Title: PRODRUGS OF AZACITIDINE 5'-PHOSPHATE

Abstract: The present disclosure relates to analogs or derivatives of azacitidine with improved pharmaceutical properties and methods for the treatment of proliferative disease utilizing said analogs or derivatives.

FIGURE 1
— as to applicant’s entitlement to apply for and be granted a patent (Rule 4.17(H))
— as to the applicant’s entitlement to claim the priority of the earlier application (Rule 4.17(Hi))
PRODRUGS OF AZACITIDINE 5'-PHOSPHATE

CROSS REFERENCE

[0001] This application claims the benefit of U.S. Provisional Application No. 61/351,789, filed June 4, 2010, which is incorporated herein by reference in its entirety.

BACKGROUND OF THE INVENTION

[0002] The present disclosure relates to analogs or derivatives of azacitidine with improved pharmaceutical properties.

SUMMARY OF THE INVENTION

[0003] Provided herein are prodrugs of azacitidine 5'-phosphate.

[0004] One embodiment provides a compound of Formula I, or pharmaceutically acceptable salt, solvate or polymorph thereof,

\[
\text{Formula I}
\]

wherein \( R \) is H or \( \text{C}_0 \text{C}_{(1-6) \text{ alkyl}} \); and

\( R' \) is H or \( \text{C}_0 \text{C}_{(1-6) \text{ alkyl}} \).

[0005] Another embodiment provides the compound of Formula I wherein \( R \) is H and \( R' \) is H. Another embodiment provides the compound of Formula I wherein \( R \) is \( \text{C}_0 \text{C}_{(1-6) \text{ alkyl}} \) and \( R' \) is H. Another embodiment provides the compound of Formula I wherein \( R \) is H and \( R' \) is \( \text{C}_0 \text{C}_{(1-6) \text{ alkyl}} \). Another embodiment provides the compound of Formula I wherein \( R \) is \( \text{C}_0 \text{C}_{(1-6) \text{ alkyl}} \) and \( R' \) is \( \text{C}_0 \text{C}_{(1-6) \text{ alkyl}} \).

[0006] One embodiment provides a compound having the Formula la:

\[
\text{Formula la}
\]

[0007] One embodiment provides a compound having the Formula lb:
Provided herein are pharmaceutical compositions comprising a compound of Formula I or a pharmaceutically acceptable salt, solvate or polymorph thereof, and a pharmaceutically acceptable excipient, wherein the compound of Formula I has the structure:

![Formula I](image)

wherein \( R \) is H or C0\(_2\)(C1-C6 alkyl); and \( R^1 \) is H or C0\(_2\)(C1-C6 alkyl).

Another embodiment provides the pharmaceutical composition comprising a compound of Formula Ia and a pharmaceutically acceptable excipient, wherein the compound of Formula Ia has the structure:

![Formula Ia](image)

Another embodiment provides the pharmaceutical composition comprising a compound of Formula Ib and a pharmaceutically acceptable excipient, wherein the compound of Formula Ib has the structure:
One embodiment provides a method for the treatment of proliferative disease in a patient comprising administration of a composition comprising a compound of Formula I, or pharmaceutically acceptable salt, solvate or polymorph thereof,

\[
\text{Formula I}
\]

wherein \( R \) is \( \text{H} \) or \( \text{C}_2(\text{C}_1-\text{C}_6 \text{ alkyl}) \); and
\( R^1 \) is \( \text{H} \) or \( \text{C}_2(\text{C}_1-\text{C}_6 \text{ alkyl}) \).

Another embodiment provides a method for the treatment of proliferative disease wherein the proliferative disease is myelodysplastic syndrome.

Another embodiment provides a method for the treatment of proliferative disease wherein the proliferative disease is selected from colon cancer, lung cancer, pancreatic cancer, gastric cancer, prostate cancer, and hepatocellular carcinoma.

**INCORPORATION BY REFERENCE**

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

**BRIEF DESCRIPTION OF THE DRAWINGS**

The features of the invention are set forth with particularity in the appended claims. A better understanding of the features of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings of which:

Figure 1 shows the LC trace for the purification of Example 1;

Figure 2 shows the MS analysis of Example 1;
Figure 3 shows the 400 MHz $^1$H NMR spectrum of Example 1;
Figure 4 shows the expanded 400 MHz $^1$H NMR spectrum of Example 1;
Figure 5 shows the D$_2$O exchange 400 MHz $^1$H NMR spectrum of Example 1; and
Figure 6 shows the effect of Example 1 on the gene expression in Calu-6 cell lines as described in Example 6.

**DETAILED DESCRIPTION OF THE INVENTION**

While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

**Azacitidine**

Azacitidine (chemical name: 4-amino-1-beta-D-ribofuranosyl1,3,5-triazin-2(1H)-one, also known as 5-azacytidine, azacytidine, ladakamycin or 5-AC; PubChem CID 9444) is a pyrrolidine nucleoside analogue of cytidine with antineoplastic activity. Azacitidine is incorporated into DNA, where it reversibly inhibits DNA methyltransferase, thereby blocking DNA methylation. Hypomethylation of DNA by azacitidine may activate tumor suppressor genes silenced by hypermethylation, resulting in an antitumor effect. This agent is also incorporated into RNA, thereby disrupting normal RNA function and impairing tRNA cytosine-5-methyltransferase activity.

![Azacitidine structure](image)

An FDA-approved form of azacitidine, marketed as Vidaza, is indicated for treatment of patients with the following French-American-British (FAB) myelodysplastic syndrome subtypes: refractory anemia (RA) or refractory anemia with ringed sideroblasts (if accompanied by neutropenia or thrombocytopenia or requiring transfusions), refractory anemia with excess blasts (RAEB), refractory anemia with excess blasts in transformation (RAEB-T), and chronic myelomonocytic leukemia (CMMoL). The recommended starting dose for the first treatment cycle, for all patients regardless of baseline hematology laboratory values, is 75 mg/m$^2$ subcutaneously or intravenously, daily for 7 days. Patients should be premedicated for nausea and vomiting.

Prodrugs are generally drug precursors that, following administration to an individual and subsequent absorption, are converted to an active, or a more active species via some process, such as conversion by a metabolic pathway. Some prodrugs have a chemical group present on the prodrug that renders it less active and/or confers solubility or some other property to the drug. Once the chemical group has been cleaved and/or
modified from the prodrug the active drug is generated. Prodrugs are often useful because, in some situations, they are easier to administer than the parent drug. They are, for instance, bioavailable by oral administration whereas the parent is not. In certain instances, the prodrug also has improved solubility in pharmaceutical compositions over the parent drug. An example, without limitation, of a prodrug would be a compound as described herein which is administered orally, subsequently subjected to a biotransformation in vivo and thus provides a therapeutically effective concentration of an active agent. For further general examples, see: Bundgaard, "Design and Application of Prodrugs" in A Textbook of Drug Design and Development, Krosgaard-Larsen and Bundgaard, Ed., 1991, Chapter 5, 113-191, which is incorporated herein by reference.

[0026] Azacitidine, once it is in the body, must first be activated by conversion into the 5'-monophosphate and then into the 5'-diphosphate and finally into the 5'-triphosphate, which is then a substrate for the DNA replication machinery. Azacitidine 5'-triphosphate incorporated into DNA is the active DNA methylation inhibitor form of the drug. The first rate limiting step in this cascade is the ATP-dependent phosphorylation of azacitidine to the monophosphorylated nucleotide (see Stressmann, C ; Lyko, F. "Modes of action of the DNA methyltransferase inhibitors azacitidine and decitabine" Int. J. Cancer 2008, 123, 8-13). Owing to the highly polar, charged nature of phosphorylated nucleosides under physiological conditions (phosphate monoester pKa values are about 1.6 and about 6.6), oral bioavailability tends to be poor (see Meier, C. cyc/oSal Phosphates as Chemical Trojan Horses for Intracellular Nucleotide and Glycosylmonophosphate Delivery-Chemistry Meets Biology, Eur. J. Org. Chem. 2006, 1081-1102).

[0027] Provided herein are prodrugs of azacitidine 5'-phosphate.

[0028] One embodiment provides a compound of Formula I, or pharmaceutically acceptable salt, solvate or polymorph thereof,

![Formula I](image)

wherein \( R \) is H or C0 _2_(C1-C6 alkyl); and

\( R^1 \) is H or C0 _2_(C1-C6 alkyl).

[0029] Another embodiment provides the compound of Formula I wherein \( R \) is H and \( R^1 \) is H. Another embodiment provides the compound of Formula I wherein \( R \) is C0 _2_(C1-C6 alkyl) and \( R^1 \) is H. Another embodiment provides the compound of Formula I wherein \( R \) is H and \( R^1 \) is C0 _2_(C1-C6 alkyl). Another embodiment provides the compound of Formula I wherein \( R \) is C0 _2_(C1-C6 alkyl) and \( R^1 \) is C0 _2_(C1-C6 alkyl).

[0030] One embodiment provides a compound having the Formula Ia:
One embodiment provides a compound having the Formula lb:

![Formula 1b](image1)

**Certain Pharmaceutical Terminology**

The term "patient", "subject" or "individual" are used interchangeably. As used herein, they refer to individuals suffering from a disorder, and the like, encompasses mammals and non-mammals. None of the terms require that the individual be under the care and/or supervision of a medical professional. Mammals are any member of the Mammalian class, including but not limited to humans, non-human primates such as chimpanzees, and other apes and monkey species; farm animals such as cattle, horses, sheep, goats, swine; domestic animals such as rabbits, dogs, and cats; laboratory animals including rodents, such as rats, mice and guinea pigs, and the like. Examples of non-mammals include, but are not limited to, birds, fish and the like. In some embodiments of the methods and compositions provided herein, the individual is a mammal. In preferred embodiments, the individual is a human.

The terms "treat," "treating" or "treatment," and other grammatical equivalents as used herein, include alleviating, abating or ameliorating a disease or condition or one or more symptoms thereof, preventing additional symptoms, ameliorating or preventing the underlying metabolic causes of symptoms, inhibiting the disease or condition, e.g., arresting the development of the disease or condition, relieving the disease or condition, causing regression of the disease or condition, relieving a condition caused by the disease or condition, or stopping the symptoms of the disease or condition, and are intended to include prophylaxis. The terms further include achieving a therapeutic benefit and/or a prophylactic benefit. By therapeutic benefit is meant eradication or amelioration of the underlying disorder being treated. Also, a therapeutic benefit is achieved with the eradication or amelioration of one or more of the physiological symptoms associated with the underlying disorder such that an improvement is observed in the individual, notwithstanding that the individual is still be afflicted with the underlying disorder. For prophylactic benefit, the compositions are administered to
an individual at risk of developing a particular disease, or to an individual reporting one or more of the physiological symptoms of a disease, even though a diagnosis of this disease has not been made.

[0034] The terms "administer," "administering", "administration," and the like, as used herein, refer to the methods that may be used to enable delivery of compounds or compositions to the desired site of biological action. These methods include, but are not limited to oral routes, intraduodenal routes, parenteral injection (including intravenous, subcutaneous, intraperitoneal, intramuscular, intravascular or infusion), topical and rectal administration. Those of skill in the art are familiar with administration techniques that can be employed with the compounds and methods described herein. In preferred embodiments, the compounds and compositions described herein are administered orally.

[0035] The terms "effective amount", "therapeutically effective amount" or "pharmaceutically effective amount" as used herein, refer to a sufficient amount of at least one agent or compound being administered which will relieve to some extent one or more of the symptoms of the disease or condition being treated. The result can be reduction and/or alleviation of the signs, symptoms, or causes of a disease, or any other desired alteration of a biological system. For example, an "effective amount" for therapeutic uses is the amount of the composition comprising a compound as disclosed herein required to provide a clinically significant decrease in a disease. An appropriate "effective" amount may differ from one individual to another. An appropriate "effective" amount in any individual case may be determined using techniques, such as a dose escalation study.

[0036] The term "acceptable" as used herein, with respect to a formulation, composition or ingredient, means having no persistent detrimental effect on the general health of the individual being treated.

[0037] The term "pharmaceutically acceptable" as used herein, refers to a material, such as a carrier or diluent, which does not abrogate the biological activity or properties of the compounds described herein, and is relatively nontoxic, i.e., the material may be administered to an individual without causing undesirable biological effects or interacting in a deleterious manner with any of the components of the composition in which it is contained.

[0038] The term "prodrug" as used herein, refers to a drug precursor that, following administration to an individual and subsequent absorption, is converted to an active, or a more active species via some process, such as conversion by a metabolic pathway. Thus, the term encompasses any derivative of a drug compound, which, upon administration to a recipient, is capable of providing, either directly or indirectly, said drug compound. Some prodrugs have a chemical group present on the prodrug that renders it less active and/or confers solubility or some other property to the drug. Once the chemical group has been cleaved and/or modified from the prodrug the active drug compound is generated. Prodrugs are often useful because, in some situations, they may be easier to administer than the parent drug. They may, for instance, be bioavailable by oral administration whereas the parent is not. Particularly favored derivatives or prodrugs are those that increase the bioavailability of the parent compounds when such prodrugs are administered to an individual (e.g., by allowing an orally administered prodrug to be more readily absorbed into the blood than the parent compound) or which enhance delivery of the parent compound to a biological compartment (e.g., the brain or lymphatic system).

[0039] The term "pharmaceutically acceptable salt" as used herein, refers to salts that retain the biological effectiveness of the free acids and bases of the specified compound and that are not biologically or otherwise undesirable. Compounds described herein may possess acidic or basic groups and therefore may react with any
of a number of inorganic or organic bases, and inorganic and organic acids, to form a pharmaceutically acceptable salt. These salts can be prepared in situ during the final isolation and purification of the compounds of the invention, or by separately reacting a purified compound in its free base form with a suitable organic or inorganic acid, and isolating the salt thus formed.

The term "pharmaceutical composition," as used herein, refers to a active pharmaceutical ingredient, optionally mixed with at least one pharmaceutically acceptable chemical component, such as, though not limited to carriers, stabilizers, diluents, dispersing agents, suspending agents, thickening agents, excipients and the like.

The term "carrier" as used herein, refers to relatively nontoxic chemical compounds or agents that facilitate the incorporation of a compound into cells or tissues.

The terms "pharmaceutical combination", "administering an additional therapy", "administering an additional therapeutic agent" and the like, as used herein, refer to a pharmaceutical therapy resulting from the mixing or combining of more than one active ingredient or prodrug and includes both fixed and non-fixed combinations of the active ingredients or prodrugs. The term "fixed combination" means that at least one of the compounds described herein, and at least one co-agent, are both administered to an individual simultaneously in the form of a single entity or dosage. The term "non-fixed combination" means that at least one of the compounds described herein, and at least one co-agent, are administered to an individual as separate entities either simultaneously, concurrently or sequentially with variable intervening time limits, wherein such administration provides effective levels of the two or more compounds in the body of the individual. These also apply to cocktail therapies, e.g. the administration of three or more active ingredients.

The terms "co-administration", "administered in combination with" and their grammatical equivalents or the like, as used herein, are meant to encompass administration of the selected therapeutic agents to a single individual, and are intended to include treatment regimens in which the agents are administered by the same or different route of administration or at the same or different times. In some embodiments the compounds described herein will be co-administered with other agents. These terms encompass administration of two or more agents to an animal so that both agents and/or their metabolites are present in the animal at the same time. They include simultaneous administration in separate compositions, administration at different times in separate compositions, and/or administration in a composition in which both agents are present. Thus, in some embodiments, the compounds of the invention and the other agent(s) are administered in a single composition. In some embodiments, compounds of the invention and the other agent(s) are admixed in the composition.

The term "metabolite," as used herein, refers to a derivative of a compound which is formed when the compound is metabolized.

The term "active metabolite," as used herein, refers to a biologically active derivative of a compound that is formed when the compound is metabolized.

The term "metabolized," as used herein, refers to the sum of the processes (including, but not limited to, hydrolysis reactions and reactions catalyzed by enzymes) by which a particular substance is changed by an organism. Thus, enzymes may produce specific structural alterations to a compound. For example, cytochrome P450 catalyzes a variety of oxidative and reductive reactions while uridine diphosphate glucuronyltransferases catalyze the transfer of an activated glucuronic-acid molecule to aromatic alcohols, aliphatic alcohols,

[0047] The term "alkyl" refers to a straight or branched hydrocarbon chain radical consisting solely of carbon and hydrogen atoms, containing no unsaturation, having from one to six carbon atoms (e.g., C₁₋₃ alkyl). In certain embodiments, an alkyl may comprise one to five carbon atoms (e.g., C₁₋₅ alkyl). In certain embodiments, an alkyl may comprise one to four carbon atoms (e.g., C₁₋₄ alkyl). In other embodiments, an alkyl may comprise one to three carbon atoms (e.g., C₁₋₃ alkyl). The alkyl is attached to the rest of the molecule by a single bond, for example, methyl (Me), ethyl (Et), -propyl, 1-methylethyl (iso-propyl), -butyl, -pentyl, 1,1-dimethylethyl (i-butyl), and the like. Unless stated otherwise specifically in the specification, an alkyl group may be optionally substituted by one or more of the following substituents: halo, cyano, nitro, thioxo, trimethylsilyl, -OR², -OC(0)-R², -N(R³)₂, -N(R³)₂, -C(0)OR², -C(0)N(R⁴)², -N(R³)C(0)OR², -N(R³)S(0)ₜ₋₇, S(0)ₜ₋₇, S(0)ₜ₋₇, S(0)ₜ₋₇ (where t is 1 or 2), N(0)ₜ₋₇, S(0)ₜ₋₇, S(0)ₜ₋₇, S(0)ₜ₋₇, S(0)ₜ₋₇ (where t is 1 or 2) where each R² is independently hydrogen, alkyl, fluorooalkyl, carbocyclic, carbocyclicalkyl, aryl, aralkyl, heterocyclyl, heterocyclylalkyl, heteroaryl or heteroaryalkyl.

[0048] In some embodiments, the compounds described herein exist as stereoisomers. For embodiments wherein the phosphorous atom is chiral, all possible stereoisomers are contemplated.

[0049] In some embodiments, the compounds described herein exist as solvates. The invention provides for methods of treating diseases by administering such solvates. The invention further provides for methods of treating diseases by administering such solvates as pharmaceutical compositions.

[0050] Solvates contain either stoichiometric or non-stoichiometric amounts of a solvent, and, in some embodiments, are formed during the process of crystallization with pharmaceutically acceptable solvents such as water, ethanol, and the like. Hydrates are formed when the solvent is water, or alcoholates are formed when the solvent is alcohol. Solvates of the compounds described herein can be conveniently prepared or formed during the processes described herein. By way of example only, hydrates of the compounds described herein can be conveniently prepared by recrystallization from an aqueous/organic solvent mixture, using organic solvents including, but not limited to, dioxane, tetrahydrofuran or methanol. In addition, the compounds provided herein can exist in unsolvated as well as solvated forms. In general, the solvated forms are considered equivalent to the unsolvated forms for the purposes of the compounds and methods provided herein.

[0051] In some embodiments, the compounds described herein exist as polymorphs. The invention provides for methods of treating diseases by administering such polymorphs. The invention further provides for methods of treating diseases by administering such polymorphs as pharmaceutical compositions.

[0052] Thus, the compounds described herein include all their crystalline forms, known as polymorphs. Polymorphs include the different crystal packing arrangements of the same elemental composition of a compound. In certain instances, polymorphs have different X-ray diffraction patterns, infrared spectra, melting points, density, hardness, crystal shape, optical and electrical properties, stability, and solubility. In certain instances, various factors such as the recrystallization solvent, rate of crystallization, and storage temperature cause a single crystal form to dominate.

**Pharmaceutical Compositions**
Provided herein are pharmaceutical compositions comprising a compound of Formula I or a pharmaceutically acceptable salt, solvate or polymorph thereof, and a pharmaceutically acceptable excipient, wherein the compound of Formula I has the structure:

![Formula I](attachment:formula1.png)

wherein $R$ is H or C0$_2$(C1-C6 alkyl); and $R^1$ is H or C0$_2$(C1-C6 alkyl).

Another embodiment provides the pharmaceutical composition comprising a compound of Formula Ia and a pharmaceutically acceptable excipient, wherein the compound of Formula Ia has the structure:

![Formula Ia](attachment:formula2.png)

Another embodiment provides the pharmaceutical composition comprising a compound of Formula Ib and a pharmaceutically acceptable excipient, wherein the compound of Formula Ib has the structure:

![Formula Ib](attachment:formula3.png)

In various embodiments, the pharmaceutical composition comprises a pharmaceutically acceptable vehicle, carrier, diluent, or excipient, or a mixture thereof.

Provided herein are pharmaceutical compositions in film-coated dosage forms, which comprise a combination of an active ingredient, and one or more tabletting excipients to form a tablet core using conventional tabletting processes and subsequently coating the core. The tablet cores can be produced using
conventional granulation methods, for example wet or dry granulation, with optional comminution of the granules and with subsequent compression and coating.

The pharmaceutical compositions provided herein may be provided in unit-dosage forms or multiple-dosage forms. Unit-dosage forms, as used herein, refer to physically discrete units suitable for administration to human and animal subjects and packaged individually as is known in the art. Each unit-dose contains a predetermined quantity of the active ingredient sufficient to produce the desired therapeutic effect, in association with the required pharmaceutical carriers or excipients. Examples of unit-dosage forms include ampules, syringes, and individually packaged tablets and capsules. Unit-dosage forms may be administered in fractions or multiples thereof. A multiple-dosage form is a plurality of identical unit-dosage forms packaged in a single container to be administered in segregated unit-dosage form. Examples of multiple-dosage forms include vials or bottles of tablets or capsules.

The pharmaceutical compositions provided herein may be administered at once, or multiple times at intervals of time. It is understood that the precise dosage and duration of treatment may vary with the age, weight, and condition of the patient being treated, and may be determined empirically using known testing protocols or by extrapolation from in vivo or in vitro test or diagnostic data. It is further understood that for any particular individual, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the formulations.

In the case wherein the patient's condition does not improve, upon the doctor's discretion the administration of the combinations may be administered chronically, that is, for an extended period of time, including throughout the duration of the patient's life in order to ameliorate or otherwise control or limit the symptoms of the patient's disease or condition. In the case wherein the patient's status does improve, upon the doctor's discretion the administration of the combinations may be given continuously or temporarily suspended for a certain length of time (i.e., a "drug holiday"). In some embodiments, once improvement of the patient's conditions has occurred, a maintenance dose is administered if necessary. Subsequently, the dosage or the frequency of administration, or both, can be reduced, as a function of the symptoms, to a level at which the improved disease, disorder or condition is retained.

Treatment dosages generally may be titrated to optimize safety and efficacy. Typically, dosage-effect relationships from in vitro studies initially can provide useful guidance on the proper doses for patient administration. Studies in animal models also generally may be used for guidance regarding effective dosages for treatment in accordance with the present disclosure. In terms of treatment protocols, it should be appreciated that the dosage to be administered will depend on several factors, including the particular agent that is administered, the route administered, the condition of the particular patient, etc. Determination of these parameters are well within the skill of the art. These considerations, as well as effective formulations and administration procedures are well known in the art and are described in standard textbooks.

The compositions provided herein may be administered alone, or in combination with one or more other active ingredients.

The pharmaceutical compositions provided herein may be formulated in various dosage forms for oral, parenteral, buccal, intranasal, epidural, sublingual, pulmonary, local, rectal, transdermal, or topical administration. The pharmaceutical compositions may also be formulated as a modified release dosage form,
including delayed-, extended-, prolonged-, sustained-, pulsatile-, controlled- and fast-, targeted-, programmed-release, and gastric retention dosage forms. These dosage forms can be prepared according to conventional methods and techniques known to those skilled in the art (see, Remington: The Science and Practice of Pharmacy, supra; Modified-Release Drug Deliver Technology, Rathbone et al., Eds., Drugs and the Pharmaceutical Science, Marcel Dekker, Inc.: New York, NY, 2002; Vol. 126).

[0064] In various embodiments, the pharmaceutical compositions provided herein may be provided in solid, semisolid, or liquid dosage forms for oral administration.

[0065] As used herein, oral administration also include buccal, lingual, and sublingual administration.

Suitable oral dosage forms include, but are not limited to, tablets, capsules, pills, troches, lozenges, pastilles, cachets, pellets, medicated chewing gum, granules, bulk powders, effervescent or non-effervescent powders or granules, solutions, emulsions, suspensions, solutions, wafers, sprinkles, elixirs, and syrups. In addition to the active ingredient(s), the pharmaceutical compositions may contain one or more pharmaceutically acceptable carriers or excipients, including, but not limited to, binders, fillers, diluents, disintegrants, wetting agents, lubricants, glidants, coloring agents, dye-migration inhibitors, sweetening agents, and flavoring agents.

[0066] In further embodiments, the pharmaceutical compositions provided herein may be provided as compressed tablets, tablet triturates, chewable lozenges, rapidly dissolving tablets, multiple compressed tablets, or enteric-coating tablets, sugar-coated, or film-coated tablets. Enteric-coated tablets are compressed tablets coated with substances that resist the action of stomach acid but dissolve or disintegrate in the intestine, thus protecting the active ingredients from the acidic environment of the stomach.

[0067] The tablet dosage forms may be prepared from the active ingredient in powdered, crystalline, or granular forms, alone or in combination with one or more carriers or excipients described herein, including binders, disintegrants, controlled-release polymers, lubricants, diluents, and/or colorants. Flavoring and sweetening agents are especially useful in the formation of chewable tablets and lozenges.

[0068] The pharmaceutical compositions provided herein may be provided as soft or hard capsules, which can be made from gelatin, methylcellulose, starch, or calcium alginate. The hard gelatin capsule, also known as the dry-filled capsule (DFC), consists of two sections, one slipping over the other, thus completely enclosing the active ingredient. The soft elastic capsule (SEC) is a soft, globular shell, such as a gelatin shell, which is plasticized by the addition of glycerin, sorbitol, or a similar polyol. The liquid, semisolid, and solid dosage forms provided herein may be encapsulated in a capsule. Suitable liquid and semisolid dosage forms include solutions and suspensions in propylene carbonate, vegetable oils, or triglycerides.

[0069] In other embodiments, the pharmaceutical compositions provided herein may be provided as non-effervescent or effervescent, granules and powders, to be reconstituted into a liquid dosage form. Pharmaceutically acceptable carriers and excipients used in the non-effervescent granules or powders may include diluents, sweeteners, and wetting agents. Pharmaceutically acceptable carriers and excipients used in the effervescent granules or powders may include organic acids and a source of carbon dioxide.

[0070] In various embodiments, the pharmaceutical compositions provided herein may be formulated as a modified release dosage form. As used herein, the term "modified release" refers to a dosage form in which the rate or place of release of the active ingredient(s) is different from that of an immediate dosage form when administered by the same route. Modified release dosage forms include delayed-, extended-, prolonged-,
sustained-, pulsatile-, controlled-, accelerated- and fast-, targeted-, programmed-release, and gastric retention
dosage forms. The pharmaceutical compositions in modified release dosage forms can be prepared using a
variety of modified release devices and methods known to those skilled in the art, including, but not limited to,
matrix controlled release devices, osmotic controlled release devices, multiparticulate controlled release devices,
ion-exchange resins, enteric coatings, multilayered coatings, microspheres, liposomes, and combinations
thereof. The release rate of the active ingredient can also be modified by varying the particle size of the active
ingredient(s). Examples of modified release include, but are not limited to, those described in U.S. Pat. Nos.:
3,845,770; 3,916,899; 3,536,809; 3,598,123; 4,008,719; 5,674,533; 5,059,595; 5,591,767; 5,120,548; 5,073,543;
5,639,476; 5,354,556; 5,639,480; 5,733,566; 5,739,108; 5,891,474; 5,922,356; 5,972,891; 5,980,945; 5,993,855;
6,045,830; 6,087,324; 6,113,943; 6,197,350; 6,248,363; 6,264,970; 6,267,981; 6,376,461; 6,419,961; 6,589,548;
6,613,358; and 6,699,500.

[0071] In other embodiments, the pharmaceutical compositions provided herein in an immediate release
dosage form are capable of releasing not less than 75 % of the therapeutically active ingredient or combination
and/or meet the disintegration or dissolution requirements for immediate release tablets of the particular
therapeutic agents or combination included in the tablet core, as set forth in USP XXII, 1990 (The United States
Pharmacopeia.).

Methods for the Treatment of Proliferative Disease

[0072] One embodiment provides a method for the treatment of proliferative disease in a patient comprising
administration of a composition comprising a compound of Formula I, or pharmaceutically acceptable salt,
solvate or polymorph thereof,

\[
\text{Formula I}
\]

wherein \( R \) is H or \( \text{C}_0 \) (C1-C6 alkyl); and
\( R' \) is H or \( \text{C}_0 \) (C1-C6 alkyl).

[0073] Another embodiment provides a method for the treatment of proliferative disease wherein the
proliferative disease is myelodysplasia syndrome.

[0074] Another embodiment provides a method for the treatment of proliferative disease wherein the
proliferative disease is selected from colon cancer, lung cancer, pancreatic cancer, gastric cancer, prostate
cancer, and hepatocellular carcinoma.

[0075] The examples and preparations provided below further illustrate and exemplify the compounds of the
present disclosure and methods of preparing such compounds. It is to be understood that the scope of the present
disclosure is not limited in any way by the scope of the following examples and preparations.
Examples

[0076] The present disclosure is further illustrated by the following examples, which should not be construed as limiting in any way. The experimental procedures to generate the data shown are discussed in more detail below. The disclosure has been described in an illustrative manner, and it is to be understood that the terminology used is intended to be in the nature of description rather than of limitation.

General Experimental Details

1. Chemical Syntheses

[0077] The synthesis of dioxaphosphinine 4 is illustrated in Scheme 1.

![Scheme 1](image)

[0078] Friedel-Crafts alkylation of 3-fluorophenol gave di(tert-butyl)-3-fluorophenol in 70% yield. Condensation of phenol 2 with formaldehyde gave alcohol 3 in 69% yield. Treatment of alcohol 3 with phosphorus trichloride, in the presence of a base, gave phosphine 4 in 87% yield.

Example 1

[0079] The synthesis of Example 1 is given in Scheme 2.

[0080] Treatment of azacytidine acetonide (5) with 4,6-di-tert-butyl-3-fluoro-2-(hydroxymethyl)phenol dioxaphosphinine (4) gave intermediate 6 which was oxidized to phosphate ester 7 with tert-butyl hydroperoxide. Removal of the acetonide group gave Example 1 (compound 8). Figure 1 shows the LC trace for the purification of Example 1. Figure 2 shows the MS analysis of Example 1, and Figures 3, 4 and 5 provide the 400 MHz ¾ NMR spectrum and the D₂O exchange ¹H NMR spectrum of Example 1.
Example 2

The synthesis of Example 2 is given in Schemes 3 and 4.

Following the procedure of Moon (Moon et al. Bull. Korean Chem. Soc. (2005) 26: 11, 1865-8), treatment of phosphate 7 with n-amyl chloroformate provides carbamate 9. Removal of the acetonide protecting group under acidic conditions provides Example 2 (compound 10). An alternative procedure is given in Scheme 4. In this procedure, acetonide protected compound 7 is treated with acid to remove the acetonide protecting group and the 2’ and 3’ hydroxyl groups protected as the acetate esters. Treatment of diester 11 with n-amyl
chloroformate provides carbamante 12. Deprotection of 12 under basic conditions provides target compound Example 2 (compound 10).

Scheme 4

II. Evaluation

Example 3 Determination of Aqueous Stability

Prepare solutions at 5 mg/mL in normal saline and 5% dextrose in water for stability analysis. Stability will be conducted at 4, 25 and 37°C in each formulation out to about 1 week of time.

Example 4 Determination of stability in human whole blood

Test compound (at various concentrations from 0.05 µM to 10 µM) is added to human whole blood. At various time points ranging from 1 min to 6 hours, an aliquot is removed and partitioned between dichloromethane and water. Analysis by LC/MS of the dichloromethane phase for test compound and azacitidine will allow for the determination of rate of release of azacitidine.

Example 5 Determination of bioavailability

Formulations of test compound and azacitidine suitable for oral dosing to rodents are prepared. After dosing by gavage, blood samples are obtained at various time points ranging from 1 min post-dose to 6 hours post-dose, and the aliquot placed into acetonitrile or dichloromethane to quench all biotransformations prior to analysis. Analysis by LC/MS of the organic phase for test compound and azacitidine will allow for the determination of bioavailability.

Example 6 Determination of activity in cell based assay

The Calu-6 cell line was selected for study. Test compound was dissolved in PBS and stored as frozen aliquots in dark tubes between dosing. The test solution was sonicated in a water bath for 15 min prior to addition to the cells. Cells were treated with either vehicle control (sham), 1 µM Example 1 (prodrug), 3 µM Example 1 (prodrug), 1 µM azacitidine (aza)), or 0.5 µM decitabine (DAC) for four consecutive days. The levels of mPVNA expression for four genes (GATA5, IL20RA, Pak6, and TCF21) was determined by QPCR. The results are provided in Figure 1.
CLAIMS

What is claimed is:

1. A compound of Formula I, or pharmaceutically acceptable salt, solvate or polymorph thereof,
   
   \[
   \text{Formula I}
   \]

   wherein \( R \) is H or \( \text{C}0_{2}(\text{C}1-\text{C}6 \text{ alkyl}) \); and
   \( R^1 \) is H or \( \text{C}0_{2}(\text{C}1-\text{C}6 \text{ alkyl}) \).

2. The compound of claim 1 wherein \( R \) is H and \( R^1 \) is H.

3. The compound of claim 1 wherein \( R \) is \( \text{C}0_{2}(\text{C}1-\text{C}6 \text{ alkyl}) \) and \( R^1 \) is H.

4. The compound of claim 1 wherein \( R \) is H and \( R^1 \) is \( \text{C}0_{2}(\text{C}1-\text{C}6 \text{ alkyl}) \).

5. The compound of claim 1 wherein \( R \) is \( \text{C}0_{2}(\text{C}1-\text{C}6 \text{ alkyl}) \) and \( R^1 \) is \( \text{C}0_{2}(\text{C}1-\text{C}6 \text{ alkyl}) \).

6. A compound having the Formula Ia:
   
   \[
   \text{Formula Ia}
   \]

7. A compound having the Formula Ib:
   
   \[
   \text{Formula Ib}
   \]

8. A pharmaceutical composition comprising a compound of any of claims 1, 6 or 7 and a pharmaceutically acceptable excipient.
9. A method for the treatment of proliferative disease in a patient comprising administration of a composition comprising a compound of Formula I, or pharmaceutically acceptable salt, solvate or polymorph thereof,

\[
\text{Formula I}
\]

wherein \( R \) is H or \( \text{C}_0 \text{C}_1\text{C}_6 \text{alkyl} \); and
\( R' \) is H or \( \text{C}_0 \text{C}_1\text{C}_6 \text{alkyl} \).

10. The method of claim 9, wherein the proliferative disease is myelodysplastic syndrome.

11. The method of claim 9, wherein the proliferative disease is selected from colon cancer, lung cancer, pancreatic cancer, gastric cancer, prostate cancer, and hepatocellular carcinoma.
A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A01N 43/04; A61 K 31/70 (2011.01)
USPC - 514/42-43

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
USPC - 514/42-43

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 206/223; 514/33.5, 110, 246; 536/28.3 (see search terms below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST (PGPB, USPT, EPAB, JPAB); Google (Google Scholar, Google Patents)
Search Terms Used: azacitidine 5'-phosphate, 5-azacytidine-5'-phosphate, produgs, azacitidine, 5-azacytidine, azacytidine, ladakamycin, 5-AC, VidaZa, 4-amino-1-beta-D-ribofuranosyl-1,3,5-triazin-2(1H)-one, proliferative, cancer, phosphate

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
<th>Date of publication</th>
</tr>
</thead>
<tbody>
<tr>
<td>US 2008/0182806 A1 (PIZZORNO) 31 July 2008 (31.07.2008) para [0018], [0022], [0039]-[0040], [0045]; claims 1, 9, 17</td>
<td>1-1</td>
<td>2008-07-31</td>
</tr>
</tbody>
</table>

Further documents are listed in the continuation of Box C.

"A" special categories of cited documents:
- "A" document defining the general state of the art which is not considered to be of particular relevance
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