Methoxycarbonyl)-1*H*-benzo[*d*]imidazol-4-yl)boronic Acid (41). A mixture of methyl 4-bromo-1*H*-benzo[*d*]imidazole-6-carboxylate (820 mg, 3.21 mmol), bis(pinanolato)diboron (1.2 g, 4.5 mmol), Pd(dppf)Cl_2 (230 mg, 0.32 mmol), and potassium acetate (630 mg, 6.4 mmol) in dioxane (30 mL) was heated at 120 °C under N_2 atmosphere for 16 h. Upon completion, the mixture was cooled, filtered through a Celite pad, and rinsed with dioxane (20 mL). The filtrate was concentrated in vacuo. The residue was diluted with water (50 mL) and extracted with ethyl acetate (3 × 50 mL). The combined organic layers were concentrated under reduced pressure to give (6-(methoxycarbonyl)-1*H*-benzo[*d*]imidazol-4-yl)boronic acid as a brown solid (650 mg, 92% crude yield). LC-MS: m/z 221 $(M+H)^+$.

Methyl 4-(6-(But-3-en-1-yl)-7-oxo-1-tosyl-6,7-dihydro-1*H*-pyrrolo[2,3-*c*]pyridin-4-yl)-1*H*-benzo[*d*]imidazole-6-carboxylate (42). A mixture of 4-bromo-6-(but-3-en-1-yl)-1-tosyl-1*H*-pyrrolo[2,3-*c*]pyridin-7(6*H*)-one (**28**, 420 mg, 1.0 mmol), (6-(methoxycarbonyl)-1*H*-benzo[*d*]imidazol-4-yl)boronic acid (**41**, 330 mg, 1.5 mmol), cesium carbonate (650 mg, 2.0 mmol), and Pd(dppf)Cl_2 (73 mg, 0.10 mmol) in dioxane/H_2O (5:1, 30 mL) was heated at 100 °C under N_2 for 3 h. The reaction mixture was concentrated in vacuo, and the residue was diluted with water (30 mL) and extracted with ethyl acetate (3 × 30 mL). The combined organic layers were concentrated under reduced pressure. The residue was purified by silica-gel chromatography (99:1 to 16:1 DCM/MeOH) to give methyl 4-(6-(but-3-en-1-yl)-7-oxo-1-tosyl-6,7-dihydro-1*H*-pyrrolo[2,3-*c*]pyridin-4-yl)-1*H*-benzo[*d*]imidazole-6-carboxylate as a white solid (400 mg, 77% yield). LC-MS: m/z 516 $(M+H)^+$.

Methyl 4-(6-(But-3-en-1-yl)-7-oxo-1-tosyl-6,7-dihydro-1*H*-pyrrolo[2,3-*c*]pyridin-4-yl)-1-methyl-1*H*-benzo[*d*]imidazole-6-carboxylate (43). To a solution of methyl 4-(6-(but-3-en-1-yl)-7-oxo-1-tosyl-6,7-dihydro-1*H*-pyrrolo[2,3-*c*]pyridin-4-yl)-1*H*-benzo[*d*]-imidazole-6-carboxylate (**42**, 290 mg, 0.56 mmol) in DMF (4 mL) were added potassium carbonate (160 mg, 1.1 mmol) and CH_3I (82 mg, 0.84 mmol). The reaction mixture was stirred at ambient temperature for 6 h, at which time it was concentrated in vacuo. The residue was diluted with H_2O (30 mL) and extracted with ethyl acetate (3 × 30 mL). The combined organic layers were concentrated to give methyl 4-(6-(but-3-en-1-yl)-7-oxo-1-tosyl-6,7-dihydro-1*H*-pyrrolo[2,3-*c*]pyridin-4-yl)-1-methyl-1*H*-benzo[*d*]imidazole-6-carboxylate (300 mg, 75% yield) as a yellow solid. LC-MS: m/z 531.1 $(M+H)^+$.

Synthesis of TAF1(2)-Tracer Probe *N*-(2-(2-((6-(6-ROX)-amidohexyl)oxy)ethoxy)ethyl)-5-(6-(but-3-en-1-yl)-7-oxo-6,7-dihydro-1*H*-pyrrolo[2,3-*c*]pyridin-4-yl)-2-methoxynicotinamide. A mixture of 4-bromo-6-(but-3-en-1-yl)-1-tosyl-1*H*-pyrrolo-[2,3-*c*]pyridin-7(6H)-one 28 (500 mg, 1.2 mmol), methyl 2-methoxy-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)nicotinate (370 mg, 1.2 mmol), Pd(dppf)Cl2 (90 mg, 0.1 mmol), and cesium carbonate (785 mg, 2.0 mmol) in 1,4-dioxane (15 mL)/water (3 mL) was heated at 110 °C for 2 h under nitrogen atmosphere. After the mixture was cooled, it was extracted with ethyl acetate (20 mL × 2). The combined organic layers were washed with brine, dried over sodium sulfate, and concentrated under reduced pressure. The residue was purified by flash column chromatography (0−20% ethyl acetate in petroleum ether) to give methyl 5-(6-(but-3-en-1-yl)-7-oxo-1-tosyl-6,7-dihydro-1*H*-pyrrolo[2,3-*c*]pyridin-4-yl)-2-methoxynicotinate (370 mg, 62%) as a yellow oil. LC-MS: m/z = 508 $(M+H)^+$

To a solution of methyl 5-(6-(but-3-en-1-yl)-7-oxo-1-tosyl-6,7-dihydro-1*H*-pyrrolo[2,3-*c*]pyridin-4-yl)-2-methoxynicotinate (370 mg, 0.7 mmol) in 1,4-dioxane (3 mL) and water (1 mL) was added sodium hydroxide (146 mg, 3.6 mmol). The reaction mixture was stirred at 75 °C for 3 h and then concentrated under reduced pressure. The residue was adjusted to pH 5 by addition of hydrochloric acid (2 N). The resulting mixture was extracted with ethyl acetate (3 × 20 mL). The combined organic layers were washed with water (20 mL) and brine (20 mL), dried over anhydrous sodium sulfate, and concentrated under reduced pressure to afford crude 5-(6-(but-3-en-1-yl)-7-oxo-6,7-dihydro-1*H*-pyrrolo[2,3-*c*]pyridin-4-yl)-2-methoxynicotinic acid as a yellow solid (220 mg, 88%). LC-MS: m/z = 340 $(M+H)^+$

To a cooled solution of *tert*-butyl *N*-[2-[2-(6-aminohexoxy)-ethoxy]ethyl]carbamate (882 mg, 2.9 mmol) in 1,4-dioxane (2.5 mL) at 0 °C was added a solution of sodium bicarbonate (730 mg, 8.7 mmol) in water (5 mL); this was followed by the addition of 9*H*-fluoren-9-ylmethyl carbonochloridate (1130 mg, 4.4 mmol) in 1,4-dioxane (2.5 mL). The reaction mixture was stirred at room temperature for 18 h, and the resulting mixture then was poured into water (20 mL) and extracted with ethyl acetate (2 × 20 mL). The combined organic layers were washed with brine, dried over sodium sulfate, and concentrated under reduced pressure. The residue was purified by flash column chromatography (0−25% ethyl acetate in petroleum ether) to give 9*H*-fluoren-9-ylmethyl *N*-[6-[2-[2-(*tert*-butoxycarbonylamino)ethoxy]ethoxy]hexyl]carbamate (262 mg, 17%) as a colorless oil. ^{1}H NMR (400 MHz, CD3OD) δ 7.82−7.78 (m, 2H), 7.68−7.65 (m, 2H), 7.45−7.35 (m, 2H), 7.34−7.30 (m, 2H), 4.62 (br s, 2H), 4.40−4.34 (m, 2H), 4.23−4.21 (m, 1H), 3.59−3.57 (m, 4H), 3.52−3.46 (m, 4H), 3.30−3.22 (m, 2H), 3.20−3.10 (m, 2H), 1.70−1.20 (m, 17H).

To a solution of 9*H*-fluoren-9-ylmethyl *N*-[6-[2-[2-(*tert*-butoxycarbonylamino)ethoxy]ethoxy] hexyl]carbamate (262 mg, 0.5 mmol) in ethyl acetate (1 mL) was added hydrochloric acid (1.0 M in ethyl acetate, 10 mL, 10.0 mmol). The mixture was stirred at 0 °C for 1 h and concentrated under reduced pressure to give 9*H*-fluoren-9-ylmethyl *N*-[6-[2-(2-aminoethoxy) ethoxy]hexyl]carbamate hydrochloride (230 mg, 100%) as a white solid. The crude product was used in the next step without further purification. LC-MS: m/z = 427 $(M+H)^+$

To a stirred solution of 9*H*-fluoren-9-ylmethyl *N*-[6-[2-(2-aminoethoxy)ethoxy]hexyl]carbamate hydrochloride (220 mg, 0.48 mmol) and DIPEA (245 mg, 1.90 mmol) in DMF (10 mL) were added 5-(6-but-3-enyl-7-oxo-1*H*-pyrrolo[2,3-*c*]pyridin-4-yl)-2-methoxy-pyridine-3-carboxylic acid (177 mg, 0.52 mmol) and HATU (271 mg, 0.71 mmol) at room temperature. The mixture was stirred at room temperature for another 2 h and quenched with the addition of water (20 mL). The resulting mixture was extracted with ethyl acetate (2 × 20 mL). The combined organic layers were washed with brine, dried over sodium sulfate, and concentrated under reduced pressure to afford crude 9*H*-fluoren-9-ylmethyl *N*-[6-[2-[2-[[5-(6-but-3-enyl-7-oxo-1*H*-pyrrolo[2,3-*c*]pyridin-4-yl)-2-methoxy-pyridine-3-carbonyl]amino]ethoxy]ethoxy]hexyl]carbamate (750 mg) as a

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brown oil. This crude was used in the next step without further purification. LC-MS: m/z = 748.3 $(M+H)^+$.

To a solution of 9*H*-fluoren-9-ylmethyl *N*-[6-[2-[2-[[5-(6-but-3-enyl-7-oxo-1*H*-pyrrolo[2,3-*c*]pyridin-4-yl)-2-methoxy-pyridine-3-carbonyl]amino]ethoxy]ethoxy]hexyl]carbamate (750 mg, 0.60 mmol) in DMF (10 mL) was added piperidine (1024 mg, 12.03 mmol). The mixture was stirred at room temperature for 10 h and then separated between ethyl acetate (30 mL) and water (10 mL). The organic layer was discarded, and the water phase was concentrated under reduced pressure to give *N*-[2-[2-(6-aminohexoxy)ethoxy]ethyl]-5-(6-but-3-enyl-7-oxo-1*H*-pyrrolo[2,3-*c*]-pyridin-4-yl)-2-methoxy-pyridine-3-carboxamide (120 mg, 38%) as a brown oil. The crude material was used in the next step without further purification.

In the dark (because of light sensitivity), *N*-[2-[2-(6-aminohexoxy)ethoxy]ethyl]-5-(6-but-3-enyl-7-oxo-1*H*-pyrrolo[2,3-*c*]-pyridin-4-yl)-2-methoxy-pyridine-3-carboxamide (100 mg, 0.19 mmol), triethylamine (385 mg, 3.80 mmol), and 6-ROX SE (300 mg, 0.48 mmol) in DMF (1.5 mL) were added to a brown bottle. The mixture was stirred at room temperature for 10 h and concentrated under reduced pressure. The residue was purified by preparative HPLC (45−67.5% ACN/0.05% ammonia in water) to give the title compound (10 mg, 5%) as a purple solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.26 (br s, 1H), 8.67 (d, J = 2.0 Hz, 1H), 8.47 (d, J = 2.0 Hz, 1H), 8.36 (br s, 1H), 8.23 (d, J = 8.0 Hz, 1H), 7.87 (d, J = 8.0 Hz, 1H), 7.65 (s, 1H), 7.01 (s, 2H), 6.70 (s, 2H), 6.49 (br s, 1H), 5.89−5.78 (m, 1H), 5.14−5.02 (m, 2H), 4.15 (s, 4H), 3.76−3.52 (m, 9H), 3.50−3.26 (m, 10H), 3.16−2.88 (m, 5H), 2.71−2.50 (m, 5H), 2.05 (d, J = 7.0 Hz, 4H), 1.88 (dd, J = 6.4, 12.4 Hz, 5H), 1.58 (br s, 6H), 1.36 (br s, 3H). LC-MS (5 to 95% acetonitrile in water with 0.03% trifluoroacetic acid over 1.5 min) retention time of 0.878 min. ESI+ found $(M+H)^+$ 1042.6.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.8b01225.

Molecular-formula strings (CSV)

Protein and assay methods, TAF1(2)-cellular-assay methods, crystallography data and refinement for **8** and **27**, kinase- and bromodomain-selectivity data for **27**, and Bliss-synergy calculation for **27** and JQ1 (PDF)

Accession Codes

Compound **1**, 5I80; compound **2**, 5I1Q; compound **8**, 6DF4; compound **27**, 6DF7.

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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

BRD4(1), first bromodomain of bromodomain-containing protein 4; BRD4(2), second bromodomain of bromodomain-containing protein 4; BRD9, bromodomain-containing protein 9; BRPF1, bromodomain- and PHD-finger-containing protein 1; CBP, CREB-binding-protein bromodomain; CECR2, cat-eye-syndrome critical-region protein 2; TAF1(1), first bromodomain of human transcription-initiation-factor TFIID subunit 1; TAF1(2), second bromodomain of human transcription-initiation-factor TFIID subunit 1

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