

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 group¹. 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 bronchodilators².

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 procedures³. 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 a 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 conditions⁴, 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 required 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 MLCK¹. Natural and synthetic MS-444 (**1**) inhibited the activity of MLCK at an IC_{50} value of $10\ \mu\text{M}$, while

Scheme

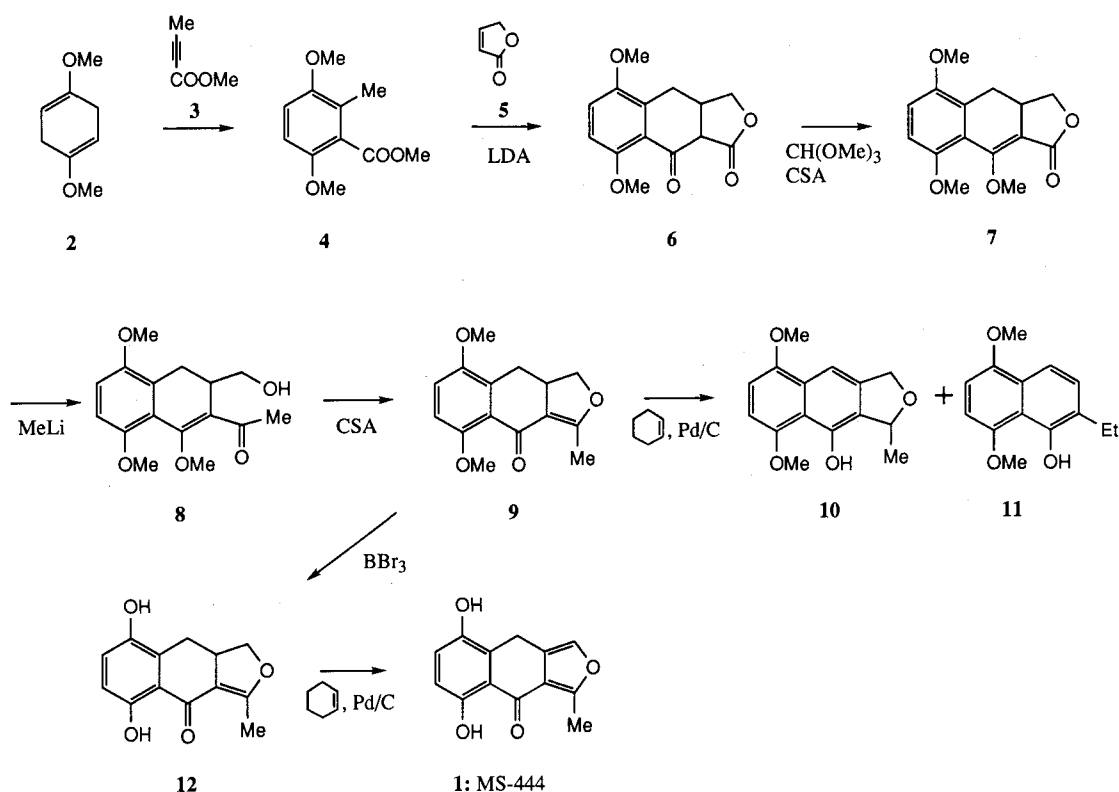


Table 1. Physico-chemical properties of compounds 1 and 6~13.

| 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.

Acknowledgment

We are grateful to Kyowa Hakko Kogyo Co., Ltd., Shikoku Chemicals Co. and Yamanouchi Pharmaceutical Co., Ltd. for the generous support of our program. We also thank Dr. SATOSHI NAKANISHI, Department of Natural Products Discovery, Kyowa Hakko Kogyo Co., Ltd. for enzyme assays.

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(Received December 17, 1996)