Synthesis and biological evaluation of new amino-alcohol-quinolines in response to non-tuberculous mycobacteria infections

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Context

Mycobacterium tuberculosis (*M. tb*) was responsible for 10.8 million infections and 1.25 million deaths worldwide in 2023 [1]. In Europe and North America, the incidence of infections with non-tuberculous mycobacteria (NTM) exceeds that of *M. tb*. [2]. NTM are predominantly found in pulmonary infections of patients with chronic obstructive pulmonary disease or bronchiectasis (Figure 1).

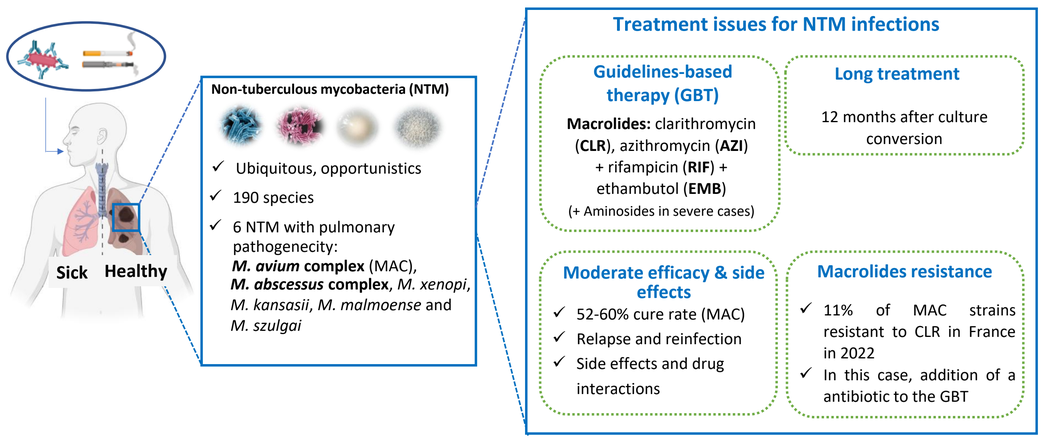


Figure 1: NTMs, generalities and current treatment issues.

Consequently, there is an urgent need to develop safer molecules and capable of countering antibiotic resistance.

Present and future antimycobacterial quinolines

The quinoline pharmacophore is found in the structure of **bedaquiline** and **mefloquine** (**MQ**), which have antimycobacterial properties. The minimum inhibitory concentration (MIC) of **MQ** on *M. abscessus* (*M. abs*) and MAC is between 32 and 4 µg/mL (Figure 2). However, it has a low selectivity index (SI) due to its cytotoxicity. Our team designed and synthesized **amino-alcohol-quinolines** (**AAQs**), analogues of **MQ**, to improve efficacity and tolerance of this class compound against NTM. A **hit A** was identified with a SI higher than that of **MQ** by a factor of 73. To further increase the SI and to establish new structure activity or toxicity relationships (SAR/STR), the secondary amine of **hit A** was replaced by a piperazine or an amide group. The alkyl chain was retained or was modified by an aryl group.

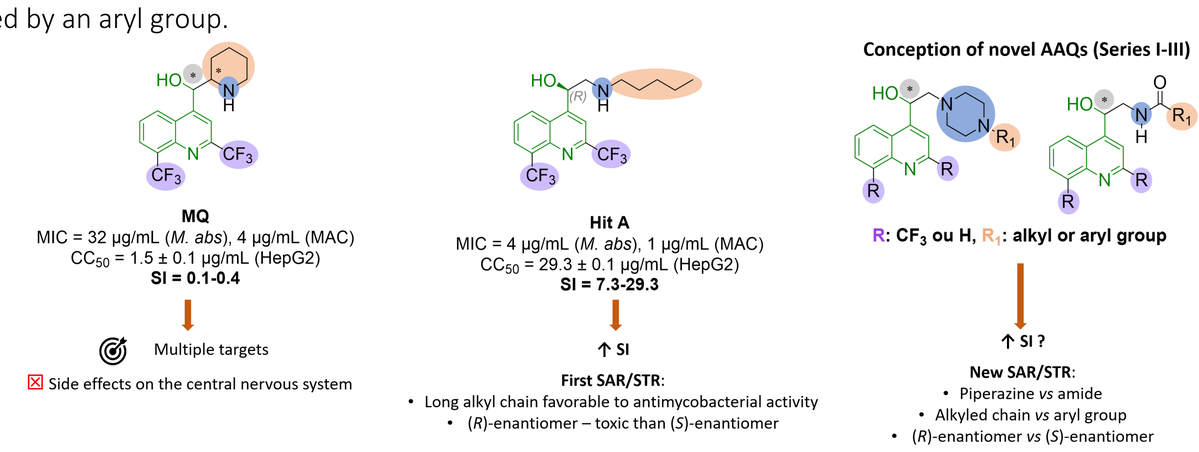


Figure 2: Antimycobacterial quinolines and development of novel **AAQs**.

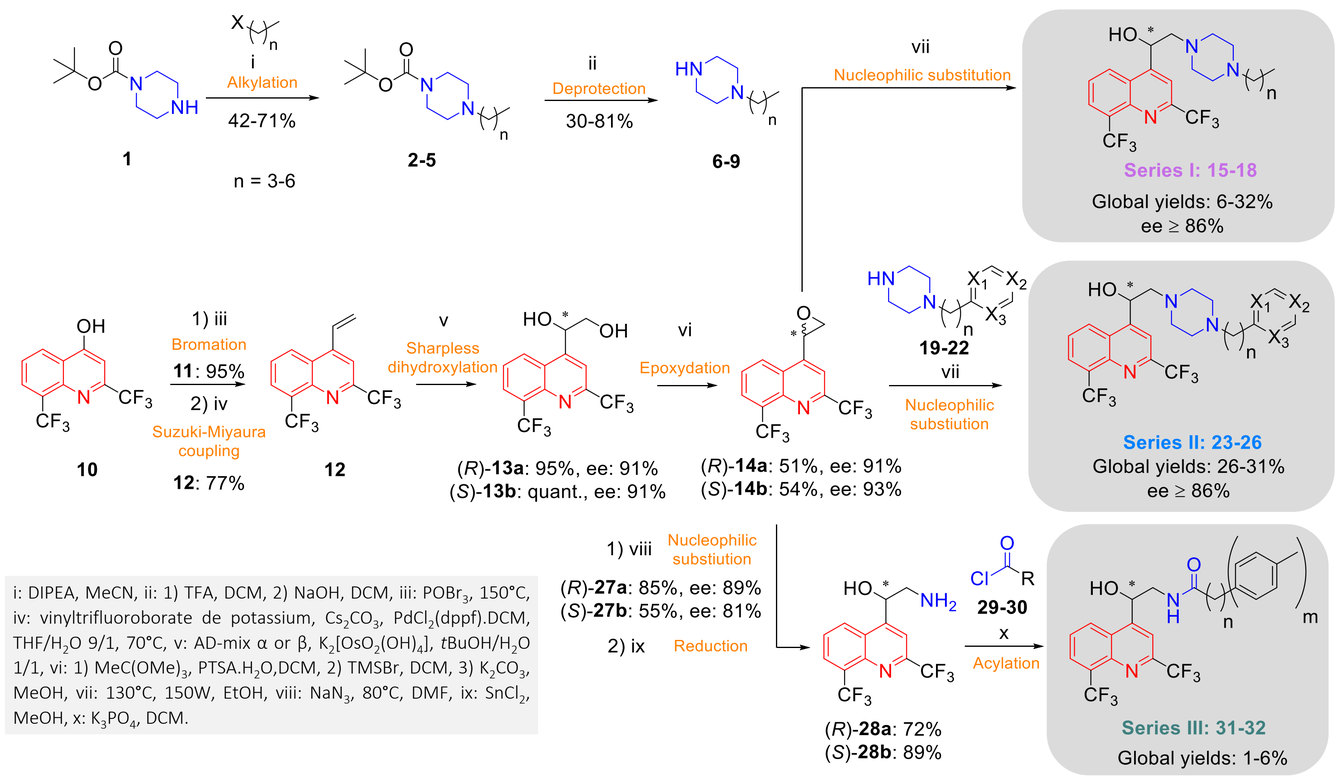
Asymmetric synthesis of AAQs

Three series of compounds (I-III) were obtained by nucleophilic substitution of epoxides **14a/b** by piperazines **6-9** (series I), **19-22** (series II) or acyl chlorides **29-30** (series III) (Scheme 1).

Alkyled piperazines **6-9** and aryled piperazine **19** were synthesized from tert-butyl piperazine-1-carboxylate **1** in two steps while aryled piperazines **20-22** are commercial.

AAQs 15-18 and **23-26** were obtained in five or seven steps with overall yields of 6-32% and ee ≥ 86%.

AAQs 31-32 of the amide series were obtained in seven steps from commercial acyl chlorides **29-30** in overall yields of 1-6%.



i: DIPEA, MeCN, ii: 1) TFA, DCM, 2) NaOH, DCM, iii: $POBr_3$, 150°C, iv: vinyltrifluoroborate de potassium, Cs_2CO_3, $PdCl_2$(dppf).DCM, THF/H_2O 9/1, 70°C, v: AD-mix α or β, $K_2[OsO_2(OH)_4]$, *t*BuOH/H_2O 1/1, vi: 1) MeC(OMe)$_3$, PTSA.H_2O,DCM, 2) TMSBr, DCM, 3) K_2CO_3, MeOH, vii: 130°C, 150W, EtOH, viii: NaN_3, 80°C, DMF, ix: $SnCl_2$, MeOH, x: K_3PO_4, DCM.

Composed	Alkyled piperazines
(*R*)-**15a**/(*S*)-**15b**	**6**: n = 3
(*R*)-**16a**/(*S*)-**16b**	**7**: n = 4
(*R*)-**17a**/(*S*)-**17b**	**8**: n = 5
(*R*)-**18a**/(*S*)-**18b**	**9**: n = 6

Composed		Aryled piperazines n	Aryled piperazines $X_{1,2,3}$
(*R*)-**23a**/(*S*)-**23b**	**19**:	2	$X_{1,3}$ = CH, X_2 = C-Me
(*R*)-**24a**/(*S*)-**24b**	**20**:	0	$X_{1,3}$ = CH, X_2 = C-Me
(*R*)-**25a**/(*S*)-**25b**	**21**:	0	X_1 = N, $X_{2,3}$ = CH
(*R*)-**26a**/(*S*)-**26b**	**22**:	0	$X_{1,3}$ = N, X_2 = CH

Composed		Acyled chlorides n	Acyled chlorides m
(*R*)-**31a**/(*S*)-**31b**	**29**:	5	0
(*R*)-**32a**/(*S*)-**32b**	**30**:	2	1

Scheme 1: Asymmetric synthesis of series I-III.

Biological results

The antimycobacterial activity of the final compounds were evaluated on two fast-growing NTMs and three slow-growing NTMs (Table 1). Compounds of series I are the most promising since the majority of them have MICs ≤ 32 µg/mL. **AAQs 17-18** with a long alkyl chain (n = 5 or 6) are more effective than those with a short aliphatic chain **15-16** (n = 3 or 4). Generally, **AAQs 15-23** are as active or more active than **MQ**, and less active than **ciprofloxacin** (**CIP**) and **CLR** except on *M. kansasii* and *M. abscessus* respectively. Compounds in the **31-32** amide series have not yet been tested on NTMs. Cell viability on HepG2 cells revealed that (*R*)-**AAQs** are less toxic than (*S*)-enantiomers and most often less toxic than reference antibiotics. The SI of **AAQs** on *M. avium* are lower than that of **hit A** (SI = 29.3), **CLR** and **CIP**. However, the SI of **AAQ 15-23** are higher than that of **MQ**. Of the two compounds with the highest SI on *M. avium*, the least cytotoxic compound (*R*)-**17a** was selected to investigate a possible synergy of action.

Table 1: *In vitro* antimycobacterial activities and cell viability of synthetized **AAQs**.

Composed	MIC (µg/mL)[a]					CC_{50} (µg/mL)[b]	SI
	Fast-growing NTMs		Slow-growing NTMs				
	M. abs R	*M. abs S*	*M. marinum*	*M. avium*	*M. kansasii*	HepG2	*M. avium*
(*R*)-**15a**	32	64	16	32	4	23.3 ± 2.1	0.7
(*S*)-**15b**	64	64	8	64	≤ 2	27.0 ± 2.8	0.4
(*R*)-**16a**	32	32	16	32	≤ 2	32.7 ± 3.0	1.0
(*S*)-**16b**	32	32	8	8	≤ 2	18.2 ± 6.1	2.3
(*R*)-**17a**	16	8	8	8	≤ 2	46.0 ± 0.5	5.8
(*S*)-**17b**	16	16	4	8	≤ 2	12.5 ± 0.3	3.1
(*R*)-**18a**	4	4	8	16	≤ 2	20.1 ± 1.8	5.0
(*S*)-**18b**	4	8	≤ 2	4	≤ 2	16.5 ± 2.9	8.3
(*R*)-**23a**	8	16	4	32	4	22.9 ± 1.2	0.7
(*S*)-**23b**	32	> 128	4	8	≤ 2	18.8 ± 1.6	2.3
(*R*)-**24a**	> 64	> 64	16	> 128	8	43.4 ± 2.0	< 0.3
(*S*)-**24b**	> 64	> 64	32	> 128	≤ 2	24.0 ± 0.04	< 0.2
(*R*)-**25a**	> 64	> 64	16	> 128	NA[d]	24.4 ± 0.3	< 0.2
(*S*)-**25b**	> 128	> 128	16	≥ 32	≤ 2	11.3 ± 0.9	≤ 0.4
(*R*)-**26a**	> 64	> 64	16	> 128	NA[d]	46.2 ± 0.5	< 0.4
(*S*)-**26b**	> 128	> 128	> 128	> 128	≤ 2	10.0 ± 0.7	< 0.1
MQ	32	16-32	4	≤ 2	≤ 2	3.6 ± 0.2	0.4
CLR	≥ 32	≥ 32	0.25	0.25	≤ 2	2.6 ± 0.6	10.4
CIP	4	4	2	≤ 2	2	18.4 ± 1.4	≥ 9.2

[a] **MIC** on *M. abs S* and *R* DSM44196, *M. marinum* DSM44344, *M. avium* ATCC700898, *M. kansasii* DSM44162. MIC determined with technical and biological duplicate,
[b] **Cell viability** is expressed as the mean ± standard deviation of a technical triplicate, [c] **SI** = **CC_{50}**/**MIC** on *M. avium* ATCC700898, [d] **NA** not applicable, [e] **ND** not determined.

Checkerboard assay against *M. avium*

In order to improve the antimycobacterial activity of (*R*)-**17a** hit compound, to reduce toxicity and to limit the risk of resistance development, the search for *in vitro* synergy of action carried out. Antibiotics of the GBT such as **CLR**, **ethambutol** (**EMB**) and **rifampicin** (**RIF**) tested with (*R*)-**17a** on the *M. avium* ATCC 700898 strain using the checkerboard method (Table 2).

Table 2: FICI calculation for **EMB**/**CLR**, (*R*)-**17a**/**EMB**, (*R*)-**17a**/**CLR** and (*R*)-**17a**/**RIF** associations.

Antibiotic associations	MIC (µg/mL) on *M. avium* ATCC 700898		FICI	Effect
	Alone antibiotic	Antibiotic associations		
CLR / **EMB**	0.12 / 4	0.06 / 4	1.5	Additive
(*R*)-**17a** / **CLR**	16 / 0.5	8 / 0.25	1	
(*R*)-**17a** / **EMB**	4 / 2	4 / 1	1.5	
(*R*)-**17a** / **RIF**	16 / 0.5	8 / 0.25	1	

FICI: fractional inhibitory coefficient index. FICI = (MIC of antibiotic A in association / MIC of antibiotic A alone) + (MIC of antibiotic B in association / MIC of antibiotic B alone). Two antibiotics have a synergistic effect when FICI ≤ 0.5, an additive effect when 0.5 < FICI < 2 and an antagonist effect when 2 ≤ FICI.

For all the antibiotic combinations tested, the FICI was equal to 1 or 1.5, demonstrating an additive effect.

Conclusion and perspectives

Twenty **AAQs** from three series were obtained and sixteen of them were evaluated biologically to identify new SAR/STR (Figure 3). A new hit was identified with a higher SI than **hit A** and an additive effect was demonstrated with the antibiotics of GBT. Later, toxicity and effectiveness of this hit will be assessed *in vivo* in a mouse model.

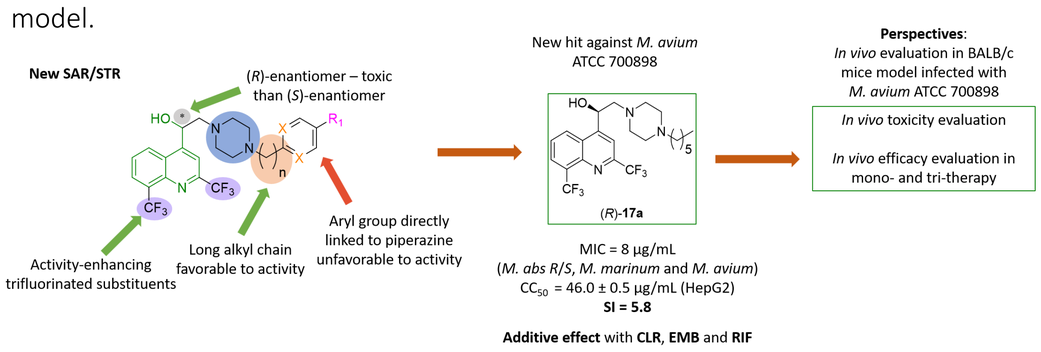


Figure 3: New SAR/STR, *in vitro* biological properties of hit (*R*)-**17a** and perspectives.

References

[1] Rapport sur la tuberculose dans le monde, OMS (**2024**). [2] Griffith, D.E. *et al*. *American Journal Respiratory and Critical Care Medicine 175*, 367-416 (**2007**).
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