BE-23372M, A NOVEL PROTEIN TYROSINE KINASE INHIBITOR

III. SYNTHESIS

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In a preceding paper, the physico-chemical properties and structural elucidation of BE-23372M, a potent novel protein tyrosine kinase inhibitor, were described. In this paper, we report the synthesis of BE-23372M from 3-(3,4-dimethoxybenzoyl)propionic acid and veratraldehyde or 3,4-diacetoxybenzaldehyde. The structure of BE-23372M was confirmed to be (*E*)-3-(3,4-dihydroxybenzylidene)-5-(3,4-dihydroxyphenyl)-2(3*H*)-furanone.

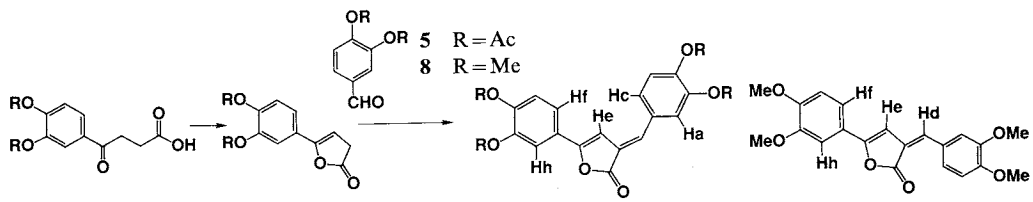
BE-23372M (**1**) is a potent protein tyrosine kinase inhibitor isolated from the mycelia of *Rhizoctonia* strain F23372. Its structure was elucidated from spectroscopic data and published in a previous paper[1]. The biological profile of this compound will be reported elsewhere in detail. In this paper, we describe the synthesis of BE-23372M.

We synthesized BE-23372M to confirm its structure and to supply BE-23372M for further biological studies because the production of BE-23372M by strain F23372 is very poor.

5-(Aryl)-3-(arylidene)-2(3*H*)-furanones have been synthesized by several groups[2~4] from sodium 3-aroylpropionate and aromatic aldehyde. Since 5-(aryl)-3-(arylidene)-2(3*H*)-furanones are reportedly unstable under acidic or basic conditions[2~4], and the double bonds of **1** might be susceptible to hydrogenation, the selection of suitable protective groups for catechol moiety was considered to be important.

First, acetyl groups were applied to protect catechol moiety during the synthesis of **1**. The demethylation of **2**[5] in refluxing hydrobromic acid - AcOH for 15 hours gave **3** in 90% yield. Acetylation and lactonization of **3** in acetic anhydride (Ac_2O) afforded enol lactone **4** in 37% yield. **4** was condensed with 3,4-diacetoxybenzaldehyde **5**[6] by sodium hydride (NaH) in Ac_2O at 80°C and **6** was obtained in 18% yield. Since NOE was observed between H_e and four aromatic protons (H_a, H_c, H_f and H_h), the *E* configuration was

Scheme 1.



2 R=Me
3 R=H

4 R=Ac
7 R=Me

6 R=Ac
9 R=Me
1 R=H, BE-23372M

10

elucidated. The low yield was probably due to the removal of the acetoxy group during the reaction. **6** was converted to BE-23372M (**1**) by treatment with aqueous NH_3 for 15 hours (52% yield). The physicochemical data of synthetic **1** were identical to those of the natural product.

Since the coupling yield was low in the case of **6**, we applied a methyl group to protect catechol moiety. M. RISCHMANN *et al.* reported one-pot synthesis of **9** from **2** and **8** in Ac_2O with NaH[4]; however, this resulted in a low yield. We synthesized **9** stepwise to determine and to improve the yield of each step. Lactonization of **2** in Ac_2O at 80°C afforded enol lactone **7** in a good yield (66%). Next, we investigated the condensation condition. Condensation of **7** and velatraldehyde **8** by NaH or potassium *tert*-butoxide (KO^{t}Bu) in CH_2Cl_2 at room temperature gave a mixture of **9** and **10** in relatively low yields (NaH 31%, KO^{t}Bu 19%). The ratios of **9** and **10** were 3.6:1 and 2.6:1, respectively. The *E* configuration of **9** was determined by NOE experiments as for **6**. Since NOE was observed between H_e and H_d and between H_e and two aromatic protons (H_f and H_h) in **10**, the configuration of **10** was elucidated as *Z*. The use of lithium diisopropylamide as a base in THF resulted in a lower yield (<10%). **7** was stable for several hours at room temperature in the reaction mixture without a base. On the other hand, treatment of **7** with KO^{t}Bu in CH_2Cl_2 afforded a complex mixture, probably because of Claisen condensation and lactone ring opening. These results suggest that **7** is relatively unstable under basic conditions.

We then utilized a weaker base, sodium acetate (NaOAc), to condense **7** and **8**. Condensation by NaOAc in Ac_2O at 80°C afforded a mixture of **9** and **10** in higher yield, as expected (57%, **9**:**10**, 8.7:1). Deprotection was carried out very carefully because the enol lactone in the structure was unstable. Treatment of the mixture of **9** and **10** with BBr_3 in CH_2Cl_2 at −78°C ~room temperature gave **1** in 71% yield. The *Z* isomer was not obtained probably because of isomerization during the reaction. Enol lactone moiety was stable under the deprotective condition.

Thus BE-23372M, (*E*)-3-(3,4-dihydroxybenzylidene)-5-(3,4-dihydroxyphenyl)-2(3*H*)-furanone (**1**), was synthesized.

Experimental

Melting points were determined with a Yanagimoto apparatus and are uncorrected. FAB-MS were obtained with a JEOL JMS-DX 300 mass spectrometer. IR spectra were determined on a HORIBA FT-200. ^{1}H NMR spectra were recorded with a Varian VXR-300 spectrometer and a JEOL JNM-EX400 spectrometer. Chemical shifts are reported relative to residual protons of deuterated NMR solvents. Silica gel (E. Merck Kieselgel 60, Art. 7734) and Sephadex LH-20 (Pharmacia) were used for column chromatography. TLC was routinely used to monitor reactions; precoated plates (E. Merck, Art. 5715) were used and the substances were detected visually or by UV absorption. The organic solutions were dried over Na_2SO_4 before vacuum evaporation.

3-(3,4-Dihydroxybenzoyl)propionic Acid (**3**)

A solution of 3-(3,4-dimethoxybenzoyl)propionic acid (**2**[5], 1.20 g, 5.04 mmol) in Ac_2O (15 ml) and hydrobromic acid (15 ml) was refluxed for 15 hours. The reaction mixture was concentrated to dryness and the residual solid was suspended in water. Collection of the solid by filtration gave **3** (954 mg, 90.0%) as a pale purple solid.

mp 150~154°C (dec.); MS *m/z* $(M+H)^+$ Calcd for $C_{10}H_{11}O_5$: 211.0606. Found: 211.0623. IR ν_{max} (KBr) cm^{-1} 3363, 3228, 1737, 1656, 1589, 887, 809; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.51 (2H, t, J=6.3 Hz), 3.09 (2H, t, J=6.3 Hz), 6.80 (1H, d, J=8.1 Hz), 7.33~7.38 (2H), 9.30 (1H, br s), 9.79 (1H, br s), 12.04 (1H, br s).

5-(3,4-Diacetoxyphenyl)-2(3*H*)-furanone (**4**)

A solution of **3** (882 mg, 4.20 mmol) in Ac_2O (10 ml) was refluxed for 3 hours and the reaction mixture was concentrated to dryness. Purification of the residual solid by silica gel chromatography (EtOAc - *n*-hexane, 1 : 2) gave **4** (430 mg, 37.1%) as a pale pink oil.

MS *m/z* (M^+) Calcd for $C_{14}H_{12}O_6$: 276.0634. Found: 276.0632. IR ν_{max} (KBr) cm^{-1} 1772, 1575; ^1H NMR (300 MHz, $CDCl_3$) δ 2.30 (3H, s), 2.31 (3H, s), 3.43 (2H, d, J=3.0 Hz), 5.78 (1H, t, J=3.0 Hz), 7.24 (1H, d, J=8.7 Hz), 7.43 (1H, d, J=2.1 Hz), 7.49 (1H, dd, J=2.1 and 8.7 Hz).

(*E*)-3-(3,4-Diacetoxybenzylidene)-5-(3,4-diacetoxyphenyl)-2(3*H*)-furanone (**6**)

4 (340 mg, 1.23 mmol) and 3,4-diacetoxybenzaldehyde (**5**[6], 273 mg, 1.85 mmol) were dissolved in Ac_2O (5 ml) under nitrogen. NaH (60% in mineral oil, 49.2 mg, 1.23 mmol) was added stirring at room temperature. The mixture was stirred for 4 hours at 80°C and concentrated to dryness. Purification of the residual oil by silica gel column chromatography (EtOAc - *n*-hexane, 3 : 4) gave a crude product. Further purification of the crude product by Sephadex LH-20 column chromatography (MeOH) gave **6** (94.3 mg, 16.0%) as a yellow solid.

mp 157~159°C; MS *m/z* (M^+) Calcd for $C_{25}H_{20}O_{10}$: 480.1056. Found: 480.1067. IR ν_{max} (KBr) cm^{-1} 1772, 1506, 1207; ^1H NMR (300 MHz, $CDCl_3$) δ 2.32 (3H, s), 2.33 (6H, s), 2.35 (3H, s), 6.84 (1H, s, H_e), 7.30 (2H, d, J=8.4 Hz), 7.39 (1H, s), 7.45 (1H, d, J=1.8 Hz, H_a), 7.48 (1H, dd, J=1.8 and 8.4 Hz, H_c), 7.59 (1H, d, J=2.4 Hz, H_h), 7.65 (1H, dd, J=2.4 and 8.4 Hz, H_f).

5-(3,4-Dimethoxyphenyl)-2(3*H*)-furanone (**7**)

To a suspension of **2** (1.50 g, 8.40 mmol) in 1,2-dichloroethane (20 ml), Ac_2O (4.76 ml, 50.4 mmol) was added at room temperature. After stirring for 15 hours at 80°C, the reaction mixture was diluted with EtOAc and the organic layer was washed with water. Removal of the solvent *in vacuo* yielded a reddish-purple oil that was purified by silica gel column chromatography (EtOAc - *n*-hexane, 1 : 2). **7** was obtained as a pale pink solid (921 mg, 66.4%); mp 106~107.5°C; MS *m/z* (M^+) Calcd for $C_{12}H_{12}O_4$: 220.0736. Found: 220.0716. IR ν_{max} (KBr) cm^{-1} 1790, 1516; ^1H NMR (300 MHz, $CDCl_3$) δ 3.42 (2H, d, J=2.7 Hz), 3.92 (6H, s), 5.65 (1H, t, J=2.7 Hz), 6.89 (1H, d, J=8.1 Hz), 7.09 (1H, d, J=1.8 Hz), 7.21 (1H, dd, J=1.8 and 8.1 Hz).

(*E,Z*)-3-(3,4-Dimethoxybenzylidene)-5-(3,4-dimethoxyphenyl)-2(3*H*)-furanone (**9**, **10**)

A mixture of **7** (40 mg, 0.182 mmol), veratraldehyde (**8**, 36.2 mg, 0.218 mmol), NaOAc (15 mg, 0.182 mmol) and Ac_2O (0.1 ml) was stirred at 80°C for 6 hours. The reaction mixture was diluted with EtOAc and the organic layer was washed with water and brine. Removal of the solvent *in vacuo* yielded a reddish-brown oil that was purified silica gel column chromatography (EtOAc - *n*-hexane, 1 : 2). A mixture of **9** and **10** was obtained as a yellow solid (38.3 mg, 57.2%, **9** : **10**, 8.7 : 1); MS *m/z* (M^+) Calcd for $C_{21}H_{20}O_6$: 368.1260. Found: 368.1243. IR ν_{max} (KBr) cm^{-1} 1770, 1629, 1515, 1275; ^1H NMR (400 MHz, $CDCl_3$) **9** δ 3.94 (3H, s), 3.96 (9H, s), 6.76 (1H, d, J=1.2 Hz, H_e), 6.92 (1H, d, J=8.4 Hz), 6.95 (1H, d, J=8.7 Hz), 7.09 (1H, d, J=2.1 Hz, H_a), 7.22 (1H, d, J=1.8 Hz, H_h), 7.29 (1H, dd, J=2.1 and 8.7 Hz, H_c), 7.33 (1H, s), 7.36 (1H, dd, 1.8 and 8.4 Hz, H_f), **10** δ 3.9~4.1 (12H), 6.37 (1H, s, H_e), 6.90 (1H, d, J=8.5 Hz), 6.92 (1H, d, J=8.5 Hz), 7.04 (1H, s, H_d), 7.17 (1H, d, J=1.9 Hz, H_h), 7.29 (1H, dd, J=1.9 and 8.5 Hz, H_f), 7.43 (1H, dd, J=1.9 and 8.5 Hz), 8.44 (1H, d, J=1.9 Hz).

(*E*)-3-(3,4-Dihydroxybenzylidene)-5-(3,4-dihydroxyphenyl)-2(3*H*)-furanone (**1**)

1) To a solution of **6** (10 mg, 0.021 mmol) in acetone (0.5 ml), 25% aqueous ammonia solution (47 μl, 0.62 mmol) was added at room temperature. After stirring for 15 hours at room temperature, the reaction mixture was acidified with 2 N HCl at 0°C. The mixture was diluted with EtOAc and the organic layer was washed with water. Removal of the solvent *in vacuo* yielded a reddish-yellow solid that was purified by Sephadex LH-20 column chromatography ($CHCl_3$ - MeOH - EtOH - H_2O, 5 : 2 : 2 : 1). **1** was obtained as a reddish orange solid (3.7 mg, 52.3%).

2) To a solution of the mixture of **9** and **10** (200 mg, 0.543 mmol) in dry CH_2Cl_2 (10 ml), BBr_3 1 M CH_2Cl_2 solution (2.72 ml, 2.72 mmol) was added at −78°C. The mixture was allowed to reach room temperature and was stirred for 15 hours. MeOH (2 ml) was added to the resulting mixture, with cooling

at −78°C. The mixture was diluted with EtOAc and the organic layer was washed with water. Removal of the solvent *in vacuo* yielded a reddish-yellow solid that was purified by Sephadex LH-20 column chromatography ($CHCl_3$ - MeOH - EtOH - H_2O, 5:2:2:1). **1** was otained as a reddish orange solid (120 mg, 70.8%).

mp 265~270°C (dec); MS *m/z* (M^+) Calcd for $C_{17}H_{12}O_6$: 312.0634. Found: 312.0640. IR ν_{max} (KBr) cm^{-1} 3412, 1752, 1599, 1518, 1458, 1344, 1302, 1173, 1119, 1069, 944; ^1H NMR (400 MHz, acetone-d_6) δ 6.93 (1H, d, J=8.3 Hz), 6.94 (1H, d, J=8.3 Hz), 7.11 (1H, s), 7.16 (1H, s), 7.25 (1H, dd, J=8.3 and 2.0 Hz), 7.28 (1H, dd, J=8.3 and 2.0 Hz), 7.33 (1H, d, J=2.0 Hz), 7.35 (1H, d, J=2.0 Hz), 8.5 (4H, br s).

^{13}C NMR (100 MHz, acetone-d_6) δ 98.9, 112.9, 116.5, 116.6, 117.5, 118.7, 121.4, 123.2, 124.8, 128.3, 134.4, 146.2×2, 148.4, 148.7, 156.7, 170.1.

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