JOURNAL OF
MEDICINAL
CHEMISTRY

Volume 40, Number 4 February 14, 1997

Communications to the Editor

Neuritogenic Effect of Epolactaene Derivatives on Human Neuroblastoma Cells Which Lack High-Affinity Nerve Growth Factor Receptors

Hideaki Kakeya, Chizuko Onozawa, Masakazu Sato,† Koshi Arai,† and Hiroyuki Osada*

Antibiotics Laboratory, The Institute of Physical and Chemical Research (RIKEN), Hirosawa 2-1, Wako-shi, Saitama 351-01, Japan, and Medicinal Research Laboratories, Taisho Pharmaceutical Co., Ltd., Yoshino-cho 1-403, Ohmiya-shi, Saitama 330, Japan

Received October 14, 1996

Neurotrophic factors such as nerve growth factor (NGF) are involved in survival, growth, and differentiation in normal and neoplastic nerve cells.[1,2] At least three receptors bearing tyrosine kinase activity for neurotrophic factors have beeen cloned;[3−5] TRK-A encodes a high-affinity receptor for NGF and also binds neurotrophin-3 (NT-3) and neurotrophin-4/5 (NT-4/5).[6−8] TRK-B encodes a receptor for brain-derived neurotrophic factor (BDNF) and also binds NT-3 and NT-4/5, and TRK-C encodes a receptor for NT-3.[9−11] Despite high sequence homology among the receptors, the developmental and physiological functions of each neurotrophic factor appear to be different, suggesting that each factor has a unique role.

In addition, it has been reported that most benign neuroblastomas express high levels of TRK-A, while malignant neuroblastomas, especially those with N-*myc* amplification, express little or no detectable TRK-A mRNA.[12−16] NGF is expected to induce the differentiation of benign neuroblastomas to neurons, but NGF has no effect on malignant neuroblastomas lacking NGF receptors. Therefore, compounds which induce the differentiation of neuroblastomas might be good candidates for pharmaceutical agents for various neurodegenerative diseases.

* Corresponding author: Tel: +81-(48)-467-9541. FAX: +81-(48)-462-4669. E-mail: antibiot@postman.riken.go.jp.
† Taisho Pharmaceutical Co., Ltd.

We have screened microbial products to discover neuritogenic compounds which induced neurite outgrowth in the human neuroblastoma cell line SH-SY5Y that lacks significant TRK family mRNAs.[17,18] During the screening, we isolated a novel, non-protein neurotrophic compound, epolactaene (Figure 1), produced by *Penicillium* sp. 1689-P, having an epoxide-containing γ-lactam ring in the molecule.[19] However, only small quantities of epolactaene could be isolated from the fungus; furthermore, the compound was unstable under light because of a labile triene group in the side chain and an epoxide fused to a γ-lactam ring. In the present paper, we prepared a number of epolactaene derivatives, 3-substituted 3-pyrrolin-2-ones possessing a double bond in the γ-lactam ring, to find compounds with more effective neuritogenic activity. In addition, we described the structure−activity relationship of 3-substituted 3-pyrrolin-2-ones on the neuritogenic effect in SH-SY5Y cells. **MT-5**, 3-acetyl-4,5-dimethyl-5-octadecyloxy-3-pyrrolin-2-one, the most effective derivative for neuritogenic activity in SH-SY5Y cells, also arrested cell cycle progression at G0/G1 like epolactaene.

Chemistry. We focused on the γ-lactam ring of epolactaene in developing derivatives that have more simple structure and potent activity. The intermediates were synthesized essentially according to the procedure of Howard *et al.*,[20] in which α,β-diketones, including aliphatic diketones, react smoothly in aqueous solution pH 7−10 at room temperature with a variety of acetamides possessing a strong electron-withdrawing group in the α-position to give 3-substituted 5-hydroxy-3-pyrrolin-2-ones. For example, as shown in Scheme 1, α,β-diketone (**1**) reacted with acetoacetamide (**2**) to give **3** and the following treatment with alcohols under acidic conditions gave 3-acetyl-4,5-dimethyl-5-(octadecyloxy)-3-pyrrolin-2-one (**MT-5**) in a moderate yield. *N*-Alkylpyrrolinones were also synthesized by a similar procedure.

Results and Discussion. Neurite outgrowth activity was measured as follows. SH-SY5Y cells were plated at a density of 3×10^3 cells per 100 μL per well on a 96-well plate which was precoated with collagen and incubated for 24 h at 37 °C in 5% CO_2 in Dulbecco's modified Eagle medium (DMEM) containing 10% fetal

S0022-2623(96)00719-4 CCC: $14.00

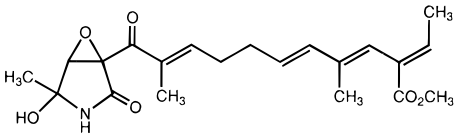
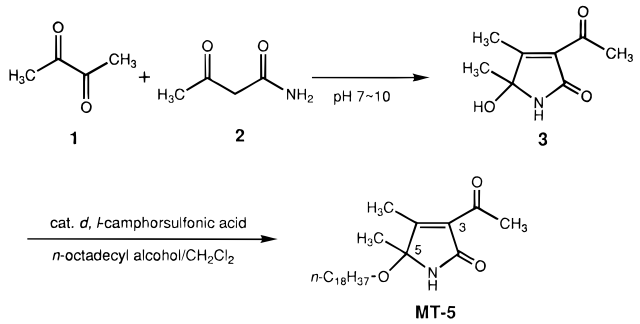


Figure 1. Structure of epoplactaene.

Scheme 1. Synthesis of 3-Acetyl-4,5-dimethyl-5-(octadecyloxy)-3-pyrrolin-2-one (MT-5)



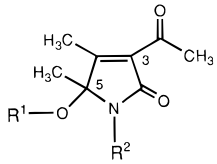
calf serum (FCS). After addition of test samples, the cells were incubated for 2 days at 37 °C in 5% CO_2. Neurite formation was measured as described by Guzwits and Cunningham.[21] In brief, cells exhibiting at least one clearly defined neurite equal to or longer than a diameter of a cell body were scored positive.

A significant portion of epolactaene-treated cells exhibited a bipolar morphology, in which two neurites extend from opposite sides of the cell body in a dose-dependent manner.[19] A series of 3-substituted 3-pyrrolin-2-ones were tested for their ability to induce neurite outgrowth in SH-SY5Y cells (Tables 1 and 2). In the case of 3-acetyl-4,5-dimethyl-5-hydroxy-*N*-alkyl-3-pyrrolin-2-ones, the compounds with a straight long chain alkyl group, *e.g.*, **MT-21**, **MT-20**, **MT-19**, and **MT-17**, showed the moderate neuritogenic activity, and **MT-21** with an octyl group was most effective within this group (Table 1). In the case of 3-acetyl-4,5-dimethyl-5-alkoxy-3-pyrrolin-2-ones, **MT-5**, which possessed an octadecyloxy group at the 5-position, induced neurite outgrowth most effectively among compounds with a straight chain alkoxy group. **MT-5** caused characteristic changes in the morphology of SH-SY5Y cells (Figure 2a). The control cells cultured without drugs extended quite a few neurites (an average of <5% neurite-bearing cells/total cells). When cells were treated with **MT-5** at concentrations of 7.4−59.4 μM, many neurites were extended from cell bodies in a dose-dependent manner (an average of 66.6% neurite-bearing cells/total cells at a dose of 59.4 μM). Neurite formation in SH-Y5Y cells was also induced by 1 mM of dibutyryl cAMP (an average of 62% neurite-bearing cells/total cells) as shown in Figure 2b. Other compounds with an electron-withdrawing moiety on the alkoxy group, *e.g.*, a nitro ethoxy group or a cyano ethoxy group, were completely inactive even at higher concentrations.

Next, we investigated the effect of the subsituted group at the 3-position of 3-pyrrolin-2-ones as shown in Table 2. Interestingly, the compounds with a cyano, a carbamoyl, a methoxycarbonyl, or a benzyl group were completely inactive in contrast to the compounds with an acetyl group at the 3-position.

Addition of 3-substituted 3-pyrrolin-2-ones such as **MT-5** and **MT-21** stopped cells from proliferating and

Table 1. Neuritogenic Effect by 3-Acetyl-4,5-dimethyl-3-pyrrolin-2-ones in SH-SY5Y Cells

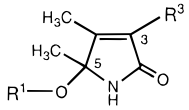


entry	compound	R^1	R^2	activity[a]
1	**MT-1**	H	H	−
2	**MT-9**	H	CH_3	−
3	**MT-10**	H	C_2H_5	−
4	**MT-15**	H	*n*-C_3H_7	−
5	**MT-16**	H	*n*-C_4H_9	−
6	**MT-21**	H	*n*-C_8H_{17}	++[b]
7	**MT-20**	H	*n*-$C_{12}H_{25}$	+[c]
8	**MT-19**	H	*n*-$C_{16}H_{33}$	+[d]
9	**MT-17**	H	*n*-$C_{18}H_{37}$	+[e]
10	**MT-3**	CH_3	H	−
11	**MT-2**	C_2H_5	H	−
12	**MT-8**	*n*-C_3H_7	H	−
13	**MT-4**	$CH(CH_3)_2$	H	−
14	**MT-6**	*n*-C_6H_{13}	H	+[f]
15	**MT-7**	*n*-$C_{14}H_{29}$	H	−
16	**MT-5**	*n*-$C_{18}H_{37}$	H	+++[g]
17	**MT-51**	$CH_2CH_2NO_2$	H	−
18	**MT-52**	CH_2CH_2CN	H	−
19	**MT-53**	CH_2Ph	H	−
20	**MT-29**	CH_2CH_2Ph	H	−
21	**MT-54**	$CH_2CH_2OCH_3$	H	−
22	**MT-22**	$CH_2CH_2CO_2CH_3$	H	−
23	**MT-24**	$CH_2CH_2CH_2ONO_2$	H	−
24	**MT-11**	CH_3	CH_3	−
25	**MT-13**	CH_3	C_2H_5	−
26	**MT-12**	C_2H_5	CH_3	−
27	**MT-14**	C_2H_5	C_2H_5	−

[a] Plus sign indicates a significant difference in the percentage of cells exhibiting a bipolar morphology at 48 h relative to 0.1% MeOH- or 0.1% DMSO-treated control cells. One plus, two plus, and three plus signs indicate that the percentage of neurite-bearing cells/total cells are 5−20%, 20−40%, and >40%, respectively. A minus sign indicates no significant difference from control. The compounds which were inactive on the neurite outgrowth were tested at concentrations which were not toxic. At least two independent experiments with triplicate samples were performed for each treatment. [b] 17.8 μM. [c] 29.5 μM. [d] 25.4 μM. [e] 118.8 μM. [f] 98.8 μM. [g] 59.4 μM.

induced them to differentiate. Thus, the effect of SH-SY5Y cells on the cell cycle progression was investigated by a flow cytometry. SH-SY5Y cells were plated at a density of 1×10^5 cells per 500 μL per well on a 24-well plate precoated with collagen. After incubation with various concentrations of **MT-5** or **MT-21** for 2 days at 37 °C, the cells were collected and stained with the 50 μg/mL of propidium iodide solution containing 0.1% sodium citrate and 0.2% Nonidet P-40. DNA histograms were obtained by using a flow cytometer equipped with an argon-ion laser at 488 nm (Figure 3). In the control cells, cells with 2C DNA (G0/G1 phase) and 4C DNA (G2/M phase) and cells between 2C and 4C (S phase) were observed. In the cells treated with **MT-5** or **MT-21**, the fraction of S-phase cells decreased while those in G0/G1 phase increased. The ratios of G1-phase cells to S-phase cells are 2.9, 7.0, and 7.1 in the cells treated with 0.1% MeOH, **MT-5**, and **MT-21**, respectively, suggesting that **MT-5** (59.4 μM) and **MT-21** (8.9 μM) significantly arrested the cell cycle at G0/G1 phase. Epolactaene also had the same effect in SH-SY5Y cells. Taxol which did not induce the neurite outgrowth caused accumulation of the M phase cells with 4C DNA

Table 2. Neuritogenic Effect by 3-Substituted 4,5-Dimethyl-3-pyrrolin-2-ones in SH-SY5Y Cells



entry	compound	R^1	R^3	activity[a]
16	**MT-5**	*n*-$C_{18}H_{37}$	$C(O)CH_3$	+++[b]
28	**MT-32**	H	CN	−
29	**MT-33**	CH_3	CN	−
30	**MT-47**	*n*-C_3H_7	CN	−
31	**MT-38**	*n*-$C_{18}H_{37}$	CN	−
32	**MT-35**	CH_2CH_2CN	CN	−
33	**MT-41**	CH_2CH_2Ph	CN	−
34	**MT-40**	H	$C(O)NH_2$	−
35	**MT-42**	CH_3	$C(O)NH_2$	−
36	**MT-48**	*n*-C_3H_7	$C(O)NH_2$	−
37	**MT-43**	*n*-$C_{18}H_{37}$	$C(O)NH_2$	−
38	**MT-45**	CH_2CH_2Ph	$C(O)NH_2$	−
39	**MT-57**	H	CO_2CH_3	−
40	**MT-58**	CH_3	CO_2CH_3	−
41	**MT-59**	*n*-$C_{18}H_{37}$	CO_2CH_3	−
42	**MT-85**	CH_3	C(O)Ph	−

[a] Plus sign indicates a significant difference in the percentage of cells exhibiting a bipolar morphology at 48 h relative to 0.1% MeOH- or 0.1% DMSO-treated control cells. Three plus indicates that the percentage of neurite-bearing cells/total cells is >40%. A minus sign indicates no significant difference from control. The compounds which were inactive on the neurite outgrowth were tested at concentrations which were not toxic. At least two independent experiments with triplicate samples were performed for each treatment. [b] 59.4 μM.

contents. The IC_{50}s of inhibitory activity on SH-SY5Y cell growth were >238 μM (**MT-5**) and >35.6 μM (**MT-21**) as determined by viable staining with the dye MTT.[22]

Our studies on the 3-substituted 3-pyrrolin-2-ones, epolactaene analogs, reveal the importance of several chemical groups in the structure for biological activity in SH-SY5Y cells. **MT-1**, **MT-38**, **MT-43**, and **MT-59** are completely inactive, but **MT-5** and **MT-21** are more effective in the neuritogenic activity in SH-SY5Y cells as shown in Tables 1 and 2. The acetyl group at the 3-position is more important, as is one straight long chain alkyl group connected to the γ-lactam ring at least. These groups may be involved in the primary recognition of the target for activity. In compounds which showed moderate neuritogenic activity, the potency of neuritogenic effect in SH-SY5Y cells is **MT-5** > **MT-21** > **MT19**, **MT-20**, but that of neuritogenic effect in rat pheochromocytoma PC12 cells is **MT-19**, **MT-20** > **MT-5**, **MT-21** (data not shown). **MT-21** revealed greater cytotoxicity than its neuritogenic effect in PC12 cells, inducing apoptotic cell death (data not shown). PC12 cells express high levels of TRK-A, while SH-SY5Y cells express little or no detectable TRK-A mRNA. The mode of action of these MT compounds might be due to the different signal transduction in PC12 cells and SH-SY5Y cells.

Recently, it has been reported that lactacystin inhibited the cell cycle progression at G0/G1 phase and induced neurite outgrowth in mouse neuroblastoma cell line Neuro 2A, through the inhibition of proteasome activity.[23−26] Lactacystin does not act on the nerve growth factor signaling pathway, at least as manifested in PC12 cells, but like **MT-21**, it is toxic to PC12 cells.[25] In addition, specific interactions between protease and

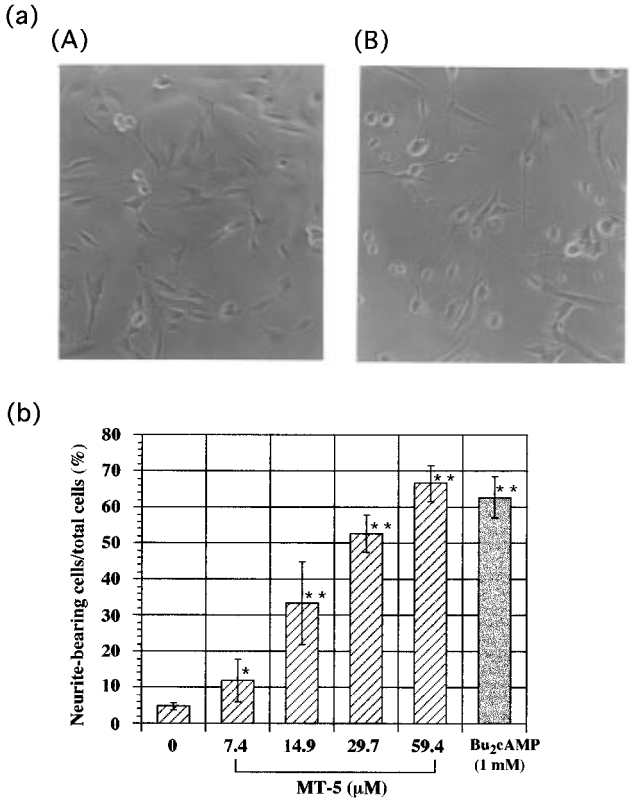


Figure 2. Neuritogenic effect by MT-5 in SH-SY5Y cells. (a) Photomicrographs of SH-SY5Y cells viewed 48 h after the following treatments: (A) 0.1% MeOH; (B) 59.4 μM MT-5. (b) Neuritogenic effect: The number of neurite-bearing cells were counted as described in the text. Bu_2cAMP indicates dibutyryl cyclic AMP. All experiments were performed in triplicate. Values are means ± SD. *p < 0.01, **p < 0.001 relative to 0.1% MeOH-treated control cells.

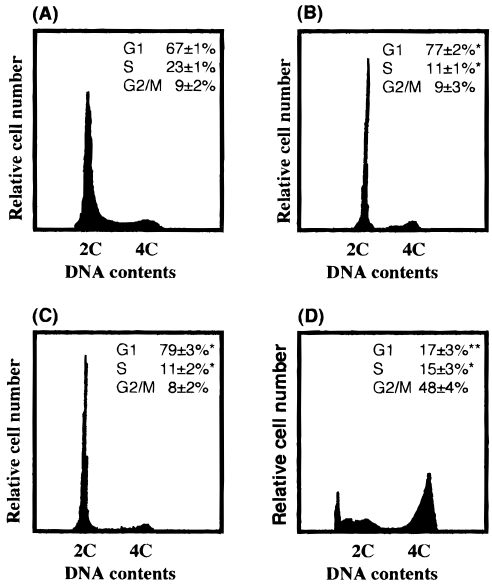


Figure 3. Flow cytometry analyses of propidium iodide-stained SH-SY5Y cells. Cells were cultured with (A) 0.1% MeOH, (B) 59.4 μM MT-5, (C) 8.9 μM MT-21, and (D) 1 μM Taxol. After treatment, the cells were analyzed as described in the text. Results are representative of three independent experiments. Values are means ± SD. *p < 0.01, **p < 0.001 relative to 0.1% MeOH-treated control cells.

protease inhibitors have been suggested to play a role in the regulation of neurite outgrowth.[27,28] However, **MT-5**, **MT-19**, **MT-20**, and **MT-21** did not inhibit the

activity of some proteases, papain, trypsin, cathepsin B, and α-chymotrypsin *in vitro*, even at much higher concentrations than the effective dose on neurite outgrowth in cultured cells (data not shown). Defining the target molecule for these compounds and clarifying the relationships between neuritogenesis and arrest of cell cycle may be useful for understanding the mechanism involved in neuronal differentiation.

Conclusion. A series of 3-substituted 3-pyrrolin-2-ones were designed as epolactaene, a novel non-protein neurotrophic factor, related analogs. The following relationships between the structures and neuritogenic activity in SH-SY5Y cells were noted: an epoxide ring fused to the γ-lactam ring in epolactaene is not always necessary for the biological activity, and at least one straight long chain alkyl group and a carbonyl group at the 3-position are required for the biological activity. The observed structure-activity relationships suggested that epolactaene and related 3-pyrrolin-2-ones analogs may act *via* acylation of one or more relevant target molecule(s) in the cell. We obtained several promising compounds, **MT-5**, **MT-19**, **MT-20**, and **MT-21** on the based on the ability to selectively induce neurite outgrowth in different cultured cells. As most neuroblastoma cell lines are derived from patients with advanced stage disease and have poor clinical prognosis, these results demonstrated a promising class of potential chemotherapeutics which can induce the differentiation of neuroblastoma tumors.

Acknowledgment. We would like to thank Drs. H. Matsui and M. Ino (St. Marianna University) for the gift of SH-SY5Y cells. We are also grateful to Dr. Phillip C. C. Liu (The University of Michigan Medical School) for the preparation of the manuscript. This work was supported in part by Special Grant for Promotion of Research from The Institute of Physical and Chemical Research (RIKEN).

Supporting Information Available: Experimental, spectral, and analytical data for 3-substituted 3-pyrrolin-2-ones (5 pages). Ordering information is given on any current masthead page.

References

(1) Barde, Y.-A. Trophic Factors and Neuronal Survival. *Neuron* **1989**, *2*, 1525−1534.
(2) Vantini, G.; Skaper, S. D. Neurotrophic Factors; From Physiology to Pharmacology? *Pharmacol. Res.* **1992**, *26*, 1−15.
(3) Martin-Zanca, D.; Hughes, S. H.; Barbacid, M. A Human Oncogene Formed by the Fusion of Truncated Tropomyosin and Protein Tyrosine Kinase Sequences. *Nature (London)* **1986**, *319*, 743−748.
(4) Klein, R.; Conway, D.; Parada, L. F.; Barbacid, M. The *TrkB* Tyrosine Protein Kinase Gene Codes for a Second Neurogenic Receptor that Lacks the Catalytic Kinase Domain. *Cell* **1990**, *61*, 647−656.
(5) Middlemas, D. S.; Lindberg, R. A.; Hunter, T. *TrkB*, a Neural Receptor Protein-Tyrosine Kinase: Evidence for a Full-Length and two Truncated Receptors. *Mol. Cell. Biol.* **1991**, *11*, 143−153.
(6) Berkemeier, L. R.; Winslow, J. W.; Kaplan, D. R.; Nikolics, K. Goeddel, D. V.; Rosenthal, A. Neurotrophin-5: A Novel Neurotrophic Factor that Activates *Trk* and *TrkB*. *Neuron* **1991**, *7*, 857−866.
(7) Kaplan, D. R.; Hempstead, B. R.; Martin-Zanca, D.; Chao, M. V.; Parada, L. F. The *Trk* Proto-Oncogene Product: A Signal Transducing Receptor for Nerve Growth Factor. *Science* **1991**, *252*, 554−558.
(8) Klein, R.; Jing, S.; Nanduri, V.; O'Rourke, E.; Barbacid, M. The *Trk* Proto-Oncogene Encodes a Receptor for Nerve Growth Factor. *Cell* **1991**, *65*, 189−197.
(9) Klein, R.; Nanduri, V.; Jing, S.; Lamballe, F.; Tapley, P.; Bryant, S.; Cordon-Cardo, C.; Jones, K. R.; Reichardt, L. F.; Barbacid, M. The *TrkB* Tyrosine Protein Kinase is a Receptor for Bain-Derived Neurotrophic Factor and Neurotrophin-3. *Cell* **1991**, *66*, 395−403.
(10) Squinto, S. P.; Stitt, T. N.; Aldrich, T. H.; Davis, S.; Bianco, S. M.; Radziejewski, C.; Glass, D. J.; Masiakowski, P.; Furth, M. E.; Valenzuela, D. M.; DiStefano, P. S.; Yancopoulos, G. D. *TrkB* Encodes a Functional Receptor for Brain-Derived Neurotrophic Factor and Neurotrophin-3 but not Nerve Growth Factor. *Cell* **1991**, *65*, 885−893.
(11) Lamballe, F.; Klein, R.; Barbacid, M. *TrkC*, a New Member of the *Trk* Family of Tyrosin Protein Kinases, is a Receptor for Neurotrophin-3. *Cell* **1991**, *66*, 967−979.
(12) Nakagawara, A.; Arima, M.; Azar, C. G.; Scavarda, N. J.; Brodeur, G. M. Inverse Relationship between *Trk* Expression and N-myc Amplification in Human Neuroblastomas. *Cancer Res.* **1992**, *52*, 1364−1368.
(13) Nakagawara, A.; Arima-Nakagawara, M.; Scavarda, N. J.; Azar, A. B.; Cantor, A. B.; Brodeur, G. M. Association between High Levels of Expression of the TRK Gene and Favorable Outcome in Human Neuroblastoma. *N. Engl. J. Med.* **1993**, *328*, 847−854.
(14) Azar, C. G.; Scavarda, N. J.; Retnolds, P.; Brodeur, G. M. Multiple Defects of the Nerve Growth Factor Receptor in Human Neuroblastomas. *Cell Growth Differ.* **1990**, *1*, 421−428.
(15) Baker, D. L.; Reddy, U. R.; Pleasure, D.; Thorpe, C. L.; Evans, A. E.; Cohen, P. S.; Ross, A. H. Analysis of Nerve Growth Factor Receptor Expression in Human Neuroblastoma and Neuroepithelioma Cell Lines. *Cancer Res.* **1989**, *49*, 4142−4146.
(16) Marchetti, D.; Perez-Polo, J. R. Nerve Growth Factor Receptors in Human Neuroblastoma Cells. *J. Neurochem.* **1987**, *49*, 475−486.
(17) Ross, R. A.; Biedler, J. L. Presence and Regulation of Tyrosinase Activity in Human Neuroblastoma Cell Variants *in vitro*. *Cancer Res.* **1985**, *45*, 1628−1632.
(18) Nakagawara, A.; Azar, C. G.; Scavarada, N. J.; Brodeur, G. M. Expression and Function of TRK-B and BDNF in Human Neuroblastomas. *Mol. Cel. Biol.* **1994**, *14*, 759−767.
(19) Kakeya, H.; Takahashi, I.; Okada, G.; Isono, K.; Osada, H. Epolactaene, a Novel Neuritogenic Compound in Human Neuroblastoma cells, Produced by a Marine Fungus. *J. Antibiot.* **1995**, *48*, 733−735.
(20) Howard, E. G.; Lindsey, R. V., Jr.; Theobald, C. W. Synthesis of 3-Substituted 5-Hydroxy-3-pyrrolin-2-ones. *J. Am. Chem. Soc.* **1959**, *81*, 4355−4358.
(21) Guzwitz, D.; Cunningham, D. D. Thrombin Modulates and Reverses Neuroblastoma Neurite Outgrowth. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 3440−3444.
(22) Mossman, T. Rapid Colorimetric Assay for Cellular Growth and Survival: Application to Proliferation and Cytotoxicity Assays. *J. Immun. Methods* **1983**, *656*, 55−63.
(23) Omura, S.; Fujimoto, T.; Otoguro, K.; Matsuzaki, K.; Moriguchi, R.; Tanaka, H.; Sasaki, Y. Lactacystin, a Novel Microbial Metabolite, Induces Neuritogenesis of Neuroblastoma Cells. *J. Antibiot.* **1991**, *44*, 113−116.
(24) Omura, S,; Matsuzaki, K.; Fujimoto, T. Kosuge, K.; Furuya, T.; Fujita, S.; Nakagawa, A. J. Structure of Lactacystin, a New Microbial Metabolite Which Induces Differentiation of Neuroblastoma Cells. *J. Antibiot.* **1991**, *44*, 117−118.
(25) Fenteany, G.; Standaert, R. F.; Reichrad, G. A.; Corey, E. J.; Schreiber, S. L. A β-Lactone Related to Lactacystin Induces Neurite Outgrowth in a Neuroblastoma Cell Line and Inhibits Cell Cycle Progression in an Osteosarcoma Cell Line. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 3358−3362.
(26) Fenteany, G.; Standaert, R. F.; Lane, W. S.; Choi, S.; Corey, E. J.; Schreiber, S. L. Inhibition of Proteasome Activities and Subunit-Specific Amino-Terminal Threonine Modification by Lactacystin. *Science* **1995**, *268*, 726−731.
(27) Monard, D. Role of Protease inhibition in Cellular Migration and Neuritic Growth. *Biochem. Pharmacol.* **1987**, *36*, 1389−1392.
(28) Saito, Y.; Kawashima, S. Enhancement of Neurite Outgrowth in PC12h Cells by a Protease Inhibitor. *Neurosci. Lett.* **1988**, *89*, 102−107.

JM960719A