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Syntheses of 3-Substituted 1-Methyl-6-phenylpyrimido[5,4-*e*]-1,2,4-triazine-5,7(1*H*,6*H*)-diones (6-Phenyl Analogs of Toxoflavin) and Their 4-Oxides, and Evaluation of Antimicrobial Activity of Toxoflavins and Their Analogs

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6-Phenyl analogs of toxoflavin {1-methyl-6-phenylpyrimido[5,4-*e*]-1,2,4-triazine-5,7(1*H*,6*H*)-diones} (7a—f) and their 4-oxides (8a—f) were synthesized by nitrosative or nitrative cyclization of the aldehyde hydrazones (6a—f) of 6-(1-methylhydrazino)-3-phenyluracil (5). Both sets of compounds, 7a—f and 8a—f, gave the corresponding 1-demethyl derivatives (10a—f) upon treatment with nucleophiles such as dimethylformamide (DMF) and acetic acid under heating. The activities of toxoflavins (1a—e), toxoflavin 4-oxides (3a—e) and their 6-phenyl analogs (7a—f and 8a—f) against a variety of bacterial and fungal strains were examined. Most of the compounds showed strong inhibitory activities against gram-positive bacteria. Among the compounds, 1c, 1d, 1e, and 3c exhibited the strongest inhibitions of *Micrococcus lutea* (0.5 μg/ml minimal growth-inhibitory concentration) and *Staphylococcus aureus 4R* (1 μg/ml), as well as *Bacillus subtilis* and *Staphylococcus aureus* (1—2 μg/ml). Most of the compounds had strong antifungal activity (2—100 μg/ml minimal growth-inhibitory concentration) against *Candida albicans* and *Saccharomyces cerevisiae*.

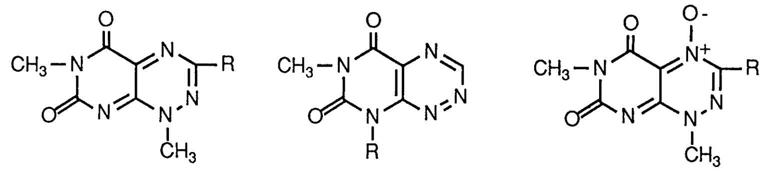
Keywords toxoflavin; toxoflavin 4-oxide; 6-phenyl analog; antibacterial activity; antifungal activity

Since the first structural report[1)] on azapteridine by Pfleiderer and Schündehütte in 1958, there has been considerable interest in synthesis[2)] and biological evaluation[3)] of the azapteridines. Several 7-azapteridines (pyrimido[5,4-*e*]-1,2,4-triazines) such as toxoflavin (xanthothricin) (**1a**),[4)] 1-demethyltoxoflavin (reumycin) (**2a**),[5)] and fervenulin (planomycin) (**2b**)[6)] have been isolated from natural sources (Chart 1). We have accomplished convenient syntheses of toxoflavin,[7)] 1-demethyltoxoflavin,[7a,8)] fervenulin[7c,d,8a,9)] and their analogs.[10)]

In connection with our studies on the syntheses and biological evaluation of toxoflavin and its derivatives and on chemical reactivities such as the transmethylation of toxoflavins into nucleophiles,[8a,9a,10a,11)] we wish to report here an efficient general method for the preparation of 6-phenyl analogs of toxoflavin {1-methyl-6-phenylpyrimido[5,4-*e*]-1,2,4-triazine-5,7(1*H*,6*H*)-diones} (**7a**—**f**) and their 4-oxides (**8a**—**f**) from the aldehyde hydrazones (**6a**—**f**) of 6-(1-methylhydrazino)-3-phenyluracil (**5**) by nitrosative or nitrative cyclization. In addition, the activities of toxoflavins (**1a**—**e**), their N-oxides (**3a**—**e**), and their analogs (**7a**—**f** and **8a**—**f**) prepared here against a variety of bacterial and fungal strain were examined.

Chemistry 6-Chloro-3-phenyluracil (**4**), which served as the starting material, was prepared by chlorination of 3-phenylbarbituric acid with phosphoryl chloride according to the reported method.[12)] The chlorine atom of **4** was replaced by using methylhydrazine in boiling ethanol to give 6-(1-methylhydrazino)-3-phenyluracil (**5**). The key intermediates, the aldehyde hydrazones (**6a**—**f**) of **5**, were prepared by trantment of **5** with appropriate aryl aldehydes in ethanol at room temperature (Chart 2 and Table I).

The hydrazones (**6a**—**f**) thus obtained were treated with excess sodium nitrite in acetic acid at 5—7 °C to afford a mixture of the corresponding toxoflavin-type compounds (**7a**—**f**) and their 4-oxides (**8a**—**f**), respectively (method A). The mixture was subjected to silica gel column chromatography and eluted with benzene–EtOAc (9 : 1) to isolate **7a**—**f** and **8a**—**f** individually. The yields of **7a**—**f** and **8a**—**f** are given in column A of Tables II and III. The structures of **7a**—**f** and **8a**—**f** were assigned on the basis of elemental analysis, satisfactory spectral data, and the expected mechanism of the reaction. Compounds **8a**—**f** showed remarkable M-16 ions with strong parent ions in their mass spectra. The above nitrosative cyclization of **6** in the presence of DEAD (diethyl azodicarboxylate) as a dehy-



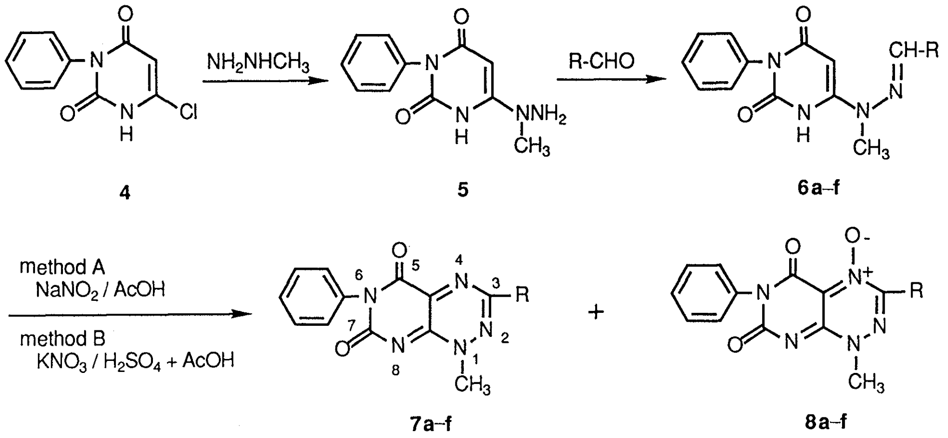
1a : toxoflavin
1b–**e**

2a : R = H: reumycin
2b : R = CH_3: fervenulin

3a–**e**

1a, 3a : R = H
1b, 3b : R = CH_3
1c, 3c : R = Ph
1d, 3d : R = 4-Cl-C_6H_4
1e, 3e : R = 3,4-Cl_2-C_6H_3

Chart 1



6a, 7a, 8a : R = Ph
6c, 7c, 8c : R = 3-Cl-C_6H_4
6e, 7e, 8e : R = 3,4-Cl_2-C_6H_3
6b, 7b, 8b : R = 4-CH_3-C_6H_4
6d, 7d, 8d : R = 4-Cl-C_6H_4
6f, 7f, 8f : R = 4-Br-C_6H_4

Chart 2

Table I. Physical Data for 6-(2-Benzylidene-1-methylhydrazino)-3-phenyluracils **6a**—**f**

Compd. No.	Yield (%)	mp (°C)[a]	IR (KBr) ν_{max} cm^{-1} C=O	NH	Formula	Analysis (%) Calcd C	H	N	Found C	H	N
6a	90	261—263	1620, 1700	3380	$C_{18}H_{16}N_4O_2$	67.48	5.03	17.48	67.59	5.00	17.22
6b	95	240—242	1620, 1695	3370	$C_{19}H_{18}N_4O_2$	68.24	5.42	16.75	68.31	5.63	16.48
6c	93	223—225	1625, 1700	3380	$C_{18}H_{15}ClN_4O_2$	60.93	4.26	15.79	60.81	4.44	15.88
6d	94	229—231	1620, 1700	3380	$C_{18}H_{15}ClN_4O_2$	60.93	4.26	15.79	61.08	4.33	15.55
6e	94	238—240	1615, 1695	3380	$C_{18}H_{14}Cl_2N_4O_2$	55.54	3.62	14.39	55.47	3.87	14.47
6f	93	265—267	1620, 1700	3400	$C_{18}H_{15}BrN_4O_2$	54.15	3.78	14.03	54.36	3.99	14.01

a) All compounds were recrystallized from EtOH and were obtained as colorless needles.

Table II. Physical Data for 1-Methyl-6-phenylpyrimido[5,4-*e*]-1,2,4-triazine-5,7-(1*H*,6*H*)-diones **7a**—**f**

Compd. No.	Yield (%)[a] A	B	mp (°C)[b]	IR (KBr) ν_{max} cm^{-1} (C=O)	Formula	Analysis (%) Calcd C	H	N	Found C	H	N
7a	16	17	245 (dec.) 323—326	1660, 1720	$C_{18}H_{13}N_5O_2$	65.25	3.96	21.14	65.36	4.08	21.07
7b	63 (24)	34	253 (dec.) >330	1665, 1720	$C_{19}H_{15}N_5O_2$	66.07	4.38	20.28	65.98	4.44	20.22
7c	60 (18)	37	233—235	1670, 1725	$C_{18}H_{12}ClN_5O_2$	59.11	3.31	19.15	58.89	3.23	19.24
7d	36	16	235 (dec.) 293—295	1670, 1720	$C_{18}H_{12}ClN_5O_2$	59.11	3.31	19.15	59.04	3.31	19.06
7e	41	16	235—237	1670, 1720	$C_{18}H_{11}Cl_2N_5O_2$	54.02	2.77	17.50	54.06	2.81	17.47
7f	40	19	244 (dec.) 320—322	1660, 1720	$C_{18}H_{12}BrN_5O_2$	52.70	2.95	17.07	52.71	2.81	16.94

a) The yields in column A were obtained by the nitrosative cyclization of **6a**—**f** and the yields given in parentheses were obtained in the presence of DEAD. The yields in column B were obtained by the nitrative cyclization of **6a**—**f**. *b*) All products were recrystallized from 40% aqueous dioxane and were obtained as orange needles.

drogenative oxidizing agent afforded the 4-oxide-type compound (**8**) exclusively. For example, in the case of dehydrogenative cyclization of **6b** and **6c**, the yields of the 4-oxides (**8b** and **8c**) in the presence of DEAE were both increased by *ca.* 20% as shown in column A of Table III.

The direct cyclizations of 6-(2-benzylidene-1-methylhydrazino)-3-phenyluracils (**6a**—**f**) were also achieved by nitration of **6a**—**f** by the reported method.[9c)] Thus, stirring of **6a**—**f** with an excess of potassium nitrate in acetic acid containing sulfuric acid at 95 °C for 3—4 h and cooling of the reaction solution gave the corresponding 4-oxides (**8a**—**f**) as the first precipitate in an almost pure state. After collection of the first crop, the filtrate was diluted with diethyl ether and allowed to stand overnight in a refrigerator to afford the second crop, which was a mixture of **7a**—**f** and **8a**—**f** (method B). The combined yields for **7a**—**f** and **8a**—**f** are shown in column B of Tables II and III.

Table III. Physical Data for 1-Methyl-6-phenylpyrimido[5,4-*e*]-1,2,4-triazine-5,7(1*H*,6*H*)-dione 4-Oxides **8a**—**f**

Compd. No.	Yield (%)[a] A	Yield (%)[a] B	mp (°C)[b]	Appearance	IR (KBr) ν_{max} cm^{-1} (C=O)	Formula	Analysis (%) Calcd C	Calcd H	Calcd N	Found C	Found H	Found N
8a	79	64	177—179	Orange prisms	1660, 1705	$C_{18}H_{13}N_5O_3 \cdot 2/3H_2O$	60.18	4.02	19.49	60.09	3.95	19.32
8b	22 (42)	30	235 (dec.) >330	Orange needles	1660, 1710	$C_{19}H_{15}N_5O_3$	63.15	4.18	19.38	62.88	4.15	19.11
8c	31 (51)	25	201—203	Yellow powder	1665, 1705	$C_{18}H_{12}ClN_5O_3$	56.62	3.16	18.34	56.59	3.10	18.48
8d	51	55	208—210	Yellow needles	1665, 1700	$C_{18}H_{12}ClN_5O_3$	56.62	3.16	18.34	56.62	3.09	18.25
8e	56	53	205—207	Yellow needles	1660, 1700	$C_{18}H_{11}Cl_2N_5O_3$	51.94	2.66	16.82	51.83	2.59	16.69
8f	50	59	209—211	Yellow needles	1660, 1705	$C_{18}H_{12}BrN_5O_3$	50.72	2.83	16.43	50.63	2.74	16.35

a) The yields in column A were obtained by the nitrosative cyclization of **6a**—**f** and the yields given in parentheses were obtained in the presence of DEAD. The yields in column B were obtained by the nitrative cyclization of **6a**—**f**. *b*) All products were recrystallized from 40% aqueous dioxane.

Table IV. Physical Data for 1-Methyl-5,7-dioxo-6-phenyl-1,4,5,6,7,8-hexahydropyrimido[5,4-*e*]-1,2,4-triazines **9a**—**f**

Compd. No.	Yield (%)	mp (°C)[a]	IR (KBr) ν_{max} cm^{-1} C=O	IR (KBr) ν_{max} cm^{-1} NH	Formula	*m*/*z*
9a	90	245 (dec.) 313—315	1610, 1700	3070, 3280	$C_{18}H_{15}N_5O_2$	331 (M^+-2), 333 (M^+)
9b	93	253 (dec.) >330	1615, 1705	3090, 3300	$C_{19}H_{17}N_5O_2$	345 (M^+-2), 347 (M^+)
9c	98	198 (dec.) 225—227	1605, 1700	3100, 3270	$C_{18}H_{14}ClN_5O_2$	365 (M^+-2), 367 (M^+), 369 (M^++2)
9d	95	235 (dec.) 294—296	1610, 1700	3100, 3300	$C_{18}H_{14}ClN_5O_2$	365 (M^+-2), 367 (M^+), 369 (M^++2)
9e	91	210 (dec.) 222—224	1610, 1705	3100, 3290	$C_{18}H_{13}Cl_2N_5O_2$	399 (M^+-2), 401 (M^+), 403 (M^++2), 405 (M^++4)
9f	89	234 (dec.) 311—313	1610, 1700	3090, 3250	$C_{18}H_{14}BrN_5O_2$	409 (M^+-2), 411 (M^+), 413 (M^++2)

a) The melting points of all compounds (obtained as brown powder) were measured without recrystallization because these compounds were unstable in all hot solvents examined.

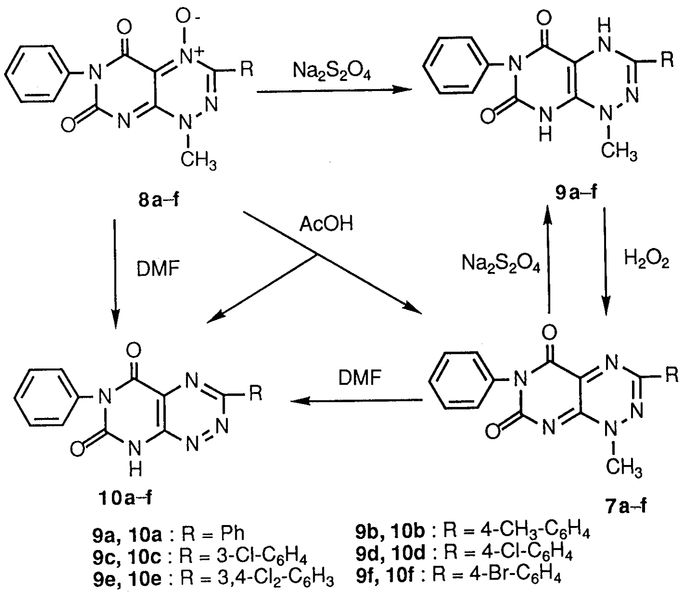


Chart 3

The assigned structures of **7a**—**f** and **8a**—**f** were confirmed by the formation of the 6-phenyl analogs of 4,8-dihydrotoxoflavins (**9a**—**f**), 1-demethyltoxoflavins (8-demethylfervenulins) (**10a**—**f**), and toxoflavins (**7a**—**f**) from toxoflavin 4-oxides (**8a**—**f**). Thus, treatment of both **7a**—**f** and **8a**—**f** with sodium dithionite in water at room temperature gave the corresponding 4,8-dihydrotoxoflavins (**9a**—**f**) (Chart 3 and Table IV). These 4,8-dihydrotoxoflavins (**9a**—**f**) were oxidized easily to toxoflavins (**7a**—**f**) in the presence of hydrogen peroxide or when they were allowed to stand in air for several days.

Previously, we have reported that toxoflavin (**1a**) and its 3-substituted derivatives readily underwent dementhylation with several nucleophiles such as *N*,*N*-dimethylformamide (DMF), dimethylacetamide, and acetic acid to give the corresponding 1-demethyltoxoflavin (**2a**) and its 3-substituted derivatives, while the nucleophiles themselves were methylated by the methyl group eliminated, and during the reactions novel radical species were observed.[8a,9a,10a,12] This transmethylation is of interest from a biological point of view. The transmethylation of 6-phenyl analogs of toxoflavin was carried out similarly. Namely, refluxing both **7a**—**f** and **8a**—**f** in DMF led to the 6-phenyl-1-demethyltoxoflavins (**10a**—**f**), whereas heating both **7a**—**f** and **8a**—**f** in acetic acid at 80 °C until the starting materials were no longer detectable led to mixtures of **7a**—**f** and **10a**—**f** in the ratio of 4 to 1, respectively (Tables V and VI).

Antimicrobial Activity and Discussion

It has been reported that toxoflavin (**1a**)[13] and fervenulin (**2b**)[14] showed wide antibacterial specta. However, no work has been done to data on the antimicrobial activities of their derivatives and analogs. Consequently, the activities of toxoflavins (**1a**—**e**), toxoflavin 4-oxides (**3a**—**e**) and their

Table V. Physical Data for 6-Phenylpyrimido[5,4-*e*]-1,2,4-triazine-5,7(6*H*,8*H*)-diones **10a**—**f**

Compd. No.	Yield (%)	mp (°C)[a]	IR (KBr) ν_{max} cm^{-1} C=O	NH	Formula	Analysis (%) Calcd C	H	N	Found C	H	N
10a	92	328—330	1690, 1735	3420	$C_{17}H_{11}N_5O_2$	64.35	3.49	22.07	64.38	3.33	22.26
10b	79	>330	1685, 1740	3430	$C_{18}H_{13}N_5O_2$	65.25	3.95	21.13	65.51	4.12	21.01
10c	75	302—304	1690, 1730	3460	$C_{17}H_{10}ClN_5O_2$	58.04	2.86	19.90	58.35	3.01	19.98
10d	87	318—320	1695, 1735	3420	$C_{17}H_{10}ClN_5O_2$	58.04	2.86	19.90	57.91	2.97	19.77
10e	87	321—323	1685, 1740	3430	$C_{17}H_9Cl_2N_5O_2$	52.87	2.34	18.13	52.67	2.12	18.40
10f	85	330—332	1690, 1735	3410	$C_{17}H_{10}BrN_5O_2$	51.53	2.54	17.67	51.66	2.39	17.42

a) All compounds were recrystallized from 40% aqueous dioxane and were obtained as dark yellow needles or powder.

Table VI. ^{1}H-NMR Spectral Data for Compounds **6a**—**f**, **7a**—**f**, **8a**—**f**, **9a**—**f**, and **10a**—**f**

Compd. No.	Chemical shifts (δ) run at 60 MHz [A, in DMSO-d_6; B, in CF_3CO_2D]
6a	A: 3.41 (3H, s, Me), 5.33 (1H, s, 5-H), 7.16—7.48 (8H, m, Ph × 2), 7.91—8.03 (2H, m, C-Ph), 7.95 (1H, s, N=CH), 10.73 (1H, s, NH)
6b	A: 2.34 (3H, s, C-Me), 3.39 (3H, s, N-Me), 5.31 (1H, s, 5-H), 7.11—7.48 (5H, m, Ph), 7.25 (2H, d, *J*=8.22 Hz, Ar-H), 7.89 (2H, d, *J*=8.22 Hz, Ar-H), 8.00 (1H, s, N=CH), 10.70 (1H, s, NH)
6c	A: 3.40 (3H, s, Me), 5.34 (1H, s, 5-H), 7.16—7.48 (7H, m, Ph and Ar-H), 7.84 (1H, m, Ar-H), 8.01 (1H, s, Ar-H), 8.24 (1H, s, N=CH), 10.98 (1H, s, NH)
6d	A: 3.41 (3H, s, Me), 5.33 (1H, s, 5-H), 7.15—7.52 (5H, m, Ph and Ar-H), 7.49 (2H, d, *J*=8.22 Hz, Ar-H), 8.02 (1H, s, N=CH), 8.06 (2H, d, *J*=8.22 Hz, Ar-H), 10.90 (1H, s, NH)
6e	A: 3.39 (3H, s, Me), 5.33 (1H, s, 5-H), 7.11—7.50 (5H, m, Ph), 7.65 (1H, d, *J*=8.22 Hz, Ar-H), 7.80 (1H, dd, *J*=1.74, 8.22 Hz, Ar-H), 7.99 (1H, s, N=CH), 8.40 (1H, d, *J*=1.74 Hz, Ar-H), 11.03 (1H, s, NH)
6f	A: 3.39 (3H, s, Me), 5.33 (1H, s, 5-H), 7.15—7.51 (5H, m, Ph), 7.61 (2H, d, *J*=8.22 Hz, Ar-H), 7.98 (2H, d, *J*=8.22 Hz, Ar-H), 8.00 (1H, s, N=CH), 10.85 (1H, s, NH)
7a	B: 4.77 (3H, s, Me), 7.30—7.73 (8H, m, Ph × 2), 8.40—8.56 (2H, m, C-Ph)
7b	B: 2.52 (3H, s, C-Me), 4.75 (3H, s, N-Me), 7.29—7.72 (5H, m, Ph), 7.48 (2H, d, *J*=8.22 Hz, Ar-H), 8.36 (2H, d, *J*=8.22 Hz, Ar-H)
7c	B: 4.73 (3H, s, Me), 7.29—7.75 (7H, m, Ph and Ar-H), 8.32—8.46 (2H, m, Ar-H)
7d	B: 4.73 (3H, s, Me), 7.22—7.71 (5H, m, Ph), 7.63 (2H, d, *J*=8.76 Hz, Ar-H), 8.42 (2H, d, *J*=8.76 Hz, Ar-H)
7e	B: 4.69 (3H, s, Me), 7.34—7.72 (5H, m, Ph), 7.71 (1H, d, *J*=8.22 Hz, Ar-H), 8.31 (1H, dd, *J*=1.80, 8.22 Hz, Ar-H), 8.54 (1H, d, *J*=1.80 Hz, Ar-H)
7f	B: 4.73 (3H, s, Me), 7.29—7.67 (5H, m, Ph), 7.80 (2H, d, *J*=8.82 Hz, Ar-H), 8.34 (2H, d, *J*=8.82 Hz, Ar-H)
8a	A: 3.95 (3H, s, Me), 7.10—7.75 (10H, m, Ph × 2); B: 4.44 (3H, s, Me), 7.11—8.00 (10H, m, Ph × 2)
8b	A: 2.39 (3H, s, C-Me), 3.94 (3H, s, N-Me), 7.02—7.73 (9H, m, Ph and Ar-H)
8c	A: 3.97 (3H, s, Me), 7.10—7.80 (9H, m, Ph and Ar-H)
8d	A: 3.96 (3H, s, Me), 7.11—7.91 (9H, m, Ph and Ar-H); B: 4.39 (3H, s, Me), 7.19—7.61 (5H, m, Ph), 7.56 (2H, d, *J*=8.82 Hz, Ar-H), 7.91 (2H, d, *J*=8.82 Hz, Ar-H)
8e	A: 3.97 (3H, s, Me), 7.10—7.55 (5H, m, Ph), 7.80—8.01 (3H, m, Ar-H)
8f	A: 3.95 (3H, s, Me), 7.10—7.61 (5H, m, Ph), 7.75 (4H, s, Ar-H)
9a	A[a]: 3.06 (3H, s, Me), 7.19—7.49 (10H, m, Ph × 2), 7.67 (1H, br s, N_4-H), 11.12 (1H, s, N_8-H); B: 3.56 (3H, s, Me), 7.15—7.67 (5H, m, Ph), 7.81 (5H, br s, Ar-H)
9b	A[a]: 2.32 (3H, s, C-Me), 3.06 (3H, s, N-Me), 7.19—7.43 (5H, m, Ph), 7.43 (2H, d, *J*=8.19 Hz, Ar-H), 7.57 (2H, d, *J*=8.19 Hz, Ar-H), 8.09 (1H, br s, N_4-H), 11.12 (1H, s, N_8-H)
9c	A[a]: 3.06 (3H, s, Me), 7.21—7.70 (9H, m, Ph and Ar-H), 8.34 (1H, br s, N_4-H), 11.13 (1H, s, N_8-H)
9d	A[a]: 3.05 (3H, s, Me), 7.21 (2H, d, *J*=8.01 Hz, Ar-H), 7.42—7.48 (5H, m, Ph), 7.68 (2H, d, *J*=8.01 Hz, Ar-H), 8.20 (1H, br s, N_4-H), 11.13 (1H, s, N_8-H)
9e	A[b]: 3.06 (3H, s, Me), 7.19—7.45 (5H, m, Ph), 7.65—7.89 (3H, m, Ar-H), 8.37 (1H, br s, N_4-H), 11.15 (1H, s, N_8-H)
9f	A[a]: 3.05 (3H, s, Me), 7.18—7.47 (5H, m, Ph), 7.61 (4H, s, Ar-H), 8.12 (1H, br s, N_4-H), 11.12 (1H, s, N_8-H)
10a	A: 7.21—7.78 (8H, m, Ph × 2), 8.36—8.52 (2H, m, C-Ph), 12.96 (1H, br, N_8-H); B: 7.37—7.73 (8H, m, Ph × 2), 8.39—8.54 (2H, m, C-Ph)
10b	A: 2.42 (3H, s, C-Me), 7.28—7.60 (5H, m, Ph), 7.42 (2H, d, *J*=8.16 Hz, Ar-H), 8.33 (2H, d, *J*=8.16 Hz, Ar-H)
10c	B: 7.32—8.48 (9H, m, Ph and Ar-H)
10d	B: 7.31—7.70 (5H, m, Ph), 7.53 (2H, d, *J*=8.22 Hz, Ar-H), 8.46 (2H, d, *J*=8.22 Hz, Ar-H)
10e	B: 7.31—7.72 (5H, m, Ph), 7.69 (1H, d, *J*=8.82 Hz, Ar-H), 8.37 (1H, dd, *J*=1.74, 8.82 Hz, Ar-H), 8.62 (1H, d, *J*=1.74 Hz, Ar-H)
10f	B: 7.35—7.98 (5H, m, Ph), 7.78 (2H, d, *J*=8.22 Hz, Ar-H), 8.40 (2H, d, *J*=8.22 Hz, Ar-H)

a) Run at 500 MHz. *b)* Run at 200 MHz.

6-phenyl analogs (**7a**—**f** and **8a**—**f**) obtained in this study against a variety of bacterial and fungal strains were evaluated by the agar dilution method.

The antibacterial activities are summarized in Tables VII and VIII. The majority of the compounds prepared here showed minimal growth-inhibitory concentration (MIC) of 0.5—100 μg/ml against many of the bacterial strains. Among the compounds, **1c**—**e**, and **3c** exhibited the strongest inhibition of *Micrococcus lutea* (0.5 μg/ml) and *Staphylococcus aureus 4R* (1 μg/ml), as well as *Bacillus subtilis* and *Staphylococcus aureus* (1—2 μg/ml). It is noteworthyl that most of the compounds have stronger antibacterial activi-

TABLE VII. Antibacterial Activities of Toxoflavins **1a**—**e** and Their 6-Phenyl Analogs **7a**—**f**

Bacteria[a]	MIC (μg/ml)										
	1a	**1b**	**1c**	**1d**	**1e**	**7a**	**7b**	**7c**	**7d**	**7e**	**7f**
Escherichia coli IFO-3301	10	2	100	>100	100	>100	>100	>100	100	>100	100
Proteus vulgaris IFO-3167	10	5	100	>100	100	>100	>100	>100	100	>100	50
Pseudomonas aeruginosa IFO-3448	>100	>100	>100	>100	>100	100	>100	>100	50	10	10
Serratia marcescens IFO-3046	50	>100	>100	>100	>100	100	20	50	10	50	10
Bacillus subtilis IFO-3007	20	10	1	1	2	10	10	20	5	100	5
Staphylococcus aureus IFO-3060	20	10	1	1	1	5	10	10	5	100	10
Staphylococcus aureus 4R	20	10	1	1	1	50	10	5	5	100	10
Micrococcus lutea IFO-3232	20	10	0.5	0.5	0.5	20	20	50	10	>100	10
Mycobacterium smegmatis IFO-3082	20	20	20	5	10	100	50	50	20	100	50

a) Culture conditions: 37 °C, 16—48 h. Medium: bouillon agar in all cases except *Mycobacterium smegmatis*, for which glucose bouillon agar was used. Method: agar dilution method.

TABLE VIII. Antibacterial Activities of Toxoflavin 4-Oxides **3a**—**e** and Their 6-Phenyl Analogs **8a**—**f**

Bacteria[a]	MIC (μg/ml)										
	3a	**3b**	**3c**	**3d**	**3e**	**8a**	**8b**	**8c**	**8d**	**8e**	**8f**
Escherichia coli IFO-3301	10	10	>100	>100	>100	100	>100	>100	>100	>100	100
Proteus vulgaris IFO-3167	20	20	100	100	>100	50	>100	>100	100	100	100
Pseudomonas aeruginosa IFO-3448	100	10	>100	>100	>100	50	>100	>100	100	100	50
Serratia marcescens IFO-3046	100	20	>100	100	100	10	10	>100	5	20	10
Bacillus subtilis IFO-3007	50	50	2	10	20	5	10	100	5	5	10
Staphylococcus aureus IFO-3060	20	20	2	10	50	5	10	20	5	5	20
Staphylococcus aureus 4R	20	10	1	20	5	10	5	10	10	2	10
Micrococcus lutea IFO-3232	10	10	0.5	100	100	10	20	>100	5	20	10
Mycobacterium smegmatis IFO-3082	10	50	50	2	20	50	100	100	20	20	50

a) Culture conditions: 37 °C, 16—48 h. Medium: bouillon agar in all cases except *Mycobacterium smegmatis*, for which glucose bouillon agar was used. Method: agar dilution method.

TABLE IX. Antifungal Activities of Toxoflavins **1a**—**e** and Their 6-Phenyl Analogs **7a**—**f**

Fungi[a]	MIC (μg/ml)										
	1a	**1b**	**1c**	**1d**	**1e**	**7a**	**7b**	**7c**	**7d**	**7e**	**7f**
Aspergillus niger IFO-4066	50	50	50	50	100	>100	100	100	100	>100	100
Penicillium chrysogenum IFO-4626	50	100	50	50	100	100	100	100	100	100	100
Rhizopus spp.	50	100	50	10	10	>100	100	50	100	100	100
Candida albicans IFO-0583	20	50	5	5	5	100	50	50	20	50	50
Saccharomyces cerevisiae IFO-0305	10	20	5	5	5	10	5	5	2	>100	5

a) Culture conditions: 28 °C, 4—5 d. Medium: glucose bouillon agar. Method: agar dilution method.

TABLE X. Antifungal Activities of Toxoflavin 4-Oxides **3a**—**e** and Their 6-Phenyl Analogs **8a**—**f**

Fungi[a]	MIC (μg/ml)										
	3a	**3b**	**3c**	**3d**	**3e**	**8a**	**8b**	**8c**	**8d**	**8e**	**8f**
Aspergillus niger IFO-4066	50	100	100	100	100	100	>100	>100	100	100	100
Penicillium chrysogenum IFO-4626	100	100	100	100	100	100	100	100	100	100	100
Rhizopus spp.	100	100	100	100	50	50	>100	100	100	100	100
Candida albicans IFO-0583	50	100	5	50	20	50	100	50	50	50	20
Saccharomyces cerevisiae IFO-0305	20	50	5	10	10	5	10	100	10	10	10

a) Culture conditions: 28 °C, 4—5 d. Medium: glucose bouillon agar. Method: agar dilution method.

ties against gram-positive bacterial strains than against gram-negative strains. Moreover, there was a marked tendency for toxoflavins (**1a**—**e**) to have more potent inhibitory activities than the toxoflavin 4-oxides (**3a**—**e**) and their 6-phenyl analogs (**7a**—**f**).

On the other hand, the antifungal activities of the compounds are shown in Tables IX and X. It seems noteworthy that most of the compounds exhibited strong inhibitory activities (2—100 μg/ml MIC) againt *Candida albicans* (yeast) and *Saccharomyces cerevisiae* (yeast) but exhibited lower inhibitory activities (*ca.* 100 μg/ml) against *Aspergillus niger* (fungus), *Penicillium chrysogenum* (fungus), and *Rhizopus* spp. (fungus).

In the case of 6-phenyl analogs of 4,8-dihydrotoxoflavins (**9a**—**f**) and 1-demethyltoxoflavins (**10a**—**f**), most of the compounds exhibited weak inhibitory activities (> 50 μg/ml MIC) against a variety of bacterial and fungal strains.

Experimental

All melting points were determined on a Yanagimoto micro hot-stage apparatus and are uncorrected. Elemental analyses were performed on a Yanagimoto MT-2 CHN Corder elemental analyzer. Infrared (IR) spectra were taken on a JASCO IRA-102 spectrophotometer. Nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on a Hitachi FT-NMR R-1500 (60 MHz), a Varian VXR-200 (200 MHz), or a Varian VXR-500 (500 MHz) instrument. Chemical shifts are given in δ (ppm) relative to $(Me)_4Si$ and coupling constants (J) are given in hertz (Hz). The following abbreviations are used: s = singlet, d = doublet, dd = doublet of doublets, m = multiplet, and br = broad. Mass spectra (MS) were obtained using a VG-70SE spectrometer with FAB ionization or a JEOL JMS 01SG-2 instrument by direct insertion at 75 eV. Reactions were monitored by analytical thin-layer chromatography (TLC) performed on TLC plates, Kiesel gel 60 F_{254} precoated, layer thickness 0.25 mm (Merck) and spots were detected under ultraviolet (UV) irradiation at 254 and 360 nm. Column chromatography was done on Kiesel gel 60 (70—230 mesh ASTM, Merck) and the developing solvents were as described below.

6-(1-Methylhydrazino)-3-phenyluracil (5) A solution of 6-chloro-3-phenyluracil (4)$^{12)}$ (40 g, 180 mmol) and methylhydrazine (28.5 ml, 540 mmol) in dry EtOH (250 ml) was heated under reflux for 1 h. After cooling, the precipitated crystalline mass was collected and recrystallized from EtOH to afford the product (**5**) (30 g, 72%) as colorless prisms, mp 236—238 °C. *Anal.* Calcd for $C_{11}H_{12}N_4O_2$: C, 56.89; H, 5.21; N, 24.13. Found: C, 56.75; H, 5.19; N, 24.11. EI-MS *m/z*: 232 (M^+, 60%), 217 ($M^+ - Me$, 7%), 144 (100%), 77 (42%). IR (KBr): 3320 (NH_2), 3300 (NH_2), 1700 (C=O), 1667 (C=O) cm^{-1}. ^{1}H-NMR (60 MHz, DMSO-d_6) δ: 3.08 (3H, s, Me), 4.74 (1H, s, 5H), 6.55 (2H, br, NH_2), 7.23—7.04 (2H, m, Ph), 7.30—7.46 (3H, m, Ph), 10.70 (1H, br, NH).

General Procedure for the Preparation of 6-(2-Benzylidene-1-methylhydrazino)-3-phenyluracils (6a—f) The respective aryl aldehyde (40 mmol) was added to a suspension of **5** (4.64 g, 20 mmol) in dry EtOH (100 ml) at room temperature with stirring. The mixture was stirred for 1.5—2 h, then the solid was filtered off by suction, washed with dry EtOH, and recrystallized from EtOH to afford the corresponding pure products (**6a**—**f**) (Tables I and VI).

General Procedure for the Preparation of 3-Substituted 1-Methyl-6-phenylpyrimido[5,4-*e*]-1,2,4-triazine-5,7(1*H*,6*H*)-diones (6-Phenyl Analogs of Toxoflavin) (7a—f) and Their 4-Oxides (8a—f) Method A: Sodium nitrite$^{15)}$ (3.1 g, 45 mmol) was added in portions to a stirred solution of **6a**—**f** (15 mmol) in acetic acid (50 ml) under cooling at 5—7 °C. The reaction mixture was then stirred at room temperature for 6h, whereupon crystals of a mixture of toxoflavin type-compounds (**7a**—**f**) and their 4-oxides (**8a**—**f**) deposited gradually.$^{16)}$ The crystals were collected by filtration and the filtrate was diluted with diethyl ether to get the second crop. The combined crystals of the first and second crops were subjected to column chromatography and eluted with benzene–EtOAc (9 : 1) to isolate **7a**—**f** and **8a**—**f** individually. All products thus isolated were further purified by recrystallization from 40% aqueous dioxane. The yields for **7a**—**f** and **8a**—**f** are given in column A of Tables II and III.

Method B: A stirred mixture of **6a**—**f** (10 mmol) and KNO_3 (1.3 g, 13 mmol) in acetic acid (50 ml) was treated with H_2SO_4 (0.5 g, 5.1 mmol) drop by drop at room temperature, and then the mixture was heated at 95 °C for 3—4 h. After cooling of the mixture, the precipitated crystals were collected by filtration to afford the 4-oxides (**8a**—**f**) in a pure state. The filtrate was diluted with diethyl ether (*ca.* 100 ml) and the solution was allowed to stand overnight in a refrigerator to afford the second crop, which was a mixture of **7a**—**f** and **8a**—**f**. The compounds were isolated individually by the procedure described above. The combined yields of **7a**—**f** and **8a**—**f** are given in column B of Tables II and III.

General Procedure for the Preparation of 3-Substituted 1-Methyl-5,7-dioxo-6-Phenyl-1,4,5,6,7,8-hexahydropyrimido[5,4-*e*]-1,2,4-triazines (6-Phenyl Analogs of 4,8-Dihydrotoxoflavin) (9a—f) The 4-oxides (**8a**—**f**) (5 mmol) were added in portions to a solution of $Na_2S_2O_4$ (2.6 g, 15 mmol) in H_2O (30 ml) ar room temperature with stirring. The solution was stirred for 10 min, then the precipitated solid was filtered off by suction, washed with H_2O, and dried in a vacuum desiccator to afford the corresponding 4,8-dihydrotoxoflavin-type compounds (**9a**—**f**) in the yields shown in Table IV. Further purification of the reduced compounds (**9a**—**f**) by recrystallization was difficult because of their unstable character in all hot solvents examined. Similarly, the reduction of the toxoflavin-type compounds (**7a**—**f**) (5 mmol) by $Na_2S_2O_4$ (2.6 g, 15 mmol) in H_2O (30 ml) gave the corresponding products (**9a**—**f**) in 92—96% yields.

General Procedure for the Preparation of 3-Substituted 1-Methyl-6-phenylpyrimido[5,4-*e*]-1,2,4-triazine-5,7(1*H*,6*H*)-diones (7a—f) by the Oxidation of the 4,8-Dihydrotoxoflavin Analogs (9a—f) A suspension of **9a**—**f** (4 mmol) in 30% H_2O_2 (20 ml) was stirred at room temperature for 20—30 min. After reaction, the precipitates were filtered off by suction, washed with EtOH, and recrystallized from 40% aqueous dioxane to afford the corresponding pure products (**7a**—**f**) in high yield. Yield: 83% for **7a**; 87% for **7b**; 85% for **7c**; 92% for **7d**; 84% for **7e**; 90% for **7f**.

General Procedure for the Preparation of 3-Substituted 6-Phenylpyrimido-[5,4-*e*]-1,2,4-triazine-5,7-(6*H*,8*H*)-diones (6-Phenyl Analogs of Reumycin) (10a—f) A solution of **8a**—**f** (3 mmol) in DMF (10 ml) was heated under reflux for 2 h. After removal of the solvent under reduced pressure, the resulting residue was triturated with EtOH to afford crystals of the corresponding demethylated compounds (**10a**—**f**). The crystals were collected by filtration and recrystallized from 40% aqueous dioxane to get the pure compounds (**10a**—**f**) in good yields as shown in Table V. Similarly, the reaction of **7a**—**f** (3 mmol) in DMF (30 ml) gave the corresponding demethylated compounds (**10a**—**f**) in 85—94% yields.

General Reaction for Deoxygenation of 3-Substituted 1-Methyl-6-phenylpyrimido[5,4-*e*]-1,2,4-triazine-5,7(1*H*,6*H*)-dione 4-Oxides (8a—f) by Acetic Acid A solution of **8a**—**f** (5 mmol) in AcOH (30 ml) was heated at 80 °C until the starting materials (**8a**—**f**) were no longer detectable (monitored by TLC, *ca.* 5—7 h). After removal of the solvent under reduced pressure, the resulting residue was triturated with EtOH to afford quantitatively a mixture of **7a**—**f** and **10a**—**f**, which was subjected to column chromatography and eluted with benzene–EtOAc (9 : 1) to isolate **7a**—**f** and **10a**—**f** individually. All products thus isolated were further purified by recrystallization from 40% aqueous dioxane. In all cases **7a**—**f** and **10a**—**f** were obtained in the ratio of 4 to 1, respectively.

Antibacterial and Antifungal Study Organisms: The microorganisms used for the assay were *Escherichia coli* IFO-3301 (gram-negative bacterium), *Proteus vulgaris* IFO-3167 (gram-negative bacterium), *Pseudomonas aeruginosa* IFO-3448 (gram-negative bacterium), *Serratia marcescens* IFO-3046 (gram-negative bacterium), *Bacillus subtilis* IFO-3007 (gram-positive bacterium), *Staphylococcus aureus* IFO-3060 (gram-positive bacterium), *Staphylococcus aureus 4R* (gram-positive bacterium), *Micrococcus lutea* IFO-3232 (gram-positive bacterium), *Mycobacterium smegmatis* IFO-3082 (gram-positive bacterium), *Aspergillus niger* IFO-4066 (fungus), *Penicillum chrysogenum* IFO-4626 (fungus), *Rhizopus* spp. (fungus) *Candida albicans* IFO-0583 (yeast), and *Saccharomyces cerevisiae* IFO-0305 (yeast).

Biological Activity Test: Antibacterial and antifungal tests were carried out by the agar dilution metod. The test bacteria were applied to bouillon agar in all cases except for *Mycobacterium smegmatis* IFO-3082, for which glucose bouillon agar was used. The test fungi were all applied to glucose bouillon agar. All media contained various concentrations of **1a**—**e**, **3a**—**e**, **7a**—**f**, or **8a**—**f** to be tested. The plates were incubated at 37 °C for 16—48 h in the case of the antibacterial tests and at 28 °C for 4—5 d in the case of the antifungal tests. Growth was observed with the naked eye.

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References and Notes

1) W. Pfleiderer and K. H. Schündehütte, *Justus Liebigs Ann. Chem.*, **615**, 42 (1958).

2) *a*) G. D. Daves, Jr., R. K. Robins, and C. C. Cheng, *J. Am. Chem. Soc.*, **83**, 3904 (1961); *b*) *Idem, ibid.*, **84**, 1724 (1962); *c*) T. K. Liao, F. Baiocchi, and C. C. Cheng, *J. Org. Chem.*, **31**, 900 (1966); *d*) F. Yoneda, K. Shinomura, and S. Nishigaki, *Tetrahydron Lett.*, **1971**, 851; *e*) E. C. Taylor and F. Sowinsky, *J. Org. Chem.*, **40**, 2321 (1975).

3) *a*) S. M. Navashin, I. N. Fomina, T. G. Terent'eva, and L. A. Shtegel'man, *Aktual. Vop. Sovrem. Onkol.* (Moscow), **1973**, 273

[*Chem. Abstr.*, **81**, 94*f* (1974)]; *b*) V. K. Akimenko, N. P. Golovchenko, and T. G. Terent'eva, *Antibiotiki* (Moscow), **19**, 1101 (1974) [*Chem. Abstr.*, **82**, 132839*h* (1975)]; *c*) V. K. Akimenko, N. P. Golovchenko, A. G. Medentsev, *Mikrobiologiya* (Moscow), **43**, 839 (1974) [*Chem. Abstr.*, **82**, 107661*m* (1975); *d*) G. G. Egorenko, L. A. Shtegel'man, V. N. Solov'ev, E. K. Berezina, V. V. Berezhinskaya, M. A. Bykova, G. V. Dolgova, and A. A. Firsov, *Antibiotiki* (Moscow), **21**, 178 (1976) [*Chem. Abstr.*, **84**, 130304*x* (1976)]; *e*) S. M. Navashin, L. P. Ivanitskaya, T. G. Terent'eva, and L. V. Egorov, *Farmatsiya* (Moscow), **30**, 55 (1981) [*Chem. Abstr.*, **95**, 73566*k* (1981)]; *f*) A. A. Firsov, S. V. Geodakyan, M. R. Lichinitser, and V. Ya. Shutka, *Antibiot. Med. Biotekhnol.* (Moscow), **30**, 604 (1985) [*Chem. Abstr.*, **103**, 189153*m* (1985)].

4) R. A. Machlowitz, W. P. Fisher, B. S. McKay, A. A. Tytell, and J. Charney, *Antibiot. Chemother.*, **4**, 259 (1954).

5) S. E. Esipov, M. N. Kolosov, and L. A. Saburova, *J. Antibiot.*, **26**, 537 (1973).

6) K. Tanabe, Y. Asahi, M. Nishikawa, T. Shima, Y. Kuwada, T. Kanazawa, and K. Ogata, *Takeda Kenkyusho Nempo*, **22**, 133 (1963) [*Chem. Abstr.*, **60**, 13242 (1964)].

7) *a*) F. Yoneda and T. Nagamatsu, *Chem. Pharm. Bull.*, **23**, 2001 (1975); *b*) *Idem*, *Synthesis*, **1975**, 177; *c*) F. Yoneda, T. Nagamatsu, and K. Shinomura, *J. Chem. Soc.*, *Perkin Trans. 1*, **1976**, 713; *d*) F. Yoneda, Y. Sakuma, T. Nagamatsu, and S. Mizumoto, *ibid.*, **1976**, 2398.

8) *a*) F. Yoneda and T. Nagamatsu, *Tetrahedron Lett.*, **1973**, 1577; *b*) F. Yoneda, M. Kawamura, T. Nagamatsu, K. Kuretani, A. Hoshi, and M. Iigo, *Heterocycles*, **4**, 1503 (1976).

9) *a*) F. Yoneda and T. Nagamatsu, *J. Heterocycl. Chem.*, **11**, 271 (1974); *b*) *Idem*, *Bull. Chem. Soc. Jpn.*, **48**, 2884 (1975); *c*) Y. Sakuma, S. Matsumoto, T. Nagamatsu, and F. Yoneda, *Chem. Pharm. Bull.*, **24**, 338 (1976).

10) *a*) F. Yoneda, T. Nagamatsu, and M. Ichiba, *J. Heterocycl. Chem.*, **11**, 83 (1974); *b*) F. Yoneda, T. Nagamatsu, K. Ogiwara, M. Kanahori, S. Nishigaki, and E. C. Taylor, *Chem. Pharm. Bull.*, **26**, 367 (1978).

11) F. Yoneda and T. Nagamatsu, *J. Am. Chem. Soc.*, **95**, 5735 (1973).

12) H. Goldner, G. Dietz, and E. Carstens, *Justus Liebigs Ann. Chem.*, **691**, 142 (1966).

13) H. E. Latuasan and W. Berends, *Biochim. Biophys. Acta*, **52**, 502 (1961).

14) C. DeBoer, A. Dietz, J. S. Evans, and R. M. Michaels, *Antibiotics Annual.*, **1959—1960**, 220.

15) In the case of the nitrosative cyclization of **6e**, 9.3 g (135 mmol) of sodium nitrite was used.

16) In order to obtain more of the 4-oxide type compound **8** than the toxoflavin-type compound **7**, the nitrosative cyclizations for **6b** and **6c** were carried out in the presence of DEAD (5.22 g, 30 mmol) as an oxidizing agent.