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(54) Titre : COMPOSITION ET UTILISATION D'UN COMPOSE D'ESTER PHENETHYLIQUE DE L'ACIDE CAFEIQUE (CAPE) POUR TRAITER LE CANCER

(54) Title: COMPOSITION AND USE OF A CAFFEIC ACID PHENETHYL ESTER (CAPE) COMPOUND TO TREAT CANCER

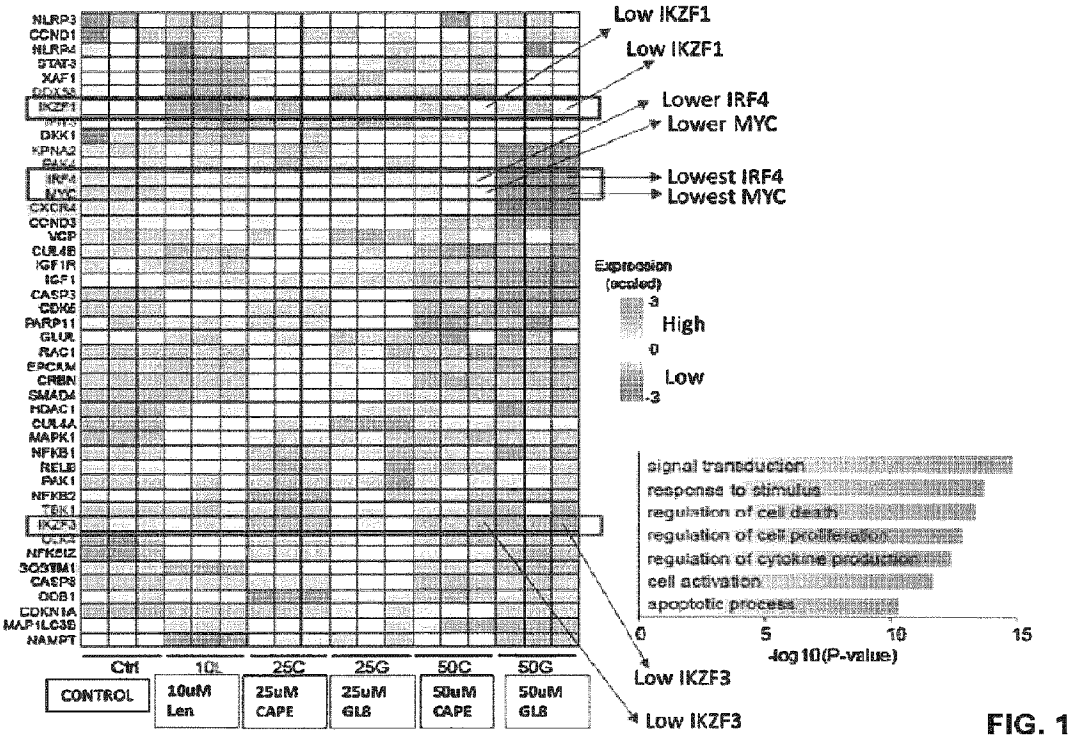

FIG. 1a

(57) Abrégé/Abstract:
Methods, compositions and uses of combination treatments for cancers are disclosed including administering to the patient a therapeutically effective amount of CAPE or CAPE analog(s), and CAPE or CAPE analog(s) in combination with a histone deacetylase inhibitor or an Immunomodulatory class of compounds or Sp1 or a MYC regulating agent, wherein administration of such combinations reduces the number or growth of cancer cells or the tumor burden or tumour growth in the patient, thereby treating the patient.

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Abstract:

Methods, compositions and uses of combination treatments for cancers are disclosed including administering to the patient a therapeutically effective amount of CAPE or CAPE analog(s), and CAPE or CAPE analog(s) in combination with a histone deacetylase inhibitor or an Immunomodulatory class of compounds or Sp1 or a MYC regulating agent, wherein administration of such combinations reduces the number or growth of cancer cells or the tumor burden or tumour growth in the patient, thereby treating the patient.

COMPOSITION AND USE OF A CAFFEIC ACID PHENETHYL ESTER (CAPE) COMPOUND TO TREAT CANCER

FIELD

The present disclosure relates to the field of cancer biology and agents and their combinations for treatment of cancer in general, and blood cancer in particular.

BACKGROUND

Rigorous scientific evaluation is in pursuit of treatments for blood cancers such as myeloma, leukemia and lymphoma. Certain caffeic acid phenethyl ester (CAPE) analogs have been found to exhibit cell growth inhibitory activity on myeloma and lymphoma cell lines that are non-responsive to lenalidomide. In addition, caffeic acid phenethyl ester has been found to decrease the level of key proteins in the cereblon pathway as disclosed in PCT Patent Application No. PCT/CA2018/000198.

Histone deacetylases (HDACs) are enzymes that remove acetyl groups from the lysine residues of histones, thereby acting as gene transcription regulators (Mitsiades et al., 2003). Since changes in histone modification is a common phenomenon in several cancers including blood cancers, HDACs remain as attractive therapeutic targets. They are generally divided into several classes including class I (HDAC1, 2, 3, and HDAC8), class II (HDAC4, 5, 6, 7, 9, and HDAC10), and class IV (HDAC11). Class III includes sirtuins, SIRT 1–7 (Subramanian et al., 2010). Many pan-HDAC inhibitors have been used for the treatment of hematologic malignancies. Vorinostat and romidepsin have gained the United States (U.S.) Food and Drug Administration (FDA) approval for the treatment of patients with refractory cutaneous T-cell lymphoma (CTCL). Combination of pan-HDAC inhibitors SAHA or panobinostat (chemical formula $C_{21}H_{23}N_3O_2$), along with proteasome inhibitor bortezomib (BTZ) have been evaluated clinically. However, side effects such as diarrhea, fatigue, nausea, thrombocytopenia, QT-interval prolongation (Subramanian et al., 2010), etc. restricts the clinical use of the combination treatments involving HDACi. In patients receiving panobinostat, the most common side effects leading to discontinuation of therapy include diarrhea, peripheral neuropathy, fatigue, thrombocytopenia, pneumonia and cardiac toxicity (Bailey, H. et al., 2015).

The Immunomodulatory drug, lenalidomide, is a less toxic and more potent derivative of thalidomide (Marriott et al., 2002) that is used in the treatment of multiple myeloma. Lenalidomide ubiquitinates IKZF1 and IKZF3, by the CRBN-CRL4 ubiquitin ligase, leading to their degradation (Lu et al., 2014; Krönke et al., 2014). IL-2 upregulation results from the downregulation of IKZF1 and IKZF3, which increases growth and activity of T and B lymphocytes (Krönke et al., 2014). Downregulation of IKZF1 and IKZF3 is necessary and sufficient for lenalidomide's cytotoxic effects. Lenalidomide can be an effective treatment for patients with relapsed or refractory multiple myeloma who are resistant to conventional chemotherapy and thalidomide treatments (Richardson et al., 2002). Although lenalidomide may rescue cytotoxic effects in many patients that do not respond to other treatment, in a phase-3 clinical study, 40% of patients with multiple myeloma who had previously undergone other forms of antimyeloma treatment did not experience a response (Dimopoulus et al., 2007). Higher incidences of deep-vein thrombosis and pulmonary embolism in lenalidomide treatment compared to placebo were observed in this study and are serious side effects of lenalidomide.

A ubiquitous zinc finger transcription factor known as the specificity protein 1 (Sp1) binds to the GC/GT rich promoter elements and regulates the gene expression of other transcription factors such as c-myc, c-Jun, Stat1, etc. (Suske, G., 1999) involved in cancer initiation and progression. Terameprocol is a semi-synthetic small molecule that acts as a transcription inhibitor by binding to the Sp1 consensus sequence and selectively reducing the transcription of genes that have promoters controlled by Sp1 (Smolewski et al., 2008). Terameprocol has been evaluated through clinical trials for treating patients with recurrent high-grade glioma (NCT02575794). In myeloma, elevation of Sp1 expression and its DNA binding activity (Näär et al., 1998) has been established; also, Sp1 has been identified as a novel therapeutic target in MM (Fulciniti et al., 2011).

Deregulation of MYC activity has been observed in several cancers, thereby contributing to disease progression, metastasis and therapeutic resistance. Direct MYC-targeted drug development has been challenging due to structural and functional challenges associated with MYC. A few potential small molecule MYC inhibitors such as MYCi361 and related analogs have recently been evaluated (Han

et al., 2019). However, small molecule inhibitors that induce epigenetic silencing, disrupt the DNA binding ability of MYC, and target proteins that modulate post-translational regulation have been employed to target MYC. Some efficacy against MYC by the inhibitors of histone deacetylases, histone methyltransferases, histone demethylases, and DNA methyltransferases, including bromodomain and extra-terminal motif (BET) bromodomains have been well documented (Allen-Petersen et al., 2019). The BET inhibitor, JQ1, has been shown to inhibit BRD4 binding at acetylated histones within the MYC promoter and enhancers, decreasing expression of MYC isoforms (Kato et al., 2016). Several BET inhibitors are being assessed, however the clinical responses have been limited, resulting in relapse (Doroshow et al., 2017).

Better treatments for cancers and blood cancers would be desirable.

SUMMARY OF THE DISCLOSURE

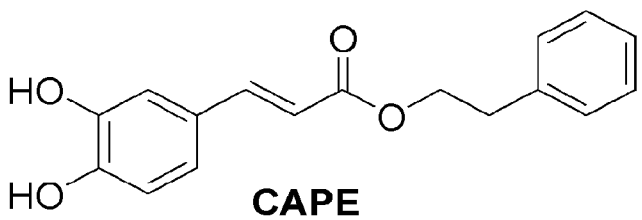
The present inventors, in one aspect of the present disclosure, demonstrate in this disclosure that CAPE and its closely related analogs downregulate a cancer gene or protein target expression, wherein administration of CAPE or a CAPE analog reduces the number or growth of cancer cells or the tumor burden or tumour growth in the patient, thereby treating the patient. Upon downregulation, the levels of a target protein or gene expression is reduced whereby the function of the target protein or gene is impaired.

In another aspect, the present disclosure relates to a method for treating a patient with cancer, the method including administering to the patient a therapeutically effective amount of a compound selected from the group consisting of CAPE or a CAPE analog in combination with a downregulating agent.

The present inventors, in one aspect of the present disclosure, demonstrate in this disclosure that CAPE and its closely related analogs (i) lower histone deacetylase, particularly HDAC1 expression at both RNA and protein levels; (ii) decrease expression of cereblon pathway genes/proteins ikaros (IKZF1), aiolos (IKZF3), IRF4, and another important gene/protein target, namely MYC; and iii) decrease expression of the specificity protein 1(Sp1).

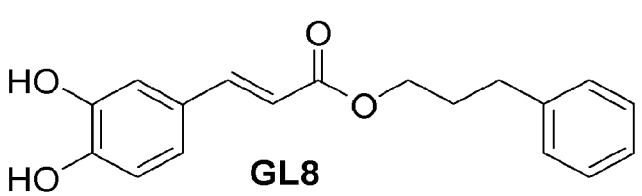
More specifically, in another aspect of the present disclosure, the data presented in this disclosure show that combining HDAC inhibitors and/or IMiDs and/or MYC targeting agents and/or Sp1 inhibitors with CAPE or potent CAPE analogs can serve as better treatment for blood cancers.

Accordingly, in one aspect, the present disclosure relates to a method for treating a patient with cancer, the method including administering to the patient a therapeutically effective amount of CAPE, having the formula:



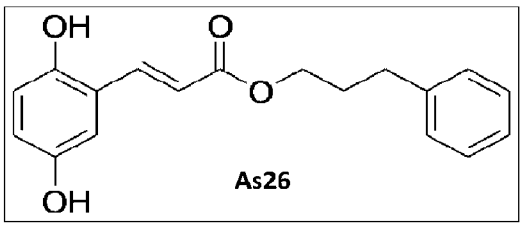
CAPE

or a CAPE analog, in combination with a histone deacetylase inhibitor, wherein administration of CAPE or CAPE analog in combination with the histone deacetylase inhibitor reduces the number of cancer cells or the tumor burden in the patient, thereby treating the patient. Also encompassed herein is a therapeutically effective amount of CAPE or CAPE analog for use in combination with a pan-histone deacetylase inhibitor (pan-HDACi) for treating a patient with cancer, wherein administration of CAPE or CAPE analog in combination with the histone deacetylase inhibitor reduces the number of cancer cells or the tumor burden in the patient, thereby treating the patient. In a particular aspect of the method or use, the HDAC is HDAC1. In a particular aspect of the method or use, the cancer is a blood cancer. In a particular aspect of the method or use, the blood cancer is myeloma, leukemia or lymphoma. In a more particular aspect of the method or use, the CAPE analog is an analog (also referred to by the present inventors as "GL8") according to the formula:



GL8

In another more particular aspect of the method or use, the CAPE analog is an analog (also referred to by the present inventors as As26) according to the formula:



As26

or a pharmaceutically acceptable salt thereof.

Exemplary HDACis include, without limitation, panobinostat, trichostatin A (chemical formula $C_{17}H_{22}N_2O_3$), entinostat (chemical formula $C_{21}H_{20}N_4O_3$), ricolinostat (chemical formula $C_{24}H_{27}N_5O_3$), romidepsin (chemical formula $C_{24}H_{36}N_4O_6S_2$), vorinostat (chemical formula $C_{14}H_{20}N2O_3$), belinostat (chemical formula $C_{15}H_{14}N_2O_4S$), and LAQ824

In another aspect, the present disclosure relates to compositions for treating cancer including CAPE or CAPE analog in combination with a histone deacetylase inhibitor panobinostat.

In another aspect, the present disclosure relates to compositions for treating cancer including CAPE or CAPE analog in combination with a histone deacetylase inhibitor trichostatin A.

In another aspect, the present disclosure relates to a method for treating a patient with cancer, the method including administering to the patient a therapeutically effective amount of CAPE or CAPE analog in combination with an Sp1 inhibitor, wherein administration of CAPE or CAPE analog in combination with the Sp1 inhibitor reduces the number of cancer cells or the tumor burden in the patient, thereby treating the patient. In one aspect, the cancer is blood cancer. In another aspect, the blood cancer is selected from the group consisting of myeloma, leukemia or lymphoma. In another aspect, the cancer is brain cancer or breast cancer. In another aspect, the compound is CAPE. In another aspect, the CAPE analog is GL8. In another aspect, the CAPE analog is As26.

In another aspect, the present disclosure relates to a method for treating a patient with cancer, the method including administering to the patient a therapeutically effective amount of CAPE or CAPE analog in combination with an MYC regulating agent, wherein administration of CAPE or CAPE analog in combination with the MYC regulating agent reduces the number of cancer cells or the tumor burden in the patient, thereby treating the patient. In one aspect, the cancer is blood cancer. In another aspect, the blood cancer is selected from the group consisting of myeloma, leukemia or lymphoma. In another aspect, the compound is CAPE. In another aspect, the CAPE analog is GL8. In another aspect, the CAPE analog is As26. In another aspect, the MYC regulating agent is a BET inhibitor. In another aspect, the BET inhibitor is a small molecule inhibitor JQ1. In another aspect, the small molecule inhibitor is MYCi361 and related analogs.

In another aspect, the present disclosure relates to a composition for treating a patient with cancer including a therapeutically effective amount of CAPE or CAPE analog in combination with a therapeutically effective amount of histone deacetylase (HDAC) inhibitor. In one aspect, the HDAC is HDAC1. In another aspect, the cancer is blood cancer. In another aspect, the blood cancer is selected from the group consisting of myeloma, leukemia or lymphoma. In another aspect, the compound is CAPE. In another aspect, the CAPE analog is GL8. In another aspect, the GL8 analog is As26. In another aspect, the HDAC inhibitor is a pan-HDAC inhibitor. In another aspect, the HDAC inhibitor is panobinostat. In another aspect, the HDAC inhibitor is trichostatin A.

In another aspect, the present disclosure relates to a composition for treating a patient with cancer including a therapeutically effective amount of CAPE or CAPE analog in combination with a therapeutically effective amount of an immunomodulatory class of compounds (IMIDs). In one aspect, the IMID is lenalidomide or pomolidomide. In another aspect, the cancer is blood cancer. In another aspect, the blood cancer is selected from the group consisting of myeloma, leukemia or lymphoma. In another aspect, the compound is CAPE. In another aspect, the CAPE analog is GL8. In another aspect, the CAPE analog is As26.

In another aspect, the present disclosure relates to a composition for treating a patient with cancer including a therapeutically effective amount of CAPE or CAPE

analog in combination with a therapeutically effective amount of an a Sp1 inhibitor. In another aspect, the cancer is blood cancer. In another aspect, the blood cancer is selected from the group consisting of myeloma, leukemia or lymphoma. In another aspect, the compound is CAPE. In another aspect, the CAPE analog is GL8. In another aspect, the GL8 analog is As26.

In another aspect, the present disclosure relates to a composition for treating a patient with cancer including a therapeutically effective amount of CAPE or CAPE analog in combination with a therapeutically effective amount of a MYC regulating agent. In another aspect, the cancer is blood cancer. In another aspect, the blood cancer is selected from the group consisting of myeloma, leukemia or lymphoma. In another aspect, the compound is CAPE. In another aspect, the CAPE analog is GL8. In another aspect, the CAPE analog is As26. In another aspect, the MYC regulating agent is a BET inhibitor. In a further aspect, the BET inhibitor is a small molecule inhibitor JQ1. In another aspect, the small molecule inhibitor is MYCi361 and related analogs.

In certain embodiments, the composition is a pharmaceutical composition. In certain embodiments, the composition is a dietary supplement. In certain embodiments, the composition includes a carrier. In certain other embodiments, the carrier is a pharmaceutically acceptable carrier.

In certain aspects of the present invention, pharmaceutically acceptable compositions are provided, wherein these compositions comprise any of the compounds or a pharmaceutically acceptable salt thereof, as described herein, and optionally comprise a pharmaceutically acceptable carrier, adjuvant or vehicle. In certain embodiments, these compositions optionally further comprise one or more additional therapeutic agents.

It will also be appreciated that certain of the compounds of the present invention can exist in free form for treatment, or where appropriate, as a pharmaceutically acceptable derivative or a prodrug thereof. According to the present invention, a pharmaceutically acceptable derivative or a prodrug includes, but is not limited to, pharmaceutically acceptable salts, esters, salts of such esters, or any other adduct or derivative which upon administration to a patient in need thereof is capable of

providing, directly or indirectly, a compound as otherwise described herein, or a metabolite or residue thereof.

As used herein, the term "pharmaceutically acceptable salt" refers to those salts which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and lower animals without undue toxicity, irritation, allergic response and the like, and are commensurate with a reasonable benefit/risk ratio. A "pharmaceutically acceptable salt" means any non-toxic salt or salt of an ester of a compound of this invention that, upon administration to a recipient, is capable of providing, either directly or indirectly, a compound of this invention or an inhibitorily active metabolite or residue thereof.

In certain aspects of the present disclosure, pharmaceutically acceptable compositions of the present disclosure can be administered to humans and other animals at a unit dose within the range 0.01-2000 mg/kg, particularly 2.5-1000 mg/kg, particularly 5-500 mg/kg, particularly 0.01-100 mg/kg, particularly 0.5-50 mg/kg, and particularly 0.4-2mg/kg, and particularly 10 mg/kg, 15 mg/kg and 20 mg/kg and this should provide a therapeutically effective dose. However, the daily dose will necessarily be varied depending upon the host treated, the particular route of administration, and the severity of the illness being treated. Accordingly, the optimum dosage may be determined by the practitioner who is treating any particular patient.

BRIEF DESCRIPTION OF THE DRAWINGS

For the purpose of illustrating the invention, the drawings show aspects of one or more embodiments of the invention. However, it should be understood that the present invention is not limited to the precise arrangements and instrumentalities shown in the drawings, wherein:

FIG. 1a and Fig. 1b are heatmap images of RNA sequencing results;

FIG. 1c is a graph showing quantification of HDAC1gene expression;

FIG. 2a and FIG. 2b are Western blots of key protein targets HDAC1, Sp1, IRF4, IKZF1 and c-MYC lowered by CAPE and its closely related analogs in comparison with the HDAC inhibitor (panobinostat) in a human myeloma cell line;

FIG. 3 is a graph showing the treatment effect of panobinostat and IMiDs (Pomalidomide or Lenalidomide) in comparison with panobinostat and CAPE analogs treatment;

FIG. 3a is a graph showing combinatorial effect of As26 with HDACi panobinostat;

FIG. 4 is a graph showing the effect of another pan-HDAC inhibitor, Trichostatin A, and potent CAPE analog As26 combination;

FIG. 5 is a graph revealing the effect of the Sp1 inhibitor TMP and potent CAPE analog As26 combination;

FIG. 6 is a Western blot of a combination treatment effect of a combination of the Sp1 inhibitor Terameprocol (TMP) and the potent CAPE analog As26 on Sp1 and IRF4 protein level; and

FIG. 6a is a Western blot of a 100uM TMP effect and IKZF1 expression.

DETAILED DESCRIPTION

Experimental Section

Cell Culture. Human myeloma cell lines JJN3 were purchased from ATCC, KMM-1 was procured from the Japanese Collection of Research Bioresources Cell Bank (JCBR, Japan). The MM1.R cell line was a kind gift from Dr. Jonathan Keats, Translational Genomics Research Institute (TGen, USA). JJN3 cells were grown in RPMI-1640 medium (Sigma Aldrich, USA) supplemented with 5% heat inactivated FBS (Gibco), 1% l-glutamine (Gibco), 5 mM HEPES, and 1% Penstrep (Sigma Aldrich, USA); KMM-1 cells in RPMI-1640 media supplemented with 10% FBS, 1% L-glutamine (Gibco) and 1% Penstrep; MM1.R cells in advanced RPMI-1640 medium with 4% FBS and 1% Glutamax, in a humidified 5% CO_2 incubator at 37 °C. Cell lines were tested regularly for mycoplasma and kept free of contamination. Lenalidomide, terameprocol (Sigma Aldrich, USA) and pomalidomide from Selleck chemicals were used.

CAPE (2) Analogs Synthesis. Analogs of CAPE were synthesized, as previously described (Sanderson et al., 2013, Selka et al., 2019). A purity of >95% has been established for all tested compounds.

Cell Growth Inhibition Studies. A 48-h cell growth inhibition assay was conducted using the PrestoBlue cell viability kit (Invitrogen, ON, Canada). Cells were plated at 10,000 cells/well in 96-well tissue culture plates. The effect of single inhibitor and inhibitor combination treatments were done using appropriate vehicle and medium (blank) controls. Cells were incubated at 37 °C in a 5% CO_2 incubator for 48 h or 72 h depending on the chosen duration of the study. At the end of the treatment, cell growth inhibition was determined by the PrestoBlue assay. In brief, 10% PrestoBlue reagent was added to untreated and treated cells in 100 μL medium in each well and incubated at 37 °C for one and a half hours. Cell growth was assessed fluorometrically at 37 ºC using the BioTek Synergy H4 Hybrid Multi-Mode microplate reader (535 nm excitation; 615 nm emission) with Gen 2.06 analyses software. Fluorescence values were corrected for any background fluorescence of the medium and test fractions by subtracting fluorescence readings of the appropriate blanks from the mean fluorescence readings of the untreated control and test wells. The percentage inhibition of cell growth was then defined as 1 − (mean test well fluorescence/mean control well fluorescence) x100.

Western/Immunoblotting Methods. Protein lysates were obtained from untreated and CAPE (**2**) analog-treated myeloma cells and the concentration of proteins was determined by the BCA method. Proteins were resolved in a 4-20% criterion gel followed by electrophoretic transfer to nitrocellulose membrane (Bio-Rad Laboratories Inc., Germany). Membranes were then blocked with 5% skimmed milk in tris-buffered saline-tween (TBS-T) for 1 h and then, after rapid TBS-T washes, incubated overnight at 4 °C with a primary antibody of interest. Goat polyclonal anti-IRF4; rabbit polyclonal anti-Ikaros (Santa Cruz Biotechnology, Santa Cruz, CA); mouse monoclonal anti-Caspase-8, rabbit anti-Caspase-3, cleaved Caspase-3, anti-Sp1 (Cell Signaling Technology, Whitby, ON), rabbit monoclonal anti-MYC and mouse monoclonal β-actin antibody (Abcam, Cambridge, MA) were the primary antibodies used for this study. Following this, the membrane was incubated with HRP conjugated secondary antibodies. Protein levels in immunoblots were detected with

an Amersham™ ECL™ Prime western blotting detection kit (GE Healthcare, ON, Canada), the imaging was performed using the ChemiDoc™ MP imaging system (Bio-Rad Laboratories, USA) and densitometry analyses were done using the Image Lab 5.0 software.

RNA Seq and Bioinformatics Analyses:

RNA from myeloma cells (treated and untreated) was isolated and checked for integrity using the Agilent Bioanalyzer 2100 system (Agilent Technologies, Santa Clara, CA, USA) and quantified using a Qubit fluorometric assay (Thermo Fisher Scientific, USA). RNA Seq was carried out at the Genome Quebec, Montreal, Canada. In brief, total RNA was quantified using a NanoDrop Spectrophotometer ND-1000 (NanoDrop Technologies, Inc.) and its integrity was assessed on a 2100 Bioanalyzer (Agilent Technologies, USA). rRNA was depleted from 250 ng of total RNA using QIAseq FastSelect (Human 96rxns). cDNA synthesis was achieved with the NEBNext RNA First Strand Synthesis and NEBNext Ultra Directional RNA Second Strand Synthesis Modules (New England BioLabs). The remaining steps of library preparation were done using the NEBNext Ultra II DNA Library Prep Kit for Illumina (New England BioLabs). Adapters and PCR primers were purchased from New England BioLabs. Libraries were quantified using the Quant-iT™ PicoGreen® dsDNA Assay Kit (Life Technologies) and the Kapa Illumina GA with Revised Primers-SYBR Fast Universal kit (Kapa Biosystems). Average size fragment was determined using a LabChip GX (PerkinElmer) instrument. All bioinformatics analyses were done by the Canadian Centre for Computational Genomics (C3G) - Montréal Node, Canada.

Experimental Results

Fig. 1a, Fig. 1b and Fig. 1c are heatmap images for RNA sequence results for gene expression profiling done using RNA isolated from untreated human myeloma cells (KMM-1) and myeloma cells treated with Lenalidomide (10uM), CAPE (25 and 50uM) and GL8 (25 and 50 uM). The key downregulated gene targets IKZF1, IKZF3, IRF4, MYC and HDAC1 in CAPE (50 uM) and GL8 (25 uM and 50 uM) treated conditions in comparison with untreated and lenalidomide treated conditions, which are related to combination therapeutic approaches, are highlighted.

FIG. 2a and FIG. 2b are Western blots of key protein targets HDAC1, Sp1, IRF4, IKZF1 and c-MYC lowered by CAPE and its closely related analogs in comparison with the HDAC inhibitor (panobinostat) in human myeloma cell lines JJN3 and MMIR. FIG. 1a is for a cell line - JJN3; where numbers 1, 5, 10 and 25 in the figure labels depict the concentration of the compounds in uM, and FIG. 2b is for cell line – MMIR.

FIG. 3 is a graph revealing the treatment effect of panobinostat and IMiDs (Pomalidomide or Lenalidomide) in comparison with panobinostat and CAPE analogs combination treatment.

Referring to FIG. 3a, in the myeloma cell line JJN3, at a concentration as low as 0.001nM, panobinostat alone demonstrated 17±3% cell growth inhibition only. However, combination treatments involving 2.5 μM As26 or 5μM As26 with 0.001nM panobinostat dramatically increased myeloma cell growth inhibition to 54.4% and 69.8% respectively.

Combining CAPE analog As26 with panobinostat can remarkably lower the dose of HDAC inhibitor, thereby reducing the adverse effects and resistance associated with administration of HDAC inhibitors alone.

FIG. 4 is a graph showing the effect of another pan-HDAC inhibitor, Trichostatin A, IC_{50} amount of potent CAPE analog As26 alone, in comparison with Trichostatin A and IC_{50} As26 combination treatment. The combination was found to provide augmented myeloma cell growth inhibition.

FIG. 5 is a graph showing the effect of the Sp1 inhibitor Terameprocol (TMP) and potent CAPE analog As26 combination. The combination was found to provide augmented myeloma cell growth inhibition, and

FIG. 6 and 6a are Western blots showing the combination treatment effect of Sp1 inhibitor TMP and the potent CAPE analog As26 on Sp1 and IRF4 protein levels. The combination was found to provide augmented myeloma cell growth inhibition.

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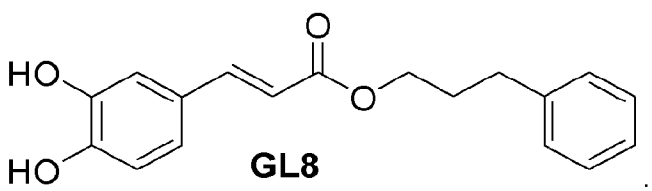
WE CLAIM:

1. A method for treating a patient with cancer, the method comprising:

   administering to the patient a therapeutically effective amount of a compound selected from the group consisting of CAPE and a CAPE analog to downregulate a cancer gene or protein target expression, wherein administration of the CAPE or the CAPE analog reduces the number or growth of cancer cells or the tumor burden or tumour growth in the patient, thereby treating the patient.
2. The method of claim 1, further comprising a downregulating agent in combination with the compound.
3. The method of claim 2, wherein the downregulating agent is a histone deacetylase (HDAC) inhibitor, wherein administration of CAPE or CAPE analog in combination with the histone deacetylase inhibitor reduces the number of cancer cells or the tumor burden in the patient, thereby treating the patient.
4. The method of claim 3, wherein the HDAC is HDAC1.
5. The method of any one of claims 1 to 3, wherein the cancer is a blood cancer.
6. The method of claim 5, wherein the blood cancer is selected from the group consisting of myeloma, leukemia or lymphoma.
7. The method of claim 5, wherein the compound is CAPE.
8. The method of claim 5, wherein the compound is a CAPE analog according to the formula



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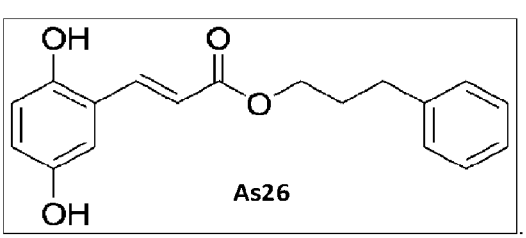
9. The method of claim 5, wherein the compound is a CAPE analog according to the formula



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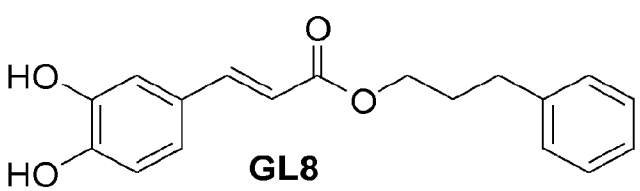
10. The method of any one of claims 3 and 5 to 9, wherein the HDAC inhibitor is a pan-HDAC inhibitor.
11. The method of any one of claims 3 and 5 to 9, wherein the HDAC inhibitor is panobinostat.
12. The method of any one of claims 3 and 5 to 9, wherein the HDAC inhibitor is trichostatin A.
13. The method of any one of claims 3 and 5 to 9, wherein the HDAC inhibitor is selected from the group consisting of entinostat, ricolinostat, romidepsin, vorinostat, belinostat, and LAQ824.
14. The method according to claim 2, wherein the downregulating agent is an Immunomodulatory class of compounds (IMIDs), wherein administration of CAPE or CAPE analog in combination with IMiD reduces the number or growth of cancer cells or the tumor burden or tumour group in the patient, thereby treating the patient.
15. The method of claim 14, wherein the cancer is a blood cancer.
16. The method of claim 15, wherein the blood cancer is selected from the group consisting of myeloma, leukemia or lymphoma.
17. The method of any one of claims 14 to 16, wherein the compound is CAPE.
18. The method of any one of claims 14 to 16, wherein the compound is a CAPE analog according to the formula:



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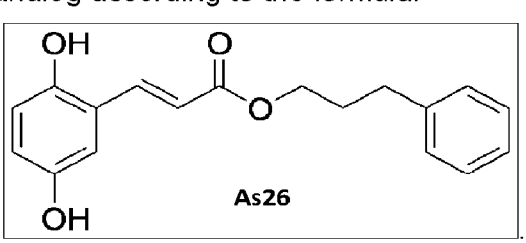
19. The method of any one of claims 14 to 16, wherein the compound is a CAPE analog according to the formula:



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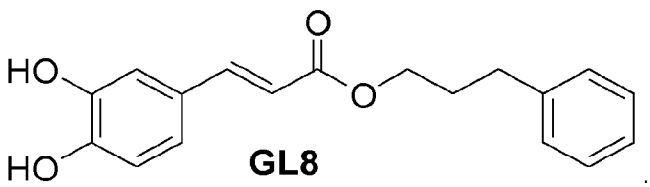
20. The method of any one of claims 14 to 19, wherein the IMID is lenalidomide or pomalidomide.

21. The method of claim 2, wherein the downregulating agent is a Sp1 inhibitor, and wherein administration of CAPE or CAPE analog in combination with Sp1 inhibitor reduces the number or growth of cancer cells or the tumor burden or tumour growth in the patient, thereby treating the patient.
22. The method of claim 21, wherein the cancer is a blood cancer.
23. The method of claim 22, wherein the blood cancer is selected from the group consisting of myeloma, leukemia or lymphoma.
24. The method of claim 21, wherein the cancer is brain cancer or breast cancer.
25. The method of any one of claims 21 to 24, wherein the compound is CAPE.
26. The method of any one of claims 21 to 24, wherein the compound is a CAPE analog according to the formula:



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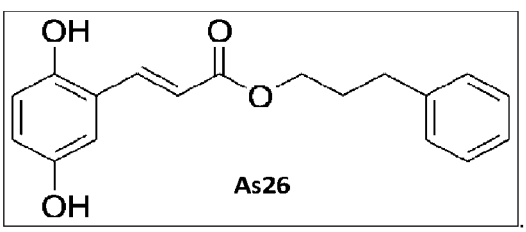
27. The method of any one of claims 21 to 24, wherein the compound is a CAPE analog according to the formula



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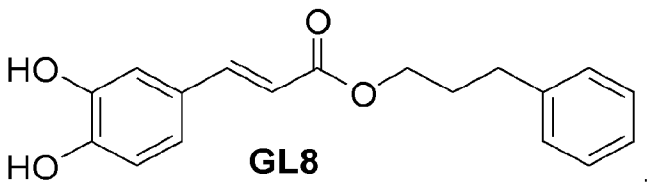
28. The method of claim 2, wherein the downregulating agent is a MYC regulating agent, wherein administration of CAPE or CAPE analog in combination with MYC regulating agent reduces the number or growth of cancer cells or the tumor burden or tumour growth in the patient, thereby treating the patient.
29. The method of claim 28, wherein the cancer is a blood cancer.
30. The method of claim 29, wherein the blood cancer is selected from the group consisting of myeloma, leukemia or lymphoma.
31. The method of any one of claims 28 to 30, wherein the compound is CAPE.
32. The method of any one of claims 28 to 30, wherein the compound is a CAPE analog according to the formula:



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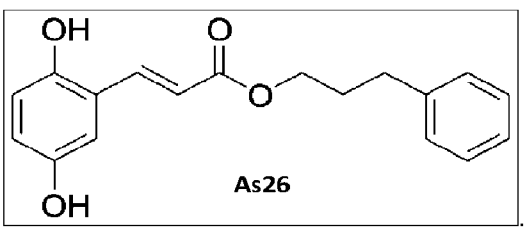
33. The method of any one of claims 28 to 30, wherein the compound is a CAPE analog according to the formula:



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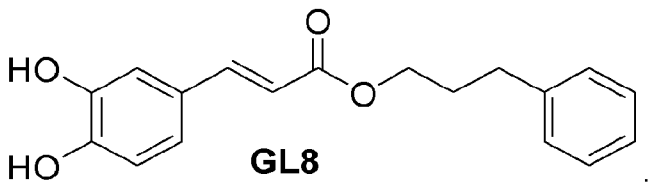
34. The method of any one of claims 28 to 33, wherein the MYC regulating agent is a BET inhibitor.
35. The method of claim 34, wherein the BET inhibitor is a small molecule inhibitor JQ1
36. The method of claim 35, wherein the small molecule inhibitor is MYCi361 and related analogs.
37. Use of a therapeutically effective amount of a compound selected from the group consisting of CAPE and a CAPE analog to downregulate a cancer gene or protein target in a patient in need thereof to reduce the number or growth of cancer cells or the tumor burden or tumour growth in the patient.
38. The use of claim 37, further comprising a downregulating agent in combination with the compound.
39. The use of claim 38, wherein the downregulating agent is a histone deacetylase (HDAC) inhibitor, wherein administration of CAPE or CAPE analog in combination with the histone deacetylase inhibitor reduces the number of cancer cells or the tumor burden in the patient.
40. The use of claim 39, wherein the HDAC is HDAC1.
41. The use of any one of claims 37 to 40, wherein the cancer is a blood cancer.
42. The use of claim 41, wherein the blood cancer is selected from the group consisting of myeloma, leukemia or lymphoma.
43. The use of claim 41, wherein the compound is CAPE.
44. The use of claim 41, wherein the compound is a CAPE analog according to the formula



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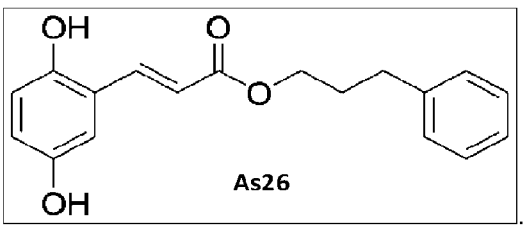
45. The use of claim 41, wherein the compound is a CAPE analog according to the formula



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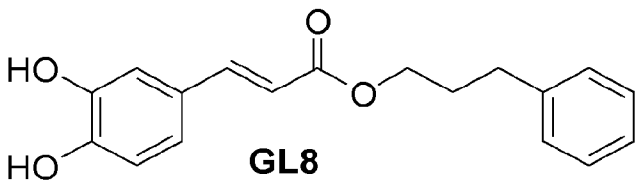
46. The use of any one of claims 39 and 41 to 45, wherein the HDAC inhibitor is a pan-HDAC inhibitor.
47. The use of any one of claims 39 and 41 to 45, wherein the HDAC inhibitor is panobinostat.
48. The use of any one of claims 39 and 41 to 45, wherein the HDAC inhibitor is trichostatin A.
49. The use of any one of claims 39 and 41 to 45, wherein the HDAC inhibitor is selected from the group consisting of entinostat, ricolinostat, romidepsin, vorinostat, belinostat, and LAQ824.
50. The use according to claim 38, wherein the downregulating agent is an Immunomodulatory class of compounds (IMIDs), wherein administration of CAPE or CAPE analog in combination with IMiD reduces the number or growth of cancer cells or the tumor burden or tumour group in the patient.
51. The use of claim 50, wherein the cancer is a blood cancer.
52. The use of claim 51, wherein the blood cancer is selected from the group consisting of myeloma, leukemia or lymphoma.
53. The use of any one of claims 50 to 52, wherein the compound is CAPE.
54. The use of any one of claims 50 to 52, wherein the compound is a CAPE analog according to the formula:



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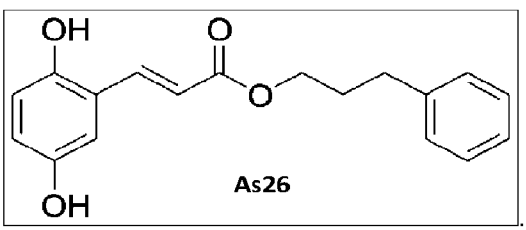
55. The use of any one of claims 50 to 52, wherein the compound is a CAPE analog according to the formula:



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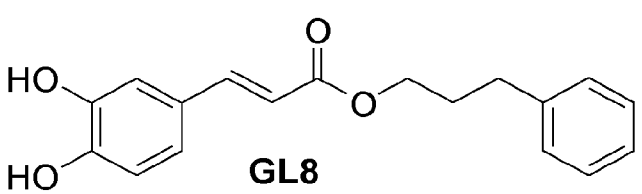
56. The use of any one of claims 50 to 52, wherein the IMID is lenalidomide or pomalidomide.
57. The use of claim 38, wherein the downregulating agent is a Sp1 inhibitor, and wherein administration of CAPE or CAPE analog in combination with Sp1 inhibitor reduces the number or growth of cancer cells or the tumor burden or tumour growth in the patient.
58. The use of claim 57, wherein the cancer is a blood cancer.
59. The use of claim 58, wherein the blood cancer is selected from the group consisting of myeloma, leukemia or lymphoma.
60. The use of claim 57, wherein the cancer is brain cancer or breast cancer.
61. The use of any one of claims 38 to 60, wherein the compound is CAPE.
62. The use of any one of claims 38 to 60, wherein the compound is a CAPE analog according to the formula:



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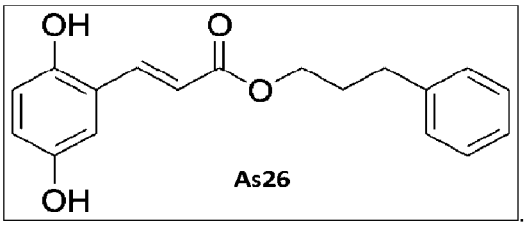
63. The use of any one of claims 38 to 60, wherein the compound is a CAPE analog according to the formula



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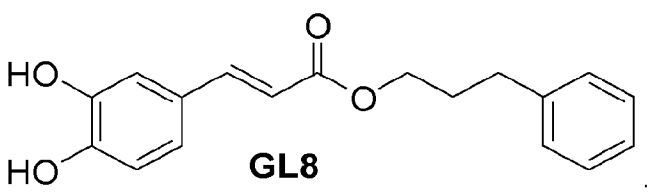
64. The use of claim 38, wherein the downregulating agent is a MYC regulating agent, wherein administration of CAPE or CAPE analog in combination with MYC regulating agent reduces the number or growth of cancer cells or the tumor burden or tumour growth in the patient.
65. The use of claim 64, wherein the cancer is a blood cancer.
66. The use of claim 64, wherein the blood cancer is selected from the group consisting of myeloma, leukemia or lymphoma.
67. The use of any one of claims 64 to 66, wherein the compound is CAPE.
68. The use of any one of claims 64 to 66, wherein the compound is a CAPE analog according to the formula:



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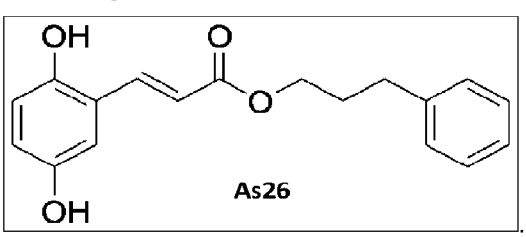
69. The use of any one of claims 64 to 66, wherein the compound is a CAPE analog according to the formula:



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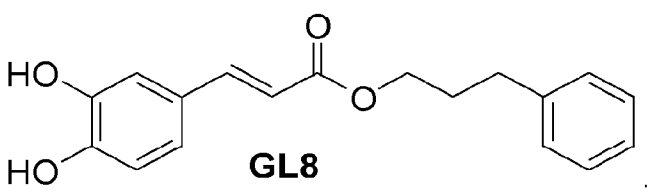
70. The use of any one of claims 64 to 66, wherein the MYC regulating agent is a BET inhibitor.
71. The use of claim 70, wherein the BET inhibitor is a small molecule inhibitor JQ1.
72. The use of claim 71, wherein the small molecule inhibitor is MYCi361 and related analogs.

73. A composition for treating a patient with cancer comprising a therapeutically effective amount of CAPE or CAPE analog in combination with a therapeutically effective amount of a downregulating agent.
74. The composition of claim 73, wherein the downregulating agent is a histone deacetylase (HDAC) inhibitor.
75. The composition of claim 74, wherein the HDAC is HDAC1.
76. The composition of any one of claims 73 to 75, wherein the cancer is blood cancer.
77. The composition of claim 76, wherein the blood cancer is selected from the group consisting of myeloma, leukemia or lymphoma.
78. The composition of any one of claims 73 to 77, wherein the compound is CAPE.
79. The composition of any one of claims 73 to 77, wherein the compound is a CAPE analog according to the formula:



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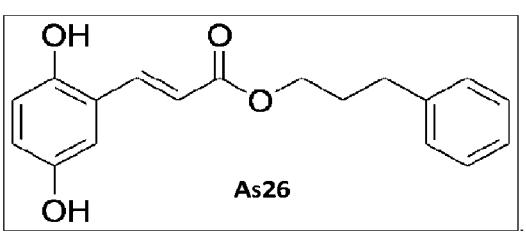
80. The composition of any one of claims 73 to 77, wherein the compound is a CAPE analog according to the formula:



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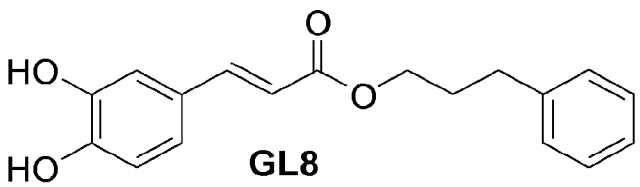
81. The composition of claim 74 and 76 to 80, wherein the HDAC inhibitor is a pan-HDAC inhibitor.
82. The composition of claim 74 and 76 to 80, wherein the HDAC inhibitor is panobinostat.
83. The composition of claim 74 and 76 to 80, wherein the HDAC inhibitor is trichostatin A.
84. The composition of claim 74, wherein the downregulating agent is an Immunomodulatory class of compounds (IMIDs).
85. The composition of claim 84, wherein the cancer is blood cancer.

86. The composition of claim 85, wherein the blood cancer is selected from the group consisting of myeloma, leukemia or lymphoma.
87. The composition of any one of claims 84 to 86, wherein the compound is CAPE.
88. The composition of any one of claims 84 to 86, wherein the compound is a CAPE analog according to the formula:



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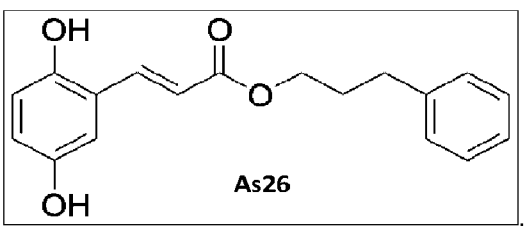
89. The composition of any one of claims 84 to 86, wherein the compound is a CAPE analog according to the formula:



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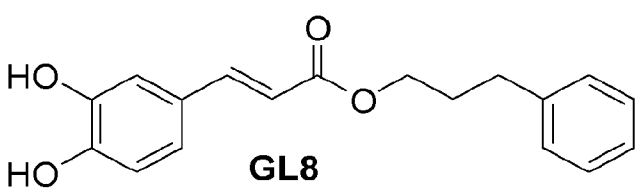
90. The composition of claim 74, wherein the downregulating agent is a Sp1 inhibitor.
91. The composition of claim 90, wherein the cancer is a blood cancer.
92. The composition of claim 91, wherein the blood cancer is selected from the group consisting of myeloma, leukemia or lymphoma.
93. The composition of claim 90, wherein the cancer is brain cancer or breast cancer.
94. The composition of any one of claims 90 to 93, wherein the compound is CAPE.
95. The composition of any one of claims 90 to 93, wherein the compound is a CAPE analog according to the formula:



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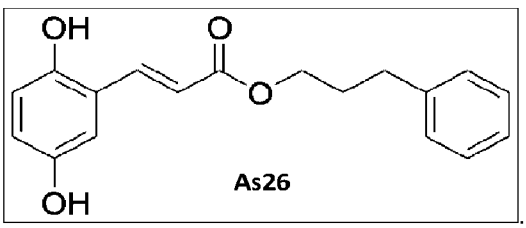
96. The composition of any one of claims 90 to 93, wherein the compound is a CAPE analog according to the formula



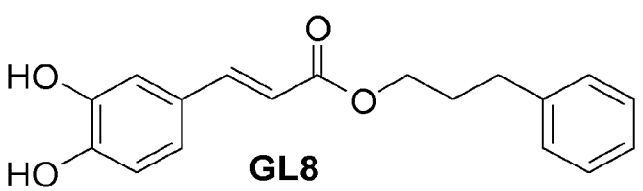
97. The composition of claim 74, wherein the downregulating agent is a MYC regulating agent.

98. The composition of claim 97, wherein the cancer is a blood cancer.

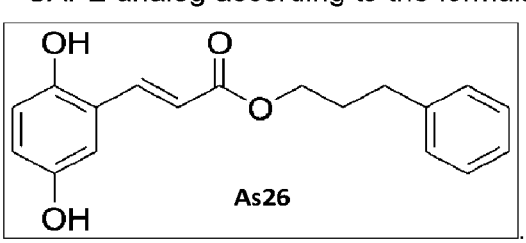
99. The composition of claim 98, wherein the blood cancer is selected from the group consisting of myeloma, leukemia or lymphoma.

100. The composition of any one of claims 97 to 99, wherein the compound is CAPE.

101. The composition of any one of claims 97 to 99, wherein the compound is a CAPE analog according to the formula:



102. The composition of any one of claims 97 to 99, wherein the compound is a CAPE analog according to the formula:



103. The composition of any one of claims 97 to 103, wherein the MYC regulating agent is a BET inhibitor.

104. The composition of claim 103, wherein the BET inhibitor is a small molecule inhibitor JQ1.

105. The composition of claim 104, wherein the small molecule inhibitor is MYCi361 and related analogs.

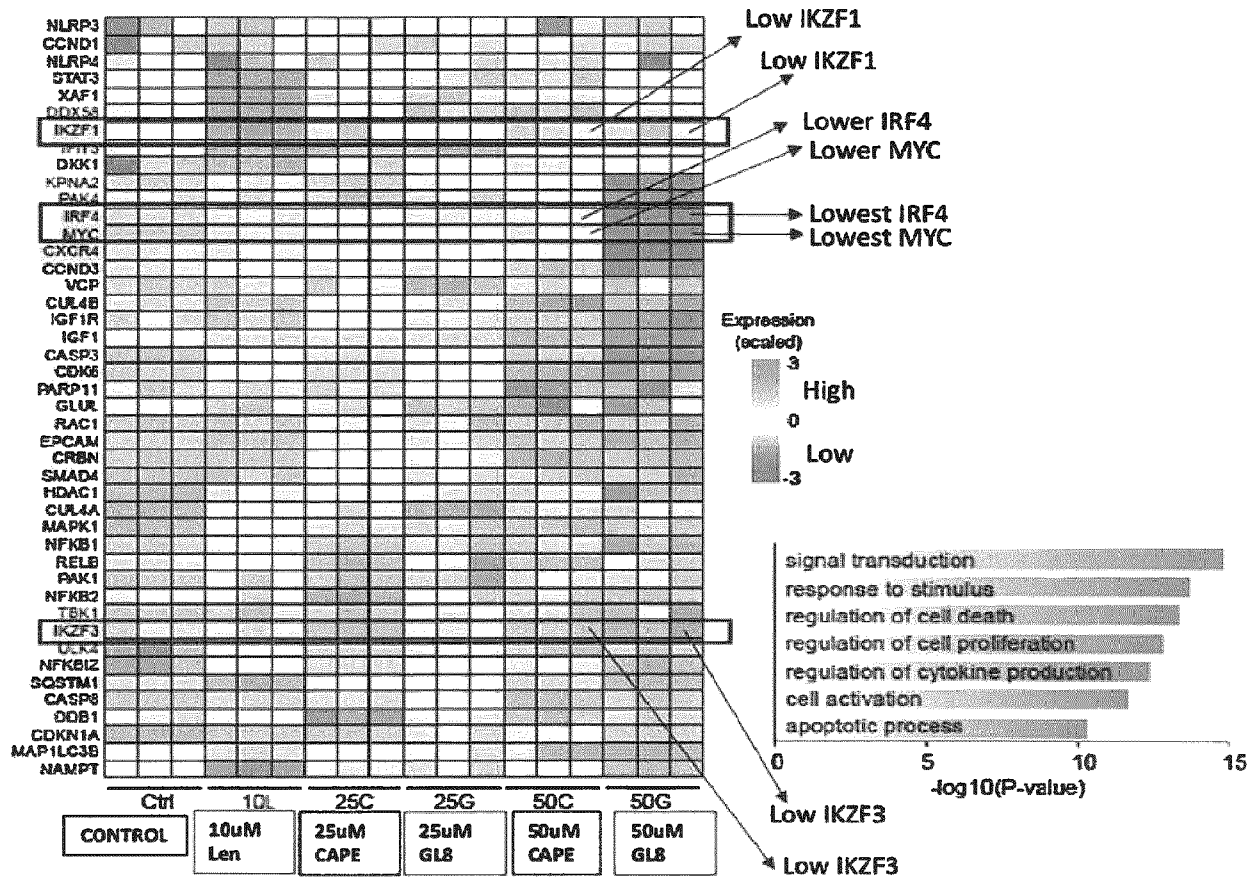

NLRP3
CCND1
NLRP4
STAT3
XAF1
DDX58
IKZF1
IFIT3
DKK1
KPNA2
PAK4
IRF4
MYC
CXCR4
CCND3
VCP
CUL4B
IGF1R
IGF1
CASP3
CDK6
PARP11
GLUL
RAC1
EPCAM
CRBN
SMAD4
HDAC1
CUL4A
MAPK1
NFKB1
RELB
PAK1
NFKB2
TBK1
IKZF3
UCK2
NFKBIZ
SQSTM1
CASP8
DDB1
CDKN1A
MAP1LC3B
NAMPT
Low IKZF1
Low IKZF1
Lower IRF4
Lower MYC
Lowest IRF4
Lowest MYC
Expression (scaled)
3
High
0
Low
-3
signal transduction
response to stimulus
regulation of cell death
regulation of cell proliferation
regulation of cytokine production
cell activation
apoptotic process
0
5
10
15
-log10(P-value)
Low IKZF3
Low IKZF3
Ctrl
10L
25C
25G
50C
50G
CONTROL
10uM Len
25uM CAPE
25uM GL8
50uM CAPE
50uM GL8

FIG. 1a

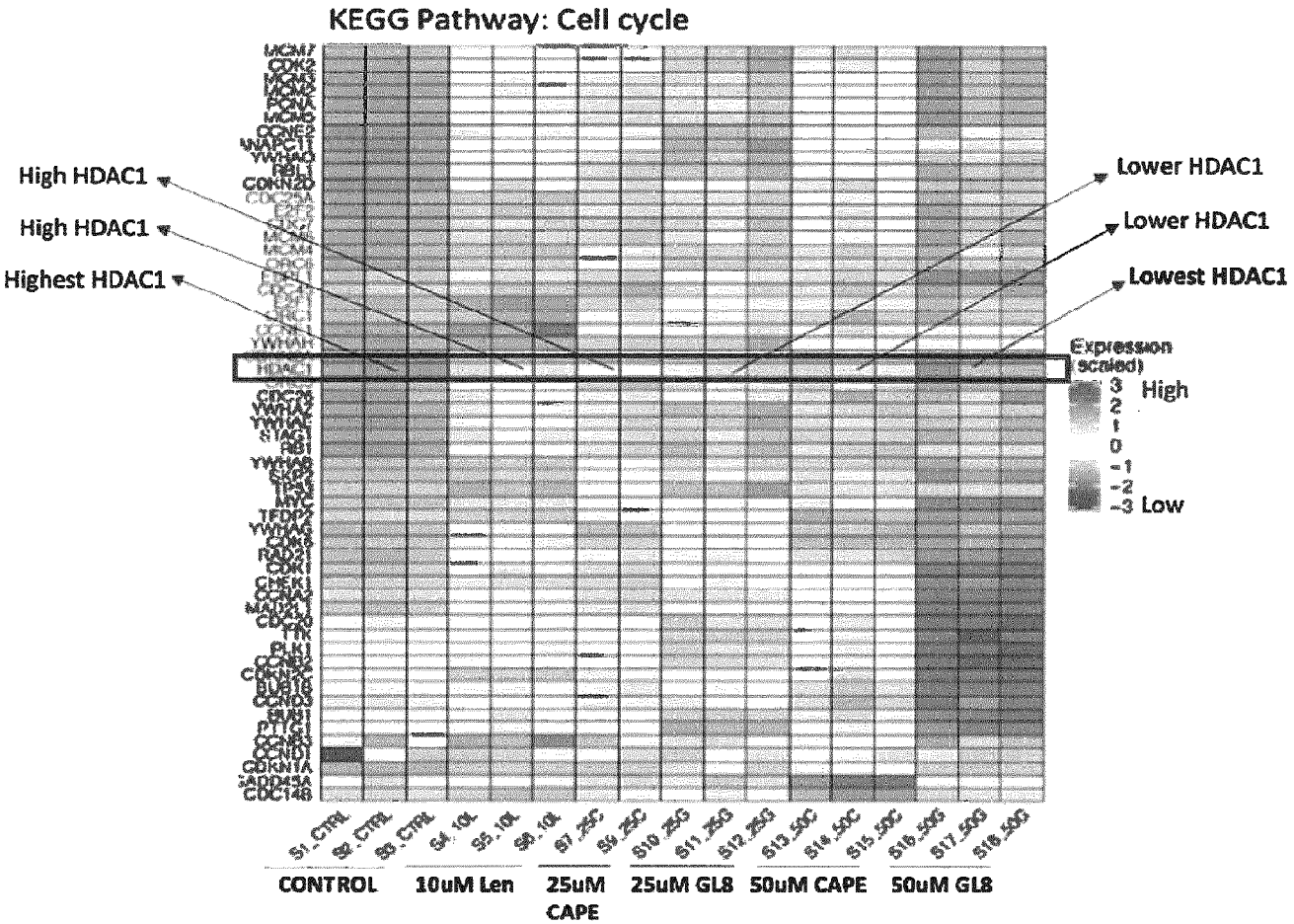

KEGG Pathway: Cell cycle
High HDAC1
High HDAC1
Highest HDAC1
Lower HDAC1
Lower HDAC1
Lowest HDAC1
Expression (scaled)
3 High
2
1
0
-1
-2
-3 Low
CONTROL
10uM Len
25uM CAPE
25uM GL8
50uM CAPE
50uM GL8

FIG. 1b

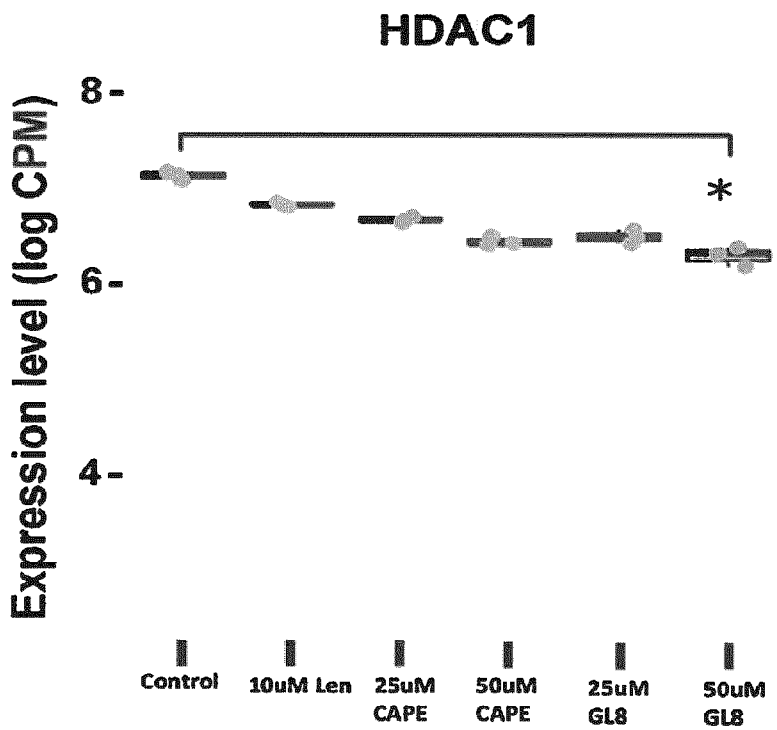


FIG. 1C (Quantification of HDAC1 Expression)

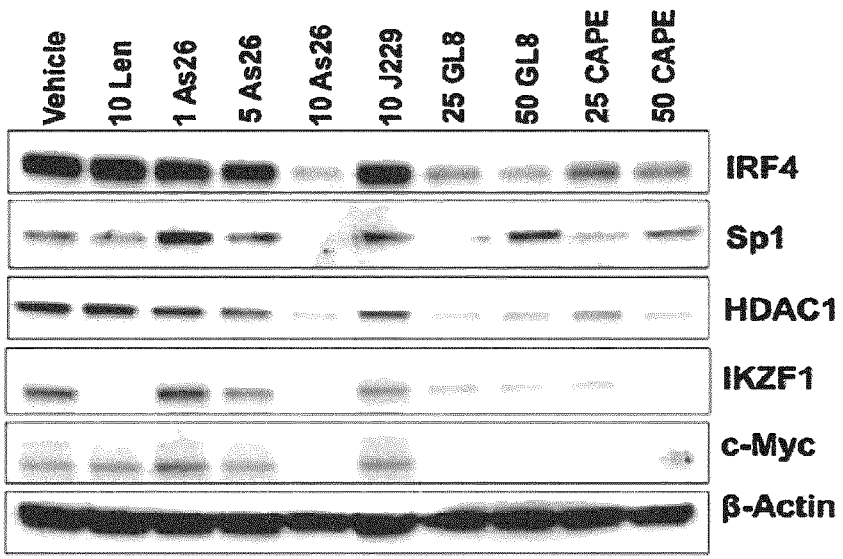

Vehicle
10 Len
1 As26
5 As26
10 As26
10 J229
25 GL8
50 GL8
25 CAPE
50 CAPE
IRF4
Sp1
HDAC1
IKZF1
c-Myc
β-Actin

FIG. 2a

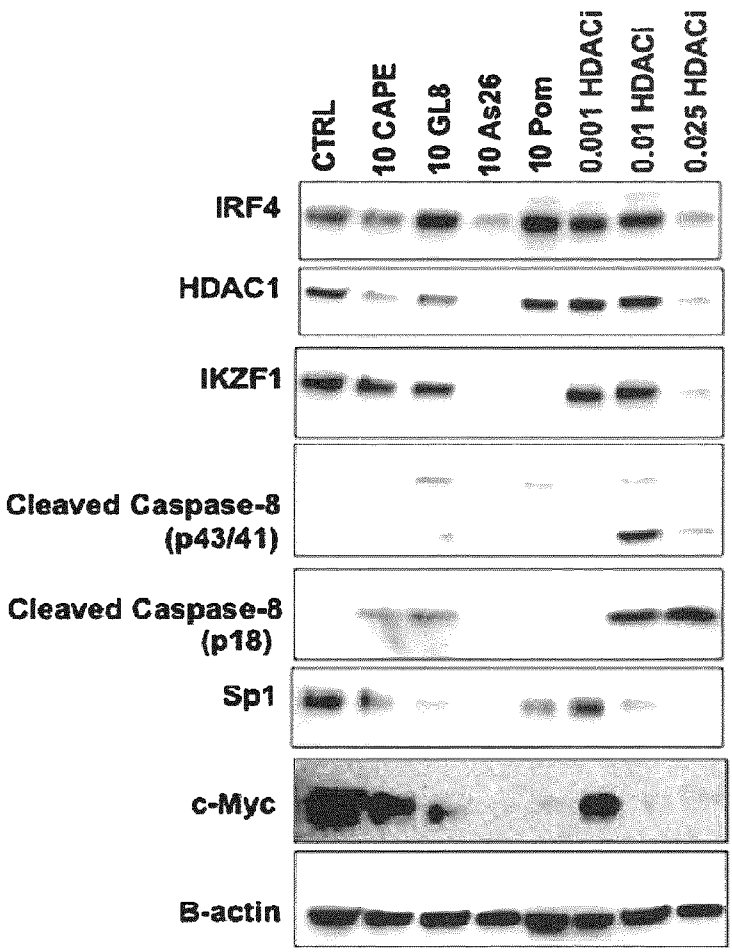

CTRL
10 CAPE
10 GL8
10 As26
10 Pom
0.001 HDACi
0.01 HDACi
0.025 HDACi
IRF4
HDAC1
IKZF1
Cleaved Caspase-8 (p43/41)
Cleaved Caspase-8 (p18)
Sp1
c-Myc
B-actin

FIG. 2b

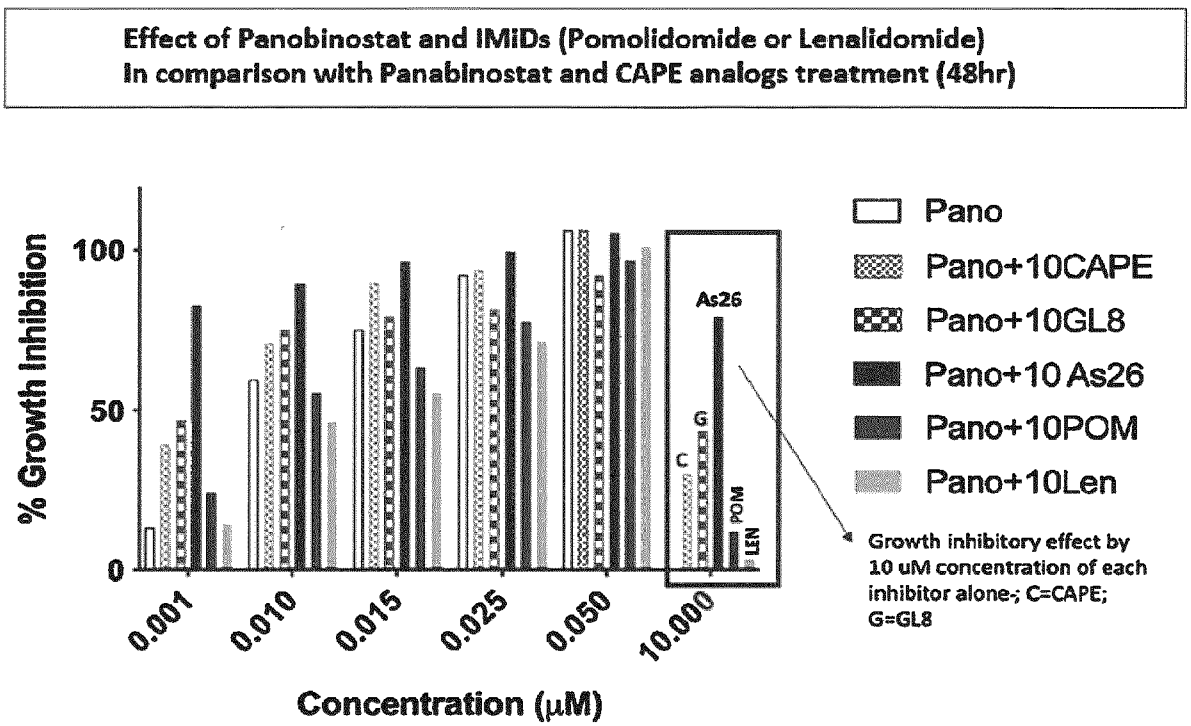

Effect of Panobinostat and IMiDs (Pomolidomide or Lenalidomide)
In comparison with Panabinostat and CAPE analogs treatment (48hr)
% Growth Inhibition
100
50
0
0.001
0.010
0.015
0.025
0.050
10.000
Concentration (μM)
As26
G
C
POM
LEN
Pano
Pano+10CAPE
Pano+10GL8
Pano+10 As26
Pano+10POM
Pano+10Len
Growth inhibitory effect by 10 uM concentration of each inhibitor alone-; C=CAPE; G=GL8

FIG. 3

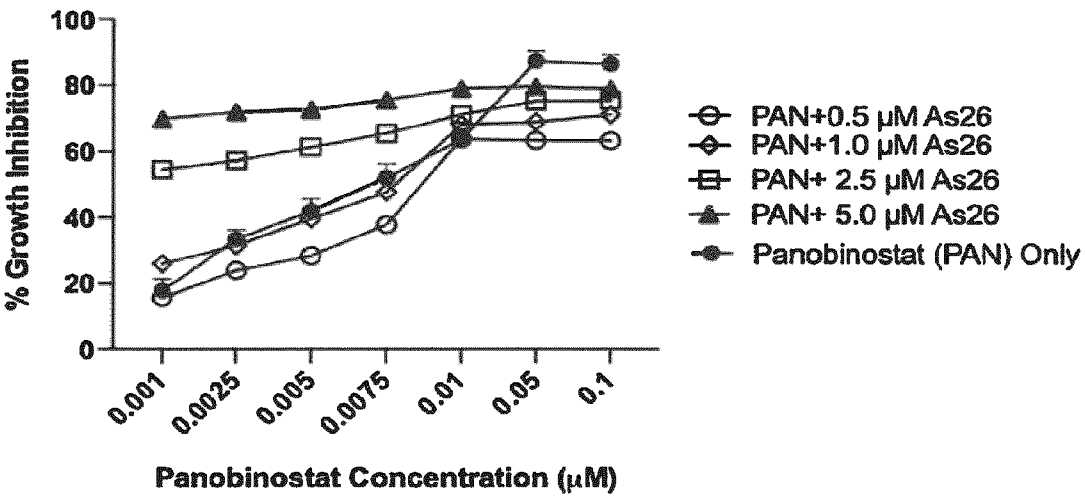


FIG. 3a: Combinatorial Effect of As26 with HDACi Panobinostat

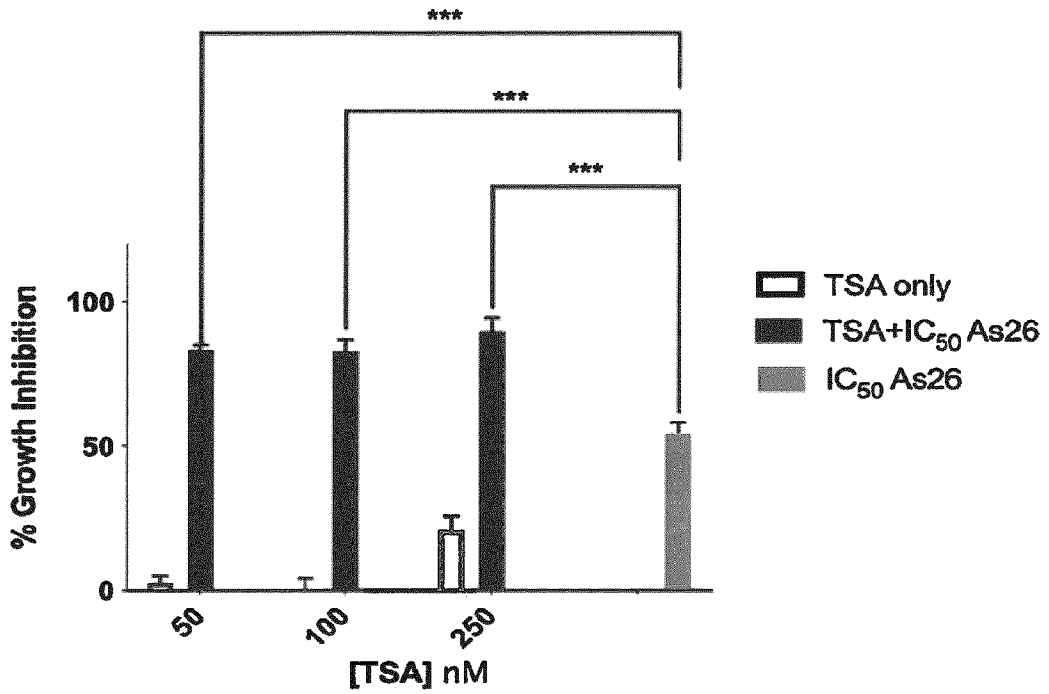

***
***
***
% Growth Inhibition
100
50
0
50
100
250
[TSA] nM
TSA only
TSA+IC50 As26
IC50 As26

FIG. 4

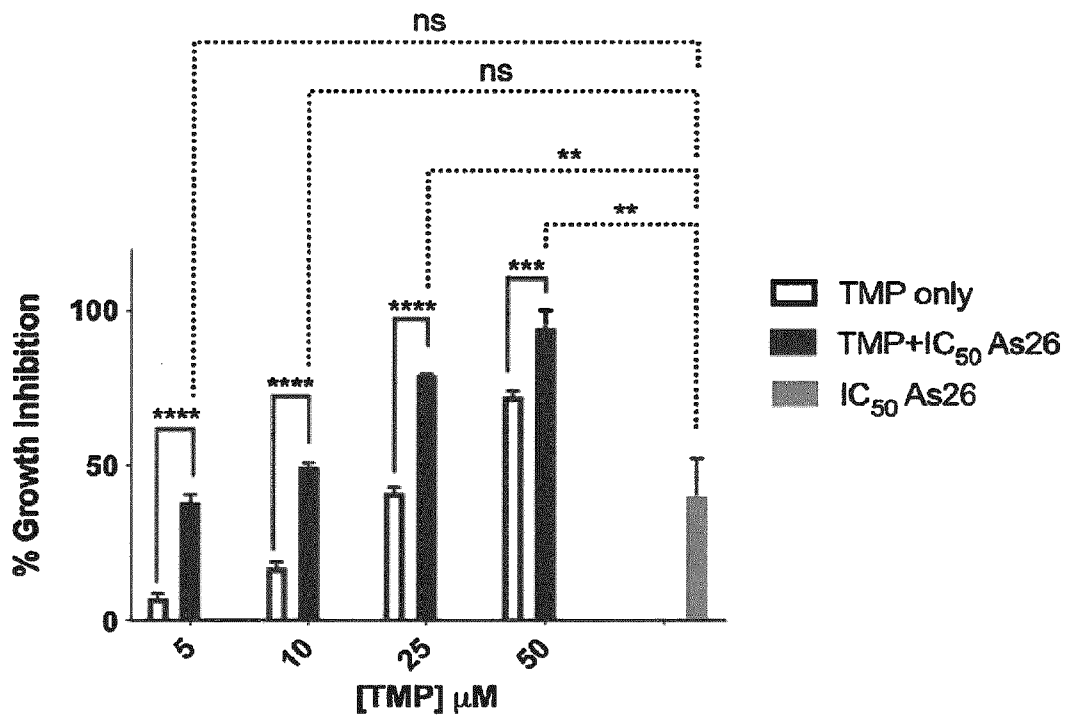

ns
ns
**
**
***
****
****
****
100
50
0
% Growth Inhibition
5
10
25
50
[TMP] μM
TMP only
TMP+IC50 As26
IC50 As26

FIG. 5

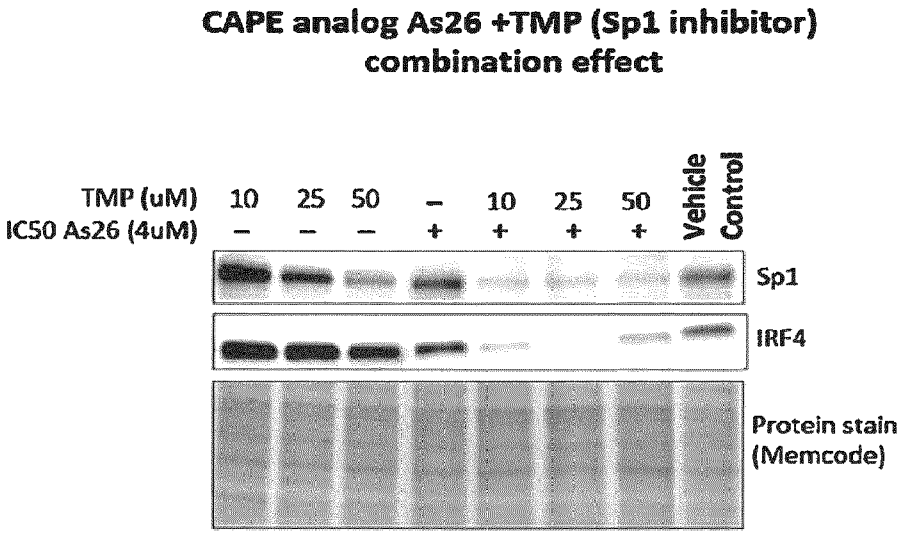

CAPE analog As26 +TMP (Sp1 inhibitor) combination effect
TMP (uM) 10 25 50 – 10 25 50 Vehicle Control
IC50 As26 (4uM) – – – + + + +
Sp1
IRF4
Protein stain (Memcode)
JJN3; 48hr

FIG. 6

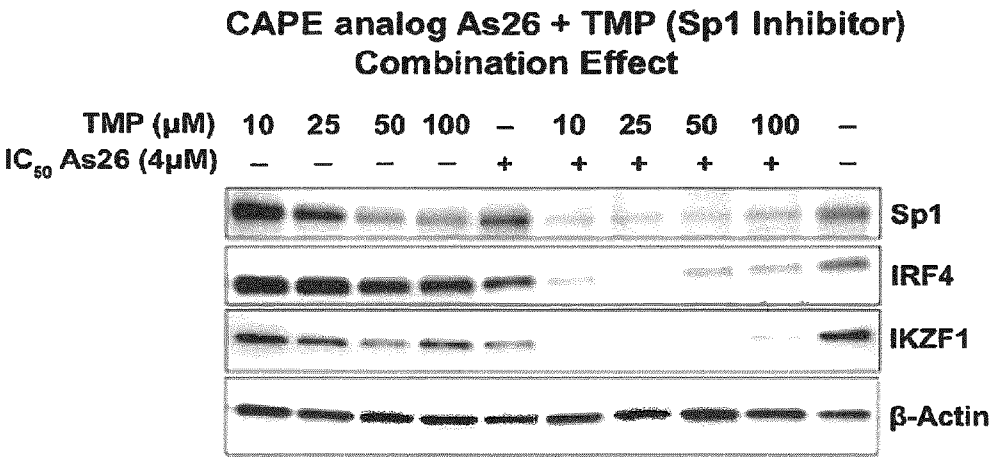


FIG. 6a (data on 100uM TMP effect and IKZF1 expression)

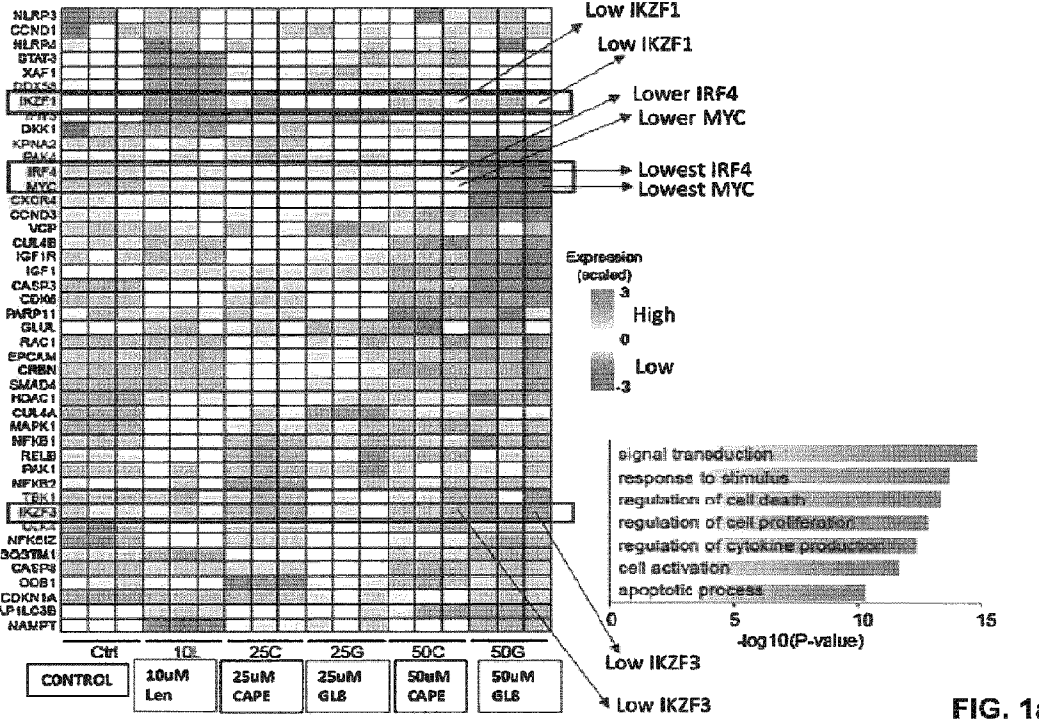

NLRP3
CCND1
NLRP4
STAT3
XAF1
DDX58
IKZF1
PYR3
DKK1
KPNA2
PAK4
IRF4
MYC
CXCR4
CCND3
VCP
CUL4B
IGF1R
IGF1
CASP3
CDK6
PARP11
GLUR
RAC1
EPCAM
CRBN
SMAD4
HDAC1
CUL4A
MAPK1
NFKB1
RELB
PAK1
NFKB2
TBK1
IKZF3
OCA2
NFKBIZ
SQSTM1
CASP8
ODB1
CDKN1A
MAP1LC3B
NAMPT
Low IKZF1
Low IKZF1
Lower IRF4
Lower MYC
Lowest IRF4
Lowest MYC
Expression (scaled)
3
High
0
Low
-3
signal transduction
response to stimulus
regulation of cell death
regulation of cell proliferation
regulation of cytokine production
cell activation
apoptotic process
0
5
10
15
-log10(P-value)
Low IKZF3
Low IKZF3
Ctrl
10C
25C
25G
50C
50G
CONTROL
10uM Len
25uM CAPE
25uM GLB
50uM CAPE
50uM GLB

FIG. 1a