Full Paper

Highly Active Potential Antituberculotics: 3-(4-Alkylphenyl)-4-thioxo-2*H*-1,3-benzoxazine-2(3*H*)-ones and 3-(4-Alkylphenyl)-2*H*-1,3-benzoxazine-2,4(3*H*)-dihiones Substituted in Ring-B by Halogen*

Karel Waisser[1], Josef Matyk[1], Jirí Kuneš[1], Rafael Doležal[1], Jarmila Kaustová[2], and Hans-Martin Dahse[3]

[1] Department of Inorganic and Organic Chemistry, Charles University in Prague, Faculty of Pharmacy in Hradec Králové, Hradec Králové, Czech Republic
[2] Regional Institute of Public Health, Department for Diagnostics of Mycobacteria, Ostrava, Czech Republic
[3] Leibniz Institute for Natural Product Research and Infection Biology – Hans Knoell Institute, Jena, Germany

A series of 6-chloro-3-(4-alkylphenyl)-4-thioxo-2*H*-1,3-benzoxazine-2(3*H*)-ones, 7-chloro-3-(4-alkylphenyl)-4-thioxo-2*H*-1,3-benzoxazine-2(3*H*)-ones, 6-bromo-3-(4-alkylphenyl)-4-thioxo-2*H*-1,3-benzoxazine-2(3*H*)-ones, 6,8-dibromo-3-(4-alkylphenyl)-4-thioxo-2*H*-1,3-benzoxazine-2(3*H*)-ones, 6-chloro-3-(4-alkylphenyl)-2*H*-1,3-benzoxazine-2,4(3*H*)-dithiones, 7-chloro-3-(4-alkylphenyl)-2*H*-1,3-benzoxazine-2,4(3*H*)-dithiones, 6-bromo-3-(4-alkylphenyl)-2*H*-1,3-benzoxazine-2,4(3*H*)-dithiones and 6,8-dibromo-3-(4-alkylphenyl)-2*H*-1,3-benzoxazine-2,4(3*H*)-dithiones was synthesized. The compounds exhibited *in-vitro* activity against *Mycobacterium tuberculosis, M. kansasii* (two strains), and *M. avium*. 6-bromo-3-(4-propylphenyl)-4-thioxo-2*H*-1,3-benzoxazin-2(3*H*)-one and 6-bromo-3-(4-propylphenyl)-2*H*-1,3-benzoxazin-2,4(3*H*)-dithione are the most active compounds against *M. tuberculosis*. The activity is similar to isoniazid (INH). The compounds under study have a broad spectrum of activity against potential pathogenic strains. The replacement of the oxo group by thioxo group of 3-(4-alkylphenyl)-2*H*-1,3-benzoxazine-2,4(3*H*)-diones often led to an improvement in the antimycobacterial activity against *M. tuberculosis*.

Keywords: Antimycobacteroal activity / Antituberculotics / Benzoxazine / Thioxo group / Tuberculostatics

Received: December 12, 2007; accepted: May 15, 2008

DOI 10.1002/ardp.200800004

Supporting Information for this article is available from the author or on the WWW under http://www.wiley-vch.de/contents/jc_2019/2008/200800004_s.pdf

Introduction

The return of tuberculosis to Europe and North America is among others a features of the period dating back to 1985. New mycobacterial diseases which are currently considered to be non-transferable to human beings have occurred (mycobacterioses produced by potentially pathogenic strains). The development of new antituberculotic agents is the principal goal of our group. We have recently studied a number of structurally different compounds, such as of pyridine [1], alkoxyphenylcarbamic acids [2], and dihydroindolethiones [3]. The replacement of the oxo group by a thioxo group usually increases the antimycobacterial activity [3]. The thioxo group is contained in several antitubercular drugs. (*e.g.* Ethioamide,

Correspondence: Karel Waisser, Charles University in Prague, Faculty of Pharmacy in Hradec Králové, Heyrovského 1203, CZ 500 05 Hradec Králové, Czech Republic.
E-mail: waisser@faf.cuni.cz
Fax: +420 495514330

Abbreviations:. gHMBC (Gradient-assisted Heteronuclear Multiple-Bond Correlation); isoniazid (INH); quantitative structure-activity relationship (QSAR)

* Dedicated to Prof. Hans-Dietrich Stachel (Munich) on the occasion of his 80th birthday.

Protionamide, derivatives of diarylthiourea). The new antimycobacterial compounds, derivatives of the ring-B-halogenated 3-(4-alkylphenyl)-2*H*-1,3-benzoxazine-2,4(3*H*)-diones, have been recently found by QSAR (Quantitative Structure-Activity Relationship) [4]. The goal of this paper is the study of the antimycobacterial activity of 3-(4-alkylphenyl)-4-thioxo-2*H*-1,3-benzoxazine-2(3*H*)-ones **1a** – **10a** and 3-(4-alkylphenyl)-2*H*-1,3-benzoxazine-2,4(3*H*)-dihiones **1b** – **10b**.

Results and discussion

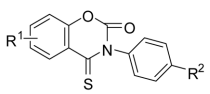
Chemistry

The synthetic pathway is illustrated in Scheme 1. The first two steps have already been published [4, 5]. The mixture of 3-(4-alkyphenyl)-4-thioxo-2*H*-1,3-benzoxazine-2(3*H*)-ones **1a** – **10a** and 3-(4-alkylphenyl)-2*H*-1,3-benzoxazine-2,4(3*H*)-dihiones **1b** – **10b** was prepared by the reaction of the halogenated 3-(4-alkylphenyl)-2*H*-1,3-benzoxazine-2,4(3*H*)-diones with phosphorus pentasulfide. The products were separated by chromatography. The frequencies in the infrared spectra within the region (1758 – 1781 cm^{1}) are characteristic for the 3-(4-alkylphenyl)-4-thioxo-2*H*-1,3-benzoxazine-2(3*H*)-ones. The location of sulfur for 3-(4-alkylphenyl)-4-thioxo-2*H*-1,3-benzoxazine-2(3*H*)-ones was elaborated by gHMBC (Gradient-assisted Heteronuclear Multiple-Bond Correlation) experiments. The correlation of the chemical shifts of carbon in the thiocarbonyl moiety with the chemical shifts of H-5 was observed. The structure of 3-(4-alkylphenyl)-2*H*-1,3-benzoxazine-2,4(3*H*)-dihiones was confirmed by NMR as well. The structure is summarized in Table 1, yield, melting point, and carbonyl frequency in IR in Table 2.

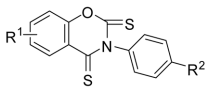
Cytotoxicity

The most active compounds, 6-bromo-3-(4-propylphenyl)-4-thioxo-2*H*-1,3-benzoxazin-2(3*H*)-one **4a** and 6-bromo-3-(4-propylphenyl)-2*H*-1,3-benzoxazin-2,4(3*H*)-dithione **4b**,

Table 1. Minimum inhibitory concentrations (MIC) of halogenated 3-(4-alkylphenyl)-4-thioxo-2*H*-1,3-benzoxazine-2(3*H*)-ones **1** to **10** and 3-(4-alkylphenyl)-2*H*-1,3-benzoxazine-2,4(3*H*)-dihiones **1b** to **10b**.



1a–10a



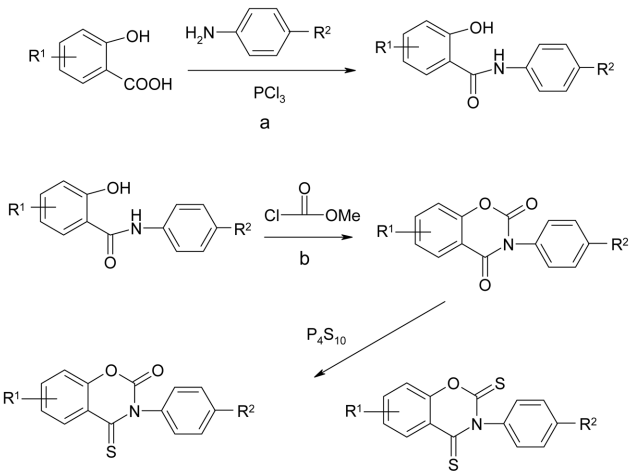
1b–10b

	Compounds		MIC (μmol/L) at an incubation time of 14 d/21 d			
	R^1	R^2	*M. tuberculosis* My 331/88	*M. kansasii* My 235/80	*M. avium* My 330/88	*M. kansasii* 6509/96
1a	6-Cl	octyl	4 / 4	4 / 4	4 / 4	4 / 8
2a	7-Cl	butyl	16 / 16	16 / 16	8 / 8	8 / 8
3a	7-Cl	hexyl	2 / 2	8 / 8	2 / 2	8 / 8
4a	6-Br	propyl	1 / 1	8 / 16	32 / 32	8 / 8
5a	6-Br	butyl	16 / 16	16 / 16	16 / 32	16 / 16
6a	6-Br	pentyl	32 / 32	32 / 32	62.5 / 62.5	32 / 32
7a	6-Br	heptyl	2 / 2	4 / 8	8 / 16	4 / 8
8a	6-Br	octyl	8 / 16	8 / 16	8 / 16	8 / 16
9a	6,8 Br_2	butyl	– / –[a)]	32 / 62.5	– / –[a)]	– / –[a)]
10a	6,8 Br_2	hexyl	16 / 32	32 / 32	– / –[a)]	32 / 32
1b	6-Cl	octyl	2 / 4	4 / 8	4 / 8	4 / 8
2b	7-Cl	butyl	8 / 8	8 / 8	4 / 8	8 / 8
3b	7-Cl	hexyl	2 / 2	8 / 8	2 / 4	8 / 8
4b	6-Br	propyl	1 / 1	8 / 16	32 / 32	8 / 8
5b	6-Br	butyl	32 / 32	32 / 32	16 / 32	16 / 16
6b	6-Br	pentyl	32 / 32	32 / 32	16 / 32	32 / 32
7b	6-Br	heptyl	2 / 4	4 / 8	8 / 8	4 / 8
8b	6-Br	octyl	8 / 8	8 / 8	8 / 16	4 / 8
9b	6,8 Br_2	butyl	– / –[a)]	– / –[a)]	– / –[a)]	62.5 / 62.5
10b	6,8 Br_2	hexyl	32 / 32	16 / 32	62.5	32 / 32
Isoniazid (isonicotinehydrazide)			1 / 1	>250 / >250	>250 / >250	8 / 8

[a)] MIC values could not be determined due to the low solubility.

Table 2. Yield, melting point and carbonyl frequency in IR.

Compound	Yield (%)	M.p. (°C)	$\nu_{C=O}$ (cm^{-1})	Compound	Yield (%)	M.p. (°C
1a	35	151–152	1773	**1b**	30	94–95
2a	30	148–150	1766.	**2b**	32	147–148
3a	32	133–134	1781	**3b**	29	117–118
4a	39	192–194	1760	**4b**	37	181–182
5a	36	148–151	1758	**5b**	40	138–140
6a	44	147–148	1772	**6b**	39	136–138
7a	37	136–137	1774	**7b**	36	114–115
8a	42	144–145	1773	**8b**	31	96–96
9a	32	218–220	1770	**9b**	40	212–215
10a	35	187–188	1758	**10b**	33	145–146



(a) Ref. 5; (b) Ref.4; Substituents are described in Table 1.

Scheme 1. Synthesis of the compounds

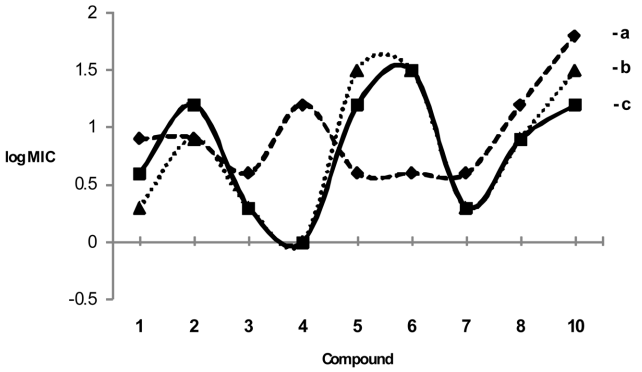


Figure 1. The demonstration of an increase of the antimycobacterial activity against *M. tuberculosis* (incubation 14 d) by replacement of the carbonyl oxygen for sulfur. (a) Benzosazinediones, (b) Benzoxazinedithiones, (c) Thioxobenzoxazinones.

were chosen for cytotoxicity testing. Both compounds showed cytotoxic properties (compound **4a** CC_{50} 10.7 µg/mL, compound **4b** CC_{50} 5.7 µg/mL).

Discussion

The structures of the products were confirmed by elemental analyses and by IR, ^{1}H and ^{13}C-NMR spectral methods. In general, the synthesized compounds possess *in-vitro* activities against all tested mycobacterial strains. The values of MICs are generally within the range 1–62.5 µmol/L, most often they range from 2–8 µmol/L. The compounds were predominantly less active than INH against *M. tuberculosis* 331/88, on the other hand, against *M. kansasii* 235/80 and *M. avium* 330/88, the compounds were more effective than INH. The replacement of the oxo group for the thioxo group in the starting 3-(4-alkylphenyl)-*2H*-1,3-benzoxazine-2,4(3*H*)-diones increases the antimycobacterial activity against *M. tuberculosis* (with exception of the butyl derivatives **2a**, **5a**, and **5b** and the pentyl derivatives **6a** and **6b**). For the graphical expression see Fig. 1. The activity of the new compounds against potentially pathogenic strains was stronger than that of INH. The activity of the starting compounds is described in the previous paper [4]. The increase of the activity against *M. tuberculosis* improves with the replacement of the first oxo group; the replacement of the second oxo group for the thioxo group has only a small effect on the increase of the activity. It is worthy to note that the monohalogenated compounds are more active than the dihalogenated. The alkyl in the position-4 on *N*-phenyl has an effect on the antimycobacterial activity as well. 6-Bromo-3-(4-propylphenyl)-4-thioxo-2*H*-1,3-benzoxazin-2(3*H*)-one **4a** and 6-bromo-3-(4-propylphenyl)-2*H*-1,3-benzoxazin-2,4(3*H*)-dithione **4b** are the most active compounds against *M. tuberculosis*. The activity is similar to INH. On the other hand, 6-chloro-3-(4-propylphenyl)-4-thioxo-2*H*-1,3-benzoxazin-2(3*H*)-one **1a** has the strongest effect against potential pathogenic strains. The newly synthesized compounds form a new, promising group of antimycobacterials with a broad spectrum of antimycobacterial activity against the potentially pathogenic strains. Substances, 6-bromo-3-(4-propylphenyl)-4-thioxo-

2*H*-1,3-benzoxazin-2(3*H*)-one **4a** and 6-bromo-3-(4-propylphenyl)-2*H*-1,3-benzoxazin-2,4(3*H*)-dithione **4b**, were chosen for preclinical testing but, unluckily, both compounds were found to be cytotoxic. (Compound **4a** CC_{50} 10.7 μg/mL, compound **4b** CC_{50} 5.7 μg/mL).

This work is a part of the research project No. MSM0021620822 of the Ministry of Education of the Czech Republic.

The authors have declared no conflict of interest.

Experimental

The melting points were determined on a Kofler apparatus (C. Reichert, Vienna, Austria). The samples for the analyses and antimycobacterial tests were dried over P_2O_5 at 61°C and 66 Pa for 24 h. Elemental analyses (C, H, N) were performed on a CHNS-O CE elemental analyzer (Fisions EA 1110, Milan, Italy) and were within ± 0.4% of the theoretical values. The IR spectra were measured in KBr pellets on a Nicolet Impact 400 apparatus (Nicolet, Madison, WI, USA); 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 ^{1}H-NMR and ^{13}C-NMR spectra of new compounds were recorded in $CDCl_3$ or DMSOd_6 solutions at ambient temperature on a Varian Mercury-Vx BB 300 spectrometer (Varian Inc., Palo Alto, CA, USA) operating at 300 MHz for ^{1}H-NMR and 75 MHz for ^{13}C-NMR. 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 ^{1}H or 39.7 for ^{13}C). The starting 3-(4-alkylphenyl)-2*H*-1,3-benzoxazine-2,4(3*H*)-diones were prepared in our previous papers [4, 5] (see Scheme 1).

Chemistry

*General procedure for the preparation of halogenated 3-(4-alkylphenyl)-4-thioxo-2H-1,3-benzoxazine-2(3H)-ones **1a–10a** and 3-(4-alkylphenyl)-2H-1,3-benzoxazine-2,4(3H)-dithiones **1b–20b***

Halogenated derivatives 3-(4-alkylphenyl)-2*H*-1,3-benzoxazine-2,4(3*H*)-diones prepared in previous paper [4] (3.8 mmol) were melted with P_4S_{10} (7.6 mmol) for 20 min (175 – 200°C). After cooling to the 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 halogenated 3-(4-alkylphenyl)-4-thioxo-2*H*-1,3-benzoxazine-2(3*H*)-one **1a – 10a** and halogenated 3-(4-alkylphenyl)-2*H*-1,3-benzoxazine-2,4(3*H*)-dithione **1b – 10b** as orange-yellow and red solids, respectively. Recrystallization from ethanol was necessary.

Microbiology

The antimycobacterial activity of compounds and isoniazid was tested *in vitro* against *Mycobacterium tuberculosis* My 331/88, *Mycobacterium avium* My 330/88, *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 using the micromethod for the determination of the minimum inhibitory concentration (MIC). The method is described in our previous paper [2, 6]. The values of MIC are summarized in Table 1.

Cytotoxicity

The experimental methods were described in our previous paper [7, 8].

References

[1] J. Adamec, R. Beckert, D. Weiss, V. Klimešová, *et al*., *Bioorg. Med. Chem.* **2007**, *15*, 2898 – 2906.

[2] K. Waisser, R. Doležal, J. Cižmárik, J. Kaustová, *Folia Microbiol.* **2006**, *51*, **21 – 24.**

[3] K. Waisser, L. Heinisch, M. Šlosárek, J. Janota, *Folia Microbiol.* **2006**, *51*, 25 – 26.

[4] K. Waisser, J. Matyk., H. Divišová, P. Husáková, *et al*., *Arch. Pharm.* **2007**, *340*, 264 – 267.

[5] K. Waisser, J. Matyk, H. Divišová, P. Husáková, *et al*., *Arch. Pharm.* **2006**, *33*, **616 – 620.**

[6] J. Adamec, K. Waisser, J. Kuneš, J. Kaustová, *Arch. Pharm.* **2005**, *338*, 385 – 389.

[7] H. M. Dahse, B. Schlegel, U. Gräfe, *Pharmazie* **2000**, *56*, 489 – 491.

[8] H. M. Dahse, U. Möllmann, K. Waisser, K. Palát, *et al*., *Folia Pharm. Univ. Carol.* **2002**, *27 – 28*, 26 – 33.

