Title: LIPOPHILIC BISPHOSPHONATES AND METHODS OF USE

Abstract: Disclosed herein are lipophilic bisphosphonate compounds and embodiments of a method for making the same. The compounds may inhibit FPPS and/or GGPPS enzymes. The compounds are useful for treating a disease, such as cancer, such as those with a KRAS mutation. In some embodiments, the compounds are used to prevent or treat a cancer, such as a lung cancer that has a KRAS mutation.

Declarations under Rule 4.17:
— of inventorship (Rule 4.17(iv))

Published:
— with international search report (Art. 21(3))
LIPOPHILIC BISPHOSPHONATES AND METHODS OF USE

CROSS REFERENCE TO RELATED APPLICATIONS
This application claims the benefit of the earlier filing date of U.S. Provisional Application No. 62/080,801, filed on November 17, 2014, the contents of which are incorporated herein by reference in its entirety.

FIELD
This application relates to lipophilic bisphosphonates and methods of their use, for example in combination with an autophagy inducer, to treat cancer.

ACKNOWLEDGMENT OF GOVERNMENT SUPPORT
This invention was made with government support under Grant Nos. R37AI048034 and R01AI048034 awarded by National Institutes of Health: National Institute of Allergy and Infectious Diseases, the National Cancer Institute Grant No. R01CA158191, and Contract No. DE-AC02-06CH1 1357 awarded by the U.S. Department of Energy. The government has certain rights in the invention.

BACKGROUND
Bisphosphonates are a class of drugs as the treatment of choice for various diseases of excessive bone resorption like osteoporosis, Paget's disease and the skeletal complications of malignancy, etc. Bisphosphonate drugs share some common properties but there are chemical, biochemical, and pharmacological differences among each bisphosphonate drugs. First generation bisphosphonates such as etidronate and clodronate act by forming "toxic-ATP" analogs that inhibit the mitochondrial adenine nucleotide translocase (ANT) in osteoclasts. They are strongly pro-apoptotic for osteoclasts and gain most of their activity by binding to bone mineral (hydroxy and carbonato-apatite). The second and third generation drugs, so-called nitrogen-containing bisphosphonates (e.g., pamidronate, alendronate, risedronate, ibandronate and zoledronate), inhibit the enzyme farnesyl-diphosphate synthase (FPPS), blocking isoprenoid bio synthesis.
SUMMARY

Currently available bisphosphonates are very polar. They enter cells, primarily macrophages and osteoclasts, via fluid-phase endocytosis. Bisphosphonates are also rapidly removed from circulation. These properties are good for a bone drug, but are undesirable for a drug that targets tumor cells in other organs. So there is a need to develop bisphosphonate to meet non-skeletal medical needs. The disclosure provides, inter alia, novel bisphosphonate compounds and methods of making and using the compounds. The disclosed compounds and methods can be used in connection with research and therapeutic applications, e.g., for treatment of cancers. In regard to certain embodiments, it has been recognized that certain structural features significantly enhance the activity of the compounds. In specific embodiments, the presence of particular alkyl substituents on a ring component in an organic bisphosphonate compound can contribute to desirable functional activity. Further variations are also provided.

The disclosure broadly provides bisphosphonate compounds and related methods of making and using such compounds. Specific embodiments concern organic bisphosphonate compounds and/or pharmaceutically acceptable salts or esters thereof. In addition, the disclosure provides other variations of bisphosphonate compounds. Particular species of functionally and/or therapeutically active bisphosphonates are also disclosed.

Pharmaceutical compositions that include one or more bisphosphonates are also disclosed. In some embodiments, the bisphosphonates are high potency bisphosphonates in one or more functional contexts.

In some embodiments, the disclosure provides an imidazole that has a molecular weight of at least 150 and less than 1,000, such as from 200 to 900, or from 300 to 800, and in certain embodiments, from 350 to 400, and that is capable of activating a γδ T cell.

Certain exemplary disclosed embodiments have a formula

\[
\begin{align*}
R^{11} & \quad O(M)_{q} \\
R^{12} & \quad X \\
Ar & \quad \text{O}(M)_{q} \\
\text{O} & \quad \text{P}(O(M)_{q} \\
\end{align*}
\]

or a pharmaceutically acceptable salt or hydrate thereof, wherein Ar is a 5-membered heteroaromatic ring; X is hydrogen, hydroxyl, SH, halogen, alkoxy, or aliphatic, particularly alkyl; each M is independently hydrogen, -(CH2)\_p-0-CO-R, -(CH2)\_p-CO-R, a cation or aliphatic, such as alkyl; each q is independently 0 or 1; R^{11} and R^{12} are independently H or aliphatic,
particularly alkyl; p is 1 to 6; r is from 1 to 6; and R is hydrogen, aryl or aliphatic, particularly alkyl. A person of ordinary skill in the art will understand that if a q is 0 for any -0(M) q moiety then the oxygen in the moiety will have a negative charge. However, in some embodiments, the compound is not (2-(lH-imidazol-1-yl)ethane-1,1-diyl)bis(phosphonic acid), (l-hydroxy-2-(lH-imidazol-4-yl)ethane-1,1-diyl)bis(phosphonic acid), sodium (1-hydroxy-2-(1-methyl-lH-imidazol-3-ium-3-yl)ethane-1,1-diyl)bis(hydrogen phosphonate), (1-hydroxy-2-(1H-1,2,3-triazol-1-yl)ethane-1,1-diyl)bis(phosphonic acid), sodium (1-hydroxy-l-(hydroxyoxidophosphoryl)-2-(1-phenyl-lH-imidazol-3-ium-3-yl)ethyl)phosphonate, sodium (1-(hydroxyoxidophosphoryl)-2-(1-phenyl-lH-imidazol-3-ium-3-yl)ethyl)phosphonate, (1-hydroxy-2-(thiazol-2-yl)ethane-1,1-diyl)bis(phosphonic acid), or 3-(2,2-diphosphonoethyl)-1-dodecyl-lH-imidazol-3-ium.

In some embodiments, M is a pharmaceutically acceptable cation. In certain examples, M is Li⁺, Na⁺, K⁺, Ca²⁺, Mg²⁺, NH₄⁺, N(R')₄⁺, where each R' independently is hydrogen or aliphatic, particularly alkyl or substituted alkyl, such as trialkyl ammonium compounds where the alkyl groups are the same or different. Certain particular examples include trimethyl ammonium, triethyl ammonium, and triethanol ammonium cations.

In certain embodiments, the compound has a formula

![Structure](image)

In some embodiments, Ar is a heteroaryl. Exemplary heteroaryl compounds include imidazole, pyrazole, pyrole, furan, thiophene, oxazole, isoxazole, thiazole, isothiazole, 1,2,3-triazole, 1,2,4-triazole, tetrazole, 1,2,3-oxadiazole, 1,2,4-oxadiazole, 1,2,5-oxadiazole, 1,3,4-oxadiazole, 1,2,3-thiadiazole, 1,2,4-thiadiazole, 1,2,5-thiadiazole or 1,3,4-thiadiazole, and in particular embodiments, Ar is a 5-membered nitrogen-containing ring, such as imidazole. The compound may have a formula selected from
wherein $R^4$, $R^6$ and $R^7$ are each independently H, aliphatic, such as alkyl or alkenyl; alkoxy; alkenoxy; haloalkyl; aryl; alkylaryl; arylalkyl; nitro; or halogen; and $R^5$ and $R^8$ are each independently a lone pair of electrons; H; aliphatic, such as alkyl or alkenyl; haloalkyl; aryl; alkylaryl; or arylalkyl.

In some embodiments, the disclosure provides compounds of the formula XA1:

$$
\begin{align*}
\text{XA1} & \\
& \quad \text{or}
\end{align*}
$$

or salts or hydrates thereof. With reference to formula XA1 X is hydrogen, hydroxyl, or halogen; each M independently is hydrogen, $-(\text{CH}_2)_p\text{O}-\text{CO-}$, $-(\text{CH}_2)_p\text{CO-}$, or aliphatic, such as alkyl; each q is independently 0 or 1; p is 1 to 6; and R is hydrogen, aryl, or aliphatic, such as alkyl; -OM can also be a salt of form $-\text{O}^-\text{A}^+$, where $\text{A}^+$ is a cation; and Z is hydrogen, aryl, aliphatic, such as alkyl. Z may be an alkyl group having a structure $X-(\text{CH}_2)_n$ where $n$ is an integer from 0 to 20, more typically 5-15, such as 8-12, and in particular examples, $n$ is 8, and X is hydrogen or halogen.

In other embodiments, $R^5$, $R^8$, or both $R^5$ and $R^8$, have a formula

$$
\begin{align*}
\text{(R}^9\text{)}_p & \\
\end{align*}
$$

where $R^9$ is aliphatic, such as alkyl or alkenyl; alkoxy; haloalkyl; NO$_2$; OH; aryl; alkenyloxy; SO$_2$R; or N(R$_{10}$)$_2$. $R^{10}$ is alkyl, and p is from 0 to 5.

A composition comprising at least one disclosed compound, or a pharmaceutically acceptable salt or hydrate thereof, and a pharmaceutically acceptable carrier also is disclosed. The composition may comprise one compound disclosed herein, or may comprise two or more
disclosed compounds. The composition may further comprise one or more additional therapeutic compounds.

Embodiments of a method for synthesizing a compound or a pharmaceutical formulation thereof also are disclosed. In one embodiment, a composition provided herein is isolated and/or purified.

The compositions disclosed herein can be used as a medicament. In an embodiment, a disclosed composition is used to prepare or manufacture a medicament, such as treatment of one or more conditions as disclosed herein (such as cancer).

Embodiments of a method for treating a medical condition comprising administering to a subject in need thereof a therapeutically effective amount of a compound or composition also are disclosed.

In an embodiment, the disclosure provides a method of selectively inhibiting an FPPS enzyme and a PvGGPPS enzyme comprising contacting the enzyme or a cell containing the enzyme with a disclosed compound, wherein the compound is capable of selectively inhibiting said FPPS enzyme and PvGGPPS enzyme.

In an embodiment, the disclosure provides a composition including a pharmaceutical formulation containing a compound of any formula herein.

In an embodiment, the disclosure provides a medicament which includes a therapeutically effective amount of one or more compositions of the disclosure. In an embodiment, the disclosure provides a method for making a medicament for treatment of a condition described herein.

A method of reducing or inhibiting growth of a cancer cell also is disclosed. Certain embodiments of the method include contacting the cancer cell with an effective amount of one or more compounds provided herein, and/or a pharmaceutical formulation thereof, for example in combination with an autophagy inducer (such as rapamycin or RAD001). Also disclosed are embodiments of a method for treating a cancer in vivo, for example by administering to a subject in need thereof a therapeutically effective amount of one or more compounds provided herein or a pharmaceutical formulation thereof, for example in combination with an autophagy inducer (such as rapamycin or RAD001). Exemplary cancers that can be treated with the disclosed embodiments include, but are not limited to, bladder cancer, lung cancer, breast cancer, melanoma cancer, colon cancer, rectal cancer, non-Hodgkin lymphoma cancer, endometrial cancer, pancreatic cancer, renal cancer, prostate cancer, leukemia cancer, and/or thyroid cancer. In one example, the cancer is one expressing a KRAS mutation.
Embodiments of a method of stimulating a T cell are disclosed. In some examples such a method includes contacting the T cell with one or more compounds provided herein, and/or a pharmaceutical formulation thereof, for example in combination with an autophagy inducer (such as rapamycin). In one embodiment, the T cell is a gamma delta (γδ) T cell.

Embodiments of a method of immunotherapeutic treatment are disclosed. In some examples such a method includes administering to a subject in need thereof a therapeutically effective amount of one or more compounds provided herein, and/or a pharmaceutical formulation thereof, for example in combination with an autophagy inducer (such as rapamycin or RADOI).

Without wishing to be bound by any particular theory, there can be discussion herein of beliefs or understandings of underlying principles or mechanisms relating to the disclosure. It is recognized that regardless of the ultimate correctness of any mechanistic explanation or hypothesis, disclosed embodiments of the disclosure can nonetheless be operative and useful.

The foregoing and other objects and features of the disclosure will become more apparent from the following detailed description, which proceeds with reference to the accompanying figures.

**BRIEF DESCRIPTION OF THE DRAWINGS**

FIG. 1A is a graph illustrating KRAS tumor cell xenografts control groups.

FIG. 1B is a graph illustrating KRAS tumor cell xenografts treated with BPH-1222 and rapamycin.

FIG. 1C is a graph illustrating KRAS tumor cell xenografts treated with rapamycin alone.

FIG. 1D is a graph illustrating KRAS tumor cell xenografts treated with BPH-1222 alone.

FIG. 2A is a graph of tumor weight versus compound illustrating the tumor volume of mice treated with rapamycin, BPH-1222 and their combination.

FIG. 2B is a photograph showing tumors from the mice from FIG. 2A. Top row: mice treated with saline (left), rapamycin +9 (center) or 9 only (right). Bottom row: mice treated with rapamycin only. Combination therapy provides a large decrease in tumor volume.

FIG. 2C is a graph illustrating the body weight of mice treated with rapamycin, BPH-1222 and their combination.
FIG. 3 is a schematic illustration of pathways involved in zoledronate analog activity in γδ T cells and in malaria parasites. Green = human cell; cyan = malaria parasite. HMG-CoA = hydroxymethyl-glutaryl coenzyme A; IPP = isopentenyl-diphosphate; DMAPP = dimethylallyl-diphosphate; FPP = farnesyl-diphosphate; GAP = glyceraldehyde-3-phosphate; HMBPP = 4-hydroxyl-3-methyl-but-2-enyl diphosphate; GGPP = geranylgeranyl-diphosphate; TNF-a = tumor necrosis factor a.

FIG. 4A provides the structures of certain exemplary disclosed compounds.

FIG. 4B is a graph of IC50 versus sidechain length illustrating the chain length dependence of enzyme and cell growth inhibition/activation and effects of the 1-OH group on HsFPPS inhibition.

FIG. 4C is a graph of IC50 versus sidechain length illustrating the chain length dependence of enzyme and cell growth inhibition/activation and effects of the 1-OH group on γδ T cell activation/ TNF-a release.

FIG. 4D is a graph of IC50 versus sidechain length illustrating the chain length dependence of enzyme and cell growth inhibition/activation and effects of the 1-OH group on PvGGPPS inhibition.

FIG. 4E is a graph of IC50 versus sidechain length illustrating the chain length dependence of enzyme and cell growth inhibition/activation and effects of the 1-OH group on intra-erythrocytic P. falciparum cell growth inhibition.

FIG. 5A is a schematic illustration of the X-ray structure of HsFPPS/bisphosphonate 5 complex (cyan, PDB ID code 4GA3) superimposed on PvGGPPS structure (purple, PDB ID code 3RBM), with the Ca rmsd over 331 residues in 1.44 Å.

FIG. 5B is a schematic illustration of the electron density map of 5 bound to HsFPPS.

FIG. 5C is a schematic illustration showing the comparison between the x-ray structures of 5 bound to HsFPPS, GPP (yellow) and FPP (green) bound to avian FPPS (PDB ID codes, 1UBX and 1UBW). The bisphosphonate 5 binds to the allylic (GPP) site. Chain elongation in FPP is blocked by F98, F99, corresponding to decreased HsFPPS inhibition by bisphosphonate inhibitors with /V-alkyl chains longer than about C15.

FIG. 5D is a schematic illustration showing the structure of HsPPPS/5 overlaid on (BPH-703) bound to PvGGPPS (PDB ID code 3RBM). The bisphosphonate, imidazolium and /V-alkyl side chain structures are quite similar. At about C11 steric repulsion ensues.

FIG. 6 is a schematic illustration of lipophilic analogs of the bone resorption drug zoledronate killing malaria parasites in a direct manner by inhibiting parasite...
geranylgeranyldiphosphate synthase, in addition to activating γδ T cells to kill parasites in an indirect (TNF-a/γδ T cells mediated) manner.

FIG. 7A provides the chemical structure of zoledronate.

FIG. 7B provides the chemical structures of a library of zoledronate analogs, side-chain length n = 0-15, X = H or OH.

FIG. 7C provides the chemical structure of BPH-1222.

FIG. 7D is a schematic illustration showing the structure of BPH-1222 binding to FPPS was determined by single crystal X-ray crystallography. Zoledronate binding is shown superimposed with BPH-1222 (zoledronate in yellow and BPH-1222 in cyan).

FIG. 8A is a graph of IC50 versus side-chain length illustrating the activity of zoledronate and its hydroxy analogs (see FIG. 10 for desoxy analogs) as determined in cell lines derived from KRAS-shp53 mouse lung cancer model (6# and L2) and control mouse embryonic fibroblasts (MEF) using MTT assay.

FIG. 8B is a graph of Ki versus side-chain length illustrating the Ki of compounds in FIG. 8A measured in vitro against human FPPS or GGPPS.

FIG. 8C provides photographs of mouse lung cancer cells (6#) treated with a single drug (FTI-277, 15 μM; GGTT-298, 15 μM; BPH-1222, 10 μM; FOH, 10 μM; GGOH, 10 μM; Ascorbic acid, 50 μM) or drug combinations as indicated for 48 hours. Images were taken under phase-contrast microscope. Scale bars, 100 μm.

FIG. 8D is a graph illustrating the cell survival of mouse lung cancer cells (L2) and embryonic fibroblasts (MEF, matched genetic background) treated with different concentrations of BPH-1222 for 3 days, measured using Cell Proliferation Reagent WST-1 from Roche.

FIG. 8E is a graph illustrating the cell survival of mouse embryonic fibroblasts transformed by KRAS-shp53 and MYCL1-shp53-shRBl treated with different concentrations of BPH-1222 for 3 days, measured using Cell Proliferation Reagent WST-1 from Roche.

FIG. 9A is a photograh of mouse lung cancer cells illustrating the FPPS/GGPPS activity and cell growth inhibited by bisphosphonates. The mouse lung cancer cells (6#) were treated with 10 μM zoledronate (CO) or its analogs (side-chain= C1-C1 1, X= OH or H) for 3 days and stained using crystal violet. Scale bars, 100 μm.

FIG. 9B is a graph of IC50 versus side-chain length illustrating the IC50 of desoxy zoledronate and its analogs determined in cell lines derived from KRAS-shp53 mouse lung cancer model (6# and L2) and control mouse embryonic fibroblasts (MEF) using MTT assay.
FIG. 9C is a graph of Ki versus side-chain length illustrating the Ki of the compounds in FIG. 9B measured in vitro against human FPPS or GGPPS.

FIG. 9D is a photograph of immunoblots illustrating mouse lung cancer cells (M3L2) that were treated with FTI-277 (15 µM), GGTI-298 (15 µM) or a combination thereof for 48 hours and whole cell lysates, analyzed by immunoblotting.

FIG. 9E is a graph illustrating the cell survival of the mouse lung cancer cells (M3L2) treated with different concentrations of FTI, GGTI or BPH-1222 for 3 days. Cell survival was measured using Cell Proliferation Reagent WST-1 from Roche.

FIG. 9F provides photographs illustrating mouse lung cancer cells (6#) that were treated with bisphosphonates (BPH-1222, 10 µM; BPH-714, 10 µM; zoledronate, 80 µM) and simvastatin (0.5 µM) alone, or in combination with other compounds (chloroquine, 30 µM; rapamycin, 0.1 µM; FOH, 10 µM; GGOH, 10 µM) for 48 hours. Images were taken under phase-contrast microscope. Scale bars, 100 µm.

FIG. 10A is a photograph of immunoblots of U20S cells expressing Flag-KRAS G12D treated with bisphosphonates (BPH-1222, 10 µM; BPH-714, 10 µM; zoledronate, 20 µM) or chloroquine (30 µM) for 48 hours. Cellular distributions of proteins (HRAS, KRAS and RAPIA) that require prenylation were examined by immunoblotting. p, pellet contains correctly prenylated proteins which bind avidly to membrane; s, supernatant contains unmodified proteins in cytoplasm. *, HRAS signal left on the membrane. **, autophagy activation determined by the presence of LC3-II (bottom band, PE-conjugated form).

FIG. 10B is a photograph of immunoblots of the cells from FIG. 10A treated with FTI-277 (15 µM), GGTI-298 (15 µM) or BPH-1222 (5, 10, 15 µM) for 48 hours. Cell lysates were separated with 15-cm SDS-PAGE and blotted with indicated antibodies. *, KRAS mobility shift was observed.

FIG. IOC is a photograph of immunoblots of mouse lung cancer cells (M3L2) treated with BPH-1222 for 48 hours and analyzed for KRAS, AKT, Caspase-3 activation and LC3 conversion. KRAS-GTP was pulled down from whole cell lysate with RAFT-1 RBD beads and immunoblotted with total KRAS antibody.

FIG. 10D is a photograph of immunoblots of human cancer cells harboring KRAS mutations (Panc-1 and MiaPaCa2) treated with BPH-1222 (10 µM) for 48 hours and analyzed in the same way as in FIG. IOC.

FIG. 10E is a photograph of immunoblots of mouse lung cancer cells (6#) treated with BPH-1222 (10 µM) for 1, 2 or 3 days illustrating induced ER stress (CHOP, BiP), autophagy...
(PE-conjugated LC3II) and apoptosis (Caspase-3) in mouse lung cancer cell (6#) in a time-dependent manner.

FIG. 11A is a schematic diagram illustrating the Mouse KRAS G12D sequence for mass spectrum analysis.

FIG. 11B is a photograph of immunoblots of mouse lung cancer cells (6#) treated with simvastatin (0.5 μM), FTI-277 (10 μM), GGTI-298 (10 μM), BPH-1222 (10 μM), or combinations (FOH, 10 μM; GGOH, 10 μM and anti-oxidants: Ascorbic acid, 10 μM; Trolox, 10 μM; Morin hydrate, 10 μM; Lipoic acid, 10 μM) for 48 hours, and lysed for immunoblotting with ER stress probes (BiP, CHOP and PERK-p).

FIG. 11C is a photograph of immunoblots of mouse lung cancer cells (6#) treated with BPH-1222 alone or together with rapamycin (0.1 μM) or chloroquine (30 μM) for 48 hours. Whole cell lysates were examined for MAPK pathway activity by immunoblotting.

FIG. 11D is a photograph of immunoblots of the same cells from FIG. 11C treated with drug combinations in the absence or presence of U0126 (10 μM) for 48 hours, and lysed for immunoblotting to check MAPK pathway activity.

FIG. 12 is a table of KRAS molecular weights (M.W.) measured by Mass spectrometry.

FIG. 13A is a graph illustrating the dose response curves of mouse lung cancer cells (6#) treated with BPH-1222 in the presence of different concentrations of chloroquine for 3 days. Cell survival was examined with Cell Proliferation Reagent WST-1. All data were collected from triplicate wells and are presented as means ± SD, n = 3.

FIG. 13B is a photograph of mouse lung cancer cell (L2, infected with 5XKB-1UCI reporter) syngeneic grafts and treated with BPH-1222 (2 mg/kg) plus either chloroquine (60 mg/kg) or rapamycin (2.5 mg/kg) for 3 weeks. Scale bars, 10 mm.

FIG. 13C is a graph illustrating the NF-κB activity in tumor grafts examined by in vivo luciferase imaging system (IVIS) immediately after the 3-week treatment.

FIG. 13D is a graph illustrating the relative mRNA levels in mouse lung cancer cells (6#) treated with BPH-1222 (10 μM) or combinations (CQ, 30 μM; Rapa, 0.1 μM) for 48 hours, and NF-KB target genes were examined by quantitative RT-PCR. All data were collected from triplicate wells and are presented as means ± SD, n = 3.

FIG. 13E provides photographs of L2 tumor samples treated with different combinations, sectioned and immuno- stained with Ki-67 and Cleaved Caspase-3 antibodies. Caspase-3 positive cells were marked with arrowheads. Scale bars, 100 μm.
FIG. 13F is a graph illustrating the percentage of Ki-67 positive cells from the samples from FIG. 13E.

FIG. 13G is a graph illustrating the percentage of Caspase-3 positive cells from the samples from FIG. 13E.

FIG. 13H is a photograph of mouse lung cancer cells (L2) syngeneic grafts treated with BPH-1222 (2 mg/kg), rapamycin (2.5 mg/kg) or combination for 3 weeks. Scale bar, 10 mm.

FIG. 13I is a photograph of immunoblots of mouse lung cancer cells (6#) treated with BPH-1222 (10 µM), rapamycin (0.1 µM) or combination for 48 hours and examined by immunoblotting.

FIG. 14A provides photographs of mouse lung cancer cell line (6#) and human lung cancer cell lines (A549 and A427) treated with BPH-1222 (10 µM), chloroquine (30 µM) or combination for 48 hours. Images were taken under phase-contrast microscope. Scale bars, 100 µm.

FIG. 14B is a photograph of immunoblots of mouse lung cancer cells (6#) treated with BPH-1222 alone or together with rapamycin (0.1 µM) or chloroquine (30 µM) for 48 hours. Whole cell lysates were examined by immunoblotting. *, non-specific bands.

FIG. 14C is a table of the pharmacokinetic profile of BPH-1222 following single dose i.v. administration (5 mg/kg) to female SD rats (n=3).

FIG. 14D is a photograph of the tumors from FVB mice carrying M3L2 cell syngeneic grafts treated with zoledronate (2 mg/kg), BPH-1222 (2 mg/kg), rapamycin (2.5 mg/kg) or combinations for 3 weeks. Each tumor was dissected and weighed. Scale bar, 10 mm.

FIG. 14E is a graph of tumor weight versus treatment illustrating the tumor weights.

FIGS. 15A-15H illustrate that combination therapy inhibits tumor growth in orthotopic graft model and KRAS-shp53 lentiviral model.

FIG. 15A provides photographs of the lungs from mouse orthotopic grafts that were induced by tail vein injection of M3L2 cells. Mice were left untreated or given treatment 16 days after the inoculation. Lungs were collected when mice reached IACUC clinical end-point.

FIG. 15B is a graph of tumor number versus treatment illustrating that the control group had 29.8 average lesions per mouse, n=8, and the treatment group had 13.5 average lesions per mouse, n=13. Tumor lesions larger than 1 mm in diameter were counted.

FIG. 15C is a graph of survival percentage versus days illustrating the Kaplan-Meier curves of mice from control group and treatment group. All survived mice were collected on day 50. Median survival time: Ctrl, 33.5 d, n=8; Treat, 37 d, n=13.
FIG. 15D provides photographs of Luciferase imaging results of one mouse from Low-Ctrl group and one mouse from Low-Treat group, to show the shrinkage of tumor after the combination therapy. Mouse lung adenocarcinomas were induced by intra-tracheally infection of KRAS-shp53 lentiviral vectors. Mice were left untreated or given treatment when luciferase signals from tumors were detectable (Low-Ctrl and Low-Treat groups, luciferase signal: $10^3$-$10^5$; High-Ctrl and High-Treat groups, luciferase signal: $>10^5$). See FIG. 16 for results from all mice.

FIG. 15E is a graph illustrating the fold changes of luciferase signal after 2-week treatment. Negative value means shrinkage in tumor size.

FIG. 15F is a graph of survival percentage versus days illustrating the Kaplan-Meier curves of mice from all groups. Median survival time: Low-Ctrl, 55 d, n=8; Low-Treat, 75.5 d, n=6; High-Ctrl, 28 d, n=8; High-Treat, 54 d, n=6.

FIG. 15G provides photographs of tumors from Low-Ctrl and Low-Treat groups that were sectioned and immuno-stained with different antibodies. Caspase-3 positive cells were marked with arrowheads. Scale bars, 100 μm (insets in 4E-BPI-p staining: scale bars, 20 μm).

FIG. 15H is a graph quantifying the results shown in FIG. 15G.

FIGS. 16A-16C provides the results from the combination therapy in the KRAS-shp53 lentiviral model. All mice carrying KRAS-shp53 tumors described in FIGS. 16A-16C were examined for tumor load by in vivo luciferase imaging system (IVIS) every 10 days during the treatment. Legend names are all derived from mouse IDs and don’t carry any real meanings.

FIG. 16A is a graph illustrating the results from the control group of untreated group, n=8.

FIG. 16B is a graph illustrating the results from the Low-Treat group, where the mice had low tumor load when treatment was started, n=8. *Two mice were collected for histology right after the treatment.

FIG. 16 C is a graph illustrating the results from the High-Treat group, where the mice had high tumor load when treatment was started, n=6.

FIG. 17 is a graph of weight versus treatment illustrating the effect of treatment with BPH-1222 and either rapamycin (rapal222) or everolimus (evel222) in a syngeneic transplantation mouse model.

FIG. 18 is a graph of weight versus treatment illustrating the effect of treatment with BPH-1222 and either rapamycin (rapal222) or everolimus (evel222) in an orthotopic model.
DETAILED DESCRIPTION

I. Terms

As used herein, the recited terms have the following meanings. All other terms and phrases used in this specification have their ordinary meanings as one of ordinary skill in the art would understand. Such ordinary meanings may be obtained by reference to technical dictionaries, such as Hawley's Condensed Chemical Dictionary 14th Edition, by R.J. Lewis, John Wiley & Sons, New York, N.Y., 2001.

References in the specification to "one embodiment," "an embodiment," etc., indicate that the embodiment described may include a particular aspect, feature, structure, moiety, or characteristic, but not every embodiment necessarily includes that aspect, feature, structure, moiety, or characteristic. Moreover, such phrases may, but do not necessarily, refer to the same embodiment referred to in other portions of the specification. Further, when a particular aspect, feature, structure, moiety, or characteristic is described in connection with an embodiment, it is within the knowledge of a person of ordinary skill in the art to affect or connect such aspect, feature, structure, moiety, or characteristic with other embodiments, whether or not explicitly described.

The singular forms "a," "an," and "the" include plural reference unless the context clearly dictates otherwise. It is further noted that the claims may be drafted to exclude any optional element. As such, this statement is intended to serve as antecedent basis for the use of exclusive terminology, such as "solely," "only," and the like, in connection with the recitation of claim elements or use of a "negative" limitation.

The term "and/or" means any one of the items, any combination of the items, or all of the items with which this term is associated. The phrase "one or more" is readily understood by a person of ordinary skill in the art, particularly when read in context of its usage. For example, one or more substituents on a phenyl ring refers to one to five, or one to four, for example if the phenyl ring is di-substituted.

Unless otherwise indicated, all numbers expressing quantities of components, molecular weights, percentages, temperatures, times, and so forth, as used in the specification or claims are to be understood as being modified by the term "about." Accordingly, unless otherwise indicated, implicitly or explicitly, the numerical parameters set forth are approximations that may depend on the desired properties sought and/or limits of detection under standard test conditions/methods. When directly and explicitly distinguishing embodiments from discussed prior art, the embodiment numbers are not approximates unless the word "about" is recited.
As will be understood by one of ordinary skill in the art, for any and all purposes, particularly in terms of providing a written description, all ranges recited herein also encompass any and all possible sub-ranges and combinations of sub-ranges thereof, as well as the individual values making up the range, particularly integer values. A recited range (e.g., weight percents or carbon groups) includes each specific value, integer, decimal, or identity within the range. Any listed range can be easily recognized as sufficiently describing and enabling the same range being broken down into at least equal halves, thirds, quarters, fifths, or tenths. As a non-limiting example, each range discussed herein can be readily broken down into a lower third, middle third and upper third, etc. As will also be understood by one skilled in the art, all language such as "up to," "at least, "greater than," "less than," "more than," "or more," and the like, include the number recited and such terms refer to ranges that can be subsequently broken down into sub-ranges as discussed above. In the same manner, all ratios recited herein also include all sub-ratios falling within the broader ratio.

A person of ordinary skill in the art will also readily recognize that where members are grouped together in a common manner, such as in a Markush group, the disclosure encompasses not only the entire group listed as a whole, but each member of the group individually and all possible subgroups of the main group. Additionally, for all purposes, the disclosure encompasses not only the main group, but also the main group absent one or more of the group members. The disclosure therefore envisages the explicit exclusion of any one or more of members of a recited group. Accordingly, provisos may apply to any of the disclosed categories or embodiments whereby any one or more of the recited elements, species, or embodiments, may be excluded from such categories or embodiments, for example, as used in an explicit negative limitation.

All references, including patent applications and patents, and sequences associated with the GenBank® Accession Numbers listed (as of November 14, 2014) are herein incorporated by reference.

**Administration:** To provide or give a subject an agent, such as one or more compounds provided herein, by any effective route. Exemplary routes of administration include, but are not limited to, injection (such as subcutaneous, intramuscular, intradermal, intraperitoneal, intratumoral, and intravenous), transdermal, intranasal, and inhalation routes.

**Autophagy:** A cellular recycling pathway in which cytoplasm and organelles are engulfed within double-membrane vesicles, autophagosomes, and fused with lysosomes for
degradation. Autophagy plays a role in cell survival and death and has been implicated in
development, aging, neurodegeneration, and cancer.

The rate or amount of autophagy increases when the cell is contacted with an autophagy
inducer. Exemplary autophagy inducers that can be used in the compositions and methods
provided herein include but are not limited to: (1) inhibitors of mTOR activation, such as
metformin, perifosine, rapamycin, everolimus, resveratrol, and tamoxifen; (2) activators of
autophagosome formation, such as MG-132, SAHA (suberoylanilide hydroxamine), trichostatin
A, valproic acid, 5-aza-cytidine, and Z-VAD-FMK. Other specific examples include
amiodarone HCl, niclosamide, verrucologen, and STF-62247 (Merck). Autophagy inducers are
administered at therapeutically effective amounts, such as at least 0.01 mg/kg, at least 0.1
mg/kg, at least 1 mg/kg, at least 2 mg/kg, at least 5 mg/kg, or at least 10 mg/kg.

Cancer: A malignant tumor characterized by abnormal or uncontrolled cell growth.
Other features often associated with cancer include metastasis, interference with the normal
functioning of neighboring cells, release of cytokines or other secretory products at abnormal
levels and suppression or aggravation of inflammatory or immunological response, invasion of
surrounding or distant tissues or organs, such as lymph nodes, etc. "Metastatic disease" refers to
cancer cells that have left the original tumor site and migrate to other parts of the body for
example via the bloodstream or lymph system. In one example, a cell targeted for removal by
the disclosed methods is a cancer cell.

Contact: Placement in direct physical association, including a solid or a liquid form.
Contacting can occur in vitro or ex vivo, for example, with a sample, or in vivo by administering
to a subject.

Increase or Decrease: A statistically significant positive or negative change,
respectively, in quantity from a control value. An increase is a positive change. In certain
examples, an increase may be at least 50%, at least 100%, at least 200%, at least 300%, at least
400% or at least 500% as compared to the control value. A decrease is a negative change. In
certain examples, a decrease may be at least 20%, at least 25%, at least 50%, at least 75%, at least
80%, at least 90%, at least 95%, at least 98%, or at least 100% decrease as compared to a control
value.

Inhibit or reduce: The slowing, halting, or reversing of something, such as the growth
or progression of a disease (such as a cancer) condition, or group of cells. In some examples,
inhibition or reduction can be a decrease of at least 20%, at least 40% at least, 60%, at least
80%, at least 90%, at least 95%, or at least 99%, for example, compared to a particular activity that occurs in the absence of a treatment or contact.

**KRAS:** OMIM 190070. Includes KRAS nucleic acid molecules and proteins. The protein product of the normal KRAS gene performs is involved in normal tissue signaling, and the mutation of a KRAS gene is a step in the development of many cancers. This proto-oncogene is implicated in many cancers, such as lung adenocarcinoma, follicular and undifferentiated thyroid cancer, acute myeloid leukemia, mucinous adenoma, ductal carcinoma of the pancreas and colorectal carcinoma. Mutations in KRAS in some examples are the result of a single amino acid substitution, and can be used to predict which subjects are not likely to respond to particular therapies, such as those that inhibit EGFR, such as erlotinib, gefitinib, panitumumab and cetuximab. KRAS mutations frequently found in neoplasms include those at exon 2 (codons 12 and 13) and exon 3 (codon 61). Mutations in KRAS codons 12 and 13 have been associated with lack of response to EGFR-targeted therapies in both CRC and NSCLC patients. In some examples, KRAS is upregulated/amplified in a cancer (such as cancers of the ovary, GI tract, uterus, and lung).

Specific Exemplary KRAS mutations that may be present in a cancer or cancer cell treated with the disclosed compositions and methods are shown below

<table>
<thead>
<tr>
<th>Variant Nucleotide Position</th>
<th>(Amino acid change)</th>
</tr>
</thead>
<tbody>
<tr>
<td>216 G&gt;T</td>
<td>(G12V)</td>
</tr>
<tr>
<td>216 G&gt;A</td>
<td>(G12D)</td>
</tr>
<tr>
<td>215 G&gt;T</td>
<td>(G12C)</td>
</tr>
<tr>
<td>219 G&gt;A</td>
<td>(G13D)</td>
</tr>
<tr>
<td>363 A&gt;T</td>
<td>(Q61L)</td>
</tr>
<tr>
<td>364 A/C; 364 A&gt;T</td>
<td>(Q61H)</td>
</tr>
</tbody>
</table>

In humans, KRAS is located at 12pl2.1, spans approximately 38 kb, and encodes a 188-amino acid residue with a molecular weight of 21.6 kDa. KRAS sequences are publically available, for example from the GenBank® sequence database (e.g., Accession Nos. NP_004976.2, NP_203524.1 and NP_067259.4 provide exemplary KRAS protein sequences, while Accession Nos. NM_004985.4 and NM_021284.6 provide exemplary KRAS nucleic acid sequences).
sequences). One of ordinary skill in the art can identify additional KRAS nucleic acid and protein sequences, including KRAS variants.

**Mammal:** This term includes both human and non-human mammals. Similarly, the term "subject" includes both human and veterinary subjects.

**Pharmacologically acceptable carriers:** Pharmacologically acceptable carriers useful in this disclosure are known to persons of ordinary skill in the art. Additionally, *Remington's Pharmaceutical Sciences*, by E. W. Martin, Mack Publishing Co., Easton, PA, 15th Edition (1975), describes compositions and formulations suitable for pharmaceutical delivery of a bisphosphonate compound provided herein.

In general, the nature of the carrier will depend on the particular mode of administration being employed. For instance, parenteral formulations usually comprise injectable fluids that include pharmaceutically and physiologically acceptable fluids such as water, physiological saline, balanced salt solutions, aqueous dextrose, glycerol or the like as a vehicle. In addition to biologically-neutral carriers, pharmaceutical compositions to be administered can contain minor amounts of non-toxic auxiliary substances, such as wetting or emulsifying agents, preservatives, and pH buffering agents and the like, for example sodium acetate or sorbitan monolaurate.

**Pharmacologically acceptable salts:** Pharmacologically acceptable salts comprise pharmaceutically-acceptable anions and/or cations. Pharmaceutically-acceptable cations include among others, alkali metal cations (e.g., Li⁺, Na⁺, K⁺), alkaline earth metal cations (e.g., Ca²⁺, Mg²⁺), non-toxic heavy metal cations and ammonium (NH₄⁺) and substituted ammonium (N(R')₄⁺, where R' is hydrogen or alkyl, i.e., including, methyl, ethyl, or hydroxyethyl, specifically, trimethyl ammonium, triethyl ammonium, and triethanol ammonium cations). Pharmaceutically-acceptable anions include among other halides (e.g., Cl⁻, Br⁻), sulfate, acetates (e.g., acetate, trifluoroacetate), ascorbates, aspartates, benzoates, citrates, and lactate.

**Subject:** Any subject that can benefit from a disclosed treatment or treatments provided herein. In one embodiment, the subject is a non-human mammalian subject, such as a monkey or other primate, mouse, rat, rabbit, pig, goat, sheep, dog, cat, horse, or cow. In another embodiment, the subject is a human subject. In some examples, the subject has a cancer, such as a cancer with a KRAS mutation (such as one at codon 12, 13 or 61).

**Effective amount or Therapeutically effective amount:** The amount of agent, such as one or more bisphosphonate compounds provided herein (for example alone or with other agents, such as an autophagy inducer), that is an amount sufficient to prevent, treat (including prophylaxis), reduce and/or ameliorate the symptoms and/or underlying causes of any of a
disorder or disease. In one embodiment, an "effective amount" is sufficient to reduce or eliminate a symptom of a disease, such as cancer (such as a cancer expressing a KRAS mutation). The therapeutically effective amount can be dependent on the subject being treated (e.g., the species or size of the subject), the severity of the disease in the recipient subject.

Determination of a therapeutically effective amount is within the capacity of persons of ordinary skill in the art.

An effective amount of a bisphosphonate compound provided herein can be determined by various methods, including generating an empirical dose-response curve, predicting potency and efficacy using modeling, and other methods used in the art. In one embodiment, a therapeutically effective amount of bisphosphonate compound provided herein is at least 0.01 mg/kg, at least 0.1 mg/kg, at least 1 mg/kg, at least 2 mg/kg, at least 5 mg/kg, or at least 10 mg/kg. Specific assays for determining the therapeutically effective amount of bisphosphonate compounds provided herein are provided herein. For example, effects on cancer cells, such as cancer cell growth, can be measured in the recipient subject.

In one embodiment, an "effective amount" of a bisphosphonate compound provided herein is an amount sufficient to reduce symptoms of a cancer, such as one that expresses a KRAS mutation, for example, by at least 10%, at least 20%, at least 50%, at least 70%, or at least 90% (as compared to no administration of the bisphosphonate compound provided herein). For example, an "effective amount" of a bisphosphonate compound provided (alone or in combination with effective amounts of other therapeutic agents, such as an autophagy inducer) herein can be an amount sufficient to reduce growth rate, size, volume, and/or metastasis of a cancer, such as one that expresses a KRAS mutation, for example, by at least 10%, at least 20%, at least 50%, at least 70%, or at least 90% (as compared to no administration of the bisphosphonate compound provided herein).

In one embodiment, an "effective amount" of a bisphosphonate compound provided herein is an amount sufficient to increase stimulation of γδ T cells in a subject, for example by at least 10%, at least 20%, at least 50%, at least 70%, or at least 90% (as compared to no administration of a bisphosphonate compound provided herein).

**Treating, Treatment, and Therapy:** Any success or indicia of success in the attenuation or amelioration of an injury, pathology or condition, including any objective or subjective parameter such as abatement, remission, diminishing of symptoms or making the condition more tolerable to the patient, slowing the rate of degeneration or decline, making the final point of degeneration less debilitating, improving a subject's physical or mental well-being,
or prolonging survival. The treatment may be assessed by objective or subjective parameters; including the results of a physical examination, blood and other clinical tests, and the like.

Tumor, neoplasia, malignancy or cancer: A neoplasm is an abnormal growth of tissue or cells which results from excessive cell division. Neoplastic growth can produce a tumor. The amount of a tumor in an individual is the "tumor burden" which can be measured as the number, volume, or weight of the tumor. A tumor that does not metastasize is referred to as "benign." A tumor that invades the surrounding tissue and/or can metastasize is referred to as "malignant." A "non-cancerous tissue" is a tissue from the same organ wherein the malignant neoplasm formed, but does not have the characteristic pathology of the neoplasm. Generally, noncancerous tissue appears histologically normal. A "normal tissue" is tissue from an organ, wherein the organ is not affected by cancer or another disease or disorder of that organ. A "cancer-free" subject has not been diagnosed with a cancer of that organ and does not have detectable cancer.

Exemplary tumors, such as cancers, that can be treated with the disclosed bisphosphonate compounds include solid tumors, such as breast carcinomas (e.g. lobular and duct carcinomas), sarcomas, carcinomas of the lung (e.g., non-small cell carcinoma, large cell carcinoma, squamous carcinoma, and adenocarcinoma), mesothelioma of the lung, colorectal adenocarcinoma, stomach carcinoma, prostatic adenocarcinoma, ovarian carcinoma (such as serous cystadenocarcinoma and mucinous cystadenocarcinoma), ovarian germ cell tumors, testicular carcinomas and germ cell tumors, pancreatic adenocarcinoma, biliary adenocarcinoma, hepatocellular carcinoma, bladder carcinoma (including, for instance, transitional cell carcinoma, adenocarcinoma, and squamous carcinoma), renal cell adenocarcinoma, endometrial carcinomas (including, e.g., adenocarcinomas and mixed Mullerian tumors (carcinosarcomas)), carcinomas of the endocervix, ectocervix, and vagina (such as adenocarcinoma and squamous carcinoma of each of same), tumors of the skin (e.g., squamous cell carcinoma, basal cell carcinoma, malignant melanoma, skin appendage tumors, Kaposi sarcoma, cutaneous lymphoma, skin adnexal tumors and various types of sarcomas and Merkel cell carcinoma), esophageal carcinoma, carcinomas of the nasopharynx and oropharynx (including squamous carcinoma and adenocarcinomas of same), salivary gland carcinomas, brain and central nervous system tumors (including, for example, tumors of glial, neuronal, and meningeal origin), tumors of peripheral nerve, soft tissue sarcomas and sarcomas of bone and cartilage, and lymphatic tumors (including B-cell and T-cell malignant lymphoma). In one example, the tumor is an adenocarcinoma.
The disclosed compounds can also be used to treat liquid tumors, such as a lymphatic, white blood cell, or other type of leukemia. In a specific example, the tumor treated is a tumor of the blood, such as a leukemia (for example acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), acute myelogenous leukemia (AML), chronic myelogenous leukemia (CML), hairy cell leukemia (HCL), T-cell prolymphocytic leukemia (T-PLL), large granular lymphocytic leukemia, and adult T-cell leukemia), lymphomas (such as Hodgkin's lymphoma and non-Hodgkin's lymphoma), and myelomas).

**Under conditions sufficient for:** A phrase that is used to describe any environment that permits a desired activity. In one example the desired activity is treatment of a cancer.

**Aliphatic:** A substantially hydrocarbon-based compound, or a radical thereof (e.g., C6H3, for a hexane radical), including alkanes, alkenes, alkynes, including cyclic versions thereof, and further including straight- and branched-chain arrangements, and all stereo and position isomers as well. Unless expressly stated otherwise, an aliphatic group contains from one to twenty-five carbon atoms; for example, from one to fifteen, from one to ten, from one to six, or from one to four carbon atoms. The term "lower aliphatic" refers to an aliphatic group containing from one to ten carbon atoms. An aliphatic chain may be substituted or unsubstituted. Unless expressly referred to as an "unsubstituted aliphatic," an aliphatic group can either be unsubstituted or substituted. An aliphatic group can be substituted with one or more substituents (up to two substituents for each methylene carbon in an aliphatic chain, or up to one substituent for each carbon of a \(-\text{C}═\text{C}-\) double bond in an aliphatic chain, or up to one substituent for a carbon of a terminal methine group). Exemplary substituents include, but are not limited to, aliphatic, alkyl, alkenyl, alkynyl, alkoxy, alkylamino, alkylthio, acyl, aldehyde, amide, amino, aminoalkyl, aryl, arylalkyl, carboxyl, cyano, cycloalkyl, dialkylamino, halo, haloaliphatic, heteroaliphatic, heteroaryl, heterocycloaliphatic, hydroxyl, o xo, sulfonamide, sulfhydroxyl, thioalkoxy, or other functionality.

**Alkyl groups** include straight-chain, branched and cyclic alkyl groups. Alkyl groups include those having from 1 to 25 carbon atoms. Alkyl groups include small alkyl groups having 1 to 3 carbon atoms. Alkyl groups include medium length alkyl groups having from 4-10 carbon atoms. Alkyl groups include long alkyl groups having more than 10 carbon atoms, particularly those having 10-25 carbon atoms. Cyclic alkyl groups include those having one or more rings. Cyclic alkyl groups include those having a 3-, 4-, 5-, 6-, 7-, 8-, 9- or 10-member carbon ring and particularly those having a 3-, 4-, 5-, 6-, or 7-member ring. The carbon rings in cyclic alkyl groups can also carry alkyl groups. Cyclic alkyl groups can include bicyclic and
tricyclic alkyl groups. Alkyl groups optionally include substituted alkyl groups. Substituted alkyl groups include among others those which are substituted with aliphatic or aryl groups, which in turn can be optionally substituted. Specific alkyl groups include methyl, ethyl, n-propyl, iso-propyl, cyclopropyl, n-butyl, s-butyl, t-butyl, cyclobutyl, n-pentyl, branched-pentyl, cyclopentyl, n-hexyl, branched hexyl, and cyclohexyl groups, all of which are optionally substituted.

**Alkoxy groups** are -O-alkyl groups, where alkyl is as defined herein.

**Alkenyl groups** include straight-chain, branched and cyclic alkenyl groups. Alkenyl groups include those having 1, 2 or more double bonds and those in which two or more of the double bonds are conjugated double bonds. Alkenyl groups include those having from 2 to 25 carbon atoms. Alkenyl groups include small alkyl groups having 2 to 3 carbon atoms. Alkenyl groups include medium length alkenyl groups having from 4-10 carbon atoms. Alkenyl groups include long alkenyl groups having more than 10 carbon atoms, particularly those having 10-25 carbon atoms. Cyclic alkenyl groups include those having one or more rings. Cyclic alkenyl groups include those in which a double bond is in the ring or in an alkenyl group attached to a ring. Cyclic alkenyl groups include those having a 3-, 4-, 5-, 6-, 7-, 8-, 9- or 10-member carbon ring and particularly those having a 3-, 4-, 5-, 6- or 7-member ring. The carbon rings in cyclic alkenyl groups can also carry alkyl groups. Cyclic alkenyl groups can include bicyclic and tricyclic alkyl groups. Alkenyl groups are optionally substituted. Substituted alkenyl groups include among others those which are substituted with aliphatic, alkyl or aryl groups, which groups in turn can be optionally substituted. Specific alkenyl groups include ethenyl, prop-1-enyl, prop-2-enyl, cycloprop-1-enyl, but-1-enyl, but-2-enyl, cyclobut-1-enyl, cyclobut-2-enyl, pent-1-enyl, pent-2-enyl, branched pentenyl, cyclopent-1-enyl, hex-1-enyl, branched hexenyl, cyclohexenyl, all of which are optionally substituted.

**Alkenoxy groups** are -O-alkenyl groups, where alkenyl is as defined herein.

**Aryl groups** include groups having one or more 5- or 6-member aromatic or heteroaromatic rings. Aryl groups can contain one or more fused aromatic rings. Heteroaromatic, or heteroaryl, rings can include one or more N, O, or S atoms in the ring. Heteroaromatic rings can include those with one, two or three N, those with one or two O, and those with one or two S, or any combination thereof. Aryl groups are optionally substituted. Substituted aryl groups include among others those which are substituted with aliphatic, alkyl or alkenyl groups, which groups in turn can be optionally substituted. Specific aryl groups include
phenyl groups, biphenyl groups, pyridinyl groups, and naphthyl groups, all of which are optionally substituted.

**Arylalkyl groups** are alkyl groups substituted with one or more aryl groups where the attachment to the rest of the molecule is through the alkyl group, and wherein the alkyl groups optionally carry additional substituents and the aryl groups are optionally substituted. Specific alkylaryl groups are phenyl-substituted alkyl groups, e.g., benzyl, and phenylethyl groups.

**Alkylaryl groups** are aryl groups substituted with one or more alkyl groups where the attachment to the rest of the molecule is through the aryl group, and wherein the alkyl groups optionally carry additional substituents and the aryl groups are optionally substituted. Specific alkylaryl groups are alkyl-substituted phenyl groups such as methylphenyl.

The rings that may be formed from two or more of any R (e.g., R1 and R2) groups herein together can be optionally substituted cycloalkyl groups, optionally substituted cycloalkenyl groups or aromatic groups. The rings may contain 3, 4, 5, 6, 7 or more carbons. The rings may be heteroaromatic in which one, two or three carbons in the aromatic ring are replaced with N, O or S. The rings may be heteroalkyl or heteroalkenyl, in which one or more CH₂ groups in the ring are replaced with O, N, NH, or S.

Optional substitution of any aliphatic, alkyl, alkenyl and aryl groups includes substitution with one or more of the following substituents: halogens, -CN, -COOR, -OR, -COR, -OCON(R), -CON(R)₂, -OCON(R)₂, -N(R)₂, -N0₂, -SR, -SO₂R, -SO₂N(R)₂ or -SOR groups. Optional substitution of aliphatic or alkyl groups includes substitution with one or more alkenyl groups, aryl groups or both, wherein the alkenyl groups or aryl groups are optionally substituted. Optional substitution of alkenyl groups includes substitution with one or more aliphatic or alkyl groups, aryl groups, or both, wherein the aliphatic, alkyl or aryl groups are optionally substituted. Optional substitution of aryl groups includes substitution of the aryl ring with one or more aliphatic, alkyl, alkenyl groups, or both, wherein the aliphatic, alkyl or alkenyl groups are optionally substituted.

Optional substituents for aliphatic, alkyl, alkenyl and aryl groups include among others:

- -COOR where R is a hydrogen or an alkyl group or an aryl group and more specifically where R is methyl, ethyl, propyl, butyl, or phenyl groups all of which are optionally substituted;

- -COR where R is a hydrogen, aliphatic, or an alkyl group or an aryl groups and more specifically where R is methyl, ethyl, propyl, butyl, or phenyl groups all of which groups are optionally substituted;
-CON(R)₂ where each R, independently of each other R, is a hydrogen, aliphatic, or an alkyl group or an aryl group and more specifically where R is methyl, ethyl, propyl, butyl, or phenyl groups all of which groups are optionally substituted, or both R groups together with the nitrogen to which they are attached, can form a ring which may contain one or more double bonds and/or one or more additional heteroatoms such as O, S or N;

-OCOOR where each R, independently of each other R, is a hydrogen, aliphatic or an alkyl group or an aryl group and more specifically where R is methyl, ethyl, propyl, butyl, or phenyl groups all of which groups are optionally substituted, or both R groups together with the nitrogen to which they are attached, can form a ring which may contain one or more double bonds and/or one or more additional heteroatoms such as O, S or N;

-N(R)₂ where each R, independently of each other R, is a hydrogen, aliphatic, or an alkyl group, acyl group or an aryl group and more specifically where R is methyl, ethyl, propyl, butyl, or phenyl or acetyl groups all of which are optionally substituted, or both R groups together with the nitrogen to which they are attached, can form a ring which may contain one or more double bonds and/or one or more additional heteroatoms such as O, S or N;

-SR, -SO₂R, or -SOR where R is an aliphatic, alkyl or aryl group and more specifically where R is methyl, ethyl, propyl, butyl, phenyl groups all of which are optionally substituted; for -SR, R can be hydrogen;

-OCOOR where R is an aliphatic, alkyl or aryl group;

-SO₂N(R)₂ where R is a hydrogen, aliphatic, alkyl or aryl group, or both R groups together with the nitrogen to which they are attached, can form a ring which may contain one or more double bonds and/or one or more additional heteroatoms such as O, S or N;

-OR where R=H, aliphatic, alkyl, aryl, or acyl; for example, R can be an acyl yielding -OCOR* where R* is a hydrogen, aliphatic or alkyl group or an aryl group and more specifically where R* is methyl, ethyl, propyl, butyl, or phenyl groups, all of which groups are optionally substituted;

Specific substituted aliphatic and alkyl groups include haloalkyl groups, particularly trihalomethyl groups and specifically trifluoromethyl groups. Specific substituted aryl groups include mono-, di-, tri-, tetra- and pentahalo-substituted phenyl groups; mono-, di-, tri-, tetra-, penta-, hexa-, and hepta-halo-substituted naphthalene groups; 3- or 4-halo-substituted phenyl groups, 3- or 4-alkyl-substituted phenyl groups, 3- or 4-alkoxy-substituted phenyl groups, 3- or 4-RCO-substituted phenyl, 5- or 6-halo-substituted naphthalene groups. More specifically, substituted aryl groups include acetylphenyl groups, particularly 4-acetylphenyl groups;
fluorophenyl groups, particularly 3-fluorophenyl and 4-fluorophenyl groups; chlorophenyl
groups, particularly 3-chlorophenyl and 4-chlorophenyl groups; methylphenyl groups,
particularly 4-methylphenyl groups, and methoxyphenyl groups, particularly 4-methoxyphenyl
groups.

In certain embodiments the term "medium length side-chain" can include side chains that
have from about 7 carbon elements to about 13 carbon elements. In other embodiments, the
term "short length side-chains" can include side chains having less than about 7 carbon
elements. In still other embodiments, the term "long length side-chains" can include side chains
having greater than 13 carbon elements.

Isomers

As to any of compound described herein, which contains one or more substituents, it is
understood, of course, that such groups do not contain any substitution or substitution patterns
that are sterically impractical and/or synthetically non-feasible. It will be appreciated that the
compounds of the disclosure can contain asymmetrically substituted carbon atoms, and may be
isolated in optically active or racemic forms. It is well known in the art how to prepare optically
active forms, such as by resolution of racemic forms or by synthesis from optically active
starting materials or by the use of enantioselective catalytic reactions. All chiral, diastereomeric,
racemic forms and all geometric isomeric forms and tautomers of a compound are intended as
part of this disclosure.

One diastereomer may display superior activity compared to another. When required,
separation of racemic materials can be achieved by high performance liquid chromatography
(HPLC) using a chiral column or by a resolution using a resolving agent such as camphonic
may also be directly synthesized using a chiral catalyst or a chiral ligand; see, for example, Mark

Certain molecules disclosed herein contain one or more ionizable groups [groups from
which a proton can be removed (e.g., -COOH) or added (e.g., amines)]. All possible ionic forms
of such molecules and salts thereof are intended to be included in the disclosure herein. With
regard to salts of the compounds herein, one of ordinary skill in the art will know how to select
from among a wide variety of available counterions those that are appropriate for preparation of
salts of this disclosure for a given application. In specific applications, the selection of a given
anion or cation for preparation of a salt may result in increased or decreased solubility of that
salt.
Compounds of the disclosure can have prodrug forms. Prodrugs of the compounds of the
disclosure are useful in the methods of this disclosure. Any compound that will be converted in vivo to provide a biologically, pharmaceutically or therapeutically active form of a compound of
the disclosure is a prodrug. Various examples and forms of prodrugs are well known in the art.

Examples of prodrugs are found, inter alia, in Design of Prodrugs, edited by H. Bundgaard,
(Academic Press, 1985); A Textbook of Drug Design and Development, edited by Krosggaard-
Larsen and H. Bundgaard, Chapter 5, "Design and Application of Prodrugs," by H. Bundgaard,
Medicinal Chemistry A Biochemical Approach, Oxford University Press, New York, pages 388-
392).

As used herein, the wavyline "\text{\~A}\text{\~A}\text{~A}" indicates the point of attachment for a group or
moiety.

II. Compounds

Certain embodiments of compounds disclosed herein have a general formula I

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\text{P} & \\
\text{O} & \\
\text{Ar} & \\
\text{X} & \\
\text{O} & \\
\text{P} & \\
\text{O} & \\
\text{Ar} & \\
\text{X} & \\
\text{O} & \\
\text{P} & \\
\text{O} & \\
\text{Ar} & \\
\text{X} & \\
\text{O} & \\
\text{P} & \\
\text{O} & \\
\text{Ar} & \\
\text{X} & \\
\text{O} & \\
\text{P} & \\
\text{O} & \\
\text{Ar} & \\
\text{X} & \\
\text{O} & \\
\text{P} & \\
\text{O} & \\
\text{Ar} & \\
\text{X} & \\
\text{O} & \\
\text{P} & \\
\text{O} & \\
\text{Ar} & \\
\text{X} & \\
\text{O} & \\
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\text{O} & \\
\text{Ar} & \\
\text{X} & \\
\text{O} & \\
\text{P} & \\
\text{O} & \\
\text{Ar} & \\
\text{X} & \\
\text{O} & \\
\text{P} & \\
\text{O} & \\ I.
\end{align*}
\]

With reference to formula I, Ar is a 5-membered heteroaromatic ring; X is hydrogen, aliphatic,
hydroxyl, SH, halogen, or alkoxy; each M is independently hydrogen, -(CH₂)p-CO-R, -
(CH₂)p-CO-R, or aliphatic, such as alkyl, where p is 1 to 6, and R is hydrogen, aryl, or aliphatic,
such as alkyl; each q is independently 0 or 1; R¹¹ and R¹² are independently H, or aliphatic, such
as alkyl; and r is from 1 to 6. X may be alkyl. -OM can also be a salt of the form -O⁻A⁺, where
A⁺ is a cation. Suitable cations include, but are not limited to, sodium, lithium, potassium,
magnesium, manganese, zinc, copper, iron, ammonium or N(R')₄⁺, where each R' independently
is hydrogen or aliphatic, particularly alkyl or substituted alkyl. The aliphatic or alkyl groups can
be the same or different. Exemplary cations include trimethyl ammonium, triethyl ammonium,
and triethanol ammonium cations. In embodiments, where the cation has more than a single
positive charge, a person of ordinary skill in the art will appreciate that the cation may be

- 25 -
associated with more than one oxygen on a phosphonate, such as \( \text{O}_2\text{P}-\text{O}^\ominus \), or the cation \( \text{M}^{+}+\text{0}^\ominus \), may be associated with two different phosphonate groups, such as \( \text{O}_2\text{P}-\text{O}^\ominus \), which may or may not be on the same molecule.

In some embodiments, the compound is not

\[
\begin{align*}
\text{HO-PO}_2\text{N}^\ominus \\
\text{HO-PO}_2\text{N}^\ominus \\
\text{HO-PO}_2\text{N}^\ominus \\
\text{HO-PO}_2\text{N}^\ominus \\
\text{HO-PO}_2\text{N}^\ominus \\
\text{HO-PO}_2\text{N}^\ominus \\
\text{HO-PO}_2\text{N}^\ominus \\
\text{HO-PO}_2\text{N}^\ominus \\
\text{HO-PO}_2\text{N}^\ominus \\
\text{HO-PO}_2\text{N}^\ominus \\
\end{align*}
\]

or a salt thereof.

In other embodiments, the compound is not

(2-(1H-imidazol-1-yl)ethane-1,1-diyl)bis(phosphonic acid);

(1-hydroxy-2-(1H-imidazol-4-yl)ethane-1,1-diyl)bis(phosphonic acid);

sodium (1-hydroxy-2-(1-methyl-1H-imidazol-3-ium-3-yl)ethane-1,1-diyl)bis(hydrogen phosphonate);

(1-hydroxy-2-(1H-1,2,3-triazol-1-yl)ethane-1,1-diyl)bis(phosphonic acid);

sodium (1-hydroxy-1-(hydroxyoxidophosphoryl)-2-(1-phenyl-1H-imidazol-3-ium-3-yl)ethyl)phosphonate;

sodium (1-(hydroxyoxidophosphoryl)-2-(1-phenyl-1H-imidazol-3-ium-3-yl)ethyl)phosphonate;

(1-hydroxy-2-(thiazol-2-yl)ethane-1,1-diyl)bis(phosphonic acid); or

3-(2,2-diphosphonoethyl)-1-dodecyl-1H-imidazol-3-ium.

In certain embodiments, the compound has a formula II
In some embodiments, Ar is imidazole, pyrazole, pyrole, furan, thiophene, oxazole, isoxazole, thiazole, isothiazole, 1,2,3-triazole, 1,2,4-triazole, tetrazole, 1,2,3-oxadiazole, 1,2,4-oxadiazole, 1,2,5-oxadiazole, 1,3,4-oxadiazole, 1,2,3-thiadiazole, 1,2,4-thiadiazole, 1,2,5-thiadiazole, or 1,3,4-thiadiazole. Ar may be a nitrogen-containing 5-membered ring, and in some examples, the attachment point of the bisphosphonate moiety to the ring is at a ring nitrogen. In certain examples, where at least one M is a negative charge, Ar is a nitrogen-containing, 5-membered heteroaromatic ring with at least one positive charge.

In some embodiments, Ar is an unsubstituted 5-membered heteroaromatic ring. In other embodiments, Ar is substituted with from 1 to as many substituents as can be accommodated by Ar, such as at least up to 4 substituents. In some embodiments, the substituent is a lipophilic substituent. Exemplary substituents for Ar include, but are not limited to, aliphatic, such as alkyl or alkenyl; alkoxy; alkenoxy; hydroxyl; halogen, such as fluoro, chloro bromo or iodo; nitro; haloalkyl; aryl; alkylaryl; arylalkyl; or a combination thereof.

In some embodiments, Ar is an imidazole, leading to compounds having formulas III-VII.

With respect to formulas III-VII, M and X are as defined above with respect to formula I; R⁴, R⁶ and R⁷ are each independently H, alkoxy, alkenoxy, haloalkyl, aryl, alkylaryl, arylalkyl, nitro, halogen or aliphatic, particularly alkyl or alkenyl; R⁵ is alkoxy, alkenoxy, haloalkyl, aryl, alkylaryl, arylalkyl or aliphatic, particularly alkyl or alkenyl; and R⁸ is a lone pair of electrons, H, haloalkyl, aryl, alkylaryl, arylalkyl or aliphatic, particularly alkyl or alkenyl. A person of
ordinary skill in the art will understand that if both nitrogen atoms in the imidazole ring are substituted, such as in formula III, or in formulas IV-VII when R^8 is other than a lone pair of electrons, then the imidazole ring has a positive charge.

In certain examples, the imidazole ring has a positive charge.

In some embodiments, if R^4, R^6, R^7 are all H, one q is 0 and the remainder are 1, each M is H, and X is OH, then R^5 is not a lone pair of electrons.

In certain embodiments, R^5 is alkyl, and may be a CMS alkyl. In particular embodiments R^5 is a C_8 alkyl.

In some examples, X is H or hydroxyl, and in particular examples, X is hydroxyl.

In certain examples, one q is 0 and the rest are 1, and each M is H.

In some examples of compounds having formulas III-VII, R^5 and/or R^8 is aryl, and may be phenyl. In some embodiments, R^5 and/or R^8 is a moiety having a structure

$$\begin{array}{c}
(R^9)_n-
\end{array}$$

wherein each R^9 independently is aliphatic, alkyl, alkoxy, haloalkyl, NO_2, OH, aryl, alkenyl, alkenyloxy, SO_2R, or N(R^10)^2, where each R^10 independently is alkyl, and n is from 0 to 5.

In some embodiments, the compound has a formula XA1:

$$\begin{array}{c}
\text{XA1}
\end{array}$$

or salts or hydrates thereof wherein: X is hydrogen, hydroxyl, or a halogen; each M independently is hydrogen, -(CH_2)_p-CO-R or -(CH_2)_p-CO-R, or aliphatic, such as alkyl, where p is 1 to 6, and R is hydrogen, aryl or aliphatic, such as alkyl; -OM can also be a salt of form -O^- A^+, where A^+ is a cation; and Z is hydrogen, aryl, aliphatic, such as alkyl. Z may be an alkyl group having a structure X-(CH_2)_n - where n is an integer from 0 to 20, such as from 5 to 15, or 8 to 12, and X is hydrogen or halogen. In certain examples, n is 8.

Exemplary compounds are provided by Table 1.
<table>
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<tr>
<th>ID</th>
<th>Side chain Length (n, OH/H)</th>
<th>Structure</th>
</tr>
</thead>
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</tr>
<tr>
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<td><img src="image2.png" alt="Structure 2" /></td>
</tr>
<tr>
<td>3</td>
<td>2, OH</td>
<td><img src="image3.png" alt="Structure 3" /></td>
</tr>
<tr>
<td>4</td>
<td>3, OH</td>
<td><img src="image4.png" alt="Structure 4" /></td>
</tr>
<tr>
<td>5</td>
<td>4, OH</td>
<td><img src="image5.png" alt="Structure 5" /></td>
</tr>
<tr>
<td>6</td>
<td>5, OH</td>
<td><img src="image6.png" alt="Structure 6" /></td>
</tr>
<tr>
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<tr>
<td>8</td>
<td>7, OH</td>
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</tr>
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</tr>
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</tr>
<tr>
<td>33</td>
<td>n, R'</td>
<td><img src="image" alt="Structure n" /></td>
</tr>
<tr>
<td>34</td>
<td>n, R, R', m</td>
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<tr>
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<td>XA3</td>
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<tr>
<td>XA5</td>
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<tr>
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</tr>
<tr>
<td>XA7</td>
<td><img src="image7" alt="Chemical Structure" /></td>
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</tbody>
</table>
For structures XA2 through XA8 listed in Table 1, \( R^1 \) is hydrogen or aliphatic, such as alkyl as described above and \( R^2 \) is independently hydrogen, hydroxyl, or halogen. In certain embodiments, \( R^2 \) can be fluorine or chlorine.

In some embodiments, the compound is selected from:

- (1-hydroxy-2-(1H-imidazol-1-yl)ethane-1,1-diyl)bis(phosphonic acid);
- hydrogen (1-hydroxy-2-(1-methyl-1H-imidazol-3-ium-3-yl)-1-phosphonoethyl)phosphonate;
- hydrogen (2-(1-ethyl-1H-imidazol-3-ium-3-yl)-1-hydroxy-1-phosphonoethyl)phosphonate;
- hydrogen (1-hydroxy-2-(1-undecyl-1H-imidazol-3-ium-3-yl)-1-phosphonoethyl)phosphonate;
- hydrogen (2-(1-butyl-1H-imidazol-3-ium-3-yl)-1-hydroxy-1-phosphonoethyl)phosphonate;
- hydrogen (1-hydroxy-2-(1-penty1-1H-imidazol-3-ium-3-yl)-1-phosphonoethyl)phosphonate;
- hydrogen (2-(1-hexyl-1H-imidazol-3-ium-3-yl)-1-hydroxy-1-phosphonoethyl)phosphonate;
- hydrogen (2-(1-heptyl-1H-imidazol-3-ium-3-yl)-1-hydroxy-1-phosphonoethyl)phosphonate;
- hydrogen (2-(1-octyl-1H-imidazol-3-ium-3-yl)-1-phosphonoethyl)phosphonate;
- hydrogen (1-hydroxy-2-(1-nonyl-1H-imidazol-3-ium-3-yl)-1-phosphonoethyl)phosphonate;
- hydrogen (2-(1-decyl-1H-imidazol-3-ium-3-yl)-1-hydroxy-1-phosphonoethyl)phosphonate;
- hydrogen (1-hydroxy-1-phosphono-2-(1-undecyl-1H-imidazol-3-ium-3-yl)ethyl)phosphonate;
hydrogen (2-(1-dodecyl-lH-imidazol-3-ium-3-yl)-l-hydroxy-l-phosphonoethyl)phosphonate;
hydrogen (1-hydroxy-l-phosphono-2-(1-tridecyl-lH-imidazol-3-ium-3-yl)ethyl)phosphonate;
hydrogen (1-hydroxy-l-phosphono-2-(1-tetradecyl-lH-imidazol-3-ium-3-yl)ethyl)phosphonate;
hydrogen (l-hydroxy-2-(l-pentadecyl-lH-imidazol-3-ium-3-yl)-l-phosphonoethyl)phosphonate;
(2-(1H-imidazol-1-yl)ethane-1,1-diyl)bis(phosphonic acid);
hydrogen (2-(1-methyl-lH-imidazol-3-ium-3-yl)-l-phosphonoethyl)phosphonate;
hydrogen (2-(1-ethyl-lH-imidazol-3-ium-3-yl)-l-phosphonoethyl)phosphonate;
hydrogen (2-(1-propyl-lH-imidazol-3-ium-3-yl)ethyl)phosphonate;
hydrogen (2-(l-butyl-lH-imidazol-3-ium-3-yl)-l-phosphonoethyl)phosphonate;
hydrogen (2-(1-pentyl-lH-imidazol-3-ium-3-yl)-l-phosphonoethyl)phosphonate;
hydrogen (2-(1-hexyl-lH-imidazol-3-ium-3-yl)-l-phosphonoethyl)phosphonate;
hydrogen (2-(1-heptyl-lH-imidazol-3-ium-3-yl)-l-phosphonoethyl)phosphonate;
hydrogen (2-(1-octyl-lH-imidazol-3-ium-3-yl)-l-phosphonoethyl)phosphonate;
hydrogen (2-(1-nonyl-lH-imidazol-3-ium-3-yl)-l-phosphonoethyl)phosphonate;
hydrogen (2-(1-decyl-lH-imidazol-3-ium-3-yl)-l-phosphonoethyl)phosphonate;
hydrogen (1-phosphono-2-(1-undecyl-lH-imidazol-3-ium-3-yl)ethyl)phosphonate;
hydrogen (2-(1-dodecyl-lH-imidazol-3-ium-3-yl)-l-phosphonoethyl)phosphonate;
hydrogen (1-phosphono-2-(1-tridecyl-lH-imidazol-3-ium-3-yl)ethyl)phosphonate;
hydrogen (1-phosphono-2-(1-tetradecyl-lH-imidazol-3-ium-3-yl)ethyl)phosphonate;
hydrogen (2-(1-pentadecyl-lH-imidazol-3-ium-3-yl)-1-phosphonoethyl)phosphonate;
or any salts thereof.

III. Methods of Using the Disclosed Compounds

The compounds disclosed herein are useful in the treatment of diseases and conditions, such as cancer. Such compounds can be administered by any routine method, in therapeutically effective amounts. In addition, more than one dose of the compound can be administered, such as every other day, every day, weekly, bi-weekly, monthly, or bi-monthly.

Disclosed herein are methods for preventing and/or treating cell proliferation diseases, such as cancer, such as a cancer with a KRAS mutation. In some embodiments, the compounds disclosed herein may be used in the prevention or treatment of adenocarcinomas, i.e. carcinoma
derived from glandular tissue or in which the tumor cells form recognizable glandular structures. Adenocarcinomas can be classified according to the predominant pattern of cell arrangement, as papillary, alveolar, etc., or according to a particular product of the cells, as mucinous adenocarcinoma. Adenocarcinomas arise in several tissues, including the colon, kidney, breast, cervix, esophagus, gastric, pancreas, prostate and lung.

The disclosed compounds can be used in the treatment or prevention of lung cancer, such as a lung cancer with a KRAS mutation, such as one at amino acid position 12, 13 or 61. Lung adenocarcinomas harboring KRAS mutations, in contrast to those with EGFR and EML4-ALK mutations, have not yet been successfully targeted. One or more of the disclosed lipophilic bisphosphonate compounds, in combination with an autophagy inducer, such as rapamycin, can be used to treat such tumors (or other KRAS mutant tumors). Lipophilic bisphosphonates inhibit both farnesyl and geranylgeranyldiphosphate synthases, effectively blocking prenylation of the KRAS and other small G-proteins critical for tumor growth and cell survival. Bisphosphonate treatment of cells initiated autophagy, and rapamycin, in addition to inhibiting the mTOR pathway, facilitated autophagy and prevented p62 accumulation-induced NF-KB activation and tumor cell proliferation.

Lung adenocarcinomas account for about 50% of all non-small cell lung cancers (NSCLC), the most common type of human malignancy and a leading cause of cancer-related mortality worldwide. There has been rapid progress in developing targeted therapies for lung adenocarcinomas over the last decade, including gefitinib and erlotinib, which target EGF receptor mutations (1, 2) and crizotinib, which targets the transforming EML4-ALK fusion gene (3). However, KRAS mutations, which are commonly found in smokers and Caucasian patients, are not effectively targeted by currently available therapeutics and have low survival rates, as well as frequent drug resistance (4). KRAS mutations at amino acid positions 12, 13 or 61 are widely found in human pancreatic, thyroid, lung and colorectal cancers (5). They typically impair GTPase activity and lead to constitutive activation of downstream signaling pathways. It is therefore difficult to develop potent KRAS mutant-specific inhibitors that can directly restore intrinsic GTPase activity, although specific inhibitors of KRAS G12C have recently been reported (6), as have attempts to interfere with mutated KRAS function by altering its membrane localization; inhibiting its downstream effectors, as well as searching for synthetic lethality (7, 8).

Farnesylation and correct membrane localization are useful for the in vivo biological activity of RAS proteins (9, 10). CAAX peptido-mimetics; farnesyltransferase and
geranylgeranyltransferase inhibitors (FTI/GGTIs); famesylthiosalicylic acid (Salirasib) -which mimics farnesylcysteine, as well as small molecule inhibitors of KRAS-PDE5 interactions have all been developed to circumvent KRAS post-translational modification and membrane anchoring (7, 11-13). Knockout mouse models support the notion that disruption of protein prenylation severely impairs lung cancer development induced by KRAS mutations (14, 15). However, there has been little success in clinical trials with these small molecule inhibitors, probably due to the existence of "cross-prenylation" (16), in which a FTI can fail due to alternative KRAS geranylgeranylation, suggesting the need for combination therapies. In addition to the protein-prenyltransferase inhibitors, there is interest in the development of compounds, such as bisphosphonates (Table 2, and U.S. Patent Nos. 7,358,361, 7,687,482, 7,745,422, 8,012,949, 8,071,573, 8,609,638, and 8,778,913, all incorporated herein by reference) that directly inhibit the biosynthesis of the two prenyldiphosphate substrates: farnesyldiphosphate (FPP) and geranylgeranyl diphosphate (GGPP), catalyzed by the respective synthases, FPP synthase (FPPS) and GGPP synthase (GGPPS).

Table 2
Exemplary Bisphosphonates

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<thead>
<tr>
<th>Name</th>
<th>Structure</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zoledronate</td>
<td><img src="image1" alt="Structure" /></td>
<td>potent inhibitor of FPPS</td>
</tr>
<tr>
<td>Ibandronate</td>
<td><img src="image2" alt="Structure" /></td>
<td>potent inhibitor of FPPS</td>
</tr>
<tr>
<td>Risedronate</td>
<td><img src="image3" alt="Structure" /></td>
<td>potent inhibitor of FPPS</td>
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</tbody>
</table>
Bisphosphonates are used to treat a variety of bone resorption diseases and function by blocking FPPS activity in osteoclasts. The lipophilic bisphosphonates disclosed herein do not bind substantially to bone mineral, but maintain inhibitory activity against both FPPS as well as GGPPS (17), both of which can provide membrane anchoring 15- and 20-carbon isoprenoid chains for KRAS post-translational modification.

Impaired protein processing, folding and trafficking usually induce ER stress and autophagy if the protective unfolded protein response (UPR) is not sufficient to clear the incorrectly processed proteins (18). Indeed, inhibitors of FPP and GGPP biosynthesis such as bisphosphonates and statins have been reported to initiate autophagy in cells (19, 20). The role of autophagy in tumorigenesis has been considered as a "double-edged sword," since it can either inhibit tumor initiation at an early stage or get adopted by tumor cells as survival mechanisms at an advanced stage (21).

### IV. Pharmaceutical Formulations

The compounds described herein can be used to prepare therapeutic pharmaceutical compositions. The compounds may be added to the compositions in the form of a salt or solvate. For example, in cases where compounds are sufficiently basic or acidic to form stable nontoxic acid or base salts, administration of the compounds as salts may be appropriate.

Examples of pharmaceutically acceptable salts are organic acid addition salts formed with acids that form a physiological acceptable anion, for example, tosylate, methanesulfonate, acetate, citrate, malonate, tartrate, succinate, benzoate, ascorbate, a-ketoglutarate, and b-glycerophosphate. Suitable inorganic salts may also be formed, including hydrochloride, halide, sulfate, nitrate, bicarbonate, and carbonate salts.
Pharmaceutically acceptable salts may be obtained using procedures known to persons of
ordinary skill in the art, for example by reacting a sufficiently basic compound, such as an
amine, with a suitable acid to provide a physiologically acceptable ionic compound. Alkali
metal (for example, sodium, potassium or lithium) or alkaline earth metal (for example, calcium)
salts of carboxylic acids can also be prepared by analogous methods.

The compounds of the formulas described herein can be formulated as pharmaceutical
compositions and administered to a mammalian host, such as a human or veterinary patient, in a
variety of forms. The forms can be specifically adapted to a chosen route of administration, e.g.,
oral or parenteral administration, by intravenous, intramuscular, topical or subcutaneous routes.

The compounds described herein may be systemically administered in combination with
a pharmaceutically acceptable vehicle, such as an inert diluent or an assimilable edible carrier.
For oral administration, compounds can be enclosed in hard or soft shell gelatin capsules,
compressed into tablets, or incorporated directly into the food of a patient's diet. Compounds
may also be combined with one or more excipients and used in the form of ingestible tablets,
buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Such
compositions and preparations typically contain at least 0.1% of active compound. The
percentage of the compositions and preparations can vary and may conveniently be from about
2% to about 60% of the weight of a given unit dosage form. The amount of active compound in
such therapeutically useful compositions is such that an effective dosage level can be obtained.

The tablets, troches, pills, capsules, and the like may also contain one or more of the
following: binders such as gum tragacanth, acacia, corn starch or gelatin; excipients such as
dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and
the like; and a lubricant such as magnesium stearate. A sweetening agent such as sucrose,
fructose, lactose or aspartame; or a flavoring agent such as peppermint, oil of wintergreen, or
cherry flavoring, may be added. When the unit dosage form is a capsule, it may contain, in
addition to materials of the above type, a liquid carrier, such as a vegetable oil or a polyethylene
glycol. Various other materials may be present as coatings or to otherwise modify the physical
form of the solid unit dosage form. For instance, tablets, pills, or capsules may be coated with
gelatin, wax, shellac or sugar and the like. A syrup or elixir may contain the active compound,
sucrose or fructose as a sweetening agent, methyl and propyl parabens as preservatives, a dye
and flavoring such as cherry or orange flavor. Any material used in preparing any unit dosage
form should be pharmaceutically acceptable and substantially non-toxic in the amounts
employed. In addition, the active compound may be incorporated into sustained-release preparations and devices.

The active compound may be administered intravenously or intraperitoneally by infusion or injection. Solutions of the active compound or its salts can be prepared in water, optionally mixed with a nontoxic surfactant. Dispersions can be prepared in glycerol, liquid polyethylene glycols, triacetin, or mixtures thereof, or in a pharmaceutically acceptable oil. Under ordinary conditions of storage and use, preparations may contain a preservative to prevent the growth of microorganisms.

Pharmaceutical dosage forms suitable for injection or infusion can include sterile aqueous solutions, dispersions, or sterile powders comprising the active ingredient adapted for the extemporaneous preparation of sterile injectable or infusible solutions or dispersions, optionally encapsulated in liposomes. The ultimate dosage form should be sterile, fluid and stable under the conditions of manufacture and storage. The liquid carrier or vehicle can be a solvent or liquid dispersion medium comprising, for example, water, ethanol, a polyol (for example, glycerol, propylene glycol, liquid polyethylene glycols, and the like), vegetable oils, nontoxic glyceryl esters, and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the formation of liposomes, by the maintenance of the required particle size in the case of dispersions, or by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thiomersal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, buffers, or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by agents delaying absorption, for example, aluminum monostearate and/or gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filter sterilization. In the case of sterile powders for the preparation of sterile injectable solutions, methods of preparation can include vacuum drying and freeze drying techniques, which yield a powder of the active ingredient plus any additional desired ingredient present in the previously sterile-filtered solutions.

For topical administration, compounds may be applied in pure form, e.g., when they are liquids. However, it will generally be desirable to administer the active agent to the skin as a composition or formulation, for example, in combination with a dermatologically acceptable carrier, which may be a solid or a liquid.
Useful solid carriers include finely divided solids such as talc, clay, microcrystalline cellulose, silica, alumina, and the like. Useful liquid carriers include water, dimethyl sulfoxide (DMSO), alcohols, glycols, or water-alcohol/glycol blends, in which a compound can be dissolved or dispersed at effective levels, optionally with the aid of non-toxic surfactants.

Adjuvants such as fragrances and additional antimicrobial agents can be added to optimize the properties for a given use. The resultant liquid compositions can be applied from absorbent pads, used to impregnate bandages and other dressings, or sprayed onto the affected area using a pump-type or aerosol sprayer.

Thickeners such as synthetic polymers, fatty acids, fatty acid salts and esters, fatty alcohols, modified celluloses, or modified mineral materials can also be employed with liquid carriers to form spreadable pastes, gels, ointments, soaps, and the like, for application directly to the skin of the user.

Examples of dermatological compositions for delivering active agents to the skin are known to the art; for example, see U.S. Patent Nos. 4,992,478 (Geria), 4,820,508 (Wortzman), 4,608,392 (Jacquet et al.), and 4,559,157 (Smith et al.). Such dermatological compositions can be used in combinations with the compounds described herein where an ingredient of such compositions can optionally be replaced by a compound described herein, or a compound described herein can be added to the composition.

Useful dosages of the compounds described herein can be determined by comparing their in vitro activity, and in vivo activity in animal models. Methods for the extrapolation of effective dosages in mice, and other animals, to humans are known to the art; for example, see U.S. Patent No. 4,938,949 (Borch et al.). The amount of a compound, or an active salt or derivative thereof, required for use in treatment will vary not only with the particular compound or salt selected but also with the route of administration, the nature of the condition being treated, and the age and condition of the patient, and will be ultimately at the discretion of an attendant physician or clinician.

The compound can be conveniently administered in a unit dosage form, for example, containing 5 to 1000 mg/m², conveniently 10 to 750 mg/m², most conveniently, 50 to 500 mg/m² of active ingredient per unit dosage form. The desired dose may conveniently be presented in a single dose or as divided doses administered at appropriate intervals, for example, as two, three, four or more sub-doses per day. The sub-dose itself may be further divided, e.g., into a number of discrete loosely spaced administrations.
**Pharmaceutical Dosage Forms**

The following formulations illustrate exemplary pharmaceutical dosage forms that may be used for the therapeutic or prophylactic administration of a compound of a formula described herein, a compound specifically disclosed herein, or a pharmaceutically acceptable salt or solvate thereof (hereinafter referred to as 'Compound X'):

<table>
<thead>
<tr>
<th></th>
<th>mg/tablet</th>
<th>mg/capsule</th>
</tr>
</thead>
<tbody>
<tr>
<td>(i)</td>
<td>Tablet 1</td>
<td></td>
</tr>
<tr>
<td>'Compound X'</td>
<td>100.0</td>
<td>10.0</td>
</tr>
<tr>
<td>Lactose</td>
<td>77.5</td>
<td>465.5</td>
</tr>
<tr>
<td>Povidone</td>
<td>15.0</td>
<td></td>
</tr>
<tr>
<td>Crockarmellose sodium</td>
<td>12.0</td>
<td></td>
</tr>
<tr>
<td>Microcrystalline cellulose</td>
<td>92.5</td>
<td></td>
</tr>
<tr>
<td>Magnesium stearate</td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td>300.0</td>
<td>600.0</td>
</tr>
</tbody>
</table>

| (ii)   | Tablet 2    |            |
| 'Compound X' | 20.0  |            |
| Microcrystalline cellulose | 410.0 |            |
| Starch  | 50.0        |            |
| Sodium starch glycolate | 15.0 |            |
| Magnesium stearate | 5.0  | 5.0        |
|        | 500.0       |            |

| (iii)  | Capsule     |            |
| 'Compound X' | 10.0  |            |
| Colloidal silicon dioxide | 1.5 |            |
| Lactose | 409.5       |            |
| Pregelatinized starch | 120.0 |            |
| Magnesium stearate | 3.0  | 3.0        |
|        | 600.0       |            |

| (iv)   | Injection 1 | mg/mL      |
| 'Compound X' (free acid form) | 1.0  |            |
| Dibasic sodium phosphate | 12.0 |            |
| Monobasic sodium phosphate | 0.7  |            |
| Sodium chloride | 4.5   |            |
| 1.0 N Sodium hydroxide solution | q.s. (pH adjustment to 7.0-7.5) |            |
| Water for injection | q.s. to 1 mL |            |

| (v)    | Injection 2 | mg/mL      |
| 'Compound X' (free acid form) | 10.0  |            |
| Monobasic sodium phosphate | 0.3   |            |
| Dibasic sodium phosphate | 1.1   |            |
| Polyethylene glycol 400 | 200.0  |            |
| 0.1 N Sodium hydroxide solution | q.s. (pH adjustment to 7.0-7.5) |            |
| Water for injection | q.s. to 1 mL |            |
**(vi) Aerosol**

<table>
<thead>
<tr>
<th>Compound</th>
<th>mg/can</th>
</tr>
</thead>
<tbody>
<tr>
<td>'Compound X'</td>
<td>20</td>
</tr>
<tr>
<td>Oleic acid</td>
<td>10</td>
</tr>
<tr>
<td>Trichloromonofluoromethane</td>
<td>5,000</td>
</tr>
<tr>
<td>Dichlorodifluoromethane</td>
<td>10,000</td>
</tr>
<tr>
<td>DichloroTetrafluoroethane</td>
<td>5,000</td>
</tr>
</tbody>
</table>

**(vii) Topical Gel 1**

<table>
<thead>
<tr>
<th>Compound</th>
<th>wt. %</th>
</tr>
</thead>
<tbody>
<tr>
<td>'Compound X'</td>
<td>5%</td>
</tr>
<tr>
<td>Carbomer 934</td>
<td>1.25%</td>
</tr>
<tr>
<td>Triethanolamine</td>
<td>q.s. (pH adjustment to 5-7)</td>
</tr>
<tr>
<td>Methyl paraben</td>
<td>0.2%</td>
</tr>
<tr>
<td>Purified water</td>
<td>q.s. to 100g</td>
</tr>
</tbody>
</table>

**(viii) Topical Gel 2**

<table>
<thead>
<tr>
<th>Compound</th>
<th>wt. %</th>
</tr>
</thead>
<tbody>
<tr>
<td>'Compound X'</td>
<td>5%</td>
</tr>
<tr>
<td>Methylcellulose</td>
<td>2%</td>
</tr>
<tr>
<td>Methyl paraben</td>
<td>0.2%</td>
</tr>
<tr>
<td>Propyl paraben</td>
<td>0.02%</td>
</tr>
<tr>
<td>Purified water</td>
<td>q.s. to 100g</td>
</tr>
</tbody>
</table>

**(ix) Topical Ointment**

<table>
<thead>
<tr>
<th>Compound</th>
<th>wt. %</th>
</tr>
</thead>
<tbody>
<tr>
<td>'Compound X'</td>
<td>5%</td>
</tr>
<tr>
<td>Propylene glycol</td>
<td>1%</td>
</tr>
<tr>
<td>Anhydrous ointment base</td>
<td>40%</td>
</tr>
<tr>
<td>Polysorbate 80</td>
<td>2%</td>
</tr>
<tr>
<td>Methyl paraben</td>
<td>0.2%</td>
</tr>
<tr>
<td>Purified water</td>
<td>q.s. to 100g</td>
</tr>
</tbody>
</table>

**(x) Topical Cream 1**

<table>
<thead>
<tr>
<th>Compound</th>
<th>wt. %</th>
</tr>
</thead>
<tbody>
<tr>
<td>'Compound X'</td>
<td>5%</td>
</tr>
<tr>
<td>White bees wax</td>
<td>10%</td>
</tr>
<tr>
<td>Liquid paraffin</td>
<td>30%</td>
</tr>
<tr>
<td>Benzyl alcohol</td>
<td>5%</td>
</tr>
<tr>
<td>Purified water</td>
<td>q.s. to 100g</td>
</tr>
</tbody>
</table>

**(xi) Topical Cream 2**

<table>
<thead>
<tr>
<th>Compound</th>
<th>wt. %</th>
</tr>
</thead>
<tbody>
<tr>
<td>'Compound X'</td>
<td>5%</td>
</tr>
<tr>
<td>Stearic acid</td>
<td>10%</td>
</tr>
<tr>
<td>Glycerylmonostearate</td>
<td>3%</td>
</tr>
<tr>
<td>Polyoxyethylene stearyl ether</td>
<td>3%</td>
</tr>
<tr>
<td>Sorbitol</td>
<td>5%</td>
</tr>
<tr>
<td>Isopropyl palmitate</td>
<td>2%</td>
</tr>
<tr>
<td>Methyl Paraben</td>
<td>0.2%</td>
</tr>
<tr>
<td>Purified water</td>
<td>q.s. to 100g</td>
</tr>
</tbody>
</table>

These formulations may be prepared by conventional procedures well known in the pharmaceutical art. It will be appreciated that the above pharmaceutical compositions may be
varied according to well-known pharmaceutical techniques to accommodate differing amounts
and types of active ingredient 'Compound X'. Aerosol formulation (vi) may be used in
conjunction with a standard, metered dose aerosol dispenser. Additionally, the specific
ingredients and proportions are for illustrative purposes. Ingredients may be exchanged for
suitable equivalents and proportions may be varied, according to the desired properties of the
dosage form of interest.

V. Additional Therapeutic Agents

The disclosed pharmaceutical compositions can further include one or more additional
therapeutic agents. Similarly, the disclosed methods can further utilize one or more additional
therapeutic agents.

Exemplary therapeutic agents that can be used in the disclosed methods (which are
administered at effective amounts) or compositions include, but are not limited to: anti-
neoplastic chemotherapeutic agents, antibiotics, alkylating agents and antioxidants, kinase
inhibitors, and other agents. Other examples include microtubule binding agents, DNA
intercalators or cross-linkers, DNA synthesis inhibitors, DNA and/or RNA transcription
inhibitors, antibodies, enzymes, enzyme inhibitors, and gene regulators. Such agents are known
in the art. Exemplary chemotherapeutic agents are described in Slapak and Kufe, Principles of
Cancer Therapy, Chapter 86 in Harrison's Principles of Internal Medicine, 14th edition; Perry et
al., Chemotherapy, Ch. 17 in Abeloff, Clinical Oncology 2nd ed., 2000 Churchill Livingstone,
Inc; Baltzer and Berkery. (eds): Oncology Pocket Guide to Chemotherapy, 2nd ed. St. Louis,
Mosby-Year Book, 1995; and Fischer Knobf, and Durivage (eds): The Cancer Chemotherapy

"Microtubule binding agent" refers to an agent that interacts with tubulin to stabilize or
destabilize microtubule formation thereby inhibiting cell division. Examples of microtubule
binding agents that can be used in conjunction with the methods and compositions provided
herein include, without limitation, paclitaxel, docetaxel, vinblastine, vindesine, vinorelbine
(navelbine), the epothilones, colchicine, dolastatin 15, nocodazole, podophyllotoxin and
rhizoxin. Analogs and derivatives of such compounds also can be used and are known to those
of ordinary skill in the art. For example, suitable epothilones and epothilone analogs are
described in International Publication No. WO 2004/018478. Taxoids, such as paclitaxel and
docetaxel, as well as the analogs of paclitaxel taught by U.S. Patent Nos. 6,610,860; 5,530,020;
and 5,912,264 can be used.
In one example the additional therapeutic agent includes one or more of: DNA and/or RNA transcription regulators, including, without limitation, actinomycin D, daunorubicin, doxorubicin and derivatives and analogs thereof. DNA intercalators and cross-linking agents that can be used include, without limitation, cisplatin, carboplatin, oxaliplatin, mitomycins, such as mitomycin C, bleomycin, chlorambucil, cyclophosphamide and derivatives and analogs thereof. DNA synthesis inhibitors suitable for use include, without limitation, methotrexate, 5-fluoro-5'-deoxyuridine, 5-fluorouracil and analogs thereof. Examples of suitable enzyme inhibitors include, without limitation, camptothecin, etoposide, formestane, trichostatin and derivatives and analogs thereof. Suitable compounds that affect gene regulation include agents that result in increased or decreased expression of one or more genes, such as raloxifene, 5-azacytidine, 5-aza-2'-deoxycytidine, tamoxifen, 4-hydroxytamoxifen, mifepristone and derivatives and analogs thereof. Kinase inhibitors include Gleevac, Iressa, and Tarceva that prevent phosphorylation and activation of growth factors.

In one example, the chemotherapy drug is epirubicin, topotecan, irinotecan, gemcitabine, iazofurine, valsparod, mitoxantrone, or Doxil (liposome encapsulated doxorubicine). In one example the drug is adriamycin, apigenin, zebularine, cimetidine, theophylline, or a derivative or analogs thereof.

In one example, the additional therapeutic agent is a biologic agent (e.g., mAb) or a small molecule, such as those shown in the table below:

<table>
<thead>
<tr>
<th>Tumor-Specific Antigen</th>
<th>Exemplary Tumors</th>
<th>Exemplary Antibody/Small Molecules</th>
</tr>
</thead>
<tbody>
<tr>
<td>HER1</td>
<td>Adenocarcinoma (<em>e.g.</em>, colorectal cancer, head and neck cancer)</td>
<td>Cetuximab, panitumamab, zalutumumab, nimotuzumab, matuzumab. Small molecule inhibitors gefitinib, erlotinib, and lapatinib can also be used.</td>
</tr>
<tr>
<td>HER2</td>
<td>breast cancer, ovarian cancer, stomach cancer, uterine cancer</td>
<td>Trastuzumab (Herceptin®), pertuzumab</td>
</tr>
<tr>
<td>CD20</td>
<td>Non-Hodgkin lymphoma</td>
<td>Tositumomab (Bexxar®); Rituximab (Rituxan, Mabthera); or Ibritumomab tiuxetan (Zevalin, for example in</td>
</tr>
<tr>
<td></td>
<td>Combination</td>
<td>Therapy</td>
</tr>
<tr>
<td>----------------</td>
<td>-----------------------------------------------------------------------------</td>
<td>-------------------------------------------------------------------------</td>
</tr>
<tr>
<td>CD25 T-cell</td>
<td>combination with yttrium-90 or indium-111 therapy</td>
<td>Daclizumab (Zenapax)</td>
</tr>
<tr>
<td>CD33 Acute</td>
<td>combination with calicheamicin therapy</td>
<td>Gemtuzumab (Mylotarg, for example in combination with calicheamicin therapy)</td>
</tr>
<tr>
<td>chronic lymphocytic</td>
<td>combination with calicheamicin therapy</td>
<td>Gemtuzumab (Mylotarg, for example in combination with calicheamicin therapy)</td>
</tr>
<tr>
<td>CD52</td>
<td>combination with calicheamicin therapy</td>
<td>Alemtuzumab (Campath)</td>
</tr>
<tr>
<td>CEA</td>
<td>combination with calicheamicin therapy</td>
<td>CEA-scan (Fab fragment, approved by FDA), coloOl</td>
</tr>
<tr>
<td>Cancer antigen</td>
<td>combination with calicheamicin therapy</td>
<td>OC125 monoclonal antibody</td>
</tr>
<tr>
<td>CD125 (CA125)</td>
<td>combination with calicheamicin therapy</td>
<td>OC125 monoclonal antibody</td>
</tr>
<tr>
<td>Alpha-fetoprotein</td>
<td>combination with calicheamicin therapy</td>
<td>ab75705 (available from Abeam) and other commercially availableAFP antibodies</td>
</tr>
<tr>
<td>Lewis Y</td>
<td>combination with calicheamicin therapy</td>
<td>B3 (Humanized)</td>
</tr>
<tr>
<td>TAG72</td>
<td>combination with calicheamicin therapy</td>
<td>B72.3 (FDA-approved monoclonal antibody)</td>
</tr>
<tr>
<td>Bevacizumab</td>
<td>combination with calicheamicin therapy</td>
<td>Bevacizumab (Avastin®)</td>
</tr>
</tbody>
</table>

In one example, the additional therapeutic agent is one or more of the following:

- Antibiotic (e.g., penicillin, ampicillin, metronidazole, tetracycline, chloramphenicol, tobramycin, cipro, and the like), anti-hypertensive drug (e.g., thiazide diuretics, ACE inhibitors, calcium channel blockers, beta blockers, and angiotensin II receptor antagonists), antidepressant (e.g., selective serotonin reuptake inhibitors (SSRIs), serotonin-norepinephrine reuptake inhibitors (SNRIs), tricyclic antidepressant (TCA), monoamine oxidase inhibitor (MAOI), buprenorphine, tryptophan, antipsychotics, and St John's wort, for example prozac), analgesics (e.g.,
acetaminophen, non-steroidal anti-inflammatory drugs (NSAIDs), COX-2 inhibitor, and opioid drugs such as morphine, codeine, and oxycodone), reproductive hormone (e.g., estrogen, testosterone, and progesterone), blood thinners (e.g., warfarin), steroid (e.g., prednisone), immunosuppressant (e.g., rapamycin, cyclosporine, and methotrexate, azathioprine, rituximab, or a steroid), or cytokine (e.g., GM-CSF) and other prescription drugs.

Exemplary additional therapeutic compounds include, but are not limited to, one or more of Abiraterone Acetate, Abitrexate (Methotrexate), Abraxane (Paclitaxel Albumin-stabilized Nanoparticle Formulation), ABVD, ABVE, ABVE-PC, AC, AC-T, Adcetris (Brentuximab Vedotin), ADE, Adriamycin (Doxorubicin Hydrochloride), Adrucil (Fluorouracil), Afinitor (Everolimus), Aldara (Imiquimod), Aldesleukin, ALeImtuzumab, Alimta (Pemetrexed Disodium), Aloxi (Palonosetron Hydrochloride), Ambochlorin (Chlorambucil), Amboclorin (Chlorambucil), Aminolevulinic Acid, Asetosazole, Aprepitant, Arimidex (Anastrozole), Aromasin (Exemestane), Arranon (Nelarabine), Arsenic Trioxide, Arzerra (Ofatumumab), Asparaginase Erwinia chrysantheami, Avastin (Bevacizumab), Axitinib, Azacitidine, BEACOPP, Bendamustine Hydrochloride, BEP, Bevacizumab, Bexarotene, Bexxar (Tositumomab and I 13I Iodine Tositumomab), Bleomycin, Bortezomib, Bosulif (Bosutinib), Bosutinib, Brentuximab, Vedotin, Cabazitaxel, Cabozantinib-S-Malate, CAF, Campath (Alemtuzumab), Camptosar (Irinotecan Hydrochloride), Capecitabine, CAPOX, Carboplatin, CARBOPLATIN-TAXOL, Carfilzomib, CeeNU (Lomustine), Cerubidine (Daunorubicin Hydrochloride), Cervarix (Recombinant HPV Bivalent Vaccine), Cetuximab, Chlorambucil, CHLORAMBUCIL-PREDNISONE, CHOP, Cisplatin, Clafen (Cyclophosphamide), Clofarabine, Clofarex (Clofarabine), Clolar (Clofarabine), CMF, Cometrix (Cabozantinib-S-Malate), COPP, Cosmegen (Dactinomycin), Crizotinib, CVP, Cyclophosphamide, Cyfos (Ifosfamide), Cytarabine, Cytarabine, Liposomal, Cytosar-U (Cytarabine), Cytoxan (Cyclophosphamide), Dacarbazine, Dacogen (Decitabine), Dactinomycin, Dasatinib, Daunorubicin Hydrochloride, Decitabine, Degarelix, Denileukin, Diftitox, Denosumab, DepoCyt (Liposomal Cytarabine), DepoFoam (Liposomal Cytarabine), Dexrazoxane Hydrochloride, Docetaxel, Doxil (Doxorubicin Hydrochloride Liposome), Doxorubicin Hydrochloride, Doxorubicin Hydrochloride Liposome, Dox-SL (Doxorubicin Hydrochloride Liposome), DTIC-Dome (Dacarbazine)Efudex (Fluorouracil), Elitek (Rasburicase), Ellence (Epirubicin Hydrochloride), Eloxatin (Oxaliplatin), Eltrombopag Olamine, Emend (Aprepitant), Enzalutamide, Epirubicin Hydrochloride, EPOCH, Erbitux (Cetuximab), Eribulin Mesylate, Erivedge (Vismodegib), Erlotinib Hydrochloride, Erwinaze (Asparaginase Erwinia chrysantheami), Etopophos (Etoposide...
Phosphate), Etoposide, Etoposide Phosphate, Evacet (Doxorubicin Hydrochloride Liposome),
Everolimus, Evista (Raloxifene Hydrochloride), Exemestane, Farem (Toremifene), Faslodex (Fulvestrant), FEC, Femara (Letrozole), Filgrastim, Fludara (Fludarabine Phosphate),
Fludarabine Phosphate, Fluoroplex (Fluorouracil), Fluorouracil, Folex (Methotrexate), Folex
5 PFS (Methotrexate), FOLFIRI, FOLFIRI-BEVACIZUMAB, FOLFIRINOX, FOLFOX, Folotyn (Pralatrexate), FU-LV, Fulvestrant, Gardasil (Recombinant HPV Quadrivalent Vaccine), Gefitinib, Gemcitabine Hydrochloride, GEMCITABINE-CISPLATIN, Gemtuzumab Ozogamicin, Gemzar (Gemcitabine Hydrochloride), Gleevec (Imatinib Mesylate), Glucarpidase, Halaven (Eribulin Mesylate), Herceptin (Trastuzumab), HPV Bivalent Vaccine, Recombinant, HPV Quadrivalent Vaccine, Recombinant, Hycamtin (Topotecan Hydrochloride), Ibritumomab Tiuxetan, ICE, Ifex (Ifosfamide), Ifosfamide, Ifofamidum (Ifosfamide), Imatinib Mesylate, Imiquimod, Inlyta (Axitinib), Ipilimumab, Iressa (Gefitinib), Irinotecan Hydrochloride, Istdox (Romidepsin), Ixabepilone, Ixempra (Ixabepilone), Jakafi (Ruxolitinib Phosphate), Jevtana (Cabazitaxel), Kepivance (Palifermin), Kyprolis
10 (Carfilzomib), Lapatinib Ditosylate, Lenalidomide, Letrozole, Leucovorin Calcium, Leukeran (Chlorambucil), Leuprolide Acetate, Levulan (Aminolevulinic Acid), Linfolizin (Chlorambucil), LipoDox (Doxorubicin Hydrochloride Liposome), Liposomal Cytarabine, Lomustine, Lupron (Leuprolide Acetate), Lupron Depot (Leuprolide Acetate), Lupron Depot-Ped (Leuprolide Acetate), Lupron Depot-3 Month (Leuprolide Acetate), Lupron Depot-4 Month (Leuprolide Acetate), Marqibo (Vincristine Sulfate Liposome), Matulane (Procarbazine Hydrochloride), Mechlorethamine Hydrochloride, Mesna, Mesnex (Mesna), Methazolastone (Temozolomide), Methotrexate, Methotrexate LPF (Methotrexate), Mexate (Methotrexate), Mexate-AQ (Methotrexate), Mitomycin C, Mitoxantrone (Mitomycin C), MOPP, Mozobil (Plertixafor), Mustargen (Mechlorethamine Hydrochloride), Mutamycin (Mitomycin C), Mylosar
15 (Azacitidine), Mylotarg (Gemtuzumab Ozogamicin), Nanoparticle Paclitaxel (Paclitaxel Albumin-stabilized Nanoparticle Formulation), Navelbine (Vinorelbine Tartrate), Nelorabine, Neosar (Cyclophosphamid), Neupogen (Filgrastim), Nexavar (Sorafenib Tosylate), Nilotinib, Novadex (Tamoxifen Citrate), Nplate (Romiplostim), Ofatumumab, Omacetaxine, Mepesuccinate, Oncaspar (Pegasparagase), Ontak (Denileukin Diftitox), Oxaliplatin, Paclitaxel, Paclitaxel Albumin-stabilized Nanoparticle Formulation, Palifermin, Palonosetron Hydrochloride, Panitumumab, Paraplat (Carboplatin), Paraplatin (Carboplatin), Pazopanib Hydrochloride, Pegaspargase, Pemetrexed Disodium, Perjeta (Pertuzumab), Pertuzumab, Platinol (Cisplatin), Platinol-AQ (Cisplatin), Plertixafor, Pralatrexate, Prednisone, Procarbazine
Hydrochloride, Proleukin (Aldesleukin), Prolia (Denosumab), Promacta (Eltrombopag Olamine), Provenge (Sipuleucel-T), Raloxifene Hydrochloride, Rasburicase, R-CHOP, R-CVP, Recombinant HPV Bivalent Vaccine, Recombinant HPV Quadrivalent Vaccine, Rapamycin, Regorafenib, Revlimid (Lenalidomide), Rheumatrex (Methotrexate), Rituxan (Rituximab), Rituximab, Romidepsin, Romiplostim, Rubidomycin (Daunorubicin Hydrochloride), Ruxolitinib Phosphate, Sclerosol Intrapleural Aerosol (Talc), Sipuleucel-T, Sorafenib Tosylate, Sprycel (Dasatinib), STANFORD V Sterile Talc Powder (Talc), Steritalc (Talc), Stivarga (Regorafenib), Sunitinib Malate, Sutent (Sunitinib Malate), Synovir (Thalidomide), Synribo (Omacetaxine Mepesuccinate), Tamoxifen Citrate, Tarabine, PFS (Cytarabine), Tarceva (Erlotinib), Taxol (Paclitaxel), Taxotere (Docetaxel), Temodar (Temozolomide), Temozolomide, Temsirolimus, Thalidomide, Thalomid (Thalidomide), Toposar (Etoposide), Topotecan Hydrochloride, Toremifene, Torisel (Temsirolimus), Tositumomab and I 131 Iodine Tositumomab, Totect (Dexrazoxane Hydrochloride), Trastuzumab, Treanda (Bendamustine Hydrochloride), Trisenox (Arsenic Trioxide), Tykerb (Lapatinib Ditosylate), Vandetanib, VAMP, Vectibix (Panitumumab), VelP, Velban (Vinblastine Sulfate), Velcade (Bortezomib), Velsar (Vinblastine Sulfate), Vemurafenib, VePesid (Etoposide), Viadur (Leuprolide Acetate), Vidaza (Azacitidine), Vinblastine Sulfate, Vincasar PFS (Vincristine Sulfate), Vincristine Sulfate, Vincristine Sulfate Liposome, Vinorelbine Tartrate, Vismodegib, Voraxaze (Glucarpidase), Vorinostat, Votrient (Pazopanib Hydrochloride), Wellcovorin (Leucovorin Calcium), Xalkori (Crizotinib), Xeloda (Capecitabine), XELOX, Xgeva (Denosumab), Xtandi (Enzalutamide), Yervoy (Ipilimumab), Zaltrap (Ziv-Aflibercept), Zelboraf (Vemurafenib), Zevalin (Ibritumomab Tiuxetan), Zindecard (Dexrazoxane Hydrochloride), Ziv-Aflibercept, Zoledronic Acid, Zolinza (Vorinostat), Zometa (Zoledronic Acid), Zytiga (Abiraterone Acetate) or an autophagy inducer.

Examples

The following Examples illustrate the disclosure and should not be construed as to narrow its scope. One skilled in the art will readily recognize that the Examples suggest many other ways in which the disclosure could be practiced. It should be understood that numerous variations and modifications may be made while remaining within the scope of the disclosure.
Example 1

**Anticancer Effects of Bisphosphonates**

L2 cells were derived from primary culture of lung tumors isolated from KRASG12D/+ mice (C57B1/6 background) infected with CA2Cre-shp53 lentiviral vector. For the xenograft model, 10^6 L2 cells were implanted into flank region at both sides. Treatment was started when decent tumor mass was palpable (day 18). Mice were randomly divided into four treatment groups (5 mice each group): saline control, BPH-1222 (2 mg/kg in saline, every other day), rapamycin (2.5 mg/kg in 75% DMSO + 25% saline, every other day) and BPH-1222 plus rapamycin. Treatment was given for total 21 days and mice were harvested after that. Tumor size was measured every week and tumor volume was calculated as: volume (mm^3) = (smallest diameter)^2 x (largest diameter)/2. Tumors were finely dissected and weighted when mice were harvested at the end of the treatment. Animal body weight was used for monitoring systemic toxicity. No significant body weight change was observed after 3-week treatment.

Tumors from saline control and Rapal222 groups were fixed and processed for pathological analysis. Tumor sections were stained with Ki67 antibody as a marker for cell proliferation (Red:ki67, blue: DAPI). Ki67 positive rate was counted at 10 independent areas. Necrotic area was quantified by Image J.

FIGS. 1A-1D and FIGS. 2A-2C illustrate the anticancer effects of bisphosphonates, such as BPH-1222, in a KRAS NSCLC xenograft model.

Example 2

**Anti-malaria Effects of Bisphosphonates**

Bisphosphonates such as zoledronate (I) activate γδ T cells by inhibiting the enzyme farnesylidiphosphate synthase (FPPS). This results in accumulation of the FPPS substrates, isopentenyldiphosphate (IPP) and dimethylallyldiphosphate (DMAPP), both of which are "phosphoantigens" that activate γδ T cells. However, zoledronate has essentially no effect on the intra-erythrocytic form of the malaria parasites, since it is poorly membrane permeable.

In contrast, lipophilic bisphosphonates (containing N-alkyl side chains) do kill the parasites. Here, the target is the *Plasmodium* geranylgeranyldiphosphate synthase (GGPPS). This enzyme is unusual in that is structurally more similar to human FPPS than human GGPPS and, unlike human GGPPS, is potently inhibited by bisphosphonates. Inhibiting GGPPS in the parasite blocks formation of protein prenylation as well as carotenoid, menaquinone and vitamin
E formation (FIG. 3), and results in "direct" parasite killing. Here, a lipophilic bisphosphonate was sought that would kill malaria parasites as well as activate γδ T cells, a possible route to malaria chemo-immunotherapy.

16 pairs of zoledronate species (1-32) shown in FIG. 4A were synthesized in which the length of the alkyl chain (n=0 through n=15 carbons) was varied and the presence or absence of the 1-OH group that is involved in bone-binding and that has been proposed (with zoledronate) to be important in γδ T cell activation. Next, all 32 compounds for human FPPS inhibition activity were tested. Potent FPPS inhibitors were those with medium length side-chains and these were about 3-10 times more potent than zoledronate itself, FIG. 4B and Table 3. As the N-alkyl chain length increased beyond C10, FPPS inhibition decreased, due possibly to the onset of steric repulsion with the highly conserved Phe 98, 99 residues in the FPPS active site that limit chain elongation.

Table 3

Enzyme inhibition together with T cell activation and P.falciparum cell growth inhibition. ND = Not Determined

<table>
<thead>
<tr>
<th>Sidechain Length (n, OH/H)</th>
<th>HsFPPS ICso (µM)</th>
<th>TNF-α ICso (µM)</th>
<th>PvFPPS ICso (µM)</th>
<th>P. falciparum ICso (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 0, OH</td>
<td>0.10</td>
<td>170</td>
<td>0.13</td>
<td>&gt;300</td>
</tr>
<tr>
<td>2 1, OH</td>
<td>0.080</td>
<td>210</td>
<td>0.15</td>
<td>&gt;300</td>
</tr>
<tr>
<td>3 2, OH</td>
<td>0.049</td>
<td>160</td>
<td>0.12</td>
<td>&gt;300</td>
</tr>
<tr>
<td>4 3, OH</td>
<td>0.034</td>
<td>190</td>
<td>0.16</td>
<td>&gt;300</td>
</tr>
<tr>
<td>5 4, OH</td>
<td>0.030</td>
<td>250</td>
<td>0.14</td>
<td>&gt;300</td>
</tr>
<tr>
<td>6 5, OH</td>
<td>0.049</td>
<td>170</td>
<td>0.12</td>
<td>97</td>
</tr>
<tr>
<td>7 6, OH</td>
<td>0.044</td>
<td>81</td>
<td>0.12</td>
<td>25</td>
</tr>
<tr>
<td>8 7, OH</td>
<td>0.040</td>
<td>47</td>
<td>0.11</td>
<td>14</td>
</tr>
<tr>
<td>9 8, OH</td>
<td>0.036</td>
<td>23</td>
<td>0.10</td>
<td>7.1</td>
</tr>
<tr>
<td>10 9, OH</td>
<td>0.080</td>
<td>23</td>
<td>0.12</td>
<td>11</td>
</tr>
<tr>
<td>11 10, OH</td>
<td>0.23</td>
<td>4.9</td>
<td>0.12</td>
<td>14</td>
</tr>
<tr>
<td>12 11, OH</td>
<td>0.21</td>
<td>4.1</td>
<td>0.33</td>
<td>17</td>
</tr>
<tr>
<td>13 12, OH</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>14 13, OH</td>
<td>4.2</td>
<td>110</td>
<td>2.7</td>
<td>44</td>
</tr>
<tr>
<td>15 14, OH</td>
<td>42</td>
<td>160</td>
<td>3.2</td>
<td>&gt;300</td>
</tr>
<tr>
<td>16 15, OH</td>
<td>60</td>
<td>280</td>
<td>7.1</td>
<td>&gt;300</td>
</tr>
<tr>
<td>17 0, H</td>
<td>0.14</td>
<td>140</td>
<td>0.16</td>
<td>&gt;300</td>
</tr>
<tr>
<td>18 1, H</td>
<td>0.19</td>
<td>210</td>
<td>0.16</td>
<td>&gt;300</td>
</tr>
<tr>
<td>19 2, H</td>
<td>0.08</td>
<td>180</td>
<td>0.17</td>
<td>&gt;300</td>
</tr>
<tr>
<td>20 3, H</td>
<td>0.066</td>
<td>200</td>
<td>0.14</td>
<td>100</td>
</tr>
</tbody>
</table>
Example 3

Effect of Chain-Length on γδ T cell activation

Next, the effects of chain-length and the presence/absence of the 1-OH group on γδ T cell activation, as determined in a TNF-a release assay, were investigated. As can be observed in Table 3 and FIG. 4C, there was a monotonic increase in activity in both series of compounds with compounds having a chain length of 4 carbons and increasing to about 11 carbons, then activity rapidly decreased with lengths of 12 or more carbons. The decrease inactivity with the longer chain species occurred at a longer chain length in cells than in FPPS inhibition, due, it is believed, to the importance of hydrophobicity with the more lipophilic species, which may facilitate cell entry. These results also indicated that there was no major difference in activity in cells due to the presence or absence of the 1-OH group.

Example 4

Binding to FPPS

To determine how these lipophilic zoledronate derivatives were bound to FPPS, the x-ray crystallographic structure of 5 (IC50 of about 30 nM) bound to human FPPS (HsFPPS), as shown in FIG. 5A (in cyan; PDB ID code 4GA3) was obtained. The electron density map is shown in FIG. 5B. Compound 5 bound into the same site as zoledronate did, with its two phosphonate groups bound to the [Mg2+]3 cluster, and there was a 0.7 Å rmsd between the [Mg2+]3 bisphosphonate and imidazole rings in the two structures. The alkyl chain extended into the GPP/FPP side-chain site, FIG. 5C (FPPS structures, PDB ID code 1UBX and 1UBW). The origin of the more potent FPPS inhibition by the N-alkylbisphosphonates over that seen with the unsubstituted species was possibly due to an enhanced hydrophobic interaction as opposed to a
purely Coulombic interaction, since the results of a solid-state NMR and quantum chemical investigation suggested that the imidazole nitrogen in zoledronate also carried a +1 charge (due to protonation), when bound to FPPS.

The inhibition of *Plasmodium vivax* GGPPS (PvGGPPS) by 1-32, as well as the direct killing of intraerythrocytic parasites by these compounds was investigated. As shown in FIGS. 4D and 4E and Table 3, several of the compounds effective in inhibiting PvGGPPS were also very effective in inhibiting *P. falciparum* growth. The correlation between PvGGPPS and cell growth inhibition was poor (R = 0.23) but improved to R = 0.90 on addition of the logP and solvation energy descriptors reported previously. The ability to inhibit FPPS as well as GGPPS with the same chain length compounds was likely due to their mimicking the FPP product (in human FPPS) or the FPP substrate (in *Plasmodium* GGPPS), together with the presence of the "third Asp" in PvGGPPS that is implicated for bisphosphonate binding to [Mg\textsuperscript{2+}] with, and as can be seen in FIG.5D, the structures of 5 bound to HsFPPS and 29 bound to PvGGPPS being very similar (rmsd=0.9Å).

In summary, a library of 31 N-alkyl analogs of the bisphosphonate drug, zoledronate, was synthesized, with and without the 1-OH group. These compounds were tested, alone with zoledronate, for activity in inhibiting human FPPS. The observed results indicated that medium chain length (C\textsubscript{4-11}) species were potent in inhibiting human FPPS, while longer chain species were less active. Without being bound to a particular theory, this may be due to a steric clash with the FPPS chain-length-determining residues Phe 98, 99. Also, the activity of all 32 compounds in γδ T cell activation was investigated. The most active species had 10+1 carbons in the N-alkyl side-chain. The increased activity of these lipophilic zoledronate-analog bisphosphonates in cells compared with zoledronate itself may be due to the improved cell uptake of the more lipophilic compounds. Additionally, the x-ray crystallographic structure of one potent inhibitor of human FPPS bound to the enzyme was determined, and indicated that the bisphosphonate and imidazole groups occupied the same position as in zoledronate bound to FPPS, as well as 29 bound to GGPPS. Furthermore, potent Vy2V52 T-cell activators that kill malaria parasites *in vitro* (and *in vivo*) were found, providing a combined chemotherapeutic approach to the development of anti-malarials in which both host innate immunity (host FPPS inhibition/γδ T cell activation/ TNF-a and granulysin-mediated killing of liver stages and merozoites) (FIG. 6) as well as direct killing (via parasite GGPPS inhibition, carotenoid, menaquinone and vitamin E biosynthesis inhibition) are targeted by a single molecule.
Example 5
Experimental Section

This example provides details on the materials and methods used for Examples 1-4 above.

Synthetic Aspects. 1 and 17 were available from previous work. The synthesis of 2-16 and 18-32 are described below.

General procedure for the synthesis of lipophilic zoledronate analogs (2-16):

A mixture of imidazole (100 mmol), alkyl bromide (100 mmol) and K2CO3 (200 mmol) in acetone (200 mL) was refluxed overnight. Upon filtration and removal of solvent, the residue was subjected to flash chromatography with ethyl acetate as eluant to give product with > 90% yield. N-alkylimidazole was stirred with methyl bromoacetate in ethyl acetate at room temperature to give the imidazolium salt which was hydrolyzed under reflux in HCl (6 M) to give the carboxylic acid in quantitative yield. A mixture of the carboxylic acid (3 mmol), H3PO3 (15 mmol), and toluene (8 mL) was heated to 80 °C with stirring. After all solids melted, POCl3 (15 mmol) was added slowly and the reaction mixture vigorously stirred at 80 °C for 5 hours. The mixture was cooled, toluene decanted and 6 M HCl (3 mL) added to the residue. The resulting solution was refluxed for 1 hour, then most of the solvent was removed in vacuo. i-PrOH (25 mL) was added to precipitate a 1-hydroxymethylene bisphosphonate as a white powder, which was filtered, washed with 2-propanol (5 x 5 mL), dried, and further purified by recrystallization in H2O/i-PrOH. In some cases, the bisphosphonate was neutralized with NaOH and crystallized as its sodium salt, from H2O/i-PrOH.
l-Hydroxy-2-(N-methyl-imidazolium-1-yl)ethylidene-1,1-bisphosphonic acid (2).

H NMR (400 MHz, D₂O) δ: 8.50 (s, 1H), 7.28 (s, 1H), 7.13 (s, 1H), 4.44 (t, J = 7.2 Hz, 2H), 3.66 (s, 3H). ³¹P NMR (162 MHz, D₂O) δ: 15.09. Anal. Calcd. for C₆H₁₂N₂O₇P₂·O.5H₂O: C, 24.42; H, 4.44; N, 9.49. Found: C, 24.36; H, 4.18; N, 9.18.

l-Hydroxy-2-(N-ethyl-imidazolium-1-yl)ethylidene-1,1-bisphosphonic acid (3).

H NMR (400 MHz, D₂O) δ: 8.60 (s, 1H), 7.32 (s, 1H), 7.25 (s, 1H), 4.48 (t, J = 7.2 Hz, 2H), 3.99 (t, J = 7.2 Hz, 2H), 1.31 (t, J = 6.8 Hz, 3H). ³¹P NMR (162 MHz, D₂O) δ: 15.15. Anal. Calcd. for C₇H₁₄N₂O₇P₂: C, 30.58; H, 5.13; N, 8.92. Found: C, 30.33; H, 5.07; N, 8.64.

l-Hydroxy-2-(N-propyl-imidazolium-1-yl)ethylidene-1,1-bisphosphonic acid (4).

H NMR (400 MHz, D₂O) δ: 8.61 (s, 1H), 7.34 (s, 1H), 7.25 (s, 1H), 3.99 (m, 2H), 1.70 (q, J = 6.8 Hz, 2H), 0.73 (t, J = 6.8 Hz, 3H). ³¹P NMR (162 MHz, D₂O) δ: 15.14. Anal. Calcd. for C₈H₁₆N₂O₇P₂: C, 35.10; H, 5.89; N, 8.19. Found: C, 35.01; H, 5.67; N, 8.24.

l-Hydroxy-2-(N-butyl-imidazolium-1-yl)ethylidene-1,1-bisphosphonic acid (5).

H NMR (400 MHz, D₂O) δ: 8.55 (s, 1H), 7.29 (s, 1H), 7.19 (s, 1H), 3.97 (t, J = 7.2 Hz, 2H), 1.66 (m, 2H), 1.07 (m, 4H), 0.64 (t, J = 7.2 Hz, 3H). ³¹P NMR (162 MHz, D₂O) δ: 15.07. Anal. Calcd. for C₁₀H₂₀N₂O₇P₂: C, 35.10; H, 5.89; N, 8.19. Found: C, 35.01; H, 5.67; N, 8.24.

l-Hydroxy-2-(N-pentyl-imidazolium-1-yl)ethylidene-1,1-bisphosphonic acid (6).

H NMR (400 MHz, D₂O) δ: 8.55 (s, 1H), 7.29 (s, 1H), 7.19 (s, 1H), 3.97 (t, J = 7.2 Hz, 2H), 1.66 (m, 2H), 1.07 (m, 4H), 0.64 (t, J = 7.2 Hz, 3H). ³¹P NMR (162 MHz, D₂O) δ: 15.07. Anal. Calcd. for C₁₀H₂₀N₂O₇P₂: C, 35.10; H, 5.89; N, 8.19. Found: C, 35.01; H, 5.67; N, 8.24.

l-Hydroxy-2-(N-hexyl-imidazolium-1-yl)ethylidene-1,1-bisphosphonic acid (7).

H NMR (400 MHz, D₂O) δ: 8.55 (s, 1H), 7.29 (s, 1H), 7.20 (s, 1H), 3.97 (t, J = 10.0 Hz, 2H), 1.64 (m, 2H), 1.07 (m, 6H), 0.64 (t, J = 7.2 Hz, 3H). ³¹P NMR (162 MHz, D₂O) δ: 15.20. Anal. Calcd. for C₁₁H₂₂N₂O₇P₂: C, 37.09; H, 6.22; N, 7.86. Found: C, 36.79; H, 6.59; N, 7.48.

l-Hydroxy-2-(N-heptyl-imidazolium-1-yl)ethylidene-1,1-bisphosphonic acid (8).

H NMR (400 MHz, D₂O) δ: 8.57 (s, 1H), 7.31 (s, 1H), 7.21 (s, 1H), 3.99 (t, J = 7.2 Hz, 2H), 1.67 (m, 2H), 1.07 (m, 8H), 0.64 (t, J = 6.8 Hz, 3H). ³¹P NMR (162
MHz, D$_2$O ) $\delta$: 15.07. Anal. Calcd. for C$_{12}$H$_{24}$N$_2$O$_7$P$_2$: C, 38.92; H, 6.53; N, 7.57. Found: C, 38.63; H, 6.50; N, 7.47.

l-Hydroxy-2-(N-octyl-imidazolium-l-yl)ethylidene-l,l-bisphosphonic acid (9).

H NMR (400 MHz, D$_2$O ) $\delta$: 8.54 (s, 1H), 7.30 (s, 1H), 7.18 (s, 1H), 4.42 (t, $J = 10.0$ Hz, 2H), 3.98 (t, $J = 6.4$ Hz, 2H), 1.67 (m, 2H), 1.07 (m, 10H), 0.64 (t, $J = 6.8$ Hz, 3H). $^{31}$P NMR (162 MHz, D$_2$O ) $\delta$: 15.27. Anal. Calcd. for C$_{18}$H$_{36}$N$_2$O$_7$P$_2$ H$_2$O: C, 45.76; H, 8.11; N, 5.93. Found: C, 5.65; H, 7.82; N, 5.69.

l-Hydroxy-2-(N-nonyl-imidazolium-l-yl)ethylidene-l,l-bisphosphonic acid (10).

H NMR (400 MHz, D$_2$O ) $\delta$: 8.56 (s, 1H), 7.03 (s, 1H), 7.20 (s, 1H), 4.44 (t, $J = 9.6$ Hz, 2H), 3.98 (t, $J = 7.2$ Hz, 2H), 1.66 (m, 2H), 1.05 (m, 12H), 0.64 (t, $J = 7.2$ Hz, 3H). $^{31}$P NMR (162 MHz, D$_2$O ) $\delta$: 15.04. Anal. Calcd. for C$_{14}$H$_{28}$N$_2$O$_7$P$_2$-0.25H$_2$O: C, 41.74; H, 7.13; N, 6.95. Found: C, 41.65; H, 6.99; N, 6.83.

l-Hydroxy-2-(N-decyl-imidazolium-l-yl)ethylidene-l,l-bisphosphonic acid (11).

H NMR (400 MHz, D$_2$O ) $\delta$: 8.55 (s, 1H), 7.30 (s, 1H), 7.20 (s, 1H), 4.46 (t, $J = 9.6$ Hz, 2H), 3.98 (t, $J = 7.2$ Hz, 2H), 1.66 (m, 2H), 1.05 (m, 14H), 0.64 (t, $J = 7.2$ Hz, 3H). $^{31}$P NMR (162 MHz, D$_2$O ) $\delta$: 15.10. Anal. Calcd. for C$_{15}$H$_{30}$N$_2$O$_7$P$_2$: C, 43.69; H, 7.33; N, 6.79. Found: C, 43.69; H, 7.47; N, 6.58.

l-Hydroxy-2-(N-undecyl-imidazolium-l-yl)ethylidene-l,l-bisphosphonic acid (12).

H NMR (400 MHz, D$_2$O ) $\delta$: 8.54 (s, 1H), 7.30 (s, 1H), 7.19 (s, 1H), 4.43 (t, $J = 9.6$ Hz, 2H), 3.98 (t, $J = 7.2$ Hz, 2H), 1.66 (m, 2H), 1.05 (m, 16H), 0.64 (t, $J = 7.2$ Hz, 3H). $^{31}$P NMR (162 MHz, D$_2$O ) $\delta$: 14.81. Anal. Calcd. for C$_{16}$H$_{32}$N$_2$O$_7$P$_2$: C, 44.44; H, 7.61; N, 6.48. Found: C, 44.35; H, 7.62; N, 6.40.

l-Hydroxy-2-(N-dodecyl-imidazolium-l-yl)ethylidene-l,l-bisphosphonic acid (13).

H NMR (400 MHz, D$_2$O ) $\delta$: 8.65 (s, 1H), 7.39 (s, 1H), 7.14 (s, 1H), 4.42 (t, $J = 9.6$ Hz, 2H), 3.98 (t, $J = 7.2$ Hz, 2H), 1.65 (m, 2H), 1.05 (m, 18H), 0.64 (t, $J = 7.2$ Hz, 3H). $^{31}$P NMR (162 MHz, D$_2$O ) $\delta$: 15.18. Anal. Calcd. for C$_{17}$H$_{34}$N$_2$O$_7$P$_2$-H$_2$O: C, 44.54; H, 7.92; N, 6.11. Found: C, 44.72; H, 7.83; N, 6.36.

l-Hydroxy-2-(N-tridecyl-imidazolium-l-yl)ethylidene-l,l-bisphosphonic acid (14).

H NMR (400 MHz, DMSO-d6) $\delta$: 9.01 (s, 1H), 7.59 (s, 2H), 4.48 (s, 2H), 4.02 (m, 2H), 1.69 (m, 2H),1.19 (m, 20H), 0.80 (t, $J = 1.2$ Hz, 3H). $^{31}$P NMR (162 MHz, D$_2$O ) $\delta$: 15.27. Anal. Calcd. for C$_{18}$H$_{36}$N$_2$O$_7$P$_2$-H$_2$O: C, 45.76; H, 8.11; N, 5.93. Found: C, 5.65; H, 7.82; N, 5.69.
1-Hydroxy-2-(N-tetradecyl-imidazolium-l-yl)ethylidene-l,l-bisphosphonic acid (15).

H NMR (400 MHz, DMSO-d6) δ: 9.01 (s, 1H), 7.59 (s, 2H), 4.48 (s, 2H), 4.02 (m, 2H), 1.69 (m, 2H), 1.19 (m, 22H), 0.80 (t, J = 12 Hz, 3H). 31P NMR (162 MHz, DMSO-d6) δ: 15.17.


1-Hydroxy-2-(N-pentadecyl-imidazolium-l-yl)ethylidene-l,l-bisphosphonic acid (16).

H NMR (400 MHz, DMSO-d6) δ: 9.01 (s, 1H), 7.59 (s, 2H), 4.48 (s, 2H), 4.02 (m, 2H), 1.69 (m, 2H), 1.19 (m, 24H), 0.80 (t, J = 12 Hz, 3H). 31P NMR (162 MHz, DMSO-d6) δ: 15.20.


General procedure for the synthesis of lipophilic deoxy-zoledronate analogs (18-32)

![Chemical structure](image)

To a solution of ethene-l,l-diylidiphosphonic acid (1 mmol) in HOAc (5 mL) was added an N-alkylimidazole (1 mmol), and the solution refluxed overnight with stirring. Upon removal of solvent, recrystallization was carried out from H2O/i-PrOH (1/10) to give the products as white powders.

2-(N-methyl-imidazolium-l-yl)ethylidene-l,l-bisphosphonic acid (18).

H NMR (400 MHz, D2O) δ: 8.56 (s, 1H), 7.34 (s, 1H), 7.18 (s, 1H), 4.41 (dt, J = 7.2, 13.2 Hz, 2H), 3.67 (s, 3H), 2.54 (tt, J = 6.4, 21.2 Hz, 1H). 31P NMR (162 MHz, D2O) δ: 16.05. Anal. Calcd. for C6H12N2O6P2: C, 26.68; H, 4.48; N, 10.37; Found: C, 26.94; H, 4.30; N, 10.32.

2-(N-ethyl-imidazolium-1-yl)ethylidene-1,1-bisphosphonic acid (19).

H NMR (400 MHz, D2O) δ: 8.68 (s, 1H), 7.40 (s, 1H), 7.31 (s, 1H), 4.47 (dt, J = 7.2, 13.2 Hz, 2H), 4.05 (t, J = 7.2 Hz, 2H), 2.58 (tt, J = 7.2, 21.6 Hz, 1H), 1.31 (t, J = 12 Hz, 3H). 31P NMR (162 MHz, D2O) δ: 16.19. Anal. Calcd. for C7H14N2O6P2: C, 29.59; H, 4.97; N, 9.86; Found: C, 29.71; H, 4.76; N, 9.90.
2-(N-propyl-imidazolium-l-yl)ethylidene-l,l-bisphosphonic acid (20).

H NMR (400 MHz, D$_2$O) $\delta$: 8.65 (s, 1H), 7.38 (s, 1H), 7.27 (s, 1H), 4.45 (dt, $J = 7.2$, 13.2 Hz, 2H), 3.96(t, $J = 6.8$ Hz, 2H), 2.58 (tt, $J = 7.2$, 21.6 Hz, 1H), 1.68 (m, 2H), 0.70 (t, $J = 1.2$ Hz, 3H). $^{31}$P NMR (162MHz, D$_2$O) $\delta$: 16.20. Anal. Calcd. for C$_8$H$_{16}$N$_2$O$_6$P$_2$·0.4H$_2$O: C, 31.46; H, 5.55; N, 9.17. Found: C, 31.25; H, 5.33; N, 8.84.

2-(N-butyl-imidazolium-l-yl)ethylidene-l,l-bisphosphonic acid (21).

H NMR (400 MHz, D$_2$O) $\delta$: 8.60 (s, 1H), 7.35 (s, 1H), 7.28 (s, 1H), 4.36 (dt, $J = 7.2$, 13.2 Hz, 2H), 3.97(t, $J = 1.2$ Hz, 2H), 2.10 (tt, $J = 6.8$, 20.4 Hz, 1H), 1.64 (m, 2H), 1.12 (m, 2H), 0.70 (t, $J = 1.6$ Hz, 3H). $^{31}$P NMR (162 MHz, D$_2$O) $\delta$: 15.36. Anal. Calcd. for C$_9$H$_{18}$N$_2$O$_6$P$_2$: C, 34.62; H, 5.81; N, 8.97. Found: C, 34.42; H, 5.92; N, 9.05;

2-(N-pentyl-imidazolium-l-yl)ethylidene-l,l-bisphosphonic acid (22).

H NMR (400 MHz, D$_2$O) $\delta$: 8.66 (s, 1H), 7.39 (s, 1H), 7.21 (s, 1H), 4.48 (dt, $J = 6.8$, 12.8 Hz, 2H), 4.00(t, $J = 1.2$ Hz, 2H), 2.59 (tt, $J = 6.4$, 20.0 Hz, 1H), 1.68 (m, 2H), 1.12 (m, 4H), 0.68 (t, $J = 1.2$ Hz, 3H). $^{31}$P NMR (162 MHz, D$_2$O) $\delta$: 16.23. Anal. Calcd. for C$_{10}$H$_{20}$N$_2$O$_6$P$_2$·0.8H$_2$O: C, 35.26; H, 6.39; N, 8.22. Found: C, 34.93; H, 6.00; N, 8.36.

2-(N-hexyl-imidazolium-l-yl)ethylidene-l,l-bisphosphonic acid (23).

H NMR (400 MHz, D$_2$O) $\delta$: 8.60 (s, 1H), 7.36 (s, 1H), 7.21 (s, 1H), 4.36 (dt, $J = 7.2$, 13.2 Hz, 2H), 3.97(t, $J = 1.2$ Hz, 2H), 2.26 (tt, $J = 6.8$, 20.4 Hz, 1H), 1.66 (m, 2H), 1.10 (m, 6H), 0.66 (t, $J = 1.2$ Hz, 3H). $^{31}$P NMR (162 MHz, D$_2$O) $\delta$: 15.34. Anal. Calcd. for C$_{11}$H$_{22}$N$_2$O$_6$P$_2$·0.8H$_2$O·PrOH: C, 37.11; H, 6.26; N, 7.87. Found: C, 7.17; H, 6.38; N, 7.88.

2-(N-heptyl-imidazolium-l-yl)ethylidene-l,l-bisphosphonic acid (24).

H NMR (400 MHz, D$_2$O) $\delta$: 8.61 (s, 1H), 7.35 (s, 1H), 7.21 (s, 1H), 4.38 (dt, $J = 7.2$, 13.2 Hz, 2H), 3.96(t, $J = 1.2$ Hz, 2H), 2.30 (tt, $J = 6.8$, 20.4 Hz, 1H), 1.66 (m, 2H), 1.08 (m, 8H), 0.66 (t, $J = 4.8$ Hz, 3H). $^{31}$P NMR (162 MHz, D$_2$O) $\delta$: 15.33. Anal. Calcd. for C$_{12}$H$_{24}$N$_2$O$_6$P$_2$: C, 40.68; H, 6.83; N, 7.91. Found: C, 40.41; H, 6.78; N, 7.59.

2-(N-octyl-imidazolium-l-yl)ethylidene-l,l-bisphosphonic acid (25).

H NMR (400 MHz, D$_2$O) $\delta$: 8.61 (s, 1H), 7.36 (s, 1H), 7.23 (s, 1H), 4.43 (dt, $J = 7.2$, 13.6 Hz, 2H), 3.96(t, $J = 1.2$ Hz, 2H), 2.45 (tt, $J = 1.6$, 21.2 Hz, 1H), 1.66 (m, 2H), 1.06 (m, 10H), 0.63 (t, $J = 6.8$ Hz, 3H). $^{31}$P NMR (162 MHz, D$_2$O) $\delta$: 15.75. Anal. Calcd. for C$_{13}$H$_{26}$N$_2$O$_6$P$_2$·0.7H$_2$O: C, 40.99; H, 7.25; N, 7.35. Found: C, 40.92; H, 7.07; N, 7.19.

2-(N-nonyl-imidazolium-l-yl)ethylidene-l,l-bisphosphonic acid (26).

H NMR (500 MHz, D$_2$O) $\delta$: 8.63 (s, 1H), 7.40 (s, 1H), 7.24 (s, 1H), 4.40 (dt, $J = 7.5$, 13.0 Hz, 2H), 4.00(t, $J = 6.0$ Hz, 2H), 2.21 (tt, $J = 1.5$, 20.0 Hz, 1H), 1.71 (m, 2H), 1.10 (m, 12H), 0.69 (t, $J = 5.1$ Hz, 3H). $^{31}$P NMR (162 MHz, D$_2$O) $\delta$: 15.34. Anal. Calcd. for C$_{14}$H$_{28}$N$_2$O$_6$P$_2$: C, 39.48; H, 6.80; N, 7.71. Found: C, 39.24; H, 6.99; N, 7.60.
$J = 1.5$ Hz, 3H). $^{31}$P NMR (202 MHz, $D_{2}O$) $\delta$: 15.75. Anal. Calcd. for $C_{4}H_{7}N_{2}NaO6P2$: C, 41.59; H, 6.79; N, 6.93. Found: C, 41.91; H, 6.85; N, 6.66.

2-(N-decyl-imidazolium-l-yl)ethylidene-l,l-bisphosphonic acid (27).

$^1$H NMR (500 MHz, $D_{2}O$) $\delta$: 8.65 (s, 1H), 7.40 (s, 1H), 7.28 (s, 1H), 4.45 (dt, $J = 7.0, 13.5$ Hz, 2H), 4.01 (t, $J = 1.5$ Hz, 2H), 2.45 (tt, $J = 1.5, 21.5$ Hz, 1H), 1.70 (m, 2H), 1.13 (m, 14H), 0.68 (t, $J = 1.5$ Hz, 3H). $^{31}$P NMR (202 MHz, $D_{2}O$) $\delta$: 15.54; Anal. Calcd. for $C_{15}H_{30}N_{2}O6P2$: C, 45.45; H, 7.63; N, 7.07. Found: C, 45.40; H, 7.56; N, 7.00.

2-(N-undecyl-imidazolium-1-yl)ethylidene-l,l-bisphosphonic acid (28).

$^1$H NMR (400 MHz, $D_{2}O$) $\delta$: 8.61 (s, 1H), 7.38 (s, 1H), 7.22 (s, 1H), 4.39 (dt, $J = 7.2, 13.6$ Hz, 2H), 3.98 (t, $J = 1.2$ Hz, 2H), 2.23 (tt, $J = 6.8, 20.4$ Hz, 1H), 1.68 (m, 2H), 1.07 (m, 16H), 0.68 (t, $J = 1.2$ Hz, 3H). $^{31}$P NMR (162 MHz, $D_{2}O$) $\delta$: 15.17; Anal. Calcd. for $C_{16}H_{32}N_{2}O6P2$: C, 43.54; H, 7.31; N, 6.35. Found: C, 43.67; H, 7.29; N, 7.43.

2-(N-dodecyl-imidazolium-1-yl)ethylidene-l,l-bisphosphonic acid (29).

$^1$H NMR (400 MHz, $D_{2}O$) $\delta$: 8.68 (s, 1H), 7.42 (s, 1H), 7.22 (s, 1H), 4.42 (dt, $J = 7.2, 13.2$ Hz, 2H), 3.43 (t, $J = 1.2$ Hz, 2H), 2.42 (tt, $J = 1.2, 21.6$ Hz, 1H), 1.65 (m, 2H), 1.04 (m, 18H), 0.68 (t, $J = 1.2$ Hz, 3H). $^{31}$P NMR (162 MHz, $D_{2}O$) $\delta$: 15.33; Anal. Calcd. for $C_{17}H_{34}N_{2}O6P2 \cdot 1.55H_{2}O$: C, 39.93; H, 6.72; N, 5.48. Found: C, 40.30; H, 7.11; N, 5.45.

2-(N-tridecyl-imidazolium-1-yl)ethylidene-l,l-bisphosphonic acid (30).

$^1$H NMR (400 MHz, $D_{2}O$) $\delta$: 8.61 (s, 1H), 7.38 (s, 1H), 7.22 (s, 1H), 4.37 (dt, $J = 1.2, 13.2$ Hz, 2H), 3.98 (t, $J = 1.2$ Hz, 2H), 2.19 (tt, $J = 1.2, 21.6$ Hz, 1H), 1.67 (m, 2H), 1.07 (m, 20H), 0.66 (t, $J = 1.2$ Hz, 3H). $^{31}$P NMR (162 MHz, $D_{2}O$) $\delta$: 15.40; Anal. Calcd. for $C_{18}H_{36}N_{2}O6P2 \cdot 3H_{2}O$: C, 47.36; H, 8.39; N, 6.14. Found: C, 47.4; H, 8.11; N, 6.05.

2-(N-tetradecyl-imidazolium-1-yl)ethylidene-l,l-bisphosphonic acid (31).

$^1$H NMR (400 MHz, $D_{2}O$) $\delta$: 8.75 (s, 1H), 7.48 (s, 1H), 7.21 (s, 1H), 4.44 (dt, $J = 1.2, 13.2$ Hz, 2H), 3.99 (t, $J = 1.2$ Hz, 2H), 2.38 (tt, $J = 1.2, 21.6$ Hz, 1H), 1.66 (m, 2H), 1.07 (m, 20H), 0.66 (t, $J = 1.2$ Hz, 3H). $^{31}$P NMR (162 MHz, $D_{2}O$) $\delta$: 15.40; Anal. Calcd. for $C_{19}H_{38}N_{2}O6P2 \cdot Q5H_{2}O$: C, 49.45; H, 8.52; N, 6.07. Found: C, 49.33; H, 8.63; N, 6.02.

2-(N-pentadecyl-imidazolium-1-yl)ethylidene-l,l-bisphosphonic acid (32).

$^1$H NMR (400 MHz, $D_{2}O$) $\delta$: 8.68 (s, 1H), 7.45 (s, 1H), 7.16 (s, 1H), 4.40 (dt, $J = 1.2, 13.2$ Hz, 2H), 3.97 (t, $J = 1.2$ Hz, 2H), 2.16 (tt, $J = 1.2, 21.6$ Hz, 1H), 1.66 (m, 2H), 1.07 (m, 24H), 0.67 (t, $J = 1.2$ Hz, 3H). $^{31}$P NMR (162 MHz, $D_{2}O$) $\delta$: 15.40; Anal. Calcd. for $C_{20}H_{40}N_{2}O6P2 \cdot O.5H_{2}O$: C, 50.52; H, 8.69; N, 5.89. Found: C, 50.30; H, 8.72; N, 5.96.
Human FPPS expression. Truncated human FPPS (6-353) was cloned into Ndel/BamHI restriction sites of pET28a vector by using forward primer (5’-CTTC ATATGAATTCCAGATTTATGCCAAGAAAAAGC AGGATTTTG-3’; SEQ ID NO: 1) and reverse primer (5’-CTTGGATCCTCAGTTTCTCCGCTTGTAGATT TTTTGCGCG-3’; SEQ ID NO: 2). pET28a-(His)6HsFPPS 6-353 was transformed into BL21(DE3) Tuner cells to ensure proper distribution of inducers across the cells. Transformed cells were spread onto LB plates with 50 µg/mL kanamycin and were incubated for 16 hours. A single colony that carried pET28a-(His)6HsFPPS 6-353 was inoculated in 100 mL LB broth with 50µg/mL kanamycin and incubated at 37°C overnight. 10 mL of inoculated cells were added into 1L LB broth with 50 µg/mL kanamycin and incubated until the OD600 reached about 0.6-0.8. Cells were induced by ImM IPTG and incubated at 24 °C for at least 16 hours, then centrifuged, and the pellets frozen at -80 °C. The cell pellets were then thawed in wash buffer (10 mM HEPES, pH 7.5, 500 mM NaCl and 35 mM imidazole) with addition of Benzonase (EMD Millipore) and EDTA-free protease cocktail (Roche). Thawed cells were sonicated (10 seconds active, 20 seconds rest, for 10 minutes) then centrifuged at 23,000 rpm for 30 minutes. The supernatant was loaded on a Ni-NTA column and eluted with 0-100 % elution buffer (10 mM HEPES, 500 mM NaCl and 500 mM imidazole). Fractions were subjected to SDS-PAGE and only pure (His)6HsFPPS was collected. The protein was digested with thrombin and dialyzed against dialysis buffer (10 mM HEPES, pH 7.4 and 150 mM NaCl) at 4 °C for 30 hours to remove the N-terminal His-tag. HsFPPS was further purified by using S200 gel filtration chromatography with storage buffer (10 mM Tris, pH 7.4 and 25 mM NaCl). The pure HsFPPS fraction was concentrated to 37 mg/mL, then quickly frozen under liquid nitrogen and stored at -80°C.

Human FPPS inhibition assays. Human FPPS inhibition assays were carried out using 96 well plates with 200 µL reaction mixture in each well. The condensation of geranyldiphosphate (100 µM final) and isopentenyldiphosphate (100 µM final) was monitored at room temperature by using a continuous spectrophotometric assay for phosphate-releasing enzymes. The reaction buffer contained 50 mM Tris-HCl (pH 7.4), 1 mM MgCl2, and 0.01 % Triton X100. The compounds investigated were pre-incubated with enzyme for 30 minutes at room temperature. The IC50 values were obtained from fitting dose-response curve using Prism 4.0 (GraphPad Software, Inc., La Jolla, CA, www.graphpad.com).
Tumor cell MTT assay

Human tumor cell line MCF-7 (breast adenocarcinoma) was obtained from the National Cancer Institute. Lung cancer cell line #4, #6 and L2 were derived from mouse primary lung adenocarcinomas which harboring KRASG12D mutation and p53 knockdown. Cell lines were cultured in RPMI-1640 or DMEM medium supplemented with 2 mM L-glutamine and 10% fetal bovine serum (Gibco, Grand Island, NY) at 37 °C in a 5% CO₂ atmosphere with 100% humidity. Compound stock solutions were typically prepared in water at a concentration of 0.02 M. A broth microdilution method was used to determine the bisphosphonate growth inhibition IC₅₀ values. Compounds were half log serial diluted using cell culture media into 96-well TC-treated round bottom plates (Corning Inc., Corning, NY). Cells were plated at a density of 5000 cells/well. Cells were then incubated under the same culture conditions for 2 days at which time an MTT (3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide) cell proliferation assay (ATCC, Manassas, VA) was performed to obtain dose response curves (Table 5).

Tumor xenograft model

Mouse lung cancer cell line L2 was used for xenograft model in C57B16/J mice. Eight-week old female mice were transplanted subcutaneously with 10⁶ tumor cells in the flank region. Tumor sizes were measured with calipers twice a week after the transplantation and treatment was started when decent tumor mass was palpable. Mice were randomly divided into four treatment groups: saline control, BPH-1222 (2mg/kg, every other day), rapamycin (2.5mg/kg, every other day) and BPH-1222 plus rapamycin. Mice were sacrificed after 21 days of treatment and tumors were dissected, weighted and analyzed. Cell proliferation was examined by Ki-67 staining on the tumor sections (FIG. 2B). Necrotic area was quantified by Image J software on H&E staining sections.

P. vivax GGPPS expression. A clone encoding P. vivax GGPPS (PlasmoDB gene ID: Pv092040) with an N-terminally His6-tagged fusion protein and a tobacco etch virus protease site was expressed in Escherichia coli BL21-codon Plus (DE3) RIL (Stratagene) at 15 °C in baffled flasks. Cells were lysed by sonication in the presence of Benzonase Nuclease (Novagen) and a protease inhibitor cocktail (Sigma), and the protein purified chromatographically by using a Ni-nitritoltriacetate resin. EDTA was added immediately to the elution fraction to 1 mM, and 5 mM DTT added after 15 minutes. The eluted protein was then concentrated and loaded onto a Sephadex S-200 gel filtration column, and fractions containing PvGGPPS collected.
**P. vivax GGPPS inhibition assays.** The *P. vivax* GGPPS inhibition assays were carried out by using 96-well plates with 200 μl of reaction mixture in each well. The condensation of geranyldiphosphate (100 μM) with isopentenyldiphosphate (100 μM) was monitored at room temperature by using a continuous spectrophotometric assay for phosphate-releasing enzymes in a reaction mixture containing 50 mM Tris-HCl (pH 7.4), 1 mM MgCl₂, and 0.01% Triton X100. The inhibitors were pre-incubated with the enzyme for 30 minutes, at room temperature. The IC₅₀ values were obtained from fitting the dose-response curve using Prism 4.0 (GraphPad Software, Inc., La Jolla, CA, www.graphpad.com).

**γδ T cell activation assays.** Vy2V52T cell activation was assessed by TNF-a release as described previously. Briefly, the CD4+ Vy2V52T cell clone, JN.23, was stimulated with bisphosphonates in the presence of the antigen presenting cell line, CP.EBV (an EBV transformed human B cell line). For TNF-arelease, supernatants were harvested 16 hours later and assayed for TNF-a levels by sandwich ELISA (R&D Systems). Concentrations required to achieve 50% of the observed maximal T cell response (EC₅₀) were obtained by using the Prism 4.0 program (Graphpad Software, La Jolla, CA, www.graphpad.com), using a sigmoidal dose-response function. Curve fitting minima for each experiment were determined using the Global Fitting technique, as implemented in Prism 4.0. Curve fitting maxima were optimized for each individual compound without the use of any constraints.

**Crystallization, data collection and refinement of the HsFPPS/5 complex.** Co-crystallization of human FPPS was carried out as follows. 34 mg/mL HsFPPS was mixed with 1 mM 5 and 2 mM MgCl₂. The mixture was then incubated at 4 °C overnight. The mixture was centrifuged and any precipitate discarded. The protein solution was then mixed with mother liquor (1.2 M Na/K phosphate, pH 5.2 and 25% glycerol) in a ratio of 1:1. Hanging drops were incubated at 18 °C. Large, hexagon-like crystals appeared in around 1-3 days and grew to maximum size in one week. The crystals were mounted, then frozen in liquid nitrogen. Diffraction data was collected at the Life Science Collaborative Access Team (LSCAT) at the Argonne National Laboratory (Argonne, IL). Data was processed by using HKL2000 and refined by using Refmac and Coot. Refined statistics are shown in Table 4. Graphics were created by using PyMOL.
### Table 4

#### Data collection and refinement statistics

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<th>Crystal (PDB ID)</th>
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#### Data collection

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<td>(I/\sigma(I))</td>
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#### Refinement

| Resolution (Å)              | 40.5-2.40       |
| (2.44-2.40)                 |                 |
| No. of reflections          | 16394 (987)     |
| \(R_{\text{work}}\) (\(\bar{R}\)) | 20.4 (26.0)   |
| \(R_{\text{free}}\) (%)    | 28.2 (35.1)     |

#### Geometry deviations

| Bond lengths (Å)            | 0.016           |
| Bond angles (°)             | 1.937           |

#### Mean B-values (Å²) / number of non-H atoms

| All refined atoms           | 43.5/2909       |
| Compound atoms              | 30.2/20         |
| \(P_{14}\) ions            | 54.4/10         |
| Mg ions                     | 20.4/3          |
| Water molecules             | 39.0/70         |

#### Ramachandran plot (%)

| Most favored               | 93.9            |
| Additionally allowed       | 6.1             |
| Generously allowed         | 0               |
| Disallowed                 | 0               |
**P. falciparum growth inhibition assays.** *P. falciparum* growth inhibition assays were carried out as described in previous work. Briefly, a *P. falciparum* culture was adjusted to 2% hematocrit, 0.5% parasitemia, then dispensed by a WellMate (Thermo) into 384 well plates (Greiner) containing the compounds (final volume 50 µL) and incubated for 72 hours. Chloroquine, artemisinin, and DMSO were used within the assay plates to serve as controls. After 3 days, a parasite lactate dehydrogenase (pLDH) assay was used to assess compound efficacy. At the end of 72 hours, the plates were frozen overnight at -20 °C. After thawing, the plates were shaken for 45 seconds at 1,700 rpm in a Mix Mate (Eppendorf) and 5 µL of the lysate transferred into the corresponding well of another plate containing 30 µL of Malstat Reagent and incubated for 2 hours. The absorbance (650 nm) was read using a Spectramax M5 (Molecular Devices). IC50 values were obtained from fitting the dose-response curve using Prism 4.0 (GraphPad Software, Inc., La Jolla, CA, www.graphpad.com).

The compounds shown in Table 5 also have potent activity against a human breast adenocarcinoma cell line.

**Table 5**

<table>
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Example 6

Materials and Methods

This example provides the materials and methods for the Examples below.

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5 Study design. 30 bisphosphonates were screened for activity against their putative targets, farnesyl diphosphate synthase (FPPS) and geranylgeranyl diphosphate synthase (GGPPS), as well as in vitro against a panel of tumor cell lines. The most effective compounds were then tested in vivo in mouse lung cancer models. In monotherapy the bisphosphonates had only moderate activity. Combination therapy was then investigated to determine if that would improve the efficacy. Chloroquine and rapamycin were selected as partner drugs, and the combinations were tested in vivo, in both syngeneic transplantation (subcutaneous and orthotopic) and lentiviral models. Tumor size was monitored 1-2 times every week during the treatment, by either palpation or in vivo luciferase imaging. At least 5 mice were used for each
group (either control or treatment). All the treatment experiments were repeated at least twice independently. Mice used for control and treatment were randomly grouped from a pool of model mice.

**Chemical reagents.** Lipophilic bisphosphonates were synthesized as described above. Protein farnesyltransferase and geranylgeranyltransferase inhibitors (FTI-277 and GGTI-298) were purchased from Calbiochem. MEK inhibitor U0126 was purchased from Cell Signaling. Farnesol (FOH) and geranylgeraniol (GGOH), ascorbic acid, Trolox, Lipoic acid, Morin hydrate and chloroquine diphosphate salt were from Sigma. Simvastatin was from Tokyo Chemical Industry Co. Rapamycin was from Alfa Aesar.

**Stable cell lines and cell survival assay.** Mouse lung cancer cell lines (6#, L2 and M3L2) were derived from primary tumors of LSL-KRAS\(^{G12D}\) mice infected with CA2Cre-shp53 lentiviral vector. L2 and M3L2 cells were derived from mouse tumors with pure C57B and pure FVB background respectively, so that they form syngeneic grafts in matched recipient mice. To monitor NF-κB activation in tumor grafts, L2 cells were stably infected with 5XKB-1UCI lentiviral vector. Human cancer cell lines (A549, A427, Panc-1 and MiaPaCa2) were from ATCC. MEFs were prepared from mouse embryo with matched genetic background and immortalized by shRNAs against p53 and Rbl. Cell survival after drug treatment was measured using Cell Proliferation Reagent (Wst-1) from Roche or MTT Cell Proliferation Assay Kit (30-1010K) from ATCC. Dose-response curves and corresponding IC50s were fitted using GraphPad Prism.

**Lentiviral vector mediated mouse lung cancer model and syngeneic graft model.** LSL-KRAS\(^{G12D/+}\) Rosa26\(^{lad/wc}\) mice were used for the lentiviral vector (CA2Cre-shp53) mediated lung cancer model. Tumor size was monitored by in vivo luciferase imaging system (IVIS 100) from Caliper Lifesciences. Syngeneic graft experiments were done by either subcutaneously transplanting \(10^6\) L2 tumor cells in the flank region of C57B mice, or tail vein injection of \(2\times10^5\) M3L2 cells into FVB mice. Subcutaneous tumor size was measured every 7 days after the transplantation. For single drug treatment, animals were given the drug every day. For combination therapies, animals were given BPH-1222 on days 1, 3, 5 and the other drug on days 2, 4, 6, alternately. BPH-1222 (2 mg/kg) and chloroquine (60 mg/kg) were diluted in PBS while rapamycin (2.5 mg/kg) in 75% DMSO and 25% PBS. All drugs were given i.p. in a volume of 100 μl. All mice studies were carried out according to the protocols that were approved by the Institutional Animal Care and Use Committee of Salk Institute.

**Cellular fractionation.** \(10^6\) Cells treated with drugs for 48 hours were washed with PBS and re-suspended in 0.5 ml 0.1M Tris HC1 (pH 7.5) with protease inhibitors for 15 minutes.
Supernatant from 10,000 g 30 minutes centrifugation was collected as cytosolic fraction. The pellet was re-suspended in 0.5 ml lysis buffer containing 1% Triton X-100 for 20 minutes and centrifuged. The supernatant was collected as membrane-bound fraction.

**Histology, immunofluorescence staining, and immunoblotting analysis.** Mouse lung tumor and syngeneic graft samples were fixed with 10% formalin, paraffin-embedded and sectioned for haematoxylin and eosin staining (H&E) and immunofluorescence staining. Elite ABC system (Vector labs) was applied where staining signal was weak. Immunoblotting analysis was performed according to standard protocols. For detecting Hdj2, Rap1A and KRAS prenylation with mobility shift assay, proteins were separated with 15-cm 8% and 13% SDS-PAGE, respectively. Antibodies were purchased from Millipore (SPC, 1:2000), Cell Signaling (phos-ERK, total ERK, phos-AKT, total AKT, phos-MEK, phos-c-RAF, Cleaved Caspase-3, CHOP, BiP, phos-PERK, LC3MI, phos-p70 S6K, phos-4E-BP1, all 1:1000), Abgent (p62, clone 2C11, 1:2000), Vector Labs (Ki-67, 1:500), and Santa Cruz Biotechnology (KRAS, HRAS, RAP1A, HDJ2, ACTIN, all 1:1000).

**Quantitative RT-PCR.** Total RNA isolated from the treated cells was reverse transcribed using Superscript III system (Invitrogen) with random primers. Quantitative PCR was performed in triplicate using 7900HT Fast Real-Time PCR system with SYBR green method (Applied Biosystems). Results were analyzed for the relative expression of mRNAs normalized against GAPDH and cyclophilin. A list of primers used for PCR is in Table 6.

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**KRAS MALDI-mass spectrum analysis.** Purified protein samples were spotted onto a MALDI target using sinapinic acid as the matrix. Spectra (averages of 200 laser shots) were...
obtained on an Applied Biosystems Voyager DE-STR instrument in linear mode with delayed extraction. The accelerating voltage was set to 25,000V and the laser repletion rate was 20 Hz. Masses were corrected using human ACTIVIN-A (MH$_{\text{ave}}^+$ = 25934.8) as an external standard.

**FPPS and GGPPS inhibition assay and crystallization of FPPS with BPH-1222.** Human FPPS and GGPPS proteins were prepared and used for enzymatic inhibition assays. Crystallization of FPPS with BPH-1222 was carried out as reported (Zhang et al., ACS Med Chem Lett 4:423-427 (2013)). Diffraction data was collected at the Life Sciences Collaborative Access Team (LS-CAT) beamline 21ID-Gat Argonne National Laboratory. Data was collected at 100K with wavelength of 0.97857Å. Data was processed using HKL3000 and refined by using CCP4. Crystallographic figures were drawn by using PyMOL (http://www.pymol.org).

Data collection and refinement statistics are shown in Table 7. After refinement, the Ramachandran statistics showed that the percentages of the most favorable and additional allowed regions are 97.6% and 2.4%, respectively.

<table>
<thead>
<tr>
<th>Table 7</th>
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Pharmacokinetics test. Pharmacokinetic studies were performed using 3 female SD rats (230-240 g body weight). Plasma concentrations were measured at 1 minute, 5 minutes, 15 minutes, 30 minutes, 1 hour, 2 hours, 4 hours, 8 hours, 24 hours, and 48 hours following a single i.v. injection of BPH-1222 at 5 mg/kg. 0.3 ml blood was taken each time. Data were analyzed using DAS2.0 software.

Statistical analysis. Statistical analyses were performed with GraphPad Prism Software. Statistical significance of the differences of tumor number and Ki-67, Cleaved Caspase-3 positive staining was evaluated using Student's unpaired two-tailed t-test. The Kaplan-Meier curves were analyzed by Log-rank test. P values less than 0.05 were considered statistically significant.

Example 7

Bisphosphonates inhibit FPPS and GGPPS activity

A library of 30 synthetic analogs of zoledronate was tested (FIGS. 7A-7D) for growth inhibition of two KRAS mutant cell lines (6#, L2) and of control mouse embryonic fibroblasts (MEF). Most anti-growth activity was found with BPH-1222, a zoledronate analog having a C» side-chain and a 1-OH group (about 1 µM ICso, FIG. 8A and FIG. 9A and 9B). Compounds with very short or very long chains inhibited growth the least while BPH-1222 and other intermediate chain length compounds had the most activity. In vitro inhibitory activities against human FPPS (Ki as low as approximately 1 nM) correlated well with activities in inhibiting cell growth, indicating FPPS was an in vivo target (FIG. 8B and Table 8 and FIG. 8C).
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<td>7, H</td>
<td>0.00088</td>
<td>0.29</td>
<td>0.9</td>
<td>1.4</td>
<td>2</td>
<td>0.25</td>
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<tr>
<td>25</td>
<td>8, H</td>
<td>0.0008</td>
<td>0.19</td>
<td>2</td>
<td>0.81</td>
<td>2.1</td>
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<td>26</td>
<td>9, H</td>
<td>0.00086</td>
<td>0.28</td>
<td>2.6</td>
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<td>2.6</td>
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<td>27</td>
<td>10, H</td>
<td>0.002</td>
<td>0.1</td>
<td>12</td>
<td>0.5</td>
<td>3.2</td>
<td>1.6</td>
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<tr>
<td>28</td>
<td>11, H</td>
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<td>0.08</td>
<td>16</td>
<td>0.58</td>
<td>3.5</td>
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<tr>
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<td>12, H</td>
<td>0.0074</td>
<td>0.07</td>
<td>65</td>
<td>30</td>
<td>101</td>
<td>2.5</td>
</tr>
<tr>
<td>30</td>
<td>13, H</td>
<td>0.007</td>
<td>0.87</td>
<td>13</td>
<td>4</td>
<td>6.7</td>
<td>2.9</td>
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<tr>
<td>31</td>
<td>14, H</td>
<td>0.06</td>
<td>0.47</td>
<td>138</td>
<td>11</td>
<td>16</td>
<td>3.3</td>
</tr>
<tr>
<td>32</td>
<td>15, H</td>
<td>0.166</td>
<td>1.8</td>
<td>68</td>
<td>1.2</td>
<td>19</td>
<td>3.8</td>
</tr>
</tbody>
</table>
To uncover how BPH-1222 binds and inhibits FPPS, we determined the structure of the BPH-1222-FPPS complex using single crystal X-ray crystallography. As shown in FIG. 7D, the bisphosphonate, head-group and imidazolium ring bind to human FPPS in essentially the same manner as does zoledronate (shown superimposed) with a 0.45 Å rmsd (root mean square deviation) for the common atoms (see Table 7 for a summary of structural and refinement statistics).

Moreover, the lipophilic bisphosphonates also inhibit human GGPPS (FIG. 8B and FIG. 9C and Table 4). Zoledronate, as well as other bisphosphonates such as risedronate and alendronate, all inhibit FPPS, but do not inhibit GGPPS, since the latter lacks the third Asp required for [Mg\(^{2+}\)] coordination to the bisphosphonate (22). With the lipophilic bisphosphonates binding to GGPPS, the loss of the additional binding interaction due to the absence of the third Asp residue is likely made up for, by increased van der Waals interactions in the hydrophobic tunnel that normally houses the allylic side-chains of the growing isoprenoid diphosphate products. The intermediate chain length species possess the best inhibitory activity for GGPPS, a \(K_i\) of -300 nM for BPH-1222. There is little difference in GGPPS enzyme inhibition activity between the 1-hydroxy and 1-desoxy species (Table 4), so in this work the focus was on BPH-1222, the N-octyl analog of zoledronate, over its desoxy analog in order to eliminate the possibility of a retro-Michael reaction (in which the hydrophobic side-chain would be lost) \textit{in vivo}.

As can be seen in Table 4, the \(K_{s}\) for FPPS inhibition by zoledronate (ID 1) and BPH-1222 (ID 9) were quite similar (1-2 nM), but the IC\(_{50}\) values for cell growth inhibition were about 10-20x lower with BPH-1222. This may correlate with the lower GGPPS \(K_i\) values (approximately 3 \(\mu\)M for zoledronate; 300 nM for BPH-1222) and with the higher clogP (the computed logarithm of the oil/water partition coefficient) value for BPH-1222 (0.25) versus zoledronate (-3.9).

Inhibition of protein farnesylation (with FTI-277), geranylgeranylation (with GGTL-298) or both had little effect on cell proliferation (FIG. 8C, and FIGS. 8D and 9E). However, there was massive cell death within 3 days with BPH-1222. Supplementation with geranylgeraniol (GGOH), but not farnesol (FOH) or anti-oxidants, substantially rescued cells from bisphosphonate-induced cell death (FIG. 8C). Similar rescue effects are seen with zoledronate (23) as well as the lipophilic pyridinium bisphosphonate BPH-714 and with simvastatin, a potent HMG-CoA reductase inhibitor that shuts down all isoprenoid biosynthesis (FIG. 9F). As noted by Goffinet \textit{et al.} (23), the chemical target for zoledronate is FPPS (not GGPPS), but the main
"biological effect" of zoledronate involves protein geranylgeranylation. Thus, dual FPPS/GGPPS-targeting bisphosphonates (such as BPH-1222) were expected to be particularly potent since formation of the FPP substrate for GGPP biosynthesis was blocked by FPPS inhibition, with GGPP production being particularly important for cell survival. While inhibition of FPPS was expected to have effects on diverse metabolic pathways (such as sterol and steroid biosynthesis), these effects were not thought to be the major ones responsible for cell death since the BPH-1222 effects were reversible upon GGOH addition.

It was observed that BPH-1222 was more toxic to cells harboring KRAS mutations, such as cell lines derived from a mouse model of KRAS-induced lung adenocarcinoma, as well as in mouse embryonic fibroblasts transformed by KRAS \textit{in vitro} (FIGS. 8D and 8E). Taken together, these results indicated that blocking protein prenylation by lipophilic bisphosphonates could be used as a targeted therapy for cancer cells that carry KRAS mutations, since potentially both FPPS as well as GGPPS could be targeted; the lipophilic bisphosphonates had much better clogP values than did more conventional bisphosphonates; and furthermore, they did not bind to bone mineral, which rapidly removed them from the circulatory system (24).

\textbf{Example 8}

\textbf{Bisphosphonates block KRAS prenylation and induces its degradation}

"KRAS addiction" has been shown in a mouse lung cancer model using inducible KRAS G12D (25), so given the observation that cells bearing KRAS mutations were more sensitive to bisphosphate treatment, there was interest in determining if the lipophilic bisphosphonates blocked KRAS prenylation. A cell fractionation assay was used to check KRAS protein prenylation status, since unprenylated KRAS proteins lose their ability to avidly associate with cell membranes and, consequently, appear in the cytosolic fraction (26). BPH-1222 treatment robustly inhibited protein farnesylation as well as protein geranylgeranylation, as indicated by HRAS degradation and RAPIA dislodgement from the membrane fraction respectively (27).

Other bisphosphonates, including BPH-714 and zoledronate, showed similar effects, but at higher concentrations (FIG. 10A). A substantial amount of KRAS was unprenylated and was also partially degraded. There was also a reduction of downstream AKT activity, induced by oncogenic KRAS expression (FIG. 10A). This result was further confirmed with HDJ2, RAPIA and KRAS electrophoretic mobility shift assay (FIG. 10B and Table 9) (28). To further verify whether cytosolic KRAS represents the unprenylated form of the protein, the molecular weight of KRAS expressed in U20S cells after BPH-1222 treatment was determined. The molecular
weights of KRAS proteins from cytosolic fraction (Supernatant) and membrane-bound fraction (Pellet) fit well with unprenylated form and farnesylated form, respectively (see FIGS. 11A and 12 for a summary). The effects of a lipophilic bisphosphonate on KRAS function was further tested in a mouse lung cancer (M3L2) and two human pancreatic cancer (Panc-1 and MiaPaCa2) cell lines carrying endogenous KRAS G12D or G12C mutations (FIGS. IOC and 10D). BPH-1222 treatment greatly reduced the amount of GTP-bound KRAS (representing the active form of the KRAS protein), and led to down-regulation of the AKT pathway as well as the activation of apoptosis as shown by increase in Caspase-3 cleavage (FIG. IOC).

Table 9. Summary of protein prenylation inhibited by various compounds.

<table>
<thead>
<tr>
<th>Cell lines</th>
<th>HD12</th>
<th>RAP1A</th>
<th>KRAS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FTI</td>
<td>GGTI</td>
<td>FTI</td>
</tr>
<tr>
<td>Mouse L2</td>
<td>Y</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Mouse M3L2</td>
<td>Y</td>
<td>N</td>
<td>Y</td>
</tr>
<tr>
<td>Mouse P53.2.1.1</td>
<td>Y</td>
<td>N</td>
<td>Y</td>
</tr>
<tr>
<td>Human U2OS (*)</td>
<td>Y</td>
<td>N</td>
<td>Y</td>
</tr>
<tr>
<td>Human MiaPaCa2</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
</tbody>
</table>

Example 9

**Bisphosphonates enhance ER stress and initiate autophagy**

Cancer cells usually exhibit a "stress phenotype" that consists of replicative stress, mitotic stress, metabolic stress, oxidative stress and proteotoxic or ER (endoplasmic reticulum) stress. They are, therefore, vulnerable to further enhancement of these stresses by chemotherapy (29). Bisphosphonates, as demonstrated above, potently block protein prenylation by eliminating the source of isoprenoid chains and lead to the accumulation of incorrectly folded proteins, inducing the so-called Unfolded Protein Response (UPR), or ER stress, as is also observed when cells are treated with HMG-CoA reductase inhibitors (30, 31).

As shown in FIG. 10E, treatment of cells with the lipophilic bisphosphonate BPH-1222 potently induced C/EBP homologous protein (CHOP) and Binding immunoglobulin protein (BiP) protein levels, the two main markers for the ER stress response (18). Notably, the ER stress response induced by this bisphosphonate was specifically due to blockade of protein prenylation, since supplementation with GGOH, but not antioxidants, completely abolished the up-regulation of CHOP, BiP as well as phospho-PERK induced by the bisphosphonate (FIG.
10B). When cells are unable to handle excessive ER stress, they activate autophagy to eliminate incorrectly processed proteins as a defense mechanism for cell survival. In tumor cells treated with BPH-1222, autophagy was induced within 2-3 days, as indicated by the accumulation of the phosphatidylethanolamine (PE)-conjugated form of LC3 (FIGS. 10A and 10E).

Example 10

Rapamycin, but not chloroquine, sensitizes tumor cells to bisphosphonates in vivo

This example describes methods used to determine if the disclosed compounds can be used in combination with inhibitors of autophagy and/or with GGPPS inhibitors.

KRAS-driven tumor cells depend on autophagy to help reduce reactive oxygen species (ROS), as well as provide substrates to fuel cell metabolism (32, 33). The hypothesis that blocking autophagy might sensitize tumor cells to bisphosphonate treatment was tested. First, the effects on cell survival of treatment with bisphosphonate and an autophagy inhibitor, chloroquine (34), was determined. Essentially additive effects of the bisphosphonate and chloroquine were observed in all the lung cancer cell lines derived from the mouse KRAS lung cancer model (with KRAS G12D and p53 knockdown), as well as in human NSCLC cell lines with KRAS mutations (A549 and A427) (FIGS. 13A and 14A).

This combination was tested in a syngeneic graft model using L2 cells derived from a mouse lung adenocarcinoma (with KRAS G12D and p53 knockdown) developed in C57B mice. Treatment was started 3 weeks after subcutaneous transplantation when the tumor mass was palpable. Surprisingly, 24 days of BPH-1222 plus chloroquine therapy only minimally suppressed tumor growth in animals. In contrast, BPH-1222 plus rapamycin (a potent autophagy inducer) showed substantially better efficacy (FIG. 13B). The results are consistent with those recently reported in pancreatic cancer (KRAS G12D and p53-/−) development where it was shown that another autophagy inhibitor, hydroxychloroquine, tended to accelerate tumor formation (35). It was observed that tumors treated with BPH-1222 plus chloroquine had elevated NF-κB activity as shown by in vivo imaging (FIG. 13C). This result was further confirmed in cultured cells treated with combination of drugs by quantitative RT-PCR of NF-κB target genes (FIG. 13D). The NF-κB activity was correlated with p62 protein accumulation in the cells, probably due to the blockage of autophagy flux by chloroquine (FIG. 14B). Indeed, high p62 protein level in cells is known to activate NF-κB through TRAF6-TBK1 pathway (36). NF-κB activity in tumors may stimulate cell proliferation (37), and this appeared to be the case in tumor samples treated with BPH-1222 plus chloroquine (FIGS. 13E and 13F), although these
tumors also showed increased number of apoptotic cells (FIGS. 13E and 13G). Without being bound to a particular theory, it is suggested that the elevated NF-κB activity and cell proliferation impaired the efficacy of bisphosphonate to reduce the size of tumors (FIG. 13B).

The results obtained in FIGS. 13B and 13E indicated that a combination therapy using a lipophilic bisphosphonate together with the autophagy inducer rapamycin might be more effective in treating KRAS induced lung adenocarcinomas. It was found that in the L2 cell syngeneic graft model, the combination of BPH-1222 with rapamycin was indeed much more effective than either single agent acting alone (FIG. 13H). Additionally, rapamycin also potently blocked phosphorylation of the two mTOR substrates (p70 S6 kinase and 4E-BP1), both of which are important for boosting metabolism in cancer cells (FIG. 13I).

Example 11

**Bisphosphonate and rapamycin combination therapy potently suppresses tumor growth in lung cancer models**

The BPH-1222 plus rapamycin combination was tested for treating lung adenocarcinoma in both the syngeneic orthotopic graft model and the lentiviral vector-mediated model, which represent the lung microenvironment. In the orthotopic model, tail vein injection of 2×10^5 M3L2 cells reproducibly generated massive lung tumors in FVB mice, resulting in a median survival of 33 days. For the treatment group, all mice (n=13) were given BPH-1222 and rapamycin alternately 16 days after the transplantation, for a total of 9 doses of each compound. BPH-1222 had good pharmacokinetic properties (FIG. 14C) and this treatment potently suppressed tumor growth, though mice survival was not significantly prolonged (37 days, n = 13, compared to 33.5 days, n = 8 in the control group, p= 0.06) due to internal bleeding in the thoracic cavity in some of the mice under treatment, even those with small tumor burdens (FIGS. 14A-C).

In the lentiviral vector-mediated model, lung tumors appeared “visible” in the luciferase imaging system at around two months after lentiviral vector infection. In the absence of any treatment, the tumors grew rapidly and all mice (n=8) died from full-blown lung cancer with a median survival of 55 days from when the tumors were first detected by imaging. A combination therapy of BPH-1222 and rapamycin was given to 12 mice around 3 months after lentiviral infection (counted as Day 1 in FIGS. 15D and IF), for a total of 16 doses of each compound. The tumor load of each mouse was monitored every 9-10 days throughout the whole treatment. The 12 mice were divided into two groups according to their tumor burden at the beginning of treatment, a high burden group (n=6, initial luciferase signal >10^5) and a low
burden group (n=6, initial luciferase signal 10^3-10^5). The combination treatment substantially delayed tumor development and prolonged mice survival in both the low burden group (median survival of 75.5 days, compared to 55 days without treatment, p=0.0009), as well as the high burden group (median survival of 54 days, compared to 28 days without treatment, p=0.02, FIG. 15F). Tumor regression was observed in most of the mice during treatment, although tumor size did increase after treatment ceased (FIGS. 15D, 15E and 17). Tumors from treated mice showed a large reduction in cell proliferation as indicated by Ki-67 staining, although apoptotic cells were rarely found (FIGS. 15G and 15H). Unlike results reported previously with an IKK2 inhibitor that suppressed tumor progression by reducing ERK signaling, there was no significant change of ERK phosphorylation after the bisphosphonate plus rapamycin treatment, in vivo (FIGS. 15G and 15H). Notably, BPH-1222 (either alone or combined with other agents) slightly increased ERK phosphorylation in cell culture conditions (FIG. 11C). A combination with an ERK inhibitor was tested to see if it might further improve treatment efficacy. However, ERK inhibitor U0126 increased KRAS protein, c-RAF, MEK and AKT phosphorylation levels under all conditions tested (FIG. 12D), perhaps due to the interruption of the ERK negative feedback loop (38).

Interestingly, zoledronate itself has been shown to potentiate the killing of osteosarcoma cells by RAD001 (a rapamycin analogue, 39) and, although less potent than BPH-1222, zoledronate still synergizes with rapamycin in vivo for killing KRAS tumors (FIGS. 14D and 14E). This indicates zoledronate plus rapamycin (or an analog) can be used for treating lung adenocarcinoma.

**Discussion**

*RAS* mutations (including *HRAS*, *KRAS* and *NRAS*) are commonly found in a variety of human cancers including lung, colon and pancreatic cancers, and in this study, the efficacy of treating lung adenocarcinomas carrying a *KRAS* mutation with a combination of a lipophilic bisphosphonate, an analog of zoledronate, with rapamycin were investigated. Bisphosphonates are a class of drugs widely used for treating osteoporosis and for preventing bone metastasis of certain cancers (40). Mechanistically, they tightly bind to bone mineral and inhibit FPPS in osteoclasts. This results in impaired protein prenylation and function, inducing cell death of osteoclasts. However, this strong binding to bone mineral (24), a desirable feature for a drug to treat bone resorption diseases, makes them less suited to treat solid tumors. Lipophilic bisphosphonates, on the other hand, do not bind to bone mineral and, in addition to inhibiting
FPPS, they also target GGPPS. Compared with the most potent commercial bisphosphonate drug, zoledronate, the lipophilic bisphosphonate BPH-1222 is more efficient in killing tumor cells with KRAS mutations both in vitro and in vivo. This enhanced efficacy is due to a combination of factors: good FPPS and GGPPS inhibition, as well as greatly enhanced lipophilicity (clogP of 0.25 for BPH-1222 versus -3.9 for zoledronate), and BPH-1222 has good pharmacokinetic properties (41, 42).

Targeting protein prenylation (including farnesylation and geranylgeranylation) has been pursued for more than 20 years, ever since researchers first found that RAS requires post-translational prenylation for its malignancy-transforming activity. FTIs and GGTIs were developed early on to kill tumor cells in vitro, however, little success has been achieved using these compounds in animals. More interestingly, responses to these inhibitors did not always correspond to RAS mutation status. This observation strongly indicated the existence of other targets (27). Herein, it is shown that KRAS prenylation and activity was largely inhibited by lipophilic bisphosphonate treatment and suggested that this was one of the major mechanisms of action of this class of compounds. However, other small G-proteins, such as RAL, RHO, RAC and CDC42, require exclusively geranylgeranylation, and all of these proteins have been shown to be involved in RAS induced transformation in a context-dependent manner (15, 43-45).

Indeed, results from this as well as other studies all supported the idea that suppression of protein geranylgeranylation was desirable for bisphosphonate mediated cytotoxicity. Based on this observation, dual-target (FPPS+GGPPS) inhibitors were thought be particularly potent in inhibiting tumor cell growth. They were also thought to be particularly toxic in vivo, so their use in combination therapies could be of interest. For examples, it has been reported that high dose treatment with dual prenyltransferase inhibitor (DPI, 1000-2000 mg/kg) led to substantial lethality (11). However, in the experiments described herein severe toxicity was not observed in animals even after treatments up to 6 weeks.

KRAS tumors cells have been shown to rely on autophagy for providing metabolic intermediates and clearing excess reactive oxygen species (ROS) (32, 33). In vitro, the additive effects of blocking protein prenylation and autophagy with chloroquine resulted in very potent tumor cell killing. However, in animals, the pro-proliferative effect induced by p62 accumulation and NF-κB activation became dominant and the combination was ineffective. In sharp contrast, the combination of a lipophilic bisphosphonate with rapamycin was far more effective because rapamycin not only facilitated autophagy, but also inhibited the mTOR pathway that is critical for the tumor cell survival. Thus, the combination of a lipophilic...
bisphosphonate plus rapamycin offers a promising therapeutic lead for treating KRAS-related lung cancers.

Example 12

Anti-PDAC effect of BPH-1222 and rapamycin combination

Pancreatic cancer, most commonly pancreatic ductal adenocarcinoma (PDAC), is the seventh cause of cancer deaths in 2012 globally and the forth in the United States. KRAS mutation is found in >90% of the PDAC patients so that anti-KRAS therapeutics is extremely attractive to this field.

To test the efficacy of a disclosed combination therapy against PDAC, a syngeneic transplantation model using mouse PDAC cell line p53.2.2.1 was used, which had KRASG12D mutation and loss of p53. About 10^6 tumor cells were transplanted subcutaneously into flank region of FVB mouse. Palpable tumors formed in 2 weeks and then combination therapy was given for additional 3 weeks. After that, mice were euthanized and tumors were isolated and weighed (FIG. 17).

Ctrl: control group receiving saline only.

Rapal222: mice were treated with BPH-1222 (1 mg/kg, i.p.) and rapamycin (2.5 mg/kg, i.p.) every other day, alternately, for total 3 weeks.

Evel222: mice were treated with BPH-1222 (1 mg/kg, i.p.) and everolimus (2.5 mg/kg, i.p.) every other day, alternately, for total 3 weeks.

The same combination therapy was also tested in orthotopic model. About 1000 PDAC p53.2.2.1 cells were orthotopically transplanted into pancreas tail of FVB mouse. Tumors were visualized on day 14 with IVIS imaging and mice were randomized into control and treatment groups. Treatment was given for 2 weeks (7 doses of BPH-1222, 1 mg/kg and 7 doses of rapamycin, 2.5 mg/kg). Mice were then euthanized and whole pancreases were isolated and weighed (FIG. 18).

References


In view of the many possible embodiments to which the principles of the disclosure may be applied, it should be recognized that the illustrated embodiments are only examples of the disclosure and should not be taken as limiting the scope of the invention. Rather, the scope of the invention is defined by the following claims. We therefore claim as our invention all that comes within the scope and spirit of these claims.
We claim:

1. A compound, having a formula

```
\[
\begin{align*}
\text{Ar} & \quad \text{X} & \quad \text{M}^i & \quad \text{q} & \quad \text{R}^{11} & \quad \text{R}^{12} & \quad \text{R}^i & \quad \text{P} & \quad \text{O}^\text{M} & \quad \text{O}^\text{M}_q \\
\text{O}^\text{P} & \quad \text{O}^\text{M}_q & \quad \text{O}^\text{M}_q & \quad \text{O}^\text{M}_q & \quad \text{O}^\text{M}_q \\
\end{align*}
\]
```

or a pharmaceutically acceptable salt or hydrate thereof, wherein

5. Ar is a 5-membered heteroaromatic ring;

X is hydrogen, aliphatic, hydroxyl, SH, halogen, or alkoxy;

each M is independently a negative charge, hydrogen, aliphatic, \(-(\text{CH}_2)_p\)-CO-R, -(\text{CH}_2)_p-CO-R, or a cation;

each q is independently 0 or 1;

10. R^{11} and R^{12} are independently H, aliphatic;

r is from 1 to 6;

p is 1 to 6; and

R is hydrogen, aliphatic, or aryl;

with the provisos the compound is not

15. (2-(1H-imidazol-1-yl)ethane-1,1-diyl)bis(phosphonic acid);

(1-hydroxy-2-(1H-imidazol-4-yl)ethane-1,1-diyl)bis(phosphonic acid);

sodium (1-hydroxy-2-(1-methyl-1H-imidazol-3-ium-3-yl)ethane-1,1-diyl)bis(hydrogen phosphonate);

sodium (1-hydroxy-2-(1H,2,3-triazol-1-yl)ethane-1,1-diyl)bis(phosphonic acid);

20. sodium (1-hydroxy-1-(hydroxyoxidophosphoryl)-2-(1-phenyl-1H-imidazol-3-ium-3-yl)ethyl)phosphonate;

sodium (1-(hydroxyoxidophosphoryl)-2-(1-phenyl-1H-imidazol-3-ium-3-yl)ethyl)phosphonate;

sodium (1-hydroxy-2-(thiazol-2-yl)ethane-1,1-diyl)bis(phosphonic acid); or

25. 3-(2,2-diphosphonoethyl)-1-dodecyl-1H-imidazol-3-ium.
2. The compound of claim 1, wherein the compound has a formula

\[
\text{Ar} \begin{array}{c}
\text{O} \\
\text{P}\text{O(M)q} \\
\text{X} \\
\text{O} \end{array} \text{O(M)q} \\
\text{O=PO(M)q} \\
\text{O(M)q}
\]

3. The compound of claim 1, wherein:

\[M \text{ is } \text{Li}^+, \text{Na}^+, \text{K}^+, \text{Ca}^{2+}, \text{Mg}^{2+}, \text{NH}_4^+ \text{ or } \text{N(R')}_4^+; \text{ and}
\]

each \text{R'} \text{ independently is hydrogen or alkyl.}

4. The compound of claim 1, wherein \( \text{Ar} \text{ is imidazole, pyrazole, pyrole, furan, thiophene, oxazole, isoxazole, thiazole, isothiazole, 1,2,3-triazole, 1,2,4-triazole, tetrazole, 1,2,3-oxadiazole, } 1,2,4-\text{oxadiazole, 1,2,5-oxadiazole, 1,3,4-oxadiazole, 1,2,3-thiadiazole, 1,2,4-thiadiazole, 1,2,5-thiadiazole or 1,3,4-thiadiazole.} \)

5. The compound of claim 1, wherein \( \text{Ar} \text{ is a 5-membered nitrogen-containing ring.} \)

6. The compound of claim 1, wherein \( \text{Ar} \text{ is imidazole.} \)

7. The compound of claim 1, wherein the compound has a formula selected from

\[
\begin{array}{c}
\text{N} \\
\text{O} \\
\text{P}\text{O(M)q} \\
\text{X} \\
\text{O} \\
\text{P}\text{O(M)q} \\
\text{O(M)q}
\end{array} \text{O(M)q} \\
\text{O(M)q} \\
\text{O(M)q} \\
\text{O(M)q} \\
\text{O(M)q} \\
\text{O(M)q} \\
\text{O(M)q}
\]

\[
\begin{array}{c}
\text{N} \\
\text{O} \\
\text{P}\text{O(M)q} \\
\text{X} \\
\text{O} \\
\text{P}\text{O(M)q} \\
\text{O(M)q}
\end{array} \text{O(M)q} \\
\text{O(M)q} \\
\text{O(M)q} \\
\text{O(M)q} \\
\text{O(M)q} \\
\text{O(M)q} \\
\text{O(M)q}
\]

\[
\begin{array}{c}
\text{N} \\
\text{O} \\
\text{P}\text{O(M)q} \\
\text{X} \\
\text{O} \\
\text{P}\text{O(M)q} \\
\text{O(M)q}
\end{array} \text{O(M)q} \\
\text{O(M)q} \\
\text{O(M)q} \\
\text{O(M)q} \\
\text{O(M)q} \\
\text{O(M)q} \\
\text{O(M)q}
\]

\[
\begin{array}{c}
\text{N} \\
\text{O} \\
\text{P}\text{O(M)q} \\
\text{X} \\
\text{O} \\
\text{P}\text{O(M)q} \\
\text{O(M)q}
\end{array} \text{O(M)q} \\
\text{O(M)q} \\
\text{O(M)q} \\
\text{O(M)q} \\
\text{O(M)q} \\
\text{O(M)q} \\
\text{O(M)q}
\]

\[
\begin{array}{c}
\text{N} \\
\text{O} \\
\text{P}\text{O(M)q} \\
\text{X} \\
\text{O} \\
\text{P}\text{O(M)q} \\
\text{O(M)q}
\end{array} \text{O(M)q} \\
\text{O(M)q} \\
\text{O(M)q} \\
\text{O(M)q} \\
\text{O(M)q} \\
\text{O(M)q} \\
\text{O(M)q}
\]

\[
\begin{array}{c}
\text{N} \\
\text{O} \\
\text{P}\text{O(M)q} \\
\text{X} \\
\text{O} \\
\text{P}\text{O(M)q} \\
\text{O(M)q}
\end{array} \text{O(M)q} \\
\text{O(M)q} \\
\text{O(M)q} \\
\text{O(M)q} \\
\text{O(M)q} \\
\text{O(M)q} \\
\text{O(M)q}
\]

\[
\begin{array}{c}
\text{N} \\
\text{O} \\
\text{P}\text{O(M)q} \\
\text{X} \\
\text{O} \\
\text{P}\text{O(M)q} \\
\text{O(M)q}
\end{array} \text{O(M)q} \\
\text{O(M)q} \\
\text{O(M)q} \\
\text{O(M)q} \\
\text{O(M)q} \\
\text{O(M)q} \\
\text{O(M)q}
\]

\[
\begin{array}{c}
\text{N} \\
\text{O} \\
\text{P}\text{O(M)q} \\
\text{X} \\
\text{O} \\
\text{P}\text{O(M)q} \\
\text{O(M)q}
\end{array} \text{O(M)q} \\
\text{O(M)q} \\
\text{O(M)q} \\
\text{O(M)q} \\
\text{O(M)q} \\
\text{O(M)q} \\
\text{O(M)q}
\]

\[
\begin{array}{c}
\text{N} \\
\text{O} \\
\text{P}\text{O(M)q} \\
\text{X} \\
\text{O} \\
\text{P}\text{O(M)q} \\
\text{O(M)q}
\end{array} \text{O(M)q} \\
\text{O(M)q} \\
\text{O(M)q} \\
\text{O(M)q} \\
\text{O(M)q} \\
\text{O(M)q} \\
\text{O(M)q}
\]

\[
\begin{array}{c}
\text{N} \\
\text{O} \\
\text{P}\text{O(M)q} \\
\text{X} \\
\text{O} \\
\text{P}\text{O(M)q} \\
\text{O(M)q}
\end{array} \text{O(M)q} \\
\text{O(M)q} \\
\text{O(M)q} \\
\text{O(M)q} \\
\text{O(M)q} \\
\text{O(M)q} \\
\text{O(M)q}
\]

R^4, R^6 and R^7 are each independently H, aliphatic, alkoxy, alkenoxy, haloalkyl, aryl, alkylaryl, arylalkyl, nitro, or halogen; and

R^5 and R^8 are each independently a lone pair of electrons, H, aliphatic, haloalkyl, aryl, alkylaryl or arylalkyl.
8. The compound of claim 1, wherein the compound has a formula \( XA_1 \) or salt or hydrate thereof, wherein:

- \( X \) is hydrogen, hydroxyl group, or a halogen;
- each \( M \), independently of other \( M \) in the compound, a hydrogen, aliphatic, \((-\text{Cl}^\text{R}_4)_p-\text{0-}\text{CO-R} \) or \(-\text{(CH2)}_p\text{-CO-R}, \) where \( p \) is 1 to 6, and \( R \) is hydrogen, aliphatic, or aryl, or \(-\text{OM} \) is a salt of form \(-0^{-}\text{A^+}, \) where \( \text{A}^+ \) is a cation; and
- \( Z \) is hydrogen, aliphatic, or aryl.

9. The compound of claim 8 wherein \( Z \) is an alkyl group having a structure

\[ X-(\text{CH}_2)_n^- \]

wherein \( n \) is an integer from 0 to 20 and \( X \) is hydrogen or halogen.

10. The compound of claim 9, wherein \( n \) is 8.

11. The compound of claim 7, wherein \( R^5, R^8 \) or both \( R^5 \) and \( R^8 \) have a formula

\[ (R^9)^p \]

\( R^9 \) is aliphatic, alkoxy, haloalkyl, NO\(_2\), OH, aryl, alkenyl, alkenyloxy, SO\(_2\)R, or

\[ \text{N}(R^{10})_2; \]

\( R^{10} \) is alkyl; and

\( p \) is from 0 to 5.

12. The compound of claim 1, wherein \( Ar \) is an imidazole and the compound has a molecular weight of at least 150 and less than 1,000, and that is capable of activating a \( \gamma\delta \) T cell.
13. The compound of claim 1, having a structure:
14. A composition comprising:

a compound having a formula

\[
\begin{array}{c}
\text{Ar} \\
\text{O} \text{P} \text{O(M)}_q \\
\text{O(M)}_q \\
\end{array}
\]

or a pharmaceutically acceptable salt or hydrate thereof, wherein

- Ar is a 5-membered heteroaromatic ring;
- X is hydrogen, aliphatic, hydroxyl, SH, halogen, or alkoxy;
- each M is independently hydrogen, aliphatic, alkyl, -(CH₂)ᵢ₋₀-CO-R, -(CH₂)ᵢ₋₀-CO-R, or a cation;
- each q is independently 0 or 1;
- R¹ and R² are independently H or aliphatic;
- r is 1 to 6;
- p is 1 to 6; and
- R is hydrogen, aliphatic, or aryl; and

a pharmaceutically acceptable carrier.

15. The composition of claim 14, wherein the compound has a formula

\[
\begin{array}{c}
\text{Ar} \\
\text{O} \text{P} \text{O(M)}_q \\
\text{O(M)}_q \\
\end{array}
\]

16. The composition of claim 14, wherein M is Li⁺, Na⁺, K⁺, Ca²⁺, Mg²⁺, NH₄⁺ or N(RV); and

each R' independently is hydrogen or alkyl.
17. The composition of claim 14, wherein the compound has a formula

\[
\begin{array}{c}
\text{Z} \quad \text{N} \\
\text{O} \quad \text{P} \quad \text{O} \quad \text{M}_{aq} \\
\text{O} \quad \text{P} \quad \text{O} \quad \text{M}_{aq} \\
\end{array}
\]

or salt or hydrate thereof, wherein:

- \( X \) is hydrogen, hydroxyl or halogen;
- each \( M \), independently of other \( M \) in the compound, is hydrogen, aliphatic, -(CH4)_p-0-CO-R or -(CH2)_p-CO-R, where \( p \) is 1 to 6, and \( R \) is hydrogen, aliphatic, or aryl, or \(-OM\) is a salt of form \(-0^+A^+\), where \( A^+ \) is a cation; and
- \( Z \) is hydrogen or aliphatic.

18. The composition of claim 14, wherein the compound is selected from

[Diagram of various chemical structures]
19. The composition of any one of claims 14-18, further comprising one or more additional therapeutic compounds.

20. The composition of claim 19, wherein the one or more additional therapeutic compounds comprise one or more of Abiraterone Acetate, Abitrexate (Methotrexate), Abraxane (Paclitaxel Albumin-stabilized Nanoparticle Formulation), ABVD, ABVE, ABVE-PC, AC, AC-T, Ad cetris (Brentuximab Vedotin), ADE, Adriamycin (Doxorubicin Hydrochloride), Adrucil (Fluorouracil), Afinitor (Everolimus), Aldara (Imiquimod), Aldesleukin, Alemtuzumab, Alimta (Pemetrexed Disodium), Alocixi (Palonosetron Hydrochloride), Ambochlorin (Chlorambucil), Amboclorin (Chlorambucil), Aminolevulinic Acid, Anastrozole, Aprepitant, Arimidex (Anastrozole), Armosin (Exemestane), Arranon (Nelarabine), Arsenic Trioxide, Arzerra (Ofatumumab), Asparaginase Erwinia chrysanthemi, Avastin (Bevacizumab), Axitinib, Azacitidine, BEACOPP, Bendamustine Hydrochloride, BEP, Bevacizumab, Bexarotene, Bexxar (Tositumomab and I 131 Iodine Tositumomab), Bleomycin, Bortezomib, Bosulif (Bosutinib), Bosutinib, Brentuximab, Vedotin, Cabazitaxel, Cabozantinib-S-Malate, CAF, Campath (Alemtuzumab), Camptosar (Irinotecan Hydrochloride), Capecitabine, CAPOX, Carboplatin, CARBOPLATIN-TAXOL, Carfilzomib, CeeNU (Lomustine), Cerubidine (Daunorubicin...
Hydrochloride), Cervarix (Recombinant HPV Bivalent Vaccine), Cetuximab, Chlorambucil, CHLORAMBUCIL-PREDNISONE, CHOP, Cisplatin, Clafen (Cyclophosphamide), Clofarabine, Clofarex (Clofarabine), Clolar (Clofarabine), CMF, Cometriq (Cabozantinib-S-Malate), COPP, Cosmegen (Dactinomycin), Crizotinib, CVP, Cyclophosphamide, Cyfos (Ifosfamide), Cytarabine, Cytarabine, Liposomal, Cytosar-U (Cytarabine), Cytoxan (Cyclophosphamide), Dacarbazine, Dacogen (Decitabine), Dactinomycin, Dasatinib, Daunorubicin Hydrochloride, Decitabine, Degarelix, Denileukin, Diftitox, Denosumab, DepoCyt (Liposomal Cytarabine), DepoFoam (Liposomal Cytarabine), Dexrazoxane Hydrochloride, Docetaxel, Doxil (Doxorubicin Hydrochloride Liposome), Doxorubicin Hydrochloride, Doxorubicin Hydrochloride Liposome, Dox-SL (Doxorubicin Hydrochloride Liposome), DTIC-Dome (Dacarbazine)Efudex (Fluorouracil), Elitek (Rasburicase), Ellence (Epirubicin Hydrochloride), Eloxdin (Oxaliplatin), Eltrombopag Olamine, Emend (Aprepitant), Enzalutamide, Epirubicin Hydrochloride, EPOCH, Erbitux (Cetuximab), Eribulin Mesylate, Erivedge (Vismodegib), Erlotinib Hydrochloride, Erwinaze (Asparaginase Erwinia chrysantheni), Etopophos (Etoposide Phosphate), Etoposide, Etoposide Phosphate, Evacet (Doxorubicin Hydrochloride Liposome), Everolimus, Evista (Raloxifene Hydrochloride), Exemestane, Fareston (Toremifene), Faslodex (Fulvestrant), FEC, Femara (Letrozole), Filgrastim, Fludara (Fludarabine Phosphate), Fludarabine Phosphate, Fluoroplex (Fluorouracil), Fluorouracil, Folex (Methotrexate), Folex PFS (Methotrexate), FOLFIRI, FOLFIRI-BEVACIZUMAB, FOLFIRINOX, FOLFOX, Folotyn (Pralatrexate), FU-LV, Fulvestrant, Gardasil (Recombinant HPV Quadrivalent Vaccine), Gefitinib, Gemcitabine Hydrochloride, GEMCrfABINE-CISPLATIN, Gemtuzumab Ozogamicin, Gemzar (Gemcitabine Hydrochloride), Gleevec (Imatinib Mesylate), Glucarpidase, Halaven (Eribulin Mesylate), Herceptin (Trastuzumab), HPV Bivalent Vaccine, Recombinant, HPV Quadrivalent Vaccine, Recombinant, Hycamtin (Topotecan Hydrochloride), Ibritumomab Tiuxetan, ICE, Ifex (Ihosfamide), Ifosfamide, Ifosfamidum (Ihosfamide), Imatinib Mesylate, Imiquimod, Inlyta (Axitinib), Ipilimumab, Iressa (Gefitinib), Irinotecan Hydrochloride, Istodax (Romidepsin),Ixabepilone, Ixempra (Ixabepilone), Jakafi (Ruxolitinib Phosphate), Jevtana (Cabazitaxel), Keoxifene (Raloxifene Hydrochloride), Kepivance (Palifermin), Kyprolis (Carfilzomib), Lapanitib Ditosylate, Lenalidomide, Letrozole, Leucovorin Calcium, Leukeran (Chlorambucil), Leuprolide Acetate, Levulan (Aminolevulinic Acid), Linfolizin (Chlorambucil), LipoDOX (Doxorubicin Hydrochloride Liposome), Liposomal Cytarabine, Lomustine, Lupron (Leuprolide Acetate), Lupron Depot (Leuprolide Acetate), Lupron Depot-Ped (Leuprolide Acetate), Lupron
Depot-3 Month (Leuprolide Acetate), Lupron Depot-4 Month (Leuprolide Acetate), Marqibo (Vincristine Sulfate Liposome), Matulane (Procarbazine Hydrochloride), Mechlorethamine Hydrochloride, Mesna, Mesnex (Mesna), Methazolastone (Temozolomide), Methotrexate, Methotrexate LPF (Methotrexate), Mexitate (Methotrexate), Mexitate-AQ (Methotrexate),

Mitomycin C, Mitozynex (Mitomycin C), MOPP, Mozobil (Plerixafor), Mustargen (Mechlorethamine Hydrochloride), Mutamycin (Mitomycin C), Mylosar (Azacitidine), Mylotarg (Gemtuzumab Ozogamicin), Nanoparticle Paclitaxel (Paclitaxel Albumin-stabilized Nanoparticle Formulation), Navelbine (Vinorelbine Tartrate), Neltarabine, Neosar (Cyclophosphamide), Neupogen (Filgrastim), Nexavar (Sorafenib Tosylate), Nilotinib, Nolvaex (Tamoxifen Citrate), Nplate (Romiplostim), Ofatumumab, Omacetaxine, Mepesuccinate, Oncaspar (Pegaspargase), Ontak (Denileukin Diftitox), Oxaliplatin, Paclitaxel, Paclitaxel Albumin-stabilized Nanoparticle Formulation, Palifermin, Palonosetron Hydrochloride, Panitumumab, Paraplatin (Carboplatin), Paraplatin (Carboplatin), Pazopanib Hydrochloride, Pegaspargase, Pemetrexed Disodium, Perjeta (Pertuzumab), Pertuzumab, Platinol (Cisplatin), Platinol-AQ (Cisplatin), Plerixafor, Pralatexate, Prednisone, Procarbazine Hydrochloride, Proleukin (Aldesleukin), Prolia (Denosumab), Promacta (El trombopag Olamine), Provenge (Sipuleucel-T), Raloxifene Hydrochloride, Rasburicase, R-CHOP, R-CVP, Recombinant HPV Bivalent Vaccine, Recombinant HPV Quadrivalent Vaccine, Rapamycin, Regorafenib, Revlimid (Lenalidomide), Rheumatrex (Methotrexate), Rituxan (Rituximab), Rituximab, Romidepsin, Romiplostim, Rubidomycin (Daunorubicin Hydrochloride), Ruxolitinib Phosphate, Sclerosol Intrapleural Aerosol (Talc), Sipuleucel-T, Sorafenib Tosylate, Sprycel (Dasatinib), STANFORD V Sterile Talc Powder (Talc), Steritalc (Talc), Stivarga (Regorafenib), Sunitinib Malate, Sutent (Sunitinib Malate), Synovir (Thalidomide), Synribo (Omacetaxine Mepesuccinate), Tamoxifen Citrate, Tarabine, PFS (Cytarabine), Tarceva (Erlotinib Hydrochloride), Targetetin (Bexarotene), Tasigna (Nilotinib), Taxol (Paclitaxel), Taxotere (Docetaxel), Temodar (Temozolomide), Temozolomide, Temsirolimus, Thalidomide, Thalomid (Thalidomide), Toposar (Etoposide), Topotecan Hydrochloride, Toremifene, Torisel (Temsirolimus), Tositumomab and I 131 Iodine Tositumomab, Totect (Dexrazoxane Hydrochloride), Trastuzumab, Treanda (Bendamustine Hydrochloride), Trisenox (Arsenic Trioxide), Tykerb (Lapatinib Ditosylate), Vandetanib, VAMP, Vectibix (Panitumumab), VelP, Velban (Vinblastine Sulfate), Velcade (Bortezomib), Velsar (Vinblastine Sulfate), Vemurafenib, VePesid (Etoposide), Viadur (Leuprolide Acetate), Vidaza (Azacitidine), Vinblastine Sulfate, Vincasar PFS (Vincristine Sulfate), Vincristine Sulfate, Vincristine Sulfate Liposome,
Vinorelbine Tartrate, Vismodegib, Voraxaze (Glucarpidase), Vorinostat, Votrient (Pazopanib Hydrochloride), Wellcovorin (Leucovorin Calcium), Xalkori (Crizotinib), Xeloda (Capecitabine), XELOX, Xgeva (Denosumab), Xtandi (Enzalutamide), Yervoy (Ipilimumab), Zaltrap (Ziv-Aflibercept), Zelboraf (Vemurafenib), Zevalin (Ibritumomab Tiuxetan), Zinecard (Dexrazoxane Hydrochloride), Ziv-Aflibercept, Zoledronic Acid, Zolinza (Vorinostat), Zometa (Zoledronic Acid), Zytiga (Abiraterone Acetate) or an autophagy inducer.

21. The composition of claim 20, wherein the one or more additional therapeutic compounds comprise rapamycin.

22. A method of treating a disease, comprising administering to a subject a composition of any one of claims 14-21, or a compound having a formula

\[
\begin{align*}
&\text{Ar is a 5-membered heteroaromatic ring;} \\
&\text{X is hydrogen, aliphatic, hydroxyl, SH, halogen, or alkoxy;} \\
&\text{each M is independently hydrogen, aliphatic, alkyl, -(CH2)_p-CO-R, -(CH2)_p-CO-R, or a cation;} \\
&\text{each q is independently 0 or 1;} \\
&\text{R}^{11} \text{ and R}^{12} \text{ are independently H or aliphatic;} \\
&\text{r is 1 to 6;} \\
&\text{p is 1 to 6; and} \\
&\text{R is hydrogen, aliphatic or aryl;} \\
&\text{and a pharmaceutically acceptable carrier.}
\end{align*}
\]

23. The method of claim 22, wherein the compound has a formula

\[
\begin{align*}
&\text{Ar is a 5-membered heteroaromatic ring;} \\
&\text{X is hydrogen, aliphatic, hydroxyl, SH, halogen, or alkoxy;} \\
&\text{each M is independently hydrogen, aliphatic, alkyl, -(CH2)_p-CO-R, -(CH2)_p-CO-R, or a cation;} \\
&\text{each q is independently 0 or 1;} \\
&\text{R}^{11} \text{ and R}^{12} \text{ are independently H or aliphatic;} \\
&\text{r is 1 to 6;} \\
&\text{p is 1 to 6; and} \\
&\text{R is hydrogen, aliphatic or aryl;} \\
&\text{and a pharmaceutically acceptable carrier.}
\end{align*}
\]
24. The composition of claim 22, wherein M is Li⁺, Na⁺, K⁺, Ca²⁺, Mg²⁺, NH₄⁺ or N(R')₄⁺; and
   each R' independently is hydrogen or alkyl.

25. The method of claim 22, wherein the compound has a formula

   \[ Z \rightleftharpoons \text{structure image} \]

   or salt or hydrate thereof, wherein:
   - X is hydrogen, hydroxyl or halogen;
   - each M, independently of other M in the compound, is hydrogen, aliphatic, -(C\(^{3-4}\))\(_p\)-O-CO-R or -(CH\(_2\))\(_p\)-CO-R, where p is 1 to 6, and R is hydrogen, aliphatic, or aryl, or -OM is a salt of form -O·A⁺, where A⁺ is a cation; and
   - Z is hydrogen or aliphatic.

26. The method of claim 22, wherein the compound is selected from

   \[ \text{structure images} \]
27. The method of any one of claims 22-26, wherein the disease is a cancer.

28. The method of any one of claims 22-26, wherein treating a disease comprises reducing growth of a cancer cell.

29. The method of claim 28, wherein reducing growth of a cancer cell comprises contacting the cancer cell with a therapeutically effective amount of the compound or composition.

30. The method of claim 27, wherein the cancer is a bladder cancer, lung cancer, breast cancer, melanoma cancer, colon cancer, rectal cancer, non-Hodgkin lymphoma cancer, endometrial cancer, pancreatic cancer, renal cancer, prostate cancer, leukemia cancer, and/or thyroid cancer.
31. The method of claim 30, wherein the cancer is lung cancer.

32. The method of any one of claims 22-26, wherein treating a disease comprises inhibiting growth of a tumor, comprising contacting the tumor with a therapeutically effective amount of the compound or composition.

33. The method of any one of claims 22-26, wherein treating a disease comprises inhibiting growth of a tumor or cancer expressing a KRAS mutation.

34. The method of any one of claims 22-26, further comprising administering to the subject a therapeutically effective amount of an autophagy inducer.

35. The method of claim 34, wherein the autophagy inducer is rapamycin.
FIG. 1C
FIG. 2C

FIG. 3

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FIG. 4A

FIG. 4B
FIG. 4C

TPN-α

IC₅₀ (µM)

0 3 6 9 12 15
Sidechain Length (n)

+OH
-OH

FIG. 4D

PvGGPPS Inhibition

IC₅₀ (µM)

0 3 6 9 12 15
Sidechain Length (n)

+OH
-OH

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FIG. 4E
FIG. 8C

FIG. 8D
FIG. 9F
FIG. 10C

FIG. 10D
FIG. 10E
Mouse KRAS G12D sequence

2A ribosomal skip

PGAPmdykdddddkMTEYKLVVVGADGVGKSALTIIQLIQNH
FVDYDPITREDYRQKVVIDGETCLLDILTDATGQEEYSAMR
DQYMRTGEGFLCVFAINTKSFEDIHHYREQIKRVKDES
VPMVLVGKCDLPSRTVDTKQAQELARLYGIPFIETSAKTR
QGVDDAFYTTLVREIRKHKEKMSKDGGKKKKKKSRTRCTVM

FIG. 11A

BPH-1222

DMSO Simva FTI GGTI FOH GGOH Ascorbic Trolox Morin Lipolic

BiP

CHOP

PERK-p

KRAS

Tubulin

FIG. 11B
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<td></td>
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<td>22989.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>22861.8</td>
<td>22849.9</td>
<td>undetected</td>
</tr>
<tr>
<td>F</td>
<td></td>
<td>(Δ=-11.9)</td>
<td></td>
</tr>
<tr>
<td>C—CH₃</td>
<td>22875.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
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<tr>
<td>C—CH₃</td>
<td>22943.9</td>
<td></td>
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</tr>
<tr>
<td>GG</td>
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**Alternative ATG**

|             | 22666.7    |       |           |        |
| CTVM        |            |       |           |        |

| C          | 22539.4    |       | 22528.2  |
| F          |            |       | (Δ=-11.2) |

| C—CH₃      | 22553.4    | 22541.2 | 22541.0  |
| F          |            | (Δ=-12.2) | (Δ=-12.4) |

| C—CH₃      | 22621.5    |       |           |        |
| GG         |            |       |           |        |

**Immunoblotting (anti-Flag)**

|             |           | | |
| Immunoblotting (anti-Flag) | | |

**FIG. 12**
FIG. 13C

FIG. 13D
FIG. 13I

FIG. 14A
**FIG. 14B**

<table>
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<tr>
<th>Parameters</th>
<th>Mean ± SD</th>
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<tr>
<td>AUC$_{0-\infty}$ (ng·min/ml)</td>
<td>835500 ± 200500</td>
</tr>
<tr>
<td>C$_{\text{max}}$(ng/ml)</td>
<td>889 ± 176</td>
</tr>
<tr>
<td>$t_{1/2}$ (min)</td>
<td>838 ± 365</td>
</tr>
<tr>
<td>Vz (ml/g)</td>
<td>7.40 ± 3.50</td>
</tr>
<tr>
<td>C$_{\text{max}}$ (ng/ml)</td>
<td>890 ± 175</td>
</tr>
</tbody>
</table>

**FIG. 14C**
FIG. 15G

FIG. 15H
FIG. 16A

FIG. 16B

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** Diagram showing the effect of DMSO and Treat on orthotopic pancreas weight.

- **Pancreas weight (g)**
- **X-axis:** DMSO and Treat
- **Y-axis:** Pancreas weight from 0 to 1000 g
- **Statistical significance:** ***, p=0.0068**

**FIG. 18**
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

C07F 9/38 (2006.01) A61K 31/675 (2006.01) A61P 35/00 (2006.01)

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

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

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

STN: Chemical Abstract Registry and CAlplus. Substructure search based on compounds of claimed formula.

PatentScope and Espacenet: Inventor and Applicant Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category*</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
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<td>X Further documents are listed in the continuation of Box C X</td>
<td>See patent family annex</td>
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</table>

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier application or patent but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed
- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- "&" document member of the same patent family

Date of the actual completion of the international search: 12 January 2016

Date of mailing of the international search report: 12 January 2016

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Form PCT/ISA/210 (fifth sheet) (July 2009)
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<td>WO 1994/020508 A1 (EISAI CO., LTD.) 15 September 1994 see Ex. No. 228 page 201; page 13 para (E) and page 55 last para; page 13-14</td>
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