In Vitro Anti-Proliferation/Cytotoxic Activity of Epingaione and its Derivatives on the Human SH-SY5Y Neuroblastoma and TE-671 Sarcoma Cells

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ABSTRACT

Epingaione (4-Methyl-1-(5-methyl-2,3,4,5-tetrahydro-[2,3']bifuranyl-5-yl)-pentan-2-one) was isolated as one of the major lipophilic secondary metabolites from the leaves and stems of Bontia daphnoides *L. The compound gave 79.24% and 50.83% anti-proliferation/cytotoxic activity on the human SH-SY5Y neuroblastoma and TE-671 sarcoma cells* in vitro *at 50 µg/mL, respectively. Epingaione was transformed into eleven derivatives under laboratory conditions using ethanol, some gave greater anti-proliferation/cytotoxic activity on the cancer cell lines tested. One of the derivatives (compound 2) with enhanced cytotoxic activity was elucidated as 5'-Ethoxy-5-methyl-5-(4-methyl-2-oxo-pentyl)-2,3,4,5-tetrahydro-5'H-[2,3']bifuranyl-2'-one. Both epingaione and compound 2 caused an accumulation of arrested or dead SH-SY5Y neuroblastoma in the m-phase of the cell cycle as revealed by the m-phase specific marker KE 67.*

Actividad Citotóxica Antiproliferativa *in vitro* de la Epingaiona y sus Derivados, Sobre el Neuroblastoma Humano SH-SY5Y y las Células del Sarcoma TE-671

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RESUMEN

La epingaiona (4-Metil-1-(5-metil-2,3,4,5-tetrahidro-[2,3']bifuranil-5-il)-pentan-2-uno) fue aislada como uno de los principales metabolitos lipofilicos secundarios de las hojas y tallos de Bontia daphnoides *L. El compuesto produjo 79.24 % y 50.83 % de actividad citotóxica/anti-proliferación sobre el neuroblastoma humano SH-SY5Y y las células del sarcoma TE-671* in vitro *a 50 µg/mL, respectivamente. La epingaiona fue transformada en once derivados en condiciones de laboratorio, utilizando etanol. Algunos produjeron mayor actividad citotóxica y antiproliferativa sobre las líneas celulares cancerosas sometidas a ensayo. Uno de los derivados (compuesto 2) de elevada actividad citotóxica fue identificado como 5'-Etoxi-5-metil-5-(4-metil-2-oxo-pentil)-2,3,4,5-tetrahidro-5'H-[2,3']bifuranil-2'-uno. Tanto la epingaiona como el compuesto 22 causaron una acumulación de neuroblastomas SH-SY5Y muertos o detenidos en la fase m del ciclo celular, según lo revela el marcador KE 67 específico de la fase m.*

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INTRODUCTION

Bontia daphnoides L is the only member of the West Indian Myoporaceae, and is known locally as; zoliv, olive bush, kidney bush and button wood. It has been used in Caribbean traditional medicine for treating nephritis, hypertension, cough and colds (1). Epingaione was isolated as one of the major secondary metabolites biosynthesized by the plant (2), with acaricidal and insecticidal activities (3, 4). Based on the well established fact that secondary metabolites are known to bind/interact with a broad spectrum of biological receptors (5), coupled with the urgent need to find new and effective anti-cancer agents, these compounds were evaluated for cytotoxic activity. In addition, significant anti-proliferation activities were found (> 90% inhibition of cellular proliferation relative to the control at 250 ppm *in vitro;* unpublished data) for the crude extracts of *B daphnoides* on the human Hep-G2 liver and MDA-MB-231 breast-oestrogen-negative tumours using the CellTiter 96^R AQ_{ueous} Non-Radioactive Cell Proliferation Assay. Thus, based on the fact that epingaione is one of the major compounds biosynthesized by *B daphnoides* it was selected for evaluation along with compound 2 and the ethanol transformed derivatives for anti-proliferation/ cytotoxic action on the human SH-SY5Y neuroblastoma and TE-671 sarcoma cell lines.

MATERIALS AND METHODS

Plant material

Leaves and stems of *Bontia daphnoides L* were collected in January 1999 from Braeton, St Catherine, Jamaica and a Voucher specimen (Accession No 34782) lodged in the Herbarium at the Faculty of Pure and Applied Sciences, The University of the West Indies, Mona Campus, Kingston 7, Jamaica, West Indies.

Preparation of crude ethanolic extract

Air dried leaves and stems weighing 500 g were pulverized and extracted with 8.0 L of ethanol (99% purity) for eight days (four days x 2). The resulting ethanolic crude extract was then concentrated *in vacuo* using a rotary evaporator to yield 30.0 g (6.0%) of a dark-green oily gum.

Isolation of Compound 1, epingaione (4-Methyl-1-(5-Methyl-2,3,4,5-tetrahydro-[2,3']bifuranyl-5-yl)-pentan-2-one) and compound 2 (5'-Ethoxy-5-methyl-5-(4-methyl-2-oxo-pentyl)-2,3,4,5-tetrahydro-5'H-[2,3']bifuranyl-2'-one

The crude residue from above was partitioned between a mixture of petroleum ether (30–50°C) and methanol-water (10:9-1; v/v; 500 mL). The petroleum ether fraction was then removed using a separatory funnel and concentrated *in vacuo* to yield 9.0 g of dark green gum from which epingaione (compound 1) was then isolated according to the method reported by Williams *et al* (4). Thus, a 5.0 g portion of the above mentioned gum was re-dissolved in petroleum ether and subjected to flash chromatograpy with a starting elution solvent system of 1.0% ethyl acetate in petroleum ether and then continued with progressively increasing proportions of ethyl acetate in petroleum ether (1.0–100%, in aliquots of 200 mL), from which 84 fractions of 20.0 mL aliquots were collected. Fractions 24–30 gave pure epingaione as a yellow oil, Rf = 0.46, while numbers 61–65 gave compound 2 as a yellow isomeric oily mixture, with Rf value of 0.22 in a solvent system of ethyl acetate in petroleum ether (30°C – 50°C) (1:4; v/v), respectively. Epingaione was revealed as a purple spot, while compound 2 gave an initial yellow colouration which turned to purple overnight (about 12 hours) when sprayed with the vanillin-H_2SO_4 reagent.

Separation of the isomers of compound 2, (5'-Ethoxy-5-methyl-5-(4-methyl-2-oxo-pentyl)2,3,4,5-tetrahydro-5'H-[2,3']bifuranyl-2'-one)

After maximal optimization of the separation conditions for the isomeric mixture of compound 2, the individual isomers were then separated from a mixture weighing 18.16 mg by semipreparative HPLC with a solvent system of 25.0% ethyl acetate in petroleum ether. The isomers were coded as *epi-2a* and *epi-2b* and eluted between 35 – 37 mins (6.63 mg, 36.5% yield) and between 41 – 46 mins (8.48 mg, 46.7% yield), respectively. Several attempts were made at crystallizing the isomers in order to determine the stereochemistry at C-5'', but without success.

Isolation of compound 3 another isomer of epingaione from the ethyl acetate fraction

The methanol-water (9:1; v/v) fraction from above was concentrated *in vacuo* to approximately 10.0 mL to which a mixture of ethyl acetate: methanol-water (10:1-9; v/v) was added. The ethyl acetate fraction was removed using a separatory funnel which was then concentrated to a yellowish-green gum weighing 11.0 g. A five-gram portion of the residue was subjected to flash chromatography from which 30 fractions of 20.0 mL were collected. Fractions, 13 – 16, 23 – 25 and 27 – 30 gave pure epingaione (Rf = 0.47, in ethyl acetate-petroleum ether (1:4;v/v)); the novel isomeric sesquiterpenoid furans (5'Ethoxy-5-methyl-5-(4-methyl-2-oxo-pentyl)-2,3,4,5-tetrahydro-5'H-[2,3']bifuranyl-2'-one) (Rf = 0.27, in ethyl acetate-petroleum ether (1:4; v/v) and compound 3 as an isomeric mixture (Rf = 0.63) in ethyl acetate-petroleum ether (1:2; v/v)), respectively.

Separation of the isomers of compound 3

Fractions 27 – 30, weighing 378 mg, was subjected to semi-preparative HPLC separation using a solvent system of 8.0% ethyl acetate in petroleum ether. This gave 77 mg of the isomeric mixture which eluted between 15–22 minutes.

The above isomers were then separated using HPLC in a solvent system of ethanol: ethyl acetate: petroleum ether (5:5:90; v/v) and coded as *epi-3a* and *epi-3b* which eluted

between 16–17 minutes (11.0 mg) and 18 minutes (12.0 mg), respectively. The elucidation of the structures of these isomers are being done in our laboratories.

Transformation and isolation of the derivatives of epingaione
Based on our comparative analyses of the ^{1}H NMR and ^{13}C data of epingaione and the two compounds (2 and 3) isolated from the extracts of *B daphnoides,* it would appear that these compounds were ethanolic transformed derivatives of epingaione. In order to justify this hypothesis, the following experiment was performed. Epingaione weighing 140.0 mg was dissolved in 14.0 mL of ethanol (99 % purity) and left under laboratory conditions (25^{o}C – 27^{o}C and 70 – 80% RH) for 81 days (Scheme 1). The mixture was monitored weekly for the formation of new compounds by Thin Layer Chromatography (TLC) in a solvent system of ethanol-petroleum ether (30^{o}C–50^{o}C) (1:4; v/v). The formation of new compounds was evident after 32 days with Rf values of 0.38 and lower. The Rf value of epingaione was 0.77. At the end of the 81-day transformation period, the mixture was then concentrated *in vacuo* to 500 µL using a rotary evaporator. Semipreparative HPLC separation was effected using a solvent system of 5.0% 2-propanol in petroleum ether (30^{o}C – 50^{o}C). The un-transformed epingaione eluted between 8.0 –14.0 mins, while the new derivatives were collected and coded as *T-epi-d-1* to *T-epi-d-11* based on their retention times. The derivatives and the time taken for their elution and weights (given in parentheses) were: *T-epi-d-1* (15 min, 6.23 mg), *T-epi-d-2* (16–17 mins, 4.71 mg), *T-epi-d-3* (18–21 mins, 18.74 mg), *T-epi-d-4* (22–23 mins, 4.64 mg), *T-epi-d-5* (24–27 mins, 10.11 mg), *T-epi-d-6* (28–30 mins, 6.06 mg), *T-epi-d-***7** (31–34 mins, 15.27 mg), *T-epi-d-8* (35 – 36 mins, 2.54 mg), *T-epi-d-9* (38–46 mins, 34.12 mg), *T-epi-d-10* (46–53 mins, 9.65 mg) and *T-epi-d-11* (54–63 mins, 6.14 mg). The major yielding fractions were assayed for anti-proliferation/cytotoxic activity on the human SH-SY5Y neuroblatsoma and TE 671 sarcoma cells.

General Chemical Experimental Procedures
Flash column and medium pressure liquid chromatographies were performed on silica gel 60 (0.03–0.07 µm mesh) using glass columns with lengths of 36.0 cm and 20.0 cm and internal diameter of 2.5 cm, respectively, at 2.0 bars. Preparative TLC was carried out on glass plates (20 x 20 cm) coated with silica gel 60 GF254 of 2.0 mm thickness. The TLC plates were visualized in UV and the resolved compounds removed and then eluted from silica using acetone and concentrated *in vacuo* using a rotary evaporator. Analytical TLC monitoring of column eluates was carried out on pre-coated silica gel 60 plates (Merck) and separations visualized by UV and vanillin-concentrated sulphuric acid (H_2SO_4) spray reagent. Analytical and semipreparative HPLC separations were performed on a Merck Hitachi 655A-12 liquid chromatograph equipped with a Shimazu SPD-2A UV/vis detector and Knauer HPLC pump K-500 operating at 2.0 bars, equipped with an ABI Applied Biosystem 785A detector, respectively. All separations were detected at 230 nm. For analytical and semipreparative separations a Diol (Lichrosorb, 250 x 5 mm, 10 µ mesh size) and silica gel columns (Lichrosorb, 250 x 16 mm of 7.0 µm mesh size), respectively, were used at a flow rate of 9.0 mL/min at a maximum pressure at 2.0 bars. All 1D (^{1}H, ^{13}C, NOE) and 2D NMRs: GHSQC (^{1}H-^{13}C correlation spectra for proton-bearing carbons); GHMQC (n $J_{c\text{-}H}$ optimized at 12 Hz in F1 for multiple bond correlations), HSQC-TOCSY (mixing time 120 ms) were recorded on Varian Unity Inova 500 MHz or 300 MHz instruments using TMS as the internal standard and $CDCl_3$ as solvent. Mass spectra (MS) were obtained on a Finnigan MAT 95 (HRFAMS) and Varian MAT 2800 (EIMS: 70 eV). The UV and CD spectra were obtained using a Hewlett-Packard 8452A diode array Spectrometer and a Jasco J-500A Spectropolarimeter at 24^{o}C, respectively. Optical rotation measurement were performed on a Perkin-Elmer 241 Polarimeter, while IR spectra were determined using a Perkin-Elmer FT-IR Paragon 1000 Spectrometer. Computer molecular mechanics modelling was performed using the MM2 force field according to the Cambridge Software package, Chem3D^{R}.

3.7. Culturing of cells
The human SH-SY5Y neuroblastoma and TE-671 sarcoma cells were cultured in plastic flasks in DMEM-HAM'S F12 medium (GIBCO) containing 10.0% fetal calf serum (FCS), tetracycline (10.0 µg/mL), penicillin (100.0 units/mL) and streptomycin (100.0 µg/mL).

3.7.1. Anti-proliferation/cytotoxic assay
Cells were seeded on 24 well plates at a density of 20 000 cells/well in 1.0 mL of the above mentioned medium and then allowed to grow for 24 hours prior to the addition of the test compounds. Cells were then cultured with serial dilutions of the different compounds ranging from 50.0 µg/mL to 12.5 µg/mL for seven and five days with the SH-SY5Y neuroblastoma and TE-671 sarcoma cells, respectively. All compounds were added to wells in 10.0 µL of DMSO to give a final DMSO concentration of 1.0% (v/v). The control wells were treated with 10.0 µL of DMSO. At the end of the cell-compound mixtures culturing periods, the total DNA content of each well was determined by means of a Fluorimetric Cyquant Cell Proliferation Assay (Molecular Probes, Eugene, OR). The assay was performed according to the instructions of the manufacturer revealing for each well a proliferation index calculated as quotient Ex480/Em520 (6). Compounds were screened in three replicates per doses and their relative anti-proliferation/cytotoxic activity expressed as mean per cent inhibition of proliferation according to the equation:

$$= \frac{\text{(mean control proliferation index) – (mean treated proliferation index)}}{\text{mean proliferation control index}} \times 100$$

IC_{50} values (concentrations of compounds required for inhibiting cellular proliferation by 50%) were determined for epingaione and the isomers of compound 2 on the basis of four concentrations which inhibited cell proliferation from 10% to 93.38% on the human SH-SY5Y neuroblastoma cells.

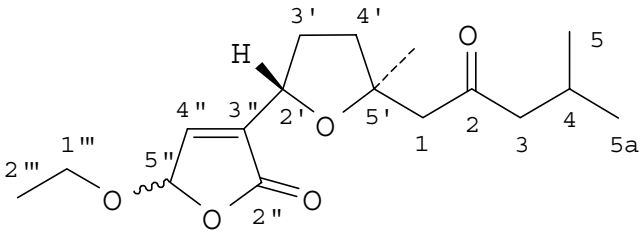
Fluorescence staining of human SH-SY5Y neuroblastoma cells with the m-phase specific marker KE 67 after treatment with epingaione and the isomers of compound 2 (epi-2a and epi-2b)

Immunostaining of the human SH-SY5Y neuroblastoma cells and staining with the m-phase specific marker KE 67 was performed according to Rosner *et al* (6) and Williams *et al* (7).

RESULTS

Structure of epingaione

Interpretation of the following spectral data: ^{1}H NMR, mass spectrum (EIMS), ^{13}C and infra red revealed that compound 1 was epingaione. EIMS (70 ev) m/z (rel Int) 250 $\mathbf{M}^+$ (5), 150 (62), 110 (100), 95 (22), 85 (75), 83 (30), 57 (84). The ^{1}H NMR data found was: (300 MHz, $CDCl_3$) δ 0.95 (6H, d, J = 6.6 Hz, Me-5, 5a-Me), 1.27 (3H, s, 5'-Me), 1.87 (2H, m H_2-3' and H_2-4'), 2.59 (1H, s, H_2-1), 2.4 (1H, s, H-4), 2.75 (2H, s, H_2-1), 4.9 (1H, m, H_2-2), 2.15 (1H, m, H-4), and 7.27 and 7.30 (2H, m, H-2'' and H-5''). ^{13}C NMR data (ppm) obtained were consistent with those reported by Chinnock *et al* (2); Hamilton *et al* (8) for epingaione, *ie* 53.70 (C-1), 209.40 (C-2), 53.70 (C-3), 24.70 (C-4), 22.44 (C-5), 22.44 (C-5a-Me), 73.51 (C-2'), 33.38 (C-3'), 37.16 (C-4'), 27.85 (C-5'-Me), 81.67 (C-5'), 143.43 (C-2''), 127.50 (C-3''), 108.72 (C-4'') and 139.34 (C-5''). The I.R. cm^{-1} (neat on NaCl disc) found were: 1680 (C=C), 1700 (C=O), and 3120, 1597 and 866 for the furanoid ring (data consistent with Williams and Caleb-Williams (3).



Compound 2

Physical data for isomer, *epi-2a* of compound 2**.**

^{1}H NMR data: (500 MHz, $CDCl_3$) δ 2.72 (1H, d, J = 15.2 Hz, H-1a), 2.62 (1H, d, J = 15.2 Hz, H-1b), 2.36 (1H, dd, J = 7.2, 16.2 Hz, H-3a), 2.32 (1H, dd, J = 6.7, 16.2 Hz, H-3b), 2.13 (1H, m, H-4), 0.92 (6H, d, J = 6.7 Hz, H_3-5, H_3-5a), 4.73 (1H, dddd, J = 0.8, 1.6, 6.8, 8.4 Hz, H-2'), 2.42 (1H, m, H-3'a), 1.86 (1H, m, H-3'b), 2.05 (1H, ddd, J = 7.1, 8.7, 12.2 Hz, H-4'a), 1.93 (1H, ddd, J = 4.4, 7.8, 12.2 Hz, H-4'b), 1.33 (3H, bs, Me-5'), 6.99 (1H, dd, J = 1.3, 1.8 Hz, H-4''), 5.86 (1H, t, J = 1.2 Hz, H-5''), 3.92 (1H, dq, J = 7.1, 9.3 Hz, H-1'''a), 3.74 (1H, dq, J = 7.1, 9.3 Hz, H-1'''b), 1.28 (3H, t, J = 7.1 Hz, H_3-2''').

EIMS (70 ev) m/z: (rel. Int.) 310 $\mathbf{M}^+$ (11), 295 (9), 268 (5), 211 (59), 166 (28), 123 (54), 95 (32), 85 (56), 57 (65), 43 (100).

HREIMS m/z: 310.1766 M^+ (calcd. for $C_{17}O_5H_{26}$, 310.1780).

^{13}C NMR data (ppm). (75 MHz, $CDCl_3$) δ 53.29 (C-1), 209.09 (C-2), 53.32 (C-3), 24.41 (C-4), 22.52 (C-5), 22.57 (C-5), 73.76 (C-2'), 31.24 (C-3'), 37.02 (C-4'), 82.46 (C-5'), 27.28 (Me-C-5'), 169.63 (C-2''), 140.40 (C-3''), 142.29 (C-4''), 101.91 (C-5''), 65.83 (C-1''') and 15.02 (C-2''').

I.R. n_{max} (cm^{-1}; KBr): 2960, 2921, 2872, 1764, 1710, 1666, 1602, 1459, 1371, 1342, 1116, 905.

$[\alpha]^{24}_D$ +11.2° (*c* 0.26, $CHCl_3$).

UV (MeCN) λ_{max} (log ε) 201 (3.88), 248 (3.49) nm.

CD (*c* 1x10^{-3}, MeCN) θ (nm) –8.95 x 10^3 (218), +6.58 x 10^3 (250), +1.68 x 10^3 (296).

Physical data for isomer, *epi-2b* of compound 2

^{1}H NMR data: (500 MHz, $CDCl_3$) δ 2.73 (1H, d, J = 15.3 Hz, H-1a), 2.63 (1H, d, J = 15.3 Hz, H-1b), 2.37 (1H, dd, J = 7.2, 16.2 Hz, H-3a), 2.32 (1H, dd, J = 6.7, 16.2 Hz, H-3b), 2.14 (1H, m, H-4), 0.92 (6H, d, J = 6.7 Hz, H_3-5, H_3-5a), 4.73 (1H, ddt, J = 1.7, 6.7, 8.4 Hz, H-2'), 2.42 (1H, m, H-3'a), 1.88 (1H, m, H-3'b), 2.06 (1H, ddd, J = 7.3, 8.7, 12.3 Hz, H-4'a), 1.95 (1H, ddd, J = 4.5, 7.6, 12.3 Hz, H-4'b), 1.35 (3H, bs, Me-5'), 6.99 (1H, t, J = 1.5 Hz, H-4''), 5.84 (1H, t, J = 1.6 Hz, H-5''), 3.95 (1H, dq, J = 7.1, 9.4 Hz, H-1'''a), 3.77 (1H, dq, J = 7.1, 9.4 Hz, H-1'''b), 1.29 (3H, t, J = 7.1 Hz, H_3-2''').

EIMS (70 ev) m/z: (rel. Int.) 310 $\mathbf{M}^+$ (11), 295 (9), 268 (5), 211 (59), 166 (28), 123 (54), 95 (32), 85 (56), 57 (65), 43 (100).

HREIMS m/z: 310.1749 M^+ (calcd. for $C_{17}O_5H_{26}$, 310.1780).

^{13}C NMR data (ppm). (75 MHz, $CDCl_3$) δ 53.24 (C-1), 208.94 (C-2), 53.51 (C-3), 24.41 (C-4), 22.53 (C-5), 22.57 (C-5), 73.54 (C-2'), 31.81 (C-3'), 37.09 (C-4'), 82.52 (C-5'), 27.26 (Me-C-5'), 169.62 (C-2''), 140.56 (C-3''), 141.76 (C-4''), 101.88 (C-5''), 66.29 (C-1''') and 15.03 (C-2''').

IR n_{max} (cm^{-1}; KBr): 2960, 2926, 2871, 1762, 1712, 1662, 1598, 1464, 1371, 1336, 1116, 905.

$[\alpha]^{24}_{D}$ -57.0° (*c* 0.27, $CHCl_3$).

UV (MeCN) λ_{max} (log *e*) 202 (3.94), 249 (3.48) nm.

CD (*c* 0.7x10^{-3}, MeCN) *q* (nm) –10.62 x 10^3 (224), -6.31 x 10^3 (246), +1.68 x 10^3 (294).

Additional remarks: ROESY correlations revealed that the relative stereochemistry of the THF-ring (1'-5') of *epi-2a* and *epi-2b* are equal.

Comparative analyses of the ^{1}H NMR and ^{13}C NMR data of the transformed derivatives revealed that *T-epi-d-3* and *T-epi-d-9* are the same as compounds 2 and 3 which were originally isolated from the ethyl acetate fraction. In addition, the ^{1}H NMR profiles obtained for the additional compounds resulting from the transformation experiments all represented derivatives of compound 2. Thus, supporting our hypothesis that compounds 2 and 3 were transformed products of epingaione. The total weights obtained for the transformed derivatives, weighing > 2.0 mg was 118.21 mg which represented a percentage transformation of 84.44% of the original epingaione after 81 days.

Anti-proliferation/cytotoxic action

From the data presented in Table 1, it is evident that epingaione and compound 2, which consisted of two isomers, *epi-2a* and *epi-2b,* and the ethanolic transformed derivatives, *T-epi*-d-3, *T-epi-d-5*, *T-epi-d-7*, *T-epi-d-9* and *T-epi-d-10* were effective inhibitors of the proliferation of both or one of the cell lines at 50.0 µg/mL and 25 µg/mL. At 25.0 µg/mL the compounds inducing > 40% inhibition of proliferation on the SH-SY5Y cells were: T-epi-d-9 (83.04 %) > *T-epi-d-7* (74.34%) > *epi-2a* (50.25%) > *T-epi-d-3* (42.38%), Table 1. However, on the TE-671 sarcoma cells the compounds inducing > 40% anti-proliferation/cytotoxic activity were: *T-epi-d-7* (87.72%) > *T-epi-d-9* (84.35%) > *T-epi-d-3* (51.58%) > *T-epi-d-10* (45.81%), Table 1. It is interesting to note that *T-epi-d-9* is an isomeric mixture with the highest anti-proliferation/cytotoxic activity on both cell lines.

When the human SH-SY5Y neuroblastoma cells were grown in the presence of 1.0 µM epingaione or the isomers of compound 2 (*epi-2a* and *epi-2b*) for two days there was an enrichment of cells synchronized in the m-phase of the cell cycle as revealed by the m-phase specific marker KE 671. The above effect was similar to that obtained for dibenzyl trisulphide (7).

Table 1: Percentage anti-proliferation/cytotoxic activity relative to the controls for epingaione, compound 2 and five of the transformed derivatives of epingaione on the human SH-SY5Y neuroblastoma and TE-671 sarcoma cells *in vitro* at 50.0 µg/mL and 25.0 µg/mL

Compounds	**Percentage anti-proliferation/cytotoxic action on the two cell lines at 50.0 µg/mL and 25.0 µg/mL**			
	SH-SY5Y neuroblastoma		**TE-671 sarcoma**	
	50.0 µg/mL	**25.0 µg/mL**	**50.0 µg/mL**	**25.0 µg/mL**
Epingaione	79.24	38.94	50.83	17.63
Compound 2				
Isomer -epi-2a	93.23	50.25	73.38	13.86
Isomer-epi-2b	75.55	27.54	60.37	28.40
Selected transformed derivatives of epingaione				
T-epi-d-3	87.71	42.38	76.14	51.58
T-epi-d-5	33.27	15.09	63.56	19.14
T-epi-d-7	93.61	74.34	84.88	87.72
T-epi-d-9	96.60	83.04	89.45	84.35
T-epi-d-10	51.29	25.12	78.85	45.81

Positive controls

1.Dibenzyl trisulphide (DTS) at 1.25µg/mL gave 90.13 % and 43.50 % anti-proliferation/cytotoxic activity on the human SH-SY5Y neuroblastoma and TE-671 sarcoma cells, respectively, *in vitro*. Patented for anti-cancer and immuno-modulatory activities.

2. Protodiocin at 10 µg/mL gave 90.22 % anti-proliferation/cytotoxic activity on the human SH-SY5Y neuroblastoma cells *in vitro*. Patented as an anti-cancer molecule.

3. Paclitaxel at 0.05 µM and 0.0325 µM gave 85.4% and 74.0% anti-proliferation/cytotoxic activity on the human SH-SY5Y neuroblastoma cells, respectively, *in vitro*.

Epingaione (compound 1) = 4-Methyl-1-(5-Methyl-2,3,4,5-tetrahydro-[2,3']bifuranyl-5-yl)-pentan-2-one.

Compound 2 = 5'-Ethoxy-5-methyl-5-(4-methyl-2-oxo-pentyl)-2,3,4,5-tetrahydro-5'H-[2,3']bifuranyl-2'-one.

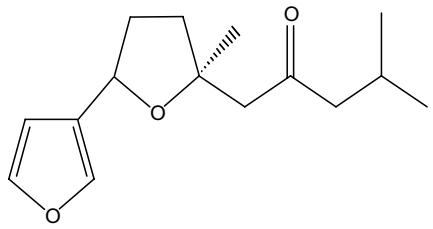
The transformed derivative; *T-epi-d-3* is equivalent to compound 2 and *T-epi-d-9* equivalent to compound 3; the structure is being elucidated in our laboratory.

IC_{50} values for epingaione = 31.0 µg/mL, epi-2a = 20.0 µg/mL and epi-2b = 39.0 µg/mL on the human SH-SY5Y cells.

DISCUSSION

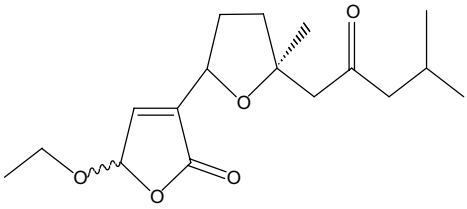
An in depth comparative analysis of the data cited in Table 1 revealed the following cell line-anti-proliferation/cytotoxic profiles of the compounds: *epi-2b*, *T-epi-d-3*, *T-epi-d-7* and *T-epi-d-9* were of comparable anti-proliferative potencies on both cell lines. Epingaione and *epi-2a* were more effective on the SH-SY5Y neuroblastoma cells while *T-epi-d-5* and *T-epi-d-10* were selective for the TE-671 sarcoma. Thus, it is evident from the above mentioned anti-proliferation/cytotoxic correlation that the structures and molecular configurations (stereochemistry) of the compounds could be important for toxicity. Thus, epingaione and its related derivative *epi-2b* were of equal toxicity on the SH-SY5Y cells at 50.0 µg/mL with inhibition values of 79.25% and 75.55%, respectively; however the isomeric counterpart *epi-2a* was significantly more potent with an inhibition value of 93.23%. Therefore, it would appear that transforming the furanoid moiety associated with C-2' in epingaione to its butenolide counterpart in compound 2 was important in governing the anti-proliferation/cytotoxic action of the compounds. In addition, it is of interest to note that the SH-SY5Y cell line was capable of detecting the single stereo-center at

C-5" which seems to differentiate the anti-proliferation/ cytotoxic selectivity between the isomers, *epi-2a* and *epi-2b* of compound 2. The other two stereo-centres at C-2' and C-5' were equivalent and consistent with that of epingaione. The TE-671 sarcoma cells were unable to reveal this stereo-selective toxicity, thus further supporting the view of Williams *et al* (9) that the SH-SY5Y neuroblatsoma cells are an effective bioassay tool for detecting natural products with anti-proliferation/cytotoxic action *in vitro*.



4-Methyl-1-(5-methyl-2,3,4,5-tetrahydro-[2,3']bifuranyl-5-yl)-pentan-2-one

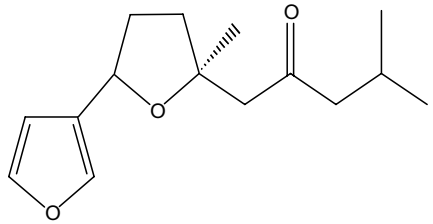
(compound 1)



5'-Ethoxy-5-methyl-5-(4-methyl-2-oxo-pentyl)-2,3,4,5-tetrahydro-5'*H*-[2,3']bifuranyl-2'-one

(compound 2)

Scheme 1



4-Methyl-1-(5-methyl-2,3,4,5-tetrahydro-[2,3']bifuranyl-5-yl)-pentan-2-one

(compound 1)

Ethanol 99% purity
25 °C–27°C
70–80% RH, 81 days

T-epi-d-1 to T-epi-d-11

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