NOVEL APPROACHES TOWARD THE ERADICATION OF RESISTANT AND PERSISTENT *M. TUBERCULOSIS* STRAINS.

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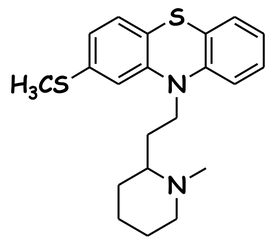
Introduction

Tuberculosis (TB), brought by *M. tuberculosis* (*Mtb*) is one of deadliest diseases that the human kind have faced throughout the centuries, and the toll of deaths caused by this disease is still remarkable.[1] Two main features characterize TB infection: drug-resistance (MDR-TB and XDR-TB) and the presence of persistent strains, that extend the duration of the therapy and make it more difficult and expensive to eradicate. Efflux pumps are known to be important means to modulate antibiotic resistance for many bacteria, and for *Mtb* in particular. Indeed, efflux pump inhibitors such as thioridazine (TZ) and verapamil (VP) have been used in combination with known antituberculars to enhance the rate of treatment.[2,3] All of these findings prompted us to prepare TZ analogues and to test them, along with a small in-house library of compounds, for their inhibitory effects on ethidium bromide (EtBr) efflux and for their synergistic effects with known antituberculars administered at sub-therapeutic concentrations. Among the compounds tested, **UPAR 174** and **UPAR 223** were found to be particularly interesting, as described herein. *M. Smegmatis* was used as a surrogate of *Mtb* for these preliminary assays.

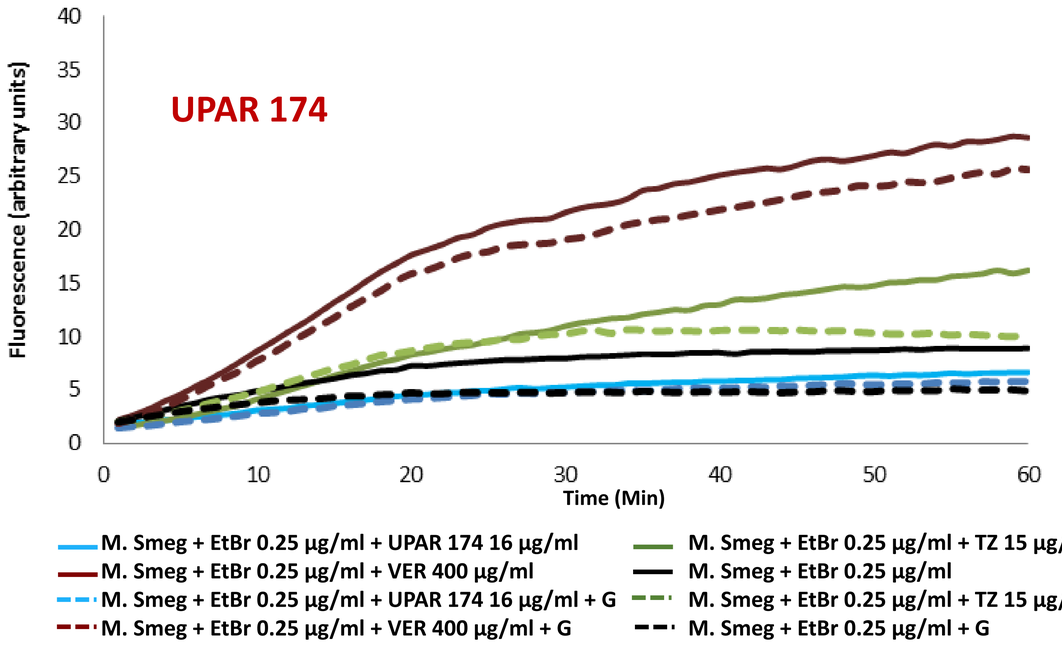
In-House Library + Thioridazine analogues → Biological screenings

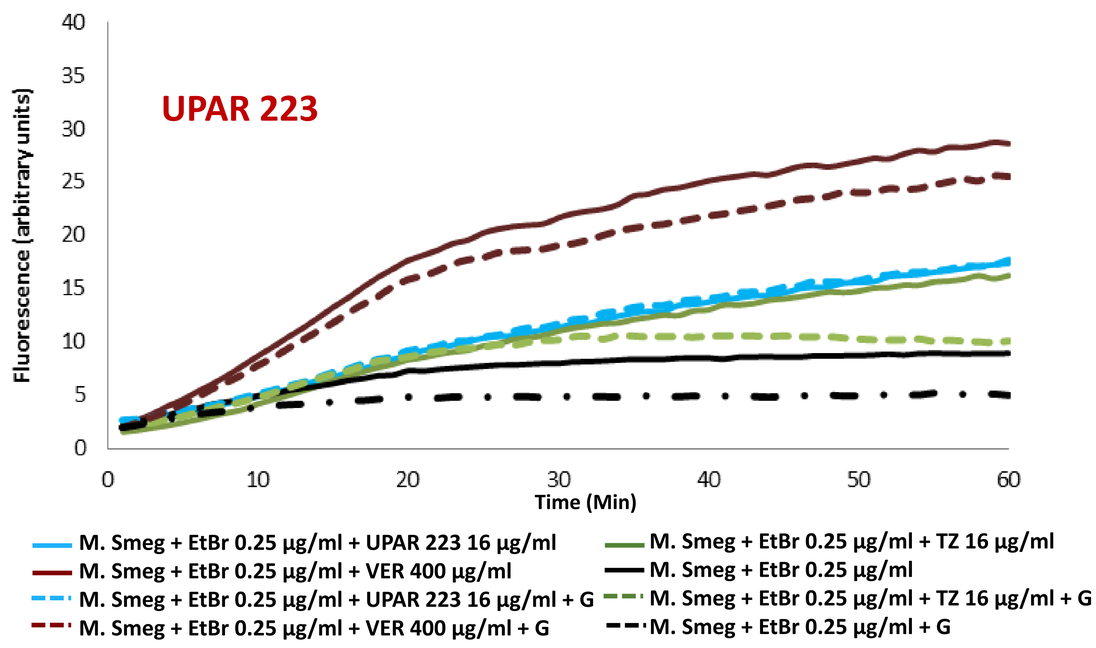


- ✓ Lack of activity toward *M. smegmatis*
- ✓ Inhibition of EtBr Efflux
- ✓ Strong synergistic effects with known drugs

Results

A . Inhibition of EtBr efflux





B. Synergistic effect of tested compounds with known antituberculars

Adjuvant	MIC RIF[a]	MIC CLA[a]	MIC OFX[a]	MIC CIP[a]	MIC EtBr[a]
No compound	16	8	0.5	0.5	25
+ UPAR 223[b]	**1 (↓16)[c]**	**0.5 (↓16)**	**1(↑2)**	**0.25**	**1.56 (↓16)**
+ UPAR 174[b]	**<0.125 (<↓128)**	**<0.125 (<↓64)**	**<0.125 (<↓64)**	**0.0312 (↓16)**	**<0.195 (↓128)**
+ VP	8	2 (↓4)	1(↑2)	0.25	1.56 (↓16)
+ TZ	2 (↓8)	2 (↓4)	0.5	0.25	6.25 (↓4)
+ CPZ	2 (↓8)	2 (↓4)	0.5	0.25	6.25 (↓4)

- **Notes**
- *M. Smegmatis* mc²-155 is used for the assay
- UPAR 174 MIC (*M. Sm.*)= 64 µg/mL
- UPAR 223 MIC (*M. Sm.*)= 128 µg/mL
- Thioridazine (TZ), verapamil (VP) and chlorpromazine (CPZ) were used as reference compounds
- [a]AntiTB agents were tested at ¼ of their MICs
- [b]Adjuvants were tested at ¼ of their MICs
- [c]Indicates how many times the MICs of the antiTB agents decreases

Conclusions and perspectives

Aim of this project was to individuate compounds that could inhibit *M. tuberculosis* efflux systems, in order to prevent and contain the spread of resistances. In this preliminary study, compounds were first tested for their capability to inhibit the growth of *M. smegmatis*, resulting either inactive or negligibly active. **UPAR 174** failed to inhibit the efflux of EtBr, while **UPAR 223** was found to inhibit the efflux of EtBr similarly to TZ, but to a much lesser extent than VP. Both compounds, however, were found to increase the activity of known antiTB drugs when tested at sub-therapeutic concentrations. Surprisingly, **UPAR 174** enhanced the activity of RIF and EtBr by more than 100 times. Experiments on *M. tuberculosis* and studies on the mechanism of action of **UPAR 174** are currently ongoing.

References 1) WHO | Global tuberculosis report **(2013)**. , *WHO*. [Online]. Available: http://www.who.int/tb/publications/global_report/en/; 2) Bhardwaj, A.K. et al. **(2012)**, *Recent Patents Anti-Infect. Drug Disc.* 7, 73–89; 3) Amaral, L. *et al.* **(2012)**, *Int. J. Antimicrob. Agents*. 39, 376-380.