Journal of Medicinal Chemistry

Article

pubs.acs.org/jmc

Structure-Based Optimization of a Small Molecule Antagonist of the Interaction Between WD Repeat-Containing Protein 5 (WDR5) and Mixed-Lineage Leukemia 1 (MLL1)

Matthäus Getlik,[†,⊥,#] David Smil,[‡,#] Carlos Zepeda-Velázquez,[†] Yuri Bolshan,[‡,▽] Gennady Poda,[†,∥] Hong Wu,[‡] Aiping Dong,[‡] Ekaterina Kuznetsova,[‡,○] Richard Marcellus,[†] Guillermo Senisterra,[‡] Ludmila Dombrovski,[‡] Taraneh Hajian,[‡] Taira Kiyota,[†] Matthieu Schapira,[‡,§] Cheryl H. Arrowsmith,[‡] Peter J. Brown,[‡] Masoud Vedadi,[‡,§] and Rima Al-awar*[,†,§]

[†]Drug Discovery Program, Ontario Institute for Cancer Research, 661 University Avenue, MaRS Centre, West Tower, Toronto, Ontario M5G 0A3, Canada

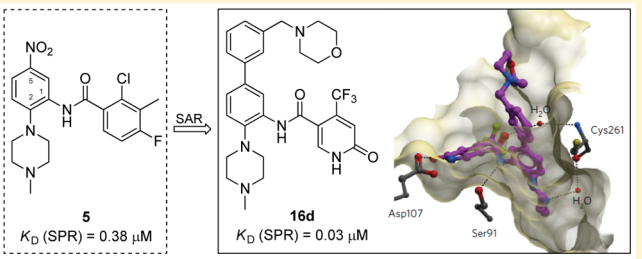
[‡]Structural Genomics Consortium, University of Toronto, 101 College Street, MaRS Centre, South Tower, Toronto, Ontario M5G 1L7, Canada

[∥]Leslie Dan Faculty of Pharmacy, University of Toronto, 144 College Street, Toronto, Ontario M5S 3M2, Canada

[§]Department of Pharmacology and Toxicology, University of Toronto, Medical Sciences Building, 1 King's College Circle, Toronto, Ontario M5S 1A8, Canada

Supporting Information

ABSTRACT: WD repeat-containing protein 5 (WDR5) is an important component of the multiprotein complex essential for activating mixed-lineage leukemia 1 (MLL1). Rearrangement of the MLL1 gene is associated with onset and progression of acute myeloid and lymphoblastic leukemias, and targeting the WDR5-MLL1 interaction may result in new cancer therapeutics. Our previous work showed that binding of small molecule ligands to WDR5 can modulate its interaction with MLL1, suppressing MLL1 methyltransferase activity. Initial structure–activity relationship studies identified *N*-(2-(4-methylpiperazin-1-yl)-5-substituted-phenyl) benzamides as potent and selective antagonists of this protein–protein interaction. Guided by crystal structure data and supported by in silico library design, we optimized the scaffold by varying the C-1 benzamide and C-5 substituents. This allowed us to develop the first highly potent (K_{disp} < 100 nM) small molecule antagonists of the WDR5-MLL1 interaction and demonstrate that *N*-(4-(4-methylpiperazin-1-yl)-3′-(morpholinomethyl)-[1,1′-biphenyl]-3-yl)-6-oxo-4-(trifluoromethyl)-1,6-dihydropyridine-3-carboxamide **16d** (OICR-9429) is a potent and selective chemical probe suitable to help dissect the biological role of WDR5.



INTRODUCTION

Mixed-lineage leukemia 1 (MLL1) is a member of the Set1 family of methyl transferases which catalyze the mono-, di-, and trimethylation of histone 3 at lysine 4 (H3K4) and facilitate transcriptional initiation. MLL1 is often deregulated in acute myeloid and lymphoblastic leukemias with a common defect being a gene translocation involving chromosome 11, leading to fusion of the MLL1 gene with other genes. The resulting MLL1 fusion proteins induce leukemia by constitutively activating the expression of HOXA9 and MEIS1, genes that are essential for self-renewal of hematopoietic stem cells and whose expression is usually down-regulated after differentiation.[1,2]

The WD repeat-containing protein 5 (WDR5) binds to MLL1 and has been shown to be crucial for the stability and methyltransferase activity of the MLL1 complex (MLL1, RbBP5, Ash2L, WDR5, and DPY-30). In the absence of any of these proteins, the catalytic activity of the core complex is significantly diminished.[3] WDR5 belongs to a family of proteins that consist of four or more 40 amino acid repeats forming a circular β-propeller structure, usually ending with a tryptophan-aspartic acid peptide (WD).[4,5] WDR5 was reported to facilitate H3K4 methylation by MLL1 through binding to the N-terminal region of histone H3 via key interactions with arginine 2. Interestingly, the conserved WDR5-interacting (WIN) motif of MLL1 binds to the same binding site in WDR5, with R3765 forming a dense hydrogen-bond network to the surrounding amino acid residues.[6,7]

Given the critical role of WDR5 within the MLL1 multiprotein complex, antagonists of the WDR5-MLL1 interaction could serve to dissociate the complex and inhibit MLL1 activity. Well-characterized antagonists of such inter-

Received: October 16, 2015

DOI: 10.1021/acs.jmedchem.5b01630
J. Med. Chem. XXXX, XXX, XXX−XXX

action would be valuable tools for advancing our understanding of the roles of this complex and the implications of MLL1 dysregulation in cancers. To that end, the small molecule inhibitor **1** (SCF-I2, Figure 1)[8] along with the linear peptide **2**

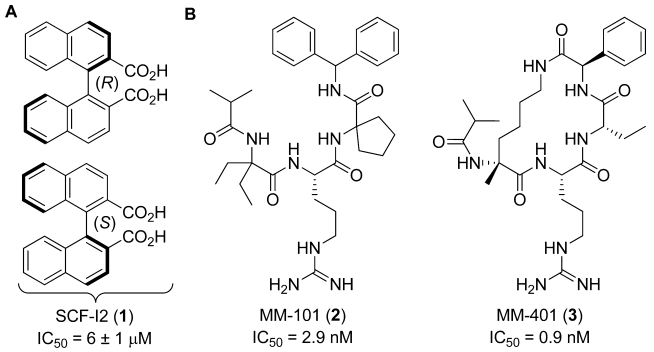


Figure 1. Chemical structures of reported WD repeat domain antagonists. (A) Racemic 1,1′-binaphthyl-2,2′-dicarboxylic acid, an inhibitor of yeast Cdc4. (B) Acyclic and cyclic peptide inhibitors that occupy the arginine binding site of WDR5.

(MM-101, Figure 1)[3] and cyclic peptide **3** (MM-401, Figure 1)[9] have been reported, with the peptides shown to directly bind the arginine binding site of WDR5 in order to disrupt its interaction with MLL1.

Compound **3** was reported as having a 0.9 nM binding affinity to WDR5 as determined using a fluorescence polarization (FP) assay and to inhibit the MLL1 methyltransferase activity (IC_{50} = 0.32 μM).[9] When tested on human leukemia cell lines, the compound was found to arrest cell growth of MV4:11, Molm13, and KOPN8 lines with a GI_{50} of 12.42, 23.97, and 29.73 μM respectively.

We had previously identified the first nonpeptide small molecule antagonists of the WDR5-MLL1 interaction[10] and have reported initial structure-activity relationship (SAR) findings for the *N*-(2-(4-methylpiperazin-1-yl)-5-substituted-phenyl) benzamide scaffold.[11] Optimization of this scaffold for potency, solubility, and selectivity resulted in the discovery of *N*-(4-(4-methylpiperazin-1-yl)-3′-(morpholinomethyl)-[1,1′-biphenyl]-3-yl)-6-oxo-4-(trifluoromethyl)-1,6-dihydropyridine-3-carboxamide **16d** (OICR-9429), the most potent small molecule antagonist of the WDR5-MLL1 interaction reported to-date, and one built on a chemotype which can potentially address the liabilities commonly associated with peptide-based compounds.[12]

Compound **16d** has significant biological activity in two separate cancer models that are dependent on WDR5/MLL1 activity. The mutated form (p30) of C/EBPα, a transcription factor that is a master regulator of myeloid gene expression programs in the hematopoietic system, interacts preferentially with WDR5, and cells with the C/EBPαp30 mutation are sensitive to **16d** as well as to WDR5 knock down.[12] Significantly, C/EBPα mutations are present in 9% of patients with acute myeloid leukemia (AML). In a separate study, gain-of-function (GOF) p53 mutants were found to upregulate chromatin regulatory genes including MLL1, and breast cancer cells with a GOF p53 mutation were sensitive to MLL1 knockdown as well as **16d**.[13] More recently, **16d** was shown to be effective at reducing N-Myc/WDR5 complex formation, N-Myc target gene expression, and neuroblastomal cell growth, thereby identifying WDR5 as a key cofactor for N-Myc-regulated transcriptional activation and tumorigenesis and as a novel therapeutic target for MYCN-amplified neuroblastomas.[14] Here, we present the structure-based design, synthesis, and biochemical evaluation of **16d** and the intermediate compounds leading to its development.

RESULTS AND DISCUSSION

Compound Synthesis and Structure−Activity Relationship Studies. Having previously leveraged the WDR5-**4** co-crystal structure (PDB code 3SMR)[10] to guide the optimization of our initial screening hit **4**, an antagonist of

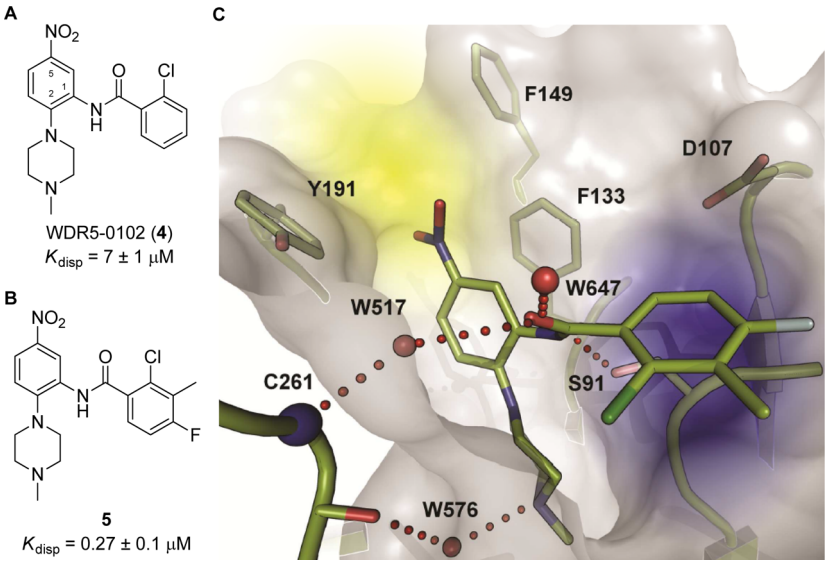
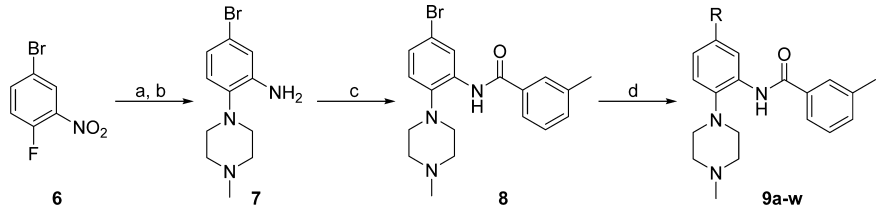


Figure 2. Chemical structures and binding mode of WDR5-MLL1 interaction antagonists. (A) Initial medium-throughput screening hit compound **4**. (B) Advanced antagonist **5** obtained following an initial round of SAR studies. (C) Crystal structure of WDR5 (gray) in complex with **5** (green; PDB code 4IA9). The antagonist resides in the MLL1 arginine-binding pocket and forms a direct H-bond to the side chain of S91 as well as water mediated H-bonds to the backbone of C261. Substituents on the benzamide add hydrophobic interactions with aliphatic side chains surrounding a hydrophobic subpocket.

DOI: 10.1021/acs.jmedchem.5b01630
J. Med. Chem. XXXX, XXX, XXX−XXX

Scheme 1. Synthesis of 5-Substituted 2-(4-Methylpiperazin-1-yl)phenyl)-3-methylbenzamides[a]



[a]Reagents and conditions: (a) 1-methylpiperazine, DIPEA, DMSO, 80 °C; (b) $Fe_{(s)}$, $CaCl_2$, water/MeOH (1:1), 100 °C; (c) 3-methylbenzoyl chloride, pyridine, CH_2Cl_2, rt; (d) boronic acid/ester, $Pd(PPh_3)_4$, Na_2CO_3, toluene/water (1:1), 115 °C.

the WDR5-MLL1 interaction with a K_{disp} = 7 ± 1 μM, we reported the development of advanced compound **5** which exhibited a 25-fold improvement in potency (Figure 2).[11] While key structural features such as the central phenyl ring, C-1 position amide, C-2 position 4-methylpiperazine, and C-5 position nitro group were maintained following the initial SAR study, introduction of a 3-methyl and 4-fluoro substituent into the benzamide portion of the scaffold was critical for improving potency to K_{disp} = 0.27 ± 0.1 μM. A co-crystal structure of WDR5-**5** (PDB code 4IA9)[11] revealed that crucial interactions of the *N*-(2-(4-methylpiperazin-1-yl)-5-nitrophenyl) benzamide scaffold with WDR5 were maintained, and the newly introduced methyl and fluoro substituents added to the potency by interacting with the surrounding hydrophobic side chains of A47, A65, Y260, I305, and L321.

From this initial work, as well as additional experiments,[15] it was apparent that even minor modifications of the 4-methylpiperazine at C-2 were not tolerated, consistent with it being a key anchor within the arginine-binding cleft of WDR5. Similarly, given the critical hydrogen bond (H-bond) formed between the C-1 amide N−H and the S91 side chain as evident in the available WDR5-**5** co-crystal structure, modifications such as reversal of the amide, methylation of the amide N−H, and replacement of the amide with a urea or sulfonamide all resulted in a complete loss of activity.[15] We therefore decided to preserve the parent 4-methylpiperazine substituent and amide linkage during all of the SAR work reported in this publication. However, we aimed to fully explore a range of substituents at C-5 and see if additional changes to the benzamide moiety could further improve the potency.

The initial published SAR efforts at the C-5 position indicated that introduction of aromatic heterocycles such as 2-furanyl or 4-pyridyl led to modest improvements in potency.[11] The co-crystal structure of WDR5 in complex with **5** revealed that this position is partially solvent exposed and that the C-5 nitro substituent forms favorable van der Waals interactions with nearby residues F133, Y191 and P173 (Figure 2C).

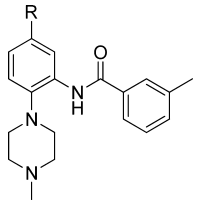
Given that the nitro group potentially reduces cellular permeability by contributing significantly to the compound's polar surface area (PSA), and because of its potential in vivo liability, we felt that this part of the molecule should be more broadly explored through generation of a focused compound collection designed to not only replace the nitro group but to also capitalize on the potential van der Waals interactions with the aforementioned aromatic residues F133 and Y191 as well as F149. With this as the goal, we introduced bulkier aromatic residues at C-5 with the intention of capturing some interactions with these aromatic side chains. In addition, the incorporation of substituents that would serve to increase overall compound solubility was considered. In order to generate the compound set, an efficient synthetic route was established which allowed for the direct functionalization of the C-5 position via Suzuki coupling as the final step (Scheme 1).

The route involved initial preparation of 5-bromo-2-(4-methylpiperazin-1-yl)aniline (**7**) by substitution of 4-bromo-1-fluoro-2-nitrobenzene (**6**) with 1-methylpiperazine, followed by iron-mediated reduction of the intermediate 1-(4-bromo-2-nitrophenyl)-4-methylpiperazine. Coupling of **7** with 3-methylbenzoyl chloride readily afforded the precursor *N*-(5-bromo-2-(4-methylpiperazin-1-yl)phenyl)-3-methylbenzamide (**8**). The 3-methyl group on the benzamide had been selected as the fixed parent substituent because it was not expected to interfere with the Suzuki reaction and was among the more potent benzamide substituents found during the initial SAR work.[11] The Suzuki couplings were then carried out on **8** using standard coupling conditions, allowing for access to a collection of derivatives (**9a−w**) (Table 1). Subsequently, the affinity of the compounds was evaluated using a pair of fluorescence polarization (FP) assays detecting the displacement of either fluorescein-labeled H3 (1−15) peptide (K_D = 167 ± 22 nM) or 9-Ala-FAM ((Ac)-ARAEVHLRK-(Ahx-Ahx)-K(5,6-FAM)) peptide (K_D = 19 ± 3 nM)[12,16] from the WDR5 binding site.[15] For each experiment, we chose a suitable binding peptide based on our estimation of compound affinity. The H3 (1−15) peptide was used when compound activity was anticipated to be above 200 nM, while the 9-Ala-FAM peptide was employed with compounds expected to have potencies below 200 nM. Binding of these peptides to WDR5 increased the FP signal which was then decreased upon displacement of these peptides by the small molecule antagonists. Reduction of signal was monitored at various compound concentrations, and $K_{displacement}$ (K_{disp}) values were calculated by fitting the data to a hyperbolic function as described in the Supporting Information.

Represented within the newly generated set were compounds containing larger heterocyclic substituents at C-5 such as the naphthyl (**9b−c**), dihydrobenzofuranyl (**9d−e**), quinolinyl (**9g−h**), benzothiophenyl (**9i−l**), and indolyl (**9m−o**) derivatives. Introduction of these groups generally results in potencies greater than that of the previously reported C-5 nitro compound (K_{disp} = 1.1 μM),[11] with K_{disp} values typically under 1 μM. Several exceptions, compounds **9b**, **9g**, **9i**, **9m**, and **9r**, all appear to have C-5 substituents featuring an L-shaped orientation with respect to the central phenyl core, resulting in an intramolecular hydrogen clash which likely diminishes the ability of these compounds to adopt the very planar binding mode required for greater potency. Compounds **8** and **9a**, functionalized at the C-5 position with relatively small, nonaromatic substituents, also show diminished potency (K_{disp} = 9.70 and 19.85 μM, respectively), pointing to the benefit of appropriate, larger aromatic groups at this position.

DOI: 10.1021/acs.jmedchem.5b01630
J. Med. Chem. XXXX, XXX, XXX−XXX

Table 1. Binding Affinities of Various 5-Substituted 2-(4-Methylpiperazin-1-yl)phenyl)-3-methylbenzamides



Compound	R	K_{disp} [μM][a,b]
8	Br	9.70
9a		19.85
9b		14.45
9c		0.88
9d		0.70
9e		0.48
9f		0.93
9g		52.50
9h		0.30
9i		1.22
9j		0.35
9k		0.61

Compound	R	K_{disp} [μM][a,b]
9l		0.84
9m		2.61
9n		0.85
9o		0.39
9p		1.13
9q		0.57
9r		15.02
9s		0.60
9t		0.71
9u		0.82
9v		1.04
9w		0.56

[a]Average of triplicate measurements. [b]FP measurement made using fluorescein-labeled H3 (1−15) peptide displacement.

To understand the binding contribution and orientation of the viable aromatic substituents, the crystal structures of WDR5 in complex with compounds **9d**, **9e**, **9h**, and **9o** were solved (PDB codes 5EAR, 5EAP, 5EAL, and 5EAM respectively). Superposition of the resultant structures reveals a high degree of alignment among the various C-5 substituents, particularly

DOI: 10.1021/acs.jmedchem.5b01630
J. Med. Chem. XXXX, XXX, XXX−XXX

with respect to the aromatic ring portion of each moiety which is directly attached to the C-5 position and which interacts in a parallel π-stacking orientation with the side chain of F133 (Figure 3).

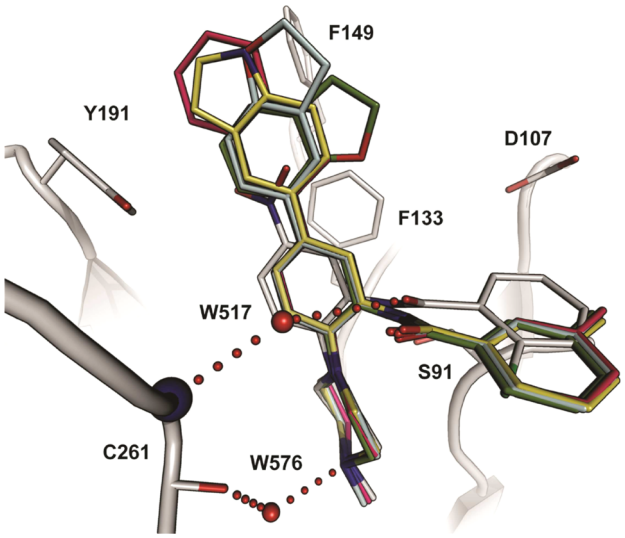


Figure 3. Structural alignment of WDR5 crystal structures in complex with **4** (gray), **9d** (green), **9e** (cyan), **9h** (pink), and **9o** (yellow). The inhibitors occupy the MLL1 binding site and form water-mediated H-bonds to residue C261 as well as a direct H-bond to S91. The phenyl rings of the heterocyclic substituents attached at the C-5 position show a high degree of alignment.

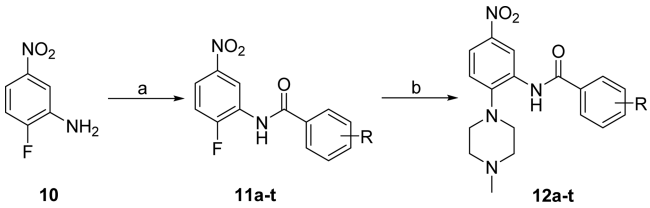
This residue also π-stacks in a similar manner with the compound's central phenyl ring, and taken together, these interactions serve to stabilize the almost planar geometry observed between the two aromatic rings. Furthermore, both the C-5 substituents of **9h** and **9o** interact in a T-shape and parallel arrangement with the side chains of F149 and Y191 respectively, presumably accounting for the relatively high potency of these compounds (K_{disp} = 0.30 and 0.39 μM, respectively). Interestingly, when comparing the potencies of the quinoline-substituted compound **9h** (K_{disp} = 0.30 μM) and naphthalene-substituted compound **9c** (K_{disp} = 0.88 μM), it is apparent that the electron-deficient quinoline substituent serves to further stabilize this interaction, consistent with previous observations that stacking between electron-poor and electron-rich aromatic rings is beneficial.[17] Introduction of a dihydrobenzofuran substituent at C-5 (compounds **9d−e**) decreased potency (K_{disp} = 0.70 and 0.48 μM, respectively), but did confer greater solubility[18] to the compounds when compared to derivatives such as **9c** and **9h**. In the case of **9d−e**, a parallel π-stacking interaction with the side chain of F133 is observed, although the furanyl portions of each substituent point toward the solvent and do not further interact with the protein.

Overall, it is apparent that the introduction of a bicyclic heterocycle at the C-5 position of the antagonist core adds favorable aromatic interactions with the nearby side chains of residues Y191, F149, and F133, ultimately resulting in greater binding affinity. Unfortunately, incorporation of these heterocycles often yields poorly soluble compounds, so the introduction of pyrrolidine and morpholine-substituted phenyl rings at C-5 was explored in order to address this issue. The resulting compounds **9p−t** displayed potencies ranging from K_{disp} = 0.57 to 15 μM and did show improved solubility[18] relative to other compounds in the series.

While attempting to identify C-5 substituents capable of conferring potency and/or solubility to the scaffold, a parallel effort was made to optimize the benzamide moiety. Co-crystal structures of WDR5 in complex with antagonists **4** and **5** revealed no direct H-bonding interaction of the benzamide substituents with the protein, and in particular, failure to engage residue D107. In order to identify suitable candidates for replacement of the phenyl derivatives that had been predominantly used at the benzamide position,[11] a chemoinformatics approach was employed. Using Accelrys Pipeline Pilot,[19] we performed an exhaustive in silico enumeration of all (benz)amides accessible using commercially available benzoyl and acid halides, benzoic acids/esters, and heterocyclic carboxylic acids (based on the eMolecules Plus database),[20] with specific consideration given to moieties that could potentially interact with the side chain of D107. The virtual focused library was reduced by applying OICR HTS filters that include calculated physical properties filters and filters to eliminate reactive moieties, entities with more than one carboxyl or acid chloride groups, and reagents from suppliers with a long lead time. About 1200 acyl halides and 9000 acids/esters were first enumerated and then docked into the WDR5-**4** co-crystal structure (PDB code 3SMR) as their *N*-(2-(4-methylpiperazin-1-yl)-5-nitrophenyl) amides using the Glide docking program.[21] The obtained docking scores were then used to rank the top 2000 compounds, of which 50 broadly representative compounds were selected for synthesis. A mix of factors such as the compound's docking score, chemotype, and anticipated synthetic difficulty were all considered during this selection, with preference given to high-scoring and more readily accessible compounds featuring relatively simple substituents (i.e., primarily those containing a limited number of heteroatoms).

In order to access the various benzamides in the target set, synthesis began by reaction of commercially available 2-fluoro-5-nitroaniline (**10**) with selected acid chlorides and benzoic acids to provide intermediate benzamides **11a−t** (Scheme 2).

Scheme 2. Synthesis of *N*-(2-(4-Methylpiperazin-1-yl)-5-nitrophenyl) Benzamides[a]



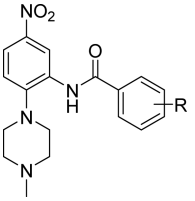
[a]Reagents and conditions: (a) (i) acid chloride, pyridine, DCM, rt; or (ii) benzoic acid, $SOCl_2$, toluene, 70 °C, then amine **10**, pyridine, DCM, rt; or (iii) benzoic acid, $POCl_3$, toluene, 70 °C, then amine **10**, pyridine, DCM, rt; (b) 1-methylpiperazine, DIPEA, DMF, 80 °C.

Acid chlorides were successfully reacted directly with **10** under basic conditions, whereas the reaction of benzoic acids using standard coupling agents such as HATU or HOBt failed to yield any product, as did in situ generation of the reactive anhydride using ethyl chloroformate. We overcame the recalcitrant reaction through use of the corresponding acid chlorides which were first prepared by refluxing the benzoic acids with either $SOCl_2$ or $POCl_3$ and then reacted with **10**

DOI: 10.1021/acs.jmedchem.5b01630
J. Med. Chem. XXXX, XXX, XXX−XXX

under basic conditions. Final elaboration of these intermediates **11a−t** was then accomplished through the addition of 1-methylpiperazine to afford benzamides **12a−t** (Table 2).

Table 2. Binding Affinities of Various *N*-(2-(4-Methylpiperazin-1-yl)-5-nitrophenyl) Benzamides

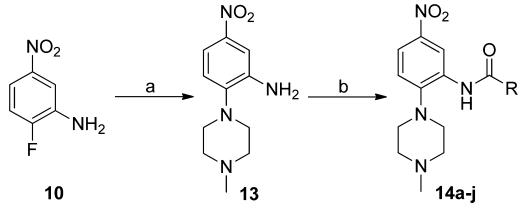


compound	R	K_{disp} [μM][a,b]
12a	2-CF_3, 5-F	>50
12b	2-CF_3, 4-OH	1.26
12c	2-Cl, 4-CF_3	13.67
12d	2-Cl, 5-CF_3	>50
12e	2-Cl, 5-Me	2.27
12f	2-Cl, 6-F	>50
12g	3-CF_3, 4-OMe	>50
12h	3-Me, 5-Me	0.29
12i	3-Me, 5-CF_3	0.85
12j	3-F, 5-CF_3	>50
12k	3-Cl, 5-Cl	0.58
12l	3-OH, 5-CF_3	0.54
12m	2-F, 5-SO_2NH_2	>50
12n	2-F, 3-F, 5-OH	0.40
12o	2-F, 3-Cl, 5-CF_3	>50
12p	2-Cl, 3-Me, 6-F	>50
12q	2-F, 3-Me, 4-F	1.36
12r	2-Me, 3-F, 5-F	2.34
12s	3-Me, 4-F, 5-Me	0.24
12t	2-F, 3-Me, 4-F, 5-Me, 6-F	0.29

[a]Average of triplicate measurements. [b]FP measurement made using fluorescein-labeled H3 (1−15) peptide displacement.

Heterocyclic carboxylic acids were coupled using a modified approach that relied on first reacting 1-methylpiperazine with commercially available **10** to afford 2-(4-methylpiperazin-1-yl)-5-nitroaniline (**13**) (Scheme 3). This intermediate was

Scheme 3. Synthesis of *N*-(2-(4-Methylpiperazin-1-yl)-5-nitrophenyl) Amides[a]

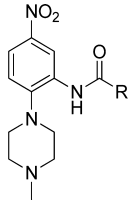


[a]Reagents and conditions: (a) 1-methylpiperazine, DIPEA, 80 °C; (b) RCO_2H, $POCl_3$, cat. DMAP, xylene, 120 °C.

subsequently reacted with the acid chlorides generated from the various heterocyclic carboxylic acids using $POCl_3$, affording the final amides **14a−j** (Table 3). All generated compounds were then evaluated in the biochemical fluorescence polarization assay as described earlier.

Compounds **12a−t** (Table 2) were intended to serve as a direct extension of compound **5**, bearing new substitution

Table 3. Binding Affinities of Various *N*-(2-(4-Methylpiperazin-1-yl)-5-nitrophenyl) Amides

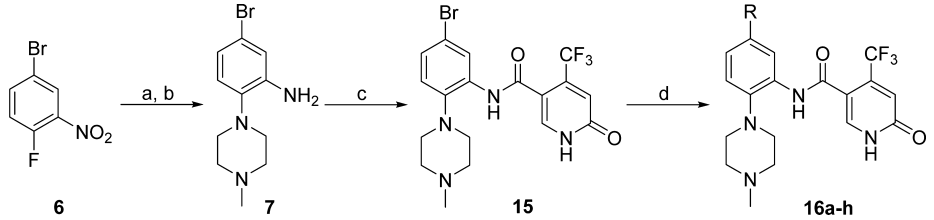


Compound	R	K_{disp} [μM][a,b]
14a		0.20
14b		>50
14c		0.77
14d		38.20
14e		>50
14f		>50
14g		>50
14h		>50
14i		13.18
14j		1.03

[a]Average of triplicate measurements. [b]FP measurement made using fluorescein-labeled H3 (1−15) peptide displacement.

DOI: 10.1021/acs.jmedchem.5b01630
J. Med. Chem. XXXX, XXX, XXX−XXX

Scheme 4. Synthesis of 5-Substituted *N*-(2-(4-Methylpiperazin-1-yl)phenyl)-6-oxo-4-(trifluoromethyl)-1,6-dihydropyridine-3-carboxamides[a]



[a]Reagents and conditions: (a) 1-methylpiperazine, DIPEA, DMSO, 80 °C; (b) $Fe_{(s)}$, $CaCl_2$, water/MeOH (1:1), 100 °C; (c) 6-oxo-4-(trifluoromethyl)-1,6-dihydropyridine-3-carboxylic acid, $SOCl_2$, 80 °C, then aniline **7**, DCM, pyridine, rt; (d) boronic acid/ester, Xphos Pd G2, Na_2CO_3, 1,4-dioxane/water (5:3), 120 °C.

patterns which we hoped would better fill the hydrophobic subpocket hitherto occupied by the 2-Cl-3-Me-4-F-phenyl moiety. Significantly, of the disubstituted compounds in the series (**12a−m**), compound **12h** is the most potent (K_{disp} = 0.29 μM) and hints at the greater capacity of the 3,5-dimethylated benzamide to occupy the hydrophobic shelf. While tri- or penta-substitution of the benzamide (compounds **12n−s** and **12t**, respectively) did not significantly improve potency, addition of a fluorine to the 4-position of **12h** was well tolerated, giving rise to **12s** with a comparable K_{disp} = 0.24 μM. The only compound bearing a substituent positioned to potentially form an H-bond with the side-chain of D107, 5-hydroxylated compound **12n**, was reasonably potent (K_{disp} = 0.40 μM) but does not likely engage in the anticipated interaction. An orthogonal analysis of the most potent compounds (K_{disp} < 0.3 μM) using surface plasmon resonance (SPR) correlated well with the observed FP values and underscored that **12h** (K_D = 0.39 μM), **12s** (K_D = 0.33 μM), and **12t** (K_D = 0.34 μM) showed no marked improvement in binding over previously reported compound **5** (K_D = 0.38 μM). Surprisingly, all appeared to bind more strongly than **9h** (K_D = 0.72 μM), the most potent compound from the initial series with a comparable FP value (K_{disp} = 0.30 μM).

The size of the heterocyclic moieties in analogs **14a−j** (Table 3), which were identified using the in silico approach, is consistent with our previous observation that larger quinolinyl or naphthyl substituents are well tolerated in the hydrophobic pocket.[11] Docking studies also suggest that the 4-hydroxyl substituents on antagonists such as **14b−c** may potentially form H-bonds to the side chain of D107 and that the non-hydroxylated aryl rings may occupy the hydrophobic pocket lined by residues A65, A47, Y260, and L321. Unfortunately, the 2-position methyl substituent on **14b** renders it inactive (presumably due to steric effects), while the activity of **14c** is little different from that of the previously reported 1-naphthyl analog,[11] suggesting that no favorable interactions are gained from the additional 4-position hydroxyl group. Moving the hydroxyl group to the 6-position only resulted in weak inhibitor **14d** despite the fact that docking predicts a flip of the entire quinolinyl moiety in order for an H-bond with D107 to be established. Introduction of pyrazolo pyridine (**14e**), benzimidazole (**14f−g**), and triazolo pyridine (**14h**) groups resulted in inactive compounds, while the benzo pyrazoles **14i−j** gave weaker antagonists. Within this heterocyclic series, only the 4-(trifluoromethyl)pyridinone compound (**14a**) showed exceptionally strong binding affinity with K_{disp} = 0.20 μM. As this was close to the detection limit of the FP assay using the H3 (1−15) peptide, a more sensitive version employing fluorescein-labeled 9-Ala-FAM peptide (K_D = 19 ± 3 nM) was developed in order to more accurately assess the potency of this compound. Upon retesting with the new assay format, **14a** was shown to have a K_{disp} = 0.079 μM, making it a very potent and suitable chemotype for further development.

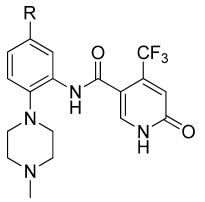
Following the discovery of the 4-(trifluoromethyl)pyridinone moiety, we turned our attention toward the synthesis of antagonists combining this feature with some of the more active or solubilizing C-5 substituents reported elsewhere in this study. To that end, we prepared compounds **16a−h** according to the route outlined in Scheme 4.

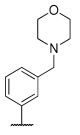
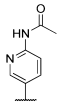
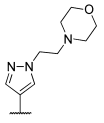
To start, the coupling of 5-bromo-2-(4-methylpiperazin-1-yl)aniline (**7**) to 6-oxo-4-(trifluoromethyl)-1,6-dihydropyridine-3-carbonyl chloride (generated from 6-oxo-4-(trifluoromethyl)-1,6-dihydropyridine-3-carboxylic acid with $SOCl_2$) produced bromide **15**, the intermediate through which the C-5 position aryl moiety was to be introduced. This was accomplished using the requisite boronic acid/ester and standard Suzuki coupling conditions, giving rise to antagonists **16a−h** (Table 4).

Because the **16a−h** antagonist set yielded many compounds with K_{disp} values <100 nM using the FP assay, we also evaluated the entire series using SPR as an orthogonal method to confirm binding affinity. The measured K_D values obtained are also presented in Table 4 and show fairly strong correlation with the recorded FP assay values. One of the principal compounds of interest is **16b**, intended to bring together the potent C-5 3-quinoline substituent described earlier with the 4-(trifluoromethyl)pyridinone moiety. By eliminating the nitro group and improving both the observed K_{disp} and K_D values, we were provided with what we considered to be a new lead compound in the overall program. As such, we first chose to screen **16b** for selectivity against a broad panel of epigenetic and nonepigenetic targets including G-protein coupled receptors (GPCRs), ion channels, transporters, and kinases to determine whether the compound was selective enough to warrant additional investigation.[22] Although **16b** was inactive against all other targets, it did inhibit receptor tyrosine kinases FLT3 and FLT4 with IC_{50} values of 0.5 and 0.6 μM, respectively, and a set of other protein kinases at a low micromolar level. We employed a structure-based approach to predict the binding mode of **16b** to a selected subset of protein kinases in DFG-in and DFG-out (DFG being the Asp-Phe-Gly motif) conformations to dial out this undesirable set of activities. Thus, docking of **16b** into the X-ray crystal structures of Abl (PDB codes 4FOC, DFG-in, and 3CS9, DFG-out), ALK (PDB code 3GXL, DFG-in), CHK2 (PDB codes 2W7X and 4A9U, both DFG-in), and KDR (PDB codes 3VO3 and 4AGD, both DFG-out) identified the 3-quinoline substituent at C-5 to be the likely hinge-binding moiety. It is known that, with exception of the PIM family kinases, eliminating the key hinge-

DOI: 10.1021/acs.jmedchem.5b01630
J. Med. Chem. XXXX, XXX, XXX−XXX

Table 4. Binding Affinities of Various *N*-(2-(4-Methylpiperazin-1-yl)phenyl)-6-oxo-4-(trifluoromethyl)-1,6-dihydropyridine-3-carboxamides



Compound	R	K_{disp} [μM][a,b]	K_D [μM][a,c]
14a	NO_2	0.08	0.04
16a		0.14	0.04
16b		0.06	0.05
16c		0.07	0.05
16d		0.06	0.03
16e		0.09	0.03
16f		0.14	0.05
16g		0.14	0.04
16h		0.13	0.21

[a]Average of triplicate measurements. [b]FP measurement made using fluorescein-labeled 9-Ala-FAM peptide displacement. [c]SPR measurement.

DOI: 10.1021/acs.jmedchem.5b01630
J. Med. Chem. XXXX, XXX, XXX−XXX

binding moiety in the ligand results in a significant reduction (or complete loss) of kinase inhibitory activity. To address the kinase inhibitory liability as well as the rather poor aqueous solubility of this compound, alternate C-5 substituents which showed acceptable activity in the initial screen (Table 1) and which could potentially prevent kinase hinge-binding while also enhancing solubility were docked into the above-mentioned kinase crystal structures. In the docking experiments with Glide, compounds **16a**, **16c**, and **16d** did not appear to interact meaningfully with the kinases, whereas compounds **16b** and **16e** were suggested to be hinge binders.

Based on these in silico results, we decided to focus our attention on the morpholine- containing compound **16d** as a probe candidate. Running the compound through the same selectivity panel as described above showed that this compound was inactive against all other targets. Compound **16d** was co-crystallized with WDR5 to yield a high-resolution structure of the WDR5-**16d** complex (PDB code 4QL1) (Figure 4).

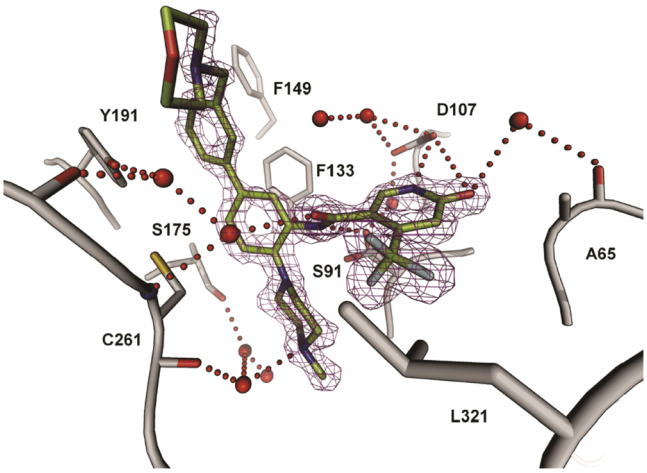


Figure 4. X-ray crystal structure of the WDR5-**16d** complex (PDB code 4QL1). Compound **16d** (green) binds in the arginine binding site of WDR5 (gray). The experimental electron density (purple mesh) of the ligand is shown at a 1.5 Å resolution ($2F_o - F_c$ map contoured at 1.5 σ). In addition to the binding interactions observed in previous derivatives, the nitrogen of the newly introduced pyridinone substituent forms a direct H-bond to the D107 side chain. A water-mediated H-bond between the pyridinone oxygen and the A65 carbonyl further stabilizes binding of the ligand. The pendant morpholino moiety points into the bulk solvent and does not interact with the protein. As a result of this solvent exposure, the electron density of the morpholine ring is not well-defined.

Recapitulated in this co-crystal structure are the direct and water-mediated H-bonds with S91 and C261, the importance of the latter clearly demonstrated through the synthesis of a **16d** analog morpholine at the C-2 position instead of methylpiperazine. Based on the fact that the WDR5 pocket recognizes highly basic arginine-containing side chains and also based on the analysis of the electrostatic potential on the Connolly surface of the pocket, we hypothesized that the positively charged nitrogen of the methylpiperazine is of paramount importance for binding to WDR5. Indeed, elimination of the protonated N1 atom of the methylpiperazine through replacement of the C-2 position substituent with morpholine leads to complete loss of antagonism. Because such a small structural change resulted in almost complete inactivation, this compound, *N*-(4-morpholino-3′-(morpholinomethyl)-[1,1′-biphenyl]-3-yl)-6-oxo-4-(trifluoromethyl)-1,6-dihydropyridine-3-carboxamide (OICR-0547), serves as a suitable negative control for biological experiments.[12]

Seen for the first time in the WDR5-**16d** structure is a new, direct H-bond to D107, with the trifluoromethyl group filling out the adjacent hydrophobic pocket by forming van der Waals contacts with residues A47, L321, and I305. This group also serves to push the entire pyridinone moiety toward the D107 face of the pocket, decreasing the bonding distance while also serving to facilitate the H-bond through an inductive electron-withdrawing effect that lengthens the pyridinone N−H bond. Finally, a water-mediated H-bond between the pyridinone oxygen and A65 confers additional stabilization to this binding pose. Elsewhere, the phenyl ring of the C-5 substituent interacts in a parallel π-stacking manner with the side chains of F133 and Y191. Compared to the C-5 quinoline of **16b**, which interacts in a T-shape arrangement with the side chain of F149, a slight rotation of the phenyl ring also enables parallel π-stacking with Y191.

Cell-Based and Pharmacokinetic Assessment. Having selected **16d** as a chemical probe candidate, the compound was evaluated for its ability to inhibit proliferation in N-terminal C/EBPα p30-expressing ($Cebpa^{p30/p30}$) human AML cells.[12] The p30-dependent increase in self-renewal was shown to require WDR5 binding. We recently reported that direct antagonism of the WDR5-MLL1 interaction with **16d** reduced the viability of primary human AML cells with N-terminal C/EBPα mutations by about 50% (mean value, n = 8) at 5 μM. This result prompted not only the adoption of **16d** as a viable in vitro chemical probe of WDR5-MLL1 interaction but also spurred us to assess the utility of this compound for in vivo applications.

Although **16d** was measured as having suboptimal Caco-2 permeability and a high efflux ratio (apparent permeability rates were P_{app} A → B = 0.1 × 10^{-6} cm/sec and P_{app} B → A = 16.8 × 10^{-6} cm/s), it was potent enough to show significant activity in cells.[10] In order to potentially extend the use of **16d** into murine in vivo models of AML,[23,24] we also undertook an assessment of the compound's stability and pharmacokinetic (PK) parameters in mice. Initial mouse liver microsomal and plasma stability assessments showed the compound to be stable, returning 85% and 100% of the parent respectively after 0.5 h. For PK studies, **16d** was administered to mice (female NOD-SCID) at 3 mg/kg IV (n = 3) and 30 mg/kg IP (n = 3), with blood collection conducted 6 times up to 24 h postinjection. The intravenous (IV) dosing protocol yielding a C_{max} of 697 ng/mL (1.26 μM) at T_{max} = 5 min (the first blood sample was drawn 5 min after the tail vein injection) and with a significant $T_{1/2}$ = 287 min (4.78 h). The compound exposure was also reasonable with an AUC = 1012 h·ng/mL, while clearance was moderate at Cl = 3233 mL/h/kg, and the V_D = 15.6 L/kg (plasma confined). The IP dosing produced a C_{max} of 3790 ng/mL (6.85 μM) at T_{max} = 15 min, with an AUC = 6813 h·ng/mL and bioavailability F = 67%. Taken together, these parameters suggest that **16d** constitutes a reasonable starting point for further optimization leading to an in vivo probe of the WDR5-MLL1 interaction and possibly an AML therapeutic.

CONCLUSION

Medium-throughput screening of the OICR 16,000 diversity set initially led to the identification of a low-micromolar WDR5 antagonist, compound **4** (K_{disp} = 7 μM).[10] Its co-crystal structure in complex with WDR5 then facilitated early SAR exploration via structure-based analog design that ultimately led

DOI: 10.1021/acs.jmedchem.5b01630
J. Med. Chem. XXXX, XXX, XXX−XXX

to the development of **5**, a reasonably potent antagonist of the WDR5-MLL1 interaction (K_{disp} = 0.27 μM, K_D = 0.38 μM).[11] Additional structural insights into the nature of the antagonist binding site and binding mode were then obtained through the WDR5-**5** complex crystal structure, allowing for further optimization of this scaffold. The SAR observed through variation of the C-5 substituent revealed several suitable moieties that improved the overall properties of the compound, although significant improvements in potency remained elusive. Design and enumeration of a focused virtual compound library of about 10,200 analogs, and subsequent docking experiments with Glide enabled the discovery of the 4-(trifluoromethyl)-pyridin-2(1*H*)-one moiety to replace the C-1 benzamide. This substituent was critical in realizing the antagonist potencies of K_{disp} < 100 nM. A chemoinformatics-supported approach was then used to select a suitable C-5 substituent from the earlier development set to pair with the 4-(trifluoromethyl)pyridin-2(1*H*)-one group, resulting in antagonist **16d.** This compound showed significant in vitro activity (K_{disp} = 64 ± 4 nM) as well as the ability to reduce the viability of primary human AML cells with N-terminal C/EBPα mutations (IC_{50} ≈ 5 μM). A reasonable pharmacokinetic profile (via IP administration) suggests that **16d** may not only be a viable chemical probe for exploring the WDR5-MLL1 interaction but also may serve as a suitable starting point for development of a therapeutic for MLL1-driven acute myeloid leukemias.

EXPERIMENTAL SECTION

Focused Library Design and Molecular Docking. Focused library design and reaction-based virtual enumeration was performed with Accelrys Pipeline Pilot (currently, BIOVIA, http://www.3ds.com/products-services/biovia/). Structures, vendor information, and prices of all commercially available building blocks were assembled in a flat SD file based on the database table dumps from eMolecules Plus (https://www.emolecules.com/info/building-blocks). These available building blocks were fed into Pipeline Pilot Markush substructure searches to yield all commercially available benzoyl and acid halides, benzoic acids and esters, and heterocyclic carboxylic acids. The lists were then used to virtually enumerate all (benz)amides accessible via commercially available reagents. OICR HTS filters were used to reduce the number of enumerated products by eliminating compounds with undesirable calculated physical properties (MW, PSA, calculated octanol−water partition coefficient, log *P*, number of aromatic rings, number of H-bond acceptors and donors, number of rotatable bonds, individual halogens counts, positively and negatively charged groups counts). As a result, about 10,200 (benz)amides were enumerated: 1200 based on available acid halides and 9000 based on available acids/esters. The obtained library was prepared with the ligprep procedure from Schrödinger, Inc. Accordingly, the molecules were ionized to pH 7 with Epik, specific chiralities were retained while all other chiral centers were enumerated, and no tautomers were generated. The resulting structures were energy minimized with OPLS_2005 force field. To avoid docking instability and to obtain the best possible results during the docking stage with Glide, 10 different conformers were generated for each molecule with the ConfGen Mixed torsional/Low-mode sampling procedure using 1 × 10^6 steps and a 21 kJ/mol energy window for saving structures. The protein grid calculation was done based on the WDR5-**4** co-crystal structure (PDB code 3SMR, resolved to 1.8 Å) with one H-bond constraint defined with the side chain of S91. Docking was performed with the Glide docking program using the SP scoring function (http://www.schrodinger.com). Each compound was subject to postdocking minimization using a set of 100 ligand poses. Out of all the poses generated for multiple conformers, one best docking pose was retained for each compound. The obtained SP Glide docking scores were used to rank the top 2000 virtual compounds that were visually inspected. The 50 best looking analogs were selected and prioritized for synthesis.

Chemistry. All oxygen- and/or moisture-sensitive reactions were carried out under a nitrogen atmosphere. Solvent removal from reaction mixtures was performed by rotary evaporation under reduced pressure at 40 °C unless otherwise noted. All reagents and laboratory grade solvents were purchased from commercial vendors and used as received, without further purification. The yields given refer to chromatographically purified and spectroscopically pure compounds, unless stated otherwise. Nuclear magnetic resonance (NMR) spectra for ^{1}H, ^{13}C, DEPTq, COSY, HSQC, and HMBC were recorded on a Bruker Avance-III 500 MHz spectrometer (500 MHz for ^{1}H, 125 MHz for ^{13}C). All ^{1}H NMR spectra were referenced relative to $SiMe_4$ through a resonance of the employed deuterated solvent or proteo impurity of the solvent; chloroform (7.26 ppm), acetone (2.05 ppm), DMSO (3.33 ppm), and methanol (3.31 ppm) for ^{1}H NMR; chloroform (77.00 ppm), acetone (29.84 ppm), DMSO (39.52 ppm), and methanol (49.00 ppm) for ^{13}C NMR. NMR data are reported as follows: chemical shifts (δ), multiplicity (br = broad, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet); coupling constant(s) (*J*) in Hz; integration. Unless otherwise noted, NMR data were collected at 25 °C. Flash column chromatography was performed using a Biotage SP1 system fitted with a KP-SIL SNAP Silica Gel (60 Å mesh) Flash Cartridge (FSKO-1107). Compound purity determination was conducted by UV absorbance at 254 nm during tandem liquid chromatography/mass spectrometry (LCMS) using a Waters Acquity separations module. All compounds reported in this publication were assessed as having ≥95% purity using this method. Compound identity was determined via low-resolution mass spectrometry (LRMS) conducted in positive ion mode using a Waters Acquity SQD mass spectrometer (electrospray ionization source) fitted with a PDA detector. Mobile phase A consisted of 0.1% formic acid in water, while mobile phase B consisted of 0.1% formic acid in acetonitrile. Two types of columns were used: Column 1, Acquity UPLC CSH C18 (2.1 × 50 mm, 130 Å, 1.7 μm; part no. 186005296); and Column 2, Acquity UPLC BEH C8 (2.1 × 50 mm, 130 Å, 1.7 μm; part no. 186002877). Column temperature was maintained at 25 °C, and the sample solution injection volume was 1 μL. Leucine Enkephalin was used as lock mass, and MassLynx 4.1 was used for data analysis. Analytical thin-layer chromatography (TLC) was performed on aluminum sheets coated with silica gel 60 F254 (0.2 mm, VWR International, Darmstadt, Germany). Visualization was accomplished with UV light and aqueous potassium permanganate ($KMnO_4$) stain followed by heating. High-resolution mass spectrometry (HRMS) was conducted using a Waters Xevo quadrupole-time-of-flight (QTOF) hybrid mass spectrometer system coupled with an Acquity ultraperformance liquid chromatography (UPLC) system.

*5-Bromo-2-(4-methylpiperazin-1-yl)aniline (**7**).* A 100 mL round-bottom flask was charged with 4-bromo-1-fluoro-2-nitrobenzene (**6**) (3.33 mL, 26.5 mmol), 1-methylpiperazine (3.28 mL, 29.2 mmol), and *N*,*N*-diisopropylethylamine (9.12 mL, 53.1 mmol) in DMSO (20 mL). The reaction turned red and was stirred for 3 h at 80 °C during which time it gradually turned brown. After cooling to 23 °C and dilution with water (80 mL), the aqueous layer was extracted with EtOAc (4 × 50 mL). The combined organic extracts were dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (0−30% MeOH/EtOAc) to afford the intermediate 1-(4-bromo-2-nitrophenyl)-4-methylpiperazine (8.22 g, 100% yield) as a red oil. Subsequently, the 1-(4-bromo-2-nitrophenyl)-4-methylpiperazine (7.94 g, 27 mmol), iron powder (7.39 g, 132 mmol), and calcium chloride (3.52 g, 32 mmol) were dissolved in a mixture of water and MeOH (1:1, 100 mL) and heated at reflux for 3 h. The solution was then basified with 1 M NaOH, diluted with brine, and extracted with EtOAc (5 × 50 mL). The combined organic extracts were dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (5−30% MeOH/EtOAc) to afford the title compound (2.75 g, 39% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 6.83 (d, *J* = 2.4 Hz, 1H), 6.80 (d, *J* = 8.3 Hz, 1H), 6.65 (dd, *J* = 2.4, 8.3 Hz, 1H), 4.97 (s, 2H), 2.76 (br s, 4H), 2.47 (br s, 4H),

DOI: 10.1021/acs.jmedchem.5b01630
J. Med. Chem. XXXX, XXX, XXX−XXX

2.22 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 144.3, 137.4, 120.9, 118.5, 116.2, 116.1, 55.0 (2), 50.1 (2), 45.8. HRMS (ESI) m/z calcd for $C_{11}H_{17}BrN_3$ $[M + H]^+$: 270.0606, found: 270.0612.

*N-(5-Bromo-2-(4-methylpiperazin-1-yl)phenyl)-3-methylbenzamide (**8**).* 5-Bromo-2-(4-methylpiperazin-1-yl)aniline (**7**) (1.5 g, 5.55 mmol) was dissolved in CH_2Cl_2 (25 mL) prior to the addition of pyridine (584 μL, 7 mmol) and 3-methylbenzoyl chloride (807 μL, 7 mmol). After 1 h at 23 °C the reaction mixture was diluted with EtOAc and 1 M NaOH, and the aqueous layer extracted with EtOAc (3 × 15 mL). The organic layers were combined, dried ($MgSO_4$), filtered, and concentrated under reduced pressure to give a brown solid. This crude product was purified by flash column chromatography on silica gel (5−30% MeOH/EtOAc) to afford the title compound (2.12 g, 93% yield) as an off-white solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.58 (s, 1H), 8.39 (d, J = 2.4 Hz, 1H), 7.74−7.71 (m, 2H), 7.49−7.45 (m, 2H), 7.30 (dd, J = 2.4, 8.6 Hz, 1H), 7.25 (d, J = 8.6 Hz, 1H), 2.86 (t, J = 4.8 Hz, 4H), 2.51 (br s, 4H), 2.43 (s, 3H), 2.24 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 164.4, 142.1, 138.2, 134.5, 134.1, 132.7, 128.8, 127.5, 126.8, 124.1, 123.0, 122.7, 116.6, 55.4 (2), 51.3 (2), 45.8, 20.9. HRMS (ESI) m/z calcd for $C_{19}H_{23}BrN_3O$ $[M + H]^+$: 388.1025, found: 388.1036.

General Procedure A for the Synthesis of Compounds 9a−w. A 5 mL reaction vial was charged with *N*-(5-bromo-2-(4-methylpiperazin-1-yl)phenyl)-3-methylbenzamide (**8**) (50 mg, 0.13 mmol), the boronic acid/ester (0.17 mmol), sodium carbonate (68 mg, 0.64 mmol), $Pd(Ph_3P)_4$ (15 mg, 0.01 mmol), and toluene/water (1:1, 2 mL). The reaction mixture was then heated at 115 °C overnight. After cooling to 23 °C, the solution was diluted with EtOAc (1 mL) and water (1 mL), and the aqueous layer extracted with EtOAc (3 × 2 mL). The combined organic layers were then dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The crude product was purified by flash column chromatography on silica gel (5−50% MeOH/EtOAc) to afford the desired compound.

*N-(5-Cyclopropyl-2-(4-methylpiperazin-1-yl)phenyl)-3-methylbenzamide (**9a**).* The title compound was prepared according to general procedure A (17 mg, 35% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.25 (br s, 1H), 8.30 (s, 1H), 7.76 (s, 1H), 7.65 (d, J = 6.6 Hz, 1H), 7.43−7.38 (m, 2H), 7.18 (d, J = 8.2 Hz, 1H), 6.84 (dd, J = 2.0, 4.3 Hz, 1H), 3.35−2.75 (m, 8H), 2.66 (br s, 3H), 2.47 (s, 3H), 1.95−1.89 (m, 1H), 0.99−0.95 (m, 2H), 0.76−0.72 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.1, 142.7, 139.0, 138.1, 135.4, 133.9, 132.7, 129.0, 128.1, 123.9, 121.4, 121.1, 116.9, 56.0 (2), 51.5, 45.5, 21.7 (2), 15.7, 9.5. HRMS (ESI) m/z calcd for $C_{22}H_{28}N_3O$ $[M + H]^+$: 350.2232, found: 350.2230.

*3-Methyl-N-(2-(4-methylpiperazin-1-yl)-5-(naphthalen-1-yl)phenyl)benzamide (**9b**).* The title compound was prepared according to general procedure A (26 mg, 44% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.32 (br s, 1H), 8.71 (d, J = 1.5 Hz, 1H), 7.99 (d, J = 8.3 Hz, 1H), 7.90 (d, J = 8.2 Hz, 1H), 7.86 (d, J = 8.2 Hz, 1H), 7.77 (s, 1H), 7.68 (d, J = 7.3 Hz, 1H), 7.53−7.38 (m, 7H), 7.27 (dd, J = 1.9, 8.1 Hz, 1H), 3.27 (br s, 4H), 2.96 (br s, 4H), 2.64 (br s, 3H), 2.46 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.2, 139.7, 139.5, 139.2, 139.1, 135.2, 134.0, 133.6, 132.9, 131.7, 129.0, 128.5, 128.1, 128.0, 127.3, 126.3, 126.1, 126.1, 125.9, 125.5, 123.8, 121.8, 121.0, 55.5 (2), 50.8, 45.0, 21.7 (2). HRMS (ESI) m/z calcd for $C_{29}H_{30}N_3O$ $[M + H]^+$: 436.2389, found: 436.2397.

*3-Methyl-N-(2-(4-methylpiperazin-1-yl)-5-(naphthalen-2-yl)phenyl)benzamide (**9c**).* The title compound was prepared according to general procedure A (9 mg, 15% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.24 (br s, 1H), 8.98 (s, 1H), 8.11 (d, J = 1.1 Hz, 1H), 7.91 (d, J = 8.1 Hz, 1H), 7.86 (d, J = 8.1 Hz, 1H), 7.81−7.79 (m, 2H), 7.69 (d, J = 7.1 Hz, 1H), 7.53−7.40 (m, 6H), 3.27 (br s, 4H), 2.97 (br s, 4H), 2.70 (br s, 3H), 2.49 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.7, 140.8, 139.3, 137.7, 137.4, 135.0, 133.8, 133.7, 133.2, 133.1, 129.1, 128.8, 128.5, 128.5, 127.9, 126.6, 126.4, 126.2, 125.6, 124.0, 123.8, 121.8, 54.3, 49.5 (2), 43.9, 29.9, 21.8 (2). HRMS (ESI) m/z calcd for $C_{29}H_{30}N_3O$ $[M + H]^+$: 436.2389, found: 436.2389.

*N-(5-(2,3-Dihydrobenzofuran-7-yl)-2-(4-methylpiperazin-1-yl)phenyl)-3-methylbenzamide (**9d**).* The title compound was prepared according to general procedure A (36 mg, 62% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.23 (br s, 1H), 8.87 (d, J = 2.0 Hz, 1H), 7.78 (s, 1H), 7.68 (d, J = 7.0 Hz, 1H), 7.50 (dd, J = 2.0, 8.3 Hz, 1H), 7.44−7.38 (m, 2H), 7.34−7.32 (m, 2H), 7.18 (dd, J = 1.1, 7.3 Hz, 1H), 6.93 (t, J = 7.6 Hz, 1H), 4.64 (t, J = 8.8 Hz, 2H), 3.35−2.80 (m, 10H), 2.65 (s, 3H), 2.47 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.3, 157.4, 139.3, 138.1, 136.7, 135.4, 133.4, 133.0, 129.0, 128.4, 128.1, 128.0, 124.9, 124.5, 123.6, 122.8, 121.4, 121.1, 120.7, 71.4, 54.7 (2), 49.3 (2), 43.8, 30.0, 21.7. HRMS (ESI) m/z calcd for $C_{27}H_{30}N_3O_2$ $[M + H]^+$: 428.2338, found: 428.2342.

*N-(5-(2,3-Dihydrobenzofuran-5-yl)-2-(4-methylpiperazin-1-yl)phenyl)-3-methylbenzamide (**9e**).* The title compound was prepared according to general procedure A (35 mg, 60% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.27 (br s, 1H), 8.79 (s, 1H), 7.79 (s, 1H), 7.71−7.67 (m, 1H), 7.51 (d, J = 1.2 Hz, 1H), 7.44−7.38 (m, 3H), 7.33−7.29 (m, 2H), 6.84 (d, J = 8.3 Hz, 1H), 4.62 (t, J = 8.5 Hz, 2H), 3.32−2.82 (m, 10H), 2.66 (s, 3H), 2.48 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.3, 160.2, 140.3, 139.3, 138.0, 135.3, 133.8, 133.1, 133.0, 129.0, 128.3, 128.0, 127.3, 124.0, 123.6, 122.7, 121.7, 118.6, 109.6, 71.7, 55.0 (2), 49.7 (2), 44.1, 29.9, 21.8. HRMS (ESI) m/z calcd for $C_{27}H_{30}N_3O_2$ $[M + H]^+$: 428.2338, found: 428.2353.

*N-(5-(2,3-Dihydrobenzo[b][1,4]dioxin-6-yl)-2-(4-methylpiperazin-1-yl)phenyl)-3-methylbenzamide (**9f**).* The title compound was prepared according to general procedure A (8 mg, 13% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.24 (br s, 1H), 8.77 (d, J = 1.5 Hz, 1H), 7.77 (s, 1H), 7.67 (d, J = 7.1 Hz, 1H), 7.44−7.39 (m, 3H), 7.32−7.27 (m, 2H), 7.17 (d, J = 2.2 Hz, 1H), 7.14 (dd, J = 2.3, 8.2 Hz, 1H), 6.92 (d, J = 8.4 Hz, 1H), 4.30 (s, 4H), 3.21 (br s, 4H), 2.96 (br s, 4H), 2.66 (br s, 3H), 2.47 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.8, 143.9, 143.9, 139.2, 136.7, 136.5, 134.8, 133.4, 133.4, 133.2, 129.0, 129.6, 123.9, 123.6. 121.5, 121.1, 120.6, 117.8, 116.2, 64.7, 64.6, 53.8 (2), 49.6, 43.8, 21.7 (2). HRMS (ESI) m/z calcd for $C_{27}H_{30}N_3O_3$ $[M + H]^+$: 444.2287, found: 444.2294.

*N-(5-(Isoquinolin-4-yl)-2-(4-methylpiperazin-1-yl)phenyl)-3-methylbenzamide (**9g**).* The title compound was prepared according to general procedure A (31 mg, 52% yield). ^{1}H NMR (500 MHz, CD_3OD) δ 9.21 (br s, 1H), 8.61 (d, J = 1.9 Hz, 1H), 8.36 (s, 1H), 8.14 (d, J = 8.0 Hz, 1H), 8.01 (d, J = 8.5 Hz, 1H), 7.77−7.67 (m, 4H), 7.44−7.41 (m, 3H), 7.29 (dd, J = 2.0, 8.1 Hz, 1H), 3.03 (t, J = 4.9 Hz, 4H), 2.68 (br s, 4H), 2.43 (s, 3H), 2.38 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 167.7, 152.9, 144.4, 142.7, 140.3, 136.0, 135.7, 135.1, 134.8, 134.6, 134.1, 132.8, 130.2, 130.1, 129.6, 129.1, 128.9, 127.7, 125.9, 125.4, 124.0, 122.5, 57.0 (2), 52.9, 46.3, 21.6 (2). HRMS (ESI) m/z calcd for $C_{28}H_{29}N_4O$ $[M + H]^+$: 437.2341, found: 437.2339.

*3-Methyl-N-(2-(4-methylpiperazin-1-yl)-5-(quinolin-3-yl)phenyl)-benzamide (**9h**).* The title compound was prepared according to general procedure A (26 mg, 44% yield). ^{1}H NMR (500 MHz, CD_3OD) δ 9.01 (d, J = 2.3 Hz, 1H), 8.61 (d, J = 2.2 Hz, 1H), 8.36 (d, J = 2.2 Hz, 1H), 7.99 (d, J = 8.5 Hz, 1H), 7.89 (d, J = 7.9 Hz, 1H), 7.72−7.67 (m, 3H), 7.57−7.47 (m, 1H), 7.43−7.37 (m, 3H), 7.29 (d, J = 8.2 Hz, 1H), 2.90 (t, J = 4.8 Hz, 4H), 2.60 (br s, 4H), 2.40 (s, 3H), 2.33 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 167.5, 150.4, 147.9, 144.4, 140.3, 135.9, 135.4, 135.1, 135.0, 134.9, 134.2, 131.2, 130.1, 129.8, 129.7, 129.1, 128.9, 128.7, 125.4, 124.8, 122.7, 120.8, 57.0 (2), 52.8, 46.4, 21.6 (2). HRMS (ESI) m/z calcd for $C_{28}H_{29}N_4O$ $[M + H]^+$: 437.2341, found: 437.2336.

*N-(5-(Benzo[b]thiophen-3-yl)-2-(4-methylpiperazin-1-yl)phenyl)-3-methylbenzamide (**9i**).* The title compound was prepared according to general procedure A (11 mg, 15% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.30 (br s, 1H), 8.82 (s, 1H), 8.03 (d, J = 8.1 Hz, 1H), 7.92 (d, J = 7.8 Hz, 1H), 7.79 (s, 1H), 7.69 (d, J = 7.7 Hz, 1H), 7.47 (s, 1H), 7.44−7.36 (m, 6H), 3.25 (br s, 4H), 2.98 (br s, 4H), 2.64 (s, 3H), 2.48 (s, 3H); ^{13}C NMR (125 MHz, CDCl3) δ 165.3, 140.9, 139.2, 139.2, 137.8, 137.4, 135.2, 134.7, 133.9, 133.0, 129.0, 128.3, 124.7, 124.6 (2C), 124.1, 123.7, 123.1 (2C), 121.7, 120.6, 55.1 (2), 50.0, 44.4, 21.7 (2). HRMS (ESI) m/z calcd for $C_{27}H_{28}N_3OS$ $[M + H]^+$: 442.1953, found: 442.1962.

*N-(5-(Benzo[b]thiophen-2-yl)-2-(4-methylpiperazin-1-yl)phenyl)-3-methylbenzamide (**9j**).* The title compound was prepared according to general procedure A (9 mg, 13% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.18 (br s, 1H), 9.01 (s, 1H), 7.84−7.77 (m, 3H), 7.68 (d, J

DOI: 10.1021/acs.jmedchem.5b01630
J. Med. Chem. XXXX, XXX, XXX−XXX

= 5.6 Hz, 1H), 7.63 (s, 1H), 7.48−7.42 (m, 3H), 7.38−7.30 (m, 3H), 3.59−2.86 (m, 8H), 2.70 (br s, 3H), 2.49 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.6, 143.1, 140.8, 139.9, 139.4, 138.0, 134.9, 133.9, 133.6, 133.2, 129.1, 128.5, 125.4, 124.8, 123.7, 123.0, 122.6, 122.5, 122.0, 121.7, 120.7, 54.4 (2), 49.4, 44.0 (2), 21.8. HRMS (ESI) m/z calcd for $C_{27}H_{28}N_3OS$ $[M + H]^+$: 442.1953, found: 442.1961.

*3-Methyl-N-(5-(5-methylbenzo[b]thiophen-2-yl)-2-(4-methylpiperazin-1-yl)phenyl)benzamide (**9k**).* The title compound was prepared according to general procedure A (14 mg, 23% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.06 (s, 1H), 8.96 (s, 1H), 7.78 (s, 1H), 7.70−7.64 (m, 2H), 7.59−7.53 (m, 2H), 7.47−7.41 (m, 3H), 7.34 (d, J = 8.2 Hz, 1H), 7.15 (dd, J = 1.3, 8.2 Hz, 1H), 3.95−2.64 (m, 11H), 2.48 (s, 3H), 2.47 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.3, 143.8, 141.2, 139.2, 137.0, 135.1, 134.5, 134.1, 133.0, 133.0, 129.0, 129.0, 128.2, 126.4, 123.8, 123.8, 122.3, 122.1, 121.7, 120.0, 117.8, 55.3 (2), 50.5, 44.5, 21.7 (2), 21.6. HRMS (ESI) m/z calcd for $C_{28}H_{30}N_3OS$ $[M + H]^+$: 456.2110, found: 456.2115.

*N-(5-(Dibenzo[b,d]thiophen-2-yl)-2-(4-methylpiperazin-1-yl)-phenyl)-3-methylbenzamide (**9l**).* The title compound was prepared according to general procedure A (26 mg, 39% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.27 (s, 1H), 8.97 (d, J = 1.4 Hz, 1H), 8.40 (d, J = 1.5 Hz, 1H), 8.28−8.24 (m, 1H), 7.91 (d, J = 8.6 Hz, 1H), 7.88−7.85 (m, 1H), 7.81 (br s, 1H), 7.75 (dd, J = 1.9, 8.4 Hz, 1H), 7.70 (d, J = 7.1 Hz, 1H), 7.50−7.46 (m, 3H), 7.45−7.40 (m, 3H), 3.26 (br s, 4H), 3.00 (br s, 4H), 2.70 (s, 3H), 2.49 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.3, 140.1, 140.1, 139.3, 139.1, 138.8, 137.4, 136.3, 135.8, 135.3, 134.3, 132.9, 129.0, 128.1, 127.0, 126.4, 124.6, 124.0, 123.2, 123.1, 123.0, 122.0, 121.6, 120.2, 118.6, 55.9 (2), 51.5, 45.7, 21.7 (2). HRMS (ESI) m/z calcd for $C_{31}H_{30}N_3OS$ $[M + H]^+$: 492.2110, found: 492.2115.

*3-Methyl-N-(5-(1-methyl-1H-indol-4-yl)-2-(4-methylpiperazin-1-yl)phenyl)benzamide (**9m**).* The title compound was prepared according to general procedure A (30 mg, 51% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.17 (br s, 1H), 8.86 (s, 1H), 7.73 (s, 1H), 7.61 (d, J = 7.0 Hz, 1H), 7.42 (dd, J = 1.9, 8.3 Hz, 1H), 7.36−7.30 (m, 3H), 7.27−7.22 (m, 2H), 7.19 (dd, J = 1.5, 7.0 Hz, 1H),7.06 (d, J = 3.2 Hz, 1H), 6.74 (d, J = 3.0 Hz, 1H), 3.77 (s, 3H), 3.18 (br s, 4H), 2.91 (br s, 4H), 2.58 (br s, 3H), 2.40 (br s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.2, 139.9, 139.9, 138.9, 137.5, 135.4, 133.7, 133.6, 132.8, 129.6, 129.0, 128.3, 126.8, 124.8, 123.7, 122.0, 121.4, 120.8, 119.6, 108.8, 100.8, 55.3, 50.4, 44.7, 33.2, 21.7. HRMS (ESI) m/z calcd for $C_{28}H_{31}N_4O$ $[M + H]^+$: 439.2498, found: 439.2496.

*3-Methyl-N-(5-(1-methyl-1H-indol-6-yl)-2-(4-methylpiperazin-1-yl)phenyl)benzamide (**9n**).* The title compound was prepared according to general procedure A (39 mg, 66% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.26 (br s, 1H), 8.86 (s, 1H), 7.74 (s, 1H), 7.64 (d, J = 6.5 Hz, 1H), 7.60 (d, J = 8.5 Hz, 1H), 7.52 (s, 1H), 7.39−7.33 (m, 4H), 7.29 (d, J = 8.2 Hz, 1H),7.02 (d, J = 3.0 Hz, 1H), 6.43 (d, J = 3.0 Hz, 1H), 3.79 (s, 3H), 3.14 (br s, 4H), 2.85 (br s, 4H), 2.57 (br s, 3H), 2.41 (br s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.3, 41.0, 139.1, 139.0, 137.4, 135.4, 134.5, 134.0, 132.9, 129.8, 129.0, 128.1, 128.2, 123.8, 123.2, 121.5, 121.2, 119.4, 118.9, 108.0, 101.0, 56.5 (2), 50.8, 45.0, 33.1 (2), 21.7. HRMS (ESI) m/z calcd for $C_{28}H_{31}N_4O$ $[M + H]^+$: 439.2498, found: 439.2496.

*N-(5-(1H-Indol-5-yl)-2-(4-methylpiperazin-1-yl)phenyl)-3-methylbenzamide (**9o**).* The title compound was prepared according to general procedure A (27 mg, 47% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.54 (s, 1H), 8.91 (d, J = 1.9 Hz, 1H), 8.35 (s, 1H), 7.87 (s, 1H), 7.78 (s, 1H), 7.72 (d, J = 7.4 Hz, 1H), 7.46 (dd, J = 1.6, 8.5 Hz, 1H), 7.39−7.32 (m, 4H), 7.24 (d, J = 8.2 Hz, 1H), 7.16 (d, J = 2.6 Hz, 1H), 6.54−6.53 (m, 1H), 2.97 (s, 4H), 2.61 (br s, 4H), 2.43 (s, 3H), 2.35 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.9, 141.0, 139.0, 138.9, 135.7, 134.8, 133.2, 132.8, 131.8, 128.8, 128.4, 128.3, 127.9, 125.2, 125.1, 123.6, 123.3, 121.2, 118.9, 111.4, 102.1, 55.0 (2), 50.2, 29.6, 21.2 (2). HRMS (ESI) m/z calcd for $C_{27}H_{28}N_4O$ $[M + H]^+$: 425.2341, found: 425.2328.

*3-Methyl-N-(4-(4-methylpiperazin-1-yl)-3′-(pyrrolidin-1-yl)-[1,1′-biphenyl]-3-yl)benzamide (**9p**).* The title compound was prepared according to general procedure A (9 mg, 15% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.45 (br s, 1H), 8.85 (d, J = 1.6 Hz, 1H), 7.77 (s, 1H), 7.70 (d, J = 7.1 Hz, 1H), 7.40−7.32 (m, 3H), 7.27−7.23 (m, 2H), 6.91 (d, J = 7.6 Hz, 1H), 6.79 (s, 1H), 6.53 (dd, J = 2.0, 8.2 Hz, 1H), 3.34−3.32 (m, 4H), 3.03 (br s, 4H), 2.71 (br s, 3H), 2.44−2.42 (m, 7H), 2.02−1.97 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.2, 148.5, 141.7, 140.6, 139.6, 139.0, 135.3, 133.9, 132.8, 129.6, 129.0, 129.0, 128.1, 128.1, 123.8, 123.1, 121.3, 118.8, 114.8, 111.1, 110.6, 55.6, 51.0 (2), 47.9, 45.2, 25.6 (2), 21.7. HRMS (ESI) m/z calcd for $C_{29}H_{35}N_4O$ $[M + H]^+$: 455.2811, found: 455.2811.

*3-Methyl-N-(4-(4-methylpiperazin-1-yl)-4′-(pyrrolidin-1-yl)-[1,1′-biphenyl]-3-yl)benzamide (**9q**).* The title compound was prepared according to general procedure A (18 mg, 29% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.45 (br s, 1H), 8.82 (d, J = 1.4 Hz, 1H), 7.80 (s, 1H), 7.72 (d, J = 7.7 Hz, 1H), 7.57−7.54 (m, 2H), 7.44−7.37 (m, 2H), 7.30 (dd, J = 2.0, 8.4 Hz, 1H), 7.27−7.25 (m, 1H), 6.63−6.60 (m, 2H), 3.33−3.31 (m, 4H), 3.08 (br s, 4H), 2.78 (br s, 3H), 2.49 (br s, 3H), 2.47 (s, 4H), 2.03−2.00 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.0, 147.6, 139.7, 138.9, 138.6, 135.4, 134.1, 132.7, 128.9, 128.1, 128.0, 127.5, 123.9, 121.6, 121.3, 117.3, 112.1, 55.9, 51.5, 47.8 (2), 45.6, 25.7 (2), 21.7. HRMS (ESI) m/z calcd for $C_{29}H_{35}N_4O$ $[M + H]^+$: 455.2811, found: 455.2815.

*3-Methyl-N-(4-(4-methylpiperazin-1-yl)-2′-(morpholinomethyl)-[1,1′-biphenyl]-3-yl)benzamide (**9r**).* The title compound was prepared according to general procedure A (27 mg, 42% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.37 (br s, 1H), 8.55 (s, 1H), 7.77 (s, 1H), 7.70 (s, 1H), 7.44−7.28 (m, 7H), 7.13 (d, J = 6.7 Hz, 1H), 3.73 (br s, 4H), 3.36−2.97 (m, 20H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.0, 142.6, 140.1, 139.2, 138.9, 135.3, 133.5, 132.8, 130.5, 130.3, 129.0, 128.0, 127.5, 127.3, 125.2, 124.0, 120.8, 120.5, 67.0, 60.1, 56.2 (2), 53.4 (2), 52.1 (2), 46.1, 21.7 (2). HRMS (ESI) m/z calcd for $C_{30}H_{37}N_4O_2$ $[M + H]^+$: 485.2917, found: 485.2924.

*3-Methyl-N-(4-(4-methylpiperazin-1-yl)-3′-(morpholinomethyl)-[1,1′-biphenyl]-3-yl)benzamide (**9s**).* The title compound was prepared according to general procedure A (15 mg, 23% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.44 (br s, 1H), 8.87 (d, J = 1.9 Hz, 1H), 7.80 (s, 1H), 7.73 (d, J = 7.3 Hz, 1H), 7.63 (s, 1H), 7.57 (d, J = 7.3 Hz, 1H), 7.45−7.31 (m, 6H), 3.78 (s, 4H), 3.65 (s, 3H), 3.11 (s, 4H), 2.82 (br s, 3H), 2.56 (br s, 4H), 2.52 (br s, 3H), 2.48 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 165.1, 140.9, 139.6, 139.0, 138.8, 135.0, 133.9, 132.8, 129.2, 128.9, 128.0, 127.4, 123.6, 123.0, 121.5, 118.7, 77.3, 77.0, 76.8, 65.6, 62.6, 55.2 (2), 52.8 (2), 50.4 (2), 44.7, 21.5 (2). HRMS (ESI) m/z calcd for $C_{30}H_{37}N_4O_2$ $[M + H]^+$: 485.2917, found: 485.2924.

*3-Methyl-N-(4-(4-methylpiperazin-1-yl)-4′-(morpholinomethyl)-[1,1′-biphenyl]-3-yl)benzamide (**9t**).* The title compound was prepared according to general procedure A (17 mg, 27% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.30 (br s, 1H), 8.85 (s, 1H), 7.79 (s, 1H), 7.69 (d, J = 7.2 Hz, 1H), 7.64 (d, J = 7.9 Hz, 2H), 7.49 (br s, 2H), 7.45−7.40 (m, 2H), 7.35 (s, 2H), 3.94−3.64 (m, 7H), 3.36−2.97 (m, 8H), 2.62 (br s, 6H), 2.48 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.2, 139.9, 139.1, 138.9, 135.2, 134.1, 132.9, 130.6, 129.2, 129.0, 128.1, 127.7, 127.6, 123.9, 122.8, 121.6, 118.6, 66.1, 62.7, 55.7 (2), 53.1 (2), 51.0 (2), 45.3, 21.8 (2). HRMS (ESI) m/z calcd for $C_{30}H_{37}N_4O_2$ $[M + H]^+$: 485.2917, found: 485.2920.

*3-Methyl-N-(5-(5-methyl-1-phenyl-1H-pyrazol-4-yl)-2-(4-methylpiperazin-1-yl)phenyl)benzamide (**9u**).* The title compound was prepared according to general procedure A (13 mg, 21% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.29 (s, 1H), 8.70 (d, J = 1.9 Hz, 1H), 7.83 (s, 1H), 7.79 (s, 1H), 7.68 (d, J = 6.8 Hz, 1H), 7.51 (s, 2H), 7.50 (s, 2H), 7.44−7.39 (m, 3H), 7.34 (d, J = 8.1 Hz, 1H), 7.22 (dd, J = 2.0, 8.2 Hz, 1H), 3.23 (br s, 4H), 2.98 (br s, 4H), 2.65 (s, 3H), 2.51 (s, 3H), 2.47 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.3, 139.6, 139.2, 139.6, 137.1, 135.8, 135.0, 133.5, 132.9, 132.6, 129.3, 129.2, 128.8, 128.2, 128.0, 125.2, 123.7, 123.3, 121.6, 121.2, 54.5, 49.7 (2), 43.7, 21.5 (2), 12.1. HRMS (ESI) m/z calcd for $C_{29}H_{32}N_5O$ $[M + H]^+$: 466.2607, found: 466.2608.

*N-(5-(1-Isopentyl-1H-pyrazol-4-yl)-2-(4-methylpiperazin-1-yl)-phenyl)-3-methylbenzamide (**9v**).* The title compound was prepared according to general procedure A (11 mg, 18% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.28 (br s, 1H), 8.72 (d, J = 1.8 Hz, 1H), 7.79 (d, J = 0.6 Hz, 1H), 7.78 (br s, 1H), 7.71 (s, 1H), 7.67 (d, J = 7.2 Hz, 1H),

DOI: 10.1021/acs.jmedchem.5b01630
J. Med. Chem. XXXX, XXX, XXX−XXX

7.45−7.40 (m, 2H), 7.28 (d, J = 8.3 Hz, 1H), 7.25 (dd, J = 2.0, 8.4 Hz, 1H), 4.16 (t, J = 7.6 Hz, 2H), 3.26 (br s, 4H), 2.94 (br s, 4H), 2.67 (br s, 3H), 2.48 (s, 3H), 1.81 (q, J = 7.1 Hz, 2H), 1.65−1.58 (m, 1H), 0.98 (s, 3H), 0.96 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.3, 139.3, 137.3, 136.6, 135.3, 134.0, 133.1, 132.1, 129.1, 128.3, 126.5, 123.5, 123.1, 122.3, 122.0, 121.5, 117.3, 54.8, 51.0, 49.4, 43.9, 39.4, 25.8, 22.5, 21.8. HRMS (ESI) m/z calcd for $C_{27}H_{35}N_5O$ $[M + H]^+$: 446.2920, found: 446.2926.

*N-(5-(6-Acetamidopyridin-3-yl)-2-(4-methylpiperazin-1-yl)-phenyl)-3-methylbenzamide (**9w**).* The title compound was prepared according to general procedure A (14 mg, 23% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.21 (br s, 1H), 9.01 (s, 1H), 8.74 (s, 1H), 8.47 (s, 1H), 8.34 (d, J = 8.8 Hz, 1H), 8.04 (dd, J = 2.0, 8.9 Hz, 1H), 7.76 (s, 1H), 7.63 (q, J = 6.2 Hz, 1H), 7.46−7.40 (m, 3H), 7.31 (dd, J = 1.8, 8.5 Hz, 1H), 3.78 (t, J = 11.5 Hz, 2H), 3.67 (d, J = 11.5 Hz, 2H), 3.15 (d, J = 13.0 Hz, 2H), 3.08 (t, J = 11.5 Hz, 2H), 2.91 (s, 3H), 2.47 (s, 3H), 2.29 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.6, 148.1, 144.9, 140.8, 139.6, 134.8, 134.6, 133.8, 133.5, 131.9, 129.2, 128.4, 123.6, 123.0, 122.7, 118.8, 117.6, 54.6 (2), 53.6, 49.2 (2), 43.9, 29.9, 25.1, 21.8. HRMS (ESI) m/z calcd for $C_{26}H_{30}N_5O_2$ $[M + H]^+$: 444.2400, found: 444.2394.

General Procedure B for the Synthesis of Compounds 12a,c,f,h,k,q,s. To a solution of commercially available 2-fluoro-5-nitroaniline (**10**) (0.100 g, 0.64 mmol) in CH_2Cl_2 (3 mL) was added the commercial acid chloride (0.83 mmol) at 23 °C. This was followed by the addition of pyridine (0.07 mL, 0.83 mmol), and the resulting solution was stirred at 23 °C for 4 h. The reaction was subsequently diluted with CH_2Cl_2 (5 mL) and washed with water (10 mL). The organic phase was then separated, dried (Na_2SO_4), filtered, and concentrated. The residue was purified by flash column chromatography on silica gel using an appropriate EtOAc/hexanes gradient as the eluent, providing the intermediate fluoro-benzamide **11**. To a solution of the fluoro-benzamide **11** (0.50 mmol) in DMF (4 mL) at 23 °C was added 1-methylpiperazine (0.072 mL, 0.65 mmol), followed by *N,N*-diisopropylethylamine (0.113 mL, 0.881 mmol). The resulting solution was then heated at 80 °C for 1 h and subsequently cooled to 23 °C. Following dilution with ethyl acetate (40 mL), the organic phase was washed with water (2 × 20 mL), separated, dried (Na_2SO_4), filtered, and concentrated. The residue was purified by trituration from EtOAc with hexanes or by flash column chromatography on silica gel using a common 5−20% MeOH/EtOAc gradient to afford the desired product.

General Procedure C for the Synthesis of Compounds 12d,e,g,j,o,p,t. To a solution of the benzoic acid (0.530 mmol) in toluene (2 mL) was added $SOCl_2$ (0.20 mL, 2.65 mmol) at 23 °C. The resulting solution was stirred vigorously and heated at 70 °C for 2 h and subsequently cooled back down to 23 °C. All of the solvent was then removed under reduced pressure and the crude acid chloride used in the subsequent reaction with no further purification. To a solution of commercially available 2-fluoro-5-nitroaniline (**10**) (0.100 g, 0.64 mmol) in CH_2Cl_2 (3 mL) was added the freshly prepared acid chloride (0.83 mmol) at 23 °C. This was followed by the addition of pyridine (0.07 mL, 0.83 mmol) and stirring of the resulting solution at 23 °C for 4 h. The reaction mixture was subsequently diluted with CH_2Cl_2 (5 mL) and washed with water (10 mL). The organic phase was then separated, dried (Na_2SO_4), filtered, and concentrated. The residue was purified by flash column chromatography on silica gel using an appropriate EtOAc/hexanes gradient as the eluent, providing the intermediate fluoro-benzamide **11**. To a solution of the fluoro-benzamide **11** (0.500 mmol) in DMF (4 mL) at 23 °C was added 1-methylpiperazine (0.072 mL, 0.650 mmol) followed by *N,N*-diisopropylethylamine (0.113 mL, 0.881 mmol). The resulting solution was heated at 80 °C for 1 h and subsequently cooled to 23 °C. Following dilution with ethyl acetate (40 mL), the organic phase was washed with water (2 × 20 mL), separated, dried (Na_2SO_4), filtered, and concentrated. The residue was purified by trituration from EtOAc with hexanes or by flash column chromatography on silica gel using a common 5−20% MeOH/EtOAc gradient to afford the desired product.

General Procedure D for the Synthesis of Compounds 12b,i,l−n,r. Identical to general procedure C, but using $POCl_3$ in place of $SOCl_2$.

*5-Fluoro-N-(2-(4-methylpiperazin-1-yl)-5-nitrophenyl)-2-(trifluoromethyl)benzamide (**12a**).* The title compound was prepared according to general procedure B (74 mg, 90% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.06 (s, 1H), 8.60 (d, J = 3.0 Hz, 1H), 8.06 (dd, J = 9.0, 2.5 Hz, 1H), 7.96 (dd, J = 8.5, 5.0 Hz, 1H), 7.69 (d, J = 7.0 Hz, 1H), 7.59 (t, J = 8.5 Hz, 1H), 7.27 (d, J = 9.0 Hz, 1H), 3.10 (br t, 4H), 2.48 (br s, 4H), 2.22 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 165.2, 164.0 (d, $^{a1}J_{C-F}$ = 250.0 Hz), 152.0, 141.7, 138.7 (d, $^{a4}J_{C-F}$ = 6.3 Hz), 130.3, 130.1 (q, $^{b3}J_{C-F}$ = 5.0 Hz), 124.0 (q, $^{b1}J_{C-F}$ = 271.3 Hz), 122.9 (q, $^{b2}J_{C-F}$ = 28.8 Hz), 122.2, 120.7, 120.0, 117.6 (d, $^{a3}J_{C-F}$ = 21.3 Hz), 116.6 (d, $^{a2}J_{C-F}$ = 25.0 Hz), 54.5 (2), 50.5 (2), 46.2. HRMS (ESI) m/z calcd for $C_{19}H_{18}F_4N_4O_3$ $[M + H]^+$: 427.1393, found: 427.1389.

*4-Hydroxy-N-(2-(4-methylpiperazin-1-yl)-5-nitrophenyl)-2-(trifluoromethyl)benzamide (**12b**).* The title compound was prepared according to general procedure D (14 mg, 15% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.59 (br s, 1H), 9.68 (s, 1H), 8.58 (s, 1H), 8.04 (dd, J = 9.0, 2.5 Hz, 1H), 7.58 (d, J = 8.5 Hz, 1H), 7.28 (d, J = 9.0 Hz, 1H), 7.18 (s, 1H), 7.13 (d, J = 8.0 Hz, 1H), 3.10 (br s, 4H), 2.51 (br s, 4H), 2.25 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 166.6, 159.4, 151.6, 141.9, 131.1, 130.9, 127.4 (q, $^1J_{C-F}$ = 193.8 Hz), 121.8 (2), 120.2, 120.1, 119.0 (2), 113.7, 54.6 (2), 50.4 (2), 46.0. HRMS (ESI) m/z calcd for $C_{19}H_{19}F_3N_4O_4$ $[M + H]^+$: 425.1437, found: 425.1438.

*2-Chloro-N-(2-(4-methylpiperazin-1-yl)-5-nitrophenyl)-4-(trifluoromethyl)benzamide (**12c**).* The title compound was prepared according to general procedure B (72 mg, 85% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.10 (br s, 1H), 8.71 (d, J = 2.5 Hz, 1H), 8.12 (s, 1H), 8.07 (dd, J = 9.0, 2.5 Hz, 1H), 7.89 (d, J = 8.0 Hz, 1H), 7.83 (d, J = 8.5 Hz, 1H), 7.27 (d, J = 9.0 Hz, 1H), 3.11 (br t, 4H), 2.49 (br s, 4H), 2.21 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 164.7, 152.2, 141.8, 137.7, 134.8, 131.3, 130.3, 128.3 (q, $^2J_{C-F}$ = 32.5 Hz), 128.3 (d, $^4J_{C-F}$ = 2.5 Hz), 126.7 (d, $^3J_{C-F}$ = 3.8 Hz), 124.1 (q, $^1J_{C-F}$ = 271.3 Hz), 122.2, 121.2, 120.0, 54.6 (2), 50.6 (2), 46.2. HRMS (ESI) m/z calcd for $C_{19}H_{18}ClF_3N_4O_3$ $[M + H]^+$: 443.1098, found: 443.1105.

*2-Chloro-N-(2-(4-methylpiperazin-1-yl)-5-nitrophenyl)-5-(trifluoromethyl)benzamide (**12d**).* The title compound was prepared according to general procedure C (65 mg, 71% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.11 (s, 1H), 8.71 (d, J = 2.5 Hz, 1H), 8.12 (s, 1H), 8.07 (dd, J = 9.0, 2.5 Hz, 1H), 7.90 (d, J = 8.5 Hz, 1H), 7.83 (d, J = 8.5 Hz, 1H), 7.28 (d, J = 8.5 Hz, 1H), 3.12 (br t, 4H), 2.50 (br t, 4H), 2.22 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 164.7, 152.2, 141.7, 137.7, 134.8, 131.3, 130.2, 128.3 (q, $^2J_{C-F}$ = 32.5 Hz), 128.3 (q, $^3J_{C-F}$ = 3.8 Hz), 126.7 (q, $^3J_{C-F}$ = 3.8 Hz), 124.1 (q, $^1J_{C-F}$ = 395.0 Hz), 122.3, 121.3, 120.0, 54.6 (2), 50.6 (2), 46.2. HRMS (ESI) m/z calcd for $C_{19}H_{18}ClF_3N_4O_3$ $[M + H]^+$: 443.1098, found: 443.1107.

*2-Chloro-5-methyl-N-(2-(4-methylpiperazin-1-yl)-5-nitrophenyl)-benzamide (**12e**).* The title compound was prepared according to general procedure C (66 mg, 88% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.83 (s, 1H), 8.67 (br s, 1H), 8.05 (dd, J = 8.5, 3.0 Hz, 1H), 7.48 (s, 1H), 7.46 (d, J = 8.5 Hz, 1H), 7.34 (d, J = 8.0 Hz, 1H), 7.29 (d, J = 9.0 Hz, 1H), 3.10 (br t, 4H), 2.49 (br t, 4H), 2.36 (s, 3H), 2.22 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 165.8, 151.8, 141.9, 137.4, 136.2, 132.4, 130.8, 130.6, 130.0 (2) 127.2, 121.9, 120.2, 54.76 (2), 50.6 (2), 46.2, 20.7. HRMS (ESI) m/z calcd for $C_{19}H_{21}ClN_4O_3$ $[M + H]^+$: 389.1392, found: 389.1390.

*2-Chloro-6-fluoro-N-(2-(4-methylpiperazin-1-yl)-5-nitrophenyl)-benzamide (**12f**).* The title compound was prepared according to general procedure B (51 mg, 68% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.33 (s, 1H), 8.54 (d, J = 2.5 Hz, 1H), 8.07 (dd, J = 9.0, 2.5 Hz, 1H), 7.59−7.53 (m, 1H), 7.46 (d, J = 8.0 Hz, 1H), 7.40 (t, J = 9.0 Hz, 1H), 7.26 (d, J = 9.0 Hz, 1H), 3.11 (br t, 4H), 2.49 (br s, 4H), 2.22 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 161.7, 159.3 (d, $^1J_{C-F}$ = 246.3 Hz), 152.2, 141.5, 132.3 (d, $^3J_{C-F}$ = 10.0 Hz), 131.4 (d, $^4J_{C-F}$ = 5.0 Hz), 129.8, 126.2, 126.0 (d, $^5J_{C-F}$ = 3.8 Hz), 122.4, 120.9, 119.9, 115.2 (d, $^2J_{C-F}$ = 21.3 Hz), 54.5 (2), 50.5 (2), 46.2. HRMS (ESI) m/z calcd for $C_{18}H_{18}ClFN_4O_3$ $[M + H]^+$: 393.1130, found: 393.1129.

DOI: 10.1021/acs.jmedchem.5b01630
J. Med. Chem. XXXX, XXX, XXX−XXX

*4-Methoxy-N-(2-(4-methylpiperazin-1-yl)-5-nitrophenyl)-3-(trifluoromethyl)benzamide (**12g**).* The title compound was prepared according to general procedure C (71 mg, 82% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.86 (s, 1H), 8.64 (d, J = 2.5 Hz, 1H), 8.28 (d, J = 9.0 Hz, 1H), 8.24 (s, 1H), 8.05 (dd, J = 9.0, 2.5 Hz, 1H), 7.48 (d, J = 8.5 Hz, 1H), 7.31 (d, J = 9.0 Hz, 1H), 4.01 (s, 3H), 3.08 (br t, 4H), 2.49 (br s, 4H), 2.22 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 163.9, 160.2, 151.9, 142.0, 134.4, 131.1, 126.9 (q, $^3J_{C-F}$ = 5.0 Hz), 123.9 (q, $^1J_{C-F}$ = 270.0 Hz), 121.7, 120.5, 120.2, 117.2 (q, $^2J_{C-F}$ = 30.0 Hz), 113.6 (2), 57.2 (2), 55.0 (2), 50.5, 46.2. HRMS (ESI) m/z calcd for $C_{20}H_{21}F_3N_4O_4$ [M + H]$^+$: 439.1593, found: 439.1595.

*3,5-Dimethyl-N-(2-(4-methylpiperazin-1-yl)-5-nitrophenyl)-benzamide (**12h**).* The title compound was prepared according to general procedure B (70 mg, 90% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.60 (s, 1H), 8.76 (d, J = 2.5 Hz, 1H), 8.03 (dd, J = 9.0, 2.5 Hz, 1H), 7.58 (s, 2H), 7.36 (d, J = 9.0 Hz, 1H), 3.07 (br t, 4H), 2.51 (br s, 4H), 2.38 (s, 6H), 2.24 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 165.5, 151.0, 142.5, 138.5, 134.4, 133.9, 131.9, 125.5 (2), 121.2, 120.6, 118.7, 55.3 (2), 50.7 (2), 46.3, 21.3 (2). HRMS (ESI) m/z calcd for $C_{20}H_{24}N_4O_3$ [M + H]$^+$: 369.1927, found: 369.1927.

*3-Methyl-N-(2-(4-methylpiperazin-1-yl)-5-nitrophenyl)-5-(trifluoromethyl)benzamide (**12i**).* The title compound was prepared according to general procedure D (4 mg, 4% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.98 (br s, 1H), 8.63 (d, J = 2.5 Hz, 1H), 8.09 (s, 2H), 8.06 (dd, J = 9.5, 3.0 Hz, 1H), 7.84 (s, 1H), 7.32 (d, J = 9.0 Hz, 1H), 4.01 (s, 3H), 3.09 (br t, 4H), 2.48 (br s, 4H), 2.21 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 164.4, 152.0, 142.0, 140.5, 135.4, 132.7, 130.9, 129.7 (q, $^2J_{C-F}$ = 32.5 Hz), 129.3, 124.4 (q, $^1J_{C-F}$ = 271.3 Hz), 122.0, 121.7, 120.6, 120.3, 55.0 (2), 50.5 (2), 46.2, 21.2. HRMS (ESI) m/z calcd for $C_{20}H_{21}F_3N_4O_3$ [M + H]$^+$: 423.1644, found: 423.1640.

*3-Fluoro-N-(2-(4-methylpiperazin-1-yl)-5-nitrophenyl)-5-(trifluoromethyl)benzamide (**12j**).* The title compound was prepared according to general procedure C (78 mg, 80% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.15 (s, 1H), 8.56 (d, J = 2.0 Hz, 1H), 8.18 (s, 1H), 8.11 (d, J = 8.5 Hz, 1H), 8.07 (dd, J = 9.0, 3.0 Hz, 1H), 8.02 (d, J = 8.5 Hz, 1H), 7.30 (d, J = 9.0 Hz, 1H), 3.12 (br t, 4H), 2.48 (br t, 4H), 2.21 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 163.2, 162.4 (d, $^{a1}J_{C-F}$ = 246.3 Hz), 152.5, 141.6, 118.1 (d, $^{a3}J_{C-F}$ = 11.3 Hz), 131.7 (q, $^{b2}J_{C-F}$ = 33.8 Hz), 130.16, 124.6, 122.5, 121.7, 121.0 (m), 120.1, 119.5 (d, $^{a2}J_{C-F}$ = 22.5 Hz), 116.7 (q, $^{b3}J_{C-F}$ = 21.3 Hz), 54.9 (2), 50.4 (2), 46.1. HRMS (ESI) m/z calcd for $C_{19}H_{18}F_4N_4O_3$ [M + H]$^+$: 427.1393, found: 427.1400.

*3,5-Dichloro-N-(2-(4-methylpiperazin-1-yl)-5-nitrophenyl)-benzamide (**12k**).* The title compound was prepared according to general procedure B (61 mg, 77% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.01 (s, 1H), 8.51 (d, J = 2.5 Hz, 1H), 8.05 (dd, J = 9.0, 2.5 Hz, 1H), 7.97 (s, 1H), 7.90 (s, 1H), 7.28 (d, J = 9.0 Hz, 1H), 3.10 (br t, 4H), 2.47 (br t, 4H), 2.21 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 163.1, 152.4, 141.6, 137.8, 134.9 (2), 131.7, 130.2, 126.9 (2), 122.4, 121.5, 120.1, 54.9 (2), 50.4 (2), 46.2. HRMS (ESI) m/z calcd for $C_{18}H_{18}Cl_2N_4O_3$ [M + H]$^+$: 409.0834, found: 409.0844.

*3-Hydroxy-N-(2-(4-methylpiperazin-1-yl)-5-nitrophenyl)-5-(trifluoromethyl)benzamide (**12l**).* The title compound was prepared according to general procedure D (8 mg, 14% yield). ^{1}H NMR (500 MHz, CD_3OD) δ 10.96 (br s, 1H), 9.91 (s, 1H), 8.64 (d, J = 2.9 Hz, 1H), 8.07 (dd, J = 2.7, 8.9 Hz, 1H), 7.72 (s, 1H), 7.64 (s, 1H), 7.33 (d, J = 9.1 Hz, 2H), 3.10 (t, J = 4.7 Hz, 4H), 2.50 (s, 4H), 2.23 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 163.6, 158.4, 151.4, 141.5, 136.5, 130.6, 130.4, 130.3, 123.7 (d, $^1J_{C-F}$ = 272.7 Hz, 1C), 121.4, 119.9 (d, $^3J_{C-F}$ = 10.0 Hz, 1C), 118.5, 115.0, 114.2 (d, $^4J_{C-F}$ = 3.7 Hz, 1C), 54.5 (2), 50.0 (2), 45.6. HRMS (ESI) m/z calcd for $C_{19}H_{20}F_3N_4O_4$ [M + H]$^+$: 425.1437, found: 425.1438.

*2-Fluoro-N-(2-(4-methylpiperazin-1-yl)-5-nitrophenyl)-5-sulfamoylbenzamide (**12m**).* The title compound was prepared according to general procedure D (26 mg, 23% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.96 (d, J = 6.9 Hz, 1H), 8.96 (d, J = 1.7 Hz, 1H), 8.37 (dd, J = 2.0, 6.7 Hz, 1H), 8.07–8.02 (m, 2H), 7.68–7.64 (m, 1H), 7.57 (s, 2H), 7.41 (d, J = 9.0 Hz, 1H), 3.10–3.07 (m, 4H), 2.60 (br s, 4H), 2.30 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 160.9, 160.7 (d, $^1J_{C-F}$ = 255.8 Hz, 1C), 150.0, 142.3, 141.0, 131.3, 131.1 (d, $^3J_{C-F}$ = 10.4 Hz, 1C), 128.9 (d, $^4J_{C-F}$ = 3.6 Hz, 1C), 122.9 (d, $^3J_{C-F}$ = 14.8 Hz, 1C), 120.9, 120.5, 117.7, 117.5 (d, $^2J_{C-F}$ = 25.0 Hz, 1C), 54.2 (2), 50.3 (2), 45.4. HRMS (ESI) m/z calcd for $C_{18}H_{21}FN_5O_5S$ [M + H]$^+$: 438.1247, found: 438.1240.

*2,3-Difluoro-5-hydroxy-N-(2-(4-methylpiperazin-1-yl)-5-nitrophenyl)benzamide (**12n**).* The title compound was prepared according to general procedure D (11 mg, 13% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.60 (br s, 1H), 8.88 (d, J = 2.5 Hz, 1H), 8.01 (dd, J = 9.0, 2.5 Hz, 1H), 7.35 (q, J = 10.0 Hz, 1H), 7.31 (d, J = 9.0 Hz, 1H), 6.73 (m, 1H), 3.08 (br t, 4H), 2.60 (br s, 4H), 2.29 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 161.3, 155.6, 150.0, 141.8 (2), 131.1, 120.5, 119.8 (2), 118.8 (d, $^2J_{C-F}$ = 18.9 Hz), 117.9, 113.2 (d, $^3J_{C-F}$ = 12.3 Hz), 111.9, 54.1 (2), 50.0 (2), 45.3. HRMS (ESI) m/z calcd for $C_{18}H_{18}F_2N_4O_4$ [M + H]$^+$: 393.1374, found: 393.1387.

*3-Chloro-2-fluoro-N-(2-(4-methylpiperazin-1-yl)-5-nitrophenyl)-5-(trifluoromethyl) benzamide (**12o**).* The title compound was prepared according to general procedure C (31 mg, 51% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.12 (br s, 1H), 8.83 (br s, 1H), 8.35 (d, J = 9.0 Hz, 1H), 8.19 (d, J = 2.8 Hz, 1H), 8.07 (dd, J = 9.0, 2.8 Hz, 1H), 7.36 (d, J = 9.0 Hz, 1H), 3.10 (br s, 4H), 2.56 (br s, 4H), 2.27 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 161.0, 157.2 (d, $^{a1}J_{C-F}$ = 250.0 Hz), 151.5, 142.2, 130.8, 127.1, 126.8 (q, $^{b2}J_{C-F}$ = 50.0 Hz), 126.6, 126.5, 123.2 (q, $^{b1}J_{C-F}$ = 295.1 Hz), 122.5, 122.1, 120.5, 119.8, 54.7 (2), 50.7 (2), 46.2. HRMS (ESI) m/z calcd for $C_{19}H_{17}ClF_4N_4O_3$ [M + H]$^+$: 461.1004, found: 461.1003.

*2-Chloro-6-fluoro-3-methyl-N-(2-(4-methylpiperazin-1-yl)-5-nitrophenyl)benzamide (**12p**).* The title compound was prepared according to general procedure C (55 mg, 59% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.29 (s, 1H), 8.54 (d, J = 2.5 Hz, 1H), 8.07 (dd, J = 9.0, 2.5 Hz, 1H), 7.52 (m, 1H), 7.31 (t, J = 9.0 Hz, 1H), 7.26 (d, J = 9.0 Hz, 1H), 3.11 (br t, 4H), 2.49 (br s, 4H), 2.37 (s, 3H), 2.22 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 162.0, 157.4 (d, $^1J_{C-F}$ = 243.8 Hz), 152.2, 141.5, 132.8, 132.7 (d, $^4J_{C-F}$ = 13.8 Hz), 131.1 (d, $^5J_{C-F}$ = 6.3 Hz), 129.8, 126.1 (d, $^2J_{C-F}$ = 22.5 Hz), 122.1, 120.9, 119.9, 114.8 (d, $^3J_{C-F}$ = 21.3 Hz), 54.6 (2), 50.4 (2), 46.2, 19.5. HRMS (ESI) m/z calcd for $C_{19}H_{20}ClFN_4O_3$ [M + H]$^+$: 407.1286, found: 407.1293.

*2,4-Difluoro-3-methyl-N-(2-(4-methylpiperazin-1-yl)-5-nitrophenyl)benzamide (**12q**).* The title compound was prepared according to general procedure B (34 mg, 86% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.78 (s, 1H), 9.00 (s, 1H), 8.02 (dd, J = 9.0, 2.5 Hz, 1H), 7.85 (q, J = 7.0 Hz, 1H), 7.41 (d, J = 9.0 Hz, 1H), 7.27 (t, J = 8.5 Hz, 1H), 3.02 (br t, 4H), 2.51 (br s, 4H), 2.25 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 163.1 (dd, $^1J_{C-F}$ = 248.8, 8.8 Hz), 161.7, 158.9 (dd, $^1J_{C-F}$ = 247.3, 8.8 Hz), 150.1, 143.0, 132.5, 129.8 (dd, $^3J_{C-F}$ = 11.3, 3.8 Hz), 121.2, 120.9, 118.9 (dd, $^3J_{C-F}$ = 11.3, 3.8 Hz), 117.2, 114.0 (t, $^2J_{C-F}$ = 21.3 Hz), 112.3 (dd, $^2J_{C-F}$ = 22.5, 2.5 Hz), 55.0 (2), 51.2 (2), 46.3, 7.4. HRMS (ESI) m/z calcd for $C_{19}H_{20}F_2N_4O_3$ [M + H]$^+$: 391.1582, found: 391.1585.

*3,5-Difluoro-2-methyl-N-(2-(4-methylpiperazin-1-yl)-5-nitrophenyl)benzamide (**12r**).* The title compound was prepared according to general procedure D (71 mg, 68% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.39 (s, 1H), 8.62 (s, 1H), 8.05 (dd, J = 2.7, 8.8 Hz, 1H), 7.33 (d, J = 8.8 Hz, 1H), 7.06–7.04 (m, 1H), 7.01–6.97 (m, 1H), 3.06 (s, 4H), 2.67 (s, 4H), 2.43 (s, 6H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 165.4 (t, $^4J_{C-F}$ = 2.9, 3.5 Hz, 1C), 162.0 (dd, $^{1,3}J_{C-F}$ = 11.4, 249.3 Hz, 1C), 160.9 (dd, $^{1,3}J_{C-F}$ = 12.7, 249.3 Hz, 1C), 147.1, 145.2, 138.5 (m), 133.6, 121.0, 120.7 (dd, $^{2,4}J_{C-F}$ = 4.1, 18.2 Hz, 1C), 120.1, 115.3, 109.5 (dd, $^{2,4}J_{C-F}$ = 3.7, 22.7 Hz, 1C), 106.2 (m), 55.4 (2), 51.8 (2), 46.0, 11.4. HRMS (ESI) m/z calcd for $C_{19}H_{21}F_2N_4O_3$ [M + H]$^+$: 391.1582, found: 391.1589.

*4-Fluoro-3,5-dimethyl-N-(2-(4-methylpiperazin-1-yl)-5-nitrophenyl)benzamide (**12s**).* The title compound was prepared according to general procedure C (49 mg, 52% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.63 (s, 1H), 8.66 (d, J = 2.5 Hz, 1H), 8.04 (dd, J = 8.5, 2.5 Hz, 1H), 7.74 (d, J = 6.5 Hz, 1H), 7.33 (d, J = 9.0 Hz, 1H), 3.08 (br t, 4H), 2.50 (br s, 4H), 2.32 (s, 3H), 2.19 (s, 6H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 164.7, 161.9 (d, $^1J_{C-F}$ = 247.5 Hz), 151.4, 142.2, 131.4, 129.9 (d, $^4J_{C-F}$ = 2.5 Hz), 129.1 (2), 129.0, 124.8 (d, $^2J_{C-F}$ = 18.9 Hz), 121.5, 120.4, 119.6, 55.2 (2), 50.6 (2), 46.3, 14.8 (2)

DOI: 10.1021/acs.jmedchem.5b01630
J. Med. Chem. XXXX, XXX, XXX–XXX

(d, $^3J_{C-F}$ = 3.8 Hz). HRMS (ESI) m/z calcd for $C_{20}H_{23}FN_4O_3$ [M + H]$^+$: 387.1832, found: 387.1834.

*2,4,6-Trifluoro-3,5-dimethyl-N-(2-(4-methylpiperazin-1-yl)-5-nitrophenyl)benzamide (**12t**).* The title compound was prepared according to general procedure C (17 mg, 22% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.19 (s, 1H), 8.55 (d, J = 2.5 Hz, 1H), 8.06 (dd, J = 8.5, 3.0 Hz, 1H), 7.29 (d, J = 8.5 Hz, 1H), 3.09 (br t, 4H), 2.51 (br s, 4H), 2.23 (s, 3H), 2.18 (s, 6H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 160.7, 159.3, 158.8, 156.5 (d, $^3J_{C-F}$ = 12.5 Hz), 154.5 (d, $^3J_{C-F}$ = 11.3 Hz), 151.9, 141.6, 129.9, 122.3, 120.5, 120.1, 111.5 (t, $^2J_{C-F}$ = 21.1 Hz), 109.4 (t, $^2J_{C-F}$ = 21.3 Hz), 54.4 (2), 50.5 (2), 46.2, 7.2 (2). HRMS (ESI) m/z calcd for $C_{20}H_{21}F_3N_4O_3$ [M + H]$^+$: 423.1644, found: 423.1653.

*2-(4-Methylpiperazin-1-yl)-5-nitroaniline (**13**).* A mixture of 2-fluoro-5-nitroaniline (**10**) (2.13 g, 13.7 mmol), 1-methylpiperazine (5 mL, 45 mmol) and *N*,*N*-diisopropylethylamine (3.6 mL, 20 mmol) was heated at 80 °C for 3 h. After cooling to 23 °C, the yellow precipitate was filtered off, washed with CH_2Cl_2, and dried under reduced pressure to afford the title compound (2.42 g, 75% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.62 (dd, J = 2.6,8.7 Hz, 1H), 7.55 (d, J = 2.6 Hz, 1H), 6.99 (d, J = 8.7 Hz, 1H), 4.11 (br s, 2H), 3.03 (br s, 4H), 2.61 (br s, 4H), 2.38 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 145.2, 144.4, 14.6, 119.2, 114.6, 109.7, 55.6 (2), 50.3 (2), 46.3. HRMS (ESI) m/z calcd for $C_{11}H_{17}N_4O_2$ [M + H]$^+$: 237.1352, found: 237.1357.

General Procedure E for the Synthesis of Compounds 14c–g,i,j. A solution of 2-(4-methylpiperazin-1-yl)-5-nitroaniline (**13**) (50 mg, 0.21 mmol) and the desired carboxylic acid (0.24 mmol) in xylene (2 mL) were heated at 120 °C for 5 min before $POCl_3$ (14 μL, 0.16 mmol) and DMAP (2.9 mg, 0.02 mmol) were added. The reaction mixture was heated at 120 °C for a further 2 h before cooling to 23 °C. The solvent was evaporated, and saturated aqueous sodium bicarbonate solution (2 mL) along with EtOAc (2 mL) were added to the residue. The aqueous layer was extracted with EtOAc (3 × 15 mL), and the combined organic extracts dried ($MgSO_4$), filtered, and concentrated. The residue was purified by flash column chromatography on silica gel (5–30% MeOH/EtOAc) or precipitation from EtOAc with hexanes to afford the desired product.

*N-(2-(4-Methylpiperazin-1-yl)-5-nitrophenyl)-6-oxo-4-(trifluoromethyl)-1,6-dihydropyridine-3-carboxamide (**14a**).* The 6-hydroxy-4-(trifluoromethyl)nicotinic acid (117 mg, 0.554 mmol) was suspended in $SOCl_2$ (1.506 mL, 20.76 mmol) to form a white slurry. The suspension was stirred at 70 °C for 2 h until the solution went clear. The remaining $SOCl_2$ was removed under reduced pressure, and the residue dried under high vacuum for 1 h. The dried residue was subsequently dissolved in CH_2Cl_2 (10 mL) and added dropwise over 20–30 min to a solution of 2-(4-methylpiperazin-1-yl)-5-nitroaniline (**13**) (109 mg, 0.461 mmol) and pyridine (0.074 mL, 0.923 mmol) in CH_2Cl_2 (5.00 mL). After 2 h the reaction mixture was diluted with saturated aqueous sodium bicarbonate solution (10 mL) and extracted with CH_2Cl_2 (5 × 10 mL) (sonication was necessary to dissolve the black solid formed). The combined organic extracts were dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (0–90% MeOH/CH_2Cl_2, 1% NH_4Ac) to afford the title compound (59 mg, 30% yield) as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.77 (s, 1H), 8.53 (d, J = 2.7 Hz, 1H), 8.04 (dd, J = 9.0, 2.8 Hz, 1H), 7.98 (s, 1H), 7.26 (d, J = 9.0 Hz, 1H), 6.82 (s, 1H), 3.34 (s, 4H), 3.10–3.04 (m, 4H), 2.24 (s, 3H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 163.06, 161.06, 151.62, 141.28, 139.55, 138.64 (q, $^2J_{C-F}$ = 32.4 Hz), 130.24, 121.97 (q, $^1J_{C-F}$ = 275.2 Hz), 121.48, 120.64, 119.49, 118.95 (q, $^3J_{C-F}$ = 5.6 Hz), 110.99, 54.03, 49.89, 45.57; ^{19}F NMR (471 MHz, DMSO-d_6) δ −61.51. HRMS (ESI) m/z calcd for $C_{18}H_{19}F_3N_5O_4$ [M + H]$^+$: 426.1389, found: 426.1380.

*3-Hydroxy-2-methyl-N-(2-(4-methylpiperazin-1-yl)-5-nitrophenyl)quinoline-4-carboxamide (**14b**).* To a solution of 3-hydroxy-2-methylquinoline-4-carboxylic acid (100 mg, 0.48 mmol) and triethlyamine (233 μL, 1.67 mmol) in EtOAc (5 mL) was added $SOCl_2$ (35 μL, 0.48 mmol) at 0 °C. The ice bath was then removed, and the solution allowed to stir at 23 °C for 1.5 h. Subsequently, 2-(4-methylpiperazin-1-yl)-5-nitroaniline (**13**) (90 mg, 0.382 mmol) was added, and the temperature was raised to 80 °C. After stirring for 1.5 h, the reaction mixture was cooled to 23 °C, and diluted with water (5 mL). The aqueous phase was extracted with EtOAc (3 × 10 mL), and the combined organic extracts were dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (5–30% MeOH/EtOAc) to afford the title compound (25 mg, 11% yield). ^{1}H NMR (500 MHz, CD_3OD) δ 9.32 (d, J = 2.7 Hz, 1H), 9.26 (d, J = 8.6 Hz, 1H), 7.96 (dd, J = 2.7, 9.0 Hz, 1H), 7.74 (dd, J = 1.1, 8.2 Hz, 1H), 7.56–7.48 (m, 1H), 7.39–7.35 (m, 1H), 7.27–7.22 (m, 2H), 3.18 (br s, 4H), 2.98 (t, J = 4.6 Hz, 4H), 2.65 (s, 3H), 2.50 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 170.8, 165.2, 161.5, 150.7, 144.8, 139.4, 135.3, 130.6, 128.1, 127.5, 127.3, 125.7, 124.3, 123.5, 120.2, 120.2, 118.9, 111.6, 55.5 (2), 51.3 (2), 46.1, 45.8. HRMS (ESI) m/z calcd for $C_{22}H_{23}N_5O_4$ [M + H]$^+$: 422.1828, found: 422.1830.

*3-Hydroxy-N-(2-(4-methylpiperazin-1-yl)-5-nitrophenyl)-quinoline-4-carboxamide (**14c**).* The title compound was prepared according to general procedure E (4 mg, 6% yield). ^{1}H NMR (500 MHz, CD_3OD) δ 9.41 (d, J = 2.7 Hz, 1H), 9.19 (d, J = 8.8 Hz, 1H), 8.52 (s, 1H), 7.97 (dd, J = 2.7 Hz, 1H), 7.80 (dd, J = 1.3, 8.2 Hz, 1H), 7.48–7.45 (m, 1H), 7.35–7.30 (m, 2H), 3.35 (s, 1H), 3.20 (br s, 4H), 3.19 (br s, 4H), 2.70 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 169.6, 163.0, 152.1, 149.6, 145.4, 141.1, 135.5, 130.4, 129.3, 128.7, 126.1, 124.6, 120.9, 120.2, 118.4, 114.8, 55.4 (2), 51.0 (2), 45.0. HRMS (ESI) m/z calcd for $C_{21}H_{22}N_5O_4$ [M + H]$^+$: 408.1672, found: 408.1666.

*6-Hydroxy-2-methyl-N-(2-(4-methylpiperazin-1-yl)-5-nitrophenyl)quinoline-4-carboxamide (**14d**).* The title compound was prepared according to general procedure E (25 mg, 23% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.31 (br s, 1H), 10.13 (s, 1H), 10.11 (s, 1H), 8.84 (s, 1H), 8.11 (dd, J = 2.7, 9.0 Hz, 1H), 7.87 (d, J = 9.0 Hz, 1H), 7.57 (s, 1H), 7.43 (d, J = 2.6 Hz, 1H), 7.34 (dd, J = 2.7, 9.0 Hz, 1H), 3.35 (br s, 4H), 3.07 (br s, 4H), 2.66 (s, 3H), 2.63 (br s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 166.3, 155.7, 154.5, 150.2, 143.0, 141.9, 139.6, 130.6, 130.2, 123.6, 122.2, 121.6, 120.2, 120.0, 119.9, 105.6, 52.4 (2), 47.7 (2), 24.4 (2). HRMS (ESI) m/z calcd for $C_{22}H_{24}N_5O_4$ [M + H]$^+$: 422.1828, found: 422.1829.

*N-(2-(4-Methylpiperazin-1-yl)-5-nitrophenyl)pyrazolo[1,5-a]-pyridine-7-carboxamide (**14e**).* The title compound was prepared according to general procedure E (12 mg, 24% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.17 (s, 1H), 9.39 (d, J = 2.7 Hz, 1H), 8.35 (d, J = 2.4 Hz, 1H), 8.10 (dd, J = 1.3, 8.8 Hz, 1H), 8.05 (dd, J = 2.9, 8.9 Hz, 1H), 8.03 (dd, J = 1.3, 7.1 Hz, 1H), 7.53–7.50 (m, 1H), 7.47 (d, J = 8.9 Hz, 1H), 7.01 (d, J = 2.5 Hz, 1H), 3.03 (br s, 4H), 2.61 (br s, 4H), 2.30 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 158.2, 149.3, 142.9, 141.3, 140.9, 132.9, 130.7, 123.8, 122.6, 120.7, 120.2, 118.2, 115.6, 98.9, 54.5 (2), 50.8 (2), 45.7. HRMS (ESI) m/z calcd for $C_{19}H_{21}N_6O_3$ [M + H]$^+$: 381.1675, found: 381.1688.

*5-Fluoro-N-(2-(4-methylpiperazin-1-yl)-5-nitrophenyl)-1H-benzo-[d]imidazole-7-carboxamide (**14f**).* The title compound was prepared according to general procedure E (7 mg, 11% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.24 (br s, 1H), 12.26 (s, 1H), 9.37(s, 1H), 8.64 (s, 1H), 8.01 (dd, J = 2.6, 8.8 Hz, 1H), 7.77 (dd, J = 2.3, 10.6 Hz, 1H), 7.71 (dd, J = 2.4, 8.5 Hz, 1H), 7.43 (d, J = 9.0 Hz, 1H), 3.02 (br s, 4H), 2.61 (br s, 4H), 2.28 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 162.4, 158.5 (d, $^1J_{C-F}$ = 237.3 Hz, 1C), 149.3, 144.4, 142.8, 136.8, 134.2 (d, $^3J_{C-F}$ = 11.5 Hz, 1C), 133.2, 122.6 (d, $^3J_{C-F}$ = 9.2 Hz, 1C), 120.5, 119.7, 115.6, 110.6 (d, $^2J_{C-F}$ = 27.1 Hz, 1C), 103.1 (d, $^2J_{C-F}$ = 27.1 Hz, 1C), 54.3 (2), 50.9 (2), 45.8. HRMS (ESI) m/z calcd for $C_{19}H_{20}FN_6O_3$ [M + H]$^+$: 399.1581, found: 399.1582.

*N-(2-(4-Methylpiperazin-1-yl)-5-nitrophenyl)-1H-benzo[d]-imidazole-5-carboxamide (**14g**).* The title compound was prepared according to general procedure E (15 mg, 19% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.85 (br s, 1H), 9.67 (br s, 1H), 8.75 (d, J = 2.5 Hz, 1H), 8.41 (s, 1H), 8.30 (br s, 1H), 8.04 (dd, J = 9.0, 2.5 Hz, 1H), 7.87 (d, J = 8.5 Hz, 1H), 7.74 (d, J = 8.5 Hz, 1H), 7.35 (d, J = 9.0 Hz, 1H), 3.09 (br t, 4H), 2.51 (br s, 4H), 2.24 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 165.9, 151.4, 144.9, 143.9, 142.3, 140.0, 131.9, 128.0, 121.2, 120.4 (2), 119.5, 113.6, 110.1, 55.1 (2), 50.7 (2), 46.2. HRMS (ESI) m/z calcd for $C_{19}H_{20}N_6O_3$ [M + H]$^+$: 381.1675, found: 381.1671.

DOI: 10.1021/acs.jmedchem.5b01630
J. Med. Chem. XXXX, XXX, XXX–XXX

*3-Methyl-N-(2-(4-methylpiperazin-1-yl)-5-nitrophenyl)-[1,2,4]-triazolo[4,3-a]pyridine-6-carboxamide (**14h**).* 3-Methyl-[1,2,4]-triazolo[4,3-*a*]pyridine-6-carboxylic acid (48.7 mg, 0.275 mmol) was suspended in $SOCl_2$ (1 mL) and stirred at 60 °C for 1 h. Excess $SOCl_2$ was removed under reduced pressure, and the residue was dried under high vacuum for 1 h. The dried residue was then dissolved in CH_2Cl_2 (3 mL) and added dropwise to a solution of 2-(4-methylpiperazin-1-yl)-5-nitroaniline (**13**) (50 mg, 0.21 mmol) and pyridine (22 μL, 0.28 mmol) in CH_2Cl_2 (2 mL). After 1.5 h, the reaction mixture was diluted with saturated aqueous sodium bicarbonate solution (5 mL) and extracted with CH_2Cl_2 (3 × 5 mL). The combined organic extracts were dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (5−30% MeOH/EtOAc) to afford the title compound (6 mg, 6% yield). ^{1}H NMR (500 MHz, CD_3OD) δ 9.14 (t, *J* = 1.3 Hz, 1H), 8.82 (d, *J* = 2.8 Hz, 1H), 8.19 (dd, *J* = 2.7, 8.9 Hz, 1H), 8.01 (dd, *J* = 1.7, 9.7 Hz, 1H), 7.88 (dd, *J* = 1.0, 9.5 Hz, 1H), 7.49 (d, *J* = 8.9 Hz, 1H), 3.62 (br s, 4H), 3.43 (br s, 2H), 3.30 (br s, 2H), 3.01 (s, 3H), 2.93 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 165.2, 150.8, 150.6, 147.5, 145.5, 133.1, 129.0, 127.7, 123.5, 123.0, 122.1, 121.9, 115.8, 115.5, 54.9 (2), 43.8 (2), 10.2. HRMS (ESI) *m*/*z* calcd for $C_{19}H_{21}N_7O_3$ $[M + H]^+$: 396.1784, found: 396.1779.

*N-(2-(4-Methylpiperazin-1-yl)-5-nitrophenyl)-1H-indazole-6-carboxamide (**14i**).* The title compound was prepared according to general procedure E (8 mg, 8% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.51 (br s, 1H), 9.80 (s, 1H), 8.70 (d, *J* = 2.8 Hz, 1H), 8.20 (d, *J* = 7.5 Hz, 2H), 8.06 (dd, *J* = 2.7, 8.9 Hz, 1H), 7.94 (d, *J* = 8.4 Hz, 1H), 7.70 (dd, *J* = 1.3, 8.4 Hz, 1H), 7.34 (d, *J* = 9.0 Hz, 1H), 3.10 (t, *J* = 5.0 Hz, 4H), 2.51 (br s, 4H), 2.23 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 165.3, 151.2, 141.6, 139.3, 133.7, 131.6, 130.9, 124.7, 121.1, 120.9, 119.9, 119.5, 119.0, 111.0, 54.6 (2), 50.1 (2), 45.7. HRMS (ESI) *m*/*z* calcd for $C_{19}H_{22}N_6O_3$ $[M + H]^+$: 381.1675, found: 381.1670.

*1-Methyl-N-(2-(4-methylpiperazin-1-yl)-5-nitrophenyl)-1H-indazole-3-carboxamide (**14j**).* The title compound was prepared according to general procedure E (31 mg, 41% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.87 (br s, 1H), 9.26 (d, *J* = 2.5 Hz, 1H), 8.26 (d, *J* = 8.0 Hz, 1H), 8.01 (dd, *J* = 9.0, 3.0 Hz, 1H), 7.83 (d, *J* = 8.5 Hz, 1H), 7.56 (t, *J* = 7.5 Hz, 1H), 7.45 (d, *J* = 8.5 Hz, 1H), 7.40 (t, *J* = 7.5 Hz, 1H), 4.23 (s, 3H), 3.04 (br t, 4H), 2.51 (br s, 4H), 2.33 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 160.2, 148.3, 143.7, 141.9, 136.3, 133.0, 127.6, 123.8, 122.5, 121.8, 121.2, 119.6, 123.8, 111.4, 55.4 (2), 51.4 (2), 46.4, 36.9. HRMS (ESI) *m*/*z* calcd for $C_{20}H_{22}N_6O_3$ $[M + H]^+$: 395.1832, found: 395.1828.

*N-(5-Bromo-2-(4-methylpiperazin-1-yl)phenyl)-6-oxo-4-(trifluoromethyl)-1,6-dihydropyridine-3-carboxamide (**15**).* The 6-hydroxy-4-(trifluoromethyl)nicotinic acid (1.890 g, 8.94 mmol) was suspended in $SOCl_2$ (24.33 mL, 335 mmol) and stirred at 80 °C for 2 h. The solution became clear and was then cooled to 23 °C. The excess $SOCl_2$ was removed under reduced pressure, and the resulting solid was dried under vacuum for 2 h. The dry residue was dissolved in CH_2Cl_2 (10 mL) and added over a 30 min period to a solution of 5-bromo-2-(4-methylpiperazin-1-yl)aniline (**7**) (2.013 g, 7.45 mmol) and pyridine (1.801 mL, 22.36 mmol) in CH_2Cl_2 (20 mL). The resulting mixture was then stirred for 12 h. The reaction was diluted with saturated aqueous sodium bicarbonate solution (100 mL), sonicated to dissolve any solid particles, and extracted with CH_2Cl_2 (4 × 50 mL). The combined organic extracts were dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (0−100%, 89% CH_2Cl_2, 10% MeOH, 1% NH_4Ac/CH_2Cl_2) to afford the title compound (366 mg, 10% yield) as a yellow solid. ^{1}H NMR (500 MHz, MeOD) δ 8.20 (d, *J* = 2.3 Hz, 1H), 7.95 (s, 1H), 7.32 (dd, *J* = 8.6, 2.3 Hz, 1H), 7.18 (d, *J* = 8.6 Hz, 1H), 6.91 (s, 1H), 2.95 (t, *J* = 4.8 Hz, 4H), 2.69 (s, 4H), 2.40 (s, 3H); ^{13}C NMR (126 MHz, MeOD) δ 164.7, 164.1, 144.2, 141.4 (q, $^2J_{C-F}$ = 33.2 Hz, 1C), 139.7, 135.1, 129.6, 126.4, 123.5, 123.3 (q, $^1J_{C-F}$ = 274.7 Hz, 1C), 120.48 (q, $^3J_{C-F}$ = 5.4 Hz, 1C), 118.5, 114.5, 56.1, 52.3, 45.9; ^{19}F NMR (471 MHz, MeOD) δ −63.94. HRMS (ESI) *m*/*z* calcd for $C_{18}H_{19}BrF_3N_4O_2$ $[M + H]^+$: 459.0643, found: 459.0647.

General Procedure F for Synthesis of Compounds 16a−h. The *N*-(5-bromo-2-(4-methylpiperazin-1-yl)phenyl)-6-oxo-4-(trifluoromethyl)-1,6-dihydropyridine-3-carboxamide (**15**) (1 equiv), boronic acid/ester (3 equiv), sodium carbonate (10 equiv), XPhos (0.2 equiv), and XPhos Pd G2 (0.2 equiv) in 1,4-dioxane and water (5:3, 0.02 M) were mixed in a 5 mL microwave vial. The mixture was stirred for 5 min, degassed, purged with N_2, and irradiated for 60 min at 120 °C. After cooling to 23 °C, all solvents were removed under reduced pressure, and the crude material purified using flash column chromatography on silica gel (0−100%, 89% CH_2Cl_2, 10% MeOH, 1% NH_4Ac/CH_2Cl_2) to afford the desired compound.

*N-(5-(2,3-Dihydrobenzofuran-5-yl)-2-(4-methylpiperazin-1-yl)-phenyl)-6-oxo-4-(trifluoromethyl)-1,6-dihydropyridine-3-carboxamide (**16a**).* The title compound was prepared according to general procedure F (50 mg, 44% yield) as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.39 (s, 1H), 8.05 (s, 1H), 7.97 (s, 1H), 7.45 (s, 1H), 7.36 (dd, *J* = 8.3, 1.8 Hz, 1H), 7.31 (d, *J* = 8.2 Hz, 1H), 7.21 (d, *J* = 8.3 Hz, 1H), 6.83 (d, *J* = 8.4 Hz, 1H), 6.82 (s, 1H), 4.56 (t, *J* = 8.7 Hz, 2H), 3.24 (t, *J* = 8.6 Hz, 2H), 2.87 (s, 4H), 2.48 (s, 4H), 2.23 (s, 3H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 161.0, 159.6, 157.6, 141.5, 137.5, 137.0 (q, $^2J_{C-F}$ = 32.5 Hz, 1C), 134.3, 130.7, 126.6, 124.5, 121.5, 121.4, 120.4 (q, $^1J_{C-F}$ = 275.1 Hz, 1C), 119.5, 118.8, 117.3 (q, $^3J_{C-F}$ = 5.5 Hz, 1C), 110.26, 107.54, 69.51, 53.20, 49.61, 44.11, 27.45; ^{19}F NMR (471 MHz, DMSO-d_6) δ −61.43. HRMS (ESI) *m*/*z* calcd for $C_{26}H_{26}F_3N_4O_3$ $[M + H]^+$: 499.1957, found: 499.1955.

*N-(2-(4-Methylpiperazin-1-yl)-5-(quinolin-3-yl)phenyl)-6-oxo-4-(trifluoromethyl)-1,6-dihydropyridine-3-carboxamide (**16b**).* The title compound was prepared according to general procedure F (31 mg, 97% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.55 (s, 1H), 9.19 (d, *J* = 2.0 Hz, 1H), 8.54 (d, *J* = 1.7 Hz, 1H), 8.24 (d, *J* = 1.5 Hz, 1H), 8.08 (d, *J* = 8.1 Hz, 1H), 8.05 (d, *J* = 8.4 Hz, 1H), 8.01 (s, 1H), 7.76 (t, *J* = 7.6 Hz, 1H), 7.69 (dd, *J* = 8.3, 1.9 Hz, 1H), 7.64 (t, *J* = 7.5 Hz, 1H), 7.33 (d, *J* = 8.3 Hz, 1H), 6.82 (s, 1H), 3.35 (s, 4H), 2.94 (s, 4H), 2.24 (s, 3H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 163.4, 161.8, 149.7, 147.2, 145.7, 140.0, 139.1 (q, $^2J_{C-F}$ = 32.7 Hz, 1C), 132.9, 132.6, 132.5, 129.9, 129.1, 128.8, 128.2, 127.5, 124.9, 123.3, 122.5 (q, $^1J_{C-F}$ = 275.2 Hz, 1C), 121.2, 119.3 (q, $^3J_{C-F}$ = 5.5 Hz, 1C), 112.1, 55.2, 51.5, 46.2; ^{19}F NMR (471 MHz, DMSO-d_6) δ −61.39. HRMS (ESI) *m*/*z* calcd for $C_{27}H_{25}F_3N_5O_2$ $[M + H]^+$: 508.1960, found: 508.1969.

*N-(5-(1H-Indol-5-yl)-2-(4-methylpiperazin-1-yl)phenyl)-6-oxo-4-(trifluoromethyl)-1,6-dihydropyridine-3-carboxamide (**16c**).* The title compound was prepared according to general procedure F (47 mg, 26% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.50 (br s, 1H), 11.15 (br s, 1H), 9.42 (s, 1H), 8.16 (s, 1H), 7.99 (s, 1H), 7.75 (s, 1H), 7.45 (d, *J* = 8.0 Hz, 1H), 7.38 (s, 1H), 7.34 (d, *J* = 8.3 Hz, 1H), 7.25 (d, *J* = 8.3 Hz, 1H), 6.84 (s, 1H), 6.50 (br s, 1H), 2.94−2.85 (m, 4H), 2.51 (br s, 4H), 2.25 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 163.1, 161.6, 143.3, 139.5, 139.1 (q, $^2J_{C-F}$ = 32.7 Hz), 137.9, 135.9, 132.8, 131.4, 128.7, 126.6, 123.9, 122.0, 120.9, 120.6, 119.5, 122.4 (q, $^1J_{C-F}$ = 275.2 Hz), 118.2, 112.46, 112.3, 102.0, 55.4 (2), 51.8 (2), 46.23. HRMS (ESI) *m*/*z* calcd for $C_{26}H_{25}F_3N_5O_2$ $[M + H]^+$: 496.1960, found: 496.1963.

*N-(4-(4-Methylpiperazin-1-yl)-3′-(morpholinomethyl)-[1,1′-biphenyl]-3-yl)-6-oxo-4-(trifluoromethyl)-1,6-dihydropyridine-3-carboxamide (**16d**).* The title compound was prepared according to general procedure F (47 mg, 26% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.95 (s, 1H), 8.64 (d, *J* = 0.9 Hz, 1H), 7.89 (s, 1H), 7.55 (s, 1H), 7.50 (d, *J* = 7.7 Hz, 1H), 7.38−7.33 (m, 2H), 7.32−7.28 (m, 2H), 6.97 (s, 1H), 3.72−3.68 (m, 4H), 3.55 (s, 2H), 2.97−2.90 (m, 4H), 2.63 (s, 4H), 2.49−2.44 (m, 4H), 2.36 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 163.6, 161.9, 140.8, 140.6, 140.0 (q, $^2J_{C-F}$ = 33.3 Hz, 1C), 139.0, 138.4, 138.3, 133.7, 128.8, 128.4, 128.1, 126.2, 123.5, 121.8 (q, $^1J_{C-F}$ = 275.4 Hz, 1C), 121.6, 119.6 (q, $^3J_{C-F}$ = 5.3 Hz, 1C), 118.6, 116.4, 114.8, 67.1, 63.5, 53.7, 52.4, 46.0, 40.9; ^{19}F NMR (471 MHz, $CDCl_3$) δ −62.33. HRMS (ESI) *m*/*z* calcd for $C_{29}H_{33}F_3N_5O_3$ $[M + H]^+$: 556.2535, found: 556.2526.

*N-(5-(6-Acetamidopyridin-3-yl)-2-(4-methylpiperazin-1-yl)-phenyl)-6-oxo-4-(trifluoromethyl)-1,6-dihydropyridine-3-carboxamide (**16e**).* The title compound was prepared according to general procedure F (47 mg, 26% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ

DOI: 10.1021/acs.jmedchem.5b01630
J. Med. Chem. XXXX, XXX, XXX−XXX

12.55 (br s, 1H), 10.60 (s, 1H), 9.48 (s, 1H), 8.55 (d, J = 2.2 Hz, 1H), 8.16 (d, J = 8.7 Hz, 1H), 8.10−8.06 (m, 1H), 8.02−7.97 (m, 2H), 7.50 (dd, J = 1.8, 8.3 Hz, 1H), 7.27 (d, J = 8.3 Hz, 1H), 6.82 (s, 1H), 2.93−2.86 (m, 4H), 2.24 (s, 3H), 2.15−2.09 (m, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.6, 163.6, 162.8, 150.9, 145.5, 143.6, 140.2, 138.4, 136.0, 133.8, 132.6, 132.0, 124.0, 123.1, 120.9, 120.9, 119.1, 113.9, 113.2, 54.7 (2), 51.0 (2), 44.4, 22.6; ^{19}F NMR (471 MHz, DMSO-d_6) δ −61.41. HRMS (ESI) m/z calcd for $C_{25}H_{26}F_3N_6O_3$ $[M + H]^+$: 515.2018, found: 515.2012.

N-(5-(6-Aminopyridin-3-yl)-2-(4-methylpiperazin-1-yl)phenyl)-6-oxo-4-(trifluoromethyl)-1,6-dihydropyridine-3-carboxamide (**16f**). The title compound was prepared by using general procedure F (154 mg, 58% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.30 (br s, 1H), 8.45 (s, 1H), 8.16 (d, J = 2.5 Hz, 1H), 8.08 (s, 1H), 8.04 (s, 1H), 7.61 (dd, J = 8.5, 2.5 Hz, 1H), 7.32 (dd, J = 8.0, 2.0 Hz, 1H), 7.22 (d, J = 8.0 Hz, 1H), 6.67 (br s, 1H), 6.53 (d, J = 8.5 Hz, 1H), 6.04 (s, 2H), 2.86 (br t, 4H), 2.48 (br s, 4H), 2.23 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 165.7, 163.6, 159.5, 145.9, 142.9 (2), 135.6, 134.4, 133.4, 124.2, 122.3, 121.1, 119.9, 117.5, 111.6, 108.5 (2), 55.4 (2), 51.8 (2), 46.2. HRMS (ESI) m/z calcd for $C_{23}H_{24}F_3N_6O_2$ $[M + H]^+$: 473.1913, found: 473.1909.

N-(4′-Amino-4-(4-methylpiperazin-1-yl)-[1,1′-biphenyl]-3-yl)-6-oxo-4-(trifluoromethyl)-1,6-dihydropyridine-3-carboxamide (**16g**). The title compound was prepared by using general procedure F (59 mg, 64% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.30 (br s, 1H), 8.42 (s, 1H), 8.06 (d, J = 2.0 Hz, 1H), 8.00 (s, 1H), 7.31−7.26 (m, 3H), 7.19 (d, J = 10.0 Hz, 1H), 6.72 (br s, 1H), 6.66−6.62 (m, 2H), 5.20 (br s, 2H), 2.86 (br t, 4H), 2.48 (br s, 4H), 2.23 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 165.7, 163.4, 148.7, 142.5, 141.6, 137.0, 133.l, 128.1, 127.6, 127.4 (2), 122.3, 120.9, 120.0, 118.2, 114.7 (2), 112.0, 55.5 (2), 51.9 (2), 46.2; ^{19}F NMR (471 MHz, DMSO-d_6) δ −61.15. HRMS (ESI) m/z calcd for $C_{24}H_{25}F_3N_5O_2$ $[M + H]^+$: 472.1960, found: 472.1952.

N-(2-(4-Methylpiperazin-1-yl)-5-(1-(2-morpholinoethyl)-1H-pyrazol-4-yl)phenyl)-6-oxo-4-(trifluoromethyl)-1,6-dihydropyridine-3-carboxamide (**16h**). The title compound was prepared according to general procedure F (42 mg, 68% yield). ^{1}H NMR (500 MHz, MeOD) δ 8.20 (d, J = 1.6 Hz, 1H), 8.01 (s, 1H), 7.98 (s, 1H), 7.80 (s, 1H), 7.38 (dd, J = 8.3, 2.0 Hz, 1H), 7.25 (d, J = 8.3 Hz, 1H), 6.92 (s, 1H), 4.29 (t, J = 6.5 Hz, 2H), 3.68−3.64 (m, 4H), 2.96 (t, J = 4.7 Hz, 4H), 2.82 (t, J = 6.5 Hz, 2H), 2.68 (s, 4H), 2.52−2.47 (m, 4H), 2.38 (s, 3H); ^{13}C NMR (126 MHz, MeOD) δ 164.8, 164.4, 143.3, 141.4 (q, $^2J_{C-F}$ = 33.2 Hz, 1C), 139.8, 137.5, 134.1, 130.8, 128.9, 123.8, 123.7, 123.4 (q, $^1J_{C-F}$ = 274.7 Hz, 1C), 122.2, 120.3 (q, $^3J_{C-F}$ = 4.5 Hz, 1C), 114.8, 67.9, 59.2, 56.3, 54.7, 52.6, 50.3, 46.0; ^{19}F NMR (471 MHz, MeOD) δ −63.80. HRMS (ESI) m/z calcd for $C_{27}H_{33}F_3N_7O_3$ $[M + H]^+$: 560.2597, found: 560.2595.

Cloning, Expression, and Purification of Human WDR5. The DNA fragment encoding human WDR5 (residues 1−334) was amplified by PCR and subcloned into pET28-LIC vector, downstream of the poly histidine coding region. The protein was expressed in *E. coli* BL21 (DE3)-V2R-pRARE2 strain by addition of 1 mM isopropyl-1-thio-D-galactopyranoside (IPTG) and incubated overnight at 15 °C. Harvested cells were resuspended in 20 mM Tris buffer (pH 7.5) supplemented with 500 mM NaCl, 5 mM imidazole, and 5% glycerol. The cells were lysed chemically, followed by sonication at a frequency of 8.5 (10 s on/10 s off) for 4 min. After clarification of the crude extract by high-speed centrifugation, the lysate was loaded onto DE52 and passed onto a Ni-NTA column. The column was washed and eluted sequentially with 20 mM Tris (pH 7.5), 500 mM NaCl, 5% glycerol, 30 mM and 250 mM imidazole. Thrombin was added while the protein was being dialyzed against 20 mM Tris buffer (pH 7.5), 2.5 mM $CaCl_2$, and 500 mM NaCl. To remove the cut his-tag and his-tagged protein, dialyzed protein solution was passed through a Ni-NTA column. Flow through was dialyzed again against 20 mM Tris buffer (pH 7.5), 2.5 mM $CaCl_2$, and 500 mM NaCl. Pure protein (>95%) was further concentrated to higher than 20 mg/mL and stored at −80 °C after flash freezing.

Fluorescence Polarization (FP) Binding Assays. H3(1−15) (ARTKQTARKSTGGKA) and 9-Ala-FAM ((Ac)-ARAEVHLRK-(Ahx-Ahx)-K(5,6-FAM)) (where Ahx represents 6 amino hexanoic acid linkers)[12,16] peptides for WDR5 were synthesized, N-terminal-labeled with isothiocyanate-fluorescein, and purified by Tufts University Core Services (Boston, MA). Compound binding assays were performed at a constant labeled peptide concentration of 30 or 20 nM for H3 (1−15) and 9-Ala-FAM respectively. WDR5 concentrations of 0.3 μM for H3 (1−15) and 0.05 μM for 9-Ala-FAM were used. For both the H3 (1−15) and 9-Ala-FAM peptides, 80 mM sodium phosphate buffer (pH 6.5), 20 mM KCl, and 0.008% Triton X-100 were used. For the H3 (1−15) peptide, FP assays were measured in 10 μL aliquots in 384-well Axygen plates using a Synergy 4 microplate reader (BioTek) with an excitation wavelength of 485 nm and emission wavelength of 528 nm. For the 9-Ala-FAM peptide, FP assays were measured in 125 μL aliquots in 96-well Microfluor plates using a ViewLux instrument (PerkinElmer) with an excitation wavelength of 480 nm and emission wavelength of 540 nm. To determine K_{disp} values, the data were fit to a hyperbolic function using Sigma Plot software (Systat Software). The K_{disp} values represent the average of quadruplicate measurements.

Crystallization and Structure Determination. Purified WDR5 protein (10 mg/mL) was mixed with each compound at a 1:5 molar ratio of protein to compound and crystallized using the sitting-drop vapor-diffusion method at 20 °C by adding 1 μL of protein solution to 1 μL of the reservoir solution containing 25% PEG [poly(ethylene glycol)] 3350, 0.1 M ammonium sulfate 0.1 M BisTris, pH 6.5 (compound **9d**), 28% PEG 2000 monomethyl ether, 0.1 M BisTris, pH 6.5 (compound **9e**), 20% PEG 5000 monomethyl ether, 0.1 M BisTris, pH 6.5 (compound **9h**), and 25% PEG3350, 0.1 M ammonium sulfate, 0.1 M BisTris, pH 6.5 (compound **9o**), respectively. Crystals were soaked in the corresponding mother liquor supplemented with 20% ethylene glycol as cryoprotectant before freezing in liquid nitrogen. Diffraction data for crystals of the WDR5-compound complexes were collected on a Rigaku FR-E SuperBright instrument or at beamline of the Canadian Macromolecular Crystallography Facility. The program suite HKL3000 was used to integrate and scale the data sets. All WDR5 co-crystal structures were determined by molecular replacement using PHASER, with coordinates from PDB entry 4IA9 as the search template. The graphic program Coot was used for manual model refinement and visualization. Refmac was used to refine the model. MolProbity was used to validate the refined structure. The structures have been deposited into the PDB under codes 3SMR, 4IA9, 5EAR, 5EAP, 5EAL, 5EAM,and 4QL1. Crystal diffraction data and refinement statistics for the structures of WDR5-**9d**, **9e**, **9h**, and **9o** complexes are shown in Supporting Information, Table 1.

Pharmacokinetics. All PK assessments were performed by InterVivo Solutions (Mississauga, Ontario, Canada). Compound **16d** was administered to mice (female NOD-SCID) at 3 mg/kg IV (n = 3) and 30 mg/kg IP (n = 3), along with an undosed control (n = 3). Blood samples were collected from all mice at 5, 15, 30 min and 1, 2, 4, 6, and 24 h. Plasma was separated from blood by centrifugation and stored at −80 °C until analysis. The pharmacokinetic parameters C_{max}, T_{max}, $t_{1/2}$, AUC, Cl, and V_D were evaluated.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.5b01630.

Synthetic procedures and compound characterization data, NMR spectra of select compounds, details of the focused library design and molecular docking, methods for cloning, expression, and purification of human WDR5, protocols for the fluorescence polarization (FP) binding assays, crystallization and structure determination methods, and how pharmacokinetic parameters were obtained (PDF)

Molecular formula strings (CSV)

DOI: 10.1021/acs.jmedchem.5b01630
J. Med. Chem. XXXX, XXX, XXX−XXX

Accession Codes

PDB codes for the structures of WDR5 bound to various antagonists are as follows: WDR5-**4**, 3SMR; WDR5-**5**, 4IA9; WDR5-**9d**, 5EAR; WDR5-**9e**, 5EAP; WDR5-**9h**, 5EAL; WDR5-**9o**, 5EAM; WDR5-**16d**, 4QL1.

AUTHOR INFORMATION

Corresponding Author

*Phone: +1 (416) 673-8577. E-mail: Rima.Alawar@oicr.on.ca.

Present Addresses

[⊥]Merck KGaA, Performance Materials, Frankfurter Str. 250, D-64289 Darmstadt, Germany.

[▽]University of Ontario Institute of Technology, Faculty of Science, 2000 Simcoe Street North, Oshawa, Ontario, L1H 7K4, Canada.

[○]Dr. Ekaterina Kuznetsova, Director of Product Development, Reaction Biology Corporation, 1 Great Valley Parkway, Suite 18, Malvern, PA 19355, United States.

Author Contributions

[#]M.G. and D.S. contributed equally to this work.

M.G., D.S., and C. Z.-V. designed and synthesized compounds. Y.B. synthesized compounds. G.P. designed compounds, enumerated and evaluated focused virtual libraries and performed docking experiments against WDR5 and protein kinases. E.K. and G.S. performed all FP assays. R.M. performed all SPR experiments. H.W. and L.D. purified and co-crystallized WDR5. A.D. solved the crystal structures. T.H. purified WDR5 for in vitro assays. T.K. performed Caco-2 studies. M.S. contributed to the design of compounds and analyzed data. C.H.A. and P.J.B. contributed to experimental design, data analysis, and editing of the paper. M.G., D.S., C. Z.-V., G.P., M.V., and R.A. reviewed data, contributed to experimental design, and wrote the paper. R.A. and M.V. were the principal investigators, designed experiments, analyzed data, and supervised the experiments.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was supported by the Ontario Institute for Cancer Research whose funding is provided by the Government of Ontario through the Ontario Ministry of Economic Development and Innovation. Additional support came from the Structural Genomics Consortium, a registered charity (no. 1097737) that receives funds from AbbVie, Bayer Pharma AG, Boehringer Ingelheim, Canada Foundation for Innovation, Eshelman Institute for Innovation, Genome Canada, Innovative Medicines Initiative (EU/EFPIA), Janssen, Merck & Co., Novartis Pharma AG, Pfizer, São Paulo Research Foundation-FAPESP, Takeda, and Wellcome Trust. Research described in this paper was performed using beamline 08ID-1 at the Canadian Light Source, which is supported by the Natural Sciences and Engineering Research Council of Canada, the National Research Council Canada, the Canadian Institutes of Health Research, the Province of Saskatchewan, Western Economic Diversification Canada, and the University of Saskatchewan. C.Z.-V. thanks CONACyT for a postdoctoral fellowship. We would also like to thank Dr. Ahmed Aman and Dr. Taira Kiyota for assistance in obtaining the HRMS of all final synthesized compounds and Dr. John R. Walker for review of the crystallography model.

ABBREVIATIONS USED

AML, acute myeloid leukemia; Ash2L, absent, small, or homeotic 2 like protein; C/EBPα, CCAAT/enhancer binding protein alpha; DFG, Asp-Phe-Gly; DIPEA, *N*,*N*-diisopropylethylamine; DMAP, 4-(*N*,*N*-dimethylamino)pyridine; DMSO, dimethyl sulfoxide; EtOAc, ethyl acetate; FP, fluorescence polarization; GOF, gain-of-function; GPCR, G-protein coupled receptor; HOXA9, homeobox A9; HRMS, high-resolution mass spectrometry; HTS, high-throughput screening; IV, intravenous; MEIS1, Meis homeobox 1; MeOH, methanol; MLL1, mixed-lineage leukemia; N-Myc, neuroblastoma-derived myelocytomatosis; PK, pharmacokinetic; PSA, polar surface area; RbBP5, retinoblastoma-binding protein 5; SAR, structure−activity relationship; SPR, surface plasmon resonance; WDR5, WD repeat-containing protein 5; WIN, WDR5-interacting

REFERENCES

(1) Wu, M.; Shu, H.-B. MLL1/WDR5 complex in leukemogenesis and epigenetic regulation. *Aizheng* **2011**, *30*, 240−246.

(2) Slany, R. K. The molecular biology of mixed-lineage leukemia. *Haematologica* **2009**, *94*, 984−993.

(3) Karatas, H.; Townsend, E. C.; Cao, F.; Chen, Y.; Bernard, D.; Liu, L.; Lei, M.; Dou, Y.; Wang, S. High-affinity, small-molecule peptidomimetic inhibitors of MLL1/WDR5 protein-protein interaction. *J. Am. Chem. Soc.* **2013**, *135*, 669−682.

(4) Bissinger, E.-M.; Heinke, R.; Sippl, W.; Jung, M. Targeting epigenetic modifiers: inhibitors of histone methyltransferases. *MedChemComm* **2010**, *1*, 114−124.

(5) Li, D.; Roberts, R. Human Genome and Diseases. WD-repeat proteins: structure characteristics, biological function, and their involvement in human diseases. *Cell. Mol. Life Sci.* **2001**, *58*, 2085−2097.

(6) Patel, A.; Dharmarajan, V.; Cosgrove, M. S. Structure of WDR5 bound to mixed lineage leukemia protein-1 peptide. *J. Biol. Chem.* **2008**, *283*, 32158−32161.

(7) Patel, A.; Vought, V. E.; Dharmarajan, V.; Cosgrove, M. S. A conserved arginine-containing motif crucial for the assembly and enzymatic activity of the mixed lineage leukemia protein-1 core complex. *J. Biol. Chem.* **2008**, *283*, 32162−32175.

(8) Orlicky, S.; Tang, X.; Neduva, V.; Elowe, N.; Brown, E. D.; Sicheri, F.; Tyers, M. An allosteric inhibitor of substrate recognition by the SCF^{Cdc4} ubiquitin ligase. *Nat. Biotechnol.* **2010**, *28*, 733−737.

(9) Cao, F.; Townsend, E. C.; Karatas, H.; Xu, J.; Li, L.; Lee, S.; Liu, L.; Chen, Y.; Ouillette, P.; Zhu, J.; Hess, J. L.; Atadja, P.; Lei, M.; Qin, Z. S.; Malek, S.; Wang, S.; Dou, Y. Targeting MLL1 H3K4 methyltransferase activity in mixed-lineage leukemia. *Mol. Cell* **2014**, *53*, 247−261.

(10) Senisterra, G.; Wu, H.; Allali-Hassani, A.; Wasney, G. A.; Barsyte-Lovejoy, D.; Dombrovski, L.; Dong, A.; Nguyen, K. T.; Smil, D.; Bolshan, Y.; Hajian, T.; He, H.; Seitova, A.; Chau, I.; Li, F.; Poda, G.; Couture, J. F.; Brown, P. J.; Al-Awar, R.; Schapira, M.; Arrowsmith, C. H.; Vedadi, M. Small-molecule inhibition of MLL1 activity by disruption of its interaction with WDR5. *Biochem. J.* **2013**, *449*, 151−159.

(11) Bolshan, Y.; Getlik, M.; Kuznetsova, E.; Wasney, G. A.; Hajian, T.; Poda, G.; Nguyen, K. T.; Wu, H.; Dombrovski, L.; Dong, A.; Senisterra, G.; Schapira, M.; Arrowsmith, C. H.; Brown, P. J.; Al-awar, R.; Vedadi, M.; Smil, D. Synthesis, optimization, and evaluation of novel small molecules as antagonists of WDR5-MLL1 interaction. *ACS Med. Chem. Lett.* **2013**, *4*, 353−357.

(12) Grebien, F.; Vedadi, M.; Getlik, M.; Giambruno, R.; Grover, A.; Avellino, R.; Skucha, A.; Vittori, S.; Kuznetsova, E.; Smil, D.; Barsyte-Lovejoy, D.; Li, F.; Poda, G.; Schapira, M.; Wu, H.; Dong, A.; Senisterra, G.; Stukalov, A.; Huber, K. V.; Schönegger, A.; Marcellus, R.; Bilban, M.; Bock, C.; Brown, P. J.; Zuber, J.; Bennett, K. L.; Al-awar, R.; Delwel, R.; Nerlov, C.; Arrowsmith, C. H.; Superti-Furga, G.

DOI: 10.1021/acs.jmedchem.5b01630
J. Med. Chem. XXXX, XXX, XXX−XXX

Pharmacological targeting of the WDR5-MLL1 interaction in C/EBPα N-terminal leukemia. *Nat. Chem. Biol.* **2015**, *11*, 571−578.
(13) Zhu, J.; Sammons, M. A.; Donahue, G.; Dou, Z.; Vedadi, M.; Getlik, M.; Barsyte-Lovejoy, D.; Al-awar, R.; Katona, B. W.; Shilatifard, A.; Huang, J.; Hua, X.; Arrowsmith, C. H.; Berger, S. L. Gain-of-function p53 mutants co-opt chromatin pathways to drive cancer growth. *Nature* **2015**, *525*, 206−211.
(14) Sun, Y.; Bell, J. L.; Carter, D.; Gherardi, S.; Poulos, R. C.; Milazzo, G.; Wong, J. W. H.; Al-Awar, R.; Tee, A. E.; Liu, P. Y.; Liu, B.; Atmadibrata, B.; Wong, M.; Trahair, T.; Zhao, Q.; Shohet, J. M.; Haupt, Y.; Schulte, J. H.; Brown, P. J.; Arrowsmith, C. H.; Vedadi, M.; MacKenzie, K. L.; Hüttelmaier, S.; Perini, G.; Marshall, G. M.; Braithwaite, A.; Liu, T. WDR5 supports an N-Myc transcriptional complex that drives a protumorigenic gene expression signature in neuroblastoma. *Cancer Res.* **2015**, *75*, 5143−5154.
(15) Data not shown. Unpublished results.
(16) In order to obtain a better signal in the FP assay used for this publication, we synthesized the described 9-Ala-FAM peptide based on the 10-Ala-FAM peptide reported in Karatas, H.; Townsend, E. C.; Bernard, D.; Dou, Y.; Wang, S. Analysis of the binding of mixed-lineage leukemia 1 (MLL1) and histone 3 peptides to WD repeat domain 5 (WDR5) for the design of inhibitors of the MLL1-WDR5 interaction. *J. Med. Chem.* **2010**, *53*, 5179−5185.
(17) Bissantz, C.; Kuhn, B.; Stahl, M. A medicinal chemist's guide to molecular interactions. *J. Med. Chem.* **2010**, *53*, 5061−5084.
(18) Qualitative finding based on the apparent solubility of the compounds in common organic solvents such as acetonitrile, methanol, and DMSO observed during the course of experimental work, measurements using SPR, and compound characterization by NMR.
(19) Currently *BIOVIA; Virtual Biosphere and Materials*. http://www.3ds.com/products-services/biovia/ (accessed February 17, 2016).
(20) *eMolecules*. http://www.emolecules.com (accessed February 17, 2016).
(21) *Glide docking program*. http://www.schrodinger.com (accessed February 17, 2016).
(22) *Cerep*. http://www.cerep.fr/cerep/users/pages/catalog/profiles/catalog.asp (accessed February 17, 2016).
(23) Zuber, J.; Radtke, I.; Pardee, T. S.; Zhao, Z.; Rappaport, A. R.; Luo, W.; McCurrach, M. E.; Yang, M.-M.; Dolan, M. E.; Kogan, S. C.; Downing, J. R.; Lowe, S. W. Mouse models of human AML accurately predict chemotherapy response. *Genes Dev.* **2009**, *23*, 877−889.
(24) McCormack, E.; Bruserud, Ø; Gjertsen, B. T. Animal models of acute myelogenous leukaemia - development, application and future perspectives. *Leukemia* **2005**, *19*, 687−706.

DOI: 10.1021/acs.jmedchem.5b01630
J. Med. Chem. XXXX, XXX, XXX−XXX