Pharmacologic Activities of 3’-Hydroxypterostilbene: Cytotoxic, Anti-Oxidant, Anti-Adipogenic, Anti-Inflammatory, Histone Deacetylase and Sirtuin 1 Inhibitory Activity

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Pharmacologic Activities of 3'-Hydroxypterostilbene: Cytotoxic, Anti-Oxidant, Anti-Adipogenic, Anti-Inflammatory, Histone Deacetylase and Sirtuin 1 Inhibitory Activity

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ABSTRACT - Purpose: Delineate the selected pharmacodynamics of a naturally occurring stilbene 3'-Hydroxypterostilbene. Objective: Characterize for the first time the pharmacodynamics bioactivity in several in-vitro assays with relevant roles in heart disease, inflammation, cancer, and diabetes etiology and pathophysiology. Methods: 3’-Hydroxypterostilbene was studied in in-vitro assays to identify possible bioactivity. Results: 3'-Hydroxypterostilbene demonstrated anti-oxidant, anti-inflammatory, cytotoxic, anti-adipogenic, histone deacetylase, and sirtuin-1 inhibitory activity. Conclusions: The importance of understanding individual stilbene pharmacologic activities were delineated. Small changes in chemical structure of stilbene compounds result in significant pharmacodynamic differences.

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INTRODUCTION

Studies on natural products continue to be evaluated for their therapeutic potential. 3’-hydroxypterostilbene, C_{16}H_{16}O_{4}, MW 272.29 g/mol, is stilbene derived through the phenylpropanoid pathway that is an analogue of resveratrol, a compound very widely studied and available as a nutraceutical (Figure 1).

![Figure 1. Chemical Structure of 3'-Hydroxypterostilbene](image)

3’-Hydroxypterostilbene is structurally related to resveratrol, which is a well-studied natural product with associated health benefits. Reportedly, 3’-hydroxypterostilbene has been isolated from Sphaerophysa salsula, which is used in Chinese folk medicine to treat hypertension (1). 3’-Hydroxypterostilbene was evaluated for its effects in-vitro and in-vivo against human cancer cell lines (COLO 205, HCT-116, and HT-29) and in nude mice (2). Researchers of this study found that 3’-Hydroxypterostilbene induced apoptosis and autophagy to inhibit cell growth. Specifically, down-regulation of phosphatidylinositol 3-kinase and mitogen-activated protein kinase was observed. In the in-vivo model of the same study, researchers found that 3'-Hydroxypterostilbene was able to reduce tumor burden in nude mice bearing COLO 205 tumor xenografts. Specifically, down-regulation of cyclooxygenase-2, matrix metallopeptidase-9, vascular endothelial growth factor, and cyclin D1, and apoptosis was observed. Tolomeo et.al. evaluated 3’-Hydroxypterostilbene for its effects in sensitive and resistant leukemia cells (3). 3’-Hydroxypterostilbene was determined to be effective at inducing apoptosis in HL60

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HUT78 cells. Additionally, apoptosis was induced by 3'-Hydroxypterostilbene, in HUT78B1 and HUT78B3, Fas-ligand resistant lymphoma cell lines, and in HL60-R and K562-ADR, multi drug-resistant leukemia cell lines. In this study it appears that 3'-Hydroxypterostilbene induces apoptosis through an intrinsic apoptotic pathway with no cytotoxic effects in normal hemopoietic stem cells.

Biomedical literature appears to have only two published studies evaluating the pharmacodynamics of 3'-Hydroxypterostilbene. Both studies evaluated the anti-cancer effects in colon cancer or leukemia cells. The objective of the study was to characterize the effects of 3'-Hydroxypterostilbene in vitro. We wanted to replicate the anti-cancer effects previously reported, and evaluate 3'-hydroypterostilbene for its potential as a therapeutic for metabolic disorders. With the growing number of metabolic disorders and use of natural products, new and effective therapies are of interest. Since the underlying causes of metabolic disorders are only in part delineated, with origins in inflammation and gene dysregulation, we take a multifaceted approach to assessing the therapeutic potential of 3'-Hydroxypterostilbene. To our knowledge, this will be first study assessing the effects of 3'-Hydroxypterostilbene in cancer cell lines of the liver and prostate, anti-oxidation, anti-adipogenesis, anti-inflammation, histone deacetylase HDAC), and sirtuin 1 (SIRT1) inhibitory activities.

METHODS

Chemicals and Reagents
3'-Hydroxypterostilbene and pterostilbene were generously provided by the Sabinsa Corporation® (Piscataway, NJ, USA). Dimethyl sulfoxide (DMSO), trypsin-ethylenediaminetetraacetic acid (Trypsin-EDTA, Catalogue No. T4174), trypsin blue, sodium bicarbonate, phosphate-buffered saline magnesium and calcium free (PBS), cell culture tested sodium carbonate, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), sodium pyruvate, Dulbecco’s Modified Eagle Medium (DMEM), McCoy’s 5A Medium, Minimum Essential Medium, RPMI 1640 medium (RPMI), and penicillin-streptomycin were purchased from Sigma-Aldrich® (St Louis, Missouri, USA). Alamar Blue® was purchased from Trek (Cleveland, OH, USA). Heat-inactivated fetal bovine serum (FBS) was purchased from Equitech-Bio Inc. (Kerrville, TX, USA). TPP, Sartdet, and BD Biosciences T25 (5 mL), T75 (15 mL), and T125 (25 mL) flasks were purchased from Central Stores (Washington State University, Pullman, WA, USA) or VWR International (West Chester, PA, USA). Assay measurements using a microplate reader used a BioTek Synergy HT Multi-Mode Microplate Reader (Winooski, VT, USA) with Gen 5 software, unless otherwise indicated.

3T3-L1 fibroblasts (murine fibroblasts), HCT-116 (human colorectal carcinoma), Hep-G2 (human hepatocellular carcinoma), HT-29 (human colorectal adenocarcinoma), MDA-MB-231 (human estrogen receptor negative breast adenocarcinoma), and PC-3 (human prostate adenocarcinoma) were purchased from American Type Culture collection (ATCC, Manassa, VA, USA).

The Adipogenesis (Catalog No. 10006908), Antioxidant (Catalog No. 709001), Cyclooxygenase Inhibitor Screening (Catalog No. 560131), Histone Deacetylase (HDAC) Activity (Catalog No. 10011563), and SIRT1 Direct Fluorescent Screening (Catalog No. 10010401) Assay Kits were purchased from Cayman Chemical Company (Ann Arbor, MI, USA). Prostaglandin E2 Biotrak™ Direct Assay Kit (RPN222) was purchased from GE Healthcare Biosciences (Pittsburgh, PA, USA).

XlogP Determinations
The partition coefficient (XLogP) was experimentally investigated using the shake flask method at ambient temperature (23 ± 2°C) with spectrophotometric analysis (4-5) and by a HPLC analytical assay (6).

For the shake flask method HPLC grade water and octanol were combined in a 2 L Erlenmeyer flask to saturate for 24 hours on a plate stirrer and allowed to separate for 24 hours. 3'-Hydroxypterostilbene was evaluated at concentrations (5, 10, and 50 mg/33 mL) with varying volumes of the saturated water and octanol in proportions of 1:1, 2:1, and 1:2 (v/v). Mixtures were rotated 100 times to facilitate sufficient mixing. Samples were allowed to separate into octanol and water entities and isolated from each other. Standard curves (0-100 μg/mL) were prepared both in HPLC grade water and octanol. A reference sample of pterostilbene with a reported partition coefficient value of 3.8 (7) was also subjected to the same treatment as 3'-Hydroxypterostilbene for comparison. All samples were assessed by spectroscopy in replicate (n = 3) and assayed in replicate (n = 4), with a peak absorbance at 325 nm.
When applying the analytical assay, samples were dried to completion using a SpeedVac® (Savant SC110 A SpeedVac® Plus Concentrator with Universal Vacuum System (UVS400), Savant Instruments, Inc, Holbrook, NY, USA). Internal standard (4-methylumbelliferone, 0.10 mL) was added to the dried samples and dried to completion. Samples were resuspended in mobile phase (0.40 mL), injected (0.05 mL) into the system, and the developed analytical method applied (6). The peak area ratios were determined for the octanol and aqueous phase taking into consideration the total volume of solvents for all standards and samples (8); concentrations of unknown samples were determined using linear regression from the standard curve and the XLogP was calculated. The retention time of 3’-Hydroxypterostilbene was noted and unweighted least squares linear regression applied to determine the XLogP for the unknown (8-11).

Cytotoxic Activity

Cell Culture

All cell line culture conditions included temperature at 37°C, 5% CO₂ atmosphere. All media used to maintain cell lines were prepared using suggested specification from the manufacturer, degassed, and filtered under aseptic conditions under a laminar flow hood. HCT-116 and HT-29 were maintained in McCoy’s 5A Medium modified with 10% FBS, penicillin-streptomycin solution (10 mL/L), and HEPES (6.0 g/L). Hep-G2 was maintained in MEM with 10% FBS and penicillin-streptomycin solution (10 mL/L). MDA-MB-231 was maintained in DMEM with 10% FBS, penicillin-streptomycin solution (10 mL/L), HEPES (2.4 g/L), and sodium pyruvate (110.4 mg/L). PC-3 was maintained in RPMI modified with 10% FBS and penicillin-streptomycin solution (10 mL/L).

Cells were seeded after counting using a trypan blue assay to predetermined optimum cell density in a 96 well plate as to avoid over confluence on day one and returned to the incubator. On day two, cells were treated with fresh media with 3’-Hydroxypterostilbene yielding final concentrations 1-250 µg/mL dissolved in DMSO and returned to the incubator.

Alamar Blue Assay

On day five, cells underwent an Alamar Blue Assay (12). Briefly, treatment media was removed and replaced with 10% Alamar Blue in fresh media. Plates were returned to the incubator and brought to room temperature. This fluorescing product was quantified using a microplate reader with excitation set at 530 nm and emission set at 590 nm (Cytofluor® 4000 fluorescence multi-well plate reader, Applied Biosystems, USA). Modeling and analysis of data was carried out using WinNonlin® Version 5.2 and Microsoft® Excel. This assay is based on the ability of the live cells to reduce resazurin to resorufin thus producing a pink fluorescing product.

Anti-oxidant Determination

All reagents were prepared according to the manuscript provided with the Antioxidant Assay Kit (13). A 6-hydroxy,2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) standard was prepared at concentrations specified as this compound is a readily used marker of anti-oxidant activity. The assay as carried out to manufacturer specifications with 3’-Hydroxypterostilbene treatments at final concentrations of 1-250 µg/mL in DMSO evaluated for antioxidant capacity. The reaction was initiated with addition of hydrogen peroxide, incubated on an orbital shaker for 5 minutes at room temperature and read using a plate reader at 750 nm. Average values were calculated and graphed to the standard as absorbance versus Trolox concentration. Unknown sample anti-oxidant capacities were calculated using unweighted least squares linear regression.

Anti-adipogenic Determination

Cell Culture

3T3-L1 fibroblasts were cultured in DMEM modified with 10% at 37°C, 5% CO₂ atmosphere. Cells were seeded on day one in a 96 well plate at 30,000 cells/well and grown to 75% confluence. All reagents were prepared according to manufacture recommendations (14). 3T3-L1s were chemically differentiated from fibroblast type cells into pre-adipose cells to mature adipose cells after stimulation with isobutyl methylxanthine (IBMX), insulin, and dexamethasone (15-16). Dexamethasone is the primary adipogenic agent used in combination with IBMX and insulin have synergistic roles in adipogenesis (15, 17). These cells replicate even if not able to differentiate, thus there is a distinct difference in morphology between those cells experiencing cytotoxic effects versus those cells that are unable to differentiate due to polyphenol treatment. On day three, growth medium was changed to induction medium [DMEM modified with 10% FBS, IBMX, insulin,
dexamethasone, and 3'-Hydroxypterostilbene]. On
day six, induction medium was changed to insulin
medium [DMEM modified with 10% FBS with 3'-
Hydroxypterostilbene]. Media was refreshed on
day eight. Treatment with 3'-Hydroxypterostilbene was
carried out at concentrations of 1-250 µg/mL. On
day ten, quantification of lipid droplet was assessed
using Oil Red O. Reagents were prepared following
manufacturer instructions. After extraction of Oil
Red O from lipid droplets, quantification of lipid
accumulation was accomplished by using a
microplate at 520 nm based on the premise that a
proportional increase in lipid accumulation and
absorbance exists.

Anti-inflammatory Determination

Cyclooxygenase Inhibition
The three day COX Inhibitor Screening Assay Kit
is used to identify COX-1 and/or COX-2 inhibitors
(18). All reagents were prepared to manufacturer
specifications and carried out accordingly. 3'-
Hydroxypterostilbene was prepared over the
concentration range of 1-250 µg/mL in DMSO.
Ibuprofen and etodolac or deracoxib, NSAID and
COX-2 specific inhibitors, respectively, were used
as controls. The assay was assessed with a plate
reader at 420 nm within 10 minutes using the plate
reader. Optical density for standards and samples
were calculated with the percent bound (%B/B_0).
A %B/B_0 versus log concentration 4-parameter
logistic curve fit was used to determine the amount
of PGs in each sample.

Histone Deacetylase (HDAC) Activity
All reagents were prepared according to the
manufacturer instructions provided with the HDAC
Activity Assay Kit (20). An acetylated substrate
was incubated with 3'-hydroxypterostilbene alone
or 3'-Hydroxypterostilbene with Trichostatin A
(known HDAC inhibitor readily used as a
reference) at specific concentrations (1-100
µg/mL). If the compound of interest is a HDAC
inhibitor, the sensitized substrate decreases in
fluorescence when treated with the HDAC
developer. This assay was read at an excitation
wavelength of 360 nm and an emission wavelength
of 460 nm to quantify HDAC activity. A linear
association was established by fluorescence versus
deaetylated standard from for each sample. HDAC
activity was determined accounting for dilution, as
one unit is defined as the amount of enzyme leading
to the formation of 1.0 nmol of deacetylated
compound per minute at 37°C.

Sirtuin 1 inhibitory Activity
All reagents were prepared according to the
manuscript provided with the SIRT1 Direct
Florescent Screening Assay Kit (21). In this assay
the p53 sequence was incubated with SIRT1
(enzyme), cosubstrate (NAD\(^+\)), and 3'-
Hydroxypterostilbene (1-250 µg/mL). The
inhibitory activity was monitored using a
fluorescence-based assay to screen for inhibitors or
activators by quantification of the sensitized
daetylated substrate. The amount of deacylation
is proportional to the amount of fluorescence, which
was able to determine if the compound is an
inhibitor or an activator. The activity of the selected
compounds can be compared to sirtinol, a known
inhibitor (21) or to resveratrol, a known activator
(22). Plates were read with excitation (350 nm) and
emission (460 nm) for this fluorescence-based assay.
The average fluorescence for each sample was
determined and the percent ratio of inhibition to
activation was calculated; thus a negative number
indicated activation of SIRT1.
STATISTICAL ANALYSIS

Data were compiled and presented as mean ± standard deviation (SD) or mean ± standard error of the mean (S.E.M.) where appropriate. Where possible, WinNonlin 5.2 modeling software was used to determine statistical significance and inhibitory concentration values. A two tailed, unpaired t-test was used to compare controls to treatments. Significance was noted p-values <0.05. Generally, significance from negative controls are indicated as * p<0.05, ** p<0.01, and *** p<0.005. Significance from positive controls are indicated as # p<0.05, ## p<0.01, and ### p<0.001.

RESULTS

XlogP Determinations

Experimental values were compared to a computer generated CLogP value using New ClogP Calculator, (University of Massachusetts at Boston General Biology Website, http://intro.bio.umb.edu/111-112/OLLM/111F98/newclogp.html, Boston, MA, USA).

The reference sample, pterostilbene, has a reported partition coefficient of 3.8 and 4.1 (7, 23). The CLogP for pterostilbene that was generated has a value of 3.69. The ADMET Predictor™ Moriguchi model of predicted partition coefficient was 2.91 for pterostilbene. According to Sabinsa® from whom we have obtained 3'-Hydroxypterostilbene, a LogD value of 3.36 is reported. The CLogP generated from the computer program determined that 3'-Hydroxypterostilbene has a CLogP of 3.39 (Figure 2). The ADMET Predictor™ Moriguchi model of predicted partition coefficient generated a value of 2.88 for 3'-Hydroxypterostilbene.

A three-parameter polynomial model was used to describe standard curves and unknown concentrations were calculated for each sample. This model was statistically a significantly better fit as determined from an F-test. The partition coefficient was determined from the logarithmic ratio of the concentration of octanol to water, adjusting for dilution and volume. The octanol and methanol p-values are 1.044E-20 and 0.047, respectively. Another objective assessment to grade the models to determine which model fits the experimental data best is Akaike Information Criterion (AIC) (24). In this assessment the lower the AIC value the better. The AIC values for the linear and quadratic models were lower for the quadratic models. For the pterostilbene the average quadratic function for the standard curve for pterostilbene in octanol can be described as \( y = 7.45x^2 + 28.43x - 14.65 \) and in water can be described as \( y = -2.58x^2 + 58.63x - 18.92 \); respectively the average correlation coefficients are 0.99 and 0.99. The average quadratic function for the standard curve for 3'-Hydroxypterostilbene in octanol can be described as \( y = -4.10x^2 + 66.10x - 24.05 \) and in water can be described as \( y = -21.93x^2 + 84.61x - 24.55 \); respectively the average correlation coefficients are 0.99 and 0.99. The experimental XLogP was determined to be 3.18. Using linear regression the LogD was determined to be 2.78. Experimentally, pterostilbene has a partition coefficient of 3.57.

Through this study the developed analytical method was successful. Differences in retention times were observed between 4-methylumbelliferone (6 minutes), 3'-Hydroxypterostilbene (15 minutes) and pterostilbene (26 minutes). The partition coefficient for 4-methylumbelliferone is reported as 1.9 (25). A relationship between retention time and LogD (R^2 = 0.98) was established which can be described as retention time = 10.44 (LogD) - 13.248.

Cytotoxicity – alamar blue assay

Cell viability assays often evaluate the percentage of cell survival. No significant differences were detected from untreated human controls over the screening concentration range; therefore, anticancer activities are evaluated. 3T3-L1 was included for the lipogenesis study. No significant differences were observed. 3'-Hydroxypterostilbene had an established concentration-dependent cytotoxic effect on the cell lines evaluated (Figure 3). The IC_{50} values for HCT 116, MDA-MB-231, PC-3, and HEPG2 are 7.62 ± 0.19 µg/mL, 18.06 ± 6.8 µg/mL, 23.29 ± 8.48 µg/mL, and 8.38 ± 0.00 µg/mL, respectively.

Antioxidant Activity

Assays that can be used to measure anti-oxidant activity include chemiluminescence, cell morphology, and carbon monoxide production (26-27). The Folin-Ciocalteu assay measures total phenolics in a sample and after, has a strong
correlation with DPPH radical scavenging activity (28). Alternatively, in this study antioxidant capacity of 3'-Hydroxypterostilbene was measured by the Trolox equivalent anti-oxidant capacity assay (27). The principle behind this method is the formation of the ferryl myoglobin (oxygen carrying protein) radical from metmyoglobin (oxidized form of myoglobin) and hydrogen peroxide, which converts the phenothiazine compound ABTS to produce a radical cation (26, 29). In the presence of anti-oxidants in the sample, oxidation of ABTS to ABTS⁺ by metmyoglobin is inhibited. The resulting chromogen has a peak absorption of 645-815 nm.
Figure 2. CLogP Values. The calculated partition coefficient of (A) 3'-Hydroxypterostilbene and reference sample (B) pterostilbene using the NewClogP Calculator.

Figure 3. Cytotoxicity of 3'-Hydroxypterostilbene. HCT 116: human colorectal carcinoma; MDA-MB-231: human breast adenocarcinoma, PC-3: human prostate adenocarcinoma, HEPG2: human hepatocellular carcinoma, 3T3L1: (normal murine fibroblasts). Data is expressed as mean ± SEM, n = 4.
Figure 4. Anti-oxidant Activity of 3'-Hydroxypterostilbene in Trolox Equivalents (µg/mL). Data are presented as mean ± SD, n=2-3. Significance from negative controls are noted as ** p<0.01 and *** p<0.005. Vehicle (DMSO) only samples had a low anti-oxidant capacity (0.14 mM ± 0.006). 3'-Hydroxypterostilbene has a concentration-dependent anti-oxidant activity over the concentration range. Significant differences were observed at 50 and 100 µg/mL from controls. Treatment at 250 µg/mL approached significance (p=0.058). At 10 µg/mL or 54.16 µg/mL (0.22 mM) Trolox equivalents of 3'-Hydroxypterostilbene indicates a 5-fold greater anti-oxidant capacity than the gold standard Trolox (Figure 4). However, at higher concentrations (100 and 250 µg/mL) 3'-Hydroxypterostilbene is equal to or less than comparable levels of Trolox (100.2 and 129.88 µg/mL, respectively).

Anti-adipogenic Activity
Cytotoxicity of 3-Hydroxypterostilbene was evaluated with no significant differences detected. In this assay, the negative control (Ctl) consisted of cells chemically differentiated and maintained, and modified with the vehicle (DMSO), which was considered to represent 100% differentiation and lipid accumulation. Genistein, the positive control, is an isoflavonoid known to have anti-adipogenic properties. Genistein was able to reduce adipogenesis at all concentrations evaluated. 3'-Hydroxypterostilbene did not appear to have a well-established concentration-effect relationship over the concentration range assessed (Figure 5), although there appears to be a reduction of induced adipogenesis over all concentrations assessed. The 50 and 100 µg/mL concentrations were able to significantly reduce adipogenesis compared to negative control. There were no significant differences between genestein and 3'-Hydroxypterostilbene at comparable concentrations. Treatment at 250 µg/mL approached significance (p=0.052).

Anti-inflammatory Activity
Inflammation is the direct reaction to stimuli, in which immunomodulators such as cyclooxygenases, prostaglandins (PG), prostacyclins, thromboxanes, leukotrienes, and lipoxygenases mediate the body’s response. Immunomodulator synthesis are inhibited by non-steroidal anti-inflammatory drugs (NSAIDs), steroids, receptor level antagonists, and lipoxygenase inhibitors (33).
Figure 5. Anti-Adipogenic Activity of 3'-Hydroxypterostilbene. Data is expressed as mean ± S.E.M., n=4. Significance from negative controls are noted as * p<0.05 and ** p<0.01.

Cyclooxygenase 1 and Cyclooxygenase 2 Inhibition
Cyclooxygenases are enzymes that are involved with the synthesis of prostaglandins, thromboxanes, and prostacyclins. 3'-Hydroxypterostilbene demonstrates both COX-1 and -2 inhibitory activities in a dose independent manner (Figure 6). For COX-1 activity, 3'-Hydroxypterostilbene (1, 10, and 250 μg/mL) had p-values that approached significance (p=0.08). Ibuprofen demonstrated a concentration dependent inhibition. Statistically significant differences were observed only in COX-1 inhibition at concentration of 10 μg/mL compared to ibuprofen at 250 μg/mL. Compared to ibuprofen, 3'-Hydroxypterostilbene appeared to have better COX-1 inhibition. COX-2 inhibition after treatment with 3'-Hydroxypterostilbene approached significance at 10 and 250 μg/mL, p=0.051 and 0.054, respectively. However, 1 μg/mL treatment of 3'-hydroxypterostilbene was significantly different from controls (p=0.037). The only significant difference between the positive control (deracoxib) and 3'-hydroxypterostilbene was at 1 μg/mL (p<0.001).

In-Vitro Inflammatory Bowel Disease
3'-Hydroxypterostilbene did not appear to have a concentration-effect relationship after induction of inflammation as quantified by the synthesis of PGE2 (Figure 7). 3'-Hydroxypterostilbene did not induce inflammation nor disrupt basal levels of PGE2 synthesis to a statistically significant extent from controls. The inhibitory effects of 3'-Hydroxypterostilbene was most apparent at 1 μg/mL but was reduced at all concentrations; although not significantly different from dexamethasone. A dose dependent effect was observed after treatments of 10 μg/mL. Dexamethasone did have an effect in reducing inflammation but did not appear to be in a dose dependent manner.

Histone Deacetylase Activity
A value below the control, Trichostatin A, a known HDAC inhibitor, indicates a reduction in HDAC activity indicating HDAC inhibition. Values above this control indicate activation of HDAC activity, as an increase in HDAC activity. 3'-
Hydroxypterostilbene inhibited the activity of HDAC and reduces HDAC activity compared to control; as such a HDAC inhibitor (Figure 8). There was a significant different between control (DMSO) and 50 µg/mL.

**Figure 6.** Cyclooxygenase Inhibition by 3'-Hydroxypterostilbene. (A) COX-1 and (B) COX-2 inhibitory activity of 3'-Hydroxypterostilbene. Data are presented as a mean ± S.E.M., n = 4. Significance from negative control is noted as * p<0.05. Significance from positive control is noted as ### p<0.001.

**Figure 7.** PGE₂ Synthesis

<table>
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<th>Concentration (µg/mL)</th>
<th>Vehicle</th>
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<th>1</th>
<th>10</th>
<th>50</th>
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**Dexamethasone** □ □ □

**3'-hydroxypterostilbene** □ □ □
Figure 7. PGE$_2$ Synthesis. Amount of PGE$_2$ synthesized after no induction and induction with TNF-α and treatment with and without 3’-Hydroxypterostilbene compared to dexamethasone. Data are presented as a mean $\pm$ S.E.M., n = 4. Significance from negative controls are expressed as * $p<0.05$ and ** $p<0.01$. Significance from positive controls are expressed as # $p<0.05$.

![HDAC Activity Graph](image)

Figure 8. HDAC Activity. Data are presented as mean $\pm$ S.E.M., n=3. Significance from negative control is indicated as * $p<0.05$.

![SIRT1 Inhibitory Activity Graph](image)

Figure 9. SIRT1 Inhibition. Data are presented as a mean $\pm$ S.E.M., n = 3.
Sirtuin 1 Inhibitory Activity.
3'-Hydroxypterostilbene acted as both an activator at mid-concentrations (10 and 50 µg/mL) evaluated and also an inhibitor at the lower (1 µg/mL) and upper (100 µg/mL) concentrations evaluated (Figure 9). The change of resveratrol from an activator to an inhibitor at higher concentrations (100 and 250 µg/mL) may be due to inherent properties of resveratrol as an unstable molecule and/or problems with solubility or a complex concentration-effect relationship.

DISCUSSION
It is likely that some of the pharmacodynamic responses are directly related to the structure of 3'-Hydroxypterostilbene. The New ClogP program uses an algorithm including the chemical moieties of the compound to determine the CLogP. Using the ADMET Predictor™ Version 5.0 the Moriguchi model of predicted partition coefficient was also employed. A disadvantage to this method is that it is an endpoint measurement not a thermodynamic measurement. The XLogP was assessed through the use of three different methods and determined to be relatively comparable with the values utilizing predictive programs and from cited sources. The partition coefficient of the reference pterostilbene was 2.91-3.69 and 3'-Hydroxypterostilbene ranged from 2.88-3.39. In particular, from the experimental data a true thermodynamic octanol-water partition coefficient is determined which is a major advantage to the HPLC assay. As an alternative, a library of samples with known partition coefficients can be assayed using the developed method for 3'-Hydroxypterostilbene. The lipophilicity may have played a role in the uptake of 3'-Hydroxypterostilbene by cells for anti-adipogenic activity. It appears that 3'-Hydroxypterostilbene was most effective at inhibiting adipogenesis at 50 µg/mL. Future studies warrant a closer look at 3'-Hydroxypterostilbene along with selected polyphenols and its effect on reducing obesity.

Concentrations of 3'-Hydroxypterostilbene were comparable to genistein in reducing adipogenesis. At the two highest concentrations it is possible that solubility may have been an issue as there was a decrease in anti-adipogenic activity by 3'-Hydroxypterostilbene. The lipophilicity may have played a role in the uptake of 3'-Hydroxypterostilbene by cells for anti-adipogenic activity. It appears that 3'-Hydroxypterostilbene was most effective at inhibiting adipogenesis at 50 µg/mL. Future studies warrant a closer look at 3'-Hydroxypterostilbene along with selected polyphenols and its effect on reducing obesity.

The expression of COX varies from tissue to tissue. COX-1 is involved in normal cell functioning typically involving mucosal integrity, while COX-2 is expressed after being induced by mitogenic stimuli (phorbol esters, lipopolysaccharides, and cytokines), suggesting involvement with neoplastic and inflammatory conditions (34). Due to COX-2’s role in the biosynthesis of PGs under acute inflammatory conditions, COX-2 is the target of NSAIDs and COX-2 inhibitors. Because of various complications with current treatments, an alternative agent for COX mediated inflammation is necessary, making phytotherapeutic agents like polyphenols potentially attractive alternatives. A reduction in inflammation can be helpful in reducing certain cancers and cardiopulmonary diseases and other chronic conditions (35). In particular, it appears that 3'-Hydroxypterostilbene
was effective at inhibiting both COX-1 and -2. In future studies it would be prudent to take a closer look at the effects at concentrations lower than 1 µg/mL to establish a concentration-effect relationship. It is possible that there are structural features of 3′-Hydoxypterostilbene that competes with cyclooxygenases to reduce inflammation.

Inhibition of PGE₂ synthesis appears to be an attractive target for management of cancer and inflammation. Oxidative stress is of clinical importance not only because oxidants are common in inflammation, but also because of disruption of mitochondrial energy metabolism. This disruption can lead to a breach of the gastrointestinal barrier function, which can cause mucosal barrier permeability and lead to the initiation and/or perturbation of mucosal inflammation. Disturbance of colonocytes may also impair the ability to combat free oxygen radicals responsible for cell damage involved in colitis. Current drug therapies including 5-aminosalicyclic acid, corticosteroids, and immunomodulators, at times exacerbate the patient’s disease (36-37). These therapies and surgery can have significant side effects and morbidity. There is a current need for new and/or adjunctive treatments to effectively manage these diseases without these side effects. Vitamins and other micronutrients, including polyphenols involved in nutrient metabolism and modulation of oxidative stress (anti-oxidants), have demonstrated importance because of their protective roles in the prevention and treatment of nutrient deficiencies and in the amelioration of disease activity in individuals with IBD (38-39). Data from this study suggests that it is possible that 3′-Hydroxypterostilbene can aid in reducing colitis by reducing inflammation and help maintain lowered levels of oxidative stress.

Histone acetylases (HATs) are enzymes that acetylate histones on lysine residues. In conjunction, histone deacetylases (HDAC) are enzymes that can remove excess acetyl groups primarily histone lysine residues. If the balance of HAT and HDAC activity is left unchecked negative long term affects may result, affecting transcription by over condensing chromatin and repressing the transcription process thereby affecting normal gene expression, thus implying roles in diseases like cancer and inflammatory diseases (20, 40-42). Sirtuins are protein deacetylases dependent on nicotinamide adenine dinucleotide (NAD+) or class III HDACs but are structurally different from class I and II HDACs as the catalytic domain requires the use of NAD as a cofactor (22, 42). Sirtuin 1 (SIRT1) activity is of particular interest as there is abundant information on the roles in regulation and/or control of metabolic activity, neurodegenerative diseases, and cell proliferation (22, 43-45). In an in-vitro model, SIRT1 regulated adipocyte and triglyceride accumulation, thus attenuating adipogenesis and increased lipolysis (44).

This study serves to investigate the action of 3′-Hydroxypterostilbene on HDAC and SIRT 1 activity. It was determined that 3′-Hydroxypterostilbene acts as an inhibitor of HDAC. Stilbenes could be activators or inhibitors. In this case, 3′-Hydroxypterostilbene acted as both an activator and an inhibitor of SIRT1 depending on concentration levels. This suggests that 3′-Hydroxypterostilbene may be further evaluated for its role in maintaining energy for mitochondrial and/or metabolic diseases as an activator at lower concentrations. While at higher concentrations, 3′-Hydroxypterostilbene may be more suitable as an inhibitor in the prevention and/or treatment of cancers, cardiovascular, and pulmonary diseases through promotion of apoptosis and/or autophagy, which is in-line with previous studies from Cheng et. al.(2); where titrating to appropriate concentration would be require.

CONCLUSIONS

In summary, 3′-Hydroxypterostilbene was demonstrated to have anti-adipogenic, anti-inflammatory, anti-oxidant, HDAC, and SIRT1 inhibitory activity for the first time. Collectively, the increased anti-oxidant, anti-adipogenic activity, cell selectivity and COX-2 specificity of 3′-Hydroxypterostilbene is in support of its activity in prevention of cardiovascular and pulmonary diseases. Coupled with HDAC and SIRT1 activity, 3′-Hydroxypterostilbene demonstrates protective effects from neurodegenerative diseases and select cancers. Further pharmacodynamic investigations of 3′-Hydroxypterostilbene in inhibition of adipogenesis, inflammation, and SIRT1 are underway based on these preliminary investigations.

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CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

REFERENCES

1. Ma, ZJ; Li, X; Li, N; Wang, JH, Stilbenes from Sphaerophyta salsula. Fitoterapia 2002, 73 (4), 313-5.
2. Cheng, TC; Lai, CS; Chung, MC; Kalyanam, N; Majeed, M; Ho, CT; Ho, YS; Pan, MH, Potent anti-cancer effect of 3'-Hydroxypterostilbene in human colon xenograft tumors. PLoS One 2014, 9 (11), e111814.
3. Tolomeo, M; Grimaudo, S; Di Cristina, A; Roberti, M; Pizzirani, D; Meli, M; Dusonchet, L; Gebbia, N; Abbadessa, V; Crosta, L; Barucchello, R; Grisolia, G; Invidiata, F; Simoni, D, Pterostilbene and 3'-Hydroxypterostilbene are effective apoptosis-inducing agents in MDR and BCR-ABL-expressing leukemia cells. The international journal of biochemistry & cell biology 2005, 37 (8), 1709-26.
15. Sun, L; Nicholson, AC; Hajjar, DP; Gotto, AM, Jr.; Han, J, Adipogenic differentiating agents regulate expression of fatty acid binding protein and CD36 in the J744 macrophage cell line. Journal of lipid research 2003, 44 (10), 1877-86.
18. COX Inhibitor Screening Assay Catalog No. 560131; Cayman Chemical Company: 2007.
21. SIRT1 Direct Fluorescent Screening Assay Kit Catalog No. 10010401; Cayman Chemical Company: 2008.
25. Miyaichi, Y; Nunomura, N; Kawata, Y; Kizu, H; Tomimori, T; Watanabe, T; Takano, A; Malla, KJ, Studies on nepalese crude drugs. XXVIII. Chemical constituents of Bhote Khair, the underground parts of Esikkemukjerjea megacarpum HARA. Chemical & pharmaceutical bulletin 2006, 54 (1), 136-8.
27. Soobrattee, MA; Neergheen, VS; Luximon-Ramma, A; Aruoma, OI; Bahorun, T, Phenolics as potential antioxidant therapeutic agents: mechanism and actions. Mutation research 2005, 579 (1-2), 200-13.
32. Park, HJ; Yang, JY; Ambati, S; Della-Fera, MA; Hausman, DB; Rayalam, S; Baile, CA, Combined effects of genistein, quercetin, and resveratrol in human and 3T3-L1 adipocytes. Journal of medicinal food 2008, 11 (4), 773-83.
35. Johnston, TP; Li, Y; Jamal, AS; Stechschulte, DJ; Dileepan, KN, Poloxamer 407-induced atherosclerosis in mice appears to be due to lipid derangements and not due to its direct effects on endothelial cells and macrophages. Mediators of inflammation 2003, 12 (3), 147-55.
43. Yamamoto, H; Schoonjans, K; Auwerx, J, Sirtuin functions in health and disease. Molecular endocrinology (Baltimore, Md 2007, 21 (8), 1745-55.
45. SIRT1; Sirtis Pharmaceuticals: 2008.