Supplemental Data

Molecular Pharmacology

Biological Characterization of an Improved Pyrrole-Based Colchicine Site Agent Identified Through Structure-based Design

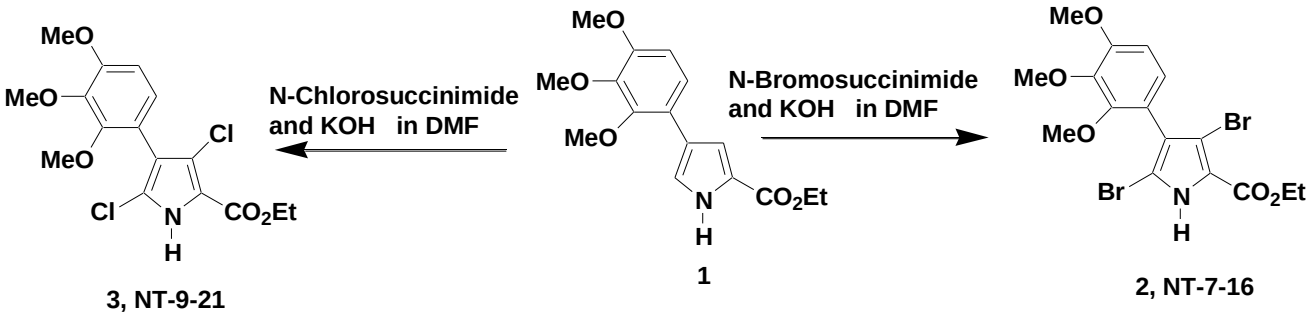
Cristina C. Rohena, Nakul Telang, Chenxiao Da, April L. Risinger, James A. Sikorski,

Glen E. Kellogg, John T. Gupton, Susan L. Mooberry

Experimental

General

Synthesis of NT-7-16 and NT-9-21



All chemicals were used as received from the manufacturer (Aldrich Chemicals and Fisher Scientific). All solvents were dried over 4 angstrom molecular sieves prior to their use. NMR spectra were obtained on either a Bruker 300 MHz spectrometer, or a Bruker 500 MHz spectrometer in either $CDCl_3$, d_6-DMSO or d_6-acetone solutions. IR spectra were recorded on a Nicolet Avatar 320 FT-IR spectrometer with an HATR attachment. High resolution mass spectra were obtained on a Shimadzu IT-TOF mass spectrometer at the University of Richmond. Low resolution GC-MS spectra were obtained on a Shimadzu QP 5050 instrument. Melting points and boiling points are uncorrected. Chromatographic purifications were carried out on a Biotage SP-1 instrument or a Biotage Isolera instrument (both equipped with a silica cartridge). Gradient elution with ethyl acetate/hexane was accomplished in both instances. The reaction products were normally eluted within the range of 4-8 column volumes of eluent with a gradient mixture of 60-80% ethyl acetate in hexane. TLC analyses were conducted on silica plates with hexane/ethyl acetate as the eluent. Purified reaction products gave TLC results, flash chromatograms, HPLC analysis, and proton/carbon NMR spectra consistent with a single, homogenous substance as exemplified by the results below.

Chromatogram of Purified NT-7-16 Sample

Biotage

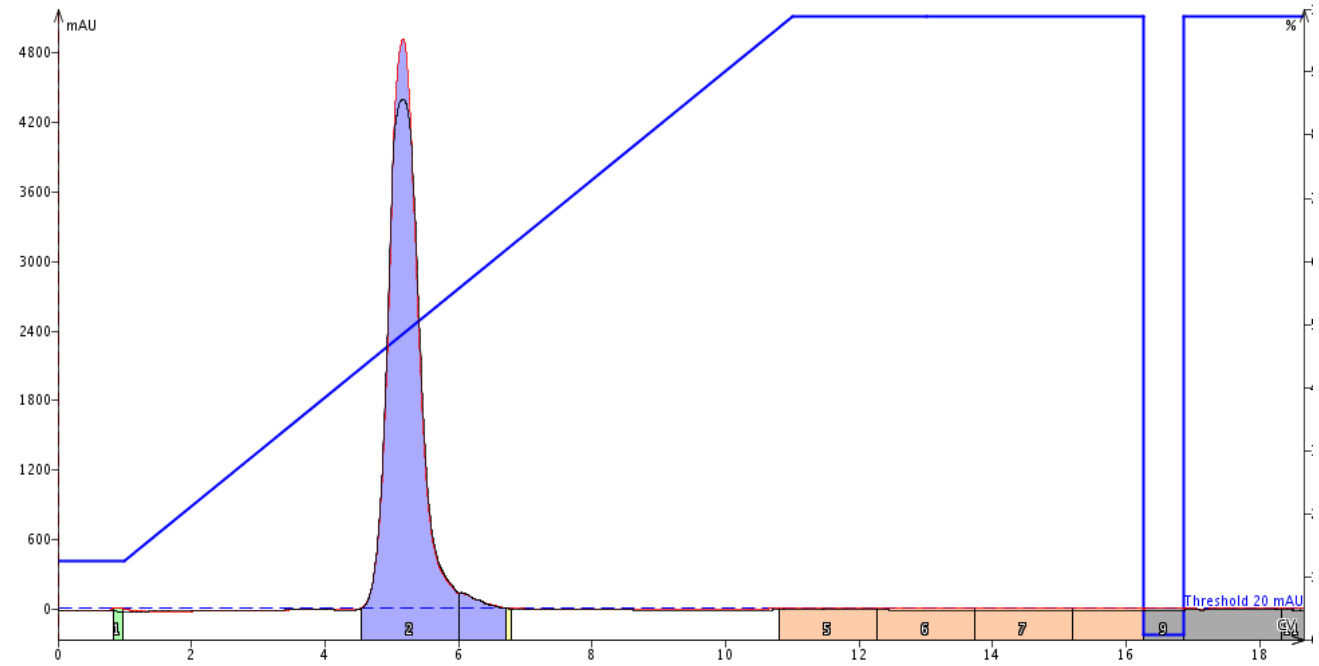
Isolera™ Fractions Report ah-2-35 purification

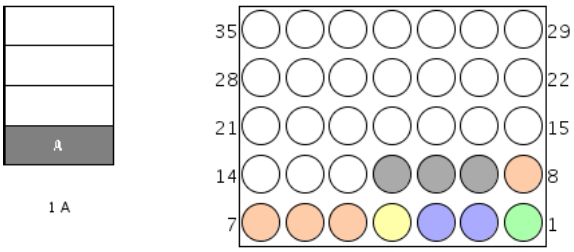
User	William	**Cartridge**	SNAP 10g
Sample Name	ah-2-35 purification	**Rack Type**	16x150 mm

Date 2015-Oct-23 08.57 **Max Fraction Volume**

22 ml

Method **Solvent A** n-Hexane **Detection**
Mode UV1+UV2 **Solvent B** Ethyl acetate **UV1 (Collection)**
254 nm





UV2 (Collection) 280 nm

HPLC Analysis of NT-7-16 on a C-18 Reverse Phase Column with a Methanol/Water Gradient

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Sample Name: dil nt-7-16

    ======================================================================
    Acq. Operator   : dsk                              Seq. Line :   4
    Acq. Instrument : Instrument 3                      Location : Vial 1
    Injection Date  : 10/30/2015 2:29:05 PM                  Inj :   1
                                                    Inj Volume : 2.0 µl
    Sequence File   : C:\Chem32\3\DATA\DEF_LC 2015-10-30 13-37-58\DEF_LC.S
    Method          : C:\CHEM32\3\DATA\DEF_LC 2015-10-30 13-37-58\DSK_TEST.M
    Last changed    : 10/30/2015 1:37:56 PM by dsk
    Method Info     : DK copy originally from Dattelbaum Hystar method for NT-7-16

    Sample Info     : dilute nt-7-16
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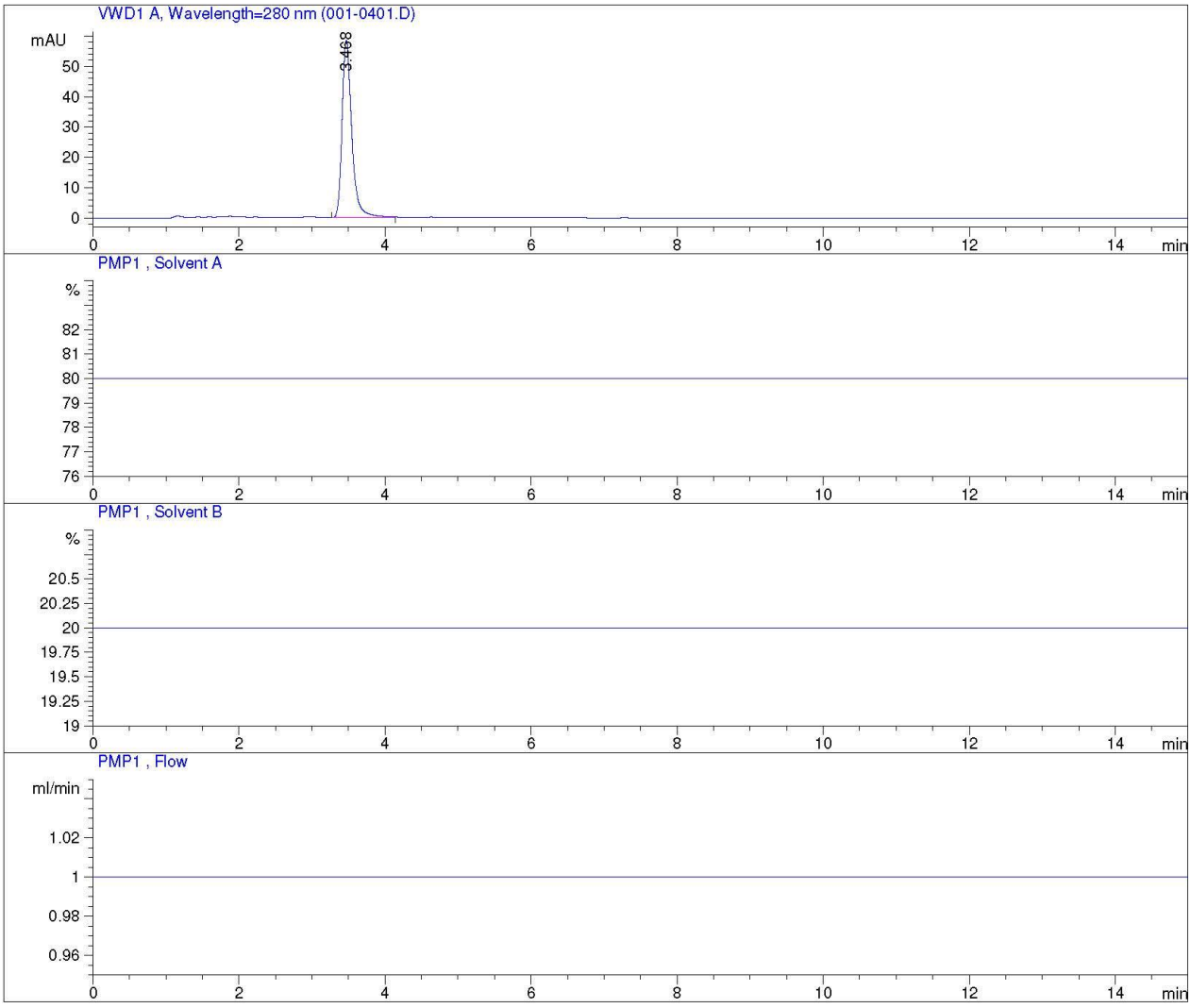

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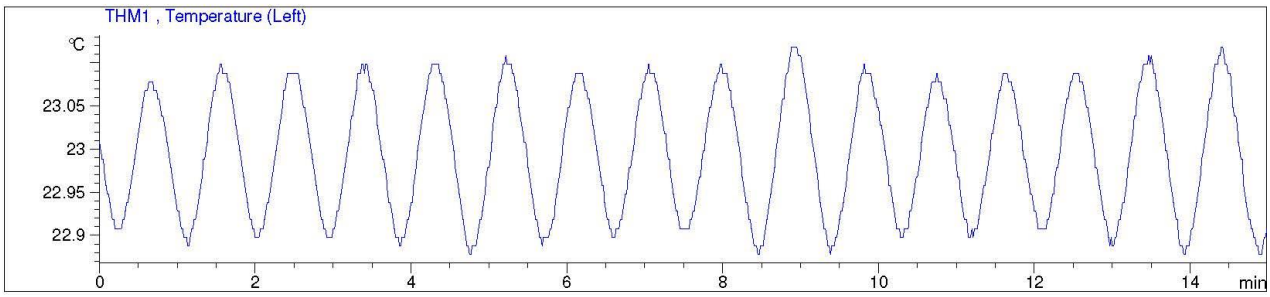

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Area Percent Report

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Sorted By : Signal
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, Wavelength=280 nm

Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	3.468	BB	0.1394	538.02875	58.47703	100.0000
Totals :				538.02875	58.47703	

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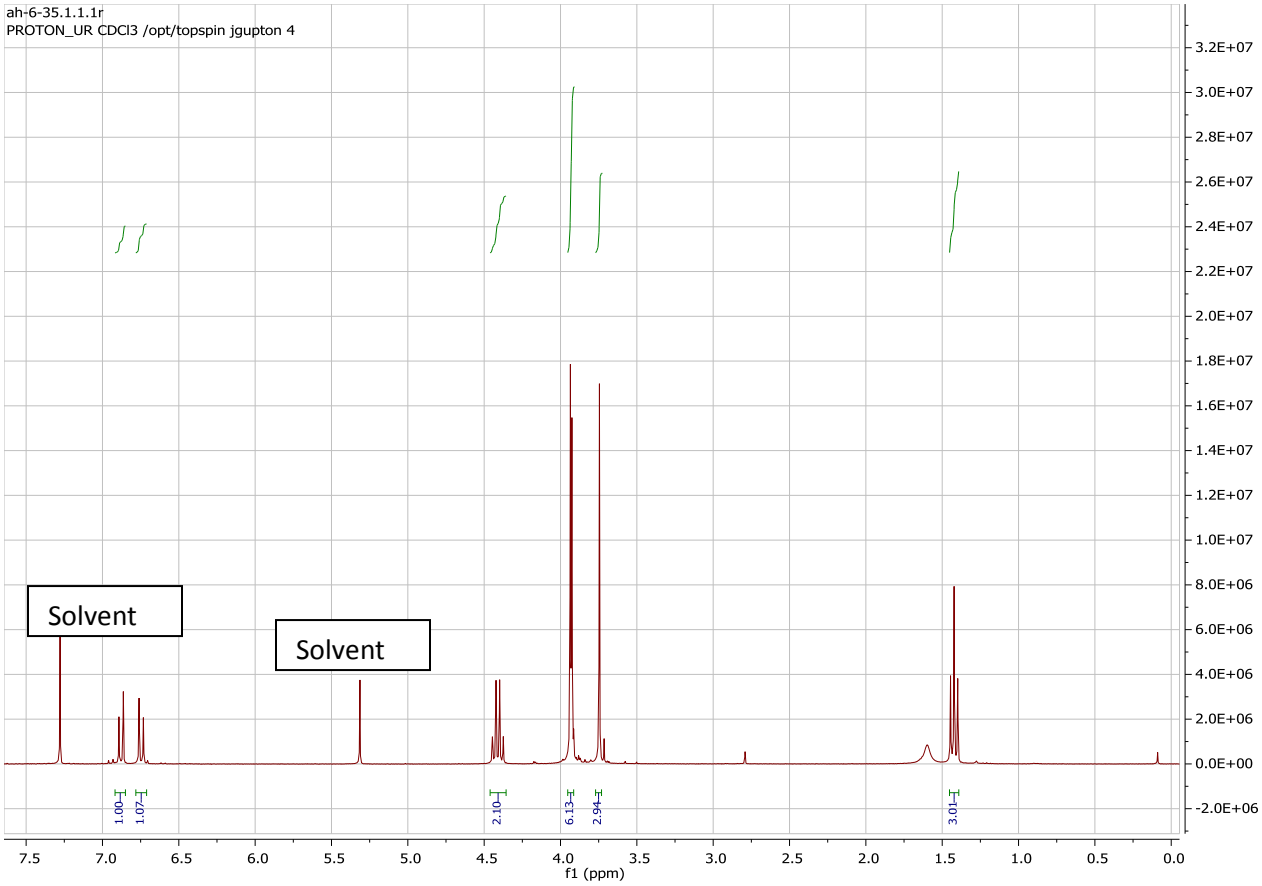
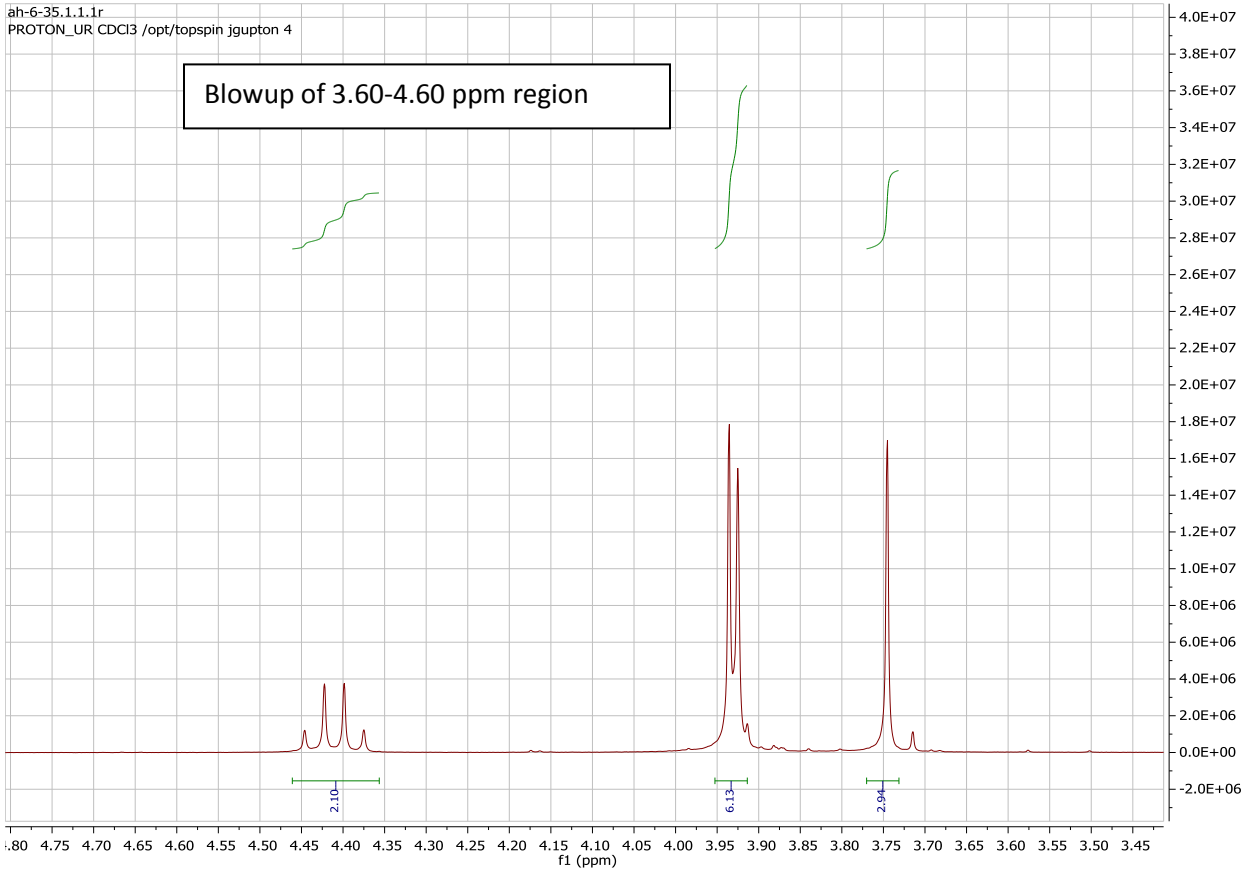
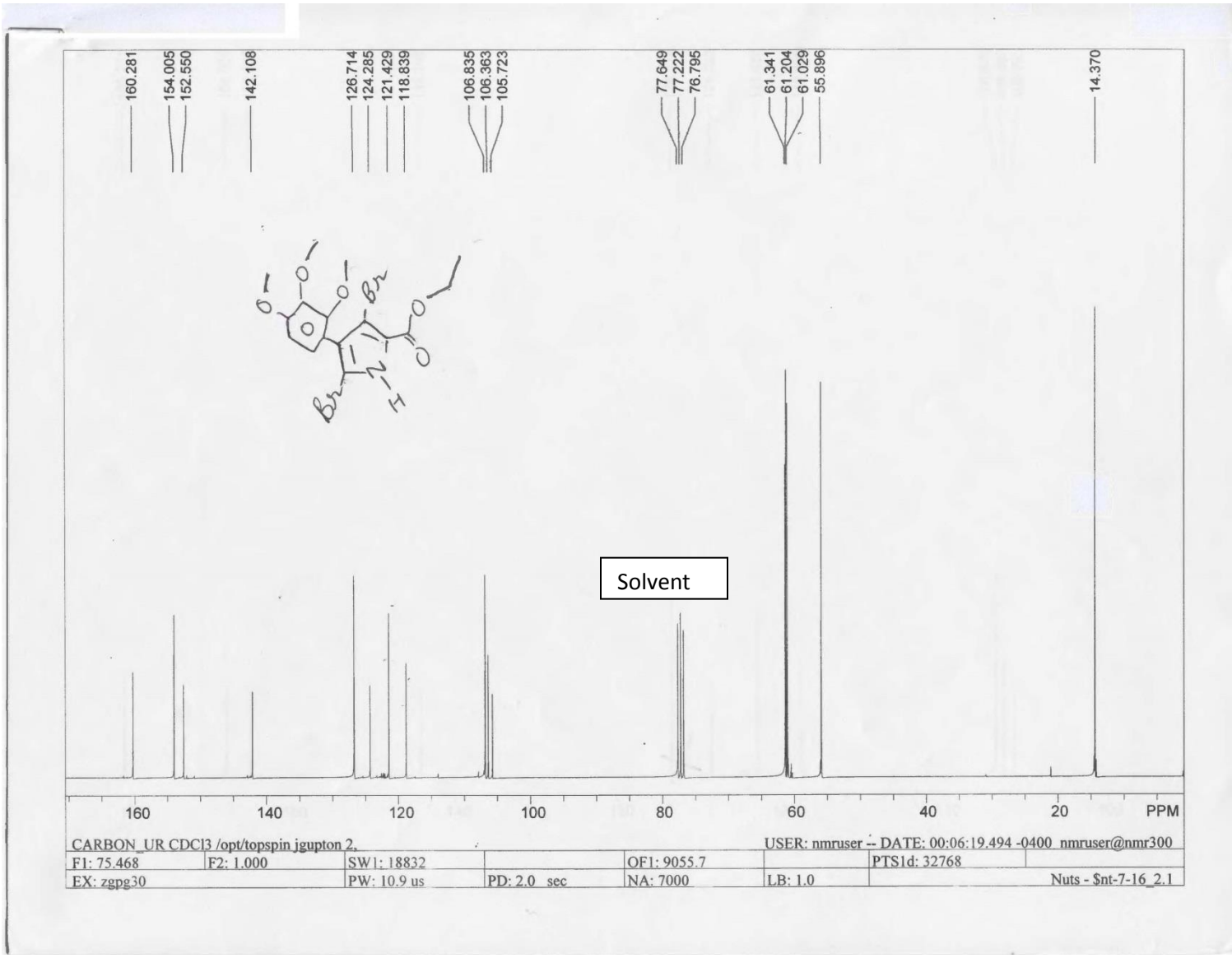
*** End of Report ***

Proton NMR of Purified NT-7-16 Sample





Carbon NMR of Purified NT-7-16 Sample



3,5-Dibromo-4-(2,3,4-trimethoxyphenyl)-1H-pyrrole-2-carboxylic acid ethyl ester (2, NT-7-16)

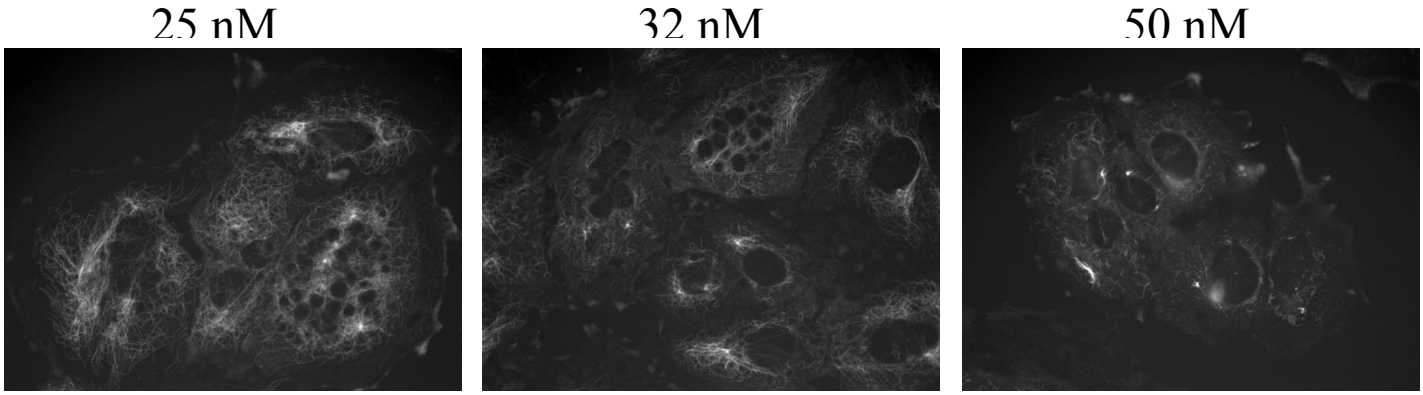
To a 100 mL round bottom flask equipped with a stir bar, was added 4-(2,3,4-trimethoxyphenyl)-1H-pyrrole-2-carboxylic acid ethyl* (0.250g, 0.820 mmol) and potassium hydroxide (0.184 g, 3.27 mmol) in 25 mL DMF. The reaction mixture was allowed to stir for 15 minutes at room temperature, after which N-bromosuccinimide (0.290 g, 1.63 mmol) was added and the reaction mixture was capped and stirred overnight at room temperature. The reaction mixture was diluted with 50 mL of water and 15 mL of sodium thiosulfate solution was added. The resulting mixture was extracted with ethyl acetate (3x15 mL) and the combined ethyl acetate phases were dried over anhydrous sodium sulfate, filtered and concentrated *in vacuo* to give an orange solid (0.315g, 84% yield). The crude residue was subjected to flash chromatography on a Biotage SP-1 instrument with a silica column in which case 0.295g (78% yield) of a tan, amorphous solid was obtained upon elution with five column volumes of a hexane/ethyl acetate gradient. This amorphous solid exhibited the following properties: mp 203 – 205 °C; ^{1}H NMR ($CDCl_3$) δ 1.42 (t, J = 7.2 Hz, 3H), 3.73 (s, 3H), 3.93 (s, 6H), 4.42 (q, J = 7.2 Hz, 2H), 6.74 (d, J = 8.4 Hz, 1H), and 6.87 (d, J = 8.4 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 160.2, 154.0, 152.5, 142.1, 126.7, 124.2, 121.4, 118.8, 106.8, 106.3, 105.7, 61.3, 61.2, 61.0, 55.8, 14.3; IR (neat) 1713 cm^{-1}; HRMS (ES, M+Na) m/z calcd for $C_{16}H_{17}NNaBr_2O_5$ 483.9366, 485.9346 found 483.9356, 485.9337.

3,5-Dichloro-4-(2,3,4-trimethoxyphenyl)-1H-pyrrole-2-carboxylic acid ethyl ester (3, NT-9-21)

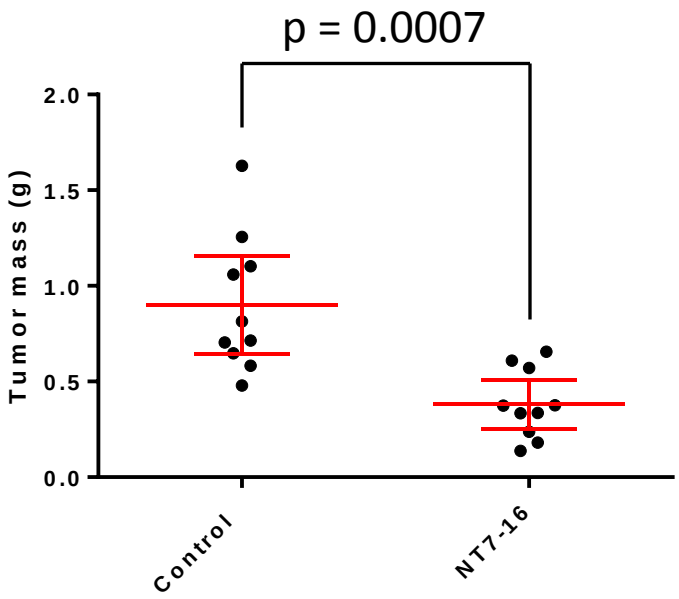
To a 100 mL round bottom flask equipped with a stir bar, was added 4-(2,3,4-trimethoxyphenyl)-1H-pyrrole-2-carboxylic acid ethyl* (0.250g, 0.820 mmol) and potassium hydroxide (0.184 g, 3.27 mmol) in 25 mL of DMF. The reaction mixture was allowed to stir for 15 minutes at room temperature, after which N-chlorosuccinimide (0.214 g, 1.63 mmol) was added and the reaction mixture was capped and stirred overnight at room temperature. The reaction mixture was diluted with 50 mL of water, and 15 mL of sodium thiosulfate solution was added and the resulting mixture was extracted with ethyl acetate (3x15 mL). The combined organic phases were dried over anhydrous sodium sulfate, filtered and concentrated to give a dark orange, amorphous solid (0.323g, 87%). The crude residue was subjected to flash chromatography on a Biotage Isolera instrument with a silica column in which case 0.256 g (69% yield) of an orange, amorphous solid was obtained upon elution with seven column volumes of a hexane/ethyl acetate gradient. This solid exhibited the following properties: m. p. 151 – 153 °C; ^{1}H NMR ($CDCl_3$) δ 1.42 (t, J = 7.2 Hz, 3H), 3.74 (s, 3H), 3.92 (s, 3H), 3.94 (s, 3H), 4.42 (q, J = 7.2 Hz, 2H), 6.74 (d, J = 8.4 Hz, 1H), 6.87 (d, J = 8.4 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 159.9, 152.8, 137.5, 127.2, 126.6, 121.8, 107.4, 104.7, 103.8, 61.4, 60.9, 56.1 and 14.3; IR (neat) 1671 cm^{-1}; HRMS (ES, M+H) m/z calcd for $C_{16}H_{18}NCl_2O_5$ 374.0557 found 374.0641.

*This compound was prepared according to the procedure of Handy and coworkers. Handy ST, Bregman H, Lewis J, and Zhang X (2003) An unusual dehalogenation in the Suzuki coupling of 4-bromopyrrole carboxylates. *Tetrahedron Lett* **44**:427-430.

Supplemental Figures



Supplemental Figure 1. Dose-dependent microtubule depolymerizing effects of NT-7-16 in A-10 cells



Supplemental Figure 2. Final tumor masses (g) were determined and the individual tumor masses (black dots) with the average tumor mass and 95% confidence intervals (red lines) were graphed. Statistical significance between the control and NT-7-16 treatment groups was determined by an unpaired t-test (p = 0.0007).