COMMUNICATION TO THE EDITOR

Synthesis and Anti-platelet Aggregation Activity of Water-soluble Staurosporine Derivatives

Sir:

Staurosporine (**1**) isolated by us from *Saccharothrix staurosporeus*,[1] is one of the most potent known inhibitors of protein kinases.[2] We remarked two kinases, protein kinase C (PKC) and myosin light chain kinase (MLCK) which staurosporine inhibits. These kinases are widely recognized to play a central role in the signal transduction pathways of secretion of hormones or cytokinins, release of neurotransmitters or growth factors, smooth muscle contractions and platelet aggregations.[3~6]

But the limited water-solubility of staurosporine hampered development studies. Thus, we initiated chemical modification of staurosporine focused on aromatic parts with hydrophilic substituents.

This report describes the synthesis, anti-platelet aggregation activity and protein kinases inhibitory effects (PKC and MLCK) of the aromatic parts-modified staurosporine derivatives; 3-, and/or 9-amino, hydroxy, hydroxymethyl and carboxyl derivatives.

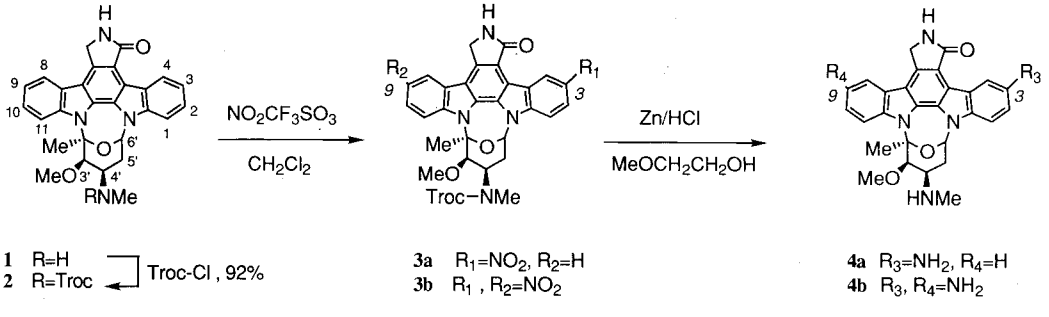
Staurosporine has a methylamino group that may interfere with electrophilic aromatic substitution. Consequently, its moiety was protected by 2,2,2-trichloroethoxycarbonyl (troc) group.[7] The synthesis of 3-amino (**4a**) and 3,9-diamino (**4b**) derivatives were accomplished nitration, followed by Zn-reduction as outlined in Scheme 1. Thus the troc-protected staurosporine (**2**) was treated with $NO_2CF_3SO_3$[8] (1.5 eq. for **3a**, 16 eq. for **3b**) in dichloromethane at $-75°C$, followed by reduction with Zn/HCl in methyl cellosolve afforded deprotected **4a** (yield 27%) or **4b** (yield 22%). Meanwhile, the nitro groups of **3a** or **3b** were also converted to desired amino functions (Scheme 1).

On the other hand, formylation of troc-staurosporine (**2**) with $Cl_2CHOCH_3/TiCl_4$[9] in CH_2Cl_2 at $0\sim25°C$ gave the diformyl intermediate (**5**) (yield 67%). Reduction of **5** with $NaBH_4$ in CH_2Cl_2 to **6**, followed by Zn/HCl reduction gave 3,9-dihydroxymethylstaurosporine (**7**) (yield 17%). Baeyer-Villiger oxidation of **5** with *m*-chloroperbenzoic acid (MCPBA) in CH_2Cl_2, then deprotection afforded 3,9-dihydroxy derivative **9** (yield 17%). **5** was oxidized with $KMnO_4$ in 1,4-dioxane-water, and then deprotected in a similar way to give 3,9-dicarboxylstaurosporine (**11**) (yield 10%) (Scheme 2). The structures of these derivatives were determined by NMR analysis, Mass spectrometry and IR spectrometry. The inhibitory effects of these compounds on protein kinases' activities and platelet aggregation were investigated. PKC and MLCK were prepared from chicken gizzard and rat brain, respectively and the activity of these protein kinases was measured as previously described.[10] Washed platelet (WP) was prepared by gel filtration method.[11] Fresh blood was obtained from guinea pig and centrifuged at $100 \times g$ for 15 minutes for the separation of platelet rich plasma (PRP). PRP was applied to the gel-filtration column and WP fraction was collected. Aggregation of WP was measured using a NBS hematracer VI (Nikoo Bioscience Co. Ltd., Japan). An aliquot of WP (6×10^8 cells/ml) was incubated with various concentrations of inhibitors for 3 minutes in the presence of 0.5% DMSO and stimulated with 1 mM U46619 for 10 minutes. The extent of aggregation was estimated quantitatively by measuring the maximal curve height. These results are shown in Table 1.

Staurosporine, **4a**, **4b**, **7** and **9** inhibited guinea pig washed platelet aggregation induced by U-46619 (1 mM) dose-dependently and IC_{50} values of these compounds were much the same. In contrast, **11** was a weak inhibitor for platelet aggregation. Activation of PKC and MLCK were important for platelet aggregation. Staurosporine, **4a** and **4b** inhibited these protein kinases with similar *Ki* values. However, the inhibitory activity of **11** for these protein kinases were reduced. Inhibitory activity of PKC and MLCK should be important for staurosporine derivatives to inhibit platelet aggregation.

The solubility was determined in distilled water by means of each staurosporine derivative hydrochloride salt which could be prepared due to the methylamino

Scheme 1.



Scheme 2.

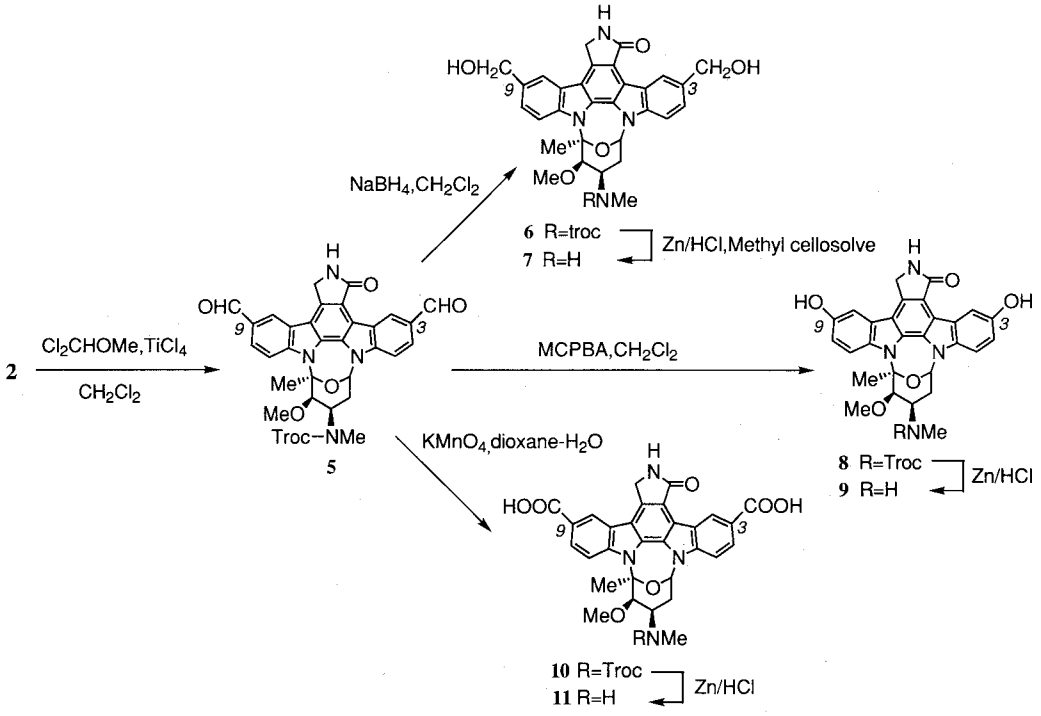


Table 1. Inhibitory effects of staurosporine and its derivatives on platelet aggregation and protein kinases.

Compounds	IC_{50} (μM)	*Ki* (nM)	
	Platelet aggregation	MLCK	PKC
1	0.15	5.0	4.4
4a	0.10	3.4	3.6
4b	0.23	4.0	9.2
7	0.10	—	—
9	0.50	—	—
11	240	150	1500

—: Not determined.

Washed platelet was prepared from guinea pig blood by gel filtration method. Washed platelet was incubated with various concentrations of staurosporine derivatives for 3 minutes and then stimulated with 1 μM U46619. MLCK was purified from chicken gizzard and it's activity was measured using MLC as substrate in the presence of various concentrations of these compounds. PKC was purified from rat brain and assayed using calponin as substrate.

function. The aromatic parts-modified derivative hydrochloride salts were more soluble than staurosporine hydrochloride (<0.5 mg/ml). The solubility of them was as follows; **4a** (200 mg/ml), **7** (1 mg/ml), **9** (10 mg/ml), and **11** (1 mg/ml). Especially, hydrochloride of **4b**, which possess the amino functionality, was highly soluble in aqueous media (>400 mg/ml).

Therefore the most interesting compound is **4b** [Analitical data of **4b**: HRFAB-MS 497.2313 $(M+H)^+$; Calcd for $C_{28}H_{29}N_6O_3$: 497.2300. IR (KBr) cm^{-1} 3330, 2940, 1665, 1608, 1570, 1488, 1460, 1450, 1400, 1340, 1315, 1285, 1235, 1142, 1128, 1100, 1068, 1040. ^1H NMR (400 MHz, DMSO-d_6) d 1.68 (3H, s, NCH_3), 2.15 (3H, s, 2′-CH_3), 2.34, 2.45 (2H, m, 5′-CH_2), 3.16 (3H, s, OCH_3), 3.19 (1H, m, 4′-CH), 3.97 (1H, d, J=3.5 Hz, 3′-CH), 4.5~5.0 (4H, br s, NH_2 × 2), 4.78 (2H, s, 7-CH_2), 6.53 (1H, d, J=6.0 Hz, 6′-CH), 6.74 (1H, dd, J=2.3, 9.0 Hz, 10-H), 6.81 (1H, dd, J=2.4, 8.6 Hz, 2-H), 7.09 (1H, d, J=2.3 Hz, 8-H), 7.23 (1H, d, J=8.6 Hz, 1-H), 7.63 (1H, d, J=9.0 Hz, 11-H), 8.33 (1H, br s, 6-NH), 8.44 (1H, d, J=2.4 Hz, 4-H), ^{13}C NMR (100 MHz, DMSO-d_6) d 29.685, 33.546, 38.984, 45.309, 50.890, 57.690, 80.105, 82.671, 91.077, 104.185, 108.404, 109.379, 112.839, 113.396, 113.677, 114.528, 115.303, 118.000, 123.631, 124.977, 126.858, 130.102, 130.207, 131.499, 132.378, 141.482, 142.278, 172.555]. **4b** shows antiplatelet aggregation activity and protein kinases (PKC and MLCK) inhibitory activity comparable to staurosporine together with high water-solubility. We can make a good tool better than staurosporine (**1**) for pharmacological study.

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