

“Prodrugs of human dihydroorotate dehydrogenase (*h*DHODH) inhibitors”

DESCRIPTION

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FIELD OF THE INVENTION

This disclosure is in the field of prodrugs of human dihydroorotate dehydrogenase (*h*DHODH) inhibitors structurally based on hydroxyazole scaffolds and methods of making and using them. In particular, the present disclosure relates to the use of such prodrugs of *h*DHODH inhibitors
10 in the treatment of cancer or viral infections.

BACKGROUND OF THE INVENTION

Human dihydroorotate dehydrogenase (*h*DHODH, EC 1.3.99.11), present in the inner mitochondrial membrane, is a flavin-dependent enzyme involved in *de novo* pyrimidine
15 biosynthesis. It catalyses the rate-limiting step in *de novo* pyrimidine biosynthesis, which converts dihydroorotate (DHO) to orotate (ORO). *h*DHODH has already been validated as a therapeutic target for the treatment of autoimmune diseases, such as rheumatoid arthritis and multiple sclerosis. Furthermore, *h*DHODH has also recently been identified as a relevant target in the treatment of triple-negative breast cancer,¹ PTEN-mutant tumors,² KRAS-driven tumors,³
20 acute myelogenous leukemia (AML),⁴ small cell lung cancer,⁵ esophageal squamous cell carcinoma,⁶ colorectal cancer,⁷ viral infection⁸ and ferroptosis-driven tumors.⁹ While the connection with AML has paved the way for completely new perspectives in the treatment of such diseases as well as in the *h*DHODH field, COVID-19 pandemic showed the importance to invest on the design of new *Broad Spectrum Antiviral Agents* (BSAAs), such as *h*DHODH
25 inhibitors.¹⁰ It is believed that both the cancer malignancies and the viral infections converge on a similar pathway of metabolic reprogramming to drive their dependence on pyrimidine synthesis and sensitivity to *h*DHODH inhibition.

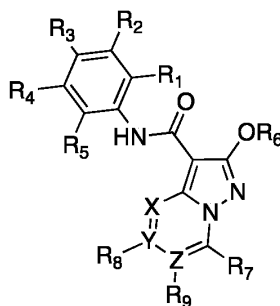
The present inventors recently identified and characterized a novel class of *h*DHODH
30 inhibitors¹¹ characterized by the presence of an acidic hydroxyazole moiety in the structure, which interacts with Arg136 at *h*DHODH subsite 2. The novel class includes, *inter alia*, compound **MEDS433** (named as compound **4** in ref.¹¹). Characterized by the presence of a 2-hydroxypyrazolo[1,5-a]pyridine moiety, **MEDS433** shows particular high potency against

- 5 *h*DHODH enzyme (*h*DHODH IC₅₀ 1.2 nM), excellent drug-like properties and *in vivo* *h*DHODH related activity in different animal models.¹² Designed to target a mitochondrial based enzyme, compound **MEDS433** owes its high potency to a great lipophilicity profile which in turn determines reduced aqueous solubility, a behaviour which could create problems in the path towards human experimentation and treatment.
- 10 The object of the present disclosure is to target and overcome the solubility problems of **MEDS433** and its analogues. The solution to the solubility problem provided by the present disclosure is a water-soluble prodrug of compound **MEDS433** and its analogues that can be administered intravenously / *per os*, thereby preventing any future formulation and administration issues.

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SUMMARY OF THE INVENTION

The present disclosure refers to a pro-drug of formula (I)

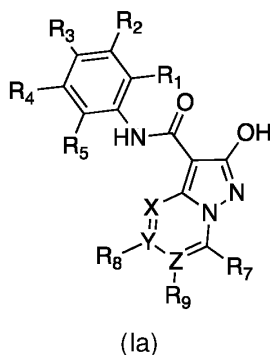


(I)

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which allows not only to improve the solubility of a hydroxyazole scaffold-based *h*DHODH inhibitor but also its bioavailability. In particular, the prodrug of formula (I) is able to release *in vivo* the corresponding hydroxyazole scaffold-based *h*DHODH inhibitor of general Formula

25 (Ia):



The disclosure refers also to the use of the pro-drug for the treatment of cancer or viral infections.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 a), b), c) shows the stability behaviour of pro-drug **1** in different mouse homogenates and **MEDS433** subsequent formation; **d)** Stability behaviour of **1** in human serum and **MEDS433** subsequent formation.

Figure 2 shows the plasma concentrations of **MEDS433** in CD1 mice after intravenous (IV) and *per os* (PO) administration of 5 and 20 mg/Kg of MEDS433, respectively.

Figure 3 shows pro-drug **2** and **MEDS433** plasma concentration in mice after IV (panel a)) and PO (panel b)) administration of pro-drug **2** at doses of 6.85 and 27.38 mg/Kg equivalent to 5 and 20 mg/Kg of **MEDS433**.

Figure 4 shows the comparison of the plasma pharmacokinetic profiles of **MEDS433** and pro-drug **2**, expressed as **MEDS433** concentrations. Drugs were administered at the doses (**MEDS433** equivalent) of 5 and 20 mg/Kg, given by IV (panel A) and PO (panel B) route, respectively.

Figure 5 shows the plasma concentrations of compound **6** in Balb/C mice after intravenous (IV) and *per os* (PO) administration of 5 and 20 mg/Kg of **6**, respectively.

5 **Figure 6** shows the plasma concentrations of compound **8** in CD1 mice after intravenous (IV) and *per os* (PO) administration of 5 and 20 mg/Kg of **8**, respectively.

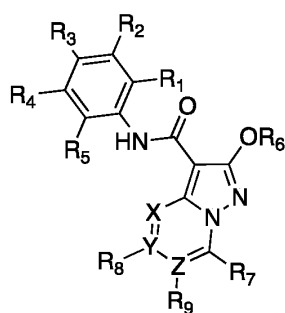
Figure 7 A) and **B)** show OCI-AML3 cells treated with indicated concentrations of **MEDS433** in combination with Dipyridamole and Fludarabine or Cladribine for 3 days. Cell apoptosis was
10 analysed by annexin V staining. The percentage of annexin positive cells is shown in the graph.

Figure 8 A) and **B)** show OCI-AML3 and THP1 cells treated with indicated concentration of **MEDS433** in combination with increased concentrations of Draflazine for 3 days; **C)** and **D)** show OCI-AML3 and THP1 cells treated with indicated concentration of **MEDS433** in
15 combination with different concentrations of JNK-IN-8 for 3 days. Cell apoptosis was analysed by annexin V staining. The percentage of annexin positive cells is shown in the graph.

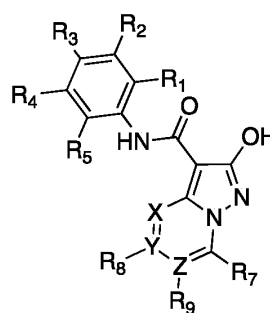
Figure 9 show SF8628 cells treated with indicated concentration of **compound 6** in combination with **A)** Panabinoestat, **B)** Abemaciclib, **C)** ONC212 through serial doses in
20 constant/variable ratios at 72 hours. Synergistic activity was analyzed using the CellTiter-Glo 2.0 and Cell Counting Kit-8 assays. Synergism and combination index are shown in the graph.

DETAILED DESCRIPTION OF THE INVENTION

A **first aspect** of the present disclosure is a prodrug of Formula (I) able to release *in vivo* the
25 corresponding hydroxyazole scaffold-based *h*DHODH inhibitor of general Formula (Ia).



Formula (I)



Formula (Ia)

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In Formulas (I) and (Ia):

R₁, R₂, R₄ and R₅ are independently selected from a hydrogen atom, a deuterium atom, a halogen atom, a linear and/or branched C₁₋₈ alkyl group, an alkyloxy group, an alkylamino group, an alkylthio group, a halo C₁₋₈ alkyl group, a halo C₁₋₈ alkyloxy group, a halo C₁₋₈ alkylamino group, a nitro group, a cyano group;

R₃ is:

- an aromatic group selected from the group consisting of: phenyl group, heteroaryl group, preferably an azinyl group such as pyridinyl group, pyrimidinyl group; phenoxy group; azinyloxy group such as pyridinyloxy group, pyrimidinyl group; phenylthio group; azinylthio group such as pyridinylthio group, pyrimidinylthio group; phenylsulfinyl group; phenylsulfonyl group; phenylsulfonylamino group; N-phenylcarbamoyl group.

The aromatic group is optionally substituted, one or two times; each substituent is independently selected from a deuterium atom, a halogen atom, a hydroxyl group, an amino group, an alkyl group, a trifluoromethyl group, a trifluoromethoxy group, an alkyloxy group, a deuterium-alkyloxy group, a halo-alkyloxy group, a nitro group, a cyano group, and an alkylamino group;

- an aliphatic group selected from the group consisting of: C₁₋₁₂ alkyl group; C₁₋₁₂ alkyloxy group; C₁₋₁₂ alkylthio group; C₁₋₁₂ alkylsulfinyl group; C₁₋₁₂ alkylsulfonyl group; C₁₋₁₂ alkylsulfonylamino group; C₁₋₁₂ alkylamino group; C₁₋₁₂ alkylaminosulfonyl group; halo C₁₋₁₂ alkyl group; halo C₁₋₁₂ alkyloxy group; halo C₁₋₁₂ alkylthio group; halo C₁₋₁₂ alkylsulfinyl group; halo C₁₋₁₂ alkylsulfonyl group; halo C₁₋₁₂ alkylsulfonylamino group; halo C₁₋₁₂ alkylamino group; halo C₁₋₁₂ alkylaminosulfonyl group.

The aliphatic group is optionally substituted, one or two times; each substituent is independently selected from a deuterium atom, a halogen atom, a hydroxyl group, an amino group, an alkyl group, a trifluoromethyl group, a trifluoromethoxy group, an alkyloxy group, a halo-alkyloxy group, a nitro group, a cyano group, and an alkylamino group;

- 5 R₇, R₈ and R₉ are independently selected from a hydrogen atom, a deuterium atom, a halogen atom, a nitro group, a cyano group, a C₁₋₄ alkyl group, a halo C₁₋₄ alkyl group, a mercapto C₁₋₄ alkyl group, an amino C₁₋₄ alkyl group, and a hydroxy C₁₋₄ alkyl group;
 X, Y and Z are independently selected from a carbon atom, and a nitrogen atom, with the proviso that when one of X, Y or Z is nitrogen, the other two positions are carbon atoms and
 10 when Y or Z are nitrogen atoms, R₈ and R₉ are not present.

According to an embodiment, at least one of R₁, R₂, R₄ and R₅ is or contains a halogen atom. A preferred halogen atom is a fluorine or a chlorine atom, more preferably a fluorine atom (F)

- 15 According to an embodiment, the alkyl group of any of R₁, R₂, R₄ and R₅ is C₁₋₄ alkyl group, preferably C₁₋₃ alkyl group. The alkyl group can be linear, branched or cyclic.

According to an embodiment at least one of R₁, R₂, R₄ and R₅ is selected from hydrogen, deuterium, -OCD₃, -OCF₃, methyl, isopropyl, methyl, 1,1,1-trifluoro-2-propanoxy.

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According to an embodiment, the alkyl group of R₃ is C₁₋₆ alkyl group, preferably C₁₋₄ alkyl group. The alkyl group can be liner, branched or cyclic.

- According to an embodiment, R₃ is selected from phenyl, deuterophenyl, ortho-
 25 trifluoromethoxyphenyl, meta-trifluoromethoxyphenyl, meta-trideuteromethoxyphenyl, meta-(2,2-difluoropropoxy)phenyl, 3-pyridinyl, phenoxy, ortho-trifluoromethylphenyl, ortho-trideuteromethylphenyl, meta-trifluoromethylphenyl, meta-trideuteromethylphenyl, meta-deuterophenyl, ortho-deuterophenyl, meta-chlorophenyl, ortho-chlorophenyl, meta-fluorophenyl, ortho-fluorophenyl, meta-hydroxyphenyl, meta-butoxyphenyl.

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According to an embodiment R₇, R₈ and R₉ are independently selected from hydrogen, deuterium, methyl, chloro, methyloxy and hydroxymethyl.

- According to an embodiment, X, Y and Z are independently selected from a carbon atom and
 35 a nitrogen atom, with the proviso that when one of X, Y or Z is nitrogen, the other two positions are carbon atoms and when Y or Z are nitrogen atoms, R₈ and R₉ are not present.

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In one embodiment, X, Z are carbon atoms, Y is carbon or nitrogen, R₇, R₈, R₉ are hydrogen, R₁, R₂, R₄, R₅ are fluorine, R₃ is a phenyl or *m*-(2,2-difluoro(C₁-C₃)alkyloxy)phenyl or *m*-(trifluoro(C₁-C₃)alkyl)phenyl or *m*-(trifluoro(C₁-C₃)alkyloxy)phenyl or *m*-butoxyphenyl.

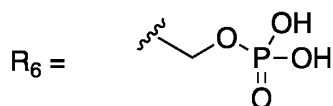
- 10 In an embodiment, X, Y, Z are carbon atoms, R₇, R₈, R₉ are hydrogen atoms, R₁, R₄ are hydrogen atoms, R₂ is a methyl, R₅ is iso-propyl, R₃ is phenyloxy.

- In an embodiment of the disclosure, one or more atoms of the formulae (I) and (Ia) can be replaced by its isotopes. Isotopes that can be incorporated into a compound of the disclosure
- 15 in a form that exceeds natural abundances and include isotopes of hydrogen, carbon, nitrogen, oxygen, phosphorous, fluorine, chlorine, and iodine, such as ²H (or chemical symbol D), ³H (or chemical symbol T), ¹¹C, ¹³C, ¹⁴C, ¹⁵N, ¹⁸O, ¹⁷O, ³¹P, ³²P, ³⁵S, ¹⁸F, ³⁶Cl, and ¹²⁵I, respectively. Such isotopically labelled compounds are useful in metabolic studies (for example based on ¹⁴C), reaction kinetic studies (for example based on ²H or ³H), detection or imaging techniques
- 20 (such as positron emission tomography (PET) or single-photon emission computed tomography (SPECT)), including drug or substrate tissue distribution assays, or in the case of radioactive treatment of patients. In particular, a ¹⁸F or ¹¹C labelled compound may be preferred for PET or SPECT studies. Further, substitution with heavier isotopes such as deuterium (i.e., ²H) may afford certain therapeutic advantages resulting from greater metabolic stability, for
- 25 example increased *in vivo* half-life or reduced dosage requirements. An isotopically labelled compound of this disclosure can generally be prepared by carrying out the procedures disclosed in the schemes or in the examples of preparations described below by substituting a non-isotopically labelled reagent with an isotopically labelled reagent.

- 30 In Formula (I), R₆ is selected from the group consisting of a linear or branched C₁₋₆ alkyl substituted with a group selected from a phosphate, a -COOH, a -CONHR₁₀, wherein R₁₀ is selected from hydrogen and a C₁₋₆ alkyl chain.

- In a preferred embodiment R₆ is a linear or branched C₁₋₆ alkyl substituted with a phosphate
- 35 group. More preferably, R₆ is a linear C₁₋₃ alkyl substituted with a phosphate group. Even more preferably R₆ is a phosphonoxymethyl group represented by formula (II):

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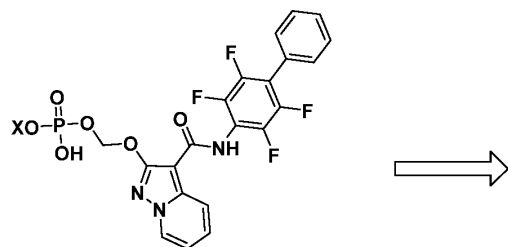


Formula (II)

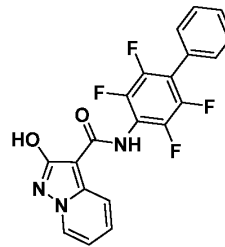
The phosphonomoxymethyl group of Formula (II) can be in the free or salified form. The salt can be an alkali metal salt, for example lithium, sodium, potassium, and cesium salt; an alkaline earth metal salt, for example calcium, magnesium or strontium salt; aluminum or zinc salt; an ammonium salt a quaternary ammonium salt having 1 to 20 carbon atoms, such as tetramethylammonium, tetraethylammonium, tetra(n-propyl)ammonium, tetra(n-butyl)ammonium, N-benzyl-N,N,N-trimethylammonium; a choline salt; a benzalkonium salt.

The ammonium salt is preferably derived from ammonia or from an organic primary, secondary or tertiary amine having 1 to 20 carbon atoms, such as ethylamine, diethylamine, triethylamine, ethyldiisopropylamine, monoethanolamine, diethanolamine, triethanolamine, dicyclohexylamine, dimethylaminoethanol, diethylaminoethanol, tris(hydroxymethyl)aminomethane (tromethamine), procaine, dibenzylamine, N-methylmorpholine, arginine, lysine, 1,2-ethylenediamine, N-methylpiperidine, N-methylglucamine, N,N-dimethyl-glucamine, N-ethyl-glucamine, 1,6-hexanediamine, glucosamine, sarcosine, serinol, 2-amino-1,3-propanediol, 3-amino-1,2-propanediol, 4-amino-1,2,3-butanetriol.

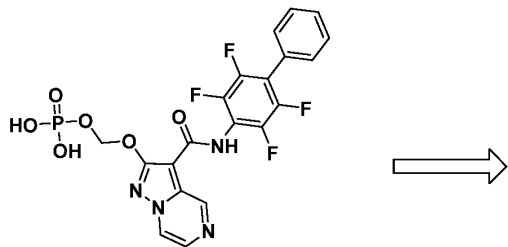
Preferred prodrugs falling within Formula (I) are compounds **1**, **2**, **2a**, **2b**, **2c**, **3**, **5** and **7** having the following structures (Scheme 1). *In vivo*, such pro-drugs yield the corresponding drugs **MEDS433**, compound **4**, compound **6** and compound **8**, according to Scheme 1 below:



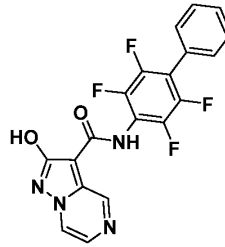
- 1) X = H;
2) X = K salt;
2a) X = Na salt;
2b) X = Tromethamine salt;
2c) X = Lysine salt;



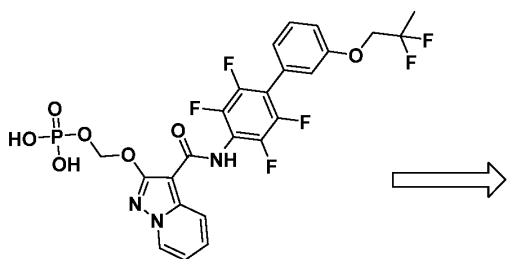
MEDS433



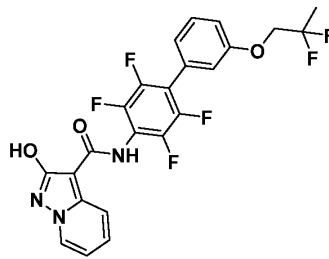
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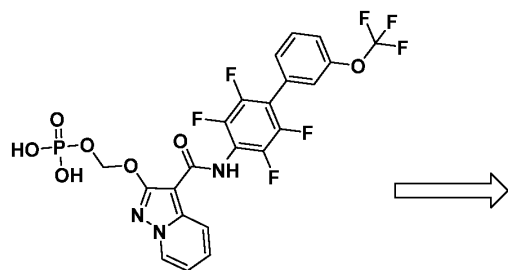
4



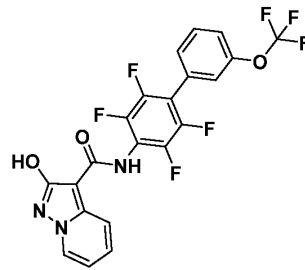
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- 5 **Scheme 1.** Prodrugs **1, 2, 2a, 2b, 2c, 3, 5** and **7** and the correspondent drugs **MEDS433, 4,**
6 and **8.**

A second aspect of the present disclosure is a pharmaceutical composition comprising a
hydroxyazole scaffold-based *h*DHODH prodrug of Formula (I) as defined above and a
10 pharmaceutically accepted carrier, excipient and/or diluent.

Another aspect of the disclosure is the use of a pro-drug of Formula (I) or a pharmaceutical
composition comprising such pro-drug as an anticancer agent, preferably for the treatment of:

- 15 a) hematologic malignancies, preferably selected from the group consisting of: acute myeloid
leukemia, myelodysplastic syndromes, chronic myeloid leukemia and other
myeloproliferative neoplasms, multiple myeloma, angioimmunoblastic T-cell lymphoma and
acute lymphoblastic leukemia, Hodgkin's and non-Hodgkin's lymphomas, IDH mutated
tumors, BRAF-mutated, RAS-mutated, PTEN-mutated and MYC/MYCN-driven hematologic
20 neoplasms;
- b) solid tumors, preferably selected from the group consisting of: osteosarcoma; Ewing
sarcoma; melanoma; BRAF mutated tumors; PTEN-mutant tumors; H3K27M-mutated
diffuse midline glioma; diffuse intrinsic pontine glioma; atypical teratoid rhabdoid tumor,
25 ependymomas; neuroendocrine tumors; multiple endocrine neoplasia type 1 (MEN1)
tumors; mutated MEN1 tumors; mutated KRAS hepatic tumors; mutated KRAS lung tumors;
mutated KRAS pancreatic tumors; mutated KRAS colon carcinoma; prostate tumors;
bladder tumors; kidney tumors; ferroptosis sensitive tumors, preferably lung cancer,
glioblastoma, breast cancer, preferably triple-negative breast cancer, pancreatic cancer,
30 hepatocellular carcinoma and colorectal cancer; IDH mutated tumors, preferably low-grade
gliomas, secondary glioblastoma, primary glioblastoma, cartilaginous and bone tumors,
fibrosarcoma, sinonasal undifferentiated carcinoma, intrahepatic cholangiocarcinoma, MYC
and MYCN driven tumors, preferably neuroblastoma, medulloblastoma, retinoblastoma,
astrocytoma, glioblastoma multiforme, castration-resistant prostate cancer, neuroendocrine
35 prostate cancer, rhabdomyosarcoma, Wilms tumors, small cell lung cancer, pancreatic

- 5 tumors; AMPK α 1_{low} expressing tumors; GPX4_{low} expressing tumors; p53 wild type and mutated cancers.

In another aspect, the present disclosure refers to the use of a compound of Formula (Ia) as an anticancer agent for the treatment of

- 10 a) hematologic malignancies, preferably selected from the group consisting of: myelodysplastic syndromes, chronic myeloid leukemia and other myeloproliferative neoplasms, multiple myeloma, angioimmunoblastic T-cell lymphoma and acute lymphoblastic leukemia, Hodgkin's and non-Hodgkin's lymphomas, IDH mutated tumors, BRAF-mutated, RAS-mutated, PTEN-mutated and MYC/MYCN-driven hematologic neoplasms;
- 15 b) solid tumors, preferably selected from the group consisting of: osteosarcoma; Ewing sarcoma; melanoma; BRAF mutated tumors; H3K27M-mutated diffuse midline glioma; diffuse intrinsic pontine glioma; atypical teratoid rhabdoid tumor, ependymomas; neuroendocrine tumors; multiple endocrine neoplasia type 1 (MEN1) tumors; mutated MEN1 tumors; mutated
- 20 KRAS hepatic tumors; mutated KRAS lung tumors; mutated KRAS pancreatic tumors; mutated KRAS colon carcinoma; prostate tumors; bladder tumors; kidney tumors; ferroptosis sensitive tumors, preferably lung cancer, glioblastoma, breast cancer, pancreatic cancer, hepatocellular carcinoma and colorectal cancer; IDH mutated tumors, preferably low-grade gliomas, secondary glioblastoma, primary glioblastoma, cartilaginous and bone tumors, fibrosarcoma,
- 25 sinonasal undifferentiated carcinoma, intrahepatic cholangiocarcinoma,; MYC and MYCN driven tumors, preferably neuroblastoma, medulloblastoma, retinoblastoma, astrocytoma, glioblastoma multiforme, castration-resistant prostate cancer, neuroendocrine prostate cancer, , rhabdomyosarcoma, Wilms tumors, small cell lung cancer, pancreatic tumors; AMPK α 1_{low} expressing tumors; GPX4_{low} expressing tumors; p53 wild type and mutated cancers.

- 30 In one embodiment, the compound of formula (I) and/o (Ia) are used for the treatment of osteosarcoma, glioblastoma, Ewing sarcoma, breast cancer, melanoma, H3K27M-mutated diffuse midline glioma, neuroblastoma, mutated KRAS lung tumors, bladder cancer, IDH mutated tumors.

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5 Another aspect of the present disclosure is a hydroxyazole scaffold-based *h*DHODH inhibitor prodrug of Formula (I) or a pharmaceutical composition containing such prodrug for use as antiviral agent, preferably for the treatment of a viral infection.

Preferably, the viral infection is caused by a DNA virus or an RNA virus. Preferably, the virus is selected from the group consisting of *Herpesviridae*, *Orthomyxoviridae*, *Paramyxoviridae*
 10 and *Coronaviridae*. More preferably, the virus is selected from the group consisting of Herpes simplex virus 1 to 8 (HSV-1-8), paramyxovirus, pneumovirus, morbillivirus, Influenza A virus, Influenza B virus, Respiratory syncytial virus (RSV), severe acute respiratory syndrome coronavirus 1 (SARS-CoV-1), severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and Middle East respiratory syndrome-related coronavirus (MERS-CoV).

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Another aspect of the disclosure is the use of a compound of formula (Ia) for the treatment of a viral infection caused by Herpes simplex virus 3-8 (HSV-3-8).

The pathologies caused by the above viruses are, for example:

- 20 - HSV1: Gingivostomatitis, keratoconjunctivitis, cutaneous herpes, genital herpes, encephalitis, herpes labialis, viral meningitis, esophagitis, pneumonia, disseminated infection, hepatitis;
- HSV2: Genital herpes, cutaneous herpes, gingivostomatitis, neonatal herpes, viral meningitis, disseminated infection, hepatitis;
- 25 - HSV3: Chickenpox, herpes zoster, disseminated herpes zoster;
- HSV4: Infectious mononucleosis, hepatitis, encephalitis, nasopharyngeal carcinoma, Hodgkin lymphoma, Burkitt lymphoma, lymphoproliferative syndromes*, oral hairy leukoplakia*, gastric cancer;
- HSV5: Cytomegalovirus mononucleosis, hepatitis, congenital cytomegalic inclusion
 30 disease, hepatitis, retinitis, pneumonia, colitis;
- HSV6: Roseola infantum, otitis media with fever, encephalitis;
- HSV7: Roseola infantum;
- HSV8: Kaposi sarcoma-associated herpesvirus;
- *Orthomyxoviridae*: influenza and lower and upper respiratory tract infections such as
 35 pneumonia;

- 5 - *Paramyxoviridae* (paramyxovirus, pneumovirus, morbillivirus): upper and lower respiratory tract infections, such as bronchiolitis and pneumonia, pancreatitis, meningitis, encephalitis;
- *Coronaviridae*: severe acute respiratory syndrome coronavirus 1 (SARS-CoV-1), severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and Middle East
- 10 respiratory syndrome-related coronavirus (MERS-CoV).

The compound of formula (I) and/or (Ia) can be used to treat any of the above listed pathologies.

- 15 Another aspect of the present disclosure is a method for the treatment of a cancer disease or a viral infection or a pathology caused by a viral infection, as above defined, comprising a step of administering an effective amount of a compound of formula (I) and/or a drug of formula (Ia) or a pharmaceutical composition comprising the same, to a subject in need thereof.
- 20 In another embodiment, the disclosure refers to a composition comprising a 2-hydroxyazole scaffold-based *h*DHODH pro-drug of Formulae (I) and/or a drug of Formula (Ia) and one or more anticancer agents, preferably selected from: Abemaciclib, Abiraterone, Acalabrutinib, Actimide, Adagrasib, Artemisinin, Artesunate Ado-Trastuzumab Emtansine, Afatinib, Alectinib, Alpelisib, Atezolizumab, Amivantamab, Amsacrine, Anastrozole, Apalutamide, Ascitiminib,
- 25 Asparaginase, Axitinib, Azacitidine, Azathioprine, 6-Azaauridine, Belzutifan, Bevacizumab, Bicalutamide, Bleomycin, Bosutinib, Brentuximab vedotin, Brigatinib, Busulfan, Cabazitaxel, Cabozantinib, Capecitabine, Capmatinib, Cyclophosphamide, Carboplatin, Carglumic acid, Carmustine, Cedazuridine, Cemiplimab-rwlc, Ceritinib, Cetuximab, Cisplatin, Chlorambucil, Cytarabine, Cladribine, Clofarabine, CNX774, Cobimetinib, Curcumin, Dabrafenib,
- 30 Dacarbazine, Dacomitinib, Dactinomycin Darolutamide, Dasatinib, Daunorubicin, Decitabine, Degarelix, Dilazep, Dihydroartemisinin, Dinutuximab, Dinutuximab beta, Dipyridamole, Docetaxel, Dordaviprone, Doxifluridine, Doxorubicin, Draflazine, Durvalumab, Epcoritamab, Enasidenib, Encorafenib, Enfortumab vedotin-ejfv, Entrectinib, Enzalutamide, EOS984 Epirubicin, Erastin, Erdafitinib, Erlotinib, Etoposide, Everolimus, ExemestaneFam-
- 35 trastuzumab deruxtecan-nxki, Fenalidomide, Feucovorin, Fludarabine, Fluorouracil, FPMINT, Flutamide, Gemcitabine, Gefitinib, Glofitamab, Hydroxyurea, , Ibrutinib, Idarubicin, Infigratinib,

- 5 Ifosfamide, Imatinib, Ipilimumab, Irinotecan, Isotretinoin, Ivosidenib, JNK-IN 8, , Larotrectinib, Lenvatinib, Lenalidomide, Letrozole, Leucovorin, Lidoflazine, Lomustine, Loncastuximab tesirine-lpy, Lorlatinib, L-alanosine, 8MDP, Masitinib, Mechlorethamine, Melphalan, Mercaptopurine, Methotrexate, Mioflazine, Mitomycin-C, Mitoxantrone, Mizoribine, Mobocertinib, Mosunetuzumab-axgb, Mycophenolic acid, Nab-paclitaxel, Nanoliposomal
- 10 irinotecan, Naxitamab, Neratinib, Nilotinib, Nilutamide, Niraparib, Nivolumab, Nitrobenzylmercaptopurine riboside (NBMPR), Obinutuzumab, Olaparib, ONC212, OR0642, Osimertinib, Oxaliplatin, Panobinostat, Paclitaxel, Pacritinib, Pazopanib, PBP 4883, Pembrolizumab, Pertuzumab, Pemetrexed, Pemigatinib, Pirtobutinib, Pyrazofurin, Pralsetinib, Ponatinib, Polatuzumab vedotin-piq, Rapadocin, Ramucirumab, Regorafenib, Relugolix,
- 15 Revlimid, Rituximab, RO7117997, Rucaparib, Sacituzumab govitecan, Selepercatinib, Selinexor, Sorafenib, Sotarasib, Sulfasalazine, Sunitinib, Talazoparib, Tamibarotene, Tamoxifen, Tazemetostat, Temozolomide, Teniposide, Tepotinib, Thioguanine, Thiotepa, Ticagrelor, Tivozanib, Tofacitinib, Topotecan, Tafasitamab-cxix, Temsirolimus, Trametinib, Trastuzumab, TRE 515, Tremelimumab, Trifluridine/Tipiracil, Treosulfan, Troglitazone,
- 20 Valrubicin, Vandetanib Venetoclax, Vemurafenib, Vinblastine, Vincristine, Vindesine and Vinorelbine, Vorasenib, Zanubrutinib, Ziv-aflibercept.

- A preferred composition comprises a pro-drug of Formula (I), preferably one or more of the pro-drugs **1, 2, 2a, 2b, 2c, 3, 5** and **7**, and one or more of Acalabrutinib, Adagrasib, Artemisinin,
- 25 Artesunate, Afatinib, Atezolizumab, Amivantamab, Ascitiminib, Axitinib, Azacitidine, Bevacizumab, Bosutinib, Busulfan, Cabozantinib Capecitabine, Carboplatin, Cemiplimab-rwlc, Cetuximab, Cisplatin, Cyclopentenyl Uracil, Cyclophosphamide, Cytarabine, Cladribine, Clofarabine, CNX774, Cobimetinib, Curcumin, Dabrafenib, Dacarbazine, Dacomitinib, Dasatinib, Daunorubicin, Decitabine, Dihydroartemisinin, Dilazep, Dipyridamole, Dinutuximab,
- 30 Docetaxel, Dordaviprone, Doxorubicin, Draflazine, Durvalumab, Enasidenib, Encorafenib, Entrectinib, Enzalutamide, EOS984, Epirubicin, Erdafitinib, Erlotinib, Etoposide, Everolimus, Fludarabine, Fluorouracil, FPMINT, Gefitinib, Gemcitabine Hydroxyurea, Ibrutinib, Idarubicin, Imatinib, Infigratinib, Ipilimumab, Irinotecan, Ivosidenib, JNK-IN 8, Lidoflazine, 8MDP ,Masitinib, Mitomycin-C, Melphalan, Mercaptopurine, Methotrexate, Mioflazine Mycophenolic
- 35 acid, Mobocertinib, Sulfasalazine, Nab-paclitaxel, Nanoliposomal irinotecan, Niraparib, Nilotinib Nivolumab, ONC212, Olaparib, OR0642, Osimertinib, Oxaliplatin, Paclitaxel,

5 Panobinostat, Pazopanib, PBP 4883, Pembrolizumab, Pemigatinib, Pralsetinib, Ponatinib, Rapadocin, Ramucirumab, Regorafenib, RO7117997, Rucaparib, Selepercatinib, Sorafenib, Sotarasib, Sunitinib, Temozolomide, Tepotinib, Thioguanine, Tivozanib, Tofacitinib, Topotecan, Temsirolimus, Trametinib, TRE 515, Tremelimumab, Troglitazone, Vandetanib, Vemurafenib, Venetoclax, Vinblastine, Vincristine, Vinorelbine, Voraseninb.

10

In another embodiment the composition comprises a pro-drug of Formula (I), preferably one or more of the pro-drugs **1**, **2**, **2a**, **2b**, **2c**, **3**, **5** and **7**, and one or more of Cetuximab, CNX774, Dabrafenib, Dihydroartemisinin, Dipyridamol, Dordaviprone, Doxorubicina, Draflazine, Enasidenib, EOS984, Erlotinib, Everolimus, FPMINT, Gemcitabine, Ibrutinib, Ivosidenib, JNK-
 15 IN-8, 8MD, Mioflazine, Nivolumab, ONC212, Panobinostat, PBP 4883, Pembrolizumab, Rapadocin, RO7117997, Sorafenib, Sulfasalazine, Tofacitinib, Temozolomide, Trametinib, Troglitazone, TRE515, Vemurafenib, Voraseninb.

In another embodiment, the disclosure refers to a composition comprising a 2-hydroxyazole
 20 scaffold-based *h*DHODH pro-drug of Formulae (I) and/or a drug of Formula (Ia) and one or more antiviral agents, preferably selected from acyclovir, adefovir, amantadine, baloxavir marboxil, bamlanivimab/etesevimab, brivudine, casirivimab/imdevimab, cidofovir, famciclovir, favipiravir, fomivirsen, foscarnet, ganciclovir, N(4)-Hydroxycytidine, indinavir, idoxuridine, laninamivir, lopinavir, molnupiravir, nelfinavir, nirmatrelvir, oseltamivir, penciclovir, permivir,
 25 ribavirin, sotrovimab, tenofovir, tenofovir alafenamide, tenofovir diisoproxilfumarate, trifluridine, telbivudine, vidarabine, sofosbuvir, nevirapine, efavirenz, raltegravir, remdesvir, rimantadine, ritonavir, ritonavir – amlanivimab, saquinavir, tixagevimab/cilgavimab, trifluridine, valganciclovir, vidarabine, zanamivir, zidovudine.

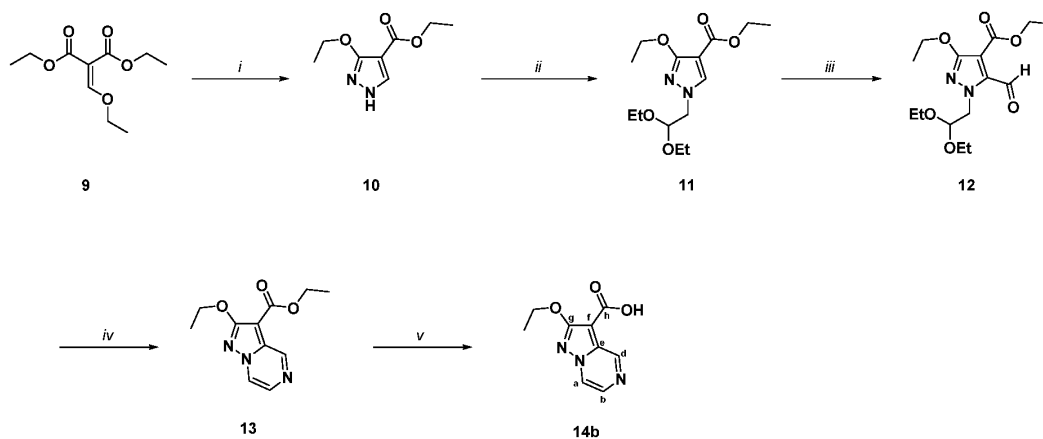
30 A preferred composition comprises a pro-drug of Formula (I), preferably one or more of the pro-drugs **1**, **2**, **2a**, **2b**, **2c**, **3**, **5** and **7**, and one or more of acyclovir, adefovir, cidofovir, famciclovir, favipiravir, foscarnet, ganciclovir, N(4)-Hydroxycytidine, idoxuridine, molnupiravir, nirmatrelvir, oseltamivir, penciclovir, ribavirin, tenofovir, sofosbuvir, remdesvir, ritonavir, valganciclovir, zidovudine.

35

- 5 The composition is used to treat a cancer or tumor or viral pathology by administration to a patient in need thereof.

Chemistry: synthesis of prodrugs 1, 2, 2a, 2b, 2c, 3, 5, 7 and drug 4.

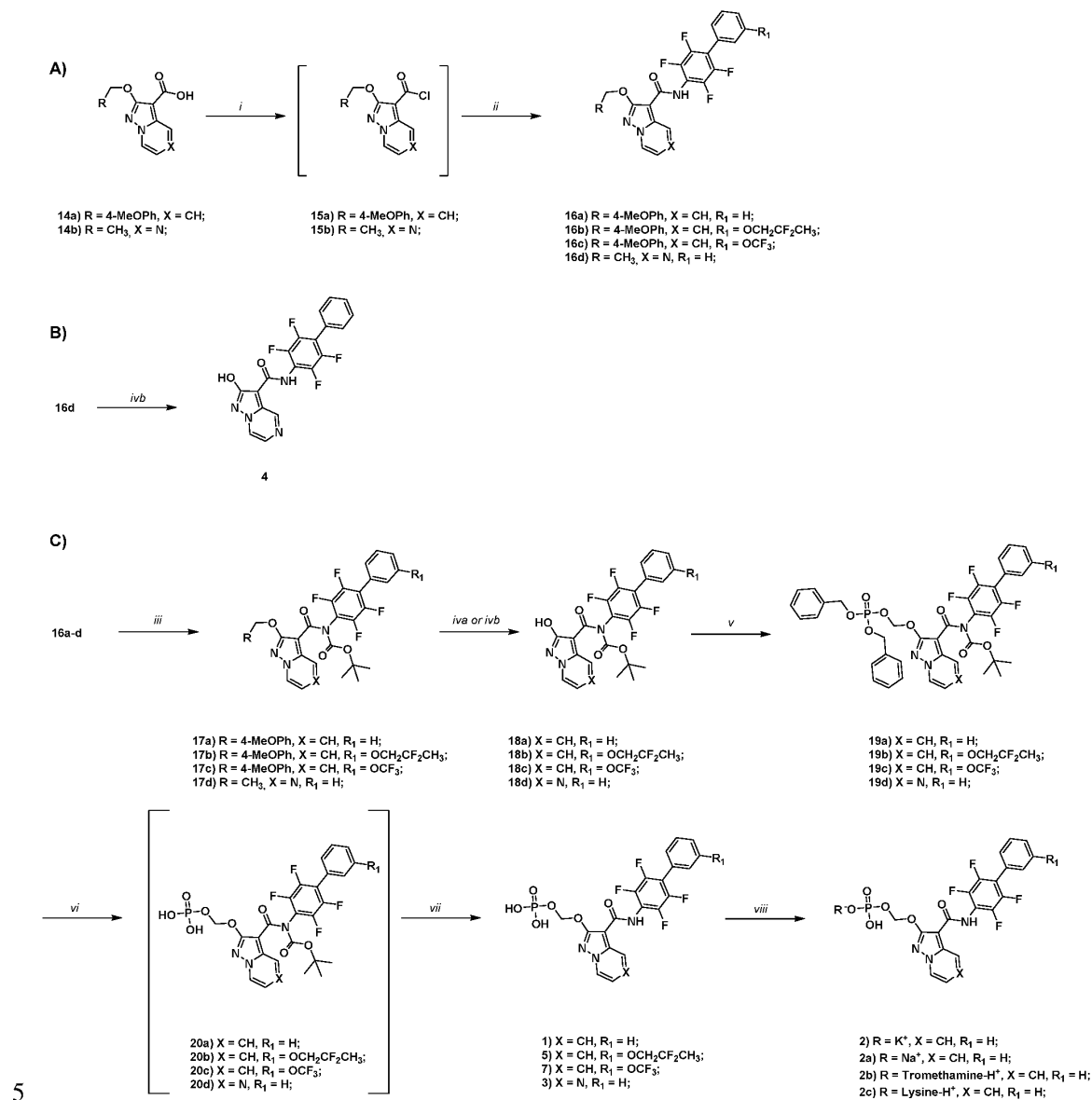
For the synthesis of prodrugs **1**, **2**, **2a**, **2b**, **2c**, **3**, **5**, **7** and drug **4** a synthetic approach was designed, improving in some case, in terms of yield and reactivity, the synthetic pathway already described in the authors previous patents.^{13,14} The starting materials are the carboxylic acids **14a-b**; while **14a** is a known compound,¹¹ for the synthesis of **14b** an original scheme was designed (Scheme 2). The ethyl 3-ethoxypyrazol-4-carboxylate **10** was initially bolted up by the reaction of compound **9** and hydrazine hydrochloride. Compound **10** was regioselectively alkylated on the nitrogen in position 1 with 2-bromo-1,1-diethoxyethane to obtain compound **11**, which undergoes a formylation step to afford **12**. In the following a two-step deprotection–cyclization protocol was used to synthesize the target ethyl 2-ethoxypyrazolo[1,5-a]pyrazine-3-carboxylate **13**, obtained in high yields. Firstly, acetal deprotection of intermediate **11** with aqueous TFA, followed by solvent evaporation and subsequent cyclization with ammonium acetate furnished the desired bicyclic product **13**. In the last step the ethyl ester present in **13** was hydrolysed in basic environment to obtain the acid **14b**.



25

- 5 **Scheme 2.** Synthesis of compound **14b**. i) $\text{NH}_2\text{NH}_2 \cdot \text{HCl}$, EtOH, reflux.; ii) Cs_2CO_3 , 2-bromo-1,1-diethoxyethane, dry DMF, reflux; iii) $n\text{BuLi}$, dry THF, -78°C , dry DMF; iv) a) TFA, H_2O , THF; b) NH_4OAc , AcOH, EtOH; v) 5 M NaOH, 60°C .

The carboxylates **14a,b** were then used for the preparation of the corresponding acyl chloride (**15a-b**) via treatment with oxalyl chloride, both used without any further purification in the
10 reaction with the lithium salt of 2,3,5,6-tetrafluoro-[1,1'-biphenyl]-4-amine or 3'-(2, 2-difluoropropoxy)-2,3,5,6-tetrafluoro-[1,1'-biphenyl]-4-amine affording the desired amides **16a-d** with a 70 - 90 % yield (Scheme 2A). Under these conditions, the benzylic or ethoxy protecting groups did not translate from the exocyclic oxygen to the endocyclic N1 nitrogen as occurred in previous synthetic scheme, thereby facilitating the following deprotection steps. At this
15 stage, compound **16d** was deprotected with BBr_3 in dry DCM to afford compound **4** in good yields (Scheme 2B). In the following step (Scheme 2C), compounds **16a-d** were then protected on the amide nitrogen with Boc to afford compounds **17a-d**. To introduce a methyl phosphate group, in the following step the oxygen protection was removed using different strategies. For compounds **17a-c**, the 4-methoxybenzyl group was removed via catalytic hydrogenation
20 conditions, while for compound **17d** the ethoxy group was removed using BBr_3 in dry DCM at 0°C . In the next step, compounds **18a-d** were alkylated with dibenzyl(chloromethyl)phosphate to afford the corresponding protected prodrugs **19a-d**. The latter were deprotected following a two steps reaction sequence: the two benzyl groups of the phosphate were removed via catalytic hydrogenation to yield compounds **20a-d**. The final prodrugs **1**, **3**, **5** and **7** were then
25 obtained by removing the Boc protection in mild acid conditions. In the following, compound **1** was then converted into the corresponding salts **2**, **2a**, **2b**, **2c** by using 1 equivalent of KOH, NaOH, tromethamine and lysine respectively.



Scheme 3A-C. Synthesis of pro-drugs **1**, **2**, **2a**, **2b**, **2c**, **3**, **5** and **7** and drug **4**. *i*) Oxalyl chloride, dry DMF, dry THF, nitrogen atmosphere; *ii*) LiHMDS, dry THF, 2,3,5,6-tetrafluoro-[1,1'-biphenyl]-4-amine for **14a** and **14c**, 3'-(2, 2-difluoropropoxy)-2,3,5,6-tetrafluoro-[1,1'-biphenyl]-4-amine for **14b**; -10 °C; *iii*) NaH, Boc₂O, THF; *iv*) a) Pd/C (9 % w/w) THF for **16a-b** or b) BBr₃,

- 5 DCM, 0 °C for **16c**; v) Cesium carbonate, dibenzyl(chloromethyl)phosphate, acetonitrile; vi) Pd/C (10 % w/w), THF; vii) TFA, DCM; viii) KOH, (or NaOH, or tromethamine, or lysine) water, lyophilization.

Solubility and stability profile

- 10 The determination of solubility in different vehicle was carried out (Table 1). Compounds solubility was evaluated initially in water and in phosphate buffered saline (PBS) at pH 7.4, after 24 hours stirring at 25 °C. While **MEDS433** confirmed its low solubility, instead **1** and its different salts **2**, **2a**, **2b**, **2c** display a better solubility, reaching values closer to 1 mM in water. In analogy to prodrug **1**, also prodrugs **5** and **7** improve the solubility of related compounds **6**
- 15 (1 µM in PBS) and **8** (8 µM in PBS¹³). For compounds **1** and **2**, the solubility in simulated gastric and intestinal fluids was also measured, to mimic the passage in the gastrointestinal tract and to observe the variation of solubility in these fluids. As expected, the solubility of the two compounds **1** and **2** became lower at acidic pH and while it is better at the intestinal pH of 6.5, still falling in the high µM range. However, their solubility remains always evaluable.

20

Table 1. Solubility at 24 h in different vehicle of **MEDS433**, its prodrugs **1**, **2**, **2a**, **2b**, **2c** and prodrugs **5** and **7**.

Vehicle	MEDS433 µM	1 µM	2 µM	2a µM	2b µM	2c µM	5 µM	7 µM
Water	2	347	1053	1390	1521	1107	742	325
PBS (pH: 7.4)	6	497	644	469	390	530	267	55
SGF ^a	n.d.	10	49	n.d.	n.d.	n.d.	n.d.	n.d.
SIF ^b	n.d.	306	338	n.d.	n.d.	n.d.	n.d.	n.d.

- 25 a) Simulated gastric fluid 37 °C (pH 1.6); b) Simulated intestinal fluid 37 °C (pH 6.5); n.d. not detected.

The analyses of the stability profile of **1** in different mouse organ homogenates and in human serum were performed to demonstrate the capacity to release active compound **MEDS433**.

- 5 The results, showed in Figure 1, demonstrate that **1** is capable of releasing **MEDS433** in liver, kidney, and intestine rapidly, while is stable in human serum until 24 hours. Like compound **1** also compounds **5** and **7** are able to release the related compounds **6** and **8** in mouse homogenates.

10 ***In vivo pharmacokinetic studies and drug releasing***

- The preclinical pharmacokinetic of **MEDS433** and compound **2** in CD1 mice after both oral and intravenous administration was studied. All treatments were well tolerated, and no clinical signs of toxicity were observed. Collected biological samples were analysed by Mass Spectrometry. The concentration time data of **MEDS433** and **2** were elaborated according to non-
- 15 compartmental analysis to obtain the pharmacokinetic profile of the drugs and the main pharmacokinetic parameters.

Table 2. MEDS433 main pharmacokinetic parameters after intravenous and oral administration

Administration route (dose)	Tissue	C _{max} (ng/mL)	T _{max} (h)	C ₀ (ng/mL)	AUC _{0-last} (ng/mL*h)	AUC _{0-inf} (ng/mL*h)	HL (h)	Cl/F (L/(h*kg))	Vd (L/Kg)	F%
IV	Plasma	15840	0.08	26616	14028	14051	3.6	0.36	1.84	
	Liver	4097	0.25		16433					
	Lung	1589	0.25		4401					
	Brain	142	0.25		519					
PO (20 mg/kg)	Plasma	2539	0.25	-	30224	30275	3.4	0.66	3.11	54
	Liver	2556	0.25		37552					
	Lung	1630	0.25		9461					
	Brain	53	0.25		708					

20

- As shown in Figure 2, which pictures concentration vs time data obtained after IV and PO administration, **MEDS433** rapidly distributes into the mouse body, within half an hour. From a visual inspection of the curves, early peak concentrations of 15840 and 2539 ng/mL can be seen, followed by a rebound of concentrations at 2 and 8 hours, suggesting that the drug
- 25 undergoes enterohepatic recirculation. After this intermediate phase, characterized by slow concentration decay, **MEDS433** disappears from plasma with a half-life of 3.6 and 3.4 hours

- 5 after IV and PO (Table 2), respectively, and with a plasmatic clearance (Cl/F of 0.36 L/(h*kg). Drug levels were measurable up to 32 and 24 hours after PO and IV, respectively. Comparing the AUC_{0-inf} obtained in the two routes of administration, the bioavailability (F) resulted 54%. **MEDS433** was mainly excreted in feces. Looking at Table 2A, can be observed how about 80 % of the drug dose was recovered in feces after the two routes of administrations.
- 10 In urine (Table 2B), the percentage of the dose recovered amounted to 13 % and 5 % after IV and PO, respectively.

Table 2A. Faecal excretion (% of dose) of **MEDS433** after intravenous and oral administration of **MEDS433** and compound **2**.

Time (h)	MEDS433 IV	MEDS433 OS	MEDS433 from 2 IV	MEDS433 from 2 OS
24	81.7 %	80.2 %	86.5 %	55.5 %
48	1.3 %	3.3 %	2.7 %	2.0 %

15

Table 2B. Urinary excretion (% of dose) of **MEDS433** after intravenous and oral administration of **MEDS433** and compound **2**.

Time (h)	MEDS433 IV	MEDS433 OS	MEDS433 from 2 IV	MEDS433 from 2 OS
24	12.6 %	2.7 %	20.7 %	-12.6 %
48	0.85 %	2.2 %	0.4 %	-0.3 %

- 5 **Table 3.** Pro-drug **2** main pharmacokinetic parameters obtained after intravenous and oral administration at doses of 6.85 mg/Kg and 27.38 mg/Kg equivalent to 5 mg/Kg and 20 mg/Kg of **MEDS433**.

Administration route (dose)	Tissue	C _{max} (ng/mL)	T _{max} (h)	C ₀ (ng/mL)	AUC _{0-last} (ng/mL*h)	AUC _{0-inf} (ng/mL*h)	HL (h)	Cl/F (L/(h*kg))	Vd (kg/L)	F%
IV	Plasma	71999	0.08	131623	25295	25314	1.1	0.27	0.42	
	Liver	985	0.25		873					
	Lung	2556	0.25		3675					
	Brain	13	0.25		-					
PO	Plasma	62	0.25	-	67	-	-	-	-	0.07
	Liver	46	0.25		-					
	Lung	1237	0.25		670					
	Brain	-	-		-					

- 10 **Table 4.** **MEDS433** main pharmacokinetic parameters obtained after intravenous and oral administration of compound **2** at doses of 6.85 mg/Kg and 27.38 mg/Kg equivalent to 5 mg/Kg and 20 mg/Kg of **MEDS433**.

Administration route (dose)	Tissue	C _{max} (ng/mL)	T _{max} (h)	C ₀ (ng/mL)	AUC _{0-last} (ng/mL*h)	AUC _{0-inf} (ng/mL*h)	HL (h)	Cl/F (L/(h*kg))	Vd (kg/L)	F%
IV	Plasma	18088	0.08	22929	20535	20581	3.8	0.24	1.35	
	Liver	11937	0.25		41066					
	Lung	6397	0.25		15325					
	Brain	249	0.25		324					
P.O.	Plasma	9440	0.25	-	105972	106257	3.6	0.19	0.97	130
	Liver	13756	0.25		184697					
	Lung	7982	0.25		30482					
	Brain	81	0.25		960					

- 15 As shown in Figure 3 (part A and B) which plots concentration vs time data of **2** obtained after IV and PO administrations, cpd **2** distributed rapidly in the body and disappears from plasma with a half-life of 1.1 h (Table 3), very rapid Cl/F of 0.27 L/(h*kg) and with measurable plasma levels up to 8 hours. Due to almost no absorption (F < 0.1%) and detectable levels only up to

5 2 hours, the half-life is not calculable after oral route. After intravenous administration, cpd **2** AUC in liver and lung were 30 and 7 times lower than in plasma, respectively. Interestingly after oral administration, cpd **2** AUC in lung was ten times higher than in plasma, but not measurable in liver. Cpd **2** was never detectable in brain (Table 3). From Figure 3A after IV administration of cpd **2**, a prompt release of **MEDS433** leading to a plasma C_0 of 22929 ng/mL
10 can be noticed. **MEDS433** is promptly released also after PO leading to a C_{max} of 9440 ng/mL at 15 min (Figure 3B and Table 4). This is expected because cpd **2** probably undergoes massive first-pass effect releasing high concentrations of **MEDS433**, then due to enterohepatic recirculation, a sustained concentration profile with bioavailability over than 100 % can be observed. Similarly, as shown after administration of **MEDS433**, even after the doses of cpd
15 **2**, **MEDS433** disappears from plasma with a half-life of 3.8 and 3.6 hours, after IV and PO route respectively and with plasma clearance slightly lower 0.24 L/(h*kg), but with measurable plasma levels up to 32 hours after both routes. After intravenous administration of cpd **2**, the experimental AUC of **MEDS433** was two times higher in liver than in plasma, slightly lower in lung and 60 times lower in brain. After oral route, **MEDS433** AUC resulted 1.7-fold higher in
20 liver than in plasma, but three-fold lower in lung and 100-fold lower in brain. Cpd **2** was never detectable in feces and urine being, as mentioned before, the drug is converted into **MEDS433** that was mainly excreted in feces. Looking at Table 2A, about 80 % and 50 % of **MEDS433** dose was recovered in feces after IV and PO, respectively. In urine (Table 2B), the percentage of the **MEDS433** recovered amounted to 20 % and 12 % after IV and PO, respectively.

25

The comparison of the pharmacokinetics of **MEDS433** after administration of the drug itself, and of **MEDS433** obtained after administration of the pro-drug **2**, shows that:

- the pharmacokinetic profiles after IV administration are substantially superimposable (Figure 4, part A), even if there is evidence of more prolonged, up to 32 hours, exposure to **MEDS433** after cpd **2**.
- important differences were observed in bioavailability. After oral administration of cpd **2** the concentration vs time curves of **MEDS433** in plasma showed similar trend, but with higher levels compared to those observed after oral administration of **MEDS433**, leading to F > 100 % vs 54 % (see Figure 4, part B).

30

- 5 • important differences were observed in the drug tissue distribution of MEDS433 in liver and lung in which AUC resulted two-five times higher after the treatment with cpd 2 than with **MEDS433**.

Antitumoral activity.

- 10 The *in vitro* activity profile of preferred compounds **MEDS433** and **6** against the above-mentioned tumors are reported in Table 5. Both falling within Formulae (Ia), **MEDS433** and **6** are released *in vivo* from the correspondent prodrug **1** and **5**, these latter falling within Formulae (I).
- 15 **Table 5.** The *in vitro* activity profile of preferred compounds **MEDS433** and **6** against different tumors.

Tumors	Cell line	MEDS433 IC ₅₀ (72h, nM)	6 IC ₅₀ (72h, nM)
<i>Osteosarcoma</i>	SAOS-2	470.4	515.6
<i>Ewing sarcoma</i>	A673	59.85	50.90
	TC71	31.87	31.93
<i>Bladder carcinoma</i>	T24	56.1	17.8
<i>IDH mutated Fibrosarcoma</i>	HT1080	Not tested	4.9
<i>Glioblastoma</i>	LN18	84.9	77.18
<i>IDH mutated Glioblastoma</i>	U87 IDH mt	Not tested	121.3
<i>Breast cancer</i>	MDA MB 231	103.7*	91.9*
<i>Melanoma</i>	A375	105.1	97.9
	SF-7761	31.09	9.26
H3K27M-mutated diffuse midline glioma	SF-8628	Not tested	112*
	LAN-5	91.93	88.52
<i>Neuroblastoma</i>	SHSY5Y	Not tested	15.6
	A549	501.74	485.9

*IC₅₀ 5 days

- 20 The obtained submicromolar IC₅₀ showed the high potency of the preferred compound MEDS433 and **6** to inhibit the tumour cellular proliferation. Notable the high potency of **6** to inhibit the proliferation of Ewing sarcoma, Neuroblastoma, Bladder carcinoma, IDH-mutated fibrosarcoma and glioblastoma and H3K27M-mutated diffuse midline glioma cell line with an IC₅₀ in the nanomolar range.

5

In vivo pharmacokinetic studies of compounds 6 and 8

In preparation to *in vivo* efficacy assay the preliminary preclinical pharmacokinetic of compound **6** in Balb/c mice after both oral and intravenous administration was studied. All treatments were well tolerated, and no clinical signs of toxicity were observed. Collected biological samples were analysed by Mass Spectrometry. The concentration time data of **6** was elaborated according to non-compartmental analysis to obtain the pharmacokinetic profile of the drugs and the main pharmacokinetic parameters.

Table 6. Compound **6** main pharmacokinetic parameters after intravenous and oral administration

Admin route	Tissue	C _{max} (ng/mL)	T _{max} (h)	C ₀ (ng/mL)	AUC _{0-last} (ng/mL*h)	AUC _{0-inf} (ng/mL*h)	HL (h)	Cl (L/h*kg)	Vd (L/kg)	F %
IV	Plasma	66300	0.25	74400	382600	435100	8.1	0.115	0.135	
	Brain	720	0.25		4636					
PO	Plasma	43130	0.25	-	473400	658300	>12.0	-	-	37.8
	Brain	670	0.25		7059					

As shown in Figure 5, which pictures concentration vs time data obtained after IV and PO administration of compound **6**, after IV route the drug appears distributed in the body within 2 hour and disappears from plasma with a half-life of 8 hour. After oral administration, there is slower absorption than IV route and enterohepatic recirculation at 2 and 4 hours, then drug disappears from plasma with a long half-life >12 hours. Drug levels were measurable up to 24 hours after both way of administrations. The main pharmacokinetic parameters are listed in **Table 6**. Comparing the compound **6** AUC_{0-inf}, obtained after the two routes of administration the bioavailability (F) results 37.8 %. The plasmatic clearance (Cl) and the volume of distribution Vd, of 0.115 L/h/Kg and 0.135 L/Kg, indicate low drug distribution in the body fluid.

- 5 Compound **6** was measurable in brain early at 15 min, and up to 24 hours after both PO and IV administration.

The preliminary preclinical pharmacokinetic of compound **8** in CD1 mice after both oral and intravenous administration was also studied. All treatments were well tolerated, and no clinical signs of toxicity were observed. Collected biological samples were analysed by Mass Spectrometry. The concentration time data of **8** was elaborated according to non-compartmental analysis to obtain the pharmacokinetic profile of the drugs and the main pharmacokinetic parameters.

Table 7. Compound **8** main pharmacokinetic parameters after intravenous and oral administration of 20 and 5 mg/kg of the drug

Admin route	Tissue	C _{max} (ng/mL)	T _{max} (h)	C ₀ (ng/mL)	AUC _{0-last} (ng/mL*h)	AUC _{0-inf} (ng/mL*h)	HL (h)	Cl (L/h*kg)	Vd (L/kg)	F %
IV	Plasma	21300	0.25	32110	48980	49090	3.7	0.102	0.54	
	Brain	430	0.25		696					
PO	Plasma	19430	0.25	-	127570	127820	3.4	-	-	65.1
	Brain	350	0.25		1643					

As shown in Figure **6**, which pictures concentration vs time data obtained after IV and PO administration of compound **8**, the drug appears rapidly distributed in the body within 1 hour, then it is probably subjected to entero-hepatic re-circulation mainly after PO (notable a rebound of concentration at 2 h) rather than after IV route, and disappears from plasma with a half-life of 3.4 and 3.7 hours, respectively. From a visual inspection of the curves, plasma levels above the LOQ (10 ng/mL) were measurable up to 32 hours after both route of administration.

The main pharmacokinetic parameters are listed in **Table 7**. Comparing the compound **8** AUC_{0-inf}, obtained after the two routes of administration the bioavailability (F) result is 65.1 %. The plasmatic clearance (Cl) and the volume of distribution Vd, of 0.101 L/h/Kg and 0.54 L/Kg, indicate moderate drug distribution in the body fluid.

- 5 Compound **8** was measurable in brain early at 15 min, and up to 8 hours after both PO and IV administration.

Use in combination

- The *in vitro* activity profile on AML cells of preferred compound **MEDS433** in combination with
10 five drugs are reported in Figure 7 (panel A and B) and in Figure 8 (panel A-D) showing the % of apoptotic cells after three days of treatment. The anti-tumour activity of **MEDS433** is potentiated by combination with registered and common drugs, able to enhance its pro-apoptotic activity. In addition, **compound 6** shows a synergistic activity also with three different anticancer drugs (Figure 9). These data support the potential use of **compound 6** in
15 combination with panabinoestat, abemaciclib and ONC212 in diffuse midline glioma and also in cancer where these medicinal products are clinical recommended.

Conclusions

- This disclosure covers prodrugs of human dihydroorotate dehydrogenase (*h*DHODH) inhibitors structurally based on hydroxyazole scaffold and methods of making and using thereof. As
20 representative compound, **2** was found able to effectively release the parent drug **MEDS433** in *in vitro* as well as *in vivo* environments. The strategy was able to successfully increase the solubility, being solubility of **2** increased x 500 in water compared to the drug **MEDS433**, as well as the bioavailability. In fact, after oral administration of **2** the concentration vs time curves
25 of **MEDS433** in plasma showed higher levels compared to those observed after oral administration of **MEDS433** itself, leading to F > 100 % vs 54 %.

- The improvement of PK parameters here demonstrated is crucial to optimize the *in vivo* treatment of aggressive solid tumors targeted by *h*DHODH inhibitors object of this disclosure. This improvement along with the potent anticancer profile demonstrated in *in vitro* cancer cell
30 line models illustrated in Table 5 represent an innovative solution to efficiently target, in association with standard of care, cancers that are still unmet medical need in oncology.

- In particular the data described herein show that solid tumors that express low levels of GPX4 (A549 and MDA MB 231 reported in Table 5)^{9, 15} can be effectively targeted by *h*DHODH inhibitors to reduce tumor burden, and that a combination of *h*DHODH inhibitors with known
35 ferroptosis inducers, such as sulfasalazine, sorafenib and artemisinin derivatives (i.e. artesunate and dihydroartemisinin), can be used to treat GPX4_{high} and ferroptosis sensitive

5 tumors. Furthermore, our data reveal a promising antiproliferative activity of our compounds in IDH mutated cancer. This evidence indicates that IDH mutated cancer are probably hyper dependent on de novo pyrimidine nucleotide synthesis. This preferential pharmacological activity could represent a new therapeutic strategy to treat IDH mutated cancer, also in combination with IDH inhibitors.

10

In addition, *h*DHODH inhibitors could be combined with other standard-of-cares in cancer therapy that can induce ferroptosis, such as chemotherapy, radiotherapy and immunotherapy.

Looking for synergic combinations to boost apoptosis in AML cells, MEDS433 was combined
15 with classical antileukemic drugs and/or drugs targeting pyrimidine salvage pathway. The combination of MEDS433 with selected drugs increased significantly the apoptotic rate, demonstrating synergistic effects.

Materials and methods

20 *Chemistry*

General methods.

All chemical reagents were obtained from commercial sources (Sigma Aldrich, Alfa Aesar, FluoroChem and BLD Pharma), and used without further purification. Thin-layer chromatography (TLC) was carried out to monitor reaction progress. Analytical grade solvents
25 (acetonitrile, diisopropyl ether, diethyl ether, dichloromethane [DCM], dimethylformamide [DMF], ethanol 99.8 % v/v, ethyl acetate [EtOAc], hexane, methanol [MeOH], petroleum ether b.p. 40 – 60 °C [*petroleum ether*], toluene), were used without further purification. When needed, solvents were dried over 4 Å molecular sieves. Tetrahydrofuran (THF) was distilled from Na and benzophenone under N₂ immediately prior to use. Thin layer chromatography
30 (TLC), on silica gel was carried out on 5 x 20 cm plates at 0.25 mm layer thickness. Anhydrous Na₂SO₄ was used as a drying agent for the organic phases. Compound purification was either achieved using flash column chromatography on silica gel (Merck Kieselgel 60, 230-400 mesh ASTM), and the eluents indicated in the procedures for each compound, or using CombiFlash Rf 200 (Teledyne Isco), with 5–200 mL/min, 200 psi (with automatic injection valve), and
35 RediSep Rf Silica columns (Teledyne Isco), with the eluents indicated in the procedures for each compound. Compounds synthesized in our laboratory generally varied between 90 %

5 and 99 % purity. Biological experiments were performed on compounds with a purity of at least 95 %. Purity was checked using two analytical methods. HPLC analyses were performed on an UHPLC chromatographic system (Perkin Elmer, Flexar). The analytical column was an UHPLC Acquity CSH Fluoro-Phenyl (2.1x100 mm, 1.7 μ m particle size, Waters). Compounds were dissolved in MeOH and injected through a 20 μ l loop. The mobile phase consisted of
10 MeOH / water with 0.1 % trifluoroacetic acid (ratio between 90 / 10 and 20 / 80, depending on the compound's retention factor). UHPLC retention times were obtained at flow rates of 0.5 mL/min, and the column effluent was monitored at 215 and 254 nm, referenced against a 360 nm wavelength. Solubility assays, and stability assays were performed on UHPLC chromatographic system (Perkin Elmer, Flexar). Melting points (m.p.) were measured on a
15 capillary apparatus (Büchi 540). Final m.p. determination was achieved by placing the sample at a temperature 10° C below the m.p. and applying a heating rate of 1° C min⁻¹. All compounds were routinely checked by ¹H and ¹³C NMR and mass spectrometry. MS spectra were performed on Waters Micromass ZQ equipped with an ESCi source for electrospray ionization mass spectra. ¹H and ¹³C NMR spectra were either performed on a JEOL ECZR600. The
20 following abbreviations are used for coupling patterns: br = broad, s = singlet, d = doublet, dd = doublet of doublets, t = triplet, q = quartet, m = multiplet. Chemical shifts (δ) are given in parts per million (ppm). In this work protons and carbons are labelled (a, b, c, d, e, f, g, h, i, m, n and o) according to Scheme 2. Values marked with an asterisk are interchangeable. Not for all the tetrafluorinated biphenyl compounds detailed ¹³C spectra, have been entirely reported
25 due to their especially complicated patterns (attributable to the multiple couplings between fluorine and carbon atoms). For some compounds, only the ¹³C signals caused by the heterocyclic substructure and non-aromatic carbons are assigned; for other compounds only the MS spectra were reported. Compound **6a** was prepared according to previously described procedures.¹¹

30

Ethyl 3-ethoxy-1H-pyrazole-4-carboxylate (**10**). Diethyl 2-(ethoxymethylene) malonate (3 g, 0.0139 mol) was added to a solution of hydrazine hydrochloride (950 mg, 13.9 mmol) in EtOH abs (30 mL). The resulting mixture was refluxed overnight, then saturated aqueous solution of NaHCO₃ (70 mL) was added. The mixture was extracted with EtOAc (2 x 50 mL) and the
35 combined organic layers were dried over Na₂SO₄ and evaporated under reduced pressure. The resulting crude product was purified by flash chromatography (eluent:

5 dichloromethane/MeOH 95/5 v/v) affording the title compound as a white solid. Yield 36%. ¹H NMR (600 MHz, *chloroform-d*), δ 1.32 (*t*, J = 7.1 Hz, 3H, -OCH₂CH₃), 1.41 (*t*, J = 7.1 Hz, 3H, -OCH₂CH₃), 4.27 (*q*, J = 7.1 Hz, 2H, -OCH₂CH₃), 4.33 (*q*, J = 7.0 Hz, 2H, -OCH₂CH₃), 7.87 (*s*, 1H, pyr-*H*), 10.45 (*br s*, 1H, -NH). ¹³C NMR (151 MHz, *chloroform-d*), δ 14.5 (C-*m*), 14.8 (C-*i*), 60.2 (C-*l*), 65.4 (C-*h*), 99.7 (C-*e*), 134.0 (C-*d*), 162.5 (C-*g*), 163.2 (C-*f*). MS (ESI⁺): 183 (M-1).

10

Ethyl 1-(2,2-diethoxyethyl)-3-ethoxy-1H-pyrazole-4-carboxylate (11). Ethyl 3-ethoxy-1H-pyrazole-4-carboxylate (**10**), 2-bromo-1,1-diethoxyethane and Cs₂CO₃ were solubilized in dry DMF. The obtained mixture was heated at 85 °C overnight, then the reaction was quenched in water (50 mL) and extracted with EtOAc (3 x 50 mL) and the combined organic layers were
15 dried over Na₂SO₄ and evaporated under reduced pressure. The resulting crude product was purified by flash chromatography (eluent: petroleum ether / EtOAc 80/20 v/v) affording the title compound as a colorless oil. Yield 68 %. ¹H NMR (600 MHz, *chloroform-d*), δ 1.14 (*t*, J = 7.0 Hz, 6H, -(OCH₂CH₃)₂), 1.31 (*t*, J = 7.1 Hz, 3H, -OCH₂CH₃), 1.42 (*t*, J = 7.1 Hz, 3H, -OCH₂CH₃), 3.37 – 3.45 (*m*, 2H, -CH(OCH₂CH₃)₂), 3.65 – 3.73 (*m*, 2H, -CH(OCH₂CH₃)₂), 3.97 (*d*, J = 5.4
20 Hz, 2H, -CH₂CH-), 4.25 (*q*, J = 7.1 Hz, 2H, -OCH₂CH₃), 4.29 (*q*, J = 7.0 Hz, 2H, -OCH₂CH₃), 4.74 (*t*, J = 5.4 Hz, -CH₂CH-), 7.72 (*s*, 1H, pyr-*H*); ¹³C NMR (151 MHz, *chloroform-d*), δ 14.5 (C-*m*), 14.8 (C-*i*), 15.4, 55.6, 59.9, 64.0, 65.3, 99.7, 100.9, 135.7, 162.2, 162.7. MS (ESI⁺): 301 (M+1).

25 *Ethyl 1-(2,2-diethoxyethyl)-3-ethoxy-5-formyl-1H-pyrazole-4-carboxylate (12)*. 2.5 M solution of butyl lithium in dry THF (650 μL, 1.612 mmol) was added to a -78°C cooled solution of **11** (440 mg, 1.466 mmol) in dry THF (15 mL). The resulting mixture was stirred at -78 °C for 15 minutes, then dry DMF (200 μL, 2.199 mmol) was added and the reaction mixture was stirred for 20 minutes. The reaction mixture was quenched in sat. aq. ammonium chloride solution
30 (150 mL) and the resulting mixture was extracted with Et₂O (2 x 50 mL) and the combined organic layers were dried over Na₂SO₄ and evaporated under reduced pressure. The resulting crude product was purified by flash chromatography (eluent: *petroleum ether* / EtOAc 95/5 v/v) affording the title compound as a colorless oil. Yield 77 %. ¹H NMR (600 MHz, *chloroform-d*), δ 1.13 (*t*, J = 7.0 Hz, 6H, -CH(OCH₂CH₃)₂), 1.37 (*t*, J = 7.1 Hz, 3H, -OCH₂CH₃), 1.44 (*t*, J = 7.0
35 Hz, 3H, -OCH₂CH₃), 3.42 – 3.49 (*m*, 2H, -CH(OCH₂CH₃)₂), 3.65 – 3.72 (*m*, 2H, -CH(OCH₂CH₃)₂), 4.32 – 4.38 (*m*, 4H, -OCH₂CH₃), 4.54 (*d*, J = 5.6 Hz, 2H, -CH₂CH-), 4.81 (*t*, J

5 = 5.6 Hz, 1H, -CH₂CH-), 10.37 (s, 1H, -COH); ¹³C NMR (151 MHz, *chloroform-d*), δ 14.4, 14.7, 15.3, 53.5, 60.9, 62.6, 65.8, 100.5, 103.0, 139.5, 161.3, 162.3, 183.4. MS (ESI⁺): 329 (M+1).

Ethyl 2-ethoxypyrazolo[1,5-a]pyrazine-3-carboxylate (13). Compound **12** (300 mg, 0.914 mmol, 1.0 eq.) was solubilized in trifluoroacetic acid (2.3 mL), water (0.7 mL), and
10 tetrahydrofuran (1.0 mL) at room temperature. The reaction mixture was stirred at 50 °C for 4 h, then was cooled to room temperature, and concentrated to give a residue, which was taken up with toluene and concentrated three times (3 x 5 mL). The resultant residue was combined with ethanol (3 mL), acetic acid (1.3 mL), and ammonium acetate (211 mg, 2.742 mmol, 3.0 eq.), and the mixture was stirred at room temperature for 20 minutes. The reaction mixture
15 was concentrated, and then water (40 mL) was added. The mixture was stirred at room temperature, and solid potassium carbonate was added portion-wise until the aqueous layer reached pH 8. Then, the aqueous layer was extracted with EtOAc (2 x 30 mL). The combined organic layers were dried over Na₂SO₄ and concentrated under reduced pressure to give a crude which was purified by flash chromatography (eluent: DCM / EtOAc 9:1 v / v) to afford the
20 title compound as a white solid. Yield 78 %. ¹H NMR (600 MHz, *chloroform-d*), δ 1.42 (t, J = 7.1 Hz, 3H, -OCH₂CH₃), 1.51 (t, J = 7.0 Hz, 3H, -OCH₂CH₃), 4.40 (q, J = 7.1 Hz, 2H, -OCH₂CH₃), 4.51 (q, J = 7.1 Hz, 2H, -OCH₂CH₃), 7.99 (d, J = 4.5 Hz, 1H, *H-a*), 8.20 (d, J = 4.3 Hz, 1H, *H-b*), 9.37 (s, 1H, *H-d*); ¹³C NMR (151 MHz, *chloroform-d*), δ 14.6, 14.8, 60.4, 66.4, 89.4, 121.6, 130.5, 136.9, 144.1, 162.3, 165.3; MS (ESI⁺): 236 (M+1).

25
2-Ethoxypyrazolo[1,5-a]pyrazine-3-carboxylic acid (14b). 5 M NaOH (5.0 eq.) was added to a solution of **13** (290 mg, 1.233 mmol) in EtOH (10 mL). The solution was stirred for 3 h at 60 °C, and then neutralized with 6 M HCl and concentrated under reduced pressure. Subsequent, a phosphate buffer solution pH 4 (40 mL) was added, and the aqueous layer was extracted
30 with EtOAc (10 x 25 mL). The combined organic layers were dried over Na₂SO₄ and concentrated under reduced pressure to give a residue which was purified by titration with diisopropyl ether to afford the title compound as white solid. Yield 76 %. ¹H NMR (600 MHz, *DMSO-d*₆), δ 1.40 (t, J = 7.0 Hz, 3H, -OCH₂CH₃), 4.43 (q, J = 7.1 Hz, 2H, -OCH₂CH₃), 8.06 (d, J = 4.5 Hz, 1H, *H-a*), 8.75 (dd, J = 4.5, 1.4 Hz, 1H, *H-b*), 9.24 (d, J = 1.4 Hz, 1H, *H-d*), 12.57
35 (s, 1H, -COOH); ¹³C NMR (151 MHz, *DMSO-d*₆), δ 14.6, 65.6, 88.6, 122.4, 130.8, 136.6, 142.8, 162.8, 164.2; MS (ESI⁻): 206 (M-1).

5

General procedure for synthesis of amide 16a - 16d.

Oxalyl chloride (2.2 eq.) and dry DMF (20 μ L) were added to a cooled (0 °C) solution of corresponding acid (**14a,b**, 1.0 eq.) in dry THF kept under a nitrogen atmosphere. The resulting mixture was stirred for 2 h at room temperature. In parallel, a 1 M THF solution of LiHMDS in
 10 (2.2 eq.) was added to a solution of appropriate aniline (2.0 eq.) in dry THF under a nitrogen atmosphere. The resulting suspension was stirred for 50 minutes at room temperature. The solution of corresponding acyl chloride (**15a,b**) was then concentrated under reduced pressure, and the residue was dissolved in dry THF (50 mL); this step was repeated three times to eliminate all gaseous residues. The corresponding acyl chloride was then dissolved
 15 in dry THF, and the solution was added to the described above solution via *cannula*. The reaction mixture was stirred at -10 °C for 30 minutes, then was quenched in ammonium saturated solution and the organic layer was separated. The aqueous solution was extracted with EtOAc (2 x 120 mL), and the combined organic layers were washed with brine, dried over Na₂SO₄, and evaporated under reduced pressure. The crude material was purified using flash
 20 chromatography.

2-((4-Methoxybenzyl)oxy)-N-(2,3,5,6-tetrafluoro-[1,1'-biphenyl]-4-yl)pyrazolo[1,5-a]pyridine-3-carboxamide (16a). The crude material was purified using flash chromatography (eluent: *petroleum ether*/EtOAc 8/2 v/v and then 7/3 v/v) to afford the title compound as a white solid.
 25 Yield 90 %. ¹H NMR (600 MHz, *chloroform-d*) δ : 3.83 (s, 3H, -OCH₃), 5.52 (s, 2H, -OCH₂Ar), 6.91 (*td*, 1H, J = 6.9, 1.0 Hz, *H-b*), 6.96 (*d*, 2H, J = 8.6 Hz, *H-n*), 7.40 – 7.51 (*m*, 8H, *aromatic protons* and *H-c*), 8.26 (*d*, 1H, J = 8.8 Hz, *H-d*), 8.31 (s, 1H, -NH), 8.34 (*d*, 1H, J = 6.8 Hz, *H-a*); ¹³C NMR (151 MHz, *chloroform-d*) δ : 55.5 (-OCH₃), 72.2 (-OCH₂Ar), 90.0 (*C-f*), 113.3 (*C-b*), 114.3 (*C-n*), 115.8 (*t*, J = 15.8 Hz, *C-s*)*, 117.9 (*t*, J = 17.3 Hz, *C-p*)*, 118.8 (*C-d*), 127.5,
 30 127.7, 128.3 (*C-l*), 128.7, 128.8 (*C-m*), 129.2, 130.3, 142.6 (*dd*, J = 248.1, 15.8 Hz, *C-r*)**, 143.2 (*C-e*), 144.1 (*dd*, J = 242.0, 15.3 Hz, *C-q*)**, 160.2 (*C-o*)***, 160.6 (*C-h*)***, 163.0 (*C-g*)***; ¹⁹F NMR (564 MHz, *chloroform-d*) δ : -144.66 (*d*, J = 25.4 Hz), -145.81 (*d*, J = 27.9 Hz); MS (ES⁺): 522 (M + 1).

35 *N-(3'-(2,2-Difluoropropoxy)-2,3,5,6-tetrafluoro-[1,1'-biphenyl]-4-yl)-2-((4-methoxybenzyl)oxy)pyrazolo[1,5-a] pyridine-3-carboxamide (16b)*. The crude product was

- 5 purified by flash chromatography (eluent: *petroleum ether* / EtOAc 8/2 then 7:3 v/v) to obtain the title compound as solid. The latter was then triturated with diisopropyl ether, and the title compound was obtained as a yellow solid (mp 114.6 - 117.0 °C). Yield 79 %. ¹H NMR (600 MHz, *chloroform-d*) δ: 1.79 (t, 3H, J = 18.8 Hz, -OCH₂CF₂CH₃), 3.83 (s, 3H, -OCH₃), 4.14 (t, 2H, J = 11.3 Hz, -OCH₂CF₂CH₃), 5.52 (s, 2H, -CH₂Ar), 6.92 (td, 1H, J = 6.9, 1.1 Hz, *H-b*), 6.95 (d, 2H, J = 8.6 Hz, *H-n*), 6.99 - 7.03 (m, 2H, *aromatic protons*), 7.09 (d, 1H, J = 7.5 Hz, *aromatic proton*), 7.40 - 7.45 (m, 2H, *aromatic protons and H-c*), 7.48 (d, 2H, J = 8.6 Hz, *H-m*), 8.25 (d, 1H, J = 8.8 Hz, *H-d*), 8.31 (s, 1H, -NH-), 8.34 (d, 1H, J = 6.9 Hz, *H-a*). ¹³C NMR (151 MHz, *chloroform-d*) δ: 21.1 (t, J = 25.6 Hz, -OCH₂CF₂CH₃), 55.5 (-OCH₃), 69.9 (t, J = 35.0 Hz, -OCH₂CF₂CH₃), 72.3 (-CH₂Ar), 90.0 (*C-f*), 113.3 (*C-b*), 114.3 (*C-n*), 115.5, 116.2 (t, J = 17.3 Hz, *C-s*)*, 116.7, 117.4 (t, J = 17.3 Hz, *C-p*)*, 118.8 (*C-d*), 121.5 (t, J = 239.6 Hz, -OCH₂CF₂CH₃), 123.8, 127.7, 128.3 (*C-a*), 128.8, 128.9, 129.9 (*C-c*), 130.3 (*C-m*), 140.9 (d, J = 255.9 Hz, *C-r*)**, 143.2 (*C-e*), 144.1 (d, J = 251.1 Hz, *C-q*)**, 158.1, 160.2, 160.5, 163.0; MS (ES⁺): 616 [M+1].
- 20 2-((4-Methoxybenzyl)oxy)-N-(2,3,5,6-tetrafluoro-3'-(trifluoromethoxy)-[1,1'-biphenyl]-4-yl)pyrazolo[1,5-a]pyridine-3-carboxamide (**16c**). The crude product was purified by flash chromatography (eluent: *petroleum ether* / EtOAc 7:3 v/v) to obtain the title compound as yellow solid (3.0 g, 4.95 mmol). Yield 65%. ¹H NMR (600 MHz, *chloroform-d*) δ: 3.83 (s, 3H, -ArOCH₃), 5.52 (s, 2H, -OCH₂Ar), 6.92 (td, 1H, J = 6.9, 1.4 Hz, *H-b*), 6.96 (d, 2H, J = 8.7 Hz, *H-v*), 7.30 - 7.34 (m, 2H, *H-c and H-n*), 7.38 - 7.44 (m, 2H, *H-r*), 7.48 (d, 1H, J = 8.7 Hz, *H-u*), 7.52 (t, 1H, J = 8.1 Hz, *H-p*)*, 8.24 - 8.27 (m, 1H, *H-q*)*, 8.32 - 8.36 (m, 2H, *H-a and -NH*). ¹³C NMR (151 MHz, *chloroform-d*) δ: 55.5 (-OCH₃), 72.3 (-CH₂Ar), 89.9 (*C-f*), 113.4 (*C-b*), 114.3 (*C-v*), 116.2 (t, J = 16.7 Hz), 116.7 (t, J = 17.5 Hz), 118.8, 120.6, (q, J = 257.8 Hz), 121.6 (*C-p*)***, 123.0 (*C-r*)***, 127.7 (*C-a*)**, 128.3 (*C-c*)**, 128.78 (*C-t*), 128.83 (*C-d*), 129.3 (*C-m*), 130.2 (*C-q*), 130.3 (*C-u*), 142.6 (d, J = 247.4 Hz), 143.2 (*C-e*), 144.1 (d, J = 246.7 Hz), 149.4 (d, J = 1.1 Hz, -OCF₃), 160.2 (*C-z*), 160.5 (*C-h*)****, 163.0 (*C-g*)****. ¹⁹F NMR (564 MHz, *chloroform-d*) δ: -145.14 (dd, 2F, J = 22.7, 9.9 Hz, *F-k*)*, -144.47 (dd, 2F, J = 21.7, 8.1 Hz, *F-j*)*, -57.75 (s, 3F, -OCF₃). MS (ES⁺): 606 [M+1].
- 35 2-Ethoxy-N-(2,3,5,6-tetrafluoro-[1,1'-biphenyl]-4-yl)pyrazolo[1,5-a]pyrazine-3-carboxamide (**16d**). The crude material was purified by flash chromatography (eluent: from DCM / EtOAc

5 9/1 v/v to 7/3 v/v) to afford the title compound as a white solid. Yield 85 %. ¹H NMR (600 MHz, *Chloroform-d*), δ 1.59 (t, J = 7.1 Hz, 3H, -OCH₂CH₃), 4.68 (q, J = 7.1 Hz, 2H, -OCH₂CH₃), 7.43 – 7.54 (m, 5H, aromatic protons), 8.07 (d, J = 4.6 Hz, 1H, *H-a*), 8.24 (dd, J = 4.6, 1.4 Hz, 1H, *H-b*), 8.36 (s, 1H, -NH), 9.65 (d, J = 1.3 Hz, 1H, *H-d*); ¹³C NMR (151 MHz, *Chloroform-d*), δ 14.9, 67.5, 91.3, 115.3 (t, J = 14.5 Hz), 118.5 (t, J = 17.1 Hz), 121.5, 127.3, 128.8, 129.3, 130.3, 131.4, 137.3, 142.6 (d, J = 248.6 Hz), 144.2 (d, J = 246.0 Hz), 144.8, 159.7, 162.6; ¹⁹F NMR (564 MHz, *DMSO-d*₆) δ: -145.69 (d, J = 21.6 Hz), -144.20 (d, J = 24.0 Hz); MS (ESI⁺): 431 (M+1).

General procedure for synthesis of compounds 17a-d.

NaH (1.1 eq.) was added to a solution of appropriate amide (**16a-d**) (1.0 eq.) in dry THF and
15 the mixture was stirred for 1 h at room temperature. In the following, a solution of di-tert-butyl dicarbonate (1.1 eq.) in 4 mL of dry THF was added and the reaction mixture was heated to reflux. After 8 h additional NaH (0.5 eq.) and di-tert-butyl dicarbonate (0.5 eq.) were added and the reaction mixture was stirred at reflux for 16 h. The reaction mixture was cooled at 0 °C with an ice bath and quenched in phosphate buffer pH = 7 (50 mL), the aqueous layer was extracted
20 with EtOAc (4 x 40 mL). The combined organic layers were washed with brine, dried over Na₂SO₄, filtered, and concentrated under reduced pressure. The crude material was purified via flash chromatography.

Tert-butyl (2-((4-methoxybenzyl)oxy)pyrazolo[1,5-a]pyridine-3-carbonyl)(2,3,5,6-tetrafluoro-
25 *[1,1'-biphenyl]-4-yl)carbamate (17a)*. The crude material was purified via flash chromatography (eluent *petroleum ether* / EtOAc 7/3 v/v) to afford the title compound as white solid. Yield 73 %. ¹H NMR (600 MHz, *chloroform-d*) δ: 1.26 (s, 9H, -CCH₃), 3.80 (s, 3H, -OCH₃), 5.45 (s, 2H, -OCH₂Ar), 6.87 – 6.94 (m, 3H, aromatic protons and *H-b*), 7.39 – 7.54 (m, 8H, aromatic protons and *H-c*), 8.00 (d, 1H, J = 8.8 Hz, *H-d*), 8.33 (d, 1H, J = 6.8 Hz, *H-a*); ¹³C NMR (151 MHz, *DMSO-d*₆) δ: 27.0, 55.1, 70.8, 83.7, 91.9, 113.7, 114.4, 116.7, 118.1 (t, J = 15.2 Hz), 119.8 (t, J = 17.1 Hz), 126.2, 128.0, 128.9, 129.1, 129.7, 130.1, 130.14, 130.19, 142.6, 143.2 (d, J = 245.3 Hz), 150.5, 159.1, 162.3, 163.1. MS (ESI⁺): 622 (M+1).

Tert-butyl (3'-(2,2-difluoropropoxy)-2,3,5,6-tetrafluoro-[1,1'-biphenyl]-4-yl)(2-((4-methoxybenzyl)oxy)pyrazolo[1,5-a]pyridine-3-carbonyl)carbamate (17b). The crude material
35 was purified via flash chromatography (eluent *petroleum ether* / EtOAc 7/3 v / v) to afford the

5 title compound as white vitreous solid (460 mg, 0.699 mmol). Yield: 92%. ¹H NMR (600 MHz, *chloroform-d*) δ: 1.27 (s, 9H, -C(CH₃)₃), 1.79 (t, 3H, J = 18.8 Hz, -OCH₂CF₂CH₃), 3.80 (s, 3H, -OCH₃), 4.16 (t, 2H, J = 11.3 Hz, -OCH₂CF₂CH₃), 5.45 (s, 2H, -OCH₂Ph), 6.87 – 6.91 (m, 3H, aromatic protons and H-b), 7.02 – 7.07 (m, 2H), 7.14 (d, 1H, J = 7.6 Hz, H-r), 7.40 – 7.46 (m, 4H, aromatic protons and H-c), 8.00 (ddd, 1H, J = 8.8, 1.4, 1.0 Hz, H-d), 8.33 (dt, J = 6.9, 1.1
10 Hz, H-a). ¹³C NMR (151 MHz, *chloroform-d*) δ: 21.1 (t, J = 26.0 Hz, -OCH₂CF₂CH₃), 27.7 (-C(CH₃)₃), 55.4 (-OCH₃), 69.9 (t, J = 34.9 Hz, -OCH₂CF₂CH₃), 71.3 (-OCH₂Ph), 84.0 (C-f), 93.3 (-C(CH₃)₃), 113.5 (C-b), 113.9 (aromatic carbon), 115.8, 116.7, 118.2 (C-d), 118.8 (t, J = 19.0 Hz), 119.7 (t, J = 17.0 Hz), 121.6 (t, J = 239.4 Hz, -CH₂CF₂CH₃), 123.9 (C-r), 128.4, 128.7, 128.8 (C-a)**, 129.1 (C-c)**, 129.3 (aromatic carbon), 130.0 (C-q), 143.8 (C-e), 144.0 (dd, 2C, J = 256.4, 10.0 Hz), 151.3 (-COOC(CH₃)₃), 158.1 (C-o), 159.6 (C-OCH₃), 163.5 (C-h)*, 163.7 (C-g)*. ¹⁹F NMR (564 MHz, *chloroform-d*) δ: -145.76 (s, 2F), -144.14 (dd, 2F, J = 21.8, 8.5 Hz), -98.00 - -97.93 (m, 2F). MS (ES⁺): 716 [M+1].

Tert-butyl (2-((4-methoxybenzyl)oxy)pyrazolo[1,5-a]pyridine-3-carbonyl)(2,3,5,6-tetrafluoro-3'-(trifluoromethoxy)-[1,1'-biphenyl]-4-yl)carbamate (17c) The crude material was purified via flash chromatography (eluent *petroleum ether* / EtOAc 7/3 v/v) to afford the title compound as vitreous white solid (2.0 g, 2.83 mmol). Yield: 78%. ¹H NMR (600 MHz, *chloroform-d*) δ: 1.27 (s, 9H, -C(CH₃)₃), 3.80 (s, 3H, -OCH₃), 5.45 (s, 2H, H-s), 6.88 – 6.93 (m, 3H, H-v and H-b), 7.32 – 7.35 (m, 1H, H-r), 7.38 (s, 1H, H-n), 7.40 – 7.46 (m, 4H, H-c, H-d, H-u), 7.55 (t, 1H, J =
20 8.0 Hz, H-p)*, 7.98 – 8.01 (m, 1H, H-q)*, 8.33 (dt, 1H, J = 6.8, 1.0 Hz, H-a). ¹³C NMR (151 MHz, *chloroform-d*) δ: 27.6 (-C(CH₃)₃), 55.4 (-OCH₃), 71.4 (C-s), 84.1 (C-f), 93.2 (C(CH₃)₃), 113.5 (C-b), 113.9 (C-u), 118.2 (C-d), 118.5 (t, J = 16.4 Hz), 119.3 (t, J = 18.4 Hz), 120.6 (q, J = 257.8 Hz), 121.9 (C-p), 123.1 (C-r), 128.4 (C-t), 128.8 (C-a)**, 128.9 (C-c)**, 129.1 (C-m)**, 129.3 (C-m)?, 129.3 (C-u), 130.2 (C-q), 143.7 (d, J = 244.2 Hz), 143.8 (C-e), 143.9 (d, J =
25 241.8 Hz), 149.4 (d, J = 1.9 Hz), 151.2 (-COOC(CH₃)₃), 159.6 (C-z), 163.4 (C-h)*, 163.6 (C-g)*. ¹⁹F NMR (564 MHz, *chloroform-d*) δ: -145.29 (s, 2F, F-k)*, -144.36 (dd, 2F, J = 21.7, 8.5 Hz, F-j)*, -57.75 (s, 3F, -OCF₃). MS (ESI) 728 [M+Na]⁺.

Tert-butyl (2-ethoxypyrazolo[1,5-a]pyrazine-3-carbonyl)(2,3,5,6-tetrafluoro-[1,1'-biphenyl]-4-yl)carbamate (17d). The crude material was purified via flash chromatography (eluent *petroleum ether* / EtOAc 5/5 v/v) to afford the title compound as vitreous white solid. ¹H NMR

- 5 (600 MHz, *DMSO-d*₆) δ: 1.35 – 1.41 (*m*, 12H, -C(CH₃)₃ and -OCH₂CH₃), 4.50 (*q*, 2H, *J* = 7.0 Hz, -OCH₂CH₃), 7.51 – 7.63 (*m*, 5H, *aromatic protons*), 8.21 (*d*, 1H, *J* = 4.5 Hz, *H-a*), 8.89 (*dd*, 1H, *J* = 4.5, 1.4 Hz, *H-b*), 9.24 (*d*, 1H, *J* = 1.4 Hz, *H-d*) ¹³C NMR (151 MHz, *DMSO-d*₆) δ: 14.3 (-OCH₂CH₃), 27.1 (-C(CH₃)₃), 66.4 OCH₂CH₃), 84.4 (-C(CH₃)₃), 92.4 (*C-f*), 117.5 (*t*, *J* = 14.5 Hz), 120.2 (*t*, *J* = 17.1 Hz), 122.9, 126.1, 128.9, 129.7, 130.1, 132.1, 136.8, 142.1, 143.1 (*d*, *J* = 248.5 Hz), 143.4 (*d*, *J* = 248.6 Hz), 150.2, 162.0, 162.6. ¹⁹F NMR (564 MHz, *DMSO-d*₆) δ: -146.73 (*dd*, 2F, *J* = 15.8 Hz), -144.11 (*dd*, 2F, *J* = 24.2, 7.7 Hz). MS (ESI⁺): 531 (M+1).

- Tert-butyl (2-hydroxypyrazolo[1,5-a]pyridine-3-carbonyl)(2,3,5,6-tetrafluoro-[1,1'-biphenyl]-4-yl)carbamate (18a)*. Palladium on carbon (Pd/C, 0.15 g, 9 % w/w) was added to a solution of compound **17a** (1.7 g, 2.7 mmol) in dry THF (40 mL) under inert atmosphere of nitrogen. The resulting mixture was vigorously stirred overnight at room temperature under a hydrogen atmosphere. The reaction mixture was filtered on silica C₁₈ and the product was eluted with MeOH to afford the title compound as white solid. Yield 81 %. ¹H NMR (600 MHz, *DMSO-d*₆) δ: 1.36 (*s*, 9H, -C(CH₃)₃), 7.09 (*t*, 1H, *J* = 6.8 Hz, *H-b*), 7.51 – 7.62 (*m*, 6H, *H-c*), 7.83 (*d*, 1H, *J* = 8.8 Hz, *H-d*), 8.67 (*d*, 1H, *J* = 6.8 Hz, *H-a*), 12.25 (*s*, 1H, -OH); ¹³C NMR (151 MHz, *DMSO-d*₆) δ: 27.1, 83.3, 91.1, 113.9, 116.3, 118.3 (*t*, *J* = 21.1 Hz), 119.3 (*t*, *J* = 24.2 Hz), 126.1, 128.8, 129.3, 129.4, 129.5, 130.0, 142.3, 144.0, 145.3, 150.5, 162.6, 163.4.

- Tert-butyl (3'-(2,2-difluoropropoxy)-2,3,5,6-tetrafluoro-[1,1'-biphenyl]-4-yl)(2-hydroxypyrazolo[1,5-a]pyridine-3-carbonyl)carbamate (18b)*. Palladium on carbon (Pd/C, 30 mg, 9 % w/w) was added to a solution of compound **17b** (340 mg, 0.48 mmol) in dry THF (20 mL) under inert atmosphere of nitrogen. The resulting mixture was vigorously stirred overnight at room temperature under a hydrogen atmosphere. The reaction mixture was filtered on silica C₁₈ and the product was eluted with MeOH to afford the title compound as white solid (380 mg, 638 mmol). Yield 99%. ¹H NMR (600 MHz, *DMSO-d*₆) δ: 1.36 (*s*, 9H, -C(CH₃)₃), 1.75 (*t*, 3H, *J* = 19.2 Hz, -OCH₂CF₂CH₃), 4.35 (*t*, 2H, *J* = 12.6 Hz, -OCH₂CF₂CH₃), 7.09 (*td*, 1H, *J* = 6.9, 1.4 Hz, *H-b*), 7.17 – 7.22 (*m*, 2H, *H-p* and *H-r*), 7.29 (*s*, 1H, *H-n*), 7.51 (*t*, 1H, *J* = 8.0 Hz, *H-c*), 7.56 – 7.60 (*m*, 1H, *H-q*), 7.83 (*d*, 1H, *J* = 8.7 Hz, *H-d*), 8.66 (*d*, 1H, *J* = 6.8 Hz, *H-a*), 12.22 (*v br s*, -OH). ¹³C NMR (151 MHz, *DMSO-d*₆) δ: 20.7 (*t*, *J* = 25.1 Hz, -OCH₂CF₂CH₃), 27.2 (-C(CH₃)₃), 68.9 (*t*, *J* = 31.5 Hz, -OCH₂CF₂CH₃), 83.5 (*C-f*), 91.2 (-C(CH₃)₃), 114.0 (*C-b*), 116.2, 116.40, 116.43, 118.6 (*t*, *J* = 13.4 Hz), 119.1 (*t*, *J* = 17.7 Hz), 122.2 (*t*, *J* = 238.9 Hz, -OCH₂CF₂CH₃),

- 5 123.2 (*C-r*), 127.5, 129.4, 129.5, 130.1, 142.4 (*C-e*), 143.2 (*dd*, 2C, *J* = 241.3, 8.9 Hz), 150.1 (-COOC(CH₃)₃), 157.8 (*C-o*), 162.7 (*C-h*)*, 163.5 (*C-g*)*. ¹⁹F NMR (564 MHz, *chloroform-d*) δ: -146.59 (*d*, 2F, *J* = 14.4 Hz), -144.08 (*d*, 2F, *J* = 17.7 Hz), -97.05 - -96.81 (*m*, 2F). MS (ESI) 594 [M-1]⁻. MS (ES⁺): 594 [M-1].
- 10 *Tert-butyl-(2-hydroxypyrazolo[1,5-a]pyridine-3-carbonyl)(2,3,5,6-tetrafluoro-3'-(trifluoromethoxy)-[1,1'-biphenyl]-4-yl)carbamate (18c)*. Palladium on carbon (Pd/C, 15 mg, 9 % w/w) was added to a solution of compound **17c** (166 mg, 0.235 mmol) in dry THF (10 mL) under inert atmosphere of nitrogen. The resulting mixture was vigorously stirred overnight at room temperature under a hydrogen atmosphere. The reaction mixture was filtered on silica
- 15 C₁₈ and the product was eluted with MeOH to afford the title compound as white solid (883 mg, 1.5mmol). Yield 56%. ¹H NMR (600 MHz, *chloroform-d*) δ: 1.44 (*s*, 9H, C(CH₃)₃), 7.01 (*td*, 1H, *J* = 7.0, 1.4 Hz, *H-b*), 7.33 – 7.36 (*m*, 1H, *H-r*), 7.37 (*s*, 1H, *H-n*), 7.44 (*d*, *J* = 7.9 Hz, 1H), 7.51 (*ddd*, *J* = 8.7, 7.1, 1.1 Hz, 1H, *H-c*)*, 7.55 (*t*, *J* = 8.0 Hz, 1H), 7.82 (*d*, *J* = 8.8 Hz, 1H, *H-d*), 8.33 (*dt*, 1H, *J* = 6.8, 1.0 Hz, *H-a*), 11.41 (*v br s*, 1H, -OH). ¹³C NMR (151 MHz, *chloroform-d*) δ:
- 20 27.8 (-C(CH₃)₃), 84.9 (*C-f*), 92.4 (-C(CH₃)₃), 114.4 (*C-b*), 118.1 (*C-d*), 119.02 (*t*, *J* = 16.5 Hz), 119.74 (*t*, *J* = 16.2 Hz), 122.0 (*C-p*), 122.3 (*q*, *J* = 257.6 Hz), 123.0 (*C-r*), 128.8 (*C-c*)**, 128.88 (*C-a*)**, 128.90 (*C-m*), 129.5 (*C-n*), 130.3 (*C-q*)***, 141.7 (*C-e*), 143.9 (*d*, *J* = 247.0 Hz), 144.1 (*d*, *J* = 248.5 Hz), 149.4 (*q*, *J* = 1.4 Hz), 151.0 (-COOC(CH₃)₃), 164.9 (*C-h*)*, 165.7 (*C-g*)*. ¹⁹F NMR (564 MHz, *chloroform-d*) δ: -144.98 (*d*, 2F, *J* = 8.5 Hz, *F-k*)*, -143.87 (*dd*, 2F, *J* = 22.6,
- 25 9.8 Hz, *F-j*)*, -57.77 (*s*, 3F, -OCF₃). MS (ESI) 586 [M+H]⁺.

- Tert-butyl (2-hydroxypyrazolo[1,5-a]pyrazine-3-carbonyl)(2,3,5,6-tetrafluoro-[1,1'-biphenyl]-4-yl)carbamate (18d)*. 1M BBr₃ solution in DCM (1200 μL, 1.161 mmol) was dissolved in dry
- 30 DCM (10 mL) under inert atmosphere. The solution was cooled at -10 °C and compound **17d** (0.200 g, 0.46 mmol) was added, and the reaction mixture was stirred for 30 minutes at -10 °C. Then, additional BBr₃ 1M solution in DCM (600 μL, 0.5807 mmol) was added and the reaction was stirred overnight. The reaction was quenched in distilled water (50 mL) and extracted with DCM (3 x 60 mL). Organic layers were collected, washed with a saturated brine
- 35 solution, dried over Na₂SO₄ and concentrated under reduced pressure to afford a pale, yellow

- 5 solid. The crude solid was triturated with diisopropyl ether to afford the title compound as a solid. MS (ESI⁺): 501 (M-1).

General procedure for synthesis of compounds 19a-d.

- Cesium carbonate (1.1 eq.) was added to a suspension of appropriate compound (**18a-d**, 1
10 eq.) in acetonitrile (30 mL), and the reaction mixture was stirred at room temperature for 40 min. Then a solution of dibenzyl(chloromethyl)phosphate (1.1 eq.) in acetonitrile (5 mL) was added, and the reaction mixture was stirred at room temperature until complete consumption of the starting material. In the following, the reaction mixture was filtered, and the filtrate was concentrated under reduced pressure. The crude material was purified by flash
15 chromatography.

- Tert-butyl (2-(((bis(benzyloxy)phosphoryl)oxy)methoxy)pyrazolo[1,5-a]pyridine-3-carbonyl)(2,3,5,6-tetrafluoro-[1,1'-biphenyl]-4-yl)carbamate (19a).* The crude material was purified by flash chromatography (eluent *petroleum ether* / EtOAc from 7:3 v/v to 5:5 v/v) to
20 afford the title compound as white solid. Yield 56 %. ¹H NMR (600 MHz, *chloroform-d*) δ: 1.44 (s, 9H, -CCH₃), 5.04 – 5.14 (m, 4H, -CH₂OPh), 6.00 (d, 2H, J = 11.9 Hz, -OCH₂OP-), 6.91 (t, 1H, J = 6.5 Hz, *H-b*), 7.22 – 7.33 (m, 10H, *aromatic protons*), 7.40 – 7.53 (m, 6H, *aromatic protons* and *H-c*), 8.00 (d, 1H, J = 8.8 Hz, *H-d*), 8.23 (d, 1H, J = 6.8 Hz, *H-a*); ¹³C NMR (151 MHz, *chloroform-d*) δ: 27.8, 69.6 (d, J = 5.3 Hz), 84.4, 88.2 (d, J = 3.9 Hz), 93.2, 113.9, 118.2
25 (t, J = 15.0 Hz), 118.4, 120.2 (t, J = 16.8 Hz), 127.2, 127.9, 128.57, 128.59, 128.8, 129.0, 129.2, 129.4, 130.3, 135.6 (d, J = 7.5 Hz), 142.9, 143.5, 144.5, 151.2, 161.1, 163.0; ³¹P NMR (243 MHz, *chloroform-d*) δ: -2.34. MS (ES⁺): 792 [M+1].

- Tert-butyl (2-(((bis(benzyloxy)phosphoryl)oxy)methoxy)pyrazolo[1,5-a]pyridine-3-carbonyl)(3',3'-difluoropropoxy)-2,3,5,6-tetrafluoro-[1,1'-biphenyl]-4-yl)carbamate (19b).* The crude material was purified by flash chromatography (eluent *petroleum ether* / EtOAc from 8:2 v/v to 6:4 v/v) to afford the title compound as white vitreous solid (240 mg, 0.272 mmol). Yield 45%. ¹H NMR (600 MHz, *chloroform-d*) δ: 1.44 (s, 9H, -C(CH₃)₃), 1.80 (t, 3H, J = 18.8 Hz, -OCH₂CF₂CH₃), 4.15 (t, 2H, J = 11.4 Hz, -OCH₂CF₂CH₃), 5.05 – 5.12 (m, 4H, -OCH₂Ph), 5.99
35 (d, 2H, J = 12.0 Hz, -OCH₂OP-), 6.90 (td, 1H, J = 7.0, 1.4 Hz, *H-b*), 7.01 (s, 1H, *H-n*), 7.03 (ddd, 1H, J = 8.3, 2.6, 0.8 Hz, *H-q*), 7.10 (d, 1H, J = 7.6 Hz, *H-p*), 7.24 – 7.31 (m, 10H, *aromatic*

5 protons), 7.41 - 7.46 (m, 2H, *H-c* and *H-r*), 7.98 - 8.01 (m, 1H, *H-d*), 8.23 (dt, 1H, *J* = 6.9, 1.0 Hz, *H-a*). ¹³C NMR (151 MHz, DMSO-*d*₆) δ: 21.1 (t, *J* = 26.0 Hz, -OCH₂CF₂CH₃), 27.8 (-C(CH₃)₃), 69.6 (d, *J* = 5.4 Hz, -OCH₂OP-), 69.9 (t, *J* = 35.0 Hz, -OCH₂CF₂CH₃), 84.4 (*C-f*), 88.2 (d, *J* = 3.7 Hz, -OCH₂OP-), 93.2 (-C(CH₃)₃), 113.9 (*C-b*), 115.8, 116.7, 117.0, 118.4, 119.7, 121.5 (t, *J* = 239.3 Hz, -OCH₂CF₂CH₃), 123.8, 127.9 (aromatic protons), 128.58, 128.60, 129.0, 129.2, 130.0, 135.6 (d, *J* = 7.3 Hz, *C-u*), 143.6 (*C-e*), 143.58 (dd, 2C, *J* = 244.7, 7.9 Hz), 151.2 (-COOC(CH₃)₃), 158.1 (*C-o*), 161.1 (*C-h*)*, 162.9 (*C-g*)*. ¹⁹F NMR (564 MHz, chloroform-*d*) δ: -145.34 (dd, 2F, *J* = 22.7, 9.1 Hz), -143.92 (d, 2F, *J* = 14.1 Hz), -98.00 - -97.83 (m, 2-OCH₂CF₂CH₃). ³¹P NMR (243 MHz, chloroform-*d*) δ: -2.36 (s). MS (ES⁺): 886 [M+1].

15 *Tert-butyl* (2-(((bis(benzyloxy)phosphoryl)oxy)methoxy)pyrazolo[1,5-*a*]pyridine-3-carbonyl)(2,3,5,6-tetrafluoro-3'-(trifluoromethoxy)-[1,1'-biphenyl]-4-yl)carbamate (**19c**). The crude material was purified by flash chromatography (eluent *petroleum ether* / EtOAc from 8:2 v/v to 6:4 v/v) to afford the title compound as white vitreous solid (644 mg, 0.735 mmol). Yield 49%. ¹H NMR (600 MHz, chloroform-*d*) δ: 1.45 (s, 9H, -C(CH₃)₃), 5.05 – 5.14 (m, 4H, -OCH₂Ph), 5.99 (d, 2H, *J* = 12.0 Hz, -OCH₂OP-), 6.91 (td, 1H, *J* = 7.0, 1.4 Hz, *H-b*), 7.25 – 7.30 (m, 10H, aromatic protons), 7.32 – 7.35 (m, 2H, *H-n* and *H-c*), 7.40 (d, 1H, *J* = 7.8 Hz, *H-r*), 7.44 (ddd, 1H, *J* = 8.8, 7.0, 1.1 Hz, *H-c*), 7.53 (dd, 1H, *J* = 8.9, 7.8 Hz), 7.99 (dt, *J* = 8.9, 1.1 Hz, *H-d*), 8.23 (dt, 1H, *J* = 6.9, 1.0 Hz, *H-a*); ¹³C NMR (151 MHz, chloroform-*d*) δ: 27.8 (-C(CH₃)₃), 69.6 (d, *J* = 5.5 Hz, -OCH₂Ph), 84.6 (*C-f*), 88.2 (d, *J* = 4.1 Hz, -OCH₂OP-), 93.2 (-C(CH₃)₃), 114.0 (*C-b*), 118.4 (*C-d*), 118.6 (t, *J* = 13.7 Hz), 119.0 (t, *J* = 15.1 Hz), 120.6 (q, *J* = 257.9 Hz, -OCF₃), 121.9 (*C-p*)*, 123.0 (*C-r*)*, 127.9 (aromatic carbons), 128.58, 128.59, 128.8 (*C-c*)**, 128.9 (*C-m*), 129.1 (*C-a*)**, 129.2 (*C-n*)**, 130.2 (*C-q*)*, 135.6 (d, *J* = 7.4 Hz, *C-u*), 143.6 (*C-e*), 143.7 (d, *J* = 245.0 Hz), 143.8 (d, *J* = 246.2 Hz), 149.4 (q, *J* = 2.1 Hz), 151.1 (COOC(CH₃)₃), 161.1 (*C-h*)****, 162.9 (*C-g*)****. ¹⁹F NMR (564 MHz, chloroform-*d*) δ: -144.82 (dd, 2F, *J* = 22.7, 9.5 Hz, *F-k*)*, -144.12 (d, 2F, *J* = 13.6 Hz, *F-j*)*, -57.75 (s, 3F, -OCF₃). ³¹P NMR (243 MHz, chloroform-*d*) δ: -2.31 (s). MS (ESI) 898 [M+Na]⁺.

Tert-butyl (2-(((bis(benzyloxy)phosphoryl)oxy)methoxy)pyrazolo[1,5-*a*]pyrazine-3-carbonyl)(2,3,5,6-tetrafluoro-[1,1'-biphenyl]-4-yl)carbamate (**19d**). The crude material was

- 5 purified by flash chromatography (eluent DCM / EtOAc from 95:5 v/v to 90: 10 v/v) to afford the title compound as white solid. MS (ESI): 791 (M-1).

General procedure for synthesis of compounds 1, 3, 5 and 7

- Palladium on carbon (Pd/C, 10 % w/w) was added to a solution of **19a-d** (1 eq.) in dry THF (15 mL). The resulting mixture was vigorously stirred under a hydrogen atmosphere for 2 h. The reaction mixture was filtered on silica C₁₈ and the product was eluted with MeOH, and concentrated under reduced pressure to afford the intermediate compounds **20a-d** that were dissolved in dry DCM (10 mL). After cooling (0 °C), a solution of TFA (5 mL) in dry DCM (15 mL) was then added and the reaction mixture was stirred at the same temperature for 3 h. In the following, the solvent was evaporated, involving toluene to facilitate the evaporation of TFA, affording the title compound as white solids.

- ((3-((2,3,5,6-Tetrafluoro-[1,1'-biphenyl]-4-yl)carbamoyl)pyrazolo[1,5-a]pyridin-2-yl)oxy)methyl dihydrogen phosphate (1)*. Yield 90 %. ¹H NMR (600 MHz, DMSO-*d*₆) δ: 5.94 (*d*, 2H, J = 14.9 Hz, -OCH₂OP-), 7.14 (*t*, 1H, J = 6.8 Hz, *H-b*), 7.49 – 7.65 (*m*, 6H, aromatic protons and *H-c*), 8.04 (*d*, 1H, J = 8.8 Hz, *H-d*), 8.76 (*t*, 1H, J = 6.9 Hz, *H-a*), 9.25 (*s*, 1H, -NH); ¹³C NMR (151 MHz, DMSO-*d*₆) δ: 87.8 (*d*, J = 5.4 Hz), 89.8, 114.1, 116.6 (*t*, J = 16.5 Hz), 117.4, 117.7 (*t*, J = 17.3 Hz), 126.6, 128.9, 129.0, 129.5, 129.7, 130.2, 142.0, 142.9 (*d*, J = 246.6 Hz), 143.2 (*d*, J = 245.9 Hz), 159.9, 160.3; ³¹P NMR (243 MHz, DMSO-*d*₆) δ: -1.60. MS (ESI): 510 (M-1).

- ((3-((3'-(2,2-Difluoropropoxy)-2,3,5,6-tetrafluoro-[1,1'-biphenyl]-4-yl)carbamoyl)pyrazolo[1,5-a]pyridin-2-yl)oxy)methyl dihydrogen phosphate (5)*. White solids. It was then crystallized with acetone. The solid was take up with H₂O (100 mL) and lyophilized. Yield 56 %. ¹H NMR (600 MHz, DMSO-*d*₆) δ: 1.76 (*t*, 3H, J = 19.2 Hz, -OCH₂CF₂CH₃), 4.36 (*t*, 2H, J = 12.5 Hz, -OCH₂CF₂CH₃), 5.95 (*d*, 2H, J = 14.7 Hz, -OCH₂OP-), 7.14 (*t*, 1H, J = 6.9 Hz, *H-b*), 7.19 (*d*, 2H, J = 7.8 Hz, *H-p* and *H-c*), 7.26 (*s*, 1H, *H-n*), 7.51 (*t*, 1H, J = 7.5 Hz, *H-q*), 7.60 (*t*, 1H, J = 7.8 Hz, *H-r*), 8.05 (*d*, 1H, J = 8.8 Hz, *H-d*), 8.76 (*d*, 1H, J = 6.8 Hz, *H-a*), 9.24 (*s*, 1H, -NH); ¹³C NMR (151 MHz, DMSO-*d*₆) δ: 20.7 (*t*, J = 25.2 Hz, -OCH₂CF₂CH₃), 68.9 (*t*, J = 31.1 Hz, -OCH₂CF₂CH₃), 87.7 (*d*, J = 5.4 Hz, -OCH₂OP-), 89.8 (*C-f*), 114.1 (*C-b*), 115.8, 116.6 (*C-d*), 116.8 (*t*, J = 15.0 Hz), 117.3 (*t*, J = 16.2 Hz), 117.4, 122.2 (*t*, J = 238.9 Hz, -OCH₂CF₂CH₃),

5 123.3, 127.9, 129.0, 129.7, 130.1, 142.0 (*C-e*), 157.8 (*C-o*), 159.8 (*C-h*)*, 160.3 (*C-g*)*. ¹⁹F NMR (564 MHz, *chloroform-d*) δ: -144.92 (*d*, 2F, *J* = 22.1 Hz), -144.80 (*d*, 2F, *J* = 23.8 Hz), -97.03 - -96.80 (*m*, 2-OCH₂CF₂CH₃). ³¹P NMR (243 MHz, *chloroform-d*) δ: -1.65 (*s*). MS (ESI⁻): 605 (*M*-1).

10 ((3-((2,3,5,6-Tetrafluoro-3'-(trifluoromethoxy)-[1,1'-biphenyl]-4-yl)carbamoyl)pyrazolo[1,5-
a]pyridin-2-yl)oxy)methyl dihydrogen phosphate (**7**). White solids. It was then crystallized with
acetone (211 mg, 354 mmol). Yield 56 %. The obtained solid was taken up with water (100 mL)
and lyophilized. ¹H NMR (600 MHz, *DMSO-d*₆) 5.94 (*d*, 2H, *J* = 14.9 Hz, -OCH₂OP-), 7.14 (*td*,
1H, *J* = 7.0, 1.4 Hz, *H-b*), 7.49 – 7.69 (*m*, 4H, *aromatic protons and H-c*), 7.72 (*t*, *J* = 8.0 Hz),
8.04 (*d*, 1H, *J* = 8.8 Hz, *H-d*), 8.76 (*t*, 1H, *J* = 6.9 Hz, *H-a*), 9.30 (*s*, 1H, -NH); ¹³C NMR (151
15 MHz, *DMSO-d*₆) δ: 87.8 (*d*, *J* = 5.0 Hz, -OCH₂OP-), 89.8 (*C-f*), 113.5, 114.1 (*C-b*), 116.0 (*t*, *J*
= 15.9 Hz, *C-i*)*, 117.4 (*C-d*), 117.6 (*t*, *J* = 14.1 Hz, *C-l*)*, 122.1 (*d*, *J* = 2.1 Hz), 123.0, 123.6,
124.6, 128.7, 129.1, 129.5, 129.7, 130.1, 142.0 (*C-e*), 148.4, 159.8, 160.3; ¹⁹F NMR (564 MHz,
*DMSO-d*₆) δ: -145.27 (*dd*, 2F, *J* = 24.2, 7.0 Hz, *F-k*)*, -144.5 (*dd*, 2F, *J* = 22.9, 7.7 Hz, *F-j*)*, -
56.77 (*s*, 3F, -OCF₃); ³¹P NMR (243 MHz, *DMSO-d*₆) δ: -1.61. MS (ESI⁻): 594 (*M*-1).

20

((3-((2,3,5,6-Tetrafluoro-[1,1'-biphenyl]-4-yl)carbamoyl)pyrazolo[1,5-*a*]pyrazin-2-yl)oxy)methyl
dihydrogen phosphate (**3**). MS (ESI⁻): 511 (*M*-1).

Potassium ((3-((2,3,5,6-tetrafluoro-[1,1'-biphenyl]-4-yl)carbamoyl)pyrazolo[1,5-*a*]pyridin-2-
25 yl)oxy)methyl hydrogen phosphate (**2**). Compound **1** (50.0 mg, 1.0 eq., 0.0978 mmol) was
dissolved in ultrapure water (50 mL). The solution was stirred and cooled to 5° C, then 9.78
mL of 0.01 M KOH solution (1.0 eq., 0.0978 mmol) was slowly added. The solution was freeze-
dried for 24 h, obtaining **2** as a white porous solid with a quantitative yield. ¹H NMR (600 MHz,
*DMSO-d*₆) δ: 5.73 (*d*, 2H, *J* = 15.7 Hz, -OCH₂OP-), 7.05 (*td*, 1H, *J* = 6.9, 1.4 Hz, *H-b*), 7.48 –
30 7.59 (*m*, 6H, *aromatic protons and H-c*), 7.93 – 7.96 (*m*, 1H, *H-d*), 8.70 (*dt*, 1H, *J* = 6.9, 1.0 Hz,
H-a), 11.20 (*v br s*, 1H, -OH); ³¹P NMR (243 MHz, *chloroform-d*) δ: -1.10 (*t*, *J* = 15.6 Hz).

Sodium ((3-((2,3,5,6-tetrafluoro-[1,1'-biphenyl]-4-yl)carbamoyl)pyrazolo[1,5-*a*]pyridin-2-
yl)oxy)methyl hydrogen phosphate. (**2a**). Compound **1** (48.6 mg, 1.0 eq., 0.095 mmol) was
35 dissolved in ultrapure water (50 mL). The solution was stirred and cooled to 5° C, then 9.5 mL
of 0.01 M NaOH solution (1.0 eq., 0.095 mmol) was slowly added. The solution was freeze-

5 dried for 24 h, obtaining **2a** as a white porous solid with a quantitative yield. ¹H NMR (600 MHz, DMSO-*d*₆) δ: 5.73 (*d*, 2H, *J* = 15.7 Hz, -OCH₂OP-), 7.05 (*td*, 1H, *J* = 6.9, 1.4 Hz, *H-b*), 7.46 – 7.61 (*m*, 6H, *aromatic protons and H-c*), 7.95 (*d*, 1H, *J* = 8.9 Hz, *H-d*), 8.69 (*d*, 1H, *J* = 6.9 Hz, *H-a*), 11.23 (*v br s*, 1H, -OH); ³¹P NMR (243 MHz, chloroform-*d*) δ: -1.07 (*t*, *J* = 15.4 Hz).

1,3-Dihydroxy-2-(hydroxymethyl)propan-2-aminium ((3-((2,3,5,6-tetrafluoro-[1,1'-biphenyl]-4-yl)carbamoyl)pyrazolo[1,5-*a*]pyridin-2-yl)oxy)methyl hydrogen phosphate (**2b**). Compound **1** (40.25 mg, 1.0 eq., 0.0787 mmol) was dissolved in EtOH (10 mL). The solution was stirred and then a solution of 2-amino-2-(hydroxymethyl)propane-1,3-diol (9.53 mg, 1.0 eq., 0.0787 mmol) in EtOH (10 mL) was slowly added. The suspension was stirred in 40°C bath for 15 min and then the solvent was evaporated to obtain **2b** as a white solid with a quantitative yield. ¹H NMR (600 MHz, DMSO-*d*₆) δ: 3.45 (*s*, 6H, (HOCH₂)₃CNH₂), 5.75 (*d*, 2H, *J* = 15.6 Hz, -OCH₂OP-), 7.06 (*td*, 1H, *J* = 6.9, 1.4 Hz, *H-b*), 7.49 – 7.60 (*m*, 6H, *aromatic protons and H-c*), 7.96 (*d*, 1H, *J* = 8.8 Hz, *H-d*), 8.70 (*dt*, 1H, *J* = 6.9, 1.0 Hz, *H-a*), 10.96 (*v br s*, 1H, -OH); ¹³C NMR (151 MHz, DMSO-*d*₆) δ: 59.4 ((HOCH₂)₃CNH₂), 60.8 ((HOCH₂)₃CNH₂), 88.5 (*d*, *J* = 6.0 Hz, -OCH₂OP-), 91.0 (*C-f*), 113.5 (*C-b*), 117.2 (*C-d*), 118.4, 119.2, 126.8, 128.3, 128.8, 129.3, 129.4, 130.1, 141.7 (*C-e*), 143.3 (*dd*, *J* = 245.3, 13.1 Hz, 2C), 160.6 (*C-h*)*, 161.2 (*C-g*)*. ¹⁹F NMR (564 MHz, chloroform-*d*) δ: -146.05 (*dd*, *J* = 24.4, 6.8 Hz), -144.40 (*dd*, *J* = 24.7, 6.3 Hz). ³¹P NMR (243 MHz, chloroform-*d*) δ: -0.67 (*t*, *J* = 15.4 Hz).

(*R*)-5-amino-5-carboxypentan-1-aminium ((3-((2,3,5,6-tetrafluoro-[1,1'-biphenyl]-4-yl)carbamoyl)pyrazolo[1,5-*a*]pyridin-2-yl)oxy)methyl hydrogen phosphate (**2c**). Compound **1** (45.05 mg, 1.0 eq., 0.0881 mmol) was dissolved in EtOH (10 mL). The solution was stirred and then a solution of 2-amino-2-(hydroxymethyl)propane-1,3-diol (12.88 mg, 1.0 eq., 0.0881 mmol) in EtOH (10 mL) was slowly added. The suspension was stirred in 40°C bath for 15 min and then the solvent was evaporated to obtain **2c** as a white solid. ¹H NMR (600 MHz, DMSO-*d*₆) δ: 1.21 – 1.85 (*m*, 6H, -CH₂CH₂CH₂-), 2.73 (*t*, 2H, *J* = 7.2 Hz, -CH₂NH₂), 3.36 – 3.42 (*m*, 1H, -CH-), 5.77 (*d*, 2H, *J* = 15.4 Hz, -OCH₂OP-), 7.07 (*td*, 1H, *J* = 6.9, 1.3 Hz, *H-b*), 7.48 – 7.59 (*m*, 6H, *aromatic protons and H-c*), 7.97 (*d*, 1H, *J* = 8.8 Hz, *H-d*), 8.70 (*d*, 1H, *J* = 6.9, *H-a*), 10.59 (*s*, 1H, -NH).

2-Hydroxy-*N*-(2,3,5,6-tetrafluoro-[1,1'-biphenyl]-4-yl)pyrazolo[1,5-*a*]pyrazine-3-carboxamide (**4**). BBr₃ 1M solution in DCM (600 μL, 0.5807 mmol) was dissolved in dry DCM (5 mL) under

- 5 inert atmosphere. The solution was cooled at -10°C and compound **16d** (0.100 g, 0.2323 mmol) was added and the reaction mixture was stirred for 30 minutes at -10°C. Then, additional BBr₃ 1M solution in DCM (600 µL, 0.5807 mmol) was added and the reaction was stirred overnight. The reaction was quenched in distilled water (50 mL) and extracted with DCM (3 x 60 mL). Organic layers were collected, washed with a saturated brine solution, dried over
- 10 Na₂SO₄ and concentrated under reduced pressure to afford a pale, yellow solid. The crude solid was triturated with diisopropyl ether to afford the title compound as pale, yellow solid. Yield 80%. ¹H NMR (600 MHz, DMSO-*d*₆), δ 7.51 – 7.60 (*m*, 5H, aromatic protons), 8.10 (*d*, *J* = 4.5 Hz, 1H, *H*-*a*), 8.75 (*dd*, *J* = 4.5, 1.3 Hz, 1H, *H*-*b*), 9.12 (*s*, 1H, -NH), 9.31 (*d*, *J* = 1.3 Hz, 1H, *H*-*c*), 13.38 (*br s*, 1H, -OH); ¹³C NMR (151 MHz, DMSO-*d*₆), δ 89.4, 116.5 (*t*, *J* = 14.8 Hz),
- 15 117.6 (*t*, *J* = 17.5 Hz), 122.1, 126.5, 128.7, 129.3, 130.0, 131.0, 136.2, 142.1, 142.8 (*dd*, *J* = 244.8, 13.2 Hz), 143.2 (*dd*, *J* = 244.6, 12.3 Hz), 159.7, 162.4; ¹⁹F NMR (564 MHz, DMSO-*d*₆) δ: -145.66 (*d*, *J* = 24.8 Hz), -145.07 (*d*, *J* = 24.3 Hz); MS (ESI⁻): 401 (M-1).

Solubility assay

- 20 Solubility was performed in different vehicles: saline phosphate buffer (PBS; Na₂HPO₄ 10 mM, KH₂PO₄ 1.8 mM, NaCl 137 mM and KCl 2.7 mM, pH 7.4), ultrapure water, simulated gastric fluid (SGF, purchased from Biorelevant and prepared according the instructions), simulated intestine fluid (SIF, purchased from Biorelevant and prepared according the instructions). For each sample, the compound was weighted (1 mg), then 1 mL of vehicle was added, and the
- 25 solution was stirred at 350 rpm for 24 hours at 25 °C (PBS and water) or 37 °C (SGF, SIF). Before the analysis, suspensions were filtered through a PTFE 0.22 µm filter and analyzed using UHPLC-UV system (Perkin Elmer, Flexar). The analytical column used was an UHPLC XBridge BEH C18 (3x75mm column XP, 2.5 µm particle size, Waters). Each analysis was performed at flow rates of 0.5 mL/min in elution gradient using water (eluent A) and Methanol
- 30 (eluent B) with 0.1 % trifluoroacetic acid (TFA) as mobile phase. The analysis started with 50 % of eluent B and the profile used was: (time min, % B) 9.50, 100 %; 12.5, 100 %; 14.5, 50 %. Quantification of the compounds was achieved by interpolation with the respective calibration curve obtained by analyzing standard sample solutions in MeOH.

- 35 *Serum stability assay*

- 5 A DMSO solution of the compound was added to human serum (sterile-filtered from human male AB plasma, Sigma-Aldrich) to obtain a concentration of 100 μ M resulting in 2 % of DMSO. The solution was incubated under stirring at 350 rpm at 37 °C for 24 h. At selected time (t_0 ; $t_{30\text{min}}$; $t_{1\text{h}}$; $t_{4\text{h}}$; $t_{24\text{h}}$), an aliquot was denatured 1 : 2 with ice-cold acetonitrile with 0.1 % of trifluoroacetic acid. Then, the obtained solution was vortexed, sonicated for 3 min and then
- 10 centrifuged at 3900rpm for 5 min. The supernatant was filtered through PTFE 0.22 μ m filter and analyzed using UHPLC-UV system (PerkinElmer, Flexar). The analytical column used was an UHPLC XBridge BEH C18 (3x75 mm column XP, 2,5 μ m particle size, Waters). The analysis was performed at flow rates of 0.5 mL/min in elution gradient using water (eluent A) and Methanol (eluent B) with 0.1 % trifluoroacetic acid (TFA) as mobile phase. The analysis
- 15 started with 50 % of eluent B and the profile used was: (time min, % B) 9.50, 100 %; 12.5, 100%; 14.5, 50 %. Quantification of the compound was achieved by interpolation with the respective calibration curve obtained by analysing standard sample solutions in MeOH.

Liver, intestine, kidney S9 Fraction homogenates preparation and stability assay

- 20 S9 fraction of mouse tissue homogenates have been prepared by the homogenization of freshly isolated organs; Mouse liver, intestine and kidneys have been isolated, cut into smaller pieces and respectively mixed with ice-cold 50mM TRIS buffer (TRIS 50 mM, KCl 150 mM, pH 7.4) to obtain a 4 ml/g of organ suspension. Each organ suspension has been homogenized using Potter-Elvehjem mortar and pestle, followed by centrifugation at 9000 g for 30 minutes
- 25 at 4 ° C to yield a supernatant fraction known as S9 fractions. Supernatants protein content was quantified using a protein Lowry assay. Aliquots of the S9 fraction were then stored at –80 °C until used. In the assay the reaction mixture was composed of mouse S9 fraction (0.5 mg protein/mL), 50 mM TRIS buffer (TRIS 50 mM, KCl 150 mM, pH 7.4), 100 μ M compound resulting in 2 % of DMSO. The solution was incubated under stirring at 350 rpm at 37 °C for
- 30 24 h. At selected time (t_0 ; $t_{30\text{min}}$; $t_{1\text{h}}$; $t_{2\text{h}}$) an aliquot was denatured 1:1 with ice-cold acetonitrile. The obtained solution was vortexed and centrifuged at 3900rpm for 10 min. The supernatant was filtered through PTFE 0.22 μ m filter and analyzed using UHPLC-UV system (Perkin Elmer, Flexar). The analytical column used was an UHPLC XBridge BEH C18 (3x75mm column XP, 2,5 μ m particle size, Waters). The analysis was performed at flow rates
- 35 of 0.5 mL/min in elution gradient using water (eluent A) and Methanol (eluent B) with 0.1 % trifluoroacetic acid (TFA) as mobile phase. The analysis started with 50 % of eluent B and the

- 5 profile used was: (time min, % B) 9.50, 100 %; 12.5, 100 %; 14.5, 50 %. Quantification of the compound was achieved by interpolation with the respective calibration curve obtained by analyzing standard sample solutions in MeOH.

Compounds under pharmacokinetic studies: sample preparation

- 10 **MEDS433** (HPLC purity > 95 %) **2** (> 95 % containing traces of **MEDS433** (around 4%)), **6** and **8** for analytical purpose, were dissolved in DMSO (1 mg/mL, stock solution), and diluted with acetonitrile for working solutions used for the analytical method set-up and the analysis of pharmacokinetic samples. For treating animal purpose, oral administration, **all compounds** were dissolved in 10 % DMSO 5 % Tween 10 % PEG400 75 % Methocell (0.5%). For
- 15 intravenous treatment, they were dissolved in DMSO 10%, Cremophor 5% e 85% saline. The stability of **2** in the vehicle used for intravenous treatment, was assessed by HPLC-HRMS before the conduction of the pharmacokinetic study. The analytical result showed that **2** is stable at 4 hours at room temperature, in fact at that time the % of **MEDS433** present is just slightly increased to 6.1 %.

20

- Test system:* mice. Seven-week-old male mice were purchased from Charles River Italia (Calco, Italy). Animals were housed at the Mario Negri Istituto di Ricerche Farmacologiche and handled under specific pathogen-free conditions in the Institute's Animal Care Facilities, which meet international standards; procedures involving animals and their care were conducted in
- 25 conformity with the following laws, regulations, and policies governing the care and use of laboratory animals: Italian Governing Law (D.lgs 26/2014; Authorization n.19/2008-A issued March 6, 2008 by Ministry of Health); Mario Negri Institutional Regulations and Policies providing internal authorization for persons conducting animal experiments (Quality Management System Certificate – UNI EN ISO 9001:2008 – Reg. N° 6121); the NIH Guide for
- 30 the Care and Use of Laboratory Animals (2011 edition) and EU directives and guidelines (EEC Council Directive 2010/63/UE). Experimental protocols have been reviewed and approved by the IRFMN Animal Care and Use Committee (IACUC), that includes members "ad hoc" for ethical issues, and by the Italian Ministry of Health (aut. Min n. 779/2022).

- 35 *Pharmacokinetic of **MEDS433**, **2**, **6** and **8**.*

5 Mice were treated with **MEDS433**, **6** and **8** at the doses of 5 mg/kg given intravenously (IV) and 20 mg/kg given by oral gavage (PO), and with **2** at 6.85 mg/kg (IV) and 27.38 mg/kg (PO), corresponding to equivalent doses of **MEDS433** of 5 mg/kg and of 20 mg/kg respectively. Blood was collected from the retro-orbital plexus under isoflurane anesthesia at the following time points:

10 IV bolus: 5, 15, 30 min and 1, 2, 4, 8, 24, 32 and 48 h, after treatment.

Oral bolus: 15, 30, 45 min and 1, 2, 4, 8, 24, 32 and 48 h, after treatment.

Four mice were used at each time point. Mice were sacrificed by cervical dislocation. Liver, heart, kidney, lung, brain and spleen were collected. To obtain plasma, blood was centrifuged at 4000 rpm for 10 min at 4 °C. To study drug excretion, urine and feces of four mice were
15 collected in fraction of 24 h (0 - 24 h and 24 - 48 h). All the biological samples collected were immediately frozen in dry ice and then stored at -20 °C until analysis of **MEDS433**, **2**, **6** and **8** by HPLC-MS/MS.

Pharmacokinetic assay.

20 **MEDS433**, **2**, **6** and **8** were quantified according to the procedures of extraction and analysis below reported.

Sample preparation

Plasma. 20 µL of plasma were spiked with 10 ng of internal standard previously published
25 (compound **5**, in ref ¹¹) in 10 µL of acetonitrile, used as (final concentration 500 ng/mL), then 50 µL of acetonitrile were added for deproteinization. After mixing by vortex and subsequent centrifugation at 4 °C for 15 minutes at 13200 rpm, the supernatants were transferred for instrumental analysis. A set of calibration points were prepared using a control plasma sample, with separate additions of **MEDS433** or **2** or **6** and **8** in the range from 0 to 30 µg/mL

30 *Liver.* 1 g of tissue was homogenized with 4 mL of methanol, after addition of 500 ng of internal standard (final concentration 500 ng/g). Tissue homogenization was carried out with a Precellys system, using 7 mL vials with a program consisting of 3x20s 6500rpm cycles separated by a 30 s pause. Finally, samples were centrifuged at 4 °C for 15 minutes at 13200 rpm, and 200 µL aliquots of the supernatants were transferred for instrumental analysis. A set
35 of calibration points were prepared using a control liver sample, with separate additions of **MEDS433** or **2** in the range from 0 to 30 µg/g.

5 *Lung.* 1 g of tissue was homogenized with 4 mL of methanol, after addition of 500 ng of internal standard (final concentration 500 ng/g). Tissue homogenization was carried out with a Precellys system, using 7 mL vials with a program consisting of 3x20s 6500rpm cycles separated by a 30s pause. Finally, samples were centrifuged at 4 °C for 15 minutes at 13200 rpm, and 200 µL aliquots of the supernatants were transferred to autosampler vials for
10 instrumental analysis. A set of calibration points were prepared using a control lung sample, with separate additions of **MEDS433** or **2** in the range from 0 to 3 µg/g.

Brain. Whole samples were weighed and spiked with 1000 ng/g of internal standard, then homogenized with 3 volumes of methanol. Tissue homogenization was carried out with a Precellys system, using 2 mL vials with a program consisting of 3x15 s 8500rpm cycles
15 separated by a 10 s pause. Finally, samples were centrifuged at 4 °C for 15 minutes at 13200 rpm, and 100 µL aliquots of the supernatants were transferred to autosampler vials for instrumental analysis. A set of calibration points were prepared using a control brain sample, with separate additions of **MEDS433** or **2** or **6** and **8** in the range from 0 to 3 µg/g.

Urine. 200 µL of urine were spiked with 100 ng of internal standard in 800 µL of methanol,
20 (final concentration 500 ng/mL). After mixing by vortex and centrifugation at 4 °C for 15 minutes at 13200 rpm, 100 µL of the supernatants were transferred to autosampler vials for instrumental analysis. A set of calibration points were prepared using a control urine sample, with separate additions of **MEDS433** or **2** in the range from 0 to 3 µg/mL.

Feces. Whole samples were weighed and homogenized with 8 volumes of methanol. Tissue
25 homogenization was carried out with a Precellys system, using 15 mL vials with a program consisting of 5x10s 4500rpm cycles separated by a 30 s pause. An aliquot of the homogenized suspension was withdrawn and spiked with 5000 ng/g of internal standard. Finally, after mixing, samples were centrifuged at 4 °C for 15 minutes at 13200 rpm, and 100 µL aliquots of the supernatants were transferred to autosampler vials for instrumental analysis. A set of
30 calibration points were prepared using a control faeces sample, with separate additions of **MEDS433** or **2** in the range from 0 to 300 µg/g.

Instrumental analysis. The analyses by HPLC-HRMS were performed for all sample extracts with the following conditions:

35

HPLC instrument: 1200 series pumps and autosampler (Agilent)

- 5 HPLC column: XBridge C18, 3.5 μ m, 100 x 2.1 mm (Waters)
 Solvent A: Ammonium formate 10 mM in water
 Solvent B: Acetonitrile
 Flow rate and inj. vol.: 200 μ L/min; 2 μ L injections
 Elution gradient: from 1 % to 99 % of B in 10 min, hold for 2 min
- 10 MS instrument: Orbitrap Q Exactive (Thermo)
 MS source: HESI, with positive ionization
 Heated capillary temperature, 320 °C
 Auxiliary heating gas temperature, 240 °C
 Spray voltage, 3500 V
- 15 MS acquisition: Full Scan MS, 150-800 Da, at 35000 resolution
 Data Dependent MS2 scans at 17500 resolution

- The detection of analytes was performed by extracting from the high resolution full scan data the chromatograms of the molecular ions ($[M+H]^+$) with a 10 ppm window, using the values indicated in Table 8. When significant molecular adducts were present ($[M+NH_4]^+$, $[M+Na]^+$, $[M+C_2H_7N]^+$) they were included in the extracted data. Calibration curves were made using linear regression with 1/x weighing. The limit of quantification (LOQ) for **MEDS433** was 5 ng/mL or 5 ng/g for all biological samples, except for feces, for which it was 20 ng/g.

- 25 **Table 8. MEDS433** and cpd **2**, with HPLC-MS retention times and measured molecular and adduct ions.

<i>Substance</i>	<i>Retention Time (min.)</i>	<i>Molecular ion</i>	<i>Molecular Adducts</i>
MEDS433	7.95	402.0860	447.1439
2	7.35	512.0629	557.1208
(IS)	8.36	416.1017	461.1595

* source fragment

Pharmacokinetic elaboration

- 30 Pharmacokinetic parameters were calculated using PK Solver (a Microsoft Excel add).

5 *Cell Cultures*

Cell lines were grown as monolayer in different growth media: in Dulbecco's Modified Eagle's Medium (Capricorn) for Triple Negative Breast Cancer (MDA-MB-231), Human Osteosarcoma (SAOS2), Human Lung Adenocarcinoma (A549), Human Melanoma (A375) and Glioblastoma Tmz-Resistant (LN-18); in Eagle's Minimum Essential Medium for Human IDH mutated (U87mt) and Human IDH mutated fibrosarcoma (HT1080); in Iscove's MDM for Ewing's sarcoma (TC-71 and A673); in RPMI 1640 medium (Biowest) for Human Neuroblastoma (LAN-5) and Eagle's Minimum Essential Medium and F12 Medium for (SH-SY5Y); in McCoy's 5a medium modified for (T24), in ReNcell NSC Maintenance Medium serum-free supplemented with 20 ng/mL EGF and 20 ng/mL FGF-2(Sigma–Aldrich) for Human Diffuse Intrinsic Pontine Glioma (SF-7761); in DMEM-High Glucose (Sigma -Aldrich) for Human Diffuse Intrinsic Pontine Glioma (SF8628). Culture media according to the manufacturer's protocols, were supplemented with 5 % or 10 % heat-inactivated Fetal Bovine Serum, L-glutamine (2 mM), penicillin (100 IU/mL) and streptomycin (100 µg/mL) and cell cultures were maintained at 37 °C, in a humidified atmosphere of 5 % CO₂. Cells were subcultured at ratio of 1 : 4 to 1 : 6 after being detached from culture flasks with 0.05 % trypsin and 0.002 % EDTA solution. All media supplements for cell cultures were purchased from Gibco BRL (Grand Island, NY). The human cell OCI-AML3 (acute monocytic leukemia) and human THP1 (acute monocytic leukemia) were cultured in complete RPMI 1640 (GIBCO BRL) supplemented with 10 % heat-inactivated fetal bovine serum (GIBCO, Invitrogen, Milan, Italy) and 1 % penicillin/streptomycin.

25

MTT Assay

Exponentially growing cells, MDA 231, SAOS2, A549, LAN-5 and A375 were seeded in triplicate in 96 well plates at density of 4x10³ or 6x10³ cells/well in 100 µL and were maintained in culture for 24 h prior to treatments in appropriate growth medium. Cells were treated with different concentration ranges of **MEDS433** and **6** (from 1 to 10000 nM). After 72 h or 5 days of treatment (exclusively for MDA MB 231), a 10 µL of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) (5 mg/mL in PBS - Sigma Chemical, Saint Louis, CO) were added to each well and incubated for 4 hours. At the end of incubation cells were lysed with 200 µL of dimethyl sulfoxide (DMSO). Absorbance values (OD) were measured at 570 nm versus 630 nm using a microplate reader (Neobiotech).

35

5 *Trypan Blue Viability assay*

For the determination of viability, TC71, A673, LN18, SF-7716, SF8628, T24, HT1080, SHSY5Y, U87mt cells were seeded at a density of 150×10^3 cells/well onto 6-well cell culture plates and allowed to adhere for 24 h. Thereafter, **MEDS433** and/or **6** were added in fresh culture medium at different concentration ranges (from 1 to 10000 nM) and cells were allowed
10 to grow for 24h, 48h, 72 h and 120 h (exclusively for SF8628). Cell viability was determined at the mentioned times, based on the Trypan blue dye exclusion method. Live and dead cell were evaluated through an automatic cell counter platform (CytoSMART – Corning).

15

Statistical analysis

All data are reported as the mean $\pm$ standard error of the mean (SD) with at least 3 experiments in cell lines. Statistical analyses were obtained using GraphPad Prism version 9 (GraphPad Software, San Diego, CA). For the determination of IC_{50} , a nonlinear regression model was
20 applied. Normality of the data set was determined by Shapiro–Wilk normality test. For multiple comparisons, one-way ANOVA tests were performed and combined with Tukey's tests for post hoc analyses. Moreover, in this case, a p-value < 0.05 was considered significant.

Annexin assay.

25 1×10^4 OCI-AML3 acute myeloid cell lines were plated in 96-well round-bottom plates and treated with **MEDS433** at 0.1 μ M, dipyridamole (Merck Life Science, Italy) at 0.1 μ M and fludarabine at 2.5 μ M or Cladribine (CliniSciences, France) at 25 nM in a volume of 200 μ L of complete medium for three days. 1×10^4 OCI-AML3 or THP1 acute myeloid cell lines were plated in 96-well round-bottom plates and treated with **MEDS433** at 0.1 μ M, and increased
30 doses of Draflazine ((CliniSciences, France) range: 10 nM to 1 μ M) or JNK-IN-8 ((Merck Life Science, Italy) range: 0.1 μ M to 1 μ M) in a volume of 200 μ L of complete medium for three days. The apoptotic assay was assessed using the Annexin V-FITC Kit purchased from Miltenyi Biotec, Italy. Cells were washed with binding buffer and centrifugated at $300 \times g$ for 10 minutes. Then, cells were stained with 10 μ L of Annexin V-FITC and incubated at dark for 15
35 minutes. After a wash, cells were resuspended in binding buffer and finally 5 μ L *propidium*

- 5 *iodide* (PI) solution was added. After the PI addition, samples were acquired on FACSVerse and analysed by Kaluza software version 2.1(Beckman Coulter Fullerton, CA).

Synergism assay.

- The synergism of compound 6 with anticancer drugs (Panabinstat – ONC212 – Abemaciclib)
10 was evaluated in SF8628 cell line. Cytotoxicity experiments in combination with 6 were conducted with serial doses in constant/variable ratios at 72 hours using the CellTiter-Glo 2.0 and Cell Counting Kit-8 assays purchased, respectively, from Promega and Sigma Aldrich. Synergism and combination index theorem were assessed using CompuSyn software¹⁶.

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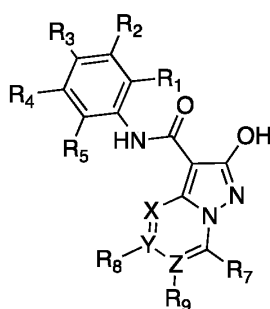
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5

CLAIMS

1. A compound of Formula (Ia):

10



Formula (Ia)

wherein R_1 , R_2 , R_4 and R_5 are independently selected from a hydrogen atom, deuterium atom,
 15 a halogen atom, a linear and/or branched C_{1-8} alkyl group, an alkyloxy group, an alkylamino group, an alkylthio group, a halo C_{1-8} alkyl group, a halo C_{1-8} alkyloxy group, a halo C_{1-8} alkylamino group, a nitro group, a cyano group;

R_3 is:

- an aromatic group selected from the group consisting of: phenyl group, heteroaryl
 20 group preferably an azinyl group such as pyridinyl group, pyrimidinyl group; phenoxy group; azinyloxy group such as pyridinyloxy group, pyrimidinyloxy group; phenylthio group; azinylthio group such as pyridinylthio group, pyrimidinylthio group; phenylsulfinyl group; phenylsulfonyl group; phenylsulfonylamino group; N-phenylcarbamoyl group.
- an aliphatic group selected from the group consisting of: C_{1-12} alkyl group; C_{1-12} alkyloxy
 25 group; C_{1-12} alkylthio group; C_{1-12} alkylsulfinyl group; C_{1-12} alkylsulfonyl group; C_{1-12} alkylsulfonylamino group; C_{1-12} alkylamino group; C_{1-12} alkylaminosulfonyl group; halo C_{1-12} alkyl group; halo C_{1-12} alkyloxy group; halo C_{1-12} alkylthio group; halo C_{1-12} alkylsulfinyl group; halo C_{1-12} alkylsulfonyl group; halo C_{1-12} alkylsulfonylamino group;
 30 halo C_{1-12} alkylamino group; halo C_{1-12} alkylaminosulfonyl group.

5

R₇, R₈ and R₉ are independently selected from a hydrogen atom, deuterium atom, a halogen atom, a nitro group, a cyano group, a C₁₋₄ alkyl group, a halo C₁₋₄ alkyl group, a mercapto C₁₋₄ alkyl group, an amino C₁₋₄ alkyl group, and a hydroxy C₁₋₄ alkyl group;

X, Y and Z are independently selected from a carbon atom, a nitrogen atom, with the proviso that when one of X, Y or Z is nitrogen, the other two positions are carbon atoms and that when Y or Z are nitrogen atoms, R₈ and R₉ are not present;

for use in the treatment of:

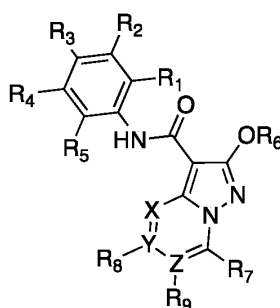
- 15 a) hematologic malignancies selected from the group consisting of: myelodysplastic syndromes, chronic myeloid leukemia and other myeloproliferative neoplasms, multiple myeloma, angioimmunoblastic T-cell lymphoma and acute lymphoblastic leukemia, Hodgkin's and non-Hodgkin's lymphomas, IDH mutated tumors, BRAF-mutated, RAS-mutated, PTEN-mutated and MYC/MYCN-driven hematologic neoplasms;
- 20 b) solid tumors selected from the group consisting of: osteosarcoma; Ewing sarcoma; melanoma; BRAF mutated tumors; H3K27M-mutated diffuse midline glioma; diffuse intrinsic pontine glioma; atypical teratoid rhabdoid tumor; ependymomas, neuroendocrine tumors; multiple endocrine neoplasia type 1 (MEN1) tumors; mutated
- 25 MEN1 tumors; KRAS-driven tumorous, preferably mutated KRAS hepatic tumors, mutated KRAS lung tumors, mutated KRAS pancreatic tumors, mutated KRAS colon carcinoma; prostate tumors; bladder tumors; kidney tumors; ferroptosis sensitive tumors preferably, lung cancer, glioblastoma, breast cancer, pancreatic cancer, hepatocellular carcinoma and colorectal cancer; IDH mutated tumors, preferably low-
- 30 grade gliomas, secondary glioblastoma, primary glioblastoma, cartilaginous and bone tumors, fibrosarcoma, sinonasal undifferentiated carcinoma, intrahepatic cholangiocarcinoma; MYC and MYCN driven tumors, preferably neuroblastoma, medulloblastoma, retinoblastoma, astrocytoma, glioblastoma multiforme, castration-resistant prostate cancer, neuroendocrine prostate cancer, , rhabdomyosarcoma,
- 35 Wilms tumors, small cell lung cancer, pancreatic tumors; AMPK α _{1low} expressing tumors; GPX4_{low} expressing tumors; p53 wild type and mutated cancers.

5

2. A composition comprising a compound of Formula (Ia) as defined in claim 1 and one or more anticancer agents, preferably selected from: Abemaciclib, Abiraterone, Acalabrutinib, Actimide, Adagrasib, Artemisinin, Artesunate Ado-Trastuzumab Emtansine, Afatinib, Alectinib, Alpelisib, Atezolizumab, Amivantamab, Amsacrine, Anastrozole, Apalutamide, Asparaginase, Axitinib, Azacitidine, Azathioprine, Belzutifan, Bevacizumab, Bicalutamide, Bleomycin, Brentuximab vedotin, Brigatinib, Busulfan, Cabazitaxel, Cabozantinib, Capecitabine, Capmatinib, Cyclophosphamide, Carboplatin, Carmustine, Cedazuridine, Cemiplimab-rwlc, Ceritinib, Cisplatin, Chlorambucil, Cytarabine, Cladribine, Clofarabine, CNX774, Cobimetinib, Dabrafenib, Dacarbazine, Dacomitinib, Dactinomycin, Darolutamide, Daunorubicin, Decitabine, Degarelix, Dilazep, Dihydroartemisinin, Dinutuximab, Dinutuximab beta, Dipyridamole, Docetaxel, Dordaviprone, Doxifluridine, Doxorubicin, Draflazine, Durvalumab, Epcoritamab, Enasidenib, Encorafenib, Enfortumab vedotin-ejfv, Entrectinib, Enzalutamide, EOS984, Epirubicin, Erastin, Erdafitinib, Erlotinib, Etoposide, Everolimus, Exemestane, , , Fam-trastuzumab deruxtecan-nxki, Fenalidomide, Feucovorin, Fludarabine, Fluorouracil, FPMINT, Flutamide, Gemcitabine, Gefitinib, Glofitamab, Hydroxyurea, , Ibrutinib, Idarubicin, Infigratinib, Ifosfamide, Ipilimumab, Irinotecan, Isotretinoin, Ivosidenib, JNK-IN-8, Larotrectinib, Lenvatinib, Lenalidomide, Letrozole, Leucovorin, Lomustine, Loncastuximab tesirine-lpy, Lorlatinib, L-alanosine, 8MDP, Mechlorethamine, Melphalan, Mercaptopurine, Methotrexate, Mioflazine, Mitomycin-C, Mitoxantrone, Mizoribine, Mobocertinib, Mosunetuzumab-axgb, Mycophenolic acid, Nab-paclitaxel, Nanoliposomal irinotecan, Naxitamab, Neratinib, Nilutamide, Niraparib, Nivolumab, Obinutuzumab, ONC212, Olaparib, Osimertinib, Oxaliplatin, Paclitaxel, Pacritinib, Panobinostat, Pazopanib, PBP 4883, Pebrolizumab, Pertuzumab, Pemetrexed, Pemigatinib, Pirtobutinib, Pralsetinib, Polatuzumab vedotin-piiq, Rapadocin, Ramucirumab, Regorafenib, Relugolix, Revlimid, Rituximab, RO7117997, Rucaparib, Sacituzumab govitecan, Selepercatinib, Selinexor, Sorafenib, Sotarasib, Sulfasalazine, Sunitinib, Talazoparib, Tamibarotene, Tamoxifen, Tazemetostat, Temozolomide, Teniposide, Tepotinib, Thioguanine, Thiotepa, Ticagrelor, Tivozanib, Tofacitinib, Topotecan, Tafasitamab-cxix, Temsirolimus, Trametinib, Trastuzumab, Tremelimumab, Trifluridine/Tipiracil, Treosulfan, Troglitazone, Valrubicin, Venetoclax, Vemurafenib, Vinblastine, Vincristine, Vindesine and Vinorelbine, Vorasenib, Zanubrutinib, Ziv-aflibercept.

- 5 3. A compound of Formula (Ia) as defined in claim 1 for use in the treatment of a viral infection caused by Herpes simplex virus 3-8 (HSV-3-8).

4. A compound of formula (I):



Formula (I)

10

wherein R₁, R₂, R₄ and R₅ are independently selected from a hydrogen atom, deuterium atom, a halogen atom, a linear and/or branched C₁₋₈ alkyl group, an alkyloxy group, an alkylamino group, an alkylthio group, a halo C₁₋₈ alkyl group, a halo C₁₋₈ alkyloxy group, a halo C₁₋₈ alkylamino group, a nitro group, a cyano group;

15

R₃ is:

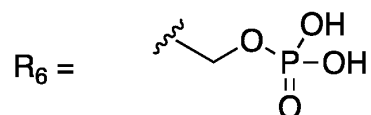
- an aromatic group selected from the group consisting of: phenyl group, heteroaryl group preferably an azinyl group such as pyridinyl group, pyrimidinyl group; phenoxy group; azinyloxy group such as pyridinyloxy group, pyrimidinyl group; phenylthio group; azinylthio group such as pyridinylthio group, pyrimidinylthio group; phenylsulfinyl group; phenylsulfonyl group; phenylsulfonylamino group; N-phenylcarbamoyl group;
- an aliphatic group selected from the group consisting of: C₁₋₁₂ alkyl group; C₁₋₁₂ alkyloxy group; C₁₋₁₂ alkylthio group; C₁₋₁₂ alkylsulfinyl group; C₁₋₁₂ alkylsulfonyl group; C₁₋₁₂ alkylsulfonylamino group; C₁₋₁₂ alkylamino group; C₁₋₁₂ alkylaminosulfonyl group; halo C₁₋₁₂ alkyl group; halo C₁₋₁₂ alkyloxy group; halo C₁₋₁₂ alkylthio group; halo C₁₋₁₂ alkylsulfinyl group; halo C₁₋₁₂ alkylsulfonyl group; halo C₁₋₁₂ alkylsulfonylamino group; halo C₁₋₁₂ alkylamino group; halo C₁₋₁₂ alkylaminosulfonyl group;

25

- 5 R₇, R₈ and R₉ are independently selected from a hydrogen atom, deuterium atom, a halogen atom, a nitro group, a cyano group, a C₁₋₄ alkyl group, a halo C₁₋₄ alkyl group, a mercapto C₁₋₄ alkyl group, an amino C₁₋₄ alkyl group, and a hydroxy C₁₋₄ alkyl group;
R₆ is selected from the group consisting of a linear or branched C₁₋₆ alkyl substituted with a group selected from a phosphate, a -COOH, a -CONHR₁₀, wherein R₁₀ is selected from
- 10 hydrogen and C₁₋₆ alkyl chain;
X, Y and Z are independently selected from a carbon atom, a nitrogen atom, with the proviso that when one of X, Y or Z is nitrogen, the other two positions are carbon atoms and when Y or Z are nitrogen atoms, R₈ and R₉ are not present.
- 15 5. A compound according to claim 4, wherein at least one of R₁, R₂, R₄ and R₅ is or contains a halogen atom, preferably a fluorine or chlorine atom, more preferably a fluorine atom.
- 20 6. A compound according to claim 4, wherein at least one of R₁, R₂, R₄ and R₅ is selected from hydrogen, -OCF₃, methyl, isopropyl, methyl, 1,1,1-trifluoro-2-propanoxy.
- 7 A compound according to any one of claims 4 to 6, wherein R₃ is selected from trifluoromethoxyphenyl, 2,2 difluoropropoxyphenyl, 3-pyridinyl, phenoxy, ortho-trifluoromethylphenyl, meta-trifluoromethylphenyl, meta-chlorophenyl, ortho-chlorophenyl,
- 25 meta-fluorophenyl, ortho-fluorophenyl, meta-hydroxyphenyl, meta-butoxyphenyl.
8. A compound according to any one of claims 4 to 7, wherein R₇, R₈ and R₉ are independently selected from hydrogen, methyl, chloro, methoxy, and hydroxymethyl.
- 30 9. A compound according to any one of claims 4 to 8, wherein X, Z are carbon atoms, Y is carbon or nitrogen, R₇, R₈, R₉ are hydrogen, R₁, R₂, R₄, R₅ are fluorine, R₃ is a phenyl or 2,2-difluoro(C₁-C₃)alkyloxyphenyl or trifluoro(C₁-C₃)alkylphenyl or trifluoro(C₁-C₃)alkyloxyphenyl.
10. A compound according to any one of claims 4 to 9, wherein X, Y, Z are carbon atoms, R₇,
- 35 R₈, R₉ are hydrogen atoms, R₁, R₄ are hydrogen atoms, R₂ is a methyl, R₅ is iso-propyl, R₃ is phenoxy.

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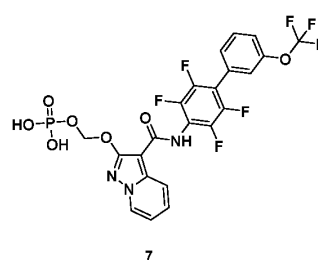
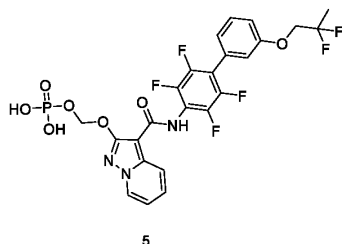
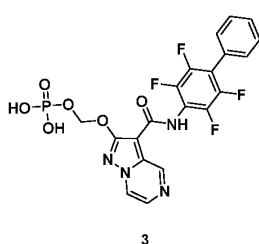
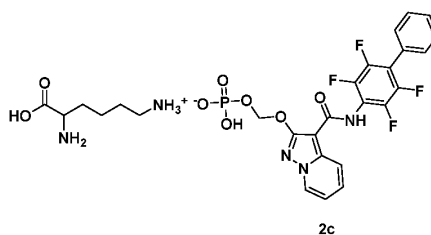
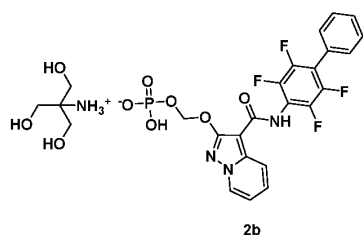
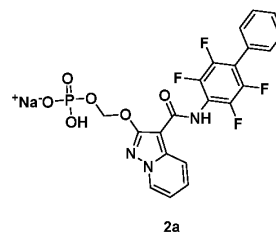
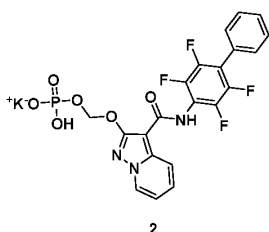
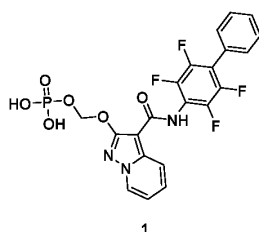
11. A compound according to any one of claims 4 to 10, wherein R_6 is a phosphonoxymethyl group represented by formula (II):



10

Formula (II)

12. A compound according to claim 11, wherein R_6 is in salified form, wherein the salt is selected from an alkali metal salt, preferably lithium, sodium, potassium, and cesium salt; an alkaline earth metal salt, preferably calcium, magnesium or strontium salt; aluminum or zinc salt; an ammonium salt; a quaternary ammonium salt having 1 to 20 carbon atoms, preferably tris(hydroxymethyl)aminomethane (tromethamine), tetramethylammonium, tetraethylammonium, tetra(n-propyl)ammonium, tetra(n-butyl)ammonium, N-benzyl-N,N,N-trimethylammonium; a lysine salt, a choline salt; a benzalkonium salt.
13. A compound according to any one of claims 4 to 12, wherein the compound is selected from the following formulas:



5

14. A pharmaceutical composition comprising a compound of Formula (I) according to any one of claims 4 to 13 and a pharmaceutically accepted carrier, excipient and/or diluent.

- 10 15. A composition comprising a compound of Formula (I) according to any one of claims 4 to 14 and one or more anticancer agents, preferably selected from: Abemaciclib, Abiraterone, Acalabrutinib, Actimide, Adagrasib, Artemisinin, Artesunate, Ado-Trastuzumab, Emtansine, Afatinib, Alectinib, Alpelisib, Atezolizumab, Amivantamab, Amsacrine, Anastrozole, Apalutamide, Asparaginase, Axitinib, Azacitidine, Azathioprine, Belzutifan, Bevacizumab, Bicalutamide, Bleomycin, Brentuximab vedotin, Brigatinib, Busulfan, Cabazitaxel, Cabozantinib, Capecitabine, Capmatinib, Cyclophosphamide, Carboplatin, Carmustine, Cedazuridine, Cemiplimab-rwlc, Ceritinib, Cisplatin, Chlorambucil, Cytarabine, Cladribine,

- 5 Clofarabine, CNX774, Cobimetinib, Dabrafenib, Dacarbazine, Dacomitinib, Dactinomycin, Darolutamide, Daunorubicin, Decitabine, Degarelix, Dilazep, Dihydroartemisinin, Dinutuximab, Dinutuximab beta, Dipyridamole, Docetaxel, Dordaviprone, Doxifluridine, Doxorubicin, Draflazine, Durvalumab, Epcoritamab, Enasidenib, Encorafenib, Enfortumab vedotin-ejfv, Entrectinib, Enzalutamide, EOS984, Epirubicin, Erastin, Erdafitinib, Erlotinib, Etoposide,
- 10 Everolimus, Exemestane, Fam-trastuzumab deruxtecan-nxki, Fenalidomide, Feucovorin, Fludarabine, Fluorouracil, FPMINT, Flutamide, Gemcitabine, Gefitinib, Glofitamab, Hydroxyurea, Ibrutinib, Idarubicin, Infigratinib, Ifosfamide, Ipilimumab, Irinotecan, Isotretinoin, Ivosidenib, JNK-IN-8, Larotrectinib, Lenvatinib, Lenalidomide, Letrozole, Leucovorin, Lomustine, Loncastuximab tesirine-lpy, Lorlatinib, L-alanosine, 8MDP, Mechlorethamine,
- 15 Melphalan, Mercaptopurine, Methotrexate, Mioflazine, Mitomycin-C, Mitoxantrone, Mizoribine, Mobocertinib, Mosunetuzumab-axgb, Mycophenolic acid, Nab-paclitaxel, Nanoliposomal irinotecan, Naxitamab, Neratinib, Nilutamide, Niraparib, Nivolumab, Obinutuzumab, Olaparib, ONC212, Osimertinib, Oxaliplatin, Paclitaxel, Pacritinib, Panobinostat, Pazopanib, PBP 4883, Pembrolizumab, Pertuzumab, Pemetrexed, Pemigatinib, Pirtobutinib, Pralsetinib, Polatuzumab
- 20 vedotin-piiq, Rapadocin, Ramucirumab, Regorafenib, Relugolix, Revlimid, Rituximab, RO7117997, Rucaparib, Sacituzumab govitecan, Selepercatinib, Selinexor, Sorafenib, Sotarasib, Sulfasalazine, Sunitinib, Talazoparib, Tamibarotene, Tamoxifen, Tazemetostat, Temozolomide, Teniposide, Tepotinib, Thioguanine, Thiotepa, Ticagrelor, Tivozanib, Tofacitinib, Topotecan, Tafasitamab-cxix, Temsirolimus, Trametinib, Trastuzumab,
- 25 Tremelimumab, Trifluridine/Tipiracil, Treosulfan, Troglitazone, Valrubicin, Venetoclax, Vemurafenib, Vinblastine, Vincristine, Vindesine and Vinorelbine, Vorasidenib, Zanubrutinib, Ziv-aflibercept.

16. A compound of Formula (I) according to any one of claims from 4 to 13 or a pharmaceutical
- 30 composition according to claim 14 or a composition according to claim 15, for use as an anticancer agent, preferably for the treatment of
 - a) hematologic malignancies, preferably selected from the group consisting of: acute myeloid leukemia, myelodysplastic syndromes, chronic myeloid leukemia and other myeloproliferative neoplasms, multiple myeloma, angioimmunoblastic T-cell lymphoma and
 - 35 acute lymphoblastic leukemia, Hodgkin's and non-Hodgkin's lymphomas, IDH mutated tumors,

5 BRAF-mutated, RAS-mutated, PTEN-mutated and MYC/MYCN-driven hematologic neoplasms;

- b) solid tumors, preferably selected from the group consisting of: osteosarcoma; Ewing sarcoma; melanoma; BRAF mutated tumors; PTEN-mutant tumors; H3K27M-mutated diffuse midline glioma; diffuse intrinsic pontine glioma; atypical teratoid rhabdoid tumor;
- 10 ependymomas, neuroendocrine tumors; multiple endocrine neoplasia type 1 (MEN1) tumors; mutated MEN1 tumors; KRAS-driven tumorous, preferably mutated KRAS hepatic tumors, mutated KRAS lung tumors, mutated KRAS pancreatic tumors, mutated KRAS colon carcinoma; prostate tumors; bladder tumors; kidney tumors; ferroptosis sensitive tumors preferably, lung cancer, glioblastoma, breast cancer, preferably triple-negative breast cancer,
- 15 pancreatic cancer, hepatocellular carcinoma and colorectal cancer; IDH mutated tumors, preferably low-grade gliomas, secondary glioblastoma, primary glioblastoma, cartilaginous and bone tumors, fibrosarcoma, sinonasal undifferentiated carcinoma, intrahepatic cholangiocarcinoma; MYC and MYCN driven tumors, preferably neuroblastoma, medulloblastoma, retinoblastoma, astrocytoma, glioblastoma multiforme, castration-resistant
- 20 prostate cancer, neuroendocrine prostate cancer, rhabdomyosarcoma, Wilms tumors, small cell lung cancer, pancreatic tumors; AMPK α ₁_{low} expressing tumors; GPX4_{low} expressing tumors; p53 wild type and mutated cancers.

17. A compound of Formula (I) according to any one of claims from 4 to 13 or a pharmaceutical

25 composition according to claim 14, for use as antiviral agent, preferably for the treatment of a viral infection caused by a DNA virus or an RNA virus, wherein the virus is selected from the group consisting of *Herpesviridae*, *Orthomyxoviridae*, *Paramyxoviridae* and *Coronaviridae*.

18. A compound of Formula (I) or a pharmaceutical composition for use according to claim

30 17, wherein the virus is selected from the group consisting of Herpes simplex virus 1 to 8 (HSV-1-8), paramyxovirus, pneumovirus, morbillivirus, Influenza A virus, Influenza B virus, Respiratory syncytial virus (RSV), severe acute respiratory syndrome coronavirus 1 (SARS-CoV-1), severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and Middle East respiratory syndrome-related coronavirus (MERS-CoV).

35

- 5 19. A composition comprising a compound of Formula (I) as defined in claim 4 and/or a
compound of formula (Ia) as defined in claim 1 and one or more antiviral agents, preferably
selected from acyclovir, adefovir, amantadine, baloxavir marboxil, bamlanivimab/etesevimab,
brivudine, casirivimab/imdevimab, cidofovir, famciclovir, favipiravir, fomivirsen, foscarnet,
ganciclovir, N(4)-Hydroxycytidine, indinavir, idoxuridine, laninamivir, lopinavir, molnupiravir,
10 nelfinavir, nirmatrelvir, oseltamivir, penciclovir, permivir, ribavirin, sotrovimab, tenofovir,
tenofovir alafenamide, tenofovir diisoproxilfumarate, trifluridine, telbivudine, vidarabine,
sofosbuvir, nevirapine, efavirenz, raltegravir, remdesvir, rimantadine, ritonavir, ritonavir –
amlanivimab, saquinavir, tixagevimab/cilgavimab, trifluridine, valacyclovir, valganciclovir,
vidarabine, zanamivir, zidovudine.

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