Supplementary data

Synthesis of tricyclic pyrano[2,3-*e*]isoindol-3-ones as the core structure of Stachybotrin A, B, and C

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General

All melting points are uncorrected, and were measured on a Büchi 535 micromelting point apparatus. IR spectra were recorded on a Perkin-Elmer Paragon 1000 Fourier transform IR spectrometer. ^{1}H-NMR spectra was measured on a JEOL JNM-EX 270 (270 MHz) and a JEOL JNM-ALS 400 (400 MHz) spectrometer with tetramethylsilane as an internal standard.

3-Hydroxy-4-(1-isopropylthio-3-methyl-3-butenyl)-5-methoxybenzoic acid ethyl ester (5)
3-Hydroxy-2-(1-isopropylthio-3-methyl-3-butenyl)-5-methoxybenzoic acid ethyl ester (6)

Sulfuryl chloride (0.05 g, 0.6 mmol) was added dropwise to a solution of isopentenyl isopropyl sulfide (0.073 g, 0.5 mmol), *s*-collidine (0.08 ml, 0.6 mmol), and 3-hydroxy-5-methoxybenzoic acid ethyl ester (**4**) (0.2 g, 0.74 mmol) in dry CH_2Cl_2 (30 ml) under dry Ar atmosphere at －50℃. The reaction mixture was stirred for 20 min at －50℃. The reaction mixture was added dropwise to a solution of NEt_3 (0.4 ml, 3 mmol) in dry cyclohexane (35 ml), precooled to 0℃, by cannula under dry Ar atmosphere. After being stirred for 1 h at 0℃, the reaction mixture was poured into ice－water (100 ml), and extracted with Et_2O (80 ml×2). The organic layer was washed with saturated NaCl (50 ml×2), dried ($MgSO_4$), and concentrated. The crude product was purified by silica gel column using a mixture of AcOEt and hexane (1:7) as an eluent to provide compound **5** (0.059 g, 35 %) as colorless oil along with regioisomer **6** (0.025 g, 15 %) as a colorless oil.

Compound 5
IR (neat) cm^{-1}: 3219, 2967, 1719, 1582, 1452, 1423, 1370, 1236, 1094, 771
^1H NMR (270 MHz, $CDCl_3$) δ : 1.15 (3H, d, *J*= 6.8 Hz), 1.23 (3H, d, *J*= 6.5 Hz), 1.38 (3H, t, *J*= 7.3 Hz), 1.75 (3H, s), 2.43－2.46 (2H,m), 2.52－2.62 (1H, m), 3.85 (3H, s), 4.35 (2H, q, *J*= 7.3 Hz), 4.56 (1H, s), 4.63 (1H, s), 7.12 (1H, s), 7.22 (1H, s)

Compound 6
IR (neat) cm^{-1}: 3177, 2973, 1765, 1718, 1610, 1463, 1367, 1330, 1233, 1158, 1036, 891, 850, 793. ^1H NMR (270 MHz, $CDCl_3$) δ : 1.08 (3H, d, *J*= 6.8 Hz), 1.21(3H, d, *J*= 6.5 Hz), 1.30 (3H, t, *J*= 7.3 Hz), 1.64 (3H, s), 2.48 (2H, d, *J*= 7.8 Hz), 2.6－2.7 (1H, m), 3.71 (3H, s), 4.25 (2H, q, *J*= 7.3 Hz), 4.6 (1H, s), 4.69 (1H, s), 5.17 (1H, t, *J*= 7.3 Hz), 6.52 (1H, d, *J*= 2.7 Hz), 6.76 (1H, d, *J*= 2.43 Hz)

4-Allyl-3-hydroxy-5-methoxybenzoic acid ethyl ester (8)
2-Allyl-3-hydroxy-5-methoxybenzoic acid ethyl ester (9)

Compound **7** (1.0 g, 4.2 mmol) was refluxed in xylene (10 ml) for 1 d. The cooled reaction mixture was concentrated in vacuo and dissolved in AcOEt (30 ml). The organic layer was washed with saturated aqueous NaCl solution (100 ml). The organic phase was dried ($MgSO_4$) and concentrated. The crude product was purified by silica gel column using a mixture of AcOEt and hexane (1:3) as an eluent to give compound **8** as

colorless oil in 87 % yield (0.87 g) along with compound **9** as a colorless oil in 7 % yield (0.07 g).

Compound 8

IR (neat) cm^{-1}: 2980, 2939, 1716, 1693, 1640, 1593, 1508, 1465, 1422, 1371, 1325, 1249, 1213, 1127, 1097, 1027, 991, 913, 866, 771. ^1H NMR (270 MHz, $CDCl_3$) δ : 1.37 (3H, t, *J* = 7.3 Hz), 3.48 (2H, d, *J* = 6.8 Hz), 3.76 (3H, s), 4.34 (2H, q, *J* = 7.3 Hz), 5.02－5.09 (2H, m), 5.9－6.05 (2H, m), 7.16 (1H, s), 7.28 (1H, s)

Compound 9

IR (neat) cm^{-1}: 2980, 1716, 1693, 1610, 1593, 1465, 1422, 1371, 1325, 1249, 1213, 1127, 1097, 1027, 913, 866, 771. ^1H NMR (270 MHz, $CDCl_3$) δ : 1.37 (3H, t, *J* = 7.3 Hz), 3.66 (2H, d, *J* = 5.9 Hz), 3.76 (3H, s), 4.34 (2H, q, *J* = 7.3 Hz), 5.02－5.09 (2H, m), 5.95－6.05 (2H, m), 6.57 (1H, s), 6.59 (1H, s)

Mannich reaction (General procedure), Table 1

A variety of phenols were dissolved in ethanol, and amine (1.5 eq) and 38% aqueous formaldehyde (1.5 eq) were added. The mixture were refluxed and monitored by TLC. After completion reaction (~8 h), the reaction mixture was concentrated in vacuo and dissolved in AcOEt. The organic layer was washed with saturated NaCl solution (100 ml). The organic phase was dried ($MgSO_4$) and concentrated. The residue was purified by flash chromatography on silica gel to yield the desired Mannich products **11a**, **11b**, **12a** and **12b**.

3-Benzyl-7-*tert*-butyl-3,4-dihydro-2*H*-benzo[*e*][1,3]oxazine (11a, R = *t*-Bu)

Yield 95 %

M.P. 72.3～75.6 ℃ (White crystal)

IR (neat) cm^{-1}: 2961, 1621, 1572, 1500, 1454, 1419, 1363, 1330, 1260, 1202, 1123, 1086, 1025, 993, 961, 874, 809, 742, 699. ^1H NMR (270MHz, $CDCl_3$) δ : 1.27 (9H, s), 3.84 (4H, s), 4.76 (2H, s), 6.76 (1H, d, *J* = 8.6 Hz), 6.85－6.86 (2H, m), 7.2－7.27 (5H, m)

3-Benzyl-7-methyl-3,4-dihydro-2*H*-benzo[*e*][1,3]oxazine (11a, R = Me)

Yield 89 %

M. P. 69.7－70.1 ℃ (White crystal)

IR (neat) cm^{-1}: 2961, 1617, 1502, 1469, 1328, 1240, 1167, 1112, 1013, 959, 875, 808, 741. ^1H NMR (270 MHz, $CDCl_3$) δ : 2.29 (3H, s), 3.91(4H, s), 4.82 (2H, s), 6.65－6.8 (3H, m), 7.29－7.35 (5H, m)

3-Benzyl-3,4-dihydro-2*H*-benzo[*e*][1,3]oxazine-7-carboxylic acid ethyl ester (11a, R = COOEt)

Yield 45 % (Colorless oil)

IR (neat) cm^{-1}: 2980, 1717, 1577, 1452, 1428, 1368, 1288, 1252, 1211, 1089, 1022, 946, 759. ^1H NMR (270 MHz, $CDCl_3$) δ : 1.37 (3H, t, *J* = 7.3 Hz), 3.88 (2H, s), 3.97 (2H, s), 4.34 (2H, q, *J* = 7.3 Hz), 4.87 (2H, s), 6.96 (1H, d, *J* = 7.8 Hz), 7.29－7.33 (5H, m), 7.51 (1H, s), 7.54 (1H, d, *J* = 7.8 Hz)

3-Benzyl-5-bromo-3,4-dihydro-2*H*-benzo[*e*][1,3]oxazine (11a, R = Br)

Yield 46 %

M.P. 71.2－75.1℃ (White crystal)

IR cm^{-1}: 2987, 1598, 1564, 1460, 1366, 1326, 1238, 1132, 1025, 988, 933, 877, 851, 775, 699. ^1H NMR (270 MHz, $CDCl_3$) δ : 3.86 (4H,s), 4.84 (2H, s), 6.74 (1H, d, *J* = 7.3 Hz), 6.97 (1H, d, *J* = 7.3 Hz), 6.98 (1H, s), 7.24－7.33 (5H, m)

3-Benzyl-7-bromo-3,4-dihydro-2*H*-benzo[*e*][1,3]oxazine (11b, R = Br)

Yield 28% (Colorless oil)

IR (neat) cm^{-1} : 2898, 1737, 1598, 1438, 1414,1366, 1325, 1214, 1130, 989, 929, 870, 796, 714, 699. ^1H NMR (270 MHz, $CDCl_3$) δ : 3.88 (2H, s), 3.95 (2H, s), 4.82 (2H, s) 6.78 (1H, d, *J* = 7.3 Hz), 7.02 (1H, dd, *J* = 8.6 Hz, 7.8 Hz), 7.11 (1H, d, *J* = 8.1 Hz), 7.31－7.4 (5H, m)

5-Bromo-2-(dimethylaminomethyl)phenol (12a, R = Br, R^1 = Me)

Yield 37 % (Colorless oil)

IR (neat) cm^{-1}: 2952, 2789, 1602, 1582, 1486, 1468, 1377, 1351, 1236, 19, 1179, 1069, 1017, 891, 849, 799. ^1H NMR (270 MHz, $CDCl_3$) δ : 2.4 (6H, s), 3.59 (3H, s), 6.8 (1H, d, *J* = 7.9 Hz), 6.88 (1H, d, *J* = 7.9 Hz), 6.98 (1H, s)

3-Bromo-2-(dimethylaminomethyl)phenol (12b, R = Br, R^1 = Me)

Yield 30 % (Colorless oil)

IR(neat) cm^{-1} : 2954, 1600, 1574, 1453, 1389, 1355, 1274, 1181, 1018, 879, 774.

^1H NMR (270 MHz, $CDCl_3$) δ : 2.36 (6H, s), 3.87 (2H, s), 6.75 (1H, dd, *J* = 5.9, 3.3 Hz), 6.99 (1H, d, *J* = 5.9 Hz), 7.0 (1H, d, *J* = 3.3 Hz)

5-Bromo-2-(dibenzylaminomethyl)phenol (12a, R = Br, R^1 = Bn)

Yield 26 % (Colorless oil)

IR(neat) cm^{-1}: 3026, 2912, 2820, 1737, 1604, 1582, 1484, 1453, 1376, 1236, 1101, 1073,

961, 894, 750, 699. ^{1}H NMR (270 MHz, $CDCl_3$) δ : 3.59 (4H, s), 3.67 (2H, s), 6.82－6.92 (2H, m), 6.99 (1H, s), 7.25－7.35 (10H, m)

3-Benzyl-7-methoxy-3,4-dihydro-2*H*-benzo[*e*][1,3]oxazine (11a, R = OMe)

Yield 57%

M.P. 55.8－56.7℃ (White crystal)

IR (neat) cm^{-1}: 2943, 2901, 2842, 1736, 1619, 1586, 1502, 1451, 1369, 1336, 1246, 1196, 1163, 1119, 1035, 966, 911, 838, 738. ^{1}H NMR (270M Hz, $CDCl_3$) δ : 3.77 (2H, s), 3.89 (3H, s), 4.86 (2H, s), 6.39 (1H, s), 6.5 (1H, d, J = 8.4 Hz), 6.8 (1H, d, J = 8.4 Hz), 7.27－7.34 (5H, m)

3-Benzyl-5-methoxy-3,4-dihydro-2*H*-benzo[*e*][1,3]oxazine (11b, R = OMe)

Yield 13 %

M.P. 54.7－57.7 ℃ (White crystal)

IR (neat) cm^{-1} : 2933, 2898, 2838, 1591, 1740, 1345, 1263, 1237, 1125, 1091, 1025, 948, 895, 773, 698. ^{1}H NMR (270 MHz, $CDCl_3$) δ : 3.7 (2H, s), 3.9 (2H, s), 4.78 (2H, s), 6.4 (1H, d, J = 8.1 Hz), 6.48 (1H, d, J = 7.8 Hz), 7.1 (1H, dd, J = 7.8, 8.6 Hz), 7.28－7.34 (5H, m)

2-Dibenzylaminomethyl-5-methoxyphenol (12a, R = OMe, R^1 = Bn)

Yield 72 % (Clorless oil)

IR (neat) cm^{-1}: 3026, 2930, 2833, 1790, 1509, 1496, 1436, 1626, 1509, 1496, 1453, 1374, 1312, 1287, 1242, 1199, 1159, 1122, 1103, 1011, 1030, 964, 834, 749, 699.

^{1}H NMR (270 MHz, $CDCl_3$) δ : 3.57 (4H,s), 3.65 (2H, s), 3.73 (3H, s), 6.35 (1H, d, J = 8.4 Hz), 6.43 (1H, s), 6.87 (1H, d, J = 8.4 Hz), 7.28－7.33 (10H, m)

Scheme 3

2-Dibenzylaminomethyl-3-hydroxy-5-methoxybenzoic acid ethyl ester (14)

A mixture of 3-hydroxy-5-methoxybenzoic acid ethyl ester **4** (3.5 g, 18 mmol), Bn_2NH (4.2 ml, 22 mmol), 38 % formaldehyde (1.4 ml, 22 mmol) was refluxed in ethanol (100 ml) for 1 d. The cooled reaction mixture was concentrated in vacuo and dissolved in AcOEt (50 ml). The organic layer was washed with saturated NaCl solution (50 ml×2). The organic phase was dried ($MgSO_4$) and concentrated. The crude product was purified by silica gel column using a mixture of AcOEt and hexane (1:7) as an eluent to give **14** as a colorless oil (0.42 g, 58 %).

IR (neat) cm^{-1} : 2936, 1716, 1613, 1586, 1496, 1451, 1330, 1205, 1098, 848.

^{1}H NMR (270 MHz, $CDCl_3$) δ : 1.38 (3H, t, J = 7.3 Hz), 3.62 (4H, s), 3.78 (3H, s), 4.06

(2H, s), 4.37 (2H, q, J= 7.3 Hz), 6.58 (1H, s), 6.82 (1H, s), 7.22－7.36 (10H, m)

3-Benzyl-7-methoxy-3,4-dihydro-2*H*-benzo[*e*][1,3]oxazine-5-carboxylic acid ethyl ester (16)

A mixture of **4** (3.0 g, 15.4 mmol), Bn_2NH (1.89 ml, 18.5 mmol), 38 % aqueous formaldehyde (1.46 ml, 18.5 mmol) was refluxed in ethanol (80 ml) for 1 d. Cooled reaction mixture was concentrated in vacuo and dissolved in AcOEt (50 ml). The organic layer was washed with saturated NaCl solution (50 ml×2). The organic phase was dried ($MgSO_4$) and concentrated. The crude product was purified by silica gel column using a mixture of AcOEt and hexane (1:6) as an eluent to give **16** (4.68 g, 93 %).

M.P. 89.5－96.4 ℃ (White crystal)

IR (KBr) cm^{-1}: 2962, 1713, 1587, 1452, 1355, 1299, 1214, 1159, 1129, 1105, 1033, 1000, 979, 897, 872, 759, 728. ^1H NMR (400 MHz, $CDCl_3$) δ : 1.38 (3H, t, J= 7.3 Hz), 3.82 (3H, s), 3.94(2H, s), 4.37 (2H, q, J= 7.3 Hz), 4.82 (2H, s), 6.58 (1H, s), 7.17 (1H, s), 7.29－7.35 (5H, m)

2-Benzyl-4-hydroxy-6-methoxy-2,3-dihydroisoindol-1-one (15)

(from compound **14**)

To a solution of **16** (9.0 g, 23 mmol) in 4.4 % formic acid－ethanol (150 ml) was added 10 %Pd/C (0.45 g) and the mixtuture was stirred for 6 h. The reaction mixture was filtered and concentrated. The organic residue was dissolved in dry THF (30 ml) was added to a suspension of NaH (1.1 g, 28 mmol) in dry THF (100 ml) at 0℃ and the mixture was stirred for 4 h at room temperature. Saturated NH_4Cl (50 ml) was added to the reaction mixture at 0℃. Water (50 ml) was added to the reaction mixture at 0℃. The mixture was extracted with $CHCl_3$ (100 ml×2) and organic layer was washed with saturated NaCl (80 ml×2), dried ($MgSO_4$), and concentrated. The crude product was recrystallized in H_2O－ethanol (1:1, 100 ml) to provide **15** (4.7 g, 75 %) as white solid.

(from compound **16**)

Oxazine **16** (20 g, 0.066 mol) was treated with conc. HCl (16.4 ml, 0.197 mol) in ethanol (200 ml) under reflux. After completion of reaction (ca. 8 h), NaOEt (4.6 g, 0.197 mol) was added. After being stired for 10 h, the mixture was filtered and the solution was concentrated in vacuo. The residue was dissolved in AcOEt (200 ml). The organic layer was washed with saturated NaCl solution (300 ml). The organic phase was dried ($MgSO_4$) and concentrated. The residue was purified by recrystallization from ethanol (200 ml) to yield **15** as a white solid in 58 % yield for two steps (10.3 g).

M.P. 180.8－181.7 ℃ (White crystal).

IR (KBr) cm^{-1}: 3061, 2928, 1650, 1613, 1453, 1357, 1156, 1082, 854, 700
^1H NMR (400 MHz, CD_3OD) δ : 3.8 (3H, s), 4.18 (2H, s), 4.78 (2H, s), 6.86 (1H, s), 6.97 (1H, s), 7.24－7.34 (5H, m)

2-Benzyl-4-(1,1-dimethyl-2-propenyloxy)-6-methoxy-2,3-dihydroisoindol-1-one (17)

Compound **15** (2.0 g, 7.4 mmol) and 1,1-dimethyl-2-propenyl isobutyl carbonate (2.07 g, 0.011 mol) were dissolved in DMF and THF (1:1) (30 ml). Tetrakis(triphenylphosphine)palladium (0.19 g, 0.16 mmol) was added to the solution and the mixture was stirred for 10 h. Reaction mixture was poured into an ice water (30 ml). The organic phase was washed with saturated NaCl solution (50 ml), dried ($MgSO_4$) and concentrated. The residue was purified by silica gel column chromatography using a mixture of AcOEt and hexane (1:3) as an eluent to give **17** as colorless oil in 68 % yield (1.7 g).

IR (neat) cm^{-1}: 2984, 1689, 1605, 1499, 1412, 1358, 1317, 1203, 1137, 1080, 993, 917,883, 854, 699. ^1H NMR (270 MHz, $CDCl_3$) δ : 1.47 (6H, s), 3.85 (3H, s), 4.13 (2H, s), 4.77 (2H, s), 5.16－5.25 (2H, m), 6.03－6.13 (2H, m), 6.77 (1H, s), 6.99 (1H, s), 7.3－7.4 (5H, m)

2-Benzyl-4-hydroxy-6-methoxy-5-(3-methylbut-2-enyl)-2,3-dihydroisoindol-1-one (18)

Compound **17** (1.7 g, 2 mmol) was refluxed in dry xylene (5 ml) for 10 h. The mixture was dissolved in AcOEt (50 ml) and washed with NaCl solution (50 ml). The organic phase was dried ($MgSO_4$) and concentrated. The residue was purified by silica gel column chromatography using a mixture of AcOEt and hexane (1:2) as an eluent to give compound **17** in 86 % yield (1.46 g).

M.P. 178.2－187.2 ℃ (White crystal)

(KBr) cm^{-1}: 3365.8, 2912, 1674, 1650, 1595, 1473, 19332, 1198, 1172, 1137, 1084, 833, 766.2, 699. ^1H NMR (270 MHz, $CDCl_3$) δ : 1.61 (3H,s), 1.71 (3H, s), 3.39 (2H, d, J = 7.3 Hz), 3.73 (3H, s), 4.12 (2H, s), 4.61 (2H, s), 5.14 (1H, t, J = 7.3 Hz), 7.09－7.18 (5H, m)

2-Benzyl-5-methoxy-8,8-dimethyl-1,2,7,8-tetrahydro-6H-2-aza-9-oxacyclopenta[*a*]naphthalene-3-one (2)

Compund **18** (0.19 g, 0.56 mmol) was dissolved in dry CH_2Cl_2 (5 ml), and $BF_3 \cdot (OEt_2)$ (0.17 ml, 1.4 mmol) was added to the solution at 0℃. The mixture was stirred for 3h, and was poured into ice water (20 ml). The mixture was extracted with AcOEt (50 ml) and the combined organic phase was washed with saturated NaCl solution (100 ml). The residue was purified by silica gel column chromatography using a mixture of AcOEt

and hexane (1:2) as an eluent to give **2** in 84% yield (0.16 g).
M.P.; 78.3－79.2 ℃ (White crystal)
IR (neat) cm^{-1}: 2930, 1689, 1610, 1472, 1366, 1320, 1158, 1110, 887.
^1H NMR (400 MHz, $CDCl_3$) δ : 1.23 (6H, s), 1.78 (2H, t, *J*= 6.8 Hz), 2.68 (2H, t, *J*= 6.8 Hz), 3.88 (3H, s), 4.12 (2H, s), 4.78 (2H, s), 6.92 (1H, s), 7.29－7.32 (5H, m)

2-Benzyl-7-hydroxy-5-methoxy-8,8-dimethyl-1,2,7,8-tetrahydro-6H-2-aza-9-oxa cyclopenta[a]naphthalen-3-one (3)

Under an argon atmosphere, $VO(acac)_2$ (3 mg, 0.011 mmol) and **17** (0.19 g, 0.7 mmol) were added to anhydrous CH_2Cl_2 (5 ml) ; then after 5 min 5N TBHP in dry CH_2Cl_2 (0.2 ml) was added. Upon completion of reaction, the solvent was removed in vacuo and the crude product was purified by flash chromatography using a mixture of AcOEt and hexane (1:1) as an eluent to give **3** in 65 % yield (0.13 g).
M.P. 78.3－79.2 ℃ (White crystal)
IR (neat) cm^{-1}: 3399, 2977, 2936, 2245, 1671, 1609, 1473, 1437, 1418, 1367, 1324, 1191, 1141, 1113, 1091, 1058, 910, 733. ^1H NMR (400 MHz, $CDCl_3$) δ : 1.26 (3H, s), 1.32 (3H, s), 2.72 (1H, dd, *J*= 5.4, 18 Hz), 2.92 (1H, dd, *J*= 5, 18 Hz), 3.81－3.85 (4H, m), 4.1 (2H, s), 4.75 (2H, s), 6.9 (1H, s), 7.25－7.33 (5H, m)