

Supplementary Information

Broad-Spectrum *In Vitro* Activity of
 $N\alpha$ -aroyl-*N*-aryl-Phenylalanine Amides
Against Non-Tuberculous Mycobacteria
and Comparative Analysis of RNA
Polymerases

Table S 1. MIC₉₀ values of a selection of AAPs against type strains of the *Mycobacterium abscessus* complex. Cell shading from green to red indicates high to low activity. The displayed values are average values of two technical replicates. For detailed information on the origin of clinical isolates see Materials & Methods section of the main manuscript.

	<i>M. abscessus</i> subsp. abscessus ATCC 19977	<i>M. abscessus</i> subsp. massiliense CCUG 48898-T	<i>M. abscessus</i> subsp. bolletii CCUG 50184-T
ID	MIC ₉₀ [μM]	MIC ₉₀ [μM]	MIC ₉₀ [μM]
CLR	1.5	0.2	0.5
MMV	6.4	9.1	4.4
1	7.3	5.7	1.9
2	4.7	4.8	2.1
3	2.1	2.8	8.5
4	2.6	4.4	3.6
5	1.5	2.3	2.3
6	5.0	4.0	2.0
7	1.8	2.0	2.1
8	1.5	1.4	1.7
9	4.8	4.4	6.5
10	2.2	2.6	9.0

Table S 2. MIC₉₀ values of a selection of AAPs against a panel of *Mycobacterium abscessus* complex clinical isolates. Cell shading from green to red indicates high to low activity. The displayed values are average values of two technical replicates. For detailed information on the origin of clinical isolates see Materials & Methods section of the main manuscript.

	<i>M. abscessus</i> subsp. abscessus Bamboo	<i>M. abscessus</i> subsp. abscessus M9	<i>M. abscessus</i> subsp. abscessus M199	<i>M. abscessus</i> subsp. abscessus M337	<i>M. abscessus</i> subsp. abscessus M404
ID	MIC ₉₀ [μM]	MIC ₉₀ [μM]	MIC ₉₀ [μM]	MIC ₉₀ [μM]	MIC ₉₀ [μM]
CLR	0.5	6.7	19.5	7.3	0.8
MMV	8.2	26.4	29.3	9.8	14.2
7	2.1	8.1	9.5	3.5	6.1
8	2.3	5.8	9.3	3.9	7.0

	<i>M. abscessus</i> subsp. abscessus M422	<i>M. abscessus</i> subsp. bolletii M232	<i>M. abscessus</i> subsp. bolletii M506	<i>M. abscessus</i> subsp. massiliense M111
ID	MIC ₉₀ [μM]	MIC ₉₀ [μM]	MIC ₉₀ [μM]	MIC ₉₀ [μM]
CLR	4.2	10.6	1.1	0.5
MMV	13.4	6.0	10.8	6.8
7	1.8	3.9	2.9	3.4
8	1.8	4.6	2.9	5.6

Table S 3. MIC₉₀ values of a selection of AAPs against type strains of the *Mycobacterium avium* complex. Cell shading from green to red indicates high to low activity. The displayed values are average values of two technical replicates. For detailed information on the origin of clinical isolates see Materials & Methods section of the main manuscript.

	<i>M. avium</i> subsp. <i>hominissuis</i> MAC109	<i>M. avium</i> subsp. <i>hominissuis</i> M. avium 11	<i>M. intracellulare</i> subsp. <i>intracellulare</i> ATCC 13950	<i>M. intracellulare</i> subsp. <i>chimaera</i> CCUG 50989
ID	MIC ₉₀ [μM]	MIC ₉₀ [μM]	MIC ₉₀ [μM]	MIC ₉₀ [μM]
CLR	1.7	0.4	0.6	0.5
MMV	> 50 ^a	3.2	3.2	2.0
1	19.0	1.7	3.4	1.4
2	14.1	1.6	1.9	1.3
3	13.5	1.4	1.2	0.8
4	14.4	1.4	1.0	0.7
5	8.5	1.2	0.9	0.7
6	9.6	1.2	1.4	0.7
7	12.5	1.0	1.2	0.6
8	6.3	0.6	0.5	0.3
9	22.6	1.5	1.7	1.1
10	9.6	1.0	1.1	0.6

^a incubation of *M. avium* subsp. *hominissuis* MAC109 with MMV did not reach 90% growth inhibition. MIC₇₅ = 6.6 μM.

Table S 4. MIC₉₀ values of a selection of AAPs against type strains of the different NTM. Cell shading from green to red indicates high to low activity. The displayed values are average values of two technical replicates. For detailed information on the origin of clinical isolates see Materials & Methods section of the main manuscript.

	<i>M. chelonae</i> ATCC 35752	<i>M. fortuitum</i> ATCC 6841	<i>M. szulgai</i> ATCC 35799	<i>M. xenopi</i> ATCC 19250	<i>M. ulcerans</i> S4018	<i>M. marinum</i> ATCC 927	<i>M. simiae</i> ^a ATCC 25275	<i>M. malmoense</i> ^a ATCC 29571	<i>M. kansasii</i> ATCC 12478
ID	MIC ₉₀ [μM]	MIC ₉₀ [μM]	MIC ₉₀ [μM]	MIC ₉₀ [μM]	MIC ₉₀ [μM]	MIC ₉₀ [μM]	MIC ₉₀ [μM]	MIC ₉₀ [μM]	MIC ₉₀ [μM]
CLR	0.1	2.9	0.2	0.1	0.12	9.1	> 100	3.4	0.4
MMV	0.8	1.5	1.6	48.8	0.47	2.2	> 100	> 100	0.5
1	0.4	2.0	2.8	24.1	0.16	9.6	> 100	> 100	2.6
2	0.3	0.9	1.4	21.2	0.09	3.2	43.0	> 100	0.6
3	0.2	1.5	1.1	6.9	0.08	2.7	54.1	16.1	0.7
4	0.4	1.7	1.8	7.0	0.13	6.5	> 100	18.2	0.7
5	0.4	1.4	0.9	3.3	0.10	2.4	45.7	> 100	0.3
6	0.5	1.5	9.0	1.8	0.05	13.9	> 100	> 100	2.8
7	0.2	0.8	0.5	8.0	0.06	1.2	> 100	2.3	0.2
8	0.2	0.6	0.5	3.3	0.02	1.4	20.9	4.9	0.2
9	0.5	1.6	1.2	10.0	0.14	3.1	> 100	> 100	0.8
10	0.4	1.0	0.7	5.2	0.08	1.9	33.5	5.8	0.5

^a In some cases 90% growth inhibition was not reached for *M. simiae* ATCC 25275 and for *M. malmoense* ATCC 29571. MIC₇₅ values are displayed:

	<i>M. simiae</i> ATCC 25275	<i>M. malmoense</i> ATCC 29571
ID	MIC ₇₅ [μM]	MIC ₇₅ [μM]
CLR	29.3	0.6
MMV	25.2	3.1
1	36.9	2.8
2	13.7	1.7
3	16.4	1.8
4	17.9	2.2
5	11.0	1.7
6	20.9	5.5
7	6.3	1.1
8	6.9	0.6
9	20.5	1.7
10	11.2	1.3

Table S 5. Alignment of RpoB primary sequences of all tested strains from position 450-600. Position numbering refers to PDB: 5UHE. Dots represent amino acid identity.

[illegible]

Table S 6. Alignment of RpoC primary sequences of all tested strains from position 500-580. Position numbering refers to PDB: 5UHE. Dots represent amino acid identity.

RpoC																
M. tuberculosis ATCC 25618/H37Rv PDB: 5UHE		M. chelonae ATCC 35752	M. fortuitum ATCC 6841	M. szulgai ATCC 35799	M. xenopi ATCC 19250	M. ulcerans S4018	M. marinum ATCC 927	M. simiae ATCC 25275	M. malmoense ATCC 29571	M. kansasii ATCC 12478	M. intracellulare ssp. chimaera CCUG 50989	M. avium ssp. hominissuis MAC109	M. abscessus ssp. abscessus ATCC 19977	M. intracellulare ssp. intracellulare ATCC 13950	E. coli ATCC 11775	
1	800	I	T	.	Y	
2	801	T	L	.	M	
3	802	I	L	.	M	
4	803	V	P	.	A	
5	804	D	E	K	.	E	K	.	.	
6	805	S	
7	806	G	
8	807	A	
9	808	T	R	
10	809	G	
11	810	N	S	
12	811	F	.	L	M	.	A	
13	812	T	A	
14	813	Q	
15	814	T	V	.	I	
16	815	R	
17	816	T	N	.	Q	
18	817	L	
19	818	A	
20	819	G	
21	820	M	
22	821	K	R	
23	822	G	
24	823	L	
25	824	V	M	
26	825	T	A	
27	826	N	K	
28	827	P	
29	828	K	D	
30	829	G	
31	830	E	S	
32	831	F	Y	.	I	
33	832	I	
34	833	P	E	
35	834	R	T	
36	835	P	
37	836	V	I	I	.	I	I	I	I	.	I	
38	837	K	T	
39	838	S	A	
40	839	S	N	
41	840	F	
42	841	R	
43	842	E	
44	843	G	
45	844	L	
46	845	T	N	
47	846	V	
48	847	L	
49	848	E	Q	
50	849	Y	
51	850	F	
52	851	I	
53	852	N	S	
54	853	T	
55	854	H	
56	855	G	
57	856	A	
58	857	R	
59	858	K	
60	859	G	
61	860	L	
62	861	A	
63	862	D	
64	863	T	
65	864	A	
66	865	L	
67	866	R	K	
68	867	T	
69	868	A	
70	869	D	N	
71	870	S	
72	871	G	
73	872	Y	
74	873	L	
75	874	T	
76	875	R	
77	876	R	
78	877	L	
79	878	V	
80	879	D	
81	880	V	
Direct drug target contacts																
Reported resistance after mutation																
7 Å distance from target bound D-AAP1																