Journal of Medicinal Chemistry

pubs.acs.org/jmc

Article

An Alkynylpyrimidine-Based Covalent Inhibitor That Targets a Unique Cysteine in NF-κB-Inducing Kinase

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Cite This: *J. Med. Chem.* 2021, 64, 10001−10018

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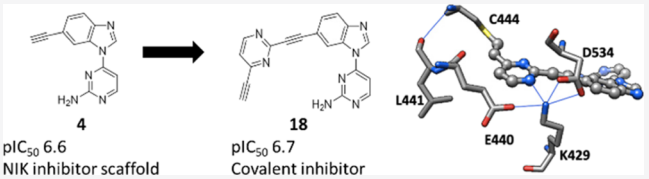
ABSTRACT: NF-κB-inducing kinase (NIK) is a key enzyme in the noncanonical NF-κB pathway, of interest in the treatment of a variety of diseases including cancer. Validation of NIK as a drug target requires potent and selective inhibitors. The protein contains a cysteine residue at position 444 in the back pocket of the active site, unique within the kinome. Analysis of existing inhibitor scaffolds and early structure−activity relationships (SARs) led to the design of C444-targeting covalent inhibitors based on alkynyl heterocycle warheads. Mass spectrometry provided proof of the covalent mechanism, and the SAR was rationalized by computational modeling. Profiling of more potent analogues in tumor cell lines with constitutively activated NIK signaling induced a weak antiproliferative effect, suggesting that kinase inhibition may have limited impact on cancer cell growth. This study shows that alkynyl heterocycles are potential cysteine traps, which may be employed where common Michael acceptors, such as acrylamides, are not tolerated.



INTRODUCTION

NF-κB-inducing kinase (NIK) modulates the noncanonical NF-κB pathway involving downstream signaling of a subset of TNF receptor family members.[1] The primary direct function of NIK is to phosphorylate IKKα homodimers, which in turn phosphorylate p100.[2] Phosphorylation of p100 results in proteolytic processing to generate the p52 subunit, which leads to transcriptional activation via p52:RelB heterodimers.[3] In the absence of stimulation, the pathway is silenced by upstream negative regulation of NIK induced by BIRC2/3 and TRAF2/3.[4,5] Upon TNF receptor family activation, NIK is released from TRAF2/3, leading to its upregulation and stabilization.[6]

NIK signaling represents a key node that mediates the survival of multiple B-cell malignancies following mutation of NIK itself or as a consequence of deletion/inactivating mutations in its upstream negative regulators (BIRC2/3 and TRAF2/3). In multiple myeloma, 17% of cases are associated with activation of NIK through mutation of these regulators,[7] while fludarabine refractory CLL shows loss of BIRC3 (25% of cases),[8] and mantle cell lymphoma shows alterations in BIRC3 and TRAF2 (10 and 6% of cases, respectively).[9] In Hodgkin lymphoma, >90% of biopsies show constitutive NIK expression.[10] NIK is therefore a potential target for small-molecule inhibitors, and identification of mutation or loss of negative regulators provides a potential means of stratifying patients for therapeutic intervention.

Small-molecule validation of inhibition of the kinase activity of NIK has been hampered by a lack of selective inhibitors. Potent aminopyrimidine-based inhibitors have been described by several groups, such as Amgen[11] and Genentech[12] (Figure 1). These inhibitors differ in the nature of the 5,6-bicyclic core and are characterized by an aminopyrimidine that binds the kinase hinge region and by a propargylic alcohol substituent on

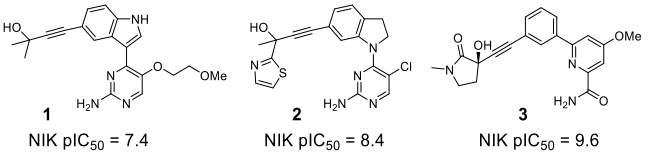


Figure 1. Representative literature NIK inhibitors.

Received: February 17, 2021
Published: July 2, 2021

https://doi.org/10.1021/acs.jmedchem.0c01249
J. Med. Chem. 2021, 64, 10001−10018

the bicyclic system, which has been shown to contribute significantly to potency.

Further structurally diverse scaffolds have since been disclosed, perhaps most notably by Genentech, including **3**.[13] Prior to the disclosure of **3**, NIK inhibitors generally possessed poor kinase selectivity. Achieving potency and selectivity in NIK inhibitors is particularly challenging because of their constitutive activity, relatively shallow binding pocket,[12,14] high ATP affinity (K_M 4 μM), and the suggestion that sustained coverage above IC_{90} concentrations is required for a reversible inhibitor to deliver efficacy.[15] Hence, it was proposed that an irreversible inhibitor of NIK that targets active site Cys444, which is unique to NIK, would deliver greatly improved selectivity and superior efficacy.

RESULTS AND DISCUSSION

The design strategy to target Cys444 started with an analysis of inhibitor bound structures from the literature, such as that of **1** (Figure 2a).[11] In this structure, the propargyl alcohol sidechain

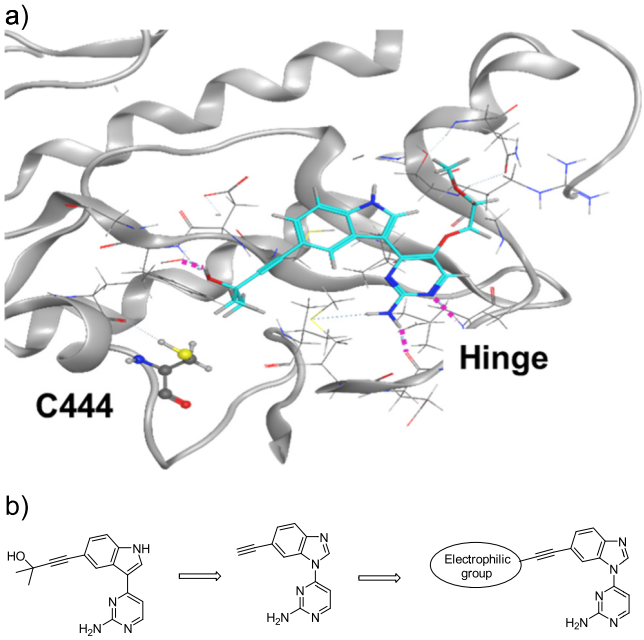


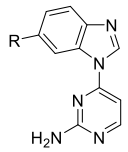
Figure 2. (a) Structure of **1** (cyan) in complex with NIK (pdb 4IDV) showing the proximity of the inhibitor to Cys444; key hydrogen bonding interactions are shown with dashed magenta lines; (b) design strategy for a covalent Cys444-targeting inhibitor.

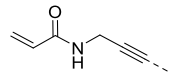
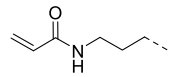
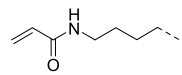
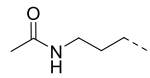
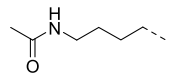
extends into the back pocket, with one of the methyl groups approaching Cys444. This suggested that the replacement of the alcohol motif with a suitable electrophilic moiety would lead to a covalent inhibitor (Figure 2b).

The hydroxyl group of **1** makes several productive interactions and significantly contributes to the potency of this and other NIK inhibitors. Nevertheless, introducing an electrophilic warhead while retaining the alcohol group would have been challenging with regard to simultaneously obtaining the correct orientations of both groups and to synthetic tractability. Accordingly, structure–activity relationship (SAR) investigations were carried out without the alcohol. Most of the work incorporated the benzimidazole head group because of its reduced lipophilicity relative to other precedented bicyclic systems such as indole and indoline.

The truncated ethynyl benzimidazole **4** established a baseline level of potency for this investigation (pIC_{50} = 6.6, Table 1). The alkyne contributes significantly to potency, with

Table 1. SAR of Amide-Based Covalent Inhibitors



	R	NIK pIC_{50} ± SD[a]
4	HC≡C---	**6.6 ± 0.15**
5	Et---	**4.1 ± 0.14**
6		**5.2 ± 0.22**
7		**3.3 ± 0.028**
8		**3.3 ± 0.12**
9		**<4.0**
10		**3.4 ± 0.14**

[a]IC_{50} values were determined after 30 min preincubation.

the corresponding ethyl derivative **5** being more than 100-fold less potent. Our initial investigations of covalent groups focused on acrylamides, which are the most commonly employed successful warheads for cysteine targeting.[16−18] Attaching an acrylamide group to the acetylene side chain (**6**) resulted in a significant loss of potency relative to the unsubstituted acetylene. Reasoning that this meant it was unlikely to be forming the desired covalent bond, it was postulated that the constrained geometry of the acetylene may prevent the acrylamide from assuming an appropriate conformation for reaction with Cys444. Acrylamides attached to two flexible alkyl side chains (**7** and **8**) were therefore explored, both of which resulted in further loss of potency.

The loss of potency of **7** and **8**, combined with the similar lack of potency for the analogous acetamides **9** and **10**, synthesized as nonreactive controls, could be rationalized on the basis that polar carboxamide functionality was not tolerated in the back pocket. It was therefore reasoned that less-polar electrophiles may be required. Of course, any Michael acceptor functionality is required to have a degree of polarity, but it was considered that alkenyl and alkynyl groups activated by conjugation with electron-deficient heterocycles might be better tolerated in the pocket while also providing sufficient reactivity to effect covalent binding on an appropriate timescale.[19] Docking of the proposed structures suggested that meta- and para-substituted pyridine/diazine heterocycles would orient vinyl or alkynyl substituents toward Cys444 (Figure 3).

Exploration of the meta-alkenyl pyridines **11** and **12** showed that these groups were tolerated, albeit with weak activity

https://doi.org/10.1021/acs.jmedchem.0c01249
J. Med. Chem. 2021, 64, 10001−10018

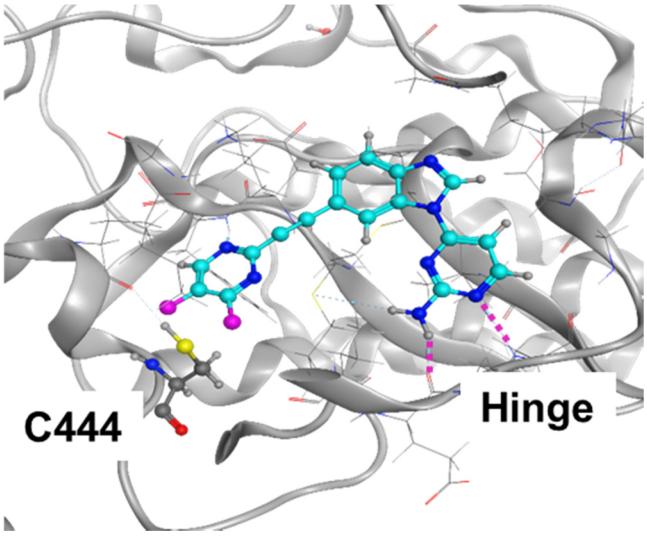


Figure 3. Modeled structure (based on 4IDV[11]) of an alkynyl heterocycle (cyan) in the back pocket of NIK. Meta- and para-positions (highlighted in magenta) are appropriate for attachment of reactive groups to target Cys444, and hinge interactions are shown with dashed magenta lines.

(Table 2). Isomer **12**, with the nitrogen at the 6-position relative to the alkyne linker, was 10-fold more potent than 4-aza analogue **11**. The meta-alkynyl pyridines showed a similar trend to the 2- and 6-aza derivatives **13** and **16** being more potent than the 4- and 5-isomers **14** and **15**. The 6-aza derivative **16** stood out as the most potent pyridine isomer (pIC_{50} = 6.6).

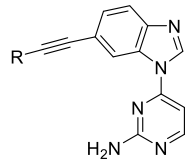
In the diazine series, these effects were reinforced, with the 2,6-pyrimidine analogues **17** and **18** showing submicromolar potency, significantly enhanced over all other diazine isomers tested (**19**, **20**, **21**, and **22**). Para-substituted derivatives **23**, **24**, and **25** showed reduced potency regardless of the heterocycle. The most potent compound in the series with the highest lipophilic ligand efficiency[20−22] was the meta-alkynyl-2,6-pyrimidine **18** (pIC_{50} 6.7, LLE 3.1).

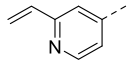
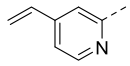
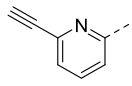
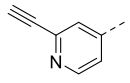
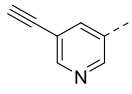
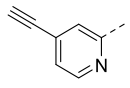
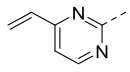
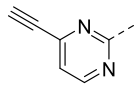
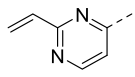
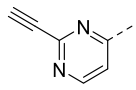
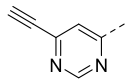
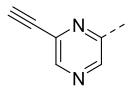
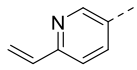
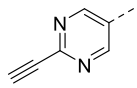
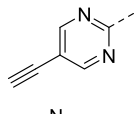
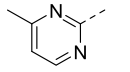
The SAR, combined with the increased potency of **18**, suggested that sub 1 μM potency was only associated with a meta-substituted, 6-aza heterocycle, as in **16**, **17**, and **18**. This led us to speculate that these compounds were binding covalently. This was consistent with the decreased potency of vinylpyridine **12**, which also fits the same structural motif but is presumably the least reactive of the four compounds in this class. Furthermore, replacing the alkyne of **18** with a methyl group (**26**) reduced potency significantly (ΔpIC_{50}−0.9).

To establish that the compounds were binding covalently, the formation of adducts of **18** with NIK protein was studied by mass spectrometry. After incubation of the compound with NIK over a time course of 24 h, intact protein analysis showed clear evidence of the formation of an adduct corresponding to the addition of a single molecule of the inhibitor (Figure 4a): 337 Da higher in mass than the apo protein. The rate of labeling correlated with the reciprocal of the protein concentration versus time, consistent with a second-order reaction (Figure 4b). Labeling of Cys444 was confirmed by peptide mapping experiments in which digestion followed by analysis of the MS/MS fragmentation spectra of the peptide containing Cys444 (Ala438-Arg451) was conducted, which clearly demonstrated the addition of the inhibitor to Cys444 (Figure 4c).

An analogous experiment carried out with the pyridine analogue **16** failed to show any appreciable labeling after 18 h

Table 2. SAR for Alkenyl and Alkynyl Heterocycles



	R	NIK pIC_{50} ± SD[a]	X logP	LLE
11		4.7 ± 0.29	4.4	0.069
12		6.1 ± 0.24	4.4	1.3
13		5.5 ± 0.14	4.0	1.6
14		4.5 ± 0.37	4.1	0.47
15		4.2 ± 0.091	4.2	-0.11
16		6.6 ± 0.16	4.2	2.4
17		5.8 ± 0.50	4.0	2.4
18		6.7 ± 0.11	3.7	3.1
19		4.4 ± 0.19	4.0	0.38
20		4.4 ± 0.36	3.7	1.0
21		4.3 ± 0.38	3.4	0.91
22		5.2 ± 0.22	2.7	2.3
23		4.1 ± 0.080	4.5	-0.23
24		4.1 ± 0.07	3.7	0.29
25		4.6 ± 0.10	3.7	0.76
26		5.8 ± 0.026	3.5	2.3

[a]IC_{50} values were determined after 30 min preincubation.

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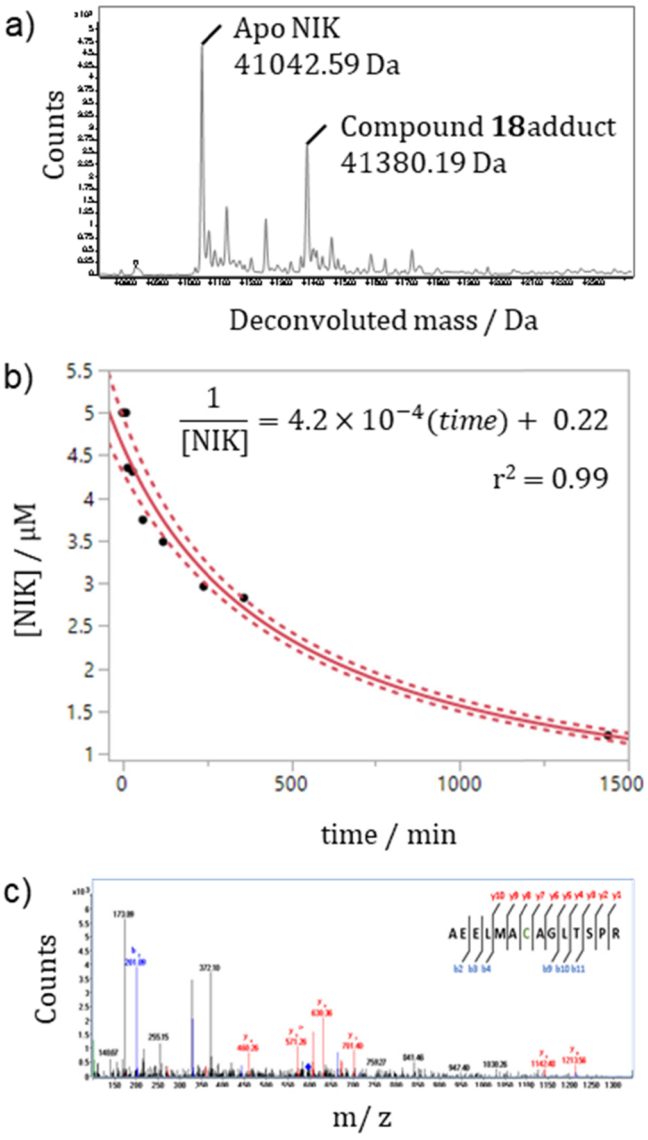

Figure 4. Characterization of covalent binding of **18** by mass spectrometry. (a) Intact protein deconvoluted mass spectrum following 6 h incubation of NIK (5 μM) with **18** (100 μM); (b) time course of covalent labeling of NIK by **18** showing the correlation between 1/[NIK] and time (the red line shows the line of best fit, and dotted lines are the 95% confidence limits); and (c) MS/MS fragmentation spectrum for peptide **A(438)EELMACAGLTSPR-(451)** showing formation of a major adduct on Cys444, detected after peptide mapping using tryptic digestion and reduction/alkylation (b-ion fragmentation, blue; y-ion fragmentation, red; and precursor ion (m/z 595.95, z = +3), blue diamond).

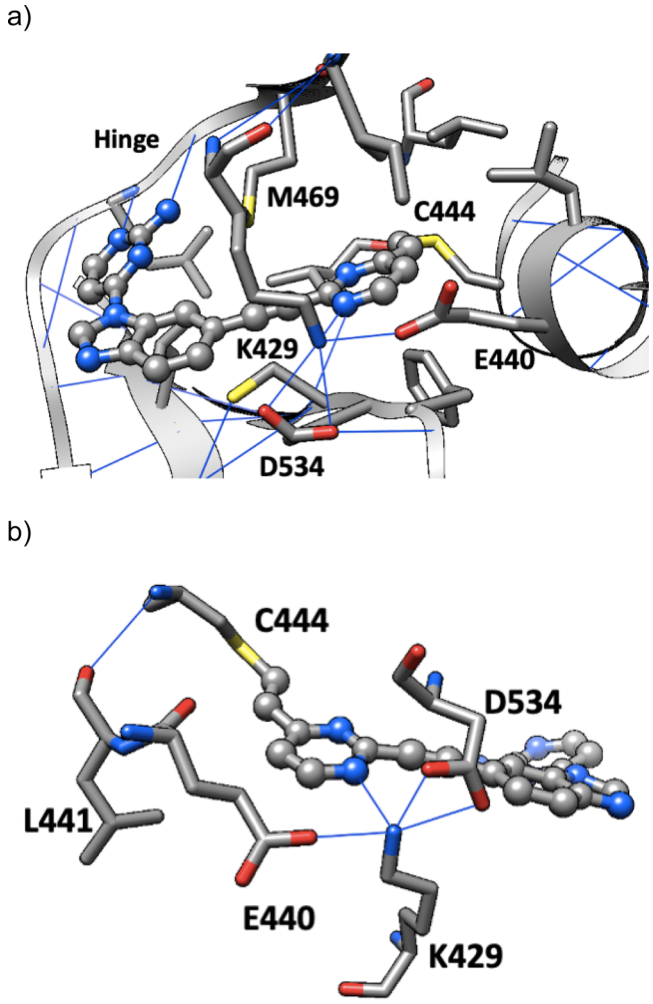

Figure 5. Molecular modeling of **18** in complex with NIK. (a) Docked structure of **18** in complex with NIK showing the compound binding to the hinge region and forming a covalent bond with Cys444; (b) interactions of the pyrimidine nitrogen that potentially increase potency and activate the ring toward covalent binding.

incubation with NIK. This observation implies that the less electron-poor pyridine system results in an alkyne that is insufficiently electrophilic to react in this context.

Docking of **18** with a NIK structure derived from 4IDV was consistent with the postulated binding mode, including the formation of a covalent bond between the terminal alkynylcarbon of **18** and Cys444, resulting in a vinylsulfide adduct (Figure 5a) and the hydrogen bonding interactions of the aminopyrimidine in the hinge region, observed for previous NIK inhibitors.[11,12] An explanation for the enhanced potency of the 6-aza analogues was also suggested. A hydrogen bond was predicted between the 6-nitrogen of **18** and the ε-nitrogen of Lys429, which is involved in a hydrogen bonding network with Glu440 and Asp534 (Figure 5b). This may play both an orienting and activating role in promoting the conjugate addition reaction in which the covalent bond is formed.

To rationalize the SAR for the positioning of the nitrogen atoms in the alkynylpyrimidine moiety, molecular mechanical Poisson−Boltzmann surface area calculations[23] for the covalent adducts with ligands **18** and **20** were performed. The results showed the stabilization of the NIK-**18** complex arising from noncovalent electrostatic interactions with an electrostatic energy of 6 kcal mol^{-1} greater than that of its 2,4-diaza isomer **20** (Table S1). These results suggest that the increased potency of the 6-aza derivatives, such as **18**, arise in part from more favorable noncovalent interactions with the protein.

However, it would be expected that the 2,6-isomer would be slightly more reactive toward conjugate addition because of the existence of a para-relationship between the alkyne and one of the nitrogen atoms, which is absent in **20**.[24] It would also be expected that the formation of a hydrogen bond between the 6-nitrogen and the charged ammonium group of Lys429, as observed in the covalent complex, should make the covalent

https://doi.org/10.1021/acs.jmedchem.0c01249
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reaction more favorable, provided similar interactions form in the reaction transition state. Together, these observations suggest that the observed SAR results from a combination of covalent and noncovalent effects (i.e., an effect on both K_i and k_{inact}).[25]

Selectivity of compound **18** showed was assessed in a panel of 140 kinases (Figure 6 and Table S2). Only 10 kinases

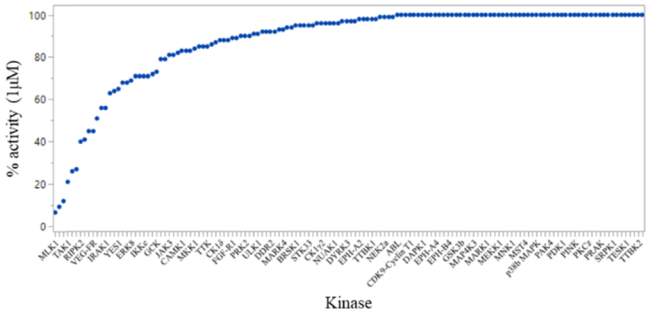


Figure 6. Kinase selectivity for compound **18**.

showed >50% inhibition at 1 μM (MLK1, HER4, SGK1, TAK1, PDGFRA, BTK, RIPK2 Aurora B, VEGFR, and SIK2). It is anticipated that because of the uniqueness of Cys444 to NIK that none of these kinases would be inhibited irreversibly meaning that the kinetic selectivity in an endogenous setting would be greater still.

Testing the more potent compounds **12**, **16**, **17**, and **18** for growth inhibition in Z-138 and Maver-1 cancer cell lines, which have constitutive NIK activation,[7] showed growth inhibition in ranges consistent with their isolated protein potency (Table 3). However, they also demonstrated

Table 3. Cellular Profile of the Potent Inhibitors and Comparison with Literature Compound 3[a]

compound	12	16	17	18	3
NIK pIC_{50}	5.8	6.5	6.4	6.7	8.0
Z-138 pGI_{50}	6.4	5.1	6.0	6.2	4.7
Maver-1 pGI_{50}	6.3	5.0	5.9	6.4	5.4
MCF-7 pGI_{50}	5.9	<5.0	6.2	6.3	<4.5
JIM-3 pGI_{50}	5.8	<5.1	5.8	6.3	<4.5

[a]Data are a mean of at least three independent determinations (for full data, see Table S2).

comparable inhibition in cell lines without constitutive NIK activation (MCF-7 and JIM-3), suggesting that these effects were NIK-independent, despite the very selective kinase profile. In comparison, literature compound **3** also demonstrated weak activity across all tumor cell lines, relative to its cell-free potency, but did show some evidence of a differential effect in NIK-activated cell lines. These results suggest that NIK inhibition does not have a strong antiproliferative phenotype.

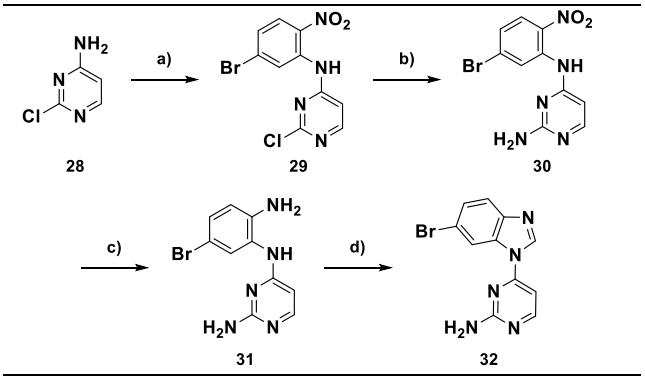
ADMET profiling of compound **18** in in vitro ADMET assays showed it to have moderate lipophilicity significantly lower than the calculated XlogP value, with relatively low solubility and permeability (Table 4). The compound had relatively high turnover in both rat and human hepatocytes.

Key analogues **4**–**10** were prepared from the common intermediate **32**, which was itself prepared using a four-step synthetic route (Scheme 1).[26] Nucleophilic aromatic substitution of 4-bromo-2-fluoro-1-nitrobenzene **27** with 2-

Table 4. ADMET Profile of Compound 18

compound	18
$logD_{7.4}$	2.8
solubility/μM	3.8
Caco2 P_{app} (A to B/B to A)/nm.s^{-1}	3.3/2.2
hep Cl_{int} (rat/human)/μL.min^{-1}.10^{-6} cells	915/382

Scheme 1. Synthesis of Key Intermediate 32[a]

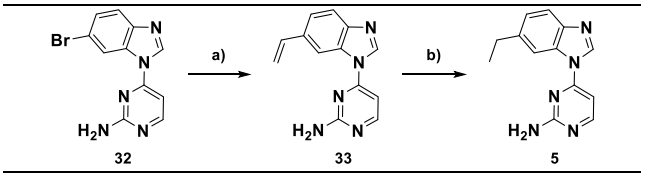


[a]Reagents and conditions: (a) x-bromo-x-flouro-x-nitrobenzene (**27**), NaH, THF, 0 °C, 1 h, and 79% (**29**); (b) NH_4OH, 33%, IPA, 110 °C, 24–48 h, 91% (**30**); (c) $SnCl_2$, AcOEt, 85 °C, 1–3 h, and 61% (**31**); (d) trimethyl orthoformate, *p*-toluene sulfonic acid, THF, 100 °C, 2–6 h, and 88% (**32**).

chloropyrimidin-4-amine **28** afforded intermediate **29** in excellent yield. Intermediate **29** was treated with ammonium hydroxide 33% solution to install the amino group at the 2-position of the pyridimine core followed by nitro reduction in the presence of tin chloride to obtain intermediate **31**, which then upon reaction with trimethyl orthoformate under acidic catalysis furnished the bromo-benzimidazole intermediate **32**.

Compound **5** was synthesized from key intermediate **32**. The vinyl group was introduced onto the 4-(6-bromo-1*H*-benzo[*d*]imidazol-1-yl)pyrimidin-2-amine **32** under Stille coupling conditions.[27] Intermediate **33** was reduced by Pd/C-catalyzed hydrogenation to give compound **5** (Scheme 2).

Scheme 2. Synthesis of Compound 5[a]

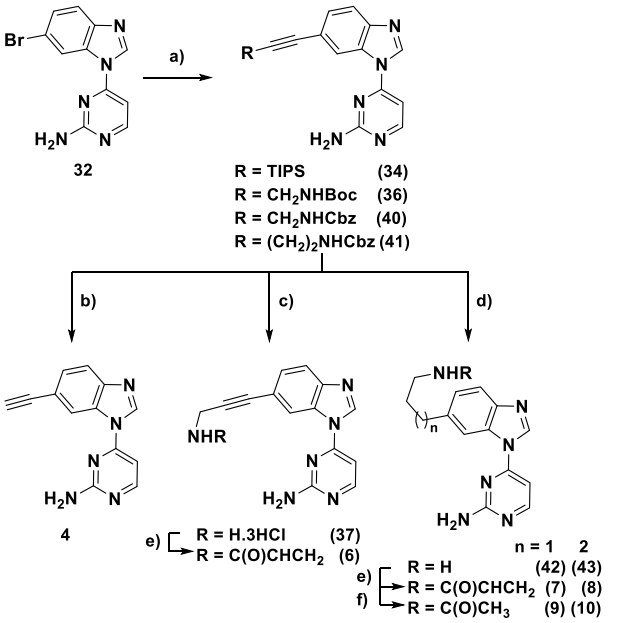


[a]Reagents and conditions: (a) $Pd(PPh_3)_4$, $Bu_3SnCHCH_2$, 1,4-dioxane, 110 °C, 1 h, and 69% (**33**); (b) Pd/(C), MeOH, H_2, 5 h, and 95% (**5**).

Compounds **4** and **6**–**10** were prepared in two to three steps from common intermediate **32** (Scheme 3). Sonogashira cross-coupling with triisopropylacetlyene, *N*-Boc-propargylamine **35**, *N*-Cbz-propargylamine **38**, and *N*-Cbz-3-butynylamine **39** gave rise to intermediates **34**, **36**, **40**, and **41**, respectively, in moderate yields. Deprotection of **34** using tetrabutylammonium fluoride or potassium fluoride gave rise to compound **4** in good yield. Deprotection of amino group **36** under acidic conditions revealed amine **37**, which was finally

https://doi.org/10.1021/acs.jmedchem.0c01249
J. Med. Chem. 2021, 64, 10001–10018

Scheme 3. Synthesis of Compounds 4 and 6−10[a]



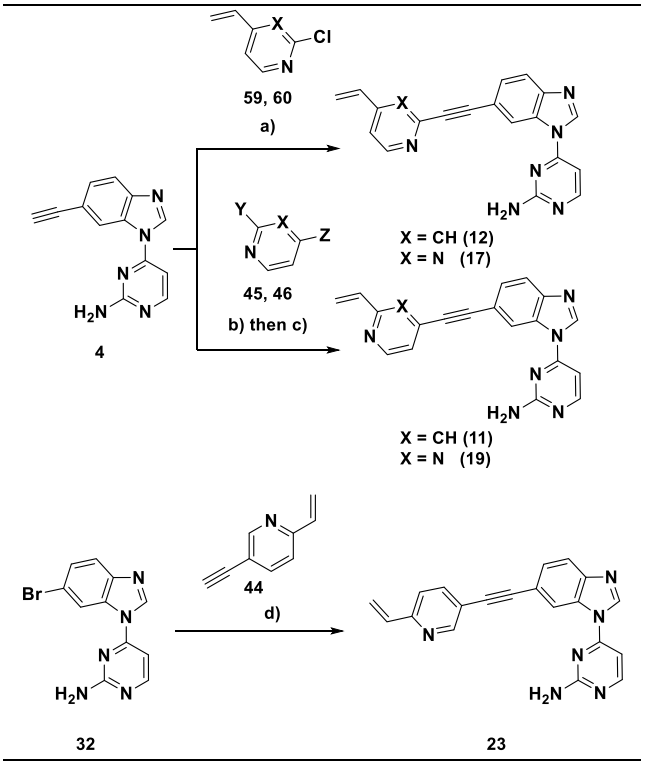
[a]Reagents and conditions: (a) triisopropylsilyl acetylene, $Pd(Ph_3)_2Cl_2$, CuI, DIPEA, DMF, 55 °C, 12 h, 90% (**34**), or, *N*-Boc-propargylamine **35**, $Pd(PPh_3)_4$, CuI, piperidine, DMF, 75 °C, 4 h, 57% (**36**) or *N*-Cbz-propargylamine **38**/ *N*-Cbz-3-butynylamine **39**, $PdCl_2(PPh_3)_2$, CuI, DIPEA, DMF, 50 °C, 14 h, and 63% (**40**) 79% (**41**); (b) TBAF, THF, 0−25 °C, 5 min or KF, DMF, 25 °C, 1−3 h, and 66% (**4**); (c) HCl 4 M, 1,4-dioxane, 25 °C, and 90 min (**37**); d) H_2, Pd(C), MeOH, 25 °C, 14 h 73% (**42**), and 66% (**43**); (e) acryloyl chloride, TEA, DCM, 0−25 °C, 2−14 h, 26% (**6**), 38% (**7**), and 37% (**8**); (f) acetyl chloride, Et_3N, DCM, 0−25 °C, 14 h, 40% (**9**), and 41% (**10**).

acylated in the presence of acryloyl chloride to obtain compound **6**. Compounds **7**−**10** were accessed from Pd-mediated hydrogenolytic deprotection of the benzyloxycarbonyl group of intermediates **40** and **41**. Compounds **7** and **8** were synthesized by acylation of the unprotected amino group on **42** and **43** with acroloyl chloride, while its acetylation furnished compounds **9** and **10**.

Reaction of alkyne **4** under standard Sonogashira cross-coupling conditions with vinyl halo heterocycles **59** and **60** (prepared as reported in section 3 of the Supporting Information, page S7, Scheme 2) gave rise to vinyl analogues **12** and **17**. Commercially available halo pyridine and pyrimidine **45** and **46** were cross-coupled to alkyne **4** under Sonogashira conditions to yield intermediates **51** and **52**, which were then subjected to Suzuki or Stille couplings to furnish desired vinyl compounds **11** and **19**. Compound **23** was synthesized by Sonogashira cross-coupling between common intermediate **32** and 5-ethynyl-2-vinylpyridine **44** (Scheme 4), which was prepared as reported in the Supporting Information, page S7, Scheme 1.

Alkynyl analogues **13**, **15**, **16**, **18**, and **25** were prepared in two-steps from alkyne **4** by reaction under standard Sonogashira cross-coupling conditions with halo-((triisopropylsilyl)ethynyl)heterocycles or halo-((trimethylsilyl)ethynyl)heterocycles **61**−**65** (prepared as reported in section 3 of the Supporting Information, page S7, Scheme 3) followed by deprotection of the silyl ethers **66**−**70** (Schemes 5 and 6). A one-pot double Sonogashira cross-coupling with commercially available halo pyridines and pyrimidines **46**, **48**, and **72** followed by triisopropylsilylacetylene gave rise to intermediates **55**−**57**, while intermediates **54** and **58** were prepared in two separate Sonogshira cross-coupling reactions, first with dihalo-pyrimidines **45** and **47** and then with triisopropylsilylacetylene. Deprotection of the alkynyl-protecting groups of intermediates **55**−**58** using TBAF or cesium fluoride[28] for the triisopropylsilyl ether and potassium carbonate for the trimethylsilyl ether gave rise to compounds **20**−**22** and **24**. Finally, the noncovalent analogue **26** was prepared by a single Sonogashira cross-coupling reaction between alkyne **4** and commercially available pyrimidine **76** (Scheme 6).

Scheme 4. Synthesis of Vinyl Analogues 11, 12, 17, 19, and 23[a]



[a]Reagents and conditions: (a) $PdCl_2(PPh_3)_2$, CuI, DIPEA, DMF, 50 °C, 14 h, 23% (**12**), and 16% (**17**); (b) Y, Z = halo **45**, **46**, $PdCl_2(PPh_3)_2$, CuI, DIPEA, DMF, 50 °C, 14 h, 87% (**51**), and 36% (**52**); (c) **51**, vinylboronic acid pinacol ester, $Pd(dppf)Cl_2.CH_2Cl_2$, Cs_2CO_3, THF, H_2O, 85 °C, 24 h, 23% (**11**) or **52**, tributylvinyltin, $Pd(PPh_3)_4$, DMF, 100 °C, 12 h, and 36% (**19**); (d) $PdCl_2(PPh_3)_2$, CuI, DIPEA, DMF, 50 °C, 14 h, and 25% (**23**).

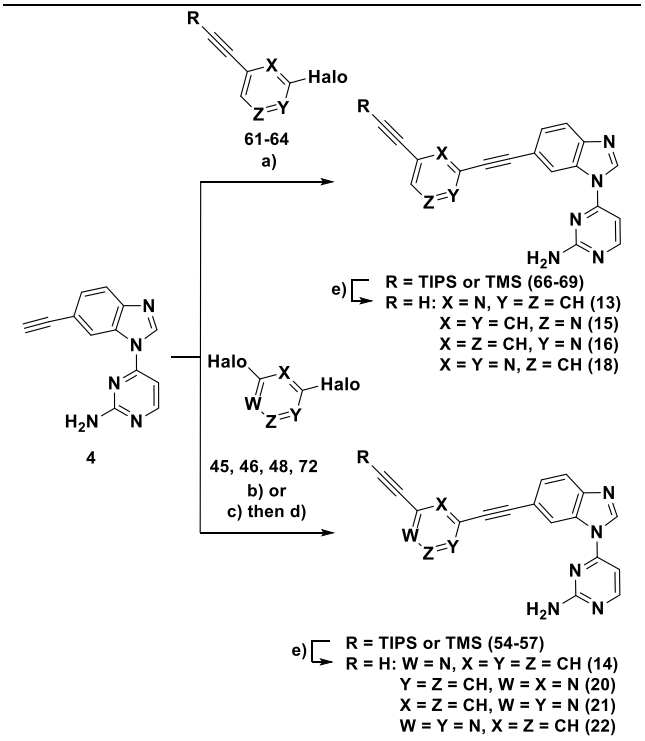
CONCLUSIONS

This work demonstrates that covalent inhibition of NIK-targeting Cys444 is feasible and provides a means of developing selective inhibitors. The current chemical series shows additional, NIK-independent effects on cancer cell growth, suggestive of additional pharmacology. Our attempts to remove the undesired activity from the compounds and further target validation studies will be the subject of a future communication.

Inhibition of NIK results in a relatively weak antiproliferative phenotype, even with covalent inhibition, suggesting that it has

https://doi.org/10.1021/acs.jmedchem.0c01249
J. Med. Chem. 2021, 64, 10001−10018

Scheme 5. Synthesis of Alkynyl Analogues 13−16, 18, and 20−22[a]



[a]Reagents and conditions: (a) Y = $PdCl_2(PPh_3)_2$, CuI, DIPEA, DMF, 50 °C, 14 h, and 54−77% (**66−69**); (b) (i). $PdCl_2(PPh_3)_2$, CuI, DIPEA, DMF, 50 °C, and 14 h and (ii). triisopropylsilyl acetylene, $PdCl_2(PPh_3)_2$, CuI, DIPEA, DMF, 50 °C, 14 h, 45% (**55**) 70% (**56**), and 54% (**57**); (c) $PdCl_2(PPh_3)_2$, CuI, DIPEA, DMF, 50 °C, 14 h, and 87% (**51**); (d) **51** triisopropylsilyl acetylene, $PdCl_2(PPh_3)_2$, CuI, DIPEA, DMF, 50 °C, 14 h, and 75% (**54**); (e) TBAF, THF, 0−25 °C, 5 min, 20−65% (**13, 15, 18, 20, 21**), or CsF, DMF, 25 °C, 1−3 h, 35% (**22**) or K_2CO_3, MeOH, 25 °C, 5 h, 83% (**14**), and 24% (**16**).

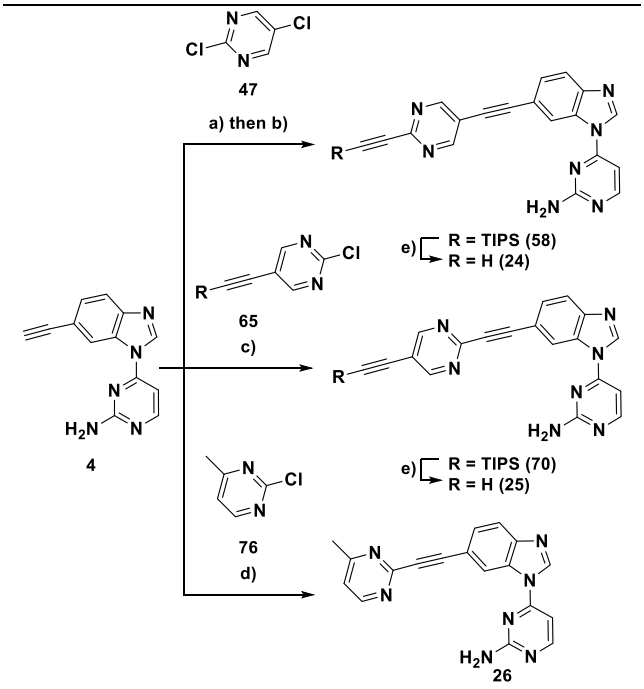
limited utility as a strategy to reduce cancer cell growth directly. Further biological studies will also be disclosed in future communications.

The successful targeting of Cys444 with alkynyl heterocycles shows that these moieties are useful covalent binding species that can be exploited in areas where more traditional acrylamide cysteine traps are not tolerated, presumably because of their differences in polarity. Further analysis of the SAR suggests that the differences in potency can be attributed to the difference in noncovalent interactions in the back pocket, suggesting that the more potent compounds gain affinity through noncovalent affinity, rather than increased reactivity. In this case, specific reaction of the compounds appears to be reinforced by the hydrogen bonds formed to the heterocycles. Compounds of this type may be of further utility in the development of covalent inhibitors of other proteins.

EXPERIMENTAL SECTION

Chemical Synthesis. Chemicals and solvents were obtained from standard suppliers (Fluorochem, Alfa Aesar, Apollo Scientific, and Sigma-Aldrich). All compounds had purity ≥95% as determined by high-performance liquid chromatography (UV detection) and ^{1}H-NMR analysis. Compounds 27, 28, 35, 45−50, 71−73, and 76 are commercially available obtained from standard suppliers.

Scheme 6. Synthesis of Alkynyl Analogues 24 and 25 and Methyl Analogue 26[a]



[a]Reagents and conditions: (a) $PdCl_2(PPh_3)_2$, CuI, DIPEA, DMF, 50 °C, 14 h, and 41% (**53**); (b) **53,** triisopropylsilyl acetylene, $PdCl_2(PPh_3)_2$, CuI, DIPEA, DMF, 50 °C, 14 h, and 62% (**58**); (c) $PdCl_2(PPh_3)_2$, CuI, DIPEA, DMF, 50 °C, 14 h, and 69% (**70**); (d) $PdCl_2(PPh_3)_2$, CuI, DIPEA, DMF, 50 °C, 14 h, and 40% (**26**); (e) TBAF, THF, 0−25 °C, 5 min, 59% (**24**), and 64% (**25**).

General Procedure a for Silyl Ether Deprotection Using TBAF. To a stirred solution of the silyl ether-protected acetylene intermediate (1 equivalent) in THF (0.14 M) at 0 °C was added tetrabutylammonium fluoride (1.2 equivalents). Upon completion of reaction, the solid precipitated out, and the solution was stirred for 5 min. Methanol (0.27 M) was added to quench the reaction, and the resulting solution was dried in vacuo, taken up in dichloromethane, and dry-loaded on silica. The crude product was purified by automated flash column chromatography.

General Procedure B for Sonogashira Cross-Coupling. A microwave vial was charged with aryl halide (1 equivalent), *bis*(triphenylphosphine)palladium(II) dichloride (0.1 equivalent), and copper(I) iodide (0.1 equivalent), sealed, and purged with vacuum and nitrogen. DMF (0.20 M) was added, and the solution was sparged with nitrogen for 5 min followed by addition of DIPEA (0.40 M) and further sparging with nitrogen for 5 min. The reaction mixture was then heated for 16 h at 55 °C. Upon completion, the reaction mixture was filtered through Celite, and the solvent was evaporated in vacuo. The crude product was then purified by automated flash column chromatography.

4-(6-Ethynyl-1*H*-Benzo[*d*]Imidazol-1-yl)Pyrimidin-2-Amine (4). 4-(6-((Triisopropylsilyl)ethynyl)-1*H*-benzo[*d*]imidazol-1-yl)-pyrimidin-2-amine 34 (53.0 mg and 0.135 mmol) was reacted with TBAF under conditions similar to those described in general procedure A. The crude product was purified by automated flash column chromatography (silica, 0−10% methanol in dichloromethane) to yield the desired compound as a white solid (21.0 mg and 8.90 μmol, 66%), m.p.: 231−233 °C. ^{1}H-NMR (DMSO-d_6): δ 4.22 (s, 1H), 7.14 (d, *J* = 5.6 Hz, 3H), 7.44 (dd, *J* = 1.6, 8.3 Hz, 1H), 7.74 (d, *J* = 8.3 Hz, 1H), 8.37 (d, *J* = 5.5 Hz, 1H), 8.74 (d, *J* = 1.5 Hz, 1H), 9.12 (s, 1H) ppm. ^{13}C-NMR (DMSO-d_6): δ 80.7, 84.8, 98.4, 118.0, 120.0, 120.6, 127.9, 131.8, 144.0, 145.0, 157.2, 160.9, 164.0

https://doi.org/10.1021/acs.jmedchem.0c01249
J. Med. Chem. 2021, 64, 10001−10018

ppm. HRMS-ESI (m/z): $[M + H]^+$ calculated for $C_{13}H_{10}N_5^+$, 236.0931; found, 236.0932.

4-(6-Ethyl-1*H*-Benzo[*d*]Imidazol-1-yl)Pyrimidin-2-Amine (5). 4-(6-Vinyl-1*H*-benzo[*d*]imidazol-1-yl)pyrimidin-2-amine 33 (50.0 mg and 0.211 mmol) was dissolved in methanol (2.1 mL) under an atmosphere of nitrogen. Palladium on carbon was added, and the reaction vessel was sparged with hydrogen for 15 min and then stirred for a further 16 h at 25 °C until the starting material was consumed by liquid chromatography mass spectrometry (LCMS) analysis. The reaction mixture was filtered through Celite and washed with methanol (20 mL), then concentrated in vacuo, and purified by automated flash column chromatography (silica, 0−10% methanol in dichloromethane) to yield the desired compound as a white solid (50.0 mg, 0.209 mmol, 95%), m.p.: 213−215 °C. ^{1}H-NMR (DMSO-d_6): δ 1.27 (t, J = 7.6 Hz, 3H), 2.79 (q, J = 7.6, 2H), 7.12 (d, J = 5.6 Hz, 1H), 7.17 (bs, 2H), 7.21 (dd, J = 1.6, 8.2 Hz, 1H), 7.64 (d, J = 8.2 Hz, 1H,), 8.38 (d, J = 5.5 Hz, 1H), 8.44 (d, J = 0.9 Hz, 1H), 8.96 (s, 1H) ppm. ^{13}C-NMR (DMSO-d_6): δ 17.0, 29.2, 98.3, 119.9, 115.9, 124.2, 132.2, 140.9, 142.0, 143.1, 157.4, 160.8, 164.0 ppm. HRMS-ESI (m/z): $[M + H]^+$ calculated for $C_{13}H_{14}N_5^+$, 240.1244; found, 240.1225.

***N*-(3-(1-(2-Aminopyrimidin-4-yl)-1*H*-Benzo[*d*]Imidazol-6-yl)-Prop-2-yn-1-yl)Acrylamide (6).** Acryloyl chloride (21.0 μL and 0.261 mmol) in dichloromethane (0.50 mL) was added to the 4-(6-(3-aminoprop-1-yn-1-yl)-1*H*-benzo[*d*]imidazol-1-yl)pyrimidin-2-amine trihydrochloride 37 (92.0 mg, 0.261 mmol) and triethylamine (146 μL, 1.04 mmol) in dichloromethane (1 mL) at 0 °C, and the mixture was stirred at 0 °C for 90 min, and then, it was stirred at 25 °C for 1 h. The reaction mixture was cooled to 0 °C, and additional acryloyl chloride (10.5 μL and 0.130 mmol) and triethylamine (36.5 μL and 0.261 mmol) in dichloromethane (0.50 mL) were added. The reaction mixture was stirred at 0 °C for 1 h and then quenched with the dropwise addition of methanol (0.50 mL), and the solvent was removed in vacuo; then, the crude product was purified using automated flash column chromatography (silica, 0−5% methanol in dichloromethane). The compound was triturated with EtOAc (2 × 2 mL) to give the title compound as an off-white solid (20.0 mg, 62.8 μmol, 26%). ^{1}H-NMR (500 MHz, DMSO-d_6): δ 4.31 (d, J = 5.4 Hz, 2H), 5.70 (dd, J = 2.2, 10.1 Hz, 1H), 6.19 (dd, J = 2.2, 17.1 Hz, 1H), 6.32 (dd, J = 10.1, 17.1 Hz, 1H), 7.18 (t, J = 8.2 Hz, 3H), 7.43 (dd, J = 1.6, 8.3 Hz, 1H), 7.78 (d, J = 8.3 Hz, 1H), 8.41 (d, J = 5.5 Hz, 1H), 8.73−8.66 (m, 2H), 9.14 (s, 1H) ppm. ^{13}C-NMR (126 MHz, DMSO-d_6): δ 29.3, 83.1, 86.6, 98.5, 118.5, 119.4, 120.6, 126.4, 127.7, 131.7, 131.9, 143.9, 144.7, 157.2, 161.0, 164.0, 164.8 ppm.

***N*-(3-(1-(2-Aminopyrimidin-4-yl)-1*H*-Benzo[*d*]Imidazol-6-yl)-Propyl)Acrylamide (7).** To a stirred solution of 4-(6-(3-aminopropyl)-1*H*-benzo[*d*]imidazol-1-yl)pyrimidin-2-amine 42 (36.0 mg, 0.134 mmol) in dichloromethane (0.45 mL) and THF (0.45 mL) at 0 °C were added acryloyl chloride (12.0 μL and 0.147 mmol) and trimethylamine (23.0 μL and 0.161 mmol). The reaction mixture was stirred at room temperature for 16 h until completion by LCMS analysis. Water and ethyl acetate were added to quench the reaction. The aqueous layer was extracted with ethyl acetate (×3), and combined organic extracts were washed with water and brine and dried over sodium sulfate and then concentrated in vacuo. The crude residue was purified by automated flash column chromatography (silica, 0−30% methanol in dichloromethane) to give the title compound as a white solid (17.0 mg, 52.8 μmol, 38%), m.p.: 202−204 °C. ^{1}H-NMR (500 MHz, CD_3OD): δ 1.89 (quint., J = 7.3 Hz, 2H), 2.79 (t, J = 7.6 Hz, 2H), 3.25−3.30 (m, 2H), 5.58−5.60 (dd, J = 3.1, 8.9 Hz, 1H), 6.14−6.22 (m, 2H), 7.01 (d, J = 5.7 Hz, 1H), 7.21 (dd, J = 1.5, 8.3 Hz, 1H), 7.58 (d, J = 8.3 Hz, 1H), 8.28−8.29 (m, 2H), 8.79 (s, 1H) ppm. ^{13}C-NMR (500 MHz, CD_3OD): δ 31.2, 33.1, 38.5, 98.3, 114.7, 118.9, 124.7, 125.1, 130.7, 131.8, 139.1, 141.2, 142.2, 157.5, 159.8, 163.7, 166.8 ppm. HRMS-ESI (m/z): $[M + H]^+$ calculated for $C_{17}H_{19}N_6O^+$, 322, 3720; found, $[M + H]^+$ 323.1615.

***N*-(4-(1-(2-Aminopyrimidin-4-yl)-1*H*-Benzo[*d*]Imidazol-6-yl)-Butyl)Acrylamide (8).** To a stirred solution of 4-(6-(4-aminobutyl)-1*H*-benzo[*d*]imidazol-1-yl)pyrimidin-2-amine 43 (70.0 mg, 0.245 mmol) in dichloromethane (0.83 mL) and THF (0.83 mL) at 0 °C were added acryloyl chloride (22.0 μL and 0.270 mmol) and trimethylamine (42.0 μL and 0.294 mmol). The reaction mixture was stirred at room temperature for 16 h until completion by LCMS analysis. Water and ethyl acetate were added to quench the reaction, and the biphasic mixture was transferred to a separating funnel. The aqueous layer was extracted with ethyl acetate (3 × 50 mL), and combined organic extracts were washed with water and brine and dried over sodium sulfate and then concentrated in vacuo. The crude residue was purified by automated flash column chromatography (silica, 0−30% methanol in dichloromethane) to give the title compound as a white solid (31.0 mg, 92.1 μmol, 37%), m.p.: 199−201 °C. ^{1}H-NMR (500 MHz, CD_3OD): 1.60−1.65 (m, 2H), 1.75−1.81 (m, 2H), 2.85−2.88 (m, 2H), 3.31−3.34 (m, 2H), 5.63−5.65 (dd, J = 4.1, 7.9 Hz, 2H), 6.21−6.23 (m, 2H), 7.08 (d, J = 5.7 Hz, 1H), 7.27 (dd, J = 1.4, 8.3 Hz, 1H), 7.65 (d, J = 8.3 Hz, 1H), 8.36 (d, J = 5.7 Hz, 2H), 8.86 (1H, s) ppm. ^{13}C-NMR (500 MHz, CD_3OD): δ 28.6, 29.1, 35.4, 38.8, 98.3, 114.6, 118.8, 124.7, 125.0, 130.7, 131.7, 139.8, 141.1, 142.1, 157.5, 159.8, 163.7, 166.8 ppm. HRMS-ESI (m/z): $[M + NH_4]^+$ calculated for $C_{18}H_{24}N_7O^+$, 354.2037; found, $[M + NH_4]^+$ 354.1969.

***N*-(3-(1-(2-Aminopyrimidin-4-yl)-1*H*-Benzo[*d*]Imidazol-6-yl)-Propyl)Acetamide (9).** To a stirred solution of 4-(6-(3-aminopropyl)-1*H*-benzo[*d*]imidazol-1-yl)pyrimidin-2-amine 42 (35.7 mg, 0.133 mmol) in dichloromethane (1.0 mL) at 0 °C were added acetyl chloride (11.0 μL and 0.1461 mmol) and DIPEA (28.0 μL and 0.159 mmol). The reaction mixture was stirred at room temperature for 16 h until completion by LCMS analysis. Water and ethyl acetate were added to quench the reaction. The aqueous layer was extracted with ethyl acetate (×3), and combined organic extracts were washed with water and brine and dried over sodium sulfate and then concentrated in vacuo. The crude residue was purified by automated flash column chromatography (silica, 0−30% methanol in dichloromethane) to give the title compound as a white solid (16.0 mg, 51.6 μmol, 40%), m.p.: 214−215 °C. ^{1}H-NMR (500 MHz, CD_3OD): δ 1.91−1.95 (m, 2H), 1.96 (s, 3H), 2.86 (t, J = 7.6 Hz, 2H), 2.87 (t, J = 7.0 Hz, 2H), 7.10 (d, J = 5.7 Hz, 1H), 7.29 (dd, J = 1.7, 8.3 Hz, 1H), 7.66 (d, J = 8.3 Hz, 1H), 8.34−8.37 (m, 2H), 8.88 (s, 1H) ppm. ^{13}C-NMR (500 MHz, CD_3OD): δ 39.09, 39.29, 96.15, 125.29, 130.63, 153.76, 162.82, 163.70, 167.13 ppm (further signals obscured by solvent, C_q not resolved). HRMS-ESI (m/z): $[M + H]^+$ calculated for $C_{16}H_{19}N_6O^+$, 311.1615; found, 311.1620.

***N*-(4-(1-(2-Aminopyrimidin-4-yl)-1*H*-Benzo[*d*]Imidazol-6-yl)-Butyl)Acetamide (10).** To a stirred solution 4-(6-(4-aminobutyl)-1*H*-benzo[*d*]imidazol-1-yl)pyrimidin-2-amine 43 (35.0 mg, 0.124 mmol) in dichloromethane (0.62 mL) at 0 °C were added acetyl chloride (11.0 μL and 0.149 mmol) and DIPEA (27.0 μL and 0.149 mmol). The reaction mixture was stirred at room temperature for 16 h until completion by LCMS analysis. Water and ethyl acetate were added to quench the reaction. The aqueous layer was extracted with ethyl acetate (3 × 50 mL), and combined organic extracts were washed with water and brine and dried over sodium sulfate and then concentrated in vacuo. The crude residue was purified by automated flash column chromatography (silica, 0−30% methanol in dichloromethane) to give the title compound as a white solid (16.0 mg, 49.3 μmol, 41%), m.p.: 224−226 °C. ^{1}H-NMR (500 MHz, CD_3OD): δ 1.57 (quint, J = 7.3 Hz, 2H), 1.75 (quint, J = 7.6 Hz, 2H), 1.93 (s, 3H), 2.86 (t, J = 7.6 Hz, 2H), 3.23 (t, J = 7.0 Hz, 2H), 7.07 (d, J = 5.7 Hz, 1H), 7.26 (dd, J = 1.3, 8.3 Hz, 1H), 7.64 (d, J = 8.3 Hz, 1H), 8.35 (bs, 1H), 8.36 (d, J = 5.7 Hz, 1H), 8.86 (s, 1H) ppm. ^{13}C-NMR (500 MHz, CD_3OD): δ 21.1, 28.6, 29.1, 35.4, 38.8, 98.3, 114.7, 118.8, 124.7, 131.7, 139.8, 141.1, 142.0, 157.5, 159.8, 163.7, 171.8 ppm. HRMS-ESI (m/z): $[M + H]^+$ calculated for $C_{17}H_{21}N_6O^+$, 325.1771; found, 325.1771.

4-(6-((2-Vinylpyridin-4-yl)Ethynyl)-1*H*-Benzo[*d*]Imidazol-1-yl)Pyrimidin-2-Amine (11). 4-(6-((2-Bromopyridin-4-yl)ethynyl)-1*H*-benzo[*d*]imidazol-1-yl)pyrimidin-2-amine 51 (100 mg, 0.256 mmol), vinylboronic acid pinacol ester (43.0 mg, 0.281 mmol), [1,1′-*bis*(diphenylphosphino)ferrocene]dichloropalladium-(II).dichloromethane (20.0 mg and 25.6 μmol), and cesium carbonate (250 mg, 0.767 mmol) were sealed in a microwave vial and purged

https://doi.org/10.1021/acs.jmedchem.0c01249
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with vacuum and nitrogen. Tetrahydrofuran (1.0 mL) and water (0.26 mL) were added, and the reaction mixture was then heated for 24 hours at 85 °C before drying in vacuo. The crude product was then purified by automated flash column chromatography (silica, 0−8% methanol in dichloromethane) yielding the desired compound as an off-white solid (20.0 mg, 59.1 μmol, 23%), m.p.: 190−192 °C. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 5.55 (dd, *J* = 1.5, 10.7 Hz, 1H), 6.34 (dd, *J* = 1.5, 17.4 Hz, 1H), 6.81−6.91 (m, 1H), 7.19 (d, *J* = 5.6 Hz, 2H), 7.22 (s, 1H), 7.32 (d, *J* = 5.5 Hz, 1H), 7.47 (dd, *J* = 1.5, 5.0 Hz, 1H), 7.72 (t, *J* = 1.2 Hz, 1H), 7.84 (dd, *J* = 0.7, 8.3 Hz, 1H), 8.40 (d, *J* = 5.5 Hz, 1H), 8.62 (dd, *J* = 0.8, 4.9 Hz, 1H), 8.92 (dd, *J* = 0.7, 1.7 Hz, 1H), 9.20 (s, 1H) ppm. ^{13}C-NMR (126 MHz, DMSO-d_6): δ 86.9, 95.1, 98.4, 117.4, 119.7, 120.4, 120.8, 123.5, 124.6, 127.8, 131.6, 132.0, 136.8, 144.3, 145.5, 150.3, 155.8, 157.2, 161.0, 164.0 ppm. HRMS-ESI (*m*/*z*): $[M + H]^+$ calculated for $C_{20}H_{15}N_6^+$, 339.1353; found, 339.1379.

4-(6-((4-Vinylpyridin-2-yl)Ethynyl)-1*H*-Benzo[*d*]Imidazol-1-yl)Pyrimidin-2-Amine (12). 2-Chloro-4-vinylpyridine 59 (40.0 mg, 0.287 mmol), 4-(6-ethynyl-1*H*-benzo[*d*]imidazol-1-yl)pyrimidin-2-amine 4 (50.0 mg and 0.213 mmol), *bis*(triphenylphosphine)-palladium(II) dichloride (18.0 mg and 28.7 μmol), and copper(I) iodide (4.60 mg and 28.7 μmol) were reacted under similar conditions to those described in general procedure B. The crude product was then purified by automated flash column chromatography (silica, 0−10% methanol in dichloromethane) yielding the desired compound as an off-white solid (22.0 mg, 65.0 μmol, and 23%), m.p.: 182−184 °C. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 5.62 (d, *J* = 11.2 Hz, 1H), 6.26 (d, *J* = 17.6 Hz, 1H), 6.79 (dd, *J* = 10.9, 17.6 Hz, 1H), 7.18 (d, *J* = 5.6 Hz, 1H), 7.21 (d, *J* = 13.2 Hz, 2H), 7.51 (dd, *J* = 1.7, 5.1 Hz, 1H), 7.59 (dd, *J* = 1.6, 8.3 Hz, 1H), 7.82 (dd, *J* = 1.2, 3.2 Hz, 1H), 7.84 (d, *J* = 0.6 Hz, 1H), 8.35−8.44 (m, 1H), 8.59 (dd, *J* = 0.8, 5.0 Hz, 1H), 8.85−8.94 (m, 1H), 9.19 (s, 1H) ppm. ^{13}C-NMR (126 MHz, $CDCl_3$): δ 89.1, 90.05, 98.4, 117.11, 120.1, 120.8, 120.9124.7, 127.8, 132.0, 134.4, 142.92, 143.80, 144.3, 145.3, 151.0, 157.25, 157.45, 161.0, 164.0 ppm. HRMS-ESI (*m*/*z*): $[M + H]^+$ calculated for $C_{20}H_{15}N_6^+$, 339.1353; found, 339.1560.

4-(6-((6-Ethynylpyridin-2-yl)Ethynyl)-1*H*-Benzo[*d*]Imidazol-1-yl)Pyrimidin-2-Amine (13). To a solution of 4-(6-((6-((triisopropylsilyl)ethynyl)pyridin-2-yl)ethynyl)-1*H*-benzo[*d*]-imidazol-1-yl)pyrimidin-2-amine 67 (92.0 mg and 0.187 mmol) in THF (2.0 mL) at 0 °C was added TBAF (1 M in THF, 0.200 mL, 0.200 mmol), and the reaction was carried out according to general procedure A. The resulting solid was purified directly by flash column chromatography (silica, 0−10% methanol in dichloromethane) to yield the desired product as a white solid (41.0 mg, 0.122 mmol, 65%); m.p. 234−236 °C. ^{1}H-NMR (500 MHz, DMSO-d_6): δ 4.43 (s, 1H), 7.17 (d, *J* = 5.6 Hz, 1H), 7.20 (s, 2H), 7.59 (dt, *J* = 1.4, 7.9 Hz, 2H), 7.72 (dd, *J* = 1.0, 7.9 Hz, 1H), 7.82 (d, *J* = 8.3 Hz, 1H), 7.90 (t, *J* = 7.8 Hz, 1H), 8.39 (d, *J* = 5.5 Hz, 1H), 8.88 (d, *J* = 1.5 Hz, 1H), 9.17 (s, 1H) ppm. ^{13}C-NMR (126 MHz, DMSO-d_6): δ 81.2, 82.9, 88.3, 90.6, 98.4, 117.3, 120.2, 120.9, 127.3, 127.7, 128.0, 132.0, 138.1, 142.6, 143.4, 144.4, 145.5, 157.2, 161.0, 164.1 ppm. HRMS-ESI (*m*/*z*): $[M + H]^+$ calculated for $C_{20}H_{13}N_6^+$, 337.1196; found, 337.1183.

4-(6-((2-Ethynylpyridin-4-yl)Ethynyl)-1*H*-Benzo[*d*]Imidazol-1-yl)Pyrimidin-2-Amine (14). A stirred solution of 4-(6-((2-((trimethylsilyl)ethynyl)pyridin-4-yl)ethynyl)-1*H*-benzo[*d*]imidazol-1-yl)pyrimidin-2-amine 54 (120 mg and 0.294 mmol) in methanol (3.0 mL) at 25 °C and potassium carbonate (5.00 mg and 36.2 μmol) was stirred at room temperature for 5 h. The solvent was then removed in vacuo, and purification by automated flash column chromatography (silica, 0−10% methanol in dichloromethane) yielded the desired product as an off-white solid (80.0 mg, 0.238 mmol, and 83%), m.p.: 187−189 °C. ^{1}H-NMR (500 MHz, DMSO-d_6): δ 4.46 (s, 1H), 7.17−7.21 (m, 2H), 7.57−7.58 (m, 1H), 7.62−7.64 (m, 2H), 7.76 (dd, *J* = 0.9, 1.7 Hz, 1H), 7.84 (dd, *J* = 0.7, 8.3 Hz, 1H), 8.40 (d, *J* = 5.5 Hz, 1H), 8.64 (dd, *J* = 0.9, 5.2 Hz, 1H), 8.93 (dd, *J* = 0.7, 1.6 Hz, 1H), 9.20 (s, 1H) ppm. ^{13}C-NMR (DMSO-d_6): δ 81.6, 82.9, 86.1, 96.2, 98.3, 117.1, 120.6, 120.9, 125.6, 127.8, 129.1, 131.7, 132.0, 142.6, 144.4, 145.6, 151.0, 157.2, 161.0, 164.0 ppm. HRMS-ESI (*m*/*z*): $[M + H]^+$ calculated for $C_{20}H_{13}N_6^+$, 337.1196; found, 337.1211.

4-(6-((5-Ethynylpyridin-3-yl)Ethynyl)-1*H*-Benzo[*d*]Imidazol-1-yl)Pyrimidin-2-Amine (15). 4-(6-((5-((Triisopropylsilyl)-ethynyl)pyridin-3-yl)ethynyl)-1*H*-benzo[*d*]imidazol-1-yl)pyrimidin-2-amine 66 (80.0 mg and 0.162 mmol) in THF (1.8 mL) at 0 °C was added, and TBAF (1 M in THF, 0.180 mL, and 0.180 mmol) was reacted under the conditions described in general procedure A. The remaining solid was purified by automated flash column chromatography (silica, 0−10% methanol in dichloromethane) to yield the desired compound as a white solid (22.0 mg, 65.4 μmol, and 41%); m.p. 248−249 °C. ^{1}H-NMR (500 MHz, DMSO-d_6): δ 4.56 (s, 1H), 7.14 (s, 2H), 7.18 (d, *J* = 5.6, 1H), 7.57 (dd, *J* = 1.6, 8.3 Hz, 1H), 7.83 (dd, *J* = 0.7, 8.3 Hz, 1H), 8.15 (t, *J* = 2.0 Hz, 1H), 8.40 (d, *J* = 5.5 Hz, 1H), 8.70 (d, *J* = 2.0 Hz, 1H), 8.82 (d, *J* = 2.0 Hz, 1H), 8.90 (dd, *J* = 0.7, 1.6 Hz, 1H), 9.18 (s, 1H) ppm. ^{13}C-NMR (126 MHz, DMSO-d_6): δ 80.0, 85.2, 85.5, 94.8, 98.3, 117.6, 119.2, 120.1, 120.3, 120.8, 127.6, 132.0, 141.3, 144.2, 145.4, 147.8, 151.6, 157.2, 161.0, 164.0 ppm. HRMS-ESI (*m*/*z*): $[M + H]^+$ calculated for $C_{20}H_{13}N_6^+$, 337.1196; found, 337.1186.

4-(6-((4-Ethynylpyrimidin-2-yl)Ethynyl)-1*H*-Benzo[*d*]-Imidazol-1-yl)Pyrimidin-2-Amine (16). 4-(6-((4-((Trimethylsilyl)ethynyl)pyridin-2-yl)ethynyl)-1*H*-benzo[*d*]imidazol-1-yl)pyrimidin-2-amine 68 (80.0 mg, 0.196 mmol) was stirred in methanol (2.30 mL) at 25 °C, and potassium carbonate (3.30 mg and 23.9 μmol) was stirred at 25 °C for 5 h. The solvent was then removed in vacuo, and the crude product was purified using automated flash column chromatography (silica, 0−10% methanol in dichloromethane) to yield the desired product as an off-white solid (16.0 mg, 47.6 μmol, and 24%), m.p.: 157−159 °C. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 3.28 (s, 1H), 5.36 (s, 2H), 6.81 (d, *J* = 5.5 Hz, 1H), 7.25 (dd, *J* = 1.5, 5.1 Hz, 1H), 7.53 (dd, *J* = 1.5, 8.3 Hz, 1H), 7.57 (d, *J* = 1.3 Hz, 1H), 7.76 (dd, *J* = 8.3, 0.7 Hz, 1H), 8.37 (d, *J* = 5.5 Hz, 1H), 8.46 (dd, *J* = 0.7, 1.5 Hz, 1H), 8.54 (dd, *J* = 0.9, 5.2 Hz, 1H), 8.58 (s, 1H) ppm. ^{13}C-NMR (126 MHz, $CDCl_3$): δ 80.3, 82.6, 83.3, 90.8, 99.6, 118.2, 118.6, 120.9, 125.0, 128.1, 129.4, 130.9, 131.05, 131.71, 142.1, 150.1, 156.9, 157.15, 160.5, 163.1 ppm. HRMS-ESI (*m*/*z*): $[M + H]^+$ calculated for $C_{20}H_{13}N_6^+$, 336.1196; found, 337.1211.

4-(6-((4-Vinylpyrimidin-2-yl)Ethynyl)-1*H*-Benzo[*d*]Imidazol-1-yl)Pyrimidin-2-Amine (17). 4-(6-Ethynyl-1*H*-benzo[*d*]imidazol-1-yl)pyrimidin-2-amine 4 (50.0 mg and 0.212 mmol) was reacted with 2-chloro-4-vinylpyrimidine 60 (39.0 mg and 0.276 mmol) under similar conditions to those described in procedure B. The crude product was purified by automated flash column chromatography twice (silica, 0−50% ethyl acetate in petroleum ether; C_{18} reversed-phase flash cartridge, 0−65% methanol in dichloromethane) to yield the title compound as a white solid (15.0 mg, 44.2 μmol, 16%); m.p. 212−214 °C. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 5.43 (s br, 2H), 5.78 (d, *J* = 10.9 Hz, 1H), 6.50 (d, *J* = 17.4 Hz, 1H), 6.77 (dd, *J* = 10.7, 17.4 Hz, 1H), 7.27 (d, *J* = 5.1 Hz, 2H), 7.69 (d, *J* = 7.6 Hz, 1H), 7.82 (d, *J* = 9.2 Hz, 1H), 8.42 (d, *J* = 4.5 Hz, 1H), 8.59−8.71 (m, 3H) ppm. ^{13}C-NMR (126 MHz, $CDCl_3$): δ 87.0, 87.2, 98.6, 115.0, 116.6, 118.2, 119.9, 123.2, 127.7, 133.8, 141.2, 152.3, 155.9, 156.7, 159.4, 162.0, 162.1 ppm. HRMS-ESI (*m*/*z*): $[M + H]^+$ calculated for $C_{19}H_{14}N_7^+$, 340.1305; found 340.1308.

4-(6-((4-Ethynylpyrimidin-2-yl)Ethynyl)-1*H*-Benzo[*d*]-Imidazol-1-yl)Pyrimidin-2-Amine (18). 4-(6-((4-((Triisopropylsilyl)ethynyl)pyrimidin-2-yl)ethynyl)-1*H*-benzo[*d*]-imidazol-1-yl)pyrimidin-2-amine 69 (30.0 mg and 60.8 μmol) in THF (0.79 mL) and TBAF (1.0 M in THF, 73.0 μL, and 73.0 μmol) at 0 °C were reacted under similar conditions to those described in general procedure A. The solvent was then removed in vacuo, and purification by automated flash column chromatography (silica, 0−10% methanol in dichloromethane) yielded the desired product as an off-white solid (8.00 mg, 23.7 μmol, 39%), m.p.: 183−185 °C. ^{1}H-NMR (500 MHz, DMSO-d_6): δ 4.90 (s, 1H), 7.18 (d, *J* = 5.5 Hz, 1H), 7.24 (bs, 1H), 7.64 (dd, *J* = 1.7, 8.4 Hz, 1H), 7.68 (d, *J* = 5.2 Hz, 2H), 7.86 (d, *J* = 8.2 Hz, 1H), 8.40 (d, *J* = 5.5 Hz, 1H), 8.96−8.87 (m, 2H), 9.21 (s, 1H) ppm. ^{13}C-NMR (126 MHz, DMSO-d^6): δ 80.9,

https://doi.org/10.1021/acs.jmedchem.0c01249
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86.4, 88.0, 88.8, 98.4, 116.3, 120.5, 121.0, 123.2, 128.4, 132.0, 144.7, 146.0, 150.1, 152.8, 157.2, 159.2, 161.0, 164.10 ppm. HRMS-ESI (*m/z*): $[M + H]^+$ calculated for $C_{19}H_{12}N_7^+$, 338.1149; found 338.1122.

4-(6-((2-Vinylpyrimidin-4-y)Ethynyl)-1*H*-Benzo[*d*]Imidazol-1-yl)Pyrimidin-2-Amine (19). To a solution of 4-(6-((2-chloropyrimidin-4-yl)ethynyl)-1*H*-benzo[*d*]imidazol-1-yl)pyrimidin-2-amine 52 (37.0 mg and 0.106 mmol) in DMF (2 mL) was added tributylvinyltin (46.0 μL and 0.16 mmol) at 25 °C. The solution was sparged with nitrogen for 5 min before addition of *tetrakis*(triphenylphosphine)palladium(0) (0.700 mg, 0.610 μmol). The mixture was further degassed for 5 min and allowed to stir at 100 °C for 12 h. The reaction mixture was filtered through a Celite pad, diluted with water, and extracted with ethyl acetate (3 × 50 mL). The combined organic layers were dried over magnesium sulfate, filtered, and concentrated in vacuo. Purification by automated flash column chromatography (silica, 0−6% methanol in dichloromethane) yielded the desired product as a yellow solid (13.0 mg, 38.3 mmol, 36%), m.p.: > 250 °C. ^{1}H-NMR (DMSO-d_6): δ 5.82 (dd, *J* = 1.80, 10.45 Hz, 1H), 6.60 (dd, *J* = 1.80, 17.26 Hz, 1H), 6.85 (dd, *J* = 10.45, 17.26 Hz, 1H), 7.19 (d, *J* = 5.60 Hz, 1H), 7.21−7.26 (brs, 2H), 7.63 (d, *J* = 5.05 Hz, 1H), 7.65 (dd, *J* = 1.60, 8.30 Hz, 1H), 7.86 (d, *J* = 8.30 Hz, 1H), 8.41 (d, *J* = 5.50 Hz, 1H), 8.88 (d, *J* = 5.05 Hz, 1H), 8.95 (d, *J* = 1.00 Hz, 1H), 9.21 (s, 1H) ppm. ^{13}C-NMR (DMSO-d_6): δ 87.1, 94.6, 98.4, 116.4, 120.7, 121.0, 122.3, 125.0, 128.2, 132.0, 136.7, 144.7, 146.0, 150.5, 157.2, 158.5, 161.1, 164.0, 164.1 ppm. HRMS-ESI (*m/z*): $[M + H]^+$ calculated for $C_{19}H_{14}N_7^+$, 340.1305; found 340.1313.

4-(6-((2-Ethynylpyrimidin-4-yl)Ethynyl)-1*H*-Benzo[*d*]-Imidazol-1-yl)Pyrimidin-2-Amine (20). 4-(6-((2-((Triisopropylsilyl)ethynyl)pyrimidin-4-yl)ethynyl)-1*H*-benzo[*d*]-imidazol-1-yl)pyrimidin-2-amine 55 (50.0 mg and 0.101 mmol) in THF (1.0 mL) and TBAF (1.0 M in THF, 0.111 mL, and 0.111 mmol) were reacted at 0 °C under conditions described in general procedure A. The crude product was purified by automated flash column chromatography (silica, 0−10% methanol in dichloromethane) to yield the desired compound (7.00 mg, 20.8 μmol, 20%), m.p.: 192−194 °C. ^{1}H-NMR (DMSO-d_6): δ 4.50 (s, 1H), 7.10 (d, *J* = 5.6 Hz, 1H), 7.21 (bs, 2H), 7.66 (dd, *J* = 1.6, 8.3 Hz, 1H), 7.78 (d, *J* = 5.2 Hz, 1H), 7.87 (d, *J* = 8.4 Hz, 1H), 8.40 (d, *J* = 5.5 Hz, 1H), 8.89 (d, *J* = 5.1 Hz, 1H), 8.96 (d, *J* = 1.6 Hz, 1H), 9.22 (s, 1H) ppm. ^{13}C-NMR (DMSO-d_6): δ 79.6, 82.4, 86.4, 95.7, 98.4, 116.1, 120.9, 121.0, 123.6, 128.3, 132.0, 144.8, 146.1, 150.8, 152.0, 157.2, 158.9, 161.0, 164.0 ppm. HRMS-ESI (*m/z*): $[M + H]^+$ calculated for $C_{19}H_{12}N_7^+$, 338.1149; found 338.1296.

4-(6-((6-Ethynylpyrimidin-4-yl)Ethynyl)-1*H*-Benzo[*d*]-Imidazol-1-yl)Pyrimidin-2-Amine (21). 4-(6-((6-((Triisopropylsilyl)ethynyl)pyrimidin-4-yl)ethynyl)-1*H*-benzo[*d*]-imidazol-1-yl)pyrimidin-2-amine 56 (197 mg and 0.399 mmol) in THF (3.9 mL) and TBAF (1 M in THF, 0.440 mL, and 0.440 mmol) were reacted at 0 °C under similar conditions to the ones reported in general procedure A. The resulting solid was purified by automated flash column chromatography (silica, 0−10% methanol in dichloromethane) to yield the desired product as a white solid (50.0 mg, 0.148 mmol, and 37%). m.p. 210−212 °C. ^{1}H-NMR (500 MHz, DMSO-d_6): δ 4.88 (s, 1H), 7.18 (d, *J* = 5.6 Hz, 1H), 7.21 (s, 2H), 7.64 (dd, *J* = 1.6, 8.3 Hz, 1H), 7.86 (dd, *J* = 0.7, 8.3 Hz, 1H), 7.93 (d, *J* = 1.4 Hz, 1H), 8.40 (d, *J* = 5.5 Hz, 1H), 8.95 (dd, *J* = 0.7, 1.7 Hz, 1H), 9.20 (d, *J* = 1.4 Hz, 1H), 9.21 (s, 1H). ^{13}C-NMR (126 MHz, DMSO-d_6): δ 80.6, 86.1, 86.1, 95.7, 97.9, 115.7, 120.4, 120.5, 126.0, 127.7, 131.5, 144.2, 145.6, 149.4, 150.5, 156.7, 159.1, 160.6, 163.5 ppm. HRMS-ESI (*m/z*): $[M + H]^+$ calculated for $C_{19}H_{12}N_7^+$, 338.1149; found 338.1181.

4-(6-((6-Ethynylpyrazin-2-yl)Ethynyl)-1*H*-Benzo[*d*]Imidazol-1-yl)Pyrimidin-2-Amine (22). To a solution of 4-(6-((6-((triisopropylsilyl)ethynyl)pyrazin-2-yl)ethynyl)-1*H*-benzo[*d*]-imidazol-1-yl)pyrimidin-2-amine 57 (62.0 mg and 0.126 mmol) in DMF (1.2 mL) at 25 °C was added cesium fluoride (23.0 mg and 0.138 mmol). The resulting solution was stirred at room temperature for 1 h. The reaction solvent was removed in vacuo, and the remaining solid was purified by automated flash column chromatography (silica, 0−10% methanol in dichloromethane) to yield the compound as a white solid (15.0 mg, 44.4 μmol, and 35%); m.p. 207−209 °C. ^{1}H-NMR (500 MHz, DMSO-d_6): δ 4.79 (s, 1H), 7.19 (d, *J* = 5.6 Hz, 1H), 7.22 (s, 2H), 7.64 (dd, *J* = 1.6, 8.4 Hz, 1H), 7.86 (d, *J* = 8.3 Hz, 1H), 8.40 (d, *J* = 5.6 Hz, 1H), 8.80 (s, 1H), 8.93 (s, 1H), 8.95 (s, 1H), 9.22 (s, 1H). ^{13}C-NMR (126 MHz, DMSO-d_6): δ 80.3, 85.4, 85.6, 94.6, 98.4, 116.6, 120. 6, 121.0, 128.1, 132.0, 138.5, 139.5, 144.6, 145.9, 146.4, 147.1, 157.2, 161.0, 164.0 ppm. HRMS-ESI (*m/z*): $[M + H]^+$ calculated for $C_{19}H_{12}N_7^+$, 338.1149; found 338.1157.

4-(6-((6-Vinylpyridin-3-yl)Ethynyl)-1*H*-Benzo[*d*]Imidazol-1-yl)Pyrimidin-2-Amine (23). 4-(6-Bromo-1*H*-benzo[*d*]imidazol-1-yl)pyrimidin-2-amine 32 (50.0 mg and 0.172 mmol), *bis*(triphenylphosphine)palladium(II) dichloride (12.0 mg and 17.2 μmol), copper(I) iodide (3.00 mg and 17.2 μmol), DMF (0.4 mL), DIPEA (0.2 mL), and 5-ethynyl-2-vinylpyridine 44 (44.0 mg, 0.344 mmol) were reacted under the conditions described in general procedure B for 16 h. The solvent was then removed in vacuo, and the crude product was purified by automated flash column chromatography (silica, 0−10% methanol in dichloromethane) to yield the desired product an off-white solid (15.0 mg, 44.3 μmol, and 25%), m.p.: 208−210 °C. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 5.28 (s, 2H) 5.55 (dd, *J* = 1.1, 10.7 Hz, 1H), 6.26 (dd, *J* = 1.1, 17.5 Hz, 1H), 6.84 (dd, *J* = 10.8, 17.5 Hz, 1H), 6.91 (d, *J* = 5.5 Hz, 1H), 7.36 (d, *J* = 8.1 Hz, 1H), 7.57 (dd, *J* = 1.5, 8.3 Hz, 1H), 7.78−7.87 (m, 2H), 8.41 (d, *J* = 0.9 Hz, 1H), 8.46 (d, *J* = 5.4 Hz, 1H), 8.65 (s, 1H), 8.76 (d, *J* = 1.5 Hz, 1H) ppm. ^{13}C-NMR (126 MHz, DMSO-d_6): δ 86.5, 94.3, 98.3, 118.0, 118.8, 120.0, 120.1, 120.7, 121.7, 127.6, 132.0, 136.7, 139.5, 144.1, 145.2, 151.9, 154.2, 157.2, 161.0, 164.0 ppm. HRMS-ESI (*m/z*): $[M + H]^+$ calculated for $C_{20}H_{15}N_6^+$, 339.1353; found 339.131349.

4-(6-((2-Ethynylpyrimidin-5-yl)Ethynyl)-1*H*-Benzo[*d*]-Imidazol-1-yl)Pyrimidin-2-Amine (24). 4-(6-((2-((Triisopropylsilyl)ethynyl)pyrimidin-5-yl)ethynyl)-1*H*-benzo[*d*]-imidazol-1-yl)pyrimidin-2-amine 58 (15.0 mg and 30.4 μmol) in anhydrous THF (1 mL) and TBAF (36.0 μL and 36.0 μmol) were reacted at 0 °C as described in general procedure A. The crude compound was then purified by automated flash chromatography purification (silica, 0−6% methanol in dichloromethane) to yield the desired compound as a yellow solid (6.00 mg, 17.8 mmol, 59%), m.p.: 229−231 °C. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 4.62 (s, 1H), 7.19 (d, *J* = 5.60 Hz, 1H), 7.18−7.20 (m, NH_2, 2H), 7.59−7.60 (dd, *J* = 1.60, 8.30 Hz, 1H), 7.85 (d, *J* = 8.25 Hz, 1H),8.39−8.40 (d, *J* = 5.55 Hz, 1H), 8.93 (d, 0.95 Hz, 1H), 9.05 (s, 2H), 9.19 (s, 1H) ppm. ^{13}C-NMR (126 MHz, $CDCl_3$): δ 81.7, 82.2, 99.8, 117.8, 118.06, 118.91, 119.0, 121.2, 127.8, 131.6, 142.3, 145.7, 149.5, 156.8, 158.8, 160.8, 163.2 ppm (C_q not resolved). HRMS-ESI (*m/z*): $[M + H]^+$ calculated for $C_{19}H_{12}N_7^+$, 338.1149; found 338.1223.

4-(6-((5-Ethynylpyrimidin-2-yl)Ethynyl)-1*H*-Benzo[*d*]-Imidazol-1-yl)Pyrimidin-2-Amine (25). 4-(6-((5-((Triisopropylsilyl)ethynyl)pyrimidin-2-yl)ethynyl)-1*H*-benzo[*d*]-imidazol-1-yl)pyrimidin-2-amine 70 (39.0 mg and 79.0 μmol) in THF (0.79 mL) and TBAF (1.0 M in THF, 87.0 μL, and 87.0 μmol) were reacted at 0 °C as described in general procedure A. The solution was stirred for 5 min before methanol (0.25 mL) was added to quench the reaction resulting in a solid precipitating out of solution. The solid was filtered and washed with dichloromethane to yield the desired compound as a brown solid (17.0 mg, 50.4 μmol, and 64%), m.p.: 243−245 °C. ^{1}H-NMR (500 MHz, DMSO-d_6): δ 4.87 (s, 1H), 7.18 (s, 1H), 7.23 (bs, 2H), 7.65 (t, *J* = 9.1 Hz, 1H), 7.86 (d, *J* = 8.5 Hz, 1H), 8.42 (s, 1H), 8.92 (d, *J* = 9.8 Hz, 1H), 9.00 (s, 1H), 9.17 (s, 1H), 9.20−9.26 (m, 1H) ppm. ^{13}C-NMR (126 MHz, DMSO-d_6): δ 80.9, 86.4, 88.0, 88.8, 98.6, 116.3, 117.0, 120.5, 121.1, 123.2, 128.4, 132.0, 144.7, 150.8, 157.2, 159.2, 160.3, 161.0, 164.1 ppm. HRMS-ESI (*m/z*): $[M + H]^+$ calculated for $C_{19}H_{12}N_7^+$, 338.1149; found 338.1218.

4-(6-((4-Methylpyrimidin-2-yl)Ethynyl)-1*H*-Benzo[*d*]-Imidazol-1-yl)Pyrimidin-2-Amine (26). 2-Bromo-4-methyl pyrimidine 76 (35.0 mg and 0.202 mmol), *bis*(triphenylphosphine)-palladium(II) dichloride (14.2 mg and 20.2 μmol), copper(I) iodide (3.80 mg and 20.2 μmol), DMF (1.2 mL), DIPEA (0.64 mL), and 4-(6-ethynyl-1*H*-benzo[*d*]imidazol-1-yl)pyrimidin-2-amine 4 (57.1 mL

https://doi.org/10.1021/acs.jmedchem.0c01249
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and 0.243 mmol) were reacted under similar conditions to the ones described in general procedure B. The crude product was purified by automated flash column chromatography (silica; 0−20% methanol in dichloromethane) to yield the title compound as a white solid (26.0 mg, 79.4 μmol, 40%), m.p. >250 °C. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.60 (s, 3H), 5.37 (s, 2H), 6.88 (dd, J = 0.8, 5.5 Hz, 1H), 7.14 (d, J = 5.2 Hz, 1H), 7.70 (dt, J = 1.2, 8.3 Hz, 1H), 7.83 (dt, J = 0.8, 8.3 Hz, 1H), 8.44 (dd, J = 0.8, 5.5 Hz, 1H), 8.62 (ddd, J = 0.9, 1.9, 3.3 Hz, 2H), 8.65 (d, J = 0.9 Hz, 1H) ppm. ^{13}C-NMR (126 MHz, $CDCl_3$): δ 24.5, 88.1, 88.3, 99.8, 117.7, 119.4, 119.6, 121.0, 128.8, 131.6, 142.4, 145.9, 153.1, 156.9, 157.1, 160.8163.3, 167.9 ppm. HRMS-ESI (*m/z*): $[M + NH_4]^+$ calculated for $C_{18}H_{14}N_7^+$, 328.1305; found 345.1571.

***N*-(5-Bromo-2-Nitrophenyl)-2-Chloropyrimidin-4-Amine (29).** To a stirred suspension of sodium hydride (60% mineral oil dispersion, 3.90 g, 90.2 mmol) in THF (100 mL) at 0 °C was added 4-amino-2-chloropyrimidine **28** (4.42 g and 34.1 mmol). The suspension was stirred for 10 min, then treated with 4-bromo-2-fluoro-1-nitrobenzene **27** (5.00 g, 22.5 mmol), stirred for 1 h, allowed to attain room temperature, and then stirred for a further 2 h. The reaction mixture was quenched by pouring ice water (500 mL) and stirred for 2 h. The yellow precipitate formed was filtered and washed with water (300 mL), then dissolved in methanol, and concentrated in vacuo. Azeotropic distillation with acetonitrile (3 × 100 mL) removed residual water to yield the title compound as a yellow powder (5.90 g, 17.9 mmol, and 79%). ^{1}H-NMR (500 MHz, DMSO-d_6): δ 6.93 (d, J = 5.8 Hz, 1H), 7.62 (dd, J = 2.1, 8.7 Hz, 1H), 7.96−7.98 (m, 2H), 8.30 (d, J = 5.8 Hz, 1H), 10.4 (s, 1H) ppm. ^{13}C-NMR (126 MHz, DMSO-d_6): δ 98.1, 125.7, 126.5, 127.6, 128.1, 136.1, 139.2, 158.4, 160.0, 163.1 ppm. LRMS *m/z* (method 2): retention time = 1.37 min, (ES^+) *m/z* = 331.1 $[M + H]^+$.

***N*^{4}-(5-Bromo-2-Nitrophenyl)Pyrimidine-2,4-Diamine (30).** Eighteen microwave vials (10−20 mL) were charged with *N*-(5-Bromo-2-nitrophenyl)-2-chloropyrimidin-4-amine **29** (520 mg per vial and 1.58 mmol). To each were added isopropanol (5.0 mL) and ammonium hydroxide (33% wt in water and 10 mL). The vials were sealed, then heated to 90 °C, stirred for 48 h, and then cooled to 0 °C in an ice bath. Once cooled, the reaction suspension was combined by pouring ice water (200 mL), stirred for 45 min, and then filtered. The orange filter cake was washed with water (3 × 200 mL) and then dried in a vacuum oven for 16 h to yield the title compound as an orange solid (8.02 g, 25.9 mmol, and 91%). ^{1}H-NMR (500 MHz, DMSO-d_6): δ 6.19 (d, J = 5.6 Hz, 1H), 6.32 (bs, 2H), 7.39 (dd, J = 2.1, 8.9 Hz, 1H), 7.95 (d, J = 3.4 Hz, 1H), 7.96 (s, 1H), 8.37 (d, J = 2.0 Hz, 1H), 9.61 (s, 1H) ppm. ^{13}C-NMR (126 MHz, DMSO-d_6): δ 107.2, 125.7, 126.5, 127.6, 128.1, 136.1, 139.2, 158.4, 160.0, 163.1 ppm. LRMS *m/z* (method 1): retention time = 1.01 min, (ES^+) *m/z* = 312.1 $[M + H]^+$.

***N*^{4}-(2-Amino-5-Bromophenyl)Pyrimidine-2,4-Diamine (31).** A stirred solution of *N*^{4}-(5-bromo-2-nitrophenyl)pyrimidine-2,4-diamine **30** (8.02 g and 25.9 mmol) in ethanol (172 mL) was treated with tin(II) chloride (17.2 g and 90.5 mmol). The reaction mixture was heated to 65 °C and stirred for 90 min and then cooled to room temperature, and the solvent was removed under reduced pressure. The orange residue was suspended in ice water, and the pH was adjusted to 10 by slow addition of saturated aqueous sodium carbonate solution. Ethyl acetate (200 mL) and Rochelle's salt (saturated aqueous sodium potassium tartrate, 150 mL) were added, and the mixture was stirred vigorously for 45 min until two phases were clearly distinguishable. The organic layer was extracted, and the aqueous layer was extracted with ethyl acetate (3 × 200 mL). Combined organic phases were dried over magnesium sulfate, filtered, and concentrated in vacuo to yield the title compound (4.40 g, 15.7 mmol, and 61%). This material was taken onto the next step without further purification. ^{1}H-NMR (500 MHz, DMSO-d_6): δ 5.04 (bs, 2H), 5.81 (d, J = 5.7 Hz, 1H), 6.05 (bs, 2H), 6.69 (d, J = 8.6 Hz, 1H), 7.03 (dd, J = 2.2, 8.5 Hz, 1H), 7.37 (d, J = 2.0 Hz, 1H), 7.77 (d, J = 5.7 Hz, 1H), 8.14 (s, 1H) ppm. ^{13}C-NMR (DMSO-d_6): δ 95.8, 106.5, 117.5, 126.4, 128.3, 128.4, 142.8, 157.0, 162.3, 163.6 ppm. LRMS *m/z* (method 1): retention time = 0.94 min, (ES^+) *m/z* = 310.2, 312.2 $[M + H]^+$.

4-(6-Bromo-1H-Benzo[*d*]Imidazol-1-yl)Pyrimidin-2-Amine (32). N4-(2-Amino-5-bromophenyl)pyrimidine-2,4-diamine **31** (4.40 g and 15.7 mmol) was dissolved in methanol (40 mL) and THF (176 mL). To this were added trimethylorthoformate (71.0 mL and 466 mmol) and para-toluenesulfonic acid (299 mg and 1.57 mmol), and the resulting solution was heated to 65 °C for 1 h. A further 26 mL of trimethylorthoformate (241 mmol) was added to the reaction mixture, which was stirred for a further 5 h until completion by LCMS. Once cooled to room temperature, the reaction mixture was neutralized by slow addition of saturated aqueous sodium bicarbonate solution. The phases were separated, and then the aqueous phase was extracted with dichloromethane (3 × 150 mL). Combined organic phases were washed with water (100 mL), dried over magnesium sulfate, filtered, and concentrated in vacuo. The crude residue obtained was purified by flash column chromatography (silica, 0−75% ethyl acetate in petroleum ether) to give compound as a white powder (4.00 g, 13.8 mmol, and 88%). 1H-NMR (500 MHz, DMSO-d6): δ 7.15 (d, J = 5.6 Hz, 1H), 7.18 (bs, 2H), 7.51 (dd, J = 1.9, 8.5 Hz, 1H), 7.72 (d, J = 8.6 Hz, 1H), 8.38 (d, J = 5.6 Hz, 1H), 8.84 (d, J = 1.9 Hz, 1H), 9.08 (s, 1H) ppm. 13C-NMR (126 MHz, DMSO-d6): δ 98.2, 117.4, 119.2, 121.9, 127.1, 133.0, 143.4, 143.9, 157.2, 160.9, 163.9 ppm. LRMS *m/z* (method 1): retention time = 1.04 min, (ES+) *m/z* = 290.1 $[M + H]^+$.

4-(6-Vinyl-1H-Benzo[*d*]Imidazol-1-yl)Pyrimidin-2-Amine (33). 4-(6-Bromo-1H-benzo[*d*]imidazol-1-yl)pyrimidin-2-amine **32** (400 mg and 1.38 mmol) was dissolved in anhydrous 1,4-dioxane (1.7 mL) in a 5 mL microwave vial. Vinyl tributyltin (605 μL and 2.07 mmol) and tetrakis(triphenylphosphine)palladium (0) (79.0 mg, 70.0 μmol) were added, and then the reaction mixture was heated to 110 °C for 16 h. The reaction mixture was filtered through Celite, the solvent was evaporated, and the crude product was dissolved in ethyl acetate and sodium hydroxide aqueous solution (2 M). The aqueous layer was extracted with ethyl acetate, and the combined organic layers were washed with water and dried over sodium sulfate and then concentrated in vacuo. The residue was purified by automated flash column chromatography (silica, 0−15% methanol in dichloromethane) to give the title compound as an off-white solid (225 mg, 0.948 mmol, and 69%). 1H-NMR (500 MHz, DMSO-d6): δ 5.31 (d, J = 11.1 Hz, 1H), 5.98 (d, J = 17.8 Hz, 1H), 6.92 (dd, J = 11.0, 17.7 Hz, 1H), 7.13 (bs, 2H), 7.15 (d, J = 5.6 Hz, 1H), 7.53 (dd, J = 1.6, 8.4 Hz, 1H), 7.71 (d, J = 8.3 Hz, 1H), 8.38 (d, J = 5.5 Hz, 1H), 8.68 (d, J = 1.2 Hz, 1H), 9.03 (s, 1H) ppm. 13C-NMR (126 MHz, DMSO-d6): δ 98.3, 114.4, 114.6, 120.2, 122.1, 132.5, 134.3, 137.7, 143.0, 144.7, 157.4, 160.9, 164.0 ppm. LRMS *m/z* (method 1): retention time = 1.07 min, (ES+) *m/z* = 238.1 $[M + H]^+$.

4-(6-((Triisopropylsilyl)Ethynyl)-1H-Benzo[*d*]Imidazol-1-yl)-Pyrimidin-2-Amine (34). 4-(6-Bromo-1H-benzo[*d*]imidazol-1-yl)-pyrimidin-2-amine **32** (500 mg, 1.72 mmol), bis-(triphenylphosphine)palladium(II) dichloride (120 mg, 0.172 mmol), and copper(I) iodide (32.0 mg, 0.172 mmol) were sealed in a microwave vial and purged with nitrogen and vacuum. DMF (8.8 mL) was added, and the solution was stirred while sparging with nitrogen for 5 min before DIPEA (4.4 mL) was added and further sparging for 5 min. TIPS acetylene (0.580 mL, 2.58 mmol) was added and sparged for 5 min before heating at 55 °C for 16 h. The reaction mixture was filtered through a Celite pad. The resulting solution was dried in vacuo before being taken up in dichloromethane and dry-loaded on silica for automated flash column chromatography (silica, 0−8% methanol in dichloromethane) to yield the product as a pale yellow solid (603 mg, 1.54 mmol, and 90%). 1H-NMR (500 MHz, $CDCl_3$): δ 1.18 (s, 21H), 5.30 (s, 2H), 6.88 (d, J = 5.5 Hz, 1H), 7.50 (dd, J = 1.5, 8.3 Hz, 1H), 7.76 (d, J = 8.3 Hz, 1H), 8.25 (s, 1H), 8.45 (d, J = 5.5 Hz, 1H), 8.64 (s, 1H) ppm. 13C-NMR (126 MHz, $CDCl_3$): δ 11.4, 18.7, 90.6, 99.9, 107.5, 117.5, 119.9, 120.7, 128.4, 135.0, 141.9, 144.9, 156.8, 160.7, 163.1 ppm. LRMS *m/z* (method 2): retention time = 1.94 min, (ES+) *m/z* = 392.3 $[M + H]^+$.

Tert-Butyl (3-(1-(2-Aminopyrimidin-4-yl)-1H-Benzo[*d*]-Imidazol-6-yl)Prop-2-yn-1-yl)Carbamate (36). 4-(6-Bromo-1H-benzo[*d*]imidazol-1-yl)pyrimidin-2-amine **32** (150 mg and 0.517 mmol), tetrakis(triphenylphosphine)palladium(0) (60.0 mg and 50.0

https://doi.org/10.1021/acs.jmedchem.0c01249
J. Med. Chem. 2021, 64, 10001−10018

μmol), copper(I) iodide (10.0 mg and 50.0 μmol), *N*-Boc-propargylamine 35 (161 mg and 1.04 mmol), and piperidine (1.07 mL, 10.9 mmol) were combined and sparged with nitrogen for 5 min. The mixture was heated in a sealed vessel at 75 °C for 4 h. The reaction mixture was allowed to cool to 25 °C, and the solvent was removed in vacuo. The residue was purified by automated flash column chromatography (silica, 1−7% methanol in dichloromethane) to give the title compound as a pale orange solid (108 mg, 0.296 mmol, and 57%); R_f 0.45 (EtOAc; NH_2 SiO_2). LCMS *m/z* (method 2): retention time = 1.16 min, (ES+) *m/z* = 365.2 $[M + H]^+$.

4-(6-(3-Aminoprop-1-yn-1-yl)-1H-Benzo[*d*]Imidazol-1-yl)-Pyrimidin-2-Amine Trihydrochloride (37). *tert*-Butyl (3-(1-(2-aminopyrimidin-4-yl)-1H-benzo[*d*]imidazol-6-yl)prop-2-yn-1-yl)-carbamate **36** (95.0 mg, 0.261 mmol) was dissolved in dioxane (3.0 mL), and HCl in dioxane (4 M, 2.0 mL) was added. The mixture was stirred at 25 °C for 90 min, and the solvent was removed in vacuo to give a beige solid (96.0 mg and 0.320 mmol), used as the crude product for the next step.

Benzyl Prop-2-yn-1-ylcarbamate (38). Propargylamine (224 μL and 3.50 mmol) and trimethylamine (537 μL and 3.85 mmol) were dissolved in dichloromethane (18 mL) and cooled to 0 °C. Benzyl chloroformate (550 μL and 3.85 mmol) was added to the solution, which was stirred at 0 °C for 2 h and then a further 14 h at room temperature until completion by thin-layer chromatography (TLC) analysis (silica, 0−20% methanol in dichloromethane). Water and dichloromethane were added to quench the reaction and then stirred for 30 min. The organic phase was washed with water (× 3), dried over magnesium sulfate, and concentrated in vacuo then purified by automated flash column chromatography (silica, 5% methanol in dichloromethane) to give the title compound as a colorless oil (556 mg, 2.94 mmol, and 84%). 1H-NMR (500 MHz, $CDCl_3$): δ 2.27 (t, J = 2.5 Hz, 1H), 4.02 (d, *J* = 3.3 Hz, 2H), 4.96 (bs, 1H), 5.15 (s, 2H), 7.32−7.40 (m, 5H) ppm. 13C-NMR (126 MHz, $CDCl_3$): δ 29.1, 66.7, 67.1, 104.9, 109.6, 120.4, 123.8, 126.1, 135.5, 136.7, 170.5 ppm.

Benzyl but-3-yn-1-ylcarbamate (39). 3-Butynylamine hydrochloride (369 mg, 3.50 mmol) and potassium carbonate (968 mg, 7.00 mmol) were dissolved in dichloromethane (18 mL) and stirred for 30 min then cooled to 0 °C. Benzyl chloroformate (550 μL and 3.85 mmol) was added to the solution, which was stirred at 0 °C for 2 h and then a further 14 h at room temperature until completion by TLC analysis (silica, 20% methanol in dichloromethane). Water and dichloromethane were added to quench and then stirred for 30 min. The organic phase was washed with water (3 × 100 mL), dried over magnesium sulfate, concentrated in vacuo, and then purified by column chromatography (silica, 10% methanol in dichloromethane) to give the title compound as a colorless oil (683 mg, 3.36 mmol, and 96%). 1H-NMR (500 MHz, $CDCl_3$): δ 2.02 (t, *J* = 2.6 Hz, 1H), 2.42−2.45 (m, 2H), 3.37−3.41 (m, 2H), 5.11−5.17 (m, 3H), 7.33−7.39 (m, 5H) ppm. 13C-NMR (126 MHz, $CDCl_3$): δ 19.9, 39.7, 66.8, 70.1, 81.4, 128.2, 128.2, 128.6, 136.4, 156.2 ppm.

Benzyl (3-(1-(2-Aminopyrimidin-4-yl)-1H-Benzo[*d*]Imidazol-6-yl)Prop-2-yn-1-yl)Carbamate (40). 4-(6-Bromo-1H-benzo[*d*]-imidazol-1-yl)pyrimidin-2-amine **32** (220 mg and 0.758 mmol), benzyl prop-2-yn-1-ylcarbamate **38** (196 mg and 1.03 mmol), bis(triphenylphosphine)palladium(II) dichloride (49.0 mg and 70.0 μmol), copper(I) iodide (13.0 mg and 70.0 μmol), DIPEA (1.7 mL), and anhydrous DMF (3.5 mL) were added together and heated to 50 °C for 40 h. The reaction mixture was cooled to room temperature, and the solvents were evaporated. The reaction crude product was then purified by automated flash column chromatography (silica, 0−10% methanol in dichloromethane) to give as an enriched sample the desired compound as a white solid (189 mg, 0.474 mmol, 63%). The compound was used for the next step without further purification.

Benzyl (4-(1-(2-Aminopyrimidin-4-yl)-1H-Benzo[*d*]Imidazol-6-yl)but-3-yn-1-yl)Carbamate (41). Benzyl (4-(1-(2-aminopyrimidin-4-yl)-1H-benzo[*d*]imidazol-6-yl)but-3-yn-1-yl)carbamate **32** (200 mg, 0.700 mmol), benzyl but-3-yn-1-ylcarbamate **39** (211 mg, 1.03 mmol), bis(triphenylphosphine)palladium(II) dichloride (49.0 mg, 70.0 μmol), copper(I) iodide (13.0 mg, 70.0 μmol), DIPEA (1.7 mL), and anhydrous DMF (3.5 mL) were added together and heated to 50 °C for 40 h. Once completion by LCMS analysis, the reaction mixture was cooled to room temperature, and the solvents were evaporated and then purified by automated flash column chromatography (silica, 0−10% methanol in dichloromethane) to give the title compound as a white solid (227 mg, 0.550 mmol, 79%). LRMS *m/z* (method 1): retention time = 1.27 min, (ES+) *m/z* = 413.1 $[M + H]^+$. The compound was used for the next step without further purification.

4-(6-(3-Aminopropyl)-1H-Benzo[*d*]Imidazol-1-yl)Pyrimidin-2-Amine (42). Benzyl (3-(1-(2-aminopyrimidin-4-yl)-1H-benzo[*d*]-imidazol-6-yl)prop-2-yn-1-yl)carbamate **40** (168 mg, 0.422 mmol) was dissolved in methanol (4.2 mL) under an atmosphere of nitrogen. Palladium on carbon was added, and the reaction vessel was sparged with hydrogen for 15 min and then stirred at 50 °C for a further 16 h until the starting material was consumed by LCMS analysis. The reaction mixture was filtered through Celite and washed with methanol (20 mL), then concentrated in vacuo, and purified by automated flash column chromatography (silica, 0−30% methanol in dichloromethane) to give the title compound as a white solid (82.0 mg, 0.306 mmol, and 73%). 1H-NMR (500 MHz, DMSO-d6): δ 1.89 (quint, *J* = 7.5 Hz, 2H), 2.71 (t, *J* = 7.2 Hz, 2H), 2.86 (t, *J* = 7.7 Hz, 2H), 3.32−3.36 (m, 4H), 7.06 (d, *J* = 5.7 Hz, 1H), 7.27 (dd, *J* = 1.4, 8.3 Hz, 1H), 7.64 (d, *J* = 8.3 Hz, 1H), 8.34−8.36 (m, 2H), 8.85 (s, 1H) ppm. 13C-NMR (126 MHz, DMSO-d6): δ 33.2, 34.6, 40.6, 98.3, 114.6, 118.8, 124.6, 131.7, 139.5, 141.1, 142.1, 157.5, 159.8, 163.7 ppm. LRMS *m/z* (method 1): retention time = 0.72 min, (ES+) *m/z* = 269.3 $[M + H]^+$.

4-(6-(4-Aminobutyl)-1H-Benzo[*d*]Imidazol-1-yl)Pyrimidin-2-Amine (43). Benzyl (4-(1-(2-aminopyrimidin-4-yl)-1H-benzo[*d*]-imidazol-6-yl)but-3-yn-1-yl)carbamate **41** (213 mg, 0.516 mmol) was dissolved in methanol (5.2 mL) under an atmosphere of nitrogen. Palladium on carbon was added, and the reaction vessel was sparged with hydrogen for 15 min and then stirred at 50 °C for a further 16 h until the starting material was consumed by LCMS analysis. The reaction mixture was filtered through Celite and washed with methanol (20 mL), then concentrated in vacuo, and then purified by automated flash column chromatography (silica, 0−30% methanol in dichloromethane) to give the title compound as a white solid (96.0 mg, 0.340 mmol, 66%). 1H-NMR (500 MHz, CD_3OD): δ 1.40−1.42 (m, 2H), 1.65−1.66 (m, 2H), 2.50−2.52 (m, 2H),2.74−2.76 (m, 2H), 7.08 (d, *J* = 5.7 Hz, 3H), 7.19−7.20 (m, 2H), 7.64 (d, *J* = 8.3 Hz, 1H), 8.32 (d, *J* = 0.7 Hz, 2H), 8.36 (d, *J* = 5.6 Hz, 1H), 8.85 (s, 1H) ppm. 13C-NMR (126 MHz, CD_3OD): δ 29.1, 32.0, 35.7, 41.0, 98.3, 114.5, 118.8, 124.7, 131.7, 139.9, 141.1, 142.1, 157.5, 159.8, 163.7 ppm. LRMS *m/z* (method 1): retention time = 1.31 min, (ES+) *m/z* = 283.1 $[M + H]^+$.

5-Ethynyl-2-Vinylpyridine (44). To a solution of 5-((triisopropylsilyl)ethynyl)-2-vinylpyridine **75** (613 mg, 2.15 mmol) in THF (21.5 mL), cooled to 0 °C was added tetrabutylammonium fluoride (1.0 M in THF, 73.0 μL, 73.0 μmol). The solution was stirred for 30 min before water (10 mL) was added to quench the reaction. The aqueous layer was then extracted with ethyl acetate (45 mL), the resulting organic layer was dried over Na_2SO_4, and the solvent was removed in vacuo. The crude product was then purified by automated flash column chromatography (silica, 0−50% ethyl acetate in petroleum ether) to yield the desired product as a pale yellow oil (225 mg, 1.74 mmol, and 81%). 1H-NMR (500 MHz, $CDCl_3$): δ 3.26 (s, 1H), 5.56 (dd, *J* = 1.1, 10.8 Hz, 1H), 6.26 (dd, *J* = 1.1, 17.5 Hz, 1H), 6.83 (dd, *J* = 10.8, 17.5 Hz, 1H), 7.32 (dd, *J* = 0.9, 8.0 Hz, 1H), 7.75 (dd, *J* = 2.2, 8.1 Hz, 1H), 8.69 (dd, *J* = 0.9, 2.1 Hz, 1H) ppm. 13C-NMR (126 MHz, $CDCl_3$): δ 80.7, 80.8, 117.7, 119.5, 120.4, 136.3, 139.5, 152.5, 155.1 ppm.

4-(6-((2-Bromopyridin-4-yl)Ethynyl)-1H-Benzo[*d*]Imidazol-1-yl)Pyrimidin-2-Amine (51). 2-Bromo-4-iodo-pyridine **45** (250 mg, 0.884 mmol) was reacted with 4-(6-ethynyl-1H-benzo[*d*]-imidazol-1-yl)pyrimidin-2-amine **4** (200 mg, 0.850 mmol), bis-(triphenylphosphine)palladium(II) dichloride (49.0 mg, 7.00 μmol), and copper(I) iodide (13.0 mg, 7.00 μmol), sealed in a microwave vial, and purged with nitrogen and vacuum. DMF (3.7 mL) was added, and the solution was stirred while sparging with nitrogen for 5

https://doi.org/10.1021/acs.jmedchem.0c01249
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min before DIPEA (1.9 mL) was added and further sparging for 5 min. The reaction mixture was stirred at 55 °C for 2 h. The reaction mixture was filtered through Celite, and the solvent was removed in vacuo to give the desired compound as a brown powder (290 mg, 0.741 mmol, and 87%). 1H-NMR (500 MHz, DMSO-d6): δ 7.20 (s, 2H), 7.38−7.76 (m, 2H), 7.77−8.04 (m, 2H), 8.23 (s, 1H), 8.28−8.50 (m, 2H), 8.94 (s, 1H), 9.22 (s, 1H) ppm. 13C-NMR (126 MHz, DMSO-d6): δ 85.5, 97.0, 98.3, 116.9, 120.7, 120.9, 125.3, 127.8, 129.3, 132.0, 133.6, 134.0, 142.1, 145.7, 157.2, 161.0, 159.8, 163.9 ppm. LRMS *m/z* (method 2): retention time = 2.06 min, (ES+) *m/z* = 391.2 $[M + H]^+$. Used without further purification.

4-(6-((2-Chloropyrimidin-4-yl)Ethynyl)-1H-Benzo[*d*]-Imidazol-1-yl)Pyrimidin-2-Amine (52). 4-(6-Ethynyl-1H-benzo-[*d*]imidazol-1-yl)pyrimidin-2-amine **4** (30.0 mg, 0.127 mmol) and 2,4-dichoropyrimidine **46** (28.0 mg, 0.191 mmol), bis-(triphenylphosphine)palladium(II) dichloride (8.91 mg, 12.7 μmol) and copper(I) iodide (2.41 mg, 12.7 μmol) were sealed in a microwave vial and purged with nitrogen and vacuum. DMF (1.0 mL) was added, and the solution was stirred while sparging with nitrogen for 5 min before DIPEA (0.50 mL) was added and further sparging for 5 min. The reaction mixture was stirred at 55 °C for 2 h. The reaction mixture was filtered through Celite, concentrated in vacuo, and purified by automated flash column chromatography (silica, 0−20% methanol in dichloromethane) to yield the desired compound as a yellow solid (16.0 mg, 46.0 μmol, and 36%). The solid was further triturated with methanol. 1H-NMR (500 MHz, DMSO-d6): δ 7.19 (d, J = 5.3 Hz, 2H), 7.19−7.28 (m, 1H), 7.67 (d, J = 7.7 Hz, 1H), 7.83 (d, J = 4.1 Hz, 1H), 7.88 (d, J = 7.9 Hz, 1H), 8.40 (brs, 1H), 8.87 (m, 1H), 9.06 (s, 1H), 9.24 (s, 1H) ppm. 13C-NMR (126 MHz, DMSO-d6): δ 86.1, 97.1, 98.4, 115.71, 115.83121.1, 123.09, 123.11, 128.4, 132.0, 144.9, 146.3, 152.9, 157.2, 161.07, 161.59, 164.0 ppm. LRMS *m/z* (method 1): retention time = 1.22 min, (ES+) *m/z* = 348.4 $[M + H]^+$.

4-(6-((2-Chloropyrimidin-5-yl)Ethynyl)-1H-Benzo[*d*]-Imidazol-1-yl)Pyrimidin-2-Amine (53). 2,5-Dichloro pyrimidine **47** (142 mg, 0.953 mmol), bis(triphenylphosphine)palladium(II) dichloride (60.0 mg, 42.0 μmol), and copper(I) iodide (16.0 mg, 42.0 μmol) were sealed in a microwave vial and purged with nitrogen and vacuum. DMF (2.0 mL) was added, and the solution was stirred while sparging with nitrogen for 5 min. DIPEA (1.0 mL) was added with further sparging for 5 min. 4-(6-ethynyl-1H-benzo[*d*]imidazol-1-yl)-pyrimidin-2-amine **4** (100 mg, 0.425 mmol) was added, and the reaction mixture was heated at 55 °C for 16 h. The resulting solution was filtered through Celite and dried in vacuo and then dissolved in dichloromethane and methanol. The filtrate was concentrated to dryness to afford the desired compound 53 as a dark red solid (60.0 mg, 0.173 mmol, 41%). The crude product was used without further purification.

4-(6-((2-((Trimethylsilyl)Ethynyl)Pyridin-4-yl)Ethynyl)-1H-Benzo[*d*]Imidazol-1-yl)Pyrimidin-2-Amine (54). 4-(6-((2-Bromopyridin-4-yl)ethynyl)-1H-benzo[*d*]imidazol-1-yl)pyrimidin-2-amine **51** (68.0 mg and 0.174 mmol) was reacted with trimethylsilylether (94.0 mg and 0.957 mmol), bis-(triphenylphosphine) palladium(II) dichloride (18.0 mg and 26.0 μmol), and copper(I) iodide (4.90 mg and 26.0 μmol), sealed in a microwave vial, and purged with nitrogen and vacuum. DMF (1.4 mL) was added, and the solution was stirred while sparging with nitrogen for 5 min before DIPEA (0.70 mL) was added with further sparging for 5 min. The reaction mixture was stirred at 55 °C for 24 h. The reaction mixture was filtered through a Celite pad, and the solvent was removed in vacuo to give the desired product 54 as a brown powder (53.3 mg, 0.130 mmol, and 75%), used as the crude product for the next step without further purification. 1H-NMR (500 MHz, $CDCl_3$): δ 0.07 (s, 9H), 6.66 (d, J = 5.5 Hz, 1H), 7.11 (dd, J = 1.6, 5.2 Hz, 1H), 7.28−7.30 (m, 1H), 7.33 (dd, J = 0.9, 1.6 Hz, 1H), 7.38−7.46 (m, 2H), 7.59 (dd, J = 0.7, 8.4 Hz, 1H), 7.77 (s, 1H), 8.21 (d, J = 5.5 Hz, 1H), 8.32 (dd, J = 0.9, 5.1 Hz, 1H), 8.44 (s, 1H) ppm. 13C-NMR (126 MHz, $CDCl_3$): δ 17.2, 80.6, 86.3, 96.1, 100.0, 103.4, 110.2, 118.4, 120.6, 121.3, 125.0, 130.8, 132.4, 142.6, 143.5, 145.8, 150.3, 157.1, 160.9, 162.9, 163.4 ppm. LRMS *m/z* (method 2): retention time = 1.58 min, (ES+) *m/z* = 410.1 $[M + H]^+$.

4-(6-((2-((Triisopropylsilyl)Ethynyl)Pyrimidin-4-yl)Ethynyl)-1H-Benzo[*d*]Imidazol-1-yl)Pyrimidin-2-Amine (55). 4-(6-Ethynyl-1H-benzo[*d*]imidazol-1-yl)pyrimidin-2-amine **4** (55.0 mg and 0.233 mmol), 2,4-dichloropyrimidine **46** (40.0 mg and 0.265 mmol), bis(triphenylphosphine)palladium(II) dichloride (30.0 mg and 42.0 μmol), and copper(I) iodide (8.30 mg and 42.0 μmol) were sealed in a microwave vial and purged with vacuum and nitrogen. DMSO (1.0 mL) was added, and the solution was sparged with nitrogen for 5 min followed by addition of DIPEA (0.50 mL) and further sparging with nitrogen for 5 min. The reaction mixture was then heated for 15 min at 100 °C in the microwave. TIPS acetylene (0.240 mL and 1.06 mmol) was added, and the reaction mixture was heated for a further 1 h. The solvent was then removed in vacuo before purification by automated flash column chromatography (silica, 0−10% methanol in dichloromethane) to yield the product as a sticky yellow solid (52.0 mg, 0.105 mmol, 45%). 1H-NMR (500 MHz, DMSO-d6): δ 1.09−1.23 (m, 21H), 7.19 (d, J = 5.5 Hz, 3H), 7.67 (dd, J = 1.6, 8.3 Hz, 1H), 7.76 (d, J = 5.1 Hz, 1H), 7.87 (d, J = 8.3 Hz, 1H), 8.40 (d, J = 5.5 Hz, 1H), 8.87 (d, J = 5.1 Hz, 1H), 8.94−8.98 (m, 1H), 9.22 (s, 1H) ppm. LRMS *m/z* (method 2): retention time = 1.86 min, (ES+) *m/z* = 494.5 $[M + H]^+$.

4-(6-((6-((Triisopropylsilyl)Ethynyl)Pyrimidin-4-yl)Ethynyl)-1H-Benzo[*d*]Imidazol-1-yl)Pyrimidin-2-Amine (56). 4-(6-Ethynyl-1H-benzo[*d*]imidazol-1-yl)pyrimidin-2-amine **4** (300 mg, 1.27 mmol), 4,6-dichloro-pyrimidine **72** (562 mg, 1.91 mmol), bis-(triphenylphosphine)palladium(II) dichloride (84.0 mg, 0.127 mmol), and copper(I) iodide (23.0 mg, 0.127 mmol) were sealed in a microwave vial and purged with nitrogen and vacuum. DMF (10 mL) was added, and the solution was stirred while sparging with nitrogen for 5 min. DIPEA (5.0 mL) was added with further sparging for 5 min before heating at 55 °C for 16 h. TIPS acetylene (1.04 mL, 5.71 mmol) was added, and the reaction mixture was heated for a further 1 h at 55 °C. The resulting solution was dried in vacuo and then dissolved in dichloromethane and methanol and filtered through Celite. The filtrate was concentrated to dryness to afford the crude compound as a dark red solid, which was purified by automated flash column chromatography (silica, 0−10% methanol in dichloro-methane) to give the desired product 56 as a yellow solid (441 mg, 0.893 mmol, and 70%); m.p. 221−223 °C. 1H-NMR (500 MHz, DMSO-d6): δ 1.12 (d, J = 6.3 Hz, 21H), 7.10−7.30 (m, 3H), 7.65 (d, J = 8.6 Hz, 1H), 7.86 (d, J = 8.3 Hz, 1H), 7.90 (s, 1H), 8.40 (d, J = 5.5 Hz, 1H), 8.96 (s, 1H), 9.19 (s, 1H), 9.22 (s, 1H) ppm. 13C-NMR (126 MHz, DMSO-d6): δ 11.0, 18.9, 86.7, 96.2, 97.7, 98.4, 104.1, 116.3, 120.9, 121.0, 126.5, 128.3, 132.0, 144.8, 146.2, 149.8, 151.1, 157.2, 159.7, 161.0, 164.0 ppm. HRMS $[M + H]^+$ predicted for $C_{28}H_{31}N_7Si$: 494.2483; found: 494.2475.

4-(6-((6-((Triisopropylsilyl)Ethynyl)Pyrazin-2-yl)Ethynyl)-1H-Benzo[*d*]Imidazol-1-yl)Pyrimidin-2-Amine (57). 4-(6-ethynyl-1H-benzo[*d*]imidazol-1-yl)pyrimidin-2-amine 4 (55.0 mg and 0.234 mmol), 2,6-dichloropyrazine **48** (102 mg and 0.685 mmol), bis(triphenylphosphine)palladium(II) dichloride (16.0 mg and 23.4 μmol), and copper(I) iodide (5.00 mg and 23.4 μmol) were sealed in a microwave vial and purged with nitrogen and vacuum. DMF (2.5 mL) was added, and the solution was stirred while sparging with nitrogen for 5 min. DIPEA (1.3 mL) was added with further sparging for 5 min before adding TIPS acetylene (0.077 mL and 0.345 mmol) heating at 55 °C for 16 h. The resulting solution was dried in vacuo and then dissolved in dichloromethane and methanol and filtered through Celite. The filtrate was concentrated to dryness to afford the crude compound as a dark red solid, which was purified by automated flash column chromatography (silica, 0−10% methanol in dichloro-methane) to give the desired compound (62.0 mg, 0.126 mmol, and 54%); m.p. decomposed >256 °C. 1H-NMR (500 MHz, DMSO-d6): δ 1.13 (m, 21H), 7.18 (d, J = 5.6 Hz, 1H), 7.19−7.27 (br s, 2H), 7.64 (dd, J = 1.6, 8.3 Hz, 1H), 7.86 (d, J = 8.3 Hz, 1H), 8.39 (d, J = 5.5 Hz, 1H), 8.76 (s, 1H), 8.90 (s, 1H), 8.95 (d, J = 1.6 Hz, 1H), 9.21 (s, 1H) ppm. 13C-NMR (126 MHz, DMSO-d6): δ 11.1, 18.9, 85.7, 94.7, 96.2, 98.4, 103.2, 116.6, 120.6, 121.0, 128.1, 132.0, 138.7, 139.5,

https://doi.org/10.1021/acs.jmedchem.0c01249
J. Med. Chem. 2021, 64, 10001−10018

144.6, 145.9, 146.4, 146.9, 157.2, 161.0, 164.0 ppm. HRMS $[M + H]^+$ predicted for $C_{28}H_{31}N_7Si$: 494.2483; found: 494.2475.

4-(6-((2-((Triisopropylsilyl)Ethynyl)Pyrimidin-5-yl)Ethynyl)-1H-Benzo[*d*]Imidazol-1-yl)Pyrimidin-2-Amine (58). 4-(6-((2-Chloropyrimidin-5-yl)ethynyl)-1H-benzo[*d*]imidazol-1-yl)pyrimidin-2-amine **53** (40.0 mg and 0.115 mmol), bis(triphenylphosphine)-palladium(II) dichloride (17.0 mg and 23.0 μmol) and copper(I) iodide (4.80 mg and 23.0 μmol) were sealed in a microwave vial and purged with nitrogen and vacuum. DMF (0.87 mL) was added, and the solution was stirred while sparging with nitrogen for 5 min. DIPEA (0.29 mL) was added and further sparging for 5 min. TIPS acetylene (56.0 μL and 0.345 mmol) was added, and the reaction mixture was heated at 55 °C for 16 h. The resulting solution was dried in vacuo, then dissolved in dichloromethane and methanol, and filtered through Celite. The filtrate was concentrated to dryness to afford the crude compound as a dark red solid, which was purified by automated flash column chromatography (silica, 0−10% methanol in dichloromethane) to give the desired compound as a white solid (35.0 mg, 70.9 μmol, and 62%). 1H-NMR: (500 MHz, DMSO-d6): δ 1.03−1.26 (m, 21H), 7.19 (d, *J* = 5.6 Hz, 1H), 7.21 (s, 1H), 7.22−7.24 (s, 2H), 7.60 (dd, *J* = 1.6, 8.3 Hz, 1H), 7.85 (d, *J* = 8.2 Hz, 1H), 8.40 (d, *J* = 5.5 Hz, 1H), 8.94 (d, *J* = 1.6 Hz, 1H), 9.03 (s, 1H), 9.20 (s, 1H) ppm.

2-Chloro-4-Vinylpyridine (59). 2-Chloro-4-bromopyridine **49** (500 mg and 2.60 mmol) was reacted with palladium(II) chloride (10.0 mg and 0.520 mmol), triphenylphosphine (40.0 mg and 15.6 μmol), cesium carbonate (2.54 g and 7.79 mmol), and potassium vinyltrifluoroborate (345 mg and 2.60 mmol) in THF (4.5 mL) and water (0.50 mL). The solution was sparged with nitrogen for 5 min before heating at 85 °C for 24 h. The reaction mixture was filtered through Celite, and the solvent was removed in vacuo. The reaction mixture was purified by automated flash column chromatography (silica, 0−20% methanol in dichloromethane) to yield the desired compound as a colorless oil (300 mg, 2.15 mmol, and 83%). 1H-NMR (500 MHz, $CDCl_3$): δ 5.48 (d, *J* = 10.8 Hz, 1H), 5.91 (d, *J* = 17.5 Hz, 1H), 6.55 (dd, *J* = 10.9, 17.6 Hz, 1H), 7.13 (dd, *J* = 1.5. 5.2 Hz, 1H), 7.23 (dt, *J* = 0.6, 1.4 Hz, 1H), 8.25 (dd, *J* = 0.7, 5.2 Hz, 1H) ppm. 13C-NMR (126 MHz, $CDCl_3$): δ 119.5, 121.3, 133.6, 148.0, 149.8, 150.0, 152.0 ppm. LRMS *m/z* (method 1): retention time = 1.28 min, (ES+) *m/z* = 140.1 $[M + H]^+$.

2-Chloro-4-Vinylpyrimidine (60). To a stirred solution 2,4-dichloropyrimidine **46** (100 mg and 0.671 mmol) in a mixture of water and dioxane (1:4 and 5.0 mL) was added vinylboronic acid pinacol ester (113 mg and 0.738 mmol), [1,1′-bis-(diphenylphosphino)ferrocene]dichloropalladium-(II).dichloromethane (55.0 mg and 67.1 μmol) and cesium carbonate (654 mg and 2.01 mmol). The resulting mixture was heated at 85 °C overnight. The solvents were removed in vacuo, and the residue was diluted with water (25 mL) and extracted with ethyl acetate (3 × 50 mL). The organic layers were combined, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The crude product was purified by column chromatography (silica, 0−30% ethyl acetate in petroleum ether) to yield the title compound as a white solid (54 mg, 0.39 mmol, and 57%). 1H NMR (500 MHz, $CDCl_3$): δ 5.80 (d, *J* = 10.6 Hz, 1H), 6.55 (d, *J* = 17.3 Hz, 1H), 6.71 (dd, *J* = 10.6, 17.3 Hz, 1H), 7.23 (d, *J* = 5.1 Hz, 1H), 8.57 (d, *J* = 5.1 Hz, 1H) ppm. 13C-NMR (126 MHz, $CDCl_3$): δ 116.3, 125.2, 133.8, 159.8, 161.6, 165.5 ppm. LRMS *m/z* (method 1): retention time = 1.07 min, (ES+) *m/z* = 141.1 $[M + H]^+$.

3-Bromo-5-((Triisopropylsilyl)Ethynyl)Pyridine (61). 3,5-Dibromopyridine **50** (1.42 g, 5.99 mmol) was stirred with bis-(triphenylphosphine)palladium(II) dichloride (140 mg, 0.200 mmol), and copper(I) iodide (38.0 mg, 0.200 mmol) was sealed in a microwave vial and purged with nitrogen and vacuum. DMF (10 mL) was added, and the solution was stirred while sparging with nitrogen for 5 min before DIPEA (5.0 mL) was added and further sparging for 5 min. TIPS acetylene (0.440 mL, 1.96 mmol) was added and sparged for 5 min before heating at 55 °C for 16 h. The crude product was then filtered through Celite, the solvent was removed in vacuo, and the product was purified using automated flash column chromatography (silica, 0−25% ethyl acetate in petroleum ether) to afford the desired product as a white powder (536 mg, 1.59 mmol, 27%). The compound was used without further purification.

2-Bromo-6-((Triisopropylsilyl)Ethynyl)Pyridine (62). 2,6-Dibromo pyridine **71** (1.42 g, 5.99 mmol) was stirred with bis(triphenylphosphine)palladium(II) dichloride (140 mg, 0.200 mmol) and copper(I) iodide (38.0 mg, 0.200 mmol), sealed in a microwave vial, and purged with nitrogen and vacuum. DMF (10 mL) was added, and the solution was stirred while sparging with nitrogen for 5 min before DIPEA (5.0 mL) was added and further sparging for 5 min. TIPS acetylene (440 μL, 1.96 mmol) was added and sparged for 5 min before heating at 55 °C for 16 h. The crude product was then filtered through Celite, the solvent was removed in vacuo, and the crude product was purified using automated flash column chromatography (silica, 0−25% ethyl acetate in petroleum ether) to afford the desired product 62 as a white powder (520 mg, 1.54 mmol, 23%). The compound was used without further purification.

2-Bromo-4-((Trimethylsilyl)Ethynyl)Pyridine (63). 2-Bromo 4-iodo-pyridine **45** (100 mg, 0.352 mmol) was reacted with TIPS acetylene (480 μL, 0.352 mmol), bis(triphenylphosphine)palladium-(II) dichloride (24.0 mg, 35.2 μmol), and copper(I) iodide (6.00 mg, 35.2 μmol), sealed in a microwave vial, and purged with nitrogen and vacuum. DMF (1.8 mL) was added, and the solution was stirred while sparging with nitrogen for 5 min before DIPEA (0.92 mL) was added and further sparging for 5 min. TIPS acetylene (480 μL, 0.352 mmol) was added and sparged for 5 min before heating at 105 °C for 15 min under microwave irradiation. The reaction mixture was filtered through Celite, the solvent was removed in vacuo, and the reaction mixture was purified by automated flash column chromatography (silica, 0−35% ethyl acetate in petroleum ether) to afford the desired product as a colorless oil (68.0 mg, 0.268 mmol, 76%). 1H-NMR: (500 MHz, $CDCl_3$): δ 0.27 (d, *J* = 3.1 Hz, 9H), 7.21−7.32 (m, 1H), 7.46−7.62 (m, 1H), 8.33 (dd, *J* = 0.8, 5.1 Hz, 1H) ppm. 13C-NMR (126 MHz, $CDCl_3$): δ 0.22100.8, 101.6, 125.4, 129.9, 134.2, 142.6, 150.3 ppm. LRMS *m/z* (method 2): retention time = 1.81 min, (ES+) *m/z* = 256.1 $[M + H]^+$.

2-Chloro-4-2-((Triisopropylsilyl)Ethynyl)Pyrimidine (64). 2,4-Dichloro-pyrimidine **46** (100 mg and 0.671 mmol) was reacted bis(triphenylphosphine)palladium(II) dichloride (47.0 mg and 67.1 μmol) and copper(I) iodide (13.0 mg and 67.1 μmol) were sealed in a microwave vial and purged with nitrogen and vacuum. DMF (3.2 mL) was added, and the solution was stirred while sparging with nitrogen for 5 min before DIPEA (1.6 mL) was added and further sparging for 5 min. TIPS acetylene (22.0 μL and 1.01 mmol) was added and sparged for 5 min before heating at 55 °C for 16 h. The reaction mixture was filtered through Celite, the solvent was removed in vacuo, and the reaction mixture was purified by automated column chromatography (silica, 0−10% ethyl acetate in petroleum ether) to furnish the desired compound as a white solid (150 mg, 0.509 mmol, and 76%). 1H-NMR (500 MHz, $CDCl_3$): δ 1.19−1.01 (m, 21H), 7.31 (d, *J* = 5.0 Hz, 1H), 8.56 (d, *J* = 5.0 Hz, 1H) ppm.13C-NMR (126 MHz, $CDCl_3$): δ 11.1, 18.5, 101.0, 102.3, 122.3, 152.9, 159.3, 161.5 ppm. LRMS *m/z* (method 2): retention time = 1.81 min, (ES+) *m/z* = 256.1 $[M + H]^+$.

2-Chloro-5-((Triisopropylsilyl)Ethynyl)Pyrimidine (65). 2,5-Dichloro-pyrimidine **47** (250 mg and 1.29 mmol) was reacted with bis(triphenylphosphine)palladium(II) dichloride (90.0 mg and 0.129 mmol) and copper(I) iodide (25.0 mg and 0.129 mmol) were sealed in a microwave vial and purged with nitrogen and vacuum. DMF (6.5 mL) was added, and the solution was stirred while sparging with nitrogen for 5 min before DIPEA (3.3 mL) was added and further sparging for 5 min. TIPS acetylene (14.0 μL and 0.646 mmol) was added and sparged for 5 min before heating at 55 °C for 16 h. Palladium was filtered through Celite, the solvent was removed in vacuo, and the crude product was not further purified, but used directly for the next step.

4-(6-((5-((Triisopropylsilyl)Ethynyl)Pyridin-3-yl)Ethynyl)-1H-Benzo[*d*]Imidazol-1-yl)Pyrimidin-2-Amine (66). 4-(6-Ethynyl-1H-benzo[*d*]imidazol-1-yl)pyrimidin-2-amine 4 (77.0 mg, 0.327 mmol), 3-bromo-5-((triisopropylsilyl)ethynyl)pyridine **61** (162 mg,

https://doi.org/10.1021/acs.jmedchem.0c01249
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0.491 mmol), bis(triphenylphosphine)palladium(II) dichloride (23.0 mg, 32.7 μmol), and copper(I) iodide (6.00 mg, 32.7 μmol) were sealed in a microwave vial and purged with nitrogen and vacuum. DMF (3.0 mL) was added, and the solution was stirred while sparging with nitrogen for 5 min. DIPEA (1.5 mL) was added and further sparging for 5 min before heating at 55 °C for 16 h. The resulting solution was dried in vacuo then dissolved in dichloromethane and methanol, and filtered through Celite. The filtrate was concentrated to dryness to afford the crude compound as a dark red solid, which was purified by automated flash column chromatography (silica, 0−10% methanol in dichloromethane) to give the desired compound as a white solid (106 mg, 0.215 mmol, and 66%); m.p. 201−202 oC. 1H-NMR (500 MHz, DMSO-d6): δ 1.13 (d, J = 4.3 Hz, 21H), 7.17 (s, 2H), 7.19 (d, J = 5.6 Hz, 1H), 7.58 (dd, J = 1.6, 8.3 Hz, 1H), 7.83 (d, J = 8.3 Hz, 1H), 8.12 (t, J = 2.1 Hz, 1H), 8.40 (d, J = 5.5 Hz, 1H), 8.67 (d, J = 2.0 Hz, 1H), 8.80 (d, J = 2.0 Hz, 1H), 8.90 (d, J = 1.5 Hz, 1H), 9.18 (s, 1H) ppm. 13C-NMR (126 MHz, DMSO-d6): δ 11.1, 18.9, 85.2, 94.8, 95.6, 98.4, 103.2, 117.7, 119.8, 120.2, 120.2, 120.8, 127.6, 132.0, 141.2, 144.2, 145.4, 151.3, 151.4, 157.2, 161.0, 164.0 ppm. HRMS $[M + H]^+$ predicted for $C_{29}H_{32}N_6Si$: 493.2530; found: 493.2524.

4-(6-((6-((Triisopropylsilyl)Ethynyl)Pyridin-2-yl)Ethynyl)-1H-Benzo[*d*]Imidazol-1-yl)Pyrimidin-2-Amine (67). 4-(6-Ethynyl-1H-benzo[*d*]imidazol-1-yl)pyrimidin-2-amine **4** (76.0 mg and 0.323 mmol), 2-bromo-6-((triisopropylsilyl)ethynyl)pyridine **62** (163 g and 0.485 mmol), bis(triphenylphosphine)palladium(II) dichloride (23.0 mg and 32.3 μmol), and copper(I) iodide (6.00 mg and 32.3 μmol) were sealed in a microwave vial and purged with nitrogen and vacuum. DMF (3.0 mL) was added and the solution stirred while sparging with nitrogen for 5 min. DIPEA (1.5 mL) was added and further sparging for 5 min before heating at 55 °C for 16 h. The resulting solution was dried in vacuo then dissolved in dichloromethane and methanol and filtered through Celite. The filtrate was concentrated to dryness to afford the crude compound as a dark red solid, which was purified by automated flash column chromatography (silica, 0−10% methanol in dichloromethane) to give the desired product as a white solid (112 mg, 0.227 mmol, and 70%); m.p. 125−126 oC. 1H-NMR (500 MHz, DMSO-d6): δ 0.95 (d, J = 4.5 Hz, 21H), 7.01 (d, J = 5.5 Hz, 1H), 7.03 (s, 2H), 7.40 (dd, J = 1.0, 7.8 Hz, 1H), 7.44 (dd, J = 1.6, 8.3 Hz, 1H), 7.55 (dd, J = 1.0, 7.8 Hz, 1H), 7.66 (dd, J = 0.7, 8.3 Hz, 1H), 7.73 (t, J = 7.8 Hz, 1H), 8.22 (d, J = 5.5 Hz, 1H), 8.72 (dd, J = 0.6, 1.7 Hz, 1H), 9.01 (s, 1H) ppm. 13C-NMR (126 MHz, DMSO-d6): δ 11.1, 18.9, 88.3, 90.7, 91.3, 98.4, 106.1, 117.3, 120.1, 120.8, 127.6, 127.8, 128.0, 132.0, 138.1, 142.9, 143.4, 145.6, 157.3, 161.0, 164.1 ppm. HRMS $[M + H]^+$ predicted for $C_{29}H_{32}N_6Si$: 493.2530; found: 493.2530.

4-(6-((4-((Trimethylsilyl)Ethynyl)Pyridin-2-yl)Ethynyl)-1H-Benzo[*d*]Imidazol-1-yl)Pyrimidin-2-Amine (68). 2-Bromo-4-((trimethylsilyl)ethynyl)pyridine **63** (68.0 mg and 0.268 mmol), 4-(6-ethynyl-1H-benzo[*d*]imidazol-1-yl)pyrimidin-2-amine **4** (94.0 mg and 0.400 mmol), bis(triphenylphosphine)palladium(II) dichloride (18.0 mg and 26.8 μmol), and copper(I) iodide (4.60 mg, 26.8 μmol) were sealed in a microwave vial and purged with vacuum and nitrogen. DMF (1.4 mL) was added, and the solution was sparged with nitrogen for 5 min followed by addition of DIPEA (0.7 mL) and further sparging with nitrogen for 5 min. The reaction mixture was then heated for 16 h minutes at 55 °C before drying in vacuo. The crude product was then purified by automated flash column chromatography (silica, 0−8% methanol in dichloromethane) yielding the desired product as an off-white solid (80.0 mg, 0.196, and 73%). 1H-NMR (500 MHz, $CDCl_3$): δ 0.27 (d, J = 3.1 Hz, 9H), 5.40 (s, 2H), 6.82 (d, J = 5.6 Hz, 1H), 7.45 (dd, J = 1.5, 8.4 Hz, 1H), 7.72 (dd, J = 0.7, 8.4 Hz, 1H), 8.04 (s, 2H), 8.40 (d, J = 5.1 Hz, 4H) 8.45−8.51 (m, 2H), 8.57 (s, 1H) ppm. LRMS *m/z* (method 1): retention time = 1.82 min, (ES+) m/z = 410.1 $[M + H]^+$.

4-(6-((4-((Triisopropylsilyl)Ethynyl)Pyrimidin-2-yl)Ethynyl)-1H-Benzo[*d*]Imidazol-1-yl)Pyrimidin-2-Amine (69). 4-(6-Ethynyl-1H-benzo[*d*]imidazol-1-yl)pyrimidin-2-amine **4** (30.0 mg and 0.127 mmol), 2-chloro-4-((triisopropylsilyl)ethynyl)pyrimidine **64** (56.0 mg and 0.191 mmol), bis(triphenylphosphine)palladium(II) dichloride (15.0 mg and 191. μmol), and copper(I) iodide (3.00 mg and 19.1 μmol) were sealed in a microwave vial and purged with vacuum and nitrogen. DMF (0.64 mL) was added, and the solution was sparged with nitrogen for 5 min followed by addition of DIPEA (0.32 mL) and further sparging with nitrogen for 5 min. The reaction mixture was then heated for 16 h minutes at 55 °C before drying in vacuo. The crude product was then purified by automated flash column chromatography (silica, 0−8% methanol in dichloromethane) yielding the desired product 69 as an off-white solid (48.0 mg, 97.2 μmol, and 77%). 1H-NMR (500 MHz, CD_3OD): δ 1.10−1.31 (m, 21H), 7.14 (d, J = 4.9 Hz, 1H), 7.53 (d, J = 5.2 Hz, 1H), 7.71 (d, J = 7.6 Hz, 1H), 7.83 (d, J = 8.4 Hz, 1H), 8.46 (s, 1H), 8.83 (d, J = 5.2 Hz, 1H), 9.07 (s, 1H), 9.12 (s, 1H) ppm. LRMS *m/z* (method 2): retention time = 1.82 min, (ES+) m/z = 494.5 $[M + H]^+$. The material was used without further deprotection.

4-(6-((5-((Triisopropylsilyl)Ethynyl)Pyrimidin-2-yl)Ethynyl)-1H-Benzo[*d*]Imidazol-1-yl)Pyrimidin-2-Amine (70). 4-(6-Ethynyl-1H-benzo[*d*]imidazol-1-yl)pyrimidin-2-amine **4** (30.0 mg and 0.127 mmol), 2-chloro-5-((triisopropylsilyl)ethynyl)pyrimidine **65** (56.0 mg and 0.191 mmol), bis(triphenylphosphine)palladium(II) dichloride (18.0 mg and 25.0 μmol), and copper(I) iodide (5.00 mg and 25.0 μmol) were sealed in a microwave vial and purged with vacuum and nitrogen. DMF (0.64 mL) was added, and the solution was sparged with nitrogen for 5 min followed by addition of DIPEA (0.32 mL) and further sparging with nitrogen for 5 min. The reaction mixture was then heated for 15 min at 100 °C in the microwave. The solvent was then removed in vacuo before purification by automated flash column chromatography (silica, 0−5% methanol in dichloromethane) to yield the desired product as a pink solid (42.5 mg, 86.1 μmol, 69%). 1H-NMR (500 MHz, DMSO-d6): δ 1.13 (m, 21H), 7.18 (d, J = 5.5 Hz, 1H), 7.23 (bs, 2H), 7.64 (dd, J = 1.7, 8.3 Hz, 1H), 7.86 (d, J = 8.3 Hz, 1H), 8.40 (d, J = 5.5 Hz, 1H), 8.91 (d, J = 1.6 Hz, 1H), 8.98 (s, 2H), 9.20 (s, 1H) ppm. 13C-NMR (126 MHz, $CDCl_3$): δ 11.4, 18.7, 90.6, 96.7, 98.6, 99.9, 107.5, 117.5, 119.9, 120.7, 128.4, 135.0, 141.9, 144.9, 148.52, 150.78, 156.8, 159.7, 160.7, 163.1 ppm. LRMS *m/z* (method 2): retention time = 2.02 min, (ES+) m/z = 494.5 $[M + H]^+$.

5-((Triisopropylsilyl)Ethynyl)Picolinaldehyde (74). 5-Chloropicolinaldehyde **73** (500 mg and 3.53 mmol), bis-(triphenylphosphine)palladium(II) dichloride (247 mg and 0.353 mmol), and copper(I) iodide (67.0 mg and 0.353 mmol) were sealed in a microwave vial and purged with vacuum and nitrogen. DMF (18 mL) was added, and the solution was sparged with nitrogen for 5 min followed by addition of DIPEA (9.0 mL) and further sparging with nitrogen for 5 min. TIPS acetylene (1.20 mL and 5.30 mmol) was added and sparged for 5 min before heating at 55 °C for 16 h before drying in vacuo. The crude product was purified by automated flash column chromatography (silica, 0−10% ethyl acetate in petroleum ether) to yield the desired product as a pale yellow oil (850 mg, 2.96 mmol, and 84%). 1H-NMR (500 MHz, $CDCl_3$): δ 1.04−1.25 (m, 21H), 7.93 (d, J = 1.4 Hz, 2H), 8.84 (t, J = 1.4 Hz, 1H), 10.09 (s, 1H) ppm. 13C-NMR (500 MHz, $CDCl_3$): δ 11.2, 18.6, 99.7, 102.7, 120.8, 124.9, 139.8, 150.9, 152.9, 192.6 ppm. LRMS *m/z* (method 2): retention time = 2.10 min, (ES+) m/z = 288.5 $[M + H]^+$.

5-((Triisopropylsilyl)Ethynyl)-2-Vinylpyridine (75). A suspension of methyltriphenylphosphonium bromide (2.00 g and 5.53 mmol) in THF (22 mL) was cooled to 0 °C and stirred for 30 min. Potassium *tert*-butoxide (620 mg and 5.53 mmol) was added, and the reaction mixture was stirred at 0 °C for 1 h before warming to room temperature. A solution of 5-((triisopropylsilyl)ethynyl)-picolinaldehyde **74** (795 mg, 2.77 mmol) was added slowly, and the solution was stirred overnight. The solution was then diluted with dichloromethane and washed with water followed by brine, before drying over sodium sulfate. The solvent was removed in vacuo, and the resulting yellow solid was purified by automated flash column chromatography (silica, 0−10% ethyl acetate in petroleum ether) to afford the desired product as a pale yellow solid (620 mg, 2.17 mmol, and 78%). 1H-NMR (500 MHz, $CDCl_3$): δ 0.82−1.10 (m, 21H), 5.41 (dd, J = 1.1, 10.8 Hz, 1H), 6.10 (dd, J = 1.2, 17.5 Hz, 1H), 6.69 (dd, J = 10.8, 17.5 Hz, 1H), 7.12−7.19 (m, 1H), 7.59 (dd, J = 2.1, 8.1

https://doi.org/10.1021/acs.jmedchem.0c01249
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Hz, 1H), 8.48−8.61 (m, 1H) ppm. LRMS *m/z* (method 1): retention time = 2.23 min, (ES+) *m/z* = 286.5 $[M + H]^+$.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jmedchem.0c01249.

Molecular formula strings (CSV)

Supplementary figures and tables, protein expression and bioassay protocols, additional compound synthesis schemes, and spectra (PDF)

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All authors have given approval to the final version of the manuscript.

Funding

We gratefully acknowledge the support of Cancer Research UK (program funding of the Drug Discovery Group, grant reference C2115/A21421 and Centre Network Accelerator Award, grant reference A20263), Astex Pharmaceuticals (alliance funding of the program), Jordan University of Science & Technology (studentship award to IA-K), Saudi Arabian Cultural Bureau (studentship award to SK), and Newcastle University (studentship awards to ABH, HL, and JVdeS).

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We gratefully acknowledge friends and collaborators at Astex Pharmaceuticals for helpful discussions during the course of this work and particularly thank Tom Davies, Tom Heightman, John Lyons, Chris Murray, Neil Thompson, Mark Wade, and Nicola Wallis.

■ ABBREVIATIONS

ADMET, absorption, distribution, metabolism and toxicology; BIRC, baculoviral inhibitor of apoptosis repeat-containing protein; DIPEA, diisopropylethylamine; DMF, dimethyl formamide; DMSO, dimethylsulfoxide; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B-cells; NIK, NF-κB-inducing kinase; NMR, nuclear magnetic resonance; SAR, structure−activity relationship; TBAF, tetrabutylammonium fluoride; THF, tetrahydrofuran; TRAF, tumor necrosis factor receptor associated factor; MS, mass spectrometry.

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https://doi.org/10.1021/acs.jmedchem.0c01249
J. Med. Chem. 2021, 64, 10001−10018