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Antimycobacterial and Antifungal Isosters of Salicylamides

A set of 40 derivatives of 3-hydroxypicolinic acid and 2-sulfanylbenzoic acid, isosteric to salicylanilides was synthesized. The compounds were evaluated for *in vitro* activity against *Mycobacterium tuberculosis, Mycobacterium kansasii* and *Mycobacterium avium*, *Candida albicans, Candida tropicalis, Candida krusei, Candida glabrata, Trichosporon beigelii, Aspergillus fumigatus, Absidia corymbifera, Trichophyton mentagrophytes* and *Microsporum gypseum*. Structure-activity relationships of antimycobacterial activity and antifungal activity against *T. mentagrophytes* and *M. gypseum* were analyzed by the Free-Wilson method. An increase in antimycobacterial activity was observed only for the sulfanylbenzoic acid derivatives, especially those with the benzyl moiety. The antifungal activity was not significant.

Keywords: Salicylanilides; Antimycobacterial activity; Fungicidal activity; Structure-activity relationships; Tuberculostatics; Atypical mycobacterial strains

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Introduction

The search for new antimycobacterially active compounds is undoubtedly one of the important directions of current pharmaceutical chemistry. Based on collaboration with H. D. Stachel, we discovered a few new groups of potential antituberculotics [1, 2]. During the research on the antimycobacterial activity of salicylanilides [3], we identified these compounds as broad spectrum antimycobacterial substances, active not only against *Mycobacterium tuberculosis,* but also against other, conditionally pathogenic strains. Salicylanilides are unlikely to become effective chemotherapeutic agents themselves due to their effect on mitochondrial oxidation. However, they can serve as structural templates for the design of potential drugs, as Macielag with coworkers have recently found that they can act as inhibitors of two-component regulatory systems in bacteria [4]. The goal of this work was to study selected isosteric modifications of salicylanilides, in particular the anilides of 3-hydroxypicolinic and 2-sulfanylbenzoic acid, and some other isosteric structures as well (the derivatives derived from N-benzylamines). Based on our preliminary studies, we assumed that the compounds isosteric to salicylanilides would also display antifungal activity [5, 6].

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Chemistry

All compounds are summarized in Scheme 1. Salicylanilides **1** were described in our previous paper [3]. Derivatives **2–5** were prepared by the treatment of 3-hydroxypicolinic acid or 2-sulfanylbenzoic acid with substituted anilines or benzylamines. The characteristics of the compounds are presented in Table 1.

Microbiology

Antimycobacterial activity of compounds **2**–**5** was tested *in vitro* against *Mycobacterium tuberculosis*, *Mycobacterium kansasii*, and *Mycobacterium avium*. In several cases, the MIC values could not be determined due to the limited solubility of the compounds. The antimycobacterial activity of compounds **1** has been published recently [3].

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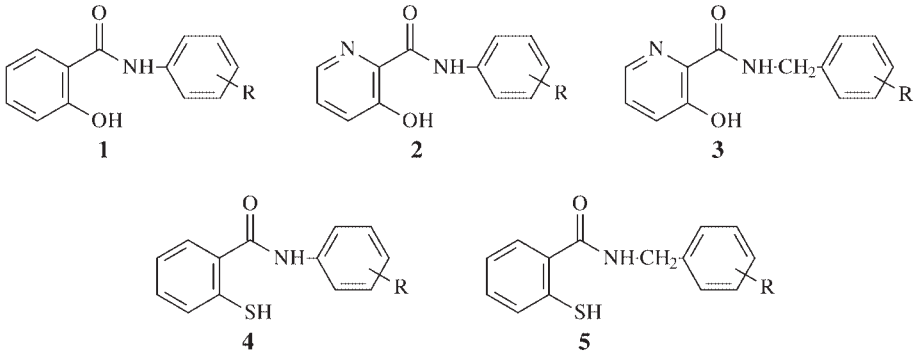

		R		R		
1	**a**	H	**f**	4-Br	**k**	3-NO_2
2	**b**	4-CH_3	**g**	4-F	**l**	4-OCH_3
3	**c**	4-Cl	**h**	3-F	**m**	$N(CH_3)_2$
4	**d**	3-Cl	**i**	4-CF_3	**n**	COOEt
5	**e**	3,4-Cl_2	**j**	4-NO_2	**o**	CN

Scheme 1

Table 1. Characteristics of the compounds.

Compound	R	Formula M_r	ν C=O (cm^{-1})	M.p. (°C) Found	Ref.
2 a	H	$C_{12}H_{10}N_2O_2$ 214.22	1658	85–86.5	88–89 [8, 9]
2 b	4-CH_3	$C_{13}H_{12}N_2O_2$ 228.25	1655	90.5–91.5	89 [8, 9]
2 c	4-Cl	$C_{12}H_9ClN_2O_2$ 248.66	1656	135–136	–
2 d	3-Cl	$C_{12}H_9ClN_2O_2$ 248.66	1670	131–132	131 [8, 9]
2 e	3,4-Cl_2	$C_{12}H_8Cl_2N_2O_2$ 283.11	1664	189–190	180 [8] 189 [9]
2 f	4-Br	$C_{12}H_9BrN_2O_2$ 293.11	1658	156	150 [8, 9]
2 g	4-F	$C_{12}H_9FN_2O_2$ 232.21	1656	135–137	–
2 i	4-CF_3	$C_{13}H_9F_3N_2O_2$ 282.22	1650	64–65	–
2 j	4-NO_2	$C_{12}H_9N_3O_4$ 259.22	1662	269–271	252–253 [8, 9]
2 k	3-NO_2	$C_{12}H_9N_3O_4$ 259.22	1668	216–218	–
2 l	4-OCH_3	$C_{13}H_{12}N_2O_3$ 244.24	1655	108–110	108 [8, 9]
3°	H	$C_{13}H_{12}N_2O_2$ 228.25	1647	88–89	84 [8, 9]
3 b	4-CH_3	$C_{14}H_{14}N_2O_2$ 242.27	1652	85–86	80–81 [8, 9]
3 c	4–Cl	$C_{13}H_{11}ClN_2O_2$ 262.69	1650	95–96	99–100 [8, 9]

Table 1. (continued).

Compound	R	Formula M_r	ν C=O (cm^{-1})	M.p. (°C) Found	Ref.
3 d	3-Cl	$C_{13}H_{11}ClN_2O_2$ 262.69	1651	60.5–61	–
3 e	3,4-Cl_2	$C_{13}H_{10}Cl_2N_2O_2$ 297.14	1648	98	98 [8, 9]
3 f	4–Br	$C_{13}H_{11}BrN_2O_2$ 307.14	1648	103–104	–
3 g	4-F	$C_{13}H_{11}FN_2O_2$ 246.24	1649	69–71	–
3 i	4-CF_3	$C_{14}H_{11}F_3N_2O_2$ 296.24	1652	76–78	–
3 j	4-NO_2	$C_{13}H_{11}N_3O_4$ 273.24	1649	116–117	128–129 [8, 9]
3 k	3-NO_2	$C_{13}H_{11}N_3O_4$ 273.24	1648	106–108	117–123 [8, 9]
3 l	4-OCH_3	$C_{14}H_{14}N_2O_3$ 258.27	1646	86–87	94 [8, 9]
4°	H	$C_{13}H_{11}NOS$ 229.29	1641	102–104	109–110 [10]
4 b	4-CH_3	$C_{14}H_{13}NOS$ 243.32	1645	124–126	130–132 [11]
4 c	4-Cl	$C_{13}H_{10}ClNOS$ 263.74	1646	113–116	120–122 [11]
4 d	3-Cl	$C_{13}H_{10}ClNOS$ 263.74	1649	118–120	–
4 e	3,4-Cl_2	$C_{13}H_9Cl_2NOS$ 298.18	1649	161–163	–
4 f	4-Br	$C_{13}H_{10}BrNOS$ 308.19	1650	102–104	–
4 g	4-F	$C_{13}H_{10}FNOS$ 247.28	1645	129,5–131	–
4 h	3-F	$C_{13}H_{10}FNOS$ 247.28	1648	87–90	–
4 l	4-OCH_3	$C_{14}H_{13}NO_2S$ 259.32	1655	149–151	–
5 a	H	$C_{14}H_{13}NOS$ 243.32	1633	209–210	108 [12]
5 b	4-CH_3	$C_{15}H_{15}NOS$ 257.35	1629	215–217	–
5 c	4-Cl	$C_{14}H_{12}ClNOS$ 277.76	1630	243–245	–
5 d	3-Cl	$C_{14}H_{12}ClNOS$ 277.76	1631	184–186	–
5 e	3,4-Cl_2	$C_{14}H_{11}Cl_2NOS$ 312.21	1631	222–224	–
5 f	4-Br	$C_{14}H_{12}BrNOS$ 322.21	1630	231–233	–
5 g	4-F	$C_{14}H_{12}FNOS$ 261.31	1628	224–226	–
5 k	3-NO_2	$C_{14}H_{12}N_2O_3S$ 288.32	1636	216–217	–

Table 2. ^{1}H and ^{13}C NMR spectra of new 3-hydroxypicolinanilides (**2**), 3-hydroxypicolinbenzylamides (**3**), 2-sulfanylbenzanilides (**4**) and 2-sulfanylbenzylamides (**5**).

Compounds	NMR, δ
2a	^{1}H NMR (300 MHz, $CDCl_3$) δ 11.96 (s, ^{1}H, OH), 9.93 (bs, ^{1}H, NH), 8.13 (dd, ^{1}H J = 4.00 Hz, J = 1.78 Hz, H6), 7.75–7.69 (m, 2H, H2′, H6′), 7.45–7.33 (m, 4H, H5, H3′, H4′, H5′), 7.23–7.15 (m, ^{1}H, H4) ^{13}C NMR (75 MHz, $CDCl_3$) δ = 166.8, 158.2, 139.5, 136.7, 131.3, 129.2, 129.0, 126.5, 124.9, 120.1
2b	^{1}H NMR (300 MHz, $CDCl_3$) δ 12.02 (bs, ^{1}H, OH), 9.86 (bs, ^{1}H, NH), 8.11 (dd, ^{1}H, J = 4.12 Hz, J = 1.65 Hz, H6), 7.64–7.56 (m AA′, BB′, 2 H, H2′, H6′), 7.41–7.32 (m, 2 H, H4, H5), 7.24–7.16 (m AA′, BB′, 2 H, H3′, H5′), 2.35 (s, 3 H, CH_3) ^{13}C NMR (75 MHz, $CDCl_3$) δ = 166.6, 158.2, 139.4, 134.6, 134.1, 131.4, 129.6, 128.8, 126.4, 120.0, 20.9
2c	^{1}H NMR (300 MHz, $CDCl_3$) δ 11.78 (s, ^{1}H, OH), 9.93 (bs, ^{1}H, NH), 8.11 (dd, ^{1}H, J = 4.23 Hz, J = 1.69 Hz, H6), 7.70–7.63 (m AA′, BB′, 2 H, H2′, H6′), 7.41–7.38 (m, 2 H, H4, H5), 7.37–7.32 (m AA′, BB′, 2 H, H3′, H5′) ^{13}C NMR (75 MHz, $CDCl_3$) δ = 166.8, 158.2, 139.5, 135.3, 131.0, 129.9, 129.2, 129.1, 126.6, 121.2
2d	^{1}H NMR (300 MHz, $CDCl_3$) δ 11.74 (s, ^{1}H, OH), 9.95 (bs, ^{1}H, NH), 8.12 (dd, 1 H, J = 4.16 Hz, J = 1.25 Hz, H6), 7.85 (t, 1 H, J = 2.08 Hz, H2′), 7.54 (ddd, ^{1}H, J = 8.12 Hz, J = 2.08 Hz, J = 0.84 Hz, H6′), 7.44–7.25 (m, 3 H, H4, H5, H5′), 7.15 (ddd, 1 H, J = 8.12 Hz, J = 2.08 Hz, J = 0.84 Hz, H4′) ^{13}C NMR (75 MHz, $CDCl_3$) δ = 166.9, 158.3, 139.6, 137.9, 134.8, 130.9, 130.1, 129.2, 126.6, 124.9, 120.1, 117.9
2e	^{1}H NMR (300 MHz, $CDCl_3$) δ 11.62 (s, ^{1}H, OH), 9.96 (bs, ^{1}H, NH), 8.12 (dd, 1 H J = 4.16 Hz, J = 1.67 Hz, H6), 7.97 (d, ^{1}H, J = 2.08 Hz, H2′), 7.54 (dd, ^{1}H, J = 8.74 Hz, J = 2.50 Hz, H6′), 7.45 (d overlapped, 1 H, J = 8.74 Hz, H5′), 7.42 (dd overlapped, 1 H, J = 8.84 Hz, J = 4.16 Hz, H5), 7.37 (dd, ^{1}H J = 8.84 Hz, J = 1.67 Hz, H4) ^{13}C NMR (75 MHz, $CDCl_3$) δ = 166.9, 158.3, 139.7, 136.2, 133.0, 130.7, 130.7, 129.4, 128.1, 126.7, 121.6, 119.1
2f	^{1}H NMR (300 MHz, $CDCl_3$) δ 11.77 (s, ^{1}H, OH), 9.93 (bs, ^{1}H, NH), 8.12 (dd, 1 H, J = 4.17 Hz, J = 1.65 Hz, H6), 7.65–7.59 (m AA′, BB′, 2 H, H3′, H5′), 7.53–7.47 (m AA′, BB′, 2 H, H2′, H6′), 7.41 (dd, 1 H, J = 8.45 Hz, J = 4.17 Hz, H5), 7.35 (dd, ^{1}H, J = 8.45 Hz, J = 1.65 Hz, H4) ^{13}C NMR (75 MHz, $CDCl_3$) δ = 166.8, 158.2, 139.6, 135.8, 132.1, 131.0, 129.2, 126.6, 121.5, 117.6
2g	^{1}H NMR (300 MHz, DMSO) δ 11.88 (bs, ^{1}H, OH), 9.93 (s, ^{1}H, NH), 8.12 (dd, ^{1}H, J = 4.12 Hz, J = 1.65 Hz, H6), 7.73–7.63 (m, 2 H, H2′, H6′), 7.45–7.33 (m, H4, H5), 7.14–7.05 (m AA′, BB′, 2 H, H3′, H5′) ^{13}C NMR (75 MHz, DMSO) δ 166.6, 161.3 and 158.1 (J = 244.5 Hz), 158.3, 139.4, 132.8 and 132.7 (J = 2.9 Hz), 131.0, 129.1, 126.7, 121.9 and 121.8 (J = 8.0 Hz), 116.0 and 115.7 (J = 22.6 Hz)
2i	^{1}H NMR (300 MHz, DMSO) δ 11.84 (bs, ^{1}H, OH), 11.20 (s, ^{1}H, NH), 8.27 (d, ^{1}H, J = 4.12 Hz, H6), 8.12–8.05 (m AA′, BB′, 2 H, H3′, H5′), 7.77–7.71 (m AA′, BB′, 2 H, H2′, H6′), 7.61 (dd, J = 8.51 Hz, J = 4.12 Hz, H5), 7.5 (dd, ^{1}H, J = 8.52 Hz, J = 1.38 Hz, H4) ^{13}C NMR (75 MHz, DMSO) δ 168.0, 157.8, 141.2, 140.3, 130.0, 126.6, 126.3, 126.1 (q, J = 3.7 Hz), 125.3 (q, J = 272.6 Hz), 124.8 (q, J = 32.1 Hz), 121.5
2j	^{1}H NMR (300 MHz, DMSO) δ 11.64 (bs, ^{1}H, OH), 11.35 (bs, 1 H, NH), 8.31–8.22 (m AA′BB′overlapped, 3 H, H6, H3′, H5′), 8.18–8.11 (m AA′, BB′, 2 H, H2′, H6′), 7.65–7.58 (m, 1 H, H5), 7.53–7.47 (m, 1 H, H4) ^{13}C NMR (75 MHz, DMSO) δ 168.1, 157.7, 143.8, 143.4, 140.4, 131.5, 130.1, 126.7, 124.8, 121.3
2k	^{1}H NMR (300 MHz, DMSO) δ 11.74 (bs, ^{1}H, NH), 11.33 (bs, 1 H, OH), 8.88 (t, 1 H, J = 2.20 Hz, H2′), 8.29–8.21 (m, 2 H, H6, H4′), 8.02–7.97 (m, ^{1}H, H6′), 7.69–7.57 (m, 2 H, H5, H5′), 7.49 (dd, ^{1}H, J = 8.52 Hz, J = 1.37 Hz, H4) ^{13}C NMR (75 MHz, DMSO) δ 168.0, 157.8, 148.0, 140.3, 138.8, 131.3, 130.2, 130.0, 127.6, 126.6, 119.3, 115.7

Table 2. (continued).

Compounds	NMR, δ
2 l	^{1}H NMR (300 MHz, $CDCl_3$) δ 12.09 (bs, 1 H, OH), 9.89 (s, 1 H, NH), 8.12 (dd, 1 H, J = 3.85 Hz, J = 1.65 Hz, H6), 7.67–7.59 (m AA′, BB′, 2H, H2′, H6′), 7.44–7.34 (m, H4, H5), 7.03–6.85 (m AA′, BB′, 2 H, H3′, H5′), 3.82 (s, 3 H, OCH_3) ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.27, 158.3, 156.9, 139.2, 131.2, 129.8, 128.9, 126.8, 121.8, 114.3, 55.5
3 a	^{1}H NMR (300 MHz, DMSO) δ 12.51 (bs, 1 H, OH), 9.76 (t, 1 H, J = 6.18 Hz, NH), 8.16 (dd, 1 H, J = 4.39 Hz, J = 1.37 Hz, H6), 7.53 (dd, 1 H, J = 8.52 Hz, J = 4.4 Hz, H5), 7.41 (dd, 1 H, J = 8.52 Hz, J = 1.38 Hz, H4), 7.37–7.19 (m, 5 H, H2′, H3′, H4′, H5′, H6′), 4.49 (d, 2 H, J = 6.59 Hz, CH_2) ^{13}C NMR (75 MHz, DMSO) δ 168.9, 157.5, 140.1, 139.0, 131.5, 129.5, 128.6, 127.7, 127.2, 126.1, 42.3
3 b	^{1}H NMR (300 MHz, $CDCl_3$) δ 12.2 (bs, 1 H, OH), 8.38 (bs, 1 H, NH), 8.03 (dd, 1 H, J = 3.57 Hz, J = 2.2 Hz, H6), 7.35–7.32 (m, 2 H, H4, H5), 7.29–7.23 (m AA′, BB′, 2H, H2′, H6′), 7.20–7.14 (m AA′, BB, 2 H, H3′, H5′), 4.6 (d, 2 H, J = 6.32 Hz, CH_2), 2.35 (s, 3 H, CH_3) ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.4, 157.8, 139.28, 137.4, 134.3, 131.3, 129.4, 128.6, 127.8, 126.3, 42.8, 21.1
3 c	^{1}H NMR (300 MHz, DMSO) δ 12.45 (bs, 1 H, OH), 9.80 (bs, 1 H, NH), 8.16 (dd, 1 H, J = 3.44 Hz, J = 1.37 Hz, H6), 7.52 (dd, 1 H, J = 7.56 Hz, J = 3.44 Hz, H5), 7.41 (dd, 1 H, J = 7.56 Hz, J = 1.38 Hz, H4), 7.38–7.32 (m, 4 H, H2′, H3′, H5′, H6′), 4.47 (d, 2 H, J = 6.31 Hz, CH_2) ^{13}C NMR (75 MHz, DMSO) δ 169, 157.5, 140.1, 138.1, 131.7, 131.4, 129.6, 129.5, 128.5, 126.2, 41.65
3 d	^{1}H NMR (300 MHz, DMSO) δ 12.41 (bs, 1 H, OH), 9.81 (t, 1 H, J = 6.32 Hz, NH), 8.16 (dd, 1 H, J = 4.39 Hz, J = 1.38 Hz, H6), 7.52 (dd, 1 H, J = 8.52 Hz, J = 4.4, H5), 7.41 (dd, 1 H, J = 8.51 Hz, J = 1.38 Hz, H4), 7.38–7.27 (m, 5 H, H2′, H4′, H5′, H6′), 4.49 (d, 2 H, J = 6.59 Hz, CH_2) ^{13}C NMR (75 MHz, DMSO) δ 169.1, 157.5, 141.6, 140.1, 133.2, 131.3, 130.5, 129.5, 127.5, 127.2, 126.3, 126.15, 41.8
3 e	^{1}H NMR (300 MHz, DMSO) δ 12.45 (bs, 1 H, OH), 9.83 (t, 1 H, J = 6.04 Hz, NH), 8.16 (dd, 1 H, J = 4.39 Hz, J = 1.37 Hz, H6), 7.62–7.49 (m, 3H, H2′, H5′, H6′), 7.41 (dd, 1 H, J = 8.38 Hz, J = 1.37 Hz, H5), 7.32 (dd, J = 8.38 Hz, J = 1.37 Hz, H4), 4.47 (d, 2 H, J = 6.32 Hz, CH_2) ^{13}C NMR (75 MHz, DMSO) δ 169.1, 157.5, 140.3, 140.2, 131.3, 131.1, 130.8, 129.8, 129.7, 129.5, 128.1, 126.2, 41.3
3 f	^{1}H NMR (300 MHz, DMSO) δ 12.44 (bs, 1 H, OH), 9.80 (t, 1 H, J = 6.32 Hz, NH), 8.16 (dd, 1 H, J = 4.12 Hz, J = 1.37 Hz, H6), 7.55–7.52 (m AA′, BB′, 2H, H2′, H6′), 7.51 (dd, 1 H, J = 6.05 Hz, J = 1.37 Hz, H5), 7.41 (dd, 1 H, J = 8.51 Hz, J = 1.37 Hz, H4), 7.32 (m AA′, BB′, 2 H, H3′, H5′), 4.45 (d, 2 H, J = 6.31 Hz, CH_2) ^{13}C NMR (75 MHz, DMSO) δ 169, 157.5, 140.1, 138.5, 131.4, 131.4, 129.9, 129.4, 126.2, 120.3, 41.7
3 g	^{1}H NMR (300 MHz, DMSO) δ 12.48 (bs, 1 H, OH), 9.78 (t, 1 H, J = 6.32 Hz, NH), 8.15 (dd, 1 H, J = 4.39 Hz, J = 1.38 Hz, H6), 7.43–7.34 (m, 4 H, H4, H5, H2′, H6′), 7.19–7.09 (m, 2 H, H3′, H5′), 4.46 (d, 2 H, J = 6.31 Hz, CH_2) ^{13}C NMR (75 MHz, DMSO) δ 168.9, 163.1 and 159.9 (J = 242.5 Hz), 157.5, 140.1, 135.2 and 135.2 (J = 2.9 Hz), 131.4, 129.8 and 129.7 (J = 8.1 Hz), 129.4, 126.1, 115.4 and 115.1 (J = 21.2 Hz)
3 i	^{1}H NMR (300 MHz, DMSO) δ 12.39 (bs, 1 H, OH), 9.88 (t, 1 H, J = 6.32 Hz, NH), 8.17 (dd, 1 H, J = 4.12 Hz, J = 1.37 Hz, H6), 7.73–7.65 (m AA′, BB′, 2 H, H3′, H5′), 7.6–7.49 (m AA′, BB′ overlapped, 3 H, H5, H2′, H6′), 7.41 (dd, 1 H, J = 8.51 Hz, J = 1.37 Hz, H4), 4.57 (d, 2 H, J = 6.32 Hz, CH_2) ^{13}C NMR (75 MHz, DMSO) δ 169.2, 157.5, 143.9, 140.2, 131.4, 129.5, 128.3, 127.8 (q, J = 31.8 Hz), 126.2, 125.5 (q, 3.8 Hz), 124.5 (q, J = 272.0 Hz), 42.0
3 j	^{1}H NMR (300 MHz, DMSO) δ 12.34 (bs, 1 H, OH), 9.93 (t, 1 H, J = 6.32 Hz, NH), 8.24–8.13 (m, 3 H, H6, H3′, H5′), 7.63–7.5 (m, 3 H, H5, H2′, H6′), 7.41 (dd, 1 H, J = 8.51 Hz, J = 1.37 Hz, H4), 4.61 (d, 2 H, J = 6.31 Hz, CH_2) ^{13}C NMR (75 MHz, DMSO) δ 169.26, 157.5, 147, 146.8, 140.2, 131.3, 129.6, 128.6, 126.2, 123.8, 41.9

Table 2. (continued).

Compounds	NMR, δ
3k	^{1}H NMR (300 MHz, DMSO) δ 12.33 (bs, 1 H, OH), 9.95 (t, 1 H, J = 6.32 Hz, NH), 8.23–8.2 (m, 1 H, ^{1}H2′), 8.18 (dd, 1 H, J = 4.12 Hz, J = 1.38 Hz, H6), 8.15–8.08 (m, 1 H, H4′), 7.8 (d, 1 H, J = 7.69 Hz, 1 H, H6′), 7.67–7.58 (m, 1 H, H5′), 7.54 (dd, J = 8.51 Hz, J = 4.12 Hz, H5), 7.41 (dd, 1 H, J = 8.51 Hz, J = 1.37 Hz, H4), 4.61 (d, 2 H, J = 6.32 Hz, CH_2) ^{13}C NMR (75 MHz, DMSO) δ 169.2, 157.5, 148, 141.4, 140.2, 134.5, 131.3, 130.2, 129.6, 126.2, 122.4, 122.3, 41.7
3l	^{1}H NMR (300 MHz, DMSO) δ 12.56 (bs, 1 H, OH), 9.67 (t, 1 H, J = 6.32 Hz, NH), 8.14 (dd, 1 H, J = 4.40 Hz, J = 1.37 Hz, H6), 7.51 (dd, 1 H, J = 8.52 Hz, J = 4.40 Hz, H5), 7.40 (dd, J = 8.52 Hz, J = 1.33 Hz, H4), 7.31–7.24 (m AA′, BB′, 2 H, H2′, H6′), 6.91–6.84 (m AA′, BB′, 2H, H3′, H5′), 4.41 (d, 2 H, J = 6.60 Hz, CH_2), 3.70 (s, 3H, OCH_3) ^{13}C NMR (75 MHz, DMSO) δ 168.8, 158.6, 157.5, 140, 131.5, 131, 129.4, 129.2, 126.1, 113.9, 55.3, 41.7
4a	^{1}H NMR (300 MHz, DMSO) δ 10.37 (bs, 1 H, NH), 7.77–7.67 (m, 2 H, H2′, H6′), 7.60 (dd, 1 H, J = 7.42 Hz, J = 1.37 Hz, H6), 7.49 (dd, 1 H, J = 7.42 Hz, J = 1.37 Hz, H3), 7.39–7.30 (m, 3 H, H3′, H4′, H5′), 7.24 (dt, 1 H, J = 7.42, J = 1.37 Hz, H4), 7.14–7.06 (m, 1 H, H5), 5.24 (bs, 1 H, SH) ^{13}C NMR (75 MHz, DMSO) δ 166.7, 139.3, 134.3, 133.3, 130.7, 128.9, 128.8, 124.9, 124.0, 120.2
4b	^{1}H NMR (300 MHz, DMSO) δ 10.30 (bs, 1 H, NH), 7.65–7.56 (m, 3 H, H6, H2′, H6′), 7.51–7.45 (m, 1 H, H3), 7.34 (dt, 1 H, J = 7.69 Hz, J = 1.37 Hz, H4), 7.23 (dt, 1 H, J = 7.70 Hz, J = 1.38 Hz, H5), 7.18–7.11 (m AA′, BB′, 2H, H3′, H5′), 5.33 (bs, 1 H, SH), 2.27 (s, 3 H, CH_3) ^{13}C NMR (75 MHz, DMSO) δ 166.5, 136.8, 134.3, 133.4, 132.9, 130.7, 129.3, 128.7, 124.9, 120.3, 120.2, 20.7
4c	^{1}H NMR (300 MHz, DMSO) δ 10.52 (bs, 1 H, NH), 7.81–7.72 (m AA′, BB′, 2 H, H2′, H6′), 7.61 (dd, 1 H, J = 7.69 Hz, J = 1.37 Hz, H6), 7.50 (dd, 1 H, J = 7.69 Hz, J = 1.37 Hz, H3), 7.43–7.37 (m AA′, BB′, 2 H, H3′, H5′), 7.35 (dt overlapped, 1 H, J = 7.69 Hz, J = 1.37 Hz, H4), 7.24 (dt, 1 H, J = 7.69 Hz, J = 1.37 Hz, H5), 5.26 (bs, 1 H, SH) ^{13}C NMR (75 MHz, DMSO) δ 166.7, 138.2, 133.9, 133.5, 130.9, 130.8, 128.9, 128.8, 127.5, 124.9, 121.7
4d	^{1}H NMR (300 MHz, DMSO) δ 10.55 (bs, 1 H, NH), 7.93 (t, 1 H, J = 1.92 Hz, H2′), 7.67–7.58 (m, 2 H, H6, H6′), 7.51 (dd, 1 H, J = 7.97 Hz, J = 1.10 Hz, H3), 7.41–7.32 (m, 2 H, H4, H5′), 7.25 (dt, 1 H, J = 7.41 Hz, J = 1.10 Hz, H5), 7.16 (ddd, 1 H, J = 7.97 Hz, J = 2.20 Hz, J = 1.10 Hz, H4′), 5.26 (bs, 1 H, SH) ^{13}C NMR (75 MHz, DMSO) δ 166.9, 140.7, 133.8, 133.5, 133.2, 131.0, 130.8, 130.6, 128.8, 125.0, 123.7, 119.5, 118.5
4e	^{1}H NMR (300 MHz, DMSO) δ 10.66 (bs, 1 H, NH), 8.11 (s, 1 H, J = 2.19 Hz, H2′), 7.71–7.57 (m, 3 H, H6, H5′, H6′), 7.51 (dd, 1 H, J = 7.42 Hz, J = 0.83 Hz, H3), 7.37 (dt, 1 H, J = 7.42 Hz, J = 1.38 Hz, H4), 7.25 (dt, 1 H, J = 7.41 Hz, J = 1.38 Hz, H5), 5.27 (bs, 1 H, SH) ^{13}C NMR (75 MHz, DMSO) δ 166.9, 139.4, 133.8, 133.5, 131.2, 131.1, 130.9, 130.9, 128.9, 125.4, 125.0, 121.2, 120.1
4f	^{1}H NMR (300 MHz, DMSO) δ 10.50 (bs, 1 H, NH), 7.75–7.67 (m AA′, BB′, 2 H, H2′, H6′), 7.61 (dd, 1 H, J = 7.42 Hz, J = 1.37 Hz, H6), 7.56–7.47 (m AA′, BB′, 2 H, H3′, H5′), 7.36 (dt, 1 H, J = 7.42 Hz, J = 1.37 Hz, H4), 7.24 (dt, 1 H, J = 7.41 Hz, J = 1.37 Hz, H5), 5.26 (bs, 1 H, SH) ^{13}C NMR (75 MHz, DMSO) δ 166.7, 138.6, 133.9, 133.5, 131.7, 130.9, 130.8, 128.8, 125.0, 122.0, 115.6
4g	^{1}H NMR (300 MHz, DMSO) δ 10.44 (bs, 1 H, NH), 7.81–7.70 (m, 2 H, H2′, H6′), 7.61 (dd, 1 H, J = 7.56 Hz, J = 1.37 Hz, H6), 7.49 (dd, 1 H, J = 7.56 Hz, J = 1.37 Hz, H3), 7.35 (dt, 1 H, J = 7.56 Hz, J = 1.37 Hz, H4), 7.25 (dt, 1 H, J = 7.56 Hz, J = 1.37 Hz, H5), 7.23–7.14 (m, 2 H, H3′, H5′), 5.25 (bs, 1 H, SH) ^{13}C NMR (75 MHz, DMSO) δ 166.5, 158.5 (d, J = 240.5 Hz), 135.6 (d, J = 2.6 Hz), 134.0, 133.4, 130.8, 128.8, 124.9, 122.0 (d, J = 7.7 Hz), 115.5 (d, J = 22.3 Hz)

Table 2. (continued).

Compounds	NMR, δ
4 h	^{1}H NMR (300 MHz, DMSO) δ 10.59 (bs, 1 H, NH), 7.80–7.65 (m, 1 H, H2′), 7.61 (dd, 1 H, J = 7.70 Hz, J = 1.38 Hz, H6), 7.54–7.45 (m, 2 H, H3, H6′), 7.44–7.32 (m, 2 H, H4, H5′), 7.25 (dt, 1 H, J = 7.70 Hz, J = 1.38 Hz, H5), 6.98–6.89 (m, 1 H, H4′), 5.26 (bs, 1 H, SH) ^{13}C NMR (75 MHz, DMSO) δ 166.9, 166.1, 162.3 (d, J = 241.1 Hz), 141.0 (d, J = 10.9 Hz), 133.7 (d, J = 23.8 Hz), 130.9 (d, J = 7.5 Hz), 130.6 (d, J = 9.7 Hz), 128.8, 125.0, 115..9 (d, J = 2.6 Hz), 110.4 (d, J = 20.9 Hz), 107.0, 106.6
4 l	^{1}H NMR (300 MHz, DMSO) δ 10.25 (bs, 1 H, NH), 7.68–7.60 (m AA′, BB′overlapped, 2 H, H2′, H6′), 7.59 (dd overlapped, 1 H, J = 7.56 Hz, J = 1.10 Hz, H6), 7.48 (dd, 1 H, J = 7.56 Hz, J = 1.10 Hz, H3), 7.33 (dt, 1 H, J = 7.56 Hz, J = 1.10 Hz, H4), 7.23 (dt, 1 H, J = 7.56 Hz, J = 1.10 Hz, H5), 7.00–6.87 (m AA′, BB′, 2 H, H3′, H5′), 5.25 (bs, 1 H, SH), 3.73 (s, 3H, OCH_3) ^{13}C NMR (75 MHz, DMSO) δ 166.2, 155.8, 134.2, 133.4, 132.4, 130.7, 130.6, 128.7, 124.9, 121.7, 114.0, 55.4
5 a	^{1}H NMR (300 MHz, DMSO) δ 9.21 (t, 1 H, J = 6.04 Hz, NH), 7.71 (dd, 1 H, J = 7.56 Hz, J = 1.10 Hz, H6), 7.65 (d, 1 H, J = 7.56 Hz, H3), 7.48–7.31 (m, 7 H, H4, H5, H2′, H3′, H4′, H5′, H6′), 4.50 (d, 1 H, J = 6.04 Hz, CH_2) ^{13}C NMR (75 MHz, DMSO) δ 167.1, 139.5, 137.1, 133.8, 131.4, 128.6, 128.2, 127.5, 127.1, 126.2, 126.0, 42.8
5 b	^{1}H NMR (300 MHz, DMSO) δ 9.16 (t, 1 H, J = 6.04 Hz, NH), 7.68 (dd, 1 H, J = 7.76 Hz, J = 1.30 Hz, H6), 7.64 (dd, 1 H, J = 7.76 Hz, J = 1.30 Hz, H3), 7.42 (dt, 1 H, J = 7.76 Hz, J = 1.30 Hz, H4), 7.30 (dt overlap, 1 H, J = 7.76 Hz, J = 1.30, H5) 7.28–7.22 (m AA′BB′overlap, 2 H, H2′, H6′), 7.17–7.12 (m AA′, BB′, 2 H, H3′, H5′), 4.44 (d, 2 H, J = 6.05 Hz, CH_2), 2.27 (s, 3 H, CH_3) ^{13}C NMR (75 MHz, DMSO) δ 167.1, 137.1, 136.5, 136.1, 133.9, 131.4, 129.1, 128.2, 127.5, 126.2, 125.9, 42.6, 20.9
5 c	^{1}H NMR (300 MHz, DMSO) δ 9.24 (t, 1 H, J = 5.77 Hz, NH), 7.71 (dd, 1 H, J = 7.83 Hz, J = 0.96 Hz, H6), 7.63 (dd, 1 H, J = 7.83 Hz, J = 0.96 Hz, H3), 7.49–7.35 (m, 5 H, H4, H2′, H3′, H5′, H6′), 7.35–7.27 (m, 1 H, H5), 4.47 (d, 2 H, J = 6.05 Hz, CH_2) ^{13}C NMR (75 MHz, DMSO) δ 167.2, 128.6, 137.1, 133.7, 131.7, 131.5, 129.4, 128.5, 128.2, 126.3, 126.0, 42.2,
5 d	^{1}H NMR (300 MHz, DMSO) δ 9.26 (t, 1 H, J = 6.05 Hz, NH), 7.71 (dd, 1 H, J = 7.69 Hz, J = 1.10 Hz, H6), 7.67–7.62 (m, 1 H, H3), 7.49–7.26 (m, 6 H, H4, H5, H2′, H4′, H5′, H6′), 4.48 (d, 2 H, J = 6.05 Hz, CH_2) ^{13}C NMR (75 MHz, DMSO) δ 167.3, 142.1, 137.0, 133.7, 133.2, 131.5, 130.5, 128.2, 127.3, 127.1, 126.3, 126.2, 126.0, 42.4
5 e	^{1}H NMR (300 MHz, DMSO) δ 9.26 (t, 1 H, J = 6.05 Hz, NH), 7.71 (dd, 1 H, J = 7.69 Hz, J = 1.10 Hz, H6), 7.66–7.58 (m, 3 H, H3, H2′, H5′), 7.48–7.28 (m, 4 H, H4, H5, H6′), 4.48 (d, 2H, J = 6.05 Hz, CH_2) ^{13}C NMR (75 MHz, DMSO) δ 167.3, 140.8, 137.0, 133.6, 131.6, 131.1, 130.8, 129.6, 129.5, 128.3, 127.9, 126.4, 126.0, 41.9
5 f	^{1}H NMR (300 MHz, DMSO) δ 9.25 (t, 1 H, J = 6.04 Hz, NH), 7.70 (dd, 1 H, J = 7.76 Hz, J = 1.10 Hz, H6), 7.65–7.60 (m, 1 H, H3), 7.57–7.50 (m AA′, BB′, 2 H, H2′, H6′), 7.44 (dt, 1 H, J = 7.76 Hz, J = 1.10, H4) 7.37–7.27 (m, 3 H, H5, H3′, H5′), 4.45 (d, 2 H, J = 6.05 Hz, CH_2) ^{13}C NMR (75 MHz, DMSO) δ 167.2, 139.0, 137.1, 133.6, 131.5, 131.4, 129.8, 129.7, 128.2, 126.3, 120.1, 42.3
5 g	^{1}H NMR (300 MHz, DMSO) δ 9.22 (t, 1 H, J = 6.04 Hz, NH), 7.70 (dd, 1 H, J = 7.69 Hz, J = 1.10 Hz, H6), 7.63 (dd, 1 H, J = 7.69 Hz, J = 1.10 Hz, H3), 7.47–7.36 (m, 3 H, H4, H2′, H6′), 7.30 (dt, 1 H, J = 7.69 Hz, J = 1.10, H5) 7.22–7.11 (m, 2 H, H3′, H5′), 4.47 (d, 2 H, J = 6.04 Hz, CH_2) ^{13}C NMR (75 MHz, DMSO) δ 167.1, 161.5 (d, J = 242.2 Hz), 137.1, 135.7 (d, J = 2.9 Hz), 133.8, 131.5, 129.5 (d, J = 8.0 Hz), 128.2, 126.3, 126.0, 115.3 (d, J = 21.1 Hz), 42.2

Table 2. (continued).

Compounds	NMR, δ
5 k	^{1}H NMR (300 MHz, DMSO) δ 9.36 (t, 1 H, J = 6.05 Hz, NH), 8.25 (t, 1 H, J = 1.65 Hz, H2′), 8.16–8.10 (m, 1 H, H6′), 7.87–7.81 (m, 1 H, H4′), 7.73 (dd, 1 H, J = 7.41 Hz, J = 1.10 Hz, H6), 7.69–7.61 (m, 2 H, H3, H5′), 7.45 (dt, 1 H, J = 7.42 Hz, J = 1.10 Hz, H4), 7.33 (dt, 1 H, J = 7.42 Hz, J = 1.10, H5), 4.62 (d, 2 H, J = 6.05 Hz, CH_2) ^{13}C NMR (75 MHz, DMSO) δ 167.4, 148.0, 141.9, 137.0, 134.3, 133.5, 131.6, 130.2, 128.3, 126.3, 126.0, 122.2, 122.1, 42.3

Compounds **1**–**5** were evaluated for *in vitro* antifungal activity against nine strains of human pathogenic fungi. With the exceptions of *Trichophyton mentagrophytes* 445 and *Microsporum gypseum 27339*, the antifungal activities of the derivatives were low and, in some cases, the values of minimum inhibitory concentration (MIC) could not be determined due to the limited solubility of the compounds. For this reason, quantitative structure-antifungal activity relationships were studied only for the above dermatophytic strains.

Calculation

Quantitative structure-activity relationships were determined by the Free-Wilson method modified according to Fujita and Ban [7].

Results and discussion

The characteristics of the compounds are presented in Table 1. The compounds were characterized by NMR and IR spectroscopy. In the IR spectra, the carbonyl vibration ν(C=O) was in the region 1650–1670 cm^{-1} for picolinanilides (**2**), in the region 1652–1646 cm^{-1} for 2-hydroxypicolinbenzylamides (**3**), in the region 1641–1655 cm^{-1} for 2-sulfanylbenzanilides (**4**), and in the region 1628–1636 cm^{-1} for *N*-benzyl-2-sulfanylbenzamides (**5**). For NMR data, see Table 2.

The antimycobacterial activity is given in Table 3. The most active compounds could be considered superior to INH, given by their broad spectrum of activity. Thus, we have found 4 groups of potentially new antituberculotics, i.e. 3-hydroxypicolinanilides **2**, N-benzyl-3-hydroxypico-

Table 3. Antimycobacterial activities (MIC(ımol/L)) of substances **1–5.**

Compounds		MIC (μmol/L) Incubation time 14 d/21 d		
	R	*M. tuberculosis*	*M. kansasii*	*M. avium*
1 a[b]	H	62.5/62.5	125/250	62.5/125
1 b[b]	4-CH_3	62.5/62.5	62.5/125	31/62.5
1 c[b]	4-Cl	31/31	31/31	31/31
1 d[b]	3-Cl	16/16	8/8	31/31
1 e[b]	3,4-Cl	28/8	4/8	16/31
1 f[b]	4-Br	16/31	16/31	31/31
1 g[b]	4-F	62.5/62.5	62.5/125	31/62.5
1 h[b]	3-F	31/31	62.5/62.5	62.5/62.5
1 i[c]	4-CF_3	8/8	16/16	16/32
1 j[b]	4-NO_2	8/16	16/16	31/31
1 k[b]	3-NO_2	16/166	2.5/*a*	31/*a*
1 l[b]	4-OCH_3	62.5/62.5	250/250	62.5/125
2 a	H	250/250	125/125	125/250
2 b	4-CH_3	125/*a*	62.5/62.5	62.5/125
2 c	4-Cl	62.5/62.5	62.5/62.5	32/62.5

Table 3. (continued).

Compounds	R	MIC (μmol/L) Incubation time 14 d/21 d *M. tuberculosis*	*M. kansasii*	*M. avium*
2d	3-Cl	62.5/62.5	62.5/62.5	32/62.5
2e	3,4-Cl_2	*a/a*	*a/a*	*a/a*
2f	4-Br	62.5/62.5	62.5/62.5	32/62.5
2g	4-F	128/125	122/125	62.5/125
2i	4-CF_3	32/62.5	62.5/62.5	62.5/62.5
2j	4-NO_2	*a/a*	>1000/>1000	*a/a*
2k	3-NO_2	*a/a*	*a*/>1000	*a/a*
2l	4-OCH_3	125/125	32/62.5	*a/a*
3°	H	500/1000	125/500	250/500
3b	4-CH_3	*a/a*	62.5/125	125/125
3c	4-Cl	125/125	32/62.5	62.5/125
3d	3-Cl	125/125	32/62.5	62.5/125
3e	3,4-Cl_2	62.5/62.5	16/32	32/62.5
3f	4-Br	*a/a*	32/62.5	32/62.5
3g	4-F	250/500	32/62.5	125/2
3i	4-CF_3	125/125	62.5/62.5	62.5/125
3j	4-NO_2	125/250	125/250	125/125
3k	3-NO_2	250/500	62.5/125	125/250
3l	4-OCH_3	*a/a*	125/125	*a/a*
4°	H	32/32	32/32	62.5/62.5
4b	4-CH_3	16/16	16/16	32/32
4c	4-Cl	32/32	16/16	32/32
4d	3-Cl	32/32	62.5/62.5	62.5/62.5
4e	3,4-Cl_2	62.5/62.5	32/32	62.5/62.5
4f	4-Br	62.5/62.5	16/16	16/32
4g	4-F	16/16	32/32	62.5/62.5
4h	3-F	32/32	62.5/62.5	62.5/62.5
4l	4-OCH_3	16/16	16/16	16/16
5a	H	8/8	2/2	8/8
5b	4-CH_3	8/8	4/4	16/16
5c	4-Cl	8/8	4/4	16/16
5d	3-Cl	8/16	4/4	8/16
5e	3,4-Cl_2	32/32	4/8	16/32
5f	4-Br	8/8	4/4	8/16
5g	4-F	8/8	2/4	8/16
5j	4-NO_2	8/16	8/8	16/16
5k	3-NO_2	8/8	8/8	16/16
INH		½	250/250	250/250

a: MIC not determined due to a low solubility of the compound
b: data were taken from Ref. [3]

linamides **3**, 2-sulfanylbenzanilides **4**, and N-benzyl-2-sulfanylbenzamides **5**. Quantitative structure-antimycobacterial activity relationships were determined by the Free-Wilson method (see Table 4). As regards the substitution on the phenyl ring in the amide part of the molecule, the introduction of lipophilic substituents, e.g. 4-bromo-, 3-chloro-, 4-chloro-, and 4-trifluoromethyl moiety led to the highest increase of antimycobacterial activity. The activity increased upon isosteric replacement of the hydroxy group in the acyl part with the sulfanyl substituent. The derivatives of picolinic acid **2** and **3**, however, were less active than the corresponding deriv-

Table 4. Structure – antimycobacterial activity relationships. Results of the Free-Wilson analysis, modification according to Fujita and Ban (incubation 14 and 21 d).

Fragment	Δ log MIC (µmol/L)					
	M. tuberculosis 331/88		*M. kansasii* 235/80		*M. avium* 330/88	
	14d	21d	14d	21d	14d	21d
OH	0	0	0	0	0	0
SH	–0.1425	–0.1728	–0.265	–0.3574	–0.0593	–0.1913
C	0	0	0	0	0	0
N	0.7708	0.8024	0.557	0.4776	0.484	0.5093
NH	0	0	0	0	0	0
$NHCH_2$	–0.0919	–0.0426	–0.4228	–0.444	–0.1889	–0.1313
H′	0	0	0	0	0	0
4′-CH_3	–0.2443	–0.2557	–0.1814	–0.0976	–0.1786	–0.2396
4′-Cl	–0.301	–0.3612	–0.2998	–0.2769	–0.2969	–0.36
3′-Cl	–0.3585	–0.3584	–0.299	–0.2762	–0.299	–0.3018
3′,4′-Cl_2	–0.2205	–0.2778	–0.4887	–0.3912	–0.2725	–0.223
4′-Br	–0.3187	–0.2919	–0.3572	–0.2769	–0.4755	–0.4202
4′-F	–0.1806	–0.1806	–0.061	0.0228	–0.1806	–0.1212
3′-F	–0.0756	–0.1004	0.2548	0.2466	0.1207	–0.0143
4′–CF3	–0.6264	–0.6069	–0.3035	–0.1595	–0.3639	–0.4003
4′-NO_2	–0.4921	–0.2643	–0.0858	–0.0668	–0.1211	–0.325
3′-NO_2	–0.2914	–0.2643	0.0098	0.0073	–0.1211	–0.1306
4′-OCH_3	–0.1566	–0.1969	0.0228	0.1092	–0.0246	–0.0072
μ_0	1.6449	1.6848	1.6717	1.7261	1.7028	1.9039
	R = 0.8363 s = 0.334 F = 5.15 n = 46	R = 0.8215 s = 0.3684 F = 4.45 n = 45	R = 0.8117 s = 0.3728 F = 4.69 n = 49	R = 0.8081 s = 0.385 F = 4.57 n = 48	R = 0.7787 s = 0.265 F = 3.52 n = 47	R = 0.8405 s = 0.2529 F = 5.33 n = 46

Table 5. Structure – antifungal activity relationships. Results of the Free-Wilson analysis, modification according to Fujita and Ban (incubation 72 and 120 h).

Fragment	Δ log MIC (µmol/L)			
	M. gypseum		*T. mentagrophytes*	
	72 h	120 h	72 h	120 h
OH	0	0	0	0
SH	0.2822	–0.0645	–0.2527	0.0251
C	0	0	0	0
N	0.5644	0.473	0.7739	0.7525
H′	0	0	0	0
4′-CH_3	8.9E-16	–0.1505	–0.1003	–2E-16
4′-Cl	–0.3011	–0.6021	–0.8151	–0.5185
3′-Cl	–0.4014	–0.4515	–0.5142	–0.4431

Table 5. (continued).

Fragment	Δ log MIC (μmol/L)			
	M. gypseum		*T. mentagrophytes*	
	72 h	120 h	72 h	120 h
3′,4′-Cl_2	–0.7208	–1.1177	–1.03	–1.0448
4′-Br	–0.4014	–0.5159	–0.7295	–0.4431
4′-F	–0.1003	–0.301	–0.2006	–0.2007
3′-F	–0.4203	–0.5159	–0.4282	–0.4431
4′-CF_3	–0.4012	–0.7524	–0.6646	–0.6687
4′-NO_2	–0.4203	–0.8173	–0.4282	–0.4431
3′-NO_2	–0.4203	–0.5159	–	–
4′-OCH_3	0.3512	0.1505	–0.1004	0.2341
μ_0	1.313	1.7099	1.6221	1.637
	n = 23	n = 20	n = 22	n = 20
	r = 0.9503	r = 0.9273	r = 0.9288	r = 0.9399
	s = 0.2113	s = 0.3218	s = 0.3062	s = 0.3121
	F = 6.45	F = 2.83	F = 4.71	F = 4.42

atives from the other groups. In all subseries studied, *N*-benzylderivatives displayed better activities than *N*-phenylderivatives. To evaluate the relationships between the structure and antifungal activity against T. mentagrophytes and M. gypseum, the Free-Wilson method was used (see Table 5). The antifungal activity of the derivatives under study is relatively low, and only the activity against the dermatophytic strains is significant. This paper completed the results of our previous paper [3], and the conclusions will be further utilized in our future work towards the development of new antituberculotics.

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Dedicated to Prof. Dr. Hans-Dietrich Stachel, München, on the occasion of his 75th birthday.

Experimental

Chemistry

The melting points were determined on a Kofler apparatus. The samples for analysis and antimycobacterial tests were dried over P_4O_{10} at 61 °C and 66 Pa for 24 h. Elemental analyses (C, H, N) were performed on a CHNS-O CE elemental analyzer (Fisions EA 1110, Milan) and were within ±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}. TLC was performed on silica gel plates precoated with a fluorescent indicator Silufol UV 254 + 366 (Kavalier Votice, Czech Republic), cyclohexane-acetone (3:1) was used as the mobile phase. The ^1H NMR and ^{13}C NMR spectra of new compounds were recorded in [D_6]DMSO solutions at ambient temperature on a Varian Mercury-Vx BB 300 spectrometer operating at 300 MHz for ^1H, and 75 MHz for ^{13}C. Chemical shifts were recorded as δ 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). The data are given in Table 2.

General Procedure for the Preparation of 3-hydoxypicolinanilides **2**, *N*-benzyl-3-hydroxypicolinamides **3**, 2-sulfanylbenzanilides **4** and, *N*-benzyl-2-sulfanylbenzamides **5:**

a suspension of a substituted acid (0.02 mol) and a substituted aniline or substituted benzylmides (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 %).

Microbiology

Antimycobacterial susceptibility testing. The following strains, obtained from the Czech National Collection of Type Cultures (CNCTC), National Institute of Public Health, Prague, were used for the evaluation of *in vitro* antimycobacterial activity: *M. tuberculosis* CNCTC My 331/8, *M. kansasii* CNCTC My 235/80, and *M. avium* CNCTC My 330/88. The antimycobacterial activity of the compounds was determined in the Sula semisynthetic medium (SEVAC, Prague). This medium (with bovine serum) is routinely used in the Czech Republic. Each strain was simultaneously inoculated into a Petri dish containing the Löwenstein-Jensen medium for the control of the 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, 31, 16, 8, 4, 2 μmol/L. The MICs were determined after incubation at 37 °C for 14 and 21 d (see Table 3). MIC was the lowest concentration of an antimycobacterially effective substance (on the above concentration scale), at which inhibition of the growth of the mycobacteria occurred.

Table 6. Antifungal activities (MIC, (μmol/L)) of salicylanilides and their isosters against nine strains of potentially pathogenic fungi.

Ozn.	R	CA	CT	CK	CG	TB	AF	AC	TM	MG
		Incubation time								
		24h 48h	24h 48h	24h 48h	24h 48h	24h 48h	24h 48h	24h 48h	72h 120h	72h 120h
1a	H	125 250	250 500	250 500	500 500	250 500	125 250	250 250	62.5 62.5	31.25 62.5
1b	4-CH_3	62.5 >250	>250 >250	>250 >250	>250 >250	>250 >250	>250 >250	>250 >250	31.25 62.5	31.25 62.5
1c	4-Cl	15.63 62.5	62.5 >125	31.25 31.25	62.5 62.5	31.25 >125	7.81 31.25	31.25 31.25	3.91 7.81	7.81 7.81
1d	3-Cl	31.25 >62.5	>62.5 >62.5	31.25 >62.5	>62.5 >62.5	>62.5 >62.5	31.25 >62.5	>62.5 >62.5	15.63 15.63	7.81 15.63
1e	3,4-Cl_2	>125 >125	>125 >125	>125 >125	>125 >125	>125 >125	>125 >125	>125 >125	3.91 3.91	3.91 3.91
1f	4-Br	15.63 62.5	>125 >125	31.25 62.5	>125 >125	31.25 >125	62.5 >125	31.25 31.25	7.81 15.63	7.81 15.63
1g	4-F	62.5 125	125 >125	125 >125	125 125	125 >125	62.5 125	125 125	31.25 31.25	15.63 31.25
1h	3-F	62.5 125	125 125	32.5 125	125 125	62.5 125	62.5 125	62.5 62.5	15.63 15.63	7.81 15.63
1i	4-CF_3	>125 >125	>125 >125	>125 >125	>125 >125	>125 >125	>125 >125	31.25 >125	3.91 3.91	3.91 3.91
1j	4-NO_2	31.25 62.5	>125 >125	62.5 62.5	62.5 125	31.25 >125	62.5 >125	62.5 62.5	15.63 15.63	7.81 7.81
1k	3-NO_2	>125 >125	>125 >125	>125 >125	>125 >125	>125 >125	>125 >125	>125 >125	>125 >125	7.81 15.63
1l	4-OCH_3	>500 >500	>500 >500	>500 >500	>500 >500	>500 >500	>500 >500	>500 >500	62.5 125	62.5 125
2a	H	125 250	500 500	250 250	125 250	500 500	250 500	250 250	125 250	62.5 125
2b	4-CH_3	125 125	250 >250	250 250	125 250	250 >250	250 250	125 125	125 125	62.5 62.5
2c	4-Cl	62.5 >125	>125 >125	62.5 >125	>125 >125	>125 >125	>125 >125	125 >125	62.5 125	31.25 62.5
2d	3-Cl	62.5 >125	>125 >125	>125 >125	62.5 >125	>125 >125	>125 >125	125 >125	62.5 >125	31.25 62.5
2e	3,4-Cl_2	>250 >250	>250 >250	>250 >250	>250 >250	>250 >250	>250 >250	>250 >250	>250 >250	>250 >250
2f	4-Br	>250 >250	>250 >250	>250 >250	>250 >250	>250 >250	>250 >250	>250 >250	>250 >250	31.25 >250
2g	4-F	62.5 125	250 >500	250 250	125 125	250 >500	125 125	125 125	125 125	62.5 62.5
2i	4-CF_3	125 250	500 500	250 500	62.5 125	500 500	125 250	125 125	125 125	62.5 62.5
2j	4-NO_2	>250 >250	>250 >250	>250 >250	>250 >250	>250 >250	>250 >250	>250 >250	>250 >250	>250 >250

Table 6. (continued).

Ozn.	R	CA	CT	CK	CG	TB	AF	AC	TM	MG
		Incubation time								
		24h	24h	24h	24h	24h	24h	24h	72h	72h
		48h	48h	48h	48h	48h	48h	48h	120h	120h
2k	3-NO_2	>250	>250	>250	>250	>250	>250	>250	>250	>250
		>250	>250	>250	>250	>250	>250	>250	>250	>250
2l	4-OCH_3	125	>500	250	125	>500	250	250	250	125
		250	>500	>500	250	>500	>500	250	250	125
4a	H	15.63	125	62.5	>125	15.63	>125	>125	31.25	31.25
		>125	>125	>125	>125	>125	>125	>125	31.25	>125
4b	4-CH_3	7.81	31.25	31.25	15.63	15.63	>250	>250	31.25	1.25
		15.63	31.25	>250	>250	31.25	>250	>250	62.53	31.25
4c	4-Cl	125	>125	>125	>125	>125	>125	>125	>125	31.25
		>125	>125	>125	>125	>125	>125	>125	>125	>125
4d	3-Cl_3	1.25	>500	>500	>500	>500	>500	>500	>500	>500
		>500	>500	>500	>500	>500	>500	>500	>500	>500
4e	3,4-Cl_2	>250	>250	>250	>250	>250	>250	>250	>250	>250
		>250	>250	>250	>250	>250	>250	>250	>250	>250
4f	4-Br	>125	>125	>125	>125	>125	>125	>125	>125	>125
		>125	>125	>125	>125	>125	>125	>125	>125	>125
4g	4-F	7.81	>62.5	>62.5	>62.5	15.63	>62.5	>62.5	15.63	>62.5
		>62.5	>62.5	>62.5	>62.5	>62.5	>62.5	>62.5	31.25	>62.5
4h	3-F	>125	>125	>125	>125	>125	>125	>125	>125	>125
		>125	>125	>125	>125	>125	>125	>125	>125	>125
4l	4-OCH_3	7.81	7.81	31.25	>250	3.91	15.63	>250	7.81	250
		>250	>250	>250	>250	7.81	>250	>250	>250	>250·

CA-*Candida albicans* ATCC 44859, **CT**-*Candida tropicalis* 156, **CK**-*Candida krusei* E28, **CG**-*Candida glabrata* 20/I, **TB**-*Trichosporon beigelii* 1188, **AF**-*Aspergillus fumigatus* 231, **AC**-*Absidia corymbifera* 272, **TM**-*Trichophyton mentagrophytes* 445, **MG**-*Microsporum gypseum 27339.*

Antifungal susceptibility testing. The compounds were dissolved in dimethylsulfoxide (DMSO)**.** Nine fungal isolates (*Trichophyton mentagrophytes 445, Microsporum gypseum, Aspergillus fumigatus 231, Absidia corymbifera 272, Candida albicans ATTCC 44859* and *Microsporum gypseum*) from patients with suspected or proven mycosis were selected for *in vitro* testing by the microdilution broth test. All strains were subcultured on the Sabouraud dextrose agar (SDA) and maintained on the same medium at 4 °C. Prior to testing, each strain was passaged onto SDA and fungal inocula were prepared by suspending yeasts or spores in sterile 0.85 % saline. The cell density was adjusted by means of the Bürker's chamber to yield a stock suspension of 5.0×10^5 CFU/mL. The final inoculum was made by 1:20 dilution of the stock suspension with the test medium. The *in vitro* antifungal activity of the compounds was determined in the RPMI 1640 medium (Sevapharma, Prague) buffered to pH 7.0 with 0.165 M morpholinopropansulfonic acid (MOPS, Serva). The results are summarized in Table 6.

Calculations

All calculations were carried out with the use of the Multireg H programme (Klemera) for Microsoft Excel.

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