Synthesis of 2,3-Bis(halomethyl)quinoxaline Derivatives and Evaluation of Their Antibacterial and Antifungal Activities

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Received December 12, 2012; accepted January 11, 2013

Quinoxaline derivatives having bis(fluoromethyl), bis(chloromethyl), or bis(iodomethyl) groups at the 2- and 3-positions, and various electron-donating/withdrawing substituents at the 6- and/or 7-positions, were synthesized. Their antibacterial and antifungal activities were evaluated by means of minimum inhibitory concentration assays. The relationships between the substituents and the antimicrobial activities of the quinoxaline derivatives indicate that the electrophilicity of the halomethyl units plays an important role in generating the antimicrobial activity.

Key words quinoxaline; antibacterial activity; antifungal activity; halomethyl

Bacteria and fungi are responsible for a variety of problems, including infectious diseases, food spoilage, and corrosion of industrial materials. To counter this, many antimicrobial agents have been developed, such as penicillin,[1] gentamicin,[2] nalidixic acid,[3] 3-iodo-2-propynyl *N*-butylcarbamate (IPBC),[4] and 5-chloro-2-methyl-4-isothiazolin-3-one (CMIT).[5] However, because bacteria can develop resistance to all commonly used antimicrobial agents, drug-resistant bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococci* (VRE) and vancomycin-resistant *Staphylococcus aureus* (VRSA) have appeared,[6–8] and so the development of new antimicrobial agents is critically required.

Nitrogen-containing heterocycles form the main component of many essential biomolecules, from DNA and RNA to coenzymes. They are thought to have high biocompatibility, and have been used as structural units within many pharmaceutical products.[9] Among the various classes of heterocyclic units, the quinoxaline ring is one of the components involved in a variety of antibiotic molecules such as hinomycin, levomycin, and actinoleutin.[10–12] Furthermore, many quinoxaline derivatives have been reported to possess anticancer, antibacterial, antifungal, antiviral, and antiprotozoal activities.[13–23]

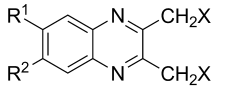
In a previous paper, we reported that the 2,3-bis(bromomethyl)quinoxaline framework is a good candidate for a novel industrial antimicrobial agent, and that the lipophilicity and electrical properties of its substituents affect the antimicrobial activity.[24] For example, we found compounds with the strong electron-withdrawing and highly lipophilic trifluoromethyl group at the 6-position showed the highest effectiveness against Gram-positive bacteria, while quinoxalines having a hydrophilic group such as CO_2H or OH at the 6-position exhibited almost no antimicrobial activity. However, an assessment of antifungal activity was less clear, with the introduction of strong electron-releasing/withdrawing substituents (*e.g.*, F, CF_3, NO_2, CN, and OCH_3) resulting in a wide antifungal spectrum. These results prompted us to study the effect of introducing various halomethyl groups at the 2- and 3-positions of these quinoxaline compounds on their antimicrobial activity.

In this paper, we synthesized quinoxaline derivatives with bis(fluoromethyl), bis(chloromethyl), and bis(iodomethyl) units at the 2- and 3-positions, as well as various substituents at the 6- and/or 7-positions (Fig. 1). Their antibacterial and antifungal activities were evaluated by means of minimum inhibitory concentration assays, and relationships between the substituents and the antimicrobial activities were studied.

Results and Discussion

Chemistry The 2,3-bis(fluoromethyl)quinoxalines **2a**–**8a** were synthesized by the reaction of the corresponding bromomethyl compounds **2c**–**8c** with potassium fluoride in the presence of 18-crown-6 in acetone, giving 18–84% yields[25] (Chart 1). The fluorination of quinoxaline derivatives bearing a strong electron-withdrawing group at the 6-position, such as **2c**–**4c**, gave many by-products and so the yields of the target compounds were low. In particular, the fluorination of **1c** (6-NO_2) afforded a complex mixture from which **1a** could not be extracted.[26]

The similar reactions of **1c**–**8c** with potassium chloride gave 2,3-bis(chloromethyl)quinoxalines **1b**–**8b** in good yields[27,28]



1a-**8a** (X = F), **1b**-**8b** (X = Cl), **1c**-**8c** (X = Br), **1d**-**8d** (X = I).

1: $R^1 = NO_2$, $R^2 = H$ **5**: $R^1 = Cl$, $R^2 = H$
2: $R^1 = CN$, $R^2 = H$ **6**: $R^1 = Br$, $R^2 = H$
3: $R^1 = CF_3$, $R^2 = H$ **7**: $R^1 = R^2 = CH_3$
4: $R^1 = F$, $R^2 = H$ **8**: $R^1 = OCH_3$, $R^2 = H$

Fig. 1. Structure of the 2,3-Bis(halomethyl)quinoxaline Derivatives

The authors declare no conflict of interest.

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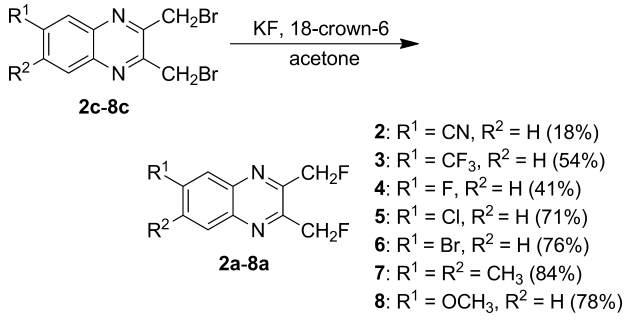

Chart 1. Synthesis of the 2,3-Bis(fluoromethyl)quinoxaline Derivatives **2a**–**8a**

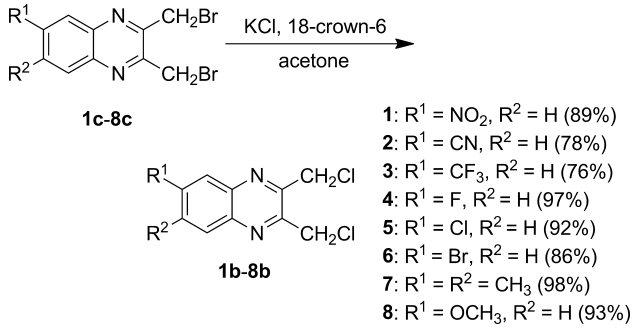

Chart 2. Synthesis of the 2,3-Bis(chloromethyl)quinoxaline Derivatives **1b**–**8b**

(Chart 2). Unlike in the fluorination reactions, the compounds having electron-withdrawing substituents at the 6-position could be chlorinated without serious side reactions. The progress of the reaction was monitored with HPLC, because TLC analysis exhibited that the *Rf* value of the product was almost the same as that of the starting material.

The 2,3-bis(iodomethyl)quinoxalines **2d**–**8d** were synthesized from **2c**–**8c** by the Finkelstein reaction,[29,30] and obtained in 36–94% yields (Chart 3). These reactions were also monitored with HPLC. As with some of the fluorination reactions, the reactions of **2d** and **3d**, which had the strong electron-withdrawing substituents CN and CF_3 respectively at the 6-position, were accompanied by many side reactions, resulting in low yields for the target compounds, and the iodination of **1c** having a nitro group at the 6-position did not afford **1d** at all.[26]

Antibacterial Activity The antibacterial activities of the newly-synthesized quinoxaline derivatives (**2a**–**8a**, **1b**–**8b**, **2d**–**8d**), as well as those of previously reported compounds (**1c**–**8c**),[24] were evaluated by means of minimum inhibitory concentration (MIC) assays; the results are summarized in Table 1. All compounds were inactive against Gram-negative bacteria. The outer membrane of Gram-negative bacteria is covered by many lipopolysaccharides, which consist mainly of hydrophilic polysaccharides.[31] Therefore, the lipophilic materials are hard to reach the surface of the outer membrane. We think that the synthesized 2,3-bis(halomethyl)quinoxaline derivatives are too lipophilic to come close to the outer membrane of Gram-negative bacteria.

For Gram-positive bacteria, while 2,3-bis(fluoromethyl)-quinoxalines **2a**–**8a** exhibited no antibacterial activity, four 2,3-bis(chloromethyl)quinoxalines (**1b**–**3b**, **8b**), five 2,3-bis(iodomethyl)quinoxalines (**2d**, **4d**–**6d**, **8d**), and all eight 2,3-bis(bromomethyl)quinoxalines (**1c**–**8c**) did. Among them, 2,3-bis(chloromethyl)-6-nitroquinoxaline (**1b**) showed the highest activity. The relationships between the substituents and the activities of quinoxaline derivatives suggest that the electrophilicity of halomethyl groups plays an important role in their antibacterial activity. That is, the lower electrophilicity of the fluoromethyl group compared with the other halomethyl groups can be interpreted as directly responsible for the inactivity of 2,3-bis(fluoromethyl) derivatives.

When the activities of the quinoxaline derivatives with 6-CN (**2b**, **2c**, **2d**) and 6-Cl (**5b**, **5c**, **5d**) substituents were compared, we found the halomethyl groups exhibited high activity in the descending order $-CH_2I > -CH_2Br > -CH_2Cl$.

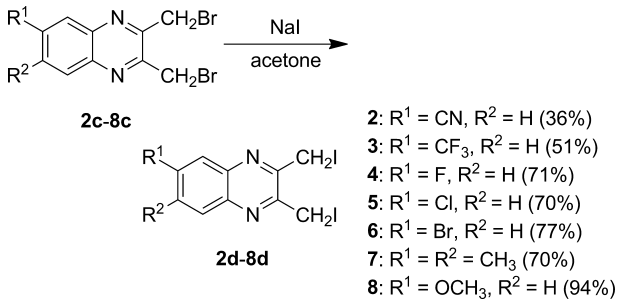

Chart 3. Synthesis of the 2,3-Bis(iodomethyl)quinoxaline Derivatives **2d**–**8d**

A similar trend ($-CH_2I \approx -CH_2Br > -CH_2Cl$) was observed in compounds containing 6-F (**4b**, **4c**, **4d**), 6-Br (**6b**, **6c**, **6d**), and 6-OCH_3 (**8b**, **8c**, **8d**) substituents. In these cases, the compounds that exhibited the highest activity possessed iodomethyl, the halomethyl group of highest electrophilicity. In contrast, the activities of 6-CF_3-substituted quinoxalines (**3b**, **3c**, **3d**) ranked as **3c** ($-CH_2Br$)>**3b** ($-CH_2Cl$), with 2,3-bis(iodomethyl)quinoxaline (**3d**) being completely inactive. These results seem to be caused by heightened electrophilicity as well, although in this case it becomes excessive: the electron-withdrawing trifluoromethyl group at the 6-position of **3d** increases the electrophilicity of the iodomethyl group so much that **3d** undergoes decomposition under the MIC assay conditions before it can exert its antibacterial activity. A similar relationship between electron-withdrawing substituents at the 6-position and antibacterial activity was also observed in the case of 6-NO_2 substituted quinoxalines (**1b**, **1c**), whose activity became the highest when the substituents at the 2- and 3-positions were chloromethyl, a less reactive substituent than bromomethyl. As with the 6-CF_3-substituted quinoxalines, the strong electron-withdrawing nitro group at the 6-position increases the electrophilicity of halomethyl groups, which would result in adequate reactivity for the chloromethyl group of **1b**, but extend too far and induce instability for the bromomethyl group of **1c**. The notion of electron-withdrawing group-induced destabilization of halomethyl groups seems to also be supported by the fact that the reaction of 2,3-bis(bromomethyl)-6-nitroquinoxaline **1c** with sodium iodide afforded complex mixtures of by-products, instead of forming the desired iodinated compounds.

Antifungal Activity The MIC values of 2,3-bis-(halomethyl)quinoxaline derivatives (**2a**–**8a**, **1b**–**8b**, **1c**–**8c**,[24]

Table 1. Antibacterial Activities of **2a**–**8a**, **1b**–**8b**, **1c**–**8c**, and **2d**–**8d**

	R^1	R^2	X	MIC (μg/mL)				
				Gram-positive		Gram-negative		
				B. s.[a)]	S. a.[b)]	E. c.[c)]	P. a.[d)]	S. m.[e)]
1b	NO_2	H	Cl	0.4	6.3	>100	>100	>100
1c	NO_2	H	Br	25	50	>100	>100	>100
2a	CN	H	F	>100	>100	>100	>100	>100
2b	CN	H	Cl	50	>100	>100	>100	>100
2c	CN	H	Br	25	25	>100	>100	>100
2d	CN	H	I	6.3	6.3	>100	>100	>100
3a	CF_3	H	F	>100	>100	>100	>100	>100
3b	CF_3	H	Cl	25	25	>100	>100	>100
3c	CF_3	H	Br	12.5	12.5	>100	>100	>100
3d	CF_3	H	I	>100	>100	>100	>100	>100
4a	F	H	F	>100	>100	>100	>100	>100
4b	F	H	Cl	>100	>100	>100	>100	>100
4c	F	H	Br	25	50	>100	>100	>100
4d	F	H	I	25	25	>100	>100	>100
5a	Cl	H	F	>100	>100	>100	>100	>100
5b	Cl	H	Cl	>100	>100	>100	>100	>100
5c	Cl	H	Br	50	50	>100	>100	>100
5d	Cl	H	I	12.5	25	>100	>100	>100
6a	Br	H	F	>100	>100	>100	>100	>100
6b	Br	H	Cl	>100	>100	>100	>100	>100
6c	Br	H	Br	25	50	>100	>100	>100
6d	Br	H	I	25	>100	>100	>100	>100
7a	CH_3	CH_3	F	>100	>100	>100	>100	>100
7b	CH_3	CH_3	Cl	>100	>100	>100	>100	>100
7c	CH_3	CH_3	Br	50	50	>100	>100	>100
7d	CH_3	CH_3	I	>100	>100	>100	>100	>100
8a	OCH_3	H	F	>100	>100	>100	>100	>100
8b	OCH_3	H	Cl	100	100	>100	>100	>100
8c	OCH_3	H	Br	25	50	>100	>100	>100
8d	OCH_3	H	I	25	100	>100	>100	>100

a) *Bacillus subtilis*. *b*) *Staphylococcus aureus*. *c*) *Escherichia coli*. *d*) *Pseudomonas aeruginosa*. *e*) *Serratia marcescens*.

2d–**8d**) against fungi are listed in Table 2. All 2,3-bis(fluoromethyl)quinoxalines (**2a**–**8a**) were inactive, as was the case for the antibacterial assays, while five 2,3-bis(chloromethyl)-quinoxalines (**1b**–**4b**, **8b**), four 2,3-bis(iodomethyl)quinoxalines (**2d**, **4d**, **5d**, **8d**), and seven 2,3-bis(bromomethyl)quinoxalines (**1c**–**6c**, **8c**) exhibited antifungal activities. These results indicate that, similarly to antibacterial activities, quinoxaline derivatives having highly electrophilic halomethyl groups tend to exhibit antifungal activities.

Detailed analyses of the relationships between the substituents at the 6-position and the antifungal activities of 2,3-bis(halomethyl)quinoxalines indicate that the compounds having an electron-withdrawing group at the 6-position showed the greatest antifungal activity. Among them, **1b** (6-NO_2), **2b** (6-CN), **2d** (6-CN), and **4d** (6-Cl) showed the highest activity (12.5 μg/mL) against *Aspergillus niger*, *Cladosporium cladosporioides*, and *Mucor spinescens*. In contrast, introduction of the moderate electron-withdrawing bromo group or strong electron-donating methoxy group at the 6-position, and of the moderate electron-donating methyl group at the 6- and 7-positions resulted in low and/or no antifungal activities.

Among the 2,3-bis(chloromethyl)quinoxalines (**1b**–**8b**), the compound with the widest antifungal spectrum was **1b** (6-NO_2), followed by **2b** (6-CN), **3b** (6-CF_3), and **4b** (6-F). This suggests that the greater the electron-withdrawing ability of the substituent at the 6-position, the wider the antifungal spectrum, due to the increase in electrophilicity at the chloromethyl moiety.

As for the quinoxaline derivatives having iodomethyl (**2d**–**8d**) and bromomethyl groups (**1c**–**8c**), the compounds with the highest and the widest-ranging activities were electron-withdrawing cyano group-substituted **2d** and **2c**, followed by fluoro-substituted **4d** and **4c**. In contrast, the compound **3d** bearing the strong electron-withdrawing trifluoromethyl group was inactive against fungi, similar to its effectiveness against bacteria. This result corroborates the notion of destabilization of iodomethyl and bromomethyl groups induced by the strong electron-withdrawing trifluoromethyl unit, which leads to the decomposition of compounds before any antifungal activity can be exerted. We think that the moderate activity of **1c** (6-NO_2) and **3c** (6-CF_3) may also be caused by similar destabilization of their bromomethyl groups.

Conclusion

Quinoxaline derivatives bearing fluoromethyl, chloromethyl, or iodomethyl groups at the 2- and 3-positions were synthesized by the reaction of corresponding 2,3-bis(bromomethyl)-quinoxaline derivatives with a metal halide (KF, KCl, or NaI). No compounds were active against Gram-negative

Table 2. Antifungal Activities of **2a**–**8a**, **1b**–**8b**, **1c**–**8c**, and **2d**–**8d**

	R^1	R^2	X	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]
1b	NO_2	H	Cl	12.5	50	100	50	50	25	100	100	50
1c	NO_2	H	Br	100	100	50	100	25	50	100	>100	50
2a	CN	H	F	>100	>100	>100	>100	>100	>100	>100	>100	>100
2b	CN	H	Cl	25	>100	12.5	50	>100	12.5	>100	100	>100
2c	CN	H	Br	50	25	25	50	25	25	>100	100	50
2d	CN	H	I	25	25	12.5	25	50	25	>100	>100	25
3a	CF_3	H	F	>100	>100	>100	>100	>100	>100	>100	>100	>100
3b	CF_3	H	Cl	50	50	50	50	>100	25	>100	>100	100
3c	CF_3	H	Br	50	50	100	50	100	25	>100	100	50
3d	CF_3	H	I	>100	>100	>100	>100	>100	>100	>100	>100	>100
4a	F	H	F	>100	>100	>100	>100	>100	>100	>100	>100	>100
4b	F	H	Cl	100	>100	25	100	>100	50	>100	>100	>100
4c	F	H	Br	50	50	50	100	50	25	100	100	50
4d	F	H	I	50	100	25	100	50	25	>100	>100	25
5a	Cl	H	F	>100	>100	>100	>100	>100	>100	>100	>100	>100
5b	Cl	H	Cl	>100	>100	>100	>100	>100	>100	>100	>100	>100
5c	Cl	H	Br	50	100	25	>100	>100	50	>100	>100	>100
5d	Cl	H	I	>100	>100	12.5	>100	>100	>100	>100	>100	>100
6a	Br	H	F	>100	>100	>100	>100	>100	>100	>100	>100	>100
6b	Br	H	Cl	>100	>100	>100	>100	>100	>100	>100	>100	>100
6c	Br	H	Br	100	100	50	>100	>100	50	>100	>100	>100
6d	Br	H	I	>100	>100	>100	>100	>100	>100	>100	>100	>100
7a	CH_3	CH_3	F	>100	>100	>100	>100	>100	>100	>100	>100	>100
7b	CH_3	CH_3	Cl	>100	>100	>100	>100	>100	>100	>100	>100	>100
7c	CH_3	CH_3	Br	>100	>100	>100	>100	>100	>100	>100	>100	>100
7d	CH_3	CH_3	I	>100	>100	>100	>100	>100	>100	>100	>100	>100
8a	OCH_3	H	F	>100	>100	>100	>100	>100	>100	>100	>100	>100
8b	OCH_3	H	Cl	>100	>100	50	>100	>100	50	>100	>100	>100
8c	OCH_3	H	Br	50	>100	50	100	100	50	>100	>100	100
8d	OCH_3	H	I	>100	>100	>100	>100	50	>100	>100	>100	>100

a) *Aspergillus niger*. *b*) *Penicillium citrinum*. *c*) *Cladosporium cladosporioides*. *d*) *Aureobasidium pullulans*. *e*) *Alternaria* sp. *f*) *Mucor spinescens*. *g*) *Gliocladium virens*. *h*) *Rhodotorula rubra*. *i*) *Saccharomyces cerevisiae*.

bacteria. All fluoromethyl derivatives were inactive against Gram-positive bacteria and fungi, while the antibacterial and antifungal properties of chloromethyl, bromomethyl, and iodomethyl quinoxalines were dependent upon the substituents at the 6-position. We propose that the antimicrobial activities of 2,3-bis(halomethyl)quinoxaline derivatives largely depend on the electrophilicity of halomethyl groups, which is in turn affected by the electrical properties of the substituent at the 6-position. When moderate electron-withdrawing substituents were introduced at the 6-position, the relative strengths of the antibacterial and antifungal activities of the halomethyl compounds became $-CH_2I \geq -CH_2Br > -CH_2Cl$: almost in the order of descending electrophilicity of the halomethyl groups. In contrast, introduction of strong electron-withdrawing groups, such as nitro and trifluoromethyl groups, at the 6-position induced the destabilization of iodomethyl groups, leading to low antimicrobial activities. To confirm the effects of substituent electrophilicity at the 2- and 3-positions on antibacterial and antifungal efficacy, synthesis of quinoxaline derivatives containing other electrophilic substituents at the 2- and 3-positions and evaluation of their antimicrobial activities is in progress.

Experimental

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 60N, 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. ^{1}H- 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 as the midpoint of $CDCl_3$ (77.16 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 and EURO EA 3000 Series. All compounds were characterized by the above techniques. Syntheses of **1c**–**8c** and evaluation of antimicrobial activities by means of minimum inhibitory concentration assay were carried out as has been described previously.[24]

General Procedure for 2,3-Bis(fluoromethyl)quinoxalines (2a–8a) A mixture of **2c**–**8c** (1.0 mmol), KF (10.0 mmol), and

18-crown-6 (4.0 mmol) in dry acetone (15 mL) was refluxed for 8 h under an argon atmosphere. Then, the solvent was evaporated, and the residue was dissolved in $CHCl_3$ (50 mL). The $CHCl_3$ layer was washed with H_2O (50 mL×3) and dried over anhydrous Na_2SO_4. The crude product was purified by column chromatography on silica gel with $CHCl_3$–acetone–EtOH (200 : 5 : 1).

6-Cyano-2,3-Bis(fluoromethyl)quinoxaline (**2a**): White powder, yield: 18%, mp 157–159°C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 5.83 and 5.84 (4H, two d, J=47 Hz), 7.98 (1H, dd, J=1.7, 8.5 Hz), 8.25 (1H, d, J=8.5 Hz), 8.52 (1H, d, J=1.7 Hz). ^{13}C-NMR (126 MHz, $CDCl_3$) δ: 83.5 (d, J_{CF}=169 Hz, CH_2), 83.6 (d, J_{CF}=169 Hz, CH_2), 114.7 (CN), 117.7 (C), 131.0 (CH), 131.8 (CH), 135.3 (CH), 140.4 (C), 142.7 (C), 151.4 (d, J_{CF}=19 Hz, C), 152.2 (d, J_{CF}=19 Hz, C). IR (KBr) cm^{-1}: 3082, 2976, 2235, 1615, 1560, 1493, 1323, 1037, 899, 846. *Anal.* Calcd for $C_{11}H_7N_3F_2$: C, 60.28; H, 3.22; N, 19.17. Found: C, 60.57; H, 3.44; N, 18.87.

2,3-Bis(fluoromethyl)-6-(trifluoromethyl)quinoxaline (**3a**): Brown oil, yield: 54%. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 5.84 and 5.85 (4H, two d, J=46 Hz), 8.01 (1H, d, J=2.7 Hz), 8.27 (1H, dd, J=2.7, 9.2 Hz), 8.47 (1H, d, J=9.2 Hz). ^{13}C-NMR (126 MHz, $CDCl_3$) δ: 83.5 (d, J_{CF}=169 Hz, CH_2), 123.5 (q, J_{CF}=274 Hz, CF_3), 126.8 (q, J_{CF}=2.4 Hz, CH), 127.4 (q, J_{CF}=3.6 Hz, CH), 130.8 (CH), 132.7 (q, J_{CF}=32 Hz, C), 140.4 (C), 142.4 (C), 150.8 (d, J_{CF}=19 Hz, C), 151.6 (d, J_{CF}=18 Hz, C). IR (KBr) cm^{-1}: 3023, 2964, 1573, 1449, 1316, 1196, 1022, 906, 844. *Anal.* Calcd for $C_{11}H_7N_2F_5\cdot 0.8\ H_2O$: C, 47.77; H, 3.13; N, 10.13. Found: C, 47.52; H, 3.08; N, 10.36.

6-Fluoro-2,3-bis(fluoromethyl)quinoxaline (**4a**): White solid, yield: 41%, mp 121–123°C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 5.80 and 5.81 (4H, two d, J=47 Hz), 7.62 (1H, dt, J=2.5, 9.0 Hz), 7.77 (1H, dd, J=2.5, 8.8 Hz), 8.15 (1H, dd, J=6.0, 9.1 Hz). ^{13}C-NMR (126 MHz, $CDCl_3$) δ: 83.6 (d, J_{CF}=169 Hz, CH_2), 83.7 (d, J_{CF}=169 Hz, CH_2), 113.0 (d, J_{CF}=22 Hz, CH), 121.6 (d, J_{CF}=25 Hz, CH), 131.6 (d, J_{CF}=11 Hz, CH), 138.7 (C), 142.3 (d, J_{CF}=13 Hz, C), 148.8 (d, J_{CF}=19 Hz, C), 150.3 (d, J_{CF}=19 Hz, C), 163.5 (d, J_{CF}=254 Hz, C). IR (KBr) cm^{-1}: 3054, 2977, 1622, 1570, 1493, 1331, 1210, 1146, 880, 821. *Anal.* Calcd for $C_{10}H_7N_2F_3\cdot 0.2H_2O$: C, 55.66; H, 3.46; N, 12.98. Found: C, 55.56; H, 3.32; N, 12.88.

6-Chloro-2,3-bis(fluoromethyl)quinoxaline (**5a**): White powder, yield: 71%, mp 100–101°C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 5.81 and 5.82 (4H, two d, J=47 Hz), 7.77 (1H, dd, J=2.0, 9.0 Hz), 8.08 (1H, d, J=9.0 Hz), 8.14 (1H, d, J=2.0 Hz). ^{13}C-NMR (126 MHz, $CDCl_3$) δ: 83.7 (d, J_{CF}=169 Hz, CH_2), 128.3 (CH), 130.6 (CH), 132.2 (CH), 137.1 (C), 140.0 (C), 141.7 (C), 149.5 (d, J_{CF}=19 Hz, C), 150.4 (d, J_{CF}=19 Hz, C). IR (KBr) cm^{-1}: 3050, 2975, 1605, 1562, 1465, 1322, 1146, 884, 827, 739. *Anal.* Calcd for $C_{10}H_7N_2F_2Cl\cdot 0.3H_2O$: C, 51.32; H, 3.27; N, 11.97. Found: C, 51.12; H, 3.10; N, 12.21.

6-Bromo-2,3-bis(fluoromethyl)quinoxaline (**6a**): White powder, yield: 76%, mp 94–95°C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 5.81 and 5.82 (4H, d, J=47 Hz), 7.91 (1H, dd, J=2.2, 9.0 Hz), 8.01 (1H, d, J=9.0 Hz), 8.33 (1H, d, J=2.2 Hz). ^{13}C-NMR (126 MHz, $CDCl_3$) δ: 83.7 (d, J_{CF}=169 Hz, CH_2), 125.3 (C), 130.7 (CH), 131.7 (CH), 134.7 (CH), 140.2 (C), 141.9 (C), 149.7 (d, J_{CF}=19 Hz, C), 150.3 (d, J_{CF}=19 Hz, C). IR (KBr) cm^{-1}: 3075, 2977, 1598, 1560, 1455, 1319, 1146, 880, 821, 583. *Anal.* Calcd for $C_{10}H_7N_2F_2Br\cdot 0.4H_2O$: C, 42.85; H, 2.80; N, 9.99. Found: C, 42.76; H, 2.91; N, 10.16.

2,3-Bis(fluoromethyl)-6,7-dimethylquinoxaline (**7a**): White powder, yield: 84%, mp 130–131°C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 2.52 (6H, s), 5.79 (4H, d, J=47 Hz), 7.88 (2H, s). ^{13}C-NMR (126 MHz, $CDCl_3$) δ: 20.6 (CH_3), 83.9 (d, J_{CF}=168 Hz, CH_2), 128.4 (CH), 140.5 (C), 142.0 (C), 148.3 (d, J_{CF}=19 Hz, C). IR (KBr) cm^{-1}: 3000, 2926, 1625, 1559, 1456, 1361, 1205, 1017, 874. *Anal.* Calcd for $C_{12}H_{12}N_2F_2$: C, 64.85; H, 5.44; N, 12.61. Found: C, 64.83; H, 5.50; N, 12.49.

2,3-Bis(fluoromethyl)-6-methoxyquinoxaline (**8a**): Pale yellow powder, yield: 78%, mp 103–104°C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 3.99 (3H, s), 5.80 and 5.81 (4H, two d, J=48 Hz), 7.41 (1H, d, J=2.7 Hz), 7.47 (1H, dd, J=2.7, 9.2 Hz), 8.00 (1H, d, J=9.2 Hz). ^{13}C-NMR (126 MHz, $CDCl_3$) δ: 56.0 (OCH_3), 83.5 (d, J_{CF}=169 Hz, CH_2), 83.6 (d, J_{CF}=169 Hz, CH_2), 106.5 (CH), 124.6 (CH), 130.3 (CH), 137.7 (C), 143.4 (C), 146.4 (d, J_{CF}=19 Hz, C), 149.4 (d, J_{CF}=19 Hz, C), 161.8 (C). IR (KBr) cm^{-1}: 3016, 2973, 1620, 1498, 1335, 1242, 1146, 1020, 839, 797. *Anal.* Calcd for $C_{11}H_{10}N_2OF_2\cdot 0.5H_2O$: C, 56.65; H, 4.75; N, 12.01. Found: C, 56.85; H, 4.48; N, 11.89.

General Procedure for 2,3-Bis(chloromethyl)quinoxalines (1b–8b) A mixture of **1c**–**8c** (1.0 mmol), KCl (10.0 mmol), and 18-crown-6 (4.0 mmol) in dry acetone (15 mL) was refluxed for 16 h under an argon atmosphere. Then, the solvent was evaporated, and the residue was dissolved in $CHCl_3$ (50 mL). The $CHCl_3$ layer was washed with H_2O (50 mL×3) and dried over anhydrous Na_2SO_4. The crude product was purified by column chromatography on silica gel with $CHCl_3$.

2,3-Bis(chloromethyl)-6-nitroquinoxaline (**1b**): White powder, yield: 89%, mp 97–99°C (decomp.). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 5.08 (4H, s), 8.26 (1H, d, J=9.0 Hz), 8.58 (1H, dd, J=2.4, 9.0 Hz), 9.01 (1H, d, J=2.4 Hz). ^{13}C-NMR (126 MHz, $CDCl_3$) δ: 43.8 (CH_2), 124.4 (CH), 125.5 (CH), 131.0 (CH), 140.5 (C), 143.9 (C), 148.6 (C), 153.0 (C), 153.8 (C). IR (KBr) cm^{-1}: 3027, 2977, 1618, 1526, 1482, 1347, 915, 849, 730. *Anal.* Calcd for $C_{10}H_7N_3O_2Cl_2\cdot 0.7H_2O$: C, 42.19; H, 2.79; N, 14.76. Found: C, 42.01; H, 3.02; N, 14.88.

2,3-Bis(chloromethyl)-6-cyanoquinoxaline (**2b**): White powder, yield: 78%, mp 162–164°C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 5.05 (4H, s), 7.97 (1H, dd, J=1.3, 8.8 Hz), 8.21 (1H, d, J=8.8 Hz), 8.48 (1H, d, J=1.3 Hz). ^{13}C-NMR (126 MHz, $CDCl_3$) δ: 43.9 (CH_2), 114.6 (CN), 117.8 (C), 130.9 (CH), 131.8 (CH), 135.1 (CH), 140.6 (C), 142.9 (C), 152.8 (C), 153.4 (C). IR (KBr) cm^{-1}: 3002, 2978, 2230, 1568, 1489, 1323, 899, 846, 706. *Anal.* Calcd for $C_{11}H_7N_3Cl_2$: C, 52.41; H, 2.80; N, 16.67. Found: C, 52.52; H, 3.08; N, 16.94.

2,3-Bis(chloromethyl)-6-(trifluoromethyl)quinoxaline (**3b**): Brown solid, yield: 76%, mp 61–62°C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 5.06 (4H, s), 7.99 (1H, dd, J=2.0, 8.8 Hz), 8.23 (1H, d, J=8.8 Hz), 8.43 (1H, d, J=2.0 Hz). ^{13}C-NMR (126 MHz, $CDCl_3$) δ: 44.0 (CH_2), 123.5 (q, J_{CF}=274 Hz, CF_3), 126.8 (q, J_{CF}=2.4 Hz, CH), 127.3 (q, J_{CF}=4.8 Hz, CH), 130.5 (CH), 132.7 (q, J_{CF}=32 Hz, C), 140.7 (C), 142.7 (C), 152.2 (C), 152.8 (C). IR (KBr) cm^{-1}: 3053, 2984, 1631, 1569, 1449, 1318, 1135, 905, 826, 707. *Anal.* Calcd for $C_{11}H_7N_2F_3Cl_2\cdot 0.6H_2O$: C, 43.19; H, 2.70; N, 9.16. Found: C, 43.13; H, 2.51; N, 9.41.

2,3-Bis(chloromethyl)-6-fluoroquinoxaline (**4b**): White powder, yield: 97%, mp 141–142°C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 5.04 (4H, s), 7.60 (1H, dt, J=2.6, 9.1 Hz), 7.73 (1H, dd, J=2.5, 9.0 Hz,), 8.11 (1H, dd, J=2.6, 9.1 Hz). ^{13}C-NMR (126 MHz, $CDCl_3$) δ: 44.1 (CH_2), 44.2 (CH_2), 112.8 (d, J_{CF}=22 Hz, CH), 121.6 (d, J_{CF}=25 Hz, CH), 131.3 (d, J_{CF}=11 Hz, CH), 138.9

(C), 142.8 (d, J_{CF}=13 Hz, C), 150.0 (C), 151.6 (C), 163.5 (d, J_{CF}=254 Hz, C). IR (KBr) cm^{-1}: 3019, 2975, 1620, 1567, 1497, 1334, 1217, 866, 841, 714. *Anal.* Calcd for $C_{10}H_7N_2FCl_2 \cdot 0.3 H_2O$: C, 47.95; H, 3.06; N, 11.18. Found: C, 47.88; H, 3.20; N, 10.93.

6-Chloro-2,3-bis(chloromethyl)quinoxaline (**5b**): White powder, yield: 92%, mp 142–143°C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 5.03 (4H, s), 7.75 (1H, dd, J=2.2, 9.0 Hz), 8.04 (1H, d, J=9.0 Hz), 8.10 (1H, d, J=2.2 Hz). ^{13}C-NMR (126 MHz, $CDCl_3$) δ: 44.1 (CH_2), 44.2 (CH_2), 128.0 (CH), 130.3 (CH), 132.2 (CH), 137.0 (C), 140.2 (C), 141.9 (C), 150.8 (C), 151.6 (C). IR (KBr) cm^{-1}: 3020, 2921, 1605, 1558, 1479, 1323, 879, 805, 731, 711. *Anal.* Calcd for $C_{10}H_7N_2Cl_3 \cdot 0.6 H_2O$: C, 44.10; H, 3.03; N, 10.29. Found: C, 44.09; H, 3.16; N, 10.11.

6-Bromo-2,3-bis(chloromethyl)quinoxaline (**6b**): White powder, yield: 86%, mp 137–138°C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 5.02 (4H, s), 7.88 (1H, dd, J=2.0, 9.0 Hz), 7.97 (1H, d, J=9.0 Hz), 8.29 (1H, d, J=2.0 Hz). ^{13}C-NMR (126 MHz, $CDCl_3$) δ: 44.1 (CH_2), 44.2 (CH_2), 125.3 (C), 130.5 (CH), 131.5 (CH), 134.7 (CH), 140.4 (C), 142.1 (C), 150.9 (C), 151.6 (C). IR (KBr) cm^{-1}: 3019, 2924, 1597, 1478, 1321, 880, 821, 727, 583. *Anal.* Calcd for $C_{10}H_7N_2Cl_2Br$: C, 39.25; H, 2.31; N, 9.14. Found: C, 39.53; H, 2.45; N, 9.09.

2,3-Bis(chloromethyl)-6,7-dimethylquinoxaline (**7b**): White powder, yield: 98%, mp 148–149°C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 2.51 (6H, s), 5.02 (4H, s), 7.84 (2H, s). ^{13}C-NMR (126 MHz, $CDCl_3$) δ: 20.6 (CH_3), 44.5 (CH_2), 128.2 (CH), 140.7 (C), 141.9 (C), 150.0 (C). IR (KBr) cm^{-1}: 3013, 2917, 1621, 1556, 1484, 1359, 1022, 873, 733. *Anal.* Calcd for $C_{12}H_{12}N_2Cl_2 \cdot 0.3H_2O$: C, 55.32; H, 4.87; N, 10.75. Found: C, 55.37; H, 4.68; N, 10.72.

2,3-Bis(chloromethyl)-6-methoxyquinoxaline (**8b**): Pale yellow powder, yield: 93%, mp 108–109°C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 3.98 (3H, s), 5.01 and 5.02 (4H, two s), 7.37 (1H, d, J=2.7 Hz), 7.46 (1H, dd, J=2.7, 9.2 Hz), 7.97 (1H, d, J=9.2 Hz). ^{13}C-NMR (126 MHz, $CDCl_3$) δ: 44.3 (CH_2), 44.4 (CH_2), 56.1 (OCH_3), 106.5 (CH), 124.6 (CH), 130.2 (CH), 137.9 (C), 143.5 (C), 147.8 (C), 150.6 (C), 161.8 (C). IR (KBr, cm^{-1}): 3004, 2963, 1613, 1445, 1327, 1224, 1020, 853, 836, 714. *Anal.* Calcd for $C_{11}H_{10}N_2OCl_2 \cdot 0.2H_2O$: C, 50.67; H, 4.02; N, 10.74. Found: C, 50.56; H, 4.28; N, 10.89.

General Procedure for 2,3-Bis(iodomethyl)quinoxalines (2d–8d) A mixture of **2c**–**8c** (0.5 mmol) and NaI (5.0 mmol) in dry acetone (10 mL) was refluxed for 2 h under an argon atmosphere. Then, the solvent was evaporated, and the residue was dissolved in $CHCl_3$ (40 mL). The $CHCl_3$ layer was washed with H_2O (40 mL×2) and dried over anhydrous Na_2SO_4. The crude product was purified by column chromatography on silica gel with $CHCl_3$.

6-Cyano-2,3-bis(iodomethyl)quinoxaline (**2d**): Brown powder, yield: 36%, mp 126–128°C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 4.82 and 4.84 (4H, two s), 7.90 (1H, dd, J=1.7, 8.8 Hz), 8.11 (1H, d, J=8.8 Hz), 8.39 (1H, d, J=1.7 Hz). ^{13}C-NMR (126 MHz, $CDCl_3$) δ: 1.5 (CH_2), 1.6 (CH_2), 114.0 (CN), 117.9 (C), 130.5 (CH), 131.3 (CH), 134.7 (CH), 140.6 (C), 142.9 (C), 154.4 (C), 155.1 (C). IR (KBr) cm^{-1}: 3034, 2957, 2227, 1554, 1489, 1441, 1356, 916, 803. *Anal.* Calcd for $C_{11}H_7N_3I_2$: C, 30.37; H, 1.62; N, 9.66. Found: C, 30.66; H, 1.57; N, 9.66.

2,3-Bis(iodomethyl)-6-(trifluoromethyl)quinoxaline (**3d**): Brown solid, yield: 51%, mp 109–111°C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 4.84 (4H, s), 7.92 (1H, dd, J=1.5, 8.8 Hz), 8.12 (1H, d, J=8.8 Hz), 8.33 (1H, d, J=1.5 Hz). ^{13}C-NMR (126 MHz, $CDCl_3$) δ: 1.8 (CH_2), 123.6 (q, J_{CF}=272 Hz, CF_3), 126.2 (q, J_{CF}=3.6 Hz, CH), 126.9 (q, J_{CF}=4.8 Hz, CH), 130.1 (CH), 132.2 (q, J_{CF}=34 Hz, C), 140.5 (C), 142.6 (C), 153.7 (C), 154.4 (C). IR (KBr) cm^{-1}: 3022, 2961, 1554, 1496, 1449, 1317, 1282, 899, 843. *Anal.* Calcd for $C_{11}H_7N_2F_3I_2$: C, 27.64; H, 1.48; N, 5.86. Found: C, 27.82; H, 1.51; N, 5.62.

6-Fluoro-2,3-bis(iodomethyl)quinoxaline (**4d**): Pale orange powder, yield: 71%, mp 148–150°C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 4.81 (4H, s), 7.54 (1H, dt, J=2.2, 9.0 Hz), 7.64 (1H, dd, J=2.2, 8.8 Hz), 8.02 (1H, dd, J=5.8, 9.0 Hz,). ^{13}C-NMR (126 MHz, $CDCl_3$) δ: 2.2 (CH_2), 2.3 (CH_2), 112.6 (d, J_{CF}=22 Hz, CH), 121.2 (d, J_{CF}=26 Hz, CH), 131.1 (d, J_{CF}=10 Hz, CH), 138.8 (C), 142.5 (d, J_{CF}=14 Hz, C), 151.4 (C), 153.1 (C), 163.2 (d, J_{CF}=254 Hz, C). IR (KBr) cm^{-1}: 3042, 2954, 1566, 1490, 1418, 1326, 1215, 894, 818. *Anal.* Calcd for $C_{10}H_7N_2FI_2$: C, 28.06; H, 1.65; N, 6.55. Found: C, 28.36; H, 1.45; N, 6.39.

6-Chloro-2,3-bis(iodomethyl)quinoxaline (**5d**): Orange powder, yield: 70%, mp 144–146°C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 4.80 (4H, s), 7.70 (1H, dd, J=2.4, 9.0 Hz), 7.95 (1H, d, J=9.0 Hz), 8.01 (1H, d, J=2.4 Hz). ^{13}C-NMR (126 MHz, $CDCl_3$) δ: 2.2 (CH_2), 2.3 (CH_2), 127.9 (CH), 130.1 (CH), 131.8 (CH), 136.6 (C), 140.1 (C), 141.8 (C), 152.3 (C), 153.2 (C). IR (KBr) cm^{-1}: 3016, 2950, 1557, 1479, 1438, 1355, 893, 832, 735. *Anal.* Calcd for $C_{10}H_7N_2ClI_2$: C, 27.02; H, 1.59; N, 6.30. Found: C, 27.19; H, 1.57; N, 6.38.

6-Bromo-2,3-bis(iodomethyl)quinoxaline (**6d**): Pale yellow powder, yield: 77%, mp 158–160°C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 4.80 (4H, s), 7.82 (1H, dd, J=1.7, 9.0 Hz), 7.88 (1H, d, J=9.0 Hz), 8.20 (1H, d, J=1.7 Hz). ^{13}C-NMR (126 MHz, $CDCl_3$) δ: 2.1 (CH_2), 2.3 (CH_2), 124.8 (CH), 130.2 (CH), 131.3 (CH), 134.3 (C), 140.3 (C), 142.1 (C), 152.4 (C), 153.2 (C). IR (KBr) cm^{-1}: 3026, 2964, 2922, 1547, 1470, 1436, 1351, 880, 830, 569. *Anal.* Calcd for $C_{10}H_7N_2BrI_2$: C, 24.57; H, 1.44; N, 5.73. Found: C, 24.57; H, 1.33; N, 5.84.

2,3-Bis(iodomethyl)-6,7-dimethylquinoxaline (**7d**): White powder, yield: 70%, mp 145–146°C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 2.48 (6H, s), 4.81 (4H, s), 7.76 (2H, s). ^{13}C-NMR (126 MHz, $CDCl_3$) δ: 3.2 (CH_2), 20.6 (CH_3), 127.9 (CH), 140.6 (C), 141.6 (C), 150.0 (C). IR (KBr) cm^{-1}: 3023, 2970, 2913, 1623, 1481, 1442, 1358, 1019, 870. *Anal.* Calcd for $C_{12}H_{12}N_2I_2$: C, 32.90; H, 2.76; N, 6.40. Found: C, 32.73; H, 2.73; N, 6.17.

2,3-Bis(iodomethyl)-6-methoxyquinoxaline (**8d**): Pale yellow powder, yield: 94%, mp 161–163°C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 3.96 (3H, s), 4.80 and 4.81 (4H, two s), 7.30 (1H, d, J=2.7 Hz), 7.40 (1H, dd, J=2.7, 9.3 Hz), 7.90 (1H, d, J=9.3 Hz). ^{13}C-NMR (126 MHz, $CDCl_3$) δ: 2.7 (CH_2), 3.1 (CH_2), 56.0 (OCH_3), 106.2 (CH), 124.3 (CH), 129.9 (CH), 137.8 (C), 143.3 (C), 149.1 (C), 152.0 (C), 161.5 (C). IR (KBr) cm^{-1}: 3021, 2959, 1617, 1492, 1443, 1354, 1226, 1020, 886, 819. *Anal.* Calcd for $C_{11}H_{10}N_2OI_2$: C, 30.03; H, 2.29; N, 6.37. Found: C, 30.25; H, 2.21; N, 6.18.

Acknowledgments This work was supported by a Grant from Seikei University.

References and Notes

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