NEW GROUPS OF POTENTIAL ANTITUBERCULOTICS: 5-ALKYLTHIO-1-ARYLTETRAZOLES*

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A series of 5-alkylthio-1-aryltetrazoles **1–14** was prepared by alkylation of the corresponding 1-aryltetrazole-5-thiols with alkyl bromides in the cyclohexane–aqueous potassium hydroxide system. The new compounds were evaluated for their activity against *Mycobacterium tuberculosis, M. kansasii, M. avium* and *M. fortuitum*. The effects of aryl and alkyl fragments on minimum inhibitory concentrations (MIC) against *M. tuberculosis* and *M. kansasii* were analyzed by Free–Wilson method. On basis of calculated fragment contributions, 5-butylthio-1-(3,4-dimethylphenyl)tetrazole (**15**) was predicted to be the most antimycobacterially active derivative in the present series studied and its activity was verified experimentally. MIC values of 30 μmol l^{-1} and 61 μmol l^{-1} were obtained for its activity against *M. tuberculosis* and *M. kansasii*, respectively.

Key words: 5-Alkylthio-1-aryltetrazoles; Antituberculotics; Phase-transfer catalysis; Structure–activity relationships.

The recent reappearence of tuberculosis represents a serious problem even in developed countries[3–6]. During the last years we have been trying to find new groups of potential antituberculotics[7–10]. The aim of the present paper is to publish information on the antimycobacterial activity of 5-alkylthio-1-phenyltetrazoles **1–14** substituted on the phenyl ring with one, two, or three methyl groups. These compounds were selected because of the presence of an alkylthio group bound to the electron deficient carbon atom. This pharmacophore was described in our previous study[11]. We also analyzed structure–antimycobacterial activity relationships.

All the 5-alkylthio-1-aryltetrazoles were prepared by alkylation of the corresponding 1-aryltetrazole-5-thiols with alkyl bromides in the cyclohexane–aqueous potassium hy-

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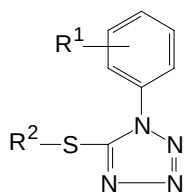
droxide system. Tetrabutylammonium iodide was used as a phase-transfer catalyst. This method gave higher yields (Table I) than the previously used alkylation performed in ethanol[12]. Only the sulfur atom is substituted under these conditions[13]. Starting 1-aryltetrazole-5-thiols were prepared by a procedure described in our previous papers[12,14].

Structure of the products was proven by IR and ^{1}H NMR spectra. The IR spectra displayed bands at 1 110–1 120 cm^{-1} and 1 010–1 050 cm^{-1} due to the 1,5-disubstituted tetrazole ring[15] (Table II). In the ^{1}H NMR spectra the protons of methyl and other alkyl groups as well as the protons of aromatic ring were observed at the expected values (Table III) with expected splitting patterns.

The compounds prepared were tested in vitro for their antimycobacterial activity against *Mycobacterium tuberculosis*, *M. kansasii*, *M. avium* and *M. fortuitum*. The minimum inhibitory concentrations (MICs) are given in Table IV. In contrast to activity against *M. tuberculosis*, the activity of the compounds under study against *M. fortuitum* was found to be very small. All the starting 1-aryltetrazole-5-thiols have no antimycobacterial activity.

Structure–Activity Relationships

The relationships between the structure and activity against *M. tuberculosis* and *M. kansasii* were analyzed. Total lipophilicity of compounds **1–14** calculated according to



1-15

	R^1	R^2		R^1	R^2
1	3-CH_3	CH_2CH_3	**9**	3,4-$(CH_3)_2$	$(CH_2)_2CH_3$
2	3-CH_3	$(CH_2)_2CH_3$	**10**	3,4-$(CH_3)_2$	$CH(CH_3)_2$
3	3-CH_3	$CH(CH_3)_2$	**11**	2,4,6-$(CH_3)_3$	CH_2CH_3
4	3-CH_3	$(CH_2)_3CH_3$	**12**	2,4,6-$(CH_3)_3$	$(CH_2)_2CH_3$
5	4-CH_3	CH_2CH_3	**13**	2,4,6-$(CH_3)_3$	$CH(CH_3)_2$
6	4-CH_3	$CH(CH_3)_2$	**14**	2,4,6-$(CH_3)_3$	$(CH_2)_3CH_3$
7	4-CH_3	$(CH_2)_3CH_3$	**15**	3,4-$(CH_3)_2$	$(CH_2)_3CH_3$
8	3,4-$(CH_3)_2$	CH_2CH_3			

TABLE I
Characteristic data of 1-aryl-5-alkylthiotetrazoles **1**, **2**, **4–14**

Compound	M.p., °C Yield, %	Formula M.w.	Calculated/Found		
			% C	% H	% N
1	34–35	$C_{10}H_{12}N_4S$	54.52	5.48	25.43
	55	220.3	54.44	5.46	25.22
2	26–27	$C_{11}H_{14}N_4S$	56.39	6.02	23.91
	68	234.3	56.41	6.23	24.14
4	27–28	$C_{12}H_{16}N_4S$	58.04	6.49	22.56
	74	248.4	57.98	6.44	22.08
5	76–77	$C_{10}H_{12}N_4S$	54.52	5.48	25.43
	99	220.3	54.34	5.28	25.29
6	54–55	$C_{11}H_{14}N_4S$	56.39	6.02	23.91
	53	234.3	56.41	6.20	23.76
7	28–30	$C_{12}H_{16}N_4S$	58.04	6.49	22.56
	73	248.4	57.80	6.50	22.57
8	76–77	$C_{11}H_{14}N_4S$	56.39	6.02	23.91
	59	234.3	56.12	6.27	23.84
9	30.5–32	$C_{12}H_{16}N_4S$	58.04	6.49	22.56
	75	248.4	58.05	6.53	22.76
10	72–73	$C_{12}H_{16}N_4S$	58.04	6.49	22.56
	81	248.4	57.99	6.41	22.75
11	115–118	$C_{12}H_{16}N_4S$	58.04	6.49	22.56
	99	248.4	58.12	6.40	22.56
12	77.5	$C_{13}H_{18}N_4S$	59.51	6.92	21.35
	83	262.4	59.60	6.91	21.54
13	43–46	$C_{13}H_{18}N_4S$	59.51	6.92	21.35
	69	262.4	59.42	6.67	21.57
14	108–108.5	$C_{14}H_{20}N_4S$	60.84	7.29	20.27
	90	276.4	60.64	7.25	20.49

Hansch and Leo[16] did not correlate with their antimycobacterial activity. The effects of aryl and alkylthio fragments on minimum inhibitory concentrations (MICs) were analyzed by Free–Wilson method[17]. Inactive compounds **11** and **14** were eliminated from calculation. The calculated fragment contributions, Δ log MIC, are presented in Table V.

It is obvious that the effect of an alkyl bound to the sulfur atom upon both antimycobacterial activities studied is more considerable than that of methyl substitution(s) on the phenyl ring. Regarding the alkylthio group, it seems that the activity against both strains increases with an increase in the hydrophobicity of the group. Of the alkyl substituents studied, butyl has the strongest effect. Regarding methyl substitutions on the phenyl ring, various aryls differ in their influence on the individual antimycobacterial activity. Of the aryls under study, it is only the strongest effect of 3,4-dimethylphenyl that both activities have in common.

The results discussed above suggest that 5-butylthio-1-(3,4-dimethylphenyl)tetrazole (**15**) should be the most antimycobacterially active derivative of the studied series. Since the compound was not included into the initial set of synthesized compounds, the compound was prepared and its activity experimentally verified. MIC values of 30 μmol l^{-1}

TABLE II
Infrared spectra of 1-aryl-5-alkylthiotetrazoles **1–15**

Compound	$\tilde{\nu}$(C–H)	Skeletal vibrations of phenyl ring	Vibrations of tetrazole ring
1	3 015, 2 995, 2 940, 2 895	1 610	1 110, 1 028
2	3 020, 2 990, 2 945, 2 895	1 610	1 110, 1 028
3	3 020, 2 990, 2 940, 2 890	1 610	1 115, 1 028
4	3 020, 2 980, 2 945, 2 890	1 610	1 120, 1 028
5	3 020, 2 995, 2 950, 2 895	1 620	1 110, 1 038
6	3 025, 2 995, 2 940, 2 885	1 620	1 110, 1 038
7	3 018, 2 978, 2 945, 2 895	1 610	1 110, 1 038
8	3 020, 2 995, 2 950, 2 895	1 610	1 115, 1 042
9	3 020, 2 985, 2 945, 2 895	1 612	1 112, 1 042
10	3 025, 2 995, 2 955, 2 895	1 615	1 115, 1 045
11	3 015, 2 995, 2 940, 2 890	1 615	1 118, 1 042
12	3 020, 2 990, 2 950, 2 895	1 610	1 118, 1 040
13	3 020, 2 990, 2 950, 2 890	1 610	1 118, 1 035
14	3 020, 2 980, 2 950, 2 890	1 610	1 118, 1 040
15	3 025, 2 990, 2 960, 2 895	1 615	1 115, 1 025

and 61 μmol l^{-1} were obtained for its activity against *M. tuberculosis* and *M. kansasii*, respectively (Table IV).

5-Alkylthio-1-aryltetrazoles studied can be considered to be novel potential antituberculotic agents.

EXPERIMENTAL AND CALCULATIONS

The melting points were determined with a Kofler apparatus and are uncorrected. The samples for analyses and antimycobacterial tests were dried over P_2O_5 at 61 °C and 66 Pa for 24 h. Elemental analyses were performed on a C,H,N analyzer (Laboratorni pristroje, Prague). The IR spectra were measured in chloroform on a Nicolet Impact 400 apparatus and the wavenumbers are given in cm^{-1}. The ^1H NMR spectra were measured with a Tesla BS 497 spectrometer (100 MHz) in $CDCl_3$ at 21 °C. Chemical shifts are given in ppm relative to tetramethylsilane as internal standard.

Chemicals. The starting 1-(3-methylphenyl)tetrazole-5-thiol, 1-(4-methylphenyl)tetrazole-5-thiol, 1-(3,4-dimethylphenyl)tetrazole-5-thiol and 1-(2,4,6-methylphenyl)tetrazole-5-thiol were prepared by the procedure reported in our previous papers[12,14].

TABLE III
^1H NMR chemical shifts of 5-alkylthio-1-aryltetrazoles **1–15**

Compound	Arom.					CH_3 on phenyl ring	Alkylthio group			
	H-2	H-3	H-4	H-5	H-6		SCH_2 or SCH	other CH_2		CH_3
1	7.38	–	7.38	7.38	7.38	2.46	3.41	–		1.47
2	7.39	–	7.39	7.39	7.39	2.46	3.38	1.83		1.06
3	7.37	–	7.37	7.37	7.37	2.46	4.11	–		1.47
4	7.38	–	7.38	7.38	7.38	2.46	3.40	1.44	1.73	0.96
5	7.44	7.32	–	7.32	7.44	2.46	3.32	–		1.48
6	7.45	7.34	–	7.34	7.45	2.45	4.14	–		1.51
7	7.47	7.34	–	7.34	7.47	2.45	3.40	1.46	1.82	0.95
8	7.33	–	–	7.28	7.28	2.34	3.40	–		1.49
9	7.33	–	–	7.29	7.29	2.35	3.34	1.80		1.05
10	7.31	–	–	7.27	7.27	2.34	4.13	–		1.50
11	–	7.02	–	7.02	–	1.93 2.37	3.37	–		1.47
12	–	7.01	–	7.01	–	1.93 2.37	3.34	1.80		1.04
13	–	7.02	–	7.02	–	1.94 2.37	4.11	–		1.47
14	–	7.01	–	7.01	–	1.93 2.37	3.37	1.45	1.75	0.93
15	7.31	–	–	7.27	7.27	2.34	3.38	1.43	1.81	0.95

TABLE IV
Minimum inhibitory concentrations (MICs, μmol l^{-1}) of 5-alkylthio-1-aryltetrazoles **1–15**

Compound	MIC, μmol l^{-1}			
	M. tuberculosis	*M. kansasii*	*M. avium*	*M. fortuitum*
1	1 000	1 000	1 000	>1 000
2	500	500	500	>1 000
3	250	250	500	1 000
4	61	125	250	500
5	500	500	1 000	1 000
6	250	500	1 000	>1 000
7	61	125	125	250
8	250	250	250	250
9	125	250	250	250
10	125	250	250	250
11	>1 000	>1 000	>1 000	>1 000
12	250	1 000	1 000	>1 000
13	125	500	1 000	500
14	>1 000	>1 000	>1 000	>1 000
15	30	61	125	250

TABLE V
Results of Free–Wilson structure–antimycobacterial activity analysis; contributions of fragments to log MIC values, Δ log MIC, for *Mycobacterium tuberculosis* (r = 0.986, s = 0.084, F = 22.7, n = 11) and *M. kansasii* (r = 0.945, s = 0.144, F = 5.5, n = 11)

Fragment		Δ log $MIC_{M.tbc.}$	Δ log $MIC_{M.kans.}$
R^1	3-CH_3	0.0905	–0.0483
	4-CH_3	0.1283	0.0645
	3,4-$(CH_3)_2$	–0.1915	–0.2025
	2,4,6-$(CH_3)_3$	–0.0409	0.2795
R^2	C_2H_5	0.3304	0.1532
	C_3H_7	0.0859	0.0817
	i-C_3H_7	–0.0271	0.0441
	C_4H_9	–0.5838	–0.4180
Common skeleton		2.2592	2.5075

General Procedure for the Preparation of 1-Aryl-5-alkylthiotetrazoles

Tetrabutylammonium iodide (50 mg, 0.135 mmol) was added to a stirred suspension consisting of 1-aryltetrazole-5-thiol (5 mmol) and alkyl bromide (5 mmol) in cyclohexane (20 ml), followed by 10 M KOH solution (10 ml). The mixture was stirred and heated at reflux for 0.5 to 4 h until the starting thiol disappeared (TLC in chloroform–acetone 3 : 1). The cyclohexane layer was washed with water, dried over Na_2SO_4, filtered, and the solvent was evaporated under reduced pressure. The solid residue was dissolved in methanol, passed through activated carbon and recrystallized from ethanol or ethanol–water. For melting points, yields, and elemental analyses see Table I. Compounds **3** and **15** were isolated as liquids. IR and NMR data for compounds **1–15** are presented in Table II and Table III, respectively.

Microbiological Evaluation

Microbiological tests were carried out on a semisynthetic Sula liquid medium with proteins (USOL, Prague). The following mycobacterial strains were applied: *Mycobacterium tuberculosis* $H_{37}Rv$, *M. kansasii* PKG 8, *M. avium* No. 80/72, and *M. fortuitum* No. 1023. The substances were added after dissolution in dimethyl sulfoxide. The resulting concentrations of the compounds in the substrate were: 7, 15, 30, 61, 125, 250, 500, and 1 000 μmol l^{-1}. The minimum inhibition concentrations (MICs) were read after 15 days of incubation at 37 °C. The results are summarized in Table IV.

Calculations

All the calculations were carried out with the use of the Multireg H program. Table V presents a survey of calculated contributions of the varied structural fragments to the activity.

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