A 10-mg portion of the protected peptide prepd as above was treated with HBr–CF_3COOH for 30 min at 25°, evapd on a rotary evaporator at 25°, and then lyophilized from AcOH giving 8 mg. The ir spectrum showed succinimide carbonyl bands at 1680 and 1730 cm^{-1} of comparable intensity to those in an equimolar mixt of the heptapeptide and succinimide.

Antibacterial Nitrofuran Derivatives. 2. 5-Nitro-2-furaldehyde Aminoacethydrazones

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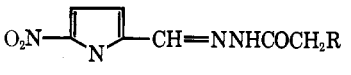
The nitrofuran derivatives used in the treatment of bacterial infections of the urinary tract show only a slight water solubility, a property which limits pharmaceutical formulations and therapeutic use. A series of 5-nitro-2-furaldehyde aminoacethydrazones with the following structure were synthesized in order to obtain new antibacterial nitrofurans with a better water solubility.



These products were prepared by condensing the 5-nitro-2-furaldehyde with the corresponding aminoacethydrazides.

Biological Results (Table I).—The acute toxicity was determined ip in mice. All compounds were tested for bacteriostatic activity *in vitro* on the following microorganisms: *Escherichia coli* 100, *Salmonella typhimurium* 1090, *Pseudomonas aeruginosa* H2, *Proteus vulgaris* OX, *Micrococcus pyogenes* SG511, *Streptococcus pyogenes* A88, *Bacillus subtilis* ATCC 9466, *Clostridium novyi*, *Mycobacterium tuberculosis* $H_{37}Ra$, *Trichophyton mentagrophytes* 1236, and *Candida albicans* 28.

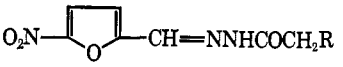
None of the compounds, or nitrofurantoin, exhibited significant activity against *Cl. novyi*, *M. tuberculosis*, *T. mentagrophytes*, and *C. albicans*. The monoalkylaminoacethydrazones (**1**–**7**) showed an *in vitro* antibacterial activity generally higher than that of nitrofurantoin. The dialkylaminoacethydrazones (**8**–**18**) showed an *in vitro* antibacterial activity comparable to that of nitrofurantoin.

The urinary excretion was determined in rats. The urinary excretion of monoalkylaminoacethydrazones was highest for the ethylamino derivative **2** and decreased with lengthening of the side chain. The dialkylamino derivatives were scarcely excreted in the urine whereas a slight excretion was observed for the *N*-dimethylamino (**15**) and pyrrolidino (**12**) derivatives. By contrast the *N'*-methylpiperazino derivative (**13**)[1] was excreted to a large extent.

Only **13** was active in experimental infections. It exhibited an activity comparable to **19** in a systemic infection of mice with *Strep. pyogenes* C 203 and a higher activity than **19** in infections of mice with *S. typhimurium* 1086 and in im infection of mice with *Staphylococcus aureus* 742. Compd **13** was active on the ascending *P. vulgaris* urinary tract infection of rats.[2,3]

TABLE I

ANTIMICROBIAL ACTIVITY OF 5-NITRO-2-FURALDEHYDE AMINOACETHYDRAZONES



No.	*E. coli*	*S. typhimurium*	*Ps. aeruginosa*	*P. vulgaris*	*M. pyogenes*	*Strep. pyogenes*	*B. subtilis*	*Drug urinary excretion*	LD_{50}, mg/kg ip
1	10	10	40	40	2.5	20	1.25	4	188[g]
2	10	10	40	20	5	20	1.25	10	143[g]
3	20	20	40	40	20	40	2.5	2	172[g]
4	20	20	40	40	5	20	1.25	3.5	170[g]
5	10	10	80	80	10	10	5	0	120[g]
6	10	20	80	40	5	10	5	0	113[g]
7	10	10	40	40	2.5	5	0.625	0	130[g]
14[a,b]	10	10	40	40	5	40	5	4.5	300[h]
15[a,c]	10	20	80	80	20	160	20	0	109[h]
8	2.5	40	80	80	10	80	10	0	150
16[a,d]	20	40	80	80	10	80	5	0	390
9	5	80	>160	>160	20	80	5	0	700
10	10	40	>160	>160	2.5	40	2.5	0	900
11	20	>160	>160	>160	20	80	10	0	800
12	20	20	80	80	20	1.25	5	2.5	250[h]
17[a,e]	80	80	80	80	40	10	2.5	0	120[h]
18[a,f]	80	160	>160	160	20	2.5	2.5	0	420[h]
13	40	40	160	80	20	2.5	20	24	315
19[i]	5	40	160	80	10	5	10	37	96

[a] While our study was in progress, A. Jujita, S. Minami, and H. Takamatsu, *Yakugaku Zasshi*, **84**, 890 (1964), reported the synthesis and antimicrobial data of these products. [b] R = $N(CH_3)_2$. [c] R = $N(C_2H_5)_2$. [d] R = $N(i\text{-}C_3H_7)_2$. [e] R = piperidino. [f] R = morpholino. [g] AcOH salt. [h] HCl salt. [i] Nitrofurantoin.

(1) Nonproprietary name, nifurpipone.

(2) L. Degen, M. Salvaterra, S. Vella, D. Nardi, and E. Massarani, *Chemotherapy*, in press.

(3) L. Degen, M. Salvaterra, and S. Vella, *ibid.*, in press.

TABLE II

AMINOACETHYDRAZIDES

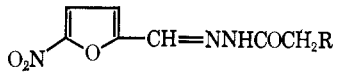
$RCH_2CONHNH_2$

R	Yield, %	Bp, °C (mm)	Mp, °C	Recrystn solvent	Formula[d]
EtNH	83	105 (0.1)			$C_4H_{11}N_3O$
n-PrNH	92		50–52	C_6H_6	$C_5H_{13}N_3O$
i-Pr-NH	84	101 (0.5)	66–68	C_6H_6	$C_5H_{13}N_3O$
n-BuNH	91		66	C_6H_6	$C_6H_{15}N_3O$
i-BuNH	95		88	C_6H_6	$C_6H_{15}N_3O$
CH_2=$CHCH_2NH$	88	115 (0.2)			$C_5H_{11}N_3O$[f]
			142–144	EtOH	$C_5H_{11}N_3O \cdot 2HCl$[e]
n-Pr_2N	92[a–c]	103 (0.3)			$C_8H_{19}N_3O$
n-Bu_2N	90[a,b]	106–107 (0.3)			$C_{10}H_{23}N_3O$
i-Bu_2N	79[a–c]	105 (0.5)			
		158 (12)			$C_{10}H_{23}N_3O$
n-Am_2N	75[a,b]	124 (0.2)			$C_{12}H_{27}N_3O$
N'-Methylpiperazino	90[c]	125–130 (0.2)	88–89	Ligroin	$C_7H_{16}N_4O$

[a] Hydrazine hydrate (0.02 mole) was used. [b] The reaction was carried out for 12 hr. [c] After distillation, the residue was crystallized from EtOAc to give N^1,N^2-bis(*N'*-methyl-*N*-piperazino acetyl)hydrazine, mp 113°. *Anal.* ($C_{14}H_{28}N_6O_2$) C, H, N; C: calcd, 53.82; found, 53.33. [d] All compds were analyzed for C, H, N. [e] Cl anal. also. [f] Not analyzed.

TABLE III

5-NITRO-2-FURALDEHYDE AMINOACETHYDRAZONES



No.	R	Yield, %	Recrystn solvent	Mp, °C	Formula[b]
1	NHMe	60	EtOAc	143	$C_8H_{10}N_4O_4$
			EtOH	151	$C_8H_{10}N_4O_4 \cdot CH_3COOH$
2	NHEt	88	EtOAc	127–128	$C_9H_{12}N_4O_4$
			EtOAc	163	$C_9H_{12}N_4O_4 \cdot CH_3COOH$
				219 dec	$C_9H_{12}N_4O_4 \cdot HCl$[c]
3	NH-*n*-Pr	82	EtOAc	130–131	$C_{10}H_{14}N_4O_4$
			i-PrOH	125	$C_{10}H_{14}N_4O_4 \cdot CH_3COOH$
4	NH-*i*-Pr	95	EtOAc	151–152	$C_{10}H_{11}N_4O_4$
			i-PrOH	148	$C_{10}H_{14}N_4O_4 \cdot CH_3COOH$
5	NH-*n*-Bu	93	EtOAc	128	$C_{11}H_{16}N_4O_4$
			EtOAc	140	$C_{11}H_{16}N_4O_4 \cdot CH_3COOH$
6	NH-*i*-Bu	88	EtOAc	138	$C_{11}H_{16}N_4O_4$
			i-PrOH	137	$C_{11}H_{16}N_4O_4 \cdot CH_3COOH$
7	$NHCH_2CH$=CH_2	94	EtOAc	125–126	$C_{10}H_{12}N_4O_4$
			i-PrOH	125	$C_{10}H_{12}N_4O_4 \cdot CH_3COOH$
8	*N*-*n*-Pr_2	60	EtOH–H_2O	126	$C_{13}H_{20}N_4O_4$
9	*N*-*n*-Bu_2	96	EtOH–H_2O	146	$C_{15}H_{24}N_4O_4$
10	*N*-*i*-Bu_2	94	EtOH	148	$C_{15}H_{24}N_4O_4$
11	*N*-*n*-Am_2	55	EtOH–H_2O	116	$C_{17}H_{28}N_4O_4$
12	Pyrrolidino	87	*i*-PrOH	158	$C_{11}H_{14}N_4O_4$
			EtOH	213	$C_{11}H_{14}N_4O_4 \cdot HCl$[c]
13	*N'*-Me-piperazino	90[a]	EtOAc	167–168	$C_{12}H_{17}N_5O_4$
			EtOAc	128–129	$C_{12}H_{17}N_5O_4 \cdot CH_3COOH$
			EtOH–H_2O	250	$C_{12}H_{17}N_5O_4 \cdot 2HCl$[c]

[a] The reaction was carried out for 90 min. See Table II, footnote *d*. [c] See Table II, footnote *e*.

Experimental Section[4]

Ethyl (Diisobutylamino)acetate.—A mixt of 6.45 g (0.05 mole) of *i*-Bu_2NH (4.2 g, 0.05 mole) of $NaHCO_3$, 25 ml of Me_2CO, and 6.1 g (0.05 mole) of ethyl chloroacetate was refluxed for 16 hr. Then the hot mixt was filtered and the residue was washed with hot Me_2CO. The solvent was evapd *in vacuo* and the residue was distd at 103° (13 mm): yield 8.05 g (75%); η^{22}°D 1.4262. *Anal.* ($C_{12}H_{15}NO_2$) C, H, N.

Ethyl (di-*n*-amylamino)acetate was prepd from *n*-Am_2NH and $ClCH_2CO_2Et$ in a similar way: yield 80%; bp 140° (12 mm); η^{20}°D 1.4369. *Anal.* ($C_{14}H_{29}NO_2$) C, H, N.

Aminoacethydrazides. General Procedure.—A mixt of 0.01 mole of ethyl aminoacetate, 0.01 mole of hydrazine hydrate, and 2 ml of EtOH was refluxed for 8 hr. EtOH was evapd *in vacuo* and the residue was treated with Et_2O. When a solid was obtained, it was filtered and crystd. When no solid was obtained, the solvent was evapd and the residue was distd below 160°. Higher temps caused formation of RCONHNHCOR derivs. The unchanged ethyl aminoacetates were recovered from Et_2O or from lower boiling fractions (Table II).

5-Nitro-2-furaldehyde Aminoacethydrazones. General Procedure.—To a soln of 0.01 mole of aminoacethydrazine in 2 ml of AcOH was added a soln of 0.01 mole of 5-nitro-2-furaldehyde in 1 ml of AcOH. The reaction was exothermic. The mixt was stirred for 30 min, poured in to Et_2O, and stirred until a solid sepd; this was filtered and crystd.

Some products pptd as acetates, others as bases. The bases were also obtd by making alkaline with Na_2CO_3 the aq solns of acetates. The HCl salts were prepd by acidifying with anhyd HCl an EtOH soln of bases (Table III).

Pharmacological Methods.—For acute toxicity NMRI albino mice (18–20 g) and for urinary excretion Wistar albino rats (200–250 g) were used.

(4) Melting points are uncorrected and were determined in open glass capillaries on a Büchi apparatus. When analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.

Acute toxicity, and antimicrobial and antifungal activity *in vitro* were determined as previously described.[5]

Urinary Excretion of the Drug.—A single oral dose of 20 mg/kg of the drug was administered by intubation and the urine of each rat was collected (in metabolic cage) after 6 hr. The urinary level was determined according to the standard cylinder plate assay[6a] modified by Degen, *et al.*[6] *B. subtilis* ATCC 9466 was used as test organism. Each drug was used as its own standard.

(5) E. Massarani, D. Nardi, L. Degen, and M. Magistretti, *J. Med. Chem.*, **9**, 617 (1966).

(6) (a) "The Pharmacopeia of the United States of America," 17th revision, U. S. P., Bethesda, Md., 1965; (b) L. Degen, M. Salvaterra, and S. Vella, *Chemotherapy*, in press.

Antibacterial Nitrofuran Derivatives. 3. 5-Nitro-2-furaldehyde Piperazinoacylhydrazones

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As a part of our investigations on nitrofuran derivatives we recently described[1] a series of water-soluble mono- and disubstituted aminoacethydrazones of 5-nitro-2-furaldehyde active as antibacterial agents.

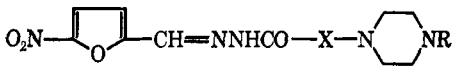
The 5-nitro-2-furaldehyde *N'*-methylpiperazinoacethydrazone **19**[2] showed the highest urinary excretion and exhibited antibacterial activity in systemic infection of mice with *Streptococcus pyogenes* and *Salmonella typhimurium*, in im infection of mice with *Staphylococcus aureus*, and on urinary *Proteus vulgaris* infection of rats.

(1) E. Massarani, D. Nardi, A. Tajana and L. Degen, *J. Med. Chem.*, **14**, 633 (1971).

(2) Nonproprietary name, nifurpipone.

These activities were comparable to or sometimes better than that of nitrofurantoin.[3-5]

The purpose of this paper was to synthesize a series of compounds with the following structure



to determine the effect of various substituents at the N atom of piperazine and the effect of the modification of the X group.

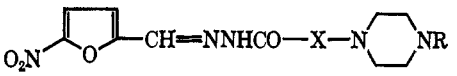
Chemistry.—The synthetic steps leading to the formation of 5-nitro-2-furaldehyde piperazinoacylhydrazones are outlined in Scheme I and are described in the Experimental Section.

The *N*-(β-hydroxyethyl)-, *N*-benzyl-, *N*-(*p*-nitrophenyl)-, *N*-acetyl-, and *N*-(diethylcarbamoyl)piperazines were prepared according to other methods previously reported.[6]

Biological Results (Table I).—The acute toxicity was determined ip in mice. All compounds were tested for bacteriostatic activity *in vitro* on the following microorganisms: *Escherichia coli* 100, *Salmonella typhimurium* 1090, *Pseudomonas aeruginosa* H2, *Proteus vulgaris* OX, *Micrococcus pyogenes* SG511, *Streptococcus pyogenes* A88, *Bacillus subtilis* ATCC 9466, *Myco-*

TABLE I

ANTIMICROBIAL ACTIVITY OF 5-NITRO-2-FURALDEHYDE N'-SUBSTITUTED PIPERAZINOACYLHYDRAZONES



No.	*E. coli*	*S. typhi murium*	*Ps. aeruginosa*	*P. vulgaris*	*M. pyogenes*	*Strep- pyogenes*	*B. subtilis*	*M. tuberculosis*	Drug urinary excre- tion	LD_M, mg/kg ip
1	80	160	>160	160	40	160	40	40	0	300
2	20	40	>160	>160	10	20	10	10	0	300
3	10	40	>160	40	10	40	5	80	18.5	260
4	10	10	>160	40	5	10	5	40	20	120
5	20	20	160	80	5	20	5	20	11.5	300
6	10	20	>160	80	5	10	5	10	18	350
7	10	40	>160	80	10	20	5	>160	0	150
8	10[c]	>160	>160	>160[c]	0.625[c]	>160	>160	>160	0	1300
9	10	>160	>160	>160	80	10	5	1.25	0	200
10	10	>160	>160	>160	10	20	5	20	0	180
11	80	>160	>160	>160	160	20	>160	40	*d*	210
12	40	160	>160	>160	10	1.25	5	20	0	270
13	20	80	160	160	10	80	10	2.5	0	180
14	80	80	80	80	20	40	5	40	0	500
15	>160	>160	>160	>160	20	2.5	40	0.31	0	>3000
16	20	80	>160	160	10	5	20	40	0	350
17	80	>160	>160	>160	10	5	10	40	0	80
18	>160	>160	>160	>160	160	>160	>160	>160	*d*	>3000
19[a]	40	40	160	80	20	2.5	20	>160	24	315
20[b]	5	40	160	80	10	5	10	>160	37	96

[a] 5-Nitro-2-furaldehyde *N'*-methylpiperazinoacethydrazone. [b] Nitrofurantoin. [c] In Difco nutrient broth. [d] Not tested.

(3) L. Degen, M. Salvaterra, S. Vella, D. Nardi, and E. Massarani, *Chemotherapy*, in press.

(4) L. Degen, M. Salvaterra, and S. Vella, *ibid.*, in press.

(5) L. Degen, M. Salvaterra, and S. Vella, *ibid.*, in press.

(6) (a) J. Kitchen and C. B. Pollard, *J. Org. Chem.*, **8**, 338 (1943); (b) J. C. Craig and R. J. Young, *Org. Syn.*, **42**, 19 (1962); (c) V. Prelog, G. J. Driza, *Collect. Czech. Chem. Commun.*, **5**, 497 (1933); *Chem. Abstr.*, **28**, 1348 (1934); (d) R. L. Bent, J. C. Dessloch, F. C. Duennebier, D. W. Fassett, D. B. Glass, T. H. James, D. B. Julian, W. R. Ruby, J. M. Snell, J. H. Sterner, J. R. Thirtle, P. W. Vittum, and A. Weissberger, *J. Amer. Chem. Soc.*, **73**, 3100 (1951); (e) G. Schorsch, U. S. Patent 2,973,362, Feb 28, 1961; *Chem. Abstr.*, **55**, 14488c (1961); (f) K. Fujii, K. Tomino, and H. Watanabe, *Yakugaku Zasshi*, **74**, 1049 (1954).