NEW GROUPS OF POTENTIAL ANTITUBERCULOTICS: BIS(1-ARYLTETRAZOL-5-YL) DISULFIDES. STRUCTURE–ACTIVITY RELATIONSHIP*

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Dedicated to Dr Milan Celadnik on the occasion of his 70th birthday.

Oxidation of 1-aryltetrazole-5-thiols afforded bis(1-aryltetrazol-5-yl) disulfides. The compounds were tested for antimycobacterial activity against *Mycobacterium tuberculosis*, *M. kansasii*, *M. avium* and *M. fortuitum*. In the case of *M. tuberculosis*, the logarithm of minimum inhibitory concentration showed a parabolic dependence on hydrophobic substituent constants. Although the compounds exhibited low to medium activity, the most active derivative, bis(4-chlorophenyltetrazol-5-yl) disulfide (*III*) was more effective against atypical strains than are the commercial tuberculostatics used as standards.

The recent reappearance of tuberculosis represents a serious problem even in industrially developed countries[1 – 3]. Therefore, search for new groups of potential tuberculostatics belongs again to important directions of pharmaceutical research. In one of our previous communications[4] we reported on an active compound whose structure was new from the viewpoint of antimycobacterial activity: bis(1-phenyltetrazol-5-yl) disulfide (*I*). We now report the synthesis and antimycobacterial activity studies of its derivatives with substituents on the phenyl ring (*II – IX*).

All the studied compounds *II – IX* were prepared by oxidation of the corresponding 1-aryltetrazole-5-thiols with hydrogen peroxide. The usual oxidation of thiols to disulfides with iodine did not give good results. The oxidation of 1-aryltetrazole-5-thiols with hydrogen peroxide gave yields higher than the described[5] preparation of bis-(1-aryltetrazol-5-yl) disulfides by oxidation of the corresponding 1-aryltetrazole-5-

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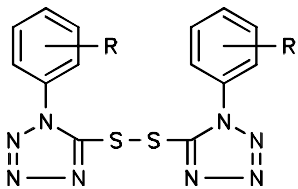
thiols with bromine. The structure of the products was verified by their IR spectra in which we followed bands due to the ν(C–H) vibrations and skeletal vibrations of substituted phenyl, and absorption maxima considered to be characteristic for 1,5-disubstituted tetrazoles[6]. The structure was also confirmed by elemental analysis. The starting 1-aryltetrazolethiols were prepared by a procedure already described in our previous papers[4,7] where their characteristics are described. All the starting 1-aryltetrazole-5-thiols have no antimycobacterial activity. Bis(1-aryltetrazol-6-yl) disulfides are also regarded as potential antimycotics[5].



I–IX

I, R = H

II–IX, for R see Table I

TABLE I

Minimum inhibitory concentration (MIC) against *Mycobacterium tuberculosis* $H_{31}Rv$, *M. kansasii* PKG 8, *M. avium* No. 80/72, *M. fortuitum* No. 1023 and the sum of hydrophobic substituent constant (π) of substituents on the phenyl rings in the series of bis(1-aryltetrazol-5-yl) disulfides (*II – IX*)

Compound	R	MIC, μmol l^{-1}				π
		M. tbc.	*M. kans.*	*M. avium*	*M. fortuitum*	
II	4-Br	250	500	1 000	>1 000	2.38
III	4-Cl	125	250	500	1 000	1.46
IV	4-F	250	>1 000	>1 000	>1 000	0.30
V	4-I	125	>1 000	>1 000	>1 000	2.52
VI	4-CH_3	250	250	>1 000	>1 000	1.20
VII	4-OCH_3	500	>1 000	>1 000	>1 000	–0.06
VIII	3,4-$(CH_3)_2$	250	250	1 000	>1 000	2.24
IX	2,4,6-$(CH_3)_3$	>1 000	>1 000	>1 000	>1 000	4.56
INH[a]		30	500	1 000	1 000	
Ethionamide[b]		60	500	1 000	>1 000	

[a] Isonicotinhydrazide. [b] 2-Ethyl-4-pyridinecarbothioamide.

EXPERIMENTAL

The melting points were determined on a Kofler block and are uncorrected. Samples for analysis and antimycobacterial tests were dried over phosphorus pentoxide at 61 °C and 66 Pa for 24 h. IR spectra were measured on a Perkin–Elmer 577 instrument using the KBr technique; wavenumbers are given in cm^{-1}.

General Procedure for Preparation of Bis(1-aryltetrazol-5-yl) Disulfides

An excess of 12% hydrogen peroxide (0.4 ml) was added dropwise at 30 °C to a stirred mixture of the corresponding 1-aryltetrazole-5-thiol (3 – 5 mmol) and ethanol (10 ml). After stirring at 60 °C for 10 min, the disulfide precipitated. The reaction mixture was cooled to 10 °C, the product collected, washed with ethanol and crystallized from ethanol.

Bis(1-(4-bromophenyl)tetrazol-5-yl) disulfide (II): m.p. 190 – 192 °C, yield 80%. For $C_{14}H_8Br_2N_8S_2$ (512.2) calculated: 32.83% C, 1.57% H, 31.20% Br, 21.88% N, 12.52% S; found: 32.81% C, 1.50% H, 31.07% Br, 21.99% N, 12.65% S. IR spectrum: 3 100, 3 060, 1 595, 1 498, 1 090, 1 030.

Bis(1-(4-chlorophenyl)tetrazol-5-yl) disulfide (III): m.p. 195 °C, yield 96% (reported[5] m.p. 195 °C).

Bis(1-(4-fluorophenyl)tetrazol-5-yl) disulfide (IV): m.p. 175 °C (reported[8] m.p. 143.5 – 144 °C), yield 57%. For $C_{14}H_8F_2N_8S_2$ (390.4) calculated: 43.07% C, 2.07% H, 28.70% N, 16.42% S; found: 43.06% C, 2.18% H, 28.97% N, 16.53% S. IR spectrum: 3 105, 3 090, 3 040, 1 603, 1 520, 1 090, 1 030.

Bis(1-(4-iodophenyl)tetrazol-5-yl) disulfide (V): m.p. 180 – 182 °C, yield 88%. For $C_{14}H_8I_2N_8S_2$ (602.2) calculated: 27.74% C, 1.33% H, 18.48% N, 10.58% S; found: 28.01% C, 1.02% H, 18.63% N, 10.81% S. IR spectrum: 3 120, 3 080, 1 605, 1 520, 1 080, 1 028.

Bis(1-(4-tolyl)tetrazol-5-yl) disulfide (VI): m.p. 180 – 182 °C (reported[5] m.p. 167 °C), yield 99%. For $C_{16}H_{14}N_8S_2$ (382.5) calculated: 50.25% C, 3.69% H, 29.30% N, 16.77% S; found: 50.12% C, 3.84% H, 29.25% N, 16.58% S. IR spectrum: 3 070, 2 940, 1 590, 1 520, 1 080, 1 030.

Bis(1-(4-methoxyphenyl)tetrazol-5-yl) disulfide (VII): m.p. 153 – 154 °C (reported[5] m.p. 219 °C), yield 94%. For $C_{16}H_{14}N_8O_2S_2$ (414.5) calculated: 46.37% C, 3.40% H, 27.04% N, 15.47% S; found: 46.44% C, 3.72% H, 27.04% N, 15.38% S. IR spectrum: 3 080, 3 015, 2 980, 2 960, 2 860, 1 610, 1 520, 1 090, 1 025.

Bis(1-(3,4-xylyl)tetrazol-5-yl) disulfide (VIII): m.p. 132 – 134 °C, yield 88%. For $C_{18}H_{18}N_8S_2$ (410.5) calculated: 52.67% C, 4.42% H, 27.30% N, 15.62% S; found: 52.32% C, 4.40% H, 27.10% N, 15.54% S. IR spectrum: 3 075, 2 990, 2 960, 1 610, 1 510, 1 075, 1 020.

Bis(1-mesityl)tetrazol-5-yl) disulfide (IX): m.p. 146.5 – 147 °C, yield 99%. For $C_{20}H_{22}N_8S_2$ (438.6) calculated: 54.77% C, 5.06% H, 25.55% N, 14.62% S; found: 54.90% C, 5.03% H, 25.54% N, 14.59% S. IR spectrum: 3 040, 3 000, 2 940, 2 880, 1 605, 1 490, 1 075, 1 035.

Microbiological Assays

Microbiological evaluation was carried out on a semisynthetic liquid protein-containing Sulas medium (UOSOL, Prague). The following mycobacterial strains were used: *Mycobacterium tuberculosis* H_{31}Rv, *M. kansasii* PKG 8, *M. avium* No. 80/72 and *M. fortuitum* No. 1023. The resulting concentration of the compounds in the substrate was 30, 60, 125, 250, 500 and 1 000 μmol l^{-1}. The minimum inhibitory concentrations were determined after 15 days of incubation at 37 °C. For the results see Table I.

Calculations

The regression equations were calculated using[9] program W-6 for Sharp PC 1211 microcomputer. As lipophilicity parameters we used the hydrophobic substituent constants for substituents bonded to phenyl according to Kuchar and Rejholec[10].

DISCUSSION

Under the in vitro experimental conditions used, all the tested compounds but derivative *IX* were active against *Mycobacterium tuberculosis*. In the search for quantitative structure–antituberculotic activity we assigned compound *IX* a minimum inhibitory activity corresponding to the next degree in the dilution scale, i.e. 2 000 μmol l^{-1}. The structure–activity relationship is then described by Eq. (*1*), where log MIC is the logarithm of minimum inhibitory concentration and π is the sum of hydrophobic constants of substituents bonded to both the phenyl rings.

$$\log \mathrm{MIC}_{\mathrm{tbc}} = 0.1327\pi^2 - 0.4567\pi + 2.6186 \qquad (1)$$

$$r = 0.927 \quad s = 0.159 \quad F = 15.47 \quad n = 8$$

An analogous calculation of the other antimycobacterial activities was not possible because of small number of active compounds.

Of the compounds tested, the chloro derivative *III* approaches most closely to the calculated lipophilicity optimum; it also shows the highest activity against the other mycobacterial strains.

In conclusion we may say that although we did find a novel group of potential tuberculostatics, the compounds obtained exhibit mostly only medium or moderate activity against *Mycobacterium tuberculosis*. However, the activity of chloro derivative *III* against other atypical strains exceeds those of the commercial tuberculostatics used as standards.

The antimycobacterial activity of bis(1-aryltetrazol-5-yl) disulfides has not been evaluated so far. However, some compounds of this group have already been described as potential antimycotics: their activity against *Aspergilus niger* and *A. flavu*s is reported[5].

The antituberculotic activity of these compounds is mostly influenced by their lipophilicity. One cannot expect any highly active antituberculotics in this group because the lipophilicity of the chloro derivative *III* nears the optimum value. The activity of the compounds studied must be connected directly with the disulfide structure because the starting 1-aryl-5-tetrazolethiols were completely inactive.

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