ChemMedChem

Supporting Information

Structure-Activity Relationship and Mode-Of-Action Studies Highlight 1-(4-Biphenylylmethyl)-1*H*-imidazole-Derived Small Molecules as Potent CYP121 Inhibitors

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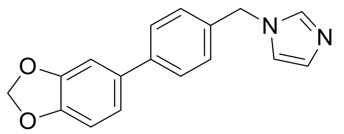
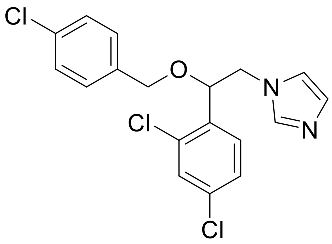
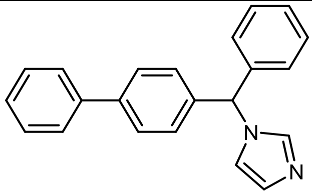
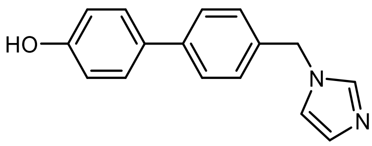
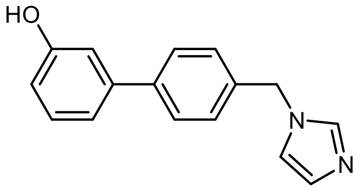
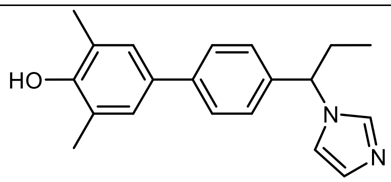
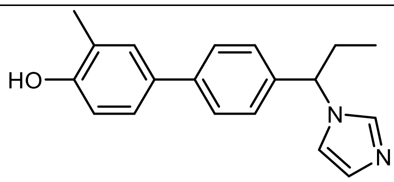
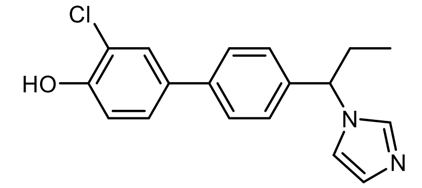
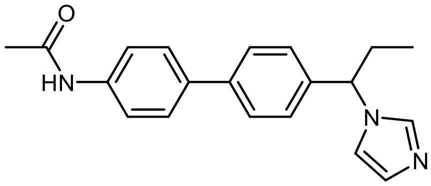
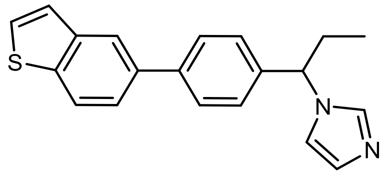
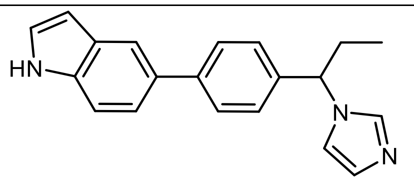
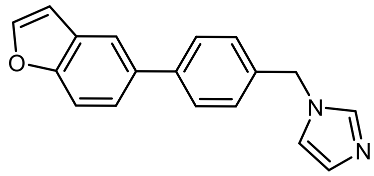
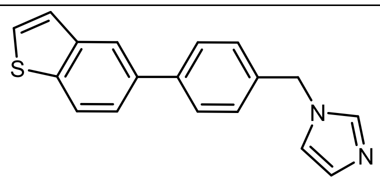
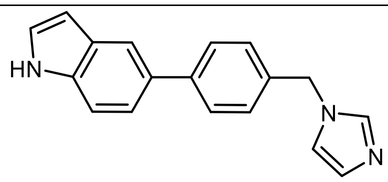
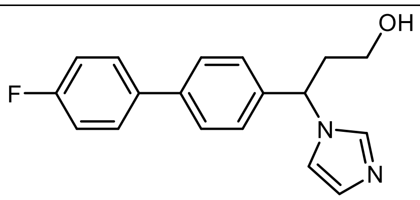
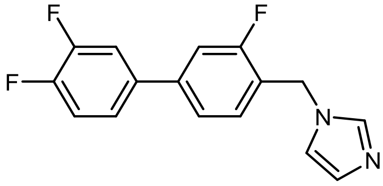
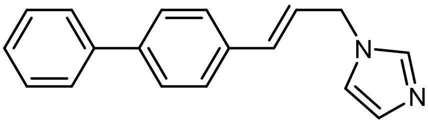
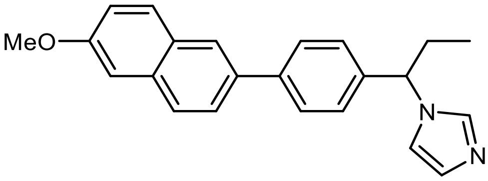
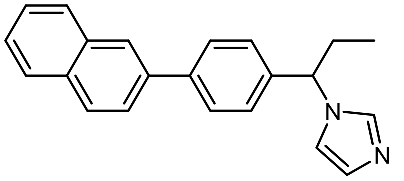
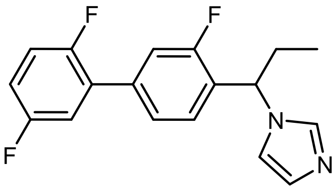
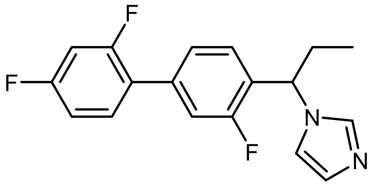
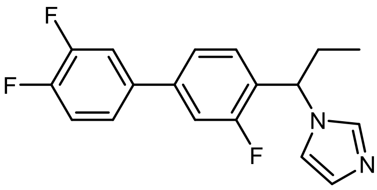
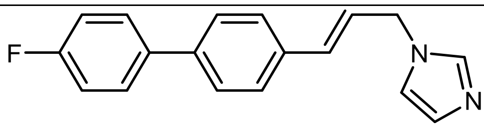
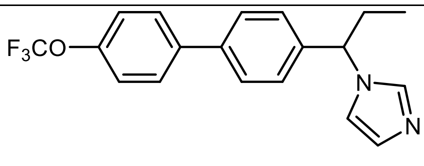
Supporting Information

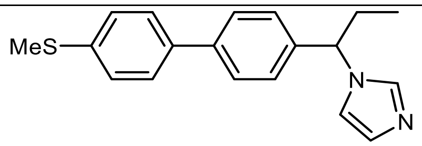
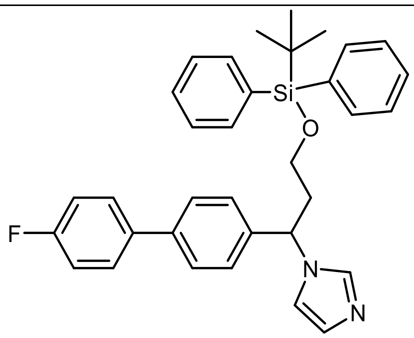
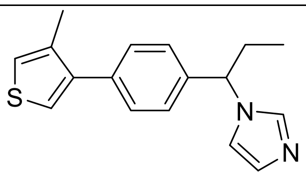
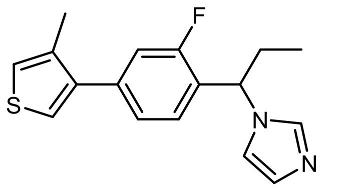
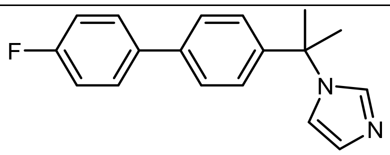
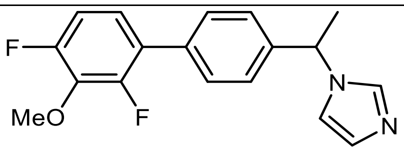
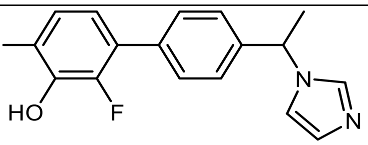
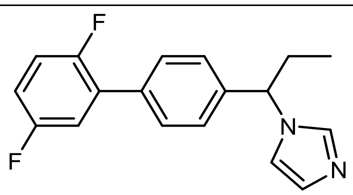
1. Screening Overview: K_D and MIC_{50} values

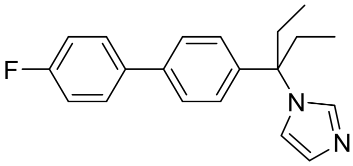
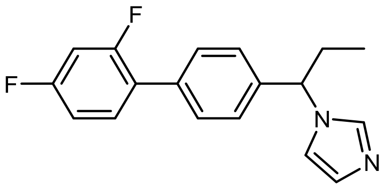
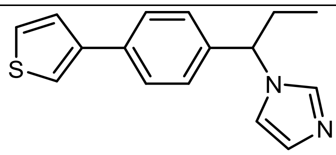
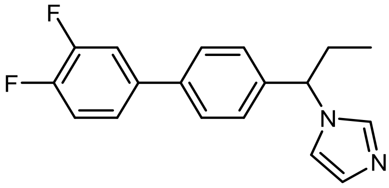
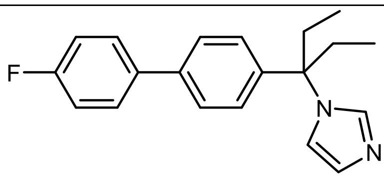
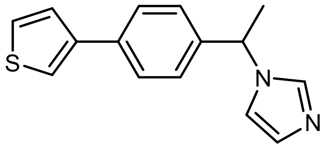
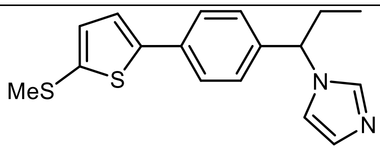
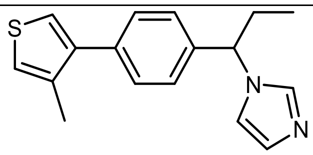
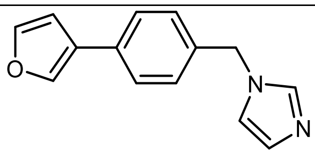
Table S1: Determined K_D values with standard deviation and MIC_{50} values against *M. bovis* BCG. K_D ± STD for compounds appearing in the main text was calculated from 4 biological replicates, for all other compounds from 2 biological replicates.

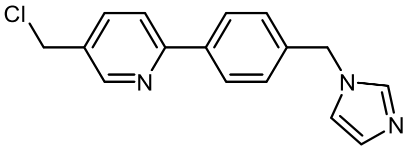
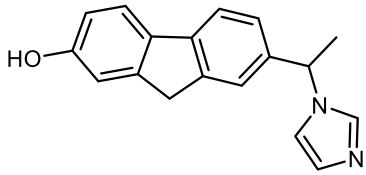
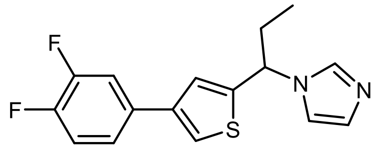
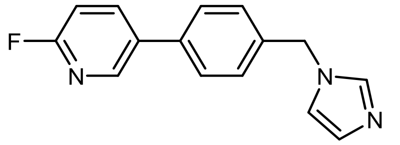
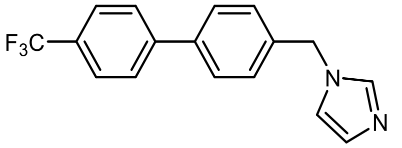
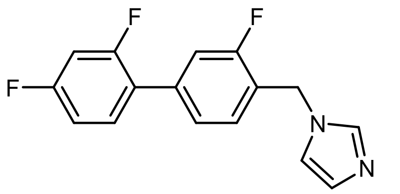
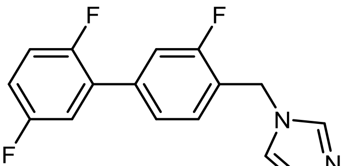
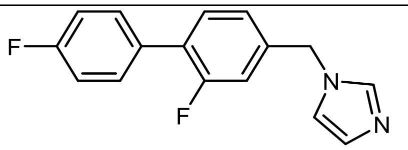
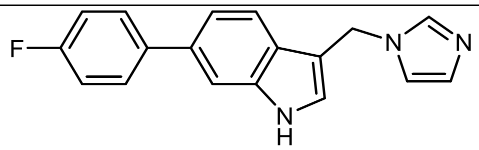
Compound	Structure	K_D ± STD [µM]	MIC_{50} [µM]
I:47		5.4 ± 1.0	4.0
Econazole		2.8 ± 0.2	12.7
L1		1.4 ± 0.22	
L2		7.4 ± 2.3	
L3		34.0 ± 8.9	
L4			
L5		6.5 ± 2.7	

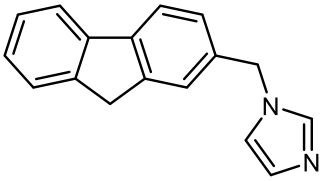
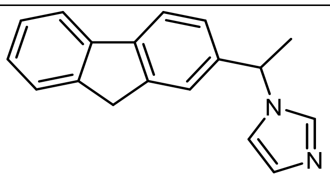
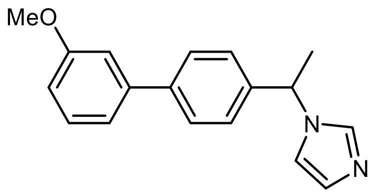
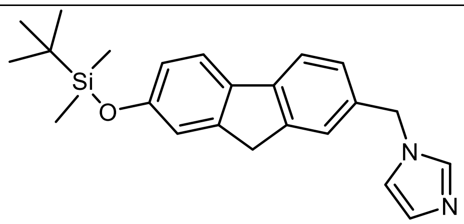
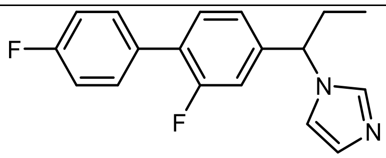
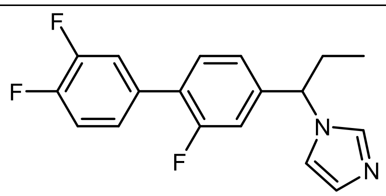
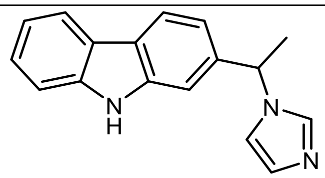
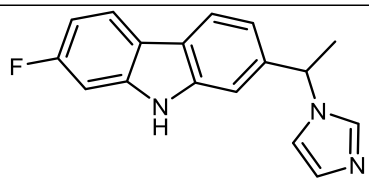
L6		19.4 ± 13.4	
L7		2.0 ± 0.44	
L8		2.7 ± 0.52	18.4
L9		2.9 ± 1.2	36.1
L10		2.9 ± 0.38	2.6
L11		1.5 ± 0.46	
L12		2.4 ± 0.19	7.6
L13		14.0 ± 2.0	

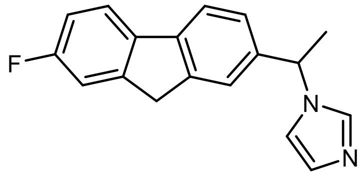
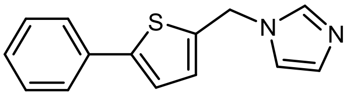
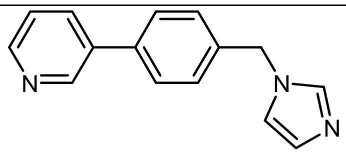
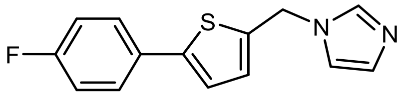
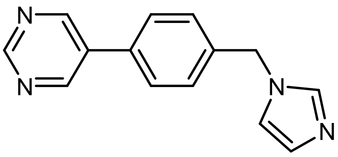
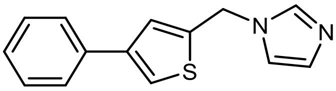
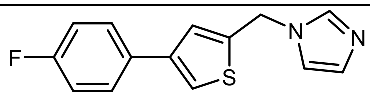
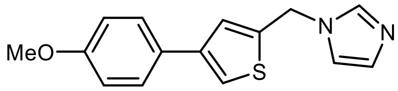
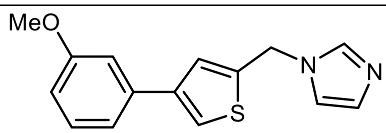
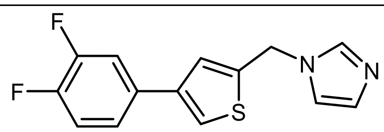
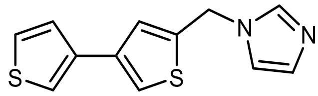
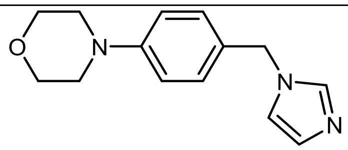
L14		6.4 ± 0.85	2.7
L15		4.3 ± 0.78	1.8
L16		0.5 ± 0.09	7.4
L17		1.4 ± 0.20	
L18		8.0 ± 1.3	
L19		4.8 ± 0.84	20.2
L20		5.0 ± 1.5	
L21		5.9 ± 0.55	1.5
L22		5.0 ± 0.49	

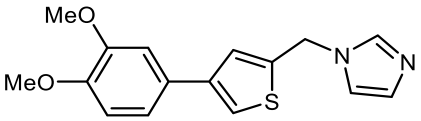
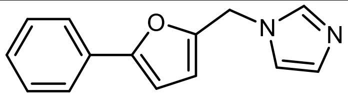
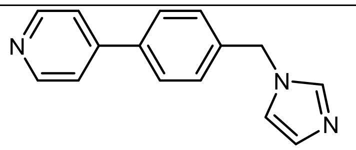
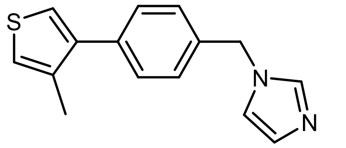
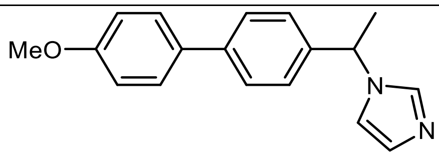
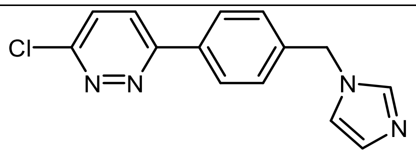
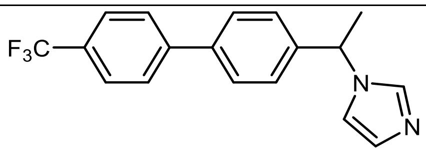
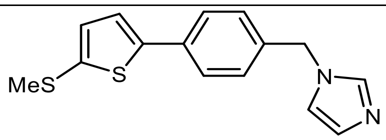
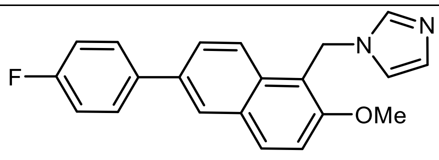
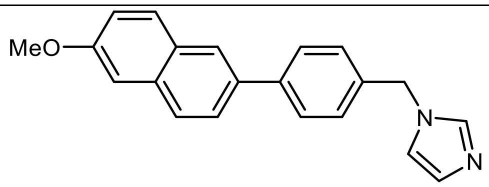
L23		3.0 ± 0.74	
L24		3.1 ± 0.73	
L25			
L26		9.0 ± 1.3	
L27		8.9 ± 0.54	
L28			
L29			
L30		7.5 ± 0.86	

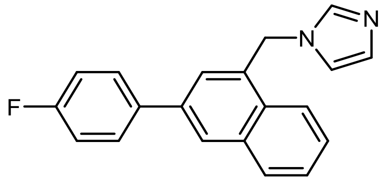
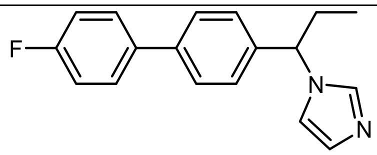
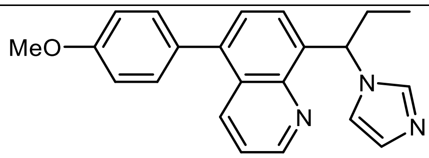
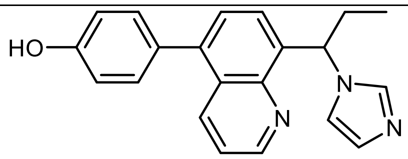
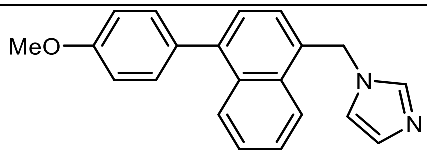
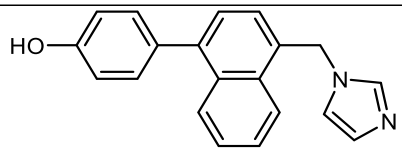
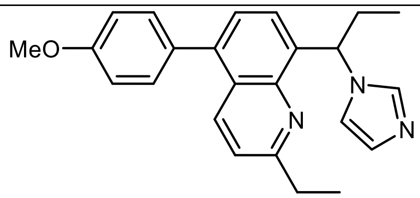
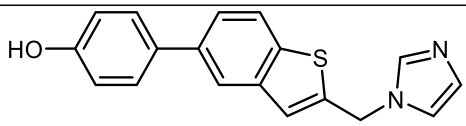
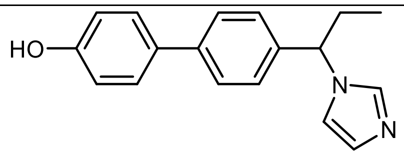
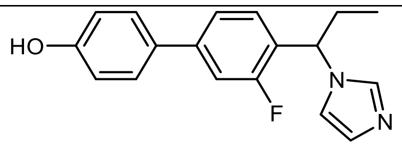
L31			
L32		8.7 ± 1.2	
L33		18.3 ± 1.7	
L34		4.1 ± 0.91	
L35		2.5 ± 0.57	
L36		16.9 ± 2.2	
L37		4.5 ± 0.88	
L38		9.4 ± 0.66	
L39		32.9 ± 11.1	

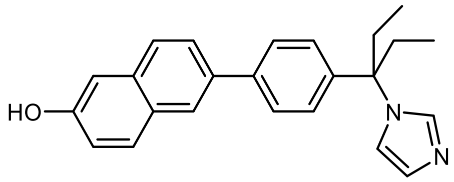
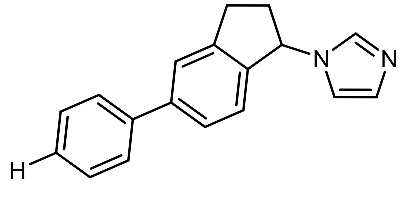
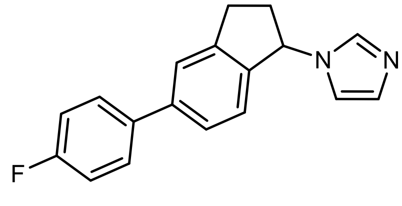
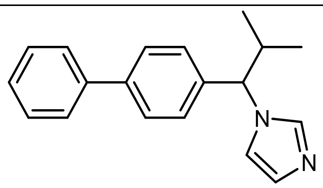
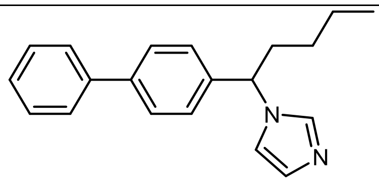
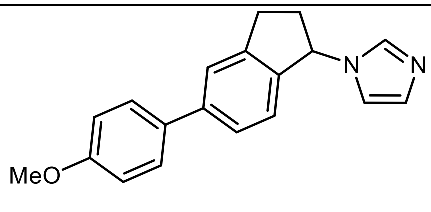
L40			
L41		5.3 ± 0.68	13.2
L42		6.3 ± 1.1	
L43			
L44		5.6 ± 0.93	2.4
L45		12.6 ± 2.0	3.4
L46		9.2 ± 1.5	2.9
L47		7.4 ± 0.80	6.0
L48		5.9 ± 2.0	6.1

L49		6.0 ± 1.1	6.7
L50		4.8 ± 0.78	
L51			
L52			
L53		5.3 ± 1.2	
L54		4.6 ± 0.70	
L55		8.7 ± 1.3	
L56		7.5 ± 0.80	

L57		17.0 ± 2.0	
L58		19.6 ± 4.4	
L59			
L60		9.1 ± 1.5	7.0
L61			
L62		28.3 ± 3.7	
L63		18.9 ± 3.1	
L64		16.0 ± 3.9	
L65		11.2 ± 1.9	
L66		5.3 ± 1.1	5.1
L67			
L68			

L69		62.7 ± 12.4	
L70		33.7 ± 5.6	
L71			
L72		21.7 ± 2.0	
L73		10.9 ± 2.0	4.5
L74			
L75		8.6 ± 2.0	8.6
L76		6.6 ± 0.97	3.2
L77		1.4 ± 0.23	14.7
L78		0.6 ± 0.17	2.4

L79		1.3 ± 0.27	29.8
L80		10.1 ± 1.5	
L81		2.5 ± 0.70	
L82		1.7 ± 0.37	
L83		1.3 ± 0.11	
L84		1.1 ± 0.22	36.4
L85		1.5 ± 0.18	
L86		4.0 ± 0.61	
L87		1.8 ± 0.32	
L88		3.8 ± 0.85	

L89		0.3 ± 0.09	21.7
L90		5.5 ± 0.9	6.9
L91		7.3 ± 1.8	10.9
L92		2.9 ± 0.59	
L93		1.9 ± 0.39	
L94		4.7 ± 0.8	3.3

2. Chemical Synthesis

2.1. Method A: Suzuki coupling

2.1.1: 4'-formyl-[1,1'-biphenyl]-4-carbonitrile (**S7b**): Synthesized according to method A using 4-bromobenzaldehyde (1.35 mmol; 250 mg) and 4-Cyanophenylboronic acid (1.75 mmol; 257 mg). Yield: 263 mg (94 %) of a white solid. R_f = 0.78 (hexane : EtOAc = 7 : 3). ^{1}H-NMR (300 MHz, CHLOROFORM-d_3): δ (ppm) = 7.70 - 7.83 (m, 6 H); 7.97 - 8.05 (m, 2 H); 10.10 (s, 1 H). ^{13}C-NMR (300 MHz, CHLOROFORM-d_3): δ (ppm) = 112.2; 118.5; 127.9; 128.0; 130.4; 132.8; 136.1; 144.1; 144.9; 191.6. LC-MS (ESI): R_t = 8.39 min; *m/z* = 415.28 $[2M+H]^+$.

2.1.2: 4-(benzo[d][1,3]dioxol-5-yl)-2-fluorobenzaldehyde (**S8b**): Synthesized according to method A using 4-bromo-2-fluorobenzaldehyde (1.35 mmol; 274 mg) and 3,4-methylenedioxyphenyl boronic acid (1.75 mmol; 290 mg). Yield: 311 mg (94 %) of a white-yellow solid. R_f = 0.47 (hexane : EtOAc = 95 : 5). ^{1}H-NMR (300 MHz, CHLOROFORM-d_3): δ (ppm) = 6.05 (s, 2 H); 6.92 (d, *J*=8.1 Hz, 1 H); 7.07 - 7.15 (m, 2 H); 7.32 (dd, *J*=11.8, 1.6 Hz, 1 H); 7.43 (dd, *J*=8.1, 1.0 Hz, 1 H); 7.87 - 7.94 (m, 1 H); 10.37 (s, 1 H). ^{13}C-NMR (300 MHz, CHLOROFORM-d_3): δ (ppm) = 101.6 (s); 107.4 (s); 108.9 (s); 114.3 (d, *J*=22.35 Hz); 121.3 (s); 122.4 (d, *J*=8.9 Hz); 122.9 (d, *J*=2.9 Hz); 129.1 (d, *J*=2.9 Hz); 132.7 (d, *J*=2.2 Hz); 148.5 (s); 148.6 (s); 149.3 (d, *J*=8.9 Hz); 164.9 (d, *J*=257.8 Hz); 186.7 (d, *J*=5.9 Hz). LC-MS (ESI): R_t = 8.38 min; *m/z* = 244.98 $[M+H]^+$.

2.2 Method B: Grignard reaction

2.2.1: 1-(3'-fluoro-4'-methoxy-[1,1'-biphenyl]-4-yl)prop-2-en-1-ol (**S6a**): Synthesized according to method B using **S6b** (1.17 mmol; 270 mg) and vinylmagnesium bromide (0.7 M in THF; 1.40 mmol; 2.0 mL). Yield: 182 mg (60 %) of a yellow solid. R_f = 0.29 (hexane : EtOAc = 8 : 2). ^{1}H-NMR (300 MHz, CHLOROFORM-d_3): δ (ppm) = 3.94 (s, 3 H); 5.21 - 5.29 (m, 2 H); 5.40 (d, *J*=17.0 Hz, 1 H); 6.09 (ddd, *J*=16.8, 10.4, 6.0 Hz, 1 H); 7.00 - 7.07 (m, 1 H); 7.29 - 7.37 (m, 2 H); 7.42 - 7.47 (m, 2 H); 7.50 - 7.57 (m, 2 H). ^{13}C-NMR (300 MHz, CHLOROFORM-d_3): δ (ppm) = 56.4 (s); 75.09 (s); 113.7 (d, *J*=2.2 Hz); 114.7 (d, *J*=19.4 Hz); 115.3 (s); 122.6 (d, *J*=3.0 Hz); 126.8 (s); 126.9 (s); 134.0 (d, *J*=6.0 Hz); 139.2 (d, *J*=1.5 Hz); 140.1 (s); 141.6 (s); 147.1 (d, *J*=10.4 Hz); 152.6 (d, *J*=244.4 Hz). LC-MS (ESI): R_t = 7.21 min; *m/z* = 241.14 $[M-OH]^+$.

2.2.2: 4'-(1-hydroxyallyl)-[1,1'-biphenyl]-4-carbonitrile (**S7a**): Synthesized according to method B using **S7b** (1.21 mmol; 250 mg) and vinylmagnesium bromide (0.7 M in THF; 1.45 mmol; 2.1 mL). Yield: 168 mg (59 %) of a yellow solid. R_f = 0.27 (hexane : EtOAc = 8 : 2). ^{1}H-NMR (300 MHz, CHLOROFORM-d_3): δ (ppm) = 5.22 - 5.32 (m, 2 H); 5.41 (d, *J*=17.0 Hz, 1 H); 6.08 (ddd, *J*=16.9, 10.4, 6.1 Hz, 1 H); 7.51 (d, *J*=8.2 Hz, 2 H); 7.60 (d, *J*=8.2 Hz, 2 H); 7.71 (dd, *J*=15.1, 8.2 Hz, 4 H). ^{13}C-NMR (300 MHz, CHLOROFORM-d_3): δ (ppm) = 75.0; 110.9; 115.6; 118.9; 127.0; 127.4; 127.7; 132.6; 138.5; 140.0; 143.1; 145.3. LC-MS (ESI): R_t = 7.10 min; *m/z* = 218.15 $[M-OH]^+$.

2.2.3: 1-(4-(benzo[d][1,3]dioxol-5-yl)-2-fluorophenyl)prop-2-en-1-ol (**S8a**): Synthesized according to method B using **S8b** (1.23 mmol; 300 mg) and vinylmagnesium bromide (0.7 M in THF; 1.47 mmol; 2.1 mL). Yield: 127 mg (38 %) of a yellow solid. R_f = 0.38 (hexane : EtOAc = 85 : 15). ^{1}H-NMR (300 MHz, CHLOROFORM-d_3): δ (ppm) = 2.14 (br. s., 1 H); 5.24 (d, *J*=10.3 Hz, 1 H); 5.40 (dd, *J*=17.1, 0.6 Hz, 1 H); 5.55 (d, *J*=5.3 Hz, 1 H); 6.05 - 6.18 (m, 1 H); 6.85 - 6.92 (m, 1 H); 7.01 - 7.08 (m, 2 H); 7.20 (dd, *J*=11.6, 1.7 Hz, 1 H); 7.31 (dd, *J*=8.0, 1.7 Hz, 1 H); 7.43 - 7.52 (m, 1 H). ^{13}C-NMR (300 MHz, CHLOROFORM-d_3): δ (ppm) = 69.1 (s); 101.3 (s); 107.5 (s); 108.6 (s); 113.7 (d, *J*=23.1 Hz); 115.4 (s); 120.6 (s); 122.6 (d, *J*=3.0 Hz); 127.7 - 128.3 (m); 133.9 (d, *J*=2.2 Hz); 138.8 (s); 142.4 (d, *J*=8.2 Hz); 147.5 (s); 148.2 (s); 160.2 (d, *J*=248.1 Hz).

2.2.4: 1-(4'-((tert-butyldimethylsilyl)oxy)-[1,1'-biphenyl]-4-yl)prop-2-en-1-ol (**S9b**): Synthesized according to method B using **S9c** (1.14 mmol; 357 mg) and vinylmagnesium bromide (0.7 M in THF; 1.37 mmol; 2.0 mL). Yield: 47 mg (47 %) of a yellow oil. R_f = 0.32 (hexane : EtOAc = 9 : 1). ^{1}H-NMR (300 MHz, CHLOROFORM-d_3): δ (ppm) = 0.21 - 0.26 (m, 6 H); 0.98 - 1.03 (m, 9 H); 5.21 - 5.28 (m, 2 H); 5.40 (dt, *J*=17.1, 1.4 Hz, 1 H); 6.10 (ddd, *J*=17.1, 10.3, 6.0 Hz, 1 H); 6.88 - 6.94 (m, 2 H); 7.40 - 7.48 (m, 4 H); 7.53 - 7.58 (m, 2 H). ^{13}C-NMR (300 MHz, CHLOROFORM-d_3): δ (ppm) = -4.4; 18.2; 25.7; 75.2; 115.2; 120.3; 126.7; 126.9; 128.0; 133.8; 140.2; 140.5; 140.9; 155.3.

2.2.5: 1-(4-(1-(tert-butyldimethylsilyl)-1H-indol-5-yl)phenyl)prop-2-en-1-ol (**S10b**): Synthesized according to method B using **S10c** (1.18 mmol; 397 mg) and vinylmagnesium bromide (0.7 M in THF; 1.42 mmol; 2.0 mL). Yield: 302 mg (70 %) of a yellow oil. R_f = 0.43 (hexane : EtOAc = 8 : 2). ^{1}H-NMR (300 MHz, CHLOROFORM-d$_3$): δ (ppm) = 0.63 (d, *J*=1.1 Hz, 6 H); 0.96 (s, 9 H); 5.19 - 5.31 (m, 2 H); 5.42 (dd, *J*=17.1, 1.4 Hz, 1 H); 6.06 - 6.20 (m, 1 H); 6.67 (d, *J*=3.2 Hz, 1 H); 7.20 - 7.24 (m, 1 H); 7.37 - 7.49 (m, 3 H); 7.57 (d, *J*=8.6 Hz, 1 H); 7.66 (d, *J*=7.5 Hz, 2 H); 7.84 (s, 1 H). LC-MS (ESI): R_t = 10.82 min; *m/z* = 346.27 [M-OH]$^+$.

2.3 Method C: CDI reaction

2.3.1 1-(1-(3'-fluoro-4'-methoxy-[1,1'-biphenyl]-4-yl)allyl)-1H-imidazole (**S6**): Synthesized according to method C using **S6a** (160 mg, 0.62 mmol) and CDI (301 mg, 1.86 mmol). Yield: 44 mg (23 %) of a colorless oil. R_f = 0.44 (EtOAc : MeOH = 98 : 2). ^{1}H-NMR (300 MHz, CHLOROFORM-d$_3$): δ[ppm] = 3.94 (s, 3 H) 5.19 (d, *J*=17.04 Hz, 1 H) 5.47 (d, *J*=10.24 Hz, 1 H) 5.84 (d, *J*=6.24 Hz, 1 H) 6.31 (ddd, *J*=16.86, 10.34, 6.33 Hz, 1 H) 6.93 (s, 1 H) 7.00 - 7.09 (m, 1 H) 7.13 (s, 1 H) 7.22 - 7.38 (m, 4 H) 7.54 (d, *J*=8.20 Hz, 2 H) 7.61 (s, 1 H). ^{13}C-NMR (75 MHz, CHLOROFORM-d$_3$): δ[ppm] = 56.3 (s), 63.3 (s), 113.7 (d, *J*=2.24 Hz), 114.7 (d, *J*=18.63 Hz), 118.6 (s), 119.5 (s), 122.6 (d, *J*=2.9 Hz), 127.2 (s), 127.9 (s), 129.0 (s), 133.3 (d, *J*=5.9 Hz), 135.5 (s), 136.6 (s), 136.9 (s), 139.9 (s), 147.3 (d, *J*=11.2 Hz), 152.6 (d, *J*=248.1 Hz). LC-MS (ESI): R_t = 2.73 min, *m/z* = 309.18 [M+H]$^+$, 241.14 [M-Imidazole]$^+$.

2.3.2 4'-(1-(1H-imidazol-1-yl)allyl)-[1,1'-biphenyl]-4-carbonitrile (**S7**): Synthesized according to method C using **S7a** (150 mg, 0.64 mmol) and CDI (310 mg, 1.92 mmol). Yield: 22 mg (34 %) of an orange oil. R_f = 0.22 (100% EtOAc). ^{1}H-NMR (300 MHz, CHLOROFORM-d$_3$): δ[ppm] = 5.20 (dd, *J*=17.00, 0.61 Hz, 1 H) 5.48 (d, *J*=10.24 Hz, 1 H) 5.85 (d, *J*=6.24 Hz, 1 H) 6.31 (ddd, *J*=16.90, 10.38, 6.33 Hz, 1 H) 6.93 (s, 1 H) 7.12 (s, 1 H) 7.30 (d, *J*=8.38 Hz, 2 H) 7.54 - 7.63 (m, 3 H) 7.64 - 7.78 (m, 4 H). ^{13}C-NMR (75 MHz, CHLOROFORM-d$_3$): δ[ppm] = 63.1, 111.3, 118.5, 118.7, 119.8, 127.7, 127.8, 128.2, 129.5, 132.7, 135.35, 136.6, 138.8, 139.4, 144.7. LC-MS (ESI): R_t = 7.70 min, *m/z* = 286.08 [M+H]$^+$, 218.01 [M-Imidazole]$^+$.

2.3.3 1-(1-(4-(benzo[d][1,3]dioxol-5-yl)-2-fluorophenyl)allyl)-1H-imidazole (**S8**): Synthesized according to method C using **S8a** (125 mg, 0.46 mmol) and CDI (224 mg, 1.38 mmol). Yield: 43 mg (29 %) of a colorless oil. R_f = 0.39 (100% EtOAc). ^{1}H-NMR (300 MHz, METHANOL-*d*$_4$): δ[ppm] = 5.16 (d, *J*=16.86 Hz, 1 H) 5.43 - 5.51 (m, 1 H) 5.99 (s, 2 H) 6.31 (d, *J*=6.05 Hz, 1 H) 6.36 - 6.50 (m, 1 H) 6.87 - 6.93 (m, 1 H) 7.02 (t, *J*=1.12 Hz, 1 H) 7.10 - 7.16 (m, 3 H) 7.27 - 7.40 (m, 2 H) 7.40 - 7.46 (m, 1 H) 7.74 (s, 1 H). ^{13}C-NMR (75 MHz, METHANOL-*d*$_4$): δ[ppm] = 58.7 (d, *J*=2.9 Hz), 102.9 (s), 108.4 (s), 109.8 (s), 115.0 (d, *J*=22.4 Hz), 119.8 (s), 120.3 (s), 122.0 (s), 124.1 (d, *J*=2.9 Hz), 125.4 (d, *J*=14.2 Hz), 129.1 (s), 130.5 (d, *J*=3.7 Hz), 134.7 (d, *J*=1.5 Hz), 136.2 (s), 138.0 (s), 145.3 (d, *J*=8.2 Hz), 149.5 (s), 150.0 (s), 163.8 (s). LC-MS (ESI): R_t = 2.95 min, *m/z* = 323.12 [M+H]$^+$, 254.99 [M-Imidazole]$^+$.

2.3.4 1-(1-(4'-((tert-butyldimethylsilyl)oxy)-[1,1'-biphenyl]-4-yl)allyl)-1H-imidazole (**S9a**): Synthesized according to method C using **S9b** (175 mg, 0.51 mmol) and CDI (249 mg, 1.53 mmol). Yield: 58 mg (29 %) of a colorless oil. R_f = 0.31 (100% EtOAc). ^{1}H-NMR (300 MHz, CHLOROFORM-d$_3$): δ[ppm] = 0.24 (s, 6 H); 1.01 (s, 9 H) 5.18 (d, *J*=17.0 Hz, 1 H); 5.45 (d, *J*=10.2 Hz, 1 H); 5.81 (d, *J*=6.3 Hz, 1 H); 6.31 (ddd, *J*=16.9, 10.4, 6.3 Hz, 1 H); 6.88 - 6.94 (m, 3 H); 7.11 (s, 1 H); 7.24 (d, *J*=8.4 Hz, 2 H); 7.43 - 7.48 (m, 2 H); 7.53 - 7.58 (m, 3 H).

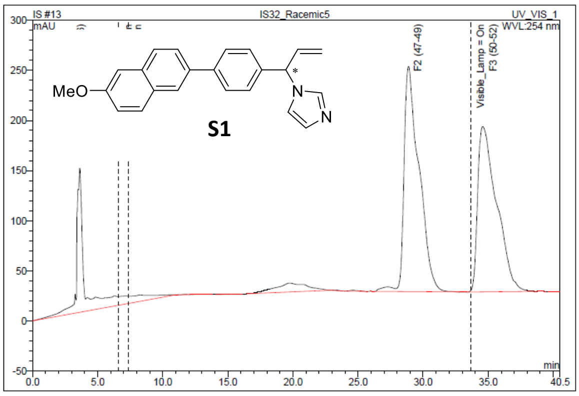
3.10 5-(4-(1-(1H-imidazol-1-yl)allyl)phenyl)-1-(tert-butyldimethylsilyl)-1H-indole (**S10a**): Synthesized according to method C using **S10b** (302 mg, 0.83 mmol) and CDI (404 mg, 2.49 mmol). Yield: 106 mg (31 %) of a yellow oil. R_f = 0.31 (100% EtOAc).

3. Synthesized Compounds: K_D and MIC_{50} values

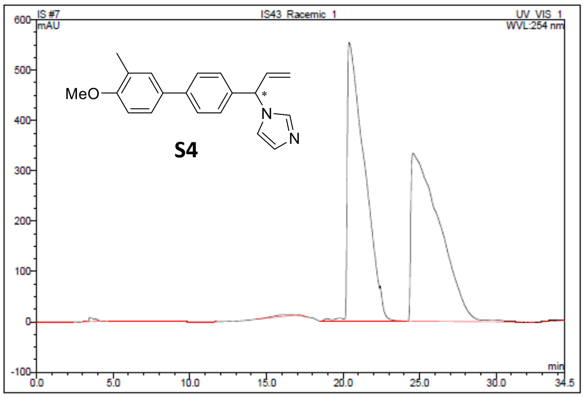
Table S3: Determined K_D values with standard deviation and MIC_{50} values against *M. bovis* BCG. K_D ± STD for compounds appearing in the main text was calculated from 4 biological replicates, for all other compounds from 2 biological replicates

Compound	K_D ± STD [µM]	MIC_{50} [µM]
S1	0.8 ± 0.8	9.7
S2	8.3 ± 1.7	49.9
S3	21.3 ± 1.1	>100
S4	5.8 ± 0.5	17.3
S5	6.7 ± 0.6	20.0
S6	7.1 ± 0.7	14.5
S7	10.0 ± 1.4	9.2
S8	4.2 ± 0.3	>30
S9	7.2 ± 0.5	>50
S10	3.5 ± 0.4	41.0
S11	5.9 ± 0.5	5.8

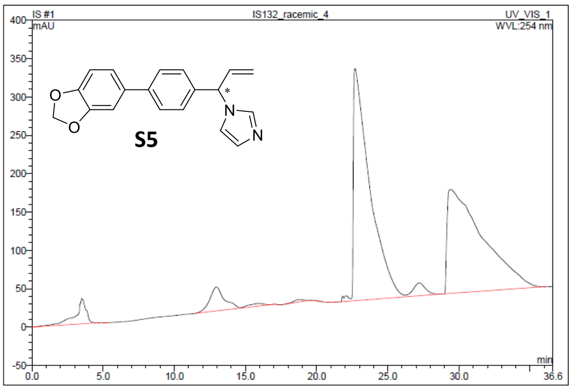
4. Enantiomer separation and affinities of enantiomers



MtBE : Ethanol = 98 : 2



MtBE : Ethanol = 98 : 2



MtBE : Ethanol = 96 : 4

Figure S4: Chromatograms of enantiomer separation with a chiral column and used solvent composition.

Table S4: Determined K_D values for the separated enantiomers.

	K_D [µM]		
	Rac.	**E1**	**E2**
S1	0.8	0.9	0.7
S4	5.8	6.1	2.6
S5	6.7	9.6	4.6

5. Complex crystal structures of L21, L44 and S2

Table S5: Data collection and refinement statistics

	CYP121 – L21	CYP121 – L44	CYP121 – S2
Data collection			
Space group	P 65 2 2	P 65 2 2	P 65 2 2
Cell dimensions			
a, *b*, *c* (Å)	77.7 77.7 264.7	77.6 77.6 264.2	77.5 77.5 263.8
α, β, γ (°)	90.0 90.0 120.0	90.0 90.0 120.0	90.0 90.0 120.0
Resolution (Å)	87.82 – 1.50	47.09 – 1.70	47.03 – 1.50
	(1.58 – 1.50)	(1.79 – 1.70)	(1.58 – 1.50)
R_{merge}	0.119 (0.801)	0.089 (0.644)	0.093 (0.686)
$I / \sigma I$	11.7 (2.4)	15.9 (3.0)	9.2 (2.0)
Completeness (%)	100.0 (100.0)	100.0 (99.8)	99.9 (99.8)
Redundancy	12.6 (13.0)	9.5 (8.7)	4.8 (5.0)
Refinement			
Resolution (Å)	67.31 – 1.50	41.53- 1.50	41.17 – 1.50
No. reflections	76683 (7490)	52828 (5143)	75864 (7436)
R_{work} / R_{free}	0.1759/0.1990	0.1705/0.1886	0.1646/0.1840
No. atoms	3592	3548	3647
Protein	3031	3013	3015
Ligand/ion	98	95	122
Water	463	440	510
B-factors	22.33	25.18	20.93
Protein	20.84	23.65	19.15
Ligand/ion	27.07	38.11	19.69
Water	30.30	32.83	31.72
R.m.s. deviations			
Bond lengths (Å)	0.008	0.005	0.011
Bond angles (°)	0.96	0.84	1.19
Occupancy of inhibitor	0.91	0.78	0.95

*Values in parentheses are for the highest-resolution shell.

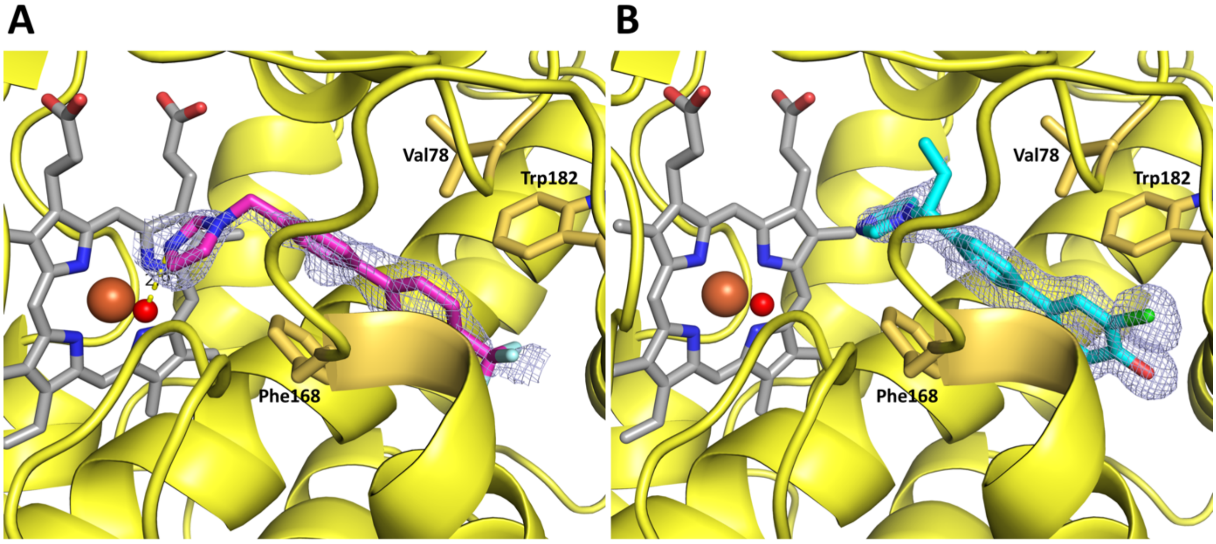


Figure S5: **A** Coordination of inhibitor **L44** (magenta) in the CYP121 crystal structure. **B** Coordination of inhibitor **S2** (cyan) in the CYP121 crystal structure. In both structures, the protein is shown as a yellow cartoon, inhibitor, heme b and interacting residues are shown as sticks. The heme iron is shown as a brown, the water ligand as a red sphere. The difference electron density maps of **L44** and **S2** ($F_O - F_C$) were contoured at 2.5σ (**L44**) and 3σ (**S2**) respectively with phases calculated from a model that was refined in the absence of **L44** or **S2** and are shown as a blue isomesh. The hydrogen bond distance between the water ligand and the imidazole nitrogen of **L44** is 2.9 Å and shown as a yellow dashed line.

6. Hydroxyl-Aspartate hydrogen bonding in the complex structure of S2

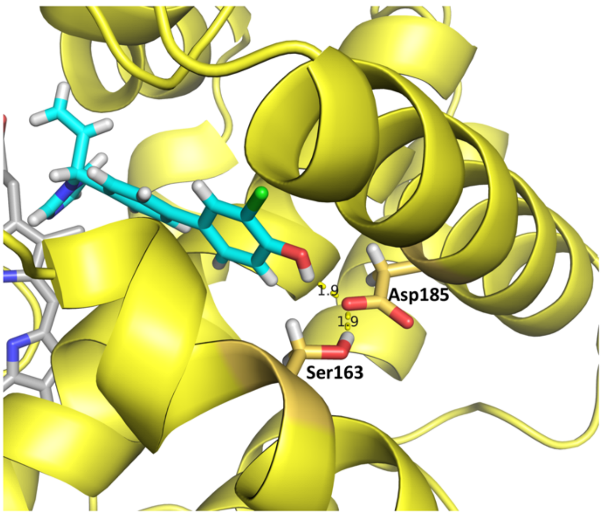


Figure S6: Observed hydrogen bonding between the para-hydroxyl group of S2 and an aspartate side chain (Asp185) of CYP121, which also engages in conserved hydrogen bonding with a serine side chain (Ser163) of an adjacent α-helix. The hydrogen bonding distance of both pairs is 1.9 Å, probably preventing the imidazole motif of S2 from reaching the heme b water ligand.

7. *In vitro* inhibiton of cYY conversion: IC_{50} values

Table S7: IC_{50} values for reference compound econazole, hit compound **I:47** and the identified potent inhibitors **L10**, **L21** and **L15**.

Compound	IC_{50} [µM]	
	Product formation	Substrate depletion
Econazole	11	8
I:47	36	34
L10	23	19
L21	24	21
L15	26	21

8. Cytotoxicity toward Macrophages

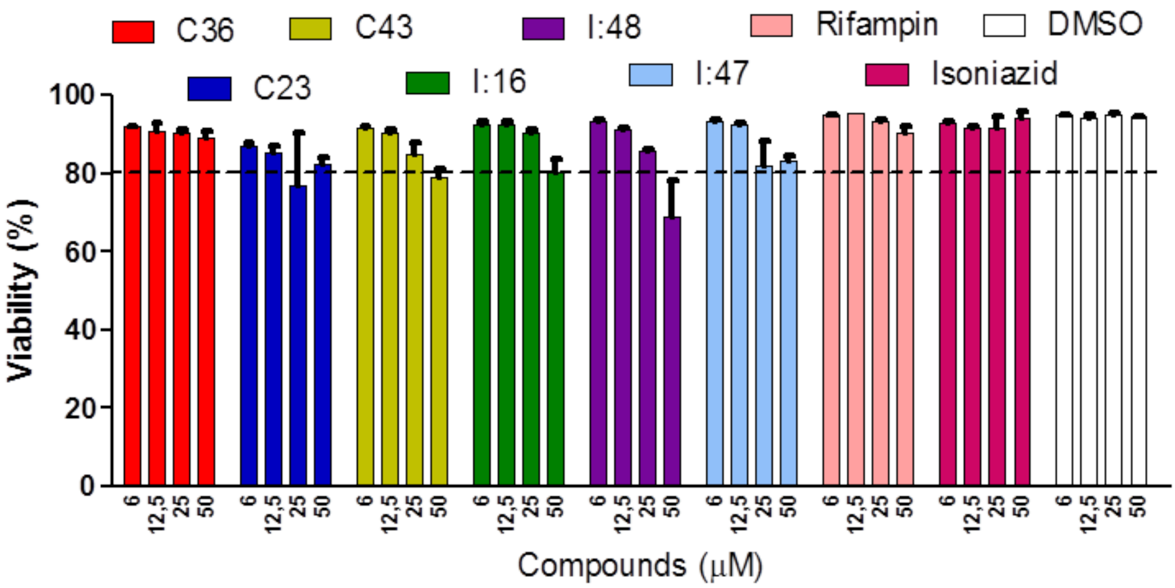
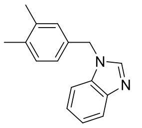
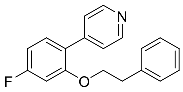
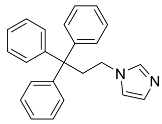
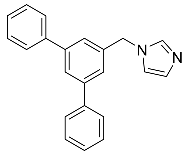
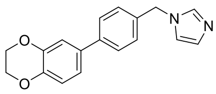
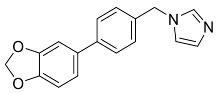


Figure S8: Cytotoxicity toward macrophages at compound concentrations of 50, 25, 12.5 and 6 µM after 48 h.

9. Intracellular Replication in Macrophages: Affinities toward CYP121 and CYP125

Table S9: Affinities toward CYP121 and CYP125 of compounds evaluated against intracellular replication in macrophages.[1,2] K_D ± STD for compounds appearing in the main text was calculated from 4 biological replicates.

Compound	Structure	K_D CYP121 [µM]	K_D CYP125 [µM]
C36		>100	1.3 ± 0.7
C23		7.2 ± 0.5	1.5 ± 0.6
C43		8.8 ± 3.0	13.1 ± 0.9
I:16		1.3 ± 0.4	no SPR response
I:48		5.3 ± 0.6	Not tested
I:47		5.4 ± 1.0	Not tested

References

(1) Brengel, C.; Thomann, A.; Schifrin, A.; Eberhard, J.; Hartmann, R. W. Discovery and Biophysical Evaluation of First Low Nanomolar Hits Targeting CYP125 of M. Tuberculosis. *ChemMedChem* **2016**, *11* (21), 2385–2391. https://doi.org/10.1002/cmdc.201600361.

(2) Brengel, C.; Thomann, A.; Schifrin, A.; Allegretta, G.; Kamal, A. A. M.; Haupenthal, J.; Schnorr, I.; Cho, S. H.; Franzblau, S. G.; Empting, M.; et al. Biophysical Screening of a Focused Library for the Discovery of CYP121 Inhibitors as Novel Antimycobacterials. *ChemMedChem* **2017**, *12* (19), 1616–1626. https://doi.org/10.1002/cmdc.201700363.