Total Synthesis of MS-444, a Myosin Light Chain Kinase Inhibitor

Sir:

MS-444 (**1**) was discovered in 1995 as an inhibitor of myosin light chain kinase (MLCK) from culture broth of *Micromonospora* sp. KY7123 by a Kyowa Hakko group[1]. As MLCK is known to be a regulatory enzyme in smooth muscle contraction, the unique 4(9*H*)-naphtho[2,3-c]furanone structure with MLCK inhibitory activity has been expected to be a new lead for useful vasodilators and bronchodilators[2].

Herein, we report the effective synthesis of MS-444 (**1**), which features a general and preparative entry into a wide variety of designed analogs for biological studies. Our strategy is based on a contiguous Michael-Dieckmann reaction.

The starting material **4** was prepared from 1,4-dimethoxybenzene by Birch reduction followed by cycloaddition of the resulting diene **2** with methyl but-2-ynoate (**3**) according to RAO's procedures[3]. The 2-methylbenzoate **4** was lithiated with LDA in THF at $-40°C$ to give the benzyl anion, which reacted with 2(5*H*)-furanone (**5**) at $-40°C$ for 6 hours. Michael-Dieckmann reaction proceeded smoothly to give the lactone **6** as a 2 : 1 mixture of keto and enol tautomers in 70% yield as determined by ^1H NMR spectrum (Table 1). This mixture was treated with trimethyl orthoformate and camphorsulfonic acid (CSA) in MeOH to provide, after recrystallization from acetone-hexane, the methyl enol ether **7** as yellow needles in 84% yield. Reaction of **7** with MeLi in CH_2Cl_2 at $-78°C$ for 1 hour provided the methyl ketone **8** as an yellow oil in 87% yield. On heating **8** with CSA in PhMe at 80°C for 2 hours, the α,β-unsaturated ketone **9** was obtained as an orange solid in almost quantitative yield.

Dehydrogenation of **9** was assayed under a variety of conditions[4], but the desired furan corresponding to di-*O*-methylated MS-444 was not detected. For an example, **9** was heated in PhMe with cyclohexene and 10% Pd-C at 180°C for 24 hours in a sealed tube to afford the undesired isomer **10** as a pale yellow oil and the decomposed compound **11** in 50% and 17% yields, respectively. These results suggested that the hydroquinone structure similar with that of the natural product might be requied for the formation of the desired furan **1**. Accordingly, before dehydrogenation, de-*O*-methylation of **9** was carried out by BBr_3 in CH_2Cl_2 at 0°C for 40 minutes to give the hydroquinone **12** as a yellow solid in 86% yield. When this was submitted to dehydrogenation by heating in PhMe with cyclohexene and 10% Pd-C at 180°C for 3 hours in a sealed tube, there was obtained the desired furan **1** as yellow crystals in 59% yield. The furan **1** was identical with natural MS-444 in all respects including enzyme inhibitory activities.

The enzyme inhibitory activities were assayed against MLCK[1]. Natural and synthetic MS-444 (**1**) inhibited the activity of MLCK at an IC_{50} value of 10 μM, while

Scheme

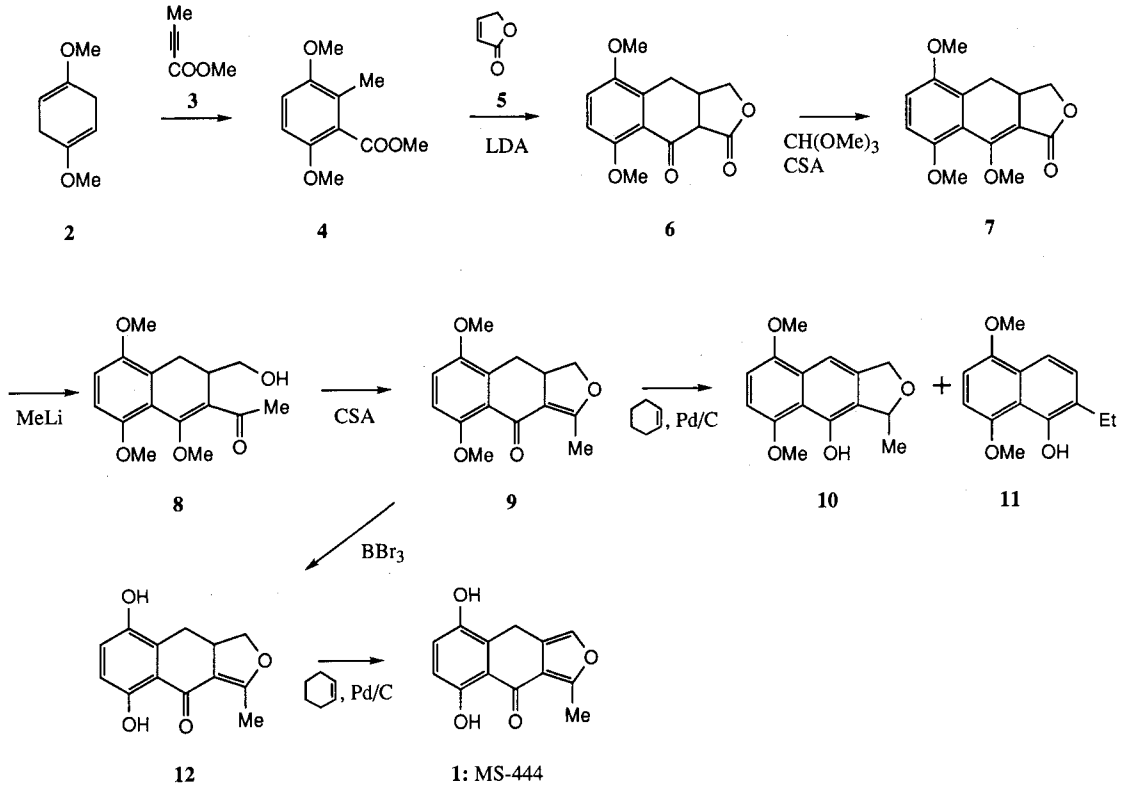

Table 1. Physico-chemical properties of compounds **1** and **6**~**13**.

No.	Mp (°C) / FAB-MS	Rf (Hexane:EtOAc=2:1)	^{1}H-NMR (400MHz; $CDCl_3$; δ ppm; *J* Hz)
1	150-152(dec.) *m/z* 231(M+H)$^+$	0.29	δ 2.71 (3H, s), 3.97 (2H, dd, *J*=1.7&0.9), 6.72 (1H, dt, *J*=8.8& 0.9), 7.15 (1H, d, *J*=8.8), 7.58 (1H, t, *J*=1.7), 8.35 (1H, s), 12.63 (1H, s)
6		< 0.1	Keto form: δ 2.86 (1H, dd, *J*=17.0&8.0), 3.14 (1H, dd, *J*=17.0& 6.0), 3.26 (1H, m), 3.66 (1H, d, *J*=8.6), 3.82 (3H, s), 3.85 (3H, s), 4.05 (1H, dd, *J*=9.2&5.0), 4.44 (1H, dd, *J*=9.2&7.0), 6.83 (1H, d, *J*=9.0), 7.05 (1H, d, *J*=9.0) Enol form: δ 2.25 (1H, dd, *J*=15.6&15.6), 3.45 (1H, dd, *J*= 15.6&6.0), 3.81 (3H, s), 3.93 (3H, s), 4.00 (1H, dd, *J*=8.4& 8.4), 4.65 (1H, t, *J*=8.4), 6.86 (1H, d, *J*=9.0), 6.95 (1H, d, *J*= 9.0), 9.56 (1H, brs)
7	137-139 *m/z* 277(M+H)$^+$	0.17	δ 2.20 (1H, dd, *J*=15.2&15.2), 3.22 (1H, ddd, *J*=9.0, 9.0&6.2), 3.36 (1H, dd, *J*=15.2&6.2), 3.81 (3H, s), 3.84 (3H, s), 3.99 (3H, s), 3.99 (1H, t, *J*=9.0), 4.58 (1H, dd, *J*=9.0&9.0), 6.84 (1H, d, *J*=9.0), 6.93 (1H, d, *J*=9.0)
8	*m/z* 293(M+H)$^+$	< 0.1	δ 2.41 (1H, t, *J*=5.2), 2.45 (1H, ddd, *J*=16.8, 7.0&0.8), 2.59 (3H, s), 3.12 (1H, m), 3.24 (1H, dd, *J*=16.8&2.4), 3.26 (1H, ddd, *J*=11.4, 6.2&5.2), 3.37 (1H, ddd, *J*=11.4, 8.4&5.2) 3.67 (3H, s), 3.80 (3H, s), 3.86 (3H, s), 6.79 (1H, dd, *J*=9.0&0.8), 6.90 (1H, d, *J*=9.0)
9	98-99 *m/z* 261(M+H)$^+$	0.13	δ 2.31 (3H, d, *J*=2.2), 2.33 (1H, dd, *J*=14.2&2.4), 3.48 (2H, m), 3.80 (3H, s), 3.89 (3H, s), 4.11 (1H, dd, *J*=9.0&9.0), 4.73 (1H, dd, *J*=9.0&9.0), 6.84 (1H, d, *J*=9.0), 6.97 (1H, d, *J*=9.0)
10	*m/z* 260(M$^+$)	0.47	δ 1.62 (3H, d, *J*=6.8), 3.94 (3H, s), 4.02 (3H, s), 5.11 (1H, d, *J*=12.2), 5.25 (1H, d, *J*=12.2), 5.55 (1H, q, *J*=6.8), 6.64 (2H, s), 7.54 (1H,s), 9.54 (1H,s)
11	61-63 *m/z* 232(M$^+$)	0.61	δ 1.27 (3H, t, *J*=6.8), 2.81 (2H, q, *J*=6.8), 3.94 (3H, s), 4.02 (3H, s) 6.58 (1H, d, *J*=10.2), 6.64 (1H, d, *J*=10.2), 7.30 (1H, d, *J*=10.2), 7.67 (1H, d, *J*=10.2), 9.67 (1H, s)
12	159-161 *m/z* 233(M+H)$^+$	0.24	δ 2.37 (3H, d, *J*=1.8), 2.41 (1H, ddd, *J*=15.6, 13.0&0.8), 3.42 (1H, dd, *J*=15.6&5.8), 3.56 (1H, m), 4.16 (1H, dd, *J*=10.4& 9.6), 4.65 (1H, s), 4.84 (1H, dd, *J*=9.6&9.6), 6.72 (1H, dd,*J*= 9.0&0.8), 6.92 (1H, d, *J*=9.0), 12.70 (1H, s)

other synthetic compounds **9**~**12** showed no significant activities.

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