Relationship between the Structure and Antimycobacterial Activity of Substituted Salicylanilides

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A series of 143 salicylanilides substituted in positions 4 and 5 and in positions 3′ and 4′ was synthesized. The compounds were evaluated for *in vitro* antimycobacterial activity against *Mycobacterium tuberculosis*, *Mycobacterium kansasii*, and *Mycobacterium avium*. To describe the structure-antimycobacterial activity relationships (QSARs), an approach based on the combination of the Free-Wilson and Hansch methods was employed (the substituent constants were used in the case of the substituents on the phenyl ring; indicator parameters were used for the substituents on the acyl moiety). The relationships between the antimycobacterial activity and physico-chemical parameters of all substituents were also explored. The quadratic representation of lipophilicity parameters did not lead to significant correlations.

Key Words: Salicylanilides; Antimycobacterial activity; Structure-activity relationships; Tuberculostatics; Atypical mycobacterial strains

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Introduction

The search for new antimycobacterial compounds is one of the most challenging tasks of current medicinal chemistry. In particular, a number of new broad-spectrum antimycobacterial substances from the class of organic sulphur compounds were synthesized by Mayer and coworkers and are reviewed in our previous paper [1]. Our own research on antimycobacterial salicylanilides was preceded by a review on the biological activity of these compounds [2]. The study of antimycobacterial activity of salicylanilides is currently of great importance, because salicylanilides are inhibitors of the bacterial two-component system [3] which is also found in mycobacteria [4]. These substances are convenient starting materials in the synthesis of antimycobacterial 3-aryl-2*H*-1,3-benzoxazin-2,4(3*H*)-diones, and also possess antimycobacterial activity. However, salicylanilides are unlikely to be effective chemotherapeutic agents themselves due to their effect on mitochondrial respiration [3]. We have previously found relatively simple SARs in the group of salicylanilides with no substitution in the acyl moiety, where the activity rises with increasing hydrophobicity and electron-accepting properties of the substituents [5, 6]. When salicylanilides were substituted in position 5, the influence of the substituents on the phenyl ring in the anilide part of the molecule was similar. However, the influence of the substituents in the acyl moiety was quite complex [7]. For this reason, we set out to synthesize a large series of compounds. The goal of this work was to assess the relationships between the antimycobacterial activity against *Mycobacterium tuberculosis*, *M. kansasii* and *M. avium*, and the structure of salicylanilides bearing substituents in positions 4 and 5, and on the phenyl ring in the anilide part of their molecules. The influence of substituents in position 4 has not yet been studied.

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Chemistry

The starting salicylanilides (**1**–**10**) were prepared by the treatment of substituted salicylic acids with the appropriate anilines in chlorobenzene.

Microbiology

Compounds **1**–**10** were tested for their *in vitro* antimycobacterial activity against *Mycobacterium tuberculosis* CNCTC My 331/88, *Mycobacterium kansasii* CNCTC My 235/80, and *Mycobacterium avium* CNCTC My 330/88 obtained from the Czech National Collection of Type Cultures (CNCTC).

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Calculation

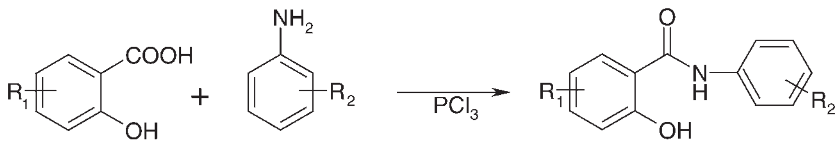
To set up the regression equations, we employed the "Multireg" programme developed by Klemera for Microsoft Excel. The values of the substituent constants and molecular refraction were taken from the literature [8, 9], with the exception of the Hammett constant for the thioamide group, which was taken from the original paper [10]. There are no significant intercorrelations between different sets of parameters.

Results and discussion

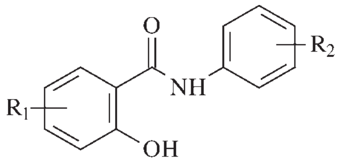
The chemical and physical data of the new salicylanilides are given in Table 1. In the spectra of the salicylanilides (**1**–**10**), the amide ν(C=O) vibrations are clearly observed. Compounds described previously [5–7] are not included in the Table. NMR spectra are given in Table 2.

Standard strains of mycobacteria from the Czech National Collection of Type Cultures (CNCTC) were employed. In several cases, the minimum inhibitory concentration could not be determined due to the limited solubility of the compounds. In general, the results suggest that the compounds under study can be considered as prospective broad-spectrum antimycobacterial substances.

In studying the relationship between the structure and antimycobacterial activity, we followed up on the conclusions drawn in our previous work [7]. The Hammett constants σ of the substituents on the phenyl ring served as one parameter, and their hydrophobic substituent constants π were used as the second parameter. However, the influence of the substituents from the acyl part of the molecule appeared to be more complex, and hence we expressed them with indicator parameters (I_n). The QSAR study of the antimycobacterial activity of salicyl-



Scheme 1. Preparation of the salicylanilides



	R₁		R₁		R₂		R₂		R₂
1	H	**7**	5-F	**a**	H	**g**	4-F	**m**	4-OCH_3
2	4-Cl	**8**	5-CH_3	**b**	4-CH_3	**h**	3-F	**n**	4-COOEt
3	4-CH_3	**9**	5-OCH_3	**c**	4-Cl	**i**	4-CF_3	**o**	4-CN
4	4-OCH_3	**10**	5-NO_2	**d**	3-Cl	**j**	4-NO_2	**p**	4-$CSNH_2$
5	5-Br			**e**	3,4-Cl_2	**k**	3-NO_2		
6	5-Cl			**f**	4-Br	**l**	4-$N(CH_3)_2$		

Scheme 2. Overview of the structures of the salicylanilides.

Table 1. Characteristics of the compounds.

Compound	R_1	R_2	Formula M_r	ν C=O [cm^{-1}]	Mp [°C] Found	Mp [°C] Ref.
2a	4-Cl	H	$C_{13}H_{10}ClNO_2$ 247.7	1634	218–220	217–220 [12]
2b	4-Cl	4-CH_3	$C_{14}H_{12}ClNO_2$ 261.7	1628	220–222	–
2c	4-Cl	4-Cl	$C_{13}H_9Cl_2NO_2$ 282.12	1611	222–223	–
2d	4-Cl	3-Cl	$C_{13}H_9Cl_2NO_2$ 282.12	1619	216–218	–
2e	4-Cl	3,4-Cl_2	$C_{13}H_8Cl_3NO_2$ 316.57	1617	221–223	221–222 [13]
2f	4-Cl	4-Br	$C_{13}H_9BrClNO_2$ 326.6	1606	220–222	–
2g	4-Cl	4-F	$C_{13}H_9FClNO_2$ 265.7	1620	205–207	–
2h	4-Cl	3-F	$C_{13}H_9FClNO_2$ 265.7	1615	226–227	–
2i	4-Cl	4-CF_3	$C_{14}H_9ClF_3NO_2$ 315.67	1607	213–216	–
2k	4-Cl	3-NO_2	$C_{13}H_9ClN_2O_4$ 292.7	1641	225–226	–
2l	4-Cl	4-$N(CH_3)_2$	$C_{15}H_{15}ClN_2O_2$ 290.8	1616	224–234	–
2m	4-Cl	4-OCH_3	$C_{14}H_{12}ClNO_3$ 277.7	1617	205–207	–
2n	4-Cl	4-COOEt	$C_{16}H_{14}ClNO_4$ 319.74	1648;1685	216–218	–
2o	4-Cl	4-CN	$C_{14}H_9ClN_2O_2$ 272.69	1648 (CN) 2227	238–240	–
3a	4-CH_3	H	$C_{14}H_{13}NO_2$ 227.26	1622	186–188	190–191 [14]
3b	4-CH_3	4-CH_3	$C_{15}H_{15}NO_2$ 241.28	1603	202–204	166–167 [14]
3c	4-CH_3	4-Cl	$C_{14}H_{12}ClNO_2$ 261.7	1623	217–219	–
3d	4-CH_3	3-Cl	$C_{14}H_{12}ClNO_2$ 261.7	1624	199–201	–
3e	4-CH_3	3,4-Cl_2	$C_{14}H_{11}Cl_2NO_2$ 296.15	1626	208–210	–
3f	4-CH_3	4-Br	$C_{14}H_{12}BrNO_2$ 306.15	1622	225–227	–
3g	4-CH_3	4-F	$C_{14}H_{12}FNO_2$ 245.25	1622	176–179	–
3h	4-CH_3	3-F	$C_{14}H_{12}FNO_2$ 245.25	1617	137–139	–
3i	4-CH_3	4-CF_3	$C_{15}H_{12}F_3NO_2$ 295.26	1618	229–231	–
3k	4-CH_3	3-NO_2	$C_{14}H_{12}N_2O_4$ 272.26	1631	216–218	120–121 [14]
3l	4-CH_3	4-$N(CH_3)_2$	$C_{16}H_{18}N_2O_2$ 270.33	1641	201–203	–

Table 1. continued.

Compound	R_1	R_2	Formula M_r	ν C=O [cm^{-1}]	Mp [°C] Found	Ref.
3m	4-CH_3	4-OCH_3	$C_{15}H_{15}NO_3$ 257.28	1628	183–185	145–146 [14]
3n	4-CH_3	4-COOEt	$C_{17}H_{17}NO_4$ 299.32	1617;1713	194–196	–
3o	4-CH_3	4-CN	$C_{15}H_{12}N_2O_2$ 252.27	1641 (CN) 2227	212–213	–
4a	4-OCH_3	H	$C_{14}H_{13}NO_3$ 243.26	1621	150–152	152–154 [15]
4b	4-OCH_3	4-CH_3	$C_{15}H_{15}NO_3$ 257.28	1614	146–147	151–152 [16]
4c	4-OCH_3	4-Cl	$C_{14}H_{12}ClNO_3$ 277.7	1617	184–186	–
4d	4-OCH_3	3-Cl	$C_{14}H_{12}ClNO_3$ 277.7	1618	165–167	–
4e	4-OCH_3	3,4-Cl_2	$C_{14}H_{11}Cl_2NO_3$ 312.15	1622	203–205	–
4f	4-OCH_3	4-Br	$C_{14}H_{12}BrNO_3$ 322.15	1617	185–187	–
4g	4-OCH_3	4-F	$C_{14}H_{12}FNO_3$ 261.25	1618	174–176	–
4h	4-OCH_3	3-F	$C_{14}H_{12}FNO_3$ 261.25	1622	182–185	–
4i	4-OCH_3	4-CF_3	$C_{15}H_{12}F_3NO_3$ 311.26	1621		–
4j	4-OCH_3	4-NO_2	$C_{14}H_{12}N_2O_5$ 288.26	1626	222–224	223–224 [16]
4k	4-OCH_3	3-NO_2	$C_{14}H_{12}N_2O_5$ 288.26	1643	189–191	194–195 [16]
4m	4-OCH_3	4-OCH_3	$C_{15}H_{15}NO_4$ 273.29	1616	163–165	164–165 [16]
4n	4-OCH_3	4-COOEt	$C_{17}H_{17}NO_5$ 315.32	1611; 1711	193–194	–
4o	4-OCH_3	4-CN	$C_{15}H_{12}N_2O_3$ 268.27	1647 (CN) 2224	218–220	–
5i	5-Br	4-CF_3	$C_{14}H_9BrF_3NO_2$ 360.13	1635	202–203	–
5n	5-Br	4-COOEt	$C_{16}H_{14}BrNO_4$ 364.19	1648; 1686	235–236.5	–
5o	5-Br	4-CN	$C_{14}H_9BrN_2O_2$ 317.14	1637 (CN) 2229	256–257	–
6i	5-Cl	4-CF_3	$C_{14}H_9ClF_3NO_2$ 315.67	1629	217–218	–
6n	5-Cl	4-COOEt	$C_{16}H_{14}ClNO_4$ 319.74	1632; 1717	212–214	–
6o	5-Cl	4-CN	$C_{14}H_9ClN_2O_2$ 272.69	1636 (CN) 2231	242–243.5	–
7i	5-F	4-CF_3	$C_{14}H_9F_4NO_2$ 299.22	1630	222–223	–
7n	5-F	4-COOEt	$C_{16}H_{14}FNO_4$ 303.28	1640; 1690	193–195	–

Table 1. continued.

Compound	R_1	R_2	Formula M_r	ν C=O [cm^{-1}]	Mp [°C] Found	Ref.
7o	5-F	4-CN	$C_{14}H_9FN_2O_2$ 256.23	1643 (CN) 2229	218–219	–
8a	5-CH_3	H	$C_{14}H_{13}NO_2$ 227.26	1629	159–161	160–161 [17]
8b	5-CH_3	4-CH_3	$C_{15}H_{15}NO_2$ 241.28	1631	189–191	167–168 [14]
8c	5-CH_3	4-Cl	$C_{14}H_{12}ClNO_2$ 261.7	1633	197–198	164–165 [17]
8d	5-CH_3	3-Cl	$C_{14}H_{12}ClNO_2$ 261.7	1646	170–172	–
8e	5-CH_3	3,4-Cl_2	$C_{14}H_{11}Cl_2NO_2$ 296.15	1644	210–212	212–213 [18]
8f	5-CH_3	4-Br	$C_{14}H_{12}BrNO_2$ 306.15	1632	204–206	210–211 [19]
8g	5-CH_3	4-F	$C_{14}H_{12}FNO_2$ 245.25	1634	166–168	–
8h	5-CH_3	3-F	$C_{14}H_{12}FNO_2$ 245.25	1634	183–184	–
8i	5-CH_3	4-CF_3	$C_{15}H_{12}F_3NO_2$ 295.26	1643	207–208	–
8j	5-CH_3	4-NO_2	$C_{14}H_{12}N_2O_4$ 272.26	1636	234–236	236–237 [14]
8k	5-CH_3	3-NO_2	$C_{14}H_{12}N_2O_4$ 272.26	1652	197–199	120–121 [14]
8l	5-CH_3	4-$N(CH_3)_2$	$C_{16}H_{18}N_2O_2$ 270.33	1616	197–199	–
8m	5-CH_3	4-OCH_3	$C_{15}H_{15}NO_3$ 257.28	1629	182–184	185–186 [19]
8n	5-CH_3	4-COOEt	$C_{17}H_{17}NO_4$ 299.32	1644; 1698	300–302	–
8o	5-CH_3	4-CN	$C_{15}H_{12}N_2O_2$ 252.27	1662 (CN) 2227	140–142	–
9a	5-OCH_3	H	$C_{14}H_{13}NO_3$ 243.26	1636	167–169	–
9b	5-OCH_3	4-CH_3	$C_{15}H_{15}NO_3$ 257.28	1634	188–190	–
9c	5-OCH_3	4-Cl	$C_{14}H_{12}ClNO_3$ 277.7	1637	209–210	–
9d	5-OCH_3	3-Cl	$C_{14}H_{12}ClNO_3$ 277.7	1642	162–164	–
9e	5-OCH_3	3,4-Cl_2	$C_{14}H_{11}Cl_2NO_3$ 312.15	1641	204–206	–
9f	5-OCH_3	4-Br	$C_{14}H_{12}BrNO_3$ 322.15	1637	223–225	214–215 [19]
9g	5-OCH_3	4-F	$C_{14}H_{12}FNO_3$ 261.25	1641	181–183	–
9h	5-OCH_3	3-F	$C_{14}H_{12}FNO_3$ 261.25	1642	178–180	–
9i	5-OCH_3	4-CF_3	$C_{15}H_{12}F_3NO_3$ 311.26	1636	207	–

Table 1. continued.

Compound	R_1	R_2	Formula M_r	ν C=O [cm^{-1}]	Mp [°C] Found	Ref.
9j	5-OCH_3	4-NO_2	$C_{14}H_{12}N_2O_5$ 288.26	1656	243–244	–
9k	5-OCH_3	3-NO_2	$C_{14}H_{12}N_2O_5$ 288.26	1642	219–221	–
9m	5-OCH_3	4-OCH_3	$C_{15}H_{15}NO_4$ 273.29	1634	188–190	–
9n	5-OCH_3	4-COOEt	$C_{17}H_{17}NO_5$ 315.32	1637; 1685	196–197	–
9o	5-OCH_3	4-CN	$C_{15}H_{12}N_2O_3$ 268.27	1644 (CN) 2224	232–233	–

Compounds described previously [5–7] were not included. For other compounds, literature references are given.

Table 2. ^1H and ^{13}C NMR spectra of the new salicylanilides.

Compound	NMR, δ
2a	^1H NMR (300 MHz, DMSO) δ 12.11 (bs, 1 H, OH), 10.37 (s, 1 H, NH), 7.97–7.91 (m, 1 H, H6), 7.72–7.65 (m, 2 H, H2′, H6′), 7.41–7.29 (m, 2 H, H3′, H5′), 7.17–7.07 (m, 1 H, H4′), 7.06–6.98 (m, 2 H, H3, H5) ^{13}C NMR (75 MHz, DMSO) δ 165.7, 159.2, 138.3, 137.6, 131.1, 129.0, 124.5, 121.1, 119.4, 117.5, 117.0
2b	^1H NMR (300 MHz, DMSO) δ 12.21 (bs, 1 H, OH), 10.31 (s, 1 H, NH), 7.98–7.93 (m, 1 H, H6), 7.61–7.53 (m AA′, BB′, 2 H, H2′, H6′), 7.20–7.12 (m, AA′, BB′, 2 H, H3′, H5′), 7.07–6.98 (m, 2 H, H3, H5), 2.27 (s, 3 H, CH_3) ^{13}C NMR (75 MHz, DMSO) δ 165.7, 159.4, 137.6, 135.7, 133.6, 130.9, 129.4, 121.2, 119.4, 117.1, 117.0, 20.7
2c	^1H NMR (300 MHz, DMSO) δ 11.94 (bs, 1 H, OH), 10.43 (s, 1 H, NH), 7.89 (d, 1 H, *J* = 8.24 Hz, H6), 7.78–7.67 (m AA′, BB′, 2 H, H2′, H6′), 7.45–7.35 (m, AA′, BB′, 2 H, H3′, H5′), 7.08–6.98 (m, 2 H, H3, H5) ^{13}C NMR (75 MHz, DMSO) δ 165.6, 158.9, 137.6, 137.4, 131.1, 128.9, 128.0, 122.5, 119.5, 117.7, 117.0
2d	^1H NMR (300 MHz, DMSO) δ 11.86 (bs, 1 H, OH), 10.45 (s, 1 H, NH), 7.91 (t, 1 H, *J* = 1.92 Hz, H2′), 7.90–7.85 (m, 1 H, H6), 7.59 (ddd, 1 H, *J* = 8.24 Hz, *J* = 1.92 Hz, *J* = 1.10 Hz, H6′), 7.38 (t, 1 H, *J* = 8.24 Hz, H5′), 7.18 (ddd, 1 H, *J* = 8.24 Hz, *J* = 1.92 Hz, 1.10 Hz, H4′), 7.06–7.00 (m, 2 H, H3, H5) ^{13}C NMR (75 MHz, DMSO) δ 165.6, 158.7, 139.9, 137.6, 133.3, 131.2, 130.6, 124.0, 120.3, 119.5, 119.2, 117.9, 116.9
2e	^1H NMR (300 MHz, DMSO) δ 11.78 (bs, 1 H, OH), 10.50 (s, 1 H, NH), 8.09 (d, 1 H, *J* = 2.19 Hz, H2′), 7.84 (d, 1 H, *J* = 8.52 Hz, H6), 7.65 (dd, 1 H, *J* = 8.79 Hz, *J* = 2.19 Hz, H5′), 7.59 (d, 1 H, *J* = 8.79 Hz, H6′), 7.05–6.99 (m, 2 H, H3, H5) ^{13}C NMR (75 MHz, DMSO) δ 165.6, 158.5, 138.6, 137.6, 131.3, 131.2, 130.8, 125.8, 121.9, 120.8, 119.5, 118.0, 116.9
2f	^1H NMR (300 MHz, DMSO) δ 11.94 (bs, 1 H, OH), 10.43 (s, 1 H, NH), 7.92–7.86 (m, 1 H, H6), 7.72–7.63 (m, AA′, BB′, 2 H, H2′, H6′), 7.59–7.50 (m AA′, BB′, 2 H, H3′, H5′), 7.07–6.98 (m, 2 H, H3, H5) ^{13}C NMR (75 MHz, DMSO) δ 165.3, 158.6, 137.5, 137.3, 131.5, 130.9, 122.6, 119.2, 117.5, 116.7, 115.9
2g	^1H NMR (300 MHz, DMSO) δ 12.02 (bs, 1 H, OH), 10.40 (s, 1 H, NH), 7.92 (d, 1 H, *J* = 8.52 Hz, H6), 7.76–7.66 (m, 2 H, H2′, H6′), 7.23–7.16 (m, 2 H, H3′, H5′), 7.05–7.00 (m, 2 H, H3, H5) ^{13}C NMR (75 MHz, DMSO) δ 165.7, 160.5 and 157.3 (*J* = 241.1 Hz), 159.3, 137.6, 134.6 and 134.6 (*J* = 2.9 Hz), 131.0, 123.1 and 123.0 (*J* = 8.0 Hz), 119.4, 117.3, 117.0, 115.7 and 115.4 (*J* = 23.4 Hz)

Table 2. continued.

Compound	NMR, δ
2h	^{1}H NMR (300 MHz, DMSO) δ 11.88 (bs, 1 H, OH), 10.48 (s, 1 H, NH), 7.90–7.85 (m, 1 H, H6), 7.70 (dt, 1 H, J = 11.54 Hz, J = 2.20 Hz, H6′), 7.49–7.33 (m, 2 H, H2′, H5′), 7.06–6.91 (m, 3H, H3, H5, H4′) ^{13}C NMR (75 MHz, DMSO) δ 165.6, 163.9 and 160.7 (J = 241.3 Hz), 158.6, 140.2 and 140.1 (J = 10.9 Hz), 137.5, 131.3, 130.7 and 130.5 (J = 9.4 Hz), 119.5, 118.0, 116.9, 116.6 and 116.5 (J = 2.6), 110.9 and 110.7 (J = 20.9 Hz), 107.7 and 107.4 (J = 26.0 Hz)
2i	^{1}H NMR (300 MHz, DMSO) δ 11.83 (bs, 1 H, OH), 10.61 (s, 1 H, NH), 7.96–7.85 (m AA′, BB′ overlapped, 3H, H6, H3′, H5′), 7.75–7.68 (m AA′, BB′, 2 H, H2′, H6′), 7.07–7.00 (m, 2 H, H3, H5) ^{13}C NMR (75 MHz, DMSO) δ 165.7, 158.5, 142.2, 137.6, 126.3 (q, J = 4.0 Hz), 124.5 (q, J = 271.4 Hz), 122.7, 124.2 (q, J = 32.1 Hz), 120.6, 119.5, 118.2, 116.9
2k	^{1}H NMR (300 MHz, DMSO) δ 11.76 (bs, 1 H, OH), 10.70 (s, 1 H, NH), 8.75 (t, 1 H, J = 2.19 Hz, H2′), 8.07–8.01 (m, 1 H, H6′), 8.00–7.94 (m, 1 H, H4′), 7.90–7.84 (m, 1 H, H6), 7.64 (t, 1 H, J = 8.24 Hz, H5′), 7.08–7.00 (m, 2 H, H3, H5) ^{13}C NMR (75 MHz, DMSO) δ 165.9, 158.6, 148.1, 139.7, 137.7, 131.3, 130.4, 126.7, 119.5, 118.8, 118.0, 116.9, 114.8
2l	^{1}H NMR (300 MHz, DMSO) δ 12.5 (bs, 1 H, OH), 10.24 (s, 1 H, NH), 8.01–7.96 (m, 1 H, H6), 7.51–7.43 (m AA′, BB′, 2 H, H2′, H6′), 7.04–6.97 (m, 2 H, H3, H5), 6.76–6.69 (m AA′, BB′, 2 H, H3′, H5′), 2.87 (s, 6 H, CH_3) ^{13}C NMR (75 MHz, DMSO) δ 165.7, 160.2, 148.0, 137.6, 130.4, 127.4, 122.8, 119.1, 117.1, 116.3, 112.6, 40.6
2m	^{1}H NMR (300 MHz, DMSO) δ 12.31 (bs, 1 H, OH), 10.29 (s, 1 H, NH), 7.99–7.94 (m, 1 H, H6), 7.63–7.54 (m AA′, BB′, 2 H, H2′, H6′), 7.04–6. 99 (m, 2 H, H3, H5), 6.97–6.90 (m AA′, BB′, H3′, H5′), 3.74 (s, 3 H, OCH_3) ^{13}C NMR (75 MHz, DMSO) δ 165.8, 159.8, 156.3, 137.6, 131.1, 130.7, 122.9, 119.3, 117.1, 116.7, 114.1, 55.4
2n	^{1}H NMR (300 MHz, DMSO) δ 11.83 (bs, 1 H, OH), 10.55 (s, 1 H, NH), 7.96–7.78 (m, 5 H, H6, H2′, H3′, H5′, H6′), 7.04–6.96 (m, 2 H, H3, H5), 4.25 (q, 1 H, J = 7.14 Hz, CH_2), 1.27 (t, 3 H, J = 7.15 Hz, CH_3) ^{13}C NMR (75 MHz, DMSO) δ165.6, 165.5, 158.5, 142.9, 137.6, 131.4, 130.4, 125.2, 120, 119.6, 118.2, 116.9, 60.7, 14.4
2o	^{1}H NMR (300 MHz, DMSO) δ 11.76 (bs, 1 H, OH), 10.62 (s, 1 H, NH), 7.94–7.76 (m, 5 H, H6, H2′, H3′, H5′, H6′), 7.05–6.99 (m, 2 H, H3, H5) ^{13}C NMR (75 MHz, DMSO) δ165.6, 158.3, 142.8, 137.6, 133.4, 131.5, 120.6, 119.6, 119.2, 118.5, 116.9, 105.9
3a	^{1}H NMR (300 MHz, DMSO) δ 11.95 (bs, 1 H, OH), 10.34 (bs, 1 H, NH), 7.93–7.88 (m, 1 H, H6), 7.74–7.65 (m, 2 H, H2′, H6′), 7.40–7.32 (m, 2 H, H3′, H5′), 7.18–7.08 (m, 1 H, H4′), 6.82–6.74 (m, 2 H, H3, H5), 2.29 (s, 3 H, CH_3) ^{13}C NMR (75 MHz, DMSO) δ 167.1, 159.3, 144.6, 138.3, 129.0, 129.0, 124.4, 121.3, 120.3, 117.7, 114.3, 21.3
3b	^{1}H NMR (300 MHz, DMSO) δ 12.04 (bs, 1 H, OH), 10.26 (s, 1 H, NH), 7.90 (d, 1 H, J = 8.51 Hz, H6), 7.60–7.52 (m, AA′, BB′, 2 H, H2′, H6′), 7.20–7.12 (m, AA′, BB′, 2 H, H3′, H5′), 6.80–6.73 (m, 2 H, H3, H5), 2.29 (s, 3 H, CH_3), 2.27 (s, 3 H, CH_3) ^{13}C NMR (75 MHz, DMSO) δ 167.1, 159.5, 144.6, 135.7, 133.5, 129.3, 128.8, 121.4, 120.2, 117.7, 114.1, 21.3, 20.8
3c	^{1}H NMR (300 MHz, DMSO) δ 11.81 (bs, 1 H, OH), 10.41 (s, 1 H, NH), 7.86 (d, 1 H, J = 8.51 Hz, H6), 7.78–7.69 (m, AA′, BB′, 2 H, H2′, H6′), 7.46–7.36 (m, AA′, BB′, 2 H, H3′, H5′), 6.82–6.74 (m, 2 H, H3, H5), 2.28 (s, 3 H, CH_3) ^{13}C NMR (75 MHz, DMSO) δ 167.1, 159.1, 144.7, 137.4, 129.1, 128.8, 128.0, 122.7, 120.3, 117.7, 114.4, 21.3

Table 2. continued.

Compound	NMR, δ
3d	^{1}H NMR (300 MHz, DMSO) δ 11.72 (bs, 1 H, OH), 10.42 (s, 1 H, NH), 7.91 (t, 1 H, *J* = 2.0 Hz, H2′), 7.85 (d, 1 H, *J* = 8.52 Hz, H6), 7.63–7.57 (m, 1 H, H6′), 7.38 (t, 1 H, *J* = 8.1 Hz, H5′), 7.20–7.15 (m, 1 H, H4′), 6.82–6.74 (m, 2 H, H3, H5), 2.29 (s, 3 H, CH_3) ^{13}C NMR (75 MHz, DMSO) δ 167.1, 158.9, 144.8, 140.0, 133.2, 130.6, 129.2, 124.0, 120.5, 120.4, 119.4, 117.7, 114.6, 21.3
3e	^{1}H NMR (300 MHz, DMSO) δ 11.63 (bs, 1 H, OH), 10.48 (s, 1 H, NH), 8.10 (d, 1 H, *J* = 2.34 Hz, H2′), 7.82 (d, 1 H, *J* = 7.69 Hz, H6), 7.66 (dd, 1 H, *J* = 8.79 Hz, *J* = 2.34 Hz, H6′), 7.58 (d, 1 H, *J* = 8.79 Hz, H5′), 6.82–6.74 (m, 2 H, H3, H5), 2.28 (s, 3 H, CH_3) ^{13}C NMR (75 MHz, DMSO) δ 167.0, 158.8, 144.8, 138.7, 131.2, 130.8, 129.2, 125.7, 122.1, 120.9, 120.4, 117.7, 114.6, 21.3
3f	^{1}H NMR (300 MHz, DMSO) δ 11.63 (bs, 1 H, OH), 10.40 (s, 1 H, NH), 7.88–7.83 (m, 1 H, H6), 7.71–7.65 (m, AA′, BB′, 2 H, H2′, H6′), 7.56–7.50 (m, AA′, BB′, 2 H, H3′, H5′), 6.81–6.75 (m, 2 H, H3, H5), 2.28 (s, 3 H, CH_3) ^{13}C NMR (75 MHz, DMSO) δ 167.0, 159.1, 144.7, 137.8, 131.8, 129.1, 123.1, 120.3, 117.7, 116.1, 114.5, 21.4
3g	^{1}H NMR (300 MHz, DMSO) δ 11.94 (bs, 1 H, OH), 10.35 (s, 1 H, NH), 7.88 (d, 1 H, *J* = 8.52 Hz, H6), 7.76–7.65 (m, 2 H, H2′, H6′), 7.26–7.14 (m, 2 H, H3′, H5′), 6.82–6.74 (m, 2 H, H3, H5), 2.29 (s, 3 H, CH_3) ^{13}C NMR (75 MHz, DMSO) δ 167.2, 160.5 and 157.3 (*J* = 241.1 Hz), 159.4, 144.7, 134.7 and 134.6 (*J* = 2.9 Hz), 128.9, 123.3 and 123.2 (*J* = 8.0 Hz), 120.2, 117.7, 115.7 and 115.4 (*J* = 22.0 Hz), 114.1, 21.3
3h	^{1}H NMR (300 MHz, DMSO) δ 11.72 (bs, 1 H, OH), 10.45 (s, 1 H, NH), 7.88–7.83 (m, 1 H, H6), 7.74–7.66 (m, 1 H, H6′), 7.50–7.32 (m, 2 H, H2′, H5′), 7.06–6.90 (m, 1 H, H4′), 6.83–6.75 (m, 2 H, H3, H5), 2.29 (s, 3 H, CH_3) ^{13}C NMR (75 MHz, DMSO) δ 167.0, 163.9 and 160.7 (*J* = 241.4 Hz), 158.8, 144.7, 140.3 and 140.1 (*J* = 11.2 Hz), 130.6 and 130.5 (*J* = 9.4 Hz), 129.2, 120.4, 117.7, 116.7 and 116.7 (*J* = 2.6 Hz), 114.7, 110.8 and 110.6 (*J* = 20.9 Hz), 107.9 nd 107.6 (*J* = 26.3 Hz), 21.3
3i	^{1}H NMR (300 MHz, DMSO) δ 11.69 (bs, 1 H, OH), 10.57 (s, 1 H, NH), 7.98–7.83 (m, 3 H, H6, H3, H5), 7.74–7.68 (m AA′, BB′, 2 H, H3′, H5′), 6.83–6.76 (m AA′, BB′, 2 H, H2′, H6′), 2.29 (s, 3 H, CH_3) ^{13}C NMR (75 MHz, DMSO) δ 167.1, 158.7, 144.8, 142.2, 129.4, 126.2 (q, *J* = 3.8 Hz), 124.6 (q, *J* = 271.4 Hz), 124.1 (q, *J* = 32.1 Hz), 120.8, 120.4, 117.7, 114.8, 21.3
3k	^{1}H NMR (300 MHz, DMSO) δ 11.60 (bs, 1 H, OH), 10.68 (s, 1 H, NH), 8.75 (t, 1 H, *J* = 2.19 Hz, H2′), 8.07 (ddd, 1 H, *J* = 8.24 Hz, *J* = 2.19 Hz, *J* = 0.83 Hz, H6′), 7.97 (ddd, 1 H, *J* = 8.24 Hz, *J* = 2.19 Hz, *J* = 0.83 Hz, H4′), 7.86 (d, 1 H, *J* = 8.52 Hz, H6), 7.65 (t, 1 H, *J* = 8.24 Hz, H5′), 6.84–6.77 (m, 2 H, H3, H5), 2.30 (s, 3 H, CH_3) ^{13}C NMR (75 MHz, DMSO) δ 167.3, 158.8, 148.1, 144.9, 139.7, 130.3, 129.3, 126.9, 120.4, 118.7, 117.7, 115.0, 114.7, 21.4
3l	^{1}H NMR (300 MHz, DMSO) δ 12.36 (bs, 1 H, OH), 10.13 (s, 1 H, NH), 7.91 (d, 1 H, *J* = 8.79 Hz, H6), 7.52–7.43 (m, AA′, BB′, 2 H, H2′, H6′), 6.80–6.67 (m, 4 H, H3, H5, H3′, H5′), 2.28 (s, 3 H, CH_3) ^{13}C NMR (75 MHz, DMSO) δ 167.2, 160.0, 147.9, 144.4, 128.3, 127.5, 123.0, 120.0, 117.8, 113.6, 112.6, 40.6, 21.3
3m	^{1}H NMR (300 MHz, DMSO) δ 12.14 (bs, 1 H, OH), 10.24 (s, 1 H, NH), 7.89 (d, 1 H, *J* = 8.51 Hz, H6), 7.62–7.53 (m, AA′, BB′, 2 H, H2′, H6′), 6.98–6.89 (m, AA′, BB′, 2 H, H3′, H5′), 6.80–6.72 (m, 2 H, H3, H5), 3.74 (s, 3 H, OCH_3), 2.28 (s, 3 H, CH_3) ^{13}C NMR (75 MHz, DMSO) δ 167.2, 159.8, 156.2, 144.6, 131.1, 128.6, 123.1, 120.1, 117.8, 114.0, 113.8, 55.4, 21.3
3n	^{1}H NMR (300 MHz, DMSO) δ 11.71 (bs, 1 H, OH), 10.56 (s, 1 H, NH), 7.99–7.92 (m AA′, BB′, 2 H, H3′, H5′), 7.91–7.83 (m, 3 H, H6, H3, H5), 6.83–6.75 (m AA′, BB′, 2 H, H2′, H6′), 4.28 (q, 2 H, *J* = 7.14 Hz, CH_2), 2.29 (s, 3 H, CH_3), 1.30 (t, 3 H, *J* = 7.14 Hz, CH_3) ^{13}C NMR (75 MHz, DMSO) δ166.9, 165.5, 158.7, 144.8, 142.9, 130.3, 129.5, 125.1, 120.5, 120.2, 117.7, 114.8, 60.7, 21.3, 14.4

Table 2. continued.

Compound	NMR, δ
3o	^{1}H NMR (300 MHz, DMSO) δ 11.56 (bs, 1 H, OH), 10.6 (s, 1 H, NH), 7.95–7.88 (m, AA′, BB′, 2 H, H3′, H5′), 7.85–7.77 (m, 3 H, H6, H3, H5), 6.83–6.76 (m AA′, BB′, 2 H, H2′, H6′), 2.29 (s, 3 H, CH_3) ^{13}C NMR (75 MHz, DMSO) δ166.9, 158.4, 144.8, 142.9, 133.4, 129.6, 120.8, 120.5, 119.2, 117.6, 115.1, 105.8, 21.3
4a	^{1}H NMR (300 MHz, DMSO) δ 12.43 (bs, 1 H, OH), 10.23 (bs, 1 H NH), 7.99 (d, 1 H, *J* = 8.79 Hz, H6), 7.72–7.63 (m, 2 H, H2′, H6′), 7.42–7.30 (m, 2 H, H3′, H5′), 7.17–7.08 (m, 1 H, H4′), 6.55 (dd, 1 H, *J* = 8.79 Hz, *J* = 2.74 HZ, H5), 6.49 (d, 1 H, *J* = 2.74 Hz, H3), 3.79 (s, 3 H, OCH_3) ^{13}C NMR (75 MHz, DMSO) δ 167.5, 164.0, 162.0, 138.3, 130.3, 128.9, 124.4, 121.5, 109.1, 106.5, 101.5, 55.6
4b	^{1}H NMR (300 MHz, DMSO) δ 12.54 (bs, 1 H, NH), 10.17 (bs, 1 H, OH), 7.99 (d, 1 H, *J* = 8.79 Hz, H6), 7.60–7.52 (m AA′, BB′, 2 H, H2′, H6′), 7.20–7.11 (m AA′, BB′, 2 H, H3′, H5′), 6.54 (dd, 1 H, *J* = 8.79 Hz, *J* = 2.47 Hz, H5), 6.49 (d, 1 H, *J* = 2.48 Hz, H3), 3.78 (s, 3 H, OCH_3), 2.27 (s, 3 H, CH_3) ^{13}C NMR (75 MHz, DMSO) δ 167.5, 164.0, 162.2, 135.7, 133.5, 130.1, 129.3, 121.6, 109.0, 106.5, 101.5, 55.6, 20.7
4c	^{1}H NMR (300 MHz, DMSO) δ 12.29 (bs, 1 H, NH), 10.30 (bs, 1 H, OH), 7.96 (d, 1 H, *J* = 8.79 Hz, H6), 7.76-7.68 (m AA′, BB′, 2 H, H2′, H6′), 7.45-7.37 (m AA′, BB′, 2 H, H3′, H5′), 6.55 (dd, 1 H, *J* = 8.79 Hz, *J* = 2.19 Hz, H5), 6.49 (d, 1 H, *J* = 2.19 Hz, H3), 3.78 (s, 3 H, OCH_3) ^{13}C NMR (75 MHz, DMSO) δ 167.4, 164.1, 161.9, 137.4, 130.4, 128.8, 128.0, 122.9, 109.1, 106.6, 101.5, 55.7
4d	^{1}H NMR (300 MHz, DMSO) δ 12.20 (bs, 1 H, NH), 10.33 (bs, 1 H, OH), 7.95 (d, 1 H, *J* = 8.79 Hz, H6), 7.89 (t, 1 H, *J* = 1.92 Hz, H2′), 7.60 (ddd, 1 H, *J* = 8.24 Hz, *J* = 1.92 Hz, *J* = 0.82 Hz, H6′), 7.37 (t, 1 H, *J* = 8.24 Hz, H5′), 7.17 (ddd, 1 H, *J* = 8.24 Hz, *J* = 1.92 Hz, *J* = 0.82 Hz, H4′), 6.56 (dd, 1 H, *J* = 8.79 Hz, *J* = 2.47 Hz, H5), 6.50 (d, 1 H, *J* = 2.47 Hz, H3), 3.78 (s, 3 H, OCH_3) ^{13}C NMR (75 MHz, DMSO) δ 167.4, 164.1, 161.7, 139.9, 133.2, 130.6, 130.5, 123.9, 120.7, 119.6, 109.3, 106.7, 101.5, 55.7
4e	^{1}H NMR (300 MHz, DMSO) δ 12.09 (bs, 1 H, NH), 10.36 (bs, 1 H, OH), 8.07 (d, 1 H, *J* = 2.47 Hz, H2′), 7.92 (d, 1 H, *J* = 8.79 Hz, H6), 7.66 (dd, 1 H, *J* = 8.79 Hz, *J* = 2.47 Hz, H5′), 7.58 (d, 1 H, *J* = 8.79 Hz, H6′), 6.55 (dd, 1 H, *J* = 8.79 Hz, *J* = 2.47 Hz, H5), 6.49 (d, 1 H, *J* = 2.47 Hz, H3), 3.78 (s, 3 H, OCH_3) ^{13}C NMR (75 MHz, DMSO) δ 167.4, 164.2, 161.6, 138.6, 131.1, 130.8, 130.6, 125.7, 122.3, 121.1, 109.3, 106.7, 101.5, 55.7
4f	^{1}H NMR (300 MHz, DMSO) δ 12.27 (bs, 1 H, NH), 10.30 (bs, 1 H, OH), 7.95 (d, 1 H, *J* = 8.79 Hz, H6), 7.70–7.63 (m AA′, BB′, 2 H, H2′, H6′), 7.57–7.50 (m AA′, BB′, 2 H, H3′, H5′), 6.55 (dd, 1 H, *J* = 8.79 Hz, *J* = 2.47 Hz, H5), 6.49 (d, 1 H, *J* = 2.47 Hz, H3), 3.78 (s, 3 H, OCH_3) ^{13}C NMR (75 MHz, DMSO) δ 167.4, 164.1, 161.8, 137.8, 131.7, 130.4, 123.2, 116.1, 109.2, 106.6, 101.5, 55.7
4g	^{1}H NMR (300 MHz, DMSO) δ 12.41 (bs, 1 H, NH), 10.26 (bs, 1 H, OH), 7.96 (d, 1 H, *J* = 8.79 Hz, H6), 7.73–7.64 (m, 2 H, H2′, H6′), 7.25–7.14 (m, 2 H, H3′, H5′), 6.55 (dd, 1 H, *J* = 8.79 Hz, *J* = 2.47 Hz, H5), 6.49 (d, 1 H, *J* = 2.47 Hz, H3), 3.78 (s, 3 H, OCH_3) ^{13}C NMR (75 MHz, DMSO) δ 167.5, 164.1, 162.1, 160.5 and 157.3 (*J* = 240.8 Hz), 134.6 and 134.6 (*J* = 2.6 Hz), 130.2, 123.5 and 123.4 (*J* = 8.0 Hz), 115.7 and 115.4 (*J* = 22.3 Hz), 108.9, 106.6, 101.5, 55.7
4h	^{1}H NMR (300 MHz, DMSO) δ 12.20 (bs, 1 H, OH), 10.35 (bs, 1 H, NH), 7.96 (d, 1 H, *J* = 8.79 Hz, H6), 7.68 (dt (*J* = 11.54 Hz, *J* = 2.20 Hz, H6′), 7.50–7.33 (m, 2 H, H2′, H5′), 6.99–6.90 (m, 1 H, H4′), 6.56 (dd, 1 H, *J* = 8.79, *J* = 2.47 Hz, H5), 6.50 (d, 1 H, *J* = 2.47 Hz, H3), 3.79 (s, 3 H, OCH_3) ^{13}C NMR (75 MHz, DMSO) δ 167.3, 164.1, 163.9 and 160.7 (*J* = 241.4 Hz), 161.6, 140.3 and 140.1 (*J* = 11.2 Hz), 130.6 and 130.4 (*J* = 11.5 Hz), 116.9 and 116.9 (*J* = 2.6 Hz), 110.8 and 110.5 (*J* = 21.2 Hz), 109.4, 108.1 and 107.8 (*J* = 26.3 Hz), 106.6, 101.5, 55.7

Table 2. continued.

Compound	NMR, δ
4 i	^{1}H NMR (300 MHz, DMSO) δ 12.14 (bs, 1 H, OH), 10.48 (s, 1 H, NH), 8.01–7.88 (m, 3 H, H6, H3′, H5′), 7.78–7.64 (m AA′, BB′, 2 H, H2′, H6′), 6.57 (dd, 1 H, *J* = 9.06 Hz, *J* = 2.47 Hz, H6), 6.51 (d, 1 H, *J* = 2.47 Hz, H8), 3.79 (s, 3 H, OCH_3) ^{13}C NMR (75 MHz, DMSO) δ 167.4, 164.2, 161.5, 142.2, 130.8, 126.2 (q, *J* = 3.8 Hz), 124.6 (q, *J* = 271.4 Hz), 124.1 (q, *J* = 32.4 Hz), 120.9, 109.5, 106.7, 101.5, 55.7
4 j	^{1}H NMR (300 MHz, DMSO) δ 10.66 (bs, 1 H, NH), 8.27–8.20 (m AA′, BB′, 2 H, H3′, H5′), 8.01–7.91 (m, 3 H, H6, H2′, H6′), 6.60–6.55 (m, 1 H, H5), 6.53–6.50 (m, 1 H, H3), 3.79 (s, 3 H, OCH_3) ^{13}C NMR (75 MHz, DMSO) δ 167.2, 164.3, 161.2, 144.9, 142.7, 131.1, 125.0, 120.4, 109.8, 106.8, 101.5, 55.7
4 k	^{1}H NMR (300 MHz, DMSO) δ 12.10 (bs, 1 H, OH), 10.55 (bs, 1 H, NH), 8.71 (t, 1 H, *J* = 2.19 Hz, H2′), 8.07 (ddd, 1 H, *J* = 8.24 Hz, *J* = 2.20 Hz, *J* = 0.82 Hz, H6′), 7.99–7.92 (m, 2 H, H6, H4′), 7.63 (t, 1 H, *J* = 8.25 Hz, H5′), 6.57 (dd, 1 H, *J* = 8.79 Hz, *J* = 2.47 Hz, H5), 6.50 (d, 1 H, *J* = 2.47 Hz, H3), 3.79 (s, 3 H, OCH_3) ^{13}C NMR (75 MHz, DMSO) δ 167.6, 164.3, 161.7, 148.1, 139.7, 130.6, 130.3, 127.0, 118.6, 115.2, 109.2, 106.7, 101.5, 55.7
4 m	^{1}H NMR (300 MHz, DMSO) δ 12.63 (bs, 1 H, OH), 10.14 (bs, 1 H, NH), 7.97 (d, 1 H, *J* = 8.79 Hz, H6), 7.59–7.52 (m AA′, BB′, 2 H, H2′, H6′), 6.97–6.89 (m AA′, BB′, 2 H, H3′, H5′), 6.53 (dd, 1 H, *J* = 8.79 Hz, *J* = 2.47 Hz, H5), 6.47 (d, 1 H, *J* = 2.47 Hz, H3), 3.78 (s, 3 H, OCH_3), 3.74 (s, 3 H, OCH_3) ^{13}C NMR (75 MHz, DMSO) δ 167.6, 164.0, 162.4, 156.2, 131.1, 129.9, 123.4, 114.0, 108.8, 106.5, 101.5, 55.6, 55.4
4 n	^{1}H NMR (300 MHz, DMSO) δ 12.15 (bs, 1 H, OH), 10.46 (s, 1 H, NH), 7.98 (d, 1 H, *J* = 8.79 Hz, H5), 7.97–7.92 (m AA′, BB′, 2 H, H3′, H5′), 7.89–7.82 (m AA′, BB′, 2 H, H2′, H6′), 6.57 (dd, 1 H, *J* = 8.79 Hz, *J* = 2.47 Hz, H6), 6.51 (d, 1 H, *J* = 2.47 Hz, H8), 4.28 (q, 2 H, *J* = 7.10 Hz, CH2), 3.79 (s, 3 H, OCH_3), 1.30 (t, 3 H, *J* = 7.10 Hz, CH_3) ^{13}C NMR (75 MHz, DMSO) δ167.3, 165.5, 164.2, 161.4, 142.9, 130.8, 130.3, 125, 120.3, 109.6, 106.7, 101.5, 60.7, 55.7, 14.4
4 o	^{1}H NMR (300 MHz, DMSO) δ 12.01 (bs, 1 H, OH), 10.5 (s, 1 H, NH), 7.94 (d, 1 H, *J* = 9.07, H5), 7.93–7.87 (m AA′, BB′, 2 H, H3′, H5′), 7.84–7.76 (m AA′, BB′, 2 H, H2′, H6′), 6.57 (dd, 1 H, *J* = 9.07 Hz, *J* = 2.47 Hz, H6), 6.51 (d, 1 H, *J* = 2.47 Hz, H8), 3.79 (s, 3 H, OCH_3) ^{13}C NMR (75 MHz, DMSO) δ167.2, 164.2, 161.2, 142.9, 133.4, 131, 120.9, 119.3, 109.7, 106.8, 105.8, 101.5, 55.7
5 i	^{1}H NMR (300 MHz, DMSO) δ 11.58 (bs, 1 H, OH), 10.59 (s, 1 H, NH), 7.95 (d, 1 H, *J* = 2.47 Hz, H6), 7.92–7.85 (m AA′, BB′, 2 H, H3′, H5′), 7.71–7.65 (m AA′, BB′, 2 H, H2′, H6′), 7.53 (dd, 1 H, *J* = 8.79 Hz, *J* = 2.47 Hz, H4), 6.93 (d, 1 H, *J* = 8.79 Hz, H3) ^{13}C NMR (75 MHz, DMSO) δ 165.2, 156.9, 142.0, 136.1, 131.7, 126.3 (q, *J* = 3.7 Hz), 124.5 (q, *J* = 271.7 Hz), 124.3 (q, 32.1 Hz), 121.1, 120.7, 119.6, 110.4
5 n	^{1}H NMR (300 MHz, DMSO) δ 11.65 (bs, 1 H, OH), 10.59 (s, 1 H, NH), 8.00 (d, 1 H, *J* = 2.47 Hz, H6), 7.98–7.92 (m AA′, BB′, 2 H, H3′, H5′), 7.71–7.65 (m AA′, BB′, 2 H, H2′, H6′), 7.65 (dd, 1 H, *J* = 8.79 Hz, *J* = 2.48 Hz, H4), 6.96 (d, 1 H, *J* = 8.79 Hz, H3), 4.28 (q, 2 H, *J* = 7.14 Hz, CH2), 1.30 (t, 3 H, *J* = 7.14 Hz, CH_3) ^{13}C NMR (75 MHz, DMSO) δ 165.5, 165.1, 156.9, 142.8, 136.1, 131.7, 130.4, 125.2, 121.1, 120.0, 119.6, 110.5, 60.7, 14.4
5 o	^{1}H NMR (300 MHz, DMSO) δ 11.52 (bs, 1 H, OH), 10.66 (s, 1 H, NH), 7.94 (d, 1 H, *J* = 2.47 Hz, H6), 7.93–7.87 (m AA′, BB′, 2 H, H3′, H5′), 7.84–7.79 (m AA′, BB′, 2 H, H2′, H6′), 7.56 (dd, 1 H, *J* = 8.79 Hz, *J* = 2.48 Hz, H4), 6.96 (d, 1 H, *J* = 8.79 Hz, H3) ^{13}C NMR (75 MHz, DMSO) δ 165.1, 156.6, 142.8, 136.0, 133.4, 131.8, 121.4, 120.6, 119.6, 119.2, 110.4, 106.0

Table 2. continued.

Compound	NMR, δ
6i	^{1}H NMR (300 MHz, DMSO) δ 10.66 (bs, 1 H, NH), 7.97–7.89 (m AA′, BB′, 2 H, H3′, H5′), 7.88 (d, 1 H, *J* = 2.74 Hz, H6), 7.75–7.68 (m AA′, BB′, 2 H, H2′, H6′), 7.46 (dd, 1 H, *J* = 8.79 Hz, *J* = 2.74 Hz, H4), 7.02 (d, 1 H, *J* = 8.79 Hz, H3) ^{13}C NMR (75 MHz, DMSO) δ 165.3, 156.5, 142.1, 133.3, 128.8, 126.3, 126.2, 124.5 (q, *J* = 271.4 Hz), 124.3 (q, *J* = 32.1 Hz), 123.0, 120.6 (d, *J* = 6.9 Hz), 120.6
6n	^{1}H NMR (300 MHz, DMSO) δ 10.65 (bs, 1 H, NH), 7.98–7.92 (m AA′, BB′, 2 H, H3′, H5′), 7.88 (d overlapped, 1 H, *J* = 2.75 Hz, H6), 7.88–7.83 (m AA′, BB′ overlapped 2 H, H2′, H6′), 7.45 (dd, 1 H, *J* = 8.79 Hz, *J* = 2.74 Hz, H4), 8.05 (d, 1 H, *J* = 8.79 Hz, H3), 4.29 (q, 2 H, *J* = 7.14 Hz, CH_2), 1.31 (t, 3 H, *J* = 7.15 Hz, CH_3) ^{13}C NMR (75 MHz, DMSO) δ 165.5, 165.1, 156.5, 142.8, 133.2, 130.4, 128.9, 125.2, 123.0, 120.6, 120.0, 119.2, 60.7, 14.4
6o	^{1}H NMR (300 MHz, DMSO) δ 11.49 (bs, 1 H, OH), 10.67 (bs, 1 H, NH), 7.94–7.88 (m AA′, BB′, 2 H, H3′, H5′), 7.85–7.79 (m AA′, BB′overlapped, 2 H, H2′, H6′), 7.82 (d overlapped, 1 H, *J* = 2.47 Hz, H6), 7.46 (dd, 1 H, *J* = 8.79 Hz, *J* = 2.47 Hz, H4), 7.02 (d, 1 H, *J* = 8.79 Hz, H3) ^{13}C NMR (75 MHz, DMSO) δ 165.2, 156.3, 142.8, 133.5, 133.2, 128.9, 123.0, 120.9, 120.6, 119.2, 119.1, 106.0
7i	^{1}H NMR (300 MHz, DMSO) δ 11.41 (bs, 1 H, OH), 10.66 (bs, 1 H, NH), 7.98–7.89 (m AA′, BB′, 2 H, H3′, H5′), 7.75–7.70 (m AA′, BB′ overlapped, 2 H, H2′, H6′), 7.68 (dd overlapped, 1 H, *J* = 9.20 Hz, *J* = 3.02 Hz, H6), 7.36–7.26 (m, 1 H, H4), 7.01 (dd, 1 H, *J* = 9.20 Hz, *J* = 4.67 Hz, H3) ^{13}C NMR (75 MHz, DMSO) δ 165.3 (d, *J* = 2.3 Hz), 155.1 (d, *J* = 235.4 Hz), 154.0, 142.1, 126.3 (q, *J* = 4.0 Hz), 124.5 (q, *J* = 271.4 Hz), 124.3 (q, *J* = 32.3 Hz), 120.7 (d, *J* = 23.2 Hz), 120.6, 119.4 (d, *J* = 6.7 Hz), 118.7 (d, *J* = 7.7 Hz), 115.3 (d, *J* = 24.6 Hz)
7n	^{1}H NMR (300 MHz, DMSO) δ 11.43 (bs, 1 H, OH), 10.64 (bs, 1 H, NH), 8.00–7.91 (m AA′, BB′, 2 H, H3′, H5′), 7.90–7.82 (m AA′, BB′, 2 H, H2′, H6′), 7.69 (dd, 1 H, *J* = 9.28 Hz, *J* = 3.16 Hz, H6), 7.35–7.25 (m, 1 H, H4), 7.01 (dd, 1 H, *J* = 9.28 Hz, *J* = 4.53 Hz, H3), 4.29 (q, 2 H, *J* = 7.14 Hz, CH_2), 1.31 (t, 3 H, *J* = 7.15 Hz, CH_3) ^{13}C NMR (75 MHz, DMSO) δ 165.5, 165.2 (d, *J* = 2.3 Hz), 155.2 (d, *J* = 235.4 Hz), 154.0, 142.8, 130.5 (d, *J* = 12.9 Hz), 125.2, 120.7 (d, *J* = 23.5 Hz), 120.0, 119.5, 118.7 (d, *J* = 7.5 Hz), 115.3 (d, *J* = 24.3 Hz), 60.7, 14.4
7o	^{1}H NMR (300 MHz, DMSO) δ 11.32 (bs, 1 H, OH), 10.69 (bs, 1 H, NH), 7.95–7.88 (m AA′, BB′, 2 H, H3′, H5′), 7.85–7.79 (m AA′, BB′, 2 H, H2′, H6′), 7.64 (dd, 1 H, *J* = 9.61 Hz, *J* = 3.29 Hz, H6), 7.35–7.25 (m, 1 H, H4), 7.02 (dd, 1 H, *J* = 9.07 Hz, *J* = 4.67 Hz, H3) ^{13}C NMR (75 MHz, DMSO) δ 165.2 (d, *J* = 2.0 Hz), 155.2 (d, *J* = 235.4 Hz), 153.7 (d, *J* = 1.7 Hz), 142.7, 133.5, 120.7 (d, *J* = 23.2 Hz), 120.6, 119.8 (d, *J* = 6.9 Hz), 119.2, 118.7 (d, *J* = 7.7 Hz), 115.4 (d, *J* = 24.3 Hz), 106.0
8g	^{1}H NMR (300 MHz, DMSO) δ 11. 56 (bs, 1 H, OH), 10.39 (bs, 1 H, NH), 7.76 (d, 1 H, *J* = 1.65 Hz, H6), 7.74–7.67 (m, 2 H, H2′, H6′), 7.27–7.14 (m, 3 H, H4, H3′, H5′), 6.87 (d, 1 H, *J* = 8.52 Hz, H3), 2.27 (s, 3 H, CH_3) ^{13}C NMR (75 MHz, DMSO) δ 166.9, 158.8 (d, *J* = 240.8 Hz), 156.7, 134.7 (d, *J* = 2.6 Hz), 134.6, 129.0, 127.9, 123.1 (d, *J* = 8.1 Hz), 117.3, 117.0, 115.6 (d, *J* = 22.3 Hz), 20.3
8h	^{1}H NMR (300 MHz, DMSO) δ 11.37 (bs, 1 H, OH), 10.48 (bs, 1 H, NH), 7.77–7.68 (m, 2 H, H2′, H6′), 7.50–7.33 (m, 2 H, H6, H5′), 7.27–7.21 (m, 1 H, H4′), 6.99–6.91 (m, 1 H, H4), 6.88 (d, 1 H, *J* = 8.24 Hz, H3), 2.26 (s, 3 H, CH_3) ^{13}C NMR (75 MHz, DMSO) δ 166.7, 162.3 (d, *J* = 241.4 Hz), 156.1, 140.3 (d, *J* = 11.2 Hz), 134.6, 130.5 (d, *J* = 9.4 Hz), 129.3, 128.0, 117.6, 117.3, 116.6 (d, *J* = 2.9 Hz), 110.7, (d, *J* = 20.9), 107.7 (d, *J* = 26.3 Hz), 20.3

Table 2. continued.

Compound	NMR, δ
8 i	^{1}H NMR (300 MHz, DMSO) δ 11.32 (bs, 1 H, OH), 10.61 (s, 1 H, NH), 7.98 (m AA′, BB′, 2 H, H3′, H5′), 7.77–7.66 (m, 3 H, H6, H2′, H6′), 7.24 (dd, 1 H, *J* = 8.24 Hz, *J* = 1.65 Hz, H4), 6.89 (d, 1 H, *J* = 8.24 Hz, H3), 2.27 (s, 3 H, CH$_3$) ^{13}C NMR (75 MHz, DMSO) δ 166.8, 155.9, 142.3, 134.6, 129.5, 128.1, 126.2 (d, *J* = 3.7 Hz), 124.6 (q, 271.4 Hz), 124.1 (q, *J* = 32.4 Hz), 120.7, 117.8, 117.2, 20.2
8 n	^{1}H NMR (300 MHz, DMSO) δ 10.60 (d, 1 H, *J* = 5.22 Hz, NH), 8.01–7.91 (m AA′, BB′, 2 H, H3′, H5′), 7.91–7.81 (m AA′, BB′, 2 H, H2′, H6′), 7.73 (s, 1 H, H6), 7.24 (dd, 1 H, *J* = 8.24 Hz, *J* = 1.93 Hz, H4), 6.90 (d, 1 H, *J* = 8.24 Hz, H3), 4.28 (q, 2 H, *J* = 7.14 Hz, CH2), 2.26 (s, 3 H, CH$_3$), 1.30 (t, 3 H, *J* = 7.14 Hz, CH$_3$) ^{13}C NMR (75 MHz, DMSO) δ 167.0, 165.5, 156.0, 143.0, 134.7, 130.4, 129.5, 128.1, 125.1, 120.1, 117.8, 117.3, 60.8, 20.3, 14.5
8 o	^{1}H NMR (300 MHz, DMSO) δ 11.20 (bs, 1 H, OH), 10.64 (bs, 1 H, NH), 7.95–7.89 (m AA′, BB′, 2 H, H3′, H5′), 7.84–7.78 (m AA′, BB′, 2 H, H2′, H6′), 7.68 (d, 1 H, *J* = 2.20 Hz, H6), 7.24 (dd, 1 H, *J* = 8.38 Hz, *J* = 2.20 Hz, H7), 6.90 (d, 1 H, *J* = 8.38 Hz, H8), 2.26 (s, 3 H, CH$_3$) ^{13}C NMR (75 MHz, DMSO) δ 166.7, 155.6, 143.0, 134.6, 133.4, 129.6, 128.1, 120.6, 119.2, 118.2, 117.2, 105.8, 20.2
9 a	^{1}H NMR (300 MHz, DMSO) δ 11.36 (bs, 1 H, OH), 10.42 (bs, 1 H, NH), 7.73–7.65 (m, 2 H, H2′, H6′), 7.50 (d, 1 H, *J* = 3.02 Hz, H6), 7.41–7.33 (m, 2 H, H3′, H5′), 7.19–7.09 (m, 1 H, H4′), 7.06 (dd, 1 H, *J* = 8.93 Hz, *J* = 3.02 Hz, H4), 6.92 (d, 1 H, *J* = 8.93 Hz, H3), 3.75 (s, 3 H, OCH$_3$) ^{13}C NMR (75 MHz, DMSO) δ 166.2, 152.4, 152.0, 138.3, 129.0, 124.4, 121.2, 120.8, 118.4, 117.7, 112.8, 55.9
9 b	^{1}H NMR (300 MHz, DMSO) δ 11.44 (bs, 1 H, OH), 10.35 (bs, 1 H, NH), 7.59–7.53 (m AA′, BB′, 2 H, H2′, H6′), 7.50 (d, 1 H, *J* = 3.30 Hz, H6), 7.20–7.14 (m AA′, BB′, 2 H, H3′, H5′), 7.05 (dd, 1 H, *J* = 8.93 Hz, *J* = 3.30 Hz, H4), 6.91 (d, 1 H, *J* = 8.93 Hz, H3), 3.75 (s, 3 H, OCH$_3$), 2.28 (s, 3 H, CH$_3$) ^{13}C NMR (75 MHz, DMSO) δ 166.2, 152.7, 152.0, 135.7, 133.5, 129.4, 121.3, 120.8, 118.4, 117.5, 112.7, 55.9, 20.7
9 c	^{1}H NMR (300 MHz, DMSO) δ 11.21 (bs, 1 H, OH), 10.47 (bs, 1 H, NH), 7.77–7.69 (m AA′, BB′, 2 H, H2′, H6′), 7.45 (d, 1 H, *J* = 3.02 Hz, H6), 7.44–7.37 (m AA′, BB′, 2H, H3′, H5′), 7.06 (dd, 1 H, *J* = 8.79 Hz, *J* = 3.02 Hz, H4), 6.92 (d, 1 H, *J* = 8.80 Hz, H3), 3.75 (s, 3 H, OCH$_3$) ^{13}C NMR (75 MHz, DMSO) δ 166.2, 152.2, 152.1, 137.4, 128.9, 128.0, 122.6, 120.8, 118.3, 117.9, 112.9, 55.9
9 d	^{1}H NMR (300 MHz, DMSO) δ 11.14 (bs, 1 H, OH), 10.50 (bs, 1 H, NH), 7.92 (t, 1 H, *J* = 1.92 Hz, H2′), 7.62–7.57 (m, 1 H, H6′), 7.44 (d, 1 H, *J* = 3.02 Hz, H6), 7.39 (t, 1 H, *J* = 8.24 Hz, H5′), 7.21–7.16 (m, 1 H, H4′), 7.06 (dd, 1 H, *J* = 8.93 Hz, *J* = 3.02 Hz, H4), 6.93 (d, 1 H, *J* = 8.93 Hz, H3), 3.74 (s, 3 H, OCH$_3$) ^{13}C NMR (75 MHz, DMSO) δ 166.2, 152.1, 152.0, 139.9, 133.3, 130.6, 124.0, 120.8, 120.4, 119.3, 118.3, 118.1, 113.0, 55.9
9 e	^{1}H NMR (300 MHz, DMSO) δ 11.06 (bs, 1H, OH), 10.56 (bs, 1 H, NH), 8.11 (d, 1 H, *J* = 2.20 Hz, H2′), 7.66 (dd, 1 H, *J* = 8.79 Hz, *J* = 2.20 Hz, H6′), 7.60 (d, 1 H, *J* = 8.79 Hz, H5′), 7.41 (d, 1 H, *J* = 3.02 Hz, H6), 7.06 (dd, 1 H, *J* = 8.93 Hz, *J* = 3.02 Hz, H4), 6.93 (d, 1 H, *J* = 8.93 Hz, H3), 3.74 (s, 3 H, OCH$_3$) ^{13}C NMR (75 MHz, DMSO) δ 166.2, 152.1, 151.9, 138.6, 131.2, 130.8, 125.7, 122.1, 120.9, 120.8, 118.3, 118.2, 113.0, 55.9
9 f	^{1}H NMR (300 MHz, DMSO) δ 10.48 (bs, 1 H, NH), 7.72–7.63 (m AA′, BB′, 2 H, H2′, H6′), 7.59–7.50 (m AA′, BB′, 2 H, H3′, H5′), 7.44 (d, 1 H, *J* = 3.02 Hz, H6), 7.05 (dd, 1 H, *J* = 8.79 Hz, *J* = 3.02 Hz, H4), 6.92 (d, 1 H, *J* = 8.79 Hz, H3), 3.74 (s, 3 H, OCH$_3$) ^{13}C NMR (75 MHz, DMSO) δ 166.1, 152.2, 152.1, 137.8, 131.8, 123.0, 120.8, 118.4, 118.0, 116.1, 112.9, 55.9
9 g	^{1}H NMR (300 MHz, DMSO) δ 11.33 (bs, 1 H, OH), 10.43 (bs, 1 H, NH), 7.77 (m, 2 H, H2′, H6′), 7.48 (d, 1 H, *J* = 3.02 Hz, H6), 7.27–7.15 (m, 2 H, H3′, H5′), 7.06 (dd, 1 H, *J* = 8.93 Hz, *J* = 3.02 Hz, H4), 6.91 (d, 1 H, *J* = 3.02 Hz, H3), 3.75 (s, 3 H, OCH$_3$) ^{13}C NMR (75 MHz, DMSO) δ 166.3, 158.9 (d, *J* = 248.8 Hz), 152.5, 152.0, 134.7 (d, *J* = 2.6 Hz), 123.2 (d, *J* = 8.0 Hz), 120.8, 118.4, 117.5 115.6 (d, *J* = 22.3 Hz), 112.7, 55.9

Table 2. continued.

Compound	NMR, δ
9h	^{1}H NMR (300 MHz, DMSO) δ 10.56 (bs, 1 H, NH), 7.77–7.67 (m, 1 H, H6′), 7.50–7.32 (m, H6, H2′, H4′, H5′), 7.06 (dd, 1 H, *J* = 9.07 Hz, *J* = 3.02 Hz, H4), 6.99–6.90 (m, 1 H, H3), 3.74 (s, 3 H, OCH_3) ^{13}C NMR (75 MHz, DMSO) δ 166.1, 162.3 (d, *J* = 241.4 Hz), 152.1, 152.0, 140.2 (d, *J* = 10.9 Hz), 130.5 (d, *J* = 9.5 Hz), 120.7, 118.4, 118.2, 116.6 (d, *J* = 2.6 Hz), 113.0, 110.7 (d, *J* = 20.9 Hz), 107.7 (d, *J* = 26.0 Hz), 55.8
9i	^{1}H NMR (300 MHz, DMSO) δ 11.12 (bs, 1 H, OH), 10.67 (bs, 1 H, NH), 7.97–7.90 (m AA′, BB′, 2 H, H3′, H5′), 7.77–7.68 (m AA′, BB′, 2 H, H2′, H6′), 7.45 (d, 1 H, *J* = 3.02 Hz, H6), 7.07 (dd, 1 H, *J* = 9.07 Hz, *J* = 3.02 Hz, H4), 6.94 (d, 1 H, *J* = 9.07 Hz, H3), 3.75 (s, 3 H, OCH_3) ^{13}C NMR (75 MHz, DMSO) δ 166.2, 159.1, 151.9, 142.2, 126.2 (q, *J* = 3.7 Hz), 124.6 (q, *J* = 271.4 Hz), 124.1 (q, *J* = 32.1 Hz), 120.8, 120.7, 118.4, 113.2, 55.8
9j	^{1}H NMR (300 MHz, DMSO) δ 10.99 (bs, 1 H, OH), 10.84 (bs, 1 H, NH), 8.30–8.20 (m, AA′, BB′, 2 H, H3′, H5′), 8.03–7.93 (m AA′, BB′, 2 H, H2′, H6′), 7.39 (d, 1 H, *J* = 3.02 Hz, H6), 7.07 (dd, 1 H, *J* = 9.06 Hz, *J* = 3.02 Hz, H4), 6.95 (d, 1 H, *J* = 9.07 Hz, H3), 3.74 (s, 3 H, OCH_3) ^{13}C NMR (75 MHz, DMSO) δ 166.1, 152.2, 151.4, 144.9, 142.8, 125.1, 120.8, 120.3, 118.9, 118.3, 113.3, 55.8
9k	^{1}H NMR (300 MHz, DMSO) δ 11.06 (bs, 1 H, OH), 10.72 (bs, 1 H, NH), 8.76 (t, 1 H, *J* = 1.92 Hz, H2′), 8.09–8.02 (m, 2 H, H4′, H6′), 7.65 (t, 1 H, *J* = 8.24 Hz, H5′), 7.44 (d, 1 H, *J* = 3.02 Hz, H6), 7.07 (dd, 1 H, *J* = 8.79 Hz, *J* = 3.02 Hz, H4), 6.94 (d, 1 H, *J* = 8.79 Hz, H3), 3.75 (s, 3 H, OCH_3) ^{13}C NMR (75 MHz, DMSO) δ 166.5, 152.1, 152.0, 148.1, 139.7, 130.3, 126.9, 120.9, 118.7, 118.4, 118.1, 114.9, 113.0, 55.9
9m	^{1}H NMR (300 MHz, DMSO) δ 11.54 (bs, 1 H, OH), 10.32 (bs, 1 H, NH), 7.61–7.54 (m AA′, BB′, 2 H, H2′, H6′), 7.51 (d, 1 H, *J* = 3.02 Hz, H6), 7.05 (dd, 1 H, *J* = 8.79 Hz, *J* = 3.02 Hz, H4), 6.98–6.92 (m AA′, BB′, 2 H, H3′, H5′), 6.90 (d, 1 H, *J* = 8.79 Hz, H3), 3.75 (s, 3 H, OCH_3), 3.74 (s, 3 H, OCH_3) ^{13}C NMR (75 MHz, DMSO) δ 166.4, 156.3, 153.0, 151.9, 131.1, 123.1, 120.8, 118.4, 117.1, 114.1, 112.4, 55.9, 55.4
9n	^{1}H NMR (300 MHz, DMSO) δ 11.13 (bs, 1 H, OH), 10.65 (bs, 1 H, NH), 7.99–7.93 (m AA′, BB′, 2 H, H3′, H5′), 7.89–7.83 (m AA′, BB′, 2 H, H2′, H6′), 7.45 (d, 1 H, *J* = 3.16 Hz, H6), 7.06 (dd, 1 H, *J* = 9.06 Hz, *J* = 3.16 Hz, H4), 6.94 (d, 1 H, *J* = 9.07 Hz, H3), 4.29 (q, 2 H, *J* = 7.14 Hz, CH_2), 3.75 (s, 3 H, OCH_3), 1.31 (t, 3 H, *J* = 7.14 Hz, CH_3) ^{13}C NMR (75 MHz, DMSO) δ 166.1, 165.5, 152.2, 151.8, 142.9, 130.4, 125.1, 120.8, 120.1, 118.4, 118.3, 113.2, 60.7, 55.8, 14.4
9o	^{1}H NMR (300 MHz, DMSO) δ 11.01 (bs, 1 H, OH), 10.69 (bs, 1 H, NH), 7.96–7.88 (m AA′, BB′, 2 H, H3′, H5′), 7.86–7.77 (m AA′, BB′, 2 H, H2′, H6′), 7.40 (d, 1 H, *J* = 3.02 Hz, H6), 7.06 (dd, 1 H, *J* = 8.92 Hz, *J* = 3.02 Hz, H4), 6.94 (d, 1 H, *J* = 8.92 Hz, H3), 3.74 (s, 3 H, OCH_3) ^{13}C NMR (75 MHz, DMSO) δ 166.1, 152.2, 151.5, 142.9, 133.5, 120.8, 120.7, 119.2, 118.7, 118.3, 113.3, 105.9, 55.8

Table 3. Minimum inhibitory concentrations of salicylanilides substituted in position 4 and 5.

Compounds	R_1	R_2	MIC (μmol/L) Incubation 14 d/21 d *M. tuberculosis*	 *M. kansasii*	 *M. avium*
1a[c]	H	H	62.5/62.5	125/250	62.5/125
1b[c]	H	4-CH_3	62.5/62.5	62.5/125	31/62.5
1c[c]	H	4-Cl	31/31	31/31	31/31
1d[c]	H	3-Cl	16/16	8/8	31/31
1e[c]	H	3,4-Cl_2	8/8	4/8	16/31

Table 3. continued.

Compounds	R_1	R_2	MIC (µmol/L) Incubation 14 d/21 d *M. tuberculosis*	*M. kansasii*	*M. avium*
1 f[c]	H	4-Br	16/31	16/31	31/31
1 g[c]	H	4-F	62.5/62.5	62.5/125	31/62.5
1 h[c]	H	3-F	31/31	62.5/62.5	62.5/62.5
1 i[d]	H	4-CF_3	8/8	16/16	16/32
1 j[c]	H	4-NO_2	8/16	16/16	31/31
1 k[c]	H	3-NO_2	16/16	62.5/a	31/a
1 l[c]	H	4-$N(CH_3)_2$	250/250	250/a	125/250
1 m[c]	H	4-OCH_3	62.5/62.5	250/250	62.5/125
1 n[d]	H	4-COOEt	16/32	32/62.5	32/32
1 o[d]	H	4-CN	32/32	62.5/125	32/62.5
1 p[d]	H	4-$CSNH_2$	32/32	32/62.5	62.5/62.5
2 a	4-Cl	H	8/16	8/16	16/16
2 b	4-Cl	4-CH_3	16/31	8/16	16/16
2 c	4-Cl	4-Cl	4/4	4/8	8/8
2 d	4-Cl	3-Cl	4/4	4/8	16/16
2 e	4-Cl	3,4-Cl_2	4/4	8/8	16/16
2 f	4-Cl	4-Br	4/4	4/4	16/16
2 g	4-Cl	4-F	16/16	16/32	32/32
2 h	4-Cl	3-F	8/16	32/32	32/32
2 i	4-Cl	4-CF_3	4/4	4/4	8/8
2 k	4-Cl	3-NO_2	8/8	16/16	16/16
2 l	4-Cl	4-$N(CH_3)_2$	a/a	a/a	16/16
2 m	4-Cl	4-OCH_3	8/16	16/31	16/16
2 n	4-Cl	4-COOEt	8/8	8/8	16/16
2 o	4-Cl	4-CN	8/8	16/16	16/32
3 a	4-CH_3	H	31/62	a/a	a/a
3 b	4-CH_3	4-CH_3	250/a	a/a	a/a
3 c	4-CH_3	4-Cl	62/a	a/a	a/a
3 d	4-CH_3	3-Cl	a/a	a/a	16/a
3 e	4-CH_3	3,4-Cl_2	4/8	8/16	8/8
3 f	4-CH_3	4-Br	a/a	a/a	a/a
3 g	4-CH_3	4-F	16/a	a/a	a/a
3 h	4-CH_3	3-F	8/16	62.5/62.5	32/62.5
3 i	4-CH_3	4-CF_3	16/a	a/a	a/a
3 k	4-CH_3	3-NO_2	4/8	31/31	31/62
3 l	4-CH_3	4-$N(CH_3)_2$	31/62	a/a	a/a
3 m	4-CH_3	4-OCH_3	16/31	a/a	62/a
3 n	4-CH_3	4-COOEt	8/8	a/a	a/a
3 o	4-CH_3	4-CN	16/16	16/32	16/32
4 a	4-OCH_3	H	16/16	a/a	62.5/125
4 b	4-OCH_3	4-CH_3	16/32	a/a	62.5/62,5
4 c	4-OCH_3	4-Cl	16/32	a/a	32/a
4 d	4-OCH_3	3-Cl	4/4	62.5/62.5	32/32
4 e	4-OCH_3	3,4-Cl_2	8/16	a/a	32/32
4 f	4-OCH_3	4-Br	32/32	a/a	a/a
4 g	4-OCH_3	4-F	16/16	a/a	32/a
4 h	4-OCH_3	3-F	8/8	62.5/a	a/a
4 i	4-OCH_3	4-CF_3	a/a	a/a	a/a

Table 3. continued.

Compounds	R_1	R_2	MIC (µmol/L) Incubation 14 d/21 d *M. tuberculosis*	*M. kansasii*	*M. avium*
4 j	4-OCH_3	4-NO_2	8/8	32/32	16/16
4 k	4-OCH_3	3-NO_2	4/4	62.5/62.5	32/32
4 m	4-OCH_3	4-OCH_3	8/8	a/a	a/a
4 n	4-OCH_3	4-COOEt	4/8	a/a	a/a
4 o	4-OCH_3	4-CN	16/16	a/a	a/a
5 a[c]	5-Br	H	16/31	31/31	31/62.5
5 b[c]	5-Br	4-CH_3	16/16	16/a	a/a
5 c[c]	5-Br	4-Cl	4/4	8/16	16/16
5 d[c]	5-Br	3-Cl	4/8	16/16	31/31
5 e[c]	5-Br	3,4-Cl_2	4/4	8/8	8/8
5 f[c]	5-Br	4-Br	4/4	8/8	16/16
5 g[c]	5-Br	4-F	8/8	8/8	31/31
5 h[c]	5-Br	3-F	8/8	8/16	31/31
5 i	5-Br	4-CF_3	1/1	2/4	1/1
5 k[c]	5-Br	3-NO_2	8/8	16/16	31/31
5 l[c]	5-Br	4-$N(CH_3)_2$	a/a	a/a	a/a
5 m[c]	5-Br	4-OCH_3	a/a	a/a	a/a
5 n	5-Br	4-COOEt	4/4	8/8	a/a
5 o	5-Br	4-CN	2/4	8/8	8/8
6 a[c]	5-Cl	H	31/31	4/4	16/31
6 b[c]	5-Cl	4-CH_3	16/31	4/4	16/16
6 c[c]	5-Cl	4-Cl	4/4	8/8	8/8
6 d[c]	5-Cl	3-Cl	4/8	4/8	8/16
6 e[c]	5-Cl	3,4-Cl_2	4/8	4/4	16/16
6 f[c]	5-Cl	4-Br	8/16	4/4	8/8
6 g[c]	5-Cl	4-F	16/16	4/4	16/16
6 h[c]	5-Cl	3-F	8/8	8/8	31/31
6 i	5-Cl	4-CF_3	2/2	1/1	8/8
6 j[c]	5-Cl	4-NO_2	4/8	4/4	8/8
6 k[c]	5-Cl	3-NO_2	8/8	8/8	16/16
6 l[c]	5-Cl	4-$N(CH_3)_2$	a/a	8/8	31/62.5
6 m[c]	5-Cl	4-OCH_3	a/a	4/8	31/31
6 n	5-Cl	4-COOEt	8/8	16/32	32/32
6 o	5-Cl	4-CN	4/8	16/16	32/32
7 a[c]	5-F	H	31/62.5	31/62.5	31/62.5
7 b[c]	5-F	4-CH_3	31/62.5	31/31	16/31
7 c[c]	5-F	4-Cl	8/8	8/8	16/16
7 d[c]	5-F	3-Cl	8/16	a/a	16/16
7 e[c]	5-F	3,4-Cl_2	4/4	4/4	8/8
7 f[c]	5-F	4-Br	4/8	4/8	8/16
7 g[c]	5-F	4-F	16/16	8/8	16/16
7 h[c]	5-F	3-F	31/31	16/16	31/31
7 i	5-F	4-CF_3	4/4	8/8	16/16
7 k[c]	5-F	3-NO_2	8/16	16/31	16/31
7 l[c]	5-F	4-$N(CH_3)_2$	31/a	a/a	a/a
7 m[c]	5-F	4-OCH_3	62.5/62.5	62.5/62.5	16/16
7 n	5-F	4-COOEt	8/16	32/32	62.5/a
7 o	5-F	4-CN	8/16	16/32	32/32

Table 3. continued.

Compounds	R_1	R_2	MIC (µmol/L) Incubation 14 d/21 d *M. tuberculosis*	*M. kansasii*	*M. avium*
8 a	5-CH_3	H	62.5/62.5	62.5/125	62.5/62.5
8 b	5-CH_3	4-CH_3	32/62.5	250/a	32/32
8 c	5-CH_3	4-Cl	8/16	32/a	32/62.5
8 d	5-CH_3	3-Cl	16/32	16/16	32/a
8 e	5-CH_3	3,4-Cl	28/16	8/16	16/a
8 f	5-CH_3	4-Br	16/32	32/a	32/32
8 g	5-CH_3	4-F	b/b	a/a	b/b
8 h	5-CH_3	3-F	b/b	a/a	b/b
8 i	5-CH_3	4-CF_3	4/8	8/16	8/16
8 j	5-CH_3	4-NO_2	16/32	8/8	16/a
8 k	5-CH_3	3-NO_2	32/32	a/a	62.5/a
8 l	5-CH_3	4-$N(CH_3)_2$	a/a	a/a	a/a
8 m	5-CH_3	4-OCH_3	125/a	a/a	62.5/a
8 n	5-CH_3	4-COOEt	>1000/>1000	>1000/>1000	>1000/>1000
8 o	5-CH_3	4-CN	500/1000	1000/1000	1000/1000
9 a	5-OCH_3	H	a/a	a/a	125/125
9 b	5-OCH_3	4-CH_3	a/a	a/a	a/a
9 c	5-OCH_3	4-Cl	62.5/a	a/a	a/a
9 d	5-OCH_3	3-Cl	62.5/62.5	a/a	a/a
9 e	5-OCH_3	3,4-Cl_2	62.5/62.5	a/a	a/a
9 f	5-OCH_3	4-Br	a/a	a/a	a/a
9 g	5-OCH_3	4-F	a/a	a/a	62.5/a
9 h	5-OCH_3	3-F	125/125	125/a	125/a
9 i	5-OCH_3	4-CF_3	16/a	a/a	a/a
9 j	5-OCH_3	4-NO_2	a/a	a/a	a/a
9 k	5-OCH_3	3-NO_2	a/a	a/a	a/a
9 m	5-OCH_3	4-OCH_3	a/a	a/a	a/a
9 n	5-OCH_3	4-COOEt	a/a	a/a	a/a
9 o	5-OCH_3	4-CN	a/a	a/a	a/a
10 a[c]	5-NO_2	H	16/31	8/16	250/250
10 b[c]	5-NO_2	4-CH_3	16/16	8/16	125/125
10 c[c]	5-NO_2	4-Cl	16/16	8/8	62.5/62.5
10 d[c]	5-NO_2	3-Cl	16/16	8/16	62.5/62.5
10 e[c]	5-NO_2	3,4-Cl_2	8/8	8/8	31/62.5
10 f[c]	5-NO_2	4-Br	16/16	8/8	31/62.5
10 g[c]	5-NO_2	4-F	16/31	8/8	125/125
10 h[c]	5-NO_2	3-F	31/31	16/16	62.5/125
10 k[c]	5-NO_2	3-NO_2	31/31	8/16	125/125
10 l[c]	5-NO_2	4-$N(CH_3)_2$	31/31	8/16	500/1000
10 m[c]	5-NO_2	4-OCH_3	31/31	8/16	250/250
	INH		4/4	500/500	500/500

[a] MIC could not be determined due to a low solubility.
[b] not tested against the given strain.
[c] data from ref. [7].
[d] data from ref. [6].

Table 4. Regression coefficients of the indicator parameters I_n in equations (1)–(3).

Equation		*M. tubeculosis.* 21 d	*M. kansasii* 21 d	*M. avium* 21 d
(1)–(3)	I(5-Br)	–0.611 (±0.106)	–0.568 (±0.121)	–0.226 (±0.120)
(1)–(3)	I(5-Cl)	–0.435 (±0.104)	–0.895 (±0.111)	–0.396 (±0.108)
(1)–(3)	I(5-F)	–0.224 (±0.109)	–0.474 (±0.118)	–0.261 (±0.113)
(1)–(3)	I(5-NO_2)	–0.209 (±0.109)	–0.619 (±0.122)	0.478 (±0.17)
(1)–(3)	I(5-CH_3)	0.212 (±0.113)	–0.019 (±0.147)	0.221 (±0.142)
(1)–(3)	I(5-OCH_3)	0.607 (±0.177)	[a] [a]	0.296 (±0.305)
(1)–(3)	I(4-Cl)	–0.545 (±0.104)	–0.578 (±0.109)	–0.390 (±0.106)
(1)–(3)	I(4-CH_3)	–0.241 (±0.120)	–0.160 (±0.172)	–0.028 (±0.167)
(1)–(3)	I(4-OCH_3)	–0.373 (±0.109)	0.019 (±0.193)	0.048 (±0.142)

[a] Evaluation hampered by the low solubility of the compounds.

anilides includes the results obtained after 21 days of incubation, as those obtained after 14 days of incubation were similar. Equations (1)–(3) are given without regression coefficients of the indicator parameters; these are summarised in Table 4. The numbers of compounds in the equations analysing structure-activity relationships against various strains are different, as in a number of cases the minimum inhibitory concentration could not be determined due to the limited solubility of the compounds.

$$\log \mathrm{MIC}_{M..tuber.'21d} = -0.498\,(\pm 0.078)\,\sigma - 0.249\,(\pm 0.050)\,\pi + I_n + 1.713\,(\pm 0.074) \quad (1)$$

$r = 0.811$ $s = 0.278$ $n = 110$ $F = 17.1$

$$\log \mathrm{MIC}_{M..kans.21d} = -0.205\,(\pm 0.100)\,\sigma - 0.336\,(\pm 0.058)\,\pi + I_n + 1.890\,(\pm 0.087) \quad (2)$$

$r = 0.797$ $s = 0.299$ $n = 89$ $F = 13.6$

$$\log \mathrm{MIC}_{M..avium\ 21d} = -0.321\,(\pm 0.084)\,\sigma - 0.262\,(\pm 0.056)\,\pi + I_n + 1.801\,(\pm 0.078) \quad (3)$$

$r = 0.765$ $s = 0.295$ $n = 94$ $F = 10.5$

Upon using the indicator parameters, the biological activity can be predicted, but only within the framework of the selection of substituents employed. Thus, we attempted to find correlations with physical and chemical parameters. A classical Hansch-type equation, using the second power of the hydrophobic parameters, was not statistically significant. Salicylanilides possess the hydroxy and amide functional groups. The influence of the substituents in the acyl part of the molecule was thus expressed with the corresponding Hammett constant values with regard to both centers, and their hydrophobic constants π^- (with regard to the hydroxy group) and molecular refraction (MR) values were used as additional parameters. The results are summarised in equations (4)–(6).

$$\log \mathrm{MIC}_{M..tuber.\ 21d} = 0.577\,(\pm 0.182)\,(\sigma_1)_{CO} - 0.777\,(\pm 0.194)\,(\sigma_1)_{OH} - 0.578\,(\pm 0.098)\,\pi_1^- - 0.494\,(\pm 0.087)\,\sigma_2 - 0.246\,(\pm 0.056)\,\pi_2 + 0.025\,(\pm 0.014)\mathrm{RM} + 1.658\,(\pm 0.073) \quad (4)$$

$r = 0.739$ $s = 0.312$ $n = 110$ $F = 20.6$

$$\log \mathrm{MIC}_{M..kans.\ 21d} = -0.680\ (\pm 0.256)\ (\sigma_1)_{CO} - 0.047\ (\pm 0.275)\ (\sigma_1)_{OH} - 0.539\ (\pm 0.136)\ \pi^{-}_{1-} - 0.202\ (\pm 0.105)\ \sigma_2 - 0.321\ (\pm 0.061)\ \pi_2 + 0.029\ (\pm 0.022)\mathrm{RM} + 1.793\ (\pm 0.084) \quad (5)$$

$r = 0.752$ $s = 0.318$ $n = 89$ $F = 17.8$

$$\log \mathrm{MIC}_{M..avium\ 21d} = 0.332\ (\pm 0.222)\ (\sigma_1)_{CO} + 0.097\ (\pm 0.238)\ (\sigma_1)_{OH} - 0.820\ (\pm 0.115)\ \pi^{-}_{1-} - 0.331\ (\pm 0.090)\ \sigma_2 - 0.254\ (\pm 0.060)\ \pi_2 + 0.074\ (\pm 0.018)\mathrm{RM} + 1.693\ (\pm 0.078) \quad (6)$$

$r = 0.700$ $s = 0.318$ $n = 94$ $F = 13.9$

The results show that the influence of the substituents on the phenyl ring in the amide part of the molecule is apparent: antitubercular activity rises with increasing values of the Hammett constants σ and the hydrophobic substituent constants π. The influence of the substituents from the acyl moiety is more complex. The correlations using the indicator parameters (1–3) are more significant than those including the substituent constants (4–6). In determining the correlation equations for the parameters of the acyl part of the molecule, we found that more significant results can be obtained upon using the hydrophobic substituent constants π^- (with regard to the hydroxy group) than the substituent constants π. The differences in the regression coefficients of the σ_m and σ_p parameters in correlations against various strains of mycobacteria are surprising. From the statistical point of view, all equations can be considered proven on the level of significance of 0.5. It has been shown [11] that upon using the twofold serial dilution scale in antimicrobial tests, the standard residual deviation up to the value of 0.3 can be explained by the methodical error in measurement. For the acyl part of the molecule, the conclusion can be drawn that the antimycobacterial activity increases with increasing lipophilicity (parameter π^-) and decreasing molecular refraction (parameter MR). The influence of the electronic parameters in the acyl part of the molecule cannot be evaluated in a simple way (parameters σ_m and σ_p). As regards the amide moiety, the activity is always favourably influenced by higher lipophilicity (parameter π) and electron-accepting properties of the substituents (parameter σ). In conclusion, even though salicylanilides are relatively simple compounds, they have a broad spectrum of antimycobacterial effect, and their structure is different from those of standard antituberculotics. Moreover, salicylanilides are superior to INH as regards their activity against conditionally pathogenic strains of mycobacteria. The mechanism of their biological activity is currently being studied at the Hans Knöll Institute at the University of Jena.

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Experimental

Melting points were determined on a Kofler block and are uncorrected. The IR spectra were measured in KBr pellets or in $CHCl_3$ solutions on a Nicolet Impact 400 apparatus; the wavenumbers are given in cm^{-1}. The NMR spectra were recorded on a Varian Mercury-Vx BB 300 spectrometer operating at 300 MHz for ^1H and 75 MHz for ^{13}C in d_6-DMSO. Chemical shifts were recorded as δ values in ppm, and were indirectly referenced to tetramethylsilane via the solvent signal (7.26 for ^1H and 77.0 for ^{13}C). The coupling constants *J* are given in Hz. Elemental analyses were done on a CHNS-O CE elemental analyser (FISONS EA1110, Milano). Analyses of the C, H, N, S content were within ±0.4 % of the theoretical values. TLC was performed on silica gel plates precoated with a fluorescent indicator, Silufol UV 254 + 366 (Kavalier, Votice, the Czech Republic), in cyclohexane/acetone 3:1, to check the purity of the products.

General procedure for the preparation of salicylanilides

A suspension of a substituted salicylic acid (0.02 mol) and a substituted aniline (0.02 mol) in chlorobenzene (100 mL) was heated under reflux in the presence of PCl_3 (0.01 mol) for three hours. The reaction mixture was filtered while hot, and the solvents evaporated. The product was crystallized from ethanol-water (yields in the range 75–90 %, Table 1).

Microbiological assay

For the evaluation of *in vitro* antimycobacterial activity of the substances, the following strains were used: *M. tuberculosis* CNCTC My 331/88, *M. kansasii* CNCTC My 235/80, *M. avium* CNCTC My 330/88, obtained from the Czech National Collection of Type Cultures (CNCTC), National Institute of Public

Health, Prague. Antimycobacterial activity of the compounds against these strains was determined in the Šula semisynthetic medium (SEVAC, Prague).

The compounds were added to the medium in dimethyl sulfoxide solutions. The following concentrations were used: 1000, 500, 250, 125, 62, 31, 16, 8, and 4 μmol/L. The minimum inhibitory concentrations were determined after incubation at 37 °C for 14 days and 21 days (see Table 3). MIC was the lowest concentration of an antimycobacterially active substance (see the above concentrations), at which the inhibition of the growth of mycobacteria occurred.

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