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Short communication

New groups of antimycobacterial agents: 6-chloro-3-phenyl-4-thioxo-2*H*-1,3-benzoxazine-2(3*H*)-ones and 6-chloro-3-phenyl-2*H*-1,3-benzoxazine-2,4(3*H*)-dithiones

Karel Waisser[a*], Jiří Gregor[a], Lenka Kubicová[a], Věra Klimešová[a], Jiří Kuneš[a,b], Miloš Macháček[a], Jarmila Kaustová[c]

[a]Department of Inorganic and Organic Chemistry, Faculty of Pharmacy, Charles University, 500 05 Hradec Králové, Czech Republic
[b]Laboratory of Structure and Interactions of Biologically Active Molecules, Faculty of Pharmacy, Charles University, 500 05 Hradec Králové, Czech Republic
[c]National Reference Laboratory for *Mycobacterium kansasii,* Regional Institute of Hygiene, 728 92 Ostrava, Czech Republic

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Abstract – Aseries of 6-chloro-3-phenyl-4-thioxo-2*H*-1,3-benzoxazine-2(3*H*)-ones **3** and a series of 6-chloro-3-phenyl-2*H*-1,3-benzoxazine-2,4(3*H*)-dithiones **4** were synthesized by melting 6-chloro-3-phenyl-2*H*-1,3-benzoxazine-2,4(3*H*)-dione and its derivatives substituted on the phenyl ring **2** with tetraphosphorus decasulfide. Compounds **2c–e**, **3** and **4** exhibited in vitro activity against *Mycobacterium tuberculosis, M. kansasii* (two strains) and *M. avium* better than or comparable to that of isoniazid. Replacement of the oxo group by a thioxo group at position 4 led to improvement in activity against *M. tuberculosis* and *M. kansasii*. The Free-Wilson method and procedure developed by the authors were used to analyse the structure–activity and structure–antimycobacterial profile relationships, respectively. © 2000 Éditions scientifiques et médicales Elsevier SAS

1,3-benzoxazine-2,4-diones / 1,3-benzoxazine-2,4-dithiones / 4-thioxo-1,3-benzoxazine-2-ones / antimycobacterial activity / quantitative structure–activity relationship / cosine coefficient

1. Introduction

The current search for new antimycobacterial agents is very urgent, as tuberculosis has become the major emerging opportunistic infection. The developing resistance to conventional antituberculotics is a stimulating factor in the research of new compounds [1–3]. In addition, also the infections caused by non-tuberculous mycobacteria, e.g., *Mycobacterium avium* complex, show a rising occurrence among children, the elderly, and HIV-infected patients [4, 5].

In our previous paper, we have found that 2-benzylthiopyridine-4-carbothioamide derivatives exhibited virtually the same in vitro activity against *M. avium* and *M. kansasii* as well as against *M. tuberculosis* [6]. A broad spectrum of antimycobacterial activity was also noted in substituted 3-phenyl-2*H*-1,3-benzoxazine-2,4(3*H*)-diones and their 6-mono- and 6,8-dihalogeno derivatives [7–10]. Furthermore, antimycobacterial data were reported for thiosalicylanilides prepared by hydrolysis of the corresponding 3-phenyl-4-thioxo-2*H*-1,3-benzoxazine-2(3*H*)-ones and 3-phenyl-2*H*-1,3-benzoxazine-2,4(3*H*)-dithiones [11]. It thus became of interest to prepare and test these thioxo analogues. Very recently, 3-(4-cyclohexylphenyl)-4-thioxo-2*H*-1,3-benzoxazine-2(3*H*)-one and 3-(4-cyclohexylphenyl)-2*H*-1,3-benzoxazine-2,4(3*H*)-dithione have been evaluated for the in vitro antimycobacterial activity [12].

In this study, a series of 6-chloro-3-phenyl-2*H*-1,3-benzoxazine-2(3*H*)-diones **2**, 6-chloro-3-phenyl-4-thioxo-2*H*-1,3-benzoxazine-2(3*H*)-ones **3** and 6-chloro-3-phenyl-2*H*-1,3-benzoxazine-2,4(3*H*)-dithiones **4** substituted on the phenyl ring were prepared. The substituents were

* Correspondence and reprints: waisser@faf.cuni.cz

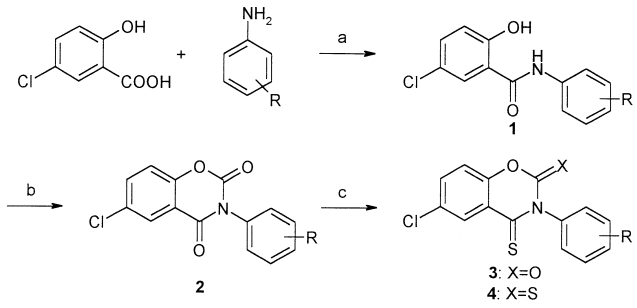

Figure 1. Synthesis of compounds **1**, **2**, **3** and **4**. Reagents: (a) PCl_3, chlorobenzene; (b) ethyl chloroformate, pyridine; (c) P_4S_{10}.

selected according to Topliss [13]. As the 4-methoxy derivative was the least active in the prior testing [9], 3-chloro derivatives were included in the present study instead. The compounds were evaluated for their in vitro activity against four *Mycobacterium* strains. In order to investigate the effects of replacement of the oxo group(s) by thioxo group(s) and substitution at the phenyl ring on the antimycobacterial activity, the Free-Wilson method [14] in the Fujita-Ban modification [15] was used. In addition, the compounds were evaluated with regard to considered therapeutic use, i.e. as broad-spectrum antimycobacterial agents. For the sake of clarity, a very brief outline of this procedure [16] is also provided. Recently, the method has been used in evaluating the substituted 3-phenyl-2*H*-1,3-benzoxazine-2,4(3*H*)-diones [7] in connection with the Hansch approach.

2. Chemistry

The synthesis of the title compounds was straightforward as illustrated in *figure 1*.

Various anilines were reacted with 5-chlorosalicylic acid in the presence of phosphorus trichloride in chlorobenzene to afford the starting 5-chlorosalicylanilides **1**. These were then cyclized with ethyl chloroformate in pyridine to the corresponding 6-phenyl-2*H*-1,3-benzoxazine-2,4(3*H*)-diones **2** [10]. Replacement of oxo group(s) was carried out by melting of the diones **2** with tetraphosphorus decasulfide. The products, 3-phenyl-6-chloro-4-thioxo-2*H*-1,3-benzoxazine-2(3*H*)-ones **3** and 3-phenyl-6-chloro-2*H*-1,3-benzoxazine-2,4(3*H*)-dithiones **4** were isolated in moderate yields by column chromatography. Chemical and physical data of the compounds **2**, **3** and **4** are shown in *table I*.

The structures of compounds **1**, **2**, **3** and **4** were confirmed by elemental analyses and by IR, ^{1}H- and ^{13}C-NMR spectral methods. All salicylanilides **1** showed

Table I. Chemical and physical data of the compounds **2**, **3** and **4**.

Compound	R	Formula	M.w.	Yield[a] (%)	M.p. (°C)	ν (C=O) (cm^{-1})	R_f[b]
			6-chloro-3-phenyl-2*H*-1,3-benzoxazine-2,4(3H)-diones **2**				
2a	H	$C_{14}H_8ClNO_3$	273.7	54	279–280[c]	1 769, 1 701	0.42
2b	4-CH_3	$C_{15}H_{10}ClNO_3$	287.7	51	250–252	1 770, 1 702	0.47
2c	4-Br	$C_{14}H_7BrClNO_3$	352.6	60	233–234[d]	1 771, 1 705	0.49
2d	4-Cl	$C_{14}H_7Cl_2NO_3$	308.1	58	216–218[e]	1 773, 1 706	0.48
2e	3,4-Cl_2	$C_{14}H_6Cl_3NO_3$	342.6	63	238–240[f]	1 763, 1 697	0.51
2f	3-Cl	$C_{14}H_7Cl_2NO_3$	308.1	57	243–245	1 770, 1 704	0.44
			6-chloro-3-phenyl-4-thioxo-2*H*-1,3-benzoxazine-2(3*H*)-ones **3**				
3a	H	$C_{14}H_8ClNO_2S$	289.7	31	232–233	1 766	0.44
3b	4-CH_3	$C_{15}H_{10}ClNO_2S$	303.8	26	242–244	1 760	0.49
3c	4-Br	$C_{14}H_7BrClNO_2S$	368.6	21	247–248	1 754	0.51
3d	4-Cl	$C_{14}H_7Cl_2NO_2S$	324.2	34	270–271	1 761	0.51
3e	3,4-Cl_2	$C_{14}H_6Cl_3NO_2S$	358.6	28	203	1 762	0.54
3f	3-Cl	$C_{14}H_7Cl_2NO_2S$	324.2	20	175–177	1 762	0.50
			6-chloro-3-phenyl-2*H*-1,3-benzoxazine-2,4(3*H*)-dithiones **4**				
4a	H	$C_{14}H_8ClNOS_2$	305.8	28	180–181	–	0.61
4b	4-CH_3	$C_{15}H_{10}ClNOS_2$	319.8	36	234–236	–	0.65
4c	4-Br	$C_{14}H_7BrClNOS_2$	384.7	47	212–214	–	0.66
4d	4-Cl	$C_{14}H_7Cl_2NOS_2$	340.2	37	212–214	–	0.66
4e	3,4-Cl_2	$C_{14}H_6Cl_3NOS_2$	374.7	31	184–186	–	0.68
4f	3-Cl	$C_{14}H_7Cl_2NOS_2$	340.2	42	178–179	–	0.65

[a] Based on immediate precursor; [b] cyclohexane/acetone 3:1; [c] ref. [20] gives m.p. 285–286 °C; [d] ref. [21] gives m.p. 203 °C; [e] ref. [21] gives m.p. 215 °C; [f] ref. [21] gives m.p. 240 °C.

Table II. ^{1}H-NMR (300 MHz, $CDCl_3$) spectra of compounds **3** and **4**.

Compound	R	H-5	H-7	H-8	H-2′	H-3′	H-4′	H-5′	H-6′	CH_3
					6-chloro-3-phenyl-4-thioxo-2*H*-1,3-benzoxazine-2(3*H*)-ones **3**					
3a	H	8.39 d (2.7)	7.66 dd (8.8, 2.7)	7.30–7.22[a] m	7.30–7.22[a] m		7.61–7.48 m, 3H		7.30–7.22[a] m	–
3b	4-CH_3	8.39 d (2.5)	7.65 dd (8.8, 2.5)	7.26 d (8.8)	7.40–7.33 m (AA′BB′)	7.17–7.10 m (AA′BB′)	–	7.17–7.10 m (AA′BB′)	7.40–7.33 m (AA′BB′)	2.44 s, 3H
3c	4-Br	8.36 d (2.5)	7.67[a] dd (8.8, 2.5)	7.27 d (8.8)	7.72–7.63[a] m (AA′BB′)	7.15–7.10 m (AA′BB′)	–	7.15–7.10 m (AA′BB′)	7.72–7.63[a] m (AA′BB′)	–
3d	4-Cl	8.37 d (2.5)	7.67 dd (8.8, 2.5)	7.27 d (8.8)	7.56–7.49 m (AA′BB′)	7.22–7.15 m (AA′BB′)	–	7.22–7.15 m (AA′BB′)	7.56–7.49 m (AA′BB′)	–
3e	3,4-Cl_2	8.35 d (2.5)	7.68 dd (8.8, 2.5)	7.27 d (8.8)	7.38 d (2.5)	–	–	7.62 d (8.5)	7.11 dd (8.5, 2.5)	–
3f	3-Cl	8.35 d (2.6)	7.66 dd (8.8, 2.6)	7.26[a] d (8.8)	7.50–7.47 m	–	7.29–7.24[a] m	7.19–7.12 m	7.50–7.47 m	–
					6-chloro-3-phenyl-2*H*-1,3-benzoxazine-2,4(3*H*)-dithiones **4**					
4a	H	8.31 d (2.5)	7.67 dd (8.8, 2.5)	7.32 d (8.8)	7.24–7.18 m		7.61–7.47 m, 3H		7.24–7.18 m	–
4b	4-CH_3	8.30 d (2.6)	7.67 dd (8.8, 2.6)	7.32[a] d (8.8)	7.40–7.34[a] m (AA′BB′)	7.12–7.06 m (AA′BB′)	–	7.12–7.06 m (AA′BB′)	7.40–7.34[a] m (AA′BB′)	2.46 s, 3H
4c	4-Br	8.29 d (2.5)	7.70–7.65[a] m	7.32 d (8.7)	7.70–7.65[a] m (AA′BB′)	7.11–7.05 m (AA′BB′)	–	7.11–7.05 m (AA′BB′)	7.70–7.65[a] m (AA′BB′)	–
4d	4-Cl	8.29 d (2.5)	7.68 dd (8.8, 2.5)	7.32 d (8.8)	7.56–7.48 m (AA′BB′)	7.18–7.10 m (AA′BB′)	–	7.18–7.10 m (AA′BB′)	7.56–7.48 m (AA′BB′)	–
4e	3,4-Cl_2	8.27 d (2.5)	7.69 dd (8.8, 2.5)	7.32[a] d (8.8)	7.32[a] d (2.2)	–	–	7.62 d (8.5)	7.07 dd (8.5, 2.2)	–
4f	3-Cl	8.28 d (2.5)	7.68 dd (8.8, 2.5)	7.32 d (8.8)	7.52–7.45 m	–	7.24–7.21 m	7.14–7.08 m	7.52–7.45 m	–

[a] Overlapping signals.

a band at 1 603–1 643 cm^{-1} in their IR spectra, which is characteristic of the amide C=O group, and 1,3-benzoxazine-2,4(3*H*)diones **2** showed characteristic absorption maxima of two C=O groups at 1 763–1 773 cm^{-1} and 1 697–1 706 cm^{-1}. Compounds **3** showed only one characteristic band of a C=O group at 1 754–1 766 cm^{-1}. The ^{1}H- and ^{13}C-NMR spectra of compounds **3** and **4** (*tables II* and *III*) are in agreement with the proposed structures. The location of sulfur was corroborated by gHMBC experiments, e.g. for compound **3f**, the carbon

Table III. ^{13}C-NMR (75 MHz, $CDCl_3$) spectra of compounds **3** and **4**.

Compound	R	δ													
		6-chloro-3-phenyl-4-thioxo-2*H*-1,3-benzoxazine-2(3*H*)-ones **3**													
3a	H	190.0,	147.8,	144.5,	138.8,	135.6,	131.5,	131.2,	129.9,	129.3,	127.7,	121.7,	118.2		
3b	4-CH_3	190.2,	147.8,	144.7,	139.5,	136.2,	135.6,	131.5,	131.3,	130.6,	127.3,	121.7,	118.2,	21.5	
3c	4-Br	189.8,	147.7,	144.3,	137.6,	135.8,	133.2,	131.7,	131.2,	129.5,	123.5,	121.6,	118.2		
3d	4-Cl	189.9,	147.7,	144.4,	137.1,	135.8,	135.4,	131.7,	131.2,	130.2,	129.2,	121.6,	118.2		
3e	3,4-Cl_2	189.5,	147.6,	144.2,	137.6,	136.0,	133.9,	133.9,	131.8,	131.5,	131.1,	130.1,	127.4,	121.5,	118.3
3f	3-Cl	189.6,	147.6,	144.2,	139.5,	135.8,	135.3,	131.6,	131.1,	130.7,	129.6,	128.3,	126.2,	121.5,	118.2
		6-chloro-3-phenyl-2*H*-1,3-benzoxazine-2,4(3*H*)-dithiones **4**													
4a	H	185.6,	176.9,	148.0,	143.0,	135.8,	132.2,	131.1,	130.0,	129.1,	127.6,	123.0,	117.9		
4b	4-CH_3	185.7,	177.1,	148.0,	140.5,	139.2,	135.7,	132.1,	131.2,	130.7,	127.2,	123.0,	117.9,	21.5	
4c	4-Br	185.4,	176.6,	148.0,	141.8,	136.0,	133.4,	132.3,	131.1,	129.5,	123.2,	122.9,	117.9		
4d	4-Cl	185.5,	176.7,	148.0,	141.3,	136.0,	135.1,	132.3,	131.1,	130.4,	129.2,	122.9,	117.9		
4e	3,4-Cl_2	185.3,	176.4,	148.0,	141.6,	136.1,	134.0,	133.6,	132.4,	131.7,	131.0,	130.0,	127.4,	122.7,	117.9
4f	3-Cl	185.3,	176.5,	148.0,	143.6,	136.0,	135.4,	132.3,	131.0,	130.9,	129.4,	128.2,	126.2,	122.8,	117.9

Table IV. Antimycobacterial activity of compounds **2**, **3** and **4** expressed as MIC (μmol.L^{-1}).

Compound	R	MIC, μmol.L^{-1} after 14/21 days			
		M. tuberculosis My 331/88	*M. avium* My 330/88	*M. kansasii* My 235/80	*M. kansasii* 6509/96
		6-chloro-3-phenyl-2*H*-1,3-benzoxazine-2,4(3*H*)-diones **2**			
2a	H	31/31	16/31	4/4	4/4
2b	4-CH_3	16/16	8/16	4/8	4/4
2c	4-Br	4/4	4/8	4/4	4/4
2d	4-Cl	4/4	8/8	4/4	4/4
2e	3,4-Cl_2	4/4	8/8	4/4	4/4
2f	3-Cl	8/16	8/8	4/4	4/4
		6-chloro-3-phenyl-4-thioxo-2*H*-1,3-benzoxazine-2(3H)-ones **3**			
3a	H	1/1	8/31	2/4	0.5/1
3b	4-CH_3	0.5/1	16/31	4/8	1/1
3c	4-Br	0.5/1	8/31	2/4	1/2
3d	4-Cl	0.5/0.5	16/16	2/4	1/1
3e	3,4-Cl_2	0.5/1	8/16	2/4	2/4
3f	3-Cl	0.5/1	16/31	2/4	1/1
		6-chloro-3-phenyl-2*H*-1,3-benzoxazine-2,4(3*H*)-dithiones **4**			
4a	H	1/1	8/16	2/4	1/1
4b	4-CH_3	0.5/0.5	16/-	2/4	0.5/1
4c	4-Br	0.5/0.5	16/16	2/8	1/2
4d	4-Cl	0.5/1	16/16	2/8	1/2
4e	3,4-Cl_2	1/2	16/16	2/4	1/1
4f	3-Cl	1/2	16/31	2/4	1/1
isoniazid		4/4	500/500	8/8	500/500

of the thiocarbonyl moiety at 189.6 ppm had a strong correlation to H-5 of the neighbouring benzene ring at 8.35 ppm, while the other carbonyl group at 144.2 ppm displayed no correlation whatsoever.

3. Biological investigation

The antimycobacterial activity of compounds **2**, **3**, **4** and isoniazid was tested in vitro against *Mycobacterium tuberculosis* My 331/88, *Mycobacterium avium* My 330/88 and *Mycobacterium kansasii* My 235/80, obtained from the Czech National Collection of Type Cultures (CNCTC), National Institute of Public Health, Prague, and a clinical isolate of *Mycobacterium kansasii* 6509/96 resistant to isoniazid using the micromethod for the determination of the minimum inhibitory concentration (MIC) (*table IV*).

4. Calculations

For the purpose of the evaluation of biologically active compounds with regard to the considered therapeutic use, a complex criterion *S* was proposed [16] as equal to the scalar (dot) product:

$$S = (\mathbf{A}, \mathbf{U}) \quad (1)$$

where the activity vector **A** represents the evaluated compound and the unit vector **U** the 'ideal' drug. The term 'complex' is given by the fact that the criterion *S* can be decomposed into two components:

$$S = g(\mathbf{A})\, k(\mathbf{A}, \mathbf{U}). \quad (2)$$

The first component $g(\mathbf{A})$, the Euclidean norm of the activity vector, is a measure of the overall potency of the evaluated compound. The other $k(\mathbf{A},\mathbf{U})$, called the cosine coefficient, is a measure of the relative similarity of the evaluated compound to the 'ideal' drug.

The following two situations are of great importance. In the case of broad-spectrum drugs, the 'ideal' drug is represented by the unit vector $\mathbf{U}_0$. Its *n* components (*n* is the number of activities taken into consideration) are given by:

$$U_{0i} = 1/\sqrt{n},\ i = 1, \cdots, n \quad (3)$$

so that the formula for calculating the complex criterion S_0 can be written as:

$$S_0 = \frac{1}{\sqrt{n}} \sum_{i=1}^{n} A_i. \quad (4)$$

In the case of the selectivity of the *s*th effect, the 'ideal' drug is represented by the unit vector $\mathbf{U}_s$, the components of which are given by:

$$U_{si} = \begin{cases} (n-1)/\sqrt{n(n-1)} & \text{for } i = s, \\ -1/\sqrt{n(n-1)} & \text{for } i \neq s,\ i = 1, \cdots, n. \end{cases} \quad (5)$$

It is noteworthy that all but the *s*th effect are considered to be undesired and the negative sign is attributed to them. The formula for calculating the complex criteria S_s (complex selectivities) can be written as:

$$S_s = \frac{1}{\sqrt{n(n-1)}}\left(nA_s - \sum_{i=1}^{n} A_i\right), \quad \text{where } s = 1, \cdots, n. \quad (6)$$

Quantitative structure–activity relationships were analysed by the Free-Wilson method [14] in the Fujita-Ban modification [15].

For the *n*-component vector of regression coefficients **a**, interpreted as contributions of respective fragment to the analysed activities, analogous measures, i.e. complex criterion *s*, the norm of the vector $g(\mathbf{a})$ and cosine coefficient $k(\mathbf{a}, \mathbf{U})$, can be introduced:

$$s = (\mathbf{a}, \mathbf{U}) = g(\mathbf{a})\, k(\mathbf{a}, \mathbf{U}). \quad (7)$$

5. Results and discussion

The antimycobacterial activities of compounds **2**, **3**, **4** and isoniazid against *Mycobacterium tuberculosis* My 331/88, *M. avium* My 330/88, *M. kansasii* My 235/80 and *M. kansasii* 6509/96 (clinical isolate resistant to isoniazid) are shown in *table IV*. In general, the synthesized compounds possess in vitro activities against all mycobacterial strains tested, better than or comparable to that of isoniazid. We cannot compare the data presented here with the previously published results of testing of compounds **2a–e** against *M. tuberculosis* $H_{37}R_v$ and *M. kansasii* PKG 8 [9] as they were obtained for different strains.

Evaluation of broad-spectrum antimycobacterial profiles of individual compounds was performed by the procedure described previously [16]. Activities, expressed as log (1/MIC), for strains of *Mycobacterium tuberculosis*, *M. kansasii* and *M. avium* obtained from the CNCTC were used for the calculation. The clinical isolate of *M. kansasii* was omitted from these calculations as it exhibits the same (to compounds **2**) or even better susceptibility (to compounds **3** and **4**) than the strain of *M. kansasii* from the CNCTC. The results of calculations are summarized in *table V*.

The norm of activity vector $g(\mathbf{A})$ is a measure of the overall antimycobacterial potency of the compound. Complex criterion S_0 is proposed for a comparison of the evaluated compound with the ideal broad-spectrum drug, i.e. compound possessing equal MICs against all strains taken into consideration. Cosine coefficient k_0 is a measure of relative similarity to the ideal drug as the influence of the overall potency is eliminated. This aspect, of course, is of greater importance in the search for selectively acting compounds. The more the cosine coefficient is close to the value of 1, the more the profile of evaluated compound relatively agrees with that of the ideal drug. As follows from *table V*, the values of cosine coefficient k_0 (0.963–1.000) justify the conclusion that the compounds represent broad-spectrum antimycobacterial agents. Detailed inspection of *table V* reveals that, in general, higher values of S_0 are found in compounds **3** and **4**, while values of k_0 closer to 1 are found in **2**. This is consistent with the fact that the replacement of one or both oxo groups by thioxo groups yielded the compounds **3** and **4** with greater activity against *M. tuberculosis* and *M. kansasii* but with lesser activity against *M. avium* than that of the compounds **2**.

For quantitative analysis of the structure–antimycobacterial activity relationships we used the method of Free-Wilson [14] as modified by Fujita-Ban [15]. In order to describe the influence of replacement of one or both oxo groups by thioxo group(s) and substitution on the phenyl ring, the unsubstituted on the phenyl ring dione **2a** was chosen as the reference compound for all the analyses. Since no data for 2-thioxo-1,3-benzoxazine-4(3*H*)-ones were available, sulfur atoms occur six times at position 2 and 12 times at position 4 of the basis set. *Table VI* shows the derived correlation equations.

These equations confirm that each fragment has a constant, independent and additive contribution to the activity of the whole molecule against *M. tuberculosis* My 331/88, clinical isolate of *M. kansassii* 6509/96 and *M. kansasii* My 235/80, in the last case at least for data after 14 days. Analyses of data for *M. avium* My 330/88 and *M. kansasii* My 235/80 after 21 days resulted in non-significant correlations.

In all cases of the significant correlations, the only significant positive contributions were those of the sulfur atom in position 4. It is also observed that the contributions of substituents on the phenyl ring are non-significant in comparison to the unsubstituted phenyl, except for *M. tuberculosis* data after 14 days. The contribution of the sulfur atom in position 2 is also non-significant in comparison to the oxygen atom. The antimycobacterial activity of 3-phenyl-2-thioxo-1,3-benzoxazine-4(3*H*)-ones

Table V. Evaluation of compounds **2**, **3** and **4** as broad-spectrum antimycobacterial agents.

Compound	R	Complex criterion S_0		Norm of vector **A** g(**A**)		Cosine coefficient k_0	
		14 days	21 days	14 days	21 days	14 days	21 days
		6-chloro-3-phenyl-2*H*-1,3-benzoxazine-2,4(3*H*)-diones **2**					
2a	H	3.292	3.126	3.354	3.21	0.982	0.974
2b	4-CH_3	3.632	3.284	3.657	3.294	0.993	0.997
2c	4-Br	4.153	3.98	4.153	3.987	1	0.998
2d	4-Cl	3.98	3.98	3.987	3.987	0.998	0.998
2e	3,4-Cl_2	3.98	3.98	3.987	3.987	0.998	0.998
2f	3-Cl	3.806	3.632	3.814	3.657	0.998	0.993
		6-chloro-3-phenyl-2*H*-4-thioxo-1,3-benzoxazine-2(3H)-ones **3**					
3a	H	4.501	3.988	4.548	4.126	0.99	0.967
3b	4-CH_3	4.327	3.814	4.458	3.959	0.971	0.963
3c	4-Br	4.675	3.988	4.752	4.126	0.984	0.967
3d	4-Cl	4.501	4.327	4.627	4.458	0.973	0.971
3e	3,4-Cl_2	4.675	4.153	4.752	4.24	0.984	0.979
3f	3-Cl	4.501	3.988	4.627	4.126	0.973	0.967
		6-chloro-3-phenyl-2*H*-1,3-benzoxazine-2,4(3*H*)-dithiones **4**					
4a	H	4.501	4.153	4.548	4.24	0.99	0.979
4b	4-CH_3	4.501	–	4.627	–	0.973	–
4c	4-Br	4.501	4.153	4.627	4.303	0.973	0.965
4d	4-Cl	4.501	3.98	4.627	4.077	0.973	0.976
4e	3,4-Cl_2	4.327	3.98	4.417	4.032	0.98	0.987
4f	3-Cl	4.327	3.814	4.417	3.913	0.98	0.975
isoniazid		1.732	1.732	2.435	2.435	0.711	0.711

is predicted not to differ substantially from that of the corresponding 2,4-diones **2**.

The Free-Wilson method was also applied to the complex criteria S_0 (*table VII*). The statistical validity of the analyses was acceptable only in the case of values calculated from the MICs after 14 days. The significant positive contributions to the broad-spectrum antimycobacterial profile were found for the sulfur atom in position 4 and the 4-bromine substitution at the phenyl ring. The non-significant correlation derived for the values based on the data after 21 days correspond to the fact that the Free-Wilson analysis of the respective MICs gave the acceptable results only in the case of *M. tuberculosis.*

We also carried out calculations based on the equation (7) using fragment contributions to the antimycobacterial activities (*table VII*). It follows from the comparison of results that the higher the statistical validity of the individual Free-Wilson equations, the better the complex criteria s_0 reproduce the contributions to complex criteria S_0 calculated by the Free-Wilson method. Although the direct information on their significance is missing, additional information can be obtained by decomposition of complex criteria for fragments s_0 into the cosine coefficient k_0 and the norm of vector g(**a**). Comparison of the two fragments with contributions significantly different from zero show that the value of the cosine coefficient of the 4-bromine substituent at the phenyl is larger than that of the sulfur atom at position 4, however, as mentioned above, the value of the cosine coefficient is less decisive in the case of broad-spectrum compounds as compared to the norm of the vector.

In conclusion, the replacement of the carbonyl moieties in 3-phenyl-1,3-benzoxazine-2,4(3*H*)-diones **2** by the thiocarbonyl group retains antimycobacterial activity. In the case of activity against *M. tuberculosis* and *M. kansasii* (clinical isolate), the corresponding thiones **3** and dithiones **4** were more potent than the parent compounds **2**, while these were more potent against *M. avium* than the compounds **3** and **4**.

6. Experimental protocols

6.1. Chemistry

The melting points were determined on a Kofler apparatus and are uncorrected. The IR spectra were taken on a Nicolet Impact 400 spectrometer in KBr pellets. The NMR spectra were recorded on a Varian Mercury-Vx BB 300 spectrometer operating at 300 MHz in deuteriochloroform. Chemical shifts were recorded as δ values and were indirectly referenced to tetramethylsilane via the

Table VI. Results of the Free-Wilson/Fujita-Ban analyses.

Fragment	Contribution[a] to activity against							
	M. tuberculosis My 331/88		*M. avium* My 330/88		*M. kansasii* My 235/80		*M. kansasii* 6509/96	
	14 days	21 days	14 days	21 days	14 days	21 days	14 days	21 days
μ[b]	1.767	1.850	2.133	1.795	2.417	2.417	2.483	2.483
At 2								
=O[c]	0	0	0	0	0	0	0	0
=S	–0.100	–0.100	–0.100	0.115	0.050	–0.050	0.050	0.050
At 4								
=O[c]	0	0	0	0	0	0	0	0
=S	1.150*	1.000*	–0.150	–0.350	0.250*	0.000	0.600*	0.450*
At phenyl								
3-H, 4-H[c]	0	0	0	0	0	0	0	0
3-H, 4-CH_3	0.300	0.200	–0.100	0.030	–0.100	–0.200	0.000	0.000
3-H, 4-Br	0.500*	0.400	0.100	0.200	0.000	–0.100	–0.100	–0.200
3-H, 4-Cl	0.500*	0.400	–0.100	0.300	0.000	–0.100	–0.100	–0.100
3,4-Cl_2	0.400*	0.200	0.000	0.300	0.000	0.000	–0.200	–0.200
3-Cl, 4-H	0.300	0.000	–0.100	0.100	0.000	0.000	–0.100	0.000
r	0.964	0.923	0.707	0.835	0.933	0.627	0.952	0.886
s	0.202	0.274	0.170	0.172	0.071	0.130	0.130	0.170
F	18.78	8.17	1.41[d]	2.97[d]	9.57	0.92[d]	13.91	5.2
n	18	18	18	17	18	18	18	18

[a] The contribution of each fragment is expressed in log (1/MIC) scale (MIC in mmol.L^{-1}). [b] Activity calculated for **2a** (reference compound). [c] Fragment on the reference compound; zero value of the contribution by definition. [d] Non-significant correlation. * A value significantly ($P < 0.05$) different from the contribution of the fragment on the reference compound.

solvent signal (7.26 for ^{1}H- and 77.0 for ^{13}C-). Coupling constants *J* are given in Hz. Elemental analyses were performed on a CHNS-O CE elemental analyser (FISONS EA 1110, Milan). Analyses of C, H, N, S, and halogens were within ± 0.4% of the theoretical values.

Column chromatography was carried out on silica gel 60 (0.040–0.063 mm) from E. Merck (Darmstadt, Germany) and TLC was performed on pre-coated silica gel plates with a fluorescent indicator Silufol UV 254 + 366 (Kavalier, Votice, Czechia), cyclohexane/acetone 3:1, to check the purity of the products.

5-Chlorosalicylanilide (**1a**), m.p. 209–211 °C (ref. [17] gives m.p. 209–211 °C), 5-chloro-4′-methylsalicylanilide (**1b**), m.p. 225–227 °C (ref. [18] gives m.p. 215–217 °C), 4′-bromo-5-chlorosalicylanilide (**1c**), m.p. 220–222 °C (ref. [17] gives m.p. 224–226.5 °C), 4′,5-dichlorosalicylanilide (**1d**), m.p. 224–226 °C (ref. [17] gives m.p. 231–232 °C), 3′,4′,5-trichlorosalicylanilide (**1e**), and m.p. 248–250 °C (ref. [17] gives m.p. 246–248 °C), 3′,5-dichlorosalicylanilide (**1f**), m.p. 216–218 °C (ref. [19] gives m.p. 216 °C) were prepared according to the method described previously [10].

6.1.1. General procedure for 6-chloro-3-phenyl-2H-1,3-benzoxazine-2,4(3H)-diones **2**

Ethyl chloroformate (5.2 g, 48 mmol) was added dropwise to a stirred solution of the salicylanilide (40 mmol) in dry pyridine (20 mL) at 0 °C. The mixture was heated on a water bath for 1 h and then poured into 5% hydrochloric acid (140 mL). After 12 h, the product was filtered off, suspended in 5% potassium hydroxide solution, filtered again, and recrystallized from ethanol (*table I*).

6.1.2. General procedure for 3-phenyl-6-chloro-4-thioxo-2H-1,3-benzoxazine-2(3H)-ones **3** *and 3-phenyl-6-chloro-2H-1,3-benzoxazine-2,4(3H)-dithiones* **4**

Benzoxazinedione **2** (3.8 mmol) was melted with P_4S_{10} (7.6 mmol) for 20 min (175–200 °C). After cooling to room temperature a 10% potassium carbonate solution (60 mL) was poured into the reaction mixture, the crude product was filtered off and dissolved in toluene (p.a., at the most 40 mL). Column chromatography on silica gel gave 3-phenyl-6-chloro-4-thioxo-*2H*-1,3-benzoxazine-2(3*H*)-one **3** and 3-phenyl-6-chloro-*2H*-1,3-benzoxazine-2,4(3*H*)-dithione **4** as orange–yellow and red solids,

Table VII. Results of the Free-Wilson/Fujita-Ban analyses of complex criterion S_0. Complex criteria s_0, norm $g(\mathbf{a})$ and cosine coefficient k_0 for fragments.

Fragment	Contribution to S_0[a]		Complex criterion s_0[b]		Norm of vector **a** $g(\mathbf{a})$[b]		Cosine coefficient k_0[b]	
	14 days	21 days	14 days	21 days	14 days	21 days	14 days	21 days
μ	3.647[c]	3.531[c]	3.647	3.500	3.676	3.534	0.992	0.990
At 2								
=O[d]	0	0	0	0	0	0	0	0
=S	–0.087	–0.084	–0.087	–0.020	0.150	0.160	–0.580	–0.125
iii								
=O[d]	0	0	0	0	0	0	0	0
=S	0.722*	0.375*	0.722	0.375	1.186	1.059	0.609	0.354
At phenyl								
3-H, 4-H	0	0	0	0	0	0	0	0
3-H, 4-CH_3	0.058	–0.167	0.058	0.017	0.332	0.284	0.175	0.060
3-H, 4-Br	0.347*	0.289	0.346	0.289	0.510	0.458	0.678	0.631
3-H, 4-Cl	0.231	0.346	0.231	0.346	0.510	0.510	0.453	0.678
3,4-Cl_2	0.231	0.289	0.231	0.289	0.400	0.361	0.578	0.801
3-Cl, 4-H	0.116	0.058	0.115	0.058	0.316	0.100	0.364	0.580
r	0.925	0.827						
s	0.189	0.230						
F	8.45	2.78[e]						
n	18	17						

[a] Calculated for data from *table V*. [b] Calculated for data from *table VI* (see the text). [c] Complex criterion S_0 calculated for **2a** (reference compound). [d] Fragment on the reference compound. [e] Non-significant correlation. * A value significantly ($P < 0.05$) different from the contribution of the fragment on the reference compound.

respectively. Recrystallization from ethanol was necessary (*table I*).

6.2. Microbiological methods

For the evaluation of the antimycobacterial activity of the substances in vitro the following strains were used: *Mycobacterium 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, and *M. kansasii* 6509/96 isolated in National Reference Laboratory for *Mycobacterium kansasii*, Ostrava. Antimycobacterial activity of the compounds against these strains was determined in Šula semisynthetic medium (SEVAC, Prague). Each strain was simultaneously inoculated into a Petri dish containing Löwenstein-Jensen medium for the control of the sterility of the inoculum and its growth. The compounds were added to the medium in a dimethyl sulfoxide solution. The following concentrations were used: 1 000, 500, 250, 125, 62, 31, 16, 8, 4, 2, 1 and 0.5 μmol.L^{-1}. The minimum inhibitory concentrations (MICs) were determined after incubation at 37 °C for 14 and 21 days. MIC was the lowest concentration of a substance at which the inhibition of the growth occurred. Isoniazid was used as standard.

6.3. Calculations

Activities were calculated as logarithms of reciprocal values of minimum inhibitory concentrations (MICs). To obtain only positive numbers, numerical values of MICs expressed in mmol.L^{-1} were taken.

Activities against *Mycobacterium tuberculosis* My 331/88, *M. avium* My 330/88 and *M. kansasii* My 235/80 (all strains from the CNCTC), form the components of vector **A** (in the given order) representing the evaluated compound. Complex criterion S_0 was calculated according to the equation (4) ($n = 3$). Its division by the norm of vector $g(\mathbf{A})$ resulted in cosine coefficient k_0.

Free-Wilson analyses were calculated with the use of the linear regression module of the ADSTAT program, version 2.00 (TriloByte, Pardubice, Czechia).

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