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DESCRIPTION

Description

BACKGROUND OF THE INVENTION

[0001] Human cells have a variety of receptors on their surfaces. G rotein-coupled receptors ("GPCR" or "GPCRs") form one of the largest protein families of transmembrane receptors. The human genome has approximately 30,000 genes, as many as 1,000 of which encode GPCRs. GPCRs have been grouped into five classes. The first class is the rhodopsin receptor family or "Class A GPCR" with 670 receptor proteins. The rhodopsin receptor family can react with various ligands including amines (alpha group), peptides (beta group), lipid-like substances (gamma group), nucleotides, and glycoproteins (delta group), and comprises a lot of drug target receptors. The second class is the secretin receptor family, and has binding domains for peptide hormones. Receptors in this family are associated with homeostasis and have been arising as important targets for drug development. The third class is the adhesion receptor family, characterized by a GPCR proteolytic site (GPS). Development of drugs targeting this family of GPCRs has not yet taken place because they exhibit various N-terminal moieties and little is known about their ligands. The fourth class is the glutamate receptor family with 22 GPCR members have so far been identified. Relatively little is known about the specificity of each protein. The last class is the Frizzled/Taste2 family that encompasses 10 Frizzled receptors for which Wnt glycoproteins serve as ligands, 5 SMO (smoothened) receptors which need no ligands, and 25 Taste2 receptors which are required for sensing various tastes. Receptors including GPCRs are also classified on the basis of the identification of endogenous ligands. Receptors bind with known endogenous compounds or are classified as orphan receptors whose endogenous ligands have not yet been identified.

[0002] GPCRs are found in a broad range of tissue and cell types and associated with many different physiological mechanisms. They are activated by a wide range of ligands, e.g., hormones such as thyroid-stimulating hormone (TSH), adrenocorticotropic hormone, glucagon and vasopressin, amines such as 5-HT, acetylcholine (muscarinic AchR), and histamines, lipids such as LPA and S1P, and signal transmitters such as amino acids, Ca^{2+}, nucleic acids, peptides and light. The wide distribution and diversity of roles that GPCRs play is evidence of their importance in various pathological diseases. Indeed, GPCRs are involved in various diseases including bronchoconstriction, hypertension, diabetes, inflammation, cell death, hormone disorders, cancer, neurotransmission and behavioral disorders. GPCRs are therefore an important area for the development of pharmaceutical products. Approximately 360 GPCRs are now considered available for drug development. Of these, 46 have already been used for drug development. There are approximately an estimated 150 Orphan GPCRs (oGPCRs). In the drug development field, cell membrane receptors act as selective sites for drug action and are responsible for 50% of all drug targets; GPCR activity modulating drugs account for 30% of the most frequently used top 100 drugs (40 billion dollars, 9% of the total drug market). Therefore, GPCRs are among the most significant targets for new drug development.

[0003] GPCRs have common structural features. They have seven hydrophobic membrane-spanning domains, each 20-30 amino acids long, which are connected by hydrophilic amino acid sequences of various lengths. The receptors have an extracellular N-terminus while the C-terminus is located in the cytoplasm. GTP-binding proteins (G proteins) act as mediators transmitting to intracellular effectors the signals that are generated by binding hormones or other chemical ligands that stimulate GPCR. After ligand binding, the GPCR intracellular domain undergoes a conformational change to allow the receptor to interact with a G protein, which in turn activates intracellular signal transmitters such as adenylate cyclase, phospholipase C or ion channels. This system generates a signaling cascade in which many secondary transmitters act in response to the binding of one ligand to GPCR. Cells use this mechanism to detect extracellular environmental changes and to properly react in response to the changes. On the whole, endogenous ligands activate receptors with the concomitant generation of

a conformational change, which allows association between the receptors and G proteins. Recent studies on the interaction between proteins have revealed that GPCRs associate with various proteins such as GRK or SH2 (Src Homology 2) domain-containing proteins, and adaptor Grb2 as well as G protein to participate in signaling transduction.

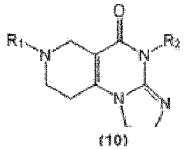
[0004] Under normal conditions, signaling transduction brings about the final result which is cell activation or suppression. In a physiological environment, GPCRs exist in equilibrium between their inactive and active states in the cell membrane. Inactive receptors cannot exert a biological response in conjunction with cellular signal transduction pathways. The receptors exhibit biological responses via a signal transduction pathway (through G proteins) only when they have structurally changed to their active form. The receptor may be stabilized into an active form by compounds such as endogenous ligands or drugs. Therefore, functional studies, such as cloning such gene families, and the identification of new ligands thereof, have the same meaning as the development of new drug candidates, that is, siRNA, antibodies, polypeptides, effectors, inhibitors, agonists, antagonists.

[0005] Development, differentiation, homeostasis, responses to stimuli, control of the cell cycle, as well as the aging and apoptosis of living organisms are mostly a result of selective expression of specific genes within cells. This is true for cellular mechanisms associated with diseases. Particularly, pathological phenomena, such as oncogenesis, are induced by gene mutations that in the end lead to changes in gene expression.

[0006] ONC201 (7-benzyl-4-(2-methylbenzyl)-1,2,6,7,8,9-hexahydroimidazo [1,2-a]pyrido [3,4-e]pyrimidin-5(1H)-one) is the founding member of a class of anti-cancer compounds called imipridones that is in Phase II clinical trials in multiple advanced cancers. Since the discovery of ONC201 as a p53-independent inducer of TRAIL gene transcription, preclinical studies have determined that ONC201 has anti-proliferative and pro-apoptotic effects against a broad range of tumor cells but not normal cells. The mechanism of action of ONC201 involves engagement of PERK-independent activation of the integrated stress response, leading to tumor upregulation of DR5 and dual Akt/ERK inactivation, and consequent Foxo3a activation leading to upregulation of the death ligand TRAIL. ONC201 is orally active with infrequent dosing in animal models, causes sustained pharmacodynamic effects, and is not genotoxic. The first-in-human ONC201 clinical trial in advanced aggressive refractory solid tumors confirmed that it is well-tolerated. In summary, the imipridone family that comprises ONC201 and its chemical analogs represent a new class of therapeutic agents. US 2014/335048 describes compound (1) and its use in the treatment of a cancer selected from colon cancer, breast cancer, glioblastoma multiforme, Mantle cell lymphoma and colorectal cancer.

BRIEF SUMMARY OF THE INVENTION

[0007] The invention is as described in the appended claims. Also provided herein are



(10)

compounds of formula **(10):** , wherein R_1 and R_2 are independently selected from H, alkyl, cycloalkyl, cycloalkylalkyl, heterocycloalkyl, heterocycloalkylalkyl, aryl, heteroaryl, arylalkyl, heteroarylalkyl, alkoxyalkyl, alkoxycarbonyl, aralkoxy, aralkylthio, and acyl radicals. Also described herein are compounds, where R_1 is CH_2Ph, and R_2 is not CH_2-(2-CH_3-Ph). When R_1 is CH_2Ph and R_2 is CH_2-(2-CH_3-Ph) (i.e., ONC201) the compound is as described in the appended claims. R_1 may be CH_2Ph and R_2 may be CH_2-(2,4-di F-Ph) (i.e., ONC206). R_1 may be CH_2Ph and R_2 may be CH_2-(4-CF_3-Ph) (i.e., ONC212). R_1 may be CH_2Ph and R_2 may be CH_2-(3,4-di F-Ph) (i.e., ONC213). R_1 may be CH_2(3,4-di-Cl-Ph and R_2 may be CH_2-(4-CF_3-Ph) (i.e., ONC234). R_1 may be CH_2-3-thienyl and R_2 may be CH_2-(4-CF_3-Ph) (i.e., ONC236).

[0008] As defined in the claims, provided herein is a compound of formula **(1)** or a pharmaceutically acceptable salt thereof, for use in treating a central nervous system cancer in a subject, wherein the central nervous system

cancer has a histone H3 mutation. The compound is ONC201. The subject has cancer. The cancer is a central nervous system tumor (e.g. a brain tumor)The cancer has a histone H3 mutation (*e.g.*, the mutation H3.3 K27M) and may also have an epigentically silenced unmethylated O(6)-methylguanine-DNA methyltransferase (MGMT) gene. The subject may have, or may be at risk of having, a psychiatric disorder, such as psychosis, schizophrenia, bipolar disorder, or major depressive disorder. The subject may have, or may be at risk of having, an infection, such as a bacterial infection,e.g. a gram-negative bacterial infectionor a gram-positive bacterial infection. The bacterial infection may be an infection of a bacteria selected from the group consisting of *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter* species, for example a *Staphylococcus* infection such as an *S. aureus* infection *(e.g.,* a methicillin-resistant *S. aureus* (MRSA) infection).

[0009] Also provided herein, but not part of the invention are methods of treating or preventing a disease, disorder, or condition in a subject in need of selective modulation of the activity of a G protein-coupled receptor (GPCR) or of a G protein-coupled receptor (GPCR) signaling pathway. Modulation includes, but is not lmited to, agonism, partial agonism, inverse agonism, partial antagonism, antagonism, bivalent modulation, or bitopic modulation. The methods may comprise administering to the subject in need of such treatment a pharmaceutical composition comprising a therapeutically effective amount a compound of formula **(10)** or an analog thereof, or a pharmaceutically acceptable salt thereof. The subject has, or is at risk of having, cancer. The subject may have, or may be at risk of having, a psychiatric disorder, such as psychosis, or schizophrenia. The subject may have, or may be at risk of having, an infection, such as a bacterial infection (for example, a gram-negative bacterial infectionor a gram-positive bacterial infection). The bacterial infection may be an infection of a bacteria selected from *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* or *Enterobacter* species. The bacterial infection may be a *Staphylococcus* infection, such as an *S. aureus* infection (*e.g.,* a methicillin-resistant *S. aureus* (MRSA) infection). The treatment regimen may comprise administering an effective amount of a therapeutic, such as compound of formula **(10),** a pharmaceutically acceptable salt thereof, or an analog thereof. The GPCR may be a Class A GPCR. The GPCR may be GPR132, GPR91, MTNR1A, GPR162, GPR137, BAI3, LGR4, PTGIR, CXCR7 or a combination thereof. The GPCR may be GPR132 (also called G2A). The GPCR may be GPR91. The GPCR may be MTNR1A. The GPCR may be CXCR7.

[0010] Also provided herein are methods of treating or preventing a disease, disorder, or condition in a subject in need of selective modulation of the activity of a dopamine receptor or of a member of a dopamine receptor signaling pathway. The methods may comprise administering to the subject in need of such treatment a pharmaceutical composition comprising a therapeutically effective amount a compound of formula **(10)** or an analog thereof, or a pharmaceutically acceptable salt thereof. The subject has, or is at risk of having, cancer. The subject may have, or may be at risk of having, a psychiatric disorder, such as psychosis or schizophrenia. The subject may have, or may be at risk of having, an infection, such as a bacterial infection, for example a gram-negative bacterial infection, or a gram-positive bacterial infection. The bacterial infection may be an infection of a bacteria selected from the group consisting of *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter* species. The bacterial infection may be a *Staphylococcus* infection, such as an *S. aureus* infection *(e.g.,* a methicillin-resistant *S. aureus* (MRSA) infection). The treatment regimen may comprise administering an effective amount of a therapeutic, such as compound of formula **(10),** a pharmaceutically acceptable salt thereof, or an analog thereof. The dopamine receptor may be from the D2-like family of dopamine receptors.

[0011] Also provided herein are methods of treating or preventing liver fibrosis or of regenerating liver tissue, comprising: administering to the subject in need of such treatment a pharmaceutical composition comprising a therapeutically effective amount a compound of formula **(10)** or a compound of formula **(100)** *(e.g.,* TIC-10), or an analog thereof, or a pharmaceutically acceptable salt thereof. The compound may be a CXCR7 agonist.

[0012] Also provided herein are methods of stimulating the immune system (*e.g.*, activating NK cells) in a subject in need thereof, comprising: administering to the subject a pharmaceutical composition comprising a therapeutically effective amount a compound of formula **(10)** or an analog thereof, or a pharmaceutically acceptable salt thereof.

The compound may be a GPR91 agonist. The compound may be ONC213. The subject has cancer and the method may be a method of cancer immunotherapy. The subject may have a viral infection (*e.g.*, HIV). The subject may have systemic lupus erythematosus. The method may further comprise administering a vaccine (*e.g.,* a cancer vaccine) to the subject, and the compound may be administered as an adjuvant.

[0013] Also provided herein are methods of identifying whether a subject having a condition is likely to be responsive to a treatment regimen described herein. The methods may comprise (i) obtaining a biological sample from the subject; (ii) measuring expression levels of at least one dopamine receptor or G protein-coupled receptor (GPCR) in the sample; (iii) comparing the levels measured in the sample to those for a pre-determined standard; and (iv) determining whether the subject is likely to be responsive to the treatment regimen, based on the levels measured in the sample to those for the pre-determined standard. The subject has, or is at risk of having, cancer. The subject may have, or may be at risk of having, a psychiatric disorderand/or an infection. The treatment regimen may further comprise administering an effective amount of a therapeutic, such as compound of formula **(10),** a pharmaceutically acceptable salt thereof, or an analog thereof. The dopamine receptor may be from the D2-like family of dopamine receptors. The GPCR may be a Class A GPCR. The GPCR may be GPR132, GPR91, MTNR1A, GPR162, GPR137, BAI3, LGR4, PTGIR, CXCR7 or a combination thereof. The GPCR may be GPR132, GPR91, MTNR1A, CXCR7 or a combination thereof. The GPCR may be GPR132.

[0014] Also provided herein are methods of assessing the effectiveness of a treatment regimen described herein, monitoring, or providing a prognosis for a subject with a condition. The methods may comprise (i) obtaining a biological sample from the subject; (ii) measuring expression levels of at least one dopamine receptor or G protein-coupled receptor (GPCR) in the sample; (iii) comparing the levels measured in the sample to those for a pre-determined standard; and (iv) determining a prognosis or determining whether the subject is responsive to the treatment regimen, based on the levels measured in the sample to those for the pre-determined standard. The methods may comprise (i) obtaining a biological sample from the subject; (ii) measuring gene copy number or mutations in at least one dopamine receptor in the sample; (iii) comparing the copy number measured or mutations found in the sample to those for a pre-determined standard; and (iv) determining whether the subject is responsive to the treatment regimen, based on the copy number measured or mutations found in the sample to those for the pre-determined standard. The subject has, or is at risk of having, cancer. The subject may have, or may be at risk of having, a psychiatric disorder, and/or an infection. The treatment regimen may comprise administering an effective amount of a therapeutic, such as compound of formula **(10),** a pharmaceutically acceptable salt thereof, or an analog thereof. The dopamine receptor may be selected from DRD2, DRD2S, DRD2L, and DRD3. The dopamine receptor may be from the D2-like family of dopamine receptors. The GPCR may bea Class A GPCR. The GPCR may be GPR132, GPR91, MTNR1A, GPR162, GPR137, BAI3, LGR4, PTGIR, CXCR7 or a combination thereof.

[0015] Also provided herein are methods for screening a potential therapeutic for a condition. The method may comprise (i) contacting at least one G protein-coupled receptor (GPCR) with a test molecule suspected of being a therapeutic for a condition; (ii) measuring the binding affinity, interaction or GPCR signalling of the test compound to the GPCR; and (iii) comparing the binding affinity, interaction or signalling of the test molecule to a pre-determined threshold. GPCR modulation or GPCR signaling modulation by the test molecule comparable to or greater than the threshold may be indicative of a therapeutic for the condition, such as cancer. The pre-determined threshold may be the GPCR modulation or GPCR signaling modulation of a therapeutic, such as a compound of formula **(10)** or a pharmaceutically acceptable salt thereof, or an analog thereof. The GPCR may be a Class A GPCR. The GPCR may be GPR132. The GPCR may be GPR132, GPR91, MTNR1A, GPR162, GPR137, BAI3, LGR4, PTGIR, CXCR7 or a combination thereof. The GPCR may be GPR132. The GPCR may be GPR91. The GPCR may be MTNR1A. The GPCR may be CXCR7.

[0016] Also provided herein are methods for screening a potential therapeutic for a condition. The method may comprise (i) contacting at least one dopamine receptor with a test molecule suspected of being a therapeutic for a condition; (ii) measuring the binding affinity, interaction or signalling of the test molecule to the at least one dopamine receptor; and (iii) comparing the binding affinity or interaction of the test molecule to a pre-determined threshold. Modulation of the dopamine receptor by the test molecule comparable to or greater than the threshold

may be indicative of a therapeutic for the condition, such as cancer. The dopamine receptor may be a member of the D2-like family of dopamine receptors. The pre-determined threshold may be the modulation of the dopamine receptor or dopamine receptor signalling by a therapeutic, such as a compound of formula **(10)** or a pharmaceutically acceptable salt thereof, or an analog thereof.

[0017] Provided herein are methods for screening a potential therapeutic for a condition. Using a processor, the method may comprise (i) using a computational docking method to model binding or interaction, if any, of one or more 3-dimensional structures (conformations) of a test molecule suspected of being a therapeutic for the condition to a 3-dimensional structure or model of at least one dopamine receptor; (ii) using the computational method to estimate the binding affinity or interaction of the test molecule structure to the structure or model of the at least one dopamine receptor; and (iii) using the computational method to compare the binding affinity or interaction of the test molecule to a pre-determined threshold, wherein modulation of the dopamine receptor by the test molecule comparable to or greater than the threshold is indicative of a therapeutic for the condition, such as cancer. The dopamine receptor may be a member of the D2-like family of dopamine receptors.

[0018] Also provided herein are methods of treating a subject having a condition. The method may comprise administering an effective amount of a therapeutic agent that targets at least one dopamine receptor or G protein-coupled receptor (GPCR). The therapeutic agent may be a neutralizing agent, an antagonist of the receptor, an agonist of the receptor, a competitive inhibitor of the receptor with respect to dopamine, or a non-competitive inhibitor of the receptor with respect to dopamine. The therapeutic agent may be selective for the D2-like family of dopamine receptors with respect to the D1-like family of dopamine receptors. The subject has, or is at risk of having, cancer. The subject may have, or may be at risk of having, a psychiatric disorder, and/or an infection. The dopamine receptor may be a member of the D2-like family of dopamine receptors. The GPCR may be a Class A GPCR. The GPCR may be GPR132, GPR91, MTNR1A, CXCR7. The GPCR may be GPR132, GPR91, MTNR1A, GPR162, GPR137, BAI3, LGR4, PTGIR, CXCR7 or a combination thereof. The therapeutic agent may be a monoclonal antibody (*e.g.*, a chimerized or humanized monoclonal antibody), polyclonal antibody (*e.g.*, a chimerized or humanized polyclonal antibody), or a bispecific antibody. The therapeutic agent may be a drug or active agent, such as an anti-cancer agent, conjugated to an antibody. The therapeutic agent may be a radioactively-conjugated antibody or a small molecule-conjugated antibody. The therapeutic agent may be a vector that expresses a recombinant antibody to the dopamine receptor or GPCR. The therapeutic agent may be a fusion protein or a peptide that targets the dopamine receptor or GPCR. The therapeutic agent may be an siRNA, shRNA, or an antisense oligonucleotide that targets the dopamine receptor or GPCR. The dopamine receptor or GPCR may be targeted by CRISPR interference.

[0019] Also provided herein are methods of treating and assessing the efficacy of a treatment in a subject having a condition. The method may comprise (i) treating the subject according to a treatment method described herein (ii) assessing as decribed herein the treatment's efficacy. The subject has, or is at risk of having, cancer. The treatment regimen may comprise administering an effective amount of a therapeutic, such as a compound of formula **(10)** or a pharmaceutically acceptable salt thereof or an analog thereof. The dosage of a therapeutic administered, the frequency of administration of the compound (*e.g.,* a compound of formula **(10)),** or both, is selected or adjusted based on the levels of gene expression or gene copy number measured or mutations found.

BRIEF DESCRIPTION OF THE DRAWINGS

[0020] The above summary, as well as the following detailed description of embodiments of the invention, will be better understood when read in conjunction with the appended drawings. It should be understood, however, that the invention is not limited to the precise arrangements and instrumentalities shown. In the drawings:

Figure **1**.Antagonism of dopamine receptors (DRD1, DRD2S, DRD2L, DRD3, DRD4, and DRD5) by ONC201.

Figure **2** illustrates soluble prolactin detected by ELISA in the peripheral blood of advanced solid tumor patients at baseline and following a single ONC201 dose (PO 125-625 mg). Sampling time points post-treatment include 6 hours, 1, 2, 7, and 21 days post-treatment.

Figure **3.** Tumor type sensitivity of the Genomic of Drug Sensitivity in Cancer program (GDSC) cell line collection. The average sensitivity was determined by average estimated IC_{50} values from cell viability assays conducted at 72 hours post-treatment. Numbers above the bar indicates indicate the number of cell lines per tumor type.

Figure **4.** ONC201 is a selective DRD2 antagonist. (A) Agonism of orphan or known GPCRs or antagonism of known GPCRs using an arrestin recruitment reporter assay (10 μM ONC201). (B) Antagonism of ligand-stimulated dopamine receptors by ONC201 using an arrestin recruitment reporter assay. Schild analysis of DRD2L antagonism by ONC201 using (C) arrestin recruitment or (D) cAMP modulation reporters.

Figure **5.** ONC201 antagonism of DRD2 is highly specific among GPCRs and other cancer drug targets. (A) Antagonism of GPCRs using an arrestin recruitment reporter assay (10 μM ONC201). Competition of ONC201-mediated antagonism of DRD2L by dopamine in (B) arrestin recruitment or (C) cAMP modulation reporters. (D) Antagonism or agonism of nuclear hormone receptors by ONC201 (2 or 20 μM) with a nuclear translocation reporter assay. (E) *In vitro* inhibition of kinase enzymatic activity by ONC201 (1 μM). (F) DRD2L antagonistic activity of ONC201 or an ONC201 linear isomer with no biological activity using an arrestin recruitment reporter assay.

Figure **6.** GBM cell lines with higher DRD2 expression are more responsive to ONC201. (A) Inhibition of NCI60 GBM cell lines as a function of ONC201 concentration. (B) Log ONC201 GI_{50} (M) vs DRD2 expression for each GBM cell line. $R^2 = 0.8707$.

Figure 7. ONC201 exhibits superior selectivity among GPCRs for DRD2 compared to other DRD2 antagonists, such as risperidone.

Figure **8.** ONC201 has a higher selectivity for tumor cells than the antipsychotic DRD2 antagonist, thioridazine.

Figure **9.** Optimization of ONC201 inhibition of DRD2 calcium flux. HEK-293T cells were transfected with expression constructs for wild-type DRD2 **(A)** or a control GPCR **(B).** DRD2-specific calcium flux inhibition was investigated at 0.1 and 1 nM dopamine, for ONC201 concentrations between 100 pM and 100 μM. 100 μM ONC201 completely inhibited DRD2 dopamine-induced calcium flux but had no effect on the control GPCR.

Figure **10.** Comparison of DRD2 inhibitors. DRD2-specific calcium flux inhibition was investigated at 1 nM dopamine, using inhibitors spiperone (squares), domperidone (triangles), and ONC201 (circles) at a range of concentrations. Data for individual assays was normalized using the no-inhibitor value (shown as 10^{-11} M) as 100% activity.

Figure **11.** Identification of DRD2 residues critical for dopamine-induced calcium flux. (A) Dopamine-induced calcium flux was assayed as before at 1 nM dopamine, across the entire DRD2 alanine-scan library. The data represent the average of three experiments. Mutant clones were considered to be deficient for calcium flux if they demonstrated flux values less than 2 standard deviations below the average calcium flux value (AV - 2SD) for the entire library. (B) The locations of the 28 mutated residues identified are indicated (green spheres) on the DRD3 crystal structure (PDB id 3PBL; Chien, E.Y. et al. (2010) Science 330:1091-5). The D2R/D3R antagonist eticlopride is shown in cyan.

Figure **12.** Identification of DRD2 residues critical for ONC201 inhibition of dopamine-induced calcium flux. (A) Dopamine-induced calcium flux was assayed as before at 1 nM dopamine but in the presence of 100 μM ONC201, across the entire DRD2 alanine-scan library. The data represent the average of three experiments normalized to the value for flux value with wild-type DRD2 (%WT). Mutant clones were considered to be critical for ONC201 inhibition if they demonstrated flux values greater than 2 standard deviations above the average calcium flux value (AV + 2SD) for the entire library. (B) The locations of the 8 mutated residues identified are indicated (red spheres) on the DRD3 crystal structure.

Figure **13.** A reference compound, (+) Butaclamol, and a test compound, ONC201 dihydrochloride, successfully competed for [^{3}H]Methylspiperone, with IC_{50} values of 2.5 nM and 21 μM, respectively.

Figure **14.** Association kinetic curves for ONC201 dihydrochloride to DRD2S receptor to determine K_{on} and K_{off}.

Figure **15.** Compound activity with the selected GPCR and Orphan GPCR Biosensor Assays. Compound was tested in antagonist and agonist mode with the desired GPCR and Orphan GPCR Biosensor Assays. For agonist assays, data was normalized to the maximal and minimal response observed in the presence of control ligand and vehicle. For antagonist assays, data was normalized to the maximal and minimal response observed in the presence of EC_{80} ligand and vehicle. The following EC_{80} concentrations were used: CCR4 Arrestin: 0.0078 μM CCL22; CHRM2 Arrestin: 26 μM Acetylcholine; and MC4R Arrestin: 0.0026 μM Melanotan II.

Figure **16.** ONC206 and ONC212 demonstrated anti-cancer efficacy across various tumor types in the NCI60 cancer cell line panel. ONC203 is an inactive negative control

Figure **17.** ONC206 is an imipridone with improved DRD2 antagonism. ONC206, an analog of ONC201, exhibits superior antagonism of D2-like dopamine receptor family, and retains highly selective antagonism of D2-like dopamine receptors compared to other antipsychotics, such as a haloperidol.

Figure **18.** Bone cancer is more responsive to ONC206 than ONC201.

Figure **19.** Ewing's sarcoma is the most ONC206 responsive bone cancer subtype.

Figure **20.** ONC206 anti-cancer efficacy is in the nanomolar range in 14 out of 16 Ewing's sarcoma cell lines. ONC206 demonstrated superior efficacy compared to ONC201 in all cell lines

Figure **21.** The imipridone ONC212 targets an orphan GPCR It is a highly selective agonist of the orphan GPCR tumor suppressor GPR132, and it does not engage DRD2.

Figure **22.** ONC212 induced cell death in cancer cells (HCT116) but not normal cells (MRC5) at nanomolar concentrations.

Figure **23.** ONC212 induces the integrated stress response and inhibits Akt/ERK phosphorylation at nanomolar concentrations and at earlier time points compared to ONC201.

Figure **24.** ONC212 demonstrates oral and IP anti-cancer efficacy in xenograft mouse models of colorectal and breast cancer.

Figure **25.** Leukemia is more responsive to ONC212 than ONC201.

Figure **26.** ONC212 demonstrates anti-cancer efficacy (and superior efficacy compared to ONC201) in the nanomolar range in 55 leukemia cell lines regardless of subtype.

Figure **27.** GPCRs agonized or antagonized (>50%) by 9 imipridones tested. Imipridones selectively target rhodopsin-like Class A GPCRs.

Figure **28.** Case study of a subject with recurrent glioblastoma (Example 16). (A) Tumor size relative to baseline (%) of total tumor burden in the subject. One cycle is 3 weeks. (B) Contrast MRI scans at baseline, 21, 27 and 36 weeks post-ONC201 initiation of one of 2 malignant lesions.

Figure **29.** ONC212 demonstrates anti-cancer effects in acute myeloid leukemia (AML) cell lines. (A) Comparison of cell viability of MV411 AML cells treated with ONC212 or cytarabine. (B) Comparison of cell viability of MOLM14, MV411 AML cells, MRC5 lung fibroblasts and Hs27a bone marrow cells treated with ONC212. (C) Cell viability of MOLM14 and MV411 AML cells treated with ONC212 (250nM) for 4, 8, 24, 48, 72 and 96h.

Figure **30.** ONC212 efficacy in ONC201-resistant AML xenograft model (MV411 AML cells (5×10^6) subcutaneously implanted in the flanks of athymic nude mice). ONC212 and ONC201 were administered orally (PO) as indicated. Tumor volume (A and B) and body weight (C) (n=10) was measured on indicated days. * represents $p < 0.05$ relative to vehicle.

Figure **31.** ONC206 efficacy in Ewing's sarcoma xenograft model (MHH-ES-1 Ewing's sarcoma cells (5×10^6)

subcutaneously implanted in the flanks of athymic nude mice). ONC206 (PO) and methotrexate (IV) were administered on day 1 and day 13 as indicated. Tumor volume (A) and body weight (B) (n=4) was measured on indicated days.

Figure **32.** ONC213 (10μM) GPCR profile using a β-arrestin recruitment reporter assay.

Figure **33.** ONC213 demonstrated *in vitro* anti-cancer potency in HCT116/RPMI8226 cancer cells similar to ONC212, but *in vitro* toxicity to normal cells was reduced compared to ONC212.

Figure **34.** ONC237 (10μM) GPCR profile using a β-arrestin recruitment reporter assay.

Figure **35.** ONC236 (10μM) GPCR profile using a β-arrestin recruitment reporter assay.

Figure **36.** ONC234 (10μM) GPCR profile using a β-arrestin recruitment reporter assay.

Figure **37.** ONC201 linear isomer (TIC-10) (10μM) GPCR profile using a β-arrestin recruitment reporter assay.

Figure **38.** Number of GPCRs hit for several imipridones.

DETAILED DESCRIPTION OF THE INVENTION

[0021] Scientific and technical terms used here are intended to have the meanings commonly understood by those of ordinary skill in the art. Such terms are found and used in context in various standard references illustratively including J. Sambrook and D. W. Russell, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press; 3rd Ed., 2001; F. M. Ausubel, Ed., Short Protocols in Molecular Biology, Current Protocols; 5th Ed., 2002; B. Alberts et al., Molecular Biology of the Cell, 4th Ed., Garland, 2002; D. L. Nelson and M. M. Cox, Lehninger Principles of Biochemistry, 4th Ed., W.H. Freeman & Company, 2004; Engelke, D. R., RNA Interference (RNAi): Nuts and Bolts of RNAi Technology, DNA Press LLC, Eagleville, PA, 2003; Herdewijn, P. (Ed.), Oligonucleotide Synthesis: Methods and Applications, Methods in Molecular Biology, Humana Press, 2004; A. Nagy, M. Gertsenstein, K. Vintersten, R. Behringer, Manipulating the Mouse Embryo: A Laboratory Manual, 3rd edition, Cold Spring Harbor Laboratory Press; Dec. 15, 2002, ISBN-10: 0879695919; Kursad Turksen (Ed.), Embryonic stem cells: methods and protocols in Methods Mol Biol. 2002;185, Humana Press; Current Protocols in Stem Cell Biology, ISBN: 9780470151808, as well as U.S. Patent No. 8,673,923.

[0022] The term "substituted" means that any one or more hydrogens on the designated atom is replaced with a selection from the indicated group, provided that the designated atom's normal valency is not exceeded, and that the substitution results in a stable compound. When a substituent is keto (i.e., =O), then 2 hydrogens on the atom are replaced. Keto substituents are not present on aromatic moieties. Ring double bonds are double bonds that are formed between two adjacent ring atoms (e.g., C=C, C=N, or N=N).

[0023] When a variable (e.g., R^4) occurs more than one time in a constituent or formula for a compound, its definition at each occurrence is independent of its definition at every other occurrence. Thus, for example, if a group is shown to be substituted with 0-3 R^4 moieties, then the group may optionally be substituted with up to three R^4 moieties and R^4 at each occurrence is selected independently from the definition of R^4. Also, combinations of substituents and/or variables are permissible, but only if such combinations result in stable compounds.

[0024] When an atom or chemical moiety is followed by a subscripted numeric range (e.g., $C_{1\text{-}6}$), it will be appreciated that this is meant to encompass each number within the range, as well as all intermediate ranges. For example, "$C_{1\text{-}6}$ alkyl" is meant to include alkyl groups with 1, 2, 3, 4, 5, 6, 1-6, 1-5, 1-4, 1-3, 1-2, 2-6, 2-5, 2-4, 2-3, 3-6, 3-5, 3-4, 4-6, 4-5, and 5-6 carbons.

[0025] The term "alkyl" includes both branched and straight-chain saturated aliphatic hydrocarbon groups having

the specified number of carbon atoms. For example, C_{1-6} alkyl is intended to include C_1, C_2, C_3, C_4, C_5, and C_6 alkyl groups. Examples of alkyl include, but are not limited to, methyl, ethyl, n-propyl, i-propyl, n-butyl, isobutyl s-butyl, t-butyl, n-pentyl, s-pentyl, neopentyl and n-hexyl. In certain cases, a straight chain or branched chain alkyl has six or fewer carbon atoms in its backbone (e.g., C_1-C_6 for straight chain, C_3-C_6 for branched chain), and in other cases, a straight chain or branched chain alkyl has four or fewer carbon atoms. Likewise, cycloalkyls have from three to eight carbon atoms in their ring structure, and in other cases, cycloalkyls have five or six carbons in the ring structure. Most preferred is C_{1-6} alkyl, particularly ethyl, methyl, isopropyl, isobutyl, n-pentyl, n-hexyl and cyclopropylmethyl.

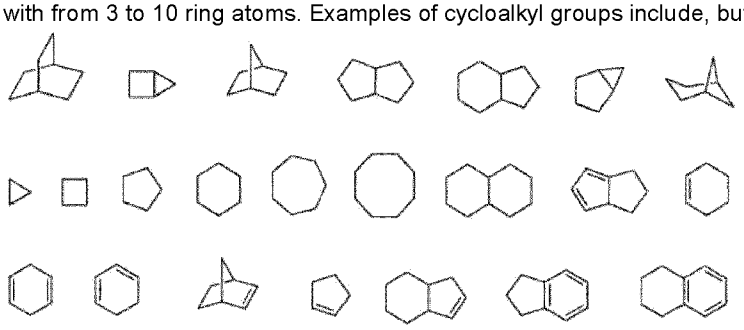
[0026] the term "substituted alkyl" means alkyl as defined above, substituted by one, two or three substituents selected from halogen, -OH, alkoxy, $-NH_2$, $-N(CH_3)_2$, -C(=O)OH, trifluoromethyl, -C=N, $-C(=O)O(C_1$-$C_4)$alkyl, $-C(=O)NH_2$, $-SO_2NH_2$, $-C(=NH)NH_2$, and $-NO_2$, preferably containing one or two substituents selected from halogen, -OH, alkoxy, $-NH_2$, trifluoromethyl, $-N(CH_3)_2$, and -C(=O)OH, more preferably selected from halogen, alkoxy and -OH. Examples of substituted alkyls include, but are not limited to, 2,2-difluoropropyl, 2-carboxycyclopentyl and 3-chloropropyl.

[0027] Unless the number of carbons is otherwise specified, "lower alkyl" is an alkyl group, as defined above, but having from one to six carbon atoms, preferably one to four, in its backbone structure. "Lower alkenyl" and "lower alkynyl" have chain lengths of 2-6 carbon atoms and preferably 2-4 carbon atoms.

[0028] "Alkenyl" includes unsaturated aliphatic groups analogous in length and possible substitution to the alkyls described above, but that contain at least one double bond. For example, the term "alkenyl" includes straight-chain alkenyl groups (e.g., ethenyl, propenyl, butenyl, pentenyl, hexenyl, heptenyl, octenyl, nonenyl, decenyl), branched-chain alkenyl groups, cycloalkenyl (e.g., alicyclic) groups (e.g., cyclopropenyl, cyclopentenyl, cyclohexenyl, cycloheptenyl, cyclooctenyl), alkyl or alkenyl substituted cycloalkenyl groups, and cycloalkyl or cycloalkenyl substituted alkenyl groups. In certain cases, a straight chain or branched chain alkenyl group has six or fewer carbon atoms in its backbone (e.g., C_2-C_6 for straight chain, C_3-C_6 for branched chain). Likewise, cycloalkenyl groups may have from three to eight carbon atoms in their ring structure, and in some embodiments, cycloalkenyl groups have five or six carbons in the ring structure. The term "C_2-C_6" includes alkenyl groups containing two to six carbon atoms. The term "C_3-C_6" includes alkenyl groups containing three to six carbon atoms.

[0029] "Alkynyl" includes unsaturated aliphatic groups analogous in length and possible substitution to the alkyls described above, but which contain at least one triple bond. For example, "alkynyl" includes straight-chain alkynyl groups (e.g., ethynyl, propynyl, butynyl, pentynyl, hexynyl, heptynyl, octynyl, nonynyl, decynyl), branched-chain alkynyl groups, and cycloalkyl or cycloalkenyl substituted alkynyl groups. In certain embodiments, a straight chain or branched chain alkynyl group has six or fewer carbon atoms in its backbone (e.g., C_2-$C_6$for straight chain, C_3-C_6 for branched chain). The term "C_2-C_6" includes alkynyl groups containing two to six carbon atoms. The term "C_3-C_6" includes alkynyl groups containing three to six carbon atoms.

[0030] The term "cycloalkyl" refers to a mono cyclic or polycyclic non-aromatic radical, where each of the atoms forming the ring (i.e. skeletal atoms) is a carbon atom. In some cases, the cycloalkyl group is saturated or partially unsaturated. In other cases, the cycloalkyl group is fused with an aromatic ring. Cycloalkyl groups include groups with from 3 to 10 ring atoms. Examples of cycloalkyl groups include, but are not limited to, the following moieties:



[0031] Monocyclic cycloalkyls include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, and cyclooctyl. Dicyclic cycloalkyls include, but are not limited to, tetrahydronaphthyl, indanyl, and tetrahydropentalene. Polycyclic cycloalkyls include adamantine and norbornane. The term cycloalkyl includes "unsaturated nonaromatic carbocyclyl" or "nonaromatic unsaturated carbocyclyl" groups, both of which refer to a nonaromatic carbocycle as defined herein, which contains at least one carbon carbon double bond or one carbon carbon triple bond.

[0032] The term "cycloalkylalkyl" refers to an alkyl group substituted by a cycloalkyl group. Example cycloalkylalkyl groups include cyclopropylalkyl, cyclohexylalkyl.

[0033] The term "heterocycloalkyl" refers to a non-aromatic heterocycle where one or more of the ring-forming atoms is a heteroatom such as an O, N, or S atom. Heterocycloalkyl groups include mono- or polycyclic (e.g., having 2, 3 or 4 fused rings) ring systems, as well as spirocycles. Example heterocycloalkyl groups include morpholino, thiomorpholino, piperazinyl, tetrahydrofuranyl, tetrahydrothienyl, 2,3-dihydrobenzofuryl, 1,3-benzodioxole, benzo-1,4-dioxane, piperidinyl, pyrrolidinyl, isoxazolidinyl, isothiazolidinyl, pyrazolidinyl, oxazolidinyl, thiazolidinyl, and imidazolidinyl. Also included in the definition of heterocycloalkyl can be moieties that have one or more aromatic rings fused (i.e., having a bond in common with) to the nonaromatic heterocyclic ring, for example quinolyl, isoquinolyl, and benzo derivatives of heterocycles. A heterocycloalkyl group having one or more fused aromatic rings are attached though either the aromatic or non-aromatic portion. Also included in the definition of heterocycloalkyl are moieties where one or more ring-forming atoms can be substituted by 1 or 2 oxo or sulfido groups. In some cases, the heterocycloalkyl group has from 1 to about 20 carbon atoms, and in further case from about 3 to about 20 carbon atoms. In some cases, a heterocycloalkyl group contains 3 to about 20, 3 to about 14, 3 to about 7, or 5 to 6 ring-forming atoms. In some cases, a heterocycloalkyl group has 1 to about 4, 1 to about 3, or 1 to 2 heteroatoms. In some cases, a heterocycloalkyl group contains 0 to 3 double bonds. In some cases, a heterocycloalkyl group contains 0 to 2 triple bonds.

[0034] The term "heterocycloalkylalkyl" refers to an alkyl group substituted by a heterocycloalkyl. Example heterocycloalkylalkyls include morpholinoalkyl and piperazinylalkyl.

[0035] The term "aryl" refers to monocyclic or polycyclic (e.g., having 2, 3 or 4 fused rings) aromatic hydrocarbons such as, phenyl, naphthyl, anthracenyl, phenanthrenyl. In some cases, an aryl group has from 6 to about 20 carbon atoms.

[0036] The term "arylalkyl" refers to an alkyl group substituted by an aryl group. Example arylalkyl groups include benzyl and phenylethyl.

[0037] The term "heteroaryl" refers to an aromatic heterocycle having at least one heteroatom ring member such as an O, S, or N atom. Heteroaryl groups include monocyclic and polycyclic (e.g., having 2, 3 or 4 fused rings) systems. A ring-forming N atom in a heteroaryl group can also be oxidized to form an N-oxo moiety. Examples of heteroaryl groups include pyridyl, N-oxopyridyl, pyrimidinyl, pyrazinyl, pyridazinyl, triazinyl, furyl, quinolyl, isoquinolyl, thienyl, imidazolyl, thiazolyl, indolyl, pyrryl, oxazolyl, benzofuryl, benzothienyl, benzthiazolyl, isoxazolyl, pyrazolyl, triazolyl, tetrazolyl, indazolyl, 1,2,4-thiadiazolyl, isothiazolyl, benzothienyl, purinyl, carbazolyl, benzimidazolyl, indolinyl. In some cases, a heteroaryl group has from 1 to about 20 carbon atoms, and in further cases from about 3 to 20 carbon atoms. In some cases, a heteroaryl group contains 3 to about 14, 3 to about 7, or 5-6 ring-forming atoms. In some cases, a heteroaryl group has 1 to about 4, 1 to about 3, or 1-2 heteroatoms.

[0038] a "heteroarylalkyl" group refers to an alkyl group substituted by a heteroaryl group. An example of a heteroarylalkyl group is pyridylmethyl.

[0039] The terms "halo" or "halogen" refer to a fluorine (F), chlorine (Cl), bromine (Br), or iodine (I) atom; preferably, F, Cl, or Br; more preferably, F or Cl. The term "perhalogenated" refers to a moiety where all hydrogens are replaced by halogens. The term "haloalkyl" refers to alkyl moieties with a halogen replacing a hydrogen on one or more carbons of the hydrocarbon backbone. C_1-C_6 haloalkyl includes a straight chain or branched alkyl with six

or fewer backbone carbon atoms and a halogen replacing a hydrogen on one or more backbone carbons.

[0040] The term "alkoxy" or "alkoxyl" includes substituted and unsubstituted alkyl, alkenyl, and alkynyl groups covalently linked to an oxygen atom. C_1-C_6 alkoxy refers to moieties having six or fewer carbon atoms in the hydrocarbon backbone. Examples of alkoxy groups (or alkoxyl radicals) include methoxy, ethoxy, isopropyloxy, propoxy, butoxy, and pentoxy groups. Preferred are (C_1-C_3) alkoxy, particularly ethoxy and methoxy. Examples of substituted alkoxy groups include halogenated alkoxy groups.

[0041] The term "hydroxy" or "hydroxyl" includes groups with an -OH or $-O^-$.

[0042] The term "pharmaceutically acceptable salts" refers to derivatives of compounds where the parent compound is modified by converting an existing acid or base moiety to its salt form. Examples of pharmaceutically acceptable salts include, but are not limited to, mineral or organic acid salts of basic residues such as amines; alkali or organic salts of acidic residues such as carboxylic acids. Pharmaceutically acceptable salts include conventional non-toxic salts of a parent compound formed, for example, from non-toxic inorganic or organic acids. Pharmaceutically acceptable salts may be synthesized from a parent compound which contains a basic or acidic moiety by conventional chemical methods. Generally, such salts can be prepared by reacting a free acid or base form of these compounds with a stoichiometric amount of the appropriate base or acid in water or in an organic solvent, or in a mixture of the two; generally, nonaqueous media like ether, ethyl acetate, ethanol, isopropanol, or acetonitrile are preferred. Lists of suitable salts can be found in Remington's Pharmaceutical Sciences, 17th ed., Mack Publishing Company, Easton, PA, 1985, p. 1418, Journal of Pharmaceutical Science, 66, 2 (1977), and P. H. Stahl and C. G. Wermuth, editors, Handbook of Pharmaceutical Salts: Properties, Selection and Use, 2nd Revised edition, Weinheim/Zürich:Wiley-VCH/VHCA (2011).

[0043] Examples of suitable inorganic acids include hydrochloric acid, sulphuric acid, phosphoric acid, or hydrobromic acid, while examples of suitable organic acids include carboxylic acid, sulpho acid, or sulphonic acid, such as acetic acid, tartaric acid, lactic acid, propionic acid, glycolic acid, malonic acid, maleic acid, fumaric acid, tannic acid, succinic acid, alginic acid, benzoic acid, 2-phenoxybenzoic acid, 2-acetoxybenzoic acid, cinnamic acid, mandelic acid, citric acid, maleic acid, salicylic acid, trifluoroacetic acid, 3-aminosalicylic acid, ascorbic acid, embonic acid, nicotinic acid, isonicotinic acid, oxalic acid, gluconic acid, amino acids, methanesulphonic acid, ethanesulphonic acid, 2-hydroxyethanesulphonic acid, ethane-1,2-disulphonic acid, benzenesulphonic acid, 4-methylbenzenesulphonic acid or naphthalene-2-sulphonic acid. Examples of suitable inorganic bases include sodium hydroxide, potassium hydroxide and ammonia, while examples of suitable organic bases include amines, e.g., tertiary amines, such as trimethylamine, triethylamine, pyridine, *N,N*-dimethylaniline, quinoline, isoquinoline, α-picoline, β-picoline, γ-picoline, quinaldine, or pyrimidine.

[0044] the term "antibody" encompasses the structure that constitutes the natural biological form of an antibody. In most mammals, including humans, and mice, this form is a tetramer and consists of two identical pairs of two immunoglobulin chains, each pair having one light and one heavy chain, each light chain comprising immunoglobulin domains V_L and C_L, and each heavy chain comprising immunoglobulin domains V_H, Cγ1, Cγ2, and Cγ3. In each pair, the light and heavy chain variable regions (V_L and V_H) are together responsible for binding to an antigen, and the constant regions (C_L, Cγ1, Cγ2, and Cγ3, particularly Cγ2, and Cγ3) are responsible for antibody effector functions. In some mammals, for example in camels and llamas, full-length antibodies may consist of only two heavy chains, each heavy chain comprising immunoglobulin domains V_H, Cγ2, and Cγ3. By "immunoglobulin (Ig)" herein is meant a protein consisting of one or more polypeptides substantially encoded by immunoglobulin genes. Immunoglobulins include but are not limited to antibodies. Immunoglobulins may have a number of structural forms, including full-length antibodies, antibody fragments, and individual immunoglobulin domains including V_H, Cγ1, Cγ2, Cγ3, V_L, and C_L.

[0045] Based on the heavy-chain constant domain amino acid sequence, intact antibodies can be assigned to different "classes." There are five-major classes (isotypes) of intact antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into "subclasses," e.g., IgG1, IgG2, IgG3, IgG4, IgA, and IgA2. The heavy-

chain constant domains that correspond to the different antibody classes are called alpha, delta, epsilon, gamma, and mu, respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known to one skilled in the art.

[0046] The terms "antibody" or "antigen-binding fragment," respectively, refer to intact molecules as well as functional fragments thereof, such as Fab, a scFv-Fc bivalent molecule, $F(ab')_2$, and Fv that are capable of specifically interacting with a desired target. In some cases, the antigen-binding fragments comprise:

1. (1) Fab, the fragment which contains a monovalent antigen-binding fragment of an antibody molecule, which can be produced by digestion of whole antibody with the enzyme papain to yield an intact light chain and a portion of one heavy chain;
2. (2) Fab', the fragment of an antibody molecule that can be obtained by treating whole antibody with pepsin, followed by reduction, to yield an intact light chain and a portion of the heavy chain; two Fab' fragments are obtained per antibody molecule;
3. (3) $(Fab')_2$, the fragment of the antibody that can be obtained by treating whole antibody with the enzyme pepsin without subsequent reduction; F(ab')2 is a dimer of two Fab' fragments held together by two disulfide bonds;
4. (4) Fv, a genetically engineered fragment containing the variable region of the light chain and the variable region of the heavy chain expressed as two chains;
5. (5) Single chain antibody ("SCA"), a genetically engineered molecule containing the variable region of the light chain and the variable region of the heavy chain, linked by a suitable polypeptide linker as a genetically fused single chain molecule; and
6. (6) scFv-Fc, is produced by fusing single-chain Fv (scFv) with a hinge region from an immunoglobulin (Ig) such as an IgG, and Fc regions.

[0047] Also provided herein is a monoclonal antibody. Also provided herein is a single chain Fv (scFv), a diabody, a tandem scFv, a scFv-Fc bivalent molecule, an Fab, Fab', Fv, $F(ab')_2$ or an antigen binding scaffold (*e.g.*, affibody, monobody, anticalin, DARPin, Knottin).

[0048] the terms "binds," "binding" or grammatical equivalents, refer to compositions, directly or indirectly, having affinity for each other. "Specific binding" is where the binding is selective between two molecules. A particular example of specific binding occurs between an antibody and an antigen. Typically, specific binding can be distinguished from non-specific when the dissociation constant (K_D) is less than about 1×10^{-5} M or less than about 1×10^{-6} M or 1×10^{-7} M. Specific binding can be detected, for example, by ELISA, immunoprecipitation, coprecipitation, with or without chemical crosslinking, and two-hybrid assays. Appropriate controls can be used to distinguish between "specific" and "non-specific" binding. "Affinity" is the strength of the binding interaction of two molecules, such as an antigen and its antibody, which is defined for antibodies and other molecules with more than one binding site as the strength of binding of the ligand at one specified binding site. Although the noncovalent attachment of a ligand to antibody or other molecule is typically not as strong as a covalent attachment, "high affinity" is for a ligand that binds to an antibody or other molecule having an affinity constant (K_a) of greater than 10^4 M^{-1}, typically 10^5-10^{11} M^{-1}; as determined by inhibition ELISA or an equivalent affinity determined by comparable techniques, such as Scatchard plots or using K_d/dissociation constant, which is the reciprocal of the K_a.

[0049] The term "selective" with respect to binding, inhibition, stimulation, or modulation means preferential binding, inhibition, stimulation, or modulation, respectively, of a first activity relative to a second activity (e.g., preferential binding of one receptor to another receptor; preferential inhibition relative to other receptors; or preferential inhibition of a mutant to a wild-type or vice versa). In some cases, binding is greater than two times more selective, greater than five times more selective, greater than ten times more selective, greater than fifty times more selective, greater than 100 times more selective, or greater than 1000 times more selective for the desired molecular target or pathway versus an undesired molecular target or pathway. In some cases, a compound will bind a first molecular target or affect a pathway by at least 2-fold, at least 5-fold, at least 10-fold, at least 20-

fold, at least 50-fold, at least 100-fold relative to a second target or pathway under the same conditions. It will be appreciated that binding to the D2-like family of dopamine receptors or a member thereof, may be selective with respect to the D1-like family of dopamine receptors or a member thereof by any of the foregoing amounts. The *in vitro* or *in vivo* activity of a molecular target or pathway may be measured by any suitable reproducible means.

[0050] The term "modulating" refers to "stimulating" or "inhibiting" an activity of a molecular target or pathway. For example, a composition modulates the activity of a molecular target or pathway if it stimulates or inhibits the activity of that target or pathway by at least 10%, by at least about 20%, by at least about 25%, by at least about 30%, by at least about 40%, by at least about 50%, by at least about 60%, by at least about 70%, by at least about 75%, by at least about 80%, by at least about 90%, by at least about 95%, by at least about 98%, or by about 99% or more relative to the activity of that molecular target or pathway under the same conditions but lacking only the presence of the composition. In another example, a composition modulates the activity of a molecular target or pathway if it stimulates or inhibits the activity of that target or pathway by at least 2-fold, at least 5 -fold, at least 10-fold, at least 20-fold, at least 50-fold, at least 100-fold relative to the activity of that target or pathway under the same conditions but lacking only the presence of the composition. The activity of a molecular target or pathway may be measured by any reproducible means. For example, the activity of a molecular target or pathway may be measured *in vitro* or *in vivo* by a suitable assay known in the art for measuring the activity. Control samples (untreated with the composition) can be assigned a relative activity value of 100%.

[0051] An antibody, antigen-binding fragment, or affinity tag may bind its target with a K_D of 0.1 nM - 10 mM, 0.1 nM - 1 mM, or within the 0.1 nM range. An antibody, antigen-binding fragment, or affinity tag may bind its target with a K_D of 0.1-2 nM, 0.1-1 nM, 0.05-1 nM, 0.1-0.5 nM, or 0.1-0.2 nM. An antibody, antigen-binding fragment, or affinity tag may bind its target directlyor indirectly, for example, binding as a secondary antibody that binds to an antibody bound to the target.

[0052] The word "label" refers to a compound or composition which is conjugated or fused directly or indirectly to a reagent such as a nucleic acid probe or an antibody and facilitates detection of the reagent to which it is conjugated or fused. The label may itself be detectable (e.g., radioisotope or fluorescent labels) or, in the case of an enzymatic label, may catalyze chemical alteration of a substrate compound or composition, which is detectable.

[0053] The term "probe" refers to synthetic or biologically produced nucleic acids that contain specific nucleotide sequences which hybridize under stringent conditions to target nucleic acid sequences. The terms "labeled probe," "nucleic acid probe operably linked to a detectable label," or "nucleic acid strand operably linked to a detectable label" refer to a probe which is prepared with a marker moiety or "detectable label" for detection. The marker moiety is attached at either the 5' end, the 3' end, internally, or a combination thereof. That is, one probe may be attached to multiple marker moieties. A preferred moiety is an identifying label such as a fluorophore. A labeled probe may also comprise a plurality of different nucleic acid sequences each labeled with one or more marker moieties. Each marker moiety may be the same or different. It may be beneficial to label different probes (*e.g.*, nucleic acid sequences) each with a different marker moiety. This can be achieved by having a single distinguishable moiety on each probe. For example, probe A is attached to moiety X and probe B is attached to moiety Y. Alternatively, probe A is attached to moieties X and Y while probe B is attached to moiety Z and W. Alternatively, probe A is attached to moieties X and Y, while probe B is attached to moieties Y and Z. All probes "A" and "B" above would be distinguishable and uniquely labeled.

[0054] By "tissue sample" is meant a collection of similar cells obtained from a tissue of a subject or patient, preferably containing nucleated cells with chromosomal material. The four main human tissues are (1) epithelium; (2) connective tissues, including blood vessels, bone and cartilage; (3) muscle tissue; and (4) nerve tissue. The tissue sample source may be solid tissue as from a fresh, frozen and/or preserved organ or tissue sample or biopsy or aspirate; blood or a blood constituent; bodily fluids such as cerebral spinal fluid, amniotic fluid, peritoneal fluid, or interstitial fluid; cells from a time in gestation or development of the subject. A tissue sample may be primary or cultured cells or cell lines. A tissue sample may contain compounds that are not naturally intermixed with the tissue in nature such as preservatives, anticoagulants, buffers, fixatives, nutrients, or antibiotics. By a "section" of a tissue sample is meant a single part or piece of a tissue sample, *e.g.*, a thin slice of tissue or cells cut from a

tissue sample. Multiple sections of tissue samples may be taken and subjected to analysis. A "cell line" refers to a permanently established cell culture that will proliferate given appropriate fresh medium and space.

Detection Methods

[0055] Provided herein are methods of detecting or measuring a target receptor *(e.g.,* a dopamine receptor or a GPCR) in a biological sample. Targets are detected by contacting the sample with a target detection reagent, *e.g.,* an antibody or fragment thereof, and a labeling reagent. The presence or absence of targets are detected by the presence or absence of the labeling reagent. In some instances, a sample is contacted with the target detection and the labeling reagents concurrently *e.g.*, the detection reagent is a primary antibody and the labeling reagent is a fluorescent dye conjugated to it. Alternatively, the biological sample is contacted with the target detection and labeling reagents sequentially, *e.g.*, the detection reagent is a primary antibody and the labeling reagent includes a secondary antibody. For example, a sample is incubated with a detection reagent, in some cases together with a labeling reagent, under conditions that allow a complex between the detection reagent (and labeling reagent) and target to form. After complex formation the sample is optionally washed one or more times to remove unbound detection reagent (and labeling reagent). When the sample is further contacted with a labeling reagent that specifically binds the detection reagent bound to the target, the sample can optionally be washed one or more times to remove unbound labeling reagent. The presence or absence of the target in the sample is then determined by detecting the labeling reagent.

[0056] The methods described here provide for detection of multiple targets in a sample. Multiple targets are identified by contacting the biological sample with additional detection reagents followed by additional labeling reagent specific for the additional detection reagents using the methods described.

[0057] A detection moiety, i.e., detectable label, is a substance used to facilitate identification and/or quantitation of a target. Detection moieties are directly observed or measured or indirectly observed or measured. Detection moieties include, but are not limited to, radiolabels that can be measured with radiation-counting devices; pigments, dyes or other chromogens that can be visually observed or measured with a spectrophotometer; spin labels that can be measured with a spin label analyzer; and fluorescent moieties, where the output signal is generated by the excitation of a suitable molecular adduct and that can be visualized by excitation with light that is absorbed by the dye or can be measured with standard fluorometers or imaging systems. The detection moiety can be a luminescent substance such as a phosphor or fluorogen; a bioluminescent substance; a chemiluminescent substance, where the output signal is generated by chemical modification of the signal compound; a metal-containing substance; or an enzyme, where an enzyme-dependent secondary generation of signal occurs, such as the formation of a colored product from a colorless substrate. The detection moiety may also take the form of a chemical or biochemical, or an inert particle, including colloidal gold, microspheres, quantum dots, or inorganic crystals such as nanocrystals or phosphors. The term detection moiety or detectable label can also refer to a "tag" or hapten that can bind selectively to a labeled molecule such that the labeled molecule, when added subsequently, is used to generate a detectable signal. For instance, one can use biotin, iminobiotin or desthiobiotin as a tag and then use an avidin or streptavidin conjugate of horseradish peroxidase (HRP) to bind to the tag, and then use a chromogenic substrate (e.g., tetramethylbenzidine) or a fluorogenic substrate such as Amplex Red or Amplex Gold (Molecular Probes, Inc.) to detect the presence of HRP. Similarly, the tag can be a hapten or antigen (e.g., digoxigenin), and an enzymatically, fluorescently, or radioactively labeled antibody can be used to bind to the tag. Numerous labels are known by those of skill in the art and include, but are not limited to, particles, fluorescent dyes, haptens, enzymes and their chromogenic, fluorogenic, and chemiluminescent substrates.

[0058] A fluorophore is a chemical moiety that exhibits an absorption maximum beyond 280 nm, and when covalently attached in a labeling reagent retains its spectral properties. Fluorophores include a pyrene, an anthracene, a naphthalene, an acridine, a stilbene, an indole or benzindole, an oxazole or benzoxazole, a thiazole or benzothiazole, a porphyrin, a cyanine, a perylene, a 4-amino-7-nitrobenz-2-oxa-1,3-diazole (NBD), a carbocyanine, a carbostyryl, a salicylate, an anthranilate, an azulene, a pyridine, a quinoline, a

borapolyazaindacene, a xanthene, an oxazine or a benzoxazine, a carbazine, a phenalenone, a coumarin, a benzofuran and benzphenalenone and derivatives thereof. oxazines include resorufins, aminooxazinones, diaminooxazines, and their benzo-substituted analogs.

[0059] When the fluorophore is a xanthene, the fluorophore may be a fluorescein, a rhodol, or a rhodamine. Fluorescein includes benzo- or dibenzofluoresceins, seminaphthofluoresceins, or naphthofluoresceins. Similarly, rhodol includes seminaphthorhodafluors. Alternatively, the fluorophore is a xanthene that is bound via a single covalent bond at the 9-position of the xanthene. Preferred xanthenes include derivatives of 3H-xanthen-6-ol-3-one, derivatives of 6-amino-3H-xanthen-3-one, or derivatives of 6-amino-3H-xanthen-3-imine. Fluorophores include xanthene (rhodol, rhodamine, fluorescein and derivatives thereof) coumarin, cyanine, pyrene, oxazine and borapolyazaindacene. In addition, the fluorophore can be sulfonated xanthenes, fluorinated xanthenes, sulfonated coumarins, fluorinated coumarins and sulfonated cyanines. The choice of fluorophore in the labeling reagent will determine the absorption and fluorescence emission properties of the labeling reagent. Physical properties of a fluorophore label include spectral characteristics (absorption, emission and stokes shift), fluorescence intensity, lifetime, polarization and photo-bleaching rate can all be used to distinguish one fluorophore from another.

[0060] Typically, a fluorophore contains one or more aromatic or heteroaromatic rings that are optionally substituted by one or more of a variety of substituents, including halogen, nitro, cyano, alkyl, perfluoroalkyl, alkoxy, alkenyl, alkynyl, cycloalkyl, arylalkyl, acyl, aryl or heteroaryl ring system, benzo, or other substituents typically present on fluorophores known in the art.

[0061] Preferably, the detection moiety is a fluorescent dye. Fluorescent dyes include, for example, Fluorescein, Rhodamine, Texas Red, Cy2, Cy3, Cy5, Cy0, Cy0.5, Cy1, Cy1.5, Cy3.5, Cy7, VECTOR Red, ELF™ (Enzyme-Labeled Fluorescence), FluorX, Calcein, Calcein-AM, CRYPTOFLUOR™'S, Orange (42 kDa), Tangerine (35 kDa), Gold (31 kDa), Red (42 kDa), Crimson (40 kDa), BHMP, BHDMAP, Br-Oregon, Lucifer Yellow, Alexa dye family, N-(6-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)caproyl) (NBD), BODIPY™, boron dipyrromethene difluoride, Oregon Green, MITOTRACKER™ Red, DiOC7 (3), DiIC18, Phycoerythrin, Phycobiliproteins BPE (240 kDa) RPE (240 kDa) CPC (264 kDa) APC (104 kDa), Spectrum Blue, Spectrum Aqua, Spectrum Green, Spectrum Gold, Spectrum Orange, Spectrum Red, NADH, NADPH, FAD, Infra-Red (IR) Dyes, Cyclic GDP-Ribose (cGDPR), Calcofluor White, Tyrosine and Tryptophan. Many fluorophores can also function as chromophores and thus they are also preferred chromophores.

[0062] In addition to fluorophores, enzymes also find use as detectable moieties. Enzymes are desirable detectable moieties because amplification of a detectable signal can be achieved resulting in increased assay sensitivity. The enzyme itself does not produce a detectable response but breaks down a substrate when it is contacted by an appropriate substrate such that the converted substrate produces a fluorescent, colorimetric or luminescent signal. Enzymes amplify a detectable signal because one enzyme on a labeling reagent can result in multiple substrates being converted to a detectable signal. This is advantageous where there is a low quantity of target present in the sample or a fluorophore does not exist that will give comparable or stronger signal than the enzyme. However, fluorophores are preferred because they do not require additional assay steps, and thus reduce the overall time to complete an assay. The enzyme substrate is selected to yield the preferred measurable product, e.g. colorimetric, fluorescent or chemiluminescence. Such substrates are extensively used in the art.

[0063] A preferred colorimetric or fluorogenic substrate and enzyme combination uses oxidoreductases such as horseradish peroxidase and a substrate such as 3,3'-diaminobenzidine (DAB) and 3-amino-9-ethylcarbazol-e (AEC), which yield a distinguishing color (brown and red, respectively). Other colorimetric oxidoreductase substrates that yield detectable products include, but are not limited to: 2,2-azino-bis(3-ethylbenzothiaz-oline-6-sulfonic acid) (ABTS), o-phenylenediamine (OPD), 3,3',5,5'-tetramethylbenzidine (TMB), o-dianisidine, 5-aminosalicylic acid, 4-chloro-1-naphthol. Fluorogenic substrates include, but are not limited to, homovanillic acid or 4-hydroxy-3-methoxyphenylacetic acid, reduced phenoxazines and reduced benzothiazines, including Amplexe Red reagent and its variants and reduced dihydroxanthenes, including dihydrofluoresceins and dihydrorhodamines including dihydrorhodamine 123. Peroxidase substrates that are tyramides represent a unique class of peroxidase

substrates in that they can be intrinsically detectable before action of the enzyme but are "fixed in place" by the action of a peroxidase in the process described as tyramide signal amplification (TSA). These substrates are extensively utilized to label targets in samples that are cells, tissues or arrays for their subsequent detection by microscopy, flow cytometry, optical scanning and fluorometry.

[0064] Additional colorimetric (and in some cases fluorogenic) substrate and enzyme combination use a phosphatase enzyme such as an acid phosphatase, an alkaline phosphatase or a recombinant version of such a phosphatase in combination with a colorimetric substrate such as 5-bromo-6-chloro-3-indolyl phosphate (BCIP), 6-chloro-3-indolyl phosphate, 5-bromo-6-chloro-3-indolyl phosphate, p-nitrophenyl phosphate, or o-nitrophenyl phosphate or with a fluorogenic substrate such as 4-methylumbelliferyl phosphate, 6,8-difluoro-7-hydroxy4-methylcoumarinyl phosphate (DiFMUP) fluorescein diphosphate, 3-0-methylfluorescein phosphate, resorufin phosphate, 9H-(1,3-dichloro-9,9-dimethylacridin-2-one-7-yl) phosphate (DDAO phosphate), or ELF 97, ELF 39 or related phosphates.

[0065] Glycosidases, in particular β-galactosidase, β-glucuronidase and β-glucosidase, are additional suitable enzymes. Appropriate colorimetric substrates include, but are not limited to, 5-bromo4-chloro-3-indolyl β-D-galactopyranoside (X-gal) and similar indolyl galactosides, glucosides, and glucuronides, o-nitrophenyl β-D-galactopyranoside (ONPG) and p-nitrophenyl β-D-galactopyranoside. Preferred fluorogenic substrates include resorufin β-D-galactopyranoside, fluorescein digalactoside (FDG), fluorescein diglucuronide and their structural variants, 4-methylumbelliferyl β-D-galactopyranoside, carboxyumbelliferyl β-D-galactopyranoside and fluorinated coumarin β-D-galactopyranosides. Additional enzymes includehydrolases such as cholinesterases and peptidases, oxidases such as glucose oxidase and cytochrome oxidases, and reductases for which suitable substrates are known.

[0066] Enzymes and their appropriate substrates that produce chemiluminescence are preferred for some assays. These include, but are not limited to, natural and recombinant forms of luciferases and aequorins. Chemiluminescence-producing substrates for phosphatases, glycosidases and oxidases such as those containing stable dioxetanes, luminol, isoluminol and acridinium esters are additionally useful. For example, the enzyme is luciferase or aequorin. The substrates are luciferine, ATP, Ca^{++} and coelenterazine.

[0067] In addition to enzymes, haptens such as biotin are useful detectable moieties. Biotin is useful as it is in an enzyme system that can further amplify a detectable signal, and it can serve as a tag in affinity chromatography for isolation purposes. For detection, an enzyme conjugate that has affinity for biotin is used, such as avidin-HRP. Subsequently, a peroxidase substrate is added to produce a detectable signal. Haptens also include hormones, naturally occurring and synthetic drugs, pollutants, allergens, affector molecules, growth factors, chemokines, cytokines, lymphokines, amino acids, peptides, chemical intermediates, or nucleotides.

[0068] In some cases, a detectable moiety is a fluorescent protein. Exemplary fluorescent proteins include green fluorescent protein (GFP), phycobiliproteins and their derivatives, luciferase or aequorin. Fluorescent proteins, especially phycobiliprotein, are particularly useful for creating tandem dye labeled labeling reagents. These tandem dyes comprise a fluorescent protein and a fluorophore to obtain a larger stokes shift where the emission spectra is farther shifted from the fluorescent protein's absorption spectra. This is particularly advantageous to detect a low amount of target in a sample where the emitted fluorescent light is maximally optimized, in other words the fluorescent protein reabsorbs little to none of the emitted light. The fluorescent protein and fluorophore function as an energy transfer pair where the fluorescent protein emits at a wavelength the fluorophore absorbs, and the fluorphore then emits at a wavelength farther from the fluorescent protein than could be obtained with only the fluorescent protein. A particularly useful combination is phycobiliproteins and sulforhodamine fluorophores, or sulfonated cyanine fluorophores; or sulfonated xanthene derivatives. Alternatively, the fluorophore is an energy donor and the fluorescent protein is an energy acceptor.

Methods of visualizing the detection moiety depend on the label.

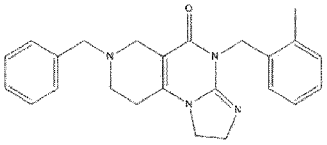
[0069] In some cases, a sample is illuminated with a light wavelength selected to give a detectable optical response, and observed with a means for detecting that response. Equipment useful for illuminating fluorescent compounds include hand-held ultraviolet lamps, mercury arc lamps, xenon lamps, lasers and laser diodes. These illumination sources are optically integrated into laser scanners, fluorescent microplate readers or standard or microfluorometers. The degree or location of signal, compared to a standard or expected response, indicates whether and to what degree the sample possesses a given characteristic or desired target.

[0070] An optical response is detected by visual inspection, or by using one of the following devices: CCD camera, video camera, photographic film, laser-scanning devices, fluorometers, photodiodes, quantum counters, epifluorescence microscopes, scanning microscopes, flow cytometers, fluorescence microplate readers, or by means for amplifying the signal such as photomultiplier tubes. When a sample is examined using a flow cytometer, examination of it optionally includes sorting portions of it according to their fluorescence response.

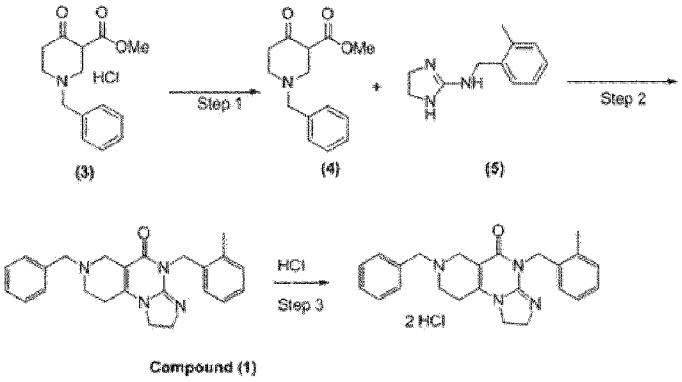
[0071] When an indirectly detectable label is used, then illuminating typically includes adding a reagent to produce a detectable signal such as a colorimetric enzyme substrate. Radioisotopes are also considered indirectly detectable where an additional reagent is not needed, rather the radioisotope is exposed to X-ray film or other mechanism to record and measure the signal. This is true for some chemiluminescent signals that are observed after exposure to film.

I. ONC201 (COMPOUND (1)), SALTS THEREOF AND SYNTHESES THEREOF

[0072] ONC201 (compound **(1))**



and its analogs, and their pharmaceutically acceptable salts, as well as syntheses for them, are provided herein. In *in vitro* models, animal models, and human clinical trials, ONC201 has broad anti-cancer activity, low toxicity including few, if any, adverse effects, low genotoxicity, and high bioavailability including orally. These features allow ONC 201 and various analogs to be well suited for a variety of applications. ONC201 can be made by the synthesis shown in Scheme **1.**



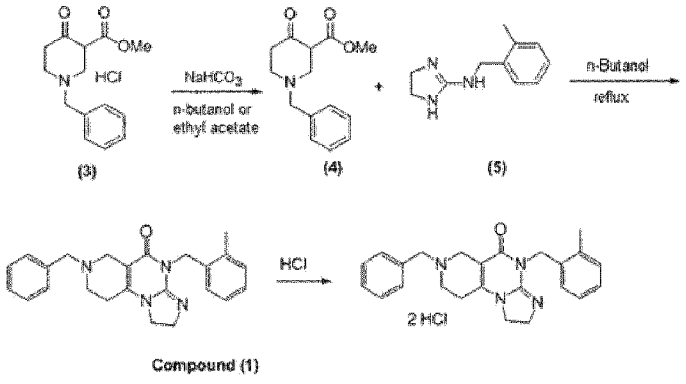
Scheme 1

[0073] Synthesis of an ONC201 dihydrochloride salt starts with commercially available intermediary N-Benzyl-3-carbomethoxy-4-piperidone hydrochloride, compound **(3).** The synthesis may include neutralizing compound **(3)** with a base (Step **1)** to produce compound **(4),** a free base. Compound **(3)** may be neutralized with an inorganic base to produce compound **(4).** Compound **(3)** may be neutralized with an organic base to produce compound **(4).** Compound **(3)** may be neutralized in the presence of an alcohol, for example, n-butanol. Compound **(3)** may be neutralized in the presence of at least one organic solvent, for example, n-butanol and/or ethyl acetate. Compound

(3) may be neutralized in the presence of a base and at least one organic solvent, for example, NaHCOs and n-butanol. Compound **(3)** may be neutralized in the presence of n-butanol and triethyl amine (Et_3N).

[0074] The synthesis may include reacting compound **(4)** with compound **(5)** (Step **2)** to produce intermediary compound **(1).** The reaction in Step **2** may include heating compound **(4)** with compound **(5).** The reaction in Step **2** may include refluxing heating compound **(4)** and compound **(5)** in the presence of a solvent. The reaction in Step **2** may include use of Dean-stark trap to remove water and/or methanol (MeOH) formed in the reaction.

[0075] An ONC201 dihydrochloride salt may be synthesized (Step **3).** This reaction (Step **3)** may include treating ONC201 with HCl in dioxane. Step **3** may include treating ONC201with 4N HCl in dioxane. The synthesis optionally includes recrystallizing the ONC201 di-salt. The ONC201 di-hydrochloride salt may be synthesized as shown in Scheme **2.**



Scheme 2

II. TNF-RELATED APOPTOSIS-INDUCING LIGAND ("TRAIL")

[0076] TRAIL protein can be assayed in a sample obtained from a subject to detect TRAIL expression induced by compounds and their salts described herein. Immunoassays can be used to assay TRAIL in a sample, including enzyme-linked immunosorbent assay (ELISA), enzyme-linked immunofiltration assay (ELIFA), flow cytometry, immunoblot, immunoprecipitation, immunohistochemistry, immunocytochemistry, luminescent immunoassay (LIA), fluorescent immunoassay (FIA), and radioimmunoassay. Assays may be used to obtain qualitative and/or quantitative results. Specific details of suitable methods for both qualitative and quantitative sample assays are described in standard references, including E. Harlow & D. Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, 1988; F. Breitling & S. Diibel, Recombinant Antibodies, John Wiley & Sons, New York, 1999; H. Zola, Monoclonal Antibodies: Preparation and Use of Monoclonal Antibodies and Engineered Antibody Derivatives, Basics: From Background to Bench, BIOS Scientific Publishers, 2000; B.K.C. Lo, Antibody Engineering: Methods and Protocols, Methods in Molecular Biology, Humana Press, 2003; F.M. Ausubel et al., Eds., Short Protocols in Molecular Biology, Current Protocols, Wiley, 2002; S. Klussman, Ed., The Aptamer Handbook: Functional Oligonucleotides and Their Applications, Wiley, 2006; Ormerod, M.G., Flow Cytometry: a practical approach, Oxford University Press, 2000; Givan, A.L., Flow Cytometry: first principles, Wiley, New York, 2001; Gorczyca, W., Flow Cytometry in Neoplastic Hematology: morphologic-immunophenotypic correlation, Taylor & Francis, 2006; Crowther, J.R., The ELISA Guidebook (Methods in Molecular Biology), Humana Press, 2000; Wild, D., The Immunoassay Handbook, 3rd Edition, Elsevier Science, 2005, and J. Sambrook and D.W. Russell, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, 3rd ed., 2001.

[0077] Protocols to assay and analyze a sample for TRAIL to detect an effect of a pharmaceutical composition are described in U.S. Patent 8,673,923 to Wafik S. El-deiry et al.

[0078] TRAIL assays may be used to monitor a subject. For example, a sample may be obtained from a subject

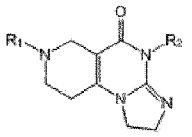
before treatment with a pharmaceutical composition and at one or more times during and/or following treatment to assess the treatment's effectiveness. A sample may be obtained from a subject at various times to assess the course or progress of disease or healing. Death receptors from circulating tumor cells may be assayed to see if a treatment described here increases the amount or type of death receptors.

[0079] Cancers treated using methods and compositions described herein are characterized by abnormal cell proliferation including pre-neoplastic hyperproliferation, cancer *in-situ,* neoplasms and metastasis. Methods and compositions described herein can be used for prophylaxis, as well as amelioration of cancer signs or symptoms. "Treatment" of a cancer in a subject includes: preventing, inhibiting or ameliorating cancer in the subject, such as slowing cancer progression or reducing or ameliorating a cancer sign or symptom. Examples of cancers include breast cancer, CNS cancers, colon cancer, ovarian cancer, prostate cancer, leukemia, lung cancer, and lymphoma.

III. COMPOUNDS OF FORMULA (10) AND SALTS THEREOF

[0080] Provided herein are compounds and salts of formula **(10)** and methods of making them. Persons skilled in the art will understand that the general principles and concepts described here in conjunction with ONC201 (compound **(1))** and its salts, including principles and concepts related to methods and pharmaceutical compositions, apply with equal force to compounds of formula **(10)** and salts thereof.

[0081] Provided herein are compounds of formula **(10):**



(10)

wherein R_1 and R_2 are independently selected from H, alkyl, aryl, cycloalkyl, cycloalkylalkyl, heterocycloalkyl, heterocycloalkylalkyl, heteroaryl, arylalkyl, heteroarylalkyl, alkoxyalkyl, alkoxycarbonyl, aralkoxy, aralkylthio, and acyl radicals. R_1 may be CH_2Ph and R_2 may be CH_2-(2-CH_3-Ph) (i.e., ONC201). R_1 may be CH_2Ph and R_2 may be CH_2-(2,4-di F-Ph) (i.e., ONC206). R_1 may be CH_2Ph and R_2 may be CH_2-(4-CF_3-Ph) (i.e., ONC212). R_1 may be CH_2Ph and R_2 may be CH_2-(3,4-di F-Ph) (i.e., ONC213). R_1 may be CH_2 (3,4-di-Cl-Ph and R_2 may be CH_2-(4-CF_3-Ph) (i.e., ONC234). R_1 may be CH_2-3-thienyl and R_2 may be CH_2-(4-CF_3-Ph) (i.e., ONC236).

[0082] R_1 and R_2 may be independently selected from the group consisting of H, C_{1-4}alkyl, C_{1-4}alkylphenyl, C_{1-4}alkylphenylketone, C_{1-4}benzyl-piperazine, C_{1-4}alkylthienyl, C_{1-4}alkylpyridinyl, C_{1-4}alkylisoxazolidinyl, C_{1-4}alkylmorpholinyl, C_{1-4}alkylthiazolyl, and C_{1-4}alkylpyrazinyl wherein C_{1-4}alkyl, C_{1-4}alkylphenyl, C_{1-4}alkylphenylketone, C_{1-4}benzylpiperazine, C_{1-4}alkylthienyl, C_{1-4}alkylpyridinyl, C_{1-4}alkylisoxazolidinyl, C_{1-4}alkylmorpholinyl, C_{1-4}alkylthiazolyl, and C_{1-4}alkylpyrazinyl are optionally substituted with C_{1-4}alkyl, C_{1-4}alkoxyl, hydroxyl, perhalogenated C_{1-4}alkyl, or halo. R_1 and/or R_2 may be a substituted or unsubstituted, arylalkyl or heteroarylalkyl. The heteroarylalkyl may be selected from C_{1-4}alkylpyrrolyl, C_{1-4}alkylfuryl, C_{1-4}alkylpyridyl, C_{1-4}alkyl-1,2,4-thiadiazolyl, C_{1-4}alkylpyrimidyl, C_{1-4}alkylthienyl, C_{1-4}alkylisothiazolyl, C_{1-4}alkylimidazolyl, C_{1-4}alkyltetrazolyl, C_{1-4}alkylpyrazinyl, C_{1-4}alkylpyrimidyl, C_{1-4}alkylquinolyl, C_{1-4}alkylisoquinolyl, C_{1-4}alkylthiophenyl, C_{1-4}alkylbenzothienyl, C_{1-4}alkylisobenzofuryl, C_{1-4}alkylpyrazolyl, C_{1-4}alkylindolyl, C_{1-4}alkylpurinyl, C_{1-4}alkylcarbazolyl, C_{1-4}alkylbenzimidazolyl, and C_{1-4}alkylisoxazolyl.

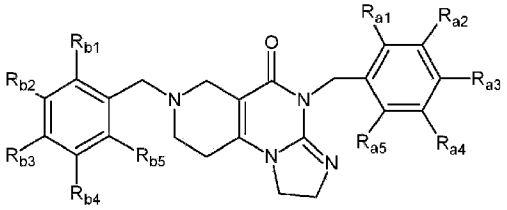
[0083] R_1 and/or R_2 may be a benzyl optionally substituted with one or more of the following substituents on the benzyl ring: X, $-CH_3$, $-NO_2$, $-OCH_3$, -CN, $-CXH_2$, $-CX_2H$, C_2-C_4 alkyl, $-CX_3$, $-CH_2(CX_3)$, $-CH(CX_3)_2$, $-C(CX_3)_3$, $-C_pX_{2p+1}$, $-OCX_3$, $-OC_pH_{2p+1}$, $-OC_pX_{2p+1}$, OR^m, SR^m, NR^mR^n, $NR^mC(O)R^n$, SOR^m, SO_2R^m, $C(O)R^m$, and $C(O)OR^m$; R^m and R^n are independently selected from H or a C_1-C_4 alkyl; and where p is an integer from 2 to 20 and X is a halogen, including F, Cl, Br, or I; preferably, F, Cl, or Br; more preferably, F or Cl.

[0084] R_1 may be selected from H, CH_3, CH_2Ph, CH_2-(4-CF_3-Ph), CH_2-(4-F-Ph), CH_2-(4-Cl-Ph), CH_2-(OCH_3-Ph), CH_2-((2-Cl)-Ph), CH_2-(2-thienyl), CH_2-(3-thienyl), CH_2-2-pyridinyl, CH_2-4-methyl-2-thiazolyl, CH_2-2-pyrazinyl, CH_2CH_2Ph, CH_2CH_2(4-N-benzylpiperazine), CH_2-(2,4-di F-Ph), CH_2-(3,4-di Cl-Ph), CH_2-(3,4-di F-Ph), CH_2-(3,5-di F-Ph), CH_2-((2-CH_3)-Ph), $CH_2CH(OH)Ph$, (4-F-Ph)-4-oxobutyl, $CH_2CH_2NHCOOC(CH_3)_3$, $CH_2CH_2CH_2NH_2$, and $CD_2C_6D_5$. R_2 may be selected from H, CH_3, CH_2Ph, CH_2-(4-CF_3-Ph), CH_2-((2-Cl)-Ph), CH_2-((2-F)-Ph), CH_2-(2-thienyl), CH_2CH_2Ph, CH_2CH_2(4-N-benzylpiperazine), CH_2-(2,4-di F-Ph), CH_2-(2,4-di Cl-Ph), CH_2-(3,4-di Cl-Ph), CH_2-(3,4-di F-Ph), CH_2-(3,5-di F-Ph), CH_2-((2-CH_3)-Ph), CH_2(2-CH_3, 4-F-Ph), CH_2-((4-OCH_3)-Ph), CH_2-(3-pyridinyl), CH_2-(3-isoxazolidinyl), CH_2CH_2-(4-morpholinyl), CH_2-(2-F, 4-CF_3-Ph), $CH_2CH(OH)Ph$, $(CH_2)_3CO$-4F-Ph, , (4-F-Ph)-4-oxobutyl, $CH_2CH_2NHCOOC(CH_3)_3$, $CH_2CH_2CH_2NH_2$, and $CD_2C_6D_5$.

[0085] R_1 may be H. R_1 may be a substituted or unsubstituted arylalkyl, *e.g.*, a benzyl (CH_2Ph) or phenylethyl group. The arylalkyl may be substituted with C_{1-4}alkyl, C_{1-4}alkoxyl, hydroxyl, perhalogenated C_{1-4}alkyl, or halo.

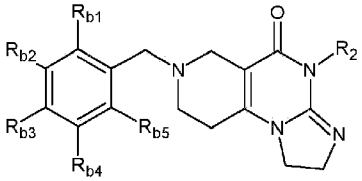
[0086] R_2 may be a substituted or an unsubstituted arylalkyl, *e.g.*, benzyl or phenylethyl. The arylalkyl may be substituted with C_{1-4}alkyl, C_{1-4}alkoxyl, hydroxyl, perhalogenated C_{1-4}alkyl, or halo. The arylalkyl may be substituted with one or more substituents selected from halo, CH_3, CF_3 or OCH_3. R_2 may be a substituted or an unsubstituted heterocycloalkylalkyl, *e.g.*, piperazinylalkyl or morpholinoalkyl; or a substituted or an unsubstituted heteroarylalkyl, *e.g.*, pyridylmethyl or isoxazolidinylmethyl. The heterocycloalkylalkyl or heteroarylalkyl may be substituted with C_{1-4}alkyl, C_{1-4}alkoxyl, hydroxyl, perhalogenated C_{1-4}alkyl, or halo, for example the heterocycloalkylalkyl or heteroarylalkyl may be substituted with at least one substituent selected from halo, CH_3, CF_3 or OCH_3.

[0087] Compound **(10)** may have the structure of formula **(80):**



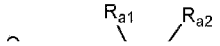
(80), wherein R_{a1}, R_{a2}, R_{a3}, R_{a4}, R_{a5}, R_{b1}, R_{b2}, R_{b3}, R_{b4}, and R_{b5} are each independently selected from the group consisting of H, X, -CH_3, -NO_2, -OCH_3, -CN, -CXH_2, -CX_2H, C_2-C_4 alkyl, -CX_3, -$CH_2(CX_3)$, -$CH(CX_3)_2$, -$C(CX_3)_3$, -C_pX_{2p+1}, -OCX_3, -OC_pH_{2p+1}, -OC_pX_{2p+1}, OR^m, SR^m, NR^mR^n, $NR^mC(O)R^n$, SOR^m, SO_2R^m, $C(O)R^m$, and $C(O)OR^m$; R^m and R^n are independently selected from H or a C_1-C_4 alkyl; and where p is an integer from 2 to 20 and X is a halogen.

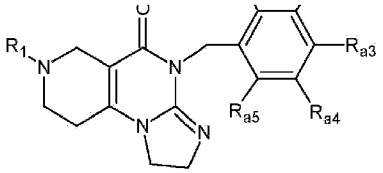
[0088] Compound **(10)** may have the structure of formula **(90)**



(90), wherein R_2 is as defined above, and wherein R_{b1}, R_{b2}, R_{b3}, R_{b4}, and R_{b5} are each independently selected from the group consisting of H, X, -CH_3, -NO_2, -OCH_3, -CN, -CXH_2, -CX_2H, C_{2-4} alkyl, -CX_3, -$CH_2(CX_3)$, -$CH(CX_3)_2$, -$C(CX_3)_3$, -C_pX_{2p+1}, -OCX_3, -OC_pH_{2p+1}, -OC_pX_{2p+1}, OR^m, SR^m, NR^mR^n, $NR^mC(O)R^n$, SOR^m, SO_2R^m, $C(O)R^m$, and $C(O)OR^m$; R^m and R^n are independently selected from H or a C_{1-4}alkyl; and where p is an integer from 2 to 20 and X is a halogen.

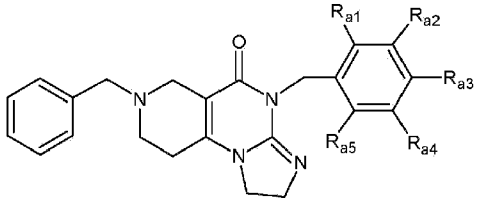
[0089] Compound **(10)** may have the structure of formula **(40)**





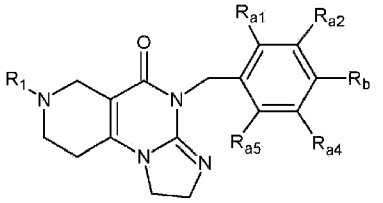
(40), where R_1 is as defined above, and where R_{a1}, R_{a2}, R_{a3}, R_{a4}, and R_{a5} are each independently selected from the group consisting of H, X, - CH_3, -NO_2, -OCH_3, -CN, -CXH_2, -CX_2H, C_{2-4}alkyl, -CX_3, -$CH_2(CX_3)$, -$CH(CX_3)_2$, -$C(CX_3)_3$, -C_pX_{2p+1}, -OCX_3, -OC_pH_{2p+1}, -OC_pX_{2p+1}, OR^m, SR^m, NR^mR^n, $NR^mC(O)R^n$, SOR^m, SO_2R^m, $C(O)R^m$, and $C(O)OR^m$; R^m and R^n are independently selected from H or a C_{1-4} alkyl; p is an integer from 2 to 20; and X is a halogen. R_1 may be Hor a substituted or unsubstituted arylalkyl, such as benzyl or phenylethyl. The arylalkyl may be substituted with C_{1-4}alkyl, C_{1-4}alkoxyl, hydroxyl, perhalogenated C_{1-4}alkyl, or halo. The benzyl may be substituted with one or more halo. The benzyl may be substituted with one or more substituents selected from halo, CH_3, CF_3, and OCH_3. For example, the benzyl is substituted with one halo, *e.g.*, F at an ortho or para position or the benzyl is substituted with two halogen, *e.g.*, F at both meta positions.

[0090] Compound **(40)** may have the structure of compound **(45):**



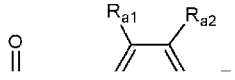
(45), where R_{a1}, R_{a2}, R_{a3}, R_{a4}, and R_{a5} are as defined above. The benzyl may be substituted with one or more halogens. The benzyl may be substituted with one or more substituents selected from halo, CH_3, CF_3, and OCH_3. R_{a1} or R_{a5} may be a halo, *e.g.,* F. Both R_{a2} and R_{a3} may be halo, *e.g.,* F.

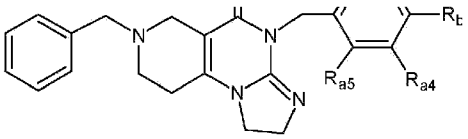
[0091] Compound **(10)** may have the structure of compound **(50)**



(50), wherein R_1 is as defined above, and wherein R_b is selected from the group consisting of H, X, -CH_3, -NO_2, -OCH_3, -CN, -CXH_2, -CX_2H, C_{2-4}alkyl, -CX_3, -$CH_2(CX_3)$, -$CH(CX_3)_2$, -$C(CX_3)_3$, -C_pX_{2p+1}, -OCX_3, -OC_pH_{2p+1}, -OC_pX_{2p+1}, OR^m, SR^m, NR^mR^n, $NR^mC(O)R^n$, SOR^m, SO_2R^m, $C(O)R^m$, and $C(O)OR^m$; R^m and R^n are independently selected from H or C_{1-4}alkyl; and where p is an integer from 2 to 20 and X is a halogen, and wherein R_{a1}, R_{a2}, R_{a4}, and R_{a5} are each independently selected from the group consisting of H, X, -CH_3, -NO_2, -OCH_3, -CN, -CXH_2, -CX_2H, C_{2-4}alkyl, -CX_3, -$CH_2(CX_3)$, - $CH(CX_3)_2$, -$C(CX_3)_3$, -C_pX_{2p+1}, -OCX_3, -OC_pH_{2p+1}, -OC_pX_{2p+1}, OR^m, SR^m, NR^mR^n, $NR^mC(O)R^n$, SOR^m, SO_2R^m, $C(O)R^m$, and $C(O)OR^m$; R^m and R^n are independently selected from H or C_{1-4} alkyl; and where p is an integer from 2 to 20 and X is a halogen. R_1 may be H. R_1 may bea substituted or unsubstituted arylalkyl, such as a benzyl or phenylethyl group. The arylalkyl may be substituted with C_{1-4}alkyl, C_{1-4}alkoxyl, hydroxyl, perhalogenated C_{1-4}alkyl, or halo. R_b may be selected from halo, CH_3, CF_3, and OCH_3. One or more of R_{a1}, R_{a2}, R_{a4}, and R_{a5} may be selected from halo, CH_3, CF_3, and OCH_3. R_{a1}, R_{a2}, R_{a4}, and R_{a5} may be H, and R_b may be selected from halo, CH_3, CF_3, and OCH_3. R_b may be a halogen, e.g., F, and R_{a1} may be CH_3. R_b may be For Cl, and R_{a2} may be For Cl. R_b may be CF_3. R_b may be OCH_3. R_b and R_{a1} may be Cl.

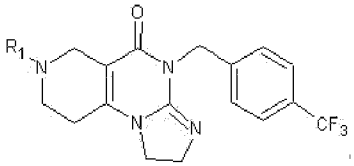
[0092] Compound **(50)** may have the structure of compound **(55):**





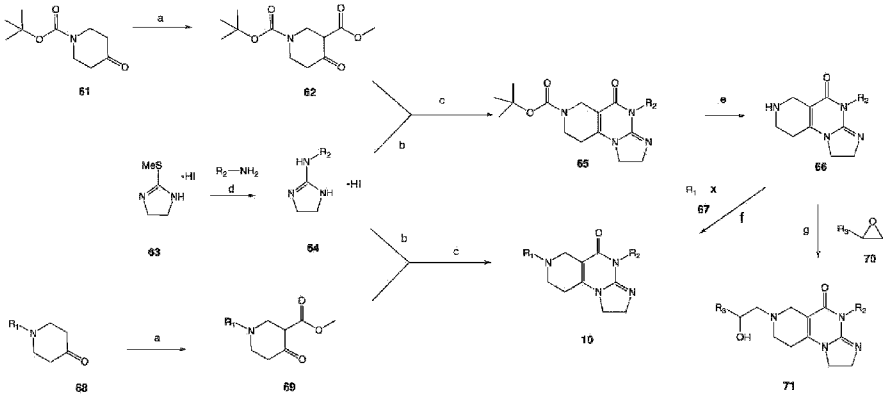
(55), where R_{a1}, R_{a2}, R_{a4}, R_{a5}, and R_b are as defined above. R_b may be selected from halo, CH_3, CF_3, and OCH_3. One or more of R_{a1}, R_{a2}, R_{a4}, and R_{a5} may be selected from halo, CH_3, CF_3, and OCH_3. R_{a1}, R_{a2}, R_{a4}, and R_{a5} may be H, and R_b may be selected from halo, CH_3, CF_3, and OCH_3. R_b may be halo, e.g., F, and R_{a1} is CH_3. R_b may be F or Cl, and R_{a2} is F or Cl. R_b may be CF_3. R_b may be OCH_3. R_b and R_{a1} may beCl.

[0093] Compound **(10)** may havethe structure of compound **(60)**



(60). R_1 may be H. R_1 may be a substituted or unsubstituted arylalkyl, such as benzyl or phenylethyl. R_1 may be a substituted or unsubstituted heterocycloalkylalkyl or a substituted or unsubstituted heteroarylalkyl, such as CH_2-(2-thienyl), CH_2-(3-thienyl), CH_2-4-methyl-2-thiazolyl, CH_2-2-pyrazinyl, CH_2CH_2(4-N-benzyl-piperazine), CH_2-(3-isoxazolidinyl), CH_2-2-pyridinyl, CH_2-3-pyridinyl, and CH_2CH_2-(4-morpholinyl). The arylalkyl may be substituted with C_{1-4}alkyl, C_{1-4}alkoxyl, hydroxyl, perhalogenated C_{1-4}alkyl, or halo. The benzyl may be substituted with one or more halogens. The benzyl may besubstituted with one or more substituents selected from halo (*e.g.*, F), CH_3, CF_3, and OCH_3. The benzyl may be substituted at the para position with a halo, CH_3, CF_3, or OCH_3 substituent. R_1 may be fluorophenyloxobutyl or hydroxyphenylethyl.

[0094] Scheme 3 illustrates the synthesis of compounds of formula **(10):**



Methods: a. NaH, dimethyl carbonate, toluene, 80°C 4 h; b. 1N NaOH/CH_2Cl_2 to convert to free base, then heat in dioxane 70 °C; c. 1-butanol/reflux 3-6 h (Dean-Stark trap) PPTS; d. dioxane 70 °C; e. HCl in dioxane -25 °C - RT to give HCl salt ; f. Na_2CO_3, DIEA 80 °C; g. NaOH/CH_2Cl_2 to make free base, then MeOH reflux, 3-5 h

Scheme 3

[0095] Compounds of formula **(10)** (i.e., imipridones) are synthesized starting from a substituted piperidone, which is converted by reaction with a substitued aminoimidazoline to give the core compound **(10).** There are two routes, one in which the R_1 substituent is present in the piperidone (*e.g.*, **68).** In that route, **(68)** is acylated with dimethyl carbonate using sodium hydride in toluene at 80 °C to form piperidone ester **(69).** Commercially available methylthioimidazoline HI salt **(63)** is reacted with an amine in dioxane at 70 °C to afford the R_2-substituted aminoimidazoline **(64)** as its HI salt. Direct reaction of **(64)** with piperidone ester **(69)** in 1-butanol at reflux with removal of water via a Dean-Stark trap over 3-6 h gives the tricylic compound **(10).** In a variant of this scheme, N-BOC protected piperidone **(61)** is converted by the same methods to BOC protected compound **(65),** which is treated with HCl in dioxane to remove the BOC group and then converted to the free base of **(66)** with 1N NaOH with extraction with methylene chloride. Subsequent treatment of **(66)** with a halide **(67)** or epoxide **(70)** affords desired compound **(10).**

[0096] Crude products may be purified by column chromatography eluting with methylene chloride:methanol or by HPLC using acetonitrile:TFA:H_2O to produce final products as either free bases or as TFA salts. Treatment of free bases with HCl in dioxane or lyophilization of TFA salts generates products **(10)** as HCl or TFA salts. Alternatively, the free base may be treated with another inorganic or organic acid to form other salts, generally selected from those known to be pharmaceutically acceptable. Salts of compound **(10)** are usually solids and examples have been crystallized from ethanol or other solvents to give high quality crystals. The tricyclic structure has been definitively confirmed in the case of compound **(1)** by an X-ray crystal structure and NMR.

[0097] Compounds described herein can be used, with or without an aminoalkyl linker (*e.g.*, compound **(33)),** to identify molecules (*e.g.*, proteins) that interact with them in a cellular context. Expression of these binding targets may be used to predict response to imipridones or analogs thereof (i.e. serve as biomarkers). These compounds can also be used to screen for structurally unrelated molecules using competition assays known in the art to identify drugs able to outcompete the target interaction with a higher affinity. In addition, these molecules may have improved drug properties or allow additional applications by altering drug properties including safety, potency, pharmacokinetics, biodistribution, or metabolism.

TABLE 1:EXAMPLES OF COMPOUNDS OF FORMULA (10)

No.	ONC Number	R_1	R_2
1	ONC201	CH_2Ph	CH_2-((2-CH_3)-Ph)
13*		CH_2Ph	CH_3
14*	ONC202	CH_2Ph	CH_2-((2-Cl)-Ph)
15*	ONC203	CH_2Ph	CH_2-(2-thienyl)
16*	ONC204	CH_2Ph	CH_2CH_2Ph
17*	ONC205	CH_2Ph	CH_2CH_2(4-N-benzyl-piperazine)
18*	ONC206	CH_2Ph	CH_2-(2,4-di F-Ph)
19*	ONC207	H	CH_2-((2-CH_3)-Ph)
20*	ONC208	CH_3	CH_2-((2-CH_3)-Ph)
21*	ONC209	CH_2CH_2Ph	CH_2-((2-CH_3)-Ph)
22*		CH_2CH_2-(4-N-benzyl-piperizine)	CH_2-((2-CH_3)-Ph)
23*		$CH_2CHOHPh$	CH_2-((2-CH_3)-Ph)
24*		$(CH_2)_3CO$-4F-Ph	CH_2-((2-CH_3)-Ph)
32*	ONC215	$CH_2CH_2NHCOOC(CH_3)_3$	CH_2-((2-CH_3)-Ph)
33*	ONC216	$CH_2CH_2CH_2NH_2$	CH_2-((2-CH_3)-Ph)
41*	ONC210	CH_2Ph	CH_2-(3,5-di F-Ph)
51*	ONC211	CH_2Ph	CH_2-(3,4-di Cl-Ph)
52*	ONC212	CH_2Ph	CH_2-(4-CF_3-Ph)
53*	ONC213	CH_2Ph	CH_2-(3,4-di F-Ph)
54*	ONC214	$CD_2C_6D_5$	CH_2-((2-CH_3)-Ph)
43*	ONC217	CH_2Ph	CH_2-(2-F-Ph)
55*	ONC218	CH_2Ph	CH_2(2-CH_3, 4-F-Ph)
56*	ONC219	CH_2Ph	CH_2-(2,4-di Cl-Ph)
57*	ONC220	CH_2Ph	CH_2-((4-OCH_3)-Ph)
34*	ONC226	CH_2Ph	CH_2-(3-pyridinyl)

No.	ONC Number	R_1	R_2
35*	ONC222	CH_2Ph	CH_2-(3-isoxazolidinyl)
36*	ONC224	CH_2Ph	CH_2CH_2-(4-morpholinyl)
37*	ONC223	CH_2Ph	CH_2-(4-CH_3-Ph)
38*	ONC221	H	CH_2-(4-CF_3-Ph)
73*	ONC227	CH_2-(4-CF_3-Ph)	CH_2-(4-CF_3-Ph)
72*	ONC225	CH_2Ph	CH_2-(2-F, 4-CF_3-Ph)
74*	ONC228	CH_2-(4-F-Ph)	CH_2-(4-CF_3-Ph)
75*	ONC229	CH_2-(OCH_3-Ph)	CH_2-(4-CF_3-Ph)
76*	ONC230	(4-F-Ph)-4-oxobutyl	CH_2-(4-CF_3-Ph)
77*	ONC231	CH_2-3-pyridyl	CH_2-(4-CF_3-Ph)
78*	ONC232	CH_2-4-methyl-2-thiazolyl	CH_2-(4-CF_3-Ph)
79*	ONC233	CH_2-2-pyrazinyl	CH_2-(4-CF_3-Ph)
81*	ONC234	CH_2-(3,4-di Cl-Ph)	CH_2-(4-CF_3-Ph)
82*	ONC235	CH_2-(4-Cl-Ph)	CH_2-(4-CF_3-Ph)
83*	ONC236	CH_2-3-thienyl	CH_2-(4-CF_3-Ph)
84*	ONC237	$CH_2CH(OH)Ph$	CH_2-(4-CF_3-Ph)
* reference compound			

IV. ASSESSING SENSITIVITY AND EFFICACY OF TREATMENT REGIMENS

[0098] Measuring expression, gene mutation, or gene copy number of a dopamine receptor or another G protein-coupled receptor (GPCR) may be used to predict response or sensitivity to a method of treatment described herein and to identify subjects likely to be responsive to a method of treatment described herein, such as treatment with a compound of formula **(10),** a pharmaceutically acceptable salt thereof, or an analog thereof. Provided herein are methods of identifying whether a subject having a condition is likely to be responsive to a treatment regimen described herein. The methods may comprise (i) obtaining a biological sample from the subject; (ii) measuring expression levels of at least one dopamine receptor or G protein-coupled receptor (GPCR) in the sample; (iii) comparing the levels measured in the sample to those for a pre-determined standard; and (iv) determining whether the subject is likely to be responsive to the treatment regimen, based on the levels measured in the sample to those for the pre-determined standard. The step of measuring an expression level of a dopamine receptor or GPCR in the sample may include the steps of (i) contacting the sample with an antibody or antigen-binding fragment that specifically binds to the receptor to form a complex of the antibody or antigen-binding fragment with the receptor; and (ii) measuring the amount of the complex. The subject has, or is at risk of having, cancer. For example, the subject may have or be at risk of having a neuro-oncology disease, a neuroendocrine tumor, meningioma, ependymoma, glioma, neuroblastoma, or diffuse intrinsic pontine glioma. The subject may have, or may be at risk of having, a psychiatric disorder, such as psychosis, bipolar disorder, and major depressive disorder. The subject may have, or may be at risk of having, an infection, such as a bacterial infection(for example, a gram-negative bacterial infection or a gram-positive bacterial infection). The bacterial infection may be an infection of a bacteria selected from the group consisting of *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter* species. The gram-positive bacterial infection may be a *Staphylococcus* infection, such as an *S. aureus* infection *(e.g.,* a methicillin-resistant *S. aureus* (MRSA) infection). The treatment regimen may comprise administering an effective amount of a therapeutic, such as a compound of formula **(10),** a pharmaceutically acceptable salt thereof, or an analog thereof.

The dopamine receptor may be from the D2-like family of dopamine receptors. The dopamine receptor may be DRD2, DRD3, or DRD4. The dopamine receptor may be DRD2, DRD3, or both. The GPCR may be a Class A GPCR. The GPCR may be GPR132. The GPCR may be selected from the group consisting of GPR132, GPR91, MTNR1A, GPR162, GPR137, BAI3, LGR4, PTGIR, CXCR7, and combinations thereof. The dopamine receptor may be DRD5, the treatment regimen may comprise administering an effective amount of a therapeutic, such as a compound of formula **(10)** or a pharmaceutically acceptable salt thereof, and an increased level of expression of DRD5 measured in the sample relative to the pre-determined standard indicates that the subject is or is not likely to be responsive to the treatment regimen.

[0099] Also provided herein are methods of assessing the effectiveness of a treatment regimen described herein, monitoring, or providing a prognosis for a subject with a condition. The methods may comprise (i) obtaining a biological sample from the subject; (ii) measuring expression levels of at least one dopamine receptor or G protein-coupled receptor (GPCR) in the sample; (iii) comparing the levels measured in the sample to those for a pre-determined standard; and (iv) determining a prognosis or determining whether the subject is responsive to the treatment regimen, based on the levels measured in the sample to those for the pre-determined standard. The step of measuring an expression level of a dopamine receptor or GPCR in the sample may include the steps of (i) contacting the sample with an antibody or antigen-binding fragment that specifically binds to the receptor to form a complex of the antibody or antigen-binding fragment with the receptor; and (ii) measuring the amount of the complex. The methods may comprise (i) obtaining a biological sample from the subject; (ii) measuring gene copy number or mutations in at least one dopamine receptor in the sample; (iii) comparing the copy number measured or mutations found in the sample to those for a pre-determined standard; and (iv) determining whether the subject is responsive to the treatment regimen, based on the copy number measured or mutations found in the sample to those for the pre-determined standard. The subject has, or is at risk of having, cancer. The cancer may be a neuro-oncology disease, or a neuroendocrine tumor. The cancer may be selected from the group consisting of meningioma, ependymoma, glioma, neuroblastoma, and diffuse intrinsic pontine glioma. The subject may have, or may be at risk of having, a psychiatric disorder, such as psychosis, bipolar disorder, and major depressive disorder. The subject may have, or may be at risk of having, an infection, such as a bacterial infection,for example, a gram-negative bacterial infectionor a gram-positive bacterial infection. The bacterial infection may be an infection of a bacteria selected from the group consisting of *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter* species. The gram-positive bacterial infection may be a *Staphylococcus* infection, such as an *S. aureus* infection *(e.g.,* a methicillin-resistant *S. aureus* (MRSA) infection). The treatment regimen may comprise administering an effective amount of a therapeutic, such as a compound of formula **(10),** a pharmaceutically acceptable salt thereof, or an analog thereof. The dopamine receptor may be selected from DRD2, DRD2S, DRD2L, and DRD3. The dopamine receptor may be from the D2-like family of dopamine receptors, or the D1-like family of dopamine receptors. The dopamine receptor may be DRD1, DRD2, DRD3, DRD4. , or DRD5. The dopamine receptor may be DRD2, DRD3, or both. The GPCR may be a Class A GPCR. The GPCR may be GPR132. The GPCR may be selected from the group consisting of GPR132, GPR91, MTNR1A, GPR162, GPR137, BAI3, LGR4, PTGIR, CXCR7, and combinations thereof.

[0100] The dopamine receptor may be DRD5, the treatment regimen may comprise administering an effective amount of a compound of formula **(10)** or a pharmaceutically acceptable salt thereof, and an increased level of expression of DRD5 measured in the sample relative to the pre-determined standard may indicate that the treatment regimen is or is not effective. The dopamine receptor may be DRD5, the treatment regimen may comprise administering an effective amount of a therapeutic, such as a compound of formula **(10)** or a pharmaceutically acceptable salt thereof, and mutation in the DRD5 gene measured in the sample may indicate that the treatment regimen is or is not effective. The dopamine receptor may be DRD5, the treatment regimen may comprise administering an effective amount of a therapeutic, such as a compound of formula **(10)** or a pharmaceutically acceptable salt thereof, and the misense mutation Q366R in the DRD5 gene measured in the sample may indicate that the treatment regimen is or is not effective.

[0101] Also provided herein are methods of identifying whether a subject having a condition is likely to be responsive to a treatment regimen described herein. The methods may comprise (i) obtaining a biological sample from the subject; (ii) measuring gene copy number or mutations in at least one dopamine receptor in the sample;

(iii) comparing the copy number measured or mutations found in the sample to those for a pre-determined standard; and (iv) determining whether the subject is likely to be responsive to the treatment regimen, based on the copy number measured or mutations found in the sample to those for the pre-determined standard. The subject has, or is at risk of having, cancer. The cancer may be a neuro-oncology disease or a neuroendocrine tumor. The cancer may be selected from the group consisting of meningioma, ependymoma, glioma, neuroblastoma, and diffuse intrinsic pontine glioma. The subject may have, or may be at risk of having, a psychiatric disorder, such as psychosis, schizophrenia, bipolar disorder, and major depressive disorder. The subject may have, or may be at risk of having, an infection, such as a bacterial infection. The infection may be a gram-negative bacterial infectionor a gram-positive bacterial infection. The bacterial infection may be an infection of a bacteria selected from the group consisting of *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter* species. The gram-positive bacterial infection may be a *Staphylococcus* infection, such as an *S. aureus* infection *(e.g.,* a methicillin-resistant *S. aureus* (MRSA) infection). The treatment regimen may comprise administering an effective amount of a therapeutic, such as a compound of formula **(10),** a pharmaceutically acceptable salt thereof, or an analog thereof. The dopamine receptor may be from the D2-like family of dopamine receptors. The dopamine receptor may be DRD1, DRD2, DRD3, DRD4, or DRD5. The dopamine receptor may be DRD2, DRD3, or both. The dopamine receptor may be DRD5, the treatment regimen may comprise administering an effective amount of a therapeutic, such as a compound of formula **(10)** or a pharmaceutically acceptable salt thereof, and mutation in the DRD5 gene measured in the sample may indicate that the subject is or is not likely to be responsive to the treatment regimen. The dopamine receptor may be DRD5, the treatment regimen may comprise administering an effective amount of a therapeutic, such as a compound of formula **(10)** or a pharmaceutically acceptable salt thereof, and the misense mutation Q366R in the DRD5 gene measured in the sample may indicate that the subject is or is not likely to be responsive to the treatment regimen.

[0102] In addition, measuring expression, post-translational modifications, or activity levels of or mutations in eIF2-α, ATF4, CHOP, DRS, or cleaved or total cytokeratin 18 may be used to predict response or sensitivity to a method of treatment described herein and to identify subjects likely to be responsive to a method of treatment described herein, such as treatment with a compound of formula **(10),** a pharmaceutically acceptable salt thereof, or an analog thereof. In addition, measuring expression, post-translational modifications, or activity levels of or mutations in eIF2-α, ATF4, CHOP, DRS, or cleaved or total cytokeratin 18 can be used to assess the effectiveness of or monitor a method of treatment described herein. Furthermore, measuring expression, post-translational modifications, or activity levels of or mutations in eIF2-α, ATF4, CHOP, DRS, or cleaved or total cytokeratin 18 can be used to screen *in vivo, in vitro,* or *in silico* for structurally unrelated anti-cancer molecules. For example, competition and other assays known in the art may be used to identify drugs able to outcompete the target interaction with a higher affinity to compare changes in those levels to the respective changes produced by a compound of formula **(10)** or an analog thereof. Assays can also be performed on living mammalian cells, which more closely approximate the effects of a particular serum level of drug in the body, or on microsomal extracts prepared from cultured cell lines.

[0103] The subject has, or is at risk of having, cancer. The treatment regimen may comprise administering an effective amount of an imipridone, such as ONC201, or an analog thereof.The treatment regimen comprises administering an effective amount of ONC201. The treatment regimen may comprise administering an effective amount of a compound of formula **(10).** The compound of formula **(10)** may be a compound of formula **(40),** *e.g.,* a compound of formula **(45);** a compound of formula **(50),** *e.g.,* a compound formula **(55),** a compound of formula **(80),** or a compound of formula **(90).** The compound of formula **(10)** may be a compound of formula **(60).** Analogs of compound **(1)** may have a structure selected from the structures of formula **(25),** formula **(26),** formula **(27),** formula **(28),** formula **(29),** formula **(30),** or formula **(31).**

[0104] Levels for a pre-determined standard can be, e.g., the average or median levels measured in samples from subjects. The levels for a pre-determined standard can be measured under the same or substantially similar experimental conditions as in measuring a sample from a subject. The levels for the pre-determined standard may be obtained from subjects who are responsive to treatment with an imipridone, such as ONC201, or an analog thereof. The pre-determined standard may be obtained from subjects who are responsive to treatment with the

compound, and if the levels in a sample from a subject are similar to those in the standard, then the subject can be classified as likely to be responsive to treatment. The levels for the pre-determined standard may be obtained from subjects who are not responsive to treatment with the compound. The pre-determined standard may be obtained from subjects who are not responsive to treatment with the compound, and if the levels in a sample from a subject are different *(e.g.,* up- or down-regulated) than in the pre-determined standard, then the subject can be classified as likely to be responsive to treatment. The levels for the pre-determined standard may be obtained from normal healthy subjects.

[0105] Immunoassays can be used to assay protein or methylation levels in a sample, including enzyme-linked immunosorbent assay (ELISA), enzyme-linked immunofiltration assay (ELIFA), flow cytometry, immunoblot, immunoprecipitation, immunohistochemistry, immunocytochemistry, luminescent immunoassay (LIA), fluorescent immunoassay (FIA), and radioimmunoassay. m^6A mRNA methylation levels can be obtained by methylated RNA immunoprecipitation (Me-RIP)) or other quantitative biochemical assays known in the art.

[0106] Nucleic acid mutations can be determined by any of a number of known procedures. For example, a biologic sample from an individual can first be obtained. Such biological samples include, but are not limited to, a bodily fluid (such as urine, saliva, plasma, or serum) or a tissue sample (such as a buccal tissue sample or buccal cell). The biologic sample can then be sequenced or scanned using known methods. For example, DNA arrays can be used to analyze at least a portion of the subject's genomic sequence. Furthermore, whole or partial genome sequence information can be used. Such sequences can be determined using standard sequencing methods including chain-termination (Sanger dideoxynucleotide), dye-terminator sequencing, and SOLID™ sequencing (Applied Biosystems). Whole genome sequences can be cut by restriction enzymes or sheared (mechanically) into shorter fragments for sequencing. DNA sequences can also be amplified using known methods such as PCR and vector-based cloning methods (*e.g., Escherichia coli*). At least a portion of a subject's genetic material (*e.g.*, DNA, RNA, mRNA, cDNA, other nucleotide bases or derivatives of these) may be scanned or sequenced using, *e.g.*, conventional DNA sequencers or chip-based technologies, to identify the presence or absence of mutations or copy number variations.

[0107] Provided herein are methods of identifying and treating a subject having a condition and who is likely to be responsive to a treatment regimen described herein. The method may comprise (i) identifying whether a subject having a condition is likely to be responsive to a treatment regimen described herein; and (ii) treating with the treatment regimen a subject determined likely to be responsive to that treatment regimen. The subject has, or is at risk of having, cancer. The treatment regimen may comprise administering an effective amount an imipridone, *e.g.*, ONC201 or an analog thereof. The treatment regimen comprises administering an effective amount of compound **(1).** The treatment regimen may comprise administering an effective amount of a compound of formula **(10).** The compound of formula **(10)** may be a compound of formula **(40),** *e.g.*, a compound of formula **(45);** a compound of formula **(50),** *e.g.,* a compound formula **(55);** a compound of formula **(80);** a compound of formula **(90);** or a compound of formula **(60).** Analogs of compound **(1)** may have a structure selected from the structures of formula **(25),** formula **(26),** formula **(27),** formula **(28),** formula **(29),** formula **(30),** or formula **(31).**

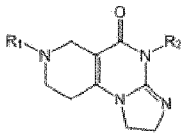
[0108] Levels for a pre-determined standard can be, e.g., the average or median levels measured in samples from subjects. The levels for a pre-determined standard can be measured under the same or substantially similar experimental conditions as in measuring a sample from a subject. The levels for the pre-determined standard may be obtained from subjects who are responsive to treatment with an imipridone, such as ONC201 or an analog thereof. The pre-determined standard may be obtained from subjects who are responsive to treatment with the compound, and if the levels in a sample from a subject are similar to those in the standard, then the subject can be classified as likely to be responsive to treatment. The levels for the pre-determined standard may be obtained from subjects who are not responsive to treatment with the compound. The pre-determined standard may be obtained from subjects who are not responsive to treatment with the compound, and if the levels in a sample from a subject are different (e.g., up- or down-regulated) than those in the pre-determined standard, then the subject can be classified as likely to be responsive to treatment. The levels for the pre-determined standard may be obtained from normal healthy subjects. Immunoassays can be used to assay protein levels in a sample.

[0109] Provided herein are methods of treating and assessing the effectiveness of a treatment in a subject having a condition. The method may comprise (i) treating the subject according to a method of treatment described herein (ii) assessing as decribed herein the effectiveness of the treatment. The subject has, or is at risk of having, cancer. The treatment regimen may comprise administering an effective amount of an imipridone, such as ONC201 or an analog thereof. The treatment regimen may comprise administering an effective amount of compound **(1).** The treatment regimen may comprise administering an effective amount of a compound of formula **(10),** for example, a compound of formula **(40),** *e.g.,* a compound of formula **(45);** a compound of formula **(50),** *e.g.,* a compound formula **(55);** a compound of formula **(80);** a compound of formula **(90);** or a compound of formula **(60).** Analogs of compound **(1)** may have a structure selected from the structures of formula **(25),** formula **(26),** formula **(27),** formula **(28),** formula **(29),** formula **(30),** or formula **(31).**

[0110] Other conditions that may be suitable for the methods described herein include Attention Deficit Disorder; Addiction; Epilepsy; Viral infection; Inflammation; Neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, Huntington's disease, Amyotrophic lateral sclerosis; Cardiovascular diseases such as coronary artery disease, cardiomyopathy, hypertensive heart disease, heart failure, pulmonary heart disease, cardiac dysrhythmias, inflammatory heart disease, endocarditis, inflammatory cardiomegaly, myocarditis, valvular heart disease, cerebrovascular disease, peripheral arterial disease, congenital heart disease, rheumatic heart disease; Diabetes; and light chain amyloidosis.

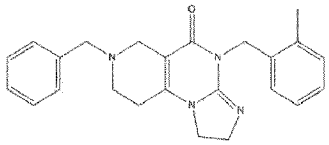
V. COMPOSITIONS

[0111] Pharmaceutical compositions are also provided, comprising compounds of formula **(10):**



(10)

or of formula **(1):**



and their pharmaceutically acceptable salts. The salt may be a pharmaceutically acceptable mono-salt of the compound, or a pharmaceutically acceptable di-salt of the compound. The salt may be a pharmaceutically acceptable mono- or multi-salt (*e.g.*, a di-salt or tri-salt) thereof selected from the group consisting of hydrochloride, hydrobromide, hydrogensulphate, sulfates, phosphates, fumarates, succinates, oxalates and lactates, bisulfates, hydroxyl, tartrate, nitrate, citrate, bitartrate, carbonate, malate, maleate, fumarate sulfonate, methylsulfonate, formate, acetate, and carboxylate. The salt may be a salt selected from the group consisting of p-toluene-sulfonate, benzenesulfonate, citrate, methanesulfonate, oxalate, succinate, tartrate, fumarate and maleate. The salt may be a salt selected from the group consisting of ammonium, sodium, potassium, calcium, magnesium, zinc, lithium, and/or with counter-ions such as methylamino, dimethylamino, diethylamino and triethylamino counter-ions. The salt may be a di-hydrochloride salt or a di-hydrobromide salt.

[0112] Compound **(1)** (ONC201) has the same chemical structure that would be revealed by structural analysis (*e.g.*, NMR, X-ray diffraction) of compound NSC 350625, available from the National Cancer Institute's Developmental Therapeutics Program Repository.

[0113] the pharmaceutical composition may include a di-salt (*e.g.,* a di-hydrochloride salt) of ONC201 or an analog thereof *(e.g.,* an imipridone). Salts (*e.g.*, di-salts or tri-salts) of an ONC201 analog can be prepared from an ONC201 analog, which can be synthesized as described herein, or using standard chemical synthetic methodology known to one of ordinary skill in the art.

[0114] The pharmaceutical composition may include at least one pharmaceutically acceptable carrier. Suitable pharmaceutically acceptable carriers, include, but are not limited to, those in Handbook of Pharmaceutical Excipients, 7th ed., edited by Raymond C. Rowe et al., American Pharmaceutical Association, Washington, USA and Pharmaceutical Press, London; and earlier editions. Exemplary pharmaceutically acceptable carriers, methods for making pharmaceutical compositions and various dosage forms, as well as administration modes are well-known in the art, for example as detailed in Pharmaceutical Dosage Forms: Tablets, edited by Larry L. Augsburger & Stephen W. Hoag., London: Informa Healthcare, 2008; and in L.V. Allen, Jr. et al., Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, 8th ed., Philadelphia, Pa.: Lippincott, Williams & Wilkins, 2004; A.R. Gennaro, Remington: The Science and Practice of Pharmacy, Lippincott Williams & Wilkins, 21st ed., 2005, particularly chapter 89; and J.G. Hardman et al., Goodman & Gilman's The Pharmacological Basis of Therapeutics, McGraw-Hill Professional, 10th ed., 2001.

[0115] Pharmacuetical compositions may be formulated for ocular administration, or topical administration. Pharmaceutical compositions may be formulated as drops, ointments, or liquids. Pharmaceutical compositions may include conventional pharmaceutical carriers such as aqueous, powdery or oily bases, thickeners.

[0116] A pharmaceutical composition may be a formulation for intravenous administrationwhich may comprise a compound of formula **(10)** or a pharmaceutically acceptable salt thereof dissolved in a solvent,such as a solvent comprising water. The intravenous formulation may include the compound or its salt in a concentration of about 0.05, about 0.25, about 0.5, about 2.5, about 5, about 25, or about 50 mg/mL. The intravenous formulation may include the compound or its salt in a concentration of from about 0.05, 0.5, or 5 mg/mL to about 1, 10, or 100 mg/mL. The intravenous formulation may include from about 0.005% 0.05%, or 0.5% to about 0.1%, 1%, or 10% of the compound or its saltsuch as about 0.05%, 0.5%, or 5% of the compound or its salt. The intravenous formulation may include a higher or a lower concentration of the compound or its salt.

[0117] The intravenous formulation may have a pH of about 3. The formulation may be adjusted to pH 3 with a phosphate buffer. The intravenous formulation may include dextrose or sodium chloride. The intravenous formulation may include the compound or its salt in a concentration of about 5 mg/mL and pH 3 and forms a stable solution. The intravenous formulation may include the compound or its salt in a concentration of about 5 mg/mL and pH < 5 and forms a stable solution. The intravenous formulation may include the compound or its salt and one or more antioxidants. The intravenous formulation may include a mixture of mono- and di-hydrochloride salts of the compound. The intravenous formulation may include the compound or its salt as a 1 % solution in a concentration of about 10 mg/mL. For example, the intravenous formulation may be a solution with a pH of about 3.3. The pH may be less than 4.0.

[0118] The pharmaceutical composition may further include a pharmaceutically acceptable carrier. A suitable pharmaceutically acceptable carrier may include an aqueous carrier, such as sterile water. The formulation may include dextrose and/or sodium. The pharmaceutically acceptable carrier may include an oil.

[0119] An intravenous formulation may comprise ONC201 or an analog thereof or a di-hydrochloride salt thereof dissolved in water at 25 mg/mL. The formulation may be adjusted to pH 3 with phosphate buffer. The formulation may include dextrose, sodium chloride or both. the formulation may include a higher or a lower concentration of the di-hydrochloride salt of ONC201 or an analog thereof. The formulation may include ONC201 or an analog thereof or a di-hydrochloride salt thereof in a concentration of about 5 mg/mL. The formulation of about 5 mg/mL may form a stable solution and pH 3. The formulation of about 5 mg/mL may have a pH < 5 and may form a stable solution. The intravenous formulation may include ONC201 or an analog thereof or a di-hydrochloride salt thereof and one or more antioxidants. The intravenous formulation may include a mixture of mono- and di-hydrochloride salts of ONC201 or an analog thereof. The intravenous formulation may include ONC201 or an analog thereof or a di-hydrochloride salt thereof as a 1 % solution in a concentration of about 10 mg/mL. For example, the intravenous formulation may be a solution having a pH of about 3.3. The pH may be less than 4.0.

[0120] The intravenous formulation may include from about 0.5% to about 10% (or from about 5 mg/mL to about 100 mg/mL) of ONC201 or an analog thereof or a di-salt thereof. The formulation may include from about 5 % (or

about 50 mg/mL) of ONC201 or an analog thereof or a di-salt thereof. The intravenous infusion rate may be slowed to decrease side effects of ONC201 or an analog thereof or a di-salt thereof.

[0121] The pharmaceutical composition may comprise about 0.1-99% of an ONC201 salt or an analog thereof; and a pharmaceutically acceptable carrier, e.g., an oil or sterile water or other aqueous carrier. The composition may comprise a mono or di-salt of ONC201 or an analog thereof in a range of from about 5% to about 50% for oral dosage forms.

[0122] A pharmaceutical composition may include an antioxidant, such as: ascorbic acid derivatives such as ascorbic acid, erythorbic acid, sodium ascorbate, thiol derivatives such as thioglycerol, cysteine, acetylcysteine, cystine, dithioerythreitol, dithiothreitol, glutathione, tocopherols, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), sulfurous acid salts such as sodium sulfate, sodium bisulfite, acetone sodium bisulfite, sodium metabisulfite, sodium sulfite, sodium formaldehyde sulfoxylate, and sodium thiosulfate, nordihydroguaiaretic acid. It should be noted that antioxidants used for aqueous formulations typically include: sodium sulphite, sodium metabisulphite, sodium formaldehyde sulphoxylate and ascorbic acid and combinations thereof, whereas antioxidants used in oilbased solutions, organic solvents, include butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and propyl gallate and combinations thereof. An antioxidant may be one or more of a flavanoid, an isoflavone, monothioglycerol, L-cysteine, thioglycolic acid, α-tocopherol, ascorbic acid 6-palmitate, dihydrolipoic acid, butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), vitamin E, propyl gallate, β-carotene, ascorbic acid. Antioxidants may typically be used in about 0.1% to 1.0% by weight, more typically about 0.2%.

[0123] The pharmaceutical composition may include an imipridone, such as ONC201 or an analog thereof, or a pharmaceutically acceptable salt thereof and at least one other therapeutic agent. For example, the other therapeutic agent may be selected from the group consisting of hormone analogs and antihormones, aromatase inhibitors, LHRH agonists and antagonists, inhibitors of growth factors, growth factor antibodies, growth factor receptor antibodies, tyrosine kinase inhibitors; antimetabolites; antitumour antibiotics; platinum derivatives; alkylation agents; antimitotic agents; tubuline inhibitors; PARP inhibitors, topoisomerase inhibitors, serine/threonine kinase inhibitors, tyrosine kinase inhibitors, protein protein interaction inhibitors, RAF inhibitors, MEK inhibitors, ERK inhibitors, IGF-1R inhibitors, ErbB receptor inhibitors, rapamycin analogs, BTK inhibitors, CRM1 inhibitors (e.g., KPT185), P53 modulators (e.g., Nutlins), antiangiogenics (e.g., axitinib, aflibercept, sorafenib, and regorafenib), amifostin, anagrelid, clodronat, filgrastin, interferon, interferon α, leucovorin, rituximab, procarbazine, levamisole, mesna, mitotane, pamidronate and porfimer, 2-chlorodesoxyadenosine, 2-fluorodesoxy-cytidine, 2-methoxyoestradiol, 2C4,3-alethine, 131-1-TM-601, 3CPA, 7-ethyl-10-hydroxycamptothecin, 16-aza-epothilone B, A 105972, A 204197, abiraterone, aldesleukin, alitretinoin, allovectin-7, altretamine, alvocidib, amonafide, anthrapyrazole, AG-2037, AP-5280, apaziquone, apomine, aranose, arglabin, arzoxifene, atamestane, atrasentan, auristatin PE, ABT-199 (Venetoclax), ABT-263 (Navitoclax), AVLB, AZ10992, ABX-EGF, AMG-479 (ganitumab), ARRY 162, ARRY 438162, ARRY-300, ARRY-142886/AZD-6244 (selumetinib), ARRY-704/AZD-8330, AR-12, AR-42, AS-703988, AXL-1717, AZD-8055, AZD-5363, AZD-6244, ARQ-736, ARQ 680, AS-703026 (primasertib), avastin, AZD-2014, azacytidine, azaepothilone B, azonafide, BAY-43-9006, BAY 80-6946, BBR-3464, BBR-3576, bevacizumab, BEZ-235, biricodar dicitrate, BCX-1777, BKM-120, bleocin, BLP-25, BMS-184476, BMS-247550, BMS-188797, BMS-275291, BMS-663513, BMS-754807, BNP-1350, BNP-7787, BIBW 2992 (afatinib, tomtovok), BIBF 1120 (vargatef), BI 836845, BI 2536, BI 6727, BI 836845, BI 847325, BI 853520, BUB-022, bleomycinic acid, bleomycin A, bleomycin B, brivanib, bryostatin-1, bortezomib, brostallicin, busulphan, BYL-719, CA-4 prodrug, CA-4, CapCell, calcitriol, canertinib, canfosfamide, capecitabine, carboxyphthalatoplatin, CCI-779, CC-115, CC-223, CEP-701, CEP-751, CBT-1 cefixime, ceflatonin, ceftriaxone, celecoxib, celmoleukin, cemadotin, CH4987655/RO-4987655, chlorotrianisene, cilengitide, ciclosporin, CDA-II, CDC-394, CKD-602, CKI-27, clofarabin, colchicin, combretastatin A4, COT inhibitors, CHS-828, CH-5132799, CLL-Thera, CMT-3 cryptophycin 52, CTP-37, CTLA-4 monoclonal antibodies, CP-461, CV-247, cyanomorpholinodoxorubicin, cytarabine, D 24851, decitabine, deoxorubicin, deoxyrubicin, deoxycoformycin, depsipeptide, desoxyepothilone B, dexamethasone, dexrazoxanet, diethylstilbestrol, diflomotecan, didox, DMDC, dolastatin 10, doranidazole, DS-7423, E7010, E-6201, edatrexat, edotreotide, efaproxiral, eflornithine, EGFR inhibitors, EKB-569, EKB-509, enzastaurin, enzalutamide, elsamitrucin, epothilone B, epratuzumab, ER-86526, erlotinib, ET-18-0CH3, ethynylcytidine, ethynyloestradiol, exatecan,

exatecan mesylate, exemestane, exisulind, fenretinide, figitumumab, floxuridine, folic acid, FOLFOX, FOLFOX4, FOLFIRI, formestane, fotemustine, galarubicin, gallium maltolate, gefinitib, gemtuzumab, gimatecan, glufosfamide, GCS-100, GDC-0623, GDC-0941 (pictrelisib), GDC-0980, GDC-0032, GDC-0068, GDC-0349, GDC-0879, G17DT immunogen, GMK, GPX-100, gp100-peptide vaccines, GSK-5126766, GSK-690693, GSK-1120212 (trametinib), GSK-2118436 (dabrafenib), GSK-2126458, GSK-2132231A, GSK-2334470, GSK-2110183, GSK-2141795, GW2016, granisetron, herceptine, hexamethylmelamine, histamine, homoharringtonine, hyaluronic acid, hydroxyurea, hydroxyprogesterone caproate, ibandronate, ibrutinib, ibritumomab, idatrexate, idenestrol, IDN-5109, IGF-1R inhibitors, IMC-1C11, IMC-A12 (cixutumumab), immunol, indisulam, interferon α-2a, interferon α-2b, pegylated interferon α-2b, interleukin-2, INK-1117, INK-128, INSM-18, ionafarnib, ipilimumab, iproplatin, irofulven, isohomohalichondrin-B, isoflavone, isotretinoin, ixabepilone, JRX-2, JSF-154, J-107088, conjugated oestrogens, kahalid F, ketoconazole, KW-2170, KW-2450, lobaplatin, leflunomide, lenograstim, leuprolide, leuporelin, lexidronam, LGD-1550, linezolid, lutetium texaphyrin, lometrexol, losoxantrone, LU 223651, lurtotecan, LY-S6AKT1, LY-2780301, mafosfamide, marimastat, mechloroethamine, MEK inhibitors, MEK-162, methyltestosteron, methylprednisolone, MEDI-573, MEN-10755, MDX-H210, MDX-447, MDX-1379, MGV, midostaurin, minodronic acid, mitomycin, mivobulin, MK-2206, MK-0646 (dalotuzumab), MLN518, motexaf in gadolinium, MS-209, MS-275, MX6, neridronate, neratinib, Nexavar, neovastat, nilotinib, nimesulide, nitroglycerin, nolatrexed, norelin, N-acetylcysteine, 06-benzylguanine, oblimersen, omeprazole, oncophage, oncoVEXGM-CSF, ormiplatin, ortataxel, OX44 antibodies, OSI-027, OSI-906 (linsitinib), 4-1BB antibodies, oxantrazole, oestrogen, panitumumab, patupilone, pegfilgrastim, PCK-3145, pegfilgrastim, PBI-1402, PBI-05204, PD0325901, PD-1 antibodies, PEG-paclitaxel, albumin-stabilized paclitaxel, PEP-005, PF-05197281, PF-05212384, PF-04691502, PHT-427, P-04, PKC412, P54, PI-88, pelitinib, pemetrexed, pentrix, perifosine, perillylalcohol, pertuzumab, PI3K inhibitors, PI3K/mTOR inhibitors, PG-TXL, PG2, PLX-4032/RO-5185426 (vemurafenib), PLX-3603/RO-5212054, PT-100, PWT-33597, PX-866, picoplatin, pivaloyloxymethylbutyrate, pixantrone, phenoxodiol O, PKI166, plevitrexed, plicamycin, polyprenic acid, porfiromycin, prednisone, prednisolone, quinamed, quinupristin, R115777, RAF-265, ramosetron, ranpirnase, RDEA-119BAY 869766, RDEA-436, rebeccamycin analogs, receptor tyrosine kinase (RTK) inhibitors, regorafenib, revimid, RG-7167, RG-7304, RG-7421, RG-7321, RG 7440, rhizoxin, rhu-Mab, rinfabate, risedronate,rituximab, robatumumab, rofecoxib, RO-31-7453, RO-5126766, RO-5068760, RPR 109881A, rubidazone, rubitecan, R-flurbiprofen, RX-0201, S-9788, sabarubicin, SAHA, sargramostim, satraplatin, SB 408075, Se-015/Ve-015, SU5416, SU6668, SDX-101, semustin, seocalcitol, SM-11355, SN-38, SN-4071, SR-27897, SR-31747, SR-13668, SRL-172, sorafenib, spiroplatin, squalamine, suberanilohydroxamic acid, sutent, T 900607, T 138067, TAK-733, TAS-103, tacedinaline, talaporf in, Tarceva, tariquitar, tasisulam, taxotere, taxoprexin, tazarotene, tegafur, temozolamide, tesmilifene, testosterone, testosterone propionate, tesmilifene, tetraplatin, tetrodotoxin, tezacitabine, thalidomide, theralux, therarubicin, thymalfasin, thymectacin, tiazofurin, tipifarnib, tirapazamine, tocladesine, tomudex, toremofin, trabectedin, TransMID-107, transretinic acid, traszutumab, tremelimumab, tretinoin, triacetyluridine, triapine, triciribine, trimetrexate, TLK-286TXD 258, tykerb/tyverb, urocidin, valrubicin, vatalanib, vincristine, vinflunine, virulizin, WX-UK1, WX-554, vectibix, xeloda, XELOX, XL-147, XL-228, XI,-281, XL-518/R-7420/GDC-0973, XL-765, YM-511, YM-598, ZD-4190, ZD-6474, ZD-4054, ZD-0473, ZD-6126, ZD-9331, ZD1839, ZSTK-474, zoledronat, zosuquidar, and combinations thereof.

[0124] The other therapeutic agent may comprise a hormone analog, an antihormone or both selected from tamoxifen, toremifene, raloxifene, fulvestrant, megestrol acetate, flutamide, nilutamide, bicalutamide, aminoglutethimide, cyproterone acetate, finasteride, buserelin acetate, fludrocortisone, fluoxymesterone, medroxy-progesterone, octreotide, and combinations thereof; one or more LHRH agonists and/or antagonists selected from goserelin acetate, luprolide acetate, triptorelin pamoate and combinations thereof and wherein the LHRH antagonists are selected from Degarelix, Cetrorelix, Abarelix, Ozarelix, Degarelix combinations thereof; one or more growth factor inhibitors selected from inhibitors of: platelet derived growth factor (PDGF), fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), insuline-like growth factors (IGF), human epidermal growth factor (HER) (such as HER2, HER3, and HER4) and hepatocyte growth factor (HGF); one or more tyrosine kinase inhibitors selected from cetuximab, gefitinib, imatinib, lapatinib and trastuzumab, and combinations thereof; one or more aromatase inhibitors selected from anastrozole, letrozole, liarozole, vorozole, exemestane, atamestane, and combinations thereof; one or more antimetabolites which are antifolates selected from methotrexate, raltitrexed, and pyrimidine analogs such as 5-fluorouracil, capecitabin and gemcitabin; one or more antimetabolites which are purine and/or adenosine analogs selected from

mercaptopurine, thioguanine, cladribine and pentostatin, cytarabine, fludarabine, and combinations thereof; one or more antitumour antibiotics selected from anthracyclins, doxorubicin, daunorubicin, epirubicin and idarubicin, mitomycin-C, bleomycin, dactinomycin, plicamycin, streptozocin and combinations thereof; one or more platinum derivatives selected from cisplatin, oxaliplatin, carboplatin and combinations thereof; one or more alkylation agents selected from estramustin, meclorethamine, melphalan, chlorambucil, busulphan, dacarbazin, cyclophosphamide, ifosfamide, temozolomide, nitrosoureas, and combinations thereof; nitrosoureas selected from carmustin, lomustin, thiotepa, and combinations thereof; antimitotic agents selected from Vinca alkaloids and taxanes. The taxanes may be selected from paclitaxel, docetaxel, and combinations thereof. The Vinca alkaloids may be selected from vinblastine, vindesin, vinorelbin, vincristine, and combinations thereof. The other therapeutic agent may comprise one or more topoisomerase inhibitors which are epipodophyllotoxinssuch as etoposide and etopophos, teniposide, amsacrin, topotecan, irinotecan, mitoxantron, and combinations thereof. The other therapeutic agent may comprise one or more serine/threonine kinase inhibitors selected from PDK 1 inhibitors, B-Raf inhibitors, mTOR inhibitors, mTORC1 inhibitors, PI3K inhibitors, dual mTOR/PI3K inhibitors, STK 33 inhibitors, AKT inhibitors, PLK 1 inhibitors, inhibitors of CDKs, Aurora kinase inhibitors, and combinations thereof. The other therapeutic agent may comprise one or more tyrosine kinase inhibitors which are PTK2/FAK inhibitors. the other therapeutic agent may comprise one or more protein protein interaction inhibitors selected from IAP, Mcl-1, MDM2/MDMX and combinations thereof. The other therapeutic agent may comprise one or more rapamycin analogs selected from everolimus, temsirolimus, ridaforolimus, sirolimus, and combinations thereof. The other therapeutic agent may compriseone or more therapeutic agents selected from amifostin, anagrelid, clodronat, filgrastin, interferon, interferon α, leucovorin, rituximab, procarbazine, levamisole, mesna, mitotane, pamidronate and porfimer, and combinations thereof. The other therapeutic agent may comprise one or more therapeutic agents selected from 2-chlorodesoxyadenosine, 2-fluorodesoxy-cytidine, 2-methoxyoestradiol, 2C4,3-alethine, 131-1-TM-601, 3CPA, 7-ethyl-10-hydroxycamptothecin, 16-aza-epothilone B, A 105972, A 204197, abiraterone, aldesleukin, alitretinoin, allovectin-7, altretamine, alvocidib, amonafide, anthrapyrazole, AG-2037, AP-5280, apaziquone, apomine, aranose, arglabin, arzoxifene, atamestane, atrasentan, auristatin PE, ABT-199 (Venetoclax), ABT-263 (Navitoclax), AVLB, AZ10992, ABX-EGF, AMG-479 (ganitumab), ARRY 162, ARRY 438162, ARRY-300, ARRY-142886/AZD-6244 (selumetinib), ARRY-704/AZD-8330, AR-12, AR-42, AS-703988, AXL-1717, AZD-8055, AZD-5363, AZD-6244, ARQ-736, ARQ 680, AS-703026 (primasertib), avastin, AZD-2014, azacytidine, azaepothilone B, azonafide, BAY-43-9006, BAY 80-6946, BBR-3464, BBR-3576, bevacizumab, BEZ-235, biricodar dicitrate, BCX-1777, BKM-120, bleocin, BLP-25, BMS-184476, BMS-247550, BMS-188797, BMS-275291, BMS-663513, BMS-754807, BNP-1350, BNP-7787, BIBW 2992 (afatinib, tomtovok), BIBF 1120 (vargatef), BI 836845, BI 2536, BI 6727, BI 836845, BI 847325, BI 853520, BUB-022, bleomycinic acid, bleomycin A, bleomycin B, brivanib, bryostatin-1, bortezomib, brostallicin, busulphan, BYL-719, CA-4 prodrug, CA-4, CapCell, calcitriol, canertinib, canfosfamide, capecitabine, carboxyphthalatoplatin, CCI-779, CC-115, CC-223, CEP-701, CEP-751, CBT-1 cefixime, ceflatonin, ceftriaxone, celecoxib, celmoleukin, cemadotin, CH4987655/RO-4987655, chlorotrianisene, cilengitide, ciclosporin, CDA-II, CDC-394, CKD-602, CKI-27, clofarabin, colchicin, combretastatin A4, COT inhibitors, CHS-828, CH-5132799, CLL-Thera, CMT-3 cryptophycin 52, CTP-37, CTLA-4 monoclonal antibodies, CP-461, CV-247, cyanomorpholinodoxorubicin, cytarabine, D 24851, decitabine, deoxorubicin, deoxyrubicin, deoxycoformycin, depsipeptide, desoxyepothilone B, dexamethasone, dexrazoxanet, diethylstilbestrol, diflomotecan, didox, DMDC, dolastatin 10, doranidazole, DS-7423, E7010, E-6201, edatrexat, edotreotide, efaproxiral, eflomithine, EGFR inhibitors, EKB-569, EKB-509, enzastaurin, enzalutamide, elsamitrucin, epothilone B, epratuzumab, ER-86526, erlotinib, ET-18-0CH3, ethynylcytidine, ethynyloestradiol, exatecan, exatecan mesylate, exemestane, exisulind, fenretinide, figitumumab, floxuridine, folic acid, FOLFOX, FOLFOX4, FOLFIRI, formestane, fotemustine, galarubicin, gallium maltolate, gefinitib, gemtuzumab, gimatecan, glufosfamide, GCS-100, GDC-0623, GDC-0941 (pictrelisib), GDC-0980, GDC-0032, GDC-0068, GDC-0349, GDC-0879, G17DT immunogen, GMK, GPX-100, gp100-peptide vaccines, GSK-5126766, GSK-690693, GSK-1120212 (trametinib), GSK-2118436 (dabrafenib), GSK-2126458, GSK-2132231A, GSK-2334470, GSK-2110183, GSK-2141795, GW2016, granisetron, herceptine, hexamethylmelamine, histamine, homoharringtonine, hyaluronic acid, hydroxyurea, hydroxyprogesterone caproate, ibandronate, ibrutinib, ibritumomab, idatrexate, idenestrol, IDN-5109, IGF-1R inhibitors, IMC-1C11, IMC-A12 (cixutumumab), immunol, indisulam, interferon α-2a, interferon α-2b, pegylated interferon α-2b, interleukin-2, INK-1117, INK-128, INSM-18, ionafamib, ipilimumab, iproplatin, irofulven, isohomohalichondrin-B, isoflavone, isotretinoin, ixabepilone, JRX-2, JSF-154, J-107088, conjugated oestrogens, kahalid F, ketoconazole, KW-2170, KW-2450, lobaplatin, leflunomide, lenograstim, leuprolide, leuporelin, lexidronam, LGD-1550, linezolid, lutetium

texaphyrin, lometrexol, losoxantrone, LU 223651, lurtotecan, LY-S6AKT1, LY-2780301, mafosfamide, marimastat, mechloroethamine, MEK inhibitors, MEK-162, methyltestosteron, methylprednisolone, MEDI-573, MEN-10755, MDX-H210, MDX-447, MDX-1379, MGV, midostaurin, minodronic acid, mitomycin, mivobulin, MK-2206, MK-0646 (dalotuzumab), MLN518, motexaf in gadolinium, MS-209, MS-275, MX6, neridronate, neratinib, Nexavar, neovastat, nilotinib, nimesulide, nitroglycerin, nolatrexed, norelin, N-acetylcysteine, 06-benzylguanine, oblimersen, omeprazole, oncophage, oncoVEXGM-CSF, ormiplatin, ortataxel, OX44 antibodies, OSI-027, OSI-906 (linsitinib), 4-1BB antibodies, oxantrazole, oestrogen, panitumumab, patupilone, pegfilgrastim, PCK-3145, pegfilgrastim, PBI-1402, PBI-05204, PDO325901, PD-1 antibodies, PEG-paclitaxel, albumin-stabilized paclitaxel, PEP-005, PF-05197281, PF-05212384, PF-04691502, PHT-427, P-04, PKC412, P54, PI-88, pelitinib, pemetrexed, pentrix, perifosine, perillylalcohol, pertuzumab, PI3K inhibitors, PI3K/mTOR inhibitors, PG-TXL, PG2, PLX-4032/RO-5185426 (vemurafenib), PLX-3603/RO-5212054, PT-100, PWT-33597, PX-866, picoplatin, pivaloyloxymethylbutyrate, pixantrone, phenoxodiol O, PKI166, plevitrexed, plicamycin, polyprenic acid, porfiromycin, prednisone, prednisolone, quinamed, quinupristin, R115777, RAF-265, ramosetron, ranpirnase, RDEA-119BAY 869766, RDEA-436, rebeccamycin analogs, receptor tyrosine kinase (RTK) inhibitors, revimid, RG-7167, RG-7304, RG-7421, RG-7321, RG 7440, rhizoxin, rhu-Mab, rinfabate, risedronate,rituximab, robatumumab, rofecoxib, RO-31-7453, RO-5126766, RO-5068760, RPR 109881A, rubidazone, rubitecan, R-flurbiprofen, RX-0201, S-9788, sabarubicin, SAHA, sargramostim, satraplatin, SB 408075, Se-015/Ve-015, SU5416, SU6668, SDX-101, semustin, seocalcitol, SM-11355, SN-38, SN-4071, SR-27897, SR-31747, SR-13668, SRL-172, sorafenib, spiroplatin, squalamine, suberanilohydroxamic acid, sutent, T 900607, T 138067, TAK-733, TAS-103, tacedinaline, talaporf in, Tarceva, tariquitar, tasisulam, taxotere, taxoprexin, tazarotene, tegafur, temozolamide, tesmilifene, testosterone, testosterone propionate, tesmilifene, tetraplatin, tetrodotoxin, tezacitabine, thalidomide, theralux, therarubicin, thymalfasin, thymectacin, tiazofurin, tipifarnib, tirapazamine, tocladesine, tomudex, toremofin, trabectedin, TransMID-107, transretinic acid, traszutumab, tremelimumab, tretinoin, triacetyluridine, triapine, triciribine, trimetrexate, TLK-286TXD 258, tykerb/tyverb, urocidin, valrubicin, vatalanib, vincristine, vinflunine, virulizin, WX-UK1, WX-554, vectibix, xeloda, XELOX, XL-147, XL-228, XL-281, XL-518/R-7420/GDC-0973, XL-765, YM-511, YM-598, ZD-4190, ZD-6474, ZD-4054, ZD-0473, ZD-6126, ZD-9331, ZD1839, ZSTK-474, zoledronat, zosuquidar, and combinations thereof.

[0125] The other therapeutic agent may comprise a steroid, including dexamethasone, prednisolone, methyl prednisolone, prednisone, hydrocortisone, triamcinolone, betamethasone, and cortivazol. The other therapeutic agent may comprise an anti-emetic such as 5-HT3 receptor agonists (*e.g.*, dolasetron, granisetron, ondansetron, tropisetron, palonosetron, and mirtazapine), dopamine agonists (*e.g.*, domperidone, olanzapine, droperidol, haloperidol, chlorpromazine, prochlorperazine, alizapride, prochlorperazine, and metoclopramide), NK1 receptor antagonists (*e.g.*, aprepitant and casopitant), antihistamines (such as cyclizine, diphenhydramine, dimenhydrinate, doxylamine, meclizine, promethazine, hydroxyzine), cannabinoids (*e.g.*, cannabis, dronabinol, nabilone, and sativex), benzodiazepines (*e.g.*, midazolam and lorazepam), anticholinergics (*e.g.*, hyoscine), trimethobenzamide, ginger, emetrol, propofol, peppermint, muscimol, and ajwain.

[0126] The other therapeutic agent may comprise an anti-cancer agent, which includes a mitotic inhibitor such as a taxane, for example a taxane selected from paclitaxel and docetaxel.

[0127] The pharmaceutical composition may include an imipridone, such as ONC201, or an analog thereof, or a pharmaceutically acceptable salt thereof; and at least one anti-cancer agent, which may include one or more of acivicin, aclarubicin, acodazole, acronine, adozelesin, aldesleukin, alitretinoin, allopurinol, altretamine, ambomycin, ametantrone, amifostine, aminoglutethimide, amsacrine, anastrozole, anthramycin, arsenic trioxide, asparaginase, asperlin, azacitidine, azetepa, azotomycin, batimastat, benzodepa, bevacizumab, bicalutamide, bisantrene, bisnafide dimesylate, bizelesin, bleomycin, brequinar, bropirimine, busulfan, cactinomycin, calusterone, capecitabine, caracemide, carbetimer, carboplatin, carmustine, carubicin, carzelesin, cedefingol, celecoxib, chlorambucil, cirolemycin, cisplatin, cladribine, crisnatol mesylate, cyclophosphamide, cytarabine, dacarbazine, dactinomycin, daunorubicin, decitabine, dexormaplatin, dezaguanine, dezaguanine mesylate, diaziquone, docetaxel, doxorubicin, droloxifene, dromostanolone, duazomycin, edatrexate, eflomithine, elsamitrucin, enloplatin, enpromate, epipropidine, epirubicin, erbulozole, esorubicin, estramustine, etanidazole, etoposide, etoprine, fadrozole, fazarabine, fenretinide, floxuridine, fludarabine, fluorouracil, flurocitabine, fosquidone, fostriecin,

fulvestrant, gemcitabine, hydroxyurea, idarubicin, ifosfamide, ilmofosine, interleukin II (IL-2, including recombinant interleukin II or rIL2), interferon α-2a, interferon α-2b, interferon α-n1, interferon α-n3, interferon β-Ia, interferon gamma-Ib, iproplatin, irinotecan, lanreotide, letrozole, leuprolide, liarozole, lometrexol, lomustine, losoxantrone, masoprocol, maytansine, mechlorethamine hydrochlride, megestrol, melengestrol acetate, melphalan, menogaril, mercaptopurine, methotrexate, metoprine, meturedepa, mitindomide, mitocarcin, mitocromin, mitogillin, mitomalcin, mitomycin, mitosper, mitotane, mitoxantrone, mycophenolic acid, nelarabine, nocodazole, nogalamycin, ormnaplatin, oxisuran, paclitaxel, pegaspargase, peliomycin, pentamustine, peplomycin, perfosfamide, pipobroman, piposulfan, piroxantrone hydrochloride, plicamycin, plomestane, porfimer, porfiromycin, prednimustine, procarbazine, puromycin, pyrazofurin, riboprine, rogletimide, safingol, semustine, simtrazene, sparfosate, sparsomycin, spirogermanium, spiromustine, spiroplatin, streptonigrin, streptozocin, sulofenur, talisomycin, tamoxifen, tecogalan, tegafur, teloxantrone, temoporfin, teniposide, teroxirone, testolactone, thiamiprine, thioguanine, thiotepa, tiazofurin, tirapazamine, topotecan, toremifene, trestolone, triciribine, trimetrexate, triptorelin, tubulozole, uracil mustard, uredepa, vapreotide, verteporfin, vinblastine, vincristine sulfate, vindesine, vinepidine, vinglycinate, vinleurosine, vinorelbine, vinrosidine, vinzolidine, vorozole, zeniplatin, zinostatin, zoledronate, zorubicin and combinations thereof.

[0128] Examples of suitable anti-cancer agents include those described Goodman and Gilman's The Pharmacological Basis of Therapeutics, 12th Ed., edited by Laurence Brunton, Bruce Chabner, Bjorn Knollman, McGraw Hill Professional, 2010.

[0129] The pharmaceutical composition may include a salt (e.g., a mono-or di- salt) of an imipridone, *e.g.*, ONC201, or an analog thereof and at least one other therapeutic agent, where the other therapeutic agent comprises an anti-angiogenic agent, for example, bevacizumab. The anti-angiogenic agent may be selected from aflibercept, axitinib, angiostatin, endostatin, 16kDa prolactin fragment, laminin peptides, fibronectin peptides, tissue metalloproteinase inhibitors (TIMP 1, 2, 3, 4), plasminogen activator inhibitors (PAI-1, -2), tumor necrosis factor α, (high dose, invitro), TGF-β1, interferons (IFN-α, -β, γ), ELR-CXC chemokines, IL-12; SDF-1; MIG; platelet factor 4 (PF-4); IP-10, thrombospondin (TSP), SPARC, 2-methoxyoestradiol, proliferin-related protein, suramin, sorafenib, regorafenib, thalidomide, cortisone, linomide, fumagillin (AGM-1470; TNP-470), tamoxifen, retinoids, CM101, dexamethasone, leukemia inhibitory factor (LIF), hedgehog inhibitor and combinations thereof.

[0130] A pharmaceutical combination may include first and second therapeutic agents in any desired proportions provided that the synergistic or cooperative effect still occurs. A synergistic pharmaceutical combination preferably contains the first and second therapeutic agents in a ratio of from about 1:9 to about 9:1. A synergistic combination may contain the first and second therapeutic agents in a ratio of from about 1:8 to about 8:1, from about 1:7 to about 7:1, from about 1:6 to about 6:1, from about 1:5 to about 5:1, from about 1:4 to about 4:1, from about 1:3 to about 3:1, or from about 1:2 to about 2:1. The synergistic combination may contain the therapeutic agents in a ratio of approximately 1:1.

[0131] The second therapeutic agent may be selected from Allopurinol, Arsenic Trioxide, Azacitidine, Bortezomib, Bevacizumab, Capecitabine, Carboplatin, Celecoxib, Chlorambucil, Clofarabine, Cytarabine, Dacarbazine, Daunorubicin HCl, Docetaxel, Doxorubicin HCl, Floxuridine, Gemcitabine HCl, Hydroxyurea, Ifosfamide, Imatinib Mesylate, Ixabepilone, Lenalidomide, Megestrol acetate, Methotrexate, Mitotane, Mitoxantrone HCl, Oxaliplatin, Paclitaxel, Pralatrexate, Romidepsin, Sorafenib, Streptozocin, Tamoxifen Citrate, Topotecan HCl, Tretinoin, Vandetanib, Vismodegib, Vorinostat, and combinations thereof.

[0132] The second therapeutic agent may comprise a small molecule multi-kinase inhibitor, *e.g.*, sorafenib or regorafenib. The second therapeutic agent may comprise a Hedgehog Pathway Inhibitor, *e.g.*, vismodegib. The second therapeutic agent may include a drug selected from Table 2 below.

Table 2: Classes Of Drugs

Classes of drugs	Examples
Purine analogs	allopurinol, oxypurinol, clofarabine, and tisopurine
Pyrimidine	5-fluorouracil, Floxuridine (FUDR), capecitabine, cytarabine, 6-azauracil (6-AU),

Classes of drugs	Examples
analogs	and gemcitabine (Gemzar)
Proteasome inhibitors	bortezomib, carfilzomib, cediranib, disulfiram, epigallocatechin-3-gallate, salinosporamide A, ONCX 0912, CEP-18770, MLN9708, epoxomicin, and MG132.
Anti-angiogenic	bevacizumab, aflibercept, sunitinib, sorafenib, pazopanib, vandetanib, cabozantinib, axitinib, ponatinib, regorafenib, ranibizumab, lapatinib, and vandetanib.
Platinum-based antineoplastic drugs	cisplatin, carboplatin, oxaliplatin, satraplatin, picoplatin, nedaplatin, and triplatin.
COX-2 inhibitors	celecoxib, valdecoxib (Bextra), parecoxib (Dynastat), lumiracoxib, etoricoxib, and rofecoxib.
Nitrogen mustards	cyclophosphamide, chlorambucil, uramustine, ifosfamide, melphalan, bendamustine, and mustine.
Alkylating agents	cyclophosphamide, mechlorethamine or mustine (HN2) (trade name Mustardgen), uramustine or uracil mustard, melphalan, chlorambucil, ifosfamide, bendamustine, carmustine, lomustine, streptozocin, and busulfan.
Anthracyclines	Daunorubicin (Daunomycin), Daunorubicin (liposomal), Doxorubicin (Adriamycin), Doxorubicin (liposomal), Epirubicin, Idarubicin, Valrubicin, and Mitoxantrone.
Taxanes	Paclitaxel (Taxol), Docetaxel (Taxotere), and albumin-bound paclitaxel (Abraxane).
Nucleotide synthesis inhibitor	methotrexate, pralatrexate, hydroxyurea, and 5-fluorodeoxyuridine, 3,4-dihydroxybenzylamine.
Bcr-abl inhibitors	imatinib, nilotinib, dasatinib, bosutinib and ponatinib.
Other	arsenic trioxide, thalidomide, revlimid, and mitotane.
Topoisomerase inhibitor	amsacrine, etoposide, etoposide phosphate, teniposide, doxorubicin, Topotecan (Hycamtin), Irinotecan (CPT-11, Camptosar), Exatecan, Lurtotecan, ST 1481, CKD 602, ICRF-193, and genistein.
HDAC inhibitors	Vorinostat (SAHA), Romidepsin (Istodax), Panobinostat (LBH589), Valproic acid (as Mg valproate), Belinostat (PXD101), Mocetinostat (MGCD0103), Abexinostat (PCI-24781), Entinostat (MS-275), SB939, Resminostat (4SC-201), Givinostat, Quisinostat (JNJ-26481585), CUDC-101, AR-42, CHR-2845, CHR-3996, 4SC-202, CG200745, ACY-1215, ME-344, sulforaphane, Kevetrin, and ATRA.
Multi-kinase inhibitors	sorafenib, regorafenib, and vandetanib.
Hormone therapies	tamoxifen, toremifene, Arimidex (anastrozole), Aromasin (exemestane), Femara (letrozole), and Fulvestrant (Faslodex).
Hedgehog signaling Inhibitors	vismodegib, BMS-833923, IPI-926, LDE-225, PF-04449913, LEQ 506, and TAK-441.
Checkpoint Inhibitors	Opdivo (nivolumab), Durvalumab (Medi4736), Keytruda (pembrolizumab, MK3475), BGB-A317, AMP-224, PDR001, REGN 281, Atezolizumab (MPDL3280A), Pidilizumab (BMS-936559, CT-011, ONO-4538), Avelumab (MSB0010718 C), Yervoy (ipilimumab), tremelimumab
BCL2 Inhibitors	AT-101, Bcl-2/xL inhibitor, Navitoclax (ABT-263), Venetoclax (ABT-199), Apogossypol, PTN1258, obatoclax, G3139

[0133] The second therapeutic agent may include drugs that target tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) receptors. The second therapeutic agent may include a recombinant TRAIL or an agonistic antibody that activates one or more TRAIL receptors. The second therapeutic agent may include one or more antibodies or recombinant TRAIL that activate signaling by DR4, DR5 or both. The second therapeutic agent may

include one or more of AMG-655, LBY-135, mapatumumab, lexatumumab, Apomab, and rhApo2L/TRAIL. The second therapeutic agent may include an active agent selected from Camptothecin, 5-FU, capecitabine, cisplatin, doxorubicin, irinotecan, paclitaxel, cisplatin, bortezomib, BH3I-2, rituximab, radiation, triterpenoids, sorafenib, gemcitabine, HDAC inhibitors, carboplatin, T-101 (a gossypol derivate), ABT-263, ABT-737, and GX-15-070 (obatoclax), vorinostat, cetuximab, panitumumab, bevacizumab, ganitumab, interferon gamma, sorafenib, XIAP antagonists, Bcl-2 antagonists, and Smac mimetics.

VI. DOSE

[0134] A pharmaceutical composition may comprise an imipridone, such as ONC201, or an analog thereof, or a pharmaceutically acceptable salt thereof in a dose ranging from about 40, 50, 60, or 100 mg to about 2000 mg; from about 4, 5, 6, or 10 mg to about 200 mg; or from about 0.4, 0.5, 0.6, or 1 mg to about 20 mg where the weight can be based on the compound in its free base form. A pharmaceutical composition may comprise an imipridone, such as ONC201, or an analog thereof, or a pharmaceutically acceptable salt thereof in a dose level ranging from about 50 mg to about 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, or 2000 mg; from about 5 mg to about 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, and 200 mg; or from about 0.5 mg to about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, and 20 mg; and/or a dose level ranging from about 40 mg to about 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, or 2000 mg; from about 4 mg to about 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, or 200 mg; or from about 0.4 mg to about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, and 20 mg; and/or a dose level ranging from about 60 mg to about 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, or 2000 mg; from about 6 mg to about 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, or 200 mg; or from about 0.6 mg to about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 mg; and/or a dose level ranging from about 100 mg to about 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900 mg, or 2000 mg; from about 10 mg to about 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, or 200 mg; or from about 1 mg to about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 mg. A pharmaceutical composition may comprise an imipridone, such as ONC201, or an analog thereof, or a pharmaceutically acceptable salt thereof in a dose level ranging from about 200 mg to about 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, or 2000 mg; from about 20 mg to about 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, or 200 mg; or from about 2 mg to about 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 mg, based on the compound in its free base form; and/or a dose level ranging from about 400 mg to about 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, or 2000 mg; from about 40 mg to about 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, or 200 mg; or from about 4 mg to about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 mg based on the compound in its free base form. A pharmaceutical composition may comprise an imipridone, such as ONC201, or an analog thereof, or a pharmaceutically acceptable salt thereof thereof in a dose level ranging from about 50 mg to about 60, 70, 80, 90, or 100 mg; from about 60 mg to about 70, 80, 90, or 100 mg; from about 70 mg to about 80, 90 or 100 mg, from about 80 mg to about 90 or 100 mg; from about 90 mg to about 100 mg; from about 5 mg to about 6, 7, 8, 9, or 10 mg; from about 6 mg to about 7, 8, 9, or 10 mg; from about 7 mg to about 8, 9 or 10 mg, from about 8 mg to about 9 or 10 mg; from about 9 mg to about 10 mg; from about 0.5 mg to about 0.6, 0.7, 0. 8, 0.9, or 1 mg; from about 0.6 mg to about 0.7, 0.8, 0.9, or 1 mg; from about 0.7 mg to about 0.8, 0.9 or 1 mg, from about 0.8 mg to about 0.9 or 1 mg; or from about 0.9 mg to about 1 mg.

[0135] A pharmaceutical composition may comprise an imipridone, such as ONC201, or an analog thereof, or a pharmaceutically acceptable salt thereof in a dose ranging from about 1 mg/kg to about 40 mg/kg; 0.1 mg/kg to about 4 mg/kg; or 0.01 mg/kg to about 0.40 mg/kg; such as a dose level ranging from about 1, 2, 3, 4, 5, 6, 7, 8, or 9 mg/kg to about 10, 20, 30, or 40 mg/kg; from about 10, 11, 12, 13, 14, 15, 16, 17, 18, or 19 mg/kg to about 20, 30, or 40 mg/kg; from about 20, 21, 22, 23, 24, 25, 26, 27, 28, or 29 mg/kg to about 30 or 40 mg/kg; from about 30, 31, 32, 33, 34, 35, 36, 37, 38, or 39 mg/kg to about 40 mg/kg; from about 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, or 0.9 mg/kg to about 1, 2, 3, or 4 mg/kg; from about 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, or 1.9 mg/kg to about

2, 3, or 4 mg/kg; from about 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, or 2.9 mg/kg to about 3 or 4 mg/kg; or from about 3.0, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, or 3.9 mg/kg to about 4 mg/kg; from about 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09 mg/kg to about 0.10, 0.20, 0.30, or 0.40 mg/kg; from about 0.10, 0.11, 0.12, 0.13, 0.14, 0.15, 0.16, 0.17, 0.18, or 0.19 mg/kg to about 0.20, 0.30, or 0.40 mg/kg; from about 0.20, 0.21, 0.22, 0.23, 0.24, 0.25, 0.26, 0.27, 0.28, or 0.29 mg/kg to about 0.30 or 0.40 0.mg/kg; or from about 0.30, 0.31, 0.32, 0.33, 0.34, 0.35, 0.36, 0.37, 0.38, or 0.39 mg/kg to about 0.40 mg/kg.

[0136] A pharmaceutical composition may comprise an imipridone, such as ONC201, or an analog thereof, or a pharmaceutically acceptable salt thereof in a dose ranging from about 37.5 mg/m^2 to about 1500 mg/m^2; from about 3.75 mg/m^2 to about 150 mg/m^2; or from about 0.4 mg/m^2 to about 15 mg/m^2 A pharmaceutical composition may comprise an imipridone, such as ONC201, or an analog thereof, or a pharmaceutically acceptable salt thereof in a dose ranging from about 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, 150, 155, 160, 165, 170, 175, 180, 185, 190, 195, 200, 205, 210, 215, 220, 225, 230, 235, 240, 245, 250, 255, 260, 265, 270, 275, 280, 285, 290, 295, 300, 305, 310, 315, 320, 325, 330, 335, 340, 345, 350, 355, 360, 365, 370, 375, 380, 385, 390, 395, 400, 405, 410, 415, 420, 425, 430, 435, 440, 445, 450, 455, 460, 465, 470, 475, 480, 485, 490, 495, 500, 505, 510, 515, 520, 525, 530, 535, 540, 545, 550, 555, 560, 565, 570, 575, 580, 585, 590, 595, 600, 605, 610, 615, 620, 625, 630, 635, 640, 645, 650, 655, 660, 665, 670, 675, 680, 685, 690, 695, 700, 705, 710, 715, 720, 725, 730, 735, 740, 745, 750, 755, 760, 765, 770, 775, 780, 785, 790, 795, 800, 805, 810, 815, 820, 825, 830, 835, 840, 845, 850, 855, 860, 865, 870, 875, 880, 885, 890, 895, 900, 905, 910, 915, 920, 925, 930, 935, 940, 945, 950, 955, 960, 965, 970, 975, 980, 985, 990, 995, 1000, 1005, 1010, 1015, 1020, 1025, 1030, 1035, 1040, 1045, 1050, 1055, 1060, 1065, 1070, 1075, 1080, 1085, 1090, 1095, 1100, 1105, 1110, 1115, 1120, 1125, 1130, 1135, 1140, 1145, 1150, 1155, 1160, 1165, 1170, 1175, 1180, 1185, 1190, 1195, 1200, 1205, 1210, 1215, 1220, 1225, 1230, 1235, 1240, 1245, 1250, 1255, 1260, 1265, 1270, 1275, 1280, 1285, 1290, 1295, 1300, 1305, 1310, 1315, 1320, 1325, 1330, 1335, 1340, 1345, 1350, 1355, 1360, 1365, 1370, 1375, 1380, 1385, 1390, 1395, 1400, 1405, 1410, 1415, 1420, 1425, 1430, 1435, 1440, 1445, 1450, 1455, 1460, 1465, 1470, 1475, 1480, 1485, 1490, 1495 mg/m^2 to about 1500 mg/m^2; from about 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, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, or 149 mg/m^2 to about 150 mg/m^2;or from about 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5, 11, 111, 11.5, 12, 12.5, 13, 13.5, 14, or 14.5 mg/m^2 to about 15 mg/m^2.

VII. DOSAGE FORMS

[0137] Suitable pharmaceutical compositions for use in the methods described herein can be formulated into a dosage form that can be administered to a patient. A pharmaceutical composition may be in the form of an oral dosage unit or parenteral dosage unit. A pharmaceutical composition may be in the form of an oral dosage unit. An oral dosage unit may be fractionated into several, smaller doses, which are administered to a subject over a predetermined period of time in order to reduce toxicity of a therapeutic agent being administered. An oral dosage unit may be administered by a tablet or capsule comprising a controlled release formulation that can include a plurality of particles, granules, pellets, minitablets or tablets. The pharmaceutical composition may be in the form of a parenteral dosage unit. The parenteral dosage unit may be selected from the group consisting of intravenous (IV), subcutaneous (SC), and intramuscular (M), rectal (PR) and transdermal dosage units. The composition may be in a dosage form selected from the group consisting of sterile solutions, suspensions, suppositories, tablets and capsules. The composition may be an oral dosage form selected from the group consisting of a tablet, caplet, capsule, lozenge, syrup, liquid, suspension and elixir. The composition may be in an oral dosage form selected from the group consisting of tablets, hard shell capsules, soft gelatin capsules, beads, granules, aggregates, powders, gels, solids and semi-solids.

[0138] Suitable forms of pharmaceutical compositions for use in the methods described herein may include dermatological compositions adapted for cutaneous topical administration. For example, dermatological compositions may include a cosmetically or pharmaceutically acceptable medium. Dermatological compositions for topical administration can include ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners, skin enhancers may be necessary or desirable and therefore may be used. Examples of suitable enhancers include ethers such as diethylene glycol monoethyl ether (available commercially as TRANSCUTOL®) and diethylene glycol monomethyl ether; surfactants such as sodium laurate, sodium lauryl sulfate, cetyltrimethylammonium bromide, benzalkonium chloride, Poloxamer (231, 182, 184), Tween (20, 40, 60, 80), and lecithin (US Pat. 4,783,450); alcohols such as ethanol, propanol, octanol, benzyl alcohol; polyethylene glycol and esters thereof such as polyethylene glycol monolaurate; amides and other nitrogenous compounds such as urea, dimethylacetamide (DMA), dimethylformamide (DMF), 2-pyrrolidone, l-methyl-2-pyrrolidone, ethanolamine, diethanolamine and triethanolamine; terpenes; alkanones; and organic acids, particularly citric acid and succinic acid. AZONE® and sulfoxides such as DMSO and C
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0MSO may also be used, but are less preferred.

[0139] The pharmaceutical composition may be in a dosage form selected from the group consisting of sustained release forms, controlled release forms, delayed release forms and response release forms.

VIII. METHODS OF USE

[0140] The compositions and methods described herein have utility in treating many disease conditions, including cancer (e.g., colorectal, brain, and glioblastoma).The compositions and methods described herein may be used to treat diseases such as ocular melanoma, desmoplastic round cell tumor, chondrosarcoma, leptomengial disease, diffuse large B-cell lymphoma, Acute Lymphoblastic Leukemia, Acute Myeloid Leukemia, Adrenocortical Carcinoma, AIDS-Related Cancers, AIDS-Related Lymphoma, Anal or Rectal Cancer, Appendix Cancer, Astrocytomas, and Atypical Teratoid/Rhabdoid Tumor. The compositions and methods described herein may be used to treat diseases such as Basal Cell Carcinoma, Basal Cell Nevus Syndrome, Gorlin-Nevus Syndrome, Bile Duct Cancer, Bladder Cancer, Bone Cancer, Osteosarcoma and Malignant Fibrous Histiocytoma, Brain Tumor, Breast Cancer, Bronchial Tumors, Burkitt Lymphoma, and Spinal Cord Tumors The compositions and methods described herein may be used to treat diseases such as Carcinoid Tumor, Carcinoma of Unknown Primary, Central Nervous System Atypical Teratoid/Rhabdoid Tumor, Leptomeningeal Disease, Central Nervous System Embryonal Tumors, Central Nervous System Lymphoma, Cervical Cancer, Chordoma, Chronic Lymphocytic Leukemia, Chronic Myelogenous Leukemia, Chronic Myeloproliferative Disorders, Colon Cancer, Colorectal Cancer, Craniopharyngioma, and Cutaneous T-Cell Lymphoma (including Sezary syndrome and mycosis fungoides (MF)). The compositions and methods described herein may be used to treat cdiseases such as Embryonal Tumors of Central Nervous System, Endometrial Cancer, Ependymoblastoma, Ependymoma, Esophageal Cancer, Ewing Sarcoma Family of Tumors, Extracranial Germ Cell Tumor, Extragonadal Germ Cell Tumor, Extrahepatic Bile Duct Cancer, and Eye Cancer, including Intraocular Melanoma and Retinoblastoma. The compositions and methods described herein may be used to treat diseases such as Gallbladder Cancer, Gastric (Stomach) Cancer, Gastrointestinal Carcinoid Tumor, Gastrointestinal Stromal Tumor (GIST), Germ Cell Tumor, Gestational Trophoblastic Tumor, and Glioma. The compositions and methods described herein may be used to treat a cancer selected from the group consisting of Hairy Cell Leukemia, Head and Neck Cancer, Hepatocellular (Liver) Cancer, Histiocytosis, Hodgkin Lymphoma, and Hypopharyngeal Cancer. The compositions and methods described herein may be used to treat diseases such as Kaposi Sarcoma and Kidney (Renal Cell) Cancer. The compositions and methods described herein may be used to treat diseases such as Langerhans Cell Histiocytosis, Laryngeal Cancer, Lip and Oral Cavity Cancer, Liver Cancer, Lung Cancer, including Non-Small Cell Lung Cancer, and Small Cell Lung Cancer, Non-Hodgkin Lymphoma, and Primary Central Nervous System Lymphoma. The compositions and methods described herein may be used to treat diseases such as Waldenström's macroglobulinemia (lymphoplasmacytic lymphoma), Malignant Fibrous Histiocytoma of Bone and Osteosarcoma, Medulloblastoma, Medulloepithelioma, Melanoma, Merkel Cell Carcinoma, Mesothelioma, Metastatic Squamous Neck Cancer with

Occult Primary, Multiple Endocrine Neoplasia Syndrome, Mouth Cancer, Multiple Myeloma/Plasma Cell Neoplasm, Mycosis Fungoides, Myelodysplastic Syndromes, complex karyotype, blastic phase leukemia, Myelodysplastic/Myeloproliferative Neoplasms, Multiple Myeloma, and Myeloproliferative Disorders. The compositions and methods described herein may be used to treat cancer. The compositions and methods described herein may be used to treat diseases such as Nasal Cavity and Paranasal Sinus Cancer, Nasopharyngeal Cancer, and Neuroblastoma. The compositions and methods described herein may be used to treat diseases such as Oral Cancer,Lip and Oral Cavity Cancer, Oropharyngeal Cancer, Osteosarcoma and Malignant Fibrous Histiocytoma of Bone, Ovarian Cancer, Ovarian Germ Cell Tumor, Ovarian Epithelial Cancer, and Ovarian Low Malignant Potential Tumor. The compositions and methods described herein may be used to treat diseases such as Pancreatic Cancer, Papillomatosis, Paranasal Sinus and Nasal Cavity Cancer, Parathyroid Cancer, Penile Cancer, Pharyngeal Cancer, Pineal Parenchymal Tumors of Intermediate Differentiation, Pineoblastoma and Supratentorial Primitive Neuroectodermal Tumors, Pituitary Tumor, Pleuropulmonary Blastoma, Pregnancy and Breast Cancer, Primary Central Nervous System Lymphoma, and Prostate Cancer. Tthe compositions and methods described herein may be used to treat a cancer selected from the group consisting of Rectal Cancer, Renal Cell (Kidney) Cancer, Renal Pelvis and Ureter, Respiratory Tract Carcinoma Involving the NUT Gene on Chromosome 15, Retinoblastoma, and Rhabdomyosarcoma. The compositions and methods described herein may be used to treat high grade prostate cancer,medium grade prostate cancer, or low grade prostate cancer. The compositions and methods described herein may be used to treat castration-resistant prostate cancer. The compositions and methods described herein may be used to treat a nervous system tumor, such as a central nervous system tumor. Tthe compositions and methods described herein may be used to treat a peripheral nervous system tumor. The compositions and methods described herein may be used to treat a paraganglioma. The compositions and methods described herein may be used to treat a pheochromocytoma.

[0141] In *in vitro* models, in animal models, and in human clinical trials compound (1) (ONC201) has broad anti-cancer activity, low toxicity including few, if any, adverse effects, low genotoxicity, and high bioavailability including oral bioavailability. These features allow ONC 201 and various analogs to be particularly well suited for pediatric patients. These features also make ONC 201 and various analogs particularly well suited for chronic therapy, for high risk patients, and to ensure long-lasting responses or stable disease or to prevent disease recurrence.

[0142] The compositions and methods described herein may be used to treat a pediatric cancer (*e.g.*, pediatric solid tumors, pediatric sarcomas, pediatric Ewing's sarcomas, pediatric gliomas, pediatric central nervous system cancers, pediatric neuroblastoma, pediatric leukemia and pediatric lymphoma).

[0143] The compositions and methods described herein may be used to treat a proliferative skin disorder such as psoriasis. The compositions and methods described herein may be used to treat a cancer selected from the group consisting of Salivary Gland Cancer, Sarcoma, Sézary Syndrome, Skin Cancer, Ocular Cancer, Skin Carcinoma, Small Intestine Cancer, Soft Tissue Sarcoma, Squamous Cell Carcinoma, Squamous Neck Cancer with Occult Primary, and Supratentorial Primitive Neuroectodermal Tumors. The compositions and methods described herein may be used to treat a cancer selected from the group consisting of T-Cell Lymphoma, Testicular Cancer, Throat Cancer, Thymoma and Thymic Carcinoma, Thyroid Cancer, Transitional Cell Cancer of the Renal Pelvis and Ureter, and Gestational Trophoblastic Tumor. The compositions and methods described herein may be used to treat a cancer selected from the group consisting of Carcinoma of Unknown Primary Site, Cancer of Unknown Primary Site, Unusual Cancers of Childhood, Transitional Cell Cancer of the Renal Pelvis and Ureter, Urethral Cancer, and Uterine Sarcoma. The compositions and methods described herein may be used to treat cancer selected from the group consisting of Vaginal Cancer and Vulvar Cancer. The compositions and methods described herein may be used to treat a cancer selected from the group consisting of Wilms Tumor and Women's Cancers.

[0144] The compositions and methods described herein may be used as a first-line therapy (sometimes called primary therapy), a second-line therapy, or a third-line therapy. The compositions and methods described herein may be used as a salvage therapy. The term "salvage therapy" means a therapeutic agent that can be taken with any regimen after a subject's initial treatment regimen has failed or after the subject's condition has not responded to an initial treatment. The compositions and methods described herein may be used as a rescue therapy such as when the compositions are used as a rescue agent to counteract the action of an initial treatmentor as a rescue

agent which is administered to a subject who has developed resistance to a standard or an initial treatment. The compositions and methods described herein may be used as a neoadjuvant therapy, which may comprise administration of one or more of the therapeutic agents described herein to a subject before a main or first line treatment. The neoadjuvant therapy may reduce the size or extent of the cancer being treated before a main or first line treatment is administered to the subject undergoing treament. The compositions and methods described herein may be used as an adjuvant therapy, which may comprise administration of one or more therapeutic agents described herein to a subject, wherein the one or more therapeutic agent that modify the effect of other therapeutic agents that are already administered to the subject or are concurrently administered to the subject or subsequently administered to the subject.

[0145] The compositions and methods described herein may exhibit reduced chance of drug-drug interactions. An imipridone, such as ONC201, or an analog thereof may be eliminated from the patient's body before it can interact with another pharmaceutically active agent.

[0146] The compositions and methods of described herein may exhibit toxicity levels that facilitates combinations with other pharamaceutical agents.

[0147] The methods and compositions described herein are not limited to a particular animal species. A subject treated according to methods and using compositions described herein, may be mammalian or non-mammalian. A mammalian subject mammal may include, but is not limited to, a human; a non-human primate; a rodent such as a mouse, rat, or guinea pig; a domesticated pet such as a cat or dog; a horse, cow, pig, sheep, goat, or rabbit. A non-mammalian subject may include, but is not limited to, a bird such as a duck, goose, chicken, or turkey. The subject may be a human. Subjects may be either gender and any age. The composition and methods may also be used to prevent cancer. The composition and methods may also be used to stimulate the immune system.

[0148] The methods and compositions described herein are not limited to a particular age of the subject. A subject treated according to methods and using compositions described herein may be over 50 years old, over 55 years old, over 60 years old, or over 65 years old. A subject treated according to methods and using compositions described herein may be under 50 years old, under 55 years old, under 60 years old, or under 65 years old.

[0149] . A subject treated according to methods and using compositions described herein may be a pediatric patient. The pediatric patient may be younger than 18 years old, younger than 17 years old, younger than 16 years old, younger than 15 years old, younger than 14 years old, is younger than 13 years old, younger than 12 years old, younger than 11 years old, younger than 10 years old, younger than 9 years old, younger than 8 years old, younger than 7 years old, younger than 6 years old, younger than 5 years old, younger than 4 years old, younger than 3 years old, younger than 2 years old, younger than 1 year old, younger than 12 months old, younger than 11 months old, younger than 10 months old, younger than 9 months old, younger than 8 months old, younger than 7 months old, younger than 6 months old, is younger than 5 months old, younger than 4 months old, younger than 3 months old, younger than 2 months old, younger than 1 month old, younger than 4 weeks old, younger than 3 weeks old, younger than 2 weeks old, younger than 1 weeks old, 7 days old, younger than 6 days old, younger than 5 days old, younger than 4 days old, younger than 3 days old, younger than 2 days old, or younger than 1 day old. The pediatric patient may be a neonate. The pediatric patient may be prematurely born.

[0150] The patient may be less than 45 kg in weight, less than 40 kg in weight, less than 35 kg in weight, less than 30 kg in weight, less than 25 kg in weight, less than 20 kg in weight, less than 15 kg in weight, less than 14 kg in weight, less than 10 kg in weight, less than 5 kg in weight, less than 4 kg in weight, less than 3 kg in weight, less than 2 kg in weight, or less than 1 kg in weight.

[0151] The subject may have received at least one prior therapeutic agent, such as at least two, at least three, or at least four prior therapeutic agents The prior therapeutic agent may be ibrutinib, bortezomib, carfilzomib, temozolomide, bevacizumab, cyclophosphamide, hydroxydaunorubicin, vincristine, prednisone, cytarabine, cisplatin, rituximab, 5-fluorouracil, oxaliplatin, leucovorin, or lenalidomide.

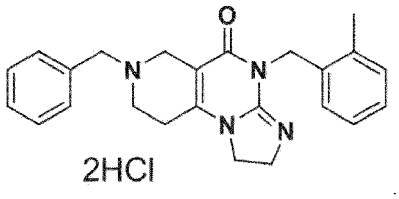
[0152] The subject may have been treated with radiation, surgery, and/or adoptive T-cell therapy.

[0153] The cancer may no longer respond to treatment with ibrutinib, bortezomib, carfilzomib, temozolomide, bevacizumab, cyclophosphamide, hydroxydaunorubicin, vincristine, prednisone, cytarabine, cisplatin, rituximab, 5-fluorouracil, oxaliplatin, leucovorin, lenalidomide, radiation, surgery, or a combination thereof.

[0154] The compositions and methods described herein may have a dose response relation in cancer cells that is different from the dose response relation of the same compositions and methods in normal cells. The dose response relation of ONC201 on proliferation and cell death in normal and tumor cells was determined by measuring cell viability following treatment with ONC201 at various concentrations for 72 hours. The tumors tested included a human colon cancer cell line (HCT116), breast tumor cell line (MDA-MB-231), and a human primary glioblastoma cell line (U87). And the normal cells tested included human foreskin fibroblasts (HFF), human fetal lung fibroblast (MRC-5) cells, and a human lung fibroblast cell line (WI-38). Doxorubicin was used as a positive control at 1 μg/mL in normal fibroblasts. Cell viability of normal cells tested was at least about 75% at about 1-5 mg/mL of ONC201, whereas viability of tumor cells was significantly lower (e.g., at or below 50%) at the same ONC201 concentration. Moreover, as ONC201 concentration increased beyond about 5 mg/mL viability of tumor cells fell to below 25%, whereas viability of normal cells remained at about 75%. Cell viability assays in human fetal lung fibroblast (MRC-5) cells were performed following 72 hour treatment with compound (1) (5 μM) or DMSO and a recovery period in complete drug-free media after treatment. Cell recovery was seen with ONC201, but not with DMSO.

[0155] The compositions and methods described herein may have utility in treating cancer in a subject, such as a human subject. The treatment method may comprise administering to a subject in need of such treatment, a pharmaceutically effective amount of an imipridone, such as ONC201, or an analog thereof, or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable carrier.

[0156] The treatment method may comprise administering to a subject in need of such treatment: (i) a first therapeutic agent including an imipridone, such as ONC201, or an analog thereof, or a pharmaceutically acceptable salt thereof in combination with (ii) a second therapeutic agent, wherein the first and the second therapeutic agents are administered either simultaneously or sequentially. The second therapeutic agent can be any suitable therapeutic agent, including any pharmaceutically active agent disclosed herein. A pharmaceutically acceptable ONC201 salt includes the di-hydrochloride salt below:



[0157] It is understood that a di-hydrochloride salt of ONC201 or an analog thereof (including a compound of formula **(10)),** or an alternative di-salt thereof apparent from the teaching of this disclosure, can be substitued for ONC201 or an analog thereof in a composition or dosing regimen described hererin.

[0158] The treatment method may comprise administering a synergistic pharmaceutical combination, either simultaneously or sequentially, to a subject in need of such treatment, wherein the synergistic pharmaceutical combination comprises (i) a first therapeutic agent comprising an imipridone, such as ONC201, or an analog thereof, or a pharmaceutically acceptable salt thereof; and (ii) a second therapeutic agent. The treatment method may comprise administering to a subject in need of such treatment, either simultaneously or sequentially, therapeutically synergistic effective amounts of the first therapeutic agent in combination with the second therapeutic agent. The treatment method may comprise administering to a subject in need of such treatment, an effective amount of the first therapeutic agent in combination with an effective amount of the second therapeutic agent, wherein the combination provides a synergistic effect in the *in vivo* treatment of a cancer sensitive to the combination, and wherein the first and the second therapeutic agents are administered either simultaneously or

sequentially. The treatment method may comprise administering to a subject in need of such treatment, an effective amount of the first therapeutic agent in combination with an effective amount of a second therapeutic agent, wherein the combination provides a synergistic effect in the *in vivo* treatment of a minimal residual disease sensitive to the combination, and wherein the first and second therapeutic agents are administered either simultaneously or sequentially. The second therapeutic agent may be given before or prior to the first therapeutic agent.

[0159] The treatment method may target a cancer selected from the group consisting of solid tumors, liquid tumors, lymphomas, leukemias, or myelomas.

[0160] The treatment method may target a solid tumor, wherein the solid tumor is selected from the group consisting of: Cervical Cancer, Endometrial Cancer, Extracranial Germ Cell Tumor; Extragonadal Germ Cell Tumor; Germ Cell Tumor; Gestational Trophoblastic Tumor; Ovarian Cancer, Ovarian Germ Cell Tumor, Ovarian Epithelial Cancer, and Ovarian Low Malignant Potential Tumor; Penile Cancer, Prostate Cancer; Pregnancy and Breast Cancer; high grade prostate cancer; medium grade prostate cancer; low grade prostate cancer; castration-resistant prostate cancer; Breast Cancer; Bile Duct Cancer; Extrahepatic Bile Duct Cancer; Gallbladder Cancer; Hepatocellular (Liver) Cancer; Kidney (Renal Cell) Cancer; Liver Cancer, Renal Cell (Kidney) Cancer, Renal Pelvis and Ureter; Basal Cell Carcinoma; Basal Cell Nevus Syndrome, Gorlin-Nevus Syndrome, Melanoma, Merkel Cell Carcinoma, Papillomatosis, Multiple Endocrine Neoplasia Syndrome; Pancreatic Cancer, Parathyroid Cancer, ocular melanoma; Eye Cancer; Retinoblastoma; Malignant Fibrous Histiocytoma; Ewing Sarcoma Family of Tumors; desmoplastic round cell tumor; chondrosarcoma, Kaposi Sarcoma, Rhabdomyosarcoma; Spinal Cord Tumors, Leptomeningeal Disease, Central Nervous System Embryonal Tumors, Chordoma, Embryonal Tumors of Central Nervous System, Ependymoblastoma, Ependymoma, Neuroblastoma; Pineal Parenchymal Tumors of Intermediate Differentiation, Pineoblastoma; Adrenocortical Carcinoma; Bone Cancer, Osteosarcoma; Malignant Fibrous Histiocytoma of Bone and Osteosarcoma; Osteosarcoma and Malignant Fibrous Histiocytoma of Bone; Carcinoid Tumor, Carcinoma of Unknown Primary, Bronchial Tumors, Lung Cancer, Pleuropulmonary Blastoma; Respiratory Tract Carcinoma Involving the NUT Gene on Chromosome 15, Astrocytomas, Atypical Teratoid/Rhabdoid Tumor; Central Nervous System Atypical Teratoid/Rhabdoid Tumor, Craniopharyngioma, Glioma, Brain cancer, Medulloblastoma, Medulloepithelioma, Supratentorial Primitive Neuroectodermal Tumors; Pituitary Tumor; Gastric (Stomach) Cancer, Gastrointestinal Carcinoid Tumor, Gastrointestinal Stromal Tumor (GIST), Bladder Cancer, Anal or Rectal Cancer, Appendix Cancer, Esophageal Cancer, Hypopharyngeal Cancer; Laryngeal Cancer, Lip and Oral Cavity Cancer, Metastatic Squamous Neck Cancer with Occult Primary, Mouth Cancer, Nasal Cavity and Paranasal Sinus Cancer, Nasopharyngeal Cancer, Oral Cancer, Lip and Oral Cavity Cancer, Oropharyngeal Cancer, Paranasal Sinus and Nasal Cavity Cancer, Pharyngeal Cancer; Head and Neck Cancer, and Mesothelioma.

[0161] The treatment method may target a lymphoma selected from the group consisting of: diffuse large B-cell lymphoma, AIDS-Related Lymphoma, Cutaneous T-Cell Lymphoma, Sezary syndrome, mycosis fungoides (MF); Histiocytosis; Burkitt Lymphoma, and Central Nervous System Lymphoma; Non-Hodgkin Lymphoma, and Primary Central Nervous System Lymphoma, Hodgkin Lymphoma, Waldenström's macroglobulinemia; Mycosis Fungoides; Primary Central Nervous System Lymphoma; lymphoplasmacytic lymphoma, and Primary Central Nervous System Lymphoma.

[0162] The treatment method may target a Non-Hodgkin's lymphoma (NHL) selected from the group consisting of: mantle cell lymphoma, diffuse large B-cell lymphoma, follicular lymphoma, marginal zone lymphoma, small lymphocytic lymphoma, lyphoplasmacytic NHL, Waldenstrom's macroglobulinaemia, and skin lymphomas.

[0163] The treatment method may target a leukemia selected from the group consisting of: Acute Lymphoblastic Leukemia (ALL), Chronic Lymphocytic Leukemia (CLL), Chronic Myeloproliferative Disorders; Hairy Cell Leukemia; Acute Myeloid Leukemia (AML); Chronic Myelogenous Leukemia (CML); and Langerhans Cell Histiocytosis.

[0164] The treatment method may target an acute leukemia selected from the group consisting of: acute lymphotyte leukemia, acute myeloid leukemia, chronic lymphoblasitc leukemia, chronic myeloid leukemia,

myelodysplastic syndrome, and myeloproliferative disease.

[0165] The treatment method may target a myeloma selected from the group consisting of: IgA myeloma; IgG myeloma; IgM myeloma; IgD myeloma; IgE myeloma; light chain myeloma; non secretory myeloma; complex karyotype, blastic phase leukemia; Multiple Myeloma/Plasma Cell Neoplasm, Multiple Myeloma, Myelodysplastic Syndromes, Myelodysplastic/Myeloproliferative Neoplasms, and Myeloproliferative Disorders.

[0166] The treatment method may target a peripheral nervous system tumor. The treatment method may target a paraganglioma. The treatment method may target a pheochromocytoma.

[0167] Treatment of cancer may comprise prevention of tumor growth in a cancer subject. Treatment of cancer may comprise prevention of formation of cancer metastases in a cancer subject. Treatment of cancer may comprise targeted treatment of minimal residual disease in a cancer subject known to have the minimal residual disease in a cancer or a subject at risk for having minimal residual disease.

[0168] This might be indicated after treatment of the primary tumor by surgery and/or after chemotherapy (radiotherapy) has been initiated or determined to be efficaceous. Disseminated tumor cells may be in their dormant state and often cannot be attacked by chemotherapy (radiotherapy). A thus treated patient seemingly is in a healed state, and refered to as "minimal residual disease." Nevertheless, the dormant tumor cells have a potential to form metastases if they become metastasising cells due to a growth stimulus after a longer dormant state.

[0169] The term "minimal residual disease" denotes a small number of cancer cells that remain in a subject during or after treatment when the subject is in remission (exhibiting no symptoms or signs of the disease). The methods described herein are preferably applied to a form of the diseases listed herein, including adult and childhood forms of these diseases.

[0170] The treatment method may be useful for treating an autoimmune disease, such as alopecia areata, antiphospholipid, autoimmune hepatits, celiac disease, diabetes type 1, Graves' disease, Guillain-Barre syndrome, Hashimoto's disease, hemolytic anemia, idiopathic thrombocytopenic purpura, inflammatory bowel disease, inflammatory myopathies, multiple sclerosis, primary biliary cirrhosis, psoriasis, rheumatoid arthritis, scleroderma, Sjögren's syndrome, systemic lupus erythematosus, and vitiligo.

[0171] The treatment method may be useful for treating autoimmune and inflammatory disorders of the peripheral nerve system such as amyotrophic lateral sclerosis (Lou Gehrig's disease), based on various causes such as metabolic disorders that include diabetes, B 12 and folate vitamin deficiencies, chemotherapy medications and medicines used to treat HIV, poisons that cause peripheral nerve damage, cancers that develop peripheral neuropathies as well as paraneoplastic syndromes, alcohol abuse, chronic kidney disease, injuries that cause compression on nerves and other lesions, infections such as Lyme disease, Guillain Barre syndrome, connective tissue disease, rheumatoid arthritis, Sjogren's syndrome, systemic lupus erythematosus, certain inflammatory conditions such as sarcoidosis, coeliac disease, hereditary diseases such as charcot marie tooth syndrome, Friedreich's ataxia, and/or idiopathic where no specific cause is found but inflammatory and/or autoimmune mechanisms are the cause of onset.

[0172] The treatment method may be useful for treating autoimmune and inflammatory disorders with ocular manifestations. Such ocular manifestations include, but are not limited to, ocular cicatricial pemphigoid, Mooren's corneal ulcer, various forms of uveitis, rheumatoid arthritis, systemic lupus erythematosus, polyarteritis nodosa, relapsing polychondritis, Wegener's granulomatosis, scleroderma, Behcet's disease, Reiter's disease, inflammatory bowel disease (ulcerative colitis and Crohn's disease) and ankylosing spondylitis, retinitis pigmentosa, macular degeneration, keratoconjunctivitis sicca, scleritis, episcleritis, keratitis, peripheral corneal ulceration, and less common entities such as choroiditis, retinal vasculitis, episcleral nodules, retinal detachments, and/or macular edema.

[0173] The treatment method may be useful for treating acute allograft rejection in transplant patients. Tthe treatment method may be useful for treating ischemic stroke. The treatment method may be useful for treating inflammatory diseases including arthritis, psoriasis, asthma, and colitis.

[0174] A therapeutic agent may include a pharmaceutically acceptable mono-salt of ONC201 , or a pharmaceutically acceptable ONC201 di-salt. As described herein, some of the analogs can be tri-salts. A therapeutic agent may include ONC201 in the form of a pharmaceutically acceptable mono- or di-salt selected from the group consisting of hydrochloride, hydrobromide, hydrogensulphate, sulfates, phosphates, fumarates, succinates, oxalates and lactates, bisulfates, hydroxyl, tartrate, nitrate, citrate, bitartrate, carbonate, malate, maleate, fumarate sulfonate, methylsulfonate, formate, acetate, and carboxylate. A therapeutic agent may include ONC201 in the form of a pharmaceutically acceptable mono-or di-salt selected from p-toluene-sulfonate, benzenesulfonate, methanesulfonate, oxalate, succinate, tartrate, citrate, fumarate and maleate. A therapeutic agent may include ONC201 in the form of a pharmaceutically acceptable mono- or di-salt having a counter ion selected from the group consisting of ammonium, sodium, potassium, calcium, magnesium, zinc, lithium, and/or with counter-ions such as methylamino, dimethylamino, diethylamino, triethylamino counter-ions, and combinations thereof. A therapeutic agent may include a compound described herein in the form of a halide di-salt, such as a di-hydrochloride salt or a dihydrobromide salt.

[0175] The second therapeutic agent may include an anti-cancer agent. The second therapeutic agent may be selected from acivicin, aclarubicin, acodazole, acronine, adozelesin, aldesleukin, alitretinoin, allopurinol, altretamine, ambomycin, ametantrone, amifostine, aminoglutethimide, amsacrine, anastrozole, anthramycin, arsenic trioxide, asparaginase, asperlin, azacitidine, azetepa, azotomycin, batimastat, benzodepa, bevacizumab, bicalutamide, bisantrene, bisnafide dimesylate, bizelesin, bleomycin, brequinar, bropirimine, busulfan, cactinomycin, calusterone, capecitabine, caracemide, carbetimer, carboplatin, carmustine, carubicin, carzelesin, cedefingol, celecoxib, chlorambucil, cirolemycin, cisplatin, cladribine, crisnatol mesylate, cyclophosphamide, cytarabine, dacarbazine, dactinomycin, daunorubicin, decitabine, dexormaplatin, dezaguanine, dezaguanine mesylate, diaziquone, docetaxel, doxorubicin, droloxifene, dromostanolone, duazomycin, edatrexate, eflomithine, elsamitrucin, enloplatin, enpromate, epipropidine, epirubicin, erbulozole, esorubicin, estramustine, etanidazole, etoposide, etoprine, fadrozole, fazarabine, fenretinide, floxuridine, fludarabine, fluorouracil, flurocitabine, fosquidone, fostriecin, fulvestrant, gemcitabine, hydroxyurea, idarubicin, ifosfamide, ilmofosine, interleukin II (IL-2, including recombinant interleukin II or rIL2), interferon α-2a, interferon α-2b, interferon α-n1, interferon α-n3, interferon β-Ia, interferon gamma-Ib, iproplatin, irinotecan, lanreotide, letrozole, leuprolide, liarozole, lometrexol, lomustine, losoxantrone, masoprocol, maytansine, mechlorethamine hydrochlride, megestrol, melengestrol acetate, melphalan, menogaril, mercaptopurine, methotrexate, metoprine, meturedepa, mitindomide, mitocarcin, mitocromin, mitogillin, mitomalcin, mitomycin, mitosper, mitotane, mitoxantrone, mycophenolic acid, nelarabine, nocodazole, nogalamycin, ormnaplatin, oxisuran, paclitaxel, pegaspargase, peliomycin, pentamustine, peplomycin, perfosfamide, pipobroman, piposulfan, piroxantrone hydrochloride, plicamycin, plomestane, porfimer, porfiromycin, prednimustine, procarbazine, puromycin, pyrazofurin, riboprine, rogletimide, safingol, semustine, simtrazene, sparfosate, sparsomycin, spirogermanium, spiromustine, spiroplatin, streptonigrin, streptozocin, sulofenur, talisomycin, tamoxifen, tecogalan, tegafur, teloxantrone, temoporfin, teniposide, teroxirone, testolactone, thiamiprine, thioguanine, thiotepa, tiazofurin, tirapazamine, topotecan, toremifene, trestolone, triciribine, trimetrexate, triptorelin, tubulozole, uracil mustard, uredepa, vapreotide, verteporfin, vinblastine, vincristine sulfate, vindesine, vinepidine, vinglycinate, vinleurosine, vinorelbine, vinrosidine, vinzolidine, vorozole, zeniplatin, zinostatin, zoledronate, zorubicin and combinations thereof.

[0176] The second therapeutic agent may be selected, from hormone analogs and antihormones, aromatase inhibitors, LHRH agonists and antagonists, inhibitors of growth factors, growth factor antibodies, growth factor receptor antibodies, tyrosine kinase inhibitors; antimetabolites; antitumour antibiotics; platinum derivatives; alkylation agents; antimitotic agents; tubuline inhibitors; PARP inhibitors, topoisomerase inhibitors, serine/threonine kinase inhibitors, tyrosine kinase inhibitors, protein protein interaction inhibitors, MEK inhibitors, ERK inhibitors, IGF-1R inhibitors, ErbB receptor inhibitors, rapamycin analogs, amifostin, anagrelid, clodronat, filgrastin, interferon, interferon α, leucovorin, rituximab, procarbazine, levamisole, mesna, mitotane, pamidronate and porfimer, 2-chlorodesoxyadenosine, 2-fluorodesoxy-cytidine, 2-methoxyoestradiol, 2C4,3-alethine, 131-1-TM-601, 3CPA, 7-

ethyl-10-hydroxycamptothecin, 16-aza-epothilone B, A 105972, A 204197, abiraterone, aldesleukin, alitretinoin, allovectin-7, altretamine, alvocidib, amonafide, anthrapyrazole, AG-2037, AP-5280, apaziquone, apomine, aranose, arglabin, arzoxifene, atamestane, atrasentan, auristatin PE, ABT-199 (Venetoclax), ABT-263 (Navitoclax), AVLB, AZ10992, ABX-EGF, AMG-479 (ganitumab), ARRY 162, ARRY 438162, ARRY-300, ARRY-142886/AZD-6244 (selumetinib), ARRY-704/AZD-8330, AR-12, AR-42, AS-703988, AXL-1717, AZD-8055, AZD-5363, AZD-6244, ARQ-736, ARQ 680, AS-703026 (primasertib), avastin, AZD-2014, azacytidine, azaepothilone B, azonafide, BAY-43-9006, BAY 80-6946, BBR-3464, BBR-3576, bevacizumab, BEZ-235, biricodar dicitrate, BCX-1777, BKM-120, bleocin, BLP-25, BMS-184476, BMS-247550, BMS-188797, BMS-275291, BMS-663513, BMS-754807, BNP-1350, BNP-7787, BIBW 2992 (afatinib, tomtovok), BIBF 1120 (vargatef), BI 836845, BI 2536, BI 6727, BI 836845, BI 847325, BI 853520, BUB-022, bleomycinic acid, bleomycin A, bleomycin B, brivanib, bryostatin-1, bortezomib, brostallicin, busulphan, BYL-719, CA-4 prodrug, CA-4, CapCell, calcitriol, canertinib, canfosfamide, capecitabine, carboxyphthalatoplatin, CCI-779, CC-115, CC-223, CEP-701, CEP-751, CBT-1 cefixime, ceflatonin, ceftriaxone, celecoxib, celmoleukin, cemadotin, CH4987655/RO-4987655, chlorotrianisene, cilengitide, ciclosporin, CDA-II, CDC-394, CKD-602, CKI-27, clofarabin, colchicin, combretastatin A4, COT inhibitors, CHS-828, CH-5132799, CLL-Thera, CMT-3 cryptophycin 52, CTP-37, CTLA-4 monoclonal antibodies, CP-461, CV-247, cyanomorpholinodoxorubicin, cytarabine, D 24851, decitabine, deoxorubicin, deoxyrubicin, deoxycoformycin, depsipeptide, desoxyepothilone B, dexamethasone, dexrazoxanet, diethylstilbestrol, diflomotecan, didox, DMDC, dolastatin 10, doranidazole, DS-7423, E7010, E-6201, edatrexat, edotreotide, efaproxiral, eflomithine, EGFR inhibitors, EKB-569, EKB-509, enzastaurin, enzalutamide, elsamitrucin, epothilone B, epratuzumab, ER-86526, erlotinib, ET-18-0CH3, ethynylcytidine, ethynyloestradiol, exatecan, exatecan mesylate, exemestane, exisulind, fenretinide, figitumumab, floxuridine, folic acid, FOLFOX, FOLFOX4, FOLFIRI, formestane, fotemustine, galarubicin, gallium maltolate, gefinitib, gemtuzumab, gimatecan, glufosfamide, GCS-100, GDC-0623, GDC-0941 (pictrelisib), GDC-0980, GDC-0032, GDC-0068, GDC-0349, GDC-0879, G17DT immunogen, GMK, GPX-100, gp100-peptide vaccines, GSK-5126766, GSK-690693, GSK-1120212 (trametinib), GSK-2118436 (dabrafenib), GSK-2126458, GSK-2132231A, GSK-2334470, GSK-2110183, GSK-2141795, GW2016, granisetron, herceptin, hexamethylmelamine, histamine, homoharringtonine, hyaluronic acid, hydroxyurea, hydroxyprogesterone caproate, ibandronate, ibritumomab, idatrexate, idenestrol, IDN-5109, IGF-1R inhibitors, IMC-1C11, IMC-A12 (cixutumumab), immunol, indisulam, interferon α-2a, interferon α-2b, pegylated interferon α-2b, interleukin-2, INK-1117, INK-128, INSM-18, ionafarnib, ipilimumab, iproplatin, irofulven, isohomohalichondrin-B, isoflavone, isotretinoin, ixabepilone, JRX-2, JSF-154, J-107088, conjugated oestrogens, kahalid F, ketoconazole, KW-2170, KW-2450, lobaplatin, leflunomide, lenograstim, leuprolide, leuporelin, lexidronam, LGD-1550, linezolid, lutetium texaphyrin, lometrexol, losoxantrone, LU 223651, lurtotecan, LY-S6AKT1, LY-2780301, mafosfamide, marimastat, mechloroethamine, MEK inhibitors, MEK-162, methyltestosteron, methylprednisolone, MEDI-573, MEN-10755, MDX-H210, MDX-447, MDX-1379, MGV, midostaurin, minodronic acid, mitomycin, mivobulin, MK-2206, MK-0646 (dalotuzumab), MLN518, motexaf in gadolinium, MS-209, MS-275, MX6, neridronate, neratinib, Nexavar, neovastat, nilotinib, nimesulide, nitroglycerin, nolatrexed, norelin, N-acetylcysteine, 06-benzylguanine, oblimersen, omeprazole, oncophage, oncoVEXGM-CSF, ormiplatin, ortataxel, OX44 antibodies, OSI-027, OSI-906 (linsitinib), 4-1BB antibodies, oxantrazole, oestrogen, panitumumab, patupilone, pegfilgrastim, PCK-3145, pegfilgrastim, PBI-1402, PBI-05204, PDO325901, PD-1 antibodies, PEG-paclitaxel, albumin-stabilized paclitaxel, PEP-005, PF-05197281, PF-05212384, PF-04691502, PHT-427, P-04, PKC412, P54, PI-88, pelitinib, pemetrexed, pentrix, perifosine, perillylalcohol, pertuzumab, PI3K inhibitors, PI3K/mTOR inhibitors, PG-TXL, PG2, PLX-4032/RO-5185426 (vemurafenib), PLX-3603/RO-5212054, PT-100, PWT-33597, PX-866, picoplatin, pivaloyloxymethylbutyrate, pixantrone, phenoxodiol O, PKI166, plevitrexed, plicamycin, polyprenic acid, porfiromycin, prednisone, prednisolone, quinamed, quinupristin, R115777, RAF-265, ramosetron, ranpirnase, RDEA-119BAY 869766, RDEA-436, rebeccamycin analogs, receptor tyrosine kinase (RTK) inhibitors, revimid, RG-7167, RG-7304, RG-7421, RG-7321, RG 7440, rhizoxin, rhu-MAb, rinfabate, risedronate,rituximab, robatumumab, rofecoxib, RO-31-7453, RO-5126766, RO-5068760, RPR 109881A, rubidazone, rubitecan, R-flurbiprofen, RX-0201, S-9788, sabarubicin, SAHA, sargramostim, satraplatin, SB 408075, Se-015/Ve-015, SU5416, SU6668, SDX-101, semustin, seocalcitol, SM-11355, SN-38, SN-4071, SR-27897, SR-31747, SR-13668, SRL-172, sorafenib, spiroplatin, squalamine, suberanilohydroxamic acid, sutent, T 900607, T 138067, TAK-733, TAS-103, tacedinaline, talaporf in, Tarceva, tariquitar, tasisulam, taxotere, taxoprexin, tazarotene, tegafur, temozolamide, tesmilifene, testosterone, testosterone propionate, tesmilifene, tetraplatin, tetrodotoxin, tezacitabine, thalidomide, theralux, therarubicin, thymalfasin, thymectacin, tiazofurin, tipifarnib, tirapazamine, tocladesine, tomudex, toremofin,

trabectedin, TransMID-107, transretinic acid, traszutumab, tremelimumab, tretinoin, triacetyluridine, triapine, triciribine, trimetrexate, TLK-286TXD 258, tykerb/tyverb, urocidin, valrubicin, vatalanib, vincristine, vinflunine, virulizin, WX-UK1, WX-554, vectibix, xeloda, XELOX, XL-147, XL-228, XL-281, XL-518/R-7420/GDC-0973, XL-765, YM-511, YM-598, ZD-4190, ZD-6474, ZD-4054, ZD-0473, ZD-6126, ZD-9331, ZD1839, ZSTK-474, zoledronat, zosuquidar, and combinations thereof.

[0177] The second therapeutic agent may be selected from tamoxifen, toremifene, raloxifene, fulvestrant, megestrol acetate, flutamide, nilutamide, bicalutamide, aminoglutethimide, cyproterone acetate, finasteride, buserelin acetate, fludrocortisone, fluoxymesterone, medroxy-progesterone, octreotide, and combinations thereof. The second therapeutic agent may be selected from LHRH agonists and LHRH antagonists. A LHRH agonist may be selected from goserelin acetate, luprolide acetate, triptorelin pamoate and combinations thereof. The second therapeutic agent may include a LHRH antagonist is selected from Degarelix, Cetrorelix, Abarelix, Ozarelix, Degarelix combinations thereof. The second therapeutic agent may include an inhibitor of a growth factor. such as inhibitors of: platelet derived growth factor (PDGF), fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), insuline-like growth factors (IGF), human epidermal growth factor (HER), hepatocyte growth factor (HGF), and combinations thereof. The human epidermal growth factor (HER) may be selected from HER2, HER3, and HER4.

[0178] The second therapeutic agent may include a tyrosine kinase inhibitor, such as cetuximab, gefitinib, imatinib, lapatinib and trastuzumab, and combinations thereof. The second therapeutic agent may include an aromatase inhibitor, such as anastrozole, letrozole, liarozole, vorozole, exemestane, atamestane, and combinations thereof.

[0179] The second therapeutic agent may include an antimetabolite. The antimetabolite may comprise an antifolate. The antifolate may be selected from methotrexate, raltitrexed, pyrimidine analogs, and combinations thereof. The antimetabolite may be a pyrimidine analog, such as 5-fluorouracil, capecitabin, gemcitabin, and combination thereof. The antimetabolite may be a purine analog or an adenosine analog, such as mercaptopurine, thioguanine, cladribine and pentostatin, cytarabine, fludarabine, and combinations thereof. The second therapeutic agent may include an antitumour antibiotic. The antitumor antibiotic may be selected from anthracyclins, doxorubicin, daunorubicin, epirubicin and idarubicin, mitomycin-C, bleomycin, dactinomycin, plicamycin, streptozocin and combinations thereof. The second therapeutic agent may include a platinum derivative, such as cisplatin, oxaliplatin, carboplatin and combinations thereof. The second therapeutic agent may include an alkylation agent, such as estramustin, meclorethamine, melphalan, chlorambucil, busulphan, dacarbazin, cyclophosphamide, ifosfamide, temozolomide, nitrosoureas, and combinations thereof. The second therapeutic agent may include a nitrosourea, such as carmustin, lomustin, thiotepa, and combinations thereof. The second therapeutic agent may include an antimitotic agent, such as one selected from Vinca alkaloids and taxanes. The taxane may be selected from paclitaxel, docetaxel, and combinations thereof. The Vinca alkaloids may be selected from vinblastine, vindesin, vinorelbin, vincristine, and combinations thereof. The second therapeutic agent may include a topoisomerase inhibitor, such as epipodophyllotoxin, for example etoposide, etopophos, teniposide, amsacrin, topotecan, irinotecan, mitoxantron, and combinations thereof. The second therapeutic agent may include a serine/threonine kinase inhibitor, such as PDK 1 inhibitors, B-Raf inhibitors, mTOR inhibitors, mTORC1 inhibitors, PI3K inhibitors, dual mTOR/PI3K inhibitors, STK 33 inhibitors, AKT inhibitors, PLK 1 inhibitors, inhibitors of CDKs, Aurora kinase inhibitors, and combinations thereof. The second therapeutic agent may include a tyrosine kinase inhibitor. The second therapeutic agent may include a PTK2/FAK inhibitor. The second therapeutic agent may include a protein protein interaction inhibitor, such as IAP, Mcl-1, MDM2/MDMX and combinations thereof. The second therapeutic agent may include a rapamycin analog, such as everolimus, temsirolimus, ridaforolimus, sirolimus, and combinations thereof. The second therapeutic agent may be selected from amifostin, anagrelid, clodronat, filgrastin, interferon, interferon α, leucovorin, rituximab, procarbazine, levamisole, mesna, mitotane, pamidronate and porfimer, and combinations thereof. The second therapeutic agent may be selected from 2-chlorodesoxyadenosine, 2-fluorodesoxy-cytidine, 2-methoxyoestradiol, 2C4,3-alethine, 131-1-TM-601, 3CPA, 7-ethyl-10-hydroxycamptothecin, 16-aza-epothilone B, A 105972, A 204197, abiraterone, aldesleukin, alitretinoin, allovectin-7, altretamine, alvocidib, amonafide, anthrapyrazole, AG-2037, AP-5280, apaziquone, apomine, aranose, arglabin, arzoxifene, atamestane, atrasentan, auristatin PE, ABT-199 (Venetoclax), ABT-263 (Navitoclax), AVLB, AZ10992, ABX-EGF, AMG-479 (ganitumab), ARRY 162, ARRY 438162, ARRY-300, ARRY-142886/AZD-

6244 (selumetinib), ARRY-704/AZD-8330, AR-12, AR-42, AS-703988, AXL-1717, AZD-8055, AZD-5363, AZD-6244, ARQ-736, ARQ 680, AS-703026 (primasertib), avastin, AZD-2014, azacytidine, azaepothilone B, azonafide, BAY-43-9006, BAY 80-6946, BBR-3464, BBR-3576, bevacizumab, BEZ-235, biricodar dicitrate, BCX-1777, BKM-120, bleocin, BLP-25, BMS-184476, BMS-247550, BMS-188797, BMS-275291, BMS-663513, BMS-754807, BNP-1350, BNP-7787, BIBW 2992 (afatinib, tomtovok), BIBF 1120 (vargatef), BI 836845, BI 2536, BI 6727, BI 836845, BI 847325, BI 853520, BUB-022, bleomycinic acid, bleomycin A, bleomycin B, brivanib, bryostatin-1, bortezomib, brostallicin, busulphan, BYL-719, CA-4 prodrug, CA-4, CapCell, calcitriol, canertinib, canfosfamide, capecitabine, carboxyphthalatoplatin, CCI-779, CC-115, CC-223, CEP-701, CEP-751, CBT-1 cefixime, ceflatonin, ceftriaxone, celecoxib, celmoleukin, cemadotin, CH4987655/RO-4987655, chlorotrianisene, cilengitide, ciclosporin, CDA-II, CDC-394, CKD-602, CKI-27, clofarabin, colchicin, combretastatin A4, COT inhibitors, CHS-828, CH-5132799, CLL-Thera, CMT-3 cryptophycin 52, CTP-37, CTLA-4 monoclonal antibodies, CP-461, CV-247, cyanomorpholinodoxorubicin, cytarabine, D 24851, decitabine, deoxorubicin, deoxyrubicin, deoxycoformycin, depsipeptide, desoxyepothilone B, dexamethasone, dexrazoxanet, diethylstilbestrol, diflomotecan, didox, DMDC, dolastatin 10, doranidazole, DS-7423, E7010, E-6201, edatrexat, edotreotide, efaproxiral, eflornithine, EGFR inhibitors, EKB-569, EKB-509, enzastaurin, enzalutamide, elsamitrucin, epothilone B, epratuzumab, ER-86526, erlotinib, ET-18-0CH3, ethynylcytidine, ethynyloestradiol, exatecan, exatecan mesylate, exemestane, exisulind, fenretinide, figitumumab, floxuridine, folic acid, FOLFOX, FOLFOX4, FOLFIRI, formestane, fotemustine, galarubicin, gallium maltolate, gefinitib, gemtuzumab, gimatecan, glufosfamide, GCS-100, GDC-0623, GDC-0941 (pictrelisib), GDC-0980, GDC-0032, GDC-0068, GDC-0349, GDC-0879, G17DT immunogen, GMK, GPX-100, gp100-peptide vaccines, GSK-5126766, GSK-690693, GSK-1120212 (trametinib), GSK-2118436 (dabrafenib), GSK-2126458, GSK-2132231A, GSK-2334470, GSK-2110183, GSK-2141795, GW2016, granisetron, herceptine, hexamethylmelamine, histamine, homoharringtonine, hyaluronic acid, hydroxyurea, hydroxyprogesterone caproate, ibandronate, ibritumomab, idatrexate, idenestrol, IDN-5109, IGF-1R inhibitors, IMC-1C11, IMC-A12 (cixutumumab), immunol, indisulam, interferon α-2a, interferon α-2b, pegylated interferon α-2b, interleukin-2, INK-1117, INK-128, INSM-18, ionafarnib, ipilimumab, iproplatin, irofulven, isohomohalichondrin-B, isoflavone, isotretinoin, ixabepilone, JRX-2, JSF-154, J-107088, conjugated oestrogens, kahalid F, ketoconazole, KW-2170, KW-2450, lobaplatin, leflunomide, lenograstim, leuprolide, leuporelin, lexidronam, LGD-1550, linezolid, lutetium texaphyrin, lometrexol, losoxantrone, LU 223651, lurtotecan, LY-S6AKT1, LY-2780301, mafosfamide, marimastat, mechloroethamine, MEK inhibitors, MEK-162, methyltestosteron, methylprednisolone, MEDI-573, MEN-10755, MDX-H210, MDX-447, MDX-1379, MGV, midostaurin, minodronic acid, mitomycin, mivobulin, MK-2206, MK-0646 (dalotuzumab), MLN518, motexaf in gadolinium, MS-209, MS-275, MX6, neridronate, neratinib, Nexavar, neovastat, nilotinib, nimesulide, nitroglycerin, nolatrexed, norelin, N-acetylcysteine, 06-benzylguanine, oblimersen, omeprazole, oncophage, oncoVEXGM-CSF, ormiplatin, ortataxel, OX44 antibodies, OSI-027, OSI-906 (linsitinib), 4-1BB antibodies, oxantrazole, oestrogen, panitumumab, patupilone, pegfilgrastim, PCK-3145, pegfilgrastim, PBI-1402, PBI-05204, PDO325901, PD-1 antibodies, PEG-paclitaxel, albumin-stabilized paclitaxel, PEP-005, PF-05197281, PF-05212384, PF-04691502, PHT-427, P-04, PKC412, P54, PI-88, pelitinib, pemetrexed, pentrix, perifosine, perillylalcohol, pertuzumab, PI3K inhibitors, PI3K/mTOR inhibitors, PG-TXL, PG2, PLX-4032/RO-5185426 (vemurafenib), PLX-3603/RO-5212054, PT-100, PWT-33597, PX-866, picoplatin, pivaloyloxymethylbutyrate, pixantrone, phenoxodiol O, PKI166, plevitrexed, plicamycin, polyprenic acid, porfiromycin, prednisone, prednisolone, quinamed, quinupristin, R115777, RAF-265, ramosetron, ranpirnase, RDEA-119/BAY 869766, RDEA-436, rebeccamycin analogs, receptor tyrosine kinase (RTK) inhibitors, revimid, RG-7167, RG-7304, RG-7421, RG-7321, RG 7440, rhizoxin, rhu-MAb, rinfabate, risedronate,rituximab, robatumumab, rofecoxib, RO-31-7453, RO-5126766, RO-5068760, RPR 109881A, rubidazone, rubitecan, R-flurbiprofen, RX-0201, S-9788, sabarubicin, SAHA, sargramostim, satraplatin, SB 408075, Se-015/Ve-015, SU5416, SU6668, SDX-101, semustin, seocalcitol, SM-11355, SN-38, SN-4071, SR-27897, SR-31747, SR-13668, SRL-172, sorafenib, spiroplatin, squalamine, suberanilohydroxamic acid, sutent, T 900607, T 138067, TAK-733, TAS-103, tacedinaline, talaporf in, Tarceva, tariquitar, tasisulam, taxotere, taxoprexin, tazarotene, tegafur, temozolamide, tesmilifene, testosterone, testosterone propionate, tesmilifene, tetraplatin, tetrodotoxin, tezacitabine, thalidomide, theralux, therarubicin, thymalfasin, thymectacin, tiazofurin, tipifarnib, tirapazamine, tocladesine, tomudex, toremofin, trabectedin, TransMID-107, transretinic acid, traszutumab, tremelimumab, tretinoin, triacetyluridine, triapine, triciribine, trimetrexate, TLK-286TXD 258, tykerb/tyverb, urocidin, valrubicin, vatalanib, vincristine, vinflunine, virulizin, WX-UK1, WX-554, vectibix, xeloda, XELOX, XL-147, XL-228, XL-281, XL-518/R-7420/GDC-0973, XL-765, YM-511, YM-598, ZD-4190, ZD-6474, ZD-4054, ZD-0473, ZD-6126, ZD-9331, ZD1839, ZSTK-474, zoledronat,

zosuquidar, and combinations thereof.

[0180] The other therapeutic agent may comprise a steroid, including dexamethasone, prednisolone, methyl prednisolone, prednisone, hydrocortisone, triamcinolone, betamethasone, and cortivazol. The other therapeutic agent may comprise an anti-emetic. Anti-emetics include, but are not limited to, 5-HT3 receptor agonists (such as dolasetron, granisetron, ondansetron, tropisetron, palonosetron, and mirtazapine), dopamine agonists (such as domperidone, olanzapine, droperidol, haloperidol, chlorpromazine, prochlorperazine, alizapride, prochlorperazine, and metoclopramide), NK1 receptor antagonists (such as aprepitant and casopitant), antihistamines (such as cyclizine, diphenhydramine, dimenhydrinate, doxylamine, meclizine, promethazine, hydroxyzine), cannabinoids (such as cannabis, dronabinol, nabilone, and sativex), benzodiazepines (such as midazolam and lorazepam), anticholinergics (such as hyoscine), trimethobenzamide, ginger, emetrol, propofol, peppermint, muscimol, and ajwain.

[0181] Pharmaceutical compositions may be administered to a subject via any suitable route of administration. The pharmaceutical composition may be administered to a subject orally, parenterally, transdermally or transmucosally. The pharmaceutical composition may be administered to a subject parenterally. The pharmaceutical composition may be administered to a subject via a parenteral route of administration selected from the group consisting of intravenous (IV), subcutaneous (SC), and intramuscular (IM). The pharmaceutical composition may be administered to a subject via a route of administration selected from rectal and transdermal. The pharmaceutical composition may be administered to a subject in a dosage form selected from the group consisting of sterile solutions, suspensions, suppositories, tablets and capsules. The pharmaceutical composition may be administered to a subject in an oral dosage form selected from the group consisting of a tablet, caplet, capsule, lozenge, syrup, liquid, suspension and elixir. The pharmaceutical composition may be administered to a subject in an oral dosage form selected from the group consisting of tablets, hard shell capsules, soft gelatin capsules, beads, granules, aggregates, powders, gels, solids and semi-solids.

[0182] The pharmaceutical composition may be administered to a subject as a dosage form selected from the group consisting of sustained release forms, controlled release forms, delayed release forms and response release forms.

[0183] The pharmaceutical composition may be administered to a subject once daily. The pharmaceutical composition may be administered to a subject accoridng to an infrequent dosing regimen (e.g., administered once per week or less frequently),or a frequent dosing regimen (e.g., administered more than once per week). The pharmaceutical composition may be administered to a subject once weekly, once every four weeks, twice a week, once every two weeks, or once every three weeks. The pharmaceutical composition may be administered to a subject in a repeated cycle of once weekly, once every two weeks, once every three weeks, once every four weeks or combinations thereof.

[0184] The treatment method may comprise administering to a subject in need of such treatment: (i) a first therapeutic agent including a compound comprising an imipridone, such as ONC201, or an analog thereof, or a pharmaceutically acceptable salt thereof in combination with (ii) a second therapeutic agent, wherein the first therapeutic agent and the second therapeutic agent are administered either simultaneously or sequentially; and further comprises assaying the expression of an endoplasmic reticulum (ER) stress response gene in a biological sample. The endoplasmic reticulum stress response gene may be selected from the group that includes, but is not limited to, C/EBP-Homologous Protein (CHOP), Activating Transcription Factor 3 (ATF3) and both CHOP and ATF3. The endoplasmic reticulum stress response gene may be selected from the group that includes, but is not limited to, ATF3, Activating Transcription Factor 4 (ATF4) CHOP, IRE1, Binding immunoglobulin protein (BiP), Eukaryotic translation initiation factor 2A (eIF2a), X-box binding protein 1 (XBP1). The biological sample may be tumor, peripheral blood mononuclear cells, or skin biopsy. The biological sample may be obtained before, during, or after drug administration. The treatment method may further comprise adjusting a dose of the first therapeutic agent to achieve induction of about 50%, 75%, 100%, 125%, 150%, 175%, 200%, 225%, 250%, 275%, 300%, 325%, 350%, 375%, 400%, 425%, 450%, 475%, 500%, 525%, 550%, 575%, 600%, or greater than 600% of one or more ER stress gene. The treatment method may further comprise adjusting a dose of the first therapeutic agent

to achieve induction of about 50% to about 100%, about 100% to about 150%, about 150% to about 200%, about 200% to about 250%, about 250% to about 300%, about 300% to about 350%, about 350% to about 400%, about 400% to about 450%, about 450% to about 500%, about 500% to about 550%, about 550% to about 600%, or greater than 600% of ER stress genes. The treatment method may further comprise adjusting a dose of the first therapeutic agent to achieve induction of about 50% to about 100%, about 100% to about 200%, about 200% to about 300%, about 300% to about 400%, about 400% to about 500%, about 500% to about 600%, or greater than 600% of ER stress genes.

[0185] The treatment method may comprise administering to a subject in need of such treatment: (i) a first therapeutic agent including a compound comprising an imipridone, such as ONC201, an analog thereof, or a pharmaceutically acceptable salt thereof in combination with (ii) a second therapeutic agent, wherein the first therapeutic agent and the second therapeutic agent are administered either simultaneously or sequentially; and further comprises assaying the expression of proteasomal activity in a biological sample. The proteasomal activity may be chymotrysin-like, trypsin-like, and/or caspase-like activity. The biological sample may be tumor, peripheral blood mononuclear cells, or skin cells. The biological sample may be obtained before, during, or after drug administration. The treatment method may further comprise adjusting the dose to achieve inhibition of about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, or about 100% of the proteasomal activity. The treatment method may further comprise adjusting the dose to achieve inhibition of at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% of the proteasomal activity The treatment method may further comprise adjusting the dose to achieve inhibition of about 20% to about 30%, about 30% to about 40%, about 40% to about 50%, about 50% to about 60%, about 60% to about 70%, about 70% to about 80%, about 80% to about 90%, or greater than 90% of the proteasomal activity.

[0186] Also provided herein are treatment methods, which comprise administering to a subject in need of such treatment a combination of a first therapeutic agent including an imipridone, such as ONC201, an analog thereof, or a pharmaceutically acceptable salt thereof (e.g., a di-salt or tri-salt) and a second therapeutic agent, the method comprising:

1. (i) administering to the subject the first therapeutic agent;
2. (ii) waiting until a predetermined waiting time has elapsed after the time of administration of the first therapeutic agent to the subject; and/or until adverse events are resolved or resolving; and
3. (iii) administering the second therapeutic agent to the subject, wherein the predetermined waiting time is chosen so as to obtain a delayed therapeutic effect of the first therapeutic agent without an increased risk of possible combined toxic effects of the first and second therapeutic agents. The predetermined waiting time may be determined based on the clearance rate of the compound of the first therapeutic agent or a metabolite thereof. The predetermined waiting time may be determined by a quantitative assessment of renal function and parameters of renal. The predetermined waiting time may be determined by an assay for the determination of renal function, wherein the assay is selected from the group consisting of serum level the compound of the first therapeutic agent or a metabolite thereof; clearance rate of the compound of the first therapeutic agent or a metabolite thereof; 24-hour urinary clearance of the compound of the first therapeutic agent or a metabolite thereof.

[0187] The predetermined waiting time may substantially equal the time required for systemic clearance of the compound of the first therapeutic agent or a metabolite thereof from the subject's body. The predetermined waiting time may substantially equal the time required for renal clearance of the compound of the first therapeutic agent or a metabolite thereof from the subject's body. The predetermined waiting time may substantially equal the time required for hepatic clearance of the compound of the first therapeutic agent or a metabolite thereof from the subject's body. The predetermined waiting time may substantially equal the time required for total clearance of the compound of the first therapeutic agent or a metabolite thereof from the subject's body. The predetermined waiting time may be about 4 hours or 1 day. The waiting time may be until C_{max} of the compound of the first therapeutic

agent has passed. The waiting time may be after most of the adverse events are resolved or are resolving. The predetermined waiting time may be about 2 days, about 3 days, about 4 days, about 5 days, about 6 days, or about 7 days. The predetermined waiting time may be a range of about 1-7 days, about 1-6 days, about 1-5 days, about 1-4 days, about 1-3 days, or about 1 to 2 days. The waiting time may be up to 3 weeks. The preceeding are considered "therapeutic time periods."

[0188] When the order of administration is reversed, timing for the administration of the first therapeutic agent can be after the C_{max} of the second therapeutic agent (i.e., the first administered drug) has passed. Administration of the first therapeutic agent can be after most or substantially all of the first administered drug has been eliminated from the body or the toxicity effects for the first administered drug are resolved or are resolving.

[0189] The treatment method may further comprise monitoring levels of the compound of the first therapeutic agent or a metabolite thereof in the subject using pharmacokinetic profiling. Monitoring levels of the compound of the first therapeutic agent or a metabolite thereof in the subject using pharmacokinetic profiling may comprise constructing a pharmacokinetic profile of the compound of the first therapeutic agent or a metabolite thereof for the subject using concentrations of the compound of the first therapeutic agent or a metabolite thereof in at least two samples obtained from the subject at time points suitable to construct a pharmacokinetic profile. When levels of the compound of the first therapeutic agent or a metabolite thereof in the subject using pharmacokinetic profiling are monitored, samples may be collected from the subject at point-of-care or point of use by sampling or self-sampling on point-of-care devices or point of use devices or on matrices suitable for storage of the samples prior to quantitation in a laboratory. Each of the point-of-care devices or point of use devices may be capable of quantitating the compound of the first therapeutic agent or a metabolite thereof. When levels of the compound of the first therapeutic agent or a metabolite thereof in the subject are monitored, one or more samples may be collected from the subject at point-of-care or point of use by biopsy device for analysis at the point-of-care or point of use devices or for storage prior to analysis by a laboratory. A biopsy may be taken after a time interval of 3-8 hours following administration the first therapeutic agent to the subject, 3-24 hours following administration of the first therapeutic agent to the subject, 8-24 hours following administration of the first therapeutic agent thereof to the subject, 2 days following administration of the first therapeutic agent to the , 3 days following administration of the first therapeutic agent to the subject, or 4 days following administration of the first therapeutic agent to the subject. A biopsy may be taken after a time interval of 1-7 days following administration of the first therapeutic agent.

[0190] The pharmacokinetic profile may include pharmacokinetic parameters suitable for guiding dosing of the first therapeutic agent for the subject being treated. The C_{max} of the first therapeutic agent following its administration to the subject may range from about 1000 ng/dL to 1500 ng/dL for a therapeutic time period. C_{max} may be less than 1500 ng/dL and greater than 85 ng/dL for a therapeutic time period. C_{max} of the first therapeutic following its administration to the subject may range from about 1000 ng/mL to 1500 ng/mL for a therapeutic time period. C_{max} may be less than 1500 ng/mL and greater than 85 ng/mL for a therapeutic time period.

[0191] Maximum concentration of the first therapeutic agent in blood (whole blood, plasma, or serum) ("C_{max}") of a subject after administering it to the subject may be a C_{max} of from about 1000, 1010, 1020, 1030, 1040, 1050, 1060, 1070, 1080, 1090, 1100, 1110, 1120, 1130, 1140, 1150, 1160, 1170, 1180, 1190, 1200, 1210, 1220, 1230, 1240, 1250, 1260, 1270, 1280, 1290, 1300, 1310, 1320, 1330, 1340, 1350, 1360, 1370, 1380, 1390, 1400, 1410, 1420, 1430, 1440, 1450, 1460, 1470, 1480, or 1490 ng/dL to about 1500 ng/dL; from about 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, or 149 ng/dL to about 150 ng/dL; or from about 10, 10.5, 11, 11.5, 120, 12.5, 13, 13.5, 14, or 14.5 ng/dL to about 15 ng/dL.

[0192] Maximum concentration of the first therapeutic agent in blood (whole blood, plasma, or serum) ("C_{max}") of the subject following its administration may be a C_{max} of from about 1000, 1010, 1020, 1030, 1040, 1050, 1060, 1070, 1080, 1090, 1100, 1110, 1120, 1130, 1140, 1150, 1160, 1170, 1180, 1190, 1200, 1210, 1220, 1230, 1240, 1250, 1260, 1270, 1280, 1290, 1300, 1310, 1320, 1330, 1340, 1350, 1360, 1370, 1380, 1390, 1400, 1410, 1420,

1430, 1440, 1450, 1460, 1470, 1480, or 1490 ng/mL to about 1500 ng/mL; from about 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, or 149 ng/mL to about 150 ng/mL; or from about 10, 10.5, 11, 11.5, 120, 12.5, 13, 13.5, 14, or 14.5 ng/mL to about 15 ng/mL.

[0193] Maximum concentration of the first therapeutic agent in blood (whole blood, plasma, or serum) ("C_{max}") of a subject following its administration may be selected from about 1000, 1010, 1020, 1030, 1040, 1050, 1060, 1070, 1080, 1090, 1100, 1110, 1120, 1130, 1140, 1150, 1160, 1170, 1180, 1190, 1200, 1210, 1220, 1230, 1240, 1250, 1260, 1270, 1280, 1290, 1300, 1310, 1320, 1330, 1340, 1350, 1360, 1370, 1380, 1390, 1400, 1410, 1420, 1430, 1440, 1450, 1460, 1470, 1480, or 1490 ng/dL. The C_{max} of the first therapeutic agent in blood (whole blood, plasma, or serum) ("C_{max}") of a subject following its administration may be selected from about 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, or 149 ng/dL. The C_{max} of the first therapeutic agent following its administration may be selected from about 10, 10.5, 11, 11.5, 120, 12.5, 13, 13.5, 14, or 14.5 ng/dL.

[0194] The C_{max} of the first therapeutic agent following its administration may be selected from about 1000, 1010, 1020, 1030, 1040, 1050, 1060, 1070, 1080, 1090, 1100, 1110, 1120, 1130, 1140, 1150, 1160, 1170, 1180, 1190, 1200, 1210, 1220, 1230, 1240, 1250, 1260, 1270, 1280, 1290, 1300, 1310, 1320, 1330, 1340, 1350, 1360, 1370, 1380, 1390, 1400, 1410, 1420, 1430, 1440, 1450, 1460, 1470, 1480, or 1490 ng/mL. The C_{max} of the first therapeutic agent following its administration may be selected from about 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, or 149 ng/mL. The C_{max} of the first therapeutic agent following its administration may be selected from about 10, 10.5, 11, 11.5, 120, 12.5, 13, 13.5, 14, or 14.5 ng/mL.

[0195] The C_{max} of the first therapeutic agent following its administration may be selected from about 85, 95, 105, 115, 125, 135, 145, 155, 165, 175, 185, 195, 205, 215, 225, 235, 245, 255, 265, 275, 285, 295, 305, 315, 325, 335, 345, 355, 365, 375, 385, 395, 405, 415, 425, 435, 445, 455, 465, 475, 485, 495, 505, 515, 525, 535, 545, 555, 565, 575, 585, 595, 605, 615, 625, 635, 645, 655, 665, 675, 685, 695, 705, 715, 725, 735, 745, 755, 765, 775, 785, 795, 805, 815, 825, 835, 845, 855, 865, 875, 885, 895, 905, 915, 925, 935, 945, 955, 965, 975, 985, 995, 1005, 1015, 1025, 1035, 1045, 1055, 1065, 1075, 1085, 1095, 1105, 1115, 1125, 1135, 1145, 1155, 1165, 1175, 1185, 1195, 1205, 1215, 1225, 1235, 1245, 1255, 1265, 1275, 1285, 1295, 1305, 1315, 1325, 1335, 1345, 1355, 1365, 1375, 1385, 1395, 1405, 1415, 1425, 1435, 1445, 1455, 1465, 1475, 1485, 1495, or 1500 ng/dL. The C_{max} of the first therapeutic agent following its administration may be selected from about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, or 149 ng/dL. The C_{max} of the first therapeutic agent following its administration may be selected from about 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5, 11, 11.5, 12, 12.5, 13, 13.5, 14, or 14.5 ng/dL.

[0196] The C_{max} of the first therapeutic agent following its administration may be selected from about 85, 95, 105, 115, 125, 135, 145, 155, 165, 175, 185, 195, 205, 215, 225, 235, 245, 255, 265, 275, 285, 295, 305, 315, 325, 335, 345, 355, 365, 375, 385, 395, 405, 415, 425, 435, 445, 455, 465, 475, 485, 495, 505, 515, 525, 535, 545, 555, 565, 575, 585, 595, 605, 615, 625, 635, 645, 655, 665, 675, 685, 695, 705, 715, 725, 735, 745, 755, 765, 775, 785, 795, 805, 815, 825, 835, 845, 855, 865, 875, 885, 895, 905, 915, 925, 935, 945, 955, 965, 975, 985, 995, 1005, 1015, 1025, 1035, 1045, 1055, 1065, 1075, 1085, 1095, 1105, 1115, 1125, 1135, 1145, 1155, 1165, 1175, 1185, 1195, 1205, 1215, 1225, 1235, 1245, 1255, 1265, 1275, 1285, 1295, 1305, 1315, 1325, 1335, 1345, 1355, 1365, 1375, 1385, 1395, 1405, 1415, 1425, 1435, 1445, 1455, 1465, 1475, 1485, 1495, or 1500 ng/mL. The

C_{max} of the first therapeutic following its administration may be selected from about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, or 149 ng/mL. The C_{max} of the first therapeutic agent following its administration may be selected from about 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5, 11, 11.5, 12, 12.5, 13, 13.5, 14, or 14.5 ng/mL.

[0197] The C_{max} of the first therapeutic agent after administering it to the subject may range from about 85 ng/dL to 1500 ng/dL; from about 8.5 ng/dL to 150 ng/dL; or from about 0.85 ng/dL to 15 ng/dL. The C_{max} of the first therapeutic agent in a subject's blood (whole blood, plasma, or serum) after its administration may be selected from about 85, 95, 105, 115, 125, 135, 145, 155, 165, 175, 185, 195, 205, 215, 225, 235, 245, 255, 265, 275, 285, 295, 305, 315, 325, 335, 345, 355, 365, 375, 385, 395, 405, 415, 425, 435, 445, 455, 465, 475, 485, 495, 505, 515, 525, 535, 545, 555, 565, 575, 585, 595, 605, 615, 625, 635, 645, 655, 665, 675, 685, 695, 705, 715, 725, 735, 745, 755, 765, 775, 785, 795, 805, 815, 825, 835, 845, 855, 865, 875, 885, 895, 905, 915, 925, 935, 945, 955, 965, 975, 985, 995, 1005, 1015, 1025, 1035, 1045, 1055, 1065, 1075, 1085, 1095, 1105, 1115, 1125, 1135, 1145, 1155, 1165, 1175, 1185, 1195, 1205, 1215, 1225, 1235, 1245, 1255, 1265, 1275, 1285, 1295, 1305, 1315, 1325, 1335, 1345, 1355, 1365, 1375, 1385, 1395, 1405, 1415, 1425, 1435, 1445, 1455, 1465, 1475, 1485, or 1495 ng/dL to about 1500 ng/dL; from about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, or 149 ng/dL to about 150 ng/dL; or from about 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5, 11, 11.5, 12, 12.5, 13, 13.5, 14, or 14.5 ng/dL to about 15 ng/dL.

[0198] The C_{max} of the first therapeutic agent following its administration may range from about 85 ng/mL to 1500 ng/mL; from about 8.5 ng/mL to 150 ng/mL; or from about 0.85 ng/mL to 15 ng/mL. The C_{max} of the first therapeutic following its administration may be selected from about 85, 95, 105, 115, 125, 135, 145, 155, 165, 175, 185, 195, 205, 215, 225, 235, 245, 255, 265, 275, 285, 295, 305, 315, 325, 335, 345, 355, 365, 375, 385, 395, 405, 415, 425, 435, 445, 455, 465, 475, 485, 495, 505, 515, 525, 535, 545, 555, 565, 575, 585, 595, 605, 615, 625, 635, 645, 655, 665, 675, 685, 695, 705, 715, 725, 735, 745, 755, 765, 775, 785, 795, 805, 815, 825, 835, 845, 855, 865, 875, 885, 895, 905, 915, 925, 935, 945, 955, 965, 975, 985, 995, 1005, 1015, 1025, 1035, 1045, 1055, 1065, 1075, 1085, 1095, 1105, 1115, 1125, 1135, 1145, 1155, 1165, 1175, 1185, 1195, 1205, 1215, 1225, 1235, 1245, 1255, 1265, 1275, 1285, 1295, 1305, 1315, 1325, 1335, 1345, 1355, 1365, 1375, 1385, 1395, 1405, 1415, 1425, 1435, 1445, 1455, 1465, 1475, 1485, or 1495 ng/mL to about 1500 ng/mL; from about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, or 149 ng/mL to about 150 ng/mL; or from about 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5, 11, 11.5, 12, 12.5, 13, 13.5, 14, or 14.5 ng/mL to about 15 ng/mL.

[0199] The total drug exposure over time, measured as the area under the curve ("AUC") of a plot of the concentration of the drug in blood (whole blood, plasma, or serum) of a subject following administration of the drug against time after administration of the drug may range from about 150 ng hr/mL to about 8000 ng hr/mL; from about 15 ng hr/mL to about 800 ng hr/mL; or from about 1.5 ng hr/mL to about 80 ng hr/mL. AUC may be less than 8000 ng hr/mL and is greater than or equal to 150 ng hr/mL. AUC may be less than 800 ng hr/mL and is greater than or equal to 15 ng hr/mL. AUC may be less than 80 ng hr/mL and is greater than or equal to 1.5 ng hr/mL.

[0200] The total drug exposure over time may be an AUC of from about 100 ng hr/mL to about 8000 ng hr/mL; from about 10 ng hr/mL to about 800 ng hr/mL; or from about 1 ng hr/mL to about 80 ng hr/mL. The total drug exposure over time may be an AUC of from about from about 150, 200, 400, 600, 800, 1000, 1200, 1400, 1600, 1800, 2000, 2200, 2400, 2600, 2800, 3000, 3200, 3400, 3600, 3800, 4000, 4200, 4400, 4600, 4800, 5000, 5200, 5400, 5600, 5800, 6000, 6200, 6400, 6600, 6800, 7000, 7200, 7400, 7600, or 7800 ng hr/mL to about 8000 ng hr/mL. The total drug exposure over time may be an AUC of from about 15, 20, 40, 60, 80, 100, 120, 140, 160, 180, 200, 220, 240, 260, 280, 300, 320, 340, 360, 380, 400, 420, 440, 460, 480, 500, 520, 540, 560, 580, 600, 620, 640, 660, 680, 700, 720, 740, 760, or 780 ng hr/mL to about 800 ng hr/mL. The total drug exposure over time may be an AUC of from about from about 1.5, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, or 78 ng hr/mL to about 80 ng hr/mL.

[0201] The total drug exposure over time may be an AUC of from about 100 ng hr/mL to about 8000 ng hr/mL, from about 10 ng hr/mL to about 800 ng hr/mL; or from about 1 ng hr/mL to about 80 ng hr/mL. The total drug exposure over time may be an AUC of from about from about 150 ng hr/mL to about 7800, 7600, 7400, 7200, 7000, 6800, 6600, 6400, 6200, 6000, 5800, 5600, 5400, 5200, 5000, 4800, 4600, 4400, 4200, 4000, 3800, 3600, 3400, 3200, 3000, 2800, 2600, 2400, 2200, 2000, 1800, 1600, 1400, 1200, 1000, 800, 600, 400, or 200 ng hr/mL. The total drug exposure over time may be an AUC of from about from about 15 ng hr/mL to about 780, 760, 740, 720, 700, 680, 660, 640, 620, 600, 580, 560, 540, 520, 500, 480, 460, 440, 420, 400, 380, 360, 340, 320, 300, 280, 260, 240, 220, 200, 180, 160, 140, 120, 100, 80, 60, 40, or 20 ng hr/mL. The total drug exposure over time may be an AUC of from about from about 1.5 ng hr/mL to about 78, 76, 74, 72, 70, 68, 66, 64, 62, 60, 58, 56, 54, 52, 50, 48, 46, 44, 42, 40, 38, 36, 34, 32, 30, 28, 26, 24, 22, 20, 18, 16, 14, 12, 10, 8, 6, 4, or 2 ng hr/mL. The total drug exposure over time may be an AUC of from about 100 ng hr/mL to about 200 ng hr/mL; from about 10 ng hr/mL to about 20 ng hr/mL; or from about 1 ng hr/mL to about 2 ng hr/mL.

[0202] The total drug exposure over time may be an AUC selected from about 100, 150, 200, 400, 600, 800, 1000, 1200, 1400, 1600, 1800, 2000, 2200, 2400, 2600, 2800, 3000, 3200, 3400, 3600, 3800, 4000, 4200, 4400, 46000, 4800, 5000, 5200, 5400, 5600, 5800, 6000, 6200, 6400, 6600, 6800, 7000, 7200, 7400, 7600, 7800, and 8000 ng hr/mL. The total drug exposure over time may be an AUC selected from about 10, 15, 20, 40, 60, 80, 100, 120, 140, 160, 180, 200, 220, 240, 260, 280, 300, 320, 340, 360, 380, 400, 420, 440, 4600, 480, 500, 520, 540, 560, 580, 600, 620, 640, 660, 680, 700, 720, 740, 760, 780, and 800 ng hr/mL. The total drug exposure over time may be an AUC selected from about 1, 15, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 460, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, and 80 ng hr/mL.

[0203] Provided herein are methods of treatment, or use of a composition to treat a disease state, which comprises administering to a subject in need of such treatment a combination of a first therapeutic agent and a second therapeutic agent, the method comprising:

1. (i) administering to the subject the first therapeutic agent including an imipridone, such as ONC201, an analog thereof, or a pharmaceutically acceptable salt thereof;
2. (ii) monitoring levels of the compound of the first therapeutic agent or a metabolite thereof in the subject using pharmacokinetic profiling; and
3. (iii) administering the second therapeutic agent conditional on the level of the first therapeutic agent in the subject. The monitoring step may include constructing a pharmacokinetic profile of the compound of the first therapeutic agent or a metabolite thereof for the subject using concentrations of the compound of the first therapeutic agent or a metabolite thereof in a plurality of samples obtained from the subject at time points suitable to construct a pharmacokinetic profile. At least two samples may be collected at point-of-care or point of use by sampling or self-sampling on point-of-care devices or point of use devices or on matrices suitable for storage of the samples prior to quantitation of the compound or a metabolite thereof by a laboratory. Each point-of-care devices or point of use devices may be capable of quantitating the compound or a metabolite thereof. The pharmacokinetic profile may include pharmacokinetic parameters suitable for guiding dosing of the compound or a salt thereof for the subject. The samples may include from 2-12 samples. The samples may be collected over a time period of up to 8 hours, up to 24 hours, up to 48 hours, or up to 72 hours. The pharmacokinetic parameters may include at least one parameter selected from the

group consisting of AUC, AUC_{inf}, T_{max}, C_{max}, time above threshold, steady state concentration, absorption rate, clearance rate, distribution rate, terminal T-1/2 or parameters drawn from noncompartmental pharmacokinetic (PK) or compartmental PK analysis, including physiological model-based compartmental PK analysis. The treatment method may further comprise generating a report including the pharmacokinetic profile of the subject. The report may include a recommendation regarding dosing based on the pharmacokinetic profile of the subject. A reduction in dosage of ONC20 1, the analog thereof, or the pharmaceutically acceptable salt thereof may be indicated to reduce risk of toxicity based on one or more pharmacokinetic parameters. The reduction in dosage of the compound or salt thereof may be indicated based on time above threshold, wherein the threshold is the drug concentration above which toxicity occurs, or one or more of AUC, AUC_{inf}, mean residence time (MRT), exponentials defining the pharmacokinetic profile, volume of distribution at steady state (Vss), volume of distribution during the terminal phase (Vz) or combination of a group of pharmacokinetic variable to adequately describe the pharmacokinetic profile. A dose adjustment of the compound or salt thereof may be indicated to increase efficacy based on one or more pharmacokinetic parameters. An increase in dosage of the compound or salt thereof may be indicated based on one or more of AUC, AUC_{inf}, MRT, exponentials defining the pharmacokinetic profile, steady state volume (Vss) of distribution, volume of distribution during the terminal phase (Vz) or combination of a group of pharmacokinetic variables to adequately describe the pharmacokinetic profile. The dose of the compound or salt thereof may be adjusted to within 5% to 25% of a desired target value. Each of the samples may be applied to the point-of-care device or the point of use device for determining the concentration of the compound or a metabolite thereof, wherein the point-of-care device or the point of use device comprises a lateral flow strip having a construction and composition such that an application of one or more of the samples to the lateral flow strip causes a fraction of the drug in the sample to bind to with a component of the lateral flow strip such that a detectable signal proportional to the concentration of the drug in the applied sample is produced. The samples may be applied to matrices suitable for storage of the samples prior to quantitation by a laboratory. The samples may be stored as dried blood spots. Drug concentrations may be measured by ELISA, LC MS MS, LC UV or LCMS. The pharmacokinetic parameters may include at least one of steady state concentration, absorption, and terminal $T_{1/2}$. At least one of the samples may be whole blood.

IX. MULTIMODAL THERAPEUTIC METHODS

[0204] Provided herein are multimodal therapeutic methods in which administration of an imipridone, such as ONC201, an analog thereof, or a pharmaceutically acceptable salt thereof to a subject in need of such treatment is supplemented by administration of other therapeutic modalities. The multimodal therapeutic method may comprise administering to a subject a pharmaceutical composition comprising an imipridone, such as ONC201, an analog thereof, or a pharmaceutically acceptable salt thereof in conjunction with radiation therapy or after radiation is determined to not have been efficacious. The multimodal therapeutic method may comprise administering to a subject a pharmaceutical composition comprising an imipridone, such as ONC201, an analog thereof, or a pharmaceutically acceptable salt thereof in conjunction with radiation therapy, wherein the pharmaceutical composition comprising theimipridone, such as ONC201, the analog thereof, or pharmaceutically acceptable salt thereof and the radiation therapy are administered concurrently or sequentially in any order. The multimodal therapeutic method may comprise administering to a subject a pharmaceutical composition comprising an imipridone, such as ONC201, an analog thereof, or a pharmaceutically acceptable salt thereof in conjunction with radiation therapy in a sequential arrangement. The multimodal therapeutic method may comprise administering to a subject in need of such treatment a pharmaceutical composition comprising an imipridone, such as ONC201, an analog thereof, or a pharmaceutically acceptable salt thereof thereof concurrently with radiation therapy. The multimodal therapeutic method may be used for the treatment of cancer. The multimodal therapeutic method may include administering to a cancer subject in need of such treatment a pharmaceutical composition comprising an imipridone, such as ONC201, an analog thereof, or a pharmaceutically acceptable salt thereof and irradiating cancer cells with a radiation beam. The multimodal therapeutic method may use the technique of conformal radiotherapy (CRT) to deliver a dose volume histogram (DVH) prescribed to a cancer subject. The multimodal

therapeutic method may use the technique of intensity modulated radiation therapy (IMRT) to deliver radiation to cancer cells. The multimodal therapeutic method may use techniques that compensate for motion of tumors in the subject during treatment (e.g., where doses of radiation must be administered to a thoracic tumor which moves as the patient breathes). For example, the multimodal therapeutic method may use Four Dimensional Computed Tomography (4D CT) scanning techniques to adjust the delivered radiation field to compensate for tumor motion over the breathing cycle.

[0205] Any suitable type of radiation, including gamma radiation which is given fractionated, IMRT (intensity modulated radiation therapy), gamma knife, proton therapy and brachytherapy can be used with the multimodal therapeutic method. Radiation therapy and administering an imipridone, such as ONC201, an analog thereof, or a pharmaceutically acceptable salt thereof can be used to treat brain tumors such as glioblastoma or disease that has metastasized to the brain from lung cancer. The multimodal therapeutic method can be used to treat lung cancer, pancreatic cancer, rectal cancer, breast cancer, sarcoma, prostate cancer, gynecological malignancies, and lymphoma. The gamma knife is used frequently to treat brain metastases. The multimodal therapeutic method may include use of proton therapy to treat cancer, including brain tumors, prostate cancer and any tumor proximate vital organs where it is very important to minimize toxicity to nearby normal tissue.

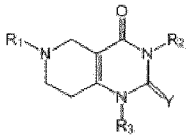
[0206] A multimodal therapeutic method may include administering to a cancer subject in need of such treatment a pharmaceutical composition comprising an imipridone, such as ONC201, an analog thereof, or a pharmaceutically acceptable salt thereof in combination with adoptive cell therapy (*e.g.*, CAR-T (JCAR 14, 15, 16, 17, KTE-C19, or CTL019); other T Cell (AFM13); or NK (CDNO-109 or NK-92)) either simultaneously or in combination.

[0207] The multimodal therapeutic method may eliminate minimal residual disease without adding to toxicity resulting from treatment by an imipridone, such as ONC201, an analog thereof, or a pharmaceutically acceptable salt thereof. The multimodal therapeutic method may improve prognosis and/or reduces adverse side-effects associated with a disease state or condition in a subject undergoing treatment.

X. ADDITIONAL IMIPRIDONE DERIVATIVES, ANALOGS, AND SALTS

[0208] Provided herein are compounds that are analogs of the compounds of formula **(10)** and methods of making them. Persons skilled in the art will understand that the general principles and concepts described above in conjunction with ONC201 and compounds of formula **(10)** and their salts, including principles and concepts related to methods and pharmaceutical compositions, apply with equal force to the following analogs and salts thereof.

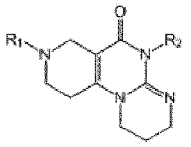
[0209] The analogs may have the structure of compound **(25):**



(25)

wherein Y is NR_4 or O, and wherein R_1, R_2, R_3, and R_4 independently represent H, alkyl, cycloalkyl, cycloalkylalkyl, carboxyl, haloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, aralkyl, hydroxyalkyl, alkoxy, aryloxy, alkoxyalkyl, alkoxycarbonyl, aralkoxy, aralkylthio, alkanoyl, mercapto, alkylthio, arylthio, alkylsulfinyl, arylsulfinyl, alkylsulfonyl, arylsulfonyl, heteroaryl, acyl, and heterocycle radicals. R_1, R_2, R_3, and R_4 may be optionally substituted Some or all hydrogens in R_1, R_2, R_3, and R_4 may be substituted by deuterium. R_1, R_2, R_3, and R_4 may be independently selected from the group consisting of H, $C_{1\text{-}4}$alkyl, $C_{1\text{-}4}$alkylphenyl, $C_{1\text{-}4}$alkylphenylketone, $C_{1\text{-}4}$benzyl-piperazine, and $C_{1\text{-}4}$alkylthienyl, wherein $C_{1\text{-}4}$alkyl, $C_{1\text{-}4}$alkylphenyl, $C_{1\text{-}4}$alkylphenylketone, and $C_{1\text{-}4}$benzylpiperazine are optionally substituted with $C_{1\text{-}4}$alkyl, hydroxyl, or halo. R_1, R_2, R_3, and R_4 may be independently selected from the group consisting of H, CH_3, CH_2Ph, CH_2-((2-Cl)-Ph), CH_2-(2-thienyl), CH_2CH_2Ph, CH_2CH_2(4-N-benzyl-piperazine), CH_2-(2,4-di F-Ph), CH_2-((2-CH_3)-Ph), $CH_2CHOHPh$, and $(CH_2)_3CO$-4F-Ph.

[0210] The analogs may have the structure of compound **(26):**



(26)

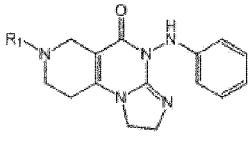
wherein R_1 and R_2 independently represent H, alkyl, cycloalkyl, cycloalkylalkyl, carboxyl, haloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, aralkyl, hydroxyalkyl, alkoxy, aryloxy, alkoxyalkyl, alkoxycarbonyl, aralkoxy, aralkylthio, alkanoyl, mercapto, alkylthio, arylthio, alkylsulfinyl, arylsulfinyl, alkylsulfonyl, arylsulfonyl, heteroaryl, acyl, and heterocycle radicals. R_1 and R_2 may be independently selected from the group consisting of H, C_{1-4}alkyl, C_{1-4}alkylphenyl, C_{1-4}alkylphenylketone, C_{1-4}benzylpiperazine, and C_{1-4}alkylthienyl, wherein C_{1-4}alkyl, C_{1-4}alkylphenyl, C_{1-4}alkylphenylketone, and C_{1-4}benzyl-piperazine are optionally substituted with C_{1-4}alkyl, C_{1-4}alkoxyl, hydroxyl, perhalogenated C_{1-4}alkyl, or halo. R_1 may be selected from the group consisting of H, CH_3, CH_2Ph, CH_2-((2-Cl)-Ph), CH_2-(2-thienyl), CH_2CH_2Ph, CH_2CH_2(4-N-benzyl-piperazine), CH_2-(2,4-di F-Ph), CH_2-((2-CH_3)-Ph), $CH_2CHOHPh$, and $(CH_2)_3CO$-4F-Ph. R_2 may be selected from the group consisting of H, CH_3, CH_2Ph, CH_2-((2-Cl)-Ph), CH_2-(2-thienyl), CH_2CH_2Ph, CH_2CH_2(4-N-benzyl-piperazine), CH_2-(2,4-di F-Ph), CH_2-((2-CH_3)-Ph), $CH_2CHOHPh$, and $(CH_2)_3CO$-4F-Ph.

[0211] R_1 may be a benzyl optionally substituted with one or more of the following substituents alone or in combination in the ortho, meta, and/or para positions of the benzyl ring: $-CH_3$, $-NO_2$, $-OCH_3$, $-CXH_2$, $-CX_2H$, $-CX_3$, $-CH_2(CX_3)$, $-CH(CX_3)_2$, $-C(CX_3)_3$, $-C_pX_{2p+1}$, $-OCX_3$, or $-OC_pX_{2p+1}$, where p is an integer from 2 to 20 and where X is a halogen including F, Cl, Br, or I; preferably, F, Cl, or Br; more preferably, F or Cl. R_2 may be a benzyl substituted with one or more of the following substituents alone or in combination in the ortho, meta, and/or para positions of the benzyl ring: $-CH_3$, $-NO_2$, $-OCH_3$, $-CXH_2$, $-CX_2H$, $-CX_3$, $-CH_2(CX_3)$, $-CH(CX_3)_2$, $-C(CX_3)_3$, $-C_pX_{2p+1}$, $-OCX_3$, or $-OC_pX_{2p+1}$, where p is an integer from 2 to 20 and where X is a halogen.

[0212] R_1 may be a H. R_1 may be a substituted or an unsubstituted arylalkyl, such as a benzyl or phenylethyl group. The arylalkyl may be substituted with C_{1-4}alkyl, C_{1-4}alkoxyl, hydroxyl, perhalogenated C_{1-4}alkyl, or halo.

[0213] R_2 may be a substituted or an unsubstituted arylalkyl, such as a benzyl or phenylethyl group. The arylalkyl may be substituted with C_{1-4}alkyl, C_{1-4}alkoxyl, hydroxyl, perhalogenated C_{1-4}alkyl, or halo. The arylalkyl may be substituted with one or more substituents selected from the group consisting of halo, $-CH_3$, $-CF_3$, and $-OCH_3$. R_2 may be a substituted or an unsubstituted heterocycloalkylalkyl, such as a morpholinoalkyl or piperazinylalkyl group. R_2 may be a substituted or an unsubstituted heteroarylalkyl, such as an isoxazolidinylmethyl or pyridylmethyl group. The heterocycloalkylalkyl or heteroarylalkyl may be substituted with C_{1-4}alkyl, C_{1-4}alkoxyl, hydroxyl, perhalogenated C_{1-4}alkyl, or halo. The heterocycloalkylalkyl or heteroarylalkyl may be substituted with one or more substituents selected from the group consisting of halo, $-CH_3$, $-CF_3$, and $-OCH_3$.

[0214] The analogs may have the structure of compound **(27):**



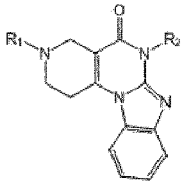
(27)

wherein R_1 is H, alkyl, cycloalkyl, cycloalkylalkyl, carboxyl, haloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, aralkyl, hydroxyalkyl, alkoxy, aryloxy, alkoxyalkyl, alkoxycarbonyl, aralkoxy, aralkylthio, alkanoyl, mercapto, alkylthio, arylthio, alkylsulfinyl, arylsulfinyl, alkylsulfonyl, arylsulfonyl, heteroaryl, acyl, and heterocycle radicals. R_1 may be selected from the group consisting of H, C_{1-4}alkyl, C_{1-4}alkylphenyl, C_{1-4}alkylphenylketone, C_{1-4}benzyl-piperazine,

and C_{1-4}alkylthienyl, wherein C_{1-4}alkyl, C_{1-4}alkylphenyl, C_{1-4}alkylphenylketone, and C_{1-4}benzyl-piperazine are optionally substituted with C_{1-4}alkyl, C_{1-4}alkoxyl, hydroxyl, perhalogenated C_{1-4}alkyl, or halo. R_1 may be selected from the group consisting of H, CH_3, CH_2Ph, CH_2-((2-Cl)-Ph), CH_2-(2-thienyl), CH_2CH_2Ph, CH_2CH_2(4-N-benzyl-piperazine), CH_2-(2,4-di F-Ph), CH_2-((2-CH_3)-Ph), $CH_2CHOHPh$, and $(CH_2)_3$CO-4F-Ph.

[0215] R_1 may be a benzyl optionally substituted with one or more of the following substituents alone or in combination in the ortho, meta, and/or para positions of the benzyl ring: -CH_3, -NO_2, -OCH_3, -CXH_2, -CX_2H, -CX_3, -$CH_2(CX_3)$, -$CH(CX_3)_2$, -$C(CX_3)_3$, -C_pX_{2p+1}, -OCX_3, or -OC_pX_{2p+1}, where p is an integer from 2 to 20 and where X is a halogen including F, Cl, Br, or I; preferably, F, Cl, or Br; more preferably, F or Cl. R_1 may be a H or a substituted or an unsubstituted arylalkyl, such as a benzyl or phenylethyl group. The arylalkyl may be substituted with C_{1-4}alkyl, C_{1-4}alkoxyl, hydroxyl, perhalogenated C_{1-4}alkyl, or halo.

[0216] The analogs may have the structure of compound **(28):**



(28)

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wherein R_1 and R_2 independently represent H, alkyl, cycloalkyl, cycloalkylalkyl, carboxyl, haloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, aralkyl, hydroxyalkyl, alkoxy, aryloxy, alkoxyalkyl, alkoxycarbonyl, aralkoxy, aralkylthio, alkanoyl, mercapto, alkylthio, arylthio, alkylsulfinyl, arylsulfinyl, alkylsulfonyl, arylsulfonyl, heteroaryl, acyl, and heterocycle radicals. R_1 and R_2 may be independently selected from the group consisting of H, C_{1-4}alkyl, C_{1-4}alkylphenyl, C_{1-4}alkylphenylketone, C_{1-4}benzyl-piperazine, and C_{1-4}alkylthienyl, wherein C_{1-4}alkyl, C_{1-4}alkylphenyl, C_{1-4}alkylphenylketone, and C_{1-4}benzyl-piperazine are optionally substituted with C_{1-4}alkyl, C_{1-4}alkoxyl, hydroxyl, perhalogenated C_{1-4}alkyl, or halo. R_1 may be selected from the group consisting of H, CH_3, CH_2Ph, CH_2-((2-Cl)-Ph), CH_2-(2-thienyl), CH_2CH_2Ph, CH_2-(2,4-di F-Ph), CH_2-((2-CH_3)-Ph), $CH_2CHOHPh$, CH_2CH_2(4-N-benzylpiperazine), and $(CH_2)_3$CO-4F-Ph. R_2 may be selected from the group consisting of H, CH_3, CH_2Ph, CH_2-((2-Cl)-Ph), CH_2-(2-thienyl), CH_2CH_2Ph, CH_2CH_2(4-N-benzyl-piperazine), CH_2-(2,4-di F-Ph), CH_2-((2-CH_3)-Ph), $CH_2CHOHPh$, and $(CH_2)_3$CO-4F-Ph. When R_1 is CH_2Ph, R_2 may not be CH_2-(2-CH_3-Ph). R_1 may be CH_2Ph and R_2 may be CH_2-(2-CH_3-Ph). R_1 may be CH_2Ph and R_2 may be CH_2-(2,4-di F-Ph). R_1 may be CH_2Ph and R_2 may be CH_2-(4-CF_3-Ph).

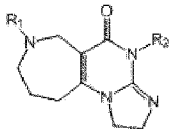
[0217] R_1 may be a benzyl optionally substituted with one or more of the following substituents alone or in combination in the ortho, meta, and/or para positions of the benzyl ring: -CH_3, -NO_2, -OCH_3, -CXH_2, -CX_2H, -CX_3, -$CH_2(CX_3)$, -$CH(CX_3)_2$, -$C(CX_3)_3$, -C_pX_{2p+1}, -OCX_3, or -OC_pX_{2p+1}, where p is an integer from 2 to 20 and where X is a halogen including F, Cl, Br, or I; preferably, F, Cl, or Br; more preferably, F or Cl. R_2 may be a benzyl substituted with one or more of the following substituents alone or in combination in the ortho, meta, and/or para positions of the benzyl ring: -CH_3, -NO_2, -OCH_3, -CXH_2, -CX_2H, - CX_3, -$CH_2(CX_3)$, -$CH(CX_3)_2$, -$C(CX_3)_3$, -C_pX_{2p+1}, -OCX_3, or -OC_pX_{2p+1}, where p is an integer from 2 to 20 and where X is a halogen.

[0218] R_1 may be a H. R_1 may be a substituted or an unsubstituted arylalkyl, such as a benzyl or phenylethyl group. The arylalkyl may be substituted with C_{1-4}alkyl, C_{1-4}alkoxyl, hydroxyl, perhalogenated C_{1-4}alkyl, or halo.

[0219] R_2 may be a substituted or an unsubstituted arylalkyl, such as a benzyl or phenylethyl group. The arylalkyl may be substituted with C_{1-4}alkyl, C_{1-4}alkoxyl, hydroxyl, perhalogenated C_{1-4}alkyl, or halo. The arylalkyl may be substituted with one or more substituents selected from the group consisting of halo, -CH_3, -CF_3, and -OCH_3. R_2 may be a substituted or an unsubstituted heterocycloalkylalkyl, such as a morpholinoalkyl or piperazinylalkyl group.

R_2 may be a substituted or an unsubstituted heteroarylalkyl, such as an isoxazolidinylmethyl or pyridylmethyl group. The heterocycloalkylalkyl or heteroarylalkyl may be substituted with C_{1-4}alkyl, C_{1-4}alkoxyl, hydroxyl, perhalogenated C_{1-4}alkyl, or halo. The heterocycloalkylalkyl or heteroarylalkyl may be substituted with one or more substituents selected from the group consisting of halo, $-CH_3$, $-CF_3$, and $-OCH_3$.

[0220] The analogs may have the structure of compound **(29):**



(29)

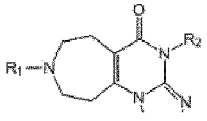
wherein R_1 and R_2 independently represent H, alkyl, cycloalkyl, cycloalkylalkyl, carboxyl, haloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, aralkyl, hydroxyalkyl, alkoxy, aryloxy, alkoxyalkyl, alkoxycarbonyl, aralkoxy, aralkylthio, alkanoyl, mercapto, alkylthio, arylthio, alkylsulfinyl, arylsulfinyl, alkylsulfonyl, arylsulfonyl, heteroaryl, acyl, and heterocycle radicals. R_1 and R_2 may be independently selected from the group consisting of H, C_{1-4}alkyl, C_{1-4}alkylphenyl, C_{1-4}alkylphenylketone, C_{1-4}benzylpiperazine, and C_{1-4}alkylthienyl, wherein C_{1-4}alkyl, C_{1-4}alkylphenyl, C_{1-4}alkylphenylketone, and C_{1-4}benzyl-piperazine are optionally substituted with C_{1-4}alkyl, C_{1-4}alkoxyl, hydroxyl, perhalogenated C_{1-4}alkyl, or halo. R_1 may be selected from the group consisting of H, CH_3, CH_2Ph, CH_2-((2-Cl)-Ph), CH_2-(2-thienyl), CH_2CH_2Ph, CH_2CH_2(4-N-benzyl-piperazine), CH_2-(2,4-di F-Ph), CH_2-((2-CH_3)-Ph), $CH_2CHOHPh$, and $(CH_2)_3CO$-4F-Ph. R_2 may be selected from the group consisting of H, CH_3, CH_2Ph, CH_2-((2-Cl)-Ph), CH_2-(2-thienyl), CH_2CH_2Ph, CH_2CH_2(4-N-benzyl-piperazine), CH_2-(2,4-di F-Ph), CH_2-((2-CH_3)-Ph), $CH_2CHOHPh$, and $(CH_2)_3CO$-4F-Ph. When R_1 is CH_2Ph, R_2 may not be CH_2-(2-CH_3-Ph). R_1 may be CH_2Ph and R_2 may be CH_2-(2-CH_3-Ph). R_1 may be CH_2Ph and R_2 may be CH_2-(2,4-di F-Ph). R_1 may be CH_2Ph and R_2 may be CH_2-(4-CF_3-Ph).

[0221] R_1 may be a benzyl optionally substituted with one or more of the following substituents alone or in combination in the ortho, meta, and/or para positions of the benzyl ring: $-CH_3$, $-NO_2$, $-OCH_3$, $-CXH_2$, $-CX_2H$, $-CX_3$, $-CH_2(CX_3)$, $-CH(CX_3)_2$, $-C(CX_3)_3$, $-C_pX_{2p+1}$, $-OCX_3$, or $-OC_pX_{2p+1}$, where p is an integer from 2 to 20 and where X is a halogen including refers to F, Cl, Br, or I; preferably, F, Cl, or Br; more preferably, F or Cl. R_2 may be a benzyl substituted with one or more of the following substituents alone or in combination in the ortho, meta, and/or para positions of the benzyl ring: $-CH_3$, $-NO_2$, $-OCH_3$, $-CXH_2$, $-CX_2H$, $-CX_3$, $-CH_2(CX_3)$, $-CH(CX_3)_2$, $-C(CX_3)_3$, $-C_pX_{2p+1}$, $-OCX_3$, or $-OC_pX_{2p+1}$, where p is an integer from 2 to 20 and where X is a halogen.

[0222] R_1 may be a H. R_1 may be a substituted or an unsubstituted arylalkyl, such as a benzyl or phenylethyl group. The arylalkyl may be substituted with C_{1-4}alkyl, C_{1-4}alkoxyl, hydroxyl, perhalogenated C_{1-4}alkyl, or halo.

[0223] R_2 may be a substituted or an unsubstituted arylalkyl, such as a benzyl or phenylethyl group. The arylalkyl may be substituted with C_{1-4}alkyl, C_{1-4}alkoxyl, hydroxyl, perhalogenated C_{1-4}alkyl, or halo. The arylalkyl may be substituted with one or more substituents selected from the group consisting of halo, $-CH_3$, $-CF_3$, and $-OCH_3$. R_2 may be a substituted or an unsubstituted heterocycloalkylalkyl, such as a morpholinoalkyl or piperazinylalkyl group. R_2 may be a substituted or an unsubstituted heteroarylalkyl, such as an isoxazolidinylmethyl or pyridylmethyl group. The heterocycloalkylalkyl or heteroarylalkyl may be substituted with C_{1-4}alkyl, C_{1-4}alkoxyl, hydroxyl, perhalogenated C_{1-4}alkyl, or halo. The heterocycloalkylalkyl or heteroarylalkyl may be substituted with one or more substituents selected from the group consisting of halo, $-CH_3$, $-CF_3$, and $-OCH_3$.

[0224] The analogs may have the structure of compound **(30):**



(30)

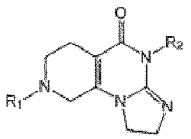
wherein R_1 and R_2 independently represent H, alkyl, cycloalkyl, cycloalkylalkyl, carboxyl, haloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, aralkyl, hydroxyalkyl, alkoxy, aryloxy, alkoxyalkyl, alkoxycarbonyl, aralkoxy, aralkylthio, alkanoyl, mercapto, alkylthio, arylthio, alkylsulfinyl, arylsulfinyl, alkylsulfonyl, arylsulfonyl, heteroaryl, acyl, and heterocycle radicals. R_1 and R_2 may be independently selected from the group consisting of H, C_{1-4}alkyl, C_{1-4}alkylphenyl, C_{1-4}alkylphenylketone, C_{1-4}benzylpiperazine, and C_{1-4}alkylthienyl, wherein C_{1-4}alkyl, C_{1-4}alkylphenyl, C_{1-4}alkylphenylketone, and C_{1-4}benzyl-piperazine are optionally substituted with C_{1-4}alkyl, C_{1-4}alkoxyl, hydroxyl, perhalogenated C_{1-4}alkyl, or halo. R_1 may be selected from the group consisting of H, CH_3, CH_2Ph, CH_2-((2-Cl)-Ph), CH_2-(2-thienyl), CH_2CH_2Ph, CH_2CH_2(4-N-benzyl-piperazine), CH_2-(2,4-di F-Ph), CH_2-((2-CH_3)-Ph), $CH_2CHOHPh$, and $(CH_2)_3CO$-4F-Ph. R_2 may be selected from the group consisting of H, CH_3, CH_2Ph, CH_2-((2-Cl)-Ph), CH_2-(2-thienyl), CH_2CH_2Ph, CH_2CH_2(4-N-benzyl-piperazine), CH_2-(2,4-di F-Ph), CH_2-((2-CH_3)-Ph), $CH_2CHOHPh$, and $(CH_2)_3CO$-4F-Ph. When R_1 may be CH_2Ph, R_2 may not be CH_2-(2-CH_3-Ph). R_1 may be CH_2Ph and R_2 may be CH_2-(2-CH_3-Ph). R_1 may be CH_2Ph and R_2 may be CH_2-(2,4-di F-Ph). R_1 may be CH_2Ph and R_2 may be CH_2-(4-CF_3-Ph).

[0225] R_1 may be a benzyl optionally substituted with one or more of the following substituents alone or in combination in the ortho, meta, and/or para positions of the benzyl ring: -CH_3, -NO_2, -OCH_3, -CXH_2, -CX_2H, -CX_3, -$CH_2(CX_3)$, -$CH(CX_3)_2$, -$C(CX_3)_3$, -C_pX_{2p+1}, -OCX_3, or -OC_pX_{2p+1}, where p is an integer from 2 to 20 and where X is a halogen including refers to F, Cl, Br, or I, preferably, F, Cl, or Br, more preferably, F or Cl. R_2 may be a benzyl substituted with one or more of the following substituents alone or in combination in the ortho, meta, and/or para positions of the benzyl ring: -CH_3, -NO_2, -OCH_3, -CXH_2, -CX_2H, - CX_3, -$CH_2(CX_3)$, -$CH(CX_3)_2$, -$C(CX_3)_3$, -C_pX_{2p+1}, -OCX_3, or -OC_pX_{2p+1}, where p is an integer from 2 to 20 and where X is a halogen.

[0226] R_1 may be a H. R_1 may be a substituted or an unsubstituted arylalkyl, such as a benzyl or phenylethyl group. The arylalkyl may be substituted with C_{1-4}alkyl, C_{1-4}alkoxyl, hydroxyl, perhalogenated C_{1-4}alkyl, or halo.

[0227] R_2 may be a substituted or an unsubstituted arylalkyl, such as a benzyl or phenylethyl group. The arylalkyl may be substituted with C_{1-4}alkyl, C_{1-4}alkoxyl, hydroxyl, perhalogenated C_{1-4}alkyl, or halo. The arylalkyl may be substituted with one or more substituents selected from the group consisting of halo, -CH_3, -CF_3, and -OCH_3. R_2 may be a substituted or an unsubstituted heterocycloalkylalkyl, such as a morpholinoalkyl or piperazinylalkyl group. R_2 may be a substituted or unsubstituted heteroarylalkyl, such as an isoxazolidinylmethyl or pyridylmethyl group. The heterocycloalkylalkyl or heteroarylalkyl may be substituted with C_{1-4}alkyl, C_{1-4}alkoxyl, hydroxyl, perhalogenated C_{1-4}alkyl, or halo. The heterocycloalkylalkyl or heteroarylalkyl may be substituted with one or more substituents selected from the group consisting of halo, -CH_3, -CF_3, and -OCH_3.

[0228] The analogs may have the structure of compound **(31):**



(31)

wherein R_1 and R_2 independently represent H, alkyl, cycloalkyl, cycloalkylalkyl, carboxyl, haloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, aralkyl, hydroxyalkyl, alkoxy, aryloxy, alkoxyalkyl, alkoxycarbonyl, aralkoxy, aralkylthio, alkanoyl, mercapto, alkylthio, arylthio, alkylsulfinyl, arylsulfinyl, alkylsulfonyl, arylsulfonyl, heteroaryl, acyl, and heterocycle radicals. R_1 and R_2 may be independently selected from the group consisting of H, C_{1-4}alkyl, C_{1-4}alkylphenyl, C_{1-4}alkylphenylketone, C_{1-4}benzylpiperazine, and C_{1-4}alkylthienyl, wherein C_{1-4}alkyl, C_{1-4}alkylphenyl, C_{1-4}alkylphenylketone, and C_{1-4}benzyl-piperazine are optionally substituted with C_{1-4}alkyl, C_{1-4}alkoxyl, hydroxyl, perhalogenated C_{1-4}alkyl, or halo. R_1 may be selected from the group consisting of H, CH_3, CH_2Ph, CH_2-((2-Cl)-

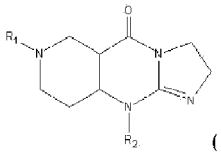
Ph), CH_2-(2-thienyl), CH_2CH_2Ph, CH_2CH_2(4-N-benzyl-piperazine), CH_2-(2,4-di F-Ph), CH_2-((2-CH_3)-Ph), $CH_2CHOHPh$, and $(CH_2)_3CO$-4F-Ph. R_2 may be selected from the group consisting of H, CH_3, CH_2Ph, CH_2-((2-Cl)-Ph), CH_2-(2-thienyl), CH_2CH_2Ph, CH_2CH_2(4-N-benzyl-piperazine), CH_2-(2,4-di F-Ph), CH_2-((2-CH_3)-Ph), $CH_2CHOHPh$, and $(CH_2)_3CO$-4F-Ph. When R_1 may be CH_2Ph, R_2 may not be CH_2-(2-CH_3-Ph). R_1 may be CH_2Ph and R_2 may be CH_2-(2-CH_3-Ph). R_1 may be CH_2Ph and R_2 may be CH_2-(2,4-di F-Ph). R_1 may be CH_2Ph and R_2 may be CH_2-(4-CF_3-Ph).

[0229] R_1 may be a benzyl optionally substituted with one or more of the following substituents alone or in combination in the ortho, meta, and/or para positions of the benzyl ring: -CH_3, -NO_2, -OCH_3, -CXH_2, -CX_2H, -CX_3, -$CH_2(CX_3)$, -$CH(CX_3)_2$, -$C(CX_3)_3$, -C_pX_{2p+1}, -OCX_3, or -OC_pX_{2p+1}, where p is an integer from 2 to 20 and where X is a halogen including F, Cl, Br, or I; preferably, F, Cl, or Br; more preferably, F or Cl. R_2 may be a benzyl substituted with one or more of the following substituents alone or in combination in the ortho, meta, and/or para positions of the benzyl ring: -CH_3, -NO_2, -OCH_3, -CXH_2, -CX_2H, - CX_3, -$CH_2(CX_3)$, -$CH(CX_3)_2$, -$C(CX_3)_3$, -C_pX_{2p+1}, -OCX_3, or -OC_pX_{2p+1}, where p is an integer from 2 to 20 and where X is a halogen.

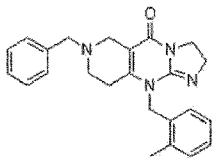
[0230] R_1 may be a H or a substituted or an unsubstituted arylalkyl, such as a benzyl or phenylethyl group. The arylalkyl may be substituted with C_{1-4}alkyl, C_{1-4}alkoxyl, hydroxyl, perhalogenated C_{1-4}alkyl, or halo.

[0231] R_2 may be a substituted or an unsubstituted arylalkyl, such as a benzyl or phenylethyl group. The arylalkyl may be substituted with C_{1-4}alkyl, C_{1-4}alkoxyl, hydroxyl, perhalogenated C_{1-4}alkyl, or halo. The arylalkyl may be substituted with one or more substituents selected from the group consisting of halo, -CH_3, -CF_3, and -OCH_3. R_2 may be a substituted or an unsubstituted heterocycloalkylalkyl, such as a morpholinoalkyl or piperazinylalkyl group. R_2 may be a substituted or an unsubstituted heteroarylalkyl, such as an isoxazolidinylmethyl or pyridylmethyl group. The heterocycloalkylalkyl or heteroarylalkyl may be substituted with C_{1-4}alkyl, C_{1-4}alkoxyl, hydroxyl, perhalogenated C_{1-4}alkyl, or halo. The heterocycloalkylalkyl or heteroarylalkyl may be substituted with one or more substituents selected from the group consisting of halo, -CH_3, -CF_3, and -OCH_3.

[0232] Provided herein are compounds of formula **(100):**



(100), wherein R_1 and R_2 are independently selected from H, alkyl, cycloalkyl, cycloalkylalkyl, heterocycloalkyl, heterocycloalkylalkyl, aryl, heteroaryl, arylalkyl, heteroarylalkyl, alkoxyalkyl, alkoxycarbonyl, aralkoxy, aralkylthio, and acyl radicals. R_1 may be CH_2Ph and R_2 may be CH_2-(2-CH_3-Ph), which is an ONC201 linear isomer (i.e., TIC-10)



TIC-10, which lacks anti-cancer activity (Jacob et al., Angew. Chem. Int. Ed., (2014) 53:6628; Wagner et al., Oncotarget (2015) 5(24): 12728). But as shown in the Examples TIC-10 is a CXCR7 agonist. CXCR7 agonists can be used for liver regeneration and preventing or treating liver fibrosis (Nature (2014) 505:97).

[0233] R_1 and R_2 may be independently selected from the group consisting of H, C_{1-4}alkyl, C_{1-4}alkylphenyl, C_{1-4}alkylphenylketone, C_{1-4}benzyl-piperazine, C_{1-4}alkylthienyl, C_{1-4}alkylpyridinyl, C_{1-4}alkylisoxazolidinyl, C_{1-4}alkylmorpholinyl, C_{1-4}alkylthiazolyl, and C_{1-4}alkylpyrazinyl wherein C_{1-4}alkyl, C_{1-4}alkylphenyl, C_{1-4}alkylphenylketone, C_{1-4}benzyl-piperazine, C_{1-4}alkylthienyl, C_{1-4}alkylpyridinyl, C_{1-4}alkylisoxazolidinyl, C_{1-}

$_4$alkylmorpholinyl, C_{1-4}alkylthiazolyl, and C_{1-4}alkylpyrazinyl are optionally substituted with C_{1-4}alkyl, C_{1-4}alkoxyl, hydroxyl, perhalogenated C_{1-4}alkyl, or halo. R_1 and/or R_2 may be a substituted or unsubstituted, arylalkyl or heteroarylalkyl. The heteroarylalkyl may be selected from C_{1-4}alkylpyrrolyl, C_{1-4}alkylfuryl, C_{1-4}alkylpyridyl, C_{1-4}alkyl-1,2,4-thiadiazolyl, C_{1-4}alkylpyrimidyl, C_{1-4}alkylthienyl, C_{1-4}alkylisothiazolyl, C_{1-4}alkylimidazolyl, C_{1-4}alkyltetrazolyl, C_{1-4}alkylpyrazinyl, C_{1-4}alkylpyrimidyl, C_{1-4}alkylquinolyl, C_{1-4}alkylisoquinolyl, C_{1-4}alkylthiophenyl, C_{1-4}alkylbenzothienyl, C_{1-4}alkylisobenzofuryl, C_{1-4}alkylpyrazolyl, C_{1-4}alkylindolyl, C_{1-4}alkylpurinyl, C_{1-4}alkylcarbazolyl, C_{1-4}alkylbenzimidazolyl, and C_{1-4}alkylisoxazolyl.

[0234] R_1 and/or R_2 may be a benzyl optionally substituted with one or more of the following substituents on the benzyl ring: X, $-CH_3$, $-NO_2$, $-OCH_3$, -CN, $-CXH_2$, $-CX_2H$, C_2-C_4 alkyl, $-CX_3$, $-CH_2(CX_3)$, $-CH(CX_3)_2$, $-C(CX_3)_3$, $-C_pX_{2p+1}$, $-OCX_3$, $-OC_pH_{2p+1}$, $-OC_pX_{2p+1}$, OR^m, SR^m, NR^mR^n, $NR^mC(O)R^n$, SOR^m, SO_2R^m, $C(O)R^m$, and $C(O)OR^m$; R^m and R^n are independently selected from H or a C_1-C_4 alkyl; and where p is an integer from 2 to 20 and X is a halogen, including F, Cl, Br, or I; preferably, F, Cl, or Br; more preferably, F or Cl.

XI. EXAMPLES

[0235] It should be understood that the description and examples below are meant for purposes of illustration only and are not meant to limit the scope of this disclosure. The examples below are meant to illustrate the embodiments disclosed and are not to be construed as being limitations to them. Additional compounds, other than those described below, may be prepared by the following reaction schemes or appropriate variations or modifications thereof.

Example 1. Synthesis of 2-Chlorobenzylamino-2-imidazoline hydriodide

[0236] To a stirred solution of 2-methylthio-2-imidazoline hydriodide (244 mg, 1.00 mMol) in dry dioxane (2.0 mL) was added 2-chlorobenzylamine (141 mg, 1.0 mMol). The reaction mixture was stirred for 90 min at 70 °C under argon. The solution was cooled to room temperature, filtered on a sintered funnel, washed with cold dioxane (2 mL) and dried under vacuum. The white solid compound 4•HI (R_2=2-chlorobenzyl) was obtained (242 mg, 72%) and used without further purification.

Example 2. Synthesis of 2-Chlorobenzylamino-2-imidazoline

[0237] To a stirred solution of 2-chlorobenzylamino-2-imidazoline hydriodide (242 mg, 0.72 mMol) in water (3 mL), was added 1.0 N sodium hydroxide (2 mL) at 7 °C. The reaction mixture was stirred for 30 min at 7 °C under argon. After that methylene chloride (5 mL) was added and the mixture stirred for another 5 min. The reaction mixture was extracted with methylene chloride (2× 2.5 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated. The resulting free base (150 mg, 100%) was obtained as a viscous liquid and was used for the next reaction without any further purification. MS(ESI) 210(M+H).

Example 3. Synthesis of Methyl-1-benzyl 4-oxo-3-piperidine carboxylate (Compound (6)).

[0238] To a stirred methyl-1-benzyl 4-oxo-3-piperidine carboxylate hydrochloride (5.7 g, 20 mMol) in ethyl acetate (50 mL), was added triethylamine (6 mL) at 7 °C. The reaction mixture was stirred for 30 min at 7 °C under an argon atmosphere. The reaction mixture was extracted with ethyl acetate (2× 50 mL) and washed with water (50 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated. The resulting free base residue (5, Ri=benzyl) as a viscous oil was used in the next reaction without any further purification MS(ESI) 248(M+H)

Reference Example 4. Synthesis of ONC202 (Compound (14))

[0239] To a solution of 2-chlorobenzylamino-2- imidazoline (150 mg, 0.72 mMol), methyl 1-benzyl 4- oxo-3-piperidine carboxylate (5, Ri=benzyl) (195 mg, 0.79 mMol) in 1-butanol (2 mL) was added PPTS (10 mg) and the mixture was stirred at room temperature for 48 h. After that the reaction mixture was refluxed at 125 °C to 130 °C for 2h. The solvents were removed under vacuum, extracted with ethyl acetate (10 mL), and washed with saturated sodium bicarbonate solution (2×10 mL) and water (10 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated. The crude free base was purified by RP HPLC (10%-40% acetonitrile / water) to give ONC902 TFA salt as a white solid (228 mg, 50% yield) MS(ESI) 407 (M+H).

[0240] The same process was used starting with different benzylamines to prepare various analogs, *e.g.,* ONC203, 204, 205, 206, 912, 210, 211, 212, 213, 214, 217, 218, 219, 220, 221, 222, 223, 224, 225, and 226.

Reference Example 5. Synthesis of ONC207 (Compound (19))

[0241] To a suspension of 60% sodium hydride (3.5 g, 88 mMol) in dry toluene (50 mL), dimethyl carbonate (4.32 g, 48.0 mMol) was added dropwise in 0.5 h at room temperature under nitrogen. After addition of a few drops of methanol, 1-tert-butoxycarbonyl-4-piperidone (4.8 g, 24 mMol) dissolved in dry toluene (20 mL) was added dropwise to the reaction mixture while stirring at 80 °C over 1h. The reaction mixture was stirred for 3 h at the same temperature and then cooled to 0 °C (ice bath) and adjusted to pH 6-6.5 with acetic acid. The resulting cold mixture was diluted with water (10mL) and adjusted to pH 8 with 5% sodium hydroxide solution. The toluene layer was separated and the aqueous layer was extracted with toluene (20 mL). The combined organic layer was dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The compound was dried in vacuum to give methyl-1-tert-butoxycarbonyl- 4-oxo-3-piperidine carboxylate (5.0 g, 80%). The compound obtained was carried to the next reaction without any further purification.

[0242] 2-methybenzylamino-2-imidazoline (190 mg, 1 mMol), methyl 1-tert-butoxycarbonyl- 4-oxo-3-piperidine carboxylate (315 mg, 1.1 mMol) in 1-butanol (2 mL) was added PPTS (10.0 mg) and the mixture was stirred at room temperature for 48 h. After that the reaction mixture was refluxed at 125 °C to 130 °C for 2h. The solvents were removed under vacuum, extracted with ethyl acetate (10 mL), washed with saturated sodium bicarbonate solution (2×10 mL) and water (10 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated. The crude free base was cleaved with 10% trifluoroacetic acid in dichloromethane, purified by RP HPLC (10%-40% acetonitrile/water) to give ONC907 (262 mg, 50%) TFA salt as a white solid MS(ESI) 297 (M+H).

Reference Example 6. Synthesis of ONC209 (Compound (21))

[0243] A mixture of ONC907 (100 mg, 0.2 mMol), phenylethyl bromide (55.0 mg, 0.28 mMol) and potassium carbonate (150 mg, 1.0 mMol) in N,N-dimethylformamide (3 mL) was heated to 70 °C for 12 h. The solvents were removed under vacuum, extracted with ethyl acetate (10 mL), and washed with water (5 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated. The crude free base was purified by RP HPLC (10%-40% acetonitrile/water) to give ONC209 (62 mg, 50%) TFA salt as a white solid MS(ESI) 401 (M+H).

[0244] The same process was used starting with different halides to give ONC215 and 214. Compounds 227, 228, 229, 230, 231, 232, 233, 234, 235, and 236 were prepared using an analogous process from Examples 1 and 5 starting with different benzylamines. Then treating the intermediate compound where R_1 is H with different halides as above.

[0245] Compound ONC216 was prepared from ONC215 by treatment with TFA.

[0246] Compound **(72)** was prepared by reacting the precursor NH compound prepared in analogy to Example 5 and treating it with styrene oxide.

Reference Example 7. Synthesis of ONC208 (Compound (20))

[0247] To a solution of 2-methylbenzylamino-2-imidazoline (190.0 mg, 1.0 mmol), methyl 1-methyl 4-oxo-3-piperidine carboxylate (185.0 mg, 1.0 mMol) in 1-butanol (2.0 mL) was added PPTS (10.0 mg) and the mixture was stirred at room temperature for 48 h. After that the reaction mixture was refluxed at 125 °C to 130 °C for 2h. The solvents were removed under vacuum, extracted with ethyl acetate (10 mL), washed with saturated sodium bicarbonate solution (2×10 mL) and water (10 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated. The crude free base was purified by HPLC 10%-40% acetonitrile and water to give ONC908 (270.0 mg, 50%) TFA salt as a white solid MS(ESI) 311 (M+H).

Example 8. Synthesis of ONC201 (Compound (1))

[0248] To a stirred 800 mL saturated NaHCOs in a 2 L round bottom flask, compound (**3**) (239.7 g, 0.845 mol, 1.6 equiv) was added in portions. n-Butanol (500 mL) was added to the resulting mixture, which was stirred for 30 min and then transferred to a separating funnel. The organic phase, containing compound (**4**), was separated and transferred to a 2 L three-neck round bottom flask equipped with mechanical stirring, N_2 inlet, a thermocouple, a condenser and a Dean-Stark trap. To the contents of the flask, Compound (**5**) (100 g, 0.528 mol, 1 equiv) and pyridinium p-toluenesulfonate (PPTS) (6.63 gm 0.026 mol, 5 mol%) were added. The resulting mixture was heated to reflux for 6 hours. Water in the reaction mixture was separated into the Dean-Stark trap as necessary. Refluxing temperature increased from 93 °C to 118 °C. Reaction progress was monitored by HPLC. When the peak area of compound (1) on HPLC remained constant with the reaction time, the reaction was stopped.

Example 9. Synthesis of Di-Salt of ONC201 (Compound (1)•2HCl)

[0249] Without isolation of the compound (**1**), the reaction mixture from Example 8 was washed with water (500 mL) and diluted with methyl tert-butyl ether (MTBE) (800 mL). The organic phase was washed with water (500 mL × 2) and transferred to a 3 L three-neck round bottom flask equipped with mechanical stirring, N_2 inlet, a thermocouple, a condenser and a Dean-Stark trap. While agitating the reaction mixture, 1 N HCl in dioxane-MTBE solution was added dropwise (4 N HCl in dioxane: 300 mL, 1.2 mol, 2.27 equiv; MTBE: 1200 mL) until no more solid precipitated out of the reaction mixture upon addition of HCl. The reaction mixture was heated to reflux at 60-65 °C for 2 hours. Water was separated into the Dean-Stark trap as necessary. Upon cooling to room temperature, the solid precipitate was filtered through a sintered glass funnel and washed with n-butanol-MTBE (1: 2, 600 mL) and MTBE (600 mL) respectively. The solid was dried in a vacuum oven at 65°C overnight (16 hours) to afford 200 g yellow solid.

[0250] To a 2 L three-neck round bottom flask equipped with mechanical stirring, N_2 inlet, a thermocouple and a condenser, the above solid (200 g) was added, followed by ethanol (1000 mL). The mixture was heated to reflux at 78°C for 2 hours. Upon cooling to room temperature, the solid was filtered through a sintered glass funnel and washed with ethanol (200 mL × 3). The wet solid was dried in the vacuum oven at 85°C for 3 days until the residual solvent met specification. 120 g of compound (2) was obtained as a white solid in a yield of 49%, with HPLC purity 99.7%.

Example 10. Activity of Imipridones

[0251] A number of imipridones were prepared based on the syntheses above. For each compound, viability of human cancer cells at 72 hours post-treatment with the compound was measured. The change in potency (relative to ONC201) was determined and shown in Table 3.

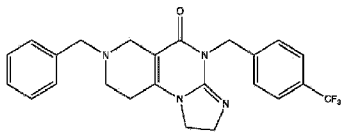
TABLE 3: RELATIVE POTENCY OF ONC201 ANALOGS

No.	Identifier	R_1	R_2	Relative Potency*
1	**ONC201**	CH_2Ph	CH_2-((2-CH_3)-Ph)	**N/A**
14**	**ONC202**	CH_2Ph	CH_2(2-Cl-Ph)	**B**
15**	**ONC203**	CH_2Ph	CH_2-(2-thienyl)	**C**
16**	**ONC204**	CH_2Ph	CH_2CH_2Ph	**C**
17**	**ONC205**	CH_2Ph	CH_2CH_2(4-N-benzylpiperazine)	**C**
18**	**ONC206**	CH_2Ph	CH_2-(2,4-di F-Ph)	**A**
19**	**ONC207**	H	CH_2-((2-CH_3)-Ph)	**B**
20**	**ONC208**	CH_3	CH_2-((2-CH_3)-Ph)	**B**
21**	**ONC209**	CH_2CH_2Ph	CH_2-((2-CH_3)-Ph)	**B**
32**	**ONC215**	$(CH_2)_3$-NH-BOC	CH_2-((2-CH_3)-Ph)	**B**
33**	**ONC216**	$(CH_2)_3$-NH_2	CH_2-((2-CH_3)-Ph)	**B**
41**	**ONC210**	CH_2Ph	CH_2-(3,5-di F-Ph)	**B**
51**	**ONC211**	CH_2Ph	CH_2-(3,4-di Cl-Ph)	**B**
52**	**ONC212**	CH_2Ph	CH_2-(4-CF_3-Ph)	**A**
53**	**ONC213**	CH_2Ph	CH_2-(3,4-di F-Ph)	**A**
54**	**ONC214**	$CD_2C_6D_5$	CH_2-((2-CH_3)-Ph)	**B**
43**	**ONC217**	CH_2Ph	CH_2(2-F-Ph)	**C**
55**	**ONC218**	CH_2Ph	CH_2(2-CH_3, 4-F-Ph)	**A**
56**	**ONC219**	CH_2Ph	CH_2-(2,4-di Cl-Ph)	**A**
57**	**ONC220**	CH_2Ph	CH_2-((4-OCH_3)-Ph)	**A**
35**	**ONC222**	CH_2Ph	CH_2-(3-isoxazolidinyl)	**C**
36**	**ONC224**	CH_2Ph	CH_2CH_2-(4-morpholinyl)	**A**
38**	**ONC221**	H	CH_2-(4-CF_3-Ph)	**A**
72**	**ONC225**	CH_2Ph	CH_2-(2-F, 4-CF_3-Ph)	**A**
37**	**ONC223**	CH_2Ph	CH_2-(4-CH_3-Ph)	**A**
34**	**ONC226**	CH_2Ph	CH_2-(3-pyridinyl)	**A**
77**	**ONC231**	CH_2-3-pyridyl	CH_2-(4-CF_3-Ph)	**A**
78**	**ONC232**	CH_2-4-methyl-2-thiazolyl	CH_2-(4-CF_3-Ph)	**B**
79**	**ONC233**	CH_2-2-pyrazinyl	CH_2-(4-CF_3-Ph)	**B**
81**	**ONC234**	CH_2-(3,4-di Cl-Ph)	CH_2-(4-CF_3-Ph)	**A**
83**	**ONC236**	CH_2-3-thienyl	CH_2-(4-CF_3-Ph)	**A**
84**	**ONC237**	$CH_2CH(OH)Ph$	CH_2-(4-CF_3-Ph)	**C**
73**	**ONC227**	CH_2-(4-CF_3-Ph)	CH_2-(4-CF_3-Ph)	**B**
74**	**ONC228**	CH_2-(4-F-Ph)	CH_2-(4-CF_3-Ph)	**A**

No.	Identifier	R_1	R_2	Relative Potency*
75**	**ONC229**	CH_2-(4-OCH_3-Ph)	CH_2-(4-CF_3-Ph)	**B**
76**	**ONC230**	4-F-Ph-4-oxobutyl	CH_2-(4-CF_3-Ph)	**A**
* Relative to the potency of ONC201; **A** Indicates a potency increase of >2-fold of ONC201; **B** Indicates potency that is within 2-fold of ONC201; and **C** Indicates a potency decrease of >2-fold of ONC201. ** Reference compound.				

Reference compound: ONC212

[0252]



[0253] The IC_{50} of ONC201 and ONC212 (5nM - 5μM, 72h) upon treatment of several acute myeloid leukemia (AML) cell lines (n=3) were determined and shown below in Table 11.

TABLE 11

AML cell line	ONC201 IC_{50} (μM)	ONC212 IC_{50} (μM)
MV411	3.25	0.01
HL60	>5	0.21
MOLM14	3.92	0.01

[0254] Cell viability of MV411 AML cells treated with ONC212 and cytarabine (5nM - 5μM, 24h) (n=3) was measured (Figure 29A). Furthermore, cell viability MOLM14, MV411 AML cells, MRC5 lung fibroblasts and Hs27a bone marrow cells treated with ONC212 (5nM - 5μM, 72h) (n=3) was measured (Figure 29B). Cell viability of MOLM14 and MV411 AML cells treated with ONC212 (250 nM) for 4, 8, 24, 48, 72 and 96h was measured. ONC212 medium was replaced by fresh medium at these time points and cell viability was determine at 96h for all samples. (n=2) (Figure 29C).

[0255] In addition, a single dose of compound **(52)** (ONC212) by oral or intraperitoneal administration to human colon cancer xenograft-bearing mice resulted in significant reduction of tumor volume compared to vehicle-treated control cohorts (Figure 24). Compound **(52)** has a wide therapeutic window, as it is well tolerated at doses at least up to 225 mg/kg in mice.

[0256] Furthermore, ONC212 demonstrated efficacy in ONC201-resistant AML xenograft model (Figure 30). MV411 AML cells (5×10^6) were subcutaneously implanted in the flanks of athymic nude. ONC212 and ONC201 were administered orally (PO) as indicated. Tumor volume (A and B) and body weight (C) (n=10) was measured on indicated days. * represents $p < 0.05$ relative to vehicle.

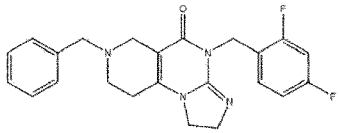
[0257] ONC212 efficacy in AML was evaluated *in vitro* and was upto 400 fold more potent compared to ONC201 (Table 11). ONC212 was also efficacious in AML cells resistant to standard of care cytarabine (Fig 29A). Despite robust improvement in efficacy ONC212 maintains a wide therapeutic window *in vitro* and is non-toxic to normal cells at efficacious concentrations (Fig 29B). An 8 hr exposure of ONC212 at 250nM was sufficient to cause robust reduction in cell viability in MOLM14 and MV411 AML cells (Fig 29C). At least 24-48h exposure was required with ONC201 for efficacy.

[0258] ONC212 efficacy was determined in a leukemia xenograft model with MV411 AML cells resistant to standard-of-care cytarabine (Fig 30). ONC212 50 mg/kg significantly reduced leukemia xenograft tumor growth with oral weekly administration while ONC201 was not efficacious in this model at similar doses (Fig 30A). Interesting, biweekly ONC212 dosing with 25 mg/kg and weekly/biweekly dosing with 5 mg/kg was not efficacious (Fig 30B). None of these ONC212 administration regimens were associated with body weight loss (Fig 30C) or gross observations.

[0259] ONC212 25 mg/kg represents NOAEL in mouse and rat non-GLP oral single dose studies which is also the efficacious dose in mouse xenograft studies. ONC212 is approximately 10 fold more toxic compared to ONC201 (NOAEL 225 mg/kg in rat non-GLP oral single dose study).

Reference compound: ONC206

[0260]



[0261] ONC206 demonstrated efficacy in a Ewing's sarcoma xenograft model (Fig. 31). MHH-ES-1 Ewing's sarcoma cells (5×10^6) were subcutaneously implanted in the flanks of athymic nude mice. ONC206 (PO) and methotrexate (IV) were administered on day 1 and day 13 as indicated. Tumor volume (Fig. 31A) and body weight (Fig. 31B) (n=4) was measured on indicated days.

[0262] In addition, the IC_{50} of ONC201 and ONC206 (5nM - 5μM, 72h) upon treatment of several cell lines (n=3) were determined and shown below in Table 11.

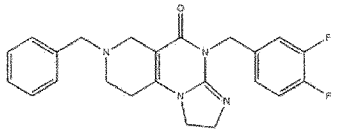
TABLE 12

Cell line	ONC201 IC_{50} (μM)	ONC206 IC_{50} (μM)
MV411 (AML)	3.25	0.2
K562 (CML)	>5	0.22
MOLM14 (AML)	3.92	0.27
MHH-ES-1 (Ewing's sarcoma)	5.65	0.61
HFF (Normal Fibroblast)		>5

[0263] ONC206 showed up to 20 fold improvement compared to ONC201 in *in vitro* potency with no in vitro toxicity to normal cells at therapeutic doses (Table 12). With ONC206, only 2-fold increased toxicity (NOAEL 125 mg/kg) was noted overall relative to ONC201 (NOAEL 225 mg/kg) in rat non-GLP oral single dose study. *In vivo* efficacy in Ewing's sarcoma model with no toxicity (Fig 31). ONC206 efficacy was comparable to chemotherapy methotrexate, but chemotherapy was associated with body weight loss.

Reference compound: ONC213

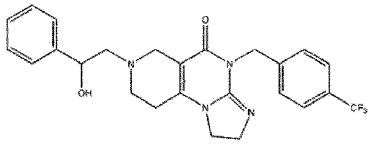
[0264]



[0265] *In vitro* profiling of GPCR activity using a hetereologous reporter assay for arrestin recruitment, a hallmark of GPCR activation, indicated that ONC213 selectively targets DRD2/3 and GPR132/91 (Figure 32). Dual targeting of DRD2/3 and GPR132/91 represents a novel strategy for anti-cancer efficacy without toxicity. ONC213 is a DRD2/3 inhibitor and a GPR132/91 agonist. DRD2/3 potency of ONC213 is more than ONC201 but less than ONC206. GPR132 potency of ONC213 is less than ONC212. Specifically, ONC213 demonstrated *in vitro* anti-cancer potency in HCT116/RPMI8226 cancer cells similar to ONC212, but *in vitro* toxicity to normal cells was reduced compared to ONC212 (Figure 33). The safety profile of ONC213 confirmed in mouse MTD study with NOAEL 75 mg/kg three times that of ONC212 (25mg/kg). The GPR91 agonist activity of ONC213 provides an opportunity for immunology, immune-oncology and hematopoietic applications (Nature Immunology 9:1261 (2008); J Leukoc Biol. 85(5):837 (May 2009)).

Reference compound: ONC237

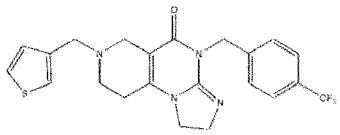
[0266]



[0267] *In vitro* profiling of GPCR activity using a hetereologous reporter assay for arrestin recruitment, a hallmark of GPCR activation, indicated that ONC237 selectively targets DRD5 and GPR132 (Figure 34). ONC237 is a GPR132 agonist and DRD5 antagonist and has reduced anticancer efficacy (IC_{50} 31.2 µM) compared to ONC201. This data show that combining GPR132 agonist activity with DRD5 (D1-like dopamine receptor) antagonist activity results in poor anti-cancer effects compared to ONC213 which combines GPR132 agonist and DRD2/3 antagonist activity.

Reference compound: ONC236

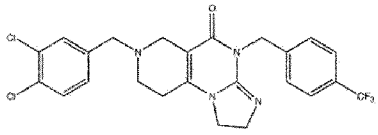
[0268]



[0269] *In vitro* profiling of GPCR activity using a hetereologous reporter assay for arrestin recruitment, a hallmark of GPCR activation, indicated that ONC236 is a highly selective GPR132 agonist (Figure 35). ONC236 has anticancer efficacy (IC_{50} 88nM) comparable to ONC212 (10nM) better than ONC206/ONC201, completeness of response is better than ONC201 but not ONC212 in HCT116 cells.

Reference compound: ONC234

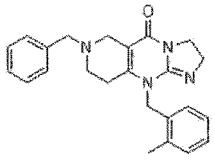
[0270]



[0271] *In vitro* profiling of GPCR activity using a hetereologous reporter assay for arrestin recruitment, a hallmark of GPCR activation, indicated that ONC234 is a broad spectrum and potent GPCR targeting small molecule (Figures 36 and 38). ONC234 hits several GPCRs including activity as an antagonist activity for adrenergic, histamine, serotonin, CHRM, CCR, DRD2/5 receptors, as well as CXCR7 agonist activity.ONC236 has anticancer efficacy (IC_{50} 234nM) similar to ONC206, completeness of response same as ONC212, and better than ONC201 in HCT116 cells.

Reference compound: QNC201 LINEAR ISOMER (TIC-10)

[0272]



(TIC-10)

[0273] *In vitro* profiling of GPCR activity using a hetereologous reporter assay for arrestin recruitment, a hallmark of GPCR activation, indicated that the ONC201 linear isomer (TIC-10) is a CXCR7 agonist (Figure 37). CXCR7 agonists can be used for liver regeneration and preventing/treating fibrosis, such as liver fibrosis (Nature 505:97 (2014)). Fibrosis is the formation of excess fibrous connective tissue in an organ or tissue, including as a result of wound healing. Examples of fibrosis includes, pulmonary fibrosis, including cystic fibrosis and idiopathic pulmonary fibrosis; radiation-induced lung injury following treatment for cancer; liver fibrosis (cirrhosis); heart fibrosis, including atrial fibrosis, endomyocardial fibrosis, and old myocardial infarction; glial scar; arthrofibrosis; Crohn's Disease; dupuytren's contracture; keloids; mediastinal fibrosis; myelofibrosis; Peyronie's disease; nephrogenic systemic fibrosis; progressive massive fibrosis; retroperitoneal fibrosis; scleroderma/systemic sclerosis; and adhesive capsulitis.

Example 11. GPCR Antagonism of ONC201

[0274] ONC201 was evaluated in a whole cell, functional assay of β-Arrestin G protein-coupled receptor (GPCR) activity that directly measures dopamine receptor activity by detecting the interaction of β-Arrestin with the activated GPCR that serves as a reporter. For each dopamine receptor (DRD1, DRD2S, DRD2L, DRD3, DRD4, and DRD5), cell lines overexpressing reporter constructs were expanded from freezer stocks. Cells were seeded in a total volume of 20 µL into white walled, 384-well microplates and incubated at 37°C prior to testing, with antagonist followed by agonist challenge at the EC_{80} concentration. Intermediate dilution of sample stocks was performed to generate 5× sample in assay buffer. 3.5 µL of 5× sample was added to cells and incubated at 37°C or room temperature for 30 minutes. Vehicle concentration was 1%. 5 µL of 6× EC_{80} agonist in assay buffer was added to cells and incubated at 37°C or room temperature for 90 or 180 minutes prior to assay readout. % Antagonism was calculated using the following formula %: Antagonism =100% × (1 - (mean RLU of test sample - mean RLU of vehicle control) / (mean RLU of EC_{80} control - mean RLU of vehicle control).

Example 12: Selective Antagonism of DRD2 by ONC201.

[0275] ONC201 is a first-in-class small molecule discovered in a phenotypic screen for p53-independent inducers of tumor selective proapoptotic pathways. Oral ONC201 is being evaluated as a new therapeutic agent in five early phase clinical trials for select advanced cancers based on pronounced efficacy in aggressive and refractory tumors

and excellent safety.

[0276] In this Example, the prediction and validation of selective direct molecular interactions between ONC201 and specific dopamine receptor family members are reported. Experimental GPCR profiling indicated that ONC201 selectively antagonizes the D2-like, but not D 1-like, dopamine receptor subfamily. Reporter assays in a heterologous expression system revealed that ONC201 selectively antagonizes both short and long isoforms of DRD2 and DRD3, with weaker potency for DRD4 and no antagonism of DRD1 or DRD5. Increased secretion of prolactin is a clinical hallmark of DRD2 antagonism by several psychiatric medications that potently target this receptor. ELISA measurements in peripheral blood of patients treated with ONC201 in the first-in-human trial with advanced solid tumors determined that 10/11 patients evaluated exhibited induction of prolactin (mean of 2-fold).

[0277] Using the TCGA database, the D2-like dopamine receptor subfamily, particularly DRD2, was found to be prevalent and selectively overexpressed in several malignancies. Preclinical reports show that DRD2 inhibition imparts antitumor efficacy, without killing normal cells, via induction of ATF4/CHOP and inhibition of Akt and ERK signaling that are all attributes of ONC201.

Methods

[0278] ONC201 dihydrochloride was obtained from Oncoceutics. Kinase inhibition assays for the kinome were performed as described (*see* Anastassiadis et al., Nat Biotech 29:1039 (2011)). GPCR arrestin recruitment and cAMP modulation reporter assays were performed as described (*see* McGuinness et al., Journal of Biomolecular Screening 14:49 (2009)). PathHunterTM (DiscoveRx) β-arrestin cells expressing one of several GPCR targets were plated onto 384-well white solid bottom assay plates (Corning 3570) at 5000 cells per well in a 20 µL volume in an appropriate cell plating reagent. Cells were incubated at 37 °C, 5% CO_2 for 18-24 h. Samples were prepared in buffer containing 0.05% fatty-acid free BSA (Sigma). For agonist mode tests, samples (5 µL) were added to pre-plated cells and incubated for 90 minutes at 37 °C, 5% CO_2. For antagonist mode tests, samples (5 µL) were added to pre-plated cells and incubated for 30 minutes at 37 °C, 5% CO_2 followed by addition of EC_{80} agonist (5 µL) for 90 minutes at 37 °C, 5% CO_2. For Schild analysis, samples (5 µL) were added to pre-plated cells and incubated for 30 minutes at 37 °C, 5% CO_2 followed by addition of serially diluted agonist (5 µL) for 90 minutes at 37 °C, 5% CO_2. Control wells defining the maximal and minimal response for each assay mode were tested in parallel. Arrestin recruitment was measured by addition of 15 µL PathHunter Detection reagent and incubated for 1-2 h at room temperature and read on a Perkin Elmer Envision Plate Reader. For agonist and antagonist tests, data was normalized for percent efficacy using the appropriate controls and fitted to a sigmoidal dose-response (variable slope), Y=Bottom + (Top-Bottom)/(1+10^((LogEC$_{50}$-X)*HillSlope)), where X is the log concentration of compound. For Schild analysis, data was normalized for percent efficacy using the appropriate controls and fitted to a Gaddum/Schild EC_{50} shift using global fitting, where Y=Bottom + (Top-Bottom)/(1+10^((LogEC-X)*HillSlope)), Antag=1+(B/(10^(-1*pA2)))^SchildSlope and LogEC=Log(EC_{50}*Antag). EC_{50} / IC_{50} analysis was performed in CBIS data analysis suite (Cheminnovation) and Schild analysis performed in GraphPad Prism 6.0.5.

Results

[0279] ONC201 is a small molecule in phase II clinical trials for select advanced cancers. It was discovered in a phenotypic screen for p53-independent inducers of the pro-apoptotic TRAIL pathway. Although the contribution of ONC201-induced ATF4/CHOP upregulation and inactivation of Akt/ERK signaling (Allen et al., Science translational medicine 5, 171ra117-171ra117 (2013)) to its anti-cancer activity has been characterized, its molecular binding target has remained elusive.

[0280] *In vitro* profiling of GPCR activity using a hetereologous reporter assay for arrestin recruitment, a hallmark of GPCR activation, indicated that ONC201 selectively antagonizes the D2-like (DRD2/3/4), but not D1-like

(DRD1/5), dopamine receptor subfamily (Reference Figures 4B and 5A). Antagonism of adrenoceptor alpha receptors or other GPCRs was not observed under the evaluated conditions. Among the DRD2 family, ONC201 antagonized both short and long isoforms of DRD2 and DRD3, with weaker potency for DRD4. Further characterization of ONC201-mediated antagonism of arrestin recruitment to DRD2L was assessed by a Gaddum/Schild EC_{50} shift analysis, which determined a dissociation constant of 2.9 μM for ONC201 that is equivalent to its effective dose in many human cancer cells (Figure 4C). Confirmatory results were obtained for cAMP modulation in response to ONC201, which is another measure of DRD2L activation (Figure 4D). The ability of dopamine to reverse the dose-dependent antagonism of up to 100 μM ONC201 suggests direct, competitive antagonism of DRD2L (Figures 5B and 5C). In agreement with the ONC201 specificity predicted by BANDIT, no significant interactions were identified between ONC201 and nuclear hormone receptors, the kinome, or other drug targets of FDA-approved cancer therapies (Figures 5D and 5E). Interestingly, a biologically inactive constitutional isomer of ONC201 (Wagner et al., Oncotarget 5:12728 (2014)) did not inhibit DRD2L, suggesting that antagonism of this receptor could be linked to its biological activity (Figure 5F). In summary, these studies establish that ONC201 selectively antagonizes the D2-like dopamine receptor subfamily, which appears to be a promising therapeutic target in oncology, and ONC201 is the first compound to exploit this treatment paradigm in several ongoing Phase II clinical studies.

Example 13 : Shotgun Mutagenesis Epitope Mapping of DRD2.

[0281] Shotgun Mutagenesis uses a high-throughput cellular expression technology to express and analyze large libraries of mutated target proteins within eukaryotic cells. Every residue in a protein is individually mutated to an alanine, or other specified residue, to assay changes in function. Proteins are expressed within standard mammalian cell lines, therefore even difficult proteins that require eukaryotic translational or post-translational processing can be mapped.

[0282] First, conditions were evaluate and identified for screening the DRD2 antagonist ONC201 with wild-type DRD2 using the Shotgun Mutagenesis screening assay. Then, a DRD2 Ala-scan library was prepared and the residues critical for ONC201 binding were mapped at single amino acid resolution using Shotgun Mutagenesis technology.

DRD2 Shotgun Mutagenesis Library:

[0283]

Parental plasmid: DRD2

Library size: 442 mutant clones (complete protein)

Mutation Strategy: Alanine scan mutagenesis

Cell type: HEK-293T

Screening Assay: Calcium flux

Epitope Tag: C-terminal V5/HIS6

[0284] Parental Construct: DNA encoding the full-length human DRD2 (Accession No: NP_000786.1; MDPLNLSWYD DDLERQNWSR PFNGSDGKAD RPHYNYYATL LTLLIAVIVF GNVLVCMAVS REKALQTTTN YLIVSLAVAD LLVATLVMPW VVYLEVVGEW KFSRIHCDIF VTLDVMMCTA SILNLCAISI DRYTAVAMPM LYNTRYSSKR RVTVMISIVW VLSFTISCPL LFGLNNADQN ECIIANPAFV VYSSIVSFYV PFIVTLLVYI KIYIVLRRRR KRVNTKRSSR AFRAHLRAPL KGNCTHPEDM KLCTVIMKSN GSFPVNRRRV EAARRAQELE MEMLSSTSPP

ERTRYSPIPP SHHQLTLPDP SHHGLHSTPD SPAKPEKNGH AKDHPKIAKI FEIQTMPNGK TRTSLKTMSR RKLSQQKEKK ATQMLAIVLG VFIICWLPFF ITHILNIHCD CNIPPVLYSA FTWLGYVNSA VNPIIYTTFN IEFRKAFLKI LHC (SEQ ID NO: 1) was subcloned into a mammalian high-expression vector. This parental construct was sequence-verified and then validated for mammalian cell expression by detection of calcium flux in response to dopamine. DNA yields from plasmid preparations have been validated for high-throughput processing.

[0285] Assay Set-up: A DRD2-specific calcium flux assay was successfully optimized for DRD2 expressed in human cells. An agonist dose-response assay was used to identify a suitable dopamine concentration for use in optimizing the inhibition of DRD2-specific calcium flux by antagonist ONC201. Subsequent dose-response inhibition assays identified a concentration of ONC201 that inhibited the DRD2 dopamine response by >95%.

Calcium Flux Assay Optimization:

[0286] Receptor Activity Assay. DRD2 activity was assessed using a published GPCR assay (Greene, T.A. et al., (2011) PLoS One 6, e20123). Briefly, HEK-293T cells were transfected with expression constructs for wild-type DRD2 or a negative control GPCR, in 384-well format. After 22 hr, calcium flux experiments were performed over a range of dopamine concentrations (300 pM - 100 nM), using a Flexstation II- 384 fluorescence reader (Molecular Devices). Data sets were analyzed and represented as percentage over baseline signal using Prism 5.0 software (GraphPad Software, Inc).

[0287] For cells expressing DRD2, but not a control GPCR, addition of dopamine resulted in increases in cellular calcium flux, measured as increased fluorescence. A dose response plot of the fluorescence peak height versus dopamine concentration demonstrated the strong dopamine-induced calcium flux (EC_{50} = 0.45 nM) in cells expressing DRD2, but not the control GPCR. This suggested that the calcium flux assay could be used to test for ONC201 inhibition.

DRD2 Calcium Flux Inhibition Assay Optimization

[0288] Following identification of the EC_{50} for dopamine in the calcium flux assay, ONC201 inhibition of DRD2-specific calcium flux was investigated at several dopamine concentrations. Using 1 nM dopamine (> 2-fold higher than the dopamine EC_{50}) with a range of ONC201 concentrations (1 nM to 100 μM), ONC201 inhibition of dopamine-induced DRD2 calcium flux was observed at the highest concentrations tested (Figure 9A), with complete inhibition by 100 μM ONC201 (IC_{50} = 21.5 μM). Inhibition of calcium flux by 100 μM ONC201 was not the result of a broad inhibition of GPCRs or of a non-specific effect on cells since ONC201 had no effect on the calcium flux activity of cells expressing a control GPCR (Figure 9B).

[0289] Analysis of a number of replicate values obtained for inhibition of DRD2 calcium flux by 100 μM ONC201 indicated a robust assay, with a Z' value of 0.61. The Z' value is a measurement of assay quality, calculated from the means and standard deviations obtained for replicate determinations of calcium flux obtained with or without ONC201.

Comparison of DRD2 inhibitors.

[0290] The ONC201 inhibition of DRD2 was compared to that by the DRD2 antagonists spiperone and domperidone (Figure 10), which have been described as inhibiting DRD2 at concentrations lower than the 100 μM required for inhibition by ONC201. These antagonists were screened at concentrations between 100 pM and 1 μM, and both showed complete inhibition of dopamine-induced calcium flux, with spiperone having an IC_{50} = 19 nM, and domperidone an IC_{50} = 47 nM. These values were consistent with previous characterizations and demonstrate

that the relatively high IC_{50} obtained for ONC201 (21.5 μM) does not result from the use of a calcium flux assay to measure DRD2 activity.

[0291] Optimal screening conditions were determined for ONC201 inhibition of DRD2-specific calcium flux in response to dopamine. These conditions give a robust response to dopamine, this response is reduced by >95% by addition of ONC201 to 100 μM, and the assay demonstrated low variability between replicates. These data indicate that the selected conditions are suitable for successful high-throughput screening. Further screening of the DRD2 mutation library was at a dopamine concentration of 1 nM and an ONC201 concentration of 100 μM.

Screening the DRD2 alanine-scan library for response to dopamine.

[0292] The DRD2 alanine-scan mutation library (and with alanines changed to serines) comprised 442 clones, covering residues 2 - 443 of the DRD2 protein, 100% of target residues. The DRD2 mutation library was first screened by calcium flux assay with dopamine (1 nM) in the absence of ONC201 to identify residues whose mutation diminished dopamine-induced calcium flux. We identified 28 amino acid residues that were critical for dopamine-induced DRD2 flux **(Figure 11).**

[0293] Residues were identified from the analysis are listed in Table 4 and shown in Figure 11. Clones were considered to be deficient for calcium flux if they demonstrated flux values less than 2 standard deviations below the average calcium flux value (AV - 2SD) for the entire library.

TABLE 4: DRD2 RESIDUES CRITICAL FOR DOPAMINE-INDUCED CALCIUM FLUX

Mutation	Calcium Flux % WT		Mutation	Calcium Flux % WT
C182A	0		S7A	15
I184A	0		W386A	15
S197A	0		S121A	16
T119A	1		I394A	16
S193A	1		E248A	19
D80A	3		V190A	20
R132A	3		Y199A	20
D114A	4		C107A	20
H393A	4		S419A	20
F198A	10		F189A	22
V83A	10		I122A	23
I377A	11		T205A	24
Y416A	12		N23A	25
C118A	14		L125A	25
			I128A	27

Screening the DRD2 alanine-scan library for ONC 201 inhibition of dopamine-induced signaling identified residues required for inhibition by ONC201.

[0294] To identify residues important for the inhibition of DRD2 by ONC201, the DRD2 alanine-scan mutation library was screened by the calcium flux assay for the ability to respond to dopamine in the presence of an inhibiting concentration of ONC201, using dopamine at 1 nM and ONC201 at 100 μM. Eight residues critical for ONC201 inhibitory activity were identified (Figure 12). All residues identified by this screen showed high calcium flux with dopamine alone (Table 5). Clones were considered to be critical for inhibition by ONC201 at 100 μM if they

demonstrated flux values greater than 2 standard deviations above the average calcium flux value (AV + 2SD) for the entire library. Also shown in Table 5 for these critical clones are the calcium flux values obtained from similar experiments performed with 250 μM ONC201 or without ONC201 (dopamine 1 nM), and in addition the % conservation of the critical residues across the 5 DRD receptors, with the residues found in each receptor.

TABLE 5: DRD2 RESIDUES CRITICAL FOR ONC201 INHIBITION OF DOPAMINE-INDUCED CALCIUM FLUX

Calcium Flux as a % of flux with WT DRD2 (100)				DRD % Conservation	DRD				
Mutation	ONC201 100 μM	ONC201 250 μM	Dopamine 1nM		1	2	3	4	5
I397A	122	89	105	20	P	**I**	T	A	P
E95A	97	39	123	100	E	**E**	E	E	E
V91A	94	58	119	40	K	**V**	V	F	K
Y192A	85	11	64	60	S	**Y**	Y	Y	S
V196A	79	22	119	40	I	**V**	V	C	I
A177S	77	26	85	40	A	**A**	T	V	D
T165A	67	28	92	20	L	**T**	A	A	L
L81A	63	20	83	100	L	**L**	L	L	L

[0295] Since the average inhibition by 100 μM ONC201 across the library was approximately 75%, we also conducted a screen at 250 μM ONC201 to determine if critical residues would be the same at higher levels of inhibition. Under this condition dopamine-induced calcium flux was inhibited by approximately 93%, and the previously identified residues **V91, E95,** and **1397** were also critical for inhibition at 250 μM ONC201 (Table 5), using the same criteria of flux values greater than 2 standard deviations above the average calcium flux value (AV + 2SD) for the library.

Conclusions:

[0296] In initial screens of the DRD2 alanine-scan mutation library by dopamine-induced calcium flux assay, 28 mutations greatly decreased calcium flux, identifying resides critical for DRD2 function. As found in a similar analysis of the GPCR CXCR4, the critical residues were distributed throughout the protein, in the predicted dopamine binding pocket, the transmembrane regions and in the cytoplasmic exposed portion of DRD2. These 28 residues are critical for either dopamine binding, signal transduction through the transmembrane domains, or G protein coupling. A detailed analysis comparable to that performed for CXCR4, as well as the structural analysis of the DRD3-eticlopride structure (Chien *et al.,* 2010), can be used to assign specific function to each DRD2 critical residue.

[0297] To identify residues important for the inhibition of DRD2 by ONC201, the DRD2 alanine-scan mutation library was screened by calcium flux assay with dopamine and 100 μM ONC201. These screens identified 8 residues as critical for ONC201 inhibition of DRD2-dependent dopamine-induced calcium flux - **L81, V91, E95, T165, A177, Y192, V196,** and **1397.** Residues **V91, E95,** and **1397** were also identified as critical for resistance to DRD2 inhibition by 250 μM ONC201, suggesting that they are key ONC201-interacting residues. These residues define a relatively large ligand binding site, which is not unexpected due to the larger size of ONC201 compared to dopamine and eticlopride. The locations of these residues are generally consistent with a role in mediating ONC201 inhibition of DRD2-dependent dopamine-induced calcium flux. Residues critical for inhibition of a GPCR taste receptor by probenecid were previously identified (Greene et al., 2011), with the location of the residues consistent with a non-competitive mechanism of inhibition. In contrast, the residues identified here for DRD2 are consistent with competitive inhibition by ONC201 at the dopamine binding site. When modeled on the structure of homologous receptor DRD3, the majority of the residues identified surround the binding pocket containing a co-crystallized antagonist eticlopride, with 5 of the 8 identified residues conserved between DRD2 and DRD3. Two of the residues appear to more distal from the putative binding site (A177 and L81) and may affect ONC201 binding

in a more allosteric fashion. Additional residues that contribute to ONC201 inhibition may be identified using DRD2 agonists with structures distinct from dopamine.

Example 14: Determination of the Association & Dissociation Rate Constants of unlabelled ONC201 Dihydrochloride on the Human D2S Receptor.

[0298] In this Example, the kon / koff rates of unlabeled ONC201 dihydrochloride on the D2S receptor was determined. The kon / koff rate estimation was performed by competitive ligand binding according to the method described in: M.R. Dowling & S.J. Charlton (2006) Brit. J. Pharmacol. 148:927-937 and H.J. Motulsky & L.C. Mahan (1984) Mol. Pharmacol. 25:1-9. Referring to this method, the kon / koff rates of the unlabeled test compounds were calculated from its Ki value (competition binding) and its effect on the binding kinetics of the radioligand (competition kinetics).

[0299] First, the IC_{50} and Ki values of ONC201 dihydrochloride, and selection of the adequate compound concentrations for the competition kinetics experiment, were determined. Then, the *k*on and *k*off rate constants of the radioligand ([^{3}H]Methylspiperone) was determined. Finally, the *k*on and *k*off rate constants of the unlabeled ONC201 dihydrochloride was determined. ONC201 dihydrochloride was tested at 8 concentrations in duplicate (n = 2) in the competition binding assay, and the IC_{50} and *K*i values were determined.

[0300] The reference compound, (+) Butaclamol, and the test compound, ONC201-2HCL, successfully competed for [^{3}H]Methylspiperone, with IC_{50} values of 2.5 nM and 21 µM, respectively. Previously, the compound ONC201-2HCL yielded a similar IC_{50} value of 16 µM. For the competition binding assay, the following 6 concentrations of ONC201-2HCL were selected: 5 / 10 / 20 / 40 / 60 / 80 µM.

[0301] The the binding kinetics of [^{3}H]Methylspiperone on the D2S receptor was determined. For this, [^{3}H]Methylspiperone (at one concentration of 0.3 nM) was incubated with the D2S receptor membranes for 12 different incubation times to measure the association rate. The non-specific binding was measured with Butaclamol (10 µM) for each incubation time. The dissociation was initiated by addition of an excess of Butaclamol (10 µM) after 60 minutes incubation of [^{3}H]Methylspiperone (0.3 nM) with the D2S receptor membranes, and the signal decrease was measured after 12 different incubation times. The experiment was performed in triplicate (n = 3) with incubation times adjusted to 0 / 30 / 60 / 80 / 120 / 180/ 240 / 300 / 360 / 420 / 480 minutes and 24 hours for the association and 2 / 5 / 8 / 10 / 15 / 20 / 25 / 30 / 40 / 60 / 120 / 180 minutes for the dissociation kinetics.

[0302] [3H]Methylspiperone displayed a k_{on} value of 2.3 × 10^8 $M^{-1}min^{-1}$ and a k_{off} value of 0.009506 min^{-1} (and thus a $t_{1/2}$ value of 73 minutes) on the D2S receptor. The K_d calculated from the results of the association / dissociation experiment (0.04 nM) is in the same range as compared to the K_d observed in the saturation experiment (0.15 nM), thereby validating the experiment.

[0303] The effect of the unlabeled ONC201-2HCl at six concentrations on the association kinetics of [^{3}H]Methylspiperone (0.3 nM) was tested. The non-specific binding was measured with Butaclamol (10 µM). The same 12 incubation times as above were used: 2 / 5 / 8 / 10 / 15 / 20 / 25 / 30 / 40 / 60 / 120 / 180 minutes. A measurement in the absence of compound was performed as negative control.

[0304] ONC201-2HCl displayed a k_{on} value of 4.1 × 10^5 $M^{-1}min^{-1}$ and a k_{off} value of 1.32 min^{-1} (and thus a $t_{1/2}$ value of 0.53 minutes) on the D2S receptor. The K_i calculated from the results of the association / dissociation experiment (3.2 µM) is in the same range as compared to the K_i observed in the saturation experiment (7 µM), thereby validating the experiment. In conclusion, **ONC201-2HCl displays a much slower association and a much faster dissociation as compared to [^{3}H]Methylspiperone.**

Reference Example 15: Bactericidal Activity of Imipridones.

Materials and Methods

[0305] Test material: ONC201 dihydrochloride; Control: Microcrystalline Cellulose.

[0306] Method: Harmonized EP/USP Microbial Examination of Nonsterile Products (Current USP <61>/<62>).

Results

[0307]

TABLE 6: VERIFICATION OF THE INOCULUM RECOVERY CONTROL AND MICROBIAL ENUMERATION TEST

1:300 with TSB Mod Dilution	Indicator Organisms Count							
	Ec	Sa	Pa	Bs	Ca (TSA)	Ab (TSA)	CA (SDA)	Ab (SDA)
Inoculum	27	31	28	52	48	21	52	20
434019	N/A	0	24	48	51	18	46	19

TABLE 7: THE VALIDATION FOR SPECIFIED MICROORGANISMS

Sample	BTGN	Ec	Pa	Sa	Ca
1:300 with TSB Mod Dilution	P	P	P	F	P

P = Pass F = Fail NA = Not Applicable; Ec = Escherichia coli ATCC# 8739; Pa = Pseudomonas aeruginosa ATCC# 9027; Sa = Staphylococcus aureus ATCC# 6538; Bs = Bacillus subtilis ATCC# 6633; Ca = Candida albicans ATCC# 10231; Ab =Aspergillus brasiliensis ATCC# 16404; BTGN = Bile Tolerant Gram Negative bacteria; Cs = Clostridium species; TSA = Trypticase Soy Agar; SDA = Sabouraud Dextrose Agar.

[0308] ONC201 dihydrochloride when tested at the 1:300 dilution with TSB Mod, did not meet the requirements of the USP <61>/<62>

[0309] Microbial Limit Suitability Test. Inhibition was observed for Staphylococcus aureus for USP<61>/<62>. Therefore, it can be assumed that the failure to isolate the inoculated microorganism is attributable to the bactericidal activity of ONC201 dihydrochloride and thus it is not likely to be contaminated with the inhibited species of microorganism.

[0310] Next, the Minimal Inhibitory Concentration (MIC) for six imipridones was determined against wild type and methicillin-resistant *Staphylococcus aureus.*

Materials and Methods

Compounds

[0311] ONC201 and ONC206 were previously solubilized at 40 mM in DMSO. ONC212, ONC207 and ONC213 were solubilized at 20 mg/mL in DMSO and an ONC201 linear isomer (TIC-10) was solubilized at 10 mg/mL in DMSO. Methicillin and/or vancomycin were evaluated in parallel as positive control antibiotics and were purchased

from Sigma-Aldrich and solubilized in deionized H_2O at a concentration of 10 mg/mL.

Bacteria

[0312] The bacterial strains employed in these assays were obtained from the American Type Culture Collection (ATCC). All bacterial strains were propagated as recommended by the ATCC. Each strain was stored as a frozen glycerol stock at -80°C and a 10 µL loop of the frozen stock was used to inoculate each culture for these assays. The strains with their classification and properties are listed in Table 8 below.

TABLE 8: STRAINS OF *STAPHYLOCOCCUS AUREUS* AND CHARACTERISTICS

ATCC #	Classification	Properties	Assay Media
29213	Gram Positive Cocci	QC Wild Type Strain	Trypticase Soy Broth (TSB)
33591		Hospital Acquired Methicillin Resistant	Nutrient Broth
700699		Hospital Acquired, MDR, Reduced Susceptibility to Vancomycin	Brain Heart Infusion Broth + 0.004 g/L Vancomycin

Minimal Inhibitory Concentration (MIC) Determination

[0313] The susceptibility of the bacterial organisms to the test compounds was evaluated by determining the MIC of each compound using a micro-broth dilution analysis according to the methods recommended by the Clinical and Laboratory Standards Institute CLSI. All microbial strains were obtained from American Type Culture Collections (ATCC) and cultured according to the supplier's recommendations. Evaluation of the susceptibility of each organism against the test compounds included a positive control antibiotic(s). For each organism, a standardized inoculum was prepared by direct suspension of freshly plated colonies in the appropriate media as indicated in Table 8 to an optical density 625 nm (OD_{625}) of 0.1 (equivalent to a 0.5 McFarland standard). The suspended inoculum was diluted to a concentration of approximately 1×10^6 colony forming units per milliliter (CFU/mL) and 100 µL placed into triplicate wells of a 96-well plate containing 100 µL of test compound serially diluted 2-fold in the appropriate broth. One hundred microliters (100 µL) of the inoculum was also added to triplicate wells containing 100 µL of two-fold serial dilutions of a positive control antibiotic and to wells containing 100 µL of media only. This dilution scheme yielded final concentrations for each microbial organism estimated to be 5×10^5 CFU/mL. Test compound concentrations ranged from a high-test of 100 to a low test of 0.2 µM using a two-fold dilution scheme. The plates were incubated for 24 or 48 hours (*Staphylococcus aureus* 700699) at 37°C and the microbial growth at each concentration of compound was determined by measuring the optical density at 625 nm on a Molecular Devices SpectraMax Plus-384 plate reader and visually by scoring the plates +/- for bacterial growth. The MIC for each compound was determined as the lowest compound dilution that completely inhibited microbial growth.

Results

[0314] Six (6) imipridones were evaluated for their ability to inhibit the growth of three strains of *Staphylococcus aureus.* ONC201, ONC207, and an ONC201 linear isomer (TIC-10) were inactive against all three strains up to a concentration of 100 µg/mL. Against wild type *Staphylococcus aureus* (ATCC 29213) the MIC of ONC206, ONC212 and ONC213 was 6.25 µg/mL, 3.13 µg/mL and 25 µg/mL, respectively. Against *Staphylococcus aureus* (ATCC 33591) the MIC of ONC206, ONC212 and ONC213 was 12.5 µg/mL, 3.13 µg/mL and 3.13 µg/mL, respectively. The activity was similar against the MDR *Staphylococcus aureus* (ATCC 700699) with all three compounds having a MIC of 12.5 µg/mL. Vancomycin, the positive control compound, was active at the expected concentration and methicillin was found to be inactive up to a concentration of 100 µg/mL against the two methicillin resistant strains

of bacteria. Data are presented in Table 9.

TABLE 9: MIC DETERMINATION OF 6 IMIPRIDONES FOR 3 *STAPHYLOCOCCUS AUREUS* STRAINS

Compound (µg/mL)	*Staphylococcus aureus* ATCC 29213				*Staphylococcus aureus* ATCC 33591				*Staphylococcus aureus* ATCC 700699 (48 hours)			
	MIC_{90}	MIC_{95}	MIC_{99}	Visual	MIC_{90}	MIC_{95}	MIC_{99}	Visual	MIC_{90}	MIC_{95}	MIC_{99}	Visual
ONC201	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
ONC206	6.25	6.25	6.25	6.25	12.5	25	>100	12.5	12.5	12.5	25	12.5
ONC207	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
ONC212	3.13	3.13	3.13	3.125	3.13	6.25	100	3.125	6.25	12.5	12.5	12.5
ONC213	12.5	12.5	25	25	3.13	6.25	100	3.125	6.25	12.5	12.5	12.5
TIC-10	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
Methicillin	---	---	---	---	>100	>100	>100	>100	>100	>100	>100	>100
Vancomycin	3.13	3.13	6.25	3.125	0.39	0.39	0.78	0.391	12.5	25	25	25

Discussion

[0315] Six (6) imipridones were evaluated for activity against 3 strains of *Staphylococcus aureus.* ONC201, ONC207, and TIC-10 were inactive against all three strains. ONC206, ONC212 and ONC213 had varying activity ranging from 3.13 µg/mL to 25 µg/mL against all three bacterial strains. Relative to vancomycin the activity of these three imipridones was equivalent or 2 to 8-fold less against strain 29213. All three of these imipridones had 10 to 30-fold less activity compared to vancomycin against strain 33591 and the activity for all three compounds was 2-fold higher than vancomycin against strain 700699.

[0316] These experiments are repeated with additional impiridones and for additional bacteria, including both Gram-positive and Gram-negative bacteria, such as those in Table 10.

TABLE 10

Organism	Condition	Gram +/Gram -
Enterococcus faecium	Noscomial bacteremia, wound infections, endocarditis, UTIs	+
Staphylococcus aureus	Bacteremia, endocarditis	+
Klebsiella pneumonia	Pneumonia, UTIs, Upper respiratory tract infections	-
Acinetobacter baumannii	Infections in ICU and burn patients; also being seen in general hospital and nursing homes	-
Pseudomonas aeruginosa	Pneumoniae, CF	-
Enterobacter cloacae	UTIs, respiratory infections	-

Example 16: Case study of ONC201 treatment in a subject with recurrent glioblastoma

[0317] This Example provides a case study of a 22 year old female with recurrent glioblastoma (unmethylated MGMT, H3.3 K27M mutant) treated with 625mg of ONC201 once every three weeks. Figure 28 (A) Tumor size relative to baseline (%) of total tumor burden in the subject. One cycle is 3 weeks. (B) Contrast MRI scans at baseline, 21, 27 and 36 weeks post-ONC201 initiation of one of 2 malignant lesions in the subject with 625mg q3w ONC201.

[0318] This invention is not limited to the exemplary embodiments shown and described, but it is intended to cover modifications within the scope of this invention as defined by the claims. Features of the disclosed embodiments may be combined. Unless specifically set forth here, the terms "a", "an" and "the" are not limited to one element but instead should be read to mean "at least one."

[0319] It is to be understood that the figures and descriptions may have been simplified to focus on elements that are relevant for a clear understanding, while eliminating, for purposes of clarity, other elements that those of ordinary skill in the art will appreciate may also comprise a portion of the invention. However, because such elements are well known in the art, and because they do not necessarily facilitate a better understanding of the invention, a description of such elements is not provided herein.

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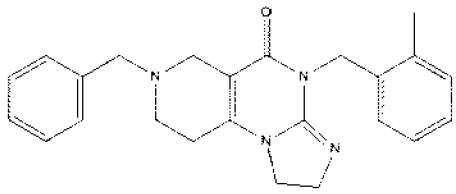
G-PROTEINKOBLET RECEPTOR (GPCR)-MODULATION VED HJÆLP AF IMIPRIDONER

Patentkrav

1. Forbindelse med formlen **(1)**



(1),

eller et farmaceutisk acceptabelt salt deraf, til anvendelse ved behandling af cancer i centralnervesystemet hos en patient, hvilken cancer i centralnervesystemet har en histon H3-mutation.

2. Forbindelse til anvendelse ifølge krav1, hvor histon H3-mutationen er H3.3 K27M.

3. Forbindelse til anvendelse ifølge krav 1 eller krav 2, hvor canceren er et gliom.

4. Forbindelse til anvendelse ifølge krav 1 eller krav 2, hvor canceren er en hjernetumor.

5. Forbindelse til anvendelse ifølge krav 1 eller krav 2, hvor canceren er et meningiom, ependymom, neuroblastom eller diffust internt pontingliom.

6. Forbindelse til anvendelse ifølge krav 1, hvor canceren har et epigent inaktiveret umethyleret O(6)-methylguanin-DNA-methyltransferase (MGMT)-gen.

7. Forbindelse til anvendelse ifølge et hvilket som helst af kravene 1-6, hvor patienten er et menneske.

8. Forbindelse til anvendelse ifølge et hvilket som helst af kravene 1-7, hvor det farmaceutisk acceptable salt er et dihydrochloridsalt.

DRAWINGS

Drawing

Figure 1

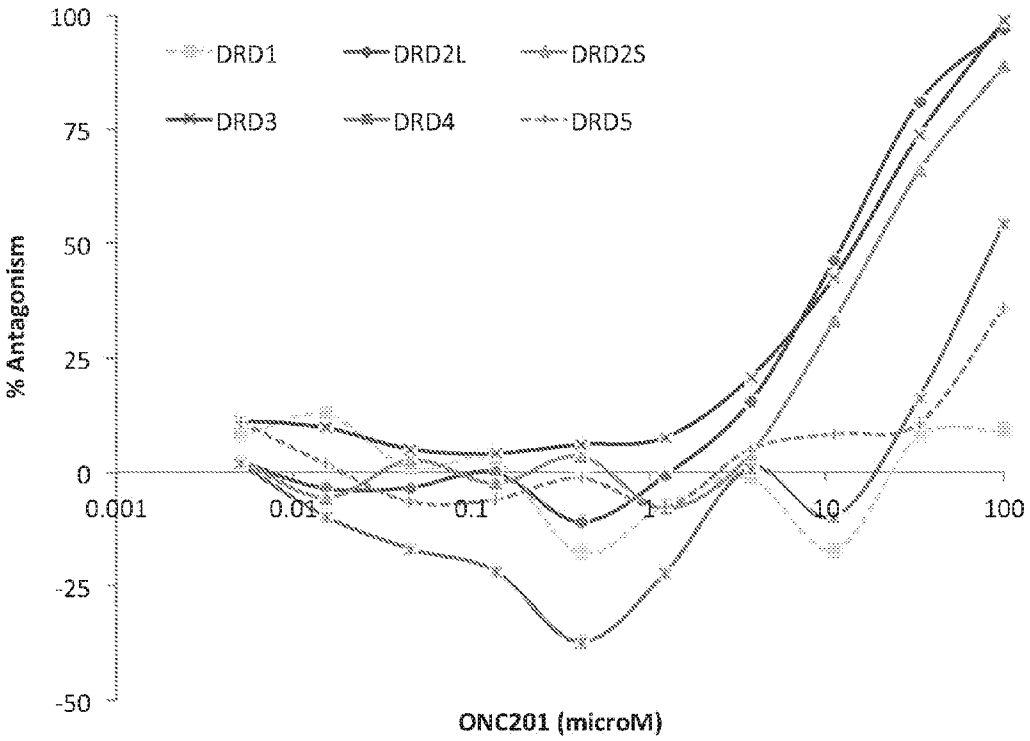
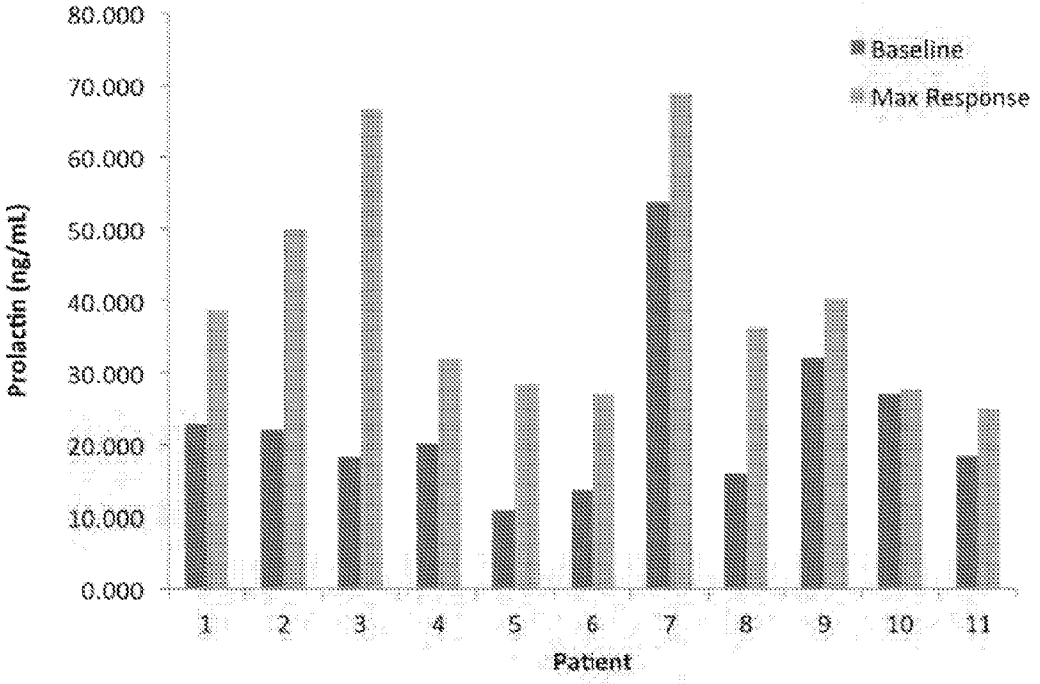


Figure 2


80.000
70.000
60.000
50.000
40.000
30.000
20.000
10.000
0.000
Prolactin (ng/mL)
Baseline
Max Response
1
2
3
4
5
6
7
8
9
10
11
Patient

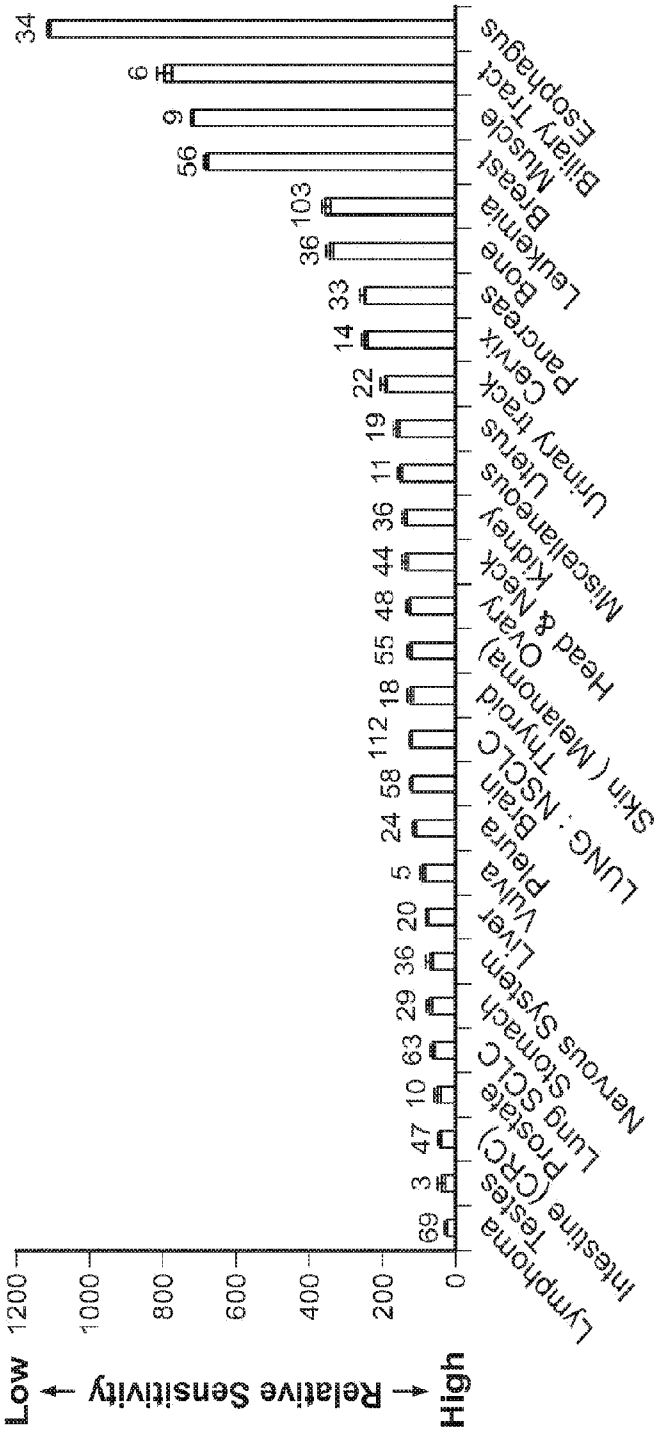

Low
Relative Sensitivity
High
1200
1000
800
600
400
200
0
69
3
47
10
63
29
36
20
5
24
58
112
18
55
48
44
36
11
19
22
14
33
36
103
56
9
6
34
Lymphoma
Testes
Intestine (CRC)
Prostate
Lung SCLC
Stomach
Nervous System
Liver
Vulva
Pleura
Brain
LUNG : NSCLC
Thyroid
Skin (Melanoma)
Ovary
Head & Neck
Kidney
Miscellaneous
Uterus
Urinary track
Cervix
Pancreas
Bone
Leukemia
Breast
Muscle
Biliary Tract
Esophagus

Figure 3

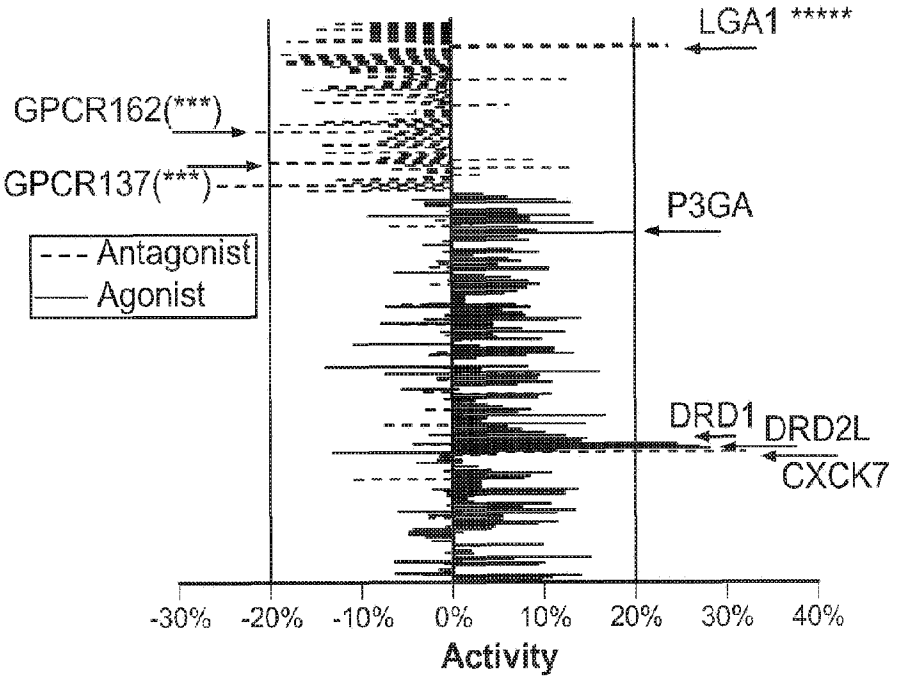


Figure 4A

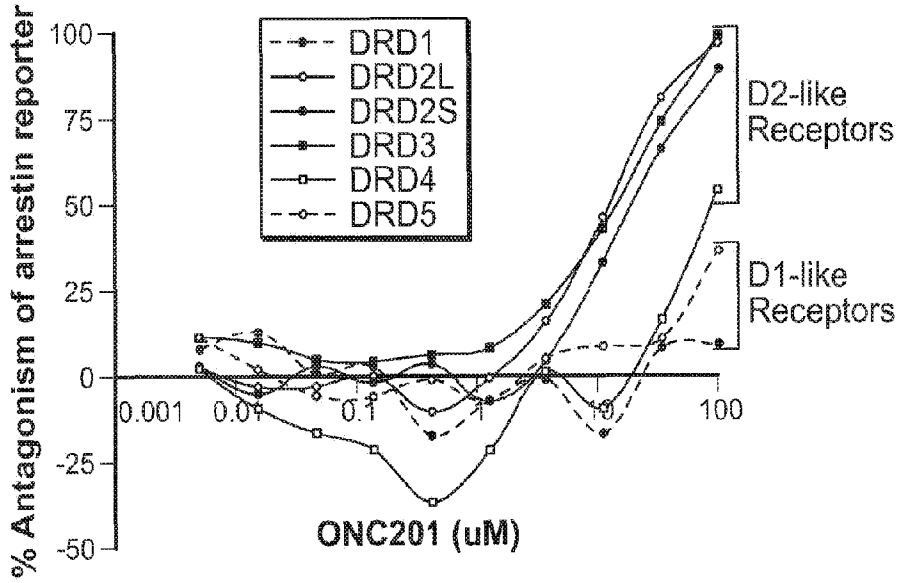


Figure 4B

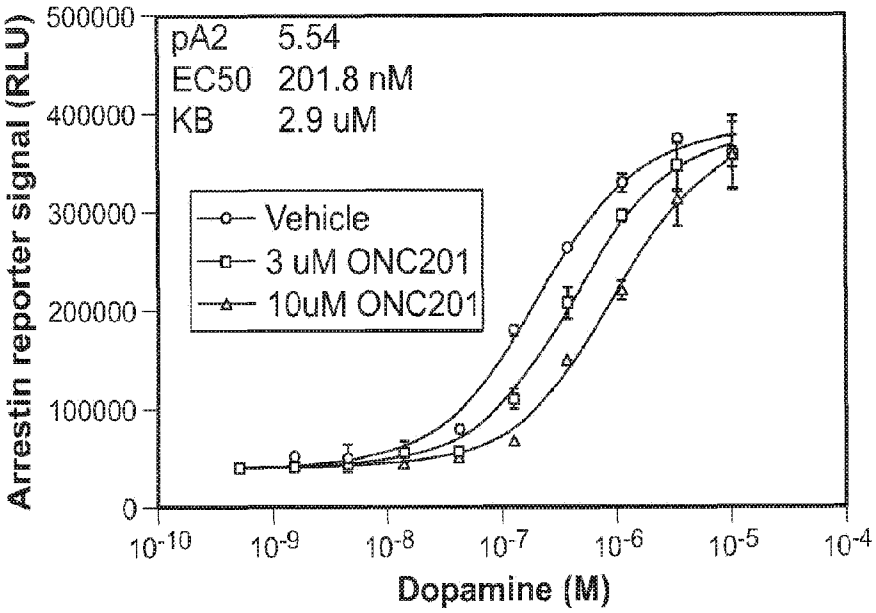


Figure 4C

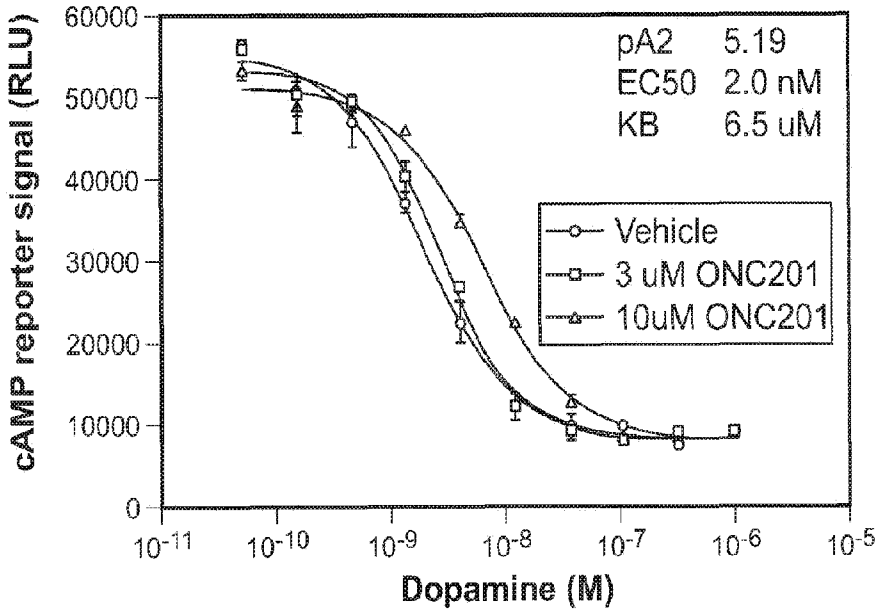


Figure 4D

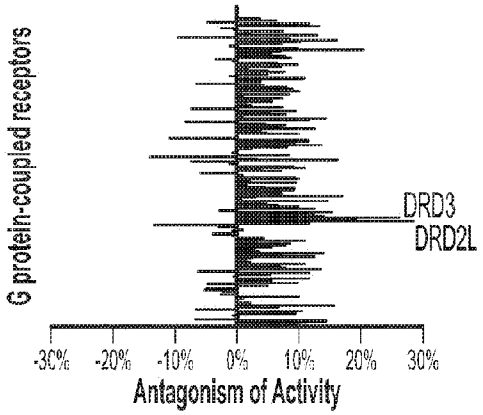


Figure 5A

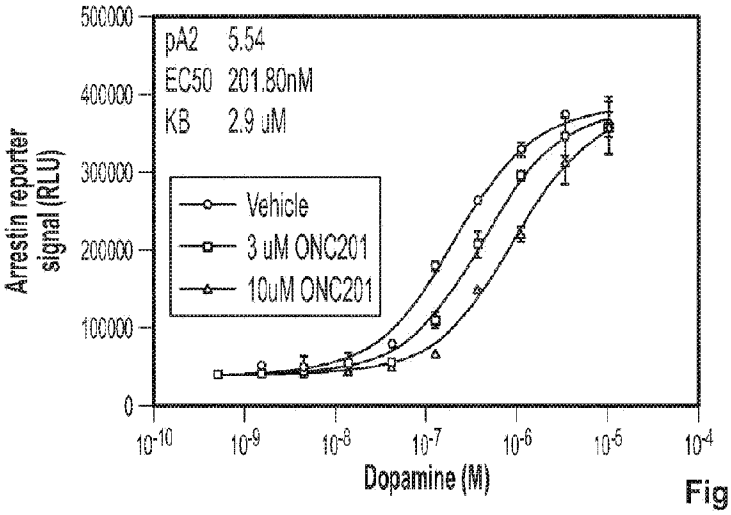


Figure 5B

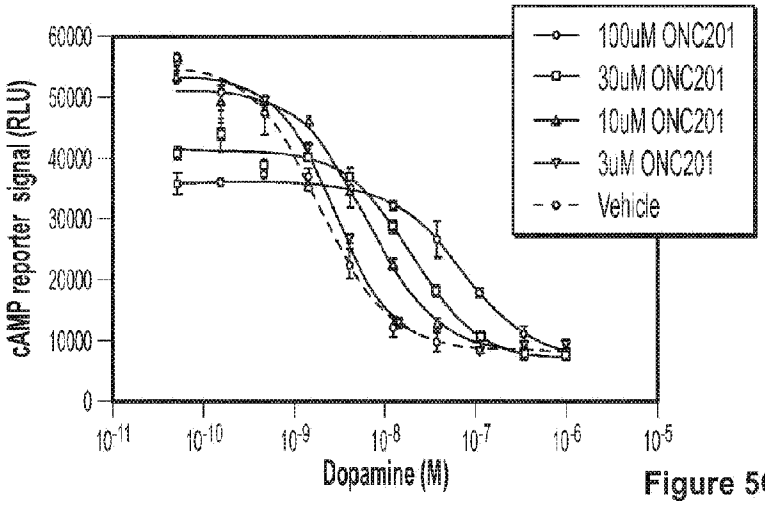


Figure 5C

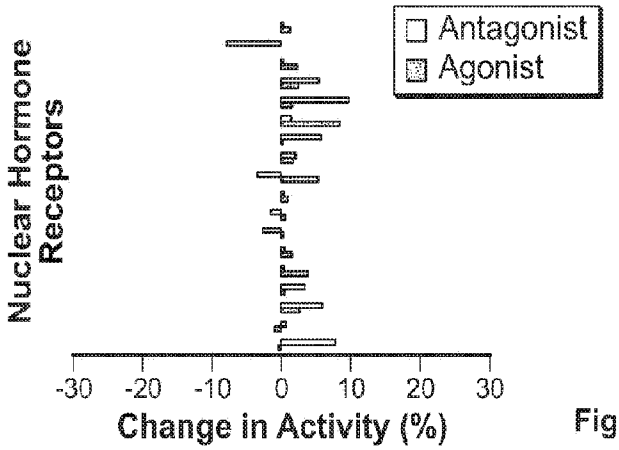


Figure 5D

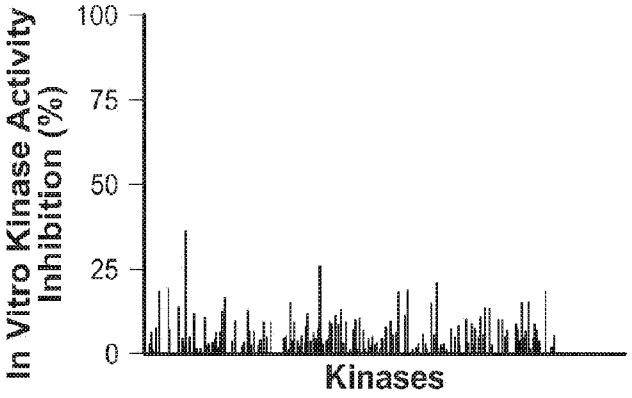


Figure 5E

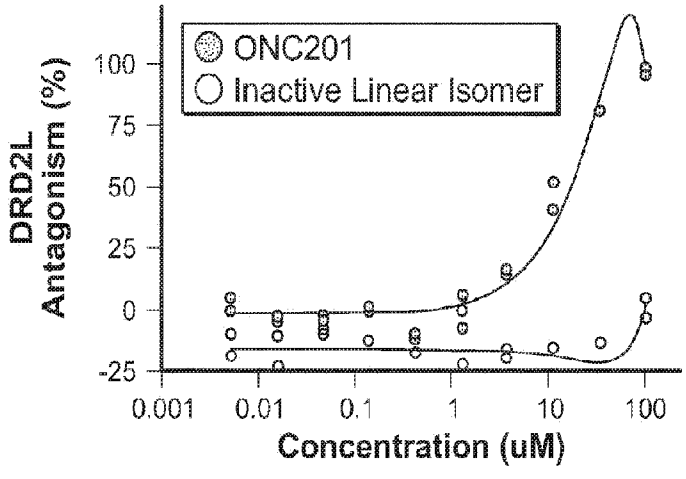
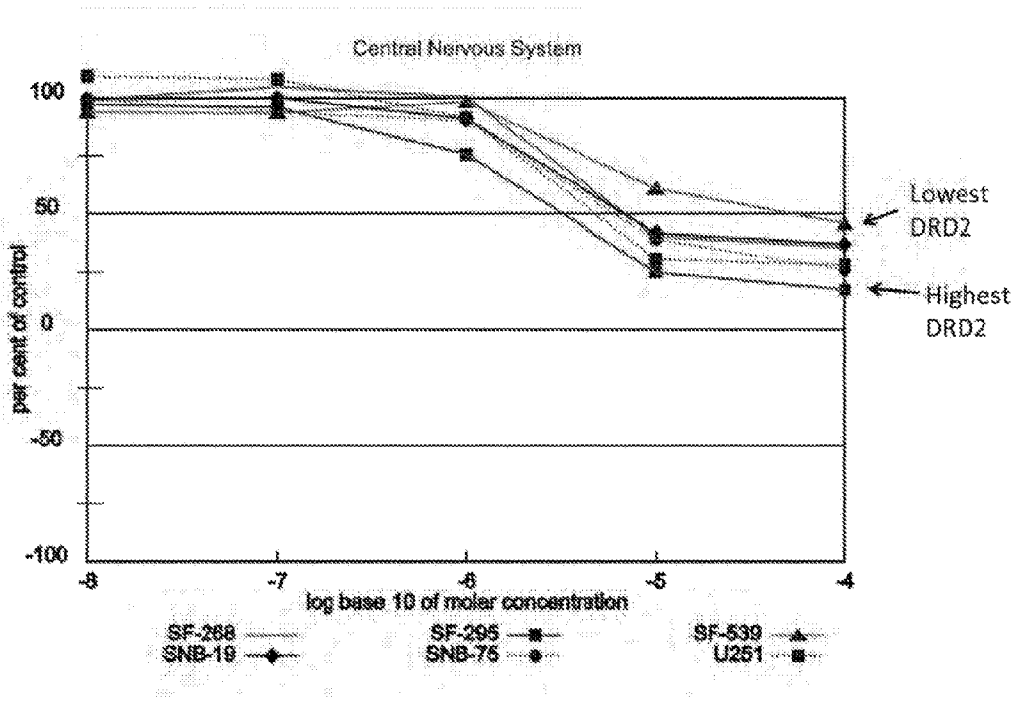


Figure 5F

Figure 6

A


Central Nervous System
100
50
0
-50
-100
per cent of control
-8
-7
-6
-5
-4
log base 10 of molar concentration
Lowest DRD2
Highest DRD2
SF-268
SNB-19
SF-295
SNB-75
SF-539
U251

B

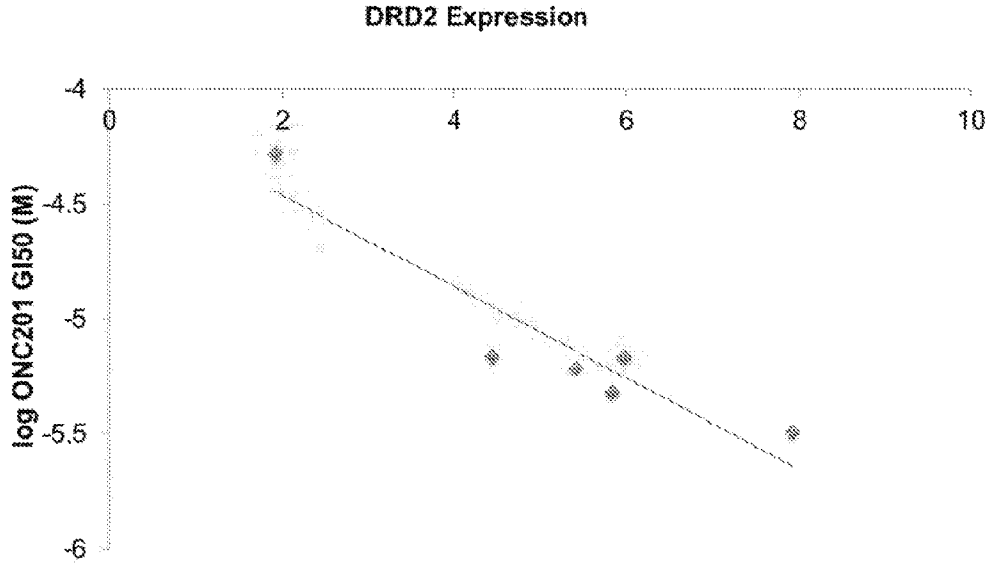
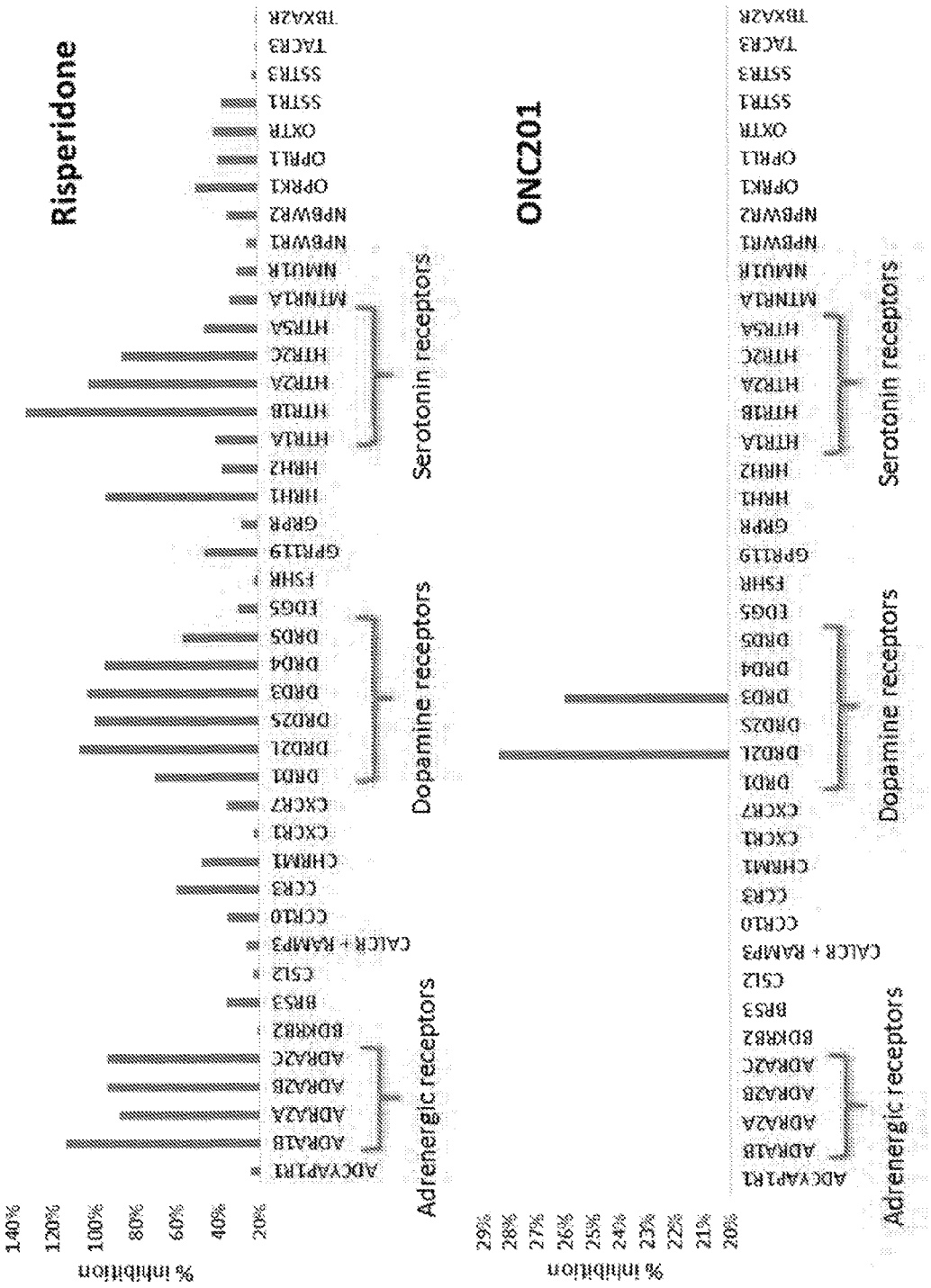

DRD2 Expression
-4
-4.5
-5
-5.5
-6
log ONC201 GI50 (M)
0
2
4
6
8
10

Figure 7


Risperidone
ONC201
% inhibition
Adrenergic receptors
Dopamine receptors
Serotonin receptors
ADCYAP1R1
ADRA1B
ADRA2A
ADRA2B
ADRA2C
BDKRB2
BRS3
CALCR + RAMP3
CCR10
CCR3
CHRM1
CXCR1
CXCR7
DRD1
DRD2L
DRD2S
DRD3
DRD4
DRD5
EDG5
FSHR
GPR119
GRPR
HRH1
HRH2
HTR1A
HTR1B
HTR2A
HTR2C
HTR5A
MTNR1A
NMUR1
NPBWR1
NPBWR2
OPRK1
OPRL1
OXTR
SSTR1
SSTR3
TACR3
TBXA2R

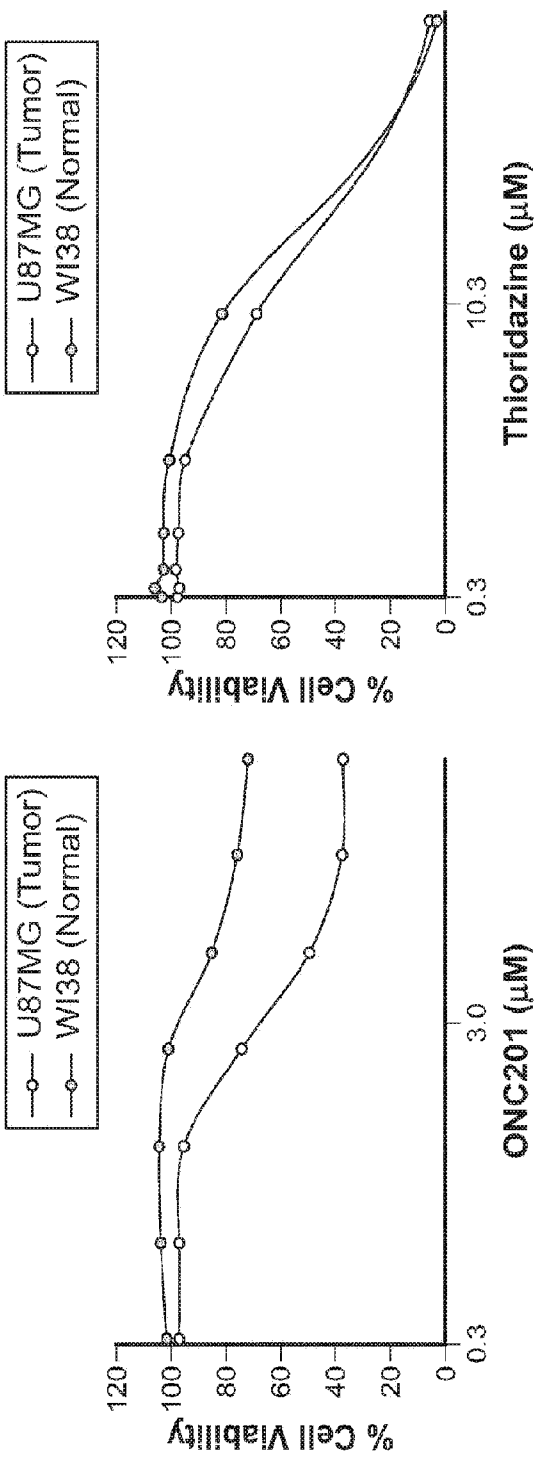

U87MG (Tumor)
WI38 (Normal)
% Cell Viability
120
100
80
60
40
20
0
0.3
3.0
ONC201 (μM)
U87MG (Tumor)
WI38 (Normal)
% Cell Viability
120
100
80
60
40
20
0
0.3
10.3
Thioridazine (μM)

Figure 8

Figure 9

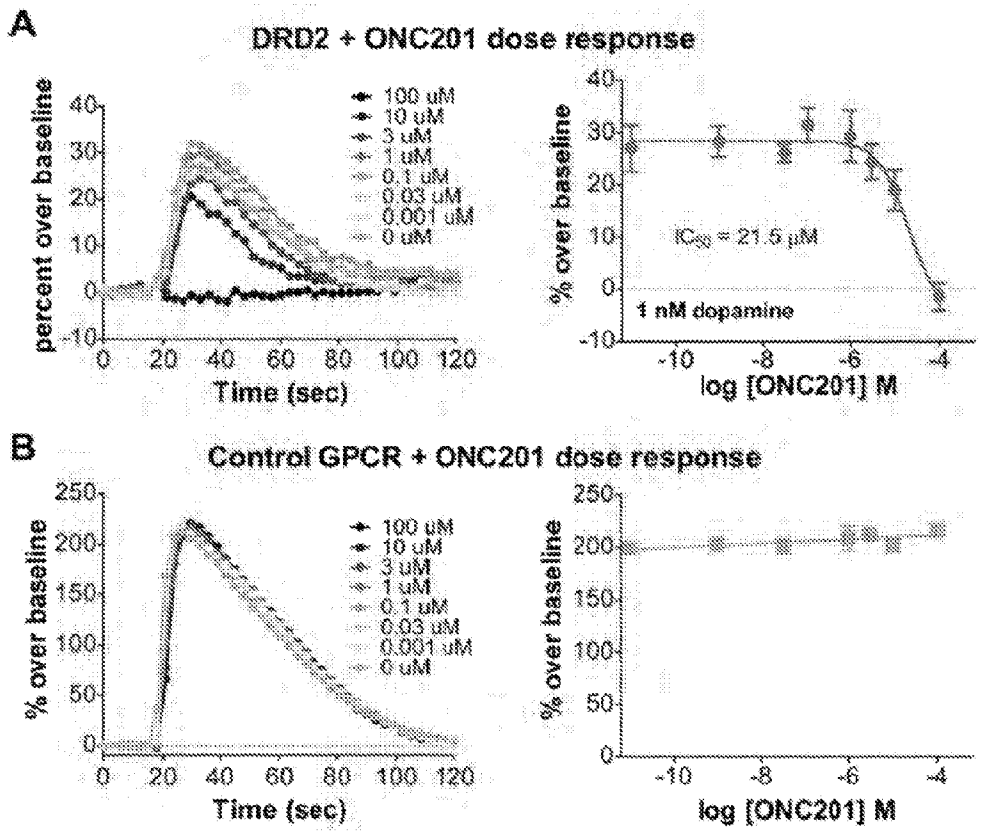


Figure 10

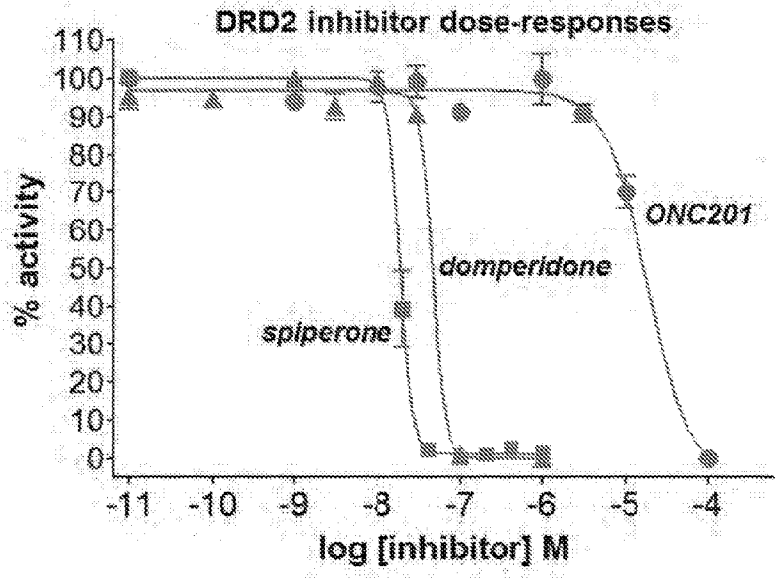
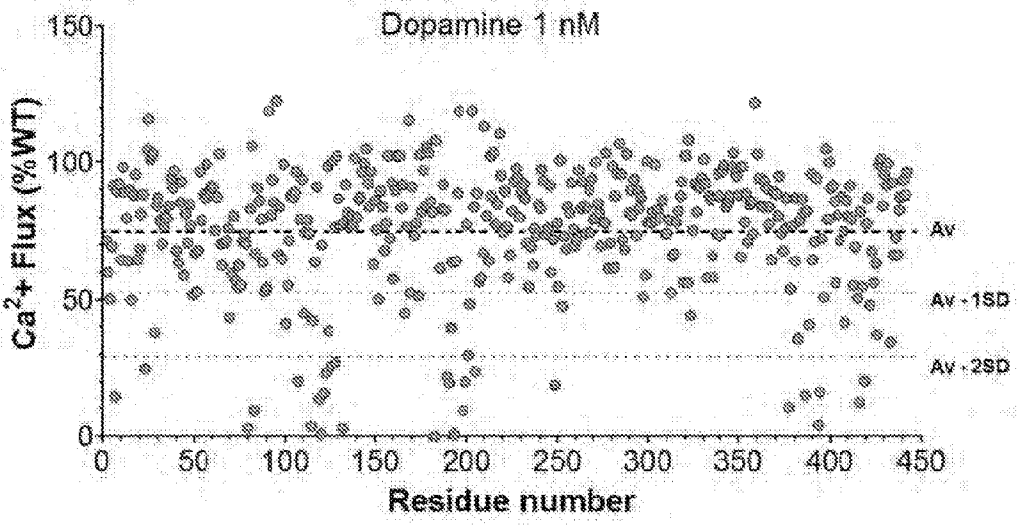


Figure 11

A


Dopamine 1 nM
Ca2+ Flux (%WT)
150
100
50
0
0
50
100
150
200
250
300
350
400
450
Residue number
Av
Av - 1SD
Av - 2SD

B

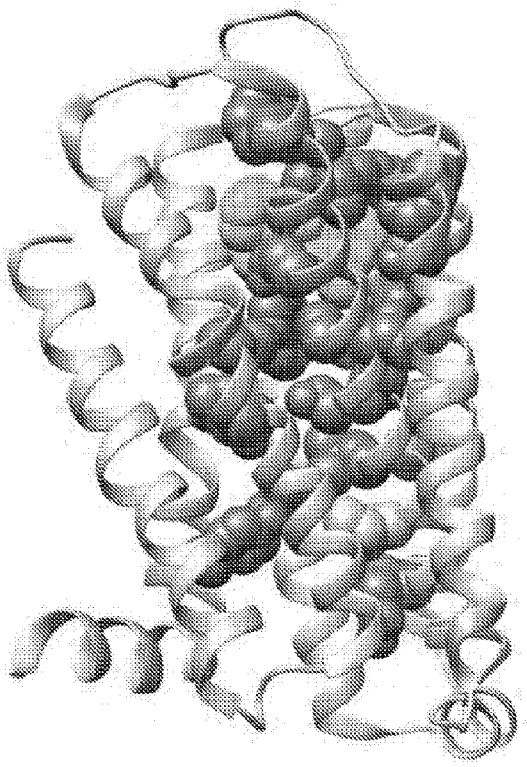
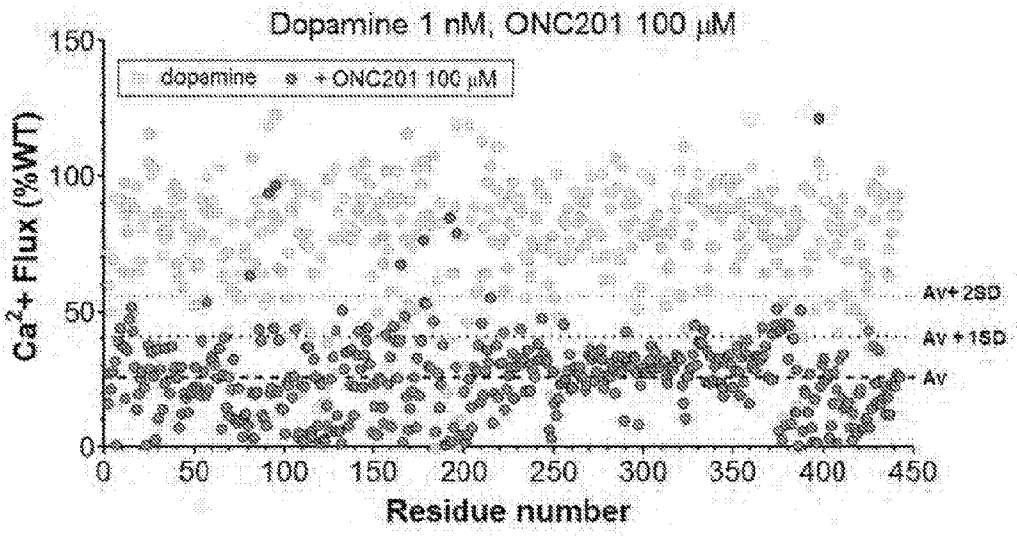
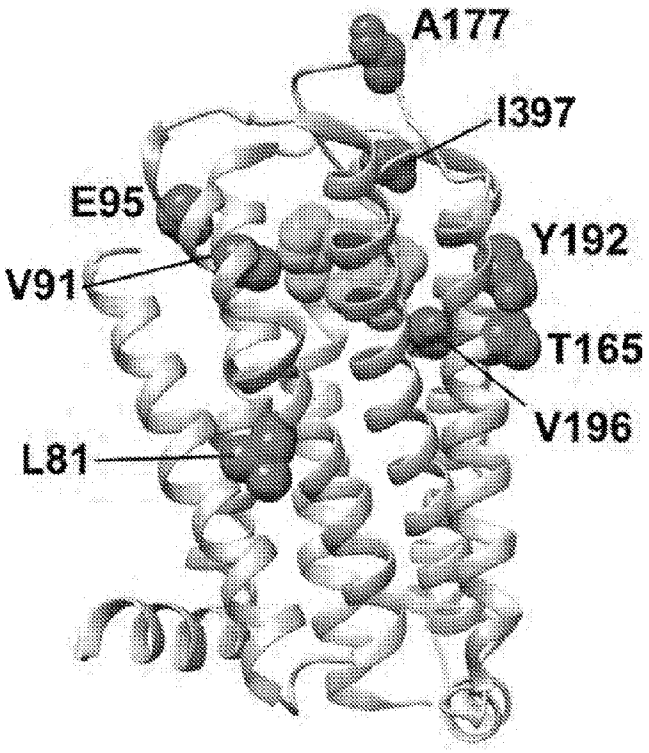


Figure 12

A


Dopamine 1 nM, ONC201 100 μM
dopamine
+ ONC201 100 μM
Ca2+ Flux (%WT)
150
100
50
0
0
50
100
150
200
250
300
350
400
450
Residue number
Av+2SD
Av+1SD
Av

B


A177
I397
E95
Y192
V91
T165
V196
L81

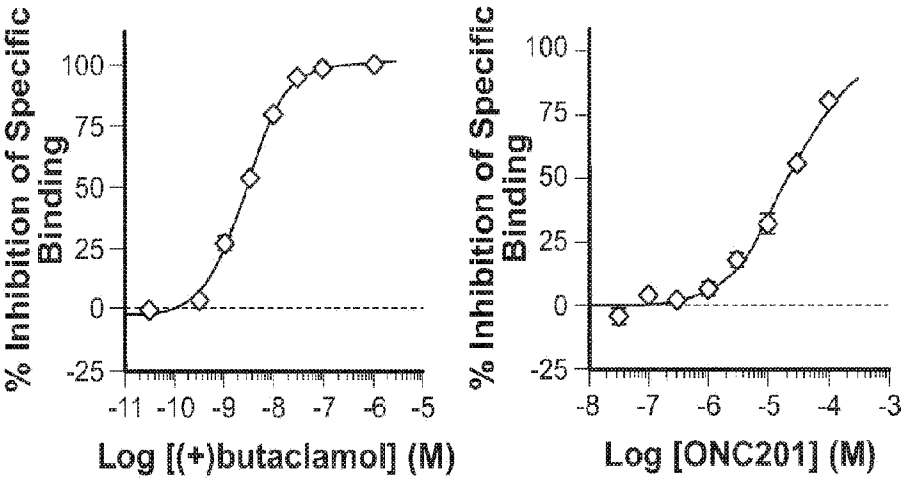


Figure 13

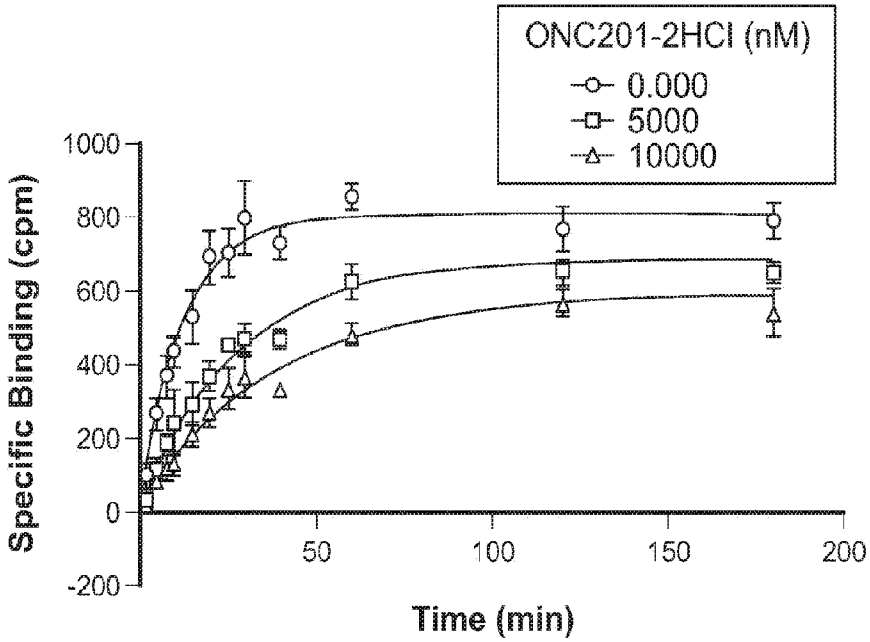


Figure 14

] Figure 15

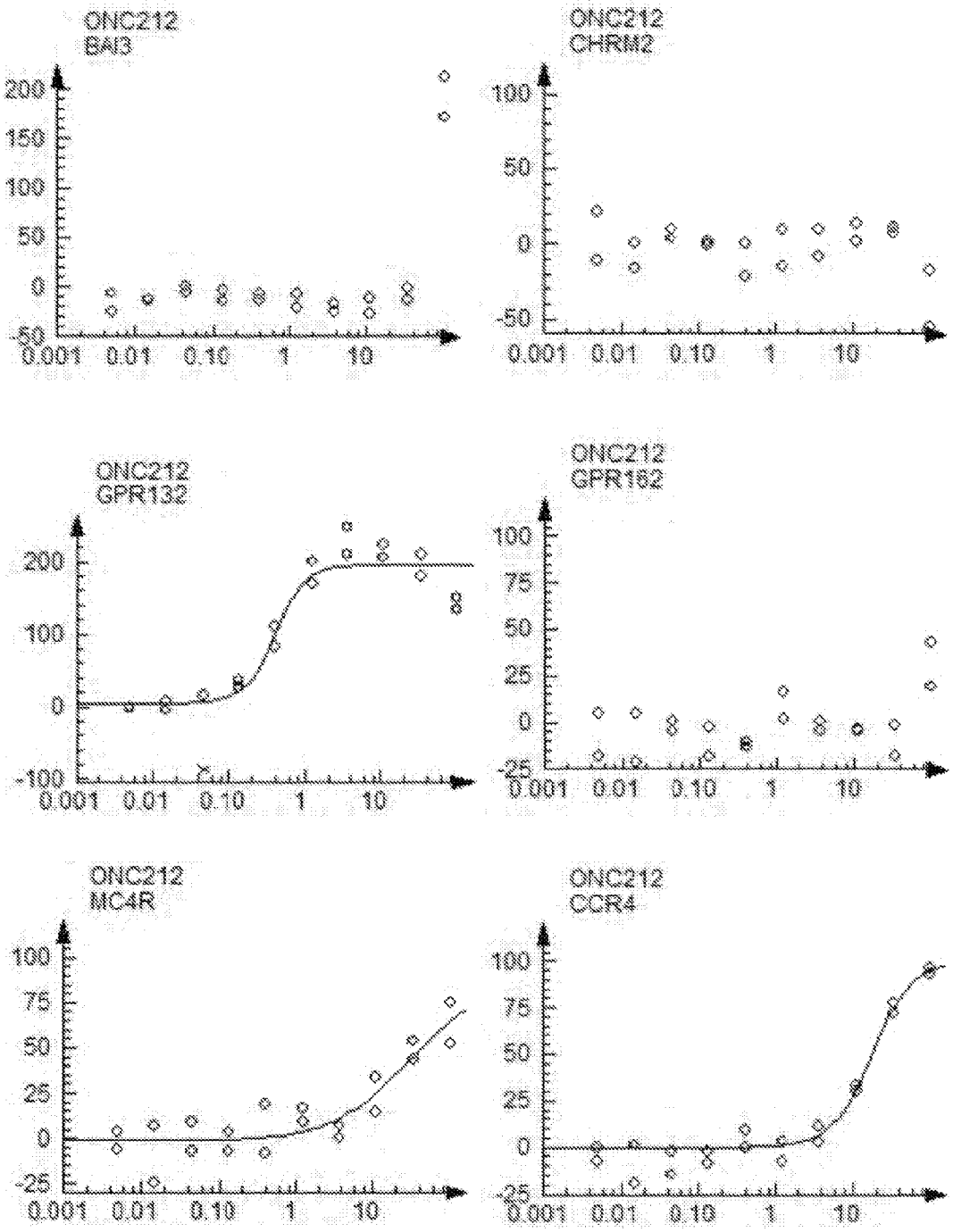
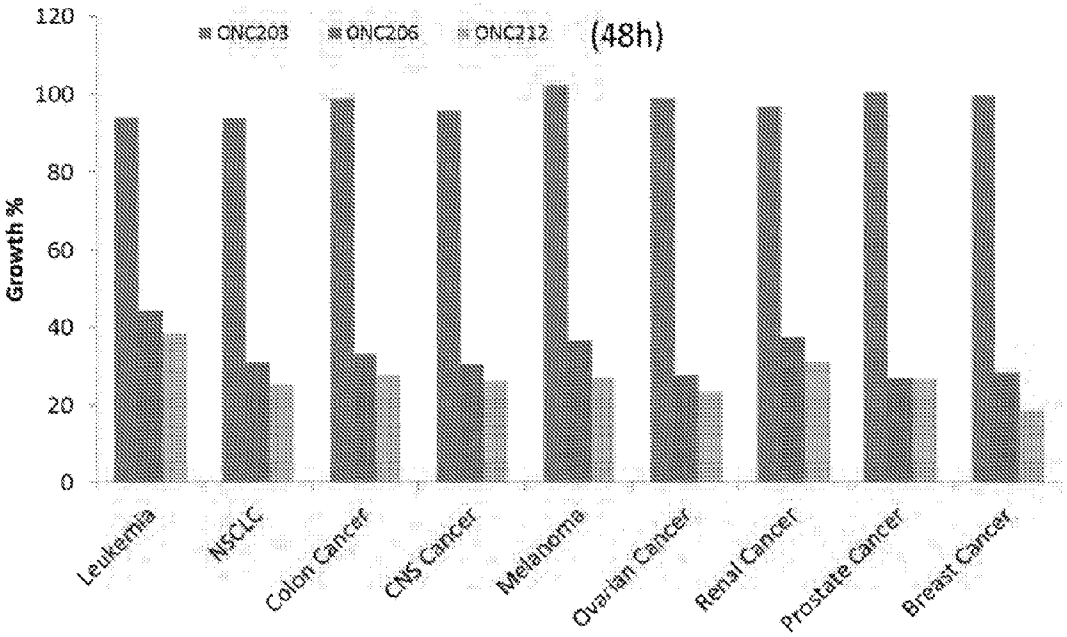

ONC212
BAI3
200
150
100
50
0
-50
0.001
0.01
0.10
1
10
ONC212
CHRM2
100
50
0
-50
0.001
0.01
0.10
1
10
ONC212
GPR132
200
100
0
-100
0.001
0.01
0.10
1
10
ONC212
GPR152
100
75
50
25
0
-25
0.001
0.01
0.10
1
10
ONC212
MC4R
100
75
50
25
0
-25
0.001
0.01
0.10
1
10
ONC212
CCR4
100
75
50
25
0
-25
0.001
0.01
0.10
1
10

Figure 16


120
100
80
60
40
20
0
Growth %
ONC203
ONC206
ONC212
(48h)
Leukemia
NSCLC
Colon Cancer
CNS Cancer
Melanoma
Ovarian Cancer
Renal Cancer
Prostate Cancer
Breast Cancer

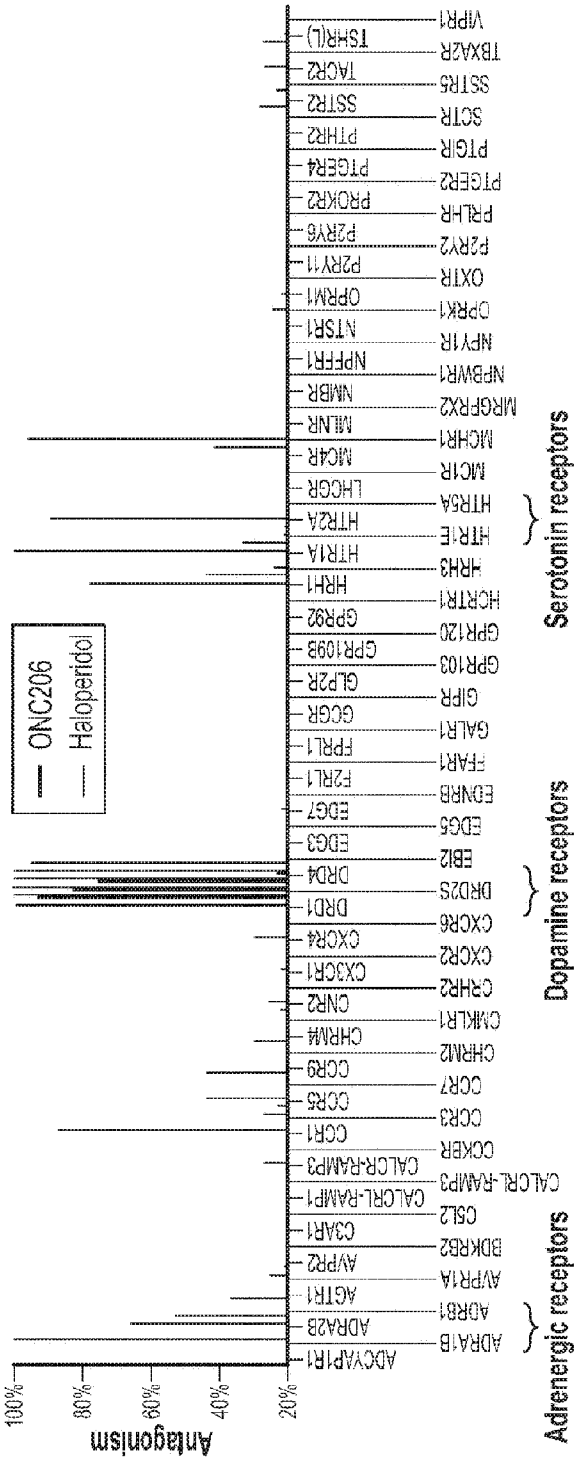

ONC206
Haloperidol
Antagonism
100%
80%
60%
40%
20%
ADCYAP1R1
ADRA1B
ADRB1
AGTR1
AVPR2
AVPR1A
BDKRB2
C3AR1
C5L2
CALCRL-RAMP1
CALCRL-RAMP3
CCKBR
CCR1
CCR3
CCR5
CCR7
CCR9
CHRM4
CHRM2
CNR2
CNR1
CRHR2
CX3CR1
CXCR2
CXCR4
CXCR6
DRD1
DRD4
DRD2S
EBI2
EDG3
EDG5
EDG7
EDNRB
F2RL1
FFAR1
FPRL1
GALR1
GCGR
GLP2R
GIPR
GPR103
GPR1098
GPR120
GPR92
HRH1
HRH3
HCRTR1
HTR1A
HTR1E
HTR2A
HTR5A
LHCGR
MC1R
MC4R
MCHR1
MLNR
MRGFRX2
NMBR
NPBWR1
NPFFR1
NPY1R
NTSR1
OPRK1
OPRM1
OXTR
P2RY11
P2RY2
P2RY6
PRLHR
PROKR2
PTGER2
PTGER4
PTGIR
PTHR2
SCTR
SSTR2
SSTR5
TACR2
TBXA2R
TSHR(L)
VIPR1
Adrenergic receptors
Dopamine receptors
Serotonin receptors

Figure 17

Figure 18

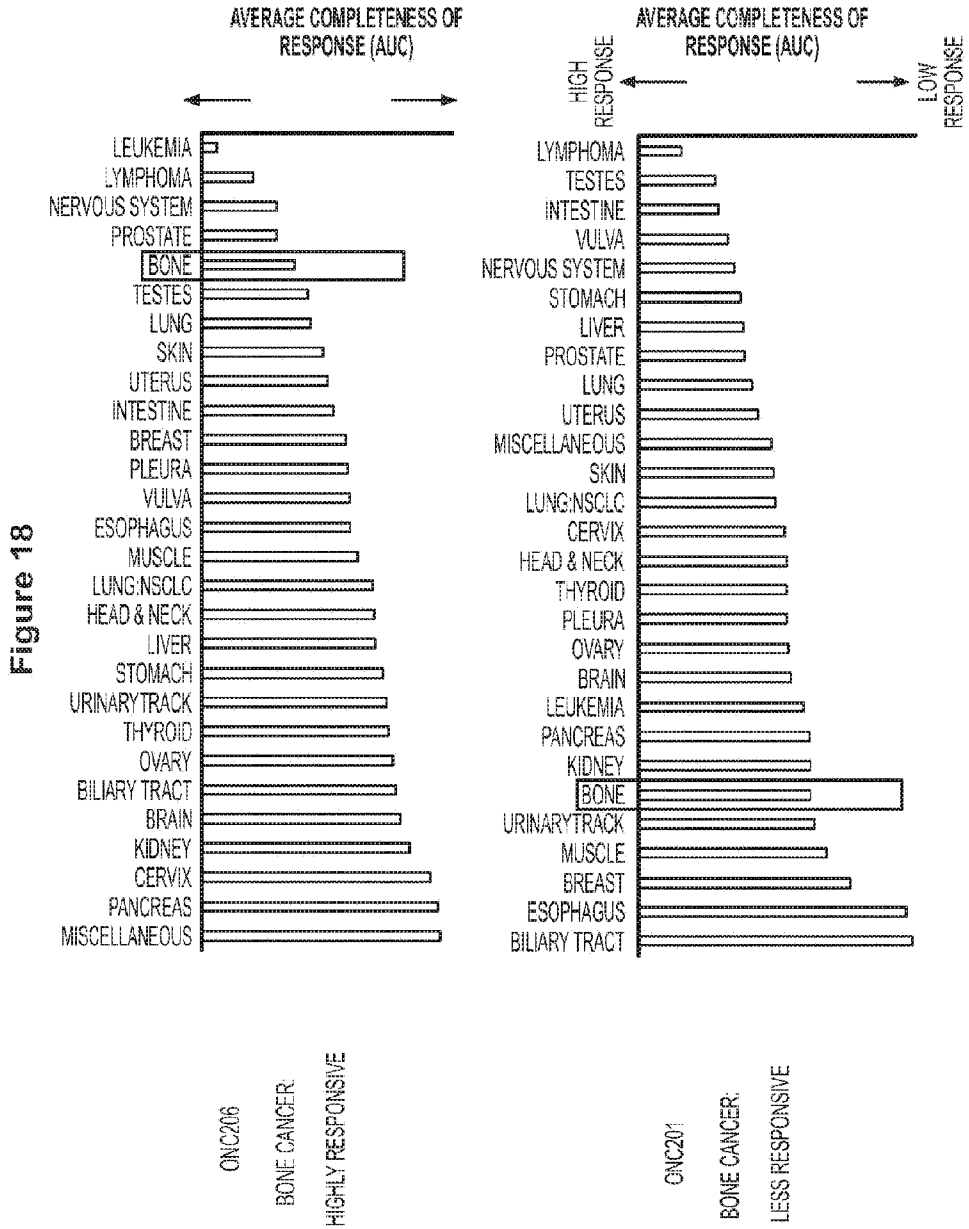
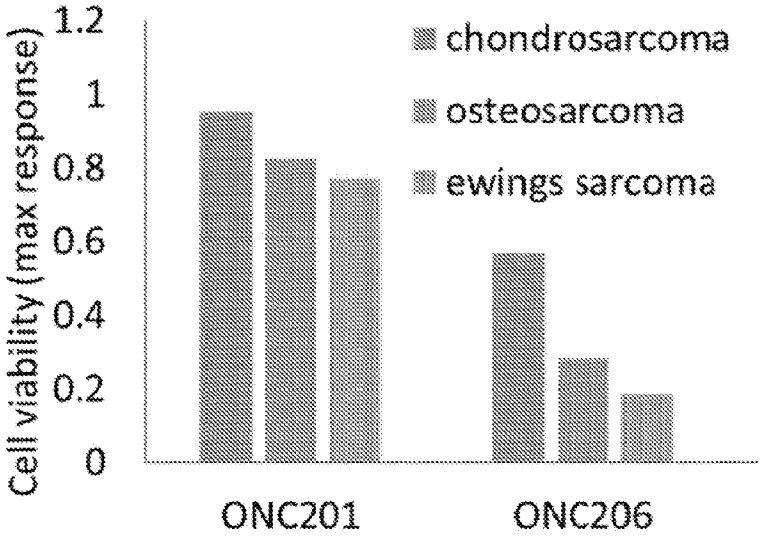

AVERAGE COMPLETENESS OF RESPONSE (AUC)
LEUKEMIA
LYMPHOMA
NERVOUS SYSTEM
PROSTATE
BONE
TESTES
LUNG
SKIN
UTERUS
INTESTINE
BREAST
PLEURA
VULVA
ESOPHAGUS
MUSCLE
LUNG:NSCLC
HEAD & NECK
LIVER
STOMACH
URINARYTRACT
THYROID
OVARY
BILIARY TRACT
BRAIN
KIDNEY
CERVIX
PANCREAS
MISCELLANEOUS
ONC206
BONE CANCER:
HIGHLY RESPONSIVE
AVERAGE COMPLETENESS OF RESPONSE (AUC)
HIGH RESPONSE
LOW RESPONSE
LYMPHOMA
TESTES
INTESTINE
VULVA
NERVOUS SYSTEM
STOMACH
LIVER
PROSTATE
LUNG
UTERUS
MISCELLANEOUS
SKIN
LUNG:NSCLC
CERVIX
HEAD & NECK
THYROID
PLEURA
OVARY
BRAIN
LEUKEMIA
PANCREAS
KIDNEY
BONE
URINARYTRACK
MUSCLE
BREAST
ESOPHAGUS
BILIARY TRACT
ONC201
BONE CANCER:
LESS RESPONSIVE

Figure 19


Cell viability (max response)
1.2
1
0.8
0.6
0.4
0.2
0
chondrosarcoma
osteosarcoma
ewings sarcoma
ONC201
ONC206

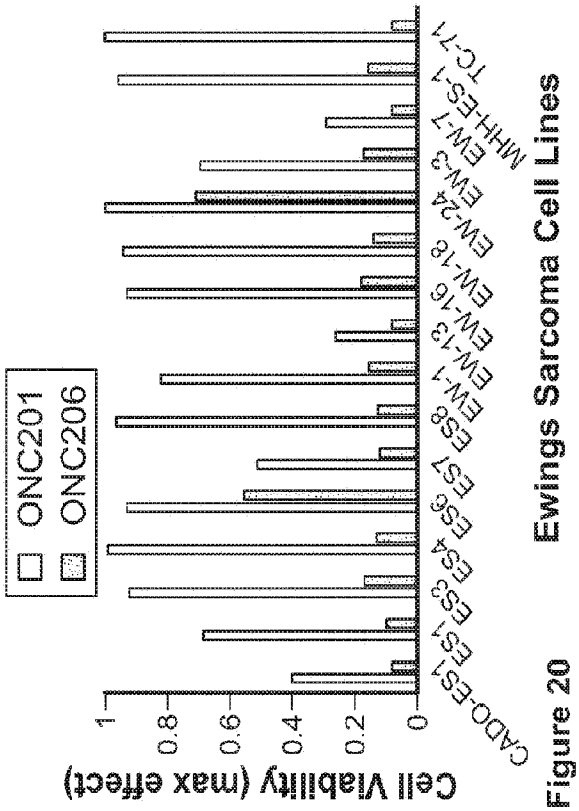
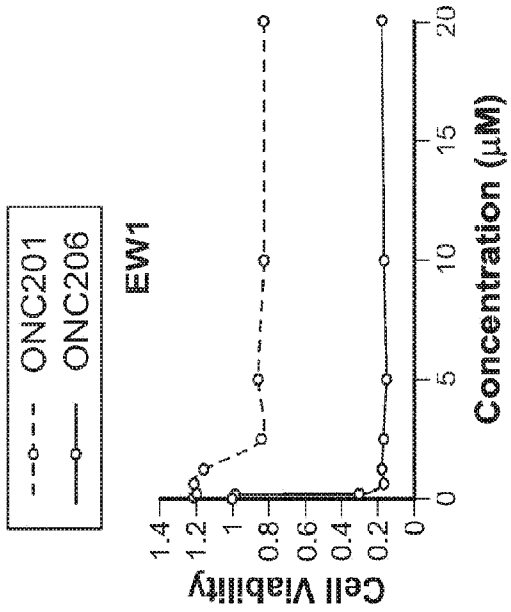

ONC201
ONC206
Cell Viability (max effect)
1
0.8
0.6
0.4
0.2
0
CADO-ES1
ES2
ES3
ES4
ES6
ES7
ES8
EW-1
EW-13
EW-16
EW-18
EW-24
EW-3
EW-7
MHH-ES-1
TC-71
Ewings Sarcoma Cell Lines

Figure 20


ONC201
ONC206
EW1
Cell Viability
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
5
10
15
20
Concentration (µM)

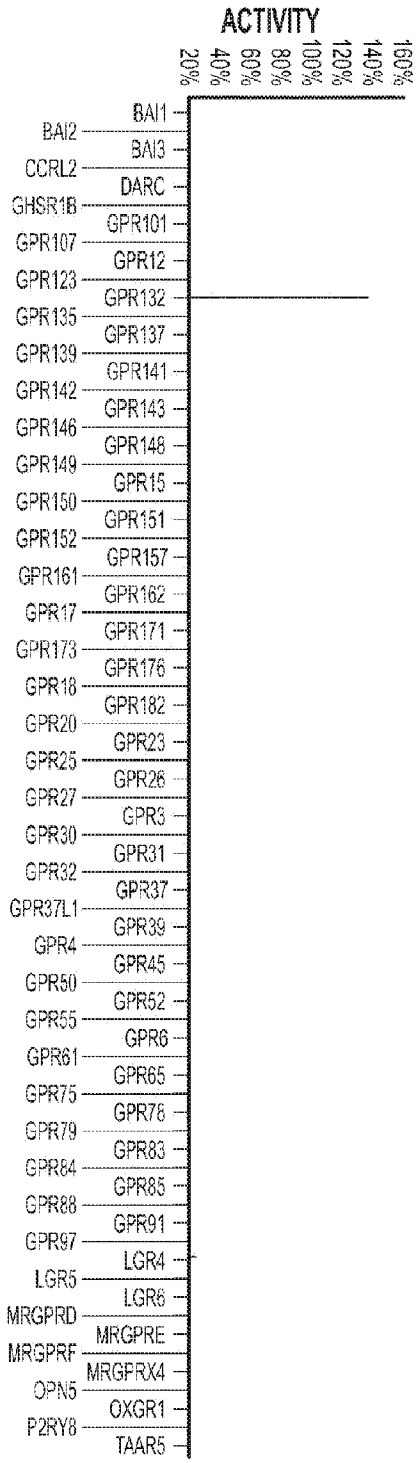

ACTIVITY
20%
40%
60%
80%
100%
120%
140%
160%
BAI1
BAI2
BAI3
CCRL2
DARC
GHSR1B
GPR101
GPR107
GPR12
GPR123
GPR132
GPR135
GPR137
GPR139
GPR141
GPR142
GPR143
GPR146
GPR148
GPR149
GPR15
GPR150
GPR151
GPR152
GPR157
GPR161
GPR162
GPR17
GPR171
GPR173
GPR176
GPR18
GPR182
GPR20
GPR23
GPR25
GPR26
GPR27
GPR3
GPR30
GPR31
GPR32
GPR37
GPR37L1
GPR39
GPR4
GPR45
GPR50
GPR52
GPR55
GPR6
GPR61
GPR65
GPR75
GPR78
GPR79
GPR83
GPR84
GPR85
GPR88
GPR91
GPR97
LGR4
LGR5
LGR6
MRGPRD
MRGPRE
MRGPRF
MRGPRX4
OPN5
OXGR1
P2RY8
TAAR5

Figure 21

Figure 22

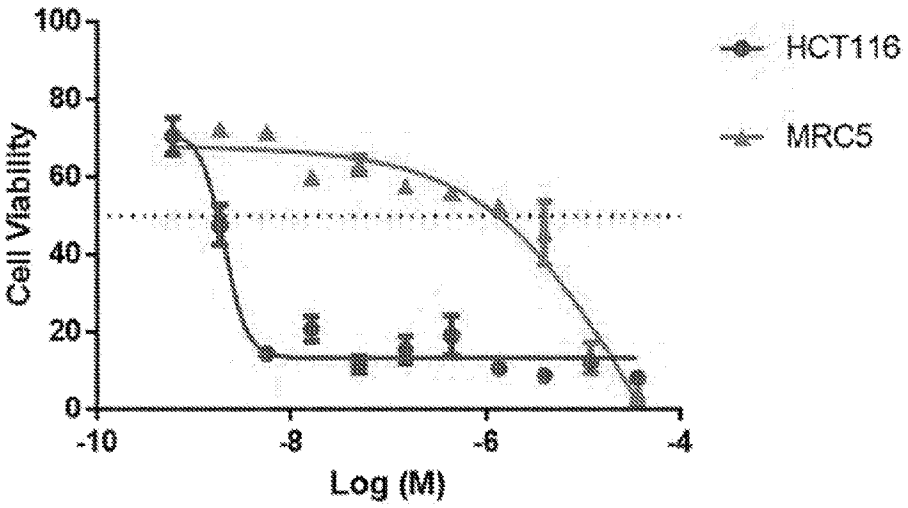
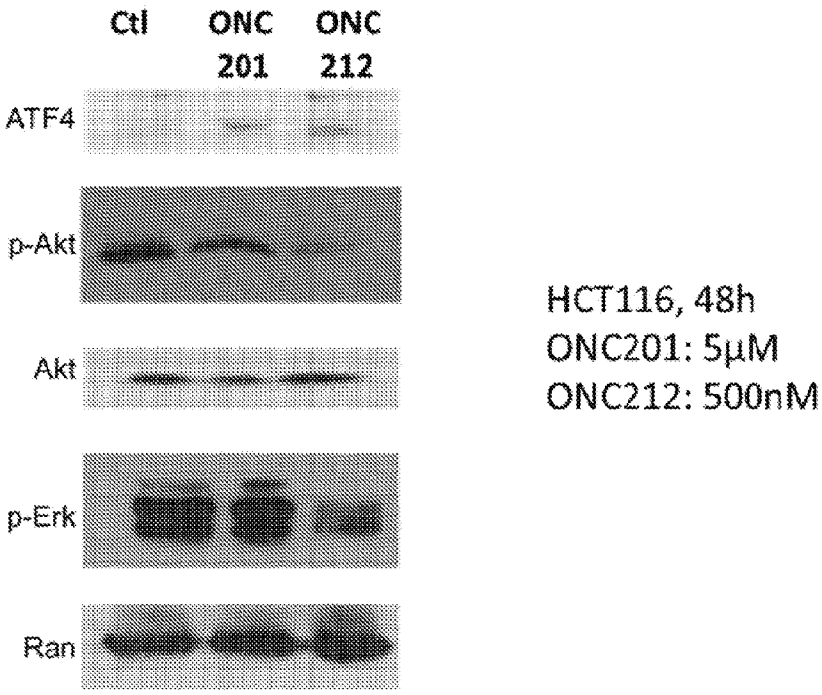


Figure 23



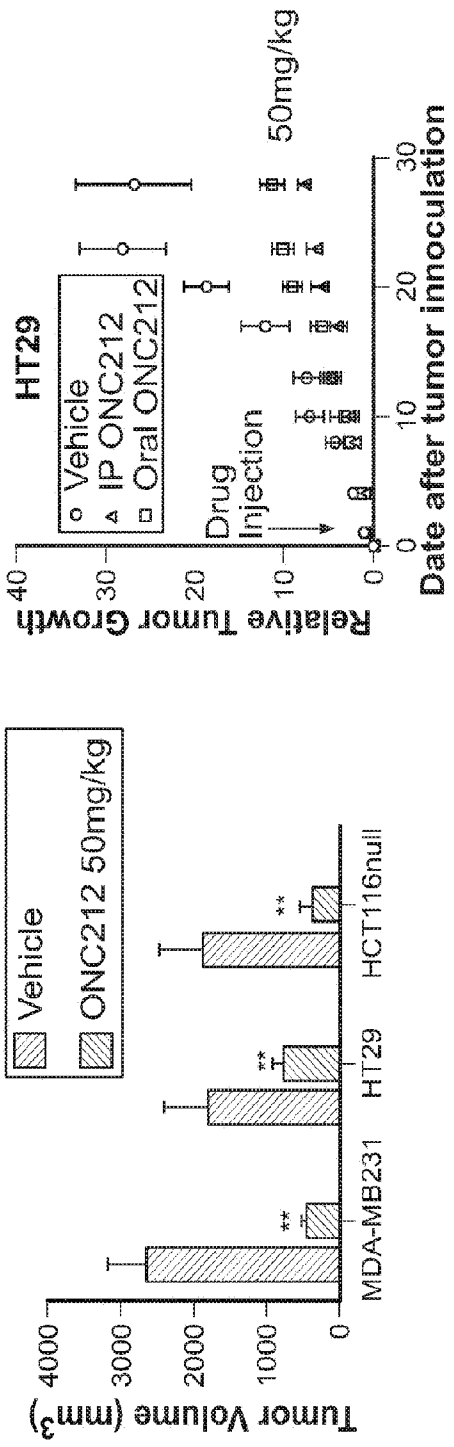

Tumor Volume (mm3)
4000
3000
2000
1000
0
Vehicle
ONC212 50mg/kg
**
**
**
MDA-MB231
HT29
HCT116null
Relative Tumor Growth
40
30
20
10
0
HT29
Vehicle
IP ONC212
Oral ONC212
50mg/kg
Drug Injection
0
10
20
30
Date after tumor innoculation

Figure 24

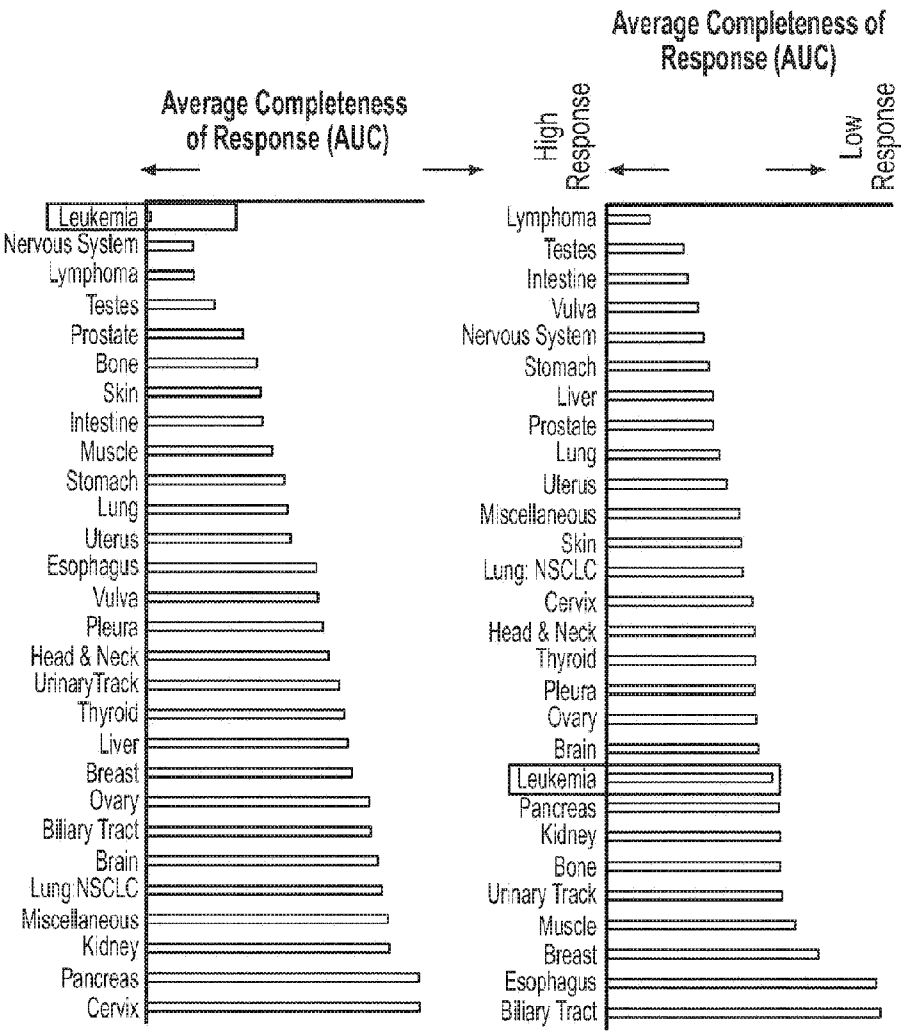
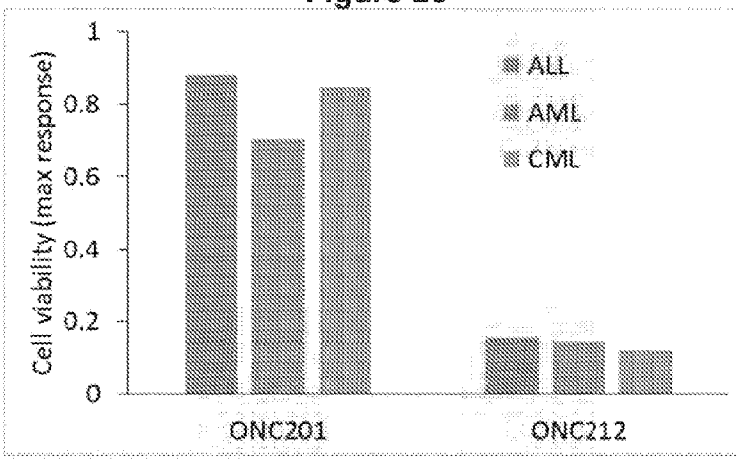
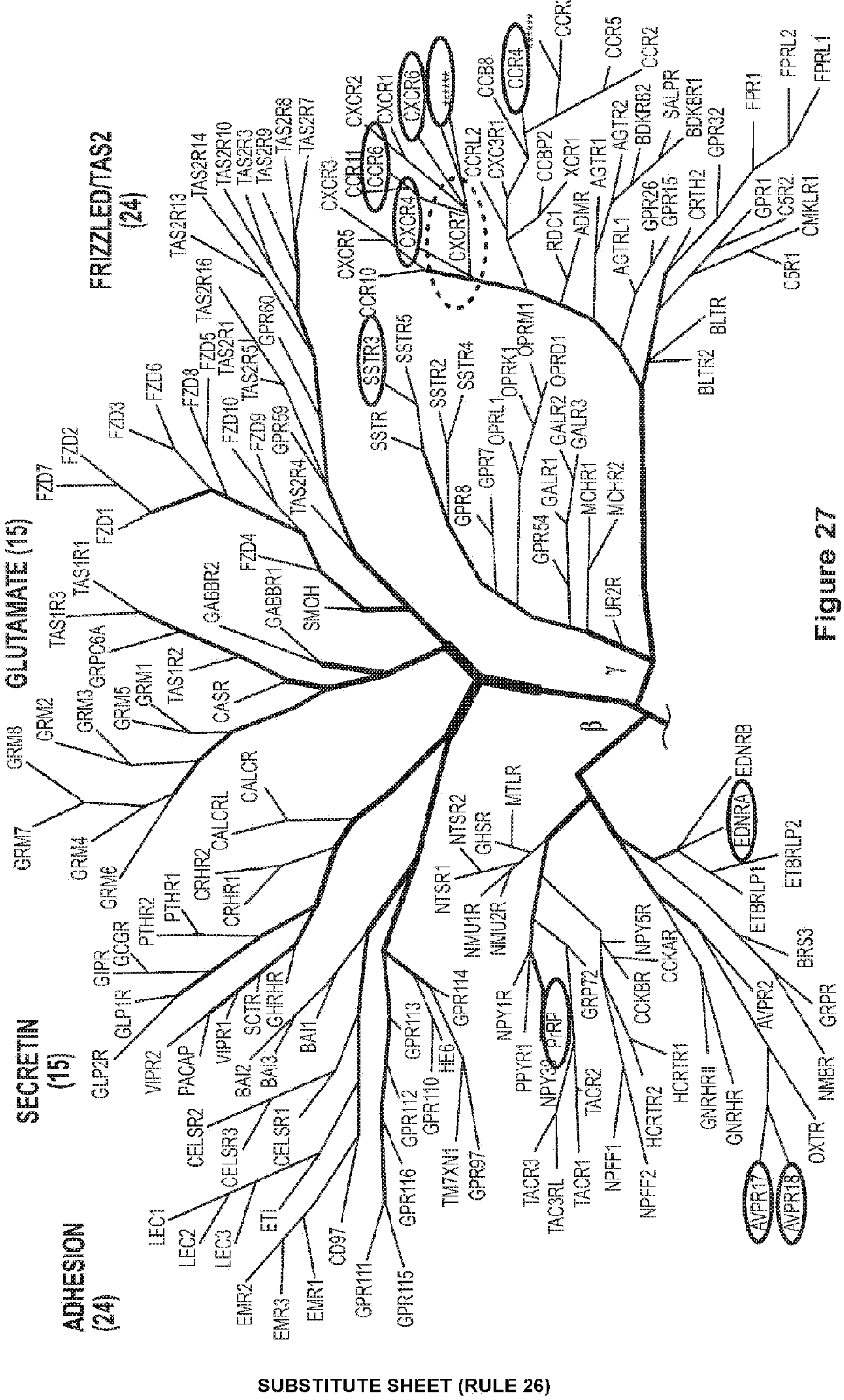

Average Completeness of Response (AUC)
High Response
Leukemia
Nervous System
Lymphoma
Testes
Prostate
Bone
Skin
Intestine
Muscle
Stomach
Lung
Uterus
Esophagus
Vulva
Pleura
Head & Neck
UrinaryTrack
Thyroid
Liver
Breast
Ovary
Biliary Tract
Brain
Lung:NSCLC
Miscellaneous
Kidney
Pancreas
Cervix
ONC212 Leukemia: Highly Responsive
Average Completeness of Response (AUC)
Low Response
Lymphoma
Testes
Intestine
Vulva
Nervous System
Stomach
Liver
Prostate
Lung
Uterus
Miscellaneous
Skin
Lung: NSCLC
Cervix
Head & Neck
Thyroid
Pleura
Ovary
Brain
Leukemia
Pancreas
Kidney
Bone
Urinary Track
Muscle
Breast
Esophagus
Biliary Tract
ONC201 Leukemia: Less Responsive

Figure 25

Figure 26


Cell viability (max response)
1
0.8
0.6
0.4
0.2
0
ALL
AML
CML
ONC201
ONC212


FRIZZLED/TAS2 (24)
GLUTAMATE (15)
SECRETIN (15)
ADHESION (24)
TAS2R13
TAS2R14
TAS2R10
TAS2R3
TAS2R9
TAS2R8
TAS2R7
TAS2R16
TAS2R1
TAS2R5
GPR60
FZD5
FZD6
FZD8
FZD10
FZD9
GPR59
TAS2R4
FZD3
FZD2
FZD7
FZD1
FZD4
SMOH
GABBR1
GABBR2
TAS1R1
TAS1R3
GRPC6A
GRM1
TAS1R2
GRM5
CASR
GRM3
GRM2
GRM8
GRM7
GRM4
GRM6
CALCR
CALCRL
CRHR2
CRHR1
PTHR2
PTHR1
GIPR
GCGR
GLP1R
GLP2R
VIPR2
PACAP
VIPR1
SCTR
GHRHR
BAI2
BAI3
BAI1
CELSR2
CELSR3
CELSR1
LEC1
LEC2
LEC3
ETI
EMR2
EMR3
EMR1
CD97
GPR111
GPR115
GPR116
GPR112
GPR110
HE6
GPR113
GPR114
TM7XN1
GPR97
CXCR5
CXCR3
CCR11
CXCR2
CXCR1
CXCR6
CCR10
CXCR4
CCR6
CXCR7
CCRL2
CXC3R1
CCB8
CCR4
CCR3
CCR5
CCR2
CCBP2
XCR1
RDC1
ADMR
AGTR1
AGTR2
BDKRB2
SALPR
BDKBR1
GPR32
AGTRL1
GPR26
GPR15
CRTH2
GPR1
C5R2
CMKLR1
C5R1
FPR1
FPRL2
FPRL1
BLTR
BLTR2
SSTR3
SSTR5
SSTR
SSTR2
SSTR4
OPRM1
OPRK1
OPRD1
OPRL1
GPR7
GPR8
GALR2
GALR3
GALR1
GPR54
MCHR1
MCHR2
UR2R
γ
β
MTLR
NTSR2
GHSR
NTSR1
NMU1R
NMU2R
NPY1R
PrRP
PPYR1
NPY2
GRP72
NPY5R
CCKBR
CCKAR
TACR2
TACR3
TAC3RL
TACR1
NPFF1
NPFF2
HCRTR2
HCRTR1
GNRHRII
GNRHR
EDNRB
EDNRA
ETBRLP2
ETBRLP1
BRS3
GRPR
AVPR2
NMBR
OXTR
AVPR1
AVPR1B
Figure 27

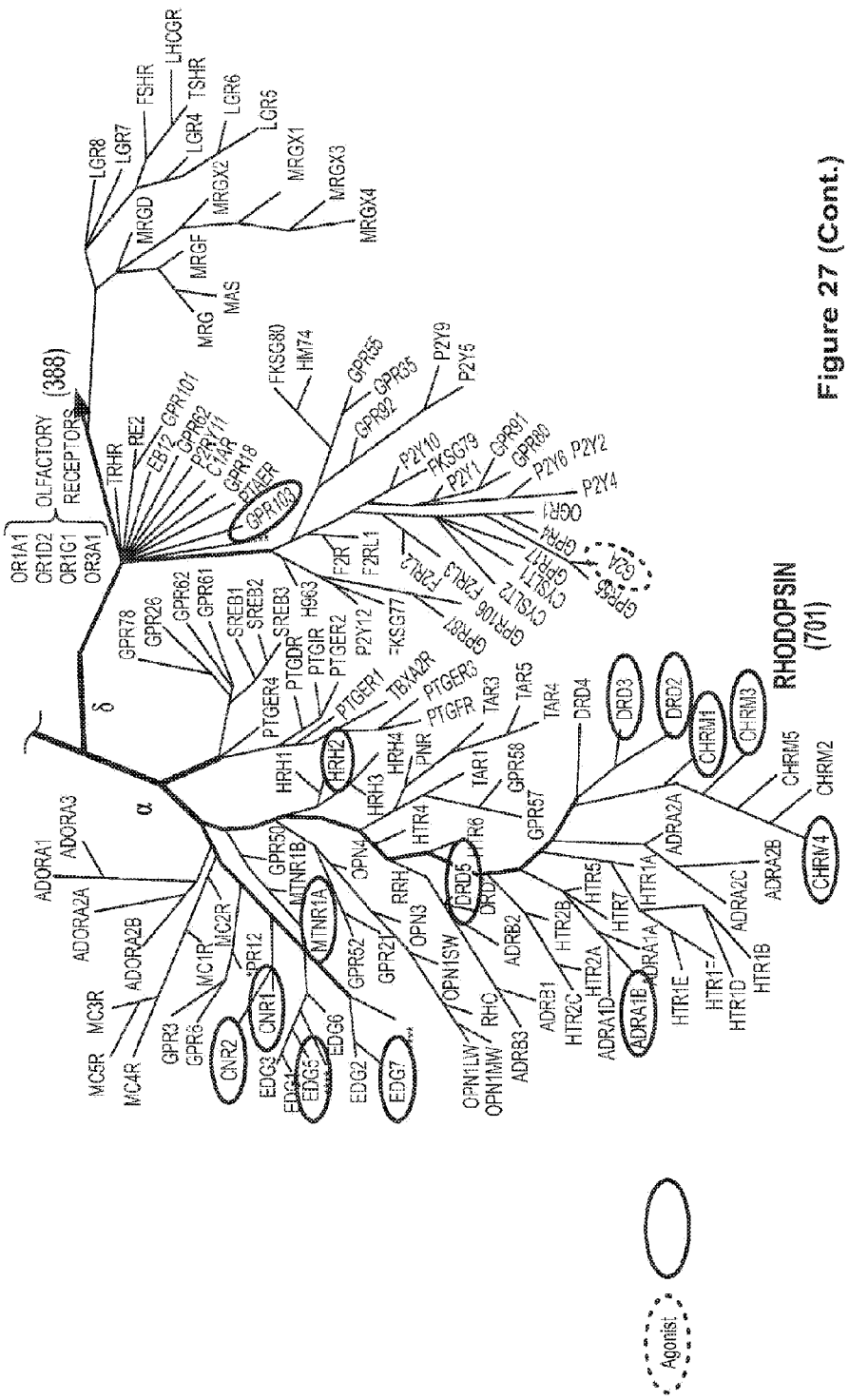
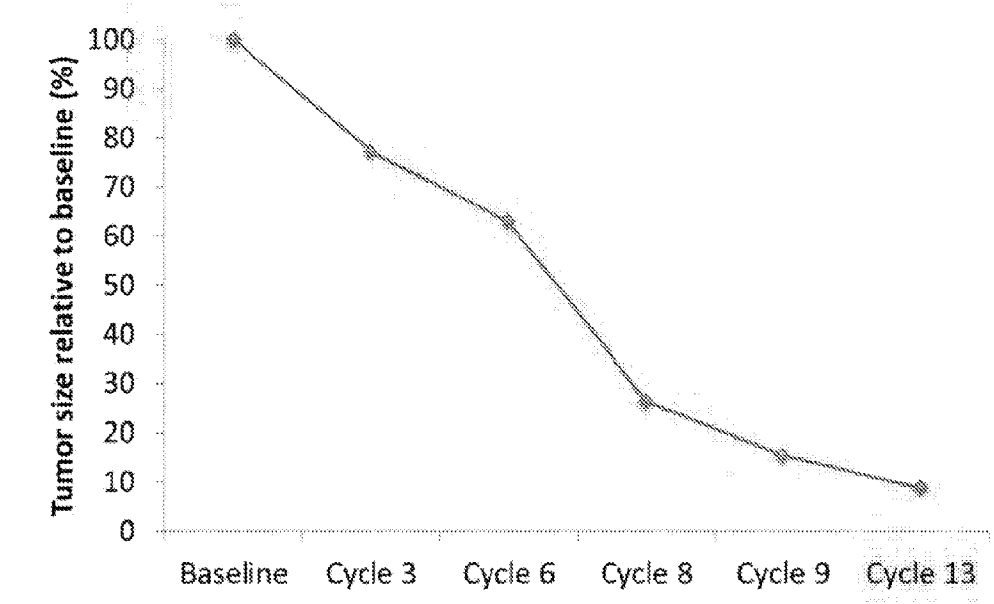


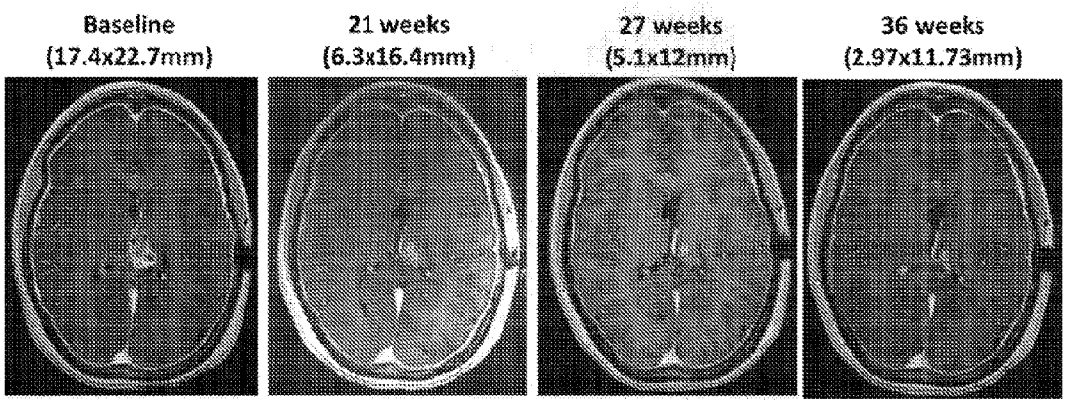
Figure 27 (Cont.)

Figure 28

A


Tumor size relative to baseline (%)
100
90
80
70
60
50
40
30
20
10
0
Baseline
Cycle 3
Cycle 6
Cycle 8
Cycle 9
Cycle 13

B


Baseline
(17.4x22.7mm)
21 weeks
(6.3x16.4mm)
27 weeks
(5.1x12mm)
36 weeks
(2.97x11.73mm)

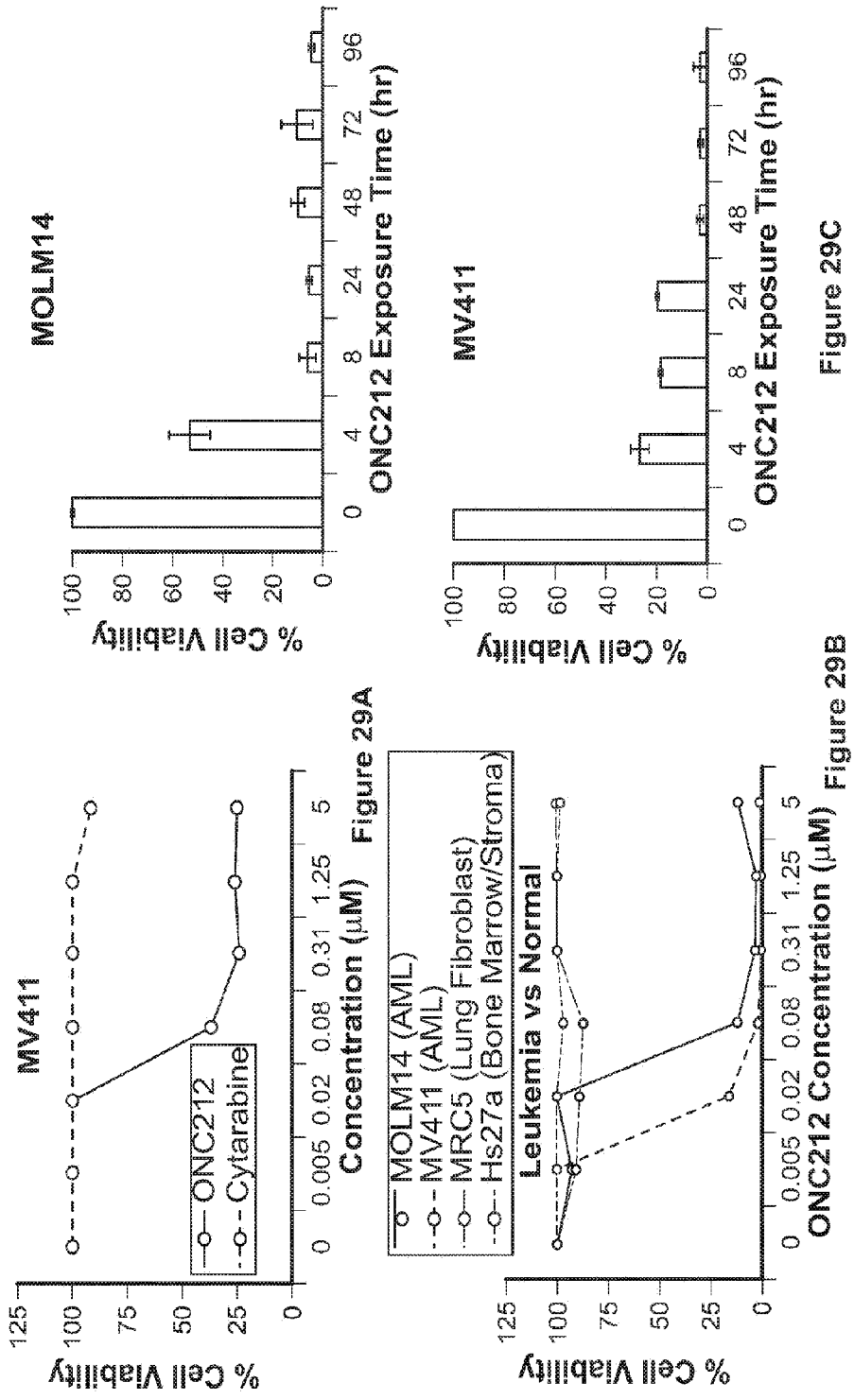


Figure 29C

Figure 29B

Figure 29A

Figure 29B

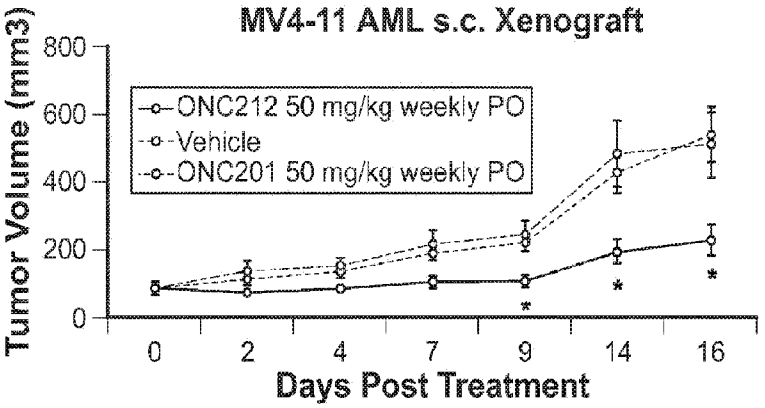


Figure 30A

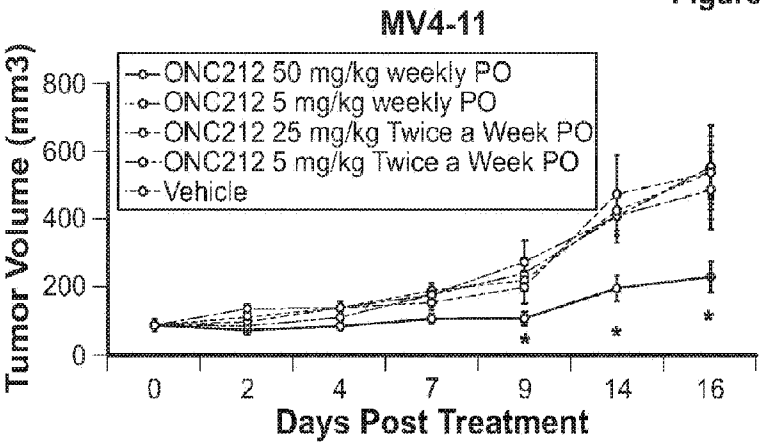


Figure 30B

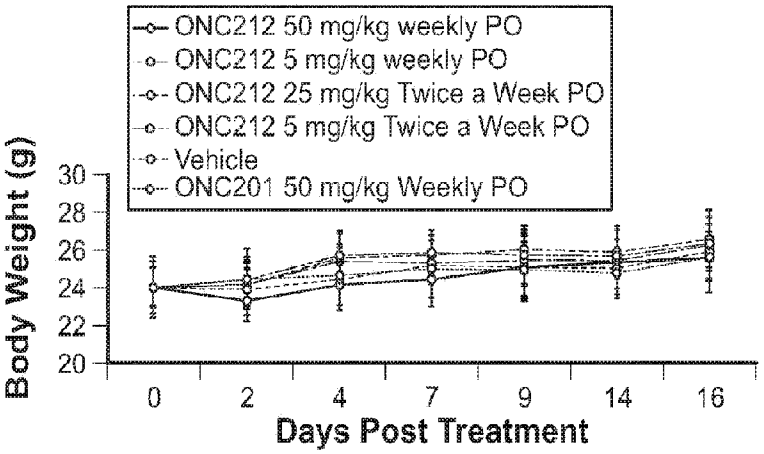
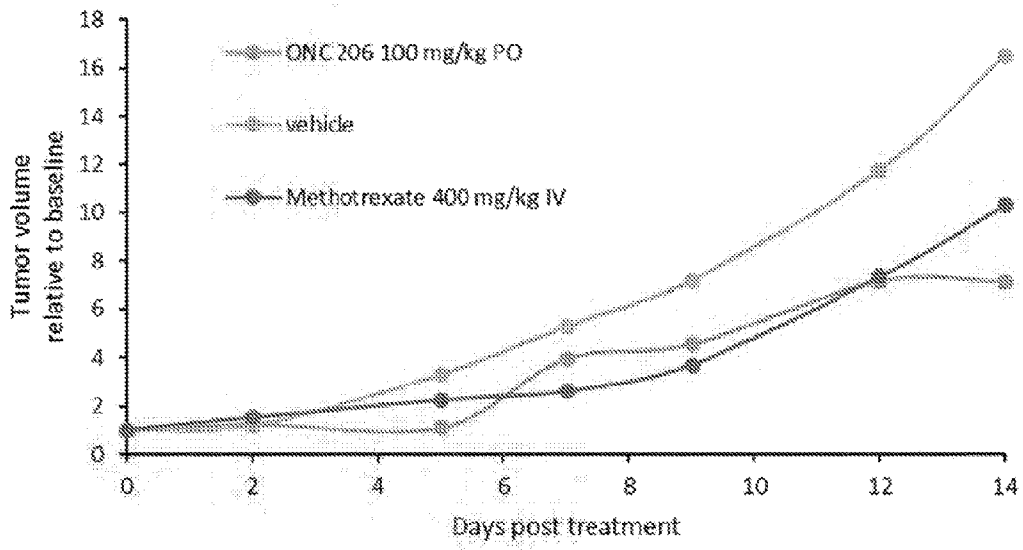


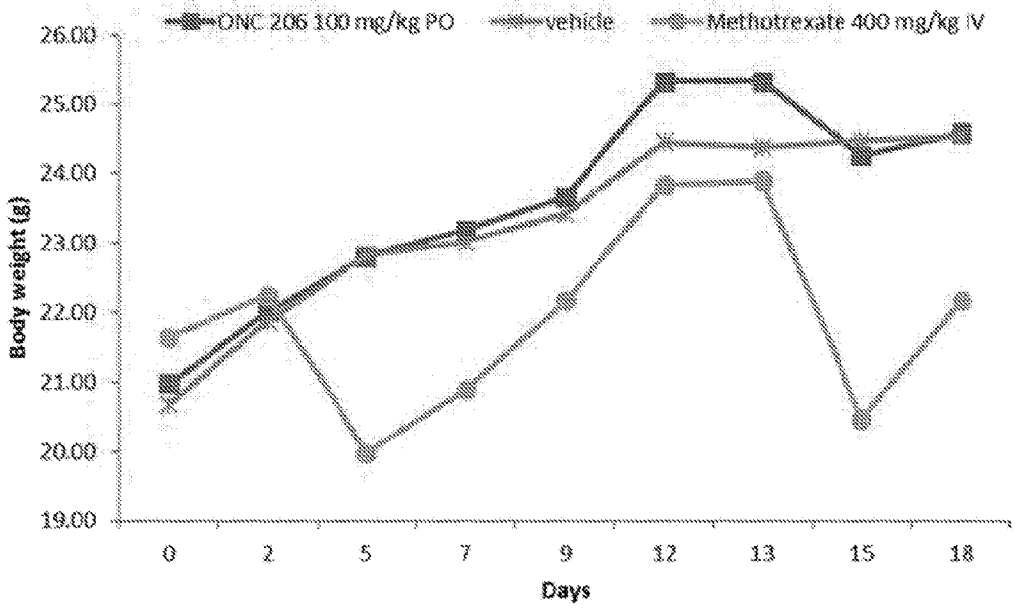
Figure 30C

Figure 31

A


ONC 206 100 mg/kg PO
vehicle
Methotrexate 400 mg/kg IV
Tumor volume relative to baseline
Days post treatment
0
2
4
6
8
10
12
14
16
18

B


ONC 206 100 mg/kg PO
vehicle
Methotrexate 400 mg/kg IV
Body weight (g)
Days
26.00
25.00
24.00
23.00
22.00
21.00
20.00
19.00
0
2
5
7
9
12
13
15
18

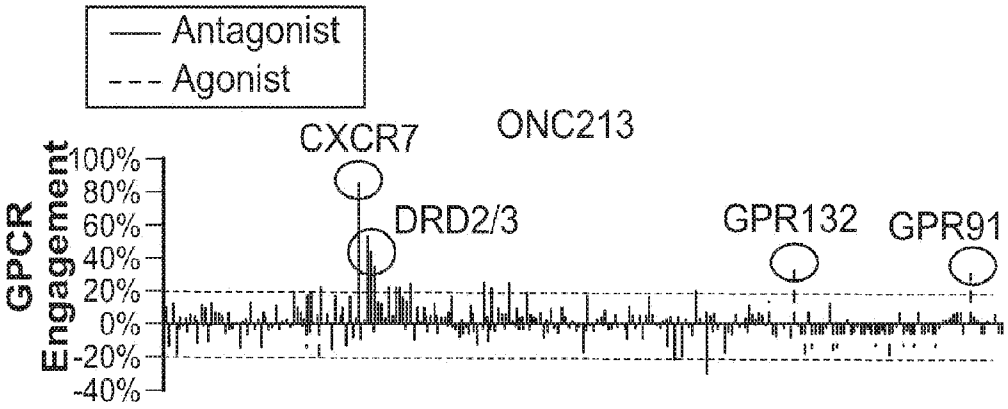

Antagonist
Agonist
ONC213
CXCR7
DRD2/3
GPR132
GPR91
GPCR Engagement
100%
80%
60%
40%
20%
0%
-20%
-40%

Figure 32

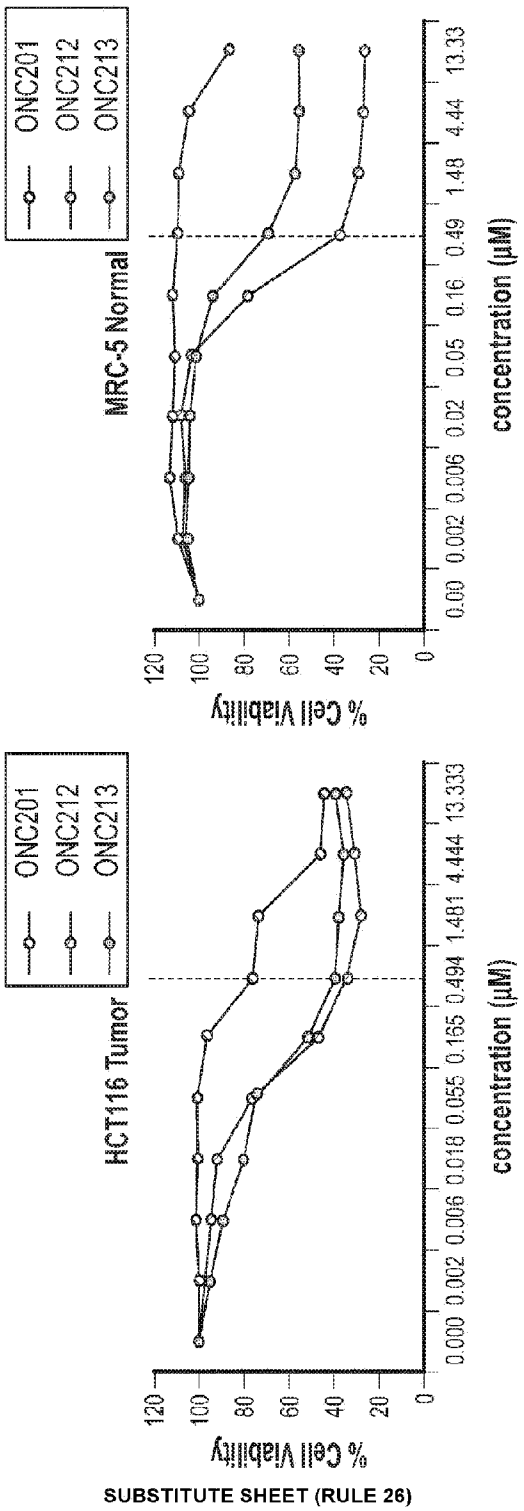

HCT116 Tumor
ONC201
ONC212
ONC213
% Cell Viability
120
100
80
60
40
20
0
0.000 0.002 0.006 0.018 0.055 0.165 0.494 1.481 4.444 13.333
concentration (µM)
MRC-5 Normal
ONC201
ONC212
ONC213
% Cell Viability
120
100
80
60
40
20
0
0.00 0.002 0.006 0.02 0.05 0.16 0.49 1.48 4.44 13.33
concentration (µM)

Figure 33

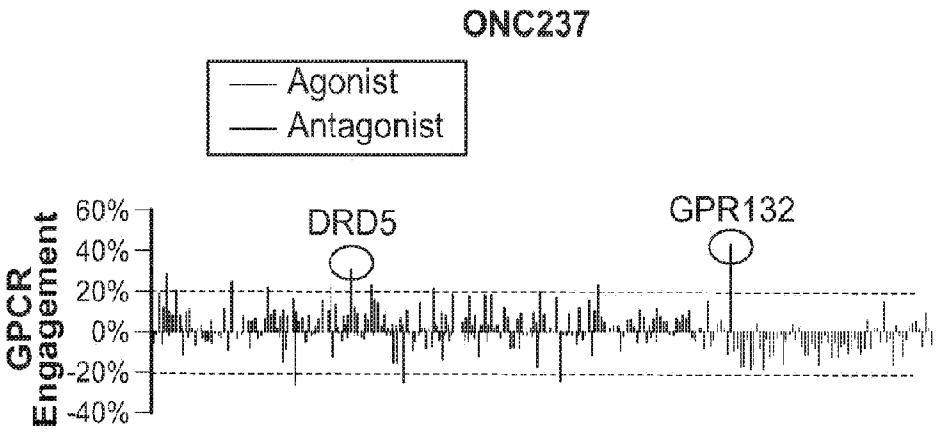


Figure 34

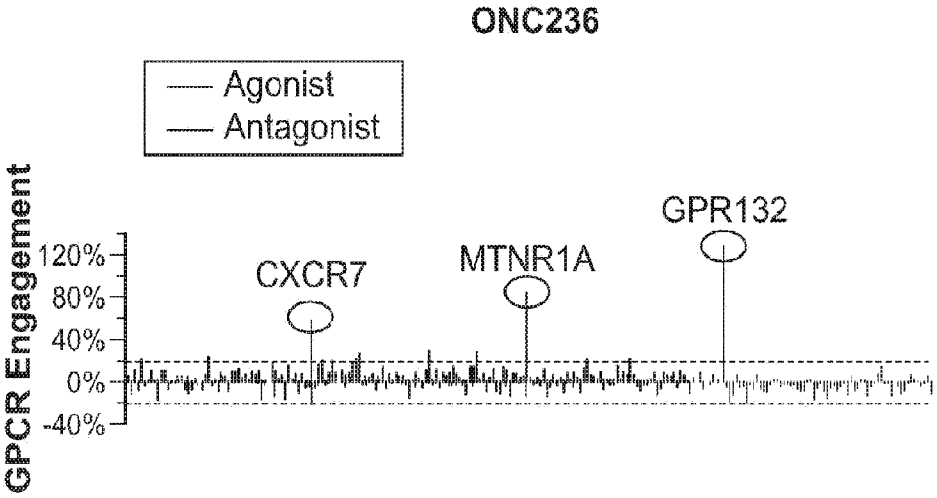


Figure 35

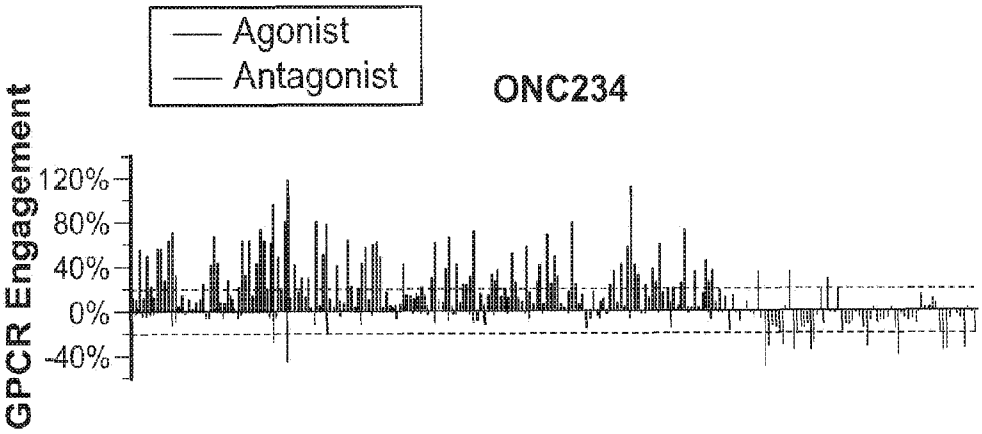


Figure 36

Linear Isomer

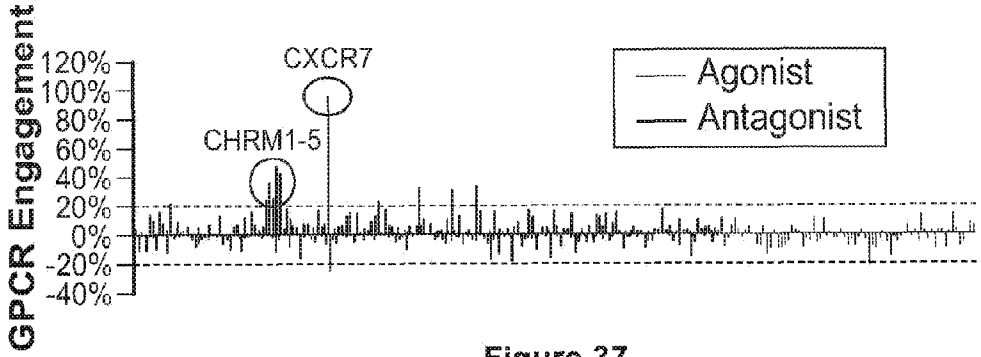
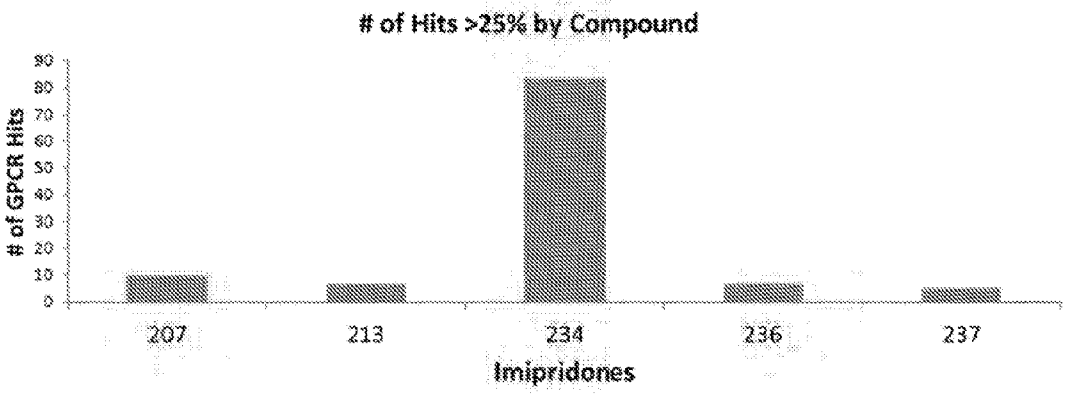


Figure 37

Figure 38


# of Hits >25% by Compound
# of GPCR Hits
90
80
70
60
50
40
30
20
10
0
207
213
234
236
237
Imipridones