[Chem. Pharm. Bull. 33(7)2854—2861(1985)]

Antibacterial Activity and Polarographic Half-Wave Reduction Potential of 2-Nitrobenzo[*b*]furans[1)]

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(Received October 9, 1984)

The antibacterial activities of a series of 2-nitrobenzo[*b*]furan derivatives against *St. aureus, B. subtilis, E. coli, Sal. typhimurium, Sal. enteritidis, Sh. flexneri, Pr. vulgaris* or *Ps. aeruginosa* were determined *in vitro*. Most of the compounds showed considerable activities against the bacteria except *Pr. vulgaris* and *Ps. aeruginosa* and one of them was about 30 times as active as nitrofurantoin against *St. aureus*. Mono- and dimethoxy derivatives (**2a**, **3a**, **4a**, **5f**) were the most active. The polarographic half-wave potentials ($E_{1/2}$) of the 2-nitrobenzo[*b*]furans at pH 7 were in a narrow range of -0.450 ± 0.04 V, whereas the $E_{1/2}$ values of regioisomeric nitrobenzo[*b*]furans were more negative (-0.560—-0.726 V). In the case of 2-nitrobenzo[*b*]furans, substituent(s) on the benzene ring had little influence on the reduction potential of the 2-nitro group, whereas the antibacterial activity depended markedly on the substituent group(s).

Keywords——2-nitrobenzo[*b*]furan; antibacterial activity; polarographic half-wave potential; nitrobenzo[*b*]furan; nitrofuran; mutagenicity test

Many kinds of heterocyclic nitro compounds show useful antibacterial activity, and 5-nitrofuran derivatives in particular have been synthetically and pharmacologically investigated[2)] for many years. Several 5-nitrofurans have been used clinically. We previously prepared 2-, 3-, 4-, 5-, 6-, or 7-nitrobenzo[*b*]furan derivatives and examined their antibacterial activity. Among these regioisomeric nitrobenzo[*b*]furans, 3-, 4-, 5-, 6-, or 7-nitrobenzo[*b*]-furans (**7a—i**) did not show any significant antibacterial activity. In contrast with **7a—i**, 2-nitrobenzo[*b*]furan derivatives showed strong antibacterial activity against several gram-positive and -negative bacteria. Therefore, thirty 2-nitrobenzo[*b*]furan derivatives (**1—6**) were synthesized[3)] and examined for antibacterial activities against *St. aureus, B. subtilis, E. coli, Sal. typhimurium, Sal. enteritidis, Sh. flexneri, Pr. vulgaris* and *Ps. aeruginosa*. Furthermore, the polarographic half-wave potentials of the 2-nitrobenzo[*b*]furans, five regioisomeric nitrobenzo[*b*]furans and other nitro compounds were determined, and the possibility of a relation between the polarographic half-wave potential and the antibacterial activity was investigated.

Experimental

Samples of the nitrobenzo[*b*]furans (**1**, **2a—h**, **3a—h**, **4a—c**, **5a—h**, **6a**, **6b**, **7a—i**) that gave the expected elemental analysis values were used for measurement of the antibacterial activity and the polarographic half-wave potential.

Antibacterial Activity——The *in vitro* antibacterial activities are reported as minimum inhibitory concentrations (MIC) in μg/ml. The authors modified Skaguis' procedure[4)] for the determination of MIC as follows. Solutions of the nitrobenzo[*b*]furans in acetone were prepared ranging in concentration from 1000 to 4 μg/ml by the twofold serial

dilution method. Each acetone solution (1 ml) was mixed with heart infusion agar medium (20 ml), then acetone was removed by warming at 37 °C for 24 h to prepare the agar medium plates to which the microorganisms were applied.

Half-Wave Potentials——The polarographic half-wave potentials were measured with a Yanagimoto P-8 polarograph instrument. Methanol solutions (2×10^{-3} M) of the 2-nitrobenzo[*b*]furans (**1**, **2a**—**d**, **2f**—**h**, **3a**—**h**, **4a**, **5b**, **5e**), five regioisomeric nitrobenzo[*b*]furans (**7a**—**e**), 1-(5-nitro-2-furfurylidenamino)hydantoin (nitrofurantoin), 5-nitro-2-furaldehyde semicarbazone (nitrofurazone), 1-nitronaphthalene and 1-nitrobiphenyl were prepared. Subsequently 0.9% NaCl aqueous solution (1 ml) and McIlvaine buffer (pH 3.0, pH 7.0 or pH 9.0, 4 ml, respectively) were added to all of the methanolic solutions (100 μl). Nitrogen was bubbled through the solutions for 15 min, and then the half-wave potential was determined at 29 °C using a dropping Hg electrode (open circuit and 70 cm mercury pressure) with $t_1 = 4.0$ s and $m = 1.90$ mg/s.

Mutagenicity Test——The mutagenicity of **3a**, which showed strong antibacterial activity, was checked by Ames' procedure[5] using *Sal. typhimurium* TA 98 and TA 100. The positive controls were *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) [3 μg in dimethylsulfoxide (DMSO) (0.1 ml)] and 2-nitrofluorene [1 μg in DMSO (0.1 ml)]. Solutions of **3a** in DMSO were prepared at concentrations of 5000, 500, 50, 5 and 0.5 μg/0.1 ml for testing.

Preparation of the Nitrobenzo[*b*]furans (7a—i)

Table I[6] shows physical data for the new nitrobenzo[*b*]furans (**7a**—**i**).

2-Acetyl-5-nitrobenzo[*b*]furan (7g)——2-Acetylbenzo[*b*]furan[7] (8 g, 0.05 mol) was treated with nitric acid (15 ml, $d = 1.42$) in acetic anhydride (130 ml) at room temperature for 3 h to give a mixture consisting of **7g** and 2-acetyl-7-nitrobenzo[*b*]furan. The mixture was recrystallized from ethanol to give pale yellow needles (**7g**, 1.7 g, 16.6%). Concentration and cooling of the mother liquor yielded the crude 7-nitro isomer, which was recrystallized from methanol and ethyl acetate to give 2-acetyl-7-nitrobenzo[*b*]furan [pale yellow needles, 2.7 g, 26.3%, mp 151 °C. Proton nuclear magnetic resonance (^{1}H-NMR) ($CDCl_3$) δ: 2.60 ($COCH_3$, 3H, s), 7.38 (3-H, 1H, s), 7.50—8.45 (phenyl protons, 3H, m). Mass spectrum (MS): 205 (M^+), 190, 159. *Anal.* Calcd for $C_{10}H_7NO_4$: C, 58.54; H, 3.44. Found: C, 58.47; H, 3.39].

2-Ethyl-5-nitrobenzo[*b*]furan (7c)——A mixture of **7g** (5.7 g, 0.028 mol) and 80% hydrazine hydrate (4.4 g, 0.07 mol) in diethyleneglycol (290 ml) was heated at 125 °C for 1.5 h and then potassium hydroxide (3.9 g, 0.07 mol) was added to the mixture at 40 °C. The reaction mixture was heated again at 140 °C for 1 h and then acidified with 12 N hydrochloric acid. The acidic aqueous solution was steam-distilled to give a yellow solid. The solid was recrystallized from ethanol to give yellow needles (**7c**, 3.7 g, 69.8%).

2-Ethyl-3,5-dinitrobenzo[*b*]furan (7a)——A mixture of **7c** (2.7 g, 0.014 mol), nitric acid (1.6 g, $d = 1.42$) and H_2SO_4 (0.5 ml, $d = 1.84$) in acetic anhydride (100 ml) was stirred at 10 °C for 3 h. The mixture was poured into ice-water to give a yellow solid, which was recrystallized from ethanol to give yellow prisms (**7a**, 1.9 g, 57.6%).

2-Ethyl-3-4'-hydroxybenzoyl-5-nitrobenzo[*b*]furan (7d)——A mixture of **7c** (2.4 g, 0.013 mol), anisoyl chloride (2.9 g, 0.017 mol) and aluminum chloride (5.9 g, 0.044 mol) in carbon disulfide (170 ml) was stirred at 25 °C for 24 h. The reaction mixture was treated in the usual manner to give a yellow powder. The powder was recrystallized from acetone–ethanol (5 : 2) to give 2-ethyl-3-4'-methoxybenzoyl-5-nitrobenzo[*b*]furan [pale yellow needles, 2.9 g, 52.7%, mp 128 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.35 (CH_3CH_2, 3H, t), 2.93 (CH_2, 2H, q), 3.91 (OCH_3, 3H, s), 6.98 (3'- and 5'-H, 2H, d), 7.58 (7-H, 1H, d), 7.83 (2'- and 6'-H, 2H, d), 8.21 (6-H, 1H, dd), 8.38 (4-H, 1H, d). MS: 305 (M^+), 310, 294, 279. *Anal.* Calcd for $C_{18}H_{15}NO_5$: C, 66.45; H, 4.65. Found: C, 66.56; H, 4.65]. 2-Ethyl-3-4'-methoxybenzoyl-5-nitrobenzo[*b*]furan (1 g, 0.003 mol) was heated with aluminum chloride (0.9 g, 0.007 mol) at 120 °C for 2 h in chlorobenzene (50 ml) to give crude **7d**, which was recrystallized from ethanol–water (1 : 4) to give colorless needles (**7d**, 0.45 g, 46.9%).

2-Ethyl-3-4'-hydroxybenzoyl-3',5'-diiodo-5-nitrobenzo[*b*]furan (7f)——A mixture of **7d** (0.5 g, 0.002 mol) and chloroiodide (0.5 g, 0.003 mol) in acetic acid (12 ml) was stirred at 25 °C for 0.5 h and then H_2O (20 ml) was added to the mixture. The mixture was heated at 75 °C for 10 min and then cooled to 0 °C to give a colorless solid. The solid was recrystallized from benzene to give colorless needles (**7f**, 0.8 g, 88.8%).

2,3-Dimethoxy-5-nitrobenzo[*b*]furan (7h)[8]——A mixture of *O*-(*p*-nitrophenyl)-methylethylketone oxime[9] (2 g, 0.01 mol) and 35% hydrochloric acid (9 ml) in ethanol (90 ml) was heated at 75 °C for 1.5 h and then the mixture was cooled at −5 °C to give a pale yellow powder. The powder was recrystallized from ethanol to give pale yellow prisms (**7h**, 0.7 g, 36.6%).

4-Nitro- and 6-Nitro-2-acetyl-7-hydroxybenzo[*b*]furan (7d and 7e)——2-Acetyl-7-methoxybenzo[*b*]furan[10] (3.8 g, 0.02 mol) was treated under conditions similar to those used for the nitration of 2-acetylbenzo[*b*]furan to give a crude mixture consisting of 4-nitro- and 6-nitro-2-acetyl-7-methoxybenzo[*b*]furan (4.1 g, 87.2%). A mixture of the above regioisomeric mixture and 47% hydrobromic acid (7.5 ml) in acetic acid (30 ml) was heated at 115 °C for 3 h. The reaction mixture was poured into H_2O (250 ml) to precipitate a crude mixture of **7b** and **7e**, which was steam-distilled to give a yellow solid. This was recrystallized from methanol to give **7e** (0.91 g, 20.5%), while **7b** was extracted from the residue and recrystallized from DMSO (colorless pale needles, 1.4 g, 31.5%).

2-Acetyl-5-carbomethoxy-4-hydroxy-7-nitrobenzo[*b*]furan (7i)——Hydrogen chloride was passed into a mixture of methyl-4-hydroxysalicylate, zinc cyanide and aluminum chloride in dry benzene to give 5-carbomethoxy-6-hydroxysalicylaldehyde (23%, mp 172 °C). A mixture of the salicylaldehyde (5.2 g, 0.027 mol), monochloroacetone

TABLE I. Physical Data for Nitrobenzo[*b*]furans (**7a—i**)

Compd. No.	mp (°C)	^{1}H-NMR (ppm)	Formula and Analysis Calcd (Found) % C H	MS
7a	133	1.42 (CH_3, 3H, t), 3.29 (CH_2, 2H, q), 7.32 (7-H, 1H, d), 8.00 (6-H, 1H, dd), 8.64 (4-H, 1H, d) ($CDCl_3$)	$C_{10}H_8N_2O_5$ 50.85 3.41 (50.67 3.53)	236 (M^+), 190, 144
7b	233	2.47 ($COCH_3$, 3H, s), 3.30 (OH, 1H, br s), 6.77 (6-H, 1H, d), 7.86 (3-H, 1H, s), 7.92 (4-H, 1H, d) (DMSO-d_6)	$C_{10}H_7NO_5$ 54.30 3.19 (54.33 3.11)	221 (M^+), 207, 192
7c	85	1.33 (CH_3, 3H, t), 2.78 (CH_2, 2H, q), 6.30 (3-H, 1H, s), 7.20 (7-H, 1H, d), 7.85 (6-H, 1H, dd), 8.08 (4-H, 1H, d) ($CDCl_3$)	$C_{10}H_9NO_3$ 62.82 4.75 (62.55 4.78)	191 (M^+), 145
7d	152	1.42 (CH_3, 3H, t), 3.10 (CH_2, 2H, q), 6.72 (OH, 1H, br s), 7.20 (3′- and 5′-H, 2H, d), 7.42 (7-H, 1H, d), 8.02 (2′- and 6′-H, 2H, d), 8.21 (6-H, 1H, dd), 8.67 (4-H, 1H, d) ($CDCl_3$)	$C_{17}H_{13}NO_5$ 65.59 4.21 (65.60 4.19)	311 (M^+), 294, 265, 190
7e	191	2.43 ($COCH_3$, 3H, s), 7.06 (4-H, 1H, d), 7.56 (5-H, 1H, d), 7.62 (3-H, 1H, s) (DMSO-d_6)	$C_{10}H_7NO_5$ 54.30 3.19 (54.21 2.97)	221 (M^+), 206, 189, 175
7f	179	1.37 (CH_3, 3H, t), 2.87 (CH_2, 2H, q), 7.58 (7-H, 1H, d), 8.23 (2′- and 6′-H, 2H, s), 8.26 (6-H, 1H, dd), 8.48 (4-H, 1H, d) ($CDCl_3$)	$C_{17}H_{11}I_2NO_5$ 37.71 2.17 (37.97 2.17)	563 (M^+), 546, 517
7g	175	2.16 ($COCH_3$, 3H, s), 7.42 (3-H, 1H, s), 7.56 (7-H, 1H, s), 8.18 (6-H, 1H, dd), 8.43 (4-H, 1H, d) ($CDCl_3$)	$C_{10}H_7NO_4$ 58.54 3.44 (58.37 3.31)	205 (M^+), 190, 159
7h	116	2.20 (3-CH_3, 3H, d), 2.42 (2-CH_3, 3H, d), 7.38 (7-H, 1H, d), 8.12 (6-H, 1H, dd), 8.30 (4-H, 1H, d) ($CDCl_3$)	$C_{10}H_9NO_3$ 62.82 4.75 (62.91 4.67)	191 (M^+), 176, 145
7i	193	2.58 ($COCH_3$, 3H, s), 4.00 ($COOCH_3$, 3H, s), 6.20 (OH, 1H, br s), 8.22 (3-H, 1H, s), 8.69 (6-H, 1H, s) (DMSO-d_6)	$C_{13}H_{11}NO_7$ 53.24 3.78 (53.10 3.62)	293 (M^+), 278, 247, 234

(2.9 g, 0.032 mol) and potassium carbonate (8.2 g, 0.059 mol) in dry acetone (200 ml) was refluxed for 3 h. After usual work-up, the product was recrystallized from ethyl acetate to give 2-acetyl-4-hydroxy-5-carbomethoxybenzo[*b*]furan [pale yellow needles, 3.2 g, 50.3%, mp 175 °C. ^{1}H-NMR (DMSO-d_6) δ: 2.42 ($COCH_3$, 3H, s), 3.72 ($COOCH_3$, 3H, s), 6.96 (7-H, 1H, d), 7.64 (6-H, 1H, d), 7.69 (3-H, 1H, s). MS: 234 (M^+), 203, 187. *Anal.* Calcd for $C_{12}H_{10}O_5$: C, 61.54; H, 4.30. Found: C, 61.31; H, 4.33]. 2-Acetyl-5-carbomethoxy-4-hydroxybenzo[*b*]furan (3 g, 0.013 mol) was treated under conditions similar to those used for the nitration of 2-acetylbenzo[*b*]furan to give a crude product, which was recrystallized from ethanol to give pale yellow prisms (**7i**, 2.9 g, 76.9%).

Results and Discussion

MIC values of the 2-nitrobenzo[*b*]furans (**1—6**) and the regioisomeric nitrobenzo[*b*]-furans (**7**) against eight strains of gram-positive and -negative bacteria are shown in Table II, in which the 2-nitrobenzo[*b*]furans are classified into six series (1—6) according to their structural types. In series 2, the 2-nitrobenzo[*b*]furans have a hydroxy or alkyloxy group at the 6-position, and the methoxy compound (**2a**) showed strong antibacterial activity against six bacteria but weak activity against *Pr. vulgaris* and *Ps. aeruginosa*. The activity decreased with increasing carbon number of the alkyloxy group, and the geranyloxy compound (**2e**) was inactive. The acidic carboxymethoxy compound (**2g**) did not show any activity. In series 3, the methoxy compound (**3a**) showed the highest activity except against *Ps.*

aeruginosa. The antibacterial activity of the acetyloxy compound (**3f**) was comparable to that of the phenol (**3h**), but the lipophilic benzoyloxy compound (**3g**) was less active than the phenol (**3h**). The 5-methoxy compound (**4a**) was also active against seven bacteria. The dimethoxy compound (**5f**) in series 5 was more active than the corresponding monomethoxy compounds (**2a**, **3a**) except against *Pr. vulgaris* and *Ps. aeruginosa*.

Attempts to increase the activity of the 7-methoxy compound (**3a**) by the introduction of a substituent at the 4-position were unsuccessful, namely, the 4-acetyl derivative (**5a**) did not show significant activity, like the other compounds (**5b**—**d**). The activities of the 2-nitrobenzo[*b*]furan derivatives (**4b**, **5g**, **5h**, **6a**, **6b**) with bromine were slightly enhanced as compared with those of the corresponding compounds without bromine.

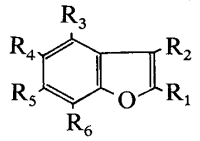
Our results suggest that the nature of the substituent group(s) does influence the antibacterial activity of 2-nitrobenzo[*b*]furans. Namely, the monomethoxy compounds (**2a**, **3a**, **4a**) show considerable antibacterial activity which decreases with increase in the length of the alkyloxy group (**2a**→**2b**→**2c**→**2d**→**2e**, **3a**→**3b**→**3c**→**3d**). The gradual decrease of activity may depend on steric hindrance or poor solubility owing to the substituent(s). Replacement of polar functional groups such as nitro, acetyl, carboxyl, bromo, hydroxyimino, acetylamino or amino on 2-nitrobenzo[*b*]furan nuclei results in somewhat lower activity against most of the microorganisms. The antibacterial activity of 5-nitrofurans is greatly influenced by substituent groups,[2g,11] but most of the 2-nitrobenzo[*b*]furan derivatives, except the compounds with a long carbon chain alkyloxy group or carboxy group, show considerable antibacterial activity. Generally speaking, the 2-nitrobenzo[*b*]furans almost all show high activity against *B. subtilis* and *E. coli*, while a low activity against *Pr. vulgaris* and *Ps. aeruginosa* except for the 6-methoxy compound (**2a**). Useful antibacterial agents have an appropriate balance of lipophilicity and hydrophilicity and a suitable polarity of the molecule for passing through the cell membrane of microorganisms. It is presumed that the polarity of the 2-nitro group and the lipophilicity of the mono- or dimethoxy group on the benzene ring in this 2-nitrobenzo[*b*]furan series may cooperatively contribute to permeation of the compounds through the cell membrane of microorganisms.

It is known that nitro compounds with antibacterial activity interfere with some reductive enzyme systems in the bacteria.[2b,12] The reduction potential of the 5-nitro group is an important factor for the antibacterial activity of 5-nitrofurans,[13] and indeed a close relationship between the polarographic half-wave potential and the antibacterial activity of 5-nitrofuran derivatives was reported.[2g] Thus, we determined half-wave potentials of the 2-nitrobenzo[*b*]furans (**1**, **2a**—**h**, **3a**—**h**, **4a**, **5b**, **5e**) and some other regioisomeric nitrobenzo[*b*]furans (**7a**—**e**) at pH 3.0, 7.0 or 9.0 to examine the possible relationship between polarographic half-wave potential and antibacterial activity. The results are shown in Table III.

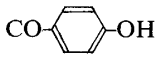
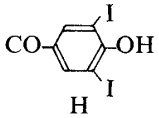
The $E_{1/2}$ values at pH 3 were distributed in a very narrow range (-0.350 ± 0.01 V) except for **3e**, **3g** and **5b**, since a proton from the medium might tend to combine with the nitro group. The distributions of the $E_{1/2}$ values at pH 7 (-0.450 ± 0.04 V) and pH 9 (-0.500 ± 0.04 V) were wider than that in the case of pH 3, and the differences among the $E_{1/2}$ values at pH 7 and pH 9 for these compounds were quite similar. The $E_{1/2}$ values (at pH 7) of 6-substituted compounds (**2**) were usually somewhat higher than those of 7-substituted compounds (**3**). Neither the length of the carbon chain of alkyloxy groups nor the number of methoxy groups on the benzene ring showed a significant correlation with the $E_{1/2}$ values.

The $E_{1/2}$ values (at pH 7) of the 2-nitrobenzo[*b*]furans except for **3g** characteristically lie in a limited range (-0.450 ± 0.04 V) regardless of variations of the substituent(s) and their position(s), and most of these 2-nitrobenzo[*b*]furans possess considerable antibacterial activity except against *Pr. vulgaris* and *Ps. aeruginosa*. The $E_{1/2}$ values (-0.450 ± 0.04 V) of the active 2-nitrobenzo[*b*]furans are close to those (-0.400——-0.350 V, at pH 7) of 5-

TABLE II. Antibacterial Activities of Minimum Inhibitory



Compound

No.	R_1	R_2	R_3	R_4	R_5	R_6
1[a]	NO_2	H	H	H	H	H
2a	NO_2	H	H	H	CH_3O	H
2b	NO_2	H	H	H	C_2H_5O	H
2c	NO_2	H	H	H	n-C_3H_7O	H
2d	NO_2	H	H	H	n-C_4H_9O	H
2e	NO_2	H	H	H	Geranyloxy[b]	H
2f	NO_2	H	H	H	$C_2H_5OOCCH_2O$	H
2g	NO_2	H	H	H	$HOOCCH_2O$	H
2h	NO_2	H	H	H	HO	H
3a	NO_2	H	H	H	H	CH_3O
3b	NO_2	H	H	H	H	C_2H_5O
3c	NO_2	H	H	H	H	n-C_3H_7O
3d	NO_2	H	H	H	H	n-C_4H_9O
3e	NO_2	H	H	H	H	$(C_2H_5)_2N(CH_2)_2O$
3f	NO_2	H	H	H	H	CH_3COO
3g	NO_2	H	H	H	H	C_6H_5COO
3h	NO_2	H	H	H	H	HO
4a	NO_2	H	H	CH_3O	H	H
4b	NO_2	H	H	Br	H	H
4c	NO_2	H	H	NO_2	H	H
5a	NO_2	H	CH_3CO	H	H	CH_3O
5b	NO_2	H	$HO\cdot N=C(CH_3)$	H	H	CH_3O
5c	NO_2	H	CH_3CONH	H	H	CH_3O
5d	NO_2	H	$HCl\cdot NH_2$	H	H	CH_3O
5e	NO_2	H	H	H	CH_3O	CH_3O
5f	NO_2	H	CH_3O	H	CH_3O	H
5g	NO_2	H	H	CH_3O	H	Br
5h	NO_2	H	H	Br	H	Br
6a	NO_2	H	Br	H	Br	HO
6b[a]	NO_2	H	Br	H	Br	CH_3COO
7a	C_2H_5	NO_2	H	NO_2	H	H
7b	$COCH_3$	H	NO_2	H	H	HO
7c	C_2H_5	H	H	NO_2	H	H
7d	C_2H_5		H	NO_2	H	H
7e	$COCH_3$	H	H	H	NO_2	HO
7f	C_2H_5		H	NO_2	H	H
7g	$COCH_3$	H	H	NO_2	H	H
7h	CH_3	CH_3	H	NO_2	H	H
7i	$COCH_3$	H	HO	$COOCH_3$	H	NO_2
	Nitrofurantoin					
	Nalidixic acid					

a) All of the 2-nitrobenzo[*b*]furans (**1**, **2a**—**h**, **3a**—**h**, **4a**—**c**, **5a**—**h**, **6a**, **6b**) were prepared by authors (ref. 3). *b*) Geranyloxy:

Nitrobenzo[*b*]furan Derivatives:
Concentration (μg/ml)

Microorganism							
Gram-positive		Gram-negative					
St. aureus	*B. subtilis*	*E. coli*	*Sal. typhimurium*	*Sal. enteritidis*	*Sh. flexneri*	*Pr. vulgaris*	*Ps. aeruginosa*
50	50	6.25	6.25	12.5	6.25	50	>50
12.5	<0.20	0.78	1.56	3.13	0.78	25	50
>50	1.56	1.56	6.25	12.5	6.25	>50	>50
>50	0.39	1.56	25	>50	25	>50	>50
12.5	0.39	12.5	>50	>50	>50	>50	>50
>50	>50	>50	>50	>50	>50	>50	>50
50	3.13	3.13	12.5	50	25	>50	>50
>50	>50	>50	>50	>50	>50	>50	>50
6.25	25	6.25	6.25	6.25	6.25	50	>50
12.5	0.40	1.56	3.13	6.25	3.13	12.5	>50
12.5	0.78	0.78	6.25	12.5	3.13	12.5	>50
25	6.25	6.25	50	>100	25	25	>100
100	12.5	12.5	>100	>100	>100	>100	>100
25	1.56	6.25	25	50	25	12.5	>50
50	6.25	1.56	3.13	3.13	3.13	25	>50
>50	12.5	6.25	6.25	12.5	6.25	>50	>50
25	1.56	1.56	3.13	6.25	1.56	25	>50
12.5	1.56	6.25	12.5	12.5	6.25	25	>50
12.5	6.25	6.25	25	>50	6.25	50	>50
50	12.5	12.5	25	25	12.5	12.5	>50
>50	0.40	12.5	>50	>50	>50	>50	>50
3.13	6.25	3.13	12.5	>12.5	6.25	>12.5	>12.5
12.5	3.13	6.25	25	>50	12.5	50	>50
50	3.13	6.25	25	50	12.5	12.5	>50
25	25	3.13	12.5	25	6.25	50	>50
<0.75	<0.20	0.39	1.56	3.13	3.13	>50	>50
6.25	3.13	3.13	6.25	>50	3.13	>50	>50
6.25	6.25	12.5	25	>50	12.5	12.5	>50
12.5	12.5	3.13	50	50	12.5	12.5	>50
25	12.5	6.25	25	>50	12.5	25	>50
>50	>50	>50	>50	>50	>50	>50	>50
>50	>50	>50	>50	>50	>50	>50	>50
>50	>50	>50	>50	>50	>50	>50	>50
25	12.5	>50	>50	>50	>50	>50	>50
>50	>50	>50	>50	>50	>50	>50	>50
6.25	3.13	>50	>50	>50	>50	>50	>50
>50	>50	>50	>50	>50	>50	>50	>50
>50	>50	>50	>50	>50	>50	>50	>50
>50	>50	>50	>50	>50	>50	>50	>50
25	12.5	6.25	12.5	12.5	6.25	50	>50
50	12.5	1.56	1.56	3.13	1.56	3.13	>50

$(CH_3)_2C=CHCH_2CH_2C(CH_3)=CHCH_2O$–.

TABLE III. Half-Wave Potentials of Nitrobenzo[*b*]furan Derivatives and Some Other Nitro Compounds

Compound	$-E_{1/2}$ (V)		
	pH 3.0	pH 7.0	pH 9.0
1	0.361	0.433	0.481
2a	0.369	0.474	0.526
2b	0.354	0.454	0.504
2c	0.361	0.480	0.532
2d	0.354	0.447	0.489
2f	0.358	0.488	0.533
2g	—	—	0.537
2h	0.348	0.458	0.535
3a	0.360	0.431	0.483
3b	0.354	0.454	0.504
3c	0.359	0.455	0.509
3d	0.350	0.435	0.488
3e	0.306	0.411	0.461
3f	0.364	0.337	0.456
3g	0.310	0.434	0.477
3h	0.340	0.410	0.453
4a	0.356	0.451	0.514
5b	0.325	0.421	0.472
5e	0.348	0.452	0.518
7a		0.560	
7b		0.696	
7c	0.588	0.726	0.805
7d		0.640	
7e		0.615	
Nitrofurantoin	0.354	0.389	0.461
Nitrofurazon	0.367	0.410	0.453
1-Nitronaphthalene	0.440	0.546	0.626
1-Nitrobiphenyl	0.398	0.551	0.630

The geranyloxy compound (**2e**) and carboxy compound (**2g**) did not dissolve sufficiently under the conditions used for the determination of $E_{1/2}$.

nitrofurans[13e] with antibacterial activity. $E_{1/2}$ values of clinically useful nitrofurantoin and nitrofurazone are similar to those of the active 5-nitrofurans in our experiments (see Table III). A substituent at the 2-position of 5-nitrofuran derivatives influences the half-wave potential of the nitro group,[14] but the half-wave potentials of the 2-nitrobenzo[*b*]furans are scarcely affected by the substituent(s) or the position(s). This is a characteristic difference between 2-nitrobenzo[*b*]furans and 5-nitrofurans.

The $E_{1/2}$ values (-0.450 ± 0.04 V, at pH 7) of the active 2-nitrobenzo[*b*]furans were considerably different from those [-0.560—-0.726 V, at pH 7 (see Table III)] of the 3-, 4-, 5-, or 6-nitrobenzo[*b*]furan derivatives (**7a**—**e**) without antibacterial activity and those (-0.77 and -0.68 V, at pH 7.4) of the 3- or 7-nitrobenzo[*b*]furan derivatives.[15] The results show that the 2-nitro group is more easily reduced than the nitro group at other positions. This may be a reason[2g,13b,15] for the high activity of the 2-nitrobenzo[*b*]furans among the regioisomeric nitrobenzo[*b*]furans.

It was difficult to find any clear relationship between the $E_{1/2}$ value at pH 7 and the antibacterial activity of the 2-nitrobenzo[*b*]furans series; namely, the $E_{1/2}$ values of **2a** and **3b** with high activities against six bacteria were very close to those of **2c** and **3c**, with low activities. Similarly, $E_{1/2}$ values of the inactive compounds (**2d**, **3d**) were also very close to

those of the active compounds (**2b**, **3a**, **3b**, **4a**).

There has been no report on the mutagenicity of nitrobenzo[*b*]furans, so the mutagenicity of **3a**, which has high antibacterial activity, was tested using *Sal. typhimurium* TA 98 and TA 100 by Ames' procedure.[5] The test microorganisms were killed at high dose [>5 μg in DMSO (0.1 ml)], but **3a** [0.5 μg in DMSO (0.1 ml)] showed nearly the same mutagenicity as 2-nitrofluorene [1 μg in DMSO (0.1 ml)] towards *Sal. typhimurium* TA 98 and as MNNG [3 μg in DMSO (0.1 ml)] towards *Sal. typhimurium* TA 100. The results suggest that further mutagenicity tests of 2-nitrobenzo[*b*]furans would be desirable.

In conclusion, the $E_{1/2}$ values of the 2-nitrobenzo[*b*]furans were generally within a narrow positive direction range (-0.450 ± 0.04 V, at pH 7), which is compatible with considerable antibacterial activity, regardless of substituent group(s), while those of 5-nitrofurans are variable with the substituent group. Therefore, the probability of finding new antibacterial agents may be higher among 2-nitrobenzo[*b*]furans than among 5-nitrofurans. However, the experimental antibacterial activity is influenced by the nature of the substituent group(s) because highly active compounds must have a good balance of polarity, lipophilicity, hydrophilicity, *etc.* Thus, selection of the substituent group is very important. The results of the present work (see Table III) suggest that an $E_{1/2}$ value more positive than -0.49 V at pH 7 may be a possible criterion in the search for new antibacterial agents containing a nitro group.

Acknowledgements The authors are indebted to Prof. Masao Okamoto and Assistant Prof. Shunsaku Ohta, Kyoto Pharmaceutical University, for valuable advice and discussions during this work.

References and Notes

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14) In the case of 5-nitrofurans, the half-wave potential of the nitro group is significantly influenced by the substituent at the 2-position; namely, the radical anion formed during the initial reduction step of the nitro group can be stabilized by a resonance effect of the 2-substituted functional group. Such nitro groups of 5-nitrofurans are easily reduced and their half-wave potentials are shifted in the positive direction (refs. 2*g*, 11).
15) Powers reported $E_{1/2}$ values of two nitrobenzo[*b*]furans at pH 7.4: 2-methyl-3-nitrobenzo[*b*]furan (-0.77 V), 2-methyl-7-nitrobenzo[*b*]furan (-0.68 V). [L. J. Powers and M. P. Mertes, *J. Med. Chem.*, **13**, 1102 (1970)].