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Original Research Article

Antimicrobial effect of salicylamide derivatives against intestinal sulfate-reducing bacteria

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ABSTRACT

Sulfate-reducing bacteria (SRB) are most likely involved in both the initiation and maintenance of inflammatory bowel disease (IBD); unfortunately present antibacterial chemotherapeutics used in the treatment of IBD have been ineffective. Thus, the antimicrobial activity of salicylamide derivatives against two different genera of intestinal SRB, *Desulfovibrio* and *Desulfomicrobium*, was investigated. Six 2-(phenylcarbamoyl)phenyl *N*-[(benzyloxy)carbonyl] alkanoates and three 2-hydroxy-*N*-[(2*S*)-1-oxo-1-(phenylamino)alkan-2-yl]benzamides showed MIC values in the range from 0.22 to 0.35 μM against *Desulfovibrio piger* Vib-7 and in the range from 0.27 to 8.52 μM against *Desulfomicrobium* sp. Rod-9, while MIC values of ciprofloxacin were 41.2 μM and 39.3 μM. The highest potency against the two strains was observed for 4-chloro-*N*-{(2*S*)-1-[(3,4-dichlorophenyl)amino]-3-methyl-1-oxobutan-2-yl}-2-hydroxybenzamide (MIC 0.22 μM and 0.27 μM). 4-Chloro-2-[(4-nitrophenyl)carbamoyl]phenyl (2*S*)-2-{[(benzyloxy)carbonyl]amino}-3-methylbutanoate showed high activity against *D. piger* Vib-7 (MIC = 0.26 μM), while 4-chloro-2-[(4-methylphenyl)carbamoyl]phenyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-(1*H*-indol-2-yl)propanoate expressed high activity against *Desulfomicrobium* sp. Rod-9 (MIC = 0.31 μM). Structure–activity relationships are discussed.

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Introduction

Ulcerative colitis (UC) is one of the two major forms of idiopathic inflammatory bowel disease (IBD) (Cummings et al., 2003). Both acute and chronic forms of the illness affect the colon and rectum and can be a highly disabling condition (Barton and Hamilton, 2010). This disease is more common in North America and Western Europe with the increasing incidence in Asia. The reported incidence is 1.2–20.3 cases per 100,000 persons per year, and the prevalence is 7.6–245 cases per 100,000 per year (Feuerstein and Cheifetz, 2014). Ulcerative colitis usually has a relapsing/remitting pattern and current medical approaches focus on treating active disease to address symptoms, to improve the quality of life and thereafter to maintain remission. Bloody diarrhoea, an urgent need to defecate and abdominal pain are the main symptoms of active disease or relapse. The treatment chosen for active disease depends not only on clinical severity, but also on the extent of disease and the person's preference (Loubinoux et al., 2000, 2002a,b; Kornbluth and Sachar, 2010). Conventional drug therapy for UC involves the use of 5-aminosalicylates (the mainstay of treatment for mild to moderate disease), corticosteroids (for patients who failed 5-aminosalicylates therapy and for acute episodes), azathioprine/6-mercaptopurine, cyclosporine and anti-tumour necrosis factor therapy (Lissner and Siegmund, 2013).

Several reports suggested the possible involvement of sulfate-reducing bacteria (SRB), a group of phylogenetically diverse anaerobic microorganisms, in both the initiation and maintenance of the disease (Loubinoux et al., 2000, 2002a,b; Zinkevich and Beech, 2000; Cummings et al., 2003). SRB such as *Desulfovibrio* and *Desulfomicrobium* genera, are normal inhabitants of the human and animal large intestine, capable of dissimilatory sulfate reduction (Gibson et al., 1991, 1993; Kushkevych, 2012a,b; Kushkevych and Moroz, 2012). Most of the SRB utilize sulfate or other sulfur compounds such as thiosulfate, sulfite and sulfur as terminal electron acceptors. The main product of SRB metabolism, hydrogen sulfide, is a compound that may act through inhibition of butyrate oxidation, the main energy source for colonocytes. In addition it is cytotoxic, mutagenic and cancerogenic to epithelial intestinal cells. All these properties of hydrogen sulfide lead to the damage of the epithelial barrier function resulting in inflammatory responses characteristic for IBD (Pitcher and Cummings, 1996; Zinkevich and Beech, 2000). Therefore the association between SRB and inflammatory bowel diseases, such as ulcerative colitis, was hypothesized (Loubinoux et al., 2002a,b; Rowan et al., 2009; Kushkevych, 2014). Unlike Crohn's disease, ulcerative colitis occurs only in the large bowel, where bacteria amount is greater than in the rest of the gut and also where the rate of passage of material is characterized by slow movement of digestive materials (Cummings et al., 2003).

An antibiotic for animal colitis, in order to be effective, should have activity against gut anaerobes. Such antibiotics that specifically target Gram-negative facultative species are not successful in IBD (Cummings et al., 2003). The benefits of antibiotic therapy in UC are mediated by different mechanisms such as decreasing the concentration of luminal bacteria, altering the composition of gut microflora, decreasing bacterial tissue invasion and decreasing bacterial translocation and systemic dissemination. Antibiotics have been prescribed for UC, however they have been largely ineffective. Therefore, it is necessary to study new antibacterial compounds in order to improve the treatment and discover alternative therapeutics (Garud and Peppercorn, 2009).

In the previous studies it was found that salicylamide-like compounds can be considered as promising antimicrobial agents (Vinsova et al., 2007; Imramovsky et al., 2009a,b, 2011; Pauk et al., 2013; Zadrazilova et al., 2015a,b). Therefore this study focused on the investigation of the antimicrobial activity of selected derivatives of 2-(phenylcarbamoyl)phenyl *N*-[(benzyloxy)carbonyl]alkanoates and 2-hydroxy-*N*-[(2*S*)-1-oxo-1-(phenylamino)alkan-2-yl]benzamides against two different genera of SRB, *Desulfovibrio* and *Desulfomicrobium*, is a follow-up paper to the previous contributions. The investigated salicylamide derivatives showed high potency against different bacterial strains as was published recently (Pauk et al., 2013; Zadrazilova et al., 2015a). Both SRB are Gram-negative strictly anaerobe genera. *Desulfovibrio piger* is usually considered as a commensal bacterium in humans. More recently, *D. piger* has attracted more interest as it was found to be the most prevalent species of SRB in faeces of patients with inflammatory bowel disease (Holt et al., 1994; Barton and Hamilton, 2010).

Materials and methods

Tested compounds

The discussed salicylamide derivatives (see Table 1) were synthesized previously (Pauk et al., 2013) by means of microwave-assisted synthesis and rearrangement described in literature (Imramovsky et al., 2006, 2009a, 2010, 2011; Pauk et al., 2013). The compounds were fully characterized by melting point, CHN analyses, IR and NMR spectroscopy (Pauk et al., 2013).

In vitro *antibacterial susceptibility testing*

The synthesized compounds were evaluated for *in vitro* antibacterial activity against the intestinal sulfate-reducing bacteria *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9 that were isolated from the healthy human large intestine as described previously (Kushkevych, 2013; Kushkevych et al., 2014). The strains have been kept in the collection of microorganisms at the Department of Molecular Biology and Pharmaceutical Biotechnology of the Faculty of Pharmacy at the University of Veterinary and Pharmaceutical Sciences Brno (Czech Republic). Ciprofloxacin (Sigma–Aldrich) was used as the standard. Prior to testing, each strain was passaged onto nutrition modified Kravtsov-Sorokin's (KS) agar medium (Kushkevych and Moroz, 2012). Bacterial inocula were prepared by suspending a small portion of bacterial colony in sterile KS liquid medium (pH 7.5). The cell density was adjusted to 0.5 McFarland units using a densitometer (Densi-La-Meter, LIAP, Latvia). The final inoculum was made to a 1:20 dilution of the suspension with KS liquid medium. Before bacterial passage in the medium, 10 mL/L of sterile Mohr's salt solution [$(NH_4)SO_4Fe(SO_4)_2{\cdot}6H_2O$] (10%) for detecting colonies of the