SHORT ARTICLES

High active potential antimycobacterial agents against *Mycobacterium avium*

Vysoce účinné potenciální antimykobakteriální látky proti *Mycobacterium avium*

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Summary

Derivatives of 3-phenyl-*2H*-1,3-benzoxazine-2,4(3*H*)--dione are active against *Mycobacterium avium* when substituted in position 7 with a methyl. One or two carbonyl groups have to be replaced with a thioxo group. High active derivatives are the compounds without substitution on the phenyl, or those substituted on the phenyl in position 3 or 4 with chlorine, bromine or a methyl. The substitution in position 3 and 4 with two atoms of chlorine lowers the activity. The compounds are active against INH resistant strains. We synthesized other 44 derivatives with a similar structure of the compounds as in the paper but substituted in position 7 with other substituents (chlorine, bromine methoxy). The activity against *M. avium* was poor. It can be concluded that a new group of compounds with an excellent activity against *M. avium* has been found.
Keywords: benzoxazine • thioxo group • *Mycobacterium avium* • antimycobacterial activity

Souhrn

Deriváty 3-fenyl-*2H*-1,3-benzoxazin-2,4(3*H*)-dionu jsou aktivní vůči *Mycobacterium avium,* pokud jsou substituovány v poloze 7 methylem. Jedna nebo dvě karbonylové skupiny musí být zaměněny za thioxo skupiny. Nejaktivnější sloučeniny nebyly substituovány na fenylu, nebo byly substituovány na fenylu v poloze 3 nebo 4 chlorem, bromem či methylem. Substituce v poloze 3 a 4 dvěma atomy chloru však aktivitu snižuje. Sloučeniny jsou aktivní vůči INH rezistentním kmenům. Připravili jsme dalších 44 derivátů s podobnou strukturou studovaným látkám, avšak s jiným substituentem v ploze 7 (chlor, brom, methoxy). Aktivita vůči *M. avium* byla nízká. Můžeme uzavřít, že se jedná o novou skupinu významně aktivních látek vůči *Mycobacterium avium.*

Klíčová slova: benzoxazin thioxo skupina • *Mycobacterium avium* • antimykobacteriání aktivita

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Introduction

The recent reappearance of tuberculosis and other infectious diseases is a serious problem worldwide. According to the World Health Organization, there were 9.4 million new tuberculosis cases in 2008 including 1.4 million cases among HIV positive people. In the year 2008, WHO also reported the highest rates of MDR-TB ever recorded[1]. Therefore, there is a great need to develop new antituberculotics and antibiotics. New mycobacterial diseases have occurred which have been recently considered noncontagious to humans (mycobacterioses produced by potentially pathogenic strains). The development of antimycobacterial drugs is usually focused on *Mycobacterium tuberculosis* and *Mycobacterium leprae.* Mycobacterial diseases due to multiresistant strains of *Mycobacterium avium* are not frequent, but often fatal in the end. The review of chemotherapy of nontuberculous mycobacterial infections is taken over from Tortoll[2]. The goal of the present paper is a search in the group of 7-methyl-3-phenyl-4-thioxo-2*H-1,3*-benzoxazine-2(3*H*)--ones for active compounds against *M. avium.*

Experimental

Chemistry

General information

The melting points were determined on a Kofler apparatus. The samples for the analyses and antimycobacterial tests were dried over P_2O_5 at 61 °C and 66 Pa for 24 h. The 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 purity of compounds was checked by TLC on silica gel plates precoated with the fluorescent indicator Silufol UV 254 + 366 (Kavalier Votice, Czech Republic), hexane-acetone (3 : 1) being used as the mobile phase. The

^{1}H NMR and ^{13}C NMR spectra of the new compounds were recorded in DMSO–d_6 solutions at ambient temperature on a Varian Mercury-Vx BB 300 spectrometer operating at 300 MHz for ^{1}H NMR and 75 MHz for ^{13}C NMR.

General procedure for the preparation of 7-methyl 3-phenyl-4-thioxo-2H-1,3-benzoxazine-2(3H)-ones and 7-methyl 3-phenyl-2H-1,3-benzoxazine-2,4(3H)-dithiones

The derivatives of 7-methyl-3-phenyl-2*H*-1,3--benzoxazine-2,4(3*H*)-diones prepared in our previous paper[3] (4 mmol) were melted with P_4S_{10} (10 mmol) for 45 minutes (180—210 °C). After cooling to room temperature, a 10% potassium carbonate solution 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 yielded substituted derivatives of 7-methyl-3-phenyl--4-thioxo-2*H*-1,3-benzoxazine-2(3*H*)-one or (**1–11**) and derivatives of 7-methyl-3-phenyl-2*H*-1,3-benzoxazine--2,4(3*H*)-dithione (**12–22**) as orange-yellow and red solids, respectively. Recrystallization from ethanol was necessary.

Microbiology

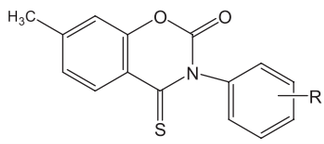
The antimycobacterial activity of the compounds was determined in the Šula semisynthetic medium (SEVAC, Prague). This medium (with bovine serum) is routinely used in the Czech Republic. Each strain was simultaneously inoculated into a Petri dish containing the Löwenstein-Jensen medium for the control of sterility of the inoculum and its growth. The compounds were added to the medium in DMSO solutions. The final concentrations were 1000, 500, 250, 125, 62.5, 32, 16, 8, 4, 2, 1 and 0.5 μmol/l. The minimum inhibitory concentration MICs were determined after incubation at 37 °C for 14 days and 21 days. The evaluation was repeated three times and the values of the minimum inhibitory concentration were the same.

Results

Chemistry

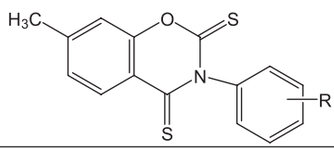
The reaction of phosphorus pentasulfide with 7-methyl-3-phenyl-2*H*-1,3-benzoxazine-2,4(3*H*)-diones was used. The product was a mixture of 7-methyl-3--phenyl-4-thioxo-2*H*-1,3-benzoxazine-2(3*H*)-one and 7--methyl-3-phenyl-2*H*-1,3-benzoxazine-2,4(3*H*)--dithione. The mixture is easily separable by chromatography on silica gel. The structures of compounds are characterized by NMR and IR spectroscopy and their purity was checked by TLC and elemental analysis. The starting compounds are described[3].

Table 1. Minimum inhibitory concentrations (MIC) in μg/l activity of 7-methyl-3-phenyl-4-thioxo-2H-1,3-benzoxazine-2(3H)-one against M. avium



Compounds	R	MIC (μmol/l)	Compounds	R	MIC (μmol/l)
1	H	2/2	**7**	4-F	2/4
2	4-CH_3	2/2	**8**	3-F	4/8
3	4-Cl	1/2	**9**	4-CF_3	8/16
4	3-Cl	2/2	**10**	4-COOEt	16/16
5	3,4-Cl_2	8/8	**11**	4-CN	62.5/62.5
6	4-Br	2/4	**INH**		> 250/> 250

Table 2. Minimum inhibitory concentrations (MIC) in μg/l activity of 7-methyl-3-phenyl-2H-1,3-benzoxazine-2,4(3H)-dithiones against M. avium



Compounds	R	MIC (μmol/l)	Compounds	R	MIC (μmol/l)
12	H	2/2	**18**	4-F	4/4
13	4-CH_3	2/2	**19**	3-F	4/4
14	4-Cl	2/2	**20**	4-CF_3	16/16
15	3-Cl	2/2	**21**	4-COOEt	16/16
16	3,4-Cl_2	4/8	**22**	4-CN	n/n
17	4-Br	2/4	**INH**		> 250/> 250

Microbiology

An INH-resistant strain of *M. avium* Czech Collection of Type Cultures (CNCTC) My 330/88 was used (identical with ATCC 12.478). The results are summarized in Table 1 and Table 2.

Findings

The highest antimycobacterial activity was shown by 7-methyl-3-phenyl-4-thioxo-*2H*-1,3-benzoxazine-2(3*H*)-one and its derivatives substituted on the phenol ring by chlorine or bromine. The activity of 7-methyl-3-(4-methylphenyl)-4-thioxo-*2H*-1,3-benzoxazine-2(3*H*)-one was admirable as well. Similar substituted derivatives in the group of 7-methyl-3-phenyl-*2H*-1,3--benzoxazine-2,4(3*H*)-dithione show also such an activity. The activity against *M. avium* of the starting 7-methyl-3-phenyl-*2H*-1,3-benzoxazine-2,4(3*H*)-dione was not significant We synthesized other 44 derivatives with a similar structure of the compounds as in the paper but substituted in position 7 with other substituents (chlorine, bromine, methoxy). The activity against *M. avium* was poor. It can be concluded that a new group of compounds with an excellent activity against *M. avium* has been found.

Conflicts of interest: none.

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