Alternative Method C.—A cold (0°) mixture of 5.6 g (0.022 mole) of D-phenylalanine methyl ester hydrochloride and 2.2 g (0.022 mole) of Et_3N in 75 ml of DMF was filtered, and to the filtrate was added 9 g (0.022 mole) of O-acetyl-N-carbobenzoxyserine *p*-nitrophenyl ester.[10] The solution was kept 2 days at 25° and evaporated to an oil which was taken up in EtOAc. This solution was washed with H_2O, 5% $NaHCO_3$ solution, and dilute HCl, then dried and evaporated to a solid. The product, O-acetyl-N-carbobenzoxyseryl-D-phenylalanine methyl ester was recrystallized from EtOAc–petroleum ether, 7.5 g (77%), mp 132–133°, $[\alpha]^{23}D$ +4.4 (*c* 2, DMF).

Anal. Calcd for $C_{23}H_{26}N_2O_7$: C, 62.43; H, 5.93; N, 6.33. Found: C, 62.44; H, 5.96; N, 6.52.

The methyl ester and O-acetyl groups were removed using an excess of 2 *N* NaOH in MeOH affording carbobenzoxyseryl-D-phenylalanine in 72% yield, mp 137–139°.

Method D.—To a cold (5°) solution of 7.9 g (0.03 mole) of carbobenzoxyhydroxyproline in 150 ml of MeCN was added 3 g of Et_3N and 7.5 g (0.03 mole) of Woodward's[5] reagent K. The mixture was stirred 1 hr at 5°, and 5 g (0.03 mole) of D-valine methyl ester hydrochloride and 3 g of Et_3N were added. The mixture was stirred 48 hr at 25°. The solvent was evaporated, the residue was taken up in EtOAc, and the solution was washed with H_2O, 5% $NaHCO_3$ solution, and dilute HCl and dried. Evaporation of the solvent left an oil which would not crystallize: yield 8.4 g (74%).

Anal. Calcd for $C_{19}H_{26}N_2O_6 \cdot H_2O$: C, 57.55; H, 7.12; N, 7.06. Found: C, 57.28; H, 7.00; N, 6.63.

Hydrolysis of the above methyl ester with NaOH in MeOH gave carbobenzoxyhydroxyprolyl-D-valine in 80% yield, mp 62–68°.

Method E.—To a cold (7°) solution of 6 g (0.0238 mole) of carbobenzoxy-D-serine hydrazide in 50 ml of glacial HOAc and 30 ml of 1 *N* HCl was added over 15 min, 1.7 g (0.025 mole) of $NaNO_2$ in 5 ml of H_2O. After an additional 5 min, the solution was diluted with 200 ml of ice–H_2O and extracted with cold (−5°) EtOAc. The EtOAc solution was washed with ice–H_2O several times and with cold 5% Na_2CO_3 solution until neutral and dried. To this filtered solution at 0° was added a cold (0°) mixture of 5.5 g (0.024 mole) of D-phenylalanine methyl ester hydrochloride and 2.5 g of Et_3N in 50 ml of DMF. The solution was kept 24 hr at 5° and washed with 5% $NaHCO_3$ solution, and dilute HCl then dried and the solvent was evaporated. Et_2O–cyclohexane was added producing a colorless solid, 4.5 g (46%), mp 77–78°.

Anal. Calcd for $C_{21}H_{24}N_2O_6$: C, 62.99; H, 6.04; N, 7.00. Found: C, 62.73; H, 6.23; N, 6.93.

The methyl ester was removed in the usual manner giving carbobenzoxy-D-seryl-D-phenylalanine, mp 138–139°.

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(10) E. D. Nicolaides and H. A. DeWald, U. S. Patent 3,164,614 (1965).

Nitrofuryl Heterocycles. VII.[1] 4-Amino-6-(5-nitro-2-furyl)-1H-pyrazolo[3,4-*d*]pyrimidines

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Fifty-two 1-alkyl-4-amino-6-(5-nitro-2-furyl)-1H-pyrazolo[3,4-*d*]pyrimidine derivatives were prepared and were found to possess excellent antibacterial activity. The most active compound was the 4-bis(2-hydroxypropyl)amino-1-methyl derivative, which showed an oral ED_{50} of about 2 mg/kg against *Staphylococcus aureus* infections in mice.

In a previous paper in this series[2] it was shown that the attachment of a condensed pyrimidine ring system at the 2 position of the nitrofuran ring would give compounds possessing exceptional antibacterial activity. That paper described the antibacterial activity of numerous 4-amino-2-(5-nitro-2-furyl)quinazoline derivatives. The present paper is concerned with the synthesis and biological evaluation of derivatives of another condensed pyrimidine system, 1-alkyl-4-amino-6-(5-nitro-2-furyl)-1H-pyrazolo[3,4-*d*]pyrimidine.

Chemistry.—The excellent procedure of Taylor and Borror[3] for condensing nitriles with 5-amino-4-cyanopyrazoles (**1**) in the presence of base to yield 6-substituted 4-aminopyrazolo[3,4-*d*]pyrimidines (**2**) was adapted to include the reaction of 2-furonitrile with **1**. The desired nitrofuran derivatives were then prepared by mixed-acid nitration of **2**. Unfortunately, this short synthesis was not applicable to the preparation of 4-substituted amino derivatives. These compounds were prepared with little difficulty, however, by the synthesis devised by Cheng and Robins.[4] The reactions are summarized in Scheme I.

Thus, aminocyanopyrazole (**1**) was acylated with 2-furoyl chloride to give amide **3** which was cyclized in hot, alkaline, peroxide solution to pyrazolopyrimidinone (**4**). Mixed-acid nitration of **4** gave the nitrofuryl derivative **5** in excellent yield. The assignment of the keto form to the oxygen function in position 4 of compounds **4** and **5**, rather than the frequently reported tautomeric hydroxy form, was based on the observation that carbonyl absorption occurred at 1650–1670 cm^{-1} in the infrared. Chlorination of **5** with PCl_5 in $POCl_3$ gave the 4-chloro compounds **6**. Displacement of the chlorine atom in **6** with a variety of amines proceeded smoothly in DMF solution to give the amino derivatives **10–46**, **49–55**, and **58** listed in Table I. Displacement of the halogen in **6** with ammonia in aqueous DMF gave **7–9**, identical by mixture melting points and infrared spectra with those obtained by the nitration of **2**.

Biological Screening Results.—The *in vitro* and

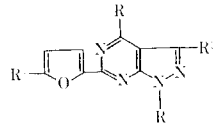
(1) For the previous paper in this series see H. R Snyder, Jr., *J. Med. Chem.*, **10**, 737 (1967).

(2) H. A. Burch, *ibid.*, **9**, 408 (1966).

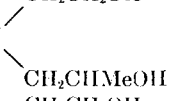
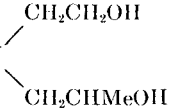
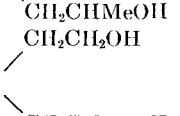
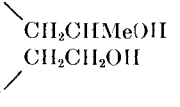
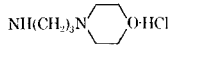
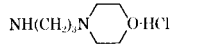
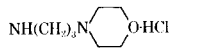
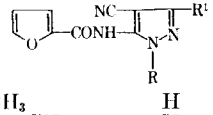
(3) E. C. Taylor and A. L. Borror, *J. Org. Chem.*, **26**, 4967 (1961).

(4) C. C. Cheng and R. K. Robins, *ibid.*, **23**, 191 (1958).

TABLE I

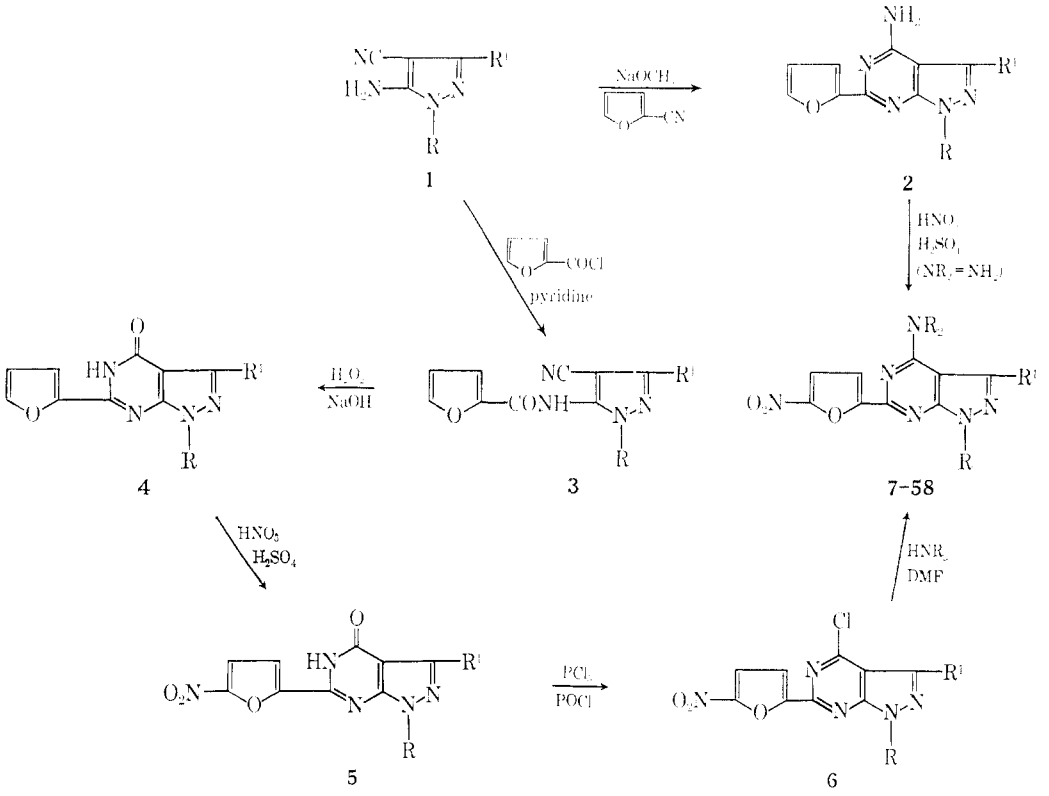


No.	R	R^1	R^2	R^3	Mp °C	Yield, %	Formula	Calcd, % C	Calcd, % H	Calcd, % N	Found, % C	Found, % H	Found, % N	*Staphylococcus aureus* MIC, μg/ml	*Staphylococcus aureus* ED_{50} (mice), mg/kg oral
2a	CH_2CH_3	H	NH_2	H	234–235	65.5	$C_{11}H_{11}N_5O$	57.63	4.84	30.55	57.42	4.99	30.46	...	...
2b	$CH_2CH_2CH_3$	H	NH_2	H	184–185	71.4	$C_{12}H_{13}N_5O$	59.25	5.39	28.79	59.28	5.38	28.59	...	...
2c	$CH_2CH_2OCH_3$	H	NH_2	H	192–193	78.6	$C_{12}H_{13}N_5O_2$	55.59	5.05	27.02	55.61	5.16	27.23	...	...
4a	CH_3	H	OH	H	283.5–285	64	$C_{10}H_8N_4O_2$	55.55	3.73	25.92	55.49	3.84	26.02	...	...
4b	CH_2CH_3	H	OH	H	260–261.5	40.6	$C_{11}H_{10}N_4O_2$	57.38	4.38	24.34	57.36	4.40	24.56	...	...
4c	$CH_2CH_2CH_3$	H	OH	H	219–220	78	$C_{12}H_{12}N_4O_2$	59.01	4.95	22.94	59.19	5.08	23.19	...	...
4d	$CH_2CH_2OCH_3$	H	OH	H	201–202	70	$C_{12}H_{12}N_4O_3$	55.38	4.65	21.53	55.46	4.58	21.41	...	...
4e	CH_3	CH_2CH_3	OH	H	290–292	72	$C_{12}H_{12}N_4O_2$	59.01	4.95	22.94	59.03	4.94	23.18	...	...
5a	CH_3	H	OH	NO_2	325 dec	53	$C_{10}H_7N_5O_4$	45.98	2.70	26.81	46.13	2.74	26.84	6	>200
5b	CH_2CH_3	H	OH	NO_2	271–272.5	54.5	$C_{11}H_9N_5O_4$	48.00	3.30	25.45	47.99	3.13	25.37	6	>100
5c	$CH_2CH_2CH_3$	H	OH	NO_2	241–242	36.5	$C_{12}H_{11}N_5O_4$	49.83	3.83	24.21	49.86	4.10	23.97	6.25	>50
5d	$CH_2CH_2OCH_3$	H	OH	NO_2	243–245	45	$C_{12}H_{11}N_5O_5$	47.21	3.63	22.95	47.17	3.61	22.92	12	...
5e	CH_3	CH_2CH_3	OH	NO_2	293–295	69	$C_{12}H_{11}N_5O_4$	49.83	3.83	24.21	49.88	3.88	24.52	6	>100
6a	CH_3	H	Cl	NO_2	211–213	72.5	$C_{10}H_6ClN_5O_3$	42.90	2.16	12.66[a]	43.15	2.35	12.64[a]	12.5	>100
6b	CH_2CH_3	H	Cl	NO_2	182.5–183.5	84.5	$C_{11}H_8ClN_5O_3$	44.99	2.74	23.85	44.94	2.71	23.91	12.5	>200
6c	$CH_2CH_2CH_3$	H	Cl	NO_2	121–122	70	$C_{12}H_{10}ClN_5O_3$	46.84	3.28	11.52[a]	46.97	3.40	11.56[a]	50	...
6d	$CH_2CH_2OCH_3$	H	Cl	NO_2	156–157	70	$C_{12}H_{10}ClN_5O_4$	44.52	3.11	21.64	44.73	3.13	21.71	>50	...
6e	CH_3	CH_2CH_3	Cl	NO_2	163–165	64	$C_{12}H_{10}ClN_5O_3$	46.84	3.28	22.76	46.91	3.43	22.86	>100	...
7	CH_2CH_3	H	NH_2	NO_2	320–321.5	27.7	$C_{11}H_{10}N_6O_3$	48.17	3.68	30.65	48.37	3.94	30.77	0.75	50
8	$CH_2CH_2CH_3$	H	NH_2	NO_2	306–307	38.7	$C_{12}H_{12}N_6O_3$	49.99	4.20	29.16	50.22	4.32	29.14	1.5	200
9	$CH_2CH_2OCH_3$	H	NH_2	NO_2	289.5–290.5	33.7	$C_{12}H_{12}N_6O_4$	47.37	3.98	27.62	47.31	4.06	27.72	1.5	33
10	CH_3	H	$NHCH_3$	NO_2	227–228	90	$C_{11}H_{10}N_6O_3$	48.17	3.68	30.65	48.39	4.00	30.73	0.19	13
11	CH_3	H	$NHCH_2CH_3$	NO_2	237–238	77	$C_{12}H_{12}N_6O_3$	49.99	4.20	29.16	50.02	4.35	29.14	0.38	35
12	CH_3	H	$NH(CH_2)_3CH_3$	NO_2	151–153	85	$C_{14}H_{16}N_6O_3$	53.16	5.10	26.57	53.09	5.10	26.47	0.012	13
13	CH_3	H	$NH(CH_2)_4CH_3$	NO_2	108–110	84	$C_{15}H_{18}N_6O_3$	54.54	5.49	25.44	54.37	5.38	25.74	0.38	21
14	CH_3	H		NO_2	209–210	73	$C_{16}H_{18}N_6O_3$	56.13	5.30	24.55	56.05	5.44	24.43	0.38	4
15	CH_3	H	$N(CH_3)_2$	NO_2	217–218	77	$C_{12}H_{12}N_6O_3$	49.99	4.20	29.16	50.07	4.34	29.20	0.38	17
16	CH_3	H	$N(CH_2CH_3)_2$	NO_2	172–173	78	$C_{14}H_{16}N_6O_3$	53.16	5.10	26.57	53.23	5.32	26.48	0.19	50
17	CH_3	H	$N(CH_2CH_2CH_3)_2$	NO_2	159–160	91	$C_{16}H_{20}N_6O_3$	55.80	5.85	24.41	55.85	5.92	24.36	0.095	35
18	CH_3	H		NO_2	202–205	83	$C_{12}H_{10}N_6O_3$	50.35	3.52	29.36	50.34	3.89	29.21	0.75	>50
19	CH_3	H		NO_2	216–217	71.5	$C_{14}H_{14}N_6O_3$	53.50	4.49	26.74	53.39	4.37	26.67	0.8	31
20	CH_3	H		NO_2	181–183	82	$C_{16}H_{18}N_6O_3$	56.13	5.30	24.55	56.30	5.19	24.62	0.75	50
21	CH_3	H	$NHCH_2CH_2OH$	NO_2	226–226.5	74	$C_{12}H_{12}N_6O_4$	47.37	3.98	27.62	47.31	3.94	27.91	0.2	19
22	CH_2CH_3	H	$NHCH_2CH_2OH$	NO_2	188–190	72	$C_{13}H_{14}N_6O_4$	49.05	4.43	26.41	49.10	4.36	26.45	0.75	25
23	$CH_2CH_2CH_3$	H	$NHCH_2CH_2OH$	NO_2	193–195	86	$C_{14}H_{16}N_6O_4$	50.60	4.85	25.29	50.71	4.74	25.25	0.75	35
24	$CH_2CH_2OCH_3$	H	$NHCH_2CH_2OH$	NO_2	202–204	92	$C_{14}H_{16}N_6O_5$	48.27	4.63	24.13	48.14	4.61	24.19	3	38
25	CH_3	CH_2CH_3	$NHCH_2CH_2OH$	NO_2	246–248	91	$C_{14}H_{16}N_6O_4$	50.60	4.85	25.29	50.67	4.83	25.27	0.8	100
26	CH_3	H	$NH(CH_2)_3OH$	NO_2	216–217	82.3	$C_{14}H_{14}N_6O_4$	53.50	4.49	26.74	53.39	4.37	26.67	0.2	100
27	CH_3	H	$NHCH_2CHMeOH$	NO_2	226–227	83.7	$C_{13}H_{14}N_6O_4$	49.05	4.43	26.41	48.99	4.57	26.39	0.38	10
28	CH_3	H	$NMeCH_2CH_2OH$	NO_2	204.5–205	84.4	$C_{13}H_{14}N_6O_4$	49.05	4.43	26.41	48.99	4.28	26.48	0.4	13
29	CH_2CH_3	H	$NMeCH_2CH_2OH$	NO_2	188–190	80	$C_{14}H_{16}N_6O_4$	50.60	4.85	25.29	50.65	4.94	25.33	3	44
30	CH_3	H	$NBuCH_2CH_2OH$	NO_2	147–148	87.5	$C_{16}H_{20}N_6O_4$	53.32	5.59	23.32	53.43	5.62	23.40	0.2	8
31	$CH_2CH_2CH_3$	H	$NBuCH_2CH_2OH$	NO_2	120–121	83	$C_{18}H_{24}N_6O_4$	55.66	6.23	21.64	55.76	6.28	21.43	0.75	35
32	CH_3	H	$N(CH_2CH_2OH)_2$	NO_2	208–209	74	$C_{14}H_{16}N_6H_5$	48.27	4.63	24.13	48.25	4.78	24.28	0.75	10

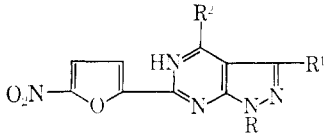
33	CH_2CH_3	H	$N(CH_2CH_2OH)_2$	NO_2	175–176	61.8	$C_{15}H_{18}N_6O_5$	49.72	5.01	23.20	49.63	5.10	23.02	1.5	20
34	$CH_2CH_2OCH_3$	H	$N(CH_2CH_2OH)_2$	NO_2	165–167	86	$C_{16}H_{20}N_6O_6$	48.97	5.14	21.42	49.04	5.18	21.45	3	65
35	CH_3	H		NO_2	218–218.5	80	$C_{15}H_{18}N_6O_5$	49.72	5.01	23.20	49.71	5.12	23.15	0.2	10
36	CH_2CH_3	H		NO_2	144–145	76	$C_{16}H_{20}N_6O_5$	51.06	5.36	22.33	51.01	5.22	22.15	0.38	7
37	$CH_2CH_2CH_3$	H		NO_2	153–154	85	$C_{17}H_{22}N_6O_5$	52.30	5.68	21.53	52.16	5.86	21.24	0.38	21
38	$CH_2CH_2OCH_3$	H		NO_2	149–151	48	$C_{17}H_{22}N_6O_6$	50.24	5.46	20.68	50.36	5.59	20.57	1.5	44
39	CH_3	H	$N(CH_2CHMeOH)_2$	NO_2	222–223.5	74.3	$C_{16}H_{20}N_6O_5$	51.06	5.36	22.33	50.60	5.44	22.29	0.095	2.2
40	CH_2CH_3	H	$N(CH_2CHMeOH)_2$	NO_2	227–229	42.5	$C_{17}H_{22}N_6O_5$	52.30	5.68	21.53	52.12	5.63	21.56	0.8	12
41	$CH_2CH_2CH_3$	H	$N(CH_2CHMeOH)_2$	NO_2	188–190	69	$C_{18}H_{24}N_6O_5$	53.45	5.98	20.78	53.30	6.23	20.64	0.38	22
42	$CH_2CH_2OCH_3$	H	$N(CH_2CHMeOH)_2$	NO_2	188–190	65	$C_{18}H_{24}N_6O_6$	51.42	5.75	19.99	51.43	5.87	20.08	0.8	10
43	CH_3	H	$NHCH_2CH_2OCH_3$	NO_2	183–184.5	49	$C_{13}H_{14}N_6O_4$	49.05	4.43	26.41	49.08	4.42	26.37	0.75	16
44	CH_2CH_3	H	$NHCH_2CH_2OCH_3$	NO_2	147–148	58	$C_{14}H_{16}N_6O_4$	50.60	4.85	25.29	50.36	4.72	25.27	1.5	29
45	$CH_2CH_2OCH_3$	H	$NHCH_2CH_2OCH_3$	NO_2	111–113	71	$C_{15}H_{18}N_6O_5$	49.72	5.01	23.20	49.85	5.08	23.23	1.5	14
46	CH_3	CH_2CH_3	$NHCH_2CH_2OCH_3$	NO_2	163–164	93	$C_{15}H_{18}N_6O_4$	52.02	5.24	24.27	52.15	5.42	23.85	3	100
47	CH_3	H	$NAcCH_2CH_2OCH_3$	NO_2	154–156	90	$C_{15}H_{16}N_6O_5$	50.00	4.48	23.23	49.74	4.54	23.12	0.75	14
48	CH_2CH_3	H	$NAcCH_2CH_2OCH_3$	NO_2	148–149	71	$C_{16}H_{18}N_6O_5$	51.33	4.85	22.45	51.28	4.96	22.62	3.1	41
49	CH_3	H	$NH(CH_2)_3OCH_3$	NO_2	141–142.5	74.5	$C_{14}H_{12}N_6O_4$	50.60	4.85	25.29	50.68	4.91	25.08	0.4	25
50	CH_3	H	$NHCHMeCH_2OCH_3$	NO_2	171–172	78	$C_{14}H_{16}N_6O_4$	50.60	4.85	25.29	50.27	4.94	25.29	0.38	42
51	CH_3	H	$NMeCH_2CH_2OCH_3$	NO_2	165–166	68	$C_{14}H_{16}N_6O_4$	50.60	4.85	25.29	50.31	4.75	25.33	0.75	12
52	CH_3	H	$N(CH_2CH_2OC_2H_5)$	NO_2	107.5–108	55.5	$C_{18}H_{24}N_6O_5$	53.45	5.98	20.78	53.19	5.91	20.81	3	16
53	CH_3	H		NO_2	280–281.5	58.5	$C_{17}H_{22}ClN_7O_4$	48.17	5.23	23.13	47.86	5.23	23.24	0.4	27
54	CH_2CH_3	H		NO_2	264–265	43.5	$C_{18}H_{24}ClN_7O_4$	49.37	5.52	22.39	49.42	5.62	22.12	1.5	93
55	$CH_2CH_2OCH_3$	H		NO_2	248–250	79	$C_{19}H_{26}ClN_7O_5$	48.77	5.60	20.96	48.74	5.64	20.68	1.5	100
56	CH_2CH_3	H	$NAcCH_2CH_2OH$	NO_2	181–183	85	$C_{15}H_{16}N_6O_5$	50.00	4.48	23.23	49.78	4.42	23.36	3	50
57	CH_2CH_3	H	$NAcCH_2CH_2OAc$	NO_2	162–164	73	$C_{17}H_{18}N_6O_6$	50.74	4.51	20.89	50.75	4.54	20.99	3	50
58	CH_3	H	$N(NH_2)CH_2CH_2OH$	NO_2	220–221	78	$C_{12}H_{13}N_7O_4$	45.14	4.10	30.88	45.20	3.98	30.89	0.2	7
															
3a	CH_3	H			171–172.5	88	$C_{10}H_8N_4O_2$	55.55	3.73	25.92	55.36	4.03	25.95	...	...
3b	CH_2CH_3	H			149.5–150.5	79	$C_{11}H_{14}N_4O_2$	57.38	4.38	24.34	57.41	4.51	24.37	...	...
3c	$CH_2CH_2CH_3$	H			115–116	89	$C_{12}H_{12}N_4O_2$	59.01	4.95	22.94	58.77	5.10	23.05	...	...
3d	$CH_2CH_2OCH_3$	H			101–103	61	$C_{12}H_{12}N_4O_3$	55.38	4.65	21.53	55.36	4.62	21.59	...	...
3e	CH_3	CH_2CH_3			145–147	70	$C_{12}H_{12}N_4O_2$	59.01	4.95	22.94	59.13	4.90	23.16	...	...
Nitrofurazone[b]														12.5	50

[a] Cl. [b] Furacin® for comparison.

SCHEME I



in vivo antibacterial properties were determined using the methods described previously.[5] The data obtained on **5–58** are summarized in Table I. The effects of substitutions at three positions in the pyrazolopyrimidine ring system were investigated. In general,



in vivo activity against *Staphylococcus aureus* infections in mice was greatest when R was methyl. The activity decreased with increasing chain length. The substitution of alkyl groups for hydrogen at R^1 caused a significant decrease in both *in vitro* and *in vivo* antibacterial activity. All pyrazolopyrimidines containing a chlorine atom or a hydroxyl group at R^2 were inactive *in vivo* at the drug levels tested. Activity was found only when an amino or a substituted-amino group was introduced at R^2. Noteworthy activity was found when the hydroxyethylamino group was introduced at R^2. Substitution of a methyl group for hydrogen on the hydroxyl carbon atom enhanced the activity. The activity was increased further by the introduction of the bis(2-hydroxyalkyl)amino group at R^2. Thus, the most active compound prepared in this series was the 4-bis(2-hydroxypropyl)amino-1-methyl analog **39** which showed an ED_{50} of about 2 mg/kg.

Other significantly active compounds in this series were **10**, **12**, **14**, **27**, **28**, **30**, **32**, **35**, **36**, **40**, **42**, **43**, **45**, **47**, **51**, **52**, and **58**. The following compounds in Table I, when subjected to toxicopathological evaluation in two dogs for a period of 30 days at a peroral dosage level of approximately 40 mg/kg daily, elicited no signs of toxicosis: **7**, **19**, **22**, **33**, **35**, **36**, **39**, and **43**.

(5) F. F. Ebetino, W. F. Carey, and B. F. Stevenson, *J. Med. Chem.*, **6**, 633 (1963).

Experimental Section

All melting points were taken on a hot stage (Mel-Temp) melting point apparatus and are corrected.

2-Methoxyethylhydrazine.—A solution of 1070 g (21.4 moles) of 100% $N_2H_4 \cdot H_2O$ was heated to 100° and the heat source was turned off. 2-Methoxyethyl chloride[6] (421 g, 4.46 moles) was added dropwise with stirring during 2.5 hr at 98–102°. The resulting solution was heated for 10 hr at 105° and allowed to cool overnight. The product was isolated by continuous Et_2O extraction of the reaction mixture during 5 days. Evaporation of the Et_2O left an oil which was distilled through a 45.7-cm Vigreux column. The fraction boiling at 83–90° (56 mm) was collected; yield 317 g (79%). An analytical sample boiled at 84° (50 mm), $n^{26.5}D$ 1.4411.

Anal. Calcd for $C_3H_{10}N_2O$: C, 39.98; H, 11.18; N, 31.08. Found: C, 39.85; H, 11.25; N, 31.07.

5-Amino-4-cyano-1-(2-methoxyethyl)pyrazole (1a).—A solution of 152 g (1.24 moles) of ethoxymethylenemalononitrile (Kay-Fries) and 112 g (1.24 moles) of 2-methoxyethylhydrazine in 1 l. of absolute EtOH was refluxed for 24 hr. After removal of the solvents *in vacuo*, the residue was taken up in a minimum of boiling C_6H_6, treated with charcoal, and filtered. Dilution of the cooled filtrate with two volumes of petroleum ether (bp 30–60°) followed by thorough chilling precipitated the product as colorless platelets melting at 110–112°, yield 132 g (64%). Recrystallization from C_6H_6–petroleum ether raised the melting point to 114–115°.

Anal. Calcd for $C_7H_{10}N_4O$: C, 50.59; H, 6.07; N, 33.72. Found: C, 50.53; H, 6.14; N, 33.92.

5-Amino-4-cyano-1-methylpyrazole (1b) was prepared from $MeNHNH_2$ (Mathieson Chemical Corp.) according to the procedure of Cheng and Robins.[7]

5-Amino-4-cyano-1-ethylpyrazole (1c).—To 122 g (1.0 mole) of ethoxymethylenemalononitrile in 1 l. of MeOH was added in portions through the condenser 60 g (1.0 mole) of $EtNHNH_2$.[8]

(6) G. M. Bennett and F. Heathcoat, *J. Chem. Soc.*, 270 (1929).

(7) C. C. Cheng and R. K. Robins, *J. Org. Chem.*, **21**, 1240 (1956).

(8) A. N. Kost and R. S. Sagitullin, *Zh. Obshch. Khim.*, **33**, 867 (1963); *Chem. Abstr.*, **59**, 8724e (1963).

When the exothermic reaction had ceased, the solution was refluxed for 1 hr, and solvents were evaporated *in vacuo* on a steam bath. The residue was crystallized from EtOAc–MeOH (5:2) (charcoal) to give the product as colorless needles melting at 159–160°, yield 49.5 g. The filtrate was chromatographed over Al_2O_3 to give 66 g of product after evaporation of the solvent. The total yield was 115.5 g (85%). Recrystallization from EtOAc raised the melting point to 163–163.5°.

Anal. Calcd for $C_5H_5N_4$: C, 52.92; H, 5.92; N, 41.15. Found: C, 52.88; H, 5.96; N, 41.33.

5-Amino-4-cyano-1-propylpyrazole (1d) was prepared from $PrNHNH_2$[8] in 36% yield by the method described for **1c**. The chromatographed material was recrystallized from *i*-PrOH (charcoal) to give the product as colorless needles melting at 159–160°.

Anal. Calcd for $C_7H_{10}N_4$: C, 55.98; H, 6.71; N, 37.31. Found: C, 56.03; H, 6.60; N, 37.15.

5-Amino-4-cyano-3-ethyl-1-methylpyrazole (1e).—To 110 g (0.74 mole) of ethylethoxymethylenemalononitrile[9] in 750 ml of absolute EtOH was added slowly through the condenser 34.5 g (0.75 mole) of $MeNHNH_2$. When the exothermic reaction had ceased, the solution was refluxed for 3 hr, then stripped of solvents *in vacuo* on a steam bath. The crystalline residue was boiled for 15 min with charcoal in a minimum of C_6H_6, filtered, and cooled, and the filtrate was diluted with two volumes of petroleum ether to give the product as colorless needles melting at 84.5–86°, yield 103 g (94%).

Anal. Calcd for $C_7H_{10}N_4$: C, 55.98; H, 6.71; N, 37.31. Found: C, 55.98; H, 6.44; N, 37.36.

4-Amino-1-ethyl-6-(2-furyl)-1H-pyrazolo[3,4-*d*]pyrimidine (2a).—A solution of 66 g (0.7 mole) of 2-furonitrile, 96 g (0.7 mole) of **1c**, and 70 g (1.3 moles) of NaOMe in 1.5 l. of *i*-PrOH was refluxed for 48 hr. The solvents were removed *in vacuo* on a steam bath and the residue was slurried with 1 kg of ice–H_2O. The crude product was filtered, washed thoroughly with H_2O, and dried at 65°. Recrystallization from *i*-PrOH (charcoal) gave the product as colorless platelets. Other derivatives of **2** were prepared from the appropriate 5-amino-4-cyanopyrazole.

4-Amino-1-ethyl-6-(5-nitro-2-furyl)-1H-pyrazolo[3,4-*d*]pyrimidine (7).—Compound **2a** (25 g) was pulverized and added in portions with stirring to 250 ml of concentrated H_2SO_4 below 10°. The temperature was lowered to −5° by means of an ice–salt bath and kept below 10°, while 50 ml of concentrated HNO_3 in 50 ml of concentrated H_2SO_4 was added dropwise during 15 min. Stirring was then continued in the cold for 1 hr. The mixture was poured over 1 kg of ice, neutralized with 20% NaOH, and diluted with H_2O to a final volume of 5 l. The crude product was filtered, washed thoroughly with H_2O to remove traces of Na_2SO_4, and air dried on the funnel. Recrystallization from DMF (charcoal) gave the product as yellow needles. Compounds **8** and **9** were prepared from the appropriate **2**.

N-(4-Cyano-1-methyl-5-pyrazolyl)-2-furamide (3a).—To a solution of 91 g (0.74 mole) of **1b** in 300 ml of pyridine was added cautiously with stirring, 101 g (0.74 mole) of 2-furoyl chloride. After the solution was heated on a steam bath for 1 hr, it was poured into 1 l. of ice–H_2O and allowed to stand overnight. The crude product was filtered, washed with cold H_2O, and dried at 65°. This material was used without further purification. Recrystallization of a sample from aqueous MeOH (charcoal) gave the product as large, colorless needles. Compounds **3b–e** were prepared from the appropriate 5-amino-4-cyanopyrazole.

6-(2-Furyl)-1-methyl-1H-pyrazolo[3,4-*d*]pyrimidin-4(5H)-one (4a).—To a warm (35–45°) solution of 62 g of NaOH in 2700 ml of H_2O was added cautiously with stirring, 230 ml of 30% H_2O_2. This was followed by the addition of 245 g (1.13 moles) of **3a** in small portions during about 1 hr. A few milliliters of EtOAc was added periodically to control frothing. The solution was then heated on a steam bath under reflux for 20 hr, chilled, and neutralized *cautiously* with AcOH. Frothing was controlled again by the addition of EtOAc. The solids were filtered, washed with cold H_2O, and dried at 65° to give 167 g of crude product. The pulverized, crude product was stirred for 15 min in 500 ml of MeCN and filtered, and the residue was dried at 65°. This separation process was repeated until an infrared spectrum of the residual solids showed an absence of nitrile absorption at 2350 cm^{-1}. Evaporation of the MeCN washings gave 29 g of recovered **3a**. The **4a** thus obtained was used without further purification. Recrystallization of a sample from $MeNO_2$ (charcoal) gave the product as colorless needles. Compounds **4b–e** were prepared from the appropriate **3**.

1-Methyl-6-(5-nitro-2-furyl)-1H-pyrazolo[3,4-*d*]pyrimidin-4(5H)-one (5a).—To 300 ml of concentrated H_2SO_4 kept below 20° was added 101 g (0.47 mole) of **4a** in small portions with stirring. A solution of 50 ml of concentrated HNO_3 in 50 ml of concentrated H_2SO_4 was added dropwise at 25–30°. The mixture was stirred at 25–30° for 1 hr following the addition and poured over 2 kg of ice. The excess acid was neutralized by the careful addition of 20% NaOH with cooling. The crude product was filtered, washed thoroughly with H_2O, and dried at 56°. Recrystallization from aqueous DMF (charcoal) gave **5a** as yellow needles. Compounds **5b–e** were prepared from the appropriate **4**.

4-Chloro-1-methyl-6-(5-nitro-2-furyl)-1H-pyrazolo[3,4-*d*]pyrimidine (6a).—To a suspension of 52 g (0.25 mole) of PCl_5 in 300 ml of $POCl_3$ was added 65 g (0.25 mole) of **5a**. The resulting suspension was refluxed with stirring for 3 hr, after which time solution was complete. The cooled solution was diluted with 500 ml of petroleum ether and filtered. After washing with petroleum ether and air drying on the funnel, the crude product was used without further purification. Recrystallization of a sample from aqueous DMF (charcoal) gave **6a** as light yellow needles. Compounds **6b–e** were prepared from the appropriate **5**.

1-Methyl-4-methylamino-6-(5-nitro-2-furyl)-1H-pyrazolo-[3,4-*d*]pyrimidine (10).—To a mixture of 42 g (0.15 mole) of **6a** in 350 ml of DMF was added 25 g (0.32 mole) of 40% aqueous $MeNH_2$. The mixture was stirred for 15 min, heated on a steam bath for 15 min, cooled, and diluted with 400 ml of H_2O. The crude solid was filtered and recrystallized from aqueous DMF (charcoal) from which the product separated as yellow needles. The remaining compounds in Table I were prepared from the appropriate **6** and amine. Other recrystallization solvents used included alcohols and MeCN.

Acetylated Derivatives.—Compounds **43**, **44**, and **22** were refluxed in Ac_2O for a few hours to give, after quenching on ice and recrystallization from MeOH, compounds **47**, **48**, and **57**, respectively. A monoacetylated derivative of **22** (**56**) was prepared by refluxing **22** in excess AcCl–AcOH solution. The position of the acetyl group was assigned arbitrarily.

Acknowledgments.—The author is indebted to Mr. W. O. Smith and Mr. B. F. Stevenson and associates for assistance in the preparation of these compounds, to Dr. G. S. Denning, Jr., for synthesizing 2-methoxyethylhydrazine, to Mr. G. Gustin and Mr. M. Tefft for the elemental analyses, to Mr. H. E. Russell and Mr. R. Freedman for the microbiological testing data, and to Dr. G. S. Murti and associates for performing the toxicopathological studies.

(9) L. Nicholl, P. J. Tarsio, and H. Blohm, U. S. Patent 2,824,121; *Chem. Abstr.*, **52**, 11909*i* (1958).