Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 17 (2007) 2939–2943
Bioorganic & Medicinal Chemistry Letters

Geldanamycin derivatives and neuroprotective effect on cultured P19-derived neurons

Sarin Tadtong,[a] Duangdeun Meksuriyen,[b] Somboon Tanasupawat,[c] Minoru Isobe[d] and Khanit Suwanborirux[a,*]

[a]*Center for Bioactive Natural Products from Marine Organisms and Endophytic Fungi (BNPME), Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok 10330, Thailand*
[b]*Department of Biochemistry, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok 10330, Thailand*
[c]*Department of Microbiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok 10330, Thailand*
[d]*Laboratory of Organic Chemistry, Graduate School of Bioagricultural Sciences, Nagoya University, Chikusa, Nagoya 464-8601, Japan*

Received 25 August 2006; revised 22 November 2006; accepted 12 December 2006
Available online 15 December 2006

Abstract—Geldanamycin (**1**), an antifungal and anticancer ansamycin, was reported as a neurotrophic and neuroprotective substance against antineoplastic drugs, paclitaxel, vincristine, and cisplatin, on cultured dorsal root ganglion neurons from chick embryos. In this study, **1** in a large quantity, together with a known 17-*O*-demethylgeldanamycin (**2**), and a new 17-*O*-demethylgeldanamycin hydroquinone (**3**) were obtained from a mangrove *Streptomyces* sp. A series of *O*-alkyl and *N*-alkyl derivatives of **1** were prepared by modification of C-17 and/or C-19 on the quinone ring and were evaluated for in vitro activity against P19-derived neurons. Compound **1** and 19-*O*-methylgeldanamycin (**7**) at a very low dose (1 nM) enhanced survival and neurite outgrowth of P19-derived neurons and prevented neurotoxicity of paclitaxel and vinblastine. Compound **7**, possessing the lowest cytotoxicity and neurotoxicity, is serving as the most promising candidate in neurodegenerative therapy against neurotoxic anticancer drugs.

Geldanamycin (**1**), a 17-substituted benzoquinoid ansamycin possessing antifungal and anticancer activities, was previously reported as the secondary metabolite produced by the terrestrial *Streptomyces hygroscopicus* var. *geldanus*.[1] Initially, **1** was thought to be a nonspecific kinase inhibitor, however, was later found to bind to the ATP binding site of the chaperone heat shock protein 90 (Hsp90), resulting in prevention of several signaling proteins from reaching their mature form, inhibiting their activity, and affecting their stability.[2] The semi-synthetic 17-allylamino-17-demethoxygeldanamycin has been currently studied in phase II clinical trial for treatment of solid tumors.[3] Interestingly, **1** prevented neurotoxic effects of anticancer drugs on cultured dorsal root ganglion neurons from chick embryo at low doses[4] and protected rat brain from focal ischemia.[5] Moreover, **1** exhibited neuroprotective ability on a variety of in vivo neurodegeneration models.[6] In order to further investigate the neuronal effect of other geldanamycin derivatives, P19-derived neurons were used as a neuronal model in our study. P19 embryonal carcinoma cell is a pluripotent stem cell line which is differentiated into neurons by retinoic acid.[7] P19-derived neurons are irreversibly postmitotic and exhibit many characteristics of mature CNS neurons containing particular neurotransmitters such as γ-aminobutyric acid[8] and acetylcholine.[9] The differentiated P19 neuroglial cultures have been used for neurotoxicity evaluation of cysteinylcatechols.[10] In this study, we succeeded in isolating **1** in a large quantity from a mangrove *Streptomyces* sp. The availability of **1** enabled us to prepare a number of geldanamycin analogs for evaluating biological effects on P19 cells and P19-derived neurons. In addition, paclitaxel and vinblastine, the anticancer agents causing irreversibly peripheral neuropathy side effect, were used as neurotoxic substances for evaluation of neuroprotective property.

The antifungal-guided fractionation of the EtOAc extract obtained from the fermentation broth of the

Keywords: Geldanamycin; 19-*O*-methylgeldanamycin; Neuroprotective effect; Cultured P19-derived neurons.
* Corresponding author. Tel.: +66 0 2218 8257; fax: +66 0 2254 5195; e-mail: skhanit@chula.ac.th

0960-894X/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2006.12.041

mangrove *Streptomyces* sp. TRA98752 led to the isolation of **1** in 1.2% yield, based on the EtOAc extract, together with a known 17-*O*-demethylgeldanamycin (**2**),[11] and a new 17-*O*-demethylgeldanamycin hydroquinone (**3**) in 0.04% yield, each. Their chemical structures were determined by extensive analyses of UV, IR, ^{1}H NMR, ^{13}C NMR, 2D NMR, and MS spectra, and comparison with those reported in the literatures.[12–14] The ^{1}H and ^{13}C NMR spectra of all compounds showed almost identical characters of the macrolactam ring and the carbamate moiety. Based on the exact mass of **3** from ESI-TOFMS which showed a pseudomolecular ion peak at *m/z* 571.2649 $[M+Na]^{+}$, its molecular formula was determined as $C_{28}H_{40}N_2O_9$, which was 2 amu greater than that of **2**. The ^{13}C NMR spectrum of **3** was different from that of **2** by the absence of two equivalent quinone carbonyl carbons (δ 183.16) and the presence of two oxygenated aromatic carbons at δ 156.18 (C-18) and δ 143.82 (C-21). Therefore, **3** was deduced as 17-*O*-demethylgeldanamycin hydroquinone which might be oxidized to **2** during the isolation process. The ^{1}H and ^{13}C NMR spectral data of **3** are shown in Table 1.

Table 1. NMR data of **3** in $CDCl_3$:DMSO-d_6 (1:1)

Position	δ_C	δ_H, mult., (*J* in Hz)
1	167.81	—
2	134.00	—
3	126.82	6.98, d (11)
4	125.98	6.51, t (11, 11)
5	136.05	5.77, t (11, 10)
6	81.38	4.25, d (10)
7	80.51	4.93, br s
8	132.84	—
9	132.47	5.73, d (9)
10	31.91	2.59, m
11	71.76	3.41, m
12	81.38	3.23, m
13	29.30	0.99, 2H, br s
14	27.73	1.70, m
15	33.22	2.24, 2H, m
16	113.57	—
17	143.82	—
18	156.18	—
19	106.36	6.89, s
20	132.84	—
21	143.82	—
1-NH	—	9.58, br s
2-CH_3	12.43	1.92, 3H, s
6-OCH_3	56.39	3.14, 3H, s
7-O*C*ONH_2	156.18	6.09, NH_2, br s
8-CH_3	12.84	1.66, 3H, s
10-CH_3	12.43	0.81, 3H, d (7)
12-OCH_3	56.21	3.22, 3H, s
14-CH_3	23.68	0.83, 3H, d (7)

mult. = multiplicity.

All isolated geldanamycins (**1**–**3**) were primarily screened for biological effects on P19-derived neurons[15] at a very low dose of 1 nM by XTT reduction assay[16] as shown in Table 2. Only **1** promoted high cell viability of the cultured neurons (% cell viability = 121), while **2** and **3** were toxic. The results suggested that the *O*-alkyl substituent at C-17 might play important roles for this remarkable effect.

Table 2. Percentages of cell viability of P19-derived neurons treated with **1** and its derivatives at a concentration of 1 nM

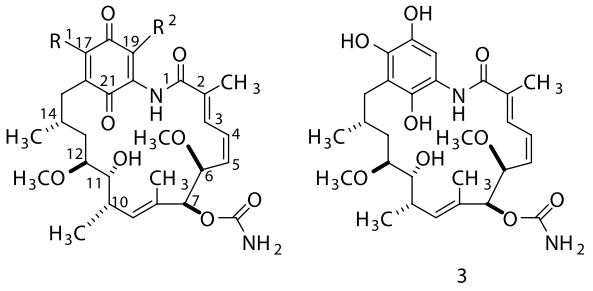
Compound	% Cell viability[a]
1	121
2	50
3	43
4	113
5	121
6	111
7	108
8	0
9	91
10	85
11	49
12	0
13	66
14	44
15	46
16	0
17	0

[a] % Cell viability for tested compounds versus control cells was calculated by the absorbance at 450 nm from three independent experiments and each experiment was run in triplicate.

Therefore, we prepared *O*-alkyl and *N*-alkyl derivatives (**4**–**17**) by modifications of the C-17 and/or C-19 on the quinone moiety of **1** (Figure 1). Three 17-*O*-alkyl-17-*O*-demethyl-geldanamycins (**4**–**6**) were obtained in approximately 70% yields from treatment of **1** with 1.4 equiv of the alkoxides generated from NaH or Na metal and the corresponding alcohols. The reaction between **1** and 3 equiv of the corresponding alkoxides gave the 17,19-di-*O*-alkyl-17-*O*-demethylgeldanamycins (**7**–**8**) in 73 and 15% yields, respectively. Addition of ammonia to the methanolic solution of **1** gave 19-aminogeldanamycin (**9**) in 73% yield. Treatment of **1** with ammonia or primary alkylamines[17] in DMF at room temperature provided the 17-alkylamino (**10**–**14**) and 17,19-di-alkylamino-17-demethoxygeldanamycins (**15**–**17**) in 43–99% yields. All compounds were characterized by UV, ^{1}H NMR, ^{13}C NMR, 2D NMR, and ESI-Q-TOFMS measurements.

All synthesized geldanamycin derivatives at 1 nM were evaluated for their effects on P19-derived neurons by XTT reduction assay to compare with **1** as shown in Table 2. All of the alkylamino derivatives (**9**–**17**) exhibited less % cell viability, indicating their neurotoxicity, while the *O*-alkyl derivatives (**4**–**7**), except the di-*O*-ethyl **8**, promoted viability of the neurons at more than 100% cell viability comparable to **1**. Therefore, the morphology of P19-derived neurons in the presence of **1** and **4**–**7** was further observed under a phase-contrast microscope. Interestingly, the neurites of these neurons possessed more branching than those in the absence of the compounds (data not shown).

As a result, **1** along with **4**–**7** were further comparatively evaluated for their neurotoxicity against P19-derived neurons and cytotoxicity against P19 cells as shown in Figures 2 and 3, respectively. Compounds **1**, **4**, **5**, **6**, and **7** exhibited neurotoxicity at IC_{50} of 1.8, 1.6, 6.7, >10, and >10 μM, and showed cytotoxicity at IC_{50} of 0.1, 0.1, 0.2, 0.5, and >10 μM, respectively. To examine



3

	R^1	R^2		R^1	R^2
1	OCH_3	H	**10**	NH_2	H
2	OH	H	**11**	NH C_2H_5	H
4	OC_2H_5	H	**12**	NH C_3H_7	H
5	OC_3H_7	H	**13**	$NHCH_2CH{=}CH_2$	H
6	$OCH_2C_6H_5$	H	**14**	$NHCH_2C_6H_5$	H
7	OCH_3	OCH_3	**15**	$NHCH_3$	$NHCH_3$
8	OC_2H_5	OC_2H_5	**16**	NH C_3H_7	NH C_3H_7
9	OCH_3	NH_2	**17**	$NH(CH_2)_3OH$	$NH(CH_2)_3OH$

Figure 1. Structures of **1** and its derivatives.

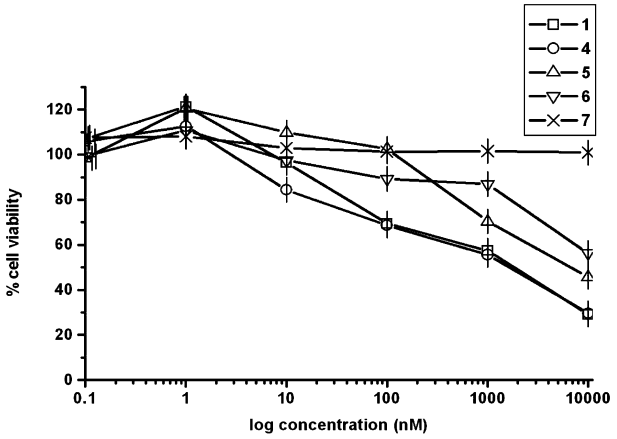


Figure 2. Neurotoxicity of **1** and **4**–**7** on cultured P19-derived neurons incubated for 18 h. % Cell viability for tested compounds versus control cells was calculated by the absorbance at 450 nm. Each point represents the average of three independent experiments ± SEM (bar) and each experiment was run in triplicate.

the neuroprotective activities of **1** and **4**–**7** at the concentration of 1 nM on cultured P19-derived neurons, the compounds were co-treated with paclitaxel at its toxic dose (IC_{50} 0.65 μM). In the presence of only paclitaxel, the neurons were remarkably degenerated and possessed shrinking neurites when observed under a phase-contrast microscope. All of the paclitaxel-treated neurons in the presence of the compounds showed 100% cell viability except that of **7**, which showed better cell viability at 120%. When observed under a phase-contrast microscope, these neurons were found survived and exhibited long-branched neurites.

Compound **7**, the 19-*O*-methylgeldanamycin which showed the highest cell viability and possessed very wide therapeutic index (TI) between neuroprotective and neurotoxic activities (TI > 10,000), was further evaluated for its neuroprotective ability by pre- and post-administration with paclitaxel (0.65 μM) and vinblastine

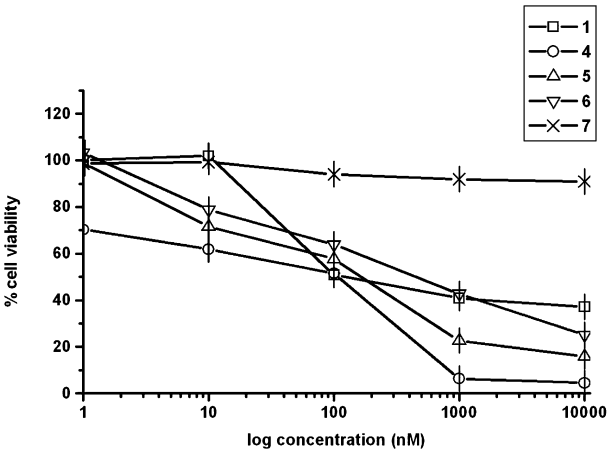


Figure 3. Cytotoxicity of **1** and **4**–**7** on cultured P19 cells incubated for 18 h. % Cell viability for tested compounds versus control cells was calculated by the absorbance at 450 nm. Each point represents the average of three independent experiments ± SEM (bar) and each experiment was run in triplicate.

(0.39 μM) compared to that of **1** (TI 1800). Both compounds showed similar effects and only the data of **7** are shown in Figure 4. Under the pre-treatment with **7** (1 nM) for 18 h and then with paclitaxel or vinblastine for further 18 h, the neurons showed impressive survival and the neurite outgrowth was found to be similar to both the co-treatment experiment as described above and the post-treatment experiment.

The above results led us to define the structure–activity relationships for neuroactivity and cytotoxicity. Four important points have been made: (i) substitutions on the quinone ring with *O*-alkyl groups at C-17 provided neuroprotective activity; (ii) the decreases in neurotoxicity and cytotoxicity were realized in substitution at C-17 with larger *O*-alkyl groups (**1** and **4**–**6**); (iii) di-*O*-alkyl substituents larger than the methyl on the quinone ring (**7**–**8**) increased neurotoxicity; and (iv) substitutions on the quinone ring with *N*-alkyl groups at C-17 and/or C-19 (**9**–**17**) resulted in potent neurotoxicity.

The mechanism(s) of action in neuroprotective activity of these compounds is not easily explained. Recently, neuroprotective activity of **1** was proposed as a consequence of the heat–shock response protecting against neurotoxic substances[6] and the suppression of the signal transduction pathway(s) involving Hsp90 that led to apoptosis.[4] The other study reported that the cancer cells maintaining Hsp90 in an activated conformation were different from the normal cells.[18] The activated Hsp90 might possibly occur in neuronal cells. Therefore, neuroprotective effects of **1** and derivatives at a low dose on neuronal cells might be a consequence of inhibition of this activated Hsp90. However, interactions of the geldanamycins at a limited dose with other specific target molecule(s) to enhance neurons' survival cannot be omitted. The mechanism(s) of the geldanamycins for neuroprotective activity need further investigation.

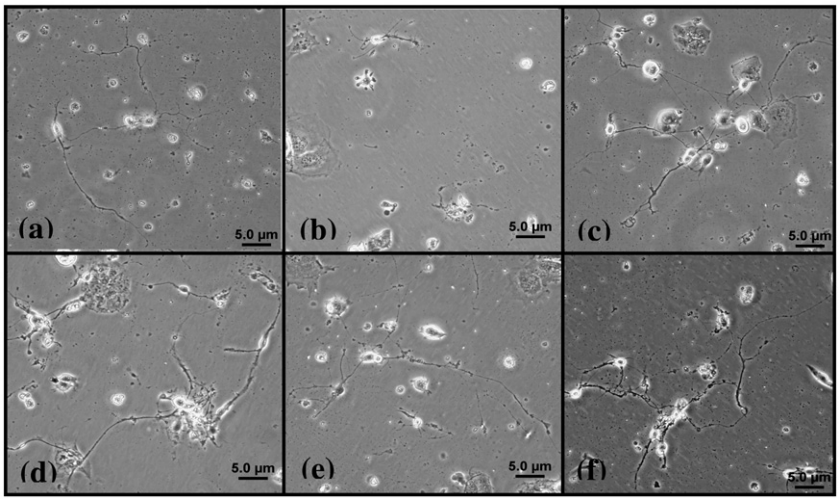


Figure 4. Neuroprotective effect of **7** on cultured P19-derived neurons. Phase-contrast micrographs of cultured P19-derived neurons incubated for 18 h and treated with: (a) no compounds, (b) 0.65 μM paclitaxel, (c) 1 nM **7**, (d) 1 nM **7** in the presence of 0.65 μM paclitaxel, (e) 1 nM **7** for 18 h and then 0.65 μM paclitaxel, and (f) 0.65 μM paclitaxel for 18 h and then 1 nM **7**. Bar = 5 μm at 100×.

In summary, **1** and its 17-*O*-alkyl derivatives at a very low dose (1 nM) enhanced the survival and neurite outgrowth of P19-derived neurons, similar to the effect of **1** on cultured dorsal root ganglion sensory neurons from chick embryos.[4] In contrast, the 17-alkylamino and 17,19-dialkylamino derivatives at the same dose caused neurotoxicity on the P19-derived neurons. The present results strongly suggest that 19-*O*-methylgeldanamycin (**7**), which has dramatically wide therapeutic index between neuroprotective and neurotoxic activities, remains the most promising for further development into neurodegenerative therapy against neurotoxic anticancer agents.

Acknowledgments

BNPME is supported by a grant for centers of excellence, Chulalongkorn University, and Commission of Higher Education's Strategic Consortia for Human Resource Development, Thailand. S. Tadtong is supported by grants from Royal Golden Jubilee (RGJ) Ph.D. program 2002 (PHD/0146/2545), Thailand Research Fund, and JASSO short-term student exchange promotion program (inbound) scholarship 2004, Japan. We thank the National Science and Technology Development Agency, Thailand, for ESI-TOFMS experiment. The Scientific and Technological Research Equipment Center of Chulalongkorn University and the Pharmaceutical Research Instrument Center of the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Thailand, are gratefully acknowledged.

Supplementary data

Supplementary data associated with this article including: fermentation procedure, extraction and isolation of geldanamycins (**1**–**3**); general synthetic procedures, % yields, spectral data of **4**–**17**; and bioactivity assay can be found, in the online version, at doi:10.1016/j.bmcl.2006.12.041.

References and notes

1. DeBoer, C.; Meulman, P. A.; Wnuk, R. J.; Peterson, D. H. *J. Antibiot.* **1970**, *23*, 442.
2. Roe, S. M.; Prodromou, C.; O'Brien, R.; Ladbury, J. E.; Piper, P. W.; Pearl, L. H. *J. Med. Chem.* **1999**, *42*, 260.
3. Cheng, H.; Cao, X.; Xian, M.; Fang, L.; Cai, T. B.; Ji, J. J.; Tunac, J. B.; Sun, D.; Wang, P. G. *J. Med. Chem.* **2005**, *48*, 645.
4. Sano, M. *Neuropharmacology* **2001**, *40*, 947.
5. Lu, A.; Ran, R.; Parmentier-Batteur, S.; Nee, A.; Sharp, F. R. *J. Neurochem.* **2002**, *81*, 355.
6. Gallo, K. A. *Chem. Biol.* **2006**, *13*, 115.
7. Jones-Villeneuve, E. M. V.; McBurney, M. W.; Rogers, K. A.; Kalnins, V. I. *J. Cell Biol.* **1982**, *94*, 253.
8. MacPherson, P. A.; McBurney, M. W. *Methods* **1995**, *7*, 238.
9. Jones-Villeneuve, E. M. V.; Rudnicki, M. A.; Harris, F.; McBurney, M. W. *Mol. Cell. Biol.* **1983**, *3*, 2271.
10. Montine, T. J.; Picklo, M. J.; Amarnath, V.; Whetsell, O.; Graham, D. G. *Exp. Neurol.* **1997**, *148*, 26.
11. Barziley, E.; Ben-Califa, N.; Supino-Rosin, L.; Kashman, Y.; Hirschberg, K.; Elazar, Z.; Neumann, D. *J. Biol. Chem.* **2004**, *279*, 6847.
12. Rinehart, K. L., Jr.; Shield, L. S. *Fortschr. Chem. Org. Naturst.* **1976**, *33*, 231.
13. Omura, S.; Nakagawa, A.; Sadekane, N. *Tetrahedron Lett.* **1979**, *20*, 4323.
14. Schnur, R. C.; Corman, M. L. *J. Org. Chem.* **1994**, *59*, 2581.
15. Parnas, D.; Lineal, M. *Int. J. Dev. Neurosci.* **1995**, *13*, 767.
16. Goodwin, C. J.; Holt, S. J.; Downes, S.; Marshall, N. J. *J. Immunol. Methods* **1995**, *179*, 95.
17. (a) Schnur, R. C.; Corman, M. L.; Gallaschun, R. J.; Cooper, B. A.; Dee, M. F.; Doty, J. L.; Muzzi, M. L.; Moyer, J. D.; DiOrio, C. I.; Barbacci, E. G.; Miller, P. E.; O'Brien, A. T.; Morin, M. J.; Foster, B. A.; Pollack, V. A.; Savage, D. M.; Sloan, D. E.; Pustilnik, L. R.; Moyer, M.

P. *J. Med. Chem.* **1995**, *38*, 3806; (b) Schnur, R. C.; Corman, M. L.; Gallaschun, R. J.; Cooper, B. A.; Dee, M. F.; Doty, J. L.; Muzzi, M. L.; DiOrio, C. I.; Barbacci, E. G.; Miller, P. E.; Pollack, V. A.; Savage, D. M.; Sloan, D. E.; Pustilnik, L. R.; Moyer, J. D.; Moyer, M. P. *J. Med. Chem.* **1995**, *38*, 3813.

18. Kamal, A.; Boehm, M. F.; Burrows, F. J. *Trends Mol. Med.* **2004**, *10*, 283.