SUPPORTING INFORMATION

# Cyanobufalins: novel cardioactive toxins from cyanobacterial blooms

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This Supporting Information consists of 38 pages including 21 Figures of NMR spectra, 2 Tables of NMR data, phytoplankton survey, procedures for acquisition of HRMS and NMR data, cytotoxicity assay procedures, NCI 60-cell line data, and a summary of the cardiomyocyte assay results.

**GLSM Whole Water Phytoplankton Enumeration**.

## HRMS analysis procedures

**General procedures for NMR analyses**
**NMR Spectroscopic data**



**Cytotoxicity (MTT) Assay for guiding fractionation and purification.**



**Sundia cytotoxicity procedure**

**Cardiomyocyte Assay Results**
**Effect of Bufalin**

Figure S23. Cell Index Time Course Plot for cyanobufalin A
Figure S24. Effect of Bufalin on Beat Rate
Table S5. ΔBeat Rate versus Bufalin concentration
Figure S25. Effect of Bufalin on Amplitude (contractility)
Table S6. Change in Amplitude (contractility) versus Bufalin concentration

**Effect of Cyanobufalin A (BSP-501).**
Figure S26. Effect of cyanobufalin A on Beat Rate
Table S7. ΔBeat Rate versus cyanobufalin A concentration
Figure S27. Effect of cyanobufalin A on Amplitude (contractility)
Table S8. Change in Amplitude (contractility) versus cyanobufalin A concentration

**GLSM Whole Water Phytoplankton Ennumeration**. The following data were generated for the Ohio Environmental Protection Agency by BSA Environmental Services, Inc. 23400 MERCANTILE RD., SUITE 8, BEACHWOOD, OH 44122 on May 8, 2012, one week prior to the harvesting period. Analysis includes the microscopic tally of various species (Genus) with calculated values for density (cells/L) and biovolume ($um^3$/L). At each sampling site *Planktothrix agardhii* is the dominant organism.

| STATION | SAMPLE DATE | SAMPLE TIME | GENUS | DIVISION | TALLY REP 1 | DENSITY (cells/L) REP 1 | TOTAL BV $um^3$/L |
|---|---|---|---|---|---|---|---|
| L-1 | 5/8/12 | 10:54 | Nitzschia acicularis | Bacillariophyta | 1 | 2.38E+05 | 4.66E+07 |
| L-1 | 5/8/12 | 10:54 | Nitzschia sp. | Bacillariophyta | 1 | 2.38E+05 | 2.57E+07 |
| L-1 | 5/8/12 | 10:54 | Stephanodiscus hantzschii | Bacillariophyta | 6 | 1.43E+06 | 8.13E+08 |
| L-1 | 5/8/12 | 10:54 | Stephanodiscus parvus | Bacillariophyta | 16 | 3.80E+06 | 3.73E+08 |
| L-1 | 5/8/12 | 10:54 | Synedra tenera | Bacillariophyta | 2 | 4.75E+05 | 5.23E+07 |
| L-1 | 5/8/12 | 10:54 | Actinastrum hantzschii | Chlorophyta | 16 | 3.80E+06 | 2.09E+08 |
| L-1 | 5/8/12 | 10:54 | Dictyosphaerium pulchellum | Chlorophyta | 8 | 3.20E+04 | 2.09E+06 |
| L-1 | 5/8/12 | 10:54 | Monoraphidium sp. | Chlorophyta | 2 | 8.00E+03 | 6.37E+05 |
| L-1 | 5/8/12 | 10:54 | Scenedesmus dimorphus | Chlorophyta | 8 | 1.90E+06 | 8.36E+07 |
| L-1 | 5/8/12 | 10:54 | Cryptomonas spp. | Cryptophyta | 4 | 9.51E+05 | 1.97E+08 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| L-1 | 5/8/12 | 10:54 | Rhodomonas sp. | Cryptophyta | 18 | 4.28E+06 | 2.24E+08 |
| L-1 | 5/8/12 | 10:54 | Planktothrix agardhii | Cyanobacteria | 14824 | 3.52E+09 | 7.47E+10 |
| L-1 | 5/8/12 | 10:54 | Pseudanabaena sp. | Cyanobacteria | 296 | 7.03E+07 | 2.76E+08 |
| | | | TOTAL | | 15202 | 3.61E+09 | 7.70E+10 |
| L-2 | 5/8/12 | 10:10 | Cyclotella sp. | Bacillariophyta | 4 | 8.56E+05 | 4.07E+08 |
| L-2 | 5/8/12 | 10:10 | Discostella pseudostelligera | Bacillariophyta | 1 | 2.14E+05 | 1.81E+07 |
| L-2 | 5/8/12 | 10:10 | Nitzschia acicularis | Bacillariophyta | 4 | 8.56E+05 | 1.77E+08 |
| L-2 | 5/8/12 | 10:10 | Nitzschia sp. | Bacillariophyta | 1 | 4.00E+03 | 1.31E+06 |
| L-2 | 5/8/12 | 10:10 | Stephanocyclus meneghiniana | Bacillariophyta | 1 | 7.26E+04 | 5.78E+07 |
| L-2 | 5/8/12 | 10:10 | Stephanodiscus parvus | Bacillariophyta | 16 | 3.42E+06 | 2.90E+08 |
| L-2 | 5/8/12 | 10:10 | Synedra cf. delicatissima | Bacillariophyta | 1 | 4.00E+03 | 4.95E+06 |
| L-2 | 5/8/12 | 10:10 | Actinastrum hantzschii | Chlorophyta | 28 | 5.99E+06 | 3.29E+08 |
| L-2 | 5/8/12 | 10:10 | Closteriopsis longissima | Chlorophyta | 1 | 7.26E+04 | 4.93E+07 |
| L-2 | 5/8/12 | 10:10 | Dictyosphaerium pulchellum | Chlorophyta | 8 | 5.81E+05 | 8.22E+06 |
| L-2 | 5/8/12 | 10:10 | Oocystis parva | Chlorophyta | 4 | 2.91E+05 | 3.04E+06 |
| L-2 | 5/8/12 | 10:10 | Scenedesmus dimorphus | Chlorophyta | 12 | 2.57E+06 | 5.91E+07 |
| L-2 | 5/8/12 | 10:10 | Scenedesmus opoliensis | Chlorophyta | 4 | 2.91E+05 | 1.37E+07 |
| L-2 | 5/8/12 | 10:10 | Cryptomonas sp. | Cryptophyta | 1 | 2.14E+05 | 1.00E+08 |
| L-2 | 5/8/12 | 10:10 | Rhodomonas sp. | Cryptophyta | 16 | 3.42E+06 | 1.79E+08 |
| L-2 | 5/8/12 | 10:10 | Planktothrix agardhii | Cyanobacteria | 19700 | 4.21E+09 | 6.21E+10 |
| L-2 | 5/8/12 | 10:10 | Pseudanabaena limnetica | Cyanobacteria | 146 | 3.12E+07 | 1.23E+08 |
| | | | TOTAL | | 19948 | 4.26E+09 | 6.39E+10 |
| L-3 | 5/8/12 | 11:34 | Aulacoseira granulata | Bacillariophyta | 15 | 4.58E+06 | 8.75E+08 |
| L-3 | 5/8/12 | 11:34 | Discostella sp. | Bacillariophyta | 8 | 2.44E+06 | 2.07E+08 |
| L-3 | 5/8/12 | 11:34 | Nitzschia acicularis | Bacillariophyta | 2 | 1.45E+05 | 3.40E+07 |
| L-3 | 5/8/12 | 11:34 | Stephanocyclus meneghiniana | Bacillariophyta | 2 | 6.11E+05 | 2.40E+08 |
| L-3 | 5/8/12 | 11:34 | Stephanodiscus hantzschii | Bacillariophyta | 7 | 2.14E+06 | 1.22E+09 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| L-3 | 5/8/12 | 11:34 | Stephanodiscus parvus | Bacillariophyta | 8 | 2.44E+06 | 1.44E+08 |
| L-3 | 5/8/12 | 11:34 | Synedra tenera | Bacillariophyta | 1 | 3.06E+05 | 3.02E+07 |
| L-3 | 5/8/12 | 11:34 | Synedra ulna | Bacillariophyta | 1 | 3.06E+05 | 4.00E+08 |
| L-3 | 5/8/12 | 11:34 | Actinastrum hantzschii | Chlorophyta | 21 | 6.42E+06 | 3.53E+08 |
| L-3 | 5/8/12 | 11:34 | Closteriopsis sp. | Chlorophyta | 1 | 3.06E+05 | 5.23E+07 |
| L-3 | 5/8/12 | 11:34 | Dictyosphaerium chlorelloides | Chlorophyta | 16 | 4.89E+06 | 3.20E+08 |
| L-3 | 5/8/12 | 11:34 | Pediastrum duplex | Chlorophyta | 7 | 2.80E+04 | 1.39E+08 |
| L-3 | 5/8/12 | 11:34 | Scenedesmus dimorphus | Chlorophyta | 2 | 8.00E+03 | 7.92E+05 |
| L-3 | 5/8/12 | 11:34 | Scenedesmus sp. | Chlorophyta | 8 | 5.81E+05 | 9.74E+06 |
| L-3 | 5/8/12 | 11:34 | Mallomonas sp. | Chrysophyta | 1 | 7.26E+04 | 5.36E+07 |
| L-3 | 5/8/12 | 11:34 | Cryptomonas sp. | Cryptophyta | 2 | 6.11E+05 | 2.25E+08 |
| L-3 | 5/8/12 | 11:34 | Rhodomonas sp. | Cryptophyta | 12 | 3.67E+06 | 1.92E+08 |
| L-3 | 5/8/12 | 11:34 | Planktothrix agardhii | Cyanobacteria | 15536 | 4.75E+09 | 6.99E+10 |
| L-3 | 5/8/12 | 11:34 | Pseudanabaena limnetica | Cyanobacteria | 188 | 5.74E+07 | 2.71E+08 |
| L-3 | 5/8/12 | 11:34 | Gymnodinium discoidale | Pyrrophyta | 2 | 8.00E+03 | 1.47E+06 |
| | | | TOTAL | | 15840 | 4.83E+09 | 7.47E+10 |

**HRMS analysis.** Accurate mass measurements were obtained with a UPLC-QTOFMS operated using an Acquity UPLC system (Waters Corporation, Milford, MA, USA) coupled with a QTOF-MS (Xevo G2 QTOF, Waters MS Technologies, Manchester, UK), controlled by MassLynx v4.1 software. MS were acquired in both positive and negative modes over the range m/z 100–1000 Da in two chanels with scan time 1s. The capillary voltages were set at 3000 V (positive mode) and 2500 V (negative mode), respectively, and the cone voltage was 20V. Nitrogen gas was used both for the nebulizer and in desolvation. The desolvation and cone gas flow rates were 600 and 20 L/h, respectively. Desolvation temperature was 250°C, and the source temperature was

100 °C. The lock mass solution of Leucine Enkephalin (1µg/mL) in acetonitrile/water (1:1) containing 0.1% formic acid was utilized as the lock mass at a flow rate 10 µL/mL, which m/z 556.2771 for positive mode and m/z 554.2615 for negative mode.

**General procedures for NMR analyses.** 1D and 2D NMR data were acquired at 298 K on a Bruker 800 MHz NMR spectrometer equipped with a triple resonance (TXI) cryoprobe. Samples were dissolved in ca. 0.6 ml of deuterated dimethylsulfoxide (DMSO-$d_6$) with deuterium serving as the lock nucleus. The NMR experiments were performed using Bruker's pulse programs at their default settings, but when necessary, some parameters including number of scans (NS), spectral width (SW), transmitter frequency offset (O1P), size of fid (TD), and delays (D[#]) were modified. $^{1}$H-$^{1}$H geminal and vicinal coupling were obtained using double quantum filtered COSY (DQF-COSY). Heteronuclear single quantum correlation (HSQC) data were acquired with $^{1}J_{CH}$ optimized for 145 Hz. The mixing time for 2D Total Correlation Spectroscopy (2D-TOCSY) was 60 ms while that for 2D Nuclear Overhauser Effect (2D-NOESY) was 500 ms. For Heteronuclear Multiple Bond Correlation (HMBC) experiments, long range $^{1}$H-$^{13}$C coupling was optimized for 8 Hz, and $^{13}$C data were acquired in proton decoupled mode. The acquired NMR data were processed using Topspin software (version 2.1, 3.2 or 3.5).

Table S1. NMR Data for Cyanobufalin B (**2**) in DMSO-$d_6$.

| No. | $\delta_H$ ($J$ in Hz) | $\delta_C$ | $^{1}$H-$^{13}$C HMBC Correlation | NOESY Correlation |
|---|---|---|---|---|
| 1 | 3.65, d (3.8) | 54.2, CH | C-2, C-5, C-10 | H-2, H-9, H-11a, H-11b |
| 2 | 3.10, d (3.8) | 56.6, CH | C-1, C-3, C-4, C-25 | H-1, H-3, H-9, H-11a |
| 3 | 4.27, s | 75.8, CH | C-1, C-2, C-4, C-25, C-26, C-27 | H-1, H-2, H-5, H-9, H-25 |
| 4 | | 36.0, qC | | |
| 5 | 1.49, ovlp | 40.8, CH | C-3, C-4, C-6, C-9, C-10, C-19, C-26 | H-3, H-6a, H-6b, H-25 |
| 6a | 2.44, m | 22.5, $CH_2$ | C-4, C-5, C-7, C-8, C-10 | H-5, H-6b, H-7, H-25, H-26 |
| 6b | 2.25, m | | C-5, C-7, C-8, C-10 | H-5, H-6a, H-7, H-25 |
| 7 | 6.11, m | 121.4, CH | C-5, C-6, C-8, C9, C-14 | H-6a, H-6b, 14-OH |

| | | | | |
|---|---|---|---|---|
| 8 | | 139.2, qC | | |
| 9 | 2.47, m | 41.4, CH | C-1, C-5, C-7, C-8, C-10, C-11 | H-1, H-3, 11a, 11b, 12a, 12b, 15a, 15b |
| 10 | | 50.0, qC | | |
| 11a | 1.72, m | 23.1, $CH_2$ | C-8, C-9, C-12, C-13 | H-1, H-9, H-11b, H-12a, H-12b, H-18 |
| 11b | 0.99, m | | C-8, C-9, C-10, C-12 | H-1, H-11a, H-12a, H-12b, H-18, H-19 |
| 12a | 1.58, td (14.0, 3.0) | 37.6, $CH_2$ | C-9, C-11, C-13, C-14, C-17, C-18 | H-9, H-11a, H-11b, H-12b, H-15a, H-16, H-17 |
| 12b | 1.48, ovlp | | C-9, C-11, C-13, C-14, C-17, C-18 | H-11a, H-11b, H-12a, H-17, H-18 |
| 13 | | 50.6, qC | | |
| 14 | | 82.9, qC | | |
| 15a | 2.99, dd (15.5, 9.7) | 50.5, $CH_2$ | C-8, C-16, C17 | H-9, H-12a, H-15b, H-16 |
| 15b | 1.96, dd (15.7, 2.6) | | C-13, C-14, C-16, C-17, C-18 | H-15a, H-16 |
| 16 | 5.00, td (9.5, 2.6) | 60.2, CH | C-14, C-15, C-17, C-22 | H-12a, H-15a, H-15b, H-17 |
| 17 | 3.09, d (9.5) | 56.8, CH | C-12, C-13, C-14, C-16, C-20, C-21, C-22 | H-12a, H-12b, H-16, H-23 |
| 18 | 0.60, 3H, s | 17.2, $CH_3$ | C-12, C-13, C-14, C-17 | H-11a, H-11b, H-12b, H-17, H-19, H-21, H-22 |
| 19 | 9.82, s | 207.8, CH | C-1, C-10 | H-6a, H-7, H-11b, H-21 |
| 20 | | 119.5, qC | | |
| 21 | 7.60, d (2.1) | 152.0, CH | C-17, C-20, C-22, C-24 | H-12a, H-12b, H-16, H-17, H-18 |
| 22 | 8.25, dd (9.8, 2.3) | 150.8, CH | C-17, C-21, C-24 | 14-OH, H-15b, H-17, H-18, H-23 |
| 23 | 6.25, d (9.8) | 112.3, CH | C-20, C-22, C-24 | H-22 |
| 24 | | 161.6, qC | | |
| 25 | 0.80, 3H, s | 25.1, $CH_3$ | C-3, C-4, C-5, C-26 | H-3, H-5, H-6a, H-6b, H-7 |
| 26 | 0.65, 3H, s | 17.1, $CH_3$ | C-3, C-4, C-5, C-25 | H-6a, H-6b, H-19 |
| 27 | | 157.0, qC | | |
| $NH_2$ | 6.75, bs | | C-27 | NH at 6.60 |
| | 6.60, bs | | C-27 | NH at 6.75 |
| 14-OH | 5.13, bs | | C-14, C-15 | |

Table S2. NMR Data for Cyanobufalin C (**3**) in DMSO-$d_6$.

| No. | $\delta_H$ ($J$ in Hz) | $\delta_C$ | $^1$H-$^{13}$C HMBC Correlation | NOESY Correlation |
|---|---|---|---|---|
| 1 | 4.59, t (5.5) | 64.2, CH | C-2, C-3, C-5, C-10, C-19 | H-5, H-9, H-11a |
| 2 | 4.80, dd (7.9, 5.7) | 80.4, CH | C-1, C-3, C-4, C-10, C-27 | H-5, H-9 |
| 3 | 4.58, d (7.6) | 82.3, CH | C-1, C-2, C-4, C-25, C-26, C-27 | H-2, H-5 |
| 4 | | 36.2, qC | | |
| 5 | 1.83, dd (12.6, 5.2) | 41.4, CH | C-3, C-4, C-6, C-9, C-10, C-26 | H-3, H-6b, H-9, H-25 |
| 6a | 2.51, m | 22.8, $CH_2$ | C-5, C-7, C-8, C-9 | H-5, H-6b, H-7, H-26 |
| 6b | 2.27, m | | C-5, C-7, C-8, C-10 | H-5, H-7, H-20 |
| 7 | 6.09, m | 120.5, CH | C-5, C-6, C-8, C-9, C-14 | H-6a, H-6b, 14-OH |
| 8 | | 139.9, qC | | |
| 9 | 2.84, m | 39.3, CH | C-10 | H-11a, H-12a, H-15a |
| 10 | | 53.3, qC | | |
| 11a | 1.85, m | 24.7, $CH_2$ | C-8, C-9, C-12, C-13 | H-1, H-11b, H-12a, H-12b, H-18 |
| 11b | 0.84, m | | C-8, C-9, C-10, C-12, C-13 | H-11a, H-12b, H-18, H-19 |
| 12a | 1.48, td (14.0, 3.0) | 38.3, $CH_2$ | C-9, C-11, C-13, C-17, C-18 | H-9, H-11a, H-11b, H-15a, H-16, H-17, H-18 |
| 12b | 1.42, dt (14.0) | | C-9, C-11, C-13, C-14, C-18 | H-11a, H-11b, H-17, H-18 |

| | | | | |
|---|---|---|---|---|
| 13 | | 50.7, qC | | |
| 14 | | 83.2, qC | | |
| 15a | 3.00, dd (15.6, 10.0) | 50.5, $CH_2$ | C-8, C-17 | H-9, H-15b, H-16 |
| 15b | 1.97, dd (15.7, 2.8) | | C-13, C-14, C-16, C-17 | 14-OH, H-15a, H-16 |
| 16 | 5.03, td (9.5, 2.8) | 60.3, CH | C-14, C-15, C-21 | H-12a, H-15a, H-17 |
| 17 | 3.09, d (9.3) | 56.8, CH | C-12, C-13, C-15, C-16, C-20, C-21, C-22 | H-12a, H-12b, H-16, H-21 |
| 18 | 0.59, 3H, s | 17.2, $CH_3$ | C-12, C-13, C-14, C-17 | H-12b, H-17, H-19, H-22 |
| 19 | 9.73, s | 205.8, CH | C-1, C-10 | H-6a, H-11b, H-18, H-26 |
| 20 | | 119.3, qC | | |
| 21 | 7.58, d (2.5) | 151.8, CH | C-17, C-20, C-22, C-24 | H-12b, H-17, H-18 |
| 22 | 8.25, dd (9.8, 2.5) | 150.5, CH | C-21, C-24 | 14-OH, H-23 |
| 23 | 6.25, d (9.8) | 112.0, CH | C-20, C-24 | H-22 |
| 24 | | 161.6, qC | | |
| 25 | 1.02, 3H, s | 29.1, $CH_3$ | C-3, C-4, C-5, C-26 | H-5, H-6b |
| 26 | 0.73, 3H, s | 18.4, $CH_3$ | C-3, C-4, C-5, C-25 | H-19 |
| 27 | | 154.8, qC | | |
| 1-OH | 5.87, d (6.0) | | C-1, C-2, C-10 | |
| 14-OH | 5.09, bs | | C-14, C-15 | |

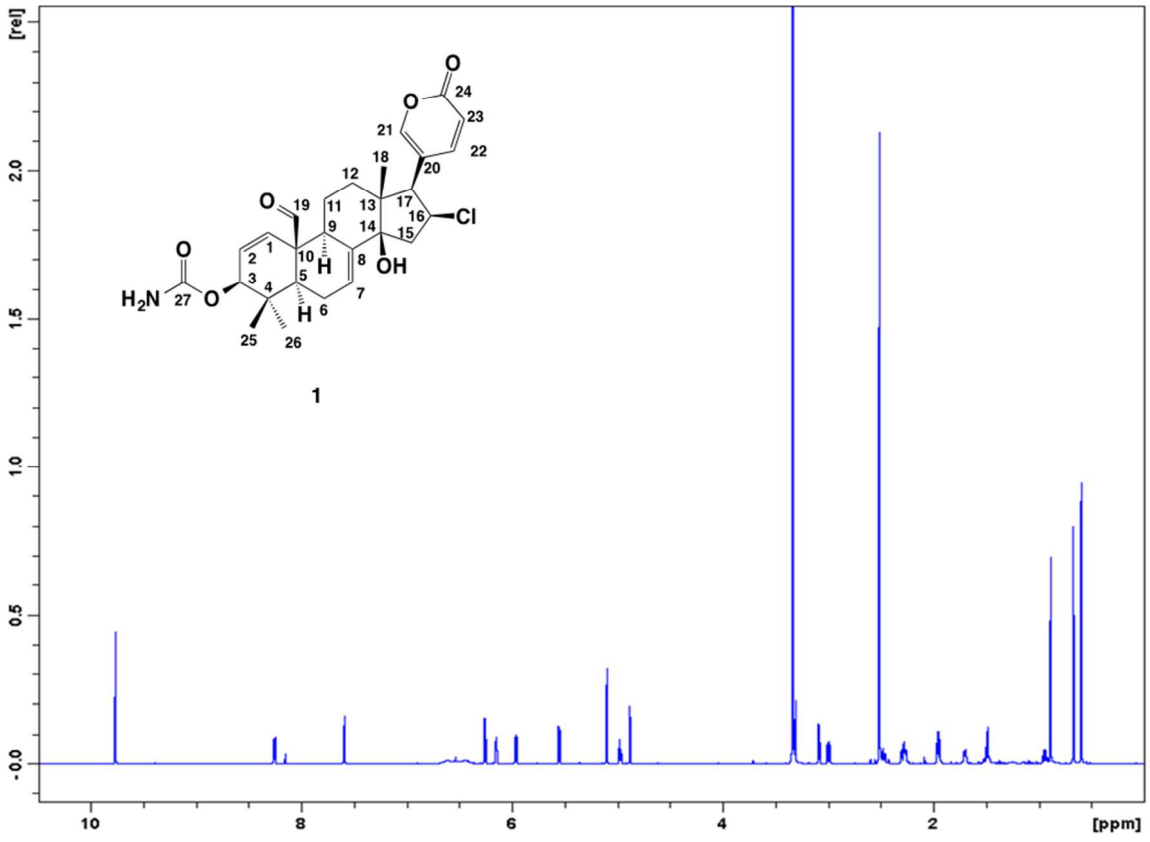



Figure S1. $^1$H NMR spectrum (800 MHz, DMSO-$d_6$) of **1**.

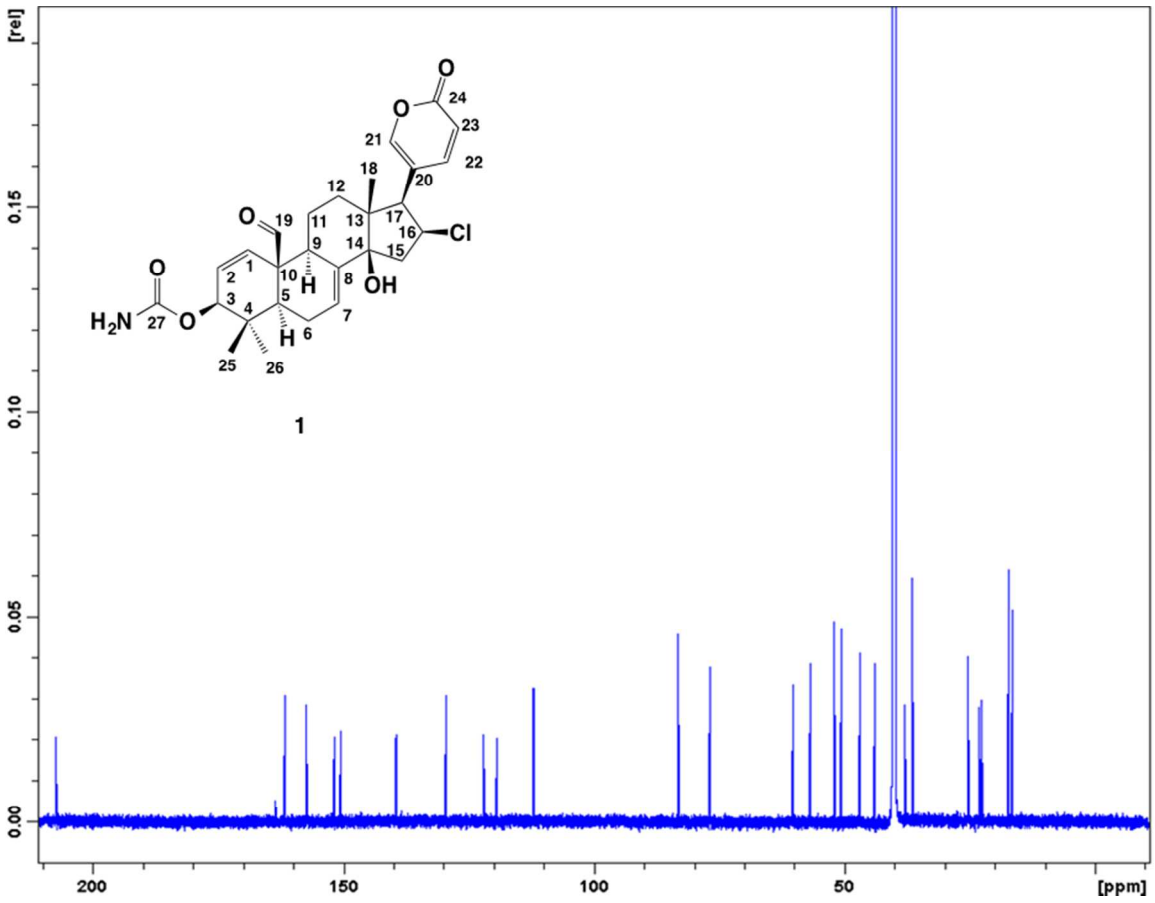



Figure S2. $^{13}$C NMR spectrum (200 MHz, DMSO-$d_6$) of **1**.

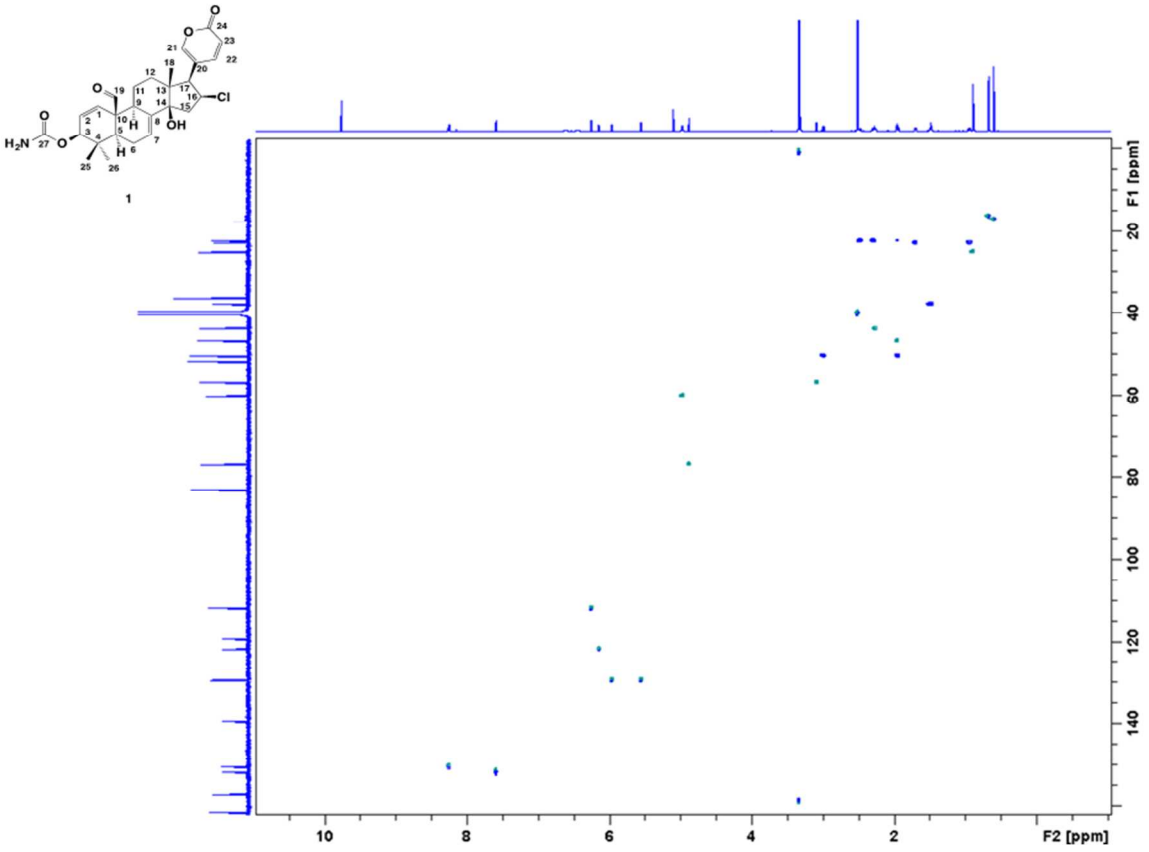



Figure S3. Multiplicity-edited HSQC of **1**.

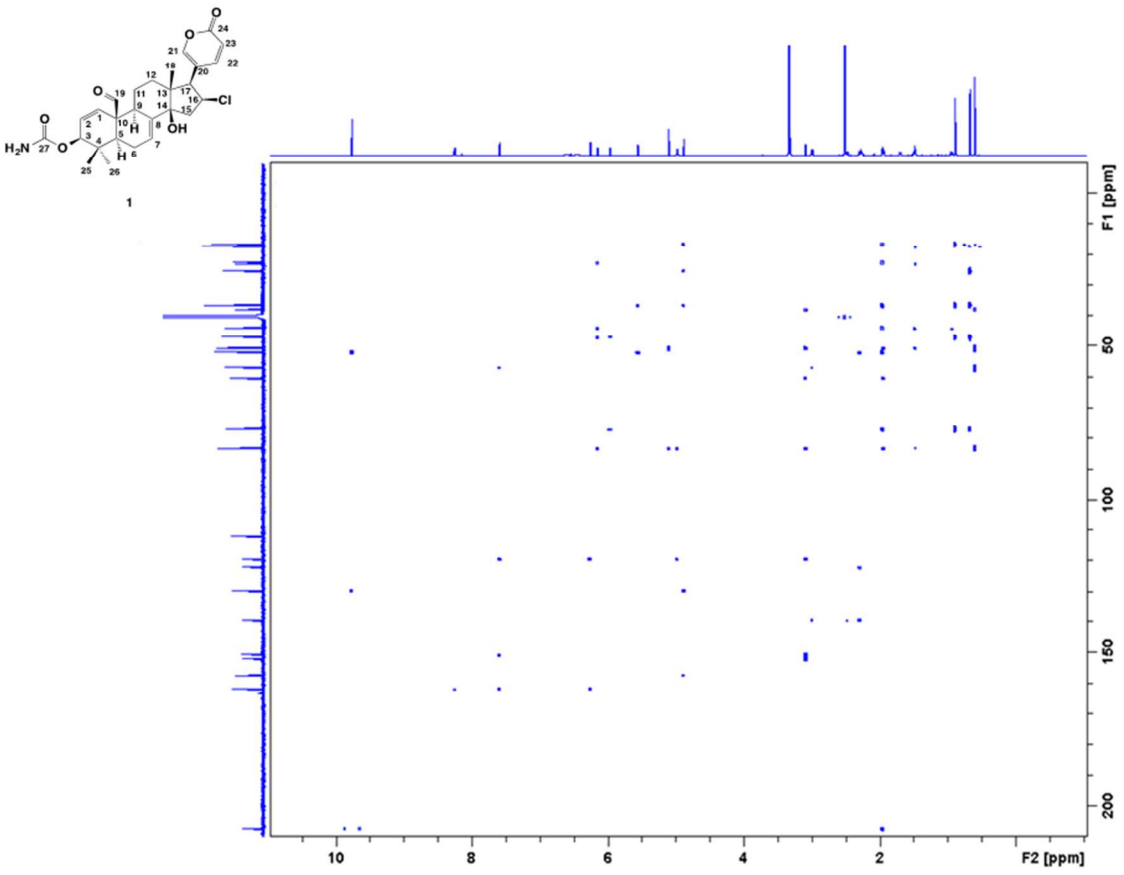



Figure S4. HMBC of **1**.

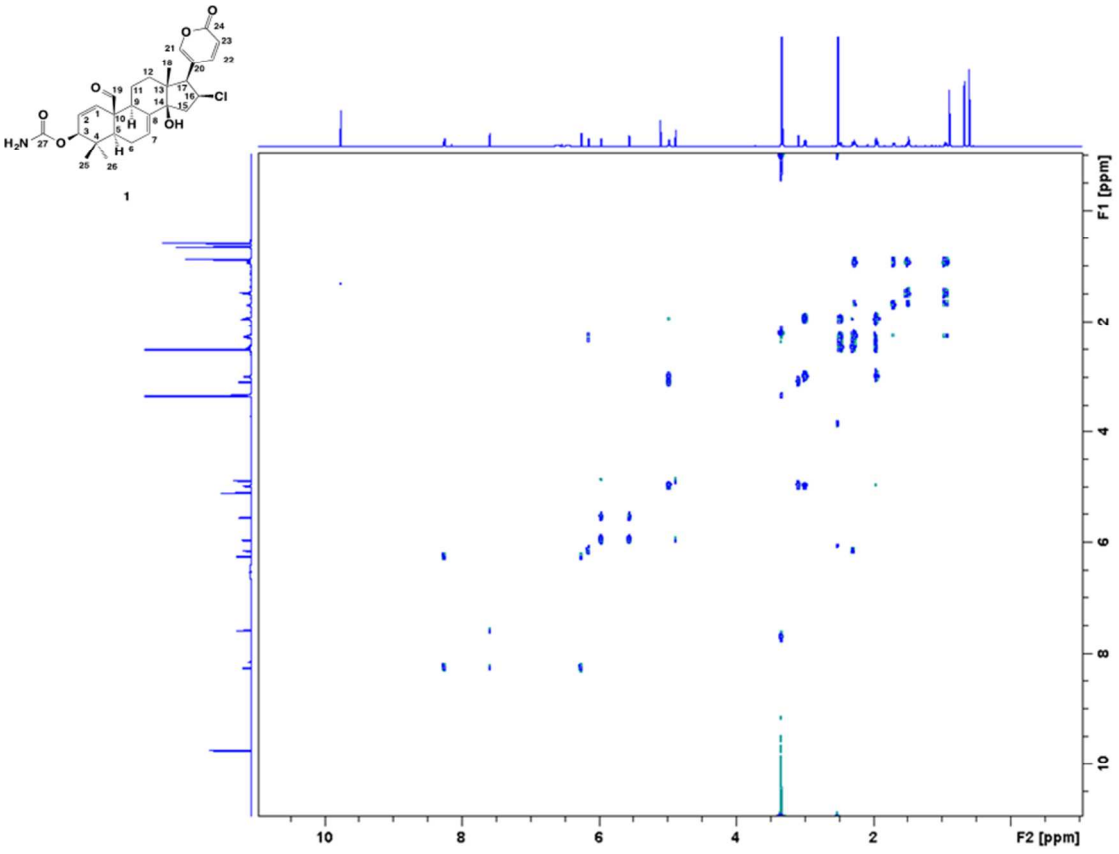



Figure S5. DQF-COSY of **1**.

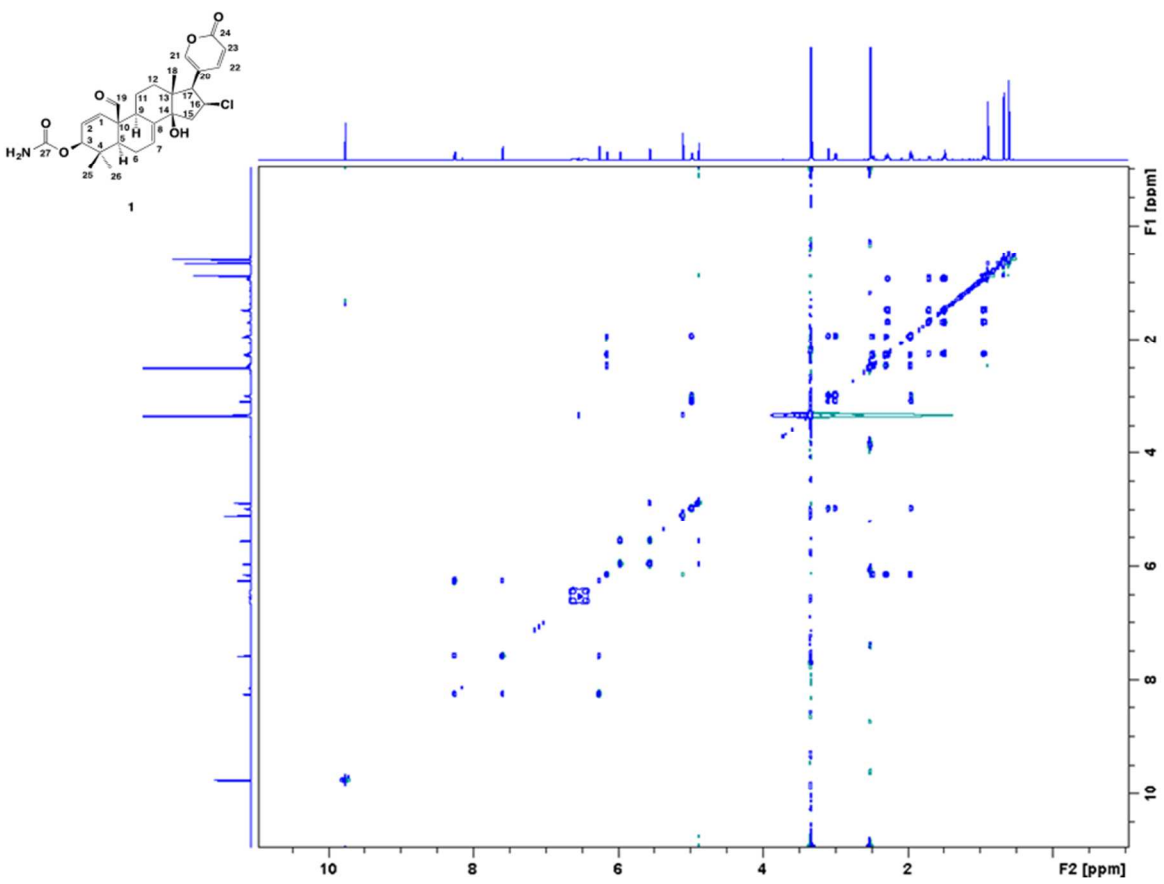



Figure S6. TOCSY of **1**.

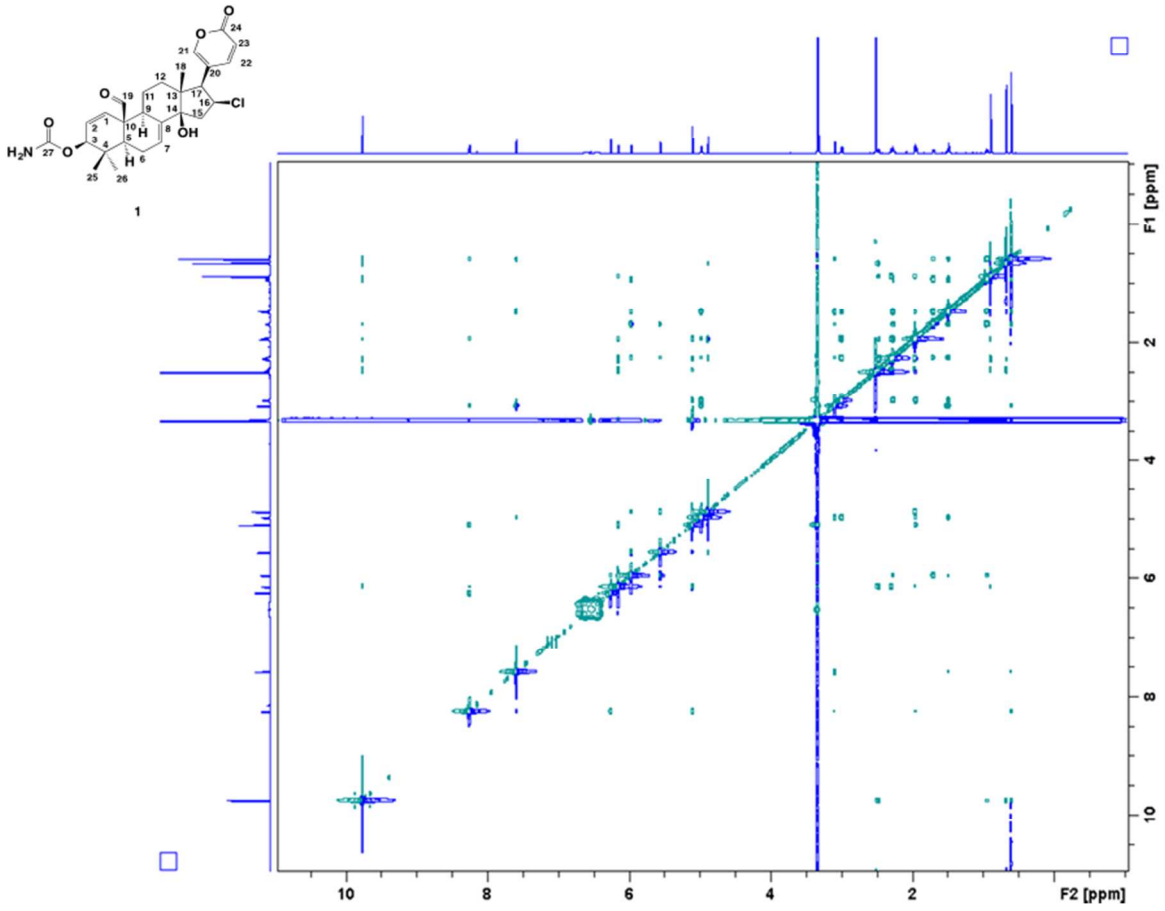



Figure S7. NOESY of **1**.

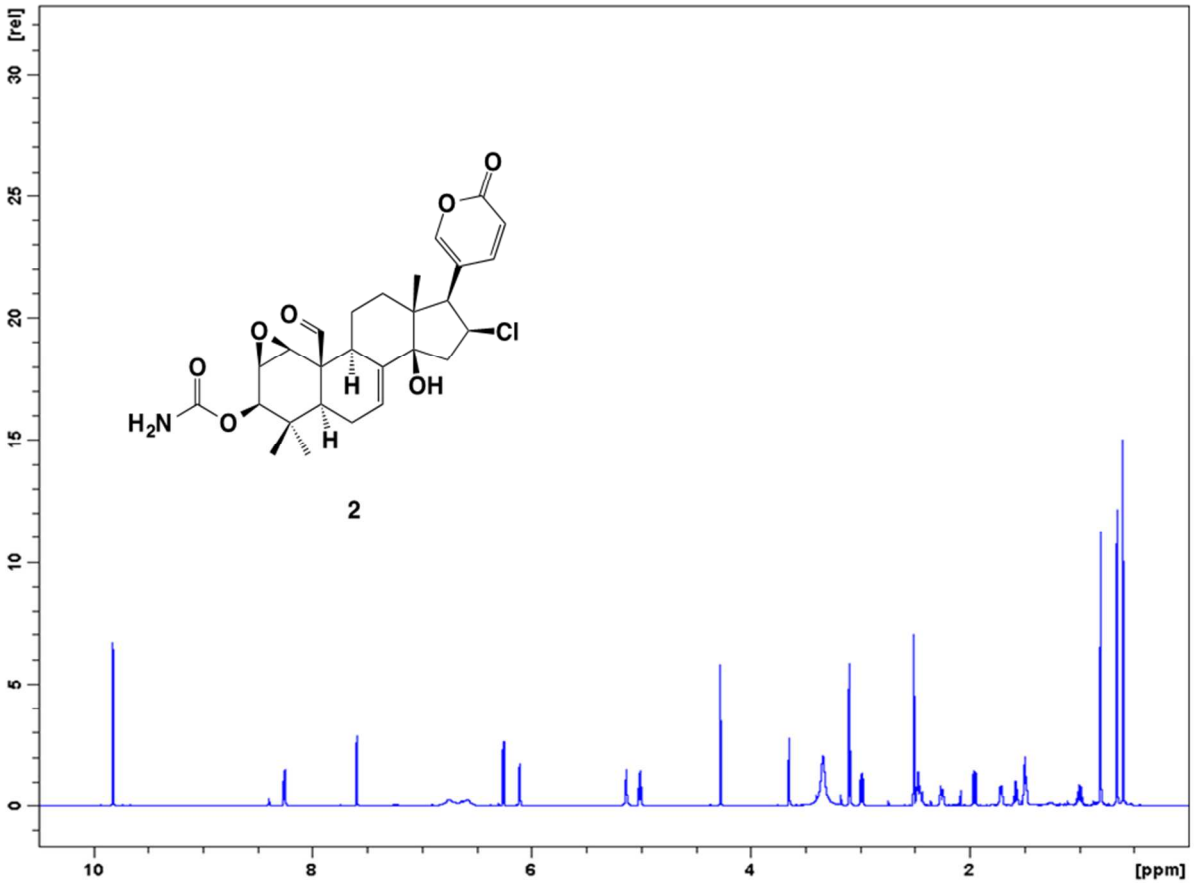



Figure S8. $^{1}$H NMR spectrum (800 MHz, DMSO-$d_6$) of **2**.

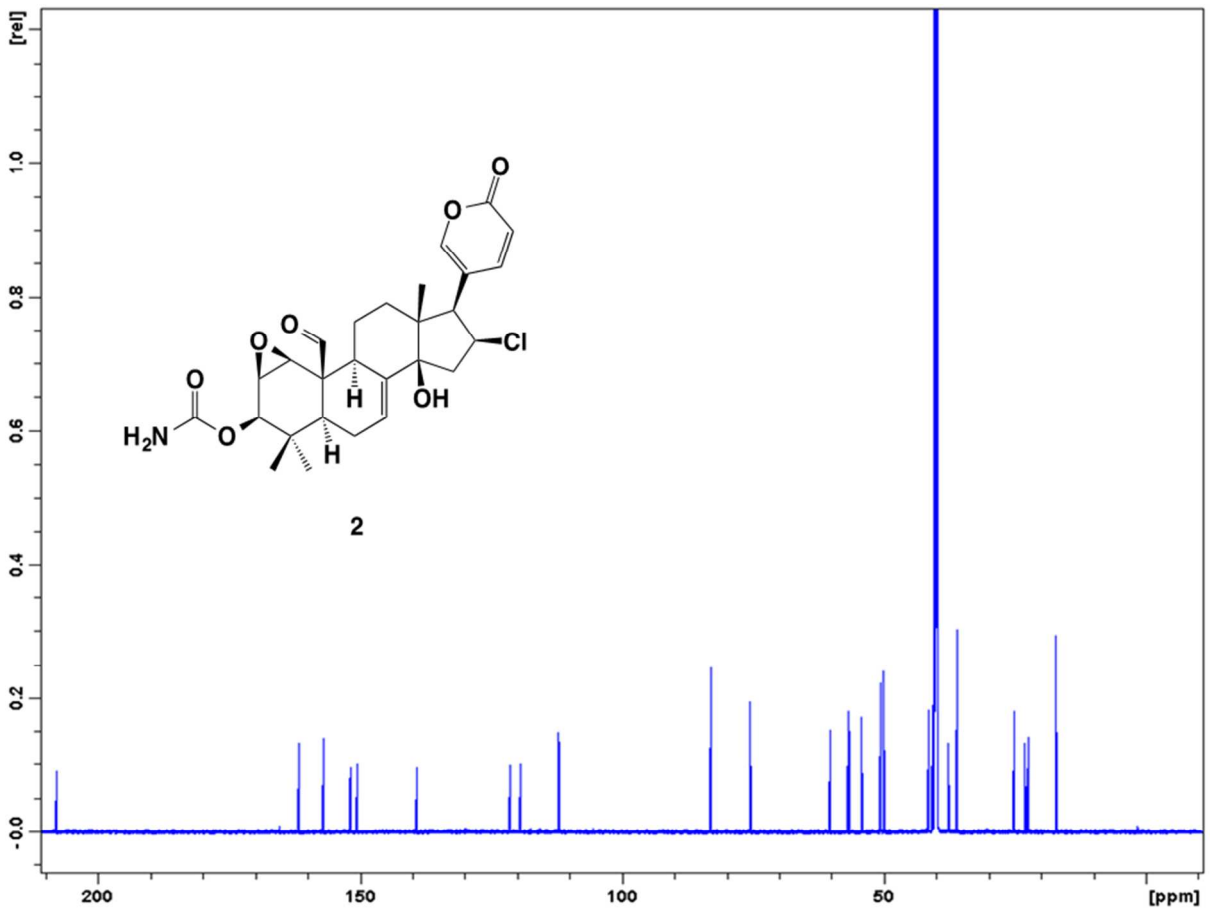



Figure S9. $^{13}$C NMR spectrum (200 MHz, DMSO-$d_6$) of **2**.

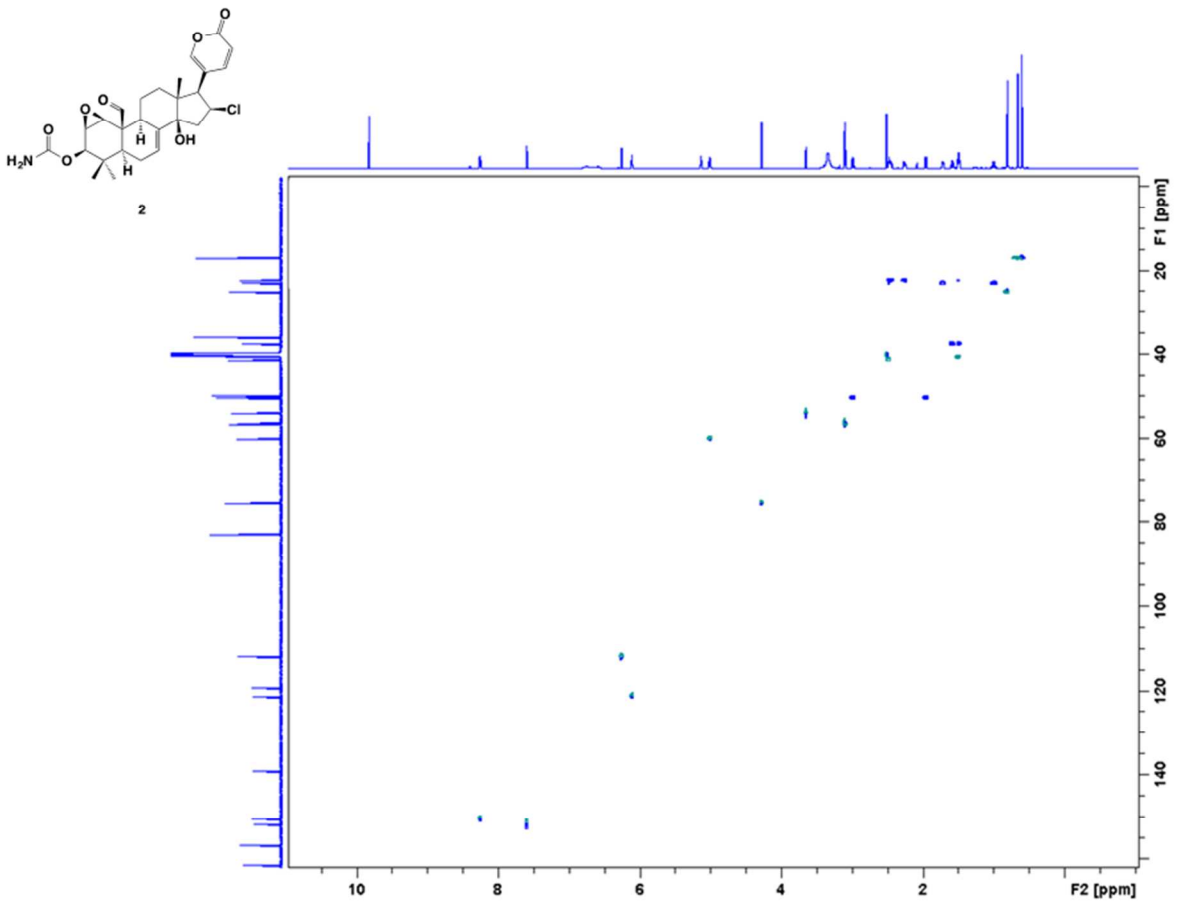



Figure S10. Multiplicity-edited HSQC of **2**.

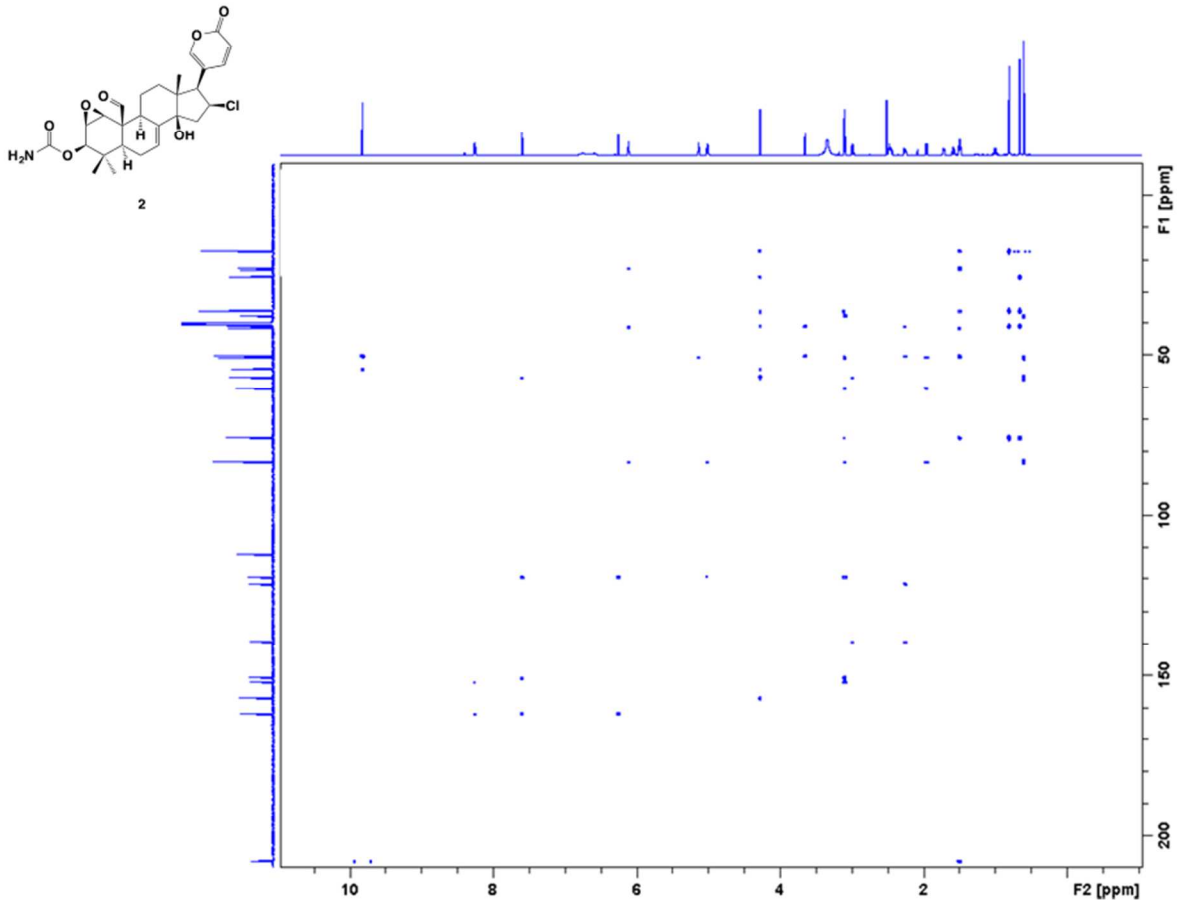



Figure S11. HMBC of **2**.

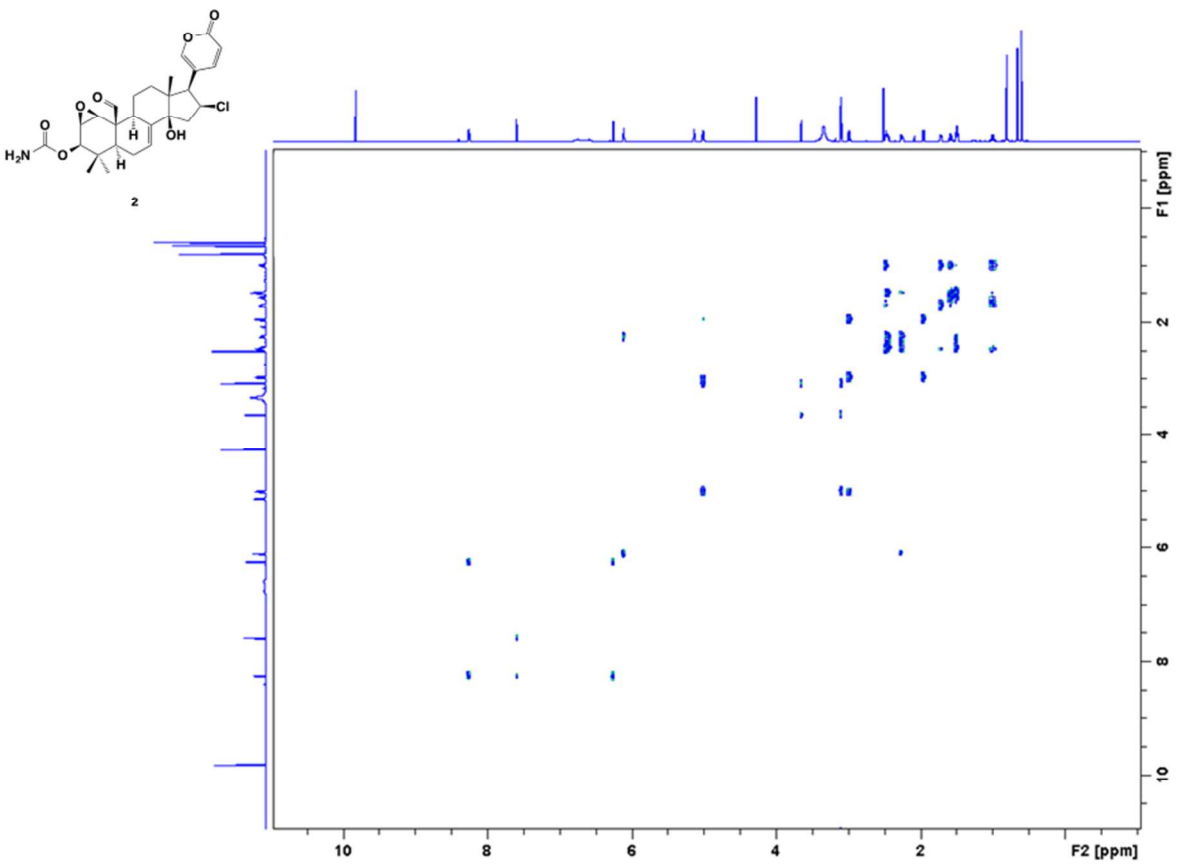



Figure S12. DFQ-COSY of **2**.

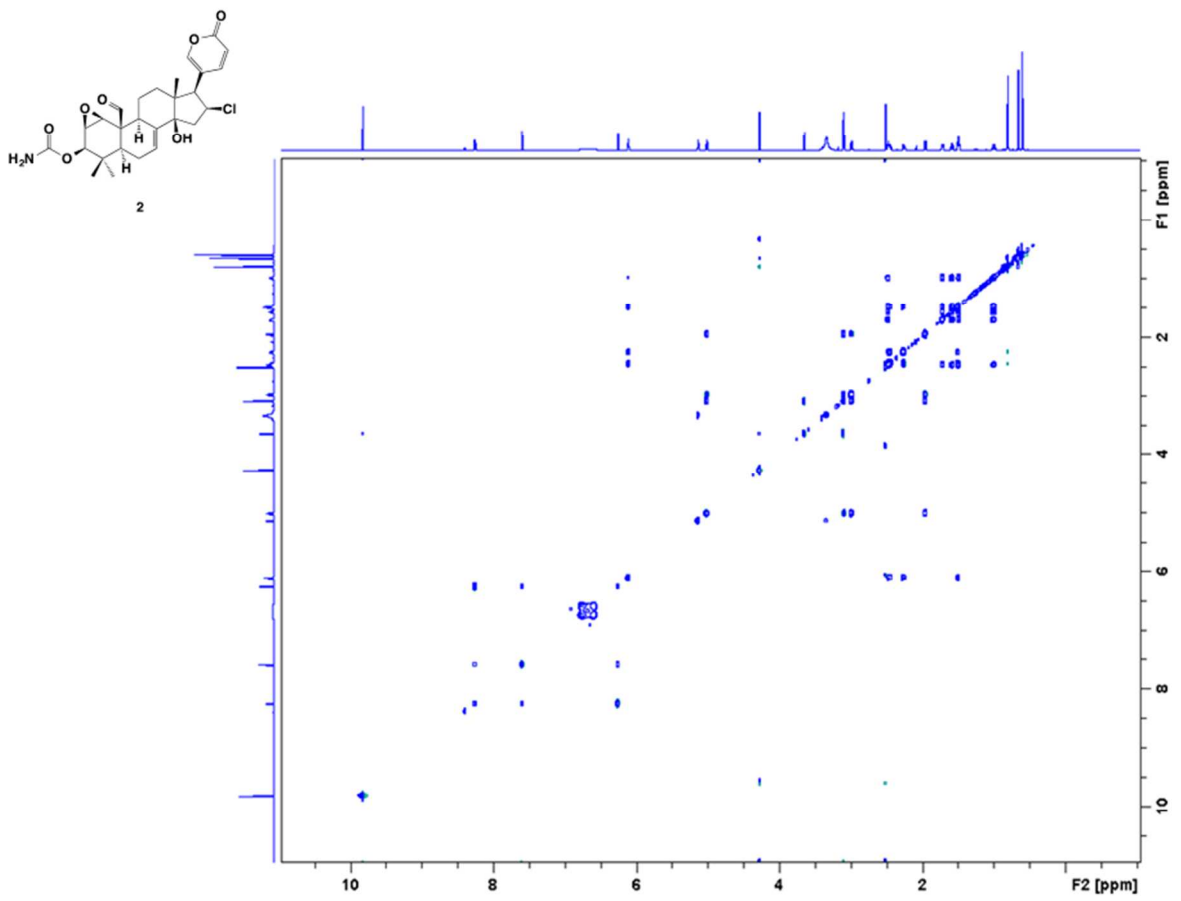



Figure S13. TOCSY of **2**.

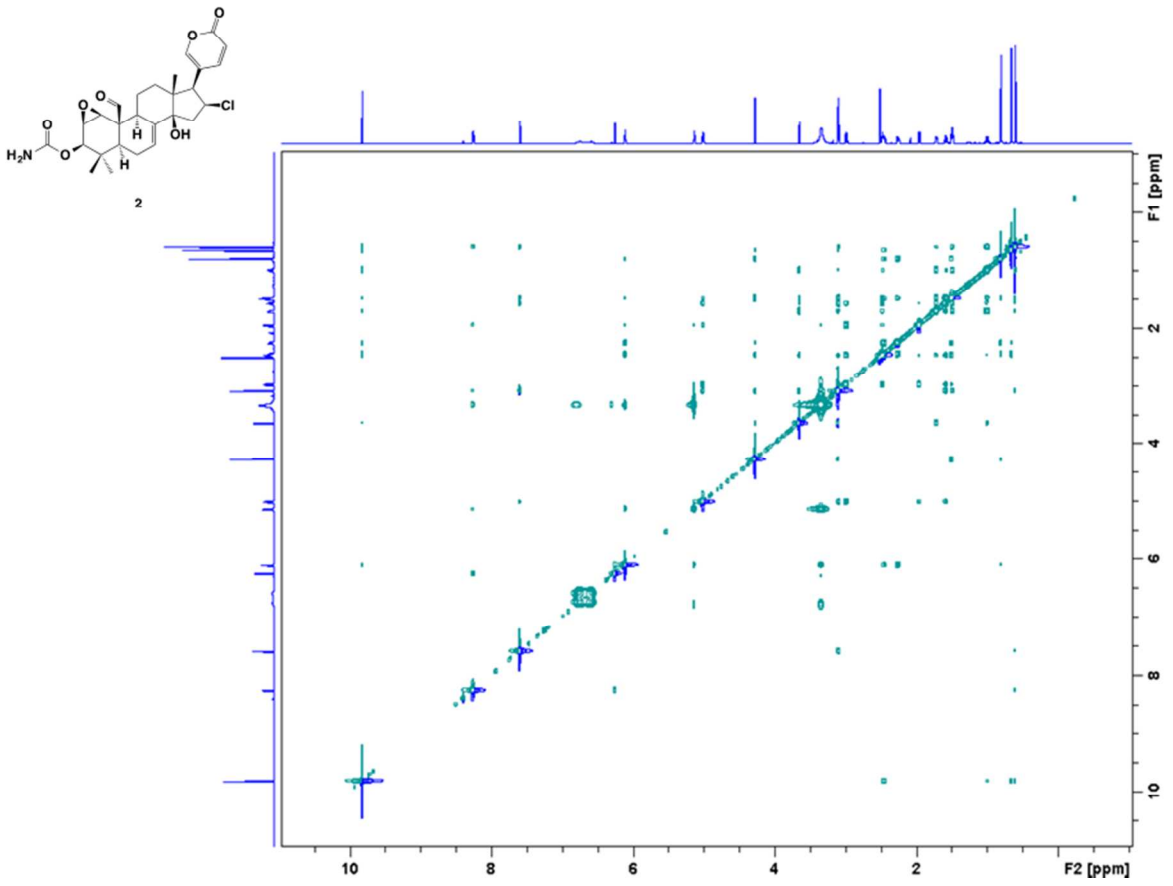



Figure S14. NOESY of **2**.

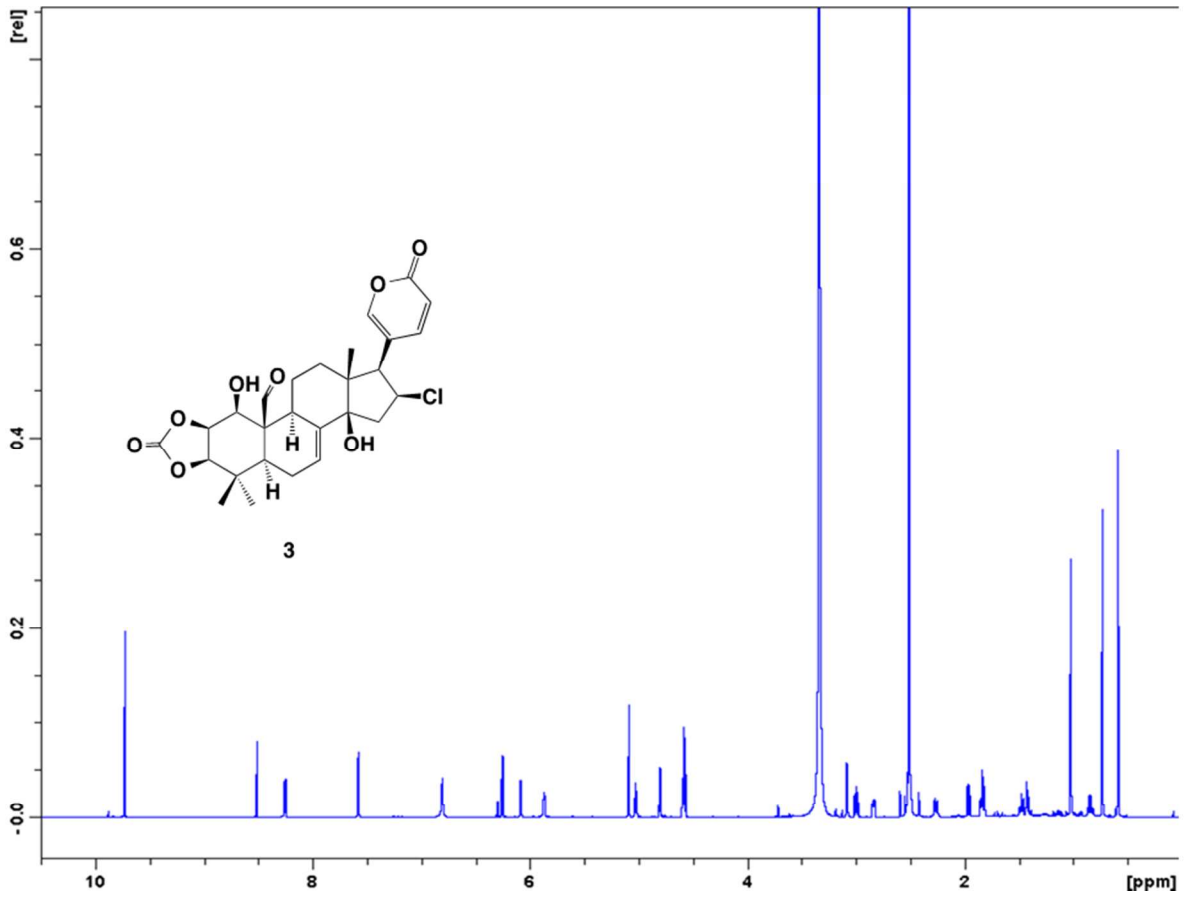



Figure S15. $^{1}$H NMR spectrum (800 MHz, DMSO-$d_6$) of **3**.

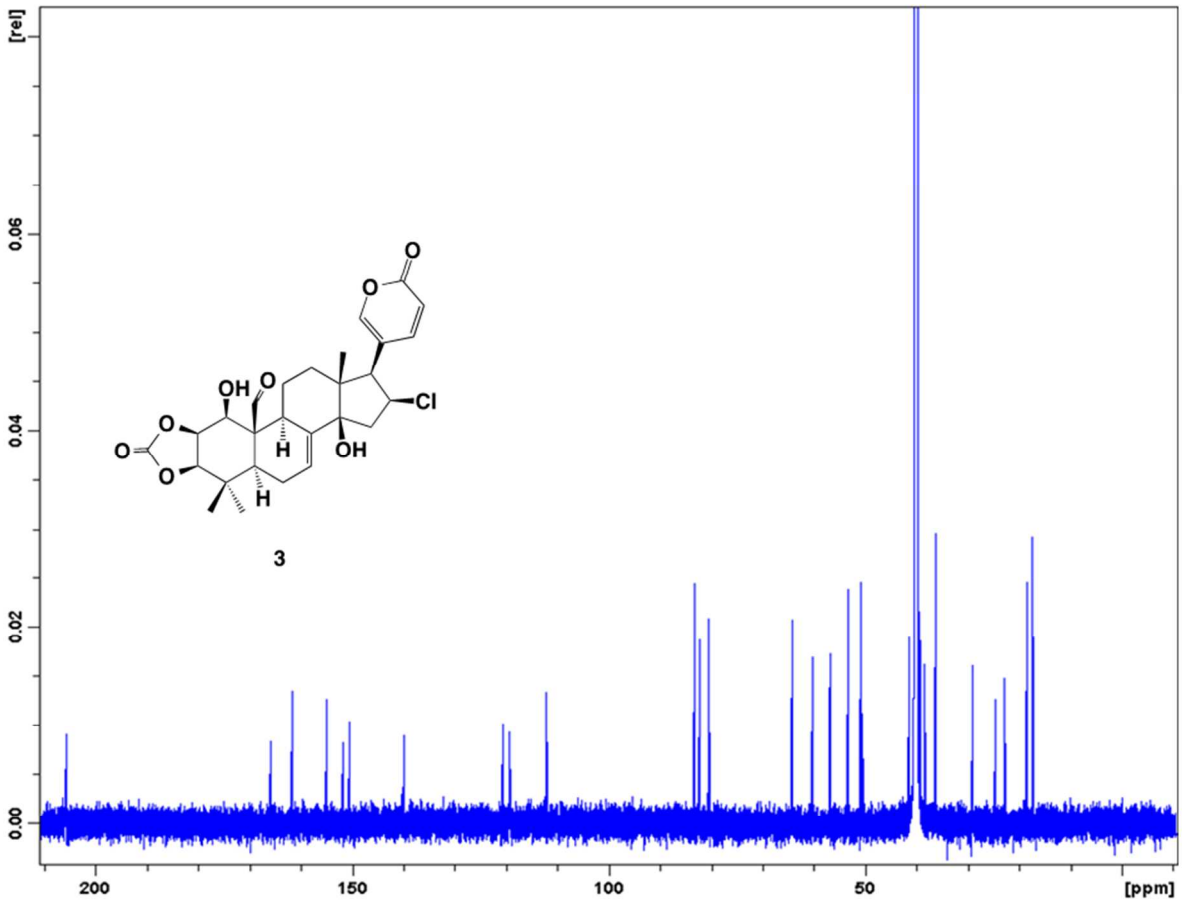



Figure S16. $^{13}$C NMR spectrum (200 MHz, DMSO-$d_6$) of **3**.

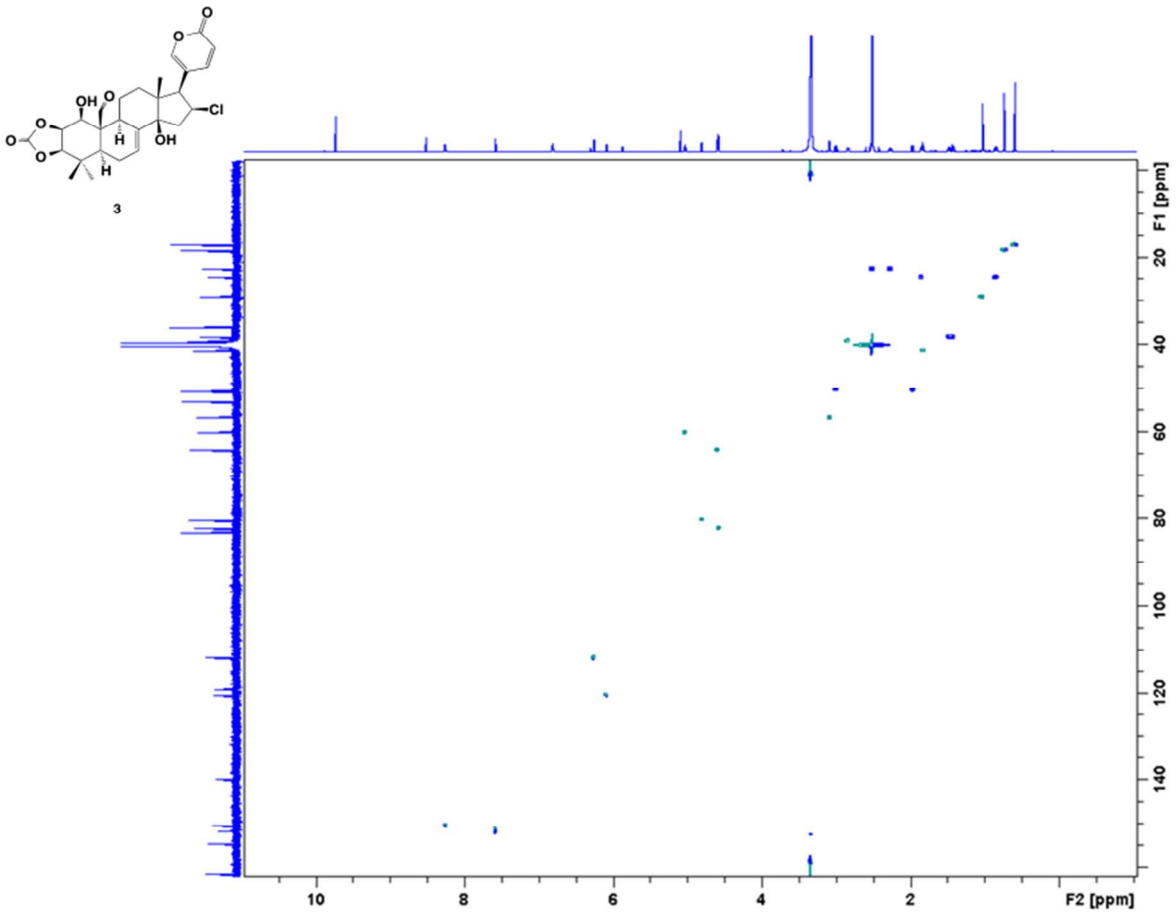



Figure S17. Multiplicity-edited HSQC of **3**.

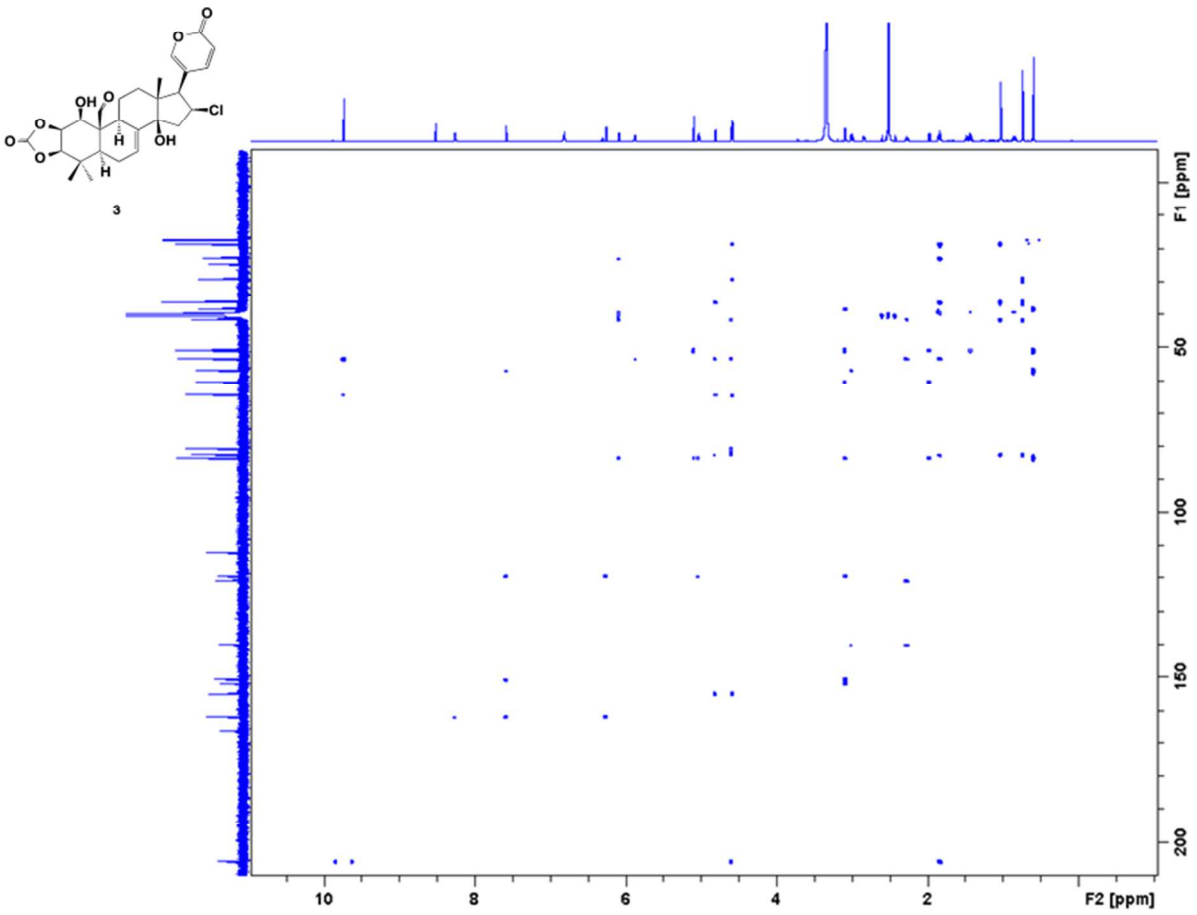



Figure S18. HMBC of **3**.

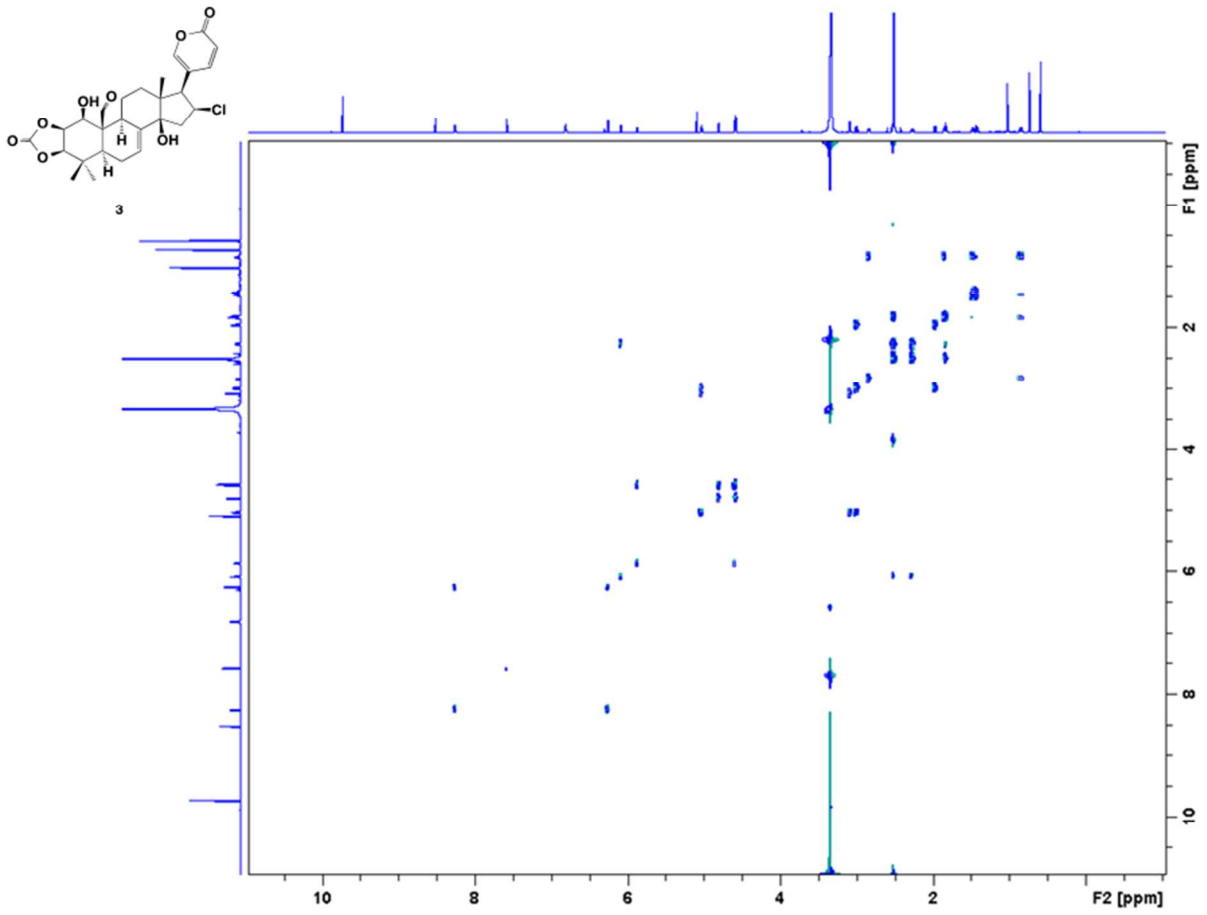



Figure S19. DFQ-COSY of **3**.

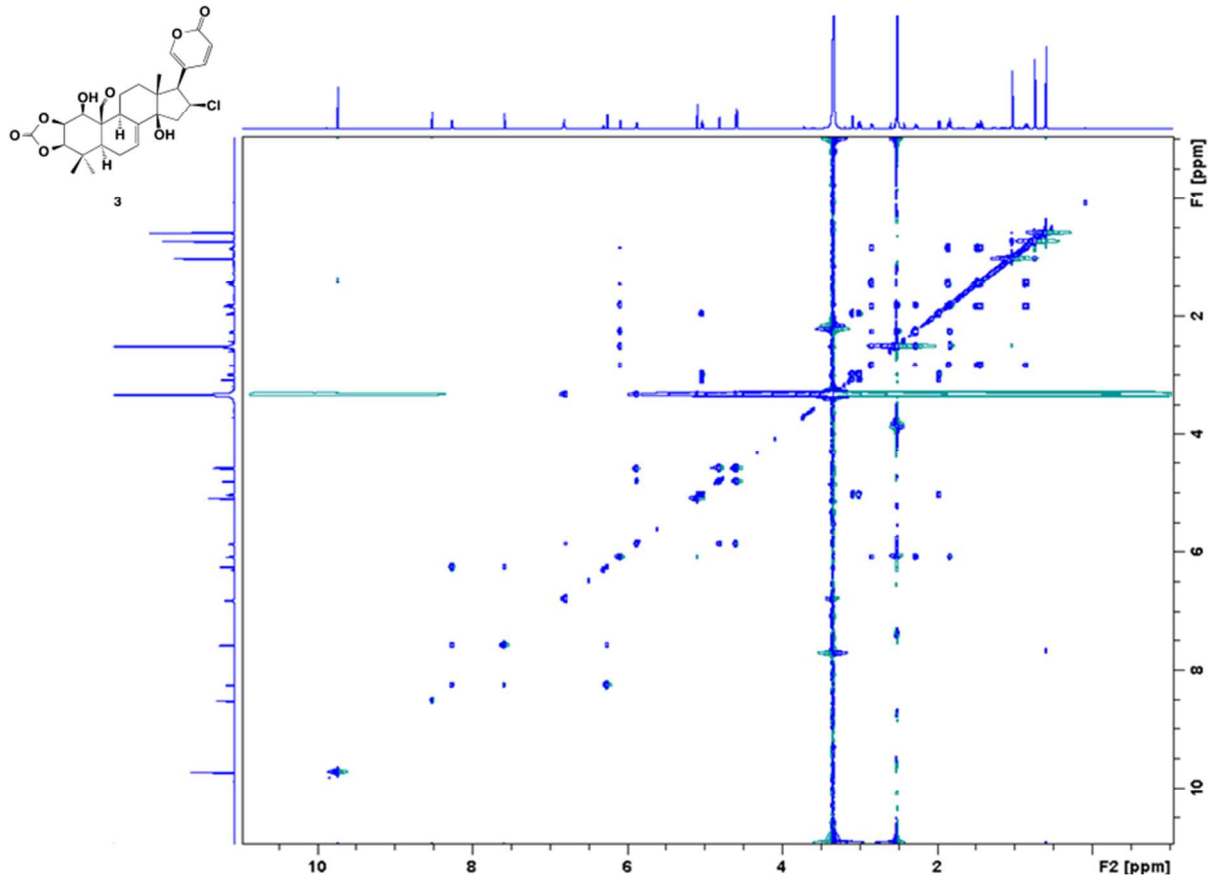



Figure S20. TOCSY of **3**.

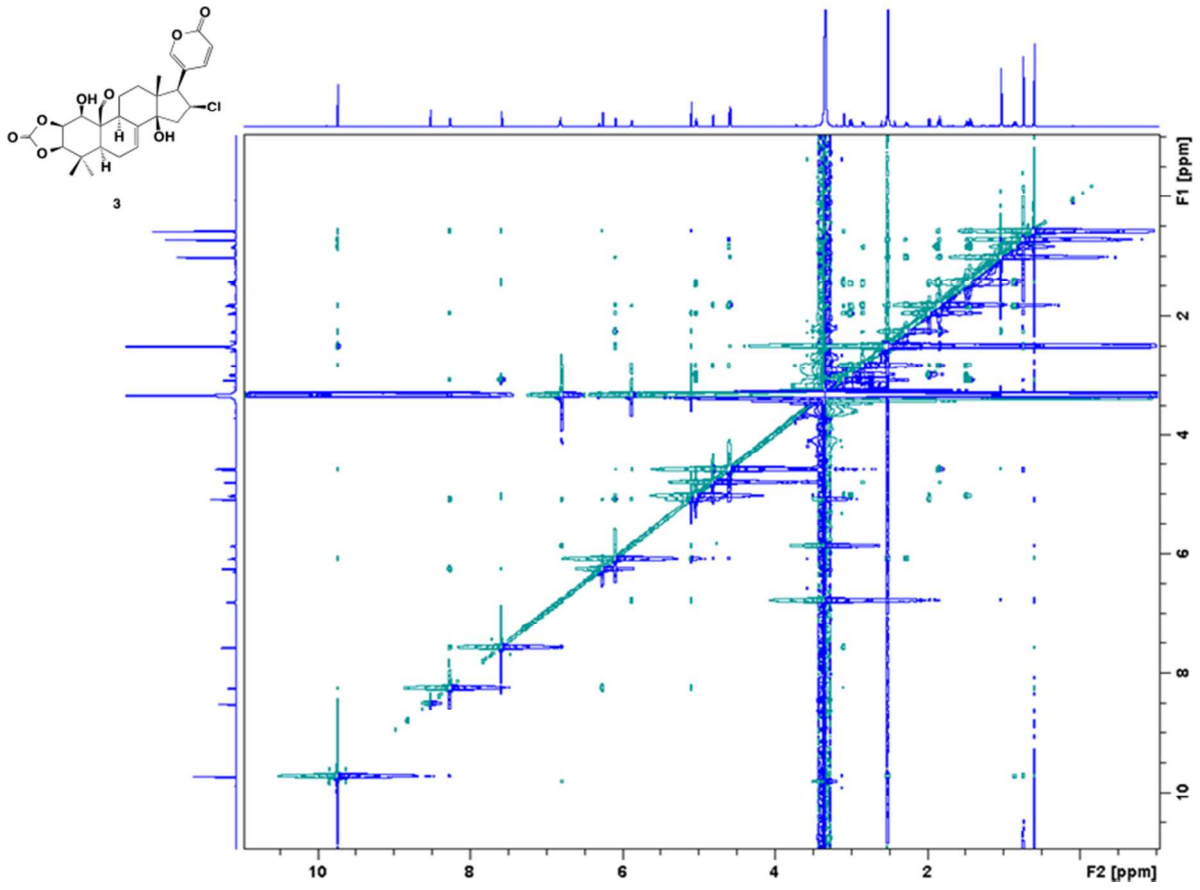



Figure S21. NOESY of **3**.

**Cytotoxicity (MTT) Assay for guiding fractionation and purification.**

To establish which materials contained the most potent components, we tested for cytotoxicity on human cancer cell lines HCT-116 (colon) and A549 (lung). Each cell line was grown in its respective growth medium and allowed to reach >80% confluence before harvesting for cytotoxicity testing. Harvesting of the cells involved removal of the spent growth medium, rinsing the cells with 10 ml PBS, trypsinization, and suspending the loosened cells in the appropriate growth medium. The harvested cells were then counted and used in the preparation of a suspension with known cell density (20,000 cells/well). Subsequently, the cells were seeded in 96-well microplates at a density of 2000 cells/well and incubated overnight at 37°C to allow them to bind to the bottom of the wells and equilibrate. Test compounds were individually suspended in 50% aqueous methanol to make 1 mg/ml test solutions. These test solutions were then serially diluted (2-fold) in 50% aqueous methanol to prepare 7 additional test solutions for each compound. Test solutions (4µl) were transferred in triplicate to distinct wells containing HCT116 or A549 cells. For the positive and negative control experiments, the cells were treated with 30 µl of methanol (100%) or 4 µl of 50% aqueous methanol, respectively. The cells were subsequently incubated at 37°C for ~72 hr before their viability was assessed by the MTT method as described previously, but with minor modifications. Briefly, 20 µl of 2.5 mg/ml MTT in PBS was added to each test well followed by incubation at 37°C for 4 hr before the growth medium was removed and 170 µl of DMSO (100%) added. After a further 5 min incubation at 37°C, optical density 540 nm was measured on a SpectraMax Plus 384 Microplate Reader integrated with SpectraMax® Pro software (Molecular Devices LLC, Sunnyvale, CA, USA) for recording and processing data. Further data processing and analysis were performed using Microsoft Excel 2010 (Redmond, WA, USA), and GraphPad Prism 5 (La Jolla, CA, USA) software.

## TABLE S3 NCI 60-Cell data for cyanobufalin A (1) [BSP 501].

National Cancer Institute Developmental Therapeutics Program
In-Vitro Testing Results

| | | | |
|---|---|---|---|
| NSC : D - 788724 / 1 | Experiment ID : 1601NS29 | Test Type : 08 | Units : Molar |
| Report Date : March 09, 2016 | Test Date : January 04, 2016 | QNS : | MC : |
| COMI : BSP_501 | Stain Reagent : SRB Dual-Pass Related | SSPL : 0YPL | |

| | | | Log10 Concentration | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Time | | Mean Optical Densities | | | | | Percent Growth | | | | | | |
| Panel/Cell Line | Zero | Ctrl | -8.6 | -7.6 | -6.6 | -5.6 | -4.6 | -8.6 | -7.6 | -6.6 | -5.6 | -4.6 | GI50 | TGI | LC50 |
| Leukemia | | | | | | | | | | | | | | | |
| CCRF-CEM | 0.516 | 2.590 | 1.826 | 0.357 | 0.319 | 0.332 | 0.330 | 63 | -31 | -38 | -36 | -36 | 3.45E-9 | 1.18E-8 | > 2.50E-5 |
| HL-60(TB) | 0.606 | 2.232 | 2.228 | 0.365 | 0.312 | 0.310 | 0.333 | 100 | -40 | -49 | -49 | -45 | 5.68E-9 | 1.30E-8 | > 2.50E-5 |
| K-562 | 0.287 | 2.093 | 1.935 | 0.284 | 0.196 | 0.180 | 0.199 | 91 | -1 | -32 | -37 | -31 | 6.98E-9 | 2.43E-8 | > 2.50E-5 |
| MOLT-4 | 0.575 | 2.513 | 1.373 | 0.386 | 0.345 | 0.308 | 0.348 | 41 | -33 | -40 | -47 | -40 | < 2.50E-9 | 9.00E-9 | > 2.50E-5 |
| RPMI-8226 | 0.974 | 2.820 | 2.699 | 0.889 | 0.741 | 0.718 | 0.743 | 93 | -9 | -24 | -26 | -24 | 6.65E-9 | 2.05E-8 | > 2.50E-5 |
| SR | 0.377 | 1.795 | 1.610 | 0.231 | 0.220 | 0.212 | 0.218 | 87 | -39 | -42 | -44 | -42 | 4.92E-9 | 1.23E-8 | > 2.50E-5 |
| Non-Small Cell Lung Cancer | | | | | | | | | | | | | | | |
| A549/ATCC | 0.574 | 2.220 | 1.832 | 0.069 | 0.049 | 0.050 | 0.054 | 76 | -88 | -91 | -91 | -91 | 3.62E-9 | 7.28E-9 | 1.47E-8 |
| EKVX | 0.884 | 2.551 | 2.260 | 0.184 | 0.101 | 0.101 | 0.082 | 83 | -79 | -89 | -89 | -91 | 3.97E-9 | 8.09E-9 | 1.65E-8 |
| HOP-62 | 0.816 | 2.018 | 1.740 | 0.055 | 0.041 | 0.044 | 0.029 | 77 | -93 | -95 | -95 | -96 | 3.60E-9 | 7.08E-9 | 1.39E-8 |
| HOP-92 | 1.420 | 1.845 | 1.784 | 1.110 | 0.934 | 0.886 | 0.880 | 86 | -22 | -34 | -38 | -38 | 5.36E-9 | 1.57E-8 | > 2.50E-5 |
| NCI-H226 | 0.910 | 2.343 | 2.220 | 0.038 | 0.023 | 0.030 | 0.020 | 91 | -96 | -97 | -97 | -98 | 4.16E-9 | 7.69E-9 | 1.42E-8 |
| NCI-H23 | 0.646 | 2.246 | 1.519 | 0.415 | 0.347 | 0.361 | 0.348 | 55 | -36 | -46 | -44 | -46 | 2.81E-9 | 1.00E-8 | > 2.50E-5 |
| NCI-H322M | 0.915 | 2.147 | 2.046 | 0.751 | 0.221 | 0.246 | 0.306 | 92 | -18 | -76 | -73 | -67 | 6.01E-9 | 1.72E-8 | 8.94E-8 |
| NCI-H460 | 0.210 | 2.284 | 0.996 | 0.055 | 0.052 | 0.048 | 0.041 | 38 | -74 | -75 | -77 | -81 | < 2.50E-9 | 5.46E-9 | 1.53E-8 |
| NCI-H522 | 1.412 | 2.864 | 1.717 | 0.567 | 0.379 | 0.429 | 0.421 | 21 | -60 | -73 | -70 | -70 | < 2.50E-9 | 4.54E-9 | 1.89E-8 |
| Colon Cancer | | | | | | | | | | | | | | | |
| COLO 205 | 0.459 | 1.573 | 1.645 | 0.402 | 0.124 | 0.132 | 0.083 | 107 | -13 | -73 | -71 | -82 | 7.46E-9 | 1.96E-8 | 1.04E-7 |
| HCC-2998 | 0.955 | 2.761 | 2.683 | 0.936 | 0.540 | 0.323 | 0.299 | 96 | -2 | -44 | -66 | -69 | 7.33E-9 | 2.38E-8 | 4.83E-7 |
| HCT-116 | 0.268 | 2.269 | 2.222 | 0.052 | 0.030 | 0.023 | 0.026 | 98 | -81 | -89 | -91 | -90 | 4.63E-9 | 8.83E-9 | 1.68E-8 |
| HCT-15 | 0.218 | 1.443 | 1.411 | 0.087 | 0.044 | 0.047 | 0.030 | 97 | -60 | -80 | -78 | -86 | 4.99E-9 | 1.04E-8 | 2.15E-8 |
| HT29 | 0.302 | 1.823 | 1.812 | 0.135 | 0.030 | 0.039 | 0.048 | 99 | -55 | -90 | -87 | -84 | 5.20E-9 | 1.09E-8 | 2.30E-8 |
| KM12 | 0.340 | 2.060 | 2.024 | 0.323 | 0.056 | 0.075 | 0.052 | 98 | -5 | -84 | -78 | -85 | 7.29E-9 | 2.23E-8 | 9.31E-8 |
| SW-620 | 0.255 | 2.004 | 1.970 | 0.173 | 0.098 | 0.082 | 0.081 | 98 | -32 | -62 | -68 | -68 | 5.85E-9 | 1.42E-8 | 1.00E-7 |
| CNS Cancer | | | | | | | | | | | | | | | |
| SF-268 | 0.610 | 2.157 | 1.811 | 0.045 | 0.063 | 0.040 | 0.045 | 78 | -93 | -90 | -94 | -93 | 3.63E-9 | 7.14E-9 | 1.40E-8 |
| SF-295 | 0.490 | 2.217 | 2.131 | 0.232 | 0.107 | 0.084 | 0.063 | 95 | -53 | -78 | -83 | -87 | 5.04E-9 | 1.10E-8 | 2.39E-8 |
| SF-539 | 0.616 | 2.247 | 2.130 | 0.132 | 0.119 | 0.140 | 0.142 | 93 | -79 | -81 | -77 | -77 | 4.44E-9 | 8.70E-9 | 1.70E-8 |
| SNB-19 | 0.786 | 2.349 | 2.347 | 0.888 | 0.081 | 0.080 | 0.086 | 100 | 7 | -90 | -90 | -89 | 8.55E-9 | 2.92E-8 | 9.67E-8 |
| SNB-75 | 0.673 | 1.404 | 1.292 | 0.372 | 0.268 | 0.212 | 0.189 | 85 | -45 | -60 | -68 | -72 | 4.63E-9 | 1.13E-8 | 5.47E-8 |
| U251 | 0.391 | 1.587 | 1.525 | 0.044 | 0.001 | 0.002 | 0.002 | 95 | -89 | -100 | -100 | -100 | 4.39E-9 | 8.21E-9 | 1.54E-8 |
| Melanoma | | | | | | | | | | | | | | | |
| LOX IMVI | 0.315 | 2.424 | 2.387 | 0.058 | 0.039 | 0.045 | 0.037 | 98 | -82 | -88 | -86 | -88 | 4.64E-9 | 8.79E-9 | 1.67E-8 |
| MALME-3M | 0.965 | 1.791 | 1.808 | 0.561 | 0.544 | 0.525 | 0.553 | 102 | -42 | -44 | -46 | -43 | 5.75E-9 | 1.28E-8 | > 2.50E-5 |
| M14 | 0.640 | 2.228 | 2.219 | 0.424 | 0.219 | 0.171 | 0.246 | 99 | -34 | -66 | -73 | -62 | 5.88E-9 | 1.39E-8 | 8.02E-8 |
| MDA-MB-435 | 0.466 | 2.331 | 2.363 | 0.392 | 0.086 | 0.095 | 0.118 | 102 | -16 | -82 | -80 | -75 | 6.88E-9 | 1.83E-8 | 8.25E-8 |
| SK-MEL-2 | 0.998 | 1.777 | 1.840 | 0.749 | 0.451 | 0.403 | 0.401 | 108 | -25 | -55 | -60 | -60 | 6.83E-9 | 1.62E-8 | 1.72E-7 |
| SK-MEL-28 | 0.583 | 1.611 | 1.677 | 0.447 | 0.374 | 0.369 | 0.337 | 106 | -23 | -36 | -37 | -42 | 6.79E-9 | 1.65E-8 | > 2.50E-5 |
| SK-MEL-5 | 1.013 | 3.088 | 2.947 | 0.185 | 0.099 | 0.088 | 0.104 | 93 | -82 | -90 | -91 | -90 | 4.41E-9 | 8.52E-9 | 1.65E-8 |
| UACC-257 | 1.318 | 2.377 | 2.285 | 0.848 | 0.246 | 0.259 | 0.236 | 91 | -36 | -81 | -80 | -82 | 5.28E-9 | 1.31E-8 | 5.14E-8 |
| UACC-62 | 0.833 | 2.694 | 2.667 | 0.490 | 0.290 | 0.250 | 0.231 | 99 | -41 | -65 | -70 | -72 | 5.56E-9 | 1.27E-8 | 5.83E-8 |
| Ovarian Cancer | | | | | | | | | | | | | | | |
| IGROV1 | 0.590 | 1.935 | 1.882 | 0.573 | 0.454 | 0.378 | 0.423 | 96 | -3 | -23 | -36 | -28 | 7.30E-9 | 2.34E-8 | > 2.50E-5 |
| OVCAR-3 | 0.419 | 1.521 | 1.287 | 0.189 | 0.048 | 0.038 | 0.032 | 79 | -55 | -89 | -91 | -92 | 4.10E-9 | 9.70E-9 | 2.29E-8 |
| OVCAR-4 | 0.633 | 1.329 | 1.121 | 0.555 | 0.224 | 0.173 | 0.167 | 70 | -12 | -65 | -73 | -74 | 4.38E-9 | 1.77E-8 | 1.31E-7 |
| OVCAR-5 | 0.776 | 1.585 | 1.587 | 0.552 | 0.311 | 0.252 | 0.309 | 100 | -29 | -60 | -68 | -60 | 6.12E-9 | 1.49E-8 | 1.19E-7 |
| OVCAR-8 | 0.570 | 2.035 | 1.918 | 0.258 | 0.089 | 0.083 | 0.092 | 92 | -55 | -84 | -85 | -84 | 4.83E-9 | 1.06E-8 | 2.32E-8 |
| NCI/ADR-RES | 0.404 | 1.420 | 1.367 | 0.298 | 0.240 | 0.269 | 0.232 | 95 | -26 | -41 | -34 | -43 | 5.85E-9 | 1.51E-8 | > 2.50E-5 |
| SK-OV-3 | 0.815 | 1.489 | 1.465 | 0.622 | 0.368 | 0.359 | 0.356 | 96 | -24 | -55 | -56 | -56 | 6.08E-9 | 1.59E-8 | 1.74E-7 |
| Renal Cancer | | | | | | | | | | | | | | | |
| 786-0 | 0.812 | 2.717 | 2.588 | 0.328 | 0.254 | 0.214 | 0.266 | 93 | -60 | -69 | -74 | -67 | 4.80E-9 | 1.02E-8 | 2.16E-8 |
| A498 | 1.095 | 1.923 | 1.941 | 0.880 | 0.658 | 0.598 | 0.510 | 102 | -20 | -40 | -45 | -53 | 6.70E-9 | 1.72E-8 | 9.30E-6 |
| ACHN | 0.449 | 1.989 | 1.178 | 0.223 | 0.124 | 0.080 | 0.128 | 47 | -50 | -72 | -82 | -71 | < 2.50E-9 | 7.62E-9 | 2.47E-8 |
| CAKI-1 | 0.722 | 2.369 | 2.266 | 0.092 | 0.080 | 0.060 | 0.050 | 94 | -87 | -89 | -92 | -93 | 4.36E-9 | 8.24E-9 | 1.56E-8 |
| RXF 393 | 0.881 | 1.510 | 1.454 | 0.545 | 0.408 | 0.374 | 0.362 | 91 | -38 | -54 | -58 | -59 | 5.20E-9 | 1.27E-8 | 1.44E-7 |
| SN12C | 0.844 | 2.715 | 1.862 | 0.693 | 0.702 | 0.651 | 0.696 | 54 | -18 | -17 | -23 | -18 | 2.87E-9 | 1.41E-8 | > 2.50E-5 |
| TK-10 | 0.872 | 1.721 | 1.586 | 0.687 | 0.079 | 0.082 | 0.048 | 84 | -21 | -91 | -91 | -94 | 5.27E-9 | 1.57E-8 | 6.46E-8 |
| UO-31 | 0.872 | 2.126 | 2.040 | 0.736 | 0.535 | 0.464 | 0.187 | 93 | -16 | -39 | -47 | -79 | 6.23E-9 | 1.80E-8 | 3.15E-6 |
| Prostate Cancer | | | | | | | | | | | | | | | |
| PC-3 | 0.620 | 2.088 | 1.793 | 0.483 | 0.330 | 0.336 | 0.383 | 80 | -22 | -47 | -46 | -38 | 4.91E-9 | 1.52E-8 | > 2.50E-5 |
| DU-145 | 0.377 | 1.817 | 1.693 | 0.036 | 0.013 | 0.009 | 0.006 | 91 | -91 | -97 | -98 | -99 | 4.22E-9 | 7.94E-9 | 1.50E-8 |
| Breast Cancer | | | | | | | | | | | | | | | |
| MCF7 | 0.307 | 1.898 | 1.768 | 0.340 | 0.215 | 0.232 | 0.189 | 92 | 2 | -30 | -24 | -38 | 7.31E-9 | 2.89E-8 | > 2.50E-5 |
| MDA-MB-231/ATCC | 0.744 | 2.054 | 2.061 | 0.921 | 0.620 | 0.599 | 0.630 | 100 | 14 | -17 | -19 | -15 | 9.51E-9 | 7.01E-8 | > 2.50E-5 |
| HS 578T | 1.201 | 2.150 | 1.639 | 1.087 | 0.970 | 0.964 | 0.918 | 46 | -10 | -19 | -20 | -24 | < 2.50E-9 | 1.68E-8 | > 2.50E-5 |
| BT-549 | 1.228 | 2.494 | 2.449 | 0.408 | 0.176 | 0.155 | 0.192 | 96 | -67 | -86 | -87 | -84 | 4.81E-9 | 9.75E-9 | 1.97E-8 |
| T-47D | 0.858 | 1.670 | 1.610 | 0.623 | 0.556 | 0.597 | 0.544 | 93 | -27 | -35 | -30 | -37 | 5.66E-9 | 1.48E-8 | > 2.50E-5 |
| MDA-MB-468 | 0.900 | 1.831 | 1.787 | 0.662 | 0.526 | 0.541 | 0.502 | 95 | -27 | -42 | -40 | -44 | 5.88E-9 | 1.51E-8 | > 2.50E-5 |

**TABLE S4 NCI 60-Cell data for cyanobufalin B (2) [BSP-517].**

National Cancer Institute Developmental Therapeutics Program
In-Vitro Testing Results

| NSC : D - 788726 / 1 | Experiment ID : 1601NS29 | Test Type : 08 | Units : Molar |
|---|---|---|---|
| Report Date : March 09, 2016 | Test Date : January 04, 2016 | QNS : | MC : |
| COMI : BSP_517 | Stain Reagent : SRB Dual-Pass Related | SSPL : 0YPL | |

| | | Log10 Concentration | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Time | Mean Optical Densities | | | | | Percent Growth | | | | | | | |
| Panel/Cell Line | Zero | Ctrl | -8.6 | -7.6 | -6.6 | -5.6 | -4.6 | -8.6 | -7.6 | -6.6 | -5.6 | -4.6 | GI50 | TGI | LC50 |
| Leukemia | | | | | | | | | | | | | | | |
| CCRF-CEM | 0.516 | 2.438 | 2.314 | 0.373 | 0.316 | 0.331 | 0.326 | 94 | -28 | -39 | -36 | -37 | 5.71E-9 | 1.47E-8 | > 2.50E-5 |
| HL-60(TB) | 0.606 | 2.327 | 2.220 | 0.461 | 0.351 | 0.339 | 0.337 | 94 | -24 | -42 | -44 | -44 | 5.88E-9 | 1.57E-8 | > 2.50E-5 |
| K-562 | 0.287 | 2.156 | 2.096 | 0.420 | 0.224 | 0.200 | 0.212 | 97 | 7 | -22 | -30 | -26 | 8.31E-9 | 4.37E-8 | > 2.50E-5 |
| MOLT-4 | 0.575 | 2.584 | 2.306 | 0.438 | 0.392 | 0.367 | 0.353 | 86 | -24 | -32 | -36 | -39 | 5.33E-9 | 1.52E-8 | > 2.50E-5 |
| RPMI-8226 | 0.974 | 2.721 | 2.654 | 1.014 | 0.737 | 0.687 | 0.725 | 96 | 2 | -24 | -29 | -26 | 7.76E-9 | 3.05E-8 | > 2.50E-5 |
| SR | 0.377 | 1.895 | 1.717 | 0.296 | 0.244 | 0.246 | 0.248 | 88 | -21 | -35 | -35 | -34 | 5.58E-9 | 1.59E-8 | > 2.50E-5 |
| Non-Small Cell Lung Cancer | | | | | | | | | | | | | | | |
| A549/ATCC | 0.574 | 2.118 | 2.059 | 0.091 | 0.060 | 0.067 | 0.054 | 96 | -84 | -90 | -88 | -91 | 4.51E-9 | 8.53E-9 | 1.61E-8 |
| EKVX | 0.884 | 2.557 | 2.548 | 0.193 | 0.110 | 0.097 | 0.082 | 99 | -78 | -88 | -89 | -91 | 4.75E-9 | 9.08E-9 | 1.74E-8 |
| HOP-62 | 0.816 | 2.043 | 1.939 | 0.069 | 0.080 | 0.069 | 0.046 | 92 | -92 | -90 | -92 | -94 | 4.21E-9 | 7.90E-9 | 1.48E-8 |
| HOP-92 | 1.420 | 1.803 | 1.758 | 1.328 | 0.895 | 0.858 | 0.823 | 88 | -7 | -37 | -40 | -42 | 6.33E-9 | 2.13E-8 | > 2.50E-5 |
| NCI-H226 | 0.910 | 2.414 | 2.279 | 0.061 | 0.040 | 0.039 | 0.030 | 91 | -93 | -96 | -96 | -97 | 4.17E-9 | 7.79E-9 | 1.45E-8 |
| NCI-H23 | 0.646 | 2.160 | 2.036 | 0.389 | 0.320 | 0.335 | 0.322 | 92 | -40 | -51 | -48 | -50 | 5.19E-9 | 1.25E-8 | . |
| NCI-H322M | 0.915 | 2.202 | 2.118 | 0.913 | 0.241 | 0.270 | 0.360 | 93 | . | -74 | -71 | -61 | 7.27E-9 | 2.49E-8 | 1.19E-7 |
| NCI-H460 | 0.210 | 2.261 | 2.209 | 0.059 | 0.067 | 0.064 | 0.056 | 97 | -72 | -68 | -70 | -74 | 4.76E-9 | 9.39E-9 | 1.85E-8 |
| NCI-H522 | 1.412 | 2.619 | 2.567 | 0.556 | 0.274 | 0.355 | 0.350 | 96 | -61 | -81 | -75 | -75 | 4.90E-9 | 1.02E-8 | 2.14E-8 |
| Colon Cancer | | | | | | | | | | | | | | | |
| COLO 205 | 0.459 | 1.622 | 1.637 | 0.516 | 0.138 | 0.122 | 0.108 | 101 | 5 | -70 | -73 | -77 | 8.52E-9 | 2.91E-8 | 1.35E-7 |
| HCC-2998 | 0.955 | 2.624 | 2.483 | 1.083 | 0.457 | 0.247 | 0.246 | 92 | 8 | -52 | -74 | -74 | 7.82E-9 | 3.36E-8 | 2.30E-7 |
| HCT-116 | 0.268 | 2.189 | 2.172 | 0.022 | 0.025 | 0.020 | 0.010 | 99 | -92 | -91 | -93 | -96 | 4.52E-9 | 8.26E-9 | 1.51E-8 |
| HCT-15 | 0.218 | 1.472 | 1.498 | 0.106 | 0.048 | 0.045 | 0.031 | 102 | -51 | -78 | -80 | -86 | 5.46E-9 | 1.16E-8 | 2.45E-8 |
| HT29 | 0.302 | 1.581 | 1.691 | 0.158 | 0.055 | 0.059 | 0.040 | 109 | -48 | -82 | -80 | -87 | 5.92E-9 | 1.24E-8 | 2.89E-8 |
| KM12 | 0.340 | 1.966 | 1.983 | 0.508 | 0.071 | 0.061 | 0.053 | 101 | 10 | -79 | -82 | -84 | 9.13E-9 | 3.26E-8 | 1.18E-7 |
| SW-620 | 0.255 | 1.909 | 1.897 | 0.151 | 0.087 | 0.079 | 0.063 | 99 | -41 | -66 | -69 | -75 | 5.61E-9 | 1.28E-8 | 5.76E-8 |
| CNS Cancer | | | | | | | | | | | | | | | |
| SF-268 | 0.610 | 2.129 | 2.121 | 0.058 | 0.073 | 0.042 | 0.039 | 100 | -90 | -88 | -93 | -94 | 4.56E-9 | 8.35E-9 | 1.53E-8 |
| SF-295 | 0.490 | 2.194 | 2.110 | 0.278 | 0.097 | 0.082 | 0.068 | 95 | -43 | -80 | -83 | -86 | 5.29E-9 | 1.22E-8 | 3.78E-8 |
| SF-539 | 0.616 | 2.307 | 2.178 | 0.156 | 0.169 | 0.146 | 0.247 | 92 | -75 | -73 | -76 | -60 | 4.48E-9 | 8.93E-9 | 1.78E-8 |
| SNB-19 | 0.786 | 2.362 | 2.405 | 1.250 | 0.180 | 0.125 | 0.118 | 103 | 29 | -77 | -84 | -85 | 1.31E-8 | 4.72E-8 | 1.39E-7 |
| SNB-75 | 0.673 | 1.386 | 1.338 | 0.372 | 0.306 | 0.213 | 0.223 | 93 | -45 | -55 | -68 | -67 | 5.14E-9 | 1.18E-8 | 8.48E-8 |
| U251 | 0.391 | 1.492 | 1.485 | 0.137 | 0.015 | 0.016 | 0.012 | 99 | -65 | -96 | -96 | -97 | 4.99E-9 | 1.01E-8 | 2.03E-8 |
| Melanoma | | | | | | | | | | | | | | | |
| LOX IMVI | 0.315 | 2.315 | 2.234 | 0.062 | 0.030 | 0.039 | 0.026 | 96 | -80 | -90 | -88 | -92 | 4.56E-9 | 8.75E-9 | 1.68E-8 |
| MALME-3M | 0.965 | 1.756 | 1.739 | 0.573 | 0.521 | 0.482 | 0.495 | 98 | -41 | -46 | -50 | -49 | 5.54E-9 | 1.27E-8 | . |
| M14 | 0.640 | 2.266 | 2.276 | 0.744 | 0.179 | 0.168 | 0.217 | 101 | 6 | -72 | -74 | -66 | 8.61E-9 | 3.02E-8 | 1.31E-7 |
| MDA-MB-435 | 0.466 | 2.295 | 2.328 | 0.747 | 0.082 | 0.111 | 0.096 | 102 | 15 | -82 | -76 | -80 | 9.93E-9 | 3.59E-8 | 1.17E-7 |
| SK-MEL-2 | 0.998 | 1.719 | 1.771 | 1.315 | 0.446 | 0.392 | 0.366 | 107 | 44 | -55 | -61 | -63 | 2.00E-8 | 6.92E-8 | 2.21E-7 |
| SK-MEL-28 | 0.583 | 1.668 | 1.725 | 0.733 | 0.415 | 0.373 | 0.402 | 105 | 14 | -29 | -36 | -31 | 1.01E-8 | 5.27E-8 | > 2.50E-5 |
| SK-MEL-5 | 1.013 | 3.135 | 3.070 | 0.218 | 0.085 | 0.087 | 0.099 | 97 | -78 | -92 | -91 | -90 | 4.63E-9 | 8.92E-9 | 1.72E-8 |
| UACC-257 | 1.318 | 2.325 | 2.233 | 1.273 | 0.203 | 0.175 | 0.145 | 91 | -3 | -85 | -87 | -89 | 6.78E-9 | 2.30E-8 | 9.37E-8 |
| UACC-62 | 0.833 | 2.774 | 2.724 | 0.983 | 0.437 | 0.366 | 0.346 | 97 | 8 | -48 | -56 | -58 | 8.44E-9 | 3.45E-8 | 4.80E-7 |
| Ovarian Cancer | | | | | | | | | | | | | | | |
| IGROV1 | 0.590 | 1.956 | 1.979 | 0.634 | 0.463 | 0.415 | 0.422 | 102 | 3 | -22 | -30 | -28 | 8.37E-9 | 3.36E-8 | > 2.50E-5 |
| OVCAR-3 | 0.419 | 1.534 | 1.589 | 0.126 | 0.056 | 0.044 | 0.033 | 105 | -70 | -87 | -89 | -92 | 5.16E-9 | 9.96E-9 | 1.92E-8 |
| OVCAR-4 | 0.633 | 1.314 | 1.323 | 0.596 | 0.254 | 0.201 | 0.185 | 101 | -6 | -60 | -68 | -71 | 7.52E-9 | 2.20E-8 | 1.64E-7 |
| OVCAR-5 | 0.776 | 1.600 | 1.571 | 0.683 | 0.400 | 0.337 | 0.338 | 96 | -12 | -48 | -57 | -56 | 6.71E-9 | 1.94E-8 | 3.86E-7 |
| OVCAR-8 | 0.570 | 1.969 | 1.944 | 0.519 | 0.099 | 0.084 | 0.097 | 98 | -9 | -83 | -85 | -83 | 7.04E-9 | 2.06E-8 | 9.01E-8 |
| NCI/ADR-RES | 0.404 | 1.346 | 1.313 | 0.266 | 0.206 | 0.232 | 0.201 | 96 | -34 | -49 | -43 | -50 | 5.67E-9 | 1.37E-8 | 2.24E-5 |
| SK-OV-3 | 0.815 | 1.505 | 1.536 | 0.682 | 0.359 | 0.423 | 0.445 | 104 | -16 | -56 | -48 | -45 | 7.06E-9 | 1.83E-8 | . |
| Renal Cancer | | | | | | | | | | | | | | | |
| 786-0 | 0.812 | 2.655 | 2.598 | 0.349 | 0.229 | 0.191 | 0.157 | 97 | -57 | -72 | -76 | -81 | 5.04E-9 | 1.06E-8 | 2.25E-8 |
| A498 | 1.095 | 1.978 | 1.967 | 1.048 | 0.695 | 0.634 | 0.583 | 99 | -4 | -37 | -42 | -47 | 7.43E-9 | 2.27E-8 | > 2.50E-5 |
| ACHN | 0.449 | 1.982 | 2.007 | 0.209 | 0.143 | 0.146 | 0.141 | 102 | -54 | -68 | -67 | -69 | 5.38E-9 | 1.13E-8 | 2.37E-8 |
| CAKI-1 | 0.722 | 2.448 | 2.322 | 0.188 | 0.117 | 0.110 | 0.100 | 93 | -74 | -84 | -85 | -86 | 4.51E-9 | 9.00E-9 | 1.80E-8 |
| RXF 393 | 0.881 | 1.555 | 1.541 | 0.650 | 0.421 | 0.433 | 0.369 | 98 | -26 | -52 | -51 | -58 | 6.08E-9 | 1.54E-8 | 2.04E-7 |
| SN12C | 0.844 | 2.689 | 2.576 | 0.724 | 0.704 | 0.663 | 0.638 | 94 | -14 | -17 | -21 | -24 | 6.36E-9 | 1.85E-8 | > 2.50E-5 |
| TK-10 | 0.872 | 1.638 | 1.631 | 0.490 | 0.055 | 0.084 | 0.051 | 99 | -44 | -94 | -90 | -94 | 5.51E-9 | 1.23E-8 | 3.32E-8 |
| UO-31 | 0.872 | 2.182 | 2.070 | 0.904 | 0.670 | 0.622 | 0.420 | 91 | 2 | -23 | -29 | -52 | 7.30E-9 | 3.11E-8 | 2.08E-5 |
| Prostate Cancer | | | | | | | | | | | | | | | |
| PC-3 | 0.620 | 2.091 | 2.054 | 0.534 | 0.336 | 0.314 | 0.361 | 97 | -14 | -46 | -49 | -42 | 6.67E-9 | 1.88E-8 | > 2.50E-5 |
| DU-145 | 0.377 | 1.860 | 1.875 | 0.019 | 0.009 | 0.009 | 0.008 | 101 | -95 | -98 | -98 | -98 | 4.55E-9 | 8.19E-9 | 1.47E-8 |
| Breast Cancer | | | | | | | | | | | | | | | |
| MCF7 | 0.307 | 1.916 | 1.876 | 0.358 | 0.222 | 0.213 | 0.214 | 97 | 3 | -28 | -31 | -30 | 7.96E-9 | 3.16E-8 | > 2.50E-5 |
| MDA-MB-231/ATCC | 0.744 | 1.945 | 1.918 | 1.175 | 0.555 | 0.547 | 0.458 | 98 | 36 | -25 | -26 | -38 | 1.48E-8 | 9.61E-8 | > 2.50E-5 |
| HS 578T | 1.201 | 2.134 | 2.067 | 1.114 | 0.987 | 0.991 | 0.945 | 93 | -7 | -18 | -18 | -21 | 6.69E-9 | 2.11E-8 | > 2.50E-5 |
| BT-549 | 1.228 | 2.554 | 2.582 | 0.441 | 0.162 | 0.105 | 0.189 | 102 | -64 | -87 | -91 | -85 | 5.14E-9 | 1.03E-8 | 2.06E-8 |
| T-47D | 0.858 | 1.682 | 1.637 | 0.693 | 0.583 | 0.626 | 0.586 | 95 | -19 | -32 | -27 | -32 | 6.15E-9 | 1.69E-8 | > 2.50E-5 |
| MDA-MB-468 | 0.900 | 1.859 | 1.807 | 0.807 | 0.463 | 0.537 | 0.493 | 95 | -10 | -49 | -40 | -45 | 6.65E-9 | 1.99E-8 | > 2.50E-5 |

**Sundia cytotoxicity procedure.** (Gibco Cat# A10491-01) supplemented with 10% fetal bovine serum (Gibco Cat# 10099-141). HUVEC was cultured in a

specifically designed medium (Allcells, Cat# H-004). Cells were plated in 96-well plates with 150 ul culture medium at the optimized cell density. 24 hours later test compounds were added and the time zero plates were measured by MTS assay as G0 reference. Cell proliferation was measured by MTS assay after compound treatment for 3 days. Compounds dilution: 20 mM stock solution in DMSO. On the day of treatment ccompounds were freshly diluted from the stock solution to a working solution in culture medium. 50ul of compound solutions was added to duplicate wells along with 150 ul of cells. These cells were cultured in a $CO_2$ incubator for 72 hours. Cell proliferation was measured by the MTS testing kit following the manufacturer's protocol.

**Cardiomyocyte Assay Results**.

Cell index plots over the time course of the assay are shown below for bufalin and cyanobufalin A.

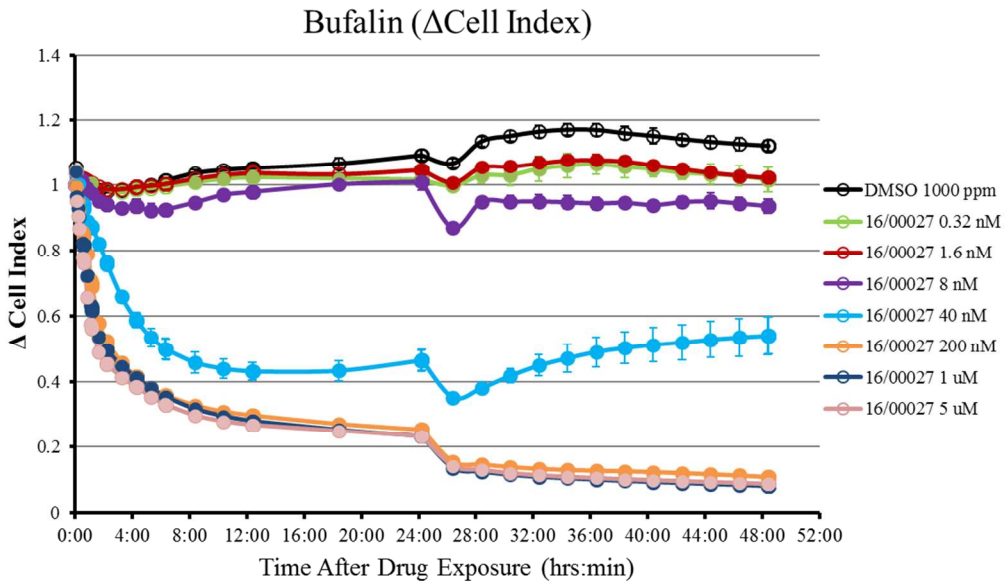



Figure S22. Cell Index Time Course Plot for Bufalin

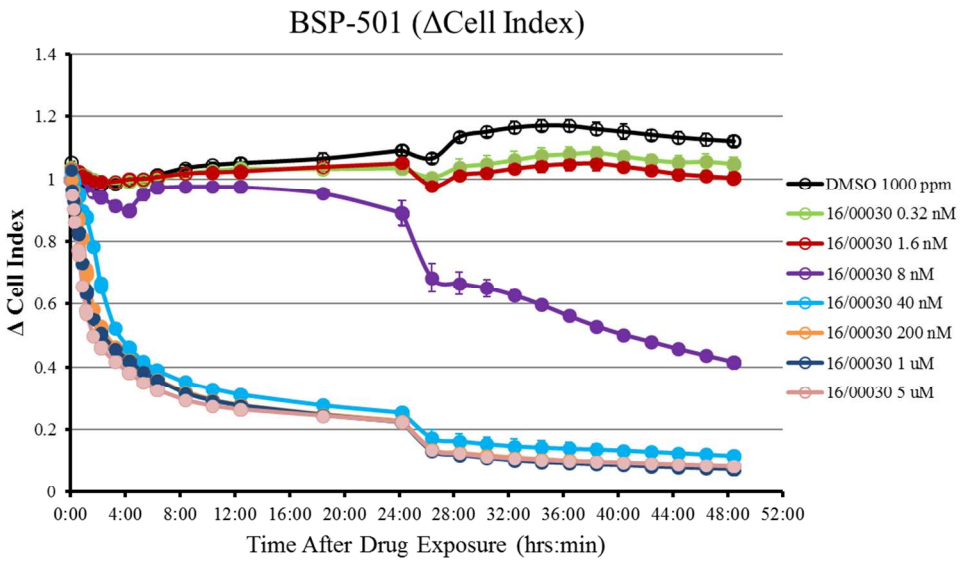
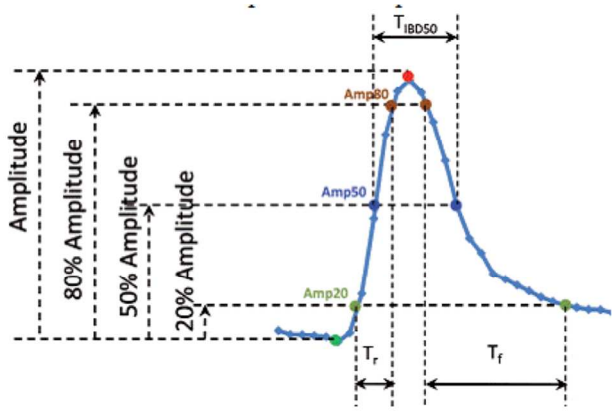
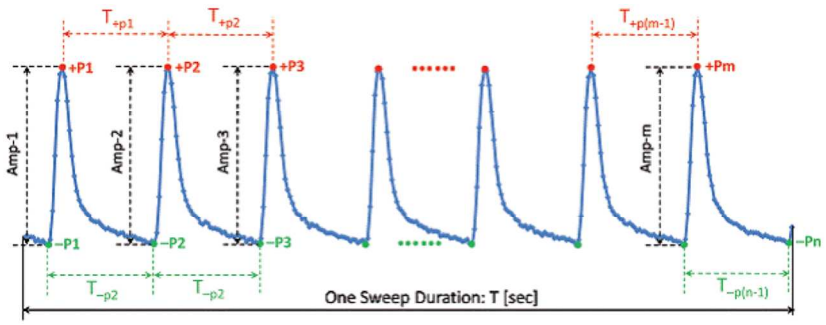



Figure S23. Cell Index Time Course Plots for Cyanobufalin A (**1**)

Beating rate is calculated by dividing 60 [sec/min] by the time [sec] from one Negative Peak to the following Negative Peak. The result is the Average of the Beating Rate (Negative Peak Period Based) plus/minus the Standard Deviation. Beating Amplitude (amplitude of the contraction) is calculated from each Negative Peak to the following Positive Peak. The result is the average of all the amplitudes (Whole Peak) in one sweep plus/minus the Standard Deviation.




Beating of cardiac myocytes appears as a transient change in the Cell Index value.




Representative beating pattern of cardiomyocyte beatings and illustration of related key parameters.

**Effect of Bufalin** Bufalin was acutely cardioactive (having effect on beating parameters) at concentration ≥ 40 nM. and acutely cytotoxic at concentrations ≥ 200 nM. Biologically significant effects on beating parameters were observed at 40 nM both acutely (where beating rate and amplitude of contraction were increased within the first few minutes to hours, followed by slowing of beat rate/loss of amplitude at longer time points ~ 6 hr). Subacute to chronic effects were observed at 8 nM as well.

Amplitude of contraction was increased after ~4 hr for at 0.32 nM and was maintained for the length of the assay. Beating rates were not significantly altered during this time/concentration.

Beating was ceased at concentrations ≥ 200 nM within the first few minutes of exposure as well.

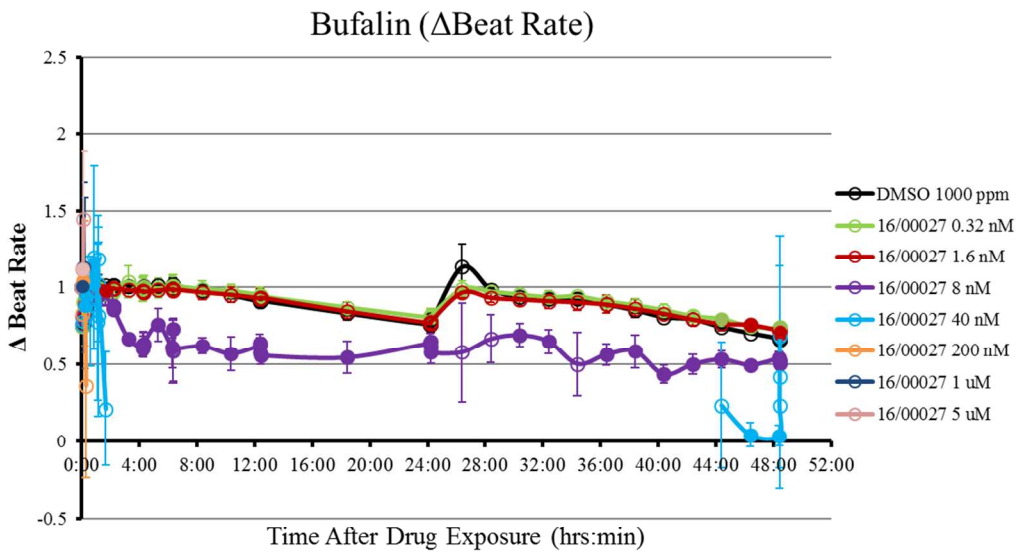



Figure S24. Effect of Bufalin on Beat Rate

**Table S5. ΔBeat Rate versus Bufalin concentration**

| TIME | DMSO | 0.32 nM | 1.6 nM | 8 nM | 40 nM | 200 nM | 1 uM | 5 uM |
|---|---|---|---|---|---|---|---|---|
| 0:07:40 | 100% | 18.0% | 4.6% | 7.5% | 2.4% | **36.5%** | 47.6% | 89.8% |
| 0:32:59 | 100% | 5.3% | 3.8% | 5.0% | 2.1% | **#N/A** | **#N/A** | **#N/A** |
| 1:06:38 | 100% | -0.1% | -2.3% | -4.2% | -21.8% | **#N/A** | **#N/A** | **#N/A** |
| 4:13:55 | 100% | -0.7% | -1.9% | **-39.3%** | **#N/A** | **#N/A** | **#N/A** | **#N/A** |
| 12:21:14 | 100% | 4.7% | 2.2% | **-31.4%** | **#N/A** | **#N/A** | **#N/A** | **#N/A** |
| 24:10:15 | 100% | 5.9% | 1.0% | -16.2% | **#N/A** | **#N/A** | **#N/A** | **#N/A** |
| 36:23:26 | 100% | 2.2% | 0.0% | **-36.8%** | **#N/A** | **#N/A** | **#N/A** | **#N/A** |
| 48:23:26 | 100% | 9.8% | 7.8% | **-19.6%** | **-94.1%** | **#N/A** | **#N/A** | **#N/A** |

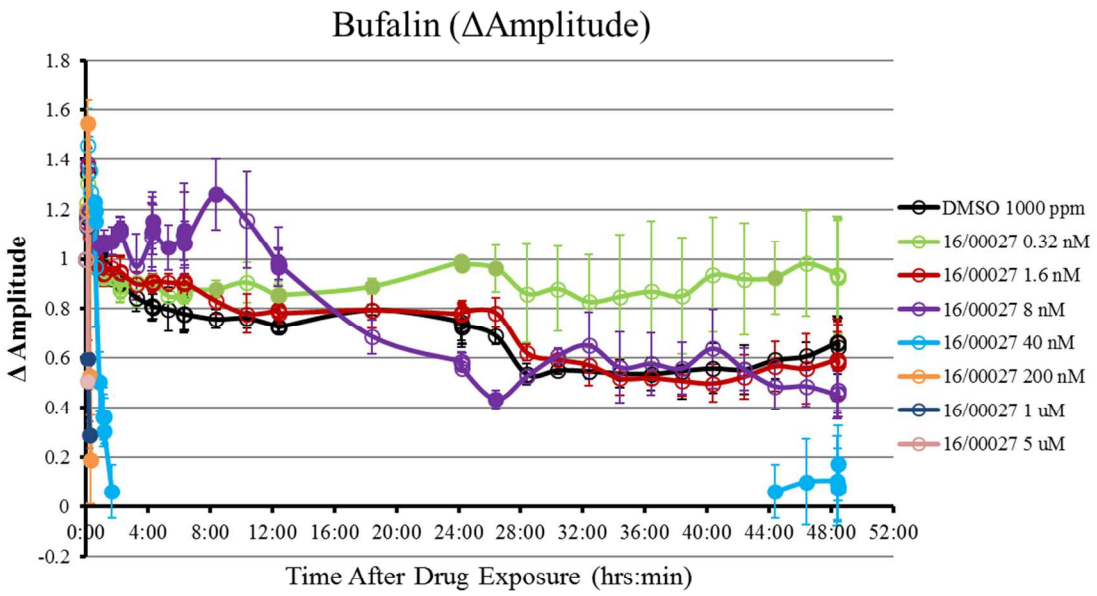



Figure S25. Effect of Bufalin on Amplitude (contractility)

**Table S6. Change in Amplitude (contractility) versus Bufalin concentration**

| TIME | DMSO | 0.32 nM | 1.6 nM | 8 nM | 40 nM | 200 nM | 1 uM | 5 uM |
|---|---|---|---|---|---|---|---|---|
| 0:07:40 | 100% | -3.0% | 1.8% | 2.8% | 8.5% | **15.1%** | **-55.4%** | **62.0%** |
| 0:32:59 | 100% | -2.0% | -0.6% | 3.1% | **20.6%** | **#N/A** | **#N/A** | **#N/A** |
| 1:06:38 | 100% | -4.4% | -2.7% | **10.5%** | **-62.5%** | **#N/A** | **#N/A** | **#N/A** |
| 4:13:55 | 100% | 11.9% | 11.2% | 33.8% | **#N/A** | **#N/A** | **#N/A** | **#N/A** |
| 12:21:14 | 100% | **16.9%** | 8.9% | **34.8%** | **#N/A** | **#N/A** | **#N/A** | **#N/A** |
| 24:10:15 | 100% | **32.4%** | 4.3% | -21.6% | **#N/A** | **#N/A** | **#N/A** | **#N/A** |
| 36:23:26 | 100% | 62.1% | -2.9% | 7.6% | **#N/A** | **#N/A** | **#N/A** | **#N/A** |
| 48:23:26 | 100% | 41.6% | -10.2% | -31.5% | **-84.3%** | **#N/A** | **#N/A** | **#N/A** |

**Effect of Cyanobufalin A (BSP-501).** Cyanobufalin A was acutely cardio-active (having effect on beating parameters) and acutely toxic at concentrations ≥ 40 nM.

A significant and sustained increase in contractility (AMP) was maintained for the length of the assay for 0.32 nM (and mostly for 1.6 nM). This MOA is line with known Na+/K+-ATPase modulators. However this effect was not see at ≥ 8 nM as this was likely where toxicity is of concern. Though not acutely cytotoxic at 8 nM, a steep drop in C.I. was observed after the second dose of BSP-501 after 24 hr. However, we did observe cardio-activity at 8 nM within the first 24 hours of exposure.

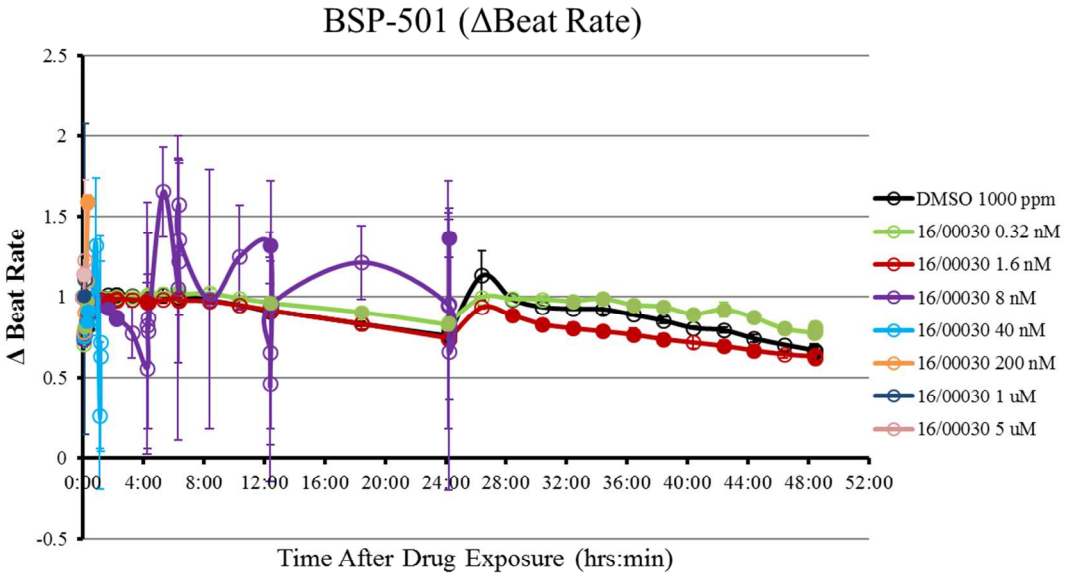



Figure S26. Effect of Cyanobufalin A (1) on Beat Rate

**Table S7. ΔBeat Rate versus Cyanobufalin A (1) concentration**

| TIME | DMSO | 0.32 nM | 1.6 nM | 8 nM | 40 nM | 200 nM | 1 uM | 5 uM |
|---|---|---|---|---|---|---|---|---|
| 0:07:40 | 100% | **6.4%** | 2.1% | 0.8% | 0.5% | 18.4% | 45.7% | 60.6% |
| 0:32:59 | 100% | 2.8% | 0.7% | 0.4% | -10.0% | **#N/A** | **#N/A** | **#N/A** |
| 1:06:38 | 100% | -1.2% | -0.9% | -3.4% | -73.6% | **#N/A** | **#N/A** | **#N/A** |
| 4:13:55 | 100% | 1.6% | -1.7% | -43.8% | **#N/A** | **#N/A** | **#N/A** | **#N/A** |
| 12:21:14 | 100% | **6.0%** | 1.1% | **45.3%** | **#N/A** | **#N/A** | **#N/A** | **#N/A** |
| 24:10:15 | 100% | **8.8%** | -2.2% | 24.9% | **#N/A** | **#N/A** | **#N/A** | **#N/A** |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 36:23:26 | 100% | **6.9%** | **-13.9%** | **#N/A** | **#N/A** | **#N/A** | **#N/A** | **#N/A** |
| 48:23:26 | 100% | **16.5%** | -5.5% | **#N/A** | **#N/A** | **#N/A** | **#N/A** | **#N/A** |

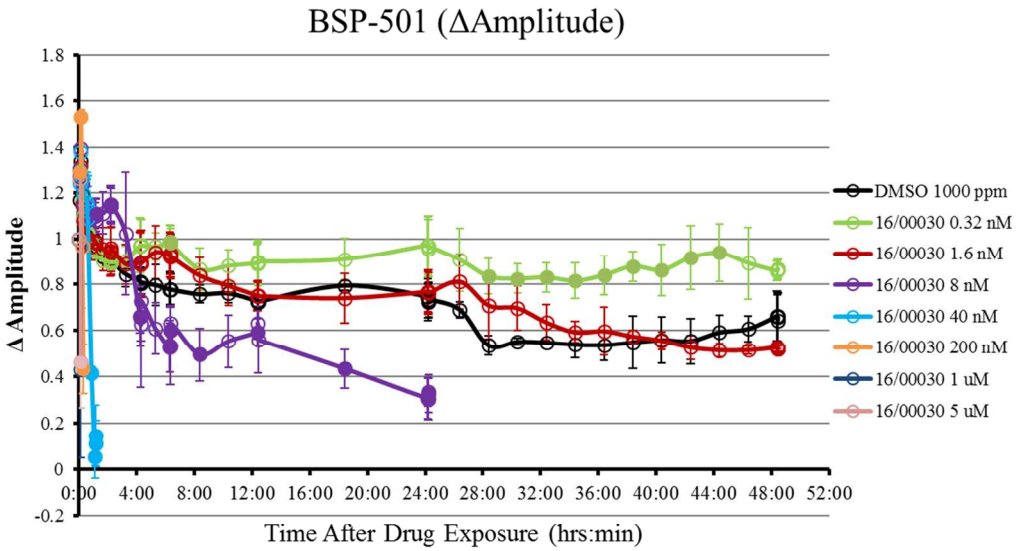



Figure S27. Effect of cyanobufalin A (**1**) on Amplitude (contractility)

Table S8. Change in Amplitude (contractility) versus Cyanobufalin A (1) concentration

| **TIME** | **DMSO** | **0.32 nM** | **1.6 nM** | **8 nM** | **40 nM** | **200 nM** | **1 uM** | **5 uM** |
|---|---|---|---|---|---|---|---|---|
| 0:07:40 | 100% | -2.7% | -1.9% | 3.9% | 2.6% | **14.0%** | -67.8% | **65.6%** |
| 0:32:59 | 100% | 0.7% | -2.1% | 7.5% | 13.4% | **#N/A** | **#N/A** | **#N/A** |
| 1:06:38 | 100% | -1.9% | 2.6% | 13.7% | **-94.4%** | **#N/A** | **#N/A** | **#N/A** |
| 4:13:55 | 100% | 19.3% | 9.7% | **-19.2%** | **#N/A** | **#N/A** | **#N/A** | **#N/A** |
| 12:21:14 | 100% | 22.7% | 3.3% | **-18.3%** | **#N/A** | **#N/A** | **#N/A** | **#N/A** |
| 24:10:15 | 100% | 30.1% | 2.8% | **-58.7%** | **#N/A** | **#N/A** | **#N/A** | **#N/A** |
| 36:23:26 | 100% | **56.9%** | 11.4% | **#N/A** | **#N/A** | **#N/A** | **#N/A** | **#N/A** |
| 48:23:26 | 100% | 29.8% | -20.5% | **#N/A** | **#N/A** | **#N/A** | **#N/A** | **#N/A** |