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Comparative Study of Activities of a Diverse Set of Antimycobacterial Agents against *Mycobacterium tuberculosis* and *Mycobacterium ulcerans*

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A library of compounds covering a broad chemical space was selected from a tuberculosis drug development program and was screened in a whole-cell assay against *Mycobacterium ulcerans*, the causative agent of the necrotizing skin disease Buruli ulcer. While a number of potent antitubercular agents were only weakly active or inactive against *M. ulcerans*, five compounds showed high activity (90% inhibitory concentration [IC_{90}], ≤1 μM), making screening of focused antitubercular libraries a good starting point for lead generation against *M. ulcerans*.

Buruli ulcer (BU) is a neglected tropical disease that is characterized by chronic necrotizing skin lesions. It is caused by *Mycobacterium ulcerans*, a slow-growing mycobacterium that produces the potent exotoxin mycolactone, which is the main virulence factor responsible for the necrotizing pathology of BU (1, 2). Standard specific treatment of BU involves 8 weeks of combination therapy with streptomycin and rifampin, which may have ototoxic, nephrotoxic, and hepatotoxic side effects (3). While streptomycin can in principal be replaced by clarithromycin or moxifloxacin (4), no replacement for rifampin is currently available (5, 6). As a consequence, antibiotic therapy of BU may become impossible if rifampin resistance in *M. ulcerans* emerges.

Since *M. ulcerans* is closely related to *Mycobacterium tuberculosis* (7), drug molecular targets may be conserved between the two species (8). Therefore, repurposing tuberculosis (TB) drug candidates for BU represents an attractive approach to overcome the problem of very limited financial resources for BU drug discovery and development. Here, we have tested a set of 83 compounds from the tuberculosis lead generation and lead optimization drug discovery programs of AstraZeneca. The set was selected based on activity against *M. tuberculosis* and on chemical diversity comprising advanced candidates and novel leads. For activity testing against *M. ulcerans*, resazurin-based assays were performed essentially as described previously (9). A good correlation between this type of metabolic activity testing and the enumeration of CFU was shown in a previous study (10). In brief, bacteria were incubated with the compounds at a concentration of 10 μM for 1 week at 30°C. Then, resazurin was added (10%, vol/vol), and the plates were further incubated overnight at 37°C followed by measurement of fluorescence intensities. Subsequently, MICs corresponding to 90% inhibition (IC_{90}) of compounds active in the prescreen were assessed by testing 2-fold serial dilutions. Values were independently determined twice using two different low-passaged African *M. ulcerans* strains (S1013 and S1047) (11).

The compound set, encompassing established tuberculosis drugs and antimicrobials as well as advanced development compounds, represented a broad chemical space of 54 clusters. Based on the IC_{90} values, the compounds were classified into six different activity categories that ranged from <0.1 μM to >10 μM. These values were compared with the corresponding values obtained earlier for *M. tuberculosis*, revealing that the activity patterns for *M. ulcerans* and *M. tuberculosis* vary substantially (Fig. 1). Only three compounds (represented by black dots in the gray-shaded area in Fig. 1), the two aminopyrazoles 9 and 10 and the pyrazolopyrimidine 11, were more active against *M. ulcerans* than they were against *M. tuberculosis*. Twenty-one compounds (rep-

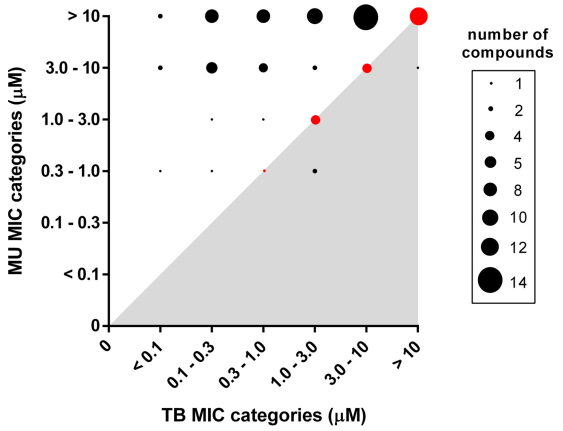

FIG 1 Comparison of activities of all 83 compounds tested on *M. ulcerans* and *M. tuberculosis* with MIC values grouped into six categories. The size of the individual circles indicates the number of compounds identified within the specific MIC range combination for *M. tuberculosis* and *M. ulcerans*. Compounds belonging to the same activity category for the two pathogens are marked in red. The three compounds showing higher activity against *M. ulcerans* than against *M. tuberculosis* are located in the gray-shaded area.

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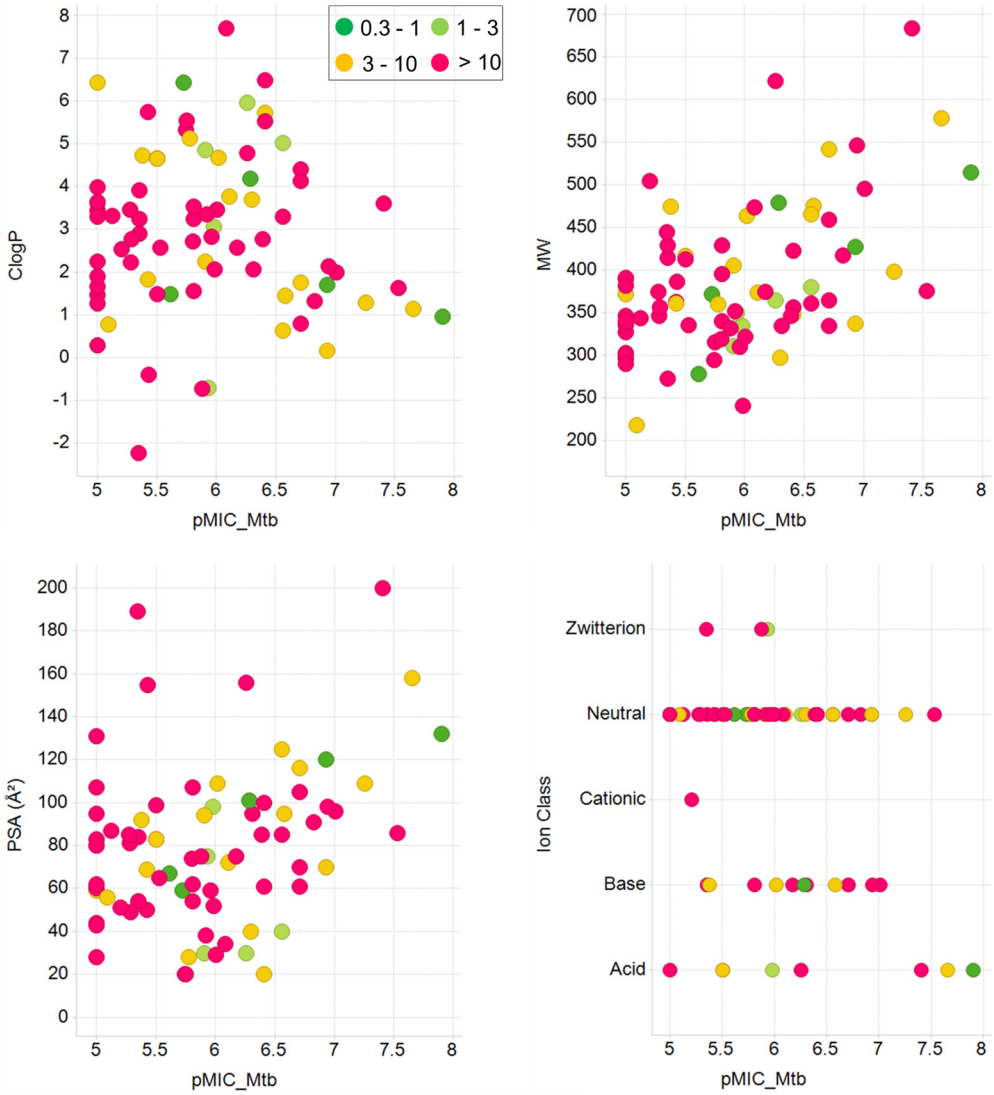
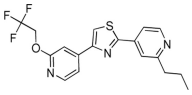
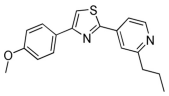
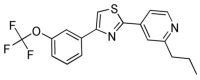
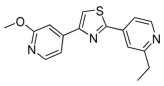
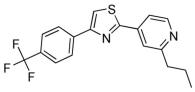
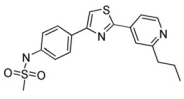
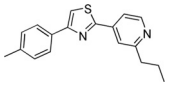
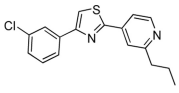
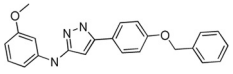
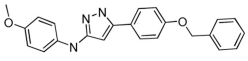
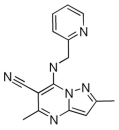
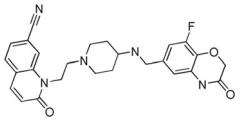
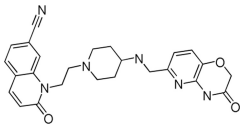


FIG 2 Physicochemical properties of the compounds analyzed in relation to activity on *M. tuberculosis* and *M. ulcerans*. Displayed are scatter plots of ClogP, molecular weight (MW), polar surface area (PSA), and ion class of all compounds tested. The *M. tuberculosis* activities are shown in log scale along the *x* axes, while the *M. ulcerans* activity ranges are depicted in different colors.

resented by red dots in Fig. 1) belonged to the same activity category for the two mycobacterial pathogens, whereas the majority (59/83, 71%) of compounds (represented by black dots in the white area) were more active against *M. tuberculosis* than they were against *M. ulcerans*. While none of the compounds tested had an IC_{90} value of ≤0.3 μM for *M. ulcerans*, 20/83 displayed activities of ≤0.3 μM for *M. tuberculosis* (Fig. 1). This lower sensitivity of *M. ulcerans* may be related to factors such as low growth rate, the extremely hydrophobic cell surface, and the production of an extracellular matrix. However, no general correlation between physicochemical properties of the compounds and differential IC_{90} values of *M. ulcerans*/*M. tuberculosis* was found (Fig. 2). *M. ulcerans* has undergone drastic genome reduction after emergence from the environmental mycobacterium *Mycobacterium marinum* (12, 13), and loss of genes and massive pseudogene accumulation may have caused loss of targets for some of the highly active anti-TB compounds.

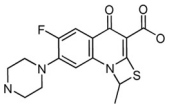
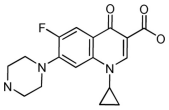
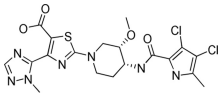
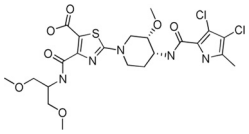
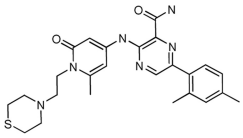
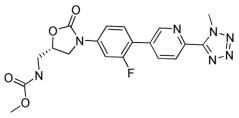
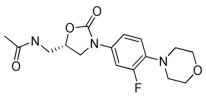
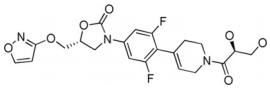
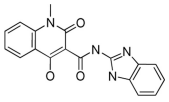
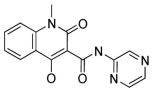
The 11 compounds with IC_{90} values of ≤3 μM against *M. ulcerans* originated from seven chemically diverse compound families of early and advanced stages of drug development (Table 1). As for the early stage compounds, 3/8 tested diarylthiazoles (Table 1, compounds 1 to 8) showed IC_{90} values of ≤3 μM against *M. ulcerans*. In *M. tuberculosis*, diarylthiazoles seem to target the two-component system PrrB/PrrA (14); they also appeared as a hit in a high-throughput screen against *Mycobacterium bovis* BCG (15). The diarylthiazole fatostatin (compound 7) acts as an inhibitor of sterol regulatory element-binding proteins (SREBPs) (16, 17). In addition, 1/2 aminopyrazoles (compound 9), the only pyrazolopyrimidine (compound 11), and 1/2 hydroxyquinolones (compound 22) tested were highly active against *M. ulcerans*. For quinolinyl pyrimidines and phenothiazines, inhibitors of the alternative type II NADH dehydrogenases in *M. tuberculosis* (18, 19), no activity against *M. ulcerans* was observed. Since the target enzymes are also present in *M. ulcerans*, the weaker activity of *M.*

TABLE 1 Structure, activity, and properties of the compound families active against *M. ulcerans*

Compound no.	Structure	*M. ulcerans* MIC (μM)	*M. tuberculosis* MIC (μM)	MW/ClogP	Compound feature/public domain ID
1		1–3	0.1–0.3	379/5.0	Fatostatin analog
2		1–3	1–3	310/4.8	Fatostatin analog ChEMBL1824662
3		1–3	0.3–1.0	364/5.9	Fatostatin analog
4		3–10	0.3–1.0	297/3.7	Fatostatin analog
5		3–10	0.3–1.0	348/5.7	Fatostatin analog
6		3–10	0.3–1.0	373/3.8	Fatostatin analog ChEMBL1824673
7		>10	1–3	294/5.3	Fatostatin ChEMBL1455549
8		>10	1–3	315/5.5	Fatostatin analog
9		0.3–1.0	1.0–3.0	371/6.4	Aminopyrazole
10		3–10	>10	371/6.4	Aminopyrazole
11		0.3–1.0	1–3	278/1.5	Pyrazolopyrimidine
12		3–10	0.1–0.3	475/1.4	GyrA_NBTI-analog
13		>10	0.1–0.3	458/0.8	GyrA_NBTI PubChem_15983305

(Continued on following page)

TABLE 1 (Continued)

Compound no.	Structure	*M. ulcerans* MIC (μM)	*M. tuberculosis* MIC (μM)	MW/ClogP	Compound feature/public domain ID
14		1–3	1–3	349/−0.7	GyrA_Fluoroquinolone Ulifloxacin ChEMBL345937
15		1–3	1–3	331/−0.7	GyrA_Fluoroquinolone Ciprofloxacin ChEMBL1077975
16		0.3–1.0	<0.1	514/1.0	GyrB_Pyrrolamide PubChem_25223515
17		3–10	<0.1	578/1.1	GyrB_Pyrrolamide PubChem_11996283
18		0.3–1.0	0.3–1.0	479/4.2	GyrB_Aminopyrazinamide
19		0.3–1.0	0.1–0.3	427/1.7	Oxazolidinone PubChem_10251911
20		3–10	0.1–0.3	337/0.2	Oxazolidinone linezolid ChEMBL126
21		3–10	0.1–0.3	465/0.6	Oxazolidinone posizolid/AZD5847 ChEMBL131854
22		1–3	1–3	333/3.1	Hydroxyquinolone PubChem_51423561
23		>10	>10	296/1.5	Hydroxyquinolone ChEMBL1310374

tuberculosis NADH dehydrogenase inhibitors against *M. ulcerans* may be attributed to reduced permeability.

Of the advanced antibacterial agents tested, the quinolones had differential activity on *M. ulcerans*. While a prulifloxacin analogue (compound 14) and ciprofloxacin (compound 15) exhibited good activity, this was not the case for two novel bacterial topoisomerase II inhibitors (NBTI; compounds 12 and 13) (20–22). Furthermore, two GyrB inhibitors, a pyrrolamide (compound 16) (23, 24), an aminopyrazinamide (compound 18) (25), and 1/3 of the tested oxazolidinones (compound 19) showed activity of ≤1 μM. The other two oxazolidinones, compounds 20 and 21, with compound 21 being a recent anti-TB clinical candidate interfering with protein translation by binding to the mycobacterial 50S ribosomal subunit (26), displayed only moderate activity (3 to 10

μM) against *M. ulcerans*. The natural product doxycycline, belonging to the family of tetracyclines, was not active against *M. ulcerans*. Members of the quinolone family targeting GyrA have already been evaluated *in vitro* and *in vivo*, and for ofloxacin, ciprofloxacin, sparfloxacin, moxifloxacin, and sitafloxacin, activity against *M. ulceran*s was shown (5, 27–31). Our results provide further evidence that the *M. ulcerans* DNA gyrase is a vulnerable target. In contrast, selected compounds of the nitroimidazole family (32, 33), including PA824 (34), did not show any activity against *M. ulcerans*, reconfirming published results (5).

Most of the compounds active against *M. ulcerans* were reported to be specific inhibitors and noncytotoxic. As a class, the diarylthiazoles (compounds 1 to 8) were shown to be inactive in cytotoxicity assays (14). Similarly, the gyrase inhibitors, such as quinolones (compounds 12 to 15) (20–22) and pyrrolocarboxamides (compound 16 and 17) (23, 24), are advanced leads from tuberculosis drug discovery programs and were demonstrated to be noncytotoxic. The other well-characterized advanced antibacterial compounds, oxazolidinones (compounds 19 to 21) (26), are specific inhibitors of bacterial protein biosynthesis and do not possess any cytotoxicity. Further profiling and structure activity relationship exploration is required to assess the full potential of these chemical classes as anti-BU candidates.

With the TB drug pipeline currently being filled with more potent and novel drug candidates, our results demonstrate that repurposing may in fact lead to the development of new treatment options for BU.

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