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Optimization of Hydantoins as Potent Antimycobacterial Decaprenylphosphoryl-β-D-Ribose Oxidase (DprE1) Inhibitors

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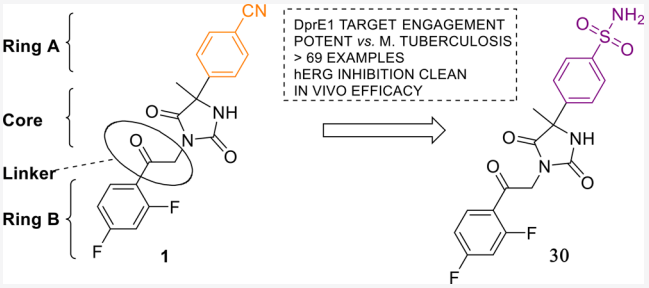
ABSTRACT: In search of novel drugs against tuberculosis, we previously discovered and profiled a novel hydantoin-based family that demonstrated highly promising in vitro potency against *Mycobacterium. tuberculosis*. The compounds were found to be noncovalent inhibitors of DprE1, a subunit of decaprenylphosphoryl-β-D-ribose-2′-epimerase. This protein, localized in the periplasmic space of the mycobacterial cell wall, was shown to be an essential and vulnerable antimycobacterial drug target. Here, we report the further SAR exploration of this chemical family through more than 80 new analogues. Among these, the most active representatives combined submicromolar cellular potency and nanomolar target affinity with balanced physicochemical properties and low human cytotoxicity. Moreover, we demonstrate in vivo activity in an acute *Mtb* infection model and provide further proof of DprE1 being the target of the hydantoins. Overall, the hydantoin family of DprE1 inhibitors represents a promising noncovalent lead series for the discovery of novel antituberculosis agents.



INTRODUCTION

Tuberculosis (TB), a disease primarily caused by the pathogen *Mycobacterium tuberculosis*, is among the top 10 causes of death worldwide while remaining the leading cause of death from a single infectious agent, as reported by the World Health Organization (WHO).[1] Around 10.0 million people developed TB globally in 2018, with an estimated 1.3 million TB deaths among HIV-negative people and an additional 300,000 deaths among HIV-positive individuals. The drugs in the current first-choice treatment regimen were identified over 60 years ago, and patients are required to take medicines for at least 6 months, even in the case of drug-sensitive infections. Pronounced side effects coupled with extended treatment regimens lead to low patient compliance and have increased the emergence of drug-resistant Mycobacteria strains. In fact, in 2018 alone, around half a million people developed TB that was resistant to rifampicin (RR-TB), the most effective first-line anti-TB drug. Moreover, 78% of these cases involve multidrug-resistant tuberculosis (MDR-TB) with resistance to at least rifampicin and isoniazid.[1] Therefore, the development of new antimycobacterial therapeutics, preferably with novel modes of action, remains an urgent need.

DprE1, a subunit of decaprenylphosphoryl-β-D-ribose-2′-epimerase, is a periplasmic protein involved in the mycobacterial cell wall biosynthesis that was shown to be a new highly promising drug target for antimycobacterial research.[2,3] The initial recognition was brought by the benzothiazinone series (BTZ), a DprE1 covalent inhibitor class.[4−7] Later on, several research groups provided insight into the DprE1 inhibitor binding mode as well as reported numerous structurally diverse compound series with either an irreversible (covalent) or reversible noncovalent binding, validating DprE1 as an attractive antimycobacterial target.[8−17] All relevant DprE1-inhibitor literature to date is summarized in a recently published comprehensive review.[18] Benzothiazinones BTZ043, azaindole AZ7371, and PBTZ-169/macozinone are the most advanced DprE1 inhibitors that have recently entered the clinical development phase (Figure 1).[19,20]

Inspired by the encouraging antimycobacterial properties of the described compounds, GSK performed a target-based high-throughput screening (HTS) campaign in search of novel DprE1inhibitors (paper under preparation). This led to the identification of the hydantoin-derived compound **1** and its several analogues (**2**−**4**) as promising hits. Recently, we

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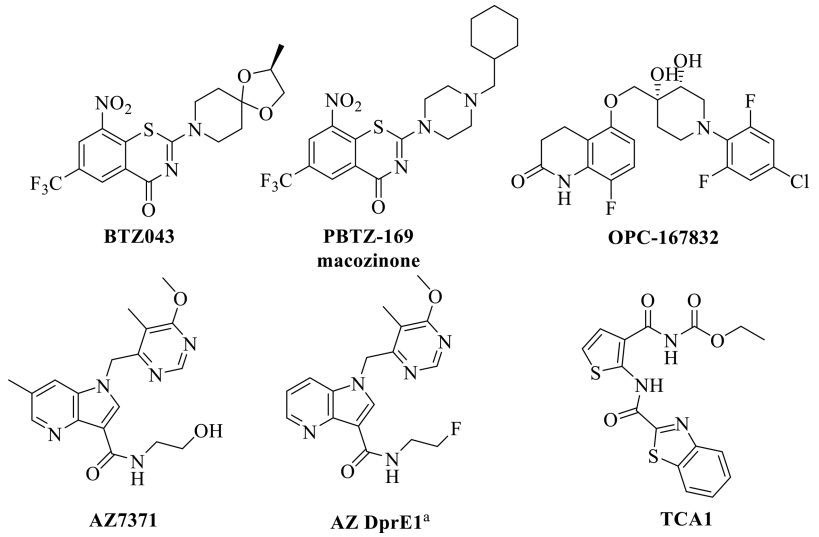


Figure 1. Selected reported DprE1 inhibitors.[18] [a]AZ DprE1 inhibitor benchmark, compound **9** in ref 11.

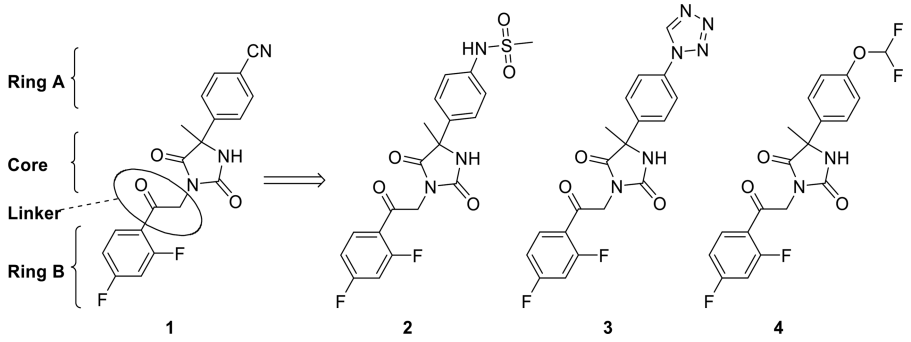
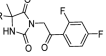


Figure 2. Most potent representatives (**1**−**4**) published previously by our team.[21] The previous findings indicate that both the hydantoin core and the acetyl linker are crucial for the potency of the series.

reported the biological profiling and initial optimization efforts on hit **1**.[21] Several potent representatives with ring A modifications obtained during this study showed promising in vitro enzyme inhibition (pIC_{50} 7−7.4) together with low micromolar whole-cell MIC values and no cytotoxicity at 100 μM in a HepG2 assay (representative compounds **1**−**4**, Figure 2 and Table 1).

Table 1. In Vitro Activity, Cytotoxicity, and Physicochemical Properties of Selected Representatives 1−4 from Our Previous Report[21]

№	R	DprE1 pIC_{50}[a]	*Mtb* MIC (μM)[b]	HepG2 IC_{50} (μM)[c]	Solubility (μM)[d]	Chrom logD[e]
1		7.0	8.3	> 100	202	4.54
2		7.0	2.5	> 100	≥ 487	3.57
3		7.3	3.1	> 100	379	3.78
4		7.4	10	> 100	85	5.63

[a]Inhibition of DprE1 enzyme. [b]MIC against *M. tuberculosis* (H37Rv), reference: isoniazid, MIC = 1.8 μM. [c]Cytotoxicity against HepG2 human Caucasian hepatocyte carcinoma. [d]Kinetic aqueous solubility (CLND). [e]Lipophilicity, chromlogD at pH = 7.4.

Earlier, we reported that the hydantoin core functions not only as a scaffold that ensures proper spatial orientation of the peripheral moieties but also appears to take part in protein interactions, crucial for the series potency.[21] Likewise, several modifications of the acetyl linker in these molecules led to significant potency loss, indicating its importance. In addition, several analogues of **1** and **4** with varied substitutions around ring B were evaluated. Several modifications were observed to be permitted in that part of the molecule, although none of the analogues showed significant potency improvement.

Overall, the obtained results suggested that the SAR around rings A and B should be further explored to optimize the potency and properties of the series. Finally, we also demonstrated in the previous publication that only the *R*-enantiomer of the hit hydantoin **1** contributes to both enzymatic and whole-cell activity. However, the assays in this manuscript were generally run with racemates for procedural simplicity.

RESULTS AND DISCUSSION

Chemistry and SAR. We reported earlier that replacing the ring A cyano moiety in compound **1** with different polar substituents seemed to be favorable for retaining antimycobacterial potency in the phenotypic MIC assay.[21] Therefore, we first focused on the introduction of additional polar groups on ring A, such as a carboxylic acid **17**, an ester **10** and amide function **16**, a urea moiety **11**, and several polar heterocycles,

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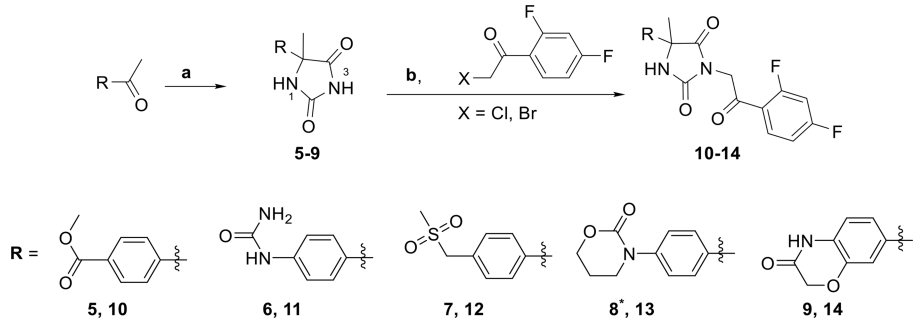
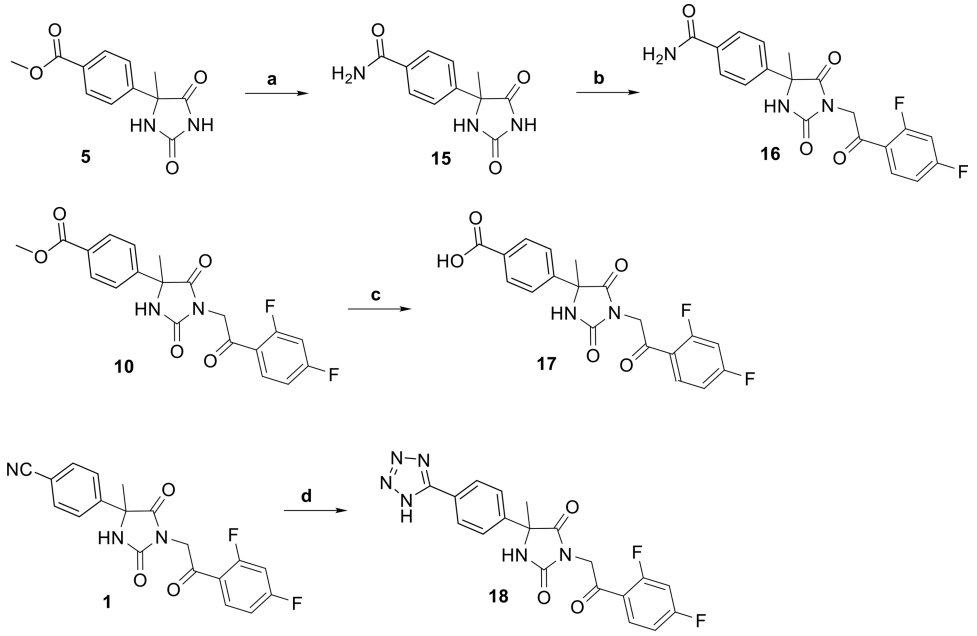
Scheme 1. General Synthetic Approach toward Analogues with a Modified *Para*-Substitution Pattern on Ring A[a]



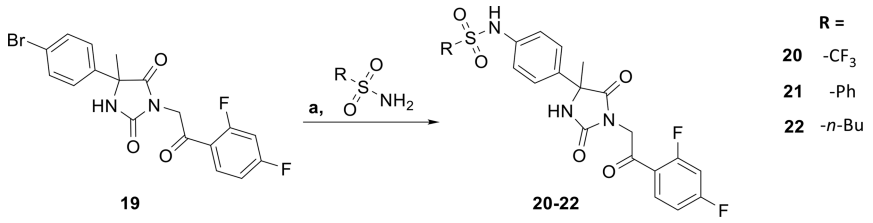
[a]Reagents and conditions: (a) KCN, $(NH_4)_2CO_3$, EtOH-H_2O, microwave irradiation 70 °C or heating 55 °C, 7−17 h; (b) K_2CO_3, DMF or acetone, rt, 24−48 h. *The main isolated reaction product was the hydantoin sodium salt **8a** used for the alkylation.

Scheme 2. Synthetic Approach toward Analogues 16−18 with a Modified *Para*-Substitution Pattern on Ring A[a]



[a]Reagents and conditions: (a) NH_4OH, 90 °C, overnight; (b) K_2CO_3, DMF or acetone, rt, 24 h; (c) LiOH, THF, H_2O, rt, 1 h; (d) NaN_3, $ZnCl_2$, *n*-PrOH, 95 °C, 24 h.

Scheme 3. Synthetic Approach to Analogues with N-Linked Sulfonamide Substituents on Ring A[a]



[a]Reagents and conditions: (a) $[PdCl(allyl)]_2$, t-BuXPhos, K_2CO_3, 2-MeTHF, 80 °C, 2 h.

including a fused bicyclic analogue of the benzene ring **14** (Schemes 1 and 2). Similar to the approach reported earlier, most analogues with ring A substitution modifications (**10**−**14**) were synthesized starting from ketones according to a modified Bucherer−Berg hydantoin cyclization,[22] followed by an alkylation as shown in Scheme 1. The ketones were either available commercially or prepared based on standard literature procedures (see the Supporting Information). The selective alkylation on the N_3-nitrogen was previously confirmed by full NMR assignment and crystal structure analysis.[21]

Intermediate **15**, bearing an amide group on ring A, was prepared by reaction of **5** with ammonia, and subsequent alkylation of the hydantoin ring resulted in final product **16** (Scheme 2). In the case of analogues **17** and **18**, the substituents on ring A seemed to be reactive in the last alkylation step. Therefore, the non-substituted acid derivative **17** was prepared by base-promoted hydrolysis of the

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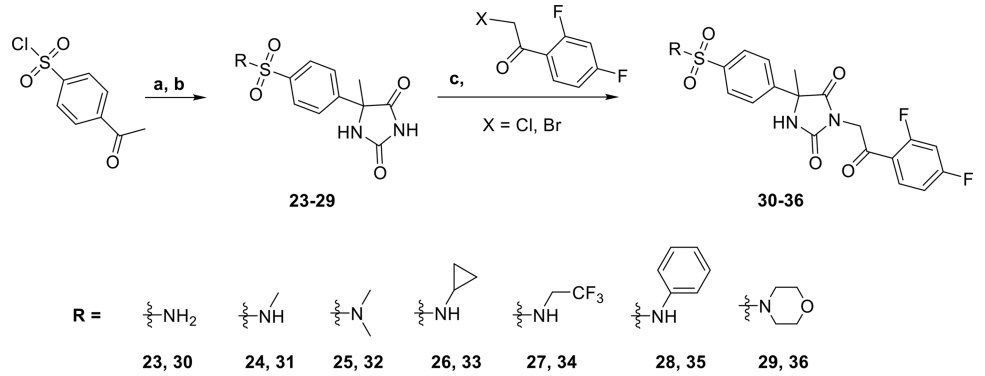
Scheme 4. Synthetic Approach to Analogues with S-Linked Sulfonamide Substituents on Ring A[a]



[a]Reagents and conditions: (a) amine·HCl, Et_3N, or amine with no base, DCM, rt, 1 h; (b) KCN, $(NH_4)_2CO_3$, EtOH-H_2O, 70 °C (MW or heating), 7−17 h; (c) K_2CO_3, acetone, rt, 24−48 h.

corresponding ester **10**. Lastly, the tetrazole ring in **18** was formed by zinc-promoted [3 + 2] cycloaddition of the nitrile in **1** with sodium azide.

Inspired by methylsulfonamide **2**'s overall activity profile (Table 1), we decided to prepare a small subseries of compounds covering analogues that are linked to ring A via either the N or S atom of the sulfonamide functionality (compounds **20**−**22** and **30**−**36**, respectively, Schemes 3 and 4). The synthetic approach shown in Scheme 1 was anticipated to be unfit for the preparation of the *N*-linked analogues. In essence, undesired alkylation of the acidic sulfonamide nitrogen was expected to occur during the final synthetic step (N-alkylation of the hydantoin moiety). Therefore, sulfonamides **20**−**22** were prepared in moderate yields from bromo-substituted precursor **19** following a literature procedure for palladium-catalyzed amidation of aryl rings (Scheme 3).[23]

Since no literature procedures were available for the late-stage introduction of S-linked sulfonamide groups, we decided to return to the strategy shown in Scheme 1 and evaluate its potential for the synthesis of analogues **30**−**36** (Scheme 4). The sulfonamide moiety was first installed by the reaction of 4-acetylbenzenesulfonyl chloride with different amines followed by applying the modified Bucherer−Berg protocol to obtain intermediates **23**−**29**. Alkylation of the hydantoin ring provided the desired products **30**−**36** in moderate to high yields. Remarkably, no alkylation of the sulfonamide moiety was observed under these conditions.

Table 2 summarizes the biological and biophysical evaluation results for the compounds that carry a polar A-ring substituent. Overall, good solubility and no detectable cytotoxicity in the HepG2 assay (IC_{50} > 100 μM) were observed.

Most polar substituents in the first subset of compounds (**10**−**14** and **16**−**18**) nonetheless led to the loss of both DprE1 inhibitory potency and whole-cell activity compared to hit **1**. Notable exceptions are methyl ester **10** and fused bicyclic analogue **14**, both retaining the overall activity profile of **1**. Surprisingly, tetrazole **18** that is closely related to a previously reported tetrazole **3** did not retain activity.

The N-linked sulfonamides **20**−**22** also demonstrated a significant reduction in DprE1 affinity and antimycobacterial potency compared to the previously reported analogue **2**, indicating that further structural diversification in this compound subset was not promising. Conversely, the presence of an inverted, S-linked sulfonamide group in **30** resulted in potent DprE1 inhibition (pIC_{50} = 7.3) and significant improvement of the whole-cell activity (MIC = 0.6 μM). The introduction of additional N-substituents on **30** (as in compounds **31**−**36**) was again found to be detrimental to cellular and DprE1 inhibitory potency. Taken together, it is likely that steric constraints in DprE1's active sites are responsible for the observed trends in affinity and activity in the N- and S-linked sulfonamide series. Since the S-linked sulfonamide derivative **30** was the most potent analogue identified at this point and the first submicromolar DprE1 inhibitor encountered in the hydantoin family, the amino-sulfonyl group of **30** was selected as a recurrent structural feature in the compound series that was subsequently prepared. Additionally, we decided to include some analogues bearing hit **1**'s 4-cyano substituted ring A for activity comparison. The substitution pattern on aryl ring B was thoroughly investigated by means of analogues **38**−**71**. For the preparation of these molecules, the same synthetic strategy was applied again (Scheme 5). Alkylation of hydantoin precursors **23** or **37** with an appropriately substituted haloacetophenone derivative provided the desired products **38**−**71** in high to moderate yields.

This series was then supplemented with compounds in which ring B was replaced by saturated or heterocyclic moieties. Compounds **72**−**88** were prepared following the general alkylation-based approach (Scheme 6), in this case relying on the appropriate alkyl halides. The aromatic ring B was changed to a simple methyl substituent in **72** or to one of several saturated ring systems in **73**−**75**. Moreover, the aryl moiety was replaced by a pyridine ring (**76**−**82**), 5-membered heterocycles (**83**−**86**), or bicyclic systems (**87** and **88**) to provide more diverse modifications in this part of the structure and to explore the physicochemical properties of novel analogues.

All the alkyl halides utilized in Schemes 5 and 6 were commercially available or prepared according to literature procedures (see the Supporting Information).

As Table 3 shows, several ring B substitution modifications were tolerated. In general, all the compounds bearing a 4-aminosulfonyl moiety on ring A showed superior enzyme affinity in comparison with the 4-cyano analogues.

Stripping off the substituents on ring B leads to a significant activity drop in **38** and **39** compared to both hit **1** and its sulfonamide analogue **30** (see Tables 1−3). Nonetheless, it

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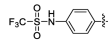
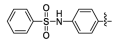
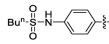
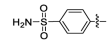
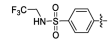
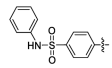
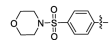
Table 2. In Vitro Activity, Cytotoxicity, and Physicochemical Properties of the Compounds with Varying Substituents at the 4-Position of Ring A

№	R	DprE1 pIC_{50}[a]	*Mtb* MIC (μM)[b]	HepG2 IC_{50} (μM)[c]	Solubility (μM)[d]	Chrom logD[e]
10		7.1	11.2	> 100	140	4.84
11		5.3	> 80	> 100	≥ 296	2.71
12		6.2	> 40	> 100	≥ 440	3.54
13		5.2	80	> 100	≥ 486	3.36
14		6.8	10	> 100	≥ 369	3.38
16		5.2	> 80	> 100	≥ 454[f]	2.68
17		4.1	> 80	> 100	≥ 282	1.49
18		5.0	> 80	> 100	≥ 511[f]	1.85
20		5.0	> 80	> 100	≥ 372	3.24
21		6.4	80	100	44	4.90
22		5.4	> 80	100	154	4.96
30		7.2	0.7	> 100	≥ 486	3.19
31		6.1	20	> 100	≥ 478	3.88
32		5.7	80	> 100	224	4.60
33		5.7	80	> 100	334	4.48
34		4.5	> 80	63.1	57	4.78
35		4.7	> 80	79.4	55	5.03
36		4.9	> 40	> 100	217	4.39

[a]Inhibition of DprE1 enzyme. [b]MIC against *M. tuberculosis* (H37Rv), reference: isoniazid, MIC = 1.8 μM. [c]Cytotoxicity against HepG2 human Caucasian hepatocyte carcinoma. [d]Kinetic aqueous solubility (CLND). [e]Lipophilicity, chromlogD at pH = 7.4. [f]Solubility determination in 5% DMSO pH 7.4 phosphate-buffered saline and quantification of DMSO stock concentration using a charged aerosol detector (CAD).

was desirable to include these analogues in the series for relative activity comparison with the corresponding compounds lacking fluorine atoms and containing other substituents or heterocycles. Compounds with a single fluorine substituent (**40**–**44**) also showed a small but consistent drop in enzymatic affinity. The presence of a 2,4-dichloro substitution pattern in **47** led to a significant drop in the whole-cell activity (MIC = 5 μM) compared to reference 30 (MIC = 0.6 μM), suggesting particular importance of fluorine substituents for the series' whole-cell activity. Although we do not have a clear rationale for this observation, it is most likely connected to small steric size and high electronegativity of the fluorine substituent. In fact, analogues with a 3,4-difluoro or 3-Cl,4-F substitution pattern (**45** and **46**, respectively) were among the most active compounds obtained (pIC_{50} = 7.2, MIC = 1.2–1.3 μM). Interestingly, 3-Br substituted compound **48** retained relatively high enzymatic potency (pIC_{50} = 7.0) with an MIC of 1.9 μM.

Compounds **52**–**60** with an electron-donating group (−Me or -OMe) in different ring positions (2-, 3-, or 4-) demonstrated lower enzymatic and whole-cell potency in comparison to the corresponding references 30 or 1.

To further investigate the influence of electron-withdrawing substituents, we first prepared a number of analogues containing $-CF_3$, -CN, $-NO_2$, or $-OCF_3$ groups in ring B (**61**–**68**). Most of the prepared compounds showed a significant drop in enzymatic and whole-cell potency. The 2-CF_3-substituted compound **61** showed an MIC value of 2.5 μM. Notably, the most potent representatives were the 3-substituted analogues **62** (3-CF_3) and **67** (3-OCF_3), which retained submicromolar whole-cell potencies (MIC = 0.6–0.9 μM). Keeping in mind the high activity of 3,4-dihalo-substituted analogues **45** and **46**, we decided to combine the two substitution patterns (3-CF_3, 4-F) in products **69** and **70**, while two CF_3 groups were simultaneously introduced in **71**. Sulfonamide **69** (3-CF_3, 4-F) retained submicromolar whole-cell potency (MIC = 0.6 μM) and enzymatic activity (pIC_{50} = 7.2 vs 7.1) compared to its closest analogue **62** (3-CF_3), while compounds **70** and **71** resulted in a substantial activity loss.

Overall, the majority of analogues with a modified substitution pattern on ring B (apart from **49**, **63**, **68**, **70**–**71**) demonstrated no detectable cytotoxicity in the HepG2 assay (IC_{50} > 100 μM).

Table 4 summarizes the biological evaluation results for compounds **72**–**88**. Methyl- or cyclopropyl-substituted compounds **72** and **73** showed a pronounced loss in both DprE1 and cellular potency, while adamantyl-containing analogue **75** retained moderate DprE1 affinity (pIC_{50} = 5.9) but did not display significant antimycobacterial activity. Introduction of a cyclohexyl ring in place of the B ring in **74**, however, preserved the potency over its phenyl analogue **38** with pIC_{50} values of 6.7 and 6.6 and MIC values of 7.5 and 10 μM, respectively. This could indicate that appropriately substituted cyclohexyl analogues may act as a potential replacement of the phenyl-type ring B in compound **30**. The introduction of a pyridine ring at this position (**76**–**81**) led to an activity drop in most compounds, and only the 2-pyridinyl-based analogues **76** and **77** retained comparable activity with the phenyl analogues **33** and **4**. Therefore, the 2-pyridinyl moiety was combined with the difluoro substitution pattern of reference 30, providing its closest heterocyclic analogue **82**. The latter retained comparable submicromolar whole-cell activity (MIC = 0.6 μM).

The 2-thiophenyl-substituted product **83** retained enzymatic and whole-cell potency compared to its direct phenyl analogue **38**, while the chlorine substituent addition to the 5-position of the thiophene ring in **84** led to further potency improvement (pIC_{50} = 7.0, MIC = 2.5 μM). The latter was, however, still inferior to the profile of reference 30. In contrast, the introduction of a (benzo)thiazole moiety in **86** and **87** was characterized by a significant loss of potency. Lastly, 2-naphthalene-containing compound **88** preserved the same whole-cell activity (MIC = 10 μM) as its phenyl analogue **38**, suggesting that the enzymatic pocket could potentially accommodate additional substituent expansion in this part of the molecule. It should be emphasized that, in the majority of

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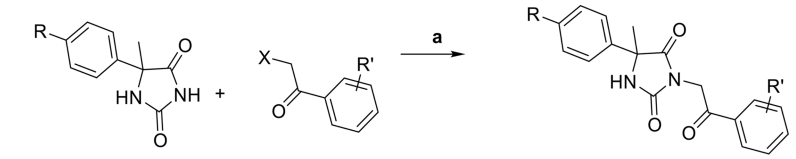
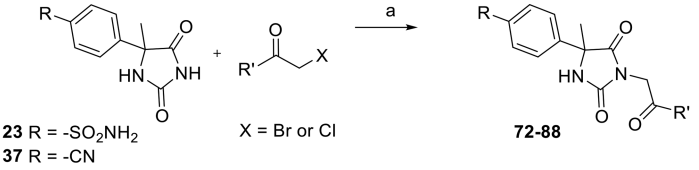
Scheme 5. Synthetic Scheme of the Synthesis of Analogues with Variable Substitution on Ring B[a]

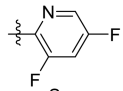
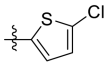
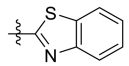
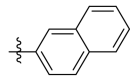


	R =	R' =
38	$-SO_2NH_2$	-H
39	-CN	-H
40	$-SO_2NH_2$	2-F
41	-CN	2-F
42	$-SO_2NH_2$	3-F
43	-CN	3-F
44	$-SO_2NH_2$	4-F
45	$-SO_2NH_2$	3,4-diF
46	$-SO_2NH_2$	3-Cl, 4-F
47	$-SO_2NH_2$	2,4-diCl
48	$-SO_2NH_2$	3-Br
49	-CN	2-OCOtBu, 4-F
50*	-CN	2-OH, 4-F
51	$-SO_2NH_2$	2-OH, 4-F
52	$-SO_2NH_2$	2-Me
53	-CN	2-Me
54	$-SO_2NH_2$	3-Me
55	-CN	3-Me
56	$-SO_2NH_2$	4-Me
57	-CN	4-Me
58	$-SO_2NH_2$	2-OMe
59	$-SO_2NH_2$	3-OMe
60	$-SO_2NH_2$	4-OMe
61	$-SO_2NH_2$	2-CF_3
62	$-SO_2NH_2$	3-CF_3
63	$-SO_2NH_2$	4-CF_3
64	$-SO_2NH_2$	3-CN
65	$-SO_2NH_2$	4-CN
66	$-SO_2NH_2$	3-NO_2
67	$-SO_2NH_2$	3-OCF_3
68	-CN	3-OCF_3
69	$-SO_2NH_2$	3-CF_3, 4-F
70	-CN	3-CF_3, 4-F
71	$-SO_2NH_2$	3,5-diCF_3

[a]Reagents and conditions: (a) K_2CO_3, acetone or DMF, rt, 24−48 h. *Compound **50** was formed by hydrolysis of **49** in LiOH solution.

Scheme 6. Synthetic Approach to Analogues with Saturated or Heterocycle Moieties Replacing Ring B[a]



	R =	R' =
72	$-SO_2NH_2$	
73	$-SO_2NH_2$	
74	$-SO_2NH_2$	
75	$-SO_2NH_2$	
76	$-SO_2NH_2$	
77	-CN	
78	$-SO_2NH_2$	
79	-CN	
80	$-SO_2NH_2$	
81	-CN	
82	$-SO_2NH_2$	
83	$-SO_2NH_2$	
84	$-SO_2NH_2$	
85	-CN	
86	$-SO_2NH_2$	
87	$-SO_2NH_2$	
88	$-SO_2NH_2$	

[a]Reagents and conditions: (a) K_2CO_3, acetone or DMF, rt, 24−48 h.

analogues, even those with increased lipophilicity, no considerable cytotoxicity was detected among the reported modifications (HepG2 IC_{50} > 100 μM), which may indicate a promising safety profile of this chemical series.

hERG Inhibition. Potential cardiotoxicity of the series was one of our primary concerns since the previously reported hit **1** and its most potent analogues **2**−**4** had all demonstrated considerable hERG potassium channel inhibition (pIC_{50} = 4.4−5.3).[21] To our satisfaction, all tested sulfonamide derivatives showed no significant hERG inhibition (pIC_{50} < 4.3), as shown in Table 5. Overall, these data support our previous findings that potential cardiotoxicity is not intrinsic to the series but rather determined by the substitution pattern on rings A and B.

Enantiomeric Separation. The enantiomers of the new reference 30 were separated by chiral HPLC, and the absolute configuration was determined by VCD analysis. The obtained results confirmed that only the R-isomer is responsible for both the enzymatic and whole-cell potency (Table 6), in agreement with our previous findings.[21]

Metabolic Stability. The in vitro metabolic stability assessment of compound **30** indicates the general stability of the compound with an intrinsic clearance value lower than 3 mL/min/g in mouse microsomes and under 0.4 mL/min/g in human microsomes, as summarized in Table 7

Evaluation against *M. tuberculosis* DprE1 Mutants. To provide a genetic link to the mechanism of action, the primary hit compound **1** was tested against three *M. tuberculosis* DprE1 mutants (E221Q, G248S, and Y314H) that were generated in-house via oligonucleotide-mediated recombineering as previously described.[11,24,25] Additionally, three spontaneous DprE1 mutants (L368P, G17C, and C387S) were provided by Stewart T. Cole (Institut Pasteur, Paris, France).[2,14] As shown in Table 8, a clear MIC-modulation of reference

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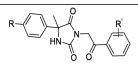
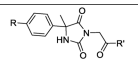
Table 3. In Vitro Activity, Cytotoxicity, and Physicochemical Properties of the Analogues with Ring B Substitution Modifications



№	R	R′	DprE1 pIC_{50}[a]	*Mtb* MIC (μM)[b]	HepG2 IC_{50} (μM)[c]	Solubility (μM)[d]	Chrom logD[e]
38	$-SO_2NH_2$	H	6.6	10	> 100	≥ 415	2.81
39	-CN	H	5.9	> 80	> 100	≥ 428[f]	4.19
40	$-SO_2NH_2$	2-F	6.7	2.5	> 100	340	2.94
41	-CN	2-F	6.1	40	> 100	≥ 312[f]	4.30
42	$-SO_2NH_2$	3-F	6.8	5.0	> 100	≥ 408	3.08
43	-CN	3-F	6.1	80	> 100	≥ 372[f]	4.39
44	$-SO_2NH_2$	4-F	6.8	2.2	> 100	≥ 387	3.03
45	$-SO_2NH_2$	3,4-diF	7.2	1.2	> 100	≥ 314	3.34
46	$-SO_2NH_2$	3-Cl, 4-F	7.2	1.3	> 100	362	3.82
47	$-SO_2NH_2$	2,4-diCl	7.1	7.5	> 100	372	4.11
48	$-SO_2NH_2$	3-Br	7.0	1.9	> 100	≥ 347[f]	3.77
49	-CN	2-OCO′Bu, 4-F	5.3	30	6.45	N.D.[h]	5.84
50	-CN	2-OH, 4-F	6.2	30	> 100	12	4.56
51	$-SO_2NH_2$	2-OH, 4-F	7.2	2.5	> 100	≥ 496	3.12
52	$-SO_2NH_2$	2-Me	7.0	2.5	> 100	≥ 311	3.30
53	-CN	2-Me	6.1	40	> 100	110[f]	4.73
54	$-SO_2NH_2$	3-Me	6.7	5.0	> 100	≥ 320[f]	3.36
55	-CN	3-Me	6.0	80[g]	> 100	50[f]	4.71
56	$-SO_2NH_2$	4-Me	6.8	10	> 100	351	3.33
57	-CN	4-Me	5.8	> 80	> 100	246[f]	4.73
58	$-SO_2NH_2$	2-OMe	5.6	> 80	> 100	≥ 473	2.98
59	$-SO_2NH_2$	3-OMe	6.9	10	> 100	≥ 417	3.00
60	$-SO_2NH_2$	4-OMe	6.5	20	> 100	≥ 392	2.86
61	$-SO_2NH_2$	$2\text{-}CF_3$	7.0	2.5	> 100	≥ 381	3.56
62	$-SO_2NH_2$	$3\text{-}CF_3$	7.1	0.9	> 100	279	3.96
63	$-SO_2NH_2$	$4\text{-}CF_3$	6.0	20	65.3	370	4.15
64	$-SO_2NH_2$	3-CN	5.9	40	> 100	≥ 482	2.40
65	$-SO_2NH_2$	4-CN	5.3	60	> 100	≥ 411	2.54
66	$-SO_2NH_2$	$3\text{-}NO_2$	6.2	20	> 100	132	2.77
67	$-SO_2NH_2$	$3\text{-}OCF_3$	7.1	0.6	> 100	≥ 438	4.14
68	-CN	$3\text{-}OCF_3$	6.2	40	69.4	127[f]	5.50
69	$-SO_2NH_2$	$3\text{-}CF_3$, 4-F	7.2	0.6	> 100	≥ 287	4.24
70	-CN	$3\text{-}CF_3$, 4-F	6.4	20	76.7	121	5.38
71	$-SO_2NH_2$	$3,5\text{-}diCF_3$	5.1	> 80	74.6	95	4.90

[a]Inhibition of the DprE1 enzyme (DprE1 assay data was generated using a modified version of the assay described, paper under preparation).[18] [b]MIC against *M. tuberculosis* (H37Rv), reference: isoniazid, MIC = 1.8 μM. [c]Cytotoxicity against HepG2 human Caucasian hepatocyte carcinoma. [d]Kinetic aqueous solubility (CLND). [e]Lipophilicity, chromlogD at pH = 7.4. [f]Solubility determination in 5% DMSO pH 7.4 phosphate-buffered saline and quantification of DMSO stock concentration using a charged aerosol detector (CAD). [g]N.D., not determined. [h]Only partial inhibition was reached.

Table 4. In Vitro Activity, Cytotoxicity, and Physicochemical Properties of the Analogues with Saturated or Heterocyclic Moieties Instead of Ring B



№	R	R′	DprE1 pIC_{50}[a]	*Mtb* MIC (μM)[b]	HepG2 IC_{50} (μM)[c]	Solubility (μM)[d]	Chrom logD[e]
72	$-SO_2NH_2$		4.0	> 80	> 100	≥ 504	0.54
73	$-SO_2NH_2$		4.5	> 80	> 100	58	1.51
74	$-SO_2NH_2$		6.7	7.5	> 100	≥ 317	3.62
75	$-SO_2NH_2$		5.9	> 80	> 100	≥ 404	4.87
76	$-SO_2NH_2$		6.3	10	> 100	≥ 405	2.03
77	-CN		5.5	> 80	> 100	≥ 292[f]	3.48
78	$-SO_2NH_2$		5.5	> 80	> 100	≥ 298	1.16
79	-CN		4.6	> 80	> 100	≥ 334[f]	2.46
80	$-SO_2NH_2$		5.6	80	> 100	≥ 439	1.16
81	-CN		4.6	> 80	> 100	≥ 310[f]	2.52
82	$-SO_2NH_2$		7.1	0.6	> 100	≥ 441	2.39
83	$-SO_2NH_2$		6.7	10	> 100	≥ 311	2.35
84	$-SO_2NH_2$		7.0	2.5	> 100	≥ 437[f]	3.52
85	-CN		6.3	80	> 100	184[f]	4.85
86	$-SO_2NH_2$		6.2	60	> 100	≥ 453	1.90
87	$-SO_2NH_2$		6.7	40[g]	> 97	≥ 339[f]	3.78
88	$-SO_2NH_2$		6.3	10	> 100	167	3.93

[a]Inhibition of DprE1 enzyme. [b]MIC against *M. tuberculosis* (H37Rv), reference: isoniazid, MIC = 1.8 μM. [c]Cytotoxicity against HepG2 human Caucasian hepatocyte carcinoma. [d]Kinetic aqueous solubility (CLND). [e]Lipophilicity, chromlogD at pH = 7.4. [f]Solubility determination in 5% DMSO pH 7.4 phosphate-buffered saline and quantification of DMSO stock concentration using a charged aerosol detector (CAD). [g]Only partial inhibition was reached.

hydantoin **1** is mainly visible in the E221Q, G248S, and Y314H mutant strains. Together with the MIC-modulation that we observed earlier in a DprE1-overexpressing strain, these data bring on additional support to our hypothesis that DprE1 is the principal target of the hydantoins in *M. tuberculosis*.[21] Moreover, the resistance profile of DprE1 mutants to compound **1** is also shared by the noncovalent reference AZ DprE1 inhibitor[11] and, to a lesser extent, with the covalent ligand TCA1 (structures shown earlier in Figure 1). While these data suggest that broader cross-resistance could be present between representatives of these three compound families, they also indicate that some overlapping interactions could be present for these families within DprE1's ligand binding site. The latter is nonetheless not straightforwardly rationalized by looking at DprE1's crystal structure: only Tyr314 lines the ligand pocket, while the other two amino acids (Gln 221 and Gly248) are at a 6−9 Å distance and are closer to the protein's outer surface.[17]

Together, the observed DprE1 inhibitory potencies, the MIC modulation observed in the DprE1-overexpression strain (reported previously),[21] and the MIC modulation against resistant mutants to other classes of DprE1 inhibitors strongly support the assignment of DprE1 as the primary driver of antimycobacterial activity in the hydantoin series.

In Vivo Therapeutic Efficacy. The two most potent compounds that were available at the time (**3** and **30**) were subsequently admitted to preclinical in vivo studies for which approval was obtained from the responsible local ethical committee. The efficacy of both compounds was determined in

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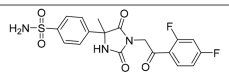
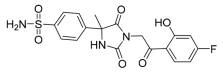
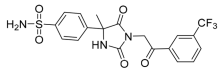
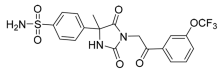
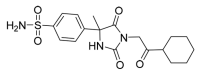
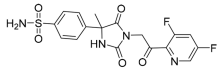
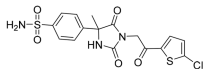
Table 5. hERG Inhibition of Selected Potent Analogues

№	Structure	hERG pIC_{50}
30		< 4.3
51		< 4.3
62		< 4.3
67		< 4.3
74		< 4.3
82		< 4.3
84		< 4.3

a C57BL/6J mouse model of acute intratracheal infection with *Mtb*. H37Rv.[26] Compounds were administered once daily per os for 4 consecutive days, starting 5 days after the infection. Moxifloxacine (100 mg/kg) was used as a positive interassay control for efficacy in these experiments. Blood samples were obtained at specific time points from treated mice to measure the levels of the assayed molecules. Lungs were harvested on day 9, 24 h after the last compound administration. The blood levels measured for both compounds and the lung microorganism burden differences (log_{10} CFU/lung) from the treated mice compared to untreated controls (day 9 after infection) are shown in Table 9 and Figure 3. No adverse clinical signs were observed in any animal.

Compound **30** demonstrated the best blood exposure with a C_{max} value of 6380 ng/mL and an AUC value of 31,400 h ng/mL. Moreover, the same compound showed the highest reduction of log_{10} CFU (0.5). Although this value reflects limited in vivo activity compared to reference moxifloxacin, it demonstrates that the hydantoin series is capable of reaching the lungs of mice after oral administration to achieve a statistically significant bacterial load reduction. The bioavailability of **3** (reflected in Table 9 by its lower AUC value and fast clearance) is significantly lower: within 6 hours, blood levels drop below the compound's MIC value. This factor most likely contributes to the absence of efficacy for this molecule. Overall, these data also show that compound optimization, especially with respect to in vivo activity, is required before the future development of the hydantoin family into a drug candidate is possible.

Table 7. Microsomal Stability of Compound 30

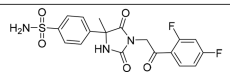
№	Structure	Mouse Cl_{int} (ml/min/g)	Human Cl_{int} (ml/min/g)
30		2.67	< 0.40

Table 8. MIC of the Primary Hit 1 against a Panel of *M. tuberculosis* DprE1-Resistant Mutants

	MIC_{mutant}/MIC_{H37Rv} ratio[a]					
compd	E221Q	G248S	Y314H	L368P	G17C	C387S
1	16	>16	16	2	1	<1
TCA1[b]	24	4	32	8	2	8
AZ DprE1[b,c]	16	>63	>63	4	2	1
BTZ043[b]	4	1	1	1	1	64

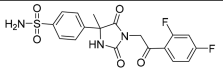
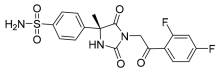
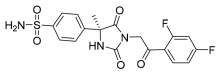
[a]A mutant strain is considered resistant if the MIC_{mutant}/MIC_{H37Rv} ratio ratios is 8 or higher. [b]The structures of reference DprE1 inhibitors AZ DprE1, TCA1, and BTZ043 are shown in Figure 1. [c]AZ DprE1 inhibitor benchmark is compound **9** in reference 11.

Table 9. Blood Exposure Levels and log_{10}CFU Reduction for Compounds 30 and 3

		blood levels		
compound	dose (mg/kg)[a]	C_{max} (ng/mL)	AUC (h ng/mL)	reduction of log_{10} CFU/lung (relative to untreated controls)
moxifloxacin	100			4.1 ± 0.3
30	200	6380	31400[b]	0.5 ± 0.1
3	170	1870	6378[c]	0.2 ± 0.1

[a]Compounds were dosed per os, once daily. [b]Compound **30**'s blood levels were found to remain above its MIC value for 24 h after single oral administration. [c]Compound **3**'s blood levels were found to be below its MIC value within 6 h after single oral administration.

Table 6. In Vitro Activity, Cytotoxicity, and Physicochemical Properties of 30 and its Enantiomers

№	Structure	DprE1 pIC_{50}[a]	*Mtb* MIC (μM)[b]	HepG2 IC_{50} (μM)[c]	Solubility (μM)[d]	Chrom logD[e]
30		7.2	0.7	> 100	≥ 486	3.19
30R		7.5	0.78	> 100	≥ 344	3.17
30S		5.1	> 80	> 100	≥ 379	3.17

[a]Inhibition of DprE1 enzyme. [b]MIC against *M. tuberculosis* (H37Rv). [c]Cytotoxicity against HepG2 human Caucasian hepatocyte carcinoma. [d]Kinetic aqueous solubility (CLND). [e]Lipophilicity, chromlogD at pH = 7.4.

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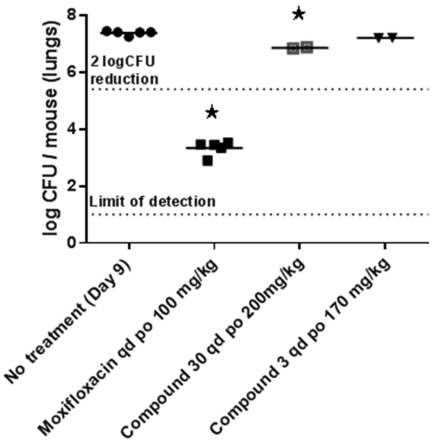

Figure 3. Antitubercular efficacy in an acute infection murine model of tuberculosis. Each point represents data from an individual mouse that received each product administered in a once a day schedule (qd). Treatment was administered for 4 days as detailed in the figure. *$p < 0.05$. ANOVA analysis with Dunnett's post test.

CONCLUSIONS

Herein, we have reported an expanded SAR exploration of a hydantoin derivative series, recently discovered by GSK. Previously, we demonstrated that these compounds are potent and selective antimycobacterials that act via inhibition of DprE1. Our medicinal chemistry research effort described herein resulted in 69 novel hydantoin analogues and led to the identification of potent representatives with high in vitro enzymatic potency (pIC_{50} 7−7.4) and whole-cell MIC values in the low micromolar range (0.6−0.9 μM). The most potent representatives were compound **30** and its close analogues **67**, **69**, and **82**. This chemical family is characterized by no appreciable cytotoxicity or cardiotoxicity (hERG), satisfactory metabolic stability, and a reasonable physicochemical profile. In vivo proof of concept for compound **30** was achieved by using an acute murine model of intratracheal infection. Although encouraging, currently available data indicate that additional research, mainly focusing on the improvement of in vivo efficacy responses, is required before preclinical development for this class of compounds can be considered successful.

EXPERIMENTAL SECTION

General Information. Laboratory reagent-grade solvents were employed unless stated otherwise. Reagents were purchased from Sigma-Aldrich, Fluorochem, Acros Organics, TCI, Enamine, or Apollo Scientific and were used without further purification unless specified otherwise. Reaction progression was monitored by TLC on silica gel and/or by UPLC−MS. Silica gel TLC analysis was performed using Polygram precoated silica gel TLC sheets SIL G/UV254 with detection by UV light (254 nm).

Structural determination and characterization of all compounds were performed with ^{1}H NMR and ^{13}C NMR spectroscopy and mass spectrometry. ^{1}H NMR (400 MHz) and ^{13}C-NMR (100 MHz) spectra were recorded on a Bruker Avance III Nanobay Ultrashield 400 or a Bruker DPX 400 spectrometer. The chemical shift (δ) values are reported in parts per million (ppm), and coupling constants are expressed in hertz (Hz). The chemical shifts δ were given relative to the residual ^{1}H and ^{13}C signals of the solvent peak as an internal standard: in ^{1}H NMR (400 MHz) δ 2.50 ppm (quin, C_2D_5HOS) for DMSO-d_6, δ 2.05 ppm (quin, C_3D_5HO) for acetone-d_6, δ 3.31 ppm (quin, CD_2HOD) for methanol-d_4; in ^{13}C NMR (101 MHz) δ 39.51 ppm (sept) for DMSO-d_6, δ 29.84 ppm (sept), δ 206.26 ppm (s) for acetone-d_6, and δ 49.00 ppm (sept) for methanol-d_4. Legend: s = singlet, d = doublet, t = triplet, q = quartet, quin = quintet, sept = septet, m = multiplet (denotes complex pattern), br = broad signal, dd = doublet of doublets, dt = doublet of triplets, td = triplet of doublets, etc.

UPLC−MS analysis was performed according to the methods A, B, or C. In all cases, ESI (electrospray ionization) was used. The quasi-molecular ions $[M + H]^+$ or $[M - H]^-$ were typically detected, unless stated otherwise. Retention time R_t is specified for each described method. Method A involved the Waters Acquity UPLC system coupled to a Waters SQ detector. A Waters Acquity UPLC BEH C18 1.7 μm, 3 mm × 50 mm column was employed. The sample concentration was 0.1 mg/mL, and the flow was 0.8 mL/min. Solvent A consisted of aqueous ammonium acetate 25 mM and 10% acetonitrile at pH 6.6, and Solvent B was acetonitrile. The method was as follows: 0.0−0.2 min A/B 99.9:0.1, 0.2−1.0 min 10:90, 1.0−1.8 min 10:90, 1.9−2.0 min 99.9:0.1 at temperature 40 °C. The UV detection was an averaged signal from the wavelength of 210 to 400 nm. In methods B and C, ESI mass spectra were obtained with an Esquire 3000 plus ion trap mass spectrometer (Bruker Daltonics) in the direct infusion mode. A Waters Acquity H-class UPLC system coupled to a waters TQD ESI mass spectrometer and a Waters TUV detector were used with a Waters Acquity UPLC BEH C18 1.7 μm 2.1′50 mm column. Solvent A consisted of water with 0.1% formic acid. Solvent B consisted of acetonitrile with 0.1% formic acid. Method B involved the following: flow 0.7 mL/min, 0.15 min isocratic elution (A/B = 95:5), then gradient elution for 1.85 min (A/B = from 95:5 to 0:100), followed by 0.25 min of isocratic elution (A/B = 0:100), then 0.75 min of isocratic elution (A/B = 95:5). Method C involved the following: flow 0.4 mL/min, 0.15 min isocratic elution (A/B = 95:5), then gradient elution for 4.85 min (A/B = from 95:5 to 0:100), followed by 0.25 min of isocratic elution (A/B = 0:100), then 0.75 min of isocratic elution (A/B = 95:5). In methods B and C, the wavelength for UV detection was 254 nm unless stated otherwise.

For the high-resolution mass spectrometry (HRMS) measurements, positive ion mass spectra were acquired using a QSTAR Elite System (AB Sciex Instruments) mass spectrometer, equipped with a turbospray source, over a mass range of 250−700.

Where necessary, flash purification was performed on a Biotage ISOLERA One or Four flash system equipped with an internal variable dual-wavelength diode array detector (200−400 nm). For normal phase purifications, Biotage SNAP (10−340 g), Sylicicle SiliaSep (4−120 g), or Götec-Labortechnik EasyVarioFlash (5−100 g) cartridges were used (flow rates of 10−100 mL/min). Reversed-phase purifications were performed with Biotage KP-C18 containing cartridges. Gradients used varied for each purification. However, typical gradients used for normal phase were a gradient of 0−100% ethyl acetate in *n*-heptane or cyclohexane or 0−15% methanol in ethyl acetate. Typically, a gradient of 5% MeCN in water to 50% MeCN in water was used for the reverse phase.

The preparative HPLC purification was conducted on an Agilent 1200 or Agilent 1100 instrument, employing either on an X-Bridge C_{18} column (19 mm × 150 mm, i.d. 5 μm packing diameter or 30 mm × 150 mm, i.d. 5 μm packing diameter) or on a SunFire C_{18} column (19 mm × 150 mm or 30 mm × 250 mm) at 35 °C. The solvents employed were as follows: A = 10 mM ammonium bicarbonate in water; B = acetonitrile ("basic" method) or A = 0.1 M formic acid in water; and B = 0.1 M formic acid in acetonitrile ("acidic" method). The purification was run as a gradient (A/B) typically from 40 to 100% over either 20 or 25 min with a flow rate of 17 mL/min (19 mm column) or 35 mL/min (30 mm column). The UV detection wavelengths were 210 and 254 nm.

Microwave radiation-assisted reactions were performed in a Biotage Initiator instrument. The initial absorption was set as "high", and 2 min of pre-stirring was applied before heating commenced.

The isolated yields are reported. Purity of final compounds was 95% or higher (verified by UPLC-MS), except for compounds **10** (>85%), **33** (>90%), **34** (>90%), **80** (>90%), and **87** (>90%). These molecules displayed low potency in the DprE1 and MIC assays, and further purification was therefore not carried out. All products were obtained as amorphous solids, and melting points were not measured.

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The following section reports the synthetic procedures and analytical data for all final compounds and some representative intermediates reported in this publication. Complementary data for the rest of the intermediate compounds can be found in the Supporting Information. Synthetic procedures that were used in the preparation of several products are summarized here as "General methods".

The literature benchmark DprE1 inhibitor ("AZ DprE1", Figure 1, *N*-(2-fluoroethyl)-1-((6-methoxy-5-methylpyrimidin-4-yl)methyl)-1*H*-pyrrolo[3,2-*b*]pyridine-3-carboxamide) was synthesized by the procedure described previously by Shirude et al.[11] TCA1 and BTZ043 were purchased from corresponding commercial sources: TCA1 (Chemexpress (Shanghai Haoyuan) Co., Ltd., Ref. HY-12904, CAS 864941-32-2) and BTZ043 (Selleck Chemicals LLC, Ref. S1097, 957217-65-1).

General Method A: Hydantoin Core Synthesis. A modified Bucherer−Berg protocol[22] was employed. The suitable ketone (1.5.0−4.0 mmol, 1.0 equiv), ammonium carbonate $(NH_4)_2CO_3$ (9.0 equiv), and potassium cyanide KCN (1.3 equiv) were dissolved in a mixture of ethanol and water (1:1) (reaction molarity ∼0.25 to 0.4 mol/L). The reaction mixture was heated at 55 °C for 18−48 h or irradiated in a microwave oven at 70 °C for 3−9 h. After the reaction was complete, the reaction mixture was cooled down to room temperature and neutralized with 6 M hydrochloric acid to pH ∼ 7 to 8. In the case of precipitate formation, the product was collected by filtration, washed with water, and dried. Otherwise, the solvent was removed under reduced pressure and the residue was diluted with water and extracted with ethyl acetate; the combined organic phase was washed with brine, dried over Na_2SO_4, filtered, and evaporated to dryness. When necessary, the product was additionally dried in the vacuum oven (40 °C, 0−10 mbar). Typically, no additional purification was performed.

General Method B: Hydantoin Core Alkylation. A mixture of the appropriate hydantoin (0.2−3.7 mmol, 1 equiv) and potassium carbonate (K_2CO_3, 1.1−2.0 equiv) was dissolved in DMF or acetone. After 10−15 min, the corresponding alkyl halide was added in a slight excess (1.1−1.5 equiv) (reaction molarity ∼0.08 to 0.2 mol/L). The reaction mixture was stirred at room temperature for 20−72 h. Upon reaction completion, the solvent was removed under reduced pressure and the residue was diluted with saturated ammonium chloride solution or water and extracted with ethyl acetate. The combined organic phase was typically washed with 1 M NaOH and brine, dried over Na_2SO_4, filtered, and evaporated under vacuum. The residue was purified by normal-phase flash chromatography on silica gel (gradient c-Hex/Hep:EtOAc = 100:0 to 10:90) or reversed-phase flash chromatography (gradient water/ACN = 90:10 to 50:50). The final product was typically lyophilized.

General Method C: Late-Stage Sulfonamide Introduction by a Coupling Reaction. 5-(4-Bromophenyl)-3-(2-(2,4-difluorophenyl)-2-oxoethyl)-5-methylimidazolidine-2,4-dione **19** (0.08−0.19 mmol, 1.0 equiv), appropriate sulfonamide (1.2−2.0 equiv), 2-di-*tert*-butylphosphino-2′,4′,6′-triisopropylbiphenyl (t-BuXPhos) (0.04 equiv), allylpalladium(II) chloride dimers ($[Pd(allyl)Cl]_2$) (0.01 equiv), and potassium carbonate (2.0 equiv) were suspended in dry 2-methyltetrahydrofuran (2-MeTHF) (6 mL) and placed in the vial, which was evacuated and backfilled with nitrogen three times. The vial was capped under nitrogen flow and stirred heated at 80 °C for 7−48 h. Then, the reaction mixture was cooled to room temperature, and 1 M hydrochloric acid solution (20 mL) was added. Subsequently, the aqueous layer was extracted with ethyl acetate (3 × 20 mL). The combined organic layers were filtered through a small Celite column, rinsed with ethyl acetate, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude residue was purified by normal-phase flash column chromatography (gradient *c*-Hex/EtOAc = 100:0 to 10:90, solid loading) and additionally by HPLC (gradient: 40−100 basic/acid) if required. The fractions, containing the desired product, were collected and evaporated under reduced pressure. The residue was dried to provide the title compound. The final product was typically lyophilized.

Compound Syntheses. *Methyl 4-(1-(2-(2,4-Difluorophenyl)-2-oxoethyl)-4-methyl-2,5-dioxoimidazolidin-4-yl)benzoate (**10**).* The title compound was prepared according to General Method B from a crude mixture of methyl 4-(4-methyl-2,5-dioxoimidazolidin-4-yl)-benzoate **5** (110 mg, 0.443 mmol) and 2-chloro-2′,4′-difluoroacetophenone (127 mg, 0.665 mmol). Yield 33% (70.1 mg, 0.148 mmol), off-white amorphous solid, purity ≥85%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.18 (s, 1H), 7.94−8.06 (m, 3H), 7.67−7.75 (m, 2H), 7.50 (ddd, J = 2.40, 9.22, 11.62 Hz, 1H), 7.28 (dt, J = 2.40, 8.40 Hz, 1H), 4.81 (d, J = 2.53 Hz, 2H), 3.87 (s, 3H), 1.79 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 189.0 (d, J = 5.1 Hz), 174.6, 165.8, 165.7 (dd, J = 255.4, 13.2 Hz), 162.4 (dd, J = 257.8, 13.5 Hz), 155.0, 144.4, 132.6 (dd, J = 11.0, 3.7 Hz), 129.4, 129.3, 126.1, 119.5 (dd, J = 13.2, 3.7 Hz), 112.8 (dd, J = 22.0, 3.6 Hz), 105.4 (t, J = 26.8 Hz), 63.3, 52.2, 47.2 (d, J = 10.2 Hz), 25.0. UPLC-MS (ESI) (A): m/z 403 $[M + H]^+$ (R_t = 1.16 min). HRMS (ESI) m/z calcd for C20H16F2N2O5 $[M + H]^+$: 425.0919; found: 425.0924.

*1-(4-(1-(2-(2,4-Difluorophenyl)-2-oxoethyl)-4-methyl-2,5-dioxoimidazolidin-4-yl)phenyl)urea (**11**).* The title compound was prepared according to General Method B from the crude mixture containing 1-(4-(4-methyl-2,5-dioxoimidazolidin-4-yl)phenyl)urea **6** (80 mg, 0.322 mmol) and 2-bromo-1-(2,4-difluorophenyl)ethanone (68.2 mg, 0.290 mmol, 0.9 equiv). Yield 22% (28.8 mg, 0.072 mmol), off-white amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.99 (s, 1H), 8.62 (s, 1H), 7.97−8.07 (m, 1H), 7.52 (ddd, J = 2.51, 9.35, 11.48 Hz, 1H), 7.40−7.46 (m, 2H), 7.34−7.40 (m, 2H), 7.30 (dt, J = 2.26, 8.41 Hz, 1H), 5.88 (s, 2H), 4.80 (d, J = 2.26 Hz, 2H), 1.72 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 189.2 (d, J = 4.4 Hz), 175.5, 165.7 (dd, J = 255.3, 12.6 Hz), 162.5 (dd, J = 257.5, 13.2 Hz), 155.9, 155.1, 140.4, 132.7 (dd, J = 11.0, 4.4 Hz), 131.6, 126.0, 119.6 (dd, J = 13.2, 3.7 Hz), 117.5, 112.8 (dd, J = 22.0, 2.9 Hz), 105.5 (t, J = 26.8 Hz), 63.0, 47.1 (d, J = 10.3 Hz), 24.4. UPLC-MS (ESI) (B): m/z 403 $[M + H]^+$, 425 $[M + Na]^+$ (R_t = 1.42 min).

*3-(2-(2,4-Difluorophenyl)-2-oxoethyl)-5-methyl-5-(4-((methylsulfonyl)methyl)phenyl)imidazolidine-2,4-dione (**12**).* The title compound was prepared according to General Method B from 5-methyl-5-(4-((methylsulfonyl)methyl)phenyl)imidazolidine-2,4-dione **7** (100 mg, 0.354 mmol) and 2-chloro-1-(2,4-difluorophenyl)-ethanone (74.3 mg, 0.390 mmol). Yield 50% (77 mg, 0.176 mmol), white amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.09 (s, 1H), 8.01 (dt, J = 6.82, 8.46 Hz, 1H), 7.57 (d, J = 8.34 Hz, 2H), 7.43−7.54 (m, 3H), 7.28 (dt, J = 2.27, 8.34 Hz, 1H), 4.80 (d, J = 2.27 Hz, 2H), 4.51 (s, 2H), 2.92 (s, 3H), 1.77 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 189.1 (d, J = 4.39 Hz), 175.0, 165.6 (dd, J = 13.17, 255.41 Hz), 162.4 (dd, J = 13.17, 258.34 Hz), 155.0, 139.4, 132.7 (dd, J = 3.66, 10.98 Hz), 131.1, 128.9, 125.8, 119.5 (dd, J = 3.66, 13.17 Hz), 112.8 (dd, J = 3.40, 22.20 Hz), 105.4 (t, J = 27.10 Hz), 63.1, 58.8, 47.2 (d, J = 10.98 Hz), 39.6 (overlaps with solvent peak), 24.7. UPLC-MS (ESI) (A) m/z 435 $[M - H]^-$ (R_t = 1.15 min).

*3-(2-(2,4-Difluorophenyl)-2-oxoethyl)-5-methyl-5-(4-(2-oxo-1,3-oxazinan-3-yl)phenyl)imidazolidine-2,4-dione (**13**).* The title compound was prepared according to General Method B from sodium 4-methyl-2,5-dioxo-4-(4-(2-oxo-1,3-oxazinan-3-yl)phenyl)imidazolidin-1-ide **8a** (76 mg, 0.244 mmol) and 2-bromo-1-(2,4-difluorophenyl)-ethanone (57.4 mg, 0.244 mmol). Yield 79% (86 mg, 0.194 mmol), white amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.10 (s, 1H), 7.95−8.07 (m, 1H), 7.46−7.57 (m, 3H), 7.41 (d, J = 8.53 Hz, 2H), 7.29 (dt, J = 2.38, 8.47 Hz, 1H), 4.73−4.90 (m, J = 2.26 Hz, 2H), 4.34 (t, J = 5.40 Hz, 2H), 3.66 (t, J = 6.15 Hz, 2H), 2.10 (quin, J = 5.71 Hz, 2H), 1.76 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 189.1 (d, J = 5.1 Hz), 175.1, 165.7 (dd, J = 255.3, 12.5 Hz), 162.5 (dd, J = 258.2, 13.9 Hz), 155.1, 151.9, 143.2, 137.0, 132.7 (dd, J = 11.0, 3.7 Hz), 126.1, 126.0, 119.5 (dd, J = 13.2, 2.9 Hz), 112.8 (dd, J = 22.0, 2.9 Hz), 105.5 (t, J = 26.8 Hz), 66.8, 63.1, 48.1, 47.2 (d, J = 11.0 Hz), 24.8, 22.0. ESI-MS (B): m/z 444 $[M + H]^+$, 466 $[M + Na]^+$ (R_t = 1.50 min).

*3-(2-(2,4-Difluorophenyl)-2-oxoethyl)-5-methyl-5-(3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)imidazolidine-2,4-dione (**14**).* The title compound was prepared according to General Method B from 5-methyl-5-(3-oxo-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazin-6-yl)-imidazolidine-2,4-dione **9** (100 mg, 0.383 mmol) and 2-chloro-

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2′,4′-difluoroacetophenone (109 mg, 0.574 mmol). Yield 44% (73.6 mg, 0.168 mmol), white amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.75 (br s, 1H), 9.06 (s, 1H), 7.99 (dt, J = 6.82, 8.59 Hz, 1H), 7.50 (ddd, J = 2.40, 9.22, 11.62 Hz, 1H), 7.29 (dt, J = 2.27, 8.46 Hz, 1H), 7.04−7.14 (m, 2H), 6.97−7.02 (m, 1H), 4.79 (d, J = 2.53 Hz, 2H), 4.58 (s, 2H), 1.70 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 189.1 (d, J = 4.4 Hz), 175.1, 165.7 (dd, J = 255.2, 12.9 Hz), 164.8, 162.4 (dd, J = 257.2, 13.9 Hz), 155.0, 143.0, 133.4, 132.6 (dd, J = 11.0, 3.9 Hz), 127.2, 120.3, 119.5 (dd, J = 13.7, 2.9 Hz), 116.1, 113.2, 112.8 (dd, J = 22.0, 2.9 Hz), 105.4 (t, J = 27.6 Hz), 66.7, 62.8, 47.1 (d, J = 10.2 Hz), 25.0. UPLC-MS (ESI) (A): m/z 416 [M + H]$^+$ (R_t = 1.19 min). HRMS (ESI) m/z calcd for $C_{20}H_{15}F_2N_3O_5$ [M + H]$^+$: 438.0872; found: 438.0885.

*4-(1-(2-(2,4-Difluorophenyl)-2-oxoethyl)-4-methyl-2,5-dioxoimidazolidin-4-yl)benzamide (**16**).* The title compound was prepared according to General Method B from 4-(4-methyl-2,5-dioxoimidazolidin-4-yl)benzamide **15** (30 mg, 0.096 mmol, ∼75% pure) and 2-bromo-1-(2,4-difluorophenyl)ethanone (33 mg, 0.141 mmol). Yield 56% (21 mg, 0.054 mmol), white amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.15 (s, 1H), 7.95−8.07 (m, 2H), 7.91 (d, J = 8.53 Hz, 2H), 7.61 (d, J = 8.28 Hz, 2H), 7.52 (ddd, J = 2.26, 9.29, 11.55 Hz, 1H), 7.44 (s, 1H), 7.29 (dt, J = 2.38, 8.47 Hz, 1H), 4.81 (d, J = 2.51 Hz, 2H), 1.77 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 189.2, 174.9, 167.4, 165.8 (dd, J = 13.21, 256.04 Hz), 162.5 (dd, J = 13.21, 258.24 Hz), 155.1, 142.3, 134.1, 132.7 (dd, J = 4.03, 11.37 Hz), 127.7, 125.6, 119.5 (dd, J = 3.30, 12.84 Hz), 112.9 (dd, J = 2.93, 22.01 Hz), 105.5 (t, J = 26.40 Hz), 63.3, 47.3 (d, J = 11.00 Hz), 24.9. UPLC-MS (ESI) (B): m/z 388 [M + H]$^+$ (R_t = 1.42 min).

*4-(1-(2-(2,4-Difluorophenyl)-2-oxoethyl)-4-methyl-2,5-dioxoimidazolidin-4-yl)benzoic acid (**17**).* Methyl 4-(1-(2-(2,4-difluorophenyl)-2-oxoethyl)-4-methyl-2,5-dioxoimidazolidin-4-yl)benzoate **10** (90 mg, 0.224 mmol) and lithium hydroxide (90 mg, 3.76 mmol) were dissolved in a mixture of THF (4 mL) and water (4,00 mL) and stirred at rt for 1 h. The mixture was acidified with 1 M aqueous HCl and extracted with AcOEt 3×. Combined organic phases were dried with sodium sulfate and evaporated. The residue was purified via flash column chromatography on the reversed phase (ACN/water 5−60%) and subsequently lyophilized to provide the title compound as a white solid. Yield 44% (38 mg, 0.098 mmol). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.09 (s, 1H), 9.17 (s, 1H), 7.92−8.10 (m, 3H), 7.65 (d, J = 8.28 Hz, 2H), 7.51 (ddd, J = 2.26, 9.29, 11.55 Hz, 1H), 7.29 (dt, J = 2.26, 8.41 Hz, 1H), 4.81 (d, J = 2.76 Hz, 2H), 1.78 (s, 3H). UPLC-MS (ESI) (B) m/z 387 [M − H]$^-$ (R_t = 1.50 min).

*5-(4-(1H-Tetrazol-5-yl)phenyl)-3-(2-(2,4-difluorophenyl)-2-oxoethyl)-5-methylimidazolidine-2,4-dione (**18**).* 4-(1-(2-(2,4-Difluorophenyl)-2-oxoethyl)-4-methyl-2,5-dioxoimidazolidin-4-yl)-benzonitrile **1** (50 mg, 0.135 mmol) was dissolved in n-PrOH (1.5 mL). Sodium azide (10.56 mg, 0.162 mmol) and zinc chloride (18.45 mg, 0.135 mmol) were added, and the reaction was stirred at 95 °C for 24 h. 5% NaOH was added. Solids were filtered and washed with 5% NaOH. The filtrate was acidified with 1 M HCl and extracted with ethyl acetate (3×). Combined organic phases were dried with sodium sulfate, evaporated, and purified via flash column chromatography on the reversed phase (ACN/water 5−50% + 0.5% formic acid). The product was lyophilized to provide the title compound. Yield 70% (39 mg, 0.095 mmol), white amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.20 (s, 1H), 8.06−8.20 (m, 2H), 7.92−8.06 (m, 1H), 7.78 (d, J = 8.53 Hz, 2H), 7.46−7.62 (m, 1H), 7.23−7.34 (m, 1H), 4.65−4.88 (m, 2H), 1.81 (s, 3H). UPLC-MS (ESI) (B) m/z 413 [M + H]$^+$ (R_t = 1.49 min).

*5-(4-Bromophenyl)-3-(2-(2,4-difluorophenyl)-2-oxoethyl)-5-methylimidazolidine-2,4-dione (**19**).* The title compound was prepared as reported previously.[21]

*N-(4-(1-(2-(2,4-Difluorophenyl)-2-oxoethyl)-4-methyl-2,5-dioxoimidazolidin-4-yl)phenyl)-1,1,1-trifluoromethanesulfonamide (**20**).* The title compound was prepared according to General Method C from 5-(4-bromophenyl)-3-(2-(2,4-difluorophenyl)-2-oxoethyl)-5-methylimidazolidine-2,4-dione **19** (35 mg, 0.083 mmol) and trifluoromethanesulfonamide (24.66 mg, 0.165 mmol, 2 equiv). Yield 27% (11 mg, 0.022 mmol), off-white amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.99 (br s, 1H), 9.05 (s, 1H), 8.00 (dt, J = 6.82, 8.59 Hz, 1H), 7.46−7.58 (m, 3H), 7.22−7.35 (m, 3H), 4.80 (d, J = 2.53 Hz, 2H), 1.74 (s, 3H). UPLC-MS (ESI) (A): m/z 490 [M − H]$^-$ (R_t = 1.02 min).

*N-(4-(1-(2-(2,4-Difluorophenyl)-2-oxoethyl)-4-methyl-2,5-dioxoimidazolidin-4-yl)phenyl)benzenesulfonamide (**21**).* The title compound was prepared according to General Method C from 5-(4-bromophenyl)-3-(2-(2,4-difluorophenyl)-2-oxoethyl)-5-methylimidazolidine-2,4-dione **19** (80 mg, 0.189 mmol) and benzenesulfonamide (35.7 mg, 0.227 mmol, 1.2 equiv). Yield 31% (31 mg, 0.059 mmol), off-white amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.46 (s, 1H), 8.94 (s, 1H), 7.99 (dt, J = 6.82, 8.59 Hz, 1H), 7.77−7.83 (m, 2H), 7.45−7.66 (m, 4H), 7.38 (d, J = 8.59 Hz, 2H), 7.28 (dt, J = 2.27, 8.46 Hz, 1H), 7.13 (d, J = 8.84 Hz, 2H), 4.76 (d, J = 2.53 Hz, 2H), 1.67 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 189.1 (d, J = 5.1 Hz), 175.1, 165.7 (dd, J = 255.6, 12.4 Hz), 162.4 (dd, J = 257.6, 11.6 Hz), 155.0, 139.6, 137.5, 134.6, 133.0, 132.6 (dd, J = 11.0, 4.2 Hz), 129.3, 126.6*, 119.5−119.6 (m), 119.5, 112.8 (dd, J = 22.6, 2.6 Hz), 105.4 (t, J = 26.6 Hz), 62.8, 47.1 (d, J = 11.0 Hz), 24.5.*Two peaks possess the identical chemical shift (proven by HSQC). UPLC-MS (ESI) (A): m/z 500 [M + H]$^+$ (R_t = 1.39 min).

*N-(4-(1-(2-(2,4-Difluorophenyl)-2-oxoethyl)-4-methyl-2,5-dioxoimidazolidin-4-yl)phenyl)butane-1-sulfonamide (**22**).* The title compound was prepared according to General Method C from 5-(4-bromophenyl)-3-(2-(2,4-difluorophenyl)-2-oxoethyl)-5-methylimidazolidine-2,4-dione **19** (80 mg, 0.189 mmol) and butane-1-sulfonamide (38.9 mg, 0.284 mmol, 1.5 equiv). Yield 34% (55 mg, 0.097 mmol), off-white amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.69 (br s, 1H), 9.01 (s, 1H), 8.01 (dt, J = 6.57, 8.59 Hz, 1H), 7.43−7.56 (m, 3H), 7.29 (dt, J = 2.40, 8.40 Hz, 1H), 7.23 (d, J = 8.84 Hz, 2H), 4.80 (d, J = 2.53 Hz, 2H), 3.04−3.14 (m, 2H), 1.73 (s, 3H), 1.58−1.69 (m, 2H), 1.35 (sxt, J = 7.43 Hz, 2H), 0.84 (t, J = 7.33 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 189.1 (d, J = 4.4 Hz), 175.2, 165.7 (dd, J = 255.4, 13.2 Hz), 162.4 (dd, J = 257.6, 13.2 Hz), 155.0, 138.2, 134.3, 132.6 (dd, J = 11.0, 4.0 Hz), 126.7, 119.5 (dd, J = 12.8, 3.2 Hz), 119.1, 112.6−113.0 (m), 105.4 (t, J = 26.6 Hz), 62.9, 50.5, 47.1 (d, J = 10.2 Hz), 25.1, 24.6, 20.6, 13.4. UPLC-MS (ESI) (A): m/z 480 [M + H]$^+$ (R_t = 1.39 min).

*4-(4-Methyl-2,5-dioxoimidazolidin-4-yl)benzenesulfonamide (**23**).* The title compound was prepared according to General Method A from 4-acetylbenzenesulfonamide **S-1** (1230 mg, 6.17 mmol). Yield 70% (1225 mg, 4.32 mmol). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.87 (br s, 1H), 8.69 (s, 1H), 7.84 (d, J = 8.59 Hz, 2H), 7.67 (d, J = 8.59 Hz, 2H), 7.37 (s, 2H), 1.68 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 176.3, 156.1, 143.6, 143.5, 126.0, 125.8, 63.8, 25.0.UPLC-MS (ESI) (A): m/z 268 [M − H]$^-$ (R_t = 0.77 min).

*4-(1-(2-(2,4-Difluorophenyl)-2-oxoethyl)-4-methyl-2,5-dioxoimidazolidin-4-yl)benzenesulfonamide (**30**).* The title compound was prepared according to General Method B from 4-(4-methyl-2,5-dioxoimidazolidin-4-yl)benzenesulfonamide **23** (200 mg, 0.743 mmol) and 2-chloro-2′,4′-difluoroacetophenone (212 mg, 1.114 mmol). Yield 53% (176 mg, 0.395 mmol), white amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.18 (br s, 1H), 8.00 (dt, J = 6.82, 8.59 Hz, 1H), 7.85−7.91 (m, 2H), 7.71−7.77 (m, 2H), 7.51 (ddd, J = 2.40, 9.22, 11.62 Hz, 1H), 7.39 (s, 2H), 7.29 (dt, J = 2.27, 8.46 Hz, 1H), 4.73−4.89 (m, J = 2.78 Hz, 2H), 1.79 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 189.0 (d, J = 4.4 Hz), 174.6, 165.7 (dd, J = 255.4, 13.3 Hz), 162.5 (dd, J = 257.6, 13.9 Hz), 155.0, 143.8, 142.9, 132.7 (dd, J = 11.3, 3.8 Hz), 126.3, 125.9, 119.5 (dd, J = 13.2, 3.1 Hz), 112.8 (dd, J = 22.0, 2.9 Hz), 105.4 (t, J = 26.7 Hz), 63.2, 47.2 (d, J = 10.2 Hz), 24.9. UPLC-MS (ESI) (A): m/z 424 [M + H]$^+$ (R_t = 1.05 min). HRMS (ESI) m/z calcd for $C_{18}H_{15}F_2N_3O_5S$ [M + H]$^+$: 446.0593; found: 446.0602.

*4-(1-(2-(2,4-Difluorophenyl)-2-oxoethyl)-4-methyl-2,5-dioxoimidazolidin-4-yl)-N-methylbenzenesulfonamide (**31**).* The title compound was prepared according to General Method B from *N*-methyl-4-(4-methyl-2,5-dioxoimidazolidin-4-yl)benzenesulfonamide **24** (97 mg, 0.342 mmol) and 2-chloro-2′,4′-difluoroacetophenone (98 mg, 0.514 mmol). Yield 62% (98 mg, 0.213 mmol), white amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.18 (br s, 1H), 8.00 (dt, J =

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6.57, 8.59 Hz, 1H), 7.82−7.86 (m, 2H), 7.75−7.80 (m, 2H), 7.46−7.55 (m, 2H), 7.28 (dt, J = 2.40, 8.40 Hz, 1H), 4.82 (d, J = 2.53 Hz, 2H), 2.43 (s, 3H), 1.79 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 189.0 (d, J = 5.1 Hz), 174.5, 165.7 (dd, J = 255.3, 12.4 Hz), 162.5 (dd, J = 257.6, 13.2 Hz), 155.0, 143.5, 139.1, 132.7 (dd, J = 11.3, 4.0 Hz), 126.9, 126.6, 119.5 (dd, J = 13.2, 2.9 Hz), 112.8 (dd, J = 22.0, 3.2 Hz), 105.4 (t, J = 27.1 Hz), 63.2, 47.3 (d, J = 11.0 Hz), 28.7, 24.9. UPLC-MS (ESI) (A): m/z 438 $[M + H]^+$ (R_t = 1.11 min). HRMS (ESI) m/z calcd for $C_{19}H_{17}F_2N_3O_5S$ $[M + H]^+$: 460.0749; found: 460.0758.

*4-(1-(2-(2,4-Difluorophenyl)-2-oxoethyl)-4-methyl-2,5-dioxoimidazolidin-4-yl)-N,N-dimethylbenzenesulfonamide (**32**).* The title compound was prepared according to General Method B, using *N*,*N*-dimethyl-4-(4-methyl-2,5-dioxoimidazolidin-4-yl)-benzenesulfonamide **25** (70 mg, 0.24 mmol) and 2-chloro-2′,4′-difluoroacetophenone (67 mg, 0.35 mmol). Yield 64% (68 mg, 0.150 mmol), white amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 9.21 (s, 1 H), 8.00 (td, J = 8.6, 6.6 Hz, 1 H), 7.78−7.89 (m, 4 H), 7.51 (ddd, J = 11.6, 9.2, 2.4 Hz, 1 H), 7.29 (td, J = 8.4, 2.4 Hz, 1 H), 4.82 (d, J = 2.5 Hz, 2 H), 2.63 (s, 6 H), 1.80 (s, 3 H). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 189.0 (d, $^3J_{CF}$ = 5.1 Hz), 174.5, 165.7 (dd,$^1J_{CF}$ = 254.7 Hz,$^3J_{CF}$ = 12.4 Hz), 162.4 (dd,$^1J_{CF}$ = 256.9 Hz,$^3J_{CF}$ = 13.9 Hz), 155.0, 144.1, 134.7, 132.6 (dd,$^3J_{CF}$ = 11.0 Hz,$^3J_{CF}$ = 4.4 Hz), 127.8, 126.7, 119.5 (dd,$^2J_{CF}$ = 13.2 Hz,$^4J_{CF}$ = 3.7 Hz), 112.8 (dd,$^2J_{CF}$ = 22.0 Hz,$^4J_{CF}$ = 2.9 Hz), 105.4 (t,$^3J_{CF}$ = 26.7 Hz), 63.2, 47.3 (d,$^4J_{CF}$ = 11.7 Hz), 37.5, 25.1. UPLC-MS (ESI) (A): m/z 452 $[M + H]^+$ (R_t = 1.23 min).

*N-Cyclopropyl-4-(1-(2-(2,4-difluorophenyl)-2-oxoethyl)-4-methyl-2,5-dioxoimidazolidin-4-yl)benzenesulfonamide (**33**).* The title compound was prepared according to General Method C from *N*-cyclopropyl-4-(4-methyl-2,5-dioxoimidazolidin-4-yl)-benzenesulfonamide **26** (100 mg, 0.323 mmol) and 2-chloro-2′,4′-difluoroacetophenone (92 mg, 0.485 mmol). Yield 75% (118 mg, 0.242 mmol), off-white amorphous solid, purity ≥90%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.19 (s, 1H), 7.95−8.05 (m, 2H), 7.84−7.90 (m, 2H), 7.75−7.82 (m, 2H), 7.50 (ddd, J = 2.40, 9.28, 11.56 Hz, 1H), 7.28 (dt, J = 2.40, 8.40 Hz, 1H), 4.82 (d, J = 2.53 Hz, 2H), 2.09 (m, 1H), 1.80 (s, 3H), 0.44−0.53 (m, 2H), 0.35−0.44 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 189.0 (d, J = 4.4 Hz), 174.5, 165.7 (dd, J = 255.3, 13.2 Hz), 162.5 (dd, J = 257.6, 13.2 Hz), 155.0, 143.6, 140.0, 132.6 (dd, J = 11.0, 4.4 Hz), 127.0, 126.5, 119.5 (dd, J = 13.2, 3.7 Hz), 112.8 (dd, J = 22.0, 3.1 Hz), 105.4 (t, J = 26.8 Hz), 63.2, 47.3 (d, J = 11.0 Hz), 24.9, 24.1, 5.1. UPLC-MS (ESI) (A): m/z 464 $[M + H]^+$ (R_t = 1.16 min).

*4-(1-(2-(2,4-Difluorophenyl)-2-oxoethyl)-4-methyl-2,5-dioxoimidazolidin-4-yl)-N-(2,2,2-trifluoroethyl)benzenesulfonamide (**34**).* The title compound was prepared according to General Method B from 4-(4-methyl-2,5-dioxoimidazolidin-4-yl)-*N*-(2,2,2-trifluoroethyl)benzenesulfonamide **27** (100 mg, 0.285 mmol) and 2-bromo-1-(2,4-difluorophenyl)ethanone (100 mg, 0.427 mmol) in acetone. Yield 16% (23.4 mg, 0.046 mmol), off-white amorphous solid, purity ≥90%. ^{1}H NMR* (400 MHz, DMSO-d_6) δ 9.20 (s, 1H), 8.68 (s, 1H), 8.00 (dt, J = 6.57, 8.59 Hz, 1H), 7.88−7.93 (m, 2H), 7.74−7.81 (m, 2H), 7.51 (ddd, J = 2.40, 9.28, 11.56 Hz, 1H), 7.29 (dt, J = 2.40, 8.40 Hz, 1H), 4.82 (d, J = 2.53 Hz, 2H), 3.73 (q, J = 9.60 Hz, 2H), 1.79 (s, 3H). *Alk position is proven by HSQC and HMBC NMR. ^{13}C NMR (101 MHz, DMSO-d_6) δ 189.0 (d, J = 5.2 Hz), 174.5, 165.7 (dd, J = 255.4, 13.2 Hz), 162.5 (dd, J = 257.6, 13.2 Hz), 155.0, 143.9, 140.5, 132.6 (dd, J = 11.0, 3.7 Hz), 126.6**, 124.3 (q, J = 278.1 Hz), 119.4 (dd, J = 13.2, 3.7 Hz), 112.8 (dd, J = 22.0, 3.2 Hz), 105.4 (t, J = 26.8 Hz), 63.2, 47.3 (d, J = 10.2 Hz), 43.3 (q, J = 34.8 Hz), 25.0. **Two peaks possess the identical chemical shift (proven by HSQC). UPLC-MS (ESI) (A): m/z 504 $[M - H]^-$ (R_t = 1.14 min).

*4-(1-(2-(2,4-Difluorophenyl)-2-oxoethyl)-4-methyl-2,5-dioxoimidazolidin-4-yl)-N-phenylbenzenesulfonamide (**35**).* The title compound was prepared according to General Method B from 4-(4-methyl-2,5-dioxoimidazolidin-4-yl)-*N*-phenylbenzenesulfonamide **28** (100 mg, 0.290 mmol) and 2-chloro-2′,4′-difluoroacetophenone (83 mg, 0.434 mmol). Yield 13% (21 mg, 0.038 mmol), off-white amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.38 (s, 1H), 9.11 (s, 1H), 7.99 (dt, J = 6.57, 8.59 Hz, 1H), 7.80−7.87 (m, 2H), 7.72 (d, J = 8.59 Hz, 2H), 7.49 (ddd, J = 2.40, 9.22, 11.62 Hz, 1H), 7.20−7.32 (m, 3H), 7.09−7.15 (m, 2H), 6.98−7.06 (m, 1H), 4.79 (d, J = 2.53 Hz, 2H), 1.75 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 189.0 (d, J = 4.4 Hz), 174.4,), 165.7 (dd, J = 255.3, 12.6 Hz), 162.4 (dd, J = 257.6, 13.2 Hz), 154.9, 143.9, 139.4, 137.6, 132.6 (dd, J = 11.1, 3.9 Hz), 129.2, 126.9, 126.7, 124.0, 119.7, 119.4 (dd, J = 13.9, 3.0 Hz), 112.8 (dd, J = 21.9, 2.9 Hz), 105.4 (t, J = 27.1 Hz), 63.2, 47.3 (d, J = 11.7 Hz), 24.8. UPLC-MS (ESI) (A): m/z 498 $[M - H]^-$ (R_t = 1.21 min).

*3-(2-(2,4-Difluorophenyl)-2-oxoethyl)-5-methyl-5-(4-(morpholinosulfonyl)phenyl)imidazolidine-2,4-dione (**36**).* The title compound was prepared according to General Method B from 5-methyl-5-(4-(morpholinosulfonyl)phenyl)imidazolidine-2,4-dione **29** (200 mg, 0.589 mmol) and 2-chloro-1-(2,4-difluorophenyl)ethanone (124 mg, 0.648 mmol). Yield 84% (243 mg, 0.492 mmol), white amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.22 (s, 1H), 7.95−8.08 (m, 1H), 7.77−7.89 (m, 4H), 7.50 (ddd, J = 2.27, 9.47, 11.49 Hz, 1H), 7.28 (dt, J = 2.15, 8.40 Hz, 1H), 4.82 (d, J = 2.27 Hz, 2H), 3.55−3.69 (m, 4H), 2.80−2.92 (m, 4H), 1.81 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 189.0 (d, J = 4.39 Hz), 174.4, 165.7 (dd, J = 13.17, 256.14 Hz), 162.5 (dd, J = 13.17, 257.61 Hz), 155.0, 144.6, 134.3, 132.6 (dd, J = 3.66, 10.98 Hz), 128.0, 126.8, 119.5 (dd, J = 3.66, 13.17 Hz), 112.8 (m, J = 2.93, 21.22 Hz), 105.4 (t, J = 27.10 Hz), 65.2, 63.2, 47.3 (d, J = 10.98 Hz), 45.9, 25.1. UPLC-MS (ESI) (A) m/z 494 $[M + H]^+$ (R_t = 1.23 min),

*4-(4-Methyl-2,5-dioxoimidazolidin-4-yl)benzonitrile (**37**).* The title compound was prepared as reported previously.[21]

*4-(4-Methyl-2,5-dioxo-1-(2-oxo-2-phenylethyl)imidazolidin-4-yl)benzenesulfonamide (**38**).* The title compound was prepared according to General Method B from 4-(4-methyl-2,5-dioxoimidazolidin-4-yl)benzenesulfonamide **23** (50 mg, 0.186 mmol) and 2-bromo-1-phenylethanone (55.4 mg, 0.279 mmol). Yield 50% (36 mg, 0.093 mmol), off-white amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.18 (br s, 1H), 8.02−8.07 (m, 2H), 7.86−7.91 (m, 2H), 7.69−7.78 (m, 3H), 7.55−7.61 (m, 2H), 7.39 (s, 2H), 4.99 (s, 2H), 1.80 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 192.1, 174.8, 155.1, 143.8, 143.0, 134.2, 133.9, 129.0, 128.1, 126.3, 125.9, 63.2, 44.6, 24.9. UPLC-MS (ESI) (A): m/z 386 $[M - H]^-$ (R_t = 0.99 min).

*4-(4-Methyl-2,5-dioxo-1-(2-oxo-2-phenylethyl)imidazolidin-4-yl)benzonitrile (**39**).* The title compound was prepared according to General Method B from 4-(4-methyl-2,5-dioxoimidazolidin-4-yl)-benzonitrile **37** (70 mg, 0.325 mmol) and 2-bromo-1-phenyl-ethanone (71.2 mg, 0.358 mmol). Yield 64% (69 mg, 0.207 mmol), white amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.20 (s, 1H), 7.92 (d, J = 8.53 Hz, 2H), 7.88 (d, J = 7.28 Hz, 1H), 7.72 (d, J = 8.53 Hz, 2H), 7.46−7.55 (m, 1H), 7.30−7.39 (m, 2H), 4.82 (s, 2H), 2.38 (s, 3H), 1.75 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 195.8, 174.4, 155.1, 144.6, 138.0, 134.4, 132.6, 132.3, 131.9, 128.9, 126.7, 126.0, 118.5, 111.0, 63.3, 46.3, 25.0, 20.6. UPLC-MS (ESI) (B) m/z 332 $[M - H]^-$ (R_t = 1.62 min).

*4-(1-(2-(2-Fluorophenyl)-2-oxoethyl)-4-methyl-2,5-dioxoimidazolidin-4-yl)benzenesulfonamide (**40**).* The title compound was prepared according to General Method B from 4-(4-methyl-2,5-dioxoimidazolidin-4-yl)benzenesulfonamide **23** (100 mg, 0.371 mmol) and 2-bromo-1-(2-fluorophenyl)ethanone (121 mg, 0.557 mmol). Yield 64% (96 mg, 0.237 mmol), white amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.18 (s, 1H), 7.84−7.94 (m, 3H), 7.71−7.80 (m, 3H), 7.32−7.49 (m, 4H), 4.82 (d, J = 2.53 Hz, 2H), 1.79 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 190.3 (d, J = 4.4 Hz), 174.6, 161.5 (d, J = 254.7 Hz), 155.0, 143.8, 142.9, 136.3 (d, J = 9.5 Hz), 130.3 (d, J = 2.2 Hz), 126.3, 125.9, 125.1 (d, J = 2.9 Hz), 122.4 (d, J = 13.2 Hz), 117.0 (d, J = 22.7 Hz), 63.2, 47.4 (d, J = 10.2 Hz), 24.8. UPLC-MS (ESI) (A): m/z 404 $[M - H]^-$ (R_t = 1.23 min).

*4-(1-(2-(2-Fluorophenyl)-2-oxoethyl)-4-methyl-2,5-dioxoimidazolidin-4-yl)benzonitrile (**41**).* The title compound was prepared according to General Method B from 4-(4-methyl-2,5-dioxoimidazolidin-4-yl)benzonitrile **37** (70 mg, 0.325 mmol) and 2-bromo-1-(2-fluorophenyl)ethanone (78 mg, 0.358 mmol). White solid, yield 64%

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(73 mg, 0.208 mmol). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.23 (s, 1H), 7.94 (d, J = 8.53 Hz, 2H), 7.89 (dt, J = 1.51, 7.53 Hz, 1H), 7.71−7.79 (m, 3H), 7.36−7.46 (m, 2H), 4.82 (d, J = 2.26 Hz, 2H), 1.78 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 190.3 (d, J = 4.40 Hz), 174.4, 161.5 (d, J = 254.57 Hz), 155.0, 144.6, 136.4 (d, J = 9.54 Hz), 132.6, 130.4 (d, J = 2.20 Hz), 126.8, 125.2 (d, J = 2.93 Hz), 122.4 (d, J = 13.21 Hz), 118.5, 117.1 (d, J = 22.74 Hz), 111.1, 63.3, 47.5 (d, J = 11.00 Hz), 24.9 UPLC-MS (ESI) (B) m/z 350 $[M - H]^-$ (R_t = 1.59 min).

*4-(1-(2-(3-Fluorophenyl)-2-oxoethyl)-4-methyl-2,5-dioxoimidazolidin-4-yl)benzenesulfonamide (**42**).* The title compound was prepared according to General Method B from 4-(4-methyl-2,5-dioxoimidazolidin-4-yl)benzenesulfonamide **23** (50 mg, 0.186 mmol) and 2-bromo-3′-fluoroacetophenone (60.4 mg, 0.279 mmol). Yield 45% (34 mg, 0.084 mmol), white amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.19 (s, 1H), 7.81−7.92 (m, 4H), 7.72−7.77 (m, 2H), 7.55−7.67 (m, 2H), 7.39 (s, 2H), 5.01 (s, 2H), 1.80 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 191.4 (d, J = 2.4 Hz), 174.7, 162.1 (d, J = 245.9 Hz), 155.1, 143.8, 142.9, 136.0 (d, J = 6.6 Hz), 131.2 (d, J = 7.3 Hz), 126.3, 125.9, 124.4 (d, J = 2.9 Hz), 121.2 (d, J = 21.2 Hz), 114.8 (d, J = 22.7 Hz), 63.2, 44.8, 24.9. UPLC-MS (ESI) (A): m/z 406 $[M + H]^+$ (R_t = 1.0 min).

*4-(1-(2-(3-Fluorophenyl)-2-oxoethyl)-4-methyl-2,5-dioxoimidazolidin-4-yl)benzonitrile (**43**).* The title compound was prepared according to General Method B from 4-(4-methyl-2,5-dioxoimidazolidin-4-yl)benzonitrile **37** (70 mg, 0.325 mmol) and 2-bromo-1-(3-fluorophenyl)ethan-1-one (78 mg, 0,358 mmol). White solid, yield 63% (72 mg, 0.205 mmol). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.25 (s, 1H), 7.94 (d, J = 8.53 Hz, 2H), 7.89 (d, J = 7.53 Hz, 1H), 7.85 (dd, J = 2.01, 9.54 Hz, 1H), 7.76 (d, J = 8.53 Hz, 2H), 7.55−7.68 (m, 2H), 5.02 (s, 2H), 1.79 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 191.4 (d, J = 2.20 Hz), 174.5, 162.2 (d, J = 245.77 Hz), 155.0, 144.6, 136.0 (d, J = 6.60 Hz), 132.6, 131.3 (d, J = 8.07 Hz), 126.7, 124.4 (d, J = 2.20 Hz), 121.2 (d, J = 21.28 Hz), 118.5, 114.8 (d, J = 22.00 Hz), 111.1, 63.3, 44.9, 25.0. UPLC-MS (ESI) (B) m/z 350 $[M - H]^-$ (R_t = 1.61 min).

*4-(1-(2-(4-Fluorophenyl)-2-oxoethyl)-4-methyl-2,5-dioxoimidazolidin-4-yl)benzenesulfonamide (**44**).* The title compound was prepared according to General Method B from 4-(4-methyl-2,5-dioxoimidazolidin-4-yl)benzenesulfonamide **23** (100 mg, 0.371 mmol) and 2-bromo-1-(4-fluorophenyl)ethanone (121 mg, 0.557 mmol). Yield 65% (98 mg, 0.242 mmol), white amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.18 (s, 1H), 8.10−8.20 (m, 2H), 7.86−7.92 (m, 2H), 7.72−7.79 (m, 2H), 7.35−7.47 (m, 4H), 4.99 (s, 2H), 1.80 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 190.8, 174.8, 165.5 (d, J = 253.2 Hz), 155.1, 143.8, 143.0, 131.3 (d, J = 9.5 Hz), 130.7 (d, J = 2.9 Hz), 126.3, 125.9, 116.1 (d, J = 22.0 Hz), 63.2, 44.6, 24.9. UPLC-MS (ESI) (A): m/z 404 $[M - H]^-$ (R_t = 1.24 min).

*4-(1-(2-(3,4-Difluorophenyl)-2-oxoethyl)-4-methyl-2,5-dioxoimidazolidin-4-yl)benzenesulfonamide (**45**).* The title compound was prepared according to General Method B from 4-(4-methyl-2,5-dioxoimidazolidin-4-yl)benzenesulfonamide **23** (75 mg, 0.279 mmol) and 2-bromo-1-(3,4-difluorophenyl)ethanone (98 mg, 0.418 mmol). Yield 54% (63.6 mg, 0.150 mmol), white amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.22 (s, 1H), 8.08−8.18 (m, 1H), 7.95 (d, J = 6.27 Hz, 1H), 7.88 (d, J = 8.53 Hz, 2H), 7.75 (d, J = 8.53 Hz, 2H), 7.61−7.71 (m, 1H), 7.41 (s, 2H), 5.02 (s, 2H), 1.80 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 190.4, 174.8, 155.1, 153.3 (dd, J = 255.3, 13.2 Hz), 149.6 (dd, J = 248.7, 13.2 Hz), 143.8, 143.0, 131.2−131.5 (m), 126.3, 126.2−126.3 (m), 126.0, 118.3 (d, J = 17.6 Hz), 117.8 (d, J = 18.3 Hz), 63.3, 44.7, 24.9. UPLC-MS (ESI) (B): m/z 424 $[M + H]^+$ (R_t = 1.50 min).

*4-(1-(2-(3-Chloro-4-fluorophenyl)-2-oxoethyl)-4-methyl-2,5-dioxoimidazolidin-4-yl)benzenesulfonamide (**46**).* The title compound was prepared according to General Method B from 4-(4-methyl-2,5-dioxoimidazolidin-4-yl)benzenesulfonamide **23** (50 mg, 0.186 mmol) and 2-bromo-3′-chloro-4′-fluoroacetophenone (70.0 mg, 0.279 mmol). Yield 65% (53 mg, 0.120 mmol), off-white amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.16−9.23 (m, 1H), 8.29 (dd, J = 2.15, 7.20 Hz, 1H), 8.08 (ddd, J = 2.27, 4.74, 8.65 Hz, 1H), 7.85−7.91 (m, 2H), 7.72−7.78 (m, 2H), 7.64 (t, J = 8.97 Hz, 1H), 7.40 (s, 2H), 5.03 (s, 2H), 1.80 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 190.4 (s), 174.7 (s), 160.6 (d, J = 255.4 Hz), 155.0 (s), 143.8 (s), 142.9 (s), 131.7 (d, J = 3.7 Hz), 131.1 (s), 129.7 (d, J = 8.8 Hz), 126.3 (s), 125.9 (s), 120.6 (d, J = 18.3 Hz), 117.6 (d, J = 22.0 Hz), 63.2 (s), 44.6 (s), 24.9 (s). UPLC-MS (ESI) (A): m/z 438 $[M - H]^-$ (R_t = 1.06 min).

*4-(1-(2-(2,4-Dichlorophenyl)-2-oxoethyl)-4-methyl-2,5-dioxoimidazolidin-4-yl)benzenesulfonamide (**47**).* The title compound was prepared according to General Method B from 4-(4-methyl-2,5-dioxoimidazolidin-4-yl)benzenesulfonamide **23** (100 mg, 0.371 mmol) and 2-bromo-2′,4′-dichloroacetophenone (100 mg, 0.371 mmol). Yield 53% (100.3 mg, 0.198 mmol), off-white amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.19 (s, 1H), 7.84−7.89 (m, 3H), 7.79 (d, J = 2.02 Hz, 1H), 7.68−7.72 (m, 2H), 7.60 (dd, J = 2.02, 8.34 Hz, 1H), 7.39 (s, 2H), 4.84 (s, 2H), 1.76 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 193.4, 174.4, 154.8, 143.8, 142.8, 137.4, 133.4, 132.0, 131.4, 130.5, 127.7, 126.2, 125.9, 63.2, 46.7, 24.9. UPLC-MS (ESI) (A): m/z 456 $[M + H]^+$ (R_t = 1.14 min). HRMS (ESI) m/z calcd for $C_{18}H_{15}Cl_2N_3O_5S$ $[M + H]^+$: 478.0002; found: 478.0024.

*4-(1-(2-(3-Bromophenyl)-2-oxoethyl)-4-methyl-2,5-dioxoimidazolidin-4-yl)benzenesulfonamide (**48**).* The title compound was prepared according to General Method B from 4-(4-methyl-2,5-dioxoimidazolidin-4-yl)benzenesulfonamide **23** (150 mg, 0.557 mmol) and 2-bromo-1-(3-bromophenyl)ethanone (232 mg, 0.836 mmol). Yield 53% (138.6 mg, 0.297 mmol), off-white amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.20 (br s, 1H), 8.19 (s, 1H), 8.03 (d, J = 7.78 Hz, 1H), 7.83−7.96 (m, 3H), 7.74 (d, J = 8.53 Hz, 2H), 7.54 (t, J = 7.91 Hz, 1H), 7.41 (br s, 2H), 5.02 (s, 2H), 1.79 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 191.5, 174.7, 155.1, 143.8, 143.0, 136.9, 135.9, 131.2, 130.8, 127.2, 126.3, 126.0, 122.3, 63.3, 44.7, 24.9. UPLC-MS (ESI) (B): m/z 466, 468 $[M + H]^+$ (R_t = 1.58 min).

*2-(2-(4-(4-Cyanophenyl)-4-methyl-2,5-dioxoimidazolidin-1-yl)-acetyl)-5-fluorophenyl Pivalate (**49**).* The title compound was prepared according to General Method B from 4-(4-methyl-2,5-dioxoimidazolidin-4-yl)benzonitrile **37** (70 mg, 0.325 mmol) and 2-(2-bromoacetyl)-5-fluorophenyl pivalate **S-11** (113 mg, 0.358 mmol). Yield 91% (134 mg, 0.297 mmol), white amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.20 (s, 1H), 8.13 (dd, J = 6.27, 8.78 Hz, 1H), 7.91 (d, J = 8.53 Hz, 2H), 7.71−7.77 (m, 2H), 7.25−7.38 (m, 2H), 4.75−4.88 (m, 2H), 1.76 (s, 3H), 1.21−1.26 (m, 9H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 190.5, 175.6, 174.4, 164.6 (d, J = 253.11 Hz), 155.0, 151.1 (d, J = 11.74 Hz), 144.6, 132.5, 132.3 (d, J = 11.01 Hz), 126.8, 124.9 (d, J = 2.93 Hz), 118.5, 113.5 (d, J = 22.01 Hz), 112.2 (d, J = 24.21 Hz), 111.0, 63.2, 46.1, 38.5, 26.6, 24.7. UPLC-MS (ESI) (B) m/z 450 $[M - H]^-$ (R_t = 1.86 min).

*4-(1-(2-(4-Fluoro-2-hydroxyphenyl)-2-oxoethyl)-4-methyl-2,5-dioxoimidazolidin-4-yl)benzonitrile (**50**).* 2-(2-(4-(4-Cyanophenyl)-4-methyl-2,5-dioxoimidazolidin-1-yl)acetyl)-5-fluorophenyl pivalate **49** (100 mg, 0.222 mmol) was dissolved in a mixture of THF (5 mL) and water (5 mL). Lithium hydroxide (21.22 mg, 0.886 mmol) was added, and the reaction mixture was stirred at rt for 30 min. Subsequently, the mixture was acidified with 1 M HCl to pH ∼5 to 6, and a precipitate formed, which was filtered, washed with water, and dried to provide the title compound. Yield 80% (65 mg, 0.177 mmol), white amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.68 (br s, 1H), 9.18 (s, 1H), 7.90−7.99 (m, 2H), 7.87 (dd, J = 6.90, 9.66 Hz, 1H), 7.73−7.80 (m, 2H), 6.76−6.85 (m, 2H), 4.83 (s, 2H), 1.78 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 192.1, 174.6, 166.4 (d, J = 253.11 Hz), 161.6 (d, J = 12.47 Hz), 155.2, 144.7, 133.0 (d, J = 11.74 Hz), 132.6, 126.8, 118.5, 118.0 (d, J = 2.20 Hz), 111.0, 107.4 (d, J = 22.74 Hz), 104.0 (d, J = 24.21 Hz), 63.2, 47.3, 25.0. UPLC-MS (ESI) (B) m/z 366 $[M - H]^-$ (R_t = 1.68 min).

*4-(1-(2-(4-Fluoro-2-hydroxyphenyl)-2-oxoethyl)-4-methyl-2,5-dioxoimidazolidin-4-yl)benzenesulfonamide (**51**).* The title compound was prepared according to General Method B from 4-(4-methyl-2,5-dioxoimidazolidin-4-yl)benzenesulfonamide **23** (100 mg, 0.371 mmol) and 2-(2-bromoacetyl)-5-fluorophenyl pivalate **S-11**

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(130 mg, 0.409 mmol). Yield 20% (32 mg, 0.076 mmol), off-white amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.16 (s, 1H), 7.83−7.94 (m, 3H), 7.76 (d, J = 8.53 Hz, 2H), 7.42 (s, 2H), 6.75−6.88 (m, 2H), 4.84 (s, 2H), 1.80 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 192.1, 174.8, 166.3 (d, J = 253.1 Hz), 161.5 (d, J = 13.2 Hz), 155.3, 143.8, 143.1, 133.0 (d, J = 12.5 Hz), 126.3, 125.9, 118.0 (d, J = 2.2 Hz), 107.4 (d, J = 22.7 Hz), 104.0 (d, J = 23.5 Hz), 63.2, 47.2, 24.9. UPLC-MS (ESI) (B): m/z 422 $[M + H]^+$ (R_t = 1.45 min).

*4-(4-Methyl-2,5-dioxo-1-(2-oxo-2-(o-tolyl)ethyl)imidazolidin-4-yl)benzenesulfonamide (**52**).* The title compound was prepared according to General Method B from 4-(4-methyl-2,5-dioxoimidazolidin-4-yl)benzenesulfonamide **23** (75 mg, 0.279 mmol) and 2-bromo-1-(*o*-tolyl)ethanone (89 mg, 0.418 mmol). Yield 40% (44.6 mg, 0.111 mmol), white amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.10 (s, 1H), 7.76−7.84 (m, 3H), 7.63 (d, J = 8.53 Hz, 2H), 7.40−7.47 (m, 1H), 7.34 (s, 2H), 7.24−7.31 (m, 2H), 4.75 (s, 2H), 2.32 (s, 3H), 1.69 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 195.8, 174.7−174.8 (m), 155.2, 143.8, 143.0, 138.0, 134.4, 132.4, 131.9, 128.9, 126.3, 126.1, 125.9, 63.2, 46.3, 24.9, 20.7. UPLC-MS (ESI) (B): m/z 402 $[M + H]^+$ (R_t = 1.48 min).

*4-(4-Methyl-2,5-dioxo-1-(2-oxo-2-(o-tolyl)ethyl)imidazolidin-4-yl)benzonitrile (**53**).* The title compound was prepared according to General Method B from 4-(4-methyl-2,5-dioxoimidazolidin-4-yl)-benzonitrile **37** (70 mg, 0.325 mmol) and 2-bromo-1-(*o*-tolyl)-ethan-1-one (76 mg, 0,358 mmol). Yield 81% (91 mg, 0.262 mmol), white amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.20 (s, 1H), 7.92 (d, J = 8.53 Hz, 2H), 7.88 (d, J = 7.28 Hz, 1H), 7.72 (d, J = 8.53 Hz, 2H), 7.46−7.55 (m, 1H), 7.30−7.39 (m, 2H), 4.82 (s, 2H), 2.38 (s, 3H), 1.75 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 195.8, 174.4, 155.1, 144.6, 138.0, 134.4, 132.6, 132.3, 131.9, 128.9, 126.7, 126.0, 118.5, 111.0, 63.3, 46.3, 25.0, 20.6. UPLC-MS (ESI) (B) m/z 346 $[M - H]^-$ (R_t = 1.67 min).

*4-(4-Methyl-2,5-dioxo-1-(2-oxo-2-(m-tolyl)ethyl)imidazolidin-4-yl)benzenesulfonamide (**54**).* The title compound was prepared according to General Method B from 4-(4-methyl-2,5-dioxoimidazolidin-4-yl)benzenesulfonamide **23** (60 mg, 0.223 mmol) and 2-bromo-1-(*m*-tolyl)ethanone (52.2 mg, 0.245 mmol). Yield 44% (39 mg, 0.097 mmol), white amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.21 (s, 1H), 7.81−7.90 (m, 4H), 7.75 (d, J = 8.53 Hz, 2H), 7.50−7.56 (m, 1H), 7.39−7.49 (m, 3H), 4.96 (s, 2H), 2.38 (s, 3H), 1.79 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 192.3, 174.9, 155.2, 143.8, 143.0, 138.5, 134.9, 134.0, 128.9, 128.6, 126.4, 126.0, 125.4, 63.3, 44.7, 24.9, 20.9. UPLC-MS (ESI) (B): m/z 402 $[M + H]^+$ (R_t = 1.54 min).

*4-(4-Methyl-2,5-dioxo-1-(2-oxo-2-(m-tolyl)ethyl)imidazolidin-4-yl)benzonitrile (**55**).* The title compound was prepared according to General Method B from 4-(4-methyl-2,5-dioxoimidazolidin-4-yl)-benzonitrile **37** (70 mg, 0.325 mmol) and 2-bromo-1-(*m*-tolyl)-ethanone (76 mg, 0.358 mmol). Yield 76% (86 mg, 0.248 mmol), white amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.23 (s, 1H), 7.94 (d, J = 8.28 Hz, 2H), 7.80−7.86 (m, 2H), 7.77 (d, J = 8.53 Hz, 2H), 7.50−7.55 (m, 1H), 7.40−7.49 (m, 1H), 4.96 (s, 2H), 2.38 (s, 3H), 1.79 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ192.2, 174.6, 155.1, 144.6, 138.5, 134.9, 134.0, 132.6, 128.9, 128.5, 126.8, 125.4, 118.5, 111.1, 63.3, 44.7, 25.0, 20.8. UPLC-MS (ESI) (B) m/z 346 $[M - H]^-$ (R_t = 1.67 min).

*4-(4-Methyl-2,5-dioxo-1-(2-oxo-2-(p-tolyl)ethyl)imidazolidin-4-yl)benzenesulfonamide (**56**).* The title compound was prepared according to General Method B from 4-(4-methyl-2,5-dioxoimidazolidin-4-yl)benzenesulfonamide **23** (50 mg, 0.186 mmol) and 2-bromo-1-(*p*-tolyl)ethanone (59.3 mg, 0.279 mmol). Yield 38% (28.6 mg, 0.071 mmol), off-white amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.19 (s, 1H), 7.95 (d, J = 8.28 Hz, 2H), 7.87−7.92 (m, 2H), 7.74−7.78 (m, 2H), 7.41 (s, 2H), 7.37−7.41 (m, 2H), 4.95 (s, 2H), 2.41 (s, 3H), 1.81 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 191.5, 174.8, 155.2, 144.9, 143.8, 143.0, 131.5, 129.5, 128.2, 126.3, 125.9, 63.2, 44.5, 24.9, 21.3. UPLC-MS (ESI) (B): m/z 402 $[M + H]^+$ (R_t = 1.50 min).

*4-(4-Methyl-2,5-dioxo-1-(2-oxo-2-(p-tolyl)ethyl)imidazolidin-4-yl)benzonitrile (**57**).* The title compound was prepared according to General Method B from 4-(4-methyl-2,5-dioxoimidazolidin-4-yl)-benzonitrile **37** (70 mg, 0.325 mmol) and 2-bromo-1-(4-methylphenyl)-ethanone (76 mg, 0.358 mmol). Yield 78% (88 mg, 0.253 mmol), white amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.22 (s, 1H), 7.90−7.97 (m, 4H), 7.77 (d, J = 8.53 Hz, 2H), 7.38 (d, J = 8.03 Hz, 2H), 4.95 (s, 2H), 2.40 (s, 3H), 1.79 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 191.5, 174.6, 155.2, 144.9, 144.7, 132.6, 131.5, 129.5, 128.3, 126.8, 118.5, 111.1, 63.3, 44.6, 25.0, 21.3. UPLC-MS (ESI) (B) m/z 346 $[M - H]^-$ (R_t = 1.73 min).

*4-(1-(2-(2-Methoxyphenyl)-2-oxoethyl)-4-methyl-2,5-dioxoimidazolidin-4-yl)benzenesulfonamide (**58**).* The title compound was prepared according to General Method B from 4-(4-methyl-2,5-dioxoimidazolidin-4-yl)benzenesulfonamide **23** (50 mg, 0.186 mmol) and 2-bromo-1-(2-methoxyphenyl)ethanone (63.8 mg, 0.279 mmol). Yield 13% (10.2 mg, 0.024 mmol), white amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.13 (s, 1H), 7.84−7.92 (m, 2H), 7.70−7.78 (m, 3H), 7.65 (ddd, J = 1.76, 7.15, 8.66 Hz, 1H), 7.40 (s, 2H), 7.24 (d, J = 8.28 Hz, 1H), 7.03−7.12 (m, 1H), 4.74 (s, 2H), 3.94 (s, 3H), 1.78 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 192.3, 174.7, 159.5, 155.3, 143.8, 143.1, 135.5, 130.2, 126.3, 125.9, 123.7, 120.8, 112.8, 63.1, 56.0, 48.4, 24.8. UPLC-MS (ESI) (B): m/z 418 $[M + H]^+$ (R_t = 1.46 min).

*4-(1-(2-(3-Methoxyphenyl)-2-oxoethyl)-4-methyl-2,5-dioxoimidazolidin-4-yl)benzenesulfonamide (**59**).* The title compound was prepared according to General Method B from 4-(4-methyl-2,5-dioxoimidazolidin-4-yl)benzenesulfonamide **23** (50 mg, 0.186 mmol) and 2-bromo-1-(3-methoxyphenyl)ethanone (63.8 mg, 0.279 mmol). Yield 48% (36.8 mg, 0.088 mmol), white amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.19 (s, 1H), 7.88 (d, J = 8.28 Hz, 2H), 7.75 (d, J = 8.53 Hz, 2H), 7.64 (d, J = 7.53 Hz, 1H), 7.46−7.54 (m, 2H), 7.40 (s, 2H), 7.28 (dd, J = 2.13, 8.16 Hz, 1H), 4.98 (s, 2H), 3.83 (s, 3H), 1.80 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 192.0, 174.8, 159.5, 155.2, 143.8, 143.0, 135.3, 130.2, 126.3, 125.9, 120.6, 120.4, 112.5, 63.2, 55.4, 44.8, 24.9. UPLC-MS (ESI) (B): m/z 418 $[M + H]^+$ (R_t = 1.45 min).

*4-(1-(2-(4-Methoxyphenyl)-2-oxoethyl)-4-methyl-2,5-dioxoimidazolidin-4-yl)benzenesulfonamide (**60**).* The title compound was prepared according to General Method B from 4-(4-methyl-2,5-dioxoimidazolidin-4-yl)benzenesulfonamide **23** (50 mg, 0.186 mmol) and 2-bromo-1-(4-methoxyphenyl)ethanone (63.8 mg, 0.279 mmol). Yield 32% (25.0 mg, 0.060 mmol), off-white amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.16 (s, 1H), 7.98−8.06 (m, 2H), 7.85−7.91 (m, 2H), 7.72−7.79 (m, 2H), 7.40 (br s, 2H), 7.05−7.12 (m, 2H), 4.92 (s, 2H), 3.86 (s, 3H), 1.79 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 190.2, 174.9, 163.9, 155.3, 143.8, 143.1, 130.5, 126.9, 126.3, 126.0, 114.2, 63.2, 55.7, 44.3, 24.9. UPLC-MS (ESI) (B): m/z 418 $[M + H]^+$ (R_t = 1.44 min).

*4-(4-Methyl-2,5-dioxo-1-(2-oxo-2-(2-(trifluoromethyl)phenyl)-ethyl)imidazolidin-4-yl)benzenesulfonamide (**61**).* The title compound was prepared according to General Method B from 4-(4-methyl-2,5-dioxoimidazolidin-4-yl)benzenesulfonamide **23** (50 mg, 0.186 mmol) and 2-bromo-1-(2-(trifluoromethyl)phenyl)ethanone (74.4 mg, 0.279 mmol). Yield 36% (30.4 mg, 0.067 mmol), white amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.23 (s, 1H), 7.77−7.92 (m, 6H), 7.71 (d, J = 8.53 Hz, 2H), 7.40 (s, 2H), 4.82 (s, 2H), 1.78 (s, 3H). ^{13}C NMR* (101 MHz, DMSO-d_6) δ 196.0, 174.4, 154.8, 143.8, 142.9, 135.7−135.8 (m), 132.8, 132.0, 128.4, 127.1 (q, J = 5.1 Hz), 126.2, 125.9, 123.3 (q, J = 273.6 Hz), 63.2, 46.8, 25.0. *Cq with $^2J_{CF}$ was not detected. UPLC-MS (ESI) (B): m/z 456 $[M + H]^+$ (R_t = 1.55 min).

*4-(4-Methyl-2,5-dioxo-1-(2-oxo-2-(3-(trifluoromethyl)phenyl)-ethyl)imidazolidin-4-yl)benzenesulfonamide (**62**).* The title compound was prepared according to General Method B from 4-(4-methyl-2,5-dioxoimidazolidin-4-yl)benzenesulfonamide **23** (75 mg, 0.279 mmol) and 2-bromo-1-(3-(trifluoromethyl)phenyl)ethanone (112 mg, 0.418 mmol). Yield 19% (24.3 mg, 0.053 mmol), white amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.24 (br s, 1H), 8.29−8.40 (m, 2H), 8.10 (d, J = 7.78 Hz, 1H), 7.89 (d, J = 8.53 Hz, 2H), 7.84 (t, J = 7.78 Hz, 1H), 7.76 (d, J = 8.53 Hz, 2H), 7.42 (s, 2H), 5.12 (s, 2H), 1.81 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ

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191.8, 174.8, 155.1, 143.8, 143.0, 134.7, 132.3, 130.6 (q, J = 3.7 Hz), 130.4, 129.7 (q, J = 32.3 Hz), 126.4, 126.0, 124.7 (q, J = 3.7 Hz), 123.7 (q, J = 272.9 Hz), 63.3, 44.9, 24.9. UPLC-MS (ESI) (B): m/z 454 $[M - H]^-$ (R_t = 1.55 min).

*4-(4-Methyl-2,5-dioxo-1-(2-oxo-2-(4-(trifluoromethyl)phenyl)-ethyl)imidazolidin-4-yl)benzenesulfonamide (**63**).* The title compound was prepared according to General Method B from 4-(4-methyl-2,5-dioxoimidazolidin-4-yl)benzenesulfonamide **23** (50 mg, 0.186 mmol) and 2-bromo-1-(4-(trifluoromethyl)phenyl)ethanone (74.4 mg, 0.279 mmol). Yield 54% (46 mg, 0.101 mmol), white amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.21 (br s, 1H), 8.24 (d, J = 8.08 Hz, 2H), 7.96 (d, J = 8.34 Hz, 2H), 7.85−7.92 (m, 2H), 7.71−7.79 (m, 2H), 7.40 (br s, 2H), 5.07 (s, 2H), 1.80 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 192.0, 174.7, 155.0, 143.8, 142.9, 137.1, 133.3 (q, J = 32.2 Hz), 129.1, 126.3, 125.9*, 123.6 (q, J = 273.0 Hz), 63.3, 44.9, 24.9. *Two peaks possess the identical chemical shift (proven by HSQC). UPLC-MS (ESI) (A): m/z 454 $[M - H]^-$ (R_t = 1.09 min).

*4-(1-(2-(3-Cyanophenyl)-2-oxoethyl)-4-methyl-2,5-dioxoimidazolidin-4-yl)benzenesulfonamide (**64**).* The title compound was prepared according to General Method B from 4-(4-methyl-2,5-dioxoimidazolidin-4-yl)benzenesulfonamide **23** (60 mg, 0.223 mmol) and 3-(2-bromoacetyl)benzonitrile (74.9 mg, 0.334 mmol). Yield 36% (32.6 mg, 0.079 mmol), off-white amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.24 (s, 1H), 8.55 (s, 1H), 8.31 (d, J = 8.03 Hz, 1H), 8.19 (d, J = 7.78 Hz, 1H), 7.89 (d, J = 8.53 Hz, 2H), 7.72−7.83 (m, 3H), 7.43 (s, 2H), 5.09 (s, 2H), 1.81 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 191.4, 174.8, 155.1, 143.8, 143.0, 137.4, 134.6, 132.5, 132.3, 130.3, 126.3, 126.0, 118.0, 112.2, 63.3, 44.8, 25.0. UPLC-MS (ESI) (B): m/z 411 $[M - H]^-$ (R_t = 1.28 min).

*4-(1-(2-(4-Cyanophenyl)-2-oxoethyl)-4-methyl-2,5-dioxoimidazolidin-4-yl)benzenesulfonamide (**65**).* The title compound was prepared according to General Method B from 4-(4-methyl-2,5-dioxoimidazolidin-4-yl)benzenesulfonamide **23** (50 mg, 0.186 mmol) and 4-(2-bromoacetyl)benzonitrile (62.4 mg, 0.279 mmol). Yield 16% (12.3 mg, 0.030 mmol), off-white amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.21 (br s, 1H), 8.19 (d, J = 8.59 Hz, 2H), 8.07 (d, J = 8.59 Hz, 2H), 7.85−7.93 (m, 2H), 7.70−7.80 (m, 2H), 7.40 (br s, 2H), 5.07 (s, 2H), 1.80 (s, 3H).^{13}C NMR (101 MHz, DMSO-d_6) δ 192.0, 174.7, 155.0, 143.8, 142.9, 137.0, 133.0, 128.8, 126.3, 125.9, 118.0, 116.1, 63.3, 44.9, 24.9. UPLC-MS (ESI) (A): m/z 411 $[M - H]^-$ (R_t = 0.97 min).

*4-(4-Methyl-1-(2-(3-nitrophenyl)-2-oxoethyl)-2,5-dioxoimidazolidin-4-yl)benzenesulfonamide (**66**).* The title compound was prepared according to General Method B from 4-(4-methyl-2,5-dioxoimidazolidin-4-yl)benzenesulfonamide **23** (60 mg, 0.223 mmol) and 3-nitrophenacyl bromide (82 mg, 0.334 mmol). Yield 48% (46.2 mg, 0.107 mmol), off-white amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.24 (s, 1H), 8.71 (s, 1H), 8.53 (dd, J = 1.51, 8.28 Hz, 1H), 8.47 (d, J = 7.78 Hz, 1H), 7.84−7.92 (m, 3H), 7.75 (d, J = 8.53 Hz, 2H), 7.42 (s, 2H), 5.14 (s, 2H), 1.80 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 191.4, 174.7, 155.1, 148.1, 143.8, 143.0, 135.1, 134.5, 130.8, 128.4, 126.3, 126.0, 122.7, 63.3, 44.9, 24.9. UPLC-MS (ESI) (B): m/z 431 $[M - H]^-$ (R_t = 1.38 min).

*4-(4-Methyl-2,5-dioxo-1-(2-oxo-2-(3-(trifluoromethoxy)phenyl)-ethyl)imidazolidin-4-yl)benzenesulfonamide (**67**).* The title compound was prepared according to General Method B from 4-(4-methyl-2,5-dioxoimidazolidin-4-yl)benzenesulfonamide **23** (60 mg, 0.223 mmol) and 2-bromo-1-(3-(trifluoromethoxy)phenyl)ethanone (95 mg, 0.334 mmol). Yield 24% (25.6 mg, 0.054 mmol), white amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.24 (s, 1H), 8.07−8.16 (m, 1H), 7.96 (s, 1H), 7.90 (d, J = 8.28 Hz, 2H), 7.72−7.80 (m, 4H), 7.43 (s, 2H), 5.07 (s, 2H), 1.81 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 191.5, 174.8, 155.1, 148.6, 143.8, 143.0, 135.9, 131.4, 127.5, 126.7, 126.3, 126.0, 120.4, 120.0 (q, J = 257.6 Hz), 63.3, 44.9, 24.9. UPLC-MS (ESI) (B): m/z 472 $[M + H]^+$ (R_t = 1.55 min).

*4-(4-Methyl-2,5-dioxo-1-(2-oxo-2-(3-(trifluoromethoxy)phenyl)-ethyl)imidazolidin-4-yl)benzonitrile (**68**).* The title compound was prepared according to General Method B from 4-(4-methyl-2,5-dioxoimidazolidin-4-yl)benzonitrile **37** (70 mg, 0.325 mmol) and 2-bromo-1-(3-(trifluoromethoxy)phenyl)ethanone (101 mg, 0.358 mmol). Yield 71% (96 mg, 0.230 mmol), white amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.25 (s, 1H), 8.05−8.15 (m, 1H), 7.90−7.98 (m, 3H), 7.70−7.79 (m, 4H), 5.06 (s, 2H), 1.79 (s, 3H).^{13}C NMR (101 MHz, DMSO-d_6) 191.5, 174.5, 155.0, 148.6 (d, J = 1.47 Hz), 144.6, 135.9, 132.6, 131.3, 127.5, 126.7, 120.4, 118.5, 120.0 (q, J = 256.80 Hz), 111.1, 63.3, 44.9, 25.0. UPLC-MS (ESI) (B) m/z 416 $[M - H]^-$ (R_t = 1.78 min).

*4-(1-(2-(4-Fluoro-3-(trifluoromethyl)phenyl)-2-oxoethyl)-4-methyl-2,5-dioxoimidazolidin-4-yl)benzenesulfonamide (**69**).* The title compound was prepared according to General Method B from 4-(4-methyl-2,5-dioxoimidazolidin-4-yl)benzenesulfonamide **23** (60 mg, 0.223 mmol) and 2-bromo-1-(4-fluoro-3-(trifluoromethyl)phenyl)-ethanone (95 mg, 0.334 mmol). Yield 80% (84.8 mg, 0.179 mmol), white amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.22 (s, 1H), 8.41−8.46 (m, 1H), 8.38 (d, J = 6.78 Hz, 1H), 7.89 (d, J = 8.53 Hz, 2H), 7.71−7.79 (m, 3H), 7.42 (s, 2H), 5.12 (s, 2H), 1.80 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 190.6, 174.8, 162.1 (dd, J = 261.9, 1.5 Hz), 155.1, 143.8, 143.0, 135.8 (d, J = 10.3 Hz), 130.9 (d, J = 2.9 Hz), 127.8−128.1 (m), 126.3, 126.0, 118.2 (d, J = 21.2 Hz), 122.1 (q, J = 272.2 Hz), 117.3 (qd, J = 33.0, 13.2 Hz), 63.3, 44.8, 24.9. UPLC-MS (ESI) (B): m/z 472 $[M - H]^-$ (R_t = 1.62 min).

*4-(1-(2-(4-Fluoro-3-(trifluoromethyl)phenyl)-2-oxoethyl)-4-methyl-2,5-dioxoimidazolidin-4-yl)benzonitrile (**70**).* The title compound was prepared according to General Method B from 4-(4-methyl-2,5-dioxoimidazolidin-4-yl)benzonitrile **37** (70 mg, 0.325 mmol) and 4-fluoro-3-(trifluoromethyl)phenacylbromide (102 mg, 0,358 mmol). Yield 63% (86 mg, 0.205 mmol), white amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.26 (s, 1H), 8.39−8.46 (m, 1H), 8.36 (d, J = 6.78 Hz, 1H), 7.94 (d, J = 8.53 Hz, 2H), 7.70−7.80 (m, 3H), 5.12 (s, 2H), 1.79 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 190.6, 174.5, 162.0 (d, J = 261.91 Hz), 155.0, 144.6, 135.8 (d, J = 11.00 Hz), 132.6, 130.8 (d, J = 3.67 Hz), 127.9, 126.7, 118.5, 118.2 (d, J = 20.54 Hz), 122.1 (q, J = 272.20 Hz), 117.3 (ddd, J = 13.21, 33.01, 66.03 Hz), 111.1, 63.3, 44.8, 25.0. UPLC-MS (ESI) (B) m/z 418 $[M - H]^-$ (R_t = 1.78 min).

*4-(1-(2-(3,5-Bis(trifluoromethyl)phenyl)-2-oxoethyl)-4-methyl-2,5-dioxoimidazolidin-4-yl)benzenesulfonamide (**71**).* The title compound was prepared according to General Method B from 4-(4-methyl-2,5-dioxoimidazolidin-4-yl)benzenesulfonamide **23** (60 mg, 0.223 mmol) and 1-(3,5-bis(trifluoromethyl)phenyl)-2-bromoethanone (112 mg, 0.334 mmol). Yield 38% (44.6 mg, 0.085 mmol), off-white amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.26 (s, 1H), 8.63 (s, 2H), 8.50 (s, 1H), 7.90 (d, J = 8.53 Hz, 2H), 7.76 (d, J = 8.53 Hz, 2H), 7.43 (s, 2H), 5.25 (s, 2H), 1.81 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 191.2, 174.7, 155.0, 143.9, 142.9, 135.9, 131.0 (q, J = 33.7 Hz), 128.8−129.2 (m), 127.2−127.5 (m), 126.3, 126.0, 122.9 (q, J = 272.9 Hz), 63.3, 45.1, 24.9. UPLC-MS (ESI) (B): m/z 522 $[M - H]^-$ (R_t = 1.68 min).

*4-(4-Methyl-2,5-dioxo-1-(2-oxopropyl)imidazolidin-4-yl)-benzenesulfonamide (**72**).* The title compound was prepared according to General Method B from 4-(4-methyl-2,5-dioxoimidazolidin-4-yl)benzenesulfonamide **23** (75 mg, 0.279 mmol) and chloroacetone (0.033 mL, 0.418 mmol). Yield 38% (34 mg, 0.105 mmol), white amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.10 (s, 1H), 7.85 (d, J = 8.53 Hz, 2H), 7.70 (d, J = 8.53 Hz, 2H), 7.39 (s, 2H), 4.33 (s, 2H), 2.16 (s, 3H), 1.74 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 201.1, 174.6, 155.1, 143.8, 143.0, 126.3, 125.9, 63.1, 47.2, 27.0, 24.8. UPLC-MS (ESI) (B): m/z 326 $[M + H]^+$ (R_t = 0.96 min).

*4-(1-(2-Cyclopropyl-2-oxoethyl)-4-methyl-2,5-dioxoimidazolidin-4-yl)benzenesulfonamide (**73**).* The title compound was prepared according to General Method B from 4-(4-methyl-2,5-dioxoimidazolidin-4-yl)benzenesulfonamide **23** (75 mg, 0.279 mmol) and 2-bromo-1-cyclopropylethanone (68.1 mg, 0.418 mmol). Yield 15% (14.8 mg, 0.042 mmol), white amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.12 (s, 1H), 7.86 (d, J = 8.53 Hz, 2H), 7.71 (d, J = 8.53 Hz, 2H), 7.40 (s, 2H), 4.48 (s, 2H), 2.11−2.20 (m, 1H), 1.75 (s, 3H), 0.95−1.04 (m, 2H), 0.84−0.93 (m, 2H). ^{13}C NMR (101

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MHz, DMSO-d_6) δ 203.0, 174.6, 155.1, 143.8, 143.0, 126.3, 125.9, 63.1, 47.1, 24.8, 18.0, 10.9. UPLC-MS (ESI) (B): m/z 352 $[M + H]^+$ (R_t = 1.18 min).

*4-(1-(2-Cyclohexyl-2-oxoethyl)-4-methyl-2,5-dioxoimidazolidin-4-yl)benzenesulfonamide (**74**).* The title compound was prepared according to General Method B from 4-(4-methyl-2,5-dioxoimidazolidin-4-yl)benzenesulfonamide **23** (90 mg, 0.334 mmol) and 2-bromo-1-cyclohexylethanone (103 mg, 0.501 mmol). Yield 69% (90.3 mg, 0.230 mmol), white amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.10 (s, 1H), 7.86 (d, J = 8.53 Hz, 2H), 7.71 (d, J = 8.53 Hz, 2H), 7.40 (s, 2H), 4.36 (s, 2H), 2.52−2.57 (m, 1H), 1.55−1.85 (m, 8H), 1.07−1.32 (m, 5H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 205.8, 174.6, 155.1, 143.8, 143.1, 126.3, 125.9, 63.1, 47.0, 45.2, 27.7, 25.3, 24.92, 24.86. UPLC-MS (ESI) (B): m/z 394 $[M + H]^+$ (R_t = 1.56 min).

*4-(1-(2-(Adamantan-1-yl)-2-oxoethyl)-4-methyl-2,5-dioxoimidazolidin-4-yl)benzenesulfonamide (**75**).* The title compound was prepared according to General Method B from 4-(4-methyl-2,5-dioxoimidazolidin-4-yl)benzenesulfonamide **23** (75 mg, 0.279 mmol) and 1-(adamantan-1-yl)-2-bromoethanone (107 mg, 0.418 mmol). Yield 27% (34.0 mg, 0.076 mmol), white amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.10 (br s, 1H), 7.86 (d, J = 8.53 Hz, 2H), 7.70 (d, J = 8.53 Hz, 2H), 7.39 (br s, 2H), 4.40 (s, 2H), 1.99 (br s, 3H), 1.77−1.82 (m, 6H), 1.74 (s, 3H), 1.62−1.71 (m, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 206.9, 174.6, 155.1, 143.7, 143.0, 126.3, 125.9, 63.1, 44.8, 42.6, 37.1, 35.8, 27.2, 24.9. UPLC-MS (ESI) (B): m/z 446 $[M + H]^+$ (R_t = 1.70 min).

*4-(4-Methyl-2,5-dioxo-1-(2-oxo-2-(pyridin-2-yl)ethyl)-imidazolidin-4-yl)benzenesulfonamide (**76**).* The title compound was prepared according to General Method B from 4-(4-methyl-2,5-dioxoimidazolidin-4-yl)benzenesulfonamide **23** (60 mg, 0.223 mmol) and 2-bromo-1-(pyridin-2-yl)ethanonehydrobromide (94 mg, 0.334 mmol). Yield 44% (38.2 mg, 0.098 mmol), white amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.21 (s, 1H), 8.79 (d, J = 4.27 Hz, 1H), 8.04−8.11 (m, 1H), 7.97−8.01 (m, 1H), 7.89 (d, J = 8.53 Hz, 2H), 7.74−7.79 (m, 3H), 7.42 (s, 2H), 5.06 (s, 2H), 1.81 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 193.3, 174.8, 155.2, 150.8, 149.5, 143.8, 143.0, 138.0, 128.8, 126.4, 125.9, 121.9, 63.3, 44.3, 24.9. UPLC-MS (ESI) (B): m/z 389 $[M + H]^+$ (R_t = 1.28 min).

*4-(4-Methyl-2,5-dioxo-1-(2-oxo-2-(pyridin-2-yl)ethyl)-imidazolidin-4-yl)benzonitrile (**77**).* The title compound was prepared according to General Method B from 4-(4-methyl-2,5-dioxoimidazolidin-4-yl)benzonitrile **37** (70 mg, 0.325 mmol) and 2-bromo-1-(pyridin-2-yl)ethanonehydrobromide (91 mg, 0.325 mmol). Yield 41% (44 mg, 0.132 mmol), white amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.24 (s, 1H), 8.78 (d, J = 4.27 Hz, 1H), 8.03−8.11 (m, 1H), 7.90−8.01 (m, 3H), 7.71−7.81 (m, 3H), 5.05 (s, 2H), 1.79 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 193.3, 174.6, 155.2, 150.8, 149.6, 144.7, 138.0, 132.6, 128.9, 126.8, 121.9, 118.6, 111.1, 63.3, 44.4, 25.0. UPLC-MS (ESI) (B) m/z 333 $[M - H]^-$ (R_t = 1.52 min).

*4-(4-Methyl-2,5-dioxo-1-(2-oxo-2-(pyridin-3-yl)ethyl)-imidazolidin-4-yl)benzenesulfonamide (**78**).* The title compound was prepared according to General Method B from 4-(4-methyl-2,5-dioxoimidazolidin-4-yl)benzenesulfonamide **23** (75 mg, 0.279 mmol) and 2-bromo-1-(pyridin-3-yl)ethanone hydrobromide (117 mg, 0.418 mmol). Yield 19% (20.1 mg, 0.052 mmol), white amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.18−9.28 (m, 2H), 8.87 (dd, J = 1.51, 4.77 Hz, 1H), 8.38 (td, J = 1.88, 8.03 Hz, 1H), 7.90 (d, J = 8.53 Hz, 2H), 7.76 (d, J = 8.53 Hz, 2H), 7.62 (dd, J = 4.89, 7.91 Hz, 1H), 7.43 (s, 2H), 5.08 (s, 2H), 1.81 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 192.1, 174.8, 155.1, 154.4, 149.4, 143.8, 143.0, 135.8, 129.5, 126.4, 126.0, 124.1, 63.3, 44.8, 24.9. UPLC-MS (ESI) (C): m/z 389 $[M + H]^+$ (R_t = 1.88 min).

*4-(4-Methyl-2,5-dioxo-1-(2-oxo-2-(pyridin-3-yl)ethyl)-imidazolidin-4-yl)benzonitrile (**79**).* The title compound was prepared according to General Method B from 4-(4-methyl-2,5-dioxoimidazolidin-4-yl)benzonitrile **37** (70 mg, 0.325 mmol) and 2-bromo-1-pyridin-3-ylethan-1-onehydrobromide (91 mg, 0.325 mmol). Yield 27% (29 mg, 0.087 mmol), white amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.26 (s, 1H), 9.21 (d, J = 1.76 Hz, 1H), 8.86 (dd, J = 1.51, 4.77 Hz, 1H), 8.37 (td, J = 1.79, 7.97 Hz, 1H), 7.95 (d, J = 8.28 Hz, 2H), 7.76 (d, J = 8.53 Hz, 2H), 7.61 (dd, J = 4.89, 7.91 Hz, 1H), 5.08 (s, 2H), 1.79 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 192.1, 174.5, 155.0, 154.4, 149.4, 144.6, 135.8, 132.7, 129.5, 126.8, 124.1, 118.5, 111.1, 63.3, 44.8, 25.0. UPLC-MS (ESI) (B) m/z 333 $[M - H]^-$ (R_t = 1.34 min).

*4-(4-Methyl-2,5-dioxo-1-(2-oxo-2-(pyridin-4-yl)ethyl)-imidazolidin-4-yl)benzenesulfonamide (**80**).* The title compound was prepared according to General Method B from 4-(4-methyl-2,5-dioxoimidazolidin-4-yl)benzenesulfonamide **23** (75 mg, 0.279 mmol) and 2-bromo-1-(pyridin-4-yl)ethanone hydrobromide (117 mg, 0.418 mmol). Yield 12% (13.2 mg, 0.034 mmol), off-white amorphous solid, purity ≥90%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.17 (s, 1H), 8.79 (d, J = 6.02 Hz, 2H), 7.79−7.85 (m, 4H), 7.67 (d, J = 8.53 Hz, 2H), 7.35 (s, 2H), 4.99 (s, 2H), 1.73 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 193.0, 174.7, 155.0, 151.0, 143.8, 142.9, 139.8, 126.3, 126.0, 121.2, 63.3, 44.9, 24.9. UPLC-MS (ESI) (B): m/z 387 $[M - H]^-$ (R_t = 1.11 min).

*4-(4-Methyl-2,5-dioxo-1-(2-oxo-2-(pyridin-4-yl)ethyl)-imidazolidin-4-yl)benzonitrile (**81**).* The title compound was prepared according to General Method B from 4-(4-methyl-2,5-dioxoimidazolidin-4-yl)benzonitrile **37** (70 mg, 0.325 mmol) and 4-(bromoacetyl) pyridinehydrobromide (91 mg, 0.325 mmol). Yield 7% (8 mg, 0.024 mmol), off-white amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.26 (s, 1H), 8.86 (d, J = 6.02 Hz, 2H), 7.94 (d, J = 8.28 Hz, 2H), 7.88−7.90 (m, 2H), 7.75 (d, J = 8.53 Hz, 2H), 5.06 (s, 2H), 1.78 (s, 3H). UPLC-MS (ESI) (B) m/z 333 $[M - H]^-$ (R_t = 1.34 min).

*4-(1-(2-(3,5-Difluoropyridin-2-yl)-2-oxoethyl)-4-methyl-2,5-dioxoimidazolidin-4-yl)benzenesulfonamide (**82**).* The title compound was prepared according to General Method B from 4-(4-methyl-2,5-dioxoimidazolidin-4-yl)benzenesulfonamide **23** (75 mg, 0.279 mmol) and 2-bromo-1-(3,5-difluoropyridin-2-yl)ethanone[21] (65.7 mg, 0.279 mmol). Yield 28% (33.2 mg, 0.078 mmol), off-white amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.21 (s, 1H), 8.72 (d, J = 2.26 Hz, 1H), 8.20 (ddd, J = 2.26, 9.04, 11.04 Hz, 1H), 7.89 (d, J = 8.53 Hz, 2H), 7.76 (d, J = 8.53 Hz, 2H), 7.41 (s, 2H), 4.99 (s, 2H), 1.80 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 189.7 (d, J = 5.1 Hz), 174.7, 161.3 (dd, J = 267.0, 7.3 Hz), 158.5 (dd, J = 278.0, 8.1 Hz), 155.1, 143.8, 143.0, 135.9−136.2 (m), 134.4 (dd, J = 24.2, 5.1 Hz), 126.3, 125.9, 114.6 (t, J = 22.0 Hz), 63.2, 44.9, 24.9. UPLC-MS (ESI) (B): m/z 425 $[M + H]^+$ (R_t = 1.28 min).

*4-(4-Methyl-2,5-dioxo-1-(2-oxo-2-(thiophen-2-yl)ethyl)-imidazolidin-4-yl)benzenesulfonamide (**83**).* The title compound was prepared according to General Method B from 4-(4-methyl-2,5-dioxoimidazolidin-4-yl)benzenesulfonamide **23** (75 mg, 0.279 mmol) and 2-bromo-1-(thiophen-2-yl)ethanone (86 mg, 0.418 mmol). Yield 39% (42.3 mg, 0.108 mmol), white amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.21 (s, 1H), 8.17−8.21 (m, 1H), 8.12−8.16 (m, 1H), 7.89 (d, J = 8.53 Hz, 2H), 7.75 (d, J = 8.53 Hz, 2H), 7.42 (s, 2H), 7.33 (dd, J = 4.02, 4.77 Hz, 1H), 4.94 (s, 2H), 1.80 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 185.3, 174.8, 155.1, 143.8, 143.0, 140.1, 136.2, 134.6, 129.1, 126.4, 125.9, 63.2, 44.3, 24.8. UPLC-MS (ESI) (B): m/z 392 $[M - H]^-$ (R_t = 1.36 min).

*4-(1-(2-(5-Chlorothiophen-2-yl)-2-oxoethyl)-4-methyl-2,5-dioxoimidazolidin-4-yl)benzenesulfonamide (**84**).* The title compound was prepared according to General Method B from 4-(4-methyl-2,5-dioxoimidazolidin-4-yl)benzenesulfonamide **23** (60 mg, 0.223 mmol) and 2-chloro-1-(5-chlorothiophen-2-yl)ethanone (47.8 mg, 0.245 mmol). Yield 43% (41 mg, 0.096 mmol), off-white amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.23 (s, 1H), 8.11 (d, J = 4.02 Hz, 1H), 7.88 (d, J = 8.53 Hz, 2H), 7.74 (d, J = 8.53 Hz, 2H), 7.36−7.47 (m, 3H), 4.93 (s, 2H), 1.79 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 185.0, 174.8, 155.0, 143.8, 142.9, 139.1, 138.9, 135.1, 129.4, 126.4, 126.0, 63.3, 43.9, 24.8. UPLC-MS (ESI) (B): m/z 428 $[M + H]^+$ (R_t = 1.54 min).

*4-(1-(2-(5-Chlorothiophen-2-yl)-2-oxoethyl)-4-methyl-2,5-dioxoimidazolidin-4-yl)benzonitrile (**85**).* The title compound was prepared according to General Method B from 4-(4-methyl-2,5-

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Table 10. Numbers of CFU/Lung Counted and Corresponding $\log_{10}$ CFU/Lung Determined

	log CFU per mouse (lungs)						
treatment	mouse 1	mouse 2	mouse 3	mouse 4	mouse 5	mean	SD
no treatment (day 9)	7.4	7.4	7.4	7.2	7.4	7.4	0.1
moxifloxacin (100 mg/kg, 4 d)	3.5	2.9	3.3	3.5	3.5	3.3	0.2
30 po (200 mg/kg, 4 d)	6.8	6.9				6.9	0.0
3 po (170 mg/kg, 4 d)	7.2	7.2				7.2	0.0

Table 11. Moxifloxacin and Compounds Evaluated Showing the Following Differences in the Lung Microorganism Burden ($\log_{10}$ CFU/Lung) with Respect to Untreated Controls (Day 9 after Infection) in the Aforementioned Experimental Conditions

compound	target dose (mg/kg)	administration	route	difference to untreated mice (log CFU)	p^a
moxifloxacin	100	once a day (days 5−8)	oral	4.1	$p < 0.05$
30	200	once a day (days 5−8)	oral	0.5	$p < 0.05$
3	170	once a day (days 5−8)	oral	0.2	$p > 0.05$

[a]ANOVA, Dunnett's post test. Compared to untreated mice, $p < 0.05$ was considered significant.

dioxoimidazolidin-4-yl)benzonitrile **37** (70 mg, 0.325 mmol) and 2-chloro-1-(5-chlorothiophen-2-yl)ethanone (69.8 mg, 0.358 mmol). Yield 62% (75 mg, 0.201 mmol), off-white amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.25 (s, 1H), 8.09 (d, J = 4.02 Hz, 1H), 7.94 (d, J = 8.28 Hz, 2H), 7.75 (d, J = 8.53 Hz, 2H), 7.40 (d, J = 4.02 Hz, 1H), 4.93 (s, 2H), 1.77 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 184.9, 174.5, 154.9, 144.5, 139.1, 138.9, 135.1, 132.7, 129.4, 126.8, 118.5, 111.1, 63.3, 43.9, 24.9. UPLC-MS (ESI) (B) m/z 372 $[M - H]^-$ (R_t = 1.69 min).

*4-(4-Methyl-2,5-dioxo-1-(2-oxo-2-(thiazol-2-yl)ethyl)-imidazolidin-4-yl)benzenesulfonamide (**86**).* The title compound was prepared according to General Method B from 4-(4-methyl-2,5-dioxoimidazolidin-4-yl)benzenesulfonamide **23** (60 mg, 0.223 mmol) and 2-bromo-1-(thiazol-2-yl)ethanone (0.047 mL, 0.334 mmol). Yield 24% (21.2 mg, 0.054 mmol), off-white amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.24 (s, 1H), 8.35 (d, J = 3.01 Hz, 1H), 8.24 (d, J = 3.01 Hz, 1H), 7.89 (d, J = 8.53 Hz, 2H), 7.75 (d, J = 8.53 Hz, 2H), 7.41 (s, 2H), 5.01 (s, 2H), 1.80 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 186.0, 174.6, 163.3, 154.9, 145.6, 143.8, 142.9, 129.1, 126.3, 125.9, 63.3, 44.1, 24.8. UPLC-MS (ESI) (B): m/z 395 $[M + H]^+$ (R_t = 1.15 min).

*4-(1-(2-(Benzo[d]thiazol-2-yl)-2-oxoethyl)-4-methyl-2,5-dioxoimidazolidin-4-yl)benzenesulfonamide (**87**).* The title compound was prepared according to General Method B from 4-(4-methyl-2,5-dioxoimidazolidin-4-yl)benzenesulfonamide **23** (60 mg, 0.223 mmol) and 1-(benzo[d]thiazol-2-yl)-2-bromoethanone (57.1 mg, 0.223 mmol). Yield 7%* (7.3 mg, 0.016 mmol), off-white amorphous solid, purity ≥90%. ^{1}H NMR (400 MHz, Acetone-d_6) δ 8.26 (ddd, J = 2.13, 3.83, 7.09 Hz, 2H), 8.17 (s, 1H), 7.93−7.99 (m, 2H), 7.83−7.89 (m, 2H), 7.70 (dquin, J = 1.38, 7.18 Hz, 2H), 6.67 (s, 2H), 5.15−5.28 (m, 2H), 1.94 (s, 3H). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.28 (s, 1H), 8.22−8.37 (m, 2H), 7.90 (d, J = 8.53 Hz, 2H), 7.76 (d, J = 8.53 Hz, 2H), 7.66−7.74 (m, 2H), 7.43 (s, 2H), 5.16 (s, 2H), 1.81 (s, 3H). UPLC-MS (ESI) (B): m/z 445, 446 $[M + H]^+$ (R_t = 1.59 min).

*4-(4-Methyl-1-(2-(naphthalen-2-yl)-2-oxoethyl)-2,5-dioxoimidazolidin-4-yl)benzenesulfonamide (**88**).* The title compound was prepared according to General Method B from 4-(4-methyl-2,5-dioxoimidazolidin-4-yl)benzenesulfonamide **23** (60 mg, 0.223 mmol) and 2-bromo-1-(naphthalen-2-yl)ethanone (83 mg, 0.334 mmol). Yield 63% (61 mg, 0.139 mmol), off-white amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.23 (s, 1H), 8.84 (s, 1H), 8.14 (d, J = 7.78 Hz, 1H), 7.96−8.10 (m, 3H), 7.90 (d, J = 8.53 Hz, 2H), 7.79 (d, J = 8.28 Hz, 2H), 7.64−7.76 (m, 2H), 7.43 (s, 2H), 5.15 (s, 2H), 1.83 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 192.1, 174.9, 155.3, 143.8, 143.1, 135.4, 132.0, 131.3, 130.6, 129.7, 129.2, 128.7, 127.8, 127.2, 126.4, 126.0, 123.2, 63.3, 44.7, 25.0. UPLC-MS (ESI) (B): m/z 438 $[M + H]^+$ (R_t = 1.56 min).

Strain and Growth Conditions. *M. tuberculosis* H37Rv (ATC25618) wild-type or mutant strains were grown in a Middlebrook 7H9-ADC broth (Difco) supplemented with 0.025% tyloxapol and on a 7H10-OADC or 7H11-OADC agar (Difco) at 37 °C. Isoniazid was purchased from Sigma-Aldrich. The DprE1 spontaneous mutants C387S, L368P, and G17C were kindly provided by Stewart T. Cole (Institut Pasteur, Paris, France). The strains carrying the point mutations E221Q, G248S,[24] and Y314H[11] in DprE1 were generated via oligonucleotide-mediated recombineering as previously described.[2,11,14,24,25]

MIC Determination. MIC determination assay was performed using a resazurin reduction assay with fluorescence readout as described previously.[27] Isoniazid was used as a positive control and rifampicin was used as a no-growth control.

Microsomal Fraction Stability. Microsomal fraction stability assays were performed as described previously.[27] The human biological samples were sourced ethically, and their research use was in accord with the terms of the informed consents.

DprE1 Enzymatic Inhibition. Expression and purification of Mt-DprE1 and cloning of Mt-DprE1 were performed as described by Batt et al.[24] Enzymatic data were generated using a modified version of the assay described in that report. The new protocol is in the process of being submitted for publication. DprE1 mutants were generated as previously described by Thulasi et al. in 2016.[25]

HepG2 Cytotoxicity Assay, Artificial Membrane Permeability (AMP), Kinetic Aqueous Solubility (CLND), and Hydrophobicity (chromlogD$_{pH7.4}$). These assays were performed as described previously.[27,28]

hERG Inhibition. Inhibition of the hERG potassium channel was determined using in vitro IonWorks patch-clamp electrophysiology as described in the literature.[29]

Therapeutic Efficacy. All animal studies were ethically reviewed and carried out in accordance with European Directive 2010/63/EU and the GSK Policy on the Care, Welfare and Treatment of Animals. Specific pathogen-free 8−10 week-old female C57BL/6 mice were purchased from Harlan Laboratories and were allowed to acclimate for 1 week. Mice were infected intratracheally with 100,000 CFU/mouse (*M. tuberculosis* H37Rv strain). Compounds were orally administered for 4 consecutive days, starting from day 5 after infection. Lungs were harvested on day 9, 24 h after the last compound administration. All lung lobes were aseptically removed, homogenized, and frozen. Homogenates were plated in 10% OADC-7H11 medium supplemented with activated charcoal (0.4%) to avoid product carry over and incubated for 18 days at 37 °C. No adverse clinical signs were observed in any animal. Blood samples were obtained at different time points from the infected mice to measure the levels of the tested compounds.

The number of CFU/mouse measured for each mouse and the differences in the lung microorganism burden (log10 CFUs/lungs) obtained in the treated mice with respect to untreated controls (day 9 after infection) were calculated. The CFU number in lungs of

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untreated mice is 7.4 log CFU. This value is included in the interval mean ± 2 SD of the values of the last experiments. Quality controls: In this experiment, moxifloxacin (100 mg/kg) was administered for 4 consecutive days starting from day 5 after infection as an interassay control. It reduced the bacterial lung number to 4.1 log CFU in comparison with the untreated mice (7.4 log CFU) (Tables 10 and 11). This quality control value is included in the accepted interval.

Vibrational Circular Dichroism. VCD analysis and assignment was performed according to an analogous protocol published previously.[30]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jmedchem.0c00107.

Additional experimental information for intermediate compounds (synthetic protocols and analytical details); references for synthetic procedures; LC-MS chromatograms for key compounds **30** (racemic mixture), **30R** (*R*-enantiomer), **30S** (*S*-enantiomer), **31**, **45**, **46**, **47**, **48**, **51**, **52**, **55**, **61**, **65**, **67**, **68**, **69**, and **82** (PDF)

Molecular formula strings for all reported final compounds (CSV)

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

μM, micromolar; 2-MeTHF, 2-methyltetrahydrofuran; AIDS, acquired immunodeficiency syndrome; ACN, acetonitrile; Cl_{int}, hepatic intrinsic clearance; CLND, chemiluminescent nitrogen detection; DCM, dichloromethane; DMF, dimethylformamide; DprE1, decaprenylphosphoryl-β-D-ribofuranose 2-oxidase; ESI, electrospray ionization; EtOH, ethanol; EtOAc, ethyl acetate; GSK, GlaxoSmithKline; HepG2, human hepatocellular carcinoma; hERG, human ether-a-go-go-related gene; HIV, human immunodeficiency virus; HPLC, high-performance liquid chromatography; HRMS, high-resolution mass spectrometry; Hz, hertz; IC_{50}, half maximal inhibitory concentration; MDR-TB, multidrug-resistant tuberculosis; MeCN, acetonitrile; MeOH, methanol; MHz, megahertz; MIC, minimum inhibitory concentration; *Mtb*, *Mycobacterium tuberculosis*; MW, microwave; NMR, nuclear magnetic resonance; *n*-PrOH, 1-propanol; ppm, parts per million; quin, quintet; RR-TB, rifampicin-resistant tuberculosis; SAR, structure−activity relationship; sept, septet; TB, tuberculosis; t-BuXPhos, 2-di-*tert*-butylphosphino-2′,4′,6′-triisopropylbiphenyl; THF, tetrahydrofuran; TLC, thin-layer chromatography; UPLC-MS, ultra-performance liquid chromatography−mass spectrometry; UV, ultraviolet; VCD, vibrational circular dichroism; WHO, World Health Organization.

■ REFERENCES

(1) World Health Organization. *Global tuberculosis report 2019*; World Health Organization: Geneva, 2019. Licence: CC BY-NC-SA 3.0 IGO. (accessed Nov 22, 2019)

(2) Makarov, V.; Manina, G.; Mikusova, K.; Mollmann, U.; Ryabova, O.; Saint-Joanis, B.; Dhar, N.; Pasca, M. R.; Buroni, S.; Lucarelli, A. P.; Milano, A.; De Rossi, E.; Belanova, M.; Bobovska, A.; Dianiskova, P.; Kordulakova, J.; Sala, C.; Fullam, E.; Schneider, P.; McKinney, J. D.; Brodin, P.; Christophe, T.; Waddell, S.; Butcher, P.; Albrethsen, J.; Rosenkrands, I.; Brosch, R.; Nandi, V.; Bharath, S.; Gaonkar, S.; Shandil, R. K.; Balasubramanian, V.; Balganesh, T.; Tyagi, S.; Grosset,

J.; Riccardi, G.; Cole, S. T. Benzothiazinones Kill Mycobacterium Tuberculosis by Blocking Arabinan Synthesis. *Science* **2009**, *324*, 801.
(3) Brecik, M.; Centárová, I.; Mukherjee, R.; Kolly, G. S.; Huszár, S.; Bobovská, A.; Kilacsková, E.; Mokošová, V.; Svetlíková, Z.; Šarkan, M.; Neres, J.; Korduláková, J.; Cole, S. T.; Mikušová, K. DprE1 Is a Vulnerable Tuberculosis Drug Target Due to Its Cell Wall Localization. *ACS Chem. Biol.* **2015**, *10*, 1631−1636.
(4) Trefzer, C.; Škovierová, H.; Buroni, S.; Bobovská, A.; Nenci, S.; Molteni, E.; Pojer, F.; Pasca, M. R.; Makarov, V.; Cole, S. T.; Riccardi, G.; Mikušová, K.; Johnsson, K. Benzothiazinones Are Suicide Inhibitors of Mycobacterial Decaprenylphosphoryl-β-d-Ribofuranose 2′-Oxidase DprE1. *J. Am. Chem. Soc.* **2012**, *134*, 912−915.
(5) Batt, S. M.; Jabeen, T.; Bhowruth, V.; Quill, L.; Lund, P. A.; Eggeling, L.; Alderwick, L. J.; Fütterer, K.; Besra, G. S. Structural Basis of Inhibition of Mycobacterium Tuberculosis DprE1 by Benzothiazinone Inhibitors. *Proc. Natl. Acad. Sci.* **2012**, *109*, 11354−11359.
(6) Tiwari, R.; Moraski, G. C.; Krchňák, V.; Miller, P. A.; Colon-Martinez, M.; Herrero, E.; Oliver, A. G.; Miller, M. J. Thiolates Chemically Induce Redox Activation of BTZ043 and Related Potent Nitroaromatic Anti-Tuberculosis Agents. *J. Am. Chem. Soc.* **2013**, *135*, 3539−3549.
(7) Neres, J.; Pojer, F.; Molteni, E.; Chiarelli, L. R.; Dhar, N.; Boy-Röttger, S.; Buroni, S.; Fullam, E.; Degiacomi, G.; Lucarelli, A. P.; Read, R. J.; Zanoni, G.; Edmondson, D. E.; De Rossi, E.; Pascal, M. R.; McKinney, J. D.; Dyson, P. J.; Riccardi, G.; Mattevi, A.; Cole, S. T.; Bindal, C. Structural Basis for Benzothiazinone-Mediated Killing of Mycobacterium Tuberculosis. *Sci. Transl. Med.* **2012**, *4*, 150ra121.
(8) Wang, F.; Sambandan, D.; Halder, R.; Wang, J.; Batt, S. M.; Weinrick, B.; Ahmad, I.; Yang, P.; Zhang, Y.; Kim, J.; Hassani, M.; Huszar, S.; Trefzer, C.; Ma, Z.; Kaneko, T.; Mdluli, K. E.; Franzblau, S.; Chatterjee, A. K.; Johnsson, K.; Johnson, K.; Mikusova, K.; Besra, G. S.; Fütterer, K.; Robbins, S. H.; Barnes, S. W.; Walker, J. R.; Jacobs, W. R., Jr.; Schultz, P. G. Identification of a Small Molecule with Activity against Drug-Resistant and Persistent Tuberculosis. *Proc. Natl. Acad. Sci. U. S. A.* **2013**, *110*, E2510−E2517.
(9) Oh, S.; Park, Y.; Engelhart, C. A.; Wallach, J. B.; Schnappinger, D.; Arora, K.; Manikkam, M.; Gac, B.; Wang, H.; Murgolo, N.; Olsen, D. B.; Goodwin, M.; Sutphin, M.; Weiner, D. M.; Via, L. E.; Boshoff, H. I. M.; Barry, C. E., III Discovery and Structure−Activity-Relationship Study of *N* -Alkyl-5-Hydroxypyrimidinone Carboxamides as Novel Antitubercular Agents Targeting Decaprenylphosphoryl-β- d -Ribose 2′-Oxidase. *J. Med. Chem.* **2018**, *61*, 9952−9965.
(10) Shirude, P. S.; Shandil, R.; Sadler, C.; Naik, M.; Hosagrahara, V.; Hameed, S.; Shinde, V.; Bathula, C.; Humnabadkar, V.; Kumar, N.; Reddy, J.; Panduga, V.; Sharma, S.; Ambady, A.; Hegde, N.; Whiteaker, J.; McLaughlin, R. E.; Gardner, H.; Madhavapeddi, P.; Ramachandran, V.; Kaur, P.; Narayan, A.; Guptha, S.; Awasthy, D.; Narayan, C.; Mahadevaswamy, J.; Vishwas, K.; Ahuja, V.; Srivastava, A.; Prabhakar, K.; Bharath, S.; Kale, R.; Ramaiah, M.; Choudhury, N. R.; Sambandamurthy, V. K.; Solapure, S.; Iyer, P. S.; Narayanan, S.; Chatterji, M. Azaindoles: Noncovalent DprE1 Inhibitors from Scaffold Morphing Efforts, Kill Mycobacterium Tuberculosis and Are Efficacious in Vivo. *J. Med. Chem.* **2013**, *56*, 9701−9708.
(11) Shirude, P. S.; Shandil, R. K.; Manjunatha, M. R.; Sadler, C.; Panda, M.; Panduga, V.; Reddy, J.; Saralaya, R.; Nanduri, R.; Ambady, A.; Ravishankar, S.; Sambandamurthy, V. K.; Humnabadkar, V.; Jena, L. K.; Suresh, R. S.; Srivastava, A.; Prabhakar, K. R.; Whiteaker, J.; McLaughlin, R. E.; Sharma, S.; Cooper, C. B.; Mdluli, K.; Butler, S.; Iyer, P. S.; Narayanan, S.; Chatterji, M. Lead Optimization of 1,4-Azaindoles as Antimycobacterial Agents. *J. Med. Chem.* **2014**, *57*, 5728−5737.
(12) Naik, M.; Humnabadkar, V.; Tantry, S. J.; Panda, M.; Narayan, A.; Guptha, S.; Panduga, V.; Manjrekar, P.; Jena, L. K.; Koushik, K.; Shanbhag, G.; Jatheendranath, S.; Manjunatha, M. R.; Gorai, G.; Bathula, C.; Rudrapatna, S.; Achar, V.; Sharma, S.; Ambady, A.; Hegde, N.; Mahadevaswamy, J.; Kaur, P.; Sambandamurthy, V. K.; Awasthy, D.; Narayan, C.; Ravishankar, S.; Madhavapeddi, P.; Reddy, J.; Prabhakar, K.; Saralaya, R.; Chatterji, M.; Whiteaker, J.; McLaughlin, B.; Chiarelli, L. R.; Riccardi, G.; Pasca, M. R.; Binda, C.; Neres, J.; Dhar, N.; Signorino-Gelo, F.; McKinney, J. D.; Ramachandran, V.; Shandil, R.; Tommasi, R.; Iyer, P. S.; Narayanan, S.; Hosagrahara, V.; Kavanagh, S.; Dinesh, N.; Ghorpade, S. R. 4-Aminoquinolone Piperidine Amides: Noncovalent Inhibitors of DprE1 with Long Residence Time and Potent Antimycobacterial Activity. *J. Med. Chem.* **2014**, *57*, 5419−5434.
(13) Panda, M.; Ramachandran, S.; Ramachandran, V.; Shirude, P. S.; Humnabadkar, V.; Nagalapur, K.; Sharma, S.; Kaur, P.; Guptha, S.; Narayan, A.; Mahadevaswamy, J.; Ambady, A.; Hegde, N.; Rudrapatna, S. S.; Hosagrahara, V. P.; Sambandamurthy, V. K.; Raichurkar, A. Discovery of Pyrazolopyridones as a Novel Class of Noncovalent DprE1 Inhibitor with Potent Anti-Mycobacterial Activity. *J. Med. Chem.* **2014**, *57*, 4761−4771.
(14) Neres, J.; Hartkoorn, R. C.; Chiarelli, L. R.; Gadupudi, R.; Pasca, M. R.; Mori, G.; Venturelli, A.; Savina, S.; Makarov, V.; Kolly, G. S.; Molteni, E.; Binda, C.; Dhar, N.; Ferrari, S.; Brodin, P.; Delorme, V.; Landry, V.; de Jesus Lopes Ribeiro, A. L.; Farina, D.; Saxena, P.; Pojer, F.; Carta, A.; Luciani, R.; Porta, A.; Zanoni, G.; De Rossi, E.; Costi, M. P.; Riccardi, G.; Cole, S. T. 2-Carboxyquinoxalines Kill *Mycobacterium Tuberculosis* through Noncovalent Inhibition of DprE1. *ACS Chem. Biol.* **2015**, *10*, 705−714.
(15) Landge, S.; Mullick, A. B.; Nagalapur, K.; Neres, J.; Subbulakshmi, V.; Murugan, K.; Ghosh, A.; Sadler, C.; Fellows, M. D.; Humnabadkar, V.; Mahadevaswamy, J.; Vachaspati, P.; Sharma, S.; Kaur, P.; Mallya, M.; Rudrapatna, S.; Awasthy, D.; Sambandamurthy, V. K.; Pojer, F.; Cole, S. T.; Balganesh, T. S.; Ugarkar, B. G.; Balasubramanian, V.; Bandodkar, B. S.; Panda, M.; Ramachandran, V. Discovery of Benzothiazoles as Antimycobacterial Agents: Synthesis, Structure−Activity Relationships and Binding Studies with Mycobacterium Tuberculosis Decaprenylphosphoryl-β-d-Ribose 2′-Oxidase. *Bioorg. Med. Chem.* **2015**, *23*, 7694−7710.
(16) Chikhale, R.; Menghani, S.; Babu, R.; Bansode, R.; Bhargavi, G.; Karodia, N.; Rajasekharan, M. V.; Paradkar, A.; Khedekar, P. Development of Selective DprE1 Inhibitors: Design, Synthesis, Crystal Structure and Antitubercular Activity of Benzothiazolylpyrimidine-5-Carboxamides. *Eur. J. Med. Chem.* **2015**, *96*, 30−46.
(17) Liu, R.; Lyu, X.; Batt, S. M.; Hsu, M.-H.; Harbut, M. B.; Vilchèze, C.; Cheng, B.; Ajayi, K.; Yang, B.; Yang, Y.; Guo, H.; Lin, C.; Gan, F.; Wang, C.; Franzblau, S. G.; Jacobs, W. R., Jr.; Besra, G. S.; Johnson, E. F.; Petrassi, M.; Chatterjee, A. K.; Fütterer, K.; Wang, F. Determinants of the Inhibition of DprE1 and CYP2C9 by Antitubercular Thiophenes. *Angew. Chem., Int. Ed.* **2017**, *56*, 13011−13015.
(18) Chikhale, R. V.; Barmade, M. A.; Murumkar, P. R.; Yadav, M. R. Overview of the Development of DprE1 Inhibitors for Combating the Menace of Tuberculosis. *J. Med. Chem.* **2018**, *61*, 8563−8593.
(19) Lupien, A.; Vocat, A.; Foo, C. S.-Y.; Blattes, E.; Gillon, J.-Y.; Makarov, V.; Cole, S. T. Optimized Background Regimen for Treatment of Active Tuberculosis with the Next-Generation Benzothiazinone Macozinone (PBTZ169). *Antimicrob. Agents Chemother.* **2018**, *62*, No. e00840.
(20) Vjecha, M. J.; Tiberi, S.; Zumla, A. Accelerating the Development of Therapeutic Strategies for Drug-Resistant Tuberculosis. *Nat. Rev. Drug Discovery* **2018**, *17*, 607−608.
(21) Rogacki, M. K.; Pitta, E.; Balabon, O.; Huss, S.; Lopez-Roman, E. M.; Argyrou, A.; Blanco-Ruano, D.; Cacho, M.; Vande Velde, C. M. L.; Augustyns, K.; Ballell, L.; Barros, D.; Bates, R. H.; Cunningham, F.; Van der Veken, P. Identification and Profiling of Hydantoins—A Novel Class of Potent Antimycobacterial DprE1 Inhibitors. *J. Med. Chem.* **2018**, *61*, 11221−11249.
(22) Bucherer, H. T.; Lieb, V. A. Über Die Bildung Substituierter Hydantoine Aus Aldehyden Und Ketonen. Synthese von Hydantoinen. *J. Prakt. Chem.* **1934**, *141*, 5−43.
(23) Rosen, B. R.; Ruble, J. C.; Beauchamp, T. J.; Navarro, A. Mild Pd-Catalyzed N -Arylation of Methanesulfonamide and Related Nucleophiles: Avoiding Potentially Genotoxic Reagents and Byproducts. *Org. Lett.* **2011**, *13*, 2564−2567.
(24) Batt, S. M.; Cacho Izquierdo, M.; Castro Pichel, J.; Stubbs, C. J.; Vela-Glez Del Peral, L.; Pérez-Herrán, E.; Dhar, N.; Mouzon, B.;

https://dx.doi.org/10.1021/acs.jmedchem.0c00107
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Rees, M.; Hutchinson, J. P.; Young, R. J.; McKinney, J. D.; Barros Aguirre, D.; Ballell, L.; Besra, G. S.; Argyrou, A. Whole Cell Target Engagement Identifies Novel Inhibitors of Mycobacterium Tuberculosis Decaprenylphosphoryl-β- d -Ribose Oxidase. *ACS Infect. Dis.* **2016**, *1*, 615−626.

(25) Warrier, T.; Kapilashrami, K.; Argyrou, A.; Ioerger, T. R.; Little, D.; Murphy, K. C.; Nandakumar, M.; Park, S.; Gold, B.; Mi, J.; Zhang, T.; Meiler, E.; Rees, M.; Somersan-Karakaya, S.; Porras-De Francisco, E.; Martinez-Hoyos, M.; Burns-Huang, K.; Roberts, J.; Ling, Y.; Rhee, K. Y.; Mendoza-Losana, A.; Luo, M.; Nathan, C. F. N-Methylation of a Bactericidal Compound as a Resistance Mechanism in Mycobacterium Tuberculosis. *Proc. Natl. Acad. Sci. U. S. A.* **2016**, *113*, E4523−E4530.

(26) Rullas, J.; Dhar, N.; McKinney, J. D.; García-Pérez, A.; Lelievre, J.; Diacon, A. H.; Hugonnet, J.-E.; Arthur, M.; Angulo-Barturen, I.; Barros-Aguirre, D.; Ballell, L. Combinations of β-Lactam Antibiotics Currently in Clinical Trials Are Efficacious in a DHP-I-Deficient Mouse Model of Tuberculosis Infection. *Antimicrob. Agents Chemother.* **2015**, *59*, 4997−4999.

(27) Pitta, E.; Rogacki, M. K.; Balabon, O.; Huss, S.; Cunningham, F.; Lopez-Roman, E. M.; Joossens, J.; Augustyns, K.; Ballell, L.; Bates, R. H.; Van der Veken, P. Searching for New Leads for Tuberculosis: Design, Synthesis, and Biological Evaluation of Novel 2-Quinolin-4-Yloxyacetamides. *J. Med. Chem.* **2016**, *59*, 6709−6728.

(28) Ballell, L.; Bates, R. H.; Young, R. J.; Alvarez-Gomez, D.; Alvarez-Ruiz, E.; Barroso, V.; Blanco, D.; Crespo, B.; Escribano, J.; González, R.; Lozano, S.; Huss, S.; Santos-Villarejo, A.; Martín-Plaza, J. J.; Mendoza, A.; Rebollo-Lopez, M. J.; Remuiñan-Blanco, M.; Lavandera, J. L.; Pérez-Herran, E.; Gamo-Benito, F. J.; García-Bustos, J. F.; Barros, D.; Castro, J. P.; Cammack, N. Fueling Open-Source Drug Discovery: 177 Small-Molecule Leads against Tuberculosis. *ChemMedChem* **2013**, *8*, 313−321.

(29) Gillie, D. J.; Novick, S. J.; Donovan, B. T.; Payne, L. A.; Townsend, C. Development of a High-Throughput Electrophysiological Assay for the Human Ether-à-Go-Go Related Potassium Channel HERG. *J. Pharmacol. Toxicol. Methods* **2013**, *67*, 33−44.

(30) Remuiñán, M. J.; Pérez-Herrán, E.; Rullás, J.; Alemparte, C.; Martínez-Hoyos, M.; Dow, D. J.; Afari, J.; Mehta, N.; Esquivias, J.; Jiménez, E.; Ortega-Muro, F.; Fraile-Gabaldón, M. T.; Spivey, V. L.; Loman, N. J.; Pallen, M. J.; Constantinidou, C.; Minick, D. J.; Cacho, M.; Rebollo-López, M. J.; González, C.; Sousa, V.; Angulo-Barturen, I.; Mendoza-Losana, A.; Barros, D.; Besra, G. S.; Ballell, L.; Cammack, N. Tetrahydropyrazolo[1,5-a]Pyrimidine-3-Carboxamide and N-Benzyl-6′,7′-Dihydrospiro[Piperidine-4,4′-Thieno[3,2-c]Pyran] Analogues with Bactericidal Efficacy against Mycobacterium Tuberculosis Targeting MmpL3. *PLoS One* **2013**, *8*, No. e60933.

https://dx.doi.org/10.1021/acs.jmedchem.0c00107
J. Med. Chem. 2020, 63, 5367−5386