Rational Design and Synthesis of Thioridazine Analogues as Enhancers of the Antituberculosis Therapy

Elisa Azzali[1], Marco Pieroni[1], Diana Machado[2], Sofia Santos Costa[2], Isabel Couto[2], Miguel Viveiros[2] and Gabriele Costantino[1]*

[1] Dipartimento di Farmacia, P4T group, University of Parma, Parco Area delle Scienze 27/A, Parma, 43124

[2]Grupo de Micobactérias, Unidade de Microbiologia Médica, Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa (IHMT/UNL), Rua da Junqueira 100, 1349-008 Lisboa, Portugal.

marco.pieroni@unipr.it

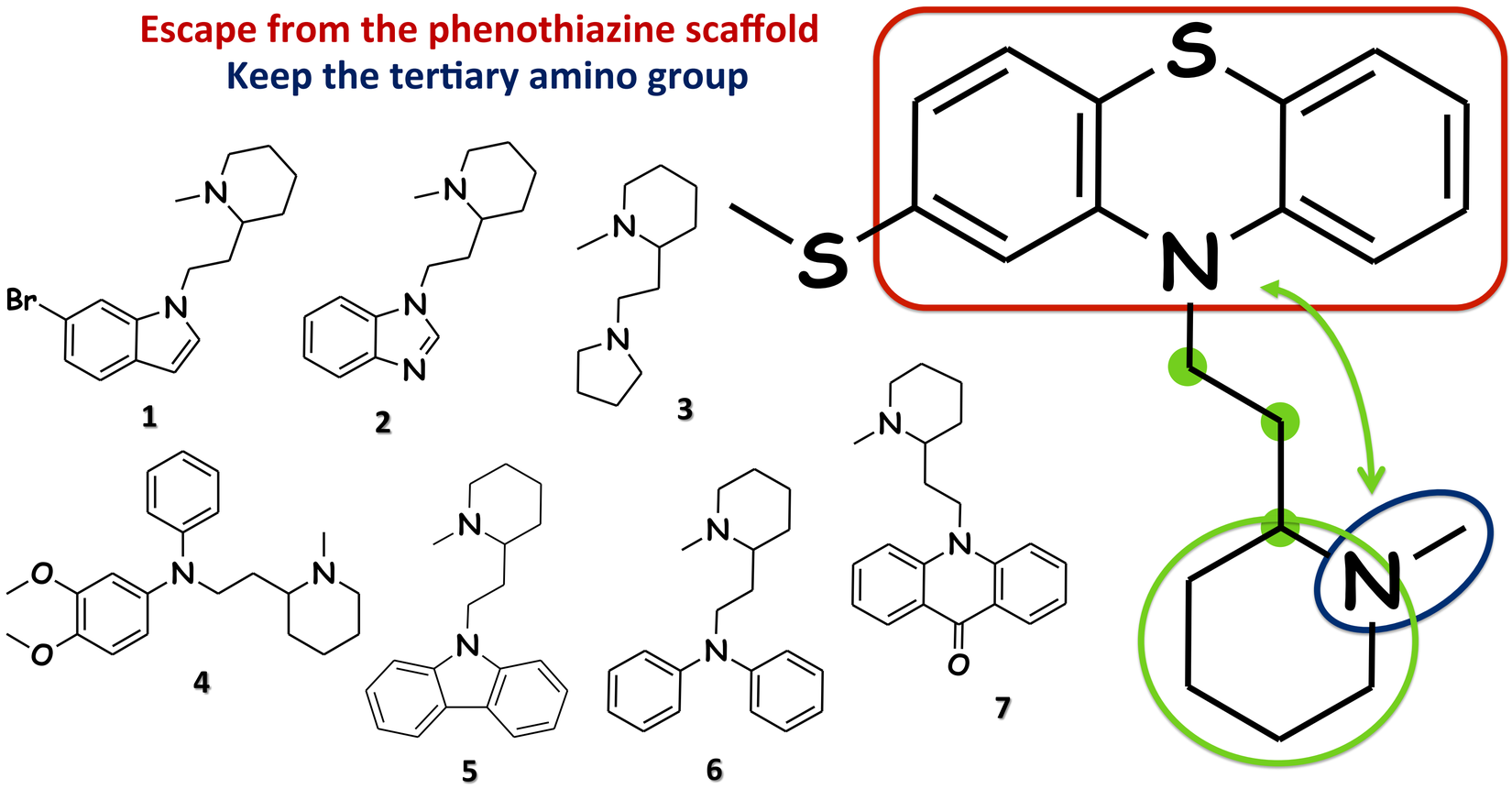
Introduction

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* infection, is one of deadliest diseases that the human kind have faced throughout the centuries. Although the many efforts toward the discovery of novel antibacterial agents, efflux pumps have been seldom investigated for their potential to prevent the pathogenesis. In fact, inhibition of efflux pumps may help to prevent the raise of drug resistance, to contain its spread, and to re-establish obsolete therapeutic options.[1] Thioridazine (TZ) is an old neuroleptic that, by virtue of its capability to inhibit bacterial and macrophage efflux mechanisms, has shown to cure drug-resistant tuberculosis when administered in combination with other anti-TB drugs.[2,3] However, its use in therapy is hampered by its general toxicity. These findings have inspired our work on the search of TZ analogues to be used as adjuvants with first-line drugs for the cure of TB, and the prevention of resistance raise.

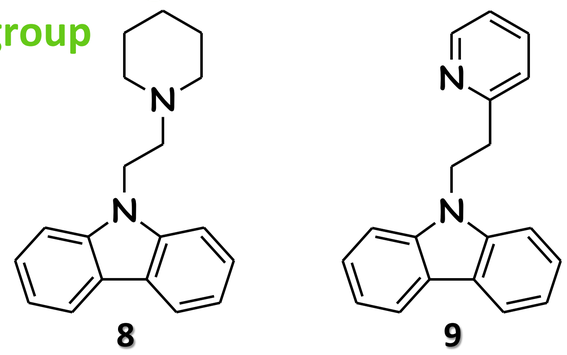
Aim of the work

Escape from the phenothiazine scaffold

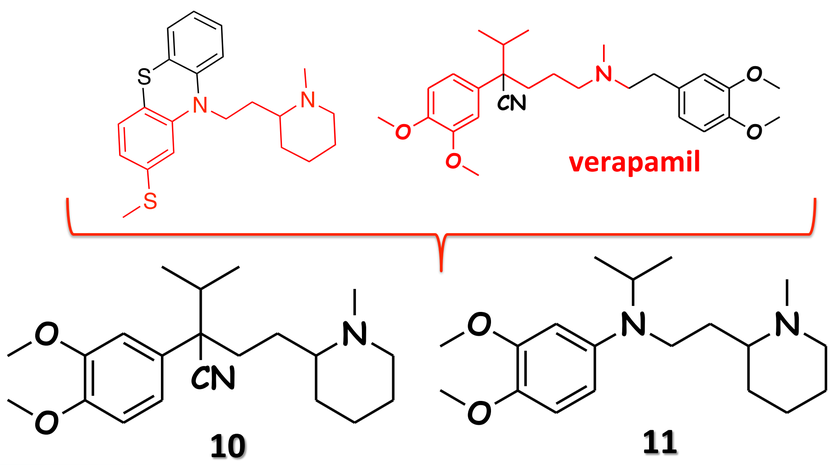
Keep the tertiary amino group



Investigate the length of the linker and the amino group



Possibility to synthesize hybrid structures with other inhibitors of efflux



Results

Table 1. Antimycobacterial (MIC), efflux inhibitory activity of the compounds against *M. smegmatis* mc²155 and *M. tuberculosis* H_{37}Rv (RFF), and cytotoxicity towards human monocyte derived macrophages (IC_{50}).

Comp	*M. smegmatis* mc²155		*M. tuberculosis* H37Rv		IC_{50} (µg/mL)
	MIC (µg/mL)[a]	RFF[b]	MIC (µg/mL)	RFF	
1	**64**	**0.81**	**>256**	**1.22**	**10.5**
2	**>256**	**15.3**	**>256**	**1.00**	**170.8**
3	>256	0.39	nd	nd	nd
4	256	0.76	nd	nd	nd
5	**256**	**0.95**	**128**	**0.33**	**8.8**
6	>64	0.61	nd	nd	nd
7	64	0.94	nd	nd	0.762
8	>256	0.08	nd	nd	nd
9	>64	0.61	nd	nd	nd
10	>256	0.72	nd	nd	nd
11	**>256**	**1.17**	**>256**	**0.84**	**84.8**
TZ	**30**	**0.82**	**15**	**0.27**	**13.78**
VP	**800**	**2.21**	**512**	**1.94**	**116.6**

[a] Determined by microdilution; [b]Relative final fluorescence based on accumulation of EtBr at 0.25 µg/mL for *M. smegmatis* and 0.5 µg/mL for *M. tuberculosis* strains; nd: not determined.
* Compounds were tested at ½ MIC in efflux assays

Compounds more active than TZ in inhibiting efflux and less toxic toward HMDM were selected for synergistic activity

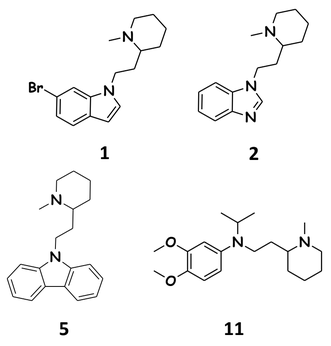


Table 2. Evaluation of the synergistic effect and determination of the modulation factor of the selected compounds with first- and second- line drugs against *M. tuberculosis* H_{37}Rv.

Comp	test conc[a]	MIC (µg/mL) against *M. tuberculosis* H37Rv (MF)[g]				
		INH[b]	RIF[c]	AMK[d]	OFX[e]	EtBr[f]
		0.1	**1**	**2**	**2**	**12.5**
1	16	0.1	**0.0625(↓16)**	1	1	12.5
	8	0.1	**0.25(↓4)**	1	1	12.5
	4	0.1	**0.5**	1	1	12.5
2	16	0.1	0.5	1	1	12.5
	8	0.1	0.5	1	1	12.5
	4	0.1	0.5	1	1	12.5
5	8	0.1	0.5	1	1	12.5
	4	0.1	1	1	1	12.5
11	16	0.1	**0.25(↓4)**	2	2	12.5
	8	0.1	**0.25(↓4)**	2	2	12.5
	4	0.1	**0.25(↓4)**	2	2	12.5
TZ	7.5	**0.00078 (↓128)**	**<0.0156 (>↓64)**	**<0.0156 (↓128)**	**<0.0156 (>↓128)**	**<0.195 (>↓64)**
	3.75	0.05	1	1	1	3.125 (↓4)
VP	64	0.05	0.25 (↓4)	1	1	3.125 (↓4)

[a] Concentration at which the compound was tested (in µg/mL). The concentrations reported were those seleceted for poster purpose; [b] Isoniazid; [c] Rifampin; [d] Amikacin; [e] Ofloxacin; [f] Ethidium bromide; TZ, thioridazine; VP, verapamil. [g] modulation factor (MIC Antibiotic without adjuvant / MIC Antibiotic with adjuvant)

Conclusions

- The phenothiazine scaffold was successfully substituted with other heterocyclics
- Compounds 1, 2, 5, 11 were more active than TZ in inhibiting efflux, both in *M. Smegmatis* and *M. Tuberculosis*
- Compounds 1, 2, 5, 11 showed low toxicity than TZ
- Compound 1 showed synergistic activity with first and second line drugs, in particular with RIF

1)Viveiros, M. et al. (2012) Inhibitor of mycobacterial efflux pumps as potential boosters for anti-tubercular drugs; Expert Reviews; 10, 983-998. 2) Martins, M. et al. (2007) The curative activity of thioridazine on mice infected with Mycobacterium tuberculosis. Vivo Athens Greece 21, 771–775
3) Abbate, E. et al. (2012) Successful alternative treatment of extensively drug-resistant tuberculosis in Argentina with a combination of linezolid, moxifloxacin and thioridazine. J. Antimicrob. Chemother. 67, 473–477