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Synthesis and antimicrobial activity of 2,3-bis(bromomethyl)quinoxaline derivatives

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ABSTRACT

We synthesized 12 derivatives of 2,3-bis(bromomethyl)quinoxaline with substituents at the 6- and/or 7-positions, and evaluated their activities against bacteria and fungi. Of the 12 compounds, nine (**1a**-**h**, **1j**, and **1k**) showed antibacterial activity. The derivative **1g**, which bears a trifluoromethyl group at the 6-position, showed the highest activity against Gram-positive bacteria, while **1c**, which has a fluoro-group at the 6-position, showed the widest antifungal activity spectrum. However, only the derivative with an ethyl ester substitution, **1k** showed activity against Gram-negative bacteria.

1. Introduction

Antimicrobial agents are essential for the treatment of a number of diseases of bacterial or fungal origin, and many such agents are used in clinically and industrially. Recently, however, bacteria have acquired drug resistance and so the development of new antimicrobial agents is required [1–3].

A number of compounds based on nitrogen-containing heterocycles show antimicrobial activity and have been developed for clinical use [4]. Among the various classes of heterocyclic units, the quinoxaline ring has frequently been used as a component of various antibiotic molecules, such as hinomycin, levomycin, and actindeutin, which inhibit the growth of Gram-positive bacteria and are active against various transplantable tumors [5–7]. In addition, many reports describe a variety of biological activities of quinoxaline derivatives including anticancer, antibacterial, antifungal, antiviral and antiprotozoal activities [8–18]. Thus, the quinoxaline ring is an important structural unit in these bioactive compounds.

Bromomethyl-substituted compounds, such as 4-bromomethyl-[2,2]-*p*-cyclophane and simple peptides with bromomethyl groups, showed antibacterial and antifungal activities [19–22]. Similarly, bromoacetyl groups can be found in industrial antimicrobial agents such as benzyl bromoacetate, 1,4-bis(bromoacetoxy)-2-butene and 2-bromo-1-(4-hydroxyphenyl)ethanone.

Based on these data, we hypothesized that the combination of a quinoxaline ring with a bromoacetyl group would produce novel compounds with antimicrobial activity. Because the bromomethyl-imino group would function in a manner chemically equivalent to the bromoacetyl group, we designed a derivative of 2,3-bis(bromomethyl)quinoxaline.

Previously, Nielsen and co-workers reported that the activity of 4′-carboxychalcones against *Staphylococcus aureus* increased when lipophilic substitutions were made [23]. Furthermore, Sanna and co-workers reported that 2-quinoxalinone derivatives bearing electron-withdrawing groups at the 6- or 7-positions showed antibacterial, antifungal, and anticancer activities [8–11]. Hence, lipophilicity and the electronic properties of the substituents affect the biological activities of these compounds.

In this paper, we report the synthesis of 2,3-bis(bromomethyl)quinoxaline derivatives **1a–l** bearing various substituents at the 6- and/or 7-positions (Fig. 1), and investigate the effect of lipophilicity and electronic properties of substituents on their antibacterial and antifungal activities. Among the synthesized quinoxaline derivatives, trifluoromethyl-substituted compound **1g** showed the highest activity against Gram-positive bacteria, while fluoro-substituted **1c** showed the broadest-acting antifungal activity.

2. Results and discussion

2.1. Synthesis

2,3-Bis(bromomethyl)quinoxaline derivatives **1a–i** were synthesized by the condensation reaction of the corresponding 1,2-phenylenediamine derivatives **2a–i** and 1,4-dibromo-2,3-butanedione **3** according to the method reported by Carta [11] (Scheme 1). The

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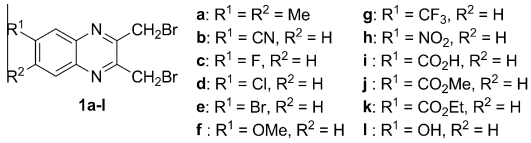
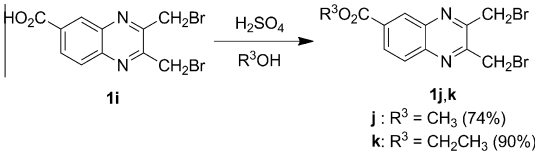


Fig. 1. Structure of the 2,3-bis(bromomethyl)quinoxaline derivatives **1a**–**l**.

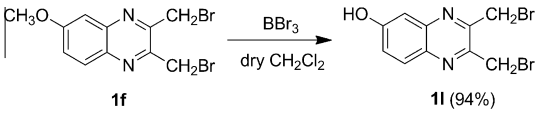
target compounds **1a–i** were obtained in 69–93% yields, and the electronic properties of the substituent at the 4-position of the 1,2-phenylenediamine derivatives did not significantly affect the efficiency of this reaction. When the reaction was completed, the compounds **1a**–**f**, **1h** and **1i** were precipitated from the reaction mixture; **1g** was not precipitated due to the difficulty of solidification. After usual work-up, all products were purified by silica gel column chromatography.

The carboxylic acid **1i** was converted into the esters **1j** and **1k**. The esterification of **1i** was carried out in methanol and ethanol in the presence of a catalytic amount of sulfuric acid, and the methyl ester **1j** and ethyl ester **1k** were obtained in good yields (Scheme 2).

The 6-hydroxyquinoxaline **1l** was synthesized by treating the methoxy compound **1f** with boron tribromide. The demethylation reaction proceeded without any side-reaction to produce an exceptional yield of **1l** (Scheme 3). Boron tribromide-assisted demethylation of the methoxy group on the benzene ring of compounds such as dimethoxytoluene and dimethoxyisopropylbenzene was complete within several hours [24,25]; however, demethylation of **1f** required 5 days for completion, indicating that cleavage of the C—O bond of the methoxy group at the 6-position of the quinoxaline ring is more difficult than in the equivalent benzene analog.



Scheme 2. Synthesis of the methyl ester **1j** and ethyl ester **1k**.



Scheme 3. Synthesis of compound **1l**.

Table 1
Antibacterial activities of **1a**–**l**.

	R^1	R^2	MIC (μg/mL)				
			Gram-positive		Gram-negative		
			B. s.[a]	S. a. [b]	E. c. [c]	P. a. [d]	S. m. [e]
1a	CH_3	CH_3	50	50	>100	>100	>100
1b	CN	H	25	25	>100	>100	>100
1c	F	H	25	50	>100	>100	>100
1d	Cl	H	50	50	>100	>100	>100
1e	Br	H	25	50	>100	>100	>100
1f	OCH_3	H	25	50	>100	>100	>100
1g	CF_3	H	12.5	12.5	>100	>100	>100
1h	NO_2	H	25	50	>100	>100	>100
1i	CO_2H	H	>100	>100	>100	>100	>100
1j	CO_2CH_3	H	50	50	>100	>100	>100
1k	$CO_2CH_2CH_3$	H	25	50	100	100	100
1l	OH	H	>100	>100	>100	>100	>100

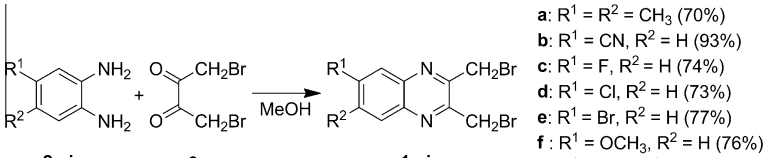
[a] *Bacillus subtilis.*
[b] *Staphylococcus aureus.*
[c] *Escherichia coli.*
[d] *Pseudomonas aerugianosa.*
[e] *Serratia marcescens.*

2.2. Antibacterial activity

The antibacterial activity of the compounds **1a**–**l** was evaluated by means of the minimum inhibitory concentration (MIC), as determined by the dilution method. The results are summarized in Table 1. Nine compounds (**1a**–**h**, **1j** and **1k**) showed antibacterial activities (MIC ⩽ 100 μg/mL), indicating that the 2,3-bis(bromomethyl)quinoxaline is the structural unit that exerts antibacterial activities. Among these nine compounds, the most active compound against Gram-positive bacteria was **1g**, which has a strong electron-withdrawing and highly lipophilic trifluoromethyl group at the 6-position (MIC = 12.5 μg/mL). However, the activity of **1b** and **1h**, which bear hydrophilic and electron-withdrawing cyano and nitro groups, respectively, was lower than that of **1g**, and almost identical to that of **1a** and **1f** which have lipophilic and electron-releasing methyl and methoxy groups, respectively (MIC = 25–50 μg/mL). These results suggest that both electron-withdrawing ability and lipophilicity of the substituents at the 6-position are need for high antibacterial activity.

The halogen-substituted compounds **1c**, **1d**, and **1e**, which have F, Cl, and Br at the 6-position, respectively, showed similar activities (MIC = 25–50 μg/mL). The order of electron-withdrawing abilities of the halogen atoms is Br < Cl < F, and the lipophilicity follows the order F < Cl < Br; it is possible, therefore, that the electronic and the lipophilic effects of these halogens on antibacterial activity may cancel each other out.

The methyl ester **1j** and ethyl ester **1k** both showed antibacterial activities, but the carboxylic acid **1i** did not. Similarly, the methoxy-substituted **1f** showed some activity, while the phenol **1l** did not. These results indicate that increasing the lipophilicity improves the antibacterial activity, but an increase in hydrophilicity decreases activity. In general, the lipophilic property of a compound plays an important role in allowing the compound to permeate the bacterial cell membrane [26]. Therefore, it is likely that the penetration of **1i** and **1l** through bacterial cell membranes is inhibited by the hydrophilic substituents of these compounds.



Scheme 1. Synthesis of the 2,3-bis(bromomethyl)quinoxaline derivatives **1a**–**i**.

Table 2
Antifungal activities of **1a–l**.

	R^1	R^2	MIC (μg/mL)								
			Mold							Yeast	
			A. n.[a]	P. c.[b]	C. c.[c]	A. p.[d]	A. s.[e]	M. s.[f]	G. v.[g]	R. r.[h]	S. c.[i]
1a	CH_3	CH_3	>100	>100	>100	>100	>100	>100	>100	>100	>100
1b	CN	H	50	25	25	50	25	25	>100	100	50
1c	F	H	50	50	50	100	50	25	100	100	50
1d	Cl	H	50	100	25	>100	>100	50	>100	>100	>100
1e	Br	H	100	100	50	>100	>100	50	>100	>100	>100
1f	OCH_3	H	50	>100	50	100	100	50	>100	>100	100
1g	CF_3	H	50	50	100	50	100	25	>100	100	50
1h	NO_2	H	100	100	50	100	25	50	100	>100	50
1i	COOH	H	>100	>100	>100	>100	>100	>100	>100	>100	>100
1j	CO_2CH_3	H	50	100	100	50	>100	25	>100	>100	50
1k	$CO_2CH_2CH_3$	H	>100	>100	>100	100	>100	100	>100	100	100
1l	OH	H	>100	>100	>100	>100	>100	>100	>100	>100	>100

[a] *Aspergillus niger*.
[b] *Penicillium citrinum*.
[c] *Cladosporium cladosporiodes*.
[d] *Aureobasidium pullelans*.
[e] *Alternaria sp*.
[f] *Mucor spinescens*.
[g] *Gliocladium virens*.
[h] *Rhodotorula rubra*.
[i] *Saccharomyces cerevisiae*.

Against Gram-negative bacteria, only the ethyl ester **1k** showed any activity. Sanna and co-workers examined the antibacterial activities of 3-ethoxycarbonyl- and 3-carboxy-2-quinoxalinone derivatives, and reported that only 3-ethoxycarbonyl-2-quinoxalinone derivatives showed activities against Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aerugianosa*) [8]. Therefore, it is possible that it is the ethoxycarbonyl group that exerts a specific action against Gram-negative bacteria. However, the methyl ester **1j** does not show any activity against Gram-negative bacteria, and so the activity against Gram-negative bacteria of **1k** may be due to the increase in the alkyl chain length.

2.3. Antifungal activity

As with the antibacterial activity, we evaluated antifungal activity by means of MIC observations, and the results are summarized in Table 2. Antifungal activity (MIC $\leqslant$ 100 μg/mL) was seen in eight of the 2,3-bis(bromomethyl)quinoxaline derivatives: **1b–h**, **1j** and **1k**. When the effects of electron-withdrawing substituents were compared (**1b** (R^1 = CN), **1g** (R^1 = CF_3), **1h** (R^1 = NO_2), **1j** (R^1 = CO_2Me), and **1k** (R^1 = CO_2Et)), the compounds with stronger electron-withdrawing groups such as **1b**, **1g** and **1h** showed wider antifungal spectra than **1j** or **1k**. Of these, **1b** exhibited the highest activity. Among the electron-releasing group-substituted compounds, **1a**, with a methyl group at the 6-position, did not show any antifungal activity, but **1f**, having a stronger electron-releasing methoxy group, showed some activity. These results suggest that, as in the case of antibacterial activity, the electronic effect of the substituent at the 6-position influences both breadth of antifungal spectrum and degree of activity, and the compounds bearing stronger electron-releasing or withdrawing substituents show improved antifungal spectra and activity.

As for the carboxylic acid derivatives, the methyl ester **1j** showed antifungal activities and spectra similar to **1b**, **1g** and **1h**. However, the more lipophilic ethyl ester **1k** exhibited lower antifungal activity and a narrower spectrum, while the carboxylic acid **1i** did not show any activity. These results indicate that moderate lipophilicity is needed to exert antifungal activities. A similar trend was also observed when compounds **1f** and **1l** were compared: the methoxy-substituted **1f** showed some activity, but the phenol **1l** did not.

When the antifungal activities of halogenated compounds **1c** (R^1 = F), **1d** (R^1 = Cl), and **1e** (R^1 = Br) were compared, activity increased in the order of **1e** < **1d** < **1c**, which is the same order as the substituent's electron-withdrawing ability. Of the synthesized compounds, fluoro-substituted **1c** showed the widest antifungal spectrum. These results indicate that the antifungal activities of the 2,3-bis(bromomethyl)quinoxaline derivatives are greater when strong electron-withdrawing and moderately lipophilic substituents are introduced at the 6-position.

3. Conclusions

We synthesized 12 kinds of 2,3-bis(bromomethyl)quinoxaline derivatives, evaluated their activities against bacteria and fungi, and found that the compounds bearing strongly electron-releasing/withdrawing and moderately lipophilic substituents showed high antimicrobial activities. Among them, trifluoromethyl-substituted **1g** showed the highest activity against Gram-positive bacteria. However, cyano-substituted **1b** and fluoro-substituted **1c** showed the greatest and widest-ranging activities against fungi respectively. These results demonstrate that 2,3-bis(bromomethyl)quinoxaline framework is a good candidate for novel industrial antimicrobial agent. The synthesis and evaluation of the antibacterial and antifungal activities of quinoxaline derivatives bearing halomethyl groups other than the bromomethyl group at the 2- and the 3-positions are in progress.

4. Experimental

4.1. General

All common reagents and solvents were obtained from Wako Pure Chemical Industries, Tokyo Chemical Industry, and Sigma–Aldrich, and used without further purification. Column chromatography was carried out using silica gel (Silica Gel 60 N, 63–210 μm, Kanto Chemical). Thin layer chromatography (TLC) was conducted on Merck Silica Gel 60 F_{254}. Melting points were determined on an SMP3 melting point apparatus (Bibby Scientific Limited) and were uncorrected. ^1H and ^{13}C NMR spectra were recorded on JEOL JNM-LA400D and JNM-ECA-500, respectively, using DMSO-d_6 and $CDCl_3$ as solvents. Chemical shifts (δ) were reported as parts per million

(ppm) relative to tetramethylsilane (TMS) as an internal standard for ^{1}H NMR and the midpoints of $CDCl_3$ (77.16 ppm) and DMSO-d_6 (39.52 ppm) for ^{13}C NMR. IR spectra were recorded with a JASCO FT/IR-470. Elemental analyses for C, H and N were performed using a Perkin Elmer 2400 analyzer series II. All compounds were characterized by the above techniques.

4.2. Preparation of 2,3-bis(bromomethyl)quinoxalines **1a–i**

A solution of equimolar amounts (7.0 mmol) of the corresponding 1,2-phenylenediamine (**2a–i**) and 1,4-dibromo-2,3-butanedione (**3**) in MeOH (10 mL) was refluxed for 2 h. After cooling to room temperature, the precipitates of **1a–f**, **1h** and **1i** were collected by suction filtration, or in the case of **1g** the solvent was evaporated. The crude product was purified by column chromatography on silica gel with $CHCl_3$ for compounds **1a–h** or 6:1 $CHCl_3$/MeOH for **1i**.

4.2.1. 2,3-Bis(bromomethyl)-6,7-dimethylquinoxaline **1a**

Yield: 70%, mp: 147 °C (decomp.). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.50 (6H, s), 4.90 (4H, s), 7.81 (2H, s). ^{13}C NMR (126 MHz, $CDCl_3$) δ: 20.6 (CH_3), 30.9 (CH_2), 128.1 (CH), 140.7 (C), 141.9 (C), 149.9 (C). IR (KBr, cm^{-1}): 3022, 2970, 2922, 1361, 861, 534. Anal. Calcd for $C_{12}H_{12}N_2Br_2$: C, 41.89; H, 3.52; N, 8.14. Found: C, 41.66; H, 3.59; N, 7.94.

4.2.2. 2,3-Bis(bromomethyl)-6-cyanoquinoxaline **1b**

Yield: 93%, mp: 157–159 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.91 (2H, s), 4.93 (2H, s), 7.95 (1H, dd, J = 1.7 and 8.8 Hz), 8.18 (1H, d, J = 8.8 Hz), 8.45 (1H, d, J = 1.7 Hz). ^{13}C NMR (126 MHz, $CDCl_3$) δ: 29.8 (CH_2), 29.9 (CH_2), 114.4 (C), 117.7 (CN), 130.8 (CH), 131.7 (CH), 135.0 (CH), 140.7 (C), 142.9 (C), 153.2 (C), 153.9 (C). IR (KBr, cm^{-1}): 3022, 2976, 2228, 1359, 912, 805, 509. Anal. Calcd for $C_{11}H_7N_3Br_2$: C, 38.74; H, 2.07; N, 12.32. Found: C, 38.79; H, 2.10; N, 12.21.

4.2.3. 2,3-Bis(bromomethyl)-6-fluoroquinoxaline **1c**

Yield: 74%, mp: 142–143 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.90 (2H, s), 4.91 (2H, s), 7.56–7.61 (1H, m), 7.68–7.71 (1H, m), 8.06–8.10 ppm (1H, m). ^{13}C NMR (126 MHz, $CDCl_3$) δ: 30.3 (CH_2), 30.4 (CH_2), 112.8 (d, J_{CF} = 22 Hz, CH), 121.5 (d, J_{CF} = 26 Hz, CH), 130.3 (d, J_{CF} = 10 Hz, CH), 138.9 (C), 142.6 (d, J_{CF} = 15 Hz, C), 150.3 (C), 151.9 (C), 163.5 (d, J_{CF} = 254 Hz, C). IR (KBr, cm^{-1}): 3025, 2973, 1330, 1225, 891, 802, 638. Anal. Calcd for $C_{10}H_7N_2FBr_2$: C, 35.96; H, 2.11; N, 8.39. Found: C, 36.12; H, 2.09; N, 8.30.

4.2.4. 2,3-Bis(bromomethyl)-6-chloroquinoxaline **1d**

Yield: 73%, mp: 149–151 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.89 (2H, s), 4.90 (2H, s), 7.74 (1H, dd, J = 2.3 and 9.0 Hz), 8.01 (1H, d, J = 9.0 Hz), 8.07 (1H, d, J = 2.3 Hz). ^{13}C NMR (126 MHz, $CDCl_3$) δ: 30.3 (CH_2), 30.4 (CH_2), 128.1 (CH), 130.4 (CH), 132.1 (CH), 137.0 (C), 140.8 (C), 141.9 (C), 151.2 (C), 152.0 (C). IR (KBr, cm^{-1}): 3029, 2972, 1358, 879, 836, 626, 547. Anal. Calcd for $C_{10}H_7N_2ClBr_2$: C, 34.27; H, 2.07; N, 7.99. Found: C, 34.41; H, 1.93; N, 7.96.

4.2.5. 6-Bromo-2,3-bis(bromomethyl)quinoxaline **1e**

Yield: 77%, mp: 142–144 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.89 (2H, s), 4.90 (2H, s), 7.87 (1H, dd, J = 1.7 and 9.0 Hz), 7.94 (1H, d, J = 9.0 Hz), 8.25 (1H, d, J = 1.7 Hz). ^{13}C NMR (126 MHz, $CDCl_3$) δ: 30.3 (CH_2), 30.4 (CH_2), 125.2 (C), 130.4 (CH), 131.5 (CH), 134.6 (CH), 140.4 (C), 142.2 (C), 151.3 (C), 152.0 (C). IR (KBr, cm^{-1}): 3076, 3028, 2972, 1356, 880, 833, 569. Anal. Calcd for $C_{10}H_7N_2Br_3 \cdot 0.4\ H_2O$: C, 29.87; H, 1.96; N, 6.97. Found: C, 29.73; H, 1.65; N, 6.90.

4.2.6. 2,3-Bis(bromomethyl)-6-methoxyquinoxaline **1f**

Yield: 76%, mp: 133–134 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.97 (3H, s), 4.88 (2H, s), 4.89 (2H, s), 7.35 (1H, d, J = 2.7 Hz), 7.44 (1H, dd, J = 2.7 and 9.3 Hz), 7.94 (1H, d, J = 9.3 Hz). ^{13}C NMR (126 MHz, $CDCl_3$) δ: 30.6 (CH_2), 30.9 (CH_2), 56.1 (OCH_3), 106.3 (CH), 124.6 (CH), 130.1 (CH), 137.9 (C), 143.5 (C), 148.1 (C), 151.0 (C), 161.7 (C). IR (KBr, cm^{-1}): 3017, 2962, 1358, 1238, 1019, 906, 835, 514. Anal. Calcd for $C_{11}H_{10}N_2OBr_2$: C, 38.18; H, 2.91; N, 8.10. Found: C, 38.28; H, 2.64; N, 8.03.

4.2.7. 2,3-Bis(bromomethyl)-6-(trifluoromethyl)quinoxaline **1g**

Yield: 69%, mp: 59–61 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.93 (2H, s), 4.94 (2H, s), 7.97 (1H, dd, J = 1.8 and 8.8 Hz), 8.20 (1H, d, J = 8.8 Hz), 8.40 (1H, d, J = 1.8 Hz). ^{13}C NMR (126 MHz, $CDCl_3$) δ: 30.7 (CH_2), 123.5 (q, J_{CF} = 274 Hz, CF_3), 126.7 (d, J_{CF} = 3.6 Hz, CH), 127.2 (d, J_{CF} = 4.8 Hz, CH), 130.5 (CH), 132.7 (q, J_{CF} = 34 Hz, C), 140.7 (C), 142.7 (C), 152.6 (C), 153.2 (C). IR (KBr, cm^{-1}): 3040, 2979, 1339, 1281, 906, 819, 668. Anal. Calcd for $C_{11}H_7N_2F_3Br_2 \cdot 0.1\ H_2O$: C, 34.25; H, 1.88; N, 7.26. Found: C, 33.95; H, 1.77; N, 7.10.

4.2.8. 2,3-Bis(bromomethyl)-6-nitroquinoxaline **1h**

Yield: 90%, mp: 113–115 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.93 (2H, s), 4.95 (2H, s), 8.23 (1H, d, J = 9.3 Hz), 8.57 (1H, dd, J = 2.4 and 9.3 Hz), 8.98 (1H, d, J = 2.4 Hz). ^{13}C NMR (126 MHz, $CDCl_3$) δ: 29.8 (CH_2), 29.9 (CH_2), 124.4 (CH), 125.5 (CH), 130.9 (CH), 140.5 (C), 143.9 (C), 148.5 (C), 153.5 (C), 154.3 (C). IR (KBr, cm^{-1}): 3084, 3036, 2980, 1536, 1364, 1330, 891, 851, 586. Anal. Calcd for $C_{10}H_7N_3O_2Br_2$: C, 33.27; H, 1.95; N, 11.64. Found: C, 33.41; H, 2.10; N, 11.69.

4.2.9. 2,3-Bis(bromomethyl)quinoxaline-6-carboxylic acid **1i**

Yield: 83%, mp: 170 °C (decomp.). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 5.06 (4H, s), 8.20 (1H, d, J = 8.8 Hz), 8.34 (1H, dd, J = 1.5 and 8.8 Hz), 8.58 (1H, d, J = 1.5 Hz). ^{13}C NMR (126 MHz, DMSO-d_6) δ: 30.0 (CH_2), 129.1 (CH), 130.3 (CH), 130.4 (CH), 133.0 (C), 140.1 (C), 142.6 (C), 152.2 (C), 153.0 (C), 166.3 (C=O). IR (KBr, cm^{-1}): 3312, 3035, 2981, 1692, 1314, 1276, 910, 826, 635. Anal. Calcd for $C_{11}H_8N_2O_2Br_2$: C, 36.72; H, 2.24; N, 7.78. Found: C, 36.92; H, 2.06; N, 7.65.

4.3. Preparation of 2,3-bis(bromomethyl)-6-alkoxycarbonylquinoxaline **1j** and **1k**

To a 20 mL solution of **1i** (1.5 mmol) in MeOH (for **1j**) or EtOH (for **1k**), 3 mL conc. H_2SO_4 was added, and the mixture was refluxed for 2 h in an argon atmosphere. Water (100 mL) was then added, and the mixture was extracted with $CHCl_3$ (50 mL × 2). The $CHCl_3$ layer was washed with H_2O (50 mL × 2) and then dried over anhydrous Na_2SO_4. After evaporation of the solvent, the residue was purified by column chromatography on silica gel with $CHCl_3$.

4.3.1. 2,3-Bis(bromomethyl)-6-methoxycarbonylquinoxaline **1j**

Yield: 74%, mp: 92–93 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.02 (3H, s), 4.93 (4H, s), 8.12 (1H, d, J = 8.8 Hz), 8.39 (1H, dd, J = 1.8 and 8.7 Hz), 8.78 (1H, d, J = 1.8 Hz). ^{13}C NMR (126 MHz, $CDCl_3$) δ: 30.1 (CH_2), 30.2 (CH_2), 52.9 (OCH_3), 129.4 (CH), 130.6 (CH), 131.7 (CH), 132.3 (C), 140.9 (C), 143.5 (C), 152.0 (C), 152.8 (C), 166.0 (C=O). IR (KBr, cm^{-1}): 3033, 2951, 1726, 1365, 1263, 911, 802, 637. Anal. Calcd for $C_{12}H_{10}N_2O_2Br_2$: C, 38.53; H, 2.69; N, 7.49. Found: C, 38.69; H, 2.61; N, 7.39.

4.3.2. 2,3-Bis(bromomethyl)-6-ethoxycarbonylquinoxaline **1k**

Yield: 90%, mp: 72–74 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.46 (3H, t, J = 7.2 Hz), 4.47 (2H, q, J = 7.2 Hz), 4.93 (4H, s), 8.12 (1H, d, J = 8.8 Hz), 8.39 (1H, dd, J = 1.9 and 8.7 Hz), 8.79 (1H, d,

J = 1.9 Hz). ^{13}C NMR (126 MHz, $CDCl_3$) δ: 14.4 (CH_3), 30.0 (CH_2), 30.1 (CH_2), 62.0 (OCH_3), 129.2 (CH), 130.7 (CH), 131.5 (CH), 132.8 (C), 140.9 (C), 143.3 (C), 152.1 (C), 152.8 (C), 165.5 (C=O). IR (KBr, cm^{-1}): 3002, 2940, 1741, 1314, 1195, 1026, 858, 808, 579. Anal. Calcd for $C_{13}H_{12}N_2O_2Br_2 \cdot 0.2\ H_2O$: C, 39.87; H, 3.19; N, 7.15. Found: C, 39.86; H, 3.20; N, 7.16.

4.4. Preparation of 2,3-bis(bromomethyl)-6-hydroxyquinoxaline **1l**

To a 10 mL solution of compound **1f** (39.8 mg/mL, 0.910 mmol) in dry CH_2Cl_2, 6 mL of 1 M BBr_3 in CH_2Cl_2 was added at −30 °C in an argon atmosphere, and the reaction mixture was stirred for 5 days at room temperature. The mixture was then cooled to −30 °C, and then 36 mL MeOH was added. After evaporation of the solvent, the residue was dissolved in 50 mL $CHCl_3$, and the $CHCl_3$ solution was washed with H_2O (50 mL × 3) and then dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure, and the residue was purified by column chromatography on silica gel with $CHCl_3$/acetone/EtOH (100/10/4 v/v/v).

Yield: 94%, mp: 140 °C (decomp.). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.87 (2H, s), 4.88 (2H, s), 7.34 (1H, d, J = 2.5 Hz), 7.41 (1H, dd, J = 2.5 and 9.2 Hz), 7.98 (1H, d, J = 9.2 Hz). ^{13}C NMR (126 MHz, $CDCl_3$) δ: 31.4 (CH_2), 31.7 (CH_2), 108.9 (CH), 124.2 (CH), 129.9 (CH), 136.2 (C), 142.8 (C), 147.1 (C), 150.8 (C), 160.0 (C). IR (KBr, cm^{-1}): 3136, 3024, 2968, 1423, 1337, 1224, 908, 834, 643. Anal. Calcd for $C_{10}H_8N_2OBr_2 \cdot 0.1\ H_2O$: C, 35.98; H, 2.48; N, 8.39. Found: C, 36.25; H, 2.45; N, 8.08.

4.5. Antimicrobial activity

4.5.1. Bacteria and fungi

The Gram-positive bacteria used were *Bacillus subtilis* (IFO 3513) and *S. aureus* (IFO 12732), and the Gram-negative were *E. coli* (IFO 3972), *P. aerugianosa* (IFO 3080) and *Serratia marcescens* (IFO 3735). The fungal species were *Aspergillus niger* (IFO 6341), *Penicillium citrinum* (IFO 6352), *Aureobasidium pullelans* (IFO 6353), *Cladosporium cladosporiodes* (IFO 6348), *Mucor spinescens* (IFO 6071), *Alternaria sp*. and *Gliocladium virens* (IFO 6355) as molds, and *Rhodotorula rubra* (IFO 0907) and *Saccharomyces cerevisiae* (IFO 0209) as yeasts.

4.5.2. Minimum inhibitory concentration assay

The minimum inhibitory concentration (MIC) was determined according to the agar plate dilution method using a microplanter (Sakuma Seisakusho, Tokyo, Japan). Compounds **1a–l** were dissolved in a water/methyl carbitol (diethylene glycol monomethyl ether) mixture at a concentration of 1 wt.% (**1**/water/methyl carbitol = 1/79/20 wt/wt/wt). Next, the solution was diluted in a glucose bouillon agar medium. Suspensions of each bacterial or fungal species were prepared that showed 0.05 of turbidity at 660 nm, and suspension of mold species were prepared that contained approximately 10^6 spore/mL. After incubation at 33 °C for 18 h (antibacterial assay), or at 33 °C for 18 h and 28 °C for 2 days (antifungal assay), the growth of the bacteria or fungus was observed. The MIC was determined as the lowest concentration of compound that completely inhibited organism growth.

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