they were tested for catalepsy using the following methods. (i) One hind leg was placed on a 3-cm high cork. (ii) One hind leg was placed on a 9-cm high cork. (iii) Rats were placed with their feet on parallel bars. (iv) Rats were placed with their feet on a vertical grid (⅜ in. mesh). The rats were considered cataleptic if no movement occurred within about 20 sec and each rat was given a score of 2 on each test. If a rat moved immediately after being placed on an object, as mentioned above, but then remained immobile, a score of 1 was given. Rats showing a high degree of catalepsy (score 7 or 8) were split into groups of four and each group dosed orally with the compounds, using a dose volume of 1 ml per rat. The rats were retested for catalepsy at intervals over the following 5.5 hr. The degree of reversal of the catalepsy induced by the compounds was assessed from the time course of the catalepsy over the 5.5-hr period. Amantadine was also tested in a similar manner for comparative purposes. The results are recorded in Table II.

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Synthesis and Structure–Activity Relationships of a Series of Antibacterially Active 5-(5-Nitro-2-furfurylidene)thiazolones, 5-(5-Nitro-2-furylpropenylidene)thiazolones, and 6-(5-Nitro-2-furyl)-4*H*-1,3-thiazinones

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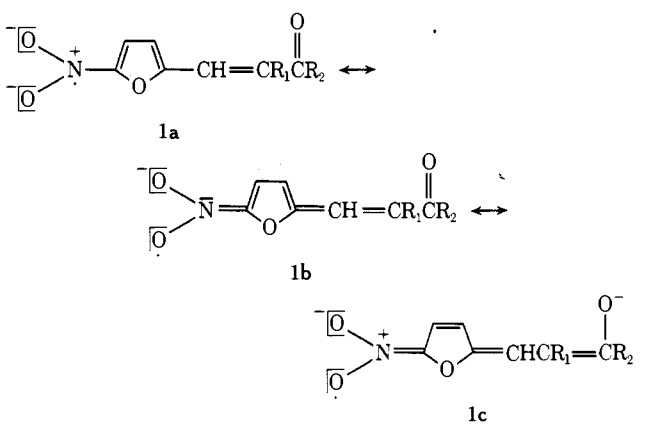
A series of 5-(5-nitro-2-furfurylidene)thiazolones, 5-(5-nitro-2-furylpropenylidene)thiazolones, and 6-(5-nitro-2-furyl)-4*H*-1,3-thiazinones was synthesized and their antibacterial activity against *Staph. aureus*, *β-haem. streptococcus*, *E. coli*, *K. aerogenes*, and *P. vulgaris* was determined. Many of the new nitrofurans showed a very high activity against all five bacteria *in vitro* and were up to 60 times as active as nitrofurantoin. The new nitrofurans were inactive in *in vivo* chemotherapeutic tests with *Sal. typhimurium* and *Sal. gallinarum*. Structure–activity studies showed that the 2-substituent may increase the antibacterial activity of a 5-nitrofuran compound by facilitating the reduction of the nitro group. Bulky substituents in the 2 position adversely affect the activity against the gram-negative bacteria, probably by sterically inhibiting the penetration of the nitrofuran into the bacteria.

5-Nitrofurans substituted at the 2 position with a wide variety of substituents are active against bacteria.[1,2] The nitro group is essential for the activity, whereas the influence of the 2-substituent on the activity is not completely understood. Nitrofurans are shown to interfere with several reductive enzyme systems in the bacteria[1,3] and there may be a correlation between the reduction potential of the nitro group and the antimicrobial activity. Sasaki[4] studied the polarographic half-wave potential for a series of nitrofurans and found that the antibacterial activity increased when the reduction potential became less negative.

The inductive and resonance effects of a substituent in the 2 position influence the half-wave potential of the nitro group. Nitrofurans with a conjugated carbonyl group in the 2 position should be easily reduced since the radical anion 1, formed during the initial reduction step, can be stabilized by conjugation[5] (1a–c, Scheme I).

In our search for nitrofurans with high antibacterial activity a series of nitrofurans with a conjugated carbonyl in the 2 position has been synthesized and examined for antibacterial activity (Table I). The relationship between

Scheme I

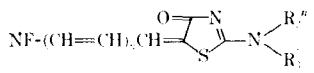


1a 1b 1c

antibacterial activity, half-wave potentials, partition coefficients, and steric effect has been studied.

Chemical Results. I. 5-(5-Nitro-2-furfurylidene)- and 5-(5-Nitro-2-furylpropenylidene)thiazolin-4-ones (2–5). **(a) Synthesis.** The nitrofurans in series 2–4 (Table I)

Table I. Chemical and Antibacterial Data of Nitrofurans **2–7**



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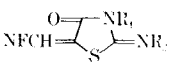

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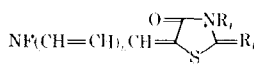

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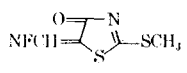

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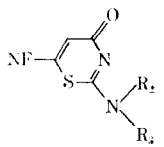

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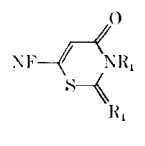

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Compd	n	R_1	R_2	R_3	R_4	Method	Yield, %	Mp, °C	Part. coeff,[b] log P	Antibacterial activity *in vitro*, MIC, μg/ml[c] St-a	β-Str	E-c	Kl-a	Pr-v
2a	0		H	H		*d*	35	290 dec	1.09 ± 0.05	0.4	0.8–1.6	0.2–0.4	0.8–1.6	3.2–6.3
2b	0		CH_3	H		B + C	71	305–306 dec	1.16 ± 0.07	0.2–0.4	0.8–1.6	0.2–0.4	0.4	1.6–3.2
2c	0		C_2H_5	H		B + C	51	280–285 dec	1.16 ± 0.03	0.2	1.6–3.2	0.2–0.4	0.8–1.6	6.3–12.5
2d	0		C_3H_7	H		B + C	64	246–249 dec	1.24 ± 0.02	0.2	6.3	1.6	6.3	25
2e	0		*i*-C_3H_7	H		B + C	64	273–275 dec	1.29 ± 0.02	0.2–0.4	>200	1.6–3.2	>200	>200
2f	0		$COCH_3$	H		B	86	292 dec	1.03 ± 0.08	1.6–3.2	6.3–12.5	0.8–1.6	6.3	25
2g	0		CH_3	$COCH_3$		B	82	244–252 dec	1.05 ± 0.06	0.1–0.2	1.6–3.2	0.1–0.2	0.8	1.6–3.2
2h	0		CH_3	CH_3		B	30	263–267 dec	1.14 ± 0.03	0.2–0.4	6.3–12.5	0.4	0.4–0.8	1.6
2i	0		C_2H_5	C_2H_5		B	35	195–197.5	1.22 ± 0.11	0.8	200–>200	6.3	12.5–50	>200
2j	1		CH_3	H		B + C	63	271–272 dec	1.35 ± 0.04	0.2	0.4–0.8	0.2	0.8	6.3
2k	1		$COCH_3$	H		B	58	Dec	0.95 ± 0.03	1.6–3.2	6.3–12.5	3.2	12.5	100–200
2l	1		CH_3	$COCH_3$		B	91	244 dec	1.21 ± 0.01	0.2–0.4	0.8–1.6	0.2–0.4	0.4–0.8	6.3
3a		CH_3	H			A (B + C)	63 (80)	247–249 dec	1.44 ± 0.05	0.8–1.6	100–200	0.4–0.8	1.6–3.2	>200
3b		C_2H_5	H			A	27	199–201 dec	1.44 ± 0.09	1.6–3.2	200–>200	3.2–6.3	>200	>200
3c		C_3H_7	H			B + C	50	161–164	1.32 ± 0.09	0.8–3.2	200–>200	6.3–12.5	>200	>200
3d		CH_3	CH_3			A	57	238–240 dec	1.42 ± 0.07	1.6	100–200	1.6–3.2	6.3	>200
3e		C_2H_5	C_2H_5			A	95	149–151	1.34 ± 0.07	0.8	200	>200	>200	>200
3f		C_3H_7	C_3H_7			A	71	100.5–103	1.32 ± 0.06	0.8	200–>200	>200	>200	>200
3g		*i*-C_3H_7	*i*-C_3H_7			A	32	134–136	1.22 ± 0.06	0.8	>200	>200	>200	>200
3h		C_3H_5	C_3H_5			A	63	126.5–127.5	1.46 ± 0.06	0.8	>200	>200	>200	>200
3i		H	Ph			B + C	69	300 dec	1.41 ± 0.04	0.2–0.4	>200	>200	>200	>200
3j		CH_3	$COCH_3$			B	86	207.5–210 dec	1.21 ± 0.09	0.4–0.8	12.5–25	3.2	25	25–50
3k		C_2H_5	$COCH_3$			B	74	191–192	1.21 ± 0.03	0.8	50	>200	>200	>200
3l		C_3H_7	$COCH_3$			B	58	189–191	1.17 ± 0.05	0.8	100	>200	>200	>200
4a	0	H			O	B + C	36	222.5–224 dec	0.63 ± 0.06	0.4	0.8	0.4	1.6–3.2	12.5–25
4b	0	CH_3			O	D	95	209–212 dec	1.78 ± 0.09	0.8–1.6	25–50	1.6	12.5	>200
4c	0	C_2H_5			O	D	93	135–136.5	2.29 ± 0.17	0.8–1.6	12.5–25	6.3–12.5	50	>200
4d	0	C_3H_7			O	D	62	114–116	2.40 ± 0.07	0.8	12.5–25	12.5–25	200–>200	>200
4e	0	*i*-C_3H_7			O	D	78	131–133	2.42 ± 0.26	0.8–1.6	25	25	>200	>200
4f	0	C_3H_5			O	D	98	138.5–140	2.22 ± 0.12	0.8–1.6	12.5–25	12.5	>200	>200
4g	0	H			S	A	97	199–200 dec	0.74 ± 0.04	0.8–1.6	3.2–6.3	1.6	12.5	6.3
4h	0	CH_3			S	A	90	188–190 dec	1.06 ± 0.03	0.4–0.8	25–50	>200	>200	>200
4i	1	H			S	A	57	233–235 dec	0.93 ± 0.04	0.8	0.8	0.8	6.3	3.2
4j	1	CH_3			O	B	35	228–229 dec	1.40 ± 0.11	0.4	50	0.8	3.2	>200
5a							15	180–182.5 dec	1.41 ± 0.04	3.2–12.5	50–100	6.3–12.5	100–200	200–>200
6a			H	H		*d*	55	Dec		12.5	25–50	1.6	25	>200
6b			CH_3	H		*d*	74	279–280 dec	0.86 ± 0.07	6.3	200–>200	0.8	6.3–12.5	100
6c			$COCH_3$	H			65	~285 dec	1.01 ± 0.06	3.2–6.3	>200	12.5–25	>200	>200
6d			CH_3	CH_3		*d*	70	230–235 dec	0.55 ± 0.08	1.6–3.2	100–200	0.8–1.6	12.5–25	100–200
7a		H			O		43	260–264 dec	0.55 ± 0.09	1.6	50	6.3	50	100
7b		CH_3			NCH_3	*d*	71	222–223.5 dec		0.8	>200	1.6	>200	>200
8	Nitrofurantoin									12.5	50	6.3	50	100

[a] NF = 5-nitro-2-furyl. [b] In octanol–water. [c] St-a = *Staph. aureus*, β-Str = *β-haem. streptococcus*, E-c = *E. coli*, Kl-a = *K. aerogenes*, Pr-v = *P. vulgaris*. [d] See ref 6.

were obtained by condensing 5-nitro-2-furfural and (*E*)-3-(5-nitro-2-furyl)acrolein, respectively, with a thiazolin-4-one or a thiazolidin-4-one derivative. The condensation was performed either in HOAc or Ac_2O in the presence of anhydrous NaOAc. When Ac_2O was used as reaction medium, NH groups present in the molecule were acetylated.

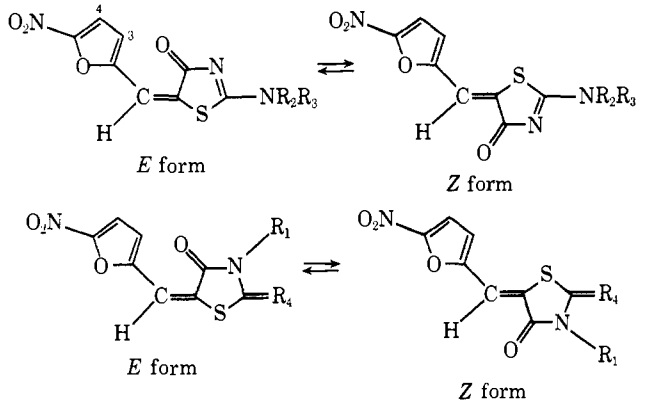
The acetyl group could in most cases be hydrolyzed in hot HOAc. However, complete hydrolysis of an iminoacetyl group could only be achieved by refluxing in 0.5 *M* EtOH-HCl, and 2-acetylamino-5-(5-nitro-2-furfurylidene)thiazolin-4-one (**2f**) could not be selectively hydrolyzed to 2-amino-5-(5-nitro-2-furfurylidene)thiazolin-4-one (**2a**). The latter compound was synthesized from thiourea and ethyl (5-nitro-2-furyl)propiolate.[6]

3-Alkyl-5-(5-nitro-2-furfurylidene)thiazolidine-2,4-diones (**4a-f**) were obtained by hydrolyzing the corresponding 2-alkylimino compounds **3d-h** in hot 5 *M* HCl.

Methylation of 5-(5-nitro-2-furfurylidene)-2-thiothiazolidin-4-one (**4g**) with diazomethane gave a mixture of 3-methyl-5-(5-nitro-2-furfurylidene)-2-thiothiazolidin-4-one (**4h**) and 2-methylthio-5-(5-nitro-2-furfurylidene)thiazolin-4-one (**5a**).

(b) Structure Determination. The nitrofurfurylidenethiazolones and nitrofurfurylidenethiazolidones (**2-5**) can exist in an *E* and a *Z* form (Scheme II). Structures were assigned by synthesizing both forms of some of the nitrofurans, **2b,h** and **3a,d**.[6] The configuration of each isomer was deduced from the nmr data, which showed that condensation of nitrofurfural with thiazolones gave the stable *Z* form of **2b,h** and **3a,d**.[6] To establish the configuration of the other nitrofurans in series **2-5** the chemical shifts of the furan protons were compared with those of the *E* and *Z* form of **2b,h** and **3a,d.** The 3-proton was found at δ 7.22-7.42 and the 4-proton at δ 7.80-7.84. This is in accordance with the chemical shifts of the furan protons of the *Z* form of **2b,h** and **3a,d,** 3 H at δ 7.22-7.29 and 4 H at δ 7.80-7.82. The chemical shifts of the furan protons of the *E* form of **2b,h** and **3a,d** were found at lower field, 3 H at δ 7.75-7.76 and 4 H at δ 7.96-8.15. The downfield shift of the furan protons in the *E* form is due to the anisotropic effect of the carbonyl group. Thus all nitrofurfurylidenethiazolones in series **2-5** have the *Z* form.

Scheme II



The nitrofurylpropenylidenethiazolones (**2j,k,l**) and nitrofurylpropenylidenethiazolidones (**4i,j**) can exist in four isomeric forms: *EE, EZ, ZE, ZZ* (Chart I).

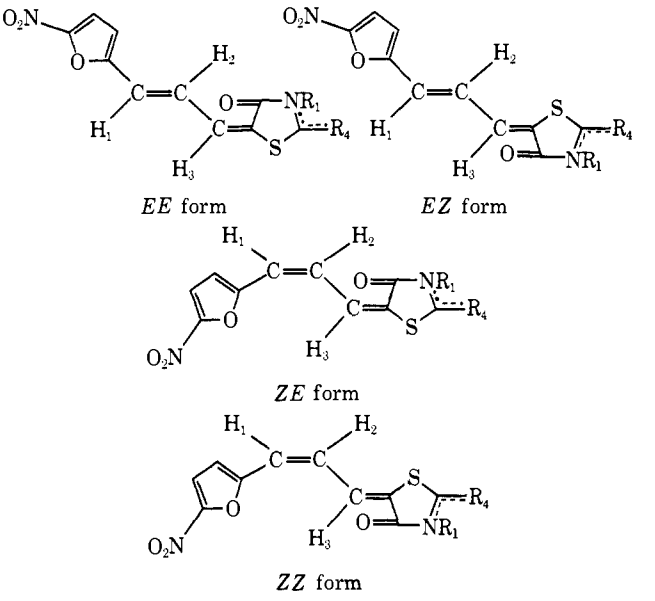
By nmr it was shown that **2j** and **4i,j** existed in the *EZ* form (Table II). The coupling constant between H_1 and H_2 was found to be 15 Hz, typical for *E* ethylene protons.[7] Further, the chemical shifts of H_1, and H_2, and H_3 (δ scale) followed the order $H_3 > H_1 > H_2$ indicating the *EZ* form, in which the C=O would deshield the H_3 proton by anisotropy.[8]

Table II. Nmr Spectra of **2j-l** and **4i,j** in DMSO-*d* (δ Value)[a]

Compd	H_1	H_2	H_3	J_{12}	J_{23}
2j	7.15	6.93	7.30	14.5	10.5
2k	7.21	7.56	7.20	9.0	2.7
2l	7.19	7.54	7.23	9.0	2.7
4i	7.28	6.95	7.39	14.5	12.0
4j	7.31	6.98	7.66	14.5	12.0

[a] For the numbering of protons, see Chart I.

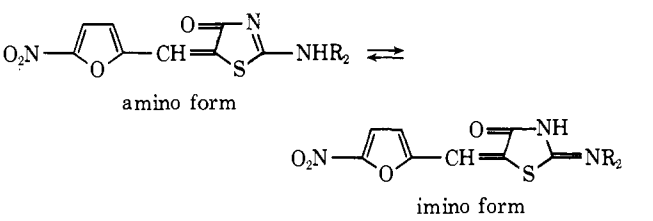
Chart I



The nmr spectra of **2k,l** indicated the *ZE* form for these compounds. The coupling constant between H_1 and H_2 was 9 Hz, which was consistent with the *Z* position for H_1 and H_2. The chemical shifts followed the order $H_2 > H_1 \sim H_3$. The low chemical shift of H_2 is in accordance with the *ZE* form in which H_2 is deshielded by the anisotropic effect of the C=O group.[8] Further evidence for the *ZE* form is obtained from the small coupling constant between H_2 and H_3 (2.7 Hz) which is accounted for by a change of the dihedral angle in the =CHCH= bond caused by the steric effect of the nitrofuran ring.[7] Thus, **2k,l** and **2j, 4i,j,** respectively, exist in different stereoisomeric forms. The reason for this is unknown.

Theoretically the nitrofurans **2a-e** can exist in an amino and an imino tautomeric form (Scheme III). 2-Aminothiazolin-4-one derivatives exist in the amino form.[9] A comparison of the ir spectra of **2a-e** with those of the 2-alkylaminothiazolin-4-one compounds shows that the tautomeric equilibrium was not changed on inserting a nitrofurfurylidene group in the 5 position.

Scheme III



2a-d crystallized in two forms, as needles and spherical crystals, whereas **2e** only crystallized as needles. The needles were obtained by recrystallization from DMSO and showed an ir frequency of C=N at 1580 cm^{-1} and of NH

Table III. Half-Wave Potentials

Compd	$-E_{1/2}$ (V vs. sce)	Compd	$-E_{1/2}$ (V vs. sce)
2a	0.21	**6b**	0.22
2b	0.23	**6c**	0.22
2f	0.24	2-Amino-5-(5-nitro-2-furyl)-	
3a	0.23	1,3,4-thiadiazole[c]	0.25
3d	0.21	*N*-(5-Nitro-2-furfurylidene)-	
3j	0.20	aminohydantoin[a]	0.26
4a	0.25	5-Nitro-2-furaldehyde semicar-	
4b	0.23	bazone[b]	0.28
4g	0.22	3-Amino-2-methyl-5-(5-nitro-	
4i	0.24	2-furyl)-1,2,4-triazole[d]	0.29
4j	0.24		

[a]Nitrofurantoin. [b]Nitrofurazone. See ref 14. [d]See ref 21.

at 3200–3400 cm^{-1}. The spherical crystals were obtained by recrystallization from DMF, CH_3CN, or acetamide and showed an ir frequency of C=N at 1620–1630 cm^{-1} and of NH at 2900–3000 cm^{-1}, indicating strong hydrogen bonds in the latter crystals. The bulkiness of the isopropyl group in **2e** may prevent the isopropylamino group from participating in the formation of hydrogen bonds.

The ir frequencies of C=N and NH in the two crystal forms of **2a–d** are in accordance with the findings for 2-alkylaminothiazolin-4-one compounds. The KBr ir spectrum of the latter showed the C=N frequency at 1620–1630 cm^{-1} and the NH frequencies at 2810 cm^{-1}, whereas the ir spectrum of a diluted $CHCl_3$ solution showed the C=N frequency at 1570–1580 cm^{-1} and the NH frequency at 3250–3485 cm^{-1}.

Further evidence for the existence of **2a–e** in the amino form was obtained from the ir spectra of 2-dialkylamino-5-(5-nitro-2-furfurylidene)thiazolin-4-one (**2h,i**) and 3-alkyl-2-alkylimino-5-(5-nitro-2-furfurylidene)thiazolidin-4-one (**3d–h**). The C=N frequency of **2a–e** at 1580 cm^{-1} is similar to that for the 2-dialkylamino-5-(5-nitro-2-furfurylidene)thiazolin-4-one (**2h,i**). The C=N frequency of the 3-alkyl-2-alkylimino-5-(5-nitro-2-furfurylidene)thiazolidin-4-ones (**3d–h**) was found at 1645–1660 cm^{-1}. However, there are no indications that the spherical crystals of **2a–d** exist in an imino form. The shift of the C=N frequency from 1580 to 1620–1630 cm^{-1} may be explained by a change in the electron distribution of the thiazolone ring in the presence of strong hydrogen bonds.

2-Alkylaminothiazolin-4-ones and 3-alkyl-2-iminothiazolidin-4-ones were interconverted by treating with acid and base.[10] No such interconversion of 2-alkylamino-5-(5-nitro-2-furfurylidene)thiazolin-4-ones (**2b–d**) and 3-alkyl-2-imino-5-(5-nitro-2-furfurylidene)thiazolidin-4-ones (**3a–c**) was observed.

2-Acetylamino-5-(5-nitro-2-furfurylidene)thiazolin-4-one (**2f**) and 2-acetylamino-5-(5-nitro-2-furylpropenylidene)thiazolin-4-one (**2k**) may also exist in two tautomeric forms, an amido and an imido form. Peresleni, *et al.*,[11] have shown that 2-acetylaminothiazolin-4-one exists in the solid state in the amido form and in solution in both forms.

A comparison of the ir spectra of **2f,k** with those of 2-*N*-methylacetylamino-5-(5-nitro-2-furfurylidene)thiazolin-4-one (**2g**) and 2-acetylimino-3-methyl-5-(5-nitro-2-furfurylidene)thiazolidin-4-one (**3j**) shows that **2f,k** exist in the amino form in the solid state.

The C=O peak of the acetyl group of **2f,k** appeared at 1700 cm^{-1} similar to that of **2g** (1695 cm^{-1}) whereas the C=O in the acetylimino group of **3j** appeared at 1670 cm^{-1}. It was not possible to determine experimentally the tautomeric equilibrium of the compounds **2f,k** in solution since they were poorly soluble.

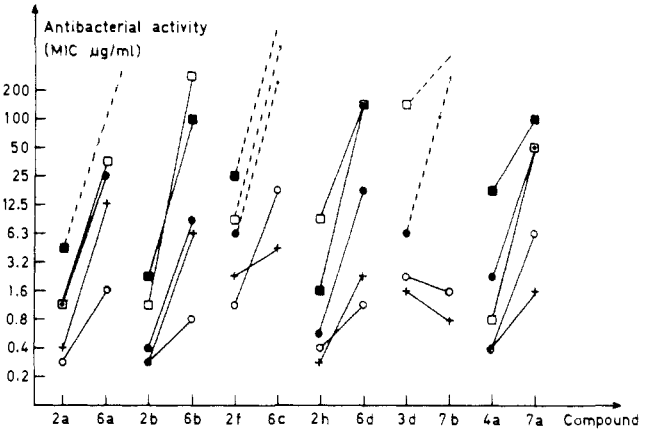


Figure 1. A comparison of the antibacterial activity of the nitrofurfurylidenethiazolones 2a,b,f,**h**, 3**d**, and 4a and the nitrofurylthiazinones **6a–d** and **7a**,b: +, *Staph. aureus*; □, *β-haem. streptococcus*; ○, *E. coli*; ●, *K. aerogenes*; ■, *P. vulgaris*.

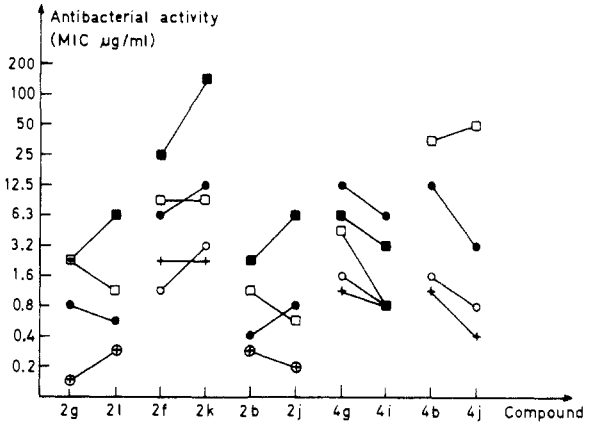


Figure 2. The influence of the bridge between the furan and thiazolone ring on the antibacterial activity: +, *Staph. aureus*; □, *β-haem. streptococcus*; ○, *E. coli*; ●, *K. aerogenes*; ■, *P. vulgaris*.

II. **6-(5-Nitro-2-furyl)-4*H*-1,3-thiazin-4-ones** (**6a–d**, **7a,b**). (a) **Synthesis.** The nitrofurans **6a–d** and **7b** were synthesized by nitrating the corresponding 6-(2-furyl)-4*H*-1,3-thiazin-4-ones with a mixture of concentrated HNO_3 and concentrated H_2SO_4 in the presence of ammonium sulfate.[6] The 6-(2-furyl)-4*H*-1,3-thiazin-4-ones were obtained by addition of thiourea derivatives to ethyl (2-furyl)propiolate.[6] **6b** was hydrolyzed in HCl to **7a**.

Physical-Chemical Results. I. Partition Coefficients. The partition coefficients (*P*) were measured in the octanol-water system and are reported as log *P* values in Table I.

Leo, *et al.*,[12] have shown that addition of a CH_2 or CH_3 group to a parent structure normally increases the logarithm of the partition coefficient by about 0.5. However, in this series of nitrofurans extension of the alkyl chains in series **2** and **3** did not lead to any increase in the partition coefficient. **3g** with two isopropyl groups has a lower partition coefficient than the corresponding methyl derivative **3d**.

However, recently Hansch, *et al.*,[13] reported divergences from the additive-constitutive nature of the partition coefficients for compounds with the alkyl group substituted to an electron-withdrawing group. The nitrofurans in series **2–4** are examples of such compounds where the alkyl groups on the thiazolin-4-one and thiazolidin-4-one compounds are exposed to electron withdrawing by the ring carbonyls. However, in the 3-alkylthiazolidine-2,4-diones in series **4** the logarithm of the partition coefficient

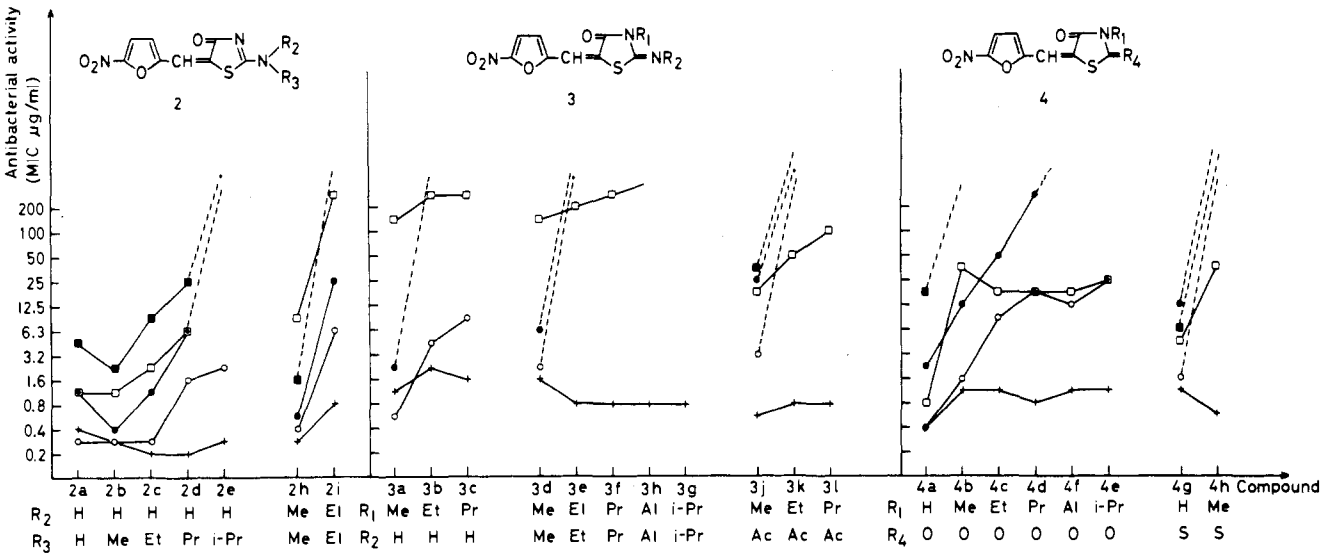


Figure 3. The change of antibacterial activity of the nitrofurans 2-4 by prolongation of their alkyl groups: +, *Staph. aureus;* □, *β-haem. Streptococcus;* ○, *E. coli;* ●, *K. aerogenes;* ■, *P. vulgaris.*

increased for every added CH_2 group. Thus, our results show that it is hazardous to make general rules for calculating partition coefficients from a parent structure.

II. Half-Wave Potentials. The polarographic half-wave potential ($E_{1/2}$) of the nitro group was determined for one nitrofuran compound in each homologous series of 2-7. The $E_{1/2}$ value of this nitrofuran is assumed to be representative of the other nitrofurans in the same homologous series. The results are reported in Table III.

The nitrofurans in series 2-7 exhibit half-wave potentials at −0.20 to −0.25 V at pH 7.2 and 37°. For comparison, the half-wave potentials of four other nitrofurans with different substituents in the 2 position were measured. These were found at −0.25 to −0.29 V. Thus, the nitrofurans in the series 2-7 are, as predicted, easily reduced.

Biological Results. I. Antibacterial Activity. The minimal inhibitory concentrations (MIC values) for new nitrofuran compounds against five different strains of bacteria representing both gram-positive and gram-positive bacteria are reported in Table I. The determinations were made by the twofold serial dilution technique.[14] Each determination was repeated three times and when divergent results were obtained, the upper and lower limits of these results represent the MIC values. Nitrofurantoin was chosen as a reference substance. The results are reported in Table I.

Among the new nitrofurans there are nine compounds, **2a-c,g,h,j,l, 4g,i,** which show a very high activity against all five bacteria in the test. They have MIC values in the region of 0.2-12.5 μg/ml. There are in addition several compounds which show a similar high activity against three or four of the bacteria strains but a lower activity against the rest. A comparison with nitrofurantoin shows that there are nitrofurans in series 2 and 4 which are up to 60 times as active as nitrofurantoin against the five bacteria in the test. The new nitrofurans are of potential value as topical antibacterial agents. They were inactive in *in vivo* chemotherapeutic tests with *Salmonella typhimurium* in mice[15] and with *Sal. gallinarum* in chicks.[16]

II. Structure-Activity Relationship. The new nitrofurans were divided into six series 2-7 according to structure (Table I). In series 2-5 a thiazolone ring is coupled *via* a -CH= or -CH=CHCH= bridge to the nitrofuran ring. In series 6 and 7 the thiazinone ring is substituted to the nitrofuran ring without bridge and the -CH= group is included in the ring. A comparison of the antibacterial activity of the corresponding derivatives in series 2 and 6 and series 4 and 7 shows that the nitrofurfurylidenethiazolone derivatives are more active than the nitrofurylthiazinone derivatives (Figure 1).

In Figure 2 the antibacterial activity of nitrofuran derivatives with different bridges is compared. Neither the length of the bridge nor its configuration change the activity noticeably. The activity is mainly dependent on the substituents on the thiazolone ring.

This is clearly shown by a comparison of the antibacterial activity of the nitrofurfurylidenethiazolones in series 2-5. In series 2 the nitrofurans have an amino group or a substituted amino group at position 2 of the thiazolone ring. Many of these nitrofurans show a very high activity against all five bacteria in our test (Table I and Figure 3). The activity decreases as the number of carbon atoms in the alkyl group increases, except in the case of *Staphylococcus aureus* against which all the nitrofurans in series 2 show about the same activity. In series 3 the nitrofurans have an alkyl group in position 3 and an imino or a substituted imino group in position 2 of the thiazolidone ring. These nitrofurans show a high activity against *Staph. aureus,* low activity against *β-haemolytic streptococcus,* and no activity at 200 μg/ml against *Proteus vulgaris.* The activity against *Escherichia coli* and *Klebsiella aerogenes* is dependent on the size of the substituents.

In series 4 the nitrofurans have a hydrogen or an alkyl group in position 3 and an oxo or thio group in position 2 of the thiazolidone ring. These nitrofurans are very active against *Staph. aureus,* whereas only the nitrofurans **4a,g,i,** with a hydrogen in position 3, are active against *P. vulgaris.* The activity against *E. coli, K. aerogenes,* and *β-haem. streptococcus* is dependent on the size of the 3-alkyl group. **4a,g,i,** with a hydrogen in position 3, are the most active nitrofurans in this series.

In series 5 the nitrofuran has a methylthio group in position 2 of the thiazolone ring. This nitrofuran, **5a,** shows moderate antibacterial activity and is thus much less active than the corresponding 2-methylamino derivative **2b.**

Our results show that the number of carbon atoms in the alkyl groups greatly influenced the activity against *P. vulgaris, K. aerogenes,* and *E. coli* and to some extent *β-haem. streptococcus* but not the activity against *Staph. aureus.*

This structure-activity relationship may be related to a change in the lipophilicity of the compounds. Hansch and others[17-19] have in several series of compounds found a correlation between antibacterial activity and lipophili-

city. However, in series 2 and 3 the lipophilicity was not increased by lengthening of the alkyl groups (Table I) and no correlation could be found in these series between antibacterial activity and lipophilicity. The lipophilicity of the nitrofurans in series 4 increased on lengthening of the alkyl chain. However, it is unlikely that the structure–activity relationship in series 4 is solely related to the lipophilicity.

An increase of the carbon chain in the 3-alkyl substituent in series 3 led to drastic changes in the antibacterial activity against *K. aerogenes* and *E. coli*. The same is true for substitution of the 3-hydrogen in **4g** with methyl group (Figure 3).

This is atypical of nitrofurans which usually exhibit a slight decrease in the activity when alkyl substituents are lengthened, as exemplified by series 2 in Figure 3.[20-24]

Space-filling models of the nitrofurans in series 2–4 show that these compounds have a very rigid structure, in contrast to other nitrofurans, *e.g.*, nitrofurantoin, nitrofuryltriazoles, **6a–d, 7a,b**, where the two rings are flexible. Steric effects might therefore be of importance for the activity of nitrofurans in series 2–5.

Steric inhibition may either prevent the nitrofurans from reacting with essential enzymes or from penetrating the bacteria. Penetration of nitrofurans into the bacteria has recently been shown at least in part to explain differences in the antibacterial spectrum of nitrofurans.[25] Further, there are indications that there is a special transport mechanism for the nitrofurans in or on the cell membrane.[26] Hence, the transport of our rigid nitrofurans into the bacteria may be sterically inhibited. However, to establish this, measurements of the penetration are needed. There is as yet no direct evidence to support the hypothesis that nitrofurans are sterically inhibited to react with essential enzymes.

The nitrofurans in series 2–7 had the half-wave potentials strongly shifted in the positive direction, and among these nitrofurans there were many with very high antibacterial activities. Thus, our results are in accordance with the postulation by Sasaki[4] that there is a relationship between antibacterial activity and half-wave potentials.

This examination shows that the 2-substituent on 5-nitrofurans may increase the antibacterial activity by facilitating the reduction of the nitro group, but if it is too bulky it may decrease the activity, probably by sterically inhibiting the penetration of the nitrofuran into the bacteria.

Experimental Section

The structures of all compounds were assigned on the basis of compatible ir, nmr, and mass spectra and satisfactory analysis. Ir spectra were measured in KBr disks or in $CHCl_3$ solution in a Unicam Sp200 spectrophotometer; nmr spectra were obtained at 60 MHz on a Perkin-Elmer T60 instrument and at 100 MHz on a Varian HA-100 instrument; mass spectra were measured at 70 eV in a LKB9000 instrument. The melting points are uncorrected. Tlc was recorded on Merck silica gel F_{254}. All compounds were analyzed for C, H, N, and S and analytical results were within ±0.4% of the calculated values.

Method A. Condensation of Nitrofurfural or Nitrofurylacrolein with Thiazolidones in Acetic Acid (3a,b,d–h, 4g–i). Nitrofurfural or nitrofurylacrolein (1 mol) was dissolved in 700 ml of concentrated HOAc. To this solution 140 g of anhydrous NaOAc and 1 mol of a thiazolidone derivative were added. The reaction mixture was warmed at 60–80° for 3 hr and thereafter cooled. The precipitate was filtered off and thoroughly washed with water to remove NaOAc. The product was recrystallized from ethanol, DMF, or a mixture of ethanol–DMF.

Method B. Condensation of Nitrofurfural or Nitrofurylacrolein with Thiazolones or Thiazolidones in Acetic Anhydride (2f–i,k,l, 3j–l, 4j). Nitrofurfural or nitrofurylacrolein (1 mol) was dissolved in 1 l. of acetic anhydride. To this solution was added 1 mol of a thiazolone or thiazolidone derivative and 140 g of anhydrous NaOAc. The mixture was warmed carefully to start the reaction and the temperature was kept at 70–80° for 3 hr. After cooling, the precipitate was filtered and thoroughly washed with water to remove sodium acetate. The product was recrystallized from acetic anhydride.

Method C. Hydrolysis of the *N*-Acetyl Group (2b–e,j, 3a,c,i, 4a). 2-Acetylalkylamino-5-(5-nitro-2-furfurylidene)thiazolin-4-ones, 2-acetylmethylamino-5-(5-nitro-2-furylpropenylidene)thiazolin-4-one, 3-acetyl-5-(5-nitro-2-furfurylidene)-2-phenyliminothiazolidin-4-one, and 3-acetyl-5-(5-nitro-2-furfurylidene)thiazolidine-2,4-dione were hydrolyzed by boiling in HOAc for 3 hr to **2b–e, 2j, 3i,** and **4a**. The reaction mixture was cooled and filtered. The products were recrystallized from DMF or a mixture of DMF and ethanol.

2-Acetylimino-3-alkyl-5-(5-nitro-2-furfurylidene)thiazolidin-4-ones were hydrolyzed by refluxing in 0.5 *M* EtOH–HCl for 40 min to **3a,c**. Attempts to hydrolyze in concentrated HOAc gave only starting material.

2-Acetylamino-5-(5-nitro-2-furfurylidene)thiazolin-4-one (**2f**) and 2-acetylamino-5-(5-nitro-2-furylpropenylidene)thiazolin-4-one (**2k**) gave a mixture of products on hydrolysis in 0.5 *M* EtOH–HCl.

Method D. Hydrolysis of 3-Alkyl-2-alkylimino-5-(5-nitro-2-furfurylidene)thiazolidin-4-ones (4b–f). 3-Alkyl-2-alkylimino-5-(5-nitro-2-furfurylidene)thiazolidin-4-one (0.04 mol) was refluxed in 100 ml of 5 *M* HCl for 5 hr. A product precipitated during the heating. After cooling the precipitate was filtered, washed with water, and recrystallized from ethanol or a mixture of DMF and water.

2-Methylthio-5-(5-nitro-2-furfurylidene)thiazolin-4-one (5a). Diazomethane (0.4 g in 40 ml of ether) was added to a mixture of 0.65 g (2.5 mmol) of 5-(5-nitro-2-furfurylidene)-2-thiothiazolidin-4-one (**4g**) in 50 ml of methanol. The reaction mixture was stirred for 3 hr at room temperature and then filtered. A product (0.4 g) was obtained which contained 61% **5a** and 39% 4h according to an nmr analysis. Repeated recrystallizations of the product in acetonitrile gave 0.1 g of pure **5a**.

2-Acetylamino-6-(5-nitro-2-furyl)-4*H*-1,3-thiazin-4-one (6c). 2-Acetylamino-6-(2-furyl)-4*H*-1,3-thiazin-4-one was nitrated according to the synthesis of **6a,b** in ref 6. The product was recrystallized from DMF.

2,3-Dihydro-6-(5-nitro-2-furyl)-4*H*-1,3-thiazine-2,4-dione (7a). 6-(2-Furyl)-2-methylamino-4*H*-1,3-thiazin-4-one (4.4 g, 0.021 mol) added to a mixture of 2.1 ml of concentrated HNO_3 and 21 ml of concentrated H_2SO_4 at −10° during 1 hr. The mixture was stirred for 30 min at −10° and then poured into ice. A yellow product, 4.1 g, precipitated which was a mixture of **6b** and **7a**. This product was boiled for 2 hr in 2 *M* HCl, and 3.3 g (65%) of **7a** was obtained. The product was recrystallized from DMF–ethanol.

Partition Coefficients. The partition coefficients (*P*) were determined in octanol–water. The octanol was of the Merck rein quality. The concentration of nitrofuran in the octanol layer was determined in a uv spectrometer and the concentration in water was obtained by difference. The volume ratio of the two phases and the amount of nitrofuran were chosen so that the absorbance of the nitrofuran had a value between 0.5 and 0.8 using a 1-cm cell. The concentration varied between 29.0×10^{-6} and 39.0×10^{-6} mmol/ml and in this range the partition coefficients were independent of the concentration. The compounds were not ionized at pH 7 (pK_a, $pK_b > 10$). The two phases were shaken for 1 min and then centrifuged. The octanol phase was carefully separated and deaerated by evacuating the uv cell to vacuum. The latter procedure was necessary to remove all air bubbles formed on the cell walls. As the nitrofurans were sensitive to light, all work was performed in a dark room. Each determination was repeated at least four times and the mean value of log $P \pm$ S.D. is reported in Table I.

Half-Wave Potentials. The polarographic half-wave potentials were measured with a PAR electrochemistry system Model 170 using 10^{-4} *M* solutions of the nitrofurans in a Tris buffer containing 10% DMF and 0.1 *M* KCl as supporting electrolyte. The Tris buffer was composed of 8.050 g of Trizma base/l. and pH was adjusted with 5 *N* HCl to 7.2. The temperature was kept at 37°. The capillary constant was 2.64 $mg^{2/3}\ sec^{-1/2}$ (open circuit and 63-cm mercury pressure), with $t_1 = 2.57$ sec and $m = 3.40$ mg $sec.^{-1}$ The reproducibility was ±0.005 V.

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Synthesis of Potential Antimalarial Agents. Preparation of Some 6-Amino-5,8-dimethoxyquinolines and the Corresponding 6-Amino-5,8-quinolinediones†

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The condensation of 2,5-dimethoxyaniline with acetylacetone and trifluoroacetylacetone gave the 5,8-dimethoxy derivatives of 2,4-dimethyl- and 4-methyl-2-(trifluoromethyl)quinoline (12 and 13), respectively. The direct preparation of 6-aminoquinolines by the cyclization of 4-(4-acetamido-2,5-dimethoxyanilino)-3-penten-2-one was unsuccessful. Also, the direct preparation of 6-nitroquinolines by condensation reactions involving 2,5-dimethoxy-4-nitroaniline was unsuccessful except for the reaction with 3-penten-2-one in the presence of arsenic acid to give 5,8-dimethoxy-2,4-dimethyl-6-nitroquinoline (10). A better method for the preparation of 10 and the corresponding 2-(trifluoromethyl) compound 11 involved the nitration of 12 and 13 in trifluoroacetic anhydride. The catalytic hydrogenation of 10 and 11 gave the corresponding 6-aminoquinolines 14 and 15. Although the condensation of 15 with 5-(diethylamino)-2-pentanone was unsuccessful, the mono- and dialkylation of 14 with 2-(diethylamino)ethyl chloride to give 16 and 17 was successful. Ether cleavage of the 5,8-dimethoxyquinolines 12 and 13 to give the 5,8-dihydroxyquinolines 22 and 23 was effected with HBr. Oxidation of 22 and 23 with dichromate gave the 5,8-quinolinediones 25 and 26. Oxidative amination of 25 with hydrazoic acid and 2-(diethylamino)ethylamine was shown to give 6-amino- and 6-[[2-(*N,N*-diethylamino)ethyl]amino]-2,4-dimethyl-5,8-quinolinediones (24 and 28), respectively. Also, the oxidative addition of *p*-chlorobenzenethiol to 26 gave both the mono- and bis(*p*-chlorophenylthio)-5,8-quinolinediones (20 and 21).

Both the 5,8-quinolinedione and 5,8-dimethoxyquinoline systems have provided compounds with antimalarial activity.[1-3] Recently, some 6-[(4-diethylamino-1-methylbutyl)amino]-5,8-dimethoxyquinolines were found to be as well tolerated by mice and canaries as chloroquine and to be as active against *Plasmodium vinckei* and the erythrocytic stages of *Plasmodium cathemerium* as primaquine. Also, no general cross resistance was observed when one of these compounds was tested against a strain of *Plasmodium berghei* fully resistant to chloroquine.[1] In this paper we wish to report our investigations on the preparation of 6-amino-5,8-quinolinediones and 6-amino-5,8-dimethoxyquinolines.

Several methods for the preparation of the 6-aminoquinolines 14 and 15 were investigated. Catalytic hydrogenation of 2,5-dimethoxy-4-nitroacetanilide with Raney nickel in EtOH gave the aniline 1, which was condensed with acetylacetone at 160° to give the 4-anilino-3-penten-2-one 4. However, the formation of 9 by treatment of 4 with a variety of reagents was unsuccessful.[4,5]

The second approach involved the condensation of the nitroaniline 2 with acetylacetone to give 5 followed by cyclization of the latter to give 10. However, only a minor amount of 5 was formed at 150°, which was attributed to the relatively low nucleophilicity of the amino group of 2 when compared with that of 1. The presence of 5 (M^+ 280) in the reaction mixture was confirmed by isolation of a sample by thick-layer chromatography. No ring closure to 10 was observed when 5 was refluxed in either diphenyl ether or toluene containing piperidine. In contrast, the condensation of 2 with trifluoroacetylacetone at 130° gave a good yield of 6, which was attributed to the increased electrophilic character of the trifluoroacetyl carbonyl group of trifluoroacetylacetone when compared with that of acetylacetone. Although mass spectral analyses indicated that a trace amount of 11 was formed by treatment of 6 (R = CF_3) with concentrated H_2SO_4 at 100°, no cyclization was observed when 6 (R = CF_3) was treated with ei-

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