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Development of amino-alcohol-quinolines targeting lung diseases caused by non-tuberculous mycobacteria

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INTRODUCTION & AIM

In Europe and North America, the incidence of infections caused by non-tuberculous mycobacteria (NTM) exceeds that of *M. tb* [1]. The effectiveness of the treatment for MAC bronchiectasis is estimated 52%, due to the development of macrolide resistance. Consequently, our team's aim is to develop safer molecules, able to overcome antibiotic resistance. The quinoline pharmacophore is found in the structure of **mefloquine** (**MQ**), an antimalarial agent that also has antimycobacterial properties (Figure 1) [2,3]. After screening of few synthesised **amino-alcohol-quinolines** (**AAQ**), as **MQ** analogues, a **hit A** was identified. To further increase the selectivity index (SI) and to establish new structure activity or toxicity relationships (SAR/STR), new **AAQ** were designed starting from **hit A** (Figure 2).

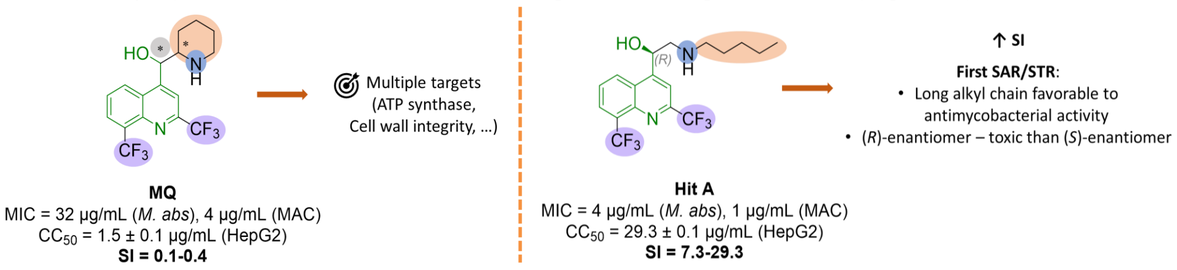


Figure 1: Antimycobacterial quinolines (**MQ** and new **AAQ**).

METHOD

Conception:

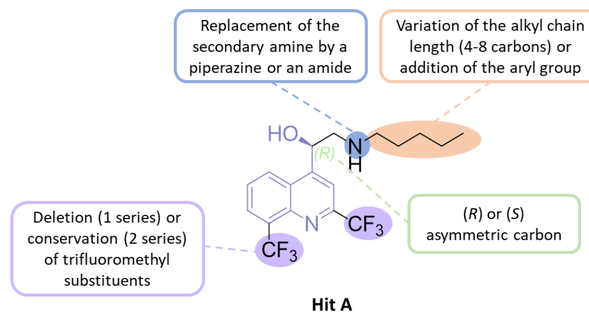
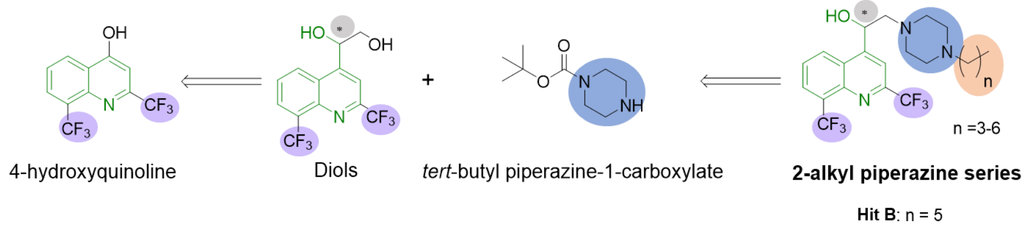


Figure 2: Development of new **AAQ**.

Synthesis: The **AAQ** were obtained using two synthesis strategies. However, only the one from series 2 with alkyl piperazine substituents is shown. The enantiopure **AAQ** were obtained through asymmetric synthesis in five or seven steps (Scheme 1).



Scheme 1: Retrosynthesis of 2-alkyl piperazine series.

***In vitro* antimycobacterial activity**

- Fast-growing NTM: rough and smooth morphotypes of *M. abscessus*
- Two clinical strains of *M. abscessus* sp. *bolletii* and sp. *massiliense*
- Slow-growing NTM: *M. marinum, M. avium* and *M. kansasii*

Cytotoxicity (MTT test)

- HepG2 cells

Selection of the **AAQ** with the highest SI (CC_{50}/MIC) → **Hit B**

Checkerboard assay

Hit B with: - Clarithromycin (CLR)
- Rifamycin (RIF)
- Ethambutol (EMB)

- The 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).

***In vivo* tolerance:** The acceptable *in vivo* dose of **hit B** was determined in BALB/c mice according to OCDE/ODCE Test Guideline n°425 [4].

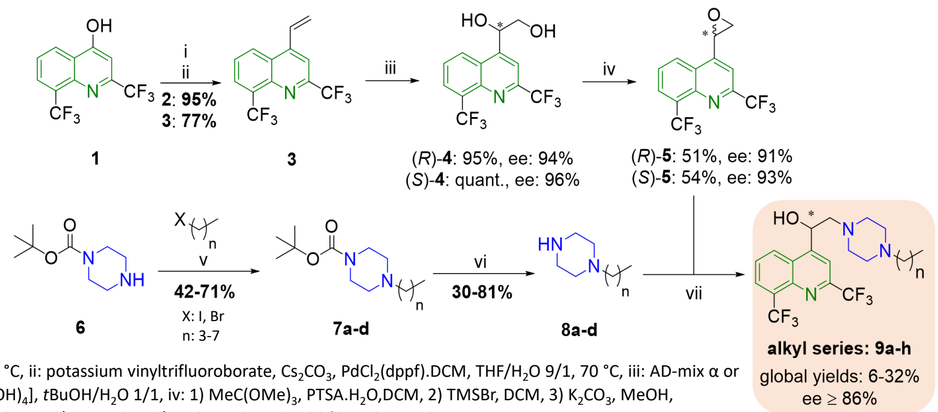
***In vivo* efficacy:** The **hit B** was administered in both mono- and triple therapy in BALB/c mice (Figure 3).

*M. avium ATCC 700898, CFU: colony-forming units

Figure 3: *In vivo* efficacy of **hit B** in BALB/c mice.

RESULTS & DISCUSSION

Twenty-eight **AAQ** were synthesised and eight compounds of series 2 with alkyl piperazine were obtained, with overall yields of 6 to 32%, and enantiomeric excesses greater than or equal to 86% (Scheme 2).



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.

Scheme 2: Synthesis of 2-alkyl piperazine series.

In vitro **antimycobacterial activity** (1 ≤ MIC ≤ 64 µg/mL) and **cytotoxicity assays** (12.5 ≤ CC_{50} ≤ 46.0 µg/mL) led to identify a new hit (**hit B**, Figure 4), the (*R*)-enantiomer with hexyle chain, and exhibited the highest SI against *M. avium* (SI = 5.8). Therefore, the **hit B** was selected for further evaluation of potential synergistic effect. For all antibiotic combinations tested (CLR/EMB, **hit B**/EMB, **hit B**/CLR and **hit B**/RIF), the FICI values were equal to 1 or 1.5, demonstrating an **additive effect** (Table 1).

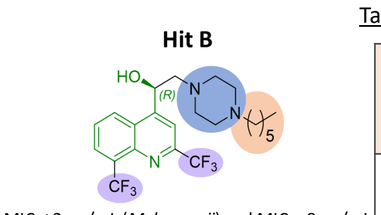


Figure 4: New hit identified.

Table 1: FICI calculation for **CLR**/**EMB**, **hit B**/**CLR**, **hit B**/**EMB** and **hit B**/**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
Hit B / CLR	16 / 0.5	8 / 0.25	1	
Hit B / EMB	4 / 2	4 / 1	1.5	
Hit B / RIF	16 / 0.5	8 / 0.25	1	

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.

The ***in vivo* safety** of **hit B** was supported by the survival of three consecutive BALB/c mice, when administered as a single dose of up to 550 mg/kg (Figure 5).

Figure 5: Administered dose and outcome in mice.

The **hit B** was administered in both mono- and triple therapy with CLR and EMB in BALB/c to evaluate its ***in vivo* efficacy**. This study was confirmed the feasibility of daily administration of **hit B** over several months of treatment.

- **Automatic lung grinding mode** is better than manual mode.
- **7H10 plating medium** is better than 7H11 selective and 7H11 non selective.

Agar plate reading was revealed a very low CFU counts (CFU ≤ 115), indicating that the infection was not sufficiently severe to highlight the potential efficacy of **hit B**.

- **10^9 CFU/mL** is the minimal nebulized inoculum load.

CONCLUSION & FUTURE WORK

- Enantioselective synthesis: **28 AAQ** were synthesised.
- *In vitro* biological evaluation: SAR/STR were established → Identification of **hit B**.
- *In vivo* toxicity: acceptable single dose of **hit B ≥ 550 mg/kg** in BALB/c mice.
- *In vivo* efficacy: too low initial infection in BALB/c mice → protocol optimization determined that a minimum nebulized *M. avium* inoculum of **10^9 CFU/mL**.

Perspectives:

- Novel pharmacomodulations: ↑ SI.
- *In vitro* anti-mycobacterial activity of **AAQ** on additional clinical strains.
- New evaluation of the *in vivo* efficacy of **hit B** with nebulized inoculum of **10^9 CFU/mL**.

REFERENCES

[1] Griffith, D.E. *et al*. *American Journal Respiratory and Critical Care Medicine 175*, 367-416 (**2007**). [2] Bermudez, L.E. *et al. J. infect. Dis. 187*, 1977-1980 (**2003**). [3] Laumaillé, P. *et al. Pharmaceuticals 12*, 91 (**2019**). [4] Test No. 425: Acute Oral Toxicity: Up-and-Down Procedure Available online: https://www.oecd.org/en/publications/test-no-425-acute-oral-toxicity-up-and-down-procedure_9789264071049-en.html (accessed on 17 January **2025**).