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Natural Product Salvianolic acid F-Inspired Divergent Synthesis of Styryl-Cinnamate Hybrid Analogues as a Premise to Investigate Anticancer Activity and its Metabolomic Profiling

Amit Shard, [a,d]KiranRawat,[c,d] Arun K. Sinha,*[a,b,d] YogendraPadwad*[c,d] and Dinesh Kumar[a]

This article is dedicated with respect to Late Professor Richard F. Heck

Abstract: *The natural product salvianolic acid F-inspired protecting-group-free synthesis of hydroxylated styryl-cinnamate hybrids (C_6-C_2-C_6-C_3 unit) has been achieved by step-economical route via sequential double C-C bond formation in one pot. The present method involves multiple reactions (Perkin-condensation/ decarboxylation-Heck cross-coupling reactions) using simple precursors (i.e. hydroxylated benzaldehyde, arylacetic acid and acrylic acid derivatives) in one pot which yields desired unnatural small hybrid molecules (**1-12**) in varying yields of 35-65% with E-selectivity under microwave irradiation whereas the reported conventional route for synthesis of salvianolic acid F itself requires six steps with an overall yield of 10.0% besides tedious separation of E/Z isomers that arise from Wittig reaction. Apart from an economical synthesis and products diversity, we herein report the potential of some hybrid molecules (**3**,**8**,**10** and **11**) with the catecholic core, to selectively inhibit glioma cells. The intrinsic mode of action (MOA) for our lead molecule involving caspase 6 and quinonemethide pathway is also reported based on ^{1}HNMR-guided metabolomic profiling. We emphatically demonstrate the role of our hybrid molecules, an analogue of salvianolic acid F, in compelling the glioma cells towards apoptosis by specifically perturbing the concentration of glutathione along with caspase 6.*

Introduction

Gliomas[1] are most common type of primary malignant brain tumors with poor prognosis. Although a significant progress is made in its molecular pathogenesis; extensive efforts are underway to evaluate novel chemotherapeutics against glioma, but there has been limited progress in developing effective therapies.[1a-b] The poor prognosis is due to high cellular and molecular heterogeneity, altered signaling pathways and intrinsic resistance.[1c] This has caught interest from various chemists and biologists to synthesize better molecules with higher yields and cytotoxic potential. Simultaneously, drug discovery invites the development of new synthetic approaches to generate structurally diverse scaffolds.[1d-e]

It is well accepted that the unique ability of organic chemists allows tailoring of many natural products,[2] which itself is an excellent pharmacy.[2a-b] This provides an extraordinary approach to unlock the full potential of an anticancer natural product like podophyllotoxin[2c]which eventually lead to the synthesis of etoposide.[2d] Similarly, many natural hybrid molecules and their synthetic analogues have shown multifarious bioactivities including anticancer property.[2b] Hybrid molecules[3] are of great therapeutic repertoire as they confer superior biological profiles (Figure 1) than their parent molecule.

Among numerous hybrid molecules reported so far, Salvianolic acid F (Figure 1), one of the natural tetrahydroxy-substituted phenolic acids[4] isolated from *Salvia multihoriza,*[5] has been subject of major scientific attention as this natural hydroxylated stilbene-cinnamate scaffold possesses numerous biological activities. [4a-b]

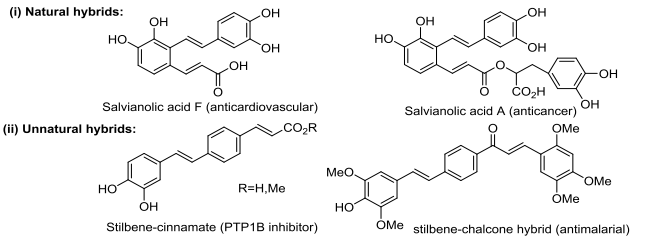

Figure 1. Structure of biologically active natural and unnatural hybrid molecules

To the best of our knowledge, salvianolic acid A and B[4b-c] have shown significant activity against glioma,[4d] however, there is no report for testing of Salvianoic acid F against it. This may be due to the fact that despite its esteemed biological profile, the synthesis of Salvianolic acid F has been an arduous task as it involves multiple steps[4a,6] reactions via Wittig reaction with requirements of additional deprotection with BBr_3[4a] leading to the formation of undesired side product. Moreover, this protocol has some shortcomings, such as using inaccessible starting materials, poor selectivity (*E/Z*),[4a] low yields (overall 10.0%)[4a] (Scheme 1) or limited substrate scope and product diversity. Thus, it is still necessary to develop efficient and protection group-free synthesis of Salvianolic acid F and their structural

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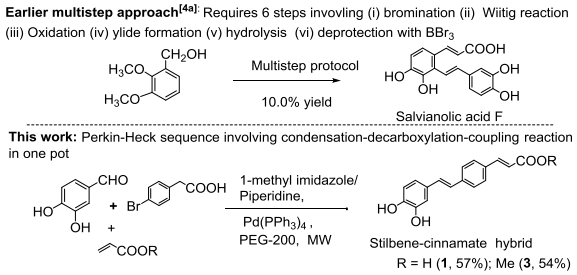
analogues (Figure 1) as many a times analogues synthesized are found more potent than the parent scaffold besides it allows short and concise synthesis of desired analogues in better yield with possibilities to conduct many additional biological activity studies.

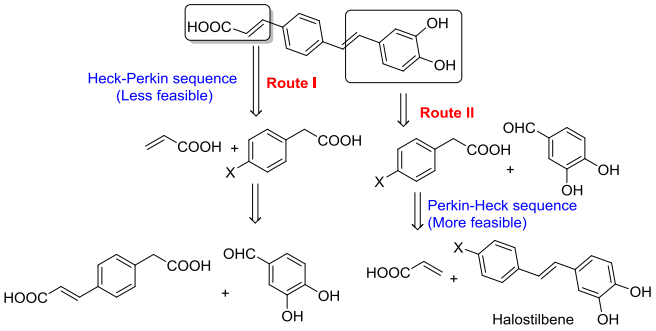


Scheme 1. Comparison of multistep prevalent protocol for natural Salvianolic acid F with our concise approach for unnatural stilbene-cinnamate in one pot.

With this notion in mind, we came across literature report[7] which revealed that unnatural stilbene-cinnamate[7a] (C_6-C_2-C_6-C_3 unit; Figure 1), an analogue of salvianloc acid F, has potent protein tyrosine phosphatase 1b (PTP1b) inhibitory[7a] activity. Interestingly, PTP1b was also reported to be a potent target for glioma.[7b] Unfortunately, the available synthetic route for such stilbene-cinnamate[7a] (Figure 1), also requires tedious seven-steps protocol via Wittig-Horner reaction along with requirements of additional protection-deprotection strategy to provide desired 3,4-dihydroxy-4'-(methoxy-carbonyl-1-ethenyl)stilbene)[7a] (see SI).

We now thoroughly investigated the possible alternative short routes for protection-group-free synthesis of such styryl-cinnamate scaffold with (*E*)-selectivity and it was visualized that above shortcomings can be fulfilled by the most acclaimed carbon-carbon cross coupling reactions i.e. palladium catalyzed Mizoroki-Heck reaction.[8] Then it was thought to join the planar centers for the synthesis of (*E*)-styryl-cinnamate(C_6-C_2-C_6-C_3 unit) by double[9] Heck coupling of dihalo substituted benzene (C_6 unit) with appropriate hydroxylated styrene (C_6-C_2 unit) and acrylate (C_3 unit) in one pot in sequential manner at two different temperatures[9a] using Pd nano-particle as a catalyst. However, problem of chemoselectivity[9a] during synthesis of dihydroxylated hybrid molecules (C_6-C_2-C_6-C_3 unit) surfaced due to possibility of formation of various side products including phenylenediacrylate or distyrylbenzene (DSB) (see SI). Another problem was propensity of hydroxylated styrenes towards polymerization apart from the fact that many styrenes such as 3,5-dimethoxy-4-hydroxystyrene (i.e. canolol)[10] are not commercially available.

In seeking a more efficient route towards step-economic synthesis of hydroxylated stilbene−cinnamate hybrids from readily available substrates (hydroxylated benzaldehyde, arylacetic acid and acrylic acid derivatives), it was equally visualized that the halogenated stilbene intermediate (Scheme 2) can act as a synthetic handle to synthesize targeted hydroxylated stilbene-cinnamates through cascade[11] Heck coupling with acrylate, by avoiding protection-deprotection strategy.[12] In this context, our group has been actively engaged in designing such cascade protocols where privileged Heck coupling[13]along with other highly useful name reactions like Suzukui/Aldol/Knoevenagel[14] remains pivotal.[13b] Therefore, keeping this in mind, two esteemed name reactions Perkin condensation (C_6-C_2-C_6 unit) and Heck coupling (C_3 unit) were designed for the generation of hydroxylated styryl-cinnamate (C_6-C_2-C_6-C_3)unit. The fundamental retro synthetic route (I or II) adopted is shown for better clarity (Scheme 2).



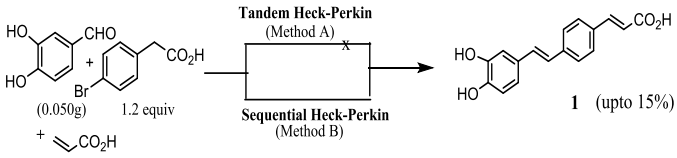
Scheme 2. Retrosynthetic analysis of hydroxylated styrylcinnamic acids, an analogue of Salvianolic acid F[4a] (Scheme 1).

In this communication, we report a rationally designed protecting-group-free (PGF) synthesis of hydroxylated styryl-cinnamate (S-C) hybrids (**1-12**) *via* sequential Perkin-Heck reactions (condensation-decarboxylation/coupling reactions) in one-pot that accomplished in shorter time under the influence of microwave irradiation[15] using polyethylene glycol (PEG) as a green solvent. The structurally diverse hybrid molecules (**1-12**) that resemble natural “Salvianolic acid F” core, were screened against C6 glioma cells *in vitro* with a conclusive report that their mode of action involves caspase 6 activity and reduction in Glutathione (GSH) concentration.

Results and Discussion

Initially, to prove the concept of two different name reactions functioning together in one-pot towards concise synthesis of styryl-cinnamic acid **1** (Scheme 3, Method A), a tandem Heck-Perkin strategy was planned. Accordingly, 4-bromophenylacetic acid (1.2 equiv.), acrylic acid (1.2 equiv.) and 3,4-dihydroxy-benzaldehyde (0.050g) were mixed in one pot with 1-methyl imidazole (MIM)/Piperidine as bases (1.5 equiv. each) and $Pd(OAc)_2$ (5 mol %) as catalyst using PEG-200 as solvent. The mixture was then microwave (MW) irradiated (at 160^{o}C) for 35 min but unfortunately the tandem reaction led to the formation of multiple side products along with trace amount of **1**. Hence, a sequential Heck-Perkin strategy (Scheme 3, Method B) was planned where 4-bromophenylacetic acid and acrylic acid were MW irradiated in presence of MIM/piperidine, $Pd(OAc)_2$ and PEG-200 to obtain intermediate 3-(4-carboxymethyl-phenyl)-acrylic acid. This was followed by sequential addition of 3,4-dihydroxybenzaldehyde in the same pot for Perkin reaction with simultaneous condensation-decarboxylation[16] under MW irradiation (160^{o}C, 35 min), yet the

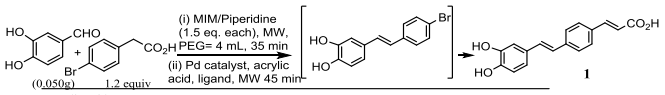
desired product 1 (Scheme 3) was obtained in 15% yield only (S.I.).



Scheme 3. Tandem Heck-Perkin and Sequential Heck and Perkin reaction towards synthesis of unnatural styryl cinnamic acids (1)

After the failure of above tandem Heck-Perkin coupling reaction and with limited success of sequential Heck-Perkin (Scheme 3) strategies, our attention moved towards sequential Perkin (condensation-decarboxylation)-Heck coupling reaction. Here, the first step involves the modified Perkin reaction[16] between 4-bromophenylacetic acid (1.2 equiv.) and 3,4-dihydroxybenzaldehyde (0.050g) in the presence of MIM/piperidine (1.5 equiv. each) and PEG-200 (4 mL) with simultaneous condensation and decarboxylation of intermediate *α*-phenylcinnamic acid into *trans*-4-bromo-3',4'-hydroxy stilbene. This was followed by Heck coupling with acrylic acid in the presence of $Pd(OAc)_2$ (5 mol %) which led to the formation of **1** in 28% yield (Table 1, entry 1). Enthused by this, different palladium catalysts were screened and amount of acrylic acid was optimized (Table 1, entries 2-8). Delightedly, $Pd(PPh_3)_4$ and acrylic acid (2 eq.) provided hydroxylated S-C hybrid **1** in 57 % yield (Table 1, entry 7) in one pot in comparison to prevalent multistep protocol.[7a]

Table 1. Optimization of reaction conditions for SequentialPerkin-Heck sequence.[a]



Entry	Acrylic acid (Equiv.)	Palladium catalyst (5 mol%)	Ligand (10 mol%)	Yield[b] (%)	Entry	Acrylic acid (Equiv.)	Palladium catalyst (5 mol%)	Ligand (10 mol%)	Yield[b] (%)
1	1.2	Pd(OAc)2	PPh3	28	5	1.2	$Pd(PPh_3)_4$		41
2	1.2	$Pd(CF_3COO)_2$	PPh_3	30	6	1.5	$Pd(PPh_3)_4$	-	45
3	1.2	$Pd(Cl)_2$	PPh_3	22					
4	1.2	$Pd(Cl)_2(PPh_3)_2$	-	34	**7**	**2.0**	**$Pd(PPh_3)_4$**	-	**57**
					8	2.5	$Pd(PPh_3)_4$		51

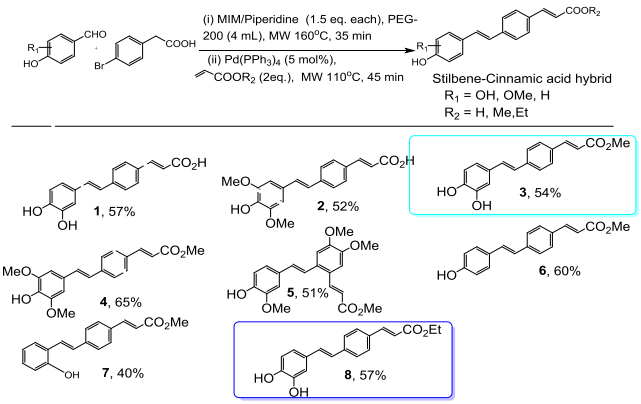
[a] CEM monomode microwave; General conditions. For step I, reaction mixture was Microwave (MW) irradiated at 150W, 160°C for 35 min (Perkin condensation). For step II, reaction mixture was MW irradiated at 110W, 110°C, for 45 min (Heck coupling).

[b] Isolated Yields.

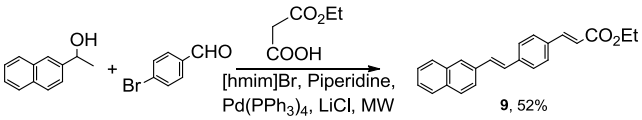
With the optimized reaction conditions in hand, the synthesis of stilbene-cinnamic acid **2** having 4 hydroxy 3,5-dimethoxy styryl (syringol)[10] substitution was also achieved in 52% yield (Table 2). Further we planned to enhance the skeletal diversity to see the effect of various substituent's in SAR studies. The methyl/ethyl esters[17] of hybrid cinnamic acids (**1-2)** were prepared by following the above sequential Perkin-Heck reaction just by replacing acrylic acid with methyl/ethyl acrylate and the desired ester hybrids (3-8, Table 2) were obtained in good yield upto 65% (4, Table 2). It is worth mentioning that hybrid molecule **5** (Table 2), a close analogue of bioactive salvianolic acid F and salvianolic acid A, an ET_AR (Endothelin-A receptor) antagonist[4e] (Figure 1), was obtained in 51% yield (Table 2) in two step instead of six steps[4a] required for synthesis of salvianolic acid F (Scheme 1).

With remarkable success towards step-economic and protection-group-free synthesis of hydroxylated hybrids S-C molecules (**1-7**, Table 2), we screened them against C6 cells (rat glioma). The preliminary screening[18] was focused on glioma[18a-b] because of two reasons. Firstly, PTP1b is one of its prime oncotargets[7b] and secondly because of the presence of ET_AR receptor on its membrane[4e] which indicates possibility of receptor-mediated endocytosis of our molecules. Antiproliferative screening of molecules **1-7** was performed by sulforhodamine B based colorimetric assay[19] and only methyl stilbene-cinnamate **3** (IC_{50} 5.4 µm) came out to be significant as compared to Vinblastine (IC_{50} 9.9 µm; Figure 2). Encouraged by the potential of **3** to target C6 that has stem cell like property;[20] the panel was also screened against four different cancer cell lines *viz.* lung (A549), colon (COLO205), cervix (HeLa) and ovary (CHOK1), but none of the hybrids could induce significant (>50%) antiproliferative activity (See S.I). However, 3,4-dihydroxy substituted ethyl stilbene-cinnamte **8** came out to be significantly active on C6 cells with an IC_{50} of 3.4 µm (Figure 2). On the other side, a non hydroxylated hybrid molecule **9** (Scheme 4) synthesized by using our recent dehydrative-Heck[13a] methodology could not exhibit potential activity on C6 cells.

Table 2. Perkin-Heck sequence led synthesis of various styryl-cinnamates.[a]



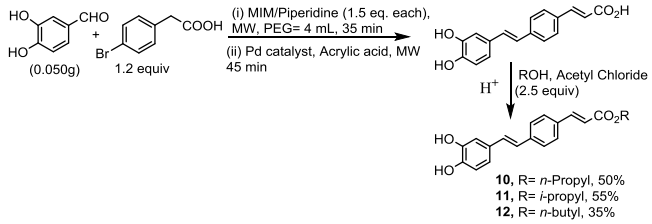
[a] CEM monomode microwave; General conditions. For step I reaction was Microwave (MW) irradiated at 150W, 160°C, 35 min. For step II reaction was MW irradiated at 110W, 110°C, for 45 min.



Scheme 4. Synthesis of styryl-cinnamate hybrid (S-C) using dhydrative-Heck methodology.

From here onwards, we thought of concentrating on the synthesis of 3,4-dihydroxy substituted styryl-cinnamate as out of nine S-C hybrids molecules (**1-9**), only methyl or ethyl substituted styryl-cinnamates (**3** and **8**) were found to be potent. However, it is imperative to mention that propyl, isopropyl and

butyl substituted acrylates are not commercially unavailable, hence the synthesis of targeted hybrid esters (**10-12**) were prepared sequentially in two steps as mentioned in scheme 5. Thus molecule **1** was prepared in large amount following Perkin and Heck protocol followed by reacting **1** with respective alcohols (excess of propyl, isopropyl or butyl alcohol) in presence of acetyl chloride with concomitant release of mineral acid. This led us to prepare the three hybrid esters **10**, **11** and **12** respectively in good yield up to 55% (Scheme 5). To our delight molecules **10** and **11** (Figure 2) also came out to be active against C6 cells (see SI) whereas *n*-butyl substituted stilbene-cinnamate (**12**) was found inactive against C6 cells.



Scheme 5. Synthesis of styryl-cinnamate (S-C) hybrids using Perkin-Heck methodology followed by esterification.

The plausible mechanism of the sequential Perkin-Heck protocol is expected to be quinone methide species.[13] Further, capitalizing on the inherent properties of 3,4-dihydroxy substituted hybrid molecule[21] and their electrophilic *ortho*-quinone methide[22] (QM) structure obtained under physiological conditions, we also proposed a plausible mode of action (see S.I). It was postulated that once the S-C hybrid molecule enter the cells, widespread occurrence of esterases[22] inside cells might cause hydrolysis of an ester bond and generate tantalizing QM's. Most importantly, QM's being strong electrophiles are vulnerable to the similar set of reactions usually committed by other anticancer drugs.[22b] It's well established that most of the anticancer drugs like mitomycin-C or platinum complexes reacts with N7 of guanine in DNA and also with other thiols containing cellular nucleophiles like glutathione (GSH). So, we hypothesized that our molecules produce QMs that on reacting with GSH reduces its availability in the cells hinting towards the activation of executioner proteins causing apoptosis.

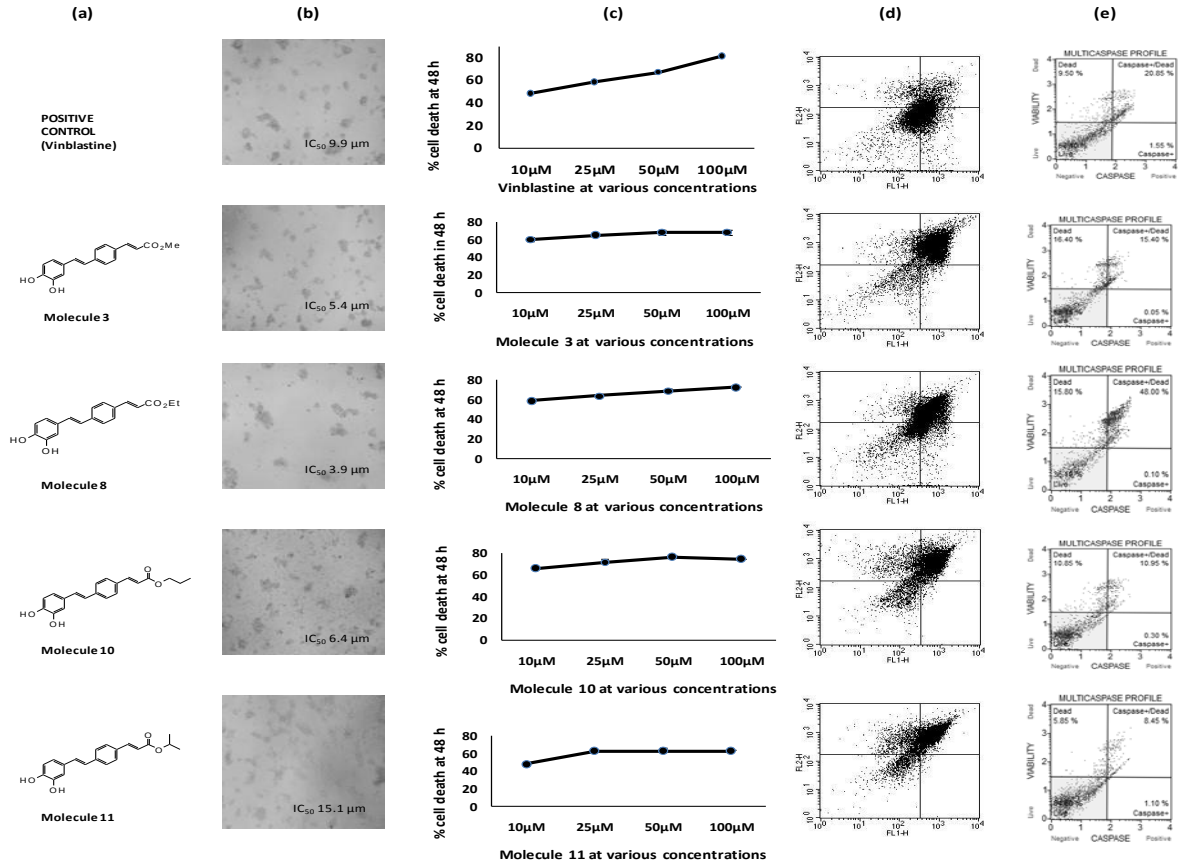


Figure 2. Cytotoxicity profile of lead hybrid molecules (**3**, **8**, **10** and **11**) compared to positive control (Vinblastine). (a) Molecules under study. (b) Image of C6 cells using Phase-contrast microscopy after 48 h insult with molecules under study. (c) Toxicity induced by Vinblastine as well as molecules **3**, **8**, **10** and **11**. C6 cells ($2x10^4$) were treated with different concentrations (10-100 µm) for 48 h before staining with 0.4% Sulforhodamine B dye. Data was analyzed by ANNOVA using STATISTICA, version 7. Graph represents the percentage toxicity of molecules since SRB staining is indicative of number of alive cells. (d) After insult with the molecules at IC_{50}, cells ($1x10^5$) were subjected to Annexin V-FITC-PI double staining. Flow Cytometry results were obtained on BD calibur for 10,000 events/sample. X-axis represents Apoptosis while Y-axis depicts necrosis. e) Cells were treated for 12 h and harvested by mechanical dislodging, resuspended in 1X Caspase Buffer with final concentration of $5x10^6$ cells/mL and proceed for staining to study Multicaspase profiling.

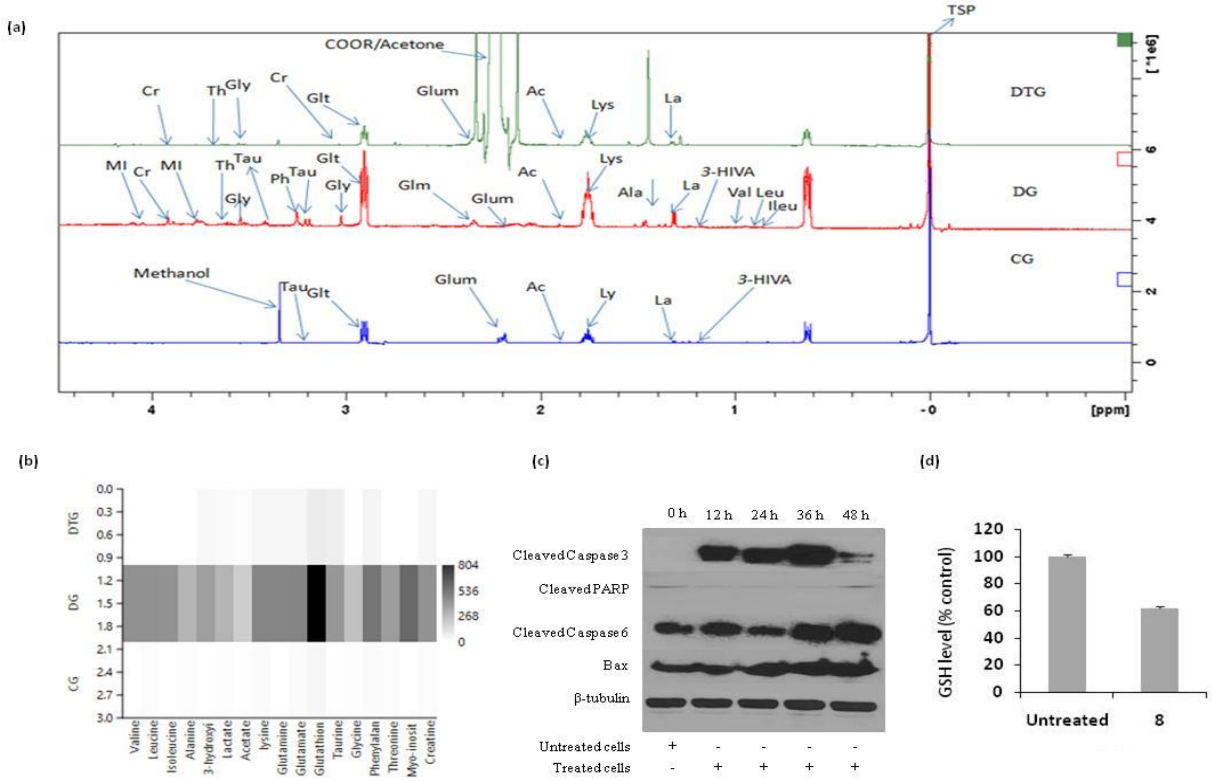


Figure 3. Determining the Mechanism of Action for our lead molecule **8**. (a) ^{1}H NMR spectra (three independent repliecates) of aqueous metabolites from three groups. The spectral regions from 0.0 to 4.5 ppm is shown (3-HIVA= 3-hydroxyisovaleric acid; La= Lactate; Ly= Lysine; Ac= Acetate; Glum= Glutamine; Glt= Glutathiona; Tau= Taurine; Val= Valine; Leu= Leucine; Ileu= Isoleucine; Ala= Alanine; Lys= Lysine; Glm= Glutamate; Gly= Glycine; Ph= Phenylalanine; Th=Threonine; Cr= Creatine; CG= Control Group; DG= Diseased Group; DTG= Drug Treated Group; TSP= 3-(Trimethylsilyl)propionic-2,2,3,3-d_4 acid sodium salt; For full spectra refer SI). (b) Matrix plot generated using Past 3.x - software to identify the differential quantitative variation in metabolites among three groups (CG= Control Group; DG= Diseased Group; DTG= Drug Treated Group; refer SI) . (c) Representative Western Blots indicate the release of activated caspase 3 upstream of caspase 6 in cells ($1x10^6$) treated at IC_{50}. Lane 1 represents untreated cells (control) while lane 2-5 indicate protein level at various time points for which insult was given. (d) Effect on intracellular GSH levels after incubation of C6 cells with 3.9 µm of molecule for 36 h, as measured by Ellman's assay. Results are presented as the mean of three independent experiments, with bars depicting the standard deviations.

In light of the cytotoxic ability (Figure 2a-c) of our lead molecules and the plausible hypothesis generated thereby, we analyzed the ability of these hybrid molecules to interfere in phosphatidylserine translocation[23] along with DNA damage. Accordingly, data generated by Propidium iodide(PI)-AnnexinV-FITC double staining[23a] indicated that all lead hybrid molecules (**3**, **8**; Table 2; **10** and **11**; Scheme 5) were able to trigger apoptosis (Figure 2d) within 24 h of incubation while molecule **8** displayed remarkable potential even at low concentration (3.9 µm; Figure 2b).

Further multicaspase study to understand if the cell death is caspase dependant or not (Figure 2e) was carried out and selectivity index (SI Index; Table 3) was calculated to understand the specificity of our lead moieties against cancer cells opposed to normal glial cells.[23b] Molecule **8** depicted highest Selectivity Index of 22 enabling us to appreciate its unique ability to specifically target cancerous cells while being less harmful to normal brain cells. This encouraged us to uptake the analysis of activated caspases by immunoblotting through **8**.

It was found that apoptosis triggered by it was through caspase 6 (Figure 3). It is well established that the proapoptotic protein, Bax, localizes to the mitochondria in response to numerous apoptotic stimuli and leads to the release of cytochrome c, which further activates the caspase cascade.[23c]

Therefore, the expression level of Bax was evaluated and it was also found to increase with time confirming upstream signalling of caspase 6 pathway. Since Poly (ADP-ribose) polymerase (PARP) is involved in repair of damaged DNA, the blot obtained for cleaved PARP indicated that **8** caused irreparable and irreversible DNA damage (Figure 3c).

Table 3. Selectivity Index (SI) of our lead molecule against normal glial cells. It was calculated as IC_{50} against normal glial cells/IC_{50} against C6 cells

Molecule under study	IC_{50} against Normal glial cells	IC_{50} against C6	Selectivity Index (SI)
Molecule **3**	142	15.4	9
Molecule **8**	86	3.9	22
Molecule **10**	103	10	10
Molecule **11**	103	39	3

In continuation to this, we tried to understand DNA damage through QM formation for which a NMR based metabolomic

study of **8** was undertaken which has become an important tool now-a-days.[24] The comparative NMR spectra (aqueous phase) acquired for 3 groups[24a] (CG= Control Group; DG= Diseased Group; DTG= Drug Treated Group) is shown in Figure 3a. We found that these metabolic profiles were highly reproducible. It was observed that the accumulation of metabolites was high in C6 cells and was significantly altered by **8**. Concentration of Glutathione (GSH; δ 2.908 ppm, m) was markedly reduced by molecule **8** after 36 h of insult, clearly indicating its role in modulating QM formation. Encouragingly, it had a pronounced effect on other metabolites as well (Figure 3b), where the concentration of Glycine (δ 3.54 ppm, s) and Lysine (δ 1.764 ppm, m) were considerably reduced. Studies have established that Lysine has significant role in histone modification at DNA level while glycine is well known marker for grade IV glioma.[24b-e] Eventually, Ellman's assay[22b] was performed and GSH reduction was validated (figure 3d). Further, due to the success of molecule **8** against C6, it was also screened against human glioma U87MG[4d] cells but a moderate cytotoxicity of 45.4% (See SI) was observed. This warrants further chemical manipulation to target human glioma.

Finally, our initial presumption is consistent with our biological results that the hybrid stilbene-cinnamates (Figure 1) which targets PTP1b enzyme as anti-diabetic agent[7a] might be potent target for glioma.[7b] It will although be premature to state whether the cluster of our bioactive hybrid molecules (**3**, **8**, **10** and **11**) would safely cross the "Valley of death[25]" or will move from "Table to be a tablet", yet the important lessons learned from this odyssey of small unnatural hybrid molecues may reinvigorate interest for future studies to tackle these recalcitrant cells for anticancer drug development.

Conclusions

In summary, a protecting-group-free, eco-sustainable and potentially economic synthesis of the hybrid styryl-cinnamates (**1**-**12**) via sequential Perkin-Heck strategies is unambiguously established wherein carbon dioxide and water are released during the course of condenstion-decarboxylation-coupling reactions in one pot. Our protocol concurrently addresses issues of simplicity and unprecedented utility by generation of S-C hybrids, which show close structural analogy to naturally occurring hybrid Salvianolic acid F. Some of these hybrid molecules (**3**, **8**, **10** and **11**) served as potent chemical warheads against glioma. The preliminary bio-activity guided syntheses of variable small S-C hybrids have inhibited the growth of C6 cells selectively without perturbing the physiology of normal glial cells. Damaging effects of these antagonists against glioma cells in vitro are manifested by superior and selective antitumor potential without causing significant distress in healthy cells. Thus our study strongly emphasizes the value of small hybrid orthologue-specific antagonists with catecholic nucleus conjugated to an ester group. The hybrid stilbene-cinnamates may act as cornerstones in future to compel the biological terrorists (glioma cells) towards apoptosis thus offering an effective method of chemotherapy. The effect of these hybrid molecules on the other metabolites and proteins as well as their role at the transcriptomic level will provide further insight into the mode of action. For the same, proteomic as well as transcriptomic studies are in progress. Further the study will be also expedited towards modification of hybrid **8**, enabling it suitable against human glioma.

Experimental Section

General Experiments. From our preliminary screening (**1**-**7**) we found that **3** could not significantly inhibit other cancer types than glioma. Since our results came positive for molecule **3** alone towards glioma (C6) cell line of rat, further designed hybrid molecules (**8**-**12**) were screened on this cell line only. All the cell lines were purchased from National Center for Cell Science (NCCS, Pune) and were cultured in respective media as suggested by NCCS. Media was purchased from Gibco, Invitrogen and cells were grown in 10% FBS (Gibco, Invitrogen) along with 1% Antibiotic-antimytotic (Gibco, Invitrogen). Cultures were incubated at 37°C with 5% CO_2 and 95% humidity till required number of cells were obtained. In order to test the efficacy of our lead molecules on primary glial[23b] cells, we isolated the cells employing standard protocol from rat pup (P0-P1). This protocol was approved by the Institutional Animal Ethical Committee (IAEC) at CSIR-IHBT in compliance with relevant State and Federal Regulations (IHBT/P2 March, 2015).

Anticancer screening. All the S-C hybrid analogues (**1-12**) were assayed for antiproliferative activity using Sulphorodamine B (SRB) assay. Linearity over a 20-fold range of cell number, sensitivity, non-destructive as well as indefinitely stable colorimetric end point and lower signal-to-noise ratio made SRB an assay of choice in our study of drug-induced cytotoxicity screening. For the same, cell monolayer in its log phase was trypsinized and final suspension of $2x10^5$/mL was prepared using DMEM. 0.1 mL (20,000cells) of this suspension was added to require number of wells of 96-well microtiter plates and incubated at 37ºC with 5% CO_2 in 95% humidified atmosphere. Insult at required concentrations was given for 48 h in triplicate following which cell monolayer was fixed with 50% Trichloroacetic acid (w/v) and subjected to colorimetric study where cells were stained with 0.1 mL of anionic/aminoxanthine 0.4 % SRB dye. Allowed 30 min staining to ensure its optimum electrostatic binding where it forms a complex with basic amino acids on live cell membrane. Thereafter, unbound dye was washed with 1% acetic acid and protein-bound dye was dissolved in 10mM Tris base [tris(hydroxymethyl)aminomethane] solution. OD_{540} was determined using microplate reader [BioTek Synergy H1 Hybrid Reader] and Percentage inhibition was calculated using following formula.

% Inhibition = [(C-S)/C]*100 where C= OD_{540} of Control and S=OD_{540} of Sample

Three-factor factorial ANNOVA was applied on this data employing STATISTICA (Version 7).

Flow Cytometry analysis. To quantify apoptosis and necrosis induced by our small-stillbene conjugates, Propidium iodide (PI) -Annexin V-FITC double staining was employed. Viable, early apoptotic as well as necrotic or late apoptotic cells were distinguished owing to differences in their plasma membrane integrity and permeability due to phosphatidylserine translocation. After treatment was given to cultured cells ($1x10^6$/mL) in 6-well plate for 36 h, the adherent cells were

suspended in PBS and subjected to Annexin V/Propidium iodide staining using Annexin V-FITC Apoptosis Detection Kit II (Becton Dickinson-BD; Biosciences Products) according to the manufacturer's protocol. Parallel to drug-treated group we also maintained vehicle control cells. The resultant green fluorescence in FL-1 channel and red fluorescence in FL-2 channel was measured by FACS Calibur flow cytometer (Becton Dickinson) thorough 10,000events/sample. Accordingly, dual negative cells in lower left quadrant show live cells while dual positive in upper right indicate mid-apoptotic stage. Single positive cells in lower right and upper left indicate early apoptotic and necrotic cells respectively. Data was collected at 36 h.

Multicaspase profiling. Muse™ Multicaspase Kit (MCH100109; Milipore, USA) was exercised for fluorescence-based facile, rapid and quantitative measurements. As directed by manufacturer, treated cells were harvested by mechanical dislodging, resuspended in 1X Caspase Buffer with final concentration of $5x10^6$cells/mL and proceeded for staining. Data Acquisition and analysis was performed on the Muse™ Cell Analyzer (version 1.3.0.0). Assay involves use of derivatized VAD peptide having Fluoromethylketone (FMK) and fluorescent moiety which is permeable and non-toxic to cells. This peptide binds to activated caspases with resulting fluorescent signal proportional to number of active caspases in cells which appears as increased signal (lower right quadrant) in the Caspase axis. On the other hand, a dead cell marker dye (7-AAD) shows increased fluorescence on Viability axis (upper left quadrant). Dual positive and dual negative results appear in upper right and lower left quadrant respectively. Protocol by Promega (USA) employing use of Apo-ONE® Homogeneous Caspase 3/7 Kit was followed on $2x10^4$cells/well of 96-well microtiter plate. Higher Florescence resulting by higher activity of caspase 3/7 on profluorescent substrate rhodamine 110 (bis-(N-CBZ-L-aspartyl-L-glutamyl-L-valyl-L-aspartic acid amide; Z-DEVD-R110) was measured at 499nm using microplate reader [BioTek Synergy H1 Hybrid Reader]. Emission maxima were obtained at 521nm and Relative Florescence Unit was calculated.$1x10^5$ cells were subjected to Pan Caspase study employing Muse analyzer and Multicaspase Kit and analyzed after 12 h of treatment.

Western Blotting. Control and treated cells were collected after 0 h, 12 h, 24 h, 36 h and 48 h. These were lysed with RIPA buffer (Sigma-aldrich, USA) along with protease inhibitry cocktail (PIC; Sigma-aldrich, USA) in the of ratio 9:1 at 4°C for 1 h. Lysates were spun at 12000 r.p.m. for 20 min to remove cellular debris. Protein concentration was determined with Bradford reagent (Sigma-aldrich, USA). Equal amounts of protein (60 µg) were subjected to electrophoresis on SDS-polyacrylamide gels and transferred to nitrocellulose membranes (Sigma) by electro-blotting. The blots were blocked with 5% Non Fat Dried milk for an hour and probed overnight at 4°C with the respective primary antibody (1:1000 dilution). Blots were then washed with PBST and probed further with secondary anti-mouse (1:15,000 dilution) or anti-rabbit antibody (1:10,000 dilution) conjugated with horseradish peroxidase. Chemiluminescence was performed with ECL substrate (Biorad, USA). The following monoclonal primary antibodies were used: caspase-3, PARP, caspase 6, Bax and β-tubulin, that were purchased from cell signalling technology (USA).

Metabolomic profiling probed by NMR. A standard extraction protocol (See SI) was followed to obtain aqueous extract. Accordingly $1x10^7$ cell pellet (normal glial cells from rat pup, C6 control and C6 cells treated with **8** for 36 h at 3.9 µM) was resuspended with 400 µL of methanol and 200 µL of chloroform. These 3 samples were sonicated for 15min and 200 µl of chloroform as well as 200 µL of distilled water was added to it. The lysates were centrifuged at 15,000g for 20min at 4°C. After centrifugation, polar layer was carefully transferred to a new micro-centrifuge tube. The samples were dried and aqueous residue was dissolved in 600 µl of NMR buffer (2mM Na_2HPO_4, 5mM NaH_2PO_4, 0.025% TSP in D_2O). For NMR analysis an aliquot of 600 µl of sample was transferred to the 5-mm NMR tube. Proton NMR (^{1}H NMR) spectra were recorded (at 300 K) on 600 MHz Bruker AVANCE III-600 spectrometer (Bruker, Karlsruhe, Germany) operating at a proton NMR frequency of 600.13 MHz. Each ^{1}H NMR spectrum consisted of 128 scans requiring 13 min and 16 s acquisition time with the following parameters: 0.11 Hz/point and relaxation delay (RD)=5 s. Pre-saturation sequence was employed to suppress residual H_2O/D_2O signal with low power selective irradiation at the H_2O frequency during the recycle delay. FIDs were Fourier transformed with LB=0 Hz. The resulting spectra were manually phased and baseline corrected, and calibrated at 0.0 ppm, using Topspin (version 3.2, Bruker). As signal intensity is absolutely proportional to molar concentration of metabolites[13], the quantification of the selected metabolites was performed by using ratio method. A known internal standard (in this case TSP) was used to determine the concentration of the targeted metabolites by using the following equation:

$$m_X = m_{ST} \times (A_X / A_{ST}) \times (MW_X / MWS_T) \times (N_{ST} / N_X)$$

where, m_X – mass of target analyte
m_{ST} – mass of TSP
A_X – Integral area of selected signal
A_{ST}- Integral area of TSP
N_X- number of protons generating integral signals
N_{ST}- Number of protons generating integral signal of TSP
MW_X- Molecular weight of target analyte
MWS_T- Molecular weight of TSP

In order to validate the lead obtained from NMR metabolomic profiling, total glutathione quantification was undertaken employing Ellman's Assay using standard protocol from Sigma Aldrich (Cat. # CS0260).

General Procedure for the Preparation of hybrid compounds (1-8) via sequencial Perkin-Heck reaction: 4-Bromophenylacetic acid (1.3 eq., 0.093g, 0.434mmol) was stirred in the presence of 1-methylimidazole (1.5 eq.) and piperidine (1.5 eq.) in the solvent PEG-200 (4 mL) to generate the carbanion. Next, the 3,4-dihydroxybenzaldehyde (1eq., 0.050g, 0.362 mmol) was added to the formerly stirred reaction mixture and the reaction mixture were microwave irradiated (MW, CEM Discover© focused) for 30-35 min (150W, 160°C) or till completion of the reaction. To the obtained intermediate 4-bromo-3',4'-hydroxy stilbene, a mixture of acrylic acid or acrylic ester (2 eq.) and $Pd(PPh_3)_4$ (5 mol%) were added in the same pot for Heck coupling reaction. Finally, the reaction mixture was again MW irradiated (110 W, 110°C, 40 min) to yield **1**. The crude reaction mixture after column purification (silica gel, hexanes:ethyl acetate) was further recrystallized in methanol to obtain the desired pure product (1-8). The compound **9** was prepared following the previously developed protocol

General Procedure for the Preparation of hybrid esters (10-12): The hybrid esters (10-12) were prepared by reacting **1** with excess of corresponding alcohols (propyl, isopropyl or butyl

alcohol) at cooled temp. in the presence of hydrochloric acid gas as generated by reaction of acetyl chloride with respective alcohol). The NMR and HRMS spectra of compounds **1-12** are given below:

Compound 1: (2*E*)-3-{4-[(*E*)-2-(3,4-dihydroxyphenyl)-ethenyl]phenyl}prop-2-enoic acid
Yield: 57%;mp: 246-248°C, R_f=0.2 (hexanes:ethyl acetate, 50:50 v/v with 1-2 drops of glacial acetic acid); ^{1}H NMR (DMSO-d_6, 300MHz):δ (ppm) 7.58 (d, *J* = 8.5 Hz, 2H), 7.52 (t, *J* = 9.3 Hz, 3H), 7.11 (d, *J* = 16.5 Hz,1H), 6.94 (d, *J* = 15.1 Hz, 1H), 6.83 (d, *J* = 8.7 Hz, 2H), 6.67 (d, *J* = 7.6 Hz, 1H), 6.46 (d, *J* = 16.5 Hz, 1H); ^{13}C NMR (75.4 MHz, DMSO-d_6):δ (ppm) 168.2, 146.4, 145.9, 144.1, 140.0, 133.1, 130.7, 129.1, 128.9, 126.9, 124.7, 119.4, 118.7, 116.2, 113.9. HRMS-ESI: m/z [M+H]+ calcd for $C_{17}H_{14}O_4$, 283.0964; found 283.0956.

Compound 2: (2*E*)-3-{4-[(*E*)-2-(4-hydroxy-3,5-dimethoxy-phenyl)ethenyl]phenyl}prop-2-enoic acid
Yield: 52%;mp: 202-205°C;R_f=0.3 (hexanes:ethyl acetate, 50:50 v/v with 1-2 drops of glacial acetic acid); ^{1}H NMR (DMSO-d_6, 300MHz):δ (ppm) 8.65 (s, 1H), 7.68 (d, *J* = 8.5 Hz, 2H), 7.60 (t, *J* = 8.5 Hz, 3H), 7.28 (q, *J* = 16.5 Hz, 2H), 6.92 (s, 2H), 6.55 (d, *J* = 15.9 Hz, 1H), 3.81 (s, 6H); ^{13}C NMR (75.4 MHz, DMSO-d_6):δ (ppm) 168.2, 148.5, 144.0, 140.0, 136.5, 133.2, 130.9, 129.2, 127.8, 126.9, 125.4, 118.8, 104.8, 56.4. HRMS-ESI: m/z [M+H]+ calcd for $C_{19}H_{18}O_5$, 327.1227; found 327.1216.

Compound 3: Methyl (2*E*)-3-{4-[(*E*)-2-(3,4-dihydroxyphenyl)-ethenyl]phenyl}prop-2-enoate
Yield: 54%; mp: 208-210°C;R_f = 0.6 (hexanes:ethyl acetate, 85:15 v/v); ^{1}H NMR (DMSO-d_6, 300MHz):δ (ppm) 9.32 (s,1H), 9.11 (s, 1H), 7.72 (d, *J* = 7.9 Hz, 3H), 7.65 (t, *J* =10.5 Hz, 3H), 7.24 (d, *J* = 15.6 Hz, 1H), 7.18 (d, *J* = 15.6 Hz, 1H), 6.96 (d, *J* = 8.7 Hz, 1H), 6.82 (d, *J* = 9.1 Hz, 1H), 6.67 (d, *J* = 16.7 Hz, 1H), 3.75 (s, 3H); ^{13}C NMR (75.4 MHz, DMSO-d_6):δ (ppm) 167.7, 146.9, 146.3, 145.1, 140.7, 133.3, 131.3, 129.6, 129.3, 127.3, 125.0, 119.9, 117.7, 116.6, 114.4, 52.3.HRMS-ESI: m/z [M+H]+ calcd for $C_{18}H_{16}O_4$, 297.1121; found. 297.1103.

Compound 4: 3-{4-[2-(4-Hydroxy-3,5-dimethoxy-phenyl)-vinyl]-phenyl}-acrylic acid methyl ester
Yield: 65%;mp: 190-192°C;R_f= 0.8 (hexanes:ethyl acetate, 85:15 v/v); ^{1}H NMR ($CDCl_3$, 300MHz):δ (ppm) 7.46 (d, *J* = 15.9 Hz, 1H), 7.51- 6.78 (m, 8H), 6.45 (d, *J* = 15.9 Hz, 1H), 5.66 (s, 1H), 3.96 (s, 6H), 3.82 (s,3H); ^{13}C NMR (75.4 MHz, $CDCl_3$):δ (ppm) 167.9, 147.6, 144.7, 139.9, 135.6, 133.6, 130.6, 128.9, 127.0, 126.2, 117.5, 103.9, 56.7, 52.1.HRMS-ESI: m/z [M+H]+ calcd for $C_{20}H_{20}O_5$, 341.1608; found. 341.1644.

Compound 5: 3-{2-[2-(4-Hydroxy-3-methoxy-phenyl)-vinyl]-4,5-dimethoxy-phenyl}-acrylic acid methyl ester
Yield: 51%;mp: 172-174°C;R_f= 0.8 (hexanes:ethyl acetate, 85:15 v/v);^{1}H NMR ($CDCl_3$, 600MHz):δ (ppm) 7.73 (s,1H), 7.28 (s,1H), 6.94- 6.91 (d, *J* = 18.0 Hz, 1H), 6.84-6.81 (m, 5H) , 6.51 (s,1H), 3.92 (s, 3H), 3.89 (s,3H), 3.82 (s,3H), 3.61 (s,3H);^{13}C NMR (150 MHz, $CDCl_3$):δ (ppm) 168.9, 149.7, 149.0, 146.8, 141.0, 136.4, 130.1, 129.2, 126.1, 122.6, 113.4, 113.2, 112.0, 108.3, 56.4, 56.3, 52.7. HRMS-ESI: m/z [M+H]+ calcd for $C_{21}H_{22}O_6$, 371.1758; found. 371.1752.

Compound 6: 3-{4-[2-(4-Hydroxy-phenyl)-vinyl]-phenyl}-acrylic acid methyl ester
Yield: 60%;mp: 182-184 °C, R_f= 0.7 (hexanes:ethyl acetate, 90:10 v/v); ^{1}H NMR ($CDCl_3$+DMSO-d_6 (3:1), 300MHz):δ (ppm) 8.10 (s, 1H), 6.88-6.51 (m, 5H), 5.95-5.90 (d, *J* = 15.0 Hz, 1H), 5.86-5.83 (m, 4 H), 5.22-5.20 (m, 2H), 4.01 (s,3H); ^{13}C NMR (75.4 MHz, ($CDCl_3$+DMSO-d_6 (3:1):δ (ppm) 170.0, 163.0, 148.0, 144.4, 138.8, 136.1, 135.2, 134.1, 129.1, 128.2, 120.6, 119.6, 118.7, 56.6. HRMS-ESI: m/z [M+H]+ calcd for $C_{18}H_{16}O_3$, 281.1308; found. 281.1326.

Compound 7: 3-{4-[2-(2-Hydroxy-phenyl)-vinyl]-phenyl}-acrylic acid methyl ester
Yield: 40%;mp: 202-205°C;R_f= 0.4 (hexanes:ethyl acetate, 85:15 v/v); ^{1}H NMR ($CDCl_3$ + DMSO-d_6, 600 MHz):δ (ppm) 8.71 (s, 1H), 7.83-7.43 (m, 3H), 7.35- 6.80 (m, 4), 6.44 (d, *J* = 15.6Hz, 1H), 6.41 (d, *J* = 15.6Hz, 1H), 3.75 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$ + DMSO-d_6):δ (ppm) 167.5, 160.2, 155.0, 153.5, 144.5, 134.7, 131.7, 128.9, 128.0, 126.6, 125.6, 124.6, 119.7, 119.4, 118.5, 116.8, 116.4, 51.7. HRMS-ESI: m/z [M+H]+ calcd for $C_{18}H_{16}O_3$, 281.1308; found. 281.1332.

Compound 8: 3-{4-[2-(3,4-Dihydroxy-phenyl)-vinyl]-phenyl}-acrylic acid ethyl ester Yield: 57%;mp: 182-184 °C;R_f = 0.7 (hexanes:ethyl acetate, 85:15 v/v); ^{1}H NMR (CD_3COCD_3, 300MHz):δ (ppm) 8.09 (s, 2H), 7.69-7.59 (m, 5H), 7.22 (d, *J* =16.2 Hz, 1H), 7.16-7.15 (m, 2H), 7.03 (d, *J* =16.2 Hz, 1H), 6.97-6.50 (m, 2H), 4.22 (q, 2H), 1.29 (t, 3H); ^{13}C NMR (75.4 MHz, CD_3COCD_3):δ (ppm) 167.5, 146.9, 146.6, 145.1, 141.4, 134.3, 131.5, 130.8, 129.8, 127.8, 126.0, 120.8, 118.7, 116.7, 114.4, 61.1, 15.0. HRMS-ESI: m/z [M+H]+ calcd for $C_{19}H_{18}O_4$, 311.1458; found. 311.0770.

Compound 9: Ethyl (2*E*)-3-{4-[(E)-2-(naphthalen-2-yl)-ethenyl]phenyl}prop-2-enoate
Yield: 52%;mp: 172-174°C; R_f = 0.8 (hexanes:ethyl acetate, 98:2 v/v); ^{1}H NMR (300 MHz, $CDCl_3$):δ (ppm) 7.89-7.77 (m, 5H), 7.74 (d, *J* = 16.0 Hz, 1H), 7.60-7.46 (m, 6H), 7.38 (d, *J* = 16.3 Hz, 1H), 7.26 (d, *J* = 16.3 Hz, 1H), 6.50 (d, *J* = 16.0 Hz, 1Hz), 4.34 (q, *J* = 7.1 Hz, 2H), 1.40 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR(75.4 MHz, $CDCl_3$):δ (ppm) 167.5, 144.5, 139.7, 134.8, 134.1, 133.6, 130.5, 128.9, 128.8, 128.5, 128.1, 127.4, 127.3, 126.8, 126.5, 123.8, 118.3. 60.9,14.7. HRMS-ESI: m/z [M+H]+ calcd for $C_{23}H_{20}O_2$, 329.1626; found. 329.1646. IR (KBr) υ 1714 cm.$^{-1}$ (ester).

Compound 10: 3-{4-[2-(3,4-Dihydroxy-phenyl)-vinyl]-phenyl}-acrylic acid propyl ester

Yield: 50%;mp: 146-148°C;R_f=0.8 (hexanes:ethyl acetate, 85:15 v/v); ^{1}H NMR (CD_3COCD_3, 300MHz):δ (ppm) 7.91 (s, 1H), 7.90 (s, 1H), 7.77-7.51 (m, 3H), 7.34-7.28 (d, *J* =18.0 Hz, 1H), 7.11-6.71 (m, 6H), 6.61-6.55 (d, *J* = 18.0 Hz, 1H), 4.15 (t, 2H), 1.8 (m, 2H), 0.96 (t, 3H); ^{13}C NMR (75.4 MHz, CD_3COCD_3, 300MHz):δ (ppm) 169.1, 147.2, 146.7, 145.9, 141.8, 134.3, 131.7, 130.9, 129.8, 127.7, 125.9, 120.8, 118.1, 116.6, 114.2, 67.4, 23.3, 10.9. HRMS-ESI: m/z [M+H]+ calcd for $C_{20}H_{20}O_4$, 325.1614; found. 325.1645.

Compound 11: 3-{4-[2-(3,4-Dihydroxy-phenyl)-vinyl]-phenyl}-acrylic acid isopropyl ester
Yield: 55%;mp: 150-152 °C;R_f= 0.7 (hexanes:ethyl acetate, 85:15 v/v); ^{1}H NMR (CD_3COCD_3, 300 MHz), δ (ppm) 8.17 (s,1H), 8.00 (s,1H): 7.68-7.59 (m, 4H), 7.28-7.16 (m, 3H), 7.03 (d, *J* = 16.0 Hz, 1H), 6.97-6.84 (m, 2H), 6.50 (d, *J* = 16.01 Hz, 1H), 5.08 (m, 1H), 1.29 (d, 6H); ^{13}C NMR (75.4 MHz, CD_3COCD_3):δ (ppm) 167.3, 147.2, 146.8, 145.1, 141.6, 134.6, 131.8, 131.1, 130.0, 128.1, 126.3, 121.0, 119.4, 116.9, 114.6, 68.6, 22.7. HRMS-ESI: m/z [M+H]+ calcd for $C_{20}H_{20}O_4$, 325.1614; found. 325.1645.

Compound 12: 3-{4-[2-(3,4-Dihydroxy-phenyl)-vinyl]-phenyl}-acrylic acid butyl ester

Yield: 35%; mp: 188-190°C;Rf = 0.9 (hexanes:ethyl acetate, 85:15 v/v); ^{1}H NMR (CD_3COCD_3, 300MHz):δ (ppm) 8.16 (s, 1H), 8.00 (s, 1H), 7.70-7.59 (m,5H), 7.22 (d, *J* = 16.3 Hz, 1H), 7.16-6.97 (m, 3 H), 6.85 (d, *J* = 8.15 Hz, 1H) ,6.55 (d, *J* = 16.3 Hz, 1H), 4.15 (t, 2H), 1.46 (m, 2H), 1.29 (s, 2H), 0.96 (t, 3H); ^{13}C NMR (75.4 MHz, CD_3COCD_3):δ (ppm) 167.7, 147.0, 146.7, 145.2, 141.5, 134.5, 131.7, 130.9, 129.9, 127.9, 126.2, 120.8, 118.8, 116.7, 114.5, 65.0, 32.0, 20.3, 14.4. HRMS-ESI: m/z [M+H]+ calcd for $C_{21}H_{22}O_4$, 339.1781; found. 339.1794.

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[1] a) P.Y. Wen, S. Kesari, *N Engl J Med.* **2008**, *359,* 492-507; b) A. Omuro, L. M. DeAngelis, *JAMA*. **2013**, *310,* 1842-1850; c) T. F. Cloughesy, W. K. Cavenee, P. S. Mischel, *Ann Rev Pathol.* **2014**, *9*, 1-25; d) M. Garcia-castro *et al., Nat. Comm.* **2015**, *6*, 6516-6518; e) T. Cloughesy *et al*., *Science Translational Medicine.* **2016**, *8*, 341ra75. DOI:10.1126/scitranslmed.aad9784.

[2] a) E. A. Crane, K. Grademan*, Angew. Chem. Int. Ed.* **2016**, *55,* 3882-3902; b) M. Decker, *Curr. Med. Chem*. **2011**, *18*, 1464-1475; c) Y. Wu, H. Zhang, Y. Zhao, J. Zhao, J. Chen, L. Li, *Org. Lett.* **2007**, 9, 1199-1202; d) T. G. Gantchev, D. J. Hunting, *Mol. Pharmacology.* **1998**, *53,* 422-428.

[3] a) N. Sharma, D. Mohanakrishnan, A. Shard, A. Sharma, Saima, A. K. Sinha, D. Sahal, *J. Med. Chem*. **2012**, *55*, 297-311; b)L. F. Tietze, H.P. Bell, S. Chandrasekhar, *Angew. Chem. Int. Ed*. **2003**, *42*, 3996-4028; c) G. Mehta., V. Sing, *Chem. Soc. Rev.* **2002**, *31,* 324–334;d) B. Meunier, *Acc. Chem. Res*. **2008**, *41*, 69-77; e) M. N. Aminake*et al*., *Bioorg. Med. Chem*. **2012**, *20*, 5277-5289; (f) P. G. Baraldi, A. N. Zaid, D. Preti, F. Fruttarolo, M.A. Tabrizi, A. Iaconinoto, M. G. Pavani**,** M. D. Carrion, C. L. Cara, R. Romagnoli, Arkivoc **2006**, (vii) 20-34.

[4] a) V. Dalla, P. Cotelle, *Tetrahedron.* **1999**, *55***,** 6923-6930; b) J. H. C. Ho, C. Y. Hong, *J. Biomed. Sci*. **2011**, *18*, 30-34; c) P. Hu, Q.L. Liang, G.A. Luo, Z.Z. Zhao, Z. H.Jiang, *Chem. Pharm. Bull*. (Tokyo) **2005**, *53*,677-683; d) Z. Wang, P. Luo, S. Dai, Z. Liu, X. Zheng, T. Chen, *Cell Mol Neurobiol*. **2013**, *33*, 921-928; e) Q. Zhang, S. Wang, Y. Yu, S. Sun, Y. Zhang, Yu. Zhang, W. Yang, S. Li, Y. Qiao, Int. J. of Mol. Sci. **2016**, 17, 1244-1259 .

[5] *S. multihoriza*, commonly known as "Danshen" in China, rich in polyphenolic acids including Salvianolic acid F, A and B, is reported for a large number of human health benefits. This patented Chinese traditional herbal plant has successfully completed Phase II clinical trials in the United States and will soon begin Phase III investigations.

[6] V. Dalla, P. Cotelle, *Tet. Lett.* **1998**, *39*, 8285-8287.

[7] a) Jung *et al*., *Bioorg. Med. Chem. Lett.* **2007**, *17*, 4481-4483; b) A. C. Navis, M. V. Eijnden, J. T. G. Schepens, R. H. V. Huijsduijnen, P. Wesseling, W. J. A. J. Hendriks, *Acta Neuropathol.* **2010**, *119,* 157-175; c) Belley *et al*., *Bioorg. Med. Chem. Lett.* **2005,** *15*, 527-530.

[8] a) V. Calo, A. Nacci, A. Monopoli, P. Cotugno, *Angew. Chem. Int. Ed.* **2009,** *48*, 6101-6103; b) J. Petrignet, A. Boudhar, G. L. Blond, J. Suffert, *Angew. Chem. Int. Ed*. **2011**, *50*, 3285-3289; c) D. H. Wang, K. M. Engle, B. F. Shi, J. Q. Yu., *Science.* **2010**, *327*, 315-319.

[9] a) X. Zhang, A. Liu, W. Chen, *Org. Lett.* **2008,** *10*, 3849-3852; b) V. Calo, A. Nacci, A. Monopoli, P. Cotugno, *Angew. Chem. Int. Ed.* **2009**, *121*, 6217-6219.

[10] H. Kuwahara, A. Kanazawa, D. Wakamatu, S. Morimura, K. Kida, T. Akaike, H. Maeda, *J. Agric. Food. Chem.* **2004,** *52,* 4380-4387.

[11] K. C. Nicolaou, D. J. Edmonds, P. G. Bulger, *Angew. Chem. Int. Ed.* **2006**, *45*, 7134-7186.

[12] a) S. L. Schreiber,*Proc. Natl. Acad. Sci. USA* **2011***, 108,* 6699-6702; b) I. S. Young, P.S. Baran, *Nat. Chem*. **2009,** *1*, 193-205.

[13] a) R. Kumar, A. Shard, R. Bharti, Y. Thopate, A. K. Sinha, *Angew. Chem. Int. Ed*. **2012**, *51*, 2636-2639; b) A. Shard, N. Sharma, R. Bharti, S. Dadhwal, R. Kumar, A. K. Sinha, *Angew. Chem. Int. Ed.* **2012**, *51*, 12250-12253; c) N. Sharma, A. Sharma, A. Shard, R. Kumar, Saima, and A. K. Sinha *Chem. Eur. J*. **2011**, *17*, 10350-10356.

[14] N. Kumar, Richa, N. H. Andhare, A. Shard, A. K. Sinha, *Chem. Eur. J.* **2013**, 19, 14798-14803.

[15] a) X. Zhang, A. Liu, W. Chen, *Org. Lett.* **2008**, *10*, 3849-3852; b) T. N. Glasnov, W. Stadlbauer, C.O. Kappe, *J. Org. Chem.* **2005***, 13,* 3864-3870*.*

[16] a) A. K. Sinha, V. Kumar, A. Sharma, A. Sharma, R. Kumar, *Tetrahedron.* **2007**,*63*, 11070-11077; b) A. K. Sinha, B. P. Joshi, A. Sharma. U.S. Patent 6,989,467 (**2006**).

[17] a) R. B. Greenwald, C. W. Gilbert, A. Pendri, C. D. Conover, J. Xia, A. Martinez, *J. Med. Chem.* **1996**, *39*, 424-431; b) A. de Jong, J. Plat, R. P. Mensink, *The Journal of Nutritional Biochemistry.* **2003**, *14*, 362-369.

[18] a) D. Kumar, M. Sukapaka, G. D. K. Babu, Y. Padwad, *Plos One.* **2015**, e0133823; b) A. Acharya, K. Rawat, K. A. Bhat, V. Patial, Y. Padwad, *Mater. Res. Express*. **2015**, *2*, 115401-115406

[19] P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. McMohan, D. Vistica, J. T. Warren, H. Bokesch, S. Kenney, M. R. Boyd*, J. Natl. Cancer Inst.* **1990**, *82,* 1107-1112.

[20] X. Zheng, G. Shen, X. Yang, W. Liu., *Cancer Res.***2007**, *67*, 3691-3697.

[21] a) K. M. Huttunen, J. Rautio, *Curr. Top. Med. Chem.* **2011,** *11*, 2265-2287; b) A. Y. Loo, K. Jain, I. Darah, *Food Chem.* **2008**, *107*, 1151-1160; c) J. B. Harborne, P. M. Dey, *Methods in plant biochemistry: A handbook of plant phenolics* Volume 1 (Academic press Inc., CA, **1989**); d) M. Koufaki, E. Theodorou, D. Galaris, L. Nousis, E. S. Katsanou, M. N. Alexis., *J. Med. Chem.* **2006**, *49*, 300-306.

[22] a) F. Dufrasne, M. Gelbcke, J. Neve, R. Kiss , J. L. Kraus, *Curr. Med. Chem.* **2011**, *18*, 3995-4011; b) N. Hulsman, J. P. Medema, C. Boss, A. Jongejan, R. Leurs, M. J. Smit, I. J. de Esch, D. Richel, M. Wijtmans, *J. Med. Chem.* **2007**, *50*, 2424-2431.

[23] a) V. A. Fadok, D. R. Voelker, P. A. Campbell, J. J. Cohen, D. L. Bratton, P. M. Henson, *J. Immunol.* **1992**,*148*, 2207-2216; b) M. Mecha, M. L. Paula, M. Leyre, H. Miriam, B. Jose, G. Carmen, *Nature Protocol Exchange*,**2014** (www.nature.com/protocolexchange/ protocols/2051#/ reagents); c) J. C. Reed, *Oncogene*. **1998**, *17,* 3225-3236.

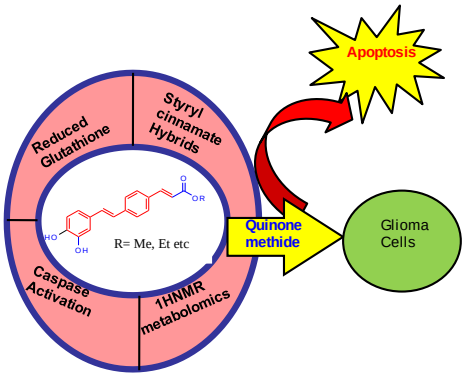
[24] a) S. J. Hur, H. W. Lee, A. H. Shin, S. J. Park, *Journal of the Korean Magnetic Resonance Society.* **2014**, *18*, 10-15R. b) Hamamoto, V. Saloura, Y. Nakamura, *Nat. Rev. Can.* **2015**, *15*, 110-124; c) A. A. Maudsley, R. K. Gupata, R. Stoyanova, N. A. Parra, B. Roy, S. Sheriff, N. Hussain, S. Behari, *Am J Neuroradiol.* **2014**, *35*, S31-36; d) I. Amelio, F. Cutruzzola, A. Antonov, M. Agostini, G. Melino, *Trends in Biochemical Sciences*. **2014**, 39, 191-198; e) M. Jain, R. Nilsson, S. Sharma, N. Madhusudhan, T. Kitami, A. L. Souza, R. Kafri, M. W. Kirschner, C. B. Clish, V. K. Mootha, *Science.* **2012**, *336*, 1040-1044; e) H. Wen , Y. J. An , W. J. Xu , K. W. Kang, S. Park, *Angew. Chem. Int. Ed. Engl.* **2015**, *127*, 1-5.

[25] S. Hoelder, P.A. Clarke, P. Workman, *Mol. Oncol.* **2012**, *6*, 155-176.

Entry for the Table of Contents

FULL PAPER

The specifically tailored catecholic styryl-cinnamates (C_6-C_2-C_6-C_3 unit), an analogue of natural salvianolic acid F designed by a novel and sustainable Perkin-Heck sequence in one pot, have enriched the diversity on ever expansive canvas of hybrid molecules with superior antagonistic profiles against glioma C6 cell line that corresponds to one of the most lethal and malignant form of brain tumors. NMR metabolomic studies of the potent hybrid molecule received after phenotypic screening revealed that these novel variants exhibited remarkable reduction in glutathione levels and also increased cleaved caspase-3 and -6 having definite role in cancer cell progression. In addition, the activation of necrotic machinery by our catecholic molecules in C6 cells is an added advantage thus helping to nip the evil in the bud and strongly obviates about its



Key Topic*

Amit Shard, Kiran Rawat, Arun K. Sinha,* Yogendra Padwad* and Dinesh Kumar

Page No. – Page No.

Title: The Natural Product Salvianolic acid F-Inspired Divergent Synthesis of Styryl-Cinnamate Hybrid Analogues as a Premise to Investigate Anticancer Activity and its Metabolomic Profiling

*one or two words that highlight the emphasis of the paper or the field of the study: Cross-coupling, Anticancer