Synthesis and biological evaluation of new piperazine-based amino-alcohol-quinolines as promising antimycobacterial drugs

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Context

Mycobacterium tuberculosis (*M. tb*) is responsible for 10.6 million infections and 1.6 million deaths worldwide in 2020.[1] However, in Europe and North America, the incidence of non-tuberculous mycobacteria (NTM) infections exceeds that of *M. tb*.[2] Among NTM with pulmonary pathogenicity, *M. abscessus* (*M. abs*) and *M. avium complex* (MAC) are the most common.[2] As for tuberculosis cure, NTM treatments require a combination of antibiotics which can induce many side effects for a long period (Figure 1).[2,3]

There is an urgent need to develop new antimycobacterial drugs that are safer, more specific to NTM and, if possible, with a new mechanism of action to limit cross-resistance.

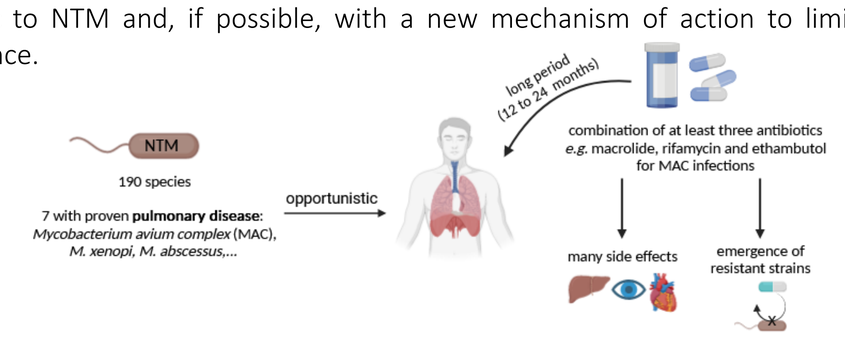


Figure 1: NTM as emerging virulent pathogens responsible for difficult-to-treat lung diseases.

Antimycobacterial quinolines

Quinoline-based pharmacophore is found in bedaquiline (BQ) and mefloquine (MQ) (Figure 2). They are active against NTM and target ATP synthase but not only in the case of **MQ**.[4,5,6] However, **BQ** and **MQ** can induce liver and central nervous system disorders respectively.[7,8] Thus, we are interested in developing new amino-alcohol-quinolines (AAQ) as **MQ** analogs to establish new structure-activity relationships (SAR) and to reduce **MQ** toxicity.

In vitro evaluations of previous series have identified the hit A. It is effective on MAC (MIC = 4 µg/mL) and has a better selectivity index (SI) than **MQ** (SI = 2.9 *vs* 0.4).[5] To increase their SI, new AAQ with piperazine core grafted with aryl or alkyl groups have been designed.

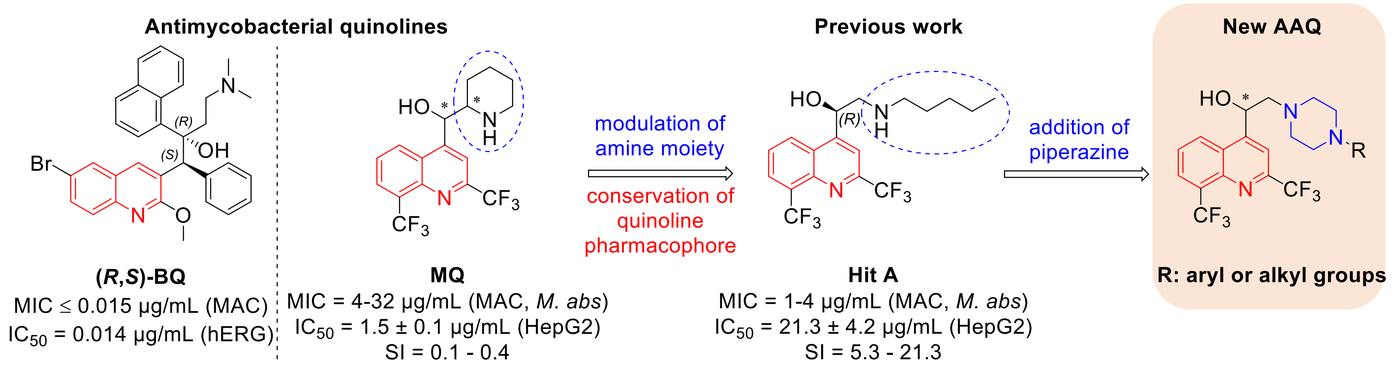
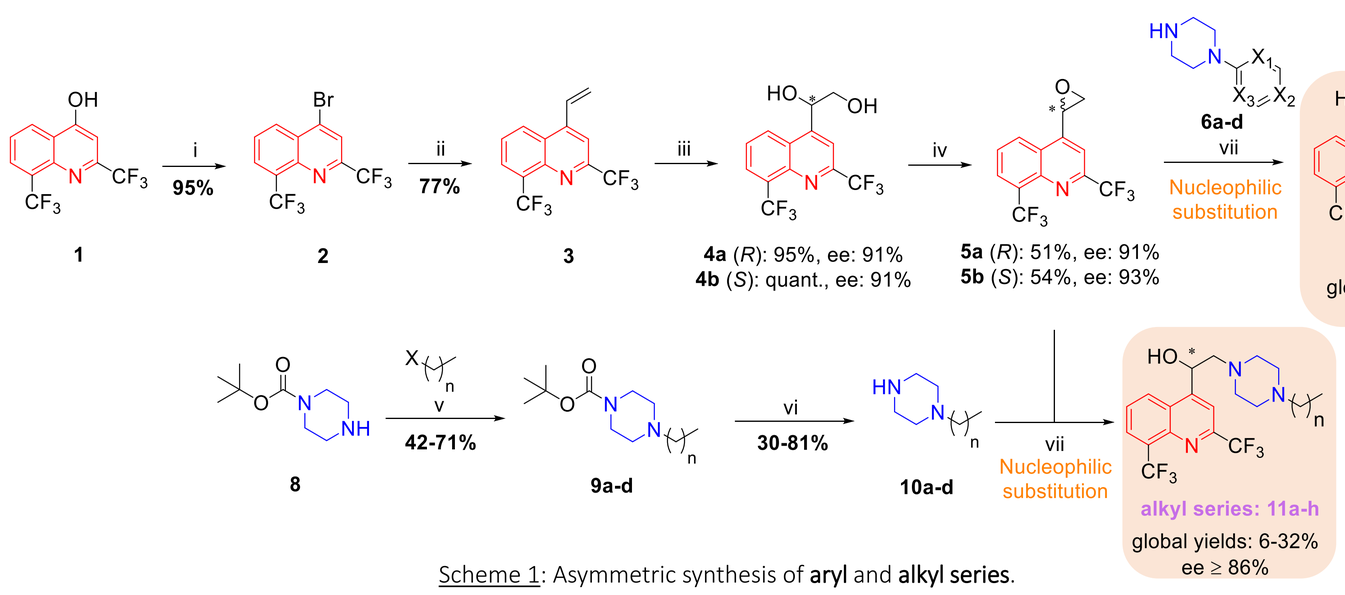


Figure 2: Known antimycobacterial quinolines and development of new amino-alcohol-quinolines.

Synthesis

To synthesise new aryl and alkyl series, two intermediates were required: i) the epoxydes **5a/b** obtained from 4-hydroxyquinoline **1** in four steps and, ii) the amines **6a-d** and **10a-d** (Scheme 1). Aryl-piperazines **6a-d** were commercially available, while alkyl-piperazines **10a-d** were obtained in two-step synthesis from *tert*-butyl piperazine-1-carboxylate **8**. Both entities were conjugated by nucleophilic substitution to afford three enantiomer pairs **7a-f** and four enantiomer pairs **11a-h** in 6 to 31 % of global yields and an enantiomeric excess (ee) greater than 86 % (Table 1).



Scheme 1: Asymmetric synthesis of **aryl** and **alkyl series**.

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

Table 1: Global yields and ee of **aryl** and **alkyl series**.

Compound	$X_{1,2,3}$	n	Global yield (%)	ee (%)
7a (*R*)	$X_{1,3}$ = CH, X_2 = C-Me	-	30	94
7b (*S*)		-	31	92
7c (*R*)	X_1 = N, $X_{2,3}$ = CH	-	28	87
7d (*S*)		-	26	86
7e (*R*)	$X_{1,3}$ = N, X_2 = CH	-	31	85
7f (*S*)		-	26	86
11a (*R*)	-	3	14	89
11b (*S*)	-		18	91
11c (*R*)	-	4	6	86
11d (*S*)	-		32	87
11e (*R*)	-	5	10	86
11f (*S*)	-		17	88
11g (*R*)	-	6	15	85
11h (*S*)	-		13	85

Physicochemical and pharmacokinetic properties

In silico physicochemical (PC) and pharmacokinetic (PK) profiles of **aryl** and **alkyl compounds** **7a-f** and **11a-h** were performed using Qikprop software (Figure 3). Lipinski's and Veber's rules were chosen for the PC data. Solubility, metabolic reactions, cardiac toxicity and permeability of Caco-2 cells parameters were selected to reflect A.D.M.E.T properties.

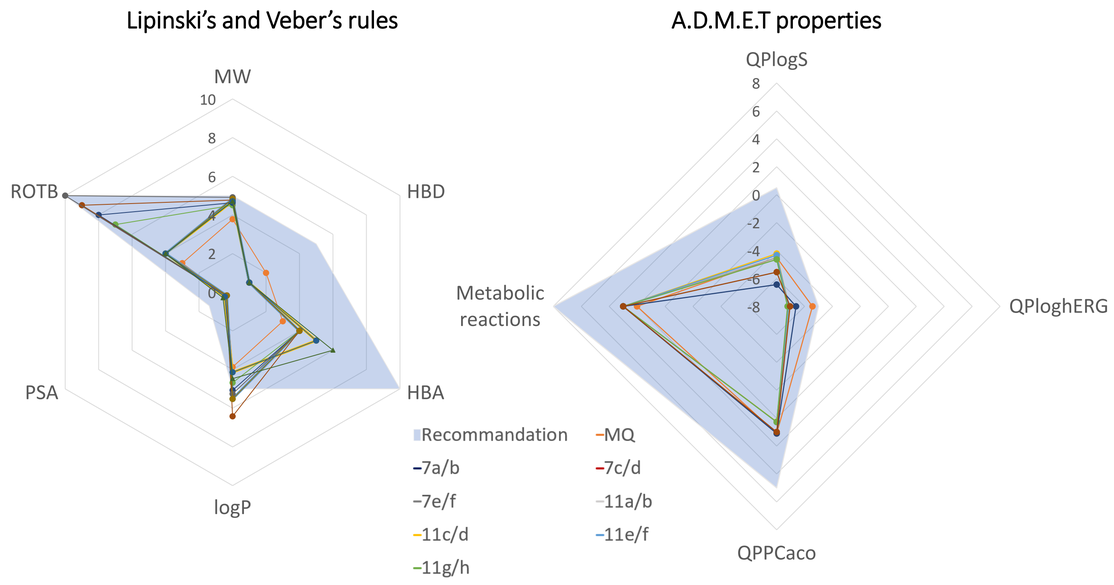


Figure 3: Radar chart of *in silico* physicochemical and pharmacokinetic properties of **aryl** and **alkyl series**.

→ Aryl and alkyl series comply with Lipinski's and Veber's rules
→ Aryl and alkyl series could be potential drug candidats

Conclusion and perspectives

Fourteen new AAQ in 6 to 32 % of global yields and 86 to 94 % of ee were obtained by a short asymmetric synthesis (5 or 7 steps).

While aryl series **7** was inactive *in vitro* against *M. abs S* and *R*, alkyl **AAQ 11** displayed anti-NTM activity from 4 to 64 µg/mL.

SI values for alkyl **AAQ 11** highlighted eight compounds AAQ 11 safer than **MQ** (calculated according their cytotoxicity on HepG2). The enantiomer pair 11g/11h is the hit of new series.

In the future, *in vitro* antimycobacterial activity on slowly growing rate NTM such as MAC or *M. xenopi*, and cytotoxicity on THP-1 cell lines for alkyl **AAQ** will be evaluated. In order to explore new SAR, further modulations focusing on the **MQ** core are planned. A few compounds with the highest SI will be selected for *in vivo* evaluation on mouse model.

Biological results

The efficacy of final compounds **7a-f** and **11a-h** was tested on *M. abs smooth* (*S*) and *rough* (*R*) phenotypes. While aryl compounds were inactive, alkyl series displayed moderate activity (Table 2). **AAQ 11g/h** with a long alkyl chain (n = 6) were more efficient than **11a-d** (n = 3 or 4). No difference in activity was observed between *S* and *R* enantiomers of the same pair. Cytotoxicity assays on HepG2 cell lines revealed that new series 7 and 11 are less toxic than MQ (IC_{50} = 26.2-96.3 *vs* 1.5 µg/mL). SI of alkyl AAQ are equal or better than those of **MQ** (SI = 0.4-5.0 *vs* 0.1-0.4).

Table 2: *In vitro* antimycobacterial activity and cytotoxicity of **aryl** and **alkyl series 7** and **11**.

Compound	$X_{1,2,3}$	n	MIC (µg/mL)[a]		IC_{50} (µg/mL)[b]	SI[c]	
			M. abs S	*M. abs R*	HepG2	*M. abs S*	*M. abs R*
7a (*R*)	$X_{1,3}$ = CH, X_2 = C-Me	-	ND[d]	ND[d]	92.3 ± 4.3	ND	ND
7b (*S*)		-	ND[d]	ND[d]	51.1 ± 0.1	ND	ND
7c (*R*)	X_1 = N, $X_{2,3}$ = CH	-	ND[d]	ND[d]	48.6 ± 2.5	ND	ND
7d (*S*)		-	> 128	> 128	39.9 ± 3.5	< 0.2	< 0.2
7e (*R*)	$X_{1,3}$ = N, X_2 = CH	-	ND[d]	ND[d]	50.6 ± 0.7	ND	ND
7f (*S*)		-	> 128	> 128	95.7 ± 1.0	< 0.1	< 0.1
11a (*R*)	-	3	64	32	58.6 ± 4.6	0.8	0.8
11b (*S*)	-		64	32-64	60.1 ± 6.3	0.4	0.4-0.8
11c (*R*)	-	4	32	32-64	70.6 ± 6.5	1.0	1.0-2.0
11d (*S*)	-		32	32	NA[e]	0.6	0.6
11e (*R*)	-	5	16	8-16	96.3 ± 1.0	2.3	1.2-2.3
11f (*S*)	-		8-16	16	26.2 ± 0.7	0.8	0.8
11g (*R*)	-	6	8	4	41.0 ± 3.7	5.0	1.3-5.0
11h (*S*)	-		4-16	4	33.5 ± 5.8	4.1	2.1-4.1
MQ			32	16-32	1.5 ± 0.1	0.1	0.1
Clarithromycin			≥ 32	≥ 32	ND[d]	ND[d]	ND[d]
Ciprofloxacin			4	4	ND[d]	ND[d]	ND[d]
Amikacin			≤ 2	≤ 2	ND[d]	ND[d]	ND[d]

[a] **MIC** *M. abs S* and *R* DSM 44196, MIC were determined with technical and biological duplicates,
[b] IC_{50} results are expressed as mean ± SD of duplicate experiments, [c] **SI** = IC_{50}/**MIC** on *M. abs S* or *R* strains,
[d] **ND** not determined, [e] **NA** not applicable.

References

[1] Rapport sur la tuberculose dans le monde, OMS (**2022**). [2] Griffith, D.E. *et al. American Journal Respiratory and Critical Care Medicine 175*, 367-416 (**2007**).
[3] Diel, R. *et al. Chest 153*, 888–921 (**2018**). [4] Brown-Eliott, B.A. *et al. Antimicrobial Agents and Chemotherapy 61* (**2017**). [5] Laumaillé, P. *et al. Pharmaceuticals 12*, 91 (**2019**). [6] Martin-Galiano, A.J. *et al.* Antimicrob. Agents and Chemother. *46*, 1680-1687 (**2002**). [7] Patel, H. *et al.* Tuberculosis, *117*, 79-84, (**2019**).
[8] Weinke, T. *et al. American Journal of Tropical Medicine and Hygiene 45*, 86-91 (**1991**).

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