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14. ABSTRACT

The purpose of this project is to determine if rescinnamine is effective against prostate cancer and treatment resistance. We found that rescinnamine is less effective against prostate cancer that against cancers from other organs. In organisms, the concentration that would effectively cause tumor growth inhibition is limited by its hypotensive activity. We are currently exploring rescinnamine derivatives that have decreased hypotensive activity, and increased tumor growth inhibition.

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Introduction

This project explores new therapeutic approaches for advanced prostate tumors. This innovative approach engages computational modeling to identify compounds that target a specific, mismatch repair protein-dependent cell death pathway. A previously identified compound, rescinnamine is being tested for its efficacy against prostate cancer, and subsequently further developed. Problems with the hypotensive activity of rescinnamine lead to the need to identify analogs that show efficacy in cell killing, while reducing side effects.

Body

As mentioned in the previous report, results with rescinnamine in animals lead to problems with the hypotensive activity of the drug. We therefore went back to the computational modeling part to predict analogs that would show improved cell killing activity while reducing side effects. This approach was described in the "alternate approaches" section of the proposal. Furthermore, we have been adding basic experiments to understand the cell death initiation by rescinnamine and other compounds in the cell.
A graduate student and several undergraduate students (as volunteers) have worked on the project.

Task 1: To demonstrate that a compound mimicking platinum-induced cell death effectively targets chemotherapy-resistant, androgen-independent prostate cancer cells

Task 1a. Growth of cell lines

All indicated cell lines (RWPE-1, PC-3, PC-3-T55, LNCaP) have been grown in the laboratory. Task 1A was completed in the previous cycle

Task 1b. MTS assays with rescinnamine

As mentioned above, we went back to computational predictions, after rescinnamine showed adverse effects in the animal study, and as outlined in our anticipated results and pitfalls. Molecular docking experiments were performed as follows:
The molecular docking was performed in four phases; structural model generation, ligand library generation, receptor grid generation and finally docking of the ligand library into the receptor grid. Models for the structures were generated using molecular dynamics as in our previous works (Vasilyeva, A et al, 2009; Vasilyeva, A et al, 2010;Salsbury. 2010). However, for this work more extensive simulations were used. The details of these simulations are reported elsewhere (Negureanu and Salsbury, 2012). In short, the simulations were four 20ns NPT all-atom simulations based on the human MSH2/6 crystal structure (Morris et al, 2009), with the (1,2)G cross-link, which is the predominate damage due to cisplatin. The structure selected was the median structure of the most populated cluster found from all-atom RMSD-

based clustering (Negureanu and Salsbury, 2012). The appropriate pdbqs file was generated with the DNA removed, so that just the protein remained, using defaults from Autodock4.
Libraries for docking were based on the core of the rescinnamine structure (Figure 7). All possible derivatives were made based on this structure (Figure 7), with R1, R2 and R3 selected from H, OH, COOH, NH_2, and OCH_3. Also, a structure without the modified phenyl ring was docked (Compound 1, Table I). The best of these compounds were suggested for chemical synthesis based on their synthetic accessiblity. We also explored larger libraries, and ones with other functional modifications, but these were not readily synthesized and not discussed herein. Autodock tools were used to generate 3D pdbq files with charges and the correct number of rotatable bonds.
The grids for docking were generated using Autodock4 with the grid centered at the position of the platinum atom in the full protein-DNA complex, however, the DNA was removed prior to grid generation. Cubic 22.5A grids were generated for electrostatics, and vdW parameters for C, S, O, N and polar H with a grid spacing of 0.375A.
The dockings were performed using Autodock4's defaults for it's Lamarckian Genetic Algorithm with a population size of 150, a maximum number of energy evaluations of 5 million and a maximum number of generations of 27000. Each derivative was subjected to 256 runs and ranked according to the best predicted Ki.

An organic chemist was included in this work who synthesized the predicted compounds. General chemistry is described as follows:
Reagents were obtained from commercial sources and used without additional purification. Extraction and flash chromatography solvents were technical grade. Flash chromatography was carried out using a Biotage SP1-B2A0/ HPFC System. Analytical thin layer chromatography (TLC) was performed on silica gel plates with C-4 Spectroline 254 indicator. Visualization was accomplished with UV light and 20% phosphomolybdic acid solution in EtOH. LC-MS, ESI-MS and HPLC solvents were HPLC grade. Melting points were determined on a Mel-Temp apparatus. ^{1}H NMR and $^{13}CNMR$ spectra were taken in commercial deuterated solvents and recorded on a Bruker Advance 300 MHz and Bruker DRX-500 spectrometer using a 5mm TBI probe equipped with z axis gradients. Probe temperature was regulated at 25^{o} C. All data was collected and processed with Topspin 1.3 using standard Bruker processing parameters. Chemical shifts (δ) are given in ppm; multiplicities are indicated by s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), dd (doublet of doublet) and br (broad).
Acryloyl reserpate (1). Dry distilled pyridine (791mg, 0.808mL, 10mmole) was added to a mixture of methyl reserpate (300mg, 0.74mmole) and acryloyl chloride (135.77mg, 0.123mL, 1.5mmole) was added and stirred under nitrogen at room temperature for 74 hr. Excess pyridine was evaporated and the residue was taken up in chloroform (50 mL) and the organic layer washed with water (3x 20 mL) and brine, the organic layer was dried with anhydrous Na_2SO_4, filtered and evaporated under reduced pressure to give a residue that was purified by flash chromatography (silica gel, chloroform/methanol, 3%, R_f 0.3) giving a solid rinsed with methanol to

afford 1 (120 mg, 35%) as white crystals : mp >260 ^{o}C; ^{1}H-NMR (300 MHz, $CDCl_3$, δ): 7.80 (s, 1H), 7.31(d, 1H, J= 8.52 Hz), 6.81 (s, 1H, J= 1.95 Hz), 6.76 (d, 1H, J= 8.53 Hz), 6.45(d, 1H, J= 17.32 Hz), 6.15 (dd, 1H, J= 17.31& 17.30 Hz), 5.87 (d, 1H, J= 10.4 Hz), 4.90-4.82 (m, 1H), 4.42 (s, 1H), 3.82 (s, 3H), 3.80 (s, 3H), 3.48 (s, 3H), 3.19-3.14 (m, 2H), 3.02-2.98 (m, 1H), 2.69- 2.60 (m, 2H), 2.49-2.39 (m,2 H), 2.26- 2.17 (m, 2H), 2.04-1.78 (m, 5H); ^{13}C-NMR (75 MHz, $CDCl_3$, δ): 172.77, 165.42, 156.10, 136.31, 130.79, 130.45, 128.63, 122.13, 118.45. 108.92, 107.92, 95.18, 77.69, 77.54, 60.68, 55.76, 53.68, 51.75, 51.72, 51.17, 48.96, 33.93, 32.25, 29.47, 24.17, 16.75; ESI-MS: m/z= 469.2 (M^+ + H); Anal. Calcd. for $C_{26}H_{32}N_2O_6.0.3H_2O$, C, 65.81; H, 6.94; N, 5.90. Found: C, 65.60; H, 6.83; N, 6.01.

General procedure for the synthesis of rescinnamine derivatives (2-7) A mixture of the aryl iodide (0.507 mmole), acryloyl reserpate (300 mg, 0.641 mmole), Et_3N (0.06 g, 0.09 mL, 0.645 mmol) and $Pd(OAc)_2$ (1.23 mg, 0.005mmole) in the presence of tri-o-tolylphosphine (4.5 mg, 0.021mmole) in acetonitrile (20 mL) was heated with stirring in a capped sealed glass tube under argon at 90^{o}C for 48hrs. After cooling, the solvent was removed under reduced pressure; the residue was dissolved in chloroform (30 mL) and washed with water (3x15 mL) and brine. The organic layer was dried with anhydrous sodium sulfate, filtered and evaporated under reduced pressure to give a residue that was purified by flash chromatography to afford a solid that was recrystallized as described below:

For 2: (R_1 = $COOCH_3$, R_2 = OCH_3): White crystals recrystallized from methanol, yield = 180 mg (44%), (chloroform/methanol 3%, R_f = 0.7), mp = 215-217 ^{o}C; ^{1}H NMR (500 MHz, $CDCl_3$, δ): 8.03 (s, 1H), 7.69 (s, 1H), 7.67-7.66 (m, 2H), 7.35 (d, 1H, J= 8.52), 7.02 (d, 1H, J= 8.77 Hz), 6.86 (s, 1H), 6.79 (d, 1H, J= 8.5 Hz), 6.40 (d, 1H, J= 15.98), 4.97-4.92 (m, 1H), 4.47 (s, 1H), 3.97 (s, 3H), 3.95 (s, 3H), 3.86(s, 3H), 3.84 (s, 3H), 3.54 (s, 3H), 3.19-3.18 (m, 2H), 3.06-3.03 (m, 1H), 2.69-2.66 (m, 1H), 2.52-2.45 (m, 3H), 2.30- 2.25 (m, 2H), 1.98-1.80 (m, 5H); ^{13}C NMR (125 MHz, $CDCl_3$, δ): 172.94, 166.41, 166.11, 160.58, 156.03, 143.66, 136.30, 133.49, 131.63, 130.43, 126.48, 122.05, 120.28, 118.55, 116.84, 112.34, 108.94, 107.85, 95.04, 65.93, 60.97, 56.26, 55.76, 53.69, 52.40, 51.76, 51.17, 48.92, 33.92, 32.25, 31.08, 29.62, 24.21, 16.75, 15.33; ESI-MS: m/z= 633.4 (M^+ + H); Anal. Calcd for $C_{35}H_{40}N_2O_9$: C, 66.44; H, 6.37; N, 4.43; Found: C, 66.16; H, 6.46; N, 4.41.

For 3 (R_1 = OCH_3, R_2 = COOH): Yellow crystals recrystallized from diethyl ether, yield = 150 mg (38%), (chloroform/methanol 8%, R_f = 0.1), mp = 228-230 ^{o}C; ^{1}H NMR (300 MHz, DMSO, δ): 10.62 (s, 1H), 7.70 (d, 1H, J= 15.97 Hz), 7.58 (d, 1H, J=7.83 Hz), 7.47 (s, 1H), 7.32 (d, 1H, J= 7.83 Hz), 7.24 (d, 1H, J= 8.55Hz), 6.82 (s, 1H), 6.79 (d, 1H, J= 15.91 Hz), 6.63 (d, 1H, J= 8.57 Hz), 4.90-4.82 (m, 1H), 4.53 (s, 1H), 3.88 (s, 3H), 3.79 (s, 3H), 3.75 (s, 3H), 3.43 (s, 3H), 3.17-3.11 (m, 3H), 2.97-2.84 (m, 3H), 2.72-2.66 (m, 1H), 2.24 (d, 1H), 1.87-1.73 (m, 6H); ^{13}C NMR (75 MHz, DMSO, δ): 171.81, 170.22, 165.82, 157.10, 155.80, 144.75, 137.20, 136.09, 129.43, 128.36, 121.26, 120.57, 118.97, 118.67, 111.83, 108.99, 105.82, 94.88, 79.40, 77.59, 76.63, 60.55, 55.99, 55.40, 53.47, 52.29, 50.73, 50.06, 48.87, 32.21, 31.29, 28.94, 23.05, 16.00; ESI-MS: m/z= 619.3 (M^+ + H); Anal. Calcd for $C_{34}H_{38}N_2O_9.2.5H_2O$: C, 61.53; H, 6.53; N: 4.22; Found: C, 61.41; H, 7.11; N, 4.00.

For 4 (R_1 = COOH, R_2 = OCH_3): Pale yellow solid recrystallized from diethyl ether, yield = 108 mg (27%), (chloroform/methanol 8%, R_f = 0.1), mp = 249-250 ^{o}C; ^{1}H

NMR (500 MHz, DMSO, δ): 10.61 (s, 1H), 8.32 (s, 1H), 7.93 (s, 1H), 7.87 (d, 1H, J= 8.57 Hz), 7.70 (d, 1H, J= 15.96 Hz), 7.24 (d, 1H, J= 8.44 Hz), 7.16 (d, 1H, J= 8.66 Hz), 6.82 (s, 1H), 6.63 (d, 1H, J= 8.34 Hz), 6.55 (d, 1H, J= 15.99 Hz), 4.81-4.79 (m, 1H), 4.49 (s, 1H), 3.87 (s, 3H), 3.80 (s, 3H), 3.75 (s, 3H), 3.42 (s, 3H), 3.14-3.13 (m, 3H), 2.94-2.85 (m, 3H), 2.68-2.66 (m, 1H), 2.21-2.16 (m, 1H), 1.99-1.74 (m, 6H); ^{13}C NMR (125 MHz, DMSO, δ): 172.07, 168.17, 166.19, 159.75, 155.79, 144.34, 137.13, 132.76, 130.86, 126.43, 123.99, 121.87, 118.53, 116.70, 113.15, 108.81, 106.24, 95.28, 79.59, 77.91, 77.02, 60.64, 56.41, 55.67, 53.97, 52.31, 51.31, 50.96, 48.69, 33.17, 32.12, 29.67, 23.67, 16.57; ESI-MS: m/z= 619.3 (M^+ + H); Anal. Calcd for $C_{34}H_{38}N_2O_9.3H_2O$: C, 60.70; H, 6.59; N, 4.16; Found: C, 60.76; H, 6.39; N, 4.07.

For 5 (R_1 = OCH_3, R_2 = OH): White flakes from chloroform, yield = 108 mg (27%), (chloroform/methanol 4%, R_f = 0.3), mp = 259-260 ^{o}C; ^{1}H NMR (300 MHz, DMSO, δ): 10.49 (s, 1H), 9.61 (s, br, 1H), 7.62 (d, 1H, J= 15.87 Hz), 7.35 (s, 1H), 7.21 (d, 1H, J= 8.48 Hz), 7.14 (d, 1H, J= 8.35Hz), 6.82-6.79 (m, 2H), 6.61 (d, 1H, J= 8.49 Hz), 6.50 (d, 1H, J= 15.89 Hz), 4.87-4.78 (m, 1H), 4.33 (s, 1H), 3.83 (s, 3H), 3.78 (s, 3H), 3.75 (s, 3H), 3.41 (s, 3H), 3.04-3.01 (m, 2H), 2.87-2.81 (m, 2H), 2.66-2.63 (m, 1H), 2.36- 2.31 (m, 2H), 2.19-2.12 (m, 1H), 2.04- 2.00 (m, 1H), 1.93-1.69 (m, 5H); ^{13}C NMR (75 MHz, DMSO, δ): 172.88, 166.49, 156.23, 148.72, 145.98, 144.79, 136.34, 130.55, 128.02, 122.22, 121.86, 118.55, 116.23, 113.18, 110.59, 109.05, 108.13, 95.20, 77.84, 65.84, 60.77, 55.99, 55.82, 53.71, 51.78, 51.19, 49.06, 34.04, 32.34, 29.69, 24.28, 16.81, 15.26; ESI-MS: m/z= 591.2 (M^+ + H).

For 6 (R_1 = OH, R_2 = OCH_3): Yellow solid recrystallized from diethyl ether, yield = 174 mg (43%), (chloroform/methanol 3%, R_f = 0.3), mp = 180-182 ^{o}C; ^{1}H NMR (300 MHz, $CDCl_3$, δ): 7.77 (s, 1H), 7.63 (d, 1H, J= 15.92 Hz), 7.36 (d, 1H, J= 8.54 Hz), 7.32 (d, 1H, J= 8.54 Hz), 7.27 (s, 1H), 6.97 (d, 1H, J= 8.56 Hz), 6.82 (s, 1H), 6.76 (d, 1H, J= 8.53 Hz), 6.33 (d, 1H, J= 15.92 Hz), 4.95-4.87 (m, 1H), 4.42 (s, 1H), 3.85 (s, 3H), 3.82 (s, 3H), 3.81 (s, 3H), 3.50 (s, 3H), 3.49-3.44 (m, 2H), 3.18-3.14 (m, 2H), 3.02-2.97 (m, 1H), 2.67-2.62 (dd, 1H), 2.49-2.39 (m, 3H), 2.33 (s, 3H), 2.04-1.78 (m, 5H).; ^{13}C NMR (125 MHz, $CDCl_3$, δ): 172.86, 168.88, 166.34, 156.18, 152.97, 143.86, 140.06, 136.38, 130.57, 127.82, 127.50, 122.21, 121.94, 118.51, 116.76, 112.35, 108.98, 107.99, 95.25, 77.87, 65.84, 60.79, 56.02, 55.82, 53.74, 51.79, 51.23, 49.04, 34.03, 32.32, 29.69, 24.25, 20.62, 16.81, 15.27; ESI-MS: m/z= 6.33.3 (M^+ + H); Anal. Calcd for $C_{35}H_{40}N_2O_9.0.25H_2O$: C, 65.97; H, 6.41; N, 4.40; Found: C, 65.74; H, 6.55; N, 4.49.

For 7 (R_1 = $OCOCH_3$, R_2 = OCH_3): Yellow solid recrystallized from diethyl ether, yield = 174 mg (43%), (chloroform/methanol 3%, R_f = 0.3), mp = 180-182 ^{o}C; ^{1}H NMR (300 MHz, $CDCl_3$, δ): 7.77 (s, 1H), 7.63 (d, 1H, J= 15.92 Hz), 7.36 (d, 1H, J= 8.54 Hz), 7.32 (d, 1H, J= 8.54 Hz), 7.27 (s, 1H), 6.97 (d, 1H, J= 8.56 Hz), 6.82 (s, 1H), 6.76 (d, 1H, J= 8.53 Hz), 6.33 (d, 1H, J= 15.92 Hz), 4.95-4.87 (m, 1H), 4.42 (s, 1H), 3.85 (s, 3H), 3.82 (s, 3H), 3.81 (s, 3H), 3.50 (s, 3H), 3.49-3.44 (m, 2H), 3.18-3.14 (m, 2H), 3.02-2.97 (m, 1H), 2.67-2.62 (dd, 1H), 2.49-2.39 (m, 3H), 2.33 (s, 3H), 2.04-1.78 (m, 5H).; ^{13}C NMR (125 MHz, $CDCl_3$, δ): 172.86, 168.88, 166.34, 156.18, 152.97, 143.86, 140.06, 136.38, 130.57, 127.82, 127.50, 122.21, 121.94, 118.51, 116.76, 112.35, 108.98, 107.99, 95.25, 77.87, 65.84, 60.79, 56.02, 55.82, 53.74, 51.79, 51.23, 49.04, 34.03, 32.32, 29.69, 24.25, 20.62, 16.81, 15.27; ESI-MS: m/z= 6.33.3 (M^+ + H); Anal. Calcd for $C_{35}H_{40}N_2O_9.0.25H_2O$: C, 65.97; H, 6.41; N, 4.40; Found: C, 65.74; H, 6.55; N, 4.49.

Propionoyl reserpate (8). Using the same procedure for 1 and substituting propionyl chloride for acryloyl chloride yields a residue that was purified by flash chromatography (silica gel, chloroform/methanol, 3%, R_f 0.2) to give a solid that was rinsed with methanol/diethylether (1:1) to afford 8 (317 mg, 56%) as a fine light yellow powder : mp = 258 ^oC; ^1H-NMR (300 MHz, $CDCl_3$, δ): 7.60 (br, s, 1H), 7.32 (d, 1H, J= 8.54 Hz), 6.82 (s, 1H), 6.76 (d, 1H, J= 8.59 Hz), 4.81-4.72 (m, 1H), 4.41 (s, 1H), 3.83 (s, 3H), 3.80 (s, 3H), 3.74-3.71 (m, 1H), 3.49 (s, 3H), 3.24-3.10 (m, 2H), 3.03-2.88 (m, 2H), 2.63-2.58 (m, 1H), 2.50-2.31 (m, 2 H), 2.28-2.12 (m, 4H), 2.03-1.75 (m, 4H), 1.17 (t, 3H, J= 1.35, 7.39 Hz); ^{13}C-NMR (75 MHz, $CDCl_3$, δ): 173.79, 172.52, 156.21, 136.42, 130.53, 122.27, 118.52, 109.02, 108.05, 95.34, 77.74, 77.32, 60.61, 55.83, 53.73, 51.78, 51.69, 51.22, 49.05, 34.01, 32.33, 29.55, 28.03, 24.25, 16.80, 9.16.; ESI-MS: m/z= 471.3 (M^+ + H); Anal. Calcd. for $C_{26}H_{34}N_2O_6C$, 66.36; H, 7.28; N, 5.95. Found: C, 66.08; H, 7.44; N, 5.87.

5-iodo-2-methoxyphenyl acetate (9). 2-Methoxyphenylacetate (28.5 g, 171.87 mmol) was added to a mixture of iodine (17.46 g, 68.75 mmol) and HIO_3 (7.18 g, 40.8 mmol) in glacial acetic acid (190 mL), chloroform (50 mL), water (65 mL) and concentrated sulfuric acid (2 mL) and this mixture was stirred for 24 h at 40^{o}C. Chloroform (50 mL) and water (30mL) were added and the mixture was washed with dilute $NaHSO_3$ (3X) and water. The organic layer was dried with magnesium sulfate and the organic solvent was removed under vacuum to leave a residue that was recrystallized from ethanol to afford 9 as white crystals (11.7 g, 23%); (chloroform/methanol 2%, R_f, 0.3), m.p = 75 °C; ^1H-NMR (300 MHz, $CDCl_3$, δ): 7.49-7.46 (dd, 1H J= 8.63, 2.15 Hz), 7.34 (d, 1H, J= 2.17 Hz), 6.73 (d, 1H, J= 8.66 Hz), 3.79 (s, 3H), 2.29 (s, 3H); ^{13}C-NMR (75 MHz, $CDCl_3$, δ): 168.58, 151.37, 140.48, 135.71, 131.62, 114.33, 81.32, 55.98, 20.55; ESI-MS: m/z= 292.9 (M^+); Anal. Calcd. for $C_9H_9IO_3.0.5CH_3COOH$ C, 37.29; H, 3.44. Found: C, 37.92; H, 3.09.

N-hydroxy-5-iodo-2-methoxybenzamide (10). Separate solutions of hydroxylamine hydrochloride (605 mg, 8.60 mmole) in methyl alcohol (75 mL) and potassium hydroxide (1.2 g, 18.81 mmole) in methanol (50 mL) are prepared at the boiling point of the solvent. Both are cooled to 30-40 °C and the one containing alkali was added with shaking to the solution of hydroxylamine; any excessive rise of temperature during the addition is prevented by occasional cooling in an ice bath. After all the alkali has been added, the mixture is allowed to stand in an ice bath for 5 min to ensure complete precipitation of potassium chloride followed by filtration. The filtrate was added to methyl 5-iodo-2-methoxybenzoate (500 mg, 1.72 mmole) and the mixture was heated to reflux for 6 h and cooled to room temperature. The mixture was acidified with glacial acetic acid until the pH was about 6 and concentrated to remove the solvents. The residue was mixed with EtOAc (100 mL) and water (80 mL) was added to get a clear solution. The organic layer was separated and the aqueous solution was extracted with EtOAc (2 x 50 mL). The combined organic phases were washed with brine, dried over anhydrous sodium sulfate, and concentrated to afford a white solid residue, recrystallized from chloroform to afford 9 as white crystals (362 mg, 73% yield) (ethyl acetate/ hexane 1:5, R_f=0.3); mp = 157-158°C; ^1H-NMR (300 MHz, DMSO, δ): 10.68 (s, 1H), 9.16 (br, s, 1H), 7.82-7.72 (m, 2H), 7.01-6.93 (d, 1H, J = 8.62 Hz), 3.81 (s, 3H); ^{13}C-NMR (75 MHz, DMSO, δ): 161.50, 156.48, 139.93, 137.55, 124.89, 114.64, 82.73, 55.85; ESI-

MS: m/z= 294.0 (M^+ + 1); Anal. Calcd. for $C_8H_8INO_3$ C, 32.79; H, 2.75; N, 4.78. Found: C, 32.82; H, 2.61; N, 4.79.

N-hydroxy-4-iodo-2-methoxybenzamide (11). This compound was prepared using the same procedure for 7 with methyl-4-iodo-2-methoxybenzoate as substrate to afford an off-white solid that was recrystallized from ethyl acetate/n-hexane to yield 10 as off-white crystals (102 mg, 21%); (ethyl acetate/ hexane 1:5, R_f=0.4); mp = 105-106°C; ^{1}H-NMR (300 MHz, $CDCl_3$, δ): 10.24 (s, br, 1H), 7.82 (d, 1H, J = 8.23 Hz), 7.41 (d, 1H, J = 8.12 Hz), 7.28 (s, 1H), 3.95 (s, 3H); ^{13}C-NMR (75 MHz, $CDCl_3$, δ): 163.13, 157.03, 132.89, 130.94, 120.77, 118.01, 99.66, 56.52; ESI-MS: m/z= 294.0 (M^+ + 1); Anal. Calcd. for $C_8H_8INO_3.0.1CH_3COOC_2H_5$ C, 33.42; H, 2.94; N, 4.64. Found: C, 33.48; H, 2.76; N, 4.57.

General procedure for synthesis of rescinnamine derivatives (12-15): A similar Heck coupling procedure without a phosphine ligand using the appropriate substituted aryl iodides affords the corresponding substituted rescinnamine derivatives (12-15).

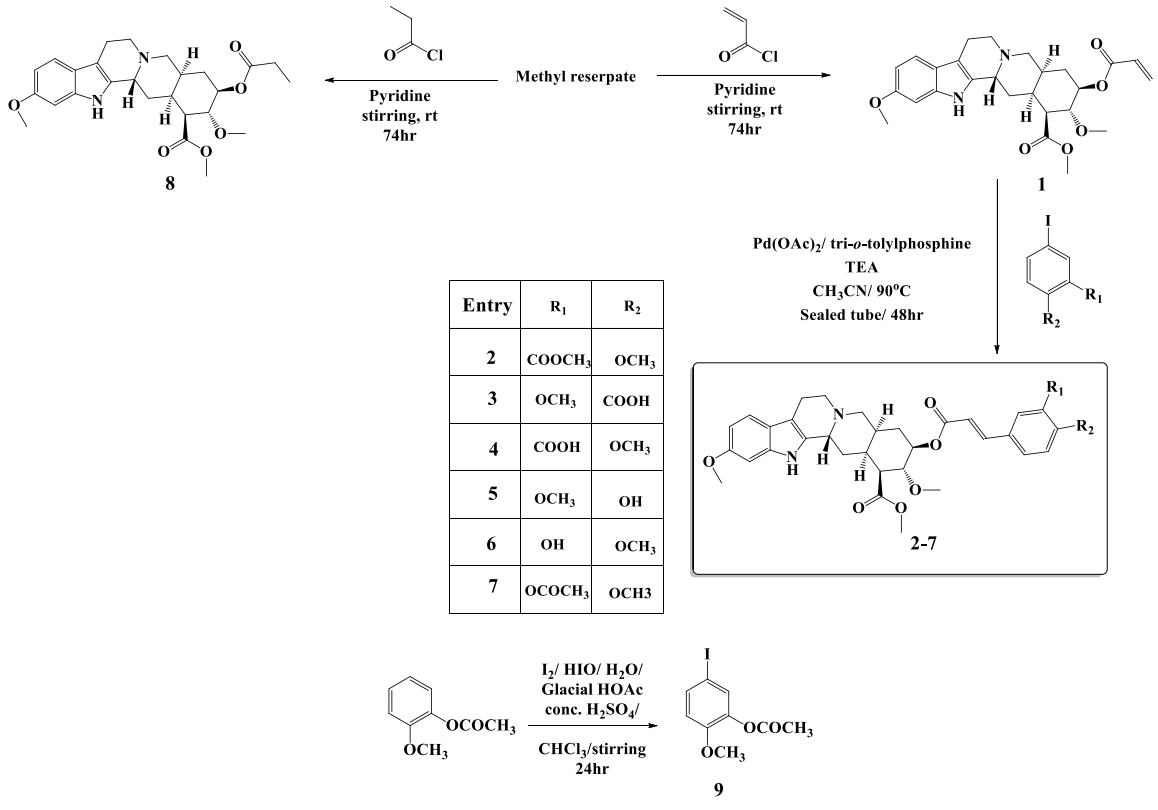
For 12: Yellowish brown solid recrystallized from diethyl ether, yield = 132 mg (35%), (chloroform/methanol 2%, R_f = 0.2), mp = 202-203 ^{o}C; ^{1}H NMR (300 MHz, $CDCl_3$, δ): 7.66 (s, 1H), 7.63 (d, J = 15.91 Hz, 1H), 7.32 (d, J = 8.54 Hz, 1H), 6.94-6.90 (m, 2H), 6.82 (s, 1H), 6.74 (d, J = 8.70 Hz, 2H), 6.27 (d, J = 15.88 Hz, 1H), 4.96-4.87 (m, 1H), 4.44 (s, 1H), 3.88 (s, 3H), 3.83 (s, 3H), 3.81 (s, 3H), 3.51 (s, 3H), 3.42-3.44 (m, 1H), 3.20-3.15 (m, 2H), 3.04-2.89 (m, 2H), 2.73-2.61(m, 1H), 2.50-2.42 (m, 2H), 2.29-2.17 (m, 2H), 2.05-1.77 (m, 4H), 1.28-1.18 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$, δ): 172.92, 166.68, 156.23, 149.42, 145.33, 136.54, 136.38, 130.51, 127.47, 122.21, 120.30, 118.53, 115.41, 113.13, 110.18, 109.05, 108.06, 95.23, 77.88, 65.84, 60.75, 55.83, 55.59, 53.75, 51.78, 51.21, 49.08, 34.04, 32.32, 29.74, 24.27, 16.81, 15.26; ESI-MS: m/z= 590 (M^+ + 1), 295 (M^+ + 2). HRMS-ESI$^+$ (m/z): $[M + H]^+$ calcd for $C_{33}H_{40}N_3O_7$, 590.2866; found, 590.2846; Anal. Calcd for $C_{33}H_{39}N_3O_7.0.8H_2O$: C, 65.61; H, 6.77; N, 6.96; Found: C, 65.27; H, 6.63; N, 6.87.

For 13: Pale yellow flakes recrystallized from diethyl ether, yield = 153 mg (41%), (chloroform/methanol 2%, R_f = 0.4), mp = 210-213 ^{o}C; ^{1}H NMR (300 MHz, $CDCl_3$, δ): 7.81 (s, br, 1H), 7.61 (d, J = 15.8.4 Hz, 1H), 7.31 (d, J = 8.54 Hz, 1H), 7.01 (d, J = 7.98 Hz, 1H), 6.96 (s, 1H), 6.82 (s, 1H), 6.75 (d, J = 8.54 Hz, 1H), 6.66 (d, J = 7.97 Hz, 1H), 6.23 (d, J = 15.83 Hz, 1H), 4.95-4.80 (m, 1H), 4.43 (s, br, 1H), 4.14 (s, 1H), 3.88 (s, 3H), 3.82 (s, 3H), 3.80 (s, 3H), 3.50 (s, 3H), 3.18-3.13 (m, 2H), 3.03-2.93 (m, 2H), 2.67-2.58 (m, 1H), 2.49-2.40 (m, 2H), 2.27-2.16 (m, 2H), 1.92-1.77 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$, δ): 172.99, 166.94, 156.15, 146.97, 145.77, 139.20, 136.36, 130.51, 128.67, 124.60, 123.34, 122.15, 118.52, 114.06, 113.39, 108.98, 107.96, 95.19, 60.77, 55.82, 55.50, 53.73, 51.82, 51.19, 49.03, 41.81, 34.01, 32.30, 30.95, 29.77, 24.24, 16.78, 13.85; ESI-MS: m/z= 590 (M^+ + 1), Anal. Calcd for $C_{33}H_{39}N_3O_7.0.6H_2O$: C, 66.01; H, 6.75; N, 7.00; Found: C, 66.11; H, 6.64; N, 6.71.

For 14: Grayish white solid, yield = 289 mg (70%), recrystallized from benzene, (chloroform/methanol 6%, R_f = 0.3), mp = 245-255 ^{o}C; ^{1}H NMR (500 MHz, DMSO, δ): 10.96 (s, 1H), 7.57 (s, 1H), 7.49 (d, 1H, J= 15.78 Hz), 7.37-7.34 (m, 2H), 7.23 (s, 1H), 6.86 (s, 1H), 6.71 (d, 1H, J= 8.49 Hz), 6.27 (d, 1H, J= 15.79 Hz), 5.06 (s, br, 1H), 5.06 (s, 1H), 4.69-4.72 (m, 1H), 3.81 (s, 3H), 3.79 (s, 3H), 3.77 (s, 3H), 3.74-3.68 (m, 2H), 3.65-3.57 (m, 1H), 3.44 (s, 3H), 3.17-3.07 (m, 1H), 2.84-2.99 (m, 1H), 2.85-2.82 (m,

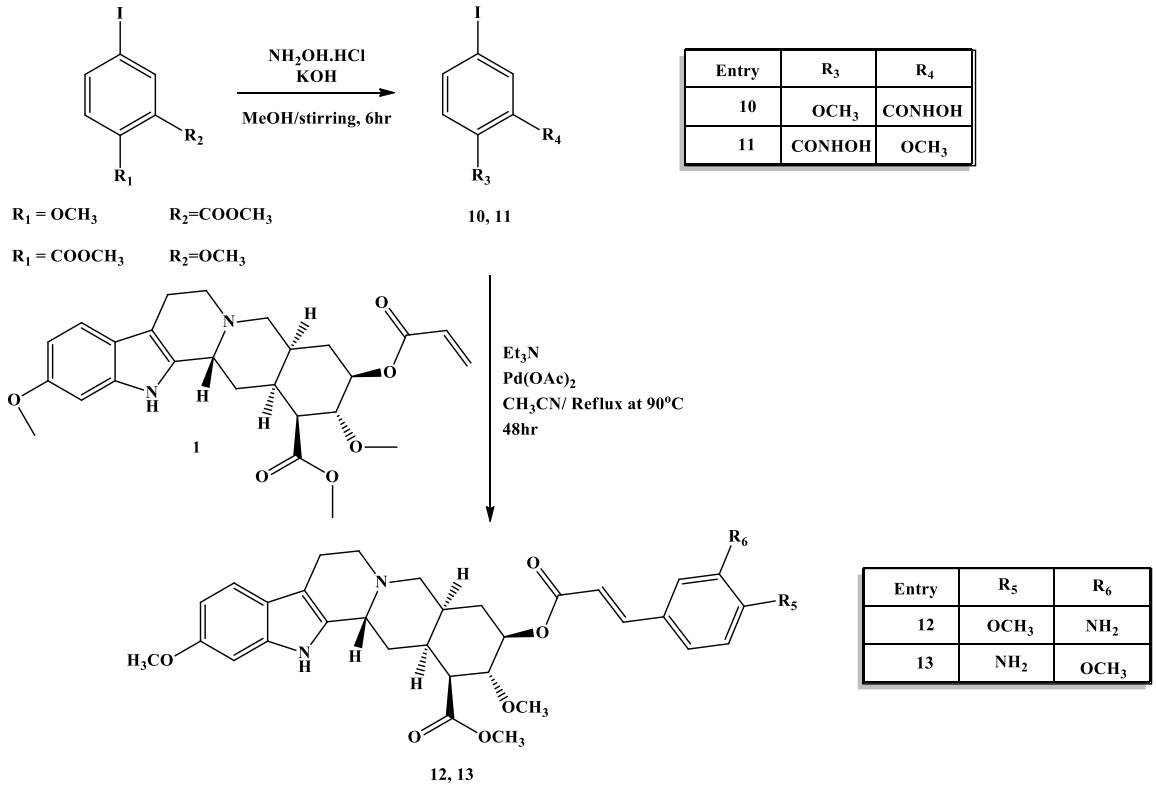
1H), 2.73-2.69 (m, 1H), 2.30-2.08 (m, 5H), 1.94-1.83 (m, 2H); ^{13}C NMR (125 MHz, DMSO, δ): 171.76, 171.24, 165.79, 157.77, 155.92, 149.02, 147.59, 145.74, 137.31, 128.29, 124.66, 122.56, 120.84, 120.64, 118.63, 118.35, 112.76, 112.08, 109.04, 105.34, 94.82, 77.12, 75.66, 60.23, 55.66, 55.25, 54.32, 51.95, 50.16, 31.27, 30.34, 28.34, 22.49, 15.49; ESI-MS: m/z= 635.2 (M^+ + H); HRMS: [M^++ H- CH_3] calcd for $C_{34}H_{39}N_2O_{10}$, 635.2605; found, 635.2582; Anal. Calcd for $C_{35}H_{40}N_2O_{10}.2.5H_2O$: C, 61.71; H, 6.25; N, 4.23; Found: C, 61.84; H, 5.87; N, 4.28.

For 15: Dark yellow solid, yield = 140 mg (35%), recrystallized from diethyl ether, (chloroform/methanol 5%, R_f = 0.1), mp = 229-231 ^{o}C; ^{1}H NMR (300 MHz, DMSO, δ): 10.49 (s, 1H), 8.96 (s, br, 1H), 7.63 (d, 1H, J= 15.81 Hz), 7.20 (d, 1H, J= 8.57 Hz), 7.06 (s, 2H), 6.80 (s, 1H), 6.62-6.54 (m, 2H), 4.89-4.76 (m, 1H), 4.35 (s, 1H), 3.82 (s, 6H), 3.79 (s, 3H), 3.75 (s, 3H), 3.42 (s, 3H), 3.10-2.97 (m, 3H), 2.89-2.79 (m, 3H), 2.69-2.60 (m, 3H), 2.42-2.14 (m, 5H); ^{13}C NMR (75 MHz, DMSO, δ): 171.61, 165.98, 155.05, 148.01, 145.61, 138.35, 136.35, 131.20, 128.52, 124.37, 121.65, 117.81, 114.96, 108.01, 106.32, 105.92, 94.77, 77.54, 76.40, 64.87, 60.05, 56.09, 55.18, 53.32, 51.74, 51.05, 50.74, 48.62, 33.19, 32.14, 29.53, 23.46, 16.44, 15.12; ESI-MS: m/z= 621.3 (M^+ + H).



Entry	R_1	R_2
2	$COOCH_3$	OCH_3
3	OCH_3	COOH
4	COOH	OCH_3
5	OCH_3	OH
6	OH	OCH_3
7	$OCOCH_3$	OCH3

Fig.1. ***Synthetic procedures for the preparation of rescinnamine derivatives (1-8). Condensation reactions between methyl reserpate and acyl chlorides to give 1 and 8. Heck coupling procedure with 1 to yield rescinnamine derivatives 2-7.***



Entry	R_3	R_4
10	OCH_3	CONHOH
11	CONHOH	OCH_3

Entry	R_5	R_6
12	OCH_3	NH_2
13	NH_2	OCH_3

Fig.2. ***Synthetic procedures for the preparation of rescinnamine derivatives (12-13). Heck coupling of synthetic hydroxamic acid aryl iodides gives the amino-substituted rescinnamine derivatives 12 and 13.***

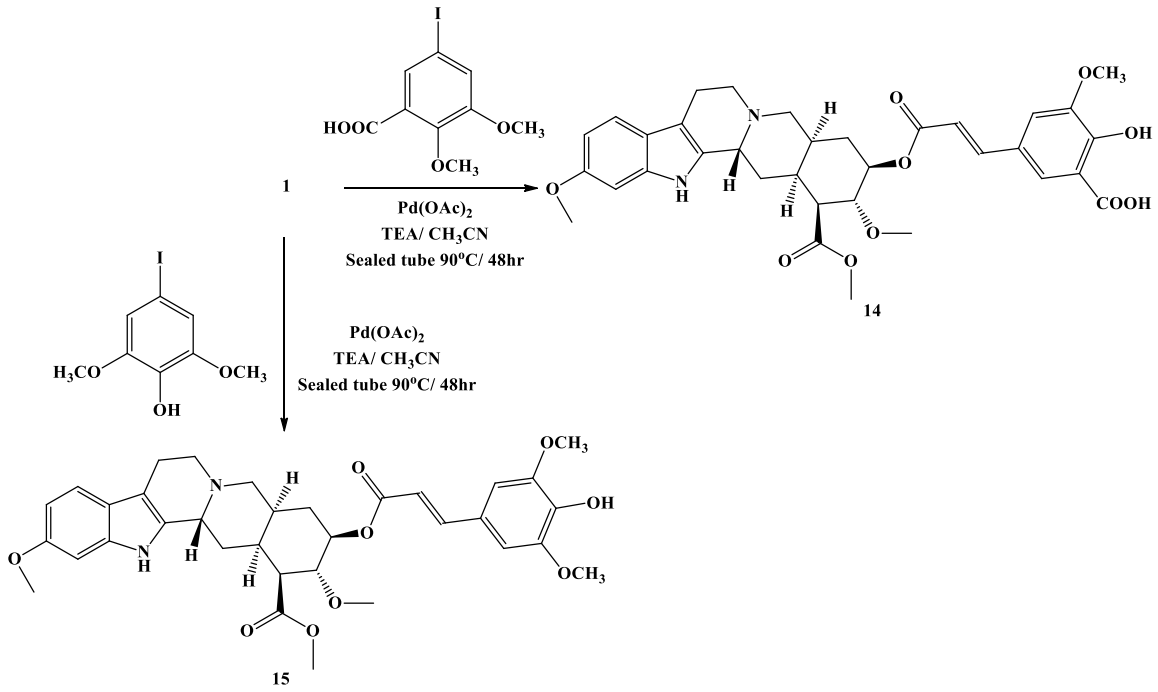


Fig. 3. ***Synthetic procedures for the preparation of rescinnamine derivatives (14-15). Heck coupling of commercially available aryl iodides to give rescinnamine derivatives 14 and 15.***

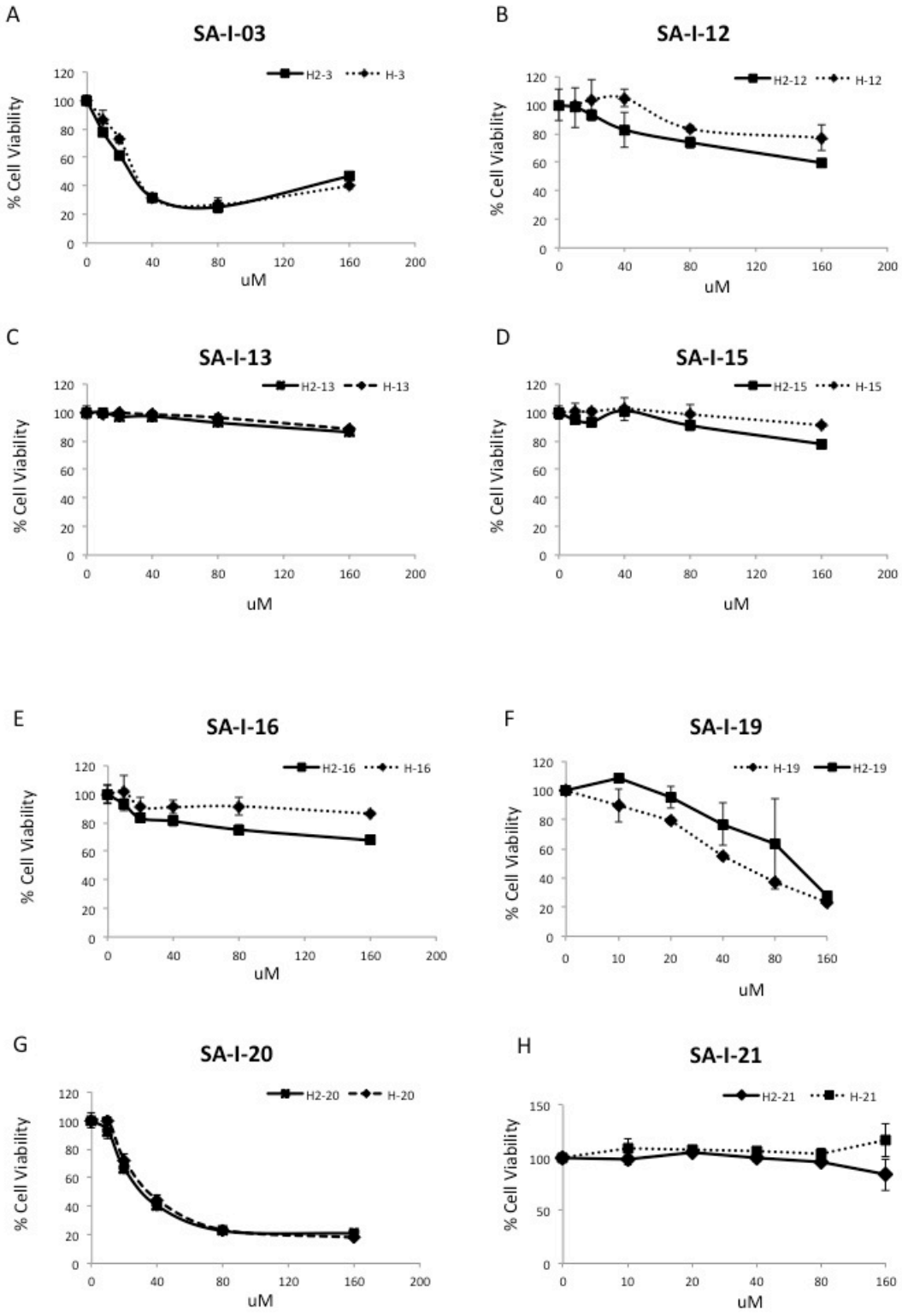
Prostate cancer cell lines were tested against these compounds as indicated in the SOW. We added HEC59, an endometrial cell line with an MSH2 deficiency and its counterpart HEC59 chr.2 with complemented MSH2 to some of the tests to determine if the new compounds show the rescinnamine-specific specificity for MSH2.

Promising lead molecules that were predicted to induce MSH2-dependent cell death and had a favorable partition coefficient were considered for chemical synthesis (Table 1).

Treatment of methyl reserpate, formed by the basic methanolysis of reserpine (Vasilyeva et al., 2010), with acryloyl chloride yields acryloyl reserpate (1) in 35% yield (Figure 3) (Pearce et al., 1989). Similar treatment of 1 with propionoyl chloride yields the saturated derivative 8 (Figure 3). Exposure of 1 to various commercial or synthetic substituted aryl iodides (9) to palladium catalyzed Heck coupling conditions gives the di-substituted rescinnamine derivatives (2-7) in 27-68% yield and coupling constant analysis shows the exclusive formation of the trans stereoisomer (Figure 3) (Ziegler et al., 1978) Condensation of the commercially available methyl esters with hydroxylamine produces the corresponding hydroxamic acids (10 and 11, Figure 4) (Hoshino et al., 2009) and Heck coupling of 1 to 10 and 11 in the absence of a phosphine ligand yields the aniline derivatives of rescinnamine (9 and 10, exclusive E stereochemistry) in 35 and 41% yield, respectively rather than the expected hydroxamic acids (Figure 4). (Patel et al.,

1977) Such results suggest either a base or palladium-mediated Lossen rearrangement of these hydroxamic acid substrates, a process that holds some literature precedence (Hoshino et al., 2009). Similar Heck coupling of 1 to commercial aryl iodides yields the tri-substituted rescinnamine derivatives 14 and 15 in 70 and 35% yield, respectively (Figure 5). Extensive mass spectrometry and two-dimensional nuclear magnetic resonance (NMR) experiments confirm the structure of 14, which lacks the expected p-methoxy group of the starting material (Figure 5). A combination of proton and carbon NMR spectroscopy, mass spectrometry and elemental analysis confirms the identity of 1-15.

Effects of rescinnamine analogs on cell viability:
We next tested these new rescinnamine analogs in a well-defined cellular system with an endometrial cell line deficient (HEC59) and proficient (via chromosome transfer, HEC59 + chr.2) for MSH2. This cell system allows to determine whether our new analogs hit their target and, generally, induce cell death. The assays identified a few compounds that induced cell killing in the micromolar range (Fig. 6 and Table 1, compounds 1, 6, 7, 13, 15 and to a lesser extent 12). However, little significant MSH2-dependence was observed for these compounds. The only compounds that demonstrated a small difference between MSH2-proficiency and deficiency were 2, 5 and 13 (Figure 6 and Table 1). No significant difference was detected when determining the IC50 values and these results are surprising in-light of our previous work. Previous studies have suggested that in addition to MSH2-dependent cell-killing due to rescinnamine and derivatives, there is off-target cell-killing as well (Vasilyeva et al., 2010). These results suggest considerable off-target killing for these classes of compounds.
Compounds 84-90 do not show significant specificity for MSH2, either, but compound 81 shows significant cell death with minor specificity for MSH2.


A
SA-I-03
H2-3
H-3
% Cell Viability
uM
B
SA-I-12
H2-12
H-12
% Cell Viability
uM
C
SA-I-13
H2-13
H-13
% Cell Viability
uM
D
SA-I-15
H2-15
H-15
% Cell Viability
uM
E
SA-I-16
H2-16
H-16
% Cell Viability
uM
F
SA-I-19
H-19
H2-19
% Cell Viability
uM
G
SA-I-20
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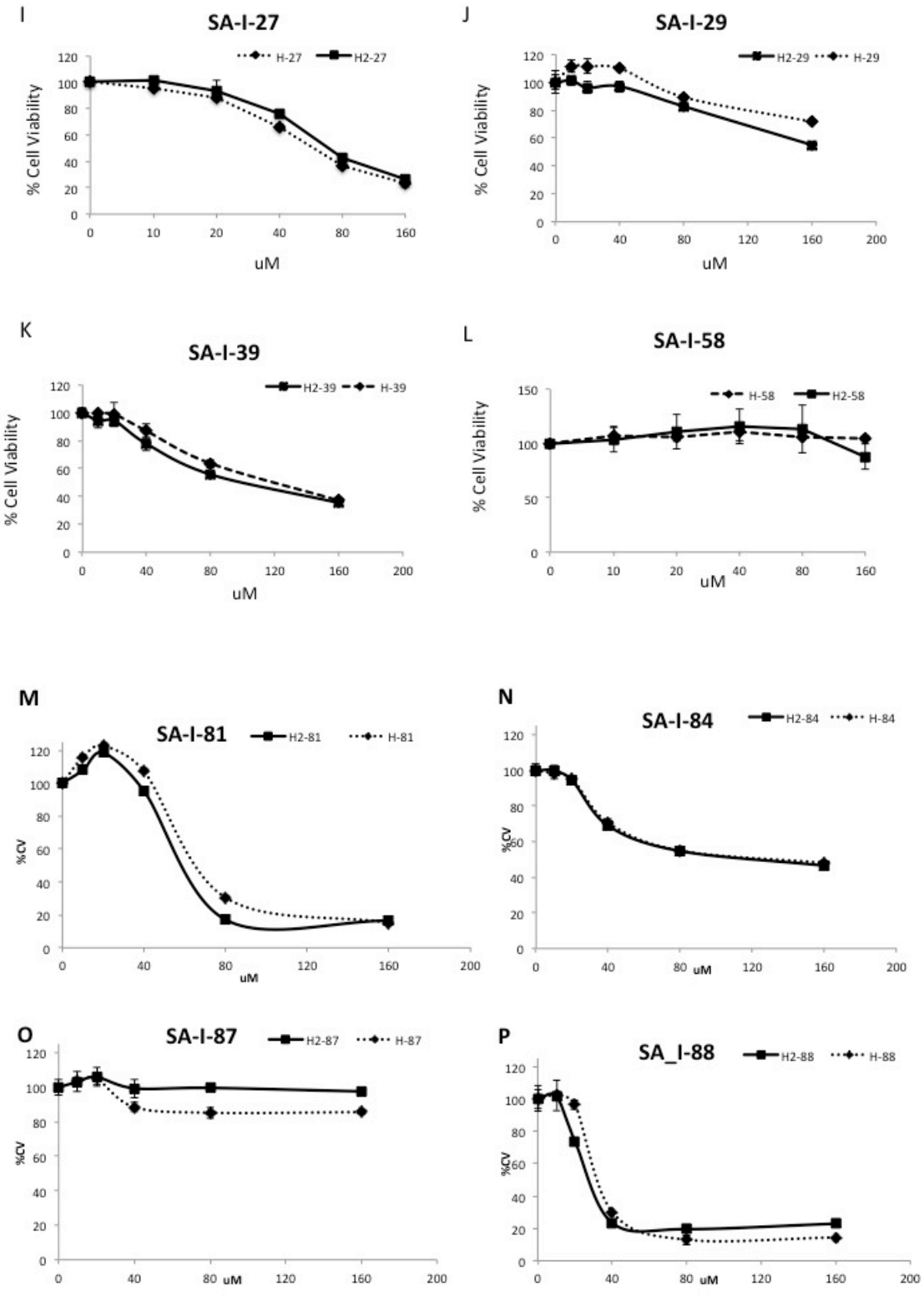
H-20
% Cell Viability
uM
H
SA-I-21
H2-21
H-21
% Cell Viability
uM


I
SA-I-27
H-27
H2-27
% Cell Viability
uM
J
SA-I-29
H2-29
H-29
% Cell Viability
uM
K
SA-I-39
H2-39
H-39
% Cell Viability
uM
L
SA-I-58
H-58
H2-58
% Cell Viability
uM
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H-84
%CV
uM
O
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%CV
uM

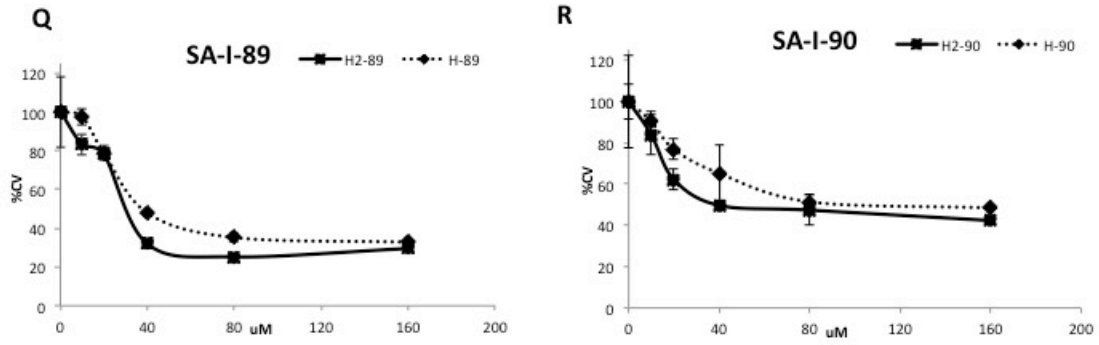
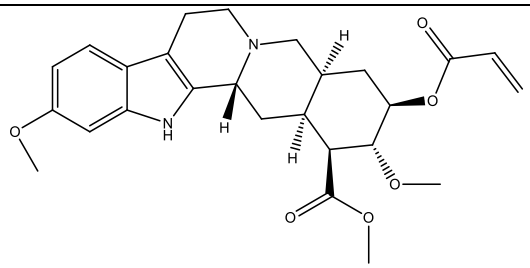
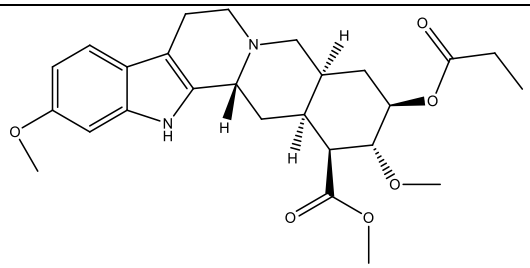
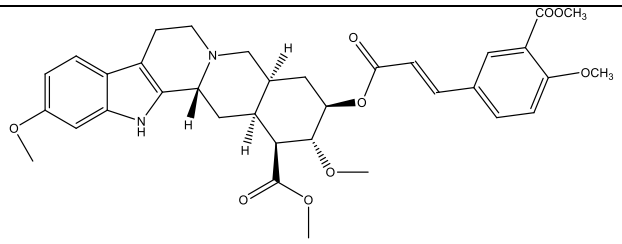
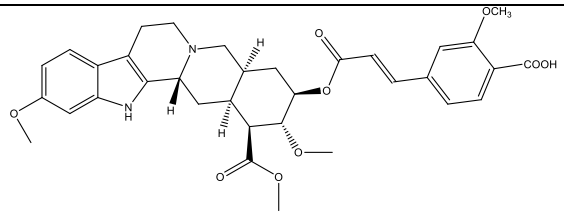
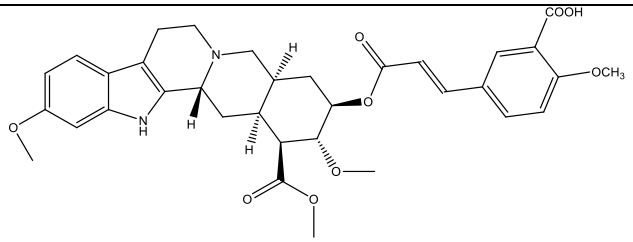
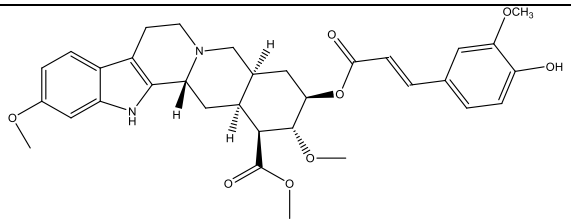
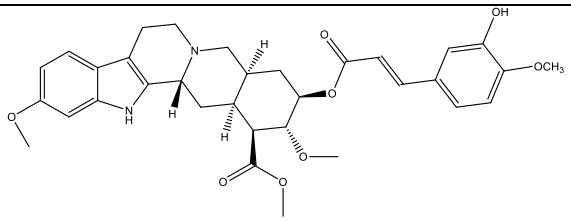
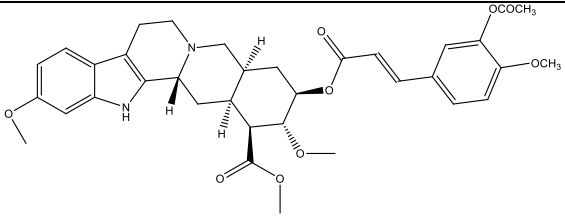
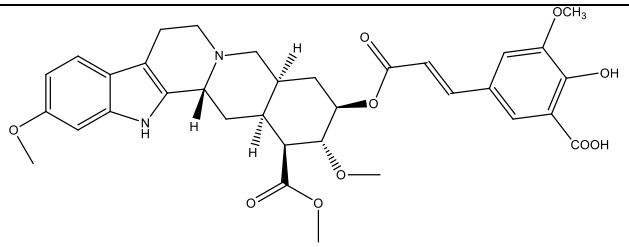
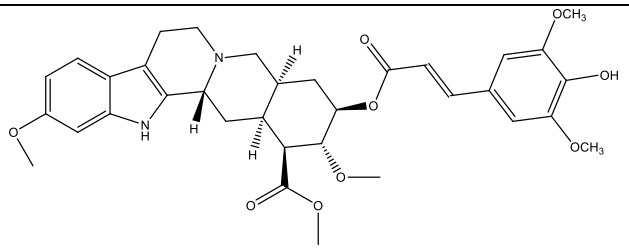
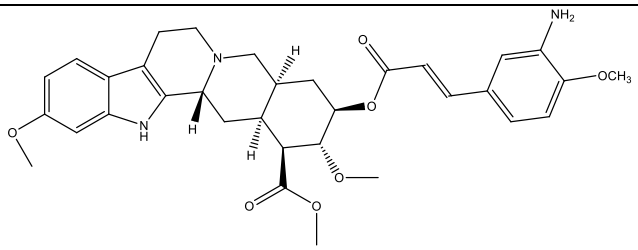
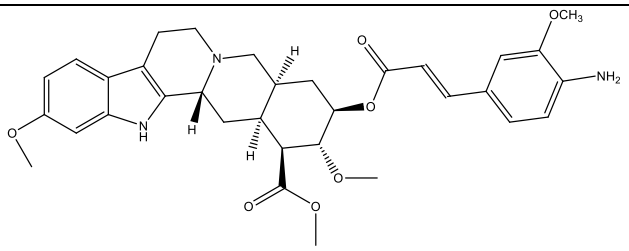
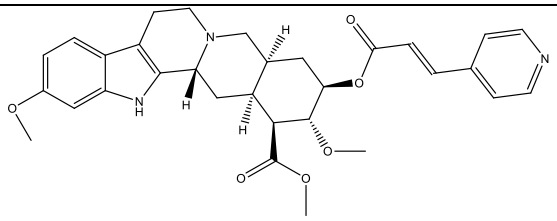
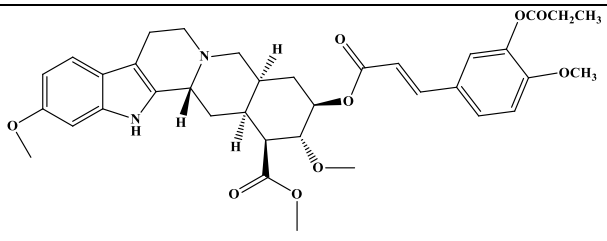
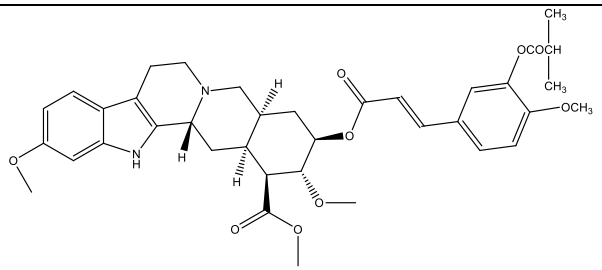
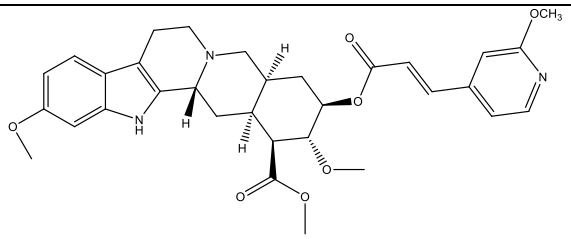
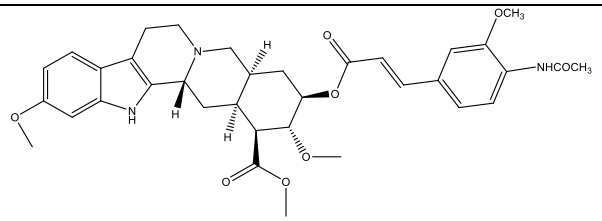
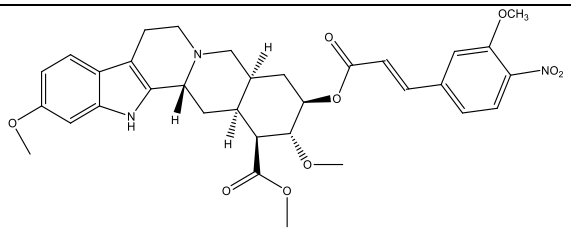


Fig. 4. ***Cell Survival of MSH2 proficient and deficient cells after treatment with rescinnamine analogs. HEC59 ("H") and its isogenic cell line containing a chromosome 2 transfer ("H2") were treated with increasing concentrations of the indicated compound (Table 1). Cell viability is graphed in dependence of concentration. CV: Cell Viability.***

Table 1: IC50 values for individual rescinnamine analogs. Where appropriate, graphs that did not reach 50& mortality were extrapolated. NA: no extrapolation possible.			
			IC50
Entry	**Compound**	MSH2 deficient	MSH2 proficient
SA-I-03		20.7	20.37
SA-I-058		NA	NA

SA-I-012		50.72	48.91
SA-I-013		163.1	1223
SA-I-015		89.29	84.23
SA-I-016		NA	NA
SA-I-020		22.32	21.29
SA-I-019		39.28	NA

SA-I-021		NA	NA
SA-I-027		47.67	54.53
SA-I-029		77.25	113.4
SA-I-039		85.73	68.38
SA-I-81		58.23	47.63
SA-I-84		37.3	35.6

SA-I-87		NA	NA
SA-I-88		31.3	22.0
SA-I-89		25.6	27.8
SA-I-90		24.1	4.8

We repeated the cell viability assay for the most promising compounds, 12, 19 and 81 and performed a caspase-3 activation assay to determine induction of apoptosis:

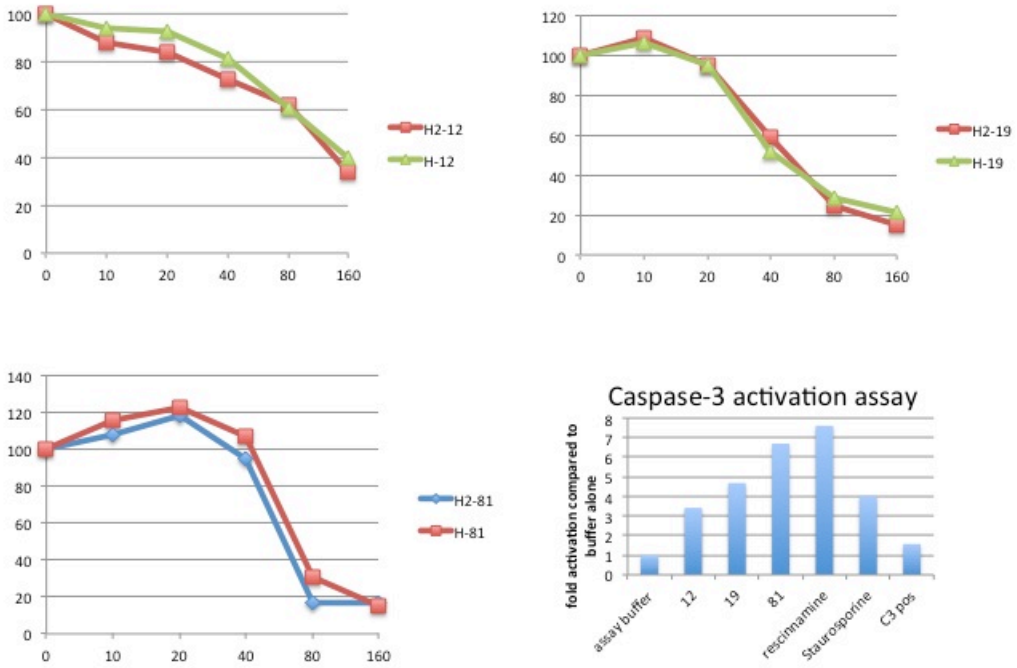


Fig. 5. ***Dose-dependent MTS assay with compounds 12, 19 and 81 in HEC59/HEC59chr.2 cells. Caspase-3 activation kit shows activation of the caspase as a measure of apoptosis. The assay was performed as recommended by the manufacturer. C3 pos is the included positive control. Staurosporine and rescinnamine are added as additional positive controls. All data are fold increase in comparison to "assay buffer", which was defined as "1". CV: Cell Viability***

While no significant specificity for MSH2 is observed, compounds 19, 81 and 88 show significant cell killing ability that is dose-dependent. Both compounds activate caspase -3, similar to rescinnamine.

We next tested the most promising compounds against prostate cancer cells, and used A2780 cells (ovarian cancer) as a control, as before.

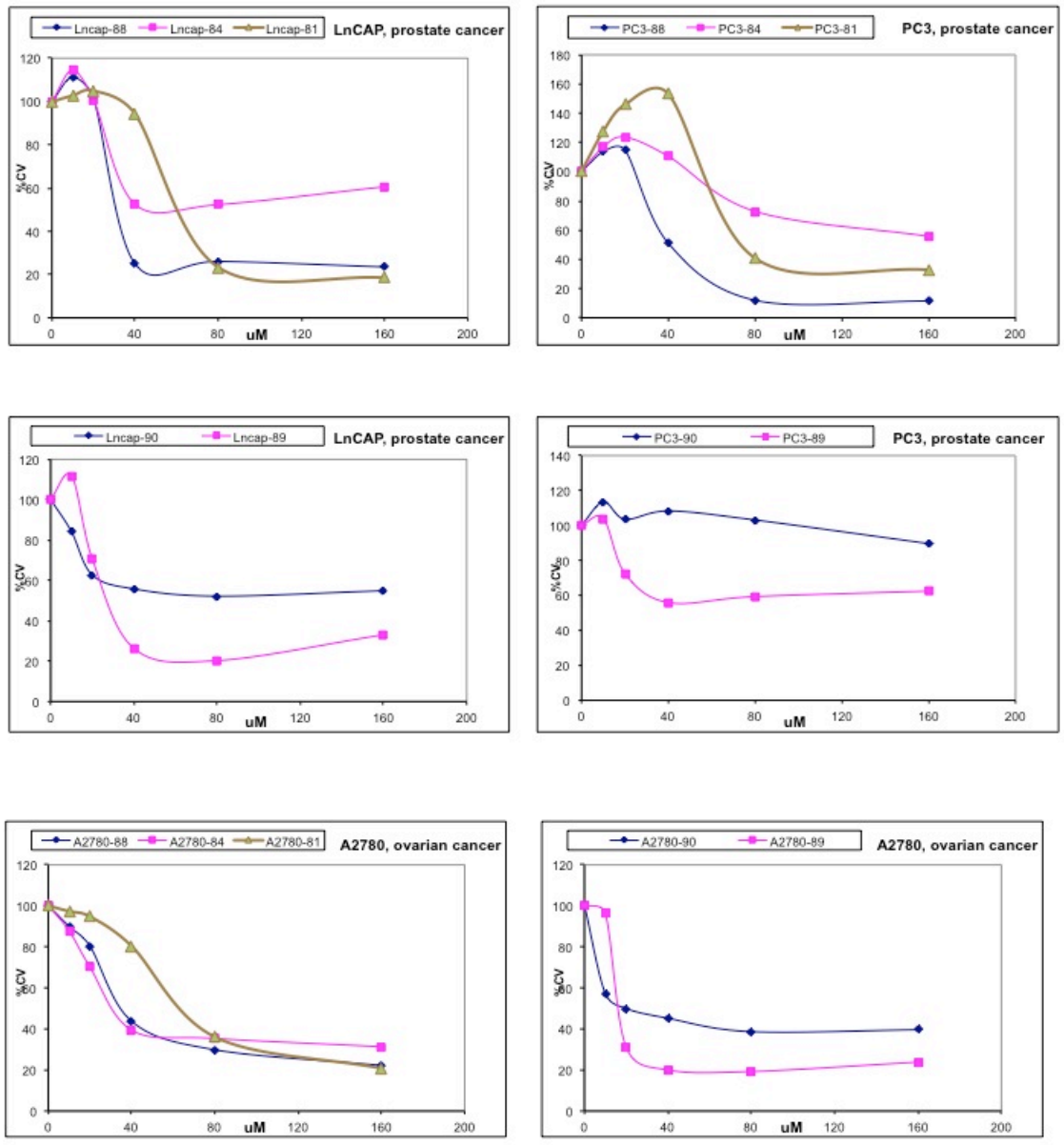


Fig. 6. ***Dose-dependent MTS assay with compounds 81, 84 and 88 in prostate cancer cells.*** *CV: Cell viability. Ovarian cancer cell line A2780 added as control.*

As was already observed in HEC59 cells, a threshold around 25 uM appears to exist beyond which cell viability is drastically reduced. For compound 81, the concentration at which this significant reduction in cell viability occurs, appears to be shifted to slightly higher concentrations, around 40 uM. Compounds 84, 89 and 90 show the worst effect on cell viability in prostate cancer cells, though they have efficacy in the ovarian cancer cell line. Even in LnCAP cells, 89 and 90 show some efficacy, which is almost entirely gone in PC3 cells. Overall, in prostate cancer cells, compound SA-I-88 exhibits the best activity, followed by SA-I-81 at this point.

Table 2: IC50 values for individual rescinnamine analogs in prostate cancer cells. Where appropriate, graphs that did not reach 50& mortality were extrapolated (in parentheses). NA: no extrapolation possible.			
	LnCAP	**PC3**	**A2780**
SA-I-81	53.8	73.4	56.8
SA-I-84	(21.5)	(63.0)	23.6
SA-I-88	22.96	38.6	31.3
SA-I-89	20.1	(18.4)	16.5
SA-I-90	(36.6)	NA	36.9

Our next step will be the determination of effects on other prostate cancer cells to determine specificity for cancer. We will also determine caspase activation to demonstrate the induction of apoptosis.
It may further be required to use these data in advanced computational docking experiments and identify additional analogs that may show even better efficacy against prostate cancer.
We will next use the most promising compounds against therapy-resistant prostate cancer cells and in combination therapy (**SOW 1c, d, but with new compounds**) to determine if efficacy can be improved and whether these compounds might be an effective way to overcome resistance.

Task 1f. Determine induction of pro-apoptotic proteins
As a control, we first performed assays on the induction of pro-apoptotic proteins with compounds known to induce or not induce the targeted response in PC3 prostatic cancer cells:

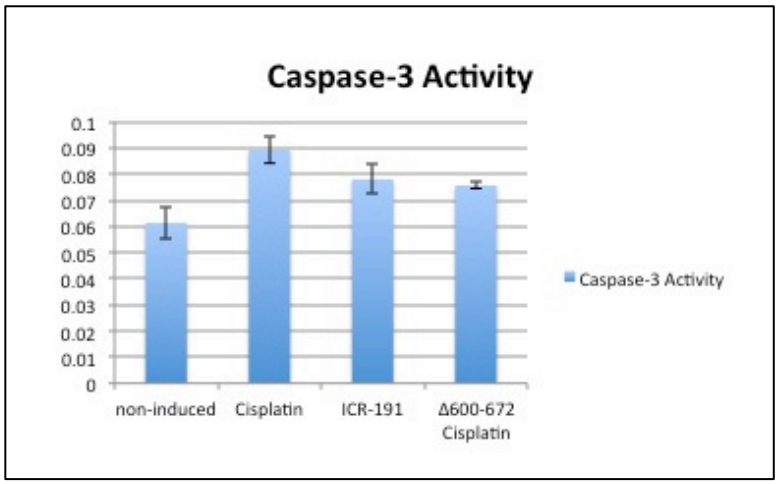


Fig. 7. ***Induction of caspase-3 as evidence of apoptotic activity in PC3 cells.*** *Cells were exposed to cisplatin and the frameshift inducer ICR-191. Delta 600-672 contains a deletion mutation in MSH2 that avoids nuclear import as part of the damage response.*

Cisplatin showed some induction of caspase-3 activity in PC3 cells. This assay will be repeated once the best new compounds have been singled out.

We added an additional experiment to our list that looked at the mechanism of MSH2 involvement in apoptotic response. Our previous observations suggested that MSH2-mediated apoptosis required nuclear import of the protein upon exposure to certain cytotoxins or genotoxins. We determined here if Cisplatin or ICR-191 induced such nuclear influx in PC3 prostatic cells:

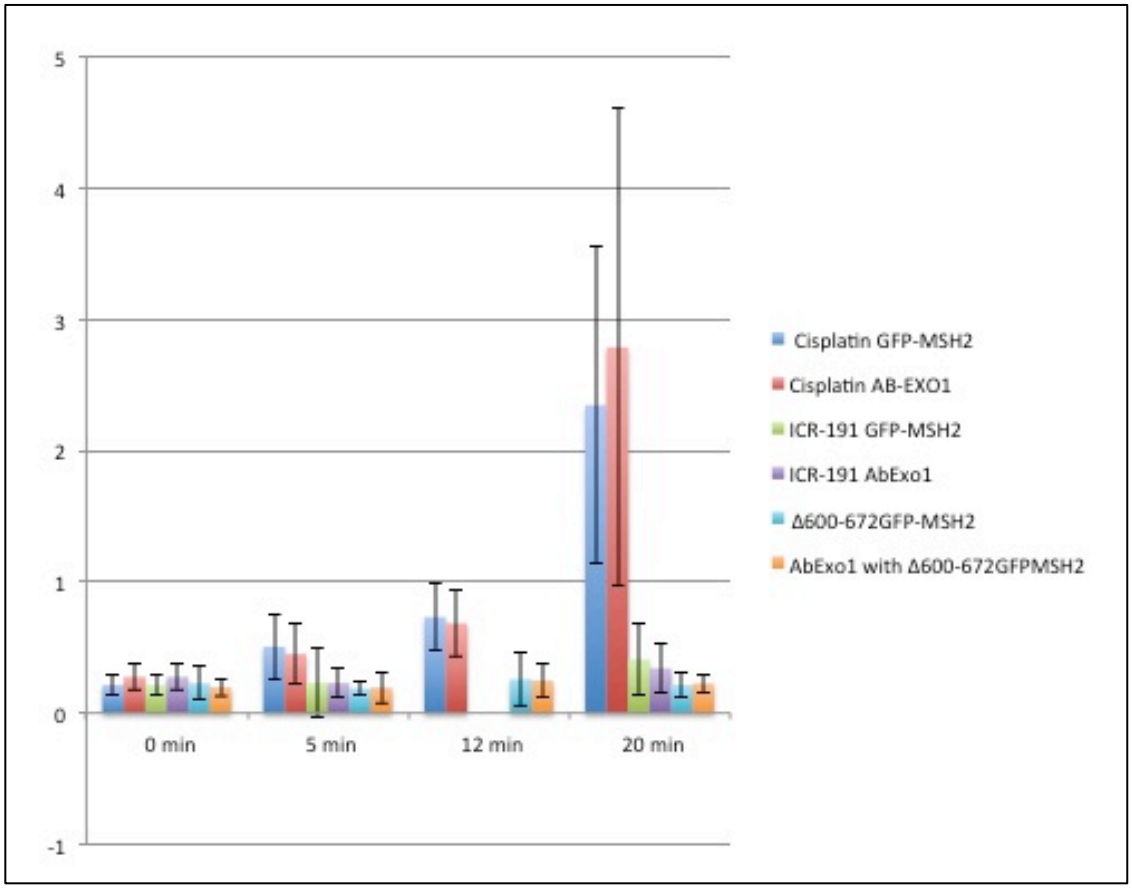


Fig. 8. Nuclear import of MSH2 and Exo1 upon treatment with cisplatin and ICR-191, respectively.

We demonstrated that only cisplatin induced nuclear influx of MSH2 upon treatment. It remains to be determined if any of our new compounds induce a similar response.

Task 2: To determine rescinnamine-induced tumor growth inhibition in hormone-refractory tumors *in vivo*

In our last progress report, we were already ahead of schedule in our proposed animal work. However, since the outcome of the animal work showed adverse effects on the mice, we have focused our efforts in this reporting period on the identification of better lead compounds, which will have to be performed in cell lines prior to moving to animals. We anticipate that in the last reporting period of

this funding mechanism, we will have a lead compound that can be moved toward animal studies.

Key Research Accomplishments

- Rescinnamine has been tested against all prostate cancer cell lines, and found not to be highly effective against prostate cancer
- We have performed computational docking and chemical synthesis to identify rescinnamine analogs that may have higher efficacy and eventually less hypotensive activity
- 17 new compounds were synthesized and tested for their effects on cell viability
- 3 of these compounds that showed best activity in pre-tests were tested against prostate cancer cells
- 2 of these compounds show significant activity against prostate cancer

Reportable Outcomes.

The following two manuscripts were supported by this project:

Jarzen, J., Diamanduros, A., Matheson, J. and **K. Scarpinato** (2013). Mismatch repair proteins in recurrent prostate cancer. Advances Clin. Chem., *in press*.

AbdelHafez, E. M.N., Diamanduros, A., Bean, J.H., Zielke, K., Crowe, B., Vasilyeva, A., Clodfelter, J.E., Aly, P.M., Abuo-Rahma, G.E.D.A.A., **Scarpinato, K.D**.*, Salsbury, F.R.*, King, S.B.* (2013). Improving rescinnamine as an inducer of MSH2-dependent apoptosis in cancer treatment. Mol. Cancer Biol., *in press*. (* corresponding authors)

Conclusions

Given our results, combined with those above, we conclude that rescinnamine itself would only be effective, if given with another drug that counteracts the hypotensive effect of the drug. Since such combination treatment would have additional problems, we decided to go with our other solution to modify rescinnamine in an effort to minimize its hypotensive activity and maximize its tumor inhibitory function. We have begun computational modeling and chemical synthesis of new rescinnamine analogs with the goal to identify such that might be effective against prostate cancer cells. At least two new compounds were found to be effective in cell culture.