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Short communication

New amino acid esters of salicylanilides active against MDR-TB and other microbes

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ABSTRACT

Eleven halogenated (*S*)-2-(phenylcarbamoyl)phenyl 2-acetamido-3-phenylpropanoates (**3a**–**3k**) were designed and synthesized as potential antimicrobial agents. They were evaluated *in vitro* against some mycobacterial, bacterial and fungal strains. These compounds were active against drug-sensitive and atypical mycobacterial strains with general MIC values from 0.25 to 16 μmol/L. The most active compounds were (*S*)-4-chloro-2-(4-(trifluoromethyl)phenylcarbamoyl)phenyl 2-acetamido-3-phenylpropanoate (**3i**) and (*S*)-4-bromo-2-(4-(trifluoromethyl)phenylcarbamoyl)phenyl 2-acetamido-3-phenylpropanoate (**3k**) which exhibited activity against MDR and XDR-TB strains with MICs from 1 to 2 μmol/L. **3k** was shown to be less cytotoxic with higher IC_{50}. Some compounds exhibited low MICs on Gram-positive bacteria (MICs $\geq$ 0.98 μmol/L) and on fungi (MICs $\geq$ 3.9 μmol/L).

1. Introduction

Salicylanilides (2-hydroxy-*N*-phenylbenzamides) have been the subject of interest in medicinal chemistry for many years [1], mainly due to their antibacterial [1,2], antiprotozoal [1,3] and antifungal activity [1,4]. They showed a wide-range of biological activities including potential anticancer efficacy [5–8], influence on interleukins production [9–12], anti-inflammatory activity [13], activity against different viruses [14–18], or effects on ion channels [19].

Increasing emergence of drug-resistant tuberculosis is alarming [20,21]. It is estimated that one third of the world's population is currently infected with *Mycobacterium tuberculosis*, and each year 8–9 million new cases develop. Every year almost 500,000 people are infected with multidrug-resistant TB (MDR-TB) and there are estimated 40,000 new cases of extensively drug-resistant TB (XDR-TB) annually [22].

Multidrug-resistant *M. tuberculosis* was defined as resistant to isoniazid (INH) and rifampicin (RIF), but it may include resistance to more antituberculotics [23]. XDR-TB was defined as a resistance to any fluoroquinolone and to at least one of three injectable drugs capreomycin, kanamycin or amikacin, in addition to MDR-TB. XDR-TB is very often untreatable. HIV co-infection with MDR-TB in immunocompromised patients is a serious challenge for the research since it is demanded a new type of drugs or prodrugs by a new mechanism of action [20].

Waisser et al. [24,25] described a good antimycobacterial activity of substituted salicylanilides against drug-sensitive *M. tuberculosis* and some atypical mycobacterial strains (*Mycobacterium avium*, *Mycobacterium kansasii*) with MICs for di- and trihalogenated salicylanilides in the range of 1–32 μmol/L for all the tested strains.

Designing of new salicylanilide derivatives based on esterification is a known trend. First recent article dealing with antimicrobial esters of salicylanilides was published in 2006 [26]. Hydrophobicity is one of the factors influencing biological activity of salicylanilide. While the presence of phenolic hydroxyls seems to be necessary for the activity, it could have irritative properties. Temporary masking of this group by esterification and resulting changes in physicochemical properties could be advantageous in a high activity, an

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improved solubility, a better bioavailability, passing through the mycobacterial cell wall and a lower toxicity. When amino acids are used for esterification, they could facilitate the possibility of targeting drugs as a drug delivery system [4,26,27].

The worldwide epidemic of antibiotic resistance is touching all people, because antibiotic-resistant bacteria are increasingly seen to be just as virulent as their sensitive counterparts. For example, the global emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) has arguably been the biggest setback in the history of antimicrobial therapy. MRSA has caused serious problems in the empirical use of the major commonly applied antibiotics. The situation in Gram-negative bacteria is no less serious, too [28].

The conditions in the fungal kingdom are quite similar. Fungi can become resistant to each of clinically used antifungal drugs by specific mechanisms. The phenomenon of multidrug-resistance was described analogously to bacteria [29].

Salicylanilide esters showed antimycobacterial activity in the range from 0.5 to 62.5 μmol/L [4,27,30,31]. Salicylanilides, their esters and some structures containing them have shown and confirmed recently their good antibacterial activity, especially on Gram-positive strains in the concentration of 0.25 μg/mL and higher [2,32–38]. About the mechanism of action – salicylanilides were identified as inhibitors of the two-component regulatory systems of bacteria by a mechanism related to the effect on uncoupling oxidative phosphorylation, but other injurious effects on prokaryote cells were described, too [2,31,32,36]. Recently, it was also found in the studies that salicylanilides are selective inhibitors of interleukin-12p40 production playing a specific role in the initiation, expansion and control of the cellular response to TB infection [10,12]. The antifungal activity of salicylanilide esters is significant for acetates and α-amino acids esters with MICs ≥ 0.49 and ≥1.95 μmol/L, respectively [4,30].

Therefore we designed and synthesized a new ester series of halogenated salicylanilides and *N*-acetyl-L-phenylalanine as potential antibacterial and antifungal agents, primarily against *M. tuberculosis* including MDR-TB strains and atypical mycobacteria.

2. Chemistry

The starting salicylanilides were selected according to their previously described high *in vitro* antimycobacterial activity [24] and the *N*-protected amino acid *N*-acetyl-L-phenylalanine due to preliminary publications about good influence of *N*-Cbz-L-phenylalanine on antimycobacterial and antifungal activity of salicylanilides [4,27].

The syntheses of salicylanilide *N*-acetyl-L-phenylalanine esters consist of two steps. At first, starting halogenated salicylanilides (**1**) were prepared routinely by the reaction of substituted salicylic acids and appropriate anilines in the presence of PCl_3 in chlorobenzene. The reaction was carried out in a microwave reactor which led to an increase of the yield and shortening the reaction time [39].

Esters of salicylanilides (**3**) with lipophilic amino acid *N*-acetyl-L-phenylalanine (**2**) were obtained by the activation with *N*,*N*′-dicyclohexylcarbodiimide (DCC) in dry *N*,*N*-dimethylformamide (DMF) (Scheme 1). This method, which was taken from peptide chemistry, has been recently published by our group [30].

All newly prepared compounds were characterised by the melting point, IR and NMR spectroscopy, elementary analysis and optical activity.

3. Pharmacology

3.1. Minimum inhibitory concentration assays

3.1.1. In vitro antimycobacterial susceptibility testing

All the prepared compounds were tested *in vitro* for their antimycobacterial activity in the Laboratory for Tuberculosis in Ostrava against *M. tuberculosis* 331/88 ($H_{37}Rv$) (dilution of the strain was 10^{-3}) and moreover for some non-tuberculous INH-resistant strains – *M. avium* (330/88, dilution 10^{-5}) and *M. kansasii* (235/80, dilution 10^{-4}). One strain was clinically isolated (*M. kansasii* 6509/96 in the dilution 10^{-5}), other strains were obtained from the Czech National Collection of Type Cultures. The micromethod for the determination of the minimum inhibitory concentration (MIC) was used.

The most active compounds **3g**, **3i** and **3k** were evaluated against MDR-TB in the similar conditions using six *M. tuberculosis* strains (dilution 10^{-3}) with different resistance patterns: 357/2005, 362/1998, 53/2009, Praha 1, Praha 131 (XDR-TB strain), and 9449/2007.

3.1.2. In vitro antibacterial susceptibility testing

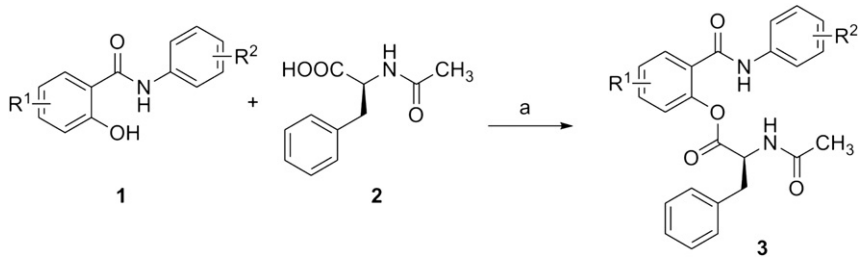
The broth microdilution test M27-A [40] was used for the investigation of *in vitro* antibacterial activity of esters (**3**) against four Gram-positive and four Gram-negative strains: *S. aureus*, methicillin-resistant *S. aureus*, *Staphylococcus epidermidis*, *Enterococcus* sp.; *Escherichia coli*, *Klebsiella pneumoniae*, EBSL-positive *K. pneumoniae*, and *Pseudomonas aeruginosa*. The method used was microdilution panel bouillon method with twofold dilution of the compounds.

3.1.3. In vitro antifungal susceptibility testing

The broth microdilution test M27-A was used for the investigation of *in vitro* antifungal activity of esters (**3**) against four yeast strains: *Candida albicans*, *Candida tropicalis*, *Candida krusei*, *Candida glabrata*, and four moulds: *Trichosporon beigelii*, *Aspergillus fumigatus*, *Absidia corymbifera*, and *Trichophyton mentagrophytes*. The method used was microdilution panel bouillon method with twofold dilution of the compounds.

3.2. In vitro cytotoxicity assay

Cytotoxicity was determined on human peripheral blood mononuclear cells (PBMC). After incubation the cell viability was



Scheme 1. Esterification of salicylanilides by *N*-acetyl-L-phenylalanine (R^1 of esters = 4-Cl, 5-Cl, 4-Br; R^2 = 3-Cl, 4-Cl, 3,4-diCl, 4-Br, 4-CF_3). Reagents and conditions: (a) DMF, DCC, −20 °C.