

Revisiting the β -lactams for Tuberculosis Therapy: A Compound-Compound Synthetic Lethality Approach

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Supplemental material

Methods

Compound handling for verification: All compounds were dissolved in DMSO at 4.096 mg/mL and stored at -80°C. To complete activity verification and determine minimum inhibitory concentrations (MICs), the compounds were pre-diluted eight times with 7H9 without Tween 80. 100 μ l of diluted compounds were transferred to Column 2 (an empty well) of the 96-well plate. 100 μ l of diluted compounds were transferred to Column 3 (pre-dispensed 100 μ l of assay media). 2-fold serial dilutions were carried out from Column 3 to Column 11, resulting in a 256 μ g/mL to 0.5 μ g/mL concentration range. 100 μ l of diluted bacterial cells (ca. 10^4 CFU) were added to Column 2 through Column 11.

Compound handling for Structure-Activity Relationship (SAR): All compounds were dissolved in DMSO at 2.048 mg/mL and stored at -80°C. To complete activity

verification and determine minimum inhibitory concentrations (MICs), the compounds were pre-diluted eight times with 7H9 without Tween 80. 100 μ l of diluted compounds were transferred to Column 2 (an empty well) of the 96-well plate. 100 μ l of diluted compounds were transferred to Column 3 (pre-dispensed 100 μ l of assay media). 2-fold serial dilutions were carried out from Column 3 to Column 11, resulting in a 128 μ g/mL to 0.25 μ g/mL concentration range. 100 μ l of diluted bacterial cells (ca. 10^4 CFU) were added to Column 2 through Column 11.

Figure legends

Figure S1. Concentration response of Meropenem (upper panel) and Clavulanic acid (lower panel) on *M. tuberculosis* H37Rv.

Figure S2. Bioavailability assay by serum inhibition titration (SIT). Vehicle (0.5% CMC) was a negative control and 10 mg/kg of INH was a positive control. Compounds 3 (217A) and 4 (217B) were tested, in the presence of 2 μ g/mL of meropenem, at two dose levels (100 and 300 mg/kg) and three time points (15, 30, and 60 min) (A and B). Compound 6 (212E) was analyzed at 300 mg/kg and 30 min in the presence of 0, 2, 4, and 8 μ g/mL of meropenem (C-F). Bacterial growth was shown as a relative fluorescence unit.

Figure S3. AmiGO 1.8 gene ontology (GO) enrichment analysis. The enriched GO terms are shaded with p-values indicated. The darker the shade, the better the p-value is. The colors of the edges between the GO terms represent the relationships: “is_a” in blue, “part_of” in lightblue, “regulates” in black, and “positively_regulates” in green.

Supplementary tables

Table S1. Structure-Activity Relationship (SAR) for *N*-arylindole chemotype

Table S2. Structure-Activity Relationship (SAR) for Benzothiophene chemotype

Table S3. Gene expression profile upon *N*-arylindole (217A) treatment as determined by RNA-seq.

Table S4. Differential expression upon *N*-arylindole (217A) treatment as analyzed by RNA-seq. Linear fold change (FC) cut off was set at ≥ 2.0 or ≤ -2.0 . Significant differentially expressed genes ($p \leq 0.05$) are highlighted in light brown (up-regulated: $n=15$) or light green (down-regulated: $n=34$).

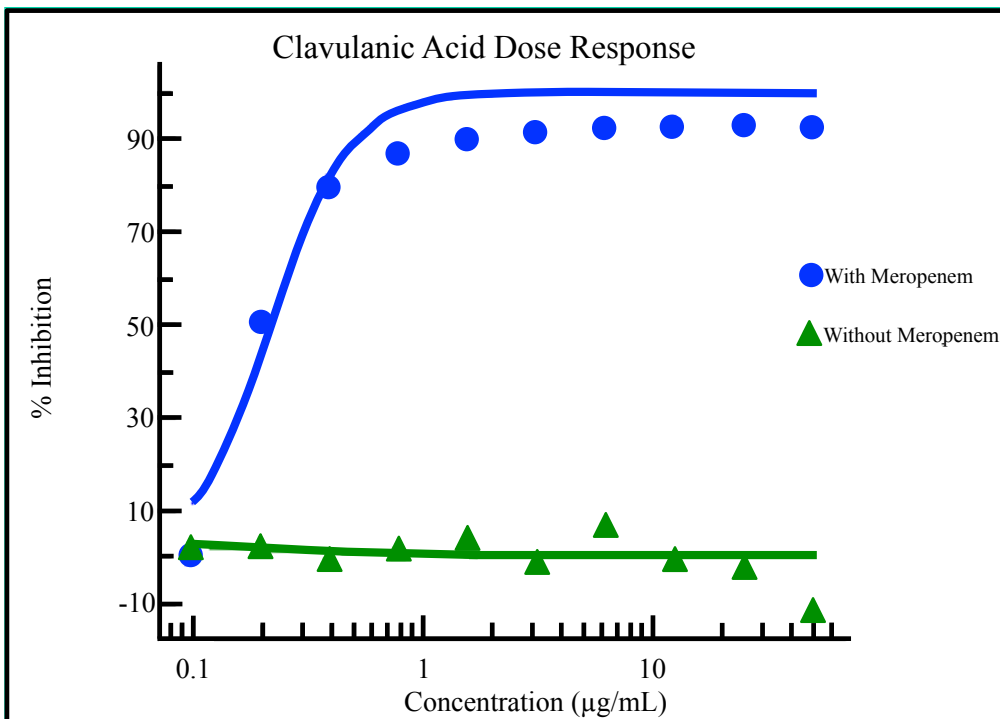
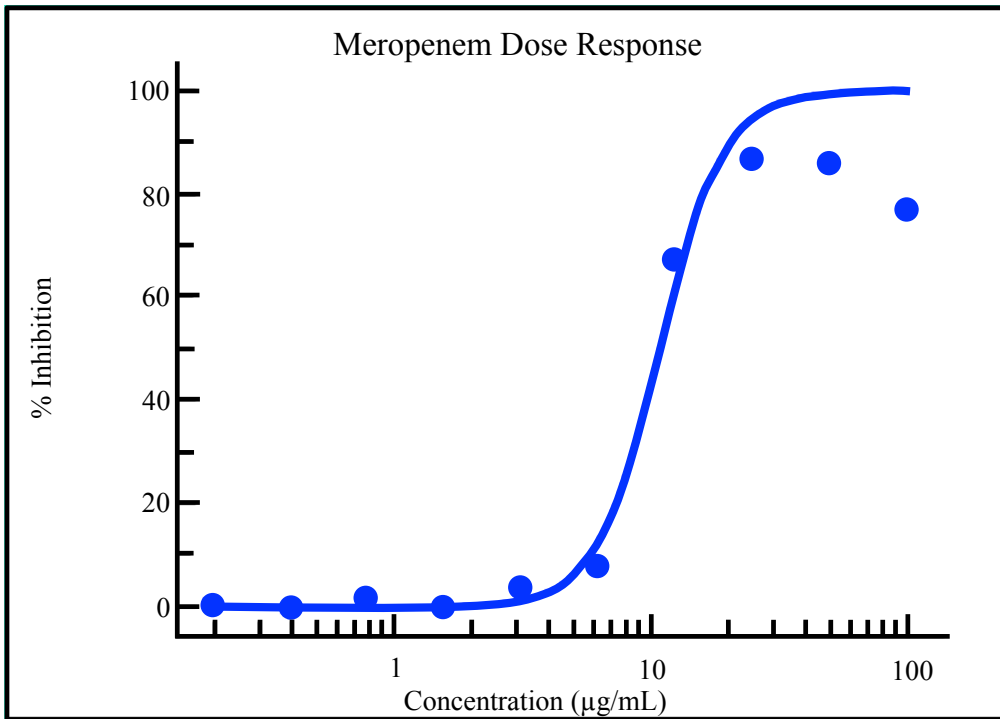


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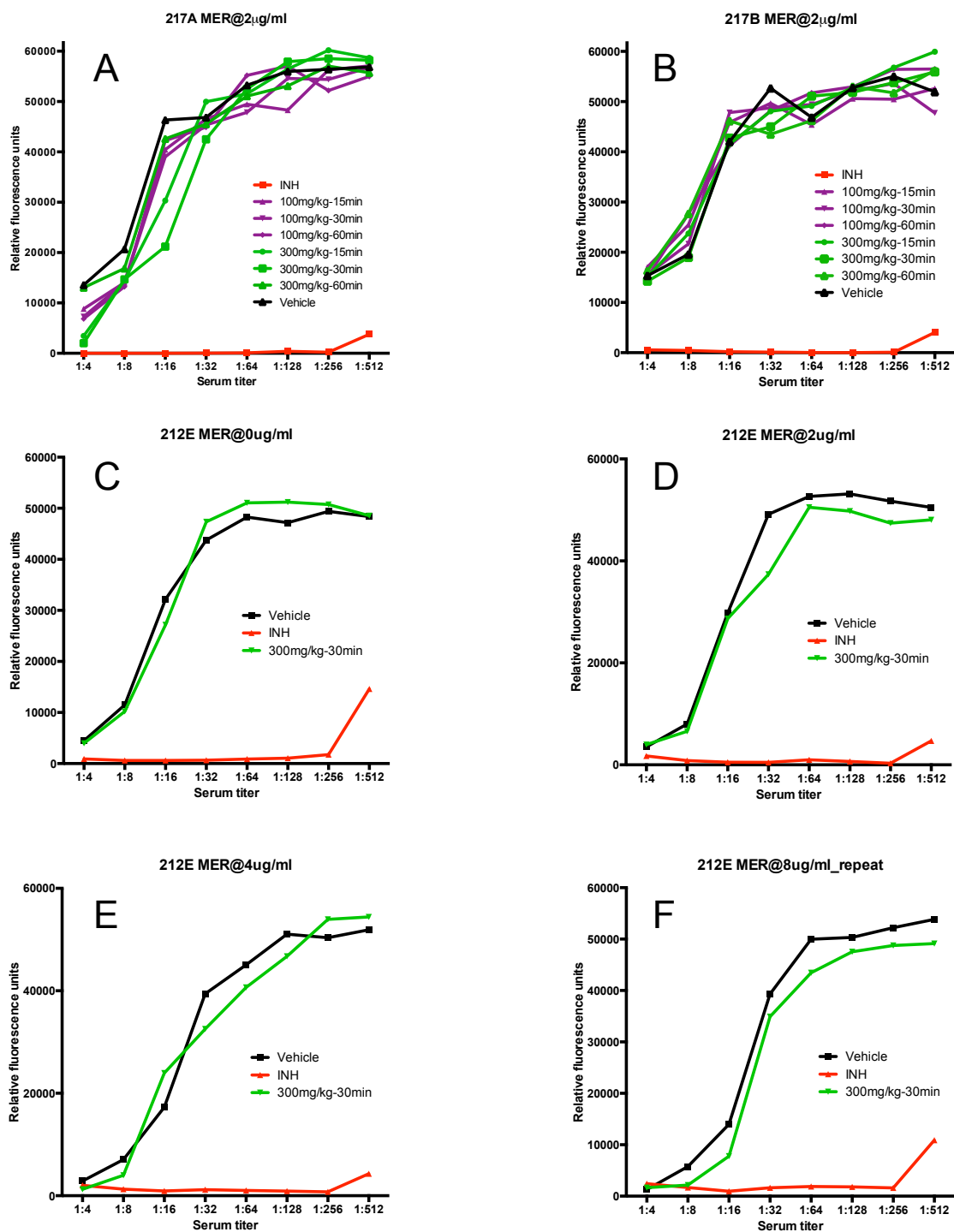
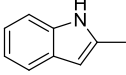
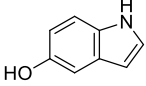
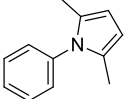
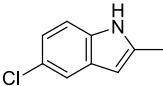
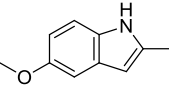
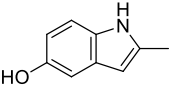
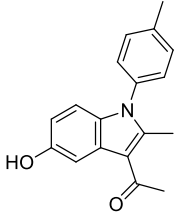
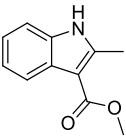
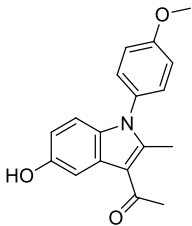
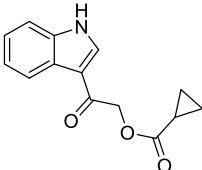


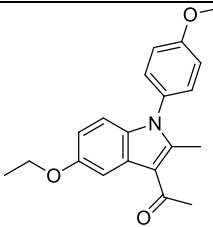
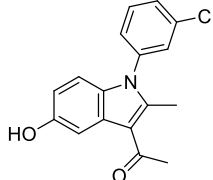
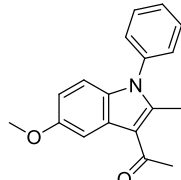
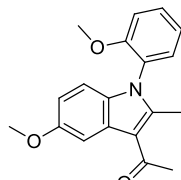
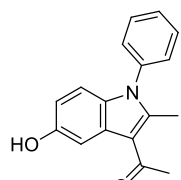
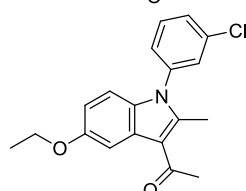
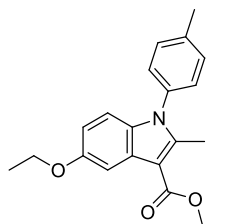
Figure S2. Bioavailability assay by serum inhibition titration (SIT). Vehicle (0.5% CMC) was a negative control and 10 mg/kg of INH was a positive control. Compounds 3 (217A)

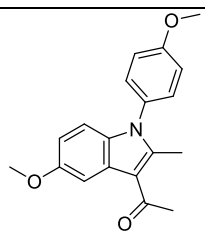
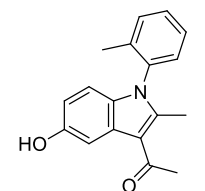
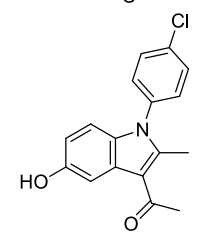
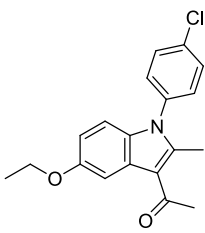
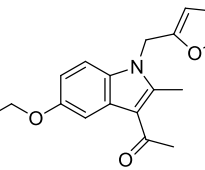
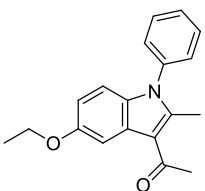
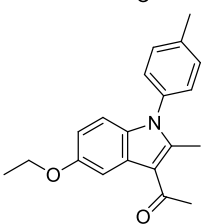
and 4 (217B) were tested, in the presence of 2 $\mu\text{g/mL}$ of meropenem, at two dose levels (100 and 300 mg/kg) and three time points (15, 30, and 60 min) (A and B). Compound 6 (212E) was analyzed at 300 mg/kg and 30 min in the presence of 0, 2, 4, and 8 $\mu\text{g/mL}$ of meropenem (C-F). Bacterial growth was shown as a relative fluorescence unit.

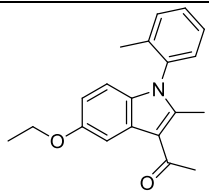
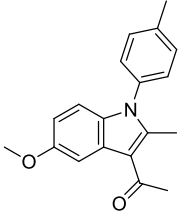
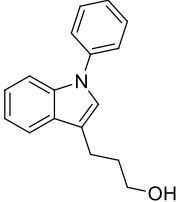
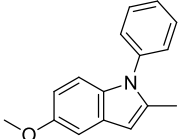
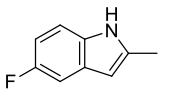
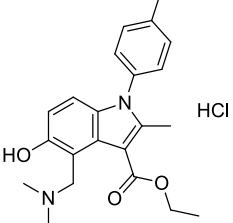
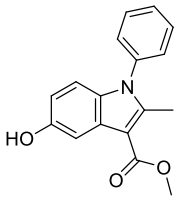
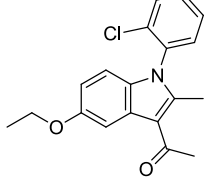
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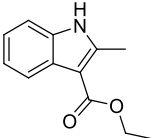
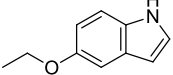
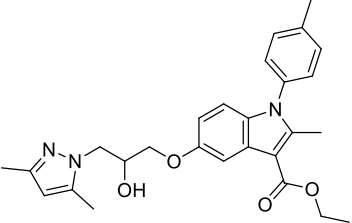
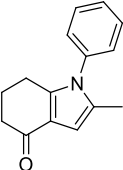
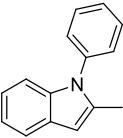
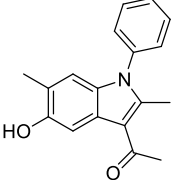
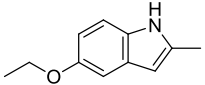
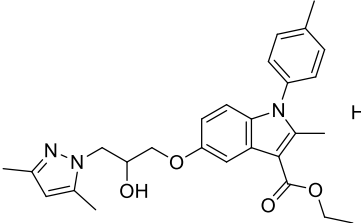
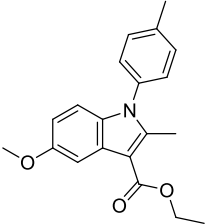
Table S1. Synthetic lethality structure-activity relationship screen on 68 *N*-arylidole derivatives in the presence of 0 or 4 $\mu\text{g/mL}$ of meropenem (MIC: $\mu\text{g/mL}$)

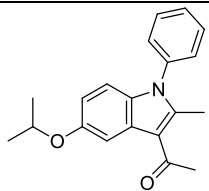
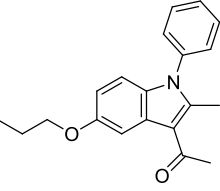
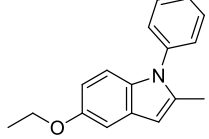
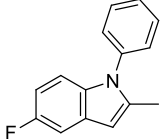
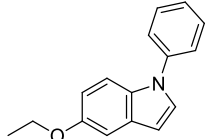
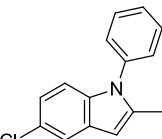
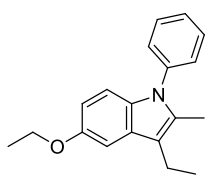
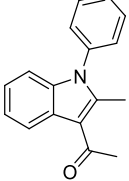
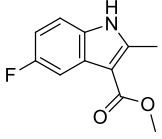
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2		16054	≥ 128	64
3		66518	64	64
4		70636	64	32
5		70642	≥ 128	≥ 128
6		83336	≥ 128	64
7		537520	≥ 128	≥ 128
8		594174	16	32
9		658914	≥ 128	≥ 128
10		665108	≥ 256	16

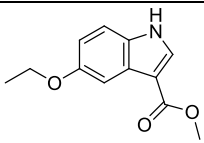
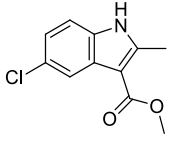
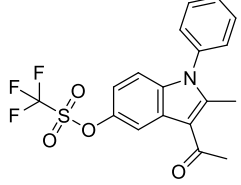
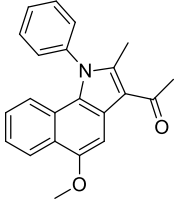
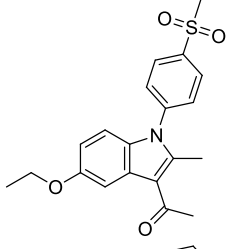
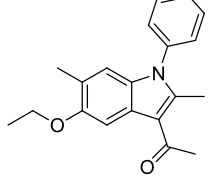
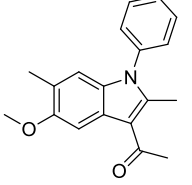
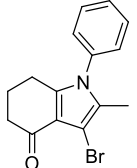
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12		708055	≥ 128	64
13		746058	≥ 256	8
14		762508	64	32
15		781247	≥ 128	64
16		804894	4	8
17		827826	16	8

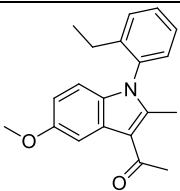
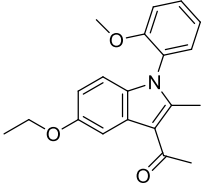
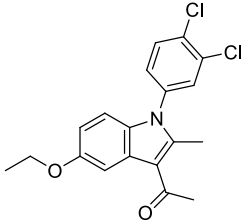
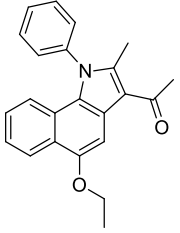
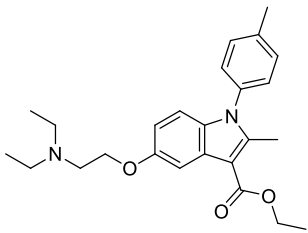
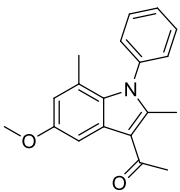
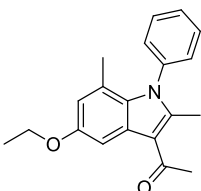
Entry	Structure	PubChem CID	MIC at 0 $\mu\text{g/mL}$ of MER	MIC at 4 $\mu\text{g/mL}$ of MER
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19		873088	≥ 128	≥ 128
20		878953	≥ 128	≥ 128
21		927090	≥ 128	64
22		931244	64	64
23		940424	≥ 128	8
24		947393	≥ 128	≥ 128

Entry	Structure	PubChem CID	MIC at 0 $\mu\text{g/mL}$ of MER	MIC at 4 $\mu\text{g/mL}$ of MER
25		949476	8	8
26		949641	≥ 128	≥ 128
27		2055004	32	16
28		2329649	32	8
29		2778715	64	64
30		2882229	8	8
31		3237314	64	64
32		3694883	16	8

Entry	Structure	PubChem CID	MIC at 0 $\mu\text{g/mL}$ of MER	MIC at 4 $\mu\text{g/mL}$ of MER
33		4072358	16	16
34		4777667	≥ 128	64
35		5175771	32	64
36		10846825	≥ 128	≥ 128
37		12682603	64	64
38		14004102	≥ 128	64
39		15157643	64	64
40		HCl 18558847	64	32
41		21234080	16	8

Entry	Structure	PubChem CID	MIC at 0 $\mu\text{g/mL}$ of MER	MIC at 4 $\mu\text{g/mL}$ of MER
42		46891659	16	16
43		46891660	≥ 128	64
44		49842879	16	8
45		49842880	≥ 128	64
46		49842881	16	16
47		49842882	64	32
48		49842883	32	2
49		49867951	≥ 128	32
50		49867952	32	32

Entry	Structure	PubChem CID	MIC at 0 $\mu\text{g/mL}$ of MER	MIC at 4 $\mu\text{g/mL}$ of MER
51		49867953	≥ 128	≥ 128
52		49867954	64	64
53		49867955	≥ 128	≥ 128
54		53308687	≥ 128	≥ 128
55		53308688	64	32
56		53308689	≥ 128	64
57		53308690	≥ 128	≥ 128
58		53308691	≥ 128	64

Entry	Structure	PubChem CID	MIC at 0 $\mu\text{g/mL}$ of MER	MIC at 4 $\mu\text{g/mL}$ of MER
59		53308692	16	8
60		53308693	32	16
61		53308694	≥ 128	64
62		53308695	≥ 128	≥ 128
63		53308696	8	4
64		53308697	≥ 128	≥ 128
65		53308698	≥ 128	64

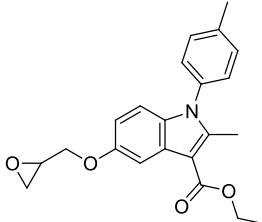
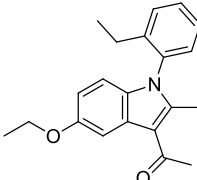
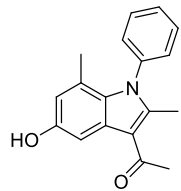
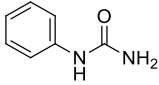
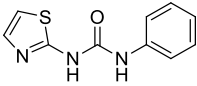
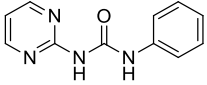
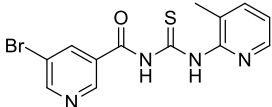
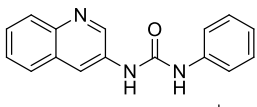
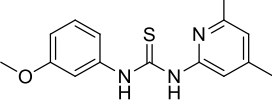
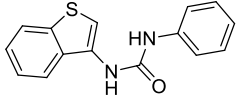
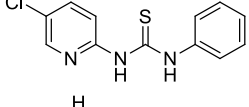
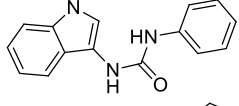
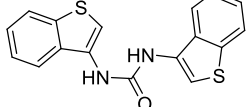
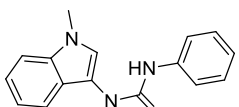
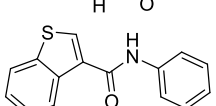
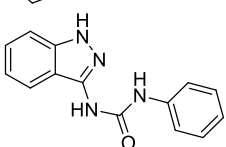
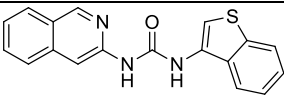
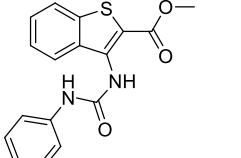
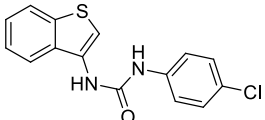
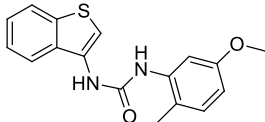
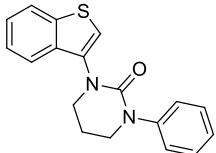
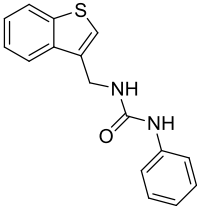
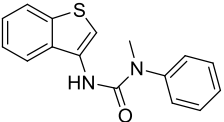
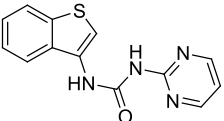
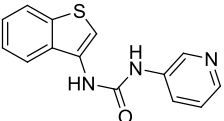
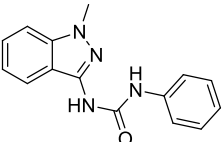
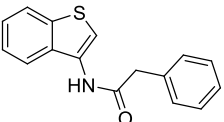
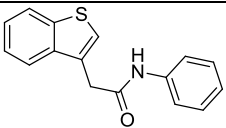
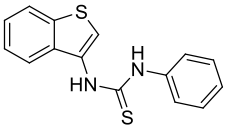
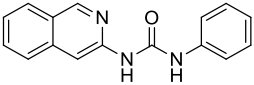
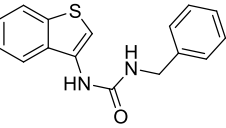
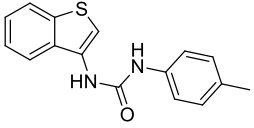
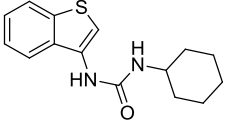
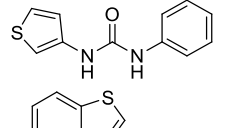
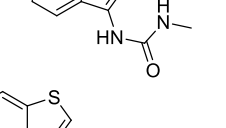
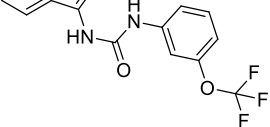
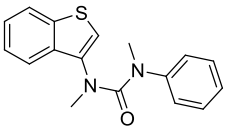
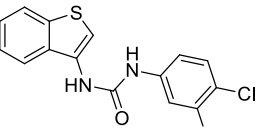
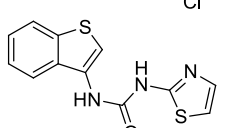
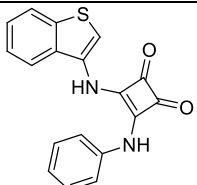
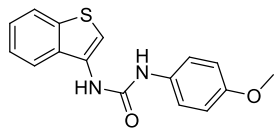
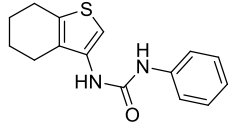
Entry	Structure	PubChem CID	MIC at 0 $\mu\text{g/mL}$ of MER	MIC at 4 $\mu\text{g/mL}$ of MER
66		53308699	32	32
67		53308700	8	4
68		53308701	≥ 128	≥ 128
69	Rifampin (RIF)	5381226	0.125	0.125
70	Isoniazid (INH)	3767	0.02	0.02

Table S2. Synthetic lethality structure-activity relationship screening on 39 bezothiophene derivatives (MIC in $\mu\text{g/mL}$ at 0, 2 and 4 $\mu\text{g/mL}$ of meropenem)

Entry	Structure	PubChem CID	MIC at 0 $\mu\text{g/mL}$ of MER	MIC at 2 $\mu\text{g/mL}$ of MER	MIC at 4 $\mu\text{g/mL}$ of MER
1		6145	$\geq 128^*$	$\geq 128^*$	$\geq 128^*$
2		283977	64	64	64
3		853562	$\geq 128^*$	$\geq 128^*$	$\geq 128^*$
4		1302558	64	64	64
5		2308931	$\geq 128^*$	$\geq 128^*$	64
6		2731492	$\geq 128^*$	$\geq 128^*$	$\geq 128^*$
7		2813497	64	2	2
8		3122100	64	32	16
9		7616028	32	64	64
10		13182769	8	2	1
11		16810982	32	32	32
12		60705040	64	64	64
13		62686029	64	$\geq 128^*$	$\geq 128^*$

Entry	Structure	PubChem CID	MIC at 0 $\mu\text{g/mL}$ of MER	MIC at 2 $\mu\text{g/mL}$ of MER	MIC at 4 $\mu\text{g/mL}$ of MER
14		66545913	$\geq 128^*$	$\geq 128^*$	$\geq 128^*$
15		66545914	64	64	64
16		66545915	2	2	2
17		66545916	4	2	2
18		66545917	64	64	$\geq 128^*$
19		66545918	$\geq 128^*$	$\geq 128^*$	$\geq 128^*$
20		66545919	32	16	16
21		66545920	$\geq 128^*$	$\geq 128^*$	$\geq 128^*$
22		66545921	64	16	4
23		66545922	$\geq 128^*$	64	64
24		66545923	32	16	16

Entry	Structure	PubChem CID	MIC at 0 $\mu\text{g/mL}$ of MER	MIC at 2 $\mu\text{g/mL}$ of MER	MIC at 4 $\mu\text{g/mL}$ of MER
25		66545924	32	32	32
26		66545925	16	16	16
27		66545926	$\geq 128^*$	$\geq 128^*$	32
28		66545927	64	16	8
29		66545928	16	4	2
30		66545929	2	2	1
31		66545930	$\geq 128^*$	$\geq 128^*$	$\geq 128^*$
32		66545931	64	32	64
33		66545932	16	16	16
34		66545933	64	64	64
35		66545934	2	2	2
36		66545935	$\geq 128^*$	$\geq 128^*$	$\geq 128^*$

Entry	Structure	PubChem CID	MIC at 0 $\mu\text{g/mL}$ of MER	MIC at 2 $\mu\text{g/mL}$ of MER	MIC at 4 $\mu\text{g/mL}$ of MER
37		66545936	64	16	16
38		66545937	$\geq 128^*$	$\geq 128^*$	64
39		66545938	8	8	4