ANTIMYCOBACTERIAL DERIVATIVES OF TETRAZOLE*

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Dedicated to Dr Miroslav Protiva on the occasion of his 70th birthday.

A synthesis of (1-phenyltetrazol-5-ylthio)acetic acid derivatives has been developed. Functional variations concern the carboxylic group and/or the 4 position of phenyl group. Most of the compounds prepared have been tested with regard to their antimycobacterial activity but the activities found are medium or low ones. However, (1-phenyltetrazol-5-ylthio)acetic acid is better than the antituberculotic isoniazid in the activity to *Mycobacterium avium*.

In one of our earlier papers[1] we looked for structural prerequisites for antituberculotic activity of tetrazole derivatives. This group of compounds was selected with respect to the fact that most of the antituberculotics currently used exhibit a low lipophilicity. Thus e.g. the values of logarithm of partition coefficient in the system of octanol–water are[2] −1·14 for isoniazid (i.e. isonicotinic acid hydrazide), 1·0 for ethionamid (i.e. 2-ethyl-thioisonicotinamide) and 0·5 for PAS (i.e. 4-amino-2-hydroxybenzoic acid). Streptomycin can also be considered a hydrophilic compound. The low lipophilicity values are obviously connected with the required pharmacokinetic properties of the compounds, viz. probably with distributions among organs (the lungs). A number of lipophilic compounds with high antimycobacterial activity in vitro are quite inefficient when tested in vivo. The four water-solvatable nitrogen atoms of the parent tetrazole ring could – in our opinion – impart the activity both in vitro and in vivo to the tetrazole derivatives.

In our first study[1] we found that the tetrazole derivatives containing a sulfur atom attached to the carbon atom of tetrazole cycle and a free electron pair at the third atom of the sulfide side chain (e.g. an oxygen atom of carbonyl group) exhibited antituberculotic activity. However, the activity of the first compounds prepared was not high. In the present paper we have decided to focus our attention to derivatives of (1-phenyltetrazol-5-ylthio)acetic acid (*XI*) with the aim of preparation of deriva-

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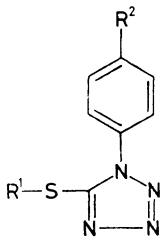
tives with modified carboxylic group and/or a bromine substituent at 4 position of the phenyl ring, and with the aim of testing of the compounds prepared with regard to their activity to *Mycobacterium tuberculosis*, *Mycobacterium kansasii*, and *Mycobacterium avium*.



I, $R^1 = R^2 = H$
II, $R^1 = H$; $R^2 = Br$
III, $R^1 = CH_2COOCH_3$; $R^2 = H$
IV, $R^1 = CH_2COOCH_3$; $R^2 = Br$
V, $R^1 = CH_2CONH_2$; $R^2 = H$
VI, $R^1 = CH_2CONH_2$; $R^2 = Br$
VII, $R^1 = CH_2CONHNH_2$; $R^2 = H$
VIII, $R^1 = CH_2CONHNH_2$; $R^2 = Br$
IX, $R^1 = CH_2CONHOH$; $R^2 = H$
X, $R^1 = CH_2CONHOH$; $R^2 = Br$
XI, $R^1 = CH_2COOH$; $R^2 = H$
XII, $R^1 = CH_2COOH$; $R^2 = Br$
XIII, $R^1 = CH_2COCl$; $R^2 = H$
XIV, $R^1 = CH_2CONHNHCONH_2$; $R^2 = H$
XV, $R^1 = CH_2CONHNHCSNH_2$; $R^2 = H$

The synthesis started from the known compounds: 1-phenyltetrazol-5-thiol (*I*) (ref.[3]) and 1-(4-bromophenyl)tetrazol-5-thiol (*II*) (ref.[4]). The reaction of their sodium salts with methyl chloroacetate gave methyl (1-phenyltetrazol-5-ylthio)-

TABLE I

The values of the minimum inhibition concentration ($\mu mol\ l^{-1}$) of some tetrazol derivatives toward *Mycobacterium tuberculosis*, *Mycobacterium kansasii*, and *Mycobacterium avium*

Compound	*Mycobacterium tuberculosis*	*Mycobacterium kansasii*	*Mycobacterium avium*
III	1 000	1 000	1 000
IV	250	1 000	1 000
V	1 000	1 000	1 000
VI	250	1 000	1 000
VII	500	1 000	1 000
VIII	250	500	1 000
IX	500	1 000	1 000
XI	1 000	1 000	125
Isoniazid	7	62	250

acetate (*III*) and methyl (1-(4-bromophenyl)tetrazol-5-ylthio)acetate (*IV*), respectively. Their structure, as well as that of the other substances prepared, was verified by means of IR spectra and elemental analysis. Thus e.g. compounds *III* and *IV* show a ν(CO) characteristic of esters (1 725 cm^{-1}, 1 724 cm^{-1}). The compounds *III* and *IV* served as starting materials for further modifications of carboxylic group. On action with ammonia the esters *III* and *IV* gave the respective amides *V* and *VI* (ν(CO) 1 680 and 1 690 cm^{-1}, respectively). On action with hydrazine the same esters gave the respective hydrazides *VII* and *VIII* (ν(CO) 1 678 and 1 680 cm^{-1}, respectively). On action with hydroxylamine, *III* and *IV* gave the respective hydroxamic acids *IX* and *X* (ν(CO) 1 670 cm^{-1}). The free carboxylic acids *XI* and *XII* were obtained either by the hydrolysis of the esters *III* and *IV*, respectively, or by the direct reaction of chloroacetic acid with the thiols *I* and *II*, respectively. On action with phosphorus pentachloride, the acid *XI* gave the acyl chloride *XIII* which, however, was not characterized and was used as a solution in toluene for further syntheses. Reaction of acyl chloride *XIII* with hydroxylamine gave the hydroxamic acid *IX*; the same starting chloride reacted with semicarbazide and thiosemicarbazide to give acylsemicarbazide *XIV* and acylthiosemicarbazide *XV*, respectively. Both these products exhibit ν(CO) values indicating the presence of carbonyl group attached to nitrogen atom.

Table I presents the values of antimycobacterial activity wherefrom it follows that the substances exhibit low or medium activities. The hydrazide *VIII* exhibits the highest efficiency to *Mycobacterium tuberculosis* and *Mycobacterium kansasii*. A surprising activity to *Mycobacterium avium* was found with the acid *XI*, this activity being higher than that of the currently used antituberculotic isoniazid. In spite of the fact that the activities of the substances prepared do not surpass those of the antituberculotics currently used (except for the activity of *XI* to *Mycobacterium avium*), the results indicate that the tetrazole derivatives studied belong to a promising group of compounds. However, it will be necessary to investigate further structural modifications so that some of the optimization procedures QSAR might be applied.

EXPERIMENTAL

The melting temperatures were determined with a Kofler apparatus and are not corrected. The samples for analyses and antimycobacterial tests were dried over P_2O_5 at 61°C at 2·7 kPa 24 h (at least). The IR spectra were measured in KBr using a Perkin–Elmer 577 apparatus, and they are given in cm^{-1}. The starting 1-phenyltetrazole-5-thiol (*I*) and 1-(4-bromophenyl)tetrazole-5-thiol (*II*) were prepared by the procedure given in our previous paper[1] and were identified by comparison of their melting points with those given in refs[3,4].

Methyl (1-Phenyltetrazol-5-ylthio)acetate (*III*)

A boiling mixture of 0·53 mol (95 g) 1-phenyltetrazole-5-thiol (*I*), 0·53 mol sodium ethoxide,

and 600 ml ethanol was treated with 0·53 mol (57·25 g) methyl chloroacetate. After 1 h refluxing, the reaction mixture was filtered, and the product separated from the filtrate was recrystallized from ethanol. Yield 63·2 g (47·6%), m.p. 85°C. For $C_{10}H_{10}N_4O_2S$ (250·3) calculated: 47·99% C, 4·03% H, 22·3% N, 12·81% S; found: 48·06% C, 3·95% H, 22·12% N, 12·53% S. IR spectrum: ν(CO) 1 725.

Methyl (1-(4-Bromophenyl)tetrazol-5-ylthio)acetate (*IV*)

The product was obtained from 1-(4-bromophenyl)tetrazole-5-thiol (*II*) in similar way as above. Yield 42%, m.p. 118°C. For $C_{10}H_9BrN_4O_2S$ (329·0) calculated: 36·48% C, 2·75% H, 17·02% N, 9·74% S; found: 35·80% C, 2·63% H, 16·97% N, 9·84% S. IR spectrum: ν(CO) 1 724.

(1-Phenyltetrazol-5-ylthio)acetamide (*V*)

A mixture of 0·01 mol (2·4 g) ester *III* and ethanol was treated with 1·5 ml aqueous ammonia. After 2 h standing, the reaction mixture was cooled to −10°C, and the separated crystals were collected by suction and recrystallized from ethanol. Yield 1·8 g (76·6%), m.p. 170°C. For $C_9H_9N_5OS$ (235·3) calculated: 45·95% C, 3·86% H, 29·77% N, 13·63% S; found: 46·01% C, 3·77% H, 30·05% N, 13·68% S. IR spectrum: ν(NH) 3 380, 3 280, 3 220, 3 180; ν(CH) 3 060, 2 980, 2 930; ν(CO) 1 680.

(1-(4-Bromophenyl)tetrazol-5-ylthio)acetamide (*VI*)

It was prepared from ester *IV* in similar way as that used above for amide *V*. Yield 56·3%, m.p. 206–208°C (ethanol). For $C_9H_8BrN_5OS$ (314·2) calculated: 34·41% C, 2·57% H, 22·29% N, 10·21% S; found: 34·28% C, 2·76% H, 21·94% N, 9·98% S. IR spectrum: ν(NH) 3 180–3 480; ν(CO) 1 690.

(1-Phenyltetrazol-5-ylthio)acetohydrazide (*VII*)

A mixture of 0·01 mol (2·4 g) ester *III* and ethanol was treated with 0·025 mol (1·25 g) hydrazine hydrate with stirring. After another 30 min stirring, the reaction mixture was cooled to −10°C, and the precipitate obtained was collected and recrystallized from ethanol. Yield 1·65 g (68·7%), m.p. 164°C. For $C_9H_{10}N_6OS$ (250·3) calculated: 43·19% C, 4·03% H, 33·58% N, 12·81% S; found: 43·13% C, 4·11% H, 33·90% N, 13·07% S. IR spectrum: ν(NH) 3 430, 3 380; ν(CH) 3 030, 2 920; ν(CO 1 678.

(1-(4-Bromophenyl)tetrazol-5-ylthio)acetohydrazide (*VIII*)

It was prepared from ester *IV* in a similar way as that used above for hydrazide *VII*. Yield 49%, m.p. 179–181°C (ethanol). For $C_9H_8BrN_6OS$ (329·2) calculated: 32·84% C, 2·75% H,25·53% N, 9·74% S; found: 32·68% C, 2·76% H, 25·78% N, 9·52% S. IR spectrum: ν(NH) 3 250–3 480; ν(CO) 1 680.

(1-Phenyltetrazol-5-ylthio)acetohydroxamic Acid (*IX*)

A) Ethanolic solution of 0·01 mol (2·4 g) ester *III* was added to ethanolic solution of 0·04 mol hydroxylamine with stirring. After 1 h refluxing, the reaction mixture was concentrated to crystallization, cooled to −10°C, and the separated crystals were collected and recrystallized from ethanol. Yield 2·0 g (79·9%), m.p. 163–164°C. For $C_9H_9N_5O_2S$ (251·3) calculated:

43·02% C, 3·61% H, 27·87% N, 12·76% S; found: 42·85% C, 3·72% H, 27·60% N, 12·55% S. IR spectrum: ν(OH) and ν(NH) 3 252, 3 160; ν(CH) 3 020, 2 995, 2 920; ν(CO) 1 670.

B) A mixture of 7·6 mmol (1·94 g) acyl chloride *XIII* and 40 ml toluene was stirred and treated with a suspension of 7·6 mmol (0·53 g) hydroxylamine hydrochloride in dimethylformamide, whereafter 15·2 mmol (1·54 g) triethylamine was added drop by drop. The precipitated triethylammonium chloride was removed by filtration, the filtrate was concentrated to crystallization, and the product was recrystallized from dimethylformamide. Yield 0·5 g (28·9%), m.p. 163—164°C. IR spectrum is identical with that of the product of the above procedure *A*).

(1-(4-Bromophenyl)tetrazol-5-ylthio)acetohydroxamic Acid (*X*)

The substance was prepared in the yield of 64% from ester *IV* by the procedure analogous to the above procedure *A*) used for the synthesis of compound *IX*. M.p. 185—187°C. For $C_9H_8Br.N_5O_2S$ (330·2) calculated: 32·74% C, 2·44% H, 21·21% N, 9·71% S; found: 32·51% C, 2·48% H, 20·86% N, 9·25% S.

(1-Phenyltetrazol-5-ylthio)acetic Acid (*XI*)

A) A mixture of 0·01 mol (2·4 g) ester *III*, 50 ml ethanol, and 20 ml 1M NaOH was refluxed 2 h, whereafter hydrochloric acid was added until pH 2, and the reaction mixture was concentrated to a half volume under reduced pressure. The separated oil was extracted with ether, the extract was evaporated, and the residue was recrystallized from a toluene–ethyl ether mixture. Yield 0·75 g (34·5%), m.p. 106—107°C.

B) A suspension of 0·06 mol (10·63 g) compound *I* and 0·06 mol (5·64 g) chloroacetic acid in water was refluxed 3·5 h, whereafter it was cooled and extracted with ether. The extract was dried with sodium sulfate, and the solvent was evaporated under reduced pressure. The dry evaporation residue was recrystallized from benzene. Yield 6·5 g (45·9%), m.p. 106—107°C. For $C_9H_8N_4O_2S$ (236·2) calculated: 45·76% C, 3·41% H, 23·72% N, 13·57% S; found: 45·87% C, 3·18% H, 23·57% N, 13·77% S. IR spectrum: ν(OH) 2 600—3 500; ν(CO) 1 700.

(1-(4-Bromophenyl)tetrazol-5-ylthio)acetic Acid (*XII*)

The substance was prepared by the procedures similar to those used for acid *XI*. Yield 35·5%, m.p. 154—155°C. For $C_9H_7BrN_4O_2S$ (315·1) calculated: 34·30% C, 2·24% H, 25·35% Br, 17·78% N, 10·17% S; found: 34·08% C, 2·22% H, 25·28% Br, 17·75% N, 10·11% S. IR spectrum: ν(OH) 2 500—3 500; ν(CO) 1 712.

(1-Phenyltetrazol-5-ylthio)acetyl Chloride (*XIII*)

A mixture of 25·4 mmol (6 g) acid *XI* and 225 ml ether was treated with 2·54 mmol (5·3 g) phosphorus pentachloride with stirring and cooling at 0°C, the stirring being continued at the same temperature for another 1 h. Then the reaction mixture was evaporated under reduced pressure until dry, and the residue was extracted with toluene. The toluene solution was used for the subsequent preparations.

(1-Phenyltetrazol-5-ylthio)acetylsemicarbazide (*XIV*)

The solution of 7·6 mmol (1·94 g) acyl chloride *XIII* in toluene was stirred and treated successively with a solution of 7·6 mmol (0·84 g) semicarbazide hydrochloride in dimethylformamide and

with 15·2 mmol (1·54 g) triethylamine. After another 30 min stirring, the separated crystals were filtered off, washed with ethanol, and recrystallized from acetonitrile. Yield 0·9 g (41·3%), m.p. 198—199°C. For $C_{10}H_{11}N_7O_2S$ (293·3) calculated: 40·95% C, 3·78% H, 33·43% N, 10·93% S; found: 40·80% C, 3·86% H, 33·25% N, 10·56% S. IR spectrum: ν(NH) 3 165, 3 380; ν(CH) 3 040, 2 995, 2 920; ν(CO) 1 675, 1 625.

(1-Phenyltetrazol-5-ylthio)acetylthiosemicarbazide (*XV*)

The solution of 7·6 mmol (1·94 g) acyl chloride *XIII* in 40 ml toluene was stirred and treated with a solution of 7·6 mmol (0·69 g) thiosemicarbazide in dimethylformamide. After 30 min stirring, the mixture was evaporated until dry. After addition of water and ether, the precipitated crystals were recrystallized from dimethylformamide. Yield 0·7 g (29·8%), m.p. 190—191°C. For $C_{10}H_{11}N_7OS_2$ (309·4) calculated: 38·82% C, 3·58% H, 31·69% N, 20·73% S; found: 38·41% C, 3·72% H, 31·54% N, 20·54% S. IR spectrum: ν(NH) 3 462, 3 260, 3 168; ν(CH) 3 020, 2 960, 2 938; ν(CO) 1 665.

Antimycobacterial Efficiency Tests

The antimycobacterial effects were evaluated with the use of the semisynthetic liquid substrate by Šula (ÚSOL, Prague). The following mycobacterial strains were applied: *Mycobacterium tuberculosis* $H_{37}Rv$, *Mycobacterium kansasii* PKG 8, *Mycobacterium avium* No. 999. The substances were added after dissolution in dimethyl sulfoxide. The resulting concentrations of the compounds in the substrate were: 7, 15, 31, 62, 125, 250, 500, and 1 000 μmol l^{-1}. The minimum inhibition concentrations were read after 15 days incubation at 37°C. The results are summarized in Table I.

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