Antimycobacterial 3-phenyl-4-thioxo-2H-1,3-benzoxazine-2(3H)-ones and 3-phenyl-2H-1,3-benzoxazine-2,4(3H)-dithiones substituted on phenyl and benzoxazine moiety in position 6

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A series of forty-five derivatives of 3-phenyl-4-thioxo-2H-1,3-benzoxazine-2(3H)-ones and forty-five derivatives of 3-phenyl-2H-1,3-benzoxazine-2,4(3H)-dithiones was synthesised. The compounds exhibited in-vitro activity against Mycobacterium tuberculosis, M. kansasii (two strains), and M. avium. The most active derivatives were more active than isonicotinhydrazide (INH). The quantitative relationships between the structure and antimycobacterial activity were calculated. The activity against M. tuberculosis increased with the lipophilicity of the substituents.

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Introduction

The prognosis that, following the millennium, tuberculosis would no longer occur in the developed world was wrong (O’Brien & Nunn, 2001). The emergence of multi-drug resistant (MDR-TB) and extensively-drug resistant (XDR-TB) strains of Mycobacterium tuberculosis is a serious problem and tuberculosis remains one of the leading infectious diseases worldwide (Dye, 2009). This unfavourable state is also being influenced by an increase in AIDS, which is often accompanied by the mycobacterial diseases and with the low standard of living of displaced persons (Aaron et al., 2004; Naidoo et al., 2011). New mycobacterial diseases are occurring which, until recently, were considered non-transferable to humans (Tortoli, 2009).

From the perspective of pharmaceutical treatment, N-benzylsalicylamides, salicylanilides, and their cyclic derivatives, benzoxazinediones, are promising classes of compounds (Matyk et al., 2005; Nemeček et al., 2009; Petrlíková et al., 2011, 2010; Waisser et al., 2006). This study is oriented towards the derivatives of benzoxazinediones in which one or both oxo groups were replaced by the thioxo group. Since the compounds are cyclic derivatives of salicylanilides, they can be expected to serve as bacterial two-component system inhibitors (Hlasta et al., 1998; Macielag et al., 1998). Benzoxazine derivatives could also target the biosynthesis of menaquinone, a polyisoprenylated naphtoquinone, that plays an important role in the mycobacterial electron transport chain (Li et al., 2010). Both types of mechanism of action are very promising since the consequent antibacterial effects
are probably different from the effects of other antibacterial drugs (Schroeder et al., 2002; van den Boogaard et al., 2009).

Theoretical

Regressions were calculated using Microsoft Excel Multireg programs. The values of the substituent \( \pi \) and \( \sigma \) constants were taken from the literature (Hansch & Leo, 1979). The stability of the QSAR models was evaluated by cross-validation (leave-one-out procedure) (Gupta et al., 2009; Golbraikh & Tropsha, 2002) in Matlab 7.0 program. The Free–Wilson method (Free & Wilson, 1964) modified by Fujita–Ban (Fujita & Ban, 1971) was used to investigate the activity contribution in the case of sulphur derivatives. Since the MIC values after 14 d and 21 d incubation correlated with each other, only the MICs after 14 d evaluation were taken for the calculations.

Experimental

Materials and methods

The melting points were determined using the Kofler apparatus (C. Reichert, Vienna, Austria) and the temperature data were corrected. The samples for analyses and antimycobacterial tests were dried over \( \text{P}_2\text{O}_5 \) at 61 °C and 66 Pa for 24 h. The elemental analyses (C, H, N, S) were performed on a CHNS-O CE elemental analyzer (Fisons EA 1110, Milan, Italy) and were within ±0.4 % of the theoretical values. The IR spectra of KBr pellets were measured on a Nicolet Impact 400 apparatus (Nicolet, Madison, WI, USA); the wavenumbers are given in cm\(^{-1}\). The purity of the compounds was verified by TLC on silica gel plates pre-coated with a fluorescent indicator Silufol UV 254 + 366 (Kavalier Votive, Czech Republic) and hexane–acetone mixture (\( \varphi_r = 3 : 1 \)) as the mobile phase. The \( ^1\text{H} \) NMR and \( ^{13}\text{C} \) NMR spectra of new compounds were recorded in DMSO-\( d_6 \) solutions at ambient temperature on a Varian Mercury-Vx BB 300 spectrometer (Varian Inc., Palo Alto, CA, USA) operating at 300 MHz for \( ^1\text{H} \) NMR and 75 MHz for \( ^{13}\text{C} \) NMR. Chemical shifts were recorded as δ values and indirectly referenced to tetramethylsilane via the solvent signal (DMSO) (2.5 for \( ^1\text{H} \) or 39.5 for \( ^{13}\text{C} \)).

General procedure for the preparation of 3-phenyl-4-thioxo-2H-1,3-benzoxazine-2(3H)-ones (I–V) and 3-phenyl-2H-1,3-benzoxazine-2,4(3H)-dithiones (VI–X)

The derivatives of 3-phenyl-2H-1,3-benzoxazine-2,4(3H)-diones (4 mmol) underwent fusion with \( \text{P}_4\text{S}_{10} \) (10 mmol) for 45 min (180–210 °C). After cooling to room temperature, a 10 % potassium carbonate solution was poured into the reaction mixture; the crude product was removed by filtration, and dissolved in toluene (p.a., a maximum of 40 mL). Column chromatography using silica gel provided substituted derivatives of 3-phenyl-4-thioxo-2H-1,3-benzoxazine-2(3H)-one (I–V) and substituted derivatives of 3-phenyl-2H-1,3-benzoxazine-2,4(3H)-dithione (VI–X) as orange-yellow and red solids, respectively. Recrystallisation from ethanol was necessary.

Antimycobacterial susceptibility testing

For the in-vitro evaluation of the antimycobacterial activity of the substances, the following strains were used: M. tuberculosis CNCTC My 331/88 (identical with H37RV and ATCC 27294), M. kansasii CNCTC My 235/80 (identical with ATCC 12 478), M. avium CNCTC My 330/88 (identical with ATCC 25291), obtained from the Czech National Collection of Type Cultures (CNCTC), National Institute of Public Health, Prague, and a clinical isolate of M. kansasii 6509/96. The antimycobacterial activity of the compounds was determined in the Sula semisynthetic medium (SEVAC, Prague). In order to control the sterility of the inoculum and its growth, a Petri dish containing the Löwenstein–Jensen medium was inoculated with each strain. The compounds were added to the medium dissolved in DMSO. The final concentrations were 1000 \( \mu \text{mol L}^{-1} \), 500 \( \mu \text{mol L}^{-1} \),

![Fig. 1.](image-url) Synthesis of 3-phenyl-4-thioxo-2H-1,3-benzoxazine-2(3H)-ones and 3-phenyl-2H-1,3-benzoxazine-2,4(3H)-dithiones.