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New derivatives of salicylamides: Preparation and antimicrobial activity against various bacterial species

Karel Pauk [a], Iveta Zadražilová [b,c,d], Aleš Imramovský [a,*], Jarmila Vinšová [e], Michaela Pokorná [c], Martina Masaříková [c,d], Alois Čížek [c,d], Josef Jampílek [b]

[a] Institute of Organic Chemistry and Technology, Faculty of Chemical Technology, University of Pardubice, Studentská 573, 532 10 Pardubice, Czech Republic
[b] Department of Chemical Drugs, Faculty of Pharmacy, University of Veterinary and Pharmaceutical Sciences, Palackého 1/3, 612 42 Brno, Czech Republic
[c] Department of Infectious Diseases and Microbiology, Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences, Palackého 1/3, 612 42 Brno, Czech Republic
[d] CEITEC VFU, University of Veterinary and Pharmaceutical Sciences, Palackého 1/3, 612 42 Brno, Czech Republic
[e] Charles University, Faculty of Pharmacy, Department of Inorganic and Organic Chemistry, Heyrovského 1203, 500 05 Hradec Králové, Czech Republic

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ABSTRACT

Three series of salicylanilides, esters of *N*-phenylsalicylamides and 2-hydroxy-*N*-[1-(2-hydroxyphenylamino)-1-oxoalkan-2-yl]benzamides, in total thirty target compounds were synthesized and characterized. The compounds were evaluated against seven bacterial and three mycobacterial strains. The antimicrobial activities of some compounds were comparable or higher than the standards ampicillin, ciprofloxacin or isoniazid. Derivatives **3f** demonstrated high biological activity against *Staphylococcus aureus* (⩽0.03 μmol/L), *Mycobacterium marinum* (⩽0.40 μmol/L) and *Mycobacterium kansasii* (1.58 μmol/L), **3g** shows activity against *Clostridium perfringens* (⩽0.03 μmol/L) and *Bacillus cereus* (0.09 μmol/L), **3h** against *Pasteurella multocida* (⩽0.03 μmol/L) and *M. kansasii* (⩽0.43 μmol/L), **3i** against methicillin-resistant *S. aureus* and *B. cereus* (⩽0.03 μmol/L). The structure–activity relationships are discussed for all the compounds.

1. Introduction

The increasing number of mycobacterial, bacterial, viral and associated fungal infections underlines the importance of searching for new antimicrobial chemotherapeutics with a target effect. Tuberculosis (TB) is a common, and in many cases lethal, infectious disease caused by various strains of mycobacteria. It is very alarming that about one-third of the world's population (two billion people) is infected with *Mycobacterium tuberculosis* (MTB) and 10% of them will progress to the active disease. The highest evidence of occurrence of tuberculosis disease is in India, China, Indonesia, Nigeria, and Bangladesh.[1] The treatment of this disease is mediated by administration of various antimicrobial chemotherapeutics, however it is generally recognized that the massive application of these chemotherapeutics is the main reason of increased antibiotic resistance among bacteria.[2,3] Moreover, the antibiotic resistance of the important Gram-positive pathogen *Staphylococcus aureus* has become one of the most challenging and persistent worldwide health problems. Methicillin-resistant *S. aureus* (MRSA) has caused life-threatening nosocomial infections for the decades and has recently become a significant threat for community acquired infections and livestock associated infections with high levels of morbidity and mortality. Methicillin resistance is connected with clinically inadequate susceptibility not only to all β-lactam antibiotics but usually also to other antimicrobial drugs (macrolides, clindamycin, fluoroquinolones).[4,5] Also veterinary clinicians face bacterial pathogens causing serious diseases in animals (e.g., *Clostridium perfringens* as a major cause of enteritis in livestock,[6] *Pasteurella multocida* causing respiratory tract infections,[7] etc.). All of these diseases are of economic significance, so new, potent and fast acting antibacterial drugs could serve as a solution not only to the economic consequences of these diseases. Therefore there is an urgent need to develop new, potent and fast acting anti-tuberculosis and anti-MRSA drugs.[8]

Variously substituted salicylanilides **3** (*N*-substituted hydroxybenzamides), see Figure 1, are well-known organic compounds with a wide range of pharmacological activities, including antimicrobial[9] and antifungal[10] effects that become more and more important. They have also found use as mollusicidal[11] or anthelmintic agents[12] in human and veterinary practice. The latest studies described this compounds as inhibitors of apicomplexan parasite *Toxoplasma gondii*.[13,14] Mechanism of their action is still under investigation. It is known that salicylanilides can serve as inhibitors of protein kinase epidermal growth factor receptor (EGFR PTK),[15] further studies extended the group of inhibitors of

* Corresponding author. Tel.: +420 466037739; fax: +420 466038004.
E-mail address: Ales.Imramovsky@upce.cz (A. Imramovský).

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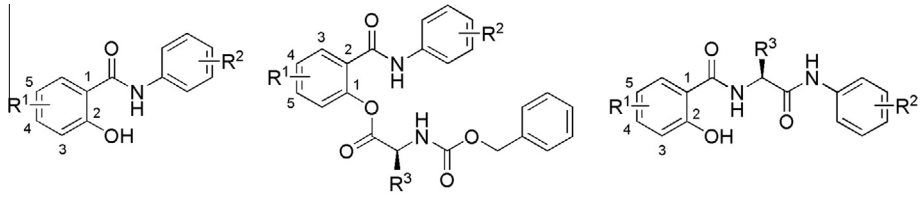

Figure 1. Discussed salicylanilide-like compounds **3**, **4**, **6** and partial numbering of their skeleton.

the new dual inhibitors of HDAC-EGFR.[16] They are generally designed to compete with ATP for binding in catalytic domain of tyrosin kinase.[17,18] The latest studies specified them also as selective inhibitors of interleukin-12p40 production that plays a specific role in initiation, expansion and control of cellular response to tuberculosis.[19,20] It was also proved that salicylanilides inhibit bacterial sortase A,[21] D-alanine–D-alanine ligase[22] and transglykosylases,[23] that is, enzymes participating in secretion of various proteins or synthesis of cell wall. Recently it was described that salicylanilides-like derivatives inhibit essential mycobacterial enzymes methionine aminopeptidase catalyzing a key step of the posttranslational modification of nascent proteins and isocitrate lyase that is indispensable for the metabolism of fatty acids.[24] In spite of the promise of salicylanilides as potential drugs, their widespread use in clinical practice was prevented by their physico-chemical properties, such as low solubility. Therefore, improvement of the physico-chemical properties of salicylanilides is an interesting and vital area of research.

An interesting and unprecedented attribute of *N*-protected amino esters of *N*-phenylsalicylamides **4**, see Figure 1, was discovered during investigation of salicylanilides modification. An unexpected rearrangement of esters of *N*-phenylsalicylamides to 2-hydroxy-*N*-[1-(2-hydroxyphenylamino)-1-oxoalkan-2-yl]benzamides **6**[25] was observed, see Figure 1. Due to their characteristic skeleton were these compounds called 'diamides'. The 'diamides' were published within preparation of a combinatorial library.[26] More recently, our research group has found them to be potential antimicrobial agents,[27] presenting in vitro antimycobacterial activity against *M. tuberculosis* and against some nontuberculous strains, such as *M. avium* and *M. kansasii*. A number of derivatives were prepared and their antimycobacterial activity was investigated. The activity of these derivatives was found to be comparable or higher than that of the starting salicylanilide compounds.[28–33]

This study is a follow-up paper to recently published articles.[27,30–33] A series of salicylanilide derivatives with general structure **3**, **4** or **6** were synthesized and evaluated against bacterial and mycobacterial strains. The structure–activity relationships between the structure and in vitro biological activities of all the evaluated compounds are discussed.

2. Results and discussion

2.1. Chemistry

The synthetic approach to preparation of 2-hydroxy-*N*-[(2*S*)-1-oxo-1-(phenylamino)alkan-2-yl]benzamides **6a–i** was mediated via the known rearrangement that was described by Imramovsky et al.[25,26] The synthetic pathway begins from substituted salicylic acid **1** and appropriate anilines **2**, see Scheme 1. This step was carried out in a microwave reactor using phosphorus trichloride in chlorobenzene[34] to give salicylanilides **3a–i** in very good yields (70–85%); these results were comparable with classical synthesis described by Waisser et al.[35] *N*-Benzyloxycarbonyl amino acids (*N*-Cbz-AA) were further esterified with prepared salicylanilides **3** by using *N*,*N*-dicyclohexylcarbodiimide activation.[36] Prepared esters[25] **4a–l** were treated by solution of hydrobromic acid in acetic acid (33%) for smooth deprotection of the *N*-benzyloxycarbonyl group. The classical deprotection using catalytical hydrogenation was also tested, but this method failed. Desired 2-hydroxy-*N*-[(2*S*)-1-oxo-1-(phenylamino)alkan-2-yl]benzamides **6a–i** were obtained by stirring hydrobromic salt **5** in chloroform in the presence of a base via the rearrangement described in literature.[25,37]

2.2. Biological activities

As discussed above, the compounds under investigation could be divided into three groups based on their chemical structure: Group I includes anilides **3a–i**; Group II contains esters **4a–l**; and Group III includes diamides **6a–i**. All compounds showed a wide range of antimicrobial activities, and some interesting structure–activity relationships were observed. Biological activity against various mycobacterial strains was published in many articles,[9,10,29,35] but the absolute novelty is the biological activity of salicylanilides and their derivatives against chosen bacterial species (*S. aureus*, *Bacillus cereus*, *P. multocida*, *C. perfringens*). All the results of antimicrobial screening are summarized in Tables 1 and 2.

2.2.1. In vitro antibacterial activity

Most of compounds were tested for their in vitro antibacterial activity against six Gram-positive bacterial strains such as *S. aureus* ATCC 29213 (SA), methicillin-resistant *S. aureus* (MRSA 63718, SA 630, SA 3202), *B. cereus* ATCC 14579 (BC) and *C. perfringens* CNCTC 5770 (CP) and against one Gram-negative bacterial strain *P. multocida* (PM). The results of the screening are summarized in Table 1. The screened salicylanilides and their derivatives (esters and diamides) showed very interesting biological activity against the mentioned strains; particularly the activity of compounds **3f–i**, **4g**, **4h**, **4j–l**, **6f**, **6h**, **6i** was comparable or higher than that of the standards. In general it can be concluded that antibacterial activity decreases as follows: Group I (salicylanilides) > Group II (esters of salicylanilides) > Group III (diamides).

Within Group I (salicylanilides) a significant antimicrobial activity was exhibited, especially by compounds **3e–h** and **3i** that contain a halogen group on the aniline ring. The presence of halogen seems to be crucial for high biological activity. These derivatives, especially compounds **3i**, **3g** and **3f**, showed activity against all tested strains. These facts support the hypothesis about the necessary presence of the halogen or trifluoromethyl moiety on the aniline ring; in the case of **3g** and **3i** the aniline ring contains two chlorines in positions $C_{(3)'}$ and $C_{(4)'}$, in the case of **3f** it contains CF_3 in position $C_{(4)'}$. Based on the results of antimicrobial effect of **3i** and **3g** it also seems that the substitution of $C_{(4)}$ position in the salicylic ring is more advantageous than that of $C_{(5)}$ position. It can be generally stated (see Table 1) that the activity within Group I increases with the increase of lipophilicity (see Fig. 2) and electron-withdrawing properties (expressed as Hammett's σ parameters) of R^2 substituents (see Fig. 3). In Figure 2 the dependence of