

## SHORT ARTICLE

# Phenyl salicylates – a new group of potential antituberculotics

## Fenylsalicyláty – nová skupina potenciálních antituberkulotik

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### Summary

Antimycobacterial activity of phenyl salicylates (salols) was studied in connection with antituberculous activity of salicylic derivatives. Phenyl salicylates are esters. Our attention was previously oriented on amides. Phenyl salicylates (salols) represent a new group of antimycobacterial compounds. They are less active than the corresponding amides. The most active compound in the group under study is substituted on phenyl in the salicylic moiety with a 4-methoxy group. The study reports a new item of information about antimycobacterial salicylic derivatives.

**Keywords:** salols • mycobacterium • antimycobacterial activity • phenyl salicylate

### Souhrn

V souvislosti se studiem protituberkulózní aktivity salicylových derivátů jsme studovali antimykobakteriální aktivitu fenyl-salicylátů (salolů). Fenylsalicyláty jsou estery. Naše pozornost byla dříve zaměřena na amidy. Fenyl-salicyláty (saloly) tvoří novou skupinu antimykobakteriálních látek. Jejich aktivita je menší než u odpovídajících amidů. Nejaktivnější látka ve studované skupině měla v salicylové části molekuly 4-methoxy skupinu. Studie přináší nové informace o antimykobakteriálních salicylových derivátech.

**Klíčová slova:** saloly • mykobakterie • antimykobakteriální aktivita • fenyl-salicyláty

### Introduction

The recent reappearance of tuberculosis and other infectious diseases is a serious problem worldwide. According to the World Health Organization, there were 9.4 million new tuberculosis cases in 2008 included 1.4 million cases among HIV positive people. In the year 2008 WHO also reported the highest rates of MDR-TB ever recorded<sup>1)</sup>. Therefore, there is a great need to develop new antituberculous and other antibiotics. For many years, our research has been oriented on exploring new active compounds. Our attention was predominantly oriented on salicylamides. Phenyl salicylate (salol) is known for its biological activity<sup>2)</sup>. Previously we used phenyl salicylates for the synthesis of antimycobacterially active salicyl amides with heterocyclic substituents on nitrogen<sup>3)</sup>. The goal of the paper consists in the preparation of some substituted phenyl salicylates and investigation of their antimycobacterial properties. Variation of substituents was on phenyl in the salicylic part of molecules.

### Experimental part

#### Chemistry

##### General information

The melting points were determined on a Kofler apparatus. The samples for the analyses and antimycobacterial tests were dried over  $P_2O_5$  at 61 °C and 66 Pa for 24 h. Elemental analyses (C, H, N) were performed on a CHNS-O CE elemental analyzer (Fisons EA 1110, Milan) and were within  $\pm 0.4$  % of the theoretical values. The IR spectra were measured in KBr pellets on a Nicolet Impact 400 apparatus; the wavenumbers are given in  $cm^{-1}$ . Crystallization of products was carried out from ethanol. The  $^1H$  NMR and  $^{13}C$  NMR spectra of new compounds were recorded in  $DMSO-d_6$  solutions at ambient temperature on a Varian Mercury-Vx BB 300 spectrometer operating at 300 MHz for  $^1H$  NMR, and 75 MHz for  $^{13}C$  NMR. Chemical shifts were recorded as  $\delta$  values in parts per million (ppm) and were indirectly referenced to tetramethylsilane via the solvent signal (2.49 for  $^1H$  or 39.7 for  $^{13}C$ ).

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*Synthetic procedures for the preparation of phenyl salicylates*

A mixture of substituted salicylic acid (1 mol) and phenol (1 mol) was heated with the presence of phosphorus oxychloride (0.38 mol) at 75–80 °C for 4 hours under a reflux condenser. Then, the reaction mixture was reduced to a molten mass and poured slowly into a solution of sodium carbonate with continuous stirring. The precipitated ester was collected on a filter and washed four times with 20 ml portions of water. The crude product was crystallized from ethanol.

**1.2.1 Phenyl 3-methylsalicylate (2)**, yield 64 %, mp. 42–43 °C, IR (νCO) 1685 cm<sup>-1</sup>; <sup>1</sup>H NMR (300 MHz, DMSO) δ 10.54 (1H, bs, OH), 7.93–7.86 (1H, m, H<sub>6</sub>), 7.56–7.44 (3H, m, H<sub>4</sub>, H<sub>3'</sub>, H<sub>5'</sub>), 7.38–7.27 (3H, m, H<sub>2'</sub>, H<sub>4'</sub>, H<sub>6'</sub>), 6.94 (1H, t, *J*=7.7 Hz, H<sub>5</sub>), 2.22 (3H, s, CH<sub>3</sub>); <sup>13</sup>C NMR (75 MHz, DMSO) δ 168.6, 159.5, 150.1, 137.4, 129.8, 128.1, 126.6, 126.4, 122.1, 119.4, 111.4, 15.6; Anal. Calcd for C<sub>14</sub>H<sub>12</sub>O<sub>3</sub> (228.25): C 73.67; H 5.30 %; Found: C 73.53; H 5.48 %.

**1.2.2. Phenyl 3-methoxysalicylate (3)**, yield 56 %, mp. 67–69 °C, IR (νCO) 1684 cm<sup>-1</sup>; <sup>1</sup>H NMR (300 MHz, DMSO) δ 10.15 (1H, bs, OH), 7.60–7.43 (3H, m, H<sub>6</sub>, H<sub>3'</sub>, H<sub>5'</sub>), 7.36–7.26 (4H, m, H<sub>4</sub>, H<sub>2'</sub>, H<sub>4'</sub>, H<sub>6'</sub>), 6.95 (1H, t, *J*=8.0 Hz, H<sub>5</sub>), 3.83 (3H, s, OCH<sub>3</sub>); <sup>13</sup>C NMR (75 MHz, DMSO) δ 167.4, 150.8, 150.3, 148.6, 129.8, 126.4, 122.1, 121.7, 119.2, 117.6, 113.4, 56.2; Anal. Calcd for C<sub>14</sub>H<sub>12</sub>O<sub>4</sub> (244.25): C 68.85; H 4.95 %; Found: C 68.47; H 5.03 %.

**1.2.3. Phenyl 4-methylsalicylate (4)**, yield 52 %, mp. 44–45 °C, IR (νCO) 1770, 1700 cm<sup>-1</sup>; <sup>1</sup>H NMR (300 MHz, DMSO) δ 10.25 (1H, bs, OH), 7.89 (1H, d, *J*=8.3 Hz, H<sub>6</sub>), 7.52–7.42 (2H, m, H<sub>3'</sub>, H<sub>5'</sub>), 7.36–7.25 (3H, m, H<sub>2'</sub>, H<sub>4'</sub>, H<sub>6'</sub>), 6.90–6.81 (2H, m, H<sub>3</sub>, H<sub>5</sub>), 2.49 (3H, s, CH<sub>3</sub>); <sup>13</sup>C NMR (75 MHz, DMSO) δ 167.3, 160.7, 150.3, 147.5, 130.7, 129.8, 126.4, 122.2, 120.9, 117.9, 110.2, 21.6; Anal. Calcd for C<sub>14</sub>H<sub>12</sub>O<sub>3</sub> (228.25): C 73.67; H 5.30 %; Found: C 73.70; H 5.51 %.

**1.2.4. Phenyl 4-chlorosalicylate (5)**, yield 54 %, mp. 57 °C, IR (νCO) 1672 cm<sup>-1</sup>; <sup>1</sup>H NMR (300 MHz, DMSO) δ 10.47 (1H, bs, OH), 8.04 (1H, d, *J*=2.5 Hz, H<sub>3</sub>), 7.70 (1H, dd, *J*=8.8 Hz, *J*=2.5 Hz, H<sub>5</sub>), 7.53–7.42 (2H, m, H<sub>3'</sub>, H<sub>5'</sub>), 7.36–7.26 (3H, m, H<sub>2'</sub>, H<sub>4'</sub>, H<sub>6'</sub>), 7.02 (1H, d, *J*=8.8 Hz, H<sub>6</sub>); <sup>13</sup>C NMR (75 MHz, DMSO) δ 165.7, 160.7, 150.3, 139.9, 132.8, 129.8, 126.4, 122.1, 119.9, 117.5, 113.2; Anal. Calcd for C<sub>13</sub>H<sub>9</sub>ClO<sub>3</sub> (248.67): C 62.79; H 3.65 %; Found: C 62.97; H 3.80 %.

**1.2.5. Phenyl 4-methoxysalicylate (6)**, yield 51 %, mp. 62 °C, IR (νCO) 1672 cm<sup>-1</sup>; <sup>1</sup>H NMR (300 MHz, DMSO) δ 10.50 (1H, bs, OH), 7.94 (1H, d, *J*=8.8 Hz, H<sub>6</sub>), 7.51–7.42 (2H, m, H<sub>3'</sub>, H<sub>5'</sub>), 7.36–7.23 (3H, m, H<sub>2'</sub>, H<sub>4'</sub>, H<sub>6'</sub>), 6.63–6.57 (2H, m, H<sub>3</sub>, H<sub>5</sub>), 3.83 (3H, s, OCH<sub>3</sub>); <sup>13</sup>C NMR (75 MHz, DMSO) δ 167.4, 165.9, 163.2, 150.3, 132.3, 129.8, 126.3, 122.2, 107.9, 105.2, 101.3, 55.9; Anal. Calcd for C<sub>14</sub>H<sub>12</sub>O<sub>4</sub> (244.25): C 68.85; H 4.95 %; Found: C 68.88; H 4.99 %.

**1.2.6. Phenyl 5-fluorosalicylate (7)**, yield 43 %, mp. 84–85 °C, IR (νCO) 1679 cm<sup>-1</sup>; <sup>1</sup>H NMR (300 MHz, DMSO) δ 10.20 (1H, bs, OH), 7.72 (1H, dd, *J*=9.1 Hz, *J*=3.3 Hz, H<sub>6</sub>), 7.54–7.41 (3H, m, H<sub>4</sub>, H<sub>3'</sub>, H<sub>5'</sub>), 7.37–7.27 (3H, m, H<sub>2'</sub>, H<sub>4'</sub>, H<sub>6'</sub>), 7.07 (1H, dd, *J*=9.1 Hz, *J*=4.4 Hz, H<sub>3</sub>); <sup>13</sup>C NMR (75 MHz, DMSO) δ 165.7

(d, *J*=2.9 Hz), 156.5, 154.8 (d, *J*=236.2 Hz), 150.3, 129.8, 126.5, 123.3 (d, *J*=23.5 Hz), 122.1, 119.4 (d, *J*=7.8 Hz), 116.2 (d, *J*=24.4 Hz), 114.2 (d, *J*=7.5 Hz); Anal. Calcd for C<sub>13</sub>H<sub>9</sub>FO<sub>3</sub> (232.21): C 67.24; H 3.91 %; Found: C 67.00; H 3.77 %.

**1.2.7. Phenyl 5-chlorosalicylate (8)**, yield 67 %, mp. 95–97 °C, IR (νCO) 1685 cm<sup>-1</sup>; <sup>1</sup>H NMR (300 MHz, DMSO) δ 10.45 (1H, bs, OH), 8.93 (1H, d, *J*=2.8 Hz, H<sub>6</sub>), 7.59 (1H, dd, *J*=8.8 Hz, *J*=2.8 Hz, H<sub>4</sub>), 7.53–7.42 (2H, m, H<sub>3'</sub>, H<sub>5'</sub>), 7.36–7.26 (3H, m, H<sub>2'</sub>, H<sub>4'</sub>, H<sub>6'</sub>), 7.07 (1H, d, *J*=8.8 Hz, H<sub>3</sub>); <sup>13</sup>C NMR (75 MHz, DMSO) δ 165.2, 158.6, 150.3, 135.4, 130.1, 129.8, 126.4, 122.9, 122.1, 119.9, 115.6; Anal. Calcd for C<sub>13</sub>H<sub>9</sub>ClO<sub>3</sub> (248.67): C 62.79; H 3.65 %; Found: C 62.90; H 3.81 %.

**1.2.8. Phenyl 5-bromosalicylate (9)**, yield 40 %, mp. 112–113 °C, IR (νCO) 1692 cm<sup>-1</sup>; <sup>1</sup>H NMR (300 MHz, DMSO) δ 10.47 (1H, bs, OH), 8.04 (1H, d, *J*=2.5 Hz, H<sub>6</sub>), 7.70 (1H, dd, *J*=8.8 Hz, *J*=2.5 Hz, H<sub>4</sub>), 7.53–7.42 (2H, m, H<sub>3'</sub>, H<sub>5'</sub>), 7.36–7.26 (3H, m, H<sub>2'</sub>, H<sub>4'</sub>, H<sub>6'</sub>), 7.02 (1H, d, *J*=8.8 Hz, H<sub>3</sub>); <sup>13</sup>C NMR (75 MHz, DMSO) δ 165.1, 159.0, 150.3, 138.2, 133.0, 129.8, 126.4, 122.1, 120.2, 116.2, 110.2; Anal. Calcd for C<sub>13</sub>H<sub>9</sub>BrO<sub>3</sub> (293.12): C 53.27; H 3.09 %; Found: C 53.42; H 2.96 %.

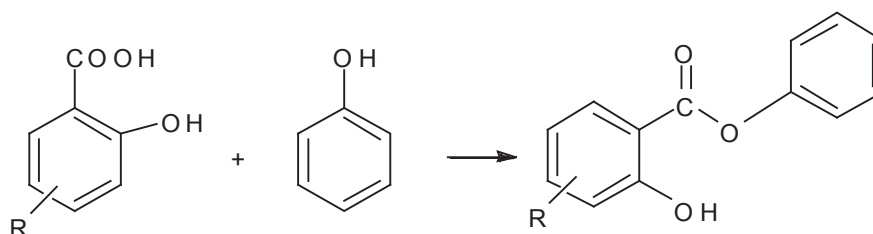
**Microbiology**

For the *in-vitro* evaluation of the antimycobacterial activity of the substances, the following strains were used: *M. tuberculosis* CNCTC My 331/88 (identical with H37RV and ATCC 27294), *M. kansasii* CNCTC My 235/80 (identical with ATCC 12 478), *M. avium* CNCTC My 330/88 (identical with – ATCC 25291), obtained from the Czech National Collection of Type Cultures (CNCTC), National Institute of Public Health, Prague, and a clinical isolate of *M. kansasii* 6509/ 96. The antimycobacterial activity of the compounds was determined in the Šula semisynthetic medium (SEVAC, Prague). Each strain was simultaneously inoculated into a Petri dish containing the Löwenstein-Jensen medium for the control of sterility of the inoculum and its growth. The compounds were added to the medium in DMSO solutions. The final concentrations were 1000, 500, 250, 125, 62.5, 32, 16, 8, 4, 2, 1, 0.5 and 0.25 μmol/l. The MICs were determined after incubation at 37 °C for 14 days and 21 days. The MIC was the lowest concentration of the antimycobacterially effective substance (on the above concentration scale), at which the inhibition of the growth of mycobacteria occurred. The evaluation was repeated three times and the values of the MIC were the same. Result are summarised in Table 1.

**Results**

Antimycobacterial activity of phenyl salicylates (salols) was studied in connection with antituberculous activity of salicylic derivatives. Phenyl salicylates are esters. Our attention was previously oriented on amides. Phenyl salicylates (salols) represent a new group of antimycobacterial compounds. They are less active than the corresponding amides. The significance of the paper consists in the preparation of some substituted phenyl salicylates and investigation of their antimycobacterial

Table 1. Overview of the phenyl salicylates and their antimycobacterial activity



Compound		MIC (μmol/l)			
		Incubation time 14d/21d			
	R	<i>M. tuberculosis</i> My 331/ 88	<i>M. avium</i> My 330/ 88	<i>M. kansasii</i> My 235/ 80	<i>M. kansasii</i> 6 509/ 96
1	H	n	n	n	n
2	3-CH <sub>3</sub>	62.5/125	250/500	250/500	250/250
3	3-OCH <sub>3</sub>	125/500	250/1000	500/1000	250/500
4	4-CH <sub>3</sub>	32/62.5	250/250	250/500	500/500
5	4-Cl	16/62.5	62.5/125	62.5/250	62.5/250
6	4-OCH <sub>3</sub>	8/32	62.5/62.5	62.5/125	62.5/125
7	5-F	32/125	125/500	250/500	125/250
8	5-Cl	62.5/125	250/500	500/500	250/500
9	5-Br	62.5/125	125/250	250/500	125/250
INH		1/1	> 250/> 250	> 250/> 250	8/8

properties. Variation of substituents was on phenyl in the salicylic part of molecules. The most active compound in the group under study is substituted on phenyl in the salicyl moiety with a 4-methoxy group. The study reports new information about antimycobacterial substances.

**Conflicts of interest:** none

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