CHEMICAL & PHARMACEUTICAL BULLETIN

Vol. 13 No. 8

August 1965

[Chem. Pharm. Bull.]
13(8) 901~906 (1965)

UDC 547.853.7 : 615.77

116. Yoshihiro Nitta, Toshio Miyamoto, Toshiyuki Nebashi,
and Fumio Yoneda : Pyrimidine Derivatives. III.*¹
The Preparation and Bacteriostatic Activity
of Benzamidopyrimidines.

(Research Laboratories, Chugai Pharmaceutical Co., Ltd.*²)

A number of studies with a view to finding medicine on the benzanilide derivatives have been reported, especially the antibacterial and anthelmintic activities of salicylanilide derivatives have been extensively studied.^{1~7)} Some of the salicylhaloanilide and halosalicylhaloanilide series were found to have strong antibacterial and antifungal activities. Moreover, certain benzanilide derivatives such as 3,4,5-trimethoxybenzanilide series⁸⁾ were found to possess nonhypnotic sedative action. Some investigations of benzamide derivatives, of which aniline part was replaced by heterocyclic amines have partially been attempted, but no systematical works on the syntheses and biological actions of heterocyclic benzamide derivatives have been done.

It is, therefore, of considerable interest to investigate the effect of heterocyclic amines on the bacteriostatic activity of benzamide derivatives, which can be obtained by benzoylation of the heterocyclic amines. In the present paper, 4-amino-2,6-dimethoxypyrimidine and its related compounds, the former being constitutional part in the molecule of sulfadimethoxine used as one of long-acting sulfa drugs, were selected for this purpose as heterocyclic amines.

Reaction of aminopyrimidines with benzoyl chlorides substituted with methyl, methoxy, chloro, bromo, and nitro groups in dry pyridine gave the corresponding benzamidopyrimidines in good yields. Although benzoylation with salicyloyl chloride failed, the desired salicylamidopyrimidines were obtained from reaction between aminopyrimidines and phenyl salicylate in α -methylnaphthalene at 200~210°. The compounds were converted into 3,5-dibromosalicylamidopyrimidines by bromination with bromine. Chemical proof of the structures of the compounds was obtained by decomposition with sulfuric acid to give 3,5-dibromosalicylic acid. Aminopyrimidines also reacted with

*¹ Part II : This Bulletin, 13, 568 (1965).

*² Takadaminami-cho, Toshima-ku, Tokyo (新田義博, 宮本俊雄, 根橋敏行, 米田文郎).

1) R.G. Taborsky, *et al.* : J. Pharm. Sci., 48, 503 (1959).

2) R.G. Taborsky, R.J. Starkey : *Ibid.*, 51, 1152 (1962).

3) *Idem* : *Ibid.*, 52, 542 (1963).

4) H. Lemaire, C.H. Schramm, A. Cahn : *Ibid.*, 50, 831 (1961).

5) R. Kimura, *et al.* : This Bulletin, 10, 1226 (1962).

6) H. Schönenberger, *et al.* : Arzneimittel-Forsch., 12, 1162 (1962).

7) R. Gönnert, E. Schraufstätter : *Ibid.*, 10, 881 (1960).

8) a) U.S. Pat. 2870145 (1959); U.S. Pat. 2870146 (1959) (C.A., 53, 10264). b) L. Vargha, *et al.* : Biochem. Pharm., 11, 639 (1962). c) E. Kasztreiner, J. Borsy, L. Vargha : *Ibid.*, 11, 667 (1962).

TABLE I. 4-Benzamido-2,6-dialkoxypyrimidines

No.	R	R'	m.p. (°C)	Crystn. solvent	Formula	Analysis (%)					
						Calcd.			Found		
						C	H	N	C	H	N
I	CH ₃	H	139	MeOH-H ₂ O	C ₁₃ H ₁₃ O ₃ N ₃	60.22	5.05	16.21	60.31	5.15	16.05
II	"	2-Cl	126	EtOH-H ₂ O	C ₁₃ H ₁₂ O ₃ N ₃ Cl	53.16	4.12	14.38	53.35	4.14	14.20
III	"	4-Cl	154~155	EtOH	C ₁₃ H ₁₂ O ₃ N ₃ Cl	53.16	4.12	14.38	53.47	4.15	14.25
IV	"	2,4-Cl ₂	144~145	MeOH-H ₂ O	C ₁₃ H ₁₁ O ₃ N ₃ Cl ₂	47.58	3.38	12.81	47.63	3.43	12.68
V	"	3,4-Cl ₂	158	"	C ₁₃ H ₁₁ O ₃ N ₃ Cl ₂	47.58	3.38	12.81	47.45	3.34	12.89
VI	"	3-Br·H ₂ O	93	EtOH-H ₂ O	C ₁₃ H ₁₄ O ₄ N ₃ Br	43.84	3.97	11.80	43.83	3.73	11.52
VII	"	4-Br	150~151	MeOH-H ₂ O	C ₁₃ H ₁₂ O ₃ N ₃ Br	46.17	3.58	12.43	46.27	3.63	12.69
VIII	"	2-CH ₃	127~128	EtOH-H ₂ O	C ₁₄ H ₁₅ O ₃ N ₃	61.53	5.53	15.38	61.92	5.74	15.43
IX	"	3-CH ₃	122	"	C ₁₄ H ₁₅ O ₃ N ₃	61.53	5.53	15.38	61.34	5.55	15.44
X	"	4-CH ₃	137	MeOH-H ₂ O	C ₁₄ H ₁₅ O ₃ N ₃	61.53	5.53	15.38	61.34	5.30	15.59
XI	"	3-NO ₂	185~186	MeOH	C ₁₃ H ₁₂ O ₃ N ₄	51.31	3.98	18.42	—	—	18.27
XII	"	4-NO ₂	184	"	C ₁₃ H ₁₂ O ₃ N ₄	51.31	3.98	18.42	51.15	4.16	18.27
XIII	"	4-OCH ₃	142~143	MeOH-H ₂ O	C ₁₄ H ₁₅ O ₄ N ₃	58.12	5.23	14.53	57.93	5.07	14.25
XIV	"	3,4-(OCH ₃) ₂	167	MeOH	C ₁₅ H ₁₇ O ₅ N ₃	56.42	5.37	13.16	56.61	5.42	13.15
XV	"	3,4,5-(OCH ₃) ₃	107	MeOH-H ₂ O	C ₁₆ H ₁₉ O ₆ N ₃	55.01	5.48	12.03	54.94	5.64	12.19
XVI	"	3-NO ₂ -4-Cl	160~162	MeOH	C ₁₃ H ₁₁ O ₃ N ₄ Cl	46.10	3.27	16.54	46.04	3.47	16.69
XVII	"	2-OH	204	MeOH-H ₂ O	C ₁₃ H ₁₃ O ₄ N ₃	56.72	4.76	15.27	57.04	4.77	15.53
XVIII	C ₂ H ₅	H·H ₂ O	76	EtOH-H ₂ O	C ₁₅ H ₁₉ O ₄ N ₃	59.00	6.27	13.76	59.47	6.61	13.85
XIX	"	2-Cl	98	"	C ₁₅ H ₁₈ O ₄ N ₃ Cl	55.99	5.01	13.06	56.15	4.82	12.65
XX	"	4-Cl	120	"	C ₁₅ H ₁₈ O ₄ N ₃ Cl	55.99	5.01	13.06	56.33	4.88	12.57
XXI	"	2,4-Cl ₂	115~116	"	C ₁₅ H ₁₇ O ₄ N ₃ Cl ₂	50.58	4.24	11.80	50.41	4.19	11.78
XXII	"	3,4-Cl ₂	140~141	"	C ₁₅ H ₁₇ O ₄ N ₃ Cl ₂	50.58	4.24	11.80	50.74	4.48	11.71
XXIII	"	4-Br	128~129	MeOH-H ₂ O	C ₁₆ H ₁₉ O ₅ N ₃ Br	49.20	4.40	11.47	49.23	4.36	11.25
XXIV	"	2-CH ₃	107~108	EtOH-H ₂ O	C ₁₆ H ₁₉ O ₅ N ₃	63.77	6.36	13.95	63.61	6.54	13.87
XXV	"	3-CH ₃	95~96	"	C ₁₆ H ₁₉ O ₅ N ₃	63.77	6.36	13.95	63.74	6.50	13.93
XXVI	"	4-CH ₃	128	MeOH-H ₂ O	C ₁₆ H ₁₉ O ₅ N ₃	63.77	6.36	13.95	63.88	6.45	14.05
XXVII	"	3-NO ₂	113~114	"	C ₁₆ H ₁₈ O ₅ N ₄	54.21	4.85	16.86	54.49	4.62	16.68
XXVIII	"	4-NO ₂	159	MeOH	C ₁₆ H ₁₈ O ₅ N ₄	54.21	4.85	16.86	54.43	4.94	17.00
XXIX	"	4-OCH ₃	116	MeOH-H ₂ O	C ₁₆ H ₁₉ O ₆ N ₃	60.55	6.04	13.24	60.87	5.67	12.96
XXX	"	3-NO ₂ -4-Cl	151	MeOH	C ₁₅ H ₁₇ O ₅ N ₄ Cl	49.12	4.12	15.28	49.11	3.98	14.99

XXXI	C ₂ H ₅	2-OH	178	MeOH-H ₂ O	C ₁₅ H ₁₇ O ₄ N ₃	59.39	5.65	13.86	59.68	5.69	13.70
XXXII	"	2-OH-3,5-Br ₂	166	EtOH-H ₂ O	C ₁₅ H ₁₅ O ₄ N ₃ Br ₂	39.07	3.28	9.11	39.60	3.35	8.80
XXXIII	C ₃ H ₇	4-Cl	84	MeOH-H ₂ O	C ₁₇ H ₂₀ O ₃ N ₃ Cl	58.37	5.76	12.01	58.50	5.42	11.93
XXXIV	"	3,4-Cl ₂	86	"	C ₁₇ H ₁₈ O ₃ N ₃ Cl ₂	53.14	5.06	10.94	53.15	4.98	11.00
XXXV	"	4-Br	79	"	C ₁₇ H ₁₈ O ₃ N ₃ Br	51.79	5.11	10.66	51.76	5.42	10.47
XXXVI	iso-C ₃ H ₇	4-Cl	102	"	C ₁₇ H ₂₀ O ₃ N ₃ Cl	58.37	5.76	12.01	58.34	5.67	11.88
XXXVII	"	2,4-Cl ₂	120	"	C ₁₇ H ₁₈ O ₃ N ₃ Cl ₂	53.14	4.98	10.94	53.41	5.21	11.20
XXXVIII	"	3,4-Cl ₂	111	"	C ₁₇ H ₁₈ O ₃ N ₃ Cl ₂	53.14	4.98	10.94	53.17	5.01	10.75
XXXIX	"	4-Br	145	"	C ₁₇ H ₂₀ O ₃ N ₃ Br	51.79	5.11	10.66	52.03	5.24	10.44
XL	"	4-CH ₃	96	"	C ₁₈ H ₂₃ O ₃ N ₃	65.63	7.04	12.76	65.92	7.19	12.64
XLI	C ₄ H ₉	4-Cl	71	"	C ₁₉ H ₂₄ O ₃ N ₃ Cl	60.39	6.40	11.12	60.09	6.47	11.20
XLII	"	3,4-Cl ₂	89	"	C ₁₉ H ₂₃ O ₃ N ₃ Cl ₂	55.35	5.62	10.19	55.53	5.72	9.89
XLIII	"	4-Br	80	"	C ₁₉ H ₂₄ O ₃ N ₃ Br	54.04	5.73	9.95	54.18	5.89	9.73
XLIV	iso-C ₄ H ₉	4-Cl	114	"	C ₁₉ H ₂₄ O ₃ N ₃ Cl	60.39	6.40	11.12	60.00	6.45	11.01
XLV	"	3,4-Cl ₂	122	"	C ₁₉ H ₂₃ O ₃ N ₃ Cl	55.35	5.62	10.19	55.51	5.81	10.18
XLVI	"	4-Br	101	"	C ₁₉ H ₂₄ O ₃ N ₃ Br	54.04	5.73	9.95	54.16	5.85	9.57

TABLE II. Nicotinamido- and Isonicotinamidopyrimidines

No.	R	X'	m.p. (°C)	Crystn. solvent	Formula	Analysis (%)					
						Calcd.			Found		
						C	H	N	C	H	N
XLVII	CH ₃		176	EtOH-H ₂ O	C ₁₂ H ₁₃ O ₃ N ₄	55.38	4.65	21.53	55.63	4.75	21.54
XLVIII	C ₂ H ₅	"	175	"	C ₁₄ H ₁₆ O ₃ N ₄	58.32	5.59	19.44	58.65	5.41	19.24
XLIX	C ₃ H ₇	"	104	MeOH-H ₂ O	C ₁₆ H ₂₀ O ₃ N ₄	60.74	6.37	17.71	60.91	6.21	17.70
L	iso-C ₃ H ₇	"	130	"	C ₁₆ H ₂₀ O ₃ N ₄	60.74	6.37	17.71	60.66	6.17	17.80
LI	iso-C ₄ H ₉	"	119	iso-Pr ₂ O	C ₁₈ H ₂₄ O ₃ N ₄	62.77	7.02	16.27	62.48	6.97	16.34
LII	CH ₃		213	MeOH-H ₂ O	C ₁₂ H ₁₃ O ₃ N ₄	55.38	4.65	21.53	55.24	4.73	21.52
LIII	C ₂ H ₅	"	164	"	C ₁₄ H ₁₆ O ₃ N ₄	58.32	5.59	19.44	58.29	5.47	19.65
LIV	C ₃ H ₇	"	116	"	C ₁₆ H ₂₀ O ₃ N ₄	60.74	6.37	17.71	60.84	6.24	17.74
LV	iso-C ₃ H ₇	" · H ₂ O	99	"	C ₁₆ H ₂₂ O ₄ N ₄	57.47	6.63	16.76	57.76	6.68	17.01
LVI	iso-C ₄ H ₉		152	"	C ₁₈ H ₂₄ O ₃ N ₄	62.77	7.02	16.27	62.91	6.77	16.60

TABLE III. Antibacterial Activity of 4-Benzamido-2,6-dialkoxypyrimidines

No.	R	R'	Minimum inhibitory concentration (mcg./ml.)				
			<i>Staph.</i> <i>aureus</i> 209P	<i>Shig.</i> <i>flexneri</i> 2a	<i>Pr.</i> <i>vulgaris</i> P-1	<i>M. tuber-</i> <i>culosis</i> H37Rv	<i>T. menta-</i> <i>grophytes</i> T-1
I	CH ₃	H	>80	—	—	>5	—
II	"	2-Cl	20	20	—	>10	—
III	"	4-Cl	5~10	5	—	>5	—
IV	"	2,4-Cl ₂	10	>20	—	>20	—
V	"	3,4-Cl ₂	>1.25	>1.25	>1.25	>1.25	>1.25
VI	"	3-Br·H ₂ O	20	>10	—	>20	—
VII	"	4-Br	5	>5	>5	>10	>10
VIII	"	2-CH ₃	20	>20	—	>20	>20
IX	"	3-CH ₃	10~20	>20	—	>20	>20
X	"	4-CH ₃	>20	>20	—	>20	>20
XI	"	3-NO ₂	20	>20	—	>5	—
XII	"	4-NO ₂	>80	—	—	>5	—
XIII	"	4-OCH ₃	>20	>20	—	>20	—
XIV	"	3,4-(OCH ₃) ₂	>80	>80	—	>10	—
XV	"	3,4,5-(OCH ₃) ₃	>20	20	—	>20	—
XVI	"	3-NO ₂ -4-Cl	>2.5	>2.5	—	>1.25	—
XVII	"	2-OH	>20	>20	>20	—	>20
XIX	C ₂ H ₅	2-Cl	10	10	>20	—	>20
XX	"	4-Cl	2.5~5	>5	>5	>5	>5
XXI	"	2,4-Cl ₂	2.5~5	>5	>5	—	>5
XXII	"	3,4-Cl ₂	1.25	>2.5	>2.5	—	>2.5
XXIII	"	4-Br	2.5~5	>5	>5	>2.5	>5
XXIV	"	2-CH ₃	10~20	>10	—	>10	>10
XXV	"	3-CH ₃	10	>20	—	>20	>20
XXVI	"	4-CH ₃	5	>5	—	>5	>20
XXVII	"	3-NO ₂	>5	>5	>5	—	>5
XXVIII	"	4-NO ₂	>80	—	>10	—	—
XXIX	"	4-OCH ₃	10	20	—	>20	—
XXX	"	3-NO ₂ -4-Cl	>5	>5	—	>5	—
XXXI	"	2-OH	>2.5	>2.5	>2.5	—	>10
XXXII	"	2-OH-3,5-Br ₂	2.5	>20	—	—	5~10
XXXIII	C ₃ H ₇	4-Cl	0.6~1.25	>2.5	>2.5	>2.5	>5
XXXIV	"	3,4-Cl ₂	>2.5	>2.5	>2.5	>5	>2.5
XXXV	"	4-Br	>2.5	>2.5	>2.5	>5	>2.5
XXXVI	iso-C ₃ H ₇	4-Cl	>5	>5	>5	>2.5	>5
XXXVII	"	2,4-Cl ₂	>2.5	>2.5	>2.5	>5	>2.5
XXXVIII	"	3,4-Cl ₂	>2.5	>2.5	>2.5	>5	>2.5
XXXIX	"	4-Br	>2.5	>2.5	>2.5	>5	>2.5
XLI	C ₄ H ₉	4-Cl	>2.5	>2.5	>2.5	>5	>2.5
XLII	"	3,4-Cl ₂	>2.5	>2.5	>2.5	>5	>2.5
XLIII	"	4-Br	>2.5	>2.5	>2.5	>5	>2.5
XLIV	iso-C ₄ H ₉	4-Cl	>2.5	>2.5	>2.5	>5	>2.5
XLV	"	3,4-Cl ₂	>2.5	>2.5	>2.5	>5	>2.5
XLVI	"	4-Br	>2.5	>2.5	>2.5	>5	>2.5
	Sulfadimethoxine		1.25~2.5	2.5	10~20	—	—
	Isoniazid		—	—	—	0.1~0.2	—
	Bithionol		—	—	—	—	0.5

TABLE V. Antibacterial Activity of Nicotinamido- and Isonicotinamidopyrimidines

No.	R	X	Minimum inhibitory concentration (mcg./ml.)				
			<i>Staph. aureus</i> 209P	<i>Shig. flexneri</i> 2a	<i>Pr. vulgaris</i> P-1	<i>M. tuberculosis</i> H37Rv	<i>T. mentagrophytes</i> T-1
XLVII	CH ₃		80	>80	—	>5	—
XLVIII	C ₂ H ₅	"	>20	>20	>20	—	>20
XLIX	C ₃ H ₇	"	>10	>10	>10	>20	>20
L	iso-C ₃ H ₇	"	>10	>10	>10	>20	>20
LI	iso-C ₄ H ₉	"	>5	>5	>5	>5	>5
LII	CH ₃		80	>80	—	>10	—
LIII	C ₂ H ₅	"	>10	>10	>20	—	>20
LIV	C ₃ H ₇	"	>10	>10	>10	>20	>20
LV	iso-C ₃ H ₇	"	>10	>10	>10	>20	>20
LVI	iso-C ₄ H ₉	"	>5	>5	>5	>5	>5

nicotinoyl chloride or isonicotinoyl chloride to give the corresponding nicotinamido- or isonicotinamidopyrimidines.

Aminopyrimidines, such as 2,6-dimethoxy-, 2,6-diethoxy-, 2,6-dipropoxy-, 2,6-diisopropoxy-, 2,6-dibutoxy-, and 2,6-diisobutoxy-4-aminopyrimidines, used as starting materials in this study, were obtained by alkoxylation of 2,6-dichloro-4-aminopyrimidine with the corresponding sodium alkoxide. New benzamido-, nicotinamido-, and isonicotinamidopyrimidine derivatives were also prepared and shown in Table I and II.

The antibacterial activities of these new compounds against *Staphylococcus aureus* 209P, *Shigella flexneri* 2a, *Proteus vulgaris* P-1, *Mycobacterium tuberculosis* H37Rv, and *Trichophyton mentagrophytes* T-1 were tested *in vitro*. The results were listed in Table III and IV. Throughout this paper, the figures given under activity refer to the minimum inhibitory concentration.

As can be seen from these data, many of these compounds clearly show activity against *Staphylococcus aureus* 209P. Some of them have slight activity against *Shigella flexneri* 2a and all of them are inactive against *Proteus vulgaris* P-1, *Mycobacterium tuberculosis* H37Rv and *Trichophyton mentagrophytes* T-1. In the course of the screening program in *Staphylococcus aureus* 209P, the relationship of bacteriostatic activity and the chemical structure is summarized as far as have been found out.

1) Introduction of halogens into the phenyl part of benzamidopyrimidines, increased the activities, especially that of chlorine at the 4- or 3,4-positions. Generally speaking, 2,6-diethoxypyrimidine was the most effective as pyrimidine moiety. But, in the case of 4-chlorobenzamide series, the maximum activity was reached by the amide of 2,6-dipropoxypyrimidine, not by that of 2,6-diethoxypyrimidine. Pyrimidine moiety having alkoxy groups higher than propoxy decreased the antibacterial activities along with solubility.

2) According to the publication^{5,6)} on the relationship of bacteriostatic activity with the chemical structure of benzanilides, only salicyloyl derivatives which have a hydroxy group at the *ortho* position show drastic activities and others have little or no effect on activities. Nevertheless, salicyloyl derivatives in this series are not always more effective than others.

3) Benzamidopyrimidines, which were effective for *Staphylococcus aureus* 209P, also showed activity in the preliminary tests against sulfanilamide resistant strain and the detailed data will be reported elsewhere. Accordingly, it is considered that their mode

of antibacterial action differs from that of sulfanilamides, although these compounds resemble structurally sulfanilamides.

Experimental*3

4-(4-Chlorobenzamido)-2,6-dimethoxypyrimidine (III)—To a solution of 1.55 g. of 4-amino-2,6-dimethoxypyrimidine in 5 ml. of dry pyridine was added dropwise with stirring and cooling an equimolar quantity of 4-chlorobenzoyl chloride. Reaction occurred in a moment when the solution was stirred vigorously at room temperature. The reaction mixture was cooled and poured into H₂O. The precipitate was collected by filtration, washed well with H₂O and dried. The crude product was recrystallized from EtOH to give 2.7 g. of colorless needles, m.p. 154~155°.

4-(2-Hydroxybenzamido)-2,6-diethoxypyrimidine (XXXI)—A mixture of 3.66 g. of 4-amino-2,6-diethoxypyrimidine, 4.8 g. of phenylsalicylate and 8 ml. of α -methylnaphthalene was heated at 200~210° for 1.5 hr. α -Methylnaphthalene and PhOH were removed by distillation under reduced pressure. The product was washed with ligroin and dried. After recrystallization from MeOH-H₂O, the pure compound was obtained as colorless needles, m.p. 178°. Yield, 1.8 g.

4-(2-Hydroxy-3,5-dibromobenzamido)-2,6-diethoxypyrimidine (XXXII)—A solution of 3.03 g. of 4-(2-hydroxybenzamido)-2,6-diethoxypyrimidine in 168 ml. of tetrahydrofuran and 112 ml. of AcOH was stirred at 15~25° during the dropwise addition of 3.20 g. of Br₂ in 10 ml. of AcOH. After the addition, stirring was continued for 5 hr. Reaction mixture was poured into 500 ml. of ice-water with stirring to give precipitate, which was collected and washed with H₂O. The precipitate was dissolved in hot AcOH and the solution was cooled to crystallize compound A, m.p. 235°, and the filtrate was diluted with H₂O to give precipitate, which crystallized from AcOH-H₂O as colorless needles (compound B), m.p. 166°. Compound A and B were refluxed for 15 min. in conc. H₂SO₄ to give 3,5-dibromosalicylic acid respectively. Accordingly, it was established that compound A was 4-(2-hydroxy-3,5-dibromobenzamido)-5-bromo-2,6-diethoxypyrimidine and compound B was 4-(2-hydroxy-3,5-dibromobenzamido)-2,6-diethoxypyrimidine.

4-Nicotinamido-2,6-dimethoxypyrimidine (XLVII)—To a solution of 1.55 g. of 4-amino-2,6-dimethoxypyrimidine in 5 ml. of dry pyridine was added 1.78 g. of nicotinoyl chloride hydrochloride with stirring. The solution was warmed at 60° for 5 min. After cooling, the reaction mixture was poured into 50 ml. of H₂O and the precipitate was collected, washed with H₂O and dried. Recrystallization from MeOH-H₂O gave 2.3 g. of pure 4-nicotinamido-2,6-dimethoxypyrimidine as fine needles, m.p. 176°.

Antibacterial Activity—Test organism: *Staphylococcus aureus* 209P, *Shigella flexneri* 2a, *Proteus vulgaris* P-1, *Mycobacterium tuberculosis* H37Rv and *Trichophyton mentagrophytes* T-1.

Culture media: Casamino acids medium was used for *Staph. aureus* 209P, *Shig. flexneri* 2a and *Pr. vulgaris* P-1. Kirchner's medium was used for *M. tuberculosis* H37Rv. Sabouraud's medium (1% glucose) was used for *T. mentagrophytes* T-1. The test compounds were dissolved in the medium above mentioned and diluted by the two fold serial dilution method and inoculated with the organisms.

Incubation: Casamino acids medium was incubated at 37° for 24 hr., Kirchner's medium was incubated at 37° for 21 days and Sabouraud's medium was incubated at 27° for 7 days. Then the minimum inhibitory concentration of test samples was estimated by comparison with sulfadimethoxine, isoniazid and bithionol.

We thank Dr. T. Akiba, Director of these laboratories, for his encouragement, and also to the members of analysis room of these laboratories for elemental analyses.

Summary

The preparation of a number of 4-benzamido-, 4-nicotinamido-, and 4-isonicotinamido-2,6-dialkoxypyrimidines was described. Some of these new compounds showed strong antibacterial activities *in vitro*, and the structure-activity relationship was discussed.

(Received December 31, 1964)

*3. All melting points were not corrected.