Synthesis and Cytotoxic Activities of *α*-Methylidene-*γ*-butyrolactones Bearing a Quinolin-4(1*H*)-one Moiety

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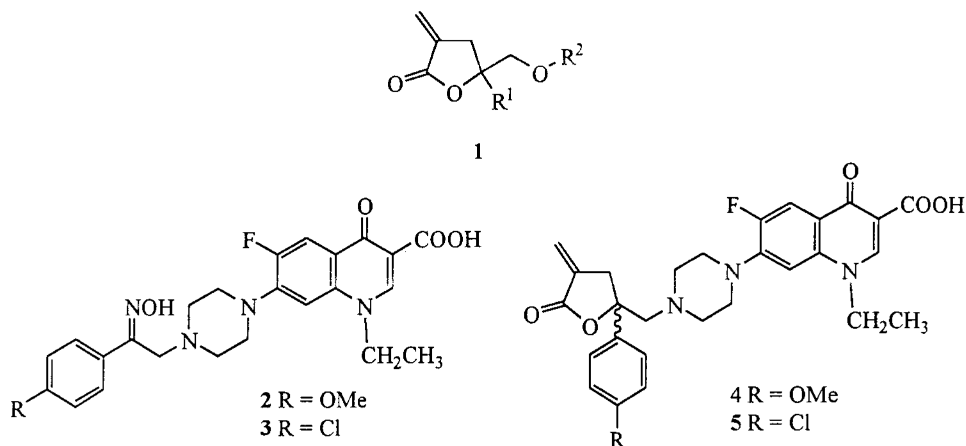
The cytotoxicities of the *α*-methylidene-*γ*-butyrolactones **4**, **5**, and **8**, which are linked to a quinolin-4(1*H*)-one moiety through a piperazine or O-atom bridge were studied. These compounds were synthesized by alkylation of 1-ethyl-6-fluoro-1,4-dihydro-7-hydroxy-4-oxoquinoline-3-carboxylic acid (**6**) followed by a *Reformatsky*-type condensation. Compounds **4**, **5**, and **8** were evaluated *in vitro* against 60 human-tumor cell lines derived from nine cancer-cell types and demonstrated not only strong growth-inhibitory activities against leukemia cancer cells, but also fairly good activities against the growth of certain solid tumors (see *Table*). The O-bridged derivatives **8a** and **8b** exhibit both cytostatic (mean log $GI_{50} = -5.20$ and -5.82, resp.) and cytocidal (mean log $LC_{50} = -4.30$ and -4.93, resp.) effects, while the piperazine-bridged analogues **4** and **5** possess only weak cytostatic (mean log $GI_{50} = -5.19$ and -4.74, resp.; mean log $LC_{50} > -4.00$) capability. Among them, **8b** is the most potent, with log $GI_{50} = -6.47$, -6.72, -6.53, and -6.52 against leukemia, SW-620 (colon), Lox IMV1, and SK-MEL-28 (melanoma) cancer cells, respectively.

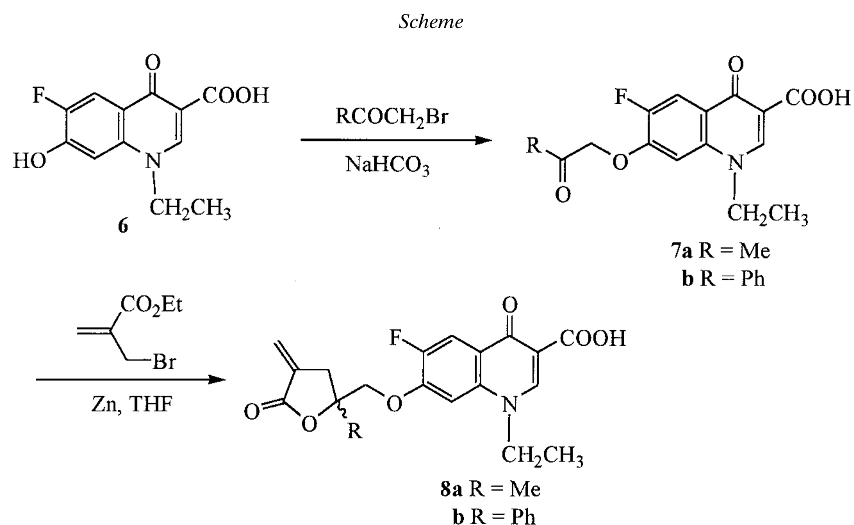
Introduction. – The *α*-methylidene-*γ*-butyrolactone moiety is a characteristic component of a large number of biologically active natural products, especially the sesquiterpene lactones [1–4]. However, the biological activity of *α*-methylidene-*γ*-butyrolactones is not confined to the complex polyfunctional sesquiterpene lactones only. For example, some simple natural *α*-methylidene-*γ*-butyrolactone-bearing butanolides [5] and even the parent *α*-methylidene-*γ*-butyrolactone (tulipaline A) [6] have significant pharmacological activities. Over the past few years, we were particularly interested in synthesizing *α*-methylidene-*γ*-butyrolactones and evaluated their cardiovascular and cytotoxic activities [7][8]. Although the enone moiety $O{=}C{-}C{=}CH_2$ of this type of lactone is essential for their biological activities, by acting as an alkylating agent through a *Michael*-type reaction with bionucleophiles or mercapto-containing enzymes [9], both *γ*-substituents R^1 and R^2 of the lactone **1** also played important roles for their pharmacological properties. For example, if R^1 is a Ph substituent, the lactone possesses more potent antiplatelet activities than if R^1 is a Me substituent, while a biphenyl counterpart is relatively inactive as a vasorelaxing agent [7]. The substituent R^2 is preferably a quinolin-2(1*H*)-one moiety, the resulting lactones being potent antiplatelet as well as cytotoxic agents [8]. Recently, we have synthesized the norfloxacin derivatives **2**–**4** and explored their antibacterial and cytotoxic activities [10] on the grounds that the quinolin-4(1*H*)-one moiety could intercalate DNA, leading to the inhibition of bacterial DNA gyrase and/or human topoisomerases [11–15]. The preliminary results indicated that the 4-methoxyphenyl

derivative **2** was a potent antibacterial agent, while its 4-chlorophenyl counterpart **3** was less active, but was more cytotoxic against the growth of renal cancers [10]. The present report describes the cytotoxicity of certain α-methylidene-γ-butyrolactones which are linked to a quinolin-4(1*H*)-one moiety by a piperazine or O-atom bridge. The reason for the choice of the quinolin-4(1*H*)-one as a carrier of the α-methylidene-γ-butyrolactone in these compounds is to avoid a serious drawback of low DNA affinity, which is common to alkylating antitumor agents.



Results and Discussion. – For the synthesis of 1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-[(2,3,4,5-tetrahydro-2-methyl-4-methylidene-5-oxofuran-2-yl)methoxy]quinoline-3-carboxylic acid (**8a**) and its phenyl analogue **8b**, the known acid **6** [16] were treated with bromoacetone or bromoacetophenone and sodium hydrogen carbonate in dry DMF to give the intermediate acids **7a** and **7b**, respectively (*Scheme*). *Reformatsky*-type

Scheme



condensation of **7a** or **7b** afforded the lactones **8a** and **8b**, respectively. Compounds **4** [10] and **5** were synthesized by alkylation of norfloxacin with 2-bromo-4′-methoxyacetophenone and 2-bromo-4′-chloroacetophenone, respectively, followed by the *Reformatsky*-type condensation.

All compounds were evaluated in *in vitro* assays against 60 human-tumor cell lines derived from nine cancer-cell types (leukemia, non-small-cell lung cancer, colon cancer, CNS cancer, melanoma, ovarian cancer, renal cancer, prostate cancer, and breast cancer; see *Table*). For each compound, dose-response curves for each cell line were measured with five different drug concentrations, and the concentration causing 50% cell growth inhibition (GI_{50}, equating to cytostatic activity) and the concentration causing 50% cell death (LC_{50}, equating to cytocidal activity) compared with the control were calculated [17]. All these compounds demonstrate potent growth-inhibitory activities against leukemia cancer cells ($\log GI_{50} < -5.0$). Compounds **4**, **8a**, and **8b** also exhibit good inhibitory activities against certain solid-tumor cell lines. However, the cytotoxic profile of these α-methylidene-γ-butyrolactones are not the same and can be classified as cytostatic (**4** and **5**; mean $\log LC_{50} > -4.00$) and cytocidal (**8a** and **8b**; mean $\log LC_{50} < -4.00$). Although the mean $\log GI_{50}$ values of **4** and **8a** are comparable (-5.19 *vs.* -5.20), the O-bridged **8a** exhibits a much lower mean $\log LC_{50}$ value than that of the piperazine-bridged **4** (-4.30 *vs.* > -4.00), indicating that only **8a** is able to kill the cells. Accordingly, the O-bridged **8b** is the most potent cytostatic (mean $\log GI_{50} = -5.82$) and cytocidal (mean $\log LC_{50} = -4.93$), while the piperazine-bridged **5** is considered only weakly cytostatic (mean $\log GI_{50} = -4.74$) without cytocidal capability (mean $\log LC_{50} > -4.00$).

Compounds **8a** and **8b** were then selected for a preliminary *in vivo* hollow-fiber assay [18]. Each compound was tested against a standard panel of 12 human-tumor cell lines including NCI-H23, NCI-H522, MDA-MB-231, MDA-MB-435, SW-620, COLO 205, LOX IMVI, UACC-62, OVCAR-3, OVCAR-5, U251, and SF-295. According to the *NCI*'s protocol, compounds with a combined intraperitoneal (IP) and subcutaneous (SC) score of 20, a SC score 8, or a net cell kill of one or more cell lines in either implant site, were referred for xenograft testing. The results were as following: **8a**, IP = 6, SC = 4, and cell kill = 0; **8b**, IP = 4, SC = 6, and cell kill = 1. Compound **8b** was able to produce a reduction in the viable cell mass below the level present at the start of the implantation.

Conclusion. – The alkylating α-methylidene-γ-butyrolactones were linked to the potential DNA-intercalating quinolin-4(1*H*)-one moiety by a piperazine or O-atom bridge with the aim to enhance the target specificity. The results of this study showed that the O-bridged derivatives **8a** and **8b**, with mean $\log LC_{50}$ values of -4.30 and -4.93, respectively, are cytocidal, while the piperazine-bridged derivatives **4** and **5** are cytostatic. Among them, **8b** shows not only potent inhibitory activities on leukemia-cancer cell lines with an average $\log GI_{50}$ of -6.47, but also good inhibitory activities on SW-620, LOX IMV1, and SK-MEL-28 cancer cells with a $\log GI_{50}$ value of -6.72, -6.53, and -6.52, respectively.

Table. *Inhibition of* in vitro *Cancer Cell Lines by α-Methylidene-γ-butyrolactones*

Cell line	log GI_{50} (log LC_{50}) [M][a])			
	4	**5**	**8a**	**8b**
Leukemia				
CCRF-CEM	– 5.38 (> – 4.0)	– 5.06 (> – 4.0)	– 5.74 (– 4.77)	– 5.69 (– 5.73)
K-562	– 5.51 (> – 4.0)	– 5.38 (> – 4.0)	– 5.57 (– 4.34)	nd[b])
MOLT-4	– 5.29 (> – 4.0)	– 5.55 (> – 4.0)	– 5.75 (– 4.88)	– 6.73 (– 6.04)
RPMI-8226	– 5.55 (> – 4.0)	– 5.51 (> – 4.0)	– 5.47 (– 4.19)	– 6.75 (– 6.01)
SR	– 5.47 (> – 4.0)	– 5.32 (> – 4.0)	– 5.55 (– 4.39)	– 6.69 (nd[b]))
Non-small-cell lung cancer				
HOP-92	– 5.24 (> – 4.0)	– 4.44 (> – 4.0)	nd[b])	– 5.77 (– 5.22)
NCI-H322M	– 4.86 (– 4.29)	– 4.71 (– 4.01)	– 4.84 (– 4.25)	– 4.92 (– 4.21)
Colon cancer				
COLO 205	– 5.47 (> – 4.0)	– 4.67 (> – 4.0)	– 5.49 (– 4.39)	– 6.72 (– 5.68)
SW-620	– 5.39 (> – 4.0)	– 4.80 (> – 4.0)	– 5.49 (– 4.39)	– 6.72 (– 5.68)
CNS cancer				
SNB-19	– 4.46 (> – 4.0)	– 4.73 (> – 4.0)	– 4.72 (– 4.24)	– 5.25 (– 4.34)
U251	– 4.91 (> – 4.0)	– 4.53 (> – 4.0)	– 5.04 (– 4.32)	– 5.82 (– 5.24)
Melanoma				
LOX IMVI	– 5.54 (> – 4.0)	– 4.78 (> – 4.0)	– 5.56 (– 4.35)	– 6.53 (– 5.43)
SK-MEL-28	– 4.74 (> – 4.0)	– 4.52 (> – 4.0)	– 5.38 (– 4.38)	– 6.52 (– 5.47)
Ovarian cancer				
IGROV1	– 5.65 (– 4.35)	– 5.03 (– 4.33)	– 5.40 (– 4.15)	– 5.74 (– 5.15)
OVCAR-5	– 4.69 (> – 4.0)	– 4.27 (> – 4.0)	– 4.77 (– 4.26)	– 5.17 (– 4.19)
Renal cancer				
SN12C	– 5.29 (> – 4.0)	– 5.42 (> – 4.0)	– 5.52 (– 4.43)	– 5.96 (– 5.31)
ACHN	– 5.29 (> – 4.0)	– 4.71 (> – 4.0)	– 5.41 (– 4.29)	– 5.95 (– 5.31)
Prostate cancer				
PC-3	– 5.24 (– 4.37)	– 4.89 (– 4.29)	– 4.95 (– 4.23)	– 5.79 (– 5.07)
DU-145	– 5.33 (> – 4.0)	– 4.85 (> – 4.0)	– 5.33 (– 4.39)	– 5.67 (– 4.90)
Breast cancer				
MDA-MB-435	– 5.10 (> – 4.0)	– 4.56 (> – 4.0)	– 5.18 (– 4.29)	– 5.69 (– 4.80)
MDA-N	– 5.20 (> – 4.0)	– 4.38 (> – 4.0)	– 5.38 (– 4.40)	– 5.79 (– 5.20)
Mean[c])	– 5.19 (> – 4.0)	– 4.74 (> – 4.0)	– 5.20 (– 4.30)	– 5.82 (– 4.93)

[a]) Data obtained from *NCI*'s *in vitro* disease-oriented tumor cell screen [17]. GI_{50}: drug molar concentration causing 50% cell-growth inhibition; LC_{50}: drug molar concentration causing 50% cell dealth.
[b]) Not determined.
[c]) Mean values over all cell lines tested. These cell lines are: leukemia (CCRF-CEM, HL-60 (TB), K-562, MOLT-4, PRMI-8226, and SR); non-small-cell lung cancer (A549/ATCC, EKVX, HOP-62, HOP-92, NCI-H226, NCI-H23, NCI-H322M, and NCI-H522); colon cancer (COLO 205, HCC-2998, HCT-116, HCT-15, HT29, KM12, and SW-620); CNS cancer (SF-268, SF-295, SF-539, SNB-19, SNB-75, and U251); melanoma (LOX IMVI, MALME-3M, M14, SK-MEL-2, SK-MEL-28, SK-MEL-5, and UACC-257); ovarian cancer (IGROV1, OVCAR-3, OVCAR-4, OVCAR-5, OVCAR-8, and SK-OV-3); renal cancer (786-0, A498, ACHN, CAKI-1, RXF 393, SN12C, TK-10, and UO-31); prostate cancer (PC-3 and DU-145); breast cancer (MCF 7, MCF 7/ ADR-RES, MDA-MB-231/ATCC, HS 578T, MDA-MB-435, MDA-N, and T-47D).

Experimental Part

General. TLC: precoated (0.2 mm) silica gel *60 F_{254}* plates from *EM Laboratories, Inc.*; detection by UV light (254 nm). M.p.: *Electrothermal IA9100* digital melting-point apparatus; uncorrected. ^{1}H-NMR Spectra: *Varian Unity-400* spectrometer at 400 MHz or *Varian Gemini-200* spectrometer at 200 MHz; chemical shifts δ in

ppm with $SiMe_4$ as an internal standard (=0 ppm), coupling constants J in Hz. Elemental analyses were carried out on a *Heraeus CHN-O-Rapid* elemental analyzer, and results were within ±0.4% of calc. values.

7-{4-{[2-(4-Chlorophenyl)-2,3,4,5-tetrahydro-4-methylidene-5-oxofuran-2-yl]methyl}piperazin-1-yl}-1-ethyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic Acid (**5**). To a soln. of 7-{4-[2-(4-chlorophenyl)-2-oxoethyl]-1-piperazinyl}-1-ethyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid [10] (0.47 g, 1 mmol) in dry THF (20 ml), activated Zn powder (0.13 g, 2 mmol), hydroquinone (6 mg), and ethyl 2-(bromomethyl)acrylate (0.26 g, 1.3 mmol) were added. The mixture was refluxed under N_2 for 3.5 h (TLC monitoring). After cooling, it was poured into an ice-cold 5% aq. HCl soln. (100 ml), neutralized with 1.0N $NaHCO_3$, and extracted with CH_2Cl_2 (3 × 60 ml). The combined CH_2Cl_2 extract was washed with H_2O, dried (Na_2SO_4), and evaporated, and the residual solid purified by column chromatography (silica gel, CH_2Cl_2/MeOH 10:1). The product was crystallized from CH_2Cl_2/EtOH 5:1: **5** (0.49 g, 91%). M.p. 248° (dec.). ^{1}H-NMR (200 MHz, CF_3COOD): 1.75 (t, J = 7.1, MeCH$_2$); 3.29 – 3.81 (m, 5 H(pip), 2 H–C(3′)); 4.05 – 4.42 (m, 3 H(pip), CH_2–N(4)); 4.86 (q, J = 7.2, MeCH_2); 6.06 (br. s, 1 H, CH_2=C(4′)); 6.62 (br. s, 1 H, CH_2=C(4′)); 7.40 – 7.59 (m, H–C(8), 4 arom. H); 8.28 (d, J = 12.4, H–C(5)); 9.30 (s, H–C(2)). Anal. calc. for $C_{28}H_{27}ClFN_3O_5$: C 62.28, H 5.04, N 7.78; found: C 62.13, H 4.98, N 7.76.

1-Ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(2-oxopropoxy)quinoline-3-carboxylic Acid (**7a**). 1-Ethyl-6-fluoro-1,4-dihydro-7-hydroxy-4-oxoquinoline-3-carboxylic acid [16] (**6**; 1.00 g, 4 mmol), $NaHCO_3$ (0.34 g, 4 mmol), and dry DMF (15 ml) were stirred at r.t. for 30 min. To this soln., bromoacetone (2.19 g, 16 mmol) was added in one portion. The resulting mixture was stirred at r.t. for 7 h (TLC monitoring). Evaporation gave a residue, which was poured into ice-water (30 ml) and extracted with CH_2Cl_2 (3 × 40 ml). The combined CH_2Cl_2 extract was washed with H_2O, dried (Na_2SO_4), and evaporated, and the residual solid crystallized from EtOH/CH_2Cl_2 5:1: **7a** (0.86 g, 74%). M.p. 255°. ^{1}H-NMR (400 MHz, CF_3COOD): 1.79 (t, J = 7.2, MeCH$_2$); 2.57 (s, MeCO); 4.92 (q, J = 7.2, MeCH_2); 5.29 (s, 2 H–C(1′)); 7.68 (d, J = 6.4, H–C(8)); 8.41 (d, J = 9.6, H–C(5)); 9.40 (s, H–C(2)). Anal. calc. for $C_{15}H_{14}FNO_5$: C 58.63, H 4.59, N 4.56; found: C 58.42, H 4.65, N 4.45.

1-Ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(2-oxo-2-phenylethoxy)quinoline-3-carboxylic Acid (**7b**). As described for **7a**, from **6** and 2-bromoacetophenone: 71% yield. M.p. 261°. ^{1}H-NMR (200 MHz, DMSO): 1.29 (t, J = 7.1, MeCH$_2$); 4.55 (q, J = 7.1, MeCH_2); 6.07 (s, 2 H–C(1′)); 7.65 (m, H–C(8), 3 arom. H); 8.07 (m, H–C(5), 2 arom. H); 8.97 (s, H–C(2)); 15.23 (br. s, COOH). Anal. calc. for $C_{20}H_{16}FNO_5$: C 65.04, H 4.37, N 3.79; found: C 64.92, H 4.34, N 3.73.

1-Ethyl-6-fluoro-1,4-dihydro-4-oxo-7-[(2,3,4,5-tetrahydro-2-methyl-4-methylidene-5-oxofuran-2-yl)methoxy]-quinoline-3-carboxylic Acid (**8a**). As described for **5**, from **7a**: 86% yield. M.p. 223°. ^{1}H-NMR (400 MHz, DMSO): 1.43 (t, J = 7.0, MeCH$_2$); 1.53 (s, Me–C(2′)); 2.88 (dt, J = 17.2, 3.0, 1 H–C(3′)); 3.14 (dt, J = 17.2, 2.4, 1 H–C(3′)); 4.43, 4.46 (2‘d’, AB type, J = 10.8, CH_2O); 4.61 (q, J = 7.2, MeCH_2); 5.77 (t, J = 2.4, 1 H, CH_2=C(4′)); 6.08 (t, J = 2.8, 1 H, CH_2=C(4′)); 7.52 (d, J = 6.8, H–C(8)); 8.02 (d, J = 11.2, H–C(5)); 8.99 (s, H–C(2)); 15.23 (br. s, COOH). Anal. calc. for $C_{19}H_{18}FNO_6$: C 60.80, H 4.83, N 3.73; found: C 60.64, H 4.74, N 3.70.

1-Ethyl-6-fluoro-1,4-dihydro-4-oxo-7-[(2,3,4,5-tetrahydro-4-methylidene-5-oxo-2-phenylfuran-2-yl)methoxy]-quinoline-3-carboxylic Acid (**8b**). As described for **5**, from **7b**: 90% yield. M.p. 212°. ^{1}H-NMR (200 MHz, DMSO): 1.38 (t, J = 6.9, MeCH$_2$); 3.22 (dt, J = 16.8, 2.8, 1 H–C(3′)); 3.68 (dt, J = 16.9, 2.6, 1 H–C(3′)); 4.53, 4.60 (2‘d’, AB type, J = 11.1, CH_2O); 4.69 (q, J = 7.2, MeCH_2); 5.81 (t, J = 2.6, 1 H, CH_2=C(4′)); 6.12 (t, J = 2.9, 1 H, CH_2=C(4′)); 7.49 (m, H–C(8), 5 arom. H); 7.99 (d, J = 11.2, H–C(5)); 9.03 (s, H–C(2)); 15.23 (br. s, COOH). Anal. calc. for $C_{24}H_{20}FNO_6$: C 65.90, H 4.61, N 3.20; found: C 65.72, H 4.56, N 3.17.

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REFERENCES

[1] K. H. Lee, I. H. Hall, E. C. Mar, C. O. Starnes, S. A. Elgebaly, T. G. Waddell, R. I. Hadgraft, C. G. Ruffner, I. Weidner, *Science (Washington, D.C.)* **1977**, *196*, 533.
[2] N. Petragnani, H. M. C. Ferraz, G. V. J. Silva, *Synthesis* **1986**, 157.
[3] H. M. R. Hoffmann, J. Rabe, *Angew. Chem., Int. Ed.* **1985**, *24*, 94.
[4] O. Spring, K. Albert, W. Gradmann, *Phytochemistry* **1981**, *20*, 1883; N. H. Fischer, T. Lu, C. L. Cantrell, J. Castaneda-Acosta, L. Quliano, S. Franzblau, *Phytochemistry* **1998**, *49*, 559; Y. Fukushi, C. Yajima, J. Mizutani, S. Tahara, *Phytochemistry* **1998**, *49*, 593; A. Ortega, R. A. Toscano, E. Maldonado, *Phytochemistry* **1998**, *49*, 1085.

[5] I. S. Chen, I. L. Lai-Yuan, C. Y. Duh, I. L. Tsai, *Phytochemistry* **1998**, *49*, 745.
[6] C. J. Cavallito, T. H. Haskell, *J. Am. Chem. Soc.* **1946**, *68*, 2332; M. T. Gette, J. E. J. James, *Arch. Dermatol*. **1990**, *126*, 203; A. Slob, *Phytochemistry* **1973**, *12*, 811; M. W. I. C. van Rossum, M. Alberda, L. H. W. van der Plas, *Phytochemistry* **1998**, *49*, 723.
[7] Y. L. Chen, T. C. Wang, K. H. Lee, Y. L. Chang, C. M. Teng, C. C. Tzeng, *Helv. Chim. Acta* **1996**, *79*, 651; Y. L. Chen, T. C. Wang, S. C. Liang, C. M. Teng, C. C. Tzeng, *Chem. Pharm. Bull*. **1996**, *44*, 1591; T. C. Wang, Y. L. Chen, S. S. Liou, Y. L. Chang, C. M. Teng, C. C. Tzeng, *Helv. Chim. Acta* **1996**, *79*, 1620; C. C. Tzeng, Y. L. Zhao, Y. L. Chen, S. S. Liou, T. C. Wang, Y. L. Chang, C. M. Teng, *Helv. Chim. Acta* **1997**, *80*, 2337; C. H. Liao, C. C. Tzeng, C. M. Teng, *Eur. J. Pharmacol.* **1998**, *349*, 107; Y. L. Zhao, T. C. Wang, Y. L. Chen, Y. L. Chang, C. M. Teng, C. C. Tzeng, *Chin. Pharm. J.* **1999**, *51*, 49; T. C. Wang, K. H. Lee, Y. L. Chen, S. S. Liou, C. C. Tzeng, *Bioorg. Med. Chem. Lett*. **1998**, *8*, 2773; Y. L. Chen, T. C. Wang, N. C. Chang, C. C. Tzeng, *Helv. Chim. Acta* **1999**, *82*, 191; K. H. Lee, B. R. Huang, C. C. Tzeng, *Bioorg. Med. Chem. Lett*. **1999**, *9*, 241; Y. L. Chen, I. L. Chen, C. C. Tzeng, T. C. Wang, *Helv. Chim. Acta* **2000**, *83*, 989.
[8] C. C. Tzeng, T. C. Wang, Y. L. Chen, C. J. Wang, Y. L. Chang, C. M. Teng, *Helv. Chim. Acta* **1997**, *80*, 1161; T. C. Wang, Y. L. Chen, S. S. Liou, W. F. Tzeng, Y. L. Chang, C. M. Teng, C. C. Tzeng, *Helv. Chim. Acta* **1998**, *81*, 1038; Y. L. Chen, T. C. Wang, K. C. Fang, N. C. Chang, C. C. Tzeng, *Heterocycles* **1999**, *50*, 453; C. C. Tzeng, I. L. Chen, Y. L. Chen, T. C. Wang, Y. L. Chang, C. M. Teng, *Helv. Chim. Acta* **2000**, *83*, 349.
[9] S. M. Kupchan, D. C. Fessler, M. A. Eakin, T. J. Giacobbe, *Science (Washington, D.C.)* **1970**, *168*, 376.
[10] K. C. Fang, Y. L. Chen, J. Y. Sheu, T. C. Wang, C. C. Tzeng, *J. Med. Chem.* **2000**, *43*, 3809.
[11] L. L. Shen, J. Baranowski, A. G. Pernet, *Biochemistry* **1989**, *28*, 3879.
[12] L. L. Shen, W. E. Kohlbrenner, D. Weigl, J. Baronowski, *J. Biol. Chem.* **1989**, *264*, 2973.
[13] J. F. Barrett, J. A. Sutcliffe, T. D. Gootz, *Antimicrob. Agents Chemother.* **1990**, *34*, 1.
[14] M. P. Wentland, G. Y. Lesher, M. Reuman, M. D. Gruett, B. Sinf, S. C. Aldous, P. H. Dorff, J. B. Rake, S. A. Coughlin, *J. Med. Chem*. **1993**, *36*, 2801.
[15] J. J. Clement, N. Burres, K. Jarvis, D. T. W. Chu, J. Swinirski, J. Alder, *Cancer Res.* **1995**, *55*, 830.
[16] C. B. Ziegler, W. V. Curran, N. A. Kuck, S. M. Harris, Y. I. Lin, *J. Heterocycl. Chem*. **1989**, *26*, 1141.
[17] A. Monks, D. Scuderio, P. Skehan, R. Shoemaker, K. Paull, D. Vistica, C. Hose, J. Langlay, P. Cronise, A. Vaigro-Wolff, M. Gray-Goodrich, H. Campbell, J. Mayo, M. Boyd, *J. Natl. Cancer Inst.* **1991**, *83*, 757.
[18] J. Plowman, D. J. Dykes, M. Hollingshead, L. Simpson-Herren, M. C. Alley, 'Human Tumor Xenograft Models in NCI Drug Development', in 'Anticancer Drug Development Guide: Preclinical Screening, Clinical Trials, and Approval', Ed. B. Teicher, Humana Press, Inc., Totowa, N.J., 1997, p. 101.

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