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The Fungal Phytotoxin Alternariol 9-Methyl Ether and Some of Its Synthetic Analogues Inhibit the Photosynthetic Electron Transport Chain

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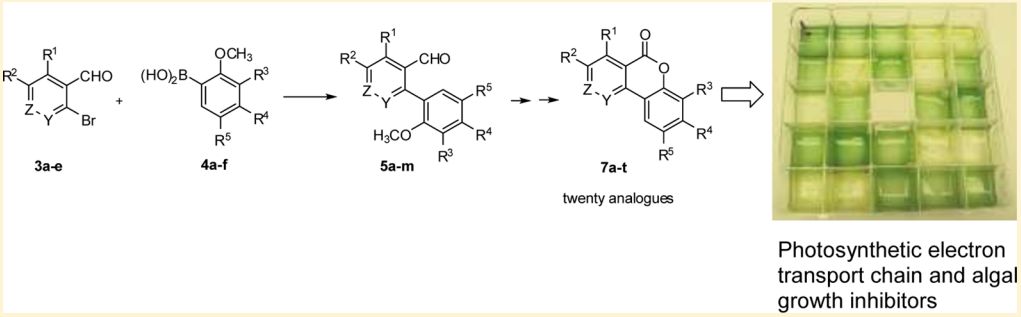
Supporting Information



ABSTRACT: Alternariol and monomethylalternariol are natural phytotoxins produced by some fungal strains, such as *Nimbya* and *Alternaria*. These substances confer virulence to phytopathogens, yet no information is available concerning their mode of action. Here we show that in the micromolar range alternariol 9-methyl ether is able to inhibit the electron transport chain (IC_{50} = 29.1 ± 6.5 μM) in isolated spinach chloroplasts. Since its effectiveness is limited by poor solubility in water, several alternariol analogues were synthesized using different aromatic aldehydes. The synthesized 6*H*-benzo[*c*]cromen-6-ones, 5*H*-chromene[4,3-*b*]pyridin-5-one, and 5*H*-chromene[4,3-*c*]pyridin-5-one also showed inhibitory properties, and three 6*H*-benzo[*c*]cromen-6-ones were more effective (IC_{50} = 12.8−22.8 μM) than the lead compound. Their addition to the culture medium of a cyanobacterial model strain was found to inhibit algal growth, with a relative effectiveness that was consistent with their activity *in vitro*. In contrast, the growth of a nonphotosynthetic plant cell culture was poorly affected. These compounds may represent a novel lead for the development of new active principles targeting photosynthesis.

Microbial phytotoxins and their synthetic analogues are a potential source of new bioactive compounds for agriculture, medicine, and the food industry.[1−7] Since the discovery of paclitaxel production by the endophytic fungus *Taxomyces andreanae* in 1993, the interest of many scientists in fungal endophytes as potential producers of bioactive compounds has increased even more, and over the past two decades many valuable substances endowed with antimicrobial, insecticidal, cytotoxic, anticancer, and other activities have been isolated from these organisms. Such bioactive compounds include alkaloids, terpenoids, steroids, quinones, isocoumarins, lignans, phenylpropanoids, phenols, and lactones.[6,8,9]

Endophytic fungi spend all or part of their life cycle inter- or intracellularly colonizing healthy tissues of their host plants.[10,11] The endophytic fungus *Alternaria* sp. causes lesions in young leaves, fruits, and stems. Depending on the intensity of the disease, the lesions can lead to a total defoliation of the plant.[12,13] All pathogenic species of *Alternaria* produce a series of mycotoxins[14,15] that have been found in many different foodstuffs including fruits, vegetables, cereals, nuts, and oil seeds.[2,16−21] Several of these metabolites have been identified as phenolic compounds with a coumarin-like structure.

Considering the interest in bioactive metabolites produced by weed pathogens as sources of novel natural herbicides, the production of toxins by *Alternaria* species was thoroughly investigated,[22] leading to the discovery of alternariol (**1**), alternariol 9-methyl ether (**2**), and alternuisol (**3**) (Figure 1),[15,23,24] for which total syntheses have also been reported.[22−29] Among *Alternaria* species, *A. alternata* is the

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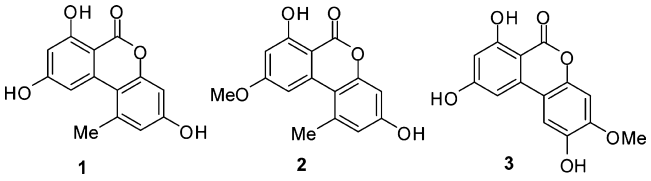

Figure 1. Structure of alternariol (**1**), alternariol 9-methyl ether (**2**), and alternuisol (**3**).

main producer of alternariol, alternariol 9-methyl ether, and some structurally related dibenzopyrone compounds.[30]

Although there are reports of genotoxic, estrogenic, and mutagenic effects in cell culture or in laboratory animals,[31] the toxicity of alternariols for humans and animals is low. Because of such low toxicity, these compounds constitute a new lead structure for the development of new herbicides for weed control. During our research on the use of natural products as models for the development of new agrochemicals,[32−35] we investigated the ability of alternariol 9-methyl ether (**2**) to inhibit the chloroplastic electron transport chain. Some alternariol analogues were also synthesized, several of which were found to share the same target. Results strengthen the hypothesis that the photosynthetic machinery represents a target of *Alternaria* mycotoxins.

RESULTS AND DISCUSSION

Alternariol 9-Methyl Ether Interferes with the Photosynthetic Process by Inhibiting the Electron Transport Chain. When increasing levels of alternariol 9-methyl ether (**2**) were added to the reaction mixture in the range from 1 to 20 μM, the light-dependent ferricyanide reduction by isolated spinach chloroplasts was progressively reduced (Figure 2), with

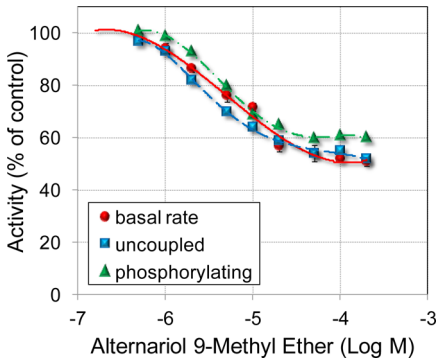

Figure 2. Effect of increasing concentrations of alternariol 9-methyl ether (**2**) on the photosynthetic electron transport chain in isolated spinach thylakoids. The Hill reaction was measured under basal, uncoupling, and phosphorylating conditions. Results were expressed as percent of untreated controls (82.1 ± 1.8, 191.9 ± 6.3, and 116.5 ± 1.9 nmol s^{-1} (mg chlorophyll)$^{-1}$, respectively) and are mean ± SE over four replicates.

an estimated concentration causing 50% inhibition (IC_{50}) of 29.1 ± 6.5 μM. This effect could result from an interaction with either the electron transport chain or the ATP synthase coupling factor, or be a consequence of uncoupling properties preventing the formation of a proton gradient across the thylakoid membrane. To discriminate among these possibilities, the activity in the presence of the same concentrations of the mycotoxin was measured under conditions that allow ATP synthesis (phosphorylating condition) and in the presence of a true uncoupler, ammonium, at a level high enough to completely dissipate the proton gradient (uncoupling condition). Results (Figure 2) showed that neither condition significantly changed the inhibition pattern, suggesting that alternariol 9-methyl ether (**2**) directly interacts with one or more of the electron carriers involved in the electron transport chain. However, when the level of the toxin was raised above 20 μM, the resulting inhibitory effect reached a plateau and did not increase further (Figure 2). The lack of a concentration−effect relationship at high rates is likely due to the poor solubility of alternariol in water.

Twenty Alternariol Analogues were Synthesized through Suzuki Condensation, Oxidation of Aldehyde, and Cyclization Reaction. To overcome this issue and to gain insight into the structure−activity relationship, some analogues were designed and synthesized. The starting aldehyde was prepared through Vilsmeyer formylation.[25,36] In this synthesis, a key stage is the formation of a C−C bond, promoting the connection of two aryl compounds by the Suzuki methodology[37,38] resulting in the formation of **5a−s**, which were then oxidized to carboxylic acids **6a−j** and **6p−s** using as the oxidizing agent sodium chlorite[25,39] or to **6k−o** using potassium permanganate[40] (Figure 3). The biaryl compounds were submitted to a further reaction for the elimination of methyl groups and formation of the lactone compounds **7a−t** (Figure 4). In some cases, low yields were obtained (**5f** (37.6%), **5n** (42.1%), **5o** (40.9%), **5p** (54.1%), and **5s** (37.6%)). However, at this stage of the research, no effort was made to improve yields since our major interest was the preparation of several analogues for biological evaluation. No clear structural aspect was evident that could account for such results, although the presence of a nitrogen atom at the *meta* position in relation to the Br moiety corresponded in most cases to a low yield at the biarylation step. This nitrogen atom might in fact interfere with the palladium oxidative addition of compound **3d**, a key step on the Suzuki coupling reaction.[37,38] The biaryl compounds were submitted to a further reaction for the elimination of methyl groups and the formation of the lactone compounds **7a−t** (Figure 4) in good yields. All compounds were fully characterized by spectroscopic data (IR, MS, ^{1}H NMR, and ^{13}C NMR).

Some Analogues Also Inhibit the Photosynthetic Electron Transport Chain and Affect the Growth of a Photoautotrophic Microalgae Culture but Not That of Heterotrophic Cultured Plant Cells. The products obtained were evaluated for their ability to interfere with the Hill reaction. The results, summarized in Table 1, showed that some of them (namely, **7a−d** and **7l−m**) are endowed with an excellent inhibitory activity, whereas others are substantially ineffective. The inhibitory potential seems related to the presence of two hydroxyl groups at positions 4 and 6 (or 5) of the first phenyl ring. Indeed, compounds sharing the whole structure but lacking these substituents showed IC_{50} values 1−2 orders of magnitude higher. The effect could be at least in part related to the greater polarity of the resulting analogues, reflecting in turn an increased solubility. Indeed, although the concentration−activity relationship profiles obtained for some of the most active compounds (**7c**, **7d**) were suggestive of a still incomplete solubilization (Figure 5), the water solubility of the synthesized analogues was in general much greater than that of alternariol 9-methyl ether. In fact, although a certain degree of lipophilicity is required to gain accessibility to the target site inside the thylakoid membranes,[41] an inadequate water

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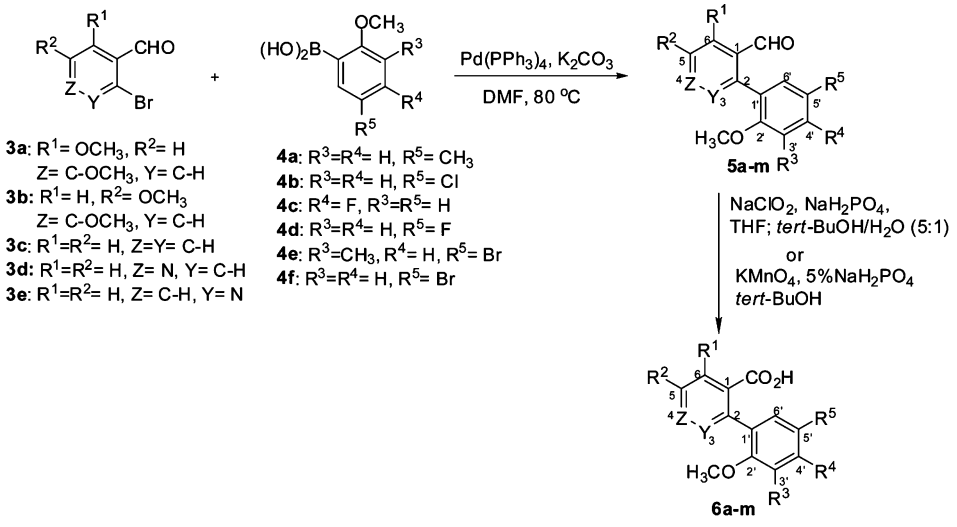

R1	R2	R3	R4	R5	Z	Y	Biaryl		Acid	
							compound	yield(%)	compound	yield (%)
OCH_3	H	H	H	CH_3	C-OCH_3	C-H	**5a**	68.0	**6a**	*
OCH_3	H	H	H	Cl	C-OCH_3	C-H	**5b**	75.5	**6b**	92.2
OCH_3	H	H	F	H	C-OCH_3	C-H	**5c**	*	**6c**	70.4
OCH_3	H	H	H	F	C-OCH_3	C-H	**5d**	71.2	**6d**	98.3
H	H	H	H	CH_3	C-H	C-H	**5e**	94.4	**6e**	61.0
H	H	H	H	Cl	C-H	C-H	**5f**	37.6	**6f**	95.8
H	H	H	F	H	C-H	C-H	**5g**	69.9	**6g**	94.0
H	H	H	H	F	C-H	C-H	**5h**	*	**6h**	68.9
H	H	H	H	Br	C-H	C-H	**5i**	*	**6i**	94.2
H	H	Br	H	CH_3	C-H	C-H	**5j**	81.5	**6j**	95.7
OCH_3	H	H	H	Br	C-OCH_3	C-H	**5k**	61.2	**6k**	90.8
H	OCH_3	H	H	F	C-OCH_3	C-H	**5l**	91.4	**6l**	89.7
H	OCH_3	H	F	H	C-OCH_3	C-H	**5m**	86.0	**6m**	91.0
H	H	H	H	F	N	C-H	**5n**	42.1	**6n**	80.9
H	H	H	H	Cl	N	C-H	**5o**	40.9	**6o**	92.8
H	H	Br	H	F	N	C-H	**5p**	54.1	**6p**	72.5
H	H	H	H	F	C-H	N	**5q**	92.9	**6q**	98.1
H	H	H	H	CH_3	C-H	N	**5r**	87.3	**6r**	86.3
H	H	Br	H	F	C-H	N	**5s**	37.6	**6s**	77.1

* Not purified and directly used in the following reaction.

Figure 3. Synthesis of biaryl acids **6** as precursors of alternariol analogues.

solubility can prevent both inhibitor translocation inside the plants and achieving high local concentrations.

Substances able to interfere with the photosynthetic electron transport chain are potentially active against photosynthetic organisms. However, several factors, including differential membrane permeability or solubility, subcellular compartmentalization, and the occurrence of metabolization or detoxification reactions, can completely abolish the inhibitory potential found *in vitro* for a given compound. To verify such a possibility, alternariol 9-methyl ether (**2**) and its analogues that had previously shown efficacy against the Hill reaction were evaluated for their ability to inhibit the photoautotrophic growth of the blue-green alga *Synechococcus*. Three inactive analogues were included in the experimental plan as negative controls. Results, presented in Table 2, clearly pointed out that all alternariols able to inhibit the electron transport chain are also capable of reducing cyanobacterial growth, whereas the negative controls were inactive. Interestingly, when the active analogues were added even at high concentration to the medium of heterotrophically growing plant cells, in most cases no inhibition took place. Significant phytotoxic effects were evident only for compound **7b**. A significant (P = 0.0013) correlation was found (Figure 6) between the concentrations able to inhibit by 50% the photosynthetic electron transport chain and those inhibiting the cyanobacterial growth.

Conclusions. The mycotoxin alternariol 9-methyl ether (**2**) was found to inhibit the photosynthetic electron transport chain in isolated spinach chloroplasts at the same concentrations at which its presence reduced the growth constant of a cyanobacterial model strain. The possible occurrence of other

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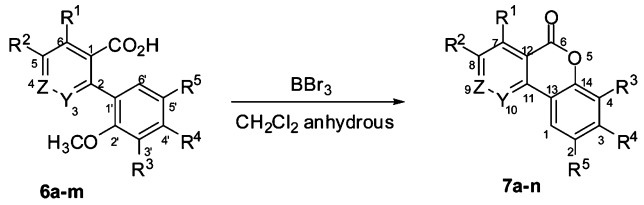

6a-m 7a-n

R_1	R_2	R_3	R_4	R_5	Z	Y	alternariol analogues	
							compound	yield (%)
OH	H	H	H	CH_3	C-OH	C-H	**7a**	34.5
OH	H	H	H	Cl	C-OH	C-H	**7b**	55.0
OH	H	H	F	H	C-OH	C-H	**7c**	48.5
OH	H	H	H	F	C-OH	C-H	**7d**	69.0
H	H	H	H	CH_3	C-H	C-H	**7e**	79.0
H	H	H	H	Cl	C-H	C-H	**7f**	93.5
H	H	H	F	H	C-H	C-H	**7g**	97.2
H	H	H	H	F	C-H	C-H	**7h**	93.3
H	H	H	H	Br	C-H	C-H	**7i**	94.7
H	H	Br	H	CH_3	C-H	C-H	**7j**	93.1
OH	H	H	H	Br	C-OCH_3	C-H	**7k**	97.3
H	OH	H	F	H	C-OH	C-H	**7l**	68.2
H	OCH_3	H	H	F	C-OCH_3	C-H	**7m**	75.4
H	H	H	H	F	N	C-H	**7n**	85.1
H	H	H	H	Cl	N	C-H	**7o**	71.5
H	H	Br	H	F	N	C-H	**7p**	35.3
H	H	H	H	F	C-H	N	**7q**	92.8
H	H	H	H	CH_3	C-H	N	**7r**	86.4
H	H	Br	H	F	C-H	N	**7s**	42.2
H	OH	H	H	F	C-OH	C-H	**7t**	86.8

Figure 4. Final stage in the synthesis of alternariol analogues **7a**–**t**.

Table 1. Concentrations of Alternariol 9-Methyl Ether (2) and of Its Analogues (7a–7t) Able to Inhibit by 50% the Basal Rate of the Photosynthetic Electron Transport in Spinach (*Spinacia oleracea* L.) Thylakoids[a]

compd	ID_{50} (μM)
2	29.1 ± 6.5
7a	22.8 ± 8.8
7b	12.8 ± 3.0
7c	19.5 ± 8.7
7d	35.7 ± 14.9
7e	>500
7f	435 ± 60
7g,h,i,j,k	>500
7l	39.7 ± 16.7
7m	31.5 ± 6.3
7n,o,p,q,r,s,t	>500
diuron	0.27 ± 0.02
hexazinone	0.11 ± 0.02
lenacil	0.08 ± 0.02

[a]Data obtained with some commercial herbicides targeting the photosynthetic apparatus are also reported. Values for untreated controls were 78.7 ± 2.3 nmol s^{-1} (mg chlorophyll)$^{-1}$.

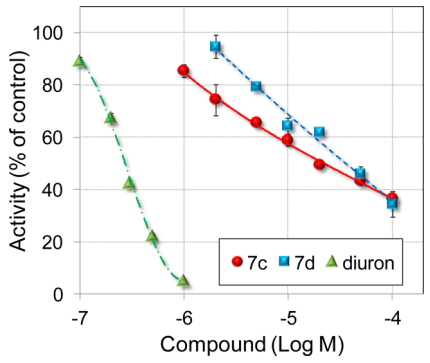

Figure 5. Effect of increasing concentrations of two active alternariol analogues on the basal rate of electron transport in spinach thylakoids. Data concerning the commercial herbicide diuron are also included.

targets cannot be ruled out, since at high levels it slightly inhibited the proliferation of dark-grown cultured plant cells. However, the effect was higher against the photosynthetically active cultures, and similar effects were shown by some alternariol analogues, whose superior activity may be related in part to higher water solubility. Our data suggests that interference with the photosynthetic apparatus may contribute to the phytotoxic activity of *Alternaria* toxins. Interestingly, the most active alternariol analogues showed IC_{50} values only 1–2 orders of magnitude higher than those of commercial herbicides targeting photosynthesis.[42] Ineffective analogues did not exert any toxic effect on the model cyanobacterial strain. Moreover, the heterotrophic growth of suspension-cultured plant cells was affected only in part, suggesting that the interaction with the photosynthetic apparatus may be their main mode of action inside the plant cell. The alternariol scaffold may thus represent a new lead for the design of active

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Table 2. Autotrophic Growth of the Cyanobacterium *Synechococcus elongatus* and Heterotrophic Growth of *Arabidopsis thaliana* Suspension Cultured Cells in the Presence of Micromolar Levels of Alternariol 9-Methyl Ether (2) and Selected Alternariol Analogues[a]

	S. elongatus				
compd	20 μM	50 μM	100 μM	200 μM	*A. thaliana* 200 μM
2	94.9 ± 0.9	96.8 ± 2.7	82.3 ± 6.6	67.9 ± 9.9	83.2 ± 7.0
7a	94.1 ± 0.5	86.9 ± 1.9	72.6 ± 1.9	56.4 ± 2.8	93.8 ± 3.3
7b	77.5 ± 0.7	71.8 ± 2.5	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
7c	96.2 ± 0.8	91.5 ± 1.3	94.3 ± 3.0	30.8 ± 5.3	95.0 ± 4.7
7d	104.9 ± 1.7	96.5 ± 1.5	90.4 ± 3.1	71.9 ± 6.2	92.2 ± 3.0
7f	106.2 ± 1.6	103.5 ± 5.2	111.5 ± 0.2	108.1 ± 1.5	88.3 ± 2.4
7h	97.3 ± 0.4	97.5 ± 1.4	108.9 ± 2.9	97.7 ± 1.5	*b*
7k	101.3 ± 2.2	107.6 ± 1.0	93.4 ± 1.3	89.4 ± 0.9	*b*
7l	99.1 ± 0.8	93.5 ± 1.1	66.1 ± 1.1	20.1 ± 0.8	86.9 ± 2.2
7m	95.1 ± 1.1	97.9 ± 2.5	80.4 ± 0.5	63.7 ± 0.8	91.0 ± 3.5
7q	107.7 ± 1.6	100.2 ± 0.9	100.4 ± 2.2	110.6 ± 1.0	*b*

[a]Growth was expressed as percentage of untreated controls (growth constant of 0.387 ± 0.005 for cyanobacteria, dry weight increase of 5.25 ± 0.03 mg d.w. mL^{-1} for plant cells). Data are means ± SE over at least three replications. [b]Not determined.

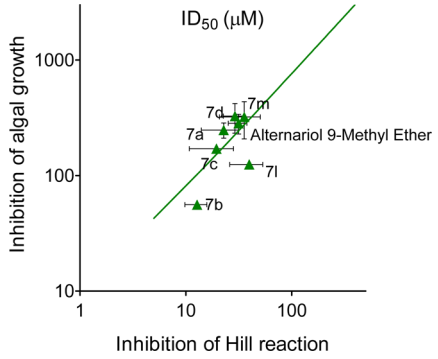

Figure 6. Relationship between the ability of alternariol analogues to inhibit the photosynthetic electron transport chain in spinach chloroplasts and their toxicity against the photosynthetic cyanobacterium *Synechococcus elongatus*.

principles capable of interfering with the photosynthetic electron transport.

EXPERIMENTAL SECTION

General Experimental Procedures. Reagents and solvents were purified as described by Perrin and Armarego (1988).[43] ^1H and ^{13}C NMR spectra were recorded on a Varian Mercury 300 spectrometer at 300 and 75 MHz using $CDCl_3$ or DMSO-d_6 or CD_3OD as solvent and TMS as internal standard. Mass spectra were recorded using a Shimadzu GCMS-QP5050A (70 eV). Infrared spectra were recorded on a Perkin Elmer Paragon 1000 FTIR spectrophotometer, with the samples prepared as a thin film on a NaCl plate, scanning from 635 to 4000 cm^{-1}. Accurate mass (HRMS) data were recorded under ESI conditions in Bruker MicroToF spectrometer (resolution = 10000 fwhm) using a lock-spray source. The lock-mass used for calibration was tetraoctylammonium bromide in the positive ion mode. Analytical thin layer chromatography analyses were conducted on aluminum-packed precoated silica gel plates. Flash chromatography was performed over silica gel (0.035−0.070 mm). Melting points were recorded in MQAPF-301 (Microquimica, Brazil). 2-Bromo-4,6-dimethoxybenzaldehyde **3a** was synthesized by Vilsmeyer formylation[25,36] of 1-bromo-3,5-dimethoxybenzene, purchased from Sigma-Aldrich (St. Louis, MO).

Synthesis. *2,4-Dimethoxy-6-(5-methyl-2-methoxyphenyl)-benzaldehyde (**5a**).* To a 25 mL two neck round-bottomed flask, equipped for reflux, 2-bromo-4,6-dimethoxybenzaldehyde (0.245 g, 1.00 mmol) in DMF (15 mL), 5-methyl-2-methoxyphenylboronic acid (0.498 g, 3.00 mmol), K_2CO_3 (0.198 g, 2.00 mmol), and $Pd(PPh_3)_4$ (0.115 g, 0.100 mmol) were added. The reaction mixture was stirred for 24 h at 80 °C. After this time, the mixture was quenched in NH_4Cl (20 mL) and extracted with ethyl acetate (3 × 30 mL). The organic phase was washed with water (5 × 30 mL), dried over $MgSO_4$, and concentrated under reduced pressure to yield the required aldehyde (**5a**) as a yellow solid (193.9 mg, 0.68 mmol). Yield: 68%; mp 119.5−120.3 °C; IR (ν_{max}) 3005, 2922, 2853, 2771, 1677, 1599, 1571, 1499, 1470, 1425, 1235, 1220, 1147, 872, 816 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 2.32 (t, J = 0.6 Hz, 3H, 5′-C$\underline{H}_3$), 3.70 (s, 3H, 2′-OC$\underline{H}_3$), 3.87 (s, 3H, 6-OC$\underline{H}_3$), 3.93 (s, 3H, 4-OC$\underline{H}_3$), 6.39 (d, J = 2.4 Hz, 1H, H5), 6.45 (d, J = 2.4 Hz, 1H, H3), 6.81 (d, J = 8.4 Hz, 1H, H3′), 7.02 (dd, J = 2.4, 0.6 Hz, 1H, H6′), 7.16 (ddd, J = 8.4, 2.4, 0.6 Hz, 1H, H4′), 9.85 (s, 1H, C$\underline{H}$O); ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.7 (5′-$\underline{C}H_3$), 55.8 (6-O$\underline{C}H_3$ and 2′-O$\underline{C}H_3$), 56.1 (4-O$\underline{C}H_3$), 98.1 (C3), 108.2 (C5), 110.6 (C1 and C3′), 128.3 (C5′), 130.1 (C4′ and C1′), 131.3 (C6′), 146.1 (C2), 154.4 (C2′), 162.3 (C6), 164.4 (C4) and 190.7 (C$\underline{H}$O); HRMS (ESI TOF-MS) calcd for $C_{17}H_{18}NaO_4^+$ 309.1097; found 309.1112.

The same procedure was used for the synthesis of biaryls (**5b−s**), whose yields are presented in Figure 3.

*2,4-Dimethoxy-6-(5-chloro-2-methoxyphenyl)benzaldehyde (**5b**).* Yellow solid; mp 127.7−128.7 °C; IR (ν_{max}) 3005, 2940, 2840, 2769, 1684, 1594, 1570, 1492, 1450, 1205, 1030, 809 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 3.70 (s, 3H, 2′-OC$\underline{H}_3$), 3.87 (s, 3H, 6-OC$\underline{H}_3$), 3.93 (s, 3H, 4-OC$\underline{H}_3$), 6.34 (d, 1H, J = 2.4 Hz, H5), 6.48 (d, 1H, J = 2.4 Hz, H3). 6.83 (d, 1H, J = 8.7 Hz, H3′), 7.17 (d, 1H, J = 2.4 Hz, H6′), 7.30 (dd, 1H, J = 8.7, 2.4 Hz, H4′), 9.97 (s, 1H, C$\underline{H}$O); ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.8 (2′-O$\underline{C}H_3$), 56.1 (6-O$\underline{C}H_3$), 56.2 (4-O$\underline{C}H_3$), 98.3 (C3), 108.4 (C5), 111.8 (C3′), 117.5 (C1), 125.7 (C5′), 129.1 (C4′), 130.0 (C6′), 130.9 (C1′), 143.5 (C2), 155.3 (C2′), 162.9 (C6), 164.6 (C4), 189.7 (C$\underline{H}$O); HRMS (ESI TOF-MS) calcd for $C_{16}H_{15}ClNaO_4^+$ 329.0551; found 329.0560.

*2,4-Dimethoxy-6-(5-fluoro-2-methoxyphenyl)benzaldehyde (**5d**).* Yellow solid; mp 105.8−106.6 °C; IR (ν_{max}) 3002, 2941, 2839, 2772, 1686, 1595, 1499, 1459, 1210, 1159, 1033 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 3.69 (s, 3H, 2′-OC$\underline{H}_3$), 3.87 (s, 3H, 6-OC$\underline{H}_3$), 3.93 (s, 3H, 4-OC$\underline{H}_3$), 6.35 (d, 1H, J = 2.4 Hz, H5), 6.50 (d, 1H, J = 2.4 Hz, H3), 6.83 (dd, 1H, J = 9.0, 4.5 Hz, H3′), 6.93 (dd, 1H, J = 8.4, 3.0 Hz, H6′), 7.04 (ddd, 1H, J = 9.0, 8.1, 3.0 Hz, H4′), 9.95 (s, 1H, C$\underline{H}$O); ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.8 (6-O$\underline{C}H_3$), 56.2 (4-O$\underline{C}H_3$), 56.2 (2′-O$\underline{C}H_3$), 98.2 (C3), 108.3 (C5), 111.5 (d, J = 8.3 Hz, C3′), 115.3 (d, J = 22.4 Hz, C4′), 117.3 (d, J = 23.8 Hz, C6′), 117.5 (C2), 130.4 (d, J = 7.7 Hz, C1′), 143.8 (C1), 152.6 (d, J = 2.2 Hz, C2′), 157.0 (d, J = 238 Hz, C5′), 162.8 (C6), 164.6 (C4), 189.9 (C$\underline{H}$O); HRMS (ESI TOF-MS) calcd for $C_{16}H_{15}FNaO_4^+$ 313.0847; found 313.0870.

*2-(2-Methoxy-5-methylphenyl)benzaldehyde (**5e**).* Colorless oil; IR (ν_{max}) 3061, 3008, 2926, 2839, 2750, 1694, 1598, 1503, 1254, 1235, 1035, 1023, 809, 772, and 744 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ

2.36 (t, 3H, J = 0.6 Hz, 5′-C$\underline{H}_3$), 3.71 (s, 3H, 2′-OC$\underline{H}_3$), 6.87 (d, 1H, J = 8.1 Hz, H3′), 7.11 (dd, 1H, J = 2.1, 0.6 Hz, H6′), 7.21 (ddd, 1H, J = 8.1, 2.1, 0.6 Hz, H4′), 7.36 (ddd, 1H, J = 7.5, 1.5, 0.6 Hz, H6), 7.47 (ddd, 1H, J = 7.8, 7.5, 1.5 Hz, H4), 7.64 (td, 1H, J = 7.5, 1.5 Hz, H5), 7.99 (ddd, 1H, J = 7.8, 1.5, 0.6 Hz, H3), 9.80 (d, 1H, J = 0.6 Hz, C$\underline{H}$O); ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.7 (5′-$\underline{C}H_3$), 55.7 (2′-O$\underline{C}H_3$), 110.8 (C3′), 126.7 (C5′), 126.8 (C3), 127.9 (C5), 130.5 (C4′), 131.4 (C6′), 132.3 (C6), 133.9 (C4), 134.2 (C2), 142.2 (C1), 154.7 (C2′), 193.0 ($\underline{C}$HO); HRMS (ESI TOF-MS) calcd for $C_{15}H_{14}NaO_2^+$ 249.0886; found 249.0872.

*2-(5-Chloro-2-methoxyphenyl)benzaldehyde (**5f**).* White solid; mp 95.1−96.3 °C; IR (ν_{max}) 3065, 3008, 2941, 2842, 2750, 1697, 1597, 1495, 1255, 1025, 811, 771, 646 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.72 (s, 3H, 2′-OC$\underline{H}_3$), 6.90 (d, 1H, J = 9.0 Hz, H3′), 7.28 (d, 1H, J = 2.7 Hz, H6′), 7.33 (ddd, 1H, J = 7.8, 1.5, 0.6 Hz, H6), 7.37 (dd, 1H, J = 9.0, 2.7 Hz, H4′), 7.50 (ddd, 1H, J = 7.8, 7.5, 1.5 Hz, H5), 7.65 (td, 1H, J = 7.5, 1.5 Hz, H4), 7.99 (ddd, 1H, J = 7.5, 1.5, 0.6 Hz, H3), 9.78 (d, 1H, J = 0.6 Hz, C$\underline{H}$O); ^{13}C NMR (75 MHz, $CDCl_3$) δ 56.0 (2′-O$\underline{C}H_3$), 112.1 (C3′), 126.2 (C5′), 127.1 (C3), 128.4 (C5), 128.7 (C1′), 129.7 (C4′), 131.2 (C6′), 131.2 (C6), 134.0 (C4), 134.2 (C2), 140.4 (C1), 155.4 (C2′), 192.2 ($\underline{C}$HO); HRMS (ESI TOF-MS) calcd for $C_{14}H_{11}ClNaO_2^+$ 269.0340; found 269.0298.

*2-(4-Fluoro-2-methoxyphenyl)benzaldehyde (**5g**).* White solid; mp 68.7−70.0 °C; IR (ν_{max}) 3066, 3008, 2940, 2839, 2749, 1690, 1597, 1507, 1449, 1279, 1192, 1151, 830, 765 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.73 (s, 3H, 2′-OC$\underline{H}_3$), 6.71 (dd, 1H, J = 10.8, 2.4 Hz, H3′), 6.80 (td, 1H, J = 8.4, 2.4 Hz, H5′), 7.24 (dd, 1H, J = 8.4, 6.6 Hz, H6′), 7.32 (ddd, 1H, J = 7.8, 1.5, 0.6 Hz, H6), 7.49 (ddd, 1H, J = 7.8, 7.5, 1.5 Hz, H5), 7.64 (td, 1H, J = 7.5, 1.5 Hz, H4), 7.99 (ddd, 1H, J = 7.5, 1.5, 0.6 Hz, H3), 9.77 (d, 1H, J = 0.6 Hz, C$\underline{H}$O); ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.9 (2′-O$\underline{C}H_3$), 99.3 (d, J = 26.3 Hz, C3′), 107.7 (d, J = 21.2 Hz, C5′), 122.9 (d, J = 3.6 Hz, C1′), 127.1 (C3), 128.1 (C5), 131.5 (C6),132.3 (d, J = 10.4 Hz, C6′), 134.0 (C4), 134.3 (C2), 141.1 (C1), 157.9 (d, J = 9.7 Hz, C2′), 164.1 (d, J = 246 Hz, C4′), 192.6 ($\underline{C}$HO); HRMS (ESI TOF-MS) calcd for $C_{14}H_{11}FNaO_2^+$ 253.0635; found 253.0634.

*2-(3-Bromo-5-methyl-2-methoxyphenyl)benzaldehyde (**5j**).* Yellow oil; IR (ν_{max}) 3060, 2996, 2927, 2849, 2750, 1693, 1595, 1469, 1419, 1266, 1237, 1196, 998, and 858 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.35 (3H, s, 5′-C$\underline{H}_3$), 3.32 (3H, s, 2′-OC$\underline{H}_3$), 7.07−7.05 (1H, m, H4′), 7.40 (1H, dd, J = 7.5, 0.6 Hz, H6), 7.47−7.45 (1H, m, H6′), 7.56−7.49 (1H, m, H5), 7.65 (1H, td, J = 7.6, 7.6, 1.5 Hz, H4), 8.03 (1H, dd, J = 7.6, 7.6, 1.1 Hz, H3), 9.83 (1H, d, J = 0.6 Hz, C$\underline{H}$O); ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.4 (5′-$\underline{C}H_3$), 60.1 (2′-O$\underline{C}H_3$), 117.3 (C3′) 127.1 (C3), 128.2 (C5), 130.9 (C6), 131.3 (C4′), 132.7 (C5′), 133.5 (C4), 134.1 (C6′), 135.4 (C2), 140.6 (C1), 152.1 (C2′), 191.6 ($\underline{C}$HO); HRMS (ESI TOF-MS) calcd for $C_{15}H_{13}BrNaO_2^+$ 326.9991; found 326.9888.

*2,4-Dimethoxy-6-(5-bromo-2-methoxyphenyl)benzaldehyde (**5k**).* Yellow solid; mp 124.8−125.3 °C; IR (ν_{max}) 3054, 3005, 2938, 2849, 2772, 1680, 1589, 1489, 1326, 1202, 1118, 1160, 1200, 803, 731 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.70 (3H, s, OC$\underline{H}_3$), 3.87 (3H, s, OC$\underline{H}_3$), 3.93 (3H, s, OC$\underline{H}_3$), 6.34 (1H, d, J = 2.3 Hz, H5), 6.50 (1H, d, J = 2.3 Hz, H3), 6.78 (1H, d, J = 8,8 Hz, H3′), 7.31 (1H, d, J = 2.5 Hz, H6′), 7.45 (1H, dd, J = 8.8, 2.5 Hz, H4′), 9.96 (1H, s, C$\underline{H}$O); ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.6 (O$\underline{C}H_3$), 55.7 (O$\underline{C}H_3$), 55.9 (O$\underline{C}H_3$), 98.1 (C3), 108.1 (C5), 112.0 (C3′), 112.7 (C1), 117.2 (C5′), 131.0 (C1′), 131.8 (C4′), 132.5 (C6′), 143.1 (C2), 155.5 (C2′), 162.6 (C6), 164.3 (C4), 189.5 ($\underline{C}$HO); HRMS (ESI TOF-MS) calcd for $C_{16}H_{15}BrNaO_4^+$ 373.0046; found 373.0126.

*3,4-Dimethoxy-6-(5-fluoro-2-methoxyphenyl)benzaldehyde (**5l**).* Colorless solid; mp 114.4−115.2 °C; IR (ν_{max}) 3077, 3063, 3023, 3008, 2997, 2939, 2886, 2858, 1677, 1593, 1511, 1496, 1460, 1263, 1251, 1176, 1023, 731 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.71 (3H, s, OC$\underline{H}_3$), 3.95 (3H, s, OC$\underline{H}_3$), 3.97 (3H, s, OC$\underline{H}_3$), 6.76 (1H, s, H3), 6.90 (1H, dd, J = 8.7, 4.4, Hz, H3′), 7.20 (1H, dd, J = 8.5, 3.1 Hz, H6′), 7.10 (1H, ddd, J = 8.7, 3.1 Hz, H4′), 7.50 (1H, s, H6), 9.62 (1H, s, C$\underline{H}$O); ^{13}C NMR (75 MHz, $CDCl_3$) δ 56.2 (O$\underline{C}H_3$), 56.3 (O$\underline{C}H_3$), 56.5 (O$\underline{C}H_3$), 108.1 (C6), 111.5 (d, J = 8.3 Hz, C3′), 112.8 (C3), 115.6 (d, J = 22.5 Hz, C4′), 118.2 (d, J = 23.5 Hz, C6′), 127.0 (C2), 127.7 (d, J = 8.0 Hz, C1′), 135.5 (C1), 148.8 (C5), 152.8 (d, J = 1.8 Hz, C2′), 153.5 (C4), 156.8 (d, J = 240.2 Hz, C5′), 191.0 ($\underline{C}$HO); HRMS (ESI TOF-MS) calcd for $C_{16}H_{15}FNaO_4^+$ 313.0847; found 313.0882.

*3,4-Dimethoxy-6-(4-fluoro-2-methoxyphenyl)benzaldehyde (**5m**).* Colorless solid; mp 117.7−118.2 °C; IR (ν_{max}) 3085, 3025, 2974, 2956, 2915, 2876, 1675, 1592, 1497, 1441, 1260, 1212, 1134, 1017, 946, 838, 728 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.74 (3H, s, OC$\underline{H}_3$), 3.95 (3H, s, OC$\underline{H}_3$), 3.97 (3H, s, OC$\underline{H}_3$), 6.71 (1H, dd, J = 10.0, 2.4 Hz, H3′), 6.76 (1H, s, H3), 6.77 (1H, td, J = 8.2, 2.4 Hz, H5′), 7.21 (1H, dd, J = 8.2, 6.7 Hz, H6′), 7.50 (1H, s, H6), 9.61 (1H, s, C$\underline{H}$O); ^{13}C NMR (75 MHz, $CDCl_3$) δ 56.2 (O$\underline{C}H_3$), 56.3 (O$\underline{C}H_3$), 56.5 (O$\underline{C}H_3$), 99.1 (d, J = 26.0 Hz, C3′), 107.3 (d, J = 24.0 Hz, C5′), 108.2 (C6), 113.2 (C3), 122.4 (d, J = 2.5 Hz, C1′), 127.3 (C2), 132.3 (d, J = 9.8 Hz, C6′), 136.1 (C1), 148.7 (C5), 153.5 (C4), 158.0 (d, J = 9.9 Hz, C2′), 163.7 (d, J = 247.2 Hz, C4′), 191.1 ($\underline{C}$HO); HRMS (ESI TOF-MS) calcd for $C_{16}H_{15}FNaO_4^+$ 313.0847; found 313.0870.

*3-(5-Fluoro-2-methoxyphenyl)isonicotinaldehyde (**5n**).* White solid; mp 105.8−106.9 °C; IR (ν_{max}) 3036, 2996, 2953, 2913, 2846, 1700, 1581, 1493, 1400, 1212, 1191, 1177, 1022, 880, 749, 714 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.69 (3H, s, 2′-OC$\underline{H}_3$), 6.92 (1H, dd, J = 9.0, 4.3 Hz, H3′), 7.06 (1H, dd, J = 8.3, 3.1 Hz, H6′), 7.13 (1H, ddd, J = 9.0, 7.9, 3.1 Hz, H4′), 7.72 (1H, dd, J = 5.0, 0.6 Hz, H5), 8.66 (3H, s, H2), 8.77 (1H, d, J = 5.0 Hz, H6), 9.79 (1H, d, J = 0.6 Hz, C$\underline{H}$O); ^{13}C NMR (75 MHz, $CDCl_3$) δ 56.1 (2′-O$\underline{C}H_3$), 112.1 (d, J = 8.2 Hz, C3′), 117.0 (d, J = 23.0 Hz C4′), 118.1 (d, J = 24.0 Hz, C6′), 119.2 (C5), 124.5 (d, J = 7.5 Hz, C1′), 133.5 (C3), 139.5 (C4), 150.2 (C6), 152.2 (C2), 153.0 (C2′), 157.3 (d, J = 241.0 Hz, C5′), 191,3 ($\underline{C}$HO); HRMS (ESI TOF-MS) calcd for $C_{13}H_{10}FNNaO_2^+$ 254.0588; found 254.0561.

*3-(5-Chloro-2-methoxyphenyl)isonicotinaldehyde (**5o**).* Colorless solid; mp 112.7−113.5 °C; IR (ν_{max}) 3046, 2979, 2946, 2874, 2762, 1695, 1600, 1504, 1469, 1399, 1276, 1150, 1028, 840 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.73 (3H, s, 2′-OC$\underline{H}_3$), 6.93 (1H, d, J = 8.8 Hz, H3′), 7.33 (1H, d, J = 2,6 Hz, H6′), 7.43 (1H, dd, J = 8.8, 2.6 Hz, H4′), 7.75 (1H, dd, J = 5.0, 0.7 Hz, H5), 8.68 (1H, s, H2), 8.80 (1H, d, J = 5.0 Hz, H6), 9.80 (d, J = 0.7 Hz, 1H, C$\underline{H}$O); ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.7 (2′-O$\underline{C}H_3$), 112.0 (C3′), 119.0 (C5), 124.6 (C1′), 126.3 (C5′), 130.4 (C4′), 130.7 (C6′), 133.0 (C3), 139.2 (C4), 149.9 (C6), 151.9 (C2), 155.2 (C2′), 190.9 ($\underline{C}$HO); HRMS (ESI TOF-MS) calcd for $C_{13}H_{10}ClNNaO_2^+$ 270.0292; found 270.0312.

*3-(3-Bromo-5-fluoro-2-methoxyphenyl)isonicotinaldehyde (**5p**).* White solid; mp 138.5−139.6 °C; IR (ν_{max}) 3075, 2939, 2857, 2750, 1709, 1595, 1572, 1498, 1463, 1416, 1317, 1280, 1257, 1233, 1209, 1178, 1029, 1001, 871, 839, 798, 739 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.34 (s, 3H, 2′-OC$\underline{H}_3$), 7.10 (dd, 1H, J = 7.8, 3.0 Hz, H4′), 7.45 (dd, 1H, J = 7.5, 3.0 Hz, H6′), 7.82 (d, 1H, J = 5.0 Hz, H5), 8.75 (s, 1H, H2), 8.88 (d, 1H, J = 5.0 Hz, H6), 9.86 (d, 1H, J = 0.9 Hz, C$\underline{H}$O); ^{13}C NMR (75 MHz, $CDCl_3$) δ 60.7 (2′-O$\underline{C}H_3$), 117.4 (d, J = 23.5 Hz, C6′), 118.5 (d, J = 10.4 Hz, C3′), 119.9 (C5), 121.9 (d, J = 25.2 Hz, C4′), 130.8 (d, J = 8.0 Hz, C1′), 132.4 (C3), 139.1 (C4), 151.1 (C6), 151.3 (C2′), 151.8 (C2), 158.8 (d, J = 247.8 Hz, C5′), 190.3 ($\underline{C}$HO); HRMS (ESI TOF-MS) calcd for $C_{13}H_9BrFNNaO_2$ 331.9693; found 331.9706.

*2-(5-Fluoro-2-methoxyphenyl)nicotinaldehyde (**5q**).* White solid; mp 130.1−131.0 °C; IR (ν_{max}) 3070, 2998, 2968, 2908, 2840, 1694, 1580, 1566, 1495, 1435, 1251, 1191, 1169, 809, 749, 716 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$), δ 3.72 (s, 3H, 2′-OC$\underline{H}_3$), 6.93 (dd, 1H, J = 9.0, 4.2 Hz, H3′), 7.17 (ddd, 1H, J = 9.0, 7.8, 3.3 Hz, H4′), 7.33 (dd, 1H, J = 8.6, 3.3 Hz, H6′), 7.45 (ddd, 1H, J = 7.8, 4.8, 0.9 Hz, H5), 8.26 (dd, 1H, J = 7.8, 1.8 Hz, H4), 8.89 (dd, 1H, J = 4.8, 1.8 Hz, H6), 9.78 (d, 1H, J = 0.9 Hz, C$\underline{H}$O); ^{13}C NMR (75 MHz, $CDCl_3$), δ 56.2 (2′-O$\underline{C}H_3$), 112.2 (d, J = 8.1 Hz, C3′), 117.6 (d, J = 22.8 Hz, C4′), 118.4 (d, J = 24.0 Hz, C6′), 123.2 (C5), 127.7 (d, J = 7.4 Hz, C1′), 130.3 (C3), 134.9 (C4), 152.8 (C2), 154.0 (C6), 157.5 (d, J = 238.6 Hz, C5′), 157.6 (C2′), 191.4 ($\underline{C}$HO); HRMS (ESI TOF-MS) calcd for $C_{13}H_{10}FNNaO_2^+$ 254.0588; found 254.0521.

*2-(2-Methoxy-5-methylphenyl)nicotinaldehyde (**5r**).* White solid; mp 63.4−64.1 °C; IR (ν_{max}) 2948, 2838, 1699, 1580, 1565, 1501, 1438, 1381, 1277, 1251, 1237, 1147, 1025, 835, 808 cm^{-1}; ^{1}H NMR

(300 MHz, $CDCl_3$) δ 2.37 (s, 3H, 5′-C$\underline{H}_3$), 3.71 (s, 3H, 2′-OC$\underline{H}_3$), 6.88 (d, 1H, J = 8.4 Hz, H3′), 7.27 (ddd, 1H, J = 8.4, 2.4, 0.6 Hz, H4′), 7.38−7.40 (m, 1H, H6′), 7.41 (ddd, 1H, J = 7.8, 4.8, 0.8 Hz, H5), 8.24 (dd, 1H, J = 7.8, 1.8 Hz, H4), 8.88 (dd, 1H, J = 4.8, 1..8 Hz, H6), 9.78 (d, 1H, J = 0.8 Hz, C$\underline{H}$O); ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.6 (5′-$\underline{C}H_3$), 55.6 (2′-O$\underline{C}H_3$), 111.0 (C3′), 122.7 (C5), 126.0 (C1′), 130.3 (C3), 131.1 (C5′), 132.0 (C4′), 132.1 (C6′), 134.7 (C4), 153.8 (C6), 154.6 (C2′), 159.0 (C2), 192.0 (C$\underline{H}$O); HRMS (ESI TOF-MS) calcd for $C_{14}H_{13}NNaO_2^+$ 250.0838; found 250.0861.

*2-(3-Bromo-5-fluoro-2-methoxyphenyl)nicotinaldehyde (**5s**).* White solid; mp 88.5−89.9 °C; IR (ν_{max}) 3075, 3003, 2939, 2865, 2824, 2744, 1702, 1579, 1567, 1467, 1449, 1419, 1383, 1265, 1257, 1233, 1196, 996, 931, 803, 741 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.34 (s, 3H, 2′-OC$\underline{H}_3$), 7.32 (dd, 1H, J = 8.1, 3.0 Hz, H4′), 7.45 (dd, 1H, J = 7.5, 3.0 Hz, H6′), 7.52 (ddd, 1H, J = 8.0, 4.8, 0.6 Hz, H5), 8.33 (dd, 1H, J = 8.0, 1.8 Hz, H4), 8.90 (dd, 1H, J = 4.8, 1.8 Hz, H6), 9.80 (d, 1H, J = 0.6 Hz, C$\underline{H}$O); ^{13}C NMR (75 MHz, $CDCl_3$) δ 61.2 (2′-O$\underline{C}H_3$), 112.2 (d, J = 8.3 Hz, C3′), 117.6 (d, J = 23.7 Hz, C6′), 122.2 (d, J = 25.2 Hz, C4′), 123.8 (C5), 129.9 (C3), 133.7 (d, J = 8.6 Hz, C1′), 135.6 (C4), 153.8 (C6), 153.9 (C2), 156.6 (C2′), 158.9 (d, J = 247.6 Hz, C5′), 190.3 (C$\underline{H}$O); HRMS (ESI TOF-MS) calcd for $C_{13}H_9BrFNNaO_2^+$ 331.9693; found 331.9732.

*2,4-Dimethoxy-6-(5-methyl-2-methoxyphenyl)benzoic Acid (**6a**).* To a round bottomed flask (50 mL), a solution of 2,4-dimethoxy-6-(5-methyl-2-methoxyphenyl)benzaldehyde (0.170 g, 0.594 mmol) in *t*-BuOH/water (15 mL, 5:1 v/v) was added. Then NaH_2PO_4 (0.214 g, 1.78 mmol), $NaClO_2$ (0.430 g, 0.594 mmol), and THF (0.50 mL, 5.35 mmol) were added, and the mixture was stirred for 3 h at room temperature. The solvents were evaporated, and the residue was extracted with DCM (3 × 10 mL). The organic extracts were combined and the organic phase was dried with anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The acid obtained was used in the subsequent stage of reaction.

The same procedure was used for the synthesis of acids (**6b−j** and **6p−s**), whose yield is presented in Figure 3.

*2,4-Dimethoxy-6-(5-chloro-2-methoxyphenyl)benzoic Acid (**6b**).* White solid; mp 123.2−123.9 °C; IR (ν_{max}) 3300−2500, 3005, 2940, 2841, 1692, 1599, 1493, 1249, 1031 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.68 (s, 3H, 2′-OC$\underline{H}_3$), 3.84 (s, 3H, 6-OC$\underline{H}_3$), 3.92 (s, 3H, 4-OC$\underline{H}_3$), 6.41 (d, 1H, J = 2.4 Hz, H5), 6.52 (d, 1H, J = 2.4 Hz, H3), 6.79 (d, 1H, J = 8.7 Hz, H3′), 7.18 (d, 1H, J = 2.7 Hz, H6′), 7.26 (dd, 1H, J = 8.7, 2.7 Hz, H4′); ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.8 (2′-O$\underline{C}H_3$), 55.8 (6-O$\underline{C}H_3$), 56.6 (4-O$\underline{C}H_3$), 98.5 (C3), 108.6 (C5), 111.9 (C3′), 114.0 (C1), 125.6 (C5′), 128.8 (C6′), 129.7 (C4′), 132.0 (C1′), 141.1 (C2), 155.1 (C2′), 159.1 (C6), 162.5 (C4), 169.5 (C$\underline{O}$OH); HRMS (ESI TOF-MS) calcd for $C_{16}H_{15}ClNaO_5^+$ 345.0500; found 345.0524.

*2,4-Dimethoxy-6-(4-fluoro-2-methoxyphenyl)benzoic Acid (**6c**).* White solid; mp 172.0−173.2 °C; IR (ν_{max}) 3500−2500, 3012, 2976, 2940, 2844, 1695, 1601, 1508, 1280, 1022, 829 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.68 (s, 3H, 2′-OC$\underline{H}_3$), 3.84 (s, 3H, 6-OC$\underline{H}_3$), 3.92 (s, 3H, 4-OC$\underline{H}_3$), 6.42 (d, 1H, J = 2.4 Hz, H5), 6.51 (d, 1H, J = 2.4 Hz, H3), 6.61 (dd, 1H, J = 10.8, 2.4 Hz, H3′), 6.69 (td, 1H, J = 8.4, 2.4 Hz, H5′), 7.15 (dd, J = 8.4, 6.9 Hz, H6′); ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.7 (2′-O$\underline{C}H_3$), 55.7 (6-O$\underline{C}H_3$), 56.5 (4-O$\underline{C}H_3$), 98.2 (C3), 99.3 (d, J = 25.7 Hz, C3′), 107.1 (d, J = 21.2 Hz, C5′), 108.8 (C5), 114.4 (C1), 126.2 (d, J = 3.4 Hz, C1′), 130.7 (d, J = 9.8 Hz, C6′), 141.3 (C2), 157.5 (d, J = 9.8 Hz, C2′), 159.0 (C6), 162.3 (C4), 164.0 (d, J = 244 Hz, C4′), 169.8 (C$\underline{O}$OH); HRMS (ESI TOF-MS) calcd for $C_{16}H_{15}FNaO_5^+$ 329.0796; found 329.0784.

*2,4-Dimethoxy-6-(5-fluoro-2-methoxyphenyl)benzoic Acid (**6d**).* White solid; mp 117.6−118.8 °C; IR (ν_{max}) 3500−2500, 3072, 3005, 2942, 2840, 1693, 1600, 1500, 1461, 1209, 1164, 1034; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.68 (s, 3H, 2′-OC$\underline{H}_3$), 3.85 (s, 3H, 6-OC$\underline{H}_3$), 3.93 (s, 3H, 4-OC$\underline{H}_3$), 6.43 (d, 1H, J = 2.4 Hz, H5), 6.52 (d, 1H, J = 2.4 Hz, H3), 6.80 (dd, 1H, J = 8.7, 4.5 Hz, H3′), 6.96 (td, 1H, J = 8.7, 3.0 Hz, H4′), 7.01 (dd, 1H, J = 8.1, 3.0 Hz, H6′); ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.8 (6-O$\underline{C}H_3$), 56.1 (2′-O$\underline{C}H_3$), 56.6 (4-O$\underline{C}H_3$), 96.1 (C1), 98.5 (C3), 108.7 (C5), 111.7 (d, J = 8.6 Hz, C3′), 114.9 (d, J = 22.4 Hz, C6′), 116.9 (d, J = 24.0 Hz, C4′), 131.8 (d, J = 7.6 Hz, C1′), 141.4 (C2), 152.5 (d, J = 2.5 Hz, C2′), 157.1 (d, J = 238 Hz, C5′), 159.1 (C6), 162.5 (C4), 168.7 (C$\underline{O}$OH); HRMS (ESI TOF-MS) calcd for $C_{16}H_{15}FNaO_5^+$ 329.0796; found 329.0778.

*2-(5-Methyl-2-methoxyphenyl)benzoic Acid (**6e**).* White solid; mp 162.9−165.0 °C; IR (ν_{max}) 3500−2500, 3060, 3002, 2924, 2834, 1690, 1599, 1503, 1296, 1279, 1251, 1237 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.35 (t, 3H, J = 0.6 Hz, 5′-C$\underline{H}_3$), 3.68 (s, 3H, 2′-OC$\underline{H}_3$), 6.77 (d, 1H, J = 8.1 Hz, H3′), 7.09 (dd, 1H, J = 2.1, 0.6 Hz, H6′), 7.13 (ddd, 1H, J = 8.1, 2.1, 0.6 Hz, H4′), 7.34 (ddd, 1H, J = 7.8, 1.5, 0.6 Hz, H6), 7.40 (td, 1H, J = 7.8, 1.5 Hz, H5), 7.57 (td, 1H, J = 7.8, 1.5 Hz, H4), 7.92 (ddd, 1H, J = 7.8, 1.5, 0.6 Hz, H3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.8 (5′-$\underline{C}H_3$), 55.3 (2′-O$\underline{C}H_3$), 110.7 (C3′), 127.3 (C3), 129.6 (C5), 130.0 (C4′/C5′), 130.3 (C1′), 130.8 (C6), 130.9 (C2), 131.8 (C6′), 132.5 (C4), 139.3 (C1), 154.3 (C2′), 173.7 (C$\underline{O}$OH); HRMS (ESI TOF-MS) calcd for $C_{15}H_{14}NaO_3^+$ 265.0835; found 265.0859.

*2-(5-Chloro-2-methoxyphenyl)benzoic Acid (**6f**).* White solid; mp 195.9−197.5 °C; IR (ν_{max}) 3500−2500, 3067, 3008, 2974, 1691, 1596, 1496, 1027, 813, 769 cm^{-1}; ^{1}H NMR (300 MHz, CD_3OD) δ 3.69 (s, 3H, 2′-OC$\underline{H}_3$), 6.93 (d, 1H, J = 9.0 Hz, H3′), 7.18 (d, 1H, J = 2.7 Hz, H6′), 7.27 (ddd, 1H, J = 7.5, 1.5, 0.6 Hz, H6), 7.28 (dd, 1H, J = 9.0, 2.7 Hz, H4′), 7.42 (td, 1H, J = 7.5, 1.5 Hz, H5), 7.57 (td, J = 7.5, 1.5 Hz, H4), 7.85 (ddd, 1H, J = 7.5, 1.5, 0.6 Hz, H3), ^{13}C NMR (75 MHz, CD_3OD) δ 54.7 (2′-O$\underline{C}H_3$), 111.8 (C3′), 125.2 (C5′), 127.4 (C3), 128.1 (C5), 129.2 (C6′), 129.3 (C6), 131.0 (C4′), 131.5 (C4), 132.4 (C1′), 132.7 (C2), 137.7 (C1), 155.4 (C2′), 170.2 (C$\underline{O}$OH); HRMS (ESI TOF-MS) calcd for $C_{14}H_{11}ClNaO_3^+$ 285.0289; found 285.0310.

*2-(4-Fluoro-2-methoxyphenyl)benzoic Acid (**6g**).* White solid; mp 151.4−152.3 °C; IR (ν_{max}) 3500−2500, 3084, 3006, 2980, 2944, 1689, 1603, 1506, 1449, 1153, 1031, 947, 840, 764 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.68 (s, 3H, 2′-OC$\underline{H}_3$), 6.61 (dd, 1H, J = 10.8, 2.4 Hz, H3′), 6.74 (td, 1H, J = 8.4, 2.4 Hz, H5′), 7.19 (dd, 1H, J = 8.4, 6.9 Hz, H6′), 7.30 (ddd, 1H, J = 7.5, 1.5, 0.3 Hz, H6), 7.42 (td, 1H, J = 7.5, 1.5 Hz, H5), 7.61 (td, 1H, J = 7.5, 1.5 Hz, H4), 7.95 (ddd, 1H, J = 7.5, 1.5, 0.3 Hz, H3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.5 (2′-O$\underline{C}H_3$), 99.2 (d, J = 25.7 Hz, C3′), 107.3 (d, J = 21.2 Hz, C5′), 126.4 (d, J = 3.5 Hz, C1′), 127.5 (C5), 130.3 (C3), 130.6 (d, J = 9.8 Hz, C6′), 130.6 (C2), 131.8 (C6), 132.7 (C4) 138.6 (C1), 157.5 (d, J = 9.8 Hz, C2′), 163.6 (d, J = 245 Hz, C4′), 173.5 (C$\underline{O}$OH); HRMS (ESI TOF-MS) calcd for $C_{14}H_{11}FNaO_3^+$ 269.0584; found 269.0602.

*2-(5-Fluoro-2-methoxyphenyl)benzoic Acid (**6h**).* White solid; mp 141.7−142.9 °C; IR (ν_{max}) 3500−2500, 3068, 3002, 2941, 2837, 1691, 1596, 1499, 1251, 1179, 1033, 807, 731 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.67 (s, 3H, 2′-OC$\underline{H}_3$), 6.79 (dd, 1H, J = 9.3, 4.5 Hz, H3′), 7.00 (dd, 1H, J = 8.1, 3.3 Hz, H6′), 7.01 (ddd, 1H, J = 9.3, 8.1, 3.3 Hz, H4′), 7.32 (ddd, 1H, J = 7.5, 1.5, 0.6 Hz, H6), 7.44 (td, 1H, J = 7.5, 1.5 Hz, H5), 7.60 (td, 1H, J = 7.5, 1.5 Hz, H4), 7.85 (ddd, 1H, J = 7.5, 1.5, 0.6 Hz, H3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.8 (2′-O$\underline{C}H_3$), 111.5 (d, J = 8.6 Hz, C3′), 114.9 (d, J = 22.3 Hz, C4′), 116.6 (d, J = 24.0 Hz, C6′), 127.9 (C5), 130.3 (C3), 130.6 (C2), 131.6 (C6), 131.7 (C1), 132.8 (C4), 138.3 (d, J = 3.3 Hz, C1′), 152.5 (d, J = 1.7 Hz, C2′), 157.3 (d, J = 237 Hz, C5′), 173.5 (C$\underline{O}$OH); HRMS (ESI TOF-MS) calcd for $C_{14}H_{11}FNaO_3^+$ 269.0584; found 269.0613.

*2-(5-Bromo-2-methoxyphenyl)benzoic Acid (**6i**).* Colorless solid; mp 211.6−212.0 °C; IR (ν_{max}) 3150−2250, 3070, 2998, 2973, 2940, 2814, 1680, 1594, 1482, 1286, 1277, 1246, 1224, 1023, 810 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.64 (3H, s, OC$\underline{H}_3$), 6.96 (1H, d, J = 8.8 Hz, H3′), 7.28 (1H, d, J = 7.6, 1.3 Hz, H6), 7.30 (1H, d, J = 2.5 Hz, H6′), 7.44 (1H, dt, J = 7.6, 7.6, 1.2 Hz, H5), 7.48 (1H, dd, J = 8.8, 2.5 Hz, H4′), 7.57 (1H, dt, J = 7.6, 7.6, 1.3 Hz, H4), 7.77 (1H, dd, J = 7.6, 1.2 Hz, H3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 56.0 (2′-O$\underline{C}H_3$), 112.3 (C5′), 113.5 (C3′), 128.2 (C5), 129.6 (C3), 131.7 (C4′), 131.7 (C1′), 132.0 (C6′), 132.2 (C6), 133.0 (C4), 133.3 (C2), 137.1 (C1), 156.0 (C2′), 169.1 (C$\underline{O}$OH); HRMS (ESI TOF-MS) calcd for $C_{14}H_{11}BrNaO_3^+$ 328.9784; found 328.9813.

*2-(3-Bromo-5-methyl-2-methoxyphenyl)benzoic Acid (**6j**).* Colorless solid; mp 184.9−186.4 °C; IR (ν_{max}) 3200−2250, 3028, 2992, 2926, 2853, 2821, 2646, 1696, 1597, 1470, 1449, 1409, 1296, 1232, 1002, 928, 751, 736 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.30 (3H, s, 5′-C$\underline{H}_3$), 3.25 (3H, s, 2′-OC$\underline{H}_3$), 7.03 (1H, s, H4′), 7.34 (1H, d, J =

7.5 Hz, H6), 7.41 (1H, s, H6′), 7.49 (1H, t, *J* = 7.5, 7.5 Hz, H5), 7.60 (1H, t, *J* = 7.5, 7.5 Hz, H4), 7.85 (1H, d, *J* = 7.5 Hz, H3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.6 (5′-$\underline{C}H_3$), 60.5 (2′-O$\underline{C}H_3$), 116.2 (C3′), 128.5 (C5), 130.1 (C3), 131.0 (C4′), 131.7 (C6), 131.9 (C4), 132.6 (C5′), 133.0 (C6′), 135.5 (C2), 137.2 (C1′), 138.0 (C1), 152.0 (C2′), 169.1 (COOH); HRMS (ESI TOF-MS) calcd for $C_{15}H_{13}BrNaO_3^+$ 342.9940; found 342.9889.

*2-(3-Bromo-5-fluoro-2-methoxyphenyl)isonicotinic Acid (**6p**).* White solid; mp 232.3−234.2 °C; IR (ν_{max}) 3446, 3078, 3034, 2948, 2862, 2420, 1916, 1721, 1602, 1573, 1465, 1423, 1394, 1283, 1218, 1194, 1174, 1103, 1073, 1044, 994, 927, 871, 851, 775, 743, 707, 670, 605, 494, 431 cm^{-1}; ^{1}H NMR (300 MHz, CD_3OD) δ 3.26 (s, 3H, 2′-O$\underline{C}H_3$), 7.32 (dd, 1H, *J* = 8.7, 3.0 Hz, H4′), 7.64 (dd, 1H, *J* = 8.1, 3.0 Hz, H6′), 7.75 (d, 1H, *J* = 5.0 Hz, H5), 8.64 (s, 1H, H2), 8.76 (d, 1H, *J* = 5.0 Hz, H6); ^{13}C NMR (75 MHz, CD_3OD) δ 60.9 (2′-O$\underline{C}H_3$), 117.3 (C3′), 117.4 (d, $J_{C,F}$ = 23.8 Hz, C6′), 120.3 (d, *J* 25.4 Hz, C4′), 123.2 (C5), 130.8 (C1′), 135.0 (C3), 139.7 (C4), 150.9 (C6), 151.5 (d, *J* = 243.5 Hz, C2′), 151.8 (C2), 158.4 (d, *J* = 243.5 Hz, C5′), 167.6 (COOH); HRMS (ESI TOF-MS) calcd for $C_{13}H_9BrFNNaO_3^+$ 347.9642; found 347.9587.

*2-(5-Fluoro-2-methoxyphenyl)nicotinic Acid (**6q**).* White solid; mp 133.8−135.2 °C; IR (ν_{max}) 3493, 3084, 2998, 2986, 2938, 2837, 2478 1743, 1684, 1578, 1498, 1459, 1438, 1285, 1247, 1185, 1179, 1090, 1042, 1025, 907, 884, 844, 813, 809, 799, 744, 713, 643, 604, 561 cm^{-1}; ^{1}H NMR (300 MHz, CD_3OD) δ 3.71 (s, 3H, 2′-O$\underline{C}H_3$), 6.98 (dd, 1H, *J* = 9.0, 4.2 Hz, H3′), 7.12 (ddd, 1H, *J* = 9.0, 8.1, 3.3 Hz, H4′), 7.18 (ddd, 1H, *J* = 8.7, 3.3, 0.3 Hz, H6), 7.50 (dd, 1H, *J* = 7.8, 5.0 Hz, H5), 8.26 (dd, 1H, *J* = 7.8, 1.8 Hz, H4), 8.70 (dd, 1H, *J* = 5.0, 1.8 Hz, H6); ^{13}C NMR (75 MHz, CD_3OD) δ 54.8 (2′-O$\underline{C}H_3$), 111.6 (d, *J* = 8.1 Hz, C3′), 115.7 (d, *J* = 22.9 Hz, C4′), 116.6 (d, *J* = 24.5 Hz, C6′), 122.6 (C5), 129.8 (C3), 130.5 (d, *J* = 7.5 Hz, C1′), 138.0 (C4), 150.8 (C6), 153.2 (C2), 154.7 (C2′), 157.2 (d, *J* = 235.8 Hz, C5′), 167.6 (COOH); HRMS (ESI TOF-MS) calcd for $C_{13}H_{10}FNNaO_3^+$ 270.0537; found 270.0588.

*2-(2-Methoxy-5-methylphenyl)nicotinic Acid (**6r**).* White solid; mp 63.4−64.1 °C; IR (ν_{max}) 3538, 3003, 2968, 2835, 2626, 2493, 1868, 1738, 1679, 1615, 1587, 1570, 1503, 1467, 1441, 1396, 1293, 1275, 1246, 1217, 1197, 1182, 1143, 1099, 1070, 1047, 1029, 952, 883, 829, 805, 790, 741, 710, 671, 639, 607, 583, 547, 517, 483 cm^{-1}; ^{1}H NMR (300 MHz, CD_3OD) δ 2.34 (s, 3H, 5′-$\underline{C}H_3$), 3.70 (s, 3H, 2′-O$\underline{C}H_3$), 6.89 (d, 1H, *J* = 8.4 Hz, H3′), 7.20 (ddd, 1H, *J* = 8.4, 2.4, 0.6 Hz, H4′), 7.23−7.26 (m, 1H, H6′), 7.46 (dd, 1H, *J* = 7.8, 5.0 Hz, H5), 8.22 (dd, 1H, *J* = 7.8, 1.8 Hz, H4), 8.67 (dd, 1H, *J* = 5.0, 1.8 Hz, H6); ^{13}C NMR (75 MHz, CD_3OD) δ 19.4 (5′-$\underline{C}H_3$), 54.3 (2′-O$\underline{C}H_3$), 110.4 (C3′), 122.1 (C5), 128.6 (C1′), 129.8 (C3), 130.1 (C5′), 130.5 (C4′), 130.6 (C6′), 137.9 (C4), 150.3 (C6), 154.8 (C2′), 155.9 (C2), 169.1 (COOH); HRMS (ESI TOF-MS) calcd for $C_{14}H_{13}NNaO_3^+$ 266.0788; found 266.0762.

*2-(3-Bromo-5-fluoro-2-methoxyphenyl)nicotinic Acid (**6s**).* White solid; mp 192.9−194.7 °C; IR (ν_{max}) 3445, 3091, 3070, 2955, 2927, 2826, 2476, 1870, 1729, 1701, 1572, 1469, 1420, 1299, 1268, 1143, 1094, 1063, 999, 933, 875, 835, 799, 776, 766, 742, 679, 655, 647, 609, 599, 457 cm^{-1}; ^{1}H NMR (300 MHz, CD_3OD) δ 3.36 (s, 3H, 2′-O$\underline{C}H_3$), 7.19 (dd, 1H, *J* = 8.4, 3.0 Hz, H4′), 7.46 (dd, 1H, *J* = 8.0, 3.0 Hz, H6′), 7.59 (dd, 1H, *J* = 8.0, 4.9 Hz, H5), 8.38 (dd, 1H, *J* = 8.0, 1.8 Hz, H4), 8.76 (dd, 1H, *J* = 4.9, 1.8 Hz, H6); ^{13}C NMR (75 MHz, CD_3OD) δ 60.4 (2′-O$\underline{C}H_3$), 116.2 (d, *J* = 24.1 Hz, C6′), 116.8 (d, *J* = 10.3 Hz, C3′), 120.2 (d, *J* = 25.8 Hz, C4′), 123.4 (C5), 129.0 (C3), 137.2 (d, *J* = 8.6 Hz, C1′), 138.7 (C4), 151.2 (C6), 151.4 (C2), 154.5 (C2′), 158.6 (d, *J* = 244.4 Hz, C5′), 167.9 (COOH); HRMS (ESI TOF-MS) calcd for $C_{13}H_9BrFNNaO_3^+$ 347.9642; found 347.9612.

*2,4-Dimethoxy-6-(5-bromo-2-methoxyphenyl)benzoic Acid (**6k**).* To a round bottomed flask (25 mL) a solution of 2,4-dimethoxy-6-(5-bromo-2-methoxyphenyl)benzaldehyde (0.120 g, 0.34 mmol) in 2 mL of *t*-BuOH was added. Then to the mixture, 2 mL of sodium hydrogen phosphate (NaH_2PO_4), pH 4.4, was added and then the solution of potassium permanganate (0.5 mol L^{-1}) (0.320 g; 2.00 mmol) under vigorous stirring. The reaction mixture was kept at room temperature for about 2 h, with the consumption of the aldehyde followed by thin layer chromatography. At the end of reaction, excess $KMnO_4$ was destroyed with saturated sodium sulfite (Na_2SO_3), the pH of the mixture was adjusted to 3 using HCl solution (2 mol L^{-1}) so as to solubilize the MnO_2 derived from the destruction of $KMnO_4$, and the mixture was subjected to extraction with ethyl acetate (3 × 30 mL). The organic phases were combined and dried with anhydrous magnesium sulfate ($MgSO_4$), filtered, and concentrated under reduced pressure to yield the required aldehyde (**6k**) as a white solid (113.0 mg; 0.31 mmol). Yield: 91%; mp 182.4−183.2 °C; IR (ν_{max}) 3213−2400, 3020, 3005, 2917, 2848, 2834, 1680, 1593, 1567, 1488, 1332, 1285, 1266, 1200, 1026, 803, 799, 621 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.67 (3H, s, O$\underline{C}H_3$), 3.84 (3H, s, O$\underline{C}H_3$), 3.91 (3H, s, O$\underline{C}H_3$), 6.41 (1H, d, *J* = 2.3 Hz, H5), 6.52 (1H, d, *J* = 2.3 Hz, H3), 6.75 (1H, d, *J* = 8.8 Hz, H3′), 7.32 (1H, d, *J* = 2.5 Hz, H6′), 7.45 (1H, dd, *J* = 8.7, 2.7 Hz, H4′); ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.7 (O$\underline{C}H_3$), 55.8 (O$\underline{C}H_3$), 56.6 (O$\underline{C}H_3$), 98.5 (C3), 108.5 (C5), 112.5 (C3′), 112.9 (C1), 113.2 (C5′), 131.8 (C4′), 132.5 (C1′), 132.6 (C6′), 140.8 (C2), 155.6 (C2′), 159.1 (C6), 162.5 (C4) and 170.0 (COOH); HRMS (ESI TOF-MS) calcd for $C_{16}H_{15}BrNaO_5^+$ 388.9995; found 388.9989.

The same procedure was used for the synthesis of acids (**6l** and **6o**), whose yield is presented in Figure 3.

*3,4-Dimethoxy-6-(5-fluoro-2-methoxyphenyl)benzoic Acid (**6l**).* Colorless solid; mp 215.2−216.2 °C; IR (ν_{max}) 3250−2500, 3068, 2998, 2964, 2936, 2837, 1675, 1596, 1518, 1499, 1460, 1417, 1347, 1262, 1212, 1170, 1097, 1025, 740 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.71 (3H, s, O$\underline{C}H_3$), 3.95 (3H, s, O$\underline{C}H_3$), 3.97 (3H, s, O$\underline{C}H_3$), 6.79 (1H, s, H3), 6.93 (1H, dd, *J* = 8.5, 4.6 Hz, H3′), 7.04 (1H, dd, *J* = 7.5, 3.0 Hz, H6′), 7.09 (1H, td, *J* = 8.5, 8.5, 3.0 Hz, H4′), 7.35 (1H, s, H6); ^{13}C NMR (75 MHz, $CDCl_3$) δ 56.2 (O$\underline{C}H_3$), 56.3 (O$\underline{C}H_3$), 56.5 (O$\underline{C}H_3$), 112.2 (d, *J* = 8.4 Hz, C3′), 113.0 (C6), 114.3 (d, *J* = 22.4 Hz, C4′), 114.8 (C3), 118.2 (d, *J* = 23.5 Hz, C6′), 124.4 (C1′), 131.4 (C1), 132.8 (d, *J* = 8.1 Hz, C2′), 147.8 (C5), 151.3 (C2), 153.2 (C4), 156.7 (d, *J* = 235.3 Hz, C5′), 168.5 (COOH); HRMS (ESI TOF-MS) calcd for $C_{16}H_{15}FNaO_5^+$ 329.0796; found 329.0884.

*3,4-Dimethoxy-6-(4-fluoro-2-methoxyphenyl)benzoic Acid (**6m**).* Colorless solid; mp 217.5−218.3 °C; IR (ν_{max}) 3250−2400, 3062, 2999, 2954, 2920, 2845, 1670, 1595, 1498, 1455, 1256, 1207, 1148, 1020 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.65 (3H, s, O$\underline{C}H_3$), 3.80 (3H, s, O$\underline{C}H_3$), 3.82 (3H, s, O$\underline{C}H_3$), 6.74 (1H, s, H3), 6.78 (1H, ddd, *J* = 8.5, 8.5, 2.5 Hz, H5′), 6.87 (1H, dd, *J* = 11.5, 2.5 Hz, H3′), 7.18 (1H, dd, *J* = 8.3, 7.0 Hz, H6′), 7.35 (1H, s, H6); ^{13}C NMR (75 MHz, $CDCl_3$) δ 56.1 (2xO$\underline{C}H_3$), 56.2 (O$\underline{C}H_3$), 99.5 (d, *J* = 25.7 Hz, C3′), 106.7 (d, *J* = 21.0 Hz, C5′), 113.1 (C6), 114.9 (C3), 124.4 (C2), 127.6 (d, *J* = 3.2 Hz, C1′), 131.0 (d, *J* = 9.9 Hz, C6′), 131.9 (C1), 147.7 (C5), 151.3 (C4), 158.1 (d, *J* = 10.2 Hz, C2′), 162.9 (d, *J* = 242.3 Hz, C4′), 168.6 (COOH); HRMS (ESI TOF-MS) calcd for $C_{16}H_{15}FNaO_5^+$ 329.0796; found 329.0824.

*3-(5-Fluoro-2-methoxyphenyl)isonicotinic Acid (**6n**).* Colorless solid; mp 248.3−250.2 °C; IR: 3022, 2973, 2939, 2834, 1704, 1595, 1494, 1477, 1284, 1252, 1217, 1032, 950, 905, 867, 821, 724 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.65 (s, 3H, 2′-O$\underline{C}H_3$), 7.03 (dd, 1H, *J* = 9.6, 4.5 Hz, H3′), 7.22−7.15 (m, 2H, H6′ and H4′), 7.66 (d, 1H, *J* = 5.0 Hz, H5), 8.56 (s, 1H, H2) 8.69 (d, 1H, *J* = 5.0 Hz, H6); ^{13}C NMR (75 MHz, DMSO-d_6) δ 56.3 (2′-O$\underline{C}H_3$), 112.6 (d, *J* = 8.4 Hz, C3′), 115.8 (d, *J* = 22.5 Hz, C4′), 117.1 (d, *J* = 24.1 Hz, C6′), 122.6 (C5), 128.2 (d, *J* = 8.1 Hz, C1′), 131.2 (C3), 140.4 (C4), 149.9 (C6), 151.7 (C2), 153.2 (C2′), 156.8 (d, *J* = 236.1 Hz, C5′), 167.9 (COOH); HRMS (ESI TOF-MS) calcd for $C_{13}H_{10}FNNaO_3^+$ 270.0537; found 270.0498.

*3-(5-Chloro-2-methoxyphenyl)isonicotinic Acid (**6o**).* White solid; mp 253.2−254.1 °C; IR: 3083, 3027, 2985, 2942, 2843, 2361, 1702, 1596, 1472, 1397, 1281, 1251, 1219, 1186, 1031, 820 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.67 (s, 3H, 2′-O$\underline{C}H_3$), 7.06 (d, 1H, *J* = 8.0 Hz, H3′), 7.35 (d, 1H, *J* = 2.6 Hz, H6′), 7.41 (dd, 1H, *J* = 8.7, 2.7 Hz, H4′), 7.66 (d, 1H, *J* = 5.0 Hz, H5), 8.55 (s, 1H, H2), 8.68 (d, 1H, *J* = 5.0 Hz, H6); ^{13}C NMR (75 MHz, DMSO-d_6) δ 56.9 (2′-O$\underline{C}H_3$), 100.1 (C3′), 100.5 (C5), 102.8 (C1′), 118.0 (C5′), 120.2 (C4′), 127.3 (C6′), 134.2 (C3), 135.9 (C4), 149.8 (C6), 163.8 (C2), 164.2 (C2′), 167.2 (COOH); HRMS (ESI TOF-MS) calcd for $C_{13}H_{10}ClNNaO_3^+$ 286.0241; found 286.0202.

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*7,9-Dihydroxy-2-methyl-6H-benzo[c]chromen-6-one (**7a**).* To a round bottomed flask (20 mL), under an atmosphere of nitrogen and at the temperature of 0 °C, a solution of 2,4-dimethoxy-6-(5-methyl-2-methoxyphenyl)benzoic acid (0.145 g, 0.480 mmol) in anhydrous DCM (5 mL) was added. To this mixture a 0.5 mol L^{-1} solution of BBr_3 (3.84 mL, 1.92 mmol) in anhydrous DCM was added slowly. After the addition, the ice bath was removed and the system was maintained under magnetic agitation for a period of 24 h at room temperature. Then, methanol (0.5 mL) was added slowly (approximately 3 min) to the system until all gas had been released and all precipitate formed had been dissolved. The solvent was evaporated, and the residue was extracted with ethyl acetate (3 × 15 mL). The organic extracts were combined, and the resulting organic phase was dried with anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The residue obtained was submitted to separation in column (hexane/ethyl acetate 7:1 v/v), resulting in the chromen-6-one (**7a**) (38.7 mg, 0.160 mmol) with 34.5% yield. Mp 281.8−283.1 °C; IR (ν_{max}) 3222, 3130, 2925, 1641, 1587, 1510, 1456, 1209, 1172 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 2.39 (s, 3H, 2-C$\underline{H}_3$), 6.44 (d, 1H, J = 2.4 Hz, H8), 7.13 (d, 1H, J = 2.4 Hz, H10), 7.28 (d, 1H, J = 8.4 Hz, H4), 7.35 (dd, 1H, J = 8.4, 2.1 Hz, H3), 7.92 (d, 1H, J = 2.1 Hz, H1), 11.34 (s, 2H, 7-O$\underline{H}$/9-O$\underline{H}$); ^{13}C NMR (75 MHz, DMSO-d_6) δ 20.0 (2-$\underline{C}H_3$), 97.7 (C12), 100.1 (C10), 102.1 (C8), 116.5 (C4), 116.8 (C13), 123.2 (C1), 131.3 (C3), 134.0 (C2), 136.1 (C11), 147.7 (C14), 163.1 (C7), 163.9 (C6), 165.4 (C9); HRMS (ESI TOF-MS) calcd for $C_{14}H_{10}NaO_4^+$ 265.0471; found 265.0477.

The compounds (**7b**−**n**) were prepared using the same procedure.

*2-Chloro-7,9-dihydroxy-6H-benzo[c]chromen-6-one (**7b**).* White solid; mp >295 °C; IR (ν_{max}) 3395, 3086, 1678, 1570, 1457, 1410, 1090 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 6.45 (d, 1H, J = 2.1 Hz, H8), 7.13 (d, 1H, J = 2.1 Hz, H10), 7.37 (d, 1H, J = 9.0 Hz, H4), 7.53 (dd, 1H, J = 9.0, 2.4 Hz, H3), 8.14 (d, 1H, J = 2.4 Hz, H1), 11.17 (s, 2H, 7-O$\underline{H}$/9-O$\underline{H}$); ^{13}C NMR (75 MHz, DMSO-d_6), δ 98.8 (C12), 102.1 (C8), 103.9 (C1), 119.9 (C4), 120.1 (C2), 124.1 (C10), 130.0 (C13), 131.3 (C3), 136.1 (C11), 149.4 (C14), 164.1 (C9), 164.4 (C7), 166.6 (C6); HRMS (ESI TOF-MS) calcd for $C_{13}H_7ClNaO_4^+$ 284.9925; found 284.9943.

*3-Fluoro-7,9-dihydroxy-6H-benzo[c]chromen-6-one (**7c**).* White solid; mp 281.8−283.1 °C; IR (ν_{max}) 3450, 3239, 1673, 1607, 1465, 1293, 1207, 1192, 6 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 6.42 (d, 1H, J = 2.1 Hz, H8), 7.08 (d, 1H, J = 2.1 Hz, H10), 7.23 (td, 1H, J = 8.7, 2.7 Hz, H2), 7.34 (dd, 1H, J = 9.3, 2.7 Hz, H4), 8.16 (dd, 1H, J = 8.7, 6.0 Hz, H1), 11.14 (s, 2H, 7-O$\underline{H}$/9-O$\underline{H}$); ^{13}C NMR (75 MHz, DMSO-d_6) δ 98.4 (C12), 101.4 (C10), 103.2 (C8), 105.3 (d, J = 25.7 Hz, C4), 113.5 (d, J = 22.3 Hz, C2), 115.4 (C11), 126.6 (d, J = 9.8 Hz, C1), 136.8 (d, J = 2.8 Hz, C13), 151.7 (d, J = 12.6 Hz, C14), 163.6 (d, J = 247 Hz, C3), 164.2 (C9), 164.5 (C7), 166.7 (C6); HRMS (ESI TOF-MS) calcd for $C_{13}H_7FNaO_4^+$ 269.0221; found 269.0218.

*2-Fluoro-7,9-dihydroxy-6H-benzo[c]chromen-6-one (**7d**).* White solid; mp 268.3−270.1 °C; IR (ν_{max}) 3445, 3301, 1676, 1579, 1467, 1185, 1102, 691 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 6.48 (d, 1H, J = 1.8 Hz, H8), 7.17 (d, 1H, J = 1.8 Hz, H10), 7.36−7.52 (m, 2H, H4/H3), 8.02 (dd, 1H, J = 9.6, 2.4 Hz, H1), 11.08 (s, 1H, 9-O$\underline{H}$), 11.27 (s, 1H, 7-O$\underline{H}$); ^{13}C NMR (75 MHz, DMSO-d_6) δ 98.8 (C12), 102.3 (C10), 103.9 (C8), 110.7 (d, J = 24.6 Hz, C1), 118.8 (d, J = 24.6 Hz, C3), 120.0 (d, J = 7.4 Hz, C4/C11), 136.5 (d, J = 4.1 Hz, C13), 147.1 (d, J = 2.0 Hz, C14), 159.6 (d, J = 239 Hz, C2), 164.2 (C9), 164.7 (C7), 166. Five (C6); HRMS (ESI TOF-MS) calcd for $C_{13}H_7FNaO_4^+$ 269.0221; found 269.0231.

*2-Methyl-6H-benzo[c]chromen-6-one (**7e**).* White solid; mp 125.2−126.5 °C; IR (ν_{max}) 3030, 2954, 2920, 2852, 1730, 1606, 1495, 1266, 1072, 1037, 807, 768 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.45 (s, 3H, 2-C$\underline{H}_3$), 7.24 (d, 1H, J = 8.4 Hz, H4), 7.27 (dd, 1H, J = 8.4, 1.2 Hz, H3), 7.56 (ddd, 1H, J = 7.8, 7.2, 1.5 Hz, H8), 7.80 (ddd, 1H, J = 7.8, 7.2, 1.5 Hz, H9), 7.82 (d, J = 1.2 Hz, H1), 8.10 (ddd, 1H, J = 7.8, 1.5, 0.6 Hz, H7), 8.39 (ddd, 1H, J = 7.8, 1.5, 0.6 Hz, H10); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.3 (2-$\underline{C}H_3$), 117.7 (C4), 117.9 (C11), 121.5 (C12), 121.8 (C10), 123.0 (C8), 129.0 (C3), 130.8 (C7), 131.6 (C1), 134.3 (C13), 134.9 (C9), 135.0 (C2), 149.6 (C14), 161.6 (C6); HRMS (ESI TOF-MS) calcd for $C_{14}H_{10}NaO_2^+$ 233.0573; found 233.0581.

*2-Chloro-6H-benzo[c]chromen-6-one (**7f**).* White solid; mp 174.8−176.4 °C; IR (ν_{max}) 3084, 3068, 1747, 1604, 1484, 1265, 1067, 1035, 811, 766, 712, 680 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.32 (d, 1H, J = 8.7 Hz, H4), 7.44 (dd, 1H, J = 8.7, 2.4 Hz, H3), 7.64 (td, 1H, J = 7.8, 1.5 Hz, H8), 7.86 (td, 1H, J = 7.8, 1.5 Hz, H9), 8.03 (d, 1H, J = 2.4 Hz, H1), 8.07 (d, 1H, J = 7.8 Hz, H7), 8.42 (dd, 1H, J = 7.8, 1.5 Hz, H10); ^{13}C NMR (75 MHz, $CDCl_3$) δ 119.4 (C4), 119.6 (C2), 121.5 (C13), 122.0 (C7), 122.9 (C1), 129.9 (C8), 130.3 (C11), 130.6 (C3), 131.0 (C10), 133.8 (C12), 135.3 (C9), 149.9 (C14), 160.8 (C6); HRMS (ESI TOF-MS) calcd for $C_{13}H_7ClNaO_2^+$ 253.0027; found 253.0018.

*3-Fluoro-6H-benzo[c]chromen-6-one (**7g**).* White solid; mp 150.5−152.0 °C; IR (ν_{max}) 3072, 1752, 1607, 1456, 1274, 1155, 1098, 875, 763, 712, 680 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.08 (td, 1H, J = 8.4, 2.4 Hz, H2), 7.09 (dd, 1H, J = 8.4, 2.4 Hz, H4), 7.58 (ddd, 1H, J = 8.1, 7.5, 1.5 Hz, H8), 7.83 (ddd, 1H, J = 8.1, 7.5, 1.5 Hz, H9), 8.04 (ddd, 1H, J = 8.1, 1.5, 0.6 Hz, H7), 8.05 (dd, 1H, J = 8.4, 7.2 Hz, H1), 8.39 (ddd, 1H, J = 8.1, 1.5, 0.6 Hz, H10); ^{13}C NMR (75 MHz, $CDCl_3$), δ 105.4 (d, J = 25.1 Hz, C4), 112.7 (d, J = 22.3 Hz, C2), 114.9 (d, J = 2.9 Hz, C13), 120.7 (C11), 121.7 (C7), 124.6 (d, J = 9.8 Hz, C1), 129.0 (C8), 130.9 (C10), 134.5 (C12), 135.3 (C9), 152.4 (d, J = 12.0 Hz, C14), 161.0 (C6), 163.7 (d, J = 250 Hz, C3); HRMS (ESI TOF-MS) calcd for $C_{13}H_7FNaO_2^+$ 237.0322; found 237.0314.

*2-Fluoro-6H-benzo[c]chromen-6-one (**7h**).* White solid; mp 148.3−149.4 °C; IR (ν_{max}) 3062, 1724, 1600, 1496, 1182, 1073, 873, 817, 764, 712, 681 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.19 (ddd, 1H, J = 9.0, 7.5, 3.0 Hz, H3), 7.35 (dd, 1H, J = 9.0, 4.5 Hz, H4), 7.63 (ddd, 1H, J = 8.1, 7.5, 1.5 Hz, H8), 7.72 (dd, 1H, J = 9.0, 3.0 Hz, H1), 7.85 (ddd, 1H, J = 8.1, 7.5, 1.5 Hz, H9), 8.03 (ddd, 1H, J = 8.1, 1.5, 0.6 Hz, H7), 8.42 (ddd, 1H, J = 8.1, 1.5, 0.6 Hz, H10); ^{13}C NMR (75 MHz, $CDCl_3$) δ 109.0 (d, J = 24.6 Hz, C1), 118.0 (d, J = 24.1 Hz, C3), 119.5 (d, J = 8.6 Hz, C4), 121.5 (C11), 122.1 (C7), 129.8 (C8/C12), 131.0 (C10), 134.2 (d, J = 2.9 Hz, C13), 135.2 (C9), 147.6 (d, J = 2.7 Hz, C14), 159.5 (d, J = 245 Hz, C2), 161.0 (C6); HRMS (ESI TOF-MS) calcd for $C_{13}H_7FNaO_2^+$ 237.0322; found 237.0336.

*2-Bromo-6H-benzo[c]chromen-6-one (**7i**).* White solid; mp 195.8−197.1 °C; IR (ν_{max}) 3065, 1733, 1717, 1599, 1480, 1437, 1287, 1399, 1260, 1212, 1062, 1033, 1017, 872, 805, 763, 709, 677 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.24 (1H, d, J = 8.8 Hz, H4), 7.69 (1H, dd, J = 8.8, 2.3 Hz, H3), 7.70 (1H, ddd, J = 8.0, 7.4, 1.2 Hz, H9), 7.94 (1H, ddd, J = 8.0, 7.4, 1.4 Hz, H8), 8.23 (1H, dd, J = 8.0, 1.2 Hz, H7), 8.48 (1H, brd, J = 8.0 Hz, H10), 8.55 (1H, d, J = 2.3 Hz, H-1); ^{13}C NMR (75 MHz, $CDCl_3$) δ 117.6 (C2), 120.2 (C10), 120.5 (C11), 121.3 (C8), 123.8 (C4), 126.8 (C12), 130.4 (C7), 130.6 (C3), 133.8 (C13), 133.9 (C1), 136.1 (C9), 150.5 (C14), 160.5 (C6); HRMS (ESI TOF-MS) calcd for $C_{13}H_7BrNaO_2^+$ 296.9522; found 296.9511.

*4-Bromo-2-methyl-6H-benzo[c]chromen-6-one (**7j**).* White solid; mp 169.4−170.9 °C; IR (ν_{max}) 3077, 2956, 2921, 2851, 1718, 1602, 1561, 1491, 1478, 1421, 1271, 1240, 1214, 1097, 1069, 1056, 1028, 846, 798, 768 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 2.39 (3H, s, 2-C$\underline{H}_3$), 7.63 (1H, d, J = 1.2 Hz, H3), 7.68 (1H, ddd, J = 8.0, 7.8, 0.8 Hz, H8), 7.93 (1H, ddd, J = 8.0, 7.8, 1.0 Hz, H9), 8.12 (1H, s, H1), 8.22 (1H, dd, J = 8.0, 1.0 Hz, H7), 8.36 (1H, d, J = 8.0 Hz, H10); ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.8 (2-$\underline{C}H_3$), 110.3 (C4), 119.7 (C10), 121.2 (C11), 123.6 (C8), 123.9 (C12), 130.5 (C1), 134.5 (C7), 135.0 (C3), 136.0 (C9), 136.1 (C13), 135.3 (C2), 145.9 (C14), 160.3 (C6); HRMS (ESI TOF-MS) calcd for $C_{14}H_9BrNaO_2^+$ 310.9678; found 310.9670.

*2-Bromo-7-hydroxy-9-methoxy-6H-benzo[c]chromen-6-one (**7k**).* White solid; mp 294.5−295.7 °C; IR (ν_{max}) 3097, 3004, 2935, 1665, 1631, 1565, 1401, 1270, 1200, 1163, 1082, 1045, 989, 813, 752 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 3.94 (3H, s, 9-OC$\underline{H}_3$), 6.64 (1H, d, J = 2.2 Hz, H8), 7,37 (1H, d, J = 8.8 Hz, H4), 7.43 (1H, d, J = 2.2 Hz, H10), 7.71 (1H, dd, J = 8.8, 2.3 Hz, H3), 8.57 (1H, d, J = 2.3 Hz, H1), 11.23 (1H, s, 7-O$\underline{H}$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 56.9 (9-O$\underline{C}H_3$), 100.1 (C12), 100.5 (C9), 102.8 (C10), 118.0 (C2), 120.0 (C4), 120.5

(C3), 127.3 (C1), 134.2 (C13), 135.9 (C11), 149.8 (C14), 163.8 (C7), 164.2 (C9), 167.2 (C6); HRMS (ESI TOF-MS) calcd for $C_{14}H_9BrNaO_4^+$ 342.9576; found 342.9579.

3-Fluoro-8,9-dihydroxy-6H-benzo[c]chromen-6-one (**7l**). Colorless solid; mp 283.8−284.4 °C; IR (ν_{max}) 3485, 3138, 3071, 2362, 1705, 1607, 1535, 1518, 1456, 1384, 1363, 1327, 1269, 1185, 1147, 1112, 1063, 980, 856, 803, 766 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.18 (1H, td, J = 8.8, 8.8, 2.6 Hz, H2), 7.27 (1H, dd, J = 9.6, 2.6 Hz, H4), 7.52 (1H, s, H10), 7.54 (1H, s, H7), 8.07 (1H, dd, J = 8.8, 6.2 Hz, H1), 10.27 (1H, s, 8-OH), 10.51 (1H, s, 9-OH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 105.0 (d, J = 24.6 Hz, C4), 108.5 (C7), 112.3 (C12), 112.7 (d, J = 24.2 Hz, C2), 114.9 (C10), 115.6 (C13), 125.2 (d, J = 9.6 Hz, C1), 128.4 (C11), 147.9 (C8), 151.6 (d, J = 12.6 Hz, C14), 154.2 (C9), 160.4 (C6), 162.7 (d, J = 244.8 Hz, C3); HRMS (ESI TOF-MS) calcd for $C_{13}H_7FNaO_4^+$ 269.0221; found 269.0202.

2-Fluoro-8,9-dimethoxy-6H-benzo[c]chromen-6-one (**7m**). White solid; mp 191.3−192.2 °C; IR (ν_{max}) 3075, 3000, 2947, 2848, 1706, 1598, 1501, 1426, 1411, 1371, 1299, 1280, 1176, 1160, 1073, 1025, 875, 805, 792 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 3.89 (3H, s, OCH_3), 4.00 (3H, s, OCH_3), 7.31 (1H, td, J = 9.0, 2.7 Hz, H3), 7.37 (1H, dd, J = 9.0, 5.1 Hz, H4), 7.53 (1H, s, H10), 7.75 (1H, s, H7), 8.27 (1H, d, J = 9.9 Hz, H1); ^{13}C NMR (75 MHz, $CDCl_3$) δ 56.7, (8-OCH_3), 57.3 (9-OCH_3), 105.7 (C10), 110.2 (d, J = 25.3 Hz, C3), 111.0 (C7), 114.3 (C12), 117.3 (d, J = 24.5 Hz, C1), 119.4 (d, J = 8.9 Hz, C4), 120.0 (d, J = 8.7 Hz, C13), 129.5 (C11), 147.4 (C14), 151.3 (C8), 156.1 (C9), 157.9 (C6), 160.7 (d, J = 60.4 Hz, C2); HRMS (ESI TOF-MS) calcd for $C_{15}H_{11}FNaO_4^+$ 297.0534; found 297.0528.

9-Fluoro-5H-chromene[4,3-c]pyridin-5-one (**7n**). White solid; mp 186.1−187.9 °C; IR (ν_{max}) 3077, 2953, 2921, 2852, 1720, 1603, 1590, 1490, 1407, 1270, 1244, 1232, 1183, 1072, 1024, 876, 818 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.28 (ddd, 1H, J = 9.1, 8.8, 2.9 Hz, H8), 7.40 (dd, 1H, J = 9.1, 4.7 Hz, H7), 7.85 (dd, 1H, J = 8.7, 2.9 Hz, H10), 8.19 (d, 1H, J = 5.0 Hz, H4), 8.92 (s, 1H, H3), 9.49 (s, 1H, H1); ^{13}C NMR (75 MHz, $CDCl_3$) δ 108.8 (d, J = 25.0 Hz, C10), 117.1 (d, J = 8.3 Hz, C11), 119.1 (d, J = 24.3 Hz, C8), 119.9 (d, J = 8.5 Hz, C7), 122.4 (C4), 127.2 (C13), 145.7 (C3), 148.2 (C12), 150.4 (C1), 159.0 (C5), 159.6 (d, J = 240.0 Hz, C9); HRMS (ESI TOF-MS) calcd for $C_{12}H_6FNNaO_2^+$ 238.0275; found 238.0278.

9-Chloro-5H-chromene[4,3-c]pyridin-5-one (**7o**). White solid; mp 193.9−194.4 °C; IR (ν_{max}) 3108, 3071, 2919, 2849, 1723, 1583, 1484, 1404, 1295, 1239, 1209, 1073, 1013, 809 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$), δ 7.35 (d, 1H, J = 8.8 Hz, H7), 7.50 (dd, 1H, J = 8.8, 2.4 Hz, H8), 8.14 (d, 1H, J = 2.4 Hz, H10), 8.17 (d, 1H, J = 5.1 Hz, H4), 8.91 (d, 1H, J = 5.1 Hz, H3), 9.51 (s, 1H, H1); ^{13}C NMR (75 MHz, $CDCl_3$), δ 117.1 (C9), 119.4 (C4), 122.1 (C10), 122.2 (C7), 126.9 (C13), 127.4 (C11), 130.8 (C14), 131.4 (C8), 145.3 (C3), 149.9 (C12), 150.2 (C1), 159.1 (C5); HRMS (ESI TOF-MS) calcd for $C_{12}H_6ClNNaO_2^+$ 253.9979; found 253.9985.

7-Bromo-9-fluoro-5H-chromene[4,3-c]pyridin-5-one (**7p**). White solid; mp 233.2−234.8 °C; IR (ν_{max}) 2921, 2850, 1757, 1581, 1567, 1470, 1425, 1419, 1299, 1269, 1221, 1173, 1103, 1075, 1050, 1031, 886, 859, 817, 787, 722, 703, 607, 589 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.92 (dd, 1H, J = 7.8, 3.0 Hz, H8), 8.07 (dd, 1H, J = 5.1, 0.9 Hz, H3), 8.49 (dd, 1H, J = 9.3, 3.0 Hz, H10), 8.91 (d, 1H, J = 5.1 Hz, H4), 9.79 (bs, 1H, H1); ^{13}C NMR (75 MHz, $CDCl_3$) δ 110.1 (d, J = 17.7 Hz, C10), 111.6 (d, J = 10.8 Hz, C7), 119.2 (d, J = 9.8 Hz, C11), 121.9 (C4), 122.4 (d, J = 27.0 Hz, C8), 127.8 (C13), 127.9 (C14), 147.4 (C3), 151.2 (C1), 158.8 (d, J = 242.8 Hz, C9), 159.2 (C5); HRMS (ESI TOF-MS) calcd for $C_{12}H_5BrFNNaO_2^+$ 315.9380; found 315.9387.

9-Fluoro-5H-chromene[4,3-b]pyridin-5-one (**7q**). White solid; mp 184.4−185.5 °C; IR (ν_{max}) 3083, 3044, 1759, 1742, 1585, 1570, 1500, 1470, 1452, 1403, 1305, 1271, 1261, 1176, 1127, 1098, 1081, 1041, 882, 817, 788, 722, 607, 594 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.29 (ddd, 1H, J = 8.7, 8.3, 3.0 Hz, H8), 7.38 (dd, 1H, J = 8.3, 4.5 Hz, H7), 7.56 (dd, 1H, J = 8.0, 4.5 Hz, H3), 8.25 (dd, 1H, J = 8.7, 3.0 Hz, H10), 8.63 (dd, 1H, J = 8.0, 1.7 Hz, H4), 9.03 (dd, 1H, J = 4.5, 1.7 Hz, H2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 105.0 (C14), 110.8 (d, J = 25.1 Hz, C10), 117.7 (C11), 119.1 (d, J = 8.0 Hz, C7), 119.8 (d, J = 25.1 Hz, C8), 124.6 (C3), 138.5 (C4), 148.9 (C13), 151.4 (C2), 156.0 (C2), 159.7 (d, J = 243.3 Hz, C9), 161.0 (C5); HRMS (ESI TOF-MS) calcd for $C_{12}H_6FNNaO_2^+$ 238.0275; found 238.0289.

9-Methyl-5H-chromene[4,3-b]pyridin-5-one (**7r**). White solid; mp 182.1−184.3 °C; IR: 3066, 3004, 2926, 2864, 1754, 1720, 1641, 1599, 1588, 1542, 1399, 1264, 1196, 1138, 1113, 1045, 823, 795, 730, 621, 606, 581 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 2.47 (s, 3H, 9-CH_3), 7.27 (d, 1H, J = 8.4 Hz, H7), 7.38 (ddd, 1H, J = 8.4, 2.1, 0.6 Hz, H8), 7.51 (dd, 1H, J = 8.0, 4.7 Hz, H3), 8.35−8.37 (m, 1H, H10), 8.61 (dd, 1H, J = 8.0, 1.8 Hz, H4), 9.00 (dd, 1H, J = 4.7, 1.8 Hz, H2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.2 (9-CH_3), 117.2 (C7), 117.7 (C11), 119.1 (C14), 123.9 (C3), 124.7 (C10), 133.4 (C8), 135.0 (C9), 138.5 (C4), 150.9 (C13), 152.2 (C12), 155.8 (C2), 161.5 (C5); HRMS (ESI TOF-MS) calcd for $C_{13}H_9NNaO_2^+$ 234.0525; found 234.0534.

7-Bromo-9-fluoro-5H-chromene[4,3-b]pyridin-5-one (**7s**). White solid; mp 213.5−214.2 °C; IR (ν_{max}) 3456, 3082, 2064, 2962, 1748, 1588, 1561, 1493, 1463, 1403, 1262, 1235, 1208, 1163, 1078, 865, 810, 786, 687, 607 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.57 (dd, 1H, J = 7.5, 3.0 Hz, H8), 7.60 (dd, 1H, J = 8.0, 4.8 Hz, H3), 8.27 (dd, 1H, J = 8.4, 3.0 Hz, H10), 8.65 (dd, 1H, J = 8.0, 1.8 Hz, H4), 9.05 (dd, 1H, J = 4.8, 1.8 Hz, H2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 110.3 (d, J = 25.1 Hz, C10), 111.5 (d, J = 9.8 Hz, C7), 117.6 (C14), 121.9 (d, J = 8.0 Hz, C11), 123.5 (d, J = 26.9 Hz, C8), 125.1 (C3), 138.6 (C4), 138.7 (C13), 150.8 (C12), 159.0 (d, J = 246.7 Hz, C9), 160.0 (C5); HRMS (ESI TOF-MS) calcd for $C_{12}H_5FBrNNaO_2^+$ 315.9378; found 315.9397.

2-Fluoro-8,9-dihydroxy-6H-benzo[c]chromen-6-one (**7t**). White solid; mp 234.1−236.0 °C; IR (ν_{max}) 3075, 3000, 2947, 2848, 1706, 1598, 1501, 1426, 1411, 1371, 1299, 1280, 1176, 1160, 1073, 1025, 875, 805, 792 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 7.28 (td, 1H, J = 8.6, 8.6, 2.7 Hz, H3), 7.38 (dd, 1H, J = 8.6, 4.8 Hz, H4), 7.56 (s, 1H, H10), 7.61 (s, 1H, H7), 7.92 (dd, J = 9.9, 2.7 Hz, H1), 10.24 (s, 2H, 8-OHand 9-OH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 105.7 (C10), 110.2 (d, J = 25.3 Hz, C3), 111.0 (C7), 114.3 (C12), 117.3 (d, J = 24.5 Hz, C1), 119.4 (d, J = 8.9 Hz, C4), 120.0 (d, J = 8.7 Hz, C13), 129.5 (C11), 147.4 (C14), 151.3 (C8), 156.1 (C9), 157.9 (C6), 160.7 (d, J = 60.4 Hz, C2); HRMS (ESI TOF-MS) calcd for $C_{13}H_7FNaO_4^+$ 269.0221; found 269.0222.

Hill Reaction Measurement. The basal rate of electron transport was measured on photosynthetically active thylakoid membranes isolated from market spinach (*Spinacia oleracea* L.) leaves by following ferricyanide reduction.[41,42] Plant material was suspended in 5 mL g^{-1} of ice-cold 20 mM Tricine-NaOH buffer, pH 8.0, containing 10 mM NaCl, 5 mM $MgCl_2$, and 0.4 M sucrose, and homogenized for 30 s in a blender at maximal speed. The homogenate was filtered through surgical gauze, and the filtrate was centrifuged at 4 °C for 1 min at 500*g*; the supernatant was further centrifuged for 10 min at 1500*g*. Pelleted chloroplasts were osmotically swollen by resuspension in sucrose-lacking buffer. The suspension was immediately diluted 1:1 with sucrose-containing buffer, kept on ice in the dark, and used within a few hours from preparation. Following proper dilution with 80% (v/v) acetone and absorbance measurement at 645 and 663 nm, the chlorophyll content of each preparation was calculated on the basis of the Arnon's formula. Aliquots of membrane preparations corresponding to 15 μg of chlorophyll were incubated at 24 °C in 1-mL cuvettes containing 20 mM Tricine-NaOH buffer, pH 8.0, 10 mM NaCl, 5 mM $MgCl_2$, 0.2 M sucrose, and 1 mM $K_3Fe(CN)_6$. The assay was initiated by exposure to saturating light (800 μmol m^{-2} s^{-1}), and the rate of ferricyanide reduction was measured at 1 min intervals for up to 20 min against an exact blank at 420 nm. Activity was calculated over the linear portion of the curve from an extinction coefficient of 1000 M^{-1} cm^{-1}. Phosphorylating conditions were obtained by adding 2 mM K_2HPO_4 and 0.5 mM ADP to the reaction mixture. The addition of 2 mM NH_4Cl allowed measurement of uncoupled activity.

Alternariol monomethyl ether from *Alternaria alternata* (A3171) was purchased from Sigma-Aldrich (St. Louis, MO), and was dissolved and diluted in DMSO. Alternariol analogues were also dissolved in DMSO so as to obtain 20 mM solutions that were then water-diluted, as appropriate. Their effect upon the photosynthetic electron transport was evaluated in parallel assays in which the compounds were added to the reaction mixture to a final concentration of 1, 2, 5, 10, 20, 50, or

100 μM. In the case of alternariol monomethyl ether (**2**), controls were added with the corresponding volume of DMSO. For each concentration, the assay was carried out in triplicate. The concentrations causing 50% inhibition (IC_{50}) were estimated utilizing the linear regression equation of activity values, expressed as percentage of untreated controls, plotted against the logarithm of inhibitor concentration. At least three points in the rectilinear part of the resulting sigmoid curve were considered. Confidence limits of IC_{50} values were computed according to Snedecor and Cochran.[44]

Cyanobacterial Cultures. *Synechococcus elongatus*, strain PCC 6301, was grown at 24 ± 1 °C under 14 h days (150 μmol m^{-2} s^{-1} PAR) and 10 h nights as described.[45] Growth was followed by measuring chlorophyll concentration.[46] Following logarithmic transformation of data, growth constants and generation times were calculated from the linear portion of each curve. Late log-grown cells were sedimented by centrifugation 5 min at 4000*g* and used to inoculate 25-well squared Petri dishes, 4.0 mL per well, to an initial density of 2.0 mg L^{-1} chlorophyll. Suitable dilutions in Bg11 medium of a 20 mM stock solution of a given compound in DMSO were added so as to obtain concentrations ranging from 20 to 200 μM. A complete randomized design with four replications (12 for untreated controls) was adopted. Growth constants were calculated and expressed as percent of the mean value for controls. Means ± SE over replicates are reported.

Plant suspension-cultured cells. *Arabidopsis thaliana* (L.) Heynh. (accession Col-0) cell suspension cultures were grown as previously described.[47] The effect of a given compound on exponentially growing cells was measured as reduction of the dry weight increase.[48] Three replications (four for untreated controls) were carried out for each treatment. Data were expressed as percentage of controls treated with DMSO alone.

■ ASSOCIATED CONTENT

Supporting Information

Spectroscopic data for alternariol analogues. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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