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A NEW ANTITUMOR AGENT : METHYL SULFONIUM PERCHLORATE OF ECHINOMYCIN

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Abstract: Newly modified-echinomycin such as S-methylated sulfonium perchlorate of echinomycin (**1**), monosulfoxide (**2**), disulfoxide (**3**) and sulfone (**4**) have been prepared and evaluated for *in vitro* biological activities of cytotoxicity against P388, B16 and SNU-16 as well as *in vivo* antitumor activity against murine leukemia P388 and melanoma B16.

Soil fungi are known to be one of the richest sources for antitumor antibiotics. Particularly *Streptomyces* has been reported to be the excellent sources for developing new tumor cell growth inhibitor. To search biologically active compounds, *Streptomyces sp.* was isolated at Chiak mountain area and cultured. A potent antitumor compound has been isolated and purified to elucidate its structure : ca. 500 mg of echinomycin (**5**) was isolated from 50 L of cultured broth. The compound has been identified as echinomycin (**5**).[1] Echinomycin is one of the leading candidates for antitumor antibiotics isolated from the cultured broth of *streptomyces sp.*.[2,3] A large molecule **5** containing two quinoxaline moieties connected with an octapeptide bridge has been thought to react as a bifunctional intercalator with DNA.[4,5] It shows a potent antitumor activity and unique action mechanism such as apoptosis, cellular signaling inhibition.[6,7] In recent years, echinomycin has been tested in phase II trial[8] for various cancer treatment.

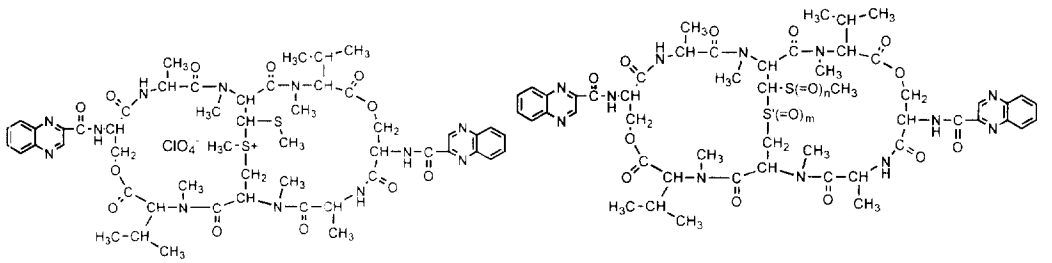

Fig.1. Structure of 1, 2, 3, and 4

2. $S(=O)_n$; n=1, $S'(=O)_m$, m=0
3. $S(=O)_n$; n=1, $S'(=O)_m$, m=1
4. $S(=O)_n$; n=2, $S'(=O)_m$, m=0

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However, a problem to be improved for clinical trial has been raised to broaden the narrow therapeutic margin as well as to reduce the toxicity. To overcome these limitations an assumption that the more polar echinomycin analogues might improve their solubility and broaden the narrow therapeutic margin as well as to reduce the toxicity by echinomycin let us prepare more polar modified echinomycin. Various modified-echinomycin such as methyl sulfonium perchlorate (**1**), monosulfoxide (**2**), disulfoxide (**3**), and sulfone (**4**) were synthesized[9] and subjected to evaluate *in vitro* cytotoxicities.[10] Their structure were elucidated by comparing their ^{1}H NMR, ^{13}CNMR, 2D NOESY, IR and Mass spectra with those from **5**.[9]

Table **1**. *In vitro* Cytotoxicity of Modified Echinomycin on Mouse and Human Tumor Cell Lines

Compound	IC_{50}(μ g/ml)[a] of Tumor cell lines		
	P388	B16	SNU16
Me-sulfonium (**1**)	8.1×10^{-6}	9.0×10^{-5}	4.9×10^{-6}
sulfoxide (**2**)	8.8×10^{-3}	3.1×10^{-1}	5.4×10^{-1}
disulfoxide (**3**)	9.8×10^{-2}	1.7×10^{0}	5.9×10^{-1}
sulfone (**4**)	6.0×10^{-2}	8.2×10^{-1}	1.1×10^{0}
echinomycin (**5**)	4.3×10^{-5}	6.5×10^{-8}	1.6×10^{-7}
adriamycin	9.6×10^{-3}	5.4×10^{-1}	1.6×10^{-1}

[a] IC_{50} was defined as the concentration that caused 50% inhibition of cell growth

With the modified echinomycin derivatives, their *in vitro* anticellular activities as well as *in vivo* antitumor activity were evaluated against P388 murine leukemia. This results were shown in Table **1**. Particularly **1** exhibits the most excellent *in vitro* cytotoxicity on P388 leukemic cells. But, the IC_{50} values of **1** against B16 mouse melanoma and SNU-16 human gastric cancer were lower than those of echinomycin. Prior to *in vivo* experiment using modified echinomycin derivatives, we examined the *in vivo* therapeutic dose-range. The compound **1** increased the life time of P388 leukemic mice at the range of test doses (0.03 mg/kg - 1 mg/kg). Besides, the compound **1** within the therapeutic dose (0.06 mg/kg - 0.5 mg/kg) revealed comparable *in vivo* antitumor activity B16 tumor bearing mice, as compared with those in echinomycin-treated group (Table **2**). Interestingly, LD_{50} value (1.05 mg/kg) of the compound **1** was at least seven times higher than that (0.15 mg/kg) of echinomycin (**5**).

Tables **2**. *In vivo* Antitumor Activity of Modified Echinomycin on Mouse Tumor bearing Mice

Compound	N[a]	P388 (ip-ip)[b]		B16 (ip-ip)[b]	
		mg/kg	MST[c] ± S.D (Day)	mg/kg	MST[c] ± S.D (Day)
control	10		26 ± 2		18 ± 3
Me-sulfoninium (**1**)	8	1	35[d] ± 12	1	28[d] ± 13
	8	0.25	34[d] ± 12	0.25	26[d] ± 2
	6	0.125	33[d] ± 7	0.125	25[d] ± 3
	6	0.0625	32 ± 3	0.0625	24[d] ± 5
	6	0.03125	31 ± 4	0.03125	23 ± 2
sulfoxide (**2**)	8	1	27 ± 4	1	20 ± 4
disulfoxide (**3**)	8	1	31 ± 1	1	17
sulfone (**4**)	8	1	25 ± 5	1	17
echinomycin (**5**)	6	0.1	27 ± 4	0.1	21 ± 2
	8	0.05	26 ± 5	0.05	23[d] ± 6
	8	0.025	33 ± 3	0.025	25[d] ± 5
	8	0.0125	32 ± 2	0.0125	24[d] ± 2
	6	0.00625	31 ± 2	0.00625	23 ± 2

[a]N = the numbers of mice

[b]Mice (BDF1) were implanted intraperitoneally (i.p) with tumor cells (day 0) and the drug was administered (mg/Kg) i.p daily (day 1 - 9).

[c]MST[1] = the mean survival day of treatment mice.

[d]P < 0.05 (significant difference between the experimental and the control groups) by Kaplan-Meier method

These data showed that the monomethylated sulfonium salt (**1**) was highly as effective against murine leukemia and melanoma as **1** with broader range of therapeutic doses. Recently, we observed the efficacy of **1** using KHH (human gastric cancer)-xenograft assay (data not shown). The cell cycle patterns and apoptotic conditions induced by **1** were quite different as compared to those of echinomycin (data not shown). Further studies on antitumor spectra and the mechanism of action (cell cycle, apoptosis) of **1** are in progress.

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References and Notes

1. a) Del, A.; Williams, D. H.; Morris, H. R.; Smith, G. A.; Feeney, J.; Robert, G. C. K.; *J. Am. Chem. Soc.*,

1975, *97*, 2497. b) Cheung, H. T.; Feeney, J.; Robert, G. C. K.; Williams, D. M.; *J. Am. Chem. Soc.*, **1978**, *100*, 46. The **5** was identified by comparing its ^{1}H NMR, ^{13}C NMR and MS spectral data with those from references.

2. Otsuka, H.; Shoji, J.; *J. Antibiotics Ser. A*, **1966**, *14* (3), 128.
3. Stephen, N.; Zelda, A.; *CRC Critical Reviews in Biochem.*, **1987**, *17*, Issue 1, 73.
4. Waring, M. J.; Wakelin, L. P. G.; *Nature*, **1974**, *252*, 653.
5. Gilbert, D. E.; Feigon, J.: *Nucleic Acids Research*, **1992**, *20* (10), 2411.
6. Kim, T. U.; Yang, S. H.; Kim, S. K.; *J. Biochem. Mol. Bio.*, **1996**, *29* (6), 489-492.
7. Kim, S. K.; Ahn, C. M.; Kim, T. U.; Choi, S. J.; Park, Y. S.; Shin, W. S.; Koh, C. M.; *Arch Pharm. Res.*,**1996**, *19* (4), 261.
8. Chang, A. Y.; Tu, I. N.; Bryan, G. T.; *Investigational New Drugs*, **1994**, *12*, 151.
9. Preparation of **2**, **3**, and **4**. Echinomycin (110 mg, 0.1 mmol) was dissolved in 7 ml of dry CH_2Cl_2. m-CPBA (23 mg, 0.13 mmol) was slowly added at 0℃. After stirring for 24 h at room temperature, the reaction mixture was poured into water (10 ml), extracted with dichloromethane (10 ml x 3), washed with water and brine, dried over $MgSO_4$, and than concentrated to give a crude mixture of products (70 mg). Preparative TLC (silicagel, 20 x 20 cm) gave a mixture of **2**, **3**, and **4**. The further purification with prep-HPLC (ODS column ; 2 x 30 cm, eluent ; MeCN/H_2O=70/30) gave pure **2** [8 mg, ^{1}H NMR ($CDCl_3$) δ : 2.43 (s, 3H, -SO-Me, cf. 2.0 in **5**), FT-IR (KBr, cm^{-1}) 1036 (-SO-), ESI-MS (m/z) 1117 $(M+H)^+$], **3** [8 mg, ^{1}H NMR ($CDCl_3$) δ : 2.58 (s, 3H, -SO-Me, cf. 2.0 in **5**), 3.45, 3.30(d, 2H, -CH_2SO-, cf. 3.36, 2.80 in **5**), ESI-MS (m/z) 1133 $(M+H)^+$], **4** [21 mg, ^{1}H NMR ($CDCl_3$) δ : 2.9 (s, 3H, -SO2-Me, cf. 2.0 in **5**), FT-IR (KBr, cm^{-1}) 1261 (-SO2-), ESI-MS (m/z) 1133 $(M+H)^+$].
Preparation of **1**. Echinomycin (20 mg, 0.018 mmol) was dissolved in $ClCH_2CH_2Cl$ (1 ml) under N_2. $AgClO_4$ (4.5 mg, 0.02 mmol) and CH_3I (40 mg) were slowly added. After stirring for 24 h at 25℃, AgI was removed by centrifugation. Concentration, followed by washing with ether (2 ml x 4) and $CHCl_3$ (2 ml x 3) respectively resulted in solid **1**, which was dissolved in MeCN, dried over $MgSO_4$, concentrated to give pure **1** (20 mg, 90%). Further purification with prep-HPLC (JAI GS-310 gel column, 2 x 50 cm, eluent ; MeCN/H_2O=70/30, ODS column, 1 x 30 cm, eluent ; CH_3CN) gave **1** [14 mg, 70%, ^{1}H NMR (CD_3CN,) δ : 2.07 (s, 3H, S-CH_3, cf. 2.0 in **5**), δ : 2.17 (s, 3H, S^+-CH_3, Me sulfonium), FT-IR (KBr, cm^{-1}) 1094, 1120, 1517, 1651, positive-mode ESI-MS (m/z) 1115 $(M+H)^+$ Calcd. for $C_{52}H_{67}N_{12}O_{12}S_2$ 1115, negative-mode ESI-MS (m/z) 102 Calcd.for ClO_4^- 101. In the NOESY spectrum of **1**, the obvious NOEs between S^+-CH_3(δ 2.17) and $S^+(CH_3)$-CH_2-(δ 3.43, 2.88) were observed. All the other protone of **1** are in accordance with those of **5** except the slight chemical shifts to the down field. The purity (ca. 96%) of **1** was determined by ^{1}H NMR and HPLC (ODS column, eluent ; MeCN/H_2O=70/30).
10. Each cancer cell line of P388 (leukemia, mouse), B16 (melanoma, mouse), and SNU16 (gastric cancer, human) was maintained in RPMI 1640 medium supplemented with 10% fetal calf serum and incubated in a humidified 5% CO_2 chamber at 37℃ (modified MTT method[6,7]). Monocellular suspension was seeded at 10^4 cells per well in 96 well plates with 100 μl of medium per well. To compare cytotoxicity between echinomycin derivatives and cytotoxic agent (adriamycin), such compounds were added at varying concentrations and cultures were incubated for 72 h in an incubator maintaining a highly humidified atmosphere, 5% CO_2 and 95% air. Fifty μl of the medium containing MTT (5 mg/ml) were added to each well. After 4h of exposure, the medium was partly decanted and the wells were washed with PBS, and then 150 μl of DMSO were added to each well to solubilize the precipitates. The plates were transferred to an ELISA reader to measure absorbance at 570 nm with a reference wave length, 630 nm. IC_{50} value, 50% inhibition of cell growth, was calculated by regression analysis (plotting the viablity versus the concentration of the test compound) using Graphpad Prism 2.0 (Graph Pad Software, Inc.). All experiments were done at least 3 times, with 6 wells for each concentrations of test agents.
11. Geran, R. I,; Greenberg, N. H.; Macdonald, M. M.; Schmacher, A. M.; *Abott, Cancer. Chemother. Rep.*, **1972**, part III *3*, 1.