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Antimycobacterial 3-aryl-2*H*-1,3-benzoxazine-2,4(3*H*)-diones

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Dedicated to Prof. Dr. Roland Mayer on the occasion of his 75th birthday

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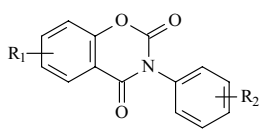
A series of 153 derivatives of 3-phenyl-2*H*-benzoxazine-2,4(3*H*)-dione substituted in position 6 or 7 on benzoxazine and on the phenyl ring was synthesized. The compounds were evaluated in vitro for antimycobacterial activity against *Mycobacterium tuberculosis, Mycobacterium kansasii* and *Mycobacterium avium*. The activity of the compounds increases with increasing hydrophobicity and electron-withdrawing properties of the substituents on the phenyl ring, whereas the effect of the substituents on the benzoxazine ring seems to be more complex.

1. Introduction

The search for new antimycobacterially active compounds is undoubtedly one of the significant directions of current pharmaceutical chemistry. A number of antimycobacterial compounds (synthesized by Mayer et al.) were summarised previously [1]. Research on antitubercular derivatives of 3-aryl-2*H*-1,3-benzoxazin-2,4(3*H*)-dione has been the central theme of our recent studies, with the pathway towards the exploration of benzoxazines having been opened up by a review published in Czech and Slovak Pharmacy [2]. The progress in the chemistry of antituberculous benzoxazines has also been reviewed recently in a review series on the development of new antitubeculotics over the last 15 years [3]. In addition to the evaluation of biological activity against *M. tuberculosis*, we also paid attention to some conditionally pathogenic strains, which are increasingly becoming a serious health problem. Originally, we assumed that electron-accepting properties of the substituents have a positive influence on the antimycobacterial activity of 3-phenyl-2*H*-benzoxazin-2,4(3*H*)-diones [4, 5]. Thus, we prepared a series of 6,8-dichloro- and 6,8-dibromo-3-phenyl-2*H*-benzoxazin-2,4(3*H*)-diones [6]. However, the results showed that the importance of hydrophobic parameters should not be underestimated, and this conclusion was further verified [7, 8]. As regards the substitution pattern of the benzoxazine ring system, the antimycobacterial activity of compounds substituted at position 7 on the benzoxazine skeleton has not yet been studied. Moreover, we assume that the mechanism of action of these compounds is different from that of standard antituberculotics. The aim of this work was to find structure-activity relationships in a series of 3-phenyl-2*H*-benzoxazin-2,4(3*H*)-diones, substituted on the phenyl ring in positions 3 and 4, and in positions 6 and 7 on the benzoxazine, including a more detailed analysis of the relationship. The results seem to indicate that while the influence of the substituents on the phenyl moiety is obvious (antimycobacterial activity increases with increasing electron-accepting properties and lipophilicity of the substituents), the influence of substitution on the benzoxazine system is somewhat more complex [9]. For this reason, we set out to prepare a large group of derivatives. A few compounds possessing exceptionally high activity were selected for preclinical evaluation.

2. Investigations, results and discussion

The substituted 3-aryl-2*H*-1,3-benzoxazin-2,4(3*H*)-diones **1**–**10** were prepared by the treatment of substituted salicylicanilides with chloroformiate. In the IR spectra, two absorption maxima of the carbonyl groups were apparent. Physical properties and NMR spectra of the compounds are presented in Tables 1 and 2, respectively.



	R_1		R_1		R_2		R_2		R_2
1	H	**7**	6-F	**a**	H	**g**	4-F	**m**	4-OCH_3
2	7-Cl	**8**	6-CH_3	**b**	4-CH_3	**h**	3-F	**n**	4-COOEt
3	7-CH_3	**9**	6-OCH_3	**c**	4-Cl	**i**	4-CF_3	**o**	4-CN
4	7-OCH_3	**10**	6-NO_2	**d**	3-Cl	**j**	4-NO_2		
5	6-Br			**e**	3,4-Cl_2	**k**	3-NO_2		
6	6-Cl			**f**	4-Br	**l**	4-N$(CH_3)_2$		

Table 1: Yields and physical properties of 3-phenyl-2*H*-benzoxazin-2,4(3*H*)-diones

Compd.	R_1	R_2	Yield (%)	Formula M_r	N–C=O (cm^{-1})	M.p. (°C)	
						Found	Ref.
2a	7-Cl	H	66	$C_{14}H_8ClNO_3$ 273.67	1771, 1697	202–204	
2b	7-Cl	4-CH_3	71	$C_{15}H_{10}ClNO_3$ 287.7	1778, 1708	180–182	
2c	7-Cl	4-Cl	46	$C_{14}H_7Cl_2NO_3$ 308.12	1773, 1708	208–209	
2d	7-Cl	3-Cl	45	$C_{14}H_7Cl_2NO_3$ 308.12	1766, 1715	199–201	
2e	7-Cl	3,4-Cl_2	43	$C_{14}H_6Cl_3NO_3$ 342.56	1773, 1712	190–192	
2f	7-Cl	4-Br	45	$C_{14}H_7BrClNO_3$ 352.57	1773, 1705	217–218	
2g	7-Cl	4-F	44	$C_{14}H_7FClNO_3$ 291.66	1769, 1712	181–183	
2h	7-Cl	3-F	46	$C_{14}H_7FClNO_3$ 291.66	1773, 1708	190–192	
2i	7-Cl	4-CF_3	47	$C_{15}H_7ClF_3NO_3$ 341.67	1773, 1705	220–222	
2k	7-Cl	3-NO_2	44	$C_{14}H_7ClN_2O_5$ 318.67	1773, 1709	180–182	
2l	7-Cl	4-$N(CH_3)_2$	61	$C_{16}H_{13}ClN_2O_3$ 316.74	1760, 1709	279–280	
2m	7-Cl	4-OCH_3	71	$C_{15}H_{10}ClNO_4$ 303.7	1774, 1705	205–207	
2n	7-Cl	4-COOEt	52	$C_{17}H_{12}ClNO_5$ 345.73	1770, 1711	180–182	
2o	7-Cl	4-CN	51	$C_{15}H_7ClN_2O_3$ 298.68	1768, 1704 (CN) 2231	256–258	
3a	7-CH_3	H	76	$C_{15}H_{11}NO_3$ 253.25	1764, 1691	237–239	239–241 [14]
3b	7-CH_3	4-CH_3	75	$C_{16}H_{13}NO_3$ 267.28	1764, 1692	239–241	
3c	7-CH_3	4-Cl	65	$C_{15}H_{10}ClNO_3$ 287.7	1765, 1705	244–245	
3d	7-CH_3	3-Cl	59	$C_{15}H_{10}ClNO_3$ 287.7	1764, 1694	174–176	
3e	7-CH_3	3,4-Cl_2	57	$C_{15}H_9Cl_2NO_3$ 322.14	1758, 1703	231–233	
3f	7-CH_3	4-Br	59	$C_{15}H_{10}BrNO_3$ 332.15	1770, 1706	238–240	
3g	7-CH_3	4-F	53	$C_{15}H_{10}FNO_3$ 271.24	1769, 1705	222–224	
3h	7-CH_3	3-F	52	$C_{15}H_{10}FNO_3$ 271.24	1767, 1701	236–238	
3i	7-CH_3	4-CF_3	50	$C_{16}H_{10}F_3NO_3$ 321.25	1771, 1707	264–266	
3k	7-CH_3	3-NO_2	51	$C_{15}H_{10}N_2O_5$ 298.25	1766, 1704	244–246	
3l	7-CH_3	4-$N(CH_3)_2$	75	$C_{17}H_{16}N_2O_3$ 296.32	1763, 1701	289–291	
3m	7-CH_3	4-OCH_3	69	$C_{16}H_{13}NO_4$ 283.28	1762, 1693	228–230	
3n	7-CH_3	4-COOEt	59	$C_{18}H_{15}NO_5$ 325.32	1769, 1707	206–206	
3o	7-CH_3	4-CN	58	$C_{16}H_{10}N_2O_3$ 278.26	1764, 1704 (CN) 2231	224–226	

Table 1: (continued)

Compd.	R_1	R_2	Yield (%)	Formula M_r	N–C=O (cm^{-1})	M.p. (°C)	
						Found	Ref.
4a	7-OCH_3	H	55	$C_{15}H_{11}NO_4$ 269.25	1760, 1685	180–181	
4b	7-OCH_3	4-CH_3	70	$C_{16}H_{13}NO_4$ 283.28	1761, 1687	200–202	
4c	7-OCH_3	4-Cl	59	$C_{15}H_{10}ClNO_4$ 303.7	1760, 1687	246–247	
4d	7-OCH_3	3-Cl	58	$C_{15}H_{10}ClNO_4$ 303.7	1766, 1702	129–132	
4e	7-OCH_3	3,4-Cl_2	57	$C_{15}H_9Cl_2NO_4$ 338.14	1763, 1692	261–263	
4f	7-OCH_3	4-Br	59	$C_{15}H_{10}BrNO_4$ 348.15	1760, 1686	256–257	
4g	7-OCH_3	4-F	61	$C_{15}H_{10}FNO_4$ 287.24	1768, 1689	212–214	
4h	7-OCH_3	3-F	56	$C_{15}H_{10}FNO_4$ 287.24	1762, 1696	234–235	
4i	7-OCH_3	4-CF_3	55	$C_{16}H_{10}F_3NO_4$ 337.25	1768, 1704	250–252	
4j	7-OCH_3	4-NO_2	49	$C_{15}H_{10}N_2O_6$ 314.25	1758, 1701	262–269	
4k	7-OCH_3	3-NO_2	48	$C_{15}H_{10}N_2O_6$ 314.25	1766, 1691	253–255	
4m	7-OCH_3	4-OCH_3	51	$C_{16}H_{13}NO_5$ 299.28	1760, 1686	216–217	
4n	7-OCH_3	4-COOEt	49	$C_{18}H_{15}NO_6$ 341.32	1763, 1703,	204–206	
4o	7-OCH_3	4-CN	48	$C_{16}H_{10}N_2O_4$ 294.26	1757, 1701 (CN) 2228	234–236	
5i	6-Br	4-CF_3	45	$C_{15}H_7BrF_3NO_3$ 386.12	1769, 1707	233,5–234	
5n	6-Br	4-COOEt	47	$C_{17}H_{12}BrNO_5$ 390.18	1773, 1709	198–200	
5o	6-Br	4-CN	48	$C_{15}H_7BrN_2O_3$ 343.14	1765; 1702 (CN) 2228	279–281	
6i	6-Cl	4-CF_3	45	$C_{15}H_7ClF_3NO_3$ 341.67	1765, 1702	246	
6n	6-Cl	4-COOEt	46	$C_{17}H_{12}ClNO_5$ 345.73	1772, 1705	194–196	
7i	6-F	4-CF_3	47	$C_{15}H_7F_4NO_3$ 325.21	1779, 1713	243–245	
7n	6-F	4-COOEt	46	$C_{17}H_{12}FNO_5$ 329.28	1773, 1711	182–183	
7o	6-F	4-CN	45	$C_{15}H_7FN_2O_3$ 282.23	1767, 1709 (CN) 2231	185–186	
8a	6-CH_3	H	71	$C_{15}H_{11}NO_3$ 253.25	1765, 1697	219–220	
8b	6-CH_3	4-CH_3	73	$C_{16}H_{13}NO_3$ 267.28	1764, 1700	198–200	
8c	6-CH_3	4-Cl	67	$C_{15}H_{10}ClNO_3$ 287.7	1765, 1705	214–216	190–191 [15]
8d	6-CH_3	3-Cl	66	$C_{15}H_{10}ClNO_3$ 287.7	1761, 1709	199–201	
8e	6-CH_3	3,4-Cl_2	55	$C_{15}H_9Cl_2NO_3$ 322.14	1763, 1706	219–221	
8f	6-CH_3	4-Br	61	$C_{15}H_{10}BrNO_3$ 332.15	1760, 1701	228–230	
8g	6-CH_3	4-F	65	$C_{15}H_{10}FNO_3$ 271.24	1768, 1709	179–180	

Table 1: (continued)

Compd.	R_1	R_2	Yield (%)	Formula M_r	N–C=O (cm^{-1})	M.p. (°C)	
						Found	Ref.
8h	6-CH_3	3-F	63	$C_{15}H_{10}FNO_3$ 271.24	1768, 1706	200–202	
8i	6-CH_3	4-CF_3	59	$C_{16}H_{10}F_3NO_3$ 321.25	1765, 1701	222–224	
8j	6-CH_3	4-NO_2	58	$C_{15}H_{10}N_2O_5$ 298.25	1762, 1699	247–248	
8k	6-CH_3	3-NO_2	56	$C_{15}H_{10}N_2O_5$ 298.25	1769, 1709	234–236	
8l	6-CH_3	4-$N(CH_3)_2$	69	$C_{17}H_{16}N_2O_3$ 296.32	1762, 1697	273–274	
8m	6-CH_3	4-OCH_3	68	$C_{16}H_{13}NO_4$ 283.28	1766, 1700	180–182	
8n	6-CH_3	4-COOEt	59	$C_{18}H_{15}NO_5$ 325.32	1763, 1712	173–175	
8o	6-CH_3	4-CN	58	$C_{16}H_{10}N_2O_3$ 278.26	1759, 1706 (CN) 2227	218–220	
9a	6-OCH_3	H	61	$C_{15}H_{11}NO_4$ 269.25	1755, 1695	227–228	
9b	6-OCH_3	4-CH_3	64	$C_{16}H_{13}NO_4$ 283.28	1757, 1693	217–218	
9c	6-OCH_3	4-Cl	55	$C_{15}H_{10}ClNO_4$ 303.7	1760, 1702	206–208	
9d	6-OCH_3	3-Cl	51	$C_{15}H_{10}ClNO_4$ 303.7	1759, 1708	199–201	
9e	6-OCH_3	3,4-Cl_2	49	$C_{15}H_9Cl_2NO_4$ 338.14	1761, 1697	236–238	
9f	6-OCH_3	4-Br	53	$C_{15}H_{10}BrNO_4$ 348.15	1759, 1693	222–224	
9g	6-OCH_3	4-F	55	$C_{15}H_{10}FNO_4$ 287.24	1758, 1697	223–224	
9h	6-OCH_3	3-F	51	$C_{15}H_{10}FNO_4$ 287.24	1756, 1709	210–212	
9i	6-OCH_3	4-CF_3	51	$C_{16}H_{10}F_3NO_4$ 337.25	1759, 1701	271–273	
9j	6-OCH_3	4-NO_2	49	$C_{15}H_{10}N_2O_6$ 314.25	1760, 1701	241–243	
9k	6-OCH_3	3-NO_2	47	$C_{15}H_{10}N_2O_6$ 314.25	1761, 1693	196–197	
9l	6-OCH_3	4-$N(CH_3)_2$	62	$C_{17}H_{16}N_2O_4$ 312,32	1756, 1692	263–267	
9m	6-OCH_3	4-OCH_3	61	$C_{16}H_{13}NO_5$ 299.28	1761, 1698	237	
9n	6-OCH_3	4-COOEt	53	$C_{18}H_{15}NO_6$ 341.32	1763, 1707	182	
9o	6-OCH_3	4-CN	52	$C_{16}H_{10}N_2O_4$ 294.26	1760, 1705 (CN) 2234	268–269	

Compounds prepared previously [7, 9, 16] are not included in the Table

Groups of compounds **1–10** were tested *in vitro* for 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). The results are summarised in Table 3. In several cases the minimum inhibitory concentration could not be determined due to the limited solubility of the compounds. The results suggest that the compounds under study can be considered as prospective wide-spectrum antimycobacterial substances.

In studying the relationship between the structure and antimycobacterial activity, we followed up on the conclusions made previously [6–9]. 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. Since the influence of the substituents from the acyl part of the molecule seemed to be more complex, we expressed them with indicator parameters (I_n). A lot of other relationships are hidden in them. Equations (1)–(6) are given without regression coefficients of the indicator variables, which are summarised in Ta-

Table 2: ^{1}H and ^{13}C NMR spectra of new benzoxazines

	NMR, δ
2a	^{1}H NMR (300 MHz, DMSO) δ 7.99 (d, 1 H, J = 8.52 Hz, H5), 7.77 (d, 1 H, J = 1.93 Hz, H8), 7.56–7.38 (m, 6 H, H6, H2′, H3′, H4′, H5′, H6′) ^{13}C NMR (75 MHz, DMSO) δ 160.3, 153.3, 147.5, 140.5, 135.2, 129.3, 129.2, 129.0, 128.7, 125.9, 116.9, 114.2
2b	^{1}H NMR (300 MHz, DMSO) δ 7.97 (d, 1 H, J = 8.51 Hz, H5), 7.76 (d, 1 H, J = 1.92 Hz, H8), 7.51 (dd, 1 H, J = 8.51 Hz, J = 1.92 Hz, H6), 7.34–7.23 (m, 4 H, H2′, H3′, H5′, H6′), 2.36 (s, 3 H, CH_3) ^{13}C NMR (75 MHz, DMSO) δ 160.3, 153.3, 147.6, 140.5, 138.5, 132.6, 129.8, 129.2, 128.4, 125.8, 116.8, 114.2, 21.0
2c	^{1}H NMR (300 MHz, DMSO) δ 7.99 (d, 1 H, J = 8.51 Hz, H5), 7.78 (d, 1 H, J = 1.92 Hz, H8), 7.62–7.56 (m AA′, BB′, 2 H, H2′, H6′), 7.53 (dd, 1 H, J = 8.51 Hz, J = 1.92 Hz, H6), 7.49–7.42 (m AA′, BB′, 2 H, H3′, H5′) ^{13}C NMR (75 MHz, DMSO) δ 160.2, 153.2, 147.4, 140.7, 134.1, 133.7, 130.7, 129.4, 129.2, 125.9, 116.9, 114.2
2d	^{1}H NMR (300 MHz, DMSO) δ 8.00 (d, 1 H, J = 8.24 Hz, H5), 7.80 (d, 1 H, J = 1.92 Hz, H8), 7.59–7.38 (m, 5 H, H6, H2′, H4′, H5′, H6′) ^{13}C NMR (75 MHz, DMSO) δ 160.2, 153.2, 147.3, 140.7, 136.5, 133.3, 131.0, 129.2, 128.9, 127.8, 126.0, 116.9, 114.1
2e	^{1}H NMR (300 MHz, DMSO) δ 8.00 (d, 1 H, J = 8.24 Hz, H5), 7.83 (d, 1 H, J = 8.51 Hz, H5′), 7.82 (d, 1 H, J = 1.92 Hz, H8), 7.78 (d, 1 H, J = 2.20 Hz, H2′), 7.55 (dd, 1 H, J = 8.24 Hz, J = 1.92 Hz, H6), 7.47 (dd, 1 H, J = 8.51 Hz, J = 2.20 Hz, H6′) ^{13}C NMR (75 MHz, DMSO) δ 160.1, 153.2, 147.2, 140.8, 135.1, 132.1, 131.5, 131.4, 130.9, 129.5, 129.3, 126.1, 117.0, 114.0
2f	^{1}H NMR (300 MHz, DMSO) δ 7.99 (d, 1 H, J = 8.38 Hz, H5), 7.78 (d, 1 H, J = 1.92 Hz, H8), 7.76–7.70 (m AA′, BB′, 2 H, H2′, H6′), 7.53 (dd, 1 H, J = 8.38 Hz, J = 1.92 Hz, H6), 7.41–7.36 (m AA′, BB′, 2 H, H3′, H5′) 13C NMR (75 MHz, DMSO) δ 160.2, 153.2, 147.3, 140.7, 134.6, 132.4, 131.0, 129.2, 125.9, 122.3, 116.9, 114.2
2g	1H NMR (300 MHz, DMSO) δ 7.99 (d, 1 H, J = 8.51 Hz, H5), 7.78 (d, 1 H, J = 1.92 Hz, H8), 7.53 (dd, 1 H, J = 8.51 Hz, J = 1.92 Hz, H6), 7.50–7.43 (m, 2 H, H2′, H6′), 7.41–7.30 (m, 2 H, H3′, H5′) ^{13}C NMR (75 MHz, DMSO) δ 163.7 and 160.5 (J = 245.3 Hz), 160.3, 153.2, 147.5, 140.6, 131.4 and 131.4 (J = 3.2 Hz), 131.0 and 130.9 (J = 8.8 Hz), 129.2, 125.9, 116.9, 116.4 and 116.1 (J = 22.9 Hz), 114.2
2h	^{1}H NMR (300 MHz, DMSO) δ 8.00 (d, 1 H, J = 8.51 Hz, H5), 7.79 (d, 1 H, J = 1.92 Hz, H8), 7.62–7.51 (m, 1 H, H6′), 7.54 (dd overlaped, 1 H, J = 8.51 Hz, J = 1.92 Hz, H6), 7.39–7.27 (m, 3 H, H2′, H4′, H5′) ^{13}C NMR (75 MHz, DMSO) δ 163.8 and 160.6 (J = 244.2 Hz), 160.1, 153.2, 147.3, 140.7, 136.7 and 136.6 (J = 10.6 Hz), 131.0 and 130.9 (J = 8.9 Hz), 129.3, 126.0, 125.3 and 125.2 (J = 2.9 Hz), 116.9, 116.3 and 116.0 (J = 24.0 Hz), 116.3 and 116.1 (J = 20.3 Hz), 114.1
2i	^{1}H NMR (300 MHz, DMSO) δ, 8.00 (d, 1 H, J = 8.38 Hz, H5), 7.96–7.88 (m AA′, BB′, 2 H, H3′, H5′), 7.8 (d, 1 H, J = 1.79 Hz, H8), 7.71–7.64 (m AA′, BB′, 2 H, H2′, H6′), 7.57–7.51 (dd, 1 H, J = 8.38 Hz, J = 1.79 Hz, H6) ^{13}C NMR (75 MHz, DMSO) δ 160.2, 153.3, 147.3, 140.8, 138.9, 130.0, 129.6 (q, J = 32.1 Hz), 129.2, 126.5 (q, J = 3,2 Hz), 126.0, 124.2 (q, J = 272.3 Hz), 116.9, 114.2
2k	^{1}H NMR (300 MHz, DMSO) δ 8.42 (t, 1 H, J = 2.01 Hz, H2′), 8.36 (ddd, 1 H, J = 7.97 Hz, J = 2.01 Hz, J = 1.10 Hz, H6′), 8.01 (d, 1 H, J = 8.52 Hz, H5), 7.92 (dt, 1 H, J = 7.96 Hz, J = 2.01 Hz, H5′), 7.86 (d, 1 H, J = 7.96 Hz, H4′), 7.82 (d, 1 H, J = 1.92 Hz, H8), 7.55 (dd, 1 H, J = 8.52, J = 1.92 Hz, H6) ^{13}C NMR (75 MHz, DMSO) δ 160.2, 153.2, 148.3, 147.3, 140.9, 136.2, 135.9, 130.9, 129.2, 126.1, 124.2, 117.0, 114.1
2l	^{1}H NMR (300 MHz, DMSO) δ 7.96 (d, 1 H, J = 8.24 Hz, H5), 7.72 (d, 1 H, J = 1.92 Hz, H8), 7.50 (dd, 1 H, J = 8.24 Hz, J = 1.92 Hz, H6), 7.19–7.11 (m AA′, BB′, 2 H, H2′, H6′), 6.81–6.72 (m AA′, BB′, 2 H, H3′, H5′), 2.94 (s, 6 H, CH_3) ^{13}C NMR (75 MHz, DMSO) δ 160.6, 153.2, 150.6, 147.9, 140.4, 129.3, 128.9, 125.7, 123.5, 116.8, 114.3, 112.3, 40.3
2m	^{1}H NMR (300 MHz, DMSO) δ 7.97 (d, 1 H, J = 8.51 Hz, H5), 7.74 (d, 1 H, J = 1.92 Hz, H8), 7.51 (dd, 1 H, J = 8.51 Hz, J = 1.92 Hz, H6), 7.34–7.27 (m AA′, BB′, 2 H, H2′, H6′), 7.07–7.00 (m AA′, BB′, 2 H, H3′, H5′), 3.41 (s, 3 H, OCH_3) ^{13}C NMR (75 MHz, DMSO) δ 160.5, 159.5, 153.3, 147.8, 140.6, 129.8, 129.3, 127.7, 125.9, 116.9, 114.5, 114.3, 55.7
2n	^{1}H NMR (300 MHz, DMSO) δ, 8.13–8.06 (m AA′, BB′, 2 H, H3′, H5′), 7.99 (d, 1 H, J = 8.51 Hz, H5), 7.79 (d, 1 H, J = 1.92 Hz, H8), 7.53 (dd, 2 H, J = 8.52 Hz, J = 1.93 Hz, H6), 7.63–7.55 (m AA′, BB′, 2 H, H2′, H6′), 4.35 (q, 2 H, J = 7.14 Hz, CH_2), 1.34 (t, 3 H, J = 7.14 Hz, CH_3) ^{13}C NMR (75 MHz, DMSO) δ_165.4, 160.2, 153.3, 147.3, 140.7, 139.4, 130.5, 130.2, 129.4, 129.2, 125.9, 116.9, 114.2, 61.2, 14.4
2o	^{1}H NMR (300 MHz, DMSO) δ 8.03 (m, 1 H, H5), 7.89–7.83 (m AA′, BB′, 2 H, H3′, H5′), 7.79–7.73 (m AA′, BB′, 2 H, H2′, H6′), 6.76 (d, 1 H, J = 1.92 Hz, H8), 6.61 (dd, 1 H, J = 8.24 Hz, J = 1.92 Hz, H6) ^{13}C NMR (75 MHz, DMSO) δ 166.1, 165.1, 144.0, 137.3, 133.6, 133.5, 131.4, 120.0, 119.5, 118.9, 117.8, 114.8, 104.6
3a	^{1}H NMR (300 MHz, DMSO) δ 7.86 (d, 1 H, J = 7.96 Hz, H5), 7.54–7.38 (m, 5 H, H2′, H3′, H4′, H5′, H6′), 7.33 (bs, 1 H H8), 7.31–7.26 (m, 1 H, H6), 2.46 (s, 3 H, CH_3) ^{13}C NMR (75 MHz, DMSO) δ 160.8, 152.8, 148.0, 147.9, 135.5, 129.2, 128.9, 128.9, 127.5, 126.6, 116.5, 112.5, 21.6
3b	^{1}H NMR (300 MHz, DMSO) δ 7.88–7.82 (m, 1 H, H5), 7.33–7.23 (m, 6 H, H6, H8, H2′, H3′, H5′, H6′), 2.45 (s, 3 H, CH_3), 2.36 (s, 3 H, CH_3) ^{13}C NMR (75 MHz, DMSO) δ 160.9, 152.8, 148.0, 147.9, 138.3, 132.9, 129.7, 128.6, 127.5, 126.5, 116.5, 112.5, 21.6, 21.0
3c	^{1}H NMR (300 MHz, DMSO) δ 7.86 (d, 1 H, J = 7.97 Hz, 1 H, H5), 7.62–7.54 (m, AA′, BB′, 2 H, H2′, H6′), 7.50–7.43 (m AA′, BB′, 2 H, H3′, H5′), 7.33 (bs, 1 H, H8), 7.28 (d, 1 H, J = 7.97 Hz, H6), 2.46 (s, 3 H, CH_3) ^{13}C NMR (75 MHz, DMSO) δ 160.7, 152.8, 148.0, 147.9, 134.4, 133.6, 130.9, 129.3, 127.5, 126.6, 116.5, 112.4, 21.6
3d	^{1}H NMR (300 MHz, DMSO) δ 7.87 (d, 1 H, J = 7.96 Hz, H5), 7.61–7.57 (m, 1 H, H2′), 7.57–7.52 (, 2 H, H6′, H5′), 7.46–7.40 (m, 1 H, H4′), 7.37–7.33 (m, 1 H, H8), 7.32–7.27 (m, 1 H, H6), 2.46 (s, 3 H, CH_3) ^{13}C NMR (75 MHz, DMSO) δ 160.7, 152.8, 148.1, 147.8, 136.8, 133.2, 130.9, 129.1, 129.0, 127.9, 127.5, 126.7, 116.6, 112.4, 21.6
3e	^{1}H NMR (300 MHz, DMSO) δ 7.87 (d, 1 H, J = 7.97 Hz, 1 H, H5), 7.81 (d, 1 H, J = 8.52 Hz, H5′), 7.81 (d, 1 H, J = 2.2 Hz, H2′), 7.48 (dd, 1 H, J = 8.52 Hz, J = 2.2 Hz, H6′), 7.35 (bs, 1h, H8), 7.32–7.27 (m, 1 H, H6), 2.46 (s, 3 H, CH_3) ^{13}C NMR (75 MHz, DMSO) δ 160.6, 152.7, 148.2, 147.7, 135.6, 131.9, 131.4, 131.3, 131.1, 129.6, 127.5, 126.8, 116.6, 112.3, 21.6

Table 2: (continued)

	NMR, δ
3f	^{1}H NMR (300 MHz, DMSO) δ 7.86 (d, 1 H, J = 7.96 Hz, H5), 7.76–7.66 (m AA′, BB′, 2 H, H2′, H6′), 7.44–7.36 (m AA′, BB′, 2 H, H3′, H5′), 7.33 (bs, 1 H, H8), 7.31–7.25 (m, 1 H, H6), 2.46 (s, 3 H, CH_3) ^{13}C NMR (75 MHz, DMSO) δ 160.7, 152.8, 148.0, 147.8, 134.8, 132.3, 131.2, 127.5, 126.6, 122.2, 116.5, 112.4, 21.6
3g	^{1}H NMR (300 MHz, DMSO) δ 7.86 (d, 1 H, J = 7.97 Hz, H5), 7.52–7.43 (m, 2 H, H6, H8), 7.39–7.24 (m, 4 H, H2′, H3′, H5′, H6′), 2.46 (s, 3 H, CH_3) ^{13}C NMR (75 MHz, DMSO) δ 163.7 and 160.4 (J = 245.1 Hz), 160.8, 152.8, 148.0, 131.7 and 131.6 (J = 2.9 Hz), 131.1 and 131.0 (J = 9.2 Hz), 127.5, 126.6, 116.5, 116.3 and 116.0 (J = 22.9 HZ), 112.4, 21.6
3h	^{1}H NMR (300 MHz, DMSO) δ 7.87 (d, 1 H, J = 7.97 Hz, H5), 7.61–7.50 (m, 1 H, H8), 7.41–7.26 (m, 5 H, H6, H2′, H4′, H5′, H6′), 2.47 (s, 3 H, CH_3) ^{13}C NMR (75 MHz, DMSO) δ 163.8 and 160.6 (J = 244.0 Hz), 160.7, 152.8, 148.1, 147.7, 137.0 and 136.8 (J = 10.6 Hz), 130.9 and 130.7 (J = 8.9 Hz), 127.5, 126.7, 125.4 and 125.3 (J = 3.2 Hz), 116.5 and 116.2 (J = 26.6 Hz), 116.5 and 116.2 (J = 24.1 Hz), 115.9, 112.4, 21.6
3i	^{1}H NMR (300 MHz, DMSO) δ 7.95–7.87 (m AA′, BB′, 2 H, H3′, H5′), 7.80 (d, 1 H, J = 8.24 Hz, H5), 7.71–7.63 (m AA′, BB′, 2 H, H2′, H6′), 6.72 (s, 1 H, H8), 6.58 (d, 1 H, J = 8.24 Hz, H6), 2.23 (s, 3 H, CH_3) ^{13}C NMR (75 MHz, DMSO) δ 166.6, 161.8, 144.0, 142.9, 129.9, 126.3 (d, J = 4.0 Hz), 124.7 (q, J = 279.1 Hz), 123.3 (q, J = 31.2 Hz), 120.1, 118.8, 118.3, 117.5, 116.4, 21.4
3k	^{1}H NMR (300 MHz, DMSO) δ 8.46–8.43 (m, 1 H, H2′), 8.37–8.32 (m, 1 H, H6′), 7.96–7.79 (m, 3 H, H5, H4′, H5′), 7.39–7.36 (m, 1 H, H8), 7.33–7.28 (m, 1 H, H6), 2.48 (s, 3 H, CH_3) ^{13}C NMR (75 MHz, DMSO) δ 160.8, 152.8, 148.3, 148.2, 147.8, 136.6, 163.1, 130.8, 127.5, 126.7, 124.4, 124.1, 116.6, 112.4, 21.6
3l	^{1}H NMR (300 MHz, DMSO) δ 7.84 (d, 1 H, J = 7.97 Hz, H5), 7.32–7.23 (m, 2 H, H6, H8), 7.18–7.11 (m AA′, BB′, 2 H, H2′, H6′), 6.79–6.73 (m AA′, BB′, 2 H, H3′, H5′), 2.93 (s, 6 H, $N(CH_3)_2$), 2.45 (s, 3 H, CH_3) ^{13}C NMR (75 MHz, DMSO) δ 161.1, 152.8, 150.5, 148.4, 147.7, 129.0, 127.5, 126.5, 123.8, 116.5, 112.5, 112.4, 40.4, 21.6
3m	^{1}H NMR (300 MHz, DMSO) δ 7.85 (d, 1 H, J = 7.97 Hz, 1 H, H5), 7.36–7.24 (m, 4 H, H6, H8, H2′, H6′), 7.08–6.99 (m AA′, BB′, 2 H, H3′, H5′), 3.80 (s, 3 H, OCH_3), 2.45 (s, 3 H, CH_3) ^{13}C NMR (75 MHz, DMSO) δ 161.0, 159.4, 152.8, 148.2, 147.9, 129.9, 128.0, 127.5, 126.6, 116.5, 114.4, 112.5, 55.6, 21.6
3n	^{1}H NMR (300 MHz, DMSO) δ, 8.11–8.05 (m AA′, BB′, 2 H, H3′, H5′), 7.87 (d, 1 H, J = 7.97 Hz, H5), 7.62–7.56 (m AA′, BB′, 2 H, H2′, H6′), 7.38–7.24 (m, 3 H, H8, H6), 4.35 (q, 2 H, J = 7.14 Hz, CH_2), 2.46 (s, 3 H, CH_3), 1.34 (t, 3 H, J = 7.14, CH_3) ^{13}C NMR (75 MHz, DMSO) δ 165.4, 160.7, 152.8, 148, 147.7, 139.7, 130.4, 130.1, 129.5, 127.4, 126.6, 116.5, 112.4, 61.2, 21.6, 14.4
3o	^{1}H NMR (300 MHz, $(CD_3)_2CO$) δ, 7.99–7.94 (m AA′, BB′, 2 H, H3′, H5′), 7.92 (d, 1 H, J = 7.97 Hz, H5), 7.75–7.70 (m AA′, BB′, 2 H, H2′, H6′), 7.33–7.24 (m, 2 H, H6, H8), 2.50 (s, 3 H, CH_3) ^{13}C NMR (75 MHz, $(CD_3)_2CO$) δ 161.2, 153.9, 149.2, 148.3, 140.6, 133.9, 131, 128.6, 127.3, 118.8, 117.2, 113.4, 113.2, 21.8
4a	^{1}H NMR (300 MHz, DMSO) δ 7.89 (d, 1 H, J = 8.79 Hz, H5), 7.54–7.37 (m, 5 H, H2′, H3′, H4′, H5′, H6′), 7.07 (d, 1 H, J = 2.47 Hz, H8), 7.02 (dd, 1 H, J = 8.79 Hz, J = 2.47 Hz, H6), 3.90 (s, 3 H, OCH_3) ^{13}C NMR (75 MHz, DMSO) δ 165.8, 160.4, 154.7, 148.0, 135.5, 129.2, 129.1, 128.9, 128.9, 113.3, 107.7, 100.6, 56.6
4b	^{1}H NMR (300 MHz, DMSO) δ 7.88 (d, 1 H, J = 8.79 Hz, H5), 7.32–7.23 (m, 4 H, H2′, H3′, H5′, H6′), 7.06 (d, 1 H, J = 2.47 Hz, H8), 7.01 (dd, 1 H, J = 8.79 Hz, J = 2.47 Hz, H6), 3.90 (s, 3 H, CH_3), 2.26 (s, 3 H, CH_3) ^{13}C NMR (75 MHz, DMSO) δ 165.7, 160.5, 154.6, 148.1, 138.3, 132.8, 129.7, 129.1, 128.6, 113.3, 107.8, 100.6, 56.6, 21.0
4c	^{1}H NMR (300 MHz, DMSO) δ 7.89 (d, 1 H, J = 8.79 Hz, H5), 7.61–7.54 (m AA′, BB′, 2 H, H2′, H6′), 7.49–7.41 (m AA′, BB′, 2 H, H3′, H5′), 7.08 (d, 1 H, J = 2.20 Hz, H8), 7.02 (dd, 1 H, J = 8.79 Hz, J = 2.20 Hz, H6), 3.91 (s, 3 H, CH_3) ^{13}C NMR (75 MHz, DMSO) δ 165.8, 160.4, 154.6, 147.9, 134.4, 133.5, 130.9, 129.3, 129.1, 113.3, 107.7, 100.6, 56.6
4d	^{1}H NMR (300 MHz, DMSO) δ 7.79 (d, 1 H, J = 8.79 Hz, H5), 7.61–7.51 (m, 3 H, H2′, H5′, H6′), 7.46–7.39 (m, 1 H, H4′), 7.09 (d, 1 H, J = 2.20 Hz, H8), 7.03 (dd, 1 H, J = 8.79 Hz, J = 2.20 Hz, H6), 3.91 (s, 3 H, OCH_3) ^{13}C NMR (75 MHz, DMSO) δ 165.9, 160.4, 154.7, 147.9, 136.8, 133.2, 130.9, 129.2, 129.1, 128.0, 113.5, 107.7, 100.7, 56.7
4e	^{1}H NMR (300 MHz, DMSO) δ 7.89 (d, 1 H, J = 8.79 Hz, H5), 7.81 (d, 1 H, J = 8.49 Hz, H5′), 7.81 (d, 1 H, J = 2.47 Hz, H2′), 7.48 (dd, 1 H, J = 8.49 Hz, J = 2.47 Hz, H6′), 7.10 (d, 1 H, J = 2.20 Hz, H8), 7.03 (dd, 1 H, J = 8.79 Hz, J = 2.20 Hz, H6), 3.91 (s, 3 H, CH_3) ^{13}C NMR (75 MHz, DMSO) δ 165.9, 160.3, 154.6, 147.7, 135.4, 131.9, 131.4, 131.3, 131.2, 129.7, 129.1, 113.5, 107.5, 100.7, 56.6
4f	^{1}H NMR (300 MHz, DMSO) δ 7.88 (d, 1 H, J = 8.79 Hz, H5), 7.75–7.68 (m AA′, BB′, 2 H, H2′, H6′), 7.43–7.36 (m AA′, BB′, 2 H, H3′, H5′), 7.08 (d, 1 H, J = 2.47 Hz, H8), 7.02 (dd, 1 H, J = 8.79 Hz, J = 2.48 Hz, H6), 3.90 (s, 3 H, CH_3) ^{13}C NMR (75 MHz, DMSO) δ 165.8, 160.3, 154.7, 147.9, 134.8, 132.3, 131.2, 129.1, 122.1, 113.4, 107.7, 100.7, 56.6
4g	^{1}H NMR (300 MHz, DMSO) δ 7.88 (d, 1 H, J = 8.79 Hz, H5), 7.51–7.43 (m, 2 H, H2′, H6′), 7.39–7.29 (m, 2 H, H3′, H5′), 7.08 (d, 1 H, J = 2.20 Hz, H8), 7.02 (dd, 1 H, J = 8.79 Hz, J = 2.20 Hz, H6), 3.91 (s, 3 H, OCH_3) ^{13}C NMR (75 MHz, DMSO) δ 165.8, 163.6 and 160.4 (J = 245.1 Hz), 160.5, 154.6, 148.1, 131.7 and 131.6 (J = 3.1 Hz), 131.2 and 131.0 (J = 9.1 Hz), 129.1, 116.2 and 115.9 (J = 22.9 Hz), 113.3, 107.7, 100.6, 56.6
4h	^{1}H NMR (300 MHz, DMSO) δ 7.89 (d, 1 H, J = 8.65 Hz, H5), 7.61–7.49 (m, 1 H, H6′), 7.40–7.27 (m, 3 H, H2′, H4′, H5′), 7.08 (d, 1 H, J = 2.34 Hz, H8), 7.03 (dd, 1 H, J = 8.65 Hz, J = 2.34 Hz, H6), 3.91 (s, 3 H, OCH_3) ^{13}C NMR (75 MHz, DMSO) δ 165.9, 163.8 and 160.6 (J = 244.0 Hz), 160.3, 154.6, 147.8, 137.0 and 136.8 (J = 10.9 Hz), 130.8 and 130.7 (J = 9.1 Hz), 129.1, 125.4 and 125.4 (J = 2.9 Hz), 116.6 and 116.3 (J = 23.8 Hz), 116.2 and 115.9 (J = 20.7 Hz), 113.4, 107.6, 100.7, 56.6
4i	^{1}H NMR (300 MHz, DMSO) δ 7.94–7.87 (m, 3 H, H5, H3′, H5′), 7.72–7.65 (m AA′, BB′, 2 H, H2′, H6′), 7.10 (d, 1 H, J = 2.20 Hz, H8), 7.03 (dd, 1 H, J = 8.79 Hz, J = 2.20 Hz, H6), 3.91 (s, 3 H, OCH_3) ^{13}C NMR (75 MHz, DMSO) δ 165.9, 160.3, 154.7, 147.8, 139.2, 130.2, 129.7 (q, J = 45.7 Hz), 129.1, 127.7 (q, J = 269.4 Hz), 126.4 (q, J = 3.8 Hz), 113.4, 107.7, 100.7, 56.6

Table 2: (continued)

	NMR, δ
4j	^{1}H NMR (300 MHz, DMSO) δ 8.41–8.34 (m AA′, BB′, 2 H, H3′, H5′), 7.91 (d, 1 H, J = 8.79 Hz, H5), 7.79–7.72 (m AA′, BB′, 2 H, H2′, H6′), 7.11 (d, 1 H, J = 2.19 Hz, H8), 7.04 (dd, 1 H, J = 8.79 Hz, J = 2.19 Hz, H6), 3.92 (s, 3 H, OCH_3) ^{13}C NMR (75 MHz, DMSO) δ 165.9, 160.2, 154.7, 147.6, 147.6, 141.3, 130.7, 129.1, 124.5, 113.4, 107.6, 100.7, 56.6
4k	^{1}H NMR (300 MHz, DMSO) δ 8.46–8.41 (m1 H, H2′), 8.37–8.30 (m, 1 H, H6′), 7.97–7.87 (m, 2 H, H5, H4′), 7.82 (t, 1 H, J = 7.96 Hz, H5′), 7.11 (d, 1 H, J = 2.20 Hz, H8), 7.04 (dd, 1 H, J = 8.79 Hz, 2.20 Hz, H6), 3.92 (s, 3 H, OCH_3) ^{13}C NMR (75 MHz, DMSO) δ 166.0, 160.4, 154.7, 148.3, 147.9, 136.6, 136.1, 130.7, 129.1, 124.4, 124.0, 113.5, 107.6, 100.7, 56.6
4l	^{1}H NMR (300 MHz, DMSO) δ 7.88 (d, 1 H, J = 8.52 Hz, H5), 7.35 (m AA′, BB′, 2 H, H2′, H6′), 7.08–6.98 (m, 4 H, H6, H8, H3′, H5′), 3.90 (s, 3 H, OCH_3), 3.80 (s, 3 H, OCH_3) ^{13}C NMR (75 MHz, DMSO) δ 165.7, 160.6, 159.4, 154.6, 148.2, 129.9, 129.1, 127.9, 114.4, 113.3, 107.8, 100.6, 56.6, 55.6
4n	^{1}H NMR (300 MHz, $CDCl_3$) δ 8.23–8.13 (m AA′, BB′, 2 H, H3′, H5′), 7.99 (d, 1 H, J = 8.79 Hz, H5), 7.45–7.33 (m AA′, BB′, 2 H, H2′, H6′), 6.92 (dd, 1 H, J = 8.79 Hz, J = 2.47 Hz, H6), 6.76 (d, 1 H, J = 2.47 Hz, H8), 4.39 (q, 2 H, J = 7.40 Hz, CH_2), 3.91 (s, 3 H, OCH_3), 1.38 (t, 3 H, J = 7.40 Hz, CH_3) ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.4, 165.5, 160, 154.6, 147.8, 138.1, 131.3, 130.8, 129.8, 128.4, 113.7, 106.9, 100.2, 61.2, 56.1, 14.3
4o	^{1}H NMR (300 MHz, DMSO) δ 8.10–7.95 (m AA′, BB′, 2 H, H3′, H5′), 7.90 (d, 1 H, J = 8.79 Hz, H5), 7.73–7.61 (m AA′, BB′, 2 H, H2′, H6′), 7.10 (d, 1 H, J = 2.10 Hz, H8), 7.03 (dd, 1 H, J = 8.79 Hz, J = 2.10 Hz, H6), 3.91 (s, 3 H, OCH_3) ^{13}C NMR (75 MHz, DMSO) δ 165.9, 160.2, 154.7, 147.6, 139.8, 133.4, 130.4, 129.1, 118.6, 113.4, 111.9, 107.6, 100.7, 56.6
5i	^{1}H NMR (300 MHz, DMSO) δ 8.09–8.07 (m, 1 H, H5), 8.05 (dd, 1 H, J = 8.51 Hz, J = 2.47 Hz, H7), 7.96–7.89 (m AA′, BB′, 2 H, H3′, H5′), 7.71–7.64 (m AA′, BB′, 2 H, H2′, H6′), 7.55–7.51 (m, 1 H, H8) ^{13}C NMR (75 MHz, DMSO) δ 159.8, 152.0, 147.2, 139.2, 138.9, 129.8, 129.6 (q, J = 38.9 Hz), 129.5, 126.5 (d, J = 3.7 Hz), 124.2 (q, J = 272.3 Hz), 119.3, 117.2, 117.0
5n	^{1}H NMR (300 MHz, DMSO) δ 8.12–8.01 (m, 4 H, H5, H7, H3′, H5′), 7.61–7.55 (m AA′, BB′, 2 H, H2′, H6′), 7.52 (d, 1 H, J = 8.79 Hz, H8), 4.35 (q, 2 H, J = 7.14 Hz, CH_2), 1.34 (t, 3 H, J = 7.14 Hz, CH_3) ^{13}C NMR (75 MHz, DMSO) δ 165.4, 159.8, 152.0, 147.2, 139.4, 139.1, 130.6, 130.2, 129.5, 129.3, 119.2, 117.2, 117.0, 61.3, 14.4
5o	^{1}H NMR (300 MHz, DMSO) δ 7.89–7.83 (m, 3 H, H5, H3′, H5′), 7.79–7.74 (m AA′, BB′, 2 H, H2′, H6′), 7.35–7.29 (m, 1 H, H7), 6.71 (d, 1 H, J = 8.79 Hz, H8) ^{13}C NMR (75 MHz, DMSO) δ 165.6, 163.6, 143.9, 135.6, 133.5, 131.7, 121.9, 120.5, 120.1, 119.5, 105.4, 104.7
6i	^{1}H NMR (300 MHz, DMSO) δ 7.99 (m, 4 H, H5, H8, H3′, H5′), 7.72–7.64 (m, 2 H, H2′, H6′), 7.63–7.58 (m, 1 H, H7) ^{13}C NMR (75 MHz, DMSO) δ 159.9, 151.5, 147.2, 138.9, 136.4, 129.9, 129.8, 129.6, 129.6 (q, J = 32.1 Hz), 126.5 (d, J = 3.1 Hz), 124.2 (q, J = 271.7 Hz), 118.0, 116.6
6n	^{1}H NMR (300 MHz, DMSO) δ 8.14–8.07 (m AA′, BB′, 2 H, H3′, H5′), 7.97–7.90 (m, 2 H, H5, H7), 7.61–7.55 (m, 3 H, H8, H2′, H6′), 4.35 (q, 2 H, J = 7.00 Hz, CH_2), 1.34 (t, 3 H, J = 7.00 Hz, CH_3) ^{13}C NMR (75 MHz, DMSO) δ 165.3, 159.9, 151.5, 147.2, 139.4, 136.3, 130.6, 130.2, 129.5, 129.3, 126.5, 119.0, 116.7, 61.2, 14.4
7i	^{1}H NMR (300 MHz, DMSO) δ 7.97–7.88 (m AA′, BB′, 2 H, H3′, H5′), 7.83–7.73 (m, 2 H, H5, H8), 7.73–7.66 (m AA′, BB′, 2 H, H2′, H6′), 7.66–7.59 (m, 1 H, H7) ^{13}C NMR (75 MHz, DMSO) δ 160.2, 158.8 (d, J = 243.7 Hz), 149.2 (d, J = 2.0 Hz), 147.4, 139.0, 130.0, 129.6 (q, J = 32.1 Hz), 126.5 (d, J = 3.8 Hz), 124.2 (q, J = 273.3 Hz), 124.1 (d, J = 24.6 Hz), 119.2 (d, J = 8.3 Hz), 116.3 (d, J = 8.6 HZ), 113.0 (d, J = 25.2 Hz)
7n	^{1}H NMR (300 MHz, DMSO) δ 8.15–8.04 (m, AA′, BB′, 2 H, H3′, H5′), 7.83–7.72 (m, 2 H, H5, H7), 7.66–7.53 (m AA′, BB′overlaped, 3 H, H8, H2′, H6′), 4.35 (q, 2 H, J = 6.87 Hz, CH_2), 1.34 (t, 3 H, J = 6.87 Hz, CH_3) ^{13}C NMR (75 MHz, DMSO) δ 165.4, 158.8 (d, J = 243.4 Hz), 160.1, 149.2 (d, J = 2.0 Hz), 147.3, 139.4, 130.5, 130.2, 129.3, 124.1 (d, J = 24.7 Hz), 119.2 (d, J = 8.3 Hz), 116.4 (d, J = 8.6 Hz), 113.0 (d, J = 25.2 Hz), 61.2, 14.4
7o	^{1}H NMR (300 MHz, DMSO) δ 8.06–8.00 (m AA′, BB′, 2 H, H3′, H5′), 7.83–7.73 (m, 2 H, H5, H7), 7.70–7.59 (m AA′, BB′ overlaped, 3 H, H8, H2′, H6′) ^{13}C NMR (75 MHz, DMSO) δ 160.0 (d, J = 2.9 Hz), 158.8 (d, J = 243.4 HZ), 157.2, 149.2 (d, J = 2.0 Hz), 147.2, 139.5, 133.6, 130.2, 124.2 (d, J = 24.6 Hz), 119.2 (d, J = 8.3 Hz), 116.3 (d, J = 8.3 Hz), 113.0 (d, J = 25.2 Hz), 112.1
8g	^{1}H NMR (300 MHz, DMSO) δ 7.79 (d, 1 H, J = 2.06 Hz, H5) , 7.67 (dd, 1 H, J = 8.52 Hz, J = 2.06 Hz, H7), 7.53–7.43 (m, 2 H, H2′, H6′), 7.39 (d overlaped, 1 H, J = 8.52 Hz, H8), 7.39–7.30 (m, 2 H, H3′, H5′), 2.40 (s, 3 H, CH_3) ^{13}C NMR (75 MHz, DMSO) δ 161.0, 162.1 (d, J = 243.3 Hz), 150.9, 147.9, 137.3, 135.1, 131.7 (d, J = 3.2 Hz) , 131.1 (d, J = 8.8 Hz), 127.1, 116.3, 116.2 (d, J = 22.8 Hz), 114.6, 20.3
8h	^{1}H NMR (300 MHz, DMSO) δ 7.81–7.78 (m, 1 H, H5) , 7.70–7.65 (m, 1 H, H7), 7.61–7.51 (m, 1 H, H2′), 7.40 (d overlaped, J = 8.51 Hz, 1 H , H8), 7.40–7.28 (m, 3 H, H4′, H5′, H6′), 2.40 (s, 3 H, CH_3) ^{13}C NMR (75 MHz, DMSO) δ 160.8, 162.1 (d, J = 242.5 Hz), 150.9, 147.7, 137.4, 137.0 (d, J = 10.5 Hz), 135.2, 127.1, 130.9 (d, J = 9.1 Hz), 125.4 (d, J = 3.2 Hz), 116.5, 116.1 (d, J = 19.7 Hz), 114.5, 20.3
8i	^{1}H NMR (300 MHz, DMSO) δ 7.95–7.88 (m AA′, BB′, 2 H, H3′, H5′), 7.82–7.78 (m, 1 H, H5), 7.73–7.65 (m, 3 H, H7, H2′, H6′), 7.41 (d, 1 H, J = 8.52 Hz, H8), 2.41 (s, 3 H, CH_3) ^{13}C NMR (75 MHz, DMSO) δ 160.9, 150.9, 147.7, 139.3, 137.4, 135.2, 130.1, 129.4 (q, J = 31.8 Hz), 128.1 (q, J = 272.3 Hz), 127.1, 126.4 (d, J = 3.7 Hz), 116.5, 114.6, 20.3
8n	^{1}H NMR (300 MHz, DMSO) δ 8.12–8.05 (m AA′, BB′, 2 H, H3′, H5′), 7.82–7.77 (m, 1 H, H5), 7.70–7.65 (m, 1 H, H7), 7.62–7.56 (m AA′, BB′, 2 H, H2′, H6′), 7.40 (d, 1 H, J = 8.24 Hz, H8), 4.35 (q, 2 H, J = 7.14 Hz, CH_2), 2.40 (s, 3 H, CH_3), 1.34 (t, 3 H, J = 7.14 Hz, CH_3) ^{13}C NMR (75 MHz, DMSO) δ 165.4, 160.8, 150.9, 147.6, 139.7, 137.4, 135.2, 130.4, 130.1, 129.5, 127.1, 116.5, 114.6, 61.2, 20.3, 14.4

Table 2: (continued)

	NMR, δ
8o	^{1}H NMR (300 MHz, DMSO) δ 8.06–7.98 (m AA′, BB′, 2 H, H3′, H5′), 7.83–7.77 (m, 1 H, H5), 7.72–7.64 (m, 3 H, H7, H2′, H6′), 7.41 (d, 1 H, J = 8.52 Hz, H8), 2.40 (s, 3 H, CH_3) ^{13}C NMR (75 MHz, DMSO) δ 160.7, 150.9, 147.5, 139.8, 137.5, 135.3, 133.5, 130.3, 127.1, 118.6, 116.5, 114.5, 111.9, 20.3
9a	^{1}H NMR (300 MHz, DMSO) δ 7.56–7.35 (m, 8 H, H5, H7, H8, H2′, H3′, H4′, H5′, H6′), 3.84 (s, 3 H, OCH_3) ^{13}C NMR (75 MHz, DMSO) δ: 160.8, 156.4, 147.9, 147.0, 135.5, 129.2, 128.9, 128.8, 124.2, 118.1, 115.4, 108.9, 56.2
9b	^{1}H NMR (300 MHz, DMSO) δ 7.48–7.40 (m, 2 H, H7, H8) , 7.40–7.35 (m, 1 H, H5), 7.32–7.25 (m, 4 H, H2′, H3′, H5′, H6′), 3.83 (s, 3 H, OCH_3), 2.36 (s, 3 H, CH_3) ^{13}C NMR (75 MHz, DMSO) δ 160.9, 156.4, 147.9, 147.0, 138.4, 132.9, 129.7, 128.5, 124.2, 118.1, 115.4, 108.9, 56.2, 21.0
9c	^{1}H NMR (300 MHz, DMSO) δ 7.63–7.55 (m AA′, BB′, 2 H, H2′, H6′) 7.50–7.43 (m AA′, BB′ overlaped, 4 H, H7, H8, H3′, H5′), 7.38 (dd, J = 2.41 Hz, J = 0.87 Hz, 1 H, H5), 3.84 (s, 3 H, OCH_3) ^{13}C NMR (75 MHz, DMSO) δ 160.8, 156.4, 147.7, 147.0, 134.4, 133.6, 130.8, 129.4, 124.3, 118.2, 115.4, 108.9, 56.2
9d	^{1}H NMR (300 MHz, DMSO) δ 7.61–7.53 (m, 3 H, H2′, H7, H8) , 7.48–7.36 (m, 4 H, H5, H4′, H5′, H6′), 3.84 (s, 3 H, OCH_3) ^{13}C NMR (75 MHz, DMSO) δ 160.7, 156.5, 147.7, 147.0, 136.9, 133.2, 130.9, 129.1, 129.0, 127.9, 124.3, 118.2, 115.3, 108.9, 56.2
9e	^{1}H NMR (300 MHz, DMSO) δ7.87–7.78 (m, 2 H, H2′, H6′), 7.53–7.42 (m, 3 H, H7, H8, H5′), 7.41–7.36 (m, 1 H, H5), 3.84 (s, 3 H, OCH_3) ^{13}C NMR (75 MHz, DMSO) δ 160.7, 156.5, 147.5, 146.9, 135.4, 131.9, 131.4, 131.3, 131.1, 129.6, 124.4, 118.2, 115.3, 108.9, 56.2
9f	^{1}H NMR (300 MHz, DMSO) δ 7.76–7.68 (m AA′, BB′, 2 H, H2′, H6′), 7.48–7.33 (m, 5 H, H5, H7, H8, H3′, H5′), 3.84 (s, 3 H, OCH_3) ^{13}C NMR (75 MHz, DMSO) δ 160.7, 156.5, 147.7, 147.0, 134.9, 132.3, 131.1, 124.3, 122.2, 118.2, 115.4, 108.9, 56.2
9g	^{1}H NMR (300 MHz, DMSO) δ 7.52–7.43 (m, 4 H, H7, H8, H2′, H6′), 7.40–7.30 (m, 3 H, H5, H3′, H5′), 3.84 (s, 3 H, OCH_3) ^{13}C NMR (75 MHz, DMSO) δ 162.5 (d, J = 244.8 Hz), 161.4, 156.9, 148.4, 147.5, 132.2 (d, J = 2.8 Hz), 131.4 (d, J = 9.1 Hz), 124.7, 118.6, 116.7 (d, J = 22.9 Hz), 115.9, 109.4, 56.7
9h	^{1}H NMR (300 MHz, DMSO) δ 7.63–7.49 (m, 1 H, H2′), 7.47–7.44 (m, 2 H, H7, H8), 7.40–7.27 (m, 4 H, H5, H4′, H5′, H6′), 3.84 (s, 3 H, OCH_3) ^{13}C NMR (75 MHz, DMSO) δ 162.2 (d, J = 244.3 Hz), 160.7, 156.5, 147.6, 147.0, 137.0 (d, J = 10.9 Hz), 130.9 (d, J = 8.9 Hz), 125.3 (d, J = 3.2 Hz), 124.3, 118.2, 116.3 (d, J = 23.5 Hz), 116.1 (d, J = 20.6 Hz), 115.3, 108.9, 56.2
9i	^{1}H NMR (300 MHz, DMSO) δ 7.95–7.88 (m AA′, BB′, 2 H, H3′, H5′), 7.73–7.65 (m AA′, BB′, 2 H, H2′, H6′), 7.49–7.45 (m, 2 H, H5, H7), 7.41–7.37 (m, 1 H, H8), 3.84 (s, 3 H, OCH_3) ^{13}C NMR (75 MHz, DMSO) δ 160.8, 156.5, 147.7, 147.1, 139.3, 130.1, 129.5 (q, J = 32.1 Hz), 126.5 (q, 3.8 Hz), 124.4, 124.2 (q, J = 272.3 Hz), 118.2, 115.4, 109.0, 56.2
9j	^{1}H NMR (300 MHz, DMSO) δ 8.43–8.35 (m AA′, BB′, 2 H, H3′, H5′), 7.80–7.72 (m AA′, BB′, 2 H, H2′, H6′), 7.51–7.44 (m, 2 H, H7, H8), 7.39 (d, J = 2.71 Hz, 1 H, H5), 3.85 (s, 3 H, OCH_3) ^{13}C NMR (75 MHz, DMSO) δ 160.7, 156.5, 147.7, 147.4, 147.0, 141.3, 130.6, 124.6, 124.4, 118.2, 115.3, 108.9, 56.2
9k	^{1}H NMR (300 MHz, DMSO) δ 8.44 (t, 1 H, J = 2.20 Hz, H2′), 8.35 (ddd, 1 H, J = 8.10 Hz, J = 2.20 Hz, J = 1.10 Hz, H4′), 7.84 (t, 1 H, J = 8.10 Hz, H5′), 7.94 (dm, 1 H, H6′), 7.40 (d, 1 H, J = 2.72 Hz, H5), 7.50–7.46 (m, 2 H, H7, H8), 3.85 (s, 3 H, OCH_3) ^{13}C NMR (75 MHz, DMSO) δ 160.8, 156.7, 148.3, 147.7, 147.0, 136.6, 136.0, 130.8, 124.4, 124.4, 124.1, 118.2, 115.3, 109.0, 56.2
9l	^{1}H NMR (300 MHz, DMSO) δ 7.46–7.33 (m, 3 H, H5, H7, H8), 7.20–7.10 (m AA′, BB′, 2 H, H2′, H6′), 6.82–6.72 (m AA′, BB′, 2 H, H3′, H5′), 3.83 (s, 3 H, OCH_3), 2.94 (s, 6 H, $N(CH_3)_2$) ^{13}C NMR (75 MHz, DMSO) δ 161.1, 156.3, 150.5, 148.3, 147.0, 128.9, 124.1, 123.8, 118.1, 115.4, 112.3, 108.9, 56.1, 40.4
9m	^{1}H NMR (300 MHz, DMSO) δ 7.45–7.41 (m, 2 H, H7, H8), 7.38–7.35 (m, 1 H, H5), 7.34–7.28 (m AA′, BB′, 2 H, H2′, H6′), 7.07–6.99 (m AA′, BB′, 2 H, H3′, H5′), 3.83 (s, 3 H, OCH_3), 3.80 (s, 3 H, OCH_3) ^{13}C NMR (75 MHz, DMSO) δ 161.0, 159.4, 156.4, 148.1, 147.0, 129.8, 128.0, 124.2, 118.1, 115.4, 114.4, 108.9, 56.2, 55.6
9n	^{1}H NMR (300 MHz, DMSO) δ 8.14–8.03 (m AA′, BB′, 2 H, H3′, H5′), 7.63–7.54 (m AA′, BB′, 2 H, H2′, H6′), 7.49–7.41 (m, 2 H, H5, H7), 7.41–7.36 (m, 1 H, H8), 4.35 (q, 2 H, J = 7.14 Hz, CH_2), 3.84 (s, 3 H, OCH_3), 1.34 (t, 3 H, J = 7.14 Hz, CH_3) ^{13}C NMR (75 MHz, DMSO) δ 165.4, 160.7, 156.5, 147.6, 147.1, 139.7, 130.5, 130.1, 129.4, 124.3, 118.2, 115.4, 108.9, 61.2, 56.2, 14.4
9o	^{1}H NMR (300 MHz, DMSO) δ 8.07–7.99 (m AA′, BB′, 2 H, H3′, H5′), 7.71–7.64 (m AA′, BB′, 2 H, H2′, H6′), 7.48–7.45 (m, 2 H, H5, H7), 7.40–7.38 (m, 1 H, H8), 3.84 (s, 3 H, OCH_3) ^{13}C NMR (75 MHz, DMSO) δ 160.6, 156.5, 147.5, 147.0, 139.8, 133.5, 130.3, 124.4, 118.6, 118.2, 115.3, 112.0, 108.9, 56.2

ble 4. The numbers of compounds in the equations analysing structure-activity relationships against various strains is different, as in a number of cases, the minimum inhibitory concentration could not be determined due to the limited solubility of the compounds.

$$\log \mathrm{MIC}_{\mathrm{M\,tuber.\,'14\,d}} = -0.661(\pm 0.083)\,\sigma - 0.221(\pm 0.049) \times \pi + \mathrm{I_n} + 1.750(\pm 0.082) \quad (1)$$

$r = 0.823 \quad s = 0.280 \quad n = 121 \quad F = 20.86$

$$\log \mathrm{MIC}_{\mathrm{M\,tuber.\,'21\,d}} = -0.672(\pm 0.084)\,\sigma - 0.262(\pm 0.049) \times \pi + \mathrm{I_n} + 1.878(\pm 0.081) \quad (2)$$

$r = 0.836 \quad s = 0.276 \quad n = 119 \quad F = 22.62$

$$\log \mathrm{MIC}_{\mathrm{M\,kans.\,14\,d}} = -0.410(\pm 0.097)\,\sigma - 0.005(\pm 0.005) \times \pi + \mathrm{I_n} + 1.570(\pm 0.081) \quad (3)$$

$r = 0.773 \quad s = 0.326 \quad n = 111 \quad F = 13.39$

Table 3: Minimum inhibitory concentrations of benzoxazines substituted in positions 6 and 7

Compd.	MIC (µmol/l) Incubation 14 d/21 d				
	R_1	R_2	*M. tuberculosis*	*M. kansasii*	*M. avium*
1a[c]	H	H	125/125	125/250	62/125
1b[c]	H	4-CH_3	62/62	125/125	31/62
1c[c]	H	4-Cl	16/31	16/16	62/62
1d[c]	H	3-Cl	31/31	8/8	31/31
1e[c]	H	3,4-Cl_2	8/8	4/4	8/16
1f[c]	H	4-Br	31/31	16/31	31/31
1g[c]	H	4-F	125/125	62/62	62/125
1h[c]	H	3-F	31/62	31/62	62/125
1i[c]	H	4-CF_3	8/8	8/16	16/16
1j[c]	H	4-NO_2	16/16	16/16	16/31
1k[c]	H	3-NO_2	16/31	31/62	16/31
1m[c]	H	4-OCH_3	62/125	125/250	31/31
1n[c]	H	4-COOEt	8/16	32/32	32/62.5
1o[c]	H	4-CN	32/32	32/62.5	32/62.5
2a	7-Cl	H	8/16	4/8	8/8
2b	7-Cl	4-CH_3	8/8	8/8	8/8
2c	7-Cl	4-Cl	4/4	4/4	16/16
2d	7-Cl	3-Cl	4/4	4/4	8/8
2e	7-Cl	3,4-Cl_2	4/4	4/8	8/8
2f	7-Cl	4-Br	8/8	4/4	8/8
2g	7-Cl	4-F	8/16	16/16	16/32
2h	7-Cl	3-F	8/16	16/16	16/16
2i	7-Cl	4-CF_3	2/2	4/4	8/8
2k	7-Cl	3-NO_2	8/8	16/16	16/16
2l	7-Cl	4-$N(CH_3)_2$	8/8	*a/a*	31/62
2m	7-Cl	4-OCH_3	*a/a*	16/62	8/8
2n	7-Cl	4-COOEt	8/8	8/8	16/32
2o	7-Cl	4-CN	8/8	8/16	16/16
3a	7-CH_3	H	31/62	62/250	31/62
3b	7-CH_3	4-CH_3	62/125	62/125	31/*a*
3c	7-CH_3	4-Cl	31/62	31/*a*	8/8
3d	7-CH_3	3-Cl	16/62	62/*a*	16/31
3e	7-CH_3	3,4-Cl_2	8/16	8/16	8/31
3f	7-CH_3	4-Br	16/62	16/*a*	16/16
3g	7-CH_3	4-F	32/32	62.5/*a*	62.5/62.5
3h	7-CH_3	3-F	8/16	*a/a*	32/32
3i	7-CH_3	4-CF_3	32/*a*	*a/a*	*a/a*
3k	7-CH_3	3-NO_2	4/8	16/16	16/*a*
3l	7-CH_3	4-$N(CH_3)_2$	31/31	*a/a*	62/62
3m	7-CH_3	4-OCH_3	16/31	125/*a*	31/62
3n	7-CH_3	4-COOEt	8/8	*a/a*	*a/a*
3o	7-CH_3	4-CN	16/16	32/32	16/32
4a	7-OCH_3	H	16/32	125/250	125/125
4b	7-OCH_3	4-CH_3	16/32	125/125	32/62.5
4c	7-OCH_3	4-Cl	16/32	62.5/62.5	32/32
4d	7-OCH_3	3-Cl	8/8	62.5/62.5	32/32
4e	7-OCH_3	3,4-Cl_2	8/16	*a/a*	32/32

Table 3: (continued)

Compd.	MIC (µmol/l) Incubation 14 d/21 d				
	R_1	R_2	*M. tuberculosis*	*M. kansasii*	*M. avium*
4f	7-OCH_3	4-Br	32/32	*a/a*	*a/a*
4g	7-OCH_3	4-F	16/32	*a/a*	62.5/62.5
4h	7-OCH_3	3-F	8/16	62,5/62,5	32/62,5
4i	7-OCH_3	4-CF_3	16/32	16/32	16/16
4j	7-OCH_3	4-NO_2	16/16	32/a	32/32
4k	7-OCH_3	3-NO_2	8/8	*a/a*	*a/a*
4m	7-OCH_3	4-OCH_3	16/16	*a/a*	*a/a*
4n	7-OCH_3	4-COOEt	2/8	*a/a*	*a/a*
4o	7-OCH_3	4-CN	32/32	62.5/125	62.5/62.5
5a[d]	6-Br	H	31/31	31/62	62/62
5b[d]	6-Br	4-CH_3	16/16	62/62	31/31
5c[d]	6-Br	4-Cl	8/8	8/8	16/16
5d[d]	6-Br	3-Cl	4/8	8/8	16/16
5e[d]	6-Br	3,4-Cl_2	4/4	4/4	16/16
5f[d]	6-Br	4-Br	4/4	8/8	16/16
5g[d]	6-Br	4-F	16/16	16/16	31/31
5h[d]	6-Br	3-F	8/16	8/8	31/31
5i	6-Br	4-CF_3	1/1	1/1	4/4
5k[d]	6-Br	3-NO_2	8/8	16/16	31/31
5n	6-Br	4-COOEt	4/4	16/16	32/32
5o	6-Br	4-CN	2/4	8/8	8/8
6a[e]	6-Cl	H	31/31	4/4	16/31
6b[e]	6-Cl	4-CH_3	16/16	4/8	8/16
6c[e]	6-Cl	4-Cl	4/4	4/4	8/8
6d[d]	6-Cl	3-Cl	8/16	4/4	8/8
6e[e]	6-Cl	3,4-Cl_2	4/4	4/4	8/8
6f[e]	6-Cl	4-Br	4/4	4/4	4/8
6g[e]	6-Cl	4-F	8/8	4/4	16/31
6h[d]	6-Cl	3-F	16/16	4/8	16/16
6i	6-Cl	4-CF_3	1/2	4/4	8/8
6j[d]	6-Cl	4-NO_2	8/8	4/4	8/8
6k[d]	6-Cl	3-NO_2	8/8	4/8	16/16
6l[d]	6-Cl	4-$N(CH_3)_2$	31/*a*	4/8	62/*a*
6m[d]	6-Cl	4-OCH_3	62/62	4/4	16/31
6n	6-Cl	4-COOEt	8/16	16/32	32/32
7a[d]	6-F	H	31/62	8/16	31/31
7b[d]	6-F	4-CH_3	16/31	8/8	16/31
7c[d]	6-F	4-Cl	8/8	8/31	8/16
7d[d]	6-F	3-Cl	8/16	4/8	16/31
7e[d]	6-F	3,4-Cl_2	8/8	4/4	8/8
7f[d]	6-F	4-Br	4/8	8/8	8/16
7g[d]	6-F	4-F	31/31	8/16	31/31
7h[d]	6-F	3-F	16/16	16/31	31/62
7i	6-F	4-CF_3	2/2	4/4	8/8
7k[d]	6-F	3-NO_2	16/31	16/31	16/31
7m[d]	6-F	4-OCH_3	31/31	16/62	16/31
7n	6-F	4-COOEt	8/16	16/16	32/32
7o	6-F	4-CN	8/16	32/32	32/62.5

Table 3: (continued)

Compd.	MIC (μmol/l) Incubation 14 d/21 d				
	R_1	R_2	*M.tuberculosis*	*M. kansasii*	*M. avium*
8a	6-CH_3	H	32/125	32/125	32/62.5
8b	6-CH_3	4-CH_3	62./62.5	32/a	62.5/62.5
8c	6-CH_3	4-Cl	16/16	16/16	16/32
8d	6-CH_3	3-Cl	4/16	4/16	16/32
8e	6-CH_3	3,4-Cl_2	8/16	8/16	8/16
8f	6-CH_3	4-Br	16/16	16/32	16/*a*
8g	6-CH_3	4-F	*b/b*	62.5/125	*b/b*
8h	6-CH_3	3-F	*b/b*	62.5/62.5	*b/b*
8i	6-CH_3	4-CF_3	4/8	8/8	8/8
8j	6-CH_3	4-NO_2	4/4	8/16	16/16
8k	6-CH_3	3-NO_2	16/32	32/32	32/32
8l	6-CH_3	4-$N(CH_3)_2$	*a/a*	*a/a*	*a/a*
8m	6-CH_3	4-OCH_3	125/125	125/125	62.5/*a*
8n	6-CH_3	4-COOEt	*a/a*	*a*/>1000	*a/a*
8o	6-CH_3	4-CN	8/16	32/32	32/32
9a	6-OCH_3	H	125/250	*a/a*	125/125
9b	6-OCH_3	4-CH_3	62.5/125	*a/a*	62.5/125
9c	6-OCH_3	4-Cl	62.5/62.5	62.5/125	62,5/125
9d	6-OCH_3	3-Cl	62,5/125	125/125	125/125
9e	6-OCH_3	3,4-Cl_2	62.5/*a*	*a/a*	*a/a*
9f	6-OCH_3	4-Br	62.5/*a*	*a/a*	*a/a*
9g	6-OCH_3	4-F	125/*a*	*a/a*	62.5/125
9h	6-OCH_3	3-F	62.5/125	125/250	125/125
9i	6-OCH_3	4-CF_3	8/8	32/32	32/32
9j	6-OCH_3	4-NO_2	32/32	*a/a*	*a/a*
9k	6-OCH_3	3-NO_2	*a/a*	*a/a*	*a/a*
9m	6-OCH_3	4-OCH_3	*a/a*	*a/a*	*a/a*
9n	6-OCH_3	4-COOEt	16/32	*a/a*	*a/a*
9o	6-OCH_3	4-CN	32/62.5	*a/a*	32/*a*
10a[d]	6-NO_2	H	62/62	31/31	500/500
10b[d]	6-NO_2	4-CH_3	62/62	31/62	250/250
10c[d]	6-NO_2	4-Cl	16/31	16/31	125/125
10d[d]	6-NO_2	3-Cl	16/31	16/16	*a/a*
10e[d]	6-NO_2	3,4-Cl_2	31/31	8/16	125/125
10f[d]	6-NO_2	4-Br	31/31	8/16	125/125
10g[d]	6-NO_2	4-F	31/31	31/62	500/500
10h[d]	6-NO_2	3-F	31/31	31/31	250/250
10k[d]	6-NO_2	3-NO_2	62/125	16/31	250/250
10l[d]	6-NO_2	4-$N(CH_3)_2$	*a/a*	125/*a*	*a/a*
10m[d]	6-NO_2	4-OCH_3	125/250	62/62	1000/ >1000
	INH		2/4	250/500	250/500

a: MIC could not be determined because of low solubility
b: the compound was not tested against the given strain
c: data from ref. [7] , d: data from ref. [9], e: data from ref. [16]

$$\log \mathrm{MIC}_{\text{M. kans. 21 d}} = -0.564(\pm 0.109)\,\sigma - 0.003(\pm 0.006) \times \pi + \mathrm{I_n} + 1.768(\pm 0.100) \quad (4)$$
$$r = 0.782 \quad s = 0.351 \quad n = 104 \quad F = 13.18$$

$$\log \mathrm{MIC}_{\text{M. avium 14 d}} = -0.272(\pm 0.064)\,\sigma - 0.247(\pm 0.039) \times \pi + \mathrm{I_n} + 1.677(\pm 0.063) \quad (5)$$
$$r = 0.891 \quad s = 0.216 \quad n = 114 \quad F = 35.65$$

$$\log \mathrm{MIC}_{\text{M. avium 21 d}} = -0.286(\pm 0.071)\,\sigma - 0.258(\pm 0.040) \times \pi + \mathrm{I_n} + 1.878(\pm 0.063) \quad (6)$$
$$r = 0.886 \quad s = 0.214 \quad n = 107 \quad F = 31.57$$

Using the indicator parameters, the biological activity can be predicted, but only within the framework of the selection of the employed substituents. Thus, we attempted to find correlations with physical and chemical parameters. The influence of the substituents on the phenyl ring was again expressed with the σ and π constants. The electronic influence of the substituents in benzoxazine B ring on the oxygen atom in position 1 and oxo group in position 4 was expressed as the corresponding Hammett constant values with regard to both centers, and the hydrophobic constant π^- (with regard to the oxygen atom) and molecular refraction (MR) values were used as additional parameters. The use of all the above parameters was necessary, as we attempted to find the most active compounds. The results are summarised in equations (7)–(12). A classical Hansch-type equation, using the second power of the hydrophobic parameters, was not statistically significant.

$$\log \mathrm{MIC}_{\text{M. tuber. 14 d}} = 0.312(\pm 0.141)\,(\sigma_1)_{CO} - 0.081(\pm 0.149)\,(\sigma_1)_{OH} - 0.709(\pm 0.081)\,\pi_1^- - 0.665(\pm 0.088)\,\sigma_2 - 0.212(\pm 0.053)\,\pi_2 + 0.030(\pm 0.011) \times \mathrm{MR} + 1.625(\pm 0.075) \quad (7)$$
$$r = 0.771 \quad s = 0.305 \quad n = 121 \quad F = 28.35$$

$$\log \mathrm{MIC}_{\text{M. tuber. 21 d}} = 0.261(\pm 0.146)\,(\sigma_1)_{CO} - 0.055(\pm 0.155)\,(\sigma_1)_{OH} - 0.730(\pm 0.084)\,\pi_1^- - 0.693(\pm 0.092)\,\sigma_2 - 0.261(\pm 0.054)\,\pi_2 + 0.027(\pm 0.012) \times \mathrm{MR} + 1.805(\pm 0.076) \quad (8)$$
$$r = 0.786 \quad s = 0.304 \quad n = 119 \quad F = 30.24$$

$$\log \mathrm{MIC}_{\text{M. kans. 14 d}} = 0.447(\pm 0.195)\,(\sigma_1)_{CO} - 0.311(\pm 0.213)\,(\sigma_1)_{OH} - 0.837(\pm 0.103)\,\pi_1^- - 0.359(\pm 0.103)\,\sigma_2 - 0.005(\pm 0.005)\,\pi_2 + 0.089(\pm 0.017) \times \mathrm{MR} + 1.329(\pm 0.084) \quad (9)$$
$$r = 0.721 \quad s = 0.347 \quad n = 111 \quad F = 18.74$$

$$\log \mathrm{MIC}_{\text{M. kans. 21 d}} = 0.412(\pm 0.224)\,(\sigma_1)_{CO} - 0.064(\pm 0.228)\,(\sigma_1)_{OH} - 0.931(\pm 0.117)\,\pi_1^- - 0.529(\pm 0.112)\,\sigma_2 - 0.003(\pm 0.005)\,\pi_2 + 0.070(\pm 0.017) \times \mathrm{MR} + 1.681(\pm 0.088) \quad (10)$$
$$r = 0.751 \quad s = 0.362 \quad n = 104 \quad F = 20.95$$

$$\log \mathrm{MIC}_{\text{M. avium. 14 d}} = 0.427(\pm 0.125)\,(\sigma_1)_{CO} - 0.627(\pm 0.132)\,(\sigma_1)_{OH} - 0.950(\pm 0.069)\,\pi_1^- - 0.268(\pm 0.072)\,\sigma_2 - 0.246(\pm 0.044)\,\pi_2 + 0.072(\pm 0.010) \times \mathrm{MR} + 1.548(\pm 0.061) \quad (11)$$
$$r = 0.850 \quad s = 0.245 \quad n = 114 \quad F = 46.43$$

$$\log \mathrm{MIC}_{\text{M. avium. 21 d}} = 0.465(\pm 0.127)\,(\sigma_1)_{CO} - 0.421(\pm 0.135)\,(\sigma_1)_{OH} - 0.972(\pm 0.071)\,\pi_1^- - 0.279(\pm 0.079)\,\sigma_2 - 0.259(\pm 0.045)\,\pi_2 + 0.058(\pm 0.010) \times \mathrm{MR} + 1.763(\pm 0.062) \quad (12)$$
$$r = 0.845 \quad s = 0.240 \quad n = 107 \quad F = 41.68$$

Table 4: Regression coefficient of indicator parameters I_n in equations (1)–(12)

Equation		*M. tuber.* 14 d	*M. tuber.* 21 d	*M. kans.* 14 d	*M. kans.* 21 d	*M. avium* 14 d	*M. avium* 21 d
(1)–(6)	I(5-Br)	−0.633 (±0.110)	−0.660 (±0.108)	−0.448 (±0.128)	−0.567 (±0.138)	−0.178 (±0.087)	−0.368 (±0.086)
(1)–(6)	I(5-Cl)	−0.536 (±0.106)	−0.548 (±0.107)	−0.834 (±0.124)	−0.892 (±0.133)	−0.405 (±0.082)	−0.504 (±0.082)
(1)–(6)	I(5-F)	−0.411 (±0.108)	−0.356 (±0.107)	−0.477 (±0.125)	−0.427 (±0.135)	−0.253 (±0.083)	−0.264 (±0.082)
(1)–(6)	I(5-NO_2)	−0.086 (±0.116)	0.099 (±0.115)	−0.072 (±0.131)	−0.153 (±0.146)	0.925 (±0.093)	0.694 (±0.095)
(1)–(6)	I(5-CH_3)	−0.191 (±0.113)	−0.185 (±0.111)	−0.099 (±0.125)	−0.040 (±0.138)	−0.125 (±0.087)	−0.201 (±0.092)
(1)–(6)	I(5-OCH_3)	0.270 (±0.110)	0.267 (±0.118)	0.548 (±0.208)	0.515 (±0.224)	0.326 (±0.096)	0.320 (±0.099)
(1)–(6)	I(4-Cl)	−0.654 (±0.113)	−0.660 (±0.109)	−0.593 (±0.125)	−0.633 (±0.135)	−0.435 (±0.083)	−0.580 (±0.082)
(1)–(6)	I(4-CH_3)	−0.138 (±0.113)	−0.082 (±0.109)	0.053 (±0.139)	0.118 (±0.183)	−0.193 (±0.087)	−0.189 (±0.092)
(1)–(6)	I(4-OCH_3)	−0.284 (±0.108)	−0.244 (±0.106)	0.333 (±0.144)	0.318 (±0.163)	0.121 (±0.090)	−0.012 (±0.088)

The study has shown that benzoxazines are broad spectrum antimycobacterial compounds, for which a mechanism of action different from that of standard antituberculotic agents can be expected. The most active derivatives are comparable to INH as regards the antimycobacterial activity against *M. tuberculosis*. More importantly, the activity of the compounds against conditionally pathogenic strains is superior to that of the drug standard. Compounds **1i**, **2c**, **2d**, **5i**, **6i**, **7i** and **8i** were selected for preclinical screening. However, they displayed substantial cytotoxicity.

3. Experimental

3.1. 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}. The bonding vibrations of the C=O group [ν(C=O)] were found in the region 1693–1716 cm^{-1} and 17641779 cm^{-1}, characteristic for 3-phenyl-2*H*-1,3-benzoxazine-2,4(3*H*)-diones. TLC was performed on silica gel plates precoated with a fluorescent indicator Silufol UV 254 + 366 (Kavalier, Votice, Czech Republic), with cyclohexane-acetone (3 : 1) as the mobile phase. The ^{1}H 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. Chemical shifts were recorded as δ values in parts per millions (ppm) and were indirectly referenced to tetramethylsilane *via* the solvent signal (2.49 for ^{1}H or 39.7 for ^{13}C). NMR data are summarised in Table 2.

The starting salicylanilides are described in previous papers [10, 11]. The title compounds **1**–**9** were synthesized from the corresponding salicylanilides. Ethyl chloroformate (5.2 g, 48 mmol) was added dropwise to a stirred solution of the salicylanilide (40 mmol) in dry pyridine (20 ml) under ice-cooling. The mixture was heated on a steam bath for 1 h and then poured into 5% hydrochloric acid (140 ml). After 24 h, the product was filtered off, suspended in 5% potassium hydroxide solution, filtered off again, and crystallized from EtOH (yield 55–65%).

3.2. Microbiology

For the *in vitro* evaluation of antimycobacterial activity of the compounds, 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 ula liquid 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 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 (or 32.5), 31, 16, 8, 4, 2, 1 μmol/l. The minimum inhibitory concentrations were determined after incubation at 37 °C for 14 and 21 days (see Table 3). MIC was the lowest concentration of an antimycobacterially effective substance (on the above-stated concentration scale), at which inhibition of the growth of the mycobacteria occurred.

3.3. Calculations

All calculations were carried out with the Multireg H programme (Klemera) for Microsoft Excel®. The values of the Hammett constants (Tables 5 and 6) were taken from ref. [12] and the values of MR from ref. [13].

Table 5: Physical parameters of substituents R_1

Substituent R_1	Hammett subst. konst. σ_m	Hammett subst. konst. σ_p	Hydrophobic subst. konst. π	Hydrophobic subst. konst. π^-	Molar refractivity MR[13]
H	0	0	0	0	1,03
6-Br	0,39	0,23	1,19	1,13	8,88
6-Cl	0,37	0,23	0,73	0,93	6,03
6-F	0,34	0,06	0,15	0,31	0,92
6-NO_2	0,71	0,78	0,02	0,45	7,36
6-CH_3	−0,07	−0,17	0,6	0,48	5,65
6-OCH_3	0,12	−0,27	−0,03	−0,12	7,87
7-Cl	0,23	0,37	0,77	1,04	6,03
7-CH_3	−0,17	−0,07	0,52	0,50	5,65
7-OCH_3	−0,27	0,12	0,12	0,12	7,87

Unless otherwise stated, the values of the substituent constants were taken from ref. [12]

Table 6: Physical parameters of substituents R_2

Substituent R_2	Hammett subst. konst. σ	Hydrophobic subst. konst. π
H	0	0
4-CH_3	−0,17	0,60
4-Br	0,23	1,19
4-OCH_3	−0,27	−0,03
4-Cl	0,23	0,73
3,4-Cl_2	0,60	1,50
3-Cl	0,37	0,77
3-F	0,34	0,22
4-F	0,06	0,15
3-NO_2	0,71	−0,05
4-NO_2	0,78	0,02
4-$N(CH_3)_2$	−0,83	−0,08
4-$COOC_2H_5$	0,45	0,62[17]
4-CF_3	0,54	1,04
4-CN	0,66	−0,33

Unless otherwise stated, the values of the substituent constants were taken from ref. [12]

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