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SYNTHESIS AND ACTIVITY OF STAUROSPORINE ANALOGS WITH A LACTONE FUNCTIONALITY

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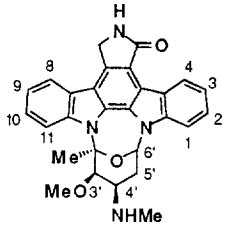
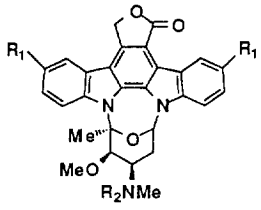
Abstract. Staurosporine analogs, whose γ-lactam functionality was converted into γ-lactone, were synthesized. The key step involves remarkably efficient ring-opening and then intramolecular cyclization reactions. In general, those compounds display potent anti-platelet aggregation activity and show higher selectivity against platelet as compared with smooth muscle contraction.

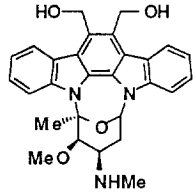
Staurosporine (**1**), a natural product was isolated by Ōmura *et al.*[1] first from *Saccharothrix staurosporeus* and found almost a decade later to be a very potent inhibitor of protein kinase C (PKC) by Tamaoki *et al.*[2]. However **1** has various pharmacological effects such as inhibition of platelet aggregation[3], inhibition of smooth muscle contraction[4], promotion of cell differentiation[5] and growth inhibition of smooth muscle cells[6], since it is completely unselective with respect to inhibition of other protein kinases (e.g.myosin light chain kinase, cyclic AMP dependent protein kinase, cdc2 kinase and tyrosine kinase etc.)[7]. We try to develop staurosporine analogs for new and novel type of anti-platelet aggregation agents. Especially, the obstacle of this development is its vasodilation activity which staurosporine naturally has. Therefore the high-specificity for platelet is demanded. We now describe the biological effect of conversion of γ-lactam into γ-lactone functionality of **1** and the key step of our synthesis of these compounds which involves a remarkably efficient reaction.



Staurosporine: 1



2: R_1=H, R_2=H
3: R_1=H, R_2=Me
4: R_1=OH, R_2=H



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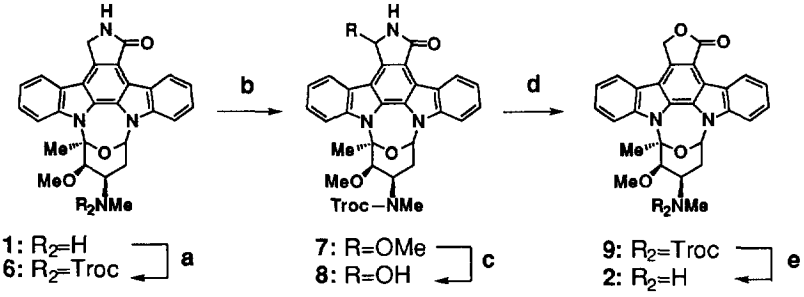
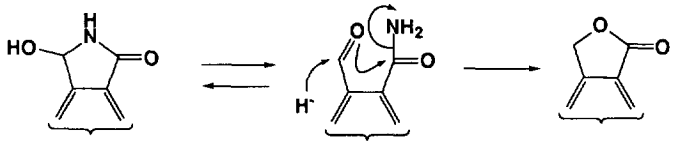
The analog (**2**), which was converted γ-lactam into γ-lactone of **1**, was synthesized in good yield in four steps (Scheme 1). **1** has a methylamino functionality that may interfere with a series of reactions for **2**. Therefore, its moiety was protected by 2,2,2-trichloroethoxycarbonyl (troc) group[8]. Treatment of troc-staurosporine (**6**) with DDQ/MeOH gave 7-methoxy product (**7**), which was hydrolyzed with HCl/H_2O-Dioxane to 7-hydroxy-troc-staurosporine (**8**). Treatment of **8** with $NaBH_4$ in Dioxane-aq.NaH_2BO_3 at 80°C afforded lactone (**9**), presumably via tautomerization from hydroxy-γ-lactam to amide-aldehyde, which was attacked at aldehyde carbon by nucleophile (H^-), then cyclization was occurred as shown in Equation 1. Then reduction with Zn/1N aq. NH_4OAc in THF provided deprotected γ-lactone (**2**).



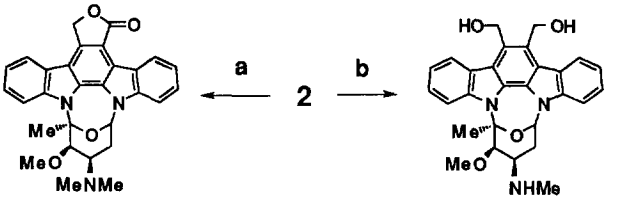
Scheme 1: (a) Troc-Cl, Et_3N, CH_2Cl_2, 0°C~rt,92%; (b) DDQ, MeOH, CH_2Cl_2, rt, 96%; (c) 1N-HCl, H_2O/Dioxane, 60-65°C, 85%; (d) $NaBH_4$, Dioxane-1M aq. NaH_2BO_3, 80°C, 72%; (e) Zn, 1M-NH_4OAc(aq.), THF, rt, 72%.



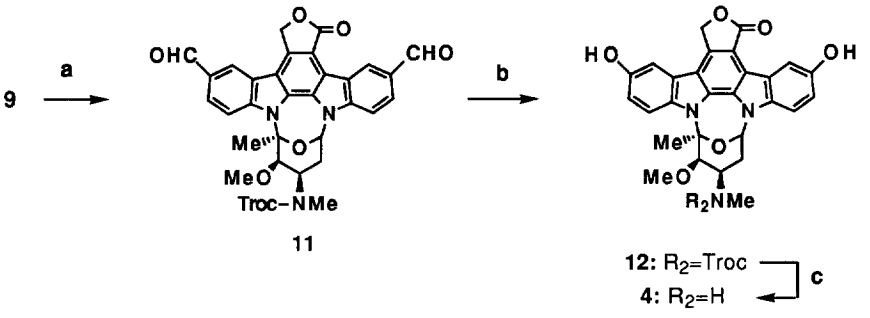
Equation 1

Methylation of **2** with $HCHO/NaBH_3CN$ gave 4'-N-Me-product (**3**). And reduction of **2** with $LiAlH_4$ in THF at room temperature gave diol (**5**) (Scheme 2).

Dihydroxy derivative (**4**) has been synthesized by the route shown in Scheme 3. Formylation of **9** with $Cl_2CHOCH_3/TiCl_4$ in dichloromethane[9] gave the diformyl intermediate (**11**). Baeyer-Villiger oxidation of **11** with H_2O_2/H_2SO_4 in MeOH, followed by Zn-reduction afforded **4**.



Scheme 2: (a) $NaBH_3CN$, 35% aq. HCHO, CH_3CN-DMF, rt, 79%; (b) $LiAlH_4$, THF, rt, 80%.



Scheme 3: (a) Cl_2CHOMe, $TiCl_4$, CH_2Cl_2, -20°C~rt, 71%; (b) H_2O_2, H_2SO_4, MeOH, rt, 32%; (c) Zn, 1M aq. NH_4OAc, THF, reflux,72%.

The analogs prepared using above methodology showed potent inhibition activity against platelet aggregation. Aggregation of platelet was measured using a NBS hematracer VI (Nikoo Bioscience Co. Ltd., Japan). Washed platelet was prepared from guinea pig blood by gel filtration method[10]. An aliquot of washed platelet ($6x10^8$cells/ml) was incubated with various concentration of inhibitors for 3 minutes and stimulated with 1μM U46619 for 10 minutes.

Table. Inhibitory effect arterial contraction, platelet aggregation and PKC activity

Compound No	IC50 values (μM)		
	Arterial contraction	Platelet aggregation	PKC activity
1	0.025	7.5	0.023
2	>30	6.8	5.8
3	>10	5.4	56
4	3.0	0.4	0.22
5	30	6.8	15.8

The IC_{50} values against platelet aggregation was 6.8μM for **2**, which was much the same as compared with that of staurosporine (7.5μM). On the other hand, the IC_{50}[11] values against smooth muscle contraction was > 30μM for **2**, which was much higher than that of staurosporine (0.025μM). Thus, the γ-lactam portion of **1** could be converted into γ-lactone such as **2** without loss of antiplatelet aggregation activity. Furthermore, **2** did not show inhibition activity against smooth muscle contraction. These results suggested that conversion of γ-lactam of staurosporine (**1**) into γ-lactone plays an important role to show platelet-selectivity. 4'-N-Methyl derivative (**3**) and diol (**5**) showed the same antiplatelet activity (IC_{50}: 5.4μM and 6.8μM, respectively) as compared with **2**. Both modification of sugar moiety and ring-opening of γ-lactone may not hve much effective for anti-platelet activity. Compound (**4**), which was substituted with hydroxy groups on aromatic portion of **2**, was improved for antiplatelet aggregation activity about by 10 times (IC_{50}: 0.4μM) than **2**. Substitution on aromatic portion may be important for augmentation of antiplatelet aggregation activity. The IC_{50}[11] for PKC,

which contains various types of isozymes, were 23nM for **1** and 220nM for **4**. Accordingly, PKC may not be important for platelet aggregation with respect to these compounds. Kinases other than PKC, probably, reflect on platelet aggregation.

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