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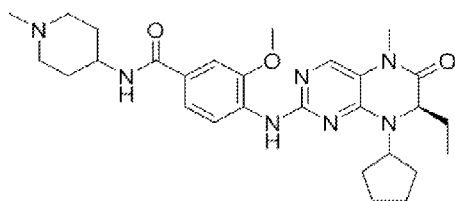
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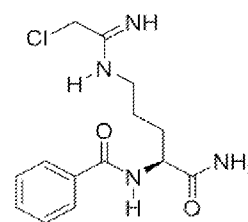
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(54) Title: CELL CULTURE CONTAINING BROMODOMAIN INHIBITORS



BI2536



Cl-amidine

FIG. 1B

(57) Abstract: Provided herein is a cell culture medium and methods of using thereof. The cell culture medium generally comprises a compound (e.g., a bromodomain inhibitor) that can enhance product titer, cell viability, cell specific productivity, and/or viable cell density of a cell culture containing the medium. Also provided herein is a method of producing a protein or polypeptide of interest (e.g., an antibody), comprising culturing cells in a culture comprising the medium.



CELL CULTURE CONTAINING BROMODOMAIN INHIBITORS

BACKGROUND OF THE INVENTION

Field of the Invention

[0001] The present invention generally pertains to a cell culture medium and methods of using thereof. The cell culture medium described herein generally comprises a compound (e.g., a bromodomain inhibitor) that can enhance product titer, cell viability, cell specific productivity, and/or viable cell density of a cell culture containing the medium.

Background Art

[0002] Over the last few decades, much research has focused on the production of recombinant proteins, *e.g.*, monoclonal antibodies, and the work has taken a variety of angles. While much work in the literature has utilized media containing sera or hydrolysates, chemically defined media were also developed in order to eliminate the problematic lot-to-lot variation of complex components (Luo and Chen, *Biotechnology and Bioengineering* 97(6):1654-1659 (2007)). An improved understanding of the cell culture has permitted a shift to chemically defined medium without compromising on growth, viability, titer, etc. To date optimized chemically defined processes have been reported with titers as high as 7.5-10 g/L (Huang *et al.*, *Biotechnology Progress* 26(5):1400-1410 (2010); Ma *et al.*, *Biotechnology Progress* 25(5):1353-1363 (2009); Yu *et al.*, *Biotechnology and Bioengineering* 108(5):1078-1088 (2011)). In general, the high titer chemically defined processes are fed batch processes with cultivation times of 11-18 days. The process intensification has been achieved without compromising product quality while maintaining relatively high viabilities. Despite these progresses, cell culture media suitable for culturing cells to produce high product titer with high cell specific productivity are still in need.

BRIEF SUMMARY OF THE INVENTION

[0003] In one aspect, the present invention is directed to a cell culture medium and method of using thereof. In one embodiment, the present invention provides a cell culture medium comprising a bromodomain inhibitor. In one embodiment, the present invention provides a cell culture medium comprising BI2536 and/or CI-amidine. In one

embodiment, the present invention also provides a cell culture comprising cells (e.g., mammalian cells) and a cell culture medium, e.g., a cell culture medium comprising a bromodomain inhibitor. In one embodiment, the cell culture or cell culture medium can be used in a batch culture, fed-batch culture, a perfusion culture, a shake flask culture, or a bioreactor. In one embodiment, the cell culture medium is a basal medium. In one embodiment, the cell culture medium is a feed medium.

[0004] Various bromodomain inhibitors can be used for the cell culture medium described herein. In a specific embodiment, the cell culture medium comprises a bromodomain inhibitor selected from the group consisting of RVX208, SGC-CBP30, JQ1, CPI-203, PFI-1, I-BET-762, OTX-015, I-BET151, bromosporine, I-CBP112, and a combination thereof. Other suitable bromodomain inhibitors are described herein.

[0005] The bromodomain inhibitor can be present in the cell culture medium in various concentrations. In one embodiment, the bromodomain inhibitor is present in the cell culture medium in a concentration ranging from about 1 μ M to about 100 μ M. In one embodiment, the bromodomain inhibitor can also be present in the cell culture medium in a concentration sufficient, when culturing mammalian cells in a fed-batch mode for 14 days (e.g., under the culturing conditions specified in the Examples section herein), (1) to produce a protein or polypeptide of interest at a titer at least about 15% (e.g., about 15% to about 100%) greater than the titer produced from a control cell culture that does not contain a bromodomain inhibitor; (2) to generate a viable cell density of at least about 20% (e.g., about 20% to about 100%) higher than that observed for a control cell culture that does not contain a bromodomain inhibitor; (3) to generate a cell specific productivity of at least about 10% (e.g., about 10% to about 60%) higher than that observed for a control cell culture that does not contain a bromodomain inhibitor; and/or (4) to achieve a cell viability of at least about 10% (e.g., about 10% to about 60%) higher than that observed for a control cell culture that does not contain a bromodomain inhibitor. Other suitable concentrations of bromodomain inhibitors are described herein.

[0006] In one embodiment, the present invention further provides a method of culturing cells, e.g., to produce a protein or polypeptide of interest. In one embodiment, the method comprises contacting the cells with a cell culture medium described herein. In one embodiment, the culturing is conducted in a batch mode, a fed-batch mode, or a perfusion mode. In a specific embodiment, the culturing is conducted in a fed-batch mode.

[0007] Various cells can be cultured using the cell culture medium described herein. In one embodiment, the cells are mammalian cells. In one embodiment, the mammalian cells comprise a polynucleotide encoding a protein or polypeptide of interest. In one embodiment, the cell culture medium comprises a bromodomain inhibitor. In one embodiment, the mammalian cells are CHO cells (e.g., CHO K1 cells). In one embodiment, the cell culture is a fed-batch culture.

[0008] In one embodiment, the present invention further provides a method of producing a protein or polypeptide of interest. In one embodiment, the method comprises culturing cells (e.g., mammalian cells as described herein) capable of producing the protein or polypeptide of interest in a culture comprising a medium described herein. In one embodiment, the method produces the protein or polypeptide of interest in a fed-batch mode. In one embodiment, the culture comprises a medium comprising a bromodomain inhibitor, and the method (1) produces the protein or polypeptide of interest (e.g., at day 14 of the culture) at a titer greater than the titer produced from a control cell culture that does not contain a bromodomain inhibitor; (2) generates a viable cell density (e.g., at day 14 of the culture) higher than that observed for a control cell culture that does not contain a bromodomain inhibitor; (3) generates a cell specific productivity (e.g., a cumulative cell specific productivity at day 14 of the culture) higher than that observed for a control cell culture that does not contain a bromodomain inhibitor; and/or (4) achieves a cell viability (e.g. at day 14 of the culture) higher than that observed for a control cell culture that does not contain a bromodomain inhibitor.

[0009] In one embodiment, the present invention further provides a method of modulating the viability the cells of a cell culture and/or the product titer of a cell culture. In one embodiment, the method comprises culturing cells (e.g., mammalian cells as described herein) in a culture comprising a medium described herein. In one embodiment, the medium comprises a bromodomain inhibitor.

BRIEF DESCRIPTION OF THE DRAWINGS/FIGURES

[0010] The foregoing summary, as well as the following detailed description of the embodiments, will be better understood when read in conjunction with the appended figures. For the purpose of illustration, the figures may describe the use of specific

embodiments. It should be understood, however, that the methods described herein are not limited to the precise embodiments discussed or described in the figures.

[0011] **FIGs. 1A and 1B** show structures of certain specific compounds described herein.

The chemical compounds shown in **FIG. 1A** are bromodomain inhibitors, including RVX208, SGC-CBP30, JQ1, CPI-203, PFI-1, I-BET-762, OTX-015, I-BET151, bromosporine, and I-CBP112. The chemical structures of BI2536 and CI-amidine are shown in **FIG. 1B**.

[0012] **FIGs. 2A-2C** show the effect on antibody titers by adding SGC-CBP30 to cell cultures at different concentrations. **FIG. 2A** presents bar graphs showing the antibody titers produced at Day 10, Day 12, and Day 14 by a fed-batch culture with SGC-CBP30 added at different concentrations on Day 8 and Day 11. **FIG. 2B** presents bar graphs showing the antibody titers produced at Day 10, Day 12, and Day 14 by a fed-batch culture with DMSO added at different volumes on Day 8 and Day 11. **FIG. 2C** presents bar graphs comparing the antibody titers produced at Day 10, Day 12, and Day 14 by fed-batch cultures: (1) cultures with SGC-CBP30 added at different concentrations on Day 8 and Day 11; (2) cultures with DMSO added at 10 uL on Day 8 and Day 11; and (3) control cultures (no SGC-CBP30 and no DMSO).

[0013] **FIGs. 3A-3D** show the effect on antibody titer, cumulative cell specific productivity, cell viability and variable cell density by adding SGC-CBP30 at 3 uM or 30 uM. For **FIGs. 3A-3D**, CHO-K1 cell line was used to produce CD40 antibody. **FIG. 3A** presents a graph comparing the CD-40 antibody titers produced over 14 days by fed-batch cultures: (1) cultures with SGC-CBP30 added at 3 uM or 30 uM on Day 8 and Day 11, (2) cultures with DMSO added at 1.5 uL or 15 uL on Day 8 and Day 11, and (3) control cultures (no SGC-CBP30 and no DMSO). **FIG. 3A** shows that antibody titers were consistently higher in cultures with SGC-CBP30 added at 3 uM or 30 uM than the titers produced by cultures with DMSO added at 1.5 uL or 15 uL or control cultures. **FIG. 3B** presents a graph comparing the cumulative cell specific productivity on Days 4, 7, 10, 12, and 14 for the different cultures (1)-(3). **FIG. 3B** shows that the cumulative cell specific productivity was also higher for cultures with SGC-CBP30 added at 3 uM or 30 uM than that observed for cultures with DMSO added at 1.5 uL or 15 uL or control cultures. **FIGs. 3C and 3D** presents graphs showing the viable cell density (VCD) and viability data of the different cultures (1)-(3).

[0014] **FIGs. 4A-4D** show the effect on antibody titer, cumulative cell specific productivity, cell viability and variable cell density by adding SGC-CBP30 at 3 uM or 30 uM. For **FIGs. 4A-4D**, CHO-K1 cell line was used to produce OSMR antibody. **FIG. 4A** presents a graph comparing the OSMR antibody titers produced over 14 days by fed-batch cultures: (1) cultures with SGC-CBP30 added at 3 uM or 30 uM on Day 8 and Day 11, (2) cultures with DMSO added at 1.5 uL or 15 uL on Day 8 and Day 11, and (3) control cultures (no SGC-CBP30 and no DMSO). **FIG. 4A** shows that antibody titers were consistently higher in cultures with SGC-CBP30 added at 3 uM or 30 uM than the titers produced by cultures with DMSO added at 1.5 uL or 15 uL or control cultures. **FIG. 4B** presents a graph comparing the cumulative cell specific productivity on Days 4, 7, 10, 12, and 14 for the different cultures (1)-(3). **FIG. 4B** shows that the cumulative cell specific productivity was also higher for cultures with SGC-CBP30 added at 3 uM or 30 uM than that observed for cultures with DMSO added at 1.5 uL or 15 uL or control cultures. **FIGs. 4C and 4D** presents graphs showing the viable cell density (VCD) and viability data of the different cultures (1)-(3).

[0015] **FIGs. 5A-5D** show the effect on antibody titer, cumulative cell specific productivity, cell viability and variable cell density by adding SGC-CBP30 at 3 uM or 30 uM. For **FIGs. 5A-5D**, CHO-K1 cell line was used to produce STX 200 antibody. **FIG. 5A** presents a graph comparing the STX-200 antibody titers produced over 14 days by fed-batch cultures: (1) cultures with SGC-CBP30 added at 3 uM or 30 uM on Day 8 and Day 11, (2) cultures with DMSO added at 1.5 uL or 15 uL on Day 8 and Day 11, and (3) control cultures (no SGC-CBP30 and no DMSO). **FIG. 5A** shows that antibody titers were consistently higher in cultures with SGC-CBP30 added at 3 uM or 30 uM than the titers produced by cultures with DMSO added at 1.5 uL or 15 uL or control cultures. **FIG. 5B** presents a graph comparing the cumulative cell specific productivity on Days 4, 7, 10, 12, and 14 for the different cultures (1)-(3). **FIG. 5B** shows that the cumulative cell specific productivity was also higher for cultures with SGC-CBP30 added at 3 uM or 30 uM than that observed for cultures with DMSO added at 1.5 uL or 15 uL or control cultures. **FIGs. 5C and 5D** present graphs showing the viable cell density (VCD) and viability data of the different cultures (1)-(3).

[0016] **FIGs. 6A-6D** show the effect on antibody titer, cumulative cell specific productivity, cell viability and variable cell density by adding various compounds at 3 uM

or 30 uM. For **FIGs. 6A-6D**, CHO-K1 cell line was used to produce CD-40 antibody. **FIGs. 6A-6B** present bar graphs comparing CD-40 antibody titers observed on Day 12 and Day 14 in fed-batch cultures: (1) cell cultures with different compounds (RVX-208, SGC-CBP30, I-BET-762, OTX015, bromosporine, PFI-1) added at 3 uM or 30 uM on Day 8 and Day 11, (2) cultures with DMSO added at 1.5 uL or 15 uL on Day 8 and Day 11, and (3) control cultures (no compound and no DMSO). **FIGs. 6A-6B** show that adding the tested compounds to the cell cultures improved both antibody titers and cell specific productivity. **FIGs. 6C-6D** present bar graphs showing the viable cell density (VCD) and viability data of the different cultures (1)-(3).

DETAILED DESCRIPTION OF THE INVENTION

I. DEFINITIONS

[0017] The term "and/or" used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. Thus, the term "and/or" as used in a phrase such as "A and/or B" herein is intended to include "A and B," "A or B," "A" (alone), and "B" (alone). Likewise, the term "and/or" as used in a phrase such as "A, B, and/or C" is intended to encompass each of the following embodiments: A, B, and C; A, B, or C; A or C; A or B; B or C; A and C; A and B; B and C; A (alone); B (alone); and C (alone).

[0018] It is understood that wherever embodiments are described with the language "comprising," otherwise analogous embodiments described in terms of "consisting of" and/or "consisting essentially of" are also provided.

[0019] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure is related. For example, the Concise Dictionary of Biomedicine and Molecular Biology, Juo, Pei-Shou, 2nd ed., 2002, CRC Press; The Dictionary of Cell and Molecular Biology, 3rd ed., 1999, Academic Press; and the Oxford Dictionary Of Biochemistry And Molecular Biology, Revised, 2000, Oxford University Press, provide one of skill with a general dictionary of many of the terms used in this disclosure.

[0020] Units, prefixes, and symbols are denoted in their Système International de Unites (SI) accepted form. Numeric ranges are inclusive of the numbers defining the range. Unless otherwise indicated, amino acid sequences are written left to right in amino to

carboxy orientation. The headings provided herein are not limitations of the various embodiments of the disclosure, which can be had by reference to the specification as a whole. Accordingly, the terms defined immediately below are more fully defined by reference to the specification in its entirety.

[0021] The term "antibody" is used to mean an immunoglobulin molecule that recognizes and specifically binds to a target, such as a protein, polypeptide, peptide, carbohydrate, polynucleotide, lipid, or combinations of the foregoing etc., through at least one antigen recognition site within the variable region of the immunoglobulin molecule. As used herein, the term encompasses intact polyclonal antibodies, intact monoclonal antibodies, antibody fragments (such as Fab, Fab', F(ab')₂, and Fv fragments), single chain Fv (scFv) mutants, multispecific antibodies such as bispecific antibodies generated from at least two intact antibodies, monovalent or monospecific antibodies, chimeric antibodies, humanized antibodies, human antibodies, fusion proteins comprising an antigen determination portion of an antibody, and any other modified immunoglobulin molecule comprising an antigen recognition site so long as the antibodies exhibit the desired biological activity. An antibody can be any of the five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, or subclasses (isotypes) thereof (*e.g.*, IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2), based on the identity of their heavy-chain constant domains referred to as alpha, delta, epsilon, gamma, and mu, respectively.

[0022] As used herein, the term "antibody fragment" refers to a portion of an intact antibody and refers to the antigenic determining variable regions of an intact antibody. Examples of antibody fragments include, but are not limited to Fab, Fab', F(ab')₂, and Fv fragments, linear antibodies, single chain antibodies, and multispecific antibodies formed from antibody fragments.

[0023] As used herein throughout, the term "alkyl" refers to a saturated aliphatic hydrocarbon including straight chain and branched chain groups. In one embodiment, the alkyl is a lower alkyl having 1 to 4 carbon atoms, *i.e.*, C₁₋₄. Whenever a numerical range; *e.g.*, "1-4", is stated herein, it implies that the group, in this case the alkyl group, may contain 1 carbon atom, 2 carbon atoms, 3 carbon atoms, up to and including 4 carbon atoms. Unless specified or otherwise obvious from context, the alkyl group is not substituted. However, In one embodiment, the alkyl group can be substituted (*e.g.*, with 1 to 5 substituent groups) when specified.

- [0024] The term "alkylene linker" as used herein refers to an alkyl linking group, *i.e.*, an alkyl group that links one atom/group to another atom/group in a molecule.
- [0025] An "alkoxy" group as used herein refers to an -O-alkyl group, wherein the alkyl can be any of those as defined herein.
- [0026] A "halogen" as used herein refers to fluorine, chlorine, bromine or iodine.
- [0027] The term "stereoisomer" as used herein includes geometric isomers, such as E or Z isomers, enantiomers, diastereomers, and the like.
- [0028] The term "stereoisomeric mixture" as used herein includes any mixture in any ratio of stereoisomers defined herein. In one embodiment, a stereoisomeric mixture includes a racemic mixture. In one embodiment, a stereoisomeric mixture includes an enantiomerically enriched mixture. In one embodiment, a stereoisomeric mixture includes a mixture of diastereomers in any ratio.
- [0029] The term "enantiomeric excess" or "ee" as used herein refers to a measure for how much of one enantiomer is present compared to the other. For a mixture of R and S enantiomers, the percent enantiomeric excess is defined as $|R - S| * 100$, where R and S are the respective mole or weight fractions of enantiomers in a mixture such that $R + S = 1$. With knowledge of the optical rotation of a chiral substance, the percent enantiomeric excess is defined as $([\alpha]_{\text{obs}}/[\alpha]_{\text{max}}) * 100$, where $[\alpha]_{\text{obs}}$ is the optical rotation of the mixture of enantiomers and $[\alpha]_{\text{max}}$ is the optical rotation of the pure enantiomer.
- [0030] The term "basal media formulation" or "basal media" as used herein refers to any cell culture media used to culture cells that has not been modified either by supplementation, or by selective removal of a certain component.
- [0031] The term "batch culture" as used herein refers to a method of culturing cells in which all the components that will ultimately be used in culturing the cells, including the medium (see definition of "medium" below) as well as the cells themselves, are provided at the beginning of the culturing process. A batch culture is typically stopped at some point and the cells and/or components in the medium are harvested and optionally purified.
- [0032] The term "bioreactor" as used herein refers to any vessel used for the growth of a mammalian cell culture. The bioreactor can be of any size so long as it is useful for the culturing of mammalian cells. Typically, the bioreactor will be at least 1 liter and can be 10, 50, 100, 250, 500, 1000, 2000, 2500, 3000, 5000, 8000, 10,000, 12,0000, 15,000,

20,000, 30,000 liters or more, or any volume in between. For example, a bioreactor will be 10 to 5,000 liters, 10 to 10,000 liters, 10 to 15,000 liters, 10 to 20,000 liters, 10 to 30,000 liters, 50 to 5,000 liters, 50 to 10,000 liters, 50 to 15,000 liters, 50 to 20,000 liters, 50 to 30,000 liters, 1,000 to 5,000 liters, or 1,000 to 3,000 liters. The internal conditions of the bioreactor, including, but not limited to pH and temperature, are typically controlled during the culturing period. The bioreactor can be composed of any material that is suitable for holding mammalian cell cultures suspended in media under the culture conditions of the present invention, including glass, plastic or metal. The term "production bioreactor" as used herein refers to the final bioreactor used in the production of the polypeptide or protein of interest. The volume of the large-scale cell culture production bioreactor is typically at least 500 liters and can be 1000, 2000, 2500, 5000, 8000, 10,000, 12,000, 15,000 liters or more, or any volume in between. For example, the large scale cell culture reactor will be between about 500 liters and about 20,000 liters, about 500 liters and about 10,000 liters, about 500 liters and about 5,000 liters, about 1,000 liters and about 30,000 liters, about 2,000 liters and about 30,000 liters, about 3,000 liters and about 30,000 liters, about 5,000 liters and about 30,000 liters, or about 10,000 liters and about 30,000 liters, or a large scale cell culture reactor will be at least about 500 liters, at least about 1,000 liters, at least about 2,000 liters, at least about 3,000 liters, at least about 5,000 liters, at least about 10,000 liters, at least about 15,000 liters, or at least about 20,000 liters. One of ordinary skill in the art will be aware of and will be able to choose suitable bioreactors for use in practicing the present invention.

[0033] The term "bromodomain inhibitor" as used herein includes an inhibitor of any protein that contains a bromodomain. Some exemplary bromodomain-containing proteins are described by Muller et. al. in "Bromodomains as therapeutic targets," *Expert Reviews in Molecular Medicine* 13:329 (2011). In one embodiment, a "bromodomain inhibitor" includes any compound that has inhibitory effect against BET (Bromodomain and extraterminal domain family), which includes BRD2, BRD3, BRD4 and BRDT. In one embodiment, a "bromodomain inhibitor" includes any compound that has inhibitory effect against a protein in human BRD families. Human BRD families are described, for example, in Hay D.A. et al., "Discovery and Optimization of Small-molecule Ligands for the CBP/p300 Bromodomains," *J. Am. Chem. Soc.* 136:9308 (2014). In one embodiment, a "bromodomain inhibitor" includes any compound that has inhibitory effect against

CBP/p300 bromodomains. Exemplary non-limiting bromodomain inhibitors are described herein.

- [0034]** The term "cell density" as used herein refers to that number of cells present in a given volume of medium.
- [0035]** The terms "culture", "cell culture" and "eukaryotic cell culture" as used herein refer to a eukaryotic cell population that is suspended in a medium (see definition of "medium" below) under conditions suitable to survival and/or growth of the cell population. As will be clear to those of ordinary skill in the art, these terms as used herein can refer to the combination comprising the mammalian cell population and the medium in which the population is suspended.
- [0036]** Unless otherwise specified or obvious from context, the term "control cell culture medium" as used herein refers to a cell culture medium that is substantially the same as the cell culture medium/media for which the control cell culture medium is used as a comparison, except that the control cell culture medium does not contain certain specified ingredient(s) (e.g., a bromodomain inhibitor as described herein) which may be present in the comparative cell culture medium/media.
- [0037]** Similarly, unless otherwise specified or obvious from context, the term "control cell culture" as used herein refers to a cell culture that is substantially the same as the cell culture(s) for which the control cell culture is used as a comparison, for example, the cells in the cultures may be derived from the same initial batch of cells, except that the control cell culture does not contain certain specified ingredient(s) (e.g., a bromodomain inhibitor as described herein) which may be present in the comparative cell culture(s). Unless otherwise specified, the cells in the control cell culture are cultured under substantially the same conditions (e.g., same temperature, duration, mode, etc.) as those used for culturing the cells in a comparative cell culture.
- [0038]** The term "fed-batch culture" as used herein refers to a method of culturing cells in which additional components are provided to the culture at some time subsequent to the beginning of the culture process. A fed-batch culture can be started using a basal medium. The culture medium with which additional components are provided to the culture at some time subsequent to the beginning of the culture process is a feed medium. The provided components typically comprise nutritional supplements for the cells which have been depleted during the culturing process. A fed-batch culture is typically stopped at

some point and the cells and/or components in the medium are harvested and optionally purified.

[0039] "Growth phase" of the cell culture refers to the period of exponential cell growth (the log phase) where cells are generally rapidly dividing. During this phase, cells are cultured for a period of time, usually between 1-4 days, and under such conditions that cell growth is maximized. The determination of the growth cycle for the host cell can be determined for the particular host cell envisioned without undue experimentation. "Period of time and under such conditions that cell growth is maximized" and the like, refer to those culture conditions that, for a particular cell line, are determined to be optimal for cell growth and division. During the growth phase, cells are cultured in nutrient medium containing the necessary additives generally at about 25°-40°C, in a humidified, controlled atmosphere, such that optimal growth is achieved for the particular cell line. Cells are maintained in the growth phase for a period of about between one and four days, usually between two to three days. The length of the growth phase for the particular cells can be determined without undue experimentation. For example, the length of the growth phase will be the period of time sufficient to allow the particular cells to reproduce to a viable cell density within a range of about 20% -80% of the maximal possible viable cell density if the culture was maintained under the growth conditions.

[0040] "Production phase" of the cell culture refers to the period of time during which cell growth has plateaued. During the production phase, logarithmic cell growth has ended and protein production is primary. During this period of time the medium is generally supplemented to support continued protein production and to achieve the desired glycoprotein product.

[0041] The term "expression" or "expresses" are used herein to refer to transcription and translation occurring within a host cell. The level of expression of a product gene in a host cell can be determined on the basis of either the amount of corresponding mRNA that is present in the cell or the amount of the protein encoded by the product gene that is produced by the cell. For example, mRNA transcribed from a product gene is desirably quantitated by northern hybridization. Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, pp. 7.3-7.57 (Cold Spring Harbor Laboratory Press, 1989). Protein encoded by a product gene can be quantitated either by assaying for the biological activity of the protein or by employing assays that are independent of such activity, such as western

blotting or radioimmunoassay using antibodies that are capable of reacting with the protein. Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, pp. 18.1-18.88 (Cold Spring Harbor Laboratory Press, 1989).

[0042] The term "hybridoma" as used herein refers to a cell created by fusion of an immortalized cell derived from an immunologic source and an antibody-producing cell. The resulting hybridoma is an immortalized cell that produces antibodies. The individual cells used to create the hybridoma can be from any mammalian source, including, but not limited to, rat, pig, rabbit, sheep, pig, goat, and human. The term also encompasses trioma cell lines, which result when progeny of heterohybrid myeloma fusions, which are the product of a fusion between human cells and a murine myeloma cell line, are subsequently fused with a plasma cell. Furthermore, the term is meant to include any immortalized hybrid cell line that produces antibodies such as, for example, quadromas (*See, e.g., Milstein et al., Nature, 537:3053 (1983)*).

[0043] The terms "medium", "cell culture medium", "culture medium", and "growth medium" as used herein refer to a solution containing nutrients which nourish growing eukaryotic cells. Typically, these solutions provide essential and non-essential amino acids, vitamins, energy sources, lipids, and trace elements required by the cell for minimal growth and/or survival. The solution can also contain components that enhance growth and/or survival above the minimal rate, including hormones and growth factors. The solution is formulated to a pH and salt concentration optimal for cell survival and proliferation. The medium can also be a "defined medium" or "chemically defined medium"--a serum-free medium that contains no proteins, hydrolysates or components of unknown composition. Defined media are free of animal-derived components and all components have a known chemical structure. One of skill in the art understands a defined medium can comprise recombinant polypeptides or proteins, for example, but not limited to, hormones, cytokines, interleukins and other signaling molecules.

[0044] The term "perfusion culture" as used herein refers to a method of culturing cells in which additional components are provided continuously or semi-continuously to the culture subsequent to the beginning of the culture process. The provided components typically comprise nutritional supplements for the cells which have been depleted during the culturing process. A portion of the cells and/or components in the medium are typically harvested on a continuous or semi-continuous basis and are optionally purified.

- [0045]** The terms "polypeptide" or "protein" as used herein refers a sequential chain of amino acids linked together via peptide bonds. The term is used to refer to an amino acid chain of any length, but one of ordinary skill in the art will understand that the term is not limited to lengthy chains and can refer to a minimal chain comprising two amino acids linked together via a peptide bond. If a single polypeptide is the discrete functioning unit and does require permanent physical association with other polypeptides in order to form the discrete functioning unit, the terms "polypeptide" and "protein" as used herein are used interchangeably. If discrete functional unit is comprised of more than one polypeptide that physically associate with one another, the term "protein" as used herein refers to the multiple polypeptides that are physically coupled and function together as the discrete unit.
- [0046]** "Recombinantly expressed polypeptide" and "recombinant polypeptide" as used herein refer to a polypeptide expressed from a host cell that has been genetically engineered to express that polypeptide. The recombinantly expressed polypeptide can be identical or similar to polypeptides that are normally expressed in the mammalian host cell. The recombinantly expressed polypeptide can also foreign to the host cell, i.e. heterologous to peptides normally expressed in the mammalian host cell. Alternatively, the recombinantly expressed polypeptide can be chimeric in that portions of the polypeptide contain amino acid sequences that are identical or similar to polypeptides normally expressed in the mammalian host cell, while other portions are foreign to the host cell. As used herein, the terms "recombinantly expressed polypeptide" and "recombinant polypeptide" also encompasses an antibody produced by a hybridoma.
- [0047]** The term "seeding" as used herein refers to the process of providing a cell culture to a bioreactor or another vessel. In one embodiment, the cells have been propagated previously in another bioreactor or vessel. In another embodiment, the cells have been frozen and thawed immediately prior to providing them to the bioreactor or vessel. The term refers to any number of cells, including a single cell.
- [0048]** The term "titer" as used herein refers to the total amount of recombinantly expressed polypeptide or protein produced by a cell culture divided by a given amount of medium volume. Titer is typically expressed in units of milligrams of polypeptide or protein per milliliter of medium or in units of grams of polypeptide or protein per liter of medium.

[0049] The term "shake flask" as used herein refers to any vessel used for the growth of a cell culture (e.g., a mammalian cell culture as described herein) that does not have an impeller.

[0050] As used in the present disclosure and claims, the singular forms "a", "an", and "the" include plural forms unless the context clearly dictates otherwise.

II. Cell culture medium and methods of using thereof

[0051] The present invention generally relates to cell culture media and methods of use thereof. A medium according to the invention can be used in a batch culture, fed-batch culture, a perfusion culture, a shake flask culture, or a bioreactor. In one embodiment, a medium of the invention is a basal medium. In another embodiment, a medium of the invention is a feed medium.

Cell culture medium comprising a bromodomain inhibitor

[0052] In one embodiment, a medium according to the invention comprises a compound such as a bromodomain inhibitor, which can enhance product titer, cell specific productivity, cell viability, and/or viable cell density in a cell culture containing the medium.

[0053] In one embodiment, a medium according to the invention comprises a bromodomain inhibitor. In one embodiment, the bromodomain inhibitor is present in the cell culture medium in a concentration ranging from about 1 uM to about 100 uM, e.g., from about 1 uM to about 75 uM, about 1 uM to about 50 uM, about 1 uM to about 30 uM, about 1 uM to about 10 uM, about 1 uM to about 3 uM, about 3 uM to about 100 uM, about 3 uM to about 75 uM, about 3 uM to about 50 uM, about 3 uM to about 30 uM, about 3 uM to about 10 uM, about 10 uM to about 100 uM, about 10 uM to about 75 uM, about 10 uM to about 50 uM, about 10 uM to about 30 uM, about 30 uM to about 100 uM, about 30 uM to about 75 uM, about 30 uM to about 50 uM, about 50 uM to about 100 uM, about 50 uM to about 75 uM, or about 75 uM to about 100 uM. In one embodiment, the bromodomain inhibitor is present in the cell culture medium in a concentration of about 1 uM, about 3 uM, about 10 uM, about 30 uM, about 50 uM, about 75 uM, or about 100 uM. In one embodiment, the bromodomain inhibitor can also be present in the cell culture medium in a concentration less than about 1 uM (e.g., about 0.5 uM, about 0.1 uM, about 0.01 uM, or any ranges between the specified values). In one

embodiment, the bromodomain inhibitor can also be present in the cell culture medium in a concentration greater than about 100 uM (e.g., about 120 uM, about 200 uM, about 400 uM, about 600 uM, about 1000 uM, or any ranges between the specified values).

[0054] In one embodiment, the bromodomain inhibitor is present in the cell culture medium in a concentration sufficient, when culturing mammalian cells in a fed-batch mode for 14 days (e.g., under the culturing conditions specified in the Examples section herein), to produce a polypeptide of interest at a titer at least about 15% (e.g., at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 100%, or at least about 200%) greater than the titer produced from a control cell culture that does not contain a bromodomain inhibitor. In one embodiment, the bromodomain inhibitor is present in the cell culture medium in a concentration sufficient, when culturing mammalian cells in a fed-batch mode for 14 days (e.g., under the culturing conditions specified in the Examples section herein), to produce a polypeptide of interest at a titer of about 15% to about 100% (e.g., about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 100%, or any ranges between the specified values) greater than the titer produced from a control cell culture that does not contain a bromodomain inhibitor.

[0055] In one embodiment, the bromodomain inhibitor is present in the cell culture medium in a concentration sufficient, when culturing mammalian cells in a fed-batch mode for 14 days (e.g., under the culturing conditions specified in the Examples section herein), to generate a viable cell density of at least about 20% (e.g., at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 100%, or at least about 200%) higher than that observed for a control cell culture that does not contain a bromodomain inhibitor. In one embodiment, the bromodomain inhibitor is present in the cell culture medium in a concentration sufficient, when culturing mammalian cells in a fed-batch mode for 14 days (e.g., under the culturing conditions specified in the Examples section herein), to generate a viable cell density of about 20% to about 100% (e.g., about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 100%, or any ranges between the specified values) higher than that observed for a control cell culture that does not contain a bromodomain inhibitor.

- [0056]** In one embodiment, the bromodomain inhibitor is present in the cell culture medium in a concentration sufficient, when culturing mammalian cells in a fed-batch mode for 14 days (e.g., under the culturing conditions specified in the Examples section herein), to generate a cell specific productivity of at least about 10% (e.g., at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or at least about 100%) higher than that observed for a control cell culture that does not contain a bromodomain inhibitor. In one embodiment, the bromodomain inhibitor is present in the cell culture medium in a concentration sufficient, when culturing mammalian cells in a fed-batch mode for 14 days (e.g., under the culturing conditions specified in the Examples section herein), to generate a cell specific productivity of about 10% to about 60% (e.g., about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, or any ranges between the specified values) greater than that observed for a control cell culture that does not contain a bromodomain inhibitor.
- [0057]** In one embodiment, the bromodomain inhibitor is present in the cell culture medium in a concentration sufficient, when culturing mammalian cells in a fed-batch mode for 14 days (e.g., under the culturing conditions specified in the Examples section herein), to achieve a cell viability of at least about 10% (e.g., at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or at least about 100%) higher than that observed for a control cell culture that does not contain a bromodomain inhibitor. In one embodiment, the bromodomain inhibitor is present in the cell culture medium in a concentration sufficient, when culturing mammalian cells in a fed-batch mode for 14 days (e.g., under the culturing conditions specified in the Examples section herein), to achieve a cell viability of about 10% to about 60% (e.g., about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, or any ranges between the specified values) greater than that observed for a control cell culture that does not contain a bromodomain inhibitor.
- [0058]** In one embodiment, the medium comprising a bromodomain inhibitor is a feed medium. In one embodiment, the bromodomain inhibitor is present in the feed medium in a sufficient concentration such that when added to a culture, the bromodomain inhibitor is present in the culture in a concentration ranging from about 1 μ M to about 100 μ M, e.g.,

from about 1 uM to about 75 uM, about 1 uM to about 50 uM, about 1 uM to about 30 uM, about 1 uM to about 10 uM, about 1 uM to about 3 uM, about 3 uM to about 100 uM, about 3 uM to about 75 uM, about 3 uM to about 50 uM, about 3 uM to about 30 uM, about 3 uM to about 10 uM, about 10 uM to about 100 uM, about 10 uM to about 75 uM, about 10 uM to about 50 uM, about 10 uM to about 30 uM, about 30 uM to about 100 uM, about 30 uM to about 75 uM, about 30 uM to about 50 uM, about 50 uM to about 100 uM, about 50 uM to about 75 uM, or about 75 uM to about 100 uM. For example, the bromodomain inhibitor can be added to reach a concentration in the culture of about 1 uM, about 3 uM, about 10 uM, about 30 uM, about 50 uM, about 75 uM, or about 100 uM. In one embodiment, the bromodomain inhibitor can be added to reach a concentration in the culture of less than about 1 uM (e.g., about 0.5 uM, about 0.1 uM, about 0.01 uM, or any ranges between the specified values). In one embodiment, the bromodomain inhibitor can be added to reach a concentration in the culture of greater than about 100 uM (e.g., about 120 uM, about 200 uM, about 400 uM, about 600 uM, about 1000 uM, or any ranges between the specified values).

[0059] In one embodiment, the bromodomain inhibitor is present in the feed medium in a sufficient concentration such that when added to a culture, the bromodomain inhibitor is present in the culture in a concentration sufficient, when culturing mammalian cells in a fed-batch mode for 14 days (e.g., under the culturing conditions specified in the Examples section herein), to produce a protein or polypeptide of interest at a titer at least about 15% (e.g., at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 100%, or at least about 200%) greater than the titer produced from a control cell culture that does not contain a bromodomain inhibitor. For example, the bromodomain inhibitor can be added to reach a concentration in the culture sufficient, when culturing mammalian cells in a fed-batch mode for 14 days (e.g., under the culturing conditions specified in the Examples section herein), to produce a protein or polypeptide of interest at a titer of about 15% to about 100% (e.g., about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 100%, or any ranges between the specified values) greater than the titer produced from a control cell culture that does not contain a bromodomain inhibitor.

[0060] In one embodiment, the bromodomain inhibitor is present in the feed medium in a sufficient concentration such that when added to a culture, the bromodomain inhibitor is present in the culture in a concentration sufficient, when culturing mammalian cells in a fed-batch mode for 14 days (e.g., under the culturing conditions specified in the Examples section herein), to generate a viable cell density of at least about 20% (e.g., at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 100%, or at least about 200%) higher than that observed for a control cell culture that does not contain a bromodomain inhibitor. For example, the bromodomain inhibitor can be added to reach a concentration in the culture sufficient, when culturing mammalian cells in a fed-batch mode for 14 days (e.g., under the culturing conditions specified in the Examples section herein), to generate a viable cell density of about 20% to about 100% (e.g., about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 100%, or any ranges between the specified values) higher than that observed for a control cell culture that does not contain a bromodomain inhibitor.

[0061] In one embodiment, the bromodomain inhibitor is present in the feed medium in a sufficient concentration such that when added to a culture, the bromodomain inhibitor is present in the culture in a concentration sufficient, when culturing mammalian cells in a fed-batch mode for 14 days (e.g., under the culturing conditions specified in the Examples section herein), to generate a cell specific productivity of at least about 10% (e.g., at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or at least about 100%) higher than that observed for a control cell culture that does not contain a bromodomain inhibitor. For example, the bromodomain inhibitor can be added to reach a concentration in the culture sufficient, when culturing mammalian cells in a fed-batch mode for 14 days (e.g., under the culturing conditions specified in the Examples section herein), to generate a cell specific productivity of about 10% to about 60% (e.g., about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, or any ranges between the specified values) greater than that observed for a control cell culture that does not contain a bromodomain inhibitor.

[0062] In one embodiment, the bromodomain inhibitor is present in the feed medium in a sufficient concentration such that when added to a culture, the bromodomain inhibitor is

present in the culture in a concentration sufficient, when culturing mammalian cells in a fed-batch mode for 14 days (e.g., under the culturing conditions specified in the Examples section herein), to achieve a cell viability of at least about 10% (e.g., at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or at least about 100%) higher than that observed for a control cell culture that does not contain a bromodomain inhibitor. For example, the bromodomain inhibitor can be added to reach a concentration in the culture sufficient, when culturing mammalian cells in a fed-batch mode for 14 days (e.g., under the culturing conditions specified in the Examples section herein), to achieve a cell viability of about 10% to about 60% (e.g., about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, or any ranges between the specified values) greater than that observed for a control cell culture that does not contain a bromodomain inhibitor.

[0063] In one embodiment, the bromodomain inhibitor is added to a cell culture medium at least one time, when culturing mammalian cells in a fed-batch mode. In one embodiment, the bromodomain inhibitor is added to the cell culture medium one time, when culturing mammalian cells in a fed-batch mode. As a non-limiting example, the bromodomain inhibitor can be added to a cell culture medium at day 5, day 6, at day 7, at day 8, at day 9, at day 10, at day 11, or at day 12, when culturing mammalian cells in a fed-batch mode for 14 days. In some embodiment, the bromodomain inhibitor can be added at day 8 or day 9 to reach a concentration in the culture sufficient, when culturing mammalian cells in a fed-batch mode for 14 days, to achieve a cell viability of about 10% to about 60% (e.g., about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, or any ranges between the specified values) greater than that observed for a control cell culture that does not contain a bromodomain inhibitor.

[0064] In one embodiment, the bromodomain inhibitor is added to a cell culture medium at least two times, when culturing mammalian cells in a fed-batch mode. In one embodiment, the bromodomain inhibitor is added to the cell culture medium two times, when culturing mammalian cells in a fed-batch mode. As a non-limiting example, the bromodomain inhibitor can be added to a cell culture medium at day 5 and day 12, at day 5 and day 11, at day 5 and day 10, at day 5 and day 9, at day 5 and day 8, at day 5 and day 7, at day 5 and day 6, at day 6 and day 12, at day 6 and day 11, at day 6 and day 10, at

day 6 and day 9, at day 6 and day 8, at day 6 and day 7, at day 7 and day 12, at day 7 and day 11, at day 7 and day 10, at day 7 and day 9, at day 7 and day 8, at day 8 and day 12, at day 8 and day 11, at day 8 and day 10, at day 8 and day 9, at day 9 and day 12, at day 9 and day 11, at day 9 and day 10, or at day 10 and day 11, when culturing mammalian cells in a fed-batch mode for 14 days. In one embodiment, the bromodomain inhibitor can be added to cell culture medium at day 8 and day 11, at day 8 and day 10, or at day 9 and day 12, when culturing mammalian cells in a fed-batch mode for 14 days. In one embodiment, the bromodomain inhibitor can be added at day 8 and day 11 to reach a concentration in the culture sufficient, when culturing mammalian cells in a fed-batch mode for 14 days (e.g., under the culturing conditions specified in the Examples section herein), to achieve a cell viability of about 10% to about 60% (e.g., about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, or any ranges between the specified values) greater than that observed for a control cell culture that does not contain a bromodomain inhibitor.

[0065] In one embodiment, the bromodomain inhibitor is added to a cell culture medium more than two times, when culturing mammalian cells in a fed-batch mode. For example, the bromodomain inhibitor can be added at least three times, at least four times, at least five times, at least six times, or at least seven times, to reach a concentration in the culture sufficient, when culturing mammalian cells in a fed-batch mode for 14 days, to achieve a cell viability of about 10% to about 60% (e.g., about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, or any ranges between the specified values) greater than that observed for a control cell culture that does not contain a bromodomain inhibitor.

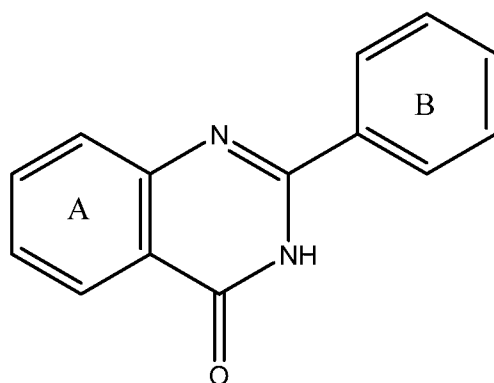
[0066] As understood by those skilled in the art, the amount of the bromodomain inhibitor present in a cell culture can vary. In one embodiment (e.g., for certain cell lines) one concentration will be useful to provide the desired cell culture modulation; in other cases (e.g., other cell lines) a higher or lower concentration that used in conjunction with a different cell line will be more useful.

[0067] In one embodiment, when a combination of bromodomain inhibitors are employed, the concentrations of each bromodomain inhibitor in the combination can be the same or they can vary relative to one another. Having provided representative concentrations, those of skill in the art can readily determine appropriate concentrations

of single and combinations of bromodomain inhibitors that can be employed in the media and methods described herein.

[0068] Bromodomain inhibitors useful for the present invention include any of those known in the art. In one embodiment, the bromodomain inhibitor is a BRD2 inhibitor (e.g., RVX-208). In one embodiment, the bromodomain inhibitor is a BRD4 inhibitor (e.g., JQ-1, CPI-203, PFI-1). In one embodiment, the bromodomain inhibitor is a BRD2, BRD3, and BRD4 inhibitor (e.g., I-BET-762, OTX015, I-BET151). In one embodiment, the bromodomain inhibitor is a broad spectrum BRD inhibitor (e.g., bromosporine). In one embodiment, the bromodomain inhibitor is a CBP/p300 bromodomain inhibitor (e.g., SGC-CBP30, I-CBP112)). Suitable concentrations of the bromodomain inhibitors in the culture medium are described herein.

[0069] In one embodiment, the cell culture medium comprises a bromodomain inhibitor



of Formula I:

(Formula I),

wherein ring A is optionally substituted with 1 to 4 R^{10} , each independently selected from the group consisting of halogen, OH, C_{1-4} alkyl, and C_{1-4} alkoxy, wherein the C_{1-4} alkyl and C_{1-4} alkoxy are optionally substituted with 1-3 halogens; and ring B is optionally substituted with 1 to 5 R^{11} each independently selected from the group consisting of OH, halogen, C_{1-4} alkyl, C_{1-4} alkoxy, wherein the C_{1-4} alkyl and C_{1-4} alkoxy are optionally substituted with 1-3 halogens or OR^{100} , wherein R^{100} is H or a C_{1-4} alkyl, optionally substituted with OH, OMe, $O(CH_2)_2-OH$, or $O(CH_2)_2-OMe$.

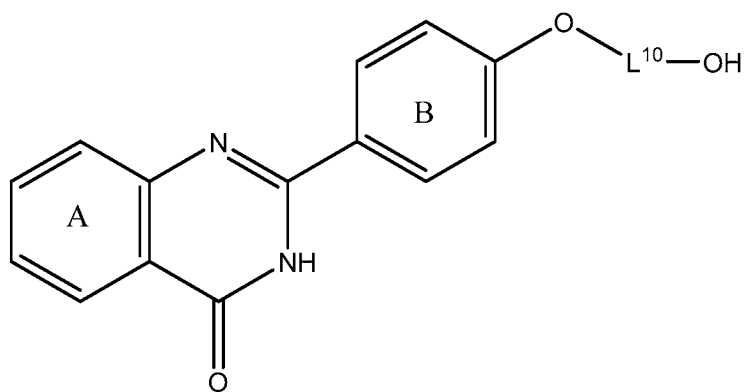
[0070] In one embodiment, the bromodomain inhibitor of Formula I can be in the form of a tautomer, a stereoisomeric mixture, or a salt. In one embodiment, the bromodomain inhibitor of Formula I has an enantiomeric purity of about 80% ee or more, e.g., about 80% ee, about 85% ee, about 90% ee, about 91% ee, about 92% ee, about 93% ee, about

94% ee, about 95% ee, about 96% ee, about 97% ee, about 98% ee, about 99% ee, about 99.5% ee or more.

[0071] In one embodiment, ring A in Formula I is not substituted with R^{10} . In another embodiment, ring A in Formula I is substituted with 1, 2, 3, or 4 R^{10} . For example, in one embodiment, ring A in Formula I is substituted with 2 R^{10} . In any of the embodiments described herein, R^{10} at each occurrence can be independently selected from the group consisting of F, Cl, OH, C_{1-4} alkyl (e.g., Me, Et, n-propyl, isopropyl, n-butyl, sec-butyl, isobutyl, or tert-butyl), and C_{1-4} alkoxy (e.g., MeO, EtO, n-propoxy, isopropoxy, n-butoxy, sec-butoxy, isobutoxy, or tert-butoxy), wherein the C_{1-4} alkyl and C_{1-4} alkoxy are not substituted with halogens.

[0072] In one embodiment, ring B in Formula I is not substituted with R^{11} . In another embodiment, ring B in Formula I is substituted with 1, 2, 3, 4, or 5 R^{11} . For example, in one embodiment, ring B in Formula I is substituted with 2 or 3 R^{11} . In any of the embodiments described herein, R^{11} at each occurrence can be independently selected from the group consisting of F, Cl, OH, C_{1-4} alkyl (e.g., Me, Et, n-propyl, isopropyl, n-butyl, sec-butyl, isobutyl, or tert-butyl), and C_{1-4} alkoxy (e.g., MeO, EtO, n-propoxy, isopropoxy, n-butoxy, sec-butoxy, isobutoxy, or tert-butoxy), wherein the C_{1-4} alkyl and C_{1-4} alkoxy are not substituted with halogens or OR^{100} .

[0073] In one embodiment, the cell culture medium comprises a bromodomain inhibitor



of Formula Ia

(Formula Ia),

wherein ring A is optionally substituted with 1 or 2 R^{10} , each independently selected from the group consisting of halogen, C_{1-4} alkyl, and C_{1-4} alkoxy;
 ring B is optionally substituted with 1 or 2 R^{11} , each independently selected from the group consisting of halogen and C_{1-4} alkyl; and
 L^{10} is a C_{2-4} alkylene linker.

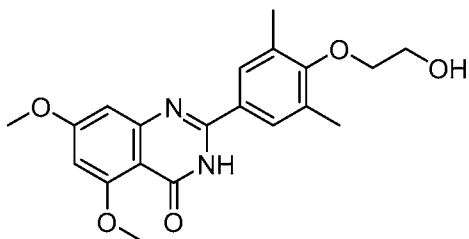
[0074] In one embodiment, the bromodomain inhibitor of Formula Ia can be in the form of a tautomer, a stereoisomeric mixture, or a salt.

[0075] In one embodiment, ring A in Formula Ia is not substituted with R^{10} . In another embodiment, ring A in Formula Ia is substituted with 1 or 2 R^{10} , each independently selected from the group consisting of F, Cl, Me, Et, OMe, and OEt. In a specific embodiment, ring A in Formula Ia is substituted with 1 or 2 methoxyl groups.

[0076] In one embodiment, ring B in Formula Ia is not substituted with R^{11} . In another embodiment, ring B in Formula Ia is substituted with 1 or 2 R^{11} , each independently selected from the group consisting of F, Cl, Me, and Et. In a specific embodiment, ring B in Formula Ia is substituted with 1 or 2 methyl groups.

[0077] In one embodiment, L^{10} is CH_2CH_2 . In one embodiment, L^{10} is $CH_2CH_2CH_2$. In one embodiment, L^{10} is $CH_2CH_2CH_2CH_2$.

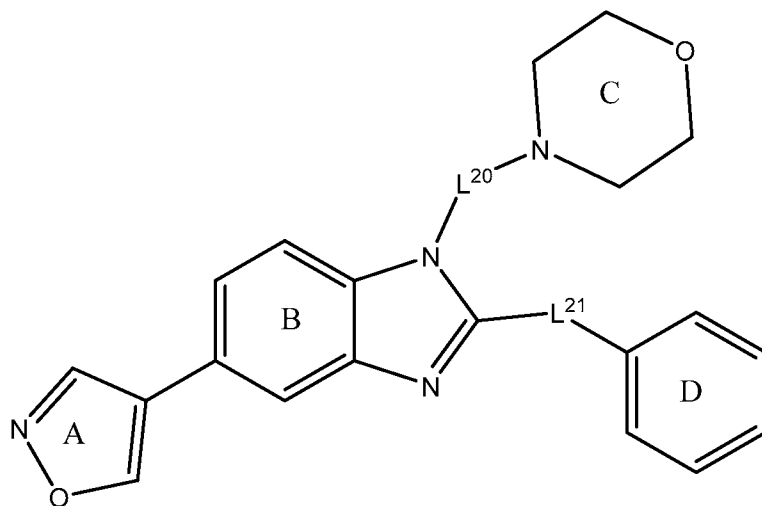
[0078] In one embodiment, the cell culture medium comprises RVX-208,



(RVX-208). In one embodiment, the only

bromodomain inhibitor present in the cell culture medium is RVX-208. In one embodiment, the cell culture medium comprises RVX-208 and at least one additional bromodomain inhibitor.

[0079] In one embodiment, the cell culture medium comprises a bromodomain inhibitor of Formula II:



(Formula II)

wherein ring A is optionally substituted with 1 or 2 R^{20} , each independently selected from the group consisting of halogen and C_{1-4} alkyl, wherein the C_{1-4} alkyl is optionally substituted with 1-3 halogens;

ring B is optionally substituted with 1-3 R^{21} , each independently selected from the group consisting of halogen and C_{1-4} alkyl, wherein the C_{1-4} alkyl is optionally substituted with 1-3 halogens;

ring C is optionally substituted with 1-3 R^{22} , each independently selected from the group consisting of halogen and C_{1-4} alkyl, wherein the C_{1-4} alkyl is optionally substituted with 1-3 halogens;

ring D is optionally substituted with 1-5 R^{23} , each independently selected from the group consisting of halogen, OH, C_{1-4} alkyl, and C_{1-4} alkoxy, wherein the C_{1-4} alkyl and C_{1-4} alkoxy are optionally substituted with 1-3 halogens;

L^{20} is a C_{2-4} alkylene linker, optionally substituted with 1 or 2 C_{1-4} alkyl, wherein the C_{1-4} alkyl is optionally substituted with 1-3 halogens; and

L^{21} is a C_{1-4} alkylene linker, optionally substituted with 1 or 2 C_{1-4} alkyl, wherein the C_{1-4} alkyl is optionally substituted with 1-3 halogens.

[0080] In one embodiment, the bromodomain inhibitor of Formula II can be in the form of a tautomer, a stereoisomeric mixture, or a salt. In one embodiment, the bromodomain inhibitor of Formula II has an enantiomeric purity of about 80% ee or more, e.g., about 80% ee, about 85% ee, about 90% ee, about 91% ee, about 92% ee, about 93% ee, about 94% ee, about 95% ee, about 96% ee, about 97% ee, about 98% ee, about 99% ee, about 99.5% ee or more.

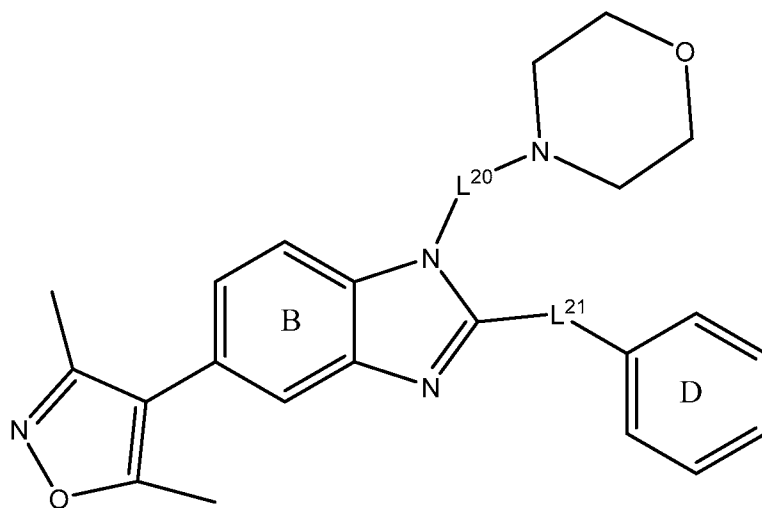
[0081] In one embodiment, ring A of Formula II is not substituted with R^{20} . In one embodiment, ring A of Formula II is substituted with 1 or 2 R^{20} . Preferably, ring A of Formula II is substituted with two methyl groups.

[0082] In one embodiment, ring B of Formula II is not substituted with R^{21} . In one embodiment, ring B of Formula II is substituted with 1, 2, or 3 R^{21} , each independently selected from the group consisting of F, Cl, Me, Et, OMe, and OEt.

[0083] In one embodiment, ring C of Formula II is not substituted with R^{22} . In one embodiment, ring C of Formula II is substituted with 1, 2, or 3 R^{22} . In one embodiment, two R^{22} can be substituted on the same carbon.

[0084] In one embodiment, ring D of Formula II is not substituted with R^{23} . In one embodiment, ring D of Formula II is substituted with 1, 2, 3, 4, or 5 R^{23} . For example, ring D of Formula II is substituted with 1 or 2 R^{23} , each independently selected from the group consisting of halogen, C_{1-4} alkyl, and C_{1-4} alkoxy.

[0085] In one embodiment, the cell culture medium comprises a bromodomain inhibitor of Formula IIa:



(Formula IIa),

wherein ring B is optionally substituted with 1 or 2 R^{21} , each independently selected from the group consisting of halogen and C_{1-4} alkyl;

ring D is optionally substituted with 1 or 2 R^{23} , each independently selected from the group consisting of halogen, C_{1-4} alkyl, and C_{1-4} alkoxy,

L^{20} is a C_{2-4} alkylene linker, optionally substituted with one methyl or ethyl group; and

L^{21} is a C_{1-4} alkylene linker, optionally substituted with one methyl or ethyl group.

[0086] In one embodiment, the bromodomain inhibitor of Formula IIa can be in the form of a tautomer, a stereoisomeric mixture, or a salt. In one embodiment, the bromodomain inhibitor of Formula IIa has an enantiomeric purity of about 80% ee or more, e.g., about 80% ee, about 85% ee, about 90% ee, about 91% ee, about 92% ee, about 93% ee, about 94% ee, about 95% ee, about 96% ee, about 97% ee, about 98% ee, about 99% ee, about 99.5% ee or more.

[0087] In one embodiment, ring B of Formula IIa is not substituted with R^{21} .

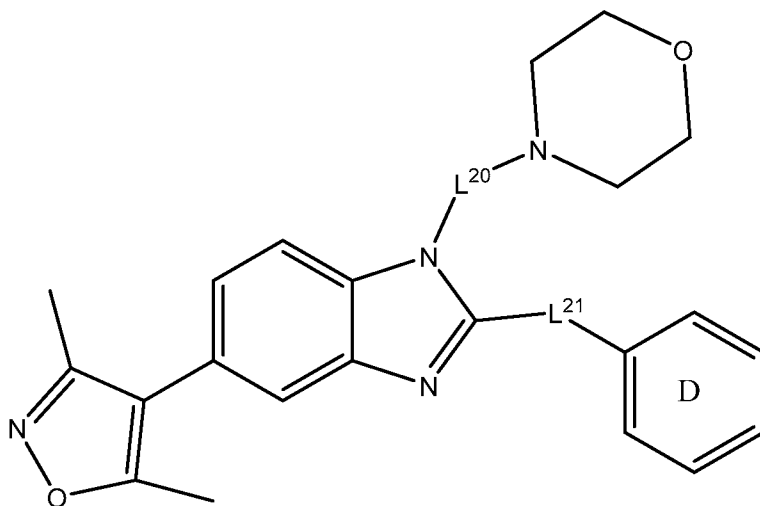
[0088] In one embodiment, ring D of Formula IIa is substituted with 1 or 2 R^{23} , each independently selected from the group consisting of halogen, C_{1-4} alkyl, and C_{1-4} alkoxy.

[0089] In one embodiment, L^{20} of Formula IIa is an unsubstituted C_{2-4} alkylene linker, e.g., CH_2CH_2 , $CH_2CH_2CH_2$, or $CH_2CH_2CH_2CH_2$. In one embodiment, L^{20} of Formula IIa

is C₂₋₄ alkylene linker which is substituted with one methyl or ethyl group, e.g., CH(Me)CH₂, wherein the CH₂ can be directly attached to the nitrogen atom of the morpholine ring or the nitrogen atom of the benzoimidazole ring.

[0090] In one embodiment, L²¹ of Formula IIa is an unsubstituted C₁₋₄ alkylene linker, e.g., CH₂, CH₂CH₂, CH₂CH₂CH₂, or CH₂CH₂CH₂CH₂. In one embodiment, L²¹ of Formula IIa is C₁₋₄ alkylene linker which is substituted with one methyl or ethyl group, e.g., CH(Me)CH₂, wherein the CH₂ can be directly attached to the carbon atom on ring D or the carbon atom of the benzoimidazole ring.

[0091] In one embodiment, the cell culture medium comprises a bromodomain inhibitor of Formula IIb:



(Formula IIb),

wherein ring D is substituted with 1 or 2 R²³, each independently selected from the group consisting of F, Cl, Me, Et, OMe, and OEt,

L²⁰ is a C₂₋₄ alkylene linker, optionally substituted with one methyl group; and

L²¹ is a C₁₋₄ alkylene linker, optionally substituted with one methyl group.

[0092] In one embodiment, the bromodomain inhibitor of Formula IIb can be in the form of a tautomer, a stereoisomeric mixture, or a salt. In one embodiment, the bromodomain inhibitor of Formula IIb has an enantiomeric purity of about 80% ee or more, e.g., about 80% ee, about 85% ee, about 90% ee, about 91% ee, about 92% ee, about 93% ee, about 94% ee, about 95% ee, about 96% ee, about 97% ee, about 98% ee, about 99% ee, about 99.5% ee or more.

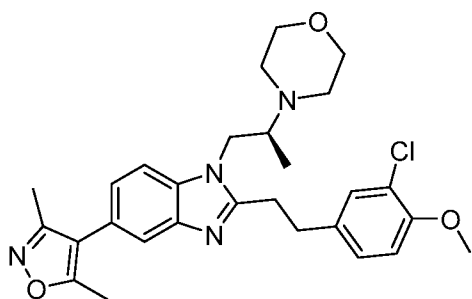
[0093] In one embodiment, ring D is substituted with 2 R²³, each independently selected from the group consisting of F, Cl, Me, Et, OMe, and OEt, wherein the two R²³ can be

ortho, meta, or para to each other. Preferably, the two R^{23} are ortho to each other, and neither of the two R^{23} is ortho to L^{21} .

[0094] In one embodiment, L^{20} of Formula IIb is an unsubstituted C_{2-4} alkylene linker, e.g., CH_2CH_2 , $CH_2CH_2CH_2$, or $CH_2CH_2CH_2CH_2$. In one embodiment, L^{20} of Formula IIb is $CH(Me)CH_2$, wherein the CH_2 can be directly attached to the nitrogen atom of the morpholine ring or the nitrogen atom of the benzoimidazole ring.

[0095] In one embodiment, L^{21} of Formula IIb is an unsubstituted C_{1-4} alkylene linker, e.g., CH_2 , CH_2CH_2 , $CH_2CH_2CH_2$, or $CH_2CH_2CH_2CH_2$. In one embodiment, L^{21} of Formula IIb is $CH(Me)CH_2$, wherein the CH_2 can be directly attached to the carbon atom of ring D or the carbon atom of the benzoimidazole ring. Preferably, L^{21} of Formula IIb is CH_2CH_2 .

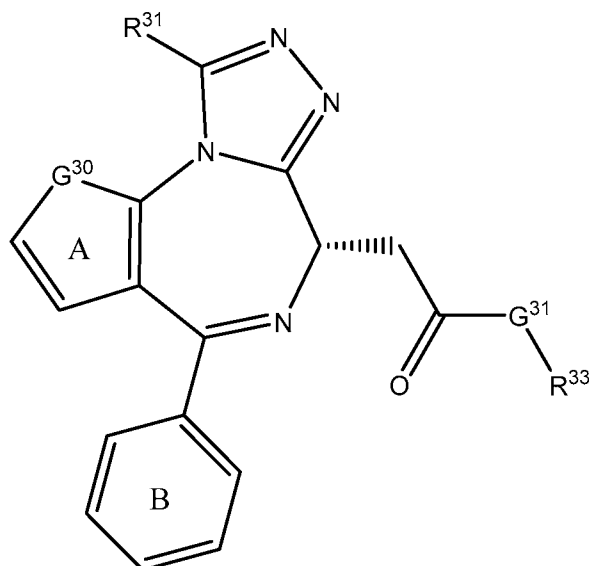
[0096] In one embodiment, the cell culture medium comprises SGC-CBP30:



(SGC-CBP30). In one embodiment, the SGC-

CBP30 has an enantiomeric purity of about 80% ee or more, e.g., about 80% ee, about 85% ee, about 90% ee, about 91% ee, about 92% ee, about 93% ee, about 94% ee, about 95% ee, about 96% ee, about 97% ee, about 98% ee, about 99% ee, about 99.5% ee or more. In one embodiment, the only bromodomain inhibitor present in the cell culture medium is SGC-CBP30. In one embodiment, the cell culture medium comprises SGC-CBP30 and at least one additional bromodomain inhibitor.

[0097] In one embodiment, the cell culture medium comprises a bromodomain inhibitor of Formula III:



(Formula III),

wherein G^{30} is S or C=C,

G^{31} is O or NR^{300} ,

ring A is optionally substituted with 1-4 R^{30} , each independently selected from the group consisting of halogen, C_{1-4} alkyl, and C_{1-4} alkoxy, wherein the C_{1-4} alkyl and C_{1-4} alkoxy are optionally substituted with 1-3 halogens;

R^{31} is H or a C_{1-4} alkyl, wherein the C_{1-4} alkyl is optionally substituted with 1-3 halogens;

ring B is optionally substituted with 1-5 R^{32} , each independently selected from the group consisting of halogen and C_{1-4} alkyl, wherein the C_{1-4} alkyl is optionally substituted with 1-3 halogens; and

R^{33} is H or a C_{1-4} alkyl, wherein the C_{1-4} alkyl is optionally substituted with 1-3 halogens, wherein R^{300} is H, a C_{1-4} alkyl optionally substituted with 1-3 halogens, or a phenyl optionally substituted with 1-5 substituents selected from the group consisting of halogen, OH, C_{1-4} alkyl, and C_{1-4} alkoxy, wherein the C_{1-4} alkyl and C_{1-4} alkoxy are optionally substituted with 1-3 halogens.

[0098] In one embodiment, the bromodomain inhibitor of Formula III can be in the form of a tautomer, a stereoisomeric mixture, or a salt. In one embodiment, the bromodomain inhibitor of Formula III has an enantiomeric purity of about 80% ee or more, e.g., about 80% ee, about 85% ee, about 90% ee, about 91% ee, about 92% ee, about 93% ee, about 94% ee, about 95% ee, about 96% ee, about 97% ee, about 98% ee, about 99% ee, about 99.5% ee or more.

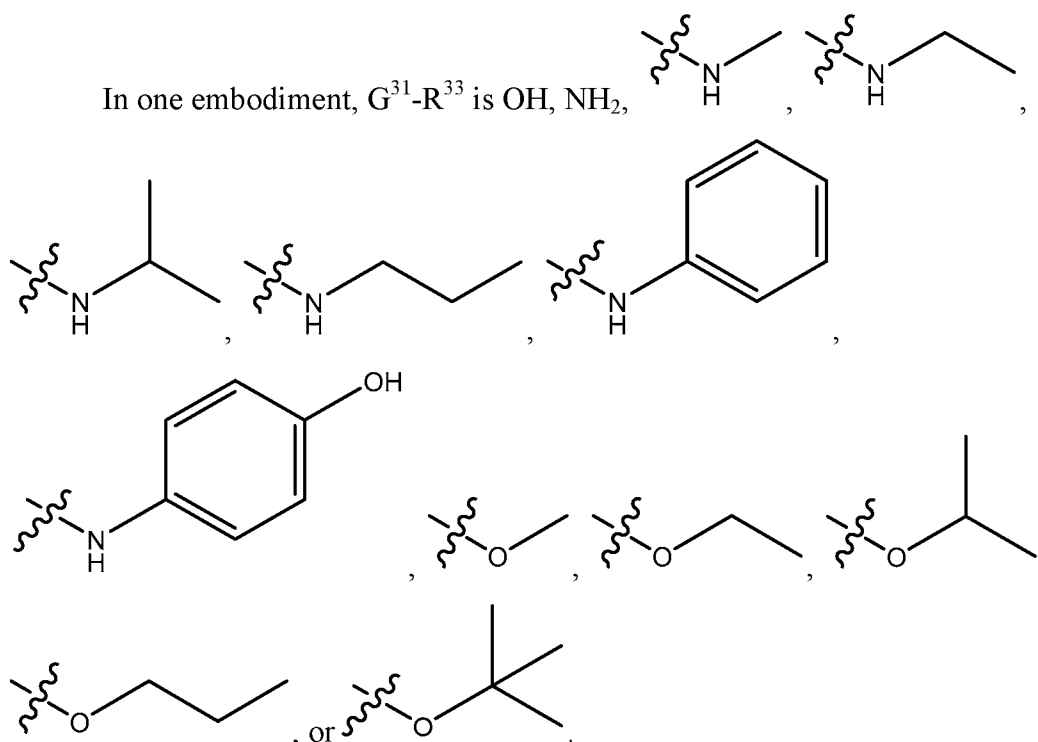
[0099] In one embodiment, G^{30} is S. In another embodiment, G^{30} is C=C.

[0100] In one embodiment, ring A of Formula III is not substituted with R^{30} . In one embodiment, ring A of Formula III is substituted with 1 or 2 R^{30} , each independently selected from the group consisting of halogen (e.g., F, Cl), C_{1-4} alkyl, and C_{1-4} alkoxy. In one embodiment, G^{30} is S and R^{30} at each occurrence is independently a halogen or a C_{1-4} alkyl. In one embodiment, G^{30} is C=C and R^{30} at each occurrence is independently a halogen, a C_{1-4} alkyl, or a C_{1-4} alkoxy. In a specific embodiment, G^{30} is S and ring A of Formula III is substituted with two methyl groups. In another specific embodiment, G^{30} is C=C and ring A of Formula III is substituted with one methoxyl group.

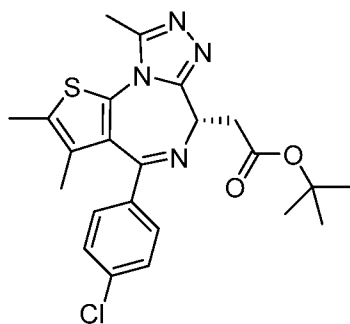
[0101] In one embodiment, R^{31} is Me.

[0102] In one embodiment, ring B of Formula III is not substituted with R^{32} . In one embodiment, ring B of Formula III is substituted with 1 or 2 R^{32} , each independently selected from the group consisting of halogen (e.g., F, Cl), C_{1-4} alkyl, and C_{1-4} alkoxy. In any of the embodiments described herein, R^{32} at each occurrence can be independently F, Cl, Me, or Et. In one embodiment, ring B of Formula III is substituted with one halogen (e.g., F, Cl). Preferably, ring B of Formula III is substituted with one Cl, e.g., at the para position to the diazepine ring.

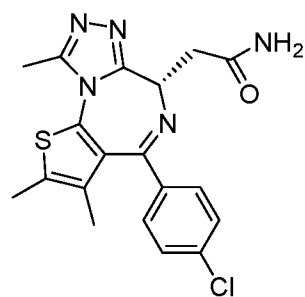
[0103] In one embodiment, G^{31} - R^{33} is OH, NH_2 ,



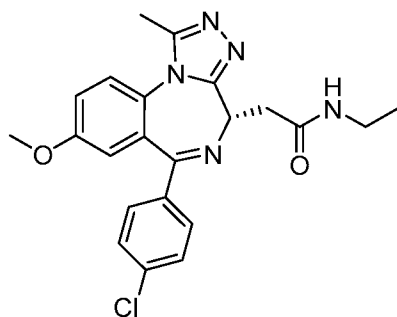
[0104] In one embodiment, the cell culture medium comprises JQ1, CPI-203, I-BET-762, and/or OTX-015:



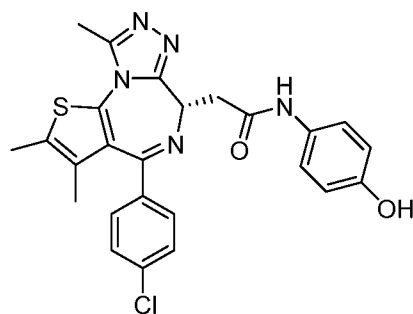
(JQ1),



(CPI-203),



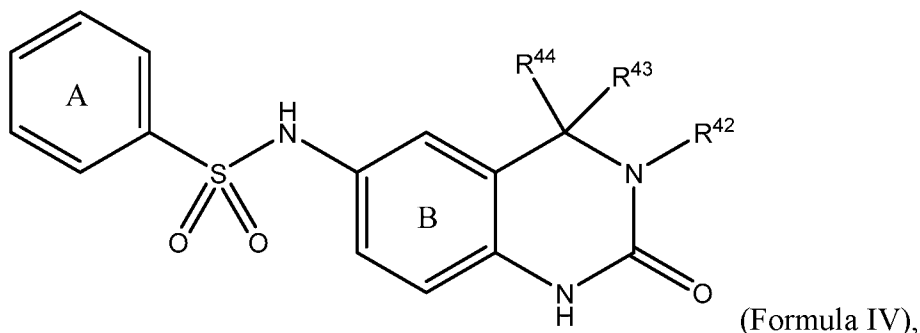
(I-BET-762),



(OTX-015). In one embodiment, JQ1, CPI-203, I-

BET-762, or OTX-015 can have an enantiomeric purity of about 80% ee or more, e.g., about 80% ee, about 85% ee, about 90% ee, about 91% ee, about 92% ee, about 93% ee, about 94% ee, about 95% ee, about 96% ee, about 97% ee, about 98% ee, about 99% ee, about 99.5% ee or more. In one embodiment, the only bromodomain inhibitor present in the cell culture medium is JQ1, CPI-203, I-BET-762, and/or OTX-015. In one embodiment, the cell culture medium comprises JQ1, CPI-203, I-BET-762, and/or OTX-015 and at least one additional bromodomain inhibitor.

[0105] In one embodiment, the cell culture medium comprises a bromodomain inhibitor of Formula IV:



wherein ring A is optionally substituted with 1-5 R^{40} , each independently selected from the group consisting of halogen, OH, C_{1-4} alkyl, and C_{1-4} alkoxy, wherein the C_{1-4} alkyl and C_{1-4} alkoxy are optionally substituted with 1-3 halogens;

ring B is optionally substituted with 1-3 R^{41} , each independently selected from the group consisting of halogen and C_{1-4} alkyl, wherein the C_{1-4} alkyl is optionally substituted with 1-3 halogens;

each of R^{42} , R^{43} , and R^{44} is independently selected from the group consisting of hydrogen and C_{1-4} alkyl, wherein the C_{1-4} alkyl is optionally substituted with 1-3 halogens.

[0106] In one embodiment, the bromodomain inhibitor of Formula IV can be in the form of a tautomer, a stereoisomeric mixture, or a salt. In one embodiment, the bromodomain inhibitor of Formula IV has an enantiomeric purity of about 80% ee or more, e.g., about 80% ee, about 85% ee, about 90% ee, about 91% ee, about 92% ee, about 93% ee, about 94% ee, about 95% ee, about 96% ee, about 97% ee, about 98% ee, about 99% ee, about 99.5% ee or more.

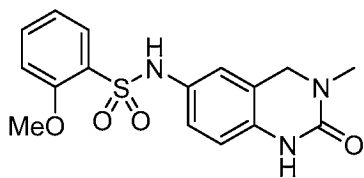
[0107] In one embodiment, ring A of Formula IV is not substituted with R^{40} . In another embodiment, ring A of Formula IV is substituted with 1 or 2 R^{40} , each independently selected from the group consisting of halogen (e.g., F, Cl), C_{1-4} alkyl (e.g., Me, Et), and C_{1-4} alkoxy (e.g., MeO). Preferably, ring A of Formula IV is substituted with one methoxyl group, e.g., at either of the ortho positions to the sulfonamide.

[0108] In one embodiment, ring B of Formula IV is not substituted with R^{41} . In one embodiment, ring B of Formula IV is substituted with 1 or 2 R^{41} , each independently selected from the group consisting of halogen (e.g., F, Cl), and C_{1-4} alkyl (e.g., Me, Et).

[0109] In one embodiment, R^{42} is Me or Et. Preferably, R^{42} is Me.

[0110] In one embodiment, R^{43} and R^{44} are each independently H or a C_{1-4} alkyl (e.g., Me, Et). For example, R^{43} and R^{44} can both be H.

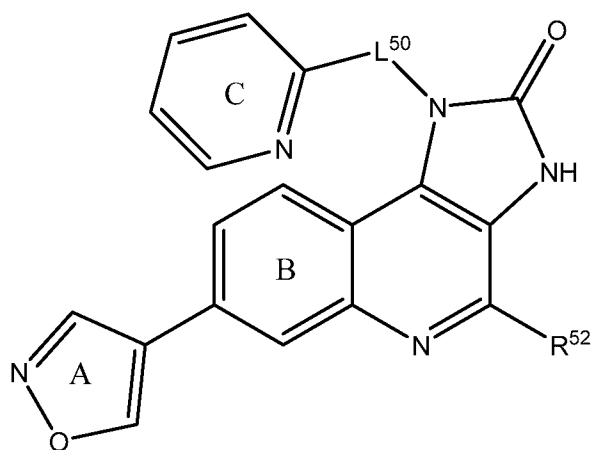
[0111] In one embodiment, the cell culture medium comprises PFI-1:



(PFI-1). In one embodiment, the only bromodomain

inhibitor present in the cell culture medium is PFI-1. In one embodiment, the cell culture medium comprises PFI-1 and at least one additional bromodomain inhibitor.

[0112] In one embodiment, the cell culture medium comprises a bromodomain inhibitor of Formula V:



(Formula V),

wherein ring A is optionally substituted with 1 or 2 R^{50} , each independently selected from the group consisting of halogen and C_{1-4} alkyl, wherein the C_{1-4} alkyl is optionally substituted with 1-3 halogens;

ring B is optionally substituted with 1-3 R^{51} , each independently selected from the group consisting of halogen, OH, C_{1-4} alkyl, and C_{1-4} alkoxy, wherein the C_{1-4} alkyl and C_{1-4} alkoxy are optionally substituted with 1-3 halogens;

R^{52} is H or a C_{1-4} alkyl, wherein the C_{1-4} alkyl is optionally substituted with 1-3 halogens;

ring C is optionally substituted with 1-4 R^{53} , each independently selected from the group consisting of halogen and C_{1-4} alkyl, wherein the C_{1-4} alkyl is optionally substituted with 1-3 halogens; and

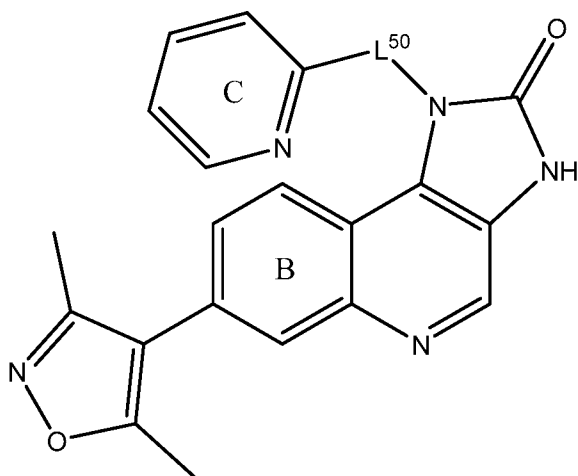
L^{50} is a C_{1-4} alkylene linker, optionally substituted with a C_{1-4} alkyl, wherein the C_{1-4} alkyl is optionally substituted with 1-3 halogens.

[0113] In one embodiment, the bromodomain inhibitor of Formula V can be in the form of a tautomer, a stereoisomeric mixture, or a salt. In one embodiment, the bromodomain inhibitor of Formula V has an enantiomeric purity of about 80% ee or more, e.g., about

80% ee, about 85% ee, about 90% ee, about 91% ee, about 92% ee, about 93% ee, about 94% ee, about 95% ee, about 96% ee, about 97% ee, about 98% ee, about 99% ee, about 99.5% ee or more.

[0114] In one embodiment, ring A of Formula V is not substituted with R^{50} . In one embodiment, ring A of Formula V is substituted with 1 or 2 R^{50} , each independently selected from the group consisting of halogen (e.g., F, Cl) and C_{1-4} alkyl (e.g., Me, Et). In one embodiment, ring A of Formula V is substituted with two methyl groups.

[0115] In one embodiment, the cell culture medium comprises a bromodomain inhibitor of Formula Va:



(Formula Va),

wherein ring B is optionally substituted with 1 or 2 R^{51} , each independently selected from the group consisting of halogen, C_{1-4} alkyl, and C_{1-4} alkoxy;

ring C is optionally substituted with 1 or 2 R^{53} , each independently selected from the group consisting of halogen and C_{1-4} alkyl; and

L^{50} is a C_{1-4} alkylene linker, optionally substituted with a Me or Et.

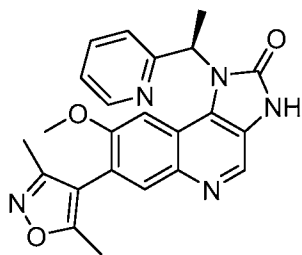
[0116] In one embodiment, the bromodomain inhibitor of Formula Va can be in the form of a tautomer, a stereoisomeric mixture, or a salt. In one embodiment, the bromodomain inhibitor of Formula Va has an enantiomeric purity of about 80% ee or more, e.g., about 80% ee, about 85% ee, about 90% ee, about 91% ee, about 92% ee, about 93% ee, about 94% ee, about 95% ee, about 96% ee, about 97% ee, about 98% ee, about 99% ee, about 99.5% ee or more.

[0117] In one embodiment, ring B of Formula Va is not substituted with R^{51} . In one embodiment, ring B of Formula Va is substituted with one C_{1-4} alkoxy group (e.g., MeO, EtO). Preferably, ring B of Formula Va is substituted with one methoxy group.

[0118] In one embodiment, ring C of Formula Va is not substituted with R^{53} . In one embodiment, ring C of Formula Va is substituted with one or two R^{53} , each independently selected from the group consisting of halogen (e.g., F, Cl) and C_{1-4} alkyl (Me, Et).

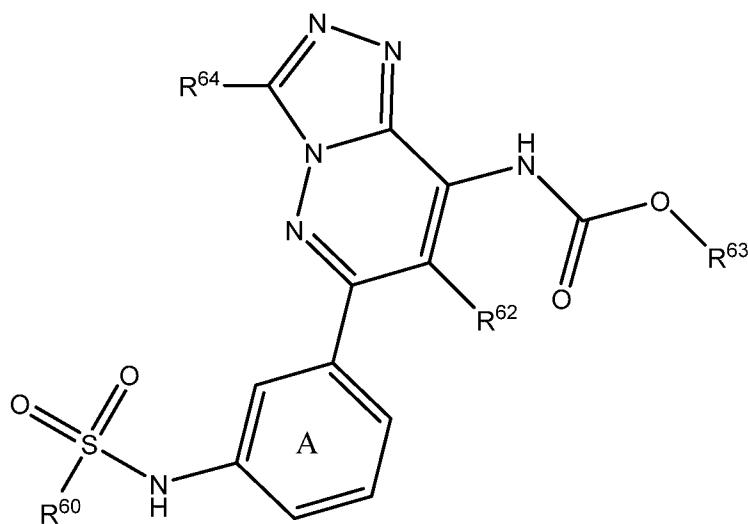
[0119] In one embodiment, L^{50} of Formula Va is an unsubstituted C_{1-4} alkylene linker, e.g., CH_2 , CH_2CH_2 , $CH_2CH_2CH_2$, or $CH_2CH_2CH_2CH_2$. In one embodiment, L^{50} of Formula Va is a C_{1-4} alkylene linker substituted with one methyl or ethyl group. In one embodiment, L^{50} of Formula Va is $CH(Me)$.

[0120] In one embodiment, the cell culture medium comprises I-BET-151:



(I-BET-151). In one embodiment, I-BET-151 can have an enantiomeric purity of about 80% ee or more, e.g., about 80% ee, about 85% ee, about 90% ee, about 91% ee, about 92% ee, about 93% ee, about 94% ee, about 95% ee, about 96% ee, about 97% ee, about 98% ee, about 99% ee, about 99.5% ee or more. In one embodiment, the only bromodomain inhibitor present in the cell culture medium is I-BET-151. In one embodiment, the cell culture medium comprises I-BET-151 and at least one additional bromodomain inhibitor.

[0121] In one embodiment, the cell culture medium comprises a bromodomain inhibitor of Formula VI:



(Formula IV),

wherein each of R^{60} and R^{63} is independently a C_{1-4} alkyl, wherein the C_{1-4} alkyl optionally substituted with 1-3 halogens;

ring A is optionally substituted with 1-4 R^{61} , each independently selected from the group consisting of halogen and C_{1-4} alkyl, wherein the C_{1-4} alkyl is optionally substituted with 1-3 halogens;

R^{62} is selected from the group consisting of H, halogen and C_{1-4} alkyl, wherein the C_{1-4} alkyl is optionally substituted with 1-3 halogens; and

R^{64} is H or a C_{1-4} alkyl, wherein the C_{1-4} alkyl is optionally substituted with 1-3 halogens.

[0122] In one embodiment, the bromodomain inhibitor of Formula VI can be in the form of a tautomer, a stereoisomeric mixture, or a salt. In one embodiment, the bromodomain inhibitor of Formula IV has an enantiomeric purity of about 80% ee or more, e.g., about 80% ee, about 85% ee, about 90% ee, about 91% ee, about 92% ee, about 93% ee, about 94% ee, about 95% ee, about 96% ee, about 97% ee, about 98% ee, about 99% ee, about 99.5% ee or more.

[0123] In one embodiment, R^{60} is a C_{1-4} alkyl (e.g., Me, Et). Preferably, R^{60} is Me.

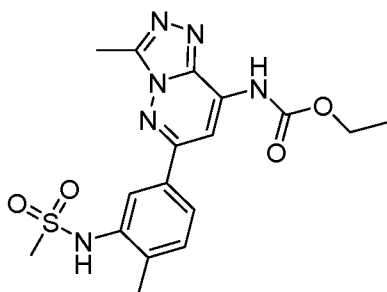
[0124] In one embodiment, R^{62} is H or a C_{1-4} alkyl (e.g., Me, Et). Preferably, R^{62} is H.

[0125] In one embodiment, R^{64} is H or a C_{1-4} alkyl (e.g., Me, Et). Preferably, R^{64} is Me.

[0126] In one embodiment, ring A of Formula VI is not substituted with R^{61} . In another embodiment, ring A of Formula VI is substituted with 1 or 2 R^{61} , each independently selected from the group consisting of halogen (e.g., F, Cl) and C_{1-4} alkyl (e.g., Me, Et). Preferably, ring A is substituted with one methyl group, e.g., at the ortho position to the sulfonamide but para to the pyridazine ring.

[0127] In one embodiment, R^{63} is a C_{1-4} alkyl. Preferably, R^{63} is Me or Et.

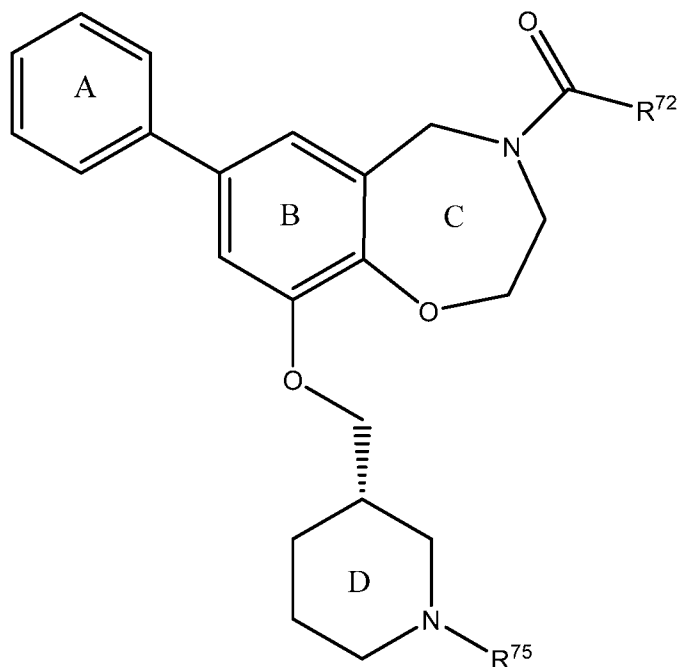
[0128] In one embodiment, the cell culture medium comprises bromosporine:



(bromosporine). In one embodiment, the only

bromodomain inhibitor present in the cell culture medium is bromosporine. In one embodiment, the cell culture medium comprises bromosporine and at least one additional bromodomain inhibitor.

[0129] In one embodiment, the cell culture medium comprises a bromodomain inhibitor of Formula VII:



(Formula VII),

wherein ring A is optionally substituted with 1-5 R^{70} , each independently selected from the group consisting of halogen, OH, C_{1-4} alkyl, and C_{1-4} alkoxy, wherein the C_{1-4} alkyl and C_{1-4} alkoxy are optionally substituted with 1-3 halogens;

ring B is optionally substituted with 1 or 2 R^{71} , each independently selected from the group consisting of halogen and C_{1-4} alkyl, wherein the C_{1-4} alkyl is optionally substituted with 1-3 halogens;

R^{72} is a C_{1-4} alkyl, wherein the C_{1-4} alkyl is optionally substituted with 1-3 halogens;

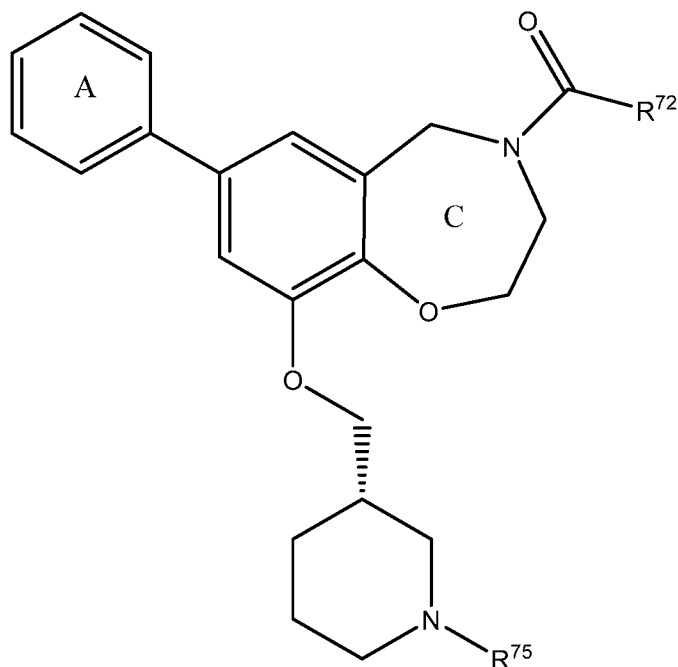
ring C is optionally substituted with 1-3 R^{73} , each independently selected from the group consisting of halogen and C_{1-4} alkyl, wherein the C_{1-4} alkyl is optionally substituted with 1-3 halogens;

ring D is optionally substituted with 1-3 R^{74} , each independently selected from the group consisting of halogen and C_{1-4} alkyl, wherein the C_{1-4} alkyl is optionally substituted with 1-3 halogens; and

R^{75} is H or a C_{1-4} alkyl, wherein the C_{1-4} alkyl is optionally substituted with 1-3 halogens.

[0130] In one embodiment, the bromodomain inhibitor of Formula VII can be in the form of a tautomer, a stereoisomeric mixture, or a salt. In one embodiment, the bromodomain inhibitor of Formula VII can have an enantiomeric purity of about 80% ee or more, e.g., about 80% ee, about 85% ee, about 90% ee, about 91% ee, about 92% ee, about 93% ee, about 94% ee, about 95% ee, about 96% ee, about 97% ee, about 98% ee, about 99% ee, about 99.5% ee or more.

- [0131] In one embodiment, ring A of Formula VII is not substituted with R^{70} . In another embodiment, ring A of Formula VII is substituted with 1 or 2 R^{70} , each independently selected from the group consisting of halogen (e.g., F, Cl), C_{1-4} alkyl (e.g., Me, Et), and C_{1-4} alkoxy (e.g., MeO, EtO). For example, ring A of Formula VII can be substituted with two methoxyl groups.
- [0132] In one embodiment, ring B of Formula VII is not substituted with R^{71} . In another embodiment, ring B of Formula VII is substituted with 1 or 2 R^{71} , each independently selected from the group consisting of halogen (e.g., F, Cl) and C_{1-4} alkyl (e.g., Me, Et). Preferably, ring B is not substituted with R^{71} .
- [0133] In one embodiment, ring C of Formula VII is not substituted with R^{73} . In another embodiment, ring C of Formula VII is substituted with 1, 2, or 3 R^{73} , each independently a C_{1-4} alkyl (e.g., Me, Et). In one embodiment, two R^{73} can be substituted on the same carbon on ring C. Preferably, ring C of Formula VII is not substituted with R^{73} .
- [0134] In one embodiment, ring D of Formula VII is not substituted with R^{74} . In another embodiment, ring D of Formula VII is substituted with 1, 2, or 3 R^{74} , each independently a halogen (e.g., F, Cl) or a C_{1-4} alkyl (e.g., Me, Et). In one embodiment, two R^{74} can be substituted on the same carbon on ring D. Preferably, ring D of Formula VII is not substituted with R^{74} .
- [0135] In one embodiment, R^{75} is H. In another embodiment, R^{75} is a C_{1-4} alkyl, preferably a methyl group.
- [0136] In one embodiment, the cell culture medium comprises a bromodomain inhibitor of Formula VIIa:



(Formula VIIa),

wherein ring A is optionally substituted with 1 or 2 R^{70} , each independently selected from the group consisting of halogen, C_{1-4} alkyl, and C_{1-4} alkoxy;

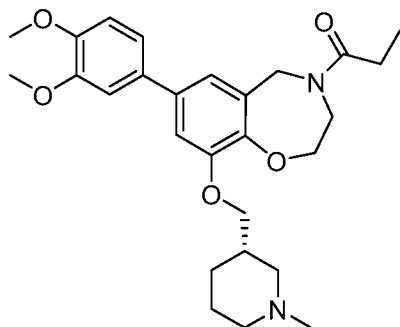
ring C is optionally substituted with 1 or 2 R^{73} , each independently a C_{1-4} alkyl; and R^{72} is a C_{1-4} alkyl; and R^{75} is H or a C_{1-4} alkyl.

[0137] In one embodiment, the bromodomain inhibitor of Formula VIIa can be in the form of a tautomer, a stereoisomeric mixture, or a salt. In one embodiment, the bromodomain inhibitor of Formula VIIa can have an enantiomeric purity of about 80% ee or more, e.g., about 80% ee, about 85% ee, about 90% ee, about 91% ee, about 92% ee, about 93% ee, about 94% ee, about 95% ee, about 96% ee, about 97% ee, about 98% ee, about 99% ee, about 99.5% ee or more.

[0138] In one embodiment, ring A of Formula VIIa is substituted with 1 or 2 R^{70} , each independently selected from the group consisting of halogen, C_{1-4} alkyl, and C_{1-4} alkoxy, ring C of Formula VIIa is not substituted with R^{73} , R^{72} is a C_{1-4} alkyl, and R^{75} is H or methyl.

[0139] In one embodiment, ring A of Formula VIIa is substituted with one or two methoxyl groups, ring C of Formula VIIa is not substituted with R^{73} , R^{72} is Et, and R^{75} is Me.

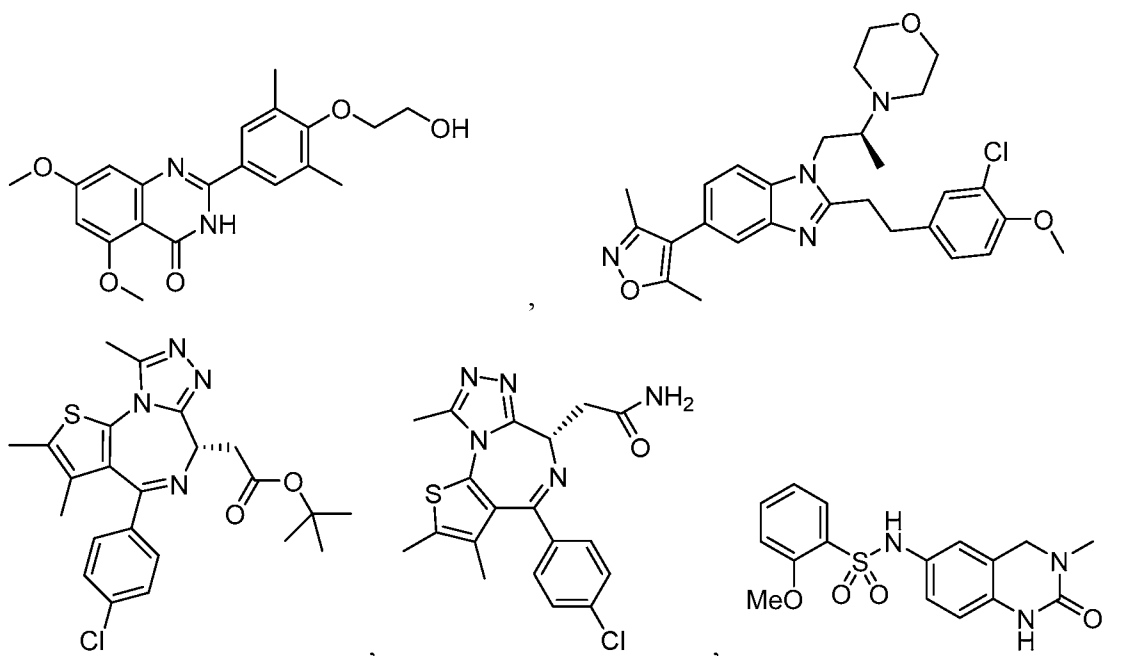
[0140] In one embodiment, the cell culture medium comprises I-CBP112:

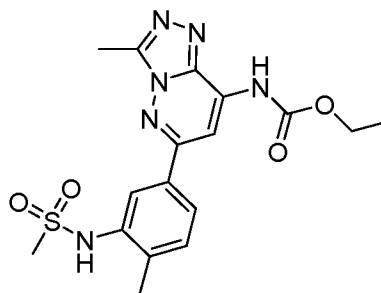
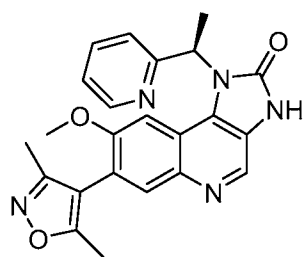
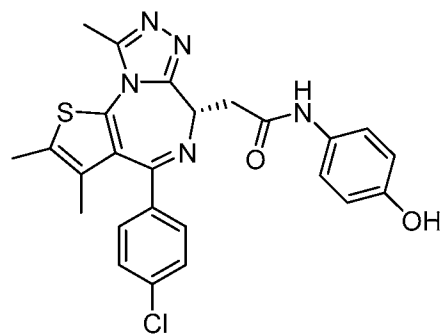
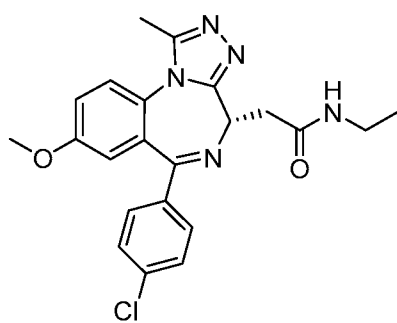


(I-CBP112). In one embodiment, I-CBP112 can have an

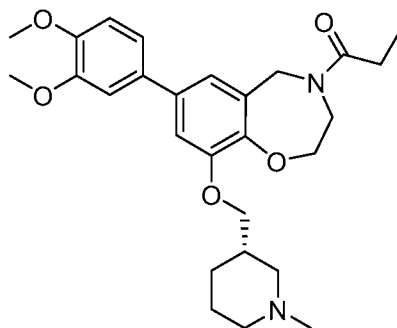
enantiomeric purity of about 80% ee or more, e.g., about 80% ee, about 85% ee, about 90% ee, about 91% ee, about 92% ee, about 93% ee, about 94% ee, about 95% ee, about 96% ee, about 97% ee, about 98% ee, about 99% ee, about 99.5% ee or more. In one embodiment, the only bromodomain inhibitor present in the cell culture medium is I-CBP112. In one embodiment, the cell culture medium comprises I-CBP112 and at least one additional bromodomain inhibitor.

[0141] In one embodiment, the cell culture medium comprises a bromodomain inhibitor selected from the group consisting of:

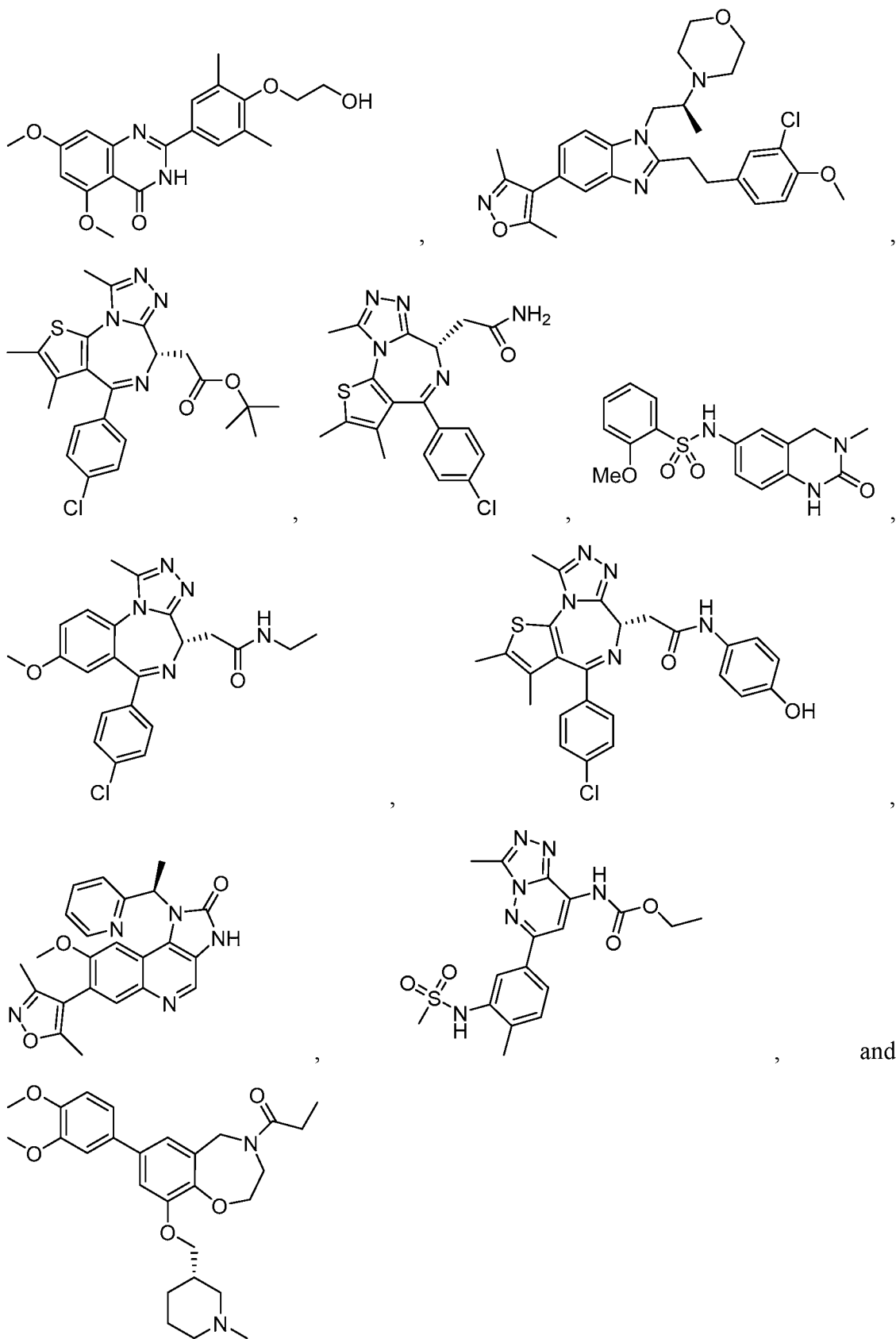




and

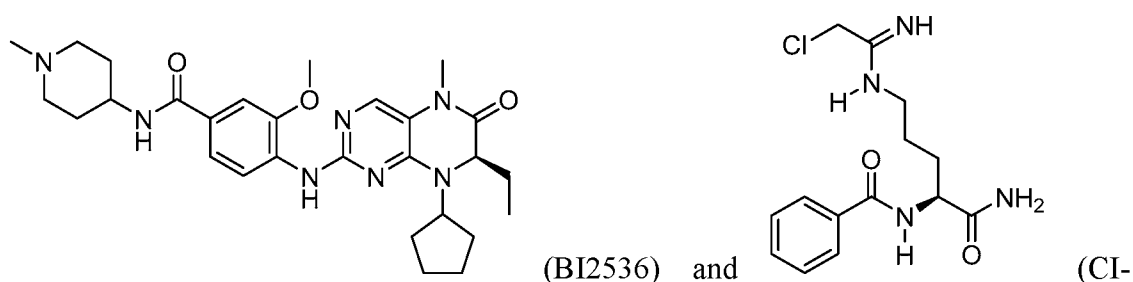


[0142] In another embodiment, cell culture medium comprises two or more bromodomain inhibitor selected from the group consisting of:



[0143] The bromodomain inhibitors described herein can be prepared by those skilled in the art. The specific compounds including RVX208, SGC-CBP30, JQ1, CPI-203, PFI-1, I-BET-762, OTX-015, I-BET151, bromosporine, I-CBP112 are known and commercially available. Analogs of these specific compounds can be prepared following similar synthetic routes. For example, compounds of Formula II can be generally synthesized by following the methods described in Hay D.A. *et al.*, "Discovery and Optimization of Small-molecule Ligands for the CBP/p300 Bromodomains," *J. Am. Chem. Soc.* 136:9308 (2014).

[0144] In certain embodiments, the cell culture medium of the present invention may not comprise a bromodomain inhibitor. For example, in one embodiment, the cell culture medium comprises BI2536 and/or CI-amidine:



In one embodiment, BI2536 and/or CI-amidine is present in the form of a tautomer, a stereoisomeric mixture, or a salt thereof. For example, CI-amidine can be present in the form of a trifluoroacetic acid (TFA) salt. In one embodiment BI2536 or CI-amidine can have an enantiomeric purity of about 80% ee or more, e.g., about 80% ee, about 85% ee, about 90% ee, about 91% ee, about 92% ee, about 93% ee, about 94% ee, about 95% ee, about 96% ee, about 97% ee, about 98% ee, about 99% ee, about 99.5% ee or more. In one embodiment, the cell culture does not comprise a bromodomain inhibitor. However, in one embodiment, the medium comprises BI2536 and/or CI-amidine and at least one bromodomain inhibitor (e.g., as described herein).

[0145] Typically, BI2536 and/or CI-amidine can be present in the cell culture medium ranging from about 1 μ M to about 100 μ M, e.g., from about 1 μ M to about 75 μ M, about 1 μ M to about 50 μ M, about 1 μ M to about 30 μ M, about 1 μ M to about 10 μ M, about 1 μ M to about 3 μ M, about 3 μ M to about 100 μ M, about 3 μ M to about 75 μ M, about 3 μ M to about 50 μ M, about 3 μ M to about 30 μ M, about 3 μ M to about 10 μ M, about 10 μ M to about 100 μ M, about 10 μ M to about 75 μ M, about 10 μ M to about 50 μ M, about 10 μ M to about 30 μ M, about 30 μ M to about 100 μ M, about 30 μ M to about 75 μ M, about 30

uM to about 50 uM, about 50 uM to about 100 uM, about 50 uM to about 75 uM, or about 75 uM to about 100 uM. In one embodiment, BI2536 and/or CI-amidine is present in the cell culture medium in a concentration of about 1 uM, about 3 uM, about 10 uM, about 30 uM, about 50 uM, about 75 uM, or about 100 uM. In one embodiment, the BI2536 and/or CI-amidine can be present can also be present in the cell culture medium in a concentration less than about 1 uM (e.g., about 0.5 uM, about 0.1 uM, about 0.01 uM, or any ranges between the specified values). In one embodiment, BI2536 and/or CI-amidine can also be present in the cell culture medium in a concentration greater than about 100 uM (e.g., about 120 uM, about 200 uM, about 400 uM, about 600 uM, about 1000 uM, or any ranges between the specified values).

[0146] In one embodiment, the medium comprising BI2536 and/or CI-amidine is a feed medium. In one embodiment, the BI2536 and/or CI-amidine is present in the feed medium in a sufficient concentration such that when added to a culture, the BI2536 and/or CI-amidine is present in the culture in a concentration ranging from about 1 uM to about 100 uM, e.g., from about 1 uM to about 75 uM, about 1 uM to about 50 uM, about 1 uM to about 30 uM, about 1 uM to about 10 uM, about 1 uM to about 3 uM, about 3 uM to about 100 uM, about 3 uM to about 75 uM, about 3 uM to about 50 uM, about 3 uM to about 30 uM, about 3 uM to about 10 uM, about 10 uM to about 100 uM, about 10 uM to about 75 uM, about 10 uM to about 50 uM, about 10 uM to about 30 uM, about 30 uM to about 100 uM, about 30 uM to about 75 uM, about 30 uM to about 50 uM, about 50 uM to about 100 uM, about 50 uM to about 75 uM, or about 75 uM to about 100 uM. Other suitable concentrations in the culture are described herein.

Cell culture composition

[0147] Certain embodiments of the present invention are directed to a cell culture composition comprising the cell culture medium described herein and cells (e.g., mammalian cells). In one embodiment, a cell culture composition according to the invention can be a batch culture, fed-batch culture or a perfusion culture. In a specific embodiment, a cell culture composition of the invention is a fed batch culture.

[0148] In one embodiment, a cell culture composition described herein comprises mammalian cells selected from the group consisting of CHO cells, HEK-293 cells, VERO cells, NS0 cells, PER.C6 cells, Sp2/0 cells, BHK cells, MDCK cells, MDBK cells, COS cells and HeLa cells. In a specific embodiment, a cell culture composition described

herein comprises CHO cells (e.g., CHO-K1 cells). In another specific embodiment, a cell culture composition described herein comprises HEK-293 cells. In another specific embodiment, a cell culture composition described herein comprises HeLa cells. In one embodiment, a cell culture composition described herein comprises hybridoma cells. In one embodiment, a cell culture composition described herein comprises non-mammalian Eukaryotic cells.

[0149] A cell culture composition described herein can comprise cells that have been adapted to grow in serum free medium, animal protein free medium or chemically defined medium. Or it can comprise cells that have been genetically modified to increase their life-span in culture. In one embodiment, the cells have been modified to express an anti-apoptotic gene. In a specific embodiment, the cells have been modified to express the bcl-xL antiapoptotic gene. Additional anti-apoptotic genes that can be used in accordance with the present invention include, but are not limited to, E1B-9K, Aven, Mcl.

[0150] In one embodiment, the cell culture comprises i) mammalian cells (e.g., mammalian cells comprising a polynucleotide encoding a protein or polypeptide of interest); and ii) a cell culture medium comprising a bromodomain inhibitor. Suitable mammalian cells, cell culture medium, protein or polypeptide of interest, and bromodomain inhibitors include any of those described herein.

Cell culturing

[0151] The present invention provides a method of culturing cells, comprising contacting the cells with a medium disclosed herein.

[0152] Cell cultures can be cultured in a batch culture, fed batch culture, shake flask, a perfusion culture, or a bioreactor. In one embodiment, a cell culture according to a method of the present invention is a batch culture. In another embodiment, a cell culture according to a method of the present invention is a fed batch culture. In one embodiment, a cell culture according to a method of the present invention is a shake flask culture. In another embodiment, a cell culture according to a method of the present invention is a bioreactor culture. In a further embodiment, a cell culture according to a method of the present invention is a perfusion culture.

[0153] In one embodiment, a cell culture according to a method of the present invention is a serum-free culture. In another embodiment, a cell culture according to a method of

the present invention is a chemically defined culture. In a further embodiment, a cell culture according to a method of the present invention is an animal protein free culture.

[0154] In one embodiment, a cell culture is contacted with a medium described herein during the growth phase of the culture. In another embodiment, a cell culture is contacted with a medium described herein during the production phase of the culture.

[0155] In one embodiment, a cell culture according to the invention is contacted with a feed medium described herein during the production phase of the culture. In one embodiment, the culture is supplemented with the feed medium between about 1 and about 25 times during production phase of the culture. In another embodiment, a culture is supplemented with the feed medium between about 1 and about 20 times, between about 1 and about 15 times, or between about 1 and about 10 times during the first time period. In a further embodiment, a culture is supplemented with the feed medium at least once, at least twice, at least three times, at least four times, at least five times, at least 6 times, at least 7 times, at least 8 times, at least 9 times, at least 10 times, at least 1 times, at least 12 times, at least 13 times, at least 14 times, at least 15 times, at least 20 times, at least 25 times. In a specific embodiment, the culture is a fed batch culture. In another specific embodiment, the culture is a perfusion culture.

[0156] A culture according to the invention can be contacted with a feed medium described herein at regular intervals. In one embodiment, the regular interval is about once a day, about once every two days, about once every three days, about once every 4 days, or about once every 5 days. In a specific embodiment, the culture is a fed batch culture. In another specific embodiment, the culture is a perfusion culture.

[0157] In one embodiment, a medium described herein is a feed medium for a fed batch cell culture. A skilled artisan understands that a fed batch cell culture can be contacted with a feed medium more than once. In one embodiment, a fed batch cell culture is contacted with a medium described herein only once. In another embodiment, a fed batch cell culture is contacted with a medium described herein more than once, for example, at least twice, at least three times, at least four times, at least five times, at least six times, at least seven times, or at least ten times.

[0158] In accordance with the present invention, the total volume of feed medium added to a cell culture should optimally be kept to a minimal amount. For example, the total volume of the feed medium added to the cell culture can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15,

20, 25, 30, 35, 40, 45 or 50% of the volume of the cell culture prior to adding the feed medium.

[0159] A culture according to the invention can be contacted with a feed medium described herein at regular intervals. In one embodiment, the regular interval is about once a day, about once every two days, about once every three days, about once every 4 days, or about once every 5 days. In a specific embodiment, the culture is a fed batch culture. In another specific embodiment, the culture is a perfusion culture.

[0160] In one embodiment, a medium described herein is a feed medium for a fed batch cell culture. A skilled artisan understands that a fed batch cell culture can be contacted with a feed medium more than once. In one embodiment, a fed batch cell culture is contacted with a medium described herein only once. In another embodiment, a fed batch cell culture is contacted with a medium described herein more than once, for example, at least twice, at least three times, at least four times, at least five times, at least six times, at least seven times, or at least ten times.

[0161] Cell cultures can be grown to achieve a particular cell density, depending on the needs of the practitioner and the requirement of the cells themselves, prior to being contacted with a medium described herein. In one embodiment, the cell culture is contacted with a medium described herein at a viable cell density of 1, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 or 99 percent of maximal viable cell density. In a specific embodiment, the medium is a feed medium.

[0162] Cell cultures can be allowed to grow for a defined period of time before they are contacted with a medium described herein. In one embodiment, the cell culture is contacted with a medium described herein at day 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 of the cell culture. In another embodiment, the cell culture is contacted with a medium described herein at week 1, 2, 3, 4, 5, 6, 7, or 8 of the cell culture. In a specific embodiment, the medium is a feed medium.

[0163] Cell cultures can be cultured in the production phase for a defined period of time. In one embodiment, the cell culture is contacted with a feed medium described herein at day 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 of the production phase.

[0164] A culture according to the invention can be maintained in production phase for between about 1 day and about 30 days. In one embodiment, a culture is maintained in production phase for between about 1 day and about 30 days, between about 1 day and about 25 days, between about 1 day and about 20 days, about 1 day and about 15 days, about 1 day and about 14 days, about 1 day and about 13 days, about 1 day and about 12 days, about 1 day and about 11 days, about 1 day and about 10 days, about 1 day and about 9 days, about 1 day and about 8 days, about 1 day and about 7 days, about 1 day and about 6 days, about 1 day and about 5 days, about 1 day and about 4 days, about 1 day and about 3 days, about 2 days and about 25 days, about 3 days and about 25 days, about 4 days and about 25 days, about 5 days and about 25 days, about 6 days and about 25 days, about 7 days and about 25 days, about 8 days and about 25 days, about 9 days and about 25 days, about 10 days and about 25 days, about 15 days and about 25 days, about 20 days and about 25 days, about 2 days and about 30 days, about 3 days and about 30 days, about 4 days and about 30 days, about 5 days and about 30 days, about 6 days and about 30 days, about 7 days and about 30 days, about 8 days and about 30 days, about 9 days and about 30 days, about 10 days and about 30 days, about 15 days and about 30 days, about 20 days and about 30 days, or about 25 days and about 30 days. In another embodiment, a culture is maintained in production phase for at least about 1 day, at least about 2 days, at least about 3 days, at least about 4 days, at least about 5 days, at least about 6 days, at least about 7 days, at least about 8 days, at least about 9 days, at least about 10 days, at least about 11 days, at least about 12 days, at least about 15 days, at least about 20 days, at least about 25 days, or at least about 30 days. In a further embodiment, a culture is maintained in production phase for about 1 day, about 2 days, about 3 days, about 4 days, about 5 days, about 6 days, about 7 days, about 8 days, about 9 days, about 10 days, about 11 days, about 12 days, about 15 days, about 20 days, about 25 days, or about 30 days.

[0165] In one embodiment of the present invention, a cell culture comprising a medium described herein can be maintained in production phase longer than a cell culture that does not comprise a bromodomain inhibitor (e.g., as described herein). A skilled artisan readily understands that an extended production phase can lead to an increase in the total amount of polypeptide produced by the cell culture. In one embodiment, a method of producing a protein or polypeptide of interest according to the present invention produces

more polypeptide than the amount produced by a method that does not comprise maintaining cells capable of producing the polypeptide in a culture comprising a bromodomain inhibitor (e.g., as described herein). In one embodiment, a method according to the present invention produces between about 5% and about 500%, about 5% and about 250%, about 5% and about 100%, about 5% and about 80%, about 5% and about 50%, about 5% and about 30%, about 10% and about 500%, about 20% and about 500%, about 30% and about 500%, about 50% and about 500%, or about 100% and about 500% more protein or polypeptide. In another embodiment, a method according to the present invention produces at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 40%, at least about 50%, at least about 70%, at least about 90%, or at least about 100% more protein or polypeptide. In another embodiment, a method according to the present invention produces at least about 2 times, three times, four times, five times or ten times more protein or polypeptide. In a specific embodiment, the protein or polypeptide is an antibody.

Method of producing a protein or polypeptide of interest

[0166] The present invention further provides a method of producing a protein or polypeptide of interest, comprising culturing cells (e.g., mammalian cells as described herein) capable of producing the protein or polypeptide of interest in a culture comprising a medium described herein; and optionally isolating the protein or polypeptide from the culture. In one embodiment, the protein or polypeptide of interest is a recombinant protein or polypeptide. In one embodiment, the protein or polypeptide of interest is an enzyme, receptor, antibody, hormone, regulatory factor, antigen, or binding agent. In a specific embodiment, the protein is an antibody.

[0167] In one embodiment, the method comprises a) providing a cell culture comprising i) mammalian cells comprising a polynucleotide encoding the protein or polypeptide of interest; and ii) a cell culture medium described herein (e.g., a cell culture medium comprising a bromodomain inhibitor (e.g., as described herein)); and b) culturing the mammalian cells under conditions that allow expression of the protein or polypeptide of interest. In one embodiment, the culturing is conducted in a batch mode, fed-batch mode or a perfusion mode. In one embodiment, the culturing is conducted in a fed-batch mode.

[0168] In one embodiment, the mammalian cells are selected from the group consisting of: CHO cells, HEK-293 cells, VERO cells, NS0 cells, PER.C6 cells, Sp2/0 cells, BHK cells, MDCK cells, MDBK cells, COS cells and HeLa cells. Other suitable mammalian cells are known in the art and can be used in embodiments described herein. In a specific embodiment, the mammalian cells are CHO cells. In a further specific embodiment, the mammalian cells are CHO-K1 cells.

[0169] In one embodiment, the method produces the protein or polypeptide of interest in a fed-batch mode. In one embodiment, the culture comprises a medium comprising a bromodomain inhibitor, and the method produces the protein or polypeptide of interest (e.g., at day 14 of the culture) at a titer greater than the titer produced from a control cell culture that does not contain a bromodomain inhibitor. In one embodiment, the method produces between about 15% and about 100%, about 15% and about 80%, about 15% and about 50%, about 15% and about 30%, about 30% and about 100%, about 30% and about 80%, about 30% and about 50%, about 50% and about 80%, or about 50% and about 100% higher titer (e.g., at day 14 of the culture) than the titer produced from a control cell culture that does not contain a bromodomain inhibitor. In another embodiment, the method produces a titer (e.g., at day 14 of the culture) at least about 15% (e.g., at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 100%, or at least about 200%) greater than the titer produced from a control cell culture that does not contain a bromodomain inhibitor. In another embodiment, the method produces at least about 2 times, three times, four times, five times or ten times higher titer. Typically, the titer produced from a control cell culture that does not contain a bromodomain inhibitor ranges from about 1g/L to about 10 g/L (e.g., about 1g/L, about 2g/L, about 3g/L, about 4g/L, about 5g/L, about 6g/L, about 7g/L, about 8g/L, about 9g/L, about 10g/L, or any ranges between the specified values).

[0170] In one embodiment, the culture comprises a medium comprising a bromodomain inhibitor, and the method generates a viable cell density (e.g., at day 14 of the culture) higher than that observed for a control cell culture that does not contain a bromodomain inhibitor. In one embodiment, the method generates a viable cell density (e.g., at day 14 of the culture) between about 20% and about 100%, about 20% and about 80%, about 20% and about 50%, about 20% and about 30%, about 30% and about 100%, about 30%

and about 80%, about 30% and about 50%, or about 50% and about 80% higher than that observed for a control cell culture that does not contain a bromodomain inhibitor. In another embodiment, the method produces at least about 20% (e.g., at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 100%, or at least about 200%) higher viable cell density (e.g., at day 14 of the culture) than that observed for a control cell culture that does not contain a bromodomain inhibitor. Typically, the viable cell density (e.g., at day 14 of the culture) that observed for a control cell culture that does not contain a bromodomain inhibitor ranges from about 5×10^6 cells/mL to about 25×10^6 cells/mL (e.g., about 5×10^6 cells/mL, about 10×10^6 cells/mL, about 15×10^6 cells/mL, about 20×10^6 cells/mL, about 15×10^6 cells/mL, or any ranges between the specified values).

[0171] In one embodiment, the culture comprises a medium comprising a bromodomain inhibitor, and the method generates a cell specific productivity (e.g., a cumulative cell specific productivity at day 14 of the culture) higher than that observed for a control cell culture that does not contain a bromodomain inhibitor. In one embodiment, the method generates a cell specific productivity (e.g., a cumulative cell specific productivity at day 14 of the culture) between about 10% and about 100%, about 10% and about 80%, about 10% and about 60%, about 10% and about 40%, about 20% and about 100%, about 20% and about 80%, about 20% and about 60%, or about 20% and about 40%, higher cell specific productivity. In another embodiment, the method generates at least about 10% (e.g., at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or at least about 100%) higher cell specific productivity (e.g., a cumulative cell specific productivity at day 14 of the culture). In one specific embodiment, the method generates a cell specific productivity (e.g., a cumulative cell specific productivity at day 14 of the culture) between about 10% and about 60% higher than that observed for a control cell culture that does not contain a bromodomain inhibitor. Typically, the cell specific productivity (e.g., a cumulative cell specific productivity at day 14 of the culture) that observed for a control cell culture that does not contain a bromodomain inhibitor ranges from about 2 pg/cell/day to about 20 pg/cell/day (e.g., about 2 pg/cell/day, about 4 pg/cell/day, about 6 pg/cell/day, about 8 pg/cell/day, about 10 pg/cell/day, about 12

pg/cell/day, about 14 pg/cell/day, about 16 pg/cell/day, about 18 pg/cell/day, about 20 pg/cell/day, or any ranges between the specified values).

[0172] In one embodiment, the culture comprises a medium comprising a bromodomain inhibitor, and the method achieves a cell viability (e.g., at day 14 of the culture) higher than that observed for a control cell culture that does not contain a bromodomain inhibitor. In one embodiment, the method achieves between about 10% and about 100%, about 10% and about 80%, about 10% and about 60%, about 10% and about 40%, about 20% and about 100%, about 20% and about 80%, about 20% and about 60%, or about 20% and about 40%, higher cell viability (e.g., at day 14 of the culture). In another embodiment, the method achieves at least about 10% (e.g., at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or at least about 100%) higher cell viability (e.g., at day 14 of the culture). In one specific embodiment, the method achieves a cell viability (e.g., at day 14 of the culture) between about 10% and about 60% higher than that observed for a control cell culture that does not contain a bromodomain inhibitor. Typically, the cell specific productivity (e.g., at day 14 of the culture) that observed for a control cell culture that does not contain a bromodomain inhibitor ranges from about 40% to about 90% (e.g., about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, or any ranges between the specified values).

[0173] In one embodiment, a method of producing a protein or polypeptide of interest according to the present invention produces a maximum protein or polypeptide titer of at least about 2 g/liter, at least about 2.5 g/liter, at least about 3 g/liter, at least about 3.5 g/liter, at least about 4 g/liter, at least about 4.5 g/liter, at least about 5 g/liter, at least about 6 g/liter, at least about 7 g/liter, at least about 8 g/liter, at least about 9 g/liter, or at least about 10 g/liter. In another embodiment, the method according to the present invention produces a maximum protein or polypeptide titer of between about 1 g/liter and about 10 g/liter, about 1.5 g/liter and about 10 g/liter, about 2 g/liter and about 10 g/liter, about 2.5 g/liter and about 10 g/liter, about 3 g/liter and about 10 g/liter, about 4 g/liter and about 10 g/liter, about 5 g/liter and about 10 g/liter, about 1 g/liter and about 5 g/liter, about 1 g/liter and about 4.5 g/liter, or about 1 g/liter and about 4 g/liter.

Method of modulating viability and/or product titer

- [0174] The present invention further provides a method of modulating the viability the cells of a cell culture and/or the product titer of a cell culture, comprising culturing cells (e.g., mammalian cells as described herein) a culture comprising a medium described herein. In one embodiment, the culturing is conducted in a batch mode, fed-batch mode or a perfusion mode. In one embodiment, the culturing is conducted in a fed-batch mode.
- [0175] In one embodiment, the cells are mammalian cells selected from the group consisting of: CHO cells, HEK-293 cells, VERO cells, NS0 cells, PER.C6 cells, Sp2/0 cells, BHK cells, MDCK cells, MDBK cells, COS cells and HeLa cells. Other suitable mammalian cells are known in the art and can be used in embodiments described herein. In a specific embodiment, the mammalian cells are CHO cells. In a further specific embodiment, the mammalian cells are CHO-K1 cells.
- [0176] In one embodiment, the culture comprises a medium comprising a bromodomain inhibitor, and the method generates a viable cell density (e.g., at day 14 of the culture) higher than that observed for a control cell culture that does not contain a bromodomain inhibitor. In one embodiment, the method generates a viable cell density (e.g., at day 14 of the culture) between about 20% and about 100%, about 20% and about 80%, about 20% and about 50%, about 20% and about 30%, about 30% and about 100%, about 30% and about 80%, about 30% and about 50%, or about 50% and about 80% higher than that observed for a control cell culture that does not contain a bromodomain inhibitor. In another embodiment, the method produces at least about 20% (e.g., at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 100%, or at least about 200%) higher viable cell density (e.g., at day 14 of the culture) than that observed for a control cell culture that does not contain a bromodomain inhibitor. Typically, the viable cell density (e.g., at day 14 of the culture) that observed for a control cell culture that does not contain a bromodomain inhibitor ranges from about 5×10^6 cells/mL to about 25×10^6 cells/mL (e.g., about 5×10^6 cells/mL, about 10×10^6 cells/mL, about 15×10^6 cells/mL, about 20×10^6 cells/mL, about 15×10^6 cells/mL, or any ranges between the specified values).
- [0177] In one embodiment, the culture comprises a medium comprising a bromodomain inhibitor, and the method achieves a cell viability (e.g., at day 14 of the culture) higher

than that observed for a control cell culture that does not contain a bromodomain inhibitor. In one embodiment, the method achieves between about 10% and about 100%, about 10% and about 80%, about 10% and about 60%, about 10% and about 40%, about 20% and about 100%, about 20% and about 80%, about 20% and about 60%, or about 20% and about 40%, higher cell viability (e.g., at day 14 of the culture). In another embodiment, the method achieves at least about 10% (e.g., at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or at least about 100%) higher cell viability (e.g., at day 14 of the culture). In one specific embodiment, the method achieves a cell viability (e.g., at day 14 of the culture) between about 10% and about 60% higher than that observed for a control cell culture that does not contain a bromodomain inhibitor. Typically, the cell specific productivity (e.g., at day 14 of the culture) that observed for a control cell culture that does not contain a bromodomain inhibitor ranges from about 40% to about 90% (e.g., about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, or any ranges between the specified values).

[0178] In one embodiment, the culture comprises a medium comprising a bromodomain inhibitor, and the method produces a product (e.g., a protein or polypeptide of interest) at a titer (e.g., at day 14 of the culture) greater than the titer produced from a control cell culture that does not contain a bromodomain inhibitor. In one embodiment, the method produces between about 15% and about 100%, about 15% and about 80%, about 15% and about 50%, about 15% and about 30%, about 30% and about 100%, about 30% and about 80%, about 30% and about 50%, about 50% and about 80%, or about 50% and about 100% higher titer (e.g., at day 14 of the culture) than the titer produced from a control cell culture that does not contain a bromodomain inhibitor. In another embodiment, the method produces a titer (e.g., at day 14 of the culture) at least about 15% (e.g., at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 100%, or at least about 200%) greater than the titer produced from a control cell culture that does not contain a bromodomain inhibitor. In another embodiment, the method produces at least about 2 times, three times, four times, five times or ten times higher titer. Typically, the titer produced from a control cell culture that does not contain a bromodomain inhibitor ranges from about 1g/L to about 10 g/L (e.g., about 1g/L, about 2g/L, about 3g/L, about

4g/L, about 5g/L, about 6g/L, about 7g/L, about 8g/L, about 9g/L, about 10g/L, or any ranges between the specified values).

[0179] In one embodiment, the culture comprises a medium comprising a bromodomain inhibitor, and the method generates a cell specific productivity (e.g., a cumulative cell specific productivity at day 14 of the culture) higher than that observed for a control cell culture that does not contain a bromodomain inhibitor. In one embodiment, the method generates a cell specific productivity (e.g., a cumulative cell specific productivity at day 14 of the culture) between about 10% and about 100%, about 10% and about 80%, about 10% and about 60%, about 10% and about 40%, about 20% and about 100%, about 20% and about 80%, about 20% and about 60%, or about 20% and about 40%, higher cell specific productivity. In another embodiment, the method generates at least about 10% (e.g., at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or at least about 100%) higher cell specific productivity (e.g., a cumulative cell specific productivity at day 14 of the culture). In one specific embodiment, the method generates a cell specific productivity (e.g., a cumulative cell specific productivity at day 14 of the culture) between about 10% and about 60% higher than that observed for a control cell culture that does not contain a bromodomain inhibitor. Typically, the cell specific productivity (e.g., a cumulative cell specific productivity at day 14 of the culture) that observed for a control cell culture that does not contain a bromodomain inhibitor ranges from about 2 pg/cell/day to about 20 pg/cell/day (e.g., about 2 pg/cell/day, about 4 pg/cell/day, about 6 pg/cell/day, about 8 pg/cell/day, about 10 pg/cell/day, about 12 pg/cell/day, about 14 pg/cell/day, about 16 pg/cell/day, about 18 pg/cell/day, about 20 pg/cell/day, or any ranges between the specified values).

[0180] In one embodiment, the cells are mammalian cells comprising a polynucleotide encoding a protein or polypeptide of interest as described herein. In one embodiment, the protein or polypeptide of interest is an enzyme, receptor, antibody, hormone, regulatory factor, antigen, or binding agent. In a specific embodiment, the protein is an antibody.

[0181] The invention further provides a conditioned cell culture medium produced by a method described herein.

[0182] In one embodiment, a conditioned cell culture medium according to the invention comprises a recombinant protein or polypeptide. In a specific embodiment, a conditioned

cell culture medium according to the invention comprises a recombinant protein or polypeptide at a titer of at least about 2 g/liter, at least about 2.5 g/liter, at least about 3 g/liter, at least about 3.5 g/liter, at least about 4 g/liter, at least about 4.5 g/liter, at least about 5 g/liter, at least about 6 g/liter, at least about 7 g/liter, at least about 8 g/liter, at least about 9 g/liter, or at least about 10 g/liter, or a titer of between about 1 g/liter and about 10 g/liter, about 1.5 g/liter and about 10 g/liter, about 2 g/liter and about 10 g/liter, about 2.5 g/liter and about 10 g/liter, about 3 g/liter and about 10 g/liter, about 4 g/liter and about 10 g/liter, about 5 g/liter and about 10 g/liter, about 1 g/liter and about 5 g/liter, about 1 g/liter and about 4.5 g/liter, or about 1 g/liter and about 4 g/liter. In another embodiment, a conditioned cell culture medium according to the invention comprises a recombinant protein or polypeptide at a higher titer than the titer obtained without the use of a medium described herein. In a specific embodiment, the protein or polypeptide is an antibody.

Polypeptides

[0183] Any polypeptide that is expressible in a host cell can be produced in accordance with the present invention. The polypeptide can be expressed from a gene that is endogenous to the host cell, or from a gene that is introduced into the host cell through genetic engineering. The polypeptide can be one that occurs in nature, or can alternatively have a sequence that was engineered or selected by the hand of man. An engineered polypeptide can be assembled from other polypeptide segments that individually occur in nature, or can include one or more segments that are not naturally occurring.

[0184] Polypeptides that can desirably be expressed in accordance with the present invention will often be selected on the basis of an interesting biological or chemical activity. For example, the present invention can be employed to express any pharmaceutically or commercially relevant enzyme, receptor, antibody, hormone, regulatory factor, antigen, binding agent, etc.

Antibodies

[0185] Given the large number of antibodies currently in use or under investigation as pharmaceutical or other commercial agents, production of antibodies is of particular interest in accordance with the present invention. Antibodies are proteins that have the ability to specifically bind a particular antigen. Any antibody that can be expressed in a

host cell can be used in accordance with the present invention. In one embodiment, the antibody to be expressed is a monoclonal antibody.

[0186] Particular antibodies can be made, for example, by preparing and expressing synthetic genes that encode the recited amino acid sequences or by mutating human germline genes to provide a gene that encodes the recited amino acid sequences. Moreover, these antibodies can be produced, *e.g.*, using one or more of the following methods.

[0187] Numerous methods are available for obtaining antibodies, particularly human antibodies. One exemplary method includes screening protein expression libraries, *e.g.*, phage or ribosome display libraries. Phage display is described, for example, U.S. Pat. No. 5,223,409; Smith (1985) *Science* 228:1315-1317; WO 92/18619; WO 91/17271; WO 92/20791; WO 92/15679; WO 93/01288; WO 92/01047; WO 92/09690; and WO 90/02809. The display of Fab's on phage is described, *e.g.*, in U.S. Pat. Nos. 5,658,727; 5,667,988; and 5,885,793.

[0188] In addition to the use of display libraries, other methods can be used to obtain an antibody. For example, a protein or a peptide thereof can be used as an antigen in a non-human animal, *e.g.*, a rodent, *e.g.*, a mouse, hamster, or rat.

[0189] In one embodiment, the non-human animal includes at least a part of a human immunoglobulin gene. For example, it is possible to engineer mouse strains deficient in mouse antibody production with large fragments of the human Ig loci. Using the hybridoma technology, antigen-specific monoclonal antibodies derived from the genes with the desired specificity can be produced and selected. *See, e.g.*, XENOMOUSE™, Green *et al.* (1994) *Nature Genetics* 7:13-21, U.S. 2003-0070185, WO 96/34096, and WO 96/33735.

[0190] In another embodiment, a monoclonal antibody is obtained from the non-human animal, and then modified, *e.g.*, humanized or deimmunized. Winter describes an exemplary CDR-grafting method that can be used to prepare humanized antibodies described herein (U.S. Pat. No. 5,225,539). All or some of the CDRs of a particular human antibody can be replaced with at least a portion of a non-human antibody. In one embodiment, it is only necessary to replace the CDRs required for binding or binding determinants of such CDRs to arrive at a useful humanized antibody that binds to an antigen.

[0191] Humanized antibodies can be generated by replacing sequences of the Fv variable region that are not directly involved in antigen binding with equivalent sequences from human Fv variable regions. General methods for generating humanized antibodies are provided by Morrison, S. L. (1985) *Science* 229:1202-1207, by Oi *et al.* (1986) *BioTechniques* 4:214, and by U.S. Pat. No. 5,585,089; U.S. Pat. No. 5,693,761; U.S. Pat. No. 5,693,762; U.S. Pat. No. 5,859,205; and U.S. Pat. No. 6,407,213. Those methods include isolating, manipulating, and expressing the nucleic acid sequences that encode all or part of immunoglobulin Fv variable regions from at least one of a heavy or light chain. Sources of such nucleic acid are well known to those skilled in the art and, for example, can be obtained from a hybridoma producing an antibody against a predetermined target, as described above, from germline immunoglobulin genes, or from synthetic constructs. The recombinant DNA encoding the humanized antibody can then be cloned into an appropriate expression vector. In one embodiment, the expression vector comprises a polynucleotide encoding a glutamine synthetase polypeptide. (*See, e.g., Porter et al., Biotechnol Prog* 26(5):1446-54 (2010).)

[0192] The antibody can include a human Fc region, *e.g.*, a wild-type Fc region or an Fc region that includes one or more alterations. In one embodiment, the constant region is altered, *e.g.*, mutated, to modify the properties of the antibody (*e.g.*, to increase or decrease one or more of: Fc receptor binding, antibody glycosylation, the number of cysteine residues, effector cell function, or complement function). For example, the human IgG1 constant region can be mutated at one or more residues, *e.g.*, one or more of residues 234 and 237. Antibodies can have mutations in the CH2 region of the heavy chain that reduce or alter effector function, *e.g.*, Fc receptor binding and complement activation. For example, antibodies can have mutations such as those described in U.S. Pat. Nos. 5,624,821 and 5,648,260. Antibodies can also have mutations that stabilize the disulfide bond between the two heavy chains of an immunoglobulin, such as mutations in the hinge region of IgG4, as disclosed in the art (*e.g., Angal et al. (1993) Mol. Immunol.* 30:105-08). *See also, e.g., U.S. 2005-0037000.*

[0193] In other embodiments, the antibody can be modified to have an altered glycosylation pattern (*i.e.*, altered from the original or native glycosylation pattern). As used in this context, "altered" means having one or more carbohydrate moieties deleted, and/or having one or more glycosylation sites added to the original antibody. Addition of

glycosylation sites to the presently disclosed antibodies can be accomplished by altering the amino acid sequence to contain glycosylation site consensus sequences; such techniques are well known in the art. Another means of increasing the number of carbohydrate moieties on the antibodies is by chemical or enzymatic coupling of glycosides to the amino acid residues of the antibody. These methods are described in, *e.g.*, WO 87/05330, and Aplin and Wriston (1981) *CRC Crit. Rev. Biochem.* 22:259-306. Removal of any carbohydrate moieties present on the antibodies can be accomplished chemically or enzymatically as described in the art (Hakimuddin *et al.* (1987) *Arch. Biochem. Biophys.* 259:52; Edge *et al.* (1981) *Anal. Biochem.* 118:131; and Thotakura *et al.* (1987) *Meth. Enzymol.* 138:350). *See, e.g.*, U.S. Pat. No. 5,869,046 for a modification that increases in vivo half-life by providing a salvage receptor binding epitope.

[0194] The antibodies can be in the form of full length antibodies, or in the form of fragments of antibodies, *e.g.*, Fab, F(ab')₂, Fd, dAb, and scFv fragments. Additional forms include a protein that includes a single variable domain, *e.g.*, a camel or camelized domain. *See, e.g.*, U.S. 2005-0079574 and Davies *et al.* (1996) *Protein Eng.* 9(6):531-7.

[0195] In one embodiment, the antibody is an antigen-binding fragment of a full length antibody, *e.g.*, a Fab, F(ab')₂, Fv or a single chain Fv fragment. Typically, the antibody is a full length antibody. The antibody can be a monoclonal antibody or a mono-specific antibody.

[0196] In another embodiment, the antibody can be a human, humanized, CDR-grafted, chimeric, mutated, affinity matured, deimmunized, synthetic or otherwise in vitro-generated antibody, and combinations thereof.

[0197] The heavy and light chains of the antibody can be substantially full-length. The protein can include at least one, and preferably two, complete heavy chains, and at least one, and preferably two, complete light chains) or can include an antigen-binding fragment (*e.g.*, a Fab, F(ab')₂, Fv or a single chain Fv fragment). In yet other embodiments, the antibody has a heavy chain constant region chosen from, *e.g.*, IgG1, IgG2, IgG3, IgG4, IgM, IgA1, IgA2, IgD, and IgE; particularly, chosen from, *e.g.*, IgG1, IgG2, IgG3, and IgG4, more particularly, IgG1 (*e.g.*, human IgG1). Typically, the heavy chain constant region is human or a modified form of a human constant region. In another embodiment, the antibody has a light chain constant region chosen from, *e.g.*, kappa or lambda, particularly, kappa (*e.g.*, human kappa).

[0198] In one embodiment, a protein or polypeptide of interest may be, but is not limited to, anti-LINGO, anti-LINGO-1 (see, e.g., U.S. Patent No. 8,425,910). Anti-LINGO-1, for example, is a fully human monoclonal antibody that targets LINGO-1, a protein expressed selectively in the central nervous system (CNS) that is known to negatively regulate axonal myelination and axonal regeneration (Mi S, et al. Nat Neurosci. 2004;7:221-8; Mi S, et al. Nat Neurosci. 2005;8:745-51). Other proteins include, but are not limited to, interferon (e.g., interferon beta 1a – AVONEX), Abciximab (REOPRO®), Adalimumab (HUMIRA®), Alemtuzumab (CAMPATH®), Basiliximab (SIMULECT®), Bevacizumab (AVASTIN®), Cetuximab (ERBITUX®), Certolizumab pegol (CIMZIA®), Daclizumab (ZENAPAX®), Eculizumab (SOLIRIS®), Efalizumab (RAPTIVA®), Gemtuzumab (MYLOTARG®), Ibritumomab tiuxetan (ZEVALIN®), Infliximab (REMICADE®), Muromonab-CD3 (ORTHOCLONE OKT3®), Natalizumab (TYSABRI®), Omalizumab (XOLAIR®), Palivizumab (SYNAGIS®), Panitumumab (VECTIBIX®), Ranibizumab (LUCENTIS®), Rituximab (RITUXAN®), Tositumomab (BEXXAR®), and/or Trastuzumab (HERCEPTIN®). In one embodiment, the protein or polypeptide of interest is Natalizumab (TYSABRI®). In one embodiment, the protein or polypeptide of interest is a blood cascade protein. Blood cascade proteins are known in the art and include, but are not limited to, Factor VII, tissue factor, Factor IX, Factor X, Factor XI, Factor XII, Tissue factor pathway inhibitor, Factor V, prothrombin, thrombin, vonWillebrandFactor, kininogen, prekallikrein, kallikrein, fibrinogen, fibrin, protein C, thrombomodulin, and antithrombin. In one embodiment, the blood cascade protein is Factor IX or Factor VIII. It should be appreciated that methods provided herein are also applicable for uses involving the production of versions of blood cascade proteins, including blood cascade proteins that are covalently bound to antibodies or antibody fragments, such as Fc. In one embodiment, the blood cascade protein is Factor IX- Fc (FIXFc) or Factor VIII – Fc (FVIII Fc). In one embodiment, one or more proteins of interest are hormones, regulatory proteins and/or neurotrophic factors. Neurotrophic factors are known in the art and include nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), neurotrophin-4 (NT-4), members of the glial cell line-derived neurotrophic factor ligands (GDNF) and ciliary neurotrophic factor (CNTF). In one embodiment, the protein or polypeptide of interest is neublastin.

[0199] In one embodiment, the protein or polypeptide of interest is an antibody.

Examples of antibodies that may be produced by the methods described herein include 3F8, 8H9, abagovomab, abciximab, actoxumab, adalimumab, adecatumumab, aducanumab, afelimomab, afutuzumab, alacizumab pegol, ALD, alemtuzumab, alirocumab, altumomab pentetate, amatuximab, anatumomab mafenatox, anifrolumab, anrukizumab (or IMA-638), apolizumab, arcitumomab, aselizumab, atinumab, atlizumab (or tocilizumab), atorolimumab, bapineuzumab, basiliximab, bavituximab, bectumomab, belimumab, benralizumab, bertilimumab, besilesomab, bevacizumab, bezlotoxumab, biciromab, bimagrumab, bivatuzumab mertansine, blinatumomab, blosozumab, brentuximab vedotin, briakinumab, brodalumab, canakinumab, cantuzumab mertansine, cantuzumab ravtansine, caplacizumab, capromab pendetide, carlumab, catumaxomab, cBR-doxorubicin immunoconjugate, cedelizumab, certolizumab pegol, cetuximab, citatuzumab bogatox, cixutumumab, clazakizumab, clenoliximab, clivatuzumab tetraxetan, conatumumab, concizumab, crenezumab, dacetuzumab, daclizumab, dalotuzumab, daratumumab, demcizumab, denosumab, detumomab, dorlimomab aritox, drozitumab, duligotumab, dupilumab, dusigitumab, ecromeximab, eculizumab, edobacomab, edrecolomab, efalizumab, efungumab, eldelumab, elotuzumab, elsilimomab, enavatuzumab, enlimomab pegol, enokizumab, enoticumab, ensituximab, epitumomab cituxetan, epratuzumab, erlizumab, ertumaxomab, etaracizumab, etrolizumab, evolocumab, exbivirumab, fanolesomab, faralimomab, farletuzumab, fasinumab, FBTa, felvizumab, fezakinumab, ficlatuzumab, figitumumab, flinvotumab, fontolizumab, foralumab, foravirumab, fresolimumab, fulranumab, futuximab, galiximab, ganitumab, gantenerumab, gavilimomab, gemtuzumab ozogamicin, gevokizumab, girentuximab, glembatumumab vedotin, golimumab, gomiliximab, guselkumab, ibalizumab, ibritumomab tiuxetan, icrucumab, igovomab, IMAB, imciromab, imgatuzumab, inclacumab, indatuximab ravtansine, infliximab, intetumumab, inolimumab, inotuzumab ozogamicin, ipilimumab, iratumumab, itolizumab, ixekizumab, keliximab, labetuzumab, lambrolizumab, lampalizumab, lebrikizumab, lemalesomab, lerdelimomab, lexatumumab, libivirumab, ligelizumab, lintuzumab, lirilumab, lodelcizumab, lorvotuzumab mertansine, lucatumumab, lumiliximab, mapatumumab, margetuximab, maslimomab, mavrilimumab, matuzumab, mepolizumab, metelimomab, milatuzumab, minretumomab, mitumomab, mogamulizumab, morolimumab,

motavizumab, moxetumomab pasudotox, muromonab-CD, nacolomab tafenatox, namilumab, naptumomab estafenatox, narnatumab, natalizumab, nebacumab, necitumumab, nerelimomab, nesvacumab, nimotuzumab, nivolumab, nofetumomab merpentan, ocaratuzumab, ocrelizumab, odulimomab, ofatumumab, olaratumab, olokizumab, omalizumab, onartuzumab, ontuxizumab, oportuzumab monatox, oregovomab, orticumab, otelixizumab, otlertuzumab, oxelumab, ozanezumab, ozoralizumab, pagibaximab, palivizumab, panitumumab, panobacumab, parsatuzumab, pascolizumab, pateclizumab, patritumab, pentumomab, perakizumab, pertuzumab, pexelizumab, pidilizumab, pinatuzumab vedotin, pintumomab, placulumab, polatuzumab vedotin, ponezumab, priliximab, pritoxaximab, pritumumab, PRO 140, quilizumab, racotumomab, radretumab, rafivirumab, ramucirumab, ranibizumab, raxibacumab, regavirumab, reslizumab, rilotumumab, rituximab, robatumumab, roledumab, romosozumab, rontalizumab, rovelizumab, ruplizumab, samalizumab, sarilumab, satumomab pendetide, secukinumab, seribantumab, setoxaximab, sevirumab, sibrotuzumab, SGN-CD19A, SGN-CD33A, sifalimumab, siltuximab, simtuzumab, sipilizumab, sirukumab, solanezumab, solitomab, sonepcizumab, sontuzumab, stamulumab, sulesomab, suvizumab, tabalumab, tacatuzumab tetraxetan, tadocizumab, talizumab, tanezumab, taplitumomab paptox, tefibazumab, telimomab aritox, tenatumomab, teneliximab, teplizumab, teprotumumab, TGN, ticilimumab (or tremelimumab), til-drakizumab, tigatuzumab, TNX-650, tocilizumab (or atlizumab), toralizumab, tositumomab, tovetumab, tralokinumab, trastuzumab, TRBS, tregalizumab, tremelimumab, tucotuzumab celmoleukin, tuvirumab, ublituximab, urelumab, urtoxazumab, ustekinumab, vantictumab, vapaliximab, vatelizumab, vedolizumab, veltuzumab, vepalimomab, vesencumab, visilizumab, volociximab, vorsetuzumab mafodotin, votumumab, zalutumumab, zanolimumab, zatuximab, ziralimumab and zolimomab aritox.

[0200] Examples of therapeutic proteins for use with the various aspects and embodiments described herein include without limitation insulin (*e.g.*, HUMULIN[®], NOVOLIN[®]), insulin human inhalation (*e.g.*, EXUBERA[®]), insulin aspart (*e.g.*, NOVOLOG[®]), insulin glulisine (*e.g.*, APIDRA[®]), insulin lispro (*e.g.*, HUMALOG[®]), isophane insulin (*e.g.*, NPH), insulin detemir (*e.g.*, LEVEMIR[®]), insulin glargine (*e.g.*, LANTUS[®]), insulin zinc extended (*e.g.*, LENTE[®], ULTRALENT[®]), pramlintide acetate

(*e.g.*, SYMLIN[®]), growth hormone (GH), somatotropin (*e.g.*, GENOTROPIN[®]), HUMATROPE[®], NORDITROPIN[®], NUTROPIN[®], OMNITROPE[®], PROTROPIN[®], SIAZEN[®], SEROSTIM[®], VALTROPIN[®]), Mecasermin (*e.g.*, INCRELEX[®]), Mecasermin rinfabate (*e.g.*, IPlex), Factor VIII (*e.g.*, BIOCLATE[®], HELIXATE[®], KOGENATE[®], RECOMBIMATE[®], REFECTO[®]), Factor IX (*e.g.*, BENEFIX[®]), Antithrombin III (*e.g.*, THROMBATE III[®]), protein C concentrate (*e.g.*, CEPROTIN[®]), β -Gluco-cerebrosidase (*e.g.*, CEREZYME[®]), β -Gluco-cerebrosidase (*e.g.*, CEREDASE[®] (purified from pooled human placenta), alglucosidase- χ , aronidase/ χ -l-iduronidase (*e.g.*, ALDURAZYME[®]), Idursulphase/Iduronate-2-sulphatase (*e.g.*, ELAPRASE[®]), Galsulphase (*e.g.*, NAGLAZYME[®]), Agalsidase- β /human χ -galactosidase A (*e.g.*, FABRAZYME[®]), χ -1-Proteinase inhibitor (*e.g.*, ARALAST[®], PROLASTIN[®]), Lactase (*e.g.*, LACTAID[®]), pancreatic enzymes (*e.g.*, ARCO-LASE[®], COTAZYM[®], CREON[®], DONNAZYME[®], PANCREASE[®], VIOKAS[®]E, ZYMASE[®]), Adenosine deaminase (*e.g.*, ADAGEN[®]), pooled immunoglobulins (*e.g.*, OCTAGAM[®]), Human albumin (*e.g.*, ALBUMARC[®], ALBUMIN[®], ALBUMINAR[®], ALBURX[®], ALBUTEIN[®], FLEXBUMIN[®], BUMINATE[®], PLASBUMIN[®]), erythropoietin, epoetin- χ (*e.g.*, EPOGEN[®], PROCRIT), darbepoetin- χ (*e.g.*, ARANESP[®]), filgrastim (*e.g.*, NEUPOGEN[®]), pegfilgrastim (*e.g.*, NEULASTA[®]), sargramostim (*e.g.*, LEUKINE[®]), oprelvekin (*e.g.*, NEUMEGA[®]), human follicle stimulating hormone (FSH) (*e.g.*, GONAL-F[®], FOLLISTIM[®]), human chorionic gonadotropin (*e.g.*, OVIDREL[®]), lutopin- χ (*e.g.*, LUVERIS[®]), type I alpha-interferon, interferon alfacon 1, consensus interferon (*e.g.*, INFERGEN[®]), interferon- χ 2a (IFN χ 2a) (*e.g.*, ROFERON-A[®]), PegInterferon- χ 2a (*e.g.*, PEGASYS[®]), Interferon- χ 2b (IFN χ 2b) (*e.g.*, INTRO A[®]), PegInterferon- χ 2b (PEG-INTRON[®]), Interferon- χ n3 (IFN χ n3), alferon N, interferon- β 1a(rIFN- β) (*e.g.*, AVONEX[®], REBIF[®]), interferon- β 1b(rIFN- β) (*e.g.*, BETASERON[®]), interferon- γ 1b(IFN γ) (*e.g.*, ACTIMMUNE[®]), aldesleukin (*e.g.*, PROLEUKIN[®]), alteplase (*e.g.*, ACTIVASE[®]), reteplase (*e.g.*, RETAVASE[®]), tenecteplase (TNKase), urokinase (*e.g.*, ABBOKINASE[®]), Factor VIIa (*e.g.*, NOVOSEVEN[®]), drotrecogin- χ (*e.g.*, XIGRIS[®]), salmon calcitonin (*e.g.*, FORTICAL[®], MIACALIN[®]), teriparatide (*e.g.*, FORTEO[®]), exenatide (*e.g.*, BYETTA[®]), octreotide (*e.g.*, SANDOSTATIN)[®], dibotermine- χ (*e.g.*, INFUSE[®]), recombinant human bone morphogenic protein 7 (*e.g.*, Osteogenic protein 1), histrelin acetate (*e.g.*, SUPPRELIN[®] LA, VANTAS[®]), palifermin (*e.g.*, KEPIVANCE[®]),

becaplermin (*e.g.*, REGRANEX[®]), trypsin (*e.g.*, GRANULEX[®]), nesiritide (*e.g.*, NATRECOR[®]), botulinum toxin type A (*e.g.*, BOTOX[®]), botulinum toxin type B (*e.g.*, MYOBLOCK[®]), collagenase (*e.g.*, Collagenase, SANTYL[®]), human deoxyribonuclease I, dornase – χ (*e.g.*, PULMOZYME[®]), hyaluronidase (*e.g.*, AMPHADASE[®]), hyaluronidase (*e.g.*, HYLENEX[®]), papin (*e.g.*, ACCUZYME[®], PANAFI[®]L), L-Asparaginase (*e.g.*, ELSPAR[®]), peg-asparaginase (*e.g.*, ONCASPARG[®]), rasburicase (*e.g.*, ELITEK[®]), lepirudin (*e.g.*, REFLUDAN[®]), bivalirudin (*e.g.*, ANGIOMAX[®]), streptokinase (*e.g.*, STREPTASE[®]), Anistreplase (*e.g.*, EMINASE[®]), bevacizumab (*e.g.*, AVASTIN[®]), cetuximab (*e.g.*, ERBITUX[®]), panitumumab (*e.g.*, VECTIBIX[®]), alemtuzumab (*e.g.*, CAMPATH[®]), rituximab (*e.g.*, RITUXAN[®]), trastuzumab (*e.g.*, HERCEPTIN[®]), abatacept (*e.g.*, ORENCIA[®]), anakinra (*e.g.*, ANTRIL[®], KINERET[®]), adalimumab (*e.g.*, HUMIRA[®]), etanercept (*e.g.*, ENBREL[®]), infliximab (*e.g.*, REMICADE[®]), alefacept (*e.g.*, AMEVIVE[®]), efalizumab (*e.g.*, RAPTIVA[®]), natalizumab (*e.g.*, TYSABRI[®]), eculizumab (*e.g.*, SOLIRIS[®]), antithymocyte globulin (*e.g.*, THYMOGLOBULIN[®]), basiliximab (*e.g.*, SIMULECT[®]), daclizumab (*e.g.*, ZENAPAX[®]), muromonab-CD3 (*e.g.*, ORTHOCLONE[®], OKT3), omalizumab (*e.g.*, XOLAIR[®]), palivizumab (*e.g.*, SYNAGIS[®]), enfuvirtide (*e.g.*, FUZEON[®]), abciximab (*e.g.*, REOPRO[®]), pegvisomant (*e.g.*, SOMAVERT[®]), crotalidae polyvalent immune Fab (*e.g.*, CROFAB[®]), digoxin immune serum (*e.g.*, DIGIFAB[®]), ranibizumab (*e.g.*, LUCENTIS[®]), denileukin Diftitox (*e.g.*, ONTAK[®]), ibritumomab tiuxetan (*e.g.*, ZEVALIN[®]), gemtuzumab ozogamicin (*e.g.*, MYLOTARG[®]), and tositumomab and I-tositumomab (*e.g.*, BEXXAR[®], BEXXAR[®] I-131).

Viruses

[0201] Additionally, the present invention also provides methods for the production of viruses using a cell culture according to methods known to those of skill in the field of virology. The viruses to be produced in accordance with the present invention can be chosen from the range of viruses known to infect the cultured cell type. For instance, when utilizing a mammalian cell culture, viruses can be chosen from the genera of orthomyxoviruses, paramyxoviruses, reoviruses, picornaviruses, flaviviruses, arenaviruses, herpesviruses, poxviruses, coronaviruses and adenoviruses. The virus used can be a wild-type virus, an attenuated virus, a reassortant virus, or a recombinant virus. In addition, instead of actual virions being used to infect the cells with a virus, an

infectious nucleic acid clone can be utilized according to infectious clone transfection methods known to those of skill in the field of virology. In one embodiment, the virus produced is an influenza virus.

Cells

[0202] Any eukaryotic cell or cell type susceptible to cell culture can be utilized in accordance with the present invention. For example, plant cells, yeast cells, animal cells, insect cells, avian cells or mammalian cells can be utilized in accordance with the present invention. In one embodiment, the eukaryotic cells are capable of expressing a recombinant protein or are capable of producing a recombinant or reassortant virus.

[0203] Non-limiting examples of mammalian cells that can be used in accordance with the present invention include BALB/c mouse myeloma line (NSO/1, ECACC No: 85110503); human retinoblasts (PER.C6 (CruCell, Leiden, The Netherlands)); monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, Graham *et al.*, *J. Gen Virol.*, 36:59 (1977)); baby hamster kidney cells (BHK, ATCC CCL 10); Chinese hamster ovary cells $\pm$ DHFR (CHO, Urlaub and Chasin, *Proc. Natl. Acad. Sci. USA*, 77:4216 (1980)); mouse sertoli cells (TM4, Mather, *Biol. Reprod.*, 23:243-251 (1980)); monkey kidney cells (CV1 ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1 587); human cervical carcinoma cells (HeLa, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); mouse mammary tumor (MMT 060562, ATCC CCL5 1); TRI cells (Mather *et al.*, *Annals N.Y. Acad. Sci.*, 383:44-68 (1982)); MRC 5 cells; FS4 cells; and a human hepatoma line (Hep G2). In one embodiment, the present invention is used in the culturing of and expression of polypeptides from CHO cell lines. In a specific embodiment, the CHO cell line is the CHO K1 cell line. In a specific embodiment, the CHO cell line comprises a vector comprising a polynucleotide encoding a glutamine synthetase polypeptide. In a further specific embodiment, the CHO cell line expresses an exogenous glutamine synthetase gene. (*See, e.g.*, Porter *et al.*, *Biotechnol Prog* 26(5):1446-54 (2010).)

[0204] Additionally, any number of commercially and non-commercially available hybridoma cell lines that express polypeptides or proteins can be utilized in accordance with the present invention. One skilled in the art will appreciate that hybridoma cell lines

might have different nutrition requirements and/or might require different culture conditions for optimal growth and polypeptide or protein expression, and will be able to modify conditions as needed.

[0205] The eukaryotic cells according to the present invention can be selected or engineered to produce high levels of protein or polypeptide, or to produce large quantities of virus. Often, cells are genetically engineered to produce high levels of protein, for example by introduction of a gene encoding the protein or polypeptide of interest and/or by introduction of control elements that regulate expression of the gene (whether endogenous or introduced) encoding the protein or polypeptide of interest.

[0206] The eukaryotic cells can also be selected or engineered to survive in culture for extended periods of time. For example, the cells can be genetically engineered to express a polypeptide or polypeptides that confer extended survival on the cells. In one embodiment, the eukaryotic cells comprise a transgene encoding the Bcl-2 polypeptide or a variant thereof. *See, e.g.,* US 7,785,880. In a specific embodiment, the cells comprise a polynucleotide encoding the bcl-xL polypeptide. *See, e.g.,* Chiang GG, Sisk WP. 2005. *Biotechnology and Bioengineering* 91(7):779-792.

[0207] The eukaryotic cells can also be selected or engineered to modify its posttranslational modification pathways. In one embodiment, the cells are selected or engineered to modify a protein glycosylation pathway. In a specific embodiment, the cells are selected or engineered to express an aglycosylated protein, *e.g.,* an aglycosylated recombinant antibody. In another specific embodiment, the cells are selected or engineered to express an afucosylated protein, *e.g.,* an afucosylated recombinant antibody.

[0208] The eukaryotic cells can also be selected or engineered to allow culturing in serum free medium.

Media

[0209] The cell culture of the present invention is prepared in any medium suitable for the particular cell being cultured. As discussed herein, a media formulation of the present invention generally comprises a compound, for example, a bromodomain inhibitor as described herein, that can have beneficial effects on cell growth and/or viability or on expression of polypeptide or protein. One of ordinary skill in the art will understand that

the media formulations of the present invention encompass both defined and non-defined media.

[0210] In one embodiment, the medium contains *e.g.*, inorganic salts, carbohydrates (*e.g.*, sugars such as glucose, galactose, maltose or fructose), amino acids, vitamins (*e.g.*, B group vitamins (*e.g.*, B12), vitamin A vitamin E, riboflavin, thiamine and biotin), fatty acids and lipids (*e.g.*, cholesterol and steroids), proteins and peptides (*e.g.*, albumin, transferrin, fibronectin and fetuin), serum (*e.g.*, compositions comprising albumins, growth factors and growth inhibitors, such as, fetal bovine serum, newborn calf serum and horse serum), trace elements (*e.g.*, zinc, copper, selenium and tricarboxylic acid intermediates), hydrolysates (hydrolyzed proteins derived from plant or animal sources), and combinations thereof. Commercially available media such as 5x-concentrated DMEM/F12 (Invitrogen), CD OptiCHO feed (Invitrogen), CD EfficientFeed (Invitrogen), Cell Boost (HyClone), BalanCD CHO Feed (Irvine Scientific), BD Recharge (Becton Dickinson), Cellvento Feed (EMD Millipore), Ex-cell CHOZN Feed (Sigma-Aldrich), CHO Feed Bioreactor Supplement (Sigma-Aldrich), SheffCHO (Kerry), Zap-CHO (Invitria), ActiCHO (PAA/GE Healthcare), Ham's F10 (Sigma), Minimal Essential Medium ([MEM], Sigma), RPMI-1640 (Sigma), and Dulbecco's Modified Eagle's Medium ([DMEM], Sigma) are exemplary nutrient solutions. In addition, any of the media described in Ham and Wallace,(1979) *Meth. Enz.*, 58:44; Barnes and Sato,(1980) *Anal. Biochem.*, 102:255; U.S. Pat. Nos. 4,767,704; 4,657,866; 4,927,762; 5,122,469 or 4,560,655; International Publication Nos. WO 90/03430; and WO 87/00195; the disclosures of all of which are incorporated herein by reference, can be used as culture media. Any of these media can be supplemented as necessary with hormones and/or other growth factors (such as insulin, transferrin, or epidermal growth factor), salts (such as sodium chloride, calcium, magnesium, and phosphate), buffers (such as HEPES), nucleosides (such as adenosine and thymidine), antibiotics (such as gentamycin), trace elements (defined as inorganic compounds usually present at final concentrations in the micromolar range) lipids (such as linoleic or other fatty acids) and their suitable carriers, and glucose or an equivalent energy source. In one embodiment the nutrient media is serum-free media, a protein-free media, or a chemically defined media. Any other necessary supplements can also be included at appropriate concentrations that would be known to those skilled in the art.

[0211] In one embodiment, the mammalian host cell is a CHO cell and a suitable medium contains a basal medium component such as a DMEM/HAM F-12 based formulation (for composition of DMEM and HAM F12 media, see culture media formulations in American Type Culture Collection Catalogue of Cell Lines and Hybridomas, Sixth Edition, 1988, pages 346-349) with modified concentrations of some components such as amino acids, salts, sugar, and vitamins, and optionally containing glycine, hypoxanthine, and thymidine; recombinant human insulin, hydrolyzed peptone, such as Primatone HS or Primatone RL (Sheffield, England), or the equivalent; a cell protective agent, such as Pluronic F68 or the equivalent pluronic polyol; gentamycin; and trace elements.

Cell Culture Processes

[0212] Various methods of preparing mammalian cells for production of proteins or polypeptides by batch and fed-batch culture are well known in the art. A nucleic acid sufficient to achieve expression (typically a vector containing the gene encoding the polypeptide or protein of interest and any operably linked genetic control elements) can be introduced into the host cell line by any number of well-known techniques. Typically, cells are screened to determine which of the host cells have actually taken up the vector and express the polypeptide or protein of interest. Traditional methods of detecting a particular polypeptide or protein of interest expressed by mammalian cells include but are not limited to immunohistochemistry, immunoprecipitation, flow cytometry, immunofluorescence microscopy, SDS-PAGE, Western blots, enzyme-linked immunosorbent assay (ELISA), high performance liquid chromatography (HPLC) techniques, biological activity assays and affinity chromatography. One of ordinary skill in the art will be aware of other appropriate techniques for detecting expressed polypeptides or proteins. If multiple host cells express the polypeptide or protein of interest, some or all of the listed techniques can be used to determine which of the cells expresses that polypeptide or protein at the highest levels.

[0213] Once a cell that expresses the polypeptide or protein of interest has been identified, the cell is propagated in culture by any of the variety of methods well-known to one of ordinary skill in the art. The cell expressing the polypeptide of interest is typically propagated by growing it at a temperature and in a medium that is conducive to the survival, growth and viability of the cell. The initial culture volume can be of any size, but is often smaller than the culture volume of the production bioreactor used in the

final production of the polypeptide or protein of interest, and frequently cells are passaged several times in bioreactors of increasing volume prior to seeding the production bioreactor. The cell culture can be agitated or shaken to increase oxygenation of the medium and dispersion of nutrients to the cells. Alternatively or additionally, special sparging devices that are well known in the art can be used to increase and control oxygenation of the culture. In accordance with the present invention, one of ordinary skill in the art will understand that it can be beneficial to control or regulate certain internal conditions of the bioreactor, including but not limited to pH, temperature, oxygenation, etc.

[0214] The cell density useful in the methods of the present invention can be chosen by one of ordinary skill in the art. In accordance with the present invention, the cell density can be as low as a single cell per culture volume. In one embodiment of the present invention, starting cell densities can range from about 2×10^2 viable cells per mL to about 2×10^3 , 2×10^4 , 2×10^5 , 2×10^6 , 5×10^6 or 10×10^6 viable cells per mL and higher.

[0215] In accordance with the present invention, a cell culture size can be any volume that is appropriate for production of polypeptides. In one embodiment, the volume of the cell culture is at least 500 liters. In other embodiments, the volume of the production cell culture is 10, 50, 100, 250, 1000, 2000, 2500, 5000, 8000, 10,000, 12,000 liters or more, or any volume in between. For example, a cell culture will be 10 to 5,000 liters, 10 to 10,000 liters, 10 to 15,000 liters, 50 to 5,000 liters, 50 to 10,000 liters, or 50 to 15,000 liters, 100 to 5,000 liters, 100 to 10,000 liters, 100 to 15,000 liters, 500 to 5,000 liters, 500 to 10,000 liters, 500 to 15,000 liters, 1,000 to 5,000 liters, 1,000 to 10,000 liters, or 1,000 to 15,000 liters. Or a cell culture will be between about 500 liters and about 30,000 liters, about 500 liters and about 20,000 liters, about 500 liters and about 10,000 liters, about 500 liters and about 5,000 liters, about 1,000 liters and about 30,000 liters, about 2,000 liters and about 30,000 liters, about 3,000 liters and about 30,000 liters, about 5,000 liters and about 30,000 liters, or about 10,000 liters and about 30,000 liters, or a cell culture will be at least about 500 liters, at least about 1,000 liters, at least about 2,000 liters, at least about 3,000 liters, at least about 5,000 liters, at least about 10,000 liters, at least about 15,000 liters, or at least about 20,000 liters.

[0216] One of ordinary skill in the art will be aware of and will be able to choose a suitable culture size for use in practicing the present invention. The production bioreactor

for the culture can be constructed of any material that is conducive to cell growth and viability that does not interfere with expression or stability of the produced polypeptide or protein.

[0217] The temperature of the cell culture will be selected based primarily on the range of temperatures at which the cell culture remains viable. For example, during the initial growth phase, CHO cells grow well at 37°C. In general, most mammalian cells grow well within a range of about 25°C to 42°C.

[0218] In one embodiment of the present invention, the temperature of the initial growth phase is maintained at a single, constant temperature. In another embodiment, the temperature of the initial growth phase is maintained within a range of temperatures. For example, the temperature can be steadily increased or decreased during the initial growth phase. Alternatively, the temperature can be increased or decreased by discrete amounts at various times during the initial growth phase. One of ordinary skill in the art will be able to determine whether a single or multiple temperatures should be used, and whether the temperature should be adjusted steadily or by discrete amounts.

[0219] The cells can be grown during the initial growth phase for a greater or lesser amount of time, depending on the needs of the practitioner and the requirement of the cells themselves. In one embodiment, the cells are grown for a period of time sufficient to achieve a viable cell density that is a given percentage of the maximal viable cell density that the cells would eventually reach if allowed to grow undisturbed. For example, the cells can be grown for a period of time sufficient to achieve a desired viable cell density of 1, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 or 99 percent of maximal viable cell density.

[0220] In another embodiment the cells are allowed to grow for a defined period of time. For example, depending on the starting concentration of the cell culture, the temperature at which the cells are grown, and the intrinsic growth rate of the cells, the cells can be grown for 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more days. In some cases, the cells can be allowed to grow for a month or more. In one embodiment, the growth phase is between about 1 day and about 20 days, about 1 day and about 15 days, about 1 day and about 14 days, about 1 day and about 13 days, about 1 day and about 12 days, about 1 day and about 11 days, about 1 day and about 10 days, about 1 day and about 9 days, about 1 day and about 8 days, about 1 day and about 7 days, about 1

day and about 6 days, about 1 day and about 5 days, about 1 day and about 4 days, about 1 day and about 3 days, about 2 days and about 15 days, about 3 days and about 15 days, about 4 days and about 15 days, about 5 days and about 15 days, about 6 days and about 15 days, about 7 days and about 15 days, about 8 days and about 15 days, about 9 days and about 15 days, about 10 days and about 15 days, about 2 days and about 20 days, about 3 days and about 20 days, about 4 days and about 20 days, about 5 days and about 20 days, about 6 days and about 20 days, about 7 days and about 20 days, about 8 days and about 20 days, about 9 days and about 20 days, about 10 days and about 20 days, or about 10 days and about 20 days. In another embodiment, the growth phase is at least about 1 day, at least about 2 days, at least about 3 days, at least about 4 days, at least about 5 days, at least about 6 days, at least about 7 days, at least about 8 days, at least about 9 days, at least about 10 days, at least about 11 days, at least about 12 days, at least about 15 days, or at least about 20 days. In a further embodiment, the growth phase is about 1 day, about 2 days, about 3 days, about 4 days, about 5 days, about 6 days, about 7 days, about 8 days, about 9 days, about 10 days, about 11 days, about 12 days, about 15 days, or about 20 days.

[0221] The cells would be grown for 0 days in the production bioreactor if their growth in a seed bioreactor, at the initial growth phase temperature, was sufficient that the viable cell density in the production bioreactor at the time of its inoculation is already at the desired percentage of the maximal viable cell density. The practitioner of the present invention will be able to choose the duration of the initial growth phase depending on polypeptide or protein production requirements and the needs of the cells themselves.

[0222] The cell culture can be agitated or shaken during the initial culture phase in order to increase oxygenation and dispersion of nutrients to the cells. In accordance with the present invention, one of ordinary skill in the art will understand that it can be beneficial to control or regulate certain internal conditions of the bioreactor during the initial growth phase, including but not limited to pH, temperature, oxygenation, etc. For example, pH can be controlled by supplying an appropriate amount of acid or base and oxygenation can be controlled with sparging devices that are well known in the art.

[0223] In one embodiment, at the end of the initial growth phase, at least one of the culture conditions is shifted so that a second set of culture conditions is applied. The shift in culture conditions can be accomplished by a change in the temperature, pH, osmolality

or chemical inductant level of the cell culture. In one embodiment, the culture conditions are shifted by shifting the temperature of the culture.

[0224] When shifting the temperature of the culture, the temperature shift can be relatively gradual. For example, it can take several hours or days to complete the temperature change. Alternatively, the temperature shift can be relatively abrupt. For example, the temperature change can be complete in less than several hours. Given the appropriate production and control equipment, such as is standard in the commercial large-scale production of polypeptides or proteins, the temperature change can even be complete within less than an hour.

[0225] The temperature of the cell culture in the subsequent growth phase will be selected based primarily on the range of temperatures at which the cell culture remains viable and expresses recombinant polypeptides or proteins at commercially adequate levels. In general, most mammalian cells remain viable and express recombinant polypeptides or proteins at commercially adequate levels within a range of about 25°C to 42°C. In one embodiment, mammalian cells remain viable and express recombinant polypeptides or proteins at commercially adequate levels within a range of about 25°C to 35°C. Those of ordinary skill in the art will be able to select appropriate temperature or temperatures in which to grow cells, depending on the needs of the cells and the production requirements of the practitioner.

[0226] In accordance with the present invention, once the conditions of the cell culture have been shifted as discussed above, the cell culture is maintained for a subsequent production phase under a second set of culture conditions conducive to the survival and viability of the cell culture and appropriate for expression of the desired polypeptide or protein at commercially adequate levels.

[0227] As discussed above, the culture can be shifted by shifting one or more of a number of culture conditions including, but not limited to, temperature, pH, osmolality, and sodium butyrate levels. In one embodiment, the temperature of the culture is shifted. According to this embodiment, during the subsequent production phase, the culture is maintained at a temperature or temperature range that is lower than the temperature or temperature range of the initial growth phase. For example, during the subsequent production phase, CHO cells express recombinant polypeptides and proteins well within a range of 25°C to 35°C.

[0228] In accordance with the present invention, the cells can be maintained in the subsequent production phase until a desired cell density or production titer is reached. In one embodiment, the cells are maintained in the subsequent production phase until the titer to the recombinant polypeptide or protein reaches a maximum. In other embodiments, the culture can be harvested prior to this point, depending on the production requirement of the practitioner or the needs of the cells themselves. For example, the cells can be maintained for a period of time sufficient to achieve a viable cell density of 1, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 or 99 percent of maximal viable cell density. In some cases, it is desirable to allow the viable cell density to reach a maximum, and then allow the viable cell density to decline to some level before harvesting the culture. In an extreme example, it can be desirable to allow the viable cell density to approach or reach zero before harvesting the culture.

[0229] In another embodiment of the present invention, the cells are allowed to grow for a defined period of time during the subsequent production phase. For example, depending on the concentration of the cell culture at the start of the subsequent growth phase, the temperature at which the cells are grown, and the intrinsic growth rate of the cells, the cells can be grown for 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more days. In some cases, the cells can be allowed to grow for a month or more. The practitioner of the present invention will be able to choose the duration of the subsequent production phase depending on polypeptide or protein production requirements and the needs of the cells themselves.

[0230] In certain cases, it can be beneficial or necessary to supplement the cell culture during the growth and/or subsequent production phase with nutrients or other medium components that have been depleted or metabolized by the cells. For example, it might be advantageous to supplement the cell culture with nutrients or other medium components observed to have been depleted. Alternatively or additionally, it can be beneficial or necessary to supplement the cell culture prior to the subsequent production phase. As non-limiting examples, it can be beneficial or necessary to supplement the cell culture with hormones and/or other growth factors, particular ions (such as sodium, chloride, calcium, magnesium, and phosphate), buffers, vitamins, nucleosides or nucleotides, trace elements (inorganic compounds usually present at very low final concentrations), amino acids, lipids, or glucose or other energy source.

- [0231] These supplementary components, including the amino acids, can all be added to the cell culture at one time, or they can be provided to the cell culture in a series of additions. In one embodiment of the present invention, the supplementary components are provided to the cell culture at multiple times in proportional amounts. In another embodiment, it can be desirable to provide only certain of the supplementary components initially, and provide the remaining components at a later time. In yet another embodiment of the present invention, the cell culture is fed continually with these supplementary components.
- [0232] In accordance with the present invention, the total volume added to the cell culture should optimally be kept to a minimal amount. For example, the total volume of the medium or solution containing the supplementary components added to the cell culture can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45 or 50% of the volume of the cell culture prior to providing the supplementary components.
- [0233] The cell culture can be agitated or shaken during the subsequent production phase in order to increase oxygenation and dispersion of nutrients to the cells. In accordance with the present invention, one of ordinary skill in the art will understand that it can be beneficial to control or regulate certain internal conditions of the bioreactor during the subsequent growth phase, including but not limited to pH, temperature, oxygenation, etc. For example, pH can be controlled by supplying an appropriate amount of acid or base and oxygenation can be controlled with sparging devices that are well known in the art.
- [0234] In certain embodiments of the present invention, the practitioner can find it beneficial or necessary to periodically monitor particular conditions of the growing cell culture. Monitoring cell culture conditions allows the practitioner to determine whether the cell culture is producing recombinant polypeptide or protein at suboptimal levels or whether the culture is about to enter into a suboptimal production phase.
- [0235] In order to monitor certain cell culture conditions, it will be necessary to remove small aliquots of the culture for analysis. One of ordinary skill in the art will understand that such removal can potentially introduce contamination into the cell culture, and will take appropriate care to minimize the risk of such contamination.
- [0236] As non-limiting example, it can be beneficial or necessary to monitor temperature, pH, cell density, cell viability, integrated viable cell density, lactate levels, ammonium levels, osmolality, or titer of the expressed polypeptide or protein. Numerous techniques

are well known in the art that will allow one of ordinary skill in the art to measure these conditions. For example, cell density can be measured using a hemacytometer, a Coulter counter, or Cell density examination (CEDEX). Viable cell density can be determined by staining a culture sample with Trypan blue. Since only dead cells take up the Trypan blue, viable cell density can be determined by counting the total number of cells, dividing the number of cells that take up the dye by the total number of cells, and taking the reciprocal. HPLC can be used to determine the levels of lactate, ammonium or the expressed polypeptide or protein. Alternatively, the level of the expressed polypeptide or protein can be determined by standard molecular biology techniques such as coomassie staining of SDS-PAGE gels, Western blotting, Bradford assays, Lowry assays, Biuret assays, and UV absorbance. It can also be beneficial or necessary to monitor the post-translational modifications of the expressed polypeptide or protein, including phosphorylation and glycosylation.

[0237] The practitioner can also monitor the metabolic status of the cell culture, for example, by monitoring the glucose, lactate, ammonium, and amino acid concentrations in the cell culture, as well as by monitoring the oxygen production or carbon dioxide production of the cell culture. For example, cell culture conditions can be analyzed by using NOVA Bioprofile 100 or 400 (NOVA Biomedical, WA). Additionally, the practitioner can monitor the metabolic state of the cell culture by monitoring the activity of mitochondria. In embodiment, mitochondrial activity can be monitored by monitoring the mitochondrial membrane potential using Rhodamine 123. Johnson LV, Walsh ML, Chen LB. 1980. *Proceedings of the National Academy of Sciences* 77(2):990-994.

Isolation of Expressed Polypeptide

[0238] In general, it will typically be desirable to isolate and/or purify proteins or polypeptides expressed according to the present invention. In one embodiment, the expressed polypeptide or protein is secreted into the medium and thus cells and other solids can be removed, as by centrifugation or filtering for example, as a first step in the purification process.

[0239] Alternatively, the expressed polypeptide can be bound to the surface of the host cell. In this embodiment, the media is removed and the host cells expressing the polypeptide or protein are lysed as a first step in the purification process. Lysis of mammalian host cells can be achieved by any number of means well known to those of

ordinary skill in the art, including physical disruption by glass beads and exposure to high pH conditions.

[0240] The polypeptide can be isolated and purified by standard methods including, but not limited to, chromatography (*e.g.*, ion exchange, affinity, size exclusion, and hydroxyapatite chromatography), gel filtration, centrifugation, or differential solubility, ethanol precipitation or by any other available technique for the purification of proteins (*See, e.g.*, Scopes, Protein Purification Principles and Practice 2nd Edition, Springer-Verlag, New York, 1987; Higgins, S. J. and Hames, B. D. (eds.), Protein Expression: A Practical Approach, Oxford Univ Press, 1999; and Deutscher, M. P., Simon, M. I., Abelson, J. N. (eds.), Guide to Protein Purification: Methods in Enzymology (Methods in Enzymology Series, Vol 182), Academic Press, 1997, all incorporated herein by reference). For immunoaffinity chromatography in particular, the protein can be isolated by binding it to an affinity column comprising antibodies that were raised against that protein and were affixed to a stationary support. Alternatively, affinity tags such as an influenza coat sequence, poly-histidine, or glutathione-S-transferase can be attached to the protein by standard recombinant techniques to allow for easy purification by passage over the appropriate affinity column. Protease inhibitors such as phenyl methyl sulfonyl fluoride (PMSF), leupeptin, pepstatin or aprotinin can be added at any or all stages in order to reduce or eliminate degradation of the polypeptide or protein during the purification process. Protease inhibitors are particularly desired when cells must be lysed in order to isolate and purify the expressed polypeptide or protein. One of ordinary skill in the art will appreciate that the exact purification technique will vary depending on the character of the polypeptide or protein to be purified, the character of the cells from which the polypeptide or protein is expressed, and the composition of the medium in which the cells were grown.

[0241] The foregoing description is to be understood as being representative only and is not intended to be limiting. Alternative methods and materials for implementing the invention and also additional applications will be apparent to one of skill in the art, and are intended to be included within the accompanying claims.

- [0242] The practice of the present invention will employ, unless otherwise indicated, conventional techniques of cell biology, cell culture, molecular biology, transgenic biology, microbiology, recombinant DNA, and immunology, which are within the skill of the art. Such techniques are explained fully in the literature. *See, for example, Molecular Cloning A Laboratory Manual*, 2nd Ed., Sambrook *et al.*, ed., Cold Spring Harbor Laboratory Press: (1989); *Molecular Cloning: A Laboratory Manual*, Sambrook *et al.*, ed., Cold Springs Harbor Laboratory, New York (1992), *DNA Cloning*, D. N. Glover ed., Volumes I and II (1985); *Oligonucleotide Synthesis*, M. J. Gait ed., (1984); Mullis *et al.* U.S. Pat. No: 4,683,195; *Nucleic Acid Hybridization*, B. D. Hames & S. J. Higgins eds. (1984); *Transcription And Translation*, B. D. Hames & S. J. Higgins eds. (1984); *Culture Of Animal Cells*, R. I. Freshney, Alan R. Liss, Inc., (1987); *Immobilized Cells And Enzymes*, IRL Press, (1986); B. Perbal, *A Practical Guide To Molecular Cloning* (1984); the treatise, *Methods In Enzymology*, Academic Press, Inc., N.Y.; *Gene Transfer Vectors For Mammalian Cells*, J. H. Miller and M. P. Calos eds., Cold Spring Harbor Laboratory (1987); *Methods In Enzymology*, Vols. 154 and 155 (Wu *et al.* eds.); *Immunochemical Methods In Cell And Molecular Biology*, Mayer and Walker, eds., Academic Press, London (1987); *Handbook Of Experimental Immunology*, Volumes I-IV, D. M. Weir and C. C. Blackwell, eds., (1986); *Manipulating the Mouse Embryo*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., (1986); and in Ausubel *et al.*, *Current Protocols in Molecular Biology*, John Wiley and Sons, Baltimore, Maryland (1989).
- [0243] General principles of antibody engineering are set forth in *Antibody Engineering*, 2nd edition, C.A.K. Borrebaeck, Ed., Oxford Univ. Press (1995). General principles of protein engineering are set forth in *Protein Engineering, A Practical Approach*, Rickwood, D., *et al.*, Eds., IRL Press at Oxford Univ. Press, Oxford, Eng. (1995). General principles of antibodies and antibody-hapten binding are set forth in: Nisonoff, A., *Molecular Immunology*, 2nd ed., Sinauer Associates, Sunderland, MA (1984); and Steward, M.W., *Antibodies, Their Structure and Function*, Chapman and Hall, New York, NY (1984). Additionally, standard methods in immunology known in the art and not specifically described are generally followed as in *Current Protocols in Immunology*, John Wiley & Sons, New York; Stites *et al.* (eds), *Basic and Clinical -Immunology* (8th ed.), Appleton & Lange, Norwalk, CT (1994) and Mishell and Shiigi (eds), *Selected Methods in Cellular Immunology*, W.H. Freeman and Co., New York (1980).

[0244] Standard reference works setting forth general principles of immunology include *Current Protocols in Immunology*, John Wiley & Sons, New York; Klein, J., *Immunology: The Science of Self-Nonself Discrimination*, John Wiley & Sons, New York (1982); Kennett, R., *et al.*, eds., *Monoclonal Antibodies, Hybridoma: A New Dimension in Biological Analyses*, Plenum Press, New York (1980); Campbell, A., "Monoclonal Antibody Technology" in Burden, R., *et al.*, eds., *Laboratory Techniques in Biochemistry and Molecular Biology*, Vol. 13, Elsevier, Amsterdam (1984), *Kuby Immunology* 4th ed. Ed. Richard A. Goldsby, Thomas J. Kindt and Barbara A. Osborne, H. Freeman & Co. (2000); Roitt, I., Brostoff, J. and Male D., *Immunology* 6th ed. London: Mosby (2001); Abbas A., Abul, A. and Lichtman, A., *Cellular and Molecular Immunology* Ed. 5, Elsevier Health Sciences Division (2005); Kontermann and Dubel, *Antibody Engineering*, Springer Verlag (2001); Sambrook and Russell, *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Press (2001); Lewin, *Genes VIII*, Prentice Hall (2003); Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Press (1988); Dieffenbach and Dveksler, *PCR Primer* Cold Spring Harbor Press (2003).

[0245] All of the references cited above, as well as all references cited herein, are incorporated herein by reference in their entireties.

EXAMPLES

Example 1 - Addition of SGC-CBP30 Enhances Protein titer

[0246] A study of the effect of SGC-CBP30 at different concentrations on protein titers in fed-batch shake flask cultures was performed. The study involved CHO K1 cell line constructed to produce CD-40 antibody. In this set of experiments, SGC-CBP30 was added to each culture on Day 8 and Day 11 of culture to reach targeted concentrations in the culture, 1 uM, 3 uM, 10 uM, 30 uM, or 100 uM. The cultures were analyzed for antibody titers.

[0247] Culture Parameters used are summarized in the table below:

Target Seed Density:	4.00E+05	cell/ml
Temp:	35	°C
Agitation:	125/800*	rpm
Humidity:	80	%
CO2:	5	%
Working Volume:	40/1*	ml

*shake flask/96-well plate

[0248] Briefly, cells were cultured in a single large shake flask (40 mL working volume) and dispensed into a 96-well plate (1 mL working volume) for treatment on day 7. SGC-CBP30 was dissolved in DMSO (10 mM). This solution was then added on Day 8 and Day 11 in the amount of 0.1 uL, 0.3 uL, 1 uL, 3 uL, or 10 uL to reach the targeted concentrations. As a control, no addition of DMSO and SGC-CBP30 on Day 8 and Day 11 was made for some of the cultures. To study the solvent effect, equal volumes of DMSO without SGC-CBP30, i.e., 0.1 uL, 0.3 uL, 1 uL, 3 uL, or 10 uL, were added on Day 8 and Day 11 to some of the cultures. Feed medium was added daily from day 3 to day 12, ranging from 2.75% to 8.25%. Samples were taken on days 10, 12, and 14 and protein titer were analyzed. Protein titers were measured by Octet® (ForteBio).

[0249] The results of the experiments were shown in FIGs. 1A-1C. As shown in FIG. 1A, the protein titers produced by cell cultures with added SGC-CBP30, as measured on Day 12 and Day 14, were higher than the titers produced by the control cultures. The enhancement is more pronounced when the SGC-CBP30 was added at a concentration of 1 uM to 30 uM. FIG. 1B shows that adding a solvent DMSO only has limited effect on antibody titers. There are only marginal enhancements from the cell cultures with 3 uL or 10 uL DMSO added. FIG. 1C further shows that adding SGC-CBP30 to cell cultures enhances protein titers when compared to the control cell cultures and the cell cultures with 10 uL DMSO added.

[0250] Table 1 below summarizes the effect of SGC-CBP30 on protein titers (ug/mL) in comparison to DMSO addition and control.

Table 1: Comparison of titers from cultures containing SGC-CBP30 or DMSO with control

	CNTL	DMSO	SGC-CBP30	% increase* over DMSO	% increase* over CNTL
1 uM	3517	3244	5127	58.04	45.78
3 uM	3517	3349	5219	55.84	48.40
10 uM	3517	3533	5612	58.84	59.56
30 uM	3517	3984	5500	38.06	56.39
100 uM	3517	4117	3356	-18.47	-4.56

* The percentage increase is calculated for SGC-CBP30.

[0251] The above results show that the addition of SGC-CBP30 is able to increase protein titers produced by cell cultures at various concentrations. The improvements in

titer in some instances reached about 60% over either cultures with DMSO added or the control cultures.

Example 2 - Addition of SGC-CBP30 Enhances Protein titers and Cell Specific Productivity

- [0252]** A study of the effect of SGC-CBP30 on viable cell density (VCD) and cell viability and protein titer in fed-batch shake flask cultures was performed. The study involved CHO K1 cell line, constructed to produce CD-40, OSMR, or STX200 proteins/antibodies, or CHO DG44 cell line, constructed to produce BART, LINGO, or Alpha-SYN proteins/antibodies. Similar to Example 1, in this set of experiments, SGC-CBP30 was added to each culture to reach targeted concentrations in the culture, 3 μ M or 30 μ M, on Day 8 and Day 11 of the culture. The cultures were studied with respect to viable cell density (VCD) and cell viability and antibody titer.
- [0253]** The culturing conditions were similar to those described for Example 1, except that in this set of experiments, a 24-well plate was used and the working volume was 5 mL and the agitation speed was 450 rpm.
- [0254]** Briefly, cells were cultured in a single large shake flask (40 mL working volume) and dispensed into a 24-well plate (5 mL working volume) for treatment on day 7. SGC-CBP30 solution in DMSO (10 mM) was then added on Day 8 and Day 11 in the amount of 1.5 μ L or 15 μ L to reach the targeted concentrations. As a control, no addition of DMSO and SGC-CBP30 on Day 8 and Day 11 was made for some of the cultures. To study the solvent effect, equal volumes of DMSO without SGC-CBP30, i.e., 1.5 μ L or 15 μ L, were added on Day 8 and Day 11 to some of the cultures. Feed medium was added daily from day 3 to day 12, ranging from 2.75% to 8.25%. Samples were taken on Day 0, Day 3, Day 4, Day 7, Day 8, Day 10, Day 12, and Day 14 and cell density, viability, and protein titers were analyzed. Cell counts were conducted on Vi-Cell™ Cell Counter (Beckman Coulter, Inc.). Protein titers were measured by Octet® (ForteBio).
- [0255]** Tables 2A-2D summarize the protein titers and cell specific productivities measured on Day 12 and Day 14. The data shown in the tables is expressed as percentage increase over the control cell culture (i.e., no DMSO and no SGC-CBP30).

Table 2A. Protein titers in cell cultures as measured on Day 14

Addition	A-Syn	BART	CD40	LINGO	OSMR	STX200	Avg	s.d.
1.5 μ L DMSO	0.1	12.5	3.8	2.8	2.0	1.0	3.7	4.5
15 μ L DMSO	2.2	9.2	17.3	5.9	1.1	6.0	6.9	5.9

3 uM SGC-CBP30	4.4	0.1	29.4	11.9	6.5	11.1	10.6	10.2
30 uM SGC-CBP30	-11.0	-12.6	35.2	-27.3	12.7	23.1	3.4	24.1

Table 2B. Cell Specific Productivity in cell cultures as measured on Day 14

Addition	A-Syn	BART	CD40	LINGO	OSMR	STX200	<u>Avg</u>	s.d.
1.5 uL DMSO	0.9	14.8	4.4	3.5	3.5	3.3	5.1	4.9
15uL DMSO	6.2	14.1	21.5	7.4	5.4	8.2	10.5	6.2
3 uM SGC-CBP30	9.5	4.4	35.1	11.9	11.0	11.7	13.9	10.8
30 uM SGC-CBP30	7.3	0.9	42.3	-11.5	20.8	26.4	14.4	19.3

Table 2C. Protein titers in cell cultures as measured on Day 12

Addition	A-Syn	BART	CD40	LINGO	OSMR	STX200	<u>Avg</u>	s.d.
1.5 uL DMSO	9.1	-3.7	-15.0	12.4	-14.7	-28.7	-6.8	15.8
15uL DMSO	-1.3	-1.0	-7.2	16.1	-11.4	-16.4	-3.6	11.3
3 uM SGC-CBP30	3.3	3.9	0.0	47.4	14.6	11.7	13.5	17.5
30 uM SGC-CBP30	4.2	-13.7	2.4	-2.2	9.3	2.7	0.5	7.9

Table 2D. Cell Specific Productivity in cell cultures as measured on Day 12

Addition	A-Syn	BART	CD40	LINGO	OSMR	STX200	<u>Avg</u>	s.d.
1.5 uL DMSO	9.9	-1.8	1.1	12.8	-13.6	-27.5	-3.2	15.1
15uL DMSO	1.3	2.0	10.4	18.0	-8.2	-14.2	1.5	11.8
3 uM SGC-CBP30	6.1	2.0	13.8	49.6	18.5	14.5	17.4	16.9
30 uM SGC-CBP30	14.0	-8.9	17.8	6.3	15.4	7.4	8.7	9.7

[0256] As shown in Tables 2A-2D, on average, antibody titers and cell specific productivity were improved in cultures with SGC-CBP30 added at 3 uM or 30 uM than those observed for cultures with DMSO added or control cultures. However, the advantageous effects observed are more pronounced in the CHO K1 cell lines. For example, in some cell lines, the cell specific productivity improvement is above 40% compared to control. The concentrations of SGC-CBP30 tested in this example may not be optimal for the CHO DG44 cell lines as tested in this example.

[0257] The results of the experiments using CHO-K1 cell lines were also shown in FIGs. 3A-3D (CD-40), 4A-4D (OSMR), and 5A-5D (STX-200). As shown in the figures, adding 3 uM or 30 uM SGC-CBP30 to cell cultures improved titers and cell specific productivities over those observed for the cell cultures with DMSO alone added or the control cultures. Further, viable cell densities and viabilities are also generally improved in cell cultures with 3 uM or 30 uM SGC-CBP30 added over the VCD and viability in the cell cultures with DMSO alone added or the control cultures.

[0258] In summary, the addition of SGC-CBP30 is able to increase protein titers and cell specific productivities at 3 uM and/or 30 uM.

Example 3 – Effect of Various Compounds on Cell Growth and Protein titer

[0259] A study of the effect of various bromodomain inhibitors, BI-2536, and CI-Amidine (as TFA salt) on viable cell density, cell viability, and protein titer in fed-batch shake flask cultures was performed. The study involved CHO K1 cell line, constructed to produce CD-40 antibodies. The compounds in this set of experiments were added to each culture to reach targeted concentrations in the culture, 3 uM or 30 uM, on Day 8 and Day 11 of the cultures. The cultures were studied with respect to viable cell density (VCD) and cell viability and antibody titer.

[0260] The same procedure of Example 1 was used for this study, except that besides SGC-CBP30, other compounds were also tested.

[0261] Briefly, cells were cultured in a single large shake flask (40 mL working volume) and dispensed into a 96-well plate (1 mL working volume) for treatment on day 7. On Day 8 and Day 11, cell cultures were added 1.5 uL or 15 uL of compound solutions in DMSO (10 mM) to reach the targeted concentrations. The compound solutions used in this study are SGC-CBP30 solution, RVX-208 solution, JQ-1 solution, PFI-1 solution, I-BET-762 solution, OTX015 solution, I-BET-151 solution, bromosporin solution, I-CBP112 solution, BI-2536 solution, or CI-Amidine solution. As a control, the compounds or DMSO were not added to some of the cultures. To study the solvent effect, equal volumes of DMSO without the tested compounds, i.e., 1.5 uL or 15 uL, were added on Day 8 and Day 11 to some of the cultures. Feed medium was added daily from day 3 to day 12, ranging from 2.75% to 8.25%. Samples were taken on Day 12, and Day 14 and cell density, viability, and protein titers were analyzed. Cell counts were

conducted on Vi-Cell™ Cell Counter (Beckman Coulter, Inc.). Protein titers were measured by Octet® (ForteBio).

- [0262] The protein titers (ug/mL) on D12 and Day 14 and cell viability and density data on Day 14 are summarized in Tables 3A and 3B. In the control cultures, the Day 12 average titer was 2225.8 mg/L and the Day 14 average titer was 2309.0 mg/L, and the percentage viable cells on Day 14 is 59.4% with the viable cell density of 11.9×10^6 cells/mL. For the DMSO treated cultures (all combined), the Day 12 average titer was 2123.4 mg/L and the Day 14 average titer was 2153.4 mg/L, and the percentage viable cells on Day 14 is 60.2% with the viable cell density of 14.5×10^6 cells/mL.
- [0263] Some of the results of the experiments were shown in FIGs. 6A-6D.
- [0264] A more detailed data is presented in Tables 3A and 3B.

Table 3A. Effects of Bromodomain Inhibitors on Titer on Day 12 and Day 14

Compound*	Compound Concentration 3uM				Compound Concentration 30uM			
	D12 Avg	D14 Avg	D12 StDev	D14 StDev	D12 Avg	D14 Avg	D12 StDev	D14 StDev
RVX-208	2034.8	2173.7	108.9	89.8	2917.5	3584.2	75.8	28.1
RVX-208	2254.7	2295.0	32.8	58.3	2836.0	3347.2	1.9	66.3
SGC-CBP30	2246.0	2404.2	80.0	14.8	2722.3	3204.9	49.3	11.6
SGC-CBP30	2323.5	2483.5	30.6	146.5	2611.7	3169.1	9.0	24.3
I-BET-762	2748.4	3198.6	65.8	8.7	2706.3	3120.1	7.9	13.5
I-BET-762	2797.2	3241.1	3.4	125.0	2643.9	3097.4	28.5	93.5
(+)-JQ1	2415.5	2788.6	213.3	174.2	2571.8	2909.0	26.6	31.8
(+)-JQ1	2265.2	2383.2	469.0	458.0	2542.7	2761.3	57.1	219.7
BI-2536	2019.3	2219.4	329.8	437.1	2494.4	2737.4	37.0	189.6
OTX015	2490.4	2947.5	157.4	112.7	2367.6	2703.4	80.4	7.5
OTX015	2826.8	3254.1	1.2	158.7	2490.5	2681.1	64.1	94.0
(+)-JQ1	2502.7	2979.5	166.4	151.3	2244.5	2679.8	51.0	183.0
Bromosporine	2713.6	3066.1	106.5	49.3	2493.9	2656.5	1.0	21.1
I-CBP112 (hydrochloride)	2106.3	2207.0	87.7	23.3	2490.7	2600.5	67.9	43.3
I-CBP112 (hydrochloride)	1921.4	1900.5	194.2	131.2	2492.5	2589.3	27.5	152.7
Bromosporine	2654.4	3018.7	5.1	29.4	2534.7	2584.2	91.3	63.1
CPI203	2242.8	2386.5	354.9	386.5	2405.7	2566.3	213.8	329.9
Cl-Amidine (TFA salt)	2103.0	2304.4	21.4	82.8	2406.2	2561.3	52.4	92.1
Bromosporine	2586.3	2866.8	167.2	37.4	2430.0	2515.5	57.6	171.3
I-BET151 (GSK1210151A)	2556.1	2818.6	252.5	303.8	2283.6	2423.7	43.3	44.2
PFI-1	2795.8	3217.0	33.9	89.1	2246.9	2277.4	12.8	21.5
PFI-1 (PF- 6405761)	2740.5	3015.9	73.2	165.5	2253.3	2417.8	23.5	114.8

* some of the compounds were tested twice.

Table 3B. Effects of Bromodomain Inhibitors on Cell Density and Viability on Day 14

VCD St Dev (e6)	Compound Concentration 3uM				Compound Concentration 30uM			
Compound*	% Viability Avg	VCD Avg (e6)	% Via StDev	VCD St Dev (e6)	% Viability Avg	VCD Avg (e6)	% Via StDev	VCD St Dev (e6)
RVX-208	62.3	12.3	1.4	1.3	75.9	23.5	1.5	2.4
RVX-208	66.9	14.3	1.0	3.0	55.7	14.5	20.5	10.8
SGC-CBP30	68.5	15.1	2.1	1.2	78.3	23.0	0.2	0.0
SGC-CBP30	66.1	13.8	3.4	0.2	80.7	25.1	1.0	0.4
I-BET-762	77.2	20.8	1.2	1.0	77.6	22.7	0.3	0.4
I-BET-762	68.9	14.9	7.6	2.1	41.8	11.5	31.7	10.8
(+)-JQ1	73.3	19.0	2.0	0.9	72.0	22.2	1.4	2.1
(+)-JQ1	51.7	9.2	23.6	7.1	58.4	12.5	15.1	7.5
BI-2536	60.4	13.8	8.4	1.6	69.1	20.5	1.3	0.0
OTX015	45.2	10.0	29.2	9.1	69.2	20.7	0.6	1.1
OTX015	70.8	15.3	8.5	2.9	73.5	22.2	1.7	0.5
(+)-JQ1	71.9	21.1	0.2	0.6	71.8	22.0	1.0	0.7
Bromosporine	75.8	22.3	0.7	0.7	71.4	20.5	1.3	1.0
I-CBP112 (hydrochloride)	36.5	5.8	19.5	4.7	40.2	11.9	28.8	10.4
I-CBP112 (hydrochloride)	59.1	11.7	3.0	2.9	63.4	14.8	2.1	1.8
Bromosporine	51.9	10.2	12.9	6.7	39.5	11.7	32.5	11.0
CPI203	67.2	12.8	9.1	6.0	70.5	22.8	2.1	0.2
Cl-Amidine (TFA salt)	36.3	6.8	21.2	5.7	62.6	16.4	1.3	0.6
Bromosporine	69.1	15.0	8.4	3.0	73.1	25.0	1.3	1.1
I-BET151 (GSK1210151A)	68.7	15.7	9.0	2.5	74.3	23.7	0.1	1.2
PFI-1	76.2	18.5	3.2	0.7	38.9	12.2	35.5	11.9
PFI-1 (PF-6405761)	62.6	13.7	8.9	6.4	47.5	12.7	31.4	11.9

* some of the compounds were tested twice.

[0265] As shown in the figures and the Tables above, the various tested compounds were shown to effective in enhancing product titers on both Day 12 and Day 14. Further, viable cell densities and cell viabilities are also improved for at least some of the compounds tested at 3 uM and/or 30 uM.

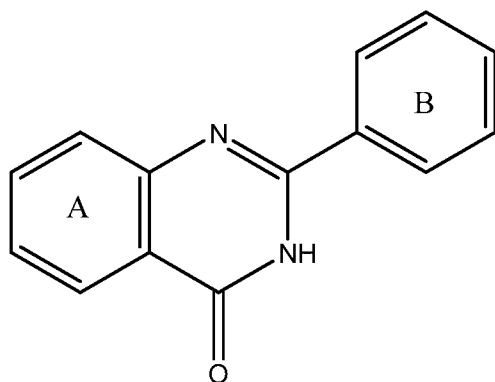
[0266] The present invention is not to be limited in scope by the specific embodiments described which are intended as single illustrations of individual aspects of the invention, and any compositions or methods which are functionally equivalent are within the scope of this invention. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and accompanying drawings. Such modifications are intended to fall within the scope of the appended claims.

[0267] All documents, articles, publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

WHAT IS CLAIMED IS:

1. A method of producing a protein or polypeptide of interest comprising
 - a) providing a cell culture comprising
 - i) mammalian cells comprising a polynucleotide encoding the protein or polypeptide of interest; and
 - ii) a cell culture medium comprising a bromodomain inhibitor; and
 - b) culturing the mammalian cells under conditions that allow expression of the protein or polypeptide of interest.
2. The method of claim 1, wherein the bromodomain inhibitor is present in the cell culture medium in a concentration ranging from about 1 uM to about 100 uM.
3. The method of claim 1 or 2, wherein the culturing step is performed in a fed-batch mode.
4. The method of any one of claims 1-3, further comprising producing the protein or polypeptide of interest at day 14 of the culture at a titer about 15% to about 100% greater than the titer produced from a control cell culture that does not contain a bromodomain inhibitor.
5. The method of any one of claims 1-4, further comprising generating a viable cell density at day 14 of the culture of about 20% to about 100% higher than that observed for a control cell culture that does not contain a bromodomain inhibitor.
6. The method of any one of claims 1-5, further comprising generating a cell specific productivity at day 14 of the culture that is about 10% to about 60% higher than that observed for a control cell culture that does not contain a bromodomain inhibitor.
7. The method of any one of claims 1-6, wherein the mammalian cells are selected from the group consisting of: CHO cells, HEK-293 cells, VERO cells, NS0 cells, PER.C6 cells, Sp2/0 cells, BHK cells, MDCK cells, MDBK cells, COS cells and HeLa cells.
8. The method of claim 7, wherein the mammalian cells are CHO cells.

9. The method of claim 8, wherein the CHO cells are CHO-K1 cells.
10. The method of any one of claims 1-9, wherein the protein or polypeptide of interest comprises an antigen binding protein.
11. The method of any one of claims 1-10, wherein the protein or polypeptide of interest comprises an antibody.
12. The method of any one of claims 1-11, wherein the protein or polypeptide of interest comprises a humanized antibody.
13. The method of any one of claims 1-12, wherein the cell culture medium comprises a bromodomain inhibitor of Formula I:



(Formula I),

wherein ring A is optionally substituted with 1 to 4 R^{10} , each independently selected from the group consisting of halogen, OH, C_{1-4} alkyl, and C_{1-4} alkoxy, wherein the C_{1-4} alkyl and C_{1-4} alkoxy are optionally substituted with 1-3 halogens; and

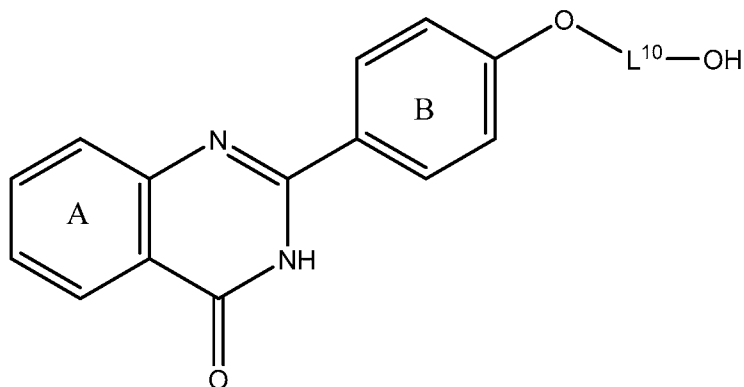
ring B is optionally substituted with 1 to 5 R^{11} each independently selected from the group consisting of OH, halogen, C_{1-4} alkyl, C_{1-4} alkoxy, wherein the C_{1-4} alkyl and C_{1-4} alkoxy are optionally substituted with 1-3 halogens or OR^{100} ,

wherein R^{100} is H or a C_{1-4} alkyl, optionally substituted with OH, OMe, $O(CH_2)_2$ -OH, or $O(CH_2)_2$ -OMe,

or a tautomer, a stereoisomeric mixture, or a salt thereof.

14. The method of claim 13, wherein the cell culture medium comprises a bromodomain inhibitor of Formula Ia:

- 88 -



(Formula Ia),

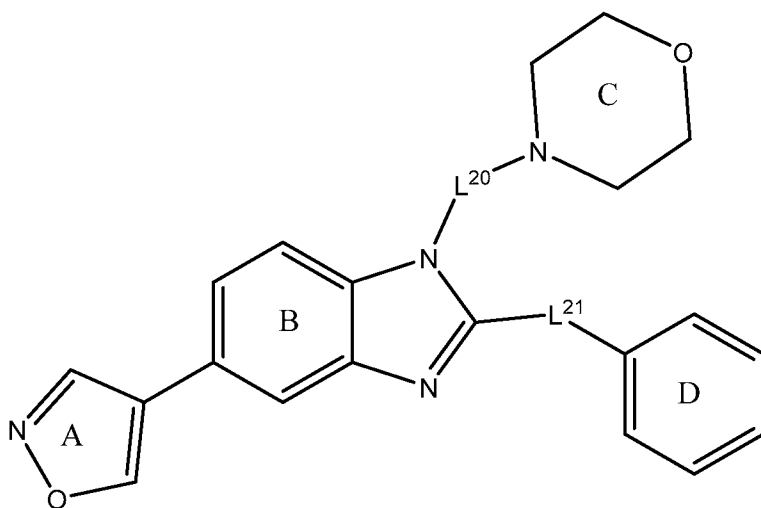
wherein ring A is optionally substituted with 1 or 2 R^{10} , each independently selected from the group consisting of halogen, C_{1-4} alkyl, and C_{1-4} alkoxy;

ring B is optionally substituted with 1 or 2 R^{11} , each independently selected from the group consisting of halogen and C_{1-4} alkyl; and

L^{10} is a C_{2-4} alkylene linker,

or a tautomer, a stereoisomeric mixture, or a salt thereof.

15. The method of claim 14, wherein ring A is substituted with 1 or 2 R^{10} , each independently selected from the group consisting of F, Cl, Me, Et, OMe, and OEt; ring B is substituted with 1 or 2 R^{11} , each independently selected from the group consisting of F, Cl, Me, and Et.
16. The method of any one of claims 1-12, wherein the cell culture medium comprises a bromodomain inhibitor of Formula II:



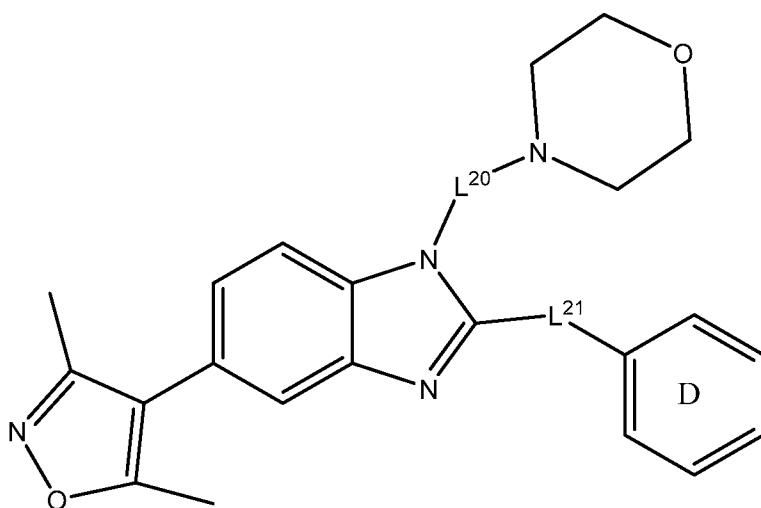
(Formula II)

ring D is optionally substituted with 1 or 2 R^{23} , each independently selected from the group consisting of halogen, C_{1-4} alkyl, and C_{1-4} alkoxy,

L^{20} is a C_{2-4} alkylene linker, optionally substituted with one methyl or ethyl group; and

L^{21} is a C_{1-4} alkylene linker, optionally substituted with one methyl or ethyl group, or a tautomer, a stereoisomeric mixture, or a salt thereof.

18. The method of claim 16, wherein the cell culture medium comprises a bromodomain inhibitor of Formula IIb:



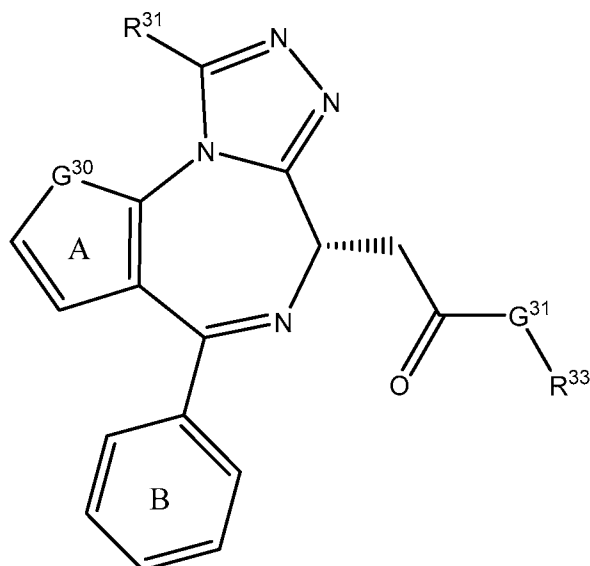
(Formula IIb)

wherein ring D is substituted with 1 or 2 R^{23} , each independently selected from the group consisting of F, Cl, Me, Et, OMe, and OEt,

L^{20} is a C_{2-4} alkylene linker, optionally substituted with one methyl group; and

L^{21} is a C_{1-4} alkylene linker, optionally substituted with one methyl group, or a tautomer, a stereoisomeric mixture, or a salt thereof.

19. The method of any one of claims 1-12, wherein the cell culture medium comprises a bromodomain inhibitor of Formula III:



(Formula III),

wherein G^{30} is S or C=C,

G^{31} is O or NR^{300} ,

ring A is optionally substituted with 1-4 R^{30} , each independently selected from the group consisting of halogen, C_{1-4} alkyl, and C_{1-4} alkoxy, wherein the C_{1-4} alkyl and C_{1-4} alkoxy are optionally substituted with 1-3 halogens;

R^{31} is H or a C_{1-4} alkyl, wherein the C_{1-4} alkyl is optionally substituted with 1-3 halogens;

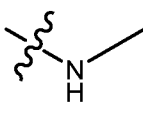
ring B is optionally substituted with 1-5 R^{32} , each independently selected from the group consisting of halogen and C_{1-4} alkyl, wherein the C_{1-4} alkyl is optionally substituted with 1-3 halogens; and

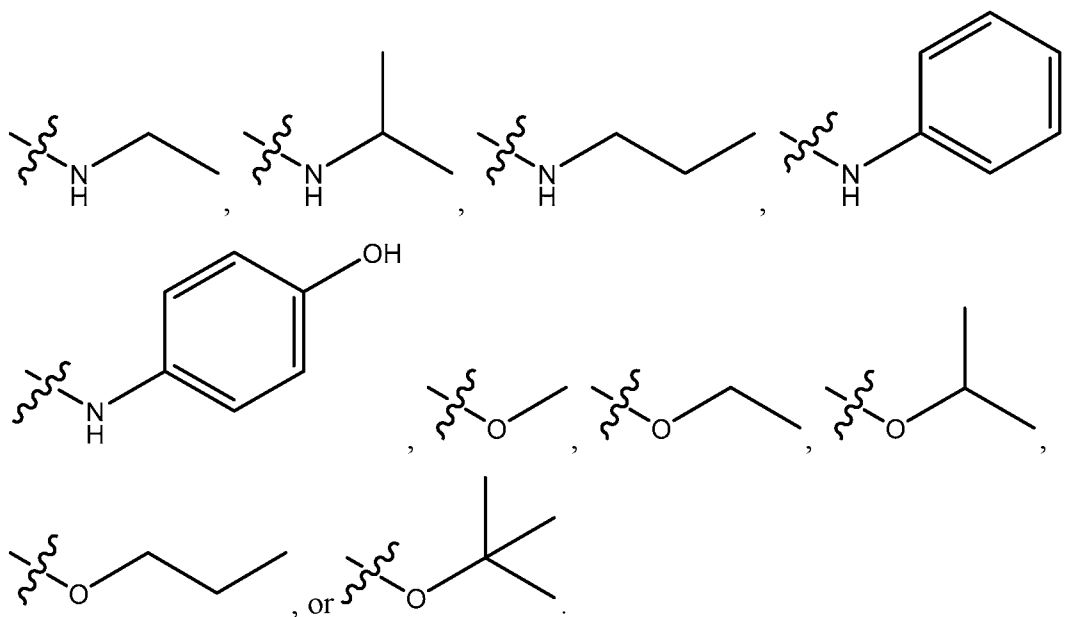
R^{33} is H or a C_{1-4} alkyl, wherein the C_{1-4} alkyl is optionally substituted with 1-3 halogens,

wherein R^{300} is H, a C_{1-4} alkyl optionally substituted with 1-3 halogens, or a phenyl optionally substituted with 1-5 substituents selected from the group consisting of halogen, OH, C_{1-4} alkyl, and C_{1-4} alkoxy, wherein the C_{1-4} alkyl and C_{1-4} alkoxy are optionally substituted with 1-3 halogens,

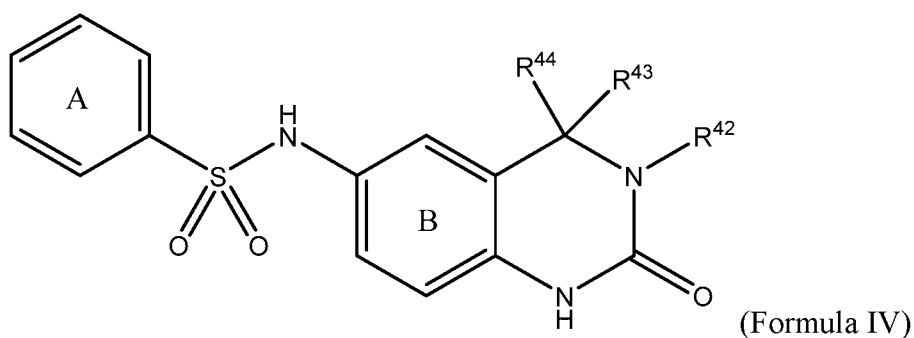
or a tautomer, a stereoisomeric mixture, or a salt thereof.

20. The method of claim 19, wherein R^{31} is Me.
21. The method of claim 19 or 20, wherein ring B is substituted with 1 or 2 R^{32} , each independently selected from the group consisting of F, Cl, Me, and Et.

22. The method of any one of claims 19-21, wherein G^{31} - R^{33} is OH, NH_2 , ,



23. The method of any one of claim 19-22, wherein G^{30} is S, and ring A is optionally substituted with 1 or 2 R^{30} , each independently selected from the group consisting of halogen and C_{1-4} alkyl.
24. The method of any one of claim 19-22, wherein G^{30} is $C=C$, and ring A is optionally substituted with 1 or 2 R^{30} , each independently selected from the group consisting of halogen, C_{1-4} alkyl, and C_{1-4} alkoxy.
25. The method of any one of claims 1-12, wherein the cell culture medium comprises a bromodomain inhibitor of Formula IV:



wherein ring A is optionally substituted with 1-5 R^{40} , each independently selected from the group consisting of halogen, OH, C_{1-4} alkyl, and C_{1-4} alkoxy, wherein the C_{1-4} alkyl and C_{1-4} alkoxy are optionally substituted with 1-3 halogens;

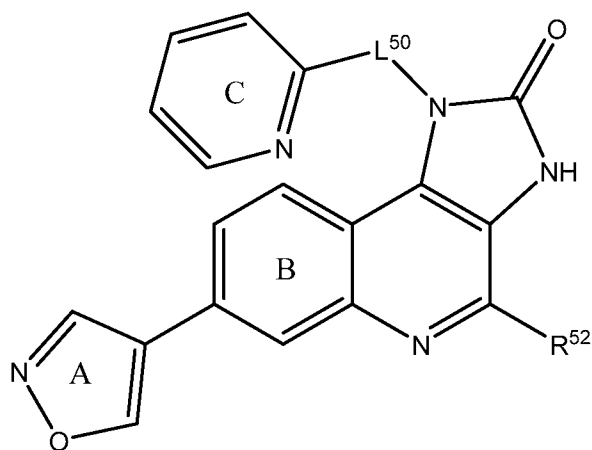
ring B is optionally substituted with 1-3 R^{41} , each independently selected from the group consisting of halogen and C_{1-4} alkyl, wherein the C_{1-4} alkyl is optionally substituted with 1-3 halogens;

each of R^{42} , R^{43} , and R^{44} is independently selected from the group consisting of hydrogen and C_{1-4} alkyl, wherein the C_{1-4} alkyl is optionally substituted with 1-3 halogens,

or a tautomer, a stereoisomeric mixture, or a salt thereof.

26. The method of claim 25, wherein ring A is optionally substituted with 1 or 2 R^{40} , each independently selected from the group consisting of halogen, C_{1-4} alkyl, and C_{1-4} alkoxy, ring B is optionally substituted with 1 or 2 R^{41} , each independently selected from the group consisting of halogen and C_{1-4} alkyl;
 R^{43} and R^{44} are both H, and
 R^{42} is Me or Et.

27. The method of any one of claims 1-12, wherein the cell culture medium comprises a bromodomain inhibitor of Formula V:



(Formula V),

wherein ring A is optionally substituted with 1 or 2 R^{50} , each independently selected from the group consisting of halogen and C_{1-4} alkyl, wherein the C_{1-4} alkyl is optionally substituted with 1-3 halogens;

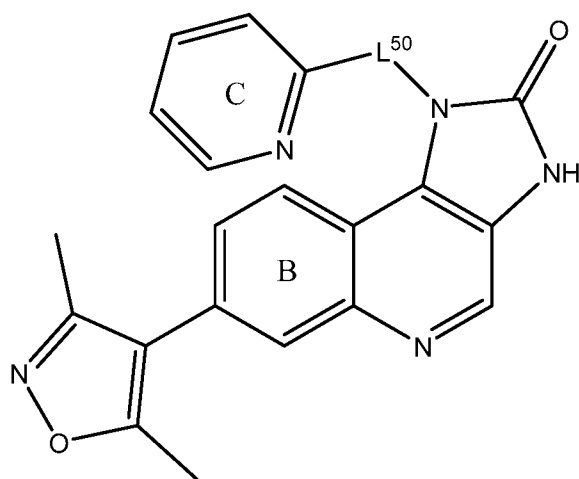
ring B is optionally substituted with 1-3 R^{51} , each independently selected from the group consisting of halogen, OH, C_{1-4} alkyl, and C_{1-4} alkoxy, wherein the C_{1-4} alkyl and C_{1-4} alkoxy are optionally substituted with 1-3 halogens;

R^{52} is H or a C_{1-4} alkyl, wherein the C_{1-4} alkyl is optionally substituted with 1-3 halogens;

ring C is optionally substituted with 1-4 R^{53} , each independently selected from the group consisting of halogen and C_{1-4} alkyl, wherein the C_{1-4} alkyl is optionally substituted with 1-3 halogens; and

L^{50} is a C_{1-4} alkylene linker, optionally substituted with a C_{1-4} alkyl, wherein the C_{1-4} alkyl is optionally substituted with 1-3 halogens,
or a tautomer, a stereoisomeric mixture, or a salt thereof.

28. The method of claim 27, wherein the cell culture medium comprises a bromodomain inhibitor of Formula Va:



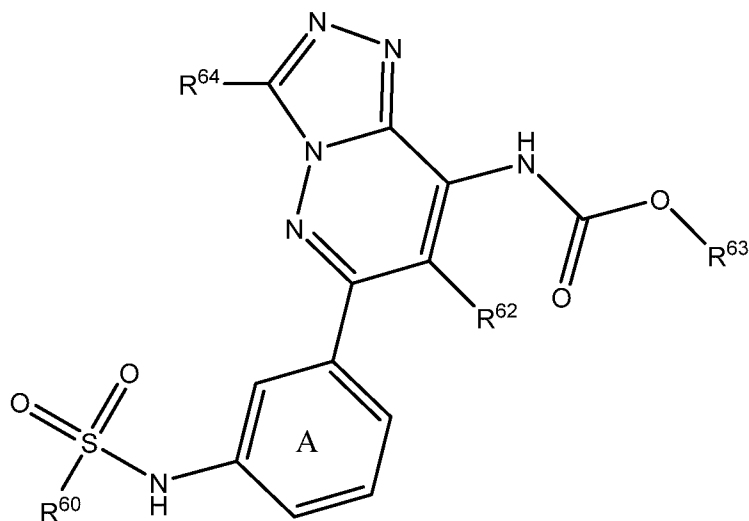
(Formula Va),

wherein ring B is optionally substituted with 1 or 2 R^{51} , each independently selected from the group consisting of halogen, C_{1-4} alkyl, and C_{1-4} alkoxy;

ring C is optionally substituted with 1 or 2 R^{53} , each independently selected from the group consisting of halogen and C_{1-4} alkyl; and

L^{50} is a C_{1-4} alkylene linker, optionally substituted with a Me or Et,
or a tautomer, a stereoisomeric mixture, or a salt thereof.

29. The method of any one of claims 1-12, wherein the cell culture medium comprises a bromodomain inhibitor of Formula VI:



wherein each of R^{60} and R^{63} is independently a C_{1-4} alkyl, wherein the C_{1-4} alkyl optionally substituted with 1-3 halogens;

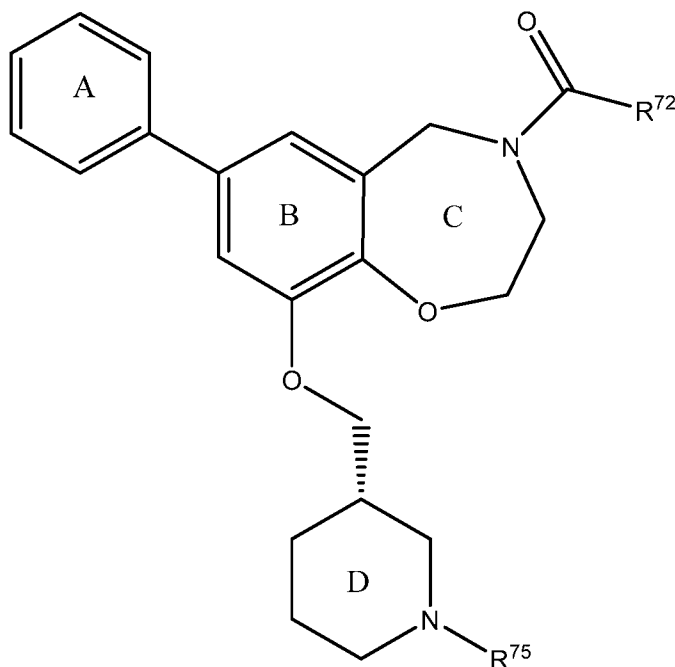
ring A is optionally substituted with 1-4 R^{61} , each independently selected from the group consisting of halogen and C_{1-4} alkyl, wherein the C_{1-4} alkyl is optionally substituted with 1-3 halogens;

R^{62} is selected from the group consisting of H, halogen and C_{1-4} alkyl, wherein the C_{1-4} alkyl is optionally substituted with 1-3 halogens; and

R^{64} is H or a C_{1-4} alkyl, wherein the C_{1-4} alkyl is optionally substituted with 1-3 halogens,

or a tautomer, a stereoisomeric mixture, or a salt thereof.

30. The method of claim 29, wherein R^{62} is H.
31. The method of claim 29 or 30, wherein R^{64} is Me.
32. The method of any one of claims 29-31, wherein R^{60} and R^{63} are independently a C_{1-4} alkyl, and ring A is optionally substituted with 1 or 2 R^{61} , each independently selected from the group consisting of halogen and C_{1-4} alkyl.
33. The method of any one of claims 1-12, wherein the cell culture medium comprises a bromodomain inhibitor of Formula VII:



(Formula VII),

wherein ring A is optionally substituted with 1-5 R^{70} , each independently selected from the group consisting of halogen, OH, C_{1-4} alkyl, and C_{1-4} alkoxy, wherein the C_{1-4} alkyl and C_{1-4} alkoxy are optionally substituted with 1-3 halogens;

ring B is optionally substituted with 1 or 2 R^{71} , each independently selected from the group consisting of halogen and C_{1-4} alkyl, wherein the C_{1-4} alkyl is optionally substituted with 1-3 halogens;

R^{72} is a C_{1-4} alkyl, wherein the C_{1-4} alkyl is optionally substituted with 1-3 halogens;

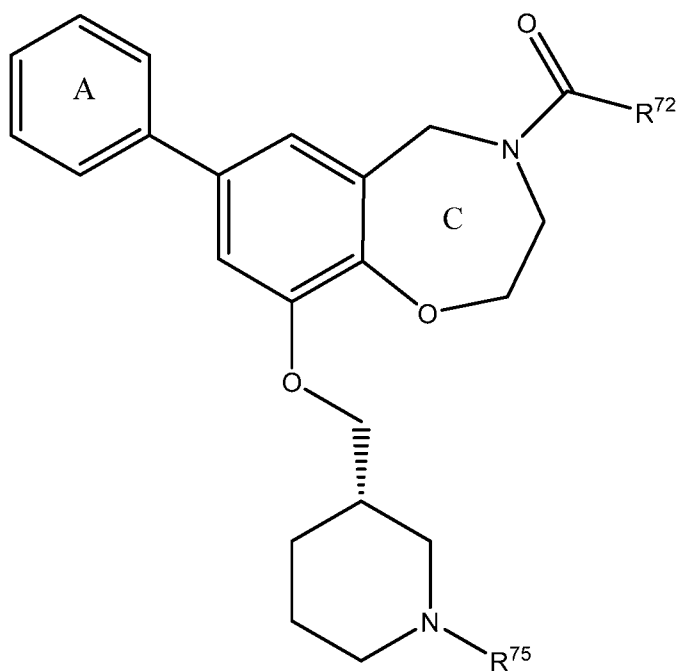
ring C is optionally substituted with 1-3 R^{73} , each independently selected from the group consisting of halogen and C_{1-4} alkyl, wherein the C_{1-4} alkyl is optionally substituted with 1-3 halogens;

ring D is optionally substituted with 1-3 R^{74} , each independently selected from the group consisting of halogen and C_{1-4} alkyl, wherein the C_{1-4} alkyl is optionally substituted with 1-3 halogens; and

R^{75} is H or a C_{1-4} alkyl, wherein the C_{1-4} alkyl is optionally substituted with 1-3 halogens,

or a tautomer, a stereoisomeric mixture, or a salt thereof.

34. The method of claim 33, wherein the cell culture medium comprises a bromodomain inhibitor of Formula VIIa:



(Formula VIIa),

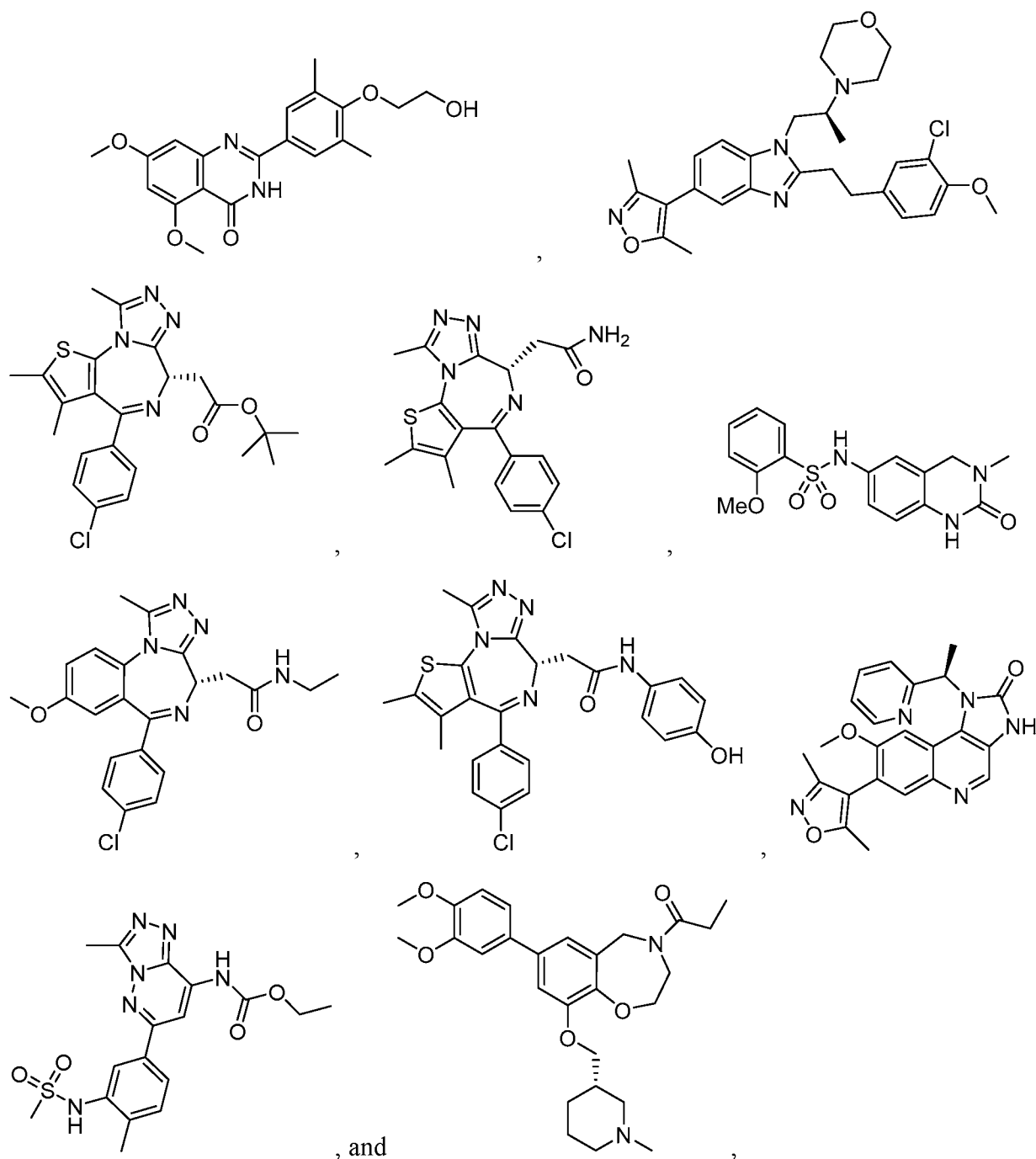
wherein ring A is optionally substituted with 1 or 2 R^{70} , each independently selected from the group consisting of halogen, C_{1-4} alkyl, and C_{1-4} alkoxy;

ring C is optionally substituted with 1 or 2 R^{73} , each independently a C_{1-4} alkyl; and

R^{72} is a C_{1-4} alkyl; and R^{75} is H or a C_{1-4} alkyl,

or a tautomer, a stereoisomeric mixture, or a salt thereof.

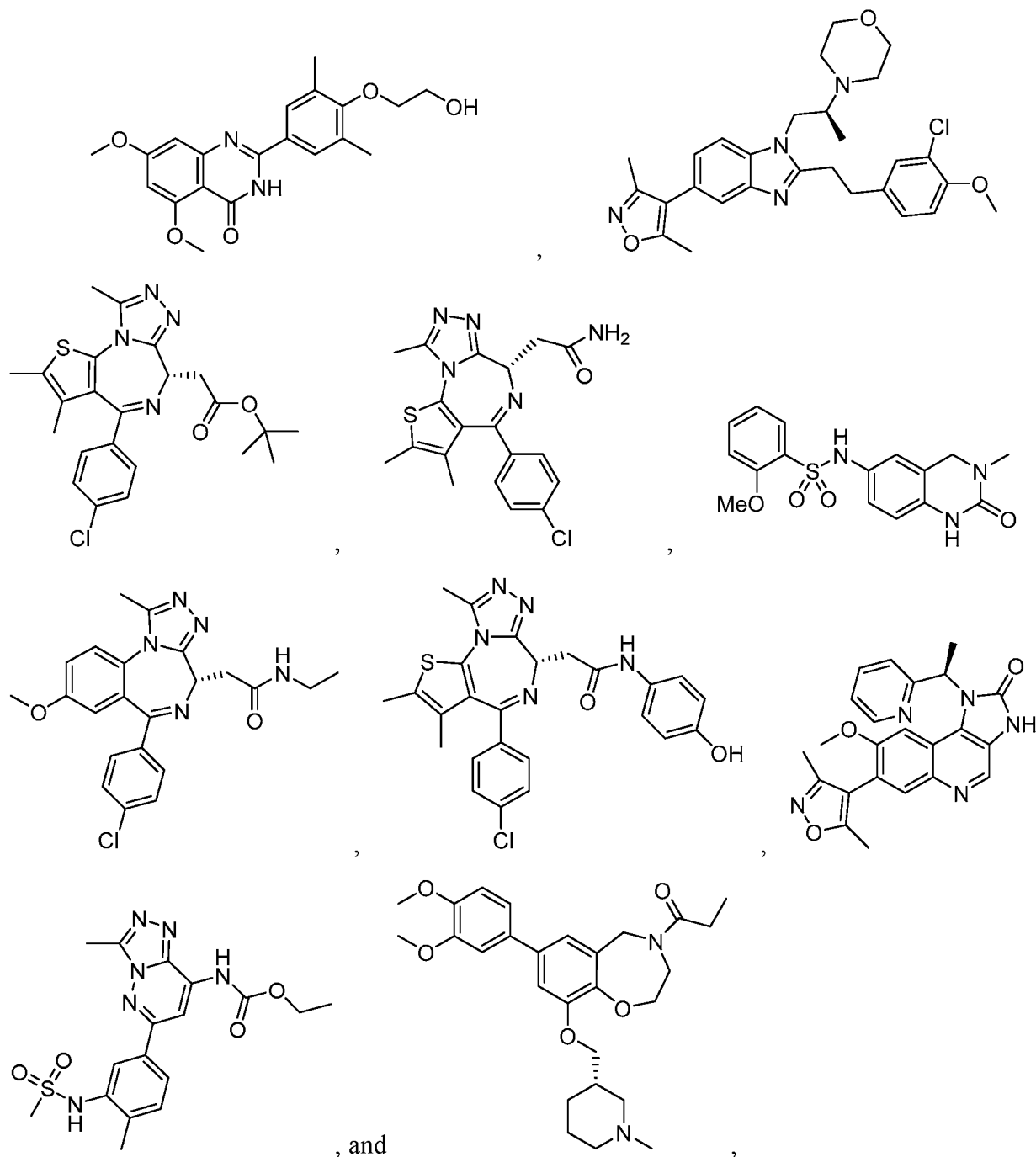
35. The method of any one of claims 1-12, wherein the cell culture medium comprises a bromodomain inhibitor selected from the group consisting of:



or a tautomer, a stereoisomeric mixture, or a salt thereof.

36. A cell culture comprising i) mammalian cells comprising a polynucleotide encoding a protein or polypeptide of interest; and ii) a cell culture medium comprising a bromodomain inhibitor.
37. The cell culture of claim 36, wherein the cell culture is suitable for culturing the mammalian cells in a fed-batch process.

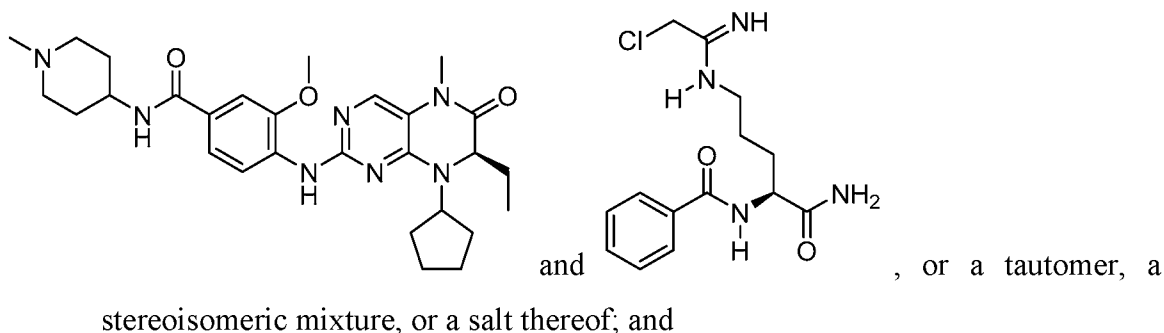
38. The cell culture of claim 36 or 37, comprising the bromodomain inhibitor at a concentration sufficient, when culturing the mammalian cells in a fed-batch mode for 14 days, to produce the protein or polypeptide of interest at a titer of about 15% to about 100% greater than the titer produced from a control cell culture that does not contain a bromodomain inhibitor.
39. The cell culture of any one of claims 36-38, comprising the bromodomain inhibitor at a concentration sufficient, when culturing the mammalian cells in a fed-batch mode for 14 days, to generate a viable cell density of about 20% to about 100% higher than that observed for a control cell culture that does not contain a bromodomain inhibitor.
40. The cell culture of any one of claims 36-39, comprising the bromodomain inhibitor at a concentration sufficient, when culturing the mammalian cells in a fed-batch mode for 14 days, to generate a cell specific productivity of about 10% to about 60% greater than that observed for a control cell culture that does not contain a bromodomain inhibitor.
41. The cell culture of claim 36 or 37, comprising the bromodomain inhibitor in a concentration ranging from about 1 μ M to about 100 μ M.
42. The cell culture of any one of claims 36-41, wherein the mammalian cells are CHO cells.
43. The cell culture of any one of claims 36-42, comprising a bromodomain inhibitor selected from the group consisting of:



or a tautomer, a stereoisomeric mixture, or a salt thereof.

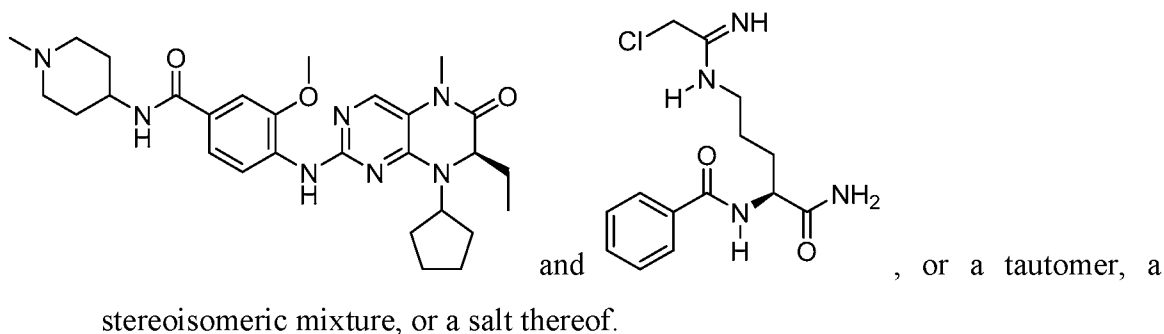
44. A method of producing a protein or polypeptide of interest comprising
- a) providing a cell culture comprising
 - i) mammalian cells comprising a polynucleotide encoding the protein or polypeptide of interest; and

ii) a cell culture medium comprising a compound selected from the group consisting of:



b) culturing the mammalian cells under conditions that allow expression of the protein or polypeptide of interest.

45. The method of claim 44, wherein the compound is present in the cell culture in a concentration ranging from about 1 μ M to about 100 μ M.
46. The method of claim 44 or 45, wherein the culturing step is performed in a fed-batch mode.
47. The method of any one of claims 44-46, wherein the mammalian cells are CHO cells.
48. A cell culture comprising
 - i) mammalian cells comprising a polynucleotide encoding the protein or polypeptide of interest; and
 - ii) a cell culture medium comprising a compound selected from the group consisting of:



49. The cell culture of claim 48, wherein the cell culture is for culturing the mammalian cells in a fed-batch process.
50. The cell culture of claim 48 or 49, wherein the mammalian cells are CHO cells.

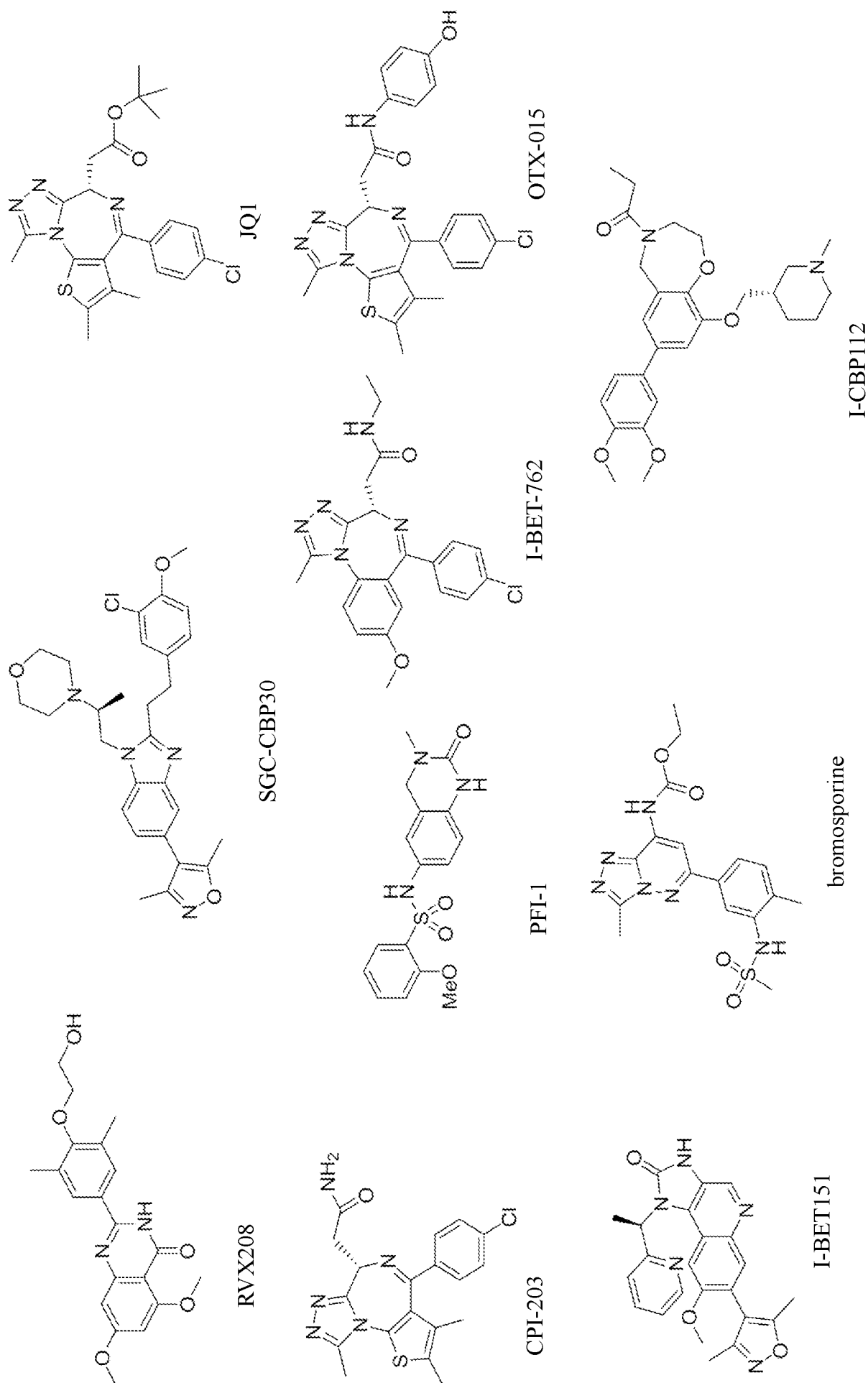
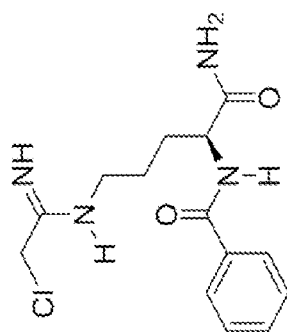
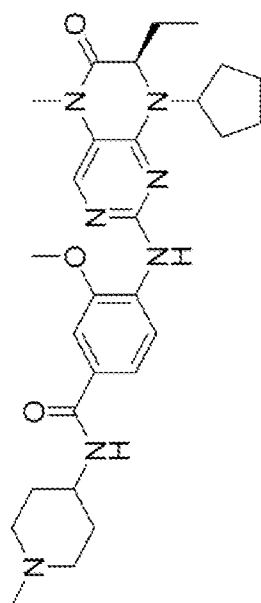


FIG. 1A

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Cl-amidine



BI2536

FIG. 1B

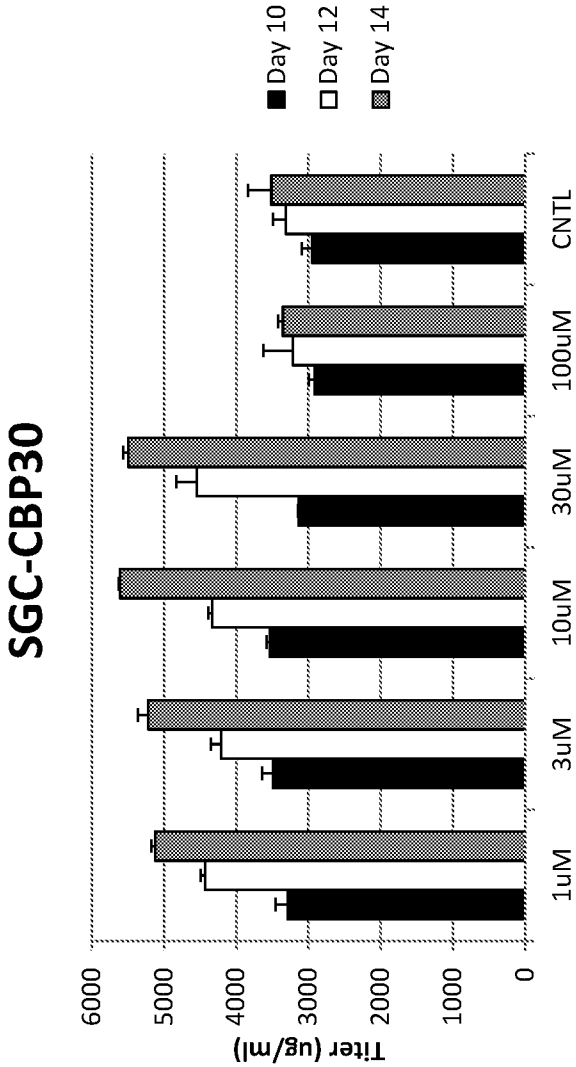


FIG. 2A

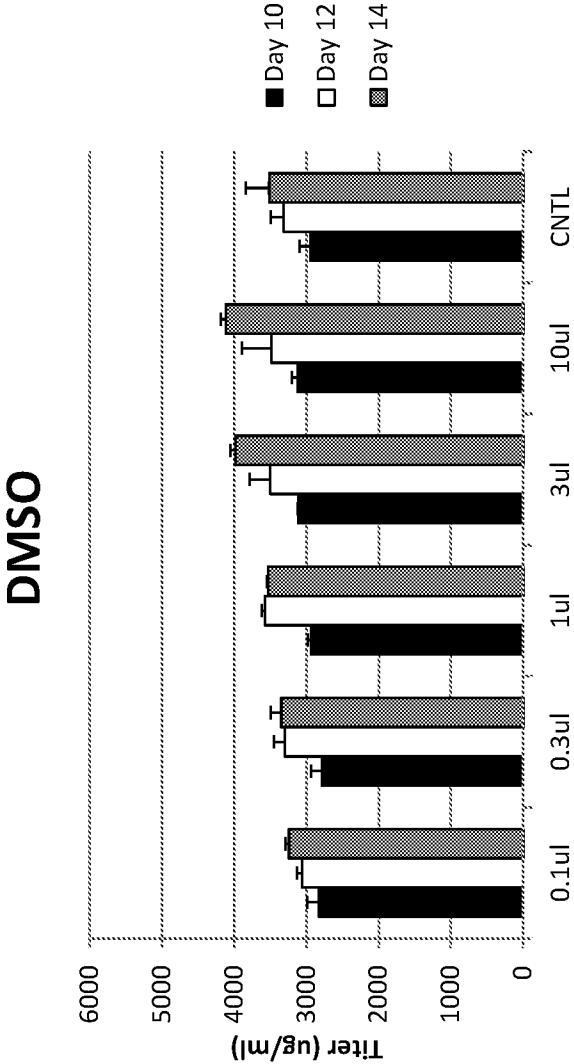


FIG. 2B

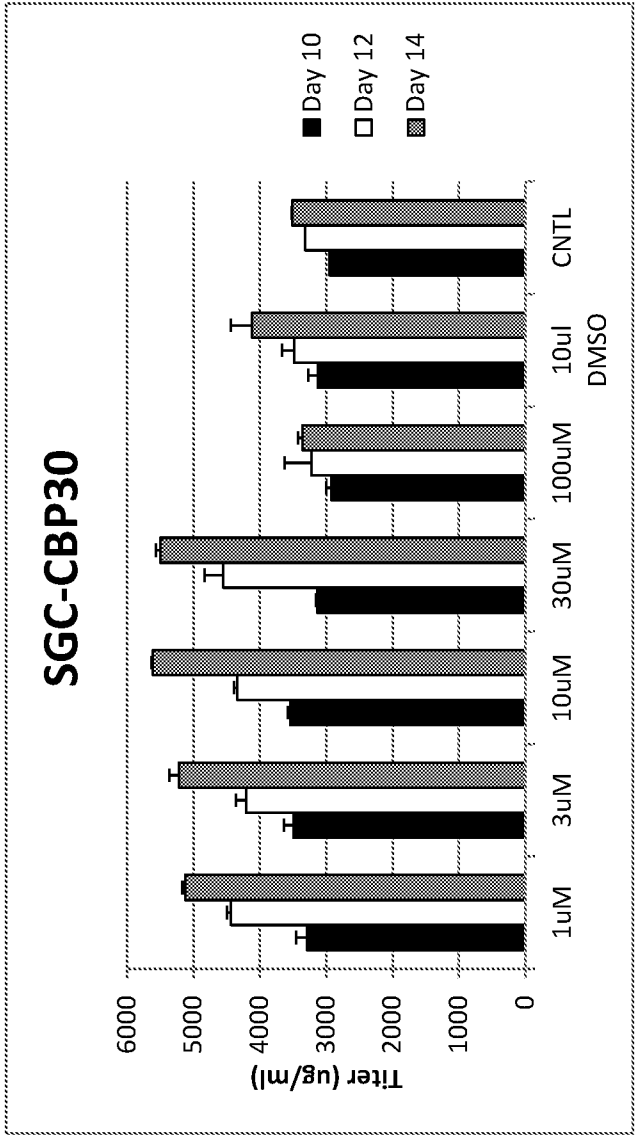


FIG. 2C

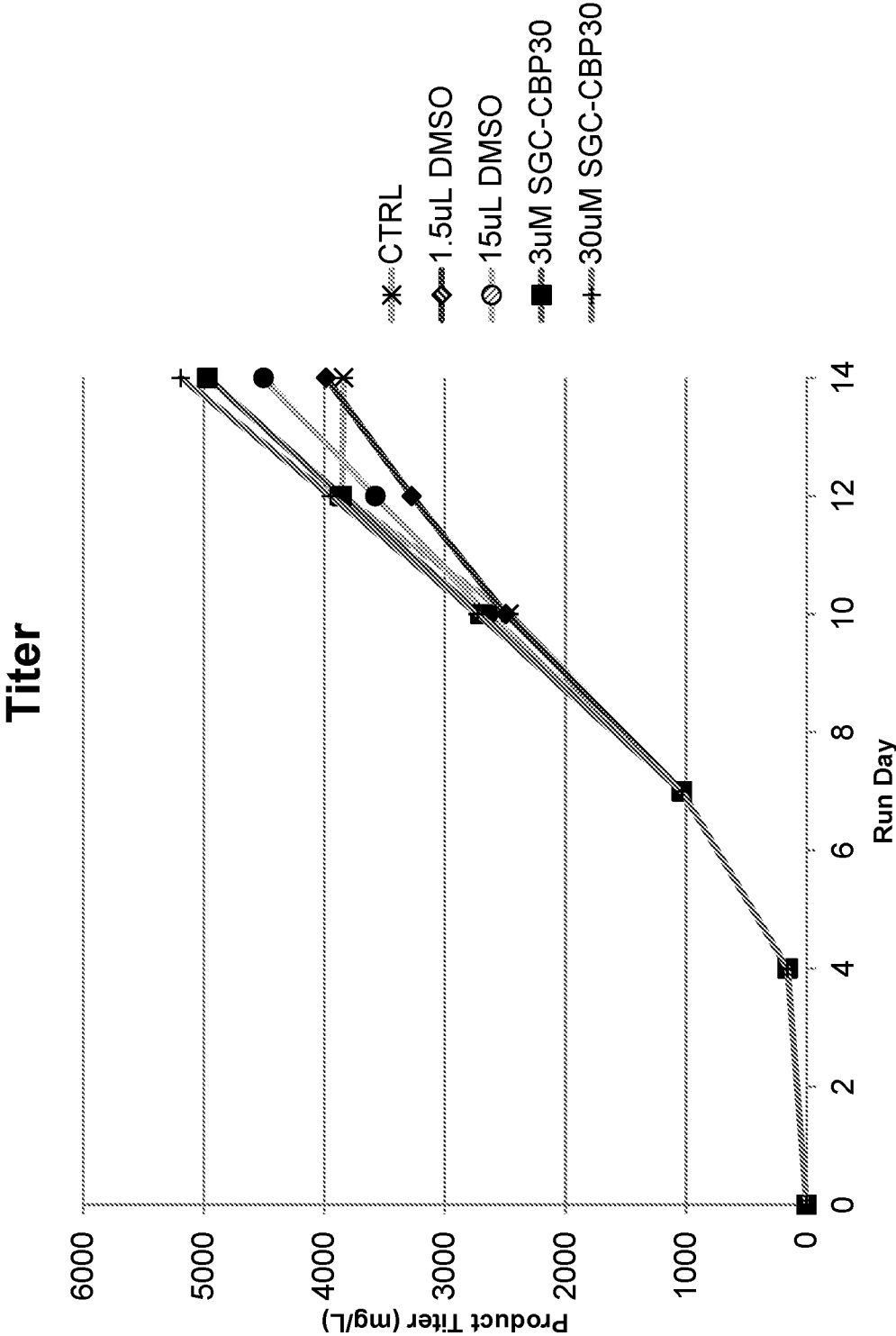


FIG. 3A

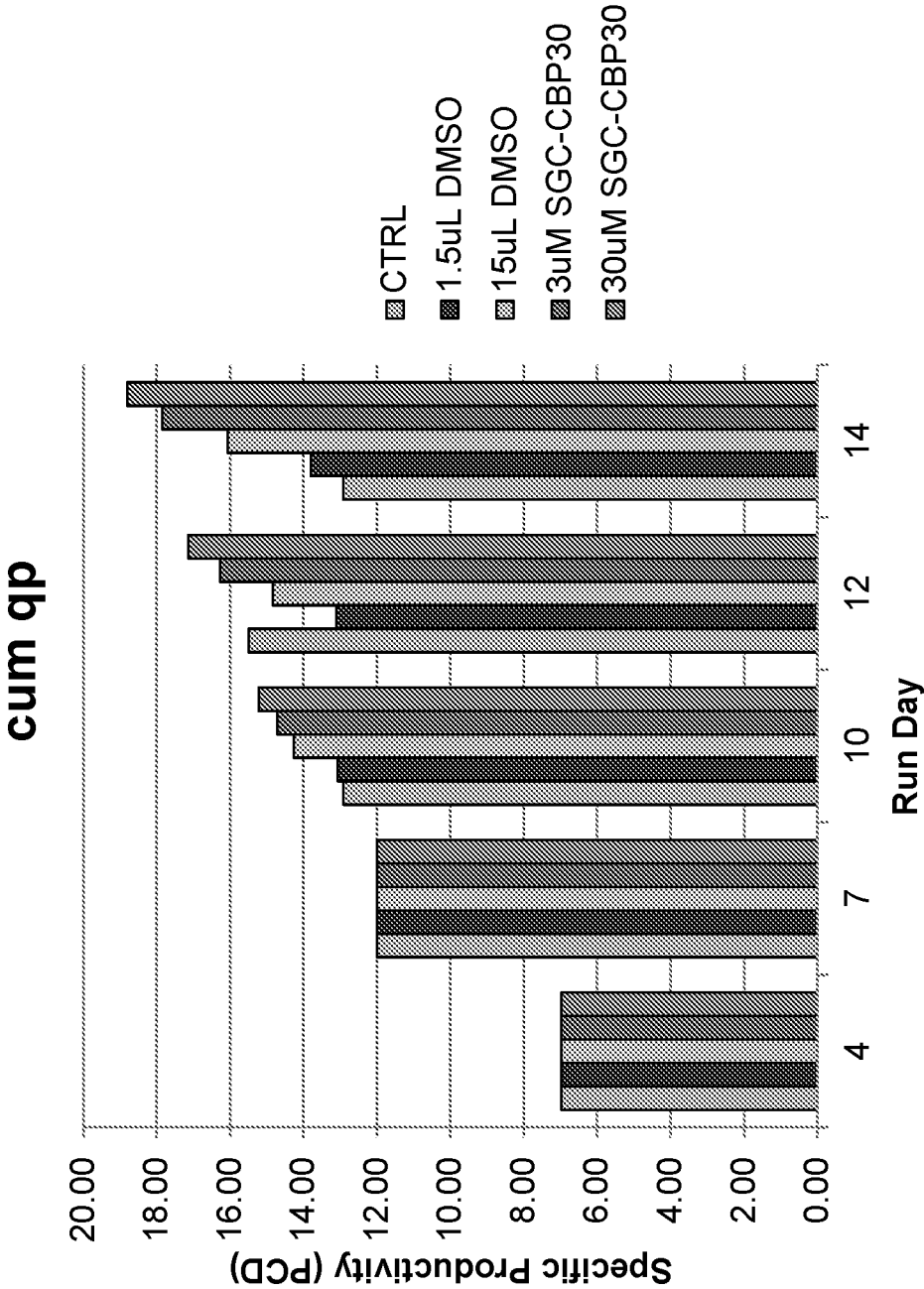


FIG. 3B

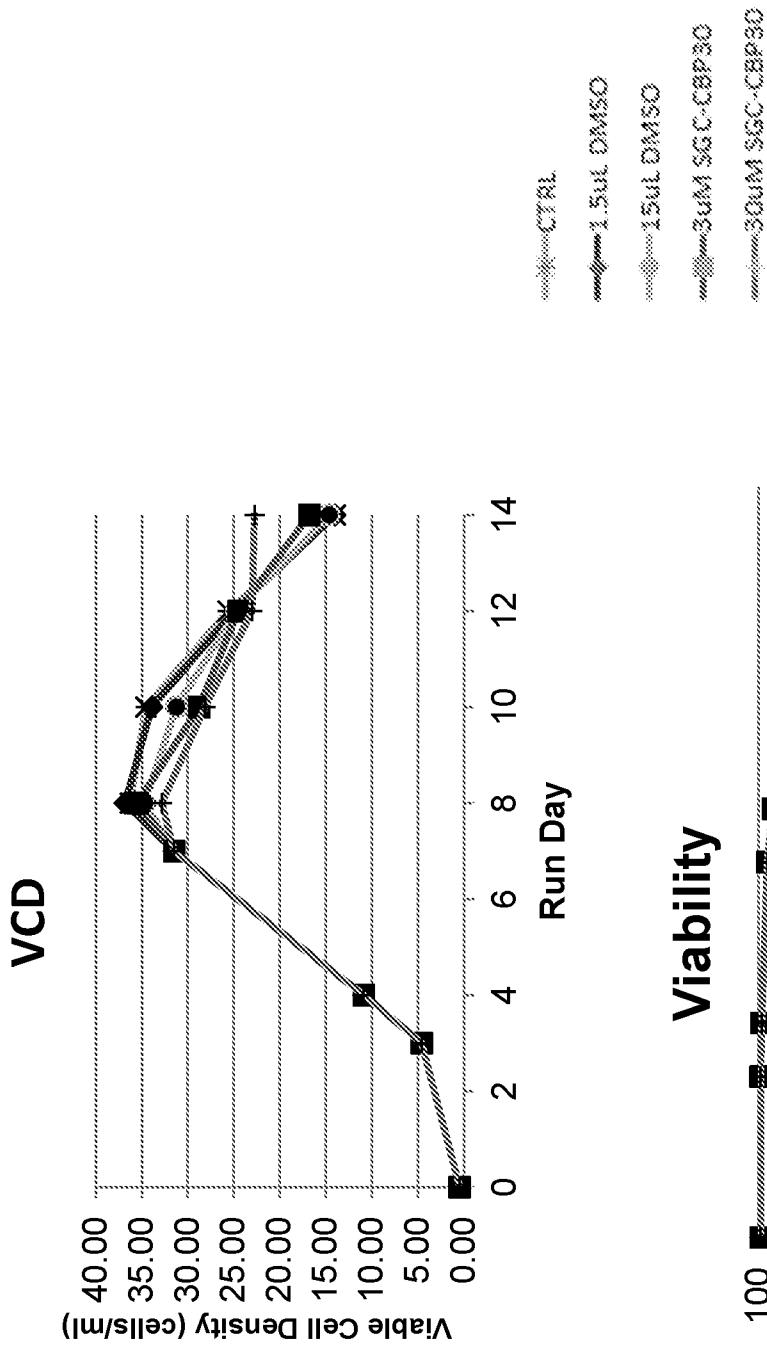


FIG. 3C

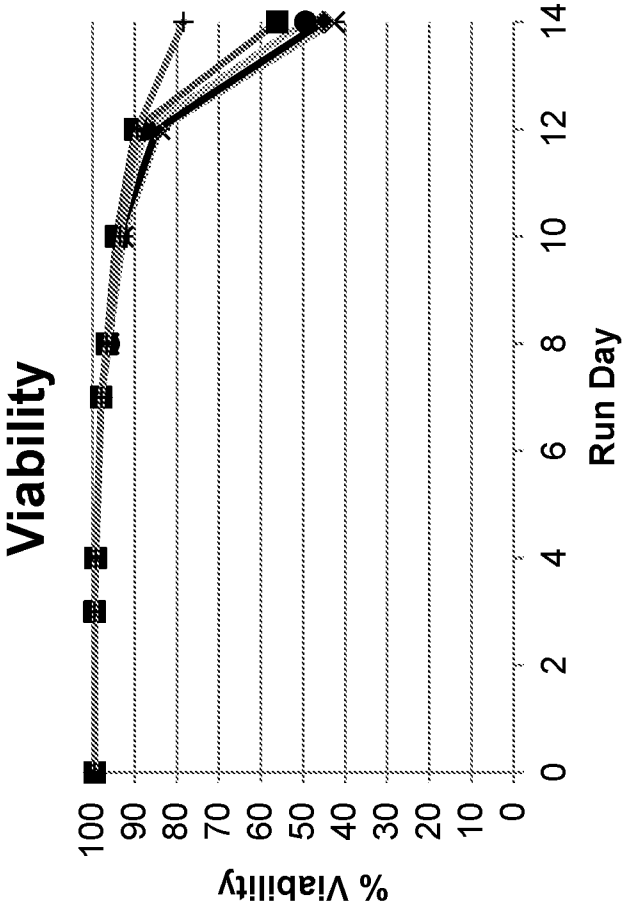


FIG. 3D

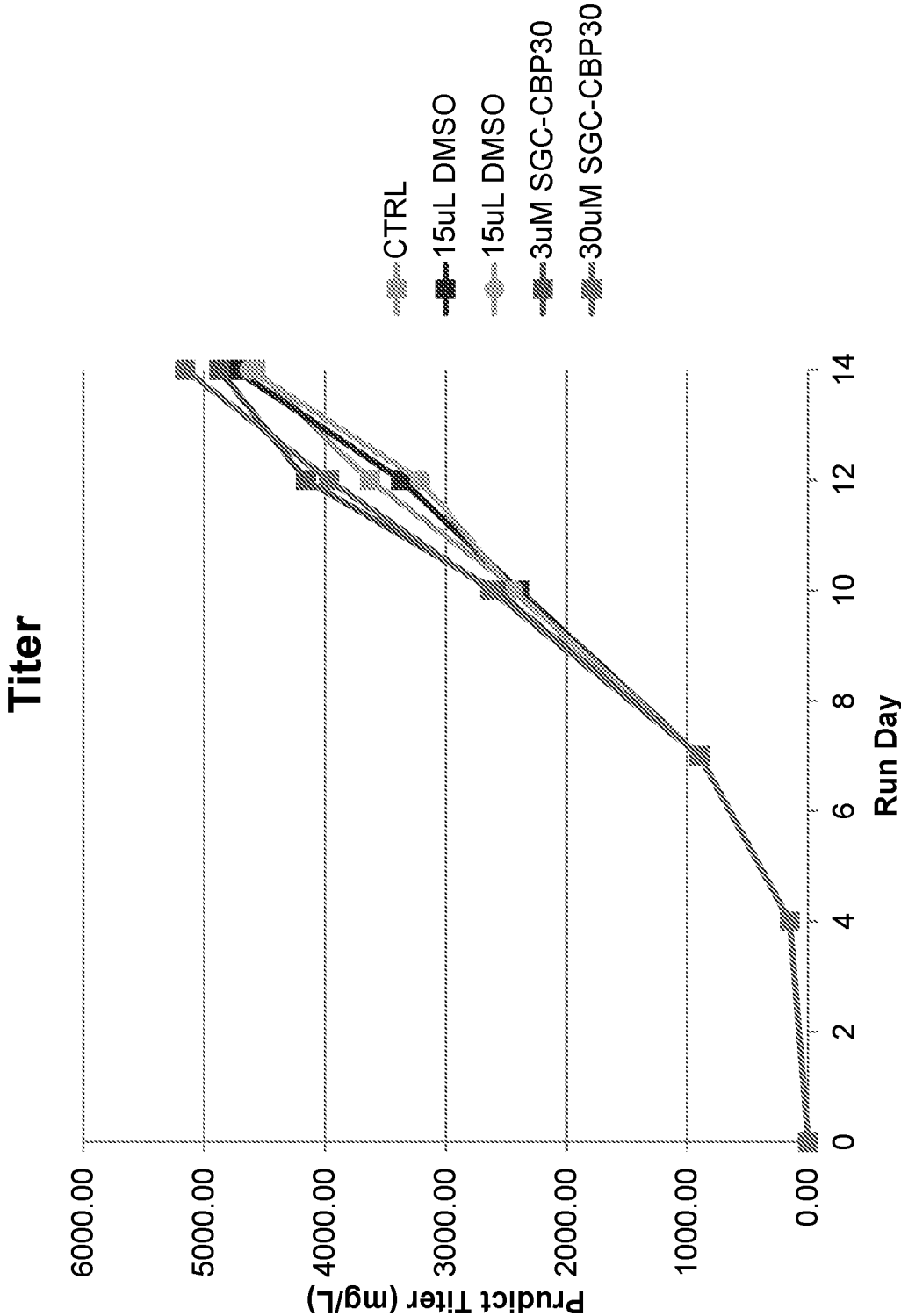


FIG. 4A

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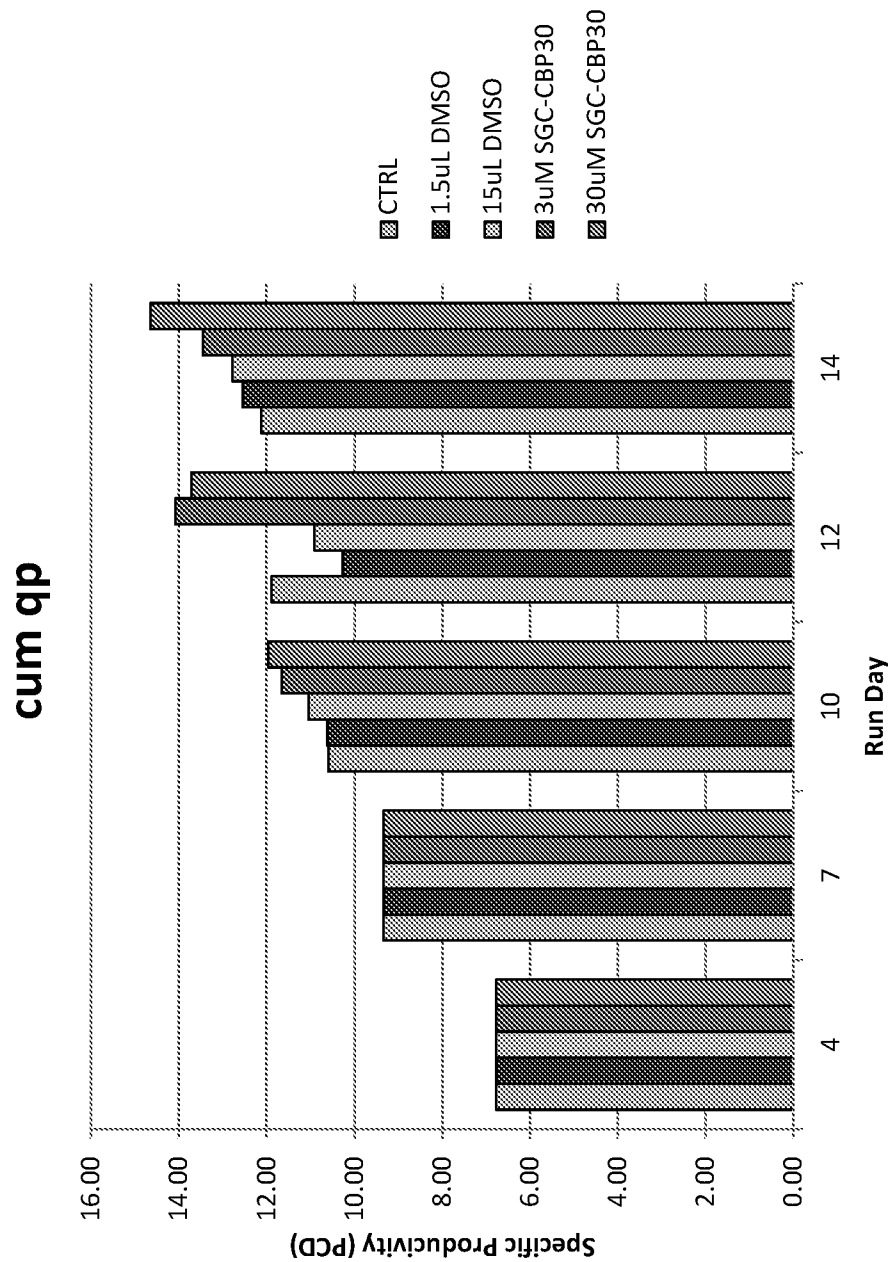


FIG. 4B

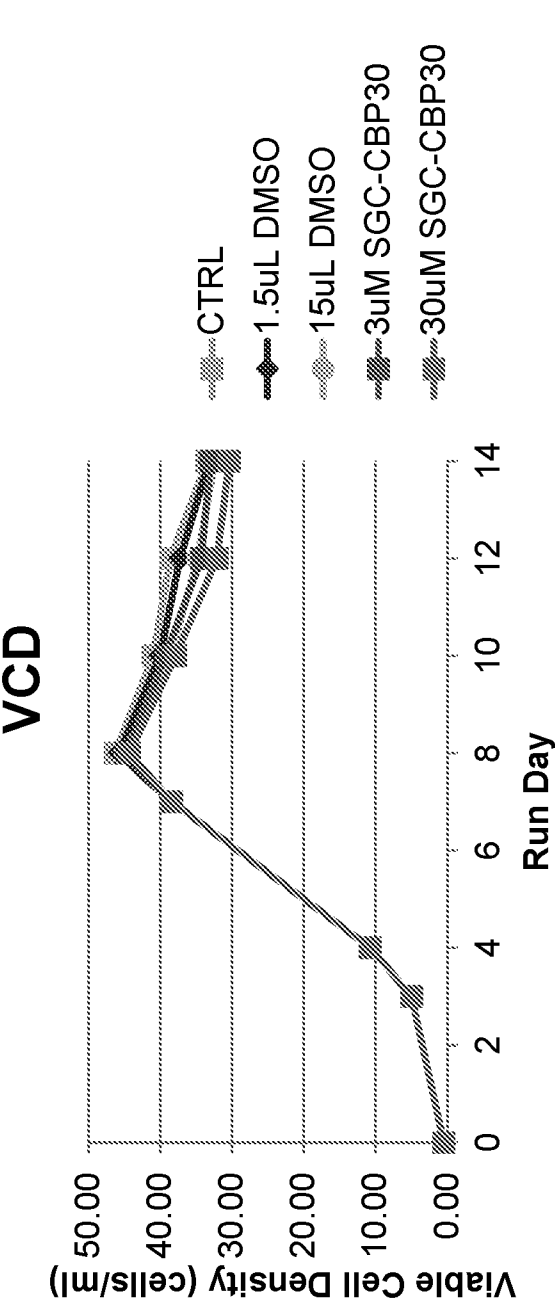


FIG. 4C

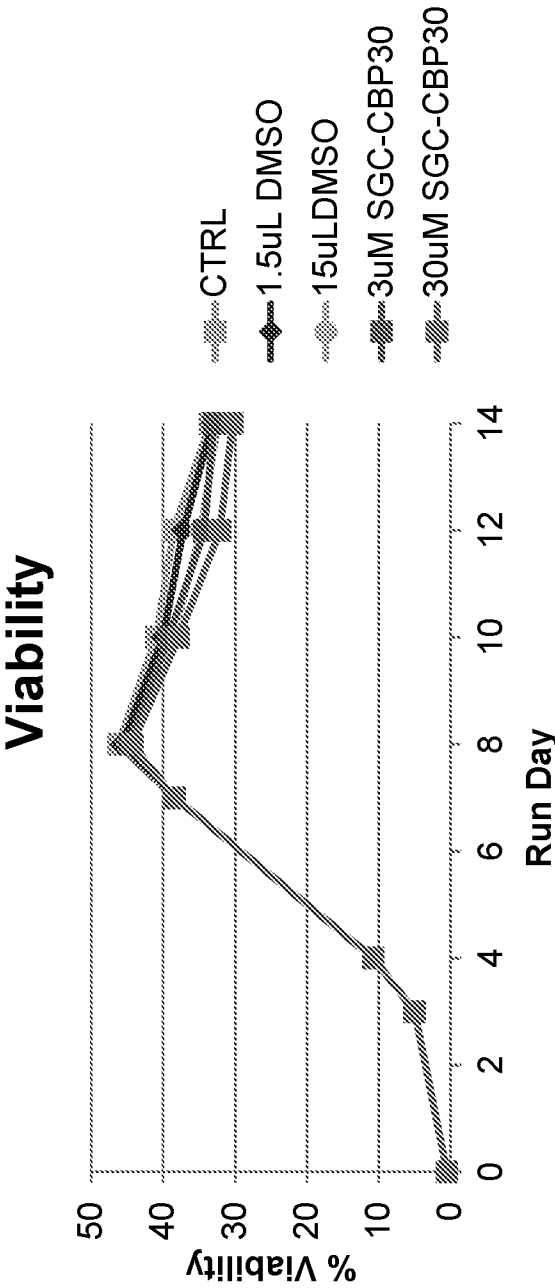


FIG. 4D

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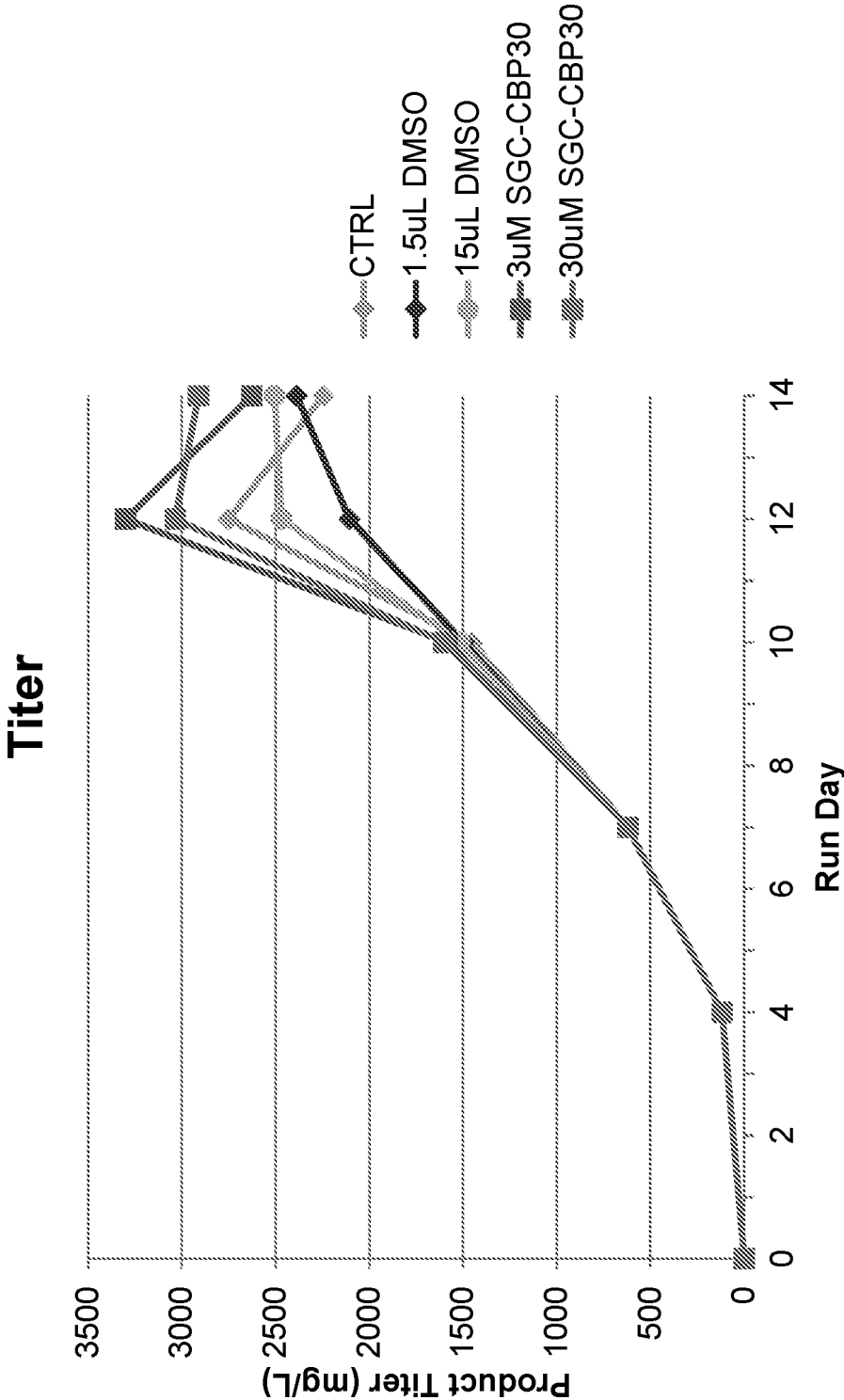


FIG. 5A

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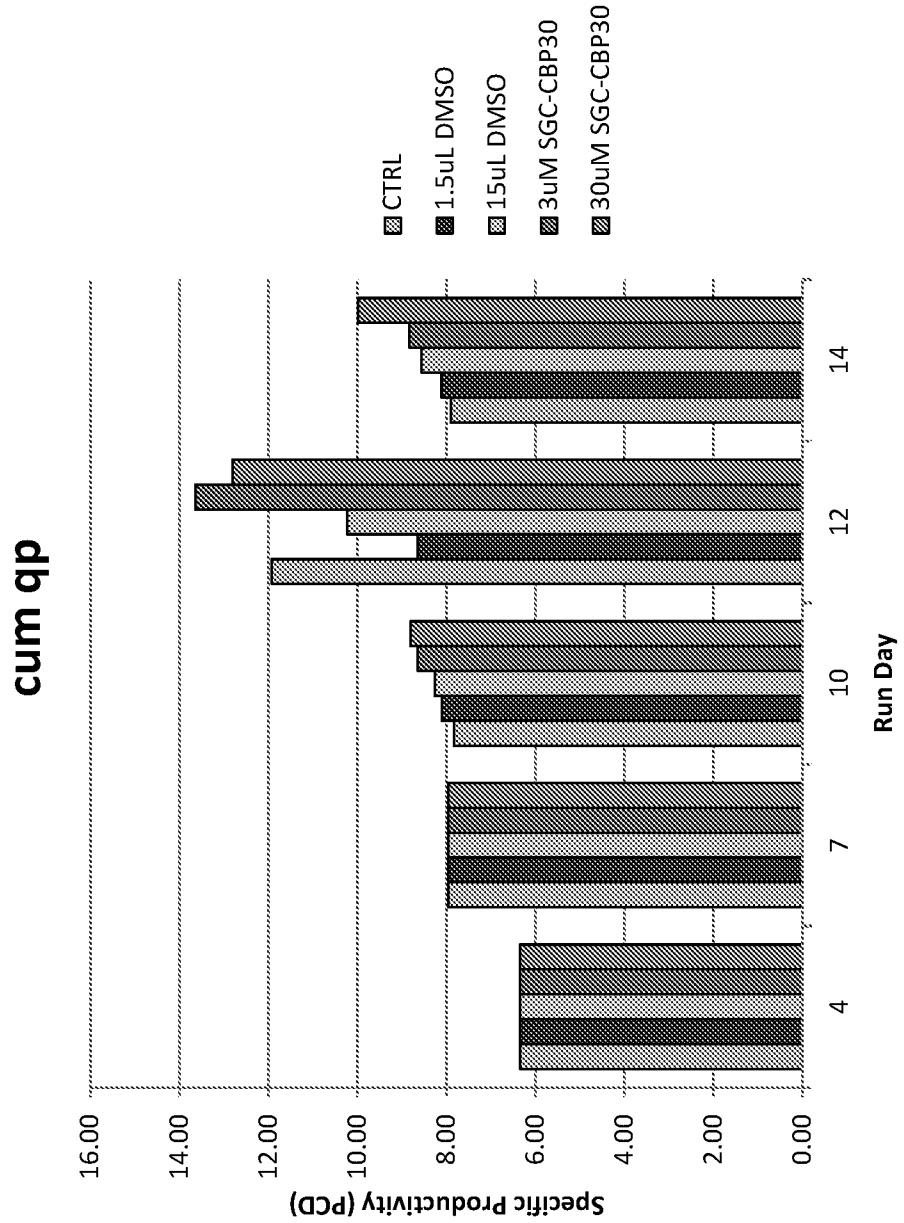


FIG. 5B

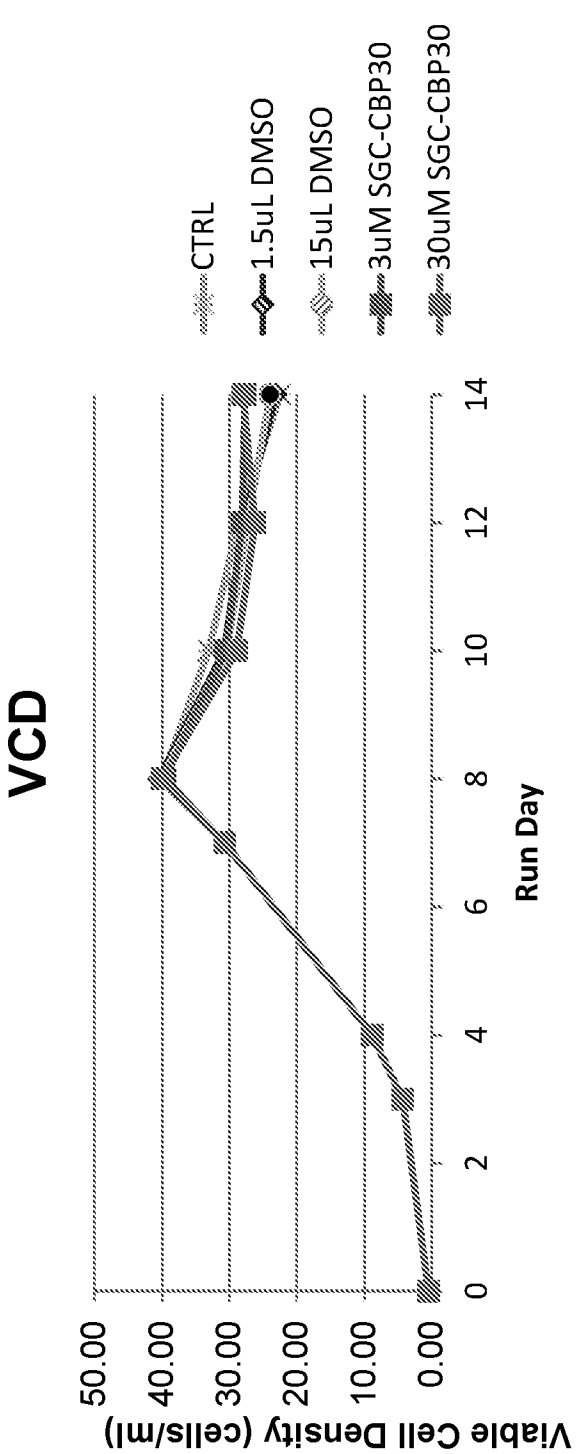


FIG. 5C

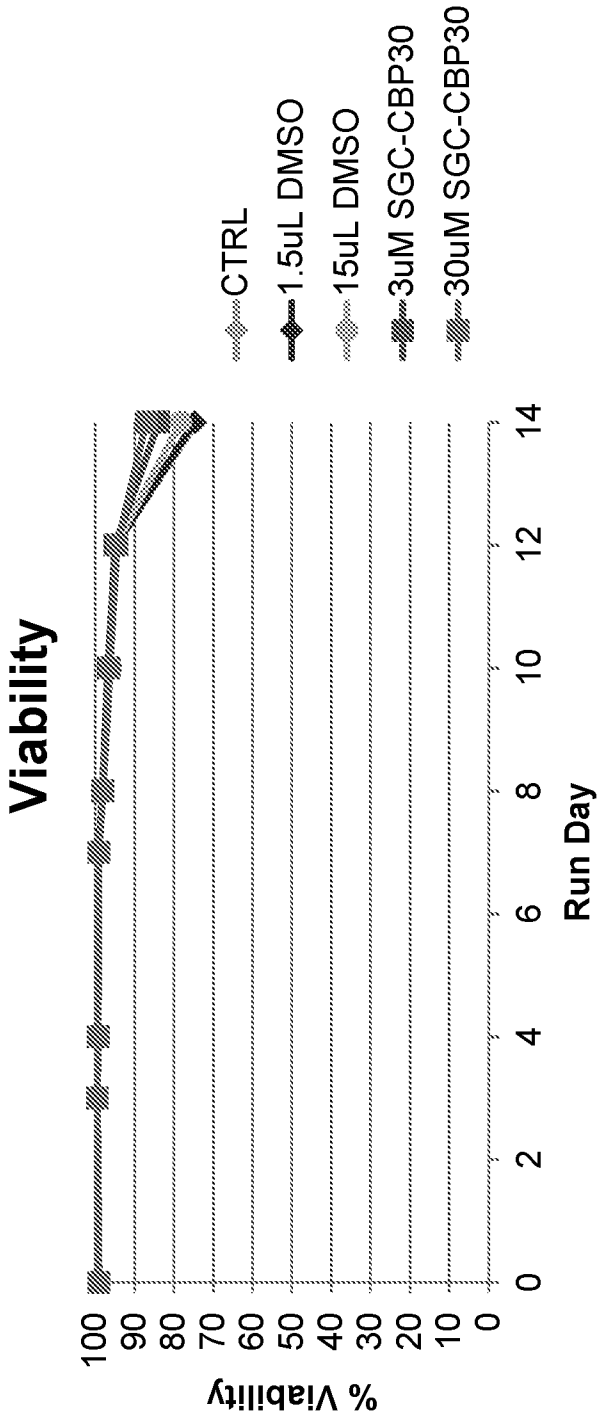


FIG. 5D

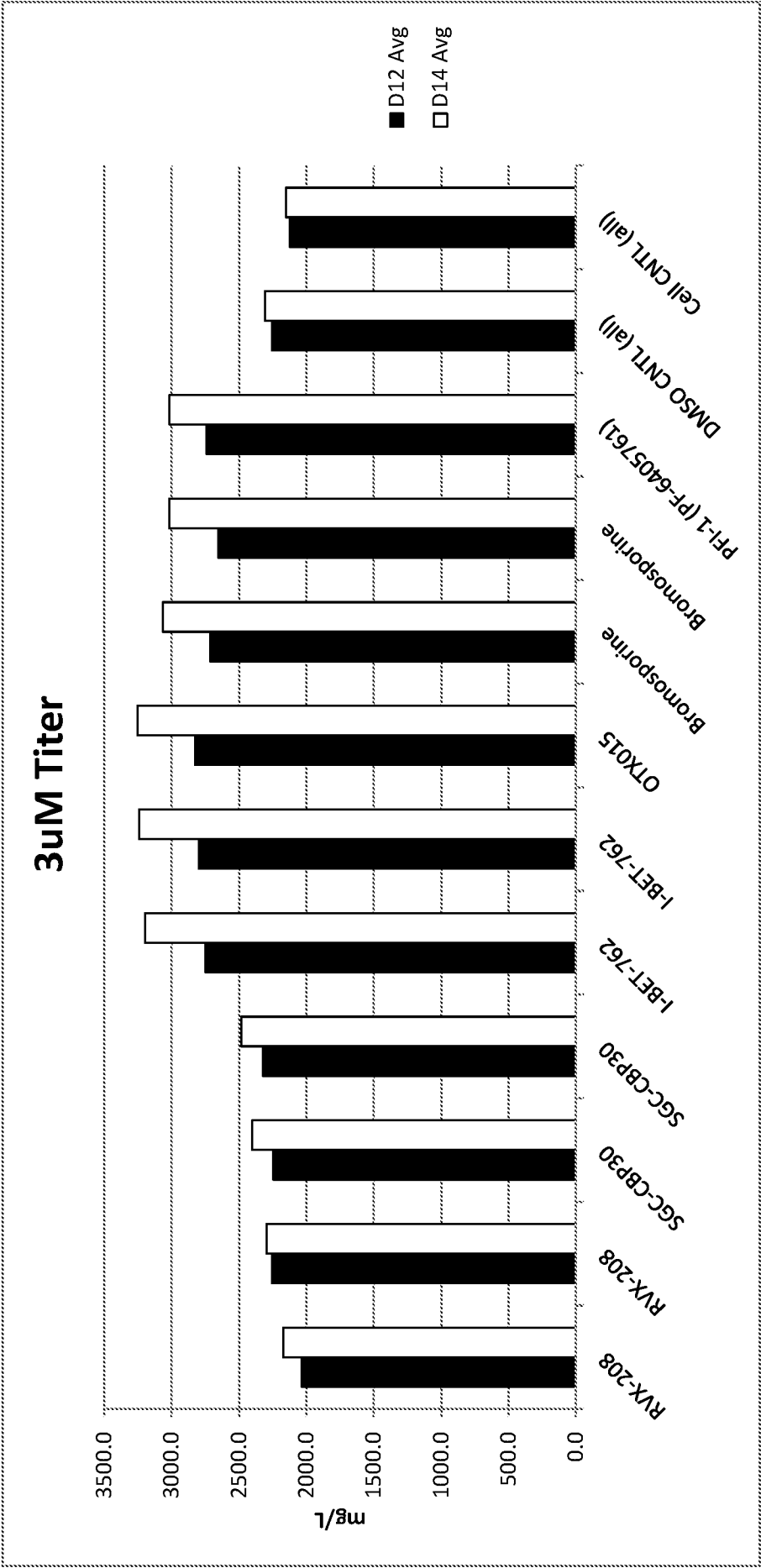


FIG. 6A

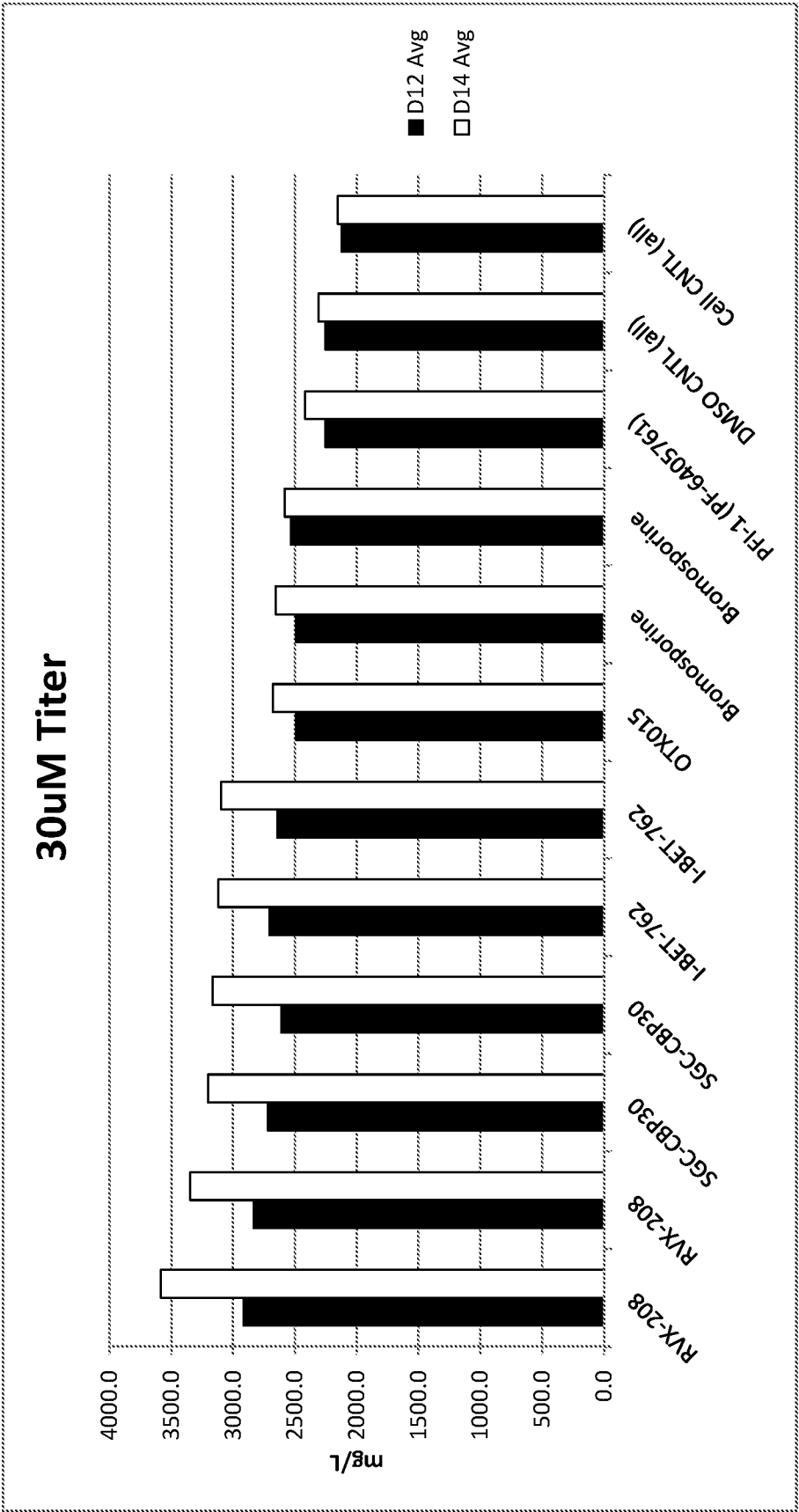


FIG. 6B

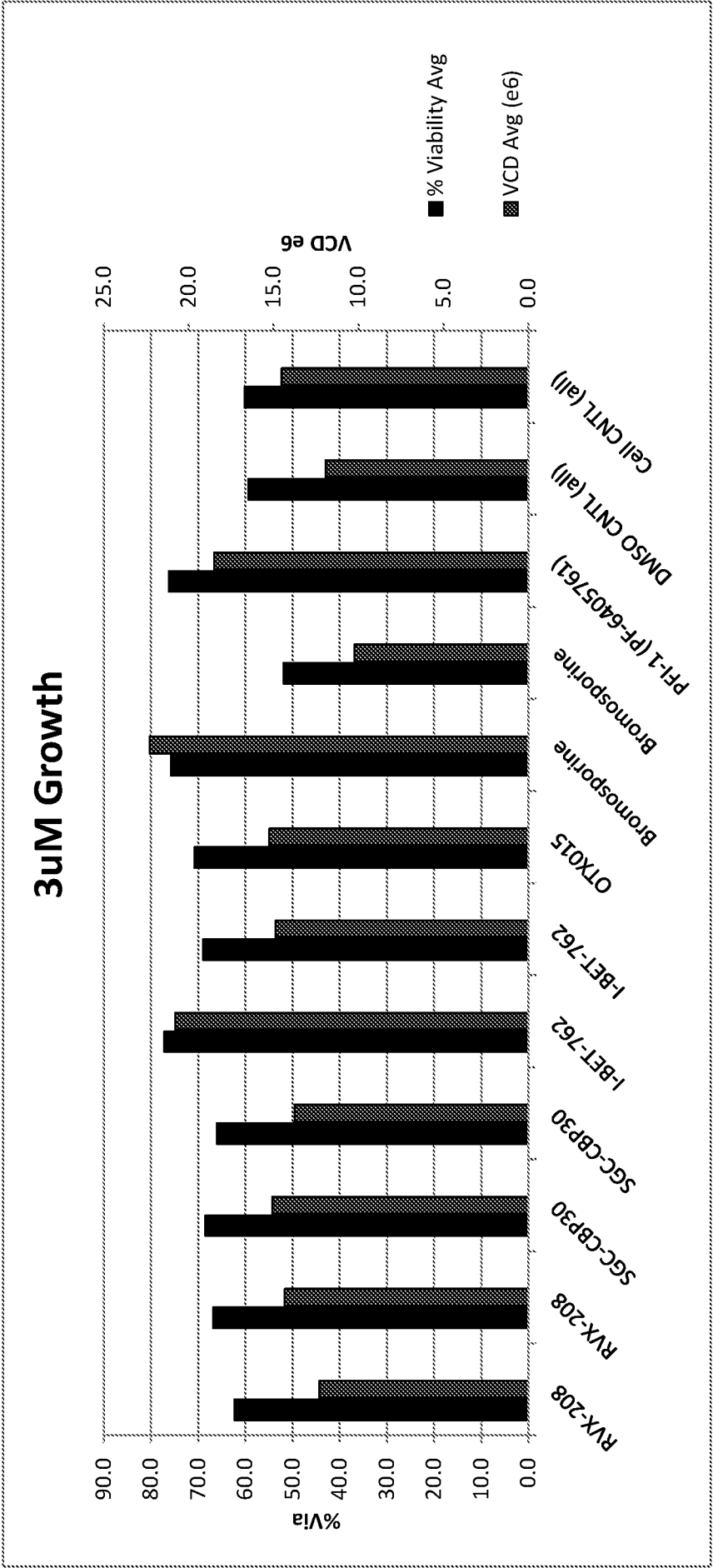


FIG. 6C

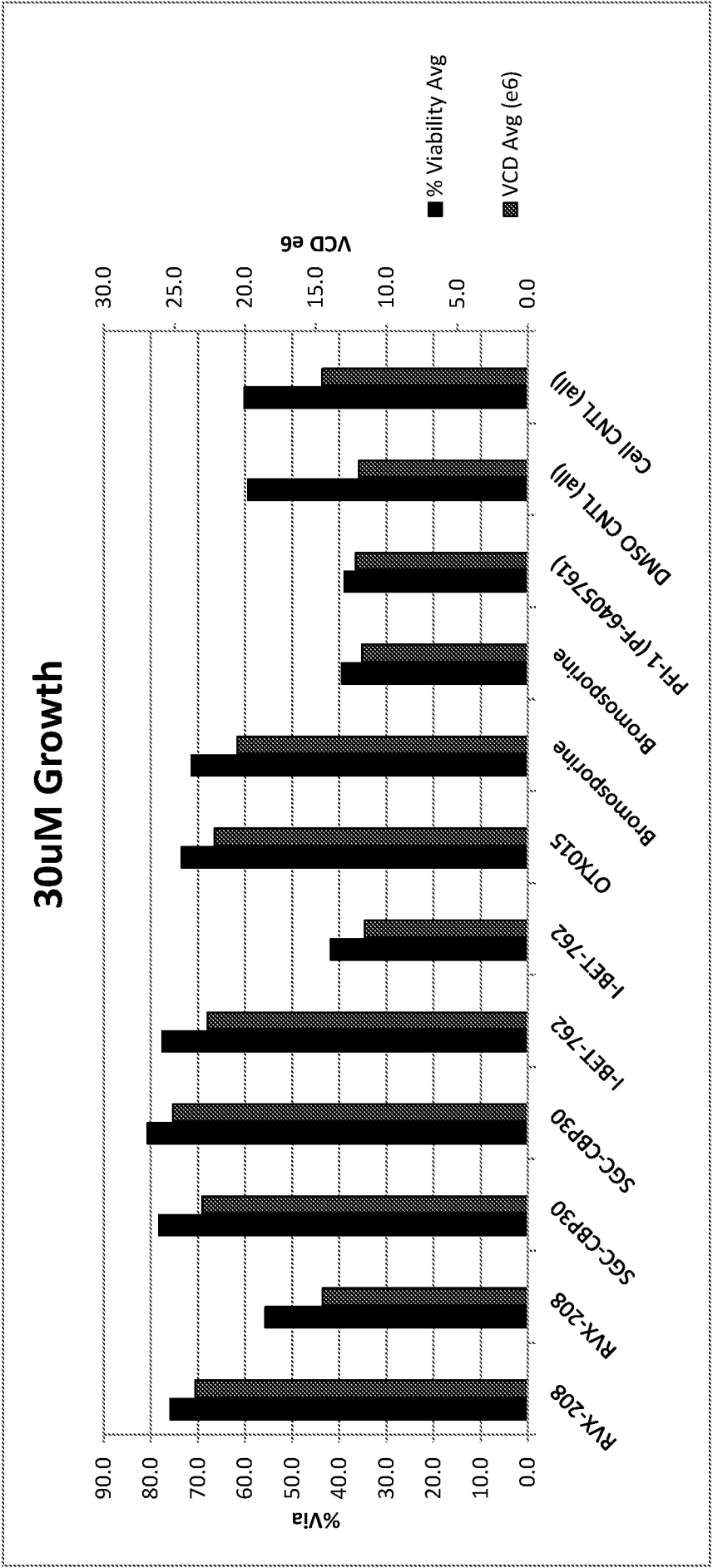


FIG. 6D

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US17/30799

A. CLASSIFICATION OF SUBJECT MATTER

IPC - A61K 31/166; C07C 237/28, 251/02 (2017.01)

CPC - A61K 31/166; C07C 237/28, 251/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2015/0342960 A1 (MEMORIAL SLOAN KETTERING CANCER CENTER) 3 December 2015; abstract; figure 6B; paragraphs [0050], [0052], [0083]-[0084], [0104], [0136]	1-2, 36, 41/36 --- 3/1-2, 37, 38/36-37, 41/37
X --- Y	US 2009/0306153 A1 (THOMPSON, PR et al) 10 December 2009; figure 12; paragraphs [0007]-[0008], [0054], [0074], [0078]	44-45, 48 --- 46/44-45, 49, 50/48-49
Y	WO 2008/130437 A2 (ARIZONA BOARD OF REGENTS FOR AND ON BEHALF OF ARIZONA STATE UNIVERSITY) 30 October 2008; paragraphs [00113], [00117], [00132]	3/1-2
Y	US 9,206,390 B2 (ABBVIE INC) 8 December 2015; figure 7; column 7, lines 1-5; column 10, lines 10-15; column 15, lines 40-45; column 22, lines 40-45; claim 31	37, 38/36-37, 41/37, 46/44-45
Y	SELVARASU, S et al. Combined In Silico Modeling and Metabolomics Analysis to Characterize Fed-Batch CHO Cell Culture. Biotechnology and Bioengineering, Vol. 109, No. 6, June 2012, pp. 1415-1429; abstract	49, 50/48-49

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

7 July 2017 (07.07.2017)

Date of mailing of the international search report

27 JUL 2017

Name and mailing address of the ISA/

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-8300

Authorized officer

Shane Thomas

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US17/30799

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 4-35, 39-40, 42-43, 47
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.