Full Paper

The Oriented Development of Antituberculotics (Part II): Halogenated 3-(4-Alkylphenyl)-1,3-benzoxazine-2,4-(3*H*)-diones*

Karel Waisser[1], Josef Matyk[1], Hana Divišová[1], Petra Husáková[1], Jirí Kuneš[1], Vera Klimešová[1], Karel Palát[1], and Jarmila Kaustová[2]

[1] Department of Inorganic and Organic Chemistry, Charles University in Prague, Faculty of Pharmacy in Hradec Králové, Heyrovského, Czech Republic
[2] Regional Institute of Public Health, Department for Diagnostic of Mycobacteria, Ostrava, Czech Republic

Based on our previous studies, 21 new halogenated 3-(4-alkylphenyl)-1,3-benzoxazine-2,4-(3*H*)-diones were synthesized by the reaction of salicylanilides and methyl-chloroformate. All compounds were screened *in vitro* against three different strains of mycobacterium, and Free-Wilson method was used to establish structure-activity relationships. 6-Bromo-3-(4-butylphenyl)-1,3-benzoxazine-2,4-(3*H*)-dione **3b** proved to be the most active compound of the series.

Keywords: Antimycobacterial activity / Antituberculotic activity / Benzoxazine / Tuberculostatics

Received: October 18, 2006; accepted: January 19, 2007

DOI 10.1002/ardp.200600002

Introduction

The return of tuberculosis to Europe and North America is one truly unpleasant development of the period dating from 1985. New mycobacterial diseases, which recently have been considered to be transferable to humans, occurred during the last years (mycobacterioses produced by potentially pathogenic strains). The development of new antituberculotic agents is the principal goal of our group. Lately, we have studied a number of structurally different compounds, such as the derivatives of salicylamides [1], pyridine [2, 3], alkoxyphenylcarbamic acids [4], tetrazoles [5, 6], dihydroindolethiones [7], and other heterocycles [8, 9]. Our research was strongly oriented to collaborate with German institutes like Technical University Dresden, Friedrich Schiller University Jena, Hans Knöll Institute Jena, and Maxmilian University in Munich. Approximately ten years ago, we studied antimycobacterial derivatives with the new pharmacophore together with Hans-Dietrich Stachel [10, 11] and for several years now, we are investigating the antimycobacterial arylbenzoxazine-2,4(3*H*)-diones [12].

The goal of this paper is the synthesis and antimycobacterial evaluation of 3-(4-alkyphenyl)-1,3-benzoxazine-2,4(3*H*)-diones, substituted in the ring B by chlorine or bromine, and to complete with this study our previous work [1]. 3-Phenyl-1,3-benzoxazine-2,4(3*H*)-diones are the cyclic carbamic derivatives of salicylanilides and the mechanism of the antimycobacterial activity of 3-phenyl-1,3-benzoxazine-2,4(3*H*)-diones is similar to that of salicylanilides. [13].

Results and discussion

Chemistry

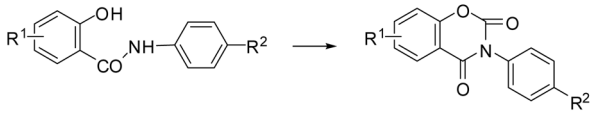
The synthesis of the title compounds is illustrated in Scheme 1. The synthesis of the starting material, halogenated *N*-(4-alkylphenyl)salicylamides, were described in the previous paper [1]. Various *N*-(4-alkylphenyl)salicylamides, being halogen substituted in ring A, were reacted with methyl-chloroformate in dry pyridine. The halogenated 3-(4-alkylphenyl)-1,3-benzoxazine-2,4(3*H*)-

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: +420 495 514-330

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

Table 1. Yield, melting point, and carbonyl frequency of 3-(4-alkylphenyl)-1,3-benzoxazine-2,4(3*H*)-diones.

Compound	Yield (%)	M. p. (°C)	$\nu_{C=O}$ (cm^{-1})	Compound	Yield (%)	M. p. (°C)	$\nu_{C=O}$ (cm^{-1})
1a	60	188 – 190	1773, 1703	**2f**	56	134 – 135	1777, 1708
1b	58	189 – 190	1771, 1705	**2g**	67	141 – 142	1777, 1708
1c	61	214 – 215	1770, 1709	**3a**	58	193 – 194	1774, 1700
1d	52	181 – 182	1773, 1701	**3b**	63	194 – 195	1770, 1702
1e	52	162 – 165	1776, 1699	**3d**	64	179 – 180	1777, 1709
1g	50	181 – 182	1773, 1702	**3e**	58	168 – 169	1774, 1701
2a	63	158 – 160	1777, 1696	**3f**	62	158 – 160	1774, 1701
2b	66	161 – 162	1776, 1708	**4b**	47	168 – 169	1774, 1701
2c	66	214 – 215	1767, 1716	**4d**	58	197 – 198	1780, 1701
2d	56	136 – 137	1777, 1708	**4e**	48	196 – 199	1781, 1703
2e	51	146 – 148	1776, 1708				



	R^1		R^2
1	6-Cl	**a**	propyl
2	7-Cl	**b**	butyl
3	6-Br	**c**	*tert*-butyl
4	6,8-Br_2	**d**	pentyl
		e	hexyl
		f	heptyl
		g	octyl

Scheme 1. Synthesis of 3-(4-alkylphenyl)-1,3-benzoxazine-2,4(3*H*)-diones.

diones were purified by crystallization from ethanol. Yield, melting point, and carbonyl frequency of the products are summarized in Table 1. Data from elemental analysis and NMR spectra are described in the Supporting Information.

Biology

In vitro antimycobacterial activity of the compounds was evaluated against *Mycobacterium tuberculosis* CNCTC My 331/88, *Mycobacterium kansasii* CNCTC My 235/80, *Mycobacterium avium* CNCTC My 330/88 and *Mycobacterium kansasii* 6 509/96 using the micromethod for the determination of minimum inhibitory concentration (MIC). All strains were obtained from the Czech National Collection of Type Cultures (CNCTC), National Institute of Public Health, Prague with the exception of *M. kansasii* 6 509/96. The minimum inhibitory concentrations are illustrated in Table 2. For the sake of comparison, we also include the MIC values of the standard isoniazide (INH).

Calculation

All calculations were carried out with the use of the Multireg H program (Klemera) for Microsoft Excel. The results of Free – Wilson method are summarized in Table 3.

Table 2. Minimum antimycobacterial inhibitory activity.

Compound			MIC (μmol/L) Incubation time 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* 6 509/ 96
1a	6-Cl	propyl	16/16	8/8	16/16	8/16
1b	6-Cl	butyl	8/8	8/8	8/8	8/16
1c	6-Cl	*tert*-butyl	8/16	8/16	16/32	8/8
1d	6-Cl	pentyl	8/4	8/16	8/16	8/16
1e	6-Cl	hexyl	8/8	8/16	8/8	8/16
1g	6-Cl	octyl	8/8	4/8	8/8	4/4
2a	7-Cl	propyl	8/8	8/16	16/16	8/16
2b	7-Cl	butyl	8/8	8/16	16/16	8/16
2c	7-Cl	*tert*-butyl	4/4	8/16	4/8	8/8
2d	7-Cl	pentyl	8/8	8/8	4/8	8/16
2e	7-Cl	hexyl	4/8	8/8	4/8	8/8
2f	7-Cl	heptyl	16/32	8/8	16/32	8/16
2g	7-Cl	octyl	8/16	8/16	8/16	8/16
3a	6-Br	propyl	16/16	8/16	16/16	8/16
3b	6-Br	butyl	4/4	8/8	8/8	8/8
3d	6-Br	pentyl	4/4	8/16	8/8	8/8
3e	6-Br	hexyl	4/8	8/16	4/8	8/8
3f	6-Br	heptyl	4/8	8/16	4/4	4/8
4b	6,8-Br_2	butyl	32/32	62.5/62.5	62.5/62.5	62.5/62.5
4d	6,8-Br_2	pentyl	16/32	16/16	62.5	32/32
4e	6,8-Br_2	hexyl	62.5/62.5	32/32	62.5	62.5/62.5
INH			1/2	250/250	250/250	8/8

Discussion

The study was incited by the previous synthesis of antimycobacterial salicylanilides. For the synthesis of 3-(4-alkylphenyl)-1,3-benzoxazine-2,4(3*H*)diones salicylanilides are reacted with chloroformate. The 1,3-benzoxazine-2,4(3*H*)diones are cyclic carbamates of salicylanilides. The structures of products were confirmed by elemental analyses and by IR, ^{1}H- and ^{13}C-NMR spectral methods. All halogenated 3-(4-alkylphenyl)-1,3-benzoxazine-2,4(3*H*)diones showed characteristic absorption maxima of two C=O groups at 1767 – 1777 cm^1 and 1696 – 1716 cm^1 with the exception of compound **3d.** Two absorption maxima of the C=O groups in this region are a characteristic feature for 1,3-benzoxazine-2,4(3*H*)diones. The other confirmation of the structure was by NMR

Table 3. Activity contribution of Free-Wilson analyzes of benzoxazinediones and statistical significants of correlations.

Parameter	Δ log MIC (μmol/L) For incubation time 14 d/21 d			
	M. tuberculosis My 331/88	*M. kansasii* My 235/80	*M. avium* *My 330/88*	*M. kansasii* 6509/96
R^1: 6-Cl	0.0209/-0.207	−0.1121[a)]	−0.0435/−0.0646	−0.1383/−0.062
7-Cl	−0.0935/−0.0842	−0.0638[a)]	−0.1504/−0.0569	−0.0696/−0.006
6-Br	−0.2664/−0.2469	0.0023[a)]	−0.2318/−0.2654	−0.1459/−0.235
6,8-Br_2	0.6204/0.6494	0.5133[a)]	0.8247/0.7043	0.7451/0.5321
R^2: propyl	0.2701/0.1744	0.0152[a)]	0.2991/0.1718	0.0322/0.1585
butyl	−0.0444/−0.1423	0.0901[a)]	0.0575/−0.0365	0.0572/0.679
tert-butyl	−0.1566/−0.0904	0.0165[a)]	−0.0458/0.1037	0.0182/−0.208
pentyl	−0.1132/−0.1423	−0.0597[a)]	−0.0926/−0.0365	−0.0178/0.0002
hexyl	−0.0382/0.0078	0.0153[a)]	−0.0962/−0.115	0.0572/0.0002
heptyl	0.1371/0.3227	0.0025[a)]	0.0483/0.054	−0.128/0.028
octyl	−0.0066/0.0596	−0.1335[a)]	−0.0458/−0.0464	−0.1318/−0.207
μ_o	0.943/1.043	0.9714[a)]	1.043/1.157	0.1015/0.1724
r	0.881/0.878	0.928[a)]	0.915/0.860	0.971/0.901
s	0.2033/0.2087	0.1253[a)]	0.211/0.238	0.1015/0.1724
F	4.24/4.12	7.57[a)]	6.25/3.48	20.42/5.25
n	21/21	21/21	21/21	21/21

[a)] Correlation is not statistically significant.

methods: The ^{1}H- and ^{13}C-NMR spectra of all compounds (see Table 1) are in the good agreement with the proposed structures. In addition, elemental analyses of all compounds correspond to the calculated values.

The values of antimycobacterial activity of the halogenated 3-(4-alkylphenyl)-1,3-benzoxazine-2,4(3*H*)-diones are shown in Table 2. For the sake of comparison, we also included the MIC values of the standard isoniazide (INH). The results revealed that the compounds exhibited *in vitro* activity against all tested mycobacterial strains. The values of MICs are generally within the range 4-62.5 μmol/L, most often between 4-8 μmol/L. The compounds were less active than INH against *M. tuberculosis* 331/88, on the other hand, the compounds possessed a better activity against *M. kansasii* 235/80 and *M. avium* 330/88 than INH. The activities of newly prepared compounds against *M. kansasii* 6509/ 96 (clinical isolate) are comparable with that of INH. The newly synthesized compounds form the new promising group of antimycobacterials with the broad spectrum of activity. It is worth to note, that the monohalogenated compounds are more active than the dihalogenated ones. The antimycobacterial activity increases if 1,3-benzoxazine-2,4(3*H*)diones are substituted in position 6 with bromine. Bromo derivatives were more active than chloro derivatives. Contrary to the starting salicylanilides, the antimycobacterial activity mostly increases when the phenyl ring is substituted by butyl. (In case of salicylanilides it was propyl). The most active in the group of halogenated 3-(4-alkylphenyl)-1,3-benzoxazine-2,4(3*H*)dione was 6-bromo-3-(4-butylphenyl)-1,3-benzoxazine-2,4(3*H*)-dione **3b**. According to Free-Wilson calculations, we can assume that 6-bromo-3-(4-*tert*-butylphenyl)-1,3-benzoxazine-2,4(3*H*)-dione will be more active than the compounds under study.

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

Experimental

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

General procedure for the preparation 3-(4-alkylphenyl)-1,3-benzoxazine-2,4(3*H*)-diones

Methyl-chloroformate (5.2 g, 48 mmol) was added dropwise to a stirred solution of the corresponding 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, and the solid was filtered off. The crude products were purified by the crystallization from EtOH-water (yields 55 – 65%).

Antimycobacterial susceptibility testing

The methods of the experiments are described in our previous paper [12]. The minimum inhibitory concentrations (MIC) were determined after incubation at 37°C for 14 and 21 days.

References

[1] K. Waisser, J. Matyk, H. Divišová, P. Husáková, *et al*., *Arch. Pharm. Chem. Life. Sci.* **2006**, *339*, 616 – 620.

[2] L. Zahajská, V. Klimešová, J. Kocí, K. Waisser, J. Kaustová, *Archiv Pharm Pharm. Med. Chem.* **2004**, *337*, 549 – 555.

[3] K. Waisser, K. Dražková, J. Kuneš, V. Klimešová, J. Kaustová, *Farmaco* **2004**, *59*, 615 – 625.

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

[5] K. Waisser, J. Adamec, R. Doležal, J. Kaustová, *Folia Microbiol.* **2005**, *50*, 195 – 197.

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

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

[8] J. Matyk, K. Waisser, K. Dražková, J. Kuneš, *et al*., *Farmaco* **2005**, *60*, 399 – 408.

[9] K. Waisser, L. Heinisch, M. Šlosárek, J. Janota, *Folia Microbiol.* **2005**, *50*, 479 – 481.

[10] H.-D. Stachel, Ž. Odlerová, K. Waisser, *Collect. Czech. Chem. Commun.* **1997**, *62*, 510 – 515,

[11] J. E. Schachtner, J. Hienaber, H. D. Stachel, K. Waisser, *Pharmazie* **1999**, *54*, 335 – 339.

[12] K. Waisser, O. Bureš, P. Holý, J. Kuneš, *et al*., *Pharmazie* **2003**, *58*, 83 – 94.

[13] K. Waisser, K. Dražková, P. Holý, K. Palát, J. Kaustová, *Folia Pharm. Univ. Carol.* **2003**, *29/30*, 17 – 20.

