# A NEW FURAN DERIVATIVE

## The Formation, Antibacterial Activity and Pharmacological Nature of 3-Amino-6-[(5-nitro-2-furyl)vinyl]-as-triazine Hydrochloride

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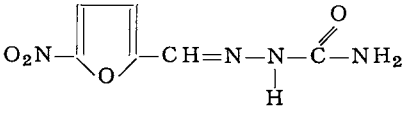
*Received for publication, July 1, 1962.*

### Introduction

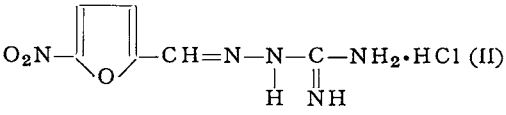
In 1944, Dodd and Stillman[1] reported that 5-nitro-2-furaldehyde semicarbazone, (nitrofurazone*) (I) had antibacterial activity against a wide spectrum of organisms including the suppurative bacteria. These studies paved the way for the use of this drug for the treatment of superficial infections.



(I)

Since then, numerous studies have been conducted on nitrofuran derivatives.

The author and his co-workers have for some time been particularly interested in the development of therapeutic agents active against the gram-negative bacteria such as the Shigella and Escherichia and in 1948[2] reported on the antibacterial activity of 5-nitro-2-furaldehydeguanylhydrazone hydrochloride** (II). This product was found to have activity comparable to that of Furacin, but it was more soluble in water.



(II)

Antibiotics such as streptomycin, chlortetracycline and chloramphenicol were discussed at approximately the same time. Because of the efficacy of these drugs, antibiotics came into the foreground and this situation undoubtedly had a retarding effect on studies with nitrofuran derivatives.

In 1951 Uoda[3] synthesized 1,5-bis-(5-nitro-2-furyl)-3-pentadienone guanylhydrazone hydrochloride*** (III), a drug recommended for therapy against dysentery and other intestinal diseases. Shortly thereafter Mintzer *et al.*[4] reported the efficacy of N-(5-nitro-2-furfurylidene)-1-amino-hydantoin, (nitrofurantoin****)(IV)against urinary tract infections.

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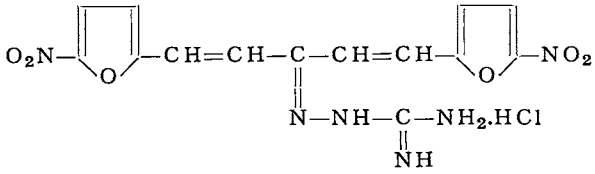
* The Eaton Laboratories trade mark name for nitrofurazone is Furacin.

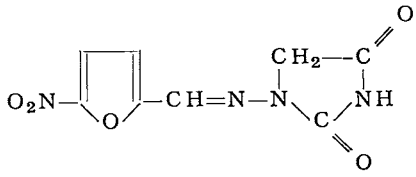
** Trade mark name for this compound is Guanofuracin.

*** Trade mark name for this compound is Panazon.

**** The Eaton Laboratories trade mark name for nitrofurantoin is Furadantoin.



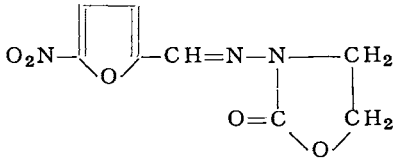
(III)



(IV)

It has been reported that since 1959, in Japan, there has been an increase in resistance of Shigella and other types of bacteria to antibiotics and there has been extensive development of cross resistance to the clinically used antibiotics. Accordingly, the efficacy of these antibiotics against dysentery bacilli and other intestinal bacteria has been decreasing. Thus the need for new chemotherapeutic agents has become more urgent.

Rogers *et al.*[6] reported that N-(5-nitro-2-furfurylidene) -amino-2-oxazolidone, (furazolidone*) (V) had broad spectrum activity. Minimal inhibitory concentrations against *Escherichia coli*, *Shigella flexneri*, *Staphylococcus aureus* and *Streptococcus pyogenes* were 0.07, 0.06, 0.39 and 11.38 mg per 100ml respectively. In our comparative tests, furazolidone was approximately eight times as active as guanofuracin against *E. coli* and *S. flexneri*.



(V)

By contrast, panazon is thirty to sixty times as active as guanofuracin against the gram-positive bacteria, but it exhibits relatively weak activity against the gram-negative bacteria. Although the *in vitro* activity of furazolidone is of the same order as that of chloramphenicol and streptomycin, it has only limited utility for the treatment of intestinal infections.

In the course of studies on the chemical nature and antibacterial activity of hydrazones of 1,5-bis-(5-nitro-2-furyl)-3-pentadienone, Miura *et al.* [6][7][8][9][10][11] observed that on heating of the guanylhydrazone the antibacterial activity was markedly increased (Table 1).

* The Eaton Laboratories trade mark name for furazolidone is Furoxone and the Takeda Chemical Co. trade mark name is Puradin.

Table 1. Effect of heating* on the antibacterial action of hydrazones of 1,5-bis-(5-nitro-2-furyl)-3-pentadienone

| Hydrazones | Test organism** | Maximal inhibiting dilution | | Change in activity*** |
|---|---|---|---|---|
| | | Before heating | After heating | |
| Phenylhydrazone | S | 64,000 | 64,000 | 1 |
| | D | 0 | 0 | — |
| | C | 0 | 0 | — |
| Semicarbazone | S | 512,000 | 128,000 | ¼ |
| | D | 640,000 | 160,000 | ¼ |
| | C | 640,000 | 640,000 | 1 |
| Thiosemicarbazone | S | 1,024,000 | 512,000 | ½ |
| | D | 64,000 | 256,000 | 4 |
| | C | 32,000 | 256,000 | 8 |
| Guanylhydrazone | S | 8,172,000 | 1,024,000 | ⅛ |
| | D | 16,000 | 2,048,000 | 128 |
| | C | 16,000 | 2,048,000 | 128 |

* Thirty minutes at 190°C in propylene glycol.

** S = *Staphylococcus aureus*; D = *Shigella flexneri*; C = *Escherichia coli*.

*** Ratio of activities after and before heating.

When heated in propylene glycol for 30 minutes at 190°C, the activity aginst *E. coli* and *S. flexneri* was increased one hundred and twenty-eight fold, whereas the activity against *S. aureus* was decreased eight fold. Under similar conditions, there was no change in the activity of the phenylhydrazone, and there was a slight decrease in the activity of the semicarbazone. Heating the thiosemicarbazone resulted in a low order increase in activity against *E. coli* and *S. flexneri* and a slight decrease in activity against *S. aureus*. These results suggested the possibility that chemical modification to new active agents took place during the heating.

In further studies on the conversion of the above guanylhydrazone by heating in formamide containing ammonia, color changes and antibacterial activities were determined (Table 2).

Table 2. Heat treatment* of 1,5-bis-(5-nitro-2-furyl)-3-pentadienone guanylhydrazone hydrochloride

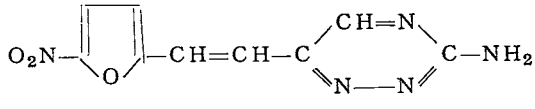
| Temp. | Time (minutes) | Color | Maximum inhibiting dilution against *Shigella flexneri* |
|---|---|---|---|
| 120° C | 0 | scarlet red | 20,000 |
| 120° C | 5 | orange red | 1,280,000 |
| 120° C | 10 | red | 2,560,000 |
| 120° C | 30 | yellow | 5,120,000 |
| 120° C | 60 | darkened yellow | 2,560,000 |
| 120° C | 120 | dark yellow | 640,000 |
| 200° C | 5 | yellow | 5,120,000 |

* In formamide containing ammonia.

Maximum antibacterial activity was observed when 1,5-bis-(5-nitro-2-furyl)-3-pentadienoneguanylhydrazone was heated at 120°C for 30 minutes, or at 200°C for 5 minutes. Several new compounds have been isolated from this reaction mixture. The most active compound has been called panfuran.

## Chemical Nature of Panfuran

Panfuran base (VI) has been identified as 3-amino-6-[(5-nitro-2-furyl)vinyl]-as-triazine




(VI)

Panfuran base is obtained as small red columnar crystals or as rhomboid crystals, decomposing at 269°C.
Panfuran hydrochloride is isolated as bright yellow columnar crystals decomposing at 235-240°C. At room temperature, it is soluble in water at concentrations of 0.2 per cent.

## *In Vitro* Antimicrobial Activity of Panfuran

In Table 3 are presented comparative studies indicating maximal dilutions possible for bacteriostatic and bactericidal activities of panfuran, guanofuracin, panazon, furazolidone, chloramphenicol, tetracycline, and kanamycin.

Table 3. Comparison of *in vitro* antibacterial activity of nitrofuran derivatives and antibiotics

(μgm/ml)

| | 1. Bacteriostatic activity<br>2. Bactericidal activity | Maximum dilution at which compounds exhibit activity | | | | |
|---|---|---|---|---|---|---|
| | | *Shigella dysenteriae* (Komagome B III strain) | *Salmonella typhi* (0) | *Esher. coli* (Gakusei strain) | *Staph. aureus* (Terashima) | *Strep. haemolyticus* (S-type) |
| Panfuran | 1 | 0.10 | 0.05 | 0.03 | 0.19 | 0.39 |
| | 2 | 0.10 | 0.05 | 0.05 | 0.39 | 0.78 |
| Guanofuracin | 1 | 7.82 | 7.82 | 7.82 | 7.82 | 7.82 |
| | 2 | 7.82 | 7.82 | 7.82 | 7.82 | 7.82 |
| Panazon | 1 | 62.50 | 125.0 | 62.50 | 0.12 | 0.98 |
| | 2 | 125.0 | 125.0 | 125.0 | 0.49 | 0.98 |
| Furazolidone | 1 | 0.98 | 0.98 | 0.98 | 1.95 | 62.50 |
| | 2 | 1.95 | 0.98 | 1.95 | 1.95 | 62.50 |
| Chloramphenicol | 1 | 0.98 | 1.95 | 1.95 | 3.91 | 0.98 |
| | 2 | 15.62 | 31.25 | 62.50 | 31.25 | 1.95 |
| Tetracycline | 1 | 1.95 | 1.56 | 3.91 | 0.98 | 0.49 |
| | 2 | 15.62 | 2.50 | 62.50 | 7.82 | 1.95 |
| Kanamycin | 1 | 3.91 | 1.56 | 15.62 | 0.49 | 31.25 |
| | 2 | 15.62 | 1.56 | 15.62 | 0.98 | 31.25 |

These results were obtained by the broth dilution procedure. Two-fold dilutions of antimicrobial agent were made in 2 ml of pH 7.0 broth. Inoculum consisted of two drops of a 1:100,000 dilution of test culture and incubation was at 37°C for 24 hours. After the tubes were examined for complete inhibition of growth(bacteriostatic determinations), aliquots were removed from clear tubes as inoculum for fresh media so as to determine whether any viable cells remained (bactericidal determinations). Except for the bacteriostatic activity of panazon against *Streptococcus pyogenes*, the superior activity of panfuran is apparent.

Panfuran exhibits outstanding activity against *E. coli* and *S. flexneri*. It is also highly significant that for the nitrofuran compounds the bacteriostatic and bactericidal concentrations are of essentially the same order. For chloramphenicol and tetracycline, much higher concentrations of antibiotic are necessary for bactericidal activity as contrasted with bacteriostatic concentrations.

The bacteriostatic activities of panfuran, panazon, kanamycin and streptomycin against a human strain of Mycobacterium tuberculosis (Kawakami strain) were compared on Kirchner's medium supplemented with serum at 10 per cent (Table 4) maximum inhibitory dilution.

Table 4. Comparison of *in vitro* activity of nitrofuran derivatives and antibiotics against *Mycobacterium tuberculosis*

| Drug dilution | Panfuran | | | | Panazon | | | | Streptomycin | | | | Kanamycin | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Weeks | | | | | | | | | | | | | | | |
| | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
| 80,000 | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| 160,000 | − | − | − | − | − | − | + | ++ | − | − | − | − | − | − | − | − |
| 320,000 | − | − | − | − | − | + | ++ | +++ | − | − | − | − | − | − | − | − |
| 640,000 | − | − | − | − | + | ++ | ++ | +++ | − | − | − | − | − | − | − | − |
| 1,280,000 | − | ± | ++ | ++ | + | ++ | +++ | +++ | − | − | ± | + | − | − | + | + |
| 2,560,000 | − | ++ | +++ | +++ | + | ++ | +++ | +++ | + | + | ++ | +++ | − | ++ | ++ | +++ |
| 5,120,000 | ± | ++ | +++ | +++ | + | ++ | +++ | +++ | ++ | ++ | +++ | +++ | ± | ++ | +++ | +++ |
| Control | ± | ++ | +++ | +++ | + | ++ | +++ | +++ | ++ | ++ | +++ | +++ | + | ++ | +++ | +++ |

After four weeks incubation, both panfuran and kanamycin were bacteriostatic at 1:640,000 dilution, whereas panazon was considerably less active.

Kimura and Kaibara[12] reported on the high order of antimicrobial activity of panfuran (Table 5).

Table 5. Antimicrobial spectrum* of panfuran

| Microorganism | Minimum inhibitory concentration (μgm/ml) |
|---|---|
| *Staphylococcus aureus* (209P) | 0.25 |
| *Staphylococcus aureus* (Terashima) | 0.25 |
| *Diplococcus pneumoniae* | 0.5 |
| *Streptococcus pyogenes* (S-43-M) | 0.25 |
| *Streptococcus pyogenes* (variant-Y) | 0.5 |
| *Bacillus subtilis* (PCL) | 0.5 |
| *Corynebacterium diphtheriae* | 0.25 |
| *Escherichia coli* (O-6) | 0.3 |
| *Escherichia coli* (O-9) | 0.5 |
| *Escherichia coli* (O-18) | 0.05 |
| *Escherichia coli* (O-55) | 0.05 |
| *Escherichia freundii* | 0.25 |
| *Aerobacter aerogenes* | 0.5 |
| Arizona | 0.05 |
| *Alkaligenes dispar* | 0.1 |
| *Klebsiella rhinosoleromatis* | 1.0 |
| *Proteus vulgaris* | 1.0 |
| *Shigella dysenteriae* (A-2) | 0.1 |

| Microorganism | Minimum inhibitory concentration (μgm/ml) |
|---|---|
| *Shigella dysenteriae* (A-5) | 0.1 |
| *Shigella dysenteriae* (A-7) | 0.25 |
| *Shigella flexneri* (B-2a) | 0.1 |
| *Shigella flexneri* (B-2b) | 0.1 |
| *Shigella sonnei* (D) | 0.05 |
| *Salmonella typhi* (T-63) | 0.1 |
| *Salmonella paratyphi* A | 0.1 |
| *Salmonella paratyphi* B | 0.1 |
| *Salmonella entritidis* | 0.01 |
| *Pseudomonas aeruginosa* | 20.0 |
| *Pseudomonas enteritis* (Kanagawa 2-XII) | 0.1 |
| *Pseudomonas enteritis* (II) | 0.01 |
| *Pseudomonas enteritis* (III) | 0.025 |
| *Pseudomonas enteritis* (IV) | 0.025 |
| *Pseudomonas enteritis* (VI) | 0.005 |
| *Pseudomonas enteritis* (VII) | 0.01 |
| *Pseudomonas enteritis* (VIII) | 0.025 |
| *Pseudomonas enteritis* (IX) | 0.025 |
| *Pseudomonas enteritis* (Kanagawa 1-II) | 0.005 |
| *Pseudomonas enteritis* (XI) | 0.025 |
| *Pseudomonas enteritis* (XII) | 0.025 |
| *Pseudomonas enteritis* (XIII) | 0.1 |
| *Pseudomonas enteritis* (XIV) | 0.05 |
| *Pseudomonas enteritis* (Kanagawa 3-XIII) | 0.005 |
| *Pseudomonas enteritis* ($N_4$-II) | 0.025 |
| *Pseudomonas enteritis* (XVIII) | 0.025 |
| *Candida albicans* | 10.0 |
| *Candida parakrusei* | 50.0 |
| *Candida tropicalis* | 25.0 |
| *Candida stellatoides* | 5.0 |
| *Candida guilliermondi* | 50.0 |
| *Candida krusei* | 50.0 |
| *Candida pseudotropicalis* | 10.0 |
| *Trichophyton mentagrophytes* (Kameda) | 2.5 |
| *Trichophyton interdigitales* (Yoshida) | 5.0 |
| *Trichophyton rubrum* (Fujisawa) | 0.6 |
| *Trichophyton rubrum* (Iura) | 1.25 |
| *Cryptococcus neoformans* | 5.0 |
| *Pseudomonas enteritis* (V) | 0.05 |

* The broth dilution method was used in these studies. Pseudomonas organisms were cultivated in 1% peptone, 3% NaCl medium, and *D. pneumoniae*, *S. pyogenes* and *C. diphtheriae* were

cultivated on heart infusion broth containing 0.5% glucose. All other bacteria were cultivated on heart infusion broth. Fungi were grown in Sabourand's medium.

It was found active against gram-positive and gram-negative bacteria and fungi belonging to the Candida, Trichophyton and Cryptococcus geners. Except for the strain of *Pseudomonas aeruginosa,* with required 20 p.p.m. for complete inhibition, all other strains of bacteria tested were inhibited by concentrations of 0.005 to 1.0 p.p.m.. It is to be noted that the Trichophyton fungi required 0.6 to 5.0 p.p.m. for complete inhibiton.

## *In Vivo* Antibacterial Activity

The *in vivo* activity of panfuran was determined with our pure strain of mice weighting approximately 20 gm each. The mice were inoculated intraperitoneally with 1,000 MLD of *Streptococcus pyogenes* and test compounds were administered subcutaneously at 3,24,48 and 72 hours after inoculation. Mice were examined daily for ten days (Table 6).

Table 6. *In vivo* activity of panfuran in *Streptococcus pyogenes* infection in mice

| Drug | Dose mg/kg | Number of treatments | Survival time (Days) | Survivors * /Total |
|---|---|---|---|---|
| Panfuran | 50 | 3 | 3,10,10,10,6 | 3/5 |
| Panfuran | 50 | 1 | 2,10,3,2,2 | 1/5 |
| Panfuran | 25 | 3 | 2,4,5,3,3 | 0/5 |
| Tetracycline | 50 | 3 | 10,10,10,10,10 | 5/5 |
| Chloramphenicol | 50 | 1 | 2,2,2,2,2 | 0/5 |
| Control | • | • | 1,1,1,1,1 | 0/5 |

* Survivors ten days after intraperitoneal inoculation of mice. Treatment subcutaneously at 3, 24, 48 hours after infection.

The control mice all died within twenty-four hours after infection. No mice survived when they were treated three times with panfuran at 25 mg/kg. One out of five mice survived when they were given one dosage of panfuran at 50 mg/kg and three out of five mice survived when they were given three treatments of panfuran at 50 mg/kg. By contrast, five out of five survived when they were given three 50 mg/kg doses of tetracycline. Chloramphenicol was ineffective with one treatment at his level.

## Pharmacological Studies

In preparation for clinical testing, pharmacological studies were made as shown in Table 7

Table 7. Results of pharmacological studies on panfuran

| *Test system* | *Activity* |
|---|---|
| Erythrocyte (*in vitro*) | Hemolytic (in 1:4,000) |
| Ehrlich's cancer cells (*in vitro*) | Inhibit growth (at 1:800 completely) |
| Isolated heart (frog) | Depress after stimulate |
| Blood vessels (toad) | No effect |
| Blood-pressure and respiration (rabbit) | Depress |
| Isolated intestine (guinea pig) | Depress the movement |
| Central nervous system (frog and mouse) | Stilmulate |
| Toxicity against mice | $LD_{50}$ • • • • 8mg/20gm (orally)<br>$LD_{50}$ • • • • 6mg/20gm (subcutaneously) |

In the course of these studies, panfuran appeared to stimulate the central nervous system but no paralysis was observed. On excessive dosage, mice showed anxiety, convulsions, and stiffness before death. Small doses stimulated the circulation, but large doses depressed the blood pressure. Temporary respiratory stimulation was noted. Large doses inhibited intestinal movement, but moderate doses had no effect. *In vitro*, it hemolysed red cells. The results indicated a moderate toxicity, the $LD_{50}$ in mice 400 mg/kg and 300 mg/kg on oral and subcutaneous administration respectively.

## Summary

A new furan derivative 3-amino-6-[(5-nitro-2-furyl)vinyl]-as-triazine hydrochloride, panfuran, is formed when 1,5-bis-(5-nitro-2-furyl)-3-pentadienone guanylhydrazone, is heated in solvent in the presence of base. This new compound exhibits a high order of activity against gram-negative and gram-positive bacteria and some fungi. *In vivo* efficacy of this product was demonstrated in mice infected

with *Streptococcus pyogenes*. The toxicity of panfuran is comparatively low and the drug appears worthy of clinical investigation.

## Literatures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