NEW AGENTS SUPRESSING *MYCOBACTERIUM ABSCESSUS*: SMALL MOLECULES AND THEIR PEPTIDE CONJUGATES

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The common environmental ***Mycobacterium abscessus*** (MA) is an important human pathogen responsible for a wide spectrum of infections, mostly in patients with an immunosuppression. **MA** has acquired the reputation of being **the most virulent and chemoresistant rapidly growing mycobacterial species**. The treatment is complicated due to the high rate of intrinsic resistance. Guidelines recommend combining a macrolide with one aminoglycoside and one other drug (linezolid, moxifloxacin, tigecycline, cefoxitin, imipenem); however, clinical efficacy of this regimen is controversial.[1] The partial drop of incidence of tuberculosis (TB) is overshadowed by an increasing incidence of nontuberculous mycobacteria including MA.
These facts justify the development of novel anti-MA drugs with improved activity, no cross-resistance, fast-action, low toxicity and good effect against intracellularly growing and persistent mycobacteria. We found **salicylanilide chloroalkyl carbamates** and **5-chloropyrazinoates** as **highly active against MA** with minimum inhibitory concentrations (**MIC**) of **≥0.2 μM**.[1]

Small molecules evaluation

We have screened a range of known and our previously and also newly prepared antimycobacterial agents' analogues also against MA: salicylanilides, their carbamates and esters with aromatic acids, Schiff bases of aminobenzoic acids, derivatives of 4-aminosalicylic acid (PAS; a second-line anti-TB drug), analogues of antimycobacterial sulphonamides, triclosan etc. Several of them inhibited MA with **MIC** from **5 μg/mL**, namely **salicylanilide**, **triclosan** and **sulfathiazole** derivatives (Fig. **1**).

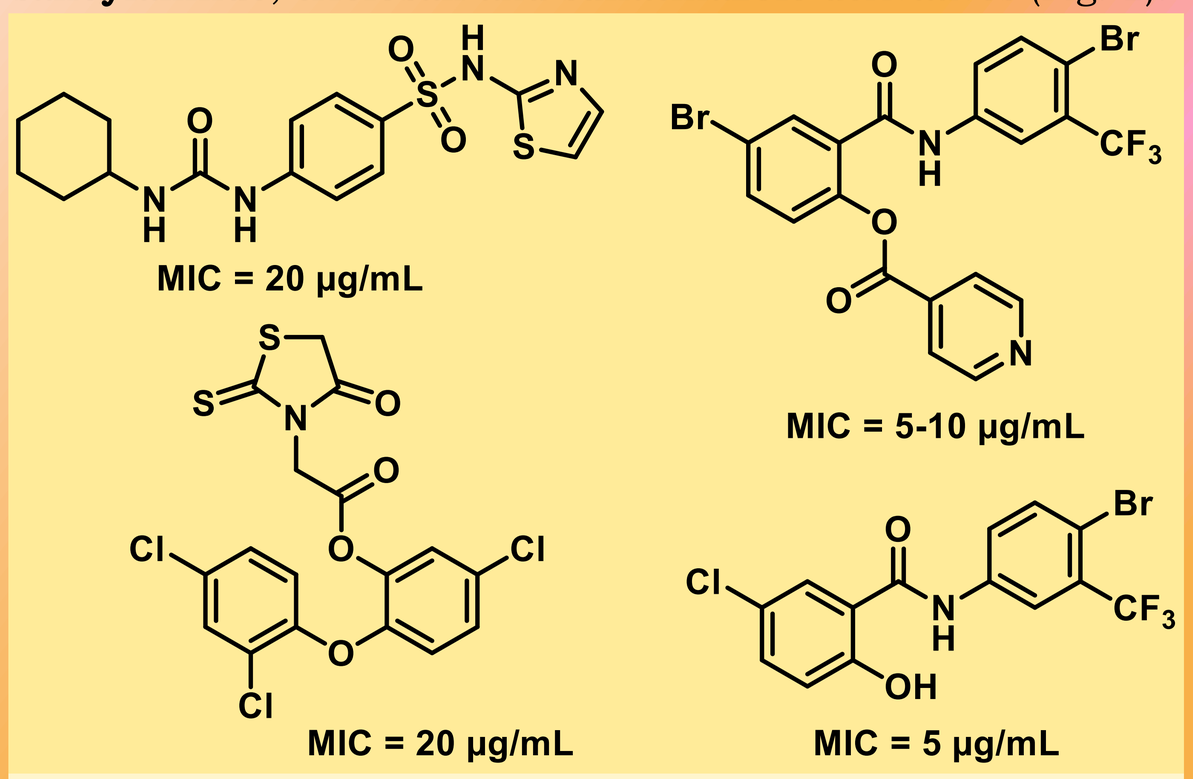
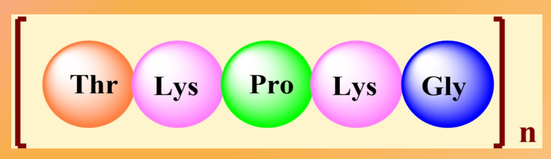


Fig. 1. Example of hits active against *Mycobacterium abscessus*

Based on these promising hits, we initiated screening of analogues and additional derivatives of the compounds summarized in Fig. **1**. The **most promising** and almost **universal anti-MA activity** was found for **salicylanilide derivatives** followed by **sulfathiazole-based scaffold**.

Peptide tuftsin carriers



Tuftsin-based carriers ([TKPKG]$_n$) have been proposed as potential **carriers for antimycobacterial agents**. They are able to overcome unfavourable properties of anti-TB drugs (low solubility and intracellular bioavailability, toxicity), enhance immune response and target macrophages, *i.e.*, increasing **cellular uptake** and **selectivity**. They are nor toxic nor immunogenic.[2]

Peptide-drug conjugates

Based on our previous positive results with conjugation of anti-TB drugs with peptide carriers,[2] we **conjugated** several **anti-MA derivatives with tuftsin-based drug delivery systems**. Peptides were synthesized *via* manual solid-phase synthesis using Fmoc/*t*-Bu strategy.[2]
We were focused on various issues:

- **various length of the carriers** ([TKPKG]$_n$, n = 1-4),
- **presence of enzyme cleavable linker** GFLG between peptide and small molecule,
- **chemical bond used for ligation** drug-carrier (oxime, amide),
- various functional groups of the small molecule used for ligation with carriers (if applicable).

Synthesis of peptide conjugate of PAS is depicted in Fig. **2**.

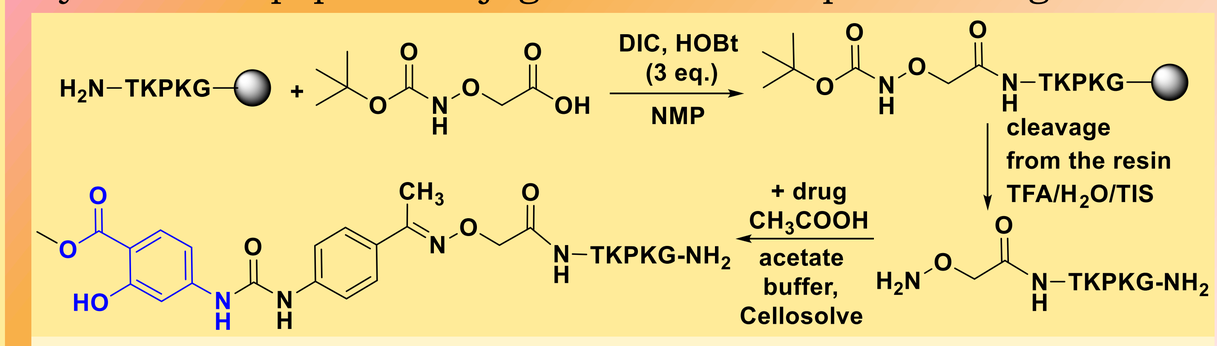


Fig. 2. Example of the peptide-drug conjugation *via* oxime bond (DIC: diisopropylcarbodiimide; HOBt: 1-hydroxybenzotriazole; NMP: *N*-methylpyrrolidone; TFA: trifluoroacetic acid; TIS: triisopropylsilane).

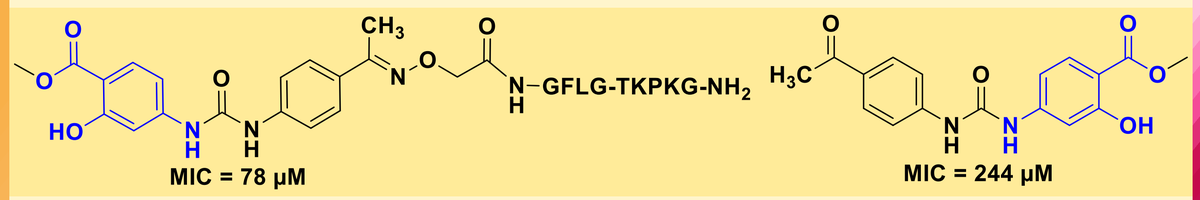


Fig. 3. The influence of peptide carrier on anti-MA activity

Conclusions

- we identified several compounds active against MA,
- we synthesized **tuftsin-based carriers** and their conjugates with anti-MA agents,
- **conjugation** can **improve** the **activity of small molecules** against MA (e.g., Fig. **3**), shorter carriers are preferred,
- majority of the peptide conjugates avoided any significant toxicity for eukaryotic cells (HepG2, MonoMac6) at 250 μM.

References

1. Baranyai Z., Krátký M. et al. *Eur. J. Med. Chem. 101*, 692 (2015).
2. Baranyai Z., Krátký M. et al. *Eur. J. Med. Chem. 133*, 152 (2017).

This work was supported by the Czech Science Foundation project No. 17-27514Y and by FIKP, Ministry of Human Capacities, 1783-3/2018/FEKUTSTRAT. This work was also supported by the project EFSA-CDN (No. CZ.02.1.01/0.0/0.0/16_019/0000841) co-funded by ERDF.