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Synthesis, Structure Revision, and Cytotoxicity of Nocarbenzoxazole G

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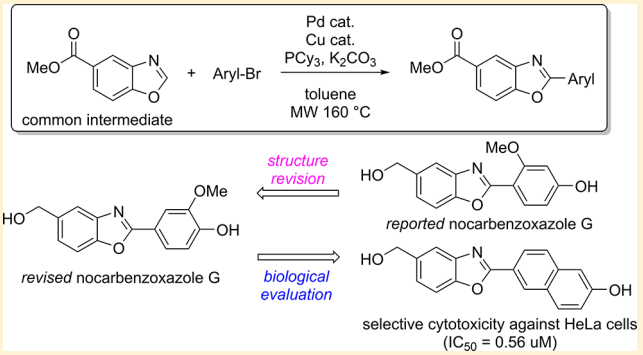
Supporting Information

ABSTRACT: The total synthesis of nocarbenzoxazoles F (**1**) and G (**2**), originally obtained from the marine-derived halophilic bacterial strain *Nocardiopsis lucentensis* DSM 44048, was achieved via a simple and versatile route involving microwave-assisted construction of a benzoxazole skeleton, followed by carbon−carbon bond formation with the corresponding aryl bromides. Unfortunately, the ^{1}H and ^{13}C NMR spectra of natural nocarbenzoxazole G did not agree with those of the synthesized compound. In particular, the spectra of the isolated and synthesized compounds showed considerable differences in the signals from the protons and carbons in the aryl group. The revised structure was validated by the total synthesis of the actual nocarbenzoxazole G (**8c**) molecule, which is a regioisomer of the compound that was reported earlier as nocarbenzoxazole G. The synthesized derivatives showed specific cytotoxicity to the human cervical carcinoma cell line, HeLa, but did not have any remarkable effect on the other cell lines.



2-Arylbenzoxazole-based compounds are one of the most important classes of heterocyclic scaffolds that exhibit remarkable biological properties such as anticonvulsant,[1] anti-inflammatory,[2] and anticancer activities.[3] These compounds show excellent activities against Gram-positive bacteria, Gram-negative bacteria, and the yeast *Candida albicans*.[4] Interestingly, a slight change in the substitution pattern of the aryl group at the C-2 position of 2-arylbenzoxazoles results in distinguishable differences in biological activities. Accordingly, various functionalized 2-arylbenzoxazoles have been studied in the field of pharmaceuticals.[5]

In 2015, Lu and co-workers isolated a new series of compounds from the halophilic bacterial strain *Nocardiopsis lucentensis* DSM 44048 and tested their cytotoxicity against a panel of human tumor cell lines.[6] These compounds were called nocarbenzoxazoles, and their structures were based on that of 2-arylbenzoxazole. Among the compounds isolated, nocarbenzoxazole F (**1**) and nocarbenzoxazole G (**2**) showed selective activities against HepG2 and HeLa cell lines. The compounds (Figure 1) were characterized using NMR spectroscopy and HRESIMS.

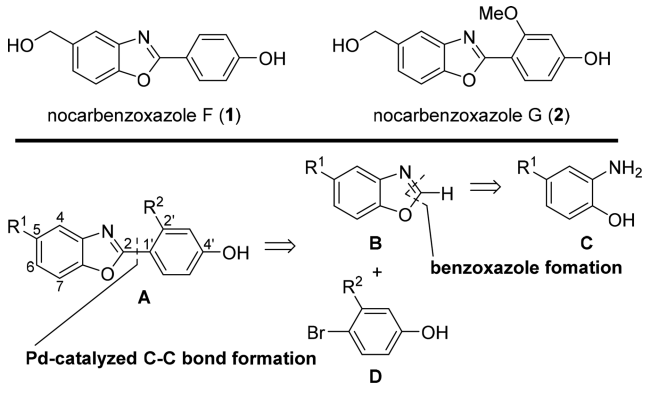


Figure 1. (Top) Originally proposed structures of nocarbenzoxazole F (**1**) and nocarbenzoxazole G (**2**). (Bottom) Retrosynthesis of 2-arylbenzoxazole.

RESULTS AND DISCUSSION

Our synthesis strategy was based on the practical and efficient construction of the 2-arylbenzoxazole framework. The

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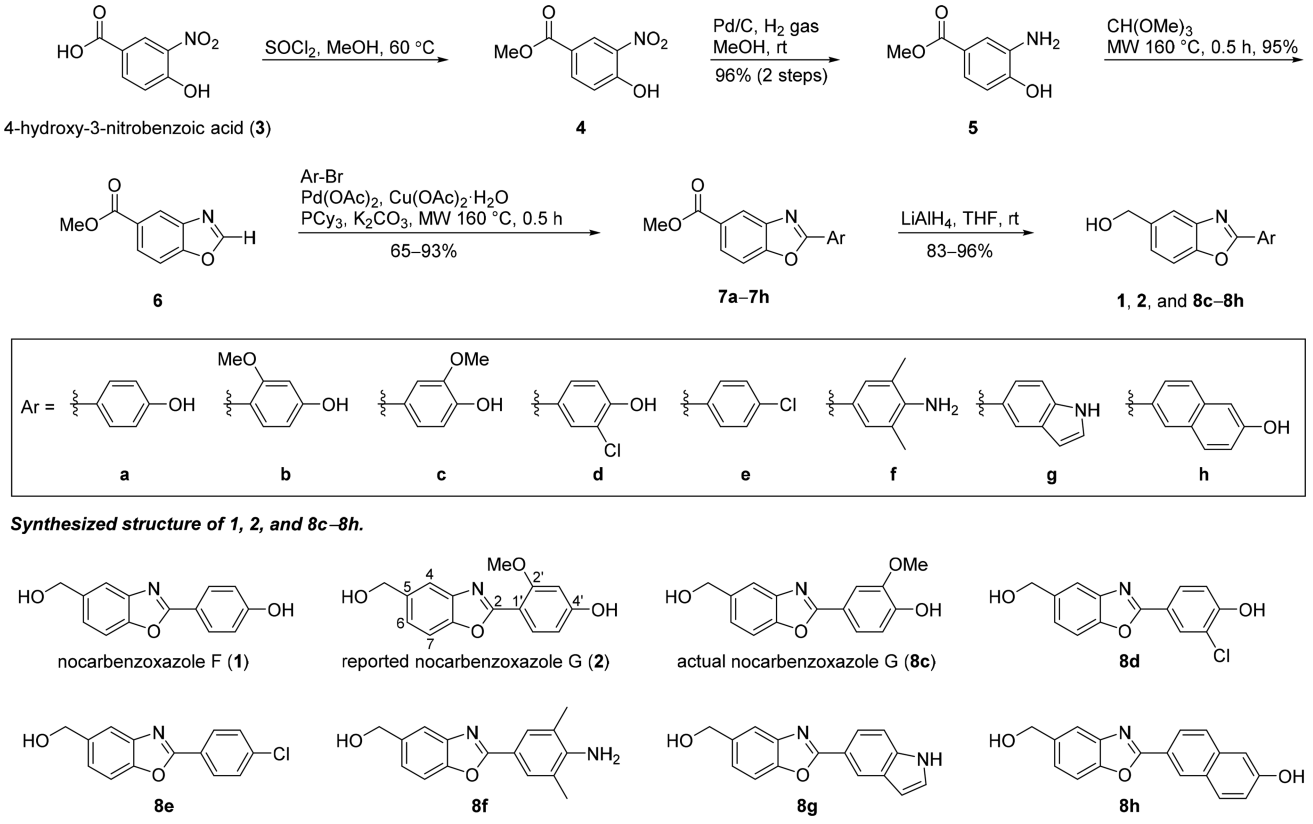
Scheme 1. Syntheses of Nocarbenzoxazoles F and G and Their Derivatives



retrosynthetic analysis of structure **A** predicts the formation of a benzoxazole core **B** and the corresponding 4-bromophenols **D**. Further retrosynthesis of **B** suggests a 2-aminophenol precursor **C**. In this study, the C−C bond formation between the benzoxazole core **B** and the aryl moiety **D** to produce the 2-arylbenzoxazole was achieved using a microwave (MW)-assisted Pd-catalyzed reaction. Owing to the efficient synthesis of the natural 2-arylbenzoxazole, this compound can contribute to the library of compounds for a structure−activity relationship (SAR) study for the investigation of the antitumor activities of these types of compounds.

The syntheses of **1**, **2**, and their derivatives (**8c**−**8h**) are described in Scheme 1. First, we focused on the synthesis of **1**. Methyl benzoate **5** was prepared from the commercially available 4-hydroxy-3-nitrobenzoic acid (**3**) using the protocol available in the literature.[7] Then, **5** was dissolved in trimethyl orthoformate and heated under microwave irradiation at 160 °C for 30 min without any additional oxidant, reductant, or catalyst. This gave 2-H benzoxazole **6** in 95% yield (Scheme 1). Next, 2-H benzoxazole **6** was treated with the corresponding bromophenol in the presence of $Pd(OAc)_2$, $Cu(OAc)_2{\cdot}H_2O$, triphenylphosphine (PPh_3), and K_2CO_3 in toluene under microwave irradiation at 160 °C for 30 min. This produced a trace amount of **7a** along with an unexpected adduct, **S1**, as the major product.[8] When the phosphine ligand was changed to tricyclohexylphosphine (PCy_3), the desired product **7a** was obtained in 67% yield. Finally, the reduction of the methyl ester in **7a** with lithium aluminum hydride afforded nocarbenzoxazole F (**1**) in 59% yield over five steps. The reported nocarbenzoxazole G (**2**) and the other derivatives (**8c**−**8h**) were synthesized similarly. Their syntheses required only two additional steps after the formation of the common intermediate **6**: (i) Pd-catalyzed C−H activation/aryl−aryl coupling with various aryl bromides (**b**−**h**) for the formation of the C−C bond between 2-H benzoxazole and the aryl moiety and (ii) reduction of the methyl ester to primary alcohol. The overall yields ranged from 54% to 90%.

The structures of **1**, **2**, and **8c**−**8h** synthesized here were determined by NMR, IR, and mass spectrometric analysis. Additionally, the data of **1** and **2** were compared with the reported data of the natural nocarbenzoxazoles F and G. The data for synthetic **1** matched the data for natural **1**. However, the spectroscopic properties of **2** synthesized in this study were different from those of natural nocarbenzoxazole G. In particular, the chemical shifts and coupling constants of the protons of the aryl group around C-2 of **2** (Figure 2, A) were clearly different in the natural compound and synthesized compound (Figure 2, B): *natural* δ_H 6.97 (d, 8.2), 7.74 (dd, 1.9 and 8.3), and 7.77 (d, 1.9); *synthetic* δ_H 6.56 (dd, 2.4 and 8.4), 6.61 (d, 2.4), and 7.94 (d, 8.4). Moreover, upon reexamining the previously reported NMR data of **2**,[6] we found that the natural product contained two signals at δ_H 7.74 (dd, 1.9 and 8.3) and 7.77 (d, 1.9) that corresponded to the C-2 carbon peak at δ_C 165.7 in the HMBC spectrum. This led us to conclude that the actual structure of the aryl group in the natural nocarbenzoxazole G should be a regioisomer with respect to the 2-methoxy group, thereby suggesting a 4-hydroxy-3-methoxyphenyl at the C-2 position. Consequently, the coupling of **6** with 4-bromo-2-methoxyphenol (**c**) followed by the reduction afforded the derivative **8c**, whose ^1H and ^{13}C NMR spectra were identical with those of natural nocarbenzoxazole G (Figure 2, C). Therefore, we achieved the total synthesis of nocarbenzoxazole G (**8c**). The revised structure of natural nocarbenzoxazole G (**8c**) is a regioisomer (with respect

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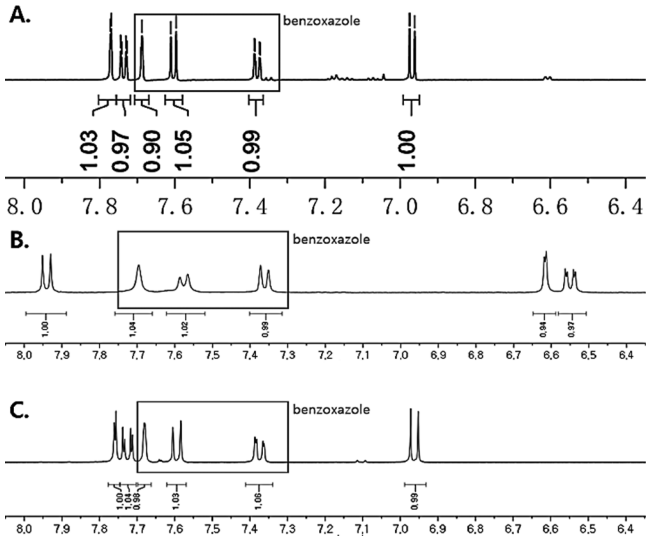

Figure 2. ^{1}H NMR spectra (δ_H 6.4−8.0) of natural nocarbenzoxazole G isolated from *Nocardiopsis lucentensis* DSM 44048 (A). Previously reported structure **2** (B) and revised structure of nocarbenzoxazole G **8c** (C).

to the methoxy group) of the originally proposed structure (**2**) of this compound.

Next, the synthesized compounds (**1**, **2**, and **8c**−**8h**) were evaluated for their cytotoxicities against a panel of human cancer cell lines, namely, HepG2 and HepB3 (liver), HeLa (cervical), MDA-MB-231 and MCF 7 (breast), and T98G (glioblastoma). Interestingly, all of the synthesized compounds specifically inhibited the growth of HeLa cells. Table 1 shows that the IC_{50} values in the CCK-8 assay with synthetic nocarbenzoxazoles **1** and **8c** were 4.7 and 5.7 μM, respectively, compared with the IC_{50} values of 14 and 1 μM in the MTT assay with the corresponding natural compounds.[9] In particular, **8h**, containing the 2-hydroxynaphth-6-yl group, showed a high cytotoxicity against the HeLa cells, with an IC_{50} value of 0.56 μM. The cytotoxic effects on the other cell lines were not remarkable because the IC_{50} values were higher than 20 μM. Further studies on the structure optimization and mechanism of their biological activities are currently in progress in our laboratory.

In summary, we have developed a facile method for the synthesis of 2-arylbenzoxazoles via a Pd-catalyzed direct arylation of 2-H benzoxazole **6** with the corresponding aryl bromides using microwave irradiation. The reduction of the methyl ester afforded the nocarbenzoxazoles and their derivatives (**1**, **2**, and **8c**−**8h**). The reexamination of the NMR data and the study of the synthesis have provided a reliable and practical route to obtain the revised structure of nocarbenzoxazole G (**8c**). Furthermore, the toxicity studies show that **8h**, containing the 2-hydroxynaphth-6-yl group, has the maximum inhibitory effect on the growth of HeLa cells. As a result, these compounds can also be investigated for their antitumor activities or other biological activities.

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were obtained on a OptiMelt MPA100 and are uncorrected. FTIR spectra were recorded on an ATR plate. ^{1}H NMR spectra were recorded on a Bruker AVANCE 400 spectrometer, and the chemical shifts are reported relative to the internal standard tetramethylsilane (0 ppm) for $CDCl_3$ and internal solvent MeOH (3.31 ppm) for CD_3OD. ^{13}C NMR spectra were obtained on the same spectrometer and referenced to the internal solvent signals (central peak is 77.16 or 49.00 ppm in $CDCl_3$ or CD_3OD, respectively). Mass spectra were recorded on a LCMS-2020 (Shimadzu Corporation) for ESIMS and an Accu TOF JMS-T100LCS (JEOL) for HRESIMS. Flash column chromatography was performed over silica gel 200−300. All reagents were weighed and handled in air at room temperature, and all reactions were performed under an air atmosphere. Unless otherwise noted, all reagents were purchased from commercial suppliers without further purification.

Preparation of Methyl 1,3-Benzoxazole-5-carboxylate (6). *Methyl 1,3-Benzoxazole-5-carboxylate (**6**).* The methyl 3-amino-4-hydroxybenzoate **3**[8] (5.50 g, 32.7 mmol) was dissolved in trimethyl orthoformate (32.7 mL) in a microwave tube. The mixture was heated under microwave irradiation at 160 °C for 30 min (200 w). After completion of the reaction, the mixture was cooled to room temperature, and the solvent was evaporated under reduced pressure. The crude residue was purified by column chromatography on silica gel to afford **6** (5.50 g, 95%) as a white solid; mp 108−109 °C; FTIR (ATR) 3102, 2999, 2951, 1701, 1604, 1434, 1288, 1116, 1049, 751, 769 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.46 (d, 1H, J = 1.2 Hz), 8.15 (s, 1H), 8.12 (dd, 1H, J = 1.6 and 8.4 Hz), 7.60 (d, 1H, J = 8.8 Hz), 3.93 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.6, 153.7, 152.9, 140.2, 127.5, 127.2, 122.8, 110.9, 52.4; ESIMS m/z 178.10 [M + H]$^+$ (calcd for $C_9H_8NO_3$,178.05).

Syntheses 7a−7h from 2-H Benzoxazole 6. *Methyl 2-(4-Hydroxyphenyl)benzo[d]oxazole-5-carboxylate (**7a**).* To a solution of **6** (354 mg, 2.00 mmol) in toluene (20.0 mL) were added 4-bromopheneol (381 mg, 2.20 mmol), $Pd(OAc)_2$(4.50 mg, 1 mol %), $Cu(OAc)_2{\cdot}H_2O$ (72.5 mg, 20 mol %), PCy_3 (280 mg, 50 mol %), and K_2CO_3(553 mg, 4.00 mmol) in a microwave tube. The mixture was heated under microwave irradiation at 160 °C for 30 min (200 W). After completion of the reaction, the mixture was cooled to room temperature (rt), and the mixture was quenched with saturated NH_4Cl solution. The resulting mixture was extracted with EtOAc. The organic layer was washed with a saturated aqueous solution of NaCl and dried with Na_2SO_4. After filtration, the mixture was

Table 1. IC_{50} Values (μM) of Nocarbenzoxazoles and Their Derivatives against a Panel of Human Tumor Cell Lines

	IC_{50} (μM)[a]					
compound	HepG2	HepB3	HeLa	MDA-MB-231	MCF7	T98G
1	>20 (16)[b]	>20	4.7 ± 0.1 (14)[b]	>20	>20	>20
2	>20	>20	2.9 ± 0.2	>20	>20	>20
8c	>20 (3)[b]	>20	5.7 ± 0.1 (1)[a]	>20	>20	>20
8d	>20	>20	11 ± 1	>20	>20	>20
8e	>20	>20	16 ± 1	>20	>20	>20
8f	>20	>20	19 ± 1	>20	>20	>20
8g	>20	>20	8.9 ± 0.3	>20	>20	>20
8h	>20	>20	0.56 ± 0.01	>20	>20	>20

[a]Data presented as a mean ± SD of three replicate measurements of the same sample. [b]Values in parentheses are from ref 6.

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concentrated under reduced pressure, and the crude residue was purified by column chromatography on silica gel to afford **7a** (361 mg, 67%) as a white solid: mp 270−274 °C; FTIR (ATR) 3059, 2921, 2850, 1722, 1606, 1433, 1290, 1120, 1170, 1085, 1064, 749 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 8.30 (d, 1H, J = 1.6 Hz), 8.11 (d, 1H, J = 8.8 Hz), 8.06 (d, 2H, J = 1.6 Hz), 7.71 (d, 1H, J = 8.8 Hz), 6.98 (d, 2H, J = 8.8 Hz), 3.95 (s, 3H); ^{13}C NMR (100 MHz, CD_3OD +$CDCl_3$) δ 168.0, 166.6, 163.0, 154.8, 143.2, 130.8 (2C), 128.2, 127.7, 121.6, 118.3, 117.0 (2C), 111.4, 52.8; ESIMS m/z 270.10 $[M + H]^+$ (calcd for $C_{15}H_{12}NO_4$, 270.08).

*Methyl 2-(4-Hydroxy-2-methoxyphenyl)benzo[d]oxazole-5-carboxylate (**7b**).* By following the procedure described above for the preparation of **7a**, the reaction with 4-bromo-2-methoxyphenol (447 mg, 2.20 mmol) instead of 4-bromophenol was performed. Purification by column chromatography afforded **7b** (389 mg, 65%) as a white solid: mp 176−179 °C; FTIR (ATR) 3222, 2921, 2845, 1730, 1618, 1501, 1429, 1292, 1192, 1083, 802, 746 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 8.42 (d, 1H, J = 1.6 Hz), 8.09 (dd, 1H, J = 1.6 and 8.4 Hz), 7.83 (dd, 1H, J = 1.6 and 8.4 Hz), 7.76 (d, 1H, J = 1.6 Hz), 7.59 (d, 1H, J = 8.4 Hz), 7.06 (d, 1H, J = 8.4 Hz), 4.03 (s, 3H), 3.96 (s, 3H); ^{13}C NMR (100 MHz, MeOD-d_3) δ 166.9, 164.6, 153.7, 149.5, 147.0, 142.4, 127.1, 126.8, 122.3, 121.6, 118.8, 115.0, 110.2, 110.0, 56.4, 52.4; ESIMS m/z 300.20 $[M + H]^+$ (calcd for $C_{16}H_{14}NO_5$, 300.09).

*Methyl 2-(4-Hydroxy-2-methoxyphenyl)benzo[d]oxazole-5-carboxylate (**7c**).* By following the procedure described above for the preparation **7a**, the reaction with 4-bromo-3-methoxyphenol (447 mg, 2.20 mmol) instead of 4-bromophenol was performed. Purification by column chromatography afforded **7c** (532 mg, 89%) as a white solid: mp 209−213 °C; FTIR (ATR) 3065, 2920, 2849, 1720, 1582, 1482, 1291, 1254, 1204, 1116, 1036, 749 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 8.31 (d, 1H, J = 1.6 Hz), 8.07 (dd, 1H, J = 1.6 and 8.4 Hz), 7.98 (d, 1H, J = 8.4 Hz), 7.68 (d, 1H, J = 8.4 Hz), 6.62 (d, 1H, J = 2.0 Hz), 6.57 (dd, 1H, J = 2.0 and 8.4 Hz), 3.97 (s, 3H), 3.95 (s, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 168.1, 165.2, 164.5, 162.2, 154.1, 143.1, 133.5, 128.0, 127.6, 121.5, 111.2, 109.3, 107.1, 100.6, 56.1, 52.8; ESIMS m/z 300.00 $[M + H]^+$ (calcd for $C_{16}H_{14}NO_5$, 300.09).

*Methyl 2-(4-Hydroxy-2-methoxyphenyl)benzo[d]oxazole-5-carboxylate (**7d**).* By following the procedure described above for the preparation of **7a**, the reaction with 4-bromo-2-chlorophenol (456 mg, 2.20 mmol) instead of 4-bromophenol was performed. Purification by column chromatography afforded **7d** (491 mg, 81%) as a white solid: mp 206−208 °C; FTIR (ATR) 3309, 2922, 2850, 1689, 1624, 1436, 1296, 1183, 1122, 1087, 1051, 765, 748 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.42 (t, 1H, J = 1.6 Hz), 8.27 (d, 1H, J = 2.0 Hz), 8.11 (d, 1H, J = 1.6 Hz), 8.09 (t, 1H, J = 2.0 Hz), 7.60 (d, 1H, J = 8.4 Hz), 7.18 (d, 1H, J = 8.4 Hz), 3.96 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$+CD_3OD) δ 166.9, 163.4, 155.3, 153.7, 142.1, 129.3, 128.2, 127.2, 127.1, 121.7, 121.1, 119.8, 117.0, 110.4, 52.4; ESIMS m/z 304.10 $[M + H]^+$ (calcd for $C_{15}H_{11}NO_4$, 304.04).

*Methyl 2-(4-Hydroxy-2-methoxyphenyl)benzo[d]oxazole-5-carboxylate (**7e**).* By following the procedure described above for the preparation **7a**, the reaction with 1-bromo-4-chlorobenzene (421 mg, 2.20 mmol) instead of 4-bromophenol was performed. Purification by column chromatography afforded **7e** (534 mg, 93%) as a white solid: mp 164−166 °C; FTIR (ATR) 2956, 2850, 1730, 1622, 1434, 1295, 1216, 1086, 1047, 1011, 829, 748 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.45 (d, 1H, J = 1.6 Hz), 8.20 (dd, 2H, J = 1.6 and 6.4 Hz), 8.13 (dd, 1H, J = 1.6 and 8.8 Hz), 7.62 (d, 1H, J = 8.8 Hz), 7.53 (dd, 2H, J = 1.6 and 6.4 Hz), 3.96 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.8, 163.5, 153.8, 142.2, 138.4, 129.5, 129.5, 129.2, 129.2, 127.4, 127.4, 125.2, 122.2, 110.5, 52.5; ESIMS m/z 288.10 $[M + H]^+$ (calcd for $C_{15}H_{11}ClNO_3$, 288.04).

*Methyl 2-(4-Hydroxy-2-methoxyphenyl)benzo[d]oxazole-5-carboxylate (**7f**).* By following the procedure described above for the preparation **7a**, the reaction with 4-bromo-2,6-dimethylaniline (440 mg, 2.20 mmol) instead of 4-bromophenol was performed. Purification by column chromatography afforded **7f** (503 mg, 85%) as a light yellow solid: mp 232−233 °C; FTIR (ATR) 3364, 2922, 2851, 1721,1617, 1483, 1286, 1227, 1172, 1093, 980, 746 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.37 (s, 1H), 8.04 (dd, 1H, J = 1.6 and 8.4 Hz), 7.87 (s, 2H), 7.55 (d, 1H, J = 8.4 Hz), 3.95 (s, 3H), 2.26 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.1, 165.6, 153.7, 146.9, 142.7, 128.4 (2C), 126.8, 126.3, 121.6, 121.1, 115.4, 110.0 (2C), 52.3, 17.6 (2C); ESIMS m/z 297.20 $[M + H]^+$ (calcd for $C_{17}H_{17}N_2O_3$, 297.13).

*Methyl 2-(4-hydroxy-2-methoxyphenyl)benzo[d]oxazole-5-carboxylate (**7g**).* By following the procedure described above for the preparation **7a**, the reaction with 5-bromoindole (431 mg, 2.20 mmol) instead of 4-bromophenol was performed. Purification by column chromatography afforded **7g** (543 mg, 93%) as a light yellow solid: mp 180−183 °C; FTIR (ATR) 3239, 2922, 2852, 1718, 1623, 1434, 1334, 1286, 1206, 1084, 762, 730 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.51 (s, 1H), 8.43 (d, 1H, J = 1.2 Hz), 8.14 (dd, 1H, J = 1.2 and 8.4 Hz), 8.09 (dd, 1H, J = 1.6 and 8.8 Hz), 7.61 (d, 1H, J = 8.4 Hz), 7.53 (d, 1H, J = 8.4 Hz), 7.32 (t, 1H, J = 1.6 Hz), 6.70 (s, 1H), 3.96 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.1, 166.1, 153.9, 142.7, 138.0, 128.1, 126.9, 126.5, 125.8, 122.0, 121.7, 121.5, 118.5, 111.8, 110.2, 104.1, 52.4; ESIMS m/z 293.20 $[M + H]^+$ (calcd for $C_{17}H_{13}N_2O_3$, 293.09).

*Methyl 2-(4-Hydroxy-2-methoxyphenyl)benzo[d]oxazole-5-carboxylate (**7h**).* By following the procedure described above for the preparation **7a**, the reaction with 6-bromo-2-naphthol (491 mg, 2.20 mmol) instead of 4-bromophenol was performed. Purification by column chromatography afforded **7h** (453 mg, 71%) as a light yellow solid: mp 198−201 °C; FTIR (ATR) 3209, 2922, 2851, 1720, 1620, 1435, 1291, 1216, 1085, 1048, 763, 747 cm^{-1}; ^1H NMR (400 MHz, CD_3OD+$CDCl_3$) δ 8.64 (s, 1H), 8.39 (d, 1H, J = 1.6 Hz), 8.16 (dd, 1H, J = 1.6 and 8.4 Hz), 8.10 (dd, 1H, J = 1.6 and 8.4 Hz), 7.87 (d, 1H, J = 8.4 Hz), 7.78 (d, 1H, J = 8.4 Hz), 7.66 (d, 1H, J = 8.8 Hz), 7.19−7.16 (m, 2H), 3.95 (s, 3H); ^{13}C NMR (100 MHz, CD_3OD +$CDCl_3$) δ 166.9, 156.9, 153.6, 142.1, 136.8, 130.8, 129.4, 128.6, 127.7, 127.1, 126.9, 126.8, 124.2, 121.5, 120.7, 119.5, 110.2, 109.3, 52.3; ESIMS m/z 320.20 $[M + H]^+$ (calcd for $C_{19}H_{14}NO_4$, 320.09).

Syntheses of 1, 2, and 8c−8h from 7a−7h. *Nocarbenzoxazole F (**1**).* To a solution of **7a** (161 mg, 0.60 mmol) in anhydrous tetrahydrofuran (6.00 mL) was slowly added $LiAlH_4$ (34.2 mg, 0.902 mmol) at 0 °C under nitrogen. The mixture was warmed to rt and stirred for 2 h. After completion of the reaction, the mixture was cooled to 0 °C and quenched with MeOH (20.0 mL). The mixture was filtered through a Celite pad and washed with EtOAc. After filtration, the mixture was concentrated under reduced pressure, and the crude residue was purified by column chromatography on silica gel to afford **1** (139 mg, 96%) as a white solid: mp 245−250 °C; FTIR (ATR) 3094, 2922, 2851, 1614, 1438, 1368, 1234, 1174, 1011, 812, 742 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 8.10 (tt, 2H, J = 4.8 and 9.6 Hz), 7.69 (d, 1H, J = 1.6 Hz), 7.61 (d, 1H, J = 8.4 Hz), 7.39 (dd, 1H, J = 1.6 and 8.4 Hz), 6.98 (tt, 2H, J = 4.8 and 9.6 Hz), 4.73 (s, 2H); ^{13}C NMR (100 MHz, CD_3OD) δ 165.6, 162.7, 151.2, 143.0, 140.0, 130.6 (2C), 125.2, 118.9, 118.3, 117.0 (2C), 111.2, 65.1; HRESIMS m/z 242.0851 $[M + H]^+$ (calcd for $C_{14}H_{12}NO_3$, 242.0817).

*4-(5-(Hydroxymethyl)benzo[d]oxazol-2-yl)-3-methoxyphenol (Reported Structure of Nocarbenzoxazole G, **2**).* By following the procedure described above for the preparation of **1**, the reaction with **7b** (179 mg, 0.604 mmol) as the starting material was performed. Purification by column chromatography afforded **2** (135 mg, 83%) as a white solid: mp 196−199 °C; FTIR (ATR) 3091, 2920, 2851, 1605, 1435, 1328, 1257, 1210, 1116, 1036, 838, 802 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 7.94 (d, 1H, J = 8.4 Hz), 7.70 (s, 1H), 7.59 (d, 1H, J = 8.4 Hz), 7.38 (d, 1H, J = 8.4 Hz), 6.61 (d, 1H, J = 2.4 Hz), 6.56 (dd, 1H, J = 2.4 and 8.4 Hz), 4.73 (s, 2H), 3.96 (s, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 167.0, 164.0, 161.9, 143.0, 139.7, 133.2, 130.8, 125.1, 118.3, 110.9, 109.1, 107.8, 100.5, 65.1, 56.1; HRESIMS m/z 272.0921 $[M + H]^+$ (calcd for $C_{15}H_{14}NO_4$, 272.0923).

*4-(5-(Hydroxymethyl)benzo[d]oxazol-2-yl)-3-methoxyphenol (Revised Structure of Nocarbenzoxazole G, **8c**).* By following the procedure described above for the preparation of **1**, the reaction with **7c** (179 mg, 0.602 mmol) as the starting material was performed.

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Purification by column chromatography afforded **8c** (153 mg, 94%) as a white solid: mp 170−173 °C; FTIR (ATR) 3272, 2936, 2900, 1597, 1503, 1305, 1262, 1226, 1124, 1028, 811, 733 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 7.75 (s, 1H), 7.73 (dd, 1H, J = 2.0 and 8.4 Hz), 7.68 (d, 1H, J = 1.2 Hz), 7.60 (d, 1H, J = 8.4 Hz), 7.38 (dd, 1H, J = 2.0 and 8.4 Hz), 6.97 (d, 1H, J = 8.4 Hz), 4.72 (s, 2H), 4.60 (brs, 1H), 3.98 (s, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 165.5, 152.0, 151.1, 149.4, 142.9, 140.0, 125.2, 122.7, 119.1, 118.3, 116.7, 111.5, 111.2, 65.0, 56.5; HRESIMS m/z 272.0951 $[M + H]^+$ (calcd for $C_{15}H_{14}NO_4$, 272.0923).

*2-Chloro-4-(5-(hydroxymethyl)benzo[d]oxazol-2-yl)phenol (**8d**).* By following the procedure described above for the preparation of **1**, the reaction with **7d** (182 mg, 0.601 mmol) as the starting material was performed. Purification by column chromatography afforded **8d** (155 mg, 94%) as a white solid: mp 223−226 °C; FTIR (ATR) 3049, 2921, 2852, 1604, 1505, 1410, 1261, 1227, 1071, 985, 813, 734 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 8.17 (d, 1H, J = 2.0 Hz), 8.01 (dd, 1H, J = 2.0 and 8.8 Hz), 7.69 (s, 1H), 7.62 (d, 1H, J = 8.4 Hz), 7.40 (dd, 1H, J = 1.2 and 8.4 Hz), 7.09 (d, 1H, J = 8.8 Hz), 4.72 (s, 2H); ^{13}C NMR (100 MHz, CD_3OD) δ 164.2, 158.2, 151.2, 142.9, 140.2, 130.4, 128.6, 125.5, 122.6, 120.1, 118.5, 118.0, 111.3, 65.0; HRESIMS m/z 276.0416 $[M + H]^+$ (calcd for for $C_{14}H_{11}ClNO_3$, 276.0427).

*2-(4-Chlorophenyl)benzo[d]oxazol-5-yl)methanol (**8e**).* By following the procedure described above for the preparation of **1**, the reaction with **7e** (172 mg, 0.598 mmol) as the starting material was performed. Purification by column chromatography afforded **8e** (151 mg, 97%) as a white solid: mp 194−196 °C; FTIR (ATR) 3319, 2919, 2850, 1597, 1478, 1403, 1259, 1199, 1087, 1008, 810, 730 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.19 (dd, 2H, J = 2.0 and 7.2 Hz), 7.76 (s, 1H), 7.56 (d, 1H, J = 8.4 Hz), 7.51 (dd, 2H, J = 1.6 and 7.2 Hz), 7.40 (dd, 1H, J = 1.6 and 8.4 Hz), 4.82 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 142.4, 138.1, 138.0, 129.5 (2C), 129.1, 129.0 (2C), 127.8, 125.8, 124.8, 118.7, 110.7, 65.5; HRESIMS m/z 260.0498 $[M + H]^+$ (calcd for $C_{14}H_{11}ClNO_2$, 260.0478).

*(2-(4-Amino-3,5-dimethylphenyl)benzo[d]oxazol-5-yl)methanol (**8f**).* By following the procedure described above for the preparation of **1**, the reaction with **7f** (178 mg, 0.603 mmol) as the starting material was performed. Purification by column chromatography afforded **8f** (140 mg, 87%) as a light yellow solid: mp 203−205 °C; FTIR (ATR) 3335, 3225, 2920, 2851, 1727, 1642, 1455, 1377, 1338, 1264, 1024, 793 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 7.75 (s, 2H), 7.62 (s, 1H), 7.54 (d, 1H, J = 8.4 Hz), 7.33 (dd, 1H, J = 1.2 and 8.4 Hz), 4.71 (s, 2H), 2.25 (s, 6H); ^{13}C NMR (100 MHz, CD_3OD +$CDCl_3$) δ 166.5, 150.8, 149.2, 143.0, 139.5, 128.7 (2C), 124.5, 122.5 (2C), 117.7, 115.2, 110.8, 65.1, 17.7 (2C); HRESIMS m/z 269.1275 $[M + H]^+$ (calcd for $C_{16}H_{17}N_2O_2$, 269.1290).

*(2-(1H-Indol-5-yl)benzo[d]oxazol-5-yl)methanol (**8g**).* By following the procedure described above for the preparation of **1**, the reaction with **7g** (175 mg, 0.60 mmol) as the starting material was performed. Purification by column chromatography afforded **8g** (149 mg, 94%) as a light yellow solid: mp 233−236 °C; FTIR (ATR) 3246, 3000, 2950, 1611, 1460, 1556, 1375, 1335, 1259, 1027, 800, 735 cm^{-1}; ^1H NMR (400 MHz, CD_3OD+$CDCl_3$) δ 8.50 (d, 1H, J = 1.6 Hz), 8.01 (dd, 1H, J = 1.6 and 8.4 Hz), 7.70 (d, 1H, J = 0.8 Hz), 7.61 (d, 1H, J = 8.4 Hz), 7.56 (d, 1H, J = 8.4 Hz), 7.38−7.35 (m, 2H), 6.63 (d, 1H, J = 0.8 Hz), 4.74 (s, 2H); ^{13}C NMR (100 MHz, CD_3OD +$CDCl_3$) δ 167.0, 150.9, 142.8, 139.5, 139.5, 129.3, 127.4, 124.8, 121.8, 121.6, 118.3, 118.0, 112.7, 111.0, 103.5, 65.0; HRESIMS m/z 265.0973 $[M + H]^+$ (calcd for $C_{16}H_{13}N_2O_2$, 265.0977).

*6-(5-(Hydroxymethyl)benzo[d]oxazol-2-yl)naphthalen-2-ol (**8h**).* By following the procedure described above for the preparation of **1**, the reaction with **7h** (191 mg, 0.60 mmol) as the starting material was performed. Purification by column chromatography afforded **8h** (155 mg, 89%) as a yellow solid: mp 216−219 °C; FTIR (ATR) 3168, 2921, 2851, 1625, 1542, 1438, 1187, 1010, 861, 795, 730 cm^{-1}; ^1H NMR (400 MHz, CD_3OD+$CDCl_3$) δ 8.63 (d, 1H, J = 0.8 Hz), 8.15 (dd, 1H, J = 2.0 and 8.4 Hz), 7.90 (d, 1H, J = 1.6 Hz), 7.80 (d, 1H, J = 2.0 Hz), 7.73 (d, 1H, J = 0.8 Hz), 7.63 (d, 1H, J = 8.4 Hz), 7.41 (dd, 1H, J = 1.6 and 8.4 Hz), 7.19−7.16 (m, 2H), 4.74 (s, 2H); ^{13}C NMR (100 MHz, CD_3OD+$CDCl_3$) δ 165.4, 158.5, 151.1, 142.8, 139.8, 138.0, 131.6, 129.1, 128.9, 128.0, 125.4, 124.8, 121.9, 120.5, 118.4, 111.2, 110.0, 64.9; HRESIMS m/z 292.0973 $[M + H]^+$ (calcd for $C_{18}H_{14}NO_3$, 292.0974).

Cell Culture. The human hepatocellular carcinoma cell lines HepG2 and Hep3B, the human cervical carcinoma cell line HeLa, the human breast carcinoma cell lines MCF7 and MDA-MB-231, and the human glioblastoma cell line T98G were purchased from the American Type Culture Collection (ATCC). The cells were routinely grown in DMEM (Gibco) and RPMI1640 (Gibco), respectively, supplemented with 10% fetal bovine serum (Gibco), 100 U/mL penicillin, and 100 μg/mL streptomycin at 37 °C in a humidified atmosphere with 5% CO_2.

Cell Proliferation Assay. Cell Counting Kit-8 (Dojindo Laboratories) was used to evaluate cell proliferation according to the manufacturer's recommendations. Briefly, exponentially growing cells were seeded in a 96-well plate at a density of 1.0×10^4 cells/well in triplicate. The next day, the cells were treated with compounds at concentrations ranging from 1.25 to 20 μM. After incubation for 48 h, 10 μL of the kit reagent was added, and the cells were incubated for an additional 1 h. Cell viability was measured by scanning with a microplate reader at 450 nm. Control cells were exposed to culture media containing 0.5% (v/v) DMSO.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.9b00072.

Copies of NMR spectra of the compounds (PDF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) (a) Bywater, W. G.; Coleman, W. R.; Kamm, O.; Merritt, H. H. *J. Am. Chem. Soc.* **1945**, *67*, 905−907. (b) Benazzouz, A.; Boraud, T.; Dubedat, P.; Boireau, A.; Stutzmann, J. M.; Gross, C. *Eur. J. Pharmacol.* **1995**, *284*, 299−307. (c) Mortimer, C. G.; Wells, G.; Crochard, J. P.; Stone, E. L.; Bradshaw, T. D.; Stevens, M. F.; Westwell, A. D. *J. Med. Chem.* **2006**, *49*, 179−185. (d) Pujar, G. V.; Synesh, C.; Purohit, M. N.; Srinivasalu, N.; Udupi, R. H. *Ind. J. Hetero. Chem.* **2008**, *17*, 387−388. (e) Siddiqui, N.; Sarafaroz, M.; Alam, M.; Ahsan, W. *Acta Polym. Pharm. Drug Res.* **2008**, *65*, 449−455. (f) Wei, C. X.; Guan, L. P.; Jia, J. H.; Chai, K. Y.; Quan, Z. S. *Arch. Pharmacal Res.* **2009**, *1*, 23−31.

(2) (a) Dunwell, D. W.; Evans, D.; Hicks, T. A. *J. Med. Chem.* **1975**, *18*, 53−58. (b) Dunwell, D. W.; Evans, D. *J. Med. Chem.* **1977**, *20*, 797−801. (c) Dannhardt, G.; Kiefer, W. *Eur. J. Med. Chem.* **2001**, *36*, 109−126. (d) Paramashivappa, P.; Phani Kumar, P.; Subba Rao, P. V.; Srinivas Rao, A. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 657−660. (e) Terzioglu, N.; Van Rijn, R.; Bakker, R. A.; De Esch, I. J. P.; Leurs, R. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5251−5256. (f) Sondhi, M. S.; Singh, N.; Kumar, A.; Lozach, A.; Meijer, L. *Bioorg. Med. Chem.* **2006**,

DOI: 10.1021/acs.jnatprod.9b00072
J. Nat. Prod. XXXX, XXX, XXX−XXX

14, 3758−3765. (g) Ampati, S.; Vidyasagar, J. V.; Swathi, K.; Sarangapani, M. *J. Chem. Pharm. Res.* **2010**, *2*, 213−219. (h) Srinivas, A.; Sagar, J. V.; Sarangapani, M. *Int. J. Pharm. Sci.* **2010**, *2*, 7−12. (i) Kablaoui, N.; Patel, S.; Shao, J.; Demian, D.; Hoffmaster, K.; Berlioz, F.; Vazquez, M. L.; Moore, W. M.; Nugent, R. A. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 907−911. (j) Park, H. J.; Kim, J.-K.; Jun, J.-G. *Bull. Korean Chem. Soc.* **2018**, *39*, 743−749.

(3) (a) Kumar, D.; Jacob, M. R.; Reynolds, M. B.; Kerwin, S. M. *Bioorg. Med. Chem.* **2002**, *10*, 3997−4004. (b) Sun, L. Q.; Chen, J.; Takaki, K.; Johnson, G. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1197−2000. (c) Wang, B.; Maghami, N.; Goodlin, L.; Smith, J. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3221−3226. (d) Aiello, S.; Wells, G.; Stone, E. L.; Kadri, H.; Bazzi, R.; Bell, D. R.; Stevens, M. F. G.; Matthews, C. S.; Bradshaw, T. D.; Westwell, A. D. *J. Med. Chem.* **2008**, *51*, 5135−5139. (e) Kamal, A.; Reddy, K. S.; Khan, M. N. A.; Shetti, R. V. C. R. N. C.; Ramaiah, M. J.; Pushpavalli, S. N. C. V. L.; Srinivas, C.; Pal-Bhadra, M.; Chourasia, M.; Sastry, G. N.; Juvekar, A.; Zingde, S.; Barkume, M. *Bioorg. Med. Chem.* **2010**, *18*, 4747−4761. (f) Wang, G.-W.; Zhou, A.-X.; Wang, J.-J.; Hu, R.-B.; Yang, S.-D. *Org. Lett.* **2013**, *15*, 5270−5273.

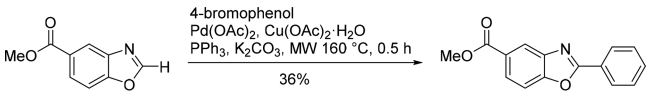
(4) (a) Kusumi, T.; Ooi, T.; Walchi, M. R.; Kakisawa, H. *J. Am. Chem. Soc.* **1988**, *110*, 2954−2958. (b) Suto, M. J.; Turner, W. R. *Tetrahedron Lett.* **1995**, *36*, 7213−7216. (c) Elnima, E.; Zubair, M. U.; Badr, A. A. *Antimicrob. Agents Chemother.* **1981**, *19*, 29−32. (d) Yalcin, I.; Sener, E.; Temiz, O.; Oren, I. *IL Farmaco* **1997**, *52*, 99−103. (e) Temiz, O.; Oren, I.; Sener, E.; Yalcin, I.; Ucarturk, N. *Farmaco* **1998**, *53*, 337−341. (f) Vinsova, J.; Horak, V.; Buchta, V.; Kaustova, J. *Molecules* **2005**, *10*, 783−793. (g) Temiz-Arpaci, O.; Yildiz, I.; Ozkan, S.; Kaynak, F.; Sener, E.; Yalcin, I. *Eur. J. Med. Chem.* **2008**, *43*, 1423−1431. (h) Ertan, T.; Yildiz, I.; Tekiner-Gulbas, B.; Bolelli, K.; Temiz-Arpacıa, O.; Ozkan, S.; Kaynak, F.; Yalcin, I.; Aki, E. *Eur. J. Med. Chem.* **2009**, *44*, 501−510. (i) Kumar, R.; Nair, R. R.; Dhiman, S. S.; Sharma, J.; Prakash, O. *Med. Chem. Res.* **2010**, *19*, 541−550. (j) Abdeen, S.; Kunkle, T.; Salim, N.; Ray, A.-M.; Mammadova, N.; Summers, C.; Stevens, M.; Ambrose, A. J.; Park, Y.; Schultz, P. G.; Horwich, A. L.; Hoang, Q. Q.; Chapman, E.; Johnson, S. M. *J. Med. Chem.* **2018**, *61*, 7345−7357.

(5) (a) Gong, B.; Hong, F.; Kohm, C.; Bonham, L.; Klein, P. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1455−1459. (b) Brown, R. N.; Cameron, R.; Chalmers, D. K.; Hamilton, S.; Luttick, A.; Krippner, G. Y.; McConnell, D. B.; Nearn, R.; Stanislawski, P. C.; Tucker, S. P.; Waston, K. G. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2051−2055. (c) Johnson, S. M.; Connelly, S.; Wilson, I.; Kelly, J. W. *J. Med. Chem.* **2008**, *51*, 260−270. (d) Connelly, S.; Choi, S.; Johnson, S. M.; Kelly, J. W.; Wilson, I. A. *Curr. Opin. Struct. Biol.* **2010**, *20*, 54−62. (e) Hunt, J. A.; Gonzalez, S.; Kallashi, F.; Hammond, M. L.; Pivnichny, J. V.; Tong, X.; Xu, S. S.; Anderson, M. S.; Chen, Y.; Eveland, S. S.; Guo, Q.; Hyland, S. A.; Milot, D. P.; Sparrow, C. P.; Wright, S. D.; Sinclair, P. J. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 1019−1022. (f) Zhao, L. J.; Zhang, L. R.; Lei, M. *Sci. China: Chem.* **2013**, *56*, 1550−1563. (g) Rajasekhar, S.; Maiti, B.; Chanda, K. *Synlett* **2017**, *28*, 521−541.

(6) Sun, M.; Zhang, X.; Hao, H.; Li, W.; Lu, C. *J. Nat. Prod.* **2015**, *78*, 2123−2127.

(7) (a) Xu, D.; Chiaroni, A.; Fleury, M. B.; Largeron, M. *J. Org. Chem.* **2006**, *71*, 6374−6381. (b) Nakamura, H.; Yasui, Y.; Ban, H. S. *J. Organomet. Chem.* **2013**, *747*, 189−194.

(8) Structure of unexpected coupling adduct **S1** and reaction condition:



(9) The results for the natural and synthetic compounds (**1** and **8c**) are slightly different from the IC_{50} values reported in ref 6. This may be due to the differences in the experimental methods (see the Experimental Section for details).

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