**Mycobacterium tuberculosis** Gyrase Inhibitors as a New Class of Antitubercular Drugs

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One way to speed up the TB drug discovery process is to search for antitubercular activity among compound series that already possess some of the key properties needed in anti-infective drug discovery, such as whole-cell activity and oral absorption. Here, we present MGIs, a new series of *Mycobacterium tuberculosis* gyrase inhibitors, which stem from the long-term efforts GSK has dedicated to the discovery and development of novel bacterial topoisomerase inhibitors (NBTIs). The compounds identified were found to be devoid of fluoroquinolone (FQ) cross-resistance and seem to operate through a mechanism similar to that of the previously described NBTI GSK antibacterial drug candidate. The remarkable *in vitro* and *in vivo* antitubercular profiles showed by the hits has prompted us to further advance the MGI project to full lead optimization.

Tuberculosis (TB), one of the oldest known infections, is still the second leading cause of mortality worldwide (1). The World Health Organization (WHO) estimated that there were 8.6 million new TB cases in 2012 and 1.3 million TB deaths, with a particularly high incidence among HIV-coinfected individuals (2).

The current standard directly observed treatment short course (DOTS) (3) consists of 2 months of treatment with isoniazid (INH), rifampin (Rf), pyrazinamide (PZA), and ethambutol (EMB) followed by 4 additional months of INH and Rf (4). Nevertheless, resistance to isoniazid, as well as to the combination of isoniazid and rifampin, a situation defining multidrug resistance (MDR), is common. Additionally, the emergence of novel strains which are resistant to these two drugs and to one of the three most commonly employed injectables has given rise to a novel category, the extensively drug-resistant (XDR) (5) strain. One step further, total drug resistance (TDR), is defined by resistance to all second-line drug classes. TDR cases have been increasingly reported in the clinic (6, 7).

Over the past decade, drug discovery and development efforts have increased, fueled by the upcoming threat of drug resistance in combination with the expansion of the HIV pandemic. These realities highlight an urgent need for more effective and tolerable treatments for drug-susceptible and drug-resistant disease in addition to latent TB infection.

To take advantage of the broad expertise of GSK in antibacterial drug discovery, a large subset of compounds representative of the wide chemical diversity generated in the GSK novel bacterial topoisomerase inhibitor (NBTI) initiative (8, 9, 10, 11) were evaluated *in vitro* against *Mycobacterium tuberculosis*. This exercise resulted in the identification of novel *M. tuberculosis* DNA gyrase inhibitors (MGIs), a new family of promising compounds with potential for the treatment of TB disease already reported in the literature as antimycobacterial and antibacterial agents (12, 13, 14, 15). Recently, AstraZeneca has reported anti-TB activity for NBTIs (16).

Here, we introduce MGIs as new advanced leads against TB as evidenced by their attractive *in vivo* and *in vitro* antitubercular profiles in addition to their lack of cross-resistance with fluoroquinolones (FQs).

**MATERIALS AND METHODS**

**General aspects and ethics statement.** All of the experiments were approved by the Diseases of the Developing World, GSK Ethical Committee. All animal studies were ethnically 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 6- to 8-week-old female C57BL/6j mice (18 to 20 g) were obtained from Harlan Interfauna (Iberica, Spain). The experiments were performed at the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC)-accredited GSK laboratory animal science animal facilities in Tres Cantos (Madrid, Spain). The mice were kept in air-conditioned facilities with 15 air changes per hour. Room temperature and relative humidity were 22 ± 3°C and 40 to 70%, respectively. The mice were accommodated in groups of up to five individuals in TecniplastH type IV cages with autoclaved dust-free corncob bedding (Panlab, Barcelona, Spain). The mice were maintained under a 12-h light/dark period. Autoclaved tap water and an irradiated pelleted diet were provided *ad libitum*. The com...
pounds used in these in vivo studies were prepared as suspensions in 1% methyl cellulose. The antitubercular standards used in the efficacy study were moxifloxacin (Seqouia Research Products Ltd.) and isoniazid (Sigma Aldrich), prepared as a solution in 20% Captisol-water and in water, respectively.

**Compound synthesis.** Compounds 1 (17), 2, and 3 (12) were prepared following a previously described synthetic pathway (see the supplemental material).

**In vitro assays.** (i) Bacterial strains and culture. *M. tuberculosis* strains (H37Rv, Beijing 1237, CDC1551, Erdman, and 8 clinical strains), *Mycobacterium canettium*, and *Mycobacterium bovis* BCG were grown at 37°C in Middlebrook 7H9 broth supplemented with 0.025% Tween 80 and 10% albumin-dextrose-catalase (ADC) or on Middlebrook 7H10 plates supplemented with 10% oleic acid-ADC (OADC). *Escherichia coli* DH5α was grown in LB broth. Ampicillin, hygromycin (Hyg), and sucrose (Suc) reagents were used for plasmid selection or recombination experiments.

(ii) DNA manipulation, plasmids, and transformation. General molecular biology procedures were used as described previously (18) or following the manufacturer’s instructions. *Escherichia coli* DH5α and *M. tuberculosis* H37Rv competent cells were prepared for electroporation as described previously (19).

(iii) MIC determination. *M. tuberculosis* H37Rv ATCC 27294 was used for all the studies and was grown and tested as described earlier (20). The MIC was determined as described previously (20).

(iv) Killing assays. Bacteria were grown at 37°C in 7H9 Middlebrook-ADC-Tween 80 to mid-exponential—exponential phase and then diluted in 10 ml fresh Middlebrook 7H-ADC-Tween 80 to 5 × 10⁷ CFU/ml. Incubation was continued after the addition of compounds at 20× MIC. At specified time points, aliquots of cultures were withdrawn, serially diluted in 7H9 Middlebrook-ADC-Tween 80, and plated on solid culture medium (7H10-OADC). Plates were then incubated at 37°C, and CFU were counted after 3 to 4 weeks.

(v) Generation rate of spontaneous resistant mutants. The MICs in solid medium (Middlebrook 7H10-OADC) were determined in 24-well plates with serial dilutions of the compound of interest. Five microliters of bacterial culture containing 10⁸ CFU/ml was added per well. Plates were incubated at 37°C for 20 days for *M. tuberculosis* H37Rv. MIC values are the minimum concentration of the compound which inhibits 90% bacterial growth.

In order to determine the generation rate of spontaneous resistant mutants to the compounds, bacteria were grown at 37°C in fresh Middlebrook 7H-9ADC-Tween 80 to the mid-exponential phase and then diluted in fresh Middlebrook 7H9-ADC-Tween 80 to 5 × 10⁷ CFU/ml. Middlebrook 7H10-OADC plates with 20× MIC of each compound were inoculated with 10⁸, 10⁷, 10⁶, and 10⁵ CFU/plate, and the plates were incubated at 37°C during 3 to 4 weeks. The frequency of appearance of resistant mutants was calculated, and isolated colonies were restreaked onto new plates with serial dilutions of the compound of interest.
with the target gyrase (8). First, a left-hand side (LHS) is responsible for important contacts with the gyrase DNA substrate. Second, a right-hand side (RHS) is embedded into the gyrase enzyme, in addition to potentially providing some bacterial target selectivity. Last, a central linker unit (CU) establishes key interactions for important contacts with the gyrase (8) and offers the possibility of modulating important physicochemical properties (solubility [CHIlogD]) that can impact human ether-à-go-go-related gene (hERG) inhibition (for hERG inhibition reviews, see reference 28).

These initial structure-activity observations, inherited from the predecessor antibacterial program, helped to schematize synthesis (details to be reported elsewhere) and to rationalize how to balance antimycobacterial potency with oral exposure, safety, and synthetic complexity. These efforts resulted in the identification of the 7-substituted-1,5-naphthyridin-2-one core as a privileged LHS, the N-ethyl-4-aminopiperidines as a linker, and monocyclic aromatic rings with different substitution patterns as the best RHS option, in contrast to NBTIs bearing bicyclic rings as the RHS (15). These three favorable features were combined in the synthesis of analogues 2 and 3 (Fig. 1).

**Antitubercular profile.** Compound 1, as the best NBTI direct screening representative, and novel synthetic entities 2 and 3 were selected to be progressed to a panel of *in vitro* and *in vivo* studies to validate their potential as antitubercular drugs. The extracellular MICs were found to be 0.5 μM for compound 1, 0.08 μM for compound 2, and <0.01 μM for compound 3 (moxifloxacin MIC, 0.15 μM). In terms of intracellular antitubercular activity against *M. tuberculosis* THP1-infected macrophages, these compounds have shown very good activity (MIC of <0.02 μM for compounds 2 and 3).

MICs of the three key MGI compounds against a panel of *M. tuberculosis* clinical and laboratory strains were then established (Tables 1 and 2) in order to have an early indication of the potential of these compounds to address MDR strains. MICs were found to be higher than those for the reference *M. tuberculosis* H37Rv strain, while still being within a range acceptable for further MDR consideration.

As a next step in compound evaluation, the bactericidal potentials of the new MGI compounds were studied by determining the time-kill kinetic curves of compounds 2 and 3 and comparing them with that of linezolid as a bacteriostatic model drug, while isoniazid and a GSK InhA direct inhibitor were used as bactericidal controls (Fig. 2). Results show that these new MGIs are bactericidal as defined by the ability of a compound to reduce 99.9% of the bacterial population in 7 days (for *in vitro* activities of fluoroquinolones, see reference 29).

In terms of the potential for spontaneously resistant mutant generation using compound 1 as a model molecule, a key consideration in any target-based program, the frequency of spontaneously resistant mutant generation was found to be $7.4 \times 10^{-8}$ mutants/CFU, a number clearly lower than that of isoniazid and similar to that of rifampin. As a direct consequence of these assays, 21 independent mutant colonies spontaneously resistant to our compounds were isolated and characterized. Sixteen of them had a single point mutation in the quinolone resistance-determining regions (QRDR) (30). Thirteen were found in gyrA, where 8 different mutations were identified (H87Q, P50T, E79G, D89N, R98H, S95G, D89G, and H52R), and 3 in gyrB, where two different mutations were observed (D495N and D495A). Only one of the mutations found in the QRDR of *gyrA* (D89N) has been pre-
previously reported (31) as being related to fluoroquinolone resistance.

More in-depth sequencing identified point mutations in *gyrA* but outside the QRDR in the remaining 5 strains. The mutations were H368R, I348S, and S178L (Fig. 3).

MICs in the spontaneous mutants were found to be between 10 and 200 times higher than in the wild type, with cross-resistance between the three described MGIs being a common finding not associated to FQs (ciprofloxacin, ofloxacin, and moxifloxacin). The only exception was found in the D89 mutants, which show a slight but noticeable cross-resistance (2- to 8-fold higher MICs for ciprofloxacin and moxifloxacin, compared to the 20- to 300-fold increase in the MICs for our compounds) (Table 3).

As a further check on the potential for cross-resistance in the clinic, five mutants resistant to FQs were also isolated and characterized. In these mutants, the most commonly encountered FQ-resistant mutations described in clinical isolates in *gyrA* (S91P, A90V, and D94G) (30, 31, 32, 33, 34, 35) were found. Of the laboratory mutants with resistance against FQs that were selected, only S91P mutants showed a slight cross-resistance with some of the lead MGIs, while D94G and A90V mutants gave rise to a hypersensitive phenotype to most of the MGIs tested.

In order to univocally correlate gene mutations with resistance, a system with a suicide plasmid vector for mutant reversal (E79G, H87Q, S95G, H368R, and I348S) was used. We used the system described by Parish and Stoker (22). Briefly, a plasmid with the wild-type *M. tuberculosis* H37Rv *gyrA* was transformed into the mutants, and a first homologous recombination took place. Hyg<sup>+</sup> recombinants were selected. A second homologous recombination occurred, and the double recombinants were Hyg<sup>+</sup>-Suc<sup>-</sup>. Afterwards, *gyrA* was amplified and sequenced, and MGI resistance was determined. As an example, in the case of the E79G mutant, 15% of the Hyg<sup>+</sup>-Suc<sup>-</sup> transformants were sensitive for our compounds, and all of them have the wild-type phenotype (Table 4; see also Fig. S1 in the supplemental material). The same process was repeated with the other mutants (H87Q, S95G, H368R, and I348S), and there was a perfect correlation between loss of mutation and reversion to sensitivity (data not shown).

Additionally, the mode of action of a representative set of NBTI and MGI compounds was evaluated in the *M. tuberculosis* DNA gyrase supercoiling assay. Determination of the concentration-dependent inhibitory effect of compounds 1, 2, and 3 yielded the following IC<sub>50</sub>s: 2.80 ± 0.65 μM, 10.98 ± 3.41 μM, and 5.4 ± 1.2 nM, respectively. Compound 2 exhibited a poorer correlation be-

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**TABLE 2** MICs of key MGI compounds against wild-type *M. tuberculosis* laboratory strains and *M. bovis* BCG

<table>
<thead>
<tr>
<th>Compound</th>
<th>MIC (μg/ml) against strain:</th>
<th>H37Rv</th>
<th>Beijing 1237</th>
<th>Canetti</th>
<th>CDC1551</th>
<th>Erdman</th>
<th>BCG</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td></td>
<td>0.04</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
<td>0.12</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>≤0.01</td>
<td>0.03</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
<td>≤0.01</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td></td>
<td>0.2</td>
<td>0.08</td>
<td>0.16</td>
<td>0.12</td>
<td>0.12</td>
<td>0.04</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td></td>
<td>0.6</td>
<td>0.3</td>
<td>0.5</td>
<td>0.3</td>
<td>0.5</td>
<td>0.16</td>
</tr>
<tr>
<td>Moxifloxacin</td>
<td></td>
<td>0.08</td>
<td>0.04</td>
<td>0.08</td>
<td>0.08</td>
<td>0.08</td>
<td>≤0.02</td>
</tr>
</tbody>
</table>

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**FIG 2** Time-kill curves of *M. tuberculosis* H37Rv in 7H9ADC medium at 20× MIC of compounds 2 and 3. Linezolid was used as a bacteriostatic model drug while isoniazid and a GSK InhA direct inhibitor were used as bactericidal controls.
between the \textit{M. tuberculosis} DNA gyrase IC$_{50}$ and the H37Rv MIC. Further studies are necessary to address this behavior. Most of the compounds with MICs against \textit{M. tuberculosis} also inhibited the supercoiling activity of \textit{M. tuberculosis} DNA gyrase, thus confirming their mode of inhibition (Fig. 4). Given the potential for toxicity due to cross-inhibition with human gyrase, a representation of this set was tested in the human topoisomerase II alpha (36). Results showed $>30$-fold more potency in \textit{M. tuberculosis} DNA gyrase than against the human enzyme.

Once the potential of the new MGI leads for MDR treatment had been clarified, compounds 1, 2, and 3 were further assessed in an acute infection mouse model of tuberculosis (25), measuring the fold reduction in CFU in the lung (Fig. 5) versus the CFU count for untreated controls. A preliminary profiling of selected compounds showed how the compounds were endowed with desirable pharmacokinetic and toxicological profiles (Table 5). The fold reductions in CFU were compared to those achieved with gold standard drugs, such as isoniazid (25 mg/kg body weight orally [p.o., once a day [u.i.d.]] and moxifloxacin (100 mg/kg p.o., u.i.d.). Both MGIs 2 (50 mg/kg p.o., twice a day [b.i.d.]) and

### Table 3

<table>
<thead>
<tr>
<th>Compound</th>
<th>H87Q</th>
<th>P50T</th>
<th>R98H</th>
<th>S95G</th>
<th>D89G</th>
<th>H52R</th>
<th>E79G</th>
<th>H368R</th>
<th>I348S</th>
<th>S178L</th>
<th>S91P</th>
<th>D94G</th>
<th>A90V</th>
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<tr>
<td>1</td>
<td>31</td>
<td>10.7</td>
<td>31</td>
<td>11</td>
<td>83</td>
<td>83</td>
<td>128</td>
<td>21</td>
<td>11</td>
<td>11</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>2</td>
<td>17</td>
<td>8.3</td>
<td>25</td>
<td>8</td>
<td>$&gt;266$</td>
<td>67</td>
<td>32</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>3</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>$&gt;2$</td>
<td>1</td>
<td>1</td>
<td>0.8</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>0.8</td>
<td>1.5</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>Moxifloxacin</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>4</td>
<td>0.8</td>
<td>1</td>
<td>1</td>
<td>NT</td>
<td>NT</td>
<td>25</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>$&gt;1.5$</td>
<td>$&gt;1.5$</td>
<td>$&gt;3.8$</td>
<td>$&gt;1.5$</td>
<td>2</td>
<td>$&gt;1.5$</td>
<td>$&gt;1.5$</td>
<td>$&gt;1.5$</td>
<td>$&gt;1.5$</td>
<td>$&gt;25$</td>
<td>74</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>INH</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>NT</td>
<td>1</td>
<td>0.8</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

\*NT, not tested.
3 (50 mg/kg subcutaneously [s.c., b.i.d.] showed a significant CFU reduction in the lungs of infected mice (>/H11022 2 log inhibition), showing potencies approaching those of the reference compounds. However, 1 (100 mg/kg p.o., b.i.d.), a compound in theory representing a good balance between in vitro anti-TB potency and pharmacokinetic profile (Table 5), failed to show activity at the maximum dose tolerated. This lack of activity might due to the poorer intracellular antitubercular profile exhibited by compound 1 than by MGIs 2 and 3.

**DISCUSSION**

As a consequence of the long-term efforts GSK has dedicated to the novel bacterial topoisomerase inhibitor (NBTI) field, new antibacterials with novel modes of action, no cross-resistance with FQs, oral drug-likeness, and good safety profiles have been identified (8, 9, 10, 11). With this exercise as a starting point for TB drug discovery, a representative set of this collection (3,000 compounds) was screened against *M. tuberculosis*, resulting in the selection of compound leads 1 to 3 for further antitubercular profiling. Compound 1 was identified as the best balanced NBTI both in terms of in vitro anti-TB potency and pharmacokinetic profile, while compounds 2 and 3 were designed as novel entities incorporating key lessons learned from the already known NBTI chemical diversity.

The compounds tested were bactericidal in vitro (10 × MIC) and showed a low rate of generation of spontaneous resistant mutants (similar to that of rifampin). As no evidence supporting the fact that MGI compounds killed *M. tuberculosis* through inhibition of the *M. tuberculosis* DNA gyrase was available to date and no assays for the TB enzyme were available at that time, as an alternative strategy, we sought to isolate laboratory resistant mutants to these compounds both to determine if there were single-point mutations in the *M. tuberculosis* DNA gyrase-resistant mutants and to test for potential cross-resistance between MGIs, NBTIs, and FQs. All 21 MGI-resistant strains isolated were found to harbor mutations in either GyrA or GyrB. Selected gyrA mutant reversion to the gyrA wild type resulted in resistant phenotype reversion to sensitive, a situation affecting the 5 mutants tested. These results strongly suggest that MGIs bind to DNA gyrase proximal to the FQ binding site and that inhibition of this enzyme is the lethal event leading to the bactericidal action of MGIs.

Interestingly, mutants with mutations outside the QRDR of

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**TABLE 4** Ratios of the MICs obtained in the different strains isolated in the reversion study of the E79G mutant for compounds 1 and 2 in relation to those for the wild-type and polymorphism in codon 79 of the gyrA gene

<table>
<thead>
<tr>
<th>Compound</th>
<th>MICmut/MIC&lt;sub&gt;H37Rv&lt;/sub&gt; ratio for strain:</th>
<th>Double crossover</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>H37Rv</td>
<td>E79G mutant</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>128</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>32</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>gyrA codon 79 polymorphism</td>
<td>GAG</td>
<td>GGG</td>
</tr>
</tbody>
</table>

<sup>a</sup> ND, not determined.

![FIG 4](image4.png) **FIG 4** Plot of the concentration-dependent inhibitory effect of a representation of MGIs and NBTIs against *M. tuberculosis* DNA gyrase expressed as pIC<sub>50</sub> versus H37Rv MIC (µM). Compounds 1, 2, and 3 are indicated as black squares.

![FIG 5](image5.png) **FIG 5** Antitubercular activity of isoniazid, moxifloxacin, and compounds 1, 2, and 3 in an acute infection murine model. Each circle represents data from an individual mouse. *, a P value of >0.05 was considered significant. Data were analyzed by a one-factor analysis of variance (ANOVA) and a Games-Howell post hoc test, since the P value for the Levene test was <0.05.
**TABLE 5 Profiles for representative NBTI/MGI compounds**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Results for compound:</th>
</tr>
</thead>
<tbody>
<tr>
<td>H37Rv MIC (μM)</td>
<td>0.5 0.08 &lt;0.01</td>
</tr>
<tr>
<td>Intracellular H37Rv MIC&lt;sub&gt;90&lt;/sub&gt; (μM)</td>
<td>1.85 &lt;0.02 0.02</td>
</tr>
<tr>
<td>M. tuberculosis DNA gyrase IC&lt;sub&gt;90&lt;/sub&gt; (μM)</td>
<td>2.80 10.98 5.40</td>
</tr>
<tr>
<td>HepG2 Tox&lt;sub&gt;50&lt;/sub&gt; (μM)</td>
<td>&gt;100 &gt;100 67.6</td>
</tr>
<tr>
<td>Chrom logD pH&lt;sub&gt;4&lt;/sub&gt; (37)</td>
<td>1.51 3.73 4.36</td>
</tr>
<tr>
<td>Solubility CLND (μM)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>354 ≥386 ≥335</td>
</tr>
<tr>
<td>In vitro CL&lt;sub&gt;i&lt;/sub&gt; (ml/min · g)</td>
<td></td>
</tr>
<tr>
<td>Mouse&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.09 3.8 20.84</td>
</tr>
<tr>
<td>Human&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;0.53 0.6 0.75</td>
</tr>
<tr>
<td>In vivo CL (ml/min/kg)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>NT 88.3 132.5</td>
</tr>
<tr>
<td>T&lt;sub&gt;max&lt;/sub&gt; (h)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.75 0.25 0.25</td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (μg/ml)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.7 2.0 0.08</td>
</tr>
<tr>
<td>DNAUC oral (μg · h/ml)/mg/kg&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.1 0.1 0.01</td>
</tr>
<tr>
<td>% F&lt;sup&gt;d&lt;/sup&gt;</td>
<td>NT 47.8 10.0</td>
</tr>
<tr>
<td>hERG XPRESS IC&lt;sub&gt;50&lt;/sub&gt; (μM) (38, 39, 40)</td>
<td>&gt;50 2.85 1.32</td>
</tr>
</tbody>
</table>

<sup>a</sup> Tox<sub>50</sub>, 50% cytotoxic concentration; CLND, chemiluminescent nitrogen detection; CL<sub>i</sub>, intrinsic clearance; CL, clearance; T<sub>max</sub>, time to maximum concentration of drug in serum; C<sub>max</sub>, maximum concentration of drug in serum; DNAUC, dose-normalized area under the blood-concentration time curve; NT, not tested.

<sup>b</sup> CLND solubility values that are within 85% of maximum possible concentration (as determined from dimethyl sulfoxide [DMSO] stock concentration).

<sup>c</sup> See supplemental material.

The results reported here resulted in the establishment of a TB-specific lead optimization effort. The current work is focused on the design of novel TB-tailored molecules that can combine the numerous factors governing successful drug design, i.e., in vitro and in vivo potency, oral exposure, an absence of cardiovascular liabilities related to hERG inhibition (10, 11), and a lack of clastogenic potential deriving from cross-activity with host topoisomerases. Further progress will be reported in due course.

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