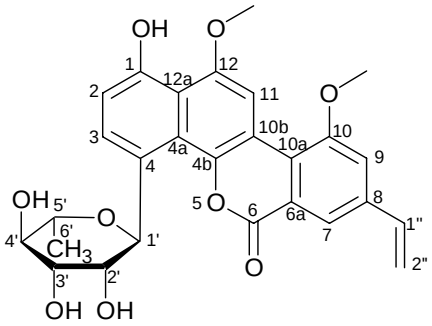
Electronic Supplementary Information for

Plasticity in Gilvocarcin-Type C-Glycoside Pathways: Discovery and Antitumoral Evaluation of Polycarcin V from *Streptomyces polyformus*

Yi-qing Li,[a,b,c] Xue-shi Huang,[a,b] Keishi Ishida,[b] Armin Maier,[d] Gerhard Kelter,[d] Yi Jiang,[c] Gundela Peschel,[b] Klaus-Dieter Menzel,[b] Ming-gang Li,[c] Meng-liang Wen,[c] Li-hua Xu,[c] Susanne Grabley,[b] Heinz-Herbert Fiebig,[d] Cheng-lin Jiang,[c] Christian Hertweck*[b,e] and Isabel Sattler*[b]

[a] *Authors contributed equally to this work.*
[b] *Leibniz Institute for Natural Product Research and Infection Biology, Hans-Knöll-Institute (HKI), Beutenbergstr. 11a, D-07745 Jena, Germany. E-mail: isabel.sattler@hki-jena.de*
[c] *The Key Laboratory for Microbial Resources of the Ministry of Education, Yunnan Institute of Microbiology, Yunnan University, Kunming 650091, China*
[d] *The Oncotest GmbH, Institut für Experimentelle Onkologie, Am Flughafen 12-14, D-79108 Freiburg, Germany*
[e] *Friedrich-Schiller-University Jena, Germany*



1

Experimental

General Procedures

Optical rotations were recorded on a Perkin-Elmer 241 polarimeter. IR spectra were recorded with a Perkin-Elmer model 298 spectrometer (KBr, disks). 1D NMR spectra (^{1}H, ^{13}C) were recorded on a Bruker DPX-300 spectrometer; 2D NMR spectra (COSY, HMBC, HMQC, NOESY) on a Bruker DRX-500 spectrometer. HRESI-MS spectra were recorded with the AMD-402 instrument of Be geometry equipped with direct inlet system (AMD Intectra). ESI-MS spectra were recorded by use of a Quattro triple quadrupole mass spectrometer (VG Biotech, Altrincham, England). HPLC/UV/MS analysis was performed on a Agilent 1100 series intrument equipped with binary pump, degasser, sample injector, column thermostat, diode array detector and LC/MSD trap MS detector. The HPLC was eqipped with a Agilent Zorbax Eclipse XDB C8 column (150 × 4.6 mm) and run with a methanol/water (0.1% formic acid) gradient (%MeOH: 0.5’ 10%, 15’ 90%, 17’ 90%, 17.5’ 100%, 22’ 100%, 23’10%, 25’ 10%). Sugar analysis was done on a Agilent 1100 HPLC system like above, but run with an MSD detector and with an Eurosphere 100-5C18 column (150 × 3.0 mm) run on an acetonitrile/water (0.1% formic acid) gradient (1 mL/min flow rate; acetonitrile: 0’ 10%, 30’ 99%,

30.1' 10%). TLC was performed on silica gel plates (Macherey-Nagel, Sil G/UV_{254}, 0.20 mm); spots were detected under a UV lamp and after staining with anisaldehyde/H_2SO_4. TLC was performed on DC Alugram SIL G/UV 254 (0.25 mm, Macherey-Nagel GmbH&Co, Germany), and staining reagents were prepared according to standard laboratory procedures. Staining was achieved through spraying onto the plate and subsequent heating. Chromatography was done on silica gel 60 (0.040-0.063 mm, Merck KgaA, Germany). PITC and L-cystine dimethyl ester were obtained from Sigma Aldrich. D-Cystine dimethyl ester was obtained from Bachem. All solvents were of gas-distilled grade.

Strain, cultivation and isolation

The producing strain YIM 33176 was isolated from a soil sample collected from the North of Vietnam and identified as a *Streptomyces polyformus* sp. nov. (as yet unvalidly published, Y. Jiang, W.J. Li, W.L. Wu, Y.X. Peng, L.F. Li, H.M. Yang, L.H. Xu and C.L. Jiang, *J. Yunnan University*, 2004, **26**, 215.). A slant culture of the strain was inoculated into 300 mL-Erlenmeyer flasks containing 100 mL of seed medium composed of yeast extract 0.4%, glucose 0.4%, malt extract 0.5%, 1 mL complex vitamins, pH 7.2 and cultured for 2 days at 28°C on a rotary shaker at 180 rpm. 3200 mL of seed culture were transferred into a 300 L-scale fermentor filled with 100 L of the medium composed of soybean flour 2%, mannitol 2%, pH 7.2 and cultured for 6 days at 28°C with reciprocal shaking at 500 rpm for upscale fermentation. The cultured broth (100 L) was filtered and the mycelium was lyophilized and eluted with MeOH. After filtration and concentration the extract was diluted with water and re-extracted with ethylacetate. After concetration, 17.4 g of crude product were obtained. This material was applied to silica gel column chromatography (40 × 790, gradient $CHCl_3$~$CHCl_3$/MeOH = 9:1) and yielded 880 mg of semi-pure material. Additional silica gel column chromatography (30 × 500, gradient $CHCl_3$~$CHCl_3$/MeOH = 9:1) yielded 87 mg of compound **1** and 63 mg of compound **2** as a yellow amorphous powder.

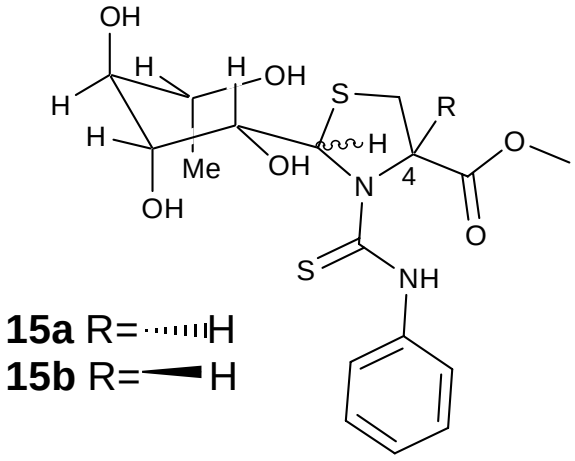
Antitumor test

Effects of the test compounds on the proliferation of human tumor cells were determined in a monolayer assay of human tumor cell lines treated with the test compounds; surviving cells were stained with a fluorescent dye. For details of the test procedure see: A.W. Dengler, J. Schulte, D.P. Berger, R. Mertelsmann, H.H. Fiebig. Development of a propidium iodide fluorescence assay for proliferation and cytotoxicity assays. *Anti-Cancer Drugs* 1995, **6**, 522. Cell lines were obtained from ATCC (Rockville, MD USA), the National Cancer Institute (Bethesda, MD, USA), DSMZ (Braunschweig, Germany), or were derived from patient tumors engrafted as a subcutaneously growing tumor in NMRI nu/nu mice. Doubling times of the cell lines ranged between 20 h +/- 1 h (CNXF 498NL) and 36 h +/- 2 h (OVXF 899L). Antitumor efficacies were described by inhibitory concentrations (i.e., IC_{70}), reflecting concentration-dependent cytotoxicity. Mean graph analysis and COMPARE analysis were carried out as described in H.H. Fiebig, T. Metz, E. Tetling, R. Vollmer, A. Korrat, N. Bausch, G. Kelter, *Proc. Am. Assoc. Cancer Res.* 2005, **46**, 3967.

Absolute stereochemistry of α-L-rhamnose moiety of polycarcin V (1).

L-Rhamnose (7.3 mg), **L**- or **D**-cysteine methyl ester (6.3 mg), and dithiothreitol (11.4 mg) were dissolved in pyridine (0.65 mL) and reacted at 60 °C for 1 h. To this reaction mixture, PITC (5.0 µL) was added and again heated at 60 °C for 1 h. The reaction mixture was afterwards directly analysed by

HPLC/MS . Polycarcin V (**1**, 0.5 mg) was dissolved in methanol (2.0 mL) containing 10% HCl and refluxed overnight. After the solvent was removed by evaporation and lyophilization, L-cysteine methyl ester (0.6 mg), and dithiothreitol (1.1 mg) were added to the residue and reacted at 60 °C for 1 h. To this reaction mixture, PITC (2.0 μL) was added and heated at 60 °C for 1 h. The retention time of the single ion peaks at *m/z* 417 (pos. mode) for the standards (3-phenylthiocarbamoyl-2-(1,2,3,4-tetrahydroxy-pentyl)-thiazolidine-4-carboxylic acid methyl ester ($C_{17}H_{24}N_2O_6S_2$, 416.11 g/mol) were 8.09 min (4*R*-D-cysteine methyl ester-PITC derivative, **15a**) and 10.09 min (4*S*-L-cysteine methyl ester-PITC derivative, **15b**). The L-cysteine methyl ester-PITC derivative of the acid hydrolysate was 10.12 min.



Polycarcin V: yellow amorphous powder; $[\alpha]_{22}^{D}$ –79° (*c* 0.4, MeOH); UV (MeOH) λ_{max} 207 (32 810), 245 (43 580), 277 (32 660), 391 nm (12 200); IR (film): 3363, 1727, 1589, 1372, 1365, 1246 cm^{-1} ; ESI-MS (neg. ion mode) *m/z* 493.7 $[M-H]^-$, 987.9 $[2M-H]^-$; ESI-MS (pos. ion mode) *m/z* 495.3 $[M+H]^+$, 1011.7 $[2M+Na]^+$; HRESI-MS *m/z* 493.1470 $[M-H]^-$ calcd *m/z* 493.1499 $[M-H]^-$ for $C_{27}H_{26}O_9$.

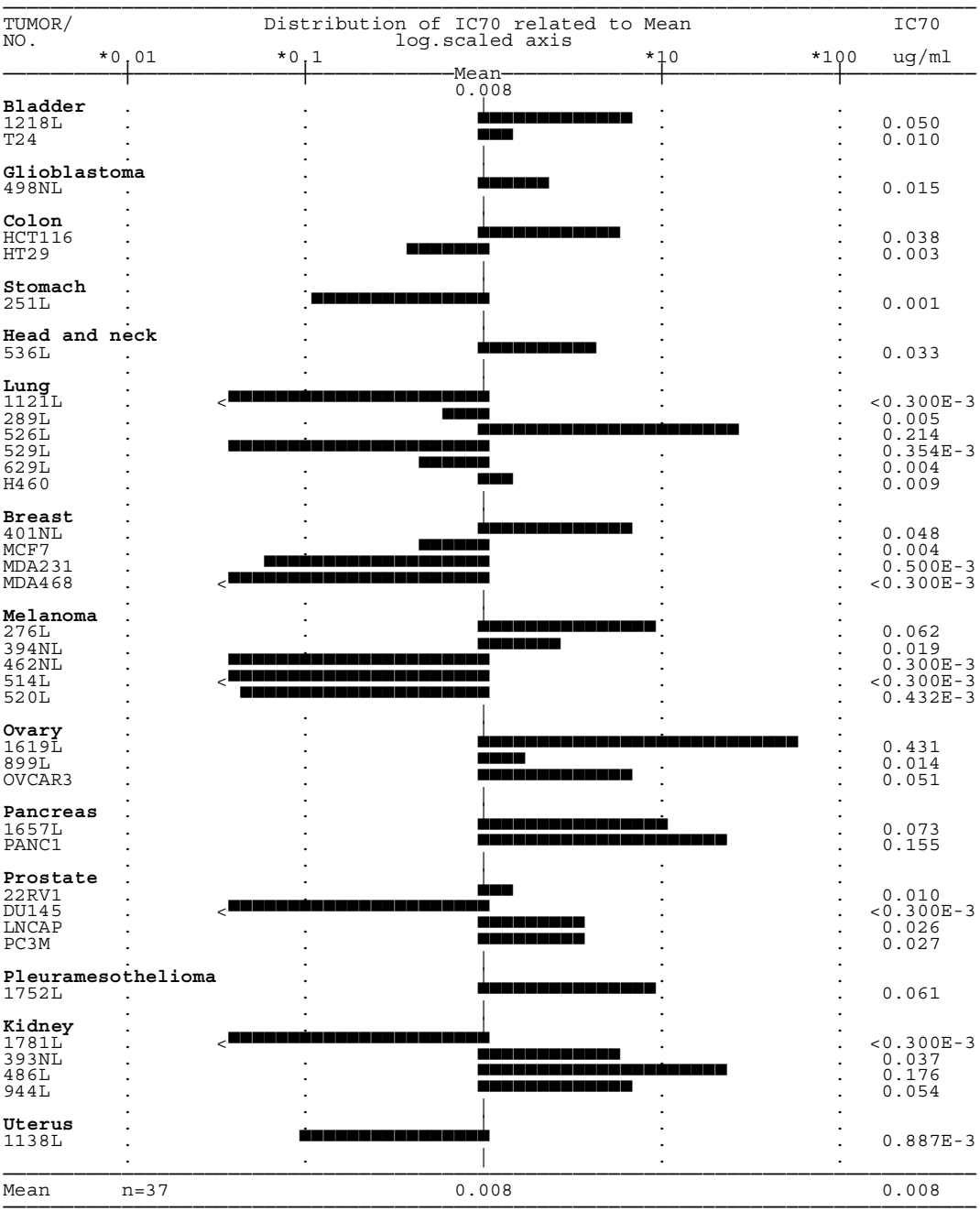
Table 1. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) spectral data of polycarcin V (**1**) in DMSO-d_6.

No	^{13}C δ	^{1}H δ int. (multiplicity, *J* in Hz)
1	152.8	-
2	112.0	6.96 1H (d; 8.4)
3	129.9	7.78 1H (d; 8.4)
4	126.7	-
4a	121.8	-
4b	141.8	-
6	159.4	-
6a	122.4	-
7	119.1	7.97 1H (d; 1.5)
8	138.8	-
9	114.6	7.73 1H (d; 1.5)
10	157.4	-
10a	122.8	-
10b	113.2	-
11	101.4	8.45 1H (s)
12	152.0	-
12a	114.9	-
10-OMe	56.7	4.16 3H (s)
12-OMe	56.5	4.11 3H (s)
1′	77.5	5.84 1H (brs)
2′	71.6	4.06 1H (dd; 5.9, 3.7)
3′	76.4	3.78 1H (ddd; 2.5, 3.7, 5.9)
4′	72.8	3.31 1H (m)
5′	74.7	3.36 1H (m)
6′	18.5	1.28 3H (d; 6.4)
1″	135.2	6.94 1H (dd; 11.0, 17.6)
		H_a 6.13 1H (d; 17.6)
2″	117.3	H_b 5.50 1H (d; 11.0)
1-OH	-	9.72 1H (s)
2’-OH	-	3.98 1H (d; 5.9)
3’-OH	-	4.42 1H (d; 5.9)
4’-OH	-	4.77 1H (d; 5.1)

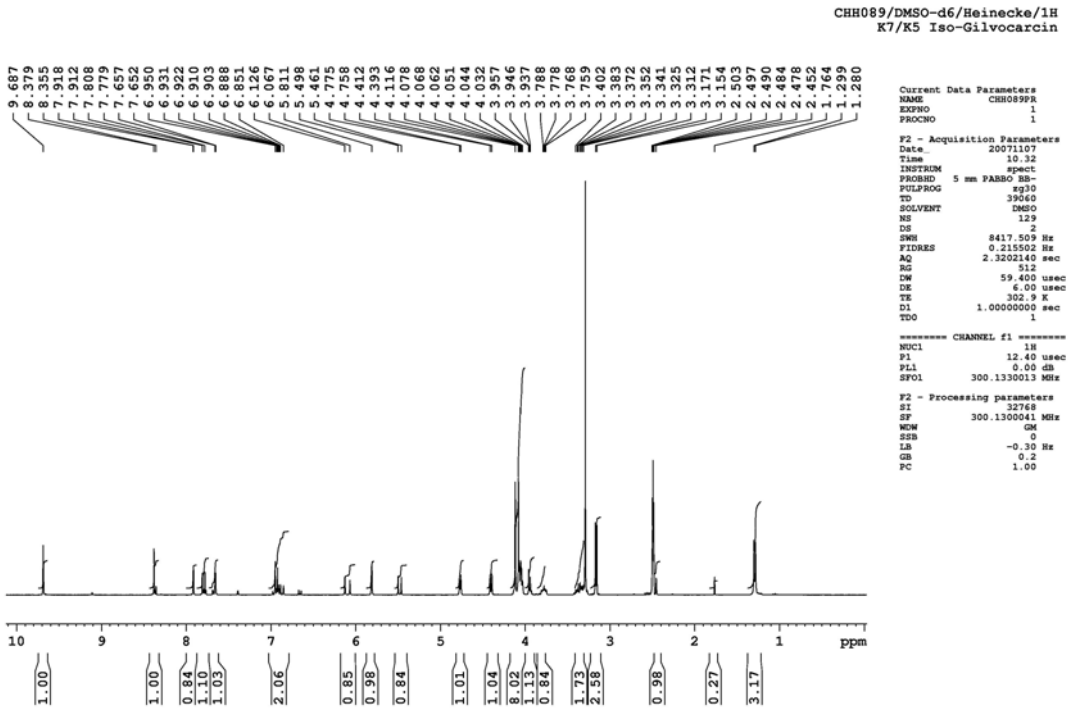
Table 2. Comparison of ^{1}H NMR spectral data (500 MHz) of the α-rhamnose moiety of polycarcin V (**1**) after H/D exchange.

No	δ_H int. (multiplicity; *J* in Hz)	
	DMSO-d_6	DMSO-d_6/ 2%MeOH-d_4
1′	5.84 1H (brs)	5.72 1H (brs)
2′	4.06 1H (dd; 5.9, 3.7)	3.99 1H (d, 3.2)
3′	3.78 1H (ddd; 2.5, 3.7, 5.9)	3.75 1H (dd, 3.2, 9.1)
4′	3.31 1H (m)	3.29 1H (t, 9.1)
5′	3.36 1H (m)	3.37 1H (dq, 9.1, 6.0)
6′	1.28 3H (d; 6.4)	1.27 3H (d, 6.0)

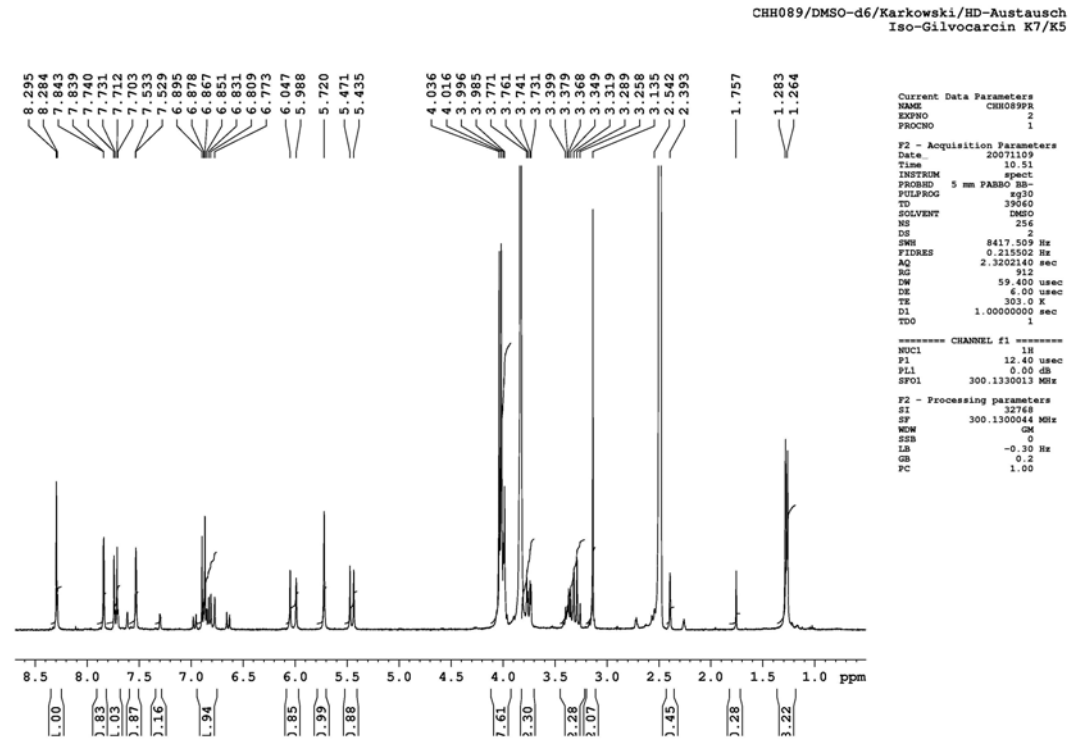
Scheme 1. Mean graph analysis of IC_{70} values for polycarcin V (**1**) tested against 37 human tumor cell lines in monolayer culture.



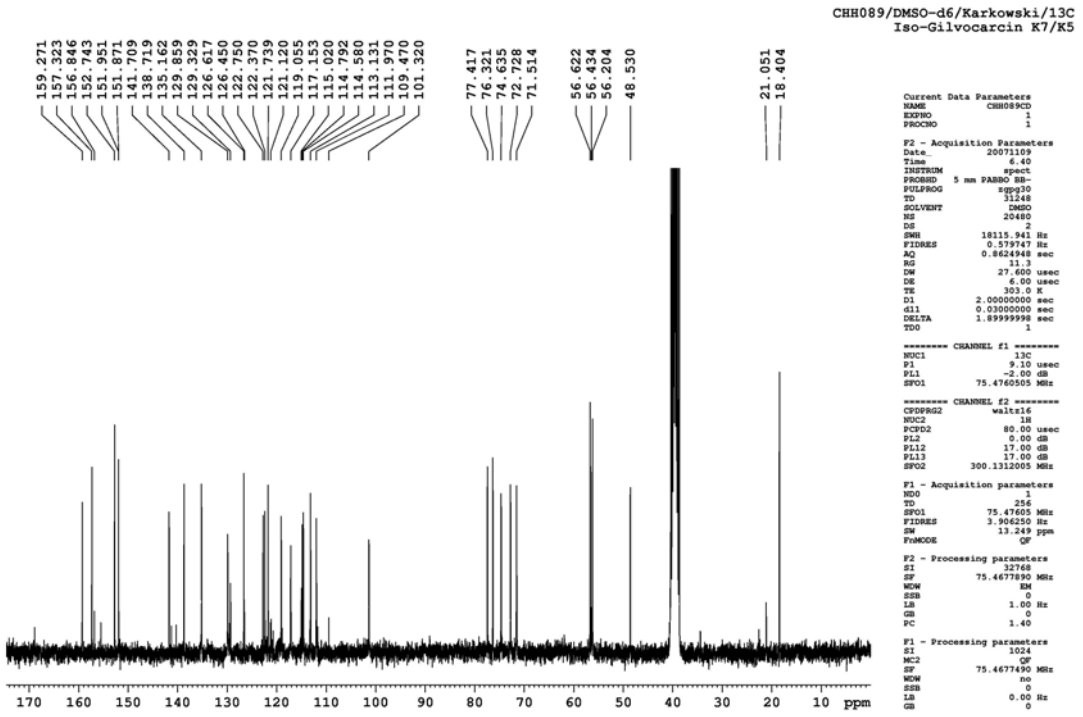
^{1}H NMR spectrum (DMSO-d_6) of polycarcin V



^{1}H NMR spectrum (HD-exchange, DMSO-d_6/2%MeOH-d_4) of polycarcin V



^{13}C NMR spectrum (DMSO-d_6) of polycarcin V



HMBC spectrum (DMSO-d_6) of polycarcin V