NOVEL ANTIBIOTICS SF2738A, B AND C, AND THEIR ANALOGS PRODUCED BY *Streptomyces* sp.

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Three new antibiotics SF2738A, B and C, and their analogs were isolated from the culture broth of *Streptomyces* sp. The antibiotics are active against Gram-positive bacteria, Gram-negative bacteria and fungi, and exhibited cytotoxic activity against P388 murine leukemia cells with IC_{50} values of 0.08, 0.25 and 7.5 μg/ml, respectively. Their structures were determined by spectral analyses and chemical conversion. Especially, the structure of SF2738A was confirmed to be (*E*)-((4-methoxy-5-methylthio-2-(2-pyridyl)pyridin-6-yl)methylene)azanol by X-ray crystallographic analysis.

In the course of our screening program for new antitumor antibiotics from microbial origin, antibiotics SF2738A (**A**), B (**B**) and C (**C**), and their analogs SF2738D (**D**), E (**E**) and F (**F**) were discovered from the culture filtrate of *Streptomyces* sp. SF2738. SF2738A, B and C showed weak activity against Gram-positive bacteria, Gram-negative bacteria and fungi, and also exhibited cytotoxic activity against P388 murine leukemia cells. The structures of these compounds were determined by spectroscopic and chemical studies coupled with a single-crystal X-ray crystallographic analysis of **A**. The six compounds possess commonly a 2,2'-dipyridyl structure. In this paper, the taxonomy and fermentation of the producing strain, isolation, physico-chemical properties, structural elucidation and biological activities of compounds SF2738 are reported.

Results and Discussion

Taxonomy of the Producing Strain

Strain SF2738 was isolated from a soil collected at Yokohama, Kanagawa, Japan. The strain showed the following morphological, cultural, physiological and chemotaxonomic characteristics. Vegetative mycelia are well developed and branched. Aerial mycelium is characterized by monopodial branching. Spore-chain morphology is classified in the Rectiflexibiles section. Spores are in chains of 30 to 50, cylindrical in shape, 0.7~0.9×0.8~1.4 μm in size and have smooth surfaces (Fig. 1). Zoospores and sporangia are not observed. Cultural characteristics on various media after incubation at 28°C for 14 days are described in Table 1. Mature aerial mass color is in the Red color-series on inorganic salts-

Fig. 1. Scanning electron micrograph of strain SF2738 cultured on inorganic salts starch agar for 2 weeks.

Bar represents 1 μm.

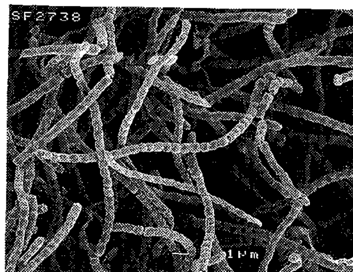


Table 1. Cultural characteristics of strain SF2738.

Medium	Growth	Reverse color	Aerial mycelium	Soluble pigment
Yeast extract - malt extract agar (ISP 2)	Good	Dark brown (3pl)	Abundant, pale pink (5cb)	None
Oatmeal agar (ISP 3)	Good	Colorless	Abundant, pearl (2ba)	None
Inorganic salts - starch agar (ISP 4)	Good	Colorless	Abundant, pearl (2ba)	Pale violet
Glycerol - asparagine agar (ISP 5)	Good	Pale brown (3ie)	Abundant, pale pink (5cb)	None
Tyrosine agar (ISP 7)	Good	Colorless	Moderate, white (a)	None
Sucrose - nitrate agar	Poor	Colorless	Scanty, white (a)	None
Glucose - asparagine agar	Moderate	Colorless	None	None
Nutrient agar	Moderate	Colorless	Scanty, white (a)	None
Calcium malate agar	Moderate	Colorless	Moderate, white (a)	None
Bennett agar	Good	Colorless	Abundant, pale pink (5cb)	None

The color codes were taken from Color Harmony Manual, 4th edition, Container Corporation of America, Chicago, Illinois, 1958.

starch agar and yeast extract-malt extract agar. Reverse side of colony is pale brown to dark brown on glycerol-asparagine agar and yeast extract - malt extract agar. No soluble pigments are formed in any media. Strain SF2738 utilizes D-glucose, D-xylose, D-fructose, D-mannitol, *myo*-inositol, sucrose and glycerol, but not L-arabinose, L-rhamnose and raffinose. The strain is aerobic and grows between 15°C and 33°C with optimum growth at around 28°C. Hydrolysis of starch, peptonization of skim milk, liquefaction of gelatin, and reduction of nitrate are positive. Coagulation of milk and formation of melanoid pigment are negative. Sodium chloride tolerance is less than 7%. Whole-cell hydrolysates contained LL-diaminopimelic acid with no characteristic sugar. The morphological and chemotaxonomic characteristics of strain SF2738 indicate that it belongs to the genus *Streptomyces* and was designated as *Streptomyces* sp. SF2738. The strain was deposited in the National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology, Japan, with an accession number of FERM P-12494.

Fermentation

Strain SF2738 on agar slant was inoculated into 20 ml of a seed medium consisting of soluble starch 2.0%, glucose 1.0%, Polypepton (Daigo Eiyo) 0.5%, wheat germ 0.6%, yeast extract 0.3%, soybean meal 0.2% and $CaCO_3$ 0.1% (pH 7.0) in a 100-ml Erlenmeyer flask. The inoculated flask was shaken on a rotary shaker (220 rpm) at 28°C for 3 days. Four ml of the seed culture were inoculated into 80 ml of a production medium composed of maltose syrup 3.0%, soybean meal 1.5%, Pharamedia (Trader Oil Mill Co., Texas, U.S.A.) 1.0%, distiller's soluble 0.5%, soybean oil 0.15%, $NaNO_3$ 0.3% and $CaCO_3$ 0.2% (pH 7.0) in 500-ml Erlenmeyer flasks. The culture was grown on a rotary shaker (220 rpm) at 28°C for 3 days. The fermentation broths of sixty-five flasks were filtered with the aid of diatomaceous earth. Antibiotics SF2738 and their analogs were monitored by measurement of cytotoxicity against P388 murine leukemia cells as well as by TLC analysis.

Isolation

The broth filtrate (4.5 liters) was adjusted to pH 9 with 6 N NaOH and extracted twice with ethyl acetate (2.5 liters). Combined ethyl acetate extracts were concentrated to give a crude powder (694 mg). The powder having five major spots detected by UV light (254 nm) on TLC was applied to a silica gel

Table 2. Physico-chemical properties of SF2738.

	SF2738A	SF2738B	SF2738C	SF2738D	SF2738E	SF2738F
Appearance	Colorless crystals	Colorless crystals	Colorless powder	Colorless powder	Colorless powder	Colorless powder
MP (°C)	174～176	140～142	104～106	135～138	123～125	145～148
Molecular formula	$C_{13}H_{13}N_3O_2S$	$C_{13}H_{13}N_3O_2S$	$C_{13}H_{14}N_2O_2S$	$C_{13}H_{11}N_3OS$	$C_{15}H_{17}N_3O_2S$	$C_{12}H_9N_3OS$
EI-MS		275 (M^+)	262 (M^+)	257 (M^+)	303 (M^+)	243 (M^+)
HREI-MS Calcd:	275.0728 (M^+)					
Found:	275.0834 (M^+)					
UV λ_{max}^{MeOH} nm ($E_{1cm}^{1\%}$)	203 (632), 242 (960), 280 (sh 530), 305 (sh 408)	204 (633), 244 (893), 286 (sh 490), 310 (sh 360)	205 (720), 219 (719), 238 (sh 495), 292 (538), 306 (sh 430)	206 (868), 236 (1,200), 288 (591), 315 (sh 410)	206 (759), 215 (sh 595), 239 (sh 450), 287 (476), 308 (sh 340)	203 (472), 248 (930), 252 (925), 272 (572), 285 (sh 445)
$\lambda_{max}^{HCl-MeOH}$ nm ($E_{1cm}^{1\%}$)	205 (627), 251 (967), 277 (sh 540), 333 (371)	206 (573), 249 (881), 275 (604), 282 (sh 585), 320 (sh 400)	211 (646), 227 (583), 260 (544), 272 (492), 306 (470), 322 (sh 400)	207 (790), 234 (914), 283 (672), 318 (sh 415), 343 (493)	206 (679), 230 (463), 263 (402), 309 (365), 335 (285)	212 (335), 241 (626), 284 (865), 311 (358), 319 (sh 335)
$\lambda_{max}^{NaOH-MeOH}$ nm ($E_{1cm}^{1\%}$)	213 (1,300), 235 (680), 285 (967)	214 (1,320), 243 (810), 276 (575)	215 (1,260), 238 (sh 500), 291 (550), 306 (sh 430)	216 (1,440), 235 (1,190), 289 (612), 315 (sh 410)	215 (1,240), 239 (sh 450), 288 (487), 307 (sh 340)	214 (1,160), 247 (939), 273 (602), 287 (sh 430)
IR (KBr) cm^{-1}	3185, 2923, 1568, 1541, 1466, 1431, 1414, 1368, 1343, 1252, 1215, 1078, 994, 795, 747	2938, 1588, 1578, 1568, 1472, 1456, 1433, 1409, 1374, 1352, 1256, 1215, 1063, 995, 961, 903, 793	3359, 2934, 1580, 1566, 1551, 1458, 1429, 1370, 1283, 1250, 1215, 1044, 916, 862, 793, 747	3085, 2946, 2934, 2240, 1566, 1532, 1468, 1414, 1368, 1256, 1215, 1067, 1057, 997, 982, 876, 793, 741	3335, 2951, 2928, 1649, 1565, 1545, 1460, 1424, 1383, 1358, 1323, 1248, 1213, 1068, 1053, 893, 797, 749	3040, 1568, 1553, 1470, 1449, 1437, 1368, 1254, 1217, 1117, 1090, 995, 972, 897, 866, 795, 742

column (50 g, Wakogel C-200, Wako Pure Chemical Industries, Ltd.) and chromatographed using chloroform (150 ml) and then using chloroform - methanol (20 : 1, 300 ml). Fractions 1 to 5 were eluted in order of low polarity to give 16.7 mg (**D**), 29.9 mg (**B** and **F**), 24.4 mg (**E**), 20.8 mg (**C**) and 221 mg (**A**) of the crude powders, respectively, after evaporation of each fraction. Further purification of the crude powders was achieved by preparative TLC (Merck, Art. No. 5744) developed with hexane - acetone (2 : 1) for crude **D**, **B**, **F** and **E**, and with chloroform - methanol (3 : 1) for crude **C** to afford pure **D** (12.8 mg), **B** (8.8 mg), **F** (2.2 mg), **E** (15.2 mg) and **C** (2.9 mg). Crude **A** was recrystallized from methanol to give pure **A** (151 mg) as colorless crystals. Additional **A** (18.9 mg) was given from the mother liquor by preparative TLC developed with chloroform - methanol (3 : 1).

Physico-chemical Properties

Antibiotics **A**, **B** and **C**, and their analogs **D**, **E** and **F** were each obtained as colorless crystals or powders. They were soluble in chloroform, ethyl acetate, acetone and methanol, but insoluble in water. Antibiotic **A** was slightly soluble in acidic water. **A**, **B**, **C**, **D**, **E** and **F** showed Rf values of 0.05, 0.79, 0.14, 0.90, 0.60 and 0.80, respectively, on TLC (chloroform - methanol, 10 : 1). These compounds exhibited positive color reactions with I_2 and Na_2MoO_4 (pale yellow) reagents, and negative with Ninhydrin reagent.

Table 3. ^{1}H NMR data of SF2738.

Proton	SF2738A	SF2738B	SF2738C
	δ M (J, Hz)	δ M (J, Hz)	δ M (J, Hz)
3-H	8.06 s	8.14 s	8.02 s
4-OCH_3	4.12 s	4.17 s	4.13 s
5-SCH_3	2.38 s	2.42 s	2.37 s
7-H or H_2	9.11 s	8.70 d (0.5)	4.95 d (4.5)
7-NOH	10.79 br	16.74 br s	—
7-OH	—	—	4.77 t (4.5)
7-NH	—	—	—
7-$NCOCH_3$	—	—	—
3′-H	8.56 ddd (8.0, 1.0, 1.0)	8.12 ddd (8.0, 1.0, 1.0)	8.45 ddd (8.0, 1.0, 1.0)
4′-H	7.87 ddd (8.0, 7.4, 1.8)	7.87 ddd (8.0, 7.4, 1.8)	7.85 ddd (8.0, 7.4, 1.8)
5′-H	7.35 ddd (7.4, 4.9, 1.0)	7.40 ddd (7.4, 4.9, 1.0)	7.36 ddd (7.4, 4.9, 1.0)
6′-H	8.67 ddd (4.9, 1.8, 1.0)	8.71 ddd (4.9, 1.8, 1.0)	8.69 ddd (4.9, 1.8, 1.0)

Proton	SF2738D	SF2738E	SF2738F
	δ M (J, Hz)	δ M (J, Hz)	δ M (J, Hz)
3-H	8.20 s	7.99 s	8.10 s
4-OCH_3	4.15 s	4.12 s	4.20 s
5-SCH_3	2.55 s	2.37 s	—
7-H or H_2	—	4.83 d (4.4)	9.13 s
7-NOH	—	—	—
7-OH	—	—	—
7-NH	—	7.25 br	—
7-$NCOCH_3$	—	2.17 s	—
3′-H	8.48 ddd (8.0, 1.0, 1.0)	8.39 ddd (8.0, 1.0, 1.0)	8.56 ddd (8.0, 1.0, 1.0)
4′-H	7.86 ddd (8.0, 7.4, 1.8)	7.87 ddd (8.0, 7.4, 1.8)	7.88 ddd (8.0, 7.4, 1.8)
5′-H	7.38 ddd (7.4, 4.9, 1.0)	7.36 ddd (7.4, 4.9, 1.0)	7.38 ddd (7.4, 4.9, 1.0)
6′-H	8.67 ddd (4.9, 1.8, 1.0)	8.70 ddd (4.9, 1.8, 1.0)	8.72 ddd (4.9, 1.8, 1.0)

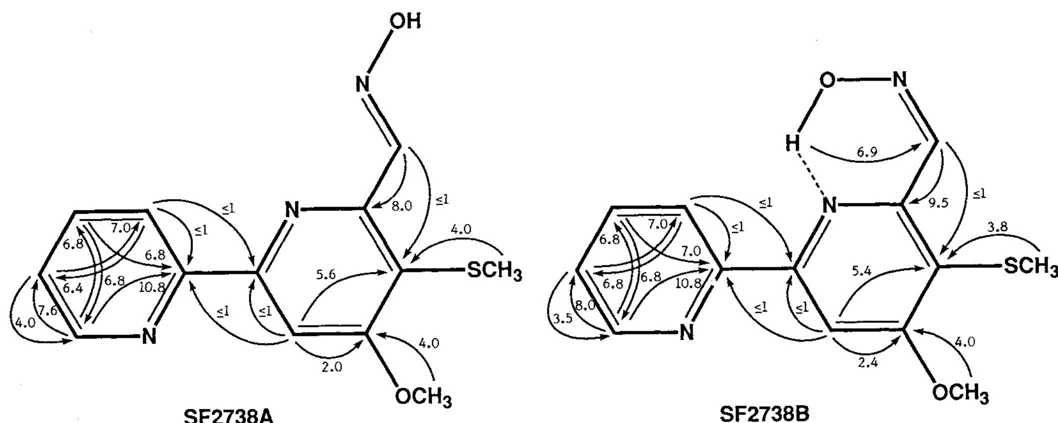
δ: ppm from TMS in $CDCl_3$.
M: Multiplicity.

Table 4. ^{13}C NMR data of SF2738.

Carbon	A		B		C		D		E		F	
	δ	M	δ	M	δ	M	δ	M	δ	M	δ	M
C-2	157.3	s	154.8	s	160.4	s	158.5	s	157.7	s	158.9	s
C-3	103.7	d	104.8	d	102.7	d	105.7	d	102.7	d	99.7	d
C-4	167.3	s	168.2	s	167.2	s	167.0	s	167.2	s	160.4	s
4-OCH_3	56.4	q	56.7	q	56.4	q	56.6	q	56.3	q	56.2	q
C-5	122.0	s	121.0	s	117.6	s	127.3	s	119.4	s	136.1	s
5-SCH_3	18.5	q	18.4	q	17.4	q	17.9	q	17.6	q	—	
C-6	152.6	s	152.5	s	154.9	s	137.6	s	155.3	s	154.2	s
C-7	147.3	d	140.2	d	62.4	t	116.5	s	43.3	t	156.2	d
7-$NCOCH_3$	—		—		—		—		169.8	s	—	
	—		—		—		—		23.5	q	—	
C-2′	155.1	s	153.1	s	155.5	s	153.8	s	155.9	s	155.6	s
C-3′	121.9	d	121.0	d	121.2	d	121.7	d	121.0	d	121.6	d
C-4′	137.2	d	137.5	d	137.0	d	137.2	d	136.9	d	137.1	d
C-5′	124.3	d	124.9	d	124.2	d	124.8	d	124.1	d	124.3	d
C-6′	148.8	d	149.5	d	149.1	d	149.1	d	149.2	d	149.0	d

δ: ppm from TMS in $CDCl_3$.
M: Multiplicity.

Fig. 2. Long range ^1H-^{13}C correlation of SF2738A and B.



Arrows represent ^1H-^{13}C coupling and the values are coupling constants in Hz. A dashed line shows a hydrogen bond.

Antibiotic A formed ultramarine colored complexes with an equal molar ferrous or ferric ions in solutions containing these ions and the complexes showed visible absorption bands with maxima at 517 nm (Fe^{2+}) and at 532 nm (Fe^{3+}), respectively. The other physico-chemical properties are summarized in Table 2, and ^1H and ^{13}C NMR data are listed in Tables 3 and 4, respectively.

Structural Elucidation

Structure of A

The molecular formula of A was established to be $C_{13}H_{13}N_3O_2S$ by high resolution EI-MS, ^1H and ^{13}C NMR spectral data. In the ^1H-^{13}C COSY spectrum, it was clarified that A possesses a methoxyl group (δ_H 4.12, δ_C 56.4) and a methylthio group (δ_H 2.38, δ_C 18.5), and that the other carbons consisting of A are all sp^2 carbon. The ^1H-^1H COSY spectrum of A indicated a sequence of 3′-H (δ 8.56), 4′-H (δ

7.87), 5'-H (δ 7.35) and 6'-H (δ 8.67) to lead to the presence of a 2-pyridyl group. Furthermore, as shown in Fig. 2, the heteronuclear multiple bond correlation spectroscopy (HMBC) and long range selective proton decoupling (LSPD) experiments revealed that **A** is composed of a tetra-substituted pyridine which is replaced by the 2-pyridyl, methoxyl, methylthio and a remaining groups at 2, 4, 5 and 6 positions, respectively. The remaining group was deduced to be an aldoxime group which means –CH=N–OH in this paper in view of the chemical shifts at 7 position (δ_H 9.11, δ_C 147.3), a presence of exchangeable proton (δ 10.79) with D_2O and of chemical conversion of **A** to **D** described later. In order to clarify the configuration of the aldoxime group and conformation for **A** in solution, NOE experiments were carried out. As a result, the strong NOE of 3-H/4-OCH_3, weak to moderate NOEs of 5-SCH_3/7-H and of 7-H/7-NOH, and no NOE of 3-H/3'-H were observed. Therefore, the aldoxime group had an *anti* configuration and the conformation of **A** in solution was deduced as shown in Fig. 2 containing a trans conformation of 2,2'-dipyridyl moiety.

Finally, the structure of **A** was confirmed to be (*E*)-((4-methoxy-5-methylthio-2-(2-pyridyl)pyridin-6-yl)methylene)azanol shown in Fig. 3 by a single-crystal X-ray diffraction analysis. The structure was solved by direct methods (MITHRIL)[1] and an ORTEP drawing of **A** is shown in Fig. 4. Antibiotic A structurally bears a close resemblance to caerulomycins A,[2~6] B,[7] C[7] and D[8] possessing a 2,2'-dipyridyl from *Streptomyces caeruleus*.

Structure of B

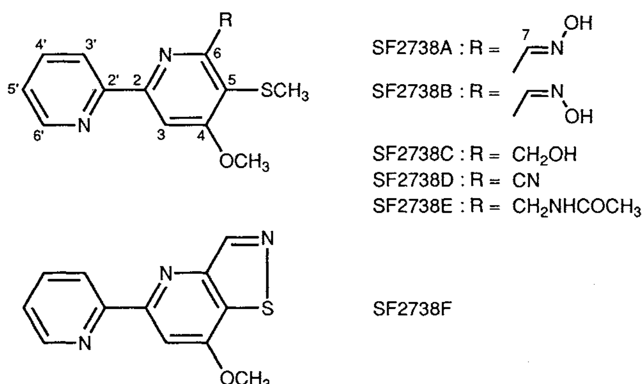
Antibiotic **B** was deduced to be an isomer of **A** by EI-MS, ^1H and ^{13}C NMR spectral data. As compared ^1H and ^{13}C NMR spectra and long range ^1H-^{13}C coupling pattern of **B** with those of **A**, it was made clear that the substitution pattern on pyridine ring of **A** is retained in **B**. Moreover, the large upfield shifts of 7-H (Δ 0.41 ppm), 3'-H (Δ 0.44 ppm) and C-7 (Δ 7.1 ppm) of **B** were observed due to a formation of hydrogen bond between 7-NOH (δ 16.74) and N^1 of the pyridine ring as shown in Fig. 2. The coupling constants ($^3J_{C\text{-}7,7\text{-}NOH}$) in **A** and in **B** were nearly 0 Hz and 6.9 Hz, respectively, indicating that the dihedral angle between C-7 and oxime proton in **B** is fixed at approximately 0° by hydrogen bonding. From these results, the structure of **B** was determined to be a geometrical isomer of **A** having the aldoxime of the *syn* configuration. In methanol solution at room temperature, **B** was converted gradually into **A**, but **A** was not.

Structures of C, D, E and F

Compounds **C**, **D**, **E** and **F** showed characteristic absorption bands in their IR and UV spectra consistent with a 2,2'-dipyridyl structure such as **A**, **B** and caerulomycins.[2,3,7,8] The NMR analyses of **C**, **D** and **E** have clarified that the structural difference of these compounds from **A** is only the substituent at 6 position. Antibiotic **C** had one methylene group (δ_H 4.95, δ_C 62.4) coupled to hydroxyl group (δ 4.77) instead of the aldoxime in **A**. Thus, the substituent at 6 position of **C** was determined to be a hydroxymethyl group.

In the case of **D**, the IR spectrum showed no band associated with a hydroxyl group, but indicated a weak absorption band at 2240 cm^{-1}. The substituent of **D** was calculated for 26 mass units by the EI-MS data, suggesting to be a nitrile or an isonitrile group. The chemical shift of this carbon in **D** was δ 116.5, so that the substituent was confirmed to be a nitrile group. Treatment of **A** with acetic anhydride under reflux produced **D** as a main product. The reaction is interpreted as the dehydration of an aldoxime to a

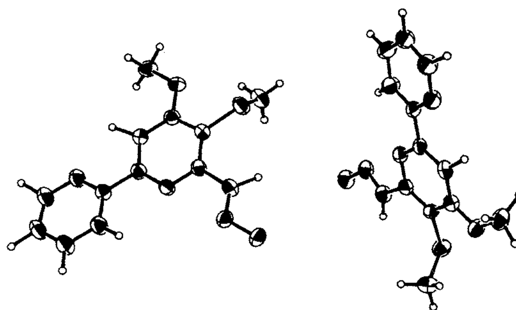
Fig. 3. The structures of SF2738.



nitrile.[4] This evidence supported the structure of **D**.

Compound **E** possessed one *N*- or *O*-acetyl group (δ_H 2.17, δ_C 23.5, 169.8) and one methylene group (δ_H 4.83, δ_C 43.3) coupled to an NH group (δ 7.25). Moreover, the IR spectrum showed an amide carbonyl stretching absorption at 1649 cm^{-1}. In the HMBC spectrum of **E**, the correlation peaks were observed between the methylene and acetyl protons and the carbonyl carbon. Therefore, the substituent of **E** was established to be an (acetylamino)methyl group. Chemical conversion of **A** to **C** or **E** will be reported in due course.

Fig. 4. Molecular structure of SF2738A in crystalline state.



By the NMR analysis of **F** using the same method as that of **A**, it was clarified that the structure of **A** except for the substituents at 5 and 6 positions was retained in **F**. Comparison of the ^1H and ^{13}C NMR spectra of **F** with those of **A** showed the absence of the protons and carbon for methylthio group, and of an exchangeable aldoxime proton. The remaining fragment having 59 mass units was estimated to be CHNS or $CHNO_2$. A C-7 of azomethine function (–CH=N–, δ_H 9.13, δ_C 156.2) should be attached to C-6 (δ 154.2) because of the observation of correlation peaks between 7-H/C-5 (δ 136.1) and C-6, and between 3-H/C-5 in the HMBC spectrum of **F**. The chemical shift of the C-5 indicated that an atom connecting to C-5 is not oxygen, but sulfur. Accordingly, the structure of **F** was deduced to be 7-methoxy-5-(2-pyridyl)-isothiazolo[4,5-*b*]pyridine shown in Fig. 3. On the other hand, treatment of **A** with acetic anhydride gave not only **D** (61%), but also **F** (34%). When heated with formic acid, **A** was converted to **F** in a good yield (90%). This reaction has been suggested to involve the nucleophilic attack to oxime nitrogen by a lone pair electron of sulfur for methylthio group, followed by the elimination of CH_3COO^- or OH^- for oxime and of CH_3^+ for methylthio group. These evidences support the structure of **F**.

Biological Activities

The MICs of **A** against bacteria and fungi are shown in Tables 5 and 6, respectively. Antibiotic **A** exhibited weak antibacterial and antifungal activity, however, showed broad antifungal spectrum against a wide range of fungi. Also, **A** was not active against *Staphylococcus aureus* 209P JC-1, *S. aureus* No. 26,

Enterococcus faecium ATCC8043, *Escherichia coli* JC-2, *Salmonella typhimurium* LT-2, *Klebsiella pneumoniae* 22#3038 and *Serratia marcescens* MB-3848 at a concentration of 100 μg/ml. As shown in Table 7, **B** showed somewhat less potent against bacteria and fungi tested than **A**. Antibiotic **C** had poor antimicrobial activity. Antibiotics **A**, **B** and **C** displayed cytotoxic activity on P388 murine leukemia cells with IC_{50} values of 0.08, 0.25 and 7.5 μg/ml, respectively. The complexes of **A** with Fe^{2+} and Fe^{3+} possessed no antimicrobial activity, whereas the cytotoxic activity of the complexes was equal to that of **A**. On the other hand, compounds **D**, **E** and **F** showed no antimicrobial activity and no cytotoxicity on P388 murine leukemia cells. When tested in mice by intraperitoneally administration of 100 mg/kg, **A** showed no signs of acute toxicity.

Table 5. Antibacterial activity of SF2738A.

Test organism	MIC (μg/ml)
Staphylococcus aureus Smith S-424	100
S. epidermidis ATCC 14990	100
S. epidermidis 109	100
Bacillus anthracis No. 119	6.25
Escherichia coli No. 29	100
E. coli W3630 RGN823	25
E. coli JR66/W677	100
Citrobacter freundii GN346	50
Salmonella typhi O-901-W	12.5
S. enteritidis No. 11	50
Shigella sonnei EW33 Type 1	25
Klebsiella pneumoniae PCI 602	100
Proteus vulgaris OX19	100
P. mirabilis GN310	100
Providencia rettgeri J-0026	25
Morganella morganii Kono	100
Pseudomonas aeruginosa MB-3829	25
P. cepacia M-0527	25
Xanthomonas maltophilia M-0627	25

MICs were determined by agar dilution method: Sensitivity disk agar-N (Nissui), 10^6 cfu/ml, 37°C, 20 hours.

Experimental

General Procedure

UV and IR spectra were recorded on a

Table 6. Antifungal activity of SF2738A.

Test organism	MIC (μg/ml)
Saccharomyces cerevisiae X2180-1A	12.5
Candida albicans TIMM 1768	50
C. albicans C-a-24	50
C. glabrata IFO 0005	25
C. glabrata IFO 0622	25
C. tropicalis IFO 0589	25
C. guilliermondii IFO 1972	50
C. krusei IFO 0584	25
C. parapsilosis IFO 0585	100
Cryptococcus neoformans Cr-1	25
C. neoformans IMC F-10	12.5
Aspergillus fumigatus Saito	25
A. fumigatus TIMM 1775	12.5
Tricophyton mentagrophytes TIMM 1189	12.5

MICs were determined by agar dilution method: Sabouraud agar, $10^5 \sim 10^6$ cfu/ml, 27°C, 72 hours.

Table 7. Antimicrobial activities of SF2738A, B and C.

Test organism	A	B	C
Staphylococcus aureus 209P	(16.7)	(24.8)	(18.8)
Bacillus subtilis ATCC 6633	(17.4)	(13.5)	—
Micrococcus luteus ATCC 9341	18.3	23.4	11.5
Escherichia coli NIHJ	16.2	—	—
E. coli K12 YW007C	21.6	(11.8)	—
Saccharomyces cerevisiae SHY3	16.2	13.6	(15.0)
Candida albicans M9001	(10.7)	(14.3)	—
C. pseudotropicalis M9035	14.8	(10.5)	—
Cryptococcus neoformans M9010	12.2	16.0	—
Debaryomyces hansenii M9011	(13.2)	(12.0)	—
Trigonopsis variabilis M9031	15.2	14.3	—
Schizosaccharomyces pombe M9025	11.6	—	—
Hansenula schneggi IAM4269	(17.0)	(15.7)	—

Samples (20 μg) were applied on 8 mm paper disk. Values represent diameters (mm) of inhibitory zone. Parentheses indicate a faint inhibitory zone.

Shimadzu UV-260 and a Shimadzu FTIR-8100 spectrophotometers, respectively. ^1H and ^{13}C NMR spectra in $CDCl_3$ were recorded on a Jeol JNM-GSX400 spectrometer using TMS as an internal standard. Mass spectra were recorded on a Hitachi M-80B mass spectrometer. MP's were determined on a Yanaco MP-S3 micro melting point apparatus and were uncorrected. TLC was done on a Kieselgel 60 F_{254} plate (Merck, Art. No. 5715).

Single-Crystal X-Ray Diffraction Analysis of **A**

A colorless prismatic crystal of SF2738A having approximate dimensions of $0.2 \times 0.2 \times 0.2$ mm was mounted on a glass fiber. All X-ray measurements were made on a Rigaku AFC5R diffractometer with graphite monochromated CuK_α radiation and a 3 KW rotating anode generater. Cell constants and an orientation matrix for data collection were obtained from a least-squares refinement using the setting angles of 22 carefully centered reflections in the 2θ range of $61.7° \sim 96.3°$.

The crystal system was monoclinic and the space group was determined to be C2/c (♯15) with unit cell dimensions: $a=43.580(3)$ Å, $b=5.937(1)$ Å, $c=21.834(3)$ Å, $\beta=107.280(8)°$, $V=5394(1)$ Å^3, $Z=16$. The data were collected at 23°C using the ω-2θ scan technique to a maximum 2θ value of 120.1°. Omega scans of several intense reflections made prior to data collection, had an average width at half-height of 0.23° with a take-off angle of 6.0°. Scans of $(1.25+0.30\tan\theta)°$ were made at a speed of 16.0°/minute in omega. The weak reflections ($I<10\sigma$) were rescanned twice and the counts were accumulated to assure good counting statistics. Stationary background counts were recorded on each side of the reflection. The ratio of peak counting time to background counting time was 2:1. Of the 4603 reflections which were collected, 4471 reflections were unique (Rint=0.026), equivalent reflections were merged. The linear absorption coefficient for CuK_α is 21.1 cm^{-1}. Azimuthal scans of several reflections indicated no need for an absorption correction. The data were corrected for Lorentz and polarization effects.

The structure was solved by direct methods which were given in the MITHRIL[1)] software package. The cell contained two independent molecules located at asymmetric position. The non-hydrogen atoms were refined anisotropically including hydrogen atoms in calculated position. The final cycle of full-matrix least-squares refinement was based on 2118 observed reflections ($I>3.00\sigma$) and 439 variable parameters and converged with an R value of 0.061. The standard deviation of an observation of unit weight was 2.17. The weighting scheme was based on counting statistics and included a factor ($p=0.03$) to downweight the intense reflections. The maximum and minimum peaks on the final difference Fourier map corresponded to 0.30 and -0.37 e^-/Å^3, respectively.

Conversion of **A** to **D** and **F**

A solution of **A** (51.0 mg, 0.185 mmol) in acetic anhydride (2 ml) was refluxed for 2 hours. To the reaction mixture was added 5 ml of methanol and stirred for 30 minutes at room temperature, and then concentrated to dryness. The residue was purified by preparative TLC (Merck, Art. No. 5744) developed with chloroform - acetone (4:1) to give **D** (29.1 mg) in 61.1% yield and **F** (15.3 mg) in 34.0% yield. The spectral data of synthetic **D** and **F** were identical with those of natural **D** and **F**, respectively.

Conversion of **A** to **F**

A solution of **A** (35.0 mg, 0.127 mmol) in formic acid (1 ml) was refluxed for 3 hours, and then concentrated to dryness. The residue was purified by preparative TLC (Merck, Art. No. 5744) developed with chloroform - acetone (4:1) to give **F** (27.9 mg) in 90.2% yield.

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