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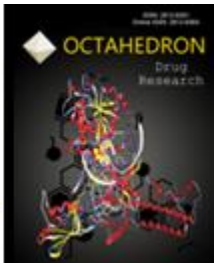

Synthesis, Molecular Modeling and Anticancer Activity of New Rescinnamine Derivatives as MMR-Inducers

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ABSTRACT

Mis-match repair proteins (MMR), such as MSH2/MSH6 have DNA dual functions, repair, and death functions. Rauwolfia alkaloids such as rescinnamine is capable to induce their death conformation lead the cancer cell to the apoptotic pathway. Cell viability assays were performed using the MTS assay on both deficient and proficient endometrial HEC59 cancer cell line. Additionally, the survival effect of 2-5, 8-10 and 14 vs rescinnamine on PC3-cancer cell line was measured by MTS assay. In this study, rescinnamine analogues 4,5,8-10 and 14 were designed as new MSH2/MSH6 inducers based on virtual fragment screening performed in the advanced Autodock4 program generating 3D pdbq files. The modeling study revealed that most of the synthesized rescinnamine derivatives show small Ki except those two analogues 1 and 3. Results show that most of reserpate intermediate and their rescinnamine analogues exhibited a promising activity as significant cytotoxic compounds but with small MSH2 dependence that may be considered potential candidate compounds for tumor treatment.

1. Introduction

Rescinnamine is a well-known pharmacologically active alkaloid;[1] it has various potent biological activities acting as an antihypertensive,[2] antipsychotic in the treatment of schizophrenia [3] and a potential anticancer agent *via* modulating P-glycoprotein-associated multidrug resistance.[4] One of the most important reasons of chemotherapy resistance is mutation of the genotoxic protein P53, which is responsible for initiation of the cytotoxicity.[5] Therefore, development of novel anticancer drugs targeting other genotoxic proteins becomes a subject of much research. In prokaryotic and eukaryotic cells, mis-match repair proteins (MMR), such as MutS and MutH, are functionalized to maintain the genetic stability of the cell by dual functions. The first function is to repair any base-base mismatch in the DNA strand and is called the repair function,[6] while the second function initiates the cellular apoptotic pathway and is called Death function.[7] The second function is promoted by DNA damage because this damage increases the potential generation of mutations causing dysfunction and disease and leads the cell to the apoptotic pathway.[8] Preliminary computational approach that examined hundreds of potential compounds targeting the death conformation of MSH2/MSH6, the major heterodimers of MutS, identified the Rauwolfia alkaloid, rescinnamine and reserpine that are supposed to give a results likewise induced by cisplatin.[9] Combination of structural and computational modeling and cell biology identified that binding those two molecules to the MSH2 protein pocket induce its death function because they mimic the binding of the protein to DNA damage which in turn leads to cell death. Here, apoptosis happens without any genotoxic insult or DNA damage.[10] Primary biological cytotoxic response for reserpine and rescinnamine was investigated in cells proficient and deficient in MSH2 and the results showed that rescinnamine is better than reserpine in cytotoxicity.[9] The hypotensive action of rescinnamine, when used in animals, inhibits the application of appropriate statistically significant tumor reduction concentrations. It is known that Rescinnamine anticancer activity is also related to its ability to induce MSH2-dependent apoptosis in cancer treatment. This first examination of the structure activity relationship of rescinnamine analogue **I** (Figure 1) shows that small changes to the structure of rescinnamine affect its activity on cell death significantly, which represents a great challenge to further work in developing derivatives of rescinnamine. Thus we aimed to modify the rescinnamine building structure toward more efficient anti-cancer agents (Figure1).

Keeping this goal in mind, the main objectives of this work are including design, synthesis, and characterization of the new modified lead rescinnamine analogues and testing their biological activity as anticancer via inducing the death conformation of MSH2.

2. Results and discussion

2.1. Chemistry

Although there are many possible operating sites, many possible R groups, in order to avoid harmonic explosion computationally, and limit our work to empirically possible compounds, we have limited ourselves to derivatives that could be readily made keeping the stable structural conformation of reserpine.

Synthesis of Rescinnamine Analogues 4 and 5

A series of new rescinnamine derivatives were semi-synthesized based on the application of palladium-catalyzed Heck reaction starting from the precursor alkaloid, reserpine, to achieve the carbon- carbon coupling between the acryloylresepate **2** with various appropriate aryl iodides, $Pd(AOc)_2$ as catalyst and TEA as a base in CH_3CN at 90^oC for 48 h (Scheme 1). Some of the aryl iodides are prepared by different synthetic

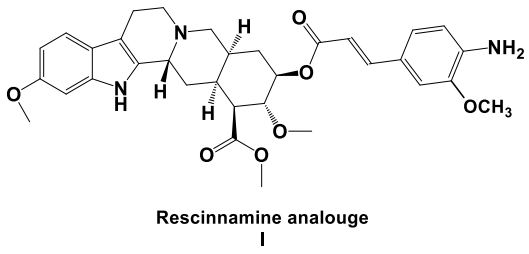
pathways. It also shows the importance of using the phosphonium ligand, in case of using an aryl bromide. Treatment of acryloyl reserpate **2** with various commercial or synthetic di-substituted aryl halides under Heck reaction conditions afforded the rescinnamine analogues **4** and **5**. The structure of the synthetic rescinnamine analogues was identified based on elemental and spectroscopic analyses. The ^{1}H NMR shows the disappearance of the doublet of doublets and appearance of two doublets for two alkene protons (C-H(2′)=C-H(3′) with a coupling constant around J_{trans} = 15 Hz that indicates the trans geometry. Also, it shows the distinctive upfield shifted singlets for the exact number of methoxy groups, in addition to the indole (–NH) and the alkene protons that are appearing at the downfield region.

2.1. Synthesis of Rescinnamine Analogue 8

The intermediate **6** was synthesized by stirring guaiacol with isobutyryl chloride to give **6** in good yield.[11] Iodination of **6** afforded the corresponding aryl iodide **7** in good yield (Scheme 2).[11] Furthermore, reaction of **7** with **2** under Heck condition yields the rescinnamine derivative **8** in low yield. The structure **8** was elucidated by the spectroscopic data and the elemental analyses. The ^{1}H NMR spectrum exhibits highly upfield doublets at δ= 1.29 ppm corresponding to two methyl protons -CH(CH$_3$)$_2$.

2.2. Synthesis of Rescinnamine Analogues 9 and 10

Replacement of the aryl halide in Heck reaction with the appropriate substituted iodopyridines produces new pyridine-substituted rescinnamine derivatives **9** and **10** in 14% and 39% yields, respectively (Scheme 3).



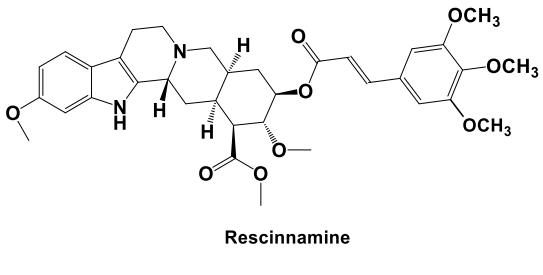


Fig. 1: Chemical Structures of rescinnamine analogue I and rescinnamine.

2.2. Synthesis of Rescinnamine Analogue 14

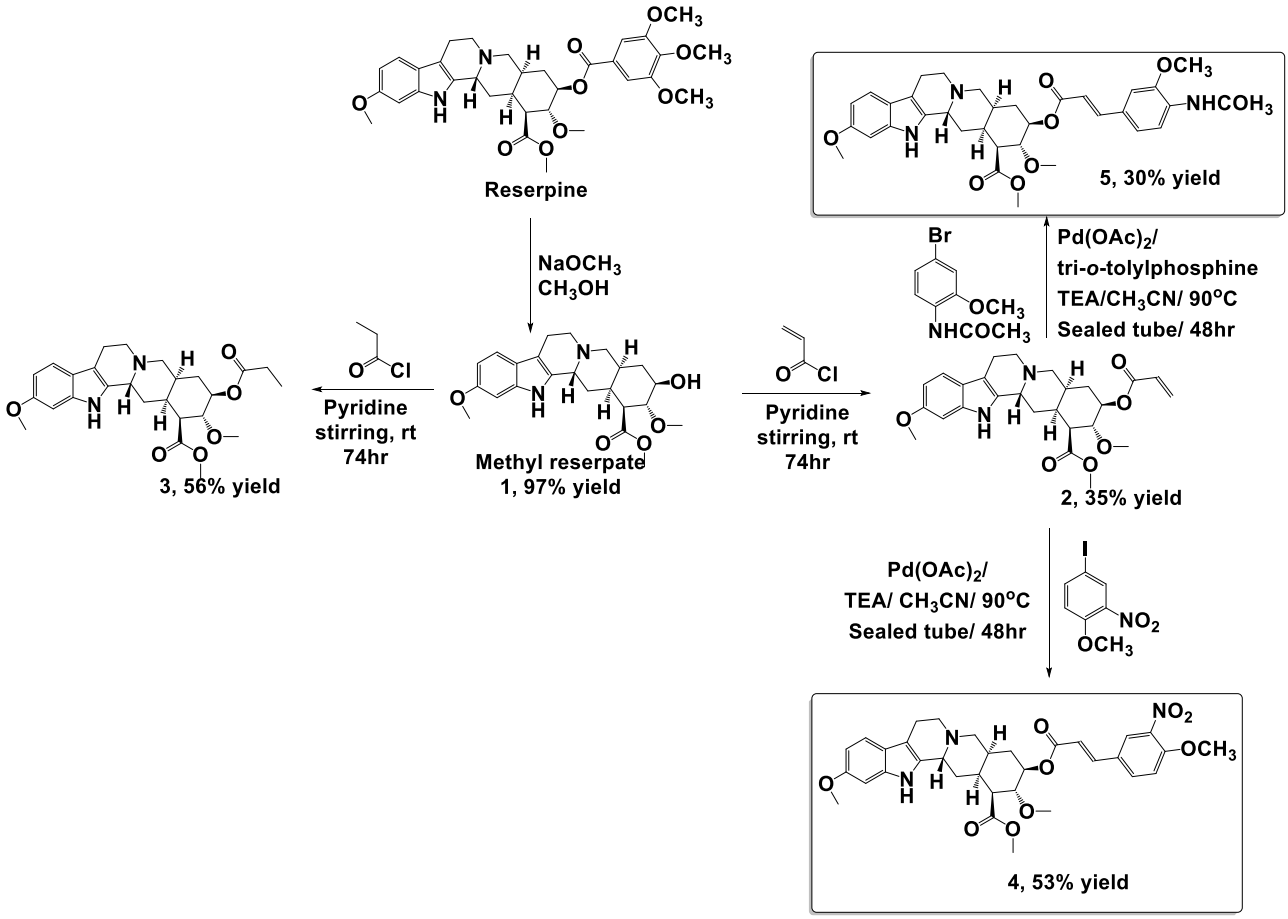
Stirring propionyl chloride with the prepared **12** afforded aryl iodide **13** in good yield.[11] The structure of **13** was confirmed by the spectroscopic data and the elemental analyses. Compound **13** was further employed in the preparation of **14** under regular Heck reaction condition in low yield (Scheme 4). The structure of **14** was confirmed by the spectroscopic data and the elemental analyses. The ^{1}H NMR shows the ethyl group appeared as a triplet at δ=1.31 ppm, *J*= 7.58 Hz for (-CH$_2$CH$_3$) and quartet at δ=2.65, *J* = 7.51 Hz for (-CH$_2$CH$_3$).

3. Molecular modeling study

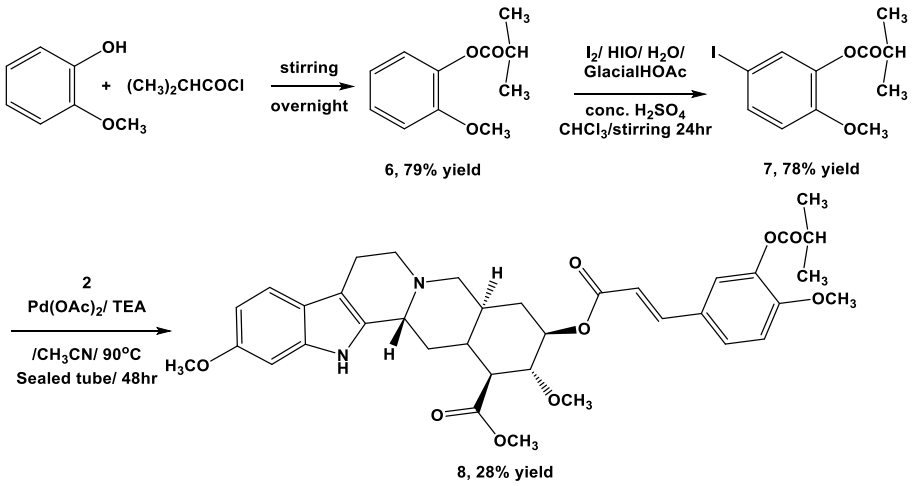
The molecular modeling study was accomplished by using structures obtained from molecular dynamics simulation along with docking in structures representing death-causing conformations via using Autodock 4. The molecular docking was performed in four phases: Structural model generation, ligand library generation, receptor grid generation and finally docking of the ligand library into the receptor grid. Libraries for docking were based on the core of the rescinnamine structure (Figure 2). The possible derivatives were made based on the structure shown in Figure 2, with R$_1$, R$_2$ and R$_3$ selected from H, OCH$_3$, NO$_2$, and OCOCH$_3$. Also, structures without the modified phenyl ring were docked;

compound **1-3**. After docking, these compounds were suggested for chemical synthesis to examine their cytotoxicity (Figure 2).

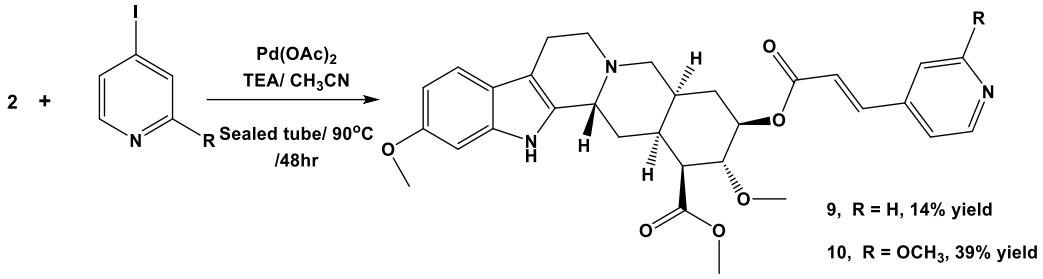
The docks were performed using Autodock 4's default settings for its Lamarckian Genetic Algorithm with a population size of 150, a maximum number of energy ratings of 5 million and a maximum number of generations of 27,000. Each derivative was subjected to 256 runs and from the estimated free energy of ligand binding (ΔG), the inhibition constant (K_i) for each ligand was calculated. K_i should be the same as the dissociation constant (K_d), the smaller the dissociation or inhibitory constant, the more tightly bound the ligand is, or the higher the affinity between ligand and protein. Most of the synthesized rescinnamine derivatives exhibit small K_i indicating significant binding to the MSH2 receptor (Table 1).



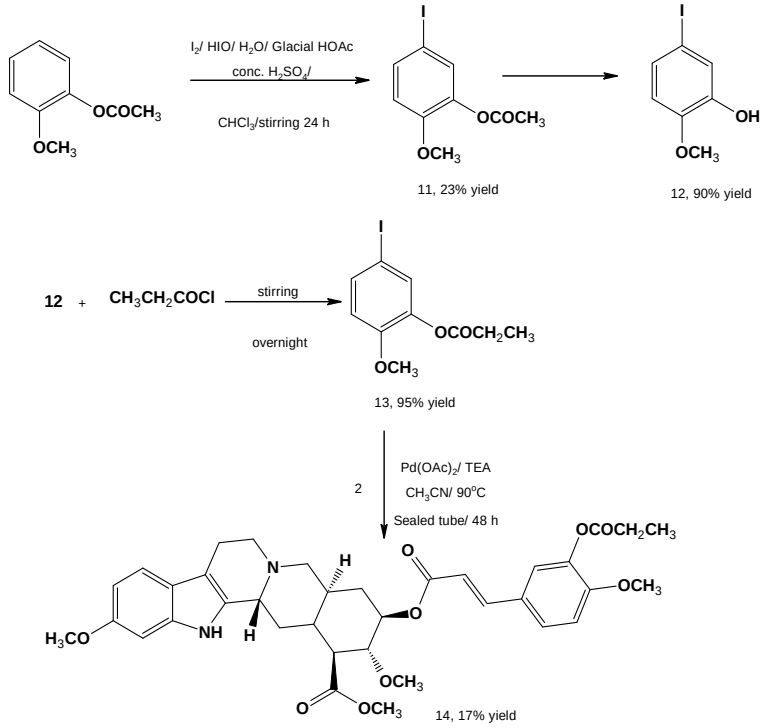
Scheme 1.



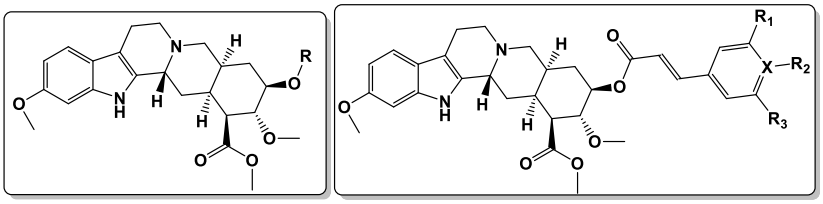
Scheme 2.



Scheme 3.



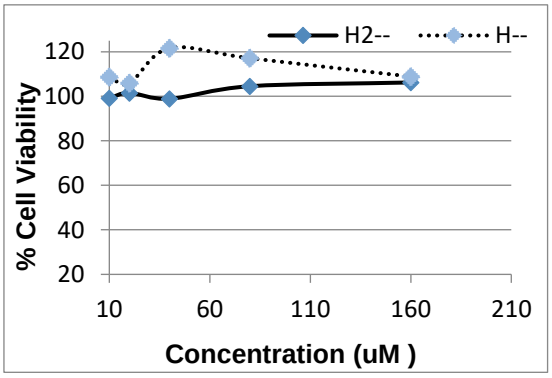
Scheme 4



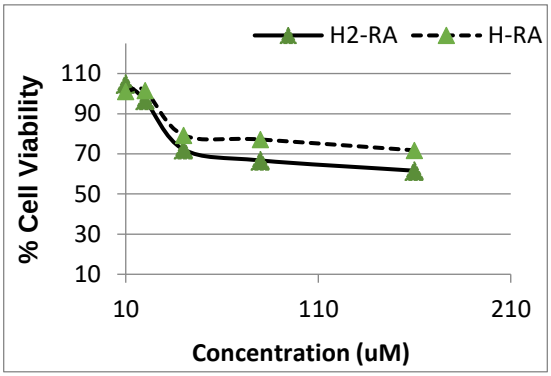
1, R = H
2, R = -COCH=CH_2
3, R = -$COCH_2CH_3$

Entry	R_1	R_2	R_3	X
4	NO_2	OCH_3	H	C
5	OCH_3	H	H	C
8	$OCOCH(CH_3)_2$	OCH_3	H	C
9	H	H	H	N
10	OCH_3	H	H	N
14	$OCOCH_2CH_3$	OCH_3	H	C

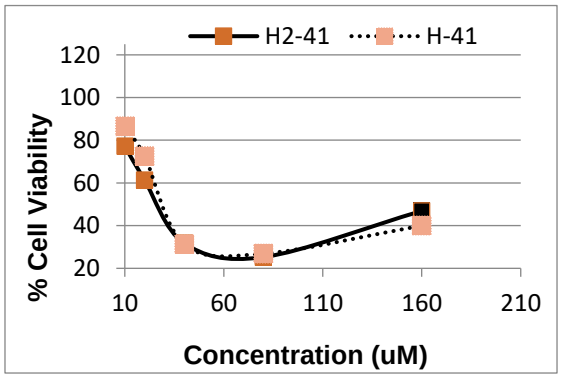
Figure 2. General structures for the docked compounds.



Control



1



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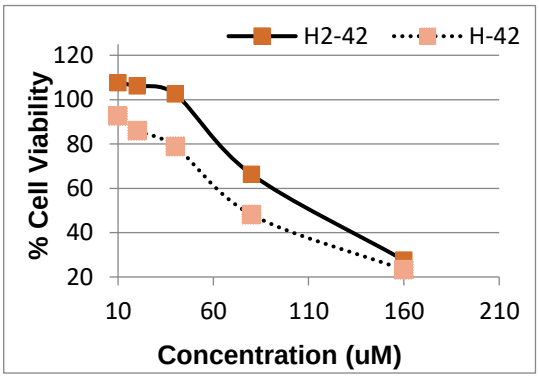

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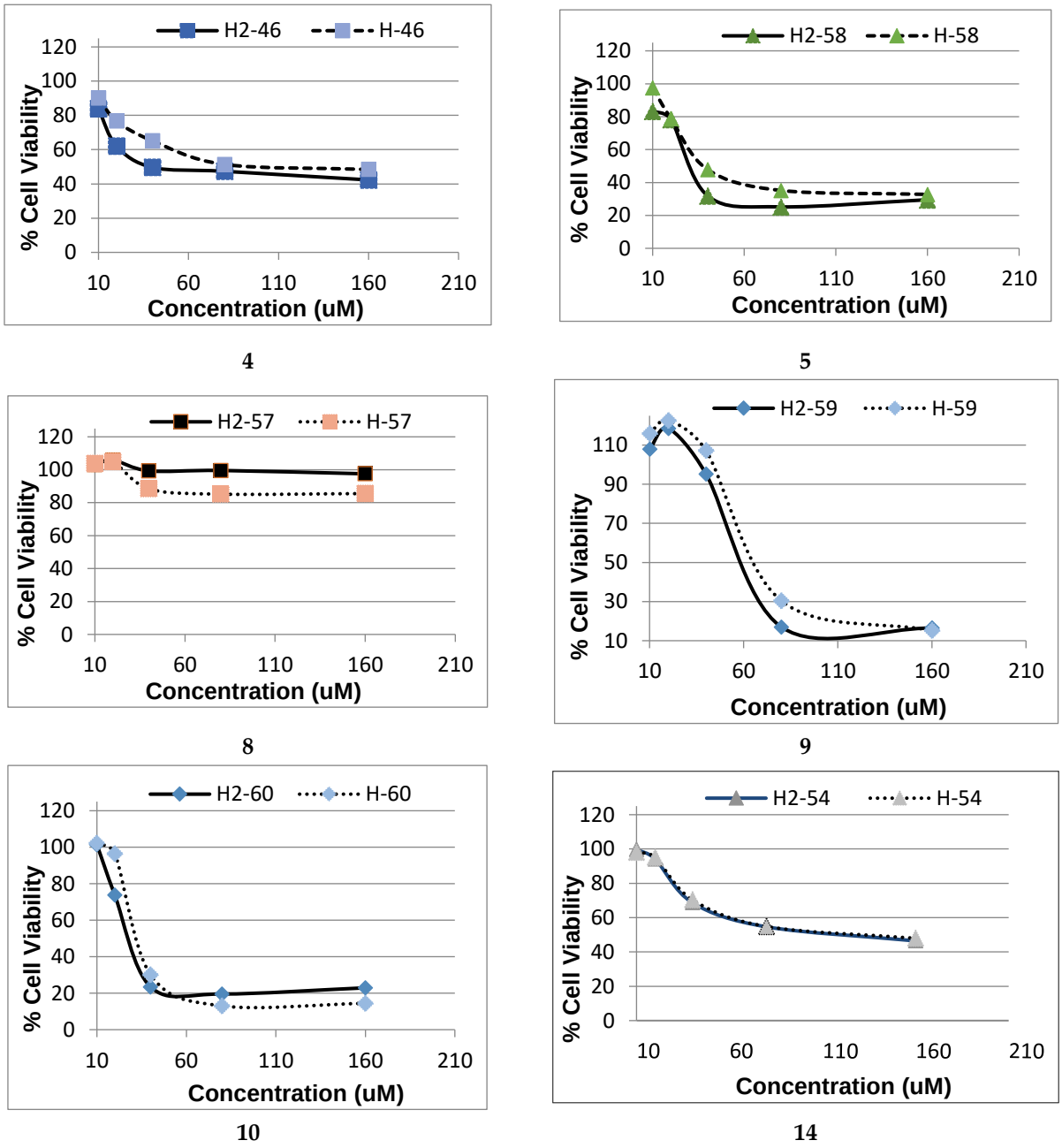

Figure 3. The survival effect of compounds rescinnamine, **2- 5, 8-10 and 14** vs rescinnamine and control on MSH2 proficient and deficient HEC59 cell line measured by MTS assay.

These computational studies can be used to determine whether rescinnamine activity can be improved by modifying functional groups on loop systems, or alternatively not being disturbed while disturbing the antihypertensive effects of rescinnamine. In this work, the relationship of structure activity is explored.

Although there are many possible functionalized sites, and many possible R-groups, to avoid a combinatorial explosion computationally, and to restrict our work to experimentally feasible compounds.

4. Screening of the Cytotoxicity

Cytotoxicity of the newly synthesized compounds was measured via a well-defined cellular system using an endometrial cell line deficient (HEC59) and proficient (via chromosome transfer, HEC59 + chr.2) for MSH2 using CellTiter 96® Aqueous One

Solution Cell Proliferation Assay (MTS). The assays on the HEC59 cells (MSH2 proficient and deficient) identified a few rescinnamine derivatives that induced cell cytotoxicity in micromolar scale including **2, 5, 9, 10** and to a lesser extent **4** and **14**, when compared to the control and rescinnamine as a reference (Figure 3). However, little significant on MSH2 has been observed for these compounds. The only compound that showed little difference between MSH2 mastery and lack was **14**. No significant difference was detected when EC50 values were detected. These results are surprising in light of previous work.[12] Previous studies have suggested that in addition to MSH2-dependent cell-cytotoxicity due to rescinnamine and derivatives, off-target cell-cytotoxicity occurs.[9] These results suggest considerable off-target cytotoxicity for these classes of compounds.

Compound 2, which lacks the extra ring system, demonstrates activity, but the saturated version of 2 (compound 3) does not lead to cell cytotoxicity 2 possibly acts through an alternative mechanism (possibly through a Michael reaction) compared to rescinnamine. The branched ester derivatives 8 was examined that showing little cytotoxicity which indicates that even small changes in the same function group affects the cytotoxic response. More interesting results is small change to the rescinnamine structure that affect its activity on cell death significantly by replacement of the phenyl ring with pyridine such as in the rescinnamine derivatives 9 and 10 that showed interested response however, with a MSH2-independent function (Figure 3).

3. EXPERIMENTAL:

Chemistry

Reagents were obtained from commercial sources and used without additional purification. Extraction and flash chromatography solvents were technical grade. LC-MS, ESI-MS and HPLC solvents were HPLC grade.

Analytical thin layer chromatography (TLC) was performed as silica gel plates with C-4 Spectroline 254 indicator. Mobile phase is the same as used in the chromatography for purification of the compounds. Visualization was accomplished with UV light and 20% phosphomolybdic acid solution in EtOH or 0.3% ninhydrin in EtOH. Solvents for extraction and purification were technical grade and used as received.

Table1. Inhibitory Constant K_i (μM) of rescinnamine analogues **1-4, 8-10** and **14**

Compound	Inhibitory Constant K_i (μM)
1	24.46
2	0.862
3	15.55
4	0.772
8	1.09
9	2.21
10	1.32
14	0.889

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), sept (septet) m (multiplet), dd (doublet of doublet) and bs (broad singlet). Flash chromatography was carried out using a BiotageSP1-B2A0/ HPFC System (single column, single path and variable wavelength). LC-MS measurements were performed on an Agilent

Technologies 1100 LC/MSD trap instrument and on GC-MS spectrometer 7890A GC 5975C MS. High resolution-mass spectra were obtained using a Thermo Scientific LTC Orbitrap mass spectrometer equipped with a heated electrospray ionization source operated in positive ion mode. IR spectrum was processed on JASCO FT/IR 460.

Table 2: EC_{50} values of the rescinnamine analogues **2- 5, 8-10 and 14**

Comp.	EC_{50} (μM)	
	MSH2 deficient	MSH2 proficient
2	20.7	20.37
3	NA	NA
4	24.06	4.87
5	25.56	27.77
8	36.47	NA
9	58.23	47.63
10	31.3	22.03
14	37.32	35.59

NA: not applicable, since data did not reach below 50%.

Preparation of Methyl reserpate 1

A suspension of reserpine (1.00 g, 1.64 mmol) and NaOMe (0.20 g, 3.61 mmol) in methanol (50 mL) was refluxed for 4 hr (more NaOMe was added 0.20 g x 2), cooled, and concentrated to one-third the volume. The solution was diluted with water (60 mL) and acidified to pH = 1 with concentrated HCl. The water layer was washed with Et_2O repeatedly and rendered basic with concentrated NH_4OH and repeatedly extracted with CH_2Cl_2 (4 X 30 mL).[13] The combined organic phases were dried with anhydrous $NaSO_4$, filtered, and concentrated to give a colorless solid (0.66 g, 97%); R_f= 0.26 ethyl acetate; mp = 230-231°C. ^{1}H NMR (300 MHz,$CDCl_3$, δ ppm): 7.67 (bs, 1H), 7.32 (d, *J* = 8.54 Hz, 1H), 6.83 (s, 1H), 6.76 (d, *J* = 8.55 Hz, 1H), 4.45-4.39 (m, 1H), 3.83 (s, Ar-OC<u>H</u>$_3$, 3H), 3.79 (s, -COOC<u>H</u>$_3$, 3H), 3.58 (s, OC<u>H</u>$_3$, 3H), 3.55-3.52 (m, 2H), 3.00-2.85 (m, 2H), 2.54-2.43 (m, 4H), 2.27-2.17 (m, 1 H), 2.04-1.95 (m, 2H), 1.83-1.74 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 173.33, 156.19, 136.38, 130.75, 122.24, 118.49, 108.98, 108.04, 95.27, 81.47, 75.23, 60.92, 55.84, 53.74, 51.72, 51.37, 51.18, 49.34, 34.55, 32.83, 32.30, 24.34, 16.79.

Preparation of acryloylreserpate 2:

Dry pyridine (791 mg, 0.808 mL, 10 mmol) was added to a mixture of methyl reserpate (300 mg, 0.74 mmol) and acryloyl chloride (135.77mg, 0.123 mL, 1.5 mmol) and stirred under nitrogen at room temperature for 74 hr. Excess pyridine was evaporated and the residue was taken up in chloroform (50 mL). The organic layer was washed with water (3x 20 mL) and brine, dried with anhydrous Na_2SO_4, filtered and evaporated under reduced pressure to give a residue that was purified by flash chromatography (silica gel, methanol/chloroform, 3%, R_f = 0.3). the obtained solid product was rinsed with methanol, the precipitated solid was filtered and dried to afford **2** (120 mg, 35%) as white crystals; mp >260 °C;[13]^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 7.80 (s, 1H), 7.31(d, 1H, *J* = 8.52 Hz), 6.81 (s, *J* = 1.95 Hz, 1H), 6.76 (d, *J* = 8.53 Hz, 1H), 6.45 (d, *J* = 17.32 Hz, 1H), 6.15 (dd, *J* = 17.31& 10.38 Hz, 1H), 5.87 (d, *J* = 10.4 Hz, 1H), 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 ppm): 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.

Preparation of propionylreserpate 3

Using the same procedures for **2**, compound **3** was prepared by using propionyl chloride instead affording a solid that was crystallized by flash chromatography (silica gel, methanol/chloroform, 3%, R_f = 0.2) giving a solid rinsed with methanol/diethylether (1:1) to give **3** (317 mg, 56%) as light yellow fine powder : mp = 258 ^oC; ^1H NMR (300 MHz, $CDCl_3$, δ ppm): 7.60 (bs, 1H), 7.32 (d, *J* = 8.54 Hz, 1H), 6.82 (s, 1H),6.76 (d, *J* = 8.59 Hz, 1H),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 ppm): 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 $C_{26}H_{34}N_2O_6$: C, 66.36; H, 7.28; N, 5.95. Found: C, 66.08; H, 7.44; N, 5.87.

Synthesis of rescinnamine derivative 4 and **5:**

A mixture of acryloylreserpate (300 mg, 0.641 mmol), the aryl iodide (0.507 mmol), TEA (0.06 g, 0.1 mL, 0.645 mmol) and $Pd(OAc)_2$ (1.23 mg, 0.005 mmol) in acetonitrile (20 mL) was heated with stirring in a capped sealed glass tube under argon at 90^{o}C for 48 hr .[15] After cooling, the solvent was removed under reduced pressure; the residue was dissolved in chloroform (30 mL) and washed with water (3 x 15 mL) and brine. The organic layer was dried using anhydrous sodium sulfate, filtered off and evaporated under reduced pressure to give a residue that was purified by flash chromatography affording a solid which rinsed with a specific solvent, mentioned below to give pure products.

For 4 (R_1 = OCH_3, R_2 = NO_2):

Yellow flakes, yield = 141 mg (53%), precipitated from diethyl ether; R_f= 0.43 methanol/chloroform 4%;mp = 213-215 ^{o}C; ^1H NMR (300 MHz, DMSO-d_6, δ ppm): 10.50 (s, 1H), 8.32 (d, *J* = 5.30 Hz, 1H), 8.07 (d, *J* = 8.84 Hz, 1H), 7.74 (d, *J* = 15.86 Hz, 1H), 7.42 (d, *J* = 8.85 Hz, 1H), 7.22 (d, *J* = 8.52 Hz, 1H), 6.80 (s, 1H), 6.72 (d, *J* = 15.96 Hz, 1H), 6.61 (d, *J* = 8.58 Hz, 1H), 4.87-4.78 (m, 1H), 4.35 (s, 1H), 3.98 (s, 3H), 3.78 (s, 3H), 3.75 (s, 3H), 3.42 (s, 3H), 3.04 (d, *J* = 8.6 Hz, 2H), 2.85 (t, *J* = 12.4 Hz, 2H), 2.66 (dd, *J* = 11.2, 4.7 Hz, 1H), 2.40 (d, *J* = 11.8 Hz, 2H), 2.16 (t, *J* = 12.6 Hz, 2H), 2.06–1.66 (m, 5H);^{13}C NMR (75 MHz, DMSO, δ ppm): 171.65, 165.47, 155.07, 152.97, 142.34, 139.65, 136.36, 134.02, 131.11, 126.76, 124.59, 121.63, 118.47, 117.81, 114.65, 108.03, 105.90, 94.76, 77.47, 76.87, 60.10, 56.97, 55.17, 53.33, 51.75, 50.99, 50.73, 48.58, 33.12, 32.11, 29.41, 23.44, 16.34; ESI-MS: *m*/*z* = 620.3(M^+ + 1); Anal. Calcd.for $C_{33}H_{37}N_3O_9$: C, 63.96; H, 6.02; N, 7.78. Found: C, 63.51; H, 6.08; N, 6.71.

For 5 (R_1 = OCH_3, R_2 = $NHCOCH_3$):

Using *N*-(4-bromo-2-methoxyphenyl) acetamide as the aryl bromide substrate with the same Heck procedures in the presence of tri-*o*-tolylphosphine (4.5 mg, 0.021 mmol) to produce white fluffy solid, yield = 121 mg (30%),[14]precipitated from methanol; R_f= 0.36 methanol/chloroform 8%;mp = 186-187 ^{o}C;^1H NMR (300 MHz, DMSO-d_6, δ ppm): 10.49 (s, 1H), 9.27 (s, 1H), 8.11 (d, *J* = 8.37 Hz, 1H), 7.67 (d, *J* = 15.88 Hz, 1H), 7.44 (s, 1H), 7.27 (d, *J* = 8.65 Hz, 2H), 7.21 (d, *J* = 8.58 Hz, 2H), 6.80 (d, *J* = 2.32 Hz, 1H), 6.71 – 6.56 (m, 2H), 4.92 – 4.77 (m, 1H), 4.33 (s, 1H), 3.92 (s, 3H), 3.79 (s, 3H), 3.75 (s, 3H), 3.42 (s, 3H), 3.02 (d, *J* = 8.5 Hz, 2H), 2.84 (d, *J* = 10.6 Hz, 2H), 2.66 (dd, *J* = 11.30, 4.56 Hz, 1H), 2.40 – 2.30 (m, 2H), 2.19 (d, *J* = 12.76 Hz, 1H), 2.12 (s, 3H), 2.02 (dd, *J* = 13.7, 5.4 Hz, 1H), 1.98-1.74 (dd, *J* = 40.8, 21.0 Hz, 5H);^{13}C NMR (75 MHz, DMSO, δ ppm): 171.68, 168.80, 165.79, 155.03, 149.05, 144.67, 136.33, 131.34, 129.92, 129.60, 121.84, 121.69, 120.69, 117.78, 116.71, 110.09, 107.98, 105.93, 94.75, 77.52, 76.65, 60.05, 55.91, 55.16, 53.29, 51.73, 51.07, 50.76, 48.63, 33.25, 32.20, 29.51, 24.00, 23.49, 16.38; ESI-MS: *m*/*z*= 632.3 (M^+ + 1); Anal. Calcd.for $C_{35}H_{41}N_3O_8.0.8H_2O$: C, 65.06; H, 6.65; N, 6.50. Found: C, 64.84; H, 6.44; N, 6.41.

Preparation of 2-methoxyphenyl isobutyrate 6:

This compound was prepared using the same procedure for **13** with guaiacol and isobutyryl chloride to give a light yellow oil (21.5 g, 79%);[11] R_f = 0.63, methanol/chloroform 5%; ^1H NMR (300 MHz, $CDCl_3$, δ ppm): 7.23 – 7.15 (m, 1H), 7.07 (dd, *J*

= 8.16, 1.78 Hz, 1H), 7.02 – 6.91 (m, 2H), 3.76 (s, 3H), 2.88 (sept, *J* = 6.89 Hz, 1H), 1.38 (d, *J* = 6.99 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 174.98, 151.35, 140.16, 126.69, 122.77, 120.69, 112.50, 55.70, 33.96, 19.03; ESI-MS: *m*/*z*= 195.1 (M^+ + H).

Preparation of 5-iodo-2-methoxyphenyl isobutyrate 7:

2-Methoxyphenylbutyrate (54.4 g, 0.17 mol) was added to a mixture of iodine (17.46 g, 0.06 mol), HIO_3 (7.18 g, 0.04 mol) 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°C. Chloroform (50 mL) and water (30 mL) were added, and the mixture was washed with dilute $NaHSO_3$ (3 x 50) and water (1 x 30). The organic layer was dried with magnesium sulfate and the organic solvent was removed under vacuum.[11] The residue was recrystallized from ethanol to afford the product as white to pink crystals (4.9 g, 78%);[11] R_f = 0.74, methanol/chloroform 5%; mp = 39-40 °C;^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 7.37 (dd, *J* = 8.63, 2.18 Hz, 1H), 7.23 (d, *J* = 2.17 Hz, 1H), 6.60 (d, *J* = 8.61 Hz, 1H), 3.67 (s, 3H), 2.77-2.68 (sept, *J* = 6.98 Hz, 1H), 1.22 (d, *J* = 7.02 Hz, 6H);^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 174.76, 151.47, 140.76, 135.50, 131.56, 114.36, 81.33, 55.99, 33.90, 19.01; ESI-MS: *m*/*z*= 321.1 (M^+ + H); Anal. Calcd. for $C_{11}H_{13}IO_3$: C, 41.27; H, 4.09. Found: C, 41.53; H, 4.14.

For 8 (R_1 = OCOCH(CH_3)$_2$, R_2 = OCH_3)

White powder, yield = 120 mg (28%), precipitated from methanol; R_f= 0.39 methanol/chloroform 5%; mp = 207-210 °C; ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 7.60-7.54 (m, 2H), 7.32– 7.19 (m, 3H), 6.90 (d, *J* = 8.48 Hz, 1H), 6.78 (s, 1H), 6.72 (d, *J* = 8.60 Hz, 1H), 6.25 (d, *J* = 15.86 Hz, 1H), 4.90-4.82 (m, 1H), 4.38 (s, 1H), 3.80 (s, 3H), 3.78 (s, 3H), 3.76 (s, 3H), 3.46 (s, 3H),3.15-3.10 (s, 2H),3.02 – 2.74 (m, 2H),2.60 (dd, *J* = 11.5, 4.7 Hz, 1H), 2.46-2.36 (m, 2H), 2.29 – 2.10 (m, 2H), 2.04 – 1.60 (m, 6H), 1.29 (d, *J* = 7.02 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 175.07, 172.86, 166.35, 156.18, 153.07, 143.94, 140.29, 136.37, 130.60, 127.71, 127.48, 122.22, 121.86, 118.51, 116.66, 112.29, 108.98, 108.02, 95.25, 77.86, 77.25, 60.80, 56.02, 55.82, 53.73, 51.79, 51.22, 49.06, 34.05, 34.00, 33.96, 32.33, 30.92, 29.70, 24.26, 19.01, 16.81; ESI-MS: *m*/*z*= 661.4 (M^+ + 1); Anal. Calcd.for $C_{37}H_{44}N_2O_9$.0.75H_2O: C, 65.91; H, 6.80; N, 4.15. Found: C, 65.57; H, 6.79; N, 4.10.

For 14 (R_1 = $OCOCH_2CH_3$, R_2 = OCH_3)

Yellowish white solid, yield = 70 mg (17%), precipitated from ethyl acetate/ n-hexane; R_f= 0.48 methanol/chloroform 4%; mp = 188-190 °C; ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 7.67-7.60 (m, 2H), 7.40-7.33 (m, 2H), 7.28 (s, 1H), 6.98 (d, *J* = 8.48Hz, 1H), 6.85 (d, *J* = 2.16 Hz, 1H), 6.79 (dd, *J* = 8.53, 2.27 Hz, 1H), 6.32 (d, *J* = 15.90 Hz, 1H), 5.06 – 4.84 (m, 1H), 4.46 (s, 1H), 3.87 (s, 3H), 3.85 (s, 3H), 3.83 (s, 3H), 3.53 (s, 3H), 3.28 – 3.14 (m, 2H), 3.13 – 2.87 (m, 2H), 2.65 (q, *J* = 7.51 Hz, 2H), 2.52-2.45 (m, 2H), 2.36-2.22 (m, 2H), 2.18 – 1.75 (m, 6H), 1.31 (t, *J* = 7.58 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 172.85, 172.42, 166.37, 165.49, 156.18, 153.03, 143.94, 140.16, 136.42, 130.51, 128.71, 127.76, 127.49, 122.19, 121.94, 118.51, 116.68, 112.32, 108.98, 107.93, 95.26, 77.86, 60.80, 56.03, 55.81, 53.76, 51.79, 51.21, 49.01, 34.01, 32.30, 29.69, 27.34, 24.22, 16.79, 9.15; ESI-MS: *m*/*z*= 647.4 (M^+ + 1); Anal. Calcd. for $C_{36}H_{42}N_2O_9$.0.6H_2O: C, 65.76; H, 6.62; N, 4.26. Found: C, 65.45; H, 6.47; N, 4.42.

General procedure for synthesis of pyridine-substituted rescinnamine derivatives 9 and 10:

The appropriate substituted iodopyridines were used with the same Heck reaction procedure[15] in the absence of a phosphine ligand afforded the following pyridine-substituted rescinnamine derivatives.

For 9 (R =H):

Brownish yellow solid, yield = 50 mg (14%)precipitated from methanol; R_f= 0.19methanol/chloroform 5%;mp = 152-153 °C;^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 8.68 (d, *J* = 6.01 Hz, pyr-2H), 7.87 (s, 1H), 7.64 (d, *J* = 16.06 Hz, 1H), 7.40 (d, *J* = 6.11 Hz, pyr- 2H), 7.34 (d, *J* = 8.54 Hz, 1H), 6.84 (d, *J* = 2.20 Hz, 1H), 6.78 (dd, *J* = 8.6, 2.2 Hz, 1H), 6.63 (d, *J* = 16.0 Hz, 1H), 5.00-4.91 (m, 1H), 4.46

(s, 1H),3.84 (s, 3H),3.82 (s, 3H),3.52 (s, 3H), 3.25-3.16 (m, 2H), 3.07-2.90 (m, 2H), 2.70-2.65 (dd, *J* = 4.63, 11.16, 1H), 2.53-2.42 (m, 2H), 2.33-2.23 (m, 2H), 2.10-1.82 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 170.89, 163.54, 163.39, 154.28, 148.63, 140.16, 139.64, 134.45, 128.90, 128.57, 126.76, 120.97, 120.21, 119.95, 116.67, 107.05, 106.10, 93.24, 76.07, 75.82, 58.88, 53.77, 51.72, 49.97, 49.29, 32.10, 30.36, 27.65, 27.59, 22.31, 14.85; ESI-MS: *m*/*z*= 546 (M^+ + 1).Anal.Calcd for $C_{31}H_{35}N_3O_6.1.9H_2O$: C, 64.21; H, 6.74; N, 7.25; Found: C, 64.69; H, 6.26; N, 6.78.

For 10 (R = OCH_3):

Brown solid, yield = 144 mg (39%), precipitated from diethyl ether; R_f= 0.16methanol/chloroform 5%;mp = 219-220 °C;^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 8.21 (d, *J* = 5.36 Hz, 1H), 7.61-7.56 (m, 2H), 7.34 (d, *J* = 8.55 Hz, 1H), 7.01 (d, *J* = 5.35 Hz, 1H), 6.87 – 6.82 (m, 2H), 6.79 (dd, *J* = 8.89, 2.24 Hz, 1H), 6.57 (d, *J* = 16.03 Hz, 1H), 5.02 – 4.87 (m, 1H), 4.47 (s, 1H), 3.98 (s, 3H), 3.85 (s, 3H), 3.83 (s, 3H), 3.53 (s, 3H), 3.28 – 3.14 (m, 2H), 3.11 – 2.88 (m, 2H), 2.67 (dd, *J* = 11.29, 4.74 Hz, 1H), 2.49 (t, *J* = 12.34 Hz, 2H), 2.33-2.23 (m, 2H), 2.17 – 1.76 (m, 5H);^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 172.77, 165.44, 165.00, 156.23, 147.57, 144.23, 142.25, 136.39, 130.38, 122.51, 122.18, 118.54, 114.60, 109.96, 109.03, 108.03, 95.24, 65.83, 60.78, 55.82, 53.73, 53.65, 51.83, 51.76, 51.20, 49.00, 34.02, 32.28, 29.59, 24.23, 16.79, 15.26; ESI-MS: *m*/*z*= 576.4 (M^+ + 1); Anal. Calcd.for $C_{32}H_{37}N_3O_7$: C, 66.77; H, 6.48; N, 7.30. Found: C, 66.06; H, 6.45; N, 7.00.

Preparation of 5-iodo-2-methoxy phenyl acetate 11

Compound **11** was synthesized using the same procedure for **7** to give a residue that was recrystallized from absolute ethanol to afford white of **11** (11.7 g, 23%); (methanol/chloroform 2%, R_f = 0.3); m.p = 75 °C; ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm):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 ppm): 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.

Preparation of 5-iodo-2-methoxy phenol 12:

The compound **11** (5 g, 17.12 mmol) was dissolved in a mixture of potassium hydroxide (5.80 g, 0.103 mol) in ethanol (25 mL) and water (19 mL) and refluxed for 2 h. After cooling to room temperature, this mixture was acidified with 10% hydrochloric acid and extracted with dichloromethane (3 x 30 mL).[11] The organic layer was dried with magnesium sulfate, the solvent was removed under vacuum and recrystallization from petroleum ether afforded product as white crystals (3.8 g, 90 %); R_f = 0.4, ethylacetate; mp: 84-85 °C. (reported 87-88 °C)[16], ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 7.23 (d, 1H *J* = 2.07 Hz), 7.17-7.14 (dd, 1H, *J* = 8.65, 2.17 Hz), 6.61 (d, 1H, *J* = 8.46 Hz), 5.26 (bs, 1H), 3.86 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 146.69, 146.60, 129.04, 123.48, 112.53, 83.01, 55.99; ESI-MS: *m*/*z*= 250.9 (M^+ + H).

Preparation of 5-iodo-2-methoxyphenyl propionate 13:

Propionyl chloride (0.72 g, 0.68 mL, 7.75 mmol) was slowly added to **12** (1.5 g, 6 mmol) over 15 min and the mixture was stirred for 24 h at room temperature. Dichloromethane was added and the solution was washed with aqueous potassium carbonate until the aqueous layer was basic.[11] The organic layer was dried with anhydrous magnesium sulfate and the solvent was removed under vacuum to afford a pure faint yellow oil (1.75 g, 95%); R_f = 0.42, chloroform; ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 7.38 (dd, *J* = 8.70, 2.22 Hz, 1H), 7.25 (d, *J* = 2.18 Hz, 1H), 6.61 (d, *J* = 8.63 Hz, 1H), 3.69 (s, 3H), 2.50 (q, *J* = 7.54 Hz, 2H), 1.17 (t, *J* = 7.55 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 172.11, 151.43, 140.62, 135.58, 131.62, 114.36, 81.33, 55.99, 27.28, 9.15; ESI-MS: *m*/*z*= 307.0 (M^+ + H); Anal. Calcd.for $C_{10}H_{11}IO_3.0.3C_2H_5OH$: C, 39.80; H, 4.03. Found: C, 40.38; H, 3.72.

Biological Investigation of cytotoxicity

Cell culture

SW416 or HEC59 cells (MSH2 deficient) and the paired cell line HEC59 chr., that restores the MSH2-deficiency via chromosome transfer have been

extensively characterized.[17] All cell lines were purchased from American Type Culture Collection (ATCC) P.O. Manassas, VA, USA. Cells were routinely cultured in standard growth media (DMEM-F12 + 10% FBS). Cells were plated in microtiter plates at an appropriate concentration in media (100 µl) and incubated overnight. Media was replaced with media containing drug and allowed to incubate for 24 hr at indicated concentrations. Untreated cells received fresh media with vehicle only. One solution reagent (CellTiter 96® Aqueous One Solution) was added to existing media (20 µl/well) and allowed to incubate 3-4 hr. A plate reader was used to record the absorbance at 490 nm. Assays were performed at least in triplicates.

Cytotoxicity assay

Cytotoxicity of the tested samples was measured using CellTiter 96® AQueous One Solution Cell Proliferation Assay (MTS) by adding a small amount of the CellTiter 96® AQueous One Solution reagent directly to culture wells, incubating for 1–4 hr and then recording the absorbance at 490nm with a 96-well plate reader. [18,19]

Reagent preparation

MTS reagent: 20 µl (CellTiter 96® Aqueous One Solution)/well.

Procedures

Xenograft. SW416 or HEC59 cells in PBS mixed with Matrigel (1:1; BD Biosciences) were subcutaneously injected into the flank of nude donor mice. Tumors were grown for up to 3 weeks. Mice were euthanized, tumors excised, minced into 3 mm pieces, and surgically implanted into the right flank of acceptor mice (10 per group). Isoflurane anesthesia was provided during tumor inoculation. Injection of compounds was started 3 days following tumor implantation to allow recovery from surgery. Compounds were given intraperitoneally, in a volume of 0.5 ml/mouse with a ¼ inch, 23-gauge needle in a 6 ml plastic syringe. Mice were monitored based on survival and body weight. Tumor size, measured by caliper, and body weight were monitored daily for 57 days, and the prolongation of median survival time after intraperitoneal injection treatment determined. Any animals showing signs of distress, unnatural movements, severe loss of appetite, severe signs of hypotension, tumor size of 1000g, or weight loss exceeding 10% before the end of the study were euthanized. Tumors were measured twice a week for each group. The photometric determination of the absorbance was performed at 490nm using a 96-well plate reader.

Calculations

Initial measurements were performed when the tumor reached 150-200 mg and the tumor volume and weight were calculated using the following equations:

Tumor Volume = length (mm) x width $(mm)^2$.

Tumor weight (in mg) = tumor weight (mg) – [length (mm) of tumor x width (mm) of $tumor^2$]/2.

The half maximal effective concentration (EC_{50}) values were calculated using GraphPad Prism 4™.[20-23] Graphs represent mean values and standard deviations.

Experimental of Molecular Docking

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 previous work.[9,12,24] However, for this work more extensive simulations were used and the details of these simulations are reported.[25] In short, the simulations were four 20ns NPT (20 nanoseconds at constant particle-number constant-pressure and constant temperature), all-atom simulations based on the human MSH2/6 crystal structure,[26] with the (1,2)G cross-link of adjacent guanine's, which is the predominate damage due to cisplatin. The structure selected was the median structure of the most populated cluster found from all-atom root-

mean square-deviation (RMSD)-based clustering.[25] The appropriate pdbqs file was generated with the DNA removed, so that just the protein remained, using defaults from Autodock4. 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 22.5Å grids were generated for electrostatics and vdW parameters for C, S, O, N and polar H with a grid spacing of 0.375Å.

CONCLUSION

This work characterizes the molecular modeling study by using the advanced Autodock4 program and then displaying the results in terms of a library concerning the ideal lead rescinnamine analogues along with calculating the K_i constant for each compound even the reserpate intermediates. The modeling study revealed that most of the synthesized rescinnamine derivatives show small K_i except those two rescinnamine analogues **1** and **3**. In addition, it extends to describe the chemical reactions required for the synthesis of a series of new rescinnamine derivatives starting from the precursor alkaloid, reserpine, it tells about how the palladium catalyzed Heck reaction conditions is employed to achieve the carbon- carbon coupling between the acryloylresepate **2** with various appropriate aryl iodides, most of the aryl iodides are prepared by different synthetic pathways. It also shows the importance of using the phosphonium ligand, in case of using an aryl bromide. The newly synthesized compounds were characterized by using various spectrometric tools; and the elemental analysis Finally, this work goes more to screen the antitumor activity. Most of reserpate intermediate and their rescinnamine analogues exhibited a promising activity as significant cytotoxic compounds but with small MSH2 dependence. Additionally, results revealed that the cell death is largely dependent on specific functional groups rather than just presence of ring systems, as **2** Thus, to elucidate the exact mechanism of these effects and the structure activity relationship, further computational modeling and cell biology studies are required.

CONFLICT OF INTEREST

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