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PYRIDO-1,2-THIAZINES AND THEIR *IN VITRO* ANTIBACTERIAL EVALUATION

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Abstract: A series of known and newly synthesized pyrido-1,2-thiazine derivatives of type **3-6** were evaluated against strains of *Mycobacterium fortuitum* (PCM 672) and *Staphylococcus aureus* (PCM 2602) The pilot experiments showed that most of the compounds in initial *in vitro* microbiological evaluation were not efficient antibacterial agents, but unexpectedly promoted replication of the microorganisms in the range of 10-50%.

Keywords: pyridothiazines, antibacterial activity

Pyridino-1,2-thiazines **I** (Figure 1), depending on the substituents R and R' and on the manner of fusion of the pyridine and 1,2-thiazine rings, are reported to have multiple biological activities including anti-inflammatory, analgesic, antitumor and antioxidant action (1-5). Previously we also described the activity of some pyrido-1,2-thiazines of type **II** (Figure 1) against *Mycobacterium tuberculosis* H37Rv in the range of 10-40% at a concentration of 12.5 μg/mL (6). This latter observation stimulated us to modify the β-dicarbonyl moiety partially incorporated in the structure of the 1,2-thiazine ring of **II** (R' = CH_3) in order to investigate the influence of this structural change on anti(myco)bacterial activity. Accordingly, we tested *in vitro* in a microbiological evaluation a series of 2-methylpyrido-1,2-thiazines of enamine type **3**, related derivatives of the triheterocyclic systems **4**, **5** and 4-methoxy derivative **6** (Scheme 1).

Chemistry

The synthesis of the enamines the **3c-e**, triheterocycles **4b**, **5b** and of compound **6** were described in our recent paper in connection with another project (6). Enamines **3c-e** were prepared by treating pyrido-1,2-thiazine **2b** with the corresponding primary amines (30-70% yield), whereas the triheterocycles **4b** (62% yield) and **5b** (18% yield) were obtained in reaction of **1b** and 1,2-dibromoethane or ethylenimine, respectively.

The (Z)-configuration of compounds **3c-e** and the structures of triheterocycles **4b**, **5b** and pyridothiazine **6** were assigned on the bases of IR, ^{1}H NMR and, in some cases (**3c**, **4b**), X-ray data (6, 7). The new enamines (**3a**,**b**,**f**,**g**) (30-60% yield) and triheterocycles **4a** (65% yield) and **5a** (12% yield) were obtained similarly (Scheme 1). The amine intermediates (H_2N-CH_2-R', Scheme 1) used in preparation of new enamines, were commercially available.

The experimental data for the preparation of new compounds **3-5** together with their physicochemical and spectral (IR, ^{1}H NMR) data, a supporting material are available on request (malinka@bf.uni.wroc.pl). Additionally, the structure of triheterocycle **5a** was confirmed by X-ray (8), the more so that the pyrido[3,2-*e*]pyrazino[1,2-*b*][1,2]-thiazine ring system of compounds **5** has not been measured yet (6).

Because the spectral data within series of new enamines **3a**,**b**,**f**,**g** did not show remarkable differences from their (E)-**3c-e** analogues described earlier (6) we also proposed the (E)-configuration for all new compounds **3**. In this context it should be noted that triheterocycles **5** can be partially considered as (Z)-analogues of (E)-enamines **3** and may be used to investigate the influence of the conformational factor on the biological action within the series. It is also worth noting that triheterocycles **4** and **5** belong to the new systems which we described in 2004 (6), and here we present the first information about their pharmacological (microbiological) properties.

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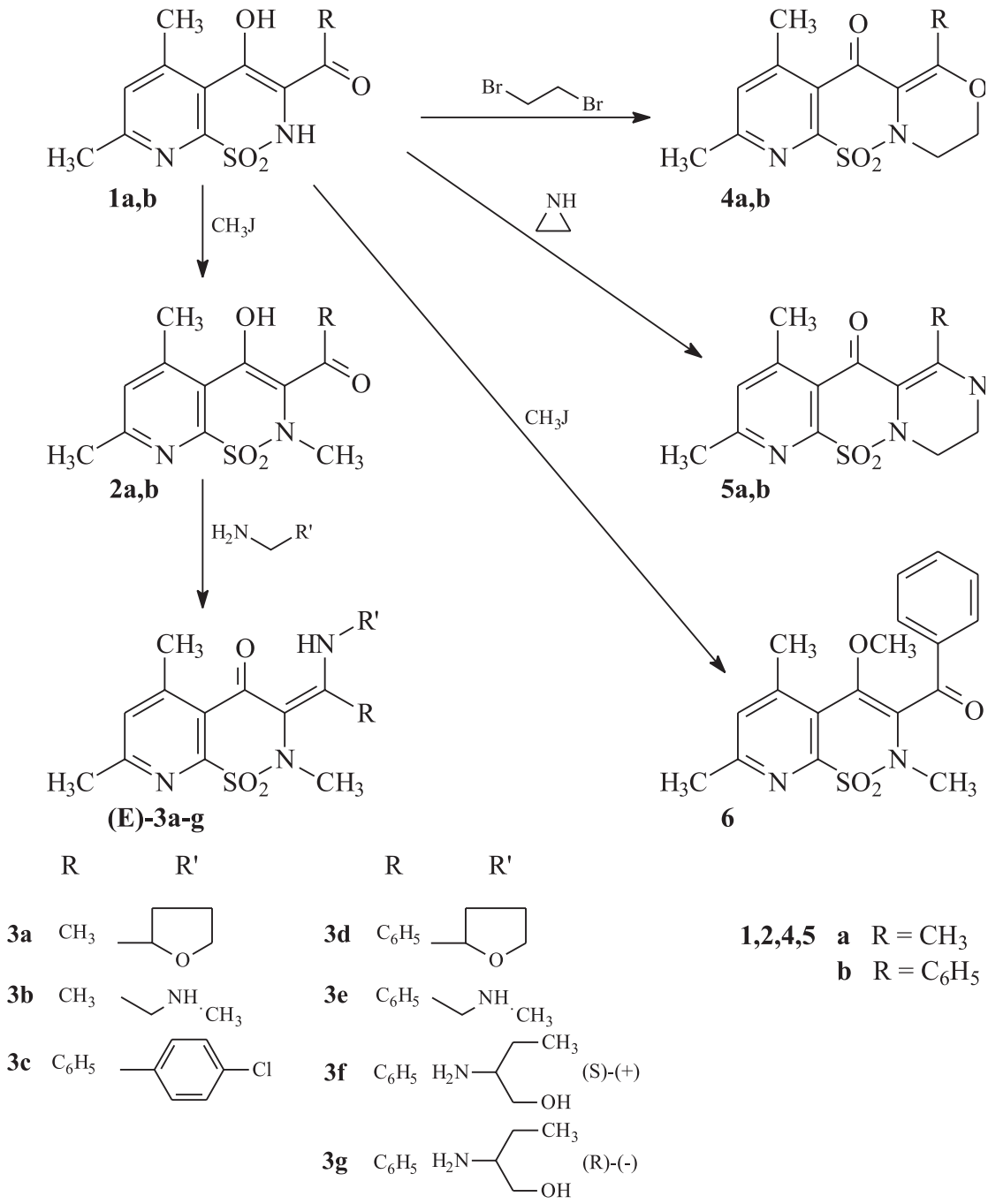


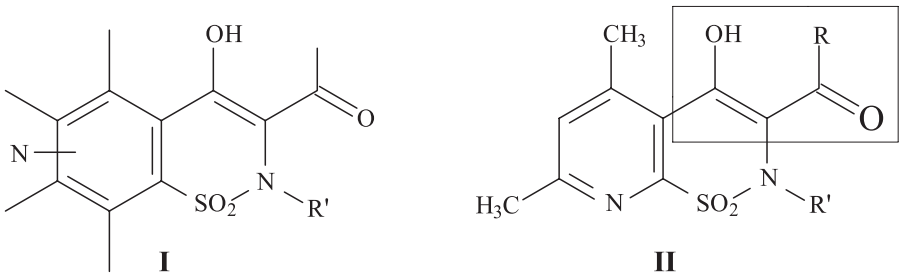
Scheme 1.

MICROBIOLOGY

Materials and Methods

Bacterial strains and growing conditions

The strains of *Mycobacterium fortuitum* (PCM 672) and *Staphylococcus aureus* (PCM 2602) (obtained from the Polish Collection of Microorganisms (PCM) of the Institute of Immunology and Experimental Therapy, Polish Academy of Sciences) were used throughout the study. Bacteria were cultivated on liquid 79 culture medium (for *M. fortuitum*), Luria-Bertani (LB) medium (for *S. aureus*) at 37°C for 24 h under aero-



R = CH_3, C_6H_5
R' = H, CH_3, $CH_2CO(NHR"$, OC_2H_5, C_6H_5)

Figure 1.

bic conditions. Then bacterial cells were diluted with the same media, respectively, to obtain suspensions of about 2×10^5 cfu/mL of each strain.

Antibacterial succeptibility test

The antibacterial activities of synthesized compounds were determined against bacterial strains by the microplate Alamar Blue assay according to Ahmed et al. (9). Stock solutions of the compounds were prepared in DMSO (1 mg/mL) and were diluted with appropriate media in the range 0.03 – 1000 µg/mL on cell culture microtitration plate. To the wells containing 100 µL of drug compound, aliquots of 100 µL of the diluted suspension of the strain were added. The control wells consisting of either bacteria only or medium only and those containing different drug concentrations (100 µL) were inoculated with 100 µL of the diluted bacterial cells. Plates were incubated at 37°C for 48 h and after that 20 µL of Alamar Blue (10× diluted) and 12.5 µL of 20% Tween 80 solutions were added to the wells and incubation was continued at 37°C for 2 h. Fluorescence was measured using Victor apparatus (Wallac, Perkin Elmer). The experiment was repeated two- or three-fold. The minimal inhibitory concentration (MIC) was defined as the lowest drug concentration which prevented a color change from blue to pink, inhibiting the bacterial growth for = 90%. The means and standard error values were determined using Statistica program.

RESULTS AND DISCUSSION

All compounds **3-6** shown in Scheme 1, except **5a**, which was isolated at a very low yield (12%), were evaluated against *Mycobacterium fortuitum* PCM 652 and *Staphylococcus aureus* PCM 2602. The literature suggests that the activity of, for example, chinolone derivatives against *M. fortuitum* correlate closely with the activity against *M. tuberculosis* (10). Therefore in our experiments the less hazardous microorganism *M. fortuitum* was used to evaluate the anti-*Mycobacterium tuberculosis* activity of our compounds **3-6**.

Isoniazide was used as a standard in the test against *Mycobacterium. fortuitum*, whereas ery-

Table 1. Effects of the compounds **3-6** on *Mycobacterium fortuitum* (PCM 672) and *Staphylococcus aureus* (PCM 2602) growth.

No.	*Mycobacterium fortuitum*		No.	*Staphylococcus aureus*	
	10% mg/mL*	10-50% mg/mL**		10% mg/mL*	10-50% mg/mL**
3c	0.45	15.6	**3a**	7.8	
3d	0.03	250***	**3b**	250	
3e	7.8	125	**3e**	31.25	
4a	0.45	125	**3f**	31.25	250
4b	0.03	250	**3g**	0.45	62.5
5b	1.95	250	**4a**	3.9	
			4b	3.9	62.5

Concentration required to increase the number of bacteria by: <10%*, ≥10 to ≤50%**, > 50%***

tromycine was a reference drug in evaluation of compounds against *Staphylococcus aureus*.

All compounds evaluated against *M. fortuitum* either demonstrated any inhibitory activity at the MIC_{50} and MIC_{90} levels, even at the maximal employed concentration (250 mg/mL), or their limited solubility prohibited an accurate determination. MIC_{50} for isoniazide was < 1 μg/mL, while the MIC_{90} vale for this drug was greater than 250 μg/mL. However, to our complete surprise, all of the compounds tested helped to stimulate the growth of the *Mycobacterium fortuitum* strain (Table 1). The best stimulant was **3d**, which promoted the growth of the microorganisms > 50%, however, the maximal effect was observed only at the highest concentration used (250 μg/mL). For the remaining compounds shown in Table 1, the maximal stimulation of growth of *M. fortuitum* was found within the range of 10-50% at different concentrations for individual compounds (e.g. **3c** – 15.6 μg/mL, **5b** – 250 mg/mL). It should be noted that the replication stimulating effect on the microorganisms at the < 10% level was observed at sub-mg/mL concentrations (0.03-0.45 μg/mL) for most of the compounds in Table 1. The other compounds (**3a**,**b**,**f**,**g**,**h**, **6**) tested against *Mycobacterium fortuitum* exhibited weak stimulation (below 10%), and only after application of the preparations at the maximal concentration used (250 μg/mL, data not shown). Compounds **3-6** were also evaluated against a strain of *Staphylococcus aureus* (PCM 2602), however, all of them were devoid of antibacterial action at the MIC_{50} as well as the MIC_{90} level, even at the maximal concentration used (250 μg/mL). $MIC_{50/90}$ values for erytromycin, used as a standard in this test, were below 1 μg/mL. However, as in the case of *M. fortuitum*, the compounds enhanced the replication of *S. aureus*. The best stimulants of growth of these microorganisms were **3g**, **4b** and **3f**, which enhanced replication of the bacteria at levels of 10-50% (Table 1). The remaining compounds shown at Table 1 (**3a**,**b**,**e**, **4a**) and the preparations **3c**, **3d** and **5b** (data not shown) revealed only weak stimulation (< 10%) observed at varied concentrations for individual compounds [e.g. 3.9 μg/mL (**4a**), 250 μg/mL (**3b**)]. The only compound which did not stimulate the growth of *S. aureus* was the non-enamine pyrido-1,2-thiazine **6**.

The mechanism by which the preparations **3-5** increase the growth of *M. fortuitum*.and *S. aureus* is unclear. On the one hand, it cannot be ruled out that the bacteria are able to utilize nitrogen-containing fragments from the enamine moieties of **3** and triheterocyle **5b** for replication. This may be partially supported by the fact that the non-enamine pyrido-1,2-thiazine **6** was the only preparation which practically did not promote the growth of microorganisms. On the other hand, stimulation was also observed in the case of trihetrocycles **4** which, similar to **6**, are devoid of enamine nitrogen.

It is also unclear if the increase in bacterial replication observed at low concentration (below 1 μg/mL; Table 1) is a consequence of an enhancement of bacterial growth by the compounds or their lack of activity to allow on the natural growth of the microorganisms.

On the basis of the above pilot data it may be concluded that modification of the β-dicarbonyl substructure of the weak antimycobacterial agents of 3-acyl-4-hydroxypyrido-1,2-thiazine type **II** (Figure 1) does not seem to offer an antibacterial specific group.

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