

# THE R. KIMURA LABORATORY

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## Antibacterial Action of Nitro-furan Derivatives

Since 1944 attempts to synthesize nitro-furan derivatives have been undertaken, quite independently from Stillman and Dodd, by Dr. H. Saikachi and his collaborators in the Pharmacy Department of Kyoto University. In one of the whole 130 derivatives, i.e. 2-(5-nitro)-furfural semicarbazone, we found in 1946 a predominant antibacterial action against grampositive as well as grammegative bacteria. The works of Stillman and Dodd were wholly unknown to us at that time.

Since then the antibacterial power in vitro, diffusibility and toxicity of about 60 among 130 synthesized nitro-furan derivatives have been examined in our Institute.

The following is a brief statement of our results:

### I. Antibacterial Action in vitro

Serial dilution of each derivative was made with bouillon (pH 6.5) and the minimal concentration of growth inhibition of aerobic bacteria was estimated.

#### 1) 5-nitro-furfural derivatives (See Table 1).

The antibacterial power of 5-nitro-furfural semicarbazone (No. 1, which is called "furacin" in the U.S.) is highest of 12 derivatives. The power of No. 2 and No. 3 is inferior to that of No. 1. But Nos. 2 and 3 are different from No. 1, and easily soluble in the water. 5-nitro-furfural phenylsemicarbazone (No. 4) shows a high titer of 1: 320,000 against tubercle bacilli, though it is not very effective, against other bacteria.

#### 2) 5-nitro-furylacrlyl derivatives (See Table 2).

The antibacterial power of 5-nitro-furylacrlyl amide (No. 13) is two times as much as that of furacin. Its solubility is so small as the latter.

#### 3) 5-nitro-furoyl derivatives (See Table 3).

5-nitro-furoylamide (No. 18) is easily soluble in water, and has almost the same antibacterial power as furacin. It is also interesting that thiazole or pyrimidine including derivatives (No. 21, 25 and 31) have strong affinity with staphylococci.

#### 4) 5-nitro-furylacrolein derivatives (See Table 4).

5-nitro-furylacrolein semicarbazone (No. 35) and 5-nitro-furylacrolein amino-guanidine (No. 36) are the most noteworthy derivatives. The former is scarcely soluble in water, but has two times or more antibacterial power as compared with furacin. The antibacterial power of the latter (No. 36) against grampositive bacteria is as strong as the former (No. 35), and it is soluble in water.

#### 5) 5-nitro-furylsulfonamide (See Table 5).

5-nitro-furylsulfonamide (No. 46) has almost the same antibacterial potency as furacin, and is soluble.

6) 5-nitro-furylsulfide (See Table 6).

5, 5'-dinitro-2, 2'-difurylsulfide (No. 52) has strong antibacterial power (1: 800,000) against staphylococci.

7) Other derivatives (See Table 7).

There are scarcely found any noteworthy derivatives as compared with furacin.

## **II. Antibacterial Action against Anaerobic Bacteria (See Table 8)**

The antibacterial power against *Clostridium tetani*, *Clostridium oedematis maligni* (*V. septique*) and *Clostridium novyi* of 8 kinds (No. 1, 2, 13, 14, 18, 19, 52 and 53) was examined with the liver-bouillon dilution of the derivatives.

The derivatives have almost the same antibacterial power as homosulfamin.

## **III. Diffusibility**

Each 5 ml of the following composition was poured in a small reagens glass:

|                             |        |
|-----------------------------|--------|
| 1.5% agar                   | 100 ml |
| 1.0% NaNO <sub>3</sub>      | 0.5 ,, |
| 0.1% methylene blue         | 3.5 ,, |
| Suspension of staphylococci | 0.1 ,, |

0.5 ml of diluted derivatives was put in the glass, and reagents glasses were kept 18-20 hours in an ice-box and 24 hours in an incubator. The inhibitory length of bacterial growth was estimated.

The result is shown in the Table 9. Nitro-furan derivatives have generally strong diffusibility like penicillin.

## **IV. Toxicity**

Toxicity of 10 derivatives, expressed in 50% lethal dose per 10 g mouse (Reed and Münch), is given in the Table 10.

5-nitro-furylacrolein semicarbazone (No. 35) is least toxic.

## **V. Fastness Gaining of Bacteria against Nitro-furan Derivatives**

The serial cultivation of susceptible strains of *Staphylococcus aureus*, *Escherichia coli communis* and *Bacillus subtilis* was conducted in the 5-nitro-furfural semicarbazone or 5-nitro-furylidene aminoguanidine containing media. Each bacterium gains fastness quickly from the 1st to the 10th generation. From the 10th generation on the resistance of the bacteria increases step by step till it reaches the climax in the 25-30th generations. The resistance of *Staphylococcus* becomes, then, 30 times larger, and that of *Escherichia coli* and *Bacillus subtilis* ca. 20 times larger as compared with the original strain. The value of fastness is remarkably inferior to that by penicillin or streptomycin. It is noteworthy that the gained fastness of bacteria against nitro-furan derivatives can not be revived, even if the bacteria are cultivated in drugfree media.

- 1) Dodd, M. C. & Stillman, W. B.: *J. Pharmacol.* **82**, 10 (1944).
- 2) Dodd, M. C.: *J. Pharmacol.* **86**, 311 (1946).
- 3) Gramer, D. L. & Dodd, M. C.: *J. Bact.* **51**, 293 (1946).
- 4) Eaton Laboratory.: *J. A. M. A.* **134** (1947).
- 5) Goi, J.: *Kokin Busshitsu Kenkyu*, **2**, 40 (1947).
- 6) Kimura, R., Higashi, N., Goi, J. & Saikachi, H.: *J. Penicillin*, **2**, 62 (1948).
- 7) William, C. W. & Dodd, M. C.: *J. Bact.* **56**, 649 (1948).
- 8) Takahashi, T., Saikachi, H. *et al.*: *Yakugaku Zasshi*, **69**, 284 (1949).
- 9) Takahashi, T., Saikachi, H. *et al.*: *Ibid.* **69**, 286 (1949).
- 10) Ikegaki, K.: *Kokin Busshitsu Kenkyu*, **2**, 442 (1949).
- 11) Ikegaki, K.: *Ibid.* **3**, 15 (1950).
- 12) Ikegaki, K.: *Yakugaku Kenkyu*, **22**, 148 (1950).
- 13) Ikegaki, K.: *Kokin Busshitsu Kenkyu*, **3**, 179 (1951).
- 14) Ikegaki, K.: *Ibid.* **3**, 199 (1951).
- 15) Ikegaki, K.: *Ibid.* **3**, 209 (1951).

Table 1. 5-nitro-furfural derivatives. (unit=10,000, R=NO<sub>2</sub>-

| Derivatives   | Bacteria | Hours | Staphylococcus aureus | Streptococcus haemolyticus | Diplococcus pneumoniae Type I | Escherichia coli communis | Eberthella typhosa | Shigella dysenteriae | Pseudomonas aeruginosa |      |      |
|---|----------|-------|-----------------------|----------------------------|-------------------------------|---------------------------|--------------------|----------------------|------------------------|------|------|
|   |          |       | 24                    | 96                         | 24                            | 96                        | 24                 | 96                   | 24                     | 96   |      |
| No. 1. R-CH=N-NH-C(=NH) <sub>2</sub>  |          | 20    | 16                    | 2                          | 1                             | 2                         | 1                  | 20                   | 16                     | 20   | 10   |
| No. 2. R-CH=N-NH-C(=NH) <sub>2</sub> ·HCl   |          | 10    | 8                     | 8                          | 4                             | 2                         | 1                  | 5                    | 2.5                    | 10   | <0.5 |
| No. 3. R-CH=N-NH-C(=NH) <sub>2</sub> ·½H <sub>2</sub> SO <sub>4</sub>   |          | 10    | 8                     | 8                          | 4                             | 2                         | 1                  | 5                    | 2.5                    | 10   | <0.5 |
| No. 4. R-CH=N-NH-C(=NH)-                              |          | 5     | 2.5                   |                            |                               |                           | <0.5               | <0.5                 | 1                      | 1    |      |
| No. 5. R-CH=N-NH-COOH   |          | 10    | 10                    |                            |                               |                           | 2.5                | 2.5                  | 5                      | 2.5  | <0.5 |
| No. 6. R-CH=N-  OH                                    |          | 2.5   | 1                     | 1                          | <0.5                          | 1                         | <0.5               | 0.5                  | <0.5                   | 0.5  | <0.5 |
| No. 7. R-CH=N-  COOH                                  |          | 2.5   | 1                     | 1                          | <0.5                          | 1                         | <0.5               | <0.5                 | <0.5                   | 0.5  | <0.5 |
| No. 8. R-CH=N-  SO <sub>2</sub> NH <sub>2</sub>       |          |       | 1                     |                            |                               |                           | <1                 | <5                   | <5                     | <5   | <0.5 |
| No. 9. R-CH=N-  SO <sub>2</sub> NHCOCH <sub>3</sub> |          |       | <1                    | <1                         |                               |                           |                    | <1                   |                        |      |      |
| No. 10. R-CH=N-NH-C(=NH) <sub>2</sub><br>S  |          |       | 5                     | <0.5                       |                               | <0.5                      |                    | 1                    | 1                      | 1    | <1   |
| No. 11. R-CH=N-  SC <sub>2</sub> H <sub>5</sub>     |          | 2.5   | 2.5                   |                            |                               | <0.5                      | <0.5               | 1                    | 1                      | 1    | <0.5 |
| No. 12. (R-CH=N-NH-CO) <sub>2</sub>   |          | 2.5   | 1                     |                            |                               | <0.5                      | <0.5               | 1                    | 1                      | <0.5 | <0.5 |

Table 2. 5-nitro-furylacyryl derivatives. (unit=10,000, R=NO<sub>2</sub>-

| Derivatives   | Hours | Bacteria | Staphylococcus aureus | Streptococcus haemolyticus | Diplococcus pneumoniae Type I | Escherichia coli communis | Enterobacter typhosa | Shigella dysenteriae | Pseudomonas aeruginosa |      |
|---|-------|----------|-----------------------|----------------------------|-------------------------------|---------------------------|----------------------|----------------------|------------------------|------|
|   |       | 24       | 96                    | 24                         | 96                            | 24                        | 96                   | 24                   | 96                     | 24   |
| No. 13. R-CH=CH-CONH <sub>2</sub>   | 40    | 20       | 4                     | 2                          | 2                             | 1                         | 40                   | 20                   | 40                     | 20   |
| No. 14. R-CH=CH-COOH  | 20    | 10       | 2                     | 1                          | 2                             | 1                         | 10                   | 5                    | 10                     | 5    |
| No. 15. R-CH=CH-CONH-                      | 16    | 10       |                       |                            |                               | 2.5                       | 1                    | 10                   | 5                      | <0.5 |
| No. 16. R-CH=CH-CONH-  Cl                  | 10    | 8        |                       | <0.5                       |                               | <0.5                      |                      | 8                    | 5                      | <0.5 |
| No. 17. R-CH=CH-CONH-  >-O-CH <sub>3</sub> | 10    | 8        |                       |                            |                               | 1                         | 0.5                  | 2.5                  | 1                      |      |

**Table 3.** 5-nitro-furoyl derivatives. (unit = 10,000, R = NO<sub>2</sub>)

**Table 4.** 5-nitro-furyl acrolein derivatives. (unit = 10,000, R = NO<sub>2</sub>)

Table 5. 5-nitro-furylsulfonamide derivatives. (unit=10,000, R=NO2c1ccc(cc1)O)

| Derivatives   | Hours | Bacteria |    | Streptococcus haemolyticus | Diplococcus pneumoniae Type I | Escherichia coli communis | Eberthella typhosa | Shigella dysenteriae | Pseudomonas aeruginosa |    |
|---|-------|----------|----|----------------------------|-------------------------------|---------------------------|--------------------|----------------------|------------------------|----|
|   |       | 24       | 96 |                            |                               |                           |                    |                      |                        |    |
| No. 46. R-SO <sub>2</sub> NH <sub>2</sub>   | 20    | 10       | <1 | <1                         | <1                            | 10                        | 10                 | 20                   | 10                     | 5  |
| No. 47. R-SO <sub>2</sub> NH- <chem>c1ccc(cc1)C(=O)OCC2=CC=C(C=C2)C3=CC=C(C=C3)C</chem> | 2.5   | 1        |    | <1                         | <1                            | 1                         | <1                 | 1                    | <1                     | <1 |
| No. 48. R-SO <sub>2</sub> NH- <chem>c1ccc(cc1)C(=O)OCC2=CC=C(C=C2)C3=CC=C(C=C3)C</chem> | 5     | 2.5      |    | <1                         | <1                            | 1                         | <1                 | 1                    | <1                     | <1 |
| No. 49. R-SO <sub>2</sub> NH- <chem>CN1C=CC=C1S\c2ccccc2</chem>                         | 1     | <1       |    |                            | 1                             | <1                        | 2.5                | 1                    | <1                     | <1 |
| No. 50. R-SO <sub>3</sub> K   | <1    | <1       |    | <1                         | <1                            | <1                        | <1                 | <1                   | 2.5                    | 1  |
|   |       |          |    |                            |                               |                           |                    |                      | <1                     | <1 |

Table 6. 5-nitrofurylsulfide derivatives. (unit=10,000, R=NO2c1ccc(cc1)S)

| Derivatives                               | Hours | Bacteria |    | Streptococcus haemolyticus | Diplococcus pneumoniae Type I | Escherichia coli communis | Eberthella typhosa | Shigella dysenteriae | Pseudomonas aeruginosa |    |
|---|-------|----------|----|----------------------------|-------------------------------|---------------------------|--------------------|----------------------|------------------------|----|
|   |       | 24       | 96 |                            |                               |                           |                    |                      |                        |    |
| No. 51. R-S- <chem>c1ccc(cc1)NO2</chem>   | 10    | 5        | 2  | 1                          |                               | 5                         | 2.5                | 5                    | 2.5                    | <1 |
| No. 52. R-S- <chem>O=[N+]([O-])NO2</chem> | 80    | 60       | <1 | <1                         |                               | 5                         | 2.5                | 5                    | 2.5                    | <1 |

Table 7. Other derivatives. (unit = 10,000, R = NO<sub>2</sub>-O-O-)

| Derivatives                                     | Bacteria | Hours | Staphylococcus aureus | Streptococcus haemolyticus | Diplococcus pneumoniae Type I | Escherichia coli communis | Eberthella typhosa | Shigella dysenteriae | Pseudomonas aeruginosa |
|---|----------|-------|-----------------------|----------------------------|-------------------------------|---------------------------|--------------------|----------------------|------------------------|
|   |          |       | 24                    | 96                         | 24                            | 96                        | 24                 | 96                   | 24                     |
| No. 53. R-C=C-COOCH <sub>3</sub>                | 5        | 2.5   |                       | <1                         | <1                            | <1                        | <1                 | <1                   | <1                     |
| No. 54. R-C=C<\COOC <sub>2</sub> H <sub>5</sub> | 1        | <1    |                       | <1                         | <1                            | <1                        | <1                 | <1                   | <1                     |
| No. 55. R-C≡N                                   | 5        | 2.5   | <1                    | <1                         | 2                             | 1                         | 10                 | 5                    | <1                     |
| No. 56. N-CH=NOH                                | 10       | 5     | 4                     | 2                          | 2                             | 1                         | 10                 | 5                    | <1                     |

Table 8. Antibacterial action of nitro-furan derivatives against anaerobic bacteria

| Derivatives  | Bacteria | Hours | Cl. tetani | V. septique | Cl. novyi |       |       |
|--|----------|-------|------------|-------------|-----------|-------|-------|
|  |          |       | 24         |             |           |       |       |
| No. 1. R-CH=N-NH-COOH <sub>2</sub>   |          | 16000 | 8000       | 16000       | 8000      | 8000  | 8000  |
| No. 2. R-CH=N-NH-C<\NH <sub>2</sub> -HCl   |          | 16000 | 16000      | 16000       | 8000      | 8000  | 4000  |
| No. 13. R-CH=CH-COOH <sub>2</sub>  |          | 16000 | 8000       | 16000       | 16000     | 8000  | <4000 |
| No. 14. R-CH=CH-COOH   |          | 8000  | 8000       | 16000       | 8000      | 8000  | 4000  |
| No. 18. R-CONH <sub>2</sub>  |          | 8000  | 4000       | 6000        | 6000      | 4000  | <4000 |
| No. 19. R-CONH-               |          | 6000  | 4000       | 8000        | 4000      | <4000 | <4000 |
| No. 52. R-S-  NO <sub>2</sub> |          | 8000  | 6000       | 6000        | <6000     | <6000 | <4000 |
| No. 53. R-CH=C-COOCH <sub>3</sub>  |          | 4000  | 4000       | 4000        | <4000     | <4000 | <4000 |

**Table 9.** Diffusibility of nitro-furan derivatives (mm)

| Derivatives                                       | Concentration | 1: 10,000 | 1: 20,000 | 1: 40,000 | 1: 80,000 | 1: 160,000 |
|---|---------------|-----------|-----------|-----------|-----------|------------|
| No. 1. R-CH=N-NH-CONH <sub>2</sub>                |               | 17.0      | 14.5      | 12.0      | 9.0       | 5.5        |
| No. 2. R-CH=N-NH-C(=NH)NH <sub>2</sub> HCl        |               | 17.0      | 14.5      | 12.5      | 9.5       | 5.5        |
| No. 13. R-CH=CH-CONH <sub>2</sub>                 |               | 18.0      | 15.5      | 13.0      | 7.5       | 4.0        |
| No. 14. R-CH=CH-COOH                              |               | 14.5      | 9.0       | 6.5       | 3.5       | 0          |
| No. 18. R-CONH <sub>2</sub>                       |               | 9.5       | 6.0       | 2.5       | 0.5       | 0          |
| No. 30. R-CONH-C(=O)CH <sub>2</sub>               |               | 14.0      | 11.0      | 8.0       | 5.0       | 1.0        |
| No. 35. R-CH=CH-CH=N-NH-CONH <sub>2</sub>         |               |           | 17.5      | 15.0      | 11.0      | 6.5        |
| No. 36. R-CH=CH-CH=N-NH-C(=NH)NH <sub>2</sub> HCl |               | 18.5      | 16.5      | 14.5      | 11.5      | 7.0        |
| No. 46. R-SO <sub>2</sub> NH <sub>2</sub>         |               |           | 14.5      |           | 9.8       |            |
| No. 52. R-S-C(=O)NO <sub>2</sub>                  |               | 23.0      | 21.5      | 19.5      | 17.0      | 15.0       |
| No. 56. R-CH=NOH                                  |               | 11.0      | 7.5       | 3.5       | 1.5       | 0          |

**Table 10.** 50% lethal dose per 10 gr mouse (mg)

|   | subcutan. | per os |
|---|-----------|--------|
| No. 1. R-CH=N-NH-CONH <sub>2</sub>                | 6.25      | 2.08   |
| No. 2. R-CH=N-NH-C(=NH)NH <sub>2</sub> HCl        | 1.2       | 1.43   |
| No. 13. R-CH=CH-CONH <sub>2</sub>                 | 3.13      | 1.88   |
| No. 14. R-CH=CH-COOH                              | 4.35      | 2.12   |
| No. 18. R-CONH <sub>2</sub>                       | 1.46      | 1.88   |
| No. 35. R-CH=CH-CH=N-NH-CONH <sub>2</sub>         | 11.11     | 9.26   |
| No. 36. R-CH=CH-CH=N-NH-C(=NH)NH <sub>2</sub> HCl | 1.0       | 1.5    |
| No. 46. R-SO <sub>2</sub> NH <sub>2</sub>         | 2.18      | 2.5    |
| No. 53. R-CH=C-COOCH <sub>2</sub> H <sub>5</sub>  | 1.25      |        |
| No. 56. R-CH=NOH                                  | 0.31      |        |