Supporting Information

Synthesis, Biological Evaluation, and Structure–Activity Relationships for 5-[(*E*)-2-Arylethenyl]-3-isoxazolecarboxylic Acid Alkyl Ester Derivatives as Valuable Antitubercular Chemotypes.

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1. HPLC purity determinations for the target compounds 6–49

Method 1. Flow rate = 1.4 mL/min; gradient eluation over 20 minutes, from 30% CH_3CN–H_2O to 100% CH_3CN with 0.05% TFA.

Method 2. Flow rate = 1.4 mL/min; gradient eluation over 20 minutes, from 10% CH_3CN–H_2O to 100% CH_3CN with 0.05% TFA.

Method 3. Flow rate = 1.4 mL/min; gradient eluation over 20 minutes, from 50% CH_3CN–H_2O to 70% CH_3CN with 0.05% TFA.

Table 1(SI). HPLC purity of the target compounds

Compd	HPLC				Compd	HPLC			
	Gradient Method	WL (nM)	t_R (min)	Purity (%)		Gradient Method	WL (nM)	t_R (min)	Purity (%)
6	2	254	15.4	98.1	**28**	1	280	7.85	98.4
7	1	254	1.91	97.9	**29**	1	280	8.48	97.9
8	1	280	9.86	97.8	**30**	1	280	9.85	97.9
9	3	254	7.54	99.1	**31**	1	280	9.63	99.3
10	1	280	10.9	99.0	**32**	1	280	8.97	96.7
11	1	280	10.6	99.7	**33**	2	254	8.85	97.2
12	1	254	10.1	98.0	**34a**	1	280	9.36	98.3
13	1	280	11.9	98.7	**34b**	1	280	10.2	97.7
14	1	280	11.3	96.9	**35**	1	280	11.2	99.1
15	1	280	11.9	98.5	**36**	1	280	10.4	99.0
16	1	280	10.1	99.8	**37**	3	254	1.51	99.5
17	1	280	11.0	99.2	**38**	3	254	4.4	97.2
18	1	280	10.7	99.4	**39**	1	280	8.1	100
19a	2	254	13.1	99.7	**40**	3	254	8.5	98.3
19b	2	254	14.0	99.2	**41**	1	280	10.6	99.8
20	1	254	10.4	97.2	**42**	1	280	11.0	99.4
21	1	280	11.8	99.2	**43**	1	280	14.3	99.2

22	1	254	12.5	98.8	**44**	1	280	14.1	99.5
23	1	280	11.9	99.5	**45**	1	280	14.0	99.5
24a	1	280	8.6	98.2	**46**	1	280	6.59	97.9
24b	2	254	13.2	98.1	**47**	2	254	13.6	95.3
25	1	280	11.8	99.5	**48**	2	254	13.9	99.1
26	1	280	10.2	99.5	**49**	1	280	11.5	99.6
27	1	280	3.09	98.5					

2. Brief description of the biological assays

Microplate Alamar Blue assay (MABA). Briefly, the test compound MICs against *Mtb* H_{37}RV (ATCC# 27294) were assessed by the MABA using rifampin, isoniazid and moxifloxacin as positive controls. Compound stock solutions were prepared in DMSO at a concentration of 12.8 mM, and the final test concentrations ranged from 128 μM to 0.5 μM. Two fold dilutions of compounds were prepared in Middlebrook 7H12 medium (7H9 broth containing 0.1% w/v casitone, 5.6 μg/mL palmitic acid, 5 mg/mL bovine serum albumin, 4 mg/mL catalase, filter-sterilized) in a volume of 100 μL in 96-well microplates (BD Optilux™,96-well Microplates , black/clear flat bottom). TB cultures (100 μL inoculum of 2 ×105 cfu/mL) was added, yielding a final testing volume of 200 μL. The plates were incubated at 37 °C. On the seventh day of incubation 12.5 μL of 20% Tween 80, and 20 μL of Alamar Blue (Invitrogen BioSource™) were added to the wells of test plate. After incubation at 37 °C for 16-24 h, fluorescence of the wells was measured (ex 530, em 590 nm). The MICs ware defined as the lowest concentration effecting a reduction in fluorescence of ≥ 90% relative to the mean of replicate bacteria-only controls.

Low-oxygen recovery assay (LORA). Briefly, a low-oxygen adapted culture of recombinant H_{37}Rv (pFCA-luxAB), expressing a *Vibrio harveyii* luciferase gene with an acetamidase promoter, was grown in a BiostatQ fermentor. Cells were collected on ice, washed in PBS, and stored at –80 °C. Circa 10^5 cfu/mL of thawed NRP cells were exposed to 2-fold serial dilutions of test compound in 7H9 broth in black 96-well plates, which were incubated 10 days anaerobically at 37 °C. Luminescence readings were obtained following a 28 h recovery in an aerobic environment (5% CO_2). The data were analyzed graphically, and the lowest concentration of test compound preventing metabolic recovery (90% reduction relative to untreated cultures) was determined as described previously.

Cytotoxicity assay. Cytotoxicity was determined by exposing different concentrations of samples to Vero cells. Samples were dissolved at 12.8 mM in DMSO. Geometric three-fold dilutions were performed in growth medium MEM (Gibco, Grand Island, NY), containing 10%

fetal bovine serum (HyClone, Logan, UT), 25 mM *N*-(2-hydroxyethyl)-piperazine-*N*'-2-ethanesulfonic acid (HEPES, Gibco), 0.2% $NaHCO_3$ (Gibco), and 2 mM glutamine (Irvine Scientific, Santa Ana, CA). Final DMSO concentrations did not exceed 1% v/v. Drug dilutions were distributed in duplicate in 96-well tissue culture plates (Becton Dickinson Labware, Lincoln Park, NJ) at a volume of 50 μL per well. An equal volume containing either 5×10^5 log phase Vero cells (CCL-81; American Type Culture Collection, Rockville, MD) was added to each well and the cultures were incubated at 37 °C in an atmosphere containing 5% of CO_2. After 72 h, cell viability was measured using the CellTiter 96 aqueous non-radioactive cell proliferation assay (Promega Corp., Madison, WI) according to the manufacturer's instructions. Absorbance at 490 nm was read in a Victor^2 multilabel reader (PerkinElmer). The IC_{50}s were determined using a curve-fitting program.

3. Activity toward other microorganisms

Table 2(SI). MICs of selected compounds toward selected microorganisms

Compd	*S. aureus*	*E. Coli*	*C. albicans*	*M. smegmatis*
	MIC (μM)			
9	> 100	> 100	> 100	> 100
30	> 100	> 100	> 100	94.4