Synthesis and Cytotoxic Activity of Several Novel N-Alkyl-Plinabulin Derivatives With Aryl Group Moieties

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Abstract
Seven novel N-alkyl-plinabulin derivatives with aryl groups moieties (nitroquinoline, 1,4-dihydroquinoline, 4-methoxybenzene, and 4-chlorobenzene) have been synthesized via aldol condensation and alkylation in one-pot, and tested for their cytotoxicity against 4 cancer cell lines (KB, HepG2, Lu, and MCF7). Compounds \((Z)-3-(((6,8\text{-dimethyl-4-oxo-1,4\text{-dihydroquinolin-2-yl}})\text{methylene})-6-((Z)-4\text{-methoxybenzylidene})-1-\text{prop-2-yn-1-yl})\text{-piperazine-2,5-dione (5a), (Z)-6-((Z)-4\text{-methoxybenzylidene})-1-(prop-2-yn-1-yl)-3-((1,6,8\text{-trimethyl-4-oxo-1,4\text{-dihydroquinolin-2-yl}})\text{methylene})\text{-piperazine-2,5-dione (5b), and (Z)-3-((Z)-4\text{-chlorobenzylidene})-1,4\text{-dimethyl-6-((8\text{-methyl-4-nitroquinolin-2-yl})\text{methylene})\text{-piperazine-2,5-dione (8)) showed strong cytotoxicity against 3 of the cancer cells lines (KB, HepG2 and Lu) with IC}_{50} values ranging from 3.04 to 10.62 \mu M. The quinoline-derived compounds had higher cytotoxic activity than the benzaldehyde derivatives. The successful synthesis of these derivatives offers useful information for the development of more potent vascular disrupting agents based on plinabulin.

Keywords
anticancer, cytotoxicity, plinabulin, VAC, quinoline, N-alkyl

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In recent years, plinabulin (NPI-2358), a synthetic analog of 2,5-diketopiperazine, has been reported to be a promising vascular disrupting agent (VDA) thus serving as an anti-cancer drug. VDAs target microtubules—the essential filamentous structure of eukaryotic cells, particularly by inducing rapid depolymerization of microtubules in highly proliferating tumor selective vascular endothelial cells, thus causing tumor vascular collapse. Historically, notable VDAs, such as colchicine and combretastatin, have been found to be effective anti-cancer drugs. On the other hand, plinabulin has a relatively favorable safety profile while still exhibiting colchicine-like tubulin depolymerizing activity. Several plinabulin derivatives showed high cytotoxicity against different cancer cell lines, for example, benzophenone derivatives were evaluated against human HT-29 colorectal cancer cells, while 5-tert-butyl-substituted imidazole analogs were not highly active. The benzene ring of the benzoyl group could induce additional π–π interaction, which could be beneficial to antiproliferation. Furan-containing derivatives tested against the human lung cancer NCI-H1460 cell line exhibited potent cytotoxic activity at the nanomolar level. Of special interest is a novel colchicine-type antimicrotubule compound: KPU-300, an agent endowed with a 2-pyridyl substituent capable of acting as a potent radiosensitizer. Currently, plinabulin is being evaluated in Phase III clinical trial in combination with docetaxel and in phase I/II clinical trial in combination with nivolumab for stage IIIb/IV non-small cell lung cancer (NCT02504489 and NCT02812667). At the same time, there has also been strong interest in the synthesis of plinabulin derivatives to explore options for heightened effectiveness and new applications. This paper reports 7 novel N-alkyl-plinabulin derivatives with aryl group moieties and their cytotoxic activity against some cancer cell lines in vitro.

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Results and Discussion

The development of plinabulin derivatives with aryl moieties is a research direction of interest due to the ability of these moieties to alter significantly the hydrophobicity and π-π interaction capacity of the compound. In this study, the moieties of choice were nitroquinoline, 1,4-dihydroquinoline, 4-methoxybenzene, and 4-chlorobenzene. The quinoline ring is commonly acknowledged for its antimalarial potency, and the quinine derivative, Chloroquine, has been the most widely used anti-malarial drug since the 1940s. The versatility of this ring system has enabled the synthesis of a large array of derivatives with diverse biological activities, notably the derivatives of quinoline-2-carbaldehyde. Promising quinoline-2-carbaldehyde and 1,4-dihydroquinoline-2-carbaldehyde derivatives previously reported in another study were applied in this work. 4-Methoxybenzene and 4-chlorobenzene, 2 of the most prevalent substructures in anticancer molecules as scanned against the National Cancer Institute’s 60 human tumor cell lines, were also utilized. The successful synthesis of these derivatives offers useful information for the development of more potent VDAs based on plinabulin. Thus, in continuation of our previous work, we have prepared new biological active plinabulin analogs. The general procedures for the synthesis of the 7 novel N-alkyl-plinabulin derivatives with different aryl moieties is outlined in Schemes 1-4.

The N-propargyl-plinabulin derivatives 5 and 6 were synthesized from 1,4-diacetyl-2,5-piperezinedione 1 through 2 steps (Schemes 1 and 2, Supplemental Figures S6-S13). In the first step (Scheme 1), the N-propargylation of the N-acetyl group leads to parallel N-propargylation. The hydrolysis of the N-acetyl group leads to parallel N-propargylation (Scheme 1). In the second step (Scheme 2), the N-propargyl-plinabulin derivatives 5 were prepared via continuous aldol reaction of aldehyde derivatives with N-propargyl-piperidinedione 3 in high yield 91% (Supplemental Figures S1 and 2). In this reaction, K₂CO₃ behaved as a Lewis base to act both for proton promotion and deprotonation. The hydrolysis of the N-acetyl group leads to parallel N-propargylation (Scheme 1). In the second step (Scheme 2), the N-propargyl-plinabulin derivatives 5 were prepared via continuous aldol reaction of aldehyde derivatives with N-propargyl-piperidinedione 3. These reactions were carried out in refluxing DMF.

Similarly, the N-propargyl-N-methyl-plinabulin derivatives 6 were synthesized from starting material 3 via continuous aldol reaction with 4-chlorobenzaldehyde and N-alkylation with methyl iodide in one-pot. This reaction was carried out in refluxing DMF in the presence of K₂CO₃ (Scheme 2). Reaction of 1,4-diacetyl-2,5-diketopiperazine 1 with 4-chlorobenzaldehyde, methyl iodide, and potassium carbonate

Scheme 1. Reagent and conditions: (i) 1.5 equiv. R¹CHO (2) 1.5 equiv. K₂CO₃, 1.5 equiv. propargyl bromide, DMF, r.t, 24 hours; (ii) 1.5 equiv. R¹CHO (2) 1.5 equiv. K₂CO₃, 1.5 equiv. MeI, DMF, r.t, 24 hours.

Scheme 2. Reagent and conditions: (i) 1.5 equiv. R²CHO (4), 1.5 equiv. K₂CO₃, DMF, reflux, 24 hours; (ii) 1.5 equiv. R²CHO (4) 1.5 equiv. K₂CO₃, 1.5 equiv. MeI, DMF, 0→80 °C, 24 hours.
in DMF at room temperature gave product 4a (Scheme 2) in 86% yield (Supplemental Figure S3). Finally, compound 4a was reacted with 4-chlorobenzaldehyde under aldol reaction conditions to provide N-alkyl product 7 in moderate yields (Supplemental Figures S14 and S15). N-propargyl-N-methyl-plinabulin (8) was obtained under aldol reaction conditions of 4a with 8-methyl-4-nitroquinoline-2-carbaldehyde in the presence of methyl iodide (Scheme 3, Supplemental Figures S16 and S17). The moving from starting 4a to 4b in the reaction with 4-methoxylbenzaldehyde provides symmetrical compound 9 (Scheme 4, Supplemental Figures S18 and 19).

The cytotoxicity evaluation results of the novel synthesized plinabulin derivatives against the epidermoid carcinoma cell line (KB), hepatoma carcinoma cell line (HepG2), lung cancer cell line (Lu), and breast carcinoma cell line (MCF-7) are presented in Table 1.

As the results shown in Table 1, compounds 5a, 5b, and 8 showed strong cytotoxicity against 3 of the cancer cells lines (KB, HepG2 and Lu). The structure–activity relationship between N-alkyl-plinabulin and cytotoxic activity was comprehensible because of the higher activity shown by compounds 5a, 5b, and 8 (one aryl = quinoline substituents) as compared to that of compounds 5 c, 6, 7, and 9 (bis aryl = benzene substituents). The quinoline-derived compounds had higher cytotoxic activity than the benzene derivatives, but substitution on the quinoline ring had no clear effect on cytotoxicity, therefore further investigations are required. Moreover, the 1,4-dihydroquinoline derivatives 5a, 5b demonstrated higher potencies against the epidermoid carcinoma cell line (KB), while quinoline derivative 8 exhibited strong cytotoxicity against hepatoma carcinoma (HepG2).

**Experimental**

**General**

All reactions were performed in appropriate oven-dried glass apparatus and under a nitrogen atmosphere. Unless otherwise stated, solvents and chemicals were obtained from commercial sources and used without further purification. Column chromatography was performed using silica gel (60Å, particle size 40-60 μm). NMR spectra were recorded on a Bruker Advance I spectrophotometer (500 MHz). Chemical shifts (δ) are given in parts per million (ppm) and coupling constants (J) in Hertz (Hz). High resolution mass spectra (HRMS) were recorded on either a Q-executive or a Q-TOF2 instrument, and IR spectra on a Perkin Elmer Spectrum Two.

**General Procedure for the Synthesis of Compounds 3, 4a, and 4b**

A solution of 1,4-diacetyl-2,5-piperazinedione (1) (1.0 equiv), 4-benzaldehyde derivatives (1.5 equiv) and propargyl bromide (1.5 equiv) or methyl iodide (1.5 equiv) in dry DMF (10 ml) was cooled to 0 °C. A solution of K₂CO₃ (1.5 equiv) in dry DMF (10 ml) was then added dropwise over 30 minutes. After the addition was complete, the mixture was allowed to reach room temperature and then stirred for 24 hours. Afterwards, the reaction mixture was added to water (50 ml) and extracted with ethyl acetate. The organic phase was washed with water and saturated bile. Drying of the organic phase (MgSO₄), filtration of the drying agent, and evaporation of the solvent in vacuo afforded crude compounds 3 or 4, which were then

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**Scheme 3.** Reagent and conditions: (i) 1.5 equiv. R²CHO (4) 1.5 equiv. K₂CO₃, DMF, reflux, 24 hours; (ii) 1.5 equiv. R²CHO (4) 1.5 equiv. K₂CO₃, 1.5 equiv. MeI, DMF, 0→80 °C, 24 hours.

**Scheme 4.** Reagent and conditions: 1.5 equiv. 4-methoxybenzaldehyde (4) 1.5 equiv. K₂CO₃, 1.5 equiv. MeI, DMF, 0→80 °C, 24 hours.
purified by silica gel column chromatography (n-hexane-EtOAc, 8:2) to obtain pure compound 3, 4a, and 4b.

(Z)-1-Acetyl-3-(4-Methoxybenzylidene)-4-(Prop-2-Yn-1-Yl)piperazine-2,5-Dione (3)
91% yield, yellow solid. 1H NMR (CDCl3, 500 MHz): 7.38 (1H, s); 7.33 (2H, dd, J = 2.0; 7.0 Hz); 6.94 (2H, dd, J = 2.0; 7.0 Hz); 4.53 (2H, s); 4.31 (2H, d, J = 2.5 Hz); 3.85 (3H, s); 2.62 (3H, s); 2.13 (1H, t, J = 2.5 Hz). 13C NMR (CDCl3, 125 MHz): 171.4; 164.8; 164.3; 160.9; 131.4 (2xC); 129.2; 127.7; 127.1; 124.5; 114.4 (2xC); 72.7; 55.4; 45.1; 33.6; 26.5. HR-ESI-MS: Found m/z 313.1179; calcd. for C17H17N2O4: 313.1183 [M + H]+.

(Z)-1-Acetyl-3-(4-Chlorobenzylidene)-4-Methylpiperazine-2,5-Dione (4a)
86% yield, yellow solid. 1H NMR (CDCl3, 500 MHz), δ (ppm): 7.40 (2H, d, J = 8.5 Hz), 7.38 (1H, s), 7.31 (2H, d, J = 8.5 Hz), 4.54 (2H, s), 2.96 (3H, s), 2.62 (3H, s). HR-ESI-MS: Found m/z 293.0687; Calcd. for C14H14ClN2O3: 293.0687 [M + H]+.

(Z)-1-Acetyl-3-(4-Methoxybenzylidene)-4-Methylpiperazine-2,5-Dione (4b)
88% yield, yellow solid. 1H NMR (CDCl3, 500 MHz), δ (ppm): 7.30 (1H, s), 7.28 (2H, dd, J = 2.0, 7.0 Hz), 6.92 (2H, dd, J = 2.0, 7.0 Hz), 4.51 (2H, s), 3.85 (3H, s), 2.96 (3H, s), 2.62 (3H, s). 13C NMR (CDCl3, 125 MHz): 171.5, 165.1, 164.1, 160.6, 131.5 (2xC), 129.8, 125.8, 124.8, 114.1 (2xC), 55.4, 45.3, 34.2, 26.6. HR-ESI-MS: Found m/z 289.1191; Calcd. for C15H17N2O4: 289.1183 [M + H]+.

Table 1. Cytotoxicity Evaluation of Novel N-Alkyl-Plinabulin Derivatives.

| Compounds | KB (µM) | HepG2 (µM) | Lu (µM) | MCF7 (µM) |
|-----------|---------|------------|--------|-----------|
| 5a        | 3.04 ± 0.12 | 5.86 ± 0.27 | 10.62 ± 0.51 | >100       |
| 5b        | 3.87 ± 0.14 | 6.41 ± 0.32 | 6.84 ± 0.35 | >100       |
| 5c        | >100 | >100 | >100 | >100 |
| 6         | >100 | >100 | >100 | >100 |
| 7         | >100 | >100 | >100 | >100 |
| 8         | 4.85 ± 0.21 | 5.14 ± 0.23 | 7.48 ± 0.36 | >100       |
| 9         | >100 | >100 | >100 | >100 |
| Ellipticine | 1.30 ± 0.08 | 1.30 ± 0.08 | 1.79 ± 0.12 | 2.11 ± 0.20 |

Ellipticine was used as a positive control.

After the addition was complete, the mixture was heated to 80 °C and stirred for 24 hours. Afterwards, the reaction mixture was added to water (50 ml) and extracted with ethyl acetate. The organic phase was washed with water and saturated bine. Drying of the organic phase (MgSO4), filtration of the drying agent, and evaporation of the solvent in vacuo afforded crude compounds 5 or 7, which were then purified by silica gel column chromatography (n-hexane-EtOAc, 82 or 83:15, v/v) to obtain pure compound 5a, 5b, 5c and 7.

(Z)-3-((6,8-Dimethyl-4-Oxo-1,4-Dihydroquinolin-2-Yl)methylene)-6-((Z)-4-Methoxybenzylidene)-1-(Prop-2-Yn-1-Yl)piperazine-2,5-Dione (5a)
38% yield, yellow solid. 1H NMR (CDCl3, 500 MHz), δ (ppm): 8.00 (2H, d, J = 8.5 Hz), 7.70 (1H, s), 7.37 (1H, s), 7.30 (2H, d, J = 8.5 Hz), 6.63 (1H, s), 6.38 (1H, s), 4.96 (2H, d, J = 2.5 Hz), 3.97 (3H, s), 2.77 (3H, s), 2.64 (3H, s), 2.22 (1H, t, J = 2.5 Hz), 2.42 (3H, s). 13C NMR (CDCl3, 125 MHz): 162.2, 159.7, 154.4, 152.8, 145.0, 135.8, 135.8, 135.6, 134.9, 133.5, 133.1, 127.1, 125.0, 119.7, 118.4, 105.8, 101.9, 77.9, 75.2, 55.7, 54.5, 21.7, 19.0. HR-ESI-MS: Found m/z 454.1753; Calcd. for C27H24N3O4: 454.1761 [M + H]+.

(Z)-6-((Z)-4-Methoxybenzylidene)-1-(Prop-2-Yn-1-Yl) methylene)piperazine-2,5-Dione (5b)
43% yield, yellow solid. 1H NMR (CDCl3, 500 MHz), δ (ppm): 7.79 (1H, s), 7.44 (1H, s), 7.42 (1H, s), 7.29 (2H, d, J = 8.5 Hz), 6.91 (2H, d, J = 8.5 Hz), 6.87 (1H, s), 6.76 (1H, s), 4.48 (2H, d, J = 2.5 Hz), 4.05 (3H, s), 3.84 (3H, s), 2.83 (3H, s), 2.49 (3H, s), 2.14 (1H, t, J = 2.5 Hz). 13C NMR (CDCl3, 125 MHz), δ (ppm): 162.4, 160.0, 159.1, 145.1, 135.9, 135.7, 133.1, 131.8, 130.9 (2xC), 127.1, 126.0, 122.3, 119.9, 118.4, 114.0 (2xC), 113.2, 102.2, 101.1, 77.7, 72.4, 55.7, 55.3, 35.6, 21.7, 19.1. HR-ESI-MS: Found m/z 454.1753; Calcd. for C28H26N3O4: 454.1761 [M + H]+.

General Procedure for the Synthesis of Compounds 5a-C and 7
A solution of compound 3 (1.0 equiv) or compound 4a (1.0 equiv) and 4-benzaldehyde derivatives (1.5 equiv) in dry DMF (5 ml) was cooled to 0 °C. A solution of K2CO3 (1.5 equiv) in dry DMF (5 ml) was then added dropwise over 30 minutes.
3-((Z)-4-Chlorobenzylidene)-6-((Z)-4-Methoxybenzylidene)1-(Prop-2-Yn-1-Y1)piperazine-2,5-Dione (6)

35% yield, yellow solid. IR (KBr) ν_max (cm⁻¹): 3336, 3072, 2926, 2851, 2132, 1734, 1683, 1700, 1613, 1583, 1558, 1336, 1139, 1088, 845. ¹H NMR (DMSO-d₆, 500 MHz), δ (ppm): 7.43 (2H, d, J = 7.5 Hz, 7.36 (2H, d, J = 7.5 Hz), 7.27 (1H, s), 7.24 (2H, d, J = 8.5 Hz), 7.00 (2H, d, J = 8.5 Hz), 6.98 (1H, s), 4.72 (2H, d, J = 2.5 Hz), 3.84 (3H, s), 2.53 (1H, t, J = 2.5 Hz). ¹³C NMR (DMSO-d₆, 125 MHz), δ (ppm): 159.6, 159.4, 157.8, 134.7, 131.4, 131.0 (2xC), 129.8 (2xC), 129.7 (2xC), 129.2, 126.8, 126.4, 121.3, 115.5, 114.8 (2xC), 78.1, 75.9, 55.8, 46.6. HR-ESI-MS: Found m/z 378.1579, calcd. for C₂₃H₂₀ClN₄O₄: 378.1574 [M + H]⁺.

3,6-Bis((Z)-4-Chlorobenzylidene)-1-Methylpiperazine-2,5-Dione (7)

36% yield, yellow solid. ¹H NMR (CDCl₃, 500 MHz), δ (ppm): 7.42-7.36 (6H, overlapped), 7.23 (1H, s), 7.22 (2H, d, J = 8.5 Hz), 7.00 (1H, s), 2.99 (3H, s). ¹³C NMR (CDCl₃, 125 MHz), δ (ppm): 159.2 (2xC = O amide), 134.8, 134.6, 132.2, 131.1, 130.9, 129.9 (2xC), 129.8 (2xC), 128.6 (2xC), 128.0 (2xC), 126.1, 119.7, 116.2, 36.7. HR-ESI-MS: Found m/z 373.0521, calcd. for C₁₉H₁₅Cl₂N₂O₂: 373.0505 [M + H]⁺.

General Procedure for the Synthesis of Compounds 6, 8, and 9

A solution of compound 3 (1.0 equiv.) or compound 4a,b (1.0 equiv.), 4-benzaldehyde derivatives (1.5 equiv.) or 8-methyl-4-nitroquinoline-2-carbaldehyde (1.5 equiv.) and methyl iodide (1.5 equiv) in dry DMF (10 ml) was cooled to 0 °C. A solution of K₂CO₃ (1.5 equiv.) in dry DMF (5 ml) was then added dropwise over 30 minutes. After the addition was complete, the mixture was heated to 100 °C and stirred for 3 hours. Afterwards, the reaction mixture was added to water (50 ml) and extracted with ethyl acetate. The organic phase was washed with water and saturated brine. Drying of the organic phase (MgSO₄), filtration of the drying agent, and evaporation of the solvent in vacuo afforded the crude compounds, which were then purified by silica gel column chromatography (n-hexane-EtOAc, 8:2 or 85:15, v/v) to obtain pure compound 6, 8, and 9.

3-((Z)-4-Chlorobenzylidene)-6-((Z)-4-Methoxybenzylidene)-4-Methyl-1-(Prop-2-Yn-1-Y1)piperazine-2,5-Dione (6)

33% yield, yellow solid. IR (KBr) ν_max (cm⁻¹): 3330, 3076, 2926, 2855, 2135, 1715, 1680, 1668, 1600, 1580, 1551, 1333, 1169, 1076, 845. ¹H NMR (DMSO-d₆, 500 MHz), δ (ppm): 7.39 (2H, dd, J = 2.0, 8.0 Hz), 7.37 (1H, s), 7.23 (2H, d, J = 8.0 Hz), 7.00 (2H, dd, J = 2.0, 8.0 Hz), 6.97 (2H, d, J = 8.0 Hz), 6.96 (1H, s), 4.75 (2H, d, J = 2.5 Hz), 3.84 (3H, s), 3.03 (3H, s), 2.55 (1H, t, J = 2.5 Hz). ¹³C NMR (DMSO-d₆, 125 MHz), δ (ppm): 159.9, 159.7, 157.7, 131.0 (2xC), 130.5, 129.5 (2xC), 127.0, 124.6, 120.6, 119.9, 117.2, 116.0, 114.9 (2xC), 114.7 (2xC), 78.1, 75.9, 55.8, 46.6, 36.6. HR-ESI-MS: Found m/z 407.1173, calcd. for C₂₃H₂₀ClN₂O₄: 407.

(Z)-3-((Z)-4-Chlorobenzylidene)-1,4-Dimethyl-6-((8-Methyl-4-Nitroquinolin-2-Yl)methylene)piperazine-2,5-Dione (8)

29% yield, yellow solid. IR (KBr) ν_max (cm⁻¹): 3047, 2924, 2855, 1735, 1680, 1616, 1583, 1487, 1334, 1165, 1089, 819, 756. ¹H NMR (DMSO-d₆, 500 MHz), δ (ppm): 7.02 (1H, s), 7.45 (1H, d, J = 8.0 Hz), 7.63 (1H, dd, J = 1.0, 8.0 Hz), 7.38-7.50 (5H, m, overlap), 7.21 (1H, s), 7.11 (1H, s), 3.02 (3H, s), 2.88 (3H, s), 2.67 (3H, s). ¹³C NMR (DMSO-d₆, 125 MHz), δ (ppm): 160.8, 160.6, 151.8, 146.7, 141.3, 137.1, 134.7, 132.2, 132.2, 131.6 (2xC), 131.2, 128.3 (2xC), 128.0, 124.4, 123.7, 121.4, 119.1, 118.6, 117.2, 36.0, 35.3, 17.7. HR-ESI-MS: Found m/z 463.1175, calcd. for C₂₄H₂₀ClN₄O₄: 463.1168 [M + H]⁺.

Cell culture and cell viability assay. The synthesized compounds were evaluated for their cytotoxicity against the 4 hoursuman cancer cell lines, epidermoid carcinoma (KB), hepatoma carcinoma (HepG2), lung cancer cell line (La) and breast carcinoma (MCF-7) obtained from the American Type Culture Collection (USA). The cells were grown in RPMI 1640 medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 µg/mL streptomycin at 37 °C in a humidified atmosphere (95% air and 5% CO2). Exponentially growing cells were used throughout the experiments. The inhibitory effects of the compounds on the growth of the cancer cell lines were determined by measuring their metabolic activity using a 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) assay. Briefly, the cell lines (1 × 10⁵ cells/mL) were treated for 3 days with a series of concentrations of the compounds (in DMSO): 0.125, 0.5, 2.0, 8.0, 32.0, and 128.0 µg/mL. After incubation, 0.1 mg MTT solution (50 µL of a 2 mg/mL solution) was added to each well, and the cells were then incubated at 37 °C for 4 hours. The plates were centrifuged at 1000 rpm for 10 minutes at room temperature, and the medium was then carefully aspirated. Dimethyl sulfoxide (150 µL) was added to each well to dissolve the formazan crystals. The plates were read immediately at 540 nm on a microplate reader (TECAN GENIOUS). All the experiments were performed 3 times, and the mean absorbance values were calculated. The results are expressed as the percentage of inhibition that produced a reduction in the absorbance by the treatment of the compounds compared to the untreated controls. A dose-response curve was generated, and the inhibitory
concentration of 50% (IC50) was determined for each compound, as well as for each cell line.

Conclusions
In conclusion, 7 novel N-alkyl-plinabulin derivatives with aryl group moieties (nitroquinoline, 1,4-dihydroquinoline, 4-methoxybenzene, and 4-chlorobenzene) were successfully synthesized via aldol condensation alkylation in one-pot. Preliminary cytotoxicity evaluation of these compounds against the KB, HepG2, Lu, and MCF7 cell lines demonstrated diverse levels of effectiveness and selectivity, thus providing useful information for the development of more potent VDAs based on plinabulin. Compounds 5a, 5b, and 8 showed strong cytotoxicity against three of the cancer cells lines (KB, HepG2 and Lu), and the quinoline-derived compounds have higher cytotoxic activity than the benzaldehyde derivatives.

Declaration of Conflicting Interests
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Supplemental Material
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