CHEMICAL TRANSFORMATION OF GILVOCARCIN V

MODIFICATION OF THE SIDE-CHAIN

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Gilvocarcin V was chemically transformed to alter its biological activities as well as its solubility by mainly focusing on the vinyl side chain. The oxirane and oxime derivatives showed slightly decreased *in vivo* antitumor activity, while the aminoethylmorpholine derivative turned out to be soluble in some organic solvents.

Since its discovery, the gilvocarcin-group of antibiotics has been attracting attention due to its intriguing antitumor activities[1] and has been a target of considerable synthetic efforts.[2] Among the congeners, gilvocarcin V (1) has been the most studied and is believed to confer its biological activity by interacting with double-stranded DNA.[3] Recently, intrinsic tendency of gilvocarcin V showing self-assemblage through intermolecular stacking was elucidated.[4] The same is believed to be true for the interaction with DNA, which affects unwinding of the DNA helices and facilitates the covalent-bond formation by photo-activation.[5]

The need for effective chemotherapeutic agents for cancer treatment is apparently ever growing. We here undertook chemical transformation studies of gilvocarcin V to improve its biological and pharmacological significance.

Among the various functional groups contained in its molecular architecture, the vinyl substituent at the C-8 position seemed appropriate for the site of modification based on the following considerations. The vinyl substituent seems suitable but not essential for biological activities, since; 1) other congeners with a simple alkyl group instead of vinyl also possess the antitumor activity, and 2) although the vinyl substituent was elucidated to be the site of the photochemically forced covalent-bond formation with DNA,[5] such an activation seems unlikely under the usual physiological conditions. Further, our preliminary experiments suggested some difficulties of selective protection of the hydroxyl groups.

In addition, we wished, if possible, to improve the solubility of the gilvocarcin antibiotics since, a major drawback of gilvocarcin V is its poor solubility in most solvents.

Materials and Methods

General

Melting points were measured on a Yanagimoto BY-1 hot-stage apparatus and are uncorrected. ^1H and ^{13}C NMR spectra were recorded on a JEOL FX-200 or a GSX-270 spectrometer. Solvent used for the measurements was $CDCl_3$ for the protected gilvocarcin derivatives and dimethylsulfoxide-d_6 for the

unprotected derivatives. ^{1}H NMR chemical shifts are reported as δ ppm relative to internal tetramethylsilane. As reported previously,[4] ^{1}H NMR chemical shifts of gilvocarcins depend on the concentration and the temperature so these data were recorded at 27°C in a concentration of *ca.* 10 mg/0.6 ml of solvent. ^{13}C NMR chemical shifts were calculated from the resonance frequency of the center peak (77.0 ppm) of the $CDCl_3$ solvent signal. UV spectra were recorded on a Shimadzu UV-160A spectrometer. Column chromatography was carried out with Kieselgel 60 (70~230 mesh, Merck). Thin layer chromatography (TLC) was carried out on precoated plates of Kieselgel 60 F_{254} (Merck, Art. 5715). Because of their insoluble nature, the deprotected products were recovered as precipitates, which were characterized by UV and ^{1}H NMR spectra, and then subjected directly to the biological assays without further purification.

Chemical Transformation

Tetra-*O*-acetyl Gilvocarcin V (**2**)

To a cooled solution of 5.28 g of **1** and a small amount (unweighed) of dimethylaminopyridine in 40 ml of dry pyridine, was added, dropwise, 45 ml of acetic anhydride over a period of 15 minutes. The mixture was stirred for 1 hour at room temperature and the reaction was examined by TLC. After confirming the disappearance of **1**, crashed ice was added to the reaction and the mixture was extracted with chloroform. The aqueous layer was acidified with 2 M HCl and was further extracted with chloroform. The combined organic extract was washed with brine and dried over anhydrous Na_2SO_4. Filtration and evaporation of the solvent under reduced pressure afforded 7.05 g of a crude product which was chromatographed over silica gel using $CHCl_3$ - MeOH (300 : 1) as eluant to yield 4.97 g of **2** (70% yield) as yellow solid.

^{1}H NMR (270 MHz, $CDCl_3$) δ 1.43 (3H, d, J=6.8 Hz), 1.52 (3H, s), 2.15 (3H, s), 2.32 (3H, s), 2.38 (3H, s), 3.96 (3H, s), 4.01 (3H, s), 4.20 (1H, dd, J=6.8, 3.9 Hz), 5.19 (1H, dd, J=3.9, 1.0 Hz), 5.39 (1H, quintet, J=6.8 Hz), 5.42 (1H, d, J=10.7 Hz), 5.92 (1H, d, J=17.6 Hz), 6.16 (1H, dd, J=3.4, 1.0 Hz), 6.53 (1H, d, J=3.4 Hz), 6.77 (1H, dd, J=17.6, 10.7 Hz), 7.16 (1H, d, J=8.3 Hz), 7.31 (1H, d, J=1.5 Hz), 8.04 (1H, d, J=8.3 Hz), 8.06 (1H, d, J=1.5 Hz), 8.45 (1H, s).

^{13}C NMR δ 16.19, 19.84, 20.62, 20.83, 21.06, 55.44, 55.55, 69.48, 77.54, 77.68, 81.14, 83.05, 104 08, 113.29, 113.99, 116.03, 119.33, 119.39, 120.00, 121.83, 122.63, 123.68, 127.21, 129.40, 134.83, 138.41, 141.11, 145.71, 150.45, 156.90, 159.50, 168.14, 169.55, 170.21, 170.27.

Anal Calcd for $C_{35}H_{34}O_{13}$: C 63.44, H 5.17.
Found: C 63.14, H 5.46.

Tetra-*O*-acetyl Gilvocarcin O (**3**)

To a solution of 482 mg of **2** in 5 ml of dichloromethane was added a solution of 100 mg of sodium acetate in 1 ml of methanol. While stirring, 380 mg of *m*-chloroperbenzoic acid was added to the mixture and the stirring was continued overnight at room temperature. The reaction was quenched by addition of sat. aq $NaHCO_3$ to make the mixture alkaline, which was then saturated with NaCl and extracted with chloroform. The organic extract was washed with brine and dried over anhydrous Na_2SO_4. After filtration and removal of solvent, the residue was chromatographed over silica gel with $CHCl_3$ - MeOH (300 : 1) as eluant to give, along with 167 mg of **2**, 208 mg of a mixture of diastereoisomeric tetra-*O*-acetyl gilvocarcin O (**3**) as yellow solid.

^{1}H NMR (270 MHz, $CDCl_3$) δ 1.43 (3H, d, J=6.3 Hz), 1.52 (3H, s), 2.15 (3H, s), 2.32 (3H, s), 2.37 (3H, s), 2.82 and 2.84 (1H, dd, J=4.2, 2.7 Hz), 3.20 and 3.22 (1H, d, J=4.2 Hz), 3.94 (3H, s), 3.95 (3H, s), 3.97 and 3.98 (1H, d, J=2.7 Hz), 4.21 (1H, dd, J=6.3, 3.9 Hz), 5.17 (1H, dd, J=3.9, 1.0 Hz), 5.39 (1H, quintet, J=6.3 Hz), 6.12 and 6.13 (1H, dd, J=3.4, 1.0 Hz), 6.47 and 6.48 (1H, d, J=3.4 Hz), 7.10 and 7.12 (1H, d, J=1.7 Hz), 7.16 (1H, d, J=8.1 Hz), 7.97 and 7.98 (1H, d, J=1.7 Hz), 8.04 (1H, d, J=8.1 Hz), 8.40 (1H, s).

^{13}C NMR δ 16.31, 19.98, 20.79, 20.94, 21.18, 51.15 and 51.24, 51.60, 55.90, 55.98, 69.64, 77.72, 78.80, 81.34, 83.25, 104.41, 112.60 and 113.09, 114.07, 119.04, 119.40 and 119.71, 120.35, 122.14 and 122.23, 123.57 and 123.60, 123.94, 127.47, 129.70, 139.68, 141.57, 145.87, 150.76, 157.46 and 157.59, 159.51, 168.40, 169.72, 170.39.

Anal Calcd for $C_{35}H_{34}O_{14}$: C 61.94, H 5.05.
Found: C 62.24, H 4.89.

Gilvocarcin O (**4**)

To a solution of 502 mg of **3** in 20 ml of tetrahydrofuran was added 3.0 ml of 1 M CH_3ONa in methanol and the mixture was stirred 30 minutes at room temperature. After confirming disappearance of **3** on TLC, sat. aq NH_4Cl was added to the reaction mixture. The yellow precipitate was recovered by filtration and washed with cooled water and cooled methanol to yield 97.1 mg of gilvocarcin O (**4**) as yellow solid (MP 251~257°C).

^1H NMR (270 MHz, DMSO-d_6) δ 1.22 (3H, d, J=6.5 Hz), 3.01 (1H, m), 3.23 (1H, m), 3.50 (1H, m), 3.84 (2H, m), 4.08 (3H, s), 4.11 (3H, s), 4.17 (1H, m), 4.46 (1H, d, J=7.0 Hz), 4.64 (1H, m), 4.80 (1H, d, J=5.2 Hz), 5.07 (1H, d, J=5.6 Hz), 6.13 (1H, d, J=5.5 Hz), 6.93 (1H, d, J=8.4 Hz), 7.46 (1H, d, J=1.6 Hz), 7.85 (1H, d, J=1.6 Hz), 8.07 (1H, d, J=8.4 Hz), 8.42 (1H, s), 9.69 (1H, s).

UV (MeOH) λ_{max} nm (ε) 201.3 (21,700), 245.0 (40,100), 277.9 (33,600), 390.8 (9,790).

Tetra-*O*-acetyl Gilvocarcin A (**5**)

To a solution of 2.67 g of **2** in a mixture of 65 ml of 1,4-dioxane and 15 ml of water was added a small amount of OsO_4 and the mixture turned black. Then 1.76 g of $NaIO_4$ was added to the reaction mixture and the mixture was stirred for 3 hours at room temperature and the reaction was examined by TLC. After confirming disappearance of **2**, sat. aq $NaHSO_3$ was added to the reaction mixture and the precipitate was extracted with chloroform. The combined organic extract was washed with sat. aq NaCl and dried over anhydrous Na_2SO_4. Filtration and removal of the solvent afforded 2.83 g of a crude product, which was chromatographed over silica gel using $CHCl_3$ as eluant to yield 2.23 g of **5** as yellow solid (83% yield).

^1H NMR (270 MHz, $CDCl_3$) δ 1.44 (3H, d, J=6.3 Hz), 1.51 (3H, s), 2.16 (3H, s), 2.33 (3H, s), 2.38 (3H, s), 3.94 (3H, s), 4.03 (3H, s), 4.20 (1H, dd, J=6.3, 3.9 Hz), 5.16 (1H, dd, J=3.9, 1.0 Hz), 5.39 (1H, quintet, J=6.3 Hz), 6.10 (1H, dd, J=3.4, 1.0 Hz), 6.38 (1H, d, J=3.4 Hz), 7.18 (1H, d, J=8.3 Hz), 7.71 (1H, d, J=1.5 Hz), 8.06 (1H, d, J=8.3 Hz), 8.40 (1H, s), 8.46 (1H, d, J=1.5 Hz), 10.05 (1H, s).

^{13}C NMR δ 16.42, 20.04, 20.85, 20.97, 21.26, 50.10, 56.49, 69.68, 77.80, 78.86, 81.40, 83.48, 104.31, 112.94, 113.61, 120.76, 121.43, 122.67, 124.03, 126.32, 127.99, 129.46, 130.36, 136.36, 143.21, 145.99, 151.18, 157.94, 159.11, 168.62, 169.75, 170.44, 170.48, 190.21.

Anal Calcd for $C_{34}H_{32}O_{14}$: C 61.44, H 4.85.
Found: C 61.29, H 5.04.

Tetra-*O*-acetyl Gilvocarcin HIM (**6**)

To a solution of 1.14 g of **5** in a mixture of 7 ml of pyridine and 3 ml of ethanol was added 352 mg of hydroxylamine hydrochloride and the mixture was stirred for 1.5 hours at room temperature. After confirming disappearance of **5** on TLC, the reaction was quenched by addition of water, and the aqueous layer was acidified with 2 M HCl, which was then saturated with NaCl and extracted with chloroform. The combined organic extract was washed with sat. aq NaCl and dried over anhydrous Na_2SO_4. Filtration and removal of the solvent afforded 1.12 g of a crude product which was chromatographed over silica gel using $CHCl_3$ - MeOH (100 : 1) as eluant to yield 898 mg of **6** as yellow solid (70% yield).

^1H NMR (270 MHz, $CDCl_3$) δ 1.44 (3H, d, J=6.6 Hz), 1.57 (3H, s), 2.16 (3H, s), 2.33 (3H, s), 2.40 (3H, s), 3.87 (3H, s), 3.95 (3H, s), 4.23 (1H, dd, J=6.7, 3.7 Hz), 5.18 (1H, dd, J=3.7, 0.9 Hz), 5.40 (1H, quintet, J=6.6 Hz), 6.13 (1H, dd, J=2.9, 0.9 Hz), 6.44 (1H, d, J=2.9 Hz), 7.17 (1H, d, J=8.1 Hz), 7.39 (1H, d, J=1.5 Hz), 7.91 (1H, d, J=1.5 Hz), 7.95 (1H, s), 8.04 (1H, d, J=8.1 Hz), 8.35 (1H, s), 8.37 (1H, s).

^{13}C NMR δ 16.26, 20.13, 20.86, 20.97, 21.24, 55.88, 69.74, 77.67, 78.82, 81.31, 83.20, 104.29, 111.28, 114.01, 119.86, 120.53, 121.27, 122.06, 123.96, 124.64, 127.70, 129.78, 133.55, 141.76, 145.82, 147.65, 150.83, 157.04, 159.28, 168.74, 170.33, 170.67.

Anal Calcd for $C_{34}H_{33}O_{14}N$: C 60.09, H 4.89, N 2.06.
Found: C 60.20, H 4.65, N 2.21.

Gilvocarcin HIM (**7**)

To a solution of 90.4 mg of **6** in 5 ml of tetrahydrofuran was added 520 μl of 1 M CH_3ONa in methanol and the mixture was stirred 15 minutes at room temperature. After confirming disappearance of **6** on TLC, sat. aq NH_4Cl was added to the reaction mixture. The yellow precipitate was recovered by filtration

and washed with cooled water and cooled methanol to yield 29.4 mg of gilvocarcin HIM (**7**) as yellow solid (MP 252~260°C).

^{1}H NMR (270 MHz, DMSO-d_6) δ 1.23 (3H, d, J=6.5 Hz), 3.48 (1H, m), 3.84 (2H, m), 4.10 (3H, s), 4.14 (3H, s), 4.49 (1H, d, J=7.0 Hz), 4.66 (1H, m), 4.78 (1H, d, J=5.2 Hz), 5.10 (1H, d, J=5.6 Hz), 6.19 (1H, d, J=5.5 Hz), 6.94 (1H, d, J=8.4 Hz), 7.82 (1H, d, J=1.6 Hz), 8.06 (1H, d, J=8.4 Hz), 8.13 (1H, d, J=1.6 Hz), 8.37 (1H, s), 8.45 (1H, s), 9.69 (1H, s), 11.63 (1H, s).

UV (MeOH) λ_{max} nm (ε) 203.2 (34,800), 249.6 (29,900), 290.7 (31,600), 395.2 (11,300), 403.9 (10,500).

Tetra-*O*-acetyl Gilvocarcin MEAM (**9**) and Tetra-*O*-acetyl Gilvocarcin H (**10**)

To a solution of 94.0 mg of **5** in 4 ml of dry benzene was added 98.9 mg of aminoethylmorpholine hydrochloride and the mixture was stirred for 4 hours. Then the reaction mixture was evaporated under reduced pressure. The residue was dissolved in a mixture of 3 ml of tetrahydrofuran and 2 ml of methanol and 30.1 mg of $NaBH_3CN$ was added to this solution. After confirming disappearance of **5** on TLC, the reaction was quenched by addition of water, and the aqueous layer was saturated with NaCl and extracted with chloroform. The combined organic extract was washed with sat. aq NaCl and dried over anhydrous Na_2SO_4. Filtration and removal of the solvent afforded 112.6 mg of a crude product, which was chromatographed over silica gel using $CHCl_3$-MeOH (100:1, 50:1, 20:1) as eluant to yield 60.6 mg of **9** (55% yield) and 30.1 mg of **10** (32%) as yellow solid.

For **9**: ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.44 (3H, d, J=6.4 Hz), 1.51 (3H, s), 2.15 (3H, s), 2.32 (3H, s), 2.37 (3H, s), 2.43 (4H, t, J=4.7 Hz), 2.52 (2H, t, J=5.9 Hz), 2.71 (2H, t, J=5.9 Hz), 3.71 (4H, t, J=4.7 Hz), 3.89 (2H, s), 3.94 (3H, s), 3.95 (3H, s), 4.21 (1H, dd, J=3.9, 6.4 Hz), 5.17 (1H, dd, J=1.0, 3.9 Hz), 5.39 (1H, quintet, J=6.4 Hz), 6.15 (1H, dd, J=1.0, 2.9 Hz), 6.51 (1H, d, J=2.9 Hz), 7.15 (1H, d, J=8.3 Hz), 7.32 (1H, d, J=1.0 Hz), 8.04 (1H, d, J=8.3 Hz), 8.43 (1H, s).

^{13}C NMR δ 16.39, 20.06, 20.88, 21.00, 21.26, 45.24, 53.26, 53.66, 56.06, 56.14, 58.13, 66.88, 69.62, 77.79, 78.81, 81.43, 83.24, 104.62, 114.31, 116.61, 119.62, 120.20, 121.08, 122.04, 122.86, 123.99, 127.38, 129.68, 141.50, 142.52, 145.81, 150.71, 157.34, 159.87, 168.33, 169.62, 170.26.

Anal Calcd for $C_{40}H_{46}O_{14}N_2$: C 61.68, H 5.95, N 3.60.
Found: C 61.54, H 5.93, N 3.55.

For **10**: ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.44 (3H, d, J=6.8 Hz), 1.53 (3H, s), 2.19 (3H, s), 2.35 (3H, s), 2.39 (3H, s), 2.68 (1H, broad), 3.68 (3H, s), 3.90 (3H, s), 4.24 (1H, dd, J=6.8, 3.9 Hz), 4.60 (2H, broad), 5.16 (1H, dd, J=3.9, 1.0 Hz), 5.39 (1H, quintet, J=6.8 Hz), 6.05 (1H, dd, J=2.9, 1.0 Hz), 6.33 (1H, d, J=2.9 Hz), 7.07 (1H, d, J=1.5 Hz), 7.14 (1H, d, J=8.3 Hz), 7.82 (1H, d, J=1.5 Hz), 7.98 (1H, d, J=8.3 Hz), 8.25 (1H, s).

^{13}C NMR δ 16.53, 20.18, 21.03, 21.14, 21.38, 55.74, 56.00, 63.99, 69.05, 77.64, 78.87, 81.43, 83.65, 104.83, 114.44, 114.45, 118.74, 119.56, 120.02, 121.61, 122.33, 123.94, 127.29, 129.62, 141.26, 143.71, 145.87, 150.47, 157.01, 159.76, 168.33, 169.82, 170.40.

Anal Calcd for $C_{34}H_{34}O_{14}$: C 61.26, H 5.14.
Found: C 61.46, H 5.01.

Gilvocarcin MEAM (**11**)

To a solution of 60.6 mg of **9** in 10 ml of methanol was added 1 ml of triethylamine and the mixture was refluxed with stirring overnight. After confirming disappearance of **9** on TLC, removal of the solvent afforded 50.6 mg of a crude product, which was recrystallized from chloroform-hexane to yield 40.2 mg of **11** as yellow solid (85%, MP 133~137°C).

^{1}H NMR (270 MHz, DMSO-d_6) δ 1.49 (3H, d, J=6.5 Hz), 2.44 (4H, m), 2.56 (2H, m), 3.68 (5H, m), 4.00 (4H, m), 4.25 (6H, s), 4.63 (1H, d, J=6.9 Hz), 4.80 (1H, m), 4.94 (1H, d, J=5.2 Hz), 5.21 (1H, d, J=5.7 Hz), 6.33 (1H, d, J=5.7 Hz), 7.08 (1H, d, J=8.3 Hz), 7.74 (1H, d, J=1.2 Hz), 8.06 (1H, d, J=1.2 Hz), 8.17 (1H, d, J=8.3 Hz), 8.60 (1H, s), 9.82 (1H, s).

UV (MeOH) λ_{max} nm (ε) 205.5 (21,200), 245.0 (41,500), 276.5 (29,700), 307.5 (11,600), 390.0 (9,840).

Gilvocarcin H (**12**)

To a solution of 212.6 mg of **10** 5 ml of tetrahydrofuran was added 1.3 ml of 1 M CH_3ONa in methanol and the mixture was stirred 30 minutes at room temperature. After confirming disappearance

of **10** on TLC, sat. aq NH_4Cl was added to the reaction mixture. The yellow precipitate was recovered by filtration and washed with cooled water and cooled methanol to yield 35.3 mg of gilvocarcin H (**12**) as yellow solid (MP 251~255°C).

^1H NMR (270 MHz, DMSO-d_6) δ 1.22 (3H, d, J=6.5 Hz), 2.61 (4H, t, J=5.0 Hz), 3.51 (1H, m), 3.87 (2H, m), 4.11 (3H, s), 4.12 (3H, s), 4.42 (1H, d, J=7.0 Hz), 4.66 (1H, m), 4.70 (2H, d, J=6.3 Hz), 4.73 (1H, d, J=5.2 Hz), 5.00 (1H, d, J=5.6 Hz), 5.49 (1H, t, J=6.3 Hz), 6.20 (1H, d, J=5.5 Hz), 6.93 (1H, d, J=8.4 Hz), 7.59 (1H, d, J=1.6 Hz), 7.94 (1H, d, J=1.6 Hz), 8.07 (1H, d, J=8.4 Hz), 8.50 (1H, s), 9.68 (1H, s).

UV (MeOH) λ_{max} nm (ε) 203.0 (29,900), 244.4 (43,500), 275.9 (33,800), 388.4 (9,280).

Biological Assays

Cytotoxicity on Tumor Cells

P388 cells were cultured in RPMI-1640 medium supplemented with 2 mM L-glutamine, 50 U/ml penicillin, 50 μg/ml streptomycin and 10% heat-inactivated fetal calf serum (FCS). The cells at 5×10^4/ml were incubated with testing agents in a humidified atmosphere of 5% CO_2 in air at 37°C for 2 days. The cytotoxic activities were essentially measured according to an automated colorimetric assay based on the production of dark blue formazan crystals by living cells incubated with the tetrazolium salt MTT.[6] The results are expressed as IC_{50} (ng/ml) values which are concentrations of the drugs that inhibit cell growth by 50%.

In Vivo Antitumor Activity

Antitumor activities of testing agents in mice were examined against P388 mouse leukemia. P388 leukemia was inoculated ip into CDF_1 mice at 10^6 cells per mice. Drug treatment was given ip on days 1, 5 and 9. Six mice were used in each test group. The effects of the drugs were evaluated on the basis of the median survival time (MST) in days. The results are expressed as T/C (%).

Results and Discussion

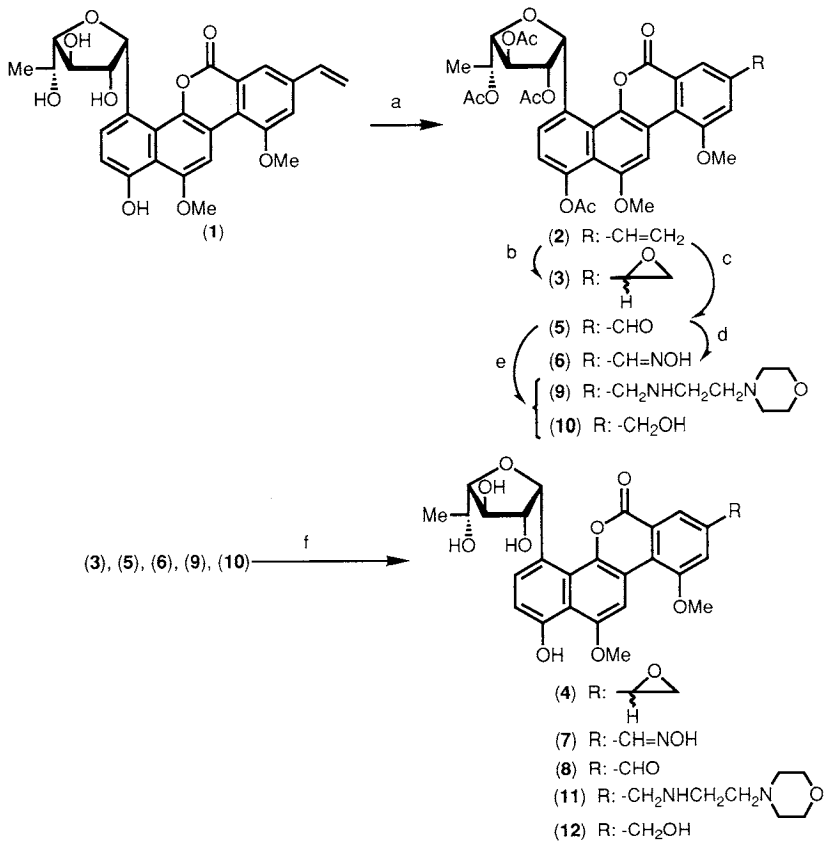
Chemistry

In order to assess the possibility of successful modification of gilvocarcin V (**1**), rather straightforward chemical transformations were pursued in this study. Initial attempts at selective protection of the hydroxyl groups were unsuccessful. Stereo-isomerization as well as rearrangement of the sugar moiety under acidic or alkaline conditions was previously described by others.[7] With these limitations, peracetylation was chosen for the protection of the hydroxyl groups. Conventional acetylation with excess acetic anhydride in pyridine afforded the tetraacetate (**2**),[8] which turned out to be soluble in most organic solvents. Chromatographic purification of **2**, followed by deprotection under several reaction conditions, gave back gilvocarcin V (**1**) in much purer state. The acetate protecting group was hydrolyzed with CH_3ONa in the earlier experiments, and the deprotection procedure was improved later by using with either triethylamine or hydrazine acetate in methanol.

Transformations carried out in this study are summarized in Scheme 1. An obvious transformation was the epoxidation of **2**, which proceeded sluggishly with *m*-chloroperbenzoic acid to afford a chromatographically unseparable mixture of the epoxide diastereoisomers (**3**). Deprotection of **3** was carried out with CH_3ONa to give gilvocarcin O (Oxiranyl) (**4**). The free epoxides (**4**) were almost insoluble in organic solvent so that the product was recovered by precipitation and filtrative collection.

The second logical manipulation was the oxidation of **2** with OsO_4 and $NaIO_4$ to give the expected aldehyde (**5**) in 83% yield. Apparently, aldehyde (**5**) seemed useful for further transformations into various functionalities. A simple way of introduction of nitrogen-functionalities seemed to derivative **5** into a mixture of the corresponding oximes (**6**). Deacetylation was carried out as described above to yield

Scheme 1.



Reagents: a) Ac_2O/py, b) *m*-chloroperbenzoic acid, CH_2Cl_2, c) OsO_4-$NaIO_4$, dioxane-H_2O, d) $NH_2OH \cdot HCl$, py-EtOH, e) aminoethylmorpholine, $NaBH_3CN$, THF-MeOH, f) CH_3ONa-MeOH or Et_3N-MeOH.

gilvocarcin HIM (HydroxyIminoMethyl) (**7**). In addition, aldehyde (**5**) was also deprotected to known gilvocarcin A (**8**).[9]

Reductive amination of **5** with aminoethylmorpholine afforded diamine (**9**) together with the by-product alcohol (**10**) formed by simple reduction. Deprotection of **9** was carried out quite smoothly with triethylamine in MeOH to afford gilvocarcin MEAM (MorpholinoEthylAminoMethyl) (**11**) in good yield. The solubility of the aminoethylmorpholine derivative (**11**) was significantly improved, being soluble in MeOH and $CHCl_3$. The by-product alcohol (**10**) was also deprotected to gilvocarcin H (Hydroxymethyl) (**12**) for comparison of the biological activities.

Biological Activities

The biological activities of the gilvocarcin derivatives were tested *in vitro* and *in vivo*. The *in vitro* cytotoxicities were assayed against leukemia P388 cells and the results are summarized in Table 1. The aminoethylmorpholine derivative (**11**) is not as active as the parent gilvocarcin V (**1**), however, the epoxide (**4**) and the oxime (**7**) showed significant activities.

The *in vivo* assays were carried out against leukemia P388 in mice and the life span (T/C%) are shown in Table 2. The aminoethylmorpholine derivative (**11**) was almost inactive and so were the by-product alcohol (**12**) and the aldehyde (**8**). The epoxide (**4**) and the oxime (**7**) were moderately active, being actually

Table 1. Cytotoxic activities of gilvocarcin derivatives.

Derivative	IC_{50} (ng/ml)
Gilvocarcin V	2
Gilvocarcin O	10
Gilvocarcin HIM	10
Gilvocarcin MEAM	5,000

Table 2. *In vivo* antitumor activities of gilvocarcin derivatives.

Derivative	Run 1		Run 2	
	Dose (mg/kg)	T/C (%)	Dose (mg/kg)	T/C (%)
Gilvocarcin V			50	155
Gilvocarcin O	40	178	30	121
	80	162	60	137
Gilvocarcin HIM	30	157	40	117
Gilvocarcin A	10	111		
Gilvocarcin MEAM	80	99		
Gilvocarcin H	24	102		
	48	115		

slightly less active than **1**.

These *in vitro* and *in vivo* results show that the vinyl group of gilvocarcin V is not necessarily essential for the antitumor activity, *i.e.* the vinyl group can be replaced with an epoxide or an oxime. This may imply possibility of other modifications of the vinyl group. A preliminary attempt to enhance possible electrostatic interactions with DNA by introducing amino functionalities seemed unsuccessful, because the diamine derivative (**11**) turned out to be less active than **1**. However, other derivatives with cationic ammonium functions remain to be examined in this context.

As to the solubility problem of **1** in organic solvents, some improvement was seen by introducing the aminoethylmorpholine group. Further elaboration may turn out to improve the solubility as well as the biological activities of gilvocarcin derivatives.

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