Targeting FabZ: Design and synthesis of potential inhibitors

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Antimicrobial resistance (AMR)

- **Antibioresistance: 1.14 M** deaths in 2021 [1]
- ***Plasmodium spp***: 597,000 deaths in 2023 [2]
- Projected deaths for 2050: **39.1 M** worldwide [3]

→ **Critical need to develop innovative and selective strategies**

The FAS-II system as target to combat AMR [4]

- Crucial for microbial lipid metabolism and membrane integrity
- Absent in humans → **Minimized risk of off-target effects**
- Composed of ubiquitous enzymes in most bacteria, parasites,…

→ **Potential for a broad-spectrum antimicrobial**

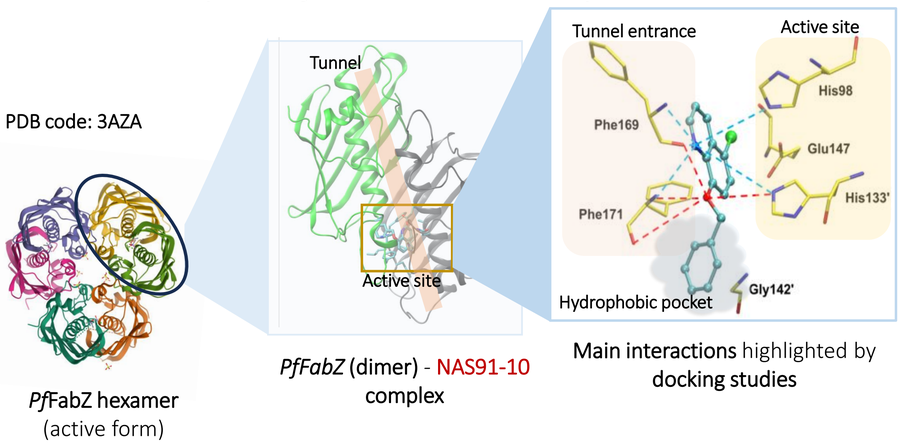
FabZ enzyme in FAS-II

β-hydroacyl-acyl carrier protein deshydratase

- Active site **highly conserved** across pathogens (*Ec*FabZ, *Pa*FabZ, *Pf*FabZ, *Yp*FabZ…) [5]
- **Some known inhibitors**: NAS-91 family, Schiff Base [5-8]
- Crystal and co-crystal structures available in the PDB

Previous work : Rational design of new FabZ inhibitors

Structural analysis of *Pf*FabZ-NAS91-10 co-crystal



*Pf*FabZ hexamer (active form)

PfFabZ (dimer) - NAS91-10 complex

Main interactions highlighted by docking studies

The three main interactions of the quinoline NAS91-10 with *Pf*FabZ **active site** [7] **are:**

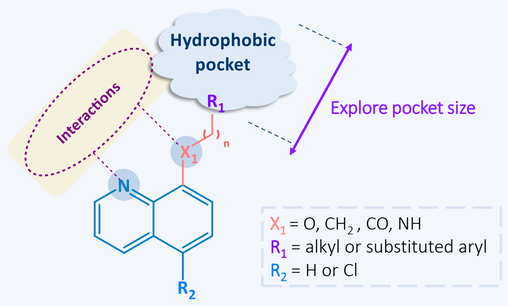
- Quinoline nitrogen and Glu147
- Oxygen and His98 and His133'

Docking studies highlighted that NAS91-10 also interacts with Phe169 and Phe171 at **the tunnel entrance**, regulating **access to the active site**.

Design and synthesis of new 8-aryl quinolines as NAS91-10 analogs to optimize interactions with *Pf*FabZ

General structure

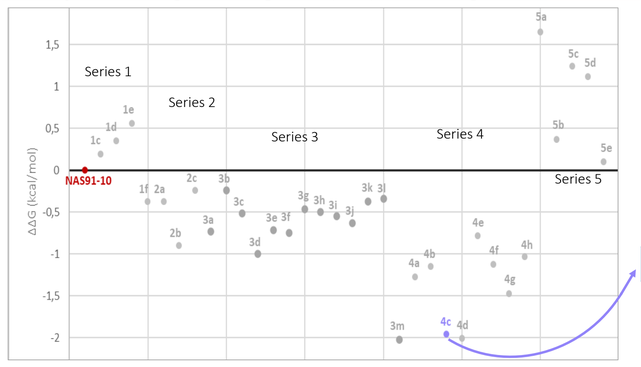
Reference NAS91-10 : X_1 = O ; R_1 = Ph ; R_2 = Cl
Biological activity :
IC_{50}(*Pf*FabZ) = 7.4 µM
IC_{50}(*Pf*) = 12 µM
IC_{50} (HepG2) = 23 µM

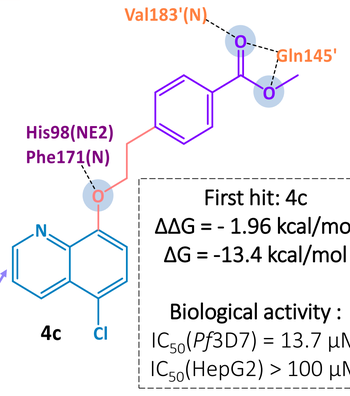


→ 37 new 8-arylquinolines (series 1-5) were synthesized.

In silico studies and *in vitro* biological assays highlighted 4c as first hit

ΔΔG-*Pf*FabZ: exogenous ligands (1-5) *vs.* endogenous NAS91-10





First hit: 4c
ΔΔG = - 1.96 kcal/mol
ΔG = -13.4 kcal/mol

Biological activity :
IC_{50}(*Pf*3D7) = 13.7 µM
IC_{50}(HepG2) > 100 µM

Docking and molecular dynamics indicate that ***Pf*FabZ-4c** forms one of the **most stable complexes compared to *Pf*FabZ-NAS91-10**.

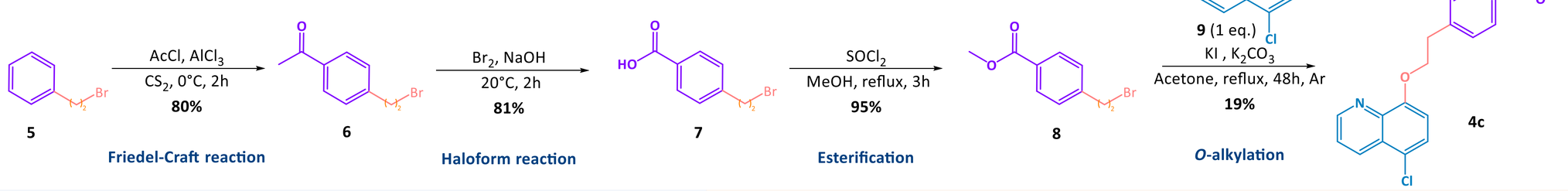
Main interactions with **active site** residues (His98) and **tunnel entrance** residues (Phe171) are maintained, while new interactions are observed (Gln145' and Val183').

Moreover, **4c** is as **active against *Pf*3D7 as** NAS91-10 with **lower cytotoxicity.**

→ Does 4c inhibit *Pf*FabZ enzyme *in vitro* ?

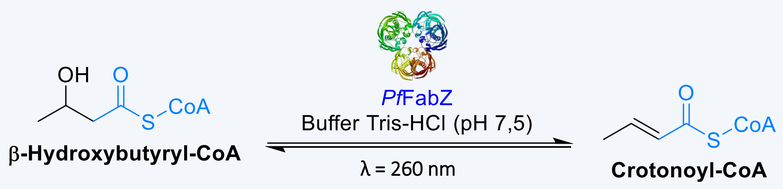
Synthesis of 4c and development of a *Pf*FabZ inhibition assay

1 New synthesis of **4c** in four steps with an **11% global yield**.



2 Design of the *Pf*FabZ enzymatic assay

Goal → High yield production of *Pf*FabZ in active oligomeric form (hexamer)

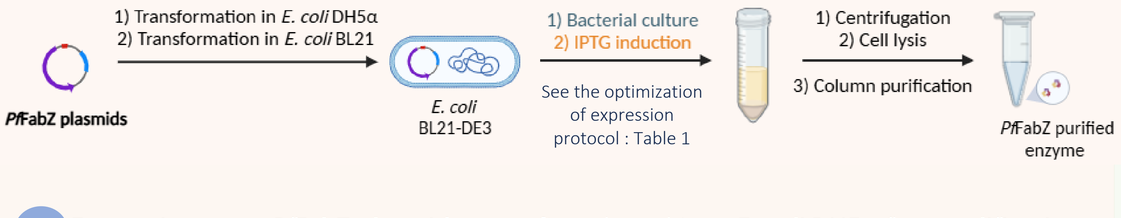


The enzymatic assay monitors the **conversion of β-hydroxybutyryl-CoA** to **crotonoyl-CoA at 260 nm.**

3 Design and optimization of *Pf*FabZ production protocol

*Pf*FabZ production involves two steps: expression and isolation

3a Expression of *Pf*FabZ → 3b Isolation of *Pf*FabZ



3a **Expression step: *Pf*FabZ plasmids** were first cloned into ***E. coli* DH5α for amplification, then transformed into *E. coli* BL21 for efficient enzyme expression.** Bacterial cultures were grown and enzyme production was induced using IPTG (Isopropyl-β-D-thiogalactopyranoside)

3b **Isolation step: bacterial cells** were **lysed**, and **His-tagged *Pf*FabZ was purified** using **Ni-NTA affinity chromatography in TNI buffer (Tris-HCl, NaCl, imidazole)**

3a Table 1: Optimization of *Pf*FabZ expression protocol

Entry	Enzyme	Bacterial cultivation: Medium	Time (h)	OD_{600nm}	Induction Time (h)	Enzyme yield*
1	*Pf*FabZ	Luria-Bertani (LB)	7	0.3 – 0.4	17	1
2	*Pf*FabZ	LB , 1% glucose, 0.4% glycerol	5	0.5 - 0.6	17	1.7
3	Truncated *Pf*FabZ			0.4 - 0.6	17	8.5
4	*Pf*FabZ				4.5	0.6
5	Truncated *Pf*FabZ					1

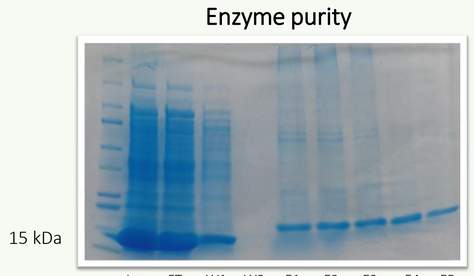
* Concentration in mg/L **determined by BCA** (BiCinchoninic acid) assays

The initial expression conditions (Entry 1) using **LB medium** yielded low ***Pf*FabZ production (1 mg/L)**. To improve yield, we optimized three key parameters: (i) the sequence of the expressed protein (***Pf*FabZ *vs* truncated *Pf*FabZ),** (ii) **bacterial cultivation conditions**, and (iii) **induction time**. In Entry 2, supplementing the culture medium with glucose and glycerol increased optical density (OD_{600nm}), reduced cultivation time and **slightly enhanced enzyme production**. **Expression of a truncated *Pf*FabZ variant** [5] (Entry 3, lacking 10 *N*-terminal amino acids) led to a fivefold increase in yield. However, reducing induction times (Entries 4 and 5) significantly decreased yield. **The optimal conditions were achieved in Entry 3, combining truncated *Pf*FabZ, supplemented medium**, and a **17-hour induction period**.

4 Analyses of *Pf*FabZ production

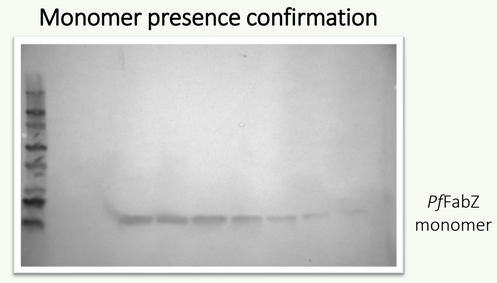
Analyses were performed under denaturing conditions using **SDS-PAGE, confirming the monomeric form of FabZ. Coomassie blue staining** demonstrated **the purity of the recovered eluates** (E_1-E_4), while Western blot **confirmed the presence** of a **His-tagged monomer** (~17 kDa) using an **anti-His-tag antibody**. In future studies, we will develop a **Native-PAGE protocol** to assess **the presence of the hexameric form of FabZ.**

Enzyme purity



Coomassie blue

Monomer presence confirmation



Western blot

Conclusion and perspectives

Previous work highlighted 4c as a potential ***Pf*FabZ inhibitor.** In this study**, 4c** was resynthesized **in four steps** with an **11% overall yield.** In parallel, optimization of the ***Pf*FabZ production protocol increased enzyme yield from 1 mg/L to 8.5 mg/L.** Future work will focus on the **structural analysis of *Pf*FabZ** using dynamic light scattering **(DLS)** to determine its **oligomeric state** and circular dichroism **(CD)** to refine its **secondary structure, followed by enzymatic inhibition assays to evaluate the effect of 4c on *Pf*FabZ.** This study will also **facilitate the production of other FabZ enzymes,** such as *Ec*FabZ and *Pa*FabZ**, and will guide the synthesis of new potential inhibitors.**

Acknowledgments : This work was supported by the ANR Astrid (projectANR-23-ASTR-0005)

1. *WHO Bacterial Priority Pathogens List 2024*, 1st ed.; World Health Organization: Geneva, **2024**. 2. *World Malaria Report 2024*, 1st ed.; World Health Organization: Geneva, **2024**. 3. Kariuki, S. *The Lancet* **2024**, *404*, 1172–1173. 4. Bibens, L.; Becker, J-P.; Dassonville-Klimpt, A.; Sonnet, P. *Pharmaceuticals*, **2023**, *16*, 425. 5. Sharma, S.K. et *al*. *Bio. Chem.*,**2003**, *278*, 45661–45671. 6. Zhang, L. et *al*. *J. Biol. Chem.*, **2008**, *283*, 5370-5379. 7. Tasdemir, D. et *al*. *J. Med. Chem.*, **2006**, *49*, 3345-33. 8. Maity, K. *et al. J. Struct. Biol.*, **2011**, *176*, 238-249.