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Isolation, synthesis, and biological activity of biphenyl and *m*-terphenyl-type compounds from *Dictyostelium* cellular slime molds

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ABSTRACT

From the fruiting bodies of *Dictyostelium polycephalum*, we obtained four aromatic compounds: dictyobiphenyl A (**1**) and B (**2**) and dictyoterphenyl A (**3**) and B (**4**). The synthesis of **1**–**4** was performed to confirm the structures and obtain sufficient material for biological evaluation. Compound **3** was the first example of nitrogen-containing natural *m*-terphenyls, and the isolation of novel classes of compounds such as **3** shows that cellular slime molds are promising sources in natural product chemistry. Moreover, dictyoterphenyl A (**3**) showed cancer cell-selective antiproliferative activity (IC_{50} 2.3–8.6 μM).

1. Introduction

The cellular slime mold *Dictyostelium discoideum* is thought to be an excellent model organism for the study of cell and developmental biology because of its simple pattern of development.[1] Vegetative cells of *D. discoideum* grow as single amoebae by eating bacteria. When starved, they initiate a developmental program of morphogenesis and gather to form a slug-shaped multicellular aggregate. This aggregate then differentiates into two distinct cell types, prespore and prestalk cells, which are precursors of spores and stalk cells, respectively. At the end of its development, the aggregate forms a fruiting body consisting of spores and a multicellular stalk.

Several small molecules including DIFs (differentiation-inducing factors)[2] and discadenine[3] have been reported as development-regulating substances of cellular slime molds. DIFs and their derivatives also exhibit many biological effects in mammalian cells such as suppression of cell growth,[4–6] induction/promotion of cell differentiation,[4a,b] promotion of glucose consumption,[5] and regulation of IL-2 production.[6] A chlorine-substituted dibenzofuran derivative AB0022A[7] and a resorcinol derivative MPBD[8] were also isolated from cellular slime molds. However, except for these compounds, there have been no reports on the secondary metabolites of cellular slime molds. Thus, we have focused on the utility of cellular slime molds as a resource for novel drug development, and we have studied the diversity of secondary metabolites of cellular slime molds.[9] We have recently isolated α-pyronoids,[9a,f] amino sugar derivatives,[9c,d] and aromatics[9b,e,g] with unique structures, and these compounds have demonstrated several biological activities such as control of cellular slime mold development. This paper reports the isolation, structure elucidation, and synthesis of new biphenyl and *m*-terphenyl-type compounds, dictyobiphenyl A (**1**), dictyobiphenyl B (**2**), dictyoterphenyl A (**3**), and dictyoterphenyl B (**4**) from the cellular slime mold, *Dictyostelium polycephalum* (Fig. 1). The selective antiproliferative activities of these compounds in several cancer cell lines are also described.

2. Results and discussion

2.1. Isolation and structural elucidation

Fruiting bodies (wet weight 571 g) of *D. polycephalum* were cultured on agar plates and extracted three times with methanol at room temperature to yield an extract (19 g), which was partitioned with ethyl acetate and water. The ethyl acetate solubles (4.0 g) were separated by repeated column chromatography over silica gel and ODS to yield **1** (3.8 mg), **2** (1.4 mg), **3** (2.0 mg), and **4** (0.5 mg).

HREIMS (*m*/*z* 243.0872 $[M]^+$) indicated that the molecular formula of **1** was $C_{14}H_{13}NO_3$ (Table 1). The ^1H NMR spectrum indicated the presence of ABX spin system (δ 7.89 (1H, dd, *J*=8.6, 2.2 Hz, H-4), 7.86 (1H, d, *J*=2.2 Hz, H-2), and 7.11 (1H, d, *J*=8.6 Hz, H-5)) and AB spin system (δ 7.38 (H-2′) and 6.86 (H-3′) (each 2H, d, *J*=8.7 Hz)) to

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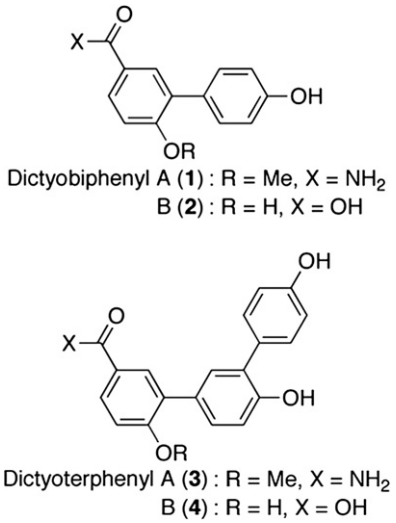


Fig. 1. Structures of dictyobiphenyl A (**1**) and B (**2**); and dictyoterphenyl A (**3**) and B (**4**).

Table 1
NMR spectral data of dictyobiphenyl A (**1**) and B (**2**)

	Dictyobiphenyl A (**1**)[a]		Dictyobiphenyl B (**2**)[b]	
	^{13}C	^{1}H	^{13}C	^{1}H
1	130.9		129.8	
2	130.6	7.86 (1H, d, *J*=2.2 Hz)	133.6	7.89 (1H, d, *J*=2.1 Hz)
3	127.5		123.2	
4	128.8	7.89 (1H, dd, *J*=8.6, 2.2 Hz)	131.0	7.79 (1H, dd, *J*=8.4, 2.1 Hz)
5	111.7	7.11 (1H, d, *J*=8.6 Hz)	116.7	6.89 (1H, d, *J*=8.4 Hz)
6	159.9		160.0	
7	169.1		170.3	
8	56.0	3.85 (3H, s)		
1′	130.0		130.4	
2′, 6′	131.5	7.38 (2H, d, *J*=8.7 Hz)	131.4	7.39 (2H, d, *J*=8.7 Hz)
3′, 5′	115.6	6.86 (2H, d, *J*=8.7 Hz)	115.9	6.82 (2H, d, *J*=8.7 Hz)
4′	157.6		157.8	

[a] 600 MHz for ^{1}H and 150 MHz for ^{13}C in acetone-d_6/methanol-d_4 (9:1). Compound **1** was not possible to dissolve in only acetone-d_6 or methanol-d_4. Acetone-d_6/methanol-d_4 (9:1) can sufficiently dissolve compound **1**.
[b] 600 MHz for ^{1}H and 150 MHz for ^{13}C in methanol-d_4.

imply a 1,2,4-trisubstituted benzene ring (ring A) and a 1,4-disubstituted benzene ring (ring B) in **1**, respectively (Fig. 2). The ^{13}C NMR spectrum displayed 12 signals, and the DEPT spectra indicated the existence of 6 quaternary carbons, 5 methine carbons, and a methoxyl carbon. The difference between the number of ^{13}C NMR signals and the number of carbon atoms in the molecular formula of **1** is two, supporting the presence of a symmetrical 1,4-disubstituted benzene ring. The HMBC correlation peaks for H-2–C-7, H-4–C-7, and H_3-8–C-6 demonstrated that the carbonyl group and the methoxy group were connected to C-1 and C-4, respectively. The HMBC correlations for H-2–C-1′ and H-2′–C-1 suggested that the benzene rings A and B were connected through the C-1–C-1′ bond. The absorption at 1654 cm^{-1} in the IR spectrum of **1** revealed the existence of an aromatic amide, and an amidocarbonyl group attached at C-3. Finally, the remaining hydroxy group attached to C-4′, elucidating the structure of **1**.

HREIMS of **2** (*m*/*z* 230.0583 [M]$^{+}$) indicated a molecular formula, $C_{13}H_{10}O_4$. The ^{1}H and ^{13}C NMR spectra of **2** were nearly identical to those of **1** (Table 1), although compound **2** lacked the signals of a methoxy group. Moreover, the absorption at 1683 cm^{-1} in the IR spectrum of **2** was observed instead of the absorption at 1654 cm^{-1} in **1**. Therefore, compound **2** had a carboxy and hydroxy groups at C-3 and C-6, respectively, in contrast to the amidocarbonyl and methoxy groups detected in **1**.

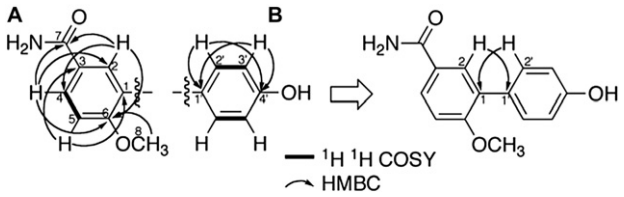


Fig. 2. Structural elucidation of dictyobiphenyl A (**1**).

^{1}H NMR spectrum of **3** revealed the presence of additional ABX spin system (*δ* 7.43 (1H, d, *J*=2.2 Hz, H-2″), 7.32 (1H, dd, *J*=8.3, 2.2 Hz, H-6″), and 6.97 (1H, d, *J*=8.3 Hz, H-5″)) along with the same two spin systems as observed in **1** (Table 2). This fact implied the existence of an additional 1,2,4-trisubstituted benzene ring (ring C). The HREIMS of **3** (*m*/*z* 335.1148 [M^{+}]) indicated a molecular formula of $C_{20}H_{17}NO_4$, which differed from that of **1** by C_6H_4O, suggesting that the ring C contained a phenolic hydroxy group. The upfield shift of the H-5″ signal revealed that a hydroxy group was attached at C-4″. The HMBC correlation peaks for H-2–C-1″, H-2″–C-1, and H-6″–C-1 indicated that the benzene rings A and C were connected through the C-1–C-1″ bond. The rings B and C were connected through the remaining C-3″–C-1′ bond. This connection was supported by the HMBC correlation between H-2′ and C-3″. Therefore, the structure of **3** was elucidated (Fig. 3).

Table 2
NMR spectral data of dictyoterphenyl A (**3**) and B (**4**)

	Dictyoterphenyl A (**3**)[a]		Dictyoterphenyl B (**4**)[b]	
	^{13}C	^{1}H	^{13}C	^{1}H
1	130.9		129.8	
2	130.6	7.92 (1H, d, *J*=2.3 Hz)	133.6	7.95 (1H, d, *J*=2.0 Hz)
3	127.5		123.4	
4	129.0	7.90 (1H, dd, *J*=8.6, 2.3 Hz)	131.0	7.80 (1H, dd, *J*=8.3, 2.0 Hz)
5	111.8	7.13 (1H, d, *J*=8.6 Hz)	116.7[c]	6.92 (1H, d, *J*=8.3 Hz)
6	160.0		160.0	
7	168.9		170.4	
8	56.1	3.87 (3H, s)		
1′	130.8		131.1	
2′, 6′	131.3	7.48 (2H, d, *J*=8.8 Hz)	131.5	7.45 (2H, d, *J*=8.7 Hz)
3′, 5′	115.7	6.86 (2H, d, *J*=8.6 Hz)	115.9	6.83 (2H, d, *J*=8.7 Hz)
4′	157.3		157.4	
1″	130.5		130.8	
2″	132.3	7.43 (1H, d, *J*=2.2 Hz)	132.4	7.45 (1H, d, *J*=2.2 Hz)
3″	128.8		129.6	
4″	154.2		154.5	
5″	116.3	6.97 (1H, d, *J*=8.3 Hz)	116.6[c]	6.90 (1H, d, *J*=8.4 Hz)
6″	129.8	7.32 (1H, dd, *J*=8.3, 2.2 Hz)	129.7	7.33 (1H, dd, *J*=8.4, 2.2 Hz)

[a] 600 MHz for ^{1}H and 150 MHz for ^{13}C in acetone-d_6/methanol-d_4 (9:1). Compound **3** was not possible to dissolve in only acetone-d_6 or methanol-d_4. Acetone-d_6/methanol-d_4 (9:1) can sufficiently dissolve compound **3**.
[b] 600 MHz for ^{1}H and 150 MHz for ^{13}C in methanol-d_4.
[c] These signals were indistinguishable.

HREIMS of **4** (*m*/*z* 322.0836 [M]$^{+}$) indicated a molecular formula of $C_{19}H_{14}O_5$. In comparison to ^{1}H NMR, ^{13}C NMR, and IR spectra of **3** and **4** (Table 2), compound **4** had a carboxy and hydroxy groups at C-3 and C-6, respectively, in contrast to the amidocarbonyl and methoxy groups observed in **3**.

2.2. Synthesis

To confirm the structures and obtain a sufficient quantity of material for performing several biological evaluations, we synthesized compounds **1**–**4**. Especially, the structures of terphenyl-type compounds **3** and **4** had to be reconfirmed because their ^{13}C NMR signals between δ_C 127 and δ_C 133 were very crowded and difficult

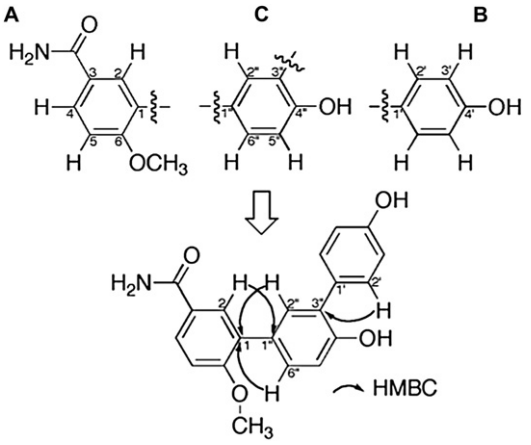
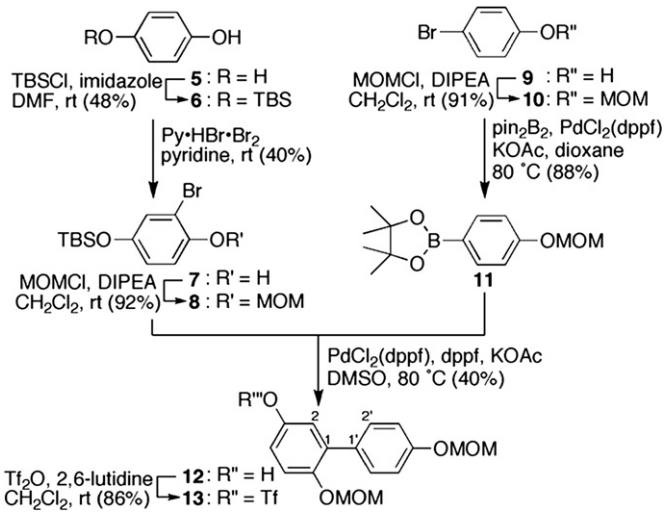
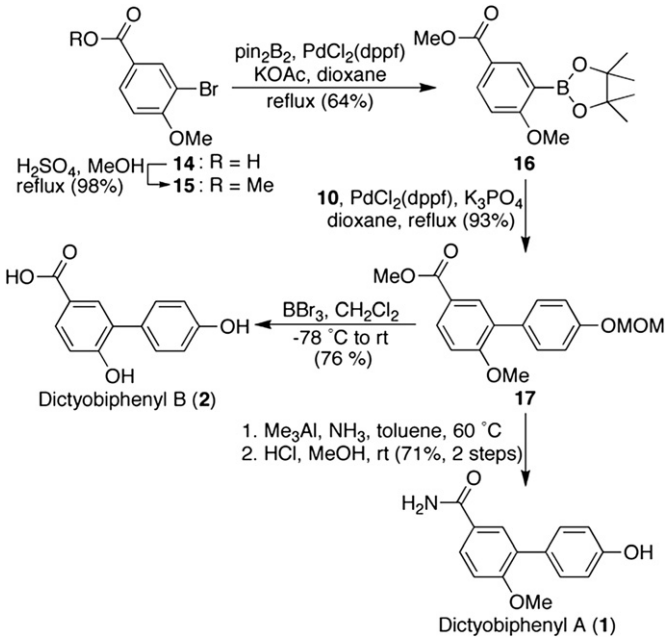


Fig. 3. Structural elucidation of dictyoterphenyl A (**3**).

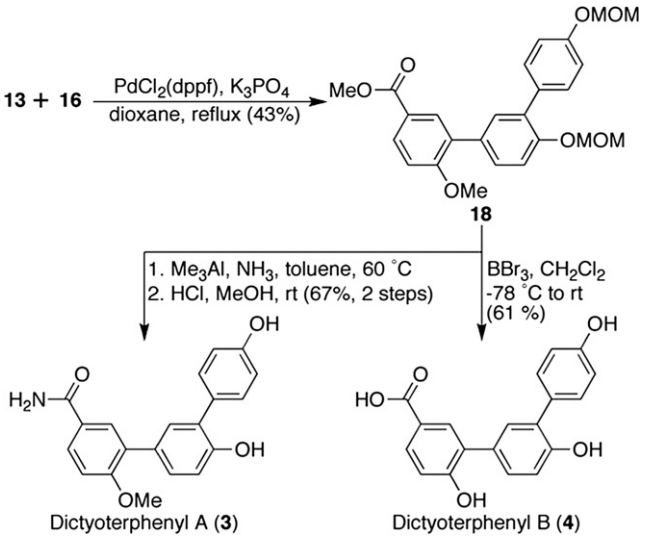
to assign. Preparation of rings B and C is shown in Scheme 1. A Suzuki–Miyaura coupling reaction was performed between **8** (the ring C precursor) and pinacolatoboronate **11** (the ring B precursor) to produce biphenyl **12** (ring C+B), which was then converted into its triflate **13**. The biphenyl skeleton of **12** was confirmed by its HMBC correlations for H-2–C-1′ and H-2′–C-1.



Scheme 1. Synthesis of the precursors of rings B and C in **1**–**4**.



Scheme 2. Synthesis of dictyobiphenyl A (**1**) and B (**2**).



Scheme 3. Synthesis of dictyoterphenyl A (**3**) and B (**4**).

In Scheme 2, dictyobiphenyl A (**1**) and B (**2**) were synthesized. A palladium-catalyzed boronation of methyl 3-bromo-4-methoxybenzoate (**15**) produced compound **16**, which corresponded to ring A. Compounds **16** and **10** (ring B (Scheme 1)) were linked by Suzuki–Miyaura coupling reaction to give biphenyl **17** (ring A+B) in good yield. Although the amidation of **17** with treatment of only ammonia was failed, the trimethylaluminum-catalyzed amidation gave a good result, and the subsequent acidic deprotection of MOM ether of **17** yielded dictyobiphenyl A (**1**). On the other hand, treatment of **17** by BBr_3 removed all MOM and methyl groups to produce dictyobiphenyl B (**2**).

In Scheme 3, the coupling reaction of **13** (ring B+C) with **16** (ring A) afforded terphenyl **18**. Dictyoterphenyl A (**3**) and B (**4**) were synthesized from **18** in a similar manner as the syntheses of **1** and **2**, respectively. All of the spectral data of synthetic **1**–**4** were identical with those of natural **1**–**4**, respectively, and thus their proposed structures were confirmed.

2.3. Biological evaluation

The antiproliferative activities of **1**–**4** were evaluated on several human cancer cell lines such as K562 leukemia, HeLa cervical carcinoma, HCT116 colon carcinoma, and MCF-7 breast adenocarcinoma cells. In addition, the effects on mouse osteosarcoma LM8 cells (cancer cells) and mouse embryo fibroblast 3T3-L1 cells (normal cells) were also evaluated. Compounds **1**, **2**, and **4** demonstrated only weak activities and their IC_{50} values were more than 20 μM for all cell lines. Dictyoterphenyl A (**3**) inhibited the proliferation of all cancer cell lines in a concentration dependent manner, and its IC_{50} values were 8.6 μM (HeLa), 2.3 μM (K562), 6.9 μM (HCT116), 5.4 μM (MCF-7), and 4.9 μM (LM8), respectively (Fig. 4). On the other hand, the proliferation of 3T3-L1 cells did not inhibit by up to 20 μM of **3**. These results indicated that dictyoterphenyl A (**3**) showed cancer cell-selective antiproliferative activity.

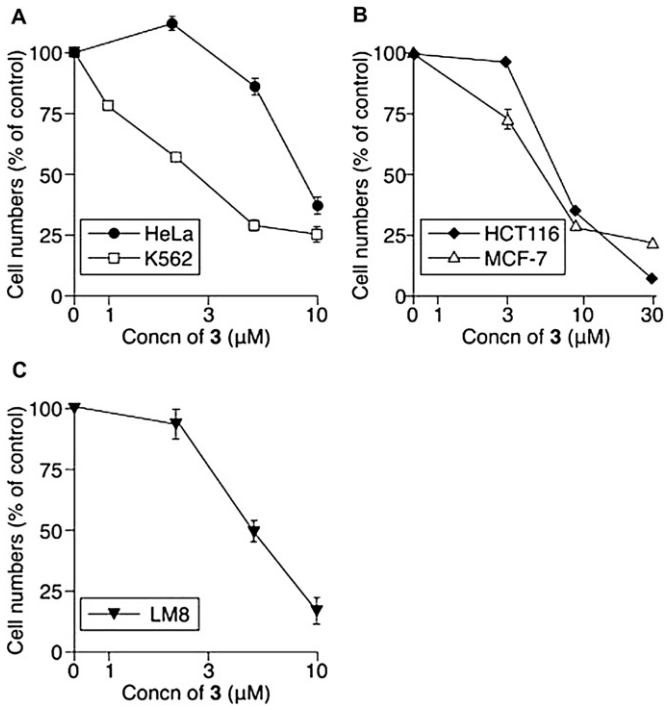


Fig. 4. Antiproliferative activity of dictyoterphenyl A (**3**) on HeLa and K562 cells (A), HCT116 and MCF-7 cells (B), and LM8 cells (C). Cells were incubated in vitro with the indicated concentrations of compound for several days, and the relative cell number was assessed. The mean values and SD (bars) of the triplicate are presented.

Although many *o*-terphenyl-type compounds have ever been reported in kingdom of fungi,[10] very few *m*-terphenyl derivatives such as dictyoterphenyl A (**3**) and B (**4**) occur naturally,[10,11] including dictyomedins previously reported by our group.[9b] In particular, the isolation of dictyoterphenyl A (**3**) described in this report represents the first-ever example for a nitrogen-containing natural *m*-terphenyl. Therefore, the isolation of novel classes of compounds, such as **3** and **4**, demonstrates that cellular slime molds are promising sources in natural product chemistry.

3. Experimental section

3.1. General methods

Analytical TLC was performed on silica gel 60 F_{254} (Merck). Column chromatography was carried out on silica gel 60 (70–230 mesh, Merck). NMR spectra were recorded on JEOL ECA-600 and AL-400. Chemical shifts for ^{1}H and ^{13}C NMR are given in parts per million (δ) relative to tetramethylsilane (δ_H 0.00) and residual solvent signals (δ_C 77.0, 29.8, and 49.0 for $CDCl_3$, acetone-d_6, and CD_3OD, respectively) as internal standards. Mass spectra were measured on JEOL JMS-700 and JMS-DX303. IR spectra were measured on JASCO FT/IR-4200. UV spectra were measured on JASCO V-550.

3.2. Organism and culture conditions

The cellular slime molds, *D. polycephalum* were kindly supplied by Dr. Hagiwara, National Science Museum, Tokyo, Japan. Spores were cultured at 22 °C with *Escherichia coli* Br on A-medium consisting of 0.5% glucose, 0.5% polypeptone, 0.05% yeast extract, 0.225% KH_2PO_4, 0.137% $Na_2HPO_4{\cdot}12H_2O$, 0.05% $MgSO_4{\cdot}7H_2O$, and 1.5% agar. When fruiting bodies had formed after 4 days, they were harvested for extraction.

3.3. Isolation of dictyobiphenyl A (1) and B (2); and dictyoterphenyl A (3) and B (4)

The cultured fruiting bodies (dry weight 92.70 g) of *D. polycephalum* were extracted three times with MeOH at room temperature to give an extract (19.4 g), which was then partitioned with EtOAc and H_2O to yield EtOAc solubles (3.99 g). The EtOAc solubles were chromatographed over silica gel, and the column was eluted with hexane/EtOAc and EtOAc/MeOH solutions with increasing polarity to afford hexane/EtOAc (1:3) eluent (fraction A, 134 mg). Fraction A was further separated by ODS column using H_2O/MeOH solvent system to give H_2O/MeOH (9:1) elutant (fraction B, 11 mg), dictyobiphenyl A (**1**) (3.8 mg), and dictyoterphenyl A (**3**) (2.0 mg). Fraction B was subjected to recycle preparative HPLC (column, JAIGEL-GS310 (ϕ 20 mm×500 mm, Japan Analytical Industry Co., Ltd.); solvent, MeOH) to give dictyobiphenyl B (**2**) (1.4 mg) and dictyoterphenyl B (**4**) (0.5 mg).

*3.3.1. Data for **1**.* Colorless amorphous solid; mp 248 °C (dec); UV (MeOH) λ_{max}, nm (log ε) 205 (4.38), 248 (4.28); IR (KBr) ν_{max} (cm^{-1}) 3205, 2926, 1654, 1604; ^{1}H NMR and ^{13}C NMR data are shown in Table 1; EIMS *m*/*z* 243 $[M]^+$ (100%), 227 (53%); HREIMS *m*/*z* 243.0872 $[M]^+$ (243.0895 calcd for $C_{14}H_{13}NO_3$).

*3.3.2. Data for **2**.* Colorless amorphous solid; mp 208–210 °C; UV (MeOH) λ_{max}, nm (log ε) 205 (4.42), 248 (4.39); IR (KBr) ν_{max} (cm^{-1}) 3277, 1683, 1605; ^{1}H NMR and ^{13}C NMR data are shown in Table 1; EIMS *m*/*z* 230 $[M]^+$ (100%), 213 (11%); HREIMS *m*/*z* 230.0583 $[M]^+$ (230.0579 calcd for $C_{13}H_{10}O_4$).

*3.3.3. Data for **3**.* Colorless amorphous solid; mp 89–90 °C; UV (MeOH) λ_{max}, nm (log ε) 205 (4.61), 248 (4.57), 293 (sh, 4.00); IR (KBr) ν_{max} (cm^{-1}) 3354, 2926, 1653, 1604; ^{1}H NMR and ^{13}C NMR data are shown in Table 2; EIMS *m*/*z* 335 $[M]^+$ (100%), 317 (16%), 277 (26%), 243 (15%); HREIMS *m*/*z* 335.1148 $[M]^+$ (335.1158 calcd for $C_{20}H_{17}NO_4$).

*3.3.4. Data for **4**.* Colorless amorphous solid; mp 212–214 °C; UV (MeOH) λ_{max}, nm (log ε) 204 (4.51), 248 (4.47), 293 (sh, 3.87); IR (KBr) ν_{max} (cm^{-1}) 3293, 1685, 1605; ^{1}H NMR and ^{13}C NMR data are shown in Table 2; EIMS *m*/*z* 322$[M]^+$ (100%), 278 (8%); HREIMS *m*/*z* 322.0836 $[M]^+$ (322.0841 calcd for $C_{19}H_{14}O_5$).

3.4. 3-Bromo-1-(*tert*-butyl(dimethylsilyloxy))-4-(methoxymethoxy)benzene (8)

To a solution of **7**[12] (2930 mg, 9.66 mmol) in dichloromethane (30 mL) were added chloromethyl methyl ether (0.95 mL, 12.5 mmol) and DIPEA (2.52 mL, 14.5 mmol) at 0 °C. After being stirred for 12 h at room temperature, the reaction mixture was poured into saturated ammonium chloride solution, and extracted with EtOAc three times. The combined organic layer was washed with water and brine, dried over sodium sulfate, and evaporated. The residue was chromatographed over silica gel eluted by hexane/EtOAc (19:1) to give **8** (3100 mg, 8.93 mmol, 92%). Data for **8**: colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.04 (1H, d, *J*=2.9 Hz), 7.00 (1H, d, *J*=8.9 Hz), 6.71 (1H, dd, *J*=8.9, 2.9 Hz), 5.15 (2H, s), 3.52 (3H, s), 0.97 (9H, s), 0.18 (6H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 151.0, 148.5, 124.7, 119.5, 117.5, 113.1, 96.0, 56.3, 25.6 (3C), 18.1, −4.5 (2C); LREIMS *m*/*z* 348 $[M+2]^+$ (100%), 346 $[M]^+$ (97%), 291 (57%), 289 (55%), 267 (43%), 260 (17%), 258 (17%), 247 (22%), 245 (21%), 165 (20%); HREIMS *m*/*z* 346.0590 $[M]^+$ (346.0600 calcd for $C_{14}H_{23}O_3Br^{79}Si$).

3.5. 1-(Methoxymethoxy)-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzene (11)

Under argon atmosphere, a solution of 1-bromo-4-(methoxymethoxy)benzene (**10**)[13] (410 mg, 1.889 mmol) in dioxane (6 mL) was added to bis(pinacolato)diboron (500 mg, 1.969 mmol), [1,1′-bis(diphenylphosphino)ferrocene]palladium(II) dichloride dichloro methane adduct (146 mg, 0.179 mmol), and potassium acetate (527 mg, 5.370 mmol) at room temperature. After being refluxed for 12 h, the reaction mixture was evaporated. The residue was chromatographed over silica gel eluted by hexane/EtOAc (19:1) to give **11** (442 mg, 1.672 mmol, 88%). Data for **11**: colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (2H, d, J=8.8 Hz), 7.02 (2H, d, J=8.8 Hz), 5.20 (2H, s), 3.47 (3H, s), 1.33 (12H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.7, 136.4 (2C), 115.4 (2C), 94.0, 83.6 (2C), 56.0, 24.8 (4C) (because a boron atom was bonded to C-4, the C-4 signal was highly broadened and not observed); LREIMS m/z 264 $[M]^+$ (100%), 234 (35%), 219 (11%); HREIMS m/z 264.1527 $[M]^+$ (264.1533 calcd for $C_{14}H_{21}O_4B$).

3.6. 4′,6-Bis(methoxymethoxy)biphenyl-3-ol (12)

Under argon atmosphere, a solution of **8** (562 mg, 1.618 mmol) and **11** (329 mg, 1.24 mmol) in DMSO (6 mL) was added to [1,1′-bis(diphenylphosphino)ferrocene]palladium(II) dichloride dichloro methane adduct (102 mg, 0.124 mmol), 1,1′-bis(diphenylphosphino) ferrocene (69.0 mg, 0.124 mmol), and potassium acetate (366 mg, 3.73 mmol) at room temperature. After being stirred for 20 h at 80 °C, the reaction mixture was poured into water, and extracted with EtOAc three times. The combined organic layer was washed with water and brine, dried over sodium sulfate, and evaporated. The residue was chromatographed over silica gel eluted by hexane/EtOAc (4:1) to give **12** (144 mg, 0.496 mmol, 40%). Data for **12**: colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 (2H, d, J=8.6 Hz), 7.06 (2H, d, J=8.6 Hz), 7.04 (1H, d, J=8.7 Hz), 6.76 (1H, d, J=3.1 Hz), 6.69 (1H, dd, J=8.7, 3.1 Hz), 5.67 (1H, br s), 5.21 (2H, s), 4.97 (2H, s), 3.51 (3H, s), 3.36 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.3, 151.0, 148.0, 132.9, 131.8, 130.5 (2C), 118.2, 117.4, 115.8 (2C), 114.6, 96.1, 94.4, 56.01, 55.99; LREIMS m/z 290 $[M]^+$ (100%), 258 (39%), 213 (41%); HREIMS m/z 290.1135 $[M]^+$ (290.1154 calcd for $C_{16}H_{18}O_5$).

3.7. 4′,6-Bis(methoxymethoxy)biphenyl-3-yl trifluoromethanesulfonate (13)

To a solution of **12** (117 mg, 0.403 mmol) in dichloromethane (3 mL) were added 2,6-lutidine (188 μL, 1.61 mmol) and trifluoromethanesulfonic anhydride (102 μL, 0.606 mmol) at 0 °C. After being stirred for 4 h at room temperature, the reaction mixture was poured into saturated sodium bicarbonate solution, and extracted with EtOAc three times. The combined organic layer was washed with water and brine, dried over sodium sulfate, and evaporated. The residue was chromatographed over silica gel eluted by hexane/EtOAc (49:1) to give **13** (146 mg, 0.346 mmol, 86%). Data for **13**: colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 (2H, d, J=8.8 Hz), 7.25 (1H, d, J=8.9 Hz), 7.20 (1H, d, J=3.0 Hz), 7.15 (1H, dd, J=8.9, 3.0 Hz), 7.13 (2H, d, J=8.8 Hz), 5.22 (2H, s), 5.13 (2H, s), 3.51 (3H, s), 3.42 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.0, 153.7, 143.9, 133.1, 130.5 (2C), 130.1, 123.3, 120.6, 118.8 (q, J=321 Hz (CF_3)), 116.4, 116.0 (2C), 95.1, 94.4, 56.3, 56.1; LREIMS m/z 422 $[M]^+$ (100%), 346 (7%), 257 (13%); HREIMS m/z 422.0636 $[M]^+$ (422.0647 calcd for $C_{17}H_{17}O_7F_3S$).

3.8. Methyl 4-methoxy-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoate (16)

Under argon atmosphere, a solution of methyl 3-bromo-4-methoxybenzoate (**15**)[14] (200 mg, 0.816 mmol) in dioxane (5 mL) was added to bis(pinacolato)diboron (228 mg, 0.898 mmol), [1,1′-bis(diphenylphosphino)ferrocene]palladium(II) dichloride dichlo romethane adduct (66.7 mg, 0.082 mmol), and potassium acetate (240 mg, 2.448 mmol) at room temperature. After being refluxed for 18 h, the reaction mixture was evaporated. The residue was chromatographed over silica gel eluted by hexane/EtOAc (9:1) to give **16** (153 mg, 0.523 mmol, 64%). Data for **16**: colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.34 (1H, d, J=2.4 Hz), 8.09 (1H, dd, J=8.7, 2.4 Hz), 6.87 (1H, d, J=8.7 Hz), 3.88 (6H, s), 1.36 (12H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.6, 166.8, 138.5, 134.5, 122.1, 109.8, 83.7 (2C), 55.9, 51.7, 24.8 (4C) (because a boron atom was bonded to C-3, the C-3 signal was highly broadened and not observed); LREIMS m/z 292 $[M]^+$ (100%), 277 (19%), 261 (14%), 249 (31%), 235 (19%), 192 (37%); HREIMS m/z 292.1469 $[M]^+$ (292.1482 calcd for $C_{15}H_{21}O_5B$).

3.9. Methyl 6-methoxy-4′-(methoxymethoxy)biphenyl-3-carboxylate (17)

Under argon atmosphere, a solution of **16** (71.2 mg, 0.291 mmol) and **10** (70.8 mg, 0.268 mmol) in dioxane (2 mL) was added to [1,1′-bis(diphenylphosphino)ferrocene]palladium(II) dichloride dichloro methane adduct (22.1 mg, 0.027 mmol), and tripotassium phosphate (175 mg, 0.824 mmol) at room temperature. After being refluxed for 24 h, the reaction mixture was evaporated. The residue was chromatographed over silica gel eluted by hexane-EtOAc (9:1) to give **17** (75.4 mg, 0.249 mmol, 93%). Data for **17**: colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98–8.02 (2H, m), 7.46 (2H, d, J=8.9 Hz), 7.09 (2H, d, J=8.9 Hz), 6.97 (1H, d, J=9.1 Hz), 5.21 (2H, s), 3.89 (3H, s), 3.86 (3H, s), 3.50 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.8, 160.1, 156.6, 132.2, 131.0, 130.6 (2C), 130.4, 130.0, 122.6, 115.8 (2C), 110.5, 94.4, 55.9, 55.7, 51.8; LREIMS m/z 302 $[M]^+$ (100%), 272 (36%), 226 (11%); HREIMS m/z 302.1141 $[M]^+$ (302.1154 calcd for $C_{17}H_{18}O_5$).

3.10. Synthesis of dictyobiphenyl A (1)

At 0 °C, 2.0 M trimethylaluminium solution in toluene (387 μL, 0.774 mmol) was added to 0.5 M ammonia solution in dioxane (774 μL, 0.387 mmol). After 45 min, a solution of **17** (11.7 mg, 0.039 mmol) in toluene (0.5 mL) was added to the reaction mixture. After being stirred for 18 h at 60 °C, the reaction mixture was poured into 1 M hydrochloric acid, and extracted with EtOAc three times. The combined organic layer was washed with saturated sodium bicarbonate solution and brine, dried over sodium sulfate, and evaporated. The residue was dissolved in 3% hydrogen chloride solution in MeOH (1.0 mL). After being stirred for 5 h at room temperature, the reaction mixture was evaporated. The residue was chromatographed over silica gel eluted by chloroform/MeOH (19:1) to give dictyobiphenyl A (**1**) (6.7 mg, 0.028 mmol, 71% (two steps)).

3.11. Synthesis of dictyobiphenyl B (2)

To a solution of **17** (18.0 mg, 0.060 mmol) in dichloromethane (1 mL) was added boron tribromide (1.0 M solution in dichloromethane) (0.6 mL, 0.600 mmol) at −78 °C. After being stirred for 30 min at −78 °C and for additional 18 h at 0 °C, the reaction mixture was poured into 0.5 M hydrochloric acid, and extracted with EtOAc three times. The combined organic layer was washed with brine, dried over sodium sulfate, and evaporated. The residue was chromatographed over silica gel eluted by chloroform/MeOH (9:1) to give dictyobiphenyl B (**2**) (10.2 mg, 0.044 mmol, 76%).

3.12. Methyl 6-methoxy-4′,4″-bis(methoxymethoxy)-*m*-terphenyl-3-carboxylate (18)

In the same manner as the synthesis of **17**, compound **18** (58.3 mg, 0.133 mmol, 43 %) was synthesized from **16** (114 mg, 0.390 mmol) and **13** (130 mg, 0.308 mmol). Data for **18**: colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99–8.03 (2H, m), 7.51 (2H, d, J=8.6 Hz), 7.48 (1H, d, J=2.3 Hz), 7.46 (1H, dd, J=8.6, 2.3 Hz), 7.26 (1H, d, J=8.6 Hz), 7.10 (2H, d, J=8.6 Hz), 6.99 (1H, d, J=8.4 Hz), 5.22 (2H, s), 5.17 (2H, s), 3.89 (3H, s), 3.88 (3H, s), 3.51 (3H, s), 3.44 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.9, 160.1, 156.3, 153.5, 132.2, 132.0, 131.9, 131.3, 130.9, 130.7 (2C), 130.5, 129.9, 129.3, 122.6, 115.7 (2C), 115.0, 110.5, 94.9, 94.4, 56.1, 56.0, 55.8, 51.9; LREIMS m/z 438 $[M]^+$ (100%), 406 (38%), 361 (19%), 330 (44%), 299 (10%); HREIMS m/z 438.1664 $[M]^+$ (438.1679 calcd for $C_{25}H_{26}O_7$).

3.13. Synthesis of dictyoterphenyl A (3)

In the same manner as the synthesis of **1**, dictyoterphenyl A (**3**) (11.4 mg, 0.034 mmol, 67% (two steps)) was synthesized from **18** (22.2 mg, 0.051 mmol).

3.14. Synthesis of dictyoterphenyl B (4)

In the same manner as the synthesis of **2**, dictyoterphenyl B (**4**) (7.6 mg, 0.024 mmol, 61%) was synthesized from **18** (17.0 mg, 0.039 mmol).

3.15. Assay for cell growth in K562, HeLa, LM8, and 3T3-L1 cells

Cells were maintained at 37 °C (5% CO_2) in tissue culture dishes filled with an appropriate medium. K562 cells were maintained in RPMI growth medium (RPMI1640 medium supplemented with 10% fetal bovine serum (FBS), 25 μg/mL penicillin, and 50 μg/mL streptomycin). HeLa and 3T3-L1 cells were in DMEM-HG (Dulbecco's modified Eagle's medium containing a high concentration (4500 mg/L) of glucose supplemented with the antibiotics and 10% FBS). LM8 cells were in MEMα (minimum essential medium alpha modification supplemented with the antibiotics and 10% FBS). K562 (2×10^4 cells/well), LM8 (2.5×10^3 cells/well), 3T3-L1 (5×10^3 cells/well), and HeLa (5×10^3 cells/well) were allowed to grow for 3 (K562, LM8, and 3T3-L1 cells) or 4 days (HeLa cells) in 12-well plates; each well filled with 1 mL of the each medium containing DMSO (0.2%) or sample compounds. The relative cell number was assessed using Alamar blue (cell number indicator) as described previously.[4c]

3.16. Assay for cell proliferation in HCT116 and MCF-7 cells

HCT116 cells were maintained at 37 °C (5% CO_2) in tissue culture dishes filled with RPMI growth medium containing 10% FBS, 1% antibiotic–antimycotic (GIBCO). MCF-7 cells were maintained at 37 °C (5% CO_2) in DMEM-HG. For the assay for cell growth, cells were incubated in a 96-well plate, each well containing 100 μL of growth medium (2.5×10^4 cells/mL for MCF-7 and HCT116) in the presence or absence of drugs. After 3 days, 10 μL of MTT reagent was added to each well, and after 1 h incubation at 37 °C (5% CO_2), absorbance at 450 nm was measured. A cell number was given as a ratio of the absorbance (% of control).

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Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.tet.2012.08.041. These data include MOL files and InChiKeys of the most important compounds described in this article.

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