Tuberculosis is the leading cause of death from infectious disease. Although the number of tuberculosis cases decreased during the 20th century, the emergence of HIV and the incidence of multiple-drug resistance have increased the difficulty of treating many new cases. Despite efforts to improve the outcome of tuberculosis care, the discovery of new antibiotics against the causative agent Mycobacterium tuberculosis has been insufficient to eradicate the disease. New and more effective drugs with novel mechanisms of action are required. We previously identified a series of novel aryl-oxadiazoles with anti-tubercular activity specific for bacteria using butyrate as a carbon source. We explored the structure activity relationship of this series. Structural modifications were performed in all domains to improve potency and physico-chemical properties. A number of compounds displayed sub-micromolar activity against M. tuberculosis utilizing butyrate, but not glucose as the carbon source. Compounds showed no or low cytotoxicity against eukaryotic cells. Three compounds were profiled in mouse pharmacokinetic studies. Plasma clearance was low to moderate but oral exposure suggested solubility-limited drug absorption in addition to first pass metabolism. The presence of a basic nitrogen in the linker slightly increased solubility, and salt formation optimized aqueous solubility. Our findings suggest that the 1,3,4-oxadiazoles are useful tools and warrant further investigation.

We recently reported the identification of a family of oxadiazoles 1–5 (Fig. 1) from a whole cell screen against M. tuberculosis using butyrate as the carbon source. The compounds were active in medium containing butyrate, but not glucose and lacked mammalian cytotoxicity. The lack of cytotoxicity and the low molecular weight prompted us to undertake structure activity relationship (SAR) investigations around this series.

Aryl-oxadiazoles, the common structural motif in compounds 1–5, have been widely applied in medicinal chemistry for the development of new drugs. Compounds containing the 1,2,4- and 1,3,4-oxadiazole motif have been evaluated against a broad spectrum of pharmacological activities, with special attention to their properties as antimicrobial and antitubercular agents. Synthetic methods for the preparation of differently functionalized 1,3,4-oxadiazoles have been recently reviewed. Compound 2 was resynthesized and compounds 13–18 and 24–41 were made in three steps by the method previously published for making compound 2, starting from the corresponding hydrazide and then...
reacting the intermediate chloride with the appropriate secondary amine. Compounds 6–9 were prepared according to the representative procedure exemplified in Scheme 1 for compound 8. Hydrazide 8a was coupled with carboxylic acid 8b using EDC and HOBt to obtain the intermediate 8c. Cyclodehydration of semicarbazide 8c by refluxing with phosphoryl chloride yielded compound 8.

In order to prepare thiadiazole 10, reaction of furan-2-carboxyhydrazide 10a with chloroacetyl chloride in the presence of N-methylmorpholine produced the intermediate acylsemicarbazide 10b (Scheme 2). Acylsemicarbazide 10b was refluxed with Lawesson’s reagent in THF to obtain the intermediate chloride 10c. Chloride replacement by 2-chloro-6-fluorobenzylamine at reflux in the presence of DIPEA and sodium iodide generated the secondary amine 10d which was treated with sodium hydride and methyl iodide to give compound 10. Compounds 11, 12 and 19 were prepared from the corresponding oxadiazole analogue to chloride 10c.
by reaction with the appropriate primary amine followed by methylation.

The synthesis of the oxadiazole 21 containing an ether group in the linker was achieved by cyclization of acylsemicarbazide 10b using phosphoryl chloride to create 21a followed by the reaction of the intermediate 21a with (2-chloro-6-fluorophenyl)methanol (Scheme 3). Amide 22 was prepared from chloride 21a in two steps, via 22a. Substitution of chloride 21a by methylation followed by amide formation using 2-chloro-6-fluorobenzoic acid in the presence of HATU and triethylamine provided the amide 22 (Scheme 3). Compound 20 was prepared by reaction of intermediate 21a with 2-chloro-6-fluorobenzylamine using DIPEA and sodium iodide.

The synthesis of compound 23 utilized the three-step route shown in Scheme 4. Commercially available 1,3,4-oxadiazol-2-one 23a was refluxed with 2-chloro-6-fluoro-phenethylamine 23b in ethanol to obtain intermediate 23c that was cyclized by heating with phosphoryl chloride. Methylation of secondary amine 23d using methyl iodide in the presence of sodium hydride in DMF afforded compound 23.

We began by evaluating mouse microsomal metabolism and thermodynamic solubility for aryl-oxadiazole hits from the screen (Fig. 1, Table 1), and learned that these compounds were all highly metabolized, and solubility varied. We then focused our attention on the most potent hit from the screen, compound 1 (previously reported MIC was 0.4 ± 0.1 μM in butyrate medium) and synthesized compounds 6–9 (Fig. 2) to answer specific structure-activity questions. In order to compare 2-thienyl- with 2-furyl-oxadiazole, the thiophene ring in compound 1 was replaced with furan 6 (Fig. 2, Table 2). These two compounds have comparable activity.

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**Scheme 3.** Synthesis of compounds 21 and 22. Reagents and conditions: (a) phosphoryl chloride; (b) (2-Chloro-6-fluorophenyl)methanol, NaH, THF; (c) MeNH2, NaI, CH3CN, reflux; (d) 2-Chloro-6-fluorobenzoic acid, HATU, Et3N, DMF.

**Scheme 4.** Synthesis of compound 23. Reagents and conditions: (a) EtOH, 100 °C; (b) POCl3, 110 °C; (c) MeI, NaH, DMF.

**Table 1.** Mouse microsomal metabolism, unbound intrinsic clearance and aqueous solubility for aryl-oxadiazoles from the screen.

| Cpd | Mouse microsome (% metabolized in 30 min) | Mouse microsome unbound intrinsic clearance (ml/min/kg) | Aqueous solubility at pH 7.4 (mg/mL) | clogP<sup>a</sup> |
|-----|----------------------------------------|-----------------------------------------------------|-----------------------------------|----------------|
| 1   | 99                                      | 3310                                      | <0.001                            | 3.9           |
| 2   | 99                                      | 1350                                     | 0.077                             | 2.5           |
| 3   | 65                                      | 1910                                     | 0.005                             | 4.4           |
| 4   | 95                                      | 1680                                     | <0.001                            | 3.7           |
| 5   | 76                                      | 332                                      | 0.018                             | 2.3           |

<sup>a</sup> clogP values are calculated from BioByte software.
in butyrate medium (7H9-Ty-BT) (Table 2). Replacements for 1-naphthyl in compound 1 were also examined, detecting that 2-naphthyl was less potent while 5-benzodioxine or phenyl retained activity. Compounds 6–9 showed neither activity under glucose conditions (7H9-Tw-OADC) nor cytotoxicity in Vero cells (Table 2).

Although aqueous solubility was low for the most lipophilic compounds 1, 3, 4, 6 and 7, a trend to improve solubility was observed when lowering calculated logP (clogP), as in compounds 2, 5, 8 and 9. The focus of SAR evaluation became improving solubility and metabolic stability while maintaining potency. With this goal, we explored the SAR around compound 2, which had a previously reported MIC of 0.8 ± 0.3 mM in butyrate medium based on the improved solubility with respect to compounds 1, 3–5. The 1,3,4-thiadiazole 10 was slightly less potent and less soluble than the 1,3,4-oxadiazole (2), so we continued the SAR evaluation using 1,3,4-oxadiazole as the heterocyclic core. Replacement of the furan ring in 2 by thiophene or phenyl led to equipotent compounds with similar solubility. The lack of both substituents at the ortho-positions, as in compounds 13–14, or the presence of a single substituent, as in compound 15, did not change the antimicrobial activity relative to compound 2, suggesting that substitution at ortho-positions is not required for activity. Nevertheless, the lack of one or both substituents at the ortho-positions decreased clogP and significantly increased solubility. Compound 16, having a fluoro group at the 4-position of the benzyl substituent, had similar potency in comparison to compound 12. However, the trifluoromethyl group at 4-position in compounds 17 and 18 substantially decreased the activity with respect to compounds 11 and 12 and negatively impacted on solubility. Replacement of the benzyl group with a cyclohexylmethyl group as in compound 19 was also well tolerated.

Table 2

| Cpd | 7H9-Ty-BT \( \text{MIC (µM)} \) | 7H9-Tw-OADC \( \text{MIC (µM)} \) | Vero \( \text{IC}_{50} \) (µM) | Mouse microsome (% metabolized) | Aqueous solubility at pH 7.4 (mg/mL) | clogP  
|-----|-------------------------------|-------------------------------|----------------|-------------------------------|-----------------------------------|-------
| 6   | 1.1 ± 0.9                      | >20                           | >100           | 100                           | <0.001                            | 3.1   
| 7   | 5.7 ± 0.2                      | >20                           | >100           | 100                           | <0.001                            | 3.9   
| 8   | 0.8 ± 0.2                      | >20                           | >100           | 99                            | 0.077                             | 2.6   
| 9   | 1.1 ± 0.2                      | >20                           | 79             | 99                            | 0.031                             | 2.7   

\( a \) Results are average ± standard deviation for at least 2 runs.
\( b \) Growth medium with butyrate as the primary carbon source.
\( c \) Growth medium with glucose as the primary carbon source.
\( d \) clogP values are calculated from BioByte software.

![Fig. 2. SAR around compound 1.](image)

![Fig. 3. Analogues of compound 2. SAR on aromatic domains and linker.](image)
resulted in complete loss of activity. As expected, the lower potent than the corresponding tetrahydroquinolines 24.

The basicity of the nitrogen is not critical for maintaining potency for the tetrahydroquinoline orientation also indicates that the indidine ring had a significant effect on activity. The clear preference showing that the site of fusion between the phenyl and the piperdine ring also impacted the activity. Indolines 27 and 28 achieved a small improvement in activity and 4-trifluoromethylphenyl 29 was more, as expected based on the position of the nitrogen atom.

When a nitrogen atom is part of the 2-phenylpyrrolidine ring as in 30–31, compounds displayed minimal activity. As expected, compounds 32–41 were not cytotoxic and did not have activity against M. tuberculosis in medium containing glucose (Fig. 4).

All the compounds we synthesized suffered from poor microsomal stability in mice (Tables 1–4) and rats (data not shown). One of the first strategies to increase metabolic stability is to reduce the overall lipophilicity. However, a correlation between microsomal metabolism and clogP could not be observed as a few compounds that lowered calculated lipophilicity did not reduce oxidative metabolism (Tables 3 and 4). On the positive side, lipophilicity apparently plays a secondary role in potency because correlation between clogP and activity was not observed (Tables 1–4).

To further investigate the impact of microsomal metabolism on clearance, three of the structurally different screening hits were selected for mouse pharmacokinetic (PK) studies (Table 5). The PK evaluation of 1, 2 and 3 was performed after intravenous (1 mg/kg) and oral (10 mg/kg) administration to mice. In order to confirm that clearance was due to oxidative metabolism, compound 2 was co-administered with 1-aminobenzotriazole (ABT), a non-selective cytochrome P450 inhibitor. Compound 2 was rapidly eliminated from the body, but clearance decreased significantly when co-administered with ABT, demonstrating that the high clearance was due to CYP-mediated metabolic oxidation. This result is consistent with the very low microsomal stability. Oral exposure was low and increased >10 fold when co-administered with ABT, proving that first pass metabolism limits exposure.

| Cpd | R | X | Y | Z | 7H9-Ty-BTabc MIC (μM) | 7H9-Tw-OADCabc MIC (μM) | Vero IC50 (μM) | Mouse microsome (% metabolized) | Aqueous solubility at pH 7.4 (mg/mL) | clogPd |
|-----|---|---|---|---|------------------------|------------------------|--------------|-------------------------------|---------------------------------------|-------|
| 10  | 2-Thiophenyl | 0.3 ± 0.5 | >20 | >20 | 75 | 99 | 0.020 | 3.3 |
| 11  | Phenyl | 0.4 ± 0.2 | >20 | >20 | 100 | 100 | 0.12 | 3.1 |
| 12  | 2-Thiophenyl | 0.5 ± 0.1 | >20 | >20 | 100 | 98 | 0.42 | 2.2 |
| 13  | 2-Furanyl | 0.6 ± 0.2 | >20 | >20 | 100 | 95 | 0.40 | 1.7 |
| 14  | Phenyl | 0.6 ± 0.2 | >20 | >20 | 98 | 100 | 0.27 | 2.4 |
| 15  | Phenyl | 0.9 ± 0.03 | >20 | >20 | 97 | ND | ND | 2.4 |
| 16  | Phenyl | 10.8 ± 3.3 | >20 | >20 | 80 | 99 | 0.009 | 3.2 |
| 17  | Phenyl | 0.7 ± 0.5 | >20 | >20 | 90 | 100 | 0.041 | 2.6 |
| 18  | Phenyl | 5.5 ± 0.4 | >20 | >20 | 90 | 100 | 0.18 | 1.5 |
| 19  | NH | 6.0 ± 0.4 | >20 | >20 | 100 | 100 | 0.15 | 2.1 |
| 20  | O | 4.0 ± 0.6 | >20 | >20 | 93 | 99 | 0.007 | 4.0 |

a Results are average ± standard deviation for at least 2 runs.

b Growth medium with butyrate as the primary carbon source.

c Growth medium with glucose as the primary carbon source.
d clogP values are calculated from BioByte software.

Fig. 4. SAR around compound 24, a hybrid structure between compounds 3 and 5.
clearance for compound 1 was low but oral exposure was much lower than expected, suggesting solubility-limited drug absorption. Compound 3 had moderate clearance and the very low oral exposure may be due to first-pass metabolism and/or solubility-limited absorption.

We tried several things to improve aqueous solubility for this series. As a general trend, the presence of a basic nitrogen in the linker increased solubility, and solubility was further increased as a carbon source. Although this series will require optimization of properties. As expected, compound as a free base had much lower limited absorption. Compound 3 had moderate clearance and the very low oral exposure may be due to first-pass metabolism and/or solubility-limited absorption.

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and glucose as carbon source, methods for microsomal stability and PK studies, as well as the PK profiles and a table of unbound fraction for compounds 1, 2, and 3) associated with this article can be found in the online version at https://doi.org/10.1016/j.bmcl.2018.04.028.

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