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1,2,4-Triazoles. VIII.[1] Synthesis and Antimicrobial Activity of 3-Alkylthio-5-pyridyl-1,2,4-triazoles and Related Compounds

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Series of 3-alkylthio-5-(2-pyridyl)-1,2,4-triazoles and 1-picolinoyl-S-alkylisothiosemicarbazides were prepared and their antimicrobial activities were tested. Of the twenty-two compounds synthesized and screened *in vitro*, 3-pentylthio-5-(2-pyridyl)-1,2,4-triazole, 1-picolinoyl-S-butylisothiosemicarbazide, 1-picolinoyl-S-pentylisothiosemicarbazide, and 1-picolinoyl-S-hexylisothiosemicarbazide were the most active against *M. tuberculosis* $H_{37}Rv$.

Keywords——1,2,4-triazoles; S-alkylisothiosemicarbazides; antimicrobial activity; antitubercular activity; antifungal activity; structure-activity relationship; thermal cyclization; S-alkylation

Formylpyridine thiosemicarbazones are known to possess fairly high antitubercular activity.[3] The tuberculostatic activity of 3-amino-5-(4-pyridyl)-1,2,4-triazole against *M. tuberculosis* has also been reported.[4] 4-Formylpyridine thiosemicarbazone and its cyclized compound, 5-(4-pyridyl)-1,2,4-triazoline-3-thione, were active against vaccinia in mice when given orally.[5] Therefore, we prepared a series of 3-alkylthio-5-pyridyl-1,2,4-triazoles, which are the alkyl derivatives of the cyclized form of formylpyridine thiosemicarbazones, and examined their antimicrobial activities.

3-Alkylthio-5-(2-pyridyl)-1,2,4-triazoles (IVa—i) were prepared by thermal cyclization of 1-picolinoyl-S-alkylisothiosemicarbazides (IIIa—i), which were obtained by reaction of 1-picolinoylthiosemicarbazide (I) with alkyl iodides in the same way as in the synthesis of the methyl derivative[6] (Table I). Compound I was prepared in higher yield by the reaction of ethyl picolinate with thiosemicarbazide in liquid ammonia or by the reaction of picolinic acid hydrazide with ammonium thiocyanate than by the reaction using potassium thiocyanate.[7] Compounds IVa—i were also prepared by alkylation of 5-(2-pyridyl)-1,2,4-triazoline-3-thione[6] with alkyl iodides in alkaline solution (Table II).

3-Pentylthio-5-(3-pyridyl)-1,2,4-triazole (V) and 3-pentylthio-5-(4-pyridyl)-1,2,4-triazole (VI) were prepared by alkylation of 5-(3-pyridyl)-1,2,4-triazoline-3-thione[8] and 5-(4-pyridyl)-1,2,4-triazoline-3-thione,[8] respectively, with pentyl iodide in alkaline solution.

The *in vitro* antimicrobial activities of 3-alkylthio-5-pyridyl-1,2,4-triazoles and 1-picolinoyl-S-alkylisothiosemicarbazides, the intermediates in the synthesis of the 1,2,4-triazoles, were tested by the broth dilution method.[9]

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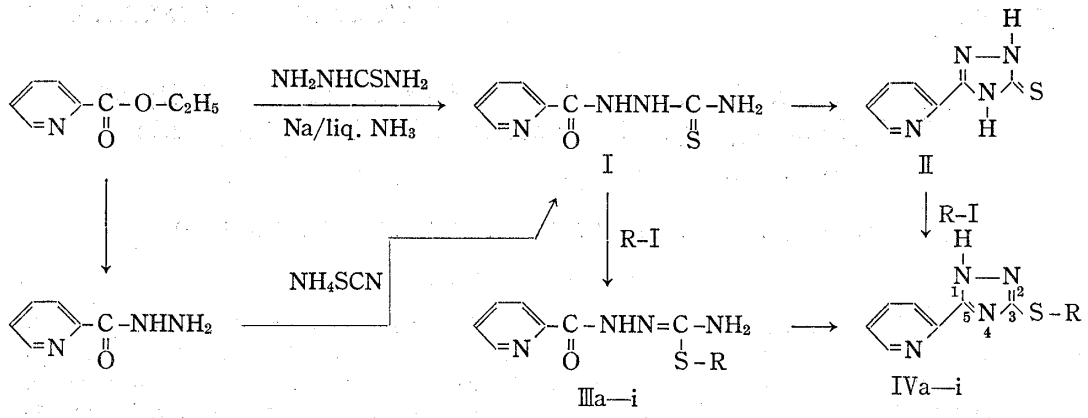
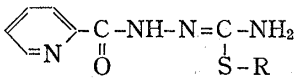


a: R=CH_3, b: R=C_2H_5, c: R=n-C_3H_7, d: R=n-C_4H_9, e: R=n-C_5H_{11}, f: R=n-C_6H_{13}, g: R=n-C_7H_{15}, h: R=n-C_8H_{17}, i: R=n-C_9H_{19}

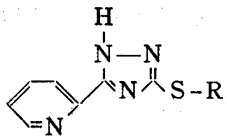
Chart 1

TABLE I. 1-Picolinoyl-S-alkylisothiosemicarbazides (IIIa—i)



Compound No.	R	Yield (%)	Appearance (recryst. solvent)	mp (°C)	Formula	Analysis (%) Calcd. (Found) C	H	N
IIIa	CH_3	81	Colorless needles (EtOH)	141—142	$C_8H_{10}N_4OS$	45.70 (45.53)	4.79 (4.99)	26.65 (26.80)
IIIb	C_2H_5	58	Colorless needles (50% EtOH)	130—132	$C_9H_{12}N_4OS$	48.20 (48.50)	5.39 (5.38)	24.98 (24.73)
IIIc	n-C_3H_7	42	Colorless needles (30% EtOH)	99—100	$C_{10}H_{14}N_4OS$	50.40 (50.12)	5.92 (5.89)	23.51 (23.69)
IIId	n-C_4H_9	69	Colorless needles (50% EtOH)	79—80	$C_{11}H_{16}N_4OS$	52.36 (52.65)	6.39 (6.38)	22.20 (21.99)
IIIe	n-C_5H_{11}	72	Colorless plates (30% EtOH)	75—76	$C_{12}H_{18}N_4OS$	54.11 (54.33)	6.81 (6.91)	21.03 (21.09)
IIIf	n-C_6H_{13}	48	Colorless plates (20% EtOH)	71—72	$C_{13}H_{20}N_4OS$	55.69 (55.41)	7.19 (7.24)	19.98 (19.74)
IIIg	n-C_7H_{15}	75	Colorless plates (isopropylether)	69—70	$C_{14}H_{22}N_4OS$	57.11 (56.98)	7.53 (7.67)	19.03 (18.94)
IIIh	n-C_8H_{17}	47	Colorless plates (isopropylether)	68—70	$C_{15}H_{24}N_4OS$	58.41 (58.67)	7.84 (7.89)	18.16 (17.85)
IIIi	n-C_9H_{19}	53	Colorless needles (isopropylether)	69—71	$C_{16}H_{26}N_4OS$	59.59 (59.35)	8.13 (8.19)	17.37 (17.13)

TABLE II. 3-Alkylthio-5-(2-pyridyl)-1,2,4-triazoles (IVa—i)



Compound No.	R	Method (reaction time)	Yield (%)	Appearance (recryst. solvent)	mp (°C)	Formula	Analysis (%) Calcd. (Found) C	H	N
IVa	CH_3	A (2.0) C (1.5)	63 75	Colorless needles (EtOH)	140—141	$C_8H_8N_4S$	49.98 (50.26)	4.19 (4.23)	29.14 (29.38)
IVb	C_2H_5	A (2.0) C (1.5)	73 80	Colorless needles (EtOH)	129—130	$C_9H_{10}N_4S$	52.41 (52.62)	4.89 (5.11)	27.16 (27.10)
IVc	n-C_3H_7	A (8.0) C (1.5)	44 59	Colorless needles (EtOH)	100—101	$C_{10}H_{12}N_4S$	54.52 (54.82)	5.49 (5.62)	25.43 (25.62)
IVd	n-C_4H_9	A (6.0) C (1.5)	70 68	Colorless needles (50% EtOH)	101—102	$C_{11}H_{14}N_4S$	56.38 (56.21)	6.02 (6.05)	23.91 (23.82)
IVe	n-C_5H_{11}	B (12) C (1.5)	67 80	Colorless needles (EtOH)	91—92	$C_{12}H_{16}N_4S$	58.04 (58.26)	6.49 (6.69)	22.56 (22.28)
IVf	n-C_6H_{13}	B (12) C (1.5)	57 60	Colorless needles (EtOH)	92—93	$C_{13}H_{18}N_4S$	59.51 (59.68)	6.92 (7.10)	21.35 (21.22)
IVg	n-C_7H_{15}	B (12) C (1.0)	79 79	Colorless needles (EtOH)	92—93	$C_{14}H_{20}N_4S$	60.84 (60.80)	7.29 (7.13)	20.27 (20.05)
IVh	n-C_8H_{17}	B (12) C (1.0)	79 72	Colorless needles (80% EtOH)	88—89	$C_{15}H_{22}N_4S$	62.03 (62.00)	7.64 (7.67)	19.29 (19.00)
IVi	n-C_9H_{19}	B (12) C (1.0)	52 81	Colorless needles (MeOH)	97—98	$C_{16}H_{24}N_4S$	63.12 (63.27)	7.95 (8.00)	18.40 (18.15)

Of the twenty-two compounds synthesized and screened, 3-pentylthio-5-(2-pyridyl)-1,2,4-triazole (IVe), 1-picolinoyl-S-butylisothiosemicarbazide (IIId), 1-picolinoyl-S-pentylisothiosemicarbazide (IIIe), and 1-picolinoyl-S-hexylisothiosemicarbazide (IIIf) were the most active against *M. tuberculosis* $H_{37}Rv$(Tables III and IV). The activities of compounds III and IV were due in part to the length of the S-alkyl chain in the molecule, and the highest activity was always found in the compounds with a pentylthio group. The compounds with activity against *M. tuberculosis* were also active against *T. asteroides* and *T. vaginalis*. None of the compounds tested showed appreciable *in vitro* activity against *S. aureus*, *E. coli*, *S. flexneri*, *P. aeruginosa*, or *C. albicans*. Mayer[10] suggested that compounds acting as antitubercular agents might have an effect on fungi. All our results are consistent with his suggest, except those on *C. albicans*.

3-Pentylthio-5-(2-pyridyl)-1,2,4-triazole(IVe) against *M. tuberculosis* was about 10 times as active as 3-pentylthio-5-(3-pyridyl)-1,2,4-triazole(V) or 3-penthylthio-5-(4-pyridyl)-1,2,4-triazole(VI). This difference in activities would be related to the characteristic structure of compound IVe. Only compound IVe has a dipyridyl-like chelating group, –N=C–C=N–, involving the nitrogen atoms of the pyridyl group and the triazole ring.[11] Moreover, com-

10) R.L. Mayer, *Rev. mèdicale France*, **1941**, 3 [*Chem. Abstr.*, **36**, 5199 (1942)].
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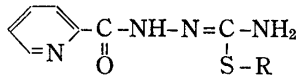
pound IVe seems to exist predominantly in the 1H form, while compounds V and VI exist predominantly in the 2H form, judging from our previous work[12] on the tautomerism of 3,5-disubstituted 1,2,4-triazoles.

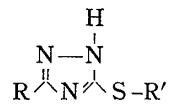
TABLE III. *In Vitro* Antimicrobial Activity (MIC)[a]



Compound No.	R	*S. aureus*	*E. coli*	*S. flexneri*	*P. aeruginosa*	*C. albicans*	*T. asteroides*	*T. vaginalis*	*M. tuberculosis*[b]
I	H	100	100	>100	>100	>100	30	10	10
IIIa	CH_3	>100	>100	>100	>100	>100	>100	>100	>100
IIIb	C_2H_5	>100	>100	>100	>100	>100	>100	>100	>100
IIIc	n-C_3H_7	>100	>100	>100	>100	>100	>100	>100	30
IIId	n-C_4H_9	>100	>100	>100	>100	>100	30	30	3
IIIe	n-C_5H_{11}	>100	>100	>100	>100	>100	30	30	3
IIIf	n-C_6H_{13}	30	30	30	>100	30	10	10	3
IIIg	n-C_7H_{15}	30	>100	>100	>100	>100	10	10	10
IIIh	n-C_8H_{17}	>100	>100	>100	>100	>100	>100	>100	>100
IIIi	n-C_9H_{19}	30	>100	>100	>100	>100	30	>100	>100

a) Minimal inhibitory concentration (μg/ml).
b) The MIC of isoniazide was 0.1.

TABLE IV. *In Vitro* Antimicrobial Activity (MIC)[a]



Compound No.	R[b]	R′	*S. aureus*	*E. coli*	*S. flexneri*	*P. aeruginosa*	*C. albicans*	*T. asteroides*	*T. vaginalis*	*M. tuberculosis*[c]
II	2-Py	H	30	>100	>100	>100	>100	30	100	30
IVa	2-Py	CH_3	>100	>100	100	>100	>100	100	100	100
IVb	2-Py	C_2H_5	>100	>100	>100	>100	>100	>100	>100	>100
IVc	2-Py	n-C_3H_7	>100	100	100	>100	100	30	30	100
IVd	2-Py	n-C_4H_9	>100	100	>100	>100	100	30	30	30
IVe	2-Py	n-C_5H_{11}	>100	30	>100	>100	>100	10	10	3
IVf	2-Py	n-C_6H_{13}	100	>100	>100	>100	>100	30	30	10
IVg	2-Py	n-C_7H_{15}	>100	>100	>100	>100	>100	100	30	10
IVh	2-Py	n-C_8H_{17}	>100	>100	>100	>100	>100	100	30	10
IVi	2-Py	n-C_9H_{19}	>100	>100	>100	>100	>100	100	30	30
V	3-Py	n-C_5H_{11}	>100	>100	>100	>100	>100	30	>30	30
VI	4-Py	n-C_5H_{11}	>100	>100	>100	>100	>100	10	>30	30

a) Minimal inhibitory concentration (μg/ml).
b) Py=Pyridyl.
c) The MIC of isoniazide was 0.1.

Experimental

All melting points were determined by the capillary method and are uncorrected.

1-Picolinoylthiosemicarbazide (I)——a) Sodium (1.0 g) was added portionwise to a stirred solution of thiosemicarbazide (3.0 g) in liquid ammonia (100 ml), cooled in a dry ice-acetone bath (−60°). To this solution was added ethyl picolinate (5.0 g), and the mixture was stirred for 3 hr. After the bath was removed,

12) S. Kubota and M. Uda, *Chem. Pharm. Bull.* (Tokyo), **23**, 955 (1975).

and the solution was allowed to evaporate at room temperature, the residual oil was dissolved in H_2O (40 ml) and neutralized with acetic acid. The precipitate was collected by filtration and recrystallized from EtOH to give colorless needles (3.62 g, 56%), mp 202—203°. *Anal.* Calcd. for $C_7H_8ON_4S$: C, 42.85; H, 4.11; N, 28.55. Found: C, 43.12; H, 3.96; N, 28.25.

b) This method is a modification of the procedure of Hoggarth.[13] A mixture of picolinic acid hydrazide (5.0 g), ammonium thiocyanate (6.6 g), 37% HCl (5 ml), and H_2O (20 ml) was refluxed for 2 hr. After being cooled, the resulting precipitate was collected by filtration and recrystallized from EtOH to give colorless needles (5.22 g, 73%), mp 202—203°.

1-Picolinoyl-S-alkylisothiosemicarbazides (IIIa—i)——To a solution of 1-picolinoylthiosemicarbazide (I, 0.01 mol) in 5% ethanolic sodium hydroxide (10 ml) was added the corresponding alkyl iodide (0.02 mol), and the mixture was stirred at room temperature overnight. Water (40 ml) was added, and the resulting precipitate was collected by filtration and recrystallized from the solvent listed in Table I.

The methylthio derivative (IIIa) was obtained by the reaction of I with methyl iodide in 1 N NaOH. The precipitate of the product was formed while the reaction proceeded (1.5 hr).

3-Alkylthio-5-(2-pyridyl)-1,2,4-triazoles (IVa—i)——Method A) A solution of the corresponding alkyl iodide (0.02 mol) in EtOH (1 ml) was added to a solution of 5-(2-pyridyl)-1,2,4-triazoline-3-thione[6] (II, 0.01 mol) in 1 N NaOH (5 ml), and the mixture was stirred at room temperature for 2—8 hr. The mixture was neutralized with 10% HCl, the resulting precipitate was collected by filtration and recrystallized from the solvent listed in Table II.

The methylthio derivative (IVa) was prepared by the reaction of II (0.01 mol) with equimolar amount of methyl iodide (0.01 mol).[6]

Method B) The corresponding alkyl iodide (0.02 mol) was added to a solution of 5-(2-pyridyl)-1,2,4-triazoline-3-thione[6] (II, 0.01 mol) in pyridine (5 ml), and the mixture was stirred at room temperature for 12 hr. Water (20 ml) was added, and the resulting precipitate was collected by filtration and recrystallized from the solvent listed in Table II.

Method C) The corresponding 1-picolinoyl-S-alkylisothiosemicarbazide (IIIa—i, 0.01 mol) was heated on an oil bath at the temperature about 20° higher than their melting point for 1—1.5 hr. At the temperature near melting point, the evolution of gas was accelerated. After being cooled, the solidified crude product was purified by recrystallization from the solvent listed in Table II.

3-Pentylthio-5-(3-pyridyl)-1,2,4-triazole (V)——This compound was prepared by the same procedure (Method A) as for IVe from 5-(3-pyridyl)-1,2,4-triazoline-3-thione.[8]

Yield 49%. mp 77—79°. *Anal.* Calcd. for $C_{12}H_{16}N_4S$: C, 58.04; H, 6.49; N, 22.56. Found: C, 58.04; H, 6.58; N, 22.39.

3-Pentylthio-5-(4-pyridyl)-1,2,4-triazole (VI)——This compound was prepared by the same procedure (Method A) as for IVe from 5-(4-pyridyl)-1,2,4-triazoline-3-thione.[8]

Yield 36%. mp 109—110°. *Anal.* Calcd. for $C_{12}H_{16}N_3S$: C, 58.04; H, 6.49; N, 22.56. Found: C, 58.00; H, 6.72; N, 22.36.

Antimicrobial Tests——The minimal inhibitory concentration (MIC) was determined by the broth-dilution method.[9] The media used were nutrient broth for *Staphylococcus aureus* TERAJIMA, *Escherichia coli* K-12, *Shigella flexneri* 2a EW-10 and *Pseudomonas aeruginosa* TSUCHIJIMA, Kirchner's medium supplemented with 10% bovine serum for *Mycobacterium tuberculosis* $H_{37}Rv$, Sabouraud Dextrose medium for *Candida albicans* ATCC 10257 and *Trichophyton asteroides*, and SYS medium[14] for *Trichomonas vaginalis* 4F.

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13) E. Hoggarth, *J. Chem. Soc.*, **1949**, 1163.
14) K. Ikeuchi, *The Journal of the Osaka City Medical Center*, **8**, 853 (1961).