Design, Synthesis, and Cytotoxic Evaluation of Novel Lupane Triterpenoid Derived Hydroxamates

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Abstract
A series of new hydroxamate derivatives of lupane triterpenoids has been designed and successfully synthesized. The synthesized compounds were evaluated for their in vitro antitumor activity using the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide-based assay against the human cancer cell lines KB and HepG2. Most of these derivatives possess at least moderate cytotoxic activity and the hydroxamate derivative compounds 3c, 3e, 7a, and 15b could be lead compounds for further optimization to develop novel anticancer agents.

Keywords
triterpenoid, betulin, betulinic acid, hydroxamate, cytotoxic activity

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Introduction
Cancer is a dangerous disease which caused about 9.6 million deaths in 2018.1 In recent decades, there has been a lot of research and development on new anticancer drugs, but there is still an urgent need for the further search and development of new improved drugs to target the inhibition of cancer cells. Many studies have shown that the molecular pathways are related to the cellular epigenome,2-4 and their dysregulation has an effect on cancer cells.5-7

Histone deacetylases (HDACs) are enzymes that are known to be overexpressed in cancer cell lines,8-13 and hydroxamate has been recognized as a zinc-binding group in the molecular structure of HDAC inhibitors. Hydroxamic acids have also been considered in the design for cancer treatment.14-17 Lead compounds of this class were approved by the Food and Drug Administration for cancer treatment, such as Zolinza (SAHA) (Figure 1) for the treatment of cutaneous T-cell lymphoma,18 Panobinostat for multiple myeloma in 2006, and Belinostat (PXD101) for peripheral T-cell lymphoma in 2014; CUDC-101, Trichostatin A, and Oxamflatin (Figure 1) are currently undergoing clinical trials against different cancer cell lines.21 Therefore, hydroxamate derivatives have been designed and synthesized as a promising class of anticancer agents.22,23

Some lupane triterpenoids, such as betulin, betulinic acid, and its derivatives, have attracted considerable attention in recent years because of their activities against cancer cell lines.24-28 Reports have been rather limited so far on the combination of a triterpenoid and hydroxamate into single molecules, as well as their screening for anticancer activity. After considering the biological potential of such compounds, we decided to construct novel triterpenoid derived hydroxamates with the expectation of their potential use as anticancer agents. Therefore, in this study, a series of novel lupane derived hydroxamates were designed and synthesized at the C-28 position of the triterpenoid. The synthesized compounds were evaluated for their cytotoxic activity against 2 human cancer cell lines (KB and HepG2).

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Results and Discussion

Chemistry

In this study, the conjugates of betulin derivatives with either hydroxylammonium chloride (NH$_2$OH.HCl) or N$_2$O-dimethylhydroxylammonium chloride (HNMeOMe.HCl) 3a-f were synthesized following the synthetic procedure illustrated in Scheme 1. In the first step, betulin (I) was reacted with acid anhydride in dichloromethane in the presence of triethylamine (TEA) at room temperature for 24 hours to give the key intermediates 28-O-acylbetulins 2a-d, which were then coupled with either NH$_2$OH.HCl or HNMeOMe.HCl in dimethylformamide (DMF) in the presence of 1 equiv benzotriazol-1-yl-oxy-tris(dimethylamino)phosphonium hexafluorophosphate (BOP) and 2 equiv 4-dimethylaminopyridine (DMAP) at room temperature for 15 hours to give the targeted compounds 3a-f.

Analogously, the reaction of 3$\beta$ acetoxy-21-oxolup-18-ene-28-oic acid (4) and betulinic acid (8) with 6-aminohexanoic acid in DMF in the presence of BOP/DMAP (1:2) at room temperature for 24 hours gave the acid intermediates 6 and 9 (Scheme 2, Scheme 3). Then, compounds 4, 6, and 9 were coupled with NH$_2$OH.HCl and HNMeOMe.HCl in the presence...
of BOP and DMAP to afford the targeted compounds 5a, b, 7a, b, and 10a, b, respectively (Scheme 2, Scheme 3, Supplemental Figures S1-S12).

Finally, the synthesis of hydroxamates 15a, b is illustrated in Scheme 4. Dicarboxylic acid 11, isolated from Schefflera octophylla (Ivy tree), was evaluated as the template for derivatization. Initial Jones oxidation (CrO₃) of acid 11 using sulfuric acid (H₂SO₄) in acetone resulted in the corresponding acid intermediate, which was subjected to decarboxylation to provide ketone 12 (Scheme 4). Next, reduction of 12 using sodium tetrahydroborate gave compound 13, which was then reacted with 6-aminohexanoic acid in the presence of a mixture BOP/DMAP (1:2) in DMF at room temperature for 24 hours to give the acid intermediate 14 (Scheme 4). Similarly, acids 6, 9, and 14 were treated with either NH₂OH.HCl or HNMeOMe.HCl under the same reaction conditions to afford the targeted compounds 15a, b in good yield. The structures of the products were determined by spectroscopic methods, such as ¹H nuclear magnetic resonance (NMR), ¹³C NMR, and infrared (IR), and mass spectrometry (MS) analysis.

**Biology**

The newly prepared compounds were subjected to cytotoxic evaluation in order to assess their biological relevance. Betulin derived hydroxamates 3a-f, the 3β-acetoxy-21-oxolup-18-ene-28-oic acid derived hydroxamates 5a, b, 7a, b, and the betulinic acid derived hydroxamates 10a, b, as well as the hydroxamates 15a, b were evaluated in vitro for their cytotoxic activity by using 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay against 2 human cancer cell lines (KB, HepG2); the results of these assays are summarized in Table 1. The results indicated that most of the tested derivatives possess at least moderate cytotoxic activity. Betulin derived hydroxamates 3c and 3e showed high cytotoxicity against both cell lines with half-maximal inhibitory concentration (IC₅₀) values ranging from 8.50 to 9.13 µM. In particular, compounds 7a and 15b showed good cytotoxicity against KB and HepG2 cell lines.

| Entry | Compounds (yield, %) | IC₅₀ (µM) KB | IC₅₀ (µM) HepG2 |
|-------|----------------------|-------------|----------------|
| 1     | 3a (57)              | 29.8        | 23.4           |
| 2     | 3b (63)              | 55.7        | 93.2           |
| 3     | 3c (61)              | 8.5         | 67.1           |
| 4     | 3d (61)              | 87.7        | 65.8           |
| 5     | 3e (63)              | 63.8        | 9.1            |
| 6     | 3f (62)              | 58.1        | 113.7          |
| 7     | 5a (59)              | 23.1        | 24.7           |
| 8     | 5b (60)              | 50.1        | 141.0          |
| 9     | 7a (53)              | 3.1         | 4.2            |
| 10    | 7b (64)              | 68.2        | 137.1          |
| 11    | 10a (61)             | 43.7        | 64.4           |
| 12    | 10b (65)             | 103.8       | 106.7          |
| 13    | 15a (54)             | 35.1        | 45.1           |
| 14    | 15b (71)             | 5.2         | 6.2            |
| 16    | Ellipticine*         | 1.3         | 1.5            |

IC₅₀, half-maximal inhibitory concentration.

*Positive control compound.
with IC\textsubscript{50} values of 3.06 µM and 4.22 µM for 7a, and 5.18 µM and 6.21 µM for 15b, respectively, pointing to the potential interest in this new class of hybrid molecules. However, comparing the cytotoxicity of compounds 3a, 3c, 5a, 7a, and 10a (RCONHOH) with compounds 3b, 3d, 5b, 7b, and 10b (RCONMeOMe), it is clear that the hydroxamic acid analogs 3a, 3c, 5a, 7a, and 10a exhibited more potent cytotoxic effects than the corresponding hydroxamates 3b, 3d, 5b, 7b, and 10b. In addition, comparing the cytotoxicity of hydroxamates via an ester bridge (3a, 3c) with hydroxamates via an amide bridge (7a, 15b) showed that the cytotoxicity of compounds 7a and 15b was higher than that of compounds 3a and 3c.

**Conclusion**

To sum up, 14 new hydroxamate derivatives of lupane triterpenoids have been designed and successfully synthesized. The synthesized compounds were evaluated using the MTT-based assay against the 2 human cancer cell lines (KB and HepG2). The results indicate that most of these derivatives possess at least moderate cytotoxic activity and compounds (3c, 3e, 7a) with hydroxamates via an amide bridge (4-(hydroxyamino)-4-oxobutanoate (7a), and 4-(hydroxyamino)-4-oxobutanoate (7a)). White solid. Yield 57%.

**Experimental**

**General: Chemistry**

The reagents were commercially available and used without further purification. Analytical thin-layer chromatography (TLC) was performed on HSGF 254 (0.15, 0.2 mm thickness). Melting points were measured on a Boetius apparatus and are uncorrected. Column chromatography was performed using silica gel (60 Å, particle size 40-60 µm). NMR spectra were recorded on a BrukerAvance (500 MHz) spectrophotometer. Chemical shifts (\(\delta\)) are given in parts per million (ppm) and coupling constants (\(J\)) in Hertz (Hz). Liquid chromatography (LC)–MS analysis was conducted on a Q-Exactive focus instrument. IR spectra were obtained using a Spectrum Two Perkin Elmer spectrophotometer using Nujol and potassium bromide (KBr) pellets.

**Syntheses**

**General procedure for preparation of conjugates between the triterpenoid and \(\text{NH}_2\text{OH.HCl}\) and \(\text{HNMeOMe.HCl}\) 3a-f, 5a, 7a,b, 10a,b, and 15a,b.** To a solution of the corresponding acid 2a-d, 4, 6, 9, and 14 (1.0 mmol) in DMF (5 mL) was added BOP (1.0 mmol) and DMAP (2.0 mmol); the mixture was stirred for 5-10 min at room temperature. \(\text{NH}_2\text{OH.HCl}\) (or \(\text{HNMeOMe.HCl}\) (2.0 mmol) was then added, and the mixture stirred at room temperature until the starting material had been consumed completely (15 hours). Afterward, water was added to the mixture, which was acidified to pH 5 with a solution of HCl 10% to induce precipitation. The crystals were removed by filtration and washed with water. The crude product was redissolved in ethyl acetate (EtOAc, 20 mL) to which water (20 mL) was added. The aqueous phase was extracted with EtOAc (20 mL \(\times\) 3). Drying (sodium sulfate), filtration of the drying agent, and removal of the solvent in vacuo afforded targeted conjugates 3a-f, 5a,b, 7a,b, 10a,b, and 15a,b respectively, which were further purified by column chromatography on silica gel using methanol in dichloromethane (0%–10%).

\(((\text{1R,3aS,5aR,5bR,9S,11aR)-9-hydroxy-5a,5b,8,8,11a-pentamethylnaphthalene-1-(prop-1-en-2-yl)-3-hydroxy-4-oxo-1-cyclopenta[a,chrysene-3a,3y]methyl-4-(hydroxyamino)-4-oxobutanoate (3a)\). White solid. Yield 57%.**

Mp: 227-229°C.

IR (KBr) cm\(^{-1}\): 3367, 2958, 2879, 1706, 1698, 1567, 1454, 1367, 1251, 1138, 1093, 883.

\(1^H\) NMR (500 MHz, dimethylsulfoxide [DMSO]-d\(_6\)) \(\delta\) (ppm): 10.39 (1H, s), 8.69 (1H, s), 4.69 (1H, s), 4.55 (1H, s), 4.27 (1H, \(d, J = 11.0\) Hz), 4.25 (1H, \(d, J = 11.0\) Hz), 3.75 (1H, \(d, J = 11.5\) Hz), 2.97-2.94 (1H, m), 2.23 (2H, \(t, J = 7.5\) Hz), 1.93-1.89 (1H, m), 1.72-1.70 (2H, m), 1.64-1.59 (5H, m), 1.56-1.53 (2H, m), 1.46-1.42 (2H, m), 1.36-1.29 (5H, m), 1.25-1.16 (6H, m), 1.07-1.05 (2H, m), 1.03 (3H, s), 0.97 (3H, s), 0.93 (3H, s), 0.87 (3H, s), 0.76 (3H, s).

\(1^C\) NMR (125 MHz, DMSO-d\(_6\)) \(\delta\) (ppm): 172.8, 168.3, 150.1, 110.2, 89.4, 77.2, 55.1, 50.4, 48.8, 47.3, 46.3, 43.4, 42.4, 40.6, 39.1, 39.0, 38.7, 38.5, 37.3, 36.9, 34.3, 34.1, 29.4, 29.1, 28.3, 27.2, 26.8, 25.0, 20.5, 19.0, 18.2, 16.1, 16.0, 15.9, 14.7.

LC–MS/MS: Found \(m/z\) 558.3437 [M + H]\(^+\), Calc. for \(C_{36}H_{59}NO_{5}\): 586.2869.

Mp: 237-239°C.

IR (KBr) cm\(^{-1}\): 2940, 2869, 1733, 1667, 1534, 1455, 1360, 1278, 1174, 1045, 883.

\(1^H\) NMR (500 MHz, deuterated chloroform [CDCl\(_3\)]) \(\delta\) (ppm): 4.68 (1H, s), 4.58 (1H, s), 4.27 (1H, \(d, J = 11.0\) Hz), 3.89 (1H, \(d, J = 11.0\) Hz), 3.72 (3H, s), 3.17 (3H, s), 2.76 (2H, s), 2.70-2.66 (2H, m), 2.45-2.41 (1H, m), 1.98-1.94 (1H, m), 1.86-1.83 (1H, m), 1.80-1.75 (2H, m), 1.71-1.53 (7H, m), 1.42-1.39 (4H, m), 1.27-1.24 (6H, m), 1.02 (3H, s), 0.98 (3H, s), 0.96 (3H, s), 0.84 (3H, s), 0.76 (3H, s).

\(1^C\) NMR (125 MHz, CDCl\(_3\)) \(\delta\) (ppm): 173.3, 171.1, 150.2, 109.7, 78.9, 62.8, 61.2, 55.3, 50.3, 48.8, 47.7, 46.3, 42.7, 40.8, 38.8, 37.6, 37.1, 34.5, 34.2, 29.8, 29.7, 29.6, 28.7, 27.9, 27.3, 27.1, 25.2, 20.8, 19.1, 18.3, 16.1, 16.0, 15.3, 14.7, 14.2.

LC–MS/MS: Found \(m/z\) 586.2869 [M + H]\(^+\), Calc. for \(C_{36}H_{59}NO_{5}\): 586.2866.

\(((\text{1R,3aS,5aR,5bR,9S,11aR)-9-hydroxy-5a,5b,8,8,11a-pentamethylnaphthalene-1-(prop-1-en-2-yl)-3-hydroxy-4-oxo-1-cyclopenta[a,chrysene-3a,3y]methyl-5-(hydroxyamino)-5-oxocaptonatoate (3c)\). White solid. Yield 61%.

Mp: 217-219°C.
IR (KBr) cm⁻¹: 3292, 2987, 2876, 1743, 1697, 1561, 1455, 1389, 1238, 1109, 1087, 776.

1H NMR (500 MHz, DMSO-d6) δ (ppm): 10.35 (1H, s), 8.69 (1H, s), 4.69 (1H, s), 4.55 (1H, s), 4.27 (1H, d, J = 11.0 Hz), 3.85 (1H, d, J = 11.0 Hz), 3.76 (2H, s), 3.54 (2H, m), 2.76 (2H, m), 2.73-2.67 (1H, m), 2.45-2.42 (4H, m), 1.97-1.93 (2H, m), 1.85-1.82 (1H, m), 1.81-1.74 (2H, m), 1.70-1.51 (6H, m), 1.41-1.37 (4H, m), 1.68 (3H, s), 1.02 (3H, s), 0.97 (3H, s), 0.93 (3H, s), 0.87 (3H, s), 0.78 (3H, s), 0.68-0.67 (1H, m).

13C NMR (125 MHz, DMSO-d6) δ (ppm): 172.3, 171.6, 150.1, 109.3, 77.9, 62.5, 60.2, 54.2, 51.3, 48.7, 47.2, 46.4, 41.7, 40.3, 37.8, 38.6, 36.5, 37.1, 34.3, 34.1, 29.7, 29.5, 29.2, 28.7, 27.6, 27.4, 27.1, 25.3, 20.7, 19.2, 18.5, 16.7, 16.0, 15.2, 14.6, 14.3.

LC–MS/MS: Found m/z 572,4257 [M + H]+, Calc. for C35H57NO5: 572,4237.

IR (KBr) cm⁻¹: 3074, 2963, 2866, 1733, 1711, 1510, 1454, 1384, 1291, 1185, 1020, 881.

1H NMR (500 MHz, CDCl3) δ (ppm): 5.76-5.74 (1H, m), 5.68-5.65 (1H, m), 4.67 (1H, s), 4.57 (1H, s), 3.90-3.83 (1H, m), 3.74 (3H, s), 3.40-3.36 (1H, m), 3.16 (3H, s), 2.95-2.88 (1H, m), 2.81-2.73 (1H, m), 2.42-2.37 (4H, m), 2.04 (1H, s), 1.97-1.94 (1H, m), 1.86-1.77 (2H, m), 1.71-1.64 (5H, m), 1.62-1.51 (4H, m), 1.42-1.34 (3H, m), 1.29-1.24 (6H, m), 0.93 (3H, s), 0.91-0.86 (3H, m), 0.81 (3H, s), 0.75 (3H, s), 0.68-0.67 (1H, m).

13C NMR (125 MHz, CDCl3) δ (ppm): 174.4, 174.3, 150.2, 124.9, 109.7, 78.9, 62.8, 55.3, 50.4, 48.8, 47.7, 46.3, 42.7, 40.8, 39.9, 39.6, 38.7, 37.6, 37.1, 35.8, 35.6, 34.5, 29.8, 29.7, 29.6, 27.9, 27.4, 27.1, 27.0, 26.2, 26.1, 25.2, 19.1, 18.3, 16.1, 16.0, 15.3, 14.7, 14.2.

LC–MS/MS: Found m/z 638,4221 [M + H]+, Calc. for C48H63NO5: 638,4279.

IR (KBr) cm⁻¹: 3435, 2948, 2856, 1735, 1647, 1673, 1381, 1246, 1025, 825.

1H NMR (500 MHz, DMSO-d6) δ (ppm): 10.31 (1H, s), 8.75 (1H, s), 4.38 (1H, dd, J = 11.5, 5.0 Hz), 3.17-3.12 (1H, m), 2.74 (1H, d, J = 11.0 Hz), 2.44 (1H, d, J = 13.5 Hz), 2.20-2.05 (2H, m), 1.99 (4H, s), 1.84-1.80 (1H, m), 1.70-1.60 (2H, m), 1.55-1.53 (2H, m), 1.47-1.38 (2H, m), 1.35-1.31 (3H, m), 1.25-1.23 (2H, m), 1.17 (5H, d, J = 6.5 Hz), 1.10 (3H, d, J = 6.5 Hz), 1.06-1.02 (3H, m), 0.98 (3H, s), 0.88 (3H, s), 0.83 (3H, s), 0.84-0.82 (1H, m), 0.79 (6H, s).

13C NMR (125 MHz, DMSO-d6) δ (ppm): 206.5, 171.9, 170.3, 170.0, 145.3, 79.8, 54.5, 51.7, 50.2, 48.1, 44.7, 44.9, 39.0, 37.8, 37.3, 36.6, 34.3, 33.1, 28.7, 27.6, 26.9, 24.6, 23.3, 20.9, 20.8, 20.1, 19.7, 17.6, 16.5, 16.4, 16.3, 15.6.

LC–MS/MS: Found m/z 528,4064 [M + H]+, Calc. for C31H49NO5: 528,4089.
(3αR,5αR,5bR,9S,11αR)-3α-((6-(hydroxyamino)-6-oxohexyl)carbamoyl)-1-isopropyl-5a,5b,8,8,11α-pentamethyl-2-oxo-3,3a,4,5,5a,5b,64,65,66-hexahydro-3aH-cyclopenta[a]chrysene-3a-carboxamide (10a). White solid. Yield 61%.

IR (KBr) cm⁻¹: 3371, 2934, 2866, 1707, 1636, 1530, 1452, 1388, 1247, 1043, 847.

C₁₃N₂O₆: 228.2930. LC–MS/MS: Found $m/z$ 285.4596 [M + H]⁺, Calc. for C₁₃H₁₄O₆N₂: 285.4553.
LC–MS/MS: Found $m/z$ 613.3348 [M + H]$^+$, Calc. for C$_{38}$H$_{64}$N$_2$O$_4$: 613.3339.

$(1R,3aS,5aR,5bR,8R,9S,11aS)-9$-hydroxy-N-(6-(hydroxymethyl)-6-oxobicyclo[3.2.1]octa-2,3,5,6-tetrahydro-3aH-cyclopenta[a]chrysene-3a-carboxamide (15a). White solid. Yield 54%. Mp: 205-207°C.

IR (KBr) cm$^{-1}$: 3362, 2976, 2953, 1714, 1687, 1538, 1475, 1321, 1269, 1108, 1089, 765.

$^1$H NMR (500 MHz, DMSO-d$_6$) $\delta$ (ppm): 10.30 (1H, s), 8.61 (1H, s), 7.50 (1H, $J = 5.0$ Hz), 4.64 (1H, s), 4.53 (1H, s), 4.28 (1H, $d$, $J = 6.0$ Hz), 3.07-3.00 (3H, m), 2.12 (2H, m), 1.92 (2H, $t$, $J = 7.0$ Hz), 1.75-1.70 (3H, m), 1.62 (3H, s), 1.48-1.42 (7H, m), 1.33 (3H, s), 1.23 (3H, s), 1.89 (3H, s), 1.16 (3H, s), 1.12-0.98 (2H, m), 0.90 (6H, s), 0.83 (3H, s), 0.79 (3H, $d$, $J = 1.5$ Hz), 0.65 (3H, s).

$^{13}$C NMR (125 MHz, DMSO-d$_6$) $\delta$ (ppm): 175.7, 169.5, 151.1, 144.1, 109.6, 89.6, 77.3, 55.4, 55.3, 50.1, 46.6, 42.4, 40.7, 38.9, 38.7, 37.2, 37.1, 34.5, 32.7, 30.8, 29.5, 29.3, 28.6, 27.0, 26.5, 25.3, 21.0, 19.5, 18.4, 16.4, 16.3, 16.2, 14.8.

LC–MS/MS: Found $m/z$ 571.4433 [M + H]$^+$, Calc. for C$_{35}$H$_{58}$N$_2$O$_4$: 571.4471.

$(1R,3aS,5aR,5bR,8R,9S,11aS)-9$-hydroxy-N-(6-(methoxy(methyl)amino)-6-oxobicyclo[3.2.1]octa-2,3,5,6-tetrahydro-3aH-cyclopenta[a]chrysene-3a-carboxamide (15b). White solid. Yield 71%. Mp: 224-226°C.

IR (KBr) cm$^{-1}$: 3374, 2933, 2866, 1697, 1637, 1530, 1452, 1375, 1296, 1198, 1040, 882.

$^1$H NMR (500 MHz, CDCl$_3$) $\delta$ (ppm): 5.67 (1H, $t$, $J = 5.5$ Hz), 4.73 (1H, s), 4.58 (1H, s), 3.67 (3H, s), 3.29 (1H, $d$, $J = 6.5$ Hz), 3.17 (3H, s), 3.07-3.02 (1H, m), 2.42 (3H, $t$, $J = 7.0$ Hz), 1.96-1.91 (2H, m), 1.78-1.70 (3H, m), 1.68 (6H, s), 1.62-1.47 (9H, m), 1.42-1.28 (6H, m), 1.25 (6H, s), 1.17-1.13 (2H, m), 0.96 (3H, s), 0.93 (3H, s), 0.89 (3H, s), 0.87 (3H, $d$, $J = 1.5$ Hz), 0.77 (3H, s), 0.61-0.65 (1H, m).

$^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ (ppm): 175.6, 171.5, 151.1, 144.1, 109.6, 89.6, 77.3, 55.4, 55.3, 50.1, 46.6, 42.4, 40.7, 38.9, 38.7, 37.2, 37.1, 34.5, 32.7, 30.8, 29.5, 29.3, 28.6, 27.0, 26.5, 25.3, 21.0, 19.5, 18.4, 16.4, 16.3, 16.2, 14.8.

Two human cancer cell lines (epidermoid carcinoma cell line KB-ATCC CCL-17 and hepatoma carcinoma cell line HepG2-ATCC HB-8065), obtained from the American Type Culture Collection (USA) ATCC, were used for cytotoxic evaluation. The cells were grown in Roswell Park Memorial Institute Medium 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% carbon dioxide). The exponentially growing cells were used throughout the experiments. The inhibitory effects of the compounds on the growth of the human cancer cell lines were determined by measuring the metabolic activity using an MTT assay. Briefly, human cancer cell lines (1 x 10$^4$ 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 media were then carefully aspirated. DMSO (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 with the untreated controls. A dose–response curve was generated, and the IC$_{50}$ value was determined for each compound as well as each cell line.

**Declaration of Conflicting Interests**

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**Supplemental Material**

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