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A NOVEL TETRAZOLE ANALOGUE OF RESVERATROL IS A POTENT ANTICANCER AGENT

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Abstract

A series of novel tetrazole analogues of resveratrol were synthesized and evaluated for their antileukemic activity against an extensive panel of human cancer cell lines and against the MV4-11 AML cell line. These molecules were designed as drug-like derivatives of the resveratrol analogue DMU-212 and its cyano derivatives. Four compounds **8g**, **8h**, **10a** and **10b** exhibited LD₅₀ values of 4.60 μ M, 0.02 μ M, 1.46 μ M, and 1.08 μ M, respectively, against MV4-11 leukemia cells. The most potent compounds, **8h** and **10b**, were also found to be active against an extensive panel of human hematological and solid tumor cell lines; compound **8h** was the most potent compound with GI₅₀ values <10 nM against more than 90% of the human cancer cell lines in the 60-cell panel. Analogs **8g**, **8h**, **10a** and **10b** were also tested for their ability to inhibit the polymerization of tubulin, and compound **8h** was found to be the most potent analogue. Molecular modeling studies demonstrated that **8h** binds to the colchicine binding site on tubulin. Thus, compound **8h** is considered to be a lead druglike molecule from this tetrazole series of compounds.

Keywords

Tetrazole analogues of resveratrol; Antileukemia activity; Anticancer activity; Tetrazolyl stilbenes; Tubulin inhibitors

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The naturally occurring *trans*-stilbene, resveratrol (**1**), is widely distributed in nature, and has received much attention since its discovery in 1939 as a constituent of the medicinal plant *Veratrum album*.^{1, 2} This likely occurred after it was shown to have cardioprotective effects as a constituent of red wine. Subsequent studies demonstrated that resveratrol had antitumor properties when applied topically to mouse skin tumors,³ and it has been shown that resveratrol has inhibitory effects on cancer initiation, promotion and progression in vitro.⁴⁻⁸ However, further studies have shown that these in vitro effects are not observed in animal and human studies,⁹ likely due to the poor bioavailability and rapid clearance of resveratrol from the systemic circulation.

The synthetic analogue, M8 (**2**) (3,3',4,4',5,5'-hexahydroxy-*trans*-stilbene) is a hyper-hydroxylated form of resveratrol that has been reported to inhibit the growth of, or induce apoptosis of, breast and colon cancer cells and leukemia, melanoma, and glioma cells.¹⁰⁻¹⁵ However, in order to improve oral bioavailability and potency of resveratrol and M8, a number of analogues have been designed that replace the phenolic hydroxy moieties with methoxy groups that can be converted in vivo to aromatic hydroxy groups via cytochrome P-450 metabolism in the liver. Such methoxylated resveratrol analogues have been shown to have improved oral bioavailability and enhanced antiproliferative effects over resveratrol via induction of apoptosis and cell cycle inhibition.^{16, 17} One such compound, DMU-212 (**3**) (*E*-3,4,5,4'-tetramethoxy stilbene) possesses enhanced apoptosis and antiproliferation activity and improved oral bioavailability in mice when compared to resveratrol and is active as an antiproliferative against a wide variety of human cancer cell types.¹⁸⁻²⁰ DMU-212 is metabolized to an active metabolite, DMU-214 (**4**), which inhibits HepG2 and breast cancer MCF cellular proliferation by inducing apoptosis and G2/M arrest through p53 and Bax/Bcl-xL upregulation.¹⁸⁻²²

A number of aromatic and heteroaromatic homologs of DMU-212 and DMU-214 have been synthesized, in which a naphthyl, quinolyl, indole, benzfuranyl or benzthiophenyl moiety has replaced one of the phenyl moieties²³⁻²⁶ in the *trans*-stilbene scaffold. Several of these resveratrol derivatives exhibited potent antiproliferation activities against a wide variety of human cancer cell lines. Such molecules have been shown to bind to the colchicine binding site on tubulin at the α/β subunit interface to inhibit tubulin polymerization. In particular, it has been shown that incorporation of a cyano group at one of the olefinic carbons of the above stilbene scaffolds affords cyanoresveratrol analogues such as **5** (cyano-DMU-214) and **6** (Fig. 1) that have nanomolar anticancer and antitubulin activity and improved binding at the colchicine binding site on tubulin²⁶⁻³⁰ when compared to their noncyano precursors. However, such compounds generally exhibit poor water solubility, which decreases their potential as clinical candidates.

Incorporation of a tetrazole moiety into a drug molecule has recently gained interest because of the favorable drug-likeness properties and metabolic stability of this structural entity.³¹⁻³³ This five membered hetero aromatic ring system is acidic in nature and is considered a bioisosteric replacement for the carboxylic acid group, since it exhibits a similar pKa value as the carboxylic acid moiety, is ionized at physiological pH and displays similar biological activity in *in vivo* studies.^{34, 35} Furthermore, tetrazole derivatives are 10 times more lipophilic than their corresponding carboxylates, which may be beneficial for improving the

concentration of drug in the systemic circulation for the desired pharmacological response.³⁶ The tetrazolyl moiety has been introduced into the structures of many modern drug entities, including anticancer, antibacterial, antiallergic, antiinflammatory, and angiotensin II antagonists, etc., and Mesenzani *et al.* have reported on tetrazolyl derivatives of the *cis*-stilbene natural product, combretastatin as antitubulin binding agents.³⁷ This study directed our attention to the design, synthesis and evaluation of the anticancer activity of tetrazolyl analogues of the *trans*-stilbene, resveratrol, which have not been previously reported.

In the present study we describe the synthesis and evaluation of novel tetrazole analogues of cyano-DMU-214 in which a tetrazolyl moiety has replaced the cyano group in **5** and **6**, and which are predicted to have improved water-solubility over their respective parent compounds. We initially compared the druglikeness characteristics (ACD/Percepta 14.1.0) for DMU-212 (**3**), DMU-214 (**4**), cyano-DMU-214 (**5**) and a proposed tetrazolyl-DMU-214 analogue **8h** (Table 1). Compounds **3-5** all showed zero violations for Lipinski compliance and lead-likeness with acceptable LogP values, predictions of high permeability in Caco-2 and CNS penetration assays, and high absorption (100%) from human intestine. However, water-solubility was predicted to be low, in the range 0.01-0.08 mg/mL. Tetrazolyl analogue **8h** (Table 1) afforded similar results as compounds **3-5** for Lipinski compliance, lead-likeness, and Caco-2 penetration/intestinal absorption assays, but was predicted to be a non-penetrant in the CNS penetration assay. Importantly, the water-solubility of **8h** was more than 1,000 times greater than the parent cyano analogue, **5**. We also determined the druglikeness of the cyano analogue **6** and its proposed tetrazole analogue **10b**. Compound **6** had one Lipinski violation and was predicted to be water insoluble (0.0002 mg/mL), whereas **10b** had no Lipinski violations and was predicted to have low water-solubility (0.06 mg/mL) with similar properties to **8h**.

We also noted that molecular modeling studies with **5** and **6** indicated that both compounds interacted at the interface of the α - and β -subunits of tubulin at the colchicine binding site, and that the cyano group in these compounds projected into an area that could easily accommodate a tetrazolyl moiety, suggesting that replacing the cyano group for a tetrazole moiety in **5** and **6** might still allow binding at the colchicine site. Thus, the rationale for the proposed studies was to determine if our previously reported cyanostilbene analogs^{26,29}, which suffer from poor drug-like properties, could be improved by replacing the cyano moiety in these molecules with a tetrazolyl moiety while still maintaining the potent antitubulin properties of the parent compound.

Based on the predictive data from our molecular modeling and druglikeness algorithm studies, we prepared two groups of tetrazolylstilbenes, **8a-8h**, and **10a** and **10b**, which were obtained by converting the nitrile group in previously reported cyanostilbenes **7a-7h**, and **9a** and **9b** into the corresponding tetrazole moiety (i.e. compounds **8a-8h** and **10a** and **10b**) to determine if this regiospecific structural change would maintain anticancer/antitubulin activity and improve druglikeness.

Results and discussion

The general procedures for the synthesis of tetrazolyl analogues **8a-8h**, and **10a** and **10b** are illustrated in Scheme 1 & 2. The synthetic strategy was based on our previous studies, which reported on a novel tributyltin azide-mediated synthesis of 1*H*-tetrazolyl-*trans*-stilbenes from cyano-*trans*-stilbenes utilizing tributyltin azide as a Lewis acid in a 1,3-dipolar [3+2] cycloaddition of azide to the cyano group of the cyanostilbene precursor.³⁵ Thus, cyanostilbenes **7a-7h** and **9a** and **9b** were individually reacted with tributyltin chloride and sodium azide in the presence of DMF at 130 °C for 12-20 h to afford the corresponding tetrazolylstilbene analogues **8a-8h**, and **10a** and **10b**, respectively, in 50-75% yield.

Anticancer activity of tetrazolylstilbene analogues **8a-8h**, and **10a** and **10b** against a panel of sixty human cancer cell lines (NCI-60 panel)

The tetrazolylstilbenes were initially evaluated for their anticancer activity against a panel of 60 human tumor cell lines at a single concentration of 10^{-5} M utilizing the procedure of Rubinstein et al.³⁸ This panel was comprised of nine subpanels of leukemia, non-small cell lung, colon, central nervous system, melanoma, ovarian, renal, prostate, and breast cancer cell lines. Growth inhibition was measured from optical density measurements of sulforhodamine B-derived color before and after 48 h exposure to test compound or control vehicle. The single-dose data reports the percent growth of the drug-treated cells relative to vehicle control (non-drug treated cells), and relative to the “time zero” number of cells. The resulting growth percent is then used to calculate both growth inhibition (values between 0 and 100) and lethality (values less than 0).

Compounds that exhibited 60% or more growth inhibition in at least eight of the cell lines at 10^{-5} M concentration were further screened at five different concentrations (i.e. 10^{-4} M, 10^{-5} M, 10^{-6} M, 10^{-7} M, and 10^{-8} M, and growth inhibition was determined as GI₅₀ values representing the molar drug concentration required to cause 50% cell growth inhibition. Single dose screening showed that only two of the tetrazolylstilbene analogues, **8h** and **10b**, exhibited more than 60% growth inhibition in at least eight cell lines from the panel of sixty cell lines (Table 2). These two compounds (**8h** and **10b**) were shown more than 60% growth inhibition or effective lethality (cancer cell killing) against NCI-H522 and NCI-H460 lung cancer cell lines, KM12 colon cancer, MDA-MB-435 and SK-MEL-5 melanoma, OVCAR-3 ovarian, A498 renal, DU-145 prostate cancer cell lines at 10 μ M (Table 2). Thus, **8h** and **10b** were further selected for a complete dose response study at five different concentrations (10^{-4} M, 10^{-5} M, 10^{-6} M, 10^{-7} M and 10^{-8} M) following 48 h of incubation with the 60 cancer cell lines in the panel.

Data from the five dose study on compounds **8h** and **10b** against the 60-human cancer cell panel are shown in Table 3, and are compared with previous data for the cyano-DMU-214 analogue **5**.²⁹ Clearly, compound **8h** was a much more effective anticancer agent than **10b**. Compound **8h** exhibited GI₅₀ values of <10 nM against 94% of the human cancer cell lines examined in the panel, and was comparable to the cyano-DMU-214 analogue **5**, which afforded GI₅₀ values of <10 nM against 87% of the cancer cell lines. Compound **10b** exhibited GI₅₀ values of <2 μ M against only 15% of the the cell lines in the panel, and

afforded nanomolar GI₅₀ values against only 3 cell lines: leukemia cell line K-562 (0.67 μ M), melanoma cell lines MDA-MB-435 (0.30 μ M) and UACC-62 (0.86 μ M).

Antileukemic activity of tetrazolyl-*trans*-stilbene analogues against MV4-11 leukemia cells

Compounds **8a-8h**, and **10a** and **10b** were screened for their antileukemia effects against MV4-11 leukemia cells in culture. The MV4-11 cell line, which was established by Rovera and associates³⁹ from the blast cells of a 10-year-old male with biphenotypic B-myelomonocytic leukemia. MV4-11 cells were seeded at 100,000 cells per well in 96 well plates. Cells were treated with various concentrations of tetrazolyl-*trans*-stilbene analogues. At 48 h post-treatment, cells were collected and stained with annexin V-fluorescein isothiocyanate (FITC) and 7-aminoactinomycin D (7-AAD) in annexin V binding buffer (10 mM HEPES, 140 mM NaCl, 2.5 mM CaCl₂). Cells were then analyzed by flow cytometry. At least 2 $\times$ 10⁴ events per condition were recorded on a BD LSR II flow cytometer. Data were analyzed using FlowJo 9.3. The viability of the treated cells (annexin V-/7-AAD-) was normalized to vehicle control. The LD₅₀ values (lethal doses) were calculated using GraphPad Prism (San Diego, CA) (Table 4 and Fig. 2) by plotting percent cell viability as a function of the concentration of the test compound and fit to a four-parameter logistic model. The data represent mean $\pm$ standard deviation of three independent experiments (Fig. 2).

Four compounds: **8g**, **8h**, **10a**, and **10b** were found to be active in the MV4-11 cell assay with LD₅₀ values of 4.60 μ M, 0.02 μ M, 1.46 μ M and 1.08 μ M, respectively (Table 4 & Fig. 2). Compound **8h** was the most potent compound in this assay with an LD₅₀ value 30 nM. Compound **8g** was approximately 170-fold less effective as an anticancer agent when compared with **8h**. Replacement of a phenyl group in the *trans*-stilbene scaffold with a 2-naphthyl moiety to afford analogues **10a** and **10b** gave LD₅₀ values 1.46 μ M and 1.08 μ M, respectively, against the MV4-11 cell line.

Tubulin binding assay results for compounds **8g**, **8h**, **10a** and **10b**

Analogues **8g**, **8h**, **10a**, and **10b** were also tested for their ability to inhibit tubulin polymerization in cell-based immunoblot assays. Briefly, polymerized tubulin in the pellet (P) and unpolymerized tubulin in the supernatant (S), after treatment (1.0 μ M and 2.0 μ M) with the above four tetrazolyl-*trans*-stilbene analogues, were evaluated by immuno-blotting using antibody against tubulin. These results are illustrated in Fig. 3. All four test compounds (**8g**, **8h**, **10a**, and **10b**) inhibited tubulin polymerization with varying potencies. Significant inhibition of tubulin exhibited with compounds **8g**, **8h**, and **10a**, at 2.0 μ M, whereas compound **10b** was significantly less active at this concentration. Compound **8h** was the more potent inhibitor of tubulin polymerization in this series of compounds.

Normal cell toxicity evaluation for lead tetrazole analogues **8h** and **10b**

The two most potent tetrazole analogues **8h** and **10b** from the MV4-11 cell assay were also evaluated for cytotoxicity against a normal stromal cell line to determine their selective

toxicity towards MV4-11 leukemia cells; the results are illustrated in Fig. 4. Both analogues afforded LD₅₀ values >50 μ M and compared to their LD₅₀ values against MV4-11 AML cells (0.02 and 1.01 μ M, respectively) were far less cytotoxic to the normal cell line.

***In silico* molecular docking studies**

Molecular modeling and docking studies were performed with compounds **4**, **5** and **8h** against full length tubulin containing α and β sub-units using Glide docking package from Schrodinger. These three compounds were chosen to determine the effect of introducing a cyano or tetrazolyl moiety into the styrene scaffold of **4** on the binding properties at the colchicine binding site, and to also determine if the tetrazolyl moiety in **8h** was functioning as a biosisostere of the cyano group in **5**. Binding energies were assessed by targeting these molecules at the colchicine binding site on the tubulin molecule. The 3-D coordinates of the tubulin structure were obtained from the RSCB protein data bank (pdb id: 1Z2B; tubulin-Vinblastine complex). Prior to the docking analysis, the tubulin structure was prepared using protein preparation wizard from Maestro (Schrodinger, Inc.)⁴⁰ with default parameters. After the protein preparation, a grid (docking box) was generated between α and β chains of the tubulin heterodimer complex (colchicine binding site).

Small molecule structures were first generated (2-D) using ChemSketch (V. 14.01). Structures were then converted to Sybyl Tripos format (3-D) using Discovery Studio (Biovia). Structures were then minimized and prepared using the LigPrep module from Maestro (Schrodinger, Inc.). Protein-ligand docking was performed using the Ligand docking module from Glide (Schrodinger, Inc.). To further predict the binding and stability of the three target compounds at the α/β interface, binding free estimates were calculated for the predicted protein-ligand complex using the Prime MM-GBSA module,⁴¹ which carries out molecular mechanics calculations using the generalized Born and surface area continuum solvation method. The Glide docking and MMGBSA indicate that molecules **5** and **8h** bind in a similar manner to the colchicine binding site and binding pose and interacting amino acid residues were similar between both molecules (Figs 5E and 5F). Replacing the cyano group with a tetrazole moiety did not alter the binding mode of these molecules. The majority of the amino acid residues interacting with **5** and **8h** were similar, including LYS326, ASN329, VAL177, ASP179, PRO325 and PRO322. Importantly, the interaction of the cyano moiety in **5** with ASN329 was also observed when the cyano moiety was replaced with a tetrazolyl moiety in structure **8h**. However, compound **5** does not interact with SER178, whereas compound **8h** does. Also, compound **5** interacts with THR221, whereas compound **8h** does not interact with this amino acid residue (Fig. 5). These subtle differences could account for the differences in activity observed between the two compounds in the *in vitro* cancer cell studies. Compound **5** exhibits three π -alkyl interactions with PRO325, PRO222, and VAL177 and demonstrates hydrogen bond interactions with LYS326, THR221, and ASN329; compound **8h** exhibits a hydrogen bond interaction with ASN329, a π -amide stack interaction with VAL177 and two π -alkyl interactions with PRO325 and PRO222. DMU-214 (**4**), on the other hand, afforded a good docking score (G score = -4.31 kcal/mol) and was predicted to bind at the interface between the α - and β -tubulin subunits (Fig. 5G), however, binding mode and interacting amino acid

residues were very different from **5** and **8h** (Fig. 5E and 5F). Although a few interacting amino acid residues were similar to those for **5** and **8h**, including ASP179, SER178, VAL353, PRO222, other amino acid interactions, i.e. GLY350 and PHE351 were unique to compound **4**. Interestingly, the binding pose of **4** differed significantly from those for **5** and **8h** (Fig. 5), which were almost superimposable. It is interesting to note that there is a slight difference in the binding free energy from MMGBSA calculations (−40.7 and −31.7 kcal/mol, respectively) for compounds **5** and **8h**, although compound **8h** exhibited greater potency as an anticancer agent than compound **5** in the *in vitro* data. This could be due to possible differences in cellular uptake kinetics and dynamics of these molecules.

Conclusions

A series of novel DMU-212 tetrazole analogues has been synthesized⁴² and evaluated for anticancer activity against a panel of NCI-60 human cancer cell lines. Compound **8h**, which is a more druglike analogue of the resveratrol derivative cyano-DMU-214 (**5**), and in which the cyano group has been replaced with a tetrazolyl moiety, was the most potent compound in the series with GI₅₀ values <10 nM against more than 90% of the solid tumor cell lines in a 60-cell human cancer cell panel. Compound **10b**, a similar tetrazolyl analogue of the naphthyl-cyanostilbene analogue **6**, was less potent than **8h**, exhibiting GI₅₀ values < 2.5 μM against more than 30% of the human cancer cell lines. Compounds **8h** and **10b** were also the most potent tetrazolyl analogues tested against the antileukemic MV4-11 leukemia cell line, with LD₅₀ values of 20 nM and 1.01 μM, respectively. Analogues **8g**, **8h**, **10a**, and **10b** were all shown to be inhibitors of tubulin polymerization in immunoblot assays, and results from molecular modeling studies were consistent with the anticancer activities of these tetrazolyl analogues of resveratrol molecules being mediated via their binding to the colchicine binding site on tubulin. Thus, regiospecific replacement of the cyano moiety in the resveratrol derivatives **5** and **6** with a tetrazole moiety maintains anticancer/antitubulin activity and improves druglikeness. Compound **8h** was a particularly potent, selective, versatile and drug-like cytotoxic agent against both hematological and solid tumor cell lines and was considered to be a lead tetrazolyl analogue of resveratrol for subsequent clinical development.

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42. **General synthetic procedure for synthesis of tetrazolyl analogues (8a-8h, 10a and 10b):** A mixture of tributyltin chloride (3 mmol) and sodium azide (3 mmol) was stirred in of dry DMF (1 ml) in a round bottom flask under argon for 20 to 30 min at room temperature, and then the appropriate precursor cyanostilbene **7a-7h**, **9a** and **9b** (1 mmol) in dry DMF was added. The mixture was heated and allowed to stir at 130 °C for 12 to 20 h. After completion of the reaction the mixture (monitored by TLC) was cooled down to room temperature, water (5 mL) was added, and stirring continued followed by addition of 1 N HCl solution to adjust the pH of the reaction mixture to 1-2, during which time the product precipitated out and was filtered off (50-75% yield). In the absence of a precipitate, the product was obtained by extracting the cooled reaction mixture with ethyl acetate, washing the organic extract with copious amounts of water, and evaporating to dryness the resulting organic liquor on a rotatory evaporator. The residue obtained was purified by column chromatography to afford the corresponding tetrazolyl analogues **8a-8h**, and **10a** and **10b**.
(Z)-5-(2-(3,5-Dimethoxyphenyl)-1-(4-methoxyphenyl)vinyl)-1H-tetrazole (8a): ¹H NMR (CDCl₃, 400 MHz): δ 7.57 (s, 1H), 7.29 (d, *J* = 8.8 Hz, 2H), 6.91 (d, *J* = 8.4 Hz, 2H), 6.35 (s, 1H), 6.03 (s, 2H), 3.77 (s, 3H), 3.58 (s, 6H); ¹³C NMR (CDCl₃, 100 MHz): δ 160.2, 159.6, 137.0, 132.5, 127.5, 114.3, 106.5, 100.3, 55.3, 55.1, 55.0, 54.8 ppm; HRMS calcd for C₁₈H₁₉N₄O₃(M+H)⁺: 339.1452, found 339.1461.
(Z)-5-(2-(3,5-Dibromophenyl)-1-(4-nitrophenyl)vinyl)-1H-tetrazole (8b): ¹H NMR (CDCl₃, 400 MHz): δ 8.26 (d, *J* = 8.8 Hz, 2H), 7.80 (s, 1H), 7.75 (s, 1H), 7.60 (d, *J* = 8.4 Hz, 2H), 7.13 (s, 2H); ¹³C NMR (CDCl₃, 100 MHz): δ 147.4, 143.8, 138.6, 135.5, 133.5, 130.6, 127.9, 125.7, 124.1, 122.4 ppm; HRMS calcd for C₁₅H₁₀N₅O₂Br₂(M+H)⁺: 449.9196, found 449.9194.
(Z)-5-(1-(3,5-Dibromophenyl)-2-(2-methoxyphenyl)vinyl)-1H-tetrazole (8c): ¹H NMR (CDCl₃, 400 MHz): δ 7.74 (s, 1H), 7.43-7.41 (m, 2H), 7.24 (s, 1H), 7.16 (s, 2H), 7.05-7.01 (m, 2H), 3.48 (s, 3H); ¹³C NMR (CDCl₃, 100 MHz): δ 149.7, 149.0, 136.2, 131.1, 130.6, 130.5, 130.3, 130.2, 128.1, 119.7, 111.6, 109.4, 55.6, 55.6 ppm; HRMS calcd for C₁₆H₁₃N₄OBr₂(M+H)⁺: 434.9451, found 434.9460.
(Z)-5-(2-(3,4-Dichlorophenyl)-1-(3,4-dimethoxyphenyl)vinyl)-1H-tetrazole (8d): ¹H NMR (CDCl₃, 400 MHz): δ 7.59 (s, 1H), 7.52 (d, *J* = 8.0 Hz, 1H), 7.23 (d, *J* = 1.2 Hz, 1H), 7.15 (d, *J* = 2.0 Hz, 1H), 6.96 (d, *J* = 8.4 Hz, 1H), 6.74 (d, *J* = 8.4 Hz, 1H), 6.54 (d, *J* = 8.0 Hz, 1H), 3.80 (s, 3H), 3.77 (s, 3H); ¹³C NMR (CDCl₃, 100 MHz): δ 149.7, 149.0, 136.2, 131.1, 130.6, 130.5, 130.3, 130.2, 128.1, 119.7, 111.6, 109.4, 55.6, 55.6 ppm; HRMS calcd for C₁₇H₁₅N₄O₂Cl₂(M+H)⁺: 377.0567, found 377.0564.
(Z)-5-(1,2-Bis(3,4,5-trimethoxyphenyl)vinyl)-1H-tetrazole (8e): ¹H NMR (CDCl₃, 400 MHz): δ 7.60 (s, 1H), 6.57 (s, 2H), 6.19 (s, 2H), 3.74 (s, 6H), 3.68 (s, 3H), 3.63 (s, 3H), 3.56 (s, 6H); ¹³C NMR (CDCl₃, 100 MHz): δ 153.9, 153.0, 152.5, 152.3, 138.0, 137.6, 134.4, 133.7, 130.4, 107.5, 106.7, 106.1, 103.7, 60.1, 60.0, 59.9, 56.1, 56.0, 55.5, 55.3 ppm; HRMS calcd for C₂₁H₂₅N₄O₆(M+H)⁺: 429.1769, found 429.1775.
(Z)-5-(1-(3,4-Dimethoxyphenyl)-2-(3,4,5-trimethoxyphenyl)vinyl)-1H-tetrazole (8f): ¹H NMR (CDCl₃, 400 MHz): δ 7.54 (s, 1H), 7.13 (s, 1H), 6.94 (d, *J* = 8.8 Hz, 1H), 6.53 (d, *J* = 8.4 Hz, 1H), 6.18 (s, 2H), 3.80 (s, 3H), 3.76 (s, 3H), 3.62 (s, 3H), 3.56 (s, 6H); ¹³C NMR (CDCl₃, 100 MHz): δ 152.5, 149.3, 148.9, 137.4, 132.5, 130.7, 119.2, 111.6, 108.9, 105.9, 60.0, 55.6, 55.5, 55.5 ppm; HRMS calcd for C₂₀H₂₃N₄O₅(M+H)⁺: 399.1663, found 399.1668.
(Z)-5-(2-(4-Methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)vinyl)-1H-tetrazole (8g): ¹H NMR (CDCl₃, 400 MHz): δ 7.48 (brs, 1H), 7.23 (d, *J* = 7.2 Hz, 2H), 6.97 (d, *J* = 7.2 Hz, 2H), 6.19 (s, 2H), 3.77 (s, 3H), 3.62 (s, 3H), 3.55 (s, 6H); ¹³C NMR (CDCl₃, 100 MHz): δ 152.5, 137.4, 132.3, 130.7, 130.6, 127.3, 123.9, 114.2, 105.9, 60.0, 55.5, 55.3 ppm; HRMS calcd for C₁₉H₂₁N₄O₄(M+H)⁺: 369.1557, found 369.1556.
(Z)-5-(2-(1H-Tetrazol-5-yl)-2-(3,4,5-trimethoxyphenyl)vinyl)-2-methoxyphenol (8h): ¹H NMR (400 MHz, DMSO-*d*₆): δ 7.48-7.50 (d, *J* = 10.8 Hz, 2H), 7.34 (s, 1H), 6.92-6.94 (d, *J* = 8.4 Hz, 1H), 6.85 (s, 2H), 3.97 (s, 3H), 3.93 (s, 6H), 3.89 (s, 3H); ¹³C NMR (100 MHz, DMSO-*d*₆): 153.5, 148.4, 145.6, 141.5, 130.5, 127.2, 122.3, 118.3, 115.1, 110.6, 109.3, 103.2, 103.1, 60.9, 56.3, 56.0 ppm; ESI (M+H)⁺: 385.
(Z)-5-(2-(4-Methoxynaphthalen-1-yl)-1-(4-nitrophenyl)vinyl)-1H-tetrazole (10a): ¹H NMR (CDCl₃, 400 MHz): δ 8.81-8.11 (m, 3H), 7.62-7.56 (m, 3H), 7.09-7.05 (m, 1H), 6.80-6.78 (m, 1H), 6.75 (s, 1H), 3.91 (s, 3H); ¹³C NMR (CDCl₃, 100 MHz): δ 155.7,

146.8, 136.8, 132.4, 127.7, 127.3, 127.2, 125.8, 124.7, 124.5, 124.3, 124.2, 123.9, 121.9, 103.9, 55.7 *ppm*, HRMS calcd for C₂₀H₁₆N₅O₃, (M+H)⁺: 374.1248, found 374.1236. **(Z)-5-(1-(3,4-Dimethoxyphenyl)-2-(naphthalen-2-yl)vinyl)-1H-tetrazole (10b)**: ¹H NMR (CDCl₃, 400 MHz): δ 7.85-7.82 (m, 1H), 7.77-7.71 (m, 3H), 7.61 (s, 1H), 7.51-7.48 (m, 2H), 7.20 (s, 1H), 6.97 (d, *J* = 8.4 Hz, 1H), 6.77 (d, *J* = 8.4 Hz, 1H), 6.59 (d, *J* = 8.8 Hz, 1H), 3.82 (s, 3H), 3.78 (s, 3H); ¹³C NMR (CDCl₃, 100 MHz): δ 149.4, 149.0, 133.0, 132.8, 132.7, 132.2, 130.9, 128.7, 127.9, 127.8, 127.5, 126.7, 126.6, 125.2, 119.5, 111.6, 109.3, 55.67, 55.6 *ppm*, HRMS calcd for C₂₁H₁₉N₄O₂, (M+H)⁺: 359.1503, found 359.1505.

Highlights

- Tetrazolylresveratrols exhibit anti-cancer activity against human cancer cells.
- Tetrazolylresveratrols are cytotoxic against MV4-11 cells but not normal cells.
- Tetrazolylresveratrols inhibit tubulin polymerization.
- Tetrazolylresveratrols interact with the colchicine binding site on tubulin.
- Lead analogue **8h** had GI₅₀ values of <10 nM in 94% of a 60-human cancer cell panel.

Figure 1:
Chemical structures of the anticancer agents: resveratrol (**1**), M8 (**2**), DMU-212 (**3**), DMU-214 (**4**), and the cyanostilbene analogues **5** and **6**.

Figure 2.
Cytotoxicity of different aromatic tetrazoles against MV4-11 leukemia cell lines

Figure 3.

In vitro tubulin binding assays for lead compounds **8g**, **8h**, **10a** and **10b**. Two different concentrations were tested for each compound; P = pellet, and S = supernatant were served as a control.

Figure 4.
Toxicity of lead tetrazole analogues **8h** and **10b** against a normal stromal cell line.

Figure 5.
Molecular docking studies of compounds **4**, **5**, and **8h**, at the colchicine binding site of α/β-tubulin

Scheme 1:

Synthesis of tetrazolylstilbene analogues **8a-8h** from precursor cyanostilbenes **7a-7h**

Scheme 2:

Synthesis of tetrazolylstilbene analogues **10a** and **10b** from precursor cyanostilbenes **9a** and **9b**

Table 1.

Physicochemical properties predicted for resveratrol analogues

Compound Structure	Lipinski Compliance	Lead-likeness	LogP	pKa	Solubility mg/ml	Caco-2 Permeability	CNS Penetration	Human Intestinal Absorption (HIA)
DMU-212 (3)	Good (0 violation)	Good (0 violation)	3.52	--	0.01	Highly permeable Pe = 244E-6 cm/s	Penetrant (-2.12)	Highly absorbed (100%)
DMU-214 (4)	Good (0 violation)	Good (0 violation)	3.16	9.3	0.08	Highly permeable Pe = 231E-6 cm/s	Penetrant (-2.09)	Highly absorbed (100%)
5	Good (0 violation)	Good (0 violation)	2.79	9.1	0.01	Highly permeable Pe = 221E-6 cm/s	Penetrant (-2.73)	Highly absorbed (100%)
8b	Good (0 violation)	Good (0 violation)	1.88	4.3	10.8	Highly permeable Pe = 5E-6 cm/s	Non-Penetrant (-4.80)	Highly absorbed (100%)
6	Good (0 violation)	Moderate (1 violation)	4.98	--	0.0002	Highly permeable Pe = 228E-6 cm/s	Penetrant (-2.99)	Highly absorbed (100%)
10b	Good (0 violation)	Good (0 violation)	4.05	9.3	0.06	Highly permeable Pe = 59E-6 cm/s	Non-Penetrant (-4.28)	Highly absorbed (100%)

Table 2.

Growth Percent^a values for nine human cancer cell lines exposed to compounds (**8f-8h**, and **10a** and **10b**) at 10 μ M

Cell line	Percentage growth inhibition				
	8f	8g	8h	10a	10b
NCI-H522 (lung)	90.18	90.33	-6.32	89.42	-2.85
NCI-H460 (lung)	105.78	110.26	2.67	102.99	14.08
KM12 (colon)	99.80	102.06	-1.73	110.52	24.53
SF-539 (CNS)	96.24	NA	-1.97	104.09	NA
MDA-MB-435 (melanoma)	102.43	100.05	-8.40	104.94	-40.96
SK-MEL-5 (melanoma)	96.38	94.10	-54.05	97.07	23.49
OVCAR-3	117.76	112.67	-8.50	112.10	-17.27
A498 (renal)	89.49	102.01	-3.50	111.68	14.49
DU-145 (prostate)	105.61	108.26	3.41	112.96	20.87

^aValues 100 mean no growth inhibition. Values between 0 and <100 represent growth percent of drug-treated cancer cells relative to vehicle control at zero time, and a negative value indicates cell lethality.

Table 3.

Antitumor activity ($GI_{50}/\mu M$)^{a,b} data for analogues **8h**, **10b**, and cyano DMU-214^c (**5**) from the five dose human cancer cell panel assay

Panel/cell line	8h $GI_{50}(\mu M)$	10b $GI_{50}(\mu M)$	5 $GI_{50}(\mu M)$
Leukemia			
CCRF-CEM	<0.01	3.33	<0.01
HL-60(TB)	<0.01	2.56	<0.01
K-562	<0.01	0.67	<0.01
MOLT-4	<0.01	4.16	<0.01
RPMI-8226	<0.01	4.02	<0.01
SR	<0.01	2.23	<0.01
Lung Cancer			
A549/ATCC	<0.01	3.80	<0.01
EKVX	NA	18.2	NA
HOP-62	<0.01	2.33	<0.01
HOP-92	<0.01	4.59	<0.01
NCI-H226	NA	>100	NA
NCI-H23	< 0.01	5.29	<0.01
NCI-H322M	NA	6.88	NA
NCI-H460	NA	2.81	NA
NCI-H522	< 0.01	1.14	<0.01
Colon Cancer			
COLO 205	0.97	2.27	2.99
HCC-2998	< 0.01	5.20	0.02
HCT-116	< 0.01	2.42	<0.01
HCT-15	< 0.01	2.92	<0.01
HT29	2.20	2.69	3.18
KM12	< 0.01	2.79	<0.01
SW-620	< 0.01	1.36	<0.01
CNS Cancer			
SF-268	< 0.01	6.55	<0.01
SF-295	5.28	1.51	0.05
SF-539	< 0.01	2.38	<0.01
SNB-19	< 0.01	7.84	<0.01
SNB-75	< 0.01	2.51	<0.01
U251	< 0.01	4.46	0.01
Melanoma			
LOX IMVI	< 0.01	5.63	<0.01
MALME-3M	NA	3.08	NA
M14	< 0.01	1.90	<0.01
MDA-MB-435	< 0.01	0.30	<0.01

Panel/cell line	8h GI ₅₀ (μ M)	10b GI ₅₀ (μ M)	5 GI ₅₀ (μ M)
SK-MEL-2	< 0.01	4.36	< 0.01
SK-MEL-28	< 0.01	8.07	1.01
SK-MEL-5	< 0.01	4.68	< 0.01
UACC-257	NA	56.6	NA
UACC-62	< 0.01	0.86	< 0.01
Ovarian Cancer			
IGROV1	< 0.01	4.23	< 0.01
OVCAR-3	< 0.01	1.69	< 0.01
OVCAR-4	< 0.01	7.78	< 0.01
OVCAR-5	NA	6.08	NA
OVCAR-8	NA	7.10	NA
NCI/ADR-RES	< 0.01	1.03	< 0.01
SK-OV-3	< 0.01	3.63	0.01
Renal Cancer			
786-0	< 0.01	5.72	< 0.01
A498	< 0.01	NA	< 0.01
ACHN	< 0.01	11.3	< 0.01
CAKI-1	NA	2.24	0.03
RXF 393	< 0.01	2.48	NA
SN12C	NA	9.97	NA
TK-10	NA	18.5	NA
UO-31	< 0.01	5.59	< 0.01
Prostate Cancer			
PC-3	< 0.01	2.36	NA
DU-145	< 0.01	3.53	NA
Breast Cancer			
MCF7	< 0.01	4.12	< 0.01
MDA-MB-231/ATCC	< 0.01	12.5	< 0.01
HS 578T	< 0.01	2.23	< 0.01
BT-549	NA	10.9	NA
T-47D	NA	28.2	NA
MDA-MB-468	< 0.01	21.5	< 0.01

NA: Not analyzed;

^a GI₅₀: concentration of drug resulting in a 50% reduction in net cell growth, as compared to cell numbers on day 0.

^b GI₅₀ values less than 10.0 and 10.0 nM were bolded.

^c Five dose NCI cancer cell screening data for compound **5** (Fig. 1) has been previously reported.²⁹

Table 4.

Cytotoxicity (LD₅₀)^a of cyanostilbene **4** and tetrazole analogues **8c**, **8g**, **8h**, **10a**, and **10b** against MV4-11 leukemia cell lines

Compound	LD ₅₀ μM (24h) ^a	LD ₅₀ μM(48h) ^a
4	-	0.04 ^b
5	-	0.04
8c	28.8	28.6
8g	4.60	4.29
8h	0.03	0.02
10a	1.46	1.39
10b	1.08	1.01

^aLD₅₀ values less than 2.0 μM are bolded;

^bMadadi et al. (2015).²⁹