Synthesis and antimicrobial activity of novel sulfonamide derivatives

MARTIN KRÁTKÝ[1], JIŘINA STOLAŘÍKOVÁ[2], JARMILA VINŠOVÁ[1]
[1]Department of Inorganic and Organic Chemistry, Faculty of Pharmacy, Charles University, Hradec Králové, Czech Republic
[2]Laboratory for Mycobacterial Diagnostics and Tuberculosis, Regional Institute of Public Health in Ostrava, Ostrava, Czech Republic

Introduction

The alarming progression of drug resistance among human pathogens justifies the development of novel antimicrobial agents. Research studies should provide new antimicrobial molecules active against, e.g., *Mycobacterium tuberculosis*, nontuberculous mycobacteria, methicillin-resistant *Staphylococcus aureus* (MRSA) and polyresistant Gram-negative species[1)].

The modification of known drugs represents an effective approach in drug design. Sulfonamides have been widely used for the therapy of various bacterial infections. However, they share some disadvantages. More recently, sulfonamides were considered useless for the treatment of tuberculosis but concomitantly useful for the therapy of some nontuberculous mycobacterial infections. Based on the findings that *M. tuberculosis* strains are susceptible *in vitro* to clinically achievable concentrations of sulfamethoxazole **1**, this molecule has been "resurrected" and novel sulfonamides have recently been reported as potential antimycobacterial agents[1, 2)]. Additionally, a wide range of urea derivatives were found to display antimycobacterial activity[3)].

That's why sulfamethoxazole-based ureas **2** and their cyclic analogues imidazolidine-2,4,5-triones **3** have been designed, synthesised, and evaluated as potential antimicrobial agents. In our previous paper[1)], we found that *N*-heptyl sulfamethoxazole-based urea displayed a significant activity against nontuberculous mycobacteria. Based on this finding, we involved also *N*-alkyl derivatives with a varying length of the alkyl chain.

Experimental methods

Sulfamethoxazole **1** was dissolved in dry acetonitrile and appropriate isocyanate (1.1 equivalent) was added. The solution was heated under reflux for 3.5 h and then stirred at ambient temperature for 8 h. In the case of decyl urea derivative, isocyanate was generated *in situ* from decylamine and triphosgene in dichloromethane in the presence of triethylamine under anhydrous conditions.

Oxalyl chloride (1.2 equivalent) was added to sulfonamide-based ureas **2** dissolved or suspended in dry tetrahydrofuran and the mixture was heated under reflux for 2 h. The crystallisation was initiated by an addition of hexane.

The synthetic pathway and the overview of designed derivatives is depicted in Figure 1. Some of the presented compounds were reported previously by our group[1)].

The *in vitro* antimycobacterial activity was evaluated against *M. tuberculosis* $H_{37}Rv$, *M. avium* and two strains of *M. kansasii*. The antibacterial activity was assayed against eight Gram-positive and Gram-negative strains: *Staphylococcus aureus*, MRSA, *Staphylococcus epidermidis*, *Enterococcus* sp.; *Escherichia coli, Pseudomonas aeruginosa* and two strains of *Klebsiella pneumoniae*. The *in vitro* antifungal activity was evaluated against four *Candida* strains, *Trichosporon asahii*, *Aspergillus fumigatus*, *Absidia corymbifera* and *Trichophyton mentagrophytes*. Cytotoxicity was determined for the human hepatocellular liver carcinoma cell line (HepG2).

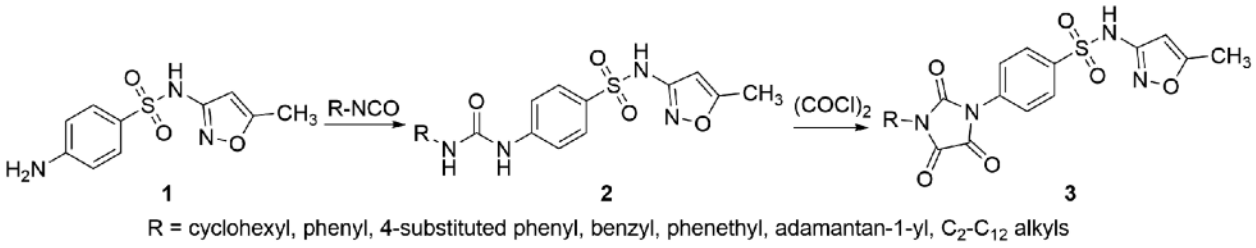

Fig. 1. Synthesis of sulfamethoxazole derivatives **2** *and* **3**

Results and discussion

Sulfamethoxazole-based ureas **2** were obtained in satisfactory yields (40–96%) similarly to corresponding imidazolidine-2,4,5-triones **3** (47–95%).

Derivatives **2** and **3** exhibited antimycobacterial activity with minimum inhibitory concentrations (MICs) of 4 μM or higher. *M. tuberculosis* showed the lowest susceptibility (MICs ≥ 62.5 μM), whereas *M. kansasii*

PharmDr. Mgr. Martin Krátký, Ph.D. (✉)
Department of Inorganic and Organic Chemistry
Faculty of Pharmacy, Charles University
Heyrovského 1203, 500 05 Hradec Králové, Czech Republic
e-mail: martin.kratky@faf.cuni.cz

was the most susceptible species. Nontuberculous mycobacteria were suppressed most effectively by 4-(3--heptylureido)-*N*-(5-methylisoxazol-3-yl)benzenesulfonamide (**2**; R = *n*-heptyl) with MICs of 4–62.5 μM. Several *n*-alkyl ureas were inactive against one or more mycobacterial strains. None of the novel derivatives **2** and **3** exceeded significantly the activity of the parent sulfamethoxazole **1** and isoniazid.

The investigation of the influence of N^3-substitution of ureas **2** on the activity for *M. tuberculosis* revealed that the highest activity is conferred by a shorter alkyl (R = ethyl, butyl), heptyl and 4-substituted phenyl. However, longer alkyls than heptyl did not produce an improvement of antitubercular activity.

The cyclisation of more efficient ureas **2** to imidazolidine-2,4,5-triones **3** resulted in derivatives with similar or lower activity whereas the modification of ureas with weaker antimycobacterial activity resulted in cyclized products **3** with beneficially decreased MIC values.

With respect to the bacterial strains, none of the derivatives significantly inhibited the growth of *P. aeruginosa*, *K. pneumoniae* and *E. coli*. Gram-positive cocci were susceptible with MICs from 125 μM. *S. aureus* exhibited the highest susceptibility with no marked difference between methicillin-susceptible and MRSA strains. Several derivatives avoided any antibacterial action. *N*-Heptyl urea **2** showed activity against *S. aureus* comparable or slightly superior to that of the parent sulfamethoxazole **1**; nevertheless, other alkyl ureas **2** were completely inactive.

With respect to antifungal properties, sulfamethoxazole derivatives **2** and **3** showed almost no antifungal activity. IC_{50} values for HepG2 cells were within the range of ≥ 103.3 μM indicating a low cytotoxicity. *N*-Heptyl urea provided sufficient selectivity indexes (> 10) for nontuberculous mycobacteria.

Conclusion

We designed and synthesized more than thirty sulfamethoxazole derivatives as potential antimicrobial agents. They were evaluated against four mycobacterial strains, a panel of Gram-positive, Gram-negative bacteria and fungi as well as for their cytotoxicity.

The highest *in vitro* activity was found against *M. kansasii*, whereas fungi and Gram-negative bacteria were practically resistant. We identified several derivatives with a promising *in vitro* activity profile.

The work was financially supported by the Research Project IGA NT 13346 (2012).

Conflicts of interest: none.

References

1. **Krátký M., Mandíková J., Trejtnar F., Buchta V., Stolaříková J., Vinšová J.** Synthesis and antimicrobial activity of sulfamethoxazole-based ureas and imidazolidine-2,4,5-triones. Chem. Pap. 2015; 69, 1108–1117.
2. **Krátký M., Vinšová J., Volková M., Buchta V., Trejtnar F., Stolaříková, J.** Antimicrobial activity of sulfonamides containing 5-chloro-2-hydroxybenzaldehyde and 5-chloro-2-hydroxybenzoic acid scaffold. Eur. J. Med. Chem. 2012; 50, 433–440.
3. **Brown J. R., North E. J., Hurdle J. G., Morisseau C., Scarborough J. S., Sun D., Kordulakova J., Scherman M. S., Jones V., Grzegorzewicz A., Crew R. M., Jackson M., Mc-Neil M. R., Lee, R. E.**. The structure-activity relationship of urea derivatives as anti-tuberculosis agents. Bioorg. Med. Chem. 2011; 19, 5585–5595.