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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 *M. tuberculosis*. The compounds were found to be non-covalent 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 sub-micromolar 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 non-covalent 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 six 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 being multidrug-resistant tuberculosis (MDR-TB) with resistance to at least rifampicin and isoniazid. 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. The initial recognition was brought by the benzothiazinone series (BTZ), a DprE1 covalent inhibitor class. 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 non-covalent binding, validating DprE1 as an attractive antimycobacterial target. All relevant DprE1-inhibitor literature to date is summarized in a recently-published comprehensive review. Benzothiazinones BTZ043, azaindole AZ7371, and PBTZ-169/macozinone are the most advanced DprE1 inhibitors that have recently entered the clinical development phase (Figure 1).
Inspired by the encouraging antimycobacterial properties of described compounds, GSK performed a target-based high-throughput screening (HTS) campaign in search of novel DprE1 inhibitors (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 reported the biological profiling and initial optimization efforts on hit 1. 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, Table 1).
Figure 2. Most potent representatives (1-4) published previously by our team.\textsuperscript{21} The previous findings indicate that both the hydantoin core and the acetyl linker are crucial for the potency of the series.

Table 1. \textit{In vitro} activity, cytotoxicity, and physicochemical properties of selected representatives 1-4 from our previous report.\textsuperscript{21}

<table>
<thead>
<tr>
<th>№</th>
<th>R</th>
<th>DprE1 pIC$_{50}$\textsuperscript{[a]}</th>
<th>\textit{Mtb} MIC (µM)\textsuperscript{[b]}</th>
<th>HepG2 IC$_{50}$ (µM)\textsuperscript{[c]}</th>
<th>Solubility (µM)\textsuperscript{[d]}</th>
<th>Chrom logD\textsuperscript{[e]}</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>NC</td>
<td>7.0</td>
<td>8.3</td>
<td>&gt; 100</td>
<td>202</td>
<td>4.54</td>
</tr>
<tr>
<td>2</td>
<td>O</td>
<td>7.0</td>
<td>2.5</td>
<td>&gt; 100</td>
<td>≥ 487</td>
<td>3.57</td>
</tr>
<tr>
<td>3</td>
<td>N=N</td>
<td>7.3</td>
<td>3.1</td>
<td>&gt; 100</td>
<td>379</td>
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</tr>
<tr>
<td>4</td>
<td>F$_3$HCO</td>
<td>7.4</td>
<td>10</td>
<td>&gt; 100</td>
<td>85</td>
<td>5.63</td>
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</tbody>
</table>

\textsuperscript{[a]}Inhibition of \textit{DprE1} enzyme; \textsuperscript{[b]}MIC against \textit{Mycobacterium tuberculosis} (H37Rv), reference: Isoniazid, MIC = 1.8 µM; \textsuperscript{[c]}Cytotoxicity against HepG2 human caucasian hepatocyte carcinoma; \textsuperscript{[d]}Kinetic aqueous solubility (CLND); \textsuperscript{[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.\textsuperscript{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.\textsuperscript{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, including a fused bicyclic analogue of the benzene ring 14 (Scheme 1-2). 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\textsuperscript{22}, followed by an alkylation as shown in Scheme 1. The ketones were either available commercially or prepared based on standard literature procedures (see Supporting Information). The selective alkylation on the N3-nitrogen was previously confirmed by full NMR assignment and crystal structure analysis.\textsuperscript{21}
Scheme 1. General synthetic approach toward analogues with a modified *para*-substitution pattern on ring A<sup>a</sup>

Reagents and conditions: (a) KCN, (NH<sub>4</sub>)<sub>2</sub>CO<sub>3</sub>, EtOH-H<sub>2</sub>O, microwave irradiation 70 °C or heating 55 °C, 7-17 hrs; (b) K<sub>2</sub>CO<sub>3</sub>, DMF or acetone, r.t., 24-48 hrs. *The main isolated reaction product was the hydantoin sodium salt 8a, used for the alkylation.*

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 17-18, the substituents on the 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 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.
Scheme 2. Synthetic approach toward analogues 16-18 with a modified \textit{para}-substitution pattern on ring A$^a$

\begin{align*}
\text{5} & \xrightarrow{a} \text{15} & \xrightarrow{b} \text{16} \\
\text{10} & \xrightarrow{c} \text{17} & \\
\text{1} & \xrightarrow{d} \text{18}
\end{align*}

$^a$Reagents and conditions: (a) NH$_4$OH, 90°C, overnight; (b) K$_2$CO$_3$, DMF or acetone, r.t., 24 hrs; (c) LiOH, THF, H$_2$O, rt, 1h; (d) NaN$_3$, ZnCl$_2$, n-PrOH, 95°C, 24h

Inspired by methylsulfonamide 2’s overall activity profile (Table 1), we decided to prepare a small sub-series of compounds, covering analogues that are linked to ring A via either the \textit{N}- or \textit{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 \textit{N}-linked analogues. In essence, undesired alkylation of the acidic sulfonamide nitrogen was expected to occur during the final synthetic step (\textit{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).$^{23}$
Scheme 3. Synthetic approach to analogues with \( N \)-linked sulfonamide substituents on ring A

\[
\begin{align*}
\text{Br} & \quad \text{O} \quad \text{N} \quad \text{N} \quad \text{O} \\
19 & \quad \text{a,} \quad \text{O} \quad \text{S} \quad \text{R} \quad \text{NH}_2 \\
\text{R} & \quad \text{S} \quad \text{O} \\
20-22 & \\
\text{R} & = \\
20 & \quad \text{CF}_3 \\
21 & \quad \text{Ph} \\
22 & \quad n \text{-Bu}
\end{align*}
\]

\(^a\)Reagents and conditions: (a) \([\text{PdCl(allyl)}]_2, \text{t-BuXPhos, K}_2\text{CO}_3, 2\text{-MeTHF}, 80 ^\circ\text{C}, 2 \text{hrs.}\)

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.

Scheme 4. Synthetic approach to analogues with \( S \)-linked sulfonamide substituents on ring A

\[
\begin{align*}
\text{Cl} & \quad \text{S} \quad \text{O} \\
21 & \quad \text{a, b} \\
\text{23-29} & \quad \text{c,} \quad \text{X} \quad \text{F} \\
\text{30-36} & \\
\text{X} & = \text{Cl, Br}
\end{align*}
\]

\(^a\)Reagents and conditions: (a) amine \( \text{HCl, Et}_3\text{N or amine with no base, DCM, r.t., 1 hr;} \) (b) \( \text{KCN, (NH}_4)_2\text{CO}_3, \text{EtOH-H}_2\text{O, 70 }^\circ\text{C (MW or heating), 7-17 hrs;} \) (c) \( \text{K}_2\text{CO}_3, \text{acetone, r.t., 24-48 hrs.} \)
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, 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.

Table 2. *In vitro* activity, cytotoxicity, and physicochemical properties of the compounds with varying substituents at the 4-position of ring A.

<table>
<thead>
<tr>
<th>№</th>
<th>R</th>
<th>DprE1 pIC$_{50}$[a]</th>
<th>$Mtb$ MIC (µM)[b]</th>
<th>HepG2 IC$_{50}$ (µM)[c]</th>
<th>Solubility (µM)[d]</th>
<th>Chrom logD[e]</th>
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<tr>
<td>10</td>
<td></td>
<td>7.1</td>
<td>11.2</td>
<td>&gt; 100</td>
<td>140</td>
<td>4.84</td>
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<tr>
<td>11</td>
<td></td>
<td>5.3</td>
<td>&gt; 80</td>
<td>&gt; 100</td>
<td>≥ 296</td>
<td>2.71</td>
</tr>
<tr>
<td>12</td>
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<td>6.2</td>
<td>&gt; 40</td>
<td>&gt; 100</td>
<td>≥ 440</td>
<td>3.54</td>
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<tr>
<td>13</td>
<td></td>
<td>5.2</td>
<td>80</td>
<td>&gt; 100</td>
<td>≥ 486</td>
<td>3.36</td>
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<tr>
<td>14</td>
<td></td>
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<td>10</td>
<td>&gt; 100</td>
<td>≥ 369</td>
<td>3.38</td>
</tr>
<tr>
<td>16</td>
<td></td>
<td>5.2</td>
<td>&gt; 80</td>
<td>&gt; 100</td>
<td>≥ 454[f]</td>
<td>2.68</td>
</tr>
<tr>
<td></td>
<td>Chemical Structure</td>
<td>DprE1 Inhibition</td>
<td>MIC (µM)</td>
<td>Cytotoxicity</td>
<td>Lipophilicity (logD)</td>
<td></td>
</tr>
<tr>
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<td>----------</td>
<td>--------------</td>
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<td></td>
</tr>
<tr>
<td>17</td>
<td><img src="image1.png" alt="Chemical Structure" /></td>
<td>4.1</td>
<td>&gt; 80</td>
<td>&gt; 100</td>
<td>≥ 282</td>
<td>1.49</td>
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<tr>
<td>18</td>
<td><img src="image2.png" alt="Chemical Structure" /></td>
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<td>&gt; 80</td>
<td>&gt; 100</td>
<td>≥ 511[1]</td>
<td>1.85</td>
</tr>
<tr>
<td>20</td>
<td><img src="image3.png" alt="Chemical Structure" /></td>
<td>5.0</td>
<td>&gt; 80</td>
<td>&gt; 100</td>
<td>≥ 372</td>
<td>3.24</td>
</tr>
<tr>
<td>21</td>
<td><img src="image4.png" alt="Chemical Structure" /></td>
<td>6.4</td>
<td>80</td>
<td>100</td>
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<tr>
<td>22</td>
<td><img src="image5.png" alt="Chemical Structure" /></td>
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<td>&gt; 80</td>
<td>100</td>
<td>154</td>
<td>4.96</td>
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<td>30</td>
<td><img src="image6.png" alt="Chemical Structure" /></td>
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<td>0.7</td>
<td>&gt; 100</td>
<td>≥ 486</td>
<td>3.19</td>
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<tr>
<td>31</td>
<td><img src="image7.png" alt="Chemical Structure" /></td>
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<td>20</td>
<td>&gt; 100</td>
<td>≥ 478</td>
<td>3.88</td>
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<td>32</td>
<td><img src="image8.png" alt="Chemical Structure" /></td>
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<td>80</td>
<td>&gt; 100</td>
<td>224</td>
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<td>80</td>
<td>&gt; 100</td>
<td>334</td>
<td>4.48</td>
</tr>
<tr>
<td>34</td>
<td><img src="image10.png" alt="Chemical Structure" /></td>
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<td>&gt; 80</td>
<td>63.1</td>
<td>57</td>
<td>4.78</td>
</tr>
<tr>
<td>35</td>
<td><img src="image11.png" alt="Chemical Structure" /></td>
<td>4.7</td>
<td>&gt; 80</td>
<td>79.4</td>
<td>55</td>
<td>5.03</td>
</tr>
<tr>
<td>36</td>
<td><img src="image12.png" alt="Chemical Structure" /></td>
<td>4.9</td>
<td>&gt; 40</td>
<td>&gt; 100</td>
<td>217</td>
<td>4.39</td>
</tr>
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</table>

1. Inhibition of DprE1 enzyme; 2. MIC against *Mycobacterium tuberculosis* (H37Rv), reference: Isoniazid, MIC = 1.8 µM; 3. Cytotoxicity against HepG2 human caucasian hepatocyte carcinoma; 4. Kinetic aqueous solubility (CLND); 5. Lipophilicity - chromlogD at pH = 7.4; 6. Solubility determination in 5% DMSO pH 7.4 phosphate buffer saline and quantification of DMSO stock concentration by Charged Aerosol Detector (CAD).

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 for cellular and DprE1 inhibitory potency. Taken together, it is likely that steric constraints in DprE1’s active site 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 sub-micromolar DprE1 inhibitor encountered in the hydantoin family, the aminosulfonyl 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.
Scheme 5. Synthetic scheme of the synthesis of analogues with variable substitution on ring B

Reagents and conditions: (a) K$_2$CO$_3$, acetone or DMF, r.t., 24-48 hrs. *Compound 50 was formed by hydrolysis of 49 in LiOH solution.

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 (87-88) 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 5-6 were commercially available or prepared according to literature procedures (see Supporting Information).

<table>
<thead>
<tr>
<th>R'</th>
<th>R</th>
<th>R'</th>
<th>R</th>
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<tbody>
<tr>
<td>-H</td>
<td>-CN</td>
<td>49</td>
<td>-CN</td>
</tr>
<tr>
<td>-H</td>
<td>-CN</td>
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<td>-CN</td>
</tr>
<tr>
<td>2-F</td>
<td>-SO$_2$NH$_2$</td>
<td>51</td>
<td>-SO$_2$NH$_2$</td>
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<tr>
<td>3-F</td>
<td>-SO$_2$NH$_2$</td>
<td>52</td>
<td>-SO$_2$NH$_2$</td>
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<tr>
<td>3-F</td>
<td>-CN</td>
<td>53</td>
<td>-CN</td>
</tr>
<tr>
<td>3-F</td>
<td>-SO$_2$NH$_2$</td>
<td>54</td>
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<tr>
<td>4-F</td>
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<td>-SO$_2$NH$_2$</td>
</tr>
<tr>
<td>3,4-diF</td>
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<td>56</td>
<td>-SO$_2$NH$_2$</td>
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<tr>
<td>3,4-diF</td>
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</tr>
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<td>2,4-diCl</td>
<td>-SO$_2$NH$_2$</td>
<td>60</td>
<td>-SO$_2$NH$_2$</td>
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*Reagents and conditions: (a) K$_2$CO$_3$, acetone or DMF, r.t., 24-48 hrs. *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

![Scheme 6](image)

\[ R = \text{-SO}_2 \text{NH}_2 \]
\[ R' = \text{Br or Cl} \]

Reagents and conditions: (a) K$_2$CO$_3$, acetone or DMF, r.t., 24-48 hrs.

Table 3. *In vitro* activity, cytotoxicity, and physicochemical properties of the analogues with ring B substitution modifications.

<table>
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<tr>
<th>No</th>
<th>R</th>
<th>R'</th>
<th>DprE1 pIC$_{50}$[a]</th>
<th><em>Mtb</em> MIC (µM)[b]</th>
<th>HepG2 IC$_{50}$ (µM)[c]</th>
<th>Solubility (µM)[d]</th>
<th>Chrom logD[e]</th>
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<tr>
<td>38</td>
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<td>H</td>
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<td>-CN</td>
<td>3-F</td>
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<td>80</td>
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<td>-SO$_2$NH$_2$</td>
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<tr>
<td>47</td>
<td>-SO₂NH₂</td>
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<td>&gt; 100</td>
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<td>&gt; 100</td>
<td>≥ 320&lt;sup&gt;[f]&lt;/sup&gt;</td>
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<td>&gt; 100</td>
<td>351</td>
<td>3.33</td>
</tr>
<tr>
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<td>-CN</td>
<td>4-Me</td>
<td>5.8</td>
<td>&gt; 80</td>
<td>&gt; 100</td>
<td>246&lt;sup&gt;[f]&lt;/sup&gt;</td>
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<td>≥ 473</td>
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<td>≥ 417</td>
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<td>4-OMe</td>
<td>6.5</td>
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<td>≥ 392</td>
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<td>2-CF₃</td>
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<td>&gt; 100</td>
<td>≥ 381</td>
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<td>3-CF₃</td>
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<td>&gt; 100</td>
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<td>4-CF₃</td>
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<td>3-CN</td>
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<td>≥ 482</td>
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<td>4-CN</td>
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<td>≥ 411</td>
<td>2.54</td>
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<td>-SO₂NH₂</td>
<td>3-NO₂</td>
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<td>20</td>
<td>&gt; 100</td>
<td>132</td>
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<td>67</td>
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<td>3-OCF₃</td>
<td>7.1</td>
<td>0.6</td>
<td>&gt; 100</td>
<td>≥ 438</td>
<td>4.14</td>
</tr>
<tr>
<td>68</td>
<td>-CN</td>
<td>3-OCF₃</td>
<td>6.2</td>
<td>40</td>
<td>69.4</td>
<td>127&lt;sup&gt;[f]&lt;/sup&gt;</td>
<td>5.50</td>
</tr>
<tr>
<td>69</td>
<td>-SO₂NH₂</td>
<td>3-CF₃, 4-F</td>
<td>7.2</td>
<td>0.6</td>
<td>&gt; 100</td>
<td>≥ 287</td>
<td>4.24</td>
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<tr>
<td>70</td>
<td>-CN</td>
<td>3-CF₃, 4-F</td>
<td>6.4</td>
<td>20</td>
<td>76.7</td>
<td>121</td>
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<tr>
<td>71</td>
<td>-SO₂NH₂</td>
<td>3,5-diCF₃</td>
<td>5.1</td>
<td>&gt; 80</td>
<td>74.6</td>
<td>95</td>
<td>4.90</td>
</tr>
</tbody>
</table>

<sup>a</sup>Inhibition of DprE1 enzyme (DprE1 assay data was generated using a modified version of the assay described, paper under preparation)<sup>18</sup>; <sup>b</sup>MIC against <i>Mycobacterium tuberculosis</i> (H37Rv),
reference: Isoniazid, MIC= 1.8 µM; ¹Cytotoxicity against HepG2 human caucasian hepatocyte carcinoma; ²Kinetic aqueous solubility (CLND); ³Lipophilicity - chromlogD at pH = 7.4; ⁴Solubility determination in 5% DMSO pH7.4 phosphate buffer saline and quantification of DMSO stock concentration by Charged Aerosol Detector (CAD); ⁵Only partial inhibition was reached; ⁶N.D. - not determined.

As Table 3 demonstrates, 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 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 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 a 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-CF₃-substituted compound 61 showed a MIC value of 2.5 µM. Notably, the most potent representatives were the 3-substituted analogues 62 (3-CF₃) and 67 (3-OCF₃), which retained sub-micromolar 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-CF₃, 4-F) in products 69-70, while two CF₃-groups were simultaneously introduced in 71. Sulfonamide 69 (3-CF₃, 4-F) retained sub-micromolar whole-cell potency (MIC = 0.6 µM) and enzymatic activity (pIC₅₀ = 7.2 vs. 7.1) compared to its closest analogue 62 (3-CF₃), 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 (IC₅₀ > 100 µM).

**Table 4.** *In vitro* activity, cytotoxicity, and physicochemical properties of the analogues with saturated or heterocyclic moieties instead of ring B.

<table>
<thead>
<tr>
<th>№</th>
<th>R</th>
<th>DprE1 pIC₅₀[a]</th>
<th>Mtb MIC (µM)[b]</th>
<th>HepG2 IC₅₀ (µM)[c]</th>
<th>Solubility (µM)[d]</th>
<th>Chrom logD[e]</th>
</tr>
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<td>72</td>
<td>-SO₂NH₂</td>
<td>4.0</td>
<td>&gt; 80</td>
<td>&gt; 100</td>
<td>≥ 504</td>
<td>0.54</td>
</tr>
<tr>
<td>73</td>
<td>-SO₂NH₂</td>
<td>4.5</td>
<td>&gt; 80</td>
<td>&gt; 100</td>
<td>58</td>
<td>1.51</td>
</tr>
<tr>
<td>74</td>
<td>-SO₂NH₂</td>
<td>6.7</td>
<td>7.5</td>
<td>&gt; 100</td>
<td>≥ 317</td>
<td>3.62</td>
</tr>
<tr>
<td>75</td>
<td>-SO₂NH₂</td>
<td>5.9</td>
<td>&gt; 80</td>
<td>&gt; 100</td>
<td>≥ 404</td>
<td>4.87</td>
</tr>
<tr>
<td>76</td>
<td>-SO₂NH₂</td>
<td>6.3</td>
<td>10</td>
<td>&gt; 100</td>
<td>≥ 405</td>
<td>2.03</td>
</tr>
<tr>
<td>77</td>
<td>-CN</td>
<td>5.5</td>
<td>&gt; 80</td>
<td>&gt; 100</td>
<td>≥ 292[f]</td>
<td>3.48</td>
</tr>
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</tr>
<tr>
<td>78</td>
<td>-SO₂NH₂</td>
<td><img src="https://via.placeholder.com/150" alt="Image" /></td>
<td>5.5</td>
<td>&gt; 80</td>
<td>&gt; 100</td>
<td>≥ 298</td>
</tr>
<tr>
<td>79</td>
<td>-CN</td>
<td><img src="https://via.placeholder.com/150" alt="Image" /></td>
<td>4.6</td>
<td>&gt;80</td>
<td>&gt; 100</td>
<td>≥ 334[^f]</td>
</tr>
<tr>
<td>80</td>
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<td>80</td>
<td>&gt; 100</td>
<td>≥ 439</td>
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<td>&gt;80</td>
<td>&gt; 100</td>
<td>≥ 310[^f]</td>
</tr>
<tr>
<td>82</td>
<td>-SO₂NH₂</td>
<td><img src="https://via.placeholder.com/150" alt="Image" /></td>
<td>7.1</td>
<td>0.6</td>
<td>&gt; 100</td>
<td>≥ 441</td>
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<tr>
<td>83</td>
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<td>10</td>
<td>&gt; 100</td>
<td>≥ 311</td>
</tr>
<tr>
<td>84</td>
<td>-SO₂NH₂</td>
<td><img src="https://via.placeholder.com/150" alt="Image" /></td>
<td>7.0</td>
<td>2.5</td>
<td>&gt; 100</td>
<td>≥ 437[^f]</td>
</tr>
<tr>
<td>85</td>
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<td>&gt; 100</td>
<td>184[^f]</td>
</tr>
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<td>86</td>
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<td>≥ 453</td>
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<tr>
<td>87</td>
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<td>6.7</td>
<td>40[^g]</td>
<td>&gt; 97</td>
<td>≥ 339[^f]</td>
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<tr>
<td>88</td>
<td>-SO₂NH₂</td>
<td><img src="https://via.placeholder.com/150" alt="Image" /></td>
<td>6.3</td>
<td>10</td>
<td>&gt; 100</td>
<td>167</td>
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</table>

[^a]: Inhibition of DprE1 enzyme;  
[^b]: MIC against *Mycobacterium 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 pH7.4 phosphate buffer saline and quantification of DMSO stock concentration by Charged Aerosol Detector (CAD);  
[^g]: Only partial inhibition was reached.

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 (pIC₅₀ = 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 pIC₅₀ values 6.7 and 6.6, and MIC values 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, sub-micromolar 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 (pIC₅₀ = 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 86-87 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 analogues, even those with increased lipophilicity, no considerable cytotoxicity was detected among the reported modifications (HepG2 IC₅₀ > 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 (pIC₅₀ = 4.4-5.3). To our satisfaction, all tested sulfonamide derivatives showed no significant hERG inhibition (pIC₅₀ < 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.

**Table 5.** hERG inhibition of selected potent analogues.

<table>
<thead>
<tr>
<th>No</th>
<th>Structure</th>
<th>hERG pIC&lt;sub&gt;50&lt;/sub&gt;</th>
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<td>&lt; 4.3</td>
</tr>
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<td><img src="structure51.png" alt="" /></td>
<td>&lt; 4.3</td>
</tr>
<tr>
<td>62</td>
<td><img src="structure62.png" alt="" /></td>
<td>&lt; 4.3</td>
</tr>
<tr>
<td>67</td>
<td><img src="structure67.png" alt="" /></td>
<td>&lt; 4.3</td>
</tr>
<tr>
<td>74</td>
<td><img src="structure74.png" alt="" /></td>
<td>&lt; 4.3</td>
</tr>
<tr>
<td>82</td>
<td><img src="structure82.png" alt="" /></td>
<td>&lt; 4.3</td>
</tr>
<tr>
<td>84</td>
<td><img src="structure84.png" alt="" /></td>
<td>&lt; 4.3</td>
</tr>
</tbody>
</table>

**Enantiomeric separation.** The enantiomers of the new reference 30 were separated by chiral HPLC, 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.\(^{21}\)
Table 6. In vitro activity, cytotoxicity, and physicochemical properties of 30 and its enantiomers.

<table>
<thead>
<tr>
<th>№</th>
<th>Structure</th>
<th>DprE1 pIC$_{50}^{[a]}$</th>
<th>Mtb MIC (µM)$^{[b]}$</th>
<th>HepG2 IC$_{50}$ (µM)$^{[c]}$</th>
<th>Solubility (µM)$^{[d]}$</th>
<th>Chrom logD$^{[e]}$</th>
</tr>
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<td>&gt; 100</td>
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<td>3.19</td>
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<td>7.5</td>
<td>0.78</td>
<td>&gt; 100</td>
<td>≥ 344</td>
<td>3.17</td>
</tr>
<tr>
<td>30S</td>
<td><img src="image" alt="Structure" /></td>
<td>5.1</td>
<td>&gt; 80</td>
<td>&gt; 100</td>
<td>≥ 379</td>
<td>3.17</td>
</tr>
</tbody>
</table>

$^{[a]}$Inhibition of DprE1 enzyme; $^{[b]}$MIC against Mycobacterium tuberculosis (H37Rv); $^{[c]}$cytotoxicity against HepG2 human caucasian hepatocyte carcinoma; $^{[d]}$kinetic aqueous solubility (CLND); $^{[e]}$lipophilicity - chromlogD at pH = 7.4.

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.

Table 7. Microsomal stability of compound 30.

<table>
<thead>
<tr>
<th>№</th>
<th>Structure</th>
<th>Mouse Cl$_{int}$ (ml/min/g)</th>
<th>Human Cl$_{int}$ (ml/min/g)</th>
</tr>
</thead>
<tbody>
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<td>2.67</td>
<td>&lt; 0.40</td>
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</tbody>
</table>

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 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 non-covalent 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 6-9 Å distance and are closer to the protein’s outer surface.\(^{17}\)

**Table 8.** MIC of the primary hit 1 against a panel of *M. tuberculosis* DprE1 resistant mutants.

<table>
<thead>
<tr>
<th>Compd</th>
<th>MIC(<em>{mutant}/\text{MIC}</em>{H37Rv}) Ratio(^{[a]})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E221Q</td>
</tr>
<tr>
<td>1</td>
<td>16</td>
</tr>
<tr>
<td>TCA1(^{[b]})</td>
<td>24</td>
</tr>
<tr>
<td>AZ DprE1(^{[b],[c]})</td>
<td>16</td>
</tr>
<tr>
<td>BTZ043(^{[b]})</td>
<td>4</td>
</tr>
</tbody>
</table>

\(^{[a]}\)A mutant strain is considered resistant if the MIC\(_{mutant}/\text{MIC}_{H37Rv}\) 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.

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 a C57BL/6J mouse model of acute intratracheal infection with *Mtb. H37Rv.* Compounds were administered once daily *per os* for four consecutive days, starting five days after the infection. Moxifloxacin (100 mg/kg) was used as a positive inter-assay 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 hours after the last compound administration. The blood levels measured for both compounds and the lung microorganism burden differences (log<sub>10</sub>CFUs/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.

**Table 9.** Blood exposure levels and log<sub>10</sub>CFU reduction for compounds 30 and 3.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose (mg/kg)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Blood Levels</th>
<th>Reduction of log&lt;sub&gt;10&lt;/sub&gt;CFUs/lung, (relative to untreated controls)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Cmax (ng/mL)</td>
<td>AUC (h*ng/mL)</td>
</tr>
<tr>
<td>Moxifloxacin</td>
<td>100</td>
<td>4.1 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>200</td>
<td>6380</td>
<td>31400&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>170</td>
<td>1870</td>
<td>6378&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Compounds were dosed *per os*, once daily; <sup>b</sup>Compound 30’s blood levels were found to remain above its MIC-value for 24 hrs after single oral administration; <sup>c</sup>Compound 3’s blood levels were found to be below its MIC-value within 6 hrs after single oral administration.
Compound 30 demonstrated the best blood exposure with a Cmax value of 6380 ng/mL and an AUC value of 31400 h*ng/mL. Moreover, the same compound showed the highest reduction of Log_{10}CFUs (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 six 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.

**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 posttest.
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 \textit{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. \textit{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 focussing on the improvement of \textit{in vivo} efficacy response, is required before preclinical development for this class of compounds can be considered successful.

EXPERIMENTAL SECTION

\textbf{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/UV$_{254}$ 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 ($\delta$) values are reported in parts per million (ppm), and coupling constants are expressed in Hertz (Hz). The chemical shifts $\delta$ 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) $\delta$ 2.50 ppm (quin, C$_2$D$_5$HOS) for DMSO-$d_6$, $\delta$ 2.05 ppm (quin, C$_3$D$_5$HO) for Acetone-$d_6$, $\delta$ 3.31 ppm (quin, CD$_3$HOD) for Methanol-$d_4$; in $^{13}$C NMR (101 MHz) $\delta$ 39.51 ppm (sept) for DMSO-$d_6$, $\delta$ 29.84 ppm (sept), $\delta$ 206.26 ppm (s) for Acetone-$d_6$, $\delta$ 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, the flow was 0.8 mL/min. Solvent A consisted of aqueous ammonium acetate 25mM 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 nm to 400 nm. In methods B and C, ESI mass spectra were obtained with an Esquire 3000plus ion trap mass spectrometer (Bruker Daltonics), using the direct infusion mode. A Waters acquity H-class UPLC
system coupled to a water TQD ESI mass spectrometer and a Waters TUV detector were used with a Waters acquity UPLC BEH C18 1.7µm 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 during 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 during 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 systems equipped with an internal variable dual-wavelength diode array detector (200-400nm). For normal phase purifications Biotage SNAP (10-340g), Sylicicle SiliaSep (4-120g) or Götec-Labortechnik EasyVarioFlash (5-100g) cartridges were used (flow rates 10-100mL/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 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 reverse-phase.

The preparative HPLC purification was conducted on the Agilent 1200 or Agilent 1100 instrument, employing either on an X-Bridge C18 column (19 mm x 150 mm, i.d 5 µm packing diameter or 30
mm x 150 mm, i.d. 5 µm packing diameter) or a SunFire C\textsubscript{18} column (19 mm x 150 mm or 30 mm x 250 mm) at 35°C. The solvents employed were: $A = 10$ mM ammonium bicarbonate in water; $B = \text{acetonitrile}$ (“basic” method) or $A = 0.1$ M formic acid in water; $B = 0.1$ M formic acid in acetonitrile (“acidic” method) respectively. The purification was run as a gradient (A:B) typically from 40 to 100% over either 20 min 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 nm 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%), 87 (>90%). These molecules displayed low potency in the DprE1- and MIC-assays, further purification was therefore not carried out. All products were obtained as amorphous solids, and melting points were not measured. 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)-1H-pyrrolo[3,2-b]pyridine-3-carboxamide was synthesized by the procedure described previously in Shirude et al.\textsuperscript{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\(^\text{22}\) was employed. The suitable ketone (1.5.0-4.0 mmol, 1.0 eq.), ammonium carbonate \((\text{NH}_4)_2\text{CO}_3\) (9.0 eq.) and potassium cyanide KCN (1.3 eq.) were dissolved in a mixture of ethanol and water (1:1) (reaction molarity \(\sim\)0.25-0.4 mol/l). The reaction mixture was heated at 55°C for 18-48 hr or irradiated in microwave oven at 70°C for 3-9 hours. After the reaction was complete, the reaction mixture was cooled down to room temperature and neutralized with 6M hydrochloric acid to pH~7-8. 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 \(\text{Na}_2\text{SO}_4\), 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 eq.) and potassium carbonate \(\text{K}_2\text{CO}_3\) (1.1-2.0 eq.) was dissolved in DMF or acetone. After 10-15 min, the corresponding alkyl halide was added in a slight excess (1.1-1.5 eq.) (reaction molarity \(\sim\)0.08-0.2 mol/l). The reaction mixture was stirred at room temperature for 20-72 hr. 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 1M NaOH, brine, dried over \(\text{Na}_2\text{SO}_4\), filtered and evaporated under vacuum. The residue was purified by normal phase flash chromatography on silicagel (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 eq.), appropriate sulfonamide (1.2-2.0 eq.), 2-di-tert-butylphosphino-2′,4′,6′-triisopropylbiphenyl (t-BuXPhos) (0.04 eq.), allylpalladium(II) chloride dimer ([Pd(allyl)Cl]₂) (0.01 eq.) and potassium carbonate (2.0 eq.) were suspended in dry 2-methyltetrahydrofuran (2-MeTHF) (6 mL) and placed in the vial, which was evacuated and backfilled with nitrogen 3 times. The vial was capped under nitrogen flow and stirred heated at 80 °C for 7-48 hrs. Then the reaction mixture was cooled to room temperature, 1M hydrochloric acid solution (20 mL) was added. Subsequently, the aqueous layer was extracted with ethyl acetate (3x20 mL). The combined organic layers were filtered through a small celite column, rinsed with ethyl acetate, dried over Na₂SO₄, filtrated 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%. ¹H NMR (400 MHz, DMSO-d₆) 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). 13C NMR (101 MHz, DMSO-d6) 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]+ (Rt = 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 eq.). Yield 22% (28.8 mg, 0.072 mmol), off-white amorphous solid. 1H NMR (400 MHz, DMSO-d6) 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). 13C NMR (101 MHz, DMSO-d6) 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]+ (Rt = 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-$d_6$) $\delta$ 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-$d_6$) $\delta$ 189.1 (d, $J = 4.39$ Hz), 175.0, 165.6 (dd, $J = 255.4$, 12.5 Hz), 162.4 (dd, $J = 13.17$, 258.3 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, $J = 3.40$, 22.2 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-$d_6$) $\delta$ 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-$d_6$) $\delta$ 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-2H-benzo[b][1,4]oxazin-6-yl)imidazolidine-2,4-dione 9 (100 mg, 0.383 mmol) and 2-chloro-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-$d_6$) $\delta$ 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-$d_6$) $\delta$ 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$_2$N$_3$O$_5$ [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, approx. 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-$d_6$) $\delta$ 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-$d_6$) $\delta$ 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 1h. The mixture was acidified with 1M aqueous HCl and extracted with AcOEt 3x. Combined organic phases were dried with sodium sulfate and evaporated. The residue was purified via flash column chromatography on 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-\(d_6\)) \(\delta\) 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) \(m/\ell\) 387 [M-H]\(^-\) (R\(_t\) = 1.50min).

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 24h. 5% NaOH was added. Solids were filtered and washed with 5% NaOH. The filtrate was acidified with 1M HCl and extracted with ethyl acetate (3x). Combined organic phases were dried with sodium sulfate, evaporated and purified via flash column chromatography on 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-\(d_6\)) \(\delta\) 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) \(m/\ell\) 413 [M+H]\(^+\) (R\(_t\) = 1.49min).

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 eq.). 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]$^-$ (Rt = 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 eq.). 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]$^+$ (Rt = 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 eq.). Yield 34% (55 mg, 0.097 mmol), off-white amorphous solid. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 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-$d_6$) $\delta$ 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-$d_6$) $\delta$ 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-$d_6$) $\delta$ 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-$d_6$) $\delta$ 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-$d_6$) $\delta$ 189.0 (d, $J$=4.4 Hz), 174.6, 165.7 (dd, $J$=255.4, 13.3 Hz), 162.5 (dd, $J$=257.6,
$\delta$ 9.18 (br. s., 1H), 8.00 (dt, $J$=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).

$\delta$ 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$_2$N$_3$O$_5$S [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-$d_6$) $\delta$ 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-$d_6$) $\delta$ ppm 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$_2$N$_3$O$_5$S [M+H]$^+$: 460.0749; found: 460.0758.
DMSO-$d_6$ $\delta$ 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, $^1J_{CF}=4.4$ 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 $\geq 90\%$. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 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-$d_6$) $\delta$ 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 $\geq 90\%$. $^1$H NMR$^*$ (400 MHz, DMSO-$d_6$) $\delta$ 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,
$11.56 \text{ Hz, 1H}$, $7.29 \text{ (dt, } J=2.40, 8.40 \text{ Hz, 1H}), 4.82 \text{ (d, } J=2.53 \text{ Hz, 2H}), 3.73 \text{ (q, } J=9.60 \text{ Hz, 2H)}, 1.79 \text{ (s, 3H).}^* \text{Alk position is proven by HSQC and HMBC NMR.}^{13} \text{C NMR (101 MHz, DMSO-d$_6$)} ~\delta\ 189.0 \text{ (d, } J=5.2 \text{ Hz)}, 174.5, 165.7 \text{ (dd, } J=255.4, 13.2 \text{ Hz)}, 162.5 \text{ (dd, } J=257.6, 13.2 \text{ Hz)}, 155.0, 143.9, 140.5, 132.6 \text{ (dd, } J=11.0, 3.7 \text{ Hz)}, 126.6^{**}, 124.3 \text{ (q, } J=278.1 \text{ Hz)}, 119.4 \text{ (dd, } J=13.2, 3.7 \text{ Hz)}, 112.8 \text{ (dd, } J=22.0, 3.2 \text{ Hz)}, 105.4 \text{ (t, } J=26.8 \text{ Hz)}, 63.2, 47.3 \text{ (d, } J=10.2 \text{ Hz)}, 43.3 \text{ (q, } J=34.8 \text{ Hz)}, 25.0. ^{**}\text{Two peaks possess the identical chemical shift (proven by HSQC).} \text{ UPLC-MS (ESI) (A): } m/z \ 504 \ [M-H]^- \ (R_t = 1.14 \text{ min}).$

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. $^1\text{H NMR (400 MHz, DMSO-d$_6$)} \delta\ 10.38 \text{ (s, 1H)}, 9.11 \text{ (s, 1H)}, 7.99 \text{ (dt, } J=6.57, 8.59 \text{ Hz, 1H}), 7.80-7.87 \text{ (m, 2H)}, 7.72 \text{ (d, } J=8.59 \text{ Hz, 2H}), 7.49 \text{ (ddd, } J=2.40, 9.22, 11.62 \text{ Hz, 1H}), 7.20-7.32 \text{ (m, 3H)}, 7.09-7.15 \text{ (m, 2H)}, 6.98-7.06 \text{ (m, 1H)}, 4.79 \text{ (d, } J=2.53 \text{ Hz, 2H)}, 1.75 \text{ (s, 3H).}^{13} \text{C NMR (101 MHz, DMSO-d$_6$)} \delta\ 189.0 \text{ (d, } J=4.4 \text{ Hz)}, 174.4, 165.7 \text{ (dd, } J=255.3, 12.6 \text{ Hz)}, 162.4 \text{ (dd, } J=257.6, 13.2 \text{ Hz)}, 154.9, 143.9, 139.4, 137.6, 132.6 \text{ (dd, } J=11.1, 3.9 \text{ Hz)}, 129.2, 126.9, 126.7, 124.0, 119.7, 119.4 \text{ (dd, } J=13.9, 3.0 \text{ Hz)}, 112.8 \text{ (dd, } J=21.9, 2.9 \text{ Hz)}, 105.4 \text{ (t, } J=27.1 \text{ Hz)}, 63.2, 47.3 \text{ (d, } J=11.7 \text{ Hz)}, 24.8. \text{ UPLC-MS (ESI) (A): } m/z \ 498 \ [M-H]^- \ (R_t = 1.21 \text{ min}).$

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% (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-$d_6$) $\delta$ 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-$d_6$) $\delta$ 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-$d_6$) $\delta$ 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-$d_6$) $\delta$ 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-$d_6$) $\delta$ 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-$d_6$) $\delta$ 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-$d_6$) $\delta$ 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-$d_6$) $\delta$ 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-$d_6$) $\delta$ 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-$d_6$) $\delta$ 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).
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]⁻ (Rt = 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. ¹H NMR (400 MHz, DMSO-d₆) 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). ¹³C NMR (101 MHz, DMSO-d₆) 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]⁺ (Rt = 1.14 min). HRMS (ESI) m/z calcd for C₁₈H₁₅Cl₂N₃O₅S [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. ¹H NMR (400 MHz, DMSO-d₆) 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). ¹³C NMR (101 MHz, DMSO-d₆) 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]⁺ (Rt = 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 (70 mg, 0.325 mmol) and 2-(2-bromoacetyl)-5-fluorophenyl pivalate (113 mg, 0.358 mmol). Yield 91% (134 mg, 0.297 mmol), white amorphous solid. \(^1\)H NMR (400 MHz, DMSO-\(_d_6\)) \(\delta\) 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-\(_d_6\)) \(\delta\) 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 (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 minutes. Subsequently the mixture was acidified with 1M HCl to pH approx. 5-6, 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-\(_d_6\)) \(\delta\) 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-\(_d_6\)) \(\delta\) 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 (100 mg, 0.371 mmol) and
2-(2-bromoacetyl)-5-fluorophenyl pivalate **S-11** (130 mg, 0.409 mmol). Yield 20% (32 mg, 0.076 mmol), off-white amorphous solid. \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 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-\(d_6\)) \(\delta\) 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-\(d_6\)) \(\delta\) 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-\(d_6\)) \(\delta\) 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-\(d_6\)) \(\delta\) 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-\(d_6\)) \(\delta\) 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.

\[
\begin{align*}
\text{\textsuperscript{1}H NMR (400 MHz, DMSO-}d_6\text{) } & \delta 9.21 (s, 1H), 7.81 - 7.90 (m, 4H), 7.75 (d, J = 8.53 Hz, 2H), \\
& 7.50 - 7.56 (m, 1H), 7.39 - 7.49 (m, 3H), 4.96 (s, 2H), 2.38 (s, 3H), 1.79 (s, 3H).
\end{align*}
\]

\[
\begin{align*}
\text{\textsuperscript{13}C NMR (101 MHz, DMSO-}d_6\text{) } & \delta 192.3, 174.9, 155.2, 143.8, 143.0, 138.5, 134.9, 134.0, 128.9, 128.6, 126.4, \\
& 126.0, 125.4, 63.3, 44.7, 24.9, 20.9. 
\end{align*}
\]

UPLC-MS (ESI) (B): \[m/z \text{ 402 [M+H]}^+ (R_t = 1.54 \text{ min})\].

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. \[
\begin{align*}
\text{\textsuperscript{1}H NMR (400 MHz, DMSO-}d_6\text{) } & \delta 9.23 (s, 1H), 7.94 (d, J = 8.28 Hz, 2H), 7.80 - 7.86 (m, 2H), 7.77 (d, J = 8.53 Hz, 2H), \\
& 7.50 - 7.55 (m, 1H), 7.40 - 7.49 (m, 1H), 4.96 (s, 2H), 2.38 (s, 3H), 1.79 (s, 3H).
\end{align*}
\]

\[
\begin{align*}
\text{\textsuperscript{13}C NMR (101 MHz, DMSO-}d_6\text{) } & \delta 192.2, 174.6, 155.1, 144.6, 138.5, 134.9, 134.0, 132.6, 128.9, 128.5, 126.8, 125.4, 118.5, 111.1, \\
& 63.3, 44.7, 25.0, 20.8. 
\end{align*}
\]

UPLC-MS (ESI) (B) \[m/z \text{ 346 [M-H]}^- (R_t = 1.67 \text{ min})\].

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.

\[
\begin{align*}
\text{\textsuperscript{1}H NMR (400 MHz, DMSO-}d_6\text{) } & \delta 9.19 (s, 1H), 7.95 (d, J = 8.28 Hz, 2H), 7.87 - 7.92 (m, 2H), \\
& 7.74 - 7.78 (m, 2H), 7.41 (s, 2H), 7.37 - 7.41 (m, 2H), 4.95 (s, 2H), 2.41 (s, 3H), 1.81 (s, 3H).
\end{align*}
\]
$^{13}$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), 7.72 - 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 $^2$J$_{CF}$ 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. ^1H NMR (400 MHz, DMSO-d6) 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). ^13C NMR (101 MHz, DMSO-d6) δ 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. ^1H NMR (400 MHz, DMSO-d6) 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). ^13C NMR (101 MHz, DMSO-d6) 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-\(d_6\)) \(\delta\) 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-\(d_6\)) \(\delta\) 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-\(d_6\)) \(\delta\) 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-\(d_6\)) \(\delta\) 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-\(d_6\)) \(\delta\) 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-\(d_6\)) \(\delta\) 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]⁻ (Rᵣ = 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. ¹H NMR (400 MHz, DMSO-d₆) 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). ¹³C NMR (101 MHz, DMSO-d₆) 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]⁺ (Rᵣ = 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. ¹H NMR (400 MHz, DMSO-d₆) 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). ¹³C NMR (101 MHz, DMSO-d₆) 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]⁻ (Rᵣ = 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-\(d_6\)) \(\delta\) 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-\(d_6\)) \(\delta\) 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-\(d_6\)) \(\delta\) 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-\(d_6\)) \(\delta\) 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-\(d_6\)) \(\delta\) 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 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), 4.48 (s, 2H), 2.11 - 2.20 (m, 1H), 1.75 (s, 3H), 0.95 - 1.04 (m, 2H), 0.84 - 0.93 (m, 2H).
13C NMR (101 MHz, DMSO-\textit{d}_6) \delta 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-\textit{d}_6) \delta 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-\textit{d}_6) \delta 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-\textit{d}_6) \delta 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-\textit{d}_6) \delta 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-$d_6$) $\delta$ 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-$d_6$) $\delta$ 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-$d_6$) $\delta$ 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-$d_6$) $\delta$ 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-$d_6$) $\delta$ 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-$d_6$) $\delta$ 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), 7.76 (d, $J$ =
8.53 Hz, 2H), 7.41 (s, 2H), 4.99 (s, 2H), 1.80 (s, 3H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 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<sub>t</sub> = 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-$d_6$) $\delta$ 9.21 (s, 1H), 8.17 - 8.21 (m, 1H), 8.12 - 8.16 (m, 1H), 8.09 (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-$d_6$) $\delta$ 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<sub>t</sub> = 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-$d_6$) $\delta$ 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-$d_6$) $\delta$ 185.0, 174.8, 155.1, 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<sub>t</sub> = 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-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-\(d_6\)) \(\delta\) 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-\(d_6\)) \(\delta\) 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-\(d_6\)) \(\delta\) 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-\(d_6\)) \(\delta\) 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 \(\geq 90\%\). \(^1\)H NMR (400 MHz, Acetone-\(d_6\)) \(\delta\) 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-
$d_\delta$ 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 mmo), 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 Middlebrook 7H9-ADC broth (Difco) supplemented with 0.025% Tyloxapol and on 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 fluorescent readout as described previously.$^{27}$ Isoniazid was used as a positive control and Rifampicin was used as a no-growth control.
**Microsomal fraction stability** assays were performed as described previously.\textsuperscript{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 \textit{et al.}\textsuperscript{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 \textit{et al.} in 2016.\textsuperscript{25}

**HepG2 cytotoxicity assay; artificial membrane permeability (AMP), kinetic aqueous solubility (CLND) and hydrophobicity (chromlogD_{\text{pH7.4}}).** These assays were performed as described previously.\textsuperscript{27,28}

**hERG inhibition.** Inhibition of the hERG potassium channel was determined using in vitro IonWorks patch-clamp electrophysiology as described in literature.\textsuperscript{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 one week. Mice were infected intratracheally with 100,000 CFU/mouse (M. tuberculosis H37Rv strain). Compounds were orally administered for four consecutive days, starting from day 5 after infection. Lungs were harvested on day 9, 24 hours 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. CFU number in lungs of untreated mice: 7.4 logCFU. 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 four consecutive days starting from day 5 after infection as an inter-assay control. It reduced 4.1 logCFU the bacterial lung number in comparison with the untreated mice (7.4 logCFU) (Tables 9, 10). This quality control value is included in the accepted interval.

**Table 10.** The numbers of cfu/lungs were counted and the corresponding log10 cfu/lungs were determined.

<table>
<thead>
<tr>
<th>logCFUs per mouse (lungs)</th>
<th>Mouse 1</th>
<th>Mouse 2</th>
<th>Mouse 3</th>
<th>Mouse 4</th>
<th>Mouse 5</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>No treatment (day 9)</td>
<td>7.4</td>
<td>7.4</td>
<td>7.4</td>
<td>7.2</td>
<td>7.4</td>
<td>7.4</td>
<td>0.1</td>
</tr>
<tr>
<td>Moxifloxacin 100 mg/kg 4d</td>
<td>3.5</td>
<td>2.9</td>
<td>3.3</td>
<td>3.5</td>
<td>3.5</td>
<td>3.3</td>
<td>0.2</td>
</tr>
<tr>
<td>30 po 200 mg/kg 4d</td>
<td>6.8</td>
<td>6.9</td>
<td></td>
<td></td>
<td></td>
<td>6.9</td>
<td>0.0</td>
</tr>
<tr>
<td>3 po 170 mg/kg 4d</td>
<td>7.2</td>
<td>7.2</td>
<td></td>
<td></td>
<td></td>
<td>7.2</td>
<td>0.0</td>
</tr>
</tbody>
</table>

**Table 11.** In the aforementioned experimental conditions, Moxifloxacin and the compounds evaluated showed the following differences in the lung microorganism burden (log10 CFUs/lungs) with respect to untreated controls (Day 9 after infection).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Target dose (mg/kg)</th>
<th>Administration</th>
<th>Route</th>
<th>Difference to untreated mice (log CFU)</th>
<th>p ¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moxifloxacin</td>
<td>100</td>
<td>Once a day (days 5-8)</td>
<td>Oral</td>
<td>4.1</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>30</td>
<td>200</td>
<td>Once a day (days 5-8)</td>
<td>Oral</td>
<td>0.5</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>3</td>
<td>170</td>
<td>Once a day (days 5-8)</td>
<td>Oral</td>
<td>0.2</td>
<td>p&gt;0.05</td>
</tr>
</tbody>
</table>

¹ ANOVA, Dunnett’s posttest. Compared to untreated mice, p<0.05 was considered significant.
Vibrational Circular Dichroism. VCD analysis and assignment was performed according to an analogous protocol published previously.\textsuperscript{30}

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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 immune deficiency syndrome; ACN, acetonitrile; Cl_{int}, hepatic intrinsic clearance; CLND, chemiluminescent nitrogen detection; DCM, dichloromethane; DMF, dimethylformamide; DprE1, decaprenylphospho-beta-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’-trisopropylbiphenyl; THF, tetrahydrofuran; TLC, Thin-Layer Chromatography; UPLC-MS, Ultra-Performance Liquid Chromatography-Mass Spectrometry; UV, ultraviolet; VCD, vibrational circular dichroism; WHO, World Health Organization.
ASSOCIATED CONTENT

Supporting information

The Supporting Information is available free of charge on the ACS Publications website. The following items are provided: additional experimental information for intermediate compounds (synthetic protocols and analytical details); references for synthetic procedures described in Supporting Information; 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, 82.

Molecular Formula Strings are available for all reported final compounds.

REFERENCES


(3) Brecik, M.; Centárová, I.; Mukherjee, R.; Kolly, G. S.; Huszár, S.; Bobovská, A.;


Fütterer, K.; Robbins, S. H.; Barnes, S. W.; Walker, J. R.; Jacobs, W. R.; Schultz, P. G.
Identification of a Small Molecule with Activity against Drug-Resistant and Persistent

(9) Oh, S.; Park, Y.; Engelhart, C. A.; Wallach, J. B.; Schnappinger, D.; Arora, K.; Manikkam,
M.; Via, L. E.; Boshoff, H. I. M.; Barry, C. E. Discovery and Structure–Activity-
Relationship Study of *N*-Alkyl-5-Hydroxypyrimidinone Carboxamides as Novel

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.;
Mahadevaswamy, J.; Vishwas, K.; Ahuja, V.; Srivastava, A.; Prabhakar, K.; Bharath, S.;
S.; Narayanan, S.; Chatterji, M. Azaindoles: Noncovalent DprE1 Inhibitors from Scaffold
Chem.* **2013**, *56* (23), 9701–9708.

Reddy, J.; Saralaya, R.; Nanduri, R.; Ambady, A.; Ravishankar, S.; Sambandamurthy, V.


(18) Chikhale, R. V.; Barmade, M. A.; Murumkar, P. R.; Yadav, M. R. Overview of the


