Design, synthesis and SAR evaluation of potential proteasomal accessory factor A (PafA) inhibitors

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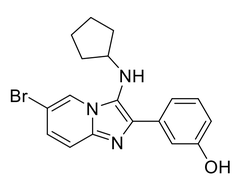
1- Introduction

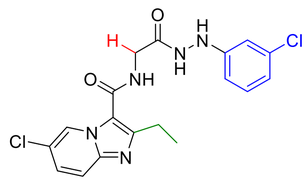
- **Tuberculosis (TB)** is an infectious disease that is a primary cause of illness and death globally and is ranked as the second leading cause of death from a single infectious agent after COVID-19.[1] According to the World Health Organisation (WHO), approximately 10.6 million people were reported to have been diagnosed with TB in 2022 whilst the number of TB deaths was 1.3 million.[1]
- Consequently, there is an increasing demand for new medications with novel mechanisms of action. **Proteasome accessory factor A (PafA)** is an attractive target due to its role in virulence and its poor sequence conservation in humans.[2] Our work focuses on the synthesis, biological activity and SAR evaluation of a novel series of potential *Mtb* PafA inhibitors.

2- Project outline

- Considering the homology between PafA and glutamine synthetase (GS) enzyme, we investigated the use of existing GS ATP-competitive inhibitors in a virtual screen in PafA. Reviewing the literature provided a series of compounds, **1** that were of interest as they are similar to compound **2**, previously synthesised in our group.[3]



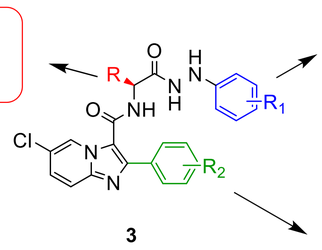
1
GS IC_{50}= 3.3 µM



2
Mtb MIC = 39 µM

- Consequently, this project began by adapting the current molecule **2**, replacing the ethyl group with a substituted aromatic ring to mirror compound **1**. This allowed three series of compounds **3** with distinct amino acid (R), hydrazine (R_1), and phenyl ring attached to scaffold (R_2) to be synthesized to enable a comprehensive SAR study.

R= Gly, Ala, Leu, Met, Phe



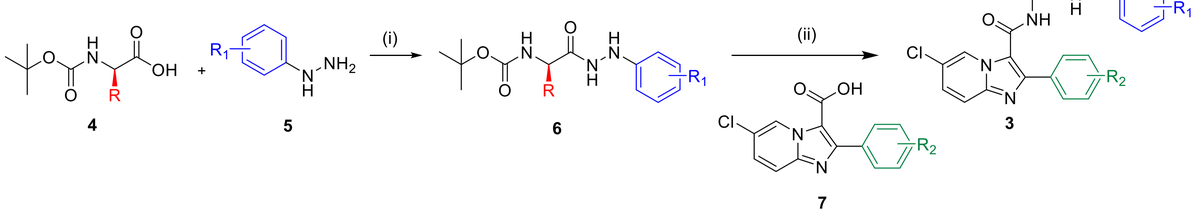
3

R_1= H, 3-Cl, 4-Cl, 3-Br, 4-Br, 3-CF_3, 4-CF_3, 3-OCF_3, 4-OCF_3

R_2= 3-Cl, 4-Cl, 3-CF_3, 4-CF_3, 3-OCH_3, 4 -OCH_3

3- Synthesis of imidazo[1,2-*a*]pyridine analogues

- This work involved two synthetic steps, starting with the synthesis of the substituted-aryl hydrazides **6** by coupling the Boc-protected amino acids **4** with the required aryl hydrazine **5.**
- The second step involved deprotection of the Boc amino acid hydrazides **6** using 4M HCl in dioxane and coupling with the carboxylic acid **7** to provide final compound **3** with moderate yields.

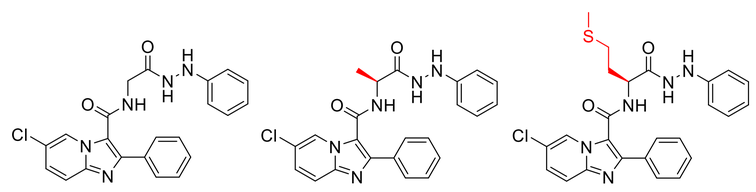


4 **5** **6** **7** **3**

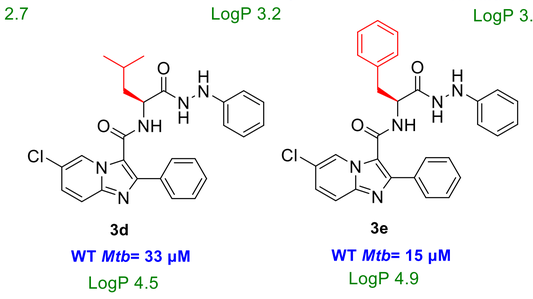
Scheme 1: Reagents and conditions (i) DIPEA, HBTU, THF, 6 h, r.t, (50% - 94%); (ii.) **7**, DIPEA, HBTU, THF, overnight, r.t, (23% - 54%).

4- Results

- 107 novel imidazo[1,2-*a*]pyridine substituted amino acid hydrazides were synthesised and screened in a resazurin microtitre assay (REMA) against different strains of *Mtb* wild-type (WT), isoniazid-resistant (INH^R), rifampicin-resistant (RIF^R) and multi-drug resistant strain (INH^R/ RIF^R).
- The results demonstrate a strong correlation between MIC values and the length of the amino acid side chain (**3a-3e**). These differences in activity are likely attributed to changes in lipophilicity as the amino acid side chain length increases.

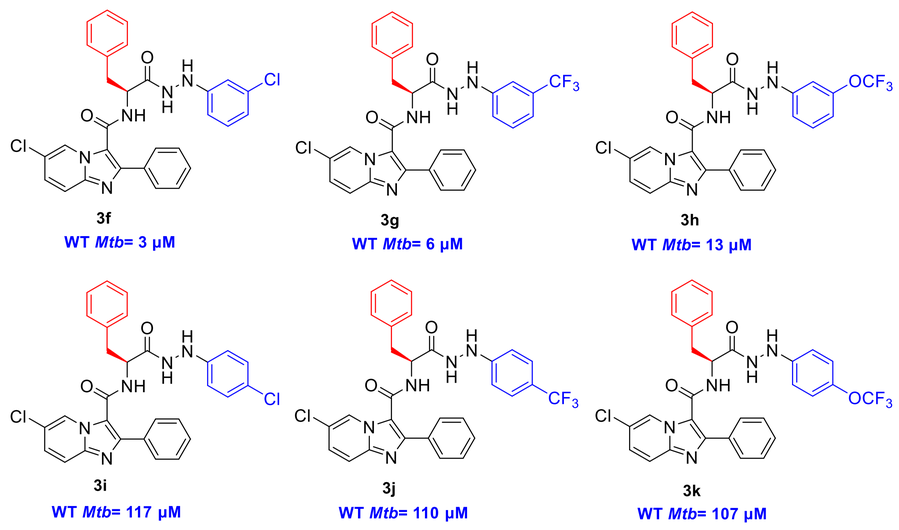


3a WT *Mtb*= 152 µM, LogP 2.7
3b WT *Mtb*= 147 µM, LogP 3.2
3c WT *Mtb*= 64 µM, LogP 3.5



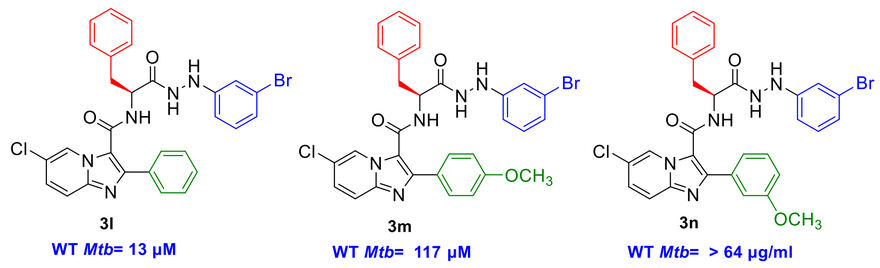
3d WT *Mtb*= 33 µM, LogP 4.5
3e WT *Mtb*= 15 µM, LogP 4.9

- The introduction of substituents in the *meta* position of hydrazine demonstrated a significant decrease in MIC **(3f – 3h)**, while changing to the *para* position leads to loss of activity. **(3i – 3k)**



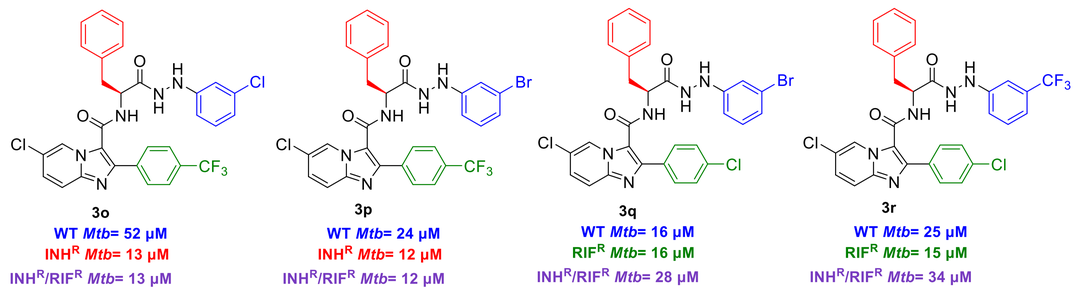
3f WT *Mtb*= 3 µM
3g WT *Mtb*= 6 µM
3h WT *Mtb*= 13 µM
3i WT *Mtb*= 117 µM
3j WT *Mtb*= 110 µM
3k WT *Mtb*= 107 µM

- Furthermore, introducing substituents at various positions around the aromatic ring connected to the imidazo[1,2-*a*]pyridine scaffold results in a diminishment of its activity. (**3l** vs **3m** and **3n**)



3l WT *Mtb*= 13 µM
3m WT *Mtb*= 117 µM
3n WT *Mtb*= > 64 µg/ml

- Interestingly, the introduction of R_2= 4-CF_3 to the aromatic ring connected to the scaffold, while resulting in a decrease in WT activity, exhibits good activity < 15 µM against INH^R and INH^R/ RIF^R strains for the phenylalanine compounds (**3o** and **3p**). Similarly, the incorporation of R_2= 4-Cl confers good activity against RIF^R strain (**3q** and **3r**).



3o WT *Mtb*= 52 µM, INH^R *Mtb*= 13 µM, INH^R/RIF^R *Mtb*= 13 µM
3p WT *Mtb*= 24 µM, INH^R *Mtb*= 12 µM, INH^R/RIF^R *Mtb*= 12 µM
3q WT *Mtb*= 16 µM, RIF^R *Mtb*= 16 µM, INH^R/RIF^R *Mtb*= 28 µM
3r WT *Mtb*= 25 µM, RIF^R *Mtb*= 15 µM, INH^R/RIF^R *Mtb*= 34 µM

5- Conclusion

- 107 novel-final compounds were successfully synthesised during this research and screened against *Mtb* strains including WT, INH^R, RIF^R and multi-drug resistant strains.
- Phenylalanine analogues exhibited the best activity, and for the phenylhydrazine moiety, the incorporation of smaller and more electronegative halogens at the *meta* position is essential for optimal activity.

References

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2. Li C, Liu S, Dong B, et al. Published online **2022**. doi:10.1021/acs.jmedchem.2c00289
3. A. K. Brown, A. K. B. Aljohani, F. M. A. Alsalem, J. L. Broadhead, J. H. Gill, Y. Lu and J. D. Sellars, *Molecules*, **2020**, **25**, 1–24.

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