[Chem. Pharm. Bull. 32(8)3291—3298(1984)]

Syntheses and Antimicrobial Activities of Five-Membered Heterocycles Having a Phenylazo Substituent[1)]

KUNIYOSHI TANAKA,* KEIZO MATSUO, AI NAKANISHI, MISAKO JO, HIRONORI SHIOTA, MIKIKO YAMAGUCHI, SAKIKO YOSHINO, and KEIKO KAWAGUCHI

Faculty of Pharmaceutical Sciences, Kinki University, 3–4–1, Kowakae, Higashi-Osaka 577, Japan

(Received March 16, 1984)

Several five-membered heterocycles having a phenylazo substituent, 3-phenylazotetronic acids (**2a—n**), 3-phenylazotetramic acids (**4a—i**), 4-phenylazo-5-isoxazolinones (**6a—g**, **8a—f**) and 5-phenylazo-4-thiazolidinones (**10a—f**, **12a—e**), were synthesized and tested for antimicrobial activities. Tetronic acid and tetramic acid derivatives (**2** and **4**) inhibited the growth of gram-positive bacteria. 5-Isoxazolinone and 4-thiazolidinone derivatives (**8** and **10**) showed inhibitory activities against fungi as well as bacteria.

Keywords——substituted phenylazo group; tetronic acid; tetramic acid; 5-isoxazolinone; 4-thiazolidinone; antimicrobial activity

In the previous papers,[2,3)] we reported the antimicrobial activities of 3-acyltetronic acids and 3-acyltetramic acids, and concluded that the tricarbonylmethane structure was essential for the actitivites. We therefore planned to synthesize tetronic acids and tetramic acids having a phenylazo substituent at the 3-position, in which one of the tricarbonyl groups is replaced by the electron-withdrawing azo group, and also to synthesize 4-phenylazo-5(4*H*)-isoxazolinone and 5-phenylazo-4-thiazolidinone derivatives as related five-membered compounds. The antimicrobial activities of these compounds were tested and the structure–activity relationships were considered.

Chemistry

3-Phenylazotetronic Acid Derivatives

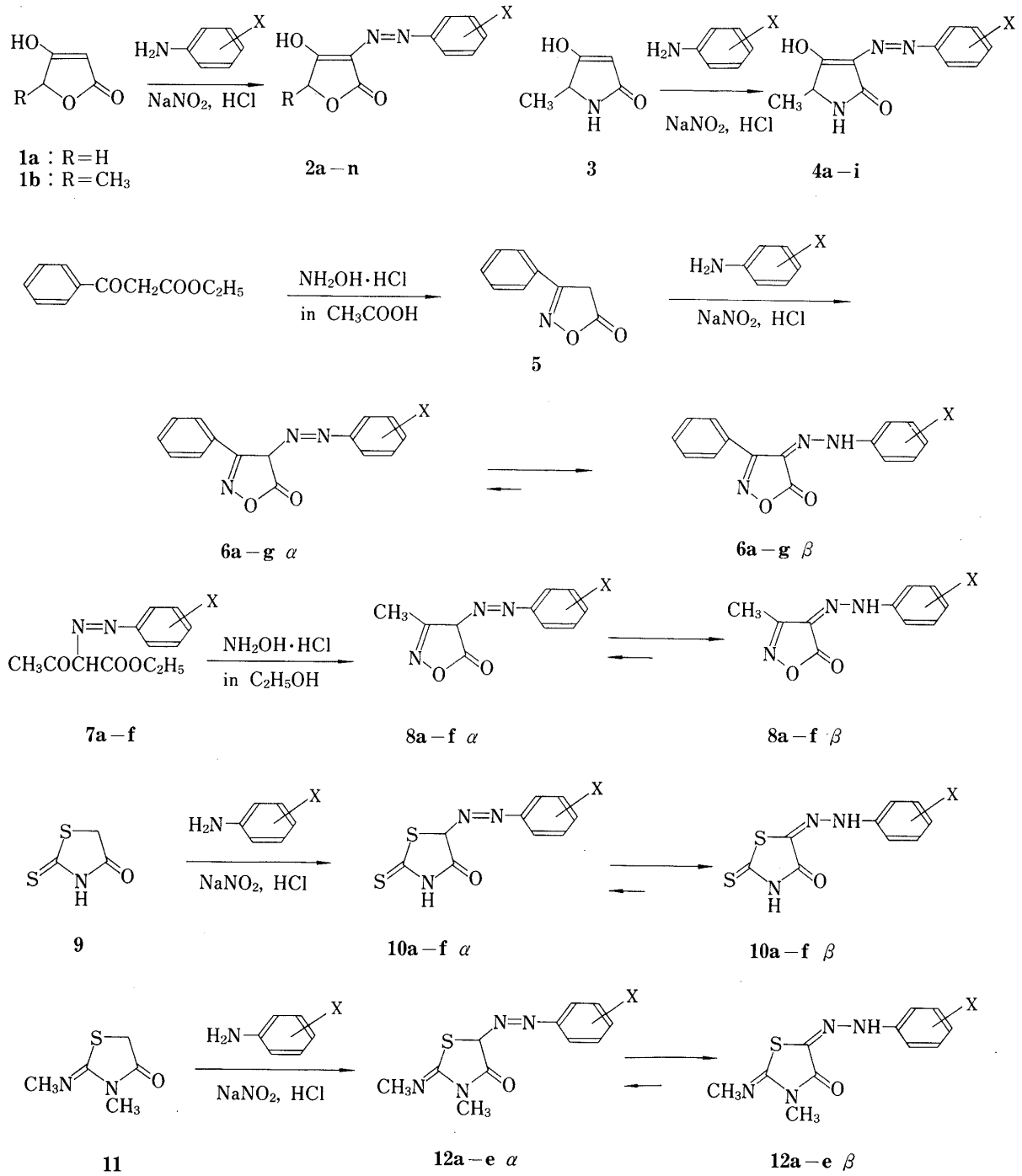
Tetronic acid (**1a**) and 5-methyltetronic acid (**1b**) were prepared by the method reported in the previous paper.[2)] Compounds **1a** and **1b** were each coupled with diazonium salts derived from substituted anilines to yield various 3-phenylazotetronic acid derivatives (**2a—n**, Chart, Table I). The structures of these compounds were confirmed by elemental analyses, and infrared (IR) and nuclear magnetic resonance (NMR) spectral data.

3-Phenylazotetramic Acid Derivatives

5-Methyltetramic acid (**3**) prepared by the known method,[3,4)] was converted to 5-methyl-3-phenylazotetramic acids (**4a—i**) by means of the azo-coupling reaction as shown in the Chart and Table II.

4-Phenylazo-5(4*H*)-isoxazolinone Derivatives

Condensation of ethyl benzoylacetate with hydroxylamine provided 3-phenyl-5(4*H*)-isoxazolinone (**5**),[5)] from which 3-phenyl-4-phenylazo-5(4*H*)-isoxazolinone derivatives (**6a—g**) were obtained by means of the azo-coupling reaction. On the other hand, as 3-methyl-



Chart

5(4*H*)-isoxazolinone was difficult to isolate from the condensation products of ethyl acetoacetate and hydroxylamine, 3-methyl-4-phenylazo-5(4*H*)-isoxazolinone derivatives (**8a**—**f**) were synthesized by condensation of ethyl 2-phenylazo-3-oxobutyrate derivatives (**7a**—**f**)[6] with hydroxylamine (Chart, Table III).[7] These compounds (**6a**—**gα**, **8a**—**fα**) were presumed to have the tautomeric structures (**6a**—**gβ**, **8a**—**fβ**) from their NMR spectra.[8,9] The NMR spectrum of **6a**, for example, showed a singlet peak at 13.30 ppm, which is consistent with the proton of =N–NH–.

5-Phenylazo-4-thiazolidinone Derivatives

5-Phenylazo-2-thioxo-4-thiazolidinone derivatives (**10a**—**f**) were prepared from 2-

TABLE I. Physical Properties of 3-Phenylazotetronic Acid Derivatives

Compound	R	X	Appearance	mp (°C)	Yield (%)	Formula	Analysis (%) Found (Calcd)		
							C	H	N
2a	H	H	Orange needles	207—208	41.7	$C_{10}H_8N_2O_3$	58.80 (58.82	3.99 3.95	14.00 13.72)
2b	CH_3	H	Orange prisms	153—155	73.2	$C_{11}H_{10}N_2O_3$	60.55 (60.26	4.62 4.55	12.84 12.79)
2c	CH_3	2-Cl	Yellow powder	174—177	79.5	$C_{11}H_9ClN_2O_3$	52.39 (52.29	3.43 3.59	10.89 11.09)
2d	CH_3	4-Cl	Yellow powder	190—192	61.3	$C_{11}H_9ClN_2O_3$	52.03 (52.29	3.66 3.59	11.32 11.09)
2e	CH_3	2,4-Cl_2	Yellow powder	184—188	20.5	$C_{11}H_8Cl_2N_2O_3$	45.76 (46.02	2.65 2.81	10.02 9.76)
2f	CH_3	3,4-Cl_2	Yellow powder	177—180	54.4	$C_{11}H_8Cl_2N_2O_3$	45.84 (46.02	2.76 2.81	10.23 9.76)
2g	CH_3	2-NO_2	Orange powder	187—191	57.8	$C_{11}H_9N_3O_5$	49.93 (50.19	3.14 3.45	15.89 15.96)
2h	CH_3	3-NO_2	Yellow powder	207—211	61.9	$C_{11}H_9N_3O_5$	50.39 (50.19	3.56 3.45	16.27 15.96)
2i	H	4-NO_2	Orange plates	236—238	62.5	$C_{10}H_7N_2O_5$	47.86 (48.20	3.69 3.83	16.71 16.86)
2j	CH_3	4-NO_2	Red powder	217—222	69.5	$C_{11}H_9N_3O_5$	50.01 (50.19	3.39 3.45	16.24 15.96)
2k	CH_3	2,6-$(CH_3)_2$	Orange prisms	140—142	47.5	$C_{13}H_{14}N_2O_3$	63.14 (63.40	5.35 5.73	11.59 11.37)
2l	CH_3	3-CF_3	Yellow powder	139—143	70.0	$C_{12}H_9F_3N_2O_3$	50.06 (50.36	2.88 3.17	9.78 9.79)
2m	CH_3	4-COOH	Yellow powder	251—255	38.3	$C_{12}H_{10}N_2O_5$	54.75 (54.97	3.74 3.84	10.86 10.68)
2n	CH_3	4-$COOC_2H_5$	Yellow powder	143—144	68.3	$C_{14}H_{14}N_2O_5$	57.98 (57.93	4.70 4.86	9.44 9.65)

thioxo-4-thiazolidinone (rhodanine) (**9**)[10] by means of the azo-coupling reaction and 3-methyl-2-methylimino-5-phenylazo-4-thiazolidinone derivatives (**12a—e**) were similarly synthesized from 3-methyl-2-methylimino-4-thiazolidinone (**11**).[11] These compounds were also presumed to have the tautomeric structures (**10a—fβ**, **12a—eβ**) from their NMR spectra (Chart, Table IV).

Biology

The synthesized compounds were tested for growth inhibitory activities against gram-positive and negative bacteria and fungi. Several 3-phenylazotetronic acids (**2**) inhibited the growth of *B. subtilis* and *S. aureus*, but did not exhibited any activities against other microorganisms (Table V). Most of the 3-phenylazotetramic acids (**4**) inhibited the growth of *B. subtilis*, but they did not show inhibitory activity against other microorganisms (Table VI). Although none of the 3-phenyl-4-phenylazo-5(4*H*)-isoxazolinone derivatives (**6a—g**) inhibited the growth of the microorganisms tested, most of the 3-methyl-4-phenylazo-5(4*H*)-isoxazolinone derivatives (**8a—f**) showed inhibitory activities against bacteria as well as fungi (Table VII). It has already been reported that **8b** has antifungal activity.[12] The 5-phenylazo-2-thioxo-4-thiazolidinone derivatives (**10a—f**) showed broad antimicrobial spectra, but 3-

Table II. Physical Properties of 3-Phenylazotetramic Acid Derivatives

Compound	X	Appearance	mp (°C)	Yield	Formula	Analysis (%) Found (Calcd)		
						C	H	N
4a	H	Yellow needles	153—155	18.8	$C_{11}H_{11}N_3O_2$	60.96 (60.82	5.16 5.10	19.14 19.34)
4b	2-Cl	Yellow needles	187—192	23.7	$C_{11}H_{10}ClN_3O_2$	52.44 (52.50	3.92 4.01	16.57 16.70)
4c	2-F	Brown powder	203—206	48.5	$C_{11}H_{10}FN_3O_2$	56.22 (56.17	4.16 4.28	17.65 17.86)
4d	2-NO_2	Orange powder	229—231	7.5	$C_{11}H_{10}N_4O_4$	50.15 (50.38	3.53 3.84	20.97 21.37)
4e	4-NO_2	Yellow powder	255—258	27.5	$C_{11}H_{10}N_4O_4$	50.21 (50.38	3.78 3.84	21.11 21.37)
4f	2-CH_3	Yellow powder	151—154	7.0	$C_{12}H_{13}N_3O_2$	62.17 (62.23	5.49 5.67	18.13 18.17)
4g	2-CF_3	Orange powder	173—175	48.2	$C_{12}H_{10}F_3N_3O_2$	50.53 (50.53	3.43 3.53	14.70 14.73)
4h	3-CF_3	Yellow powder	189—193	43.8	$C_{12}H_{10}F_3N_3O_2$	50.55 (50.53	3.45 3.53	14.51 14.73)
4i	2-OCH_3	Orange powder	203—206	71.7	$C_{12}H_{13}N_3O_3$	58.17 (58.29	5.17 5.30	17.02 17.00)

Table III. Physical Properties of 4-Phenylazo-5(4*H*)-isoxazolinone Derivatives

Compound	X	Appearance	mp (°C)	Yield (%)	Formula	Analysis (%) Found (Calcd)		
						C	H	N
6a	H	Orange prisms	169—172 (lit.[5] 166)	26.2	$C_{15}H_{11}N_3O_2$	67.81 (67.92	4.16 4.18	15.45 15.84)
6b	2-Cl	Orange needles	157—160	34.7	$C_{15}H_{10}ClN_3O_2$	60.30 (60.11	3.31 3.36	13.92 14.02)
6c	4-Cl	Yellow flocks	234—237	55	$C_{15}H_{10}ClN_3O_2$	59.84 (60.11	3.22 3.06	14.15 14.02)
6d	2-NO_2	Orange powder	179—180	32.2	$C_{15}H_{10}N_4O_4$	58.24 (58.07	3.12 3.25	17.95 18.06)
6e	4-NO_2	Yellow flocks	265—268	77.4	$C_{15}H_{10}N_4O_4$	58.18 (58.07	3.13 3.25	17.96 18.06)
6f	2-CF_3	Yellow needles	138—142	55.3	$C_{16}H_{10}F_3N_3O_2$	57.75 (57.66	2.96 3.02	12.52 12.61)
6g	3-CF_3	Yellow flocks	195—199	37.5	$C_{16}H_{10}F_3N_3O_2$	57.86 (57.66	2.96 3.02	12.46 12.61)
8a	H	Yellow plates	192—194 (lit.[6] 192)	64.6	$C_{10}H_9N_3O_2$	59.23 (59.11	4.34 4.46	20.53 20.68)
8b	2-Cl	Yellow prisms	167—168 (lit.[12] 164—166)	34.7	$C_{10}H_8ClN_3O_2$	50.33 (50.54	3.24 3.39	17.74 17.68)
8c	4-Cl	Yellow flocks	183—184 (lit.[7] 186)	66.6	$C_{10}H_8ClN_3O_2$	50.34 (50.54	3.23 3.39	17.61 17.68)
8d	2-F	Yellow prisms	145—148	71.2	$C_{10}H_8FN_3O_2$	54.52 (54.30	3.86 3.65	18.95 19.00)
8e	4-NO_2	Yellow powder	196—200	41.2	$C_{10}H_8N_4O_4$	48.16 (48.39	3.61 3.25	22.10 22.57)
8f	3-CF_3	Yellow plates	204—208	36.4	$C_{11}H_8F_3N_3O_2$	48.66 (48.72	3.04 2.97	15.50 15.49)

TABLE IV. Physical Properties of 5-Phenylazo-4-thiazolidinone Derivatives

Compound	X	Appearance	mp (°C)	Yield (%)	Formula	Analysis (%) Found (Calcd)		
						C	H	N
10a	H	Brown needles	243—247	59.6	$C_9H_7N_3OS_2$	45.66 (45.55	2.89 2.97	17.49 17.71)
10b	2-Cl	Orange needles	240—243	24.0	$C_9H_6ClN_3OS_2$	39.46 (39.78	2.08 2.23	15.26 15.46)
10c	4-Cl	Orange granulus	>250	25.7	$C_9H_6ClN_3OS_2$	39.91 (39.78	2.24 2.23	15.60 15.46)
10d	2-F	Orange needles	225—228	23.2	$C_9H_6FN_3OS_2$	42.25 (42.34	2.32 2.37	16.48 16.46)
10e	2-NO_2	Orange needles	224—228	64.2	$C_9H_6N_4O_3S_2$	38.22 (38.29	2.05 2.14	20.03 19.85)
10f	4-NO_2	Orange needles	>250	45.5	$C_9H_6N_4O_3S_2 \cdot 1/2H_2O$	37.50 (37.11	2.44 2.42	19.19 19.23)
12a	H	Yellow needles	218—221	32.9	$C_{11}H_{12}N_4OS$	53.30 (53.21	4.83 4.87	22.57 22.56)
12b	2-Cl	Orange needles	188—191	37.6	$C_{11}H_{11}ClN_4OS$	46.89 (46.73	3.95 3.92	19.58 19.82)
12c	4-Cl	Yellow needles	236—240	30.7	$C_{11}H_{11}ClN_4OS$	46.83 (46.73	3.83 3.92	19.65 19.82)
12d	2-NO_2	Orange needles	217—219	78.8	$C_{11}H_{11}N_5O_3S$	44.99 (45.05	3.48 3.78	23.77 23.88)
12e	4-NO_2	Yellow powder	>250	64.9	$C_{11}H_{11}N_5O_3S$	45.13 (45.05	3.75 3.78	23.81 23.88)

TABLE V. Antimicrobial Activities of 3-Phenylazotetronic Acid Derivatives (MIC: μg/ml)

Microorganism	Compound						
	2a	**2b**	**2c**	**2d**	**2e**	**2f**	**2g**
B. subtilis IFO-3513	12.5	3.12	1.56	50	6.25	50	3.12
S. aureus IFO-3061	>100	100	50	100	100	50	25

Microorganism	Compound						
	2h	**2i**	**2j**	**2k**	**2l**	**2m**	**2n**
B. subtilis IFO-3513	6.25	12.5	0.78	>100	1.56	>100	0.78
S. aureus IFO-3061	100	>100	50	>100	50	>100	100

methyl-2-methylimino-4-thiazolidinone derivatives (**12a—e**) were inactive (Table VIII).

The above mentioned results led us to conclude that the nature of the five-membered heterocycle in phenylazo derivatives has a significant effect on the antimicrobial activities; the

TABLE VI. Antimicrobial Activities of 3-Phenylazotetramic Acid Derivatives (MIC: μg/ml)

Microorganism	Compound								
	4a	**4b**	**4c**	**4d**	**4e**	**4f**	**4g**	**4h**	**4i**
B. subtilis IFO-3513	100	3.12	3.12	0.39	1.56	100	6.25	12.5	>100
S. aureus IFO-3061	>100	>100	>100	>100	>100	>100	>100	>100	>100

activity spectra of isoxazolinone and thiazolidinone derivatives (**8** and **10**) were broader than those of tetronic acid and tetramic acid derivatives (**2** and **4**). In addition, substituents on the heterocycles also affected the activity; that is, in contrast to 3-methyl-4-phenylazo-5(4*H*)-isoxazolinone and 5-phenylazo-2-thioxo-4-thiazolidinone derivatives (**8** and **10**), which showed remarkable inhibitory activities, 3-phenyl-5(4*H*)-isoxazolinone and 3-methyl-2-methylimino-4-thiazolidinone derivatives (**6** and **12**) were all inactive. On the other hand, it is noteworthy that the inhibitory activity was enhanced by chloro and nitro substituents on the benzene nucleus of the phenylazo group.

Experimental

Melting points were determined on a Yanagimoto micro-melting point apparatus, model MP-S3, and are uncorrected. IR spectra were measured in Nujol mulls with a Hitachi infrared spectrophotometer, model 260-30, and NMR spectra were measured with a JEOL JNM-FX 200 (200 MHz) spectrometer using tetramethylsilane as an internal standard.

3-Phenylazotetronic Acid (2a)——A solution of aniline (0.7 g, 2 mmol) in 6.5 ml of 1 N HCl was cooled to 0 °C and diazotized with $NaNO_2$ (0.16 g, in 2 ml H_2O). Tetronic acid (**1a**) (0.2 g, 2 mmol) dissolved in 2 ml of H_2O containing K_2CO_3 (0.28 g, 2.02 mmol) was added dropwise to the diazotized solution with stirring and ice-cooling. The mixture was allowed to stand for 1 h, then the orange-colored precipitates were filtered off, washed well with water, and recrystallized from glacial acetic acid to yield **2a** as orange plates. 0.17 g (41.7%). mp 207—208 °C. IR ν_{max}^{Nujol} cm^{-1}: 3295 (OH), 1780 (CO), 1690 (C=C), 1605, 1555 (aromatic). NMR (DMSO-d_6) δ: 4.67 (2H, s, CH_2), 7.28—7.64 (5H, m, aromatic), 13.1 (1H, br s, OH). Other derivatives (**2b**—**n**) were prepared similarly and are listed in Table I.

5-Methyl-3-phenylazotetramic Acid (4a)——5-Methyltetramic acid (**3**) (0.5 g, 4.42 mmol) dissolved in 5 ml of H_2O containing K_2CO_3 (0.62 g, 4.47 mmol) was slowly added to a solution of benzenediazonium chloride (prepared from 0.41 g aniline) with stirring and ice-cooling. The mixture was allowed to stand for several hours, then the separated oil was extracted with $CHCl_3$. The organic layer was washed with saturated brine and dried over Na_2SO_4. The solvent was removed *in vacuo* to yield a gummy residue (0.8 g), which was purified by silica gel column chromatography using $CHCl_3$ as an eluent and by recrystallization from AcOEt. Yellow needles, 0.18 g (18.8%). mp 153—155 °C. IR ν_{max}^{Nujol} cm^{-1}: 3100 (OH, NH), 1670 (CONH), 1590, 1525 (aromatic). NMR ($CDCl_3$) δ: 1.46 (3H, d, J=8 Hz, CH_3), 4.03 (1H, q, J=8 Hz, CH), 7.2—7.4 (6H, aromatic, CONH), 13.4 (1H, s, OH). Other derivatives (**4b**—**i**) were similarly synthesized and are listed in Table II.

3-Phenyl-4-phenylazo-5(4*H*)-isoxazolinone (6a)——3-Phenyl-5(4*H*)-isoxazolinone (**5**) (0.32 g, 2 mmol) dissolved in 2 ml of 1 N NaOH solution was added to the diazotized solution [prepared from aniline (0.2 g, 2 mmol)] with stirring and ice-cooling. The mixture was allowed to stand for half an hour, then the yellow precipitates were filtered off, washed with water and recrystallized from EtOH to yield yellow prisms. 0.14 g (26.2%). mp 169—172 °C. IR ν_{max}^{Nujol} cm^{-1}: 3170 (=N–NH–), 1704 (CO), 1660 (C=N), 1595, 1580, 1545 (aromatic). NMR ($CDCl_3$) δ: 7.28—8.12 (10H, m, aromatic), 13.30 (1H, s, =N–NH–). Other derivatives (**6b**—**g**) were similarly synthesized and are listed in Table III.

3-Methyl-4-phenylazo-5(4*H*)-isoxazolinone (8a)——A solution of benzenediazonium chloride prepared from aniline (5.72 g, 61 mmol) was added dropwise to a solution containing ethyl acetoacetate (8 g, 61 mmol in EtOH 180 ml) and NaOAc (27.2 g in H_2O 48 ml) with stirring and ice-cooling to yield ethyl 3-oxo-2-phenylazobutyrate (**7a**, 7.65 g, 53.6%). $NH_2OH \cdot HCl$ (2.34 g, 33.7 mmol) and NaOAc (4.43 g, 54 mmol) dissolved in H_2O (63 ml) were added dropwise to a boiling solution of **7a** (7.65 g, 32.7 mmol) in EtOH (63 ml) with stirring and heating. Refluxing was continued for half an hour, then the reaction mixture was cooled, yielding yellow precipitates which were filtered off,

TABLE VII. Antimicrobial Activities of 3-Methyl-4-phenylazo-5(4*H*)-isoxazolinone Derivatives (MIC: μg/ml)

Compound	Microorganism[a]														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
8a	>100	>100	>100	>100	>100	1.56	12.5	>100	>100	100	>100	100	6.25	>100	25
8b	1.56	>100	>100	>100	0.39	0.20	0.39	>100	>100	3.25	>100	3.12	3.12	100	6.25
8c	>100	>100	>100	>100	1.56	0.78	12.5	>100	>100	12.5	>100	3.12	6.25	100	100
8d	25	>100	>100	>100	1.56	0.39	3.12	12.5	>100	12.5	100	6.25	6.25	25	25
8e	6.25	25	100	>100	0.78	0.78	0.78	25	25	6.25	>100	3.12	6.25	12.5	100
8f	>100	>100	>100	>100	>100	3.12	>100	>100	>100	100	>100	6.25	>100	>100	50

a) 1, *Bacillus subtilis*, IFO-3513; 2, *Staphylococcus aureus*, IFO-3061; 3, *Escherichia coli*, IFO-12734; 4, *Pseudomonus aeruginosa*, IFO-3080; 5, *Aspergillus niger*, IFO-6341; 6, *Penicillium citrinum*, IFO-6352; 7, *Cladosporium herbarum*, IFO-6348; 8, *Mucor spinescens*, IFO-6350; 9, *Rhodorula rubra*, IFO-0907; 10, *Pyricularia oryzae*, IFO-5279; 11, *Pellicularia sasakii*, IFO-6330; 12, *Helminthosporium sigmoideum*, IFO-4867; 13, *Botrytis cinerea*, Kyoto; 14, *Collectorichum lagenerium*, IFO-6207; 15, *Sclerotinia sclerotiorum*, IFO-4876.

TABLE VIII. Antimicrobial Activities of 5-Phenylazo-2-thioxo-4-thiazolidinone Derivatives (MIC: μg/ml)

Compound	Microorganism[a]														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
10a	50	50	>100	>100	>100	50	25	100	25	25	100	12.5	50	25	>100
10b	50	25	>100	>100	>100	50	25	100	12.5	12.5	25	25	50	12.5	100
10c	50	12.5	>100	>100	100	12.5	12.5	1.56	50	50	100	6.25	100	50	100
10d	25	25	>100	>100	>100	50	25	>100	25	25	25	12.5	50	25	100
10e	25	12.5	>100	>100	>100	>100	25	>100	100	25	25	100	100	50	50
10f	25	12.5	>100	>100	>100	100	12.5	12.5	50	12.5	100	25	25	50	50

a) The kinds of microorganisms were the same as listed in Table VII.

washed with 40% EtOH and recrystallized from AcOEt. Yellow plates, 4.29 g (64.6%). mp 198—201 °C. IR ν_{max}^{Nujol} cm^{-1}: 3200 (=N–NH), 1710 (CO), 1675 (C=N), 1595, 1565 (aromatic). NMR ($CDCl_3$) δ: 2.32 (3H, s, CH_3), 7.28—7.42 (5H, m, aromatic), 12.65 (1H, s, =N–NH–). Other derivatives (**8b—f**) were similarly synthesized and are listed in Table III.

5-Phenylazo-2-thioxo-4-thiazolidinone (10a)——An aqueous solution containing rhodanine (**9**) (1.0 g, 7.52 mmol) and K_2CO_3 (1.05 g, 7.6 mmol) was added to the diazotized solution prepared from aniline (0.7 g, 7.52 mmol) with stirring and ice-cooling to yield precipitates, which were recrystallized from MeOH. Brown needles, 2.12 g (59.6%). mp 242—247 °C. IR ν_{max}^{Nujol} cm^{-1}: 3400 (CONH), 3240 (=N–NH–), 1700 (CONH), 1600, 1520 (aromatic). NMR (DMSO-d_6) δ: 7.00—7.28 (5H, m, aromatic), 10.8 (1H, s, =N–NH–), 13.6 (1H, br s, CONH). Other derivatives (**10b—f**) were similarly synthesized and are listed in Table IV.

3-Methyl-2-methylimino-5-phenylazo-4-thiazolidinone (12a)——A solution of 3-methyl-2-methylimino-4-thiazolidinone (**11**) (1 g, 6.94 mmol) and K_2CO_3 (0.97 g, 7.01 mmol) in H_2O was added dropwise with stirring to a solution of benzenediazonium chloride prepared from aniline (0.65 g, 6.94 mmol), and the whole was kept for 1 h at 0 °C. The product was filtered off, washed well with H_2O and recrystallized from EtOH. Yellow needles, 0.57 g (32.9%). mp 218—221 °C. IR ν_{max}^{Nujol} cm^{-1}: 3250 (=N–NH–), 1690 (CONH), 1660 (C=N–), 1605, 1560 (aromatic). NMR (DMSO-d_6) δ: 3.12 and 3.13 (each 3H, s, CH_3NCO and $CH_3N=C$), 6.95—7.24 (5H, m, aromatic), 10.32 (1H, s, =N–NH–). Other derivatives (**12b—e**) were similarly synthesized and are listed in Table IV.

Antimicrobial Activity Test——The minimum inhibitory concentration (MIC, μg/ml) was measured as follows; bouillon agar (9 ml) was mixed with 1 ml of an aqueous solution containing a test compound (dissolved in small amounts of *N,N*-dimethylformamide and acetone) at various concentrations. The agar was then poured into a Petri dish. After solidification, the agar was streaked with test organism suspension and incubated at 33 °C for 18—20 h. The MIC for each compound was defined as the lowest concentration inhibiting the growth of the test organisms.

Acknowledgement The authors are indebted to Dr. Kazuo Konishi and Mr. Kiyoshi Okamoto, Pesticide Research Laboratories, Agricultural Chemical Division, Takeda Chemical Industries Ltd., for carrying out antimicrobial activity tests. Thanks are also due to Mr. Masami Kan, Chemical Research Laboratories, Central Research Division, Takeda Chemical Industries Ltd., for microanalysis and to Mrs. Toshie Minematsu, Faculty of Pharmaceutical Sciences, Kinki University, for NMR spectral measurements.

References and Notes

1) The work was presented at the Annual Meeting of the Kinki Branch of the Pharmaceutical Society of Japan, Kobe, Nov., 1981.
2) K. Tanaka, K. Matsuo, Y. Nakaizumi, Y. Morioka, Y. Takashita, Y. Tachibana, Y. Sawamura, and S. Kohda, *Chem. Pharm. Bull.*, **27**, 1901 (1979).
3) K. Matsuo, I. Kitaguchi, Y. Takata, and K. Tanaka, *Chem. Pharm. Bull.*, **28**, 2494 (1980).
4) T. P. C. Mulholland, R. Foster, and D. B. Haydock, *J. Chem. Soc., Perkin Trans. 1*, **1972**, 2121.
5) L. Claisen and W. Zedal, *Chem. Ber.*, **24**, 140 (1891).
6) C. Bülow and P. Neber, *Chem. Ber.*, **45**, 3732 (1912).
7) H. G. Garg, *J. Org. Chem.*, **27**, 1045 (1962).
8) L. A. Summers and D. J. Shields, *Chem. Ind.* (London), **1964**, 1264.
9) K. Tabei, E. Kawashima, and T. Kato, *Chem. Pharm. Bull.*, **29**, 244 (1981).
10) C. E. Redemann, R. N. Icke, and G. A. Alles, "Organic Syntheses," Coll. Vol. III, ed. by A. H. Blatt, John Wiley & Sons Inc., New York, 1955, p. 763.
11) R. Andreasch, *Monatsh. Chem.*, **8**, 407 (1888).
12) Imperial Chemical Industries, Ltd., Brit. Patent 1049103 (1966) [*Chem. Abstr.*, **66**, 4408 (1967)].