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Supporting Information

Synthesis of Green Emitting Coumarin Bioconjugate for the Selective Determination of Flu Antigen

Ram Prasad Bhusal, Pyo Yun Cho,[†] Soon-Ai Kim, Hyun Park,[†,*] and Hak Sung Kim[‡,*]

College of Pharmacy, Wonkwang University, Iksan, Jeonbuk 570-749, Korea
[†]Department of Infection Biology, School of Medicine, Wonkwang University, Iksan, Jeonbuk 570-749, Korea
[*]E-mail: hyunpk@wku.ac.kr
[‡]College of Pharmacy, Institute of Pharmaceutical research and development, Wonkwang University, Iksan, Jeonbuk 570-749, Korea. [*]E-mail: hankidad@wku.ac.kr
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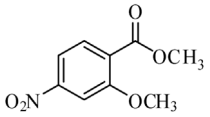
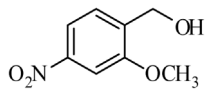
Synthesis of methyl-2-methoxy-4-nitrobenzoate (1):



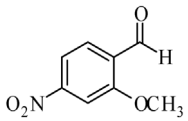
To a stirred solution of 2-hydroxy-4-nitrobenzoic acid (2.00 g, 10.92 mmol) in acetone (60 mL) at room temperature was successively added K_2CO_3 (7.54 g, 54.60 mmol) and dimethyl sulfate (2.29 mL, 24.02 mmol). The reaction mixture was refluxed for 4 hours and allowed to cool to the room temperature. After the acetone was evaporated, the residue was partitioned between ethyl acetate (30 mL) and water (30 mL). The separated organic layer washed with brine (10 mL) and dried over anhydrous sodium sulfate, and concentrated in vacuo to give the methyl-2-methoxy-4-nitrobenzoate **1** (2.28 g, 99%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.89 (d, 1H, J = 8.7 Hz), 7.83 (dd, 1H, J = 8.7 Hz), 7.81 (d, 1H, J = 1.8 Hz), 3.99 (s, 3H), 3.93 (s, 3H).

Synthesis of (2-methoxy-4-nitrophenyl)methanol (2):



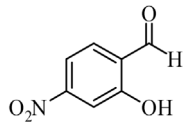
To a stirred solution of methyl-2-methoxy-4-nitrobenzoate (2.17 g, 10.27 mmol) in THF (50 mL) at 0 °C was added DIBAL (34 mL, 33.1 mmol) dropwise and stirred for 1 hour. Methanol (34 mL) was added to it. The reaction mixture was diluted with ethyl acetate (20 mL) and was filtered. The filtrate was concentrated in vacuo to give (2-methoxy-4-nitrophenyl)methanol **2** (1.79 g, 95%).

Synthesis of 2-methoxy-4-nitrobenzaldehyde (3):



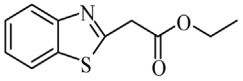
To a stirred solution of (2-methoxy-4-nitrophenyl)methanol (1.66 g, 9.06 mmol) in dichloromethane (40 mL) was added pyridinium dichromate (13.6 g, 82.10 mmol). The reaction mixture was stirred at 40 °C till the completion of reaction and was filtered through the column of silica using ethyl acetate and the filtrate was concentrated in vacuo to give **3** (1.54 g, 94%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.52 (s, 1H), 7.98 (d, 1H, J = 8.7 Hz), 7.88 (dd, 1H, J = 8.9 Hz), 7.85 (d, 1H, J = 1.8 Hz), 4.05 (s, 3H).

Synthesis of 2-hydroxy-4-nitrobenzaldehyde (4):



To a stirred solution of 2-methoxy-4-nitrobenzaldehyde (1.49 g, 8.26 mmol) in dichloromethane (35 mL) was added boron tribromide (12.3 mL, 12.3 mmol) dropwise at −78 °C. The reaction mixture was allowed to cool to the room temperature and stirred till the completion of reaction. The residue was partitioned between the water (30 mL) and ethyl acetate (30 mL). The separated organic layer was washed with brine (10 mL) and dried over anhydrous sodium sulfate, and was concentrated in vacuo to give 2-hydroxy-4-nitrobenzaldehyde **4** (1.32 g, 96%).

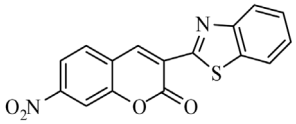
Synthesis of ethyl 2-(benzo[*d*]thiazol-2-yl)acetate (5):



A mixture of ethyl cyanoacetate (1.48 mL, 13.89 mmol) and 2-aminothiophenol (1.5 mL, 13.89 mmol) was stirred for 2 h at 120 °C. The reaction mixture was allowed to cool to the room temperature. The pure yellow oil obtained after the column chromatography using hexane and ethyl acetate (2:1) was ethyl 2-(benzo[*d*]thiazol-2-yl)acetate **5** (2.45 g, 80%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.03 (d, 1H, J = 8.2 Hz), 7.87 (d, 1H, J = 8.2 Hz), 7.49 (t, 1H, J = 7.6 Hz), 7.40 (t, 1H, J = 7.6 Hz), 4.26 (q, 2H, J = 7.3 Hz), 4.21 (s, 2H),

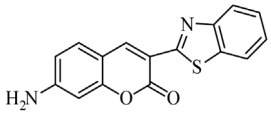
1.30 (t, 3H, J = 7.3 Hz).

Synthesis of 3-(benzo[*d*]thiazol-2-yl)-7-nitro-2*H*-chromen-2-one (6):



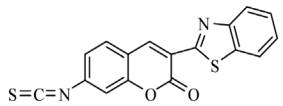
To a stirred solution of 2-hydroxy-4-nitrobenzaldehyde (1.30 g, 7.77 mmol) in ethanol (15 mL) was successfully added 2(2-benzothiazolyl)acetic acid ethyl ester (1.72 g, 7.77 mmol) and piperidine (1.53 mL, 15.54 mmol). The reaction mixture was refluxed for 2 hours and was filtered to give residue 3-(benzo[*d*]thiazol-2-yl)-7-nitro-2H-chromen-2-one **6** (2.06 g, 82%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.13 (s, 1H), 8.28 (d, 1H, J = 1.80 Hz), 8.23 (dd, 1H, J = 8.25 Hz), 8.12 (d, 1H, J = 8.25 Hz), 8.01 (d, 1H, J = 8.25 Hz), 7.90 (d, 1H, J = 8.25 Hz), 7.56-7.59 (m, 1H), 7.46-7.49 (m, 1H).

Synthesis of 7-amino-3-(benzo[*d*]thiazol-2-yl)-2*H*-chromen-2-one (7):



The mixture of 3-(benzo[*d*]thiazol-2-yl)-7-nitro-2*H*-chromen-2-one (500 mg, 1.54 mmol), tetrabutylammonium bromide (1 g) and tin (ll) chloride dihydrate (1.04 g, 4.62 mmol) was stirred for 3 h at 90 °C. The residue was partitioned between water (20 mL) and ethyl acetate (20 mL). The seperated ethyl acetate layer was washed with brine (10 mL) and dried over anhydrous sodium sulfate, and was concentrated under vacuo and purified by column chromatograpy using ethyl acetate and hexane (3:1) to give 7-amino-3-(benzo[*d*]thiazol-2-yl)-2H-chromen-2-one **7** (133 mg, 30%). ^{1}H NMR (500 MHz, CD_3OD) 8.83 (s, 1H), 7.87-7.91 (m, 2H), 7.40-7.45 (m, 2H), 7.30 (t, 1H, J = 8.2 Hz), 6.62 (dd, 1H, J = 8.7 Hz), 6.47 (d, 1H, J = 1.8 Hz).

Synthesis of 3-(benzo[*d*]thiazol-2-yl)-7-isothiocyanato-2*H*-chromen-2-one (8):



To a stirred solution of 3-(benzo[*d*]thiazol-2-yl)-7-nitro-2*H*-chromen-2-one (100 mg, 0.34 mmol) in DCM (2 mL) was successively added 1,1'-thiocarbonyldi-2(1*H*)-pyridone (158 mg, 0.68 mmol) and stirred for 2 hour at room temperature. The reaction mixture was diluted with ethyl acetate, washed with 1 N HCl (10 mL), H_2O (10 mL) and brine (10 mL) respectively and dried over anhydrous sodium sulfate, and was concentrated under vacuo and purified by column chromatography using hexane and ethyl acetate (2:1) to give 3-(benzo[*d*]thiazol-2-yl)-7-isothiocyanato-2*H*-chromen-2-one **8** (68 mg, 60%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.10 (s, 1H), 8.11 (d, 1H, J = 8.25 Hz), 7.99 (d, 1H, J = 7.75 Hz), 7.71 (d, 1H, J = 8.25 Hz), 7.53-7.57 (m, 1H), 7.44-7.47 (m, 1H), 7.24 (d, 1H, J = 1.85 Hz), 7.22 (d, 1H, J = 1.85 Hz); HR-MS (ESI): *m/z* calcd for $C_{17}H_8N_2O_2S_2$ $[M+H]^+$: calcd 337.0027; Found: 337.0102.