Electronic Supplementary Information

Synthesis of 1-*O*-acetylbritannilactone Analogues from *Inula britannica* and *in vitro* Evaluation of their Anticancer Potential

Jiang-Jiang Tang,[a†] Shuai Dong,[a†] Yang-Yang Han,[b] Ming Lei[b] and Jin-Ming Gao [a,c *]

[a] *Shaanxi Engineering Center of Bioresource Chemistry & Sustainable Utilization, College of Science, Northwest A&F University, Yangling 712100, China.*

[b] *College of Life Sciences, Northwest A&F University, Yangling 712100, China.*

[c] *Department of Chemistry and Chemical Engineering, Baoji College of Arts and Sciences, Baoji 721007, P.R. China.*

[†] *These authors contributed equally to this work.*

* **Corresponding authors**: E-mail address: jinminggao@nwsuaf.edu.cn (JMG).

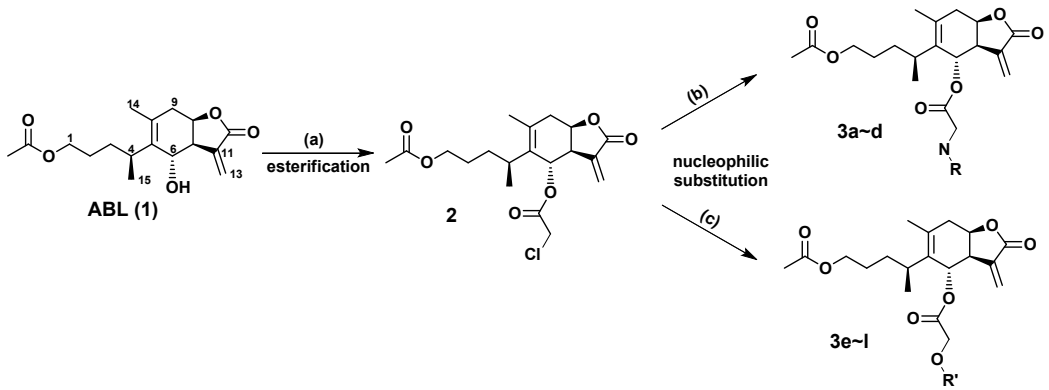
Table of Contents for Supporting Information

Title	Page
1. Experimental section	S2-S12
2. Table S1. Crystallographic parameters for OABL.	S13
3. Figure S1. ^{1}H NMR Michael acceptor assay of OABL in DMSO-*d*6.	S14
4. Figure S2. ^{1}H NMR Michael acceptor assay of **4a** in DMSO-*d*6.	S14
5. Figure S3. ^{1}H NMR spectra of adduct **5** in $CDCl_3$.	S15
6. Figure S4. ^{1}C NMR spectra of adduct **5** in $CDCl_3$	S15
7. Figure S5. NOESY spectra of adduct **5** in $CDCl_3$.	S16
8. References	S17

1. Experimental section

1.1 Chemistry

1.1.1 Materials and methods. All NMR spectra were recorded on a 400 MHz or 500 MHz Bruker NMR spectrometer in $CDCl_3$ with TMS as internal standard for protons and solvent signals as internal standard for carbon spectra. Chemical shift values are mentioned in δ (ppm) and coupling constants (*J*) are given in Hz. Mass spectra were recorded on an ESI-Thermo Fisher LTQ Fleet instrument spectrometer (Thermo Scientific). Analytical HPLC was performed on a Waters 1525 series with UV detection at 215 or 254 nm along with evaporative light scattering detection (ELSD), Method 1 = Agilent TC-C18, 5 µm, 4.6×250 mm, 10 min gradient, 80%MeOH:20%H_2O to 100%MeOH for compounds **2, 4a** ; Method 2 = Agilent TC-C18, 5 µm, 4.6×250 mm, 20 min gradient, 50%MeOH:50%H_2O to 100%MeOH for the remaining compounds **3a-l**, **4b-4h**. Column chromatography (CC) was performed over silica gel (200-300 mesh, Qingdao Marine Chemical Ltd.). The progress of all reactions was monitored by TLC on 2 cm×5 cm precoated silica gel GF_{254} plates of thickness of 0.25 mm (Qingdao Marine Chemical Group, Co.). Spots were visualized UV light (254, 365 nm) and/or by staining with 5% phosphomolybdic acid followed by heating. All commercially available solvents and reagents were freshly purified and dried by standard techniques prior to use.



Scheme 1. Synthetic route of compounds **3a–l**. Reagents and conditions: (a) chloroacetic anhydride, DMAP, Et_3N, CH_2Cl_2, 0 °C to rt, 30 min, 99%; (b) R-NH, CH_3CN, 0 °C to rt, 24 h, 77%–99%; (c) R-OH, K_2CO_3, NaI, TEBA, TEA, acetone, rt, 6–24 h, 33%–99%.

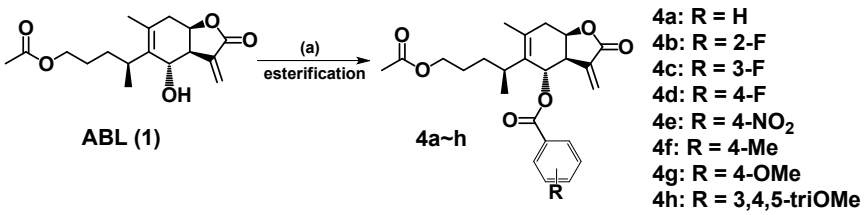
1.1.2 General procedure for the synthesis of 2 and 3a–l (Scheme 1): To a suspension

of chloroacetic anhydride (0.15 mmol, 1.5 eq.), EtN_3 (0.15 mmol, 1.5 eq.) and DMAP (0.01 mmol, 0.1 eq.) in anhydrous CH_2Cl_2 (1 mL) in an ice-bath was added ABL (0.1 mmol, 1 eq.) in anhydrous CH_2Cl_2 (1 mL) solution. After completion of the reaction for 30 min at room temperature, ice water (2 mL) was added to the solvent and stirred for 20 min, then extracted with CH_2Cl_2, dried and filtered. After removal of the solvent, the crude product was purified by silica gel chromatography (EtOAc/PE) to afford compound **2** in 99% yield. The spectrum data of **2** was referred to the published paper.[1] Then, compound **2** was transformed to the corresponding analogues **3a–l** by nucleophilic substitution.

For **3a–d**, a solution of **2** (1 eq.) in dry CH_3CN, was added at 0 ^oC R-NH (2.5 eq.) in THF. The reaction mixture was stirred at 25 ^oC for 24 h. After completion of the reaction, water was added to the solvent, extracted with CH_2Cl_2. After removal of the solvent, the crude product was purified by silica gel chromatography (EtOAc/PE) to afford compound **3a-3d** in good yields from 77% to 99%.

For **3e–l**, a solution of **2** (1 eq.) and NaI (0.2 eq.) in dry acetone stirred for 30 min was added R-OH (3 eq.), benzyltriethylammonium chloride (TEBA, 0.1 eq.) and triethanolamine (TEA, 1 eq.). The reaction mixture was stirred at 25 ^oC and monitored by TLC. After completion of the reaction, water was added to the solution, then extracted with CH_2Cl_2, dried and filtered. After removal of the solvent, the crude product was purified by silica gel chromatography (EtOAc/PE) to afford desired compound **3e–l** in yields from 33% to 99%.

1.1.3 General procedure for the synthesis of 4a–h (Scheme 2): To a solution of **ABL** (1 eq.) in dry pyridine, was added ArCOCl (1.05 eq.) at 0 ^oC in anhydrous CH_2Cl_2. The reaction mixture was stirred at 25 ^oC for 30 min. After completion of the reaction, ice water was added to the solvent and stirred for 20 min, then extracted with CH_2Cl_2. After removal of the solvent, the crude product was purified by silica gel chromatography (EtOAc/PE) to afford compound **4a–h** almost quantitatively.



Scheme 2. Synthetic route of compounds **4a–h**. Reagents and conditions: (a) ArCOCl, pyridine, rt, 30 min, 80%–99%.

1.1.4 ^{1}H NMR Michael Acceptor Assays: A previously described NMR assay was performed with modifications to measure the reactivity of OABL towards thiols.[2] In brief, OABL or **4a** (0.05 mmol) was dissolved in DMSO-*d*6 (500 μL) in a standard 5 mm NMR tube. The ^{1}H NMR spectrum was recorded on a Bruker 500 MHz NMR at 25 °C. β-mercaptoethanol (7.8 mg, 0.1 mmol, 2 equiv.) and Et_3N (10.1 mg, 0.1 mmol, 2 equiv.) were pre-weighed into a microcentrifuge tube. The solution of OABL or **4a** from the NMR tube was then added to the pre-weighed mixture and mixed. This solution was then replaced in the NMR tube and additional spectra were acquired at the time points shown (in Figure S1 and S2).

1.2 Biological Activities Evaluation

1.2.1 Cell Culture: HCT116 (human colorectal cancer) cell line was originally obtained from Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences. HeLa (human cervix cancer) and SGC-7901 (human gastric cancer) cell lines were granted by Prof. Lei group of college of life sciences, Northwest A&F University. The three cell lines were grown in RPMI-1640 (Gibco) containing 10% (v/v) thermally inactivated fetal bovine serum (FBS), penicillin (100 KU/L) and streptomycin (100 KU/L) at 37 ^{o}C in a 5% CO_2 humidified incubator.

1.2.2 Cytotoxicity (SRB) Assay: *In vitro* cytotoxicity was assessed by using the SRB colorimetric assay.[3] Briefly, 100 μL of HCT116, HeLa and SGC-7901 cells containing 2.5×10^4 cells/mL was added to each well of the 96-well flat plates and allowed to attach for 24 h. Then the medium was replaced by fresh medium, and cells were incubated with various concentrations of the test compounds. After incubation for

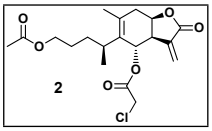
an additional 72 h at 37 ^{o}C, sulforhodamine B solution 0.4% (w/v) in 1% (v/v) acetic solution was added to each well, and bound sulforhodamine B was subsequently solubilized with 10 mM Tris base (pH 10.0), and the absorbance was read at 560 nm using an Epoch (Bio-Tek) microplate reader. The percentage of cell viability was calculated relative to control wells designated as 100% viable cells. The IC_{50} values were measured by sigmoidal fit using Origin 7.5 software.

To determine the important role of the α-methylene-γ-lactone motif for **4a**-induced cytotoxicity in HeLa cells, the effects of N-acetyl cysteine (NAC) were examined by SRB assay. HeLa cells were plated in a 96-well microtiterplate at the above-mentioned density of cells in a final volume of 100 μL medium. The cells were co-administered with 1 mM NAC and increasing concentrations of **4a** for 72 h, the cell viability was assessed.

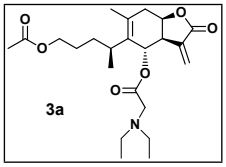
1.2.3 Apoptosis Assay: The apoptosis in HeLa cells was detected with an annexin V-FITC/PI apoptosis detection kit (KeyGEN Biotech, China) as described previously.[4] After the treatment of OABL and **4a** at the indicated concentrations for 72 h, cells were harvested, washed with ice-cold PBS and then labeled with annexin V-FITC/PI according to the manufacturer's instructions. After cells were resuspended in 400 μL of binding buffer, flow cytometric analysis (FACS Calibur; Becton-Dickinson, San Jose, CA, US) was performed. A total of 10000 cells were acquired per sample and data were analyzed using Cellquest software (Becton Dickinson).

1.2.4 Western Blotting Analysis: HeLa cells were treated with the indicated concentrations of Vp-16 and **4a** for 48 h, cell pellets were collected and lysed with RIPA lysis buffer containing 0.5 mM phenylmethylsulfonyl fluoride (PMSF) and protease inhibitors cocktail. The protein concentration of cell samples was analyzed by using BCA method. Equal amount proteins of each sample were electrophoresed on 10% SDS-PAGE gel and electrotransferred onto NC membrane. After incubation with appropriate primary and secondary antibodies, protein blots were detected by using ECL solution and ChemiDoc XRS+ imaging system (Bio-Rad, USA). β-actin was used as loading control.

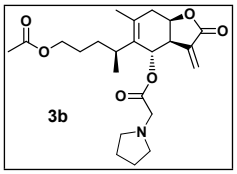
1.2.5 Cell Cycle Analysis: The cell cycle arrest in HeLa cells was detected with PI staining assay (Sigma) as described previously.[1] After treatment with tested compound **4a** at the indicated concentrations for 24 h, cells were centrifuged and fixed in 70% ethanol at 4 ^{o}C overnight and subsequently resuspended in PBS containing 100 µL RNase A and 400 µL PI. Cellular DNA content, for cell cycle distribution analysis, was measured using a FACSCalibur flow cytometer and analyzed using Modfit LT 3.0 software. Twenty thousand events were collected per sample. Mean values from three independent experiments are presented.



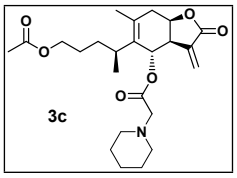
1-*O*-acetyl-6-*O*-chloracetylbritannilactone (2): White solid. Yield: 99%; HPLC: t_R = 4.338 min, purity > 99.9% @ ELSD, 99.9% @ 215 nm. The NMR and ESI-MS spectrum data of **2** was referred to the published paper.[1]



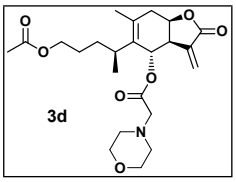
1-*O*-acetyl-6-*O*-(2-(diethylamino)acetyl)britannilactone (3a): Yellow oil. Yield: 77%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.36 (d, *J* = 2.7 Hz, 1H, H-13a), 5.94 (d, *J* = 2.3 Hz, 1H, H-13b), 5.25 (d, *J* = 1.8 Hz, 1H, H-6), 4.92 (ddd, *J* = 7.7, 3.7, 2.1 Hz, 1H, H-8), 3.98 – 3.86 (m, 2H, H-1), 3.49 – 3.44 (m, 2H, H-7), 3.28 (d, *J* = 3.1 Hz, 2H，-C*H*$_2$COO-6), 2.64 (ddd, *J* = 14.2, 7.1, 1.5 Hz, 6H, H-4, H-9a，$C_2H_6C_2H_4$N-), 2.48 (dd, *J* = 16.2, 2.1 Hz, 1H, H-9b), 2.02 (s, 3H, AcO-1), 1.79 (s, 3H, H-14), 1.29 – 1.22 (m, 2H, H-2b, H-3a), 1.04 (t, *J* = 7.2 Hz, 7H, H-3b，$C_2H_6C_2H_4$N-), 0.86 (d, *J* = 6.9 Hz, 3H, H-15). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.64, 171.34, 169.60, 136.30, 134.12, 132.06, 125.23, 74.99, 69.28, 64.35, 54.20, 47.80, 43.11, 34.71, 33.21, 31.20, 26.62, 21.09, 20.64, 18.67, 12.47. ESI-MS: 421.75 $[M+H]^+$; HPLC: t_R = 16.00 min, purity > 99.9% @ ELSD.



1-*O*-acetyl-6-*O*-(2-(pyrrolidin-1-yl)acetyl)britannilactone (3b): Yellow oil. Yield: 90%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.36 (d, *J* = 2.7 Hz, 1H, H-13a), 5.94 (d, *J* = 2.3 Hz, 1H, H-13b), 5.29 – 5.25 (m, 1H, H-6), 4.96 – 4.90 (m, 1H, H-8), 3.97 – 3.87 (m, 2H, H-1), 3.51 – 3.46 (m, 2H, H-7), 3.37 (dd, *J* = 42.0, 16.8 Hz, 2H, -C*H*$_2$COO-6), 2.67 (ddd, *J* = 11.8, 9.2, 5.0 Hz, 6H, H-4, H-9a, $C_2H_4C_2H_4$N-), 2.50 (d, *J* = 2.1 Hz, 1H, H-9b), 2.03 (s, 3H, AcO-1), 1.86 – 1.82 (m, 4H, $C_2H_4C_2H_4$N-), 1.79 (s, 3H, H-14), 1.42 – 1.33 (m, 1H, H-2a), 1.31 – 1.20 (m, 2H, H-2b, H-3a), 1.07 – 0.96 (m, 1H, H-3b), 0.86 (d, *J* = 6.9 Hz, 3H, H-15). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.35, 169.59, 136.32, 134.36, 131.89, 125.25, 77.36, 75.00, 69.65, 64.34, 56.60, 53.99, 43.05, 34.68, 33.18, 31.22, 26.61, 23.92, 21.10, 20.66, 18.65. ESI-MS: 419.93 $[M+H]^+$; HPLC: t_R = 15.14 min, purity = 97.4% @ ELSD.

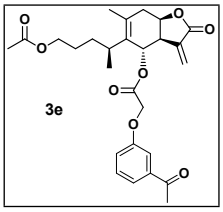


1-*O*-acetyl-6-*O*-(2-(piperidin-1-yl)acetyl)britannilactone (3c): Yellow oil. Yield: 95%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.36 (d, *J* = 2.7 Hz, 1H，H-13a), 5.94 (d, *J* = 2.3 Hz, 1H, H-13b), 5.33 – 5.18 (m, 1H，H-6), 4.92 (dd, *J* = 7.8, 1.6 Hz, 1H，H-8), 3.92 (dd, *J* = 13.0, 6.3 Hz, 2H，H-1), 3.50 – 3.44 (m, 1H，H-7), 3.13 (q, *J* = 16.7 Hz, 2H, -C*H*$_2$COO-6), 2.73 – 2.60 (m, 2H, H-4, H-9a), 2.54 – 2.39 (m, 5H，H-9b, $CH_2C_2H_4C_2H_4$N-), 2.02 (s, 3H, AcO-1), 1.80 (s, 3H, H-14), 1.60 (dt, *J* = 11.1, 5.6 Hz, 6H，C*H*$_2C_2H_4C_2H_4$N-), 1.41 (dd, *J* = 11.7, 5.9 Hz, 1H, H-2a), 1.28 – 1.17 (m, 2H, H-2b, H-3a), 1.08 – 0.95 (m, 1H，H-3b), 0.86 (d, *J* = 6.9 Hz, 3H，H-15). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.33, 170.86, 169.59, 136.33, 134.16, 131.99, 125.20, 74.99, 69.39, 64.34, 60.26, 54.38, 43.11, 34.67, 33.19, 31.21, 26.60, 25.93, 23.95, 21.08, 20.63, 18.64. ESI-MS: 433.80 $[M+H]^+$; HPLC: t_R = 16.62 min, purity > 99.9% @ ELSD.

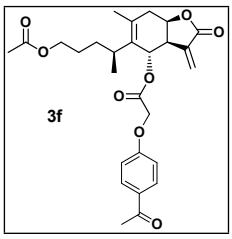


1-*O*-acetyl-6-*O*-(2-morpholinoacetate)britannilactone (3d): Yellow oil. Yield: 99%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.36 (d, *J* = 2.7 Hz, 1H, H-13a), 5.93 (d, *J* = 2.3 Hz, 1H, H-13b), 5.25 (d, *J* = 1.8 Hz, 1H, H-6), 4.92 (ddd, *J* = 7.7, 3.7, 2.1 Hz, 1H, H-8), 3.92 (dt, *J* = 12.9, 5.8 Hz, 2H, H-1), 3.73 (t, *J* = 4.6 Hz, 4H,$OC_2H_4C_2H_4$N-), 3.49 – 3.43 (m, 1H, H-7), 3.22 – 3.10 (m, 2H, -C*H*$_2$COO-6), 2.67 (ddd, *J* = 11.3, 6.7, 3.5 Hz, 2H，H-4, H-9a), 2.56 (q, *J* = 4.5 Hz, 4H，$OC_2H_4C_2H_4$N-), 2.48 (dd, *J* = 16.2, 2.1 Hz, 1H, H-9b), 2.02 (d, *J* = 1.8 Hz, 3H, AcO-1), 1.78 (s, 3H, H-14), 1.37 (d, *J* = 11.7 Hz, 1H, H-2a), 1.25 (ddd, *J* = 9.0, 7.2, 5.2 Hz, 2H, H-2b, H-3a), 1.00 (d, *J* = 10.2 Hz, 1H, H-3b),

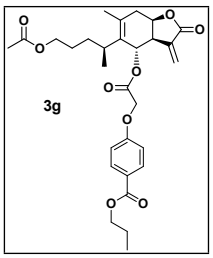
0.85 (d, *J* = 6.9 Hz, 3H, H-15). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.31, 170.27, 169.49, 136.25, 134.37, 131.85, 125.26，74.88, 69.63, 66.85, 64.30, 59.62, 53.38, 43.12, 34.65, 33.16, 31.20, 26.59, 21.07, 20.64, 18.66. ESI-MS: 435.81 $[M+H]^+$; HPLC: t_R = 12.20 min, purity > 99.9% @ ELSD.



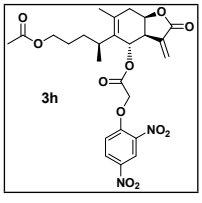
1-*O*-acetyl-6-*O*-(2-(3-acetylphenoxy)acetyl)britannilactone (3e): Yellow oil. Yield: 99%; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.59 (d, *J* = 7.8 Hz, 1H), 7.43 – 7.38 (m, 2H), 7.17 – 7.11 (m, 1H), 6.37 (d, *J* = 2.7 Hz, 1H, H-13a), 5.90 (d, *J* = 2.2 Hz, 1H, H-13b), 5.34 (d, *J* = 1.8 Hz, 1H, H-6), 4.84 (ddd, *J* = 7.7, 3.7, 2.1 Hz, 1H, H-8), 4.67 (s, 2H, -C*H*$_2$COO-6), 3.93 (ddd, *J* = 17.0, 10.9, 4.5 Hz, 2H, H-1), 3.50 – 3.44 (m, 1H, H-7), 2.71 – 2.63 (m, 1H, H-4), 2.59 (s, 3H, C*H*$_3$COC$_6$H$_4$OCH$_2$COO-6), 2.55 – 2.40 (m, 2H, H-9), 2.04 (s, 3H, AcO-1), 1.78 (s, 3H, H-14), 1.40 (dd, *J* = 11.4, 5.9 Hz, 1H, H-2a), 1.31 – 1.20 (m, 2H, H-2b, H-3a), 1.00 (dd, *J* = 24.5, 14.2 Hz, 1H, H-3b), 0.88 (s, 3H, H-15).^{13}C NMR (125 MHz, $CDCl_3$) δ 197.56, 171.30, 169.37, 168.72, 158.09, 138.74, 136.09, 135.00, 131.55, 130.09, 125.38, 122.57, 120.63, 112.26, 77.36, 74.70, 70.48, 65.38, 64.30, 43.06, 34.66, 33.17, 31.26, 26.80, 26.65, 21.09, 20.67, 18.67. ESI-MS: 507.20 $[M+Na]^+$; HPLC: t_R = 15.9 min, purity > 99.9% @ ELSD.



1-*O*-acetyl-6-*O*-(2-(4-acetylphenoxy)acetyl)britannilactone (3f): Yellow oil. Yield: 33%; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.94 (d, *J* = 8.9 Hz, 2H, $CH_3COC_6H_4OCH_2COO$-6), 6.91 (d, *J* = 8.9 Hz, 2H, $CH_3COC_6H_4OCH_2COO$-6), 6.38 (d, *J* = 2.6 Hz, 1H，H-13a), 5.93 (d, *J* = 2.3 Hz, 1H, H-13b), 5.34 (d, *J* = 1.7 Hz, 1H, H-6), 4.81 – 4.76 (m, 1H, H-8), 4.68 (s, 2H, -C*H*$_2$COO-6), 3.98 – 3.87 (m, 2H, H-1), 3.48 – 3.42 (m, 1H, H-7), 2.67 (d, *J* = 4.9 Hz, 1H, H-4), 2.56 (s, 3H, C*H*$_3$COC$_6$H$_4$OCH$_2$COO-6), 2.53 – 2.40 (m, 2H, H-9), 2.03 (s, 3H, AcO-1), 1.78 (s, 3H, H-14), 1.45 – 1.35 (m, 2H, H-2), 1.08 – 0.93 (m, 2H, H-3), 0.88 (s, 3H, H-15). ^{13}C NMR (126 MHz, $CDCl_3$) δ 182.70, 170.04, 169.28, 158.90, 131.37, 130.66, 114.43, 74.45, 70.43, 65.16, 64.14, 50.97, 42.96, 34.53, 33.03, 31.12, 26.52, 20.97, 20.56, 18.57. ESI-MS: 507.06 $[M+Na]^+$; HPLC: t_R = 15.5 min, purity > 99.9% @ ELSD.

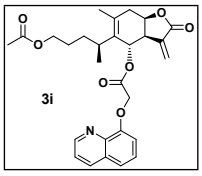


1-*O*-acetyl-6-*O*-(2-(4-(propoxycarbonyl)acetyl)britannilactone (3g): Colorless oil. Yield: 99%; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.99 (d, *J* = 8.9 Hz, 2H), 6.89 (s, 1H), 6.87 (s, 1H), 6.36 (d, *J* = 2.6 Hz, 1H, H-12a), 5.91 (d, *J* = 2.3 Hz, 1H, H-13b), 5.33 (d, *J* = 1.7 Hz, 1H, H-6), 4.75 (ddd, *J* = 7.6, 3.5, 2.2 Hz, 1H, H-8), 4.66 (s, 2H, -C*H*$_2$COO-6), 4.24 (t, *J* = 6.7 Hz, 2H), 3.97 – 3.85 (m, 2H, H-1), 3.45 – 3.38 (m, 1H, H-7), 2.65 (ddd, *J* = 9.4, 7.0, 4.8 Hz, 1H, H-4), 2.43 (qd, *J* = 16.2, 2.4 Hz, 2H, H-9), 2.02 (s, 3H, AcO-1), 1.81 – 1.73 (m, 5H, H-14), 1.43 – 1.33 (m, 1H), 1.30 – 1.18 (m, 2H, H-2b, H-3a), 1.00 (dd, *J* = 13.2, 5.8 Hz, 4H, H-3b), 0.86 (d, *J* = 6.9 Hz, 3H, H-15). ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.26, 169.27, 168.43, 166.16, 161.24, 135.96, 135.03, 131.75, 131.48, 125.39, 124.40, 114.12, 74.59, 70.44, 66.57, 65.28, 64.24, 43.02, 34.60, 33.12, 31.22, 26.61, 22.24, 21.04, 20.62, 18.65, 10.61. ESI-MS: 551.14 $[M+Na]^+$; HPLC: t_R = 19.2 min, purity > 99.9% @ ELSD.

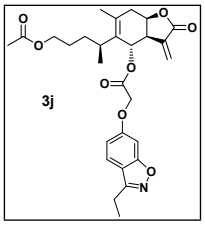


1-*O*-acetyl-6-*O*-(2-(2,4-dinitrophenoxy)acetyl)britannilactone (3h): Yellow oil. Yield: 47%; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.77 (d, *J* = 2.6 Hz, 1H), 8.41 (dd, *J* = 9.2, 2.7 Hz, 1H), 7.06 (d, *J* = 9.2 Hz, 1H), 6.40 (d, *J* = 2.5 Hz, 1H, H-13a), 5.92 (d, *J* = 2.1 Hz, 1H, H-13b), 5.35 (d, *J* = 1.3 Hz, 1H, H-6), 4.96 – 4.91 (m, 1H, H-8),

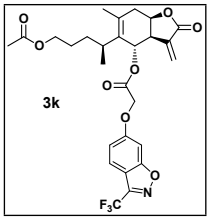
4.90 – 4.82 (m, 2H, -CH_2COO-6), 3.93 (ddd, *J* = 17.3, 10.8, 4.7 Hz, 2H, H-1), 3.50 (dd, *J* = 7.6, 1.8 Hz, 1H, H-7), 2.72 – 2.66 (m, 1H, H-4), 2.63 (d, *J* = 16.5 Hz, 1H, H-9a), 2.52 (dd, *J* = 16.3, 1.7 Hz, 1H, H-9b), 2.03 (d, *J* = 4.0 Hz, 3H, AcO-1), 1.80 (s, 3H, H-14), 1.40 (dd, *J* = 11.4, 6.7 Hz, 1H, H-2a), 1.31 – 1.20 (m, 2H, H-2b, H-3a), 1.00 (d, *J* = 10.3 Hz, 1H, H-3b), 0.85 (d, *J* = 6.9 Hz, 3H, H-15). ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.30, 169.14, 166.46, 155.25, 141.34, 139.68, 135.82, 131.13, 128.89, 125.62, 122.21, 114.56, 74.45, 71.37, 66.60, 64.21, 45.91, 43.14, 34.66, 33.14, 31.23, 26.64, 21.08, 20.71, 18.78. ESI-MS: 551.14 $[M+Na]^+$; HPLC: t_R = 16.3 min, purity > 99.9% @ ELSD.



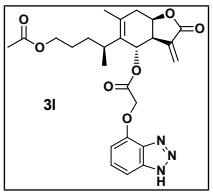
1-*O*-acetyl-6-*O*-(2-(quinolin-8-yloxy)acetyl)britannilactone (3i): Colorless oil. Yield: 75%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.95 (dd, *J* = 4.2, 1.7 Hz, 1H), 8.15 (dd, *J* = 8.3, 1.6 Hz, 1H), 7.46 (dd, *J* = 8.3, 4.2 Hz, 2H), 7.41 (t, *J* = 7.9 Hz, 1H), 6.93 (dd, *J* = 7.5, 1.1 Hz, 1H), 6.33 (d, *J* = 2.7 Hz, 1H， H-13a), 5.88 (d, *J* = 2.3 Hz, 1H, H-13b), 5.31 (d, *J* = 1.9 Hz, 1H, H-6), 5.03 – 4.92 (m, 2H，-CH_2COO-6), 4.52 (ddd, *J* = 7.7, 3.6, 2.1 Hz, 1H，H-8), 3.38 – 3.31 (m, 1H，H-7), 2.57 (dd, *J* = 6.9, 2.0 Hz, 1H，H-4), 2.28 – 2.13 (m, 2H，H-9), 2.00 (s, 3H，AcO-1), 1.62 (s, 3H, H-14), 1.39 – 1.28 (m, 1H，H-2a), 1.21 (ddd, *J* = 10.1, 8.4, 4.8 Hz, 2H, H-2b, H-3a), 0.95 (d, *J* = 10.1 Hz, 1H，H-3b), 0.81 (d, *J* = 6.9 Hz, 3H, H-15). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.28, 169.36, 168.87, 136.13, 134.80, 131.31, 126.31, 74.68, 69.95, 64.30, 63.85, 58.74, 50.77, 49.30, 46.01, 43.50, 43.14, 34.61, 33.08, 31.22, 29.75, 26.54, 21.01, 20.58, 18.69. ESI-MS: 493.82 $[M+H]^+$; HPLC: t_R = 14.5 min, purity > 99.9% @ ELSD.



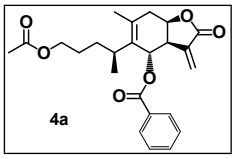
1-*O*-acetyl-6-*O*-(2-((3-ethylbenzo[*d*]isoxazol-6-yl)oxy)acetyl)britannilactone (3j): Colorless oil. Yield: 92%; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.54 (d, *J* = 8.7 Hz, 1H), 6.97 (d, *J* = 2.4 Hz, 1H), 6.88 (dd, *J* = 8.7, 2.4 Hz, 1H), 6.36 (d, *J* = 2.7 Hz, 1H, H-13a), 5.92 (d, *J* = 2.3 Hz, 1H, H-13b), 5.32 (d, *J* = 1.8 Hz, 1H, H-6), 5.29 (s, 1H), 4.75 (ddd, *J* = 7.7, 3.7, 2.1 Hz, 1H, H-8), 4.64 (s, 2H), 3.97 – 3.85 (m, 2H, H-1), 3.68 (d, *J* = 5.3 Hz, 1H), 3.46 – 3.41 (m, 1H, H-7), 2.92 (q, *J* = 7.6 Hz, 2H, H-4), 2.69 – 2.61 (m, 1H), 2.51 (dd, *J* = 16.2, 2.7 Hz, 1H, H-9a), 2.40 (dd, *J* = 16.2, 2.1 Hz, 1H, H-9b), 2.02 (s, 3H, AcO-1), 1.76 (s, 3H, H-14), 1.42 (t, *J* = 7.6 Hz, 3H), 1.38 (dd, *J* = 8.1, 4.0 Hz, 1H), 1.24 (ddd, *J* = 9.3, 7.4, 4.1 Hz, 2H, H-2b, H-3a), 0.99 (d, *J* = 10.1 Hz, 1H, H-3b), 0.86 (d, *J* = 6.9 Hz, 3H, H-15). ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.28, 169.32, 168.88, 168.01, 155.66, 151.48, 136.20, 135.97, 134.93, 131.51, 125.40, 119.82, 112.17, 96.81, 77.41, 77.16, 76.91, 74.63, 70.93, 70.33, 66.20, 64.25, 45.88, 43.00, 34.58, 33.11, 31.19, 26.59, 22.21, 21.06, 20.62, 18.64, 10.98. ESI-MS: 533.97 $[M+Na]^+$; HPLC: t_R = 17.4 min, purity = 99.5% @ ELSD.



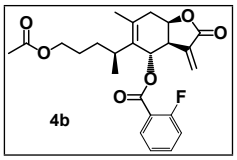
1-*O*-acetyl-6-*O*-(2-((3-(trifluoromethyl)benzo[*d*]isoxazol-6-yl)oxy)acetyl)britannilactone (3k): Yellow oil. Yield: 40%; ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.49 (s, 1H), 7.76 (dd, *J* = 9.2, 1.9 Hz, 1H), 6.58 (dd, *J* = 9.2, 2.5 Hz, 1H), 6.39 (t, *J* = 2.8 Hz, 2H, H-13a), 5.94 (d, *J* = 2.2 Hz, 1H, H-13b), 5.35 (d, *J* = 1.6 Hz, 1H, H-6), 4.88 (dd, *J* = 4.8, 3.0 Hz, 1H, H-8), 4.68 (d, *J* = 1.7 Hz, 2H), 3.98 – 3.87 (m, 2H, H-1), 3.49 (dd, *J* = 6.6, 3.0 Hz, 1H, H-7), 2.68 (d, *J* = 5.2 Hz, 1H, H-4), 2.58 – 2.44 (m, 2H, H-9), 2.04 (d, *J* = 4.8 Hz, 3H, AcO-1), 1.79 (s, 3H, H-14), 1.40 (dd, *J* = 11.3, 7.2 Hz, 1H), 1.32 – 1.17 (m, 2H, H-2b, H-3a), 1.00 (d, *J* = 10.6 Hz, 1H, H-3b), 0.87 (dd, *J* = 14.6, 7.1 Hz, 3H, H-15). ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.38, 169.29, 167.71, 167.56, 165.92, 135.87, 135.27, 132.89, 131.41, 125.63, 109.59, 108.97, 101.98, 74.56, 70.96, 70.86, 65.26, 64.27, 45.89, 43.07, 42.50, 34.63, 33.14, 31.22, 26.62, 21.09, 20.69, 18.72. ESI-MS: 553.09 $[M+H]^+$; HPLC: t_R = 17.9 min, purity = 99.8% @ ELSD.



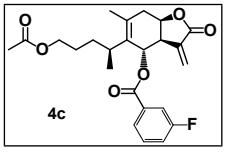
1-*O*-acetyl-6-*O*-(2-((1*H*-benzo[*d*][1,2,3]triazol-4-yl)oxy)acetyl)britannilactone (3l): Yellow oil. Yield: 75%; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.00 (d, *J* = 8.5 Hz, 1H), 7.76 (d, *J* = 8.4 Hz, 1H), 7.56 (t, *J* = 7.6 Hz, 1H), 7.42 (t, *J* = 7.7 Hz, 1H), 6.37 (d, *J* = 2.6 Hz, 1H, H-13a), 5.90 (d, *J* = 2.2 Hz, 1H, H-13b), 5.30 (d, *J* = 1.4 Hz, 1H, H-6), 5.17 (q, *J* = 16.2 Hz, 2H, -C*H*$_2$COO-6), 4.90 (ddd, *J* = 7.5, 3.5, 2.1 Hz, 1H, H-8), 3.98 – 3.86 (m, 2H, H-1), 3.48 – 3.44 (m, 1H, H-7), 2.63 (m, 1H, H-4), 2.53 (d, *J* = 2.8 Hz, 1H, H-9a), 2.44 (dd, *J* = 16.2, 1.9 Hz, 1H, H-9b), 2.03 (s, 3H, AcO-1), 1.77 (s, 3H, H-14), 1.38 (s, 1H, H-2a), 1.23 (dd, *J* = 10.1, 4.8 Hz, 2H, H-2b, H-3a), 0.97 (d, *J* = 10.2 Hz, 1H, H-3b), 0.76 (s, 3H, H-15). ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.28, 169.27, 166.90, 143.60, 135.98, 135.37, 131.25, 128.48, 127.56, 125.41, 125.14, 120.20, 109.81, 77.41, 77.16, 76.91, 74.61, 74.58, 71.03, 64.24, 42.84, 34.61, 33.09, 31.23, 26.59, 21.07, 20.69, 18.49. ESI-MS: 506.07 $[M+Na]^+$; HPLC: t_R = 16.4min, purity > 99.9% @ ELSD.



1-*O*-acetyl-6-*O*-benzoylbritannilactone (4a): Colorless oil. Yield: 95%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 – 7.93 (m, 2H), 7.58 (t, *J* = 7.4 Hz, 1H), 7.45 (t, *J* = 7.7 Hz, 2H), 6.42 (d, *J* = 2.7 Hz, 1H，H-13a), 6.03 (d, *J* = 2.3 Hz, 1H, H-13b), 5.45 (d, *J* = 1.8 Hz, 1H，H-6), 4.97 (ddd, *J* = 7.8, 3.7, 2.1 Hz, 1H, H-8), 4.03 – 3.87 (m, 2H, H-1), 3.68 – 3.59 (m, 1H, H-7), 2.83 (dd, *J* = 16.1, 2.7 Hz, 1H, H-9a), 2.78 – 2.66 (m, 1H, H-4), 2.56 (dd, *J* = 16.1, 2.1 Hz, 1H, H-9b), 2.04 (s, 3H, AcO-1), 1.86 (s, 3H, H-14), 1.46 – 1.38 (m, 1H, H-2a), 1.35 – 1.26 (m, 2H, H-2b, H-3a), 1.14 – 1.04 (m, 1H, H-3b), 0.89 (d, *J* = 6.9 Hz, 3H, H-15). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.36, 169.68, 166.48, 136.41, 134.13, 133.48, 132.37, 130.11, 129.66 (d), 128.67 (d), 125.24, 76.84, 75.08, 69.95, 64.39, 53.57, 43.01, 34.86, 33.30, 31.20, 26.68, 21.12, 20.75, 18.80. ESI-MS: 451.2 $[M+K]^+$; HPLC: t_R = 6min, purity = 99.9% @ ELSD.

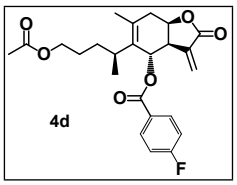


1-*O*-acetyl-6-*O*-(2-fluorobenzoyl)britannilactone (4b): Colorless oil. Yield: 99%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 (ddd, *J* = 32.6, 9.2, 4.5 Hz, 2H), 7.53 (d, *J* = 2.1 Hz, 1H), 7.52 (ddd, *J* = 13.6, 6.0, 2.4 Hz, 2H), 7.20 (td, *J* = 7.6, 3.8 Hz, 2H), 6.39 (d, *J* = 2.6 Hz, 1H，H-13a), 5.99 (d, *J* = 2.3 Hz, 1H, H-13b), 5.47 (d, *J* = 1.7 Hz, 1H，H-6), 4.97 (ddd, *J* = 7.7, 3.7, 2.1 Hz, 1H, H-8), 4.00 – 3.85 (m, 2H, H-1), 3.66 – 3.59 (m, 1H, H-7), 2.83 (dd, *J* = 16.1, 2.6 Hz, 1H, H-9a), 2.74 – 2.64 (m, 1H, H-4), 2.51 (dd, *J* = 16.1, 2.0 Hz, 1H, H-9b), 2.02 (s, 3H, AcO-1), 1.81 (s, 3H, H-14), 1.46 – 1.35 (m, 1H, H-2a), 1.34 – 1.19 (m, 2H, H-2b, H-3a), 1.14 – 0.98 (m, 1H, H-3b), 0.89 (d, *J* = 6.9 Hz, 3H, H-15). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.45, 169.69, 164.38, 163.30, 136.25, 135.35, 134.91, 134.49, 132.74, 131.99, 125.22, 117.26, 117.04, 75.11, 70.03, 64.38, 42.99, 34.64, 33.20, 31.14, 26.59, 21.01, 20.61, 18.68. ESI-MS: 453.11 $[M+Na]^+$; HPLC: t_R = 17.0 min, purity = 97.5% @ ELSD.

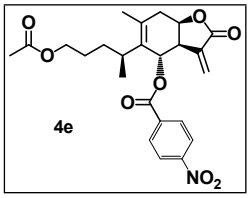


1-*O*-acetyl-6-*O*-(3-fluorobenzoyl)britannilactone (4c): Colorless oil. Yield: 99%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (dd, *J* = 6.1, 5.1 Hz, 1H), 7.63 (ddd, *J* = 9.2, 2.5, 1.5 Hz, 1H), 7.47 – 7.40 (m, 1H), 7.33 – 7.27 (m, 1H), 6.43 (d, *J* = 2.7 Hz, 1H, H-13a), 6.03 (d, *J* = 2.3 Hz, 1H, H-13b), 5.44 (d, *J* = 1.8 Hz, 1H, H-6), 4.97 (ddd, *J* = 7.8, 3.7, 2.1 Hz, 1H, H-8), 4.02 – 3.89 (m, 2H, H-1), 3.66 – 3.60 (m, 1H, H-7), 3.49 (s, 1H), 2.81 (dd, *J* = 16.2, 2.6 Hz, 1H, H-9a), 2.73 (ddd, *J* = 9.7, 7.0, 4.6 Hz, 2H, H-4), 2.57 (dd, *J* = 16.2, 2.1 Hz, 1H, H-9a), 2.04 (s, 3H, AcO-1), 1.87 (d, *J* = 0.6 Hz, 3H, H-14), 1.48 – 1.36 (m, 1H, H-2a), 1.35 – 1.21 (m, 2H, H-2b, H-3a), 1.14 – 1.02 (m, 1H, H-3b), 0.88 (d, *J* = 6.9 Hz, 3H, H-15). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.42, 169.62, 165.41, 163.94, 136.31, 134.46, 132.17, 130.34, 125.42,

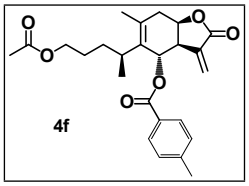
120.69, 120.47, 116.65, 116.42, 74.97, 70.41, 64.39, 43.00, 34.86, 33.29, 31.22, 26.68, 21.11, 20.77, 18.80. ESI-MS: 453.22 $[M+Na]^+$; HPLC: t_R = 17.8 min, purity = 98.2% @ ELSD.



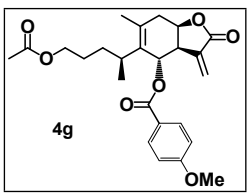
1-*O*-acetyl-6-*O*-(4-fluorobenzoyl)britannilactone (4d): Colorless oil. Yield: 99%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 – 7.94 (m, 2H), 7.12 (ddd, *J* = 8.7, 7.7, 4.3 Hz, 2H), 6.41 (d, *J* = 2.7 Hz, 1H, H-13a), 6.02 (d, *J* = 2.3 Hz, 1H, H-13b), 5.43 (d, *J* = 1.8 Hz, 1H, H-6), 4.96 (ddd, *J* = 7.8, 3.7, 2.1 Hz, 1H, H-8), 4.01 – 3.87 (m, 2H, H-1), 3.66 – 3.58 (m, 1H, H-7), 2.85 – 2.76 (m, 1H, H-9a), 2.75 – 2.67 (m, 2H, H-4), 2.56 (dd, *J* = 16.2, 2.1 Hz, 1H, H-9b), 2.04 (d, *J* = 3.1 Hz, 3H, AcO-1), 1.85 (d, *J* = 0.7 Hz, 3H, H-14), 1.47 – 1.35 (m, 1H, H-2a), 1.34 – 1.21 (m, 2H, H-2b, H-3a), 1.14 – 1.02 (m, 1H, H-3b), 0.87 (d, *J* = 6.9 Hz, 3H, H-15). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.40, 169.64, 165.53, 164.81, 136.35, 134.24, 132.91, 132.26, 132.17, 126.35, 125.30, 115.96, 115.74, 75.02, 70.14, 64.37, 43.00, 34.84, 33.27, 31.19, 26.66, 21.09, 20.73, 18.77. ESI-MS: 453.20 $[M+Na]^+$; HPLC: t_R = 17.6 min, purity = 98.7% @ ELSD.



1-*O*-acetyl-6-*O*-(4-nitrobenzoyl)britannilactone (4e): White solid. Yield: 93%; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.86 (d, *J* = 8.1 Hz, 2H), 7.24 (d, *J* = 8.2 Hz, 2H), 6.41 (d, *J* = 2.6 Hz, 1H, H-13a), 6.02 (d, *J* = 2.2 Hz, 1H, H-13b), 5.43 (d, *J* = 1.6 Hz, 1H, H-6), 4.96 (ddd, *J* = 7.6, 3.5, 2.1 Hz, 1H, H-8), 3.95 (ddd, *J* = 17.3, 10.9, 4.7 Hz, 2H, H-1), 3.66 – 3.59 (m, 1H, H-7), 2.83 (dd, *J* = 16.1, 2.8 Hz, 1H, H-9a), 2.76 – 2.64 (m, 1H, H-4), 2.55 (dd, *J* = 16.1, 1.9 Hz, 1H, H-9b), 2.04 (s, 3H, AcO-1), 1.85 (s, 3H, H-14), 1.47 – 1.37 (m, 1H, H-2a), 1.34 – 1.22 (m, 2H, H-2b, H-3a), 1.14 – 1.04 (m, 1H, H-3b), 0.87 (t, *J* = 8.2 Hz, 3H, H-15). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.24, 169.61, 166.45, 144.17, 136.41, 133.87, 132.40, 129.59, 129.21, 127.31, 125.04, 75.04, 69.70, 64.30, 42.94, 34.78, 33.22, 31.12, 26.60, 20.99, 20.61, 18.67; ESI-MS: 480.14 $[M+Na]^+$; HPLC: t_R = 19.8 min, purity > 99.9% @ ELSD.

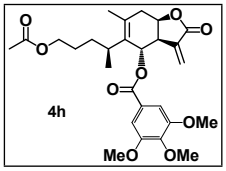


1-*O*-acetyl-6-*O*-(4-methylbenzoyl)britannilactone (4f): White solid. Yield: 98%;^{1}H NMR (500 MHz, $CDCl_3$) 8.27 (d, *J* = 8.8 Hz, 2H), 8.15 – 8.09 (m, 2H), 6.43 (d, *J* = 2.6 Hz, 1H, H-13a), 6.03 (d, *J* = 2.3 Hz, 1H, H-13b), 5.47 (d, *J* = 1.7 Hz, 1H, H-6), 4.98 (ddd, *J* = 7.7, 3.7, 2.1 Hz, 1H, H-8), 4.02 – 3.85 (m, 2H, H-1), 3.66 – 3.60 (m, 1H, H-7), 2.85 – 2.69 (m, 2H, H-9a, H-4), 2.59 (dd, *J* = 16.2, 2.0 Hz, 1H, H-9b), 2.03 (s, 3H, AcO-1), 1.87 (s, 3H, H-14), 1.49 – 1.38 (m, 1H, H-2a), 1.29 (ddd, *J* = 10.9, 7.3, 3.0 Hz, 2H, H-2b, H-3a), 1.24 (s, 3H), 1.07 (d, *J* = 10.5 Hz, 1H, H-3b), 0.86 (dd, *J* = 12.6, 7.0 Hz, 3H, H-15).^{13}C NMR (126 MHz, $CDCl_3$) δ 171.16, 169.26, 164.56, 150.76, 136.10, 135.34, 134.79, 131.86, 130.68, 125.33, 123.71, 74.63, 70.98, 64.17, 42.91, 34.79, 33.17, 31.14, 29.70, 26.58, 20.97, 20.71, 18.71; ESI-MS: 449.15 $[M+Na]^+$; HPLC: t_R = 17.7 min, purity > 99.9% @ ELSD.

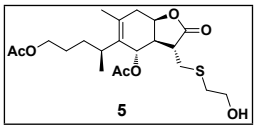


1-*O*-acetyl-6-*O*-(4-methoxybenzoyl)britannilactone (4g): White solid. Yield: 80%; ^{1}H NMR (500 MHz, $CDCl_3$) 7.98 – 7.82 (m, 2H), 6.98 – 6.87 (m, 2H), 6.40 (d, *J* = 2.7 Hz, 1H, H-13a), 6.02 (d, *J* = 2.3 Hz, 1H, H-13b), 5.42 (d, *J* = 1.8 Hz, 1H, H-6), 4.96 (ddd, *J* = 7.7, 3.7, 2.1 Hz, 1H, H-8), 4.02 – 3.88 (m, 2H, H-1), 3.86 (s, 3H), 3.66 – 3.60 (m, 1H, H-7), 2.82 (dd, *J* = 16.1, 2.7 Hz, 1H, H-9a), 2.71 (d, *J* = 1.7 Hz, 1H, H-4), 2.55 (dd, *J* = 16.1, 2.1 Hz, 1H, , H-9b), 2.03 (d, *J* = 1.2 Hz, 5H, AcO-1), 1.85 (s, 3H, H-14), 1.42 (s, 1H, H-2a), 1.29 (dt, *J* = 6.8, 3.5 Hz, 2H, H-2b, H-3a), 1.08 (d, *J* =

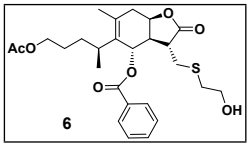
9.9 Hz, 1H, H-3b), 0.88 (d, *J* = 6.9 Hz, 3H, H-15). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.21, 169.61, 166.11, 163.71, 136.43, 133.75, 132.48, 131.61, 125.00, 122.41, 113.81, 75.05, 69.57, 64.29, 55.51, 42.96, 34.78, 33.22, 31.11, 26.60 , 20.99, 20.60, 18.67, 14.21; ESI-MS: 465.09 $[M+Na]^+$; HPLC: t_R = 18.6 min, purity > 99.9% @ ELSD.



1-*O*-acetyl-6-*O*-(3,4,5-trimethoxybenzoyl)britannilactone (4h): Yellow solid. Yield: 83%;^{1}H NMR (500 MHz, $CDCl_3$) δ 7.20 (s, 2H), 6.41 (d, *J* = 2.6 Hz, 1H, H-13a), 6.01 (d, *J* = 2.3 Hz, 1H, H-13b), 5.42 (d, *J* = 1.6 Hz, 1H, H-6), 4.96 (ddd, *J* = 7.6, 3.5, 2.1 Hz, 1H, H-8), 4.01 – 3.91 (m, 2H, H-1), 3.89 (d, *J* = 2.5 Hz, 9H), 3.64 – 3.60 (m, 1H, H-7), 2.81 – 2.69 (m, 2H, H-9a, H-4), 2.56 (dd, *J* = 16.1, 2.0 Hz, 1H, H-9b), 2.03 (d, *J* = 3.4 Hz, 3H, AcO-1), 1.86 (s, 3H, H-14), 1.43 (dd, *J* = 11.3, 6.4 Hz, 1H, H-2a), 1.34 – 1.22 (m, 2H, H-2b, H-3a), 1.14 – 1.03 (m, 1H, H-3b), 0.90 (d, *J* = 6.9 Hz, 3H, H-15). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.29, 169.57, 166.18, 153.20, 142.98, 136.46, 134.09, 132.43, 125.18, 125.05, 107.14, 75.01, 70.26, 64.34, 61.06, 56.48, 43.06, 34.87, 33.31, 31.25, 26.69, 21.08, 20.76, 18.84; ESI-MS: 525.13 $[M+Na]^+$; HPLC: t_R = 18.3 min, purity > 99.9% @ ELSD.



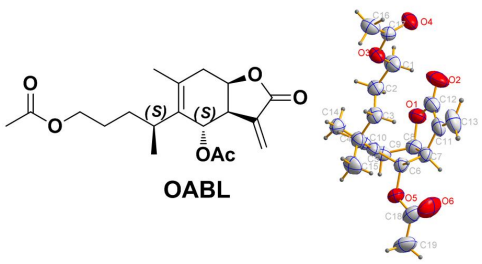
1,6-*O*,*O*-diacetyl-13-(2-hydroxyethylthiol) britannilactone (5): Colorless oil. Yield: 99%; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.38 (d, *J* = 1.7 Hz, 1H, H-6), 4.94 (ddd, *J* = 8.0, 5.2, 2.4 Hz, 1H, H-8), 4.04 (t, *J* = 6.4 Hz, 2H, H-1), 3.78 (td, *J* = 5.8, 4.7 Hz, 2H), 3.08 (dd, *J* = 13.4, 4.1 Hz, 1H, H-7), 2.97 – 2.92 (m, 1H), 2.87 (dd, *J* = 13.4, 7.5 Hz, 1H), 2.80 (td, *J* = 6.1, 2.8 Hz, 2H), 2.77 – 2.67 (m, 2H), 2.50 (td, *J* = 7.1, 4.2 Hz, 1H, H-9a), 2.38 (dd, *J* = 16.9, 2.4 Hz, 1H, H-9b), 2.06 (s, 3H, AcO-1), 2.03 (s, 3H, AcO-6), 1.81 (s, 3H, H-14), 1.62 – 1.54 (m, 1H, H-2a), 1.49 – 1.34 (m, 2H, H-2b, H-3a), 1.31 – 1.24 (m, 1H, H-3b), 0.92 (d, *J* = 6.9 Hz, 3H, H-15). ^{13}C NMR (125 MHz, $CDCl_3$) δ 176.80, 171.41, 170.95, 134.55, 131.37, 77.48, 77.16, 76.84, 75.89, 67.86, 64.31, 60.96, 44.81, 43.63, 36.48, 35.09, 33.56, 33.18, 31.59, 26.85, 21.40, 21.14, 20.40, 18.27. HPLC: t_R = 12.3min, purity > 99.9% @ ELSD.



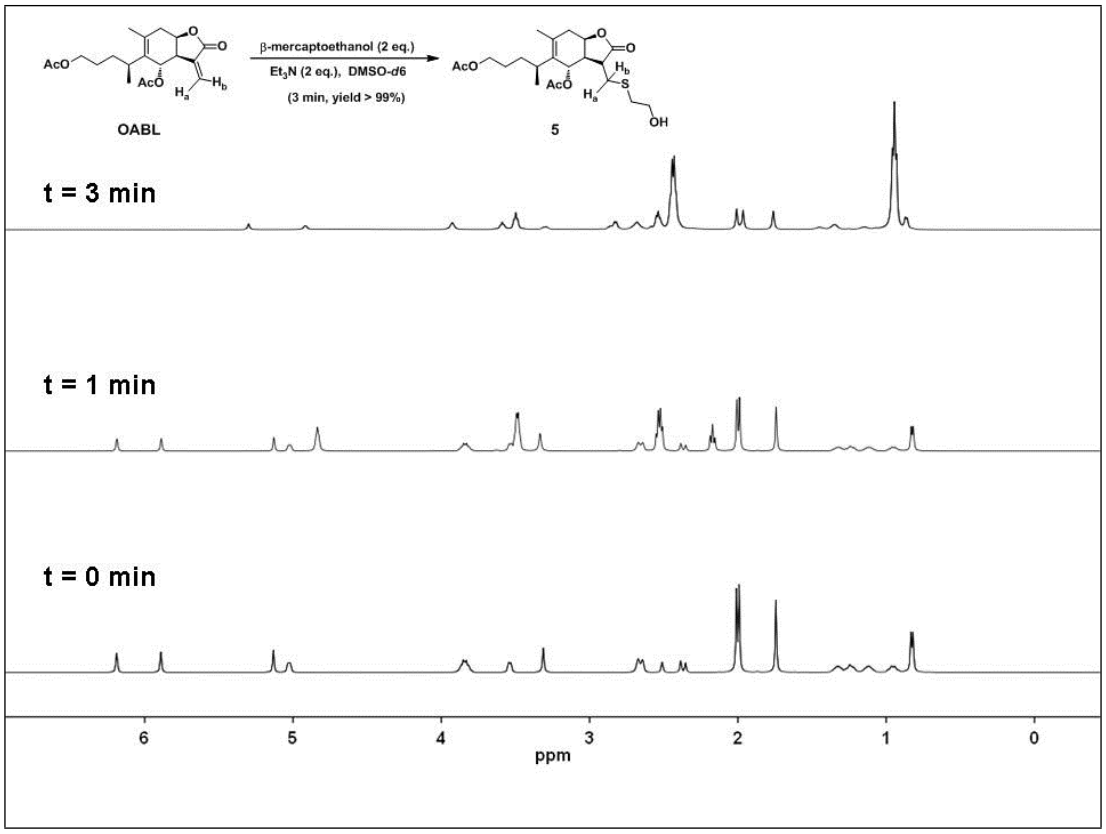
1-*O*-acetyl-6-*O*-benzoyl-13-(2-hydroxyethylthiol) britannilactone (6): Colorless oil. Yield: 99%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.94 (d, *J* = 7.3 Hz, 2H), 7.66 (t, *J* = 7.3 Hz, 1H), 7.53 (t, *J* = 7.3 Hz, 2H), 5.57 (s, 1H, H-6), 4.98 (m, 1H, H-8), 3.93 (t, *J* = 6.0 Hz, 2 H, H-1), 3.61 (m, 2H), 3.01 (dd, *J* = 9.6, 6.2 Hz, 1H, H-7), 2.89 (d, *J* = 6.9 Hz, 2H, H-13), 2.66 – 2.80 (m, 4H), 2.00 (s, 3H, AcO-1), 1.82 (s, 3H, H-14), 1.42 – 1.50 (m, 1H, H-2a), 1.32 – 1.40 (m, 2H, H-2b, H-3a), 1.14 – 1.26 (m, 1H, H-3b), 0.84 (d, *J* = 6.9 Hz, 3H, H-15). ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.78, 170.35, 165.07, 134.73, 133.34, 131.19, 129.92, 129.17 (d), 128.74 (d), 75.76, 64.59, 63.69, 60.77, 44.36, 43.84, 34.81, 34.10, 33.70, 31.57, 26.85, 26.22, 20.65 (d), 18.82.

2. Table S1. Crystal data and structure refinement for OABL.

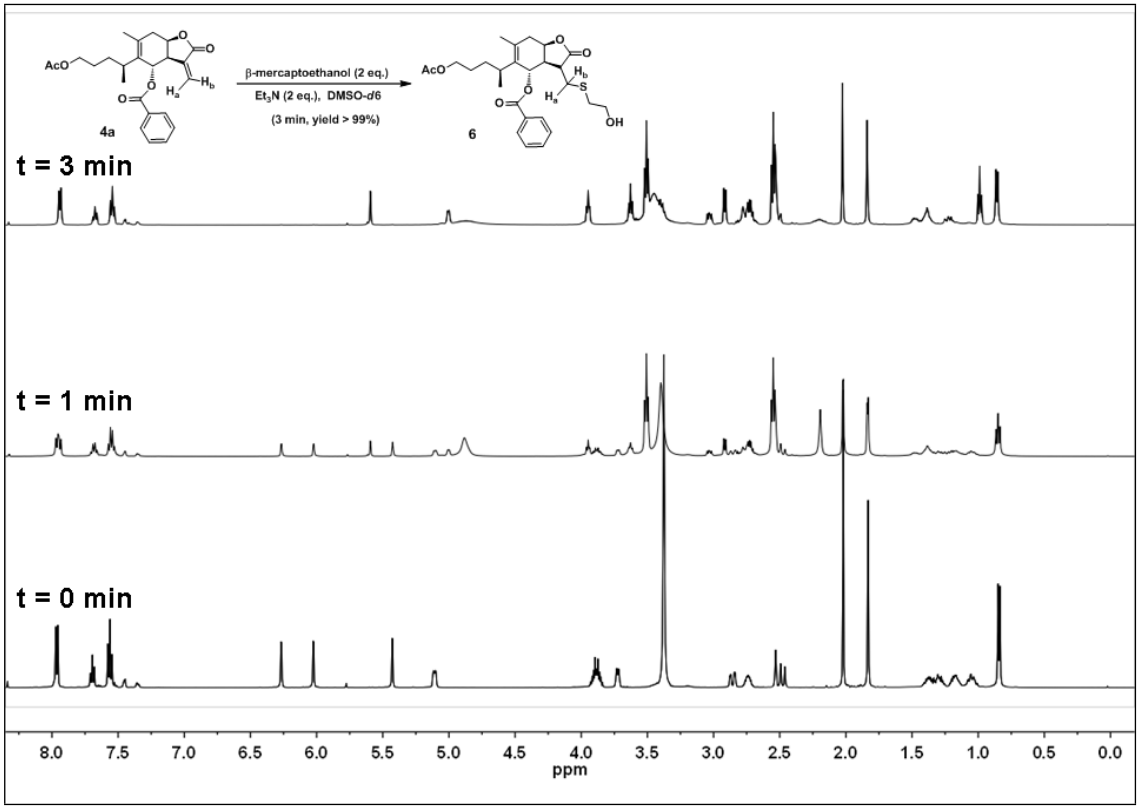
Empirical formula	$C_{19}H_{26}O_6$
Formula weight	350.40
Temperature/K	293(2)
Crystal system	orthorhombic
Space group	$P2_12_12_1$
a/Å	7.80161(14)
b/Å	15.0614(3)
c/Å	16.6418(3)
α /°	90
β /°	90
γ /°	90
Volume/Å^3	1955.46(6)
Z	4
ρ_{calc}mg/mm^3	1.190
m/mm^{-1}	0.726
F(000)	752.0
Crystal size/mm^3	0.32 × 0.31 × 0.29
Radiation	CuKα (λ = 1.54184)
2Θ range for data collection	7.918 to 141.584°
Index ranges	-8 ≤ h ≤ 9, -18 ≤ k ≤ 18, -20 ≤ l ≤ 20
Reflections collected	19655
Independent reflections	3742 [R_{int} = 0.0377, R_{sigma} = 0.0206]
Data/restraints/parameters	3742/0/239
Goodness-of-fit on F^2	1.037
Final R indexes [I>=2σ(I)]	R_1 = 0.0392, wR_2 = 0.1120
Final R indexes [all data]	R_1 = 0.0411, wR_2 = 0.1151
Largest diff. peak/hole / e Å^{-3}	0.21/-0.14
Flack parameter	0.08(9)



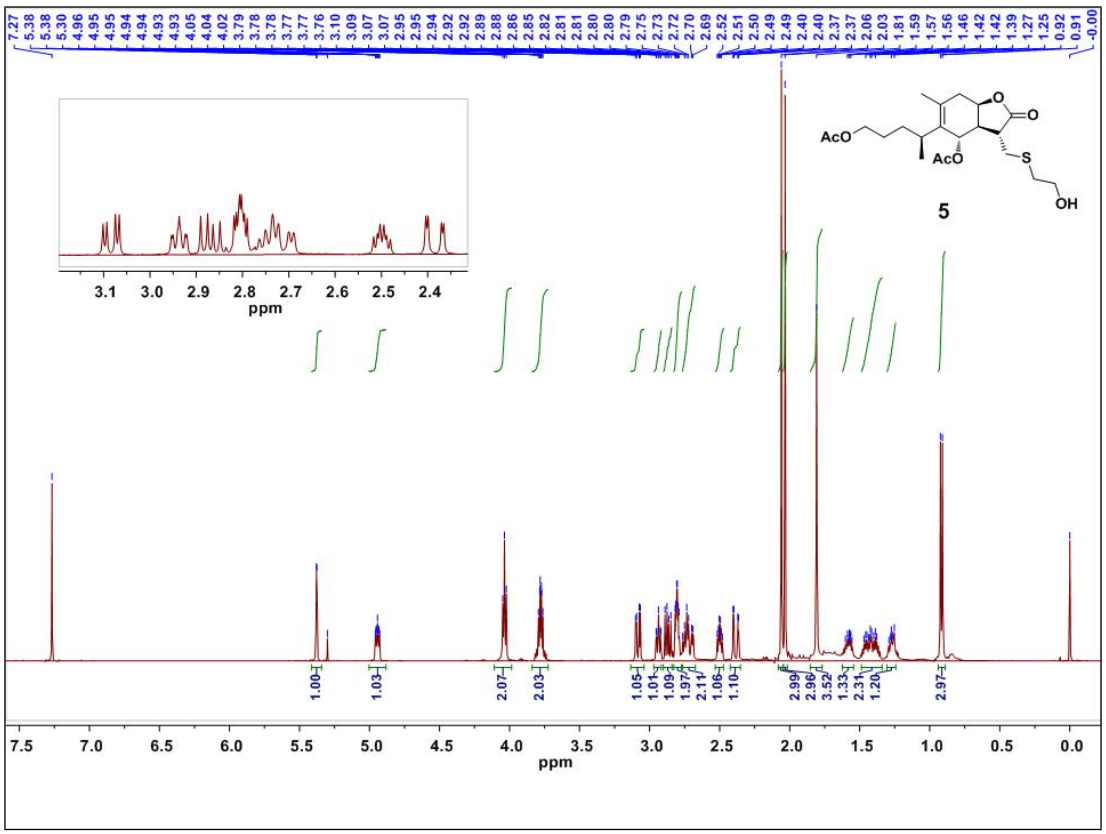
OABL



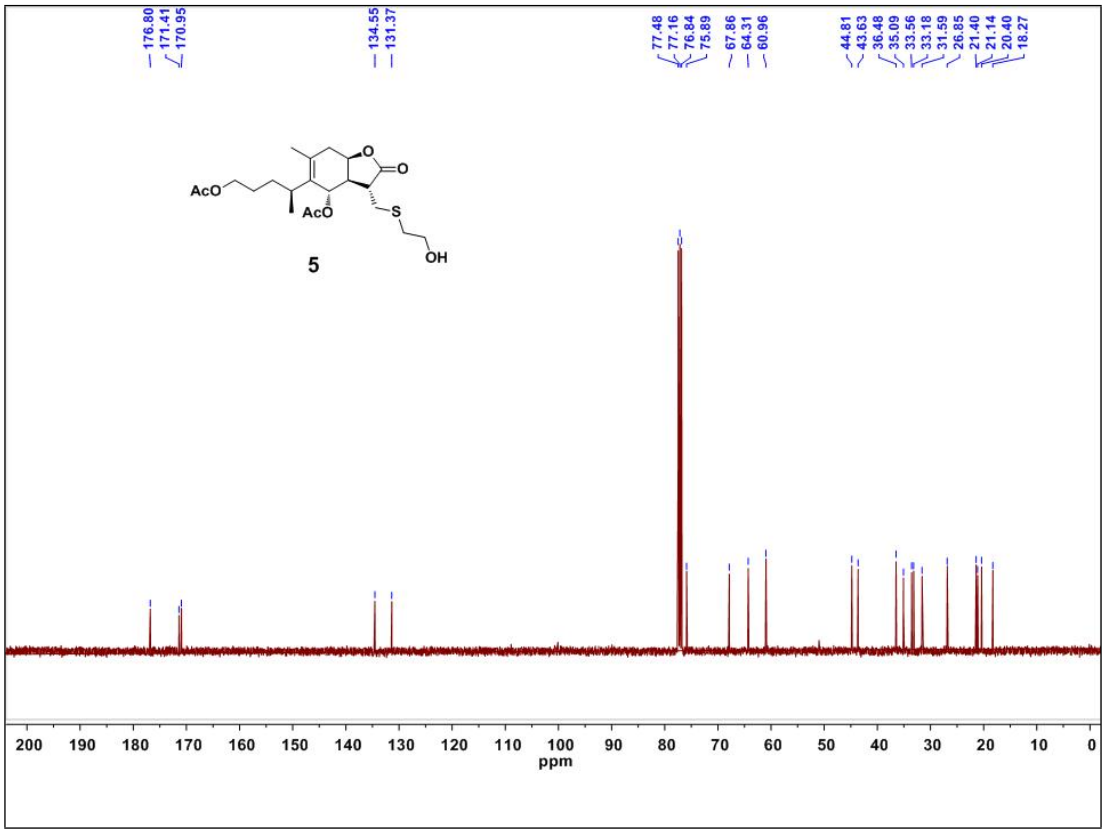
3. Figure S1. ^{1}H NMR Michael acceptor assay of OABL in DMSO-*d*6.



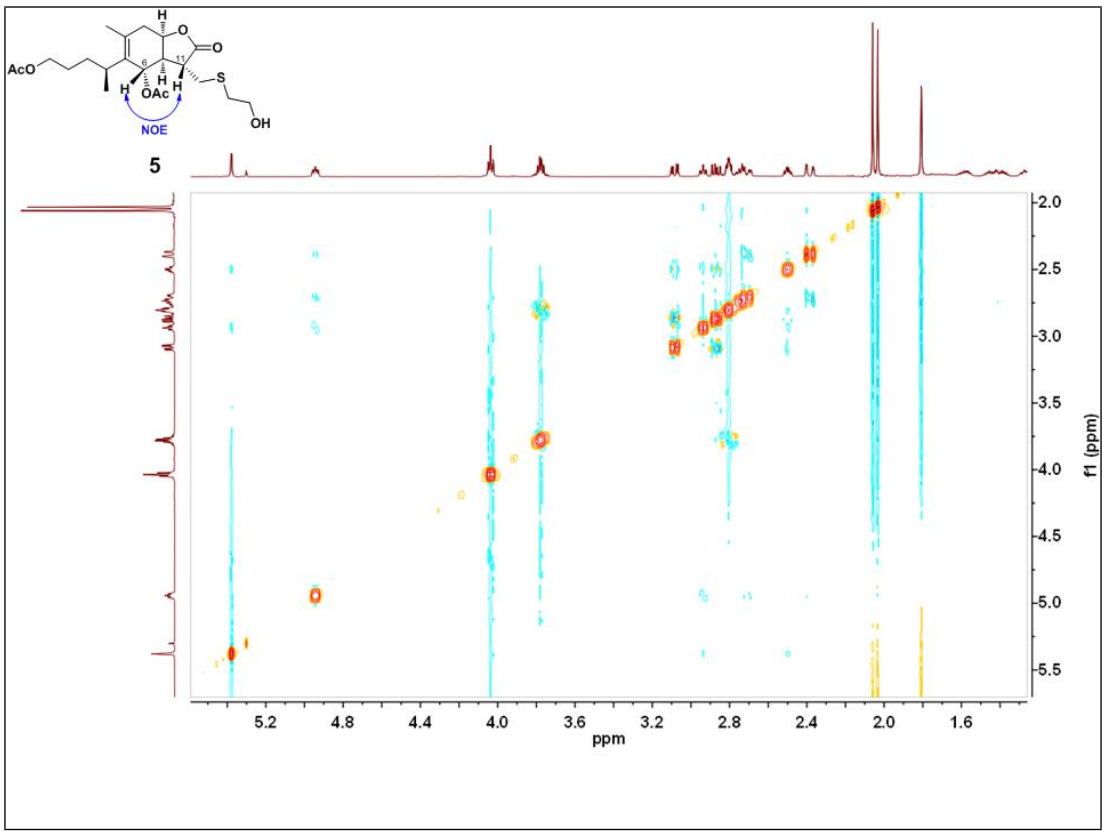
4. Figure S2. ^{1}H NMR Michael acceptor assay of **4a** in DMSO-*d*6.



5. Figure S3. ^{1}H NMR spectra of adduct **5** in $CDCl_3$**.**



6. Figure S4. ^{13}C NMR spectra of adduct **5** in $CDCl_3$**.**



7. Figure S5. NOESY spectra of adduct **5** in $CDCl_3$**.**

8. References

1. S. Dong, J.-J. Tang, C.-C. Zhang, J.-M. Tian, J.-T. Guo, Q. Zhang, H. Li and J.-M. Gao, *Eur. J. Med. Chem.*, 2014, **80**, 71-82.
2. C. Avonto, O. Taglialatela-Scafati, F. Pollastro, A. Minassi, V. Di Marzo, L. De Petrocellis and G. Appendino, *Angew. Chem. Int. Ed. Engl.*, 2011, **50**, 467-471.
3. P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. McMahon, D. Vistica, J. T. Warren, H. Bokesch, S. Kenney and M. R. Boyd, *J. Natl. Cancer I.*, 1990, **82**, 1107-1112.
4. J.-J. Tang, G.-J. Fan, F. Dai, D.-J. Ding, Q. Wang, D.-L. Lu, R.-R. Li, X.-Z. Li, L.-M. Hu, X.-L. Jin and B. Zhou, *Free Radic. Biol. Med.*, 2011, **50**, 1447-1457.