Bioorganic & Medicinal Chemistry 21 (2013) 6574–6581

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

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

ARTICLE INFO

Article history:
Received 3 July 2013
Revised 12 August 2013
Accepted 14 August 2013
Available online 24 August 2013

Keywords:
Salicylamide derivatives
Antibacterial
Antimycobacterial

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).

0968-0896/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmc.2013.08.029

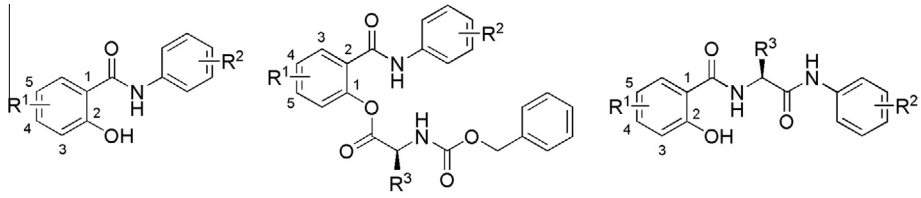


Figure 1. Discussed salicylanilide-like compounds **3**, **4**, **6** and partial numbering of their skeleton.

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]

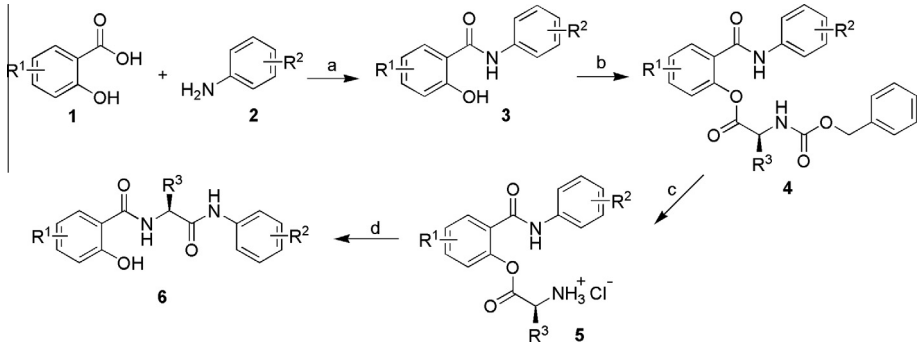
2.2. Biological activities

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



Scheme 1. Synthesis of 2-hydroxy-*N*-[(2*S*)-1-oxo-1-(phenylamino)alk-2-yl]benzamides. Reagents and conditions: (a) PCl_3, PhCl, MW, 400 W, 20 min; (b) DCC, DMF, −10 °C, 2 h; ***N*-Cbz-AA** (c) 33% HBr/AcOH, RT, 30 min; (d) TEA, $CHCl_3$, RT, 30 min.

Table 1
Structure of discussed compounds, their calculated lipophilicity (log*P*) and in vitro antibacterial activity (MIC) of compounds compared to ampicillin (APC) and ciprofloxacin (CPX) standards

Comp.	R^1	R^2	R^3	log*P*[a]	MIC (μmol/L)						
					SA	MRSA 63718	SA 630	SA 3202	BC	CP	PM
3a	5-Cl	H	—	4.24	16.15	32.30	16.15	16.15	32.30	>1034	1.01
3b	5-Cl	4-CH_3	—	4.72	>978	>978	>978	>978	30.56	>978	1.91
3c	5-Cl	4-OCH_3	—	4.20	>921	>921	>921	>921	>921	>921	28.80
3d	5-Cl	4-Cl	—	4.82	28.35	56.71	28.35	56.71	14.17	14.17	3.54
3e	5-Cl	4-Br	—	5.00	0.76	0.76	0.76	0.76	0.38	3.06	0.19
3f	5-Cl	4-CF_3	—	5.08	⩽0.03	0.10	0.20	0.20	0.10	1.58	0.20
3g	5-Cl	3,4-Cl	—	5.43	0.79	0.77	0.77	0.77	0.09	⩽0.03	0.19
3h	5-Cl	4-NO_2	—	3.91	0.85	1.71	0.85	1.71	0.85	1.71	⩽0.03
3i	**4-Cl**	**3,4-Cl**	—	**5.64**	**⩽0.03**	**0.05**	**⩽0.03**	**0.05**	**⩽0.03**	**0.20**	**0.20**
4a	4-Cl	4-H	(*S*)-iso-Pr	5.21	>517	>517	>517	>517	>517	>517	>517
4b	4-Cl	4-CH_3	(*S*)-Me	4.53	34.26	>548	>548	>548	7.13	>548	2.14
4c	4-Cl	4-CH_3	(*S*)-iso-Pr	5.16	32.31	129	129	64.63	32.31	>517	4.04
4d	4-Cl	4-CH_3	(*S*)-Bn	6.57	29.46	58.93	29.46	29.46	29.46	29.46	7.36
4e	4-Cl	4-CH_3	(*R*)-CH_2-indolyl	6.50	27.48	54.97	54.97	54.97	27.48	109	6.87
4f	4-Cl	4-OCH_3	(*S*)-iso-Pr	5.27	>501	>501	>501	>501	250	>501	62.62
4g	4-Cl	4-Br	(*S*)-CH_2-*c*Hx	7.20	3.25	1.62	1.62	1.62	1.62	3.25	0.40
4h	4-Cl	4-CF_3	(*S*)-iso-Pr	5.84	3.64	0.91	0.91	0.91	0.91	1.82	1.82
4i	4-Cl	4-NO_2	(*S*)-iso-Pr	5.03	3.80	3.80	3.80	3.80	7.60	3.80	0.95
4j	5-Cl	4-Br	(*S*)-iso-Pr	5.81	3.57	3.57	1.78	1.78	1.78	3.57	0.89
4k	5-Cl	3,4-Cl	(*S*)-iso-Pr	6.42	1.81	0.22	0.45	0.22	0.45	0.90	0.90
4l	**5-Cl**	**3,4-Cl**	**(*S*)-Bn**	**7.20**	**1.67**	**0.20**	**0.20**	**0.20**	**0.41**	**1.67**	**0.83**
6a	5-Cl	4-CH_3	(*S*)-Me	3.88	>769	>769	>769	>769	>769	>769	>769
6b	5-Cl	4-CH_3	(*S*)-iso-Pr	4.45	88.68	>709	>709	88.68	22.17	88.68	88.68
6c	5-Cl	4-CH_3	(*S*)-Bn	5.25	>626	>626	>626	>626	>626	>626	>626
6d	5-Cl	4-CH_3	(*R*)-CH_2-indolyl	5.31	>571	>571	>571	>571	>571	>571	>571
6e	5-Cl	4-OCH_3	(*S*)-iso-Pr	4.16	>679	>679	>679	>679	>679	>679	>679
6f	5-Cl	4-CF_3	(*S*)-iso-Pr	4.98	4.82	9.64	4.82	4.82	2.41	4.82	>617
6g	4-Cl	4-Br	(*S*)-iso-Pr	5.24	9.39	18.79	9.39	9.39	2.34	9.39	>601
6h	4-Cl	3,4-Cl	(*S*)-iso-Pr	5.72	2.40	4.81	2.40	2.40	1.20	38.48	>615
6i	**4-Cl**	**3,4-Cl**	**(*S*)-Bn**	**5.89**	**1.07**	**2.15**	**2.15**	**1.07**	**1.07**	**34.50**	**>552**
APC	—	—	—	—	5.72	>45.79	>45.79	>45.79	>45.79	<0.72	1.43
CPX	—	—	—	—	>48.29	>48.29	>48.29	>48.29	—	—	—

[a] Calculated using the sw. ACD/Percepta ver. 2012; SA = *Staphylococcus aureus* ATCC 29213, MRSA = methicillin-resistant *S. aureus* 63718 or SA 630 or SA 3202 (National Institute of Public Health, Prague, Czech Republic), BC = *Bacillus cereus* ATCC 14579, CP = *Clostridium perfringens* CNCTC 5770, PM = *Pasteurella multocida* (clinical isolate).

antibacterial activity against *S. aureus* (**2A**), against *B. cereus* (**2B**) and against *C. perfringens* (**2C**) expressed as log (1/MIC [mol/L]) on lipophilicity is illustrated. Compound **3h** was excluded due to the different character of the nitro moiety in comparison with the halogen moieties. Figure 2A and B illustrate typical activity of lipophilicity, while in Figure 2C a linear curve with lipophilicity optimum at log*P* ca. 5.4 is shown.

Within Group II (esters of salicylanilides **4**) more complex relationships can be found. The basic requirement for antimicrobial activity is the substitution of the salicylic ring (R^1) by a lipophilic moiety. If R^2 = 4-CF_3 substitution of compound **4h** is considered bioisosteric to R^2 = 3,4-Cl substitution of compound **4k**, that is, both compounds differ from each other only by the position of R^1 substitution, it can be stated that compounds substituted by chlorine in $C_{(5)}$ position of the salicylic ring have higher antibacterial potential than substituted by chlorine in $C_{(4)}$ position of the salicylic ring. The same observation was mentioned above. Also the R^2 substituent has substantial influence. As discussed above, the activity is increased especially by the lipophilicity of R^2 moiety and its electron-withdrawing properties. R^3 substituent can be considered as the last structure parameter influencing activity within Group II. This substitution increases the overall lipophilicity and bulkiness of the target compounds. R^3 is probably responsible for the increase of activity within the series of 4-Cl/4-CH_3

Table 2
In vitro antimycobacterial activity (MIC) of selected the compounds in comparison with isoniazid (INH) standard

Comp.	MIC (μmol/L)		
	MS	MM	MK
3a	64.60	64.60	16.15
3b	>978	61.13	30.56
3d	28.35	7.08	7.08
3e	24.49	12.24	6.12
3f	12.67	⩽0.40	1.58
3h	13.67	3.42	⩽0.43
3i	12.64	3.16	3.16
4b	>548	68.53	17.13
4c	259	259	129
4d	236	118	58.93
4e	110	110	13.74
4f	251	251	62.62
4g	26.06	26.06	13.03
4h	29.14	14.57	7.28
4i	30.42	7.60	7.60
4j	57.16	14.29	14.29
4k	29.10	14.55	7.27
4l	26.76	26.76	13.38
6b	>709	355	88.68
6f	77.14	77.14	38.57
6g	150	150	75.17
6h	76.97	76.97	76.97
6i	69.00	138	34.50
INH	117	467	29.17

MS = *Mycobacterium smegmatis* ATCC 700084, MM = *M. marinum* CAMP 5644, MK = *M. kansasii* DSM 44162.

derivatives (**4b**–**e**); with the increase of lipophilicity/bulkiness the activity increases, see Table 2. Based on this observation, bulky substituents (benzyl, methylindolyl, methylcyclohexyl) seem to be advantageous, see Figure 4. Probably the methylcyclohexyl fragment increases the antimicrobial activity of compound **4g** in comparison with compound **4j**.

It can be stated that diamides (Group III) possessed the lowest activity within the discussed groups. Compounds **6h** and **6i** were found as the most effective within Group III, which underlines the significance of the substitution of $C_{(4)}$ position of the salicylic ring in comparison with $C_{(5)}$ position for high antibacterial activity, compare **6h** and **6f**. As mentioned above, the decrease of lipophilicity and electron-withdrawing properties of the R^2 substituent causes the decrease of activity: 4-CF_3 (**6f**) >>> 4-CH_3 (**6b**) > 4-OCH_3 (**6e**). Lipophilic and bulky R^3 substituents optimize properties, and consequently antimicrobial activity is increased, compare **6h** and **6i**. Generally it can be concluded that antimicrobial activity increases with increasing lipophilicity as is illustrated in Figure 5A and B. Only for *C. perfringens* the dependence of activity on lipophilicity can be observed with an optimum at log*P* ca. 5, that is, activity decreases with a decrease or an increase of lipophilicity, see Figure 5C.

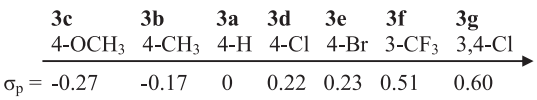


Figure 3. Increase of general antimicrobial activity within group I in dependence on Hammett's σ parameter (calculated by ACD/Percepta ver. 2012).

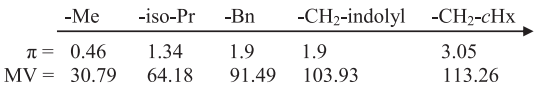


Figure 4. Increase of general antimicrobial activity owing to R^3 substituent in dependence on distributive parameter π and molar volume MV (cm^3) (both calculated using ACD/Percepta ver. 2012).

2.2.2. In vitro antimycobacterial activity

The genus *Mycobacterium* consists of a closely related group of fast and slow-growing species. *M. tuberculosis* causes one of the most serious human infections, tuberculosis. Difficulties should be considered while studying *M. tuberculosis*—especially a slow growth rate and the requirement of working in high containment biosafety facilities. To lower risks and make manipulation in the laboratory easier, surrogate model pathogens for *M. tuberculosis* can be used in laboratory studies. *M. smegmatis* is an ideal representative of a fast-growing nonpathogenic microorganism particularly useful in studying basic cellular processes of special relevance to pathogenic mycobacteria. *M. marinum* is very closely related to *M. tuberculosis* and is the cause of TB-like infections in poikilothermic organisms, especially frogs and fish. *M. marinum* is a good model for studying especially because of lower risk for laboratory workers, genetic relatedness and similar pathology to human TB.[38–40] However, because of *M. tuberculosis*, the pathogenic role of nontuberculous mycobacteria (NTM) in humans was overshadowed for a long time. *M. kansasii*, the most virulent of the NTM, causes nontuberculous mycobacterial lung infections which are very common nowadays and can be indistinguishable from tuberculosis.[41] That is the reason why *M. smegmatis*, *M. marinum* and *M. kansasii* were chosen as model species for screening of prospective antimycobacterial drugs to control mycobacterial diseases.

The evaluation of the in vitro antimycobacterial activity of the compounds was performed against *M. smegmatis* ATCC 700084 (MS), *M. marinum* CAMP 5644 (MM) and *M. kansasii* DSM 44162 (MK). All the results are shown in Table 2. Most of the tested compounds showed antimycobacterial activity against the three mentioned strains comparable or higher than the standard isoniazid. Compound **3f** demonstrated the highest activity against *M. marinum* (⩽0.40 μmol/L), and compound **3h** showed the highest activity against *M. kansasii* (⩽0.43 μmol/L). The activity of compounds from Group III was lower than the activity of compounds from

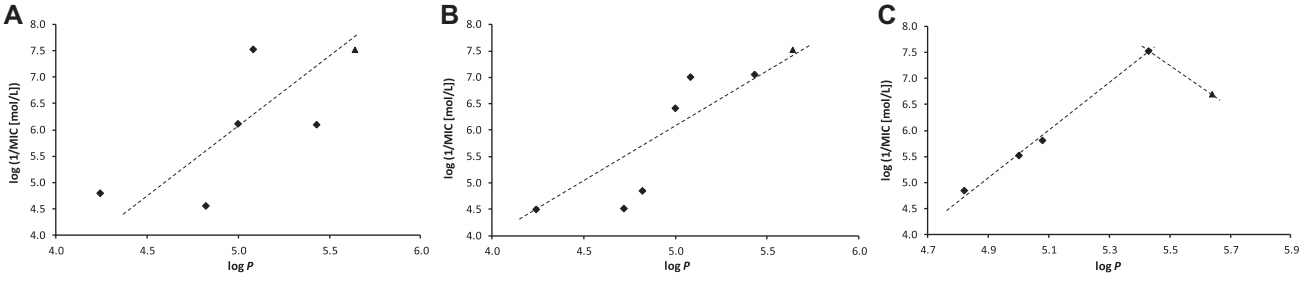


Figure 2. Dependence of in vitro antibacterial activity log (1/MIC [mol/L]) against *S. aureus* (A), against *B. cereus* (B) and against *C. perfringens* (C) on lipophilicity (log*P* calculated by ACD/Percepta ver. 2012) of studied compounds of group I, exclusive of **3h** (rhombs = 5-Cl, triangle = 4-Cl).

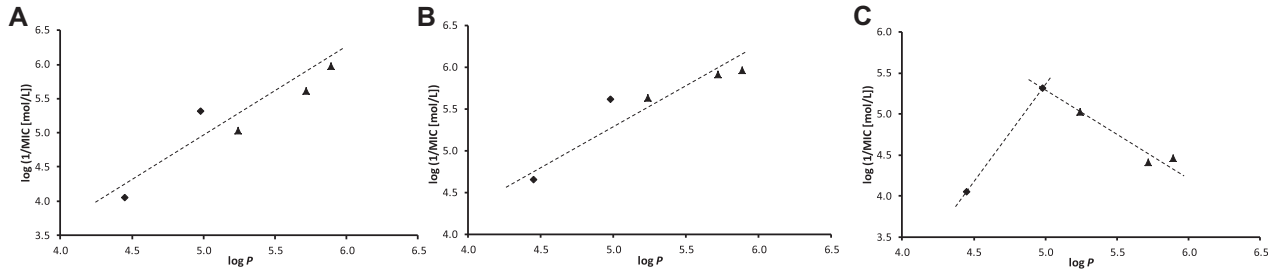


Figure 5. Dependence of in vitro antibacterial activity log (1/MIC [mol/L]) against *S. aureus* (A), against *B. cereus* (B) and against *C. perfringens* (C) on lipophilicity (log*P* calculated by ACD/Percepta ver. 2012) of studied compounds of group III, exclusive of **3h** (rhombs = 5-Cl, trianglev4-Cl).

Group II, which in its turn was lower than that of Group I. Compounds **3f**, **3h**, **3i** and **4i** demonstrated the highest activity against all the three strains.

Within Group I the following SAR can be found for all the three mycobacterial strains: the activity is significantly influenced by R^2 substituents with electron-withdrawing effect (4-CF_3, 4-NO_2, 3,4-Cl), while lipophilicity plays only a secondary role. It seems that $C_{(5)}$ position of chlorine in the salicylic ring is more advantageous than $C_{(4)}$ position contrary to the results of antibacterial screening.

It is important to note that within Group II compounds substituted by chlorine in $C_{(4)}$ position of the salicylic ring showed more significant activity than $C_{(5)}$ positional isomers. Nevertheless, as mentioned above, the electron-withdrawing effect of R^2 substituents seems to be more important than lipophilicity. A bulky and/or lipophilic moiety as R^3 substituent (methylindolyl, methylcyclohexyl) is more advantageous than the benzyl ring.

Based on the presented antimycobacterial data it is not possible to determine whether $C_{(4)}$ or $C_{(5)}$ substitution of the position of the salicylic ring is more advantageous within Group III. Antimycobacterial activity is significantly influenced by electron-withdrawing substituents (4-CF_3). Both isopropyl and benzyl are convenient R^3 substituents.

3. Conclusion

Thirty-two new salicylanilides, esters of *N*-phenylsalicylamides and 2-hydroxy-*N*-[1-(2-hydroxyphenylamino)-1-oxoalkan-2-yl]benzamides, were prepared. 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. Compounds **3f–i**, **4g**, **4h**, **4k**, **4l**, **6i** demonstrated the highest antibacterial activity against six Gram-positive/negative bacterial strains, and compounds **3f**, **3h**, **3i** and **4i** showed the highest activity against all three mycobacterial strains. Compound **3f** demonstrated high biological activity against *S. aureus* ($\leqslant$0.03 μmol/L), *M. marinum* ($\leqslant$0.40 μmol/L) and *M. kansasii* (1.58 μmol/L); **3g** against *C. perfringens* (<0.03 μmol/L) and *B. cereus* (0.09 μmol/L); compound **3h** against *P. multocida* ($\leqslant$0.03 μmol/L) and *M. kansasii* ($\leqslant$0.43 μmol/L); and compound **3i** against methicillin-resistant *S. aureus* and *B. cereus* ($\leqslant$0.03 μmol/L). The structure–activity relationships are discussed for all the compounds. As regards antibacterial activity, generally it can be stated that the R^1 substitution in the *para* position to the carboxamide moiety is important together with an R^2 lipophilic and electron-withdrawing moiety and a bulky R^3 substituent. For antimycobacterial activity especially R^1 substitution in the *meta* position to the carboxamide moiety is important for high activity. Also the electron-withdrawing substituent, such as $R^2 = NO_2$, CF_3 or 2Cl, and the bulky R^3 substituent are important.

4. Experimental

4.1. General

The chemicals were purchased from commercial sources (Sigma Aldrich, Acros Organics, TCI, Merck). Commercial grade reagents were used without further purification. Reactions were monitored by means of thin layer chromatography plates coated with 0.2 mm Silica Gel 60 F254 (Merck). TLC plates were visualized by UV irradiation (254 nm). The products were purified by crystallization, by means of column chromatography employing Silica Gel 60 (Merck). All melting points were determined on Melting Point B-540 apparatus (Buchi, Germany) and are uncorrected. NMR spectra were measured in DMSO-d_6 solutions (if not specified otherwise) on a Bruker Avance 400 apparatus at 400.13 MHz (^{1}H) and 100.62 MHz (^{13}C). The chemical shifts δ are given in ppm, relating to tetramethylsilane (TMS) as an internal standard. The coupling constants (J) are reported in hertz (Hz). Elemental analysis (C, H, N) was performed on a Thermo Scientific Flash 2000 Organic elemental analyser.

4.2. General procedures

4.2.1. Preparation of salicylamides (3a–i)

A mixture of substituted salicylic acid **1** (0.1 mol), appropriate amine **2** (0.1 mol) and phosphorus trichloride (0.05 mol) in chlorobenzene (250 ml) was stirred under microwave irradiation in the microwave reactor (400 W input power) for 20 min. Reaction mixture was then left in the fridge overnight. The precipitate was collected by filtration and recrystallized from ethanol to give salicylamide in sufficient purity.

4.2.2. Preparation of amino acid salicylanilide esters (4a–l)

N-Benzyloxycarbonyl protected amino acid (0.01 mol) and substituted salicylanilide **3** (0.01 mol) were dissolved in dry DMF (45 mL). The solution was cooled to−10 °C, and *N*,*N*-dicyclohexylcarbodiimide (0.011 equiv) was added in three portions for more than 1 h. The mixture was then stirred for 3 h at the same temperature and stored at 4 °C for 20 h. Precipitated *N*,*N*-dicyclohexylurea was removed by filtration, and the solvent was evaporated in vacuo. The crude product was crystallized from ethyl acetate–hexane.

4.2.3. Preparation of diamides (6a–i) via rearrangement

A 33% solution of hydrogen bromide in acetic acid (10 ml) was slowly added to *N*-benzyloxycarbonyl-protected ester **4** (1 mmol) while stirring. The suspension was stirred at room temperature for 30 min. During this time, the suspension turned into a clear brown solution, and evolution of carbon dioxide was observed. When the gas evolution ceased, dry diethyl ether (50 ml) was added. The precipitate was collected by filtration, washed with diethyl ether and dried. Obtained hydrobromide

salts **5** were suspended in dry chloroform (10 ml), and triethylamine (0.95 mmol) was added at room temperature. After 30 min of stirring, insoluble material was filtered off, and the filtrate was purified by column chromatography (ethyl acetate–hexan 1:1).

4.3. In vitro antibacterial susceptibility testing

The synthesized compounds were evaluated for in vitro antibacterial activity against Gram-positive strains (*B. cereus* ATCC 14579, *C. perfringens* CNCTC 5770), one Gram-negative strain (clinical isolate of *P. multocida*) and representatives of multidrug-resistant bacteria (clinical isolates of methicillin-resistant *S. aureus* (MRSA) 63718, SA 630 and SA 3202 obtained from the National Institute of Public Health, Prague, Czech Republic). *S. aureus* ATCC 29213 was used as a reference and quality control strain. Ampicillin (Sigma, Germany) and ciprofloxacin (Sigma, Germany) were used as the standards. Prior to testing, each strain was cultured on nutrient agar (Oxoid, UK) with 5% of ovine blood, and bacterial inocula were prepared by suspending a small portion of the bacterial colony in sterile phosphate buffered saline (pH 7.2–7.3). The cell density was adjusted to 0.5 McFarland units using a densitometer (Densi-La-Meter, LIAP, Latvia). The final inoculum was made by 1:20 dilution of the suspension with the Mueller–Hinton broth (MH broth). The compounds were dissolved in DMSO (Sigma, Germany), and the final concentration of DMSO in the MH broth (Oxoid, UK) did not exceed 2.5% of the total solution composition. The broth dilution micro-method modified according to NCCLS guidelines[42,43] in MH broth was used to determine the minimum inhibitory concentration (MIC). Drug-free controls, sterility controls and controls consisted of MH broth and DMSO alone were included. The determination of results was performed visually after 24 h of static incubation in the darkness at 37 °C in an aerobic atmosphere (anaerobic atmosphere for *C. perfringens*). The MICs were defined as the lowest concentration of the compound at which no visible bacterial growth was observed.

4.4. In vitro antimycobacterial susceptibility testing

The evaluation of in vitro antimycobacterial activity of the compounds was performed against *M. smegmatis* ATCC 700084, *M. marinum* CAMP 5644 and *M. kansasii* DSM 44162. The broth dilution micro-method in Middlebrook 7H9 medium (Difco, USA) supplemented with ADC Enrichment (BBL, USA) was used to determine the minimum inhibitory concentration (MIC) as previously described.[44] The tested compounds were dissolved as described above in Section 4.3. Isoniazid (Sigma, Germany) was used as a reference antibacterial drug. Bacterial inocula were prepared by transferring colonies from culture to sterile water. The cell density was adjusted to 0.5 McFarland units using a densitometer (Densi-La-Meter, LIAP, Latvia). The final inoculum was made by 1:1000 dilution of the suspension with sterile water. Drug-free controls, sterility controls and controls consisted of medium and DMSO alone were included. The determination of results was performed visually after 3 days of static incubation in the darkness at 37 °C in an aerobic atmosphere for *M. smegmatis*, after 7 days of static incubation in the darkness at 37 °C in an aerobic atmosphere for *M. kansasii* and after 21 days of static incubation in the darkness at 28 °C in an aerobic atmosphere for *M. marinum*. The MICs were defined as the lowest concentration of the compound at which no visible bacterial growth was observed.

4.5. Characterization of prepared compounds

4.5.1. 4-Chloro-2-(phenylcarbamoyl)phenyl (2*S*)-2-{[(benzyloxy)carbonyl]amino}-3-methylbutanoate (4a)

White solid, yield 34%, mp 125–127 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.48 (1H, br s, N*H*), 7.86 (1H, d, J = 8.38 Hz, CH-N*H*), 7.73–7.62 (4H, m, Ar-*H*), 7.36–7.30 (7H, m, Ar-*H*), 7.19–7.15 (1H, m, Ar-*H*), 7.09–7.06 (1H, m, Ar-*H*), 5.04 (2H, s, OCH_2), 4.18–4.12 (1H, m, C*H*-NH), 2.18–2.12 (1H, m, $CH(CH_3)_2$), 0.80–0.70 (6H, m, $(CH_3)_2$). ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.8, 163.1, 157.1, 146.7, 139.5, 137.4, 132.7, 131.5, 130.7, 129.2, 129.1, 129.0, 128.5, 128.5, 125.6, 124.4, 120.2, 66.3, 60.5, 29.9, 19.6, 18.4. Anal. Calcd for $C_{26}H_{25}ClN_2O_5$ (480.94): C, 64.93; H, 5.24; N, 5.82. Found: C, 64.89; H, 5.19; N, 5.75.

4.5.2. 4-Chloro-2-[(4-methylphenyl)carbamoyl]phenyl (2*S*)-2-{[(benzyloxy)carbonyl]amino}-propanoate (4b)

White solid, yield 50%, mp 121–125 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.16 (1H, s, N*H*), 7.99 (1H, d, J = 2.4 Hz, Ar-*H*), 7.45 (2H, m, CH-N*H*, Ar-*H*), 7.38–7.30 (6H, m, Ar-*H*), 7.12–7.06 (3H, m, Ar-*H*), 5.38 (1H, d, J = 8.4 Hz, Ar-*H*), 5.09–5.12 (1H, m, OCH*H*), 5.00–4.96 (1H, m, OC*H*H), 4.44–4.38 (1H, m, C*H*-NH), 2.30 (3H, d, J = 6.5 Hz, CH_3), 2.27 (3H, s, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.4, 162.1, 156.4, 145.6, 135.9, 134.9, 134.5, 132.0, 131.5, 130.4, 129.6, 129.4, 128.5, 128.2, 128.0, 124.3, 120.4, 67.2, 59.5, 30.5, 20.9. Anal. Calcd for $C_{25}H_{23}ClN_2O_5$ (466.91): C, 64.31; H, 4.97; N, 6.00. Found: C, 64.25; H, 5.10; N, 5.88.

4.5.3. 4-Chloro-2-[(4-methylphenyl)carbamoyl]phenyl (2*S*)-2-{[(benzyloxy)carbonyl]amino}-3-methylbutanoate (4c)

White solid, yield 40%, mp 129–132 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.16 (1H, s, N*H*), 7.99 (1H, d, J = 2.4 Hz, Ar-*H*), 7.47–7.43 (2H, m, CH-N*H*, Ar-*H*), 7.38–7.30 (6H, m, Ar-*H*), 7.15–7.04 (3H, m, Ar-*H*), 5.38 (1H, d, J = 8.4 Hz, Ar-*H*), 5.09–5.05 (1H, m, OCH*H*), 5.00–4.96 (1H, m, OC*H*H), 4.45–4.39 (1H, m, C*H*-NH), 2.35–2.29 (1H, m, $CH(CH_3)_2$), 2.27 (3H, s, CH_3), 0.97 (3H, d, J = 6.8 Hz, CH_3), 0.86 (3H, d, J = 6.7 Hz, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.4, 162.1, 156.4, 145.6, 135.9, 134.9, 134.5, 132.0, 131.5, 130.4, 129.6, 129.4, 128.5, 128.2, 128.0, 124.3, 120.4, 67.2, 59.5, 30.5, 20.9, 19.2, 17.3. Anal. Calcd for $C_{27}H_{27}ClN_2O_5$ (494.97): C, 65.52; H, 5.50; N, 5.66. Found: C, 65.40; H, 5.36; N, 5.50.

4.5.4. 4-Chloro-2-[(4-methylphenyl)carbamoyl]phenyl (2*S*)-2-{[(benzyloxy)carbonyl]amino}-3-phenylpropanoate (4d)

White solid, yield 16%, mp 145–147 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.42 (1H, br s, N*H*), 8.02 (1H, d, J = 8.08 Hz, CH-N*H*), 7.75 (1H, d, J = 2.55 Hz, Ar-*H*), 7.66–7.64–7.57 (3H, m, Ar-*H*), 7.32–7.19 (11H, m, Ar-*H*), 7.15–7.09 (2H, m, Ar-*H*), 4.96 (2H, s, OCH_2), 4.47–4.43 (1H, m, C*H*-NH), 3.25–3.17 (1H, m, CH*H*), 2.95–2.85 (1H, m, C*H*H), 2.24 (3H, s, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.7, 162.8, 156.6, 146.9, 137.9, 137.4, 136.9, 133.5, 132.3, 131.7, 130.8, 129.7, 129.4, 128.9, 128.8, 128.4, 128.2, 128.0, 127.1, 125.5, 120.5, 66.1, 56.3, 36.5, 21.1. Anal. Calcd for $C_{31}H_{27}ClN_2O_5$ (543.00): C, 68.57; H, 5.01; N, 5.16. Found: C, 68.40; H, 4.99; N, 5.03.

4.5.5. 4-Chloro-2-[(4-methylphenyl)carbamoyl]phenyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-(1*H*-indol-2-yl)propanoate (4e)

White solid, yield 40%, mp 162–164 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.85 (1H, br s, N*H*), 10.44 (1H, br s, Ar-N*H*), 7.75 (1H, d, J = 2.36 Hz, CH-N*H*), 7.66–7.61 (3H, m, Ar-*H*), 7.48–7.32 (3H, m, Ar-*H*), 7.21–7.04 (5H, m, Ar-*H*), 6.91 (1H, m, Ar-*H*), 4.38–4.32 (1H, m, CH-N*H*), 3.30 (1H, m, CH*H*), 3.01 (1H, m, C*H*H), 4.15

(1H, m, C*H*-NH), 2.25 (3H, s, CH_3), 1.32 (9H, m, (CH_3)$_3$). ^{13}C NMR (100 MHz, DMSO-d_6): δ 171.3, 162.9, 156.1, 147.0, 136.9, 136.6, 133.5, 132.4, 131.6, 130.7, 129.7, 129.3, 127.5, 125.5, 124.4, 121.6, 120.5, 119.0, 118.6, 112.0, 110.3, 79.0, 55.4, 28.3, 26.6, 21.1. Anal. Calcd for $C_{30}H_{30}ClN_3O_5$ (548.03): C, 65.75; H, 5.52; N, 7.67. Found: C, 65.66; H, 5.49; N, 7.58.

4.5.6. 4-Chloro-2-[(4-methoxyphenyl)carbamoyl]phenyl (2*S*)-2-{[(benzyloxy)carbonyl]amino}-3-methylbutanoate (4f)

White solid, yield 36%, mp 107.5–108.7 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.06 (1H, s, N*H*), 7.75 (1H, d, J = 2.0 Hz, Ar-*H*), 7.50–7.46 (2H, m, CH-N*H*, Ar-*H*), 7.42–7.36 (1H, m, Ar-*H*), 7.38–7.26 (5H, m, Ar-*H*), 7.10–7.06 (1H, m, Ar-*H*), 6.85–6.79 (2H, m, Ar-*H*), 5.32 (1H, d, J = 8.4 Hz, Ar-*H*), 5.10–5.06 (1H, m, OCH*H*), 5.01–4.97 (1H, m, OC*H*H), 4.44–4.38 (1H, m, C*H*-NH), 3.76 (3H, s, CH_3), 2.28–2.24 (1H, m, C*H*(CH$_3$)$_2$), 0.99 (3H, d, J = 6.4 Hz, CH_3), 0.88 (3H, d, J = 6.4 Hz, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.5, 162.0, 156.7, 156.4, 135.9, 132.1, 131.6, 130.5, 130.3, 129.8, 128.5. 128.2. 128.1. 124.3. 122.2, 114.1, 67.3, 59.6, 55.4, 30.5, 24.9, 19.2, 17.4. Anal. Calcd for $C_{27}H_{27}ClN_2O_6$ (510.97): C, 63.47; H, 5.33; N, 5.48. Found: C, 63.40; H, 5.25; N, 5.38.

4.5.7. 4-Chloro-2-[(4-chlorophenyl)carbamoyl]phenyl (2*S*)-2-{[(benzyloxy)carbonyl]amino}-3-cyclohexylpropanoate (4g)

White solid, yield 43%, mp 140–142 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.62 (1H, br s, N*H*), 7.89 (1H, d, J = 7.08 Hz, CH-N*H*), 7.74–7.62 (4H, m, Ar-*H*), 7.55–7.47 (2H, m, Ar-*H*), 7.37–7.20 (6H, m, Ar-*H*), 5.04 (2H, s, OCH_2), 4.26–4.22 (1H, m, C*H*-NH), 1.59–1.43 (7H, m, cyclohexyl), 1.28–1.01 (4H, m, cyclohexyl), 0.63 (2H, m, CHCH_2). ^{13}C NMR (100 MHz, DMSO-d_6): δ 171.8, 163.1, 156.8, 138.8, 137.5, 132.4, 132.1, 131.7, 130.8, 129.1, 128.9, 128.5, 128.3, 125.5, 122.1, 116.1, 66.2, 52.3, 38.0, 33.7, 33.4, 31.7, 26.5, 26.2, 25.9. Anal. Calcd for $C_{30}H_{30}BrClN_2O_5$ (613.93): C, 58.69; H, 4.93; N, 4.56. Found: C, 58.59; H, 4.53; N, 4.44.

4.5.8. 4-Chloro-2-[(4-trifluoromethylphenyl)carbamoyl]phenyl (2*S*)-2-{[(benzyloxy)carbonyl]amino}-3-methylbutanoate (4h)

White solid, yield 22%, mp 135–137.4 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.84 (1H, s, N*H*), 7.89 (2H, d, J = 8.6 Hz, Ar-*H*), 7.85 (1H, d, J = 7.5 Hz, Ar-*H*), 7.69 (2H, d, J = 8.5, Ar-*H*), 7.77 (1H, d, J = 2.6 Hz, CH-N*H*), 7.35–7.32–7.28 (6H, m, Ar-*H*), 7.20 (1H, d, J = 8.9 Hz, Ar-*H*), 5.02 (2H, s, OCH_2), 4.15 (1H, t, J = 7.0 Hz, C*H*-NH), 2.15–2.11 (1H, m, C*H*(CH$_3$)$_2$), 0.86 (3H, d, J = 6.9 Hz, CH_3), 0.84 (3H, d, J = 6.7 Hz, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.2, 163.1, 156.5, 146.1, 142.4, 136.9, 131.7, 131.3, 130.2, 128.6, 128.4, 128.9, 128.1, 127.9, 126.0, 125.1, 123.8, 119.6, 65.8, 60.0, 29.4, 19.0, 17.9. Anal. Calcd for $C_{27}H_{24}F_3ClN_2O_5$ (548.94): C, 59.08; H, 4.41; N, 5.10. Found: C, 58.99; H, 4.40; N, 5.03.

4.5.9. 4-Chloro-2-[(4-nitrophenyl)carbamoyl]phenyl (2*S*)-2-{[(benzyloxy)carbonyl]amino}-3-methylbutanoate (4i)

Yellowish solid, yield 36%, mp 135.6–137.4 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.09 (1H, s, N*H*), 8.24 (2H, d, J = 9.0 Hz, Ar-*H*), 7.93 (2H, d, J = 9.3 Hz, Ar-*H*), 7.86 (1H, d, J = 7.9, N*H*-CH), 7.80 (1H, d, J = 2.5 Hz, Ar-*H*), 7.71–7.65 (1H, m, Ar-*H*), 7.35–7.31 (5H, m, Ar-*H*), 7.22 (1H, d, J = = 8.7 Hz, Ar-*H*), 5.03 (2H, s, OCH_2), 4.15 (1H, t, J = 7.4 Hz, C*H*-NH), 2.15–2.12 (1H, m, C*H*(CH$_3$)$_2$), 0.87 (3H, d, J = 6.8 Hz, CH_3), 0.84 (3H, d, J = 6.9 Hz, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.3, 163.3, 156.4, 146.1, 145.0, 142.7, 131.5, 130.4, 128.7, 128.4, 127.8. 125.1. 124.9. 124.9, 120.1, 119.4, 99.6, 65.7, 59.9, 29.4, 19.1, 17.9. Anal. Calcd for $C_{26}H_{24}ClN_3O_7$ (525.94): C, 59.38; H, 4.60; N, 7.99. Found: C, 59.22; H, 4.51; N, 7.89.

4.5.10. 5-Chloro-2-[(4-bromophenyl)carbamoyl]phenyl (2*S*)-2-{[(benzyloxy)carbonyl]amino}-3-methylbutanoate (4j)

White solid, yield 34%, mp 160–163 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.59 (1H, br s, N*H*), 7.87 (1H, d, J = 8.38 Hz, CH-N*H*), 7.70–7.65 (3H, m, Ar-*H*), 7.51–7.49 (3H, m, Ar-*H*), 7.36–7.28 (6H, m, Ar-*H*), 5.04 (2H, s, OCH_2), 4.17–4.14 (1H, m, C*H*-NH), 2.16–2.16 (1H, m, C*H*(CH$_3$)$_2$), 0.88–0.83 (6H, m, (CH_3)$_2$). ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.6, 163.6, 157.1, 148.5, 138.9, 137.4, 135.6, 132.1, 131.0, 139.9, 129.0, 128.5, 128.4, 126.9, 123.7, 122.1, 116.0, 66.3, 60.5, 29.9, 19.6, 18.4. Anal. Calcd for $C_{26}H_{24}BrClN_2O_5$ (559.84): C, 55.78; H, 4.32; N, 5.00. Found: C, 55.69; H, 4.25; N, 4.15.

4.5.11. 5-Chloro-2-[(3,4-dichlorophenyl)carbamoyl]phenyl (2*S*)-2 {[(benzyloxy)carbonyl]amino}-3-methylbutanoate (4k)

White solid, yield 18.4%, mp 132–134 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.74 (1H, br s, N*H*), 8.06 (1H, s, Ar-*H*), 7.87 (1H, d, J = 7.56 Hz, CH-N*H*), 7.70 (1H, d, J = 8.82, Ar-*H*), 7.59 (2H, s, Ar-*H*), 7.54–7.52 (1H, m, Ar-*H*), 7.35–7.30 (6H, m, Ar-*H*), 5.03 (2H, s, OCH_2), 4.18–4.15 (1H, m, C*H*-NH), 2.17–2.13 (1H, m, C*H*(CH$_3$)$_2$), 0.89–0.83 (6H, m, (CH_3)$_2$). ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.6, 164.0, 157.1, 148.6, 139.5, 137.4, 135.9, 131.5, 131.2, 131.0, 129.6, 128.9, 128.5, 128.4, 127.0, 125.9, 123.7, 121.5, 120.3, 66.3, 60.5, 29.9, 19.6, 18.4. Anal. Calcd for $C_{26}H_{23}Cl_3N_2O_5$ (549.83): C, 56.80; H, 4.22; N, 5.09. Found: C, 56.75; H, 4.19; N, 4.03.

4.5.12. 5-Chloro-2-[(3,4-dichlorophenyl)carbamoyl]phenyl (2*S*)-2 {[(benzyloxy)carbonyl]amino}-3-phenylpropanoate (4l)

White solid, yield 36%, mp 145–147 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.73 (1H, br s, N*H*), 8.09 (1H, s, Ar-*H*), 8.02 (1H, d, J = 7.66 Hz, CH-N*H*), 7.74 (1H, d, J = 8.08 Hz, Ar-*H*), 7.64–7.53 (3H, m, Ar-*H*), 7.31–7.22 (11H, m, Ar-*H*), 4.94 (2H, s, OCH_2), 4.52–4.46 (1H, m, C*H*-NH), 3.22–3.19 (1H, m, CH*H*), 2.95–2.89 (1H, m, C*H*H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.6, 163.9, 156.5, 148.8, 139.5, 137.8, 137.4, 136.1, 131.6, 131.2, 129.7, 129.1, 128.9, 128.9, 128.4, 127.8, 127.2, 127.0, 125.9, 123.7, 121.7, 120.5, 66.1, 56.2, 36.5. Anal. Calcd for $C_{30}H_{23}Cl_3N_2O_5$ (597.87): C, 60.27; H, 3.88; N, 4.69. Found: C, 60.20; H, 3.70; N, 4.66.

Acknowledgements

This work was financially supported by the Ministry of Education, Youth and Sports of the Czech Republic (Project SGFChT 02 UPa), by IGA VFU Brno, Project No. 65/2012/FVL, and by the project CEITEC (Central European Institute of Technology) (CZ.1.05/1.1.00/02.0068) from the European Regional Development Fund.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2013.08.029.

References and notes

1. Laughon, E. L. *Curr. Top. Med. Chem.* **2007**, *7*, 463.
2. Levy, S. B. *Adv. Drug Delivery Rev.* **2005**, *57*, 1446.
3. Poole, K. *Curr. Opin. Microbiol.* **2001**, *4*, 500.
4. Kallen, A. J.; Mu, Y.; Bulens, S.; Reingold, A.; Petit, S.; Gershman, K.; Ray, S. M.; Harrison, L. H.; Lynfield, R.; Dumyati, G.; Townes, J. M.; Schaffner, W.; Patel, P. R.; Fridkin, S. K. *JAMA* **2010**, *304*, 641.
5. Liu, C.; Bayer, A.; Cosgrove, S. E.; Daum, R. S.; Fridkin, S. K.; Gorwitz, R. J.; Kaplan, S. L.; Karchmer, A. W.; Levine, D. P.; Murray, B. E.; Rybak, M. J.; Talan, D. A.; Chambers, H. F. *Clin. Infect. Dis.* **2011**, *52*, 285.

6. Uzal, F. A.; McClane, B. A. *Vet. Microbiol.* **2011**, *153*, 37.
7. Kofteridis, D. P.; Christofaki, M.; Mantadakis, E.; Maraki, S.; Drygiannakis, I.; Papadakis, J. A.; Samonis, G. *Int. J. Infect. Dis.* **2009**, *13*, e81.
8. Baquero, F.; Coque, T. M.; de la Cruz, F. *Antimicrob. Agents Chemother.* **2011**, *55*, 3649.
9. Singh, H.; Singh, A. K.; Sharma, S.; Iyer, R. N. *J. Med. Chem.* **1977**, *20*, 826.
10. Waisser, K.; Peřina, M.; Holý, P.; Pour, M.; Bureš, O.; Kuneš, J.; Klimešová, V.; Buchta, V.; Kubanová, P.; Kaustová, J. *Arch. Pharm. (Weinheim)* **2003**, *336*, 322.
11. Nawwar, G. *Arch. Pharm. (Weinheim)* **1994**, *327*, 201.
12. Sjogren, E. B.; Rider, M. A.; Nelson, P. H.; Bingham, S.; Poulton, A. L.; Emanuel, M. A.; Komuniecki, R. *J. Med. Chem.* **1991**, *34*, 3295.
13. Fomovska, A.; Wood, R. D.; Mui, E.; Dubey, J. P.; Ferreira, L. R.; Hickman, M. R.; Lee, P. J.; Leed, S. E.; Auschwitz, J. M.; Welsh, W. J.; Sommerville, C.; Woods, S.; Roberts, C.; McLeod, R. *J. Med. Chem.* **2012**, *55*, 8375.
14. Stec, J.; Fomovska, A.; Afanador, G. A.; Muench, S. P.; Zhou, Y.; Lai, B. S.; El Bissati, K.; Hickman, M. R.; Lee, P. J.; Leed, S. E.; Auschwitz, J. M.; Sommervile, C.; Woods, S.; Roberts, C. W.; Rice, D.; Prigge, S. T.; McLeod, R.; Kozikowski, A. P. *ChemMedChem* **2013**, *8*, 1138.
15. Kamatb, S.; Buolamwini, J. K. *Med. Res. Rev.* **2006**, *26*, 569.
16. Zuo, M.; Zheng, Y. W.; Lu, S. M.; Li, Y.; Zhang, S. Q. *Bioorg. Med. Chem.* **2012**, *20*, 4405.
17. Liechti, C.; Sequin, U.; Bold, G.; Furet, P.; Meyer, T.; Traxler, P. *Eur. J. Med. Chem.* **2004**, *39*, 11.
18. Guo, L.; Wang, Q. L.; Jiang, Q. Q.; Jiang, J. J.; Jiang, Y. B. *J. Org. Chem.* **2007**, *72*, 9947.
19. Brown, M. E.; Fitzner, J. N.; Stevens, T.; Chin, W.; Wright, C. D.; Boyce, J. P. *Bioorg. Med. Chem.* **2008**, *16*, 8760.
20. Boyce, J. P.; Brown, M. E.; Chin, W.; Fitzner, J. N.; Paxton, R. J.; Shen, M.; Stevens, T.; Wolfson, F.; Wright, C. D. *Bioconjugate Chem.* **2008**, *19*, 1775.
21. Chenna, B. C.; Shinkre, B. A.; King, J. R.; Lucius, A. L.; Narayana, S. V. L.; Velu, S. E. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 380.
22. Triola, G.; Wetzel, S.; Ellinger, B.; Koch, M. A.; Hubel, J.; Rauh, D.; Waldmann, H. *Bioorg. Med. Chem.* **2009**, *17*, 1079.
23. Cheng, T.-J.; Wu, Y.-T.; Yang, S.-T.; Lo, K.-H.; Chen, S.-K.; Chen, Y.-H.; Huang, W.-I.; Yuan, C.-H.; Guo, C.-W.; Huang, L.-Y.; Chen, K.-T.; Shih, H.-W.; Cheng, Y.-S.; Cheng, W.-C.; Wong, C.-H. *Bioorg. Med. Chem.* **2010**, *18*, 8512.
24. Kratký, M.; Vinšová, J.; Novotná, E.; Mandiková, J.; Wsól, V.; Trejtnar, F.; Ulmann, V.; Stolařiková, J.; Fernandes, S.; Bhat, S.; Liu, J. O. *Tuberculosis* **2012**, *92*, 434.
25. Imramovský, A.; Vinšová, J.; Férriz, J. M.; Kuneš, J.; Pour, M.; Doležal, M. *Tetrahedron Lett.* **2006**, *47*, 5007.
26. Imramovský, A.; Férriz, J. M.; Pauk, K.; Krátký, M.; Vinšová, J. *J. Comb. Chem.* **2010**, *12*, 414.
27. Imramovský, A.; Peško, M.; Králová, K.; Vejsová, M.; Stolařiková, J.; Vinšová, J.; Jampílek, J. *Molecules* **2011**, *16*, 2414.
28. Skála, P.; Macháček, M.; Vejsová, M.; Kubicová, L.; Kuneš, J.; Waisser, K. *J. Heterocycl. Chem.* **2009**, *46*, 873.
29. Waisser, K.; Matyk, J.; Divišová, H.; Husaková, P.; Kuneš, J.; Klimešová, V.; Palát, K., Jr.; Kaustová, J. *Arch. Pharm.* **2007**, *340*, 264. Weinheim, Germany.
30. Vinšová, J.; Imramovský, A.; Buchta, V.; Čečkova, M.; Doležal, M.; Štaud, F.; Jampílek, J.; Kaustová, J. *Molecules* **2007**, *12*, 1.
31. Imramovský, A.; Vinšová, J.; Férriz, J. M.; Buchta, V.; Jampílek, J. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 348.
32. Imramovský, A.; Vinšova, J.; Férriz, J. M.; Doležal, R.; Jampílek, J.; Kaustová, J.; Kunc, F. *Bioorg. Med. Chem.* **2009**, *17*, 3572.
33. Férriz, J. M.; Vavrová, K.; Kunc, F.; Imramovský, A.; Stolaříková, J.; Vavřiková, E.; Vinšová, J. *Bioorg. Med. Chem.* **2010**, *18*, 1054.
34. Veverková, E. *Monatsch. Chem.* **2003**, *134*, 1215.
35. Bodansky, M. *Principles of Peptide Synthesis*, 1st ed.; Springer: New York, 1993.
36. Waisser, K.; Hladuvková, J.; Kuneš, J.; Kubicová, L.; Klimešová, V.; Karajannis, P.; Kaustová, J. *Chem. Pap.* **2001**, *55*, 121.
37. Vinšová, J.; Imramovský, A.; Kratký, M.; Férriz, J. M.; Palát, K.; Lyčka, A.; Růžička, A. *Tetrahedron Lett.* **2010**, *51*, 23–26.
38. Acharya, N.; Varshney, U. *J. Mol. Biol.* **2002**, *318*, 1251.
39. Broussard, G. W.; Ennis, D. G. *Comp. Biochem. Physiol. C* **2007**, *145*, 45.
40. Valente, W. J.; Pienaar, E.; Fast, A.; Fluitt, A.; Whitney, S. E.; Fenton, R. J.; Barletta, R. G.; Chacon, O.; Viljoen, H. J. *Chem. Eng. Sci.* **1944**, *2009*, 64.
41. Matveychuk, A.; Fuks, L.; Priess, R.; Hahim, I.; Shitrit, D. *Respir. Med.* **2012**, *106*, 1472.
42. National Committee for Clinical Laboratory Standards *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically, 5th Edition: Approved Standard M7–A5*; NCCLS: Wayne, PA, USA, 2000.
43. National Committee for Clinical Laboratory Standards *Performance Standards for Antimicrobial Susceptibility Testing: 12th Informational Supplement M100–S12*; NCCLS: Wayne, PA, USA, 2002.
44. Schwalbe, R.; Steele-Moore, L.; Goodwin, A. C. *Antimicrobial Susceptibility Testing Protocols*; CRC Press: Boca Raton, FL, USA, 2007.