Journal of Medicinal Chemistry

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Discovery and Optimization of 2*H*-1λ^2-Pyridin-2-one Inhibitors of Mutant Isocitrate Dehydrogenase 1 for the Treatment of Cancer

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Cite This: *J. Med. Chem.* 2021, 64, 4913−4946 Read Online

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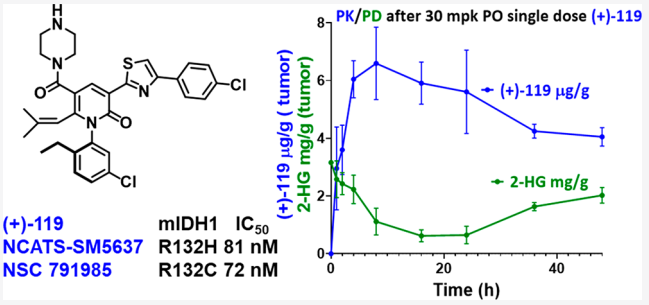
ABSTRACT: Neomorphic mutations in isocitrate dehydrogenase 1 (IDH1) are oncogenic for a number of malignancies, primarily low-grade gliomas and acute myeloid leukemia. We report a medicinal chemistry campaign around a 7,7-dimethyl-7,8-dihydro-2*H*-1λ^2-quinoline-2,5(6*H*)-dione screening hit against the R132H and R132C mutant forms of isocitrate dehydrogenase (IDH1). Systematic SAR efforts produced a series of potent pyrid-2-one mIDH1 inhibitors, including the atropisomer (+)-**119** (**NCATS-SM5637**, **NSC 791985**). In an engineered mIDH1-U87-xenograft mouse model, after a single oral dose of 30 mg/kg, 16 h post dose, between 16 and 48 h, (+)-**119** showed higher tumoral concentrations that corresponded to lower 2-HG concentrations, when compared with the approved drug AG-120 (ivosidenib).



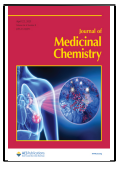
INTRODUCTION

Over the past decade, mutant isocitrate dehydrogenases 1 (mIDH1) and 2 (mIDH2) have emerged as important targets for treating a range of malignancies.[1,2] Normal (wild-type, WT) IDH1 or IDH2 protein catalyzes the conversion of isocitrate to α-ketoglutarate (α-KG, also called 2-oxoglutarate, 2-OG), using $NADP^+$ as a cofactor.[3] Critical work has demonstrated that some acute myelogenous leukemias (AMLs) and gliomas possess heterozygous somatic mutations of IDH1 at position R132 and IDH2 at R140 or R172.[4−6] Analysis of clinical samples has shown that the majority of low-grade gliomas and ∼20% of AML have IDH1 or IDH2 mutations, and a range of other solid tumors such as chondrosarcoma, cholangiocarcinoma, colon, pancreatic, and prostate cancer have also been found to carry IDH1/2 mutations, although to a lesser degree.[1,7]

The canonical mutations to IDH1 and IDH2 confer a neomorphic (gain-of-function) activity. While WT IDH1 produces α-KG, α-KG is the substrate of mIDH1, which in a pseudo-reverse reaction using NADPH produces *R*-2-hydroxyglutarate (2-HG).[8,9] An analysis of serum 2HG levels in AML patients bearing IDH1 or IDH2 mutants showed a clear elevation (median 2HG of 3004 ng/mL) compared to patients with WT IDH (median 2HG of 61 ng/mL).[10] This 2-HG "oncometabolite" has been shown to play a role in modifying cellular behavior.[11−13] For example, 2-HG inhibits α-KG-dependent enzymes,[14,15] and direct evidence exists for inhibition of α-KG-dependent histone and DNA demethylases by 2-HG leading to elevated histone methylation, with consequent impact on gene expression and cell differentiation.[16]

Both mIDH1 and mIDH2 are attractive therapeutic targets due to their genetic gain-of-function, which confers an oncogenic role that is amenable to detection via both tumor gene sequencing and metabolite (2-HG) measurement.[17] Moreover, specific inhibition of mIDH1/2 should not produce target-related clinical side effects as WT IDH's function would be unaffected. In 2017, only after ten years since the first reports of the role of IDH1/2 mutations in cancer, the FDA approved the mIDH2 inhibitor enasidenib from Agios/Celgene for treatment of relapsed or refractory AML (R/R AML).[18] In 2018, the FDA granted priority approval to Agios' mIDH1 inhibitor AG-120 (ivosidenib) for patients with R/R

Received: January 6, 2021
Published: April 6, 2021



https://doi.org/10.1021/acs.jmedchem.1c00019
J. Med. Chem. 2021, 64, 4913−4946

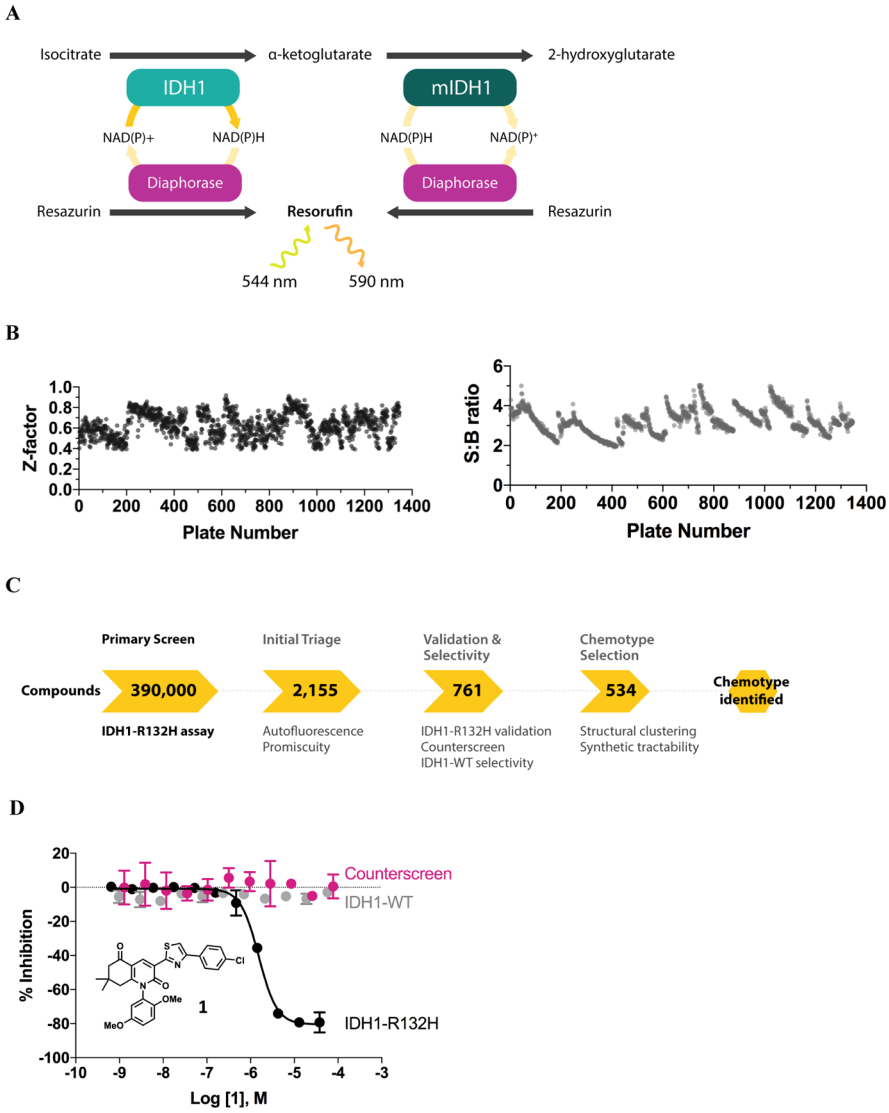


Figure 1. (A) Schematic for primary biochemical qHTS assay; (B) assay performance as measured by Z' factor and S/B ratio; (C) workflow for the identification of small-molecule inhibitors of mutant IDH1; and (D) dose-dependent activity of hit **1** against R132H mIDH1, IDH-WT, and in a readout interference counterscreen.

AML harboring IDH1 mutations as measured by the Abbott RealTime IDH1 assay.[19] In May 2019, ivosidenib was approved as first-line treatment for AML with IDH1 mutation in patients who are at least 75 years old or who have comorbidities that preclude the use of intensive induction chemotherapy.[19] Agios continues to take ivosidenib through multiple clinical trials, notably, toward AML in combination with the chemotherapeutic drug azacytidine and toward glioma and cholangiocarcinoma in patients harboring mIDH1.[20] Agios is also advancing its pan mIDH oral inhibitor AG-881 (vorasidenib) in trials with patients with advanced solid tumors (including gliomas) and low-grade glioma.[20,21] In addition to this, Novartis and Bayer have also advanced IDH inhibitors IDH305 and BAY1436032 mIDH1 to clinical trials.[22,23] In 2019, an updated succinct review of all reported classes of preclinical and clinical small-molecule mIDH inhibitors was published.[24] It included Agios' tool mIDH1 inhibitor AG-5198,[25] probe molecules from Sanofi and GlaxoSmithKline,[26,27] and CNS penetrant FT-2102 from Forma Therapeutics, Inc.[28]

We had been involved in an early assay development and screening program that produced the phenyl-glycine analogue ML309 as a potent inhibitor of R132H mIDH1.[29,30] We also developed a panel of biochemical and cell-based assays to enable inhibitor discovery campaigns and aid comparison to known mIDH1 probe and experimental therapeutic inhibitors.[31] Here, we fully describe the hit-to-lead medical chemistry campaign based on a 7,7-dimethyl-7,8-dihydro-2*H*-1λ^2-quinoline-2,5(6*H*)-dione mIDH1 inhibitor chemical series that also emerged as a screening hit besides the chemotype that was optimized to ML309. Optimization led to the discovery, separation, and biological characterization of stable atropisomers from a 1-arylpyridin-2(1*H*)-one subseries. Exemplary analogue (+)-**119** is used to demonstrate 2-HG reduction in a pharmacokinetic/pharmacodynamic (PK/PD) model with ivosidenib as a comparator molecule.

Our project was supported by the National Cancer Institute's (NCI) Experimental Therapeutics (NExT) Program facilitated within the NCI Chemical Biology Consortium (CBC). The goal of CBC's NExT Program is to bring together government, industry, and academic groups to enable a team-

https://doi.org/10.1021/acs.jmedchem.1c00019
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Scheme 1. General Analogue Synthesis

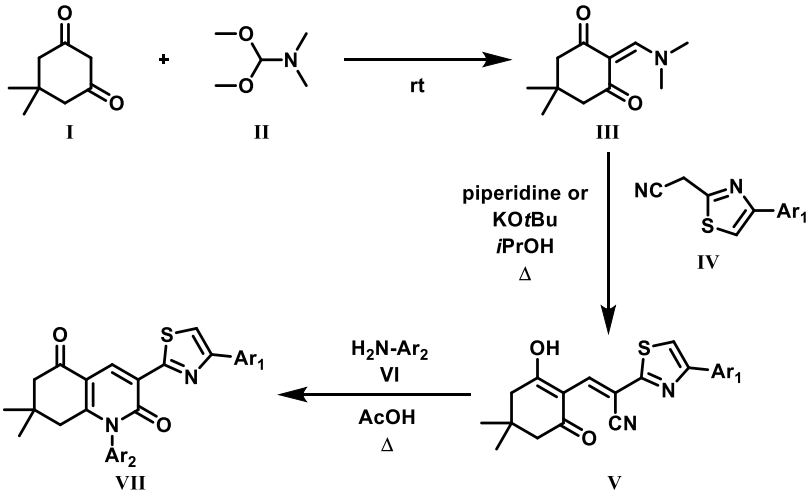
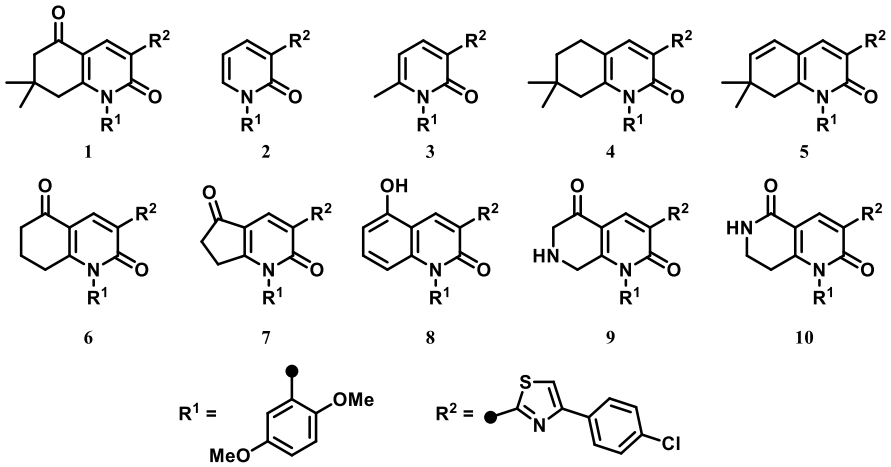


Table 1. Core Modifications and SAR



biochemical	IDH1 R132H		IDH1 R132C	
compound	IC_{50} (μM) ± SD[a]	% inhibition ± SD at 38 μM	IC_{50} (μM) ± SD[a]	% inhibition ± SD at 38 μM
1	1.6 ± 0.2	84 ± 7	1.0 ± 0.3	85 ± 9
2	>38		>38	
3	>38		>38	
4	3.6 ± 0.5	87 ± 3		64 ± 5
5	3.0 ± 0.3	96 ± 4	1.7 ± 0.2	76 ± 2
6		62 ± 1		43 ± 1
7		38 ± 1		29 ± 4
8		35 ± 0		36 ± 2
9	8.0 ± 2.4	75 ± 15		32 ± 3
10	2.3 ± 0.0	84 ± 1		65 ± 4

[a]IC_{50} values were determined utilizing the diaphorase- and resazurin-coupled R132H and R132C mIDH1 assays (average of $N = 3$). IC_{50} values are reported only for compounds where ≥75% inhibition of enzyme activity was observed at the highest concentration tested (38 μM).

science approach toward the discovery and development of novel therapeutics (https://next.cancer.gov).

RESULTS AND DISCUSSION

A diaphorase/resazurin-coupled assay for the IDH1 R132H mutation was developed and utilized to screen our in-house collection of nearly 390,000 small molecules (Figure 1A). In this quantitative high-throughput screen (qHTS), the assay was performed at six concentrations ranging from 76 μM to 2 nM. The inhibition associated with each well was computed from the endpoint and normalized against control wells [no enzyme as the positive control and dimethyl sulfoxide (DMSO) as the negative control]. The percent inhibition at each of the concentrations of inhibitor tested was plotted and modeled using a four-parameter logistic fit using in-house software to determine the compound IC_{50} values. There were nearly 600 1536-well plates in the primary qHTS, which included library plate sets and a couple of DMSO plates inserted in the beginning and end of the screening for data correction purposes. The assay was performed well with an

https://doi.org/10.1021/acs.jmedchem.1c00019
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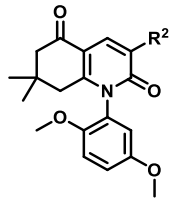
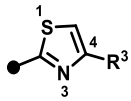
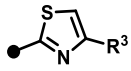
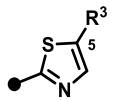
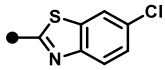
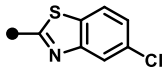
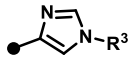
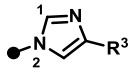
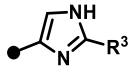
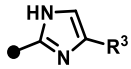
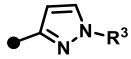
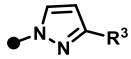
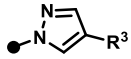
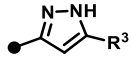
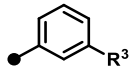
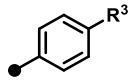
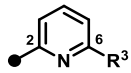
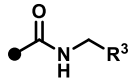
Table 2. Thiazole Substitution SAR



	Biochemical		IDH1 R132H		IDH1 R132C	
Compd	R^2	R^3	IC_{50}(μM) ± SD[a]	% inhibition ± SD at 38 μM	IC_{50}(μM) ± SD[a]	% inhibition ± SD at 38 μM
1		4-Cl-Ph	1.6 ± 0.2	84 ± 7	1.0 ± 0.3	85 ± 9
11		Ph	1.2 ± 0.1	77 ± 2	-	59 ± 3
12		4-Cl-Ph	> 38	-	> 38	-
13		-	-	50 ± 1	2.5 ± 1.6	94 ± 21
14		-	> 38	-	> 38	-
15		4-Cl-Ph	-	41 ± 14	> 38	-
16		Ph	-	59 ± 5	-	44 ± 6
17		Ph	2.1 ± 0.1	75 ± 3	-	39 ± 5
18		4-Cl-Ph	2.2 ± 0.1	82 ± 1	2.5 ± 0.2	77 ± 21
19		Ph	3.1 ± 0.2	84 ± 7	-	46 ± 2
20		Ph	-	71 ± 2	-	41 ± 2
21		Ph	-	31 ± 7	> 38	-
22		Ph	-	47 ± 6	-	41 ± 10
23		4-Cl-Ph	> 38	-	> 38	-
24		Ph	> 38	-	> 38	-
25		4-Cl-Ph	-	67 ± 3	-	46 ± 6
26		4-Cl-Ph	-	35 ± 1	> 38	-

[a]IC_{50} values were determined utilizing the diaphorase- and resazurin-coupled R132H and R132C mIDH1 assays (average of $N = 3$). IC_{50} values are reported only for compounds where ≥75% inhibition of enzyme activity was observed at the highest concentration tested (38 μM).

average Z' factor of 0.60 and an S/B ratio around 3.2 (Figure 1B). Analysis of these dose−response curves resulted in 2155 compounds that showed inhibition over 30% at the highest testing concentration. Among these 2155 inhibitors, 769 were identified as high-quality inhibitors with single or dual

https://doi.org/10.1021/acs.jmedchem.1c00019
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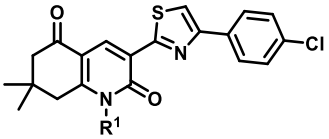
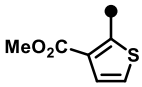
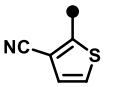
Table 3. Pyridone Aryl Substituent SAR



Biochemical		IDH1 R132H		IDH1 R132C	
Compd	R^1	IC_{50}(μM) ± SD[a]	% inhibition ± SD at 38 μM	IC_{50}(μM) ± SD[a]	% inhibition ± SD at 38 μM
1	2,5-$(OMe)_2$Ph	1.6 ± 0.2	84 ± 7	1.0 ± 0.3	85 ± 9
27	Ph	-	47 ± 3	-	38 ± 2
28	2-OMe-Ph	2.4 ± 0.5	76 ± 3	-	65 ± 2
29	2-OEt-Ph	0.51 ± 0.03	88 ± 6	0.41 ± 0.03	81 ± 3
30	2-O*i*Pr-Ph	1.1 ± 0.2	80 ± 2	0.47 ± 0.05	77 ± 1
31	2-Me-Ph	-	74 ± 4	-	64 ± 2
32	2-Et-Ph	2.0 ± 0.1	91 ± 1	0.87 ± 0.28	83 ± 7
33	2-*i*Pr-Ph	-	62 ± 3	-	61 ± 6
34	2-Ph-Ph	-	59 ± 2	-	67 ± 2
35	2-OH-Ph	-	71 ± 1	-	61 ± 3
36	2-OPh-Ph	-	30 ± 1	-	60 ± 2
37	2-OBn-Ph	-	38 ± 2	-	61 ± 2
38	2-NH_2-Ph	-	57 ± 0	-	42 ± 2
39	2-NHMe-Ph	-	59 ± 3	-	43 ± 1
40	2-NMe_2-Ph	-	56 ± 4	-	57 ± 7
41	2-CO_2H-Ph	4.6 ± 0.3	96 ± 5	11 ± 1	105 ± 7
42	2-F-Ph	-	65 ± 5	-	49 ± 3
43	2-Cl-Ph	1.3 ± 0.1	83 ± 1	-	70 ± 1
44	2-CF_3-Ph	0.27 ± 0.00	96 ± 2	0.20 ± 0.01	87 ± 2
45	2-OCF_3-Ph	0.57 ± 0.04	98 ± 2	0.28 ± 0.02	78 ± 13
46	2-Py	-	47 ± 2	-	39 ± 1
47	2-OMeBn	-	61 ± 12	-	53 ± 1
48	α-MeBn	> 38	-	-	34 ± 1
49	*i*Pr	-	31 ± 8	-	33 ± 1
50	cyclohexyl	> 38	-	> 38	-
51		0.47 ± 0.01	94 ± 1	0.47 ± 0.01	83 ± 4
52		0.26 ± 0.02	100 ± 1	0.25 ± 0.02	90 ± 2

[a]IC_{50} values were determined utilizing the diaphorase- and resazurin-coupled R132H and R132C mIDH1 assays (average of $N = 3$). IC_{50} values are reported only for compounds where ≥75% inhibition of enzyme activity was observed at the highest concentration tested (38 μM).

asymptote curves and greater than 50% inhibition (Figure 1C), representing 0.2% of the chemical library.

The 2155 hit compounds identified from the screen were thoroughly evaluated for both potency and percent inhibition; the autofluorescent compounds and promiscuous inhibitors (compounds that show activity in more than 20% of assays within historical screening data at NCATS) were eliminated from follow-up examination. A total of 761 prioritized hits were retested and screened in 12-pt dose−response in the primary screening assay to reconfirm the activity. The compounds were further tested in a readout interference assay and IDH1-WT selectivity assay to refine and triage initial screening hits (assay hit triage workflow shown in Figure 1C). After structural clustering analysis and assessment of synthetic tractability to eliminate compounds that did not offer good starting points for hit-to-lead optimization, a 7,7-dimethyl-7,8-dihydro-2*H*-1λ^2-quinoline-2,5(6*H*)-dione chemotype (representative hit **1**, Figure 1) with good starting potency and selectivity over WT IDH1 was identified. As shown in Figure 1D, compound **1** displayed inhibition with an IC_{50} of 1.6 μM against R132H mIDH1, with no readout interference and off-target activity against WT IDH1. In addition to the R132H mutant,[32,33] we also developed the analogous assay for the R132C mIDH1, a frequent mutation in AML and intracranial chondrosarcoma,[34,35] and we sought to develop a class of inhibitors capable of targeting both mutations (**1** had an IC_{50} of 1.0 μM against R132C mIDH1).

Despite the moderate structural complexity of hit **1**, it was readily accessible via an efficient one-pot, multicomponent coupling (Scheme 1). Combining dione **I** and acetal **II** well resulted in quick conversion to reactive vinylogous amide **III**. The addition of a base (piperidine or KO*t*Bu) to nitrile **IV** generated an anion that when added to intermediate **III**, afforded intermediate **V** by a Michael addition−elimination sequence. Finally, incorporation of aniline Ar_2NH_2 **VI** provided the pyridinone ring system through cyclodehydration. This last two-step sequence was sufficiently tolerant of diverse substitution on both nitrile **IV** and aniline **VI** and was

https://doi.org/10.1021/acs.jmedchem.1c00019
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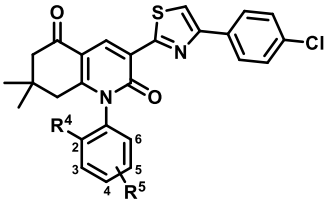
Table 4. Pyridone Aryl Substituent SAR Continued



biochemical			IDH1 R132H		IDH1 R132C	
compd	R^4	R^5	IC_{50} (μM) ± SD[a]	% inhibition ± SD at 38 μM	IC_{50} (μM) ± SD[a]	% inhibition ± SD at 38 μM
1	OMe	5-OMe	1.6 ± 0.2	84 ± 7	1.0 ± 0.3	85 ± 9
53	OMe	3-OMe		64 ± 17		35 ± 3
54	OMe	4-OMe	>38		>38	
55	OMe	6-OMe	2.4 ± 0.1	87 ± 2	1.6 ± 0.2	75 ± 7
56	OMe	3-CO_2H	14 ± 1	82 ± 2		65 ± 2
57	OMe	4-CO_2H	>38			43 ± 6
58	OMe	5-CO_2H	12 ± 1	108 ± 2	14 ± 1	86 ± 4
59	OMe	6-CO_2H	0.91 ± 0.06	102 ± 3	2.0 ± 0.1	108 ± 1
60	OMe	6-Me	2.1 ± 0.1	81 ± 2		72 ± 1
61	OMe	3-N (Py)	1.0 ± 0.1	97 ± 1	1.3 ± 0.2	80 ± 1
62	OMe	5-N (Py)	>38		>38	
63	OEt	5-OEt	0.16 ± 0.01	85 ± 1	0.064 ± 0.004	75 ± 1
64	OEt	6-OEt	0.39 ± 0.03	76 ± 4	0.16 ± 0.02	86 ± 7
65	Et	6-Me	0.79 ± 0.18	89 ± 1	0.50 ± 0.09	86 ± 3
66	Et	6-Et	0.69 ± 0.05	89 ± 2	0.24 ± 0.05	82 ± 4

[a]IC_{50} values were determined utilizing the diaphorase- and resazurin-coupled R132H and R132C mIDH1 assays (average of $N = 3$). IC_{50} values are reported only for compounds where ≥75% inhibition of enzyme activity was observed at the highest concentration tested (38 μM).

conducted in one pot, requiring only a single solvent exchange from isopropanol to acetic acid.

We initiated the optimization of the 7,8-dihydroquinoline-2,5(1*H*,6*H*)-dione ring system by first establishing the essential components necessary for activity in this region. The cyclohexanone ring proved was necessary as monocyclic analogues **2** and **3** were completely inactive. The carbonyl functionality appeared to play a lesser role as des-carbonyl analogues **4** and **5** were only slightly less potent and/or efficacious against the R132H and the R132C IDH1 mutant enzymes (Table 1). The removal of the methyl groups (**6**), contraction of the ring (**7**), and aromatization (**8**) all resulted in significant losses in activity. We also incorporated a heteroatom into this ring system with analogues **9** and **10**, and while amine **9** was less potent, lactam **10** displayed comparable activity to the hit, showing that the amide functional group was well-tolerated. In addition to focusing primarily on target potency, we closely monitored the activity of our molecules against WT IDH1. Fortunately, none of the molecules in this series showed activity against WT IDH1 (IC_{50} > 57 μM) nor did most of the compounds in the remainder of the hit-to-lead campaign described below. This brief survey of the core structure−activity relationships (SARs) highlighted the importance of the geminal methyl groups, and the six-membered ring as requirements for biochemical potency.

The next structural element investigated was the thiazole ring (Table 2). Given its proximity to the carbon center that acts as a nucleophile during the conversion of **III** to **V**, as shown above in Scheme 1, many of the modifications to the thiazole ring required developing new synthetic sequences which resulted in synthetic challenges and poor throughput; as a result, Table 2 has analogues that lack the *para*-chloro substituent present in the hit. The des-chloro compound **11** was essentially equipotent to **1** against R132H mIDH1 but lost efficacy toward the R132C mutant. Substitution at the 4-position of the thiazole in the form of a biaryl linkage seemed optimal in contrast to either substitution at the 5-position or modification to a fused ring system, with analogues **12**−**14** displaying significant losses in activity. Replacement of the thiazole with a variety of diazoles **15**−**22** had a dichotomic effect on mIDH1 potency with imidazoles **17** and **18**, pyrazoles **19** and **20**, maintaining activity, and the remainder of the analogues (**15**, **16**, and **21**−**26**) experiencing significant losses in biochemical potency. This SAR led us to believe that maintaining a nitrogen positioned akin to the thiazole nitrogen (3-position highlighted in **1**, Table 2) was important, while a nitrogen at the 2-position abrogated activity (**16**, **20**, and **21**). Further supporting this observation, expansion of the ring to a benzene (**23**−**24**; meta- and para-substitution) proved unfavorable with complete loss in mIDH1 inhibitory activity, while substitution with a 2,6-disubstituted pyridine retained some activity (**25**). In an attempt to open the thiazole ring, we synthesized amide **26**, but this proved to be a detrimental variation of the thiazole. Thus, the thiazole segment, much like the cyclohexanone moiety, was not amenable to significant modification.

Tables 3 and 4 showcase the SAR exploration of the aryl group attached to the pyridone nitrogen (R^1). Synthetically, this region was readily modifiable since it was made from the last step in Scheme 1; thus, it was extensively investigated. Given the steric congestion about the pyridinone aryl linkage, we posited that the orientation of the pyridinone orthogonal or perpendicular to the arene may be optimal, especially given the 2-methoxy substituent present in the hit molecule. Along these lines, removal of the two methoxy groups in the form of analogue **27** resulted in a loss of activity against both mutant forms of IDH1 (Table 3). Installation of a variety of ortho-

https://doi.org/10.1021/acs.jmedchem.1c00019
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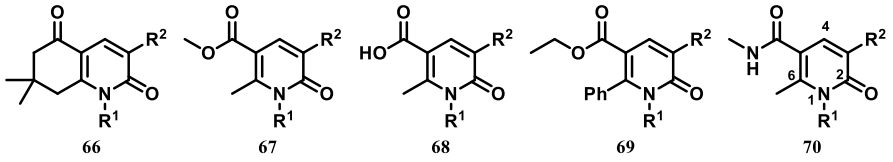
Table 5. Ring-Opened Core Modifications



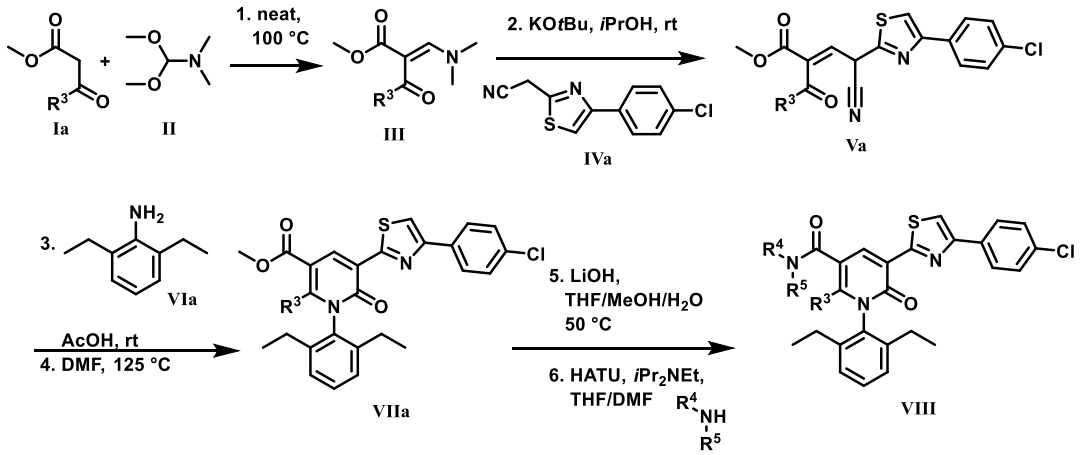
66 67 68 69 70

R^1 = 2,6-diethylphenyl
R^2 = 4-(4-chlorophenyl)thiazol-2-yl

biochemical	IDH1 R132H		IDH1 R132C	
core	IC_{50} (μM) ± SD[a]	% inhibition ± SD at 38 μM	IC_{50} (μM) ± SD[a]	% inhibition ±SD at 38 μM
1	1.6 ± 0.2	84 ± 7	1.0 ± 0.3	85 ± 4
10	2.3 ± 0.0	84 ± 1		65 ± 4
66	0.69 ± 0.05	89 ± 2	0.24 ± 0.05	82 ± 4
67	>38		>38	
68	7.2 ± 0.5	102 ± 1		72 ± 3
69	>38		>38	
70	1.5 ± 0.0	87 ± 3		64 ± 5

[a]IC_{50} values were determined utilizing the diaphorase- and resazurin-coupled R132H and R132C mIDH1 assays. IC_{50} is reported only for compounds with >75% inhibition at 38 μM, the highest concentration tested.

Scheme 2. General Amide Synthesis

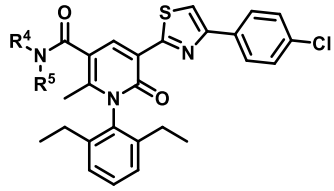


substituents regained target potency in the instance of small alkoxy substituents (**28**−**30**) and an ethyl substituent (**32**). The *ortho*-methyl-substituted compound **31** was active but did not show inhibition beyond 75% at the highest concentration. Analogues with larger R^1 groups, such as 2-*i*Pr (**33**) and 2-Ph (**34**), or with free hydroxyl (**35**) and larger alkoxy groups (**36** and **37**), or nitrogen-containing substituents (**38**−**40**) provided only partial inhibition of the mutant enzymes even at the top concentration tested (38 μM). Interestingly, the only polar substituent to maintain activity was the 2-carboxylic acid analogue **41**. With 2-halo substitutions, a smaller 2-F (**42**) lost activity against both IDH1 mutants, but a 2-Cl (**43**) was equipotent to **1** for R132H, emphasizing the need of an ortho-substitution large enough to force the pyridone ring to be orthogonal to the central bicyclic core and restrict rotation. Notably, both 2-trifluoromethyl (**44**) and 2-trifluoromethoxy (**45**) analogues exhibited improved mIDH1 activity over **1**, reiterating that varying electronic character was tolerated at the 2-position. Further modifications by extension with a carbon spacer (**48**) or conversion to alkyl groups (**49** and **50**) were not tolerated. A handful of heterocyclic replacements such as a 2-pyridyl ring (**46**) were also evaluated; most heterocycles (analogues not shown) showed diminished activities, whereas 2,3-disubstituted thiophenes, such as compounds **51** and **52**, served as viable replacements.

Probing further substitution of the pyridinone arene, we aimed to develop a more complete understanding of this region of the pharmacophore (Table 4). By keeping the 2-OMe group constant and walking the second methoxy group around the remaining positions of the ring (**53**−**55**), the importance of the relative 2,5- and 2,6-substituent arrangements became apparent. A similar scan was conducted with a polar carboxylic acid group (**56**−**59**). The 2-OMe,6-CO_2H-disubstituted compound **59** showed potent inhibition of both R132H and R132C mutant isoforms. Within this subset (**1** and **53**−**59**), we also noticed that the placement of a substituent at the 4-position led to a profound reduction of activity (**54** and **57**). A 2-OMe,6-Me compound (**60**) was also potent indicating that an alkyl group could also be tolerated at the 6-position. Despite the synthetic challenges associated with substituting the phenyl for a pyridine given the diminished nucleophilic reactivity of amino pyridines in the conversion of **V** to **VII** (Scheme 1), we synthesized analogues **61** and **62**. These analogues demonstrated that this change was only tolerated if the pyridine nitrogen was in the 3-position, next to the 2-OMe substituent (**61**). We then moved on to change the

https://doi.org/10.1021/acs.jmedchem.1c00019
J. Med. Chem. 2021, 64, 4913−4946

Table 6. Amide Substitution SAR



	biochemical		IDH1 R132H		IDH1 R132C	
compd	R^4	R^5	IC_{50} (μM) ± SD[a]	% inhibition ± SD at 38 μM	IC_{50} (μM) ± SD[a]	% inhibition ± SD at 38 μM
70	Me	H	1.5 ± 0.0	87 ± 3		64 ± 5
71	Me	Me	1.2 ± 0.1	98 ± 3	2.0 ± 0.1	86 ± 1
72	*i*Pr	H	2.8 ± 0.2	47 ± 1		54 ± 2
73	*t*Bu	H		65 ± 5		49 ± 4
74	*i*Bu	H	>38		0.81 ± 0.05	56 ± 1
75	CH_2CH_2OH	H	0.42 ± 0.00	104 ± 3	2.0 ± 0.1	96 ± 2
76	CH_2CH_2OMe	H	2.1 ± 0.0	92 ± 2	0.73 ± 0.10	102 ± 5
77	CH_2CO_2H	H	1.2 ± 0.0	106 ± 2	11 ± 1	87 ± 4
78	$CH_2CH_2NH_2$	H	0.38 ± 0.00	97 ± 1	2.0 ± 0.1	89 ± 1
79	CH_2CH_2NHMe	H	0.87 ± 0.06	101 ± 3	0.32 ± 0.02	96 ± 2
80	CH_2CONH_2	H	1.6 ± 0.1	105 ± 3	3.8 ± 0.0	77 ± 14
81	$CH_2CH_2CH_2NHMe$	H	1.0 ± 0.1	104 ± 3	0.91 ± 0.06	95 ± 0
82	Ph	H		40 ± 21		69 ± 11
83	CH_2Ph	H	>38			41 ± 1
84	cyclohexyl	H	>38			31 ± 7
85	*N*-piperidine			51 ± 0		65 ± 3
86	*N*-morpholine		4.2 ± 0.0	82 ± 4	4.1 ± 0.3	71 ± 2
87	*N*-piperazine		0.21 ± 0.00	103 ± 3	1.5 ± 0.0	93 ± 2
88	*N*-4-Me-piperazine		2.9 ± 0.2	88 ± 1	1.2 ± 0.1	86 ± 3

[a]IC_{50} values were determined utilizing the diaphorase- and resazurin-coupled mIDH1 R132H and R132C assays. IC_{50} is reported for compounds with >75% inhibition at 38 μM, the highest concentration tested.

2-methoxy substituent to a 2-ethoxy substituent and evaluated the 2,5- and 2,6-diOEt compounds **63** and **64**. Both compounds showed sub-micromolar IC_{50}s. This remarkable breakthrough in IC_{50} < 1.0 μM was also observed with a 2-ethyl group when it was combined with a 6-methyl or ethyl in **65** and **66**, respectively.

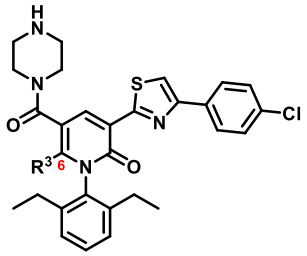
Our earlier SAR study of the bicyclic dihydroquinolinedione core in hit molecule **1** had revealed that most changes reduced potency, but lactam **10** maintained a similar activity against the R132H IDH1 mutant (Table 1). This observation provided an attractive opportunity to both substitute the amide and open the ring to reveal new opportunities for SAR exploration in the northwestern region of the chemotype. To that end, we evaluated the ring-opened variants of the lactam, as shown in Table 5; this was done with the symmetrical 2,6-diethylphenyl substituent at the R^1 position from lead **66**. This was a strategic decision as we realized that the possibility of restricted rotation around N-R^1 could lead to atropisomers (vide infra), adding one more variable to our SAR analysis. Compared to ketone **66**, ester (**67** and **69**) and acid (**68**) variants lost much, if not all mIDH1 target potency. However, amide **70** only experienced a small drop in R132H activity and did not show >75% inhibition at 38 μM against R132C (Table 5). This analogue proved pivotal in providing an additional SAR handle for a new amide-substituted pyridone series.

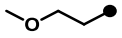
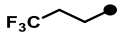
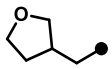
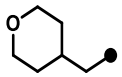
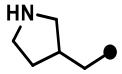
With only moderate variations to our initial multicomponent coupling, we were able to develop a modular synthesis tolerant of significant modification to both the amide and the pyridinone substituents (Scheme 2). The initial condensation between keto ester **Ia** and *N*,*N*-dimethylformamide (DMF) dimethyl acetal **II** was carried out by heating the two components without any solvent to provide intermediate **III** which was then condensed with functionalized nitrile **IVa** under basic conditions. After the addition of aniline **VIa** to ketonitrile **Va** and stirring with acetic acid, we discovered that further heating was required to efficiently drive pyridinone formation in **VIIa**. Finally, ester hydrolysis and HATU-mediated amide couplings were utilized to efficiently access a diversity of ring-opened amide analogues (**VIII**).

Based on this synthetic route, we surveyed a structurally diverse set of amides, as depicted in Table 6. The tertiary dimethylamide **71** displayed a similar activity to that of compound **70**, but larger alkyl substituents at the R^4 position provided analogues (**72**−**74**) with decreased mIDH1 activity. Incorporation of either a nitrogen or oxygen atom at or near the termini of an alkyl chain at the R^4 position (**75**−**81**) either maintained or even provided a boost in potency. Most noteworthy among these were ethanaloamide **75** and 2-aminoethyl carboxamide **78**, which exhibited threefold improvements in potency compared to analogue **70**. Secondary amides such as **82**, **83**, and **84** with large ring substituents such as phenyl, benzyl, and cyclohexyl, respectively, lost activity. We also evaluated cyclic tertiary amides (**85**−**88**) and found that piperidine analogue **85**'s activity was attenuated with ∼50−65% inhibition at the highest concentration tested. The placement of distal heteroatoms (N and O) in morpholine **86** and *N*-Me-piperazine **88** maintained good potency, while *NH*-piperazine **87** was sevenfold more potent in mIDH1 R132H inhibition compared to analogue **70**. Importantly, piperazine **87** also was more stable in a single point rat liver

https://doi.org/10.1021/acs.jmedchem.1c00019
J. Med. Chem. 2021, 64, 4913−4946

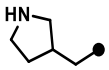
Table 7. Pyridinone 6-Position SAR



Biochemical		IDH1 R132H		IDH1 R132C	
Compd	R^3	$IC_{50}(\mu M) \pm SD^a$	% inhibition ± SD at 38 μM	$IC_{50}(\mu M) \pm SD^a$	% inhibition ± SD at 38 μM
87	Me	0.21 ± 0.00	103 ± 3	1.5 ± 0.0	93 ± 2
89	*n*Pr	0.075 ± 0.000	97 ± 2	0.10 ± 0.01	82 ± 13
90	*i*Pr	0.42 ± 0.00	102 ± 4	0.39 ± 0.05	92 ± 3
91	*i*Bu	0.11 ± 0.01	98 ± 2	0.12 ± 0.03	83 ± 14
92		0.044 ± 0.003	101 ± 1	0.058 ± 0.010	97 ± 11
93		0.055 ± 0.004	107 ± 2	0.049 ± 0.003	96 ± 3
94		0.14 ± 0.01	102 ± 6	0.34 ± 0.04	92 ± 8
95		0.24 ± 0.00	101 ± 3	0.60 ± 0.07	82 ± 12
96		0.075 ± 0.000	105 ± 3	0.13 ± 0.01	91 ± 1
97		1.1 ± 0.1	93 ± 2	0.98 ± 0.07	79 ± 3
98		10 ± 1	81 ± 2		52 ± 3

[a]IC_{50} values were determined utilizing the diaphorase- and resazurin-coupled mIDH1 R132H and R132C assays. IC_{50} is reported for compounds with >75% inhibition at 38 μM, the highest concentration tested.

Table 8. In Vitro Drug-like Properties of Analogues with >10-fold Increase in R132C mIDH1 Potency over Compound 87

		IDH1 R132H	IDH1 R132C	Single Point RLM ($t_{1/2}$, min)	PAMPA (10^{-6} cm/s)	Solubility (μg/mL)
Compd	R^3	IC_{50} Fold improvement over 87	IC_{50} Fold improvement over 87			
89	*n*Pr	2.8	15	27	134	<1
91	*i*Bu	1.9	13	17	16	<1
92		4.8	26	14	225	<1
93		3.8	31	17	43	<1
98		2.8	12	7	24	<1

microsomal stability assay (**70**: $t_{1/2}$ = 1.5 min; **87**: $t_{1/2}$ > 30 min).[36] Additionally, **87** had better aqueous kinetic solubility than methyl amide **70** (**70**: [M] < 1 μM; **87**: [M] = 6.7 μM). These results led us to maintain this substitution while we examined the SAR of the pyridinone 6-substituent.

Our evaluation of the pyridinone 6-substituent R^3 is presented in Table 7. The *n*-propyl analogue **89** was ∼3-fold more potent toward R132H mIDH1 but with a marked 15-fold improvement toward mR132C. While this trend did not carry to the α-branched isopropyl **90**, it emerged again with analogues **91**−**93** with β-branching. Indeed, isobutylene **92** and cyclopropylmethyl **93** had benchmark potencies with IC_{50} < 100 nM for both IDH1 mutants. The replacement of the methyl group on the *n*-propyl side chain in **89** with an electron-donating methoxy (**94**) or electron-withdrawing trifluoromethyl group (**95**) led to a comparative reduction in potency. Within cyclic ethers, the racemic tetrahydrofuran (THF) **96** was more potent than the larger tetrahydropyran **97**. Analogous pyrrolidine **98** (racemic) proved to be much less potent with IC_{50} ∼ 10 μM. We postulated that perhaps, steric congestion to this region of the pharmacophore helps position the piperazine away from R^3 (as suggestively drawn in

https://doi.org/10.1021/acs.jmedchem.1c00019
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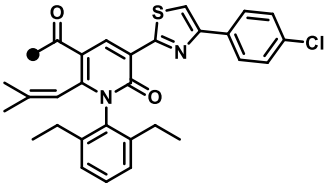
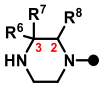
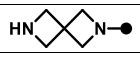
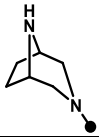
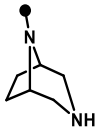
Table 9. Piperazine SAR



Biochemical					IDH1 R132H	IDH1 R132C
Compd	Carbonyl Substitution	R^6	R^7	R^8	$IC_{50}(\mu M) \pm SD^a$	$IC_{50}(\mu M) \pm SD^b$
92		H	H	H	0.044 ± 0.003	0.058 ± 0.010
99[c]		Me	H	H	0.044 ± 0.003	0.046 ± 0.003
100[c]		H	H	Me	0.039 ± 0.003	0.053 ± 0.006
101[c]		Et	H		0.10 ± 0.01	0.072 ± 0.005
102[c]		*n*Pr			0.13 ± 0.01	0.082 ± 0.014
103		CH_2-CH_2			0.078 ± 0.005	0.048 ± 0.006
104[c]		CH_2-OH	H		0.049 ± 0.003	0.060 ± 0.013
105		O (ketone)		H	0.035 ± 0.002	0.039 ± 0.003
106[c]		CF_3			0.36 ± 0.02	0.13 ± 0.00
107[c]		CN	H		0.16 ± 0.01	0.056 ± 0.010
108[c]		CO_2H			0.055 ± 0.004	0.064 ± 0.004
109[c]		$CONH_2$			0.094 ± 0.000	0.042 ± 0.000
110					0.24 ± 0.00	0.064 ± 0.021
111					0.094 ± 0.000	0.060 ± 0.007
112					0.062 ± 0.004	0.049 ± 0.003

[a]IC_{50} values were determined utilizing the diaphorase- and resazurin-coupled mIDH1 R132H and R132C assays. [b]Compounds showed >97% inhibition at 38 μM, the highest concentration tested. [c]Compounds showed >85% inhibition at 38 μM, the highest concentration tested. [d]Compounds are racemic.

Table 7); we suspect that the amide is also not coplanar to the pyridine pi-system. It could be that such a conformation is more desirable toward R132C activity and results in the >10-fold increase in potency observed in analogues **89**, **91**−**93**, and **96** compared to the methyl analogue **87**. We assessed in vitro drug-like properties in this set (Table 8); isobutylene **92** had a desirable balance between potency, rat liver microsome stability, and permeability. Thus, it was held at the R^3 position for further SAR studies. We also noticed that all compounds had low aqueous kinetic solubility.

Upon optimizing the R^3 substituent, the NR^4R^5 site (see **VIII** in Scheme 2) was further explored (Table 9). A subset of analogues with diverse groups at the piperazine 3-position such as methyl **99**, ethyl **101**, propyl **102**, cyclopropyl **103**, hydroxymethyl **104**, ketone **105**, trifluoromethyl **106**, cyano **107**, acid **108**, and amide **109** maintained their potency against both IDH mutants, showcasing that electron-donating and -withdrawing groups were tolerated at this position. The 2-methyl piperazine **100** was also potent. Furthermore, heterobicyclic systems also produced analogues **110**−**112** with nanomolar activity. Although these substitutions were tolerated for enzymatic inhibition, these additions typically did not provide additional benefits with respect to their in vitro drug-like properties [parallel artificial membrane permeability assay (PAMPA), rat and mouse microsome stability, and solubility] nor in vivo PK profiles (data not shown). Another noteworthy observation was that the analogues described in Tables 7−10 were at least 1000-fold selective for mIDH1s over WT IDH1.

Finally, our SAR studies shifted back to the pyridinone 1-arene (Table 10). Analogous to our earlier studies, an ortho substituent proved essential. The complete removal of all substituents in **113** led to a 7.5-fold drop in R132H potency and a more significant 55-fold drop in R132C potency. The presence of one *ortho* ethyl in **114** was 1−3-fold less potent compared to its diethyl counterpart **92**. While the combination of ethyl,methyl-di-*ortho* substitutions in **115** led to a potency drop, especially toward the R132C mutant, the ethyl-chloro combination in **116** was equipotent to **92**. Keeping the *ortho*-ethyl group constant, we shifted to the meta position (R^{10}) and found that methyl **117**, methoxy **118**, and chloro **119** maintained their potency across both mutants. While it was apparent that steric congestion around the pyridone seemed critical for activity, we anticipated that hindered rotation around the pyridone *N*-aryl bond likely led to the existence of atropisomers that could have dissimilar activities. As two representative examples, we separated the atropisomers in **118** and **119** and discovered that they had a ∼3- and 3−8-fold

https://doi.org/10.1021/acs.jmedchem.1c00019
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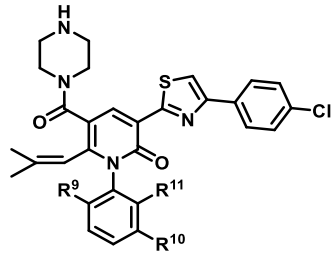
Table 10. SAR at the Pyridinone N1-Aryl Ring



	biochemical			IDH1 R132H	IDH1 R132C	
compd	R^9	R^{10}	R^{11}	IC_{50} (μM) ± SD[a]	IC_{50} (μM)± SD[a]	
92	Et	H	Et	0.044 ± 0.003	0.058 ± 0.010	
113	H	H	H	0.33 ± 0.00	3.2 ± 0.6	
114	Et	H	H	0.056 ± 0.004	0.16 ± 0.01	
115	Et	H	Me	0.27 ± 0.00	2.2 ± 0.2	
116	Et	H	Cl	0.044 ± 0.003	0.055 ± 0.004	
117	Et	Me	H	0.044 ± 0.003	0.11 ± 0.02	
118	Et	OMe	H	0.114 ± 0.007	0.075 ± 0.000	
(+)-**118**				0.12 ± 0.02	0.13 ± 0.03	
(−)-**118**				0.42 ± 0.05	0.67 ± 0.04	
119	Et	Cl	H	0.114 ± 0.007	0.10 ± 0.01	
(+)-**119**				0.081 ± 0.005	0.072 ± 0.005	**NCATS-SM5637, NSC 791985**
(−)-**119**				0.25 ± 0.04	0.54 ± 0.10	
AG-120				0.065 ± 0.009	0.063 ± 0.015	

[a]IC_{50} values were determined utilizing the diaphorase- and resazurin-coupled R132H and R132C mIDH1 assays. All compounds showed >80% inhibition at 38 μM, the highest concentration tested.

Table 11. 2-HG Levels (% of Vehicle Control) in U87-mIDH1 (R132H) Cells after 48 h Incubation with the Compound Compared to DMSO and Drug-like Properties

	% 2-HG[a]		IDH1 R132H[b]	single point	PAMPA	solubility
compd	100 nM	500 nM	IC_{50} (μM) ± SD	RLM ($t_{1/2}$, min)	(10^{-6} cm/s)	(μg/mL)
91	33 ± 25	12 ± 6.4	0.11 ± 0.01	17	16	<1
92	13 ± 3.1	16 ± 1.1	0.044 ± 0.003	14	225	<1
99	11 ± 5.5	8 ± 2.3	0.044 ± 0.003	25	213	<1
100	14 ± 5.1	11 ± 5.3	0.039 ± 0.003	17	40	<1
103	17 ± 3.8	14 ± 0.81	0.078 ± 0.005	12	48	<1
105	23 ± 4.2	13 ± 3.1	0.035 ± 0.002	2	275	<1
108	29 ± 16	10 ± 8.6	0.055 ± 0.004	23	<1	16
111	11 ± 3.1	10 ± 3.4	0.094 ± 0.000	>30	89	<1
112	32 ± 8.4	18 ± 2.8	0.062 ± 0.004	9	99	<1
118	40 ± 1.7	19 ± 3.7	0.114 ± 0.007	>30	549	<1
(−)-**119**	ND	ND	0.25 ± 0.04	>30	255	<1
(+)-**119**	5.9 ± 0.16	4.1 ± 0.17	0.081 ± 0.005	>30	434	<1

[a]Values represent % 2-HG after 48 h incubation with an inhibitor, where 100% is normalized to 2-HG levels in the DMSO-treated control. [b]The enzymatic R132H IC_{50} has been reintroduced in the table to visualize the entire data set for all compounds in this table. ND: Not Determined.

difference in the R132H and R132C biochemical potencies, respectively.

Having developed several biochemically potent mIDH1 inhibitors, for a representative set of compounds, we next assessed cellular activity in U87 cells, a cell line of glioblastoma origin engineered to express the IDH1 R132H mutation. Inhibition of 2-HG production, secreted from cells into media, was measured by mass spectrometry 48 h after the addition of compounds at 100 and 500 nM (Table 11). Analogue (+)-**119** lowered 2-HG levels by more than 94% at 100 nM. Dose−response curves for atropisomer (+)-**119**, the corresponding unseparated mixture of atropisomers **119**, and the approved drug AG-120 are shown in Figure 2. (+)-**119** was more potent at reducing 2-HG levels than **119**, even though their biochemical IC_{50}s toward R132H mIDH1 were similar (114 and 81 nM). Notably, in this cellular assay, (+)-**119** appears 100 times more potent (<0.7 nM) than in the biochemical enzyme inhibition assay and more potent than AG-120 which had a comparable biochemical potency.

For the compounds in Table 11, an analysis of drug-like properties using our in-house high throughput assays showed that analogues **111**, **118**, (−)-**119**, and (+)-**119** had promising stability in rat liver microsomes with $t_{1/2}$ > 30 min, high passive permeability, and low aqueous kinetic solubility. From this subset, based on its potent cellular activity, we chose (+)-**119** to showcase the chemical series in PK/PD studies.

To directly assess target engagement in cells, we used a high-throughput CETSA platform that uses a split NanoLuciferase

https://doi.org/10.1021/acs.jmedchem.1c00019
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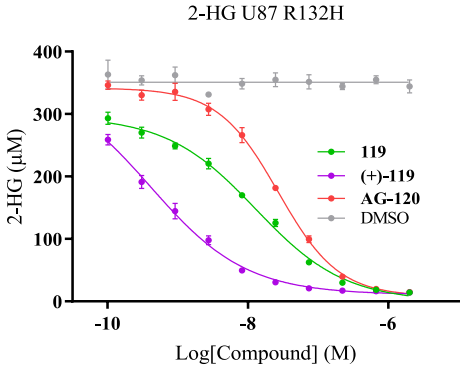
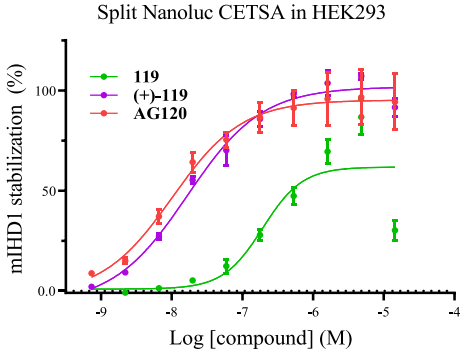


	IC_{50} (nM)
119	13.04 ± 3.56
(+)-119	0.43 ± 0.40
AG-120	27.23 ± 4.16

Figure 2. Dose-dependent reduction of 2-HG in U87-mIDH1 (R132H) cells (N = 3).

(SplitNanoLuc) reporter to detect soluble protein in cells.[37] After tagging the 15-amino acid sequence 86b from NanoLuc to the C-terminus of R132H mIDH1 (termed IDH1(R132H)-86b) and transient expression in HEK293T cells, soluble protein was measured by complementation of the larger 11S fragment to 86b. This reconstitutes NanoLuciferase activity and allows its quantification using the substrate furimazine. Dose−response testing measured the ability of compounds to increase the thermal stability of IDH1(R132H)-86b when cells were heated to 56 °C for 3.5 min. (+)-**119** and AG-120 were equipotent in this assay, whereas the corresponding mixture **119** had a 10-fold greater EC_{50} (Figure 3).

We devised a scalable route to (+)-**119** and related analogues to enable in vivo testing (Scheme 3). Commencing with a similar multicomponent coupling with dicarbonyl 6,6-dimethyldihydro-2*H*-pyran-2,4(3*H*)-dione **Ic**, a multicomponent coupling reaction efficiently assembled **VIIb** which upon



	EC_{50} (nM)
119	250.22 ± 223.70
(+)-119	18.02 ± 9.67
AG-120	12.47 ± 10.19

Figure 3. Dose-dependent stabilization of NanoLuc-tagged R132H mIDH1 in HEK293 cells (N = 3).

lactone hydrolysis and in situ elimination of the tertiary alcohol provided the sensitive intermediate **VIIc**. We observed proximity-promoted deleterious cyclization back to lactone **VIIb** under a variety of neutralization conditions as well during the subsequent amide coupling. Careful neutralization with acetic acid to neutral pH and subsequent removal via wash steps was necessary following lactone hydrolysis. The amide bond formation also had to adhere to a strict order of addition of HATU, iPr$_2$NEt, and then the Boc-protected piperazine to furnish **VIIIa** in 95% yield. Subsequently, separation of atropisomers proved optimal at the stage of the protected piperazine **VIIIa** with the use of a CHIRAL OD-H column. Final deprotection, which necessitated short reaction times with a significant excess (35 equiv) of trifluoroacetic acid in order to minimize relactonization, followed by neutralization of the TFA salt by aqueous sodium bicarbonate provided the pure atropisomer (+)-**119**. The stereochemical configuration of the atropisomer was proved by X-ray analysis of a single crystal of the intermediate **VIIb** (Supporting Information) that was formed during attempts to form a HCl salt of (+)-**119** (**NCATS-SM5637**, **NSC 791985**).

A single dose of 30 mg/kg (+)-**119** administered orally to CD1 mice led to good exposure in the plasma with a C_{max} ~3.34 μM and AUC_{last} of 24,371 h·ng/g. It also crossed the blood brain barrier with an AUC of 6996 h·ng/g and a brain/plasma ratio of 28% (Figure 4).

We compared the activities of (+)-**119** and AG-120 in vivo, using the U87 mIDH1 (R132H) xenograft to assess PK/PD responses (Figure 5). After a single 30 mg/kg p.o. dose, AG-120 reached a higher plasma C_{max} than (+)-**119** but after 8 h, (+)-**119** displayed higher plasma concentrations. Similarly, 4 h after dosing, concentrations of (+)-**119** in tumor were higher than AG-120. (+)-**119** had very slow clearance from tumor with sustained average concentrations of 9.5, 7.2, and 6.8 μM at 24, 36, and 48 h, respectively, compared to 1.6, 1.4, and 0.77 μM for AG-120. These higher sustained tumoral concentrations of (+)-**119** appear to be responsible for the greater reduction in tumor 2-HG, as compared to AG-120, from the 16 h time point onward. If this promising activity translates to the clinical setting, it could provide a therapeutic advantage over AG-120 (see the Supporting Information for profiling data on (+)-**119**).

CONCLUSIONS

An extended medicinal chemistry campaign starting with a 7,8-dihydro-2*H*-1λ^2-quinoline-2,5(6*H*)-dione chemotype led to the discovery of new amide-substituted pyridones as potent R132H and R132C mIDH1 inhibitors. The lead compound **119** had nanomolar activities against both IDH1 mutants and lowered the mIDH1-catalyzed product 2-HG in cells. The single atropisomer (+)-**119** (**NCATS-SM5637**, **NSC 791985**) was shown to engage mIDH1 in cells and reduce 2-HG production more potently that **119** (13-fold in HEK293 engagement assay and 30-fold in U87 2-HG assay). In a PK/PD model, after a single oral dose at 30 mg/kg, (+)-**119** showed sustained tumor concentrations (7 μM after 48 h) and greater reduction in 2-HG levels at later time points (16−48 h) than the approved mIDH1 drug AG-120. Further head-to-head comparative animal studies in various mIDH1-driven cancer models with key compounds from this chemical series, such as **NCATS-SM5637** (**NSC 791985**), and AG-120 are ongoing and will be reported in due course.

https://doi.org/10.1021/acs.jmedchem.1c00019
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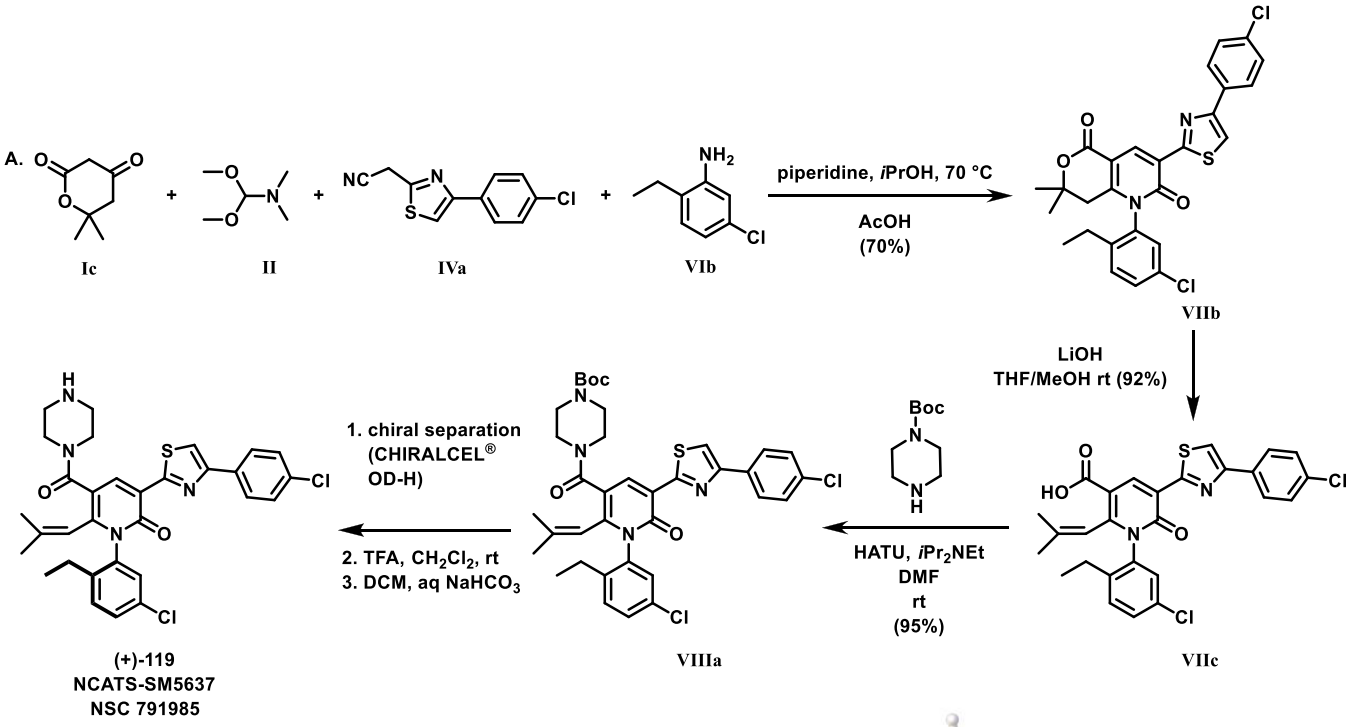
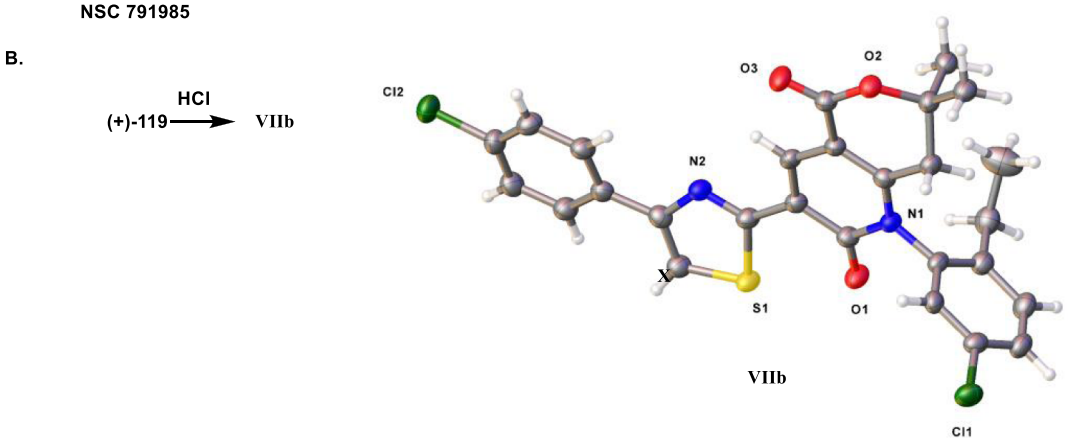
Scheme 3. (A) Synthesis of Isobutylene Analogue (+)-119 Including Separation of Atropisomers; (B) Formation of VIIb from (+)-119 and Its X-ray Coordinates of Its Single Crystal





PK parameters	Brain	Plasma
T_{max} (h)	6	4
C_{max} (ng/g or ng/mL)	594.67	2013.33
C_{max} (μmol/kg or μM)	1.00	3.39
AUC_{last} (h*ng/g or mL)	6996 ± 797	24371 ± 779
AUC_{last} ratio (P/B)	3.52 ± 0.43	
C_{max} ratio (P/B)	3.68 ± 0.13	

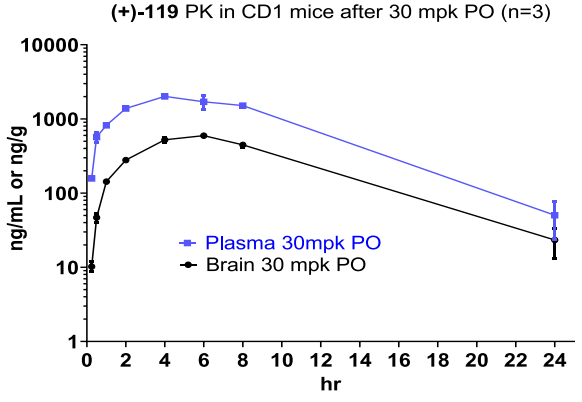


Figure 4. PK parameters and conc vs time profiles in brain and plasma of CD1 mice (n = 3) after a single 30 mpk oral dose of (+)-**119** formulated as a solution in 20% PEG300, 40% of Solutol solution (30% w/w in water), and 40% DI water.

https://doi.org/10.1021/acs.jmedchem.1c00019
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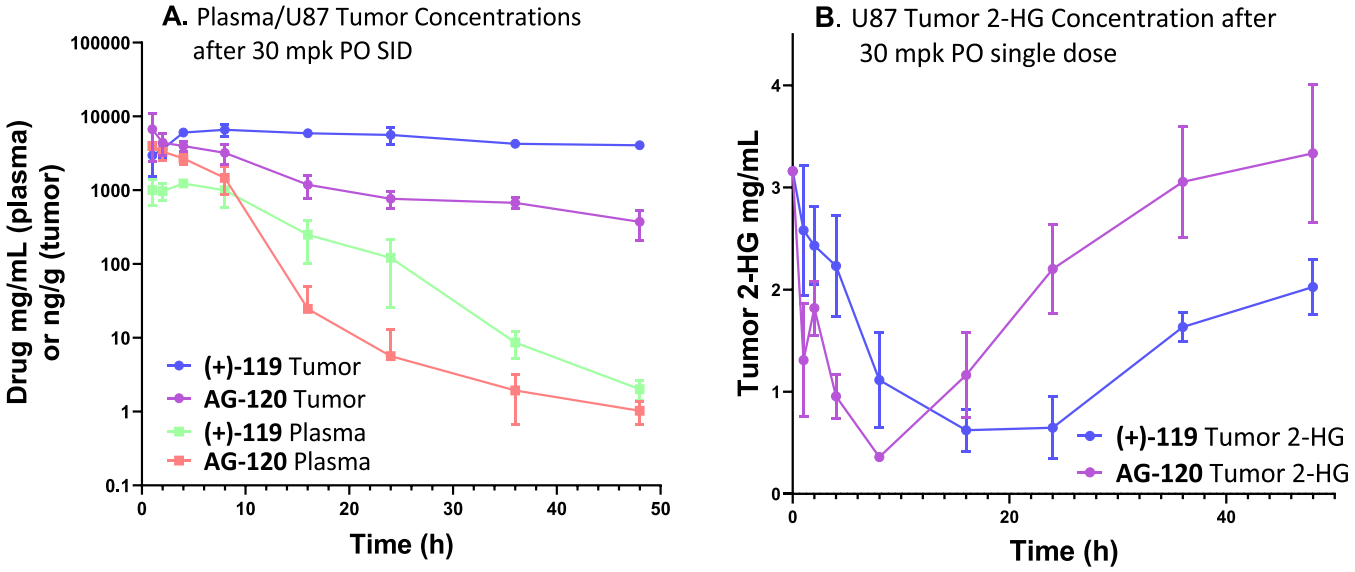

Figure 5. PK/PD in a U87 R132H mIDH-based xenograft mouse model: (A) Concentrations of (+)-**119** or AG-120 in plasma and tumor after administration of a single 30 mpk PO dose. (B) Concentrations of 2-HG in tumor.

EXPERIMENTAL SECTION

Materials and Methods: Biology. *mIDH1-R132H and mIDH-R132C Enzyme Assay.* Pubchem AID 624002. Enzyme buffer was dispensed into black, solid 1536-well plates at 3 μL/well in 20 mM Tris buffer, pH 7.5, containing final concentrations of 10 mM $MgCl_2$, 20 mM NaCl, 0.001% Tween 20, 0.05% BSA, 2 mM β-ME, and 5.2 nM IDH1 R132H. Then, 23 nL of compounds or DMSO was delivered to each well using a pin tool. The inherent compound fluorescence was measured at this point at 590 nm on a ViewLux plate reader (Ex 525, Em 598, bodipy filter); this is test for assay interference as we measure resorufin (em 590 nm in the end). The substrate buffer (3 μL; 20 mM Tris buffer, pH 7.5, containing final concentrations of 10 mM $MgCl_2$, 20 mM NaCl, 0.001% Tween 20, 0.05% BSA, 0.008 mM NADPH, and 1 mM α-ketoglutarate) was added to start the enzymatic reaction, and the plate was briefly spun at 270*g*. After the reaction was allowed to progress for 80 min at room temperature, the remaining NADPH was detected with the diaphorase/resazurin-coupled system. A total of 3 μL of detection buffer (20 mM Tris buffer, pH 7.5, containing final concentrations of 10 mM $MgCl_2$, 20 mM NaCl, 0.001% Tween 20, 0.05% BSA, 0.53 μM diaphorase and 0.012 mM resazurin) was added, and after 5 min, the fluorescence at 590 nm was measured on an Envision plate reader (Ex 544, Em 590, bodipy filter, 10 flashes). The % activity was determined from the corrected fluorescence values. The activity was normalized with 5.2 nM R132H IDH1 (no inhibitor) as 0% inhibition and the activity with no enzyme as 100% inhibition.

We also measured for assay interference by measuring dose-dependent activity against the diaphorase/resazurin-coupled system by repeating the assay mentioned above in the absence of mIDH1. This will flag false positives that may inhibit the NADPH to $NADP^+$ conversion.

Concentration−response curves were fitted to the signals arising from the resulting fluorescence. The concentration−response curves were then classified based on curve quality (r2), response magnitude, and degree of measured activity, and compounds were subsequently categorized based on their curve class. Active inhibitors showed concentration-dependent increases in fluorescence, concordant with a decrease in IDH1 R132H activity and less substrate NADPH utilization. Inactive compounds showed no effect on the fluorescence signal relative to the DMSO control.

Cellular 2-HG Production Assay. 2-HG secreted from cells was quantified as previously described with modifications. U87-R132H cells were cultured in Dulbecco's modified Eagle medium (DMEM) high glucose with glutamine supplemented with 10% FBS, 100 units/mL penicillin, and 100 μg/mL streptomycin. 8000 cells/well/100 μL were seeded into clear, tissue culture-treated 96-well plates (Corning) in 100 μL culture medium excluding wells along the edge (which instead were filled with culture medium only) and incubated overnight at 37 °C, 5% CO_2, 90% RH. Culture medium was aspirated and replaced with 100 μL of culture medium containing either a vehicle (DMSO) or mIDH1 inhibitor. Assays were established with an inhibitor tested at two concentrations 500 and 100 nM or as seven-point dose response. After 48 h, 75 μL of the culture medium was collected and snap-frozen on dry ice. Samples were analyzed by LC−MS for 2-HG concentrations using Rapidfire/Mass Spectrometry (Quintara). Remaining cells were assessed for viability by adding 100 μL of fresh media and 50 μL of CellTiter-Glo reagent (Promega). Luminescence was read using a ViewLux High-throughput CCD imager (PerkinElmer).

SplitLuc CETSA. The mIDH1(R132H) open reading frame was cloned into a pcDNA3.1(+) backbone using the NheI and EcoRI restriction sites. A 15 amino acid tag (86b, Gly-Ser-HiBiT-Gly-Ser) was fused in-frame to the carboxy terminus. HEK293T cells (obtained from ATCC; CRL-1573) were cultured in DMEM (4.5 g/L glucose) with 10% fetal bovine serum, 6 mM L-glutamine, 1 mM sodium pyruvate, 50 U/mL penicillin, and 50 μg/mL streptomycin. Cells were grown at 37 °C in a humidified incubator maintained at 5% CO_2. Cells were transfected in T75 flasks using a reverse transfection procedure, where 9 mL of complexes (45 μL Lipofectamine 2000 and 22.5 μg DNA) was combined with 10 mL of HEK293T cell suspension (1×10^6 cells/mL, 10 million cells total). After 24 h, cells were harvested by trypsinization, resuspended at 1×10^6 cells/mL (DPBS with $CaCl_2$ and $MgCl_2$ plus 1 g/L glucose), and dispensed (15 μL cells/well) into 384-well PCR plates (Roche) using a Multidrop Combi (Thermo Fisher). Compounds (63 nL) or DMSO vehicle control (63 nL) were subsequently pinned using a pin tool (GNF) and incubated for 1 h at 37 °C. Plates were sealed and heated at 56 °C for 3.5 min and cooled to 25 °C using an AB qPCR machine (Roche) with a ramp speed of 1.5 °C/s for the heating phase and max ramp rate for the cooling phase. Three microliters of 6% NP40 was added per well and incubated for 30 min to allow cell lysis followed by the addition of 11S (purified from *Escherichia coli*) and furimazine (Promega) substrate (at final concentrations of 100 nM and 0.5×, respectively). Samples were analyzed for luminescence intensity using a ViewLux reader.

Kinetic Solubility Assay. Pion's patented μSOL assay was used for kinetic solubility determination. In this assay, the classical saturation shake-flask solubility method was adapted as previously described.[38]

https://doi.org/10.1021/acs.jmedchem.1c00019
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Test compounds were prepared in 10 mM DMSO stock and diluted to a final drug concentration of 150 μM in the aqueous solution (pH 7.4, 100 mM phosphate buffer). Samples were incubated at room temperature for 6 h and vacuum-filtered using Tecan Te-Vac to remove any precipitates. The concentration of the compound in the filtrate was measured via UV absorbance (λ: 250−498 nm). The unknown drug concentration was determined by comparing the fully solubilized reference plate which contained 17 μM of the compound dissolved in spectroscopically pure *n*-propanol. All compounds were tested in duplicates. The kinetic solubility (μg/mL) of compounds was calculated using the μSOL Evolution software. The three controls used were albendazole (low solubility), phenazolpyridine (moderate solubility), and furosemide (high solubility).[39]

Rat Liver Microsome Stability Assay. Single time point microsomal stability was determined in a 96-well HTS format. Sample preparation was automated using a Tecan EVO 200 robot. A high-resolution LC/MS (Thermo Q Exactive) instrument was used to measure the percentage of compound remaining after incubation using a previously described method.[40] Six standard controls were tested in each run: buspirone and propranolol (for short half-life), loperamide and diclofenac (for short to medium half-life), and carbamazepine and antipyrine (for long half-life). 10 mM DMSO stock solutions of the drugs were first diluted to 10 μM in 1:2 MeCN/DI H_2O and then further diluted to 1 μM in assay buffer. Briefly, the incubate consisted of 0.5 mg/mL microsomal protein, 1.0 μM drug concentration, and NADPH regeneration system (containing 0.650 mM $NADP^+$, 1.65 mM glucose 6-phosphate, 1.65 mM $MgCl_2$, and 0.2 unit/mL G6PDH) in 100 mM phosphate buffer at pH 7.4. The incubation was carried out at 37 °C for 15 min.[36] The reaction was quenched by adding 555 μL of acetonitrile (~1:2 ratio) containing 0.28 μM albendazole (internal standard). Sample acquisition and data analysis were done using a previously described method.[40]

Parallel Artificial Membrane Permeability Assay. The stirring double-sink PAMPA method (patented by pION Inc.) was employed to determine the permeability of compounds via PAMPA as published before.[41] The PAMPA lipid membrane consisted of an artificial membrane of a proprietary lipid mixture and dodecane (pION Inc.), optimized to predict gastrointestinal tract passive permeability. The lipid was immobilized on a plastic matrix of a 96-well "donor" filter plate placed below a 96-well "acceptor" plate. pH 7.4 solution was used in both donor and acceptor wells. The test articles, stocked in 10 mM DMSO solutions, were diluted to 0.05 mM in aqueous buffer (pH 7.4), and the concentration of DMSO was 0.5% in the final solution. During the 30 min permeation period at room temperature, the test samples in the donor compartment were stirred using the Gutbox Technology (pION Inc.) to reduce the aqueous boundary layer. The test article concentrations in the donor and acceptor compartments were measured using a UV plate reader (Nano Quant, Infinite 200 PRO, Tecan Inc., Männedorf, Switzerland). Permeability calculations were performed using pION Inc. software and were expressed in units of 10^{-6} cm/s. Compounds with a low or weak UV signal were analyzed using high-resolution LC/MS (Thermo QExactive). The three controls used were ranitidine (low permeability), dexamethasone (moderate permeability), and verapamil (high permeability).

Mouse PK Studies. Studies were conducted by Pharmaron. Fed male CD1 mice (sourced from Si Bei Fu LaboratoryAnimal Technology Co. Ltd.), approximately 6−8 weeks of age and weight of approximately 25−30 g, were dosed with (+)-**119** at 30 mpk dose PO. The formulation was a solution at 3 mg/mL in 20% PEG300, 40% of Solutol solution (30% w/w in water), and 40% DI water. This was prepared prior to dosing a cohort of N = 24 mice. Plasma and whole brain were collected from N = 3 mice at 15 min, 30 min, 1 h, 2 h, 4 h, 6 h, 84, and 24 h postdose. Approximately, 0.200 mL of blood was collected via the heart puncture at each time point. Blood samples were then transferred into plastic microcentrifuge tubes containing heparin−Na as an anticoagulant. Samples were then centrifuged at 4000*g* for 5 min at 4 °C to obtain plasma. Plasma samples were then stored in polypropylene tubes, quickly frozen, and kept at −75 °C until analyzed by LC/MS/MS. The following PK parameters were calculated for plasma and brain: T_{max}, C_{max}, and AUC_{24h}. Animals were also monitored during the in-life phase by once daily cageside observations; no adverse clinical signs were noted as part of the PK report.

PK/PD. A PK/PD study was performed in 6-week old female athymic nude mice (nu/nu NCr). Mice were housed in microisolator cages with food and water provided ad libitum. All work was performed on an approved Institutional Animal Care and Use Committee protocol in AAALACi accredited facilities with automated 12 on−12 off light cycles. Mice were implanted subcutaneously with the U87MG human glioblastoma cell line (1 × 107 cells/mouse), containing the IDH1 R132H mutation. On the day of tumor staging (~200 mg), mice were randomized into 3 groups of 32 mice each. These groups were treated with a single oral dose of either vehicle (10% NMP, 40% PEG300, 25% [30% Solutol in water], 25% deionized water), compound (+)-**119** at 30 mg/kg, or AG120 at 30 mg/kg. Mice were anesthetized with isoflurane by inhalation and the tumors and K_2EDTA anticoagulated blood were collected from cohorts of 4 mice per group at 1, 2, 4, 8, 16, 24, 36, and 48 h post dose. Plasma was obtained by centrifugation and all samples were flash-frozen until analysis. (+)-**119** and AG120 in plasma and tumor tissue and 2-HG in tumor were quantified using validated LC−MS/MS methods.

Use of Animal Subjects. All animal studies included as part of this manuscript were performed in accordance with institutional guidelines as defined by Institutional Animal Care and Use Committee (IACUC).

EXPERIMENTAL SECTION CHEMISTRY

General Methods for Chemistry. All air- or moisture-sensitive reactions were performed under positive pressure of nitrogen with oven-dried glassware. Anhydrous solvents or reagents such as dichloromethane, DMF, acetonitrile, methanol, and triethylamine were purchased from Sigma-Aldrich. To follow most chemical reactions, LC/MS of reaction aliquots was analyzed using a gradient of 4−100% acetonitrile (containing 0.025% trifluoroacetic acid) and water (containing 0.05% trifluoroacetic acid) with a 4.5 min run time at a flow rate of 1 mL/min in an Agilent Extend-C18 column (3.5 μm, 4.6 × 100 mm) at a temperature of 50 °C using an Agilent diode array detector. Confirmation of molecular formulae was accomplished using electrospray ionization in the positive mode with the Agilent Masshunter software (version B.02).

Preparative purification was performed on a Waters semi-preparative HPLC system. The column used was a Phenomenex Luna C18 (5 μm, 30 × 75 mm) at a flow rate of 45 mL/min. The mobile phase consisted of acetonitrile and water (each containing 0.1% trifluoroacetic acid). A gradient of 10−50% acetonitrile over 8 min was used during the purification. Fraction collection was triggered by UV detection (220 nM). This purification method is referred to as **Standard Acidic Gradient Method**.

Purity of all final compounds was ≥95% as determined on an Agilent LC/MS (Agilent Technologies, Santa Clara, CA) using a 7 min gradient of 4−100% acetonitrile (containing 0.025% trifluoroacetic acid) and water (containing 0.05% trifluoroacetic acid) with an 8 min run time at a flow rate of 1 mL/min. A Phenomenex Luna C18 column (3 μm, 3 × 75 mm) was used at a temperature of 50 °C using an Agilent diode array detector. Mass determination was performed using an Agilent 6130 mass spectrometer with electrospray ionization in the positive mode.

^{1}H NMR spectra were recorded on Varian 400 MHz spectrometers. Chemical shifts are reported in ppm with a nondeuterated solvent (DMSO-h_6 at 2.50 ppm) as an internal standard for DMSO-d_6 solutions. High-resolution mass spectra (HRMS) were recorded on an Agilent 6210 Time-of-Flight LC/MS system.

https://doi.org/10.1021/acs.jmedchem.1c00019
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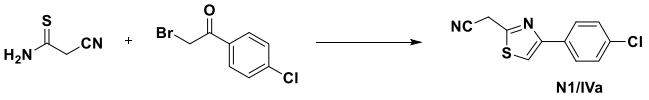
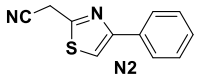
Synthetic Procedures. *Synthesis of Nitrile Intermediates (Scheme 1, Intermediate **IV**).*



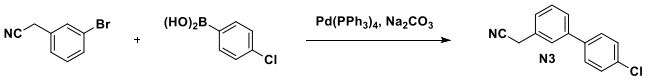
N1/IVa

*Method 1: 2-(4-(4-Chlorophenyl)thiazol-2-yl)acetonitrile (N1 or **IVa** in Schemes 2 and 3).* To a solution of 2-bromo-1-(4-chlorophenyl)ethanone (2.33 g, 10 mmol) in ethanol (25 mL) was added 2-cyanoethanethioamide (1 g, 10 mmol). The reaction mixture was heated at reflux for 15.5 h. The reaction mixture was cooled to 0 °C. A precipitate formed and was removed by filtration, washed with hexanes, and subsequently dried under vacuum. The product, 2-(4-(4-chlorophenyl)thiazol-2-yl)acetonitrile is a brown powder; LCMS: m/z $(M + H)^+$ = 235.0; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.88−7.77 (m, 2H), 7.48 (s, 1H), 7.44−7.35 (m, 2H), 4.17 (s, 2H).



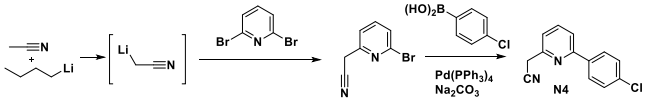
N2

*2-(4-Phenylthiazol-2-yl)acetonitrile (N2, Intermediate for Compound **11**).* Synthesized by Method 1 substituting 2-bromo-1-phenylethanone as a starting material. Following the reaction, the mixture was concentrated and purified via silica gel chromatography (0−30% EtOAc/hexanes). The product is a red-orange solid (1.53 g, 77%); LCMS: m/z $(M + H)^+$ = 201.1.



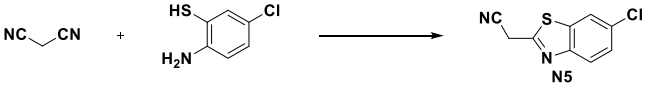
N3

*Method 2: 2-(4′-Chloro-[1,1′-biphenyl]-3-yl)acetonitrile (N3, Intermediate for Compound **23**).* In a microwave vial, 2-(3-bromophenyl)acetonitrile (300 mg, 1.53 mmol), (4-chlorophenyl)-boronic acid (287 mg, 1.84 mmol), tetrakis(triphenylphosphine)-palladium(0) (88 mg, 0.077 mmol), 2 M aqueous sodium carbonate solution (2.3 mL), and dimethoxyethane (10 mL) were combined. The reaction mixture was heated in a microwave with stirring at 140 °C for 1 h. The reaction mixture was diluted with water and extracted with CH_2Cl_2 (2 × 10 mL); the organic layers were combined, dried with magnesium sulfate, concentrated, and purified via silica gel chromatography (0−25% EtOAc/hexanes) to afford 2-(4′-chloro-[1,1′-biphenyl]-3-yl)acetonitrile (298 mg, 86%); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.62−7.38 (m, 7H), 7.37−7.28 (m, 1H), 3.82 (t, J = 0.7 Hz, 2H).



N4

*2-(6-(4-Chlorophenyl)pyridin-2-yl)acetonitrile (N4, Intermediate for Compound **25**).* Method 3: 2-(6-Bromopyridin-2-yl)acetonitrile: A solution of *n*-butyllithium in hexanes (1.6 M, 17.4 mL, 27.9 mmol) was added slowly to a solution of acetonitrile (1.5 mL, 28.7 mmol) in THF (40 mL) at −78 °C. A precipitate formed. The slurry was stirred at this temperature for 30 min. A solution of 2,6-dibromopyridine (2 g, 8.4 mmol) in THF (10 mL) was added slowly to the slurry. The reaction mixture was stirred at −78 °C for 45 min. The mixture was allowed to warm slowly to rt over 30 min. The reaction mixture was diluted with water and extracted with EtOAc (2 × 10 mL); the organic layers were combined, dried with magnesium sulfate, concentrated, and purified via silica gel chromatography (0−40% EtOAc/hexanes) to afford 2-(6-bromopyridin-2-yl)acetonitrile (1.65 g, 99%) as a yellow oil that solidified upon cooling; LCMS: m/z $(M + H)^+$ = 197.0. Method 2 was used to afford 2-(6-(4-chlorophenyl)-pyridin-2-yl)acetonitrile N4; LCMS: m/z $(M + H)^+$ = 229.1.

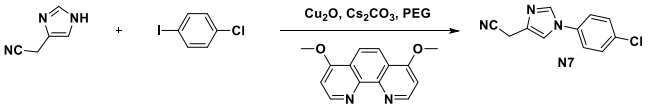


N5

*Method 4: 2-(6-Chlorobenzo[d]thiazol-2-yl)acetonitrile (N5, Intermediate for Compound **13**).* Malononitrile (65 mg, 0.98 mmol) and 2-amino-5-chlorobenzenethiol (157 mg, 0.98 mmol) were heated at 50 °C for 4 h and at reflux for 1 h in a mixture of EtOH and AcOH. The reaction mixture was concentrated under a stream of air, and 2-(6-chlorobenzo[d]thiazol-2-yl)acetonitrile was used without further purification.

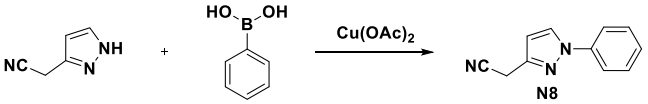
N6

*2-(5-Chlorobenzo[d]thiazol-2-yl)acetonitrile (N6, Intermediate for Compound **14**).* 2-(5-Chlorobenzo[d]thiazol-2-yl)acetonitrile (N6, intermediate for compound **14**) was prepared according to Method 4, however refluxing was conducted overnight followed by heating in a microwave at 120 °C for 1 h and at 150 °C for 1 h. The reaction mixture was concentrated under a stream of air, and 2-(5-chlorobenzo[d]thiazol-2-yl)acetonitrile was used without further purification; LCMS: m/z $(M + H)^+$ = 209.0.



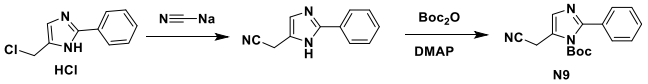
N7

*Method 5: 2-(1-(4-Chlorophenyl)-1H-imidazol-4-yl)acetonitrile (N7, Intermediate for Compound **15**).* A mixture of 2-(1*H*-imidazol-4-yl)acetonitrile (150 mg, 1.4 mmol), 1-chloro-4-iodobenzene (467 mg, 1.96 mmol), 4,7-dimethoxy-1,10-phenanthroline (101 mg, 0.42 mmol), copper(I) oxide (20 mg, 0.14 mmol), cesium carbonate (776 mg, 2.38 mmol), PEG (250 mg), and DMSO (1.5 mL) was heated with stirring at 110 °C for 24 h. The reaction mixture was diluted with water, 0.1 N HCl, and EtOAc and extracted (2×). The organic layers were combined, dried with magnesium sulfate, concentrated, and purified via reverse-phase chromatography (C18) (5−100% acetonitrile/water [0.1% TFA]) to afford 2-(1-(4-chlorophenyl)-1*H*-imidazol-4-yl)acetonitrile (35 mg, 12%) as a yellow oil; LCMS: m/z $(M + H)^+$ = 218.0.



N8

*Method 6: (2-(1-Phenyl-1H)-pyrazol-3-yl)acetonitrile (N8, Intermediate for Compound **19**).* A mixture of copper(II) acetate (382 mg, 2.1 mmol), 2-(1*H*-pyrazol-3-yl)acetonitrile (150 mg, 1.4 mmol), phenylboronic acid (341 mg, 2.8 mmol), triethylamine (0.390 mL, 2.8 mmol), pyridine (0.227 mL, 2.8 mmol), 4 Å molecular sieves (500 mg), and dichloromethane (10 mL) was heated at 55 °C overnight. The reaction mixture was filtered, extracted (DCM/1 N HCl), dried with magnesium sulfate, and concentrated, and 2-(1-phenyl-1*H*)-pyrazol-3-yl)acetonitrile was used without further purification; LCMS: m/z $(M + H)^+$ = 184.1.



N9

Method 7: 2-(2-Phenyl-1H-imidazol-5-yl)acetonitrile. A mixture of 5-(chloromethyl)-2-phenyl-1*H*-imidazole hydrochloride (197 mg, 0.86 mmol) and sodium cyanide (127 mg, 2.58 mmol) in DMSO (3 mL) was stirred at rt overnight. The reaction mixture was diluted with water and saturated aqueous sodium bicarbonate solution, extracted (EtOAc × 2), dried with magnesium sulfate, and concentrated, and 2-(2-phenyl-1*H*-imidazol-5-yl)acetonitrile was used without further purification.

*Method 8: tert-Butyl 5-(Cyanomethyl)-2-phenyl-1H-imidazole-1-carboxylate (N9, Intermediate for Compound **17**).* A mixture of 2-(2-phenyl-1*H*-imidazol-5-yl)acetonitrile (50 mg, 0.27 mmol), Boc_2O (0.070 mL, 0.3 mmol), and DMAP (trace) in acetonitrile (3 mL) and sodium cyanide (127 mg, 2.58 mmol) in DMSO (3 mL) was stirred at rt for 40 min and concentrated under a stream of air. *tert*-Butyl 5-(cyanomethyl)-2-phenyl-1*H*-imidazole-1-carboxylate was used without further purification; LCMS: m/z $(M + H)^+$ = 284.1 (weak).

https://doi.org/10.1021/acs.jmedchem.1c00019
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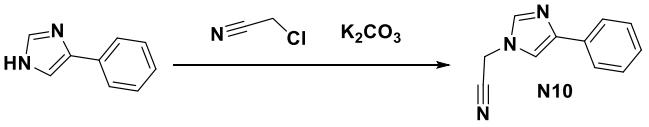
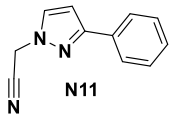
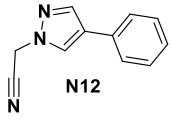


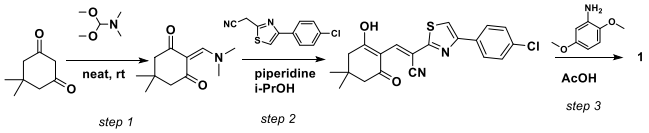
*Method 9: 2-(4-Phenyl-1H-imidazol-1-yl)acetonitrile (N10, Intermediate for Compound **16**).* A mixture of 4-phenyl-1*H*-imidazole (250 mg, 1.7 mmol), chloroacetonitrile (0.22 mL, 3.5 mmol), and potassium carbonate (1.2 g, 8.7 mmol) in DMF (8 mL) was stirred at rt for 22 h. The reaction mixture was diluted with water, extracted (EtOAc × 2), dried with magnesium sulfate, and concentrated to afford 2-(4-phenyl-1*H*-imidazol-1-yl)acetonitrile as a brown solid which was used without further purification; LCMS: m/z $(M + H)^+$ = 184.1.



*2-(3-Phenyl-1H-pyrazol-1-yl)acetonitrile (N11, Intermediate for Compound **20**).* 2-(3-Phenyl-1*H*-pyrazol-1-yl)acetonitrile (N11, intermediate for compound **20**) was synthesized by Method 9; LCMS: m/z $(M + H)^+$ = 184.1 (weak).



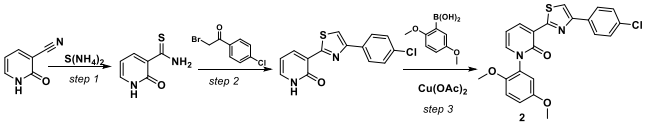
*2-(4-Phenyl-1H-pyrazol-1-yl)acetonitrile (N12, Intermediate for Compound **21**).* 2-(4-Phenyl-1*H*-pyrazol-1-yl)acetonitrile (N12, intermediate for compound **21**) was synthesized by Method 9; LCMS: m/z $(M + H)^+$ = 184.1 (weak).



*Method 10: 3-(4-(4-Chlorophenyl)thiazol-2-yl)-1-(2,5-dimethoxyphenyl)-7,7-dimethyl-7,8-dihydroquinoline-2,5(1H,6H)-dione (**1**).* Step 1: In a vial, 5,5-dimethylcyclohexane-1,3-dione (0.100 g, 0.713 mmol) and DMF-DMA (0.096 mL, 0.713 mmol) were mixed and stirred well (no solvent) for 5 min. The reaction mixture became yellow oil.

Step 2: To the mixture were added *i*-PrOH (2.55 mL), 2-(4-(4-chlorophenyl)thiazol-2-yl)acetonitrile (167 mg, 0.713 mmol), and piperidine (0.071 mL, 0.713 mmol). The reaction mixture was allowed to stir at rt for 3 h. The solid turned into solution. After 3 h, a precipitate formed at which point the solvent was removed by blowing down under a stream of air with mild heating at 30 °C.

Step 3: To the resulting residue were added acetic acid (1 mL) and 2,5-dimethoxyaniline (109 mg, 0.713 mmol). The reaction mixture was stirred for 15 min at rt, a precipitate formed almost immediately. The solvent was removed by blowing down under a stream of air with mild heating at 30 °C. The crude mixture was diluted with DMSO and purified by reverse-phase chromatography (**Standard Acidic Gradient Method**) to afford as a TFA salt, 3-(4-(4-chlorophenyl)-thiazol-2-yl)-1-(2,5-dimethoxyphenyl)-7,7-dimethyl-7,8-dihydroquinoline-2,5(1*H*,6*H*)-dione LCMS: m/z $(M + H)^+$ = 521.1; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.04 (s, 1H), 8.22 (s, 1H), 8.12−8.05 (m, 2H), 7.58−7.50 (m, 2H), 7.23 (d, J = 9.2 Hz, 1H), 7.13 (dd, J = 9.1, 3.1 Hz, 1H), 7.05 (d, J = 3.0 Hz, 1H), 3.75 (s, 3H), 3.71 (s, 3H), 2.62 (d, J = 17.7 Hz, 1H), 2.46 (d, J = 2.9 Hz, 2H), 2.22 (d, J = 17.7 Hz, 1H), 0.99 (s, 3H), 0.94 (s, 3H); HRMS (ESI): m/z $(M + H)^+$ calcd for $C_{28}H_{26}ClN_2O_4S$, 521.1296; found, 521.1321. Retention time = 3.858 min.

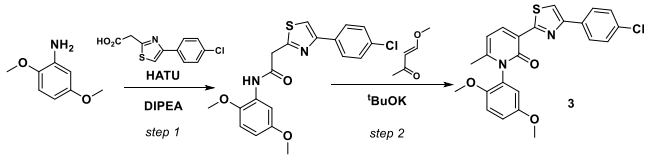


*3-(4-(4-Chlorophenyl)thiazol-2-yl)-1-(2,5-dimethoxyphenyl)-pyridin-2(1H)-one (**2**).* Step 1: 2-Oxo-1,2-dihydropyridine-3-carbothioamide: Ammonium sulfide (0.509 mL, 2.99 mmol) was added to a solution of 2-oxo-1,2-dihydropyridine-3-carbonitrile (211 mg, 1.757 mmol) in methanol (14 mL). The reaction mixture was heated in a microwave at 130 °C for 2 h. The mixture stood overnight at rt and crystals formed. The mixture was further cooled to 0 °C for 4 h. The methanol was poured off and the solid was triturated with methanol and used as is in the following step. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.60 (s, 1H), 11.31 (s, 1H), 9.98 (s, 1H), 8.93 (dd, J = 7.4, 2.2 Hz, 1H), 7.77 (dd, J = 6.2, 2.3 Hz, 1H), 6.52 (dd, J = 7.4, 6.2 Hz, 1H).

Step 2: 3-(4-(4-Chlorophenyl)thiazol-2-yl)pyridin-2(1*H*)-one: To 2-oxo-1,2-dihydropyridine-3-carbothioamide (124 mg, 0.804 mmol) in ethanol (2 mL) was added 2-bromo-1-(4-chlorophenyl)ethanone (188 mg, 0.804 mmol). The reaction mixture was heated at reflux for 17.5 h. The reaction mixture was cooled to rt and diluted with hexanes. The solid was removed by filtration, washed with hexanes, and dried on high vacuum. The product (213 mg, 65%) is a red-brown powder. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.10 (s, 1H), 8.28 (dd, J = 7.2, 2.1 Hz, 1H), 7.80 (s, 1H), 7.76−7.68 (m, 2H), 7.30 (s, 1H), 7.20−7.11 (m, 2H), 6.15 (dd, J = 7.2, 6.3 Hz, 1H).

Step 3: 3-(4-(4-Chlorophenyl)thiazol-2-yl)-1-(2,5-dimethoxyphenyl)pyridin-2(1*H*)-one (Table 1; compound **2**): To a mixture of 3-(4-(4-chlorophenyl)thiazol-2-yl)pyridin-2(1*H*)-one (60 mg, 0.208 mmol), copper(II) acetate (56.6 mg, 0.312 mmol), and 2,5-dimethoxyphenylboronic acid (76 mg, 0.416 mmol) were added 1,4-dioxane (2 mL) and pyridine (0.2 mL). The reaction mixture was sealed and heated at 80 °C for 60 h. The mixture was filtered with thiol resin, washed with EtOAc, and concentrated. The crude mixture was diluted with DMSO and purified by reverse-phase chromatography (**Standard Acidic Gradient Method**) to afford as a TFA salt, 3-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2,5-dimethoxyphenyl)-7,7-dimethyl-7,8-dihydroquinoline-2,5(1*H*,6*H*)-dione. LCMS: m/z $(M + H)^+$ = 425.1; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.70 (dd, J = 7.3, 2.0 Hz, 1H), 8.16 (s, 1H), 8.07 (d, J = 8.6 Hz, 2H), 7.78 (dd, J = 6.6, 2.0 Hz, 1H), 7.51 (d, J = 8.6 Hz, 2H), 7.17 (dd, J = 8.5, 1.3 Hz, 1H), 7.10−7.02 (m, 2H), 6.59 (t, J = 6.9 Hz, 1H), 3.73 (s, 3H), 3.68 (s, 3H); HRMS (ESI): m/z $(M + H)^+$ calcd for $C_{22}H_{18}ClN_2O_3S$, 425.0721; found, 425.0723. Retention time = 3.787 min.

*3-(4-(4-Chlorophenyl)thiazol-2-yl)-1-(2,5-dimethoxyphenyl)-6-methylpyridin-2(1H)-one (**3**).*



Step 1: 2-(4-(4-Chlorophenyl)thiazol-2-yl)-*N*-(2,5-dimethoxyphenyl)acetamide: To a mixture of 2-(4-(4-chlorophenyl)-thiazol-2-yl)acetic acid (155 mg, 0.611 mmol) in CH_2Cl_2 (3 mL) were added HATU (348 mg, 0.916 mmol), DIPEA (320 μL, 1.833 mmol), and 2,5-dimethoxyaniline (103 mg, 0.672 mmol). The reaction mixture was stirred for 1 h at room temperature. The reaction mixture was diluted with water and CH_2Cl_2 and extracted (2 × 15 mL); the organic layers were combined, dried with magnesium sulfate, concentrated, and purified via silica gel chromatography to afford 2-(4-(4-chlorophenyl)thiazol-2-yl)-*N*-(2,5-dimethoxyphenyl)-acetamide as a solid; LCMS: m/z $(M + H)^+$ = 389.

Step 2: 3-(4-(4-Chlorophenyl)thiazol-2-yl)-1-(2,5-dimethoxyphenyl)-6-methylpyridin-2(1*H*)-one (Table 1; Compound **3**): A mixture of 2-(4-(4-chlorophenyl)thiazol-2-yl)-*N*-(2,5-dimethoxyphenyl)-acetamide (74 mg, 0.190 mmol), (*E*)-4-methoxybut-3-en-2-one (38.8 μL, 0.381 mmol), and potassium 2-methylpropan-2-olate (21.35 mg, 0.190 mmol) was heated at 80 °C for 3.5 h. The reaction mixture was diluted with methanol/CH_2Cl_2/water and to alleviate the emulsion, it is acidified with 1 N HCl, extracted with EtOAc, and concentrated. The crude mixture was diluted with DMSO and purified by reverse-phase chromatography (**Standard Acidic Gradient Method**) to afford as a TFA salt, 3-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2,5-dimethoxyphenyl)-6-methylpyridin-2(1*H*)-one. LCMS:

https://doi.org/10.1021/acs.jmedchem.1c00019
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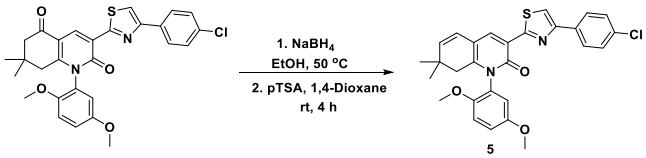
m/z (M + H)$^+$ = 521.1; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.61 (d, J = 7.4 Hz, 1H), 8.15−8.03 (m, 3H), 7.55−7.47 (m, 2H), 7.18 (d, J = 9.1 Hz, 1H), 7.07 (dd, J = 9.1, 3.1 Hz, 1H), 6.98 (d, J = 3.1 Hz, 1H), 6.59 (dd, J = 7.5, 0.9 Hz, 1H), 3.74 (s, 3H), 3.69 (s, 3H), 2.03 (d, J = 0.8 Hz, 3H); HRMS (ESI): m/z (M + H)$^+$ calcd for $C_{23}H_{20}ClN_2O_3S$, 439.0878; found, 439.0897. Retention time = 3.822 min.

*3-(4-(4-Chlorophenyl)thiazol-2-yl)-1-(2,5-dimethoxyphenyl)-7,7-dimethyl-5,6,7,8-tetrahydroquinolin-2(1H)-one (**4**).* To a solution of 3-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2,5-dimethoxyphenyl)-7,7-dimethyl-7,8-dihydroquinoline-2,5(1*H*,6*H*)-dione (35 mg, 0.067 mmol) compound **1** in THF (2 mL) was added lithium aluminum hydride (0.101 mL, 0.101 mmol). The reaction mixture was stirred at rt for 1 h and then heated at 60 °C for 12 h. The reaction mixture was cooled to 0 °C and quenched with methanol and a bit of water, diluted with EtOAc, and extracted (2 × 25 mL); the organic layers were combined, dried with magnesium sulfate, and concentrated. The crude mixture was diluted with DMSO and purified by reverse-phase chromatography (**Standard Acidic Gradient Method**) to afford as a TFA salt, 3-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2,5-dimethoxyphenyl)-7,7-dimethyl-5,6,7,8-tetrahydroquinolin-2(1*H*)-one. LCMS: m/z (M + H)$^+$ = 507.2; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.48 (s, 1H), 8.13−8.00 (m, 3H), 7.54−7.45 (m, 2H), 7.16 (d, J = 9.1 Hz, 1H), 7.05 (dd, J = 9.1, 3.0 Hz, 1H), 6.87 (d, J = 3.0 Hz, 1H), 3.73 (d, J = 1.4 Hz, 3H), 3.66 (s, 3H), 2.73 (t, J = 6.5 Hz, 2H), 2.09 (d, J = 17.8 Hz, 1H), 1.78 (d, J = 17.8 Hz, 1H), 1.47 (t, J = 6.7 Hz, 2H), 0.88 (s, 3H), 0.84 (s, 3H); HRMS (ESI): m/z (M + H)$^+$ calcd for $C_{28}H_{28}ClN_2O_3S$, 507.1504; found, 507.1509. Retention time = 4.196 min.



*3-(4-(4-Chlorophenyl)thiazol-2-yl)-1-(2,5-dimethoxyphenyl)-7,7-dimethyl-7,8-dihydroquinolin-2(1H)-one (**5**).* Step 1: To a solution of 3-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2,5-dimethoxyphenyl)-7,7-dimethyl-7,8-dihydroquinoline-2,5(1*H*,6*H*)-dione (200 mg, 0.384 mmol) compound **1** in EtOH (10 mL) was added sodium borohydride (29.0 mg, 0.768 mmol). Gas evolution was observed. The reaction mixture was stirred at 50 °C for 12 h. The reaction mixture was cooled to rt, diluted with water and EtOAc, and extracted (2 × 25 mL); the organic layers were combined, dried with magnesium sulfate, and concentrated. The crude mixture was taken to the next step without further purification.

Step 2: To a solution of 3-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2,5-dimethoxyphenyl)-5-hydroxy-7,7-dimethyl-5,6,7,8-tetrahydroquinolin-2(1*H*)-one (38 mg, 0.073 mmol) in 1,4-dioxane (10 mL) was added pTSA (1.38 mg, 0.1 equiv). The reaction mixture was stirred at rt for 4 h. The organic solvent was concentrated. The crude mixture was diluted with DMSO and purified by reverse-phase chromatography (**Standard Acidic Gradient Method**) to afford as a TFA salt, 3-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2,5-dimethoxyphenyl)-7,7-dimethyl-7,8-dihydroquinolin-2(1*H*)-one. LCMS: m/z (M + H)$^+$ = 505.2; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.60 (s, 2H), 8.01−8.23 (m, 6H), 7.54 (m, 2H), 7.18 (d, J = 8.61 Hz, 3H), 7.18−7.30 (m, 2H), 7.06−7.18 (m, 2H), 7.00 (d, J = 3.13 Hz, 2H), 6.47 (d, J = 9.78 Hz, 2H), 5.63 (d, J = 9.78 Hz, 1H), 3.73 (d, J = 19.96 Hz, 7H), 2.37−2.48 (m, 2H), 2.14 (s, 1H), 0.84−1.14 (m, 7H); HRMS (ESI): m/z (M + H)$^+$ calcd for $C_{28}H_{26}ClN_2O_3S$, 505.1347; found, 505.1351. Retention time = 4.152 min.

*3-(4-(4-Chlorophenyl)thiazol-2-yl)-1-(2,5-dimethoxyphenyl)-7,8-dihydroquinoline-2,5(1H,6H)-dione (**6**).* This compound was prepared using Method 10 using cyclohexane-1,3-dione in step 1 to afford 3-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2,5-dimethoxyphenyl)-7,8-dihydroquinoline-2,5(1*H*,6*H*)-dione as a TFA salt. LCMS: m/z (M + H)$^+$ = 493; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.04 (s, 1H), 8.21 (d, J = 1.2 Hz, 1H), 8.11−8.02 (m, 2H), 7.57−7.46 (m, 2H), 7.21 (d, J = 9.1 Hz, 1H), 7.14−7.02 (m, 2H), 3.74 (d, J = 1.1 Hz, 3H), 3.70 (s, 3H), 2.51 (d, J = 1.2 Hz, 2H), 2.47 (m, 4H); HRMS (ESI): m/z (M + H)$^+$ calcd for $C_{26}H_{22}ClN_2O_4S$, 493.0983; found, 493.0973. Retention time = 3.741 min.

*3-(4-(4-Chlorophenyl)thiazol-2-yl)-1-(2,5-dimethoxyphenyl)-6,7-dihydro-1H-cyclopenta[b]pyridine-2,5-dione (**7**).* This compound was prepared using Method 10 using cyclopentane-1,3-dione in step 1 to afford 3-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2,5-dimethoxyphenyl)-7,8-dihydroquinoline-2,5(1*H*,6*H*)-dione as a TFA salt. LCMS: m/z (M + H)$^+$ = 479.1; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.77 (s, 1H), 8.27 (s, 1H), 8.14 (d, J = 8.61 Hz, 2H), 7.54 (d, J = 8.61 Hz, 2H), 7.27 (d, J = 9.00 Hz, 1H), 7.07−7.22 (m, 2H), 3.76 (d, J = 12.91 Hz, 5H), 2.77−2.92 (m, 1H), 2.55−2.77 (m, 3H); HRMS (ESI): m/z (M + H)$^+$ calcd for $C_{25}H_{20}ClN_2O_4S$, 479.0827; found, 479.0840. Retention time = 3.672 min.

*3-(4-(4-Chlorophenyl)thiazol-2-yl)-1-(2,5-dimethoxyphenyl)-5-hydroxyquinolin-2(1H)-one (**8**).* To a solution of 3-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2,5-dimethoxyphenyl)-7,8-dihydroquinoline-2,5(1*H*,6*H*)-dione (25 mg, 0.051 mmol) compound **6** in 1,4-dioxane (3 mL) was added DDQ (17.27 mg, 0.076 mmol). The reaction mixture was stirred at 70 °C for 12 h. The organic solvent was concentrated. The crude mixture was diluted with DMSO and purified by reverse-phase chromatography (**Standard Acidic Gradient Method**) to afford as a TFA salt, 3-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2,5-dimethoxyphenyl)-5-hydroxyquinolin-2(1*H*)-one. LCMS: m/z (M + H)$^+$ = 491.2; HRMS (ESI): m/z (M + H)$^+$ calcd for $C_{26}H_{20}ClN_2O_4S$, 491.0827; found, 491.0817. Retention time = 3.619 min.

*3-(4-(4-Chlorophenyl)thiazol-2-yl)-1-(2,5-dimethoxyphenyl)-7,8-dihydro-1,7-naphthyridine-2,5(1H,6H)-dione (**9**).* This compound was prepared using Method 10 using *tert*-butyl 3,5-dioxopiperidine-1-carboxylate in step 1 followed by Boc deprotection after step 3 using TFA to afford 3-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2,5-dimethoxyphenyl)-7,8-dihydro-1,7-naphthyridine-2,5(1*H*,6*H*)-dione as a TFA salt. LCMS: m/z (M + H)$^+$ = 494.1; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.03 (s, 1H), 8.30 (s, 1H), 8.14−8.07 (m, 2H), 7.59−7.52 (m, 2H), 7.29 (d, J = 9.2 Hz, 1H), 7.19 (dd, J = 9.2, 3.1 Hz, 1H), 7.06 (d, J = 3.1 Hz, 1H), 3.98−3.79 (m, 2H), 3.76 (s, 3H), 3.74 (s, 3H); HRMS (ESI): m/z (M + 2H)$^{2+}$ calcd for $C_{25}H_{22}ClN_3O_4S$, 493.0821; found, 493.0863. Retention time = 3.148 min.

*3-(4-(4-Chlorophenyl)thiazol-2-yl)-1-(2,5-dimethoxyphenyl)-7,8-dihydro-1,6-naphthyridine-2,5(1H,6H)-dione (**10**).* This compound was prepared using Method 10 using *tert*-butyl 2,4-dioxopiperidine-1-carboxylate in step 1 followed by Boc deprotection using TFA to afford 3-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2,5-dimethoxyphenyl)-7,8-dihydro-1,6-naphthyridine-2,5(1*H*,6*H*)-dione as a TFA salt. LCMS: m/z (M + H)$^+$ = 494.2; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.06 (s, 1H), 8.20 (s, 1H), 8.13−8.04 (m, 2H), 7.92 (s, 1H), 7.57−7.47 (m, 2H), 7.21 (dd, J = 8.8, 0.8 Hz, 1H), 7.15−7.06 (m, 2H), 3.74 (s, 3H), 3.71 (s, 3H), 3.42−3.30 (m, 2H), 2.73−2.60 (m, 1H), 2.40 (dt, J = 17.6, 6.5 Hz, 1H); HRMS (ESI): m/z (M + Na)$^+$ calcd for $C_{25}H_{20}ClN_3NaO_4S$, 516.0755; found, 516.0768. Retention time = 3.822 min.

*1-(2,5-Dimethoxyphenyl)-7,7-dimethyl-3-(4-phenylthiazol-2-yl)-7,8-dihydroquinoline-2,5(1H,6H)-dione (**11**).* This compound was prepared using Method 10 using 2-(4-phenylthiazol-2-yl)acetonitrile N2 in step 2 to afford 1-(2,5-dimethoxyphenyl)-7,7-dimethyl-3-(4-phenylthiazol-2-yl)-7,8-dihydroquinoline-2,5(1*H*,6*H*)-dione as a TFA salt. LCMS: m/z (M + H)$^+$ = 487.2; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.14 (s, 1H), 8.25 (s, 1H), 8.18−8.11 (m, 2H), 7.57 (dd, J = 8.3, 7.0 Hz, 2H), 7.50−7.41 (m, 1H), 7.33 (d, J = 9.1 Hz, 1H), 7.23 (dd, J = 9.1, 3.1 Hz, 1H), 7.14 (d, J = 3.0 Hz, 1H), 3.85 (s, 3H), 3.81 (s, 3H), 2.72 (d, J = 17.8 Hz, 1H), 2.64−2.49 (m, 2H), 2.31 (d, J = 17.7 Hz, 1H), 1.09 (s, 3H), 1.03 (s, 3H); HRMS (ESI): m/z (M + Na)$^+$ calcd for $C_{28}H_{26}N_2NaO_4S$, 509.1505; found, 509.1529. Retention time = 3.718 min.

*3-(5-(4-Chlorophenyl)thiazol-2-yl)-1-(2,5-dimethoxyphenyl)-7,7-dimethyl-7,8-dihydroquinoline-2,5(1H,6H)-dione (**12**).* This compound was prepared using Method 10 using 2-(5-(4-chlorophenyl)-thiazol-2-yl)acetonitrile in step 2 to afford 3-(5-(4-chlorophenyl)-thiazol-2-yl)-1-(2,5-dimethoxyphenyl)-7,7-dimethyl-7,8-dihydroquinoline-2,5(1*H*,6*H*)-dione as a TFA salt. LCMS: m/z (M + H)$^+$ =

https://doi.org/10.1021/acs.jmedchem.1c00019
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521.2; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.03 (d, J = 4.7 Hz, 1H), 8.49 (s, 1H), 7.84−7.75 (m, 2H), 7.64−7.54 (m, 2H), 7.32 (d, J = 9.2 Hz, 1H), 7.26−7.17 (m, 1H), 7.13 (d, J = 3.1 Hz, 1H), 3.84 (s, 3H), 3.79 (s, 3H), 2.71 (d, J = 17.7 Hz, 1H), 2.54 (d, J = 3.1 Hz, 2H), 2.30 (d, J = 17.8 Hz, 1H), 1.08 (s, 3H), 1.02 (s, 3H); HRMS (ESI): m/z (M + H)$^+$ calcd for $C_{26}H_{24}ClN_2O_4S$, 495.114; found, 495.1159. Retention time = 3.787 min.

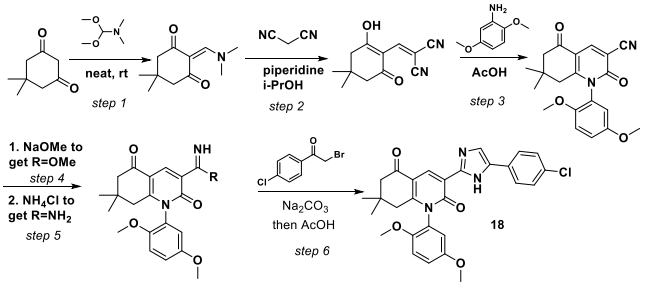
*3-(6-Chlorobenzo[d]thiazol-2-yl)-1-(2,5-dimethoxyphenyl)-7,7-dimethyl-7,8-dihydroquinoline-2,5(1H,6H)-dione (**13**).* This compound was prepared using Method 10 using 2-(6-chlorobenzo[*d*]-thiazol-2-yl)acetonitrile N5 in step 2 to afford 3-(6-chlorobenzo[*d*]-thiazol-2-yl)-1-(2,5-dimethoxyphenyl)-7,7-dimethyl-7,8-dihydroquinoline-2,5(1*H*,6*H*)-dione as a TFA salt. LCMS: m/z (M + H)$^+$ = 495.1; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.14 (s, 1H), 8.27 (d, J = 2.1 Hz, 1H), 8.08 (d, J = 8.7 Hz, 1H), 7.56 (dd, J = 8.7, 2.2 Hz, 1H), 7.25 (d, J = 9.1 Hz, 1H), 7.18−7.05 (m, 2H), 3.75 (s, 3H), 3.72 (s, 3H), 2.64−2.60 (m, 2H), 2.35−2.17 (m, 2H), 1.00 (s, 3H), 0.94 (s, 3H); HRMS (ESI): m/z (M + H)$^+$ calcd for $C_{26}H_{24}ClN_2O_4S$, 495.114; found, 495.1138. Retention time = 3.795 min.

*3-(5-Chlorobenzo[d]thiazol-2-yl)-1-(2,5-dimethoxyphenyl)-7,7-dimethyl-7,8-dihydroquinoline-2,5(1H,6H)-dione (**14**).* This compound was prepared using Method 10 using 2-(5-chlorobenzo[*d*]-thiazol-2-yl)acetonitrile N6 in step 2 to afford 3-(5-chlorobenzo[*d*]-thiazol-2-yl)-1-(2,5-dimethoxyphenyl)-7,7-dimethyl-7,8-dihydroquinoline-2,5(1*H*,6*H*)-dione as a TFA salt. LCMS: m/z (M + H)$^+$ = 495.1; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.15 (s, 1H), 8.20−8.12 (m, 2H), 7.46 (dd, J = 8.5, 2.0 Hz, 1H), 7.25 (d, J = 9.1 Hz, 1H), 7.18−7.05 (m, 2H), 3.75 (s, 3H), 3.72 (s, 3H), 2.64 (d, J = 17.8 Hz, 1H), 2.24 (d, J = 17.8 Hz, 1H), 1.00 (s, 3H), 0.94 (s, 3H); HRMS (ESI): m/z (M + Na)$^+$ calcd for $C_{28}H_{25}ClN_2NaO_4S$, 543.1116; found, 543.113. Retention time = 3.811 min.

*3-(1-(4-Chlorophenyl)-1H-imidazol-4-yl)-1-(2,5-dimethoxyphenyl)-7,7-dimethyl-7,8-dihydroquinoline-2,5(1H,6H)-dione (**15**).* This compound was prepared using Method 10 using 2-(1-(4-chlorophenyl)-1*H*-imidazol-4-yl)acetonitrile N7 in step 2 to afford 3-(1-(4-chlorophenyl)-1*H*-imidazol-4-yl)-1-(2,5-dimethoxyphenyl)-7,7-dimethyl-7,8-dihydro-quinoline-2,5(1*H*,6*H*)-dione as a TFA salt. LCMS: m/z (M + H)$^+$ = 504.1; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.65 (s, 1H), 8.38 (d, J = 1.4 Hz, 1H), 8.20 (d, J = 1.4 Hz, 1H), 7.75−7.62 (m, 2H), 7.59−7.48 (m, 1H), 7.18 (d, J = 9.1 Hz, 1H), 7.07 (dd, J = 9.1, 3.0 Hz, 1H), 6.94 (d, J = 3.0 Hz, 1H), 3.72 (s, 3H), 3.67 (s, 3H), 2.63 (dd, J = 3.7, 1.9 Hz, 1H), 2.38 (d, J = 8.1 Hz, 1H), 2.31−2.26 (m, 1H), 0.96 (s, 3H), 0.91 (s, 3H); HRMS (ESI): m/z (M + H)$^+$ calcd for $C_{28}H_{27}ClN_3O_4$, 504.1685; found, 504.1678. Retention time = 3.343 min.

*1-(2,5-Dimethoxyphenyl)-7,7-dimethyl-3-(4-phenyl-1H-imidazol-1-yl)-7,8-dihydroquinoline-2,5(1H,6H)-dione (**16**).* This compound was prepared using Method 10 using 2-(4-phenyl-1*H*-imidazol-1-yl)acetonitrile N10 in step 2 to afford 1-(2,5-dimethoxyphenyl)-7,7-dimethyl-3-(4-phenyl-1*H*-imidazol-1-yl)-7,8-dihydroquinoline-2,5(1*H*,6*H*)-dione as a TFA salt. LCMS: m/z (M + H)$^+$ = 470.2; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.71 (s, 1H), 8.35 (s, 1H), 8.27 (s, 1H), 7.88−7.80 (m, 2H), 7.41 (dd, J = 8.3, 7.1 Hz, 2H), 7.33−7.19 (m, 2H), 7.12 (dd, J = 9.1, 3.1 Hz, 1H), 7.02 (d, J = 3.1 Hz, 1H), 3.74 (s, 3H), 3.73 (s, 3H), 2.60 (d, J = 17.6 Hz, 1H), 2.44 (d, J = 4.1 Hz, 2H), 2.19 (d, J = 17.5 Hz, 1H), 0.99 (s, 3H), 0.94 (s, 3H); HRMS (ESI): m/z (M + H)$^+$ calcd for $C_{28}H_{28}N_3O_4$, 470.2074; found, 470.2095. Retention time = 3.02 min.

*1-(2,5-Dimethoxyphenyl)-7,7-dimethyl-3-(2-phenyl-1H-imidazol-4-yl)-7,8-dihydroquinoline-2,5(1H,6H)-dione (**17**).* This compound was prepared using *tert*-butyl 5-(cyanomethyl)-2-phenyl-1*H*-imidazole-1-carboxylate N9 in step 2 followed by treatment with TFA to afford 1-(2,5-dimethoxyphenyl)-7,7-dimethyl-3-(2-phenyl-1*H*-imidazol-4-yl)-7,8-dihydroquinoline-2,5(1*H*,6*H*)-dione as a TFA salt. LCMS: m/z (M + H)$^+$ = 470.2; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.71 (s, 1H), 8.01 (d, J = 7.6 Hz, 2H), 7.45 (d, J = 31.3 Hz, 3H), 7.18 (d, J = 9.1 Hz, 1H), 7.08 (dd, J = 9.1, 3.0 Hz, 2H), 6.94 (d, J = 3.0 Hz, 1H), 3.73 (s, 3H), 3.68 (s, 3H), 2.66−2.55 (m, 1H), 2.40 (d, J = 8.2 Hz, 1H), 2.29 (p, J = 1.9 Hz, 1H), 2.13 (d, J = 17.5 Hz, 1H), 0.97 (s, 3H), 0.92 (s, 3H); HRMS (ESI): m/z (M + H)$^+$ calcd for $C_{28}H_{28}N_3O_4$, 470.2074; found, 470.2054. Retention time = 2.59 min.



*1-(2,5-Dimethoxyphenyl)-7,7-dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydro-quinoline-3-carbonitrile dione (**18**).* Step 1: Similar to Method 10; Step 2: Similar to Method 10 with stirring only for 1 h. Also, aniline was added prior to concentration; Step 3: Acetic acid was added and stirred overnight at rt. The reaction mixture was diluted with water and DCM and extracted (2×); the organic layers were combined, dried with magnesium sulfate, concentrated, and purified via silica gel chromatography (10−100% EtOAc/hexanes) to afford 1-(2,5-dimethoxyphenyl)-7,7-dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carbonitrile (70% on 2.85 mmol scale); LCMS: m/z (M + H)$^+$ = 353.1. Step 4: 1-(2,5-Dimethoxyphenyl)-7,7-dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboximidamide: To a mixture of 1-(2,5-dimethoxyphenyl)-7,7-dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carbonitrile (60 mg, 0.170 mmol) in MeOH (1 mL) was added sodium methanolate (398 μL, 1.703 mmol) in MeOH. The reaction mixture was stirred at 45 °C for 45 min and then ammonia hydrochloride (91 mg, 1.703 mmol) and AcOH (1.5 mL) were added. The reaction mixture was stirred at 60 °C for 12 h. The reaction mixture was diluted with water and DCM and extracted (2×); the organic layers were combined, dried with magnesium sulfate, concentrated, and purified via silica gel chromatography (10−100% EtOAc/hexanes) to afford 1-(2,5-dimethoxyphenyl)-7,7-dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboximidamide; LCMS: m/z (M + H)$^+$ = 370.1. Step 5: To a mixture of 1-(2,5-dimethoxyphenyl)-7,7-dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboximidamide (50 mg, 0.135 mmol) in THF (1 mL) were added 2-bromo-1-(4-chlorophenyl)ethan-1-one (135 μL, 0.135 mmol) and sodium bicarbonate (11.37 mg, 0.135 mmol). The reaction mixture was stirred at 70 °C for 2 h and then AcOH (7.75 μL, 0.135 mmol) was added. The reaction mixture was stirred at 70 °C for 2 h. The solvent was removed by blowing down under a stream of air with mild heating at 30 °C. The crude mixture was diluted with DMSO and purified by reverse-phase chromatography (**Standard Acidic Gradient Method**) to afford as a TFA salt, 3-(5-(4-chlorophenyl)-1*H*-imidazol-2-yl)-1-(2,5-dimethoxyphenyl)-7,7-dimethyl-7,8-dihydroquinoline-2,5(1*H*,6*H*)-dione. LCMS: m/z (M + H)$^+$ = 504.1; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.78 (s, 1H), 7.88 (d, J = 8.2 Hz, 2H), 7.63 (s, 1H), 7.38 (d, J = 8.5 Hz, 2H), 7.20 (d, J = 9.2 Hz, 1H), 7.15−6.95 (m, 2H), 3.71 (d, J = 13.8 Hz, 6H), 2.66−2.54 (m, 2H), 2.42 (d, J = 4.5 Hz, 2H), 0.98 (s, 3H), 0.92 (s, 3H); HRMS (ESI): m/z (M + H)$^+$ calcd for $C_{28}H_{27}ClN_3O_4$, 504.1685; found, 504.1709. Retention time = 3.171 min.

*1-(2,5-Dimethoxyphenyl)-7,7-dimethyl-3-(1-phenyl-1H-pyrazol-3-yl)-7,8-dihydroquinoline-2,5(1H,6H)-dione (**19**).* This compound was prepared using Method 10 using 2-(1-phenyl-1*H*-pyrazol-3-yl)acetonitrile N8 in step 2 to afford 1-(2,5-dimethoxyphenyl)-7,7-dimethyl-3-(1-phenyl-1*H*-pyrazol-3-yl)-7,8-dihydroquinoline-2,5-(1*H*,6*H*)-dione as a TFA salt. LCMS: m/z (M + H)$^+$ = 470.2; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.68 (s, 1H), 8.51 (d, J = 2.5 Hz, 1H), 7.94−7.86 (m, 2H), 7.56−7.47 (m, 2H), 7.37−7.28 (m, 1H), 7.20 (d, J = 9.1 Hz, 1H), 7.16−7.05 (m, 2H), 6.98 (d, J = 3.0 Hz, 1H), 3.75 (s, 3H), 3.71 (s, 3H), 2.56 (d, J = 17.6 Hz, 1H), 2.41 (d, J = 7.3 Hz, 2H), 2.14 (d, J = 17.6 Hz, 1H), 0.98 (s, 3H), 0.93 (s, 3H); HRMS (ESI): m/z (M + H)$^+$ calcd for $C_{28}H_{28}N_3O_4$, 470.2074; found, 470.2094. Retention time = 3.539 min.

*1-(2,5-Dimethoxyphenyl)-7,7-dimethyl-3-(3-phenyl-1H-pyrazol-1-yl)-7,8-dihydroquinoline-2,5(1H,6H)-dione (**20**).* This compound

https://doi.org/10.1021/acs.jmedchem.1c00019
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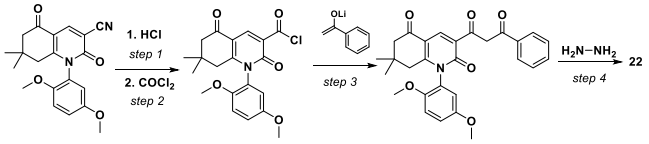
was prepared using Method 10 using 2-(3-phenyl-1*H*-pyrazol-1-yl)acetonitrile N11 in step 2 to afford 1-(2,5-dimethoxyphenyl)-7,7-dimethyl-3-(3-phenyl-1*H*-pyrazol-1-yl)-7,8-dihydroquinoline-2,5-(1*H*,6*H*)-dione as a TFA salt. LCMS: m/z $(M + H)^+$ = 470.2; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.64 (d, J = 2.6 Hz, 1H), 8.51 (s, 1H), 7.96−7.88 (m, 2H), 7.50−7.41 (m, 2H), 7.41−7.32 (m, 1H), 7.22 (d, J = 9.1 Hz, 1H), 7.12 (dd, J = 9.1, 3.1 Hz, 1H), 7.04 (d, J = 3.0 Hz, 1H), 6.96 (d, J = 2.6 Hz, 1H), 3.75 (s, 3H), 3.72 (s, 3H), 2.57 (d, J = 17.5 Hz, 1H), 2.44 (d, J = 4.2 Hz, 2H), 2.16 (d, J = 17.6 Hz, 1H), 0.99 (s, 3H), 0.94 (s, 3H); HRMS (ESI): m/z $(M + H)^+$ calcd for $C_{28}H_{28}N_3O_4$, 470.2074; found, 470.2074. Retention time = 3.66 min.

*1-(2,5-Dimethoxyphenyl)-7,7-dimethyl-3-(4-phenyl-1H-pyrazol-1-yl)-7,8-dihydroquinoline-2,5(1H,6H)-dione (**21**).* This compound was prepared using Method 10 using 2-(4-phenyl-1*H*-pyrazol-1-yl)acetonitrile N12 in step 2 to afford 1-(2,5-dimethoxyphenyl)-7,7-dimethyl-3-(4-phenyl-1*H*-pyrazol-1-yl)-7,8-dihydroquinoline-2,5-(1*H*,6*H*)-dione as a TFA salt. LCMS: m/z $(M + H)^+$ = 470.2; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.95 (d, J = 0.8 Hz, 1H), 8.44 (s, 1H), 8.25 (d, J = 0.8 Hz, 1H), 7.67−7.59 (m, 2H), 7.41−7.31 (m, 2H), 7.26−7.16 (m, 2H), 7.12 (dd, J = 9.1, 3.1 Hz, 1H), 7.04 (d, J = 3.1 Hz, 1H), 3.75 (s, 3H), 3.72 (s, 3H), 2.62−2.50 (m, 1H), 2.43 (d, J = 4.1 Hz, 2H), 2.17 (d, J = 17.6 Hz, 1H), 0.99 (s, 3H), 0.94 (s, 3H); HRMS (ESI): m/z $(M + H)^+$ calcd for $C_{28}H_{28}N_3O_4$, 470.2074; found, 470.208. Retention time = 3.608 min.



*1-(2,5-Dimethoxyphenyl)-7,7-dimethyl-3-(5-phenyl-1H-pyrazol-3-yl)-7,8-dihydroquinoline-2,5(1H,6H)-dione (**22**).* Step 1: A mixture of 1-(2,5-dimethoxyphenyl)-7,7-dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carbonitrile (60 mg, 0.17 mmol) (intermediate after step 3 in the synthesis of compound **18**) in concentrated HCl (3 mL) was heated at 80 °C for 22 h. The reaction mixture was diluted with water, extracted (DCM/MeOH × 3), dried with magnesium sulfate, and concentrated to afford 1-(2,5-dimethoxyphenyl)-7,7-dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxylic acid which was used without further purification; LCMS: m/z $(M + H)^+$ = 372.1. Step 2: To the acid (28 mg, 0.075 mmol) in DCM (3 mL) were added a drop of DMF and oxalyl chloride (0.033 mL, 0.38 mmol). The reaction mixture was stirred at rt for 1.2 h. The reaction mixture was concentrated under a stream of argon, rediluted with DCM, and reconcentrated to afford 1-(2,5-dimethoxyphenyl)-7,7-dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carbonyl chloride; this was verified by LCMS that showed the formation of the methyl ester on the addition of MeOH to an aliquot from the reaction.

Step 3: To a solution of acetophenone (0.026 mL, 0.23 mmol) in THF (1 mL) that had been cooled to −78 °C was added a solution of LiHMDS (1 M THF, 0.225 mL, 0.225 mmol) slowly. The reaction mixture was continued to stir at this temperature for 1 h (faint yellow solution) at which point a solution of 1-(2,5-dimethoxyphenyl)-7,7-dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carbonyl chloride (**VI**) (0.075 mmol) in THF (1.5 mL) was added. The reaction mixture became more yellow and was allowed to warm slowly for 1.5 h. The reaction mixture turned from yellow to red (likely red is doubly deprotonated trione). Step 4: Hydrazine (3 equiv) in ethanol was added and stirring resumed for 1 h. Acetic acid (3 drops) was added, and the reaction mixture turned from red to yellow along with the formation of a precipitate. The reaction mixture was heated at 50 °C for 1 h and stood at rt for 1 week. The crude mixture was diluted with DMSO and purified by reverse-phase chromatography (**Standard Acidic Gradient Method**) to afford as a TFA salt, 1-(2,5-dimethoxyphenyl)-7,7-dimethyl-3-(5-phenyl-1*H*-pyrazol-3-yl)-7,8-dihydroquinoline-2,5(1*H*,6*H*)-dione. LCMS: m/z $(M + H)^+$ = 470.2; ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.14 (s, 1H), 8.48 (s, 1H), 7.81 (d, J = 7.4 Hz, 1H), 7.74−7.66 (m, 1H), 7.45−7.31 (m, 1H), 7.27 (dd, J = 11.1, 6.7 Hz, 2H), 7.19 (d, J = 8.9 Hz, 2H), 7.08 (d, J = 9.4 Hz, 1H), 7.01−6.93 (m, 1H), 6.47 (s, 0H), 3.73 (s, 3H), 3.69 (s, 3H), 2.51 (s, 3H), 2.14 (d, J = 17.8 Hz, 1H), 0.97 (s, 3H), 0.92 (s, 3H); HRMS (ESI): m/z $(M + H)^+$ calcd for $C_{28}H_{28}N_3O_4$, 470.2074; found, 470.2073. Retention time = 3.428 min.

*3-(4′-Chloro-[1,1′-biphenyl]-3-yl)-1-(2,5-dimethoxyphenyl)-7,7-dimethyl-7,8-dihydroquinoline-2,5(1H,6H)-dione (**23**).* This compound was prepared using Method 10 using 2-(4′-chloro-[1,1′-biphenyl]-3-yl)acetonitrile N3 in step 2 to afford 3-(4′-chloro-[1,1′-biphenyl]-3-yl)-1-(2,5-dimethoxyphenyl)-7,7-dimethyl-7,8-dihydroquinoline-2,5(1*H*,6*H*)-dione as a TFA salt. LCMS: m/z $(M + H)^+$ = 514.2; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.12 (s, 1H), 7.99−7.93 (m, 1H), 7.79−7.60 (m, 4H), 7.56−7.45 (m, 3H), 7.19 (d, J = 9.1 Hz, 1H), 7.08 (dd, J = 9.1, 3.1 Hz, 1H), 6.97 (d, J = 3.0 Hz, 1H), 3.74 (s, 3H), 3.71 (s, 3H), 2.97 (s, 3H), 2.60−2.49 (m, 1H), 2.47−2.32 (m, 2H), 2.14 (d, J = 17.5 Hz, 1H), 0.98 (s, 3H), 0.94 (s, 3H); HRMS (ESI): m/z $(M + H)^+$ calcd for $C_{31}H_{29}ClNO_4$, 514.178; found, 514.1777. Retention time = 3.835 min.

*3-([1,1′-Biphenyl]-4-yl)-1-(2,5-dimethoxyphenyl)-7,7-dimethyl-7,8-dihydroquinoline-2,5(1H,6H)-dione (**24**).* This compound was prepared using Method 10 using 2-([1,1′-biphenyl]-4-yl)acetonitrile in step 2 to afford 3-([1,1′-biphenyl]-4-yl)-1-(2,5-dimethoxyphenyl)-7,7-dimethyl-7,8-dihydroquinoline-2,5(1*H*,6*H*)-dione as a TFA salt. LCMS: m/z $(M + H)^+$ = 480.2; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.09 (s, 1H), 7.83−7.75 (m, 2H), 7.74−7.65 (m, 4H), 7.51−7.42 (m, 2H), 7.40−7.31 (m, 1H), 7.20 (d, J = 9.2 Hz, 1H), 7.08 (dd, J = 9.1, 3.1 Hz, 1H), 6.98 (d, J = 3.0 Hz, 1H), 3.74 (s, 3H), 3.72 (s, 3H), 2.45−2.33 (m, 2H), 2.14−2.10 (m, 2H), 0.99 (s, 3H), 0.94 (s, 3H); HRMS (ESI): m/z $(M + H)^+$ calcd for $C_{31}H_{30}NO_4$, 480.2169; found, 480.2176. Retention time = 3.719 min.

*3-(6-(4-Chlorophenyl)pyridin-2-yl)-1-(2,5-dimethoxyphenyl)-7,7-dimethyl-7,8-dihydroquinoline-2,5(1H,6H)-dione (**25**).* This compound was prepared using Method 10 using 2-(6-(4-chlorophenyl)-pyridin-2-yl)acetonitrile N4 in step 2 to afford 3-(6-(4-chlorophenyl)-pyridin-2-yl)-1-(2,5-dimethoxyphenyl)-7,7-dimethyl-7,8-dihydroquinoline-2,5(1*H*,6*H*)-dione as a TFA salt. LCMS: m/z $(M + H)^+$ = 515.1; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.94 (s, 1H), 8.27 (dd, J = 7.2, 1.6 Hz, 1H), 8.16 (d, J = 8.6 Hz, 2H), 7.95−7.85 (m, 2H), 7.59 (d, J = 8.6 Hz, 2H), 7.19 (d, J = 9.1 Hz, 1H), 7.08 (dd, J = 9.1, 3.0 Hz, 1H), 6.99 (d, J = 3.0 Hz, 1H), 3.73 (s, 3H), 3.70 (s, 3H), 2.58 (d, J = 17.6 Hz, 1H), 2.41 (d, J = 7.2 Hz, 2H), 2.15 (d, J = 17.6 Hz, 1H), 0.98 (s, 3H), 0.93 (s, 3H); HRMS (ESI): m/z $(M + H)^+$ calcd for $C_{30}H_{28}ClN_2O_4$, 515.1732; found, 515.175. Retention time = 3.8 min.

*N-(4-Chlorobenzyl)-1-(2,5-dimethoxyphenyl)-7,7-dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide (**26**).* This compound was prepared using Method 10 using *N*-(4-chlorobenzyl)-2-cyanoacetamide in step 2 to afford *N*-(4-chlorobenzyl)-1-(2,5-dimethoxyphenyl)-7,7-dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide as a TFA salt. LCMS: m/z $(M + H)^+$ = 495.2; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.55 (t, J = 6.0 Hz, 1H), 8.79 (s, 1H), 7.40−7.29 (m, 2H), 7.30 (d, J = 8.5 Hz, 2H), 7.19 (d, J = 9.1 Hz, 1H), 7.09 (dd, J = 9.1, 3.1 Hz, 1H), 6.99 (d, J = 3.0 Hz, 1H), 4.54−4.38 (m, 2H), 3.72 (s, 3H), 3.69 (s, 3H), 2.59 (d, J = 17.8 Hz, 1H), 2.42 (d, J = 2.2 Hz, 2H), 2.17 (d, J = 17.8 Hz, 1H), 0.96 (s, 3H), 0.90 (s, 3H); HRMS (ESI): m/z $(M + H)^+$ calcd for $C_{27}H_{28}ClN_2O_5$, 495.1681; found, 495.1691. Retention time = 3.467 min.

*3-(4-(4-Chlorophenyl)thiazol-2-yl)-7,7-dimethyl-1-phenyl-7,8-dihydroquinoline-2,5 (1H,6H)-dione (**27**).* This compound was prepared using Method 10 using aniline in step 3 to afford N4-(4-((1*H*-tetrazol-5-yl)methoxy)-3-chlorophenyl)-N6-([1,1′-biphenyl]-4-yl)pyrimidine-4,6-diamine as a TFA salt. LCMS: m/z $(M + H)^+$ = 461; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.06 (s, 1H), 8.22 (s, 1H), 8.13−8.05 (m, 2H), 7.67−7.51 (m, 5H), 7.47−7.40 (m, 2H), 2.45 (s, 2H), 2.42 (s, 2H), 0.96 (s, 6H); HRMS (ESI): m/z $(M + H)^+$ calcd for $C_{26}H_{22}ClN_2O_2S$, 461.1085; found, 461.1086. Retention time = 3.853 min.

*3-(4-(4-Chlorophenyl)thiazol-2-yl)-1-(2-methoxyphenyl)-7,7-dimethyl-7,8-dihydroquinoline-2,5(1H,6H)-dione (**28**).* This compound was prepared using Method 10 using 2-methoxyaniline in step 3 to afford 3-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2-methoxy-

https://doi.org/10.1021/acs.jmedchem.1c00019
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phenyl)-7,7-dimethyl-7,8-dihydroquinoline-2,5(1*H*,6*H*)-dione as a TFA salt. LCMS: *m*/*z* (M + H)$^+$ = 491; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.05 (s, 1H), 8.22 (s, 1H), 8.13−8.04 (m, 2H), 7.61−7.49 (m, 3H), 7.37 (dd, *J* = 7.7, 1.6 Hz, 1H), 7.31 (dd, *J* = 8.5, 1.2 Hz, 1H), 7.17 (td, *J* = 7.6, 1.2 Hz, 1H), 3.77 (s, 3H), 2.61−2.51 (m, 2H), 2.250−2.20 (m, 2H), 0.98 (s, 3H), 0.94 (s, 3H); HRMS (ESI): *m*/*z* (M + H)$^+$ calcd for $C_{27}H_{24}ClN_2O_3S$, 491.1191; found, 491.1203. Retention time = 3.86 min.

3-(4-(4-Chlorophenyl)thiazol-2-yl)-1-(2-ethoxyphenyl)-7,7-dimethyl-7,8-dihydroquinoline-2,5(1H,6H)-dione (**29**). This compound was prepared using Method 10 using 2-ethoxyaniline in step 3 to afford 3-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2-ethoxyphenyl)-7,7-dimethyl-7,8-dihydroquinoline-2,5(1*H*,6*H*)-dione as a TFA salt. LCMS: *m*/*z* (M + H)$^+$ = 505.1; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.04 (s, 1H), 8.20 (s, 1H), 8.07 (d, *J* = 8.7 Hz, 2H), 7.57−7.48 (m, 3H), 7.34 (dd, *J* = 7.7, 1.7 Hz, 1H), 7.27 (d, *J* = 8.4 Hz, 1H), 7.14 (t, *J* = 7.6 Hz, 1H), 4.06 (q, *J* = 6.9 Hz, 2H), 2.60 (d, *J* = 17.8 Hz, 1H), 2.50 (d, *J* = 16.2 Hz, 1H), 2.40 (d, *J* = 16.3 Hz, 1H), 2.16 (d, *J* = 17.7 Hz, 1H), 1.11 (t, *J* = 6.9 Hz, 3H), 0.97 (s, 3H), 0.93 (s, 3H); HRMS (ESI): *m*/*z* (M + H)$^+$ calcd for $C_{28}H_{26}ClN_2O_3S$, 505.1347; found, 505.1354. Retention time = 3.927 min.

3-(4-(4-Chlorophenyl)thiazol-2-yl)-1-(2-isopropoxyphenyl)-7,7-dimethyl-7,8-dihydroquinoline-2,5(1H,6H)-dione (**30**). This compound was prepared using Method 10 using 2-isopropoxyaniline in step 3 to afford 3-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2-isopropoxyphenyl)-7,7-dimethyl-7,8-dihydroquinoline-2,5(1*H*,6*H*)-dione as a TFA salt. LCMS: *m*/*z* (M + H)$^+$ = 519.2; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.03 (d, *J* = 1.3 Hz, 1H), 8.19 (d, *J* = 1.4 Hz, 1H), 8.07 (d, *J* = 8.6 Hz, 2H), 7.56−7.46 (m, 3H), 7.34 (d, *J* = 7.7 Hz, 1H), 7.29 (d, *J* = 8.4 Hz, 1H), 7.12 (t, *J* = 7.0 Hz, 1H), 4.63 (h, *J* = 6.0 Hz, 1H), 2.62 (d, *J* = 17.7 Hz, 1H), 2.50 (d, *J* = 16.1 Hz, 1H), 2.40 (d, *J* = 16.3 Hz, 1H), 2.15 (d, *J* = 17.7 Hz, 1H), 1.13 (d, *J* = 5.9 Hz, 3H), 1.04 (d, *J* = 6.0 Hz, 3H), 0.97 (s, 3H), 0.93 (s, 3H); HRMS (ESI): *m*/*z* (M + H)$^+$ calcd for $C_{29}H_{28}ClN_2O_3S$, 519.1504; found, 519.1492. Retention time = 4.026 min.

3-(4-(4-Chlorophenyl)thiazol-2-yl)-7,7-dimethyl-1-(o-tolyl)-7,8-dihydroquinoline-2,5(1H,6H)-dione (**31**). This compound was prepared using Method 10 using o-toluidine in step 3 to afford 3-(4-(4-chlorophenyl)thiazol-2-yl)-7,7-dimethyl-1-(*o*-tolyl)-7,8-dihydroquinoline-2,5(1*H*,6*H*)-dione as a TFA salt. LCMS: *m*/*z* (M + H)$^+$ = 475.1; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.07 (s, 1H), 8.21 (s, 1H), 8.07 (d, *J* = 8.2 Hz, 2H), 7.60−7.39 (m, 5H), 7.32 (d, *J* = 7.6 Hz, 1H), 2.56 (d, *J* = 17.8 Hz, 1H), 2.46 (d, *J* = 8.1 Hz, 2H), 2.12 (d, *J* = 17.9 Hz, 1H), 2.00 (s, 3H), 0.97 (s, 3H), 0.94 (s, 3H); HRMS (ESI): *m*/*z* (M + H)$^+$ calcd for $C_{27}H_{24}ClN_2O_2S$, 475.1242; found, 475.1249. Retention time = 3.934 min.

3-(4-(4-Chlorophenyl)thiazol-2-yl)-1-(2-ethylphenyl)-7,7-dimethyl-7,8-dihydroquinoline-2,5(1H,6H)-dione (**32**). This compound was prepared using Method 10 using 2-ethylaniline in step 3 to afford 3-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2-ethylphenyl)-7,7-dimethyl-7,8-dihydroquinoline-2,5(1*H*,6*H*)-dione as a TFA salt. LCMS: *m*/*z* (M + H)$^+$ = 489.2; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.08 (s, 1H), 8.22 (s, 1H), 8.13−8.05 (m, 2H), 7.58−7.49 (m, 4H), 7.49−7.41 (m, 1H), 7.32 (d, *J* = 7.7 Hz, 1H), 2.63−2.50 (m, 1H), 2.49−2.40 (m, 2H), 2.34 (dd, *J* = 15.1, 7.5 Hz, 1H), 2.32−2.19 (m, 1H), 2.14 (d, *J* = 17.9 Hz, 1H), 1.08 (t, *J* = 7.6 Hz, 3H), 0.98 (s, 3H), 0.94 (s, 3H); HRMS (ESI): *m*/*z* (M + H)$^+$ calcd for $C_{28}H_{26}ClN_2O_2S$, 489.1398; found, 489.1417. Retention time = 4.023 min.

3-(4-(4-Chlorophenyl)thiazol-2-yl)-1-(2-isopropylphenyl)-7,7-dimethyl-7,8-dihydroquinoline-2,5(1H,6H)-dione (**33**). This compound was prepared using Method 10 using 2-isopropylaniline in step 3 to afford 3-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2-isopropylphenyl)-7,7-dimethyl-7,8-dihydroquinoline-2,5(1*H*,6*H*)-dione as a TFA salt. LCMS: *m*/*z* (M + H)$^+$ = 503.1; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.07 (s, 1H), 8.20 (s, 1H), 8.07 (d, *J* = 8.3 Hz, 2H), 7.64−7.49 (m, 4H), 7.46−7.37 (m, 1H), 7.28 (d, *J* = 7.4 Hz, 1H), 2.63 (d, *J* = 17.9 Hz, 1H), 2.55−2.38 (m, 3H), 2.08 (d, *J* = 17.9 Hz, 1H), 1.14 (d, *J* = 6.8 Hz, 3H), 1.06 (d, *J* = 6.8 Hz, 3H), 0.97 (s, 3H), 0.92 (s, 3H); HRMS (ESI): *m*/*z* (M + H)$^+$ calcd for $C_{29}H_{28}ClN_2O_2S$, 503.1555; found, 503.1558. Retention time = 4.121 min.

1-([1,1′-Biphenyl]-2-yl)-3-(4-(4-chlorophenyl)thiazol-2-yl)-7,7-dimethyl-7,8-dihydroquinoline-2,5(1H,6H)-dione (**34**). This compound was prepared using Method 10 using [1,1′-biphenyl]-2-amine in step 3 to afford 1-([1,1′-biphenyl]-2-yl)-3-(4-(4-chlorophenyl)thiazol-2-yl)-7,7-dimethyl-7,8-dihydroquinoline-2,5-(1*H*,6*H*)-dione as a TFA salt. LCMS: *m*/*z* (M + H)$^+$ = 537.2; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.92 (s, 1H), 8.22 (s, 1H), 8.13−7.99 (m, 2H), 7.75−7.58 (m, 2H), 7.58−7.44 (m, 2H), 7.24 (qq, *J* = 7.1, 3.8, 3.0 Hz, 7H), 2.56 (d, *J* = 18.0 Hz, 1H), 2.40 (s, 1H), 2.18 (d, *J* = 16.2 Hz, 1H), 2.00 (d, *J* = 18.0 Hz, 1H), 0.91 (s, 3H), 0.48 (s, 3H); HRMS (ESI): *m*/*z* (M + H)$^+$ calcd for $C_{32}H_{26}ClN_2O_2S$, 537.139; found, 537.1398. Retention time = 4.037 min.

3-(4-(4-Chlorophenyl)thiazol-2-yl)-1-(2-hydroxyphenyl)-7,7-dimethyl-7,8-dihydroquinoline-2,5(1H,6H)-dione (**35**). This compound was prepared using Method 10 using 2-aminophenol in step 3 to afford 3-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2-hydroxyphenyl)-7,7-dimethyl-7,8-dihydro-quinoline-2,5(1*H*,6*H*)-dione as a TFA salt. LCMS: *m*/*z* (M + H)$^+$ = 477.1; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.14 (s, 1H), 9.03 (s, 1H), 8.20 (s, 1H), 8.07 (d, *J* = 8.4 Hz, 2H), 7.52 (d, *J* = 8.3 Hz, 2H), 7.41−7.33 (m, 1H), 7.24 (dd, *J* = 7.9, 1.6 Hz, 1H), 7.07 (d, *J* = 8.2 Hz, 1H), 6.99 (t, *J* = 7.6 Hz, 1H), 2.57 (d, *J* = 17.8 Hz, 1H), 2.45 (s, 2H), 2.29 (d, *J* = 17.7 Hz, 1H), 0.97 (s, 3H), 0.94 (s, 3H); HRMS (ESI): *m*/*z* (M + H)$^+$ calcd for $C_{26}H_{22}ClN_2O_3S$, 477.1034; found, 477.1046. Retention time = 3.642 min.

3-(4-(4-Chlorophenyl)thiazol-2-yl)-7,7-dimethyl-1-(2-phenoxyphenyl)-7,8-dihydroquinoline-2,5(1H,6H)-dione (**36**). This compound was prepared using Method 10 using 2-phenoxyaniline in step 3 to afford 3-(4-(4-chlorophenyl)thiazol-2-yl)-7,7-dimethyl-1-(2-phenoxyphenyl)-7,8-dihydroquinoline-2,5(1*H*,6*H*)-dione as a TFA salt. LCMS: *m*/*z* (M + H)$^+$ = 553.1; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.99 (s, 1H), 8.20 (s, 1H), 8.05 (d, *J* = 8.3 Hz, 2H), 7.60−7.48 (m, 4H), 7.34 (dt, *J* = 18.2, 7.6 Hz, 3H), 7.08 (dt, *J* = 7.6, 3.3 Hz, 2H), 6.98 (d, *J* = 8.0 Hz, 2H), 2.68 (d, *J* = 17.8 Hz, 1H), 2.53−2.35 (m, 3H), 1.01 (s, 3H), 0.89 (s, 3H); HRMS (ESI): *m*/*z* (M + H)$^+$ calcd for $C_{32}H_{26}ClN_2O_3S$, 553.1347; found, 553.1347. Retention time = 4.048 min.

1-(2-(Benzyloxy)phenyl)-3-(4-(4-chlorophenyl)thiazol-2-yl)-7,7-dimethyl-7,8-dihydroquinoline-2,5(1H,6H)-dione (**37**). This compound was prepared using Method 10 using 2-(benzyloxy)aniline in step 3 to afford 1-(2-(benzyloxy)phenyl)-3-(4-(4-chlorophenyl)-thiazol-2-yl)-7,7-dimethyl-7,8-dihydroquinoline-2,5(1*H*,6*H*)-dione as a TFA salt. LCMS: *m*/*z* (M + H)$^+$ = 567.1; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.02 (s, 1H), 8.21 (s, 1H), 8.07 (d, *J* = 8.5 Hz, 2H), 7.58−7.49 (m, 3H), 7.43−7.34 (m, 2H), 7.19 (dd, *J* = 7.4, 3.7 Hz, 6H), 5.22−5.09 (m, 2H), 2.58 (d, *J* = 17.8 Hz, 1H), 2.46 (d, *J* = 16.1 Hz, 1H), 2.35 (d, *J* = 16.2 Hz, 1H), 2.17 (d, *J* = 17.9 Hz, 1H), 0.95 (s, 3H), 0.82 (s, 3H); HRMS (ESI): *m*/*z* (M + H)$^+$ calcd for $C_{33}H_{28}ClN_2O_3S$, 567.1504; found, 567.1516. Retention time = 3.99 min.

1-(2-Aminophenyl)-3-(4-(4-chlorophenyl)thiazol-2-yl)-7,7-dimethyl-7,8-dihydroquinoline-2,5(1H,6H)-dione (**38**). This compound was prepared using Method 10 using benzene-1,2-diamine in step 3 to afford 1-(2-aminophenyl)-3-(4-(4-chlorophenyl)thiazol-2-yl)-7,7-dimethyl-7,8-dihydroquinoline-2,5(1*H*,6*H*)-dione as a TFA salt. LCMS: *m*/*z* (M + H)$^+$ = 476.1; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.00 (s, 1H), 8.18 (s, 1H), 8.07 (d, *J* = 8.2 Hz, 2H), 7.52 (d, *J* = 8.2 Hz, 2H), 7.18 (t, *J* = 7.8 Hz, 1H), 6.96 (d, *J* = 7.1 Hz, 1H), 6.83 (d, *J* = 8.1 Hz, 1H), 6.66 (t, *J* = 7.5 Hz, 1H), 5.30 (s, 2H), 2.57 (d, *J* = 17.7 Hz, 1H), 2.47 (s, 1H), 2.35 (d, *J* = 16.3 Hz, 1H), 2.25 (d, *J* = 17.7 Hz, 1H), 0.96 (s, 6H); HRMS (ESI): *m*/*z* (M + H)$^+$ calcd for $C_{26}H_{23}ClN_3O_2S$, 476.1194; found, 476.1207. Retention time = 3.721 min.

3-(4-(4-Chlorophenyl)thiazol-2-yl)-7,7-dimethyl-1-(2-(methylamino)phenyl)-7,8-dihydroquinoline-2,5(1H,6H)-dione (**39**). This compound was prepared using Method 10 using N1-methylbenzene-1,2-diamine in step 3 to afford 3-(4-(4-chlorophenyl)-thiazol-2-yl)-7,7-dimethyl-1-(2-(methylamino)phenyl)-7,8-dihydroquinoline-2,5(1*H*,6*H*)-dione as a TFA salt. LCMS: *m*/*z* (M + H)$^+$ = 490.1; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.01 (d, *J* = 1.5 Hz, 1H), 8.19 (d, *J* = 1.4 Hz, 1H), 8.12−8.02 (m, 2H), 7.59−7.47 (m, 2H),

https://doi.org/10.1021/acs.jmedchem.1c00019
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7.37−7.27 (m, 1H), 7.01 (dd, J = 7.7, 1.6 Hz, 1H), 6.80−6.64 (m, 2H), 5.51 (q, J = 4.7 Hz, 1H), 2.69−2.51 (m, 5H), 2.40−2.30 (m, 1H), 2.13 (d, J = 17.7 Hz, 1H), 0.94 (d, J = 7.9 Hz, 6H); HRMS (ESI): m/z $(M + H)^+$ calcd for $C_{27}H_{25}ClN_3O_2S$, 490.1351; found, 490.136. Retention time = 3.845 min.

3-(4-(4-Chlorophenyl)thiazol-2-yl)-1-(2-(dimethylamino)-phenyl)-7,7-dimethyl-7,8-dihydroquinoline-2,5(1H,6H)-dione (**40**). This compound was prepared using Method 10 using N1,N1-dimethylbenzene-1,2-diamine in step 3 to afford 3-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2-(dimethylamino)phenyl)-7,7-dimethyl-7,8-dihydroquinoline-2,5(1*H*,6*H*)-dione as a TFA salt. LCMS: m/z $(M + H)^+$ = 504.2; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.06 (d, J = 1.4 Hz, 1H), 8.21 (d, J = 1.5 Hz, 1H), 8.13−8.02 (m, 2H), 7.57−7.42 (m, 2H), 7.24 (ddd, J = 7.7, 5.0, 1.5 Hz, 3H), 7.15 (td, J = 7.5, 1.4 Hz, 1H), 2.52 (d, J = 1.4 Hz, 7H), 2.44 (s, 1H), 2.33−2.26 (m, 2H), 0.94 (d, J = 3.8 Hz, 6H); HRMS (ESI): m/z $(M + H)^+$ calcd for $C_{28}H_{27}ClN_3O_2S$, 504.1507; found, 504.1516. Retention time = 4.019 min.

2-(3-(4-(4-Chlorophenyl)thiazol-2-yl)-7,7-dimethyl-2,5-dioxo-5,6,7,8-tetrahydro-quinolin-1(2H)-yl)benzoic Acid (**41**). This compound was prepared using Method 10 using 2-aminobenzoic acid in step 3 to afford 2-(3-(4-(4-chlorophenyl)thiazol-2-yl)-7,7-dimethyl-2,5-dioxo-5,6,7,8-tetrahydroquinolin-1(2*H*)-yl)benzoic acid as a TFA salt. LCMS: m/z $(M + H)^+$ = 505.1; ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.21 (s, 1H), 9.04 (s, 1H), 8.19 (s, 1H), 8.13 (d, J = 7.5 Hz, 1H), 8.07 (d, J = 8.6 Hz, 2H), 7.80 (s, 1H), 7.68 (t, J = 7.5 Hz, 1H), 7.52 (d, J = 8.6 Hz, 2H), 7.47 (d, J = 7.6 Hz, 1H), 2.66−2.55 (m, 1H), 2.41−2.27 (m, 2H), 2.14 (d, J = 17.7 Hz, 1H), 0.97 (s, 3H), 0.89 (s, 3H); HRMS (ESI): m/z $(M + H)^+$ calcd for $C_{27}H_{22}ClN_2O_4S$, 505.0983; found, 505.1001. Retention time = 3.523 min.

3-(4-(4-Chlorophenyl)thiazol-2-yl)-1-(2-fluorophenyl)-7,7-dimethyl-7,8-dihydroquinoline-2,5(1H,6H)-dione (**42**). This compound was prepared using Method 10 using 2-fluoroaniline in step 3 to afford 3-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2-fluorophenyl)-7,7-dimethyl-7,8-dihydroquinoline-2,5(1*H*,6*H*)-dione as a TFA salt. LCMS: m/z $(M + H)^+$ = 479.1; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.06 (s, 1H), 8.23 (s, 1H), 8.07 (d, J = 8.4 Hz, 2H), 7.71−7.49 (m, 5H), 7.49−7.42 (m, 1H), 2.61 (d, J = 17.8 Hz, 1H), 2.48 (d, J = 2.0 Hz, 2H), 2.31 (d, J = 17.7 Hz, 1H), 0.99 (s, 3H), 0.94 (s, 3H); HRMS (ESI): m/z $(M + H)^+$ calcd for $C_{26}H_{21}ClFN_2O_2S$, 479.0991; found, 479.0991. Retention time = 3.885 min.

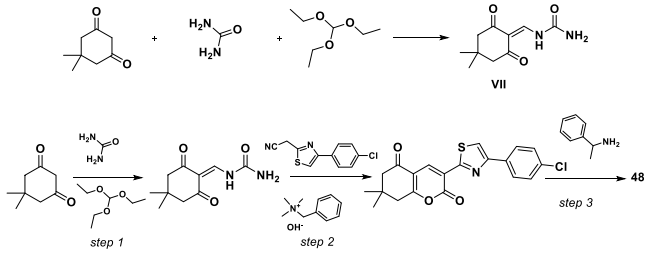
1-(2-Chlorophenyl)-3-(4-(4-chlorophenyl)thiazol-2-yl)-7,7-dimethyl-7,8-dihydroquinoline-2,5(1H,6H)-dione (**43**). This compound was prepared using Method 10 using 2-chloroaniline in step 3 to afford 1-(2-chlorophenyl)-3-(4-(4-chlorophenyl)thiazol-2-yl)-7,7-dimethyl-7,8-dihydroquinoline-2,5(1*H*,6*H*)-dione as a TFA salt. LCMS: m/z $(M + H)^+$ = 495.1; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.07 (s, 1H), 8.23 (s, 1H), 8.07 (d, J = 8.5 Hz, 2H), 7.83−7.76 (m, 1H), 7.63 (dd, J = 3.4, 1.9 Hz, 3H), 7.53 (d, J = 8.3 Hz, 2H), 2.60 (d, J = 17.8 Hz, 1H), 2.47 (s, 2H), 2.12 (d, J = 17.8 Hz, 1H), 0.98 (s, 3H), 0.95 (s, 3H); HRMS (ESI): m/z $(M + H)^+$ calcd for $C_{26}H_{21}Cl_2N_2O_2S$, 495.0695; found, 495.0716. Retention time = 3.956 min.

3-(4-(4-Chlorophenyl)thiazol-2-yl)-7,7-dimethyl-1-(2-(trifluoromethyl)phenyl)-7,8-dihydroquinoline-2,5(1H,6H)-dione (**44**). This compound was prepared using Method 10 using 2-(trifluoromethyl)aniline in step 3 to afford 3-(4-(4-chlorophenyl)-thiazol-2-yl)-7,7-dimethyl-1-(2-(trifluoromethyl)phenyl)-7,8-dihydroquinoline-2,5(1*H*,6*H*)-dione as a TFA salt. LCMS: m/z $(M + H)^+$ = 529.1; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.05 (d, J = 1.5 Hz, 1H), 8.23 (d, J = 1.6 Hz, 1H), 8.10−8.01 (m, 3H), 7.97 (t, J = 7.8 Hz, 1H), 7.84 (t, J = 7.8 Hz, 1H), 7.72 (d, J = 7.9 Hz, 1H), 7.57−7.48 (m, 2H), 2.70−2.55 (m, 2H), 2.42−2.27 (m, 1H), 2.10 (d, J = 17.9 Hz, 1H), 0.98 (s, 3H), 0.90 (s, 3H); HRMS (ESI): m/z $(M + H)^+$ calcd for $C_{27}H_{21}ClF_3N_2O_2S$, 529.0959; found, 529.0976. Retention time = 3.936 min.

3-(4-(4-Chlorophenyl)thiazol-2-yl)-7,7-dimethyl-1-(2-(trifluoromethoxy)phenyl)-7,8-dihydroquinoline-2,5(1H,6H)-dione (**45**). This compound was prepared using Method 10 using 2-(trifluoromethoxy)aniline in step 3 to afford 3-(4-(4-chlorophenyl)-thiazol-2-yl)-7,7-dimethyl-1-(2-(trifluoromethoxy)phenyl)-7,8-dihydroquinoline-2,5(1*H*,6*H*)-dione as a TFA salt. LCMS: m/z $(M + Na)^+$ = 545.1; HRMS (ESI): m/z $(M + H)^+$ calcd for $C_{27}H_{20}ClF_3N_2NaO_3S$, 567.0727; found, 567.0755. Retention time = 3.96 min.

3-(4-(4-Chlorophenyl)thiazol-2-yl)-7,7-dimethyl-1-(pyridin-2-yl)-7,8-dihydroquinoline-2,5(1H,6H)-dione (**46**). This compound was prepared using Method 10 using pyridin-2-amine in step 3 to afford 3-(4-(4-chlorophenyl)thiazol-2-yl)-7,7-dimethyl-1-(pyridin-2-yl)-7,8-dihydroquinoline-2,5(1*H*,6*H*)-dione as a TFA salt. LCMS: m/z $(M + H)^+$ = 462.1; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.07 (s, 1H), 8.72 (ddd, J = 4.9, 1.9, 0.8 Hz, 1H), 8.24 (s, 1H), 8.16 (td, J = 7.7, 1.9 Hz, 1H), 8.12−8.06 (m, 2H), 7.73−7.62 (m, 2H), 7.58−7.50 (m, 2H), 2.60 (d, J = 17.8 Hz, 1H), 2.47 (s, 2H), 2.12 (d, J = 17.8 Hz, 1H), 0.98 (s, 3H), 0.96 (s, 3H); HRMS (ESI): m/z $(M + H)^+$ calcd for $C_{25}H_{21}ClN_3O_2S$, 462.1038; found, 462.1057. Retention time = 3.723 min.

3-(4-(4-Chlorophenyl)thiazol-2-yl)-1-(2-methoxybenzyl)-7,7-dimethyl-7,8-dihydroquinoline-2,5(1H,6H)-dione (**47**). This compound was prepared using Method 10 using (2-methoxyphenyl)-methanamine in step 3 to afford 3-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2-methoxybenzyl)-7,7-dimethyl-7,8-dihydroquinoline-2,5(1*H*,6*H*)-dione as a TFA salt. LCMS: m/z $(M + H)^+$ = 505.1; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.03 (s, 1H), 8.20 (s, 1H), 8.07 (d, J = 8.6 Hz, 2H), 7.52 (d, J = 8.6 Hz, 2H), 7.27 (t, J = 7.9 Hz, 1H), 7.08 (d, J = 8.3 Hz, 1H), 6.83 (t, J = 7.5 Hz, 1H), 6.60 (d, J = 7.6 Hz, 1H), 5.39 (s, 2H), 3.88 (s, 3H), 2.85 (s, 2H), 2.46 (d, J = 11.6 Hz, 2H), 0.94 (s, 6H); HRMS (ESI): m/z $(M + H)^+$ calcd for $C_{28}H_{26}ClN_2O_3S$, 505.1347; found, 505.1334. Retention time = 3.997 min.



3-(4-(4-Chlorophenyl)thiazol-2-yl)-7,7-dimethyl-1-(1-phenylethyl)-7,8-dihydroquinoline-2,5(1H,6H)-dione (**48**). Step 1 1-((4,4-Dimethyl-2,6-dioxocyclohexylidene)methyl)urea: To a solution of triethyl orthoformate (0.89 mL, 5.35 mmol) and urea (214 mg, 3.57 mmol) in DMF (1.5 mL) was added isopropanol (10 mL). The resulting solution was heated at 80 °C for 2 h and cooled to 0 °C. A white precipitate formed and was removed by filtration (washed with water and hexanes). 1-((4,4-Dimethyl-2,6-dioxocyclohexylidene)-methyl)urea was isolated as a white solid (480 mg, 64%); LCMS: m/z $(M + H)^+$ = 211.1.

Step 2 3-(4-(4-Chlorophenyl)thiazol-2-yl)-7,7-dimethyl-7,8-dihydro-2*H*-chromene-2,5(6*H*)-dione: A mixture of 1-((4,4-dimethyl-2,6-dioxocyclohexylidene)methyl)urea (40 mg, 0.19 mmol), nitrile **1** (54 mg, 0.23 mmol), and a solution of benzyltrimethylammonium hydroxide (40% in MeOH, 0.113 mL, 0.285 mmol) in DMF/MeOH (1:1−1 mL) was heated at 140 °C for 1 h 20 min. Upon cooling, the mixture was diluted with water, acidified at 0 °C with 1 N HCl, stirred overnight, and filtered to afford a brown solid (3-(4-(4-chlorophenyl)thiazol-2-yl)-7,7-dimethyl-7,8-dihydro-2*H*-chromene-2,5(6*H*)-dione, 61 mg, 91%); LCMS: m/z $(M + H)^+$ = 386.0.

Step 3 (**48**): A solution of 3-(4-(4-chlorophenyl)thiazol-2-yl)-7,7-dimethyl-7,8-dihydro-2*H*-chromene-2,5(6*H*)-dione (34 mg, 0.088 mmol) and 1-phenylethan-1-amine (20.6 mg, 0.17 mmol) in DMF (1.0 mL) was heated at 150 °C for 2 h. The crude mixture was diluted with DMSO and purified by reverse-phase chromatography (**Standard Acidic Gradient Method**) to afford as a TFA salt, 3-(4-(4-chlorophenyl)thiazol-2-yl)-7,7-dimethyl-1-(1-phenylethyl)-7,8-dihydroquinoline-2,5(1*H*,6*H*)-dione. LCMS: m/z $(M + H)^+$ = 489.1; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.00 (s, 1H), 8.19 (s, 1H), 8.09−8.02 (m, 2H), 7.55−7.48 (m, 2H), 7.34 (t, J = 7.5 Hz, 2H), 7.30−7.19 (m, 3H), 3.06 (m, 1H), 2.69−2.51 (m, 2H), 2.13 (d, J = 17.7 Hz, 2H),1.93 (d, J = 6.9 Hz, 3H), 0.95 (s, 3, H), 0.94 (s, 3H); HRMS

https://doi.org/10.1021/acs.jmedchem.1c00019
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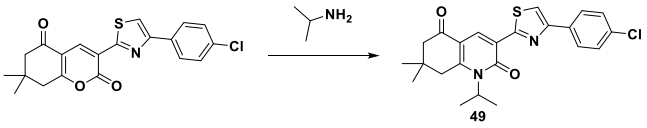
(ESI): m/z (M + H)$^+$ calcd for $C_{28}H_{26}ClN_2O_2S$, 489.1398; found, 489.1375. Retention time = 4.042 min.



*3-(4-(4-Chlorophenyl)thiazol-2-yl)-1-isopropyl-7,7-dimethyl-7,8-dihydroquinoline-2,5(1H,6H)-dione (**49**).* This compound was prepared according to the method used to make compound **48** using isopropylamine in step 3 to afford 3-(4-(4-chlorophenyl)thiazol-2-yl)-1-isopropyl-7,7-dimethyl-7,8-dihydroquinoline-2,5(1*H*,6*H*)-dione as a TFA salt. LCMS: m/z (M + H)$^+$ = 427.1; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.94 (s, 1H), 8.21 (s, 1H), 8.12−8.03 (m, 2H), 7.57−7.48 (m, 2H), 4.81 (br s, 1H), 3.08 (s, 2H), 2.45 (s, 2H), 1.60 (d, J = 6.7 Hz, 6H), 1.08 (s, 6H); HRMS (ESI): m/z (M + H)$^+$ calcd for $C_{23}H_{24}ClN_2O_2S$, 427.1242; found, 427.1242. Retention time = 3.952 min.

*3-(4-(4-Chlorophenyl)thiazol-2-yl)-1-cyclohexyl-7,7-dimethyl-7,8-dihydroquinoline-2,5(1H,6H)-dione (**50**).* This compound was prepared using Method 10 using cyclohexanamine in step 3 to afford 3-(4-(4-chlorophenyl)thiazol-2-yl)-1-cyclohexyl-7,7-dimethyl-7,8-dihydroquinoline-2,5(1*H*,6*H*)-dione as a TFA salt. LCMS: m/z (M + H)$^+$ = 467.2; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.93 (s, 1H), 8.19 (s, 1H), 8.05 (d, J = 8.3 Hz, 2H), 7.51 (d, J = 8.4 Hz, 2H), 4.35−4.20 (m, 1H), 2.73−2.66 (m, 3H), 2.54−2.39 (m, 2H), 1.81 (d, J = 12.8 Hz, 2H), 1.68 (t, J = 14.3 Hz, 3H), 1.43 (d, J = 13.2 Hz, 2H), 1.22 (d, J = 9.9 Hz, 2H), 1.07 (s, 6H); HRMS (ESI): m/z (M + H)$^+$ calcd for $C_{26}H_{28}ClN_2O_2S$, 467.1555; found, 467.1555. Retention time = 4.193 min.

*Methyl 2-(3-(4-(4-Chlorophenyl)thiazol-2-yl)-7,7-dimethyl-2,5-dioxo-5,6,7,8-tetrahydro-quinolin-1(2H)-yl)thiophene-3-carboxylate (**51**).* This compound was prepared using Method 10 using methyl 2-aminothiophene-3-carboxylate in step 3 to afford methyl 2-(3-(4-(4-chlorophenyl)thiazol-2-yl)-7,7-dimethyl-2,5-dioxo-5,6,7,8-tetrahydroquinolin-1(2*H*)-yl)thiophene-3-carboxylate as a TFA salt. LCMS: m/z (M + H)$^+$ = 525.07; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.34 (s, 1H), 8.02−7.95 (m, 2H), 7.61 (d, J = 5.7 Hz, 1H), 7.58 (s, 1H), 7.50 (t, J = 6.0 Hz, 1H), 7.45−7.39 (m, 2H), 3.71 (s, 3H), 2.61−2.45 (m, 4H), 1.08 (d, J = 9.4 Hz, 6H). Aliphatic region complicated significantly by amide rotamers; HRMS (ESI): m/z (M + H)$^+$ calcd for $C_{26}H_{22}ClN_2O_4S_2$, 525.0704; found, 525.071. Retention time = 3.812 min.

*2-(3-(4-(4-Chlorophenyl)thiazol-2-yl)-7,7-dimethyl-2,5-dioxo-5,6,7,8-tetrahydroquinolin-1(2H)-yl)thiophene-3-carbonitrile (**52**).* This compound was prepared using Method 10 using 2-aminothiophene-3-carbonitrile in step 3 to afford 2-(3-(4-(4-chlorophenyl)thiazol-2-yl)-7,7-dimethyl-2,5-dioxo-5,6,7,8-tetrahydroquinolin-1(2*H*)-yl)thiophene-3-carbonitrile as a TFA salt. LCMS: m/z (M + H)$^+$ = 492.1; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.04 (s, 1H), 8.27 (s, 1H), 8.08 (d, J = 8.7 Hz, 2H), 8.04 (d, J = 5.7 Hz, 1H), 7.65 (d, J = 5.7 Hz, 1H), 7.53 (d, J = 8.6 Hz, 2H), 2.68 (d, J = 17.8 Hz, 2H), 2.51 (m, 2H), 1.02 (s, 3H), 1.00 (s, 3H); HRMS (ESI): m/z (M + H)$^+$ calcd for $C_{25}H_{19}ClN_3O_2S_2$, 492.0602; found, 492.0606. Retention time = 3.776 min.

*3-(4-(4-Chlorophenyl)thiazol-2-yl)-1-(2,3-dimethoxyphenyl)-7,7-dimethyl-7,8-dihydroquinoline-2,5(1H,6H)-dione (**53**).* This compound was prepared using Method 10 using 2,3-dimethoxyaniline in step 3 to afford 3-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2,3-dimethoxyphenyl)-7,7-dimethyl-7,8-dihydroquinoline-2,5(1*H*,6*H*)-dione as a TFA salt. LCMS: m/z (M + H)$^+$ = 521.13; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.36 (s, 1H), 8.00 (d, J = 7.8 Hz, 2H), 7.57 (d, J = 0.9 Hz, 1H), 7.42 (d, J = 7.7 Hz, 2H), 7.26 (s, 1H), 7.12 (d, J = 8.4 Hz, 1H), 6.81 (d, J = 7.9 Hz, 1H), 3.97 (d, J = 0.6 Hz, 3H), 3.76 (d, J = 0.9 Hz, 3H), 2.53−2.36 (m, 4H), 1.07 (t, J = 9.8 Hz, 6H). Aliphatic region complicated significantly by amide rotamers; HRMS (ESI): m/z (M + H)$^+$ calcd for $C_{28}H_{26}ClN_2O_4S$, 521.1296; found, 521.1272. Retention time = 3.908 min.

*3-(4-(4-Chlorophenyl)thiazol-2-yl)-1-(2,4-dimethoxyphenyl)-7,7-dimethyl-7,8-dihydroquinoline-2,5(1H,6H)-dione (**54**).* This compound was prepared using Method 10 using 2,4-dimethoxyaniline in step 3 to afford 3-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2,4-dimethoxyphenyl)-7,7-dimethyl-7,8-dihydroquinoline-2,5(1*H*,6*H*)-dione as a TFA salt. LCMS: m/z (M + H)$^+$ = 521.13; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.33 (s, 1H), 8.03−7.96 (m, 2H), 7.56 (s, 1H), 7.42 (dd, J = 8.8, 2.2 Hz, 2H), 7.11 (d, J = 9.1 Hz, 1H), 6.70−6.64 (m, 2H), 3.90 (s, 3H), 3.78 (s, 3H), 2.55−2.45 (m, 3H), 2.34 (d, J = 17.9 Hz, 1H), 1.06 (d, J = 6.4 Hz, 6H). Aliphatic region complicated significantly by amide rotamers; HRMS (ESI): m/z (M + H)$^+$ calcd for $C_{28}H_{26}ClN_2O_4S$, 521.1296; found, 521.1308. Retention time = 3.882 min.

*3-(4-(4-Chlorophenyl)thiazol-2-yl)-1-(2,6-dimethoxyphenyl)-7,7-dimethyl-7,8-dihydroquinoline-2,5(1H,6H)-dione (**55**).* This compound was prepared using Method 10 using 2,6-dimethoxyaniline in step 3 to afford 3-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2,6-dimethoxyphenyl)-7,7-dimethyl-7,8-dihydroquinoline-2,5(1*H*,6*H*)-dione as a TFA salt. LCMS: m/z (M + H)$^+$ = 521.13; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.05 (s, 1H), 8.22 (s, 1H), 8.13−8.04 (m, 2H), 7.58−7.49 (m, 3H), 6.91 (d, J = 8.6 Hz, 2H), 3.75 (s, 6H), 2.52−2.46 (m, 3H), 2.35 (d, J = 17.9 Hz, 1H), 0.96 (s, 6H); HRMS (ESI): m/z (M + H)$^+$ calcd for $C_{28}H_{26}ClN_2O_4S$, 521.1296; found, 521.1318. Retention time = 3.864 min.

*3-(3-(4-(4-Chlorophenyl)thiazol-2-yl)-7,7-dimethyl-2,5-dioxo-5,6,7,8-tetrahydroquinolin-1(2H)-yl)-2-methoxybenzoic Acid (**56**).* This compound was prepared using Method 10 using 3-amino-2-methoxybenzoic acid in step 3 to afford 3-(3-(4-(4-chlorophenyl)-thiazol-2-yl)-7,7-dimethyl-2,5-dioxo-5,6,7,8-tetrahydroquinolin-1(2*H*)-yl)-2-methoxybenzoic acid as a TFA salt. LCMS: m/z (M + H)$^+$ = 535.1; ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.33 (s, 1H), 9.08 (s, 1H), 8.24 (s, 1H), 8.13−8.04 (m, 2H), 7.93 (dd, J = 7.8, 1.8 Hz, 1H), 7.64 (d, J = 7.4 Hz, 1H), 7.58−7.49 (m, 2H), 7.42 (t, J = 7.8 Hz, 1H), 3.62 (s, 3H), 2.57 (d, J = 17.8 Hz, 1H), 2.53 (s, 1H), 2.46−2.40 (m, 1H), 2.32 (d, J = 17.8 Hz, 1H), 0.99 (s, 3H), 0.96 (s, 3H); HRMS (ESI): m/z (M + H)$^+$ calcd for $C_{28}H_{24}ClN_2O_5S$, 535.1089; found, 535.1078. Retention time = 3.604 min.

*4-(3-(4-(4-Chlorophenyl)thiazol-2-yl)-7,7-dimethyl-2,5-dioxo-5,6,7,8-tetrahydroquinolin-1(2H)-yl)-3-methoxybenzoic Acid (**57**).* This compound was prepared using Method 10 using 4-amino-3-methoxybenzoic acid in step 3 to afford 4-(3-(4-(4-chlorophenyl)-thiazol-2-yl)-7,7-dimethyl-2,5-dioxo-5,6,7,8-tetrahydroquinolin-1(2*H*)-yl)-3-methoxybenzoic acid as a TFA salt. LCMS: m/z (M + H)$^+$ = 535.1; ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.37 (s, 1H), 9.06 (s, 1H), 8.23 (s, 1H), 8.12−8.05 (m, 2H), 7.75 (d, J = 7.8 Hz, 2H), 7.53 (dd, J = 8.2, 6.3 Hz, 3H), 3.84 (s, 3H), 2.62−2.50 (m, 2H), 2.49−2.40 (m, 1H), 2.22 (d, J = 17.7 Hz, 1H), 0.99 (s, 3H), 0.94 (s, 3H); HRMS (ESI): m/z (M + H)$^+$ calcd for $C_{28}H_{24}ClN_2O_5S$, 535.1089; found, 535.1099. Retention time = 3.643 min.

*3-(3-(4-(4-Chlorophenyl)thiazol-2-yl)-7,7-dimethyl-2,5-dioxo-5,6,7,8-tetrahydroquinolin-1(2H)-yl)-4-methoxybenzoic Acid (**58**).* This compound was prepared using Method 10 using 3-amino-4-methoxybenzoic acid in **step 3** to afford 3-(3-(4-(4-chlorophenyl)-thiazol-2-yl)-7,7-dimethyl-2,5-dioxo-5,6,7,8-tetrahydroquinolin-1(2*H*)-yl)-4-methoxybenzoic acid as a TFA salt. LCMS: m/z (M + H)$^+$ = 535.1; ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.01 (s, 1H), 9.05 (s, 1H), 8.23 (s, 1H), 8.15 (dd, J = 8.7, 2.2 Hz, 1H), 8.12−8.05 (m, 2H), 7.94 (d, J = 2.2 Hz, 1H), 7.57−7.51 (m, 2H), 7.41 (d, J = 8.8 Hz, 1H), 3.86 (s, 3H), 2.62 (d, J = 17.9 Hz, 1H), 2.55−2.49 (m, 1H), 2.47−2.39 (m, 1H), 2.19 (d, J = 17.8 Hz, 1H), 0.99 (s, 3H), 0.94 (s, 3H); HRMS (ESI): m/z (M + H)$^+$ calcd for $C_{28}H_{24}ClN_2O_5S$, 535.1089; found, 535.1112. Retention time = 3.596 min.

*2-(3-(4-(4-Chlorophenyl)thiazol-2-yl)-7,7-dimethyl-2,5-dioxo-5,6,7,8-tetrahydroquinolin-1(2H)-yl)-3-methoxybenzoic Acid (**59**).* This compound was prepared using Method 10 using 2-amino-3-methoxybenzoic acid in **step 3** to afford 2-(3-(4-(4-chlorophenyl)-thiazol-2-yl)-7,7-dimethyl-2,5-dioxo-5,6,7,8-tetrahydroquinolin-1(2*H*)-yl)-3-methoxybenzoic acid as a TFA salt. LCMS: m/z (M + H)$^+$ = 535.1; ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.19 (s, 1H), 9.05 (s, 1H), 8.21 (s, 1H), 8.12−8.04 (m, 2H), 7.76−7.65 (m, 2H), 7.61−7.50 (m, 3H), 3.81 (s, 3H), 2.58−2.49 (m, 2H), 2.37 (d, J = 2.8 Hz, 2H), 0.97 (s, 3H), 0.94 (s, 3H); HRMS (ESI): m/z (M + H)$^+$ calcd

https://doi.org/10.1021/acs.jmedchem.1c00019
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for $C_{28}H_{24}ClN_2O_5S$, 535.1089; found, 535.1099. Retention time = 3.547 min.

*3-(4-(4-Chlorophenyl)thiazol-2-yl)-1-(2-methoxy-6-methylphenyl)-7,7-dimethyl-7,8-dihydroquinoline-2,5(1H,6H)-dione (**60**).* This compound was prepared using Method 10 using 2-methoxy-6-methylaniline in **step 3** to afford 3-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2,3-dimethoxyphenyl)-7,7-dimethyl-7,8-dihydroquinoline-2,5-(1*H*,6*H*)-dione as a TFA salt. LCMS: m/z $(M + H)^+$ = 505.1; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.08 (s, 1H), 8.23 (s, 1H), 8.13−8.02 (m, 2H), 7.58−7.42 (m, 3H), 7.16−7.03 (m, 2H), 3.74 (s, 3H), 2.48−2.37 (m, 2H), 2.269 (m, 2H), 1.99 (s, 3H), 0.96 (s, 3H), 0.95 (s, 3H); HRMS (ESI): m/z $(M + H)^+$ calcd for $C_{28}H_{26}ClN_2O_3S$, 505.1347; found, 505.135. Retention time = 3.963 min.

*3-(4-(4-Chlorophenyl)thiazol-2-yl)-1-(2-methoxypyridin-3-yl)-7,7-dimethyl-7,8-dihydroquinoline-2,5(1H,6H)-dione (**61**).* This compound was prepared using Method 10 using 2-methoxypyridin-3-amine in **step 3** to afford 3-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2-methoxypyridin-3-yl)-7,7-dimethyl-7,8-dihydroquinoline-2,5(1*H*,6*H*)-dione as a TFA salt. LCMS: m/z $(M + H)^+$ = 492.11; ^{1}H NMR (400 MHz, CD_3OD): δ 9.04 (s, 1H), 8.39−8.38 (m, 1H), 8.22 (s, 1H), 8.07 (d, J = 8.4 Hz, 2H), 7.91−7.89 (m, 1H), 7.52 (d, J = 8.4 Hz, 2H), 7.27 (d, J = 8.4 Hz, 1H), 3.86 (s, 3H), 3.66−3.53 (m, 1H), 3.47−3.32 (m, 1H), 2.57 (d, J = 20 Hz, 1H), 2.23 (d, J = 20 Hz, 1H), 1.19−0.94 (m, 6H). Aliphatic region complicated significantly by amide rotamers; HRMS (ESI): m/z $(M + H)^+$ calcd for $C_{26}H_{23}ClN_3O_3S$, 492.1143; found, 492.116. Retention time = 3.836 min.

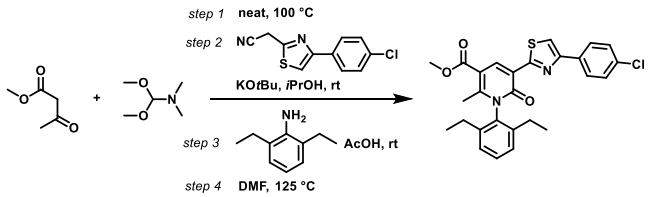
*3-(4-(4-Chlorophenyl)thiazol-2-yl)-1-(4-methoxypyridin-3-yl)-7,7-dimethyl-7,8-dihydroquinoline-2,5(1H,6H)-dione (**62**).* This compound was prepared using Method 10 using 4-methoxypyridin-3-amine in **step 3** to afford 3-(4-(4-chlorophenyl)thiazol-2-yl)-1-(4-methoxypyridin-3-yl)-7,7-dimethyl-7,8-dihydroquinoline-2,5(1*H*,6*H*)-dione as a TFA salt. LCMS: m/z $(M + H)^+$ = 492.11; ^{1}H NMR (400 MHz, CD_3OD): δ 9.01 (s, 1H), 8.21−8.19 (m, 2H), 8.08−8.06 (m, 2H), 7.88−7.86 (m, 1H), 7.52 (d, J = 8.4 Hz, 2H), 6.48 (d, J = 8.4 Hz, 1H), 3.76 (s, 3H), 3.68−3.64 (m, 1H), 2.77−2.73 (m, 2H), 2.41−2.41 (m, 1H), 1.20−0.96 (m, 6H). Aliphatic region complicated significantly by amide rotamers; HRMS (ESI): m/z $(M + H)^+$ calcd for $C_{26}H_{23}ClN_3O_3S$, 492.1143; found, 492.1148. Retention time = 3.133 min.

*3-(4-(4-Chlorophenyl)thiazol-2-yl)-1-(2,5-diethoxyphenyl)-7,7-dimethyl-7,8-dihydroquinoline-2,5(1H,6H)-dione (**63**).* This compound was prepared using Method 10 using 2,5-diethoxyaniline in **step 3** to afford 3-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2,5-diethoxyphenyl)-7,7-dimethyl-7,8-dihydroquinoline-2,5(1*H*,6*H*)-dione as a TFA salt. LCMS: m/z $(M + H)^+$ = 549.16; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.34 (s, 1H), 8.03−7.97 (m, 2H), 7.57 (s, 1H), 7.42 (d, J = 8.5 Hz, 2H), 7.04 (d, J = 1.6 Hz, 2H), 6.78−6.75 (m, 1H), 4.10−3.95 (m, 4H), 2.50 (d, J = 18.4 Hz, 3H), 2.36 (d, J = 18.0 Hz, 1H), 1.42 (t, J = 7.0 Hz, 3H), 1.19 (t, J = 7.0 Hz, 3H), 1.07 (t, J = 7.3 Hz, 6H). Aliphatic region complicated significantly by amide rotamers; HRMS (ESI): m/z $(M + H)^+$ calcd for $C_{30}H_{30}ClN_2O_4S$, 549.1603; found, 549.1609. Retention time = 4.023 min.

*3-(4-(4-Chlorophenyl)thiazol-2-yl)-1-(2,6-diethoxyphenyl)-7,7-dimethyl-7,8-dihydroquinoline-2,5(1H,6H)-dione (**64**).* This compound was prepared using Method 10 using 2,6-diethoxyaniline in **step 3** to afford 3-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2,6-diethoxyphenyl)-7,7-dimethyl-7,8-dihydroquinoline-2,5(1*H*,6*H*)-dione as a TFA salt. LCMS: m/z $(M + H)^+$ = 549.16; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.34 (s, 1H), 8.03−7.97 (m, 2H), 7.56 (s, 1H), 7.45−7.34 (m, 3H), 6.70 (dd, J = 8.5, 3.8 Hz, 2H), 4.06 (hd, J = 9.8, 7.2 Hz, 4H), 2.49 (s, 2H), 2.40 (s, 2H), 1.22 (t, J = 7.0 Hz, 6H), 1.07 (d, J = 11.2 Hz, 6H). Aliphatic region complicated significantly by amide rotamers; HRMS (ESI): m/z $(M + H)^+$ calcd for $C_{30}H_{30}ClN_2O_4S$, 549.161; found, 549.1609. Retention time = 4.023 min.

*3-(4-(4-Chlorophenyl)thiazol-2-yl)-1-(2-ethyl-6-methylphenyl)-7,7-dimethyl-7,8-dihydroquinoline-2,5(1H,6H)-dione (**65**).* This compound was prepared using Method 10 using 2-ethyl-6-methylaniline in **step 3** to afford 3-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2-ethyl-6-methylphenyl)-7,7-dimethyl-7,8-dihydroquinoline-2,5(1*H*,6*H*)-dione as a TFA salt. LCMS: m/z $(M + H)^+$ = 503.2; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.10 (s, 1H), 8.22 (s, 1H), 8.08 (d, J = 8.6 Hz, 2H), 7.53 (d, J = 8.6 Hz, 2H), 7.44 (t, J = 7.6 Hz, 1H), 7.34 (t, J = 8.0 Hz, 2H), 2.50 (d, J = 2.8 Hz, 2H), 2.32 (d, J = 14.0 Hz, 3H), 2.21−2.10 (m, 1H), 1.96 (s, 3H), 1.16−1.01 (m, 3H), 0.95 (s, 6H); HRMS (ESI): m/z $(M + H)^+$ calcd for $C_{29}H_{28}ClN_2O_2S$, 503.1555; found, 503.1562. Retention time = 4.101 min.

*3-(4-(4-Chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-7,7-dimethyl-7,8-dihydroquinoline-2,5(1H,6H)-dione (**66**).* This compound was prepared using Method 10 using 2-(4-(4-chlorophenyl)-thiazol-2-yl)acetonitrile in **step 2** and 2,6-diethylaniline in **step 3** to afford 3-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-7,7-dimethyl-7,8-dihydroquinoline-2,5(1H,6H)-dione as a TFA salt. LCMS: m/z $(M + H)^+$ = 517.2; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.11 (s, 1H), 8.23 (s, 1H), 8.13−8.05 (m, 2H), 7.59−7.47 (m, 3H), 7.38 (d, J = 7.7 Hz, 2H), 2.51 (s, 2H), 2.40−2.26 (m, 4H), 2.16 (dq, J = 15.0, 7.5 Hz, 2H), 1.07 (t, J = 7.5 Hz, 6H), 0.95 (s, 6H); HRMS (ESI): m/z $(M + H)^+$ calcd for $C_{30}H_{30}ClN_2O_2S$, 517.1711; found, 517.1699. Retention time = 4.187 min.



*Method 11: Methyl 5-(4-(4-Chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-2-methyl-6-oxo-1,6-dihydropyridine-3-carboxylate (**67**).* Step 1: In a vial, methyl 3-oxobutanoate (0.385 mL, 3.57 mmol) and DMF−DMA (0.474 mL, 3.57 mmol) were mixed and heated well at 100 °C for 15 min. The reaction mixture became red oil.

Step 2: To the mixture were added *i*-PrOH (40 mL), 2-(4-(4-chlorophenyl)thiazol-2-yl)acetonitrile N1/**IVa** (837 mg, 3.57 mmol), and potassium *tert*-butoxide (400 mg, 3.57 mmol). The reaction mixture was allowed to stir at rt for 2 h at which point the solvent was removed.

Step 3: To the resulting residue were added acetic acid (30 mL) and 2,6-dimethylaniline (646 μL, 3.9 mmol). The reaction mixture was stirred for 15 min and the mixture was diluted with water and extracted (EtOAc × 2). The organic layers were combined (not dried with magnesium sulfate) and concentrated.

Step 4: The residue was taken up in DMF (40 mL) and heated at 125 °C for 1.5 h. The reaction mixture was diluted with water and EtOAc and extracted (2×); the organic layers were combined, dried with Na_2SO_4, and concentrated. The crude mixture was diluted with DMSO and purified by reverse-phase chromatography (**Standard Acidic Gradient Method**) to afford as a TFA salt, methyl 5-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-2-methyl-6-oxo-1,6-dihydropyridine-3-carboxylate (1.05 g, 60%); LCMS: m/z $(M + H)^+$ = 493.1; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.14 (s, 1H), 8.22 (s, 1H), 8.12−8.03 (m, 2H), 7.59−7.44 (m, 3H), 7.36 (d, J = 7.7 Hz, 2H), 3.89 (s, 3H), 3.31 (s, 7H), 2.61−2.50 (m, 1H), 2.47−2.34 (m, 3H), 2.33−2.07 (m, 8H), 1.05 (t, J = 7.5 Hz, 6H), 1.00 (t, J = 7.6 Hz, 0H), 0.82 (s, 1H); HRMS (ESI): m/z $(M + H)^+$ calcd for $C_{27}H_{26}ClN_2O_3S$, 493.1347; found, 493.1338. Retention time = 4.247 min.

*5-(4-(4-Chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-2-methyl-6-oxo-1,6-dihydropyridine-3-carboxylic Acid (**68**).* To a solution of methyl 5-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-2-methyl-6-oxo-1,6-dihydropyridine-3-carboxylate (1.0 g, 2.03 mmol, **compound 67**) in THF (10 mL) and MeOH (10 mL) was added lithium hydroxide (0.168 g, 14.22 mmol) and the mixture became yellow. The mixture was stirred for 1 h at 70 °C, concentrated with a stream of air, and diluted with DCM. The pH of the aqueous layer was adjusted to pH 7 using 1 N HCl, the mixture was extracted with 2 × 25 mL DCM, and the organic layers were dried with Na_2SO_4 and concentrated. The crude mixture was diluted with DMSO and purified by reverse-phase chromatography (**Standard Acidic Gradient Method**) to afford 5-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2,6-

https://doi.org/10.1021/acs.jmedchem.1c00019
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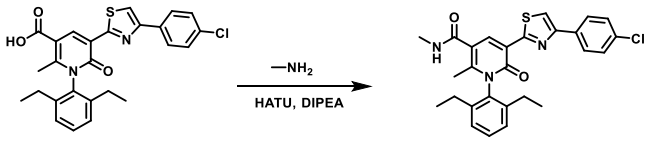
diethylphenyl)-2-methyl-6-oxo-1,6-dihydropyridine-3-carboxylic acid; LCMS: m/z (M + H)$^+$ = 478.7; ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.33 (s, 1H), 9.17 (s, 1H), 8.19 (s, 1H), 8.11−8.02 (m, 2H), 7.57−7.42 (m, 3H), 7.35 (d, J = 7.7 Hz, 2H), 2.34−2.19 (m, 5H), 2.14 (dq, J = 15.1, 7.5 Hz, 2H), 1.04 (t, J = 7.6 Hz, 6H); HRMS (ESI): m/z (M + H)$^+$ calcd for $C_{26}H_{24}ClN_2O_3S$, 479.1191; found, 479.1186. Retention time = 3.835 min.

*Ethyl 5-(4-(4-Chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-6-oxo-2-phenyl-1,6-dihydro-pyridine-3-carboxylate (**69**).* This compound was prepared using Method 11 using ethyl 3-oxo-3-phenylpropanoate in **step 1** to afford ethyl 5-(4-(4-chlorophenyl)-thiazol-2-yl)-1-(2,6-diethylphenyl)-6-oxo-2-phenyl-1,6-dihydropyridine-3-carboxylate. LCMS: m/z (M + H)$^+$ = 569.2; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.16 (s, 1H), 8.28 (s, 1H), 8.15−8.02 (m, 2H), 7.62−7.46 (m, 2H), 7.26−7.01 (m, 8H), 3.91 (q, J = 7.1 Hz, 2H), 2.60−2.48 (m, 1H), 2.47−2.15 (m, 6H), 1.04 (t, J = 7.5 Hz, 6H), 0.78 (t, J = 7.1 Hz, 3H); HRMS (ESI): m/z (M + H)$^+$ calcd for $C_{33}H_{30}ClN_2O_3S$, 569.166; found, 569.1643. Retention time = 4.366 min.



*Method 12: 5-(4-(4-Chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-N,2-dimethyl-6-oxo-1,6-dihydropyridine-3-carboxamide (**70**).* To a solution of 5-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-2-methyl-6-oxo-1,6-dihydropyridine-3-carboxylic acid, **compound 68** (250 mg, 0.522 mmol), in DMF (volume: 5 mL) were added 2-(3*H*-[1,2,3]triazolo[4,5-*b*]pyridin-3-yl)-1,1,3,3-tetramethylisouronium hexafluorophosphate(V) (397 mg, 1.044 mmol), *N*-ethyl-*N*-isopropylpropan-2-amine (365 μL, 2.088 mmol), and methanamine (1044 μL, 2.088 mmol). The mixture became yellow and the reaction mixture was stirred for 6 h at 60 °C, diluted with water, extracted with 3 × 10 mL DCM, and washed with brine. The organic layer was dried and concentrated. The crude mixture was diluted with DMSO and purified by reverse-phase chromatography (**Standard Acidic Gradient Method**) to afford 5-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-*N*,2-dimethyl-6-oxo-1,6-dihydropyridine-3-carboxamide; LCMS: m/z (M + H)$^+$ = 492.1; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.73 (s, 1H), 8.54 (d, J = 4.7 Hz, 1H), 8.18 (s, 1H), 8.13−8.05 (m, 2H), 7.56−7.48 (m, 2H), 7.46 (dd, J = 8.3, 7.1 Hz, 1H), 7.34 (d, J = 7.7 Hz, 2H), 2.96 (s, 1H), 2.78 (d, J = 4.5 Hz, 3H), 2.27 (dt, J = 15.2, 7.6 Hz, 2H), 2.14 (dq, J = 15.1, 7.5 Hz, 2H), 2.01 (s, 3H), 1.05 (t, J = 7.6 Hz, 6H); HRMS (ESI): m/z (M + H)$^+$ calcd for $C_{27}H_{27}ClN_3O_2S$, 492.1507; found, 492.1506. Retention time = 3.75 min.

*5-(4-(4-Chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-N,N,2-trimethyl-6-oxo-1,6-dihydropyridine-3-carboxamide (**71**).* This compound was prepared using Method 12 using dimethylamine to afford 5-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-*N*,*N*,2-trimethyl-6-oxo-1,6-dihydropyridine-3-carboxamide. LCMS: m/z (M + H)$^+$ = 506.1; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.58 (s, 1H), 8.19 (s, 1H), 8.10 (d, J = 8.6 Hz, 2H), 7.56−7.47 (m, 2H), 7.49−7.41 (m, 1H), 7.35 (s, 1H), 7.33 (s, 1H), 3.01 (s, 3H), 2.96 (s, 3H), 2.29 (dq, J = 15.2, 7.6 Hz, 2H), 2.16 (dq, J = 15.0, 7.5 Hz, 2H), 1.84 (s, 3H), 1.06 (t, J = 7.6 Hz, 6H); HRMS (ESI): m/z (M + H)$^+$ calcd for $C_{28}H_{29}ClN_3O_2S$, 506.1664; found, 506.1659. Retention time = 3.819 min.

*5-(4-(4-Chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-N-isopropyl-2-methyl-6-oxo-1,6-dihydropyridine-3-carboxamide (**72**).* This compound was prepared using Method 12 using propan-2-amine to afford 5-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-*N*-isopropyl-2-methyl-6-oxo-1,6-dihydropyridine-3-carboxamide. LCMS: m/z (M + H)$^+$ = 520.1; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.68 (s, 1H), 8.49 (d, J = 7.5 Hz, 1H), 8.20 (s, 1H), 8.10 (d, J = 8.5 Hz, 2H), 7.54 (d, J = 8.5 Hz, 2H), 7.48 (dd, J = 8.2, 7.1 Hz, 1H), 7.37 (s, 1H), 7.35 (s, 1H), 4.06 (dq, J = 13.4, 6.7 Hz, 1H), 2.32 (dq, J = 15.2, 7.6 Hz, 2H), 2.18 (dq, J = 15.1, 7.5 Hz, 2H), 2.00 (s, 3H), 1.20 (d, J = 6.6 Hz, 6H), 1.08 (t, J = 7.5 Hz, 6H); HRMS (ESI): m/z (M + H)$^+$ calcd for $C_{29}H_{31}ClN_3O_2S$, 520.182; found, 520.1817. Retention time = 3.916 min.

*N-(tert-Butyl)-5-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-2-methyl-6-oxo-1,6-dihydropyridine-3-carboxamide (**73**).* This compound was prepared using Method 12 using 2-methylpropan-2-amine to afford *N*-(*tert*-butyl)-5-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-2-methyl-6-oxo-1,6-dihydropyridine-3-carboxamide. LCMS: m/z (M + H)$^+$ = 534.1; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.68 (s, 1H), 8.49 (d, J = 7.5 Hz, 1H), 8.20 (s, 1H), 8.10 (d, J = 8.5 Hz, 2H), 7.54 (d, J = 8.5 Hz, 2H), 7.48 (dd, J = 8.2, 7.1 Hz, 1H), 7.37 (s, 1H), 7.35 (s, 1H), 4.06 (dq, J = 13.4, 6.7 Hz, 1H), 2.32 (dq, J = 15.2, 7.6 Hz, 2H), 2.18 (dq, J = 15.1, 7.5 Hz, 2H), 2.00 (s, 3H), 1.20 (d, J = 6.6 Hz, 6H), 1.08 (t, J = 7.5 Hz, 6H); HRMS (ESI): m/z (M + H)$^+$ calcd for $C_{30}H_{33}ClN_3O_2S$, 534.1977; found, 534.1983. Retention time = 4.032 min.

*5-(4-(4-Chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-N-isobutyl-2-methyl-6-oxo-1,6-dihydropyridine-3-carboxamide (**74**).* This compound was prepared using Method 12 using 2-methylpropan-1-amine to afford 5-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-*N*-isobutyl-2-methyl-6-oxo-1,6-dihydropyridine-3-carboxamide. LCMS: m/z (M + H)$^+$ = 534.2; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.70 (s, 1H), 8.61 (t, J = 5.8 Hz, 1H), 8.18 (d, J = 0.6 Hz, 1H), 8.07 (d, J = 8.5 Hz, 2H), 7.52 (d, J = 8.5 Hz, 2H), 7.46 (dd, J = 8.2, 7.1 Hz, 1H), 7.35 (s, 1H), 7.33 (s, 1H), 3.08 (dd, J = 6.8, 5.8 Hz, 2H), 2.29 (dq, J = 15.2, 7.6 Hz, 2H), 2.15 (dq, J = 15.1, 7.5 Hz, 2H), 2.00 (s, 3H), 1.83 (dt, J = 13.4, 6.7 Hz, 1H), 1.08 (t, J = 7.5 Hz, 6H), 0.91 (d, J = 6.6 Hz, 6H); HRMS (ESI): m/z (M + H)$^+$ calcd for $C_{30}H_{33}ClN_3O_2S$, 534.1977; found, 534.1974. Retention time = 4.012 min.

*5-(4-(4-Chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-N-(2-hydroxyethyl)-2-methyl-6-oxo-1,6-dihydropyridine-3-carboxamide (**75**).* This compound was prepared using Method 12 using 2-aminoethan-1-ol to afford 5-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-*N*-(2-hydroxyethyl)-2-methyl-6-oxo-1,6-dihydropyridine-3-carboxamide. LCMS: m/z (M + H)$^+$ = 522.1; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.71 (s, 1H), 8.61 (t, J = 5.6 Hz, 1H), 8.18 (d, J = 0.6 Hz, 1H), 8.15−8.00 (m, 2H), 7.56−7.49 (m, 2H), 7.46 (dd, J = 8.2, 7.1 Hz, 1H), 7.35 (s, 1H), 7.33 (s, 1H), 3.53 (t, J = 6.1 Hz, 2H), 3.35−3.30 (m, 2H), 3.14 (s, 1H), 2.29 (dd, J = 15.1, 7.6 Hz, 2H), 2.15 (dq, J = 15.1, 7.5 Hz, 2H), 2.00 (s, 3H), 1.08−1.02 (m, 6H); HRMS (ESI): m/z (M + H)$^+$ calcd for $C_{28}H_{29}ClN_3O_3S$, 522.1613; found, 522.1627. Retention time = 3.505 min.

*5-(4-(4-Chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-N-(2-methoxyethyl)-2-methyl-6-oxo-1,6-dihydropyridine-3-carboxamide (**76**).* This compound was prepared using Method 12 using 2-methoxyethan-1-amine to afford 5-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-*N*-(2-methoxyethyl)-2-methyl-6-oxo-1,6-dihydropyridine-3-carboxamide. LCMS: m/z (M + H)$^+$ = 536.1; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.71 (d, J = 4.7 Hz, 2H), 8.21 (s, 1H), 8.11 (d, J = 8.6 Hz, 2H), 7.54 (d, J = 8.6 Hz, 2H), 7.48 (dd, J = 8.2, 7.1 Hz, 1H), 7.37 (s, 1H), 7.35 (s, 1H), 3.50 (td, J = 5.4, 1.3 Hz, 2H), 3.47−3.39 (m, 2H), 3.31 (s, 3H), 2.39−2.26 (m, 2H), 2.18 (dq, J = 15.1, 7.5 Hz, 2H), 2.02 (s, 3H), 1.08 (t, J = 7.5 Hz, 6H); HRMS (ESI): m/z (M + H)$^+$ calcd for $C_{29}H_{31}ClN_3O_3S$, 536.1769; found, 536.1774. Retention time = 3.789 min.

*(5-(4-(4-Chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-2-methyl-6-oxo-1,6-dihydro-pyridine-3-carbonyl)glycine (**77**).* Step 1: Followed Method 12 using methyl glycinate.

Step 2: To a solution of the crude acid from step 1 in 1:1 THF/MeOH (~0.3 mM) was added lithium hydroxide (~10 equiv) and stirred for 1 h at 70 °C. The mixture was concentrated with a stream of air and diluted with DCM. The pH of the aqueous layer was adjusted to pH 7 using 1 N HCl and extracted with 2× DCM; the organic layers were dried with Na_2SO_4 and concentrated. The crude mixture was diluted with DMSO and purified by reverse-phase chromatography (**Standard Acidic Gradient Method**) to afford compound 77. LCMS: m/z (M + H)$^+$ = 536.1; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.69 (s, 1H), 9.00 (t, J = 5.9 Hz, 1H), 8.77 (s, 1H), 8.21 (d, J = 0.6 Hz, 1H), 8.14−8.07 (m, 2H), 7.57−7.52 (m, 2H),

https://doi.org/10.1021/acs.jmedchem.1c00019
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7.49 (dd, J = 8.2, 7.2 Hz, 1H), 7.37 (d, J = 7.7 Hz, 2H), 3.96 (d, J = 5.9 Hz, 2H), 2.32 (dq, J = 15.2, 7.6 Hz, 2H), 2.18 (dq, J = 15.1, 7.5 Hz, 2H), 2.08 (s, 3H), 1.08 (td, J = 7.6, 0.6 Hz, 6H); HRMS (ESI): m/z (M + H)$^+$ calcd for $C_{28}H_{27}ClN_3O_4S$, 536.1405; found, 536.1417. Retention time = 3.491 min.

*N-(2-Aminoethyl)-5-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-2-methyl-6-oxo-1,6-dihydropyridine-3-carboxamide (**78**).* This compound was prepared using Method 12 using ethane-1,2-diamine to afford *N*-(2-aminoethyl)-5-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-2-methyl-6-oxo-1,6-dihydropyridine-3-carboxamide as a TFA salt. LCMS: m/z (M + H)$^+$ = 521.1; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.86 (s, 1H), 8.77 (t, J = 5.6 Hz, 1H), 8.23 (d, J = 0.6 Hz, 1H), 8.12 (d, J = 8.4 Hz, 2H), 7.81 (s, 2H), 7.55 (d, J = 8.4 Hz, 2H), 7.53−7.45 (m, 1H), 7.38 (s, 1H), 7.36 (s, 1H), 3.51 (q, J = 6.1 Hz, 2H), 3.04 (q, J = 5.9 Hz, 2H), 2.31 (dd, J = 15.2, 7.5 Hz, 2H), 2.17 (dq, J = 15.1, 7.5 Hz, 2H), 2.08 (s, 3H), 1.13−1.04 (m, 6H); HRMS (ESI): m/z (M + H)$^+$ calcd for $C_{28}H_{30}ClN_4O_2S$, 517.1711; found, 521.1773. Retention time = 2.669 min.

*5-(4-(4-Chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-2-methyl-N-(2-(methylamino)-ethyl)-6-oxo-1,6-dihydropyridine-3-carboxamide (**79**).* This compound was prepared using Method 12 using *tert*-butyl(2-aminoethyl) (methyl)carbamate to afford *tert*-butyl(2-(5-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-2-methyl-6-oxo-1,6-dihydro-pyridine-3-carboxamido)ethyl)(methyl)carbamate followed by Boc deprotection using TFA in DCM to afford 5-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-2-methyl-*N*-(2-(methylamino)ethyl)-6-oxo-1,6-dihydropyridine-3-carboxamide as a TFA salt. LCMS: m/z (M + H)$^+$ = 535.1; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.86 (s, 1H), 8.83 (t, J = 5.7 Hz, 1H), 8.44 (s, 1H), 8.23 (s, 1H), 8.12 (d, J = 8.5 Hz, 2H), 7.56 (d, J = 8.5 Hz, 2H), 7.54−7.45 (m, 1H), 7.39 (s, 1H), 7.37 (s, 1H), 3.55 (q, J = 6.0 Hz, 2H), 3.14 (s, 2H), 2.70−2.63 (m, 3H), 2.31 (dq, J = 15.2, 7.6 Hz, 2H), 2.17 (dq, J = 15.1, 7.5 Hz, 2H), 2.09 (s, 3H), 1.08 (t, J = 7.5 Hz, 6H); HRMS (ESI): m/z (M + H)$^+$ calcd for $C_{29}H_{32}ClN_4O_2S$, 535.1929; found, 535.1938. Retention time = 2.712 min.

*N-(2-Amino-2-oxoethyl)-5-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-2-methyl-6-oxo-1,6-dihydropyridine-3-carboxamide (**80**).* This compound was prepared using Method 12 using 2-aminoacetamide to afford *N*-(2-amino-2-oxoethyl)-5-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-2-methyl-6-oxo-1,6-dihydropyridine-3-carboxamide. LCMS: m/z (M + H)$^+$ = 535.1; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.80 (d, J = 7.6 Hz, 2H), 8.21 (s, 1H), 8.11 (d, J = 8.5 Hz, 2H), 7.54 (d, J = 8.6 Hz, 1H), 7.51−7.43 (m, 2H), 7.36 (d, J = 7.7 Hz, 2H), 7.06 (s, 2H), 3.84 (d, J = 5.8 Hz, 2H), 2.32 (dq, J = 15.2, 7.6 Hz, 2H), 2.18 (dq, J = 15.2, 7.5 Hz, 2H), 2.08 (s, 3H), 1.08 (t, J = 7.5 Hz, 6H); HRMS (ESI): m/z (M + H)$^+$ calcd for $C_{28}H_{28}ClN_4O_3S$, 535.1565; found, 535.155. Retention time = 3.378 min.

*5-(4-(4-Chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-2-methyl-N-(3-(methylamino)propyl)-6-oxo-1,6-dihydropyridine-3-carboxamide (**81**).* This compound was prepared using Method 12 using *tert*-butyl (3-aminopropyl)(methyl)carbamate followed by boc deprotection using TFA in DCM to afford 5-(4-(4-chlorophenyl)-thiazol-2-yl)-1-(2,6-diethylphenyl)-2-methyl-*N*-(3-(methylamino)-propyl)-6-oxo-1,6-dihydropyridine-3-carboxamide as a TFA salt. LCMS: m/z (M + H)$^+$ = 549.1; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.76 (d, J = 2.8 Hz, 2H), 8.34 (s, 1H), 8.23 (s, 1H), 8.11 (d, J = 8.5 Hz, 2H), 7.55 (d, J = 8.5 Hz, 2H), 7.52−7.46 (m, 1H), 7.38 (s, 1H), 7.36 (s, 1H), 3.34 (q, J = 6.5, 6.1 Hz, 2H), 2.99 (p, J = 6.6 Hz, 2H), 2.60 (t, J = 5.4 Hz, 3H), 2.31 (dq, J = 15.2, 7.6 Hz, 2H), 2.17 (dq, J = 15.1, 7.5 Hz, 2H), 2.05 (s, 3H), 1.86 (p, J = 7.0 Hz, 2H), 1.08 (t, J = 7.5 Hz, 6H); HRMS (ESI): m/z (M + H)$^+$ calcd for $C_{30}H_{33}ClN_4NaO_2S$, 571.1905; found, 571.1919. Retention time = 2.708 min.

*5-(4-(4-Chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-2-methyl-6-oxo-N-phenyl-1,6-dihydropyridine-3-carboxamide (**82**).* This compound was prepared using Method 12 using aniline to afford 5-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-2-methyl-6-oxo-*N*-phenyl-1,6-dihydropyridine-3-carboxamide. LCMS: m/z (M + H)$^+$ = 554.1; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.63 (s, 1H), 8.88 (s, 1H), 8.23 (s, 1H), 8.12 (d, J = 8.6 Hz, 2H), 7.74 (d, J = 7.9 Hz, 2H), 7.57−7.45 (m, 3H), 7.43−7.32 (m, 4H), 7.18−7.09 (m, 1H), 2.42−2.28 (m, 2H), 2.21 (dq, J = 15.1, 7.5 Hz, 2H), 2.09 (s, 3H), 1.10 (t, J = 7.5 Hz, 6H); HRMS (ESI): m/z (M + H)$^+$ calcd for $C_{32}H_{29}ClN_3O_2S$, 554.1664; found, 554.1682. Retention time = 4.043 min.

*N-Benzyl-5-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-2-methyl-6-oxo-1,6-dihydropyridine-3-carboxamide (**83**).* This compound was prepared using Method 12 using phenylmethanamine to afford *N*-benzyl-5-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-2-methyl-6-oxo-1,6-dihydropyridine-3-carboxamide. LCMS: m/z (M + H)$^+$ = 567.7; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.13 (t, J = 5.9 Hz, 1H), 8.77 (s, 1H), 8.18 (s, 1H), 8.12−8.04 (m, 2H), 7.57−7.41 (m, 3H), 7.41−7.21 (m, 7H), 4.48 (d, J = 5.9 Hz, 2H), 2.22 (ddq, J = 56.8, 15.1, 7.5 Hz, 4H), 2.02 (s, 3H), 1.05 (t, J = 7.5 Hz, 6H); HRMS (ESI): m/z (M + H)$^+$ calcd for $C_{33}H_{31}ClN_3O_2S$, 568.182; found, 568.1842. Retention time = 3.966 min.

*5-(4-(4-Chlorophenyl)thiazol-2-yl)-N-cyclohexyl-1-(2,6-diethylphenyl)-2-methyl-6-oxo-1,6-dihydropyridine-3-carboxamide (**84**).* This compound was prepared using Method 12 using cyclohexanamine to afford 5-(4-(4-chlorophenyl)thiazol-2-yl)-*N*-cyclohexyl-1-(2,6-diethylphenyl)-2-methyl-6-oxo-1,6-dihydropyridine-3-carboxamide. LCMS: m/z (M + H)$^+$ = 567.7; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.64 (s, 1H), 8.46 (d, J = 7.7 Hz, 1H), 8.17 (s, 1H), 8.11−8.04 (m, 2H), 7.56−7.41 (m, 3H), 7.33 (d, J = 7.7 Hz, 2H), 3.72 (dd, J = 7.4, 3.6 Hz, 0H), 2.30 (dq, J = 15.2, 7.6 Hz, 2H), 2.15 (dq, J = 15.1, 7.5 Hz, 2H), 1.97 (s, 3H), 1.88 (d, J = 9.6 Hz, 2H), 1.71 (s, 2H), 1.58 (d, J = 13.0 Hz, 1H), 1.35−1.22 (m, 4H), 1.05 (t, J = 7.5 Hz, 6H); HRMS (ESI): m/z (M + H)$^+$ calcd for $C_{32}H_{35}ClN_3O_2S$, 560.2133; found, 560.2134. Retention time = 4.151 min.

*3-(4-(4-Chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-6-methyl-5-(piperidine-1-carbonyl)pyridin-2(1H)-one (**85**).* This compound was prepared using Method 12 using piperidine to afford 3-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-6-methyl-5-(piperidine-1-carbonyl)pyridin-2(1*H*)-one. LCMS: m/z (M + H)$^+$ = 546.1; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.58 (d, J = 0.8 Hz, 1H), 8.22 (d, J = 0.9 Hz, 1H), 8.17−8.06 (m, 2H), 7.53 (d, J = 8.5 Hz, 2H), 7.51−7.46 (m, 1H), 7.38 (s, 1H), 7.36 (s, 1H), 3.65 (s, 2H), 3.45 (s, 4H), 2.39−2.15 (m, 4H), 1.88 (d, J = 0.8 Hz, 3H), 1.63 (s, 4H), 1.12−1.05 (m, 6H); HRMS (ESI): m/z (M + H)$^+$ calcd for $C_{31}H_{33}ClN_3O_2S$, 546.1977; found, 546.1998. Retention time = 4.064 min.

*3-(4-(4-Chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-6-methyl-5-(morpholine-4-carbonyl)pyridin-2(1H)-one (**86**).* This compound was prepared using Method 12 using morpholine to afford 3-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-6-methyl-5-(morpholine-4-carbonyl)pyridin-2(1*H*)-one. LCMS: m/z (M + H)$^+$ = 548.1; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.60 (s, 1H), 8.19 (s, 1H), 8.10 (d, J = 8.6 Hz, 2H), 7.51 (d, J = 8.5 Hz, 2H), 7.48−7.43 (m, 1H), 7.35 (s, 1H), 7.33 (s, 1H), 3.62 (m, 8H), 2.36−2.12 (m, 4H), 1.86 (d, J = 1.6 Hz, 3H), 1.11−1.01 (m, 6H); HRMS (ESI): m/z (M + Na)$^+$ calcd for $C_{30}H_{30}ClN_3NaO_3S$, 570.1589; found, 570.1605. Retention time = 3.787 min.

*3-(4-(4-Chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-6-methyl-5-(piperazine-1-carbonyl)pyridin-2(1H)-one (**87**).* This compound was prepared using Method 12 using piperazine to afford 3-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-6-methyl-5-(piperazine-1-carbonyl)pyridin-2(1*H*)-one as a TFA salt. LCMS: m/z (M + H)$^+$ = 547.1; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.56 (s, 1H), 8.19 (s, 1H), 8.16−8.03 (m, 2H), 7.57−7.36 (m, 3H), 7.34 (d, J = 7.7 Hz, 2H), 3.57 (s, 2H), 3.28 (s, 4H), 2.70 (d, J = 14.8 Hz, 1H), 2.52 (p, J = 1.9 Hz, 0H), 2.26 (dt, J = 15.2, 7.4 Hz, 2H), 2.17 (s, 3H), 1.85 (s, 3H), 1.06 (t, J = 7.5 Hz, 6H); HRMS (ESI): m/z (M + H)$^+$ calcd for $C_{30}H_{32}ClN_4O_2S$, 547.1929; found, 547.1942. Retention time = 2.691 min.

*N-Benzyl-5-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-2-methyl-6-oxo-1,6-dihydropyridine-3-carboxamide (**88**).* This compound was prepared using Method 12 using 1-methylpiperazine to afford *N*-benzyl-5-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-2-methyl-6-oxo-1,6-dihydropyridine-3-carboxamide as a TFA salt. LCMS: m/z (M + H)$^+$ = 561.2; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.70 (s, 1H), 8.23 (s, 1H), 8.12 (d, J = 8.6 Hz, 2H), 7.54 (d, J =

https://doi.org/10.1021/acs.jmedchem.1c00019
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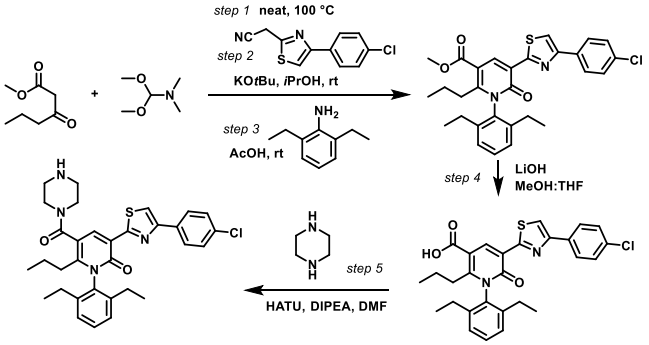
8.6 Hz, 2H), 7.52−7.47 (m, 1H), 7.38 (s, 1H), 7.37 (s, 1H), 3.42 (m, 8H), 2.84 (s, 3H), 2.39−3.15 (m, 4H), 1.90 (s, 3H), 1.09 (t, J = 7.5 Hz, 6H); HRMS (ESI): m/z (M + H)$^+$ calcd for $C_{31}H_{34}ClN_4O_2S$, 561.2086; found, 561.2076. Retention time = 2.74 min.



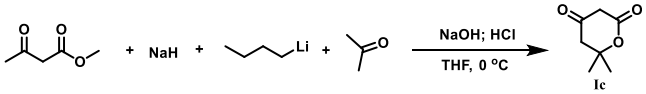
*Method 13: 3-(4-(4-Chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-5-(piperazine-1-carbonyl)-6-propylpyridin-2(1H)-one (**89**).* Steps 1−3: A mixture of ethyl 3-oxohexanoate (0.674 g, 4.26 mmol) and 1,1-dimethoxy-*N*,*N*-dimethylmethanamine (0.566 mL, 4.26 mmol) was stirred for 1 h at 100 °C. The mixture was diluted with IPA (10 mL). 2-(4-(4-Chlorophenyl)thiazol-2-yl)acetonitrile (1.00 g, 4.26 mmol) and K^{t}OBu (0.478 g, 4.26 mmol) were added and stirred at 50 °C for 3 h. The solvent was removed. To the residue were added 2,6-diethylaniline (0.772 mL, 4.69 mmol) and AcOH (6.10 mL, 107 mmol) and sonicated to a homogeneous mixture. The reaction mixture was stirred at 70 °C for 4 h, cooled to room temperature, diluted with EtOAc, and washed with water. The organic layer was dried, concentrated, and purified by column chromatography to afford ethyl 5-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-6-oxo-2-propyl-1,6-dihydro-pyridine-3-carboxylate; LCMS: m/z (M + H)$^+$ = 535.3.

Step 4−5: To a solution of ethyl 5-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-6-oxo-2-propyl-1,6-dihydropyridine-3-carboxylate (1.5 g, 2.8 mmol) in THF (10 mL) and MeOH (10 mL) was added lithium hydroxide (0.470 g, 19.6 mmol). The reaction mixture was stirred at 60 °C for 3 h. The solvent was removed by rotovap, diluted with DCM, quenched with 1 N HCl, washed with brine (2 × 50 mL), dried over $MgSO_4$, and concentrated to afford the 5-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-6-oxo-2-propyl-1,6-dihydropyridine-3-carboxylic acid; LCMS: m/z (M + H)$^+$ = 507.0. Without further purification, to the crude acid (1.00 g, 1.97 mmol) were added 2-(3*H*-[1,2,3]triazolo[4,5-*b*]pyridin-3-yl)-1,1,3,3-tetramethylisouronium hexafluorophosphate(V) (1.125 g, 2.96 mmol), DMF (5 mL), *N*-ethyl-*N*-isopropylpropan-2-amine (0.689 mL, 3.94 mmol), and piperazine (0.255 g, 2.96 mmol) (mixture became yellow). The reaction mixture was stirred for 1 h at rt, diluted with water, extracted with 3 × 10 mL of DCM, and washed with brine. The organic layer was dried and concentrated. The crude mixture was diluted with DMSO and purified by reverse-phase chromatography (**Standard Acidic Gradient Method**) to afford as a TFA salt, 3-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-5-(piperazine-1-carbonyl)-6-propylpyridin-2(1*H*)-one; LCMS: m/z (M + H)$^+$ = 575.1; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.54 (d, J = 2.1 Hz, 1H), 8.18 (d, J = 2.0 Hz, 1H), 8.09 (d, J = 7.8 Hz, 2H), 7.49 (dd, J = 16.9, 7.7 Hz, 4H), 7.34 (d, J = 7.9 Hz, 2H), 3.63 (s, 1H), 3.55 (s, 1H), 3.28 (d, J = 4.1 Hz, 2H), 3.28 (s, 4H), 2.81 (s, 1H), 2.70 (s, 5H), 2.61 (s, 1H), 2.29 (dt, J = 15.3, 7.3 Hz, 4H), 2.14 (s, 4H), 1.27 (s, 2H), 1.07 (t, J = 7.4 Hz, 8H), 0.60 (t, J = 7.1 Hz, 4H); HRMS (ESI): m/z (M + H)$^+$ calcd for $C_{32}H_{36}ClN_4O_2S$, 575.2242; found, 575.2223. Retention time = 2.804 min.

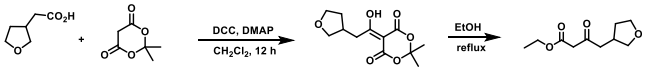
*3-(4-(4-Chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-6-isopropyl-5-(piperazine-1-carbonyl)pyridin-2(1H)-one (**90**).* This compound was prepared via Method 13 using methyl 4-methyl-3-oxopentanoate in **step 1** to afford 3-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-6-isopropyl-5-(piperazine-1-carbonyl)pyridin-2(1*H*)-one as a TFA salt. LCMS: m/z (M + H)$^+$ = 575.2; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.79 (s, 1H), 8.68 (s, 1H), 8.23 (d, J = 0.6 Hz, 1H), 8.19−8.10 (m, 2H), 7.59−7.51 (m, 2H), 7.49 (t, J = 7.7 Hz, 1H), 7.37 (dd, J = 7.6, 4.5 Hz, 2H), 4.08 (d, J = 14.1 Hz, 1H), 3.78 (d, J = 14.7 Hz, 1H), 3.73−3.61 (m, 1H), 3.60−3.48 (m, 1H), 3.49−3.38 (m, 1H), 3.26−3.13 (m, 1H), 3.10 (d, J = 6.0 Hz, 1H), 2.43−2.29 (m, 2H), 2.25 (td, J = 15.0, 7.5 Hz, 1H), 2.11 (dd, J = 14.9, 7.4 Hz, 1H), 1.10 (q, J = 7.1 Hz, 14H); HRMS (ESI): m/z (M + H)$^+$ calcd for $C_{32}H_{36}ClN_4O_2S$, 575.2242; found, 575.2225. Retention time = 2.79 min.

*3-(4-(4-Chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-6-isobutyl-5-(piperazine-1-carbonyl)pyridin-2(1H)-one (**91**).* This compound was prepared via Method 13 using methyl 5-methyl-3-oxohexanoate in **step 1** to afford 3-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-6-isobutyl-5-(piperazine-1-carbonyl)pyridin-2(1*H*)-one as a TFA salt. LCMS: m/z (M + H)$^+$ = 589.2; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.93 (s, 1H), 8.73 (d, J = 0.7 Hz, 1H), 8.24 (d, J = 0.7 Hz, 1H), 8.18−8.10 (m, 2H), 7.59−7.52 (m, 2H), 7.49 (d, J = 7.6 Hz, 1H), 7.37 (d, J = 7.7 Hz, 1H), 4.06 (s, 1H), 3.81 (s, 1H), 3.64 (m, 2H), 3.16 (m, 5H), 2.45−1.91 (m, 3H), 1.34 (dq, J = 13.5, 6.7 Hz, 1H), 1.10 (d, J = 7.9 Hz, 8H), 0.63 (d, J = 6.5 Hz, 6H); HRMS (ESI): m/z (M + H)$^+$ calcd for $C_{33}H_{38}ClN_4O_2S$, 589.2399; found, 589.2381. Retention time = 1.982 min.

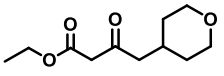
Synthesis of Intermediates.



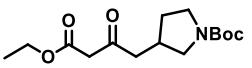
*6,6-Dimethyldihydro-2H-pyran-2,4(3H)-dione (**Ic**).* To NaH (1.92 g, 80 mmol) in anhydrous THF (200 mL) was added, at 0 °C, methyl acetoacetate (9.28 g, 80 mmol) dropwise. After 10 min of stirring, *n*-BuLi (32 mL, 2.5 M solution in hexanes, 80 mmol) was added dropwise, and the orange solution was stirred at 0 °C for 10 more min. Dry acetone (7.5 mL, 82 mmol) was added at once, and the mixture was stirred for 10 min at 0 °C. NaOH (80 mL, 2.5 M solution in water) was then added, and the mixture was stirred for 12 h at room temperature, whereupon it was acidified (2.5 M HCl solution) and extracted with ether (3 × 200 mL). The organic layer was washed with brine and dried with Na_2SO_4. After filtration, the solvent was evaporated with rotovap. The residue was dissolved in a minimum of CH_2Cl_2 and precipitated with pentane as a brownish solid, yield (62%) mp 126−127 °C. ^{1}H NMR (400 MHz, $CDCl_3$): 1.48 (s, 6H); 2.66 (s, 2H); 3.40 (s, 2H).



Method D: Ethyl 3-Oxo-4-(tetrahydrofuran-3-yl)butanoate. To a solution of 2-(tetrahydrofuran-3-yl)acetic acid (1.00 g, 7.68 mmol) in DCM (10 mL) were added DMAP (1.408 g, 11.53 mmol) and DCC (2.378 g, 11.53 mmol) and then 2,2-dimethyl-1,3-dioxane-4,6-dione (1.107 g, 7.68 mmol) was added and the mixture was stirred for 12 h at room temperature. The insoluble urea was then removed by filtration over Celite, and the solvent was evaporated under reduced pressure. The crude product was redissolved in ethyl acetate and washed with 1 M HCl. The organic phase was dried over Na_2SO_4, filtered, and evaporated. The crude product was used without further purification. The crude product was dissolved in ethanol (20 mL) and refluxed for 6 h. The organic solvent was evaporated under reduced pressure and purified by column chromatography to afford ethyl 3-oxo-4-(tetrahydrofuran-3-yl) butanoate.



Ethyl 3-Oxo-4-(tetrahydro-2H-pyran-4-yl)butanoate. This compound was prepared using Method 14 using 2-(tetrahydro-2*H*-pyran-4-yl)acetic acid to afford ethyl 3-oxo-4-(tetrahydro-2*H*-pyran-4-yl)butanoate.



tert-Butyl 3-(4-Ethoxy-2,4-dioxobutyl)pyrrolidine-1-carboxylate. This compound was prepared using Method 14 using 2-(1-(*tert*-

https://doi.org/10.1021/acs.jmedchem.1c00019
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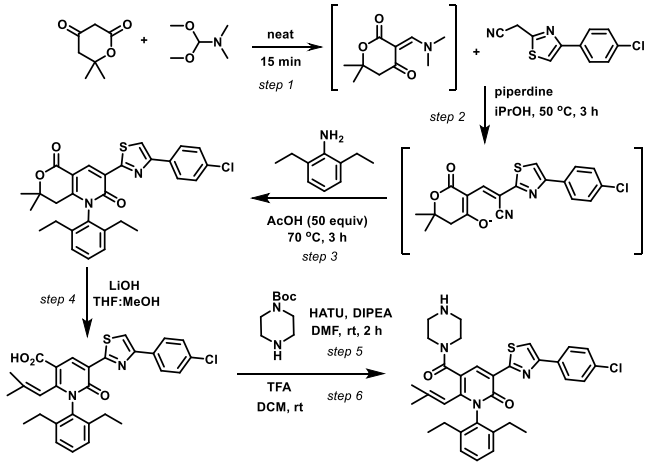
butoxycarbonyl)pyrrolidin-3-yl)acetic acid to afford *tert*-butyl 3-(4-ethoxy-2,4-dioxobutyl)pyrrolidine-1-carboxylate.



*Method 15: 3-(4-(4-Chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-6-(2-methylprop-1-en-1-yl)-5-(piperazine-1-carbonyl)-pyridin-2(1H)-one (**92**).* Steps 1−3: A mixture of 6,6-dimethyldihydro-2*H*-pyran-2,4(3*H*)-dione (3.03 g, 21.30 mmol) and 1,1-dimethoxy-*N*,*N*-dimethylmethanamine (2.83 mL, 21.30 mmol) was stirred for 15 min at room temperature. The reaction mixture became a yellow solid. To the mixture were added 2-propanol (50 mL), 2-(4-(4-chlorophenyl)thiazol-2-yl)acetonitrile (5.00 g, 21.3 mmol), and piperidine (8.4 mL, 85 mmol) and then sonicated to get a homogeneous mixture. The mixture was stirred at 70 °C for 4 h. The solvent was removed by rotovap. To the residue were added 2,6-diethylaniline (3.86 mL, 23.4 mmol) and acetic acid (61.00 mL, 1065 mmol) and the reaction mixture was sonicated to dissolve solids. The mixture was stirred at 70 °C for 4 h and cooled to room temperature and diluted with DCM and washed with water, sat $NaHCO_3$ solution, and brine solution. The organic layer was dried over Na_2SO_4 and concentrated. The crude product was purified by column chromatography (10:90 EA/Hex to 100% EA) to afford the product, 3-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-7,7-dimethyl-7,8-dihydro-2*H*-pyrano[4,3-*b*]pyridine-2,5(1*H*)-dione; LCMS: m/z (M + H)$^+$ = 520.2.

Step 4: To a solution of 3-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-7,7-dimethyl-7,8-dihydro-1*H*-pyrano[4,3-*b*]pyridine-2,5-dione (3.5 g, 6.74 mmol) in THF (25 mL) and MeOH (25 mL) was added lithium hydroxide (1.13 g, 47.2 mmol) and the reaction mixture was stirred at room temperature for 1 h. The solvent was removed and diluted with DCM and quenched with acetic acid (2.70 mL, 47.2 mmol). The organic layer was washed several times with brine and dried over Na_2SO_4 and concentrated to afford the crude 5-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-2-(2-methylprop-1-en-1-yl)-6-oxo-1,6-dihydropyridine-3-carboxylic acid; LCMS: m/z (M + H)$^+$ = 519.2. Yield ∼95%. The crude acid was used in the next step without further purification.

Steps 5 and 6: To a solution of 5-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-2-(2-methylprop-1-en-1-yl)-6-oxo-1,6-dihydropyridine-3-carboxylic acid (2.0 g, 3.85 mmol) were added HATU (2.198 g, 5.78 mmol), DMF (volume: 10 mL), DIPEA (1.346 mL, 7.71 mmol), and piperazine (1.077 g, 5.78 mmol). The reaction mixture was stirred for 1 h at rt, diluted with water, extracted with 3 × 10 mL of DCM, and washed with brine. The organic layer was dried and concentrated. The crude product (without further purification) was diluted with DCM (10 mL) and TFA (10.39 mL, 135 mmol) and stirred for 1 h at rt. The solvent was concentrated under vacuum, and the crude mixture was diluted with DMSO and purified by reverse-phase chromatography (**Standard Acidic Gradient Method**) to afford as a TFA salt, 3-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-6-(2-methylprop-1-en-1-yl)-5-(piperazine-1-carbonyl)pyridin-2(1*H*)-one; LCMS: m/z (M + H)$^+$ = 587.2; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.91 (s, 1H), 8.08−8.02 (m, 2H), 7.95 (s, 1H), 7.53−7.43 (m, 3H), 7.39−7.29 (m, 2H), 5.39 (s, 1H), 4.38 (s, 1H), 3.78 (s, 2H), 3.52 (d, J = 29.4 Hz, 3H), 3.24 (s, 1H), 2.31 (d, J = 61.9 Hz, 4H), 1.67 (dd, J = 21.3, 1.1 Hz, 6H), 1.31 (s, 2H), 1.26−1.06 (m, 6H). Aliphatic region complicated significantly by amide rotamers; HRMS (ESI): m/z (M + H)$^+$ calcd for $C_{33}H_{36}ClN_4O_2S$, 587.2242; found, 587.2243. Retention time = 2.781 min.

*3-(4-(4-Chlorophenyl)thiazol-2-yl)-6-(cyclopropylmethyl)-1-(2,6-diethylphenyl)-5-(piperazine-1-carbonyl)pyridin-2(1H)-one (**93**).* This compound was prepared using Method 13 using ethyl 4-cyclopropyl-3-oxobutanoate in **step 1** to afford 3-(4-(4-chlorophenyl)thiazol-2-yl)-6-(cyclopropylmethyl)-1-(2,6-diethylphenyl)-5-(piperazine-1-carbonyl)pyridin-2(1*H*)-one as a TFA salt. LCMS: m/z (M + H)$^+$ = 587.2; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.79 (br s, 2H), 8.73 (s, 1H), 8.21 (s, 1H), 8.16−8.07 (m, 2H), 7.55−7.44 (m, 3H), 7.34 (d, J = 7.7 Hz, 2H), 4.07 (m, 1H), 3.74 (m, 1H), 3.51 (m, 3H), 3.12 (m, 3H), 2.35 (m, 2H), 2.12 (m, 5H), 1.07 (t, J = 7.5 Hz, 6H), 0.52−0.12 (m, 2H), −0.05 (m, 1H), −0.27 (m, 1H); HRMS (ESI): m/z (M + H)$^+$ calcd for $C_{26}H_{28}ClN_{14}O$, 587.2254; found, 587.2255. Retention time = 2.803 min.

*3-(4-(4-Chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-6-(2-methoxyethyl)-5-(piperazine-1-carbonyl)pyridin-2(1H)-one (**94**).* This compound was prepared using Method 13 using methyl 5-methoxy-3-oxopentanoate in **step 1** to afford 3-(4-(4-chlorophenyl)-thiazol-2-yl)-1-(2,6-diethylphenyl)-6-(2-methoxyethyl)-5-(piperazine-1-carbonyl)pyridin-2(1*H*)-one as a TFA salt. LCMS: m/z (M + H)$^+$ = 591.2; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.74 (d, J = 0.5 Hz, 2H), 8.24 (d, J = 0.5 Hz, 1H), 8.18−8.10 (m, 2H), 7.58−7.47 (m, 3H), 7.39 (s, 1H), 7.37 (s, 1H), 4.04 (s, 1H), 3.85−3.43 (m, 3H), 3.16 (d, J = 35.4 Hz, 6H), 3.02 (d, J = 0.6 Hz, 3H), 2.85 (s, 1H), 2.33 (t, J = 1.9 Hz, 2H), 2.17 (s, 2H), 1.11 (s, 7H). Aliphatic region complicated significantly by amide rotamers; HRMS (ESI): m/z (M + H)$^+$ calcd for $C_{32}H_{36}ClN_4O_3S$, 591.2191; found, 591.2195. Retention time = 3.741 min.

*3-(4-(4-Chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-5-(piperazine-1-carbonyl)-6-(3,3,3-trifluoropropyl)pyridin-2(1H)-one (**95**).* This compound was prepared using Method 13 using ethyl 6,6,6-trifluoro-3-oxohexanoate in **step 1** to afford 3-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-5-(piperazine-1-carbonyl)-6-(3,3,3-trifluoropropyl)pyridin-2(1*H*)-one as a TFA salt. LCMS: m/z (M + H)$^+$ = 629.2; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.77 (m, 3H), 8.24 (s, 1H), 8.16−8.08 (m, 2H), 7.55−7.47 (m, 3H), 7.38 (d, J = 7.7 Hz, 2H), 3.72 (br m, 5H), 3.17 (br m, 2H), 2.36 (dt, J = 15.1, 7.6 Hz, 2H), 2.02 (br m, 3H), 1.08 (t, J = 7.5 Hz, 6H); HRMS (ESI): m/z (M + H)$^+$ calcd for $C_{32}H_{33}ClF_3N_4O_2S$, 629.1959; found, 629.195. Retention time = 2.851 min.

*3-(4-(4-Chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-5-(piperazine-1-carbonyl)-6-((tetrahydrofuran-3-yl)methyl)pyridin-2(1H)-one (**96**).* This compound was prepared using Method 13 using ethyl 3-oxo-4-(tetrahydrofuran-3-yl)butanoate in **step 1** to afford 3-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-5-(piperazine-1-carbonyl)-6-((tetrahydrofuran-3-yl)methyl)pyridin-2(1*H*)-one as a TFA salt. LCMS: m/z (M + H)$^+$ = 617.2; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.68 (s, 1H), 8.18 (s, 1H), 8.10−8.05 (m, 2H), 7.48−7.41 (m, 3H), 7.31 (d, J = 7.7 Hz, 2H), 3.98 (s, 1H), 3.72 (s, 1H), 3.46 (d, J = 17.9 Hz, 2H), 3.07−2.95 (m, 6H), 2.57−2.49 (m, 1H), 2.16−1.90 (m, 6H), 1.71 (d, J = 21.7 Hz, 1H), 1.59 (d, J = 10.6 Hz, 2H), 1.38−135 (m, 1H), 1.02 (d, J = 9.1 Hz, 6H); HRMS (ESI): m/z (M + H)$^+$ calcd for $C_{34}H_{38}ClN_4O_3S$, 617.2348; found, 617.2332. Retention time = 2.722 min.

*3-(4-(4-Chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-5-(piperazine-1-carbonyl)-6-((tetrahydro-2H-pyran-4-yl)methyl)pyridin-2(1H)-one (**97**).* This compound was prepared using Method 13 using ethyl 3-oxo-4-(tetrahydro-2*H*-pyran-4-yl)butanoate in **step 1** to afford 3-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-5-(piperazine-1-carbonyl)-6-((tetrahydro-2*H*-pyran-4-yl)methyl)pyridin-2(1*H*)-one as a TFA salt. LCMS: m/z (M + H)$^+$ = 631.2; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.67 (s, 1H), 8.19 (s, 1H), 8.08 (d, J = 8.6 Hz, 2H), 7.51−7.41 (m, 3H), 7.33 (d, J = 7.7 Hz, 2H), 4.00 (s, 1H), 3.67−3.60 (m, 3H), 2.95 (td, J = 11.6, 2.4 Hz, 2H), 2.37−2.23 (m, 2H), 2.22−1.98 (m, 4H), 1.22−0.88 (m, 16H); HRMS (ESI): m/z (M + Na)$^+$ calcd for $C_{35}H_{39}ClN_4NaO_3S$, 653.2324; found, 653.2336. Retention time = 2.746 min.

https://doi.org/10.1021/acs.jmedchem.1c00019
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*3-(4-(4-Chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-5-(piperazine-1-carbonyl)-6-(pyrrolidin-3-ylmethyl)pyridin-2(1H)-one (**98**).* This compound was prepared using Method 13 using *tert*-butyl 3-(4-ethoxy-2,4-dioxobutyl)pyrrolidine-1-carboxylate in **step 1** followed by Boc deprotection using TFA in DCM to afford 3-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-5-(piperazine-1-carbonyl)-6-(pyrrolidin-3-ylmethyl)pyridin-2(1*H*)-one as a TFA salt. LCMS: m/z $(M + H)^+$ = 616.2; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.96 (br s, 2H), 8.74 (s, 1H), 8.54 (br s, 2H), 8.24 (s, 1H), 8.16−8.01 (m, 2H), 7.60−7.44 (m, 3H), 7.37 (d, J = 7.7 Hz, 2H), 4.19−2.56 (m, 10H), 2.44−1.94 (m, 5H), 1.63 (dd, J = 101.3, 45.9 Hz, 5H), 1.39−1.15 (m, 1H), 1.10 (t, J = 7.5 Hz, 6H); HRMS (ESI): m/z $(M + H)^+$ calcd for $C_{29}H_{34}ClF_3N_9O$, 616.2521; found, 616.2524. Retention time = 2.321 min.

*3-(4-(4-Chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-5-(3-methylpiperazine-1-carbonyl)-6-(2-methylprop-1-en-1-yl)pyridin-2(1H)-one (**99**).* This compound was prepared using Method 15 using *tert*-butyl 2-methylpiperazine-1-carboxylate in **step 5** to afford 3-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-5-(3-methylpiperazine-1-carbonyl)-6-(2-methylprop-1-en-1-yl)pyridin-2(1*H*)-one as a TFA salt. LCMS: m/z $(M + H)^+$ = 601.1; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.73 (d, J = 9.4 Hz, 1H), 8.25 (s, 1H), 8.09 (dd, J = 8.9, 2.3 Hz, 2H), 7.53 (dd, J = 8.7, 2.3 Hz, 2H), 7.43 (t, J = 7.7 Hz, 1H), 7.29 (dd, J = 22.5, 7.7 Hz, 2H), 5.30 (s, 1H), 4.55 (dd, J = 32.5, 13.8 Hz, 1H), 4.33−4.23 (m, 1H), 3.72−3.48 (m, 1H), 3.23−2.62 (m, 3H), 2.42−2.24 (m, 2H), 2.16−1.97 (m, 2H), 1.63−1.52 (m, 6H), 1.24 (dd, J = 12.0, 6.4 Hz, 1H), 1.11 (dt, J = 11.5, 7.3 Hz, 6H), 0.98 (t, J = 7.5 Hz, 3H); HRMS (ESI): m/z $(M + H)^+$ calcd for $C_{34}H_{38}ClN_4O_2S$, 601.2399; found, 601.2395. Retention time = 2.805 min.

*3-(4-(4-Chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-5-(2-methylpiperazine-1-carbonyl)-6-(2-methylprop-1-en-1-yl)pyridin-2(1H)-one (**100**).* This compound was prepared using Method 15 using *tert*-butyl 3-methylpiperazine-1-carboxylate in **step 5** to afford 3-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-5-(3-methylpiperazine-1-carbonyl)-6-(2-methylprop-1-en-1-yl)pyridin-2(1*H*)-one as a TFA salt. LCMS: m/z $(M + H)^+$ = 601.1; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.70 (s, 1H), 8.25 (s, 1H), 8.13−8.05 (m, 2H), 7.53 (dd, J = 8.9, 2.3 Hz, 2H), 7.42 (t, J = 7.7 Hz, 1H), 7.35−7.21 (m, 2H), 5.28 (s, 1H), 4.94 (s, 1H), 3.59 (d, J = 14.1 Hz, 1H), 3.27−2.93 (m, 5H), 2.42−2.21 (m, 2H), 2.10 (dp, J = 26.2, 7.5 Hz, 2H), 1.63−1.47 (m, 6H), 1.38−1.19 (m, 1H), 1.17−1.06 (m, 6H), 0.99 (t, J = 7.5 Hz, 3H); HRMS (ESI): m/z $(M + H)^+$ calcd for $C_{34}H_{38}ClN_4O_2S$, 601.2399; found, 601.2388. Retention time = 2.824 min.

*3-(4-(4-Chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-5-(3-ethylpiperazine-1-carbonyl)-6-(2-methylprop-1-en-1-yl)pyridin-2(1H)-one (**101**).* This compound was prepared using Method 15 using *tert*-butyl 2-ethylpiperazine-1-carboxylate in **step 5** to afford 3-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-5-(3-ethylpiperazine-1-carbonyl)-6-(2-methylprop-1-en-1-yl)pyridin-2(1*H*)-one as a TFA salt. LCMS: m/z $(M + H)^+$ = 615.1; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.75 (s, 1H), 8.25 (s, 1H), 8.14−8.05 (m, 2H), 7.57−7.50 (m, 2H), 7.47−7.40 (m, 1H), 7.37−7.22 (m, 2H), 5.29 (s, 1H), 4.67−4.52 (m, 1H), 3.72−3.64 (m, 1H), 3.23−3.05 (m, 2H), 3.01−2.61 (m, 2H), 2.45−2.20 (m, 2H), 2.14−1.99 (m, 2H), 1.66−1.51 (m, 9H), 1.16−1.06 (m, 3H), 1.02−0.94 (m, 3H), 0.87−0.79 (m, 2H); HRMS (ESI): m/z $(M + H)^+$ calcd for $C_{35}H_{40}ClN_4O_2S$, 615.2555; found, 615.2525. Retention time = 2.848 min.

*3-(4-(4-Chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-6-(2-methylprop-1-en-1-yl)-5-(3-propylpiperazine-1-carbonyl)pyridin-2(1H)-one (**102**).* This compound was prepared using Method 15 using *tert*-butyl 2-propylpiperazine-1-carboxylate in **step 5** to afford 3-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-6-(2-methylprop-1-en-1-yl)-5-(3-propylpiperazine-1-carbonyl)pyridin-2(1*H*)-one as a TFA salt. LCMS: m/z $(M + H)^+$ = 629.3; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.75 (s, 1H), 8.25 (s, 1H), 8.16−8.05 (m, 2H), 7.53 (dt, J = 9.7, 2.9 Hz, 2H), 7.49−7.38 (m, 1H), 7.29 (dd, J = 22.4, 7.9 Hz, 2H), 5.28 (s, 1H), 4.66−4.31 (m, 2H), 3.72−3.67 (m, 1H), 3.23−2.94 (m, 2H), 2.89−2.52 (m, 2H), 2.43−2.19 (m, 2H), 2.15−1.99 (m, 2H), 1.65−1.50 (m, 6H), 1.37 (d, J = 1.2 Hz, 1H),, 1.16−1.05 (m, 4H), 1.04−0.82 (m, 8H); HRMS (ESI): m/z $(M + H)^+$ calcd for $C_{36}H_{39}ClFN_4O_3$, 629.2689; found, 629.2701. Retention time = 2.889 min.

*3-(4-(4-Chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-6-(2-methylprop-1-en-1-yl)-5-(4,7-diazaspiro[2.5]octane-7-carbonyl)-pyridin-2(1H)-one (**103**).* This compound was prepared using Method 15 using *tert*-butyl 4,7-diazaspiro[2.5]octane-4-carboxylate in **step 5** to afford 3-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-6-(2-methylprop-1-en-1-yl)-5-(4,7-diazaspiro[2.5]octane-7-carbonyl)pyridin-2(1*H*)-one as a TFA salt. LCMS: m/z $(M + H)^+$ = 613.3; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.71 (s, 1H), 8.41 (s, 1H), 8.29 (d, J = 8.1 Hz, 2H), 7.83 (d, J = 8.2 Hz, 2H), 7.48−7.21 (m, 3H), 5.33 (s, 1H), 4.43−4.33 (m, 1H), 3.70−3.37 (m, 3H), 3.07−2.90 (m, 2H), 2.71−2.59 (m, 2H), 2.44−1.90 (m, 4H), 1.58 (d, J = 10.3 Hz, 6H), 1.26−0.72 (m, 9H); HRMS (ESI): m/z $(M + H)^+$ calcd for $C_{35}H_{38}ClN_4O_2S$, 613.2399; found, 613.2427. Retention time = 2.85 min.

*3-(4-(4-Chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-5-(3-(hydroxymethyl)piperazine-1-carbonyl)-6-(2-methylprop-1-en-1-yl)pyridin-2(1H)-one (**104**).* This compound was prepared using Method 15 using *tert*-butyl 2-(hydroxymethyl)piperazine-1-carboxylate in **step 5** to afford 3-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-5-(3-(hydroxymethyl)piperazine-1-carbonyl)-6-(2-methylprop-1-en-1-yl)pyridin-2(1*H*)-one as a TFA salt. LCMS: m/z $(M + H)^+$ = 617.2; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.73 (s, 1H), 8.25 (s, 1H), 8.09 (d, J = 8.5 Hz, 2H), 7.53 (d, J = 8.5 Hz, 2H), 7.43 (t, J = 7.7 Hz, 1H), 7.29 (dd, J = 20.5, 7.7 Hz, 2H), 5.29 (s, 1H), 4.63−4.53 (m, 1H), 3.73−3.39 (m, 3H), 3.21−2.63 (m, 2H), 2.44−2.18 (m, 4H), 2.16−1.99 (m, 2H), 1.63−1.51 (m, 7H), 1.16−1.05 (m, 3H), 0.98 (t, J = 7.5 Hz, 3H); HRMS (ESI): m/z $(M + H)^+$ calcd for $C_{34}H_{38}ClN_4O_3S$, 617.2348; found, 617.2376. Retention time = 2.743 min.

*4-(5-(4-(4-Chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-2-(2-methylprop-1-en-1-yl)-6-oxo-1,6-dihydropyridine-3-carbonyl)-piperazin-2-one (**105**).* This compound was prepared using Method 15 using *tert*-butyl 2-oxopiperazine-1-carboxylate in **step 5** to afford 4-(5-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-2-(2-methylprop-1-en-1-yl)-6-oxo-1,6-dihydropyridine-3-carbonyl)piperazin-2-one as a TFA salt. LCMS: m/z $(M + H)^+$ = 601.1; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.70 (d, J = 8.2 Hz, 1H), 8.24 (d, J = 1.8 Hz, 1H), 8.17−7.97 (m, 3H), 7.58−7.36 (m, 4H), 5.23 (s, 1H), 3.89−3.79 (s, 2H), 3.26−2.95 (m, 4H), 2.41−2.00 (m, 4H), 1.61−1.42 (m, 7H), 1.20−0.86 (m, 6H); HRMS (ESI): m/z $(M + H)^+$ calcd for $C_{33}H_{34}ClN_4O_3S$, 601.2035; found, 601.2049. Retention time = 3.612 min.

*3-(4-(4-Chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-6-(2-methylprop-1-en-1-yl)-5-(3-(trifluoromethyl)piperazine-1-carbonyl)pyridin-2(1H)-one (**106**).* This compound was prepared using Method 15 using *tert*-butyl 2-(trifluoromethyl)piperazine-1-carboxylate in **step 5** to afford 3-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-6-(2-methylprop-1-en-1-yl)-5-(3-(trifluoromethyl)piperazine-1-carbonyl)pyridin-2(1*H*)-one as a TFA salt. LCMS: m/z $(M + H)^+$ = 655.2; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.75 (s, 1H), 8.24 (s, 1H), 8.10 (dd, J = 9.1, 2.5 Hz, 2H), 7.57−7.48 (m, 2H), 7.43 (td, J = 7.7, 4.6 Hz, 1H), 7.30 (dd, J = 18.6, 7.0 Hz, 2H), 5.25 (s, 1H), 4.52−4.44 (m, 1H), 4.15−3.98 (m, 1H), 3.23−2.56 (m, 5H), 2.38−2.17 (m, 2H), 2.16−1.99 (m, 2H), 1.56 (d, J = 11.2 Hz, 6H), 1.16−0.91 (m, 6H); HRMS (ESI): m/z $(M + H)^+$ calcd for $C_{34}H_{35}ClF_3N_4O_2S$, 655.2116; found, 655.214. Retention time = 3.952 min.

*4-(5-(4-(4-Chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-2-(2-methylprop-1-en-1-yl)-6-oxo-1,6-dihydropyridine-3-carbonyl)-piperazine-2-carbonitrile (**107**).* This compound was prepared using Method 15 using *tert*-butyl 2-cyanopiperazine-1-carboxylate in **step 5** to afford 4-(5-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-2-(2-methylprop-1-en-1-yl)-6-oxo-1,6-dihydropyridine-3-carbonyl)-piperazine-2-carbonitrile as a TFA salt. LCMS: m/z $(M + H)^+$ = 612.2; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.67 (d, J = 8.6 Hz, 1H), 8.24 (s, 1H), 8.09 (dd, J = 8.3, 1.7 Hz, 2H), 7.58−7.48 (m, 2H), 7.42 (t, J = 7.6 Hz, 1H), 7.29 (dd, J = 17.7, 7.3 Hz, 2H), 5.24 (d, J = 8.0 Hz, 1H), 4.45−4.12 (m, 1H), 3.23−2.97 (m, 2H), 2.93−2.63 (m, 4H), 2.44−2.20 (m, 2H), 2.20−2.01 (m, 2H), 1.67−1.50 (m, 6H),

https://doi.org/10.1021/acs.jmedchem.1c00019
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1.11 (t, J = 12.8, 7.4, Hz, 3H), 1.00 (t, J = 7.4 Hz, 3H); HRMS (ESI): m/z (M + H)$^+$ calcd for $C_{34}H_{35}ClN_5O_2S$, 612.2195; found, 612.22. Retention time = 3.776 min.

*4-(5-(4-(4-Chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-2-(2-methylprop-1-en-1-yl)-6-oxo-1,6-dihydropyridine-3-carbonyl)-piperazine-2-carboxylic Acid (**108**).* This compound was prepared using Method 15 using 1-(*tert*-butyl) 2-methyl piperazine-1,2-dicarboxylate in **step 5** followed by ester hydrolysis using LiOH in THF/MeOH and Boc deprotection using TFA in DCM to afford 4-(5-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-2-(2-methylprop-1-en-1-yl)-6-oxo-1,6-dihydropyridine-3-carbonyl)piperazine-2-carboxylic acid as a TFA salt. LCMS: m/z (M + H)$^+$ = 631.1; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.75 (s, 1H), 8.25 (s, 1H), 8.09 (dd, J = 8.2, 1.3 Hz, 2H), 7.53 (dd, J = 8.6, 2.3 Hz, 2H), 7.43 (td, J = 7.7, 5.6 Hz, 1H), 7.36−7.18 (m, 2H), 5.28 (s, 1H), 4.58−4.48 (m, 1H), 4.20−4.04 (m, 1H), 3.87−3.57 (m, 1H), 3.12−2.62 (m, 2H), 2.44−2.17 (m, 2H), 2.20−1.99 (m, 2H), 1.63−1.49 (m, 6H), 1.24−1.05 (m, 3H), 1.03−0.94 (m, 3H); HRMS (ESI): m/z (M + H)$^+$ calcd for $C_{27}H_{28}Cl\ N_{14}O_3$, 631.214; found, 631.2151. Retention time = 3.038 min.

*4-(5-(4-(4-Chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-2-(2-methylprop-1-en-1-yl)-6-oxo-1,6-dihydropyridine-3-carbonyl)-piperazine-2-carboxamide (**109**).* This compound was prepared using Method 15 using piperazine-2-carboxamide in **step 5** to afford 4-(5-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-2-(2-methylprop-1-en-1-yl)-6-oxo-1,6-dihydropyridine-3-carbonyl)-piperazine-2-carboxamide as a TFA salt. LCMS: m/z (M + H)$^+$ = 630.3; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.76 (s, 1H), 8.26 (s, 1H), 8.09 (dd, J = 8.0, 6.0 Hz, 2H), 7.53 (dd, J = 8.0, 6.2 Hz, 2H), 7.43 (t, J = 7.7 Hz, 1H), 7.37−7.20 (m, 2H), 5.29 (d, J = 9.5 Hz, 1H), 4.72−4.55 (m, 1H), 4.02−3.72 (m, 1H), 3.66−3.54 (m, 1H), 3.17−2.99 (m, 2H), 2.82 (t, J = 13.0 Hz, 1H), 2.68−2.62 (m, 1H), 2.42−2.22 (m, 2H), 2.15−1.99 (m, 2H), 1.67−1.50 (m, 6H), 1.11 (t, J = 7.5 Hz, 3H), 1.05−0.91 (m, 3H); HRMS (ESI): m/z (M + H)$^+$ calcd for $C_{34}H_{34}ClF_5N_3O$, 630.2305; found, 630.2311. Retention time = 2.753 min.

*3-(4-(4-Chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-6-(2-methylprop-1-en-1-yl)-5-(2,6-diazaspiro[3.3]heptane-2-carbonyl)-pyridin-2(1H)-one (**110**).* This compound was prepared using Method 15 using *tert*-butyl 2,6-diazaspiro[3.3]heptane-2-carboxylate in **step 5** to afford 3-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-6-(2-methylprop-1-en-1-yl)-5-(2,6-diazaspiro[3.3]heptane-2-carbonyl)pyridin-2(1H)-one as a TFA salt. LCMS: m/z (M + H)$^+$ = 599.2; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.69 (d, J = 4.2 Hz, 1H), 8.23 (d, J = 8.1 Hz, 1H), 8.07 (dd, J = 8.7, 2.3 Hz, 2H), 7.56−7.49 (m, 2H), 7.42 (t, J = 7.7 Hz, 1H), 7.29 (d, J = 7.6 Hz, 2H), 5.30 (s, 1H), 4.08 (t, J = 11.4 Hz, 4H), 2.70−2.59 (m, 2H), 2.24−2.09 (m, 6H), 1.58 (s, 6H), 1.08−1.02 (m, 6H). HRMS (ESI): m/z (M + H)$^+$ calcd for $C_{34}H_{36}ClN_4O_2S$, 599.2242; found, 599.225. Retention time = 2.749 min.

*5-((1R,5S)-3,8-Diazabicyclo[3.2.1]octane-3-carbonyl)-3-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-6-(2-methylprop-1-en-1-yl)pyridin-2(1H)-one (**111**).* This compound was prepared using Method 15 using *tert*-butyl (1R,5S)-3,8-diazabicyclo[3.2.1]-octane-8-carboxylate in **step 5** to afford 5-((1R,5S)-3,8-diazabicyclo-[3.2.1]octane-3-carbonyl)-3-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-6-(2-methylprop-1-en-1-yl)pyridin-2(1H)-one as a TFA salt. LCMS: m/z (M + H)$^+$ = 613.2; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.73 (s, 1H), 8.24 (s, 1H), 8.15−8.04 (m, 2H), 7.58−7.48 (m, 2H), 7.42 (t, J = 7.7 Hz, 1H), 7.29 (dd, J = 26.3, 7.7 Hz, 2H), 5.23 (s, 1H), 4.44 (d, J = 14.0 Hz, 1H), 4.14−3.86 (m, 2H), 3.61−3.34 (m, 2H), 2.96 (d, J = 14.0 Hz, 1H), 2.65 (p, J = 1.9 Hz, 1H), 2.44−2.27 (m, 2H), 2.15−1.97 (m, 2H), 1.96−1.71 (m, 3H), 1.55 (dd, J = 10.4, 1.3 Hz, 6H), 1.14 (t, J = 7.7 Hz, 3H), 0.96 (t, J = 7.5 Hz, 3H); HRMS (ESI): m/z (M + Na)$^+$ calcd, for $C_{35}H_{37}ClN_4NaO_2S$, 635.2218; found, 635.2206. Retention time = 2.823 min.

*5-(3,8-Diazabicyclo[3.2.1]octane-8-carbonyl)-3-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-6-(2-methylprop-1-en-1-yl)pyridin-2(1H)-one (**112**).* This compound was prepared using Method 15 using *tert*-butyl (1R,5S)-3,8-diazabicyclo[3.2.1]-octane-3-carboxylate in **step 5** to afford 5-(3,8-diazabicyclo[3.2.1]-octane-8-carbonyl)-3-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2,6-diethylphenyl)-6-(2-methylprop-1-en-1-yl)pyridin-2(1H)-one as a TFA salt. LCMS: m/z (M + H)$^+$ = 613.2; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.22 (s, 1H), 8.08 (d, J = 8.3 Hz, 2H), 7.52 (d, J = 8.3 Hz, 2H), 7.41 (t, J = 7.7 Hz, 2H), 7.27 (dd, J = 13.8, 7.7 Hz, 2H), 5.24 (s, 1H), 3.98−3.92 (m, 2H), 3.25−3.05 (m, 4H), 2.78−2.53 (m, 4H), 2.37−2.02 (m, 4H),1.90 (s, 3H), 1.55 (s, 3H), 1.04−0.97 (m, 6H); HRMS (ESI): m/z (M + H)$^+$ calcd for $C_{35}H_{38}ClN_4O_2S$, 613.2399; found, 613.2389. Retention time = 2.798 min.

*3-(4-(4-Chlorophenyl)thiazol-2-yl)-6-(2-methylprop-1-en-1-yl)-1-phenyl-5-(piperazine-1-carbonyl)pyridin-2(1H)-one (**113**).* This compound was prepared using Method 15 using aniline in **step 3** to afford 3-(4-(4-chlorophenyl)thiazol-2-yl)-6-(2-methylprop-1-en-1-yl)-1-phenyl-5-(piperazine-1-carbonyl)pyridin-2(1H)-one as a TFA salt. LCMS: m/z (M + H)$^+$ = 531.2; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.82 (s, 1H), 8.06−8.01 (m, 2H), 7.93 (d, J = 1.8 Hz, 1H), 7.61−7.49 (m, 3H), 7.48−7.42 (m, 2H), 7.37 (s, 1H), 7.19 (s, 1H), 5.59 (s, 1H), 4.25 (s, 2H), 3.84 (s, 2H), 3.64 (d, J = 18.3 Hz, 2H), 3.14 (s, 2H), 1.63 (dd, J = 9.4, 1.1 Hz, 6H). Aliphatic region complicated significantly by amide rotamers; HRMS (ESI): m/z (M + H)$^+$ calcd for $C_{29}H_{28}ClN_4O_2S$, 531.1616; found, 531.1631. Retention time = 2.583 min.

*3-(4-(4-Chlorophenyl)thiazol-2-yl)-1-(2-ethylphenyl)-6-(2-methylprop-1-en-1-yl)-5-(piperazine-1-carbonyl)pyridin-2(1H)-one (**114**).* This compound was prepared using Method 15 using 2-ethylaniline in **step 3** to afford 3-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2-ethylphenyl)-6-(2-methylprop-1-en-1-yl)-5-(piperazine-1-carbonyl)pyridin-2(1H)-one as a TFA salt. LCMS: m/z (M + H)$^+$ = 559.2; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.86 (s, 1H), 8.04 (d, J = 8.7 Hz, 2H), 7.94 (s, 1H), 7.53−7.47 (m, 2H), 7.45 (d, J = 8.6 Hz, 2H), 7.44−7.00 (m, 2H), 5.62−5.42 (m, 1H), 4.35−4.25 (m, 2H), 3.90−3.50 (m, 4H), 3.35−3.10 (m, 2H), 2.50−2.25 (m, 2H), 1.67−1.57 (m, 6H), 1.25−1.05 (m, 3H). Aliphatic region complicated significantly by amide rotamers; HRMS (ESI): m/z (M + H)$^+$ calcd for $C_{31}H_{32}ClN_4O_2S$, 559.1929; found, 559.1937. Retention time = 2.675 min.

*3-(4-(4-Chlorophenyl)thiazol-2-yl)-1-(2-ethyl-6-methylphenyl)-6-(2-methylprop-1-en-1-yl)-5-(piperazine-1-carbonyl)pyridin-2(1H)-one (**115**).* This compound was prepared using Method 15 using 2-ethyl-6-methylaniline in **step 3** to afford 3-(4-(4-chlorophenyl)-thiazol-2-yl)-1-(2-ethyl-6-methylphenyl)-6-(2-methylprop-1-en-1-yl)-5-(piperazine-1-carbonyl)pyridin-2(1H)-one as a TFA salt. LCMS: m/z (M + H)$^+$ = 533.2; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.95 (s, 2H), 8.71 (d, J = 6.5 Hz, 1H), 8.22 (s, 1H), 8.12 (d, J = 8.6 Hz, 2H), 7.52 (t, J = 8.4 Hz, 2H), 7.43 (t, J = 7.6 Hz, 1H), 7.33 (dd, J = 15.7, 8.1 Hz, 2H), 3.57 (d, J = 87.5 Hz, 4H), 3.16 (t, J = 47.1 Hz, 4H), 2.20 (t, J = 39.9 Hz, 1H), 1.98 (s, 3H), 1.90 (s, 3H), 1.08 (t, J = 7.5 Hz, 3H). Aliphatic region complicated significantly by amide rotamers; HRMS (ESI): m/z (M + H)$^+$ calcd for $C_{29}H_{30}ClN_4O_2S$, 533.1773; found, 533.1792. Retention time = 2.6 min.

*1-(2-Chloro-6-ethylphenyl)-3-(4-(4-chlorophenyl)thiazol-2-yl)-6-(2-methylprop-1-en-1-yl)-5-(piperazine-1-carbonyl)pyridin-2(1H)-one (**116**).* This compound was prepared using Method 15 using 2-chloro-6-ethylaniline in **step 3** to afford 1-(2-chloro-6-ethylphenyl)-3-(4-(4-chlorophenyl)thiazol-2-yl)-6-(2-methylprop-1-en-1-yl)-5-(piperazine-1-carbonyl)pyridin-2(1H)-one as a TFA salt. LCMS: m/z (M + H)$^+$ = 593.2; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.88 (s, 1H), 8.03 (d, J = 8.7 Hz, 2H), 7.94 (s, 1H), 7.55−7.39 (m, 5H), 5.55−5.35 (m, 1H), 4.40−4.20 (m, 1H), 3.85−3.70 (m, 1H), 3.60−3.45 (m, 2H), 3.35−3.10 (m, 4H), 2.60−2.25 (m, 2H), 1.74−1.63 (m, 6H), 1.30−1.05 (m, 3H). Aliphatic region complicated significantly by amide rotamers; HRMS (ESI): m/z (M + H)$^+$ calcd for $C_{31}H_{31}Cl_2N_4O_2S$, 593.1539; found, 593.1528. Retention time = 4.187 min.

*3-(4-(4-Chlorophenyl)thiazol-2-yl)-1-(2-ethyl-5-methylphenyl)-6-(2-methylprop-1-en-1-yl)-5-(piperazine-1-carbonyl)pyridin-2(1H)-one (**117**).* This compound was prepared using Method 15 using 2-ethyl-5-methylaniline in **step 3** to afford 3-(4-(4-chlorophenyl)-thiazol-2-yl)-1-(2-ethyl-5-methylphenyl)-6-(2-methylprop-1-en-1-yl)-5-(piperazine-1-carbonyl)pyridin-2(1H)-one as a TFA salt. LCMS:

https://doi.org/10.1021/acs.jmedchem.1c00019
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m/z (M + H)$^+$ = 573.2; ^{1}H NMR (400 MHz, CD_3OD): δ 8.86 (s, 1H), 8.04 (d, J = 8.4 Hz, 2H), 7.94 (s, 1H), 7.45 (d, J = 8.8 Hz, 2H), 7.37−7.27 (m, 2H), 7.17−7.03 (m, 1H), 5.66−5.41 (m, 1H), 4.42−4.20 (m, 1H), 3.91−3.74 (m, 1H), 3.65−3.49 (m, 2H), 3.25−3.13 (m, 3H), 2.50−2.18 (m, 6H), 1.71−1.61 (m, 6H), 1.23−1.05 (m, 3H). Aliphatic region complicated significantly by amide rotamers; HRMS (ESI): m/z (M + H)$^+$ calcd for $C_{32}H_{34}ClN_4O_2S$, 573.2086; found, 573.2082. Retention time = 2.735 min.

*3-(4-(4-Chlorophenyl)thiazol-2-yl)-1-(2-ethyl-5-methoxyphenyl)-6-(2-methylprop-1-en-1-yl)-5-(piperazine-1-carbonyl)pyridin-2(1H)-one (**118**).* This compound was prepared using Method 15 using 2-ethyl-5-methoxyaniline in **step 3** to afford 3-(4-(4-chlorophenyl)thiazol-2-yl)-1-(2-ethyl-5-methoxyphenyl)-6-(2-methylprop-1-en-1-yl)-5-(piperazine-1-carbonyl)pyridin-2(1*H*)-one as a TFA salt. LCMS: m/z (M + H)$^+$ = 589.3; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.69 (s, 1H), 8.24 (s, 1H), 8.10 (d, J = 8.2 Hz, 2H), 7.57−7.50 (m, 3H), 7.04 (d, J = 7.2 Hz, 2H), 5.53 (s, 1H), 3.78 (s, 3H), 3.72−3.67 (m, 2H), 3.45−3.37 (m, 2H), 3.20−3.01 (m, 2H), 2.65 (q, J = 1.9 Hz, 2H), 2.09−2.03 (m, 2H), 1.61−1.52 (m, 6H), 1.15−0.90 (m, 3H); HRMS (ESI): m/z (M + H)$^+$ calcd for $C_{32}H_{34}ClN_4O_3S$, 589.2035; found, 589.2024. Retention time = 2.709 min.

In Method 15, after step 5, using a preparative CHIRALCEL ODH Column and a hexanes/EtOH/DEA (70/30/0.04) mobile phase at a flow rate of 35 mL/min, Boc-**55** could be separated into two isomers with ee >98% and ee 80.9%. These were subjected to Boc deprotection with TFA to yield (+)-**118** and (−)-(**118**), respectively.

(+)-118. ^{1}H NMR (400 MHz, chloroform-*d*): δ 8.82 (bs, 1H), 7.93 (d, J = 8.6 Hz, 1H), 7.58 (s, 1H), 7.44−7.36 (m, 3H), 7.30 (d, J = 8.6 Hz, 1H), 6.98 (dd, J = 8.7, 2.7 Hz, 1H), 6.68−6.46 (m, 1H, rotameric), 5.60−5.28 (m, 1H, rotameric), 4.16−2.84 (m, 9H), 2.42−2.02 (m, 2H), 1.70−1.60 (m, 6H), 1.16−0.81 (m, 3H); HRMS (ESI): m/z (M + H)$^+$ calcd for $C_{32}H_{34}ClN_4O_3S$, 589.2035; found, 589.2021. Retention time = 2.582 min.

(−)-118. ^{1}H NMR (400 MHz, chloroform-*d*): δ 8.81 (bs, 1H), 7.93 (d, J = 8.6 Hz, 1H), 7.57 (s, 1H), 7.44−7.34 (m, 3H), 7.30 (d, J = 8.6 Hz, 1H), 6.97 (dd, J = 8.7, 2.7 Hz, 1H), 6.67−6.49 (m, 1H, rotameric), 5.61−5.29 (m, 1H, rotameric), 4.14−2.86 (m, 9H), 2.41−2.03 (m, 2H), 1.71−1.61 (m, 6H), 1.17−0.80 (m, 3H); HRMS (ESI): m/z (M + Na)$^+$ calcd for $C_{32}H_{33}ClN_4NaO_3S$, 611.1854; found, 611.1875. Retention time = 2.685 min.

*1-(5-Chloro-2-ethylphenyl)-3-(4-(4-chlorophenyl)thiazol-2-yl)-6-(2-methylprop-1-en-1-yl)-5-(piperazine-1-carbonyl)pyridin-2(1H)-one (**119**).* This compound was prepared using Method 15 using 2-ethyl-5-chloroaniline in **step 3** to afford 1-(5-chloro-2-ethylphenyl)-3-(4-(4-chlorophenyl)thiazol-2-yl)-6-(2-methylprop-1-en-1-yl)-5-(piperazine-1-carbonyl)pyridin-2(1*H*)-one as a TFA salt. LCMS: m/z (M + H)$^+$ = 593.2; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.60 (s, 1H), 8.21 (s, 1H), 8.09 (d, J = 8.2 Hz, 2H), 7.70−7.26 (m, 5H), 5.50−5.26 (m, 1H, rotameric), 3.67−3.55 (m, 1H), 3.41−3.11 (m, 3H), 2.78−2.53 (m, 4H), 2.37−2.02 (m, 2H), 1.57 (s, 3H), 1.55 (s, 3H), 1.15−0.91 (m, 3H); HRMS (ESI): m/z (M + H)$^+$ calcd for $C_{31}H_{31}Cl_2N_4O_2S$, 593.1533; found, 593.1539. Retention time = 2.747 min.

Synthesis of Isobutylene Analogues (+)-119 and (−)-119 as per Scheme 3. *1-(5-Chloro-2-ethylphenyl)-3-(4-(4-chlorophenyl)-thiazol-2-yl)-7,7-dimethyl-7,8-dihydro-2H-pyrano[4,3-b]pyridine-2,5(1H)-dione (**VIIb**).* A mixture of 6,6-dimethyldihydro-2*H*-pyran-2,4(3*H*)-dione **Ic** (6.06 g, 42.6 mmol) and 1,1-dimethoxy-*N*,*N*-dimethylmethanamine **II** (5.66 mL, 42.6 mmol) was stirred for 15 min at rt. The reaction mixture became a yellow solid. To the mixture were added 2-propanol (100 mL) followed by 2-(4-(4-chlorophenyl)-thiazol-2-yl)acetonitrile N1/**IVa** (10.0 g, 42.6 mmol) and piperidine (16.9 mL, 170 mmol). The mixture was stirred at 70 °C for 4 h and brought to rt, and the solvent was removed by rotovap. To the residue were added 5-chloro-2-ethylaniline (6.63 g, 42.6 mmol) and acetic acid (122.0 mL, 2130 mmol). The mixture was stirred at 70 °C for 4 h, cooled to room temperature, diluted with DCM, and washed with water and brine. The organic layer was dried over Na_2SO_4 and concentrated. The crude product was purified by column chromatography (10:90 EA/Hex to 100% EA) to afford the product, 1-(5-chloro-2-ethylphenyl)-3-(4-(4-chlorophenyl)thiazol-2-yl)-7,7-dimethyl-7,8-dihydro-2*H*-pyrano[4,3-*b*]pyridine-2,5(1*H*)-dione **VIIb** as a yellow solid; LCMS: m/z (M + H)$^+$ = 525.0. Yield ∼75%.

*tert-Butyl 4-(1-(5-Chloro-2-ethylphenyl)-5-(4-(4-chlorophenyl)-thiazol-2-yl)-2-(2-methylprop-1-en-1-yl)-6-oxo-1,6-dihydropyridine-3-carbonyl)piperazine-1-carboxylate (**VIIIa**).* To a solution of **VIIb** (10 g, 19 mmol) in THF (100 mL) and MeOH (100 mL) was added lithium hydroxide (2.3 g, 95 mmol) and the reaction mixture was stirred at rt for 1 h. The solvent was removed; the reaction mixture was diluted with DCM and quenched with acetic acid (5.45 mL, 95 mmol). The organic layer was washed several times with brine, dried over Na_2SO_4, and concentrated to afford crude 1-(5-chloro-2-ethylphenyl)-5-(4-(4-chlorophenyl)thiazol-2-yl)-2-(2-methylprop-1-en-1-yl)-6-oxo-1,6-dihydropyridine-3-carboxylic acid **VIIc**; LCMS: m/z (M + H)$^+$ = 525.0. Yield ∼95%.

To a solution of the crude unpurified acid **VIIc** in DMF (25 mL) were added HATU (10.9 g, 28.5 mmol), *N*-ethyl-*N*-isopropylpropan-2-amine (9.97 mL, 57.1 mmol), and *tert*-butyl piperazine-1-carboxylate (5.32 g, 28.5 mmol). The reaction mixture became yellow and it was stirred for 1 h at rt, diluted with water, extracted with 3 × 100 mL of DCM, and washed with brine. The organic layer was dried over Na_2SO_4 and concentrated. The organic solvent was concentrated and purified by reverse-phase flash chromatography (100% water to 100% acetonitrile in 0.1% TFA) to afford **VIIIa**. It was dissolved in DCM (100 mL) and washed with sat aq $NaHCO_3$ (3 × 100 mL) solution to remove any residual TFA (this step was important to prevent conversion of **VIIIa** back to **VIIb**), dried over Na_2SO_4, and filtered; the organic solvent was removed by rotovap and then dried under high vacuum to afford pure **VIIIa**, LCMS: m/z (M + H)$^+$ = 693.0. Yield ∼90%.

Atropisomer Separation. Using preparative CHIRALCEL ODH Column and a hexanes/EtOH (70/30) mobile phase at a flow rate of 1 mL/min, **VIIIa** clearly separated into two isomers and ee >98% that were designated as **1st_pos** (ee > 98%) and **2nd_neg** (ee 98.0%).

*(S)-1-(5-Chloro-2-ethylphenyl)-3-(4-(4-chlorophenyl)thiazol-2-yl)-6-(2-methylprop-1-en-1-yl)-5-(piperazine-1-carbonyl)pyridin-2(1H)-one ((+)-**119**).* To a solution of active atropisomer **1st_pos** (5.00 g, 7.21 mmol) in DCM (50 mL) was added 2,2,2-trifluoroacetic acid (19.44 mL, 252.4 mmol), and the reaction mixture was stirred for 1 h at rt. The solvent was concentrated, and the material was purified by reverse-phase flash chromatography (gradient of 100% water to 100% acetonitrile in 0.1% TFA) to provide (+)-**119**, the TFA salt. This was dissolved in DCM (100 mL) and washed with sat aq $NaHCO_3$ (3 × 100 mL) to afford the free base. The organic layer was dried over Na_2SO_4 concentrated by rotovap and dried under high vacuum to afford pure (+)-**119** aka **NCATS-SM5637**, **NSC 791985**. Yield ∼92%, LCMS: m/z (M + H)$^+$ = 593.0. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.61 (s, 1H), 8.23 (s, 1H), 8.14−8.05 (d, J = 8.2 Hz, 2H), 7.70−7.35 (m, 5H), 5.50−5.30 (2 br s 1H, rotameric), 3.64−3.54 (m, 1H), 3.65−3.10 (m, 3H), 2.80−2.56 (m, 4H), 2.22−2.05 (m, 2H), 1.62−1.55 (m, 6H), 1.13−0.95 (m, 3H); HRMS (ESI): m/z (M + H)$^+$ calcd for $C_{31}H_{31}Cl_2N_4O_2S$, 593.1539; found, 593.1561. Retention time = 2.747 min. 98% ee as determined by HPLC analysis using CHIRALCEL OD-H column, 10% *i*-PrOH in hexane, 0.5 mL/min 254 nm, t_r (min):16.4 (major), t_r 18.6 (minor).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jmedchem.1c00019.

Molecular formula strings of all compounds (CSV)

Spectroscopic data (^{1}H NMR, LC/MS) for representative compounds (PDF)

https://doi.org/10.1021/acs.jmedchem.1c00019
J. Med. Chem. 2021, 64, 4913−4946

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https://doi.org/10.1021/acs.jmedchem.1c00019
J. Med. Chem. 2021, 64, 4913−4946

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank Heather Baker, Danielle Bougie, Elizabeth Fernandez, Misha Itkin, Zina Itkin, Christopher LeClair, William Leister, Crystal McKnight, and Paul Shinn for assistance with chemical purification and compound management. This research was supported by the Molecular Libraries Initiative of the National Institutes of Health Roadmap for Medical Research Grant U54MH084681 and the Intramural Research Program of the National Center for Advancing Translational Sciences, National Institutes of Health. This project has also been funded, in part, with Federal funds from the National Cancer Institute, National Institutes of Health, under the Chemical Biology Consortium contract no. HHSN261200800001E. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services or does mention of trade names, commercial products, or organizations that imply endorsement by the U.S. Government.

■ ABBREVIATIONS

AAALACi, American Association for Accreditation of Laboratory Animal Care international; ATCC, American Type Culture Collection; bodipy, boron difluoride dipyrromethene; CBC, Chemical Biology Consortium; CCD, charged-coupled device; CETSA, cellular thermal shift assay; DI, deionized; DMEM, Dulbecco's modified Eagle medium; DPBS, Dulbecco's phosphate-buffered saline; EtOAc, ethyl acetate; G6PDH, glucose-6-phosphate dehydrogenase; HATU, hexafluorophosphate azabenzotriazole tetramethyl uronium; 2-HG, *R*-2-hydroxyglutarate; IDH1, isocitrate dehydrogenase 1; KG, ketoglutarate; KO^{t}Bu, potassium *tert*-butoxide; mIDH1, mutant isocitrate dehydrogenase 1; NCATS, National Center for Advancing Translational Sciences; NExT, Institute's (NCI) Experimental Therapeutics; NSC, (Cancer Chemotherapy) National Service Center; qHTS, quantitative high-throughput screen; RH, relative humidity; RLM, rat liver microsomes

■ REFERENCES

(1) Cairns, R. A.; Mak, T. W. Oncogenic isocitrate dehydrogenase mutations: mechanisms, models, and clinical opportunities. *Cancer Discovery* **2013**, *3*, 730−741.

(2) Dang, L.; Yen, K.; Attar, E. C. IDH mutations in cancer and progress toward development of targeted therapeutics. *Ann. Oncol.* **2016**, *27*, 599−608.

(3) Molenaar, R. J.; Radivoyevitch, T.; Maciejewski, J. P.; van Noorden, C. J. F.; Bleeker, F. E. The driver and passenger effects of isocitrate dehydrogenase 1 and 2 mutations in oncogenesis and survival prolongation. *Biochim. Biophys. Acta, Rev. Cancer* **2014**, *1846*, 326−341.

(4) Yan, H.; Parsons, D. W.; Jin, G.; McLendon, R.; Rasheed, B. A.; Yuan, W.; Kos, I.; Batinic-Haberle, I.; Jones, S.; Riggins, G. J.; Friedman, H.; Friedman, A.; Reardon, D.; Herndon, J.; Kinzler, K. W.; Velculescu, V. E.; Vogelstein, B.; Bigner, D. D. IDH1 and IDH2 mutations in gliomas. *N. Engl. J. Med.* **2009**, *360*, 765−773.

(5) Mardis, E. R.; Ding, L.; Dooling, D. J.; Larson, D. E.; McLellan, M. D.; Chen, K.; Koboldt, D. C.; Fulton, R. S.; Delehaunty, K. D.; McGrath, S. D.; Fulton, L. A.; Locke, D. P.; Magrini, V. J.; Abbott, R. M.; Vickery, T. L.; Reed, J. S.; Robinson, J. S.; Wylie, T.; Smith, S. M.; Carmichael, L.; Eldred, J. M.; Harris, C. C.; Walker, J.; Peck, J. B.; Du, F.; Dukes, A. F.; Sanderson, G. E.; Brummett, A. M.; Clark, E.; McMichael, J. F.; Meyer, R. J.; Schindler, J. K.; Pohl, C. S.; Wallis, J. W.; Shi, X.; Lin, L.; Schmidt, H.; Tang, Y.; Haipek, C.; Wiechert, M. E.; Ivy, J. V.; Kalicki, J.; Elliott, G.; Ries, R. E.; Payton, J. E.; Westervelt, P.; Tomasson, M. H.; Watson, M. A.; Baty, J.; Heath, S.; Shannon, W. D.; Nagarajan, R.; Link, D. C.; Walter, M. J.; Graubert, T. A.; DiPersio, J. F.; Wilson, R. K.; Ley, T. J. Recurring mutations found by sequencing an acute myeloid leukemia genome. *N. Engl. J. Med.* **2009**, *361*, 1058−1066.

(6) Ward, P. S.; Patel, J.; Wise, D. R.; Abdel-Wahab, O.; Bennett, B. D.; Coller, H. A.; Cross, J. R.; Fantin, V. R.; Hedvat, C. V.; Perl, A. E.; Rabinowitz, J. D.; Carroll, M.; Su, S. M.; Sharp, K. A.; Levine, R. L.; Thompson, C. B. The common feature of leukemia-associated IDH1 and IDH2 mutations is a neomorphic enzyme activity converting alpha-ketoglutarate to 2-hydroxyglutarate. *Cancer Cell* **2010**, *17*, 225−234.

(7) Losman, J.-A.; Kaelin, W. G., Jr. What a difference a hydroxyl makes: mutant IDH, (R)-2-hydroxyglutarate, and cancer. *Genes Dev.* **2013**, *27*, 836−852.

(8) Dang, L.; White, D. W.; Gross, S.; Bennett, B. D.; Bittinger, M. A.; Driggers, E. M.; Fantin, V. R.; Jang, H. G.; Jin, S.; Keenan, M. C.; Marks, K. M.; Prins, R. M.; Ward, P. S.; Yen, K. E.; Liau, L. M.; Rabinowitz, J. D.; Cantley, L. C.; Thompson, C. B.; Vander Heiden, M. G.; Su, S. M. Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. *Nature* **2010**, *465*, 966.

(9) Dang, L.; White, D. W.; Gross, S.; Bennett, B. D.; Bittinger, M. A.; Driggers, E. M.; Fantin, V. R.; Jang, H. G.; Jin, S.; Keenan, M. C.; Marks, K. M.; Prins, R. M.; Ward, P. S.; Yen, K. E.; Liau, L. M.; Rabinowitz, J. D.; Cantley, L. C.; Thompson, C. B.; Vander Heiden, M. G.; Su, S. M. Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. *Nature* **2009**, *462*, 739−744.

(10) DiNardo, C. D.; Propert, K. J.; Loren, A. W.; Paietta, E.; Sun, Z.; Levine, R. L.; Straley, K. S.; Yen, K.; Patel, J. P.; Agresta, S.; Abdel-Wahab, O.; Perl, A. E.; Litzow, M. R.; Rowe, J. M.; Lazarus, H. M.; Fernandez, H. F.; Margolis, D. J.; Tallman, M. S.; Luger, S. M.; Carroll, M. Serum 2-hydroxyglutarate levels predict isocitrate dehydrogenase mutations and clinical outcome in acute myeloid leukemia. *Blood* **2013**, *121*, 4917−4924.

(11) Losman, J.-A.; Looper, R. E.; Koivunen, P.; Lee, S.; Schneider, R. K.; McMahon, C.; Cowley, G. S.; Root, D. E.; Ebert, B. L.; Kaelin, W. G., Jr. (R)-2-hydroxyglutarate is sufficient to promote leukemogenesis and its effects are reversible. *Science* **2013**, *339*, 1621−1625.

(12) Turcan, S.; Rohle, D.; Goenka, A.; Walsh, L. A.; Fang, F.; Yilmaz, E.; Campos, C.; Fabius, A. W. M.; Lu, C.; Ward, P. S.; Thompson, C. B.; Kaufman, A.; Guryanova, O.; Levine, R.; Heguy, A.; Viale, A.; Morris, L. G. T.; Huse, J. T.; Mellinghoff, I. K.; Chan, T. A. IDH1 mutation is sufficient to establish the glioma hypermethylator phenotype. *Nature* **2012**, *483*, 479−483.

(13) Hao, Z.; Cairns, R. A.; Inoue, S.; Li, W. Y.; Sheng, Y.; Lemonnier, F.; Wakeham, A.; Snow, B. E.; Dominguez-Brauer, C.; Ye, J.; Larsen, D. M.; Straley, K. S.; Tobin, E. R.; Narayanaswamy, R.; Gaulard, P.; Mak, T. W. Idh1 mutations contribute to the development of T-cell malignancies in genetically engineered mice. *Proc. Natl. Acad. Sci. U.S.A.* **2016**, *113*, 1387−1392.

(14) Xu, W.; Yang, H.; Liu, Y.; Yang, Y.; Wang, P.; Kim, S.-H.; Ito, S.; Yang, C.; Wang, P.; Xiao, M.-T.; Liu, L.-x.; Jiang, W.-q.; Liu, J.; Zhang, J.-y.; Wang, B.; Frye, S.; Zhang, Y.; Xu, Y.-h.; Lei, Q.-y.; Guan, K.-L.; Zhao, S.-m.; Xiong, Y. Oncometabolite 2-hydroxyglutarate is a competitive inhibitor of alpha-ketoglutarate-dependent dioxygenases. *Cancer Cell* **2011**, *19*, 17−30.

(15) Chowdhury, R.; Yeoh, K. K.; Tian, Y. M.; Hillringhaus, L.; Bagg, E. A.; Rose, N. R.; Leung, I. K. H.; Li, X. S.; Woon, E. C. Y.; Yang, M.; McDonough, M. A.; King, O. N.; Clifton, I. J.; Klose, R. J.; Claridge, T. D. W.; Ratcliffe, P. J.; Schofield, C. J.; Kawamura, A. The oncometabolite 2-hydroxyglutarate inhibits histone lysine demethylases. *EMBO Rep.* **2011**, *12*, 463−469.

(16) Lu, C.; Ward, P. S.; Kapoor, G. S.; Rohle, D.; Turcan, S.; Abdel-Wahab, O.; Edwards, C. R.; Khanin, R.; Figueroa, M. E.; Melnick, A.; Wellen, K. E.; O'Rourke, D. M.; Berger, S. L.; Chan, T. A.; Levine, R. L.; Mellinghoff, I. K.; Thompson, C. B. IDH mutation impairs histone demethylation and results in a block to cell differentiation. *Nature* **2012**, *483*, 474−478.

https://doi.org/10.1021/acs.jmedchem.1c00019
J. Med. Chem. 2021, 64, 4913−4946

(17) Yen, K. E.; Bittinger, M. A.; Su, S. M.; Fantin, V. R. Cancer-associated IDH mutations: biomarker and therapeutic opportunities. *Oncogene* **2010**, *29*, 6409−6417.
(18) U.S. Food and Drug Administration. FDA Granted Regular Approval to Enasidenib for the Treatment of Relapsed or Refractory AML. https://www.fda.gov/drugs/resources-information-approved-drugs/fda-granted-regular-approval-enasidenib-treatment-relapsed-or-refractory-aml (accessed 2021-03-22).
(19) U.S. Food and Drug Administration. FDA Approves Ivosidenib for Relapsed or Refractory Acute Myeloid Leukemia. https://www.fda.gov/drugs/resources-information-approved-drugs/fda-approves-ivosidenib-relapsed-or-refractory-acute-myeloid-leukemia (accessed 2021-03-22).
(20) U.S. National Library of Medicine. Search Results in ClnicalTrial.gov with AG-120 Ivosidenib. https://clinicaltrials.gov/ct2/results?term=AG-120+AND+Ivosidenib&map=ES (accessed 2021-03-21).
(21) Konteatis, Z.; Artin, E.; Nicolay, B.; Straley, K.; Padyana, A. K.; Jin, L.; Chen, Y.; Narayaraswamy, R.; Tong, S.; Wang, F.; Zhou, D.; Cui, D.; Cai, Z.; Luo, Z.; Fang, C.; Tang, H.; Lv, X.; Nagaraja, R.; Yang, H.; Su, S.-S. M.; Sui, Z.; Dang, L.; Yen, K.; Popovici-Muller, J.; Codega, P.; Campos, C.; Mellinghoff, I. K.; Biller, S. A. Vorasidenib (AG-881): A first-in-class, brain-penetrant dual inhibitor of mutant IDH1 and 2 for treatment of glioma. *ACS Med. Chem. Lett.* **2020**, *11*, 101−107.
(22) Fujii, T.; Khawaja, M. R.; DiNardo, C. D.; Atkins, J. T.; Janku, F. Targeting isocitrate dehydrogenase (IDH) in cancer. *Discovery Med.* **2016**, *21*, 373−380.
(23) Pusch, S.; Krausert, S.; Fischer, V.; Balss, J.; Ott, M.; Schrimpf, D.; Capper, D.; Sahm, F.; Eisel, J.; Beck, A.-C.; Jugold, M.; Eichwald, V.; Kaulfuss, S.; Panknin, O.; Rehwinkel, H.; Zimmermann, K.; Hillig, R. C.; Guenther, J.; Toschi, L.; Neuhaus, R.; Haegebart, A.; Hess-Stumpp, H.; Bauser, M.; Wick, W.; Unterberg, A.; Herold-Mende, C.; Platten, M.; von Deimling, A. Pan-mutant IDH1 inhibitor BAY 1436032 for effective treatment of IDH1 mutant astrocytoma in vivo. *Acta Neuropathol.* **2017**, *133*, 629−644.
(24) Golub, D.; Iyengar, N.; Dogra, S.; Wong, T.; Bready, D.; Tang, K.; Modrek, A. S.; Placantonakis, D. G. Mutant isocitrate dehydrogenase inhibitors as targeted cancer therapeutics. *Front. Oncol.* **2019**, *9*, 417.
(25) Rohle, D.; Popovici-Muller, J.; Palaskas, N.; Turcan, S.; Grommes, C.; Campos, C.; Tsoi, J.; Clark, O.; Oldrini, B.; Komisopoulou, E.; Kunii, K.; Pedraza, A.; Schalm, S.; Silverman, L.; Miller, A.; Wang, F.; Yang, H.; Chen, Y.; Kernytsky, A.; Rosenblum, M. K.; Liu, W.; Biller, S. A.; Su, S. M.; Brennan, C. W.; Chan, T. A.; Graeber, T. G.; Yen, K. E.; Mellinghoff, I. K. An inhibitor of mutant IDH1 delays growth and promotes differentiation of glioma cells. *Science* **2013**, *340*, 626−630.
(26) Okoye-Okafor, U. C.; Bartholdy, B.; Cartier, J.; Gao, E. N.; Pietrak, B.; Rendina, A. R.; Rominger, C.; Quinn, C.; Smallwood, A.; Wiggall, K. J.; Reif, A. J.; Schmidt, S. J.; Qi, H.; Zhao, H.; Joberty, G.; Faelth-Savitski, M.; Bantscheff, M.; Drewes, G.; Duraiswami, C.; Brady, P.; Groy, A.; Narayanagari, S.-R.; Antony-Debre, I.; Mitchell, K.; Wang, H. R.; Kao, Y.-R.; Christopeit, M.; Carvajal, L.; Barreyro, L.; Paietta, E.; Makishima, H.; Will, B.; Concha, N.; Adams, N. D.; Schwartz, B.; McCabe, M. T.; Maciejewski, J.; Verma, A.; Steidl, U. New IDH1 mutant inhibitors for treatment of acute myeloid leukemia. *Nat. Chem. Biol.* **2015**, *11*, 878−886.
(27) Deng, G.; Shen, J.; Yin, M.; McManus, J.; Mathieu, M.; Gee, P.; He, T.; Shi, C.; Bedel, O.; McLean, L. R.; Le-Strat, F.; Zhang, Y.; Marquette, J.-P.; Gao, Q.; Zhang, B.; Rak, A.; Hoffmann, D.; Rooney, E.; Vassort, A.; Englaro, W.; Li, Y.; Patel, V.; Adrian, F.; Gross, S.; Wiederschain, D.; Cheng, H.; Licht, S. Selective inhibition of mutant isocitrate dehydrogenase 1 (IDH1) via disruption of a metal binding network by an allosteric small molecule. *J. Biol. Chem.* **2015**, *290*, 762−774.
(28) Caravella, J. A.; Lin, J.; Diebold, R. B.; Campbell, A.-M.; Ericsson, A.; Gustafson, G.; Wang, Z.; Castro, J.; Clarke, A.; Gotur, D.; Josephine, H. R.; Katz, M.; Kershaw, M.; Yao, L.; Toms, A. V.; Barr, K. J.; Dinsmore, C. J.; Walker, D.; Ashwell, S.; Lu, W. Structure-based design and identification of FT-2102 (Olutasidenib), a potent mutant-selective IDH1 inhibitor. *J. Med. Chem.* **2020**, *63*, 1612−1623.
(29) Davis, M.; Pragani, R.; Popovici-Muller, J.; Gross, S.; Thorne, N.; Salituro, F.; Fantin, V.; Straley, K.; Su, M.; Dang, L.; Simeonov, A.; Shen, M.; Boxer, M. B. ML309: A potent inhibitor of R132H mutant IDH1 capable of reducing 2-hydroxyglutarate production in U87 MG glioblastoma cells. *Probe Reports from the NIH Molecular Libraries Program*: Bethesda (MD), 2010.
(30) Davis, M. I.; Gross, S.; Shen, M.; Straley, K. S.; Pragani, R.; Lea, W. A.; Popovici-Muller, J.; DeLaBarre, B.; Artin, E.; Thorne, N.; Auld, D. S.; Li, Z.; Dang, L.; Boxer, M. B.; Simeonov, A. Biochemical, cellular, and biophysical characterization of a potent inhibitor of mutant isocitrate dehydrogenase IDH1. *J. Biol. Chem.* **2014**, *289*, 13717−13725.
(31) Urban, D. J.; Martinez, N. J.; Davis, M. I.; Brimacombe, K. R.; Cheff, D. M.; Lee, T. D.; Henderson, M. J.; Titus, S. A.; Pragani, R.; Rohde, J. M.; Liu, L.; Fang, Y.; Karavadhi, S.; Shah, P.; Lee, O. W.; Wang, A.; McIver, A.; Zheng, H.; Wang, X.; Xu, X.; Jadhav, A.; Simeonov, A.; Shen, M.; Boxer, M. B.; Hall, M. D. Assessing inhibitors of mutant isocitrate dehydrogenase using a suite of pre-clinical discovery assays. *Sci. Rep.* **2017**, *7*, 12758.
(32) Patel, K. P.; Ravandi, F.; Ma, D.; Paladugu, A.; Barkoh, B. A.; Medeiros, L. J.; Luthra, R. Acute myeloid leukemia with IDH1 or IDH2 mutation: frequency and clinicopathologic features. *Am. J. Clin. Pathol.* **2011**, *135*, 35−45.
(33) Waitkus, M. S.; Diplas, B. H.; Yan, H. Biological role and therapeutic potential of IDH mutations in cancer. *Cancer Cell* **2018**, *34*, 186−195.
(34) Gross, S.; Cairns, R. A.; Minden, M. D.; Driggers, E. M.; Bittinger, M. A.; Jang, H. G.; Sasaki, M.; Jin, S.; Schenkein, D. P.; Su, S. M.; Dang, L.; Fantin, V. R.; Mak, T. W. Cancer-associated metabolite 2-hydroxyglutarate accumulates in acute myelogenous leukemia with isocitrate dehydrogenase 1 and 2 mutations. *J. Exp. Med.* **2010**, *207*, 339−344.
(35) Arai, M.; Nobusawa, S.; Ikota, H.; Takemura, S.; Nakazato, Y. Frequent IDH1/2 mutations in intracranial chondrosarcoma: a possible diagnostic clue for its differentiation from chordoma. *Brain Tumor Pathol.* **2012**, *29*, 201−206.
(36) Di, L.; Kerns, E. H.; Gao, N.; Li, S. Q.; Huang, Y.; Bourassa, J. L.; Huryn, D. M. Experimental design on single-time-point high-throughput microsomal stability assay. *J. Pharm. Sci.* **2004**, *93*, 1537−1544.
(37) Martinez, N. J.; Asawa, R. R.; Cyr, M. G.; Zakharov, A.; Urban, D. J.; Roth, J. S.; Wallgren, E.; Klumpp-Thomas, C.; Coussens, N. P.; Rai, G.; Yang, S. M.; Hall, M. D.; Marugan, J. J.; Simeonov, A.; Henderson, M. J. A widely-applicable high-throughput cellular thermal shift assay (CETSA) using split Nano Luciferase. *Sci. Rep.* **2018**, *8*, 9472.
(38) Avdeef, A.; Bendels, S.; Tsinman, O.; Tsinman, K.; Kansy, M. Solubility-excipient classification gradient maps. *Pharm. Res.* **2007**, *24*, 530−545.
(39) Sun, H.; Shah, P.; Nguyen, K.; Yu, K. R.; Kerns, E.; Kabir, M.; Wang, Y.; Xu, X. Predictive models of aqueous solubility of organic compounds built on a large dataset of high integrity. *Bioorg. Med. Chem.* **2019**, *27*, 3110−3114.
(40) Shah, P.; Kerns, E.; Nguyen, D.-T.; Obach, R. S.; Wang, A. Q.; Zakharov, A.; McKew, J.; Simeonov, A.; Hop, C. E. C. A.; Xu, X. An automated high-throughput metabolic stability assay using an integrated high-resolution accurate mass method and automated data analysis software. *Drug Metab. Dispos.* **2016**, *44*, 1653−1661.
(41) Sun, H.; Nguyen, K.; Kerns, E.; Yan, Z.; Yu, K. R.; Shah, P.; Jadhav, A.; Xu, X. Highly predictive and interpretable models for PAMPA permeability. *Bioorg. Med. Chem.* **2017**, *25*, 1266−1276.

https://doi.org/10.1021/acs.jmedchem.1c00019
J. Med. Chem. 2021, 64, 4913−4946