BU-4704, A NEW MEMBER OF THE XANTHOCILLIN CLASS

MITSUAKI TSUNAKAWA, NORIYUKI OHKUSA, SEIKICHI KOBARU, YUKIO NARITA, SHINJI MURATA, YOSUKE SAWADA and TOSHIKAZU OKI

Bristol-Myers Squibb Research Institute, 2-9-3 Shimo-meguro, Meguro-ku, Tokyo 153, Japan

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Aspergillus sp. No. FA2692, isolated from a bark of *Quercus mongolica* collected in Shiga-kogen, Nagano Prefecture, Japan, was found to produce a new antibacterial and antifungal compound designated as BU-4704. The structure of BU-4704 was determined to be 1-(4-methoxyphenyl)-4-(4-hydroxysulfonyloxyphenyl)-2,3-diisocyano-1,3-butadiene, the xanthocillin class of antibiotics[1~3]. This paper deals with the structure and antimicrobial activity of BU-4704.

BU-4704 was produced in 500-ml Erlenmeyer flasks using medium composed of mannitol 1.0%, corn meal (Sakura meal Co.) 2.0%, peptone (Mikuni Kagaku Co.) 0.5%, $CaCO_3$ 0.3% and NaCl 0.2%, pH 7.0. The flasks were shaken on a rotary shaker (200 rpm) at 28°C for 5 days. The antibiotic activity in the fermentation broth was estimated by the paper disc-agar plate method using *Bacillus subtilis* PCI 219 as the test organism. The fermentation broth (20 liters) was extracted with 10 liters of butanol. The BuOH was removed by evaporation *in vacuo* and the aqueous concentrate was applied on a column of Diaion HP-20 (600 ml). The column was washed with 1 liter of water and 2 liters of methanol-water (1:1). The active material was then eluted with 3 liters of acetone-water (4:1). The active eluate was evaporated *in vacuo* to afford a crude solid of BU-4704 (10.2 g). As BU-4704 was labile to light, all subsequent operations were conducted in a dark room. The solid was charged on a silica gel column (600 ml) developed with ethyl acetate-methanol (9:1~3:1). The active fractions were combined and concentrated to give a semi-pure sample (1.14 g). The material (380 mg) was purified by reversed phase silica gel chromatography (YMC-gel, ODS-60, AM type, 800 ml) developed with methanol-0.15% KH_2PO_4 (7:3, pH 3.5). The active eluate was kept standing overnight at 5°C yielding crystalline BU-4704 monopotassium salt.

BU-4704 monopotassium salt was obtained as off-white needles (MP 165~170°C (dec.)) and optically inactive. It was readily soluble in DMSO, slightly soluble in methanol and ethyl acetate but practically insoluble in chloroform, ether and water. It gave positive reactions to iodine and sulfuric acid, but negative to ninhydrin. Upon exposure to fluorescent light at room temperature, it was gradually inactivated because of structure change as determined by UV spectrum and HPLC analysis. It showed a single spot at Rf 0.23 (CH_2Cl_2-MeOH, 6:1) and 0.37 (EtOAc-MeOH, 6:1) on a silica gel TLC plate. The molecular formula of BU-4704 salt was determined to be $C_{19}H_{13}N_2O_5SK$ based on the elemental analysis (Calcd for $C_{19}H_{13}N_2O_5SK$: C 54.29, H 3.10, N 6.67, S 7.62. Found: C 54.41, H 3.20, N 6.84, S 7.33) and high-resolution negative FAB-MS (Calcd for $C_{19}H_{13}N_2O_5S$ *m/z* 381.0545 $(M-H)^-$, Found *m/z* 381.0544). The UV spectrum of BU-4704 salt showed absorption maxima at 232 nm (ε 17,200), 283 (sh, 11,000) and 356 (61,900) in methanol and no shift was observed at acidic and alkaline pHs. The IR spectrum (KBr) showed absorption bands of isonitrile (2120 cm^{-1})[1], aromatic (1610 and 1510 cm^{-1}) and sulfate (1300~1200 cm^{-1})[4] groups. The ^{13}C NMR (Table 1) showed 14 carbon

Table 1. ^{13}C (100 MHz) and ^1H NMR (400 MHz) spectra of BU-4704 in DMSO-d_6.

Carbon No.	Chemical shift (ppm)	
	δ_C	δ_H
1	128.0 (d)	7.20 (1H, s)
2	115.4 (s)	
3	116.2 (s)	
2,3-NC	172.9 (s)	
4	127.7 (d)	7.18 (1H, s)
1′	124.3 (s)	
2′,6′	131.7 (d)	7.87 (2H, d, J=9.0 Hz)
3′,5′	114.5 (d)	7.10 (2H, d, J=9.0 Hz)
4′	160.8 (s)	
4′-OCH_3	55.4 (q)	3.84 (3H, s)
1″	126.3 (s)	
2″,6″	130.8 (d)	7.80 (2H, d, J=9.0 Hz)
3″,5″	120.1 (d)	7.31 (2H, d, J=9.0 Hz)
4″	155.3 (s)	

Correspondence should be addressed to JUN OKUMURA, Bristol-Myers Squibb Research Institute, 2-9-3 Shimo-meguro, Meguro-ku, Tokyo 153, Japan.

Fig. 1. Structure of BU-4704.

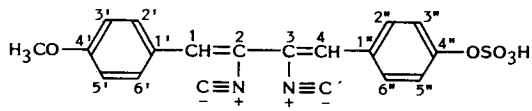

Table 2. Antimicrobial spectrum of BU-4704.

Test organism	MIC (μg/ml)
Staphylococcus aureus FDA 209P JC-1	<0.1
S. aureus Smith	1.6
Bacillus subtilis ATCC 6633	<0.1
Escherichia coli Juhl	1.6
Klebsiella pneumoniae ATCC 10031	0.4
Pseudomonas aeruginosa A9843A	>50
Candida albicans A9540	>50
Cryptococcus neoformans D49	3.1
Trichophyton mentagrophytes no. 4329	25

signals with 5 signals at δ 172.9, 131.7, 130.8, 120.1 and 114.5 being almost twice more intensive than the other signals. The ^{1}H NMR (Table 1) reveals the presence of two sets of *para*-substituted benzene rings (H-2′, 3′, 5′, 6′ and H-2″, 3″, 5″, 6″), two isolated olefin protons (H-1 and H-4) and a *O*-methyl group (C-4′). The analytical and spectroscopic data suggested that the structure of BU-4704 was closely related to that of xanthocillin monomethyl ether[1], except for the presence of a sulfate group in the former and a hydroxyl group in the latter. In the long range ^{1}H-^{13}C COSY experiments, a quaternary low-field sp^2 carbon (δ 160.8, C-4′) possessed correlations to a O–CH_3 (δ 3.84), H-2′ and H-6′ (δ 7.87) which in turn coupled to C-1 (δ 128.0). Another low-field carbon (δ 155.3, C-4″) was connected to H-2″ and H-6″ (δ 7.80) which coupled to C-4 (δ 127.7). On the basis of these findings, the structure of BU-4704 was deduced to be 1-(4-methoxyphenyl)-4-(4-hydroxysulfonyloxyphenyl)-2,3-diisocyano-1,3-butadiene (Fig. 1).

The antimicrobial spectrum of BU-4704 is shown in Table 2. MICs were determined by the agar dilution method using nutrient agar medium (Eiken Kagaku Co.) for aerobic bacteria and yeast morphology agar for fungi. The inoculum size was adjusted to 10^{-5} cells/ml for aerobic bacteria and 10^{-6} cells/ml for fungi. BU-4704 strongly inhibits the growth of Gram-positive and -negative bacteria and fungi.

BU-4704 showed potent cytotoxic activity against human colon carcinoma HCT-116 and murine melanoma B16-F10 cells with the IC_{50} values of 0.63 μg/ml and 4.3 μg/ml, respectively.

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