

# Synthesis and Inhibitory Activities against Colon Cancer Cell Growth and Proteasome of Alkylresorcinols

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**ABSTRACT:** We have identified alkylresorcinols (ARs) as the major active components in wheat bran against human colon cancer cell growth (HCT-116 and HT-29) using a bioassay-guided approach. To further study the structure–activity relationships, 15 ARs and their intermediates (1–15) were synthesized expediently by the modified Wittig reaction in aqueous media, and six 5-alkylpyrogallols and their analogues (16–21) were prepared by the general Grignard reaction. The synthetic AR analogues were evaluated for activities against the growth of human colon cancer cells HCT-116 and HT-29 and the chymotrypsin-like activity of the human 20S proteasome. Our results found that (1) AR C13:0 and C15:0 (13 and 14) had the greatest inhibitory effects in human colon cancer cells HCT-116 and HT-29, while decreasing or increasing the side chain lengths diminished the activities; (2) two free *meta*-hydroxyl groups at C-1 and C-3 on the aromatic ring of the AR analogues greatly contributed to their antitumor activity; (3) the introduction of a third hydroxyl group at C-2 (20 and 21) into the aromatic ring of the AR analogues yielded no significant enhancement in activity against HCT-116 cells and decimated the effects against HT-29 cells, but dramatically increased the activity against the chymotrypsin-like activity of the human 20S proteasome; and (4) AR C11:0 (12) was found to have the greatest effect in a series of AR C9:0–C17:0 against the chymotrypsin-like activity of the human 20S proteasome.

**KEYWORDS:** 5-alkylresorcinol analogues, synthesis, colon cancer cells, proteasome, growth inhibition

## INTRODUCTION

Colorectal cancer (CRC) is a significant cause of morbidity and mortality in the United States and throughout the world. It is the third most common type of cancer in both men and women and the second leading cause of cancer death in developed countries.<sup>1</sup> Epidemiological studies have indicated that dietary cereal fibers may reduce the risk of colon cancer, with wheat bran (WB) showing greater chemopreventative properties over oat and corn bran.<sup>2–6</sup>

Most human and animal studies show a correlation between WB consumption and the reduction of the risk of colon cancer, and there are several theories of the mechanism by which WB may prevent colon tumor development. For instance, the high fiber content of WB has been proposed as a means of lowering cancer risk by either modification of metabolism and carcinogen formation or by simply diluting carcinogens in the colon by increasing fecal bulk.<sup>7</sup> Conversely, studies in our group and in others have indicated the importance of the lipids and lipid-soluble components in WB in chemoprevention.<sup>8–10</sup>

It has been reported that removal of WB oil from WB increased colon tumorigenesis, whereas fortification of the defatted WB diet with WB oil significantly increased its inhibitory activity.<sup>11</sup> In addition, our group explored the inhibitory effects of WB oil on tumorigenesis in *Apc*<sup>min/+</sup> mice and found that the group treated with 2% WB oil had significantly fewer tumors in the small intestine than those in the control group ( $p < 0.0001$ ), with 35.6% inhibition.<sup>9</sup>

On the basis of the apparent anticancer potency of WB oil, a recent study in our group has been focused on identifying and isolating its active constituents. We identified 14 alk(en)-ylresorcinols, including C17:0–C25:0, from the most active

fraction of WB oil against human colon cancer cells HCT-116 and HT-29 using a bioassay-guided fractionation approach.<sup>12</sup> The biological evaluation for the naturally occurring alkylresorcinols (ARs) C17:0–C25:0 against colon cancer cell growth indicated that increasing the length of the side chain diminished the effects.<sup>12</sup> However, previous studies have shown ARs with shorter side chains as more effective inducers of cytotoxicity of cancer cell lines *in vitro* and inhibitors of cancer formation in nude mice *in vivo*.<sup>13</sup> ARs with shorter chain lengths were not isolated from WB oil in our previous work, which prompted the current investigation, a synthesis and exploration of the inhibitory activities of AR analogues.

The critical step in synthesis of ARs is formation of the C–C bond between the aromatic ring and the alkyl side chain. The most common approach has employed Grignard techniques starting from 3,5-dimethoxybenzaldehyde. For example, Arisawa et al. synthesized a series of the short to moderate alkyl chain ARs, including C9:0–C19:0, using a Grignard reaction.<sup>14</sup> Because the Grignard techniques are time-consuming and highly require an inert atmosphere, Parikka et al. developed an expedient approach for formation of the C–C bond between the aromatic ring and the alkyl side chain using a modified Wittig reaction in an aqueous media, and subsequently synthesized various long chain ( $\geq$ C15) ARs, including C15:0–C25:0.<sup>15</sup>

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We here described the expedient synthesis for the short to moderate length alkyl chain ARs, C9:0–C17:0, using the modified Wittig reaction in aqueous media instead of the general Grignard approaches. Alternatively, the 5-alkylpyrogallols were prepared by classic Grignard reaction in the present study. Meanwhile, the current study is especially focused on bioactivity of the synthesized alkylresorcinol analogues in both cell-based assays and proteasome inhibition experiments, and the structure–activity relationships are discussed.

## MATERIALS AND METHODS

**General.** All reagents and solvents were of commercial quality and used without further purification. The substituted benzaldehydes, alkylbromides, and all other reagents were obtained from commercial sources (Sigma-Aldrich, Fisher Scientific). Anhydrous reactions were carried out in oven-dried glassware under a nitrogen atmosphere unless otherwise noted. Analytical (250  $\mu\text{m}$  thickness, 2–25  $\mu\text{m}$  particle size) and preparative TLC plates (2000  $\mu\text{m}$  thickness, 2–25  $\mu\text{m}$  particle size) were purchased from Sigma (St. Louis, MO) and Sorbent Technologies (Atlanta, GA), respectively. Column chromatography (CC) was performed on silica gel (60  $\text{\AA}$ , 40–63  $\mu\text{m}$ ). All of the synthesized compounds were fully characterized by  $^1\text{H}$  and/or  $^{13}\text{C}$  NMR experiments together with LCMS analysis.  $^1\text{H}$ ,  $^{13}\text{C}$  NMR, and two-dimensional NMR spectra were recorded on a Bruker 600 MHz or a Bruker 700 MHz spectrometer. Multiplicities are indicated by s (singlet), d (doublet), t (triplet), q (quartet), and br (broad). The  $^{13}\text{C}$  NMR spectra are proton decoupled. LC/PDA/MS analysis was carried out with a Thermo-Finnigan Spectra System, which consisted of an Accela high speed MS pump, an Accela refrigerated autosampler, an Accela photodiode array (PDA) detector, and an LCQ Fleet ion trap mass detector (Thermo Electron, San Jose, CA) incorporated with an atmospheric pressure chemical ionization (APCI) interface. A Gemini-NX C<sub>18</sub> column (50 mm  $\times$  2.0 mm i.d., 3  $\mu\text{m}$ , Phenomenex) was used for the analysis of the reaction products with a flow rate of 0.2 mL/min. The binary mobile phase system consisted of 5% aqueous methanol with 0.1% formic acid as A and 95% aqueous methanol with 0.1% formic acid as B.

**General Procedure A for the Synthesis of 5-Alkenylresorcinol Dimethyl Ethers (1–5) via Modified Wittig Approach.** A solution of PPh<sub>3</sub> (1.2 equiv) and long-chain alkyl bromides (1.0 equiv) in toluene (50 mL) was heated to reflux overnight. After being cooled to room temperature (rt), hexane (50 mL) was added. The mixture was filtered, and the solid was washed with a mixture of hexane/ethyl acetate (10:1) three times. The final solid was dried in vacuo to give alkyltriphenylphosphonium salts (yield: 100%).

To a solution of 3,5-dimethoxybenzaldehyde (1.0 equiv) and alkyltriphenylphosphonium salts (1.2 equiv) in DMSO/H<sub>2</sub>O (10:1) at room temperature was added potassium carbonate (2.0 equiv). The mixture was heated to reflux overnight. After being cooled to room temperature, the mixture was poured into water, and extracted with EtOAc. The organic layers were combined, washed with water and brine, dried over Na<sub>2</sub>SO<sub>4</sub>, and filtered. The filtrate was concentrated under reduced pressure to give a red residue, which was subjected to column chromatography (CC) (hexane/ethyl acetate = 40:1) on silica gel to produce the title compounds 1–5.

**1,3-Dimethoxy-5-(non-1-enyl)benzene (1, *E/Z* = 1.8/1).** Procedure A was followed by using 3,5-dimethoxybenzaldehyde (3.6 g, 21.7 mmol) and 1-bromooctane (5.0 g, 26.0 mmol) in DMSO/water (44 mL, 10:1). The residue was subjected to CC (H/E = 40:1) to give the title compound 1,3-dimethoxy-5-(non-1-enyl)benzene (1) (4.5 g, yield: 80%) as a colorless oil: (E)-1,  $^1\text{H}$  NMR (600 MHz, CDCl<sub>3</sub>)  $\delta$  6.34 (1H, t, *J* = 2.2 Hz, H-2), 6.51 (2H, d, *J* = 2.2 Hz, H-4/6), 6.31 (1H, d, *J* = 15.7 Hz, H-1'), 6.22 (1H, dt, *J* = 15.7, 7.0 Hz, H-2'), 2.20 (2H, q, *J* = 7.0 Hz, H-3'), 1.45 (2H, m, H-4'), 1.33–1.24 (8H, m, ranged from H-5' to H-8'), 0.88 (3H, t, *J* = 6.9 Hz, H-9'), and 3.80 (6H, s, OMe-1/3); (Z)-1,  $^1\text{H}$  NMR (600 MHz, CDCl<sub>3</sub>)  $\delta$  6.36 (1H, t, *J* = 2.2 Hz, H-2), 6.44 (2H, d, *J* = 2.2 Hz, H-4/6), 6.34 (1H, d, *J* = 11.6 Hz, H-1'), 5.66 (1H, dt, *J* = 11.6, 7.5 Hz, H-2'), 2.34 (2H, q, *J* = 7.5 Hz, H-3'), 1.45 (2H, m, H-4'), 1.33–1.24 (8H, m, ranged from

H-5' to H-8'), 0.88 (3H, t, *J* = 6.9 Hz, H-9'), and 3.80 (6H, s, OMe-1/3); positive APCIMS, *m/z* 263 [M + H]<sup>+</sup>.

**1,3-Dimethoxy-5-(undec-1-enyl)benzene (2, *E/Z* = 1.4/1).** Procedure A was followed by using 3,5-dimethoxybenzaldehyde (2.0 g, 12.1 mmol) and 1-bromodecane (3.2 g, 14.5 mmol) in DMSO/water (33 mL, 10:1). The residue was subjected to CC (H/E = 40:1) to give the title compound 1,3-dimethoxy-5-(undec-1-enyl)benzene (2) (3.0 g, yield: 85%) as a white solid: (E)-2,  $^1\text{H}$  NMR (700 MHz, CDCl<sub>3</sub>)  $\delta$  6.32 (1H, t, *J* = 2.2 Hz, H-2), 6.50 (2H, d, *J* = 2.2 Hz, H-4/6), 6.30 (1H, d, *J* = 15.8 Hz, H-1'), 6.21 (1H, dt, *J* = 15.8, 6.8 Hz, H-2'), 2.18 (2H, q, *J* = 7.0 Hz, H-3'), 1.44 (2H, m, H-4'), 1.33–1.24 (12H, m, ranged from H-5' to H-10'), 0.87 (3H, t, *J* = 6.9 Hz, H-11'), and 3.79 (6H, s, OMe-1/3); (Z)-2,  $^1\text{H}$  NMR (700 MHz, CDCl<sub>3</sub>)  $\delta$  6.35 (1H, t, *J* = 2.2 Hz, H-2), 6.43 (2H, d, *J* = 2.2 Hz, H-4/6), 6.33 (1H, d, *J* = 11.6 Hz, H-1'), 5.65 (1H, dt, *J* = 11.6, 7.5 Hz, H-2'), 2.32 (2H, q, *J* = 7.5 Hz, H-3'), 1.44 (2H, m, H-4'), 1.33–1.24 (12H, m, ranged from H-5' to H-10'), 0.87 (3H, t, *J* = 6.9 Hz, H-11'), and 3.79 (6H, s, OMe-1/3); positive APCIMS, *m/z* 291 [M + H]<sup>+</sup>.

**1,3-Dimethoxy-5-(tridec-1-enyl)benzene (3, *E/Z* = 1.6:1).** Procedure A was followed by using 3,5-dimethoxybenzaldehyde (2.0 g, 12.1 mmol) and 1-bromododecane (3.6 g, 14.4 mmol) in DMSO/water (33 mL, 10:1). The residue was subjected to CC (H/E = 40:1) to give the title compound 1,3-dimethoxy-5-(tridec-1-enyl)benzene (3) (3.5 g, yield: 90%) as a white solid: (E)-3,  $^1\text{H}$  NMR (700 MHz, CDCl<sub>3</sub>)  $\delta$  6.32 (1H, t, *J* = 2.2 Hz, H-2), 6.50 (2H, d, *J* = 2.2 Hz, H-4/6), 6.30 (1H, d, *J* = 15.8 Hz, H-1'), 6.21 (1H, dt, *J* = 15.8, 6.8 Hz, H-2'), 2.18 (2H, q, *J* = 6.8 Hz, H-3'), 1.44 (2H, m, H-4'), 1.32–1.24 (16H, m, ranged from H-5' to H-12'), 0.88 (3H, t, *J* = 6.9 Hz, H-13'), and 3.79 (6H, s, OMe-1/3); (Z)-3,  $^1\text{H}$  NMR (700 MHz, CDCl<sub>3</sub>)  $\delta$  6.35 (1H, t, *J* = 2.2 Hz, H-2), 6.43 (2H, d, *J* = 2.2 Hz, H-4/6), 6.33 (1H, d, *J* = 11.6 Hz, H-1'), 5.65 (1H, dt, *J* = 11.6, 7.3 Hz, H-2'), 2.32 (2H, q, *J* = 7.3 Hz, H-3'), 1.44 (2H, m, H-4'), 1.32–1.24 (16H, m, ranged from H-5' to H-12'), 0.88 (3H, t, *J* = 6.9 Hz, H-13'), and 3.79 (6H, s, OMe-1/3); positive APCIMS, *m/z* 319 [M + H]<sup>+</sup>.

**1,3-Dimethoxy-5-(pentadec-1-enyl)benzene (4, *E/Z* = 1.1/1).** Procedure A was followed by using 3,5-dimethoxybenzaldehyde (2.0 g, 12.1 mmol) and 1-bromotetradecane (4.0 g, 14.4 mmol) in DMSO/water (33 mL, 10:1). The residue was subjected to CC (H/E = 40:1) to give the title compound 1,3-dimethoxy-5-(pentadec-1-enyl)benzene (4) (3.8 g, yield: 90%) as a white solid: (E)-4,  $^1\text{H}$  NMR (700 MHz, CDCl<sub>3</sub>)  $\delta$  6.32 (1H, t, *J* = 2.2 Hz, H-2), 6.49 (2H, d, *J* = 2.2 Hz, H-4/6), 6.30 (1H, d, *J* = 15.7 Hz, H-1'), 6.21 (1H, dt, *J* = 15.7, 7.0 Hz, H-2'), 2.18 (2H, q, *J* = 7.0 Hz, H-3'), 1.44 (2H, m, H-4'), 1.32–1.24 (20H, m, ranged from H-5' to H-14'), 0.87 (3H, t, *J* = 6.9 Hz, H-15'), and 3.78 (6H, s, OMe-1/3); (Z)-4,  $^1\text{H}$  NMR (700 MHz, CDCl<sub>3</sub>)  $\delta$  6.35 (1H, t, *J* = 2.2 Hz, H-2), 6.43 (2H, d, *J* = 2.2 Hz, H-4/6), 6.33 (1H, d, *J* = 11.6 Hz, H-1'), 5.65 (1H, dt, *J* = 11.6, 7.5 Hz, H-2'), 2.32 (2H, q, *J* = 7.5 Hz, H-3'), 1.44 (2H, m, H-4'), 1.32–1.24 (20H, m, ranged from H-5' to H-14'), 0.87 (3H, t, *J* = 6.9 Hz, H-15'), and 3.78 (6H, s, OMe-1/3); positive APCIMS, *m/z* 347 [M + H]<sup>+</sup>.

**1,3-Dimethoxy-5-(heptadec-1-enyl)benzene (5, *E/Z* = 1.5/1).** Procedure A was followed by using 3,5-dimethoxybenzaldehyde (166 mg, 1.0 mmol) and 1-bromoheptadecane (365 mg, 1.2 mmol) in DMSO/water (2.2 mL, 10:1). The residue was subjected to CC (H/E = 40:1) to give the title compound 1,3-dimethoxy-5-(heptadec-1-enyl)benzene (5) (300 mg, yield: 80%) as a white solid: (E)-5,  $^1\text{H}$  NMR (600 MHz, CDCl<sub>3</sub>)  $\delta$  6.32 (1H, t, *J* = 2.2 Hz, H-2), 6.50 (2H, d, *J* = 2.2 Hz, H-4/6), 6.30 (1H, d, *J* = 15.7 Hz, H-1'), 6.21 (1H, dt, *J* = 15.7, 7.0 Hz, H-2'), 2.18 (2H, q, *J* = 7.0 Hz, H-3'), 1.44 (2H, m, H-4'), 1.32–1.24 (24H, m, ranged from H-5' to H-16'), 0.88 (3H, t, *J* = 7.0 Hz, H-17'), and 3.79 (6H, s, OMe-1/3); (Z)-5,  $^1\text{H}$  NMR (600 MHz, CDCl<sub>3</sub>)  $\delta$  6.35 (1H, t, *J* = 2.2 Hz, H-2), 6.43 (2H, d, *J* = 2.2 Hz, H-4/6), 6.33 (1H, d, *J* = 11.6 Hz, H-1'), 5.65 (1H, dt, *J* = 11.6, 7.5 Hz, H-2'), 2.32 (2H, q, *J* = 7.5 Hz, H-3'), 1.44 (2H, m, H-4'), 1.32–1.24 (24H, m, ranged from H-5' to H-16'), 0.88 (3H, t, *J* = 7.0 Hz, H-17'), and 3.79 (6H, s, OMe-1/3); positive APCIMS, *m/z* 375 [M + H]<sup>+</sup>.

**General Procedure B for the Synthesis of 5-Alkylresorcinol Dimethyl Ethers (6–10) by Hydrogenation.** Methylated 5-alkylresorcinols were dissolved into a mixture of chloroform/methanol (1:1) at room temperature. To this solution was added Pd/

C (10% w/w) under N<sub>2</sub>. The mixture was flushed with H<sub>2</sub> three times, and stirred at room temperature under H<sub>2</sub> for 24 h. After the reaction was completed, the mixture was filtered through a short pad of silica gel. The filtrate was evaporated under reduced pressure to give the desired compounds 6–10.

**1,3-Dimethoxy-5-nonylbenzene (6).** Procedure B was followed by using 1,3-dimethoxy-5-(non-1-enyl)benzene (3.8 g, 14.5 mmol) and Pd/C (10% w/w, 380 mg) in a mixture of chloroform/methanol (20 mL, 1:1). The resulting filtration was evaporated to give the title compound 1,3-dimethoxy-5-nonylbenzene (6) (3.3 g, yield: 85%) as a colorless oil: <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) δ 6.30 (1H, t, *J* = 2.2 Hz, H-2), 6.35 (2H, d, *J* = 2.2 Hz, H-4/6), 2.54 (2H, t, *J* = 7.7 Hz, H-1'), 1.60 (2H, m, H-2'), 1.33–1.26 (12H, m, ranged from H-3' to H-8'), 0.88 (3H, t, *J* = 6.8 Hz, H-9'), and 3.78 (6H, s, OMe-1/3); positive APCIMS, *m/z* 265 [M + H]<sup>+</sup>.

**1,3-Dimethoxy-5-undecylbenzene (7).** Procedure B was followed by using 1,3-dimethoxy-5-(undec-1-enyl)benzene (3.0 g, 10.3 mmol) and Pd/C (10% w/w, 300 mg) in a mixture of chloroform/methanol (20 mL, 1:1). The resulting filtration was evaporated to give the title compound 1,3-dimethoxy-5-undecylbenzene (7) (2.6 g, yield: 86%) as a white solid: <sup>1</sup>H NMR (700 MHz, CDCl<sub>3</sub>) δ 6.29 (1H, t, *J* = 2.2 Hz, H-2), 6.34 (2H, d, *J* = 2.2 Hz, H-4/6), 2.53 (2H, t, *J* = 7.6 Hz, H-1'), 1.59 (2H, m, H-2'), 1.33–1.24 (16H, m, ranged from H-3' to H-10'), 0.87 (3H, t, *J* = 6.9 Hz, H-11'), and 3.77 (6H, s, OMe-1/3); positive APCIMS, *m/z* 293 [M + H]<sup>+</sup>.

**1,3-Dimethoxy-5-tridecylbenzene (8).** Procedure B was followed by using 1,3-dimethoxy-5-(tridec-1-enyl)benzene (3.6 g, 11.3 mmol) and Pd/C (10% w/w, 360 mg) in a mixture of chloroform/methanol (20 mL, 1:1). The resulting filtration was evaporated to give the title compound 1,3-dimethoxy-5-tridecylbenzene (8) (3.3 g, yield: 91%) as a white solid: <sup>1</sup>H NMR (700 MHz, CDCl<sub>3</sub>) δ 6.29 (1H, t, *J* = 2.2 Hz, H-2), 6.34 (2H, d, *J* = 2.2 Hz, H-4/6), 2.53 (2H, t, *J* = 7.7 Hz, H-1'), 1.59 (2H, m, H-2'), 1.33–1.24 (20H, m, ranged from H-3' to H-12'), 0.87 (3H, t, *J* = 6.9 Hz, H-13'), and 3.77 (6H, s, OMe-1/3); positive APCIMS, *m/z* 321 [M + H]<sup>+</sup>.

**1,3-Dimethoxy-5-pentadecylbenzene (9).** Procedure B was followed by using 1,3-dimethoxy-5-(pentadec-1-enyl)benzene (4.0 g, 11.6 mmol) and Pd/C (10% w/w, 400 mg) in a mixture of chloroform/methanol (20 mL, 1:1). The resulting filtration was evaporated to give the title compound 1,3-dimethoxy-5-pentadecylbenzene (9) (3.3 g, yield: 80%) as a white solid: <sup>1</sup>H NMR (700 MHz, CDCl<sub>3</sub>) δ 6.29 (1H, t, *J* = 2.2 Hz, H-2), 6.34 (2H, d, *J* = 2.2 Hz, H-4/6), 2.53 (2H, t, *J* = 7.7 Hz, H-1'), 1.59 (2H, m, H-2'), 1.33–1.24 (24H, m, ranged from H-3' to H-14'), 0.88 (3H, t, *J* = 6.9 Hz, H-15'), and 3.77 (6H, s, OMe-1/3); positive APCIMS, *m/z* 349 [M + H]<sup>+</sup>.

**1,3-Dimethoxy-5-heptadecylbenzene (10).** Procedure B was followed by using 1,3-dimethoxy-5-(heptadec-1-enyl)benzene (4.0 g, 10.7 mmol) and Pd/C (10% w/w, 400 mg) in a mixture of chloroform/methanol (20 mL, 1:1). The resulting filtration was evaporated to give the title compound 1,3-dimethoxy-5-heptadecylbenzene (10) (3.2 g, yield: 80%) as a white solid: <sup>1</sup>H NMR (700 MHz, CDCl<sub>3</sub>) δ 6.29 (1H, t, *J* = 2.2 Hz, H-2), 6.34 (2H, d, *J* = 2.2 Hz, H-4/6), 2.53 (2H, t, *J* = 7.7 Hz, H-1'), 1.59 (2H, m, H-2'), 1.33–1.24 (28H, m, ranged from H-3' to H-16'), 0.88 (3H, t, *J* = 6.9 Hz, H-17'), and 3.77 (6H, s, OMe-1/3); positive APCIMS, *m/z* 377 [M + H]<sup>+</sup>.

**General Procedure C for the Demethylation by BBr<sub>3</sub>.** Methyl ethers (1.0 equiv) were dissolved in dichloromethane (DCM) (50 mL). A solution of BBr<sub>3</sub> in DCM (1 M, 2.0–3.5 equiv) was added at 0 °C slowly. After the addition was completed, the reaction solution was allowed to warm to room temperature overnight and quenched by water (50 mL). The organic phase solvent was washed with water and brine, dried over Na<sub>2</sub>SO<sub>4</sub>, and filtered. The filtrate was evaporated under reduced pressure to give a residue, which was purified by column chromatography or pre-TLC to give the desired compounds.

**5-*n*-Nonylresorcinol (11).** Procedure C was followed by using 1,3-dimethoxy-5-nonylbenzene (3.6 g, 13.6 mmol) and a solution of BBr<sub>3</sub> in DCM (1M, 34.1 mL, 34.1 mmol). The residue was purified by column chromatography (DCM/MeOH = 50:1) to give the title compound 5-*n*-nonylresorcinol (11) (2.7 g, yield: 84%) as a white solid: <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>+CD<sub>3</sub>OD) δ 6.15 (1H, t, *J* = 2.0 Hz,

H-2), 6.21 (2H, d, *J* = 2.0 Hz, H-4/6), 2.45 (2H, t, *J* = 7.7 Hz, H-1'), 1.54 (2H, m, H-2'), 1.27–1.24 (12H, m, ranged from H-3' to H-8'), and 0.87 (3H, t, *J* = 6.9 Hz, H-9'); <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>+CD<sub>3</sub>OD) δ 156.6 (s, C-1/3), 100.1 (d, C-2), 108.0 (d, C-4/6), 146.1 (s, C-5), 35.8 (t, C-1'), 31.0 (t, C-2'), 29.2 (t, C-3'), 29.5–29.3 (t, ranged from C-4' to C-6'), 31.9 (t, C-7'), 22.7 (t, C-8'), and 14.1 (q, C-9'); positive APCIMS, *m/z* 237 [M + H]<sup>+</sup>.

**5-*n*-Undecylresorcinol (12).** Procedure C was followed by using 1,3-dimethoxy-5-undecylbenzene (2.3 g, 7.9 mmol) and a solution of BBr<sub>3</sub> in DCM (1 M, 15.8 mL, 15.8 mmol). The residue was purified by column chromatography (DCM/MeOH = 30:1) to give the title compound 5-*n*-undecylresorcinol (12) (2.0 g, yield: 90%) as a white solid: <sup>1</sup>H NMR (700 MHz, CDCl<sub>3</sub>+CD<sub>3</sub>OD) δ 6.14 (1H, t, *J* = 2.2 Hz, H-2), 6.20 (2H, d, *J* = 2.2 Hz, H-4/6), 2.43 (2H, t, *J* = 7.7 Hz, H-1'), 1.53 (2H, m, H-2'), 1.26–1.22 (16H, m, ranged from H-3' to H-10'), and 0.86 (3H, t, *J* = 6.9 Hz, H-11'); <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>+CD<sub>3</sub>OD) δ 156.9 (s, C-1/3), 100.1 (d, C-2), 107.6 (d, C-4/6), 145.9 (s, C-5), 35.9 (t, C-1'), 31.1 (t, C-2'), 29.3 (t, C-3'), 29.7–29.3 (t, ranged from C-4' to C-8'), 31.9 (t, C-9'), 22.6 (t, C-10'), and 14.1 (q, C-11'); positive APCIMS, *m/z* 265 [M + H]<sup>+</sup>.

**5-*n*-Tridecylresorcinol (13).** Procedure C was followed by using 1,3-dimethoxy-5-tridecylbenzene (3.0 g, 9.4 mmol) and a solution of BBr<sub>3</sub> in DCM (1 M, 18.8 mL, 18.8 mmol). The residue was purified by column chromatography (DCM/MeOH = 30:1) to give the title compound 5-*n*-tridecylresorcinol (13) (2.5 g, yield: 91%) as a white solid: <sup>1</sup>H NMR (700 MHz, CDCl<sub>3</sub>+CD<sub>3</sub>OD) δ 6.12 (1H, t, *J* = 2.2 Hz, H-2), 6.18 (2H, d, *J* = 2.2 Hz, H-4/6), 2.43 (2H, t, *J* = 7.7 Hz, H-1'), 1.53 (2H, m, H-2'), 1.26–1.22 (20H, m, ranged from H-3' to H-12'), and 0.85 (3H, t, *J* = 6.8 Hz, H-13'); <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>+CD<sub>3</sub>OD) δ 157.0 (s, C-1/3), 100.0 (d, C-2), 107.5 (d, C-4/6), 145.8 (s, C-5), 35.8 (t, C-1'), 31.1 (t, C-2'), 29.3 (t, C-3'), 29.8–29.3 (t, ranged from C-4' to C-10'), 31.9 (t, C-11'), 22.6 (t, C-12'), and 14.1 (q, C-13'); positive APCIMS, *m/z* 293 [M + H]<sup>+</sup>.

**5-*n*-Pentadecylresorcinol (14).** Procedure C was followed by using 1,3-dimethoxy-5-pentadecylbenzene (2.6 g, 7.5 mmol) and a solution of BBr<sub>3</sub> in DCM (1 M, 14.9 mL, 14.9 mmol). The residue was purified by column chromatography (DCM/MeOH = 30:1) to give the title compound 5-*n*-pentadecylresorcinol (14) (2.2 g, yield: 90%) as a white solid: <sup>1</sup>H NMR (700 MHz, CDCl<sub>3</sub>+CD<sub>3</sub>OD) δ 6.07 (1H, t, *J* = 2.2 Hz, H-2), 6.14 (2H, d, *J* = 2.2 Hz, H-4/6), 2.39 (2H, t, *J* = 7.7 Hz, H-1'), 1.49 (2H, m, H-2'), 1.24–1.18 (24H, m, ranged from H-3' to H-14'), and 0.82 (3H, t, *J* = 6.8 Hz, H-15'); <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>+CD<sub>3</sub>OD) δ 157.2 (s, C-1/3), 99.8 (d, C-2), 107.2 (d, C-4/6), 145.6 (s, C-5), 35.8 (t, C-1'), 31.1 (t, C-2'), 29.2 (t, C-3'), 29.7–29.3 (t, ranged from C-4' to C-12'), 31.8 (t, C-13'), 22.6 (t, C-14'), and 14.0 (q, C-15'); positive APCIMS, *m/z* 321 [M + H]<sup>+</sup>.

**5-*n*-Heptadecylresorcinol (15).** Procedure C was followed by using 1,3-dimethoxy-5-heptadecylbenzene (3.0 g, 7.9 mmol) and a solution of BBr<sub>3</sub> in DCM (1 M, 19.9 mL, 19.9 mmol). The residue was purified by column chromatography (DCM/MeOH = 30:1) to give the title compound 5-*n*-heptadecylresorcinol (15) (2.7 g, yield: 95%) as a white solid: <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>+CD<sub>3</sub>OD) δ 6.13 (1H, t, *J* = 2.2 Hz, H-2), 6.19 (2H, d, *J* = 2.2 Hz, H-4/6), 2.43 (2H, t, *J* = 7.7 Hz, H-1'), 1.52 (2H, m, H-2'), 1.30–1.24 (28H, m, ranged from H-3' to H-16'), and 0.86 (3H, t, *J* = 6.7 Hz, H-17'); <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>+CD<sub>3</sub>OD) δ 157.2 (s, C-1/3), 99.9 (d, C-2), 107.3 (d, C-4/6), 145.7 (s, C-5), 35.9 (t, C-1'), 31.1 (t, C-2'), 29.3 (t, C-3'), 29.7–29.4 (t, ranged from C-4' to C-14'), 31.9 (t, C-15'), 22.7 (t, C-16'), and 14.1 (q, C-17'); positive APCIMS, *m/z* 349 [M + H]<sup>+</sup>.

**General Procedure D for the Synthesis of the Secondary Alcohols by Grignard Reaction.** A flame-dried 100 mL three-necked flask, equipped with a condenser, was charged with magnesium turnings (4.04 equiv), a small piece of iodine, and 20 mL of dry THF under N<sub>2</sub>. A few drops of a solution of alkylbromide in THF were added to trigger the reaction, then the reaction mixture was heated to 60 °C, and the rest of alkylbromide (4.0 equiv) in THF (5 mL) was added dropwise in 15 min and stirred for another 5 h until most of magnesium turnings were consumed. The gray solution was cooled to 0 °C followed by addition of a solution of 5-hydroxyvanillin (1.0 equiv) in THF (2 mL) slowly. After being stirred for 15 min at 0 °C,

the reaction mixture was allowed to warm to room temperature during 2 h, and then quenched with saturated  $\text{NH}_4\text{Cl}$  aqueous solution (20 mL). The water layer was extracted with ethyl acetate (20 mL  $\times$  3). The organic layers were combined, washed with water (20 mL  $\times$  2) and brine (20 mL  $\times$  1), dried over  $\text{Na}_2\text{SO}_4$ , and filtered. The filtrate was concentrated under reduced pressure to give a residue, which was subjected to column chromatography (C/M = 20:1) to afford the desired compounds 16 and 17.

**5-(1-Hydroxytridecyl)-3-methoxybenzene-1,2-diol (16).** Procedure D was followed by using 5-hydroxyvanillin (203 mg, 1.2 mmol) and 1-bromododecane (1.2 g, 5.0 mmol) under Grignard condition. The residue was subjected to column chromatography (C/M = 20:1) to give the title compound 5-(1-hydroxytridecyl)-3-methoxybenzene-1,2-diol (16) (340 mg, yield: 85%) as a yellow solid:  $^1\text{H}$  NMR (700 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  6.44 (1H, d,  $J$  = 1.6 Hz, H-4), 6.47 (1H, d,  $J$  = 1.6 Hz, H-6), 4.41 (1H, t,  $J$  = 6.8 Hz, H-1'), 1.72 (1H, m, H-2'a), 1.62 (1H, m, H-2'b), 1.54 (1H, m, H-3'a), 1.35 (1H, m, H-3'b), 1.30–1.25 (18H, m, ranged from H-4' to H-12'), 0.89 (3H, t,  $J$  = 7.0 Hz, H-13'), and 3.82 (3H, s, OMe-1);  $^{13}\text{C}$  NMR (175 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  149.5 (s, C-1), 134.3 (s, C-2), 146.3 (s, C-3), 107.8 (d, C-4), 137.4 (s, C-5), 102.5 (d, C-6), 75.5 (d, C-1'), 40.1 (t, C-2'), 27.0 (t, C-3'), 30.8–30.5 (t, ranged from C-4' to C-10'), 33.1 (t, C-11'), 23.7 (t, C-12'), 14.4 (q, C-13'), and 56.6 (s, OMe-1); positive APCIMS,  $m/z$  339  $[\text{M} + \text{H}]^+$ .

**5-(1-Hydroxypentadecyl)-3-methoxybenzene-1,2-diol (17).** Procedure D was followed by using 5-hydroxyvanillin (203 mg, 1.2 mmol) and 1-bromotetradecane (1.3 g, 5.0 mmol) under Grignard condition. The residue was subjected to column chromatography (C/M = 20:1) to give the title compound 5-(1-hydroxypentadecyl)-3-methoxybenzene-1,2-diol (17) (382 mg, yield: 87%) as a white solid:  $^1\text{H}$  NMR (700 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  6.44 (1H, d,  $J$  = 1.6 Hz, H-4), 6.47 (1H, d,  $J$  = 1.6 Hz, H-6), 4.41 (1H, t,  $J$  = 6.8 Hz, H-1'), 1.71 (1H, m, H-2'a), 1.62 (1H, m, H-2'b), 1.54 (1H, m, H-3'a), 1.35 (1H, m, H-3'b), 1.30–1.25 (22H, m, ranged from H-4' to H-14'), 0.89 (3H, t,  $J$  = 7.0 Hz, H-15'), and 3.82 (3H, s, OMe-1);  $^{13}\text{C}$  NMR (175 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  149.5 (s, C-1), 134.3 (s, C-2), 146.3 (s, C-3), 107.8 (d, C-4), 137.4 (s, C-5), 102.5 (d, C-6), 75.4 (d, C-1'), 40.1 (t, C-2'), 27.0 (t, C-3'), 30.8–30.5 (t, ranged from C-4' to C-12'), 33.1 (t, C-13'), 23.7 (t, C-14'), 14.4 (q, C-15'), and 56.6 (s, OMe-1); positive APCIMS,  $m/z$  367  $[\text{M} + \text{H}]^+$ .

**General Procedure E for the Hydrogenation of the Secondary Alcohols by Pd/C.** A solution of the secondary alcohols in HOAc (10 mL) was flushed with  $\text{N}_2$  three times followed by addition of 10% palladium on carbon (20% w/w). The system was replaced twice by  $\text{H}_2$  and stirred at 40 °C overnight under  $\text{H}_2$ . The mixture was cooled to room temperature and filtered. The filtrate was evaporated under reduced pressure, and the residue was applied to column chromatography (H/E = 10:1) to give the desired compounds 18 and 19.

**3-Methoxy-5-tridecylbenzene-1,2-diol (18).** Procedure E was followed by using 5-(1-hydroxytridecyl)-3-methoxybenzene-1,2-diol (380 mg, 1.1 mmol) and Pd/C (100 mg, 20% w/w) in HOAc at 40 °C. The residue was applied to column chromatography (H/E = 10:1) to give the title compound 3-methoxy-5-tridecylbenzene-1,2-diol (18) (250 mg, yield: 60%) as a yellow solid:  $^1\text{H}$  NMR (700 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  6.27 (1H, d,  $J$  = 1.5 Hz, H-4), 6.28 (1H, d,  $J$  = 1.5 Hz, H-6), 2.44 (2H, t,  $J$  = 7.6 Hz, H-1'), 1.55 (2H, m, H-2'), 1.32–1.25 (20H, m, ranged from H-3' to H-12'), 0.89 (3H, t,  $J$  = 6.9 Hz, H-13'), and 3.79 (3H, s, OMe-1);  $^{13}\text{C}$  NMR (175 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  149.5 (s, C-1), 133.0 (s, C-2), 146.3 (s, C-3), 109.8 (d, C-4), 135.0 (s, C-5), 104.8 (d, C-6), 36.8 (t, C-1'), 32.9 (t, C-2'), 30.3 (t, C-3'), 30.8–30.5 (t, ranged from C-4' to C-10'), 33.1 (t, C-11'), 23.7 (t, C-12'), 14.4 (q, C-13'), and 56.6 (s, OMe-1); positive APCIMS,  $m/z$  323  $[\text{M} + \text{H}]^+$ .

**3-Methoxy-5-pentadecylbenzene-1,2-diol (19).** Procedure E was followed by using 5-(1-hydroxypentadecyl)-3-methoxybenzene-1,2-diol (382 mg, 1.0 mmol) and Pd/C (100 mg, 20% w/w) in HOAc at 40 °C. The residue was applied to column chromatography (H/E = 10:1) to give the title compound 3-methoxy-5-pentadecylbenzene-1,2-diol (19) (240 mg, yield: 67%) as a yellow solid:  $^1\text{H}$  NMR (700 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  6.27 (1H, d,  $J$  = 1.5 Hz, H-4), 6.28 (1H, d,  $J$  = 1.5 Hz, H-

6), 2.44 (2H, t,  $J$  = 7.6 Hz, H-1'), 1.55 (2H, m, H-2'), 1.32–1.25 (24H, m, ranged from H-3' to H-14'), 0.89 (3H, t,  $J$  = 6.9 Hz, H-15'), and 3.79 (3H, s, OMe-1);  $^{13}\text{C}$  NMR (175 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  149.5 (s, C-1), 133.0 (s, C-2), 146.3 (s, C-3), 109.8 (d, C-4), 135.0 (s, C-5), 104.7 (d, C-6), 36.8 (t, C-1'), 32.9 (t, C-2'), 30.3 (t, C-3'), 30.8–30.5 (t, ranged from C-4' to C-12'), 33.1 (t, C-13'), 23.7 (t, C-14'), 14.4 (q, C-15'), and 56.6 (s, OMe-1); positive APCIMS,  $m/z$  351  $[\text{M} + \text{H}]^+$ .

**5-Tridecylbenzene-1,2,3-triol (20).** Procedure C was followed by using 3-methoxy-5-tridecylbenzene-1,2-diol (118 mg, 0.37 mmol) and a solution of  $\text{BBr}_3$  in DCM (1 M, 1.28 mL, 1.28 mmol). The residue was purified by pre-TLC (DCM/MeOH = 15:1) to give the title compound 5-tridecylbenzene-1,2,3-triol (20) (54 mg, yield: 48%) as a yellow solid:  $^1\text{H}$  NMR (700 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  6.16 (2H, s, H-4/6), 2.37 (2H, t,  $J$  = 7.6 Hz, H-1'), 1.53 (2H, m, H-2'), 1.33–1.25 (20H, m, ranged from H-3' to H-12'), and 0.89 (3H, t,  $J$  = 6.9 Hz, H-13');  $^{13}\text{C}$  NMR (175 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  146.8 (s, C-1/3), 131.9 (s, C-2), 108.3 (d, C-4/6), 135.2 (s, C-5), 36.6 (t, C-1'), 32.8 (t, C-2'), 30.3 (t, C-3'), 30.8–30.5 (t, ranged from C-4' to C-10'), 33.1 (t, C-11'), 23.7 (t, C-12'), and 14.4 (q, C-13'); positive APCIMS,  $m/z$  309  $[\text{M} + \text{H}]^+$ .

**5-Pentadecylbenzene-1,2,3-triol (21).** Procedure C was followed by using 3-methoxy-5-pentadecylbenzene-1,2-diol (225 mg, 0.64 mmol) and a solution of  $\text{BBr}_3$  in DCM (1M, 2.25 mL, 2.25 mmol). The residue was purified by pre-TLC (DCM/MeOH = 15:1) to give the title compound 5-pentadecylbenzene-1,2,3-triol (21) (90 mg, yield: 50%) as a yellow solid:  $^1\text{H}$  NMR (700 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  6.16 (2H, s, H-4/6), 2.37 (2H, t,  $J$  = 7.6 Hz, H-1'), 1.52 (2H, m, H-2'), 1.32–1.25 (24H, m, ranged from H-3' to H-14'), and 0.89 (3H, t,  $J$  = 6.9 Hz, H-15');  $^{13}\text{C}$  NMR (175 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  146.8 (s, C-1/3), 131.9 (s, C-2), 108.3 (d, C-4/6), 135.2 (s, C-5), 36.6 (t, C-1'), 32.8 (t, C-2'), 30.3 (t, C-3'), 30.8–30.5 (t, ranged from C-4' to C-12'), 33.1 (t, C-13'), 23.7 (t, C-14'), and 14.4 (q, C-15'); positive APCIMS,  $m/z$  337  $[\text{M} + \text{H}]^+$ .

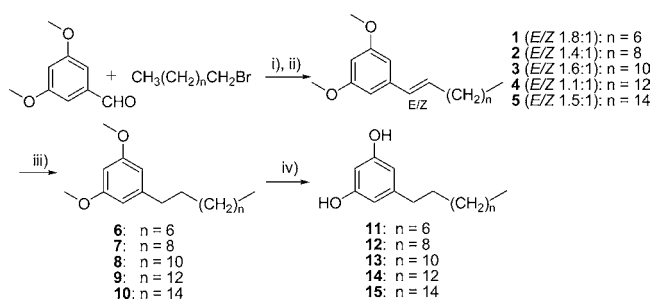
**Growth Inhibition of Human Colon Cancer Cells.** Cell growth inhibition was determined by a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay.<sup>16</sup> Human colon cancer cells HCT-116 and HT-29 (3000 cells/well) were plated in 96-well microtiter plates and allowed to attach for 24 h at 37 °C. The test compounds 1–21 (in DMSO) were added to cell culture medium to desired final concentrations (0–50  $\mu\text{M}$ ; final DMSO concentrations for control and treatments were 0.1%). After the cells were cultured for 24 h, the medium was aspirated, and cells were treated with 200  $\mu\text{L}$  of fresh medium containing 2.41 mmol/L MTT. After incubation for 3 h at 37 °C, the medium containing MTT was aspirated, 100  $\mu\text{L}$  of DMSO was added to solubilize the formazan precipitate, and the plates were shaken gently for an hour at room temperature. Absorbance values were derived from the plate reading at 550 nm on a microtiter plate reader. The reading reflected the number of viable cells and was expressed as a percentage of viable cells in the control. Both HCT-116 and HT-29 cells were cultured in McCoy's 5A medium. All of the above media were supplemented with 10% fetal bovine serum, 1% penicillin/streptomycin, and 1% glutamine, and the cells were kept in a 37 °C incubator with 95% humidity and 5%  $\text{CO}_2$ .

**Proteasome Assay.** Proteasome assay reagents were purchased from Boston Biochem, an R&D Systems Manufacturer, in Cambridge, MA. The effects of ARs analogues (11–21) were assayed following the manufacturer's recommended protocol. The reaction mixture was comprised of human 20S proteasome (2 nM final), the fluorogenic substrate Suc-Leu-Leu-Val-Tyr-AMC (Suc-LLVY-AMC, 32.5  $\mu\text{M}$  final), and the proteasome activator PA28 (5 nM final). The ARs analogues (11–21) were tested for proteasome inhibition by incubation with reaction mixture over a range of concentrations flanking the putative  $\text{IC}_{50}$  values. Cleavage of the fluorogenic substrate Suc-LLVY-AMC was measured on a Biotek fluorometer at an excitation/emission of 360 nm/460 nm. The velocity of the reaction, measured in  $\Delta\text{RFU}$  (360/460 nm)/min, was plotted against the log-concentration of the inhibitor to determine the respective  $\text{IC}_{50}$  values. The known proteasome inhibitor, lactacystin, was used as a positive control for assay validity.

## RESULTS

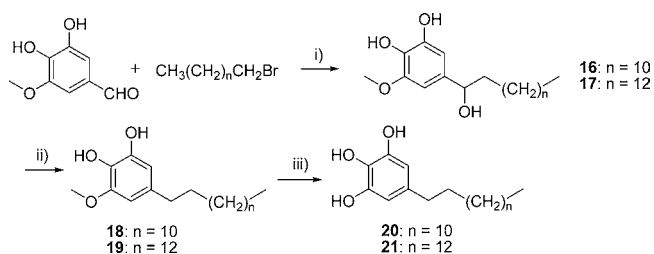
**Synthesis.** Five short to moderate length side chain ARs C9:0–C17:0 (**11–15**), together with 10 related intermediates (**1–10**), were synthesized judiciously in the current study using a modified Wittig reaction in aqueous media instead of the general Grignard approaches. The synthesis of short chain ARs C9:0–C13:0 in our efforts, however, extended the application scopes of the modified Wittig approach, which was described for the preparation of long chain ( $\geq$ C15) ARs.<sup>15</sup> In addition, our findings are potentially provocative in our discovered application limitation of the modified Wittig reaction for the synthesis of the 5-alkylpyrogallols from 5-hydroxyvanillin, which is very close to 3,5-dimethoxybenzaldehyde. The modified Wittig reaction of 5-hydroxyvanillin and appropriate alkyltriphenylphosphonium salts failed to produce the target intermediates in our case. Consequentially, we returned to the general Grignard procedure to obtain the key intermediates (**16** and **17**) for the 5-alkylpyrogallols (**20** and **21**) in good yields ( $\geq$ 85%).

In summary, Wittig reaction of 3,5-dimethoxybenzaldehyde with nonstabilized alkyltriphenylphosphonium salts in aqueous DMSO solution under  $K_2CO_3$  produced 1,3-dimethoxy-5-alkenylbenzenes (**1–5**), as a mixture of the geometric isomers (Figure 1).<sup>15</sup> Subsequently, reduction of the geometric isomers



**Figure 1.** Synthesis of 5-alk(en)ylresorcinol analogues (**1–15**). Reagents and conditions: (i)  $PPh_3$ , toluene, reflux, 18 h; (ii)  $K_2CO_3$ , DMSO/ $H_2O$  (10:1), 130 °C, 18 h; (iii) Pd/C (10%),  $CHCl_3/MeOH$  (1:1), rt, 18 h; (iv)  $BBr_3$ , DCM, 0 °C to rt, 18 h.

by palladium on carbon (Pd/C) gave 1,3-dimethoxy-5-alkylbenzenes (**6–10**). Consequently, 1,3-dimethoxy-5-alkylbenzenes were treated with  $BBr_3$  to generate 5-alkylresorcinols (**11–15**). On the other hand, Grignard reaction of 5-hydroxyvanillin with the long chain alkyl bromides afforded 5-(1-hydroxyalkyl)-3-methoxycatechols (**16** and **17**) (Figure 2). The alcohols were directly hydrogenated using Pd/C in acetic acid without a further dehydration-reduction step to give 3-methoxy-5-alkylcatechols (**18** and **19**). As a result, the



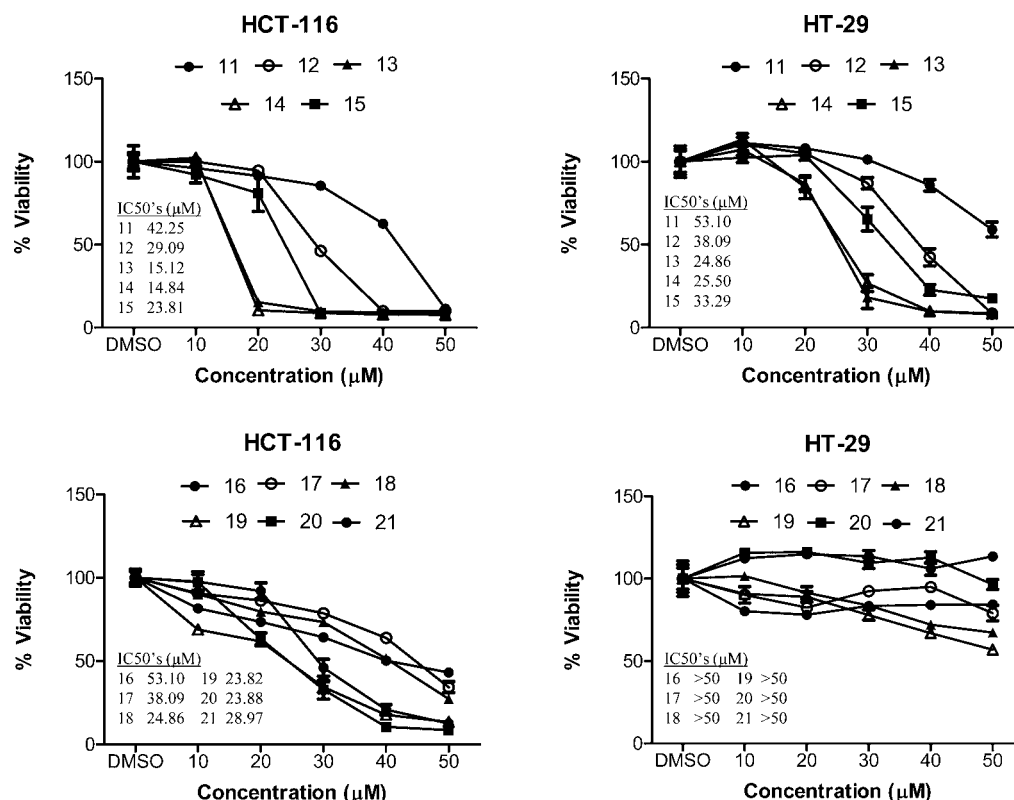
**Figure 2.** Synthesis of 5-alkylpyrogallols analogues (**16–21**). Reagents and conditions: (i) Mg, THF, 60 °C, 5 h; 0 °C to rt, 2 h; (ii) Pd/C (10%), HOAc, 40 °C, 18 h; (iii)  $BBr_3$ , DCM, 0 °C to rt, 2 h.

demethylation of 3-methoxy-5-alkylcatechols by  $BBr_3$  produced 5-alkylpyrogallols (**20** and **21**). All of the synthetic compounds (**1–21**) were fully characterized by their  $^1H$  and/or  $^{13}C$  NMR and further confirmed through LC/MS analysis.

**Human Colon Cancer Cell Growth Inhibition.** We already revealed that increasing the length of the side chain of ARs would diminish the inhibitory effects against human colon cancer cells HCT-116 and HT-29, based on the bioassay data of the naturally occurring ARs C17:0–C25:0 in WB oil.<sup>6</sup> To further delve into the structure–activity relationships between ARs and chemoprevention, 21 ARs and their related analogues (**1–21**) were synthesized and evaluated for growth inhibitory activities against human colon cancer cells (HCT-116 and HT-29) using MTT assays (Figure 3). AR C9:0–C17:0 (**11–15**) showed obvious growth inhibitory activities against human colon cancer cells HCT-116 with  $IC_{50}$  values of 42.25, 29.09, 15.12, 14.84, and 23.81  $\mu M$ , respectively, and also against human colon cancer cells HT-29 with  $IC_{50}$  values of 53.10, 38.09, 24.86, 25.50, and 33.29  $\mu M$ , respectively, indicating the relationship between the side chain lengths of ARs and antitumor activities ( $IC_{50}$ ) was nonlinear. AR C13:0 and C15:0 (**13** and **14**) were found to have the greatest inhibitory effects in both cell lines, while decreasing or increasing the side chain lengths diminished the activities, suggesting that a lipophilic alkyl side chain of 13 or 15 carbons in length yielded the optimal inhibitory activities against cancer cells HCT-116 and HT-29.

Having realized the influence of the lipophilic side chain of ARs on activities, we also investigated the hydroxyl groups on the aromatic ring. All AR dimethyl ethers (**6–10**) showed little to no activities against cancer cells HCT-116 and HT-29, with  $IC_{50}$  values greater than 100  $\mu M$ , versus according ARs (**11–15**), displaying good to moderate inhibition against cancer cells, suggesting the two free *meta*-hydroxyl groups at C-1 and C-3 on the aromatic ring played an important role in contributing to activity. Addition of a third hydroxyl group to the aromatic ring at C-2 in ARs was performed, and the final products, 5-tridecylpyrogallol (**20**) and 5-pentadecylpyrogallols (**21**), as well as their intermediates (**16–19**) were obtained (Figure 2). MTT assays, however, indicated 5-alkylpyrogallols (**20** and **21**) were less active against human colon cancer cells HCT-116 than corresponding AR C13:0 and C15:0 (**13** and **14**), as observed from the  $IC_{50}$  values of 23.88 and 28.97  $\mu M$  for **20** and **21**, respectively, versus  $IC_{50}$  values of 15.12 and 14.84  $\mu M$  for **13** and **14**. In cancer cells HT-29, two 5-alkylpyrogallols **20** and **21** showed no activity, as compared to AR C13:0 and C15:0 (**13** and **14**). In addition, two intermediates **18** and **19**, with methylation at hydroxyl group of C-1 on the aromatic ring, showed no significant inhibitory activity against cancer cells HCT-116 and HT-29.

**Inhibition of Proteasome Activity.** AR C9:0–C17:0 (**11–15**) and their related analogues (**16–21**) were screened for chymotrypsin-like proteasome inhibition (Table 1). AR **11–15** showed moderate inhibitory activity against the proteasome, with  $IC_{50}$  values of 12.63, 11.71, 31.88, 33.36, and 26.61  $\mu M$ , respectively. C11:0 (**12**) was found to have the greatest effect with  $IC_{50}$  of 11.71  $\mu M$ , suggesting that a lipophilic alkyl side chain of 11 carbons in length produced the optimal inhibitory activity against the proteasome. Surprisingly, two 5-alkylpyrogallols **20** and **21** showed around 6-fold more potency against the proteasome, with  $IC_{50}$  values of 5.13 and 4.28  $\mu M$ , respectively, than the corresponding ARs C13:0 and C15:0, indicating a third hydroxyl group on the aromatic ring



**Figure 3.** Cell growth inhibition in human colon cancer cell lines HCT-116 and HT-29 by synthetic ARs and their related analogues (11–21) (AR analogues (1–10) showed IC<sub>50</sub> values >100 µM against human colon cancer cells HCT-116 and HT-29, and their specific data were not shown here).

**Table 1.** Inhibition of the Human 20S Chymotrypsin-like Proteasome Activities by Synthetic ARs and Their Related Analogues (11–21)

compounds <sup>a</sup>	<i>n</i>	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	inhibition of proteasome activities (IC <sub>50</sub> µM)
11(C9:0)	6	H	H	H	12.63 ± 0.91
12(C11:0)	8	H	H	H	11.71 ± 0.94
13(C13:0)	10	H	H	H	31.88 ± 4.51
14(C15:0)	12	H	H	H	33.36 ± 4.22
15(C17:0)	14	H	H	H	26.61 ± 0.85
16	10	CH <sub>3</sub>	OH	OH	15.10 ± 1.72
17	12	CH <sub>3</sub>	OH	OH	11.47 ± 1.17
18	10	CH <sub>3</sub>	OH	H	13.26 ± 1.78
19	12	CH <sub>3</sub>	OH	H	27.49 ± 2.83
20	10	H	OH	H	5.13 ± 0.60
21	12	H	OH	H	4.28 ± 0.82
lactacystin <sup>b</sup>					2.84 ± 0.46

<sup>a</sup>All ARs analogues (11–21) showed apparent inhibitory activities toward human colon cancer cells HCT-116, and further screening for proteasome inhibition was carried out in the current study.

<sup>b</sup>Lactacystin is a known proteasome inhibitor.

increased inhibition. Methylation of the hydroxyl group at C-1 on the aromatic ring in 5-alkylpyrogallols decreased the activity, as observed in analogues 18 and 19 with IC<sub>50</sub> values of 13.26 and 27.49 µM, respectively, versus 5-alkylpyrogallols 20 and 21.

## DISCUSSION

In conjunction with tumor cell growth inhibition assays, investigations of proteasome inhibitors have emerged as viable means of assaying anticancer activity of a given compound. The human 20S proteasome is the catalytic core of a proteinase complex implicated in several critical cellular functions including the ATP-dependent degradation of poly ubiquitinated proteins. The proteasome also controls levels of proteins critical for cell cycle control, including p53, p27, and cyclin B, which has led to the study of proteasome inhibitors as a conduit for cancer therapy.<sup>17</sup> The current study evaluated all synthetic AR analogues (1–21) for growth inhibitory activities against human colon cancer cells HCT-116 and HT-29. Furthermore, the active AR analogues against cancer cells HCT-116 (11–21) were chosen to screen for human 20S proteasome inhibition.

Our MTT assays found that C13:0 and C15:0 (13 and 14) had the greatest inhibitory effects against cancer cells HCT-116 and HT-29 while decreasing or increasing the side chain lengths diminished potency. We also demonstrated that two free *meta*-hydroxyl groups at C-1 and C-3 on the aromatic ring of ARs greatly contributed to antitumor activity, and the replacement of hydroxyl groups with methoxy groups destroyed such an activity, whereas the screening for proteasome inhibitory activity indicated that C11:0 (12) had the greatest effect from AR C9:0 to C17:0. The optimal side chain length shifted slightly to 11 carbons in the proteasome assay instead of the favorable 13 or 15 carbons in lengths for the MTT assays.

As far as alkylpyrogallols and their intermediates (16–21) were concerned, hydroxylation at C-2 (20 and 21) or methylation of the hydroxyl group at C-1 (18 and 19) yielded no significant enhancement of activity against HCT-116 cells

and even destroyed the effects against HT-29 cells, while all 5-alkylpyrogallols analogues showed good chymotrypsin-like proteasome inhibitor, especially two 5-alkylpyrogallols **20** and **21** displaying 6-fold more potency than the corresponding AR C13:0 and C15:0. These examples suggest that the proteasome is the target of analogues **16–21** in p53 wild-type HCT-116 cells, and the lack of growth inhibition in HT-29 cells could be attributed to the mutant p53 distinction of this cell line.<sup>18,19</sup> The tumor suppressor p53 is an important molecule involved in regulating cellular response to exogenous and endogenous stress. Inhibition of proteasome activity would lead to p53 accumulation and subsequent cell apoptosis and/or cell cycle arrest, a result not noted in the proliferative HT-29 cell line.<sup>20</sup> Boronic chalcone derivatives were assayed for such an effect as their abilities to inhibit in vitro cancer activity and disrupt p53 were compared. HCT-116 cells with or without p53 were compared, and it was found that AM114 preferentially killed p53+ cells over p53-/- . Biochemical analysis also showed that AM114 significantly inhibited proteasome activity, and this led to the accumulation of p53 and other ubiquitinated proteins in whole cells. This indicated AM114 was exerting its cytotoxic effect in cancer cells through inhibition of the proteasome.<sup>21</sup> Similar tests can be applied to our compounds **16–21** to elucidate their mechanisms of inhibition in future research.

In conclusion, 15 ARs and their intermediates (**1–15**) were synthesized expediently by the modified Wittig reaction in aqueous media, and six 5-alkylpyrogallols and their analogues (**16–21**) were prepared by the general Grignard reaction. The putative chemopreventative properties of the synthesized AR analogues were explored via assays inhibiting human colon cancer cell growth and chymotrypsin-like proteasome activity. It was shown that AR C13:0 and C15:0 (**13** and **14**) were the most effective inhibitors of cancer cells HCT-116 and HT-29, and decreasing or increasing the length of the side chain decreased potency. An increase in potency was observed from the ARs with two free *meta*-hydroxyl groups at C-1 and C-3 on the aromatic ring than related methylated derivatives, while the introduction of a third hydroxyl group at C-2 (**20** and **21**) on the aromatic ring did not significantly increase inhibition of cancer cell growth. Conversely, the addition of a third hydroxyl group on the aromatic ring greatly increased activity against the chymotrypsin-like activity of the human 20S proteasome. Finally, C11:0 (**12**) was found to have the greatest potency against the proteasome in the series of AR C9:0–C17:0. The current study has clearly shown that certain AR analogues have anticancer activity. The exact mechanisms are still unknown, but the synthesis and subsequent bioassays employed here have supported the important role of ARs in chemoprevention. Many studies have shown that ARs have a variety of bioactivities and can be used as the biomarkers to reflect whole grain wheat and rye intake.<sup>13,22–24</sup> It is worthwhile to further develop ARs as novel cancer preventive agents.

## AUTHOR INFORMATION

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## Notes

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