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Antimicrobial activities of 2-arylthio-*N*-alkylmaleimides

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Key words: Antimicrobial activity; 2-Arylthio-*N*-alkylmaleimide; Antibacterial activity; Antifungal activity; Minimum inhibitory concentration

SUMMARY

A variety of 2-arylthio-*N*-alkylmaleimides were prepared, and their antimicrobial activities were examined. Almost all of these compounds exhibited antibacterial activity against Gram-positive bacteria such as *Bacillus subtilis* and *Staphylococcus aureus*. Some compounds such as 2-(halogeno-phenyl)-thio-*N*-methylmaleimides (4, 5, 6, 8 and 10) and 2-(2-carbamoylphenyl)thio-*N*-methylmaleimide(35) exhibited antibacterial activity against *Escherichia coli*. All compounds tested were inactive against *Pseudomonas aeruginosa* except 2-(2-carbamoylphenyl)thio-*N*-methylmaleimide(35) which was marginally active. Activities against Gram-positive bacteria were not due to the effect of the substituent on the benzene ring, except in the instances 2-carboxy, 2-carbomethoxy, 2-amino groups and alkyl chains, however, activities against Gram-negative bacteria were due to phenylthio and the alkyl substituents. Some of 2-arylthio-*N*-alkylmaleimides were examined for their antifungal activities using eight strains of fungi, and they showed activity against these.

INTRODUCTION

The bactericidal and fungicidal properties of *N*-substituted maleimides and several of their derivatives are well known [3]. It has been reported that some of these compounds are useful as industrial biocides, antifouling agents [9–11] or agricultural fungicides [2, 12]. A variety of maleimides were prepared and examined for their antimicrobial activities as reported previously [4–6, 13, 14]. We reported the preparation of 2-(substituted)thio maleimides and their activities against bacteria and fungi [7]. In these studies, we found that 2-phenylthio-*N*-methylmaleimide or 2-benzylthio-*N*-methylmaleimide showed good antimicrobial activity, but 2-alkylthio-*N*-(substituted)maleimides showed no activity. Recently, we reported the preparation of 2-arylthio-*N*-alkylmaleimides [8]. In this paper, we report the antimicrobial activities of these 2-arylthio-*N*-alkylmaleimides.

MATERIALS AND METHODS

Chemicals

All the 2-arylthio-*N*-alkylmaleimides were prepared by the method reported previously [8]. *N*-Alkylmaleimides were reacted with thiophenols to give 2-arylthio-*N*-alkylsuccinimides. These succinimides were chlorinated, followed by dehydrochlorination to give the compounds tested. *N*-Methylmaleimide, *N*-ethylmaleimide and various thiophenols were purchased from Tokyo Kasei Kogyo Co. Other *N*-alkylmaleimides were prepared from the reaction of maleic anhydride and various alkylamines by standard methods [1]. All of these compounds were identified by IR and NMR [8]. The microanalyses of these compounds were in agreement with the calculated values: C ± 0.25%, H ± 0.05%. All the test chemicals used for GLC and TLC measurement were analytically pure [8]. *N*-Methylmaleimide was used as a reference compound in bactericidal activity tests.

Microorganisms

The following filamentous fungi were used: *Aspergillus niger* FERM S-1 (ATCC 6275); *Penicillium citrinum* FERM S-5 (ATCC 9849); *Rhizopus stolonifer* FERM S-7 (ATCC 10404); *Cladosporium cladosporioides* FERM S-8; *Aureobasidium pullulans* FERM S-9; *Geotrichum candidum* IFO 4598.

The following yeasts were used: *Rhodotorula rubra* JCM 3785 (ATCC 4054); *Saccharomyces cerevisiae* JCM 2220 (ATCC 9804).

The following bacteria were used: *Bacillus subtilis* IFO 3134 (ATCC 6633); *Staphylococcus aureus* IFO 13276 (ATCC 6538); *Escherichia coli* IFO 3806 (ATCC 11246); *Pseudomonas aeruginosa* IFO 12689 (ATCC 1015). (The

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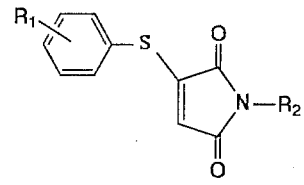
TABLE 1

Bacteriostatic activity of 2-arylthio-*N*-alkylmaleimides



Compound No.	R_1	R_2	MIC ($\mu g \cdot ml^{-1}$)							
			B. subtilis (ATCC 6633)		*S. aureus* (ATCC 6538)		*E. coli* (ATCC 11246)		*P. aeruginosa* (ATCC 10145)	
			8 h	24 h	8 h	24 h	8 h	24 h	8 h	24 h
1	H	Me	<6.25	<6.25	<6.25	12.5–25	50–100	>100	>100	>100
2	2-Me	Me	<6.25	<6.25	<6.25	12.5–25	>100	>100	>100	>100
3	4-Me	Me	<6.25	<6.25	<6.25	12.5–25	50–100	>100	>100	>100
4	2-Cl	Me	<6.25	<6.25	6.25–12.5	12.5–25	25–50	>100	>100	>100
5	3-Cl	Me	<6.25	<6.25	6.25–12.5	12.5–25	25–50	>100	>100	>100
6	4-Cl	Me	<6.25	<6.25	<6.25	12.5–25	25–50	>100	>100	>100
7	2-Br	Me	<6.25	<6.25	<6.25	25–50	>100	>100	>100	>100
8	3-Br	Me	<6.25	<6.25	<6.25	12.5–25	25–50	>100	>100	>100
9	4-Br	Me	<6.25	<6.25	<6.25	12.5–25	>100	>100	>100	>100
10	4-F	Me	<6.25	<6.25	6.25–12.5	12.5–25	12.5–25	>100	>100	>100
11	2-OMe	Me	<6.25	12.5–25	<6.25	12.5–25	>100	>100	>100	>100
12	3-OMe	Me	<6.25	6.25–12.5	6.25–12.5	25–50	>100	>100	>100	>100
13	4-OMe	Me	<6.25	6.25–12.5	<6.25	25–50	>100	>100	>100	>100
14	4-NO_2	Me	<6.25	12.5–25	12.5–25	12.5–25	>100	>100	>100	>100
15	2,4-Me	Me	<6.25	<6.25	<6.25	25–50	>100	>100	>100	>100
16	3,4-Me	Me	<6.25	<6.25	<6.25	12.5–25	>100	>100	>100	>100
17	2,5-Me	Me	<6.25	<6.25	<6.25	12.5–25	>100	>100	>100	>100
18	2,6-Me	Me	6.25–12.5	12.5–25	6.25–12.5	12.5–25	>100	>100	>100	>100
19	3,4-Cl	Me	<6.25	<6.25	12.5–25	25–50	>100	>100	>100	>100
20	2,5-Cl	Me	<6.25	<6.25	6.25–12.5	25–50	50–100	>100	>100	>100
21	2,6-Cl	Me	<6.25	25–50	<6.25	50–100	>100	>100	>100	>100
22	2-NH_2	Me	>100	>100	>100	>100	>100	>100	>100	>100
23	4-NH_2	Me	6.25–12.5	12.5–25	6.25–12.5	25–50	>100	>100	>100	>100
24	4-OH	Me	<6.25	12.5–25	<6.25	25–50	50–100	>100	>100	>100
25	2-CO_2H	Me	50–100	>100	50–100	>100	>100	>100	>100	>100
26	2-CO_2Me	Me	12.5–25	>100	50–100	>100	>100	>100	>100	>100
27	4-OSO_2Me	Me	<6.25	6.25–12.5	6.25–12.5	25–50	>100	>100	>100	>100
28	4-OCOMe	Me	<6.25	6.25–12.5	6.25–12.5	25–50	50–100	>100	>100	>100
29	2-NHCOMe	Me	<6.25	12.5–25	12.5–25	50–100	50–100	>100	>100	>100
30	2-NHCOPh	Me	<6.25	12.5–25	6.25–12.5	>100	>100	>100	>100	>100
31	2-$NHCOCH_2Br$	Me	6.25–12.5	25–50	25–50	50–100	>100	>100	>100	>100
32	2-$NHCOCF_3$	Me	<6.25	25–50	25–50	50–100	>100	>100	>100	>100
33	2-$NHCOCH_3$	Et	<6.25	25–50	12.5–25	25–50	>100	>100	>100	>100
34	4-NHCOMe	Me	<6.25	12.5–25	6.25–12.5	50–100	>100	>100	>100	>100
35	2-$CONH_2$	Me	<6.25	<6.25	25–50	>100	12.5–25	25–50	50–100	>100
36	2-CONHMe	Me	<6.25	<6.25	12.5–25	50–100	>100	>100	>100	>100
37	2-CONH-^{t}Bu	Me	<6.25	<6.25	12.5–25	50–100	>100	>100	>100	>100
38	2-CONH-Octyl	Me	<6.25	<6.25	6.25–12.5	12.5–25	>100	>100	>100	>100
39	2-CONH-Ph	Me	50–100	50–100	>100	>100	>100	>100	>100	>100
40	2-CONH-Naphtyl	Me	>100	>100	>100	>100	>100	>100	>100	>100

TABLE 1 (Continued)

Compound No.	R_1	R_2	MIC ($\mu g \cdot ml^{-1}$)							
			B. subtilis (ATCC 6633)		*S. aureus* (ATCC 6538)		*E. coli* (ATCC 11246)		*P. aeruginosa* (ATCC 10145)	
			8 h	24 h	8 h	24 h	8 h	24 h	8 h	24 h
41	2-CONH-CH_2Ph	Me	<6.25	<6.25	12.5–25	25–50	>100	>100	>100	>100
42	2-CONHCH_2CF_3	Me	<6.25	6.25–12.5	>100	>100	>100	>100	>100	>100
43	H	Et	<6.25	<6.25	<6.25	12.5–25	50–100	>100	>100	>100
44	H	n-Pr	<6.25	<6.25	6.25–12.5	12.5–25	>100	>100	>100	>100
45	H	i-Pr	<6.25	<6.25	<6.25	12.5–25	>100	>100	>100	>100
46	H	n-Bu	<6.25	<6.25	<6.25	12.5–25	>100	>100	>100	>100
47	H	i-Bu	<6.25	<6.25	<6.25	12.5–25	>100	>100	>100	>100
48	H	t-Bu	<6.25	6.25–12.5	<6.25	12.5–25	>100	>100	>100	>100
49	H	Hexyl	<6.25	<6.25	<6.25	6.25–12.5	>100	>100	>100	>100
50	H	Octyl	<6.25	<6.25	<6.25	<6.25	>100	>100	>100	>100
51	H	cyc–Hexyl	<6.25	<6.25	6.25–12.5	12.5–25	>100	>100	>100	>100

meaning of the code numbers used for the species is as follows: FERM, Fermentation Research Institute, Agency Industrial, Science and Technology, Tsukuba, Japan; IFO, Institute for Fermentation, Osaka, Japan; JCM, Japan Collection of Microorganisms, Institute of Physical and Chemical Research, Wako, Japan; ATCC, The American Type Culture Collection, 12301, Parklawn Drive, Rockville, Maryland 20852, U.S.A.)

Test for bacteriostatic activity

All of the compounds tested were dissolved in *N,N*-dimethylformamide (DMF). A standard test medium was prepared by dissolving meat extract (10.0 g), polypeptone (10.0 g, Kyokuto Seiyaku Co.) and sodium chloride (0.5 g) in 1000 ml of distilled water, and the pH of the medium was adjusted to 7.2–7.4 using 1 M aqueous sodium hydroxide solution before autoclaving at 120 °C for 15 min. A mixture of maleimide solution (0.5 ml), standard medium (9.2 ml) and bacterial suspension (0.3 ml) was incubated at 37 °C. Concentrations of the maleimides were prepared at 6.25, 12.5, 25, 50 and 100 μg ml^{-1}. After 8 h and 24 h, the turbidities at 660 nm of the mixtures were measured. When the turbidity was less than 0.1, the concentration value was designated as minimum inhibitory concentration (MIC). When no maleimide was added (blank test), the turbidity of the mixture was about 1.0. When the turbidities were 1.0 and 0.1, the bacterial contents were $>10^7$ ml^{-1} and 10^1–10^2 ml^{-1}, respectively. Turbidities were approximately zero at zero time.

Test for bactericidal activity

Distilled water (9.9 ml) was placed in a test tube and sterilized. Maleimide sample solution (0.1 ml) in DMF was added to the test tube. Sample concentrations were prepared at appropriate values. A mixture of sample solution and bacterial suspension (0.1 ml) (bacteria contents, 10^5–10^7 ml^{-1}) was incubated at 37 °C. This suspension (0.1 ml) was mixed with sterilized water (9.9 ml). The resulting suspension (0.1 ml) was inoculated onto agar. The agar was incubated at 37 °C. Bacteria were quantitated after 1, 2, 4 and 24 h. The number of colonies appearing on agar was counted as the method of quantitation.

Test for growth inhibitory activity against fungi

All the test chemicals were dissolved in DMF and subjected to the following assay. Standard medium was prepared by dissolving potato extract (4.0 g, Nissui Seiyaku Co.), glucose (20.0 g) and agar (15.0 g) in 1000 ml of distilled water and autoclaved at 121 °C for 15 min. The pH of the medium was at 5.6 ± 0.1. The standard medium (9.5 ml) was placed in a test tube and sterilized. The maleimide sample (0.5 ml solution) was added to the test tube. The concentrations of the maleimides tested were 6.25, 12.5, 25, 50 and 100 μg ml^{-1}. The test solutions were dispensed onto agar plates having a diameter of 90 mm. The plates were incubated at 28 °C for 4 days. The degrees of mycelial growth were observed, and were classified according to the area occupied on the plate, such that 1 = no mycelial growth, 2 = <10% of the area

of the palate covered, 3 = 11–30% covered, 4 = >30% covered. The lowest maleimide concentration at which fungal growth was inhibited (classification 1) was defined as the minimum inhibitory concentration (MIC).

RESULTS AND DISCUSSION

Table 1 shows the MICs of various 2-arylthio-*N*-alkylmaleimides against cultured bacteria. All of the compounds, except 2-(2-aminophenyl)thio-*N*-methylmaleimide (22) and 2-[2-(*N*-naphthylcarbamoyl)phenyl]-thio-*N*-methylmaleimide (40), inhibited growth of Gram-positive bacteria, such as *B. subtilis* and *S. aureus* at low concentrations. This result suggested that the substituent on the phenylthio moiety and the length of the *N*-alkyl-chain had no effect on activity. In the previous study concerning the structure-activity relationship of *N*-(aryl-alkyl)maleimides, a similar tendency was observed [15]. 2-(2-Carboxyphenyl)thio-*N*-methylmaleimide (25), 2-(2-carbomethoxyphenyl)thio-*N*-methylmaleimide (26) and 2-[2-(*N*-phenylcarbamoyl)phenyl]thio-*N*-methylmaleimide (39) were less active than other 2-arylthio-*N*-alkylmaleimides against Gram-positive bacteria. Comparison of the activities of the compound (22) and 2-(4-aminophenyl)thio-*N*-methylmaleimide (23) against Gram-positive bacteria is interesting. Although each compound has the same ring substituent (-NH_2), their activities against Gram-positive bacteria were quite different. It was presumed that the position of the substituent in a specific compound had an effect on the activity against Gram-positive bacteria. When amino, carboxy or carbomethoxy groups were connected at the 2-position of the phenyl moiety, the activities of the 2-arylthio-*N*-methylmaleimides against Gram-positive bacteria decreased. From the comparative results of compound (39) or (40) with other 2-[2-*N*-(substituted)carbamoyl)phenyl]thio-*N*-methylmaleimides, it was considered that substituents on the carbamoyl group had an effect on the activity against Gram-positive bacteria. When the carbamoyl group was substituted with an alkyl group or a benzyl group, these compounds showed activity. However, when the carbamoyl group was substituted with a benzene ring, activities of these compounds decreased or disappeared.

Activities of 2-arylthio-*N*-alkylmaleimides against Gram-negative bacteria were generally weak. However, 2-phenylthio-*N*-methylmaleimide (1) and 2-phenylthio-*N*-ethylmaleimide (43) showed marginal activity against *E. coli*. These results showed that an increase of *N*-alkyl chain length decreased the activity against *E. coli*. Interestingly, 2-(chlorophenyl)thio-*N*-methylmaleimides (4), (5), (6) and 2-(3-bromophenyl)thio-*N*-methylmaleimide (8) and 2-(4-fluorophenyl)thio-*N*-methylmaleimide (10) showed activity at comparatively low concentrations. We found that substitution of one hydrogen atom on the benzene nucleus at the phenylthio moiety with fluorine was most effective in increasing activity against *E. coli*. 2-(Di-

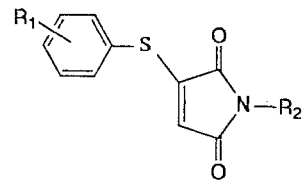
TABLE 2

Bactericidal activity of 2-phenylthio-*N*-methylmaleimide (1) and 2-phenylthio-*N*-ethylmaleimide (43)

Compound	*B. subtilis* (ATCC 6633)					*E. coli* (ATCC 11246)				
	Concentration ($\mu g \cdot ml^{-1}$)	Numbers of cells (ml^{-1})				Concentration ($\mu g \cdot ml^{-1}$)	Numbers of cells (ml^{-1})			
		1 h	2 h	4 h	24 h		1 h	2 h	4 h	24 h
1	0.39	3.5×10^5	3.6×10^5	5.4×10^5	$>10^6$	12.5	$>10^6$	$>10^6$	$>10^6$	$>10^6$
	0.78	1.0×10^2	$<10^2$	$<10^2$	$<10^2$	25	$>10^6$	$>10^6$	1.1×10^6	$>10^6$
	1.57	$<10^2$	$<10^2$	$<10^2$	$<10^2$	50	1.6×10^6	5.4×10^5	9.7×10^4	6.2×10^4
	3.13	$<10^2$	$<10^2$	$<10^2$	$<10^2$	100	1.4×10^6	8.4×10^4	3.0×10^4	8.0×10^2
43	0.39	3.4×10^3	6.0×10^3	8.3×10^3	1.0×10^4	12.5	$>10^6$	$>10^6$	$>10^6$	$>10^6$
	0.78	2.0×10^2	1.0×10^2	$<10^2$	$<10^2$	25	$>10^6$	$>10^6$	2.4×10^6	$>10^6$
	1.57	$<10^2$	$<10^2$	$<10^2$	$<10^2$	50	2.4×10^6	1.6×10^6	4.6×10^5	4.6×10^5
	3.13	$<10^2$	$<10^2$	$<10^2$	$<10^2$	100	6.2×10^5	2.4×10^5	2.2×10^4	3.0×10^2
MMA	0.39	1.9×10^6	$>10^6$	$>10^6$	$>10^6$	12.5	$>10^6$	$>10^6$	1.7×10^6	4.5×10^4
	0.78	2.9×10^5	8.1×10^5	1.0×10^6	1.4×10^6	25	$>10^6$	$>10^6$	1.3×10^6	1.4×10^4
	1.57	1.0×10^2	$<10^2$	$<10^2$	$<10^2$	50	1.9×10^6	6.4×10^4	$<10^2$	$<10^2$
	3.13	$<10^2$	$<10^2$	$<10^2$	$<10^2$	100	1.4×10^6	2.2×10^4	$<10^2$	$<10^2$
Control	–	8.6×10^6	8.8×10^6	9.1×10^6	9.6×10^6	–	2.3×10^7	2.7×10^7	2.8×10^7	$>10^7$

TABLE 3

Antifungal activity of 2-arylthio-*N*-alkylmaleimides



Compound No.	R_1	R_2	MIC ($\mu g \cdot ml^{-1}$)							
			A. n	*P. c*	*R. s*	*G. c*	*C. c*	*A. p*	*R. r*	*S. c*
1	H	Me	25	50	50	25	12.5	25	12.5	25
3	4-Me	Me	50	50	100	50	50	25	12.5	25
5	3-Cl	Me	25	50	50	50	12.5	50	12.5	12.5
10	4-F	Me	50	50	50	25	6.25	12.5	12.5	12.5
43	H	Et	25	12.5	100	25	6.25	50	12.5	25

A. n: Aspergillus niger (FERM S-1); *R. s: Rhizopus stolonifer* (FERM S-7); *C. c: Cladosporium cladosporioides* (FERM S-8); *R. r: Rhodotorula rubra* (JCM 3785); *P. c: Penicillium citrinum* (FERM S-5); *G. c: Geotrichum candidum* (IFO 4598); *A. p: Aureobasidium pullulans* (FERM S-9); *S. c: Saccharomyces cerevisiae* (JCM 2220).

chlorophenyl)thio-*N*-methylmaleimides (19)–(21) were inactive against *E. coli*. It is assumed that the activities of these compounds against *E. coli* decreased as a function of increased halogenation of the phenylthio moiety. When the polarity of the substituent on a benzene nucleus at the phenylthio moiety is large, the 2-arylthio-*N*-alkylmaleimides showed no activity. 2-(2-Carbamoylphenyl)thio-*N*-methylmaleimide (35) was an exceptional compound and was effective against *E. coli*. Though the activity was weak, only this compound displayed any activity against *P. aeruginosa*.

Compound (1) and (43), which showed good bacteriostatic activity, were examined for their bactericidal activities against *B. subtilis* and *E. coli*. Table 2 shows the test results. *N*-Methylmaleimide (NMM) was used as a reference compound. Compounds (1) and (43) killed *B. subtilis* at concentration of 1 μg/ml within 4 h. These compounds were more effective than *N*-methylmaleimide within a short period. It was assumed that the effect of (1) and (43) against *B. subtilis* was due to a bactericidal effect rather than a bacteriostatic effect. These compounds did not show bactericidal activity at 24 h against *E. coli* at a concentration less than 100 μg/ml. The minimum bactericidal concentration of *N*-methylmaleimide against *E. coli* at 24 h was 50 μg/ml. This result showed that the introduction of a phenylthio moiety at the 2-position of *N*-methylmaleimide decreased its bactericidal activity against *E. coli*. The MICs of some 2-arylthio-*N*-alkylmaleimides are summarized in Table 3. All of the compounds inhibited the growth of filamentous fungi. Especially, the compounds showed activity against yeasts. The antifungal activities of these compounds were similar to those of other maleimides such as *N*-arylmaleimides and *N*-alkylmaleimides.

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