L. Labinsky, and B. Price for pharmacological lab work; and William Baldy for biochemical lab work.

Supplementary Material Available: ^{1}H NMR LIS data for 5 (at 90 MHz); chemical shifts and coupling constants derived therefrom for the aliphatic protons. ^{1}H NMR (270 MHz) data, chemical shifts and coupling constants, for the aliphatic protons of **7c** and **7d**. Additional experimental procedure for the stereoselective L-Selectride reduction of *N*-phenylimines (4 pages). Ordering information is given on any current masthead page.

1-Oxacephalosporins: Enhancement of β-Lactam Reactivity and Antibacterial Activity

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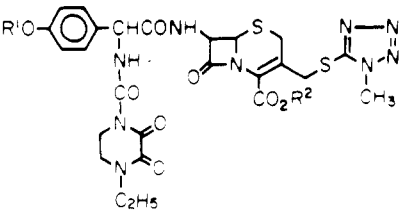
The effect of replacement of sulfur in the cephem nucleus by oxygen upon the β-lactamase stability, infrared carbonyl frequency of the β-lactam ring, and antibacterial activity was investigated. The replacement reduced the stability of β-lactam compounds to β-lactamases, increased the IR frequencies, and enhanced the intrinsic antibacterial activity against bacterial strains without β-lactamase. The instability of 1-oxacephalosporins to β-lactamases, in other words, high reactivity to the enzymes, seemed to be due to the enhanced chemical reactivity of their β-lactam rings which was indicated by their higher IR β-lactam carbonyl frequencies. Based on a view that acylation of the enzyme by β-lactam compounds occurred in both cases of β-lactamase hydrolysis and target enzyme inhibition, the suggestion was made that one of the factors which conferred the higher intrinsic antibacterial activity on 1-oxacephalosporins was their high reactivity to the target enzyme(s), as was the case with β-lactamases.

Several reports have described the synthesis of 1-carba- and 1-oxacephalosporins.[1-5] Christensen and his coworkers reported that the 1-oxa analogue of cefamandole had higher antibacterial activity than cefamandole, although 1-oxa analogues of cephalothin and cefoxitin tended to reduce the activity.[4,5] Narisada and his colleagues published the synthesis of several 1-oxacephalosporins and showed that 1-oxa congeners, including 1-oxacephalothin and 1-oxacefamandole, had four- to eightfold more antibacterial potency against sensitive bacterial strains than the corresponding cephalosporins.[2,6]

In order to study in more detail the effect of substitution of the sulfur atom in cephalosporins with oxygen upon the biological activities, we selected several cephalosporins and their 1-oxa congeners and measured their β-lactamase stability and antibacterial activity. Morin et al. assumed that high infrared β-lactam carbonyl frequency indicated high acylating power, that is, high reactivity of the β-lactam ring.[7a] Thus, we compared the infrared carbonyl frequencies of 1-oxacephalosporins and 1-sulfur congeners and correlated them with the susceptibility to β-lactamases and antibacterial activity.

Synthesis of the New Compounds. Amine **10a** was acylated with succinimino trifluoromethylthioacetate to give **13a**, which was treated with trifluoroacetic acid in anisole to yield acid **4a** (Scheme I). Preparation of the starting oxacephems **10b**, **11**, and **12** has already been reported from our laboratories.[2,6] The amine **10b**[2] was converted similarly into acid **4b** via **13b**. Acylation of the amine **11**[6] with 1(1*H*)-tetrazolylacetyl chloride and subsequent treatment of the resulting **14** with trifluoroacetic acid in anisole yielded 1-oxacefazoline (**5b**). The amine **12**[6] was acylated with 4-bromo-2-oxobutyroyl bromide to give **15**, which on treatment with thiourea was converted into the aminothiazole derivative **16**. On treatment of the latter with trifluoroacetic acid in anisole, the desired acid **6b** was obtained. Acylation of the amine **12** with 2-[4-(mesylamino)phenyl]-2-(*Z*)-[(dichloroacetoxy)imino]acetyl chloride and subsequent hydrolysis yielded the oximino compound **17**, which was treated with trifluoroacetic acid and anisole to give the acid **7b**. Cefoperazone (**18**)[8] was



18, R^1=H, R^2=H (cefoperazone)

19, R^1=H, R^2=$CHPh_2$

20, R^1=NH_2CO–, R^2=$CHPh_2$

8a, R^1=NH_2CO–, R^2=H

converted into benzhydryl ester **19**. Carbamoylation of the 4-hydroxy group of **19** proceeded smoothly to produce **20**, which on treatment with trifluoroacetic acid in anisole gave the acid **8a**.

Results

Susceptibility to β-Lactamase Hydrolysis. The susceptibility of nine pairs of cephalosporins and their 1-oxa congeners to six β-lactamases from Gram-negative bacteria was examined as shown in Table I. In most cases, 1-oxacephalosporins were more susceptible to β-lactamases

(1) R. N. Guthikonda, L. D. Cama, and B. G. Christensen, *J. Am. Chem. Soc.*, **96**, 7584 (1974).
(2) M. Narisada, H. Onoue, and W. Nagata, *Heterocycles*, **7**, 839 (1977).
(3) S. Wolfe, J.-B. Ducep, K.-C. Tin, and S.-L. Lee, *Can. J. Chem.*, **52**, 3996 (1974).
(4) L. D. Cama and B. G. Christensen, *J. Am. Chem. Soc.*, **96**, 7582 (1974).
(5) R. A. Firestone, J. L. Fahey, N. S. Maciejewicz, G. S. Patel, and B. G. Christensen, *J. Med. Chem.*, **20**, 551 (1977).
(6) M. Narisada, T. Yoshida, H. Onoue, M. Ohtani, T. Okada, T. Tsuji, I. Kikkawa, N. Haga, H. Satoh, H. Itani, and W. Nagata, *J. Med. Chem.*, **22**, 757 (1979).
(7) (a) R. B. Morin, B. G. Jackson, R. A. Mueller, E. R. Lavagnino, W. B. Scanlon, and S. L. Andrews, *J. Am. Chem. Soc.*, **91**, 1401 (1969); (b) R. M. Sweet and L. F. Dahl, *ibid.*, **92**, 5489 (1970).
(8) I. Saikawa, S. Takano, C. Yoshida, O. Takashima, K. Momonoi, S. Kuroda, M. Komatsu, T. Yasuda, and Y. Kodama, Belgian Patent 837 682 (1976); *Chem. Abstr.*, **87**, 6002*v* (1977).

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Table I. β-Lactamase Susceptibility of Cephalosporins and 1-Oxa Congeners

source of β-lactamase	class[a]	relative hydrolysis rate[b] 1a/1b	2a/2b	3a/3b	4a/4b	5a/5b	6a/6b	7a/7b	8a/8b	9a/9b
E. coli 6	Ib	360/1200	57/260	14/39	750/910	78/150	120/360	2.2/5.5	5.1/10	*c*
E. cloacae 214	Ia	130/250	39/95	1.2/7.5	200/210	82/42	36/47	0.079/0.36	2.3/13	
P. vulgaris 31	Ic	410/710	39/330	200/300	160/500	370/1200	250/770	170/330	16/34	20/86
E. coli W3110 RTEM	IIIa	39/560	0.75/12	55/370	1.5/18	20/620	17/350	34/290	59/110	0.96/19
Klebsiella sp. 363	IV	120/630	4.2/9.6	130/360	6.2/100	130/380	41/240	35/350	3.1/0.69	6.3/80
E. cloacae 53	IVa	15/140	0.70/26	20/100	1.1/29	28/330	4.9/68	14/450	1.5/0.55	2.7/81

[a] Richmond's class. [b] β-Lactamases were partially purified. Substrate concentration employed was 100 μM. Hydrolysis rates were relative to an arbitrary value of 100 for cephaloridine. 2a = cephalexin; 3a = cefamandole; 5a = cefazolin. [c] Hydrolysis was not detected.

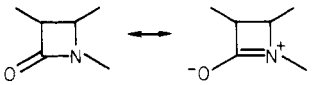
Table II. Infrared Carbonyl Frequency of β-Lactam[a]

no.	IR frequency of β-lactam carbonyl, cm^{-1} a	b
1[b]	1773.1[c]	1778.2[c]
4	1774.0	1784.8
6	1781.0	1788.3
7	1781.9	1789.4
8	1781.0	1789.0
9[b]	1770.0[c]	1779.2[c]

[a] Samples were dissolved in dimethyl sulfoxide (stored over molecular sieves) to give final concentrations of 0.017–0.03 M. Preparation of sample was carried out in N_2 gas to eliminate moisture. Spectra were calibrated by H_2O gas bands. [b] Sodium salt. [c] Reference 14.

than their corresponding cephalosporins, regardless of the enzyme class. Of 52 combinations of β-lactamases and antibiotics there were only three exceptions in which cephalosporin was more susceptible than its 1-oxa congeners. The exceptions were combinations of *Enterobacter cloacae* 214 and the pair of **5a** and **5b**, those of *Klebsiella* sp. 363 and the pair of **8a** and **8b**, and those of *E. cloacae* 53 and the pair of **8a** and **8b**. Since we used β-lactamases from several sources and antibiotics with a variety of side chains as model compounds, the phenomenon described above can probably be generalized to other β-lactamases and antibiotics.

Infrared Carbonyl Frequency of the β-Lactam Ring. The infrared carbonyl frequency shift of the β-lactam ring is considered to indicate the contribution of resonance as in eq 1.[7] Thus, infrared frequencies of the



high frequency low frequency (1)

β-lactam carbonyl of some of the cephalosporins and 1-oxa congeners in Table I were determined (Table II). In all six pairs, 1-oxacephalosporins showed a higher frequency than the corresponding cephalosporins. This indicates that replacement of the sulfur atom in cephalosporin by oxygen decreases the resonance of the β-lactam ring.

Antibacterial Activity. Table III gives minimum inhibitory concentrations (MIC's) of cephalosporins and 1-oxa congeners for six Gram-negative bacteria. Since compound **2b** was very unstable and decomposed during MIC determination,[2] its true MIC values could not be obtained. Except for the pair **6a** and **6b**, 1-oxacephalosporins had two- to eightfold lower MIC values for sensitive bacterial strains than the corresponding cephalosporins, although they were less active against β-lactamase-producing strains because of their higher susceptibility to the enzymes.

Discussion

The effects of substitution of the sulfur atom in the cephalosporin nucleus by oxygen upon the reactivity to β-lactamases, antibacterial activity, and infrared carbonyl frequency were investigated. As model compounds, we selected nine pairs of cephalosporins and their 1-oxa congeners with a variety of side chains (Chart I). The side chains at positions 3 and 7 have pronounced effects upon the susceptibility to β-lactamases and antibacterial activity of the antibiotics.[9,10] Thus, in order to eliminate the

(9) M. H. Richmond and R. B. Sykes, *Adv. Microbiol. Physiol.*, **9**, 31 (1973).

Scheme I

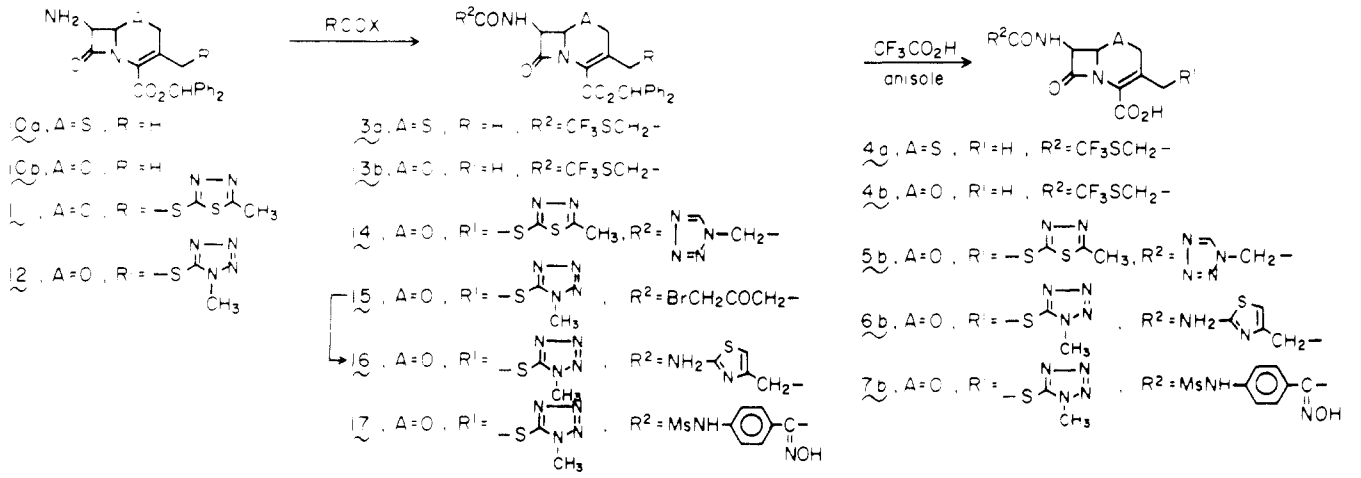
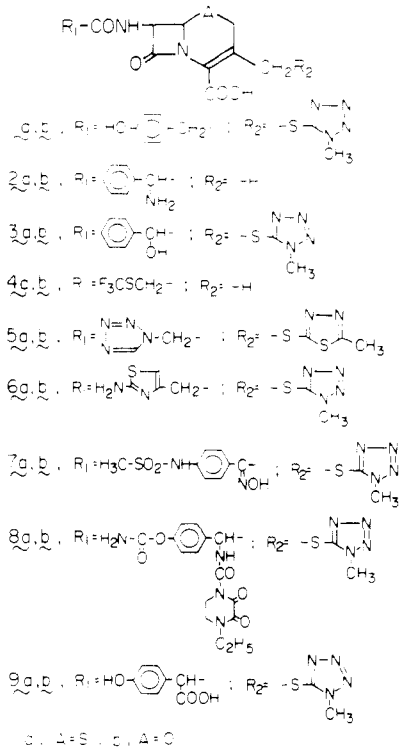


Chart I



side-chain effects, each comparison in this study was made between cephalosporin and its 1-oxa congener which had the same side chain.

In most cases, 1-oxacephalosporins were more susceptible to β-lactamases than the corresponding cephalosporins regardless of the structure of the side chain and enzyme properties (Table I). If the substitution of sulfur by oxygen changes the three-dimensional conformation of the antibiotic resulting in alteration of the stability to β-lactamases, the conformational change will make some antibiotics more labile and others more stable. However, 1-oxa congeners are generally more unstable. 5-Thioglucose and adenosine 5′-(3-thiotriphosphate) inhibit the activities of α-glucan phosphorylase and adenylate cyclase, respectively.[11,12] These facts suggest that exchange of oxygen and sulfur causes no drastic conformational change of the compounds, and this is probably also the case with cephalosporins. Accordingly, the decreased stability of 1-oxacephalosporins probably cannot be explained by a conformational change.

Morin et al. reported that infrared carbonyl frequency of the β-lactam ring was an indicator of acylating power (the higher the frequency, the better the acylating agent).[7] According to Morin's assumption, Indelicato et al. demonstrated the correlation between the pseudo-first-order rate of β-lactam ring hydrolysis at pH 10 and the infrared frequency of the β-lactam carbonyl (the higher the frequency, the faster the hydrolysis rate).[13] Recently, Narisada et al. also observed the same correlation using 7β-[(*p*-hydroxyphenyl)acetyl]cephalosporins and their 1-oxa congeners.[14] In this study we found a correlation between the lability to β-lactamases and the infrared frequency by comparing the cephalosporins and the corresponding 1-oxacephalosporins (Tables I and II). Together with the observations made by other investigators this correlation seems to show that instability of the 1-oxacephalosporins to β-lactamases is a reflection of the high chemical reactivity of their β-lactam rings. This conclusion is also supported by the fact that the 1-oxa congener of cephalexin, **2b**, was decomposed in a slightly alkaline solution with a half-life of 3 h by intramolecular aminolysis of the β-lactam ring.[2]

β-Lactam antibiotics are thought to kill bacteria by inhibiting the transpeptidase, the enzyme which catalyzes the cross-linkage of peptidoglycan, the terminal stage of cell-wall biosynthesis.[15] Once the antibiotic is bound to the enzyme, the β-lactam amide bond is cleaved with concomitant acylation and, as a consequence, the enzyme is inactivated. In fact, others have revealed that β-lactam antibiotics inhibit some carboxypeptidases by forming an ester bond with the serine residue in the active site, although the enzymes are not indispensable to bacteria.[16,17] *Bacillus cereus* β-lactamase is also covalently labeled by

(10) J. A. Webber and J. L. Ott, "Structure–Activity Relationships among the Semisynthetic Antibiotics", D. Perlman, Ed., Academic Press, New York, 1977, pp 161–237.
(11) H. P. Bär, L. P. Simonson, and F. Eckstein, *FEBS Lett.*, **41**, 199 (1974).
(12) M. Ariki and T. Fukui, *J. Biochem. (Tokyo)*, **81**, 1017 (1977).
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(16) J.-M. Frère, C. Duez, J.-M. Ghuysen, and J. Vandekerkhove *FEBS Lett.*, **70**, 257 (1976).
(17) N. Georgopapadakou, S. Hammarström, and J. L. Strominger, *Proc. Natl. Acad. Sci. U.S.A.*, **74**, 1009 (1977).

Table III. Minimum Inhibitory Concentrations (MIC) of Cephalosporins and 1-Oxa Congeners

organism	MIC, μg/mL							
	1a/1b	3a/3b	4a/4b	5a/5b	6a/6b	7a/7b	8a/8b	9a/9b
E. coli H	0.78/0.39	0.20/0.05	25/3.13	1.56/0.78	0.05/0.10	0.78/0.78	⩽0.013/⩽0.013	3.13/0.20
E. coli NIH JC-2	3.13/0.78	0.39/0.05	50/6.25	1.56/0.78	0.10/0.20	0.78/0.78	0.1/⩽0.013	3.13/0.20
K. pneumoniae	1.56/0.78	0.39/0.05	50/6.25	3.13/1.56	0.10/0.20	1.56/0.78	0.05/⩽0.013	3.13/0.20
P. mirabilis PR-4	1.56/0.39	0.78/0.10	50/25	3.13/1.56	0.10/0.20	6.25/1.56	1.56/0.2	3.13/0.39
E. coli 73[a]	25/>100	25/>100	>100/100	25/>100	1.56/>100	50/>100	12.5/12.5	50/50
Klebsiella sp. 363[a]	100/>100	>100/100	>100/>100	>100/>100	6.25/100	>100/>100	>100/12.5	50/100

[a] β-Lactamase-producing strain.

6β-bromopenicillanic acid, an active-site-directed β-lactamase inhibitor, through serine-44, suggesting that the acylated enzyme is formed as an intermediate during the catalytic reaction of β-lactamase.[18] These facts suggest a similarity between the mode of transpeptidase inhibition by β-lactam compounds and that of β-lactamase hydrolysis of β-lactam compounds. Thus, the higher antibacterial activities of 1-oxa congeners shown in Table III may be partly ascribed to the enhanced reactivities of their β-lactam rings as observed in the reaction with β-lactamases. (Note that the antibiotic which is unstable to β-lactamases does not necessarily have high antibacterial activity to sensitive bacterial strains because side chains have pronounced effects upon β-lactamase stability and antibacterial activity. However most side-chain effects are eliminated when a comparison is made between the cephalosporin and its 1-oxa congener.) The reason the 1-oxa congener with an aminothiazole side chain, **6b**, is less active is not clear.

Since 1-oxacephalosporins are labile to β-lactamase hydrolysis, they are less active against β-lactamase-producing strains. This problem can be solved by introducing proper substituents which protect antibiotics from β-lactamase hydrolysis as described in another of our papers.[19]

The outer membrane permeability of an antibiotic is another factor which determines its antibacterial activity. It should be very interesting to study which penetrates the outer membrane more easily, cephalosporins or 1-oxa congeners. This problem is under investigation.

Experimental Section

Chemistry. NMR spectra were measured on a Varian T-60 or A-60 instrument using tetramethylsilane as the internal reference. IR spectra were measured on a Jasco DS-403G spectrometer. Silica gel used for column chromatography was deactivated by the addition of 10%, w/w, water.

Materials. Cephaloridine, cephalexin, and cefamandole were obtained from Shionogi & Co., Ltd., Japan. Cefazolin was purchased from Fujisawa Pharmaceutical Co., Japan. Other compounds used in this study were synthesized by colleagues at Shionogi Research Laboratory as follows.

Synthesis. 1a,[14] **1b**,[14] **2b**,[2] **3b**,[6] **6a**,[20] **7a**,[21] **8b**,[22] **9a**,[6,14] and **9b**[14] were synthesized as reported in the literature.

4a. To a stirred solution of the hydrochloride of **10a** (100 mg, 0.24 mmol) in dry DMF (1 mL) were added triethylamine (33.5 μL,0.24 mmol) and succinimino trifluoromethylthioacetate (61.7 mg, 0.24 mmol) at room temperature under nitrogen. After the solution was stirred for 7.5 h at room temperature, an additional amount (18.5 mg, 0.07 mmol) of the latter reagent was added and stirring was continued for an additional 23 h. The reaction mixture was poured into water (5 mL) and extracted with ethyl acetate. The extract was washed with water, dried, and evaporated under reduced pressure to give a yellow syrup. Chromatography on silica gel by elution with a 1:1 mixture of benzene and ethyl acetate gave 137 mg of crude **13a** containing some amounts of the reagent and solvents: NMR ($CDCl_3$) δ 2.17 (s, 3 H, CH_3), 3.10 and 3.47 (AB-type q, 2 H, J = 19 Hz, C_2 H), 3.68 (s, 2 H, CF_3SCH_2CO), 5.05 (d, 1 H, J = 5.0 Hz, C_6 H), 5.82 (dd, 1 H, J = 9.0 and 5.0 Hz, C_7 H), 7.00 (s, 1 H, $CHPh_2$), 7.1–7.7 (m, 10 H, aromatic protons); IR ($CHCl_3$) 3390, 1785, 1723, 1691 cm^{-1}.

(18) V. Knott-Hunziker, S. G. Waley, B. S. Orlek, and P. G. Sammes, *FEBS Lett.*, **99**, 59 (1979).
(19) T. Yoshida, *Philos. Trans. R. Soc. London, Ser. B*, **289**, 231 (1980).
(20) M. Numata, I. Minamida, M. Yamaoka, M. Shiraishi, T. Miyawaki, H. Akimoto, K. Naito, and M. Kida, *J. Antibiot.*, **31**, 1262 (1978).
(21) T. Takaya, T. Masugi, H. Takasugi, and H. Kochi, German Offen. 2 604 207 (1976); *Chem. Abstr.*, **86**, 55465*m* (1977).
(22) M. Narisada, T. Tsuji, M. Yoshioka, H. Matsumura, Y. Hamashima, S. Hayashi, and W. Nagata, German Offen. 2 739 448 (1978); *Chem. Abstr.*, **89**, 6330*s* (1978).

To a stirred solution of crude **13a** (137 mg, ~0.24 mmol) in dry methylene chloride (2.5 mL) was added anisole (0.24 mL) and trifluoroacetic acid (0.24 mL) at 0 °C under nitrogen. After the mixture was stirred for 2.25 h at 0° C, the solvent and trifluoroacetic acid was removed under reduced pressure below 30 °C. The resulting precipitates were filtered off and washed with methylene chloride to give **4a** (48 mg) as colorless needles, mp 174–178 °C. The filtrate was extracted with cold sodium bicarbonate solution, and the aqueous layer was acidified with 6 N hydrochloric acid to pH 1.5 and extracted with ethyl acetate. The organic layer was washed with water, dried with sodium sulfate, and evaporated. The residue was triturated with ether to give **4a** (21 mg) as pale yellow needles: mp 162–169 °C; total yield from **10a**, 81%; NMR (acetone-d_6) δ 2.13 (s, 3 H, CH_3), 3.32 and 3.75 (AB-type q, 2 H, J = 19 Hz, C_2 H), 4.00 (s, 2 H, CF_3SCH_2CO), 5.17 (d, 1 H, J = 4.5 Hz, C_6 H), 5.78 (dd, 1 H, J = 8.5 and 4.5 Hz, C_7 H), 8.27 (d, 1 H, J = 8.5 Hz, CONH); IR (Nujol) 3260, 1785, 1707, 1665 cm^{-1}; UV max (EtOH) 259 nm (ϵ 5600).

4b. To a stirred solution of amine **10b** (150 mg, 0.41 mmol) in dry DMF (1.7 mL) was added succinimino trifluoromethylthioacetate (0.41 mmol) at room temperature under nitrogen. After the mixture had been stirred for 18 h at room temperature, it was poured into cold water (10 mL) and extracted with ethyl acetate. The extract was washed with water, dried, and evaporated under reduced pressure to give **13b** (224 mg, 96%) as a foam: NMR ($CDCl_3$) δ 2.00 (s, 3 H, CH_3), 3.67 (s, 2 H, CF_3CH_2CO), 4.25 (s, 2 H, C_2 H), 5.03 (d, 1 H, J = 3.5 Hz, C_6 H), 5.62 (dd, 1 H, J = 8.5 and 3.5 Hz, C_7 H), 6.87 (s, 1 H, $CHPh_2$), 7.1–7.7 (m, 10 H, aromatic protons); IR ($CHCl_3$) 3410, 1797, 1727, 1693 cm^{-1}.

To a stirred solution of **13b** (200 mg, 0.4 mmol) in dry methylene chloride (4 mL) was added anisole (0.4 mL) and trifluoroacetic acid (0.4 mL) at 0 °C under nitrogen. After the mixture was stirred for 1.75 h at 0 °C, the solvent and trifluoroacetic acid were removed under reduced pressure below 30 °C. The residual syrup was dissolved in ethyl acetate and extracted with cold sodium bicarbonate solution, and the aqueous layer was acidified with 6 N hydrochloric acid to pH 1.8 and then extracted with ethyl acetate. The organic layer was washed with water, dried with sodium sulfate, and evaporated. The residue was triturated with ether to give **4b** (83 mg, 61%) as colorless needles: mp 144–147 °C; $[\alpha]^{22}_D$ −45.5° (acetone, c 0.477); NMR (acetone-d_6) δ 2.03 (s, 3 H, CH_3), 4.00 (s, 2 H, CF_3SCH_2CO), 4.47 (s, 2 H, C_2 H), 5.25 (d, 1 H, J = 3.5 Hz, C_6 H), 5.67 (dd, 1 H, J = 9 and 3.5 Hz, C_7 H), 8.10 (1 H, d, J = 9 Hz, CONH); IR (Nujol) 3300, 1790, 1708, 1665 cm^{-1}; UV max (EtOH) 261 nm (ϵ 7900). Anal. Calcd for $C_{11}H_{11}O_5N_2SF_3$: C, 38.82; H, 3.26; N, 8.23; S, 9.42. Found: C, 38.62; H, 3.50; N, 8.00; S, 9.58.

5b. A mixture of 1(1*H*)-tetrazolylacetic acid (25 mg, 0.195 mmol), oxalyl chloride (50 μL, 0.585 mmol), and a catalytic amount of dimethylformamide suspended in benzene (2.0 mL) was stirred at room temperature for 2 h, and the resulting solution was concentrated under reduced pressure to give the corresponding acid chloride. Amine **11** (75 mg, 0.16 mmol) dissolved in acetone (1.5 mL) and methylene chloride (3.0 mL) was treated with the acid chloride in the presence of pyridine (15.7 μL, 0.195 mmol) at 0–25 °C for 1 h. After usual workup of the reaction mixture, the product was chromatographed on preparative thin-layer plates (Merck silica gel, 0.5-mm thick). The major band eluted with ethyl acetate was cut off and extracted with methylene chloride to give **14** (38 mg, 41%) as a foam: NMR ($CDCl_3$) δ 2.73 (s, 3 H, CH_3), 4.38 (s, 2 H, C_2 H), 4.60 (s, 2 H, CH_2S), 5.10 (d, 1 H, J = 4 Hz, C_6 H), 5.37 (s, 2 H, NCH_2CO), 5.70 (dd, 1 H, J = 8 and 4 Hz, C_7 H), 6.95 (s, 1 H, $CHPh_2$), 8.13 (d, 1 H, J = 8 Hz), 9.00 (s, 1 H, N=CH–N); IR ($CHCl_3$) 1795 and 1712 cm^{-1}.

Treatment of **14** (38 mg) with trifluoroacetic acid (0.16 mL) and anisole (0.08 mL) in methylene chloride (0.8 mL) at 0 °C for 1 h and subsequent evaporation of the solvent and trifluoroacetic acid under reduced pressure afforded the crude product. Trituration of it with a mixture of pentane and ether gave **5b** (14 mg, 51%) as an amorphous powder: IR (KBr) 3400 (br), 1789, 1703 cm^{-1}.

6b. To a stirred solution of diketone (84 μL, 1.1 mmol) in methylene chloride (1 mL) cooled at −30 °C was added bromine (84 mg, 1.05 mmol), and the stirring was continued for 1 h. The resulting solution of 4-bromo-3-oxobutyroyl bromide was added to a stirred solution of amine **12** (191 mg, 0.4 mmol) and triethylamine (41 mg, 0.4 mmol) in dry methylene chloride (2 mL) at −20 °C. After the mixture was stirred for 20 min, it was poured into 5% phosphoric acid solution and extracted with methylene chloride. The extract was washed with water, dried, and concentrated under reduced pressure. The residue was chromatographed on silica gel by elution with a 2:1 mixture of methylene chloride and ethyl acetate to give **15** (195 mg, 76%): NMR ($CDCl_3$) δ 3.72 (s, 2 H, $BrCH_2$), 3.82 (s, 3 H, CH_3), 4.03 (s, 2 H, $COCH_2CO$), 4.27 (s, 2 H, CH_2S), 4.82, 4.88 (AB q, 2 H, J = 13 Hz, C_2 H), 5.1 (d, 1 H, J = 4.0 Hz, C_6 H), 5.72 (dd, 1 H, J = 4 and 10 Hz, C_7 H), 6.58 (d, 1 H, J = 10 Hz, CONH), 6.95 (s, 1 H, $CHPh_2$), 7.3–7.67 (m, 10 H aromatic protons).

A mixture of **15** (185 mg, 0.29 mmol), thiourea (44 mg, 0.58 mmol), sodium bicarbonate (37 mg, 0.44 mmol), THF (1 mL), and water (0.5 mL) was stirred at room temperature for 1 h. The mixture was poured into water and extracted with ethyl acetate. The extract was washed with water, dried, and concentrated under reduced pressure. The residue was chromatographed on silica gel by elution with a 1:20 mixture of methanol and chloroform to give **16** (113 mg, 62%) as a colorless powder: NMR ($CDCl_3$) δ 3.47 (br s, 2 H, CH_2CO), 3.77 (s, 3 H, CH_3), 4.17 (s, 2 H, SCH_2), 4.66, 4.47 (AB q, 2 H, J = 14 Hz, C_2 H), 4.97 (d, 1 H, J = 4 Hz, C_6 H), 5.63 (dd, 1 H, J = 4.0 and 9.0 Hz, C_7 H), 5.63 (br, 1 H, NH), 6.15 (s, 1 H, thiazole C_5 H), 6.83 (s, 1 H, $CHPh_2$), 7.2–7.6 (m, 11 H, aromatic protons), 7.83 (d, 1 H, J = 9 Hz, CONH).

Treatment of **16** (113 mg, 0.18 mmol) with trifluoroacetic acid (0.5 mL) in anisole (0.5 mL) and workup in the same way as described for the preparation of **5b** afforded the trifluoroacetate of **6b** (90 mg, 55.9%): mp 165–175 °C dec, NMR (CD_3OD) δ 3.70 (s, 2 H, CH_2CO), 4.03 (s, 3 H, CH_3), 4.30 (br s, 2 H, SCH_2), 4.89 (br s, 2 H, C_2 H), 5.20 (d, 1 H, J = 4.0 Hz, C_6 H), 5.63 (d, 1 H, J = 4.0 Hz, C_7 H), 6.70 (s, 1 H, thiazole C_5 H); IR max (Nujol) 3275, 1792, 1697, 1670, 1200 cm^{-1}.

7b. To an ice-cooled suspension of 2-[4-(mesylamino)-phenyl]-2-(*Z*)-[(dichloroacetoxy)imino]acetic acid (221 mg, 0.6 mmol) in methylene chloride (2 mL) was added phosphorus pentachloride (187 mg, 0.9 mmol), and the resulting mixture was stirred at room temperature for 30 min. The solution was concentrated and the residual acid chloride was added portionwise to an ice-cooled, stirred solution of amine **12** (198 mg, 0.4 mmol) and pyridine (49 μL, 0.6 mmol) in dry methylene chloride (8 mL). The resulting mixture was stirred at 0 °C for an additional 50 min and then poured into 5% hydrochloric acid and extracted with ethyl acetate. The extract was successively washed with water, 5% sodium bicarbonate solution, and water and then dried and concentrated under reduced pressure. The residue was chromatographed on silica gel by elution with a 1:25 mixture of methanol and chloroform to give **17** (250 mg, 87%) as a colorless foam: NMR (acetone-d_6) δ 3.03 (s, 3 H, SO_2CH_3), 3.92 (s, 3 H, NCH_3), 4.33 (br s, 2 H, SCH_2), 4.76 (br s, 2 H, C_2 H), 5.33 (d, 1 H, J = 4.0 Hz, C_6 H), 5.94 (dd, 1 H, J = 4 and 7.5 Hz, C_7 H), 5.62 (br s, 1 H, SO_2NH), 6.96 (s, 1 H, $CHPh_2$), 7.1–8.0 (m, 16 H, aromatic protons); IR (Nujol) 1780, 1720, 1660, 1155 cm^{-1}.

To an ice-cooled suspension of **17** (180 mg, 0.25 mmol) in anisole (0.6 mL) and methylene chloride (3 mL) was added trifluoroacetic acid (0.6 mL), and the resulting mixture was stirred for 50 min. The mixture was concentrated under reduced pressure. The residue was triturated successively with petroleum ether and ether to give **7b** (138 mg, ~100%) as a light brown powder: IR (KBr) 1785, 1717, 1665, 1332, 1155 cm^{-1}.

8a. Cefoperazone (**18**;[8] 1.4 g, 2.2 mmol) dissolved in a 10:1 mixture of methylene chloride and methanol (50 mL) was treated with an excess of diphenyldiazomethane (4 mmol), and the resulting mixture was stirred at room temperature for 2 h. After the solvent was evaporated under reduced pressure, the residue was triturated with ether to give the crude **19** (1.9 g), which showed only one spot on the thin-layer chromatogram. In order to obtain a highly pure sample, the crude material was chromatographed on thick-layer plates (Merck silica gel, 2-mm thick) by elution with a 20:1 mixture of chloroform and methanol. The main band was cut off and extracted with a 10:1 mixture of methylene chloride and methanol to give **19** (1.1 g, 74%).

A stirred solution of pure **19** (230 mg, 0.29 mmol) dissolved in methylene chloride (3 mL) was treated with trichloroacetyl isocyanate (162 mg, 0.86 mmol) at 0 °C for 1.5 h and with an ad-

ditional amount (324 mg, 1.72 mmol) of the reagent at 0 °C for 0.5 h. The resulting mixture was poured into ice–water and separated. The methylene chloride solution was dried with magnesium sulfate, and the solvent was evaporated under reduced pressure to give a trichloroacetylcarbamoyl derivative. It was dissolved in a 20:1 mixture of chloroform and methanol (1 mL) and applied to a silica gel (10 g) packed in a column. After this had stood for 2 h, it was eluted with the same solvent mixture to give **20** (230 mg, 95%) as a foam: NMR (a 5:1 mixture of $CDCl_3$ and CD_3OD) 1.13 (t, 3 H, J = 7 Hz, CH_3), 3.2–4.4 (m, 10 H, 5 CH_2), 3.77 (s, 3 H, CH_3), 5.5–6.0 (m, 3 H, C_6 H and C_7 H, NCHCO), 6.95 (s, 1 H, $CHPh_2$), 7.05 (d, 2 H, J = 8 Hz), 7.2–7.6 (m, 12 H, aromatic protons), 10.00 (d, 1 H, J = 7 Hz, CONH), carbamoylamino protons were deuterated; IR ($CHCl_3$) 3500, 1780, 1712, 1683 cm^{-1}.

A solution of **20** (220 mg, 0.26 mmol) dissolved in methylene chloride (2 mL) was treated with anisole (0.4 mL) and trifluoroacetic acid (0.4 mL) at 0 °C for 1 h and, after dilution of the reaction mixture with benzene (5 mL), the solvent was removed under reduced pressure to yield a crude product, which was triturated successively with petroleum ether and ethyl acetate to produce **8a** (140 mg, 78%) as an amorphous powder: IR (KBr) 3300, 1785, 1720, 1680 cm^{-1}.

Biochemistry. Bacterial Stains. *E. cloacae* 214, *E. cloacae* 53, and *E. coli* W3110 RTEM were kindly supplied by M. H. Richmond (University of Bristol, England). Other strains were from Shionogi Research Laboratories stocks.

Preparation of Crude Extracts. Constitutive β-lactamase producers, *E. coli* 6, *E. cloacae* 214, *E. cloacae* 53, *E. coli* W3110 RTEM, and *Klebsiella* sp. 363, were grown statically in nutrient phosphate broth (Nissui, Japan) at 37 °C for 16 h. Cultures were harvested by centrifugation at 3000*g* for 15 min at 4 °C. The pellets were washed once with 0.1 M potassium phosphate buffer (pH 7.0) and then suspended in the same buffer for ultrasonic disruption.

Overnight culture of the inducible β-lactamase producer, *Proteus vulgaris* 31, in nutrient phosphate broth was diluted tenfold with the same broth and grown for 2 h in a shaker at 37 °C. Penicillin G was added to the culture to a final concentration of 100 μg/mL, and bacteria were grown for another 2 h. The culture was harvested by centrifugation, washed once with the phosphate buffer, and suspended in the same buffer for ultrasonic disruption.

Bacteria were disintegrated by ultrasonication (Sonicator-150, Ohtake, Japan) for 2 min in an ice–water bath. After centrifugation at 33000*g* for 30 min at 4 °C to remove cell debris, the supernatant fluids were passed through a millipore membrane filter, 0.22 μm (Millipore Corp.) and stocked at −78 °C until used.

Purification of β-Lactamases. β-Lactamases produced from *E. coli* W3110 RTEM and *Klebsiella* sp. 363 were partially purified using DEAE-Sephadex A-25. Crude extracts dialyzed against 0.01 M Na_2HPO_4–KH_2PO_4 (pH 8.0) were loaded onto a DEAE-Sephadex A-25 column equilibrated against the same buffer. A gradient was constructed from 180 mL of 0.01 M (pH 8.0) and 0.5 M (pH 6.2) phosphate buffer. β-Lactamases from *E. coli* 6, *E. cloacae* 214, *P. vulgaris* 31, and *E. cloacae* 53 were partially purified using CM-Sephadex C-50 as described previously.[23]

Active fractions were pooled, dialyzed against 0.1 M potassium phosphate buffer (pH 7.0), and stocked at −78 °C until used.

Assay of β-Lactamase. Potassium phosphate buffer of 0.1 M (pH 7.0) was used in the assay. β-Lactamase activity was determined at 30 °C by spectrophotometric assay, using the change in optical density at a definite wavelength in the ultraviolet region as described previously.[24] The difference of the absorption coefficient of the β-lactam upon hydrolysis was obtained from the spectrum of the β-lactam compound before and after the complete hydrolysis by β-lactamase. The enzyme was diluted with the buffer containing 0.001% gelatin. A 0.2-mL portion of the enzyme solution was added to 2 mL of substrate solution (final concentration of substrate was 100 μM), and the reaction mixture was incubated in a 1-cm cuvette in a Hitachi (Japan) spectrophotometer Model 200-20, through which water of 30 °C was circulated. The decrease of optical density was recorded with a Hitachi recorder 200. The hydrolysis rate was calculated from the slope of the recorded line.

Susceptibility Tests. Antibacterial activity was determined by the agar dilution method using sensitivity test agar (Eiken, Japan). An overnight culture of bacteria in tryptosoy broth (Eiken, Japan) was diluted to about 10^6 cells/mL with the same broth and inoculated with an inoculating device onto agar containing serial twofold dilutions of an antibiotic. Organisms were incubated at 37 °C for 18–20 h. The minimum inhibitory concentration (MIC) of an antibiotic was defined as the lowest concentration that inhibited visible growth.

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Piperazinylquinoxalines with Central Serotoninmimetic Activity

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Regioselective syntheses of substituted 2-chloroquinoxalines and derived 2-(1-piperazinyl)quinoxalines are described. Selectivity in regards to serotonin reuptake blocking and serotoninmimetic activities of the piperazinylquinoxalines is reported. In general, introduction of a 6-substituent into the piperazinylquinoxaline enhanced serotonin reuptake blocking activity and diminished serotoninmimetic activity. Unsubstituted and 3-hydroxypiperazinylquinoxalines had primarily serotoninmimetic activity.

Recognition that serotonin plays an important role in the physiology of the normal mammalian central nervous system (CNS) as well as in certain pathological states has stimulated the search for novel serotonin-like agents. A previous publication[1] described a series of piperazinylpyrazine derivatives having potent and selective central serotoninmimetic activity. In this work we report the syntheses of some piperazinylquinoxalines[2] and their evaluation as serotonin agonists and neuronal serotonin-reuptake inhibitors.

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(2) Some of these compounds have been disclosed in the patent literature: German Offen. 2433397 (1975); *Chem. Abstr.* **1975**, *82*, 156377*g*.

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