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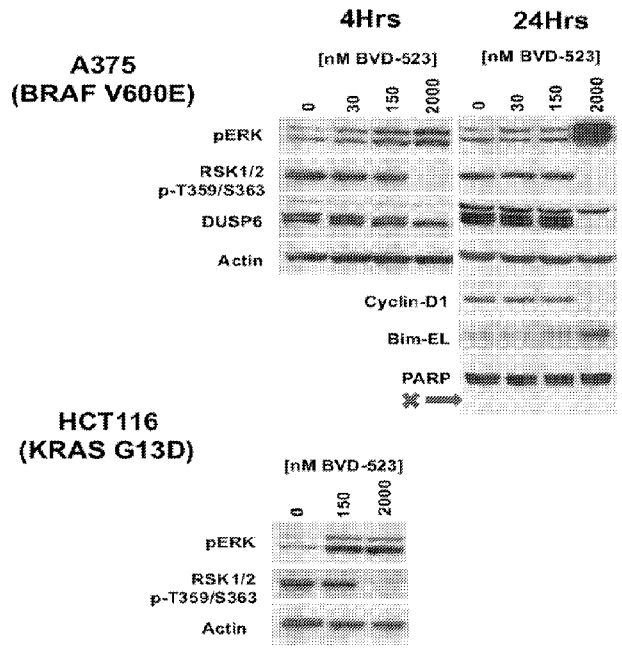
[Continued on next page]

(54) Title: CANCER TREATMENTS USING COMBINATIONS OF MTOR AND ERK INHIBITORS

FIG. 1



(57) Abstract: The present invention provides, inter alia, methods, kits, and pharmaceutical compositions for treating or ameliorating the effects of a cancer in a subject in need thereof. The method comprises administering to the subject an effective amount of (i) a first anti-cancer agent, which is BVD-523 or a pharmaceutically acceptable salt thereof and (ii) a second anti-cancer agent, which is a mTOR inhibitor or a pharmaceutically acceptable salt thereof, to treat or ameliorate the effects of the cancer. Additional methods for effecting cancer cell death are also provided.

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CANCER TREATMENTS USING COMBINATIONS OF MTOR AND ERK INHIBITORS

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims benefit of U.S. Patent Application Serial No. 61/918,986, filed on December 20, 2013 which application is incorporated by reference herein in its entirety.

FIELD OF INVENTION

[0002] The present invention provides, *inter alia,* methods, pharmaceutical composition and kits for treating or ameliorating the effects of a cancer in a subject using a first anti-cancer agent, which is BVD-523, an ERK1/2 inhibitor, or a pharmaceutically acceptable salt thereof and a second anti-cancer agent, which is a mammalian target of rapamycin (mTOR) inhibitor or a pharmaceutically acceptable salt thereof.

BACKGROUND OF THE INVENTION

[0003] Cancer is a leading cause of death worldwide, accounting for 7.6 million deaths (around 13% of all deaths) in 2008. Lung, stomach, liver, colon and breast cancer cause the most cancer deaths each year. According to the World Health Organization, deaths from cancer worldwide are projected to continue rising, with an estimated 13.1 million deaths in 2030.

[0004] Recently, mTOR has emerged as a critical effector in cell signaling pathways commonly deregulated in human cancers. Some success

has been reported with mTOR and PI3K/Akt inhibitor combinations. However, to date, there have been no reports of success using an mTOR inhibitor in combination with an ERK inhibitor to treat cancer.

[0005] Accordingly, there is a need, *inter alia*, to find new drug combinations for targeting molecular pathways that lead to uncontrolled cell proliferation and cancer. The present invention is directed to meeting these and other needs.

SUMMARY OF THE INVENTION

[0006] One embodiment of the present invention is a method of treating or ameliorating the effects of a cancer in a subject in need thereof. The method comprises administering to the subject an effective amount of (i) a first anti-cancer agent, which is BVD-523 or a pharmaceutically acceptable salt thereof and (ii) a second anti-cancer agent, which is a mTOR inhibitor or a pharmaceutically acceptable salt thereof, to treat or ameliorate the effects of the cancer.

[0007] Another embodiment of the present invention is a method of treating or ameliorating the effects of a cancer in a subject in need thereof. The method comprises administering to the subject an effective amount of (i) a first anti-cancer agent, which is BVD-523 or a pharmaceutically acceptable salt thereof and (ii) a second anti-cancer agent, which is selected from the group consisting of rapamycin, dactolisib, and pharmaceutically acceptable salts thereof, to treat or ameliorate the effects of the cancer.

[0008] A further embodiment of the present invention is a method of effecting cancer cell death. The method comprises contacting a cancer cell

with an effective amount of (i) a first anti-cancer agent, which is BVD-523 or a pharmaceutically acceptable salt thereof and (ii) a second anti-cancer agent, which is a mTOR inhibitor or a pharmaceutically acceptable salt thereof.

[0009] An additional embodiment of the present invention is a kit for treating or ameliorating the effects of a cancer in a subject in need thereof. The kit comprises an effective amount of (i) a first anti-cancer agent, which is BVD-523 or a pharmaceutically acceptable salt thereof and (ii) a second anti-cancer agent, which is a mTOR inhibitor or a pharmaceutically acceptable salt thereof, packaged together with instructions for their use.

[0010] Another embodiment of the present invention is a pharmaceutical composition for treating or ameliorating the effects of a cancer in a subject in need thereof. The pharmaceutical composition comprises a pharmaceutically acceptable diluent or carrier and an effective amount of (i) a first anti-cancer agent, which is BVD-523 or a pharmaceutically acceptable salt thereof and (ii) a second anti-cancer agent, which is a mTOR inhibitor or a pharmaceutically acceptable salt thereof, wherein administration of the first and second anti-cancer agents provides a synergistic effect compared to administration of either anti-cancer agent alone.

BRIEF DESCRIPTION OF THE DRAWINGS

[0011] FIG. 1 shows that both direct ERK substrate phosphorylation and known effector pathways are modulated following acute and prolonged treatment with BVD-523 *in vitro*. Western blots were performed using a variety of antibodies to detect changes in whole-cell lysates of cancer lines exposed to BVD-523. In the A375 BRAF mutant cell line (a human melanoma

cell line) and in the HCT116 KRAS mutant cell line (a human colorectal carcinoma cell line), phosphorylation of ERK-dependent residues (T359/S363) in RSK 1 and 2 proteins was reduced after 4 hours of treatment with BVD-523 at micromolar concentrations. Following 24 hours of treatment, direct substrate inhibition was maintained in BRAF mutant cell lines, and the MAPK feedback phosphatase DUSP6 was greatly reduced, suggesting durable and nearly complete MAPK pathway inhibition. Lastly, consistent with cytostatic effects of BVD-523 across multiple cell line backgrounds, the MAPK effector and G1/S-cell-cycle determinant gene cyclin-D1 was greatly reduced after 24 hours of treatment. In the A375 cell line, while the apoptosis effector and ERK substrate Bim-EL was increased following prolonged treatment, increased apoptosis was not observed, consistent with a lack of PARP cleavage, as well as other observations (not shown) that additional factors influence the capacity for BVD-523 to induce cell death.

[0012] FIG. 2 shows the results of single agent proliferation assays in HCT116 isogenic cells in McCoy's 5A containing either 10% FBS or 1% charcoal-stripped FBS (CS-FBS). Proliferation results are shown for treatment with BYL719 (FIG.2A), BKM120 (FIG. 2B), INK128 (FIG. 2C), PF-004691502 (FIG. 2D), BVD-523 (FIG. 2E), SCH772984 (FIG. 2F), Paclitaxel (FIG. 2G), and GDC-0941 (FIG. 2H).

[0013] FIG. 3 shows the results of the combination of BVD-523 and BYL719 in parental HCT116 and HCT116 PIK3CA (+/-) cells. FIG. 3A shows a dose matrix showing inhibition (%) for the combination in parental HCT116 cells. FIG. 3B shows Loewe excess for the combination in 3A and FIG. 3C shows Bliss excess for the combination in 3A. FIG. 3D shows a dose matrix

showing inhibition (%) for the combination in HCT116 PIK3CA (+/-) cells. FIG. 3E shows Loewe excess for the combination in 3D and FIG. 3F shows Bliss excess for the combination in 3D. FIG. 3G – FIG. 3H show the results of single agent proliferation assays for the combination in 3A. FIG. 3I – FIG. 3J show the results of single agent proliferation assays for the combination in 3D.

[0014] FIG. 4 shows the results of the combination of SCH772984 and BYL719 in parental HCT116 and HCT116 PIK3CA (+/-) cells. FIG. 4A shows a dose matrix showing inhibition (%) for the combination in parental HCT116 cells. FIG. 4B shows Loewe excess for the combination in 4A and FIG. 4C shows Bliss excess for the combination in 4A. FIG. 4D shows a dose matrix showing inhibition (%) for the combination in HCT116 PIK3CA (+/-) cells. FIG. 4E shows Loewe excess for the combination in 4D and FIG. 4F shows Bliss excess for the combination in 4D. FIG. 4G – FIG. 4H show the results of single agent proliferation assays for the combination in 4A. FIG. 4I – FIG. 4J show the results of single agent proliferation assays for the combination in 4D.

[0015] FIG. 5 shows the results of the combination of BVD-523 and BKM120 in parental HCT116 and HCT116 PIK3CA (+/-) cells. FIG. 5A shows a dose matrix showing inhibition (%) for the combination in parental HCT116 cells. FIG. 5B shows Loewe excess for the combination in 5A and FIG. 5C shows Bliss excess for the combination in 5A. FIG. 5D shows a dose matrix showing inhibition (%) for the combination in HCT116 PIK3CA (+/-) cells. FIG. 5E shows Loewe excess for the combination in 5D and FIG. 5F shows Bliss excess for the combination in 5D. FIG. 5G – FIG. 5H show the results of single agent proliferation assays for the combination in 5A. FIG. 5I – FIG. 5J show the results of single agent proliferation assays for the combination in 5D.

[0016] FIG. 6 shows the results of the combination of SCH772984 and BKM120 in parental HCT116 and HCT116 PIK3CA (+/-) cells. FIG. 6A shows a dose matrix showing inhibition (%) for the combination in parental HCT116 cells. FIG. 6B shows Loewe excess for the combination in 6A and FIG. 6C shows Bliss excess for the combination in 6A. FIG. 6D shows a dose matrix showing inhibition (%) for the combination in HCT116 PIK3CA (+/-) cells. FIG. 6E shows Loewe excess for the combination in 6D and FIG. 6F shows Bliss excess for the combination in 6D. FIG. 6G – FIG. 6H show the results of single agent proliferation assays for the combination in 6A. FIG. 6I – FIG. 6J show the results of single agent proliferation assays for the combination in 6D.

[0017] FIG. 7 shows the results of the combination of BVD-523 and INK128 in parental HCT116 and HCT116 PIK3CA (+/-) cells. FIG. 7A shows a dose matrix showing inhibition (%) for the combination in parental HCT116 cells. FIG. 7B shows Loewe excess for the combination in 7A and FIG. 7C shows Bliss excess for the combination in 7A. FIG. 7D shows a dose matrix showing inhibition (%) for the combination in HCT116 PIK3CA (+/-) cells. FIG. 7E shows Loewe excess for the combination in 7D and FIG. 7F shows Bliss excess for the combination in 7D. FIG. 7G – FIG. 7H show the results of single agent proliferation assays for the combination in 7A. FIG. 7I – FIG. 7J show the results of single agent proliferation assays for the combination in 7D.

[0018] FIG. 8 shows the results of the combination of SCH772984 and INK128 in parental HCT116 and HCT116 PIK3CA (+/-) cells. FIG. 8A shows a dose matrix showing inhibition (%) for the combination in parental HCT116 cells. FIG. 8B shows Loewe excess for the combination in 8A and FIG. 8C shows Bliss excess for the combination in 8A. FIG. 8D shows a dose matrix

showing inhibition (%) for the combination in HCT116 PIK3CA (+/-) cells. FIG. 8E shows Loewe excess for the combination in 8D and FIG. 8F shows Bliss excess for the combination in 8D. FIG. 8G – FIG. 8H show the results of single agent proliferation assays for the combination in 8A. FIG. 8I – FIG. 8J show the results of single agent proliferation assays for the combination in 8D.

[0019] FIG. 9 shows the results of the combination of BVD-523 and PF-004691502 in parental HCT116 and HCT116 PIK3CA (+/-) cells. FIG. 9A shows a dose matrix showing inhibition (%) for the combination in parental HCT116 cells. FIG. 9B shows Loewe excess for the combination in 9A and FIG. 9C shows Bliss excess for the combination in 9A. FIG. 9D shows a dose matrix showing inhibition (%) for the combination in HCT116 PIK3CA (+/-) cells. FIG. 9E shows Loewe excess for the combination in 9D and FIG. 9F shows Bliss excess for the combination in 9D. FIG. 9G – FIG. 9H show the results of single agent proliferation assays for the combination in 9A. FIG. 9I – FIG. 9J show the results of single agent proliferation assays for the combination in 9D.

[0020] FIG. 10 shows the results of the combination of SCH772984 and PF-004691502 in parental HCT116 and HCT116 PIK3CA (+/-) cells. FIG. 10A shows a dose matrix showing inhibition (%) for the combination in parental HCT116 cells. FIG. 10B shows Loewe excess for the combination in 10A and FIG. 10C shows Bliss excess for the combination in 10A. FIG. 10D shows a dose matrix showing inhibition (%) for the combination in HCT116 PIK3CA (+/-) cells. FIG. 10E shows Loewe excess for the combination in 10D and FIG. 10F shows Bliss excess for the combination in 10D. FIG. 10G – FIG. 10H show the results of single agent proliferation assays for the combination

in 10A. FIG. 10I – FIG. 10J show the results of single agent proliferation assays for the combination in 10D.

[0021] FIG. 11 shows a comparison of single agent proliferation responses in parental HCT116 and HCT116 PIK3CA (+/-). Proliferation results are shown for treatment with BYL719 (FIG. 11A), BKM120 (FIG. 11B), INK128 (FIG. 11C), PF-004691502 (FIG. 11D), BVD-523 (FIG. 11E), and SCH772984 (FIG. 11F).

[0022] FIG. 12 shows results of focused concentration combination assays in the HCT116 PIK3CA (+/-) isogenic cell line pair. FIG. 12A shows viability and Bliss scores for combinations with BVD-523 in parental HCT116 cells. FIG. 12B shows viability and Bliss scores for combinations with BVD-523 in HCT116 PIK3CA (+/-) cells. FIG. 12C shows viability and Bliss scores for combinations with SCH772984 in parental HCT116 cells. FIG. 12D shows viability and Bliss scores for combinations with SCH772984 in HCT116 PIK3CA (+/-) cells.

[0023] FIG. 13 shows the results of the combination of BVD-523 and BYL719 in parental DLD-1 and DLD-1 PIK3CA (+/-) cells. FIG. 13A shows a dose matrix showing inhibition (%) for the combination in parental DLD-1 cells. FIG. 13B shows Loewe excess for the combination in 13A and FIG. 13C shows Bliss excess for the combination in 13A. FIG. 13D shows a dose matrix showing inhibition (%) for the combination in DLD-1 PIK3CA (+/-) cells. FIG. 13E shows Loewe excess for the combination in 13D and FIG. 13F shows Bliss excess for the combination in 13D. FIG. 13G – FIG. 13H show the results of single agent proliferation assays for the combination in 13A.

FIG. 13I – FIG. 13J show the results of single agent proliferation assays for the combination in 13D.

[0024] FIG. 14 shows the results of the combination of SCH772984 and BYL719 in parental DLD-1 and DLD-1 PIK3CA (+/-) cells. FIG. 14A shows a dose matrix showing inhibition (%) for the combination in parental DLD-1 cells. FIG. 14B shows Loewe excess for the combination in 14A and FIG. 14C shows Bliss excess for the combination in 14A. FIG. 14D shows a dose matrix showing inhibition (%) for the combination in DLD-1 PIK3CA (+/-) cells. FIG. 14E shows Loewe excess for the combination in 14D and FIG. 14F shows Bliss excess for the combination in 14D. FIG. 14G – FIG. 14H show the results of single agent proliferation assays for the combination in 14A. FIG. 14I – FIG. 14J show the results of single agent proliferation assays for the combination in 14D.

[0025] FIG. 15 shows the results of the combination of BVD-523 and BKM120 in parental DLD-1 and DLD-1 PIK3CA (+/-) cells. FIG. 15A shows a dose matrix showing inhibition (%) for the combination in parental DLD-1 cells. FIG. 15B shows Loewe excess for the combination in 15A and FIG. 15C shows Bliss excess for the combination in 15A. FIG. 15D shows a dose matrix showing inhibition (%) for the combination in DLD-1 PIK3CA (+/-) cells. FIG. 15E shows Loewe excess for the combination in 15D and FIG. 15F shows Bliss excess for the combination in 15D. FIG. 15G – FIG. 15H show the results of single agent proliferation assays for the combination in 15A. FIG. 15I – FIG. 15J show the results of single agent proliferation assays for the combination in 15D.

[0026] FIG. 16 shows the results of the combination of SCH772984 and BKM120 in parental DLD-1 and DLD-1 PIK3CA (+/-) cells. FIG. 16A shows a dose matrix showing inhibition (%) for the combination in parental DLD-1 cells. FIG. 16B shows Loewe excess for the combination in 16A and FIG. 16C shows Bliss excess for the combination in 16A. FIG. 16D shows a dose matrix showing inhibition (%) for the combination in DLD-1 PIK3CA (+/-) cells. FIG. 16E shows Loewe excess for the combination in 16D and FIG. 16F shows Bliss excess for the combination in 16D. FIG. 16G – FIG. 16H show the results of single agent proliferation assays for the combination in 16A. FIG. 16I – FIG. 16J show the results of single agent proliferation assays for the combination in 16D.

[0027] FIG. 17 shows the results of the combination of BVD-523 and INK128 in parental DLD-1 and DLD-1 PIK3CA (+/-) cells. FIG. 17A shows a dose matrix showing inhibition (%) for the combination in parental DLD-1 cells. FIG. 17B shows Loewe excess for the combination in 17A and FIG. 17C shows Bliss excess for the combination in 17A. FIG. 17D shows a dose matrix showing inhibition (%) for the combination in DLD-1 PIK3CA (+/-) cells. FIG. 17E shows Loewe excess for the combination in 17D and FIG. 17F shows Bliss excess for the combination in 17D. FIG. 17G – FIG. 17H show the results of single agent proliferation assays for the combination in 17A. FIG. 17I – FIG. 17J show the results of single agent proliferation assays for the combination in 17D.

[0028] FIG. 18 shows the results of the combination of SCH772984 and INK128 in parental DLD-1 and DLD-1 PIK3CA (+/-) cells. FIG. 18A shows a dose matrix showing inhibition (%) for the combination in parental DLD-1

cells. FIG. 18B shows Loewe excess for the combination in 18A and FIG. 18C shows Bliss excess for the combination in 18A. FIG. 18D shows a dose matrix showing inhibition (%) for the combination in DLD-1 PIK3CA (+/-) cells. FIG. 18E shows Loewe excess for the combination in 18D and FIG. 18F shows Bliss excess for the combination in 18D. FIG. 18G – FIG. 18H show the results of single agent proliferation assays for the combination in 18A. FIG. 18I – FIG. 18J show the results of single agent proliferation assays for the combination in 18D.

[0029] FIG. 19 shows the results of the combination of BVD-523 and PF-004691502 in parental DLD-1 and DLD-1 PIK3CA (+/-) cells. FIG. 19A shows a dose matrix showing inhibition (%) for the combination in parental DLD-1 cells. FIG. 19B shows Loewe excess for the combination in 19A and FIG. 19C shows Bliss excess for the combination in 19A. FIG. 19D shows a dose matrix showing inhibition (%) for the combination in DLD-1 PIK3CA (+/-) cells. FIG. 19E shows Loewe excess for the combination in 19D and FIG. 19F shows Bliss excess for the combination in 19D. FIG. 19G – FIG. 19H show the results of single agent proliferation assays for the combination in 19A. FIG. 19I – FIG. 19J show the results of single agent proliferation assays for the combination in 19D.

[0030] FIG. 20 shows the results of the combination of SCH772984 and PF-004691502 in parental DLD-1 and DLD-1 PIK3CA (+/-) cells. FIG. 20A shows a dose matrix showing inhibition (%) for the combination in parental DLD-1 cells. FIG. 20B shows Loewe excess for the combination in 20A and FIG. 20C shows Bliss excess for the combination in 20A. FIG. 20D shows a dose matrix showing inhibition (%) for the combination in DLD-1 PIK3CA (+/-)

cells. FIG. 20E shows Loewe excess for the combination in 20D and FIG. 20F shows Bliss excess for the combination in 20D. FIG. 20G – FIG. 20H show the results of single agent proliferation assays for the combination in 20A. FIG. 20I – FIG. 20J show the results of single agent proliferation assays for the combination in 20D.

[0031] FIG. 21 shows a comparison of single agent proliferation responses in parental DLD-1 and DLD-1 PIK3CA (+/-). Proliferation results are shown for treatment with BYL719 (FIG. 21A), BKM120 (FIG. 21B), INK128 (FIG. 21C), PF-004691502 (FIG. 21D), BVD-523 (FIG. 21E), and SCH772984 (FIG. 21F).

[0032] FIG. 22A shows Lowe Volumes for the combinations tested. FIG. 22B shows Bliss Volumes for the combinations tested. FIG. 22C shows Synergy Scores for the combinations tested.

[0033] FIG. 23 shows the results of the combination of BVD-523 and SCH772984. FIG. 23A shows a dose matrix showing inhibition (%) for the combination in A375 cells. FIG. 23B – FIG. 23C show the results of single agent proliferation assays for the combination in 23A. FIG. 23D shows Loewe excess for the combination in 23A and FIG. 23E shows Bliss excess for the combination in 23A.

DETAILED DESCRIPTION OF THE INVENTION

[0034] One embodiment of the present invention is a method of treating or ameliorating the effects of a cancer in a subject in need thereof. The method comprises administering to the subject an effective amount of (i) a first anti-cancer agent, which is BVD-523 or a pharmaceutically acceptable

salt thereof and (ii) a second anti-cancer agent, which is a mTOR inhibitor or a pharmaceutically acceptable salt thereof, to treat or ameliorate the effects of the cancer.

[0035] As used herein, the terms "treat," "treating," "treatment" and grammatical variations thereof mean subjecting an individual subject to a protocol, regimen, process or remedy, in which it is desired to obtain a physiologic response or outcome in that subject, *e.g.*, a patient. In particular, the methods and compositions of the present invention may be used to slow the development of disease symptoms or delay the onset of the disease or condition, or halt the progression of disease development. However, because every treated subject may not respond to a particular treatment protocol, regimen, process or remedy, treating does not require that the desired physiologic response or outcome be achieved in each and every subject or subject population, *e.g.*, patient population. Accordingly, a given subject or subject population, *e.g.*, patient population may fail to respond or respond inadequately to treatment.

[0036] As used herein, the terms “ameliorate”, "ameliorating" and grammatical variations thereof mean to decrease the severity of the symptoms of a disease in a subject.

[0037] As used herein, a “subject” is a mammal, preferably, a human. In addition to humans, categories of mammals within the scope of the present invention include, for example, farm animals, domestic animals, laboratory animals, etc. Some examples of farm animals include cows, pigs, horses, goats, etc. Some examples of domestic animals include dogs, cats, etc. Some

examples of laboratory animals include primates, rats, mice, rabbits, guinea pigs, etc.

[0038] In the present invention, cancers include both solid tumor cancers and hematologic cancers. Non-limiting examples of solid tumor cancers include adrenocortical carcinoma, anal cancer, bladder cancer, bone cancer (such as osteosarcoma), brain cancer, breast cancer, carcinoid cancer, carcinoma, cervical cancer, colon cancer, endometrial cancer, esophageal cancer, extrahepatic bile duct cancer, Ewing family of cancers, extracranial germ cell cancer, eye cancer, gallbladder cancer, gastric cancer, germ cell tumor, gestational trophoblastic tumor, head and neck cancer, hypopharyngeal cancer, islet cell carcinoma, kidney cancer, large intestine cancer, laryngeal cancer, leukemia, lip and oral cavity cancer, liver tumor/cancer, lung tumor/cancer, lymphoma, malignant mesothelioma, Merkel cell carcinoma, mycosis fungoides, myelodysplastic syndrome, myeloproliferative disorders, nasopharyngeal cancer, neuroblastoma, oral cancer, oropharyngeal cancer, osteosarcoma, ovarian epithelial cancer, ovarian germ cell cancer, pancreatic cancer, paranasal sinus and nasal cavity cancer, parathyroid cancer, penile cancer, pituitary cancer, plasma cell neoplasm, prostate cancer, rhabdomyosarcoma, rectal cancer, renal cell cancer, transitional cell cancer of the renal pelvis and ureter, salivary gland cancer, Sezary syndrome, skin cancers (such as cutaneous t-cell lymphoma, Kaposi's sarcoma, mast cell tumor,and melanoma), small intestine cancer, soft tissue sarcoma, stomach cancer, testicular cancer, thymoma, thyroid cancer, urethral cancer, uterine cancer, vaginal cancer, vulvar cancer, and Wilms' tumor.

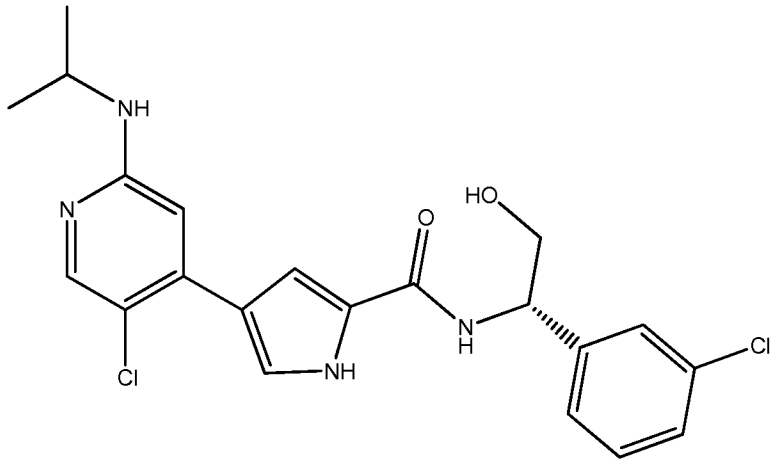
[0039] A preferred set of cancers that may be treated according to the present invention include autonomic ganglia cancer, biliary tract cancer, breast cancer, endometrial cancer, gastrointestinal tract cancer, haematopoietic and lymphoid cancer, kidney cancer, liver cancer, lung cancer, oesophagial cancer, ovarian cancer, pancreatic cancer, prostate cancer, skin cancer, soft tissue cancer, stomach cancer, thyroid cancer, upper aerodigestive tract cancer, and urinary tract cancer. Another preferred set of cancers that may be treated according to the present invention include oesophagus cancer, skin cancer, biliary tract cancer, large intestine cancer, endometrial cancer, lung cancer, urinary tract cancer, liver cancer, and kidney cancer. An additional preferred set of cancers that may be treated according to the present invention include brain cancer, colon cancer, leukemia, non-Hodgkin's lymphoma, and multiple myeloma. Preferably, the brain cancer is glioblastoma multiforme (GBM).

[0040] Examples of hematologic cancers according to the present invention include, but are not limited to, leukemias, such as adult/childhood acute lymphoblastic leukemia, adult/childhood acute myeloid leukemia, chronic lymphocytic leukemia, chronic myelogenous leukemia, and hairy cell leukemia, lymphomas, such as AIDS-related lymphoma, cutaneous T-cell lymphoma, adult/childhood Hodgkin lymphoma, mycosis fungoides, adult/childhood non-Hodgkin lymphoma, primary central nervous system lymphoma, Sézary syndrome, cutaneous T-cell lymphoma, and Waldenstrom macroglobulinemia, as well as other proliferative disorders such as chronic myeloproliferative disorders, Langerhans cell histiocytosis, multiple

myeloma/plasma cell neoplasm, myelodysplastic syndromes, and myelodysplastic/myeloproliferative neoplasms.

[0041] A preferred set of hematologic cancers that may be treated according to the present invention include Adult Acute Megakaryoblastic Leukemia (M7), Adult Acute Minimally Differentiated Myeloid Leukemia (M0), Adult Acute Monoblastic Leukemia (M5a), Adult Acute Monocytic Leukemia (M5b), Adult Acute Myeloblastic Leukemia With Maturation (M2), Adult Acute Myeloblastic Leukemia Without Maturation (M1), Adult Acute Myeloid Leukemia With 11q23 (MLL) Abnormalities, Adult Acute Myeloid Leukemia With Del(5q), Adult Acute Myeloid Leukemia With Inv(16)(p13;q22), Adult Acute Myeloid Leukemia With t(16;16)(p13;q22), Adult Acute Myeloid Leukemia With t(8;21)(q22;q22), Adult Acute Myelomonocytic Leukemia (M4), Adult Erythroleukemia (M6a), Adult Pure Erythroid Leukemia (M6b), Recurrent Adult Acute Myeloid Leukemia, and Untreated Adult Acute Myeloid Leukemia.

[0042] In the present invention, BVD-523, a preferred ERK1/2 inhibitor, corresponds to a compound according to formula (I):



and pharmaceutically acceptable salts thereof. BVD-523 may be synthesized according to the methods disclosed, *e.g.*, in U.S. Patent No. 7,354,939. Enantiomers and racemic mixtures of both enantiomers of BVD-523 are also contemplated within the scope of the present invention. BVD-523's mechanism of action is believed to be, *inter alia*, unique and distinct from certain other ERK1/2 inhibitors, such as SCH772984. For example, SCH772984 inhibits autophosphorylation of ERK (Morris *et al.*, 2013), whereas BVD-523 still allows for the autophosphorylation of ERK while still inhibiting ERK. (See, *e.g.*, FIG. 1). Thus, BVD-523, while being an excellent stand alone drug, is also a good drug to partner with other inhibitors of different nodes in the molecular pathway leading to cancer.

[0043] As used herein, a "mTOR inhibitor" means those substances that (i) directly interact with mTOR, *e.g.* by binding to mTOR and (ii) decrease the expression or the activity of mTOR. Non-limiting examples of mTOR inhibitors according to the present invention include zotarolimus (AbbVie), umirolimus (Biosensors), temsirolimus (Pfizer), sirolimus (Pfizer), sirolimus NanoCrystal (Elan Pharmaceutical Technologies), sirolimus TransDerm (TransDerm), sirolimus-PNP (Samyang), everolimus (Novartis), biolimus A9 (Biosensors), ridaforolimus (Ariad), rapamycin, TCD-10023 (Terumo), DE-109 (MacuSight), MS-R001 (MacuSight), MS-R002 (MacuSight), MS-R003 (MacuSight), Perceiva (MacuSight), XL-765 (Exelixis), quinacrine (Cleveland BioLabs), PKI-587 (Pfizer), PF-04691502 (Pfizer), GDC-0980 (Genentech and Piramed), dactolisib (Novartis), CC-223 (Celgene), PWT-33597 (Pathway Therapeutics), P-7170 (Piramal Life Sciences), LY-3023414 (Eli Lilly), INK-128 (Takeda), GDC-0084 (Genentech), DS-7423 (Daiichi Sankyo), DS-3078

(Daiichi Sankyo), CC-115 (Celgene), CBLC-137 (Cleveland BioLabs), AZD-2014 (AstraZeneca), X-480 (Xcovery), X-414 (Xcovery), EC-0371 (Endocyte), VS-5584 (Verastem), PQR-401 (Piqur), PQR-316 (Piqur), PQR-311 (Piqur), PQR-309 (Piqur), PF-06465603 (Pfizer), NV-128 (Novogen), nPT-MTOR (Biotica Technology), BC-210 (Biotica Technology), WAY-600 (Biotica Technology), WYE-354 (Biotica Technology), WYE-687 (Biotica Technology), LOR-220 (Lorus Therapeutics), HMPL-518 (Hutchison China MediTech), GNE-317 (Genentech), EC-0565 (Endocyte), CC-214 (Celgene), and ABTL-0812 (Ability Pharmaceuticals).

[0044] In another aspect of this embodiment, the method further comprises administering to the subject at least one additional therapeutic agent effective for treating or ameliorating the effects of the cancer. The additional therapeutic agent may be selected from the group consisting of an antibody or fragment thereof, a cytotoxic agent, a toxin, a radionuclide, an immunomodulator, a photoactive therapeutic agent, a radiosensitizing agent, a hormone, an anti-angiogenesis agent, and combinations thereof.

[0045] As used herein, an “antibody” encompasses naturally occurring immunoglobulins as well as non-naturally occurring immunoglobulins, including, for example, single chain antibodies, chimeric antibodies (*e.g.*, humanized murine antibodies), and heteroconjugate antibodies (*e.g.*, bispecific antibodies). Fragments of antibodies include those that bind antigen, (*e.g.*, Fab', $F(ab')_2$, Fab, Fv, and rIgG). See also, *e.g.*, Pierce Catalog and Handbook, 1994-1995 (Pierce Chemical Co., Rockford, Ill.); Kuby, J., Immunology, 3rd Ed., W.H. Freeman & Co., New York (1998). The term antibody also includes bivalent or bispecific molecules, diabodies, triabodies,

and tetrabodies. The term "antibody" further includes both polyclonal and monoclonal antibodies.

[0046] Examples of therapeutic antibodies that may be used in the present invention include rituximab (Rituxan), Cetuximab (Erbitux), bevacizumab (Avastin), and Ibritumomab (Zevalin).

[0047] Cytotoxic agents according to the present invention include DNA damaging agents, antimetabolites, anti-microtubule agents, antibiotic agents, etc. DNA damaging agents include alkylating agents, platinum-based agents, intercalating agents, and inhibitors of DNA replication. Non-limiting examples of DNA alkylating agents include cyclophosphamide, mechlorethamine, uramustine, melphalan, chlorambucil, ifosfamide, carmustine, lomustine, streptozocin, busulfan, temozolomide, pharmaceutically acceptable salts thereof, prodrugs, and combinations thereof. Non-limiting examples of platinum-based agents include cisplatin, carboplatin, oxaliplatin, nedaplatin, satraplatin, triplatin tetranitrate, pharmaceutically acceptable salts thereof, prodrugs, and combinations thereof. Non-limiting examples of intercalating agents include doxorubicin, daunorubicin, idarubicin, mitoxantrone, pharmaceutically acceptable salts thereof, prodrugs, and combinations thereof. Non-limiting examples of inhibitors of DNA replication include irinotecan, topotecan, amsacrine, etoposide, etoposide phosphate, teniposide, pharmaceutically acceptable salts thereof, prodrugs, and combinations thereof. Antimetabolites include folate antagonists such as methotrexate and premetrexed, purine antagonists such as 6-mercaptopurine, dacarbazine, and fludarabine, and pyrimidine antagonists such as 5-fluorouracil, arabinosylcytosine, capecitabine, gemcitabine, decitabine, pharmaceutically

acceptable salts thereof, prodrugs, and combinations thereof. Anti-microtubule agents include without limitation vinca alkaloids, paclitaxel (Taxol®), docetaxel (Taxotere®), and ixabepilone (Ixempra®). Antibiotic agents include without limitation actinomycin, anthracyclines, valrubicin, epirubicin, bleomycin, plicamycin, mitomycin, pharmaceutically acceptable salts thereof, prodrugs, and combinations thereof.

[0048] Cytotoxic agents according to the present invention also include an inhibitor of the PI3K/Akt pathway. Non-limiting examples of an inhibitor of the PI3K/Akt pathway include A-674563 (CAS # 552325-73-2), AGL 2263, AMG-319 (Amgen, Thousand Oaks, CA), AS-041164 (5-benzo[1,3]dioxol-5-ylmethylene-thiazolidine-2,4-dione), AS-604850 (5-(2,2-Difluoro-benzo[1,3]dioxol-5-ylmethylene)-thiazolidine-2,4-dione), AS-605240 (5-quinoxilin-6-methylene-1,3-thiazolidine-2,4-dione), AT7867 (CAS # 857531-00-1), benzimidazole series, Genentech (Roche Holdings Inc., South San Francisco, CA), BML-257 (CAS # 32387-96-5), CAL-120 (Gilead Sciences, Foster City, CA), CAL-129 (Gilead Sciences), CAL-130 (Gilead Sciences), CAL-253 (Gilead Sciences), CAL-263 (Gilead Sciences), CAS # 612847-09-3, CAS # 681281-88-9, CAS # 75747-14-7, CAS # 925681-41-0, CAS # 98510-80-6, CCT128930 (CAS # 885499-61-6), CH5132799 (CAS # 1007207-67-1), CHR-4432 (Chroma Therapeutics, Ltd., Abingdon, UK), FPA 124 (CAS # 902779-59-3), GS-1101 (CAL-101) (Gilead Sciences), GSK 690693 (CAS # 937174-76-0), H-89 (CAS # 127243-85-0), Honokiol, IC87114 (Gilead Science), IPI-145 (Intellikine Inc.), KAR-4139 (Karus Therapeutics, Chilworth, UK), KAR-4141 (Karus Therapeutics), KIN-1 (Karus Therapeutics), KT 5720 (CAS # 108068-98-0), Miltefosine, MK-2206 dihydrochloride (CAS # 1032350-

13-2), ML-9 (CAS # 105637-50-1), Naltrindole Hydrochloride, OXY-111A (NormOxys Inc., Brighton, MA), perifosine, PHT-427 (CAS # 1191951-57-1), PI3 kinase delta inhibitor, Merck KGaA (Merck & Co., Whitehouse Station, NJ), PI3 kinase delta inhibitors, Genentech (Roche Holdings Inc.), PI3 kinase delta inhibitors, Incozen (Incozen Therapeutics, Pvt. Ltd., Hydrabad, India), PI3 kinase delta inhibitors-2, Incozen (Incozen Therapeutics), PI3 kinase inhibitor, Roche-4 (Roche Holdings Inc.), PI3 kinase inhibitors, Roche (Roche Holdings Inc.), PI3 kinase inhibitors, Roche-5 (Roche Holdings Inc.), PI3-alpha/delta inhibitors, Pathway Therapeutics (Pathway Therapeutics Ltd., South San Francisco, CA), PI3-delta inhibitors, Cellzome (Cellzome AG, Heidelberg, Germany), PI3-delta inhibitors, Intellikine (Intellikine Inc., La Jolla, CA), PI3-delta inhibitors, Pathway Therapeutics-1 (Pathway Therapeutics Ltd.), PI3-delta inhibitors, Pathway Therapeutics-2 (Pathway Therapeutics Ltd.), PI3-delta/gamma inhibitors, Cellzome (Cellzome AG), PI3-delta/gamma inhibitors, Cellzome (Cellzome AG), PI3-delta/gamma inhibitors, Intellikine (Intellikine Inc.), PI3-delta/gamma inhibitors, Intellikine (Intellikine Inc.), PI3-delta/gamma inhibitors, Pathway Therapeutics (Pathway Therapeutics Ltd.), PI3-delta/gamma inhibitors, Pathway Therapeutics (Pathway Therapeutics Ltd.), PI3-gamma inhibitor Evotec (Evotec), PI3-gamma inhibitor, Cellzome (Cellzome AG), PI3-gamma inhibitors, Pathway Therapeutics (Pathway Therapeutics Ltd.), PI3K delta/gamma inhibitors, Intellikine-1 (Intellikine Inc.), PI3K delta/gamma inhibitors, Intellikine-1 (Intellikine Inc.), pictilisib (Roche Holdings Inc.), PIK-90 (CAS # 677338-12-4), SC-103980 (Pfizer, New York, NY), SF-1126 (Semafore Pharmaceuticals, Indianapolis, IN), SH-5, SH-6, Tetrahydro Curcumin, TG100-115 (Targegen Inc., San Diego, CA), Triciribine,

X-339 (Xcovery, West Palm Beach, FL), XL-499 (Evotech, Hamburg, Germany), pharmaceutically acceptable salts thereof, and combinations thereof.

[0049] In the present invention, the term "toxin" means an antigenic poison or venom of plant or animal origin. An example is diphtheria toxin or portions thereof.

[0050] In the present invention, the term "radionuclide" means a radioactive substance administered to the patient, *e.g.*, intravenously or orally, after which it penetrates via the patient's normal metabolism into the target organ or tissue, where it delivers local radiation for a short time. Examples of radionuclides include, but are not limited to, I-125, At-211, Lu-177, Cu-67, I-131, Sm-153, Re-186, P-32, Re-188, In-114m, and Y-90.

[0051] In the present invention, the term "immunomodulator" means a substance that alters the immune response by augmenting or reducing the ability of the immune system to produce antibodies or sensitized cells that recognize and react with the antigen that initiated their production. Immunomodulators may be recombinant, synthetic, or natural preparations and include cytokines, corticosteroids, cytotoxic agents, thymosin, and immunoglobulins. Some immunomodulators are naturally present in the body, and certain of these are available in pharmacologic preparations. Examples of immunomodulators include, but are not limited to, granulocyte colony-stimulating factor (G-CSF), interferons, imiquimod and cellular membrane fractions from bacteria, IL-2, IL-7, IL-12, CCL3, CCL26, CXCL7, and synthetic cytosine phosphate-guanosine (CpG).

[0052] In the present invention, the term "photoactive therapeutic agent" means compounds and compositions that become active upon exposure to light. Representative examples of photoactive therapeutic agents are disclosed, *e.g.*, in U.S. Patent Application Serial No. 2011/0152230 A1, "Photoactive Metal Nitrosyls For Blood Pressure Regulation And Cancer Therapy."

[0053] In the present invention, the term "radiosensitizing agent" means a compound that makes tumor cells more sensitive to radiation therapy. Examples of radiosensitizing agents include misonidazole, metronidazole, tirapazamine, and trans sodium crocetinate.

[0054] In the present invention, the term "hormone" means a substance released by cells in one part of a body that affects cells in another part of the body. Examples of hormones include, but are not limited to, prostaglandins, leukotrienes, prostacyclin, thromboxane, amylin, antimullerian hormone, adiponectin, adrenocorticotropic hormone, angiotensinogen, angiotensin, vasopressin, atriopeptin, brain natriuretic peptide, calcitonin, cholecystokinin, corticotropin-releasing hormone, encephalin, endothelin, erythropoietin, follicle-stimulating hormone, galanin, gastrin, ghrelin, glucagon, gonadotropin-releasing hormone, growth hormone-releasing hormone, human chorionic gonadotropin, human placental lactogen, growth hormone, inhibin, insulin, somatomedin, leptin, liptropin, luteinizing hormone, melanocyte stimulating hormone, motilin, orexin, oxytocin, pancreatic polypeptide, parathyroid hormone, prolactin, prolactin releasing hormone, relaxin, renin, secretin, somatostain, thrombopoietin, thyroid-stimulating hormone, testosterone,

dehydroepiandrosterone, androstenedione, dihydrotestosterone, aldosterone, estradiol, estrone, estriol, cortisol, progesterone, calcitriol, and calcidiol.

[0055] Some compounds interfere with the activity of certain hormones or stop the production of certain hormones. These hormone-interfering compounds include, but are not limited to, tamoxifen (Nolvadex®), anastrozole (Arimidex®), letrozole (Femara®), and fulvestrant (Faslodex®). Such compounds are also within the meaning of hormone in the present invention.

[0056] As used herein, an "anti-angiogenesis" agent means a substance that reduces or inhibits the growth of new blood vessels, such as, *e.g.*, an inhibitor of vascular endothelial growth factor (VEGF) and an inhibitor of endothelial cell migration. Anti-angiogenesis agents include without limitation 2-methoxyestradiol, angiostatin, bevacizumab, cartilage-derived angiogenesis inhibitory factor, endostatin, IFN-α, IL-12, itraconazole, linomide, platelet factor-4, prolactin, SU5416, suramin, tasquinimod, tecogalan, tetrathiomolybdate, thalidomide, thrombospondin, thrombospondin, TNP-470, ziv-aflibercept, pharmaceutically acceptable salts thereof, prodrugs, and combinations thereof.

[0057] In an additional aspect of this embodiment, administration of the first and second anti-cancer agents provides a synergistic effect compared to administration of either anti-cancer agent alone. As used herein, "synergistic" means more than additive. Synergistic effects may be measured by various assays known in the art, including but not limited to those disclosed herein.

[0058] Another embodiment of the present invention is a method of treating or ameliorating the effects of a cancer in a subject in need thereof. The method comprises administering to the subject an effective amount of (i) a first anti-cancer agent, which is BVD-523 or a pharmaceutically acceptable salt thereof and (ii) a second anti-cancer agent, which is selected from the group consisting of rapamycin, dactolisib, and pharmaceutically acceptable salts thereof, to treat or ameliorate the effects of the cancer.

[0059] Suitable and preferred subjects and various types of cancer are as disclosed herein. In this embodiment, the methods may be used to treat the cancers disclosed above, including those cancers with the mutational backgrounds identified above. Methods of identifying such mutations are also as set forth above.

[0060] In another aspect of this embodiment, the BVD-523 or a pharmaceutically acceptable salt thereof is administered in the form of a pharmaceutical composition further comprising a pharmaceutically acceptable carrier or diluent.

[0061] In a further aspect of this embodiment, the rapamycin, dactolisib or a pharmaceutically acceptable salt thereof is administered in the form of a pharmaceutical composition further comprising a pharmaceutically acceptable carrier or diluent.

[0062] In an additional aspect of this embodiment, the method further comprises administering at least one additional therapeutic agent, preferably an inhibitor of the PI3K/Akt pathway, as disclosed herein.

[0063] In a further aspect of this embodiment, administration of the first and second anti-cancer agents provides a synergistic effect compared to administration of either anti-cancer agent alone.

[0064] A further embodiment of the present invention is a method of effecting cancer cell death. The method comprises contacting a cancer cell with an effective amount of (i) a first anti-cancer agent, which is BVD-523 or a pharmaceutically acceptable salt thereof and (ii) a second anti-cancer agent, which is a mTOR inhibitor or a pharmaceutically acceptable salt thereof.

[0065] Suitable and preferred mTOR inhibitors are as disclosed herein In this embodiment, effecting cancer cell death may be accomplished in cancer cells having various mutational backgrounds and/or that are characterized as disclosed above. Methods of identifying such mutations are also as set forth above.

[0066] In addition, the methods of this embodiment may be carried out in vitro or in vivo, and may be used to effect cancer cell death in cells of the types of cancer disclosed herein.

[0067] In one aspect of this embodiment, the cancer cell is a mammalian cancer cell. Preferably, the mammalian cancer cell is obtained from a mammal selected from the group consisting of humans, primates, farm animals, and domestic animals. More preferably, the mammalian cancer cell is a human cancer cell.

[0068] In another aspect of this embodiment, the method further comprises contacting the cancer cell with at least one additional therapeutic agent, preferably an inhibitor of the PI3K/Akt pathway, as disclosed herein. In

this embodiment, "contacting" means bringing the BVD-523, mTOR inhibitor, and optionally one or more additional therapeutic agents into close proximity to the cancer cells. This may be accomplished using conventional techniques of drug delivery to mammals or in the in vitro situation by, *e.g.*, providing BVD-523, mTOR inhibitor and optionally other therapeutic agents to a culture in which the cancer cells are located.

[0069] In a further aspect of this embodiment, contacting the cancer cell with the first and second ant-cancer agents provides a synergistic effect compared to contacting the cancer cell with either anti-cancer agent alone.

[0070] An additional embodiment of the present invention is a kit for treating or ameliorating the effects of a cancer in a subject in need thereof. The kit comprises an effective amount of (i) a first anti-cancer agent, which is BVD-523 or a pharmaceutically acceptable salt thereof and (ii) a second anti-cancer agent, which is a mTOR inhibitor or a pharmaceutically acceptable salt thereof, packaged together with instructions for their use.

[0071] The kits may also include suitable storage containers, *e.g.*, ampules, vials, tubes, etc., for each anti-cancer agent of the present invention (which may *e.g.*, may be in the form of pharmaceutical compositions) and other reagents, *e.g.*, buffers, balanced salt solutions, etc., for use in administering the anti-cancer agents to subjects. The anti-cancer agents of the invention and other reagents may be present in the kits in any convenient form, such as, *e.g.*, in a solution or in a powder form. The kits may further include a packaging container, optionally having one or more partitions for housing the pharmaceutical composition and other optional reagents.

[0072] Suitable and preferred subjects and mTOR inhibitors are as disclosed herein. In this embodiment, the kit may be used to treat the cancers disclosed above, including those cancers with the mutational backgrounds identified herein. Methods of identifying such mutations are as set forth above.

[0073] In one aspect of this embodiment, the kit further comprises at least one additional therapeutic agent, preferably an inhibitor of the PI3K/Akt pathway, as disclosed herein.

[0074] In a further aspect of this embodiment, administration of the first and second anti-cancer agents provides a synergistic effect compared to administration of either anti-cancer agent alone.

[0075] Another embodiment of the present invention is a pharmaceutical composition for treating or ameliorating the effects of a cancer in a subject in need thereof. The pharmaceutical composition comprises a pharmaceutically acceptable diluent or carrier and an effective amount of (i) a first anti-cancer agent, which is BVD-523 or a pharmaceutically acceptable salt thereof and (ii) a second anti-cancer agent, which is a mTOR inhibitor or a pharmaceutically acceptable salt thereof, wherein administration of the first and second anti-cancer agents provides a synergistic effect compared to administration of either anti-cancer agent alone.

[0076] Suitable and preferred subjects and mTOR inhibitors are as disclosed herein. The pharmaceutical compositions of the invention may be used to treat the cancers disclosed above, including those cancers with the mutational backgrounds identified herein. Methods of identifying such mutations are also as set forth above.

[0077] In one aspect of this embodiment, the pharmaceutical composition further comprises at least one additional therapeutic agent, preferably an inhibitor of the PI3K/Akt pathway, as disclosed herein.

[0078] The pharmaceutical compositions according to the present invention may be in an unit dosage form comprising both anti-cancer agents. In another aspect of this embodiment, the first anti-cancer agent is in a first unit dosage form and the second anti-cancer agent is in a second unit dosage form, separate from the first.

[0079] The first and second anti-cancer agents may be co-administered to the subject, either simultaneously or at different times, as deemed most appropriate by a physician. If the first and second anti-cancer agents are administered at different times, for example, by serial administration, the first anti-cancer agent may be administered to the subject before the second anti-cancer agent. Alternatively, the second anti-cancer agent may be administered to the subject before the first anti-cancer agent.

[0080] In the present invention, an "effective amount" or a “therapeutically effective amount” of an anti-cancer agent of the invention including pharmaceutical compositions containing same that are disclosed herein is an amount of such agent or composition that is sufficient to effect beneficial or desired results as described herein when administered to a subject. Effective dosage forms, modes of administration, and dosage amounts may be determined empirically, and making such determinations is within the skill of the art. It is understood by those skilled in the art that the dosage amount will vary with the route of administration, the rate of excretion, the duration of the treatment, the identity of any other drugs being

administered, the age, size, and species of mammal, *e.g.*, human patient, and like factors well known in the arts of medicine and veterinary medicine. In general, a suitable dose of an agent or composition according to the invention will be that amount of the agent or composition, which is the lowest dose effective to produce the desired effect. The effective dose of an agent or composition of the present invention may be administered as two, three, four, five, six or more sub-doses, administered separately at appropriate intervals throughout the day.

[0081] A suitable, non-limiting example of a dosage of BVD-523 or an mTOR inhibitor disclosed herein is from about 1 mg/kg to about 2400 mg/kg per day, such as from about 1 mg/kg to about 1200 mg/kg per day, 75 mg/kg per day to about 300 mg/kg per day, including from about 1 mg/kg to about 100 mg/kg per day. Other representative dosages of such agents include about 1 mg/kg, 5 mg/kg, 10 mg/kg, 15 mg/kg, 20 mg/kg, 25 mg/kg, 30 mg/kg, 35 mg/kg, 40 mg/kg, 45 mg/kg, 50 mg/kg, 60 mg/kg, 70 mg/kg, 75 mg/kg, 80 mg/kg, 90 mg/kg, 100 mg/kg, 125 mg/kg, 150 mg/kg, 175 mg/kg, 200 mg/kg, 250 mg/kg, 300 mg/kg, 400 mg/kg, 500 mg/kg, 600 mg/kg, 700 mg/kg, 800 mg/kg, 900 mg/kg, 1000 mg/kg, 1100 mg/kg, 1200 mg/kg, 1300 mg/kg, 1400 mg/kg, 1500 mg/kg, 1600 mg/kg, 1700 mg/kg, 1800 mg/kg, 1900 mg/kg, 2000 mg/kg, 2100 mg/kg, 2200 mg/kg, and 2300 mg/kg per day. In one preferred embodiment, temsirolimus, an mTOR inhibitor, is administered once a week in a 25 mg dose infused over 30-60 minutes. The effective dose of BVD-523 or an mTOR inhibitor disclosed herein may be administered as two, three, four, five, six or more sub-doses, administered separately at appropriate intervals throughout the day.

[0082] BVD-523, the mTOR inhibitors, or pharmaceutical compositions containing the same of the present invention may be administered in any desired and effective manner: for oral ingestion, or as an ointment or drop for local administration to the eyes, or for parenteral or other administration in any appropriate manner such as intraperitoneal, subcutaneous, topical, intradermal, inhalation, intrapulmonary, rectal, vaginal, sublingual, intramuscular, intravenous, intraarterial, intrathecal, or intralymphatic. Further, BVD-523, the mTOR inhibitors, or pharmaceutical compositions containing the same of the present invention may be administered in conjunction with other treatments. BVD-523, the mTOR inhibitors, or pharmaceutical compositions containing the same of the present invention may be encapsulated or otherwise protected against gastric or other secretions, if desired.

[0083] The pharmaceutical compositions of the invention comprise one or more active ingredients, *e.g.* anti-cancer agents, in admixture with one or more pharmaceutically-acceptable diluents or carriers and, optionally, one or more other compounds, drugs, ingredients and/or materials. Regardless of the route of administration selected, the agents/compounds of the present invention are formulated into pharmaceutically-acceptable dosage forms by conventional methods known to those of skill in the art. See, *e.g.*, Remington, The Science and Practice of Pharmacy (21st Edition, Lippincott Williams and Wilkins, Philadelphia, PA.).

[0084] Pharmaceutically acceptable diluents or carriers are well known in the art (see, *e.g.*, Remington, The Science and Practice of Pharmacy (21st Edition, Lippincott Williams and Wilkins, Philadelphia, PA.) and The National

Formulary (American Pharmaceutical Association, Washington, D.C.)) and include sugars (*e.g.*, lactose, sucrose, mannitol, and sorbitol), starches, cellulose preparations, calcium phosphates (*e.g.*, dicalcium phosphate, tricalcium phosphate and calcium hydrogen phosphate), sodium citrate, water, aqueous solutions (*e.g.*, saline, sodium chloride injection, Ringer's injection, dextrose injection, dextrose and sodium chloride injection, lactated Ringer's injection), alcohols (*e.g.*, ethyl alcohol, propyl alcohol, and benzyl alcohol), polyols (*e.g.*, glycerol, propylene glycol, and polyethylene glycol), organic esters (*e.g.*, ethyl oleate and tryglycerides), biodegradable polymers (*e.g.*, polylactide-polyglycolide, poly(orthoesters), and poly(anhydrides)), elastomeric matrices, liposomes, microspheres, oils (*e.g.*, corn, germ, olive, castor, sesame, cottonseed, and groundnut), cocoa butter, waxes (*e.g.*, suppository waxes), paraffins, silicones, talc, silicylate, etc. Each pharmaceutically acceptable diluent or carrier used in a pharmaceutical composition of the invention must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the subject. Diluents or carriers suitable for a selected dosage form and intended route of administration are well known in the art, and acceptable diluents or carriers for a chosen dosage form and method of administration can be determined using ordinary skill in the art.

[0085] The pharmaceutical compositions of the invention may, optionally, contain additional ingredients and/or materials commonly used in pharmaceutical compositions. These ingredients and materials are well known in the art and include (1) fillers or extenders, such as starches, lactose, sucrose, glucose, mannitol, and silicic acid; (2) binders, such as

carboxymethylcellulose, alginates, gelatin, polyvinyl pyrrolidone, hydroxypropylmethyl cellulose, sucrose and acacia; (3) humectants, such as glycerol; (4) disintegrating agents, such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, sodium starch glycolate, cross-linked sodium carboxymethyl cellulose and sodium carbonate; (5) solution retarding agents, such as paraffin; (6) absorption accelerators, such as quaternary ammonium compounds; (7) wetting agents, such as cetyl alcohol and glycerol monostearate; (8) absorbents, such as kaolin and bentonite clay; (9) lubricants, such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, and sodium lauryl sulfate; (10) suspending agents, such as ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth; (11) buffering agents; (12) excipients, such as lactose, milk sugars, polyethylene glycols, animal and vegetable fats, oils, waxes, paraffins, cocoa butter, starches, tragacanth, cellulose derivatives, polyethylene glycol, silicones, bentonites, silicic acid, talc, salicylate, zinc oxide, aluminum hydroxide, calcium silicates, and polyamide powder; (13) inert diluents, such as water or other solvents; (14) preservatives; (15) surface-active agents; (16) dispersing agents; (17) control-release or absorption-delaying agents, such as hydroxypropylmethyl cellulose, other polymer matrices, biodegradable polymers, liposomes, microspheres, aluminum monostearate, gelatin, and waxes; (18) opacifying agents; (19) adjuvants; (20) wetting agents; (21) emulsifying and suspending agents; (22), solubilizing agents and emulsifiers, such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl

benzoate, propylene glycol, 1,3-butylene glycol, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor and sesame oils), glycerol, tetrahydrofuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan; (23) propellants, such as chlorofluorohydrocarbons and volatile unsubstituted hydrocarbons, such as butane and propane; (24) antioxidants; (25) agents which render the formulation isotonic with the blood of the intended recipient, such as sugars and sodium chloride; (26) thickening agents; (27) coating materials, such as lecithin; and (28) sweetening, flavoring, coloring, perfuming and preservative agents. Each such ingredient or material must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the subject. Ingredients and materials suitable for a selected dosage form and intended route of administration are well known in the art, and acceptable ingredients and materials for a chosen dosage form and method of administration may be determined using ordinary skill in the art.

[0086] The pharmaceutical compositions of the present invention suitable for oral administration may be in the form of capsules, cachets, pills, tablets, powders, granules, a solution or a suspension in an aqueous or non-aqueous liquid, an oil-in-water or water-in-oil liquid emulsion, an elixir or syrup, a pastille, a bolus, an electuary or a paste. These formulations may be prepared by methods known in the art, *e.g.*, by means of conventional pan-coating, mixing, granulation or lyophilization processes.

[0087] Solid dosage forms for oral administration (capsules, tablets, pills, dragees, powders, granules and the like) may be prepared, *e.g.*, by mixing the active ingredient(s) with one or more pharmaceutically-acceptable

diluents or carriers and, optionally, one or more fillers, extenders, binders, humectants, disintegrating agents, solution retarding agents, absorption accelerators, wetting agents, absorbents, lubricants, and/or coloring agents. Solid compositions of a similar type may be employed as fillers in soft and hard-filled gelatin capsules using a suitable excipient. A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared using a suitable binder, lubricant, inert diluent, preservative, disintegrant, surface-active or dispersing agent. Molded tablets may be made by molding in a suitable machine. The tablets, and other solid dosage forms, such as dragees, capsules, pills and granules, may optionally be scored or prepared with coatings and shells, such as enteric coatings and other coatings well known in the pharmaceutical-formulating art. They may also be formulated so as to provide slow or controlled release of the active ingredient therein. They may be sterilized by, for example, filtration through a bacteria-retaining filter. These compositions may also optionally contain opacifying agents and may be of a composition such that they release the active ingredient only, or preferentially, in a certain portion of the gastrointestinal tract, optionally, in a delayed manner. The active ingredient can also be in microencapsulated form.

[0088] Liquid dosage forms for oral administration include pharmaceutically-acceptable emulsions, microemulsions, solutions, suspensions, syrups and elixirs. The liquid dosage forms may contain suitable inert diluents commonly used in the art. Besides inert diluents, the oral compositions may also include adjuvants, such as wetting agents, emulsifying

and suspending agents, sweetening, flavoring, coloring, perfuming and preservative agents. Suspensions may contain suspending agents.

[0089] The pharmaceutical compositions of the present invention for rectal or vaginal administration may be presented as a suppository, which may be prepared by mixing one or more active ingredient(s) with one or more suitable nonirritating diluents or carriers which are solid at room temperature, but liquid at body temperature and, therefore, will melt in the rectum or vaginal cavity and release the active compound. The pharmaceutical compositions of the present invention which are suitable for vaginal administration also include pessaries, tampons, creams, gels, pastes, foams or spray formulations containing such pharmaceutically-acceptable diluents or carriers as are known in the art to be appropriate.

[0090] Dosage forms for the topical or transdermal administration include powders, sprays, ointments, pastes, creams, lotions, gels, solutions, patches, drops and inhalants. The active agent(s)/compound(s) may be mixed under sterile conditions with a suitable pharmaceutically-acceptable diluent or carrier. The ointments, pastes, creams and gels may contain excipients. Powders and sprays may contain excipients and propellants.

[0091] The pharmaceutical compositions of the present invention suitable for parenteral administrations may comprise one or more agent(s)/compound(s) in combination with one or more pharmaceutically-acceptable sterile isotonic aqueous or non-aqueous solutions, dispersions, suspensions or emulsions, or sterile powders which may be reconstituted into sterile injectable solutions or dispersions just prior to use, which may contain suitable antioxidants, buffers, solutes which render the formulation isotonic

with the blood of the intended recipient, or suspending or thickening agents. Proper fluidity can be maintained, for example, by the use of coating materials, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants. These pharmaceutical compositions may also contain suitable adjuvants, such as wetting agents, emulsifying agents and dispersing agents. It may also be desirable to include isotonic agents. In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption.

[0092] In some cases, in order to prolong the effect of a drug (*e.g.*, pharmaceutical formulation), it is desirable to slow its absorption from subcutaneous or intramuscular injection. This may be accomplished by the use of a liquid suspension of crystalline or amorphous material having poor water solubility.

[0093] The rate of absorption of the active agent/drug then depends upon its rate of dissolution which, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally-administered agent/drug may be accomplished by dissolving or suspending the active agent/drug in an oil vehicle. Injectable depot forms may be made by forming microencapsule matrices of the active ingredient in biodegradable polymers. Depending on the ratio of the active ingredient to polymer, and the nature of the particular polymer employed, the rate of active ingredient release can be controlled. Depot injectable formulations are also prepared by entrapping the drug in liposomes or microemulsions which are compatible

with body tissue. The injectable materials can be sterilized for example, by filtration through a bacterial-retaining filter.

[0094] The formulations may be presented in unit-dose or multi-dose sealed containers, for example, ampules and vials, and may be stored in a lyophilized condition requiring only the addition of the sterile liquid diluent or carrier, for example water for injection, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the type described above.

[0095] The present invention provides combinations shown to enhance the effects of ERK inhibitors. Herein, applicants have also shown that the combination of different ERK inhibitors is likewise synergistic. Therefore, it is contemplated that the effects of the combinations described herein can be further improved by the use of one or more additional ERK inhibitors. Accordingly, some embodiments of the present invention include one or more additional ERK inhibitors.

[0096] The following examples are provided to further illustrate the methods of the present invention. These examples are illustrative only and are not intended to limit the scope of the invention in any way.

EXAMPLES

Example 1

BVD-523 altered markers of MAPK kinase activity and effector function

[0097] For Western blot studies, HCT116 cells (5 x 10^6) were seeded into 10 cm dishes in McCoy's 5A plus 10% FBS. A375 cells (2.5 x 10^6) were seeded into 10 cm dishes in DMEM plus 10% FBS. Cells were allowed to

adhere overnight prior to addition of the indicated amount of test compound (BVD-523) or vehicle control. Cells were treated for either 4 or 24 hours before isolation of whole-cell protein lysates, as specified below. Cells were harvested by trypsinisation, pelleted and snap frozen. Lysates were prepared with RIPA (Radio-Immunoprecipitation Assay) buffer, clarified by centrifugation and quantitated by bicinchoninic acid assay (BCA) assay. 20-50 µg of protein was resolved by SDS-PAGE electrophoresis, blotted onto PVDF membrane and probed using the antibodies detailed in Table 1 (for the 4-hour treatment) and Table 2 (for the 24-hour treatment) below.

Table 1 – Antibody Details

Antigen	Size (kDa)	Supplier	Cat No	Dilution	Incubation / Block Conditions	Secondary
pRSK1/2 pS380	90	Cell Signaling	9335	1:1000	o/n 4˚C 5% BSA	anti-rabbit
pRSK1/2 pS380	90	Cell Signaling	11989	1:2000	o/n 4˚C 5% BSA	anti-rabbit
pRSK-T359/S363	90	Millipore	04-419	1:40000	o/n 4˚C 5% BSA	anti-rabbit
Total RSK	90	Cell Signaling	9333	1:1000	o/n 4˚C 5% BSA	anti-rabbit
pErk 1/2	42/44	Cell Signaling	9106S	1:500	o/n 4˚C 5% milk	anti-mouse
Total ERK	42/44	Cell Signaling	9102	1:2000	o/n 4˚C 5% milk	anti-rabbit
pMEK1/2	45	Cell Signaling	9154	1:1000	o/n 4˚C 5% BSA	anti-rabbit
Total MEK	45	Cell Signaling	9126	1:1000	o/n 4˚C 5% BSA	anti-rabbit
pS6-pS235	32	Cell Signaling	2211S	1:3000	o/n 4˚C 5% milk	anti-rabbit
Total S6	32	Cell Signaling	2217	1:2000	o/n 4˚C 5% milk	anti-rabbit
DUSP6	48	Cell Signaling	3058S	1:1000	o/n 4˚C 5% BSA	anti-rabbit
Total CRAF	73	BD Bio-sciences	610152	1:2000	o/n 4˚C 5% milk	anti-mouse
pCRAF-Ser338	73	Cell Signaling	9427	1:1000	o/n 4˚C 5% BSA	anti-rabbit
pRB (Ser780)	105	Cell Signaling	9307	1:2000	o/n 4˚C 5% BSA	anti-rabbit
β-Actin	42	Sigma	A5441	1:500,000	o/n 4˚C 5% milk	anti-mouse

Table 2 – Antibody details

Antigen	Size (kDa)	Supplier	Cat No	Dilution	Incubation / Block Conditions	Secondary
pRB (Ser780)	105	Cell Signaling	9307	1:2000	o/n 4˚C 5% BSA	anti-rabbit
CCND1	34	Abcam	ab6152	1:500	o/n 4˚C 5% milk	anti-mouse
Bim-EL	23	Millipore	AB17003	1:1000	o/n 4˚C 5% BSA	anti-rabbit
Bim-EL	23	Cell Signaling	2933	1:1000	o/n 4˚C 5% BSA	anti-rabbit
BCL-xL	30	Cell Signaling	2762	1:2000	o/n 4˚C 5% BSA	anti-rabbit
PARP	116/89	Cell Signaling	9542	1:1000	o/n 4˚C 5% milk	anti-rabbit
Cleaved Caspase 3	17,19	Cell Signaling	9664X	1:1000	o/n 4˚C 5% milk	anti-rabbit
DUSP6	48	Cell Signaling	3058S	1:1000	o/n 4°C 5% BSA	anti-rabbit
pRSK1/2 pS380	90	Cell Signaling	9335	1:1000	o/n 4°C 5% BSA	anti-rabbit
pRSK1/2 pS380	90	Cell Signaling	11989	1:2000	o/n 4°C 5% BSA	anti-rabbit
pRSK-T359/S363	90	Millipore	04-419	1:40000	o/n 4°C 5% BSA	anti-rabbit
Total RSK	90	Cell Signaling	9333	1:1000	o/n 4°C 5% BSA	anti-rabbit
pErk 1/2	42/44	Cell Signaling	9106S	1:500	o/n 4°C 5% milk	anti-mouse
Total ERK	42/44	Cell Signaling	9102	1:2000	o/n 4°C 5% milk	anti-rabbit
B-Actin	42	Sigma	A5441	1:500,000	o/n 4°C 5% milk	anti-mouse

[0098] FIG. 1 shows Western blot analyses of cells treated with BVD-523 at various concentrations for the following: 1) MAPK signaling components in A375 cells after 4 hours; 2) cell cycle and apoptosis signaling in A375 24 hours treatment with various amounts of BVD-523; and 3) MAPK signaling in HCT-116 cells treated for 4 hours. The results show that acute and prolonged treatment with BVD-523 in RAF and RAS mutant cancer cells in-vitro affects both substrate phosphorylation and effector targets of ERK kinases. The concentrations of BVD-523 required to induce these changes is typically in the low micromolar range.

[0099] Changes in several specific activity markers are noteworthy. First, the abundance of slowly migrating isoforms of ERK kinase increase following BVD-523 treatment; modest changes can be observed acutely, and increase following prolonged treatment. While this could indicate an increase in enzymatically active, phosphorylated forms of ERK, it remains noteworthy that multiple proteins subject to both direct and indirect regulation by ERK remain "off" following BVD-523 treatment. First, RSK1/2 proteins exhibit reduced phosphorylation at residues that are strictly dependent on ERK for protein modification (T359/S363). Second, BVD-523 treatment induces complex changes in the MAPK feedback phosphatase, DUSP6: slowly migrating protein isoforms are reduced following acute treatment, while total protein levels are greatly reduced following prolonged BVD-523 treatment. Both of these findings are consistent with reduced activity of ERK kinases, which control DUSP6 function through both post-translational and transcriptional mechanisms. Overall, despite increases in cellular forms of

ERK that are typically thought to be active, it appears likely that cellular ERK enzyme activity is fully inhibited following either acute or prolonged treatment with BVD-523.

[0100] Consistent with these observations, effector genes that require MAPK pathway signaling are altered following treatment with BVD-523. The G1/S cell-cycle apparatus is regulated at both post-translational and transcriptional levels by MAPK signaling, and cyclin-D1 protein levels are greatly reduced following prolonged BVD-523 treatment. Similarly, gene expression and protein abundance of apoptosis effectors often require intact MAPK signaling, and total levels of Bim-EL increase following prolonged BVD-523 treatment. As noted above, however, PARP protein cleavage and increased apoptosis were not noted in the A375 cell background; this suggests that additional factors may influence whether changes in BVD-523/ERK-dependent effector signaling are translated into definitive events such as cell death and cell cycle arrest.

[0101] Consistent with the cellular activity of BVD-523, marker analysis suggests that ERK inhibition alters a variety of molecular signaling events in cancer cells, making them susceptible to both decreased cell proliferation and survival.

[0102] In sum, FIG. 1 shows that BVD-523 inhibits the MAPK signaling pathway and may be more favorable compared to RAF or MEK inhibition in this setting.

[0103] Finally, properties of BVD-523 may make this a preferred agent for use as an ERK inhibitor, compared to other agents with a similar activity. It is known that kinase inhibitor drugs display unique and specific interactions

with their enzyme targets, and that drug efficacy is strongly influenced by both the mode of direct inhibition, as well as susceptibility to adaptive changes that occur following treatment. For example, inhibitors of ABL, KIT, EGFR and ALK kinases are effective only when their cognate target is found in active or inactive configurations. Likewise, certain of these inhibitors are uniquely sensitive to either secondary genetic mutation, or post-translational adaptive changes, of the protein target. Finally, RAF inhibitors show differential potency to RAF kinases present in certain protein complexes and/or subcellular localizations. In summary, as ERK kinases are similarly known to exist in diverse, variable, and complex biochemical states, it appears likely that BVD-523 may interact with and inhibit these targets in a fashion that is distinct and highly preferable to other agents.

Example 2

BVD-523/mTOR inhibitor combinations are effective in inhibiting the growth of cancer cell lines in vitro

[0104] Cancer cell lines are maintained in cell culture under standard media and serum conditions.

[0105] For all combination studies, U87MG (human glioblastomas) cells are seeded into triplicate 96-well plates at a cell density of 1500 cells/well in McCoy's 5A Medium plus 10% fetal bovine serum (FBS). A375 cells (BRAF V600 E human malignant melanoma) are seeded at a density of 3000 cells/well in Dulbecco's Modified Eagle Medium (DMEM) plus 10% FBS. Cells are allowed to adhere overnight prior to addition of test compound or vehicle control.

[0106] For rapamycin (an mTOR inhibitor) studies, the following combinations are tested using a 10 x 8 dose matrix: rapamycin (ranging from 1-1000 nM) with BVD-0523 (ranging from 0 to 10 μM), rapamycin (ranging from 1-1000 nM) with dabrafenib (ranging from 0 to 1 μM), and rapamycin (ranging from 1-1000 nM) with trametinib (ranging from 0 to 0.010 μM). The final concentration of DMSO is 0.2%. The compounds are incubated with the cells for 96 hours.

[0107] For dactolisib (another mTOR inhibitor) studies, the following combinations are tested using a 10 x 8 dose matrix: dactolisib (ranging from 0.1 nM-100 nM) with BVD-0523 (0 to 10 μM), dactolisib (ranging from 0.1 nM-100 nM) with dabrafenib (ranging from 0 to 1 μM), and dactolisib (ranging from 0.1 nM-100 nM) with trametinib (ranging from 0 to 0.1 μM). The final concentration of DMSO is 0.2%. The compounds are incubated with the cells for 96 hours.

[0108] Next, Alamar Blue 10% (v/v) is added and incubated with the cells for 4 hours prior to reading on a fluorescent plate reader. After reading Alamar Blue, the medium/Alamar Blue mix is flicked off, 100 μl of CellTiter-Glo/PBS (1:1) is added, and the plates are processed as per the manufacturer's instructions (Promega, Madison, WI). Media only background values are subtracted before the data is analyzed.

Caspase-Glo 3/7 assays

[0109] In brief, U87MG cells are seeded in triplicate in white 96-well plates at a cell density of 5000 cells/well in McCoy's 5A plus 10% FBS. A375 cells are seeded at a density of 5000 cells/well in DMEM plus 10% FBS. Cells are allowed to adhere overnight prior to addition of test compound or vehicle

control. The final concentration of DMSO is 0.2%, and 800 nM staurosporine is included as a positive control. 24 and 48 hour assay incubation periods are used. Then, Caspase-Glo® 3/7 50% (v/v) is added, plates are mixed for 5 minutes on an orbital shaker and incubated for 1 hour at room temperature prior to reading on a luminescent plate reader. Media only background values are subtracted before the data is analysed.

Data Analysis

[0110] The combination data may be presented as dose-response curves generated in GraphPad Prism (plotted using % viability relative to DMSO only treated controls).

[0111] Predicted fractional inhibition values for combined inhibition are calculated using the equation C_{bliss} =A + B – (A x B) where A and B are the fractional inhibitions obtained by drug A alone or drug B alone at specific concentrations. C_{bliss} is the fractional inhibition that would be expected if the combination of the two drugs is exactly additive. C_{bliss} values are subtracted from the experimentally observed fractional inhibition values to give an 'excess over Bliss' value. Excess over Bliss values greater than 0 indicate synergy, whereas values less than 0 indicate antagonism. Excess over Bliss values may be plotted as heat maps ± SD.

[0112] It is expected that the combinations of rapamycin or dactolisib with BVD-523 will be effective in inhibiting the growth of A375 and U87MG cells. Dose response curves will be obtained. It is expected that the IC_{50} of BVD-523 in these cell lines will be approximately 150 nM. It is also expected that the IC_{50} of rapamycin and dactolisib in these cell lines will be

approximately 225 nM (Lu, X., *et al.*, 2011) and 20 nM (Mukherjee, B., *et al.*, 2012) (Roper, J., *et al.*, 2011), respectively.

Example 3

BVD-523/mTOR inhibitor combinations are effective in inhibiting the growth of cancer cell lines in vivo

Mice

[0113] Female athymic nude mice (Crl:NU(Ncr)-Foxn/^{nu}, Charles River) are nine weeks old with a body weight (BW) range of about 15 to about 30 grams on Day 1 of the study. The animals are fed *ad libitum* water (reverse osmosis, 1 ppm Cl), and NIH 31 Modified and Irradiated Lab Diet® consisting of 18.0% crude protein, 5.0% crude fat, and 5.0% crude fiber. The mice are housed on irradiated Enrich-o'cobs™ Laboratory Animal Bedding in static microisolators on a 12-hour light cycle at 20-22°C (68-72°F) and 40-60% humidity. The recommendations of the *Guide for Care and Use of Laboratory Animals* with respect to restraint, husbandry, surgical procedures, feed and fluid regulation, and veterinary care are complied with.

In Vivo Implantation and Tumor Growth

[0114] U87MG human glioblastomas are cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 units/mL penicillin G sodium, 100 µg/mL streptomycin sulfate, and 25 µg/mL gentamicin. The cells are grown in tissue culture flasks in a humidified incubator at 37°C, in an atmosphere of 5% CO_2 and 95% air.

[0115] The U87MG cells used for implantation are harvested during exponential growth and resuspended in 50% Matrigel (BD Biosciences): 50% phosphate buffered saline at a concentration of 2.5 x 10^7 cells/mL. On the

day of tumor implant, each test mouse is injected subcutaneously in the right flank with 5 x 10^6 cells (0.2 mL cell suspension), and tumor growth is monitored as the average size approaches the target range of 100 to 150 mm^3. Tumors are measured in two dimensions using calipers, and volume is calculated using the formula:

$$\text{Tumor Volume } (mm^3) = (w^2 \times l) / 2$$

where w = width and l = length, in mm, of the tumor. Tumor weight may be estimated with the assumption that 1 mg is equivalent to 1 mm^3 of tumor volume.

[0116] Ten days after tumor implantation, designated as Day 1 of the study, the animals are sorted into sixteen groups, each described below.

Treatment

[0117] On Day 1 of the study, mice are sorted into groups each consisting of fifteen mice and one group consisting of ten mice, and dosing is initiated. All doses are given by oral gavage (p.o.). The rapamycin/dactolisib doses are to be given once daily (qd) until study end (qd to end), whereas the vehicle and BVD-523 doses are to be given twice daily (bid) until study end (bid to end). For bid dosing, dosing is initiated in the afternoon of Day 1, so that one dose is given on the first day ("first day 1 dose").

Controls

[0118] One group receives 1% CMC vehicle p.o. bid to end, and serves as the control group for calculation of %TGD. Another group received temozolomide, which is dissolved in deionized water. It is administered at 100 mg/kg (p.o.) per day, once daily from day 1 to day 5 of the treatment (qd x 5).

Monotherapy Treatments

[0119] Four groups receive either rapamycin at 10 or 100 mg/kg or dactolisib at 40 or 100 mg/kg. Two groups receive 50 or 100 mg/kg BVD-523 p.o. bid to end.

Combination Treatments

[0120] Each one of two groups receives a combination of 50 mg/kg BVD-523 with 10 or 100 mg/kg of rapamycin. Two other groups receive 100 mg/kg BVD-523 with 10 or 100 mg/kg of rapamycin. Two additional groups will receive 50 mg/kg BVD-523 with 40 or 100 mg/kg dactolisib, and another two groups will receive 100 mg/kg BVD-523 with 40 or 100 mg/kg dactolisib.

Endpoint and Tumor Growth Delay (TGD) Analysis

[0121] Tumors are measured using calipers twice per week, and each animal is euthanized when its tumor reaches the pre-determined tumor volume endpoint of 2000 mm^3 or on the final day, whichever comes first. Animals that exit the study for tumor volume endpoint are documented as euthanized for tumor progression (TP), with the date of euthanasia. The time to endpoint (TTE) for analysis is calculated for each mouse by the following equation:

$$TTE = [\log_{10}(\text{endpoint volume}) - b] / m$$

where TTE is expressed in days, endpoint volume is expressed in mm^3, b is the intercept, and m is the slope of the line obtained by linear regression of a log-transformed tumor growth data set. The data set consists of the first observation that exceeds the endpoint volume used in analysis and the three consecutive observations that immediately precede the attainment of this

endpoint volume. The calculated TTE is usually less than the TP date, the day on which the animal is euthanized for tumor size. Animals with tumors that do not reach the endpoint volume are assigned a TTE value equal to the last day of the study. Any animal classified as having died from NTR (non-treatment-related) causes due to accident (NTRa) or due to unknown etiology (NTRu) are excluded from TTE calculations (and all further analyses). Animals classified as TR (treatment-related) deaths or NTRm (non-treatment-related death due to metastasis) are assigned a TTE value equal to the day of death.

[0122] Treatment outcome is evaluated from TGD, defined as the increase in the median TTE in a treatment group compared to the control group:

$$TGD = T - C,$$

expressed in days, or as a percentage of the median TTE of the control group:

$$\%TGD = [(T-C) / C] \times 100$$

where:

T = median TTE for a treatment group, and

C = median TTE for the designated control group.

Criteria for Regression Responses

[0123] Treatment efficacy may be determined from the incidence and magnitude of regression responses observed during the study. Treatment may cause partial regression (PR) or complete regression (CR) of the tumor in an animal. In a PR response, the tumor volume is 50% or less of its Day 1

volume for three consecutive measurements during the course of the study, and equal to or greater than 13.5 mm^3 for one or more of these three measurements. In a CR response, the tumor volume is less than 13.5 mm^3 for three consecutive measurements during the course of the study. An animal with a CR response at the termination of the study is additionally classified as a tumor-free survivor (TFS). Animals are monitored for regression responses.

Toxicity

[0124] Animals are weighed daily on Days 1-5, then twice per week until completion of the study. The mice are observed frequently for overt signs of any adverse, TR side effects, and clinical signs are recorded when observed. Individual BW loss is monitored as per protocol, and any animal whose weight exceeds the limits for acceptable BW loss is euthanized. Group mean BW loss also is monitored as per protocol. Dosing is to be suspended in any group that exceeds the limits for acceptable mean BW loss. If mean BW recovers, then dosing is to be resumed in that group, but at a lower dosage or less frequent dosing schedule. Acceptable toxicity for the maximum tolerated dose (MTD) is defined as a group mean BW loss of less than 20% during the study and not more than 10% TR deaths. A death is classified as TR if attributable to treatment side effects as evidenced by clinical signs and/or necropsy, or may also be classified as TR if due to unknown causes during the dosing period or within 14 days of the last dose. A death is classified as NTR if there is no evidence that death is related to treatment side effects. NTR deaths may be further characterized based on cause of death. A death is classified as NTRa if it results from an accident or

human error. A death is classified as NTRm if necropsy indicates that it may result from tumor dissemination by invasion and/or metastasis. A death is classified as NTRu if the cause of death is unknown and there is no available evidence of death related to treatment side effects, metastasis, accident or human error, although death due to treatment side effects cannot be excluded.

Statistical and Graphical Analyses

[0125] Prism (GraphPad) for Windows 3.03 is used for graphical presentations and statistical analyses.

[0126] The logrank test, which evaluates overall survival experience, is used to analyze the significance of the differences between the TTE values of two groups. Logrank analysis includes the data for all animals in a group except those assessed as NTR deaths. Two-tailed statistical analyses are conducted at significance level $P = 0.05$. The statistical tests are not adjusted for multiple comparisons. Prism summarizes test results as not significant (ns) at $P > 0.05$, significant (symbolized by "*") at $0.01 < P < 0.05$, very significant ("**") at $0.001 < P \leq 0.01$, and extremely significant ("***") at $P \leq 0.001$. Groups with regimens above the MTD are not evaluated statistically.

[0127] A scatter plot is constructed to show TTE values for individual mice, by group. Group mean tumor volumes are plotted as a function of time. When an animal exits the study due to tumor size, the final tumor volume recorded for the animal is included with the data used to calculate the mean volume at subsequent time points. Error bars (when present) indicate one standard error of the mean (SEM). Tumor growth plots exclude the data for NTR deaths, and are truncated after 50% of the assessable animals in a

group exit the study or after the second TR death in a group, whichever comes first. Kaplan-Meier plots show the percentage of animals in each group remaining in the study versus time. The Kaplan-Meier plot and logrank test share the same TTE data sets. Percent mean BW changes from Day 1 are calculated for each group for each day of BW measurement, and are plotted as a function of time. BW plots exclude the data for NTR deaths, and are truncated after 50% of the assessable animals in a group exit the study.

Results

[0128] It is expected that the combinations of rapamycin or dactolisib with BVD-523 are effective against U87MG cell-derived tumors and that the results are statistically significant. It is also expected that the side effects associated with BVD-523 combination treatment are minimal.

Example 4

Cell culture studies of PI3K-MTOR and ERK inhibitors

Single Agent Proliferation Assay

[0129] Cells were seeded in 96-well plates at the densities and media conditions indicated in Table 3 in McCoy's 5A containing either 10% FBS or 1% charcoal-stripped FBS (CS-FBS), and allowed to adhere overnight prior to addition of compound or vehicle control. Compounds were prepared from DMSO stocks to give the desired final concentrations The final DMSO concentration was constant at 0.1%. Test compounds were incubated with the cells for 72h at 37°C, 5% CO_2 in a humidified atmosphere. CellTiter-Glo® reagent (Promega, Madison, WI) was added according to manufacturer's instructions and luminescence detected using the BMG FLUOstar plate reader (BMG Labtech, Ortenberg, Germany). The average media only

background value was deducted and the data analysed using a 4-parameter logistic equation in GraphPad Prism (GraphPad Software, La Jolla, CA).

Combination Proliferation Assay

[0130] Cells were seeded into triplicate 96-well plates at the densities indicated in Table 3 in McCoy's 5A media containing 2.5% FBS and allowed to adhere overnight prior to addition of test compound or vehicle control. Combinations were tested using either a 10x8 or for the follow-up HCT116 study a 3x1 dose matrix.

[0131] Test compounds were incubated with the cells for 72h at 37°C, 5% CO_2 in a humidified atmosphere. CellTiter-Glo® reagent (Promega, Madison, WI) was added according to manufacturer's instructions and luminescence detected using the BMG FLUOstar plate reader (BMG Labtech, Ortenberg, Germany). The average media only background value was deducted and the data analysed.

[0132] For the 10x8 combination assays the combination interactions across the dose matrix were determined by the Loewe Additivity and Bliss independence models using Chalice™ Combination Analysis Software (Horizon Discovery Group, Cambridge, MA) as outlined in the user manual (available at chalice.horizondiscovery.com/chalice-portal/documentation/analyzer/home.jsp). Synergy is determined by comparing the experimentally observed level of inhibition at each combination point with the value expected for additivity, which is derived from the single-agent responses along the edges of the matrix. Potential synergistic interactions were identified by displaying the calculated excess inhibition over that predicted as being additive across the dose matrix as a heat map, and by

reporting a quantitative 'Synergy Score' based on the Loewe model. The single agent data derived from the combination assay plates were presented as dose-response curves generated in GraphPad Prism (GraphPad Software, La Jolla, CA) (plotted using percentage viability relative to DMSO only treated controls).

[0133] The 3x1 combination assay follow-up experiment was analysed using the Bliss additivity model in Microsoft Excel as follows: first, predicted fractional inhibition values for combined inhibition were calculated using the equation $C_{bliss} = A + B - (A \times B)$ where A and B are the fractional inhibitions obtained by drug A alone or drug B alone at specific concentrations. (C_{bliss} is the fractional inhibition that would be expected if the combination of the two drugs were exactly additive). C_{bliss} values were then subtracted from the experimentally observed fractional inhibition values to give an 'excess over Bliss' value which were plotted as heat maps ± SD. Excess of Bliss values greater than 0 indicate synergy, whereas values less than 0 indicate antagonism.

Table 3 - Cell Line Seeding Density and Growth Media

Cell Line	Seeding Density in 10% FBS (cells/well)	Seeding Density in 1% CS-FBS (cells/well)	Seeding Density in 2.5% FBS (cells/well)
HCT116 Parental	1000	3000	2000
HCT116 PIK3CA (+/-)	3000	4500	7500
DLD-1 Parental	-	-	2000
DLD-1 PIK3CA (+/-)	-	-	3000

[0134] The aim of this study was to assess the effects on cell viability of combining ERK inhibitors with a panel of PI3K-MTOR inhibitors (Table 4) in HCT116 and DLD1 cell line pairs that are isogenic for the presence or absence of PIK3CA activating mutations. (Table 5).

Table 4 – Description of PI3K-MTOR Inhibitors Studied

Inhibitor	Selectivity
BYL719	PI3K α-selective inhibitor
BKM120	Pan-PI3K (α/β/δ/γ) inhibitor
INK128	mTOR inhibitor
PF-04691502	PI3K/mTOR dual inhibitor

Table 5 – Description of Cell Lines Studied

Cell Line	Description
HCT116 Parental	Heterozygous parental cells containing one mutant PIK3CA allele (H1047R) and one wild type allele
HCT116 PIK3CA (+/-)	Knock out of mutant KRAS allele in heterozygous parental cells Knock-out of PIK3CA mutant allele (H1047R) in heterozygous parental cells
DLD-1 Parental	Heterozygous parental cells containing one mutant PIK3CA allele (E545K) and one wild type allele
DLD-1 PIK3CA (+/-)	Knock-out of PIK3CA mutant allele (E545K) in heterozygous parental cells

[0135] Initial single agent assays were performed in the HCT116 isogenic cells in order to select appropriate concentration ranges to use in the combination assays (FIG. 2, Table 6). As high levels of serum can potentially mask interactions between targeted agents and specific mutant genotypes, due to an excess of growth factors, these assays were performed under both standard (10% FBS) and reduced serum conditions (1% charcoal-stripped FBS).

Table 6 – Single agent IC50 values (μM) for each compound in the HCT116 PIK3CA (+/-) isogenic cell line pair

Compound	HCT116 Parental		HCT116 PIK3CA (+/-)	
	10% FBS	1% CS-FBS	10% FBS	1% CS-FBS
BYL719	n.d.	n.d.	n.d.	n.d.
BKM120	0.62	0.53	0.52	1.03
INK128	0.05	0.03	0.07	0.02
PF-04691502	0.29	0.07	0.42	1.54
BVD-523	0.17	0.02	0.12	0.01
SCH772984	0.14	0.03	0.08	0.01
Paclitaxel	0.002	0.003	0.002	0.003
GDC-0941	1.53	0.06	n.d.	n.d.

[0136] Although, there were apparent differences in the calculated IC_{50} values between the two serum conditions, a reliable interpretation of these differences was confounded by the poor levels of cell growth and compromised cell health (microscopic observations) under the reduced serum conditions. As an intermediate to these conditions, all the combination assays were therefore performed in medium containing 2.5% serum.

[0137] Combination interactions between two compounds were assessed across a matrix of concentrations using the Loewe Additivity and Bliss Independence Models with Chalice™ Bioinformatics Software (Horizon Discovery Group, Cambridge, MA). Chalice™ enables potential synergistic interactions to be identified by displaying the calculated excess inhibition over that predicted as being additive across the dose matrix as a heat map, and by reporting a quantitative 'Synergy Score' based on the Loewe model.

[0138] BVD-523 showed strong synergistic interactions with BYL719, BKM120 and PF04691502, and modestly synergistic with BKM120, in the parental HCT116 cell line, which carries the PIK3CA mutation. Potential

synergies were also observed in the HCT116 isogenic cell line lacking the PIK3CA mutation, however, the strength and/or windows of synergy tended to be smaller relative to the parental line.

[0139] A similar pattern of results was seen with a second benchmark ERK inhibitor SCH772984 in this HCT116 isogenic pair supporting the notion that these synergies are specifically related to inhibition of ERK and not due to an off-target effect. (FIG. 3 – FIG. 11)

[0140] These results were confirmed in the HCT116 isogenics in a repeat experiment using a narrower range of inhibitor concentrations. (FIG. 12) BVD-523 and SCH772984 also showed a similar pattern of potentially synergistic interactions in the DLD-1 isogenic cells. (FIG. 13 – FIG. 21) However, in contrast to the HCT116 cells, synergies were weaker and there was little difference in the magnitude of synergy between the cell line lacking the PIK3CA mutation relative to the parental line. (FIG. 22)

[0141] In summary, these results suggest synergistic interactions between BVD-523 and PI3K-MTOR pathway inhibitors in cancer cell lines that are either wild type or mutated for PIK3CA.

[0142] Single agent dose-response curves in 2.5% serum were derived from the combination assay plates. IC_{50} values are a mean derived from n=4 separate combinations. A comparison of the single agent dose responses derived from the combination assay data in the HCT116 isogenics showed that the cell line lacking the PIK3CA mutation was more sensitive to BVD-523 relative to the parental line that contained the mutation. A similar result was seen with SCH772984. This may indicate that PIK3CA mutation status is a

potential biomarker for predicting response to single agent BVD-523 treatment. (Table 7)

Table 7 – Differential sensitivity to ERK inhibition in HCT116 isogenics

	IC_{50} (μM)	
	HCT116 Parental	HCT116 PIK3CA (+/-)
BVD-523	0.13	0.04
SCH772984	0.21	0.03

Example 5

Combination Interactions Between ERK inhibitors

[0143] RAF mutant melanoma cell line A375 cells were cultured in DMEM with 10% FBS and seeded into triplicate 96-well plates at an initial density of 2000 cells per well. Combination interactions between ERK inhibitors BVD-523 and SCH772984 were analized after 72 hours as described above in Example 4. Viability was determined using CellTiter-Glo® reagent (Promega, Madison, WI) according to manufacturer's instructions and luminescence was detected using the BMG FLUOstar plate reader (BMG Labtech, Ortenberg, Germany).

[0144] Visualization of the Loewe and Bliss 'excess inhibition' heat maps suggested that the combination of BVD-523 and SCH772984 was mainly additive with windows of potential synergy in mid-range doses (FIG. 23).

[0145] In summary, these results suggest that interactions between BVD-523 and SCH772984 are at least additive, and in some cases synergistic.

Documents

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[0146] All documents cited in this application are hereby incorporated by reference as if recited in full herein.

[0147] Although illustrative embodiments of the present invention have been described herein, it should be understood that the invention is not limited

to those described, and that various other changes or modifications may be made by one skilled in the art without departing from the scope or spirit of the invention.

WHAT IS CLAIMED IS:

1. A method of treating or ameliorating the effects of a cancer in a subject in need thereof comprising administering to the subject an effective amount of (i) a first anti-cancer agent, which is BVD-523 or a pharmaceutically acceptable salt thereof and (ii) a second anti-cancer agent, which is a mTOR inhibitor or a pharmaceutically acceptable salt thereof, to treat or ameliorate the effects of the cancer.

2. The method according to clam 1, wherein the subject is a mammal.

3. The method according to claim 2, wherein the mammal is selected from the group consisting of humans, primates, farm animals, and domestic animals.

4. The method according to claim 2, wherein the mammal is a human.

5. The method according to claim 1, wherein the mTOR inhibitor is selected from the group consisting of zotarolimus (AbbVie), umirolimus (Biosensors), temsirolimus (Pfizer), sirolimus (Pfizer), sirolimus NanoCrystal (Elan Pharmaceutical Technologies), sirolimus TransDerm (TransDerm), sirolimus-PNP (Samyang), everolimus (Novartis), biolimus A9 (Biosensors), ridaforolimus (Ariad), rapamycin, TCD-10023 (Terumo), DE-109 (MacuSight), MS-R001 (MacuSight), MS-R002 (MacuSight), MS-R003 (MacuSight), Perceiva (MacuSight), XL-765 (Exelixis), quinacrine (Cleveland BioLabs), PKI-587 (Pfizer), PF-04691502 (Pfizer), GDC-0980 (Genentech and Piramed), dactolisib (Novartis), CC-223 (Celgene), PWT-33597 (Pathway Therapeutics), P-7170 (Piramal Life Sciences), LY-3023414 (Eli Lilly), INK-

128 (Takeda), GDC-0084 (Genentech), DS-7423 (Daiichi Sankyo), DS-3078 (Daiichi Sankyo), CC-115 (Celgene), CBLC-137 (Cleveland BioLabs), AZD-2014 (AstraZeneca), X-480 (Xcovery), X-414 (Xcovery), EC-0371 (Endocyte), VS-5584 (Verastem), PQR-401 (Piqur), PQR-316 (Piqur), PQR-311 (Piqur), PQR-309 (Piqur), PF-06465603 (Pfizer), NV-128 (Novogen), nPT-MTOR (Biotica Technology), BC-210 (Biotica Technology), WAY-600 (Biotica Technology), WYE-354 (Biotica Technology), WYE-687 (Biotica Technology), LOR-220 (Lorus Therapeutics), HMPL-518 (Hutchison China MediTech), GNE-317 (Genentech), EC-0565 (Endocyte), CC-214 (Celgene), ABTL-0812 (Ability Pharmaceuticals), and

6. The method according to claim 1, wherein the cancer is selected from the group consisting of hematologic cancer and solid tumor cancers.

7. The method according to claim 1, wherein the cancer is selected from the group consisting of autonomic ganglia cancer, biliary tract cancer, breast cancer, endometrial cancer, gastrointestinal tract cancer, haematopoietic and lymphoid cancer, kidney cancer, liver cancer, lung cancer, oesophagial cancer, ovarian cancer, pancreatic cancer, prostate cancer, skin cancer, soft tissue cancer, stomach cancer, thyroid cancer, upper aerodigestive tract cancer, and urinary tract cancer.

8. The method according to claim 1, wherein the cancer is selected from the group consisting of oesophagus cancer, skin cancer, biliary tract cancer, large intestine cancer, endometrial cancer, lung cancer, urinary tract cancer, liver cancer, and kidney cancer.

9. The method according to claim 1, wherein the cancer is selected from the group consisting of brain cancer, colon cancer, leukemia, non-Hodgkin's lymphoma, and multiple myeloma.

10. The method according to claim 9, wherein the brain cancer is glioblastoma multiforme (GBM).

11. The method according to claim 6, wherein the hematologic cancer is selected from the group consisting of Adult Acute Megakaryoblastic Leukemia (M7), Adult Acute Minimally Differentiated Myeloid Leukemia (M0), Adult Acute Monoblastic Leukemia (M5a), Adult Acute Monocytic Leukemia (M5b), Adult Acute Myeloblastic Leukemia With Maturation (M2), Adult Acute Myeloblastic Leukemia Without Maturation (M1), Adult Acute Myeloid Leukemia With 11q23 (MLL) Abnormalities, Adult Acute Myeloid Leukemia With Del(5q), Adult Acute Myeloid Leukemia With Inv(16)(p13;q22), Adult Acute Myeloid Leukemia With t(16;16)(p13;q22), Adult Acute Myeloid Leukemia With t(8;21)(q22;q22), Adult Acute Myelomonocytic Leukemia (M4), Adult Erythroleukemia (M6a), Adult Pure Erythroid Leukemia (M6b), Recurrent Adult Acute Myeloid Leukemia, and Untreated Adult Acute Myeloid Leukemia.

12. The method according to claim 1 further comprising administering to the subject at least one additional therapeutic agent selected from the group consisting of an antibody or fragment thereof, a cytotoxic agent, a toxin, a radionuclide, an immunomodulator, a photoactive therapeutic agent, a radiosensitizing agent, a hormone, an anti-angiogenesis agent, and combinations thereof.

13. The method according to claim 12, wherein the additional therapeutic agent is an inhibitor of the PI3K/Akt pathway.

14. The method according to claim 13, wherein the inhibitor of the PI3K/Akt pathway is selected from the group consisting of A-674563 (CAS # 552325-73-2), AGL 2263, AMG-319 (Amgen, Thousand Oaks, CA), AS-041164 (5-benzo[1,3]dioxol-5-ylmethylene-thiazolidine-2,4-dione), AS-604850 (5-(2,2-Difluoro-benzo[1,3]dioxol-5-ylmethylene)-thiazolidine-2,4-dione), AS-605240 (5-quinoxilin-6-methylene-1,3-thiazolidine-2,4-dione), AT7867 (CAS # 857531-00-1), benzimidazole series, Genentech (Roche Holdings Inc., South San Francisco, CA), BML-257 (CAS # 32387-96-5), CAL-120 (Gilead Sciences, Foster City, CA), CAL-129 (Gilead Sciences), CAL-130 (Gilead Sciences), CAL-253 (Gilead Sciences), CAL-263 (Gilead Sciences), CAS # 612847-09-3, CAS # 681281-88-9, CAS # 75747-14-7, CAS # 925681-41-0, CAS # 98510-80-6, CCT128930 (CAS # 885499-61-6), CH5132799 (CAS # 1007207-67-1), CHR-4432 (Chroma Therapeutics, Ltd., Abingdon, UK), FPA 124 (CAS # 902779-59-3), GS-1101 (CAL-101) (Gilead Sciences), GSK 690693 (CAS # 937174-76-0), H-89 (CAS # 127243-85-0), Honokiol, IC87114 (Gilead Science), IPI-145 (Intellikine Inc.), KAR-4139 (Karus Therapeutics, Chilworth, UK), KAR-4141 (Karus Therapeutics), KIN-1 (Karus Therapeutics), KT 5720 (CAS # 108068-98-0), Miltefosine, MK-2206 dihydrochloride (CAS # 1032350-13-2), ML-9 (CAS # 105637-50-1), Naltrindole Hydrochloride, OXY-111A (NormOxys Inc., Brighton, MA), perifosine, PHT-427 (CAS # 1191951-57-1), PI3 kinase delta inhibitor, Merck KGaA (Merck & Co., Whitehouse Station, NJ), PI3 kinase delta inhibitors, Genentech (Roche Holdings Inc.), PI3 kinase delta inhibitors, Incozen (Incozen Therapeutics, Pvt. Ltd., Hydrabad,

India), PI3 kinase delta inhibitors-2, Incozen (Incozen Therapeutics), PI3 kinase inhibitor, Roche-4 (Roche Holdings Inc.), PI3 kinase inhibitors, Roche (Roche Holdings Inc.), PI3 kinase inhibitors, Roche-5 (Roche Holdings Inc.), PI3-alpha/delta inhibitors, Pathway Therapeutics (Pathway Therapeutics Ltd., South San Francisco, CA), PI3-delta inhibitors, Cellzome (Cellzome AG, Heidelberg, Germany), PI3-delta inhibitors, Intellikine (Intellikine Inc., La Jolla, CA), PI3-delta inhibitors, Pathway Therapeutics-1 (Pathway Therapeutics Ltd.), PI3-delta inhibitors, Pathway Therapeutics-2 (Pathway Therapeutics Ltd.), PI3-delta/gamma inhibitors, Cellzome (Cellzome AG), PI3-delta/gamma inhibitors, Cellzome (Cellzome AG), PI3-delta/gamma inhibitors, Intellikine (Intellikine Inc.), PI3-delta/gamma inhibitors, Intellikine (Intellikine Inc.), PI3-delta/gamma inhibitors, Pathway Therapeutics (Pathway Therapeutics Ltd.), PI3-delta/gamma inhibitors, Pathway Therapeutics (Pathway Therapeutics Ltd.), PI3-gamma inhibitor Evotec (Evotec), PI3-gamma inhibitor, Cellzome (Cellzome AG), PI3-gamma inhibitors, Pathway Therapeutics (Pathway Therapeutics Ltd.), PI3K delta/gamma inhibitors, Intellikine-1 (Intellikine Inc.), PI3K delta/gamma inhibitors, Intellikine-1 (Intellikine Inc.), pictilisib (Roche Holdings Inc.), PIK-90 (CAS # 677338-12-4), SC-103980 (Pfizer, New York, NY), SF-1126 (Semafore Pharmaceuticals, Indianapolis, IN), SH-5, SH-6, Tetrahydro Curcumin, TG100-115 (Targegen Inc., San Diego, CA), Triciribine, X-339 (Xcovery, West Palm Beach, FL), XL-499 (Evotech, Hamburg, Germany), pharmaceutically acceptable salts thereof, and combinations thereof.

15. The method according to claim 1, wherein administration of the first and second anti-cancer agents provides a synergistic effect compared to administration of either anti-cancer agent alone.

16. A method of treating or ameliorating the effects of a cancer in a subject in need thereof comprising administering to the subject an effective amount of (i) a first anti-cancer agent, which is BVD-523 or a pharmaceutically acceptable salt thereof and (ii) a second anti-cancer agent, which is selected from the group consisting of rapamycin, dactolisib, and pharmaceutically acceptable salts thereof, to treat or ameliorate the effects of the cancer.

17. The method according to clam 16, wherein the subject is a mammal.

18. The method according to claim 17, wherein the mammal is selected from the group consisting of humans, primates, farm animals, and domestic animals.

19. The method according to claim 17, wherein the mammal is a human.

20. The method according to claim 16, wherein the BVD-523 or a pharmaceutically acceptable salt thereof is administered in the form of a pharmaceutical composition further comprising a pharmaceutically acceptable carrier or diluent.

21. The method according to claim 16, wherein the rapamycin, dactolisib or a pharmaceutically acceptable salt thereof is administered in the form of a pharmaceutical composition further comprising a pharmaceutically acceptable carrier or diluent.

22. The method according to claim 16, wherein the cancer is selected from the group consisting of hematologic cancer and solid tumor cancers.

23. The method according to claim 16, wherein the cancer is selected from the group consisting of autonomic ganglia cancer, biliary tract cancer, breast cancer, endometrial cancer, gastrointestinal tract cancer, haematopoietic and lymphoid cancer, kidney cancer, liver cancer, lung cancer, oesophagial cancer, ovarian cancer, pancreatic cancer, prostate cancer, skin cancer, soft tissue cancer, stomach cancer, thyroid cancer, upper aerodigestive tract cancer, and urinary tract cancer.

24. The method according to claim 16, wherein the cancer is selected from the group consisting of oesophagus cancer, skin cancer, biliary tract cancer, large intestine cancer, endometrial cancer, lung cancer, urinary tract cancer, liver cancer, and kidney cancer.

25. The method according to claim 16, wherein the cancer is selected from the group consisting of brain cancer, colon cancer, leukemia, non-Hodgkin's lymphoma, and multiple myeloma.

26. The method according to claim 25, wherein the brain cancer is glioblastoma multiforme (GBM).

27. The method according to claim 22, wherein the hematologic cancer is selected from the group consisting of Adult Acute Megakaryoblastic Leukemia (M7), Adult Acute Minimally Differentiated Myeloid Leukemia (M0), Adult Acute Monoblastic Leukemia (M5a), Adult Acute Monocytic Leukemia (M5b), Adult Acute Myeloblastic Leukemia With Maturation (M2), Adult Acute

Myeloblastic Leukemia Without Maturation (M1), Adult Acute Myeloid Leukemia With 11q23 (MLL) Abnormalities, Adult Acute Myeloid Leukemia With Del(5q), Adult Acute Myeloid Leukemia With Inv(16)(p13;q22), Adult Acute Myeloid Leukemia With t(16;16)(p13;q22), Adult Acute Myeloid Leukemia With t(8;21)(q22;q22), Adult Acute Myelomonocytic Leukemia (M4), Adult Erythroleukemia (M6a), Adult Pure Erythroid Leukemia (M6b), Recurrent Adult Acute Myeloid Leukemia, and Untreated Adult Acute Myeloid Leukemia.

28. The method according to claim 16 further comprising administering to the subject at least one additional therapeutic agent selected from the group consisting of an antibody or fragment thereof, a cytotoxic agent, a toxin, a radionuclide, an immunomodulator, a photoactive therapeutic agent, a radiosensitizing agent, a hormone, an anti-angiogenesis agent, and combinations thereof.

29. The method according to claim 28, wherein the additional therapeutic agent is an inhibitor of the PI3K/Akt pathway.

30. The method according to claim 29, wherein the inhibitor of the PI3K/Akt pathway is selected from the group consisting of A-674563 (CAS # 552325-73-2), AGL 2263, AMG-319 (Amgen, Thousand Oaks, CA), AS-041164 (5-benzo[1,3]dioxol-5-ylmethylene-thiazolidine-2,4-dione), AS-604850 (5-(2,2-Difluoro-benzo[1,3]dioxol-5-ylmethylene)-thiazolidine-2,4-dione), AS-605240 (5-quinoxilin-6-methylene-1,3-thiazolidine-2,4-dione), AT7867 (CAS # 857531-00-1), benzimidazole series, Genentech (Roche Holdings Inc., South San Francisco, CA), BML-257 (CAS # 32387-96-5), CAL-120 (Gilead

Sciences, Foster City, CA), CAL-129 (Gilead Sciences), CAL-130 (Gilead Sciences), CAL-253 (Gilead Sciences), CAL-263 (Gilead Sciences), CAS # 612847-09-3, CAS # 681281-88-9, CAS # 75747-14-7, CAS # 925681-41-0, CAS # 98510-80-6, CCT128930 (CAS # 885499-61-6), CH5132799 (CAS # 1007207-67-1), CHR-4432 (Chroma Therapeutics, Ltd., Abingdon, UK), FPA 124 (CAS # 902779-59-3), GS-1101 (CAL-101) (Gilead Sciences), GSK 690693 (CAS # 937174-76-0), H-89 (CAS # 127243-85-0), Honokiol, IC87114 (Gilead Science), IPI-145 (Intellikine Inc.), KAR-4139 (Karus Therapeutics, Chilworth, UK), KAR-4141 (Karus Therapeutics), KIN-1 (Karus Therapeutics), KT 5720 (CAS # 108068-98-0), Miltefosine, MK-2206 dihydrochloride (CAS # 1032350-13-2), ML-9 (CAS # 105637-50-1), Naltrindole Hydrochloride, OXY-111A (NormOxys Inc., Brighton, MA), perifosine, PHT-427 (CAS # 1191951-57-1), PI3 kinase delta inhibitor, Merck KGaA (Merck & Co., Whitehouse Station, NJ), PI3 kinase delta inhibitors, Genentech (Roche Holdings Inc.), PI3 kinase delta inhibitors, Incozen (Incozen Therapeutics, Pvt. Ltd., Hydrabad, India), PI3 kinase delta inhibitors-2, Incozen (Incozen Therapeutics), PI3 kinase inhibitor, Roche-4 (Roche Holdings Inc.), PI3 kinase inhibitors, Roche (Roche Holdings Inc.), PI3 kinase inhibitors, Roche-5 (Roche Holdings Inc.), PI3-alpha/delta inhibitors, Pathway Therapeutics (Pathway Therapeutics Ltd., South San Francisco, CA), PI3-delta inhibitors, Cellzome (Cellzome AG, Heidelberg, Germany), PI3-delta inhibitors, Intellikine (Intellikine Inc., La Jolla, CA), PI3-delta inhibitors, Pathway Therapeutics-1 (Pathway Therapeutics Ltd.), PI3-delta inhibitors, Pathway Therapeutics-2 (Pathway Therapeutics Ltd.), PI3-delta/gamma inhibitors, Cellzome (Cellzome AG), PI3-delta/gamma inhibitors, Cellzome (Cellzome AG), PI3-delta/gamma inhibitors, Intellikine

(Intellikine Inc.), PI3-delta/gamma inhibitors, Intellikine (Intellikine Inc.), PI3-delta/gamma inhibitors, Pathway Therapeutics (Pathway Therapeutics Ltd.), PI3-delta/gamma inhibitors, Pathway Therapeutics (Pathway Therapeutics Ltd.), PI3-gamma inhibitor Evotec (Evotec), PI3-gamma inhibitor, Cellzome (Cellzome AG), PI3-gamma inhibitors, Pathway Therapeutics (Pathway Therapeutics Ltd.), PI3K delta/gamma inhibitors, Intellikine-1 (Intellikine Inc.), PI3K delta/gamma inhibitors, Intellikine-1 (Intellikine Inc.), pictilisib (Roche Holdings Inc.), PIK-90 (CAS # 677338-12-4), SC-103980 (Pfizer, New York, NY), SF-1126 (Semafore Pharmaceuticals, Indianapolis, IN), SH-5, SH-6, Tetrahydro Curcumin, TG100-115 (Targegen Inc., San Diego, CA), Triciribine, X-339 (Xcovery, West Palm Beach, FL), XL-499 (Evotech, Hamburg, Germany), pharmaceutically acceptable salts thereof, and combinations thereof.

31. The method according to claim 16, wherein administration of the first and second anti-cancer agents provides a synergistic effect compared to administration of either anti-cancer agent alone.

32. A method of effecting cancer cell death comprising contacting a cancer cell with an effective amount of (i) a first anti-cancer agent, which is BVD-523 or a pharmaceutically acceptable salt thereof and (ii) a second anti-cancer agent, which is a mTOR inhibitor or a pharmaceutically acceptable salt thereof.

33. The method according to clam 32, wherein the cancer cell is a mammalian cancer cell.

34. The method according to claim 33, wherein the mammalian cancer cell is obtained from a mammal selected from the group consisting of humans, primates, farm animals, and domestic animals.

35. The method according to claim 33, wherein the mammalian cancer cell is a human cancer cell.

36. The method according to claim 32, wherein the mTOR inhibitor is selected from the group consisting of zotarolimus (AbbVie), umirolimus (Biosensors), temsirolimus (Pfizer), sirolimus (Pfizer), sirolimus NanoCrystal (Elan Pharmaceutical Technologies), sirolimus TransDerm (TransDerm), sirolimus-PNP (Samyang), everolimus (Novartis), biolimus A9 (Biosensors), ridaforolimus (Ariad), rapamycin, TCD-10023 (Terumo), DE-109 (MacuSight), MS-R001 (MacuSight), MS-R002 (MacuSight), MS-R003 (MacuSight), Perceiva (MacuSight), XL-765 (Exelixis), quinacrine (Cleveland BioLabs), PKI-587 (Pfizer), PF-04691502 (Pfizer), GDC-0980 (Genentech and Piramed), dactolisib (Novartis), CC-223 (Celgene), PWT-33597 (Pathway Therapeutics), P-7170 (Piramal Life Sciences), LY-3023414 (Eli Lilly), INK-128 (Takeda), GDC-0084 (Genentech), DS-7423 (Daiichi Sankyo), DS-3078 (Daiichi Sankyo), CC-115 (Celgene), CBLC-137 (Cleveland BioLabs), AZD-2014 (AstraZeneca), X-480 (Xcovery), X-414 (Xcovery), EC-0371 (Endocyte), VS-5584 (Verastem), PQR-401 (Piqur), PQR-316 (Piqur), PQR-311 (Piqur), PQR-309 (Piqur), PF-06465603 (Pfizer), NV-128 (Novogen), nPT-MTOR (Biotica Technology), BC-210 (Biotica Technology), WAY-600 (Biotica Technology), WYE-354 (Biotica Technology), WYE-687 (Biotica Technology), LOR-220 (Lorus Therapeutics), HMPL-518 (Hutchison China MediTech),

GNE-317 (Genentech), EC-0565 (Endocyte), CC-214 (Celgene), ABTL-0812 (Ability Pharmaceuticals), and pharmaceutically acceptable salts thereof, and combinations thereof.

37. The method according to claim 32, wherein the cancer is selected from the group consisting of hematologic cancer and solid tumor cancers.

38. The method according to claim 32, wherein the cancer is selected from the group consisting of autonomic ganglia cancer, biliary tract cancer, breast cancer, endometrial cancer, gastrointestinal tract cancer, haematopoietic and lymphoid cancer, kidney cancer, liver cancer, lung cancer, oesophagial cancer, ovarian cancer, pancreatic cancer, prostate cancer, skin cancer, soft tissue cancer, stomach cancer, thyroid cancer, upper aerodigestive tract cancer, and urinary tract cancer.

39. The method according to claim 32, wherein the cancer is selected from the group consisting of oesophagus cancer, skin cancer, biliary tract cancer, large intestine cancer, endometrial cancer, lung cancer, urinary tract cancer, liver cancer, and kidney cancer.

40. The method according to claim 32, wherein the cancer is selected from the group consisting of brain cancer, colon cancer, leukemia, non-Hodgkin's lymphoma, and multiple myeloma.

41. The method according to claim 40, wherein the brain cancer is glioblastoma multiforme (GBM).

42. The method according to claim 37, wherein the hematologic cancer is selected from the group consisting of Adult Acute Megakaryoblastic Leukemia

(M7), Adult Acute Minimally Differentiated Myeloid Leukemia (M0), Adult Acute Monoblastic Leukemia (M5a), Adult Acute Monocytic Leukemia (M5b), Adult Acute Myeloblastic Leukemia With Maturation (M2), Adult Acute Myeloblastic Leukemia Without Maturation (M1), Adult Acute Myeloid Leukemia With 11q23 (MLL) Abnormalities, Adult Acute Myeloid Leukemia With Del(5q), Adult Acute Myeloid Leukemia With Inv(16)(p13;q22), Adult Acute Myeloid Leukemia With t(16;16)(p13;q22), Adult Acute Myeloid Leukemia With t(8;21)(q22;q22), Adult Acute Myelomonocytic Leukemia (M4), Adult Erythroleukemia (M6a), Adult Pure Erythroid Leukemia (M6b), Recurrent Adult Acute Myeloid Leukemia, and Untreated Adult Acute Myeloid Leukemia.

43. The method according to claim 32 further comprising contacting the cancer cell with at least one additional therapeutic agent selected from the group consisting of an antibody or fragment thereof, a cytotoxic agent, a toxin, a radionuclide, an immunomodulator, a photoactive therapeutic agent, a radiosensitizing agent, a hormone, an anti-angiogenesis agent, and combinations thereof.

44. The method according to claim 43, wherein the additional therapeutic agent is an inhibitor of the PI3K/Akt pathway.

45. The method according to claim 44, wherein the inhibitor of the PI3K/Akt pathway is selected from the group consisting of A-674563 (CAS # 552325-73-2), AGL 2263, AMG-319 (Amgen, Thousand Oaks, CA), AS-041164 (5-benzo[1,3]dioxol-5-ylmethylene-thiazolidine-2,4-dione), AS-604850 (5-(2,2-Difluoro-benzo[1,3]dioxol-5-ylmethylene)-thiazolidine-2,4-dione), AS-605240

(5-quinoxilin-6-methylene-1,3-thiazolidine-2,4-dione), AT7867 (CAS # 857531-00-1), benzimidazole series, Genentech (Roche Holdings Inc., South San Francisco, CA), BML-257 (CAS # 32387-96-5), CAL-120 (Gilead Sciences, Foster City, CA), CAL-129 (Gilead Sciences), CAL-130 (Gilead Sciences), CAL-253 (Gilead Sciences), CAL-263 (Gilead Sciences), CAS # 612847-09-3, CAS # 681281-88-9, CAS # 75747-14-7, CAS # 925681-41-0, CAS # 98510-80-6, CCT128930 (CAS # 885499-61-6), CH5132799 (CAS # 1007207-67-1), CHR-4432 (Chroma Therapeutics, Ltd., Abingdon, UK), FPA 124 (CAS # 902779-59-3), GS-1101 (CAL-101) (Gilead Sciences), GSK 690693 (CAS # 937174-76-0), H-89 (CAS # 127243-85-0), Honokiol, IC87114 (Gilead Science), IPI-145 (Intellikine Inc.), KAR-4139 (Karus Therapeutics, Chilworth, UK), KAR-4141 (Karus Therapeutics), KIN-1 (Karus Therapeutics), KT 5720 (CAS # 108068-98-0), Miltefosine, MK-2206 dihydrochloride (CAS # 1032350-13-2), ML-9 (CAS # 105637-50-1), Naltrindole Hydrochloride, OXY-111A (NormOxys Inc., Brighton, MA), perifosine, PHT-427 (CAS # 1191951-57-1), PI3 kinase delta inhibitor, Merck KGaA (Merck & Co., Whitehouse Station, NJ), PI3 kinase delta inhibitors, Genentech (Roche Holdings Inc.), PI3 kinase delta inhibitors, Incozen (Incozen Therapeutics, Pvt. Ltd., Hydrabad, India), PI3 kinase delta inhibitors-2, Incozen (Incozen Therapeutics), PI3 kinase inhibitor, Roche-4 (Roche Holdings Inc.), PI3 kinase inhibitors, Roche (Roche Holdings Inc.), PI3 kinase inhibitors, Roche-5 (Roche Holdings Inc.), PI3-alpha/delta inhibitors, Pathway Therapeutics (Pathway Therapeutics Ltd., South San Francisco, CA), PI3-delta inhibitors, Cellzome (Cellzome AG, Heidelberg, Germany), PI3-delta inhibitors, Intellikine (Intellikine Inc., La Jolla, CA), PI3-delta inhibitors, Pathway Therapeutics-1 (Pathway Therapeutics

Ltd.), PI3-delta inhibitors, Pathway Therapeutics-2 (Pathway Therapeutics Ltd.), PI3-delta/gamma inhibitors, Cellzome (Cellzome AG), PI3-delta/gamma inhibitors, Cellzome (Cellzome AG), PI3-delta/gamma inhibitors, Intellikine (Intellikine Inc.), PI3-delta/gamma inhibitors, Intellikine (Intellikine Inc.), PI3-delta/gamma inhibitors, Pathway Therapeutics (Pathway Therapeutics Ltd.), PI3-delta/gamma inhibitors, Pathway Therapeutics (Pathway Therapeutics Ltd.), PI3-gamma inhibitor Evotec (Evotec), PI3-gamma inhibitor, Cellzome (Cellzome AG), PI3-gamma inhibitors, Pathway Therapeutics (Pathway Therapeutics Ltd.), PI3K delta/gamma inhibitors, Intellikine-1 (Intellikine Inc.), PI3K delta/gamma inhibitors, Intellikine-1 (Intellikine Inc.), pictilisib (Roche Holdings Inc.), PIK-90 (CAS # 677338-12-4), SC-103980 (Pfizer, New York, NY), SF-1126 (Semafore Pharmaceuticals, Indianapolis, IN), SH-5, SH-6, Tetrahydro Curcumin, TG100-115 (Targegen Inc., San Diego, CA), Triciribine, X-339 (Xcovery, West Palm Beach, FL), XL-499 (Evotech, Hamburg, Germany), pharmaceutically acceptable salts thereof, and combinations thereof.

46. The method according to claim 32, wherein contacting the cancer cell with the first and second anti-cancer agents provides a synergistic effect compared to contacting the cancer cell with either anti-cancer agent alone.

47. The method according to claim 32, wherein the method is carried out in vitro.

48. The method according to claim 32, wherein the method is carried out in vivo.

49. A kit for treating or ameliorating the effects of a cancer in a subject in need thereof comprising an effective amount of (i) a first anti-cancer agent, which is BVD-523 or a pharmaceutically acceptable salt thereof and (ii) a second anti-cancer agent, which is a mTOR inhibitor or a pharmaceutically acceptable salt thereof, packaged together with instructions for their use.

50. The kit according to clam 49, wherein the subject is a mammal.

51. The kit according to claim 50, wherein the mammal is selected from the group consisting of humans, primates, farm animals, and domestic animals.

52. The kit according to claim 50, wherein the mammal is a human.

53. The kit according to claim 49, wherein the mTOR inhibitor is selected from the group consisting of zotarolimus (AbbVie), umirolimus (Biosensors), temsirolimus (Pfizer), sirolimus (Pfizer), sirolimus NanoCrystal (Elan Pharmaceutical Technologies), sirolimus TransDerm (TransDerm), sirolimus-PNP (Samyang), everolimus (Novartis), biolimus A9 (Biosensors), ridaforolimus (Ariad), rapamycin, TCD-10023 (Terumo), DE-109 (MacuSight), MS-R001 (MacuSight), MS-R002 (MacuSight), MS-R003 (MacuSight), Perceiva (MacuSight), XL-765 (Exelixis), quinacrine (Cleveland BioLabs), PKI-587 (Pfizer), PF-04691502 (Pfizer), GDC-0980 (Genentech and Piramed), dactolisib (Novartis), CC-223 (Celgene), PWT-33597 (Pathway Therapeutics), P-7170 (Piramal Life Sciences), LY-3023414 (Eli Lilly), INK-128 (Takeda), GDC-0084 (Genentech), DS-7423 (Daiichi Sankyo), DS-3078 (Daiichi Sankyo), CC-115 (Celgene), CBLC-137 (Cleveland BioLabs), AZD-2014 (AstraZeneca), X-480 (Xcovery), X-414 (Xcovery), EC-0371 (Endocyte), VS-5584 (Verastem), PQR-401 (Piqur), PQR-316 (Piqur), PQR-311 (Piqur),

PQR-309 (Piqur), PF-06465603 (Pfizer), NV-128 (Novogen), nPT-MTOR (Biotica Technology), BC-210 (Biotica Technology), WAY-600 (Biotica Technology), WYE-354 (Biotica Technology), WYE-687 (Biotica Technology), LOR-220 (Lorus Therapeutics), HMPL-518 (Hutchison China MediTech), GNE-317 (Genentech), EC-0565 (Endocyte), CC-214 (Celgene), ABTL-0812 (Ability Pharmaceuticals), and pharmaceutically acceptable salts thereof.

54. The kit according to claim 53, wherein the mTOR inhibitor is selected from the group consisting of rapamycin, dactolisib, and pharmaceutically acceptable salts thereof.

55. The kit according to claim 49, wherein the cancer is selected from the group consisting of hematologic cancer and solid tumor cancers.

56. The kit according to claim 49, wherein the cancer is selected from the group consisting of autonomic ganglia cancer, biliary tract cancer, breast cancer, endometrial cancer, gastrointestinal tract cancer, haematopoietic and lymphoid cancer, kidney cancer, liver cancer, lung cancer, oesophagial cancer, ovarian cancer, pancreatic cancer, prostate cancer, skin cancer, soft tissue cancer, stomach cancer, thyroid cancer, upper aerodigestive tract cancer, and urinary tract cancer.

57. The kit according to claim 49, wherein the cancer is selected from the group consisting of oesophagus cancer, skin cancer, biliary tract cancer, large intestine cancer, endometrial cancer, lung cancer, urinary tract cancer, liver cancer, and kidney cancer.

58. The kit according to claim 49, wherein the cancer is selected from the group consisting of brain cancer, colon cancer, leukemia, non-Hodgkin's lymphoma, and multiple myeloma.

59. The kit according to claim 58, wherein the brain cancer is glioblastoma multiforme (GBM).

60. The kit according to claim 55, wherein the hematologic cancer is selected from the group consisting of Adult Acute Megakaryoblastic Leukemia (M7), Adult Acute Minimally Differentiated Myeloid Leukemia (M0), Adult Acute Monoblastic Leukemia (M5a), Adult Acute Monocytic Leukemia (M5b), Adult Acute Myeloblastic Leukemia With Maturation (M2), Adult Acute Myeloblastic Leukemia Without Maturation (M1), Adult Acute Myeloid Leukemia With 11q23 (MLL) Abnormalities, Adult Acute Myeloid Leukemia With Del(5q), Adult Acute Myeloid Leukemia With Inv(16)(p13;q22), Adult Acute Myeloid Leukemia With t(16;16)(p13;q22), Adult Acute Myeloid Leukemia With t(8;21)(q22;q22), Adult Acute Myelomonocytic Leukemia (M4), Adult Erythroleukemia (M6a), Adult Pure Erythroid Leukemia (M6b), Recurrent Adult Acute Myeloid Leukemia, and Untreated Adult Acute Myeloid Leukemia.

61. The kit according to claim 49 further comprising at least one additional therapeutic agent selected from the group consisting of an antibody or fragment thereof, a cytotoxic agent, a toxin, a radionuclide, an immunomodulator, a photoactive therapeutic agent, a radiosensitizing agent, a hormone, an anti-angiogenesis agent, and combinations thereof.

62. The kit according to claim 61, wherein the additional therapeutic agent is an inhibitor of the PI3K/Akt pathway.

63. The kit according to claim 62, wherein the inhibitor of the PI3K/Akt pathway is selected from the group consisting of A-674563 (CAS # 552325-73-2), AGL 2263, AMG-319 (Amgen, Thousand Oaks, CA), AS-041164 (5-benzo[1,3]dioxol-5-ylmethylene-thiazolidine-2,4-dione), AS-604850 (5-(2,2-Difluoro-benzo[1,3]dioxol-5-ylmethylene)-thiazolidine-2,4-dione), AS-605240 (5-quinoxilin-6-methylene-1,3-thiazolidine-2,4-dione), AT7867 (CAS # 857531-00-1), benzimidazole series, Genentech (Roche Holdings Inc., South San Francisco, CA), BML-257 (CAS # 32387-96-5), CAL-120 (Gilead Sciences, Foster City, CA), CAL-129 (Gilead Sciences), CAL-130 (Gilead Sciences), CAL-253 (Gilead Sciences), CAL-263 (Gilead Sciences), CAS # 612847-09-3, CAS # 681281-88-9, CAS # 75747-14-7, CAS # 925681-41-0, CAS # 98510-80-6, CCT128930 (CAS # 885499-61-6), CH5132799 (CAS # 1007207-67-1), CHR-4432 (Chroma Therapeutics, Ltd., Abingdon, UK), FPA 124 (CAS # 902779-59-3), GS-1101 (CAL-101) (Gilead Sciences), GSK 690693 (CAS # 937174-76-0), H-89 (CAS # 127243-85-0), Honokiol, IC87114 (Gilead Science), IPI-145 (Intellikine Inc.), KAR-4139 (Karus Therapeutics, Chilworth, UK), KAR-4141 (Karus Therapeutics), KIN-1 (Karus Therapeutics), KT 5720 (CAS # 108068-98-0), Miltefosine, MK-2206 dihydrochloride (CAS # 1032350-13-2), ML-9 (CAS # 105637-50-1), Naltrindole Hydrochloride, OXY-111A (NormOxys Inc., Brighton, MA), perifosine, PHT-427 (CAS # 1191951-57-1), PI3 kinase delta inhibitor, Merck KGaA (Merck & Co., Whitehouse Station, NJ), PI3 kinase delta inhibitors, Genentech (Roche Holdings Inc.), PI3 kinase delta inhibitors, Incozen (Incozen Therapeutics, Pvt. Ltd., Hydrabad,

India), PI3 kinase delta inhibitors-2, Incozen (Incozen Therapeutics), PI3 kinase inhibitor, Roche-4 (Roche Holdings Inc.), PI3 kinase inhibitors, Roche (Roche Holdings Inc.), PI3 kinase inhibitors, Roche-5 (Roche Holdings Inc.), PI3-alpha/delta inhibitors, Pathway Therapeutics (Pathway Therapeutics Ltd., South San Francisco, CA), PI3-delta inhibitors, Cellzome (Cellzome AG, Heidelberg, Germany), PI3-delta inhibitors, Intellikine (Intellikine Inc., La Jolla, CA), PI3-delta inhibitors, Pathway Therapeutics-1 (Pathway Therapeutics Ltd.), PI3-delta inhibitors, Pathway Therapeutics-2 (Pathway Therapeutics Ltd.), PI3-delta/gamma inhibitors, Cellzome (Cellzome AG), PI3-delta/gamma inhibitors, Cellzome (Cellzome AG), PI3-delta/gamma inhibitors, Intellikine (Intellikine Inc.), PI3-delta/gamma inhibitors, Intellikine (Intellikine Inc.), PI3-delta/gamma inhibitors, Pathway Therapeutics (Pathway Therapeutics Ltd.), PI3-delta/gamma inhibitors, Pathway Therapeutics (Pathway Therapeutics Ltd.), PI3-gamma inhibitor Evotec (Evotec), PI3-gamma inhibitor, Cellzome (Cellzome AG), PI3-gamma inhibitors, Pathway Therapeutics (Pathway Therapeutics Ltd.), PI3K delta/gamma inhibitors, Intellikine-1 (Intellikine Inc.), PI3K delta/gamma inhibitors, Intellikine-1 (Intellikine Inc.), pictilisib (Roche Holdings Inc.), PIK-90 (CAS # 677338-12-4), SC-103980 (Pfizer, New York, NY), SF-1126 (Semafore Pharmaceuticals, Indianapolis, IN), SH-5, SH-6, Tetrahydro Curcumin, TG100-115 (Targegen Inc., San Diego, CA), Triciribine, X-339 (Xcovery, West Palm Beach, FL), XL-499 (Evotech, Hamburg, Germany), pharmaceutically acceptable salts thereof, and combinations thereof.

64. The kit according to claim 49, wherein administration of the first and second anti-cancer agents provides a synergistic effect compared to administration of either anti-cancer agent alone.

65. A pharmaceutical composition for treating or ameliorating the effects of a cancer in a subject in need thereof, the pharmaceutical composition comprising a pharmaceutically acceptable diluent or carrier and an effective amount of (i) a first anti-cancer agent, which is BVD-523 or a pharmaceutically acceptable salt thereof and (ii) a second anti-cancer agent, which is a mTOR inhibitor or a pharmaceutically acceptable salt thereof, wherein administration of the first and second anti-cancer agents provides a synergistic effect compared to administration of either anti-cancer agent alone.

66. The pharmaceutical composition according to clam 65, wherein the subject is a mammal.

67. The pharmaceutical composition according to claim 66, wherein the mammal is selected from the group consisting of humans, primates, farm animals, and domestic animals.

68. The pharmaceutical composition according to claim 66, wherein the mammal is a human.

69. The pharmaceutical composition according to claim 65, wherein the mTOR inhibitor is selected from the group consisting of zotarolimus (AbbVie), umirolimus (Biosensors), temsirolimus (Pfizer), sirolimus (Pfizer), sirolimus NanoCrystal (Elan Pharmaceutical Technologies), sirolimus TransDerm (TransDerm), sirolimus-PNP (Samyang), everolimus (Novartis), biolimus A9

(Biosensors), ridaforolimus (Ariad), rapamycin, TCD-10023 (Terumo), DE-109 (MacuSight), MS-R001 (MacuSight), MS-R002 (MacuSight), MS-R003 (MacuSight), Perceiva (MacuSight), XL-765 (Exelixis), quinacrine (Cleveland BioLabs), PKI-587 (Pfizer), PF-04691502 (Pfizer), GDC-0980 (Genentech and Piramed), dactolisib (Novartis), CC-223 (Celgene), PWT-33597 (Pathway Therapeutics), P-7170 (Piramal Life Sciences), LY-3023414 (Eli Lilly), INK-128 (Takeda), GDC-0084 (Genentech), DS-7423 (Daiichi Sankyo), DS-3078 (Daiichi Sankyo), CC-115 (Celgene), CBLC-137 (Cleveland BioLabs), AZD-2014 (AstraZeneca), X-480 (Xcovery), X-414 (Xcovery), EC-0371 (Endocyte), VS-5584 (Verastem), PQR-401 (Piqur), PQR-316 (Piqur), PQR-311 (Piqur), PQR-309 (Piqur), PF-06465603 (Pfizer), NV-128 (Novogen), nPT-MTOR (Biotica Technology), BC-210 (Biotica Technology), WAY-600 (Biotica Technology), WYE-354 (Biotica Technology), WYE-687 (Biotica Technology), LOR-220 (Lorus Therapeutics), HMPL-518 (Hutchison China MediTech), GNE-317 (Genentech), EC-0565 (Endocyte), CC-214 (Celgene), ABTL-0812 (Ability Pharmaceuticals), and pharmaceutically acceptable salts thereof.

70. The pharmaceutical composition according to claim 65, wherein the mTOR inhibitor is selected from the group consisting of rapamycin, dactolisib, and pharmaceutically acceptable salts thereof,

71. The pharmaceutical composition according to claim 65, wherein the cancer is selected from the group consisting of hematologic cancer and solid tumor cancers.

72. The pharmaceutical composition according to claim 65, wherein the cancer is selected from the group consisting of autonomic ganglia cancer,

biliary tract cancer, breast cancer, endometrial cancer, gastrointestinal tract cancer, haematopoietic and lymphoid cancer, kidney cancer, liver cancer, lung cancer, oesophagial cancer, ovarian cancer, pancreatic cancer, prostate cancer, skin cancer, soft tissue cancer, stomach cancer, thyroid cancer, upper aerodigestive tract cancer, and urinary tract cancer.

73. The pharmaceutical composition according to claim 65, wherein the cancer is selected from the group consisting of oesophagus cancer, skin cancer, biliary tract cancer, large intestine cancer, endometrial cancer, lung cancer, urinary tract cancer, liver cancer, and kidney cancer.

74. The pharmaceutical composition according to claim 65, wherein the cancer is selected from the group consisting of brain cancer, colon cancer, leukemia, non-Hodgkin's lymphoma, and multiple myeloma.

75. The pharmaceutical composition according to claim 74, wherein the brain cancer is glioblastoma multiforme (GBM).

76. The pharmaceutical composition according to claim 71, wherein the hematologic cancer is selected from the group consisting of Adult Acute Megakaryoblastic Leukemia (M7), Adult Acute Minimally Differentiated Myeloid Leukemia (M0), Adult Acute Monoblastic Leukemia (M5a), Adult Acute Monocytic Leukemia (M5b), Adult Acute Myeloblastic Leukemia With Maturation (M2), Adult Acute Myeloblastic Leukemia Without Maturation (M1), Adult Acute Myeloid Leukemia With 11q23 (MLL) Abnormalities, Adult Acute Myeloid Leukemia With Del(5q), Adult Acute Myeloid Leukemia With Inv(16)(p13;q22), Adult Acute Myeloid Leukemia With t(16;16)(p13;q22), Adult Acute Myeloid Leukemia With t(8;21)(q22;q22), Adult Acute Myelomonocytic

Leukemia (M4), Adult Erythroleukemia (M6a), Adult Pure Erythroid Leukemia (M6b), Recurrent Adult Acute Myeloid Leukemia, and Untreated Adult Acute Myeloid Leukemia.

77. The pharmaceutical composition according to claim 65 further comprising at least one additional therapeutic agent selected from the group consisting of an antibody or fragment thereof, a cytotoxic agent, a toxin, a radionuclide, an immunomodulator, a photoactive therapeutic agent, a radiosensitizing agent, a hormone, an anti-angiogenesis agent, and combinations thereof.

78. The pharmaceutical composition according to claim 77, wherein the additional therapeutic agent is an inhibitor of the PI3K/Akt pathway.

79. The pharmaceutical composition according to claim 78, wherein the inhibitor of the PI3K/Akt pathway is selected from the group consisting of A-674563 (CAS # 552325-73-2), AGL 2263, AMG-319 (Amgen, Thousand Oaks, CA), AS-041164 (5-benzo[1,3]dioxol-5-ylmethylene-thiazolidine-2,4-dione), AS-604850 (5-(2,2-Difluoro-benzo[1,3]dioxol-5-ylmethylene)-thiazolidine-2,4-dione), AS-605240 (5-quinoxilin-6-methylene-1,3-thiazolidine-2,4-dione), AT7867 (CAS # 857531-00-1), benzimidazole series, Genentech (Roche Holdings Inc., South San Francisco, CA), BML-257 (CAS # 32387-96-5), CAL-120 (Gilead Sciences, Foster City, CA), CAL-129 (Gilead Sciences), CAL-130 (Gilead Sciences), CAL-253 (Gilead Sciences), CAL-263 (Gilead Sciences), CAS # 612847-09-3, CAS # 681281-88-9, CAS # 75747-14-7, CAS # 925681-41-0, CAS # 98510-80-6, CCT128930 (CAS # 885499-61-6), CH5132799 (CAS # 1007207-67-1), CHR-4432 (Chroma Therapeutics, Ltd.,

Abingdon, UK), FPA 124 (CAS # 902779-59-3), GS-1101 (CAL-101) (Gilead Sciences), GSK 690693 (CAS # 937174-76-0), H-89 (CAS # 127243-85-0), Honokiol, IC87114 (Gilead Science), IPI-145 (Intellikine Inc.), KAR-4139 (Karus Therapeutics, Chilworth, UK), KAR-4141 (Karus Therapeutics), KIN-1 (Karus Therapeutics), KT 5720 (CAS # 108068-98-0), Miltefosine, MK-2206 dihydrochloride (CAS # 1032350-13-2), ML-9 (CAS # 105637-50-1), Naltrindole Hydrochloride, OXY-111A (NormOxys Inc., Brighton, MA), perifosine, PHT-427 (CAS # 1191951-57-1), PI3 kinase delta inhibitor, Merck KGaA (Merck & Co., Whitehouse Station, NJ), PI3 kinase delta inhibitors, Genentech (Roche Holdings Inc.), PI3 kinase delta inhibitors, Incozen (Incozen Therapeutics, Pvt. Ltd., Hydrabad, India), PI3 kinase delta inhibitors-2, Incozen (Incozen Therapeutics), PI3 kinase inhibitor, Roche-4 (Roche Holdings Inc.), PI3 kinase inhibitors, Roche (Roche Holdings Inc.), PI3 kinase inhibitors, Roche-5 (Roche Holdings Inc.), PI3-alpha/delta inhibitors, Pathway Therapeutics (Pathway Therapeutics Ltd., South San Francisco, CA), PI3-delta inhibitors, Cellzome (Cellzome AG, Heidelberg, Germany), PI3-delta inhibitors, Intellikine (Intellikine Inc., La Jolla, CA), PI3-delta inhibitors, Pathway Therapeutics-1 (Pathway Therapeutics Ltd.), PI3-delta inhibitors, Pathway Therapeutics-2 (Pathway Therapeutics Ltd.), PI3-delta/gamma inhibitors, Cellzome (Cellzome AG), PI3-delta/gamma inhibitors, Cellzome (Cellzome AG), PI3-delta/gamma inhibitors, Intellikine (Intellikine Inc.), PI3-delta/gamma inhibitors, Intellikine (Intellikine Inc.), PI3-delta/gamma inhibitors, Pathway Therapeutics (Pathway Therapeutics Ltd.), PI3-delta/gamma inhibitors, Pathway Therapeutics (Pathway Therapeutics Ltd.), PI3-gamma inhibitor Evotec (Evotec), PI3-gamma inhibitor, Cellzome (Cellzome AG), PI3-

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80. The pharmaceutical composition according to claim 65, which is in a unit dosage form comprising both anti-cancer agents.

81. The pharmaceutical composition according to claim 65 in which the first anti-cancer agent is in a first unit dosage form and the second anti-cancer agent is in a second unit dosage form, separate from the first.

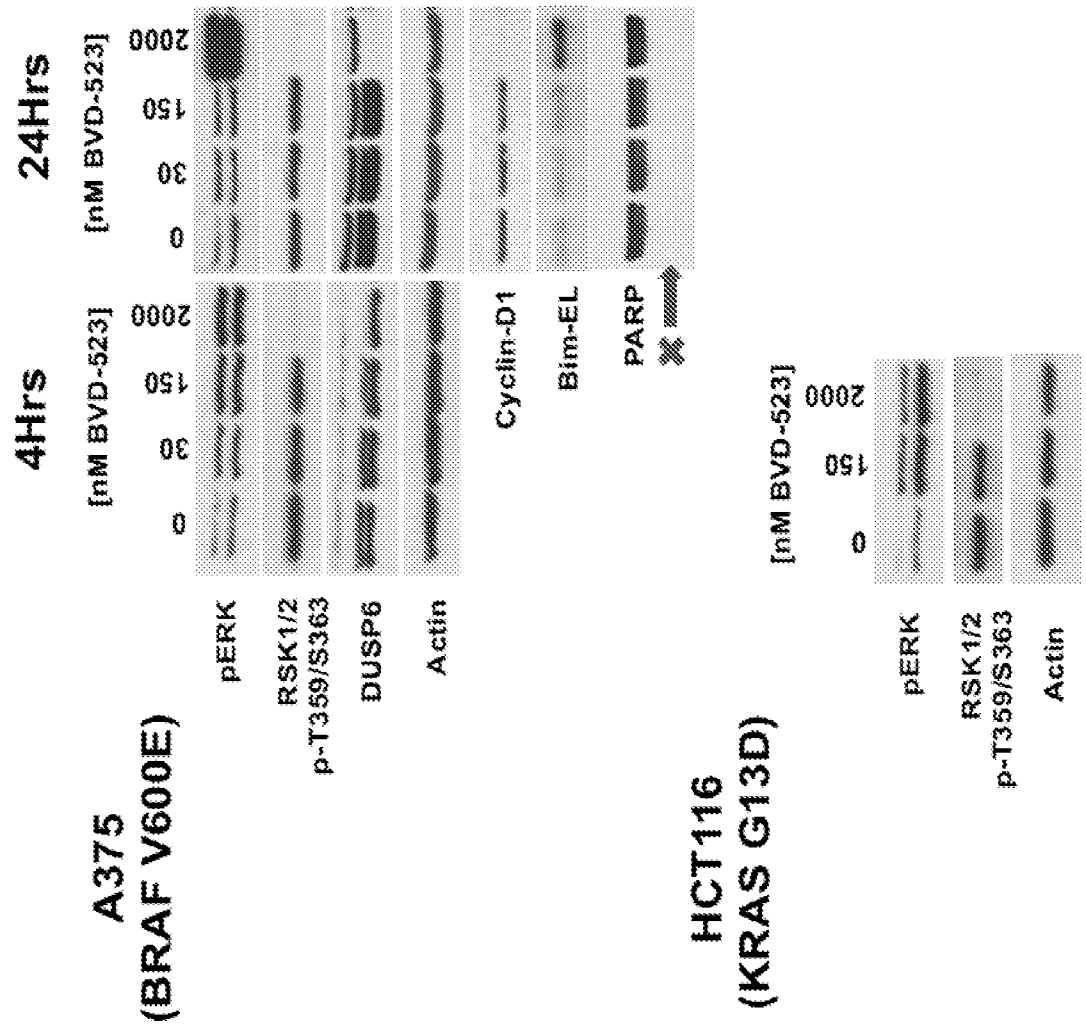
82. The pharmaceutical composition according to claim 65, wherein the first and second anti-cancer agents are co-administered to the subject.

83. The pharmaceutical composition according to claim 65, wherein the first and second anti-cancer agents are administered to the subject serially.

84. The pharmaceutical composition according to claim 83, wherein the first anti-cancer agent is administered to the subject before the second anti-cancer agent.

85. The pharmaceutical composition according to claim 83, wherein the second anti-cancer agent is administered to the subject before the first anti-cancer agent.

FIG. 1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Bim-EL
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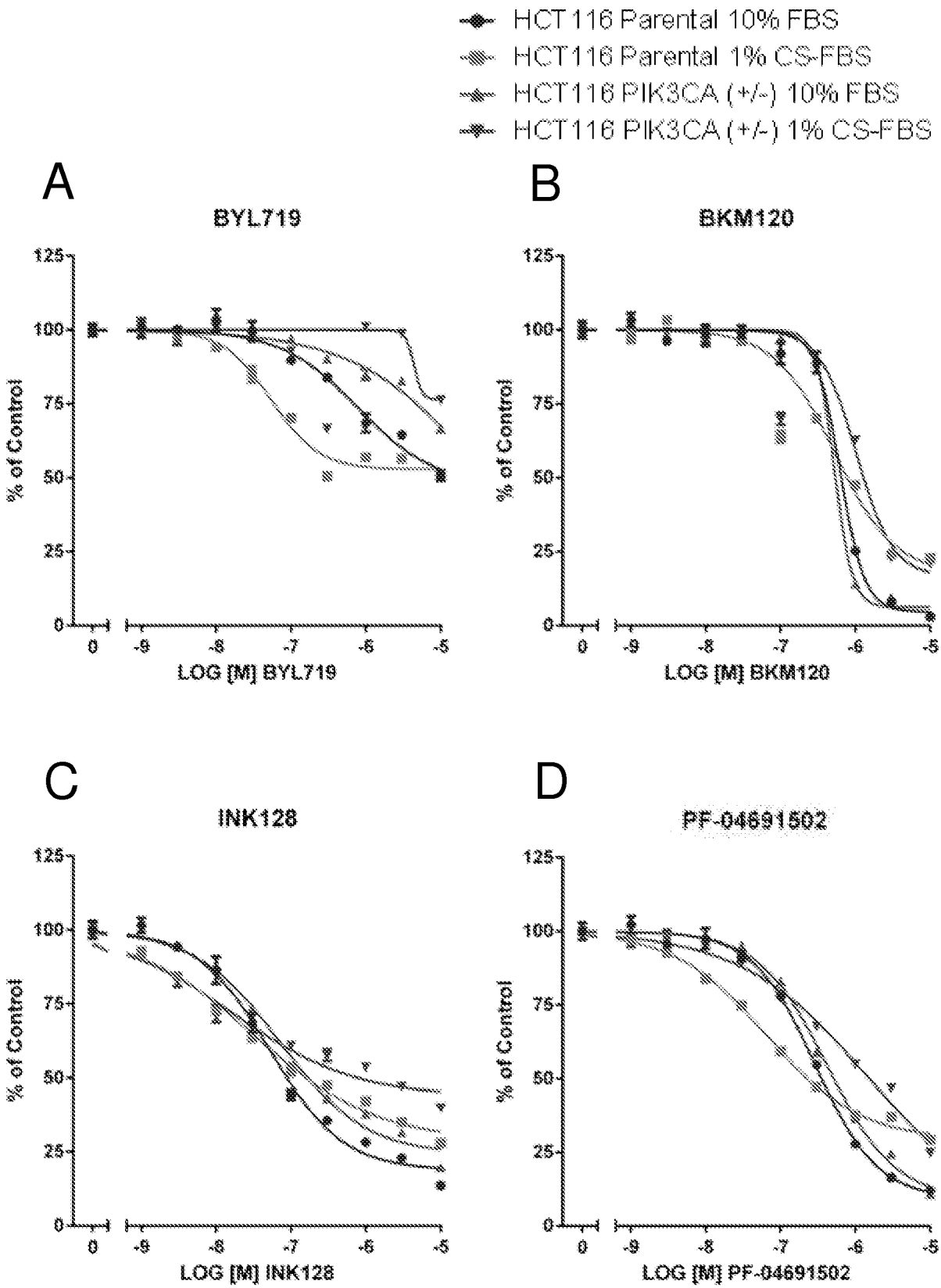
FIG. 2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BYL719
% of Control
LOG [M] BYL719
B
BKM120
% of Control
LOG [M] BKM120
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% of Control
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FIG. 2, Continued

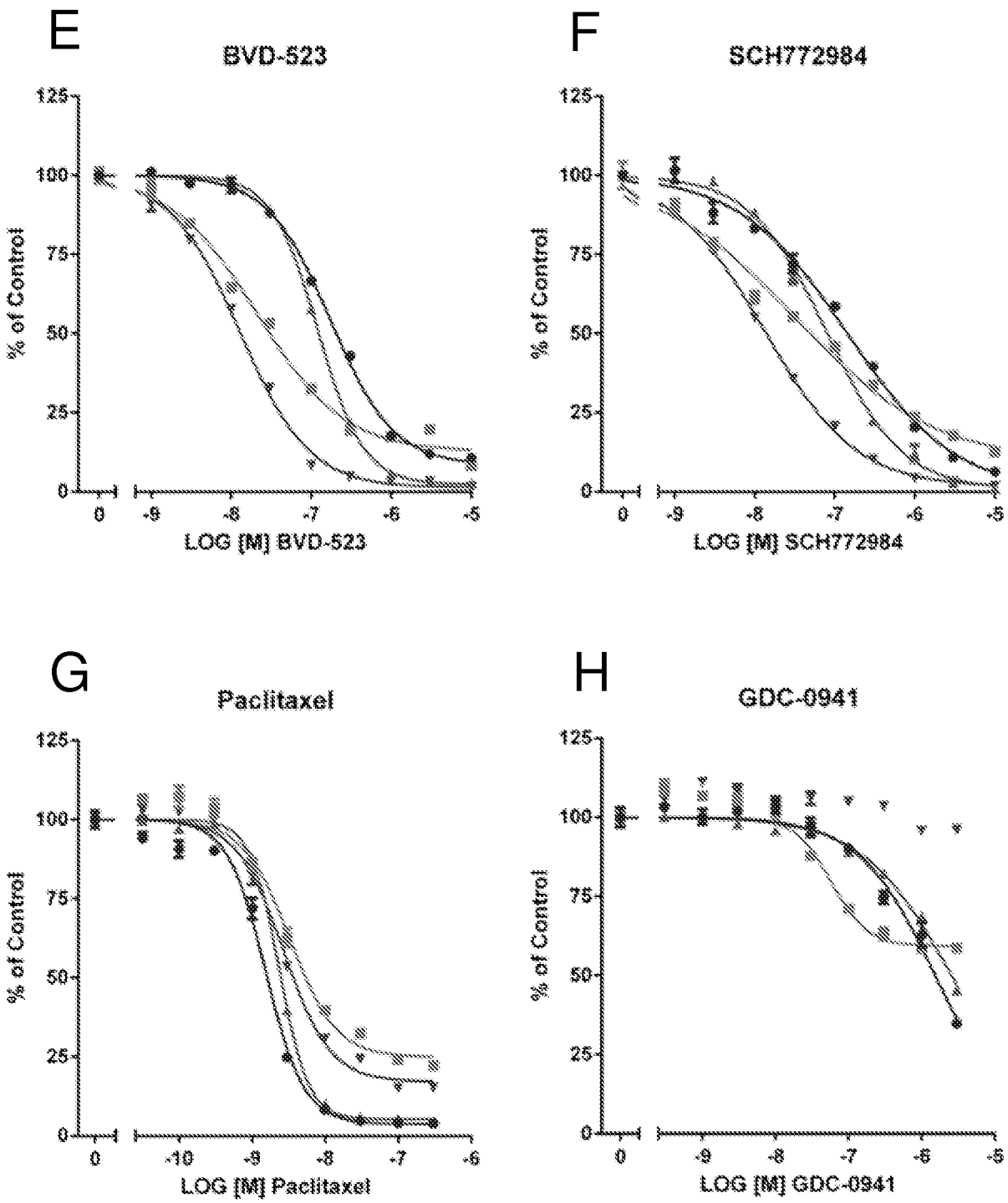

FIG. 3

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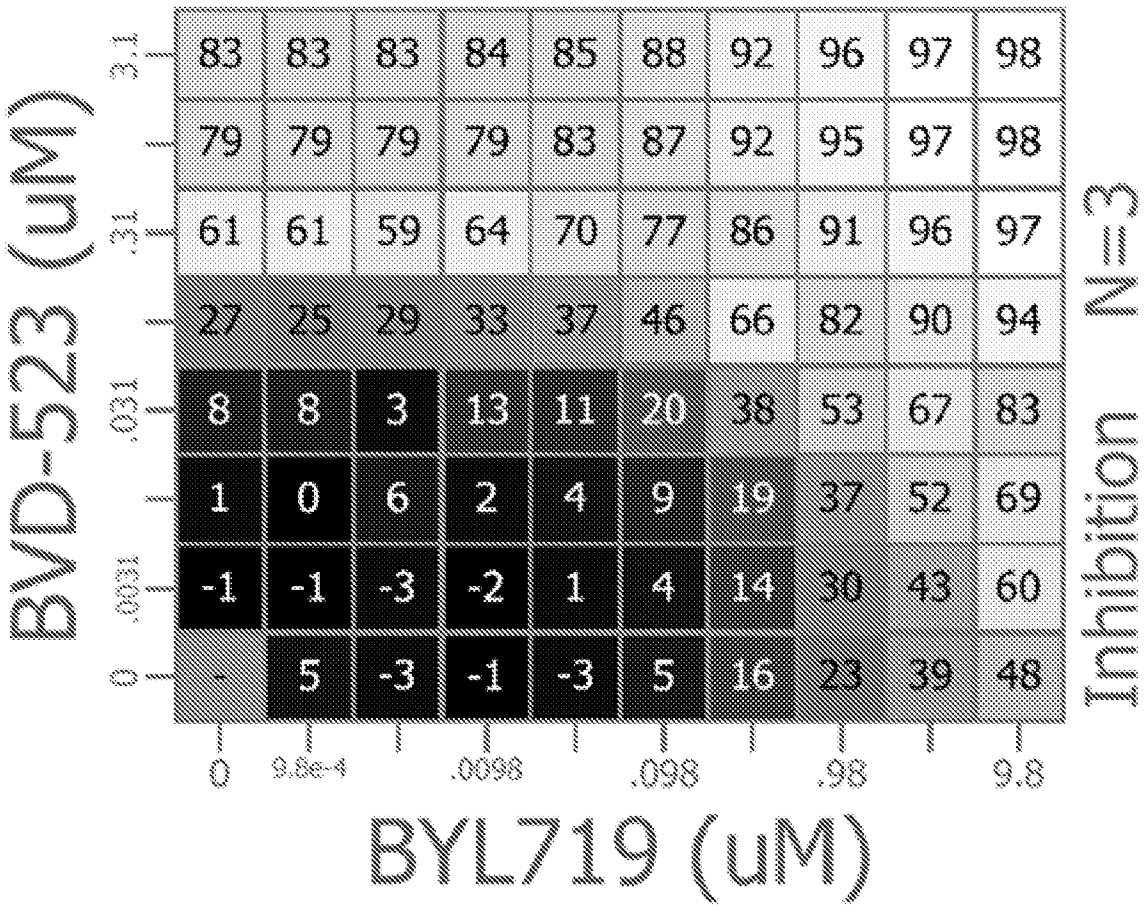
BVD-523 (uM)
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.31
.031
.0031
0
83 83 83 84 85 88 92 96 97 98
79 79 79 79 83 87 92 95 97 98
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27 25 29 33 37 46 66 82 90 94
8 8 3 13 11 20 38 53 67 83
1 0 6 2 4 9 19 37 52 69
-1 -1 -3 -2 1 4 14 30 43 60
- 5 -3 -1 -3 5 16 23 39 48
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BYL719 (uM)
Inhibition N=3

FIG. 3, Continued

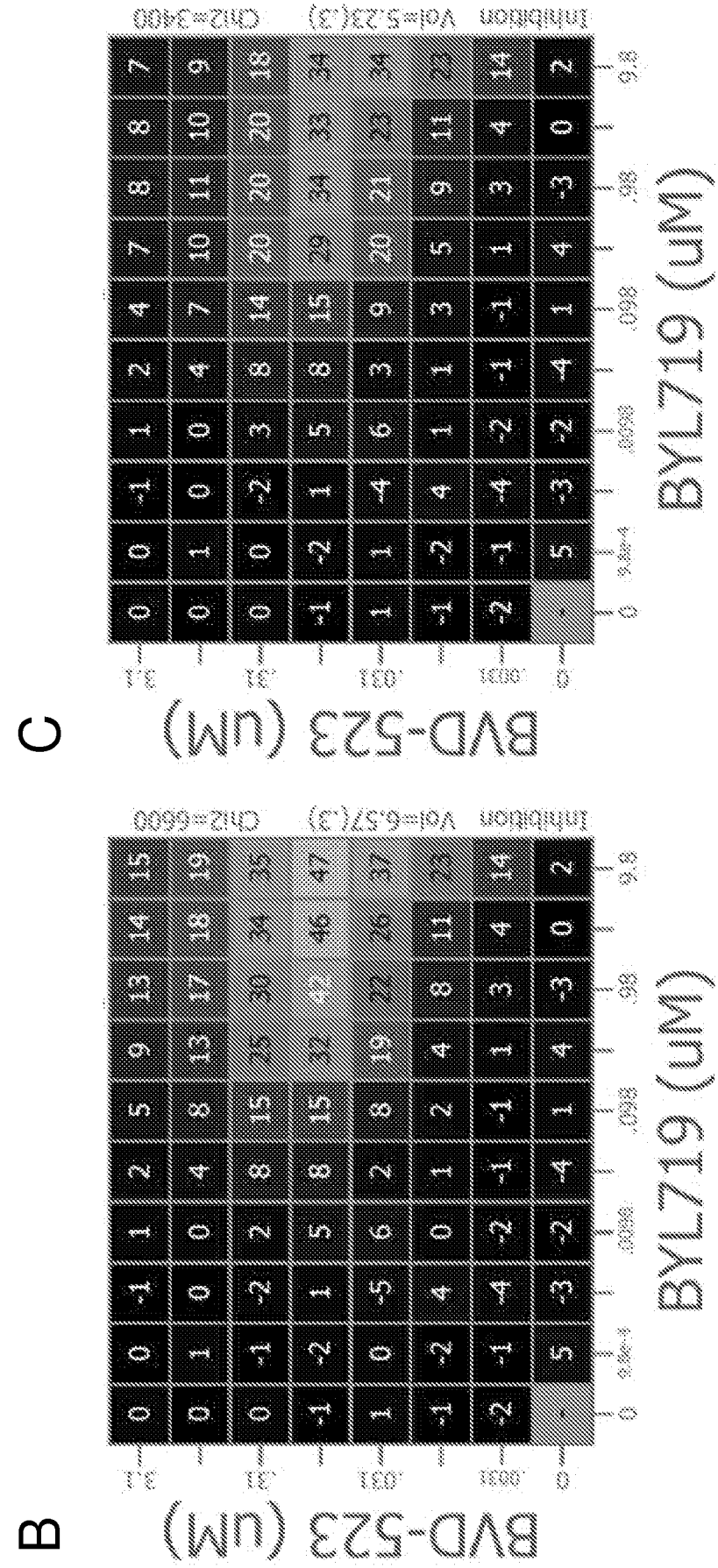


FIG. 3, Continued

D

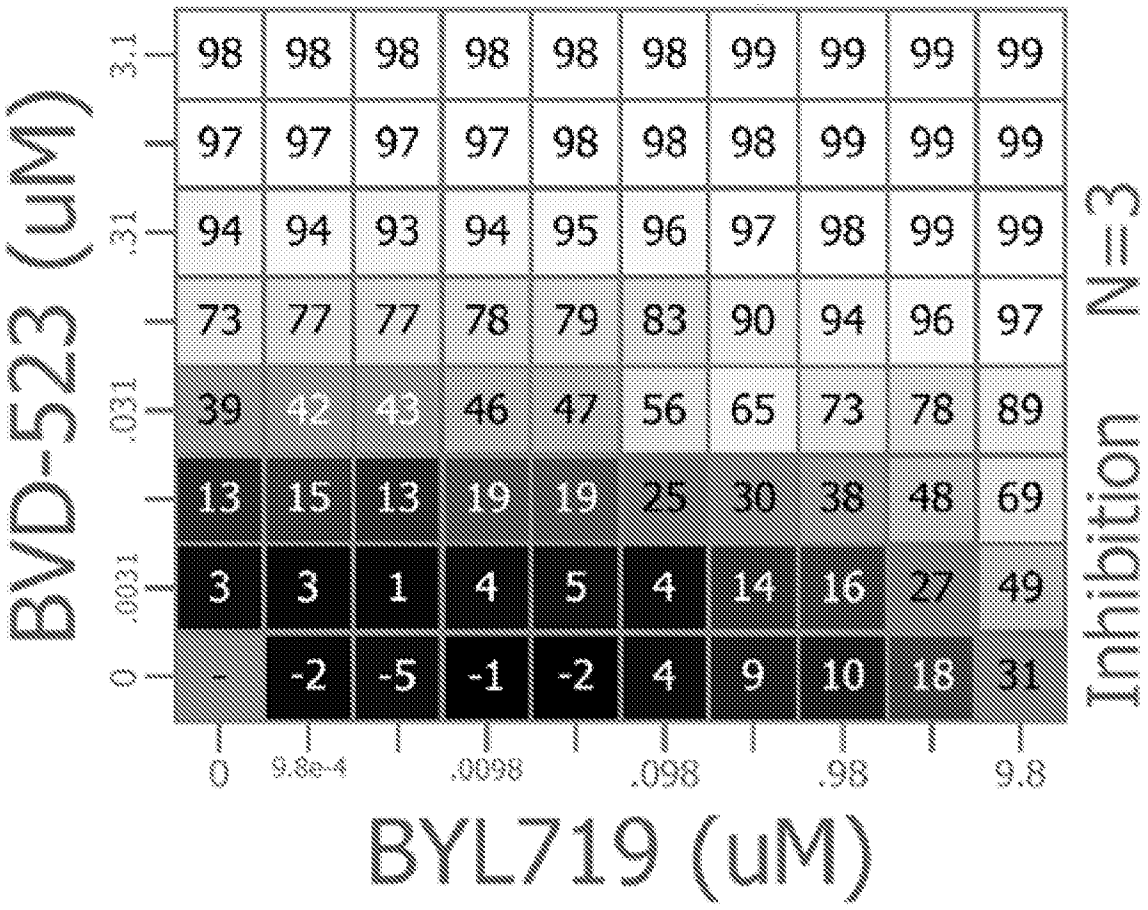


FIG. 3, Continued

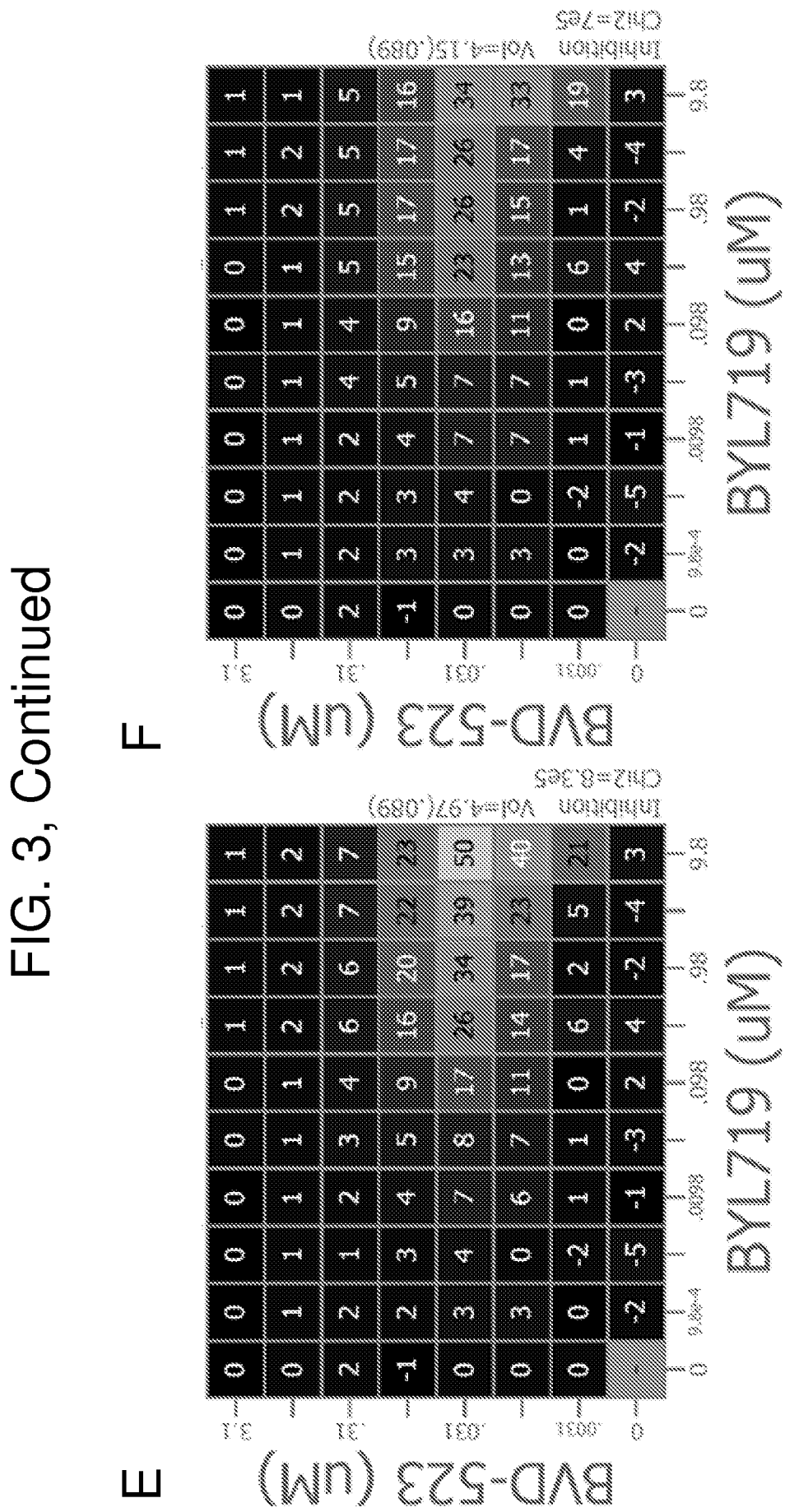
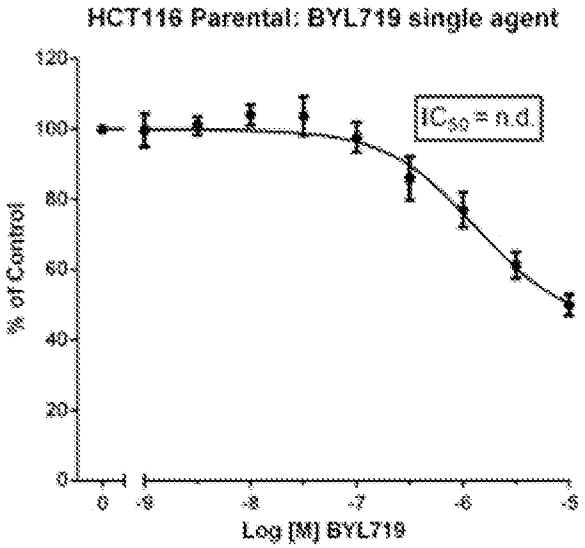
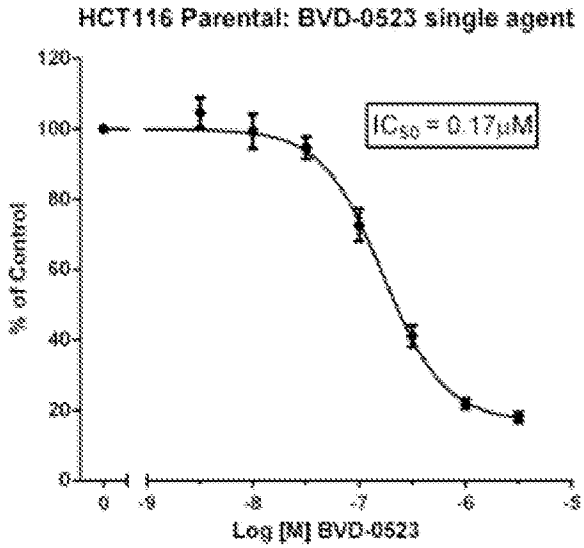


FIG. 3, Continued

G



H



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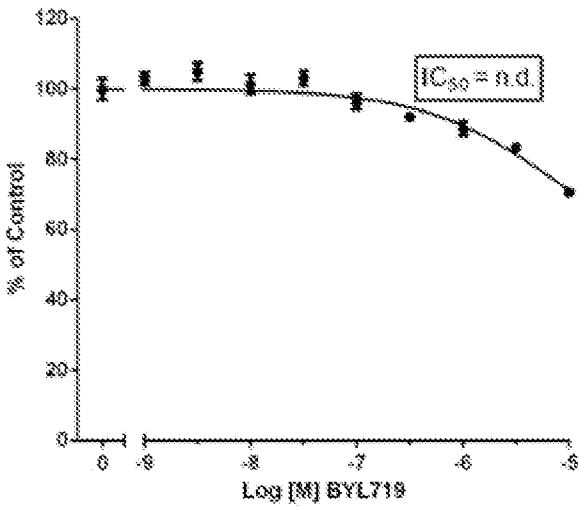


J

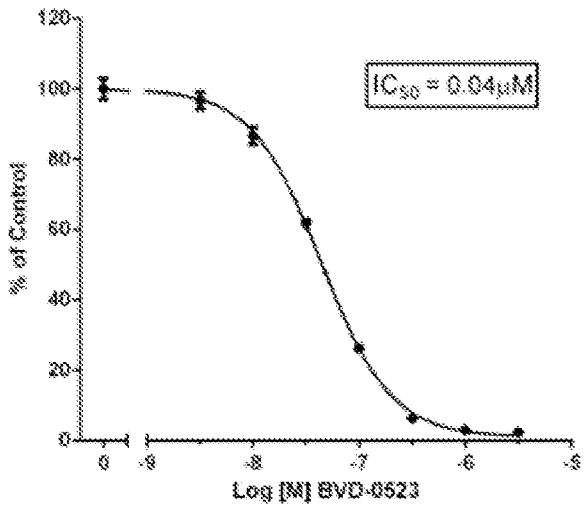


FIG. 4

A

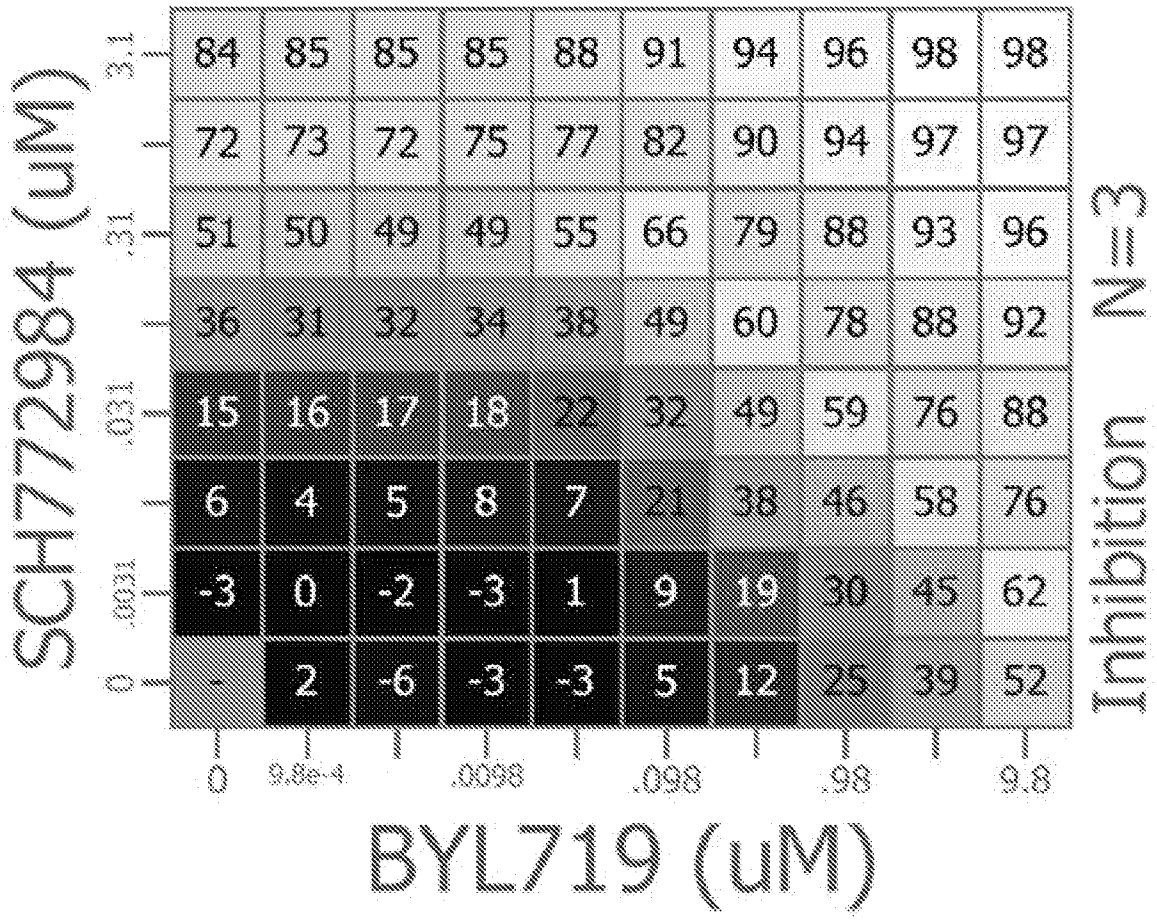

SCH772984 (uM)
3.1
.31
.031
.0031
0
84 85 85 85 88 91 94 96 98 98
72 73 72 75 77 82 90 94 97 97
51 50 49 49 55 66 79 88 93 96
36 31 32 34 38 49 60 78 88 92
15 16 17 18 22 32 49 59 76 88
6 4 5 8 7 21 38 46 58 76
-3 0 -2 -3 1 9 19 30 45 62
- 2 -6 -3 -3 5 12 25 39 52
0
9.8e-4
.0098
.098
.98
9.8
BYL719 (uM)
Inhibition N=3

FIG. 4, Continued

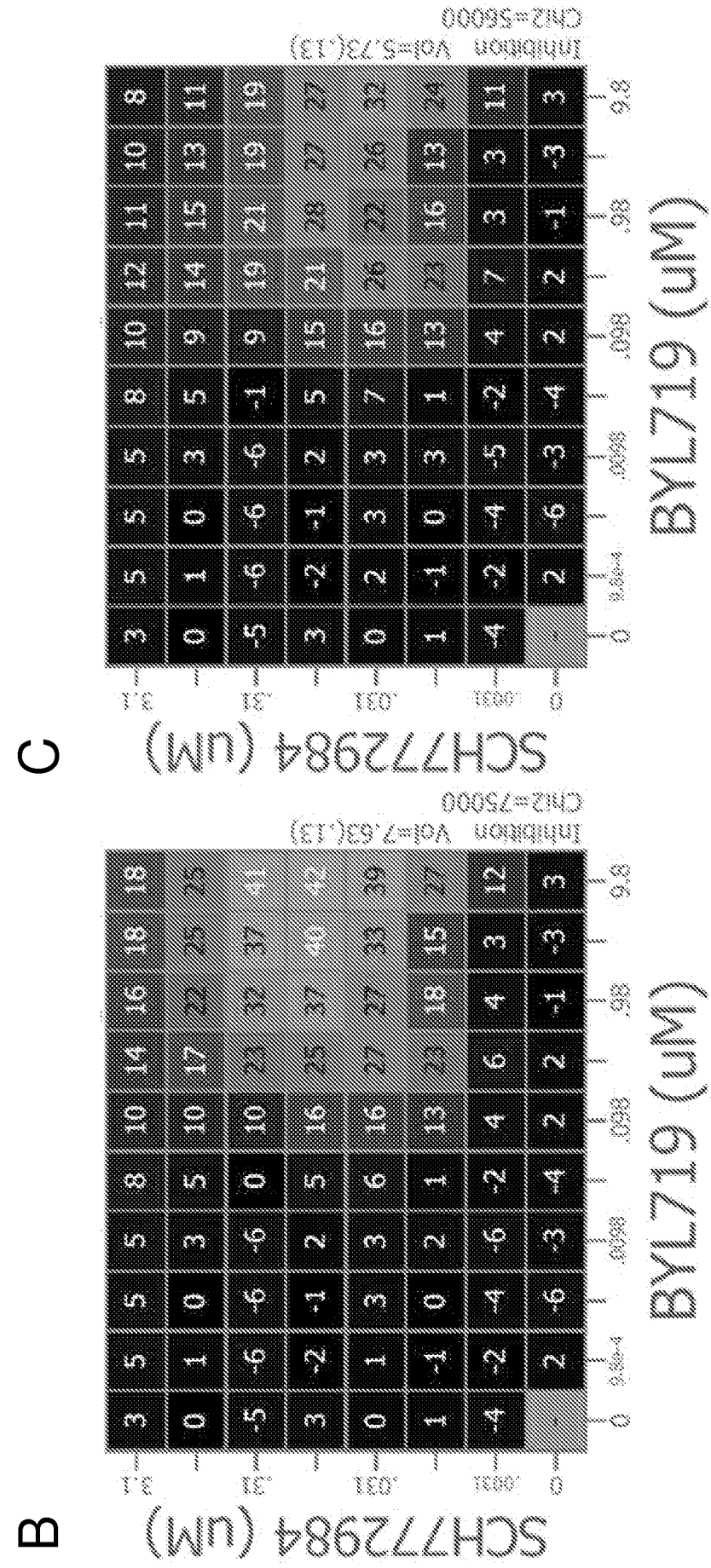


FIG. 4, Continued

D

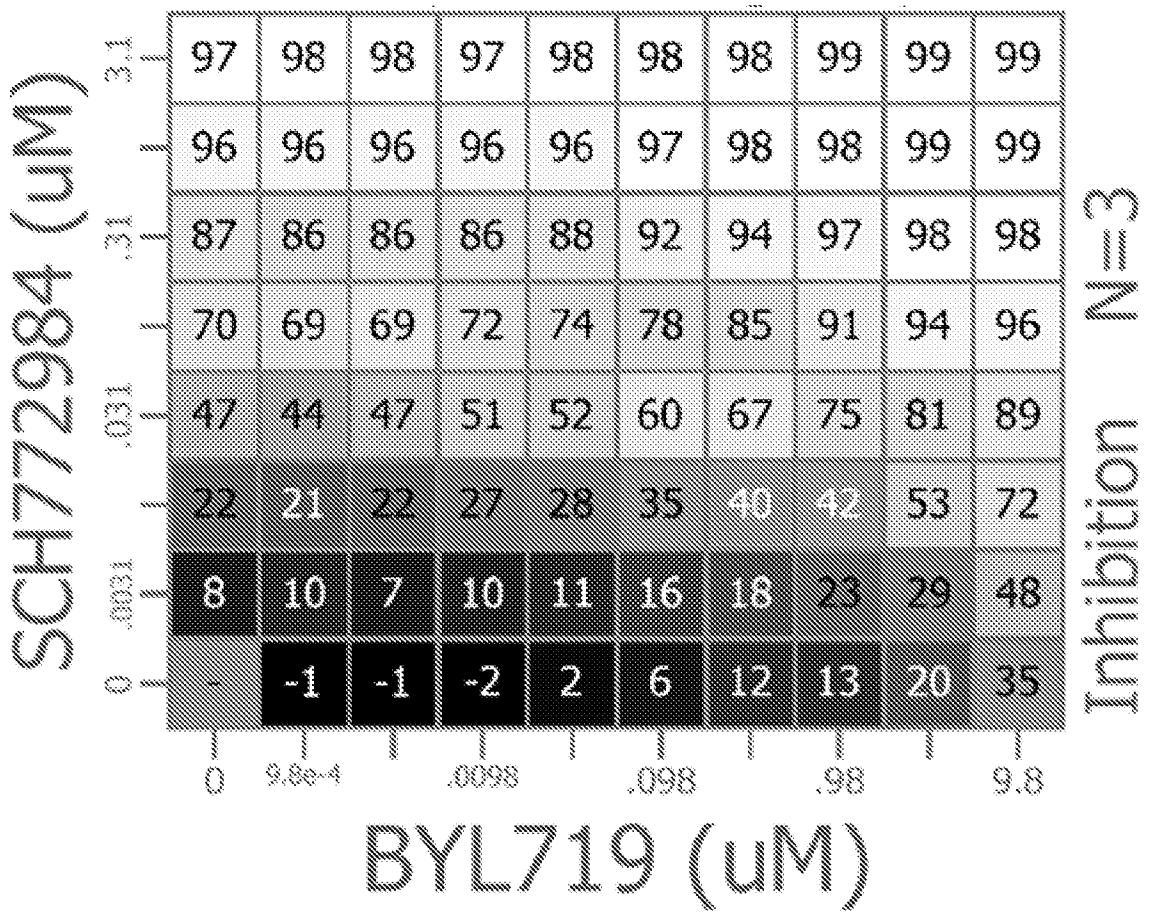


FIG. 4, Continued

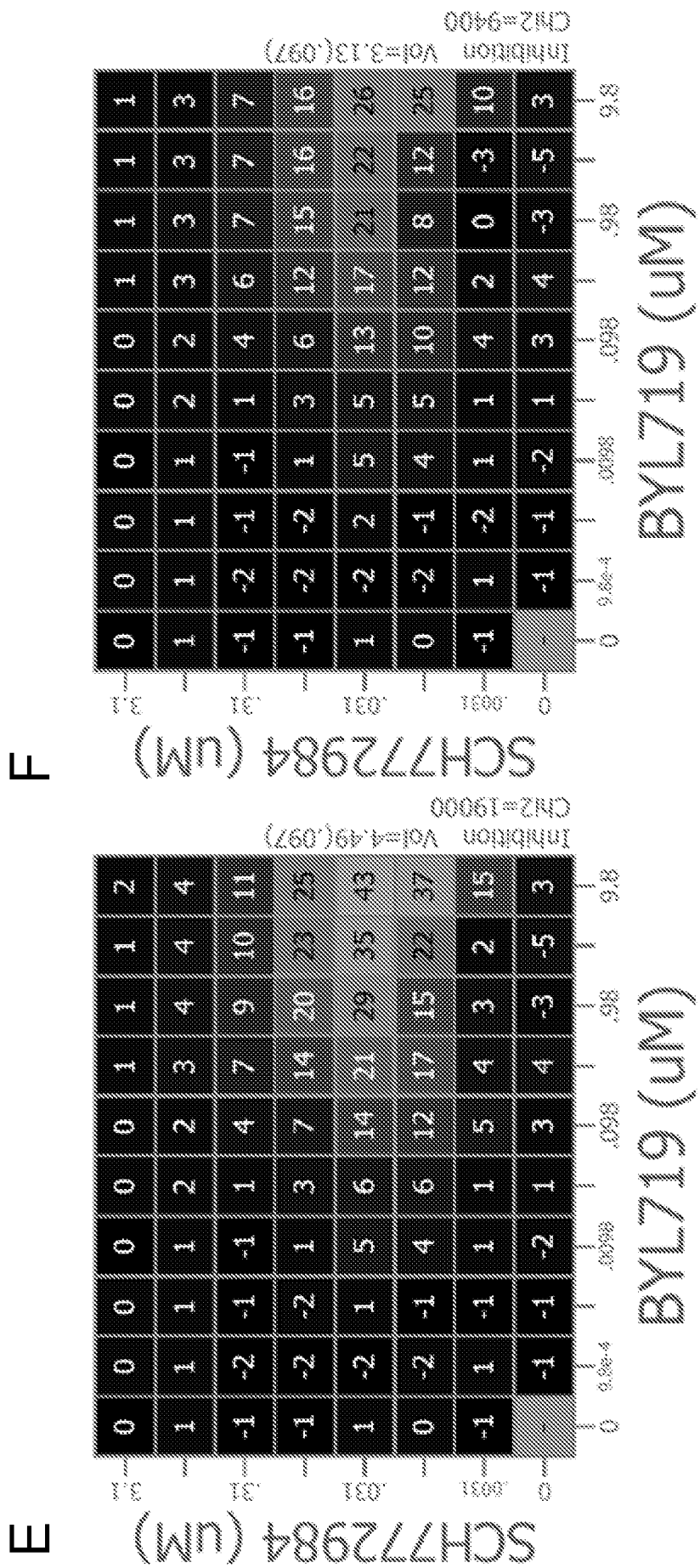
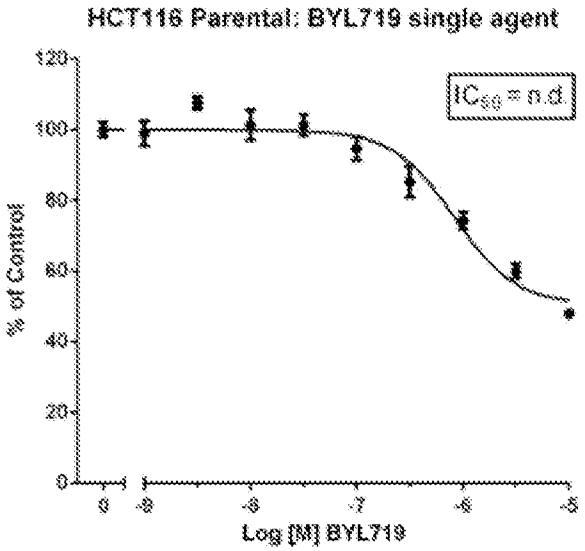
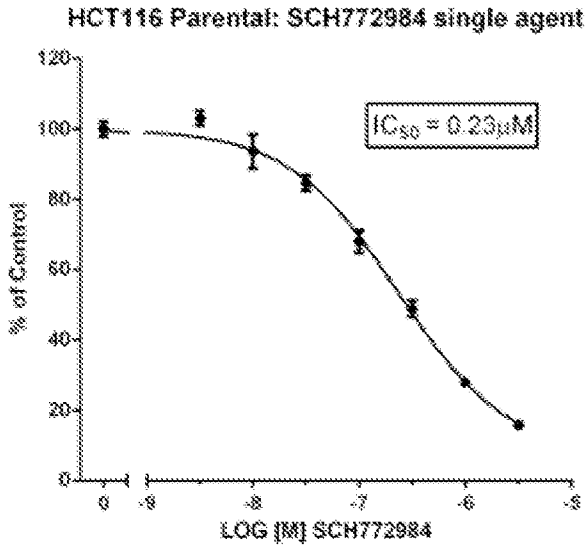


FIG. 4, Continued

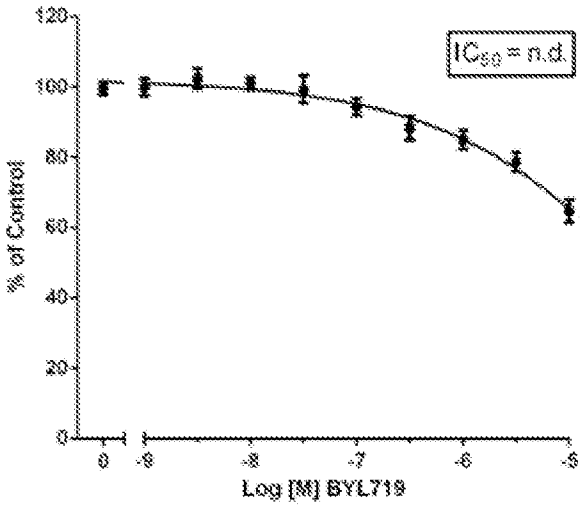
G



H



I



J

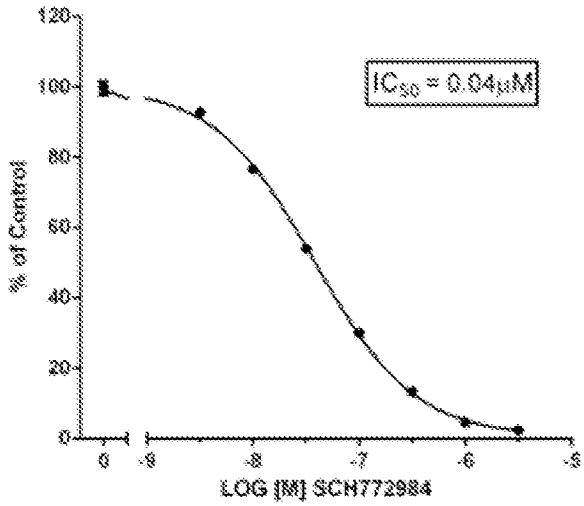


FIG. 5

A

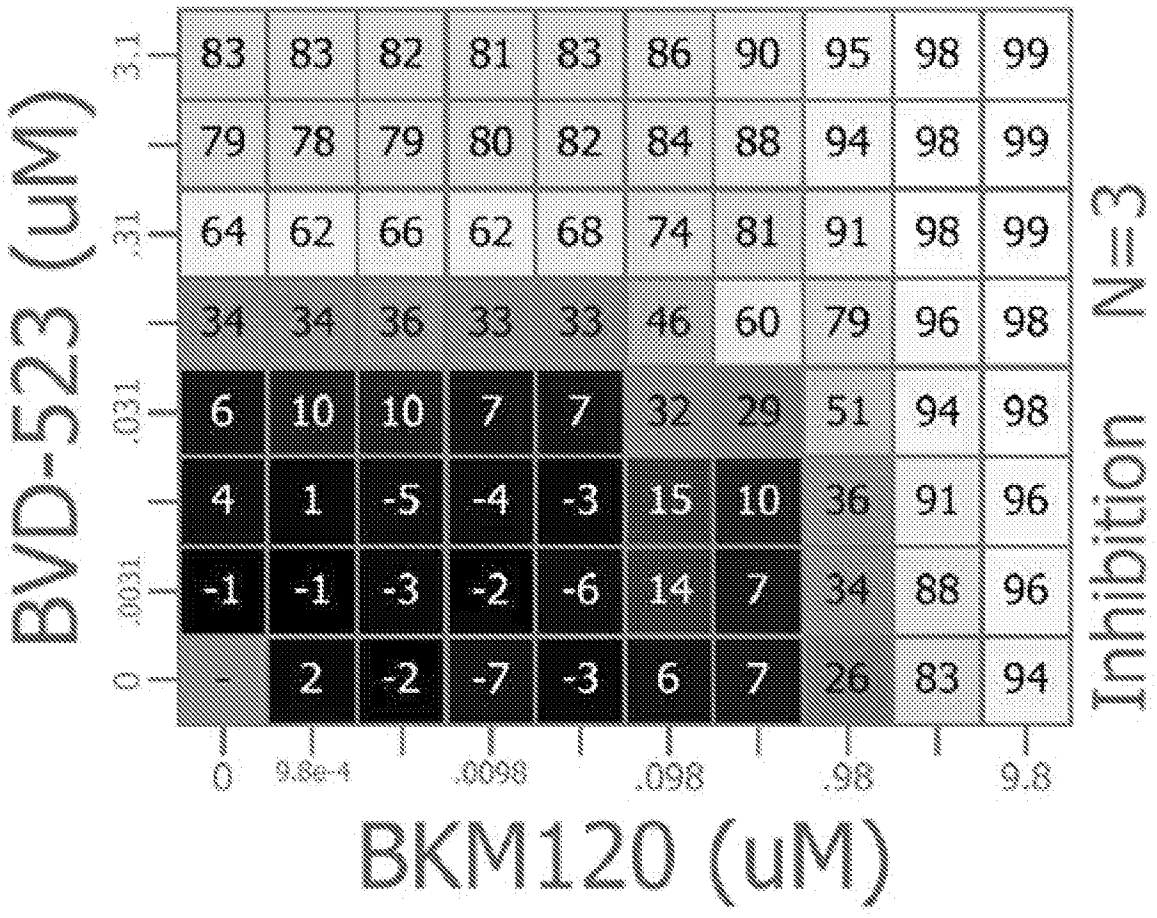

BVD-523 (uM)
3.1
.31
.031
.0031
0
83 83 82 81 83 86 90 95 98 99
79 78 79 80 82 84 88 94 98 99
64 62 66 62 68 74 81 91 98 99
34 34 36 33 33 46 60 79 96 98
6 10 10 7 7 32 29 51 94 98
4 1 -5 -4 -3 15 10 36 91 96
-1 -1 -3 -2 -6 14 7 34 88 96
- 2 -2 -7 -3 6 7 26 83 94
0
9.8e-4
.0098
.098
.98
9.8
BKM120 (uM)
Inhibition N=3

FIG. 5, Continued

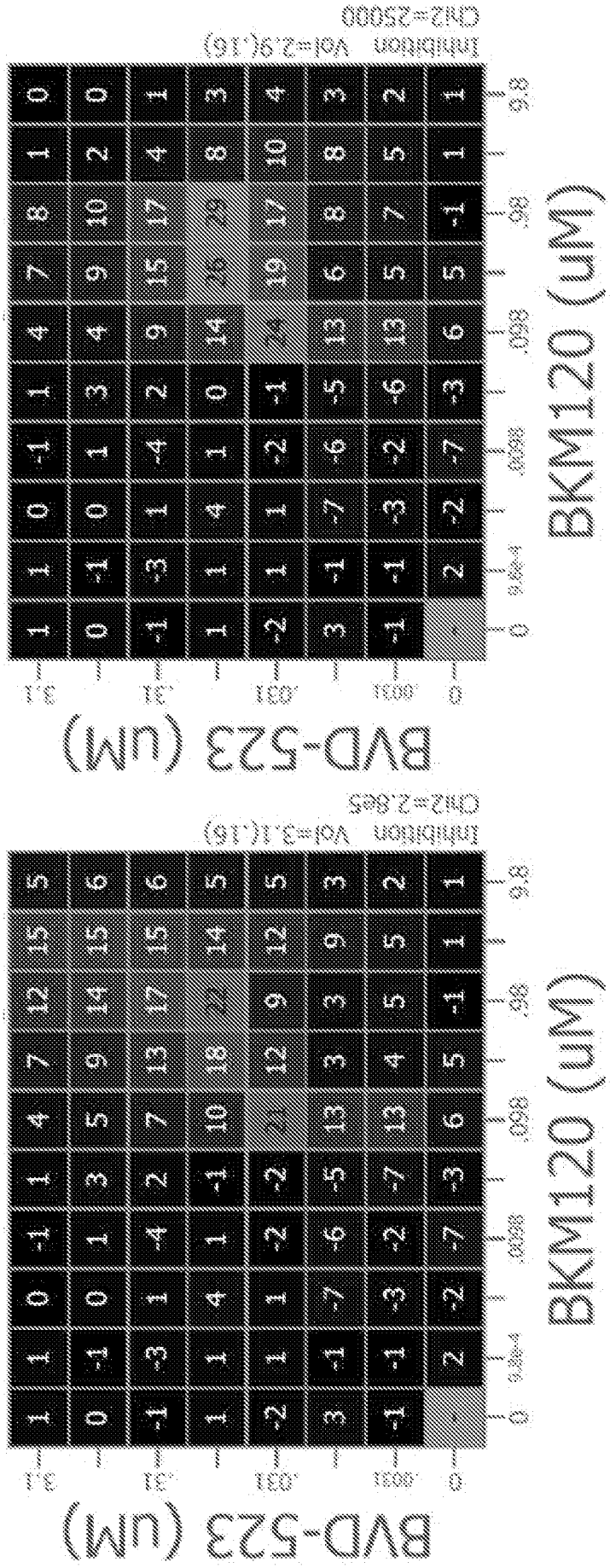


FIG. 5, Continued

D

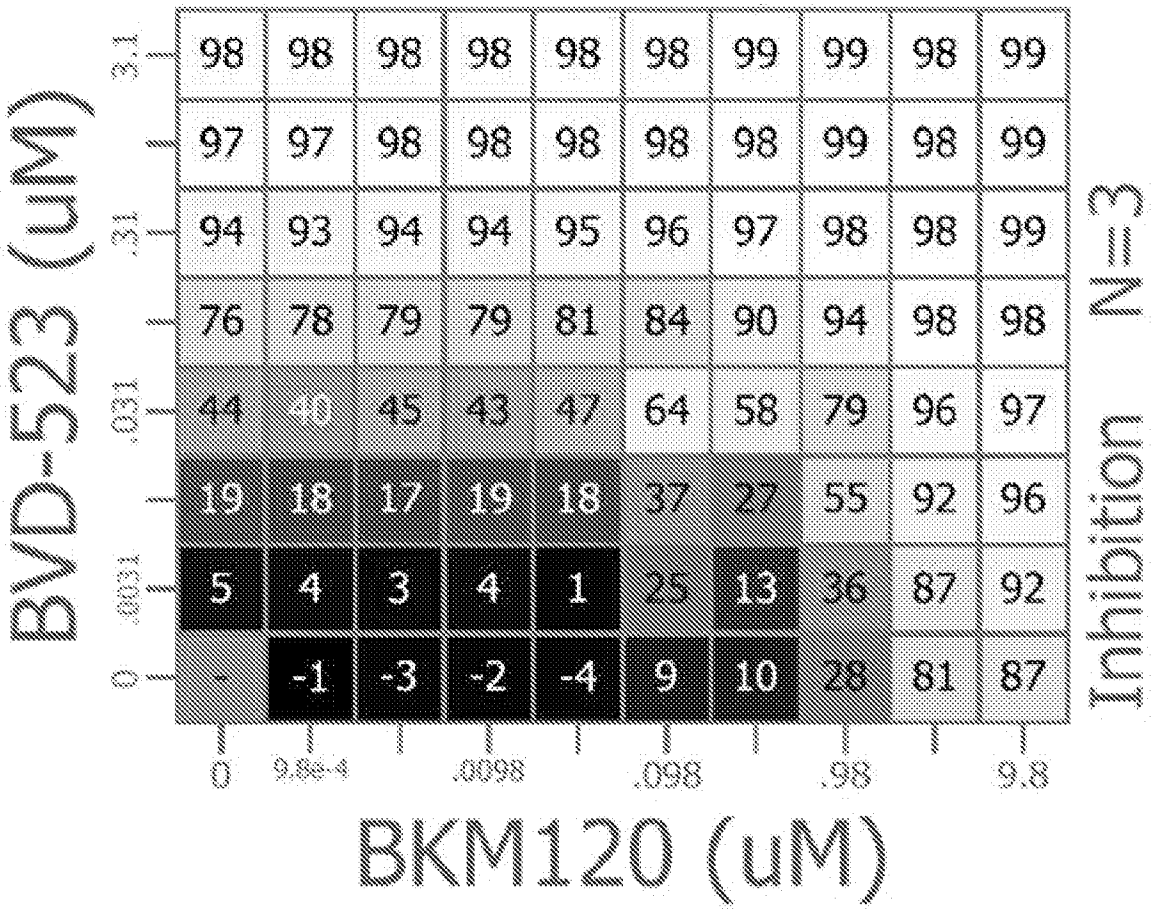
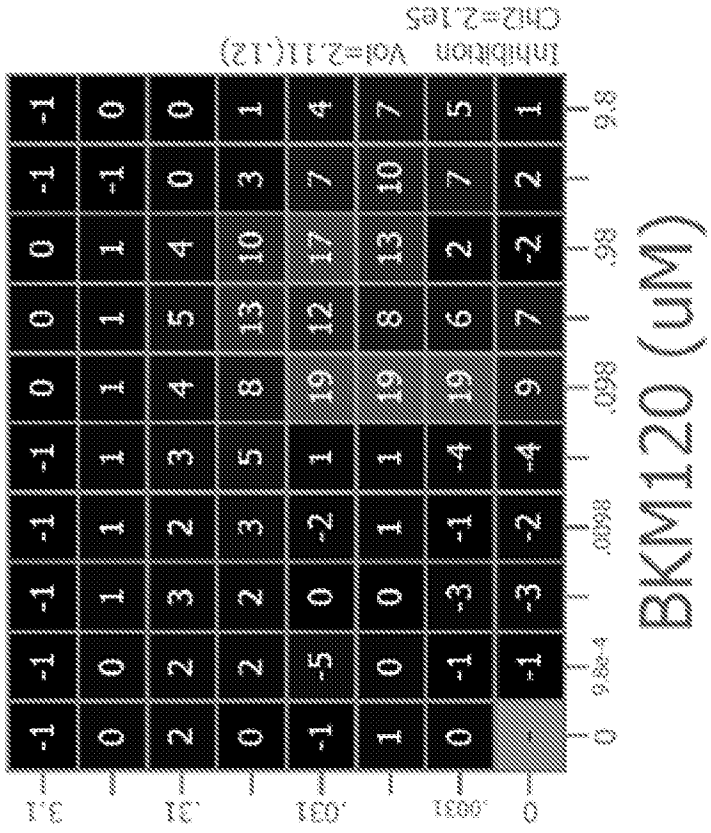


FIG. 5, Continued



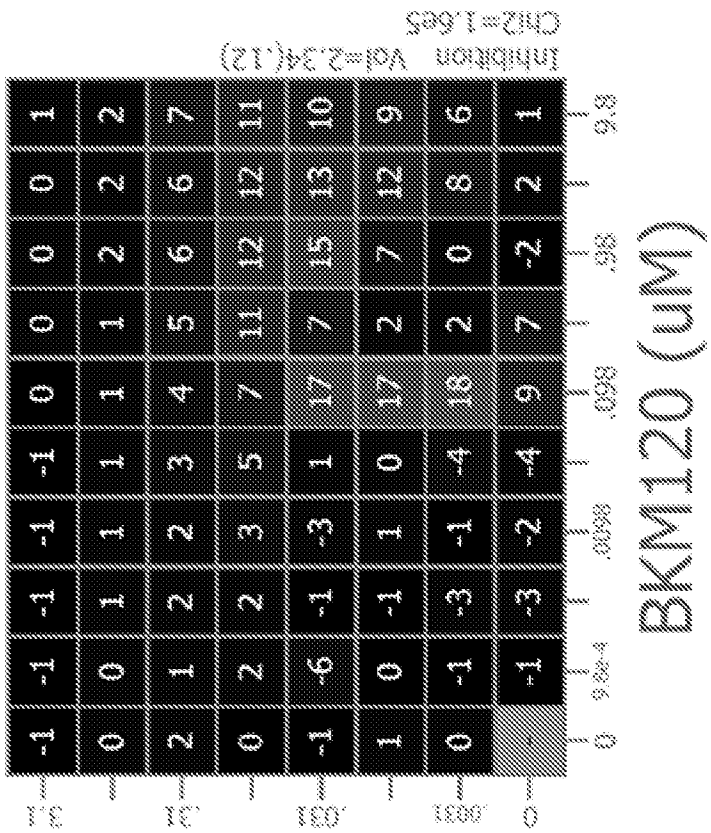
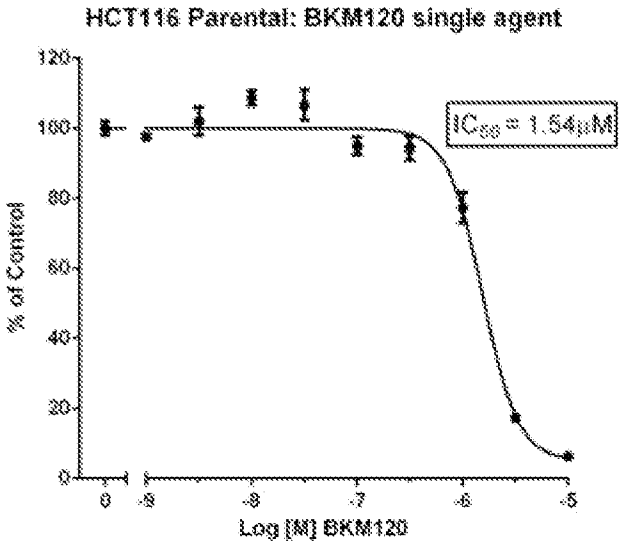
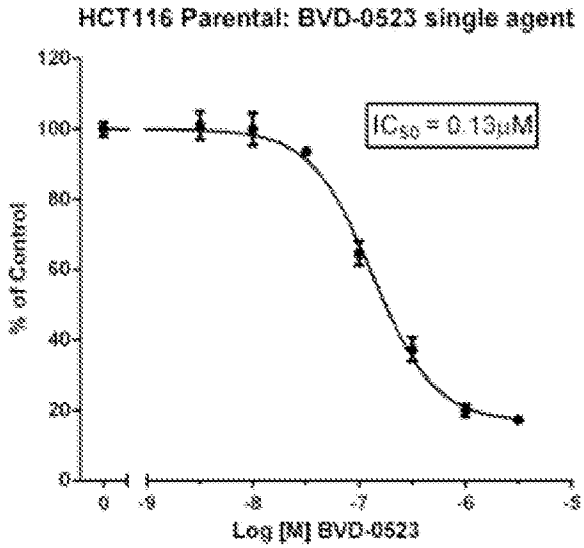


FIG. 5, Continued

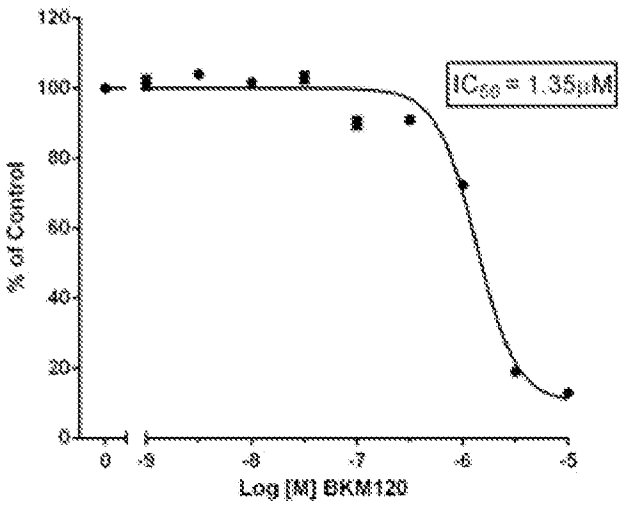
G



H



I



J

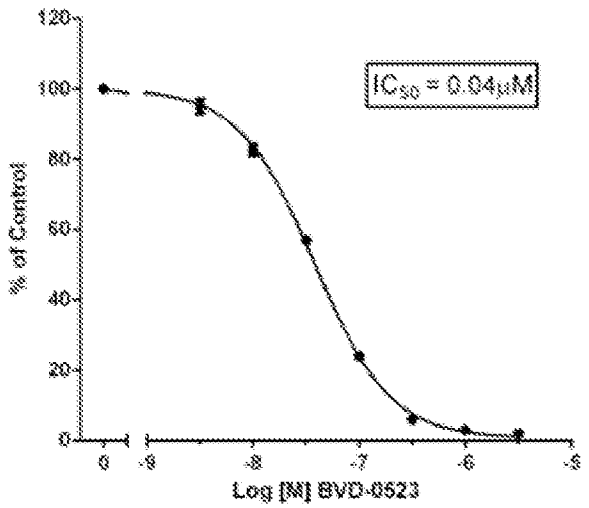


FIG. 6

A

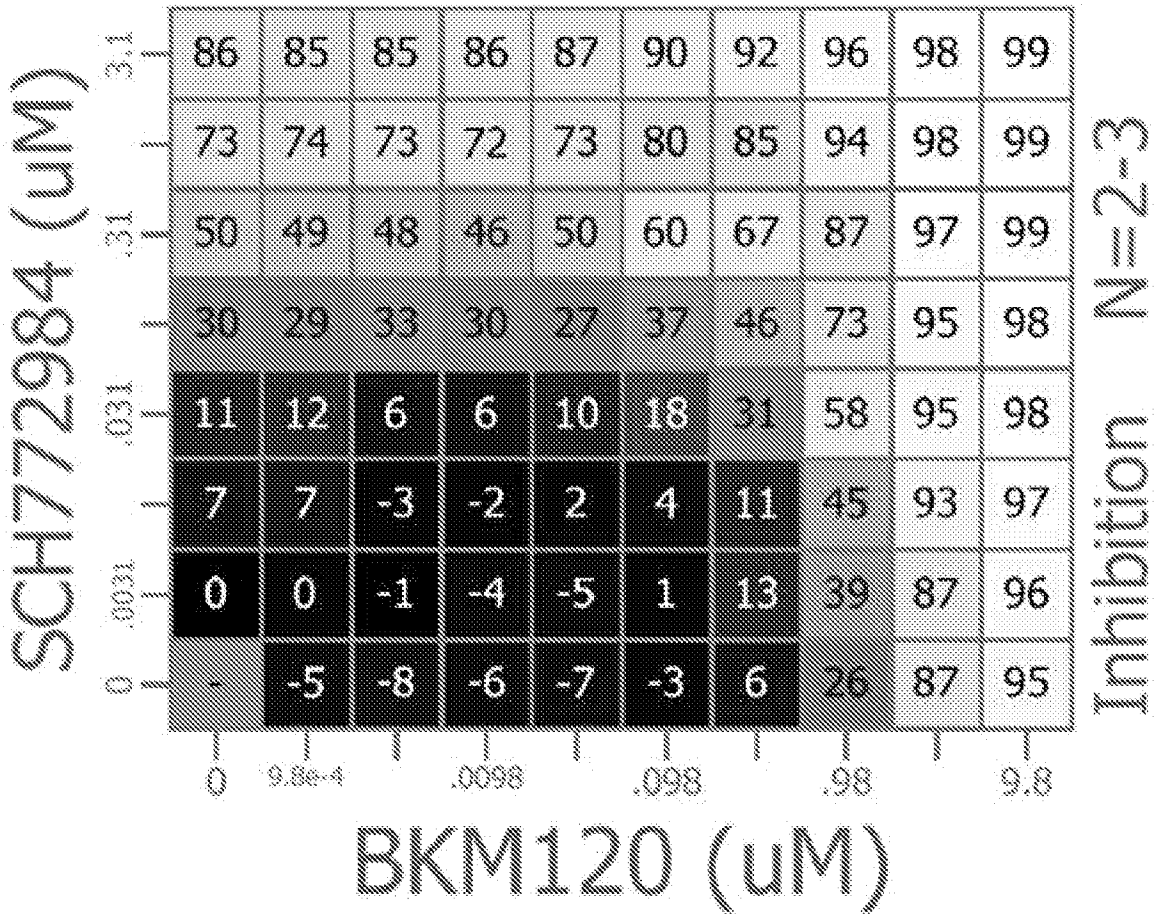

SCH772984 (uM)
3.1
.31
.031
.0031
0
86 85 85 86 87 90 92 96 98 99
73 74 73 72 73 80 85 94 98 99
50 49 48 46 50 60 67 87 97 99
30 29 33 30 27 37 46 73 95 98
11 12 6 6 10 18 31 58 95 98
7 7 -3 -2 2 4 11 45 93 97
0 0 -1 -4 -5 1 13 39 87 96
- -5 -8 -6 -7 -3 6 26 87 95
0
9.8e-4
.0098
.098
.98
9.8
BKM120 (uM)
Inhibition N=2-3

FIG. 6, Continued

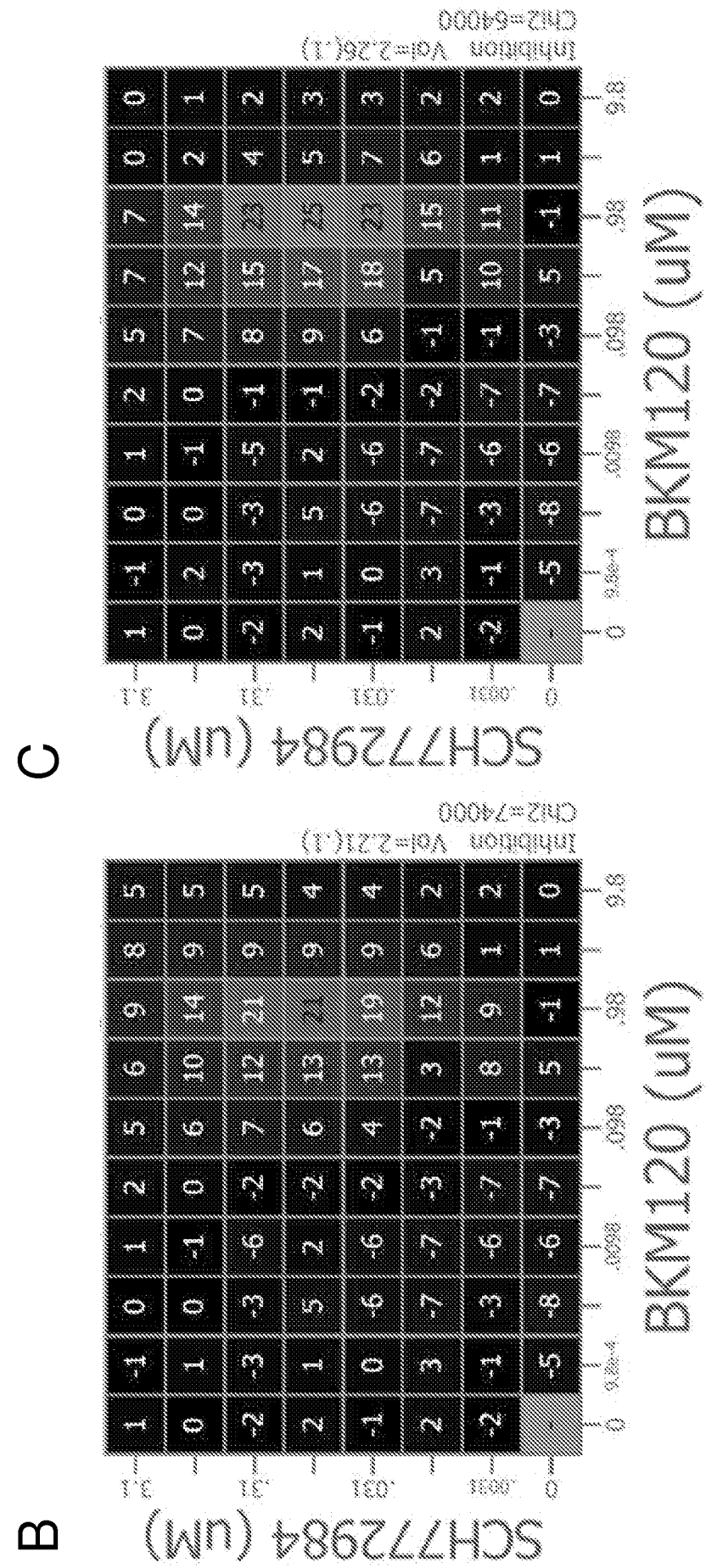


FIG. 6, Continued

D

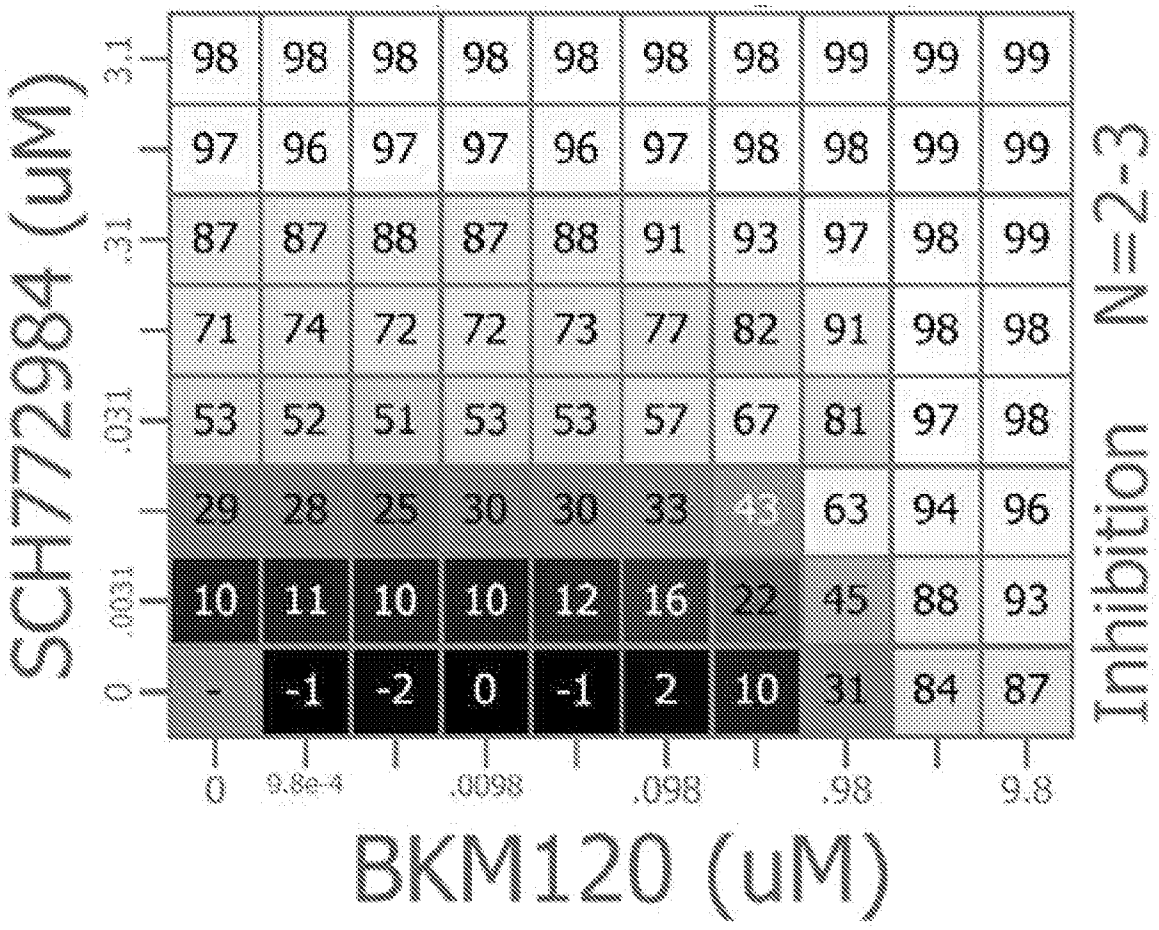


FIG. 6, Continued

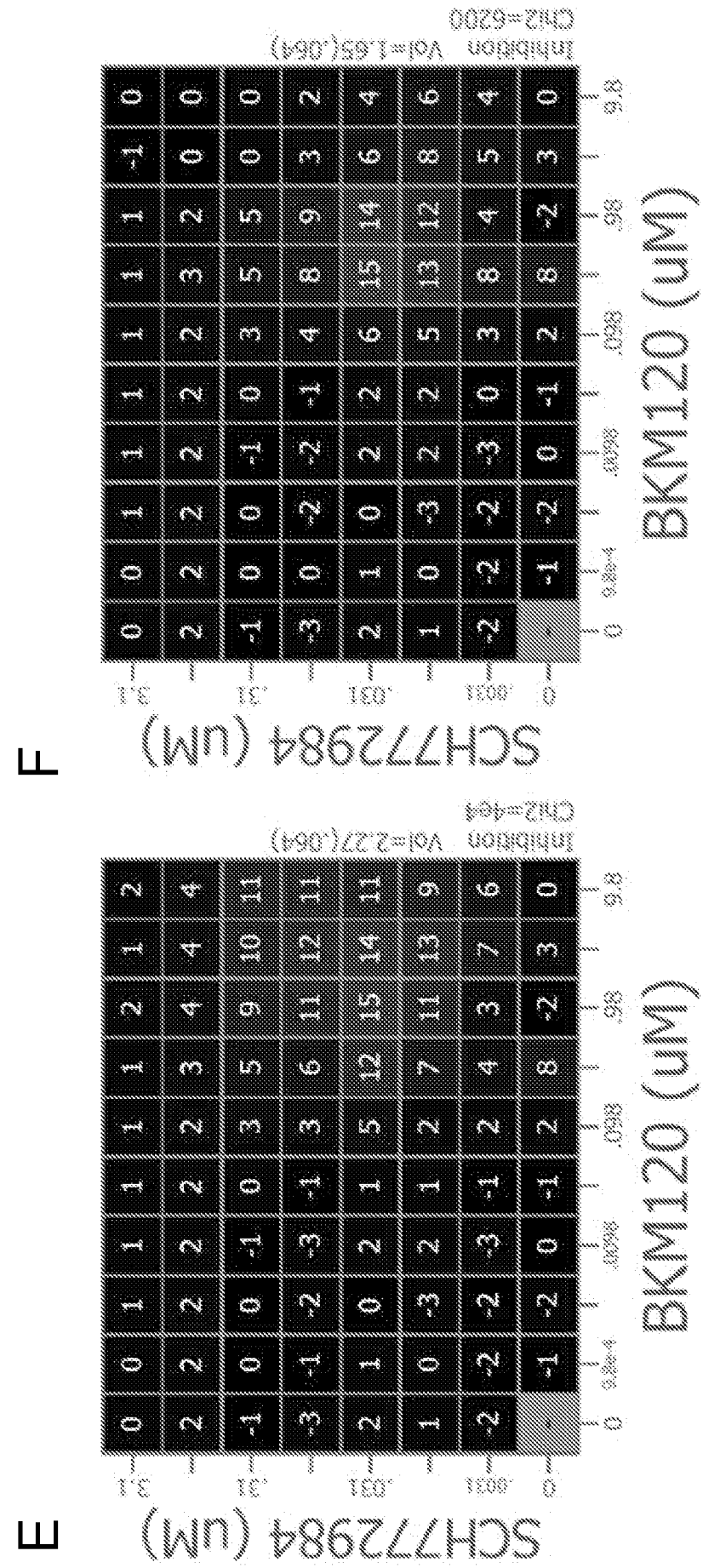
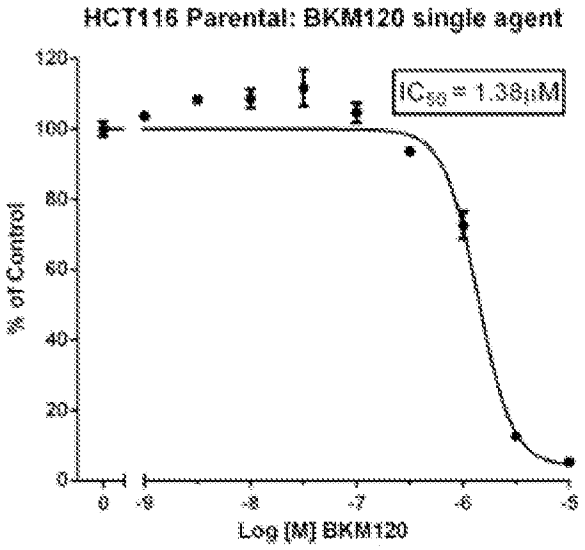
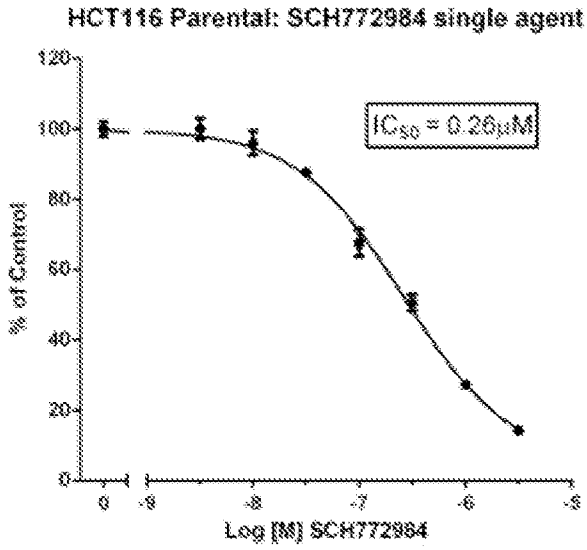


FIG. 6, Continued

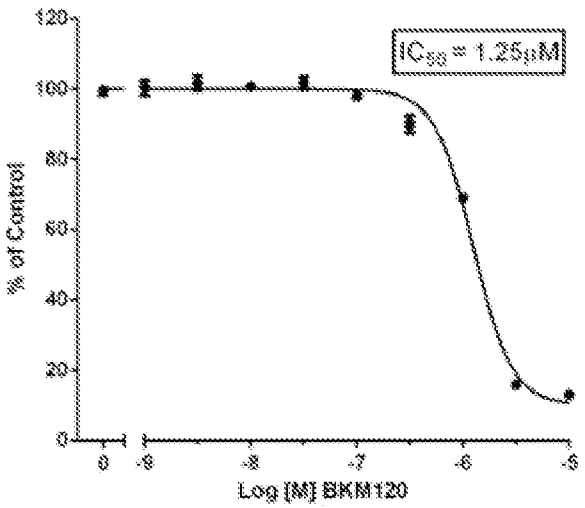
G



H



I



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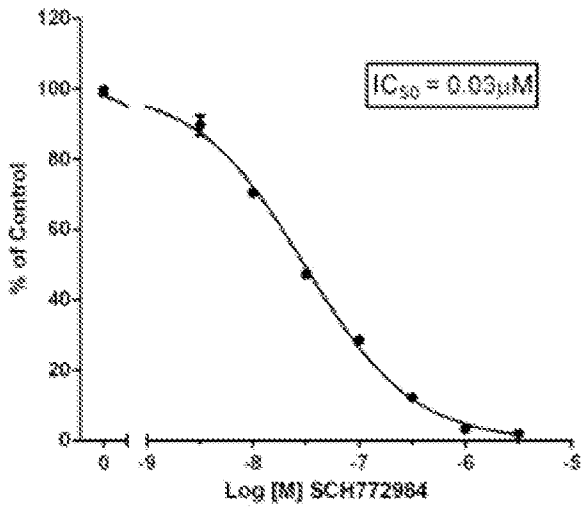


FIG. 7

A

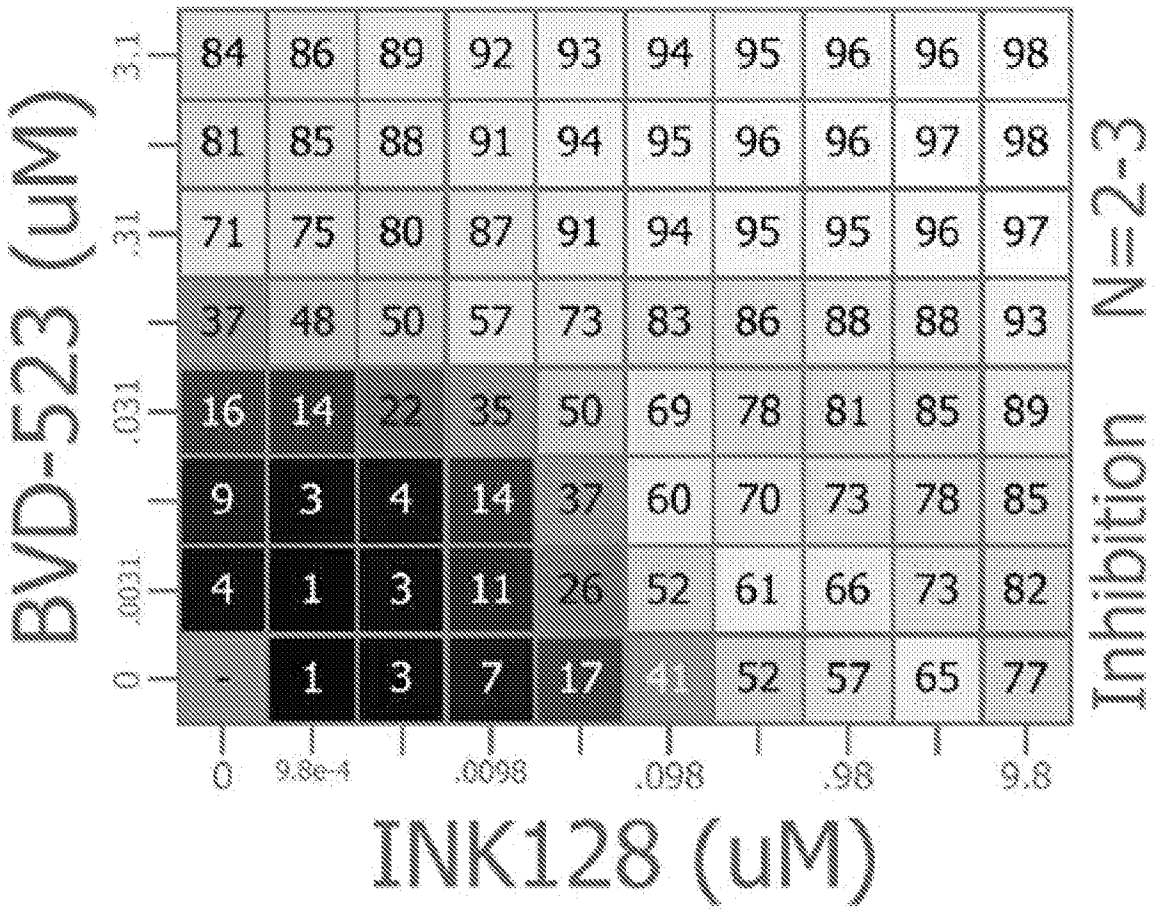

BVD-523 (uM)
3.1
.31
.031
.0031
0
84 86 89 92 93 94 95 96 96 98
81 85 88 91 94 95 96 96 97 98
71 75 80 87 91 94 95 95 96 97
37 48 50 57 73 83 86 88 88 93
16 14 22 35 50 69 78 81 85 89
9 3 4 14 37 60 70 73 78 85
4 1 3 11 26 52 61 66 73 82
- 1 3 7 17 41 52 57 65 77
0
9.8e-4
.0098
.098
.98
9.8
INK128 (uM)
Inhibition N=2-3

FIG. 7, Continued

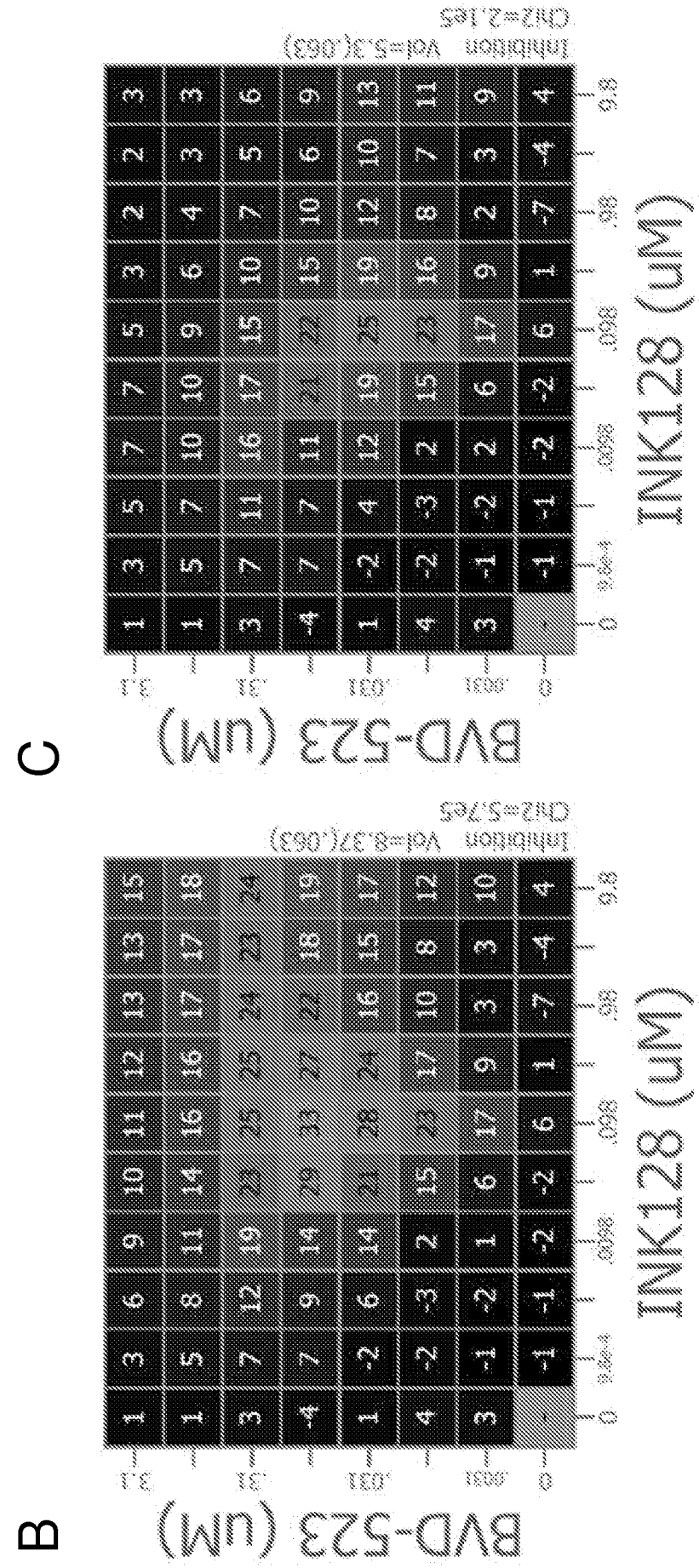


FIG. 7, Continued

D

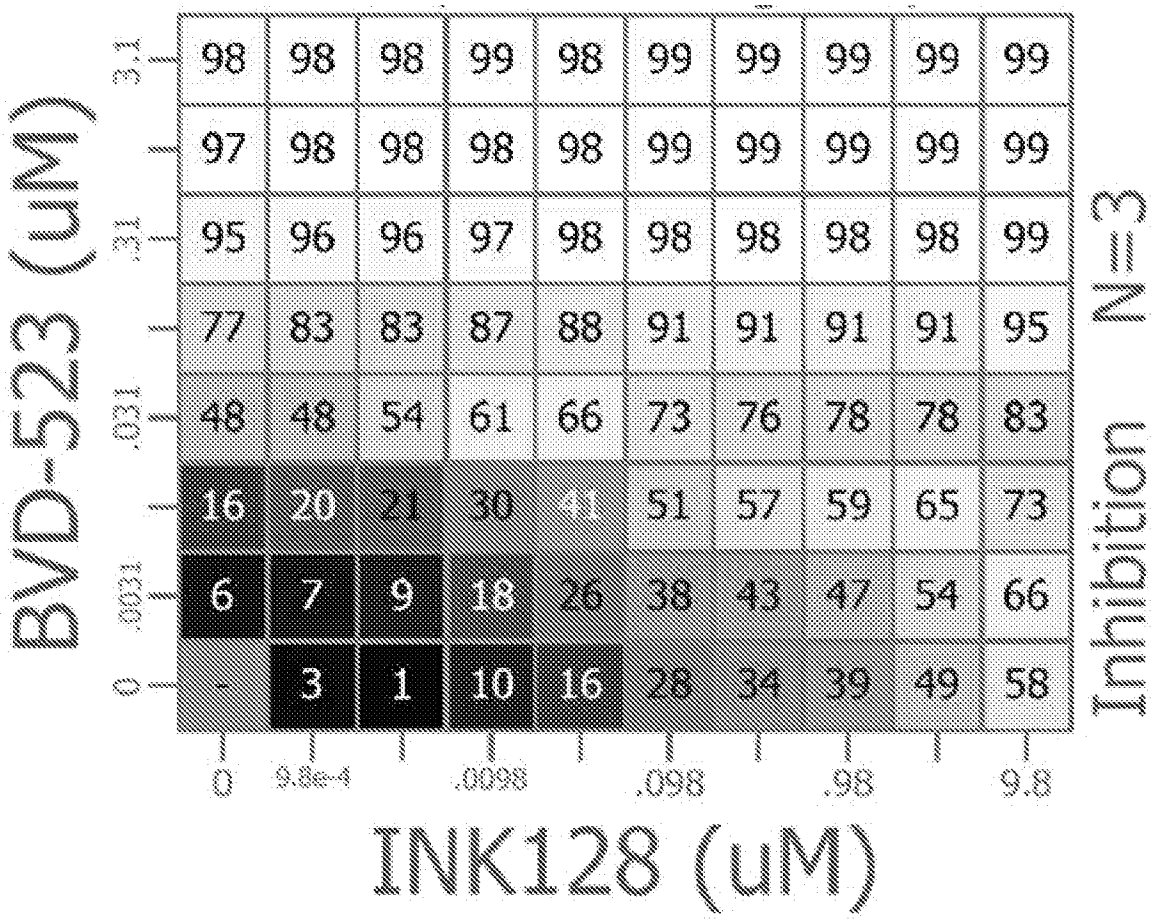


FIG. 7, Continued

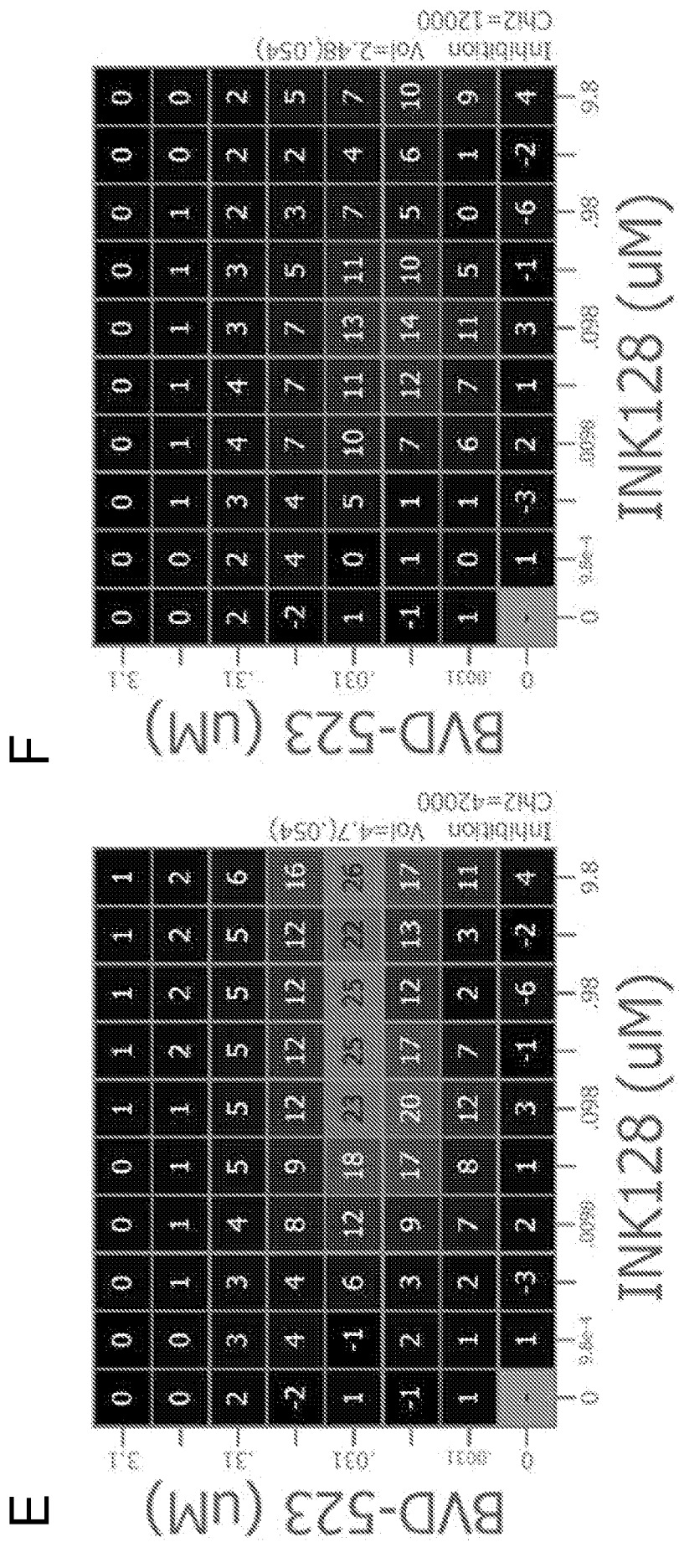
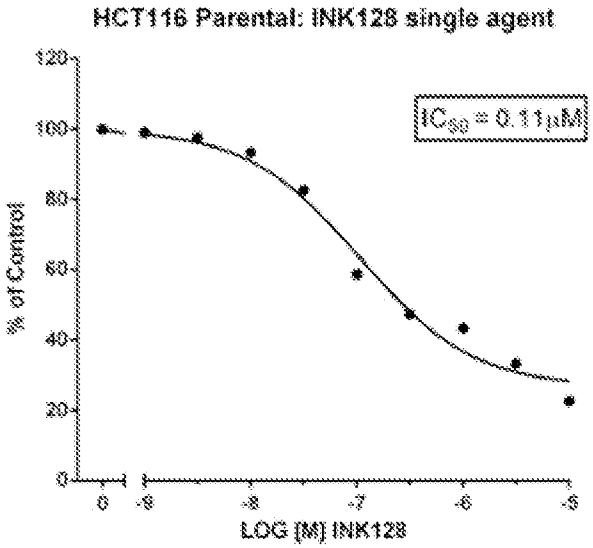
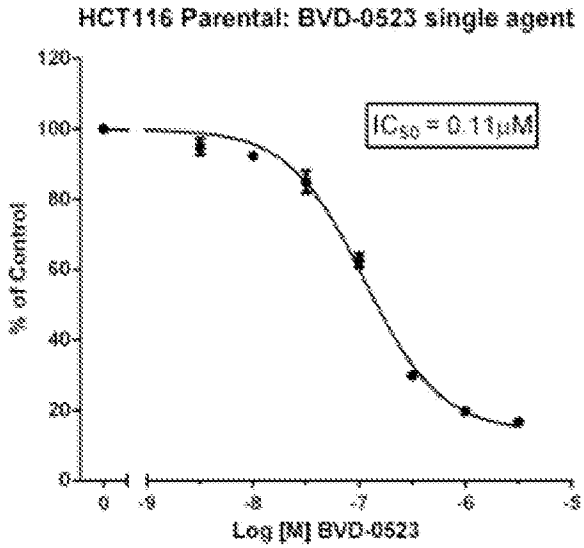


FIG. 7, Continued

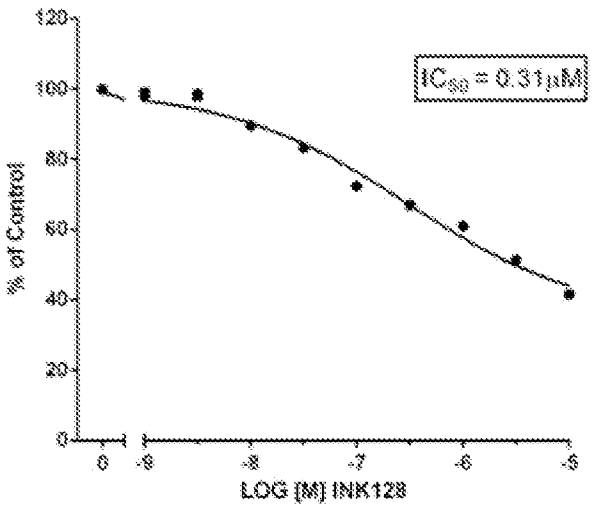
G



H



I



J

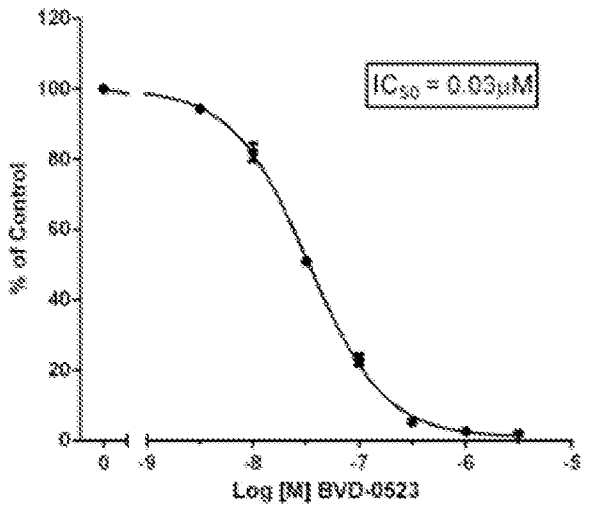


FIG. 8

A

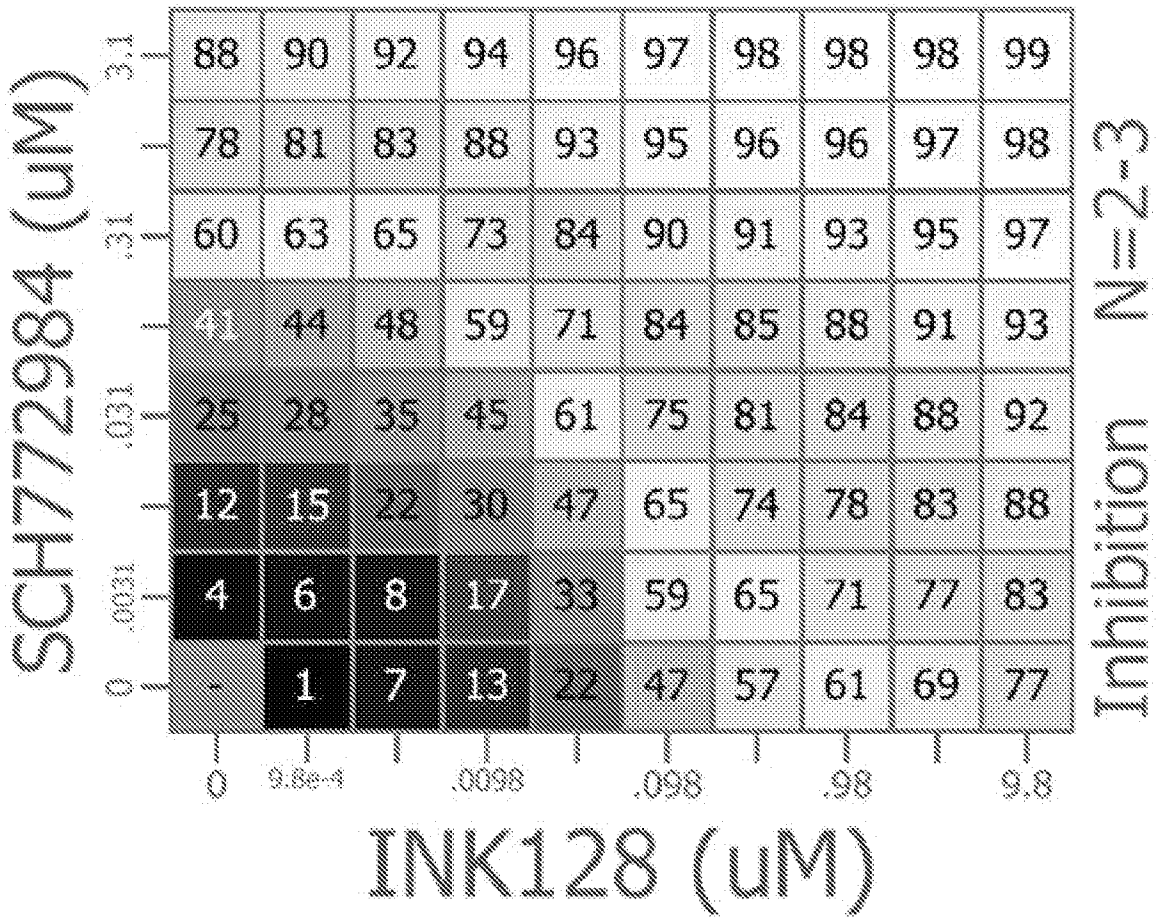

SCH772984 (uM)
3.1
.31
.031
.0031
0
88 90 92 94 96 97 98 98 98 99
78 81 83 88 93 95 96 96 97 98
60 63 65 73 84 90 91 93 95 97
44 44 48 59 71 84 85 88 91 93
25 28 35 45 61 75 81 84 88 92
12 15 22 30 47 65 74 78 83 88
4 6 8 17 33 59 65 71 77 83
- 1 7 13 22 47 57 61 69 77
0
9.8e-4
.0098
.098
.98
9.8
INK128 (uM)
Inhibition N=2-3

FIG. 8, Continued

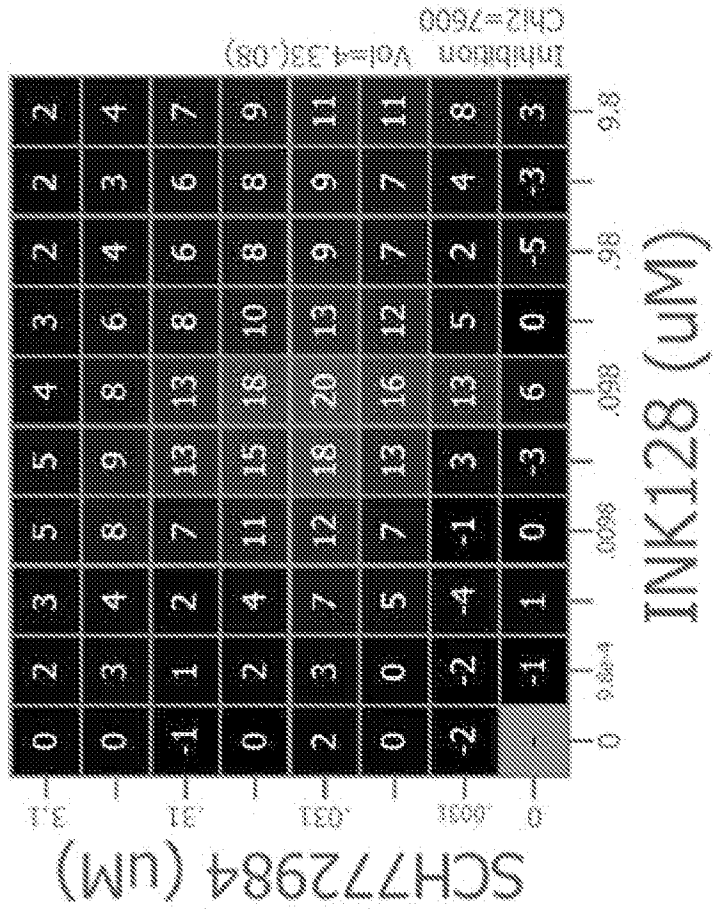


FIG. 8, Continued

D

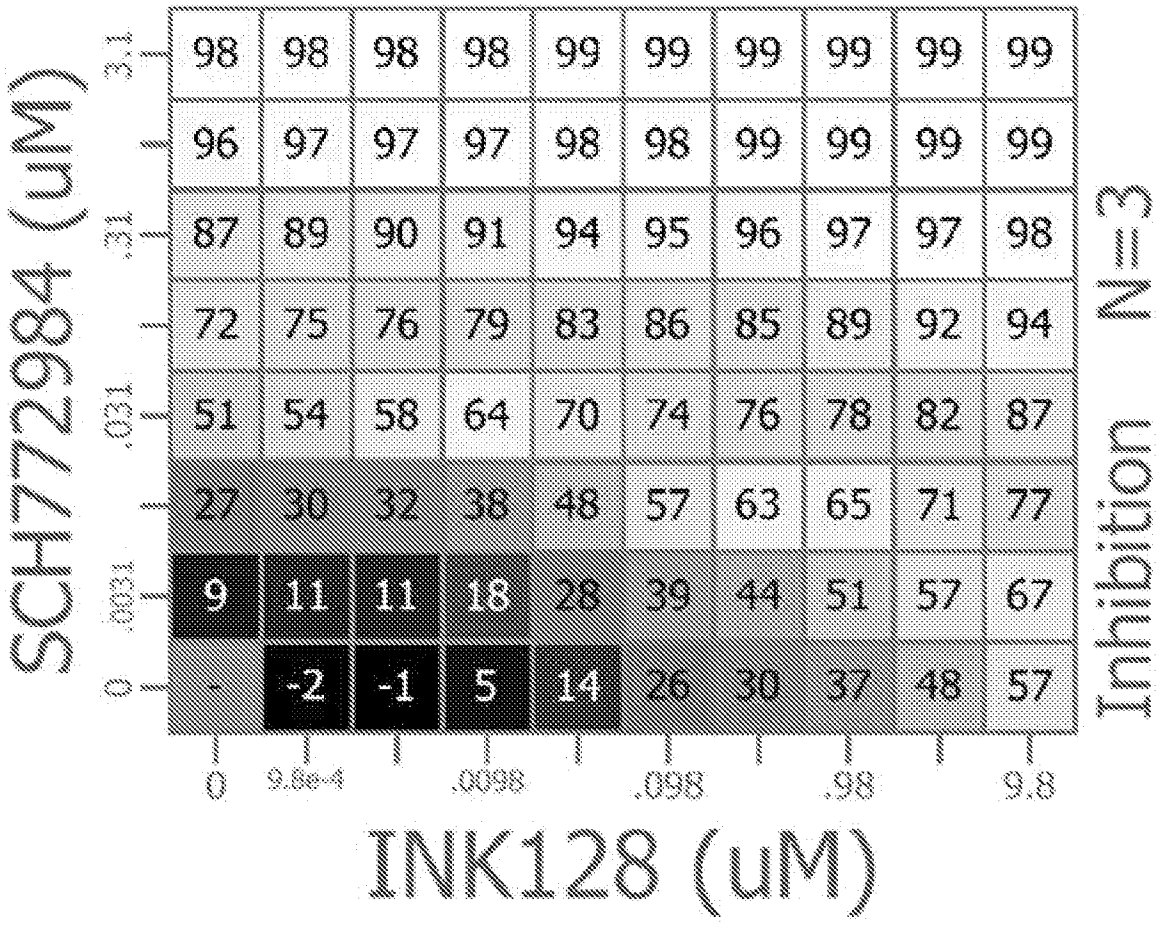


FIG. 8, Continued

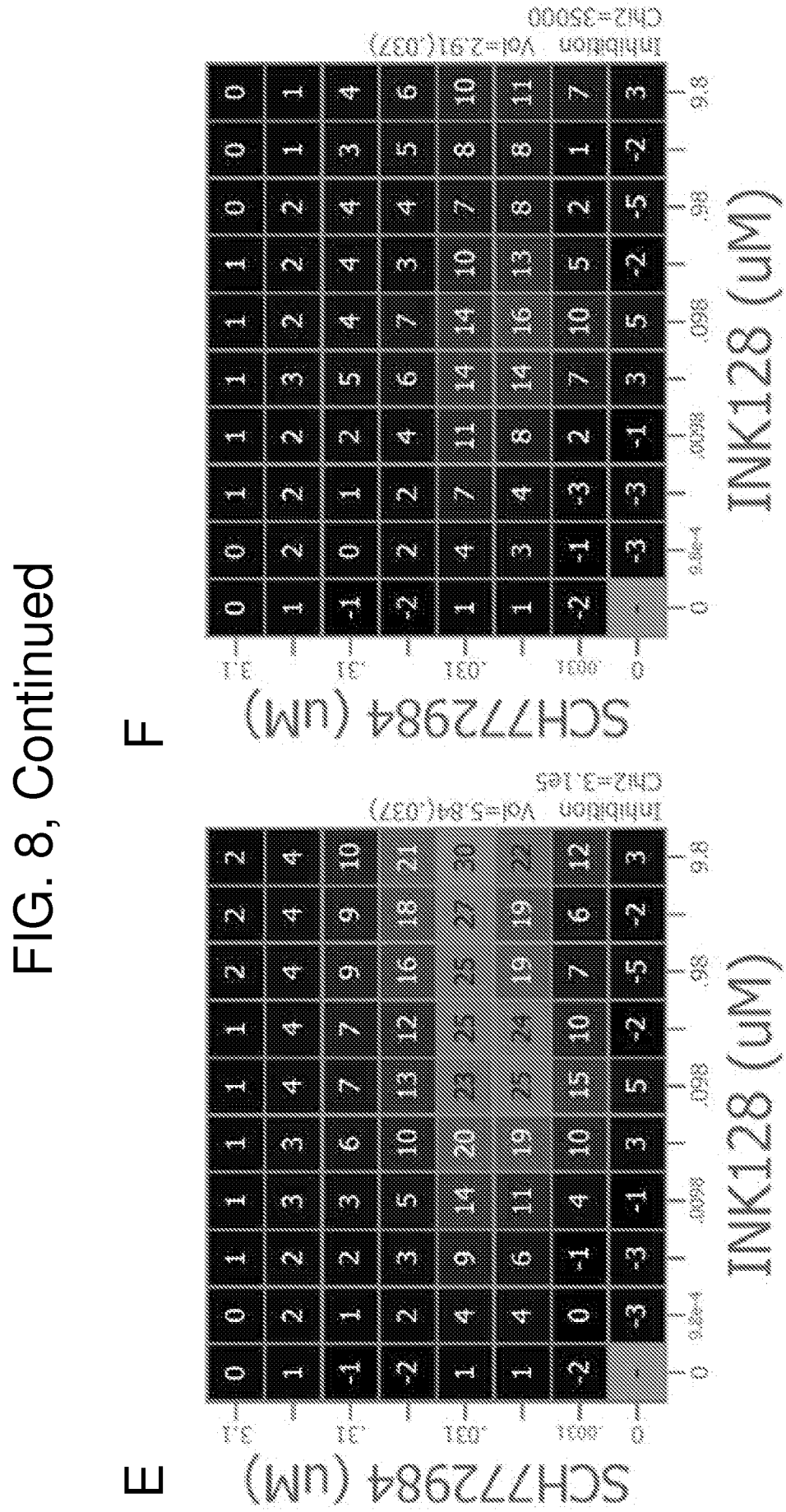
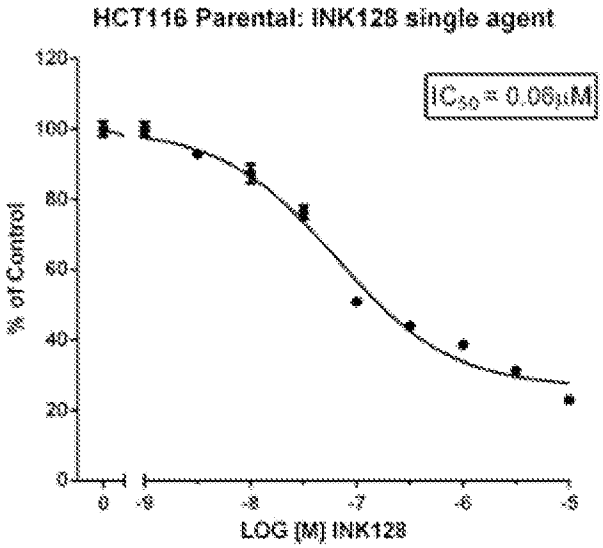
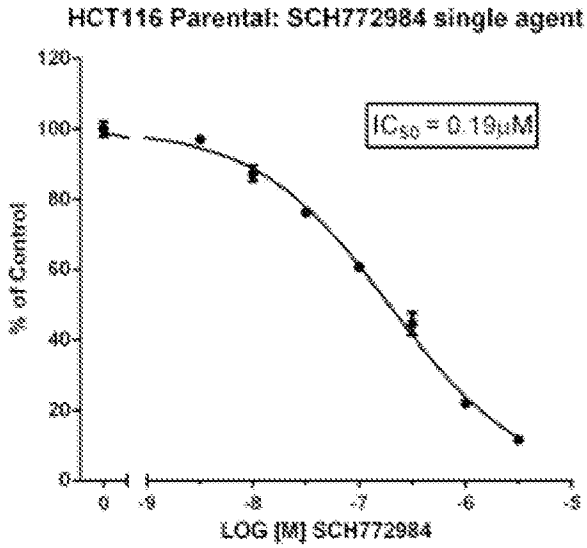


FIG. 8, Continued

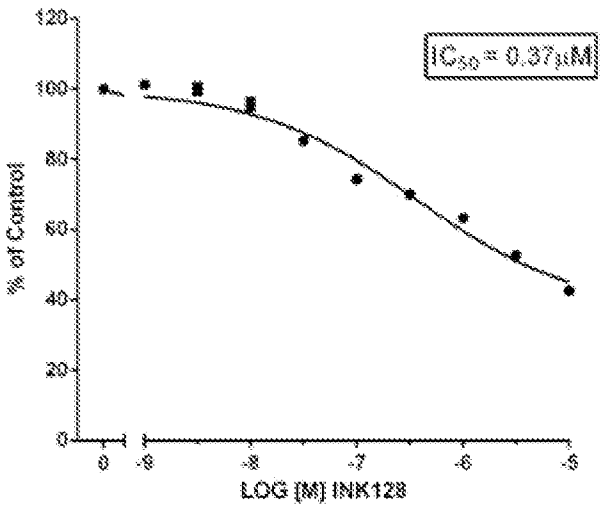
G



H



I



J

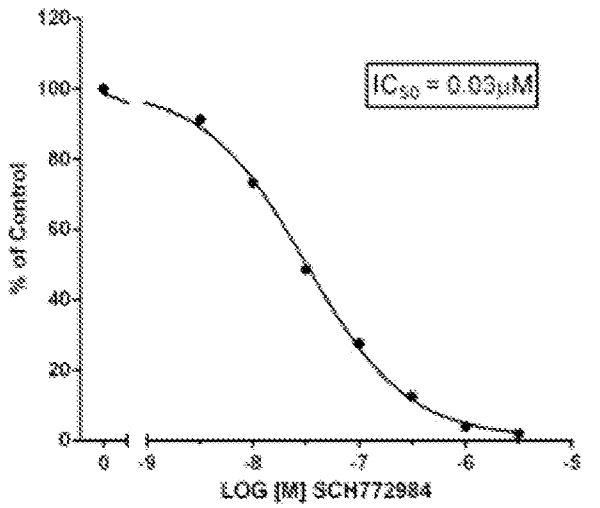


FIG. 9

A

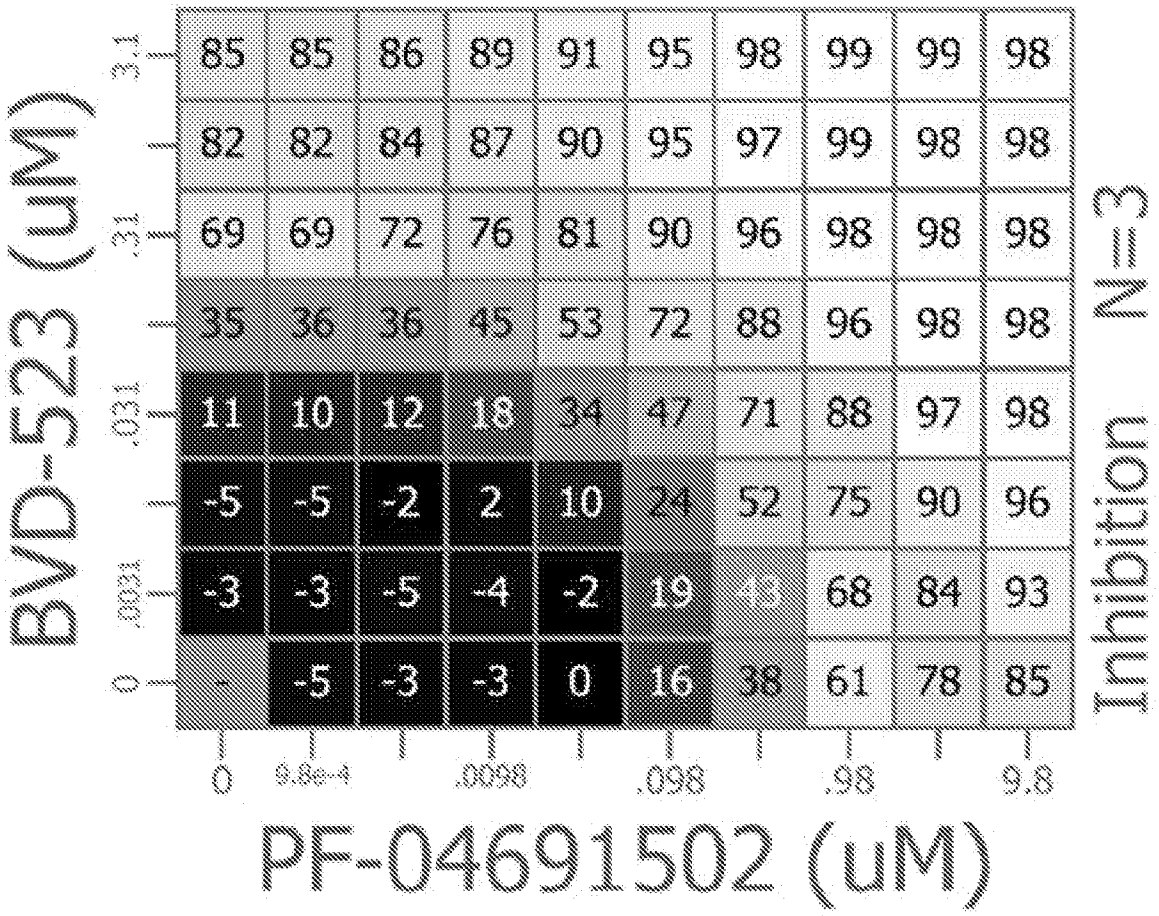

BVD-523 (uM)
3.1
.31
.031
.0031
0
85 85 86 89 91 95 98 99 99 98
82 82 84 87 90 95 97 99 98 98
69 69 72 76 81 90 96 98 98 98
35 36 36 45 53 72 88 96 98 98
11 10 12 18 34 47 71 88 97 98
-5 -5 -2 2 10 24 52 75 90 96
-3 -3 -5 -4 -2 19 48 68 84 93
- -5 -3 -3 0 16 38 61 78 85
0 9.8e-4 .0098 .098 .98 9.8
PF-04691502 (uM)
Inhibition N=3

FIG. 9, Continued

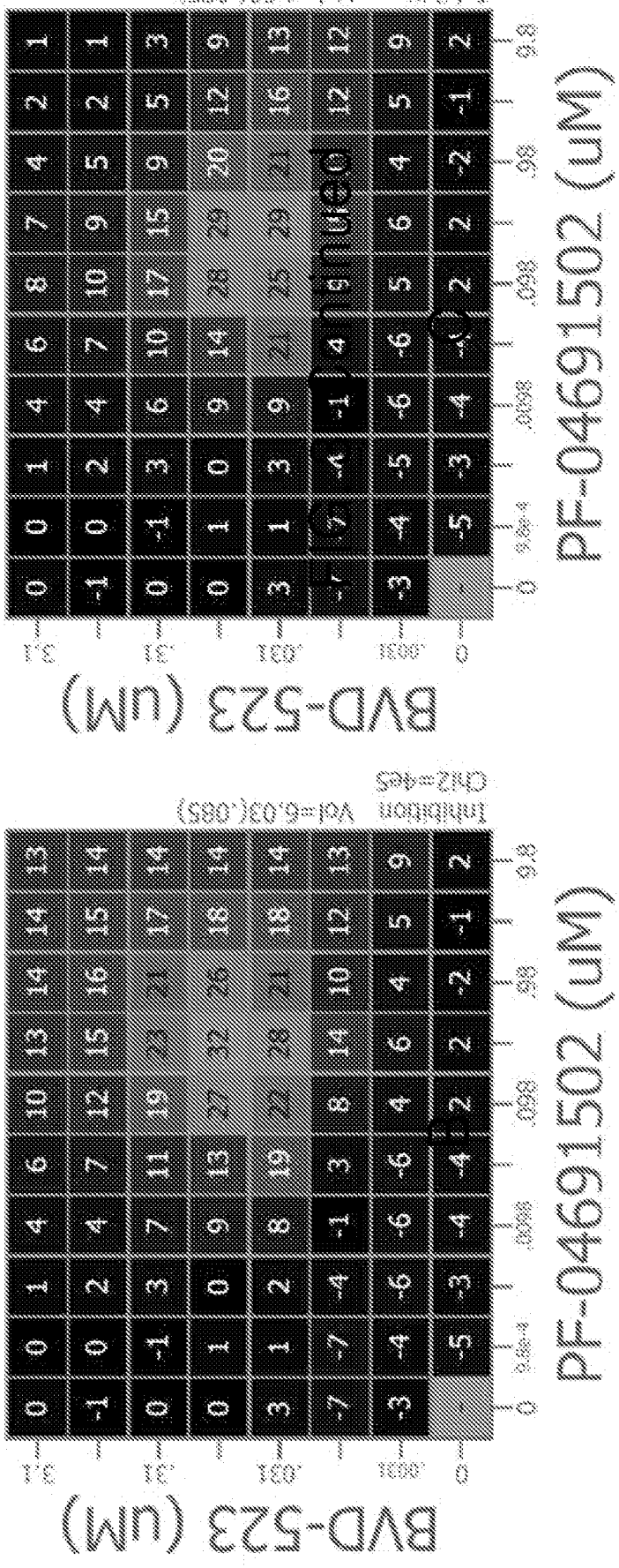


FIG. 9, Continued

D

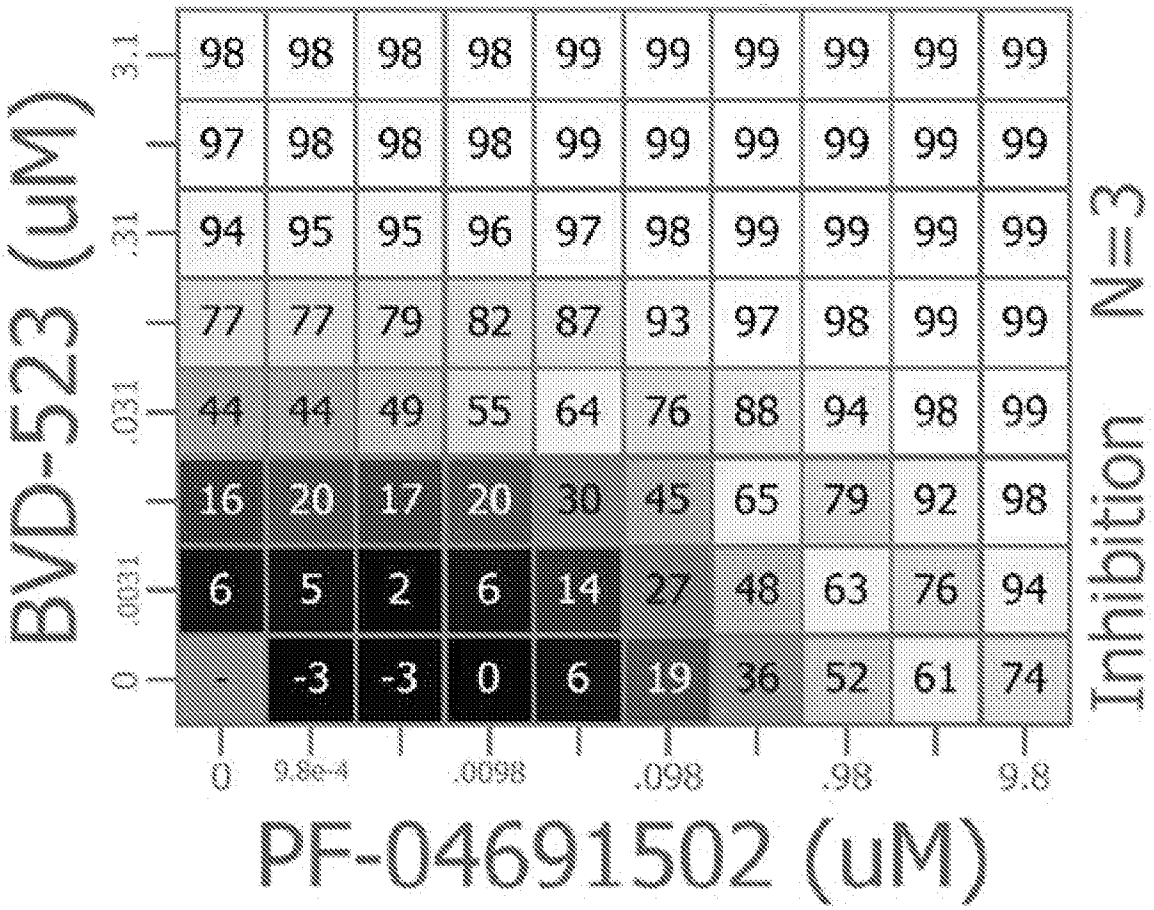


FIG. 9, Continued

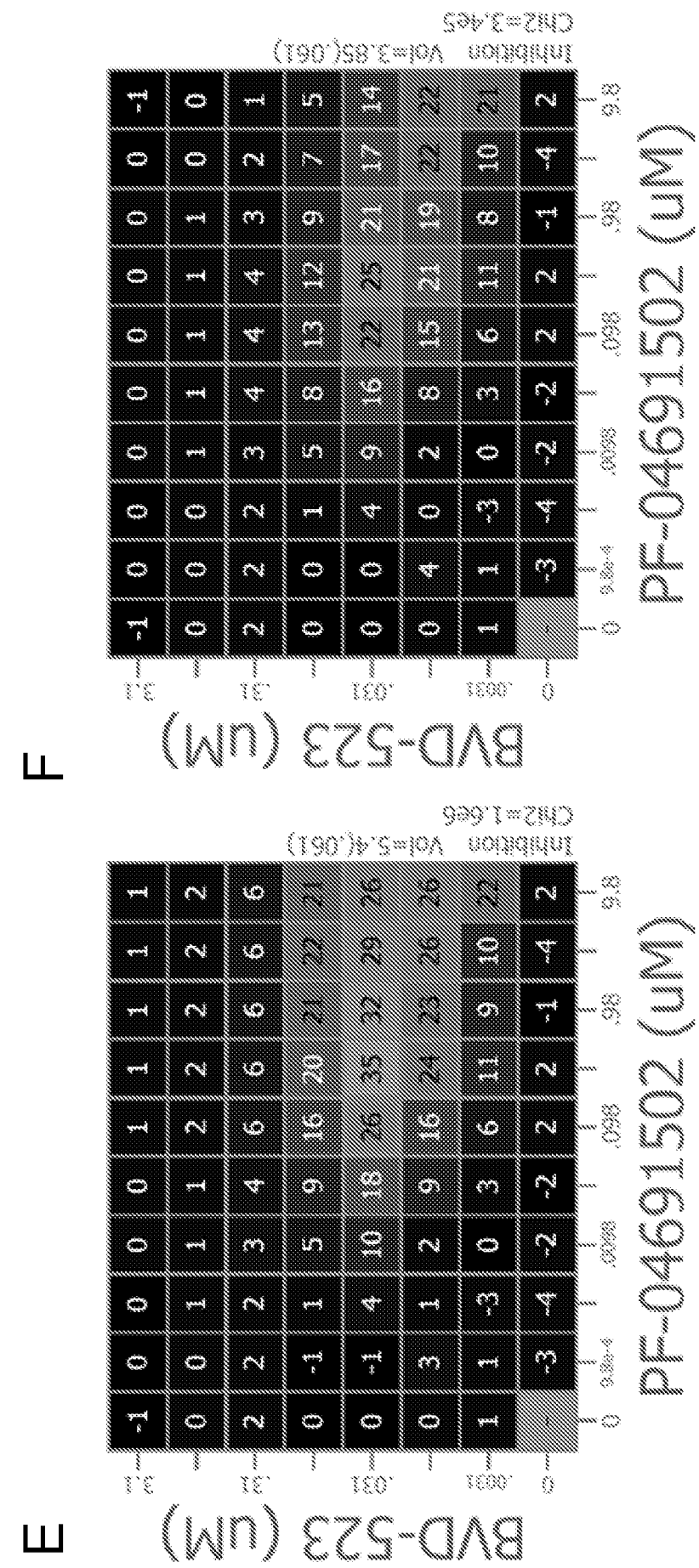
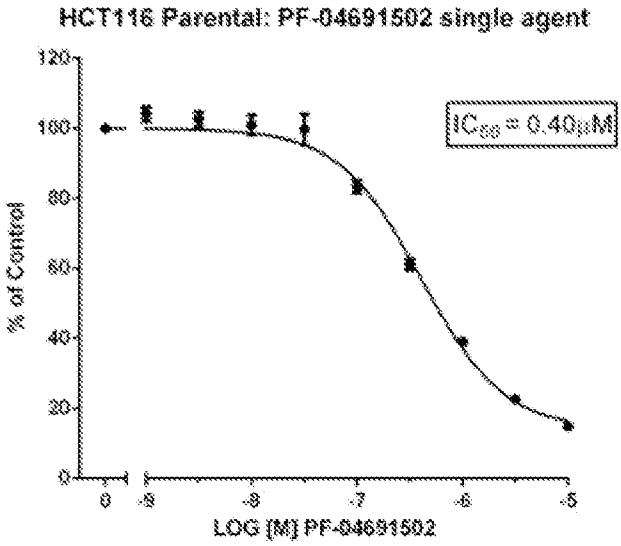
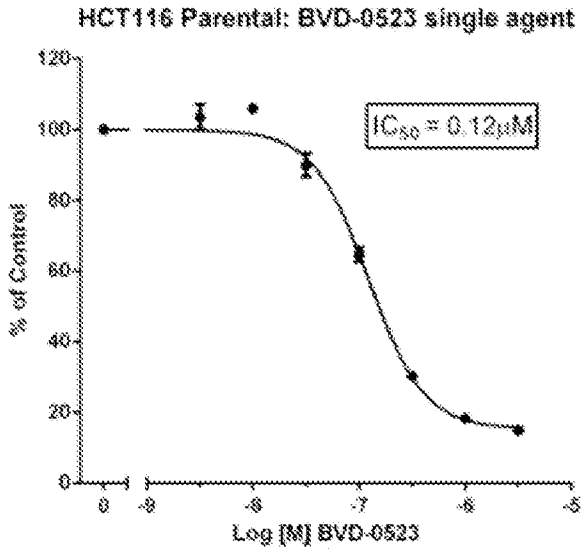


FIG. 9, Continued

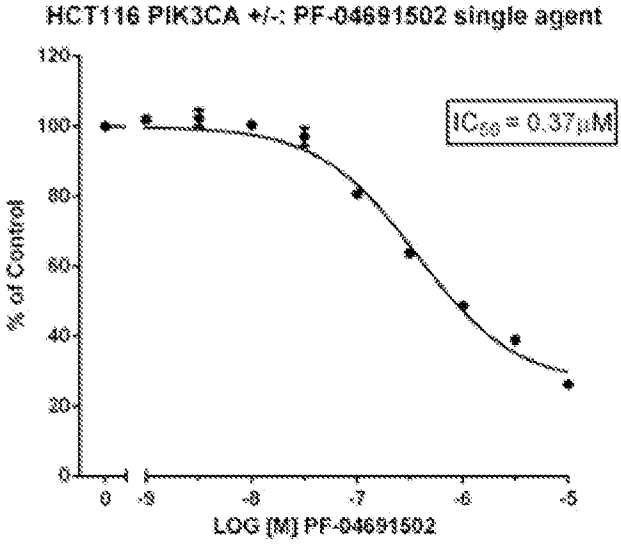
G



H



I



J

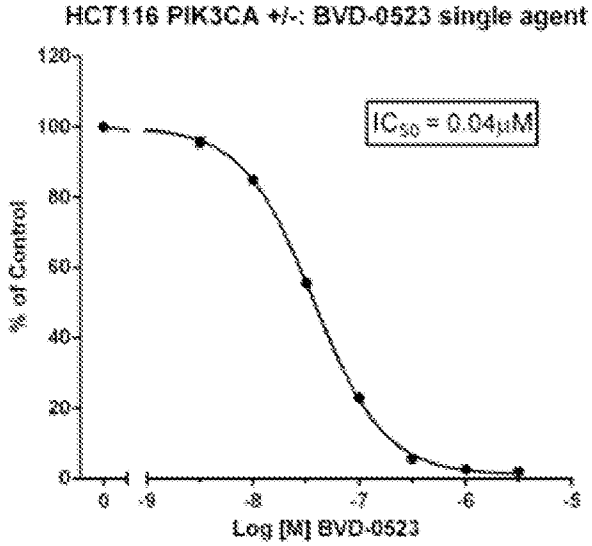


FIG. 10

A

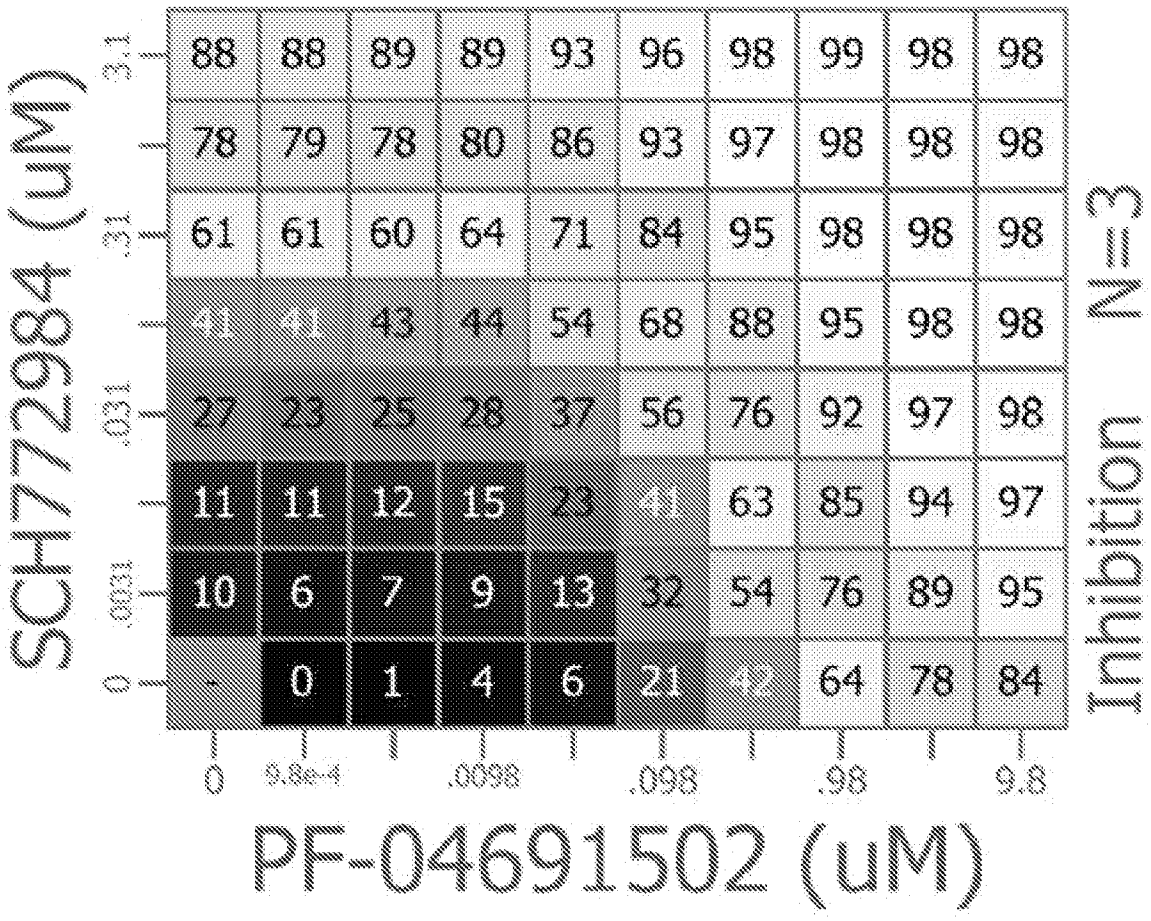

SCH772984 (uM)
3.1
.31
.031
.0031
0
88 88 89 89 93 96 98 99 98 98
78 79 78 80 86 93 97 98 98 98
61 61 60 64 71 84 95 98 98 98
41 41 43 44 54 68 88 95 98 98
27 23 25 28 37 56 76 92 97 98
11 11 12 15 23 41 63 85 94 97
10 6 7 9 13 32 54 76 89 95
- 0 1 4 6 21 42 64 78 84
0
9.8e-4
.0098
.098
.98
9.8
PF-04691502 (uM)
Inhibition N=3

FIG. 10, Continued

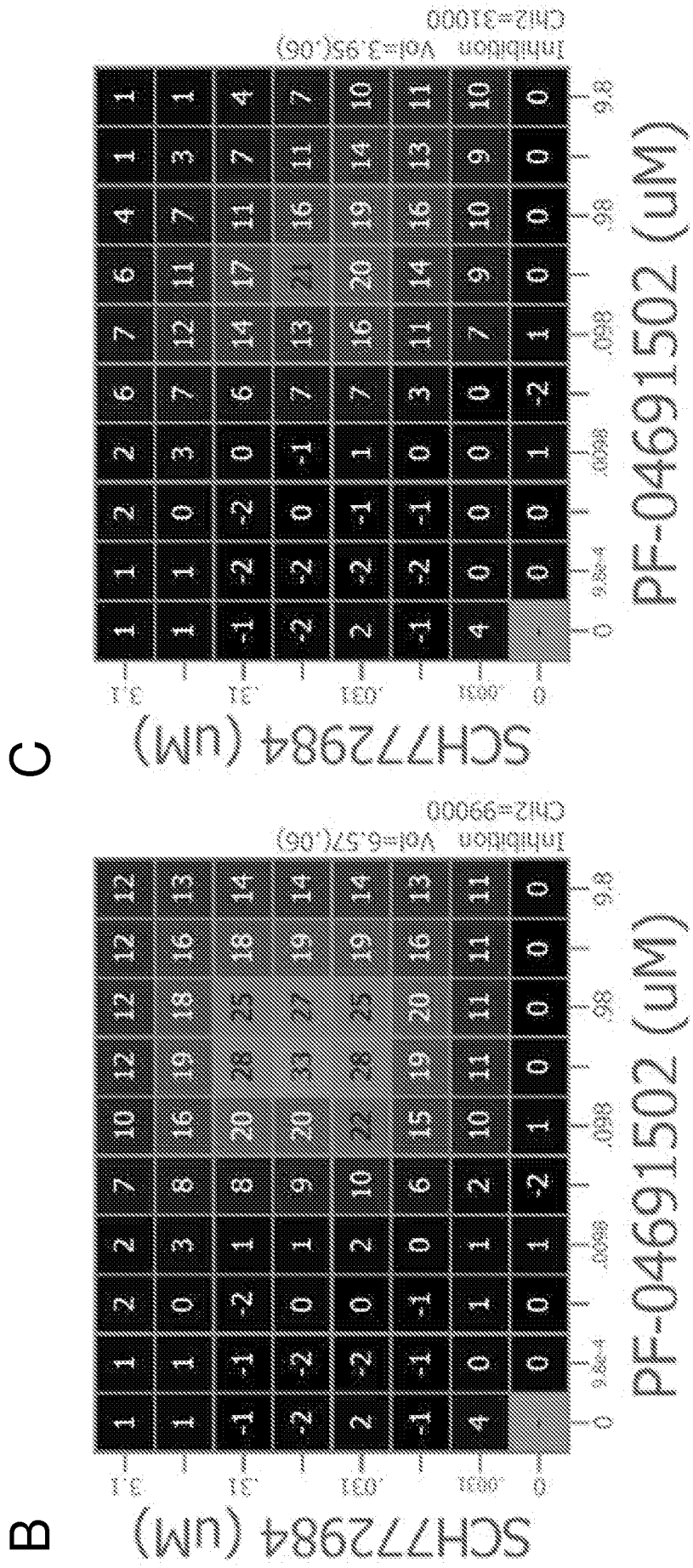


FIG. 10, Continued

D

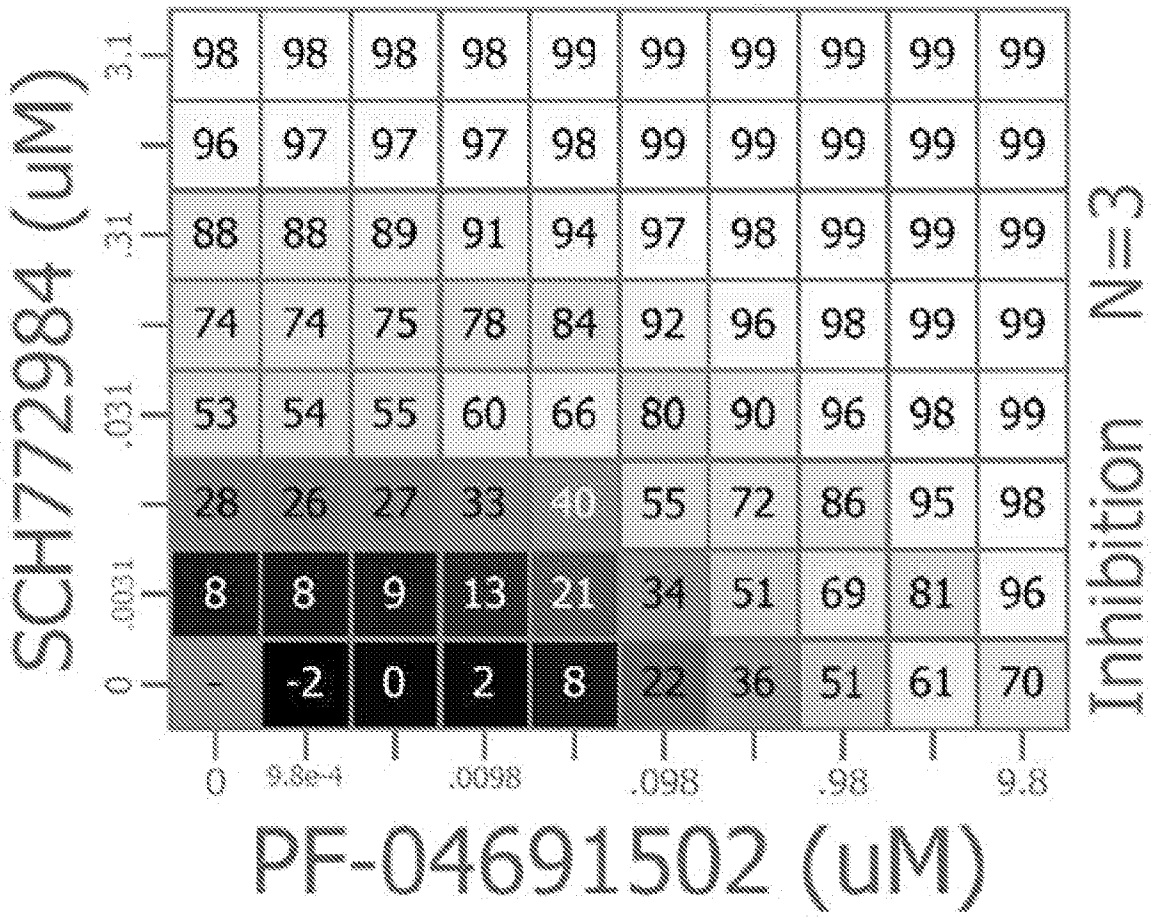


FIG. 10, Continued

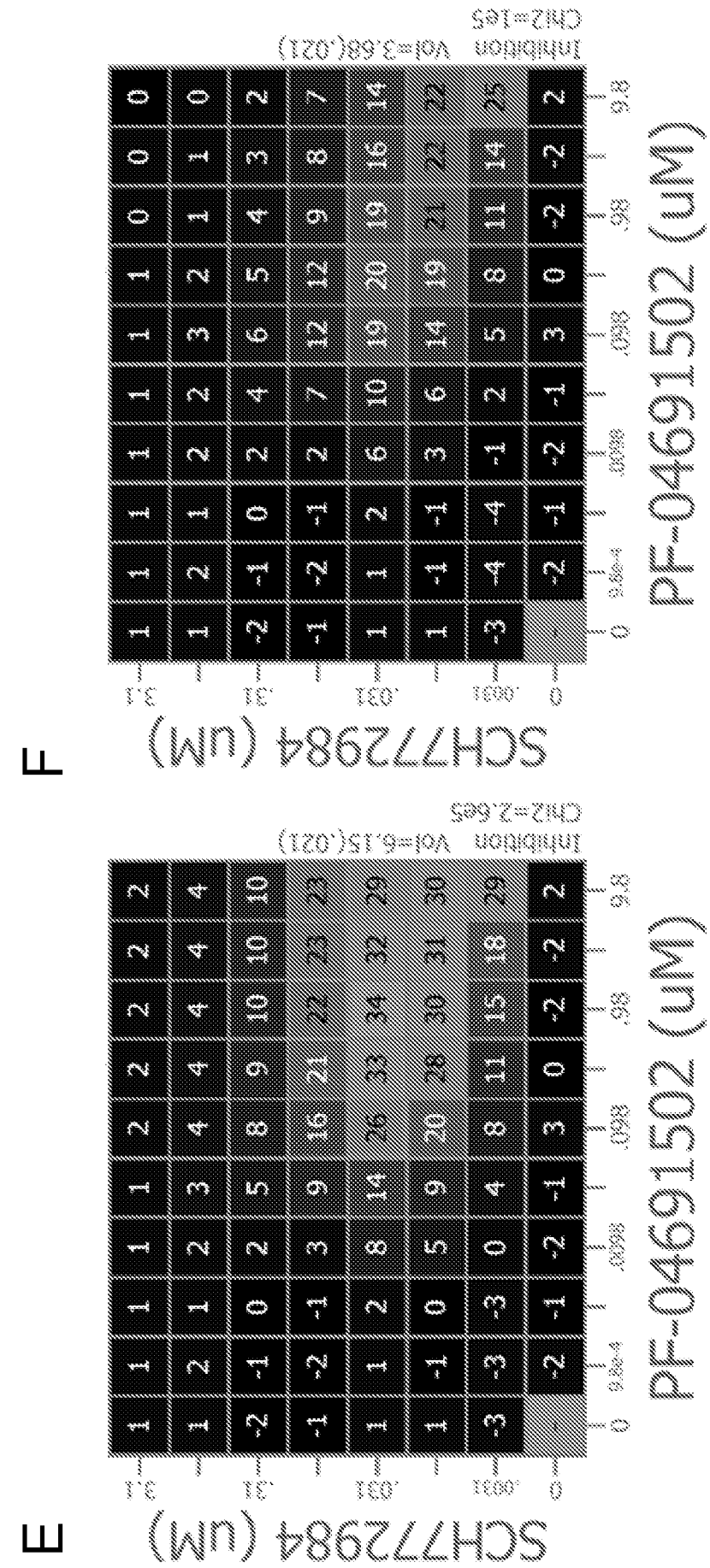
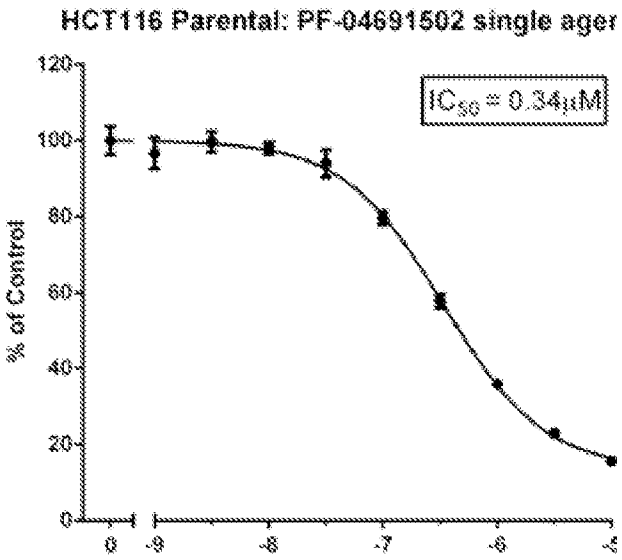
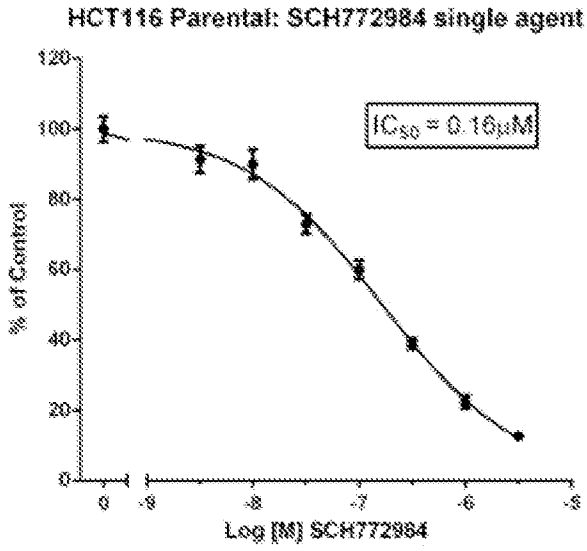


FIG. 10, Continued

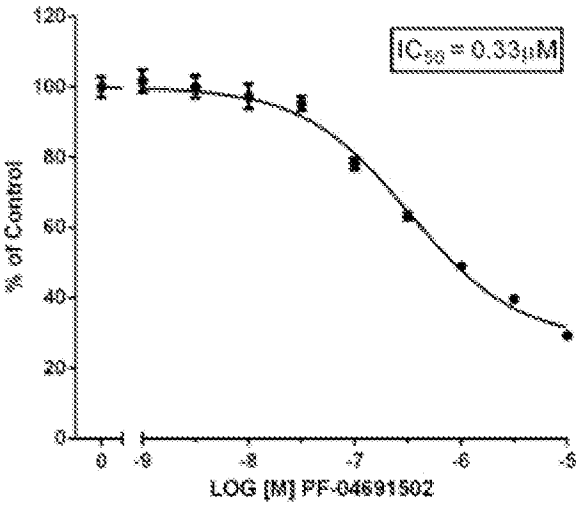
G



H



I



J

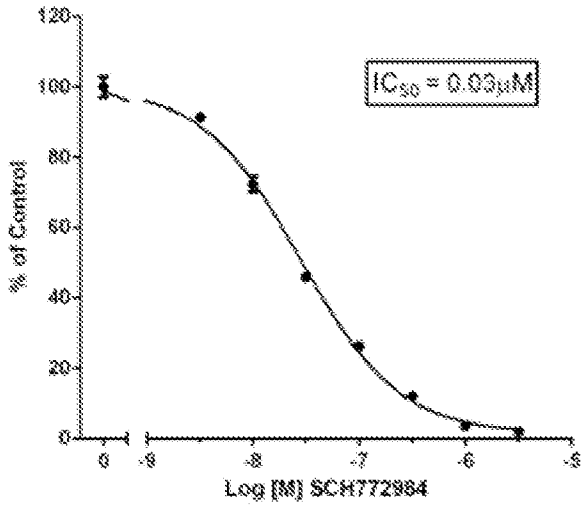


FIG. 11

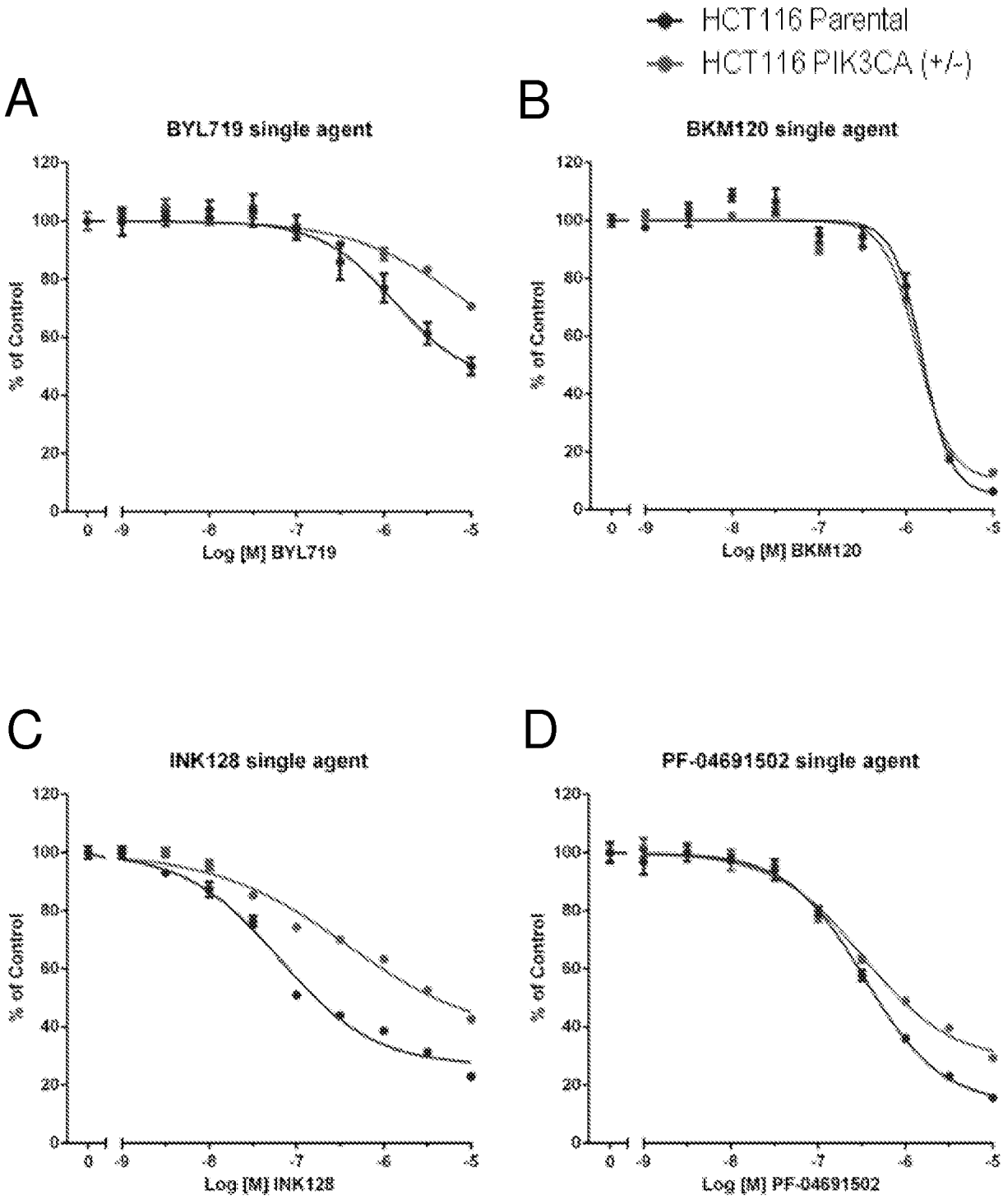
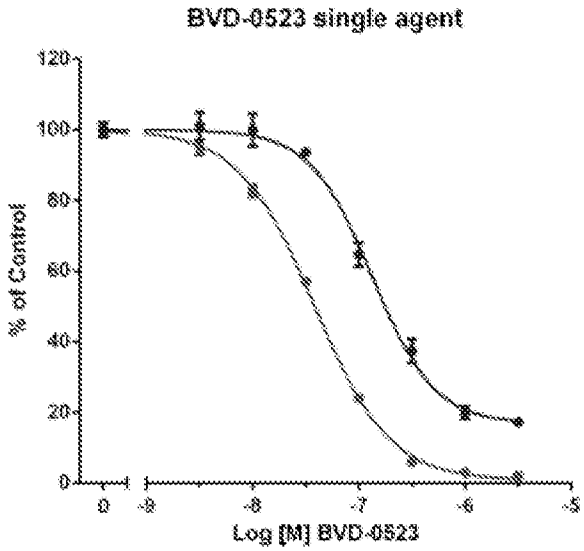

HCT116 Parental
HCT116 PIK3CA (+/-)
A
BYL719 single agent
% of Control
Log [M] BYL719
B
BKM120 single agent
% of Control
Log [M] BKM120
C
INK128 single agent
% of Control
Log [M] INK128
D
PF-04691502 single agent
% of Control
Log [M] PF-04691502

FIG. 11, Continued

E



F

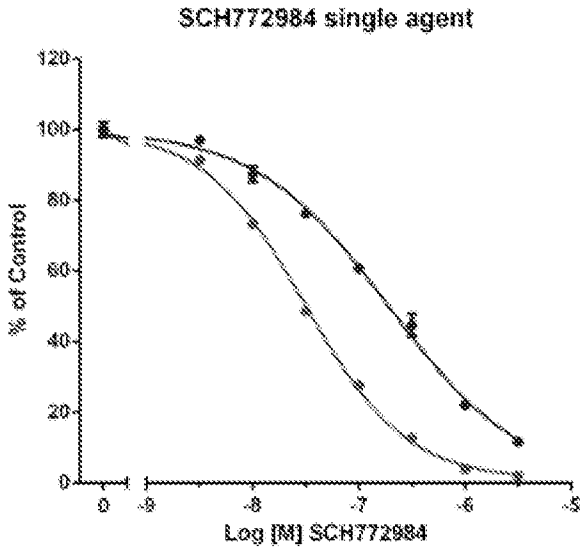


FIG. 12

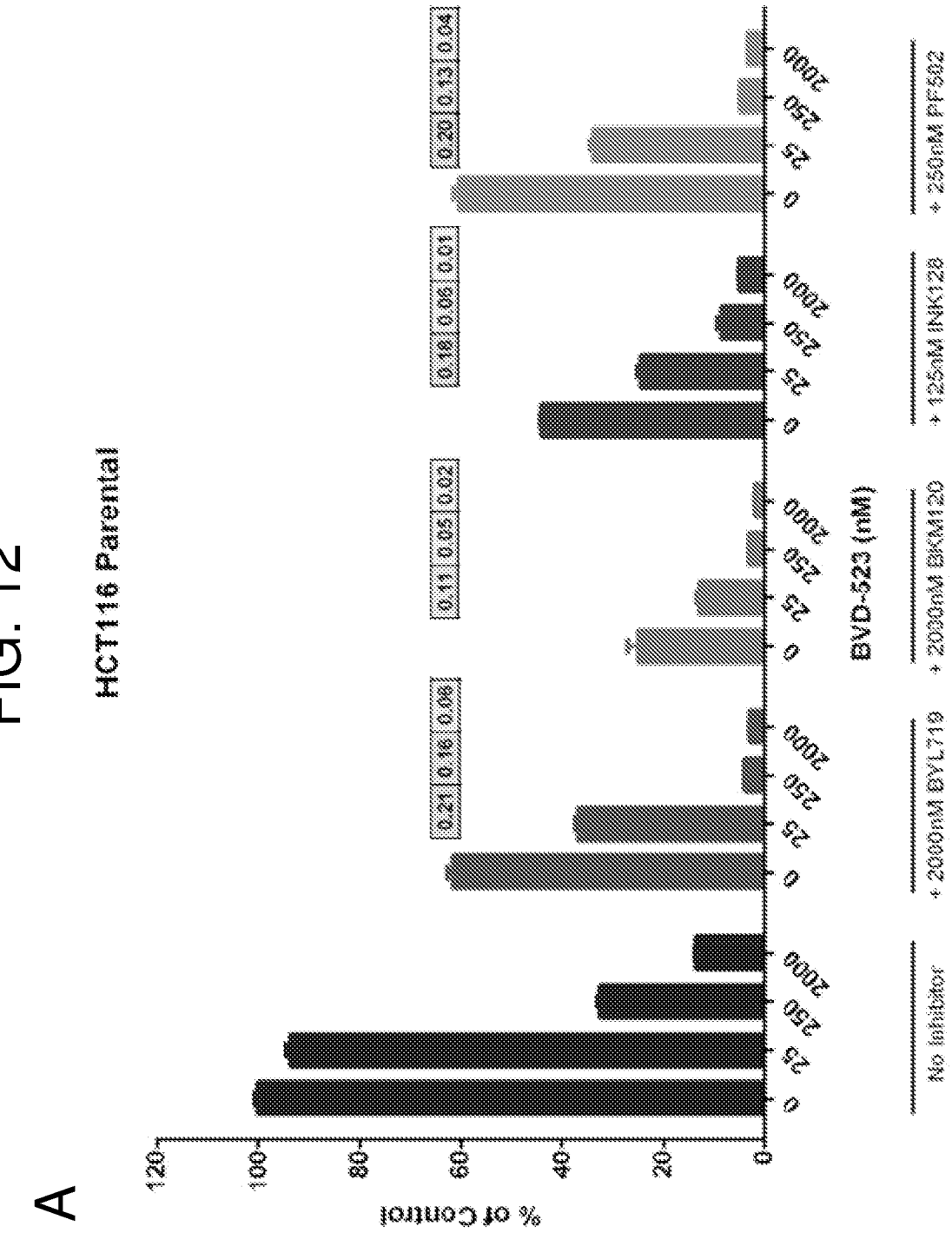

A
HCT116 Parental
% of Control
120
100
80
60
40
20
0
0.21 0.16 0.09
0.11 0.05 0.02
0.18 0.06 0.01
0.20 0.13 0.04
0
25
250
2000
BVD-523 (nM)
No Inhibitor
+ 2000nM BYL719
+ 2000nM BKM120
+ 125nM INK128
+ 250nM PF502

FIG. 12, Continued

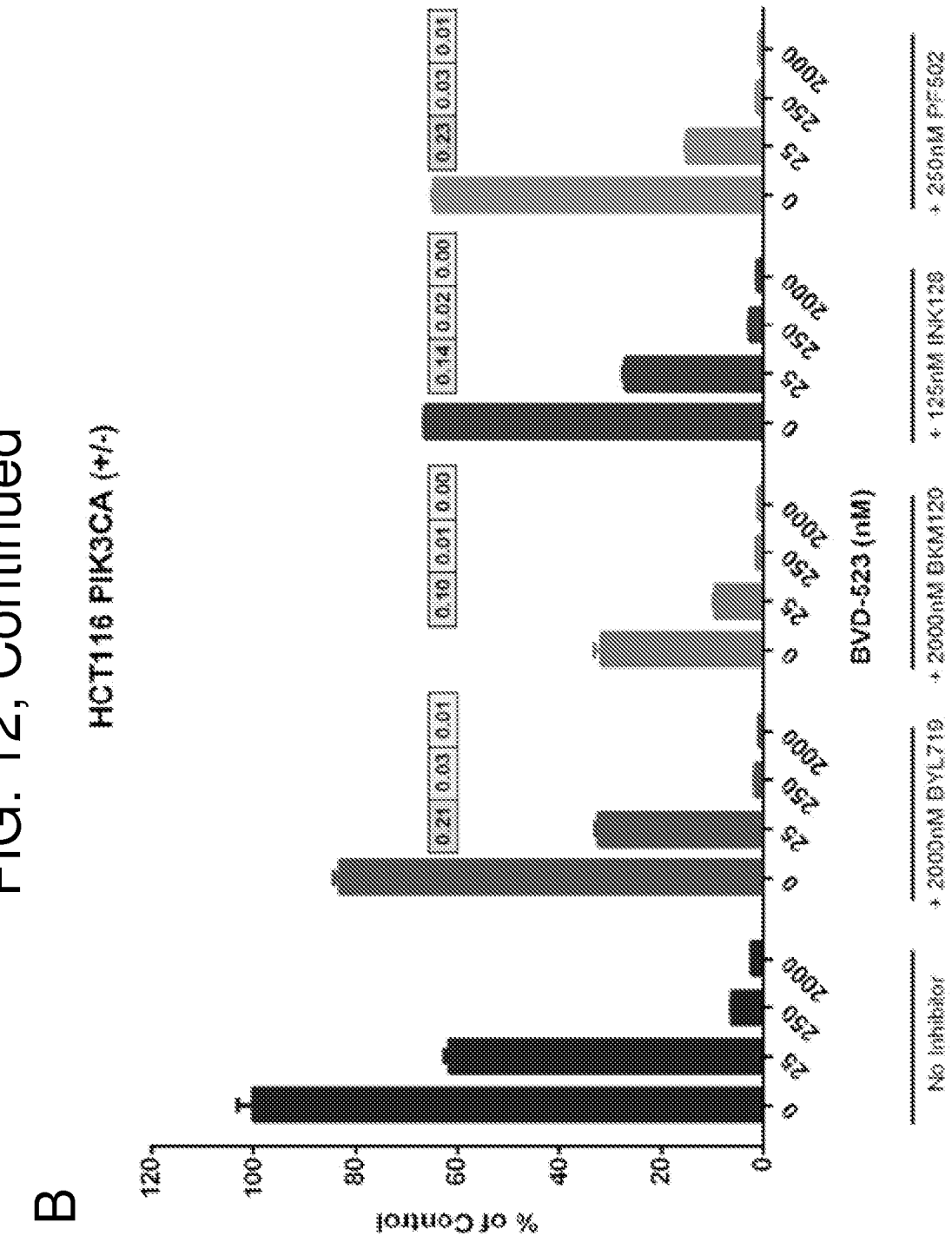


FIG. 12, Continued

C

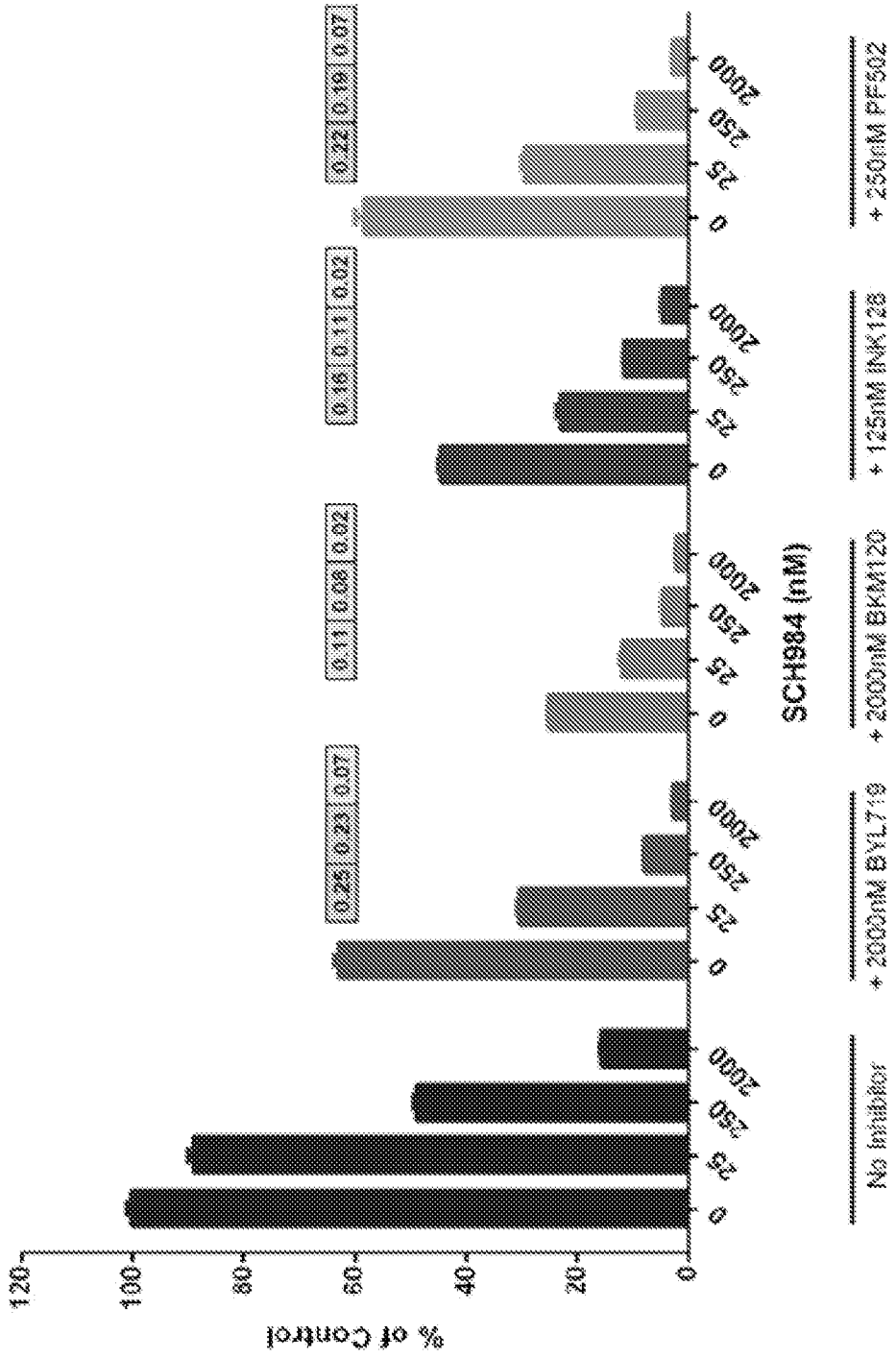


FIG. 12, Continued

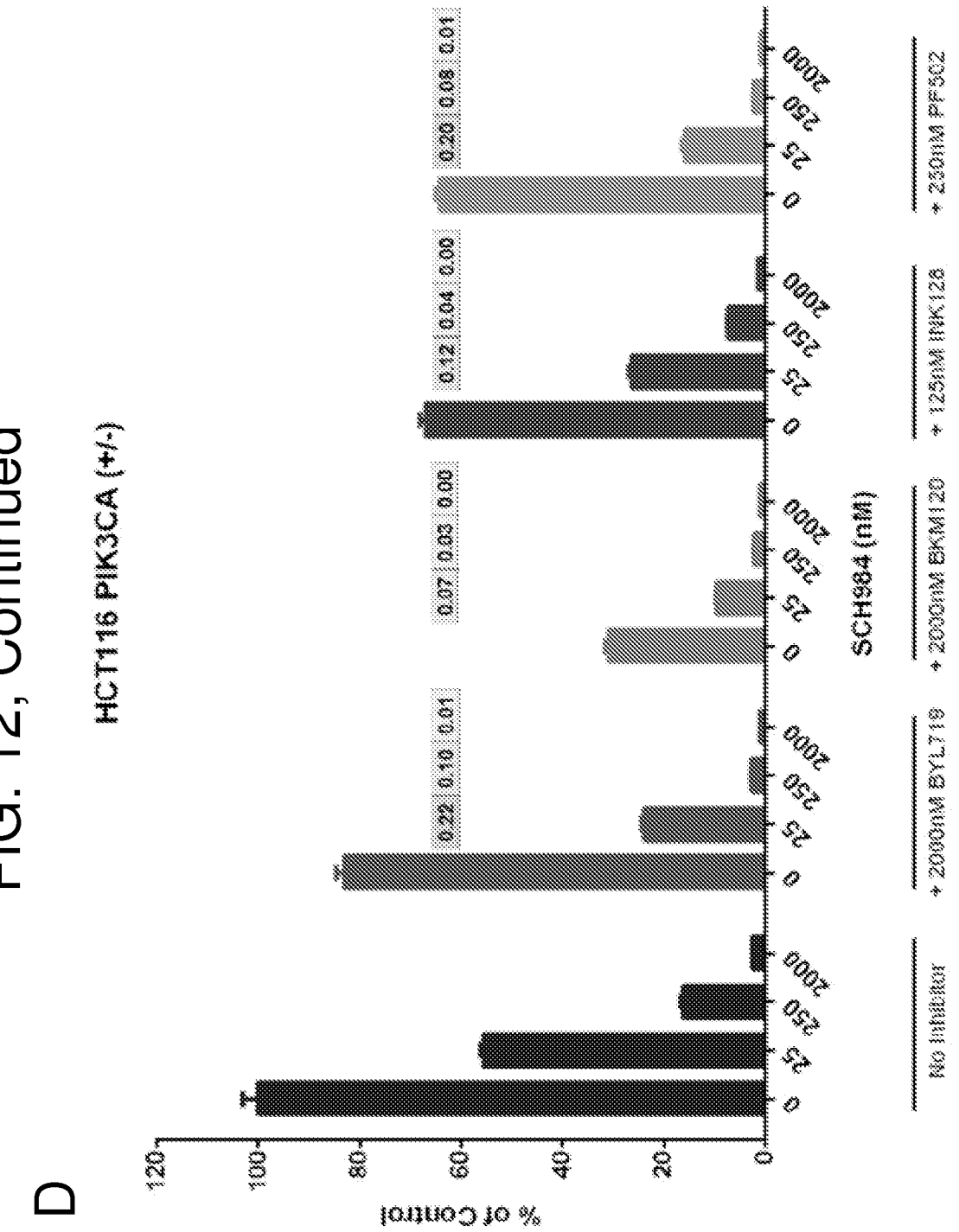


FIG. 13

A

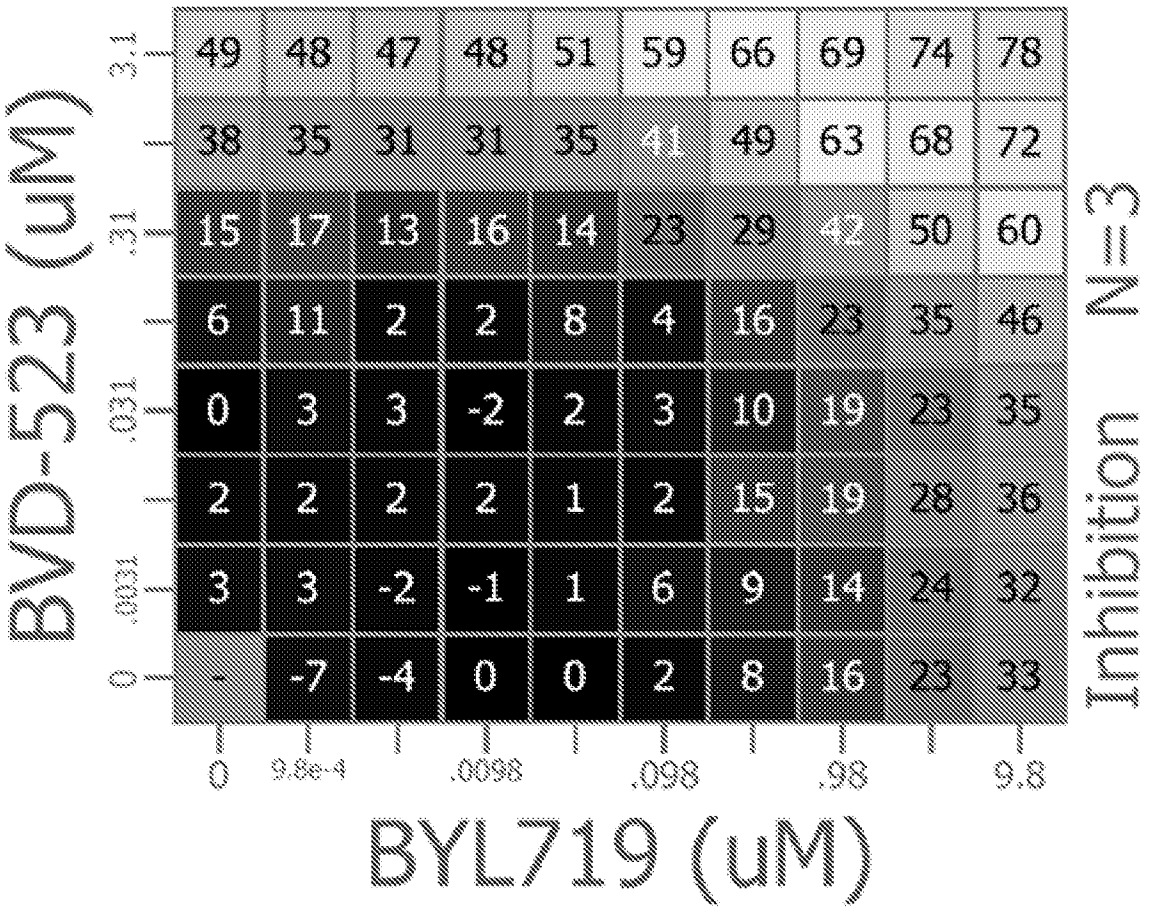

BVD-523 (uM)
3.1
.31
.031
.0031
0
49 48 47 48 51 59 66 69 74 78
38 35 31 31 35 41 49 63 68 72
15 17 13 16 14 23 29 42 50 60
6 11 2 2 8 4 16 23 35 46
0 3 3 -2 2 3 10 19 23 35
2 2 2 2 1 2 15 19 28 36
3 3 -2 -1 1 6 9 14 24 32
- -7 -4 0 0 2 8 16 23 33
Inhibition N=3
0
9.8e-4
.0098
.098
.98
9.8
BYL719 (uM)

FIG. 13, Continued

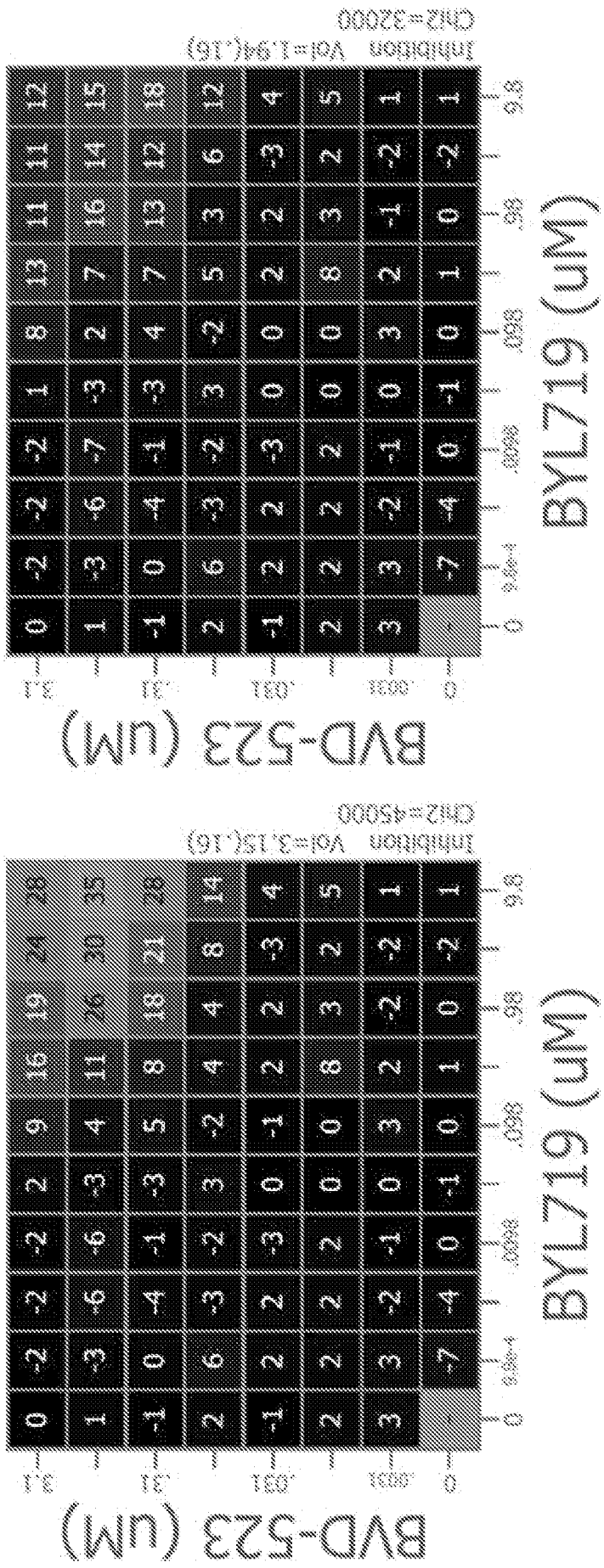


FIG. 13, Continued

D

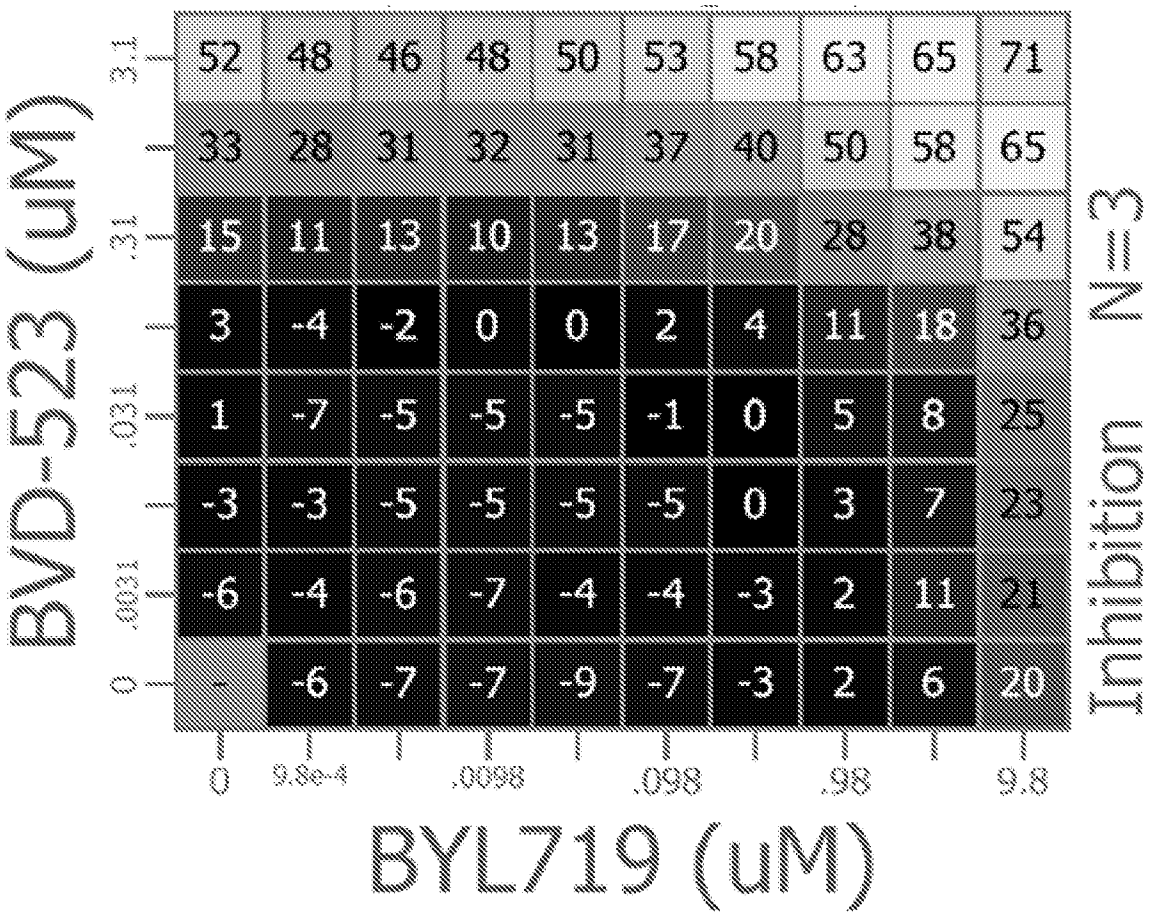


FIG. 13, Continued

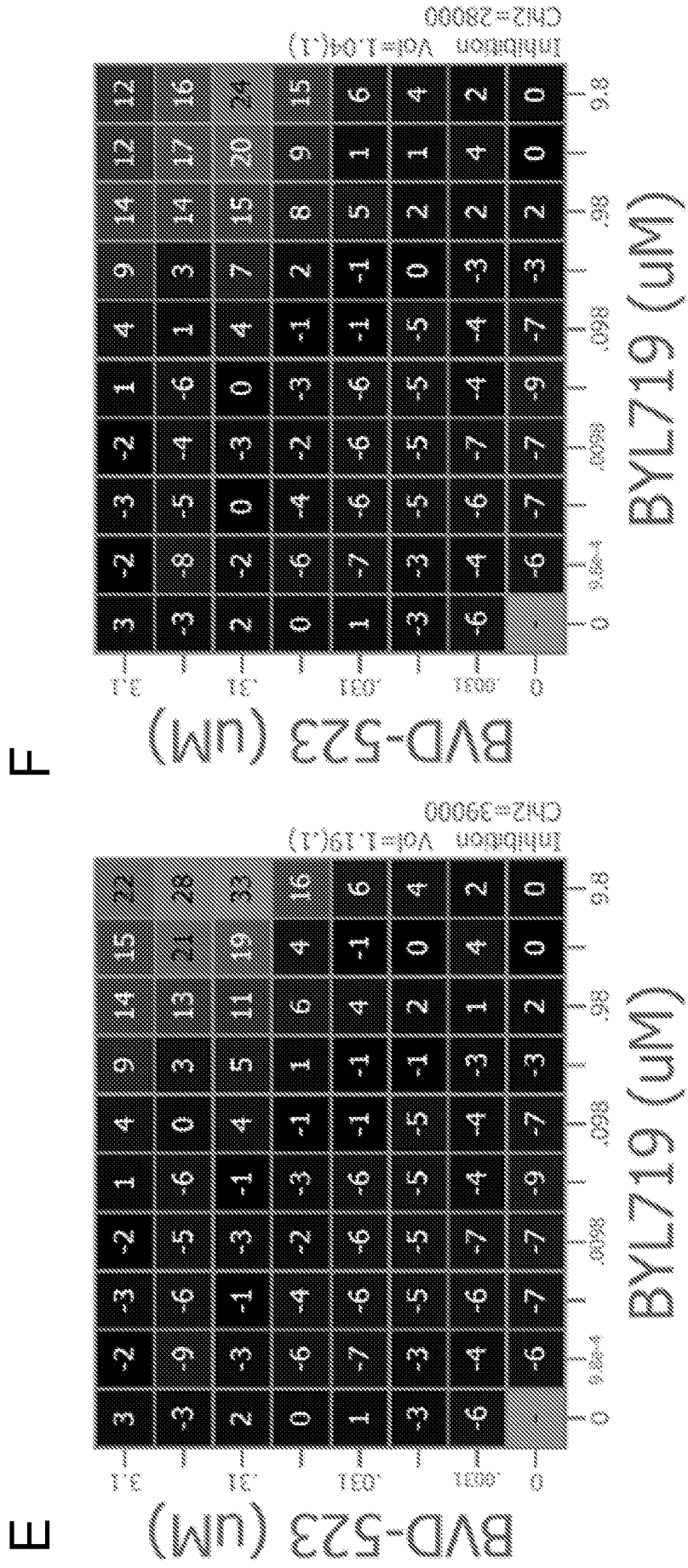
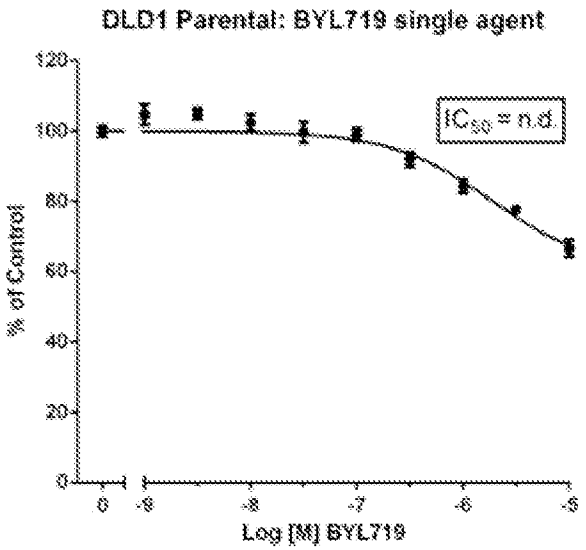
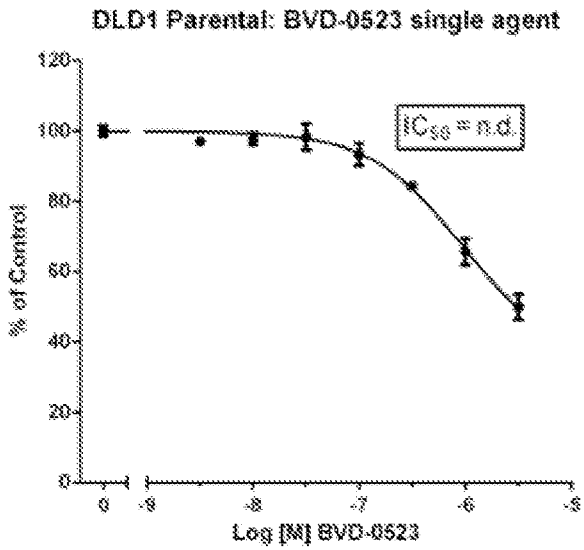


FIG. 13, Continued

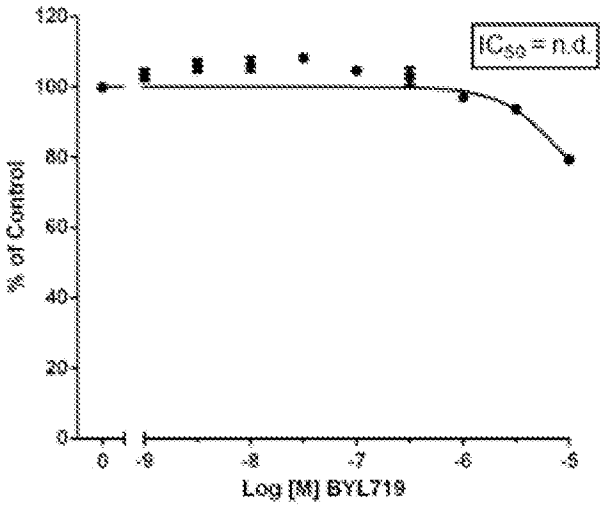
G



H



I



J

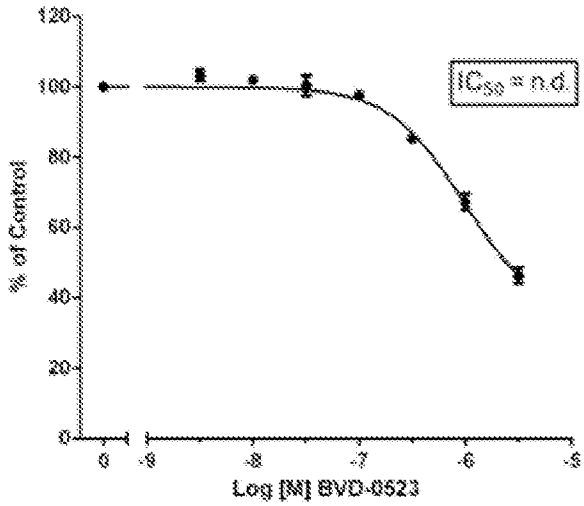


FIG. 14

A

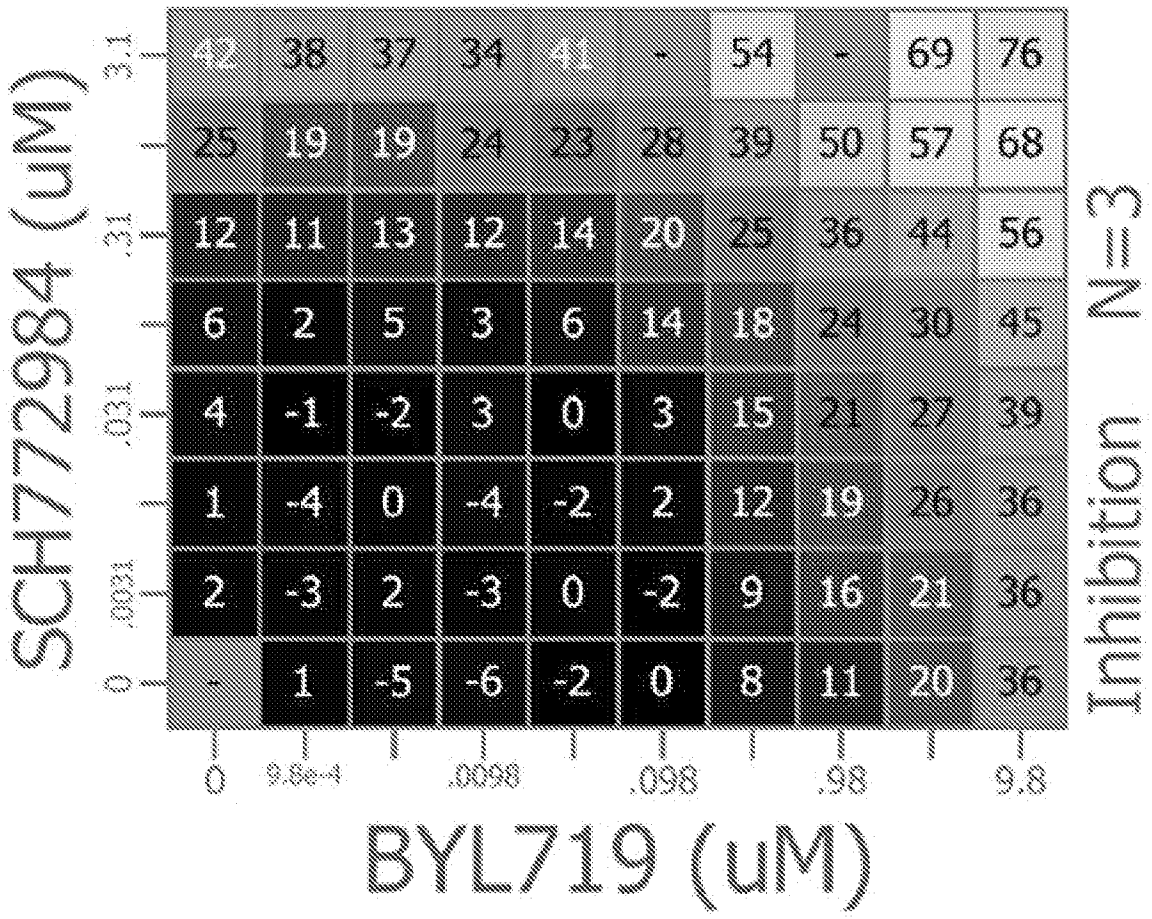

SCH772984 (uM)
3.1
.31
.031
.0031
0
42 38 37 34 41 - 54 - 69 76
25 19 19 24 23 28 39 50 57 68
12 11 13 12 14 20 25 36 44 56
6 2 5 3 6 14 18 24 30 45
4 -1 -2 3 0 3 15 21 27 39
1 -4 0 -4 -2 2 12 19 26 36
2 -3 2 -3 0 -2 9 16 21 36
- 1 -5 -6 -2 0 8 11 20 36
0
9.8e-4
.0098
.098
.98
9.8
BYL719 (uM)
Inhibition N=3

FIG. 14, Continued

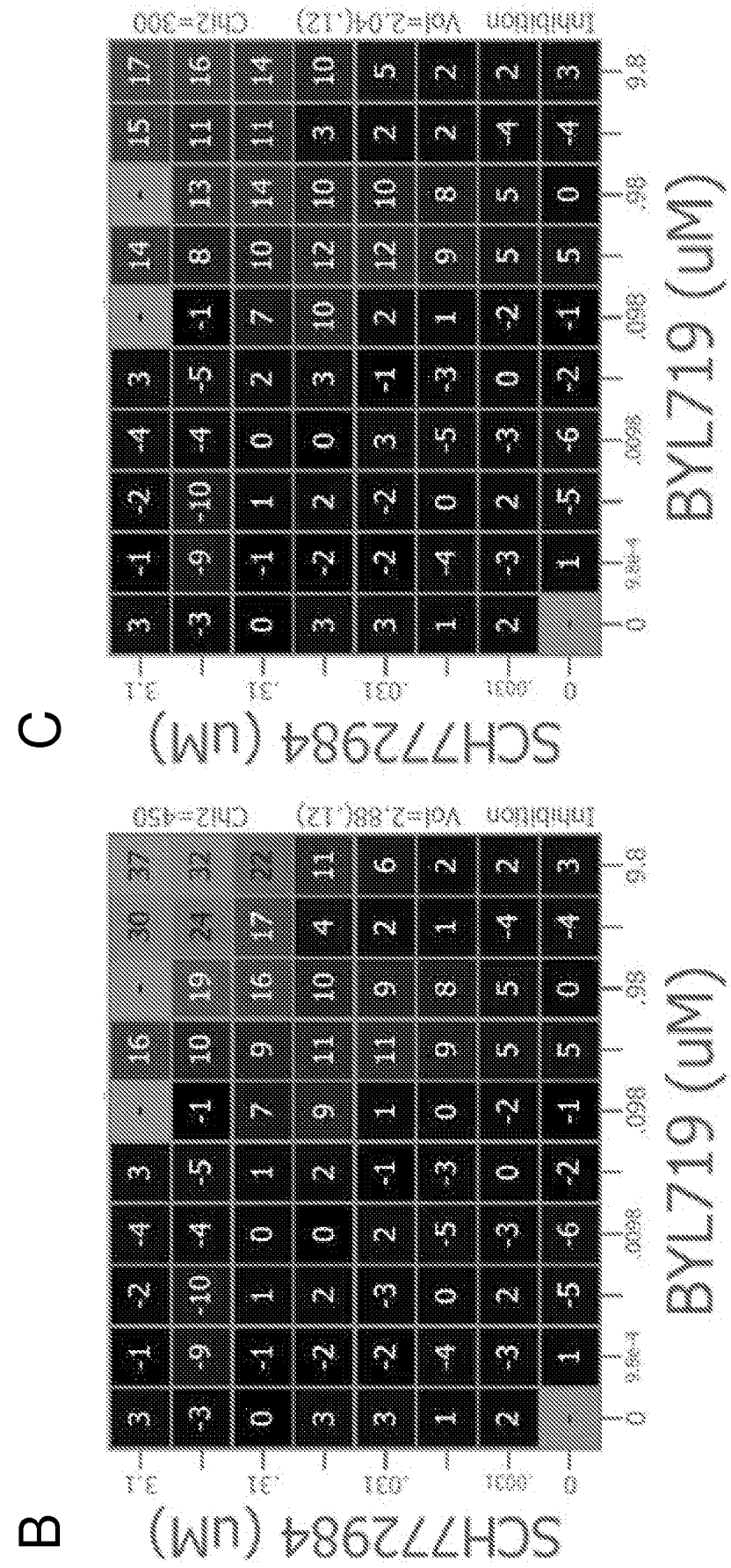


FIG. 14, Continued

D

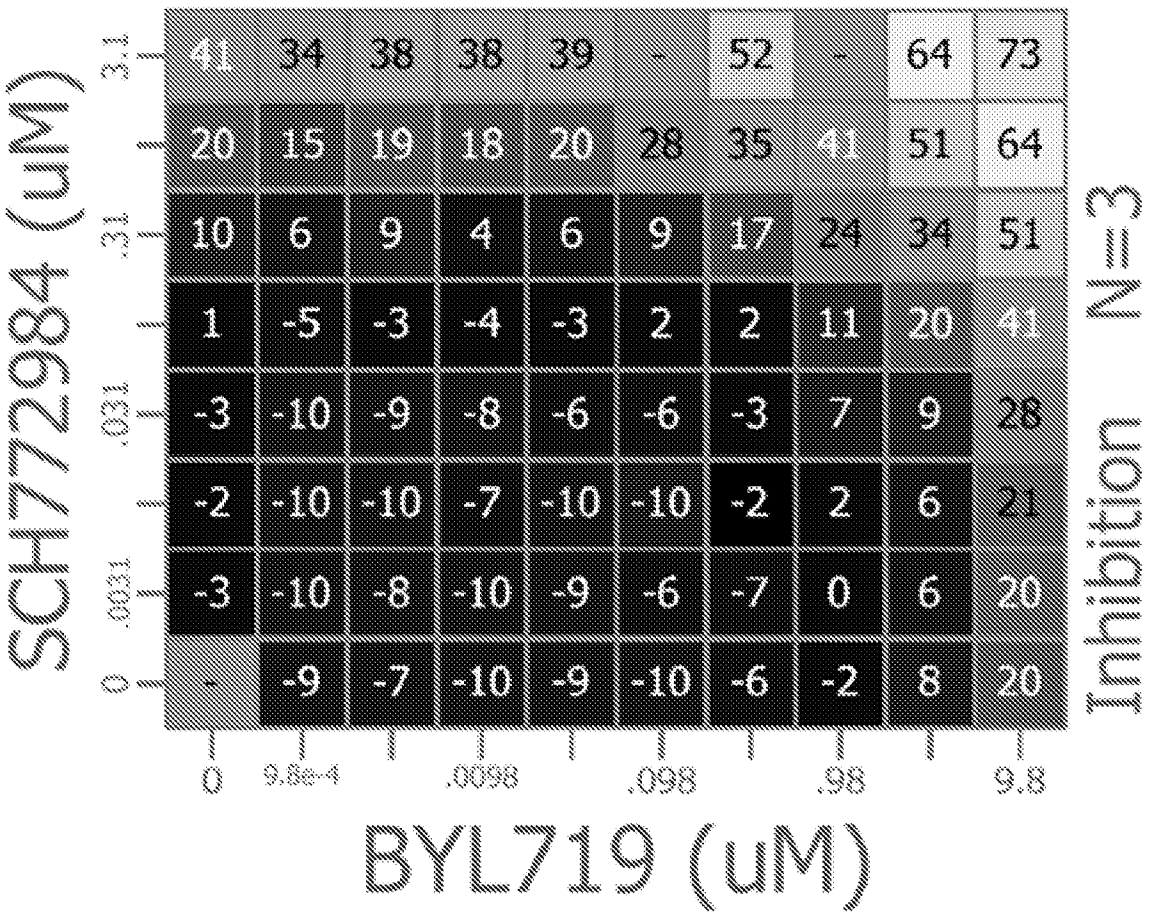


FIG. 14, Continued

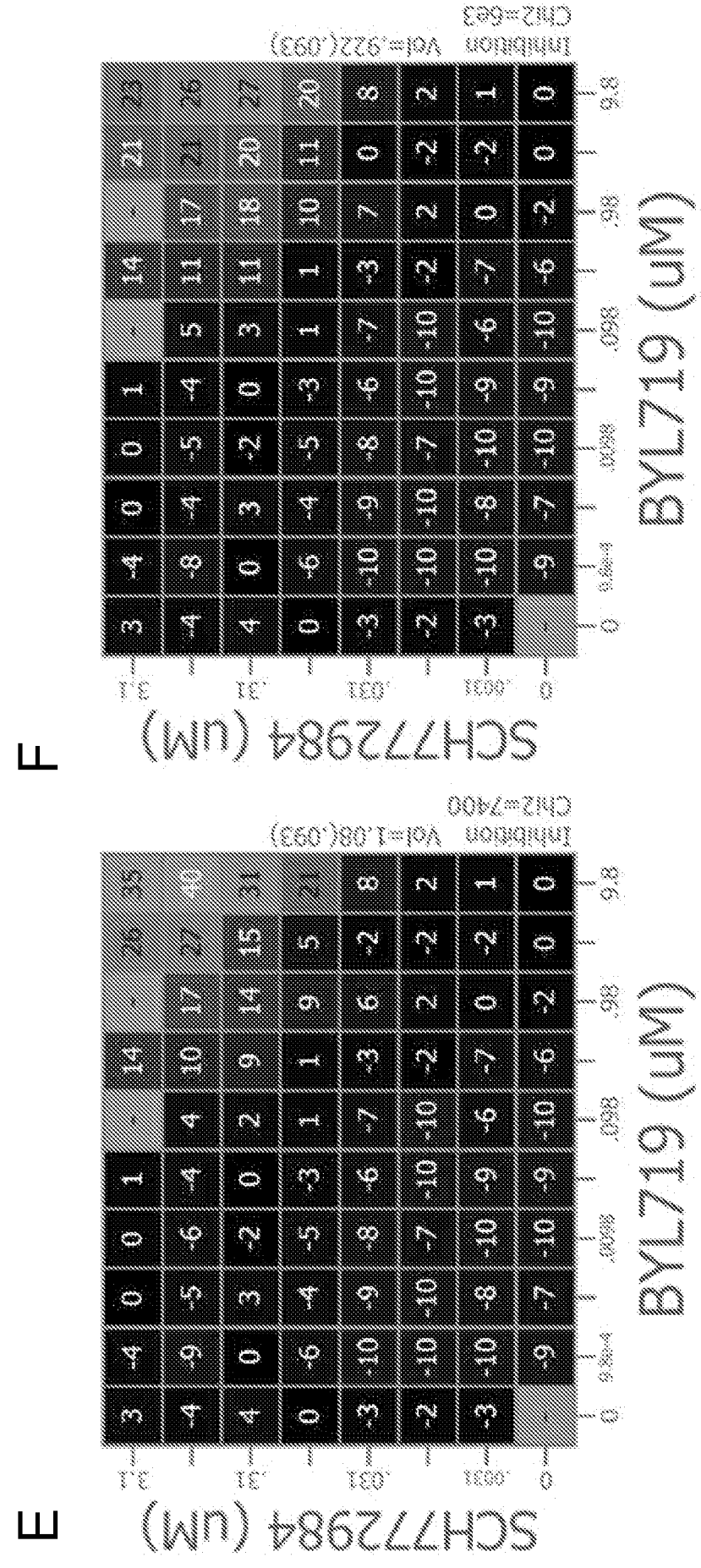
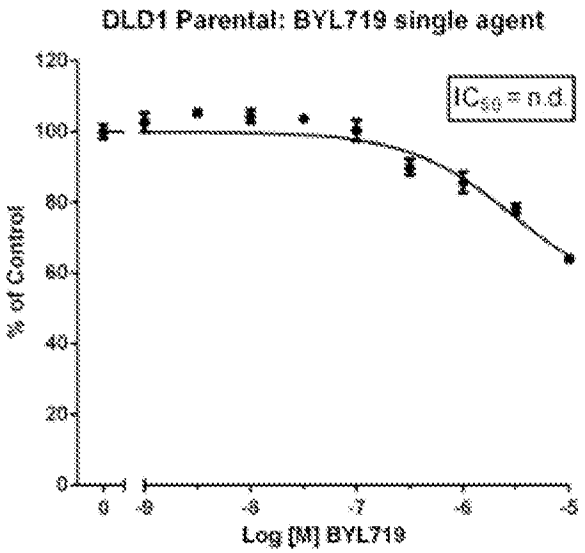
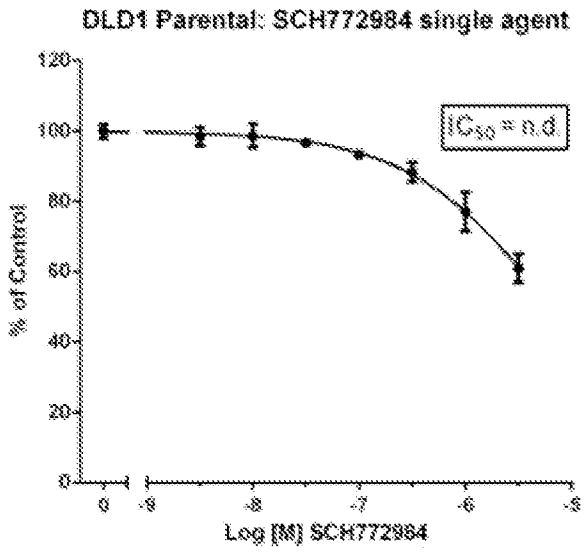


FIG. 14, Continued

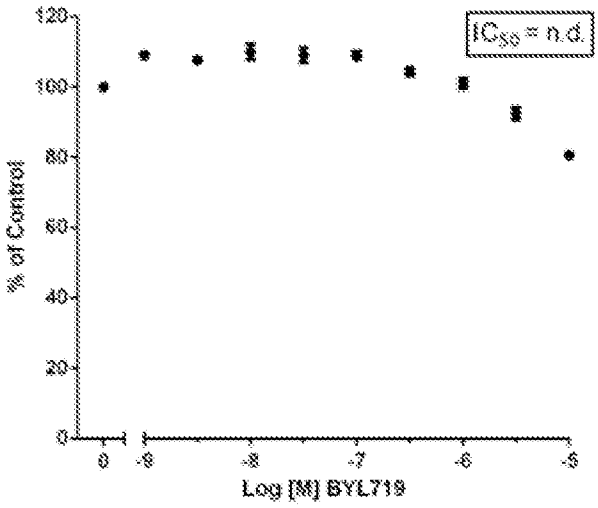
G



H



I



J

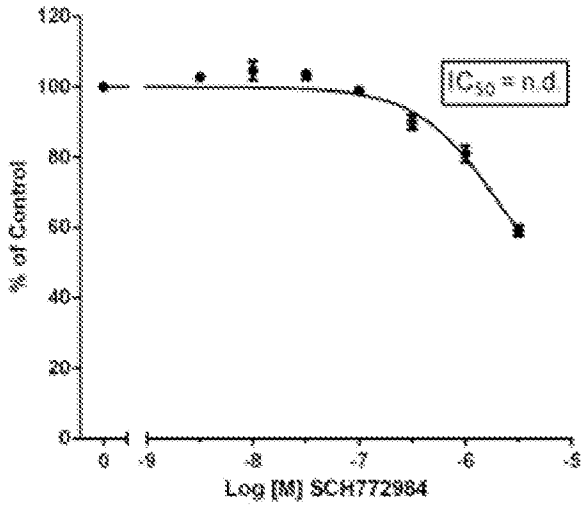


FIG. 15

A

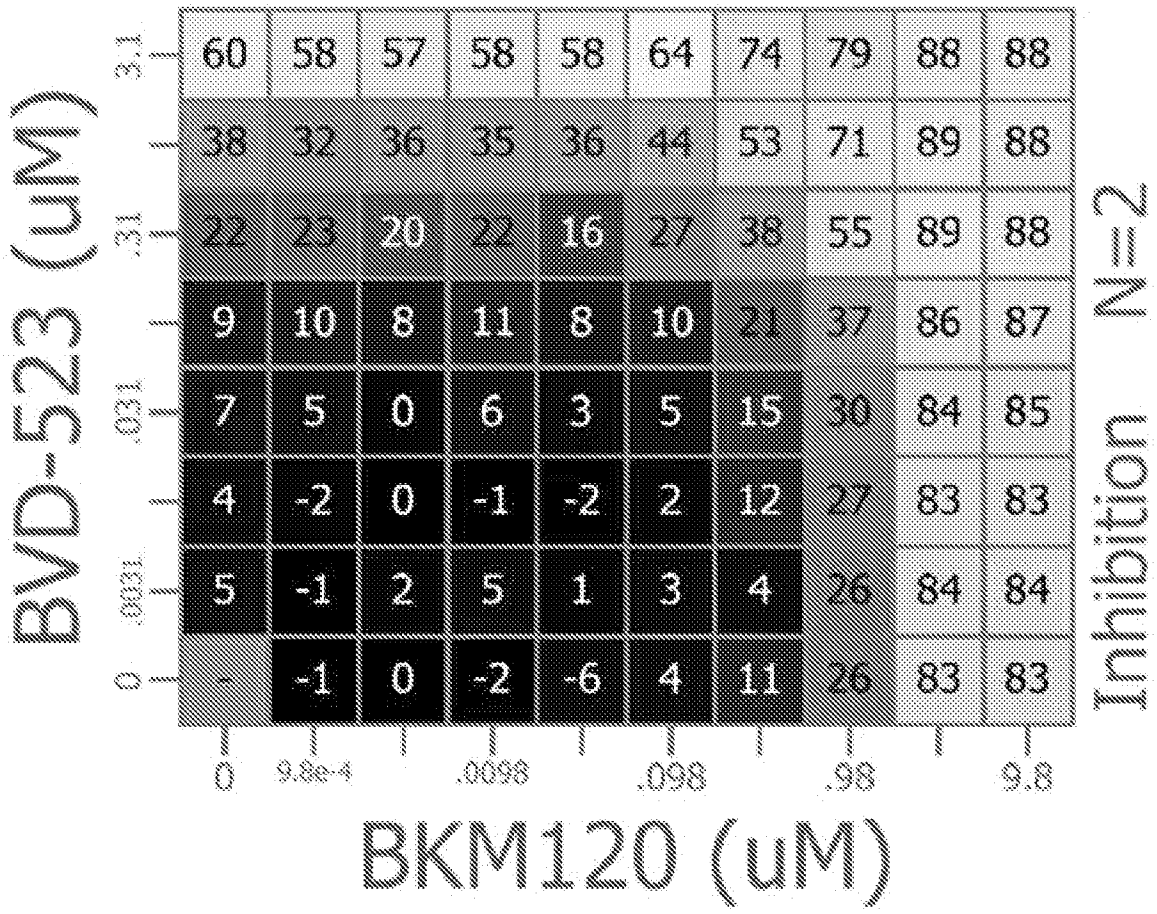

BVD-523 (uM)
3.1
.31
.031
.0031
0
60 58 57 58 58 64 74 79 88 88
38 32 36 35 36 44 53 71 89 88
22 23 20 22 16 27 38 55 89 88
9 10 8 11 8 10 21 37 86 87
7 5 0 6 3 5 15 30 84 85
4 -2 0 -1 -2 2 12 27 83 83
5 -1 2 5 1 3 4 26 84 84
- -1 0 -2 -6 4 11 26 83 83
0 9.8e-4 .0098 .098 .98 9.8
BKM120 (uM)
Inhibition N=2

FIG. 15, Continued

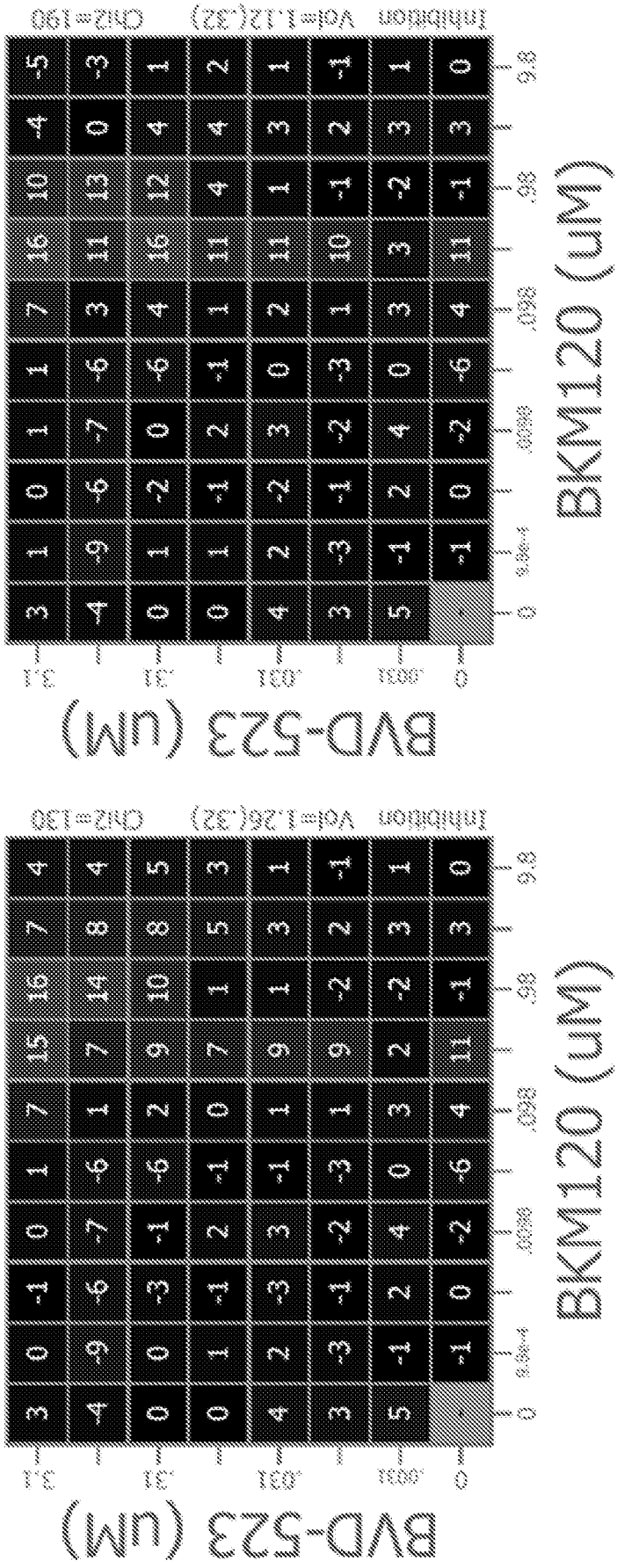


FIG. 15, Continued

D

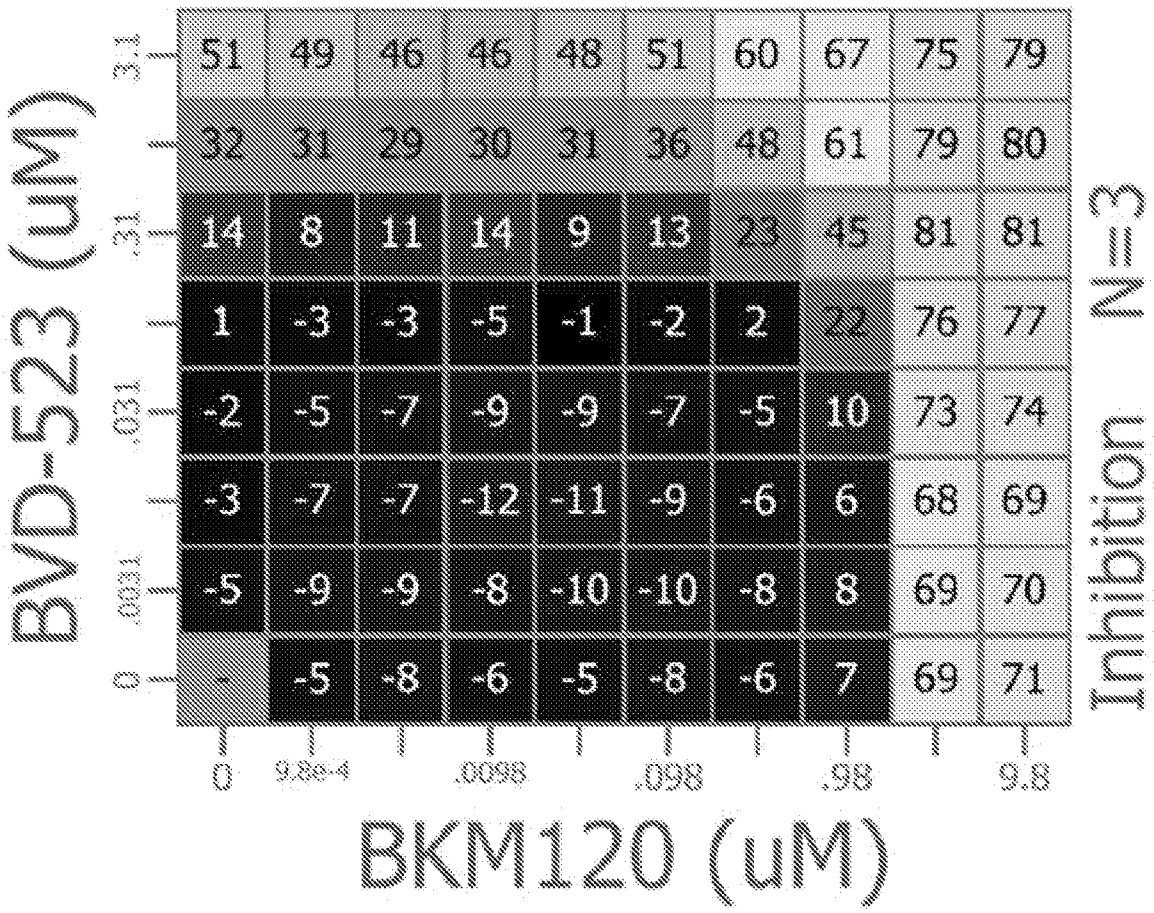


FIG. 15, Continued

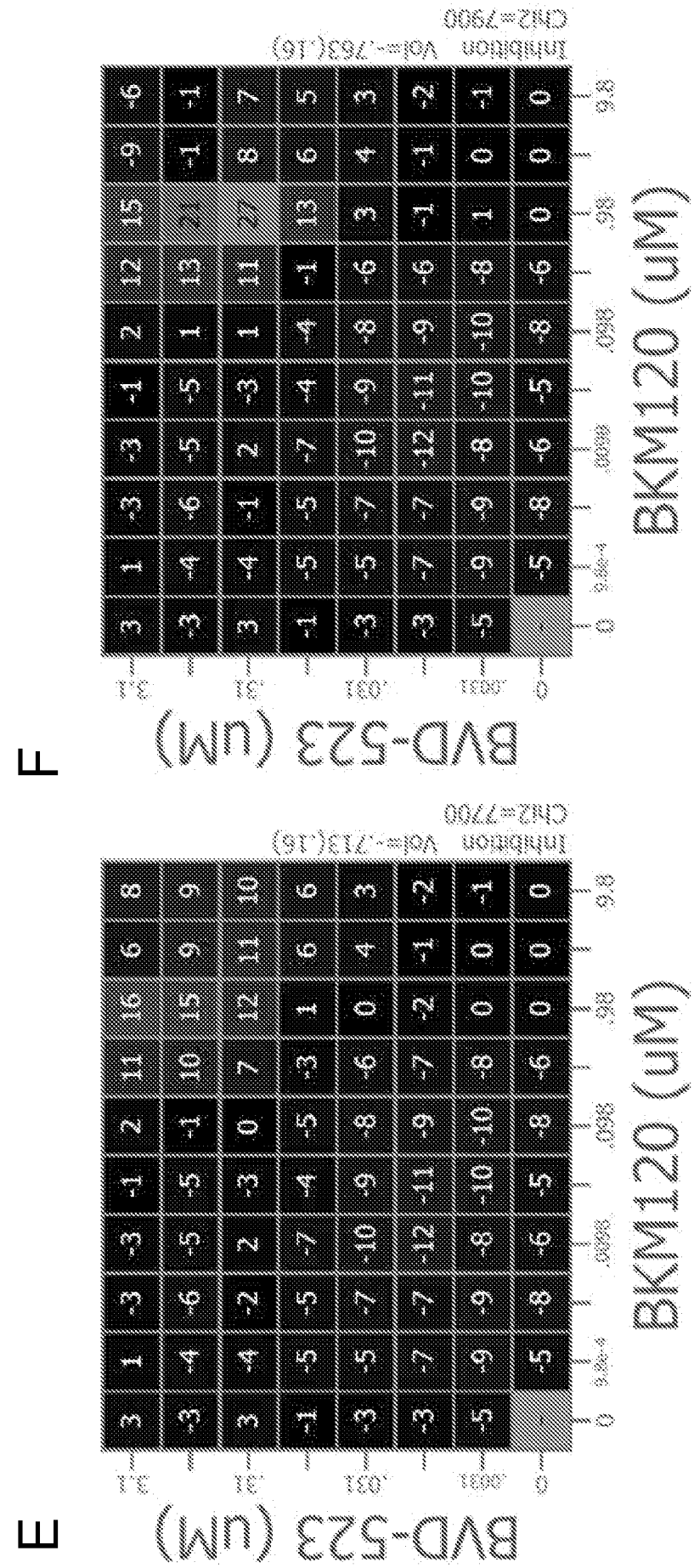
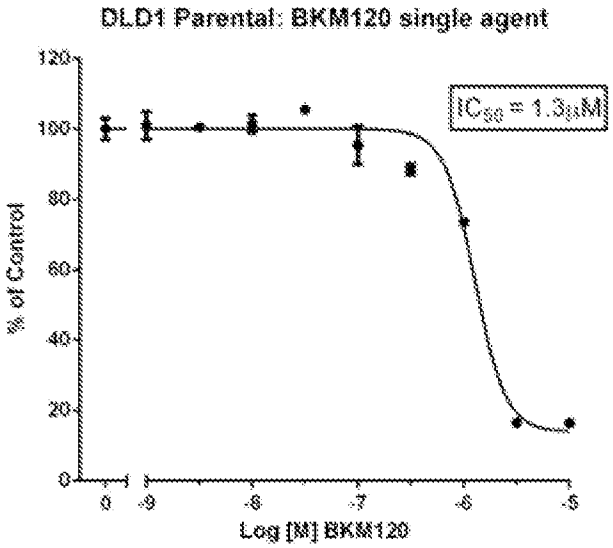
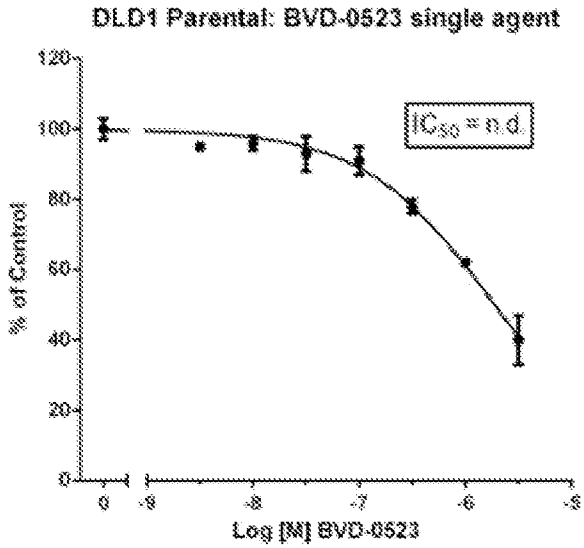


FIG. 15, Continued

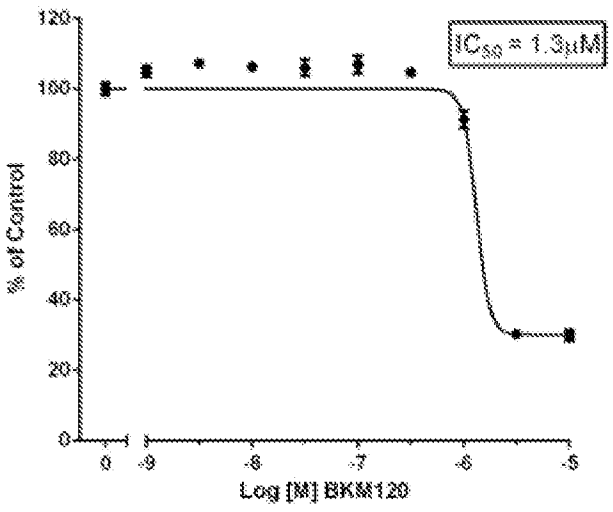
G



H



I



J

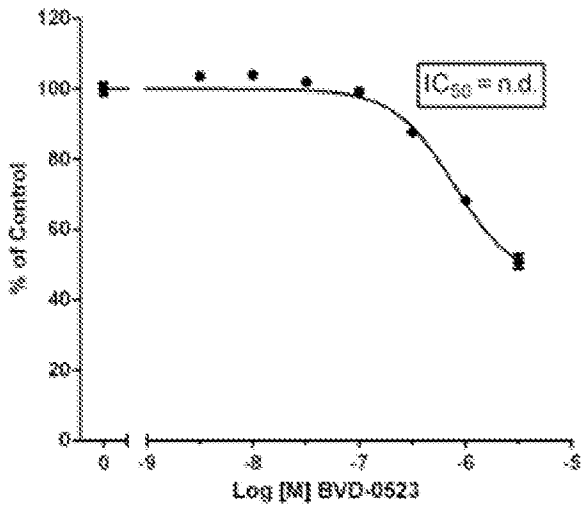


FIG. 16

A

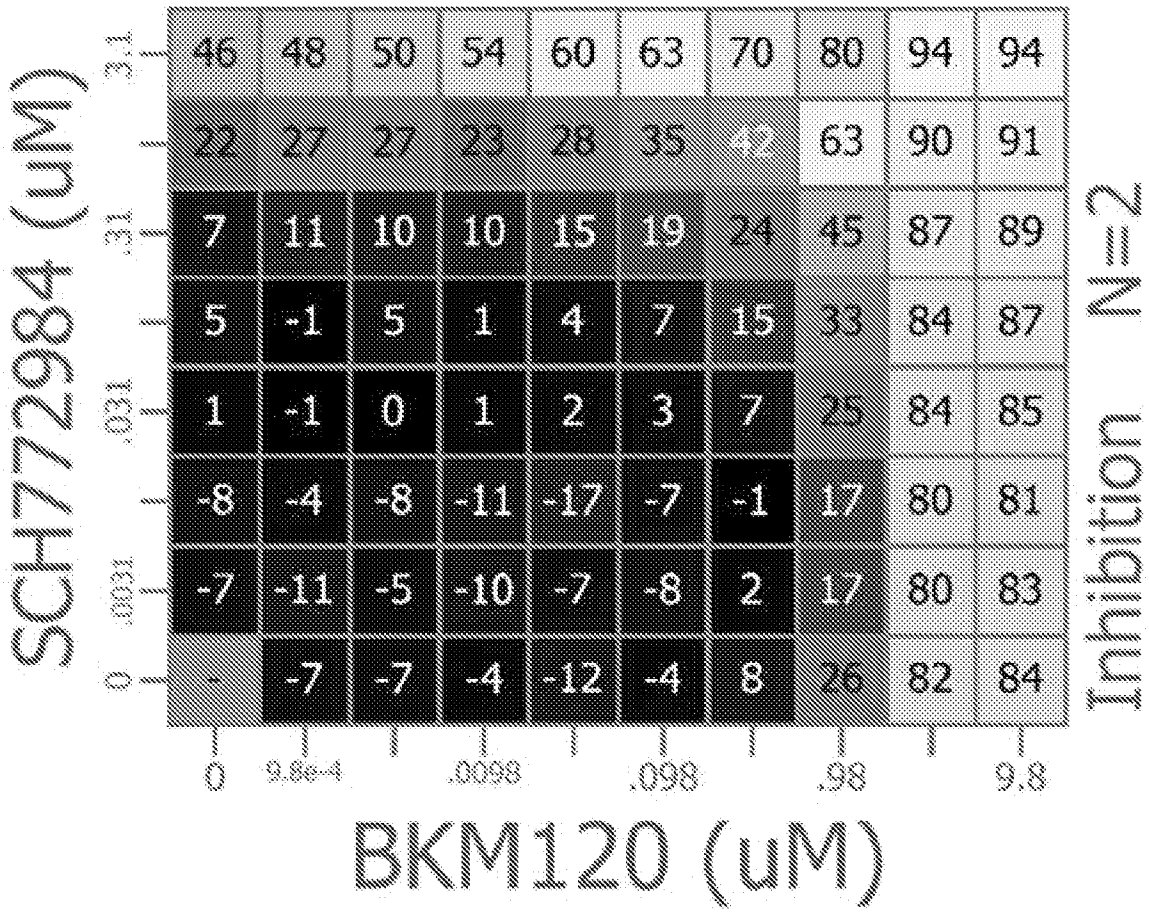

SCH772984 (uM)
3.1
.31
.031
.0031
0
46 48 50 54 60 63 70 80 94 94
22 27 27 23 28 35 42 63 90 91
7 11 10 10 15 19 24 45 87 89
5 -1 5 1 4 7 15 33 84 87
1 -1 0 1 2 3 7 25 84 85
-8 -4 -8 -11 -17 -7 -1 17 80 81
-7 -11 -5 -10 -7 -8 2 17 80 83
- -7 -7 -4 -12 -4 8 26 82 84
Inhibition N=2
0 9.8e-4 .0098 .098 .98 9.8
BKM120 (uM)

FIG. 16, Continued

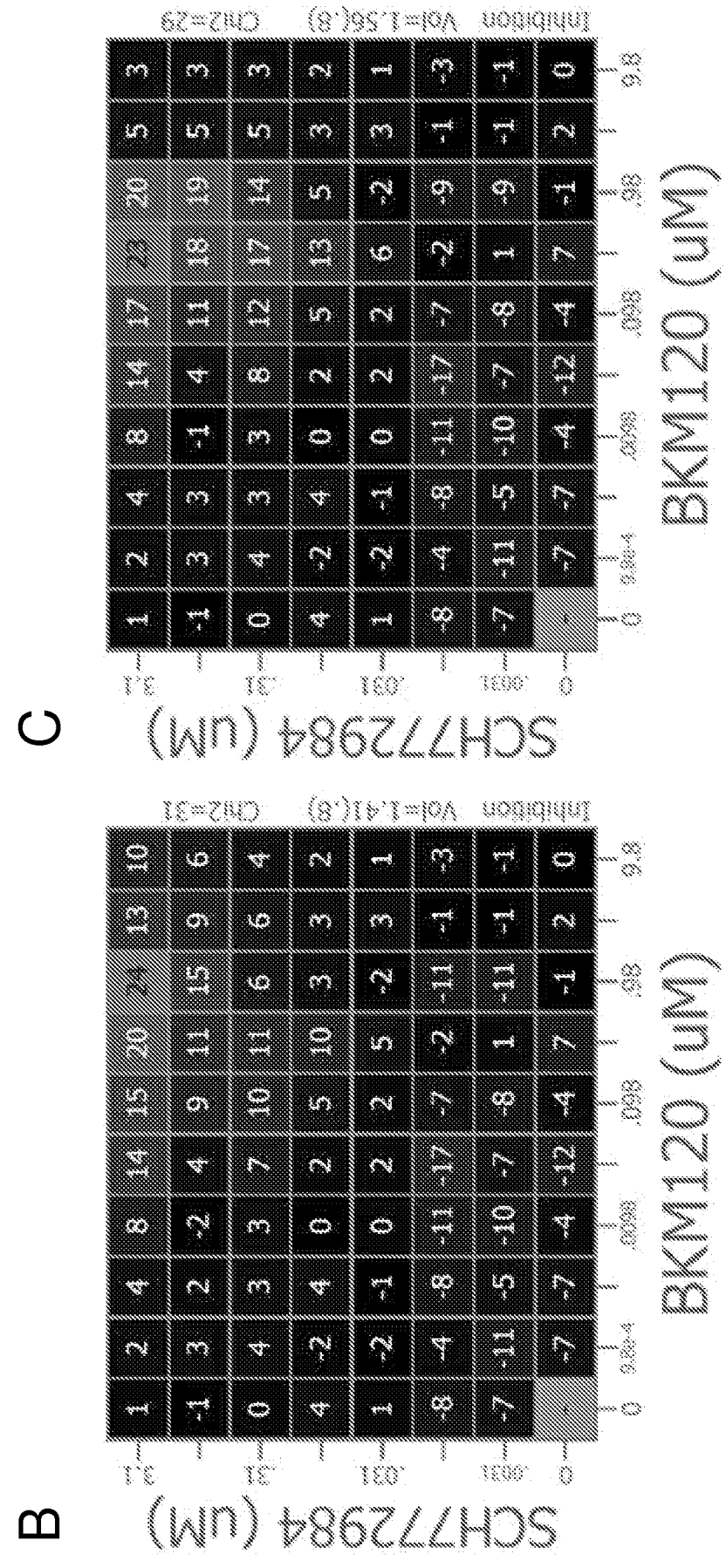


FIG. 16, Continued

D

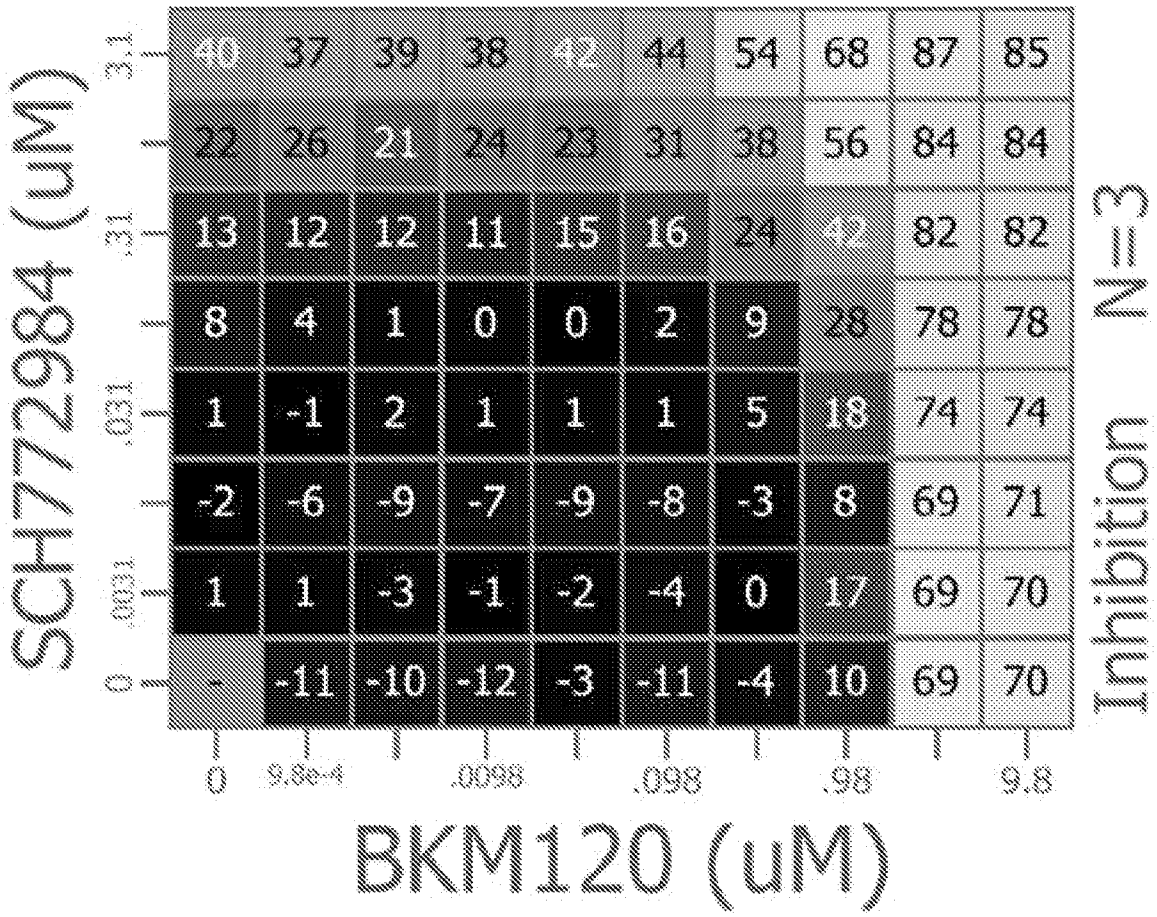


FIG. 16, Continued

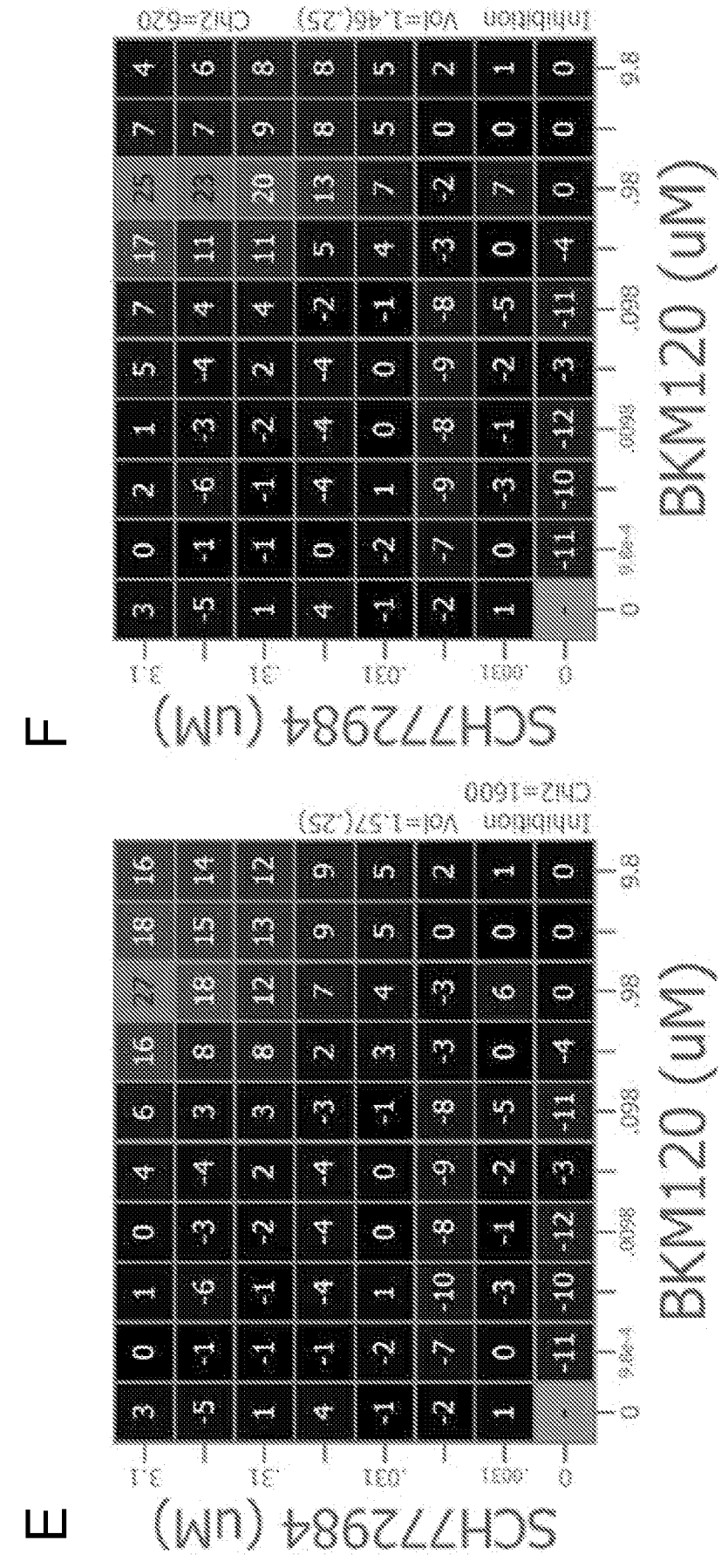
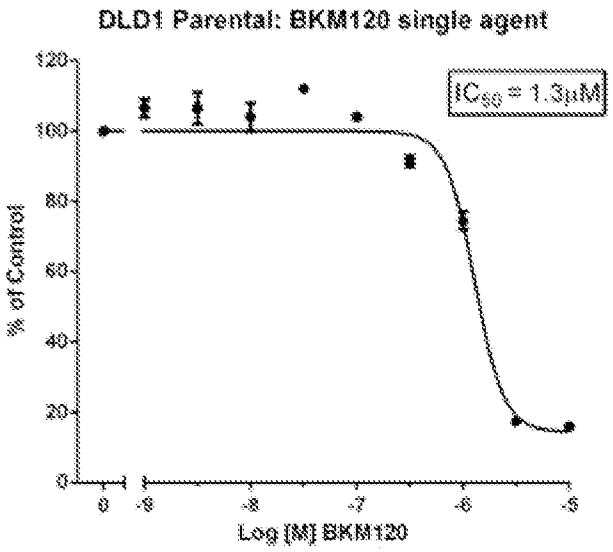
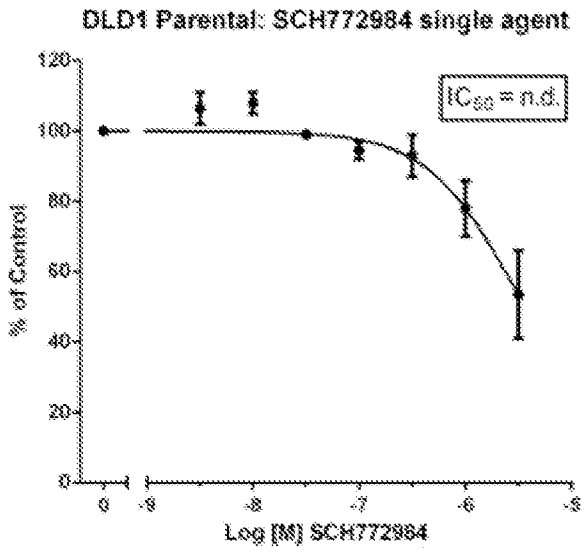


FIG. 16, Continued

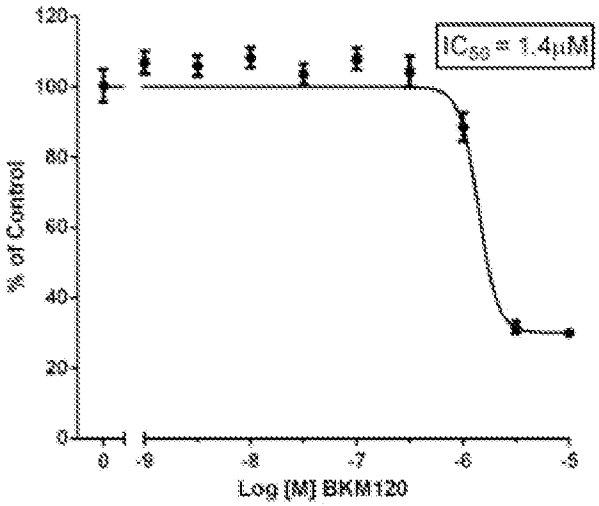
G



H



I



J

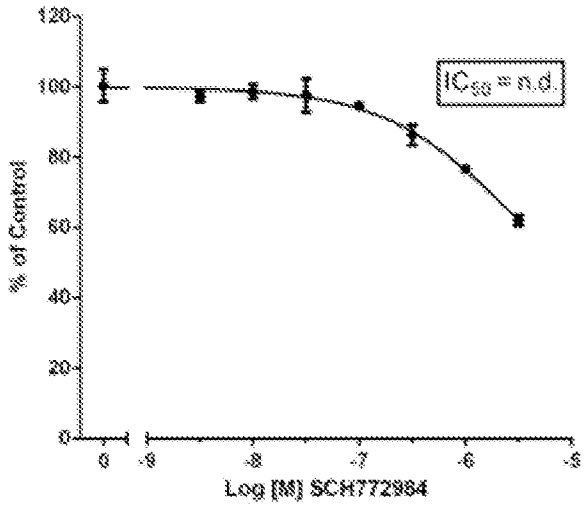


FIG. 17

A

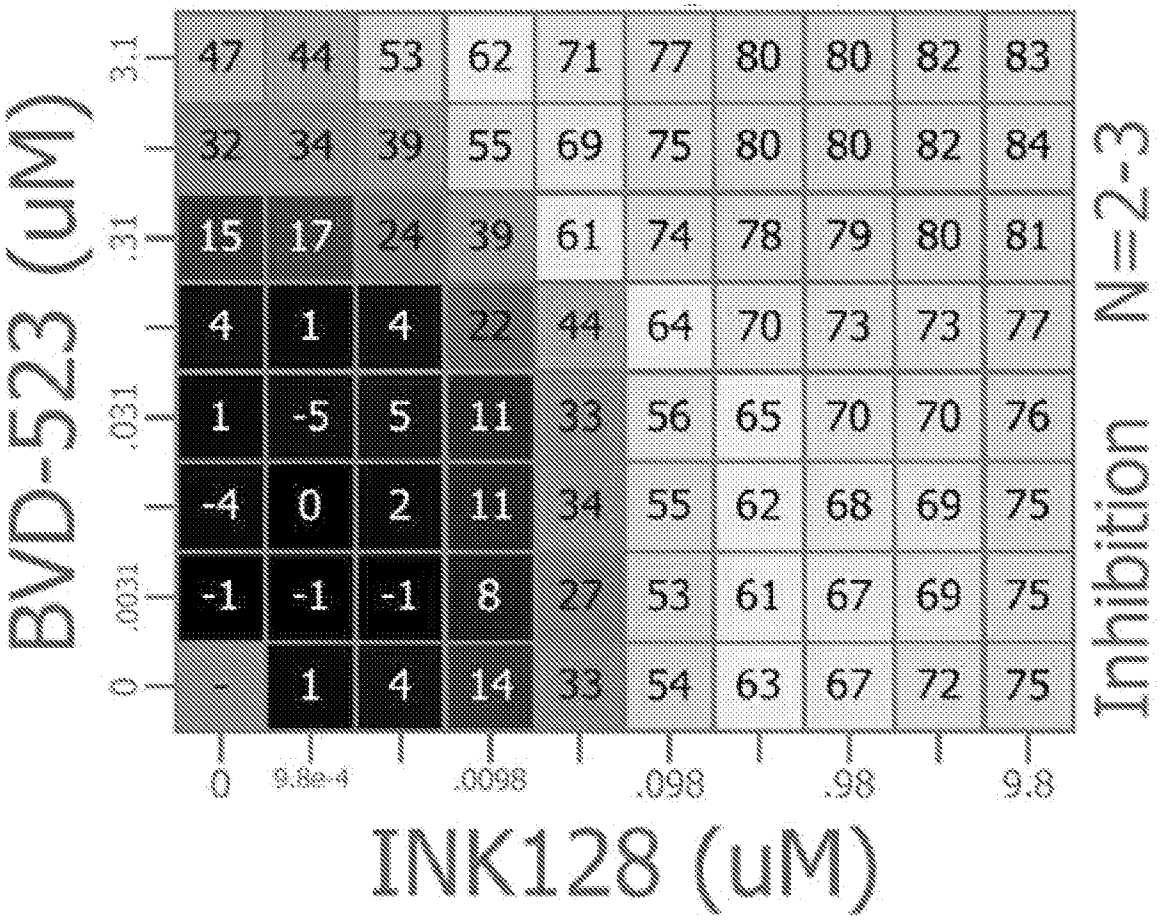

BVD-523 (uM)
3.1
.31
.031
.0031
0
47 44 53 62 71 77 80 80 82 83
32 34 39 55 69 75 80 80 82 84
15 17 24 39 61 74 78 79 80 81
4 1 4 22 44 64 70 73 73 77
1 -5 5 11 33 56 65 70 70 76
-4 0 2 11 34 55 62 68 69 75
-1 -1 -1 8 27 53 61 67 69 75
- 1 4 14 33 54 63 67 72 75
0
9.8e-4
.0098
.098
.98
9.8
INK128 (uM)
Inhibition N=2-3

FIG. 17, Continued

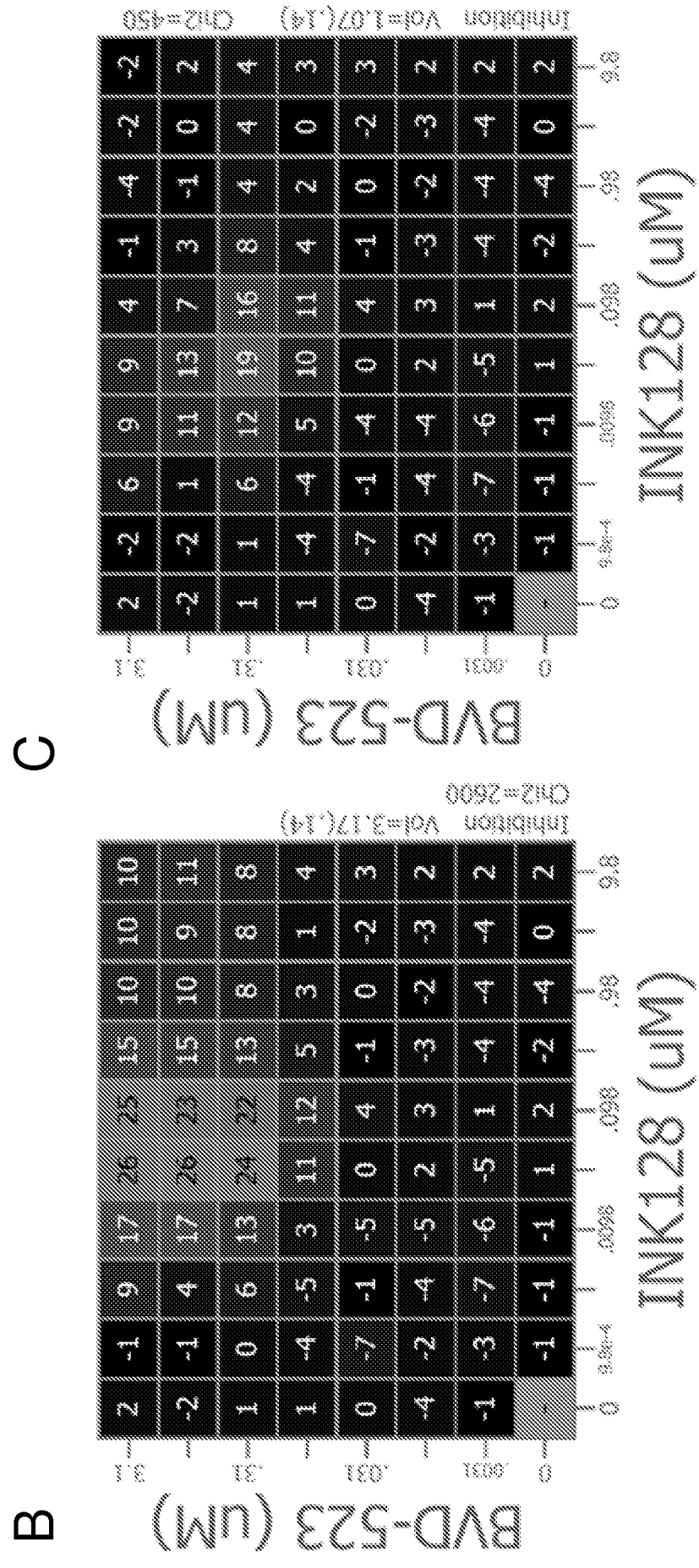


FIG. 17, Continued

D

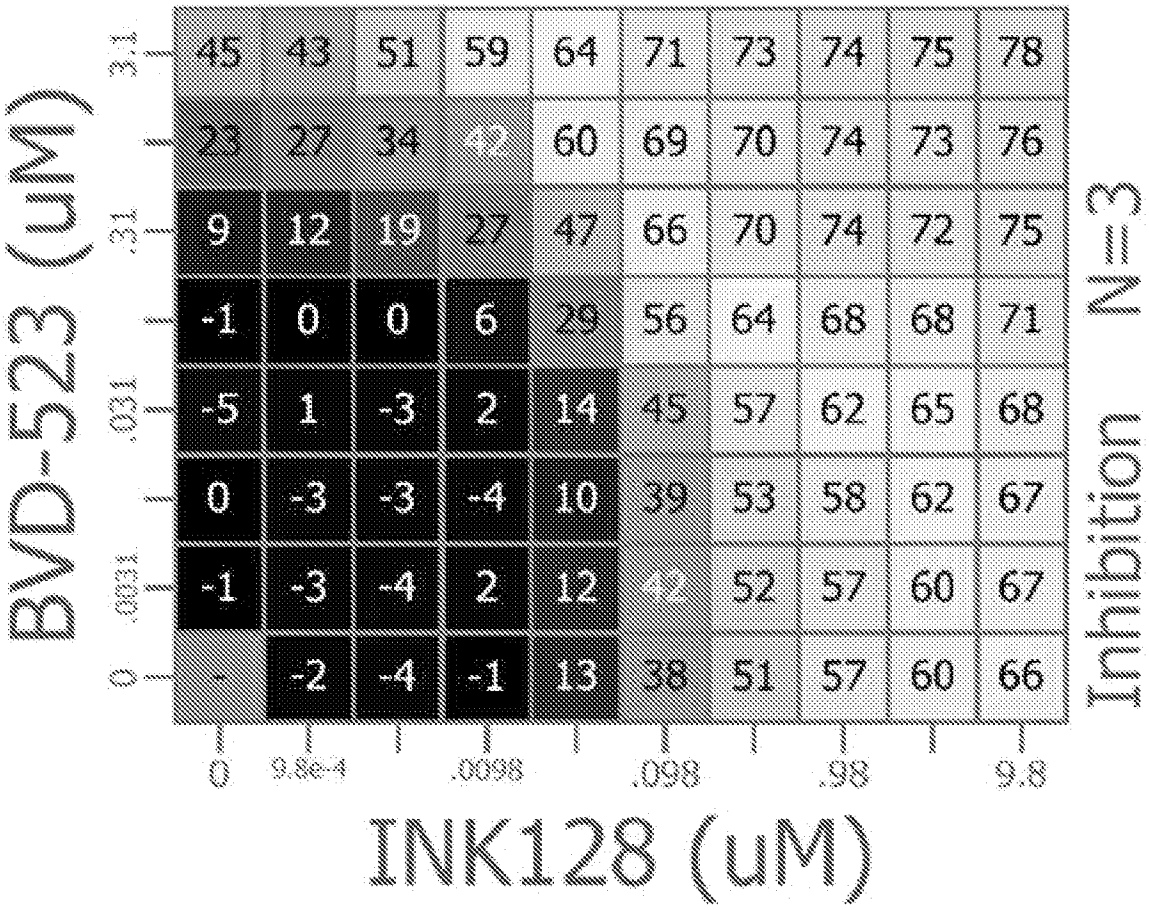


FIG. 17, Continued

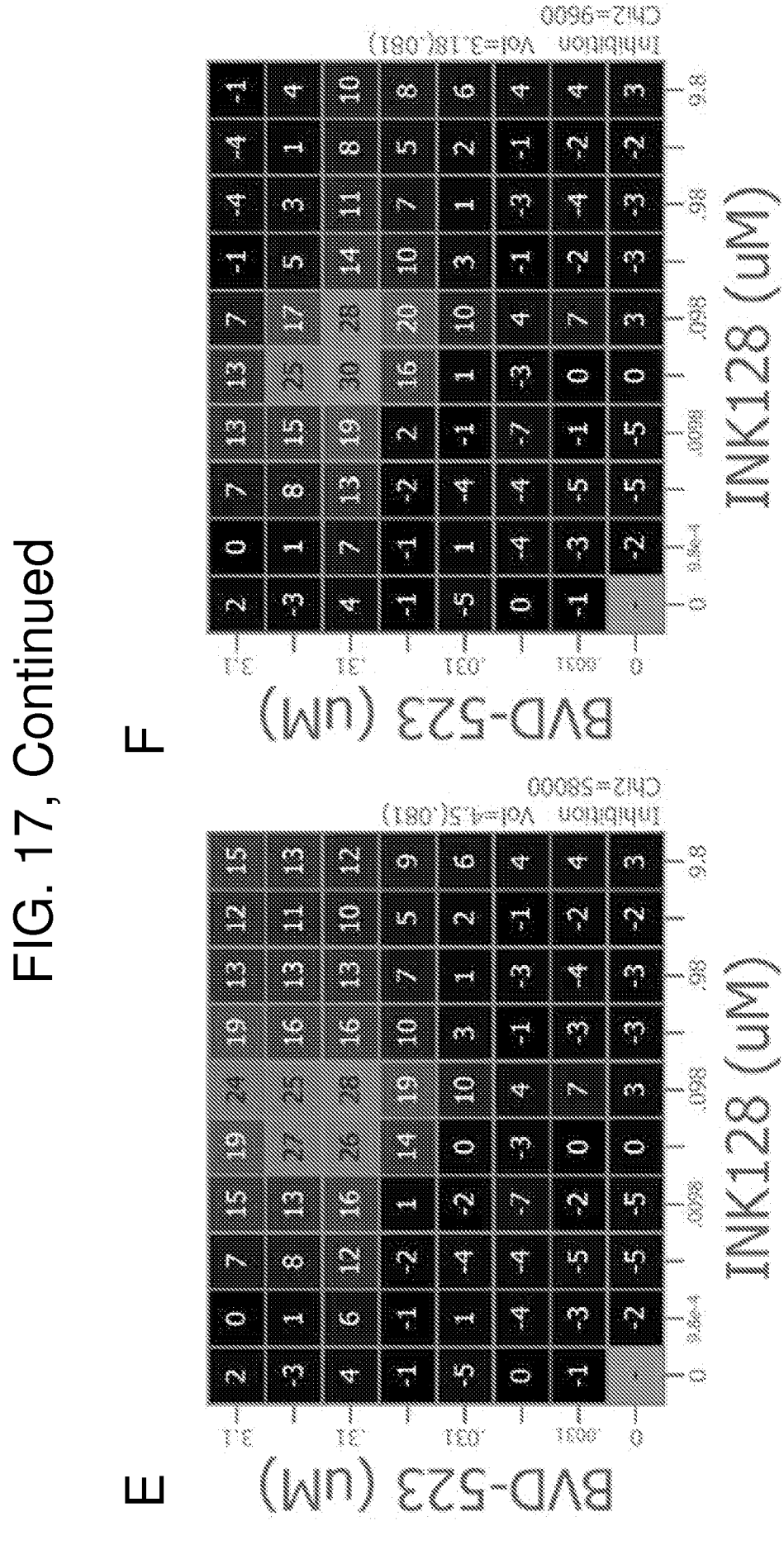
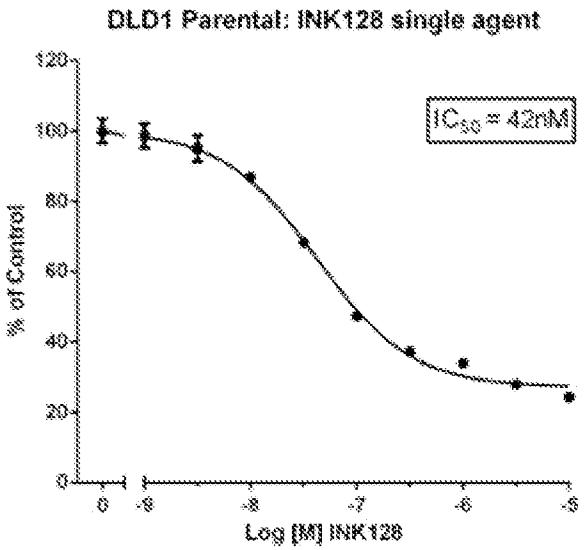
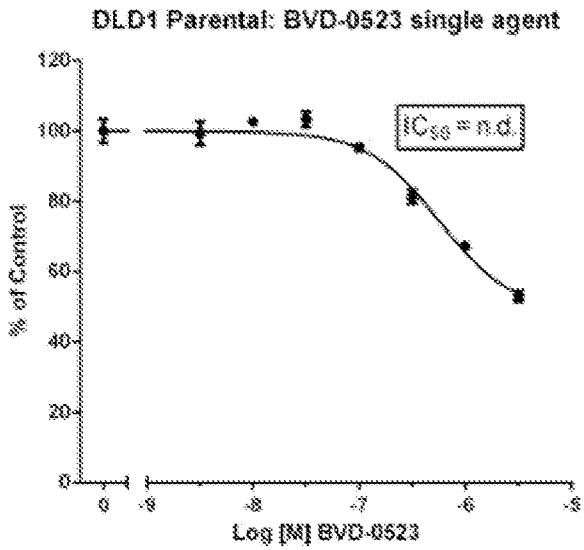


FIG. 17, Continued

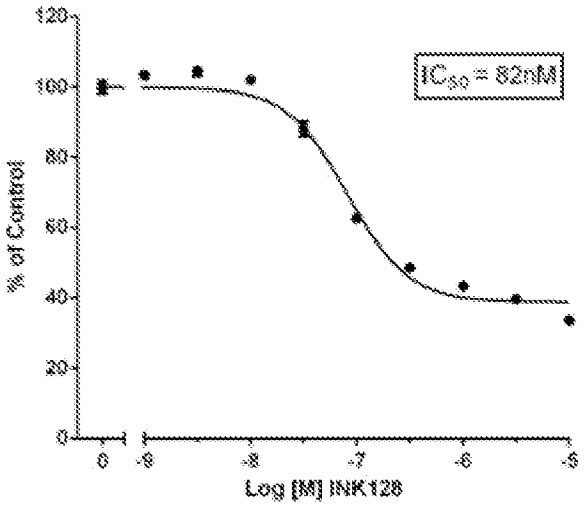
G



H



I



J

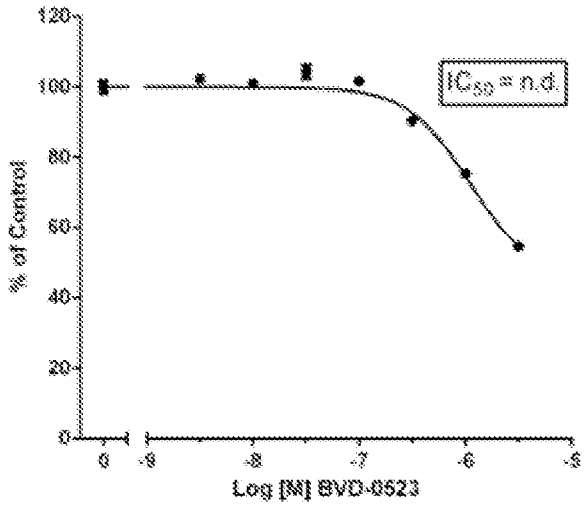


FIG. 18

A

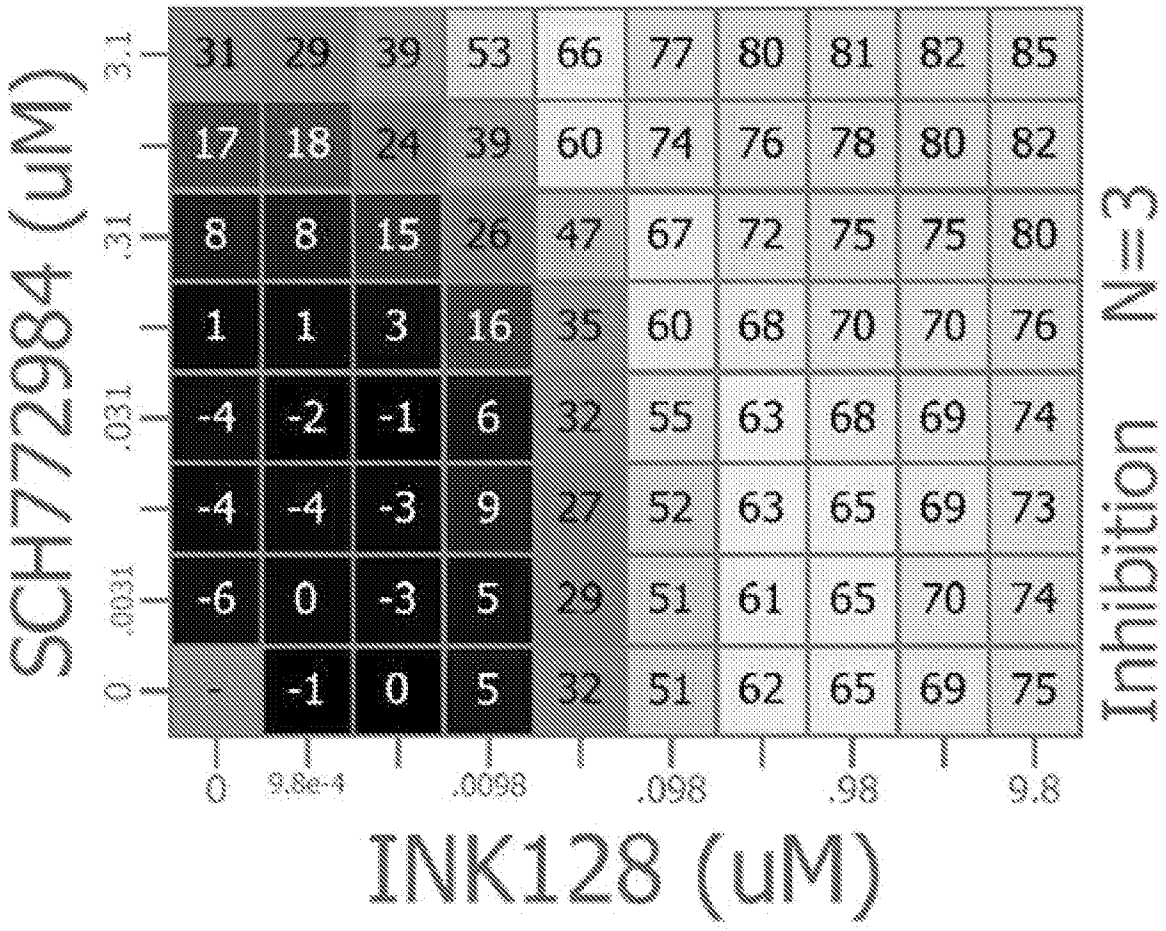

SCH772984 (uM)
3.1
.31
.031
.0031
0
31 29 39 53 66 77 80 81 82 85
17 18 24 39 60 74 76 78 80 82
8 8 15 26 47 67 72 75 75 80
1 1 3 16 35 60 68 70 70 76
-4 -2 -1 6 32 55 63 68 69 74
-4 -4 -3 9 27 52 63 65 69 73
-6 0 -3 5 29 51 61 65 70 74
- -1 0 5 32 51 62 65 69 75
0
9.8e-4
.0098
.098
.98
9.8
INK128 (uM)
Inhibition N=3

FIG. 18, Continued

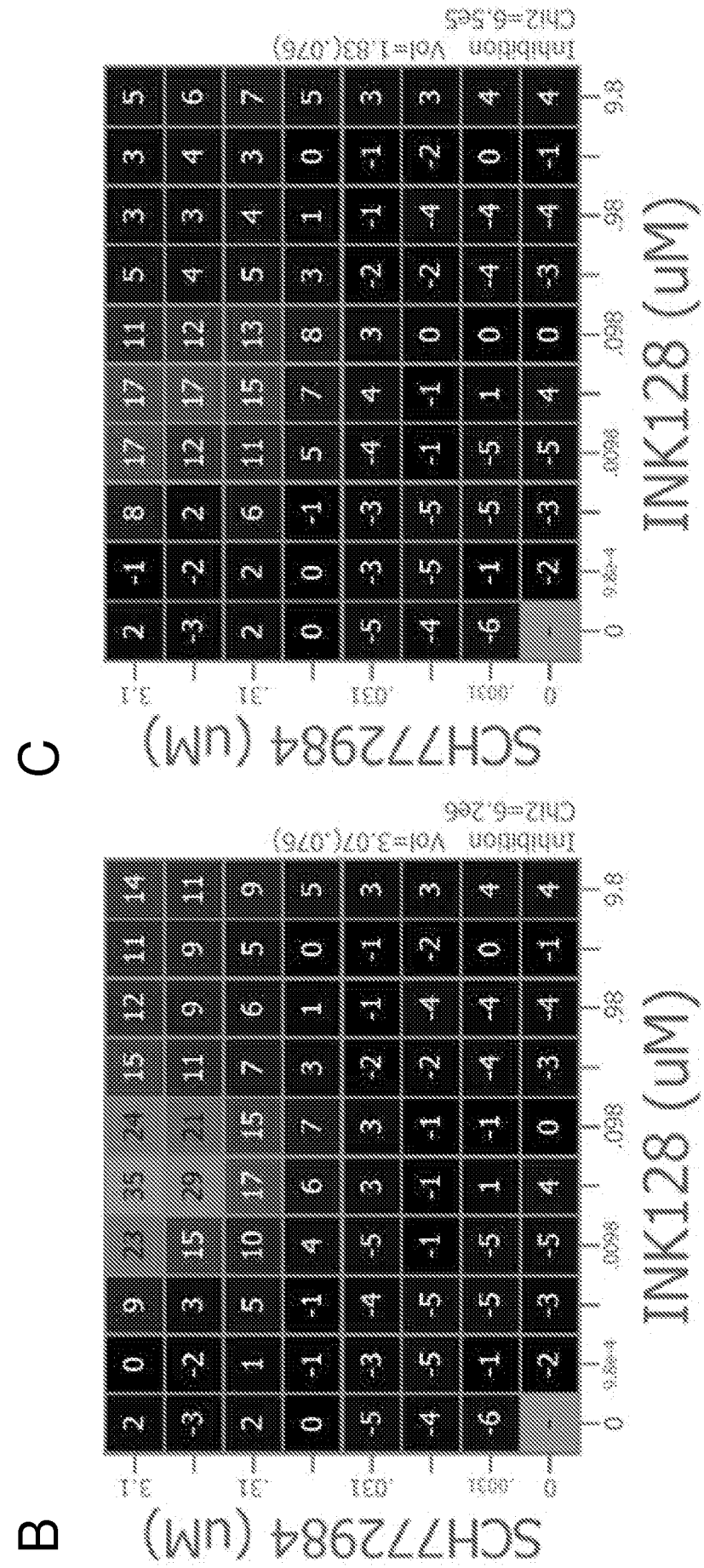


FIG. 18, Continued

D

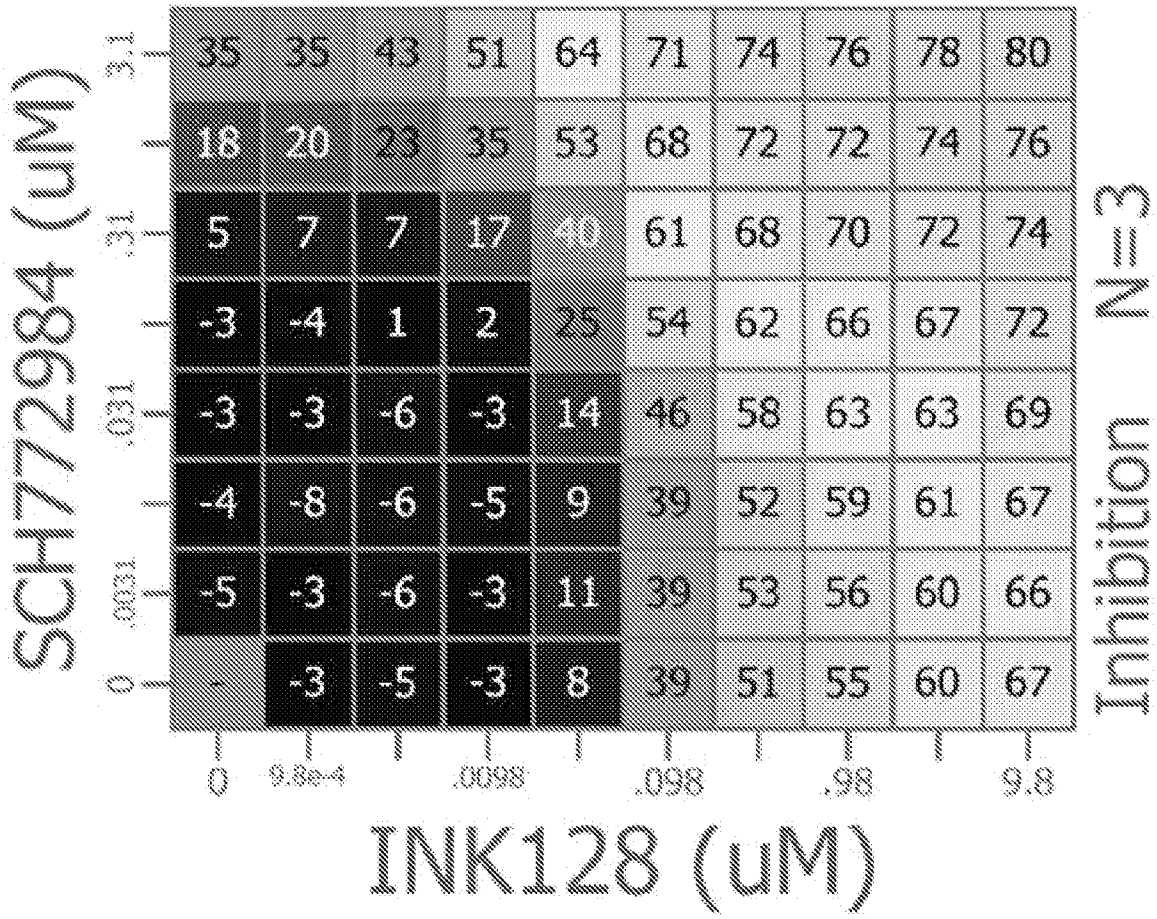


FIG. 18, Continued

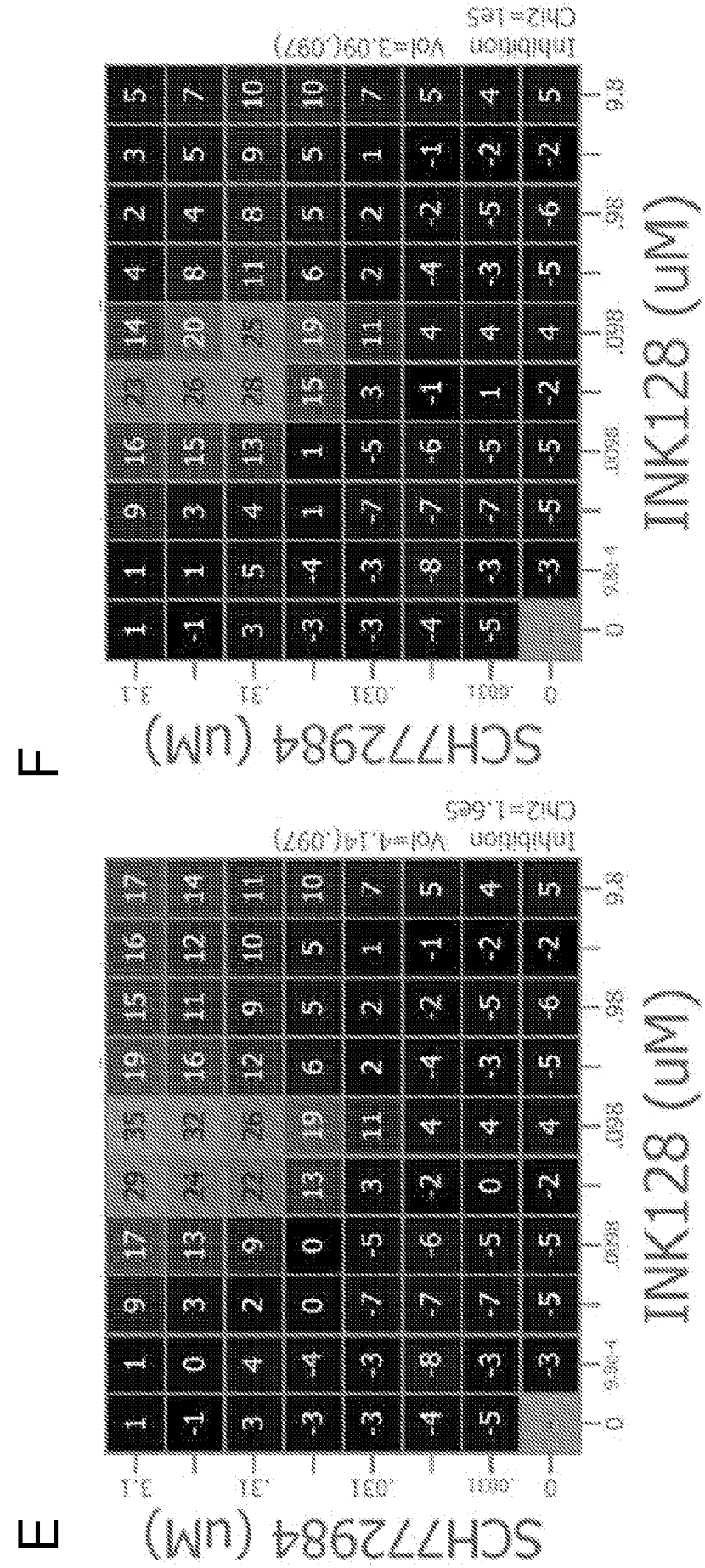
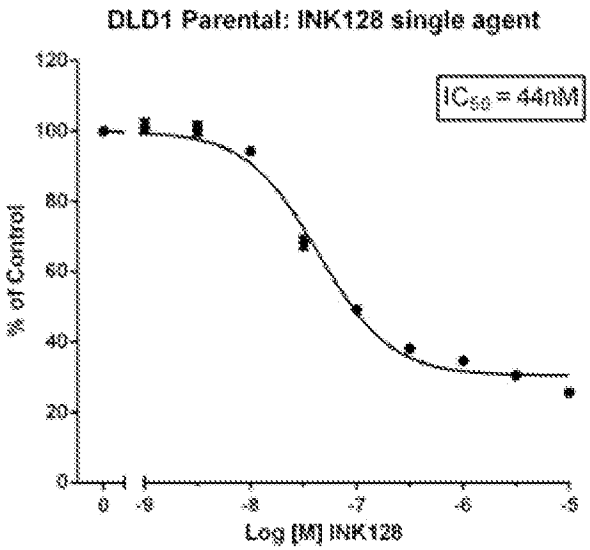
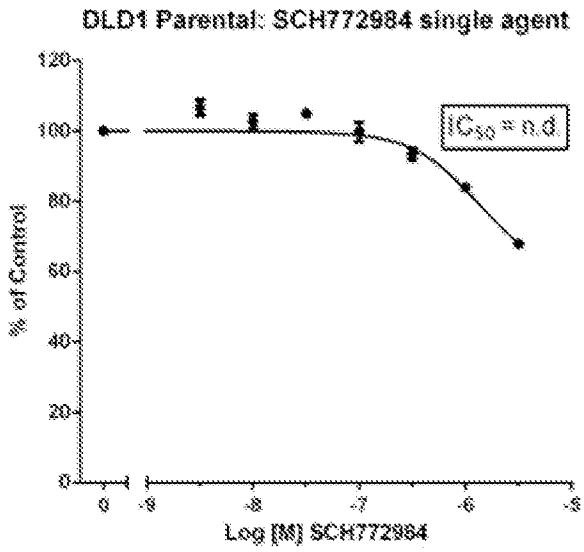


FIG. 18, Continued

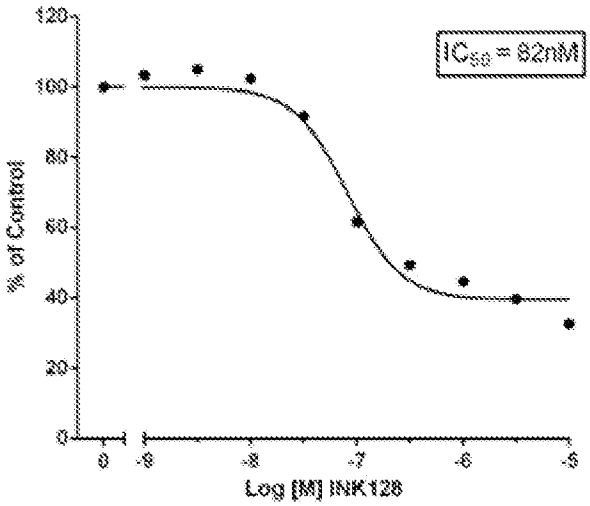
G



H



I



J

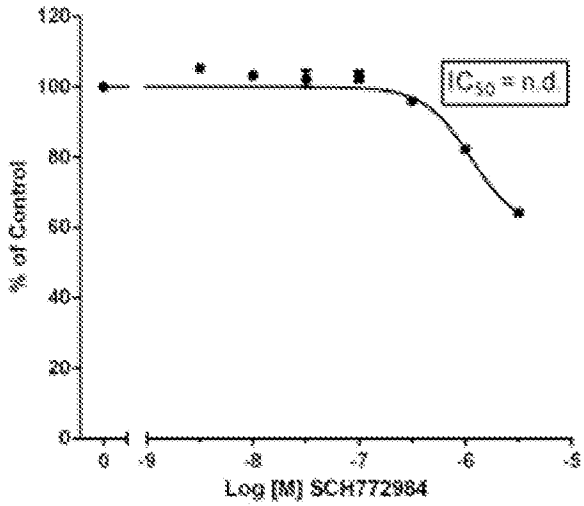


FIG. 19

A

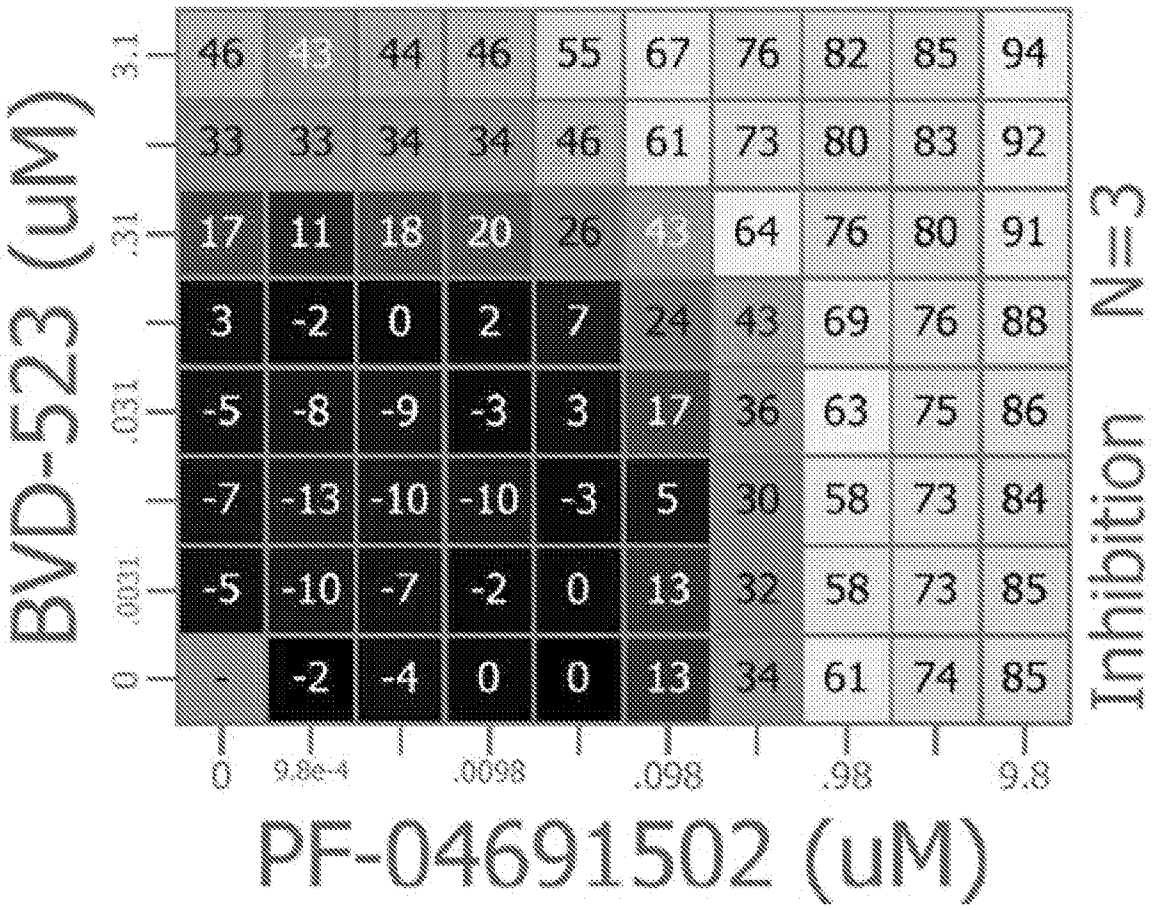

BVD-523 (uM)
3.1
.31
.031
.0031
0
46 45 44 46 55 67 76 82 85 94
33 33 34 34 46 61 73 80 83 92
17 11 18 20 26 43 64 76 80 91
3 -2 0 2 7 24 43 69 76 88
-5 -8 -9 -3 3 17 36 63 75 86
-7 -13 -10 -10 -3 5 30 58 73 84
-5 -10 -7 -2 0 13 32 58 73 85
- -2 -4 0 0 13 34 61 74 85
0
9.8e-4
.0098
.098
.98
9.8
PF-04691502 (uM)
Inhibition N=3

FIG. 19, Continued

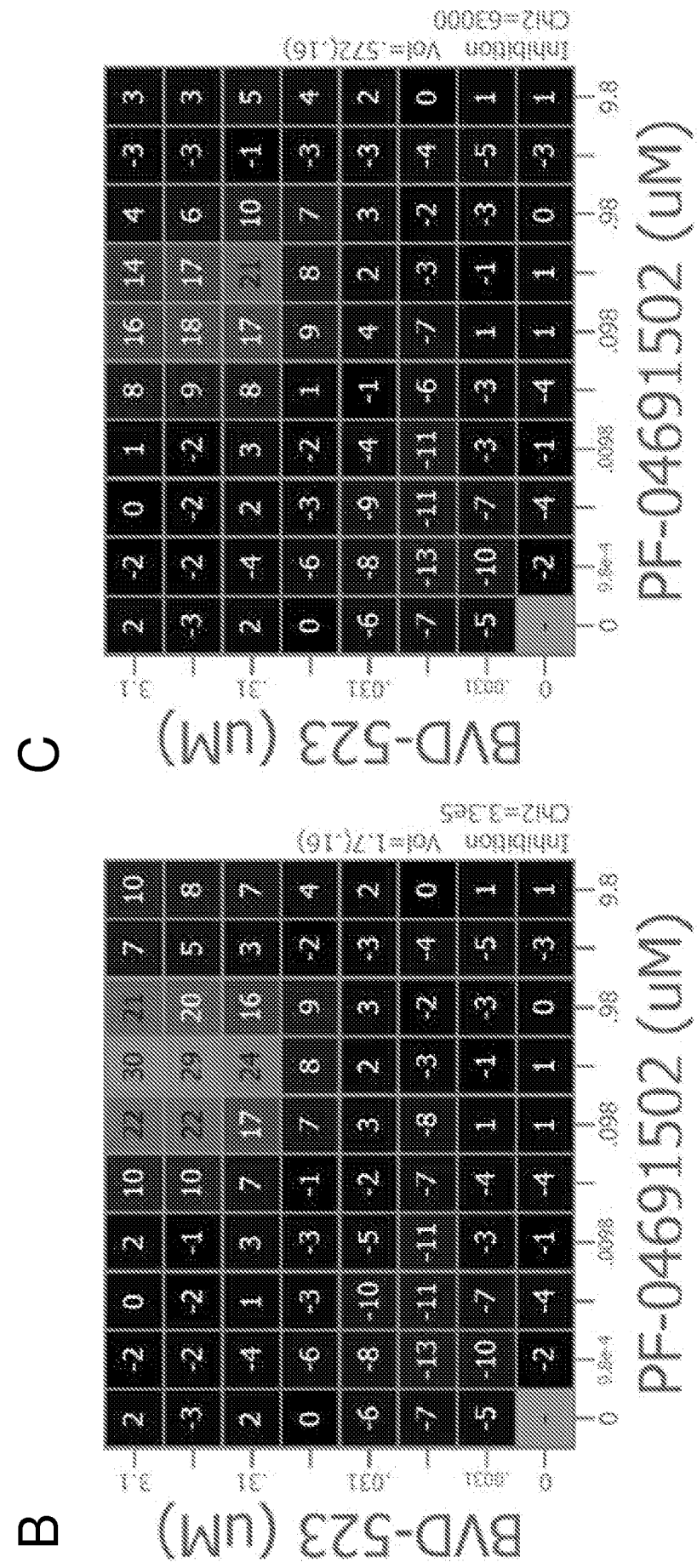


FIG. 19, Continued

D

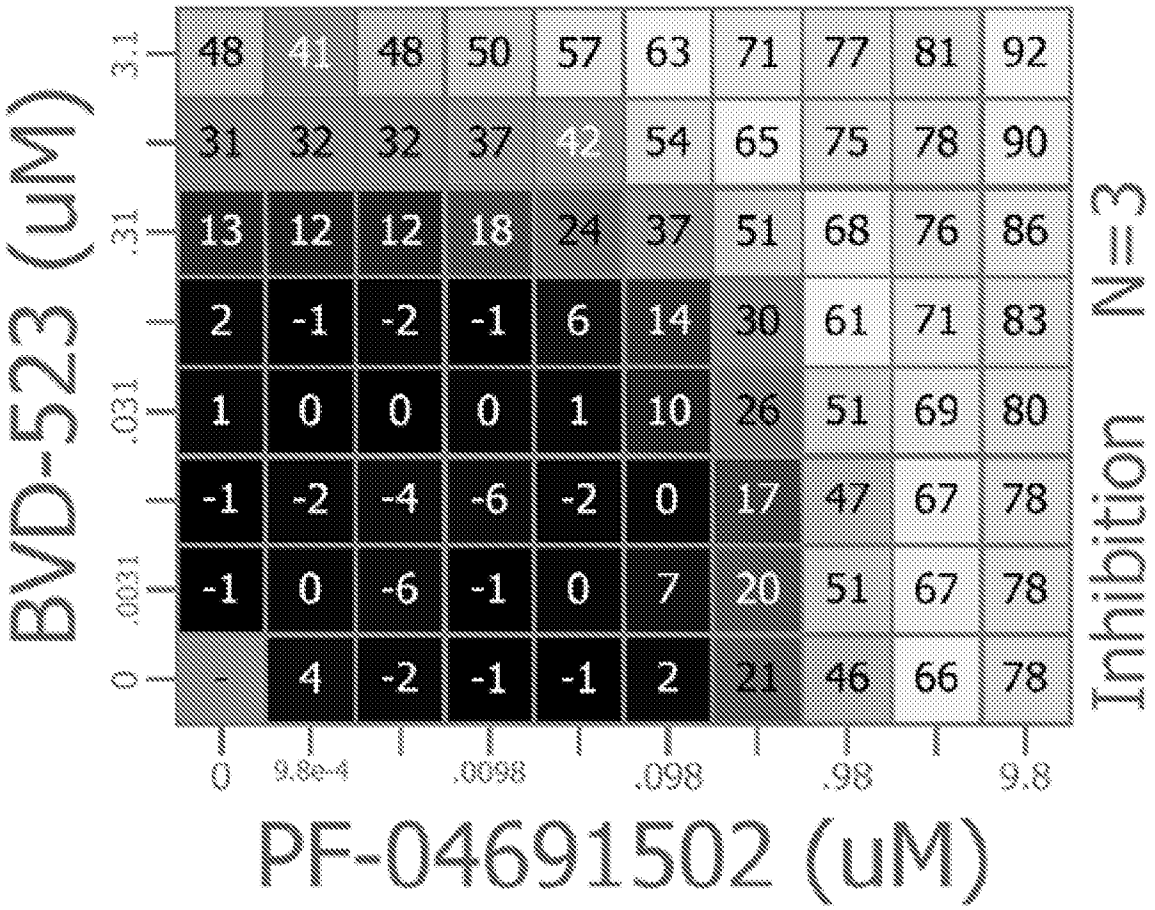


FIG. 19, Continued

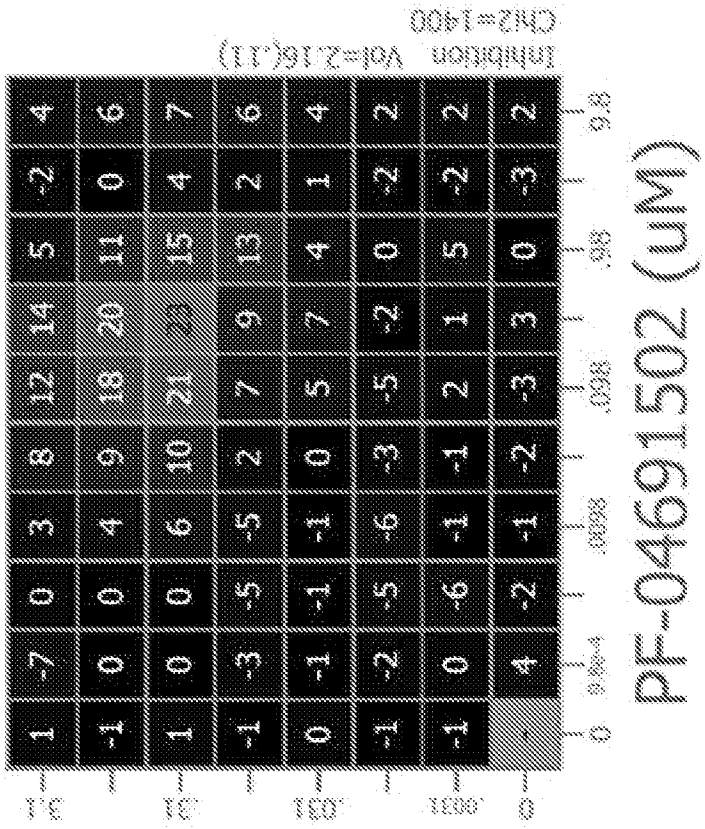
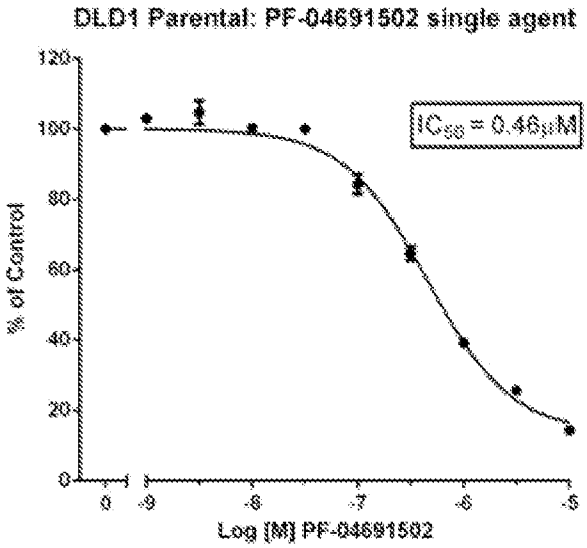
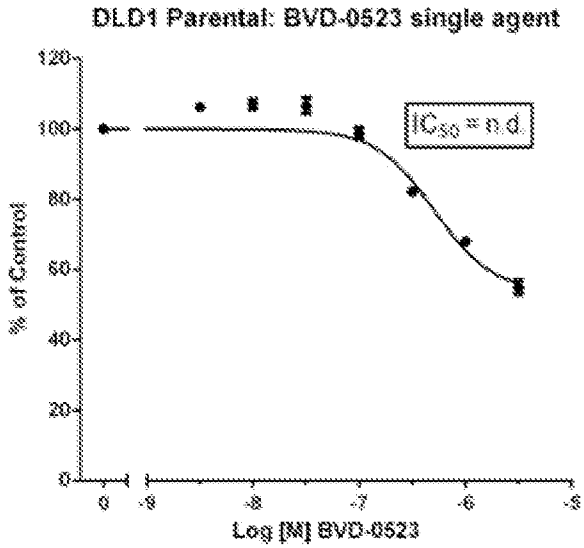


FIG. 19, Continued

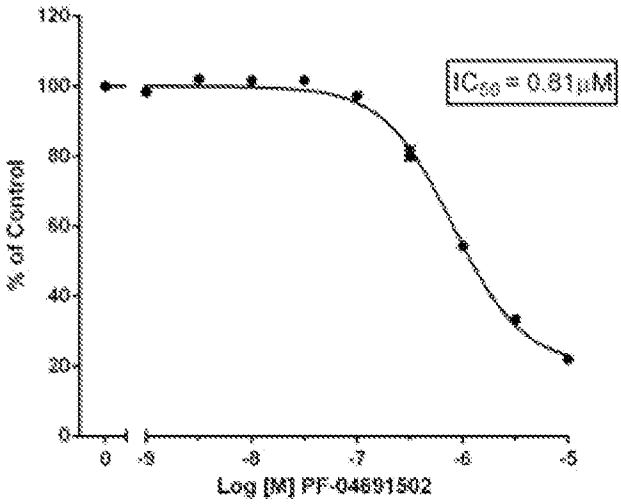
G



H



I



J

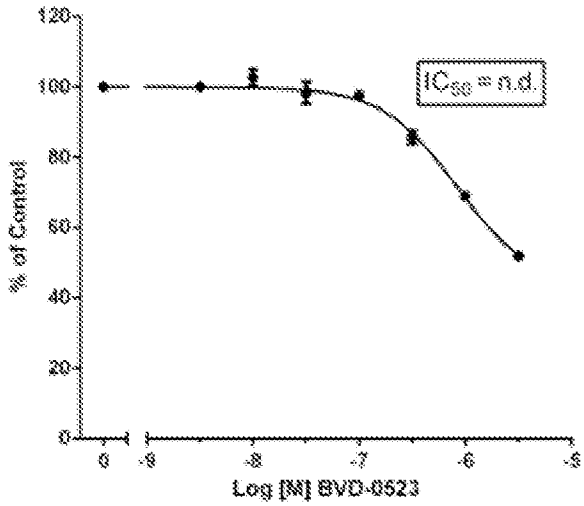


FIG. 20

A

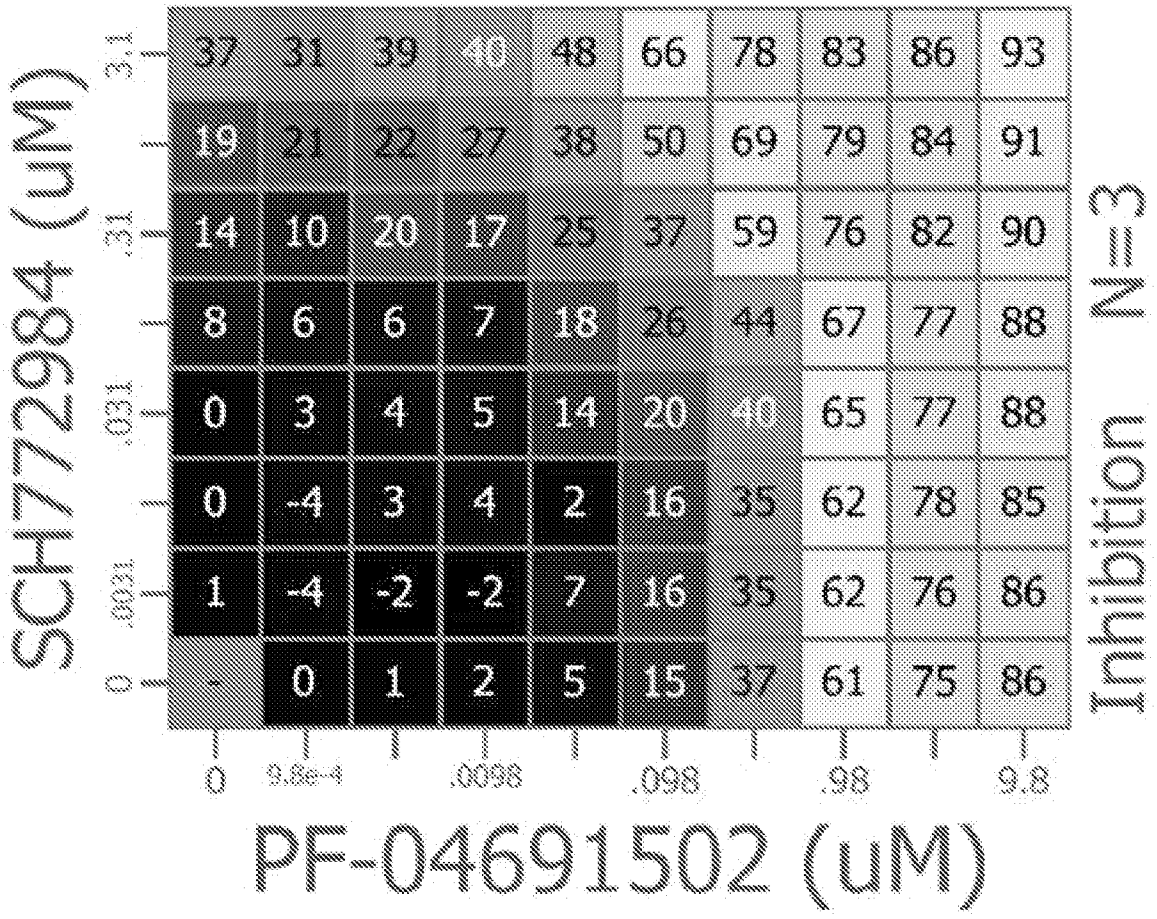

SCH772984 (uM)
3.1
.31
.031
.0031
0
37 31 39 40 48 66 78 83 86 93
19 21 22 27 38 50 69 79 84 91
14 10 20 17 25 37 59 76 82 90
8 6 6 7 18 26 44 67 77 88
0 3 4 5 14 20 40 65 77 88
0 -4 3 4 2 16 35 62 78 85
1 -4 -2 -2 7 16 35 62 76 86
- 0 1 2 5 15 37 61 75 86
0
9.8e-4
.0098
.098
.98
9.8
PF-04691502 (uM)
Inhibition N=3

FIG. 20, Continued

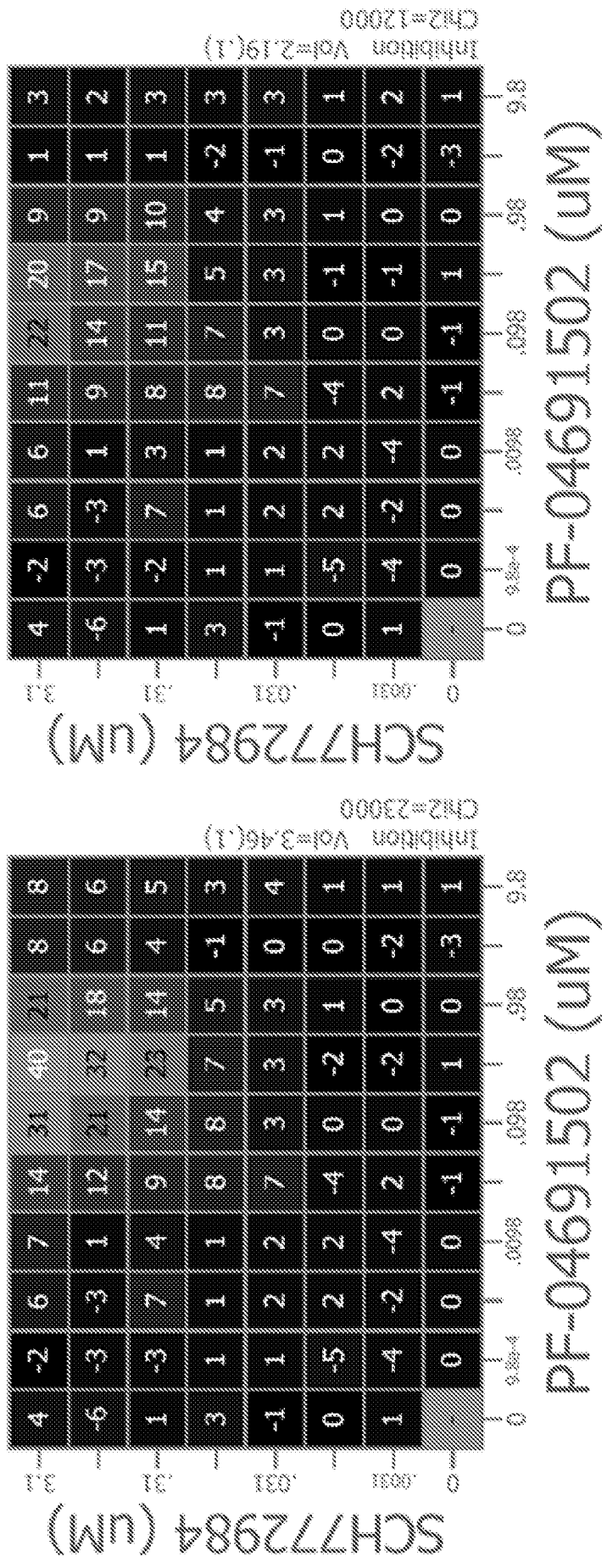


FIG. 20, Continued

D

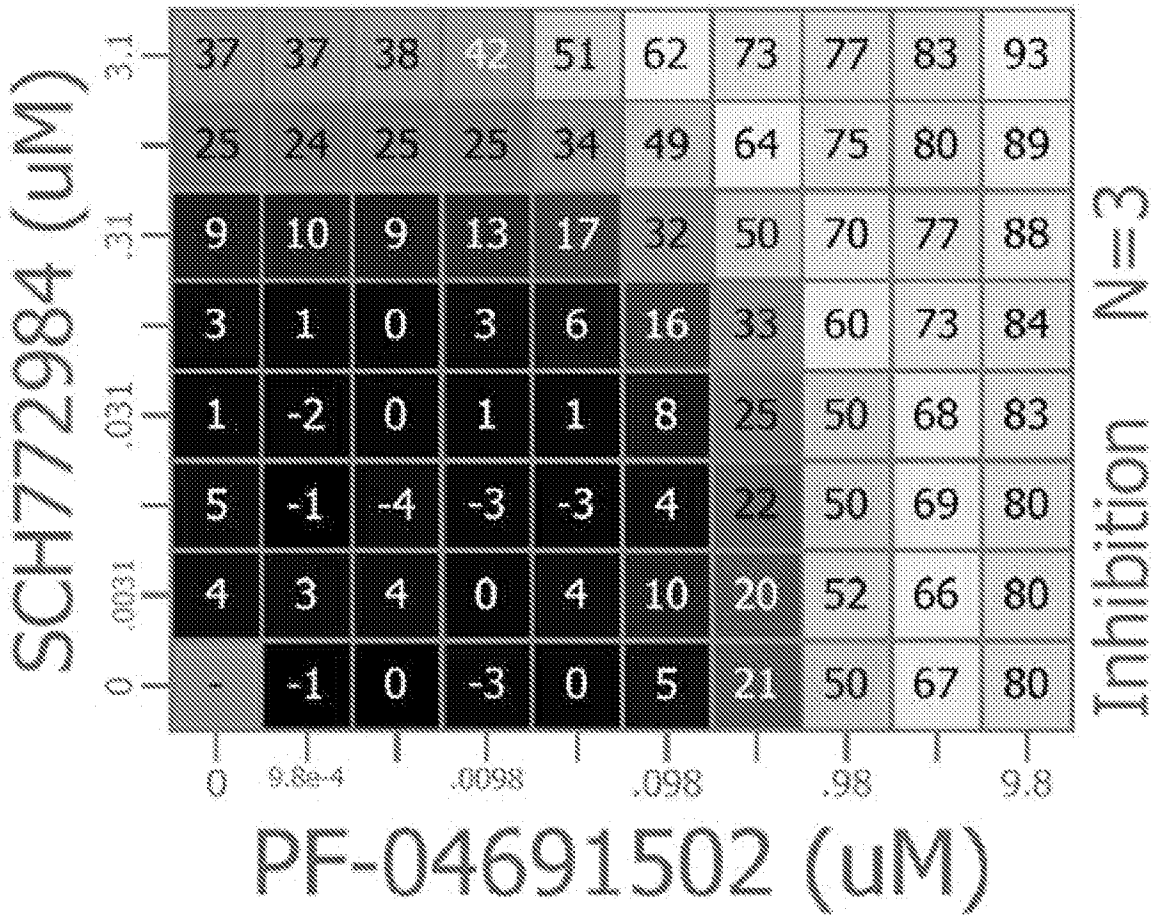


FIG. 20, Continued

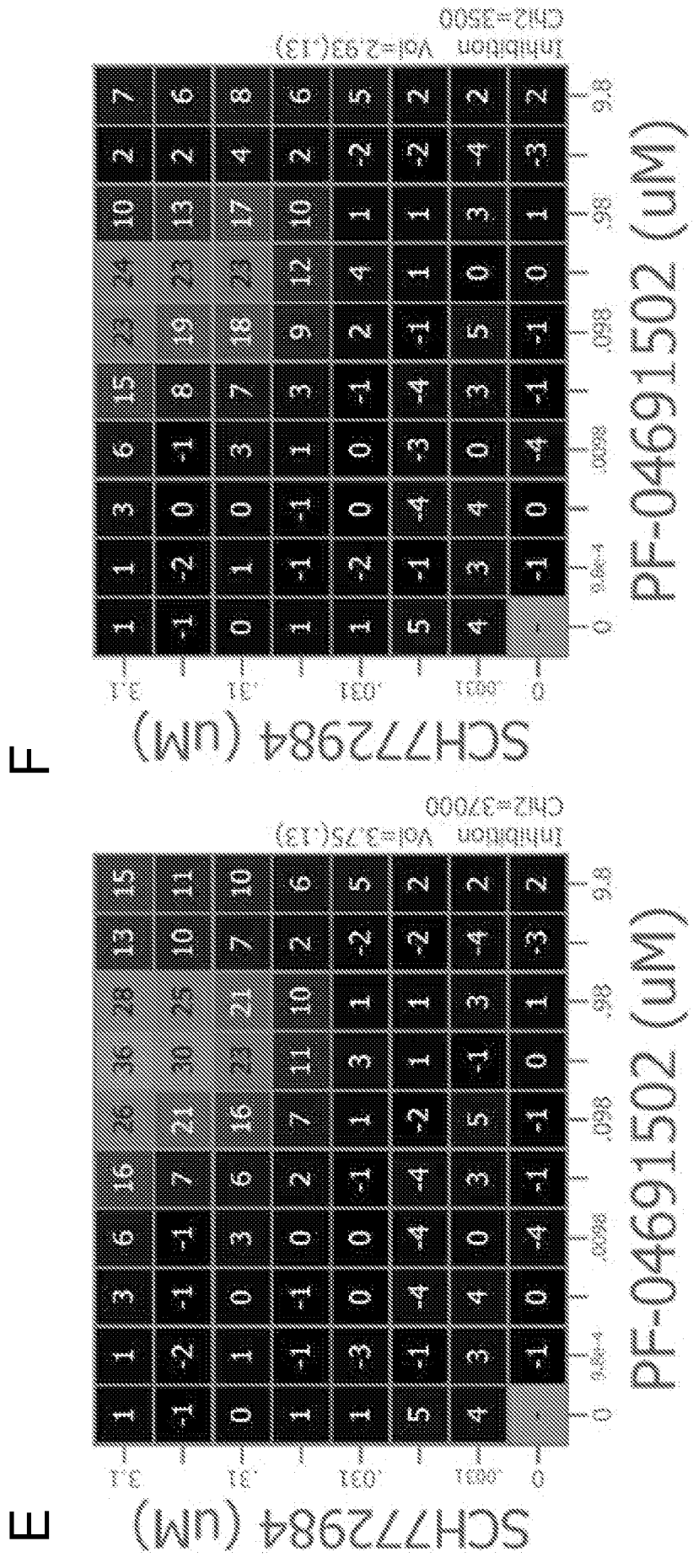
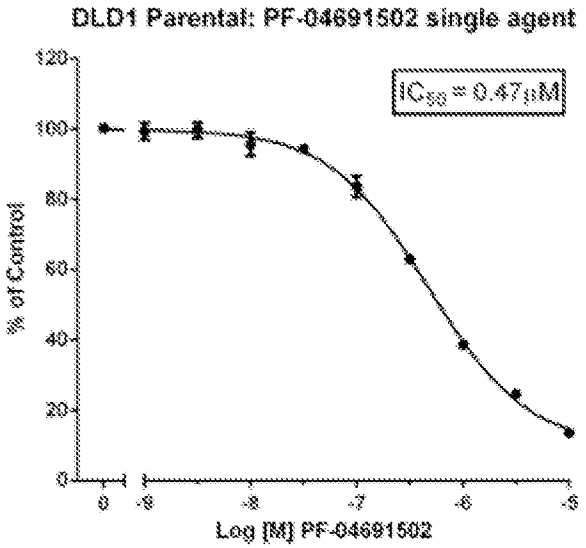
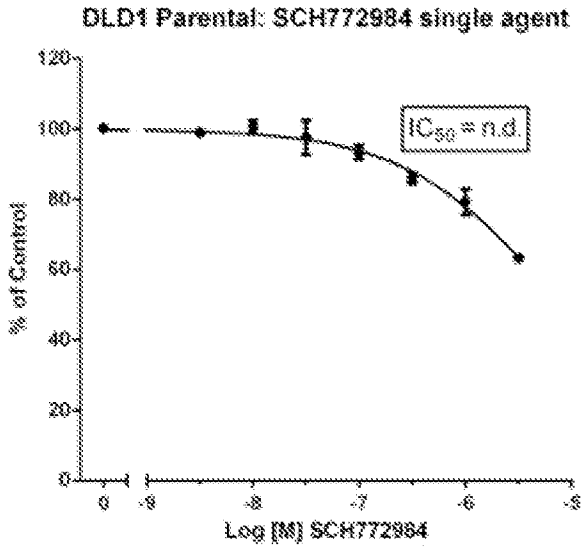


FIG. 20, Continued

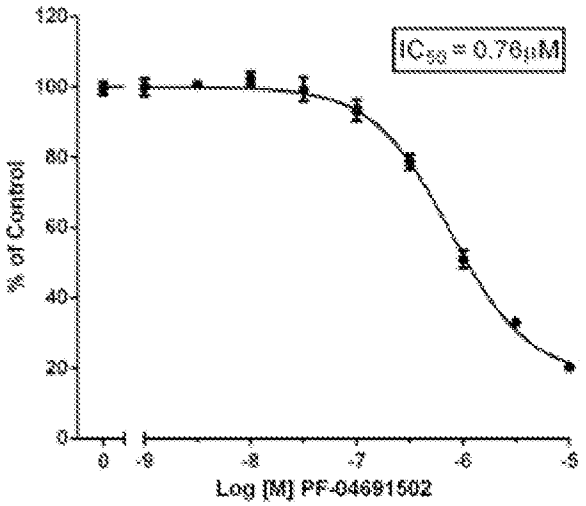
G



H



I



J

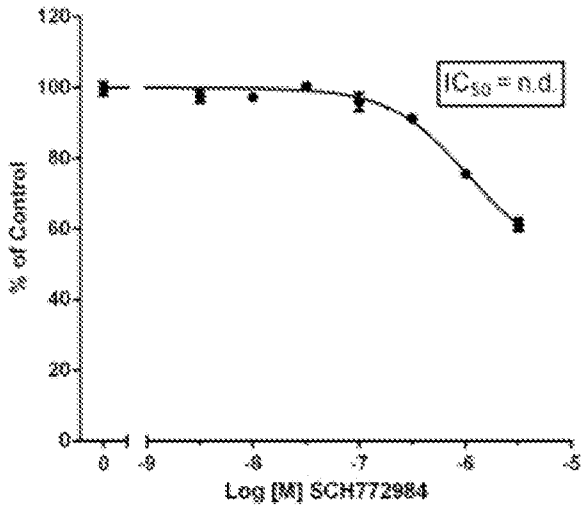


FIG. 21

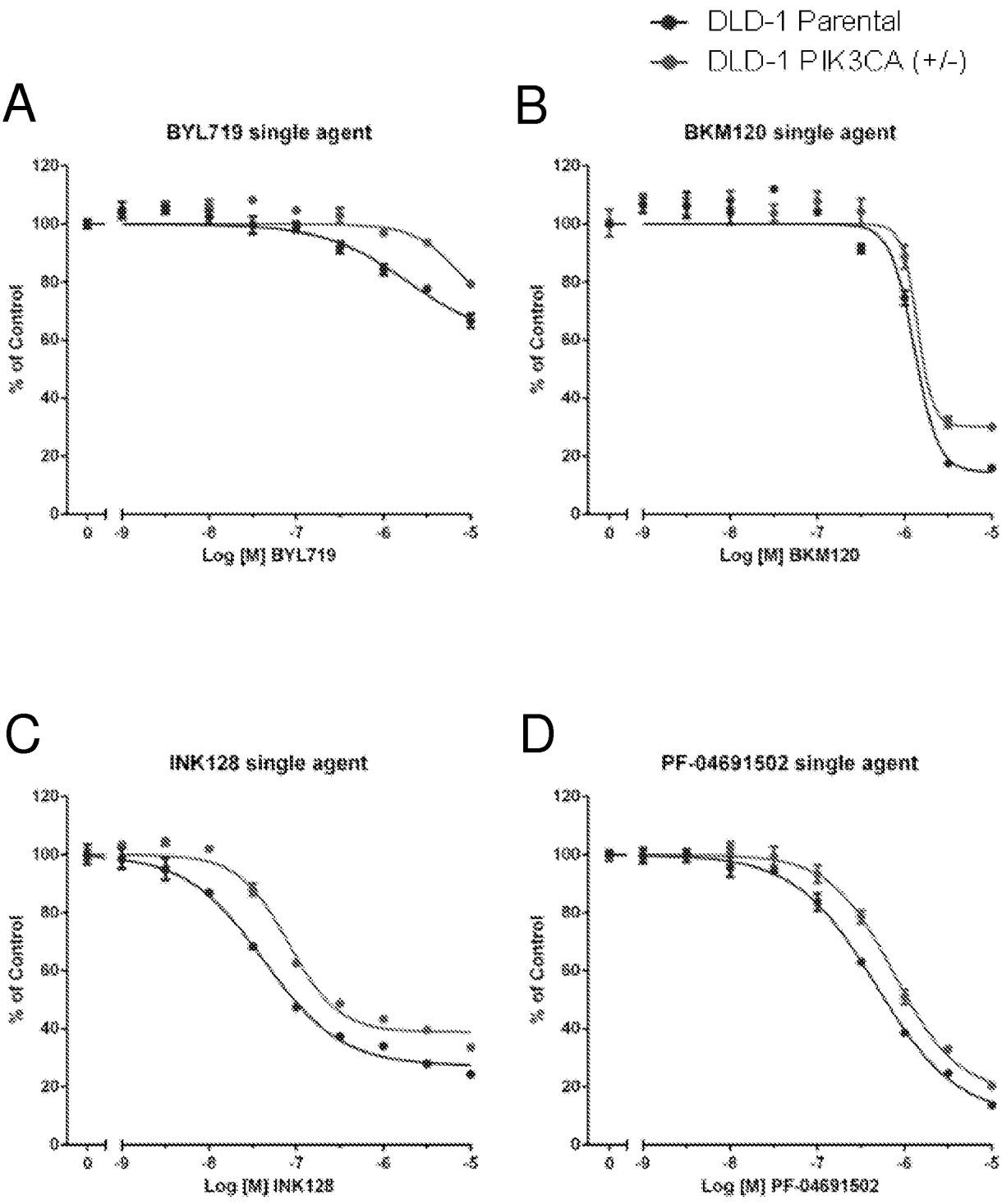
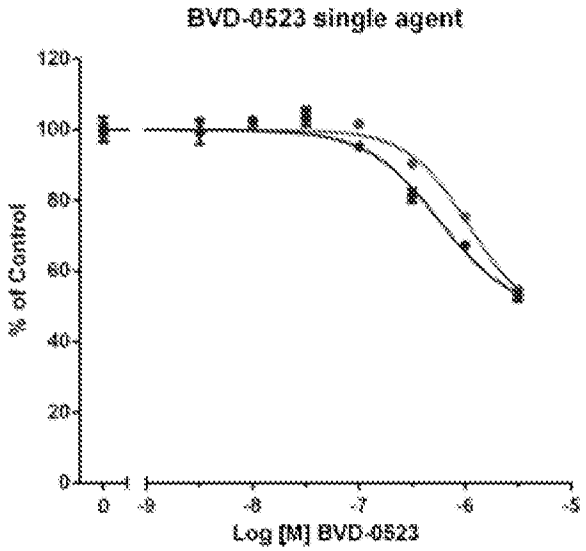

DLD-1 Parental
DLD-1 PIK3CA (+/-)
A
BYL719 single agent
% of Control
Log [M] BYL719
B
BKM120 single agent
% of Control
Log [M] BKM120
C
INK128 single agent
% of Control
Log [M] INK128
D
PF-04691502 single agent
% of Control
Log [M] PF-04691502

FIG. 21, Continued

E



F

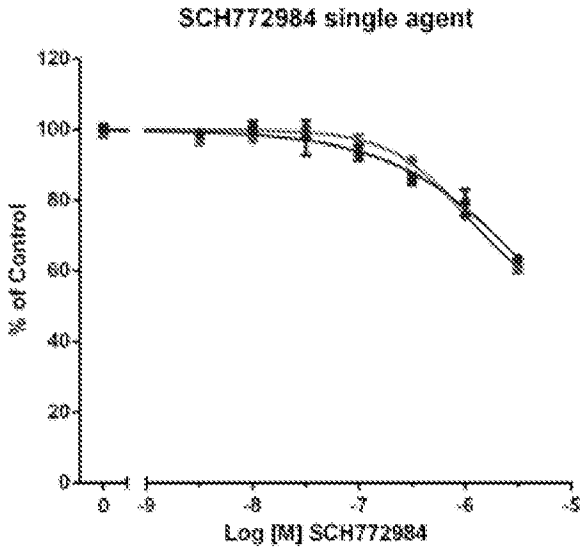


FIG. 22

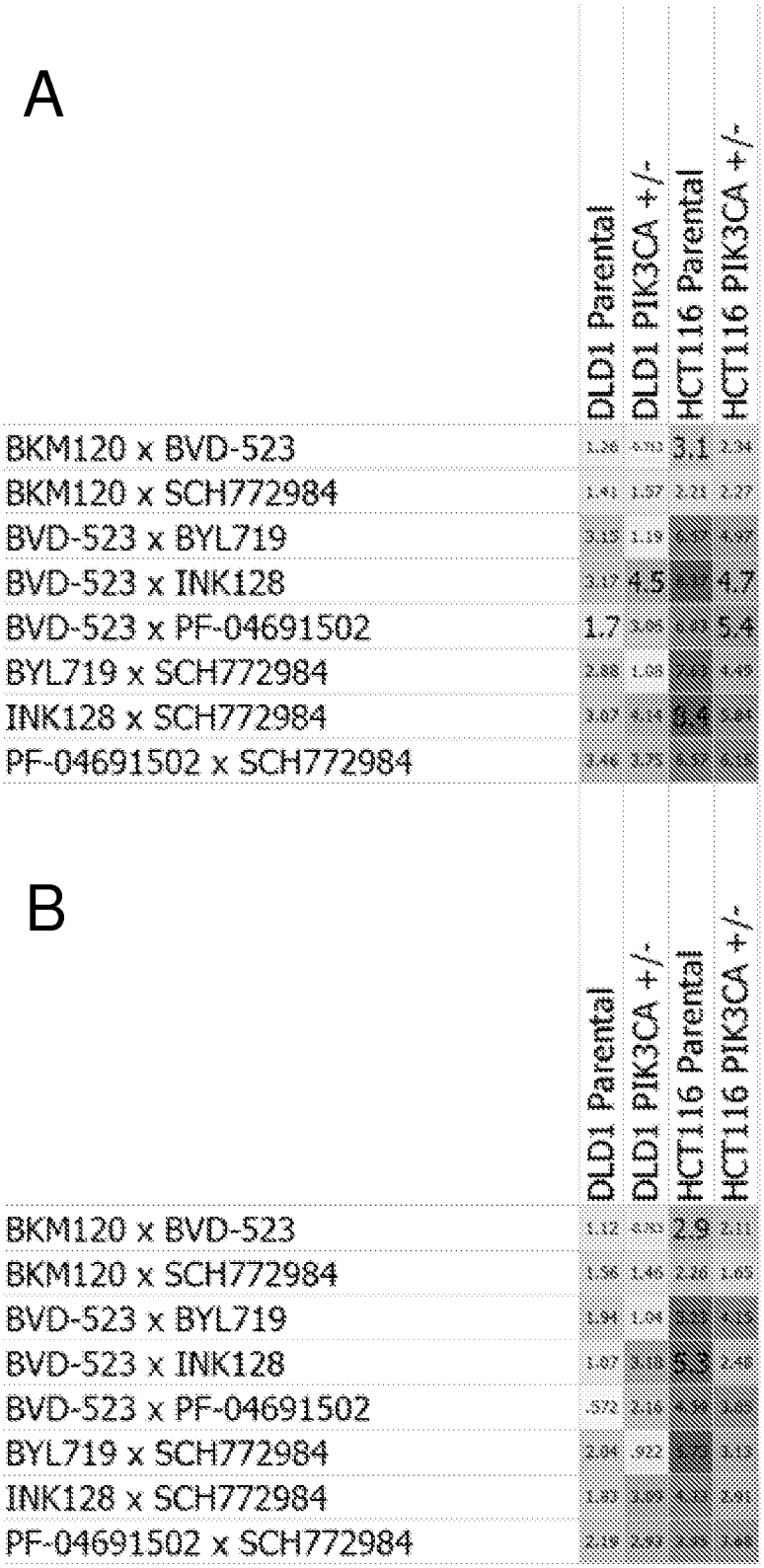

A
DLD1 Parental
DLD1 PIK3CA +/-
HCT116 Parental
HCT116 PIK3CA +/-
BKM120 x BVD-523
BKM120 x SCH772984
BVD-523 x BYL719
BVD-523 x INK128
BVD-523 x PF-04691502
BYL719 x SCH772984
INK128 x SCH772984
PF-04691502 x SCH772984
1.7
4.5
5.4
B
DLD1 Parental
DLD1 PIK3CA +/-
HCT116 Parental
HCT116 PIK3CA +/-
BKM120 x BVD-523
BKM120 x SCH772984
BVD-523 x BYL719
BVD-523 x INK128
BVD-523 x PF-04691502
BYL719 x SCH772984
INK128 x SCH772984
PF-04691502 x SCH772984

FIG. 22, Continued

C

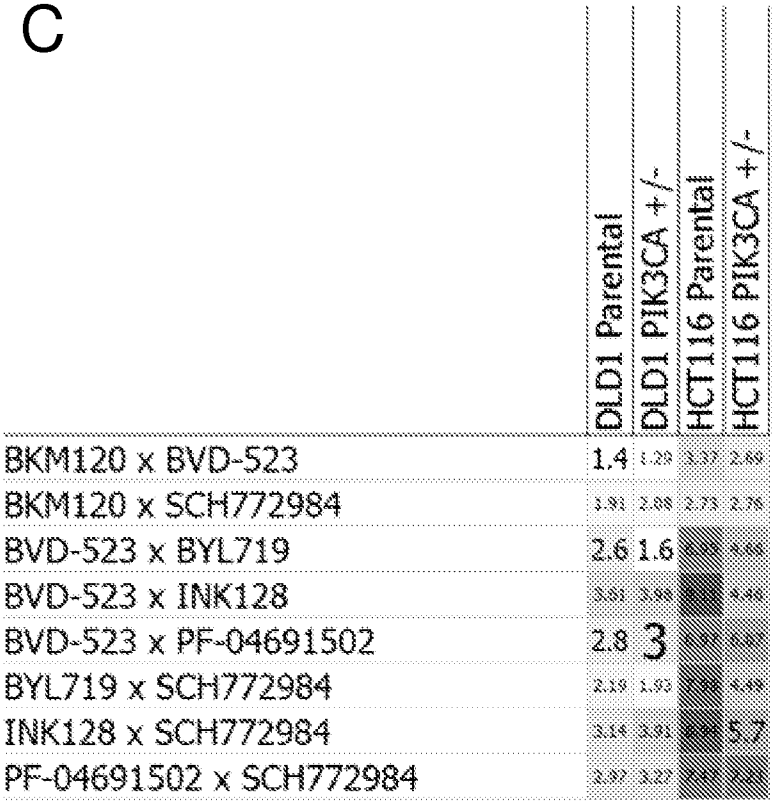
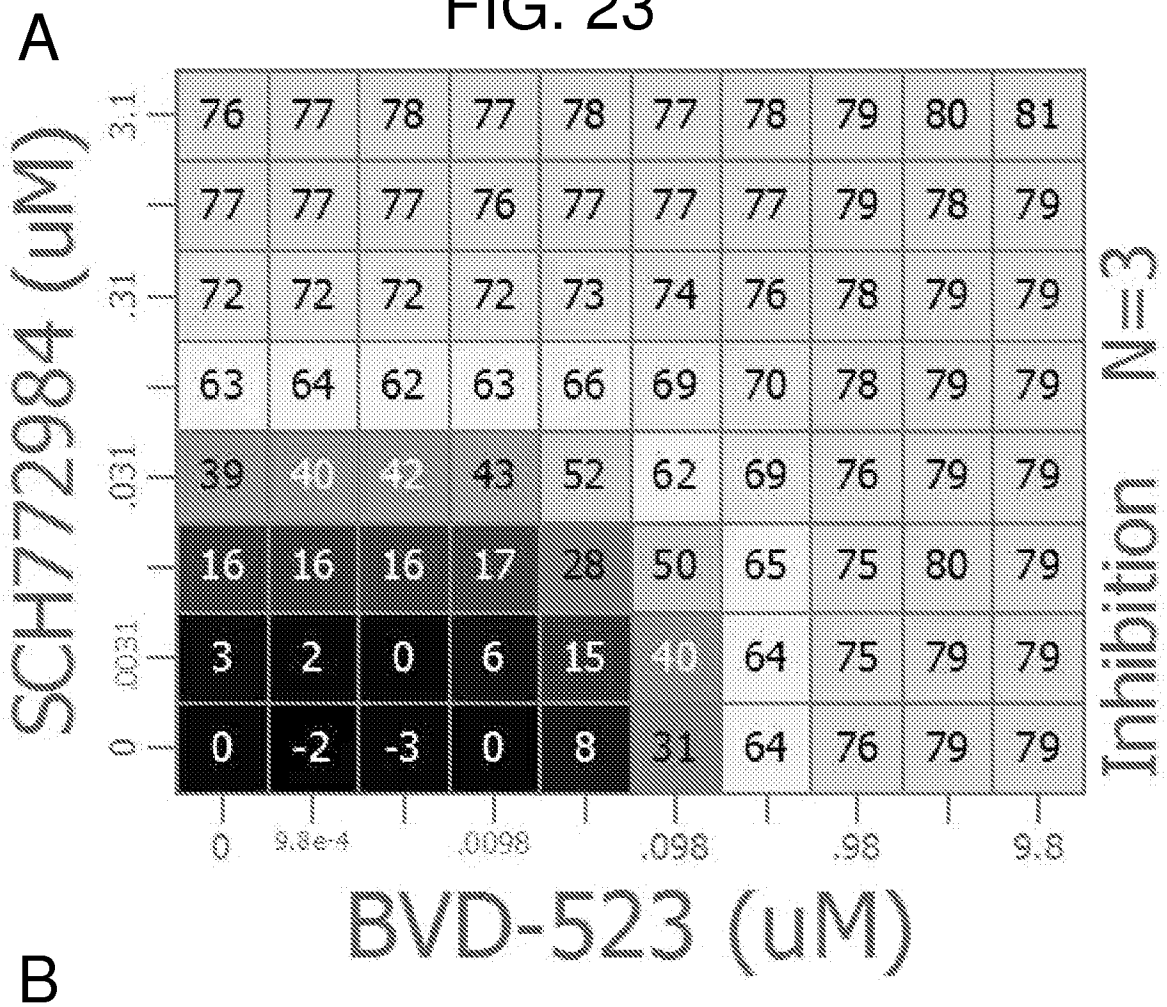
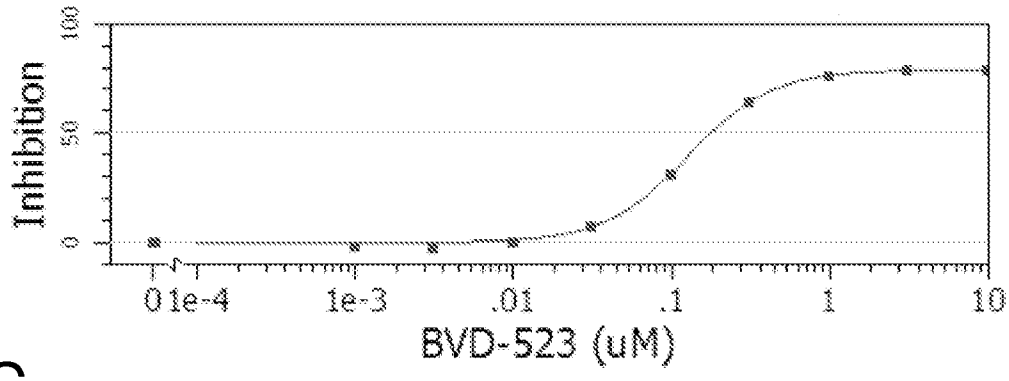


FIG. 23


A
SCH772984 (uM)
3.1
1
.31
1
.031
1
.0031
0
76 77 78 77 78 77 78 79 80 81
77 77 77 76 77 77 77 79 78 79
72 72 72 72 73 74 76 78 79 79
63 64 62 63 66 69 70 78 79 79
39 40 42 43 52 62 69 76 79 79
16 16 16 17 28 50 65 75 80 79
3 2 0 6 15 40 64 75 79 79
0 -2 -3 0 8 31 64 76 79 79
0 9.8e-4 .0098 .098 .98 9.8
BVD-523 (uM)
Inhibition N=3


B
Inhibition
100
50
0
0 1e-4 1e-3 .01 .1 1 10
BVD-523 (uM)

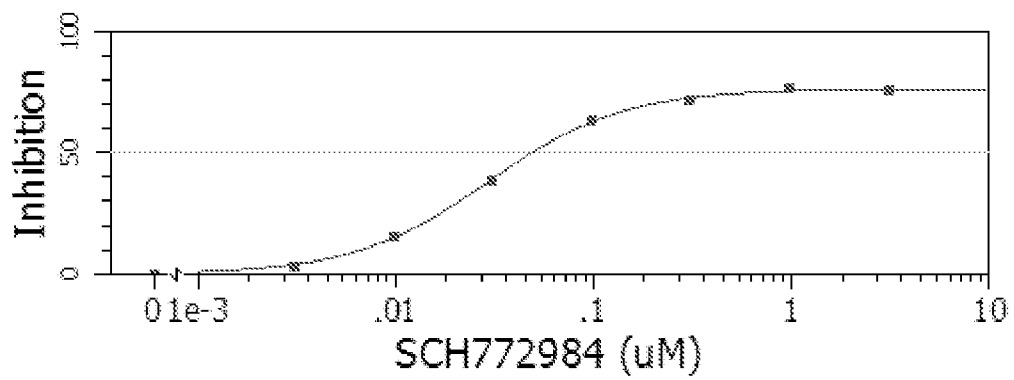
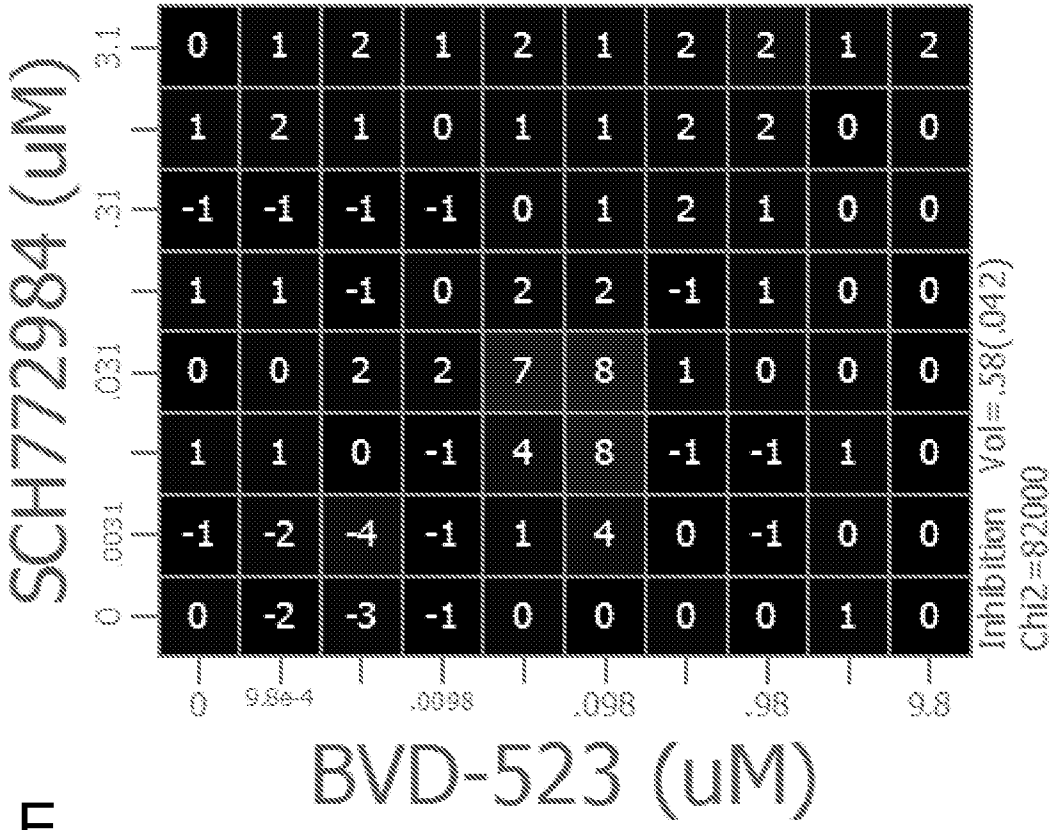
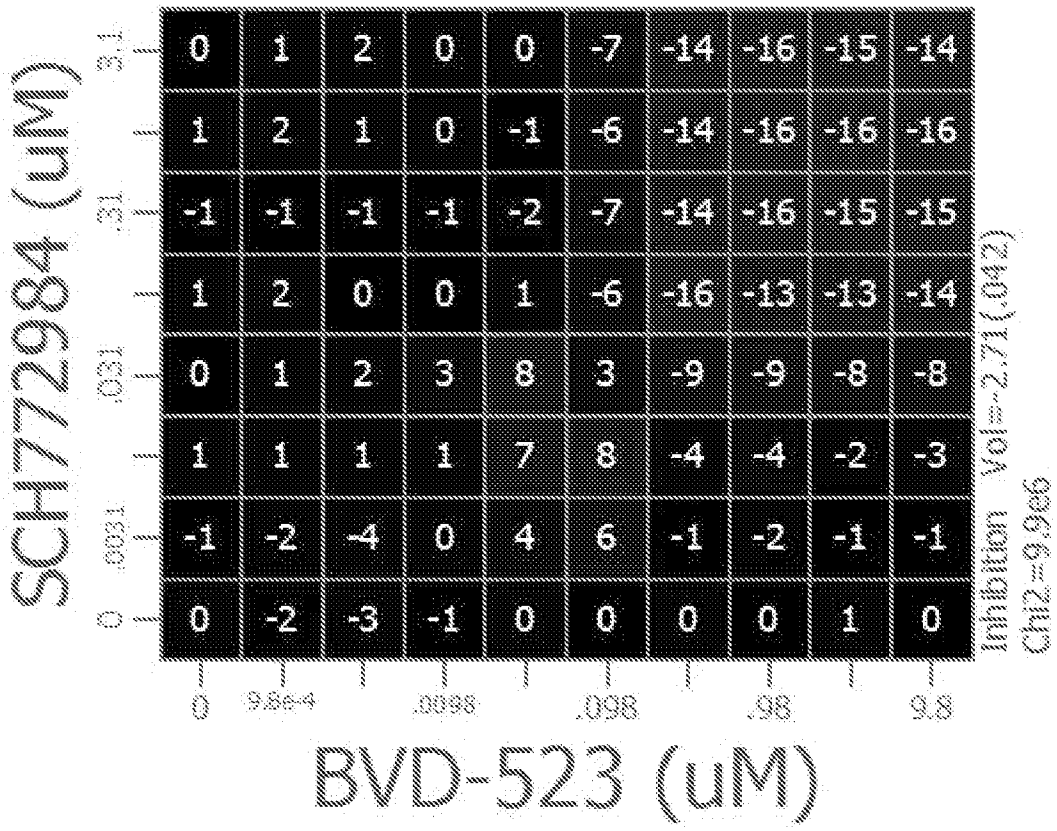

C
Inhibition
100
50
0
0 1e-3 .01 .1 1 10
SCH772984 (uM)

FIG. 23, Continued

D



E



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2014/071731

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

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

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

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

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

See Extra Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-48

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2014/071731

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 31/4439 (2015.01)
CPC - A61K 31/4439 (2015.04)
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8) - A61K 31/4439; C07D 401/04, 403/04; C07F 9/6558 (2015.01)
CPC - A61K 31/4439; C07D 401/04, 403/04; C07F 9/65583 (2015.04) (keyword delimited)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 514/343 (keyword delimited)

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

Orbit, Google Patents, Google Scholar.
Search terms used: Cancer, BVD-523, mTOR, treat, PI-3, inhibitor

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2006/0106069 A1 (MARTINEZ-BOTELLA et al) 18 May 2006 (18.05.2006) entire document	1-12, 15-28, 31-43, 46-48
---		---
Y		13, 14, 29, 30, 44, 45
Y	WO 2012/118978 A1 (THE REGENTS OF THE UNIVERSITY OF COLORADO A BODY CORPORATE et al) 07 September 2012 (07.09.2012) entire document	13, 14, 29, 30, 44, 45

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
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Date of the actual completion of the international search	Date of mailing of the international search report
14 April 2015	14 MAY 2015

Name and mailing address of the ISA/US	Authorized officer:
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2014/071731

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees need to be paid.

Group I: Claims 1-48 are drawn to methods of treating or ameliorating the effects of cancer in a subject, and methods of effecting cancer cell death.

Group II: Claims 49-85 are drawn to a kits and pharmaceutical compositions for treating or ameliorating the effects of cancer in a subject.

The inventions listed in Groups I and II do not relate to a single general inventive concept under PCT Rule 13.1, because under PCT Rule 13.2 they lack the same or corresponding special technical features for the following reasons:

The special technical features of Group I, methods of treating or ameliorating the effects of cancer in a subject, and methods of effecting cancer cell death, are not present in Group II; and the special technical features of Group II, kits and pharmaceutical compositions for treating or ameliorating the effects of cancer in a subject, are not present in Group I.

Groups I and II share the technical features of a method of treating or ameliorating the effects of a cancer in a subject in need thereof comprising administering to the subject an effective amount of (i) a first anti-cancer agent, which is BVD-523 or a pharmaceutically acceptable salt thereof and (ii) a second anti-cancer agent, which is a mTOR inhibitor or a pharmaceutically acceptable salt thereof. However, these features do not represent a contribution over the prior art.

Specifically, US 2006/0106069 A1 to Martinez-Botella et al. teaches a method of treating or ameliorating the effects of a cancer in a subject in need thereof (Abstract; Para. [0012]) comprising administering to the subject an effective amount of (i) a first anti-cancer agent, which is BVD-523 or a pharmaceutically acceptable salt thereof (Paras. [0011] and [0012]; see Table 1 below Para. [0038] at Formula I-9; see Applicant's Specification, Para. [0042], ...BVD-523, a preferred ERK1/2 inhibitor, corresponds to a compound according to formula (I):...; the shown formula (I) in the Applicant's Specification corresponds to Formula I-9) and (ii) a second anti-cancer agent, which is a mTOR inhibitor or a pharmaceutically acceptable salt thereof (Paras. [0011] and [0012]; Para. [0162], Other examples of agents the inhibitors of this invention may also be combined with include...rapamycin...; see Applicant's Specification, Para. [0043], Non-limiting examples of mTOR inhibitors according to the present invention include...rapamycin...).

The inventions listed in Groups I and II therefore lack unity under Rule 13 because they do not share a same or corresponding special technical feature.