Synthesis and Antiangiogenic Activity of Staurosporine Derivatives

Sir:

Staurosporine (**1**), a natural product first isolated by ŌMURA *et al.*[1] from *Saccharothrix aerocolonigenes* subsp. *staurosporeus* AM-2282[2], was found to be a very potent inhibitor of protein kinase C (PKC) by TAMAOKI *et al.*[3]. Subsequently, **1** was found to have various pharmacological effects such as inhibition of platelet aggregation[4], smooth muscle concentration[5], and angiogenesis[6].

We tried to develop staurosporine analogs for new and novel types of antiangiogenic agents. Thus, we focused on chemical modification of the amino sugar moiety.

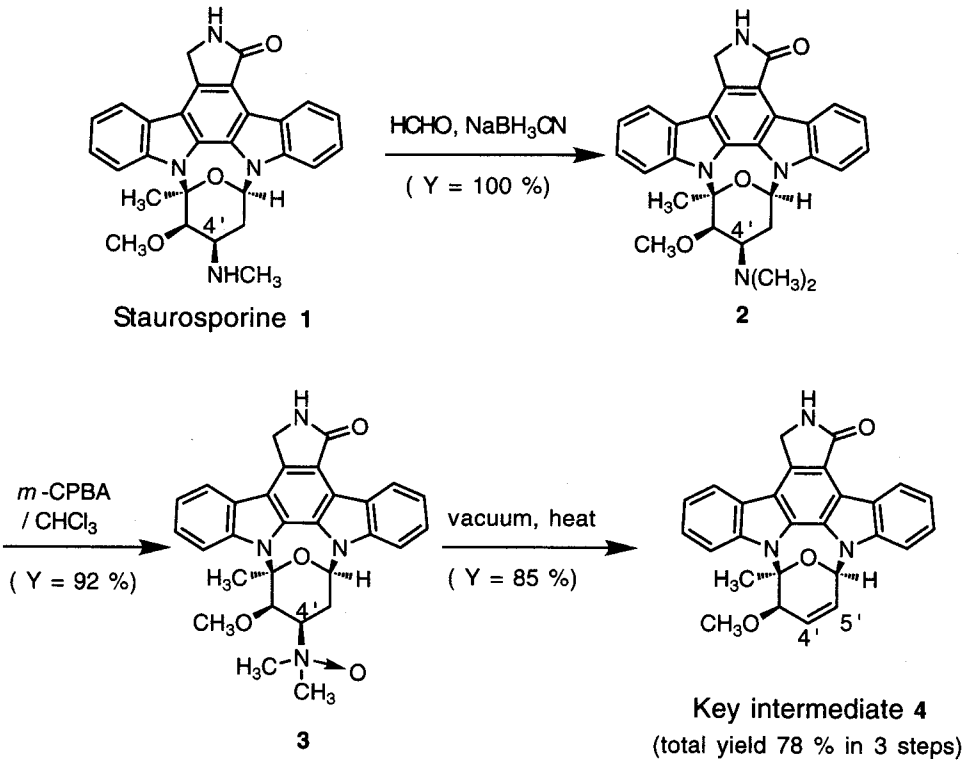
This report describes the synthesis and antiangiogenic activity of staurosporine derivatives with modified amino sugar moieties.

The synthesis of a 3′,4′-olefin (**4**) derivative was accomplished by reductive *N*-methylation (aq. HCHO, $NaBH_3CN$) and *N*-oxidation (*m*-CPBA, $CHCl_3$) followed by Hofmann degradation (160°C, 1 mmHg)[7] with a 78% overall yield in 3 steps, as outlined in Scheme 1. Next, dihydroxylation (OsO_4, NMO) of **4** produced **5**, which was identical to MLR-52[8], with a 65% yield. Protection of **5** with 1,1′-thiocarbonyldiimidazole in THF resulted a 4′,5′-*O*-*cyclo*-thiocarbonate (**6**) with a 60% yield. Selective reduction (n-Bu_3SnH, AIBN, 47% yield)[9] of **6** to a 4′-(*S*)-hydroxy derivative (**7**) followed by Moffatt oxidation[10] to ketone (**8**) (80% yield) and stereoselective reduction (K-selectride, 50% yield) produced a 4′-(*R*)-hydroxy derivative (**9**), which was identical to RK-286c[11] (Scheme 2).

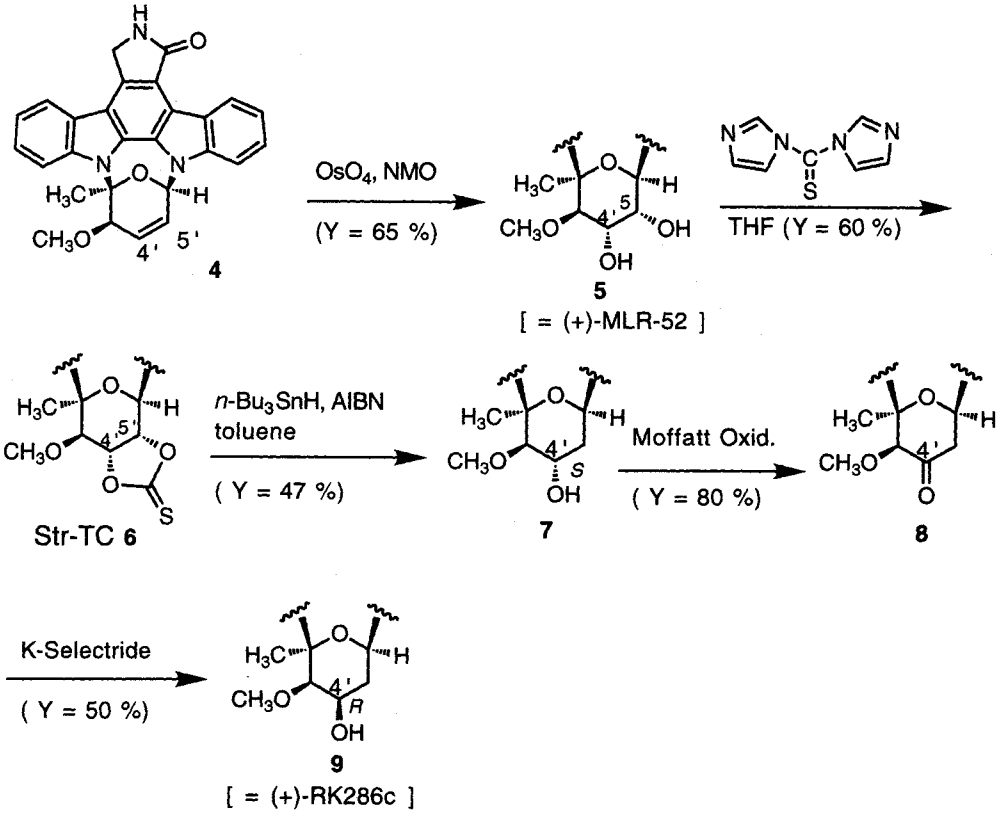
On the other hand, hydroboration of **4** [1) BH_3 2) H_2O_2-NaOH] gave the 5′-(*R*)-hydroxy intermediate (**10**) with a 80% yield. Moffatt oxidation[10] of **10** to 5′-ketone (**11**) (88% yield) and stereoselective reduction (K-selectride, 61% yield) produced a 5′-(*S*)-hydroxy derivative (**12**). Protection of **10** with 1,1′-thiocarbonyldiimidazole in THF resulted **13** with a 58% yield. Reduction of **13** with n-Bu_3SnH and AIBN produced a 4′-de-*N*-methylamino derivative (**14**) with a 63% yield (Scheme 3). The structures of these derivatives were determined by NMR analysis, IR, and mass spectrometry.

Antiangiogenic activities of these compounds were investigated by the sandwich method[12]. Calf pulmonary artery endothelial cells (CPAE) were suspended in a collagen gel mixture of 8 vol. Cellgen (Type I collagen, Koken), 1 vol. reconstructive buffer containing 50 mM NaOH, 260 mM $NaHCO_3$, and 200 mM HEPES, and 1 vol.

Scheme 1.



Scheme 2.



Scheme 3.

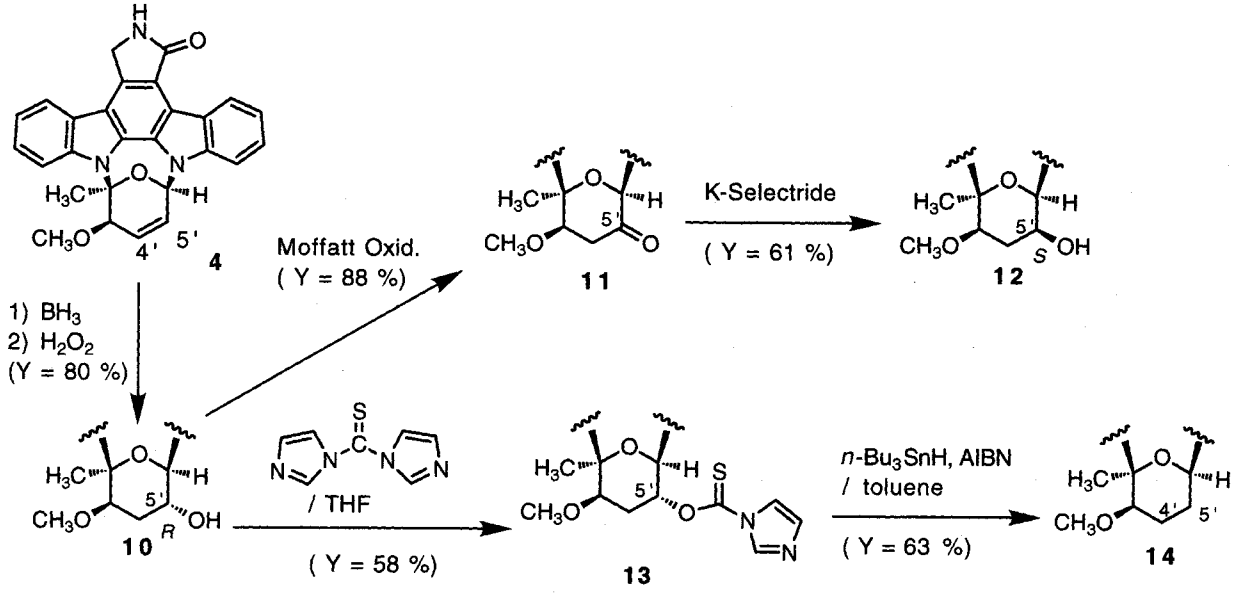


Table 1. Antiangiogenic effect of staurosporine derivatives.

Compound	Antiangiogenesis MIC : μM [A] (IC_{50} : μM)	Cytotoxicity (IC_{50} : μM)		Specificity [a]	
		CPAE [B]	HeLa [C]	[B]/[A]	[B]/[C]
1	<0.0013 (0.006)	0.0011	0.0084	>0.79	0.13
2	>2.1	>2.1	0.055	-	>38
3	0.32	1.6	7.3	5.0	0.22
4	>2.3	0.30	5.6	<0.13	0.053
5	0.043	>0.21	0.019	>5.0	>11
6	0.016 (0.42)	2.0	0.35	125	5.7
7	0.09	1.1	1.0	12	1.0
8	5.5	3.6	4.0	0.66	0.91
9	0.18	0.62	0.26	3.5	2.4
10	0.028	0.22	0.027	8.0	8.2
11	0.055	0.055	0.27	1.0	0.20
12	0.11	0.22	0.073	2.0	3.0
13	<0.04 (0.22)	3.0	1.4	>85	2.1
14	0.73	16	1.1	22	15

[a] B/A level was specific for the activity
(A: Minimum Inhibitory concentration, B: cytotoxicity)

10-fold concentration of EAGLE's minimum essential medium (MEM, Gibco) at pH 7.4, and adjusted to 2.5×10^5 cells/ml. One hundred μl of collagen-cell mixtures was poured into a 96-well multiple well plate (Sumiron, Sumitomo) and incubated at 37°C in a CO_2 incubator for 30 minutes. After gelation, 100 μl of 20% FBS-MEM medium with sample was challenged and incubated at 37°C in a CO_2 incubator for 3 days. Angiostatic activity was evaluated by the observation of the morphological change. The results are summarized in Table 1.

Staurosporine (**1**) showed strong antiangiogenic activity, IC_{50}=6 nM; however, it also showed strong non-specific cytotoxicity. It doesn't show selective toxicity.

The derivatives prepared using the above methodology gave decreased antiangiogenic activity, but some of them had significantly decreased cytotoxicity, and showed prominent selective toxicity. Derivatives **6**, **7**, **10**, **13**, and **14** showed high specificity (B/A) levels. **6** and **13** showed especially strong antiangiogenic activity, IC_{50}=0.42 and 0.22 μM, respectively. The cytotoxicity was very weak, IC_{50}=2.0 and 3.0 μM, respectively. The specificity (B/A) level was increased in both cases.

Moreover, **13** inhibited the tumor angiogenesis caused by tumor inoculation in syngenic mice *in vivo*. Therefore, the most interesting compound is **13**. Analytical data of **13**: HR-MS calcd. for $C_{31}H_{25}N_5O_4S$ 563.1627, found 563.1631; IR (KBr) cm^{-1} 3128, 2939, 1679, 1635; ^1H NMR (400 MHz, CD_3OD) δ 1.91 (1H, dd, J=2.0, 15.0 Hz, 4'-H), 2.39 (3H, s, 2'-CH_3), 2.32~2.46 (1H, m, 4'-H), 3.84 (3H, s, OCH_3), 4.08 (1H, dd, J=3.6, 12.2 Hz, 3'-H), 4.93 (2H, s, 7-H_2), 6.21 (1H, s, 5'-H), 6.51 (1H, s, 6'-H), 7.11 (1H, s, imidazole), 7.26~7.59 (5H, m, aromatic), 7.76 (1H, s, imidazole), 7.86 (2H, m, aromatic), 8.48 (1H, s, imidazole), 9.30 (1H, d, J=7.9 Hz, aromatic).

Therefore, we are very interested in testing the new staurosporine derivative **13** as an antiangiogenic agent.

Further details of its mode of action will be reported elsewhere.

ZHUORONG LI
TOSHIAKI SUNAZUKA
RINTARO YAMADA
YUMIKO KATO
AKIKO ENOMOTO
MASAHIKO HAYASHI
YOSHIHIRO HARIGAYA
SATOSHI ŌMURA*

Research Center for Biological Function,
The Kitasato Institute, and Kitasato University,
5-9-1 Shirokane, Minato-ku, Tokyo 108, Japan

(Received December 9, 1999)

References

1) ŌMURA, S.; Y. IWAI, A. HIRANO, A. NAKAGAWA, J. AWAYA, H. TSUCHIYA, Y. TAKAHASHI & R. MASUMA: A new alkaloid AM-2282 of streptomyces origin taxonomy, fermentation, isolation and preliminary characterization. J. Antibiotics 30: 275~282, 1977
2) TAKAHASHI, Y.; M. SHINOSE, A. SEINO, Y. IWAI & S. ŌMURA: Transfer of saturosporine-producing strain *Streptomuces staurosporeus* AM-2282 to the genus *Saccharothrix aerocolonigenes*. Actinomycetologica 9: 19~26, 1995
3) TAMAOKI, T.; H. NOMOTO, I. TAKAHASHI, Y. KATO, M. MORIMOTO & F. TOMITA: Staurosporine, a potent inhibitor of phospholipid/Ca^{++} dependent protein kinase. Biochem. Biophys. Res. Commun. 135: 397~402, 1986
4) ŌMURA, S.; Y. SASAKI, Y. IWAI & H. TAKESHIMA: Staurosporine, a potentially important gift from a microoraganism. J. Antibiotics 48: 535~548, 1995
5) SETO, M.; Y. SASAKI & Y. SASAKI: Alternation in the myosin phosphorylation pattern of smooth muscle by phorbol ester. Am. J. Physiol. 259: C769~C774, 1990
6) OIKAWA, T.; M. SHIMAMURA & H. ASHINO: Inhibition of angiogenesis by staurosporine, a potent protein kinase inhibitor. J. Antibiotics 45: 1155~1160, 1992
7) JONES, P. A. & E. K. ROWLEY: Chemical modification of erythromycin antibiotics, I. 3′-De(dimethylamino)erythromycin A and B. J. Org. Chem. 33: 665~670, 1968
8) MCALPINE, J. B.; J. P. KARWOWSKI, M. JACKSON, M. M. MULLALLY, J. E. HOCHLOWSKI, U. PREMACHANDRAN & N. S. BURRES: MLR-52, (4′-Demethylamino-4′,5′-dihydroxystaurosporine). A new inhibitor of protein kinase C with immunosuppressive activity. J. Antibiotics 47: 281~288, 1994
9) LARTEY, P. A.; R. FAGHIH, T. PAGANO, H. N. NELLANS, A. PATERSEN & J. J. PLATTNER: Stereoselective deoxygenation of erythromycin A at C-12: Effect of structure and conformation on prokinetic activity. J. Antibiotics 48: 730~732, 1995
10) NAGAMITSU, T.; T. SUNAZUKA, H. TANAKA, S. ŌMURA, P. A. SPRENGELER & A. B. SMITH, III: Total synthesis of (+)-lactacystin. J. Am. Chem. Soc. 118: 3584~3590, 1996
11) OSADA, H. ; H. TAKAHASHI, K. TSUNODA, H. KUSAKABE & K. ISONO: A new inhibitor of protein kinase C. RK-286c (4′-demethylamino-4′-hydroxystaurosporine). I. Screening, taxonomy, fermentation and biological activity. J. Antibiotics 43: 163~167, 1990
12) KANAYASU, T.; J. NAKANO-HAYASHI, N. ASUWA, I. MORITA, T. ISHII, H. ITO & S. MAROTA: Leukotoriene C4 stimulates angiogenesis in bovine carotid artery endothelial cells *in vitro*. Biochem. Biophys. Res. Comm. 159: 572~578, 1989