Structure–Activity Relationship (SAR) Studies on Oxazolidinone Antibacterial Agents. 3.[1] Synthesis and Evaluation of 5-Thiocarbamate Oxazolidinones

Ryukou TOKUYAMA,* Yoshiei TAKAHASHI, Yayoi TOMITA, Masatoshi TSUBOUCHI, Nobuhiko IWASAKI, Noriyuki KADO, Eiichi OKEZAKI, and Osamu NAGATA

Research and Development Division, Hokuriku Seiyaku Co., Ltd., 37–1–1, Inokuchi, Katsuyama, Fukui 911–8555, Japan.
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A series of 5-thiocarbamate oxazolidinones was prepared and tested for *in vitro* and *in vivo* antibacterial activities. The results of *in vitro* antibacterial activity indicated that the 5-thiocarbamate group was a suitable substituent for the activity by the 5-moderate hydrophilicity. The compounds within a favorable log *P* value range were found to have potent *in vitro* antibacterial activity against gram-positive bacteria including methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE). Compounds 3a and 4h were superior to linezolid in both *in vitro* and *in vivo* potency and were considered to be hopeful compounds. We also discuss the pharmacokinetic properties of several compounds in mice.

Key words oxazolidinone; antibacterial activity; structure–activity relationship; 5-thiocarbamate oxazolidinone

Several antibiotics have been prescribed and found to be very effective on various infectious disorders. However, the appearance of multi-drug-resistant gram-positive bacteria, in particular, methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE) is causing a serious menace. Moreover, the emergence of vancomycin-resistant MRSA can be anticipated in the foreseeable future. For the treatment of these intractable infections, a new anti-infectious agent is needed in clinical.

Oxazolidinone antibacterial agents[2] are a new class of synthetic antibacterial agents with activity against gram-positive bacteria. Their mode of action has been found to inhibit the protein synthesis in the initial stage.[3] Because of this unique mode of action, oxazolidinone is not cross-resistant with other types of antibiotics. Linezolid (**1**),[4] which was discovered by Pharmacia group, is well known as the first promising candidate of oxazolidinone and works effectively against numerous serious gram-positive human pathogens caused by MRSA and VRE.

In our preceding paper,[1] we described that 5-thiourea compounds **2a** and **2b** exhibited better *in vitro* antibacterial activities than linezolid. We also indicated that both the calculated log *P* value and the balance between 5-hydrophobic (or hydrophilic) substituent and hydrophilic (or hydrophobic) substituents on benzene ring would be important factors in the development of 5-thiocarbonyl oxazolidinone antibacterial agents. However, the *in vivo* activities of **2a** and **2b** were not sufficient. Thus, we converted the 5-thiourea group into 5-thiocarbamate oxazolidinones in order to find candidates with good *in vitro* and *in vivo* activities.

In this paper, we describe our SAR study on a series of 5-thiocarbamate oxazolidinones.

Chemistry 5-Thiocarbamate oxazolidinones were prepared as shown in Charts 2 and 3. Compounds **3a**—**e** were synthesized from compound **5**[1a] by treatment with the corresponding alcohols as shown in Chart 2.

(4′-Substituted)phenyl-5-thiocarbamate oxazolidinones **4** were prepared as shown in Chart 3 from key intermediates **7**, which were easily derived from **6** by the usual method.[1b] The intermediates **7** were treated with carbon disulfide followed by ethyl chloroformate to give isothiocyanates **8**. The thiocarbamate derivatives **4** were prepared from **8** by treatment with methanol. The physicochemical data of compounds **3** and **4** are shown in the experimental section.

Results and Discussion

All of the oxazolidinone derivatives were tested for antibacterial activity against both standard (*Staphylococcus aureus* SMITH) and clinically isolated strains [*S. aureus* HPC1360 (MRSA), *S. aureus* HPC428 (MRSA), *Enterococcus faecium* HPC1322 and *E. casseliflavus* HPC1310 (VRE)]. Their minimum inhibitory concentrations (MICs μg/ml) are shown in Tables 1 and 2. The data of linezolid (**1**) and vancomycin were used as reference compounds.

We investigated the influence of side chain at 5-position on

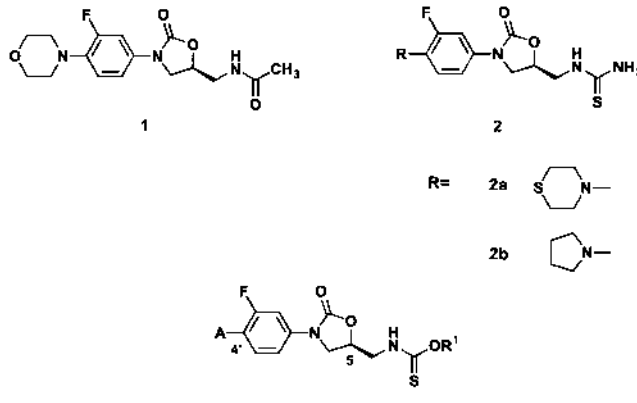


Chart 1

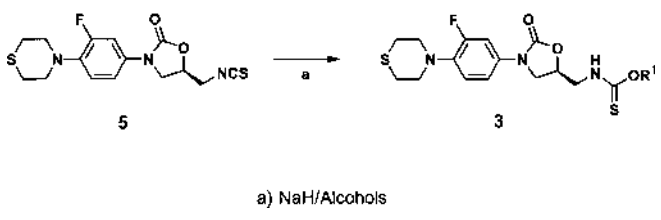
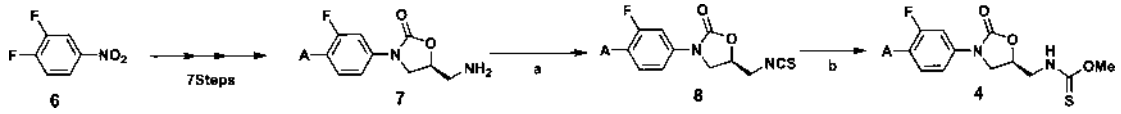


Chart 2

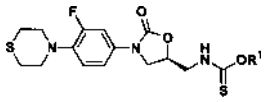
* To whom correspondence should be addressed. e-mail: organ-hs@mitene.or.jp



a) 1) CS_2 2) $ClCO_2Et$ b) NaH/MeOH

Chart 3

Table 1. *In Vitro* Antibacterial Activities of 5-Substituted Oxazolidinones



No.	R^1	MICs (μg/ml)[a]				
		Standard strain	Clinical isolates			
		S. aureus SMITH	*S. aureus* HPC1360[b]	*S. aureus* HPC428[b]	*E. faecium* HPC1322	*E. casseliflavus* HPC1310[c]
3a	Me	1.56	1.56	0.78	0.78	1.56
3b	Et	3.13	3.13	3.13	1.56	6.25
3c	*n*-Pr	50	6.25	50	3.13	12.5
3d	iso-Pr	>50	>50	>50	12.5	>50
3e	*cyc*Hex	>50	>50	>50	>50	>50
2a		0.78	1.56	0.78	0.78	0.78
2b		0.39	0.39	0.39	0.39	0.39
1		6.25	6.25	3.13	3.13	6.25
Vancomycin		0.78	0.78	0.78	0.78	12.5

a) Inoculum size, one loopful of 10^6 CFU/ml. *b*) MRSA. *c*) VRE.

5-thiocarbamate derivatives for antibacterial activities. The results of antibacterial activities are summarized in Table 1. The activity of compound **3a** showed 4 times stronger than that of linezolid (**1**). However, the introduction of lengthened alkyl groups or cycloalkyl group at R^1 position decreased antibacterial activities. Thus, we focused *O*-methyl thiocarbamate group at 5-position, and synthesized some 4′-substituted oxazolidinones in this series. The antibacterial activities and hydrophobic parameter (calculated log *P* value) of 5-thiocarbamate oxazolidinones are summarized in Table 2.

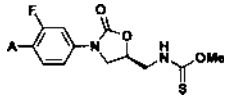
In our previous paper,[1b] we reported that the balance between 5-hydrophobic (or hydrophilic) substituent and hydrophilic (or hydrophobic) substituents on the benzene ring is one of the important factors in antibacterial activity in the 5-thiocarbonyl oxazolidinones. In this series, the prominent decrease of antibacterial activity was not observed. We assume that 5-*O*-methyl thiocarbamate group would be suitable for various substituents on the benzene ring because of its moderate hydrophilic substituent (πa=0.30) compared with 5-thiourea (πa=−0.66) or 5-dithiocarbamate (πa=0.88) groups.[1b]

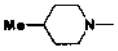
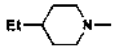
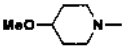
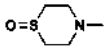
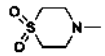
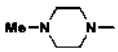
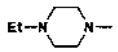
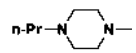
We described earlier[1b] that the favorable calculated log *P* value for antibacterial activity in the case of 5-thiocarbonyl oxazolidinones was −1 to +2. In this series, we also measured the calculated log *P* value.[5] The compounds within the favorable calculated log *P* value range provided stable antibacterial activities as we expected. Among them, compounds **4b** (calculated log *P* value: 1.51), **4h** (1.01), and **4i** (0.73) showed stronger *in vitro* activities than linezolid (**1**). On the contrary, compound **4e**, whose calculated log *P* value was 3.10, showed weak activity. It was recently reported that the azole analogues at 4′-position have interesting levels of antibacterial activity in 5-acetamide oxazolidinones.[6] Compounds **4n** and **4p** also showed strong *in vitro* activities. The activities of compounds **4b**, **4h**, **4i**, **4n** and **4p** were 8—16 times stronger than that of linezolid (**1**).

***In Vivo* Activity** The compounds that exhibited more potent *in vitro* antibacterial activity than linezolid (**1**) were actually tested for *in vivo* antibacterial activity against gram-positive bacteria (*S. aureus* SMITH). The ED_{50} (mg/kg) value of the oral route was determined based on the survival rates on day 7 after infection in mice. Among the oxazolidinones, compounds **3a** and **4h** exhibited higher *in vivo* activities than linezolid (**1**) as shown in Table 3. The data showed 5—6 fold increases in oral activity compared with linezolid (**1**). Moreover, the thiocarbamate derivative **3a** showed stronger *in vivo* activity than the corresponding thiourea derivative **2a**. To learn the reason why the activity of **3a** was stronger than that of **2a**, we examined their pharmacokinetic profiles. (Table 4)

It was found that the plasma concentration of compound **3a** immediately disappeared. The plasma concentrations of *S*-oxide and *S*,*S*-dioxide, however, reached the maximum of 3.48 μg/ml at 1.0 h and 4.92 μg/ml at 6.0 h, respectively, after oral dosing of compound **3a**. Both of them showed greatly higher serum concentrations compared to unchanged compound **3a**, suggesting that the metabolites might be responsible for *in vivo* activity in the mouse systemic infectious model. On the other hand, compound **2a**, which had 5-thiourea moiety, might be similarly metabolized to *S*-oxide or *S*,*S*-dioxide. But the *in vitro* activity of *S*-oxide was weak.[1b]

Table 2. *In Vitro* Antibacterial Activities and Hydrophilic Parameter of 5-Thiocarbamate Oxazolidinones



No.	A	Hydrophilic parameter	MICs (μg/ml)[a]				
			Standard strain	Clinical isolates			
		Calculated log P[b]	*S. aureus* SMITH	*S. aureus* HPC1360[c]	*S. aureus* HPC428[c]	*E. faecium* HPC1322	*E. casseliflavus* HPC1310[d]
4a		0.94	3.13	3.13	3.13	1.56	1.56
4b		1.51	0.39	0.78	0.39	0.39	0.78
4c		2.07	3.13	3.13	1.56	1.56	3.13
4d		2.57	3.13	3.13	3.13	1.56	3.13
4e		3.10	6.25	6.25	6.25	6.25	6.25
4f		1.05	3.13	1.56	3.13	1.56	3.13
4g		2.64	3.13	1.56	1.56	1.56	3.13
3a		1.01	1.56	1.56	0.78	0.78	1.56
4h		−1.00	0.78	0.78	0.78	0.39	0.78
4i		−0.73	0.78	0.78	0.78	0.39	0.78
4j[e]		0.45	1.56	1.56	1.56	0.78	1.56
4k		0.89	1.56	1.56	1.56	0.78	0.78
4l		1.43	1.56	1.56	1.56	0.78	1.56
4m		1.96	3.13	1.56	1.56	0.78	1.56
4n		1.86	0.78	0.39	0.39	0.39	0.39
4o		0.30	1.56	1.56	0.78	0.78	0.78
4p		0.76	0.78	0.78	0.39	0.39	0.39
2a			0.78	1.56	0.78	0.78	0.78
2b			0.39	0.39	0.39	0.39	0.39
1			6.25	6.25	3.13	3.13	6.25
Vancomycin			0.78	0.78	0.78	0.78	12.5

a) Inoculum size, one loopful of 10^6 CFU/ml. *b*) Ref. 5). *c*) MRSA. *d*) VRE. *e*) Ref. 7).

Table 3. *In Vivo* Activity in Mouse Systemic Infectious Model

Compound	MIC[a] (μg/ml)	ED_{50}[b] (mg/kg/dose)	[95% confidence limits]
3a	1.56	1.34	[0.92—1.91]
4b	0.39	>5.00	
4h	0.78	1.15	[0.81—1.56]
2a	0.78	14.3[c]	[8.16—27.0]
2b	0.39	>20.0[c]	
1	6.25	7.03	[4.90—10.1]
		6.80[c]	[4.60—10.1]

a) MIC for *S. aureus* SMITH. *b*) ED_{50}: 50% effective dose (calculated on day 7 by Probit method). *c*) Non-fasted. Animals: male ICR mice, 4-week-old, fasted. Bacterial strain: *Staphylococcus aureus* SMITH. Treatment: Mice were infected intraperitoneally with bacterial suspension. One and four hours after infection, the compounds were administered orally to animals.

Table 4. Pharmacokinetic Profiles of Unchanged **3a** and Its Metabolites in Serum after a Single Administration to Mice at a Dose of 20 mg/kg

Compound		C_{max} (μg/ml)	T_{max} (h)	$AUC_{0-\infty}$ (μg·h/ml)	$T_{1/2}$ (h)
3a	*S*,*S*-Dioxide	4.92	6.00	30.8[a]	57.8
	S-Oxide	3.48	1.00	3.9	1.82
	Unchanged	0.071	0.25	0.046[b]	1.14
2a	Unchanged	1.10	0.25	2.40	1.33
1	Unchanged	24.8	0.083	50.7	1.66

Each value represents the mean of four mice. *a*) The value of *S*-dioxide was $AUC_{0-8\,h}$. *b*) The value of unchanged **3a** was $AUC_{0-4\,h}$.

Table 5. Physical Data for Compounds **3**

No.	Yield (%)[a]	mp (°C) (Recryst. Solv.)	Formula	Analysis (%) Calcd (Found)			$[\alpha]_D^{20}$ DMSO (c=0.1)
				C	H	N	
3a	39	141.5—143 (EtOH)	$C_{16}H_{20}FN_3O_3S_2$	49.85 (49.58	5.23 5.05	10.90 10.82)	−25.9
3b	78	103.5—104.5 (iso-PrOH)	$C_{17}H_{22}FN_3O_3S_2$	51.11 (51.20	5.55 5.67	10.52 10.38)	−23.1
3c	69	124—125 (iso-PrOH)	$C_{18}H_{24}FN_3O_3S_2$	52.28 (52.22	5.85 5.86	10.16 10.12)	−30.9
3d	39	164—166 (iso-Pr_2O–iso-PrOH)	$C_{18}H_{24}FN_3O_3S_2$	52.28 (52.06	5.85 5.56	10.16 10.01)	−32.1
3e	32	150—152 (MeOH)	$C_{21}H_{28}FN_3O_3S_2$	55.61 (55.49	6.22 5.97	9.26 9.07)	−26.9

a) Yields were calculated from compounds **5**.

Table 6. Physical Data for Compounds **3**

No.	^{1}H-NMR (in DMSO-d_6)[a] δ (ppm)
3a	2.73 (4H, t, J=5 Hz), 3.26 (4H, t, J=5 Hz), 3.70—3.85 (3H, m), 3.92 (3H, s), 4.09 (1H, t, J=9 Hz), 4.75—4.93 (1H, m), 7.07 (1H, t, J=9.5 Hz), 7.17 (1H, dd, J=9.5, 2.5 Hz), 7.40 (1H, dd, J=14.5, 2.5 Hz), 9.10 (1H, br s)
3b	1.26 (3H, t, J=7.5 Hz), 2.73 (4H, t, J=5 Hz), 3.25 (4H, t, J=5 Hz), 3.70—3.90 (3H, m), 4.09 (1H, t, J=9 Hz), 4.43 (2H, q, J=7.5 Hz), 4.75—4.90 (1H, m), 7.07 (1H, t, J=9 Hz), 7.16 (1H, dd, J=9, 2.5 Hz), 7.39 (1H, dd, J=14.5, 2.5 Hz), 9.04 (1H, br s)
3c	0.91 (3H, t, J=7.5 Hz), 1.67 (2H, q, J=7.5 Hz), 2.73 (4H, t, J=5 Hz), 3.25 (4H, t, J=5 Hz), 3.75—3.90 (3H, m), 4.09 (1H, t, J=9 Hz), 4.34 (2H, t, J=7.5 Hz), 4.80—4.90 (1H, m), 7.07 (1H, t, J=8.5 Hz), 7.17 (1H, dd, J=8.5, 2.5 Hz), 7.39 (1H, dd, J=14.5, 2.5 Hz), 9.06 (1H, br s)
3d	1.27 (6H, d, J=6 Hz), 2.73 (4H, t, J=5.5 Hz), 3.25 (4H, t, J=5.5 Hz), 3.60—3.85 (3H, m), 4.09 (1H, t, J=9 Hz), 4.60—4.90 (1H, m), 5.44 (1H, sep, J=6 Hz), 7.07 (1H, t, J=9.5 Hz), 7.16 (1H, dd, J=9.5, 2.5 Hz), 7.40 (1H, dd, J=14.5, 2.5 Hz), 8.96 (1H, br s)
3e	1.20—1.90 (10H, m), 2.73 (4H, t, J=5 Hz), 3.25 (4H, t, J=5 Hz), 3.70—3.85 (3H, m), 4.09 (1H, t, J=9 Hz), 4.70—4.90 (1H, m), 5.23 (1H, br s), 7.07 (1H, t, J=9 Hz), 7.16 (1H, dd, J=9, 2.5 Hz), 7.39 (1H, dd, J=15, 2.5 Hz), 8.99 (1H, br s)

a) Measured at 100 °C.

In conclusion, 5-thiocarbamate oxazolidinones which had both a good balance of hydrophilic parameters (πa, πb) and favorable calculated log P value (−1 to +2) were synthesized and their antibacterial activity were evaluated. Among them, compounds **3a** and **4h** showed good *in vitro* and *in vivo* activities compared with linezolid. These compounds are thus expected to be effective candidates for numerous gram-positive infections.

Experimental

Melting points were measured with a Yanagimoto melting point apparatus and are uncorrected. Elemental analyses were measured with a Yanagimoto MT-5 elemental analysis apparatus, and were within ±0.4% of calculated values. ^{1}H-NMR spectra were measured with a JEOL A-500 (500 MHz) or JEOL JNM-LA300 (300 MHz) spectrometer using tetramethylsilane as an internal standard. High-resolution mass spectra were measured on a JEOL DX-300 mass spectrometer. Specific optical rotations were measured on a JASCO DIP-370 polarimeter. Column chromatography was carried out with silica gel [Kieselgel 60 (Merck)]. TLC was conducted on 0.25 mm precoated silica gel plates ($60F_{254}$, Merck). All extracted solvents were dried over Na_2SO_4 and the solvent was evaporated *in vacuo*.

***O*-Methyl (*S*)-[[3-[3-Fluoro-4-(4-thiomorpholinyl)phenyl]-2-oxo-5-oxazolidinyl]methyl]thiocarbamate (3a)** To a solution of NaH (60% in oil, 0.53 g, 13.3 mmol) in MeOH (44 ml), a mixture of **5**[1a] (4.41 g, 12.5 mmol) was added under ice cooling, followed by stirring at room temperature for 3 h. Then the reaction mixture was poured into ice water and adjusted to pH 7 with dilute hydochloric acid. The precipitates were collected by filtration and washed with water to afford **3a** as pale brown crystals. The physicochemical data are listed in Tables 5 and 6.

Compounds **3b**—**e** were respectively prepared from **5** in a similar manner. The physicochemical data are listed in Tables 5 and 6.

(*S*)-5-Aminomethyl-3-[3-fluoro-4-(4-methyl-1-piperazinyl)phenyl]oxazolidine-2-one (7k) A mixture of (*R*)-5-azidomethyl-3-[3-fluoro-4-(4-methyl-1-piperazinyl)phenyl]oxazolidine-2-one (11.2 g, 27.1 mmol), which was prepared from compound **6** by the usual method,[1b] triphenylphosphine (7.82 g, 29.8 mmol) and H_2O (4.8 ml, 271 mmol) in tetrahydrofuran (THF) (170 ml) was heated at 40 °C for 17 h. After cooling, the reaction mixture was diluted with water and dilute hydrochloric acid and extracted with AcOEt. The aqueous layer was made alkaline with aqueous NaOH and extracted with AcOEt. The extract was washed with water, dried and concentrated to afford **7k** (4.55 g, 54%) as pale yellow amorphous. $[\alpha]_D^{20}$ −34.0° (c=0.1, DMSO). ^{1}H-NMR (DMSO-d_6) δ: 1.89 (2H, br s), 2.22 (3H, s), 2.46 (4H, t, J=5 Hz), 2.79 (1H, dd, J=14, 5 Hz), 2.84 (1H, dd, J=14, 5 Hz), 2.98 (4H, t, J=5 Hz), 3.81 (1H, dd, J=9, 6 Hz), 4.01 (1H, t, J=9 Hz), 4.54—4.61 (1H, m), 7.03 (1H, t, J=8.5 Hz), 7.18 (1H, dd, J=8.5, 2 Hz), 7.46 (1H, dd, J=15.5, 2 Hz).

Compounds **7l**—**p** were respectively prepared from the corresponding **6** in a similar manner.

(*S*)-5-Aminomethyl-3-[4-(4-ethyl-1-piperazinyl)-3-fluorophenyl]oxazolidine-2-one (**7l**): Colorless prisms, 87%, mp: 104—105.5 °C (AcOEt–iso-propyl ether (iso-Pr_2O)). $[\alpha]_D^{20}$ −37.0° (c=0.1, DMSO). ^{1}H-NMR (DMSO-d_6) δ: 1.02 (3H, t, J=7.5 Hz), 1.52 (2H, br s), 2.38 (2H, q, J=7.5 Hz), 2.51 (4H, t, J=5 Hz), 2.79 (1H, dd, J=13.5, 5 Hz), 2.84 (1H, dd, J=13.5, 5 Hz), 2.98 (4H, t, J=7.5 Hz), 3.81 (1H, dd, J=9, 6.5 Hz), 4.01 (1H, t, J=9 Hz), 4.50—4.60 (1H, m), 7.03 (1H, t, J=9 Hz), 7.18 (1H, dd, J=9, 2.5 Hz), 7.46 (1H, dd, J=15, 2.5 Hz). *Anal.* Calcd for $C_{16}H_{23}FN_4O_2$: C, 59.61; H, 7.19; N, 17.38. Found: C, 59.46; H, 7.17; N, 17.37.

(*S*)-5-Aminomethyl-3-[3-fluoro-4-(4-propyl-1-piperazinyl)phenyl]oxazolidine-2-one (**7m**): Pale brown crystals, 89%, mp: 93—95 °C (AcOEt–iso-Pr_2O). $[\alpha]_D^{20}$ −37.9° (c=0.1, DMSO). ^{1}H-NMR (DMSO-d_6) δ: 0.88 (3H, t, J=7.5 Hz), 1.46 (2H, sextet, J=7.5 Hz), 1.53 (2H, br s), 2.29 (2H, t, J=7.5 Hz), 2.50 (4H, t, J=5 Hz), 2.79 (1H, dd, J=14, 5 Hz), 2.85 (1H, dd, J=14, 5 Hz), 2.98 (4H, t, J=5 Hz), 3.81 (1H, dd, J=9, 6.5 Hz), 4.01 (1H, t, J=9 Hz), 4.55—4.65 (1H, m), 7.03 (1H, t, J=9 Hz), 7.18 (1H, dd, J=9, 2.5 Hz), 7.46 (1H, dd, J=15, 2.5 Hz). *Anal.* Calcd for $C_{17}H_{25}FN_4O_2$: C, 60.70; H, 7.49; N, 16.65. Found: C, 60.47; H, 7.38; N, 16.55.

(*S*)-5-Aminomethyl-3-[3-fluoro-4-(1-pyrrolyl)phenyl]oxazolidine-2-one (**7n**): Pale brown amorphous, 95%.$[\alpha]_D^{20}$ −21.9° (*c*=0.1, DMSO). ^{1}H-NMR (DMSO-d_6) δ: 1.75 (2H, br s), 2.82 (1H, dd, *J*=14, 5 Hz), 2.89 (1H, dd, *J*=14, 5 Hz), 3.92 (1H, dd, *J*=9, 6 Hz), 4.11 (1H, t, *J*=9 Hz), 4.60—4.70 (1H, m), 6.26 (2H, t, *J*=2 Hz), 7.09 (2H, q, *J*=2 Hz), 7.43 (1H, dd, *J*=9, 2.5 Hz), 7.56 (1H, t, *J*=9 Hz), 7.69 (1H, dd, *J*=14, 2.5 Hz).

(*S*)-5-Aminomethyl-3-[3-fluoro-4-(1-pyrazolyl)phenyl]oxazolidine-2-one (**7o**): Colorless crystals, 74%, mp: 127—127.5 °C (2-propanol (iso-PrOH)). $[\alpha]_D^{20}$ −51.9° (*c*=0.1, DMSO). ^{1}H-NMR (DMSO-d_6) δ: 1.54 (2H, br s), 2.83 (1H, dd, *J*=13.5, 5 Hz), 2.90 (1H, dd, *J*=13.5, 5 Hz), 3.91 (1H, dd, *J*=9, 6 Hz), 4.12 (1H, t, *J*=9 Hz), 4.60—4.70 (1H, m), 6.55 (1H, t, *J*=2.5 Hz), 7.45 (1H, dd, *J*=9, 2.5 Hz), 7.73 (1H, dd, *J*=14.5, 2.5 Hz), 7.80—8.00 (2H, m), 8.13 (1H, t, *J*=2.5 Hz). *Anal.* Calcd for $C_{13}H_{13}FN_4O_2$: C, 56.52 H, 4.74; N, 20.28. Found: C, 56.51; H, 4.81; N, 20.29.

(*S*)-5-Aminomethyl-3-[3-fluoro-4-(1-imidazolyl)phenyl]oxazolidine-2-one (**7p**): Colorless oil, 67%, $[\alpha]_D^{20}$ −44.7° (*c*=0.1, DMSO). ^{1}H-NMR (DMSO-d_6) δ: 1.55 (2H, br s), 2.82 (1H, dd, *J*=14, 5 Hz), 2.89 (1H, dd, *J*=14, 5 Hz), 3.90 (1H, dd, *J*=9, 5.5 Hz), 4.12 (1H, t, *J*=9 Hz), 4.60—4.70 (1H, m), 7.11 (1H, s), 7.47 (1H, dd, *J*=9, 2.5 Hz), 7.50 (1H, s), 7.64 (1H, t, *J*=9 Hz), 7.74 (1H, dd, *J*=13.5, 2.5 Hz), 7.96 (1H, s)

(*R*)-[[3-[3-Fluoro-4-(4-methyl-1-piperazinyl)phenyl]-2-oxo-5-oxazolidinyl]methyl]isothiocyanate (8k) A mixture of **7k** (1.00 g, 3.24 mmol), carbon disulfide (0.40 ml, 6.49 mmol) and Et_3N (0.46 ml, 3.24 mmol) in THF (10 ml) was stirred at 0 °C for 3 h. Ethyl chloroformate (0.31 ml, 3.24 mmol) was added dropwise to the mixture, and stirred at the same temperature for 1 h. The reaction mixture was extracted with AcOEt. The extract was washed with brine, dried and concentrated to afford **8k** (0.70 g, 62%) as colorless crystals. Recrystallization from a mixture of THF and iso-Pr_2O afforded colorless prisms. mp: 127—127.5 °C. $[\alpha]_D^{20}$ −159.0° (*c*=0.1, DMSO). ^{1}H-NMR ($CDCl_3$) δ: 2.36 (3H, s), 2.60 (4H, t, *J*=4.5 Hz), 3.10 (4H, t, *J*=4.5 Hz), 3.80—3.88 (2H, m), 3.95 (1H, dd, *J*=14.5, 5.5 Hz), 4.15 (1H, t, *J*=9 Hz), 4.78—4.85 (1H, m), 6.96 (1H, t, *J*=9 Hz), 7.11 (1H, dd, *J*=9, 2 Hz), 7.42 (1H, dd, *J*=14, 2 Hz). *Anal.* Calcd for $C_{16}H_{19}FN_4O_2S$: C, 54.84; H, 5.47; N, 15.99. Found: C, 54.83; H, 5.41; N, 15.84.

Compounds **8l**—**p** were respectively prepared from the corresponding **7** in a similar manner.

(*R*)-[[3-[4-(4-Ethyl-1-piperazinyl)-3-fluorophenyl]-2-oxo-5-oxazolidinyl]-methyl]isothiocyanate (**8l**): Colorless prisms, 75%, mp: 86.5—87.5 °C (AcOEt–iso-Pr_2O). $[\alpha]_D^{20}$ −151.2° (*c*=0.1, DMSO). ^{1}H-NMR (DMSO-d_6) δ: 1.02 (3H, t, *J*=7.5 Hz), 2.38 (2H, q, *J*=7.5 Hz), 2.51 (4H, t, *J*=5 Hz), 2.99 (4H, t, *J*=5 Hz), 3.78 (1H, dd, *J*=9, 5.5 Hz), 4.02 (1H, dd, *J*=15.5, 5 Hz), 4.10 (1H, dd, *J*=15.5, 3 Hz), 4.17 (1H, t, *J*=9 Hz), 4.90—5.00 (1H, m), 7.05 (1H, t, *J*=9 Hz), 7.18 (1H, dd, *J*=9, 2.5 Hz), 7.45 (1H, dd, *J*=15, 2.5 Hz). *Anal.* Calcd for $C_{17}H_{21}FN_4O_2S$: C, 56.03; H, 5.81; N, 15.37. Found: C, 56.09; H, 5.76; N, 15.46.

(*R*)-[[3-[3-Fluoro-4-(4-propyl-1-piperazinyl)phenyl]-2-oxo-5-oxazolidinyl]methyl]isothiocyanate (**8m**): Colorless crystals, 76%, mp: 93—94 °C (AcOEt–iso-Pr_2O). $[\alpha]_D^{20}$ −141.6° (*c*=0.1, DMSO). ^{1}H-NMR (DMSO-d_6) δ: 0.88 (3H, t, *J*=7.5 Hz), 1.46 (2H, sextet, *J*=7.5 Hz), 2.29 (2H, t, *J*=7.5 Hz), 2.50 (4H, t, *J*=4.5 Hz), 2.99 (4H, t, *J*=4.5 Hz), 3.78 (1H, dd, *J*=9.5, 6 Hz), 4.02 (1H, dd, *J*=15.5, 5 Hz), 4.10 (1H, dd, *J*=15.5, 3.5 Hz), 4.17 (1H, t, *J*=9.5 Hz), 4.90—5.00 (1H, m), 7.05 (1H, t, *J*=9 Hz), 7.18 (1H, dd, *J*=9, 2.5 Hz), 7.45 (1H, dd, *J*=15.5, 2.5 Hz). *Anal.* Calcd for $C_{18}H_{23}FN_4O_2S$: C, 57.12; H, 6.13; N, 14.80. Found: C, 57.02; H, 6.13; N, 14.75.

(*R*)-[[3-[3-Fluoro-4-(1-pyrrolyl)phenyl]-2-oxo-5-oxazolidinyl]methyl]-isothiocyanate (**8n**): Pale brown crystals, 65%. mp: 129—129.5 °C (iso-PrOH). $[\alpha]_D^{20}$ −168.6° (*c*=0.1, DMSO). ^{1}H-NMR (DMSO-d_6) δ: 3.88 (1H, dd, *J*=9, 5.5 Hz), 4.06 (1H, dd, *J*=15, 5.5 Hz), 4.14 (1H, dd, *J*=15, 3.5 Hz), 4.26 (1H, t, *J*=9 Hz), 4.95—5.05 (1H, m), 6.27 (2H, t, *J*=2.5 Hz), 7.11 (2H, t, *J*=2.5 Hz), 7.44 (1H, dd, *J*=9, 2.5 Hz), 7.59 (1H, t, *J*=9 Hz), 7.69 (1H, dd, *J*=14, 2.5 Hz). *Anal.* Calcd for $C_{15}H_{12}FN_3O_2S$: C, 56.77; H, 3.81; N, 13.24. Found: C, 56.51; H, 3.97; N, 12.92.

Table 7. Physical Data for Compounds **4**

No.	Yield (%)[a]	mp (°C) (Recryst. Solv.)	Formula	Analysis (%) Calcd (Found) C	H	N	$[\alpha]_D^{20}$ DMSO (*c*=0.1)
4a	64	Amorphous	$C_{15}H_{18}FN_3O_3S$	339.10529[c] (339.10536)			−27.0
4b	48	147—148.5 (EtOH)	$C_{16}H_{20}FN_3O_3S$	54.38 (54.27	5.70 5.75	11.89 11.91)	−26.9
4c	14	117—118 (iso-PrOH)	$C_{17}H_{22}FN_3O_3S$	55.57 (55.35	6.03 6.24	11.44 11.33)	−29.1
4d	53	135—136 (iso-PrOH)	$C_{18}H_{24}FN_3O_3S$	56.67 (56.67	6.34 6.24	11.02 10.91)	−25.9
4e	46	112—114 (iso-PrOH)	$C_{19}H_{26}FN_3O_3S$	57.70 (57.70	6.63 6.74	10.62 10.53)	−24.1
4f	81	112—113.5 (iso-PrOH)	$C_{18}H_{24}FN_3O_4S$	54.39 (54.19	6.09 6.21	10.57 10.47)	−24.1
4g	82	124.5—125.5 (iso-PrOH)	$C_{18}H_{24}FN_3O_3S$	56.67 (56.55	6.34 6.50	11.02 10.82)	−29.0
4h	57	206—207 (CH_3CN)	$C_{16}H_{20}FN_3O_4S_2$	47.87 (48.04	5.02 5.00	10.47 10.51)	−25.0
4i	55	Oil	$C_{16}H_{20}FN_3O_5S_2$	417.08284[c] (417.08123)			−23.9
4j	37	125—127 (iso-PrOH)	$C_{16}H_{20}FN_3O_4S$	52.02 (52.10	5.46 5.27	11.37 11.31)	−28.1
4k	36	119.5—121.5 (iso-PrOH–iso-Pr_2O)	$C_{17}H_{23}FN_4O_3S$	53.39 (53.20	6.06 5.94	14.65 14.50)	−30.1
4l	36	122—123 (iso-PrOH)	$C_{18}H_{25}FN_4O_3S$	54.53 (54.29	6.36 6.10	14.13 14.02)	−19.1
4m	63	128.5—129.5 (iso-PrOH)	$C_{19}H_{27}FN_4O_3S$	55.59 (55.36	6.63 6.57	13.65 13.57)	−25.0
4n	83	141.5—142.5 (iso-PrOH)	$C_{16}H_{16}FN_3O_3S$	55.00 (54.97	4.62 4.68	12.03 11.86)	−33.9
4o	85	127—127.5 (AcOEt–iso-Pr_2O)	$C_{15}H_{15}FN_4O_3S$	51.42 (51.47	4.32 4.30	15.99 16.00)	−51.9
4p	65	Amorphous	$C_{15}H_{15}FN_4O_3S$	350.08489[c] (350.08382)			−32.1

a) Yields were calculated from the corresponding compounds **8**. *b*) Elemental analyses were preformed on crystalline samples only. *c*) High-resolution mass data.

Table 8. Physical Data for Compounds **4**

No.	^{1}H-NMR (in DMSO-d_6)[a] δ (ppm)
4a	2.29 (2H, quint, J=7.5 Hz), 3.70—3.80 (2H, m), 3.86 (4H, td, J=8.5, 2 Hz), 3.86—3.90 (1H, m), 3.89 (3H, s), 4.07 (1H, t, J=9 Hz), 4.85—4.90 (1H, m), 6.54 (1H, dd, J=9, 9 Hz), 7.09 (1H, dd, J=9, 2.5 Hz), 7.32 (1H, dd, J=14.5, 2.5 Hz), 9.30 (1H, br s)
4b	1.85—1.95 (4H, m), 3.20—3.30 (4H, m), 3.70—3.85 (3H, m), 3.92 (3H, s), 4.05 (1H, t, J=9 Hz), 4.75—4.85 (1H, m), 6.74 (1H, t, J=9 Hz), 7.07 (1H, dd, J=9, 2.5 Hz), 7.30 (1H, dd, J=15.5, 2.5 Hz), 9.10 (1H, br s)
4c	1.50—1.60 (2H, m), 1.60—1.70 (4H, m), 2.90—3.00 (4H, m), 3.75—3.85 (3H, m), 3.92 (3H, s), 4.08 (1H, t, J=9 Hz), 4.80—4.90 (1H, m), 7.02 (1H, t, J=9 Hz), 7.14 (1H, dd, J=9, 2.5 z), 7.38 (1H, dd, J=14.5, 2.5 Hz), 9.10 (1H, br s)
4d	0.95 (3H, d, J=6.5 Hz), 1.25—1.40 (2H, m), 1.45—1.55 (1H, m), 1.65—1.75 (2H, m), 2.60—2.75 (2H, m), 3.25—3.35 (2H, m), 3.70—3.85 (3H, m), 3.92 (3H, s), 4.08 (1H, t, J=9 Hz), 4.80—4.90 (1H, m), 7.02 (1H, t, J=9 Hz), 7.14 (1H, dd, J=9, 2.5 Hz), 7.37 (1H, dd, J=15, 2.5 Hz), 9.10 (1H, br s)
4e	0.89 (3H, t, J=7.5 Hz), 1.25—1.35 (5H, m), 1.70—1.75 (2H, m), 2.66 (2H, t, J=11.5 Hz), 3.30 (2H, d, J=11.5 Hz), 3.70—3.85 (3H, m), 3.92 (3H, s), 4.08 (1H, t, J=9 Hz), 4.80–4.90 (1H, m), 7.02 (1H, t, J=9 Hz), 7.14 (1H, dd, J=8, 2.5 Hz), 7.37 (1H, dd, J=15, 2.5 Hz), 9.10 (1H, br s)
4f	1.56—1.65 (2H, m), 1.85—1.95 (2H, m), 2.75—2.85 (2H, m), 3.15—3.25 (2H, m), 3.27 (3H, s), 3.30—3.40 (1H, m), 3.75—3.85 (3H, m), 3.89 (3H, s), 4.10 (1H, t, J=9 Hz), 4.80—4.90 (1H, m), 7.05 (1H, t, J=9 Hz), 7.14 (1H, dd, J=9, 2.5 Hz), 7.43 (1H, dd, J=14.5, 2.5 Hz), 9.39 (1H, br s)
4g	1.55—1.65 (4H, m), 1.70—1.80 (4H, m), 3.25—3.35 (4H, m), 3.70—3.85 (3H, m), 3.92 (3H, s), 4.06 (1H, t, J=9 Hz), 4.75—4.90 (1H, m), 6.92 (1H, t, J=9 Hz), 7.08 (1H, dd, J=9, 2.5 Hz), 7.31 (1H, dd, J=16, 2.5 Hz), 9.10 (1H, br s)
4h	2.80 (2H, dt, J=14, 3 Hz), 3.20 (2H, td, J=14, 3 Hz), 3.21 (2H, dt, J=14, 4 Hz), 3.60 (2H, t, J=14 Hz), 3.75—3.85 (3H, m), 3.92 (3H, s), 4.10 (1H, t, J=9 Hz), 4.80—4.90 (1H, m), 7.10—7.20 (2H, m), 7.44 (1H, dd, J=14.5, 2.5 Hz), 9.11 (1H, br s)
4i	3.19 (4H, t, J=5.5 Hz), 3.51 (4H, t, J=5.5 Hz), 3.80—3.90 (3H, m), 3.92 (3H, s), 4.10 (1H, t, J=9 Hz), 4.80—4.90 (1H, m), 7.15—7.25 (2H, m), 7.43 (1H, dd, J=13.5, 2.5 Hz), 9.10 (1H, br s)
4j	3.00 (4H, t, J=5 Hz), 3.73 (4H, t, J=5 Hz), 3.75—3.85 (3H, m), 3.92 (3H, s), 4.09 (1H, t, J=9 Hz), 4.75-4.95 (1H, m), 7.04 (1H, t, J=9 Hz), 7.17 (1H, dd, J=9, 3 Hz), 7.41 (1H, dd, J=14.5, 3 Hz), 9.10 (1H, br s)
4k	2.23 (3H, s), 2.47 (4H, t, J=5 Hz), 3.01 (4H, t, J=5 Hz), 3.70—3.85 (3H, m), 3.92 (3H, s), 4.08 (1H, t, J=9 Hz), 4.80—4.90 (1H, m), 7.02 (1H, t, J=9 Hz), 7.15 (1H, dd, J=9, 2.5 Hz), 7.39 (1H, dd, J=15.5, 2.5 Hz), 9.10 (1H, br s)
4l	1.02 (3H, t, J=7 Hz), 2.40 (2H, q, J=7 Hz), 2.52 (4H, t, J=5 Hz), 3.01 (4H, t, J=5 Hz), 3.70—3.85 (3H, m), 3.92 (3H, s), 4.08 (1H, t, J=9 Hz), 4.80—4.90 (1H, m), 7.02 (1H, t, J=9 Hz), 7.15 (1H, dd, J=9, 2.5 Hz), 7.39 (1H, dd, J=15.5, 2.5 Hz), 9.10 (1H, br s)
4m	0.88 (3H, t, J=7.5 Hz), 1.47 (2H, sextet, J=7.5 Hz), 2.31 (2H, t, J=7.5 Hz), 2.51 (4H, t, J=5 Hz), 3.01 (4H, t, J=5 Hz), 3.70—3.85 (3H, m), 3.92 (3H, s), 4.08 (1H, t, J=9 Hz), 4.80—4.90 (1H, m), 7.02 (1H, t, J=9 Hz), 7.15 (1H, dd, J=9, 2.5 Hz), 7.39 (1H, dd, J=15, 2.5 Hz), 9.10 (1H, br s)
4n	3.75—3.90 (3H, m), 3.93 (3H, s), 4.18 (1H, t, J=9 Hz), 4.85—4.95 (1H, m), 6.26 (2H, t, J=2.5 Hz), 7.06 (2H, q, J=2.5 Hz), 7.38 (1H, dd, J=9, 2.5 Hz), 7.54 (1H, t, J=9 Hz), 7.64 (1H, dd, J=14, 2.5 Hz), 9.13 (1H, br s)
4o	3.75—3.90 (3H, m), 3.93 (3H, s), 4.20 (1H, t, J=9 Hz), 4.85—4.95 (1H, m), 6.51 (1H, t, J=2 Hz), 7.43 (1H, dd, J=9, 2 Hz), 7.67 (1H, dd, J=14, 2 Hz), 7.72 (1H, d, J=2 Hz), 7.77 (1H, t, J=9 Hz), 8.06 (1H, t, J=2 Hz), 9.13 (1H, br s)
4p	3.80—3.90 (3H, m), 3.95 (3H, s), 4.19 (1H, t, J=9 Hz), 4.95—5.00 (1H, m), 7.11 (1H, s), 7.44 (1H, dd, J=9, 2.5 Hz), 7.50 (1H, s), 7.65 (1H, t, J=9 Hz), 7.73 (1H, dd, J=13.5, 2.5 Hz), 7.97 (1H, s), 9.33 (1H, br s)

a) Measured at 100 °C.

(*R*)-[[3-[3-Fluoro-4-(1-pyrazolyl)phenyl]-2-oxo-5-oxazolidinyl]methyl]-isothiocyanate (**8o**): Colorless prisms, 81%, mp: 139.5—141 °C (AcOEt). $[\alpha]_D^{20}$ −178.4° (c=0.1, DMSO). ^{1}H-NMR (DMSO-d_6) δ: 3.90 (1H, dd, J=9, 6 Hz), 4.06 (1H, dd, J=15.5, 5 Hz), 4.15 (1H, dd, J=15.5, 3 Hz), 4.27 (1H, t, J=9 Hz), 4.95—5.05 (1H, m), 6.55 (1H, s), 7.48 (1H, dd, J=9, 2.5 Hz), 7.73 (1H, dd, J=14, 2.5 Hz), 7.76 (1H, s), 7.81 (1H, t, J=9 Hz), 8.14 (1H, t, J=2.5 Hz). *Anal.* Calcd for $C_{14}H_{11}FN_4O_2S$: C, 52.82; H, 3.48; N, 17.60. Found: C, 52.87; H, 3.53; N, 17.49.

(*R*)-[[3-[3-Fluoro-4-(1-imidazolyl)phenyl]-2-oxo-5-oxazolidinyl]methyl]-isothiocyanate (**8p**): Pale yellow oil, 78%. $[\alpha]_D^{20}$ −144.5° (c=0.1, DMSO). ^{1}H-NMR (DMSO-d_6) δ: 3.89 (1H, dd, J=9.5, 5.5 Hz), 4.06 (1H, dd, J=15, 5.5 Hz), 4.14 (1H, dd, J=15, 3.5 Hz), 4.28 (1H, t, J=9 Hz), 5.00—5.10 (1H, m), 7.12 (1H, s), 7.49 (1H, dd, J=8.5, 2.5 Hz), 7.63 (1H, s), 7.67 (1H, t, J=8.5 Hz), 7.74 (1H, dd, J=13.5, 2.5 Hz), 7.97 (1H, s).

***O*-Methyl (*S*)-[[3-[3-Fluoro-4-(4-methyl-1-piperazinyl)phenyl]-2-oxo-5-oxazolidinyl]methyl]thiocarbamate (4k)** To a solution of NaH (60 wt% in oil, 0.18 g, 7.53 mmol) in MeOH (5 ml), a mixture of **8k** (1.32 g, 3.77 mmol) in MeOH (5 ml) was added under ice cooling, followed by stirring at room temperature for 17 h. Then the reaction mixture was poured into ice water and adjusted to pH 7 with dilute hydochloric acid. The precipitates were collected by filtration and washed with water to afford **4k** as colorless crystals. The physicochemical data are listed in Tables 7 and 8.

Compounds **4** were respectively prepared from the corresponding **8** in a similar manner. The physicochemical data are listed in Tables 7 and 8.

***In Vitro* Antibacterial Test** These studies were conducted according to the method of the Japan Society of Chemotherapy.[8] The MICs (μg/ml) were determined by an agar dilution method with Muller–Hinton agar (MHA, Difco Laboratories, Detroit, Mich). Bacterial suspensions for inocula were prepared by diluting overnight cultures of organisms to give a final concentration of 10^6 CFU/ml, and one loopful (5 μl) of an inoculum, corresponding to about 5×10^3 CFU per spot was inoculated on drug-containing agar plates. The plates were incubated for 18—24 h at 37 °C. The MIC was defined as the lowest drug concentration that prevented visible growth of bacteria.

***In Vivo* Antibacterial Test** Four week old male ICR mice (18—21 g body weight) were infected intraperitoneally with bacterial suspension. The bacterium used for infection was *S. aureus* Smith (3—7×10^7). Following infection, graded doses of compounds were administered orally to mice in groups of 10 each. The ED_{50}, including 95% confidence limits, was calculated by the probit method[9] from the survival rates on day 7 after infection.

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References and Notes

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