

PYRAZOLO DERIVATIVES AS HUMAN DIHYDROOROTATE DEHYDROGENASE
(HDHODH) INHIBITORS FOR USE AS ANTIVIRALS

FIELD OF THE INVENTION

The present invention relates to human dihydroorotate dehydrogenase (*h*HDHODH) inhibitors for use as antivirals. The *h*HDHODH inhibitors for use according to the invention are effective as antivirals by triggering in the host cell pyrimidine starvation due to inhibition of *h*HDHODH. The *h*HDHODH inhibitors for use according to the invention are effective against a broad spectrum of both RNA and DNA viruses, including, *inter alia*, SARS-CoV-2 and other important human viral pathogens.

BACKGROUND OF THE INVENTION

The social and economic long terms effects of the COVID-19 pandemic are unpredictable, however strategic decisions made now and in the coming months will be some of the most important made in generations. After many outbreaks of emerging and re-emerging RNA viruses, such as SARS, Influenza virus, MERS, Ebola, and Zika, strategic actions recommended to better prepare for other viral disease outbreaks, such as investments on *Broad Spectrum Antiviral Agents* (BSAAs), remain inadequate and this has led to the present crisis scenario. Among BSAAs, which are advantageously able to be rapidly adapted from

a pandemic event to another, *Host-Targeting Antivirals* (HTAs) work by interfering with cellular biochemical pathways commonly used by different viruses for the replication, thus overcoming virus specificity and potential viral mutagenesis.

Human dihydroorotate dehydrogenase (*hDHODH*, EC 1.3.99.11) present in the inner mitochondrial membrane, is a flavin-dependent enzyme involved in *de novo* pyrimidine biosynthesis. It catalyzes the rate-limiting step in *de novo* pyrimidine biosynthesis, which converts dihydroorotate (DHO) to orotate (ORO). *hDHODH* has already been validated as a therapeutic target for the treatment of autoimmune diseases, such as rheumatoid arthritis and multiple sclerosis. Furthermore, *hDHODH* 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 acute myelogenous leukemia (AML) and viral infection.⁴ The connection with AML has paved the way for completely new perspectives in the treatment of the disease as well as in the *hDHODH* field. Presumably, all of these diverse malignancies converge on a similar pathway of metabolic reprogramming to drive their dependence on pyrimidine synthesis and sensitivity to *DHODH*-inhibition.

The inventors recently identified and characterized a novel class of *hDHODH* inhibitors⁵, including, *inter alia*,

compound **1** (named as compound **4** in ref.⁶), which has a particularly elevated potency toward the hDHODH enzyme (hDHODH IC₅₀ 1.2 nM) and excellent drug-like properties.

SUMMARY OF THE INVENTION

The inventors have now unexpectedly found that compound **1** and its derivatives illustrated in formulae (I) to (V) below are also potent and broad-spectrum antiviral agents, including anti-SARS-CoV-2. The research carried out by the present inventors was dedicated to investigate the use of compound **1** and its derivatives as *Broad Spectrum Antiviral Agent* (BSAA), as well as to refine the Structure Activity Relationship (SAR) of this class of hDHODH inhibitors. As it will be illustrated in detail in the following, the tested hDHODH inhibitors for use according to the present invention advantageously showed high activity *in vitro*. In particular, compound **1** is superior to brequinar in terms of antiviral potency and safety profile, as it is able to block viral replication at concentrations that are 1 log digit lower than those obtained with brequinar. Given its ability to inhibit SARS-CoV-2 replication with EC₅₀ 74 nM and an incredibly effective SI (>7900, CC₅₀ >500 µM), compound **1** has one of the most potent and safer *in vitro* profile so far obtained against SARS-CoV-2 replication in E6 cells. Compound **1** was also investigated as BSAA against other viruses and showed similar potencies.

To pave the way to future preclinical and clinical applications, the inventors also carried out some studies concerning the pKs, the half-life *per os* and *i.v.*, the *in vivo* toxicity and metabolism, as well as the *in vitro* activity against some selected pathogenic viruses of compounds for use according to the invention. Such studies are illustrated in the following. The compounds were tested against the following viruses: Herpes simplex 1 and 2, Influenza Virus and some pathogenic viruses for the respiratory tract such as Respiratory Syncytial Virus (RSV), one of the main causes of infant's hospitalization and mortality, as well as severe acute respiratory syndrome corona virus 2 (SARS-CoV-2), belonging to the same family of viruses responsible for severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS).

DETAILED DESCRIPTION OF THE INVENTION

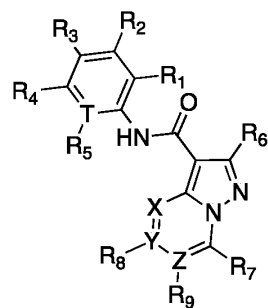
The present invention relates to a class of hDHODH inhibitors, based on an unusual carboxylic group bioisostere, the 2-hydroxypyrazolo[1,5-a]pyridine, for use as antiviral agents, i.e. as inhibitors of virus replication.

2-Hydroxypyrazolo[1,5-a]pyridine is a system that is still relatively unexplored in the literature. The present description reports its scaffold-hopping use as

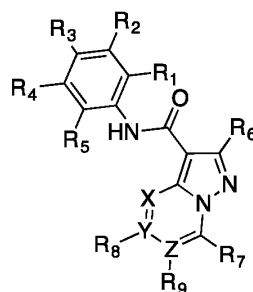
a bioisostere of a carboxylic function (often present in DHODH inhibitor structures) in preparing two series of derivatives. In the first series, besides investigating the moiety itself (compounds **2-5**, Figure 1A), the inventors have also investigated the effect of introducing a chloro and a methyl group into the pyridine ring in order to improve its lipophilic interaction with *h*DHODH subsite 4 (compounds **2**, **3** and **4**, Figure 1) or replacing a pyridine with a tetrahydrogenated pyridine (piperidine) moiety as in compound **5**. The second ring of the biphenylic scaffold was also subject of investigation by inserting either polar (compounds **6**, **8**, **9**, **14**) or lipophilic (compounds **7**, **10-13**, **15-17**) moieties.

In the second series (compounds **18**, **23**, **24**, **26-37**, Figure 2A and 2B), the inventors have replaced the biphenyl substituent with a more flexible diphenyl ether in order to improve its pharmacokinetic properties and provide more drug-like compounds. The theoretical design, synthesis, SAR, biological assays, physicochemical characterization and preliminary ADME profiles of the compounds are presented and discussed fully hereinafter.

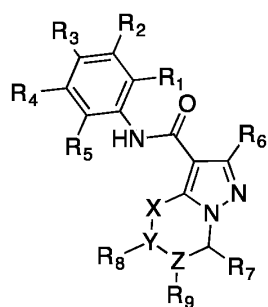
Accordingly, a first aspect of the present invention is a 2-hydroxypyrazolo[1,5-*a*]pyridine scaffold-based *h*DHODH inhibitor of Formula (I), (II), (III), (IV) or (V) illustrated below, for use in inhibiting viral replication or for use as an antiviral agent.



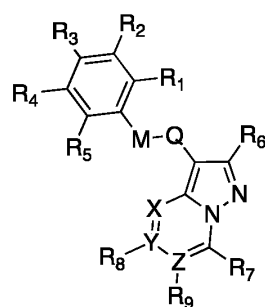
Formula (I)



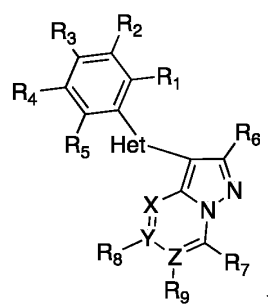
Formula (II)



Formula (III)



Formula (IV)



Formula (V)

In Formulae (I) to (V) above:

R₁, R₂, R₄ and R₅ are independently selected from a hydrogen atom, a halogen atom, a alkyl group, a alkyloxy group, a cycloalkyloxy group, an alkylthio group, a halo alkyl group, a halo alkyloxy group, a nitro group, a cyano group, and alkylamino group;

R₃ is selected from an optionally substituted phenyl group, heteroaryl group, pyridinyl group, piperidinyl group, phenoxy group, pyridinoxy group, piperidinyl group, phenylthio group, azinyl group, phenylsulfonyl group, phenylsulfinyl group, phenylsulfonamino group, alkyl group, alkyloxy group, alkylthio group, halo alkyl group, and halo alkyloxy group;

R₇, R₈ and R₉ are independently selected from a hydrogen atom, a halogen atom, a nitro group, a cyano group, a halo alkyl group, a thio alkyl group, an amino alkyl group, a alkyl group, and a hydroxy alkyl group;

R₆ is selected from a alkyloxy group, a halogen atom, an acyloxy group, a monophosphate group, a hydroxyl group, a thiol group, an amino group, or a salt thereof;

X, Y and Z are independently selected from a carbon atom, a nitrogen atom, an oxygen atom and a sulfur atom, with the proviso that when one of X, Y or Z is nitrogen, oxygen or sulfur, the other two positions are carbon atoms;

in Formula (I), T is a carbon atom or a heteroatom like nitrogen atom, with the proviso that when T is a nitrogen atom, R⁵ in Formula (I) does not exist;

in Formula (IV), M is selected from a carbon sp² atom, a nitrogen sp³ atom, a nitrogen sp² atom, a carbonyl group and a sulfonyl group;

in Formula (IV), Q is selected from a carbon sp² atom, a carbonyl group, a thiocarbonyl group, a sulfonyl group, a polihalogenate-C₂-alkylchain, a carbonylamino group, an aminocarbonyl group, a nitrogen sp² atom and a nitrogen sp³ atom;

with the provisos that when M is a carbon sp² atom, Q is a carbon sp² atom; when M is a nitrogen sp³ atom, Q is selected from a carbonyl group, a thiocarbonyl group, a sulfonyl group, a polihalogenate-C₂-alkylchain, a carbonylamino group, an aminocarbonyl group; when M is a nitrogen sp² atom, Q is a nitrogen sp² atom; when M is a carbonyl group or sulfonyl group, Q is a nitrogen sp³ atom;

in Formula (V), Het is selected from azoles, such as an imidazole, a pyrazole, an oxazole, a thiazole, a triazole, an oxadiazole, a thiadiazole, a tetrazole.

According to a preferred embodiment, at least one of R₁, R₂, R₄ and R₅ is or contains a halogen atom. A preferred

halogen atom is a fluorine atom (F). In a particularly preferred embodiment, all of R₁, R₂, R₄ and R₅ are fluorine (F).

Preferred alkyls in the definition of R₁, R₂, R₄, R₅ are C1-C6 alkyls, more preferably C1-C4 alkyls. Alkyls are either linear or branched.

Particularly preferred meanings for R₁, R₂, R₄ and/or R₅ are H (hydrogen), F (fluorine), Cl (chlorine), -CH₃, -CH(CH₃)₂, -O-CH(CH₃)₂, -o-cyclobutyl, -O-CH(CH₃)(CH₂CH₃), -O-CH(CH₂CH₃)₂, -O-CH(CH₃)(CH₂CH₂CH₃).

Preferred alkyls in the definitions of R₃ are C1-C12 alkyls. Alkyls are either linear or branched.

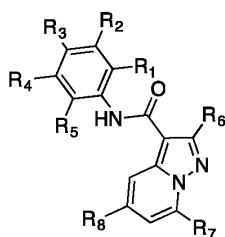
Particularly preferred meanings for R₃ are optionally substituted phenyl, phenoxy, thiophenol, morpholine, thiophene, pyridine and indole radicals. Suitable substituents are for example halogen atoms (e.g., F or Cl), alkyl or alkoxy groups (such as methyl, methoxy, ethyl ethoxy, propyl, propoxy); haloalkyl or haloalkoxy groups (such as trifluoroalkyl, trifluoroalkoxy, difluoroalkyl, difluoroalkoxy, fluoroalkyl, fluoroalkoxy, in which alkyl is preferably methyl, ethyl, propyl or butyl); -OH; oxyketones and oxyalcohols (such as oxypropanone, oxypropanol).

Preferred alkyls in the definitions of R_7 , R_8 and R_9 are C1-C6 alkyls, more preferably C1-C4 alkyls. Alkyls are either linear or branched.

In the definition of R_6 , if R_6 is OH or a monophosphate, preferred salts are Na^+ , K^+ Ca or Cs^{2+} salts.

In all of the aforementioned embodiments, a preferred C₁-C₄ alkyl group as R_7 and R_8 is a methyl group while both X, Y and Z are carbon sp² atoms.

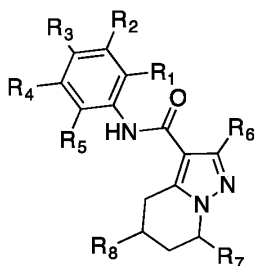
Formula (Ia) below illustrates a preferred embodiment of Formula (I) in which X=Y=Z are sp² C atoms and R_9 is a proton:



Formula (Ia)

Preferred compounds falling within the scope of Formulae (I) and (Ia) are compounds **1-4**, **6-18**, **23-24**, **26-37**, **40-43** illustrated in Figures 1 and 2.

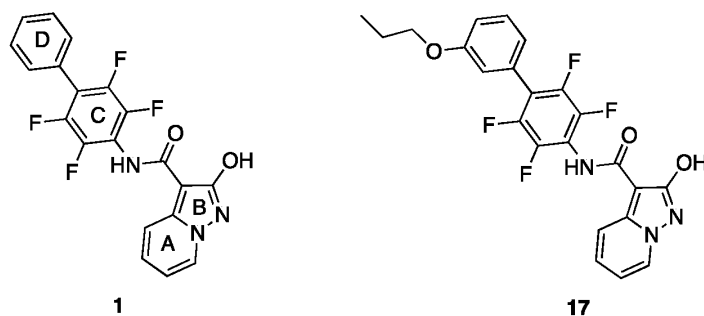
Formula (IIa) below illustrates a preferred embodiment of Formula (II) in which X=Y=Z are sp³ C atoms and R_9 is a proton:



Formula (IIa)

Preferred compounds falling within Formulae (I) to (V) are compounds **1** to **43** illustrated in Figures 1A, 1B, and Figures 2A, 2B and 2C.

Particularly preferred inhibitors for use according to the invention are compounds **1** and **17**, having the structural formulae depicted below:



Particularly preferred compounds **1** (also designated as "MEDS433") and **17** show brequinar-like hDHODH potency levels *in vitro* and are superior in terms of antiviral potency and selectivity blocking the viral replication

at concentrations that are 1 log digit lower than those achieved in experiments with brequinar.

A second aspect of the present invention is an antiviral pharmaceutical composition comprising a 2-hydroxypyrazolo[1,5-a]pyridine scaffold-based *h*DHODH inhibitor of Formulae (I) to (V) as defined above as the antiviral agent, and a pharmaceutically acceptable carrier, excipient and/or diluent.

A third aspect of the present invention is a 2-hydroxypyrazolo[1,5-a]pyridine scaffold-based *h*DHODH inhibitor of the general formulae (I) to (V) as defined above for use in the therapeutic treatment of a virus infection in a subject, wherein the virus is preferably pathogenic.

In all of the aforementioned aspects of the present invention, the virus is a DNA virus or an RNA virus. Preferably, the virus is selected from the group consisting of *Herpesviridae*, *Orthomyxoviridae*, *Paramyxoviridae* and *Coronaviridae*. More preferably, the virus is selected from the group consisting of Herpes simplex virus 1 (HSV-1), Herpes simplex virus 2 (HSV-2), 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).

The following experimental part is provided by way of illustration only and is not intended to limit the scope of the invention as defined by the appended claims.

In the experimental part, reference is made to the following figures:

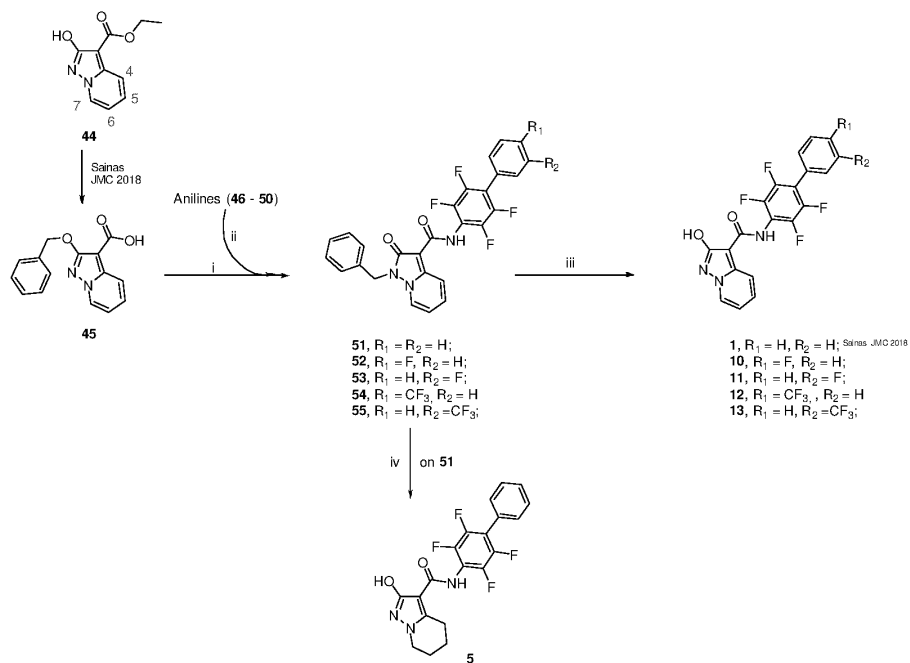
Figures 1A, 1B and Figures 2A, 2B, 2C showing the structures of compounds **1 - 43** which are the preferred compounds for use according to the invention.

Figure 3 includes two diagrams showing that the antiviral activity of compound **1** against RSV is rescued by uridine and orotic acid.

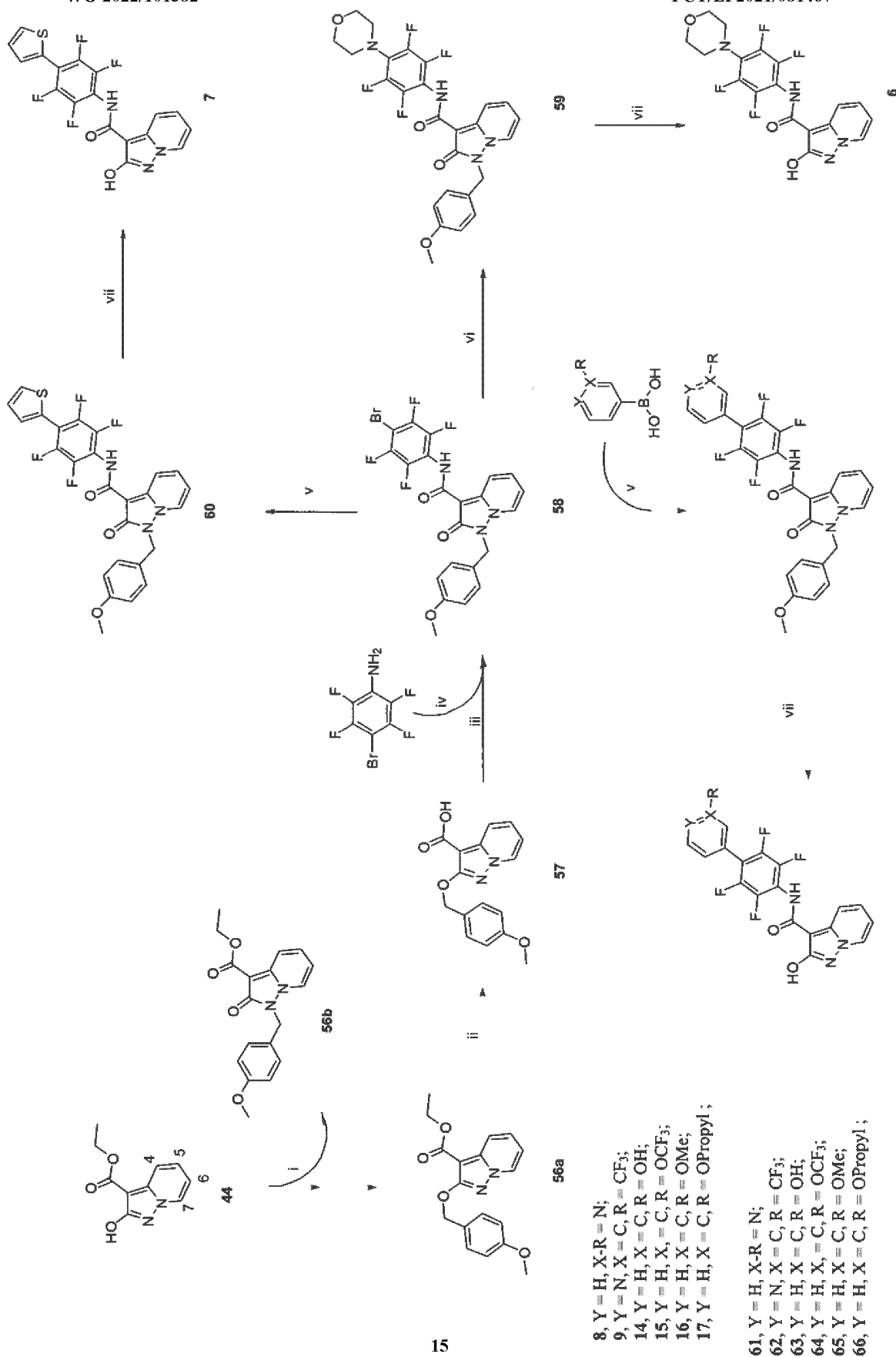
Figure 4 is a graph showing that compound **1** inhibits SARS-CoV-2 replication in Vero E6 cells.

EXPERIMENTAL PART

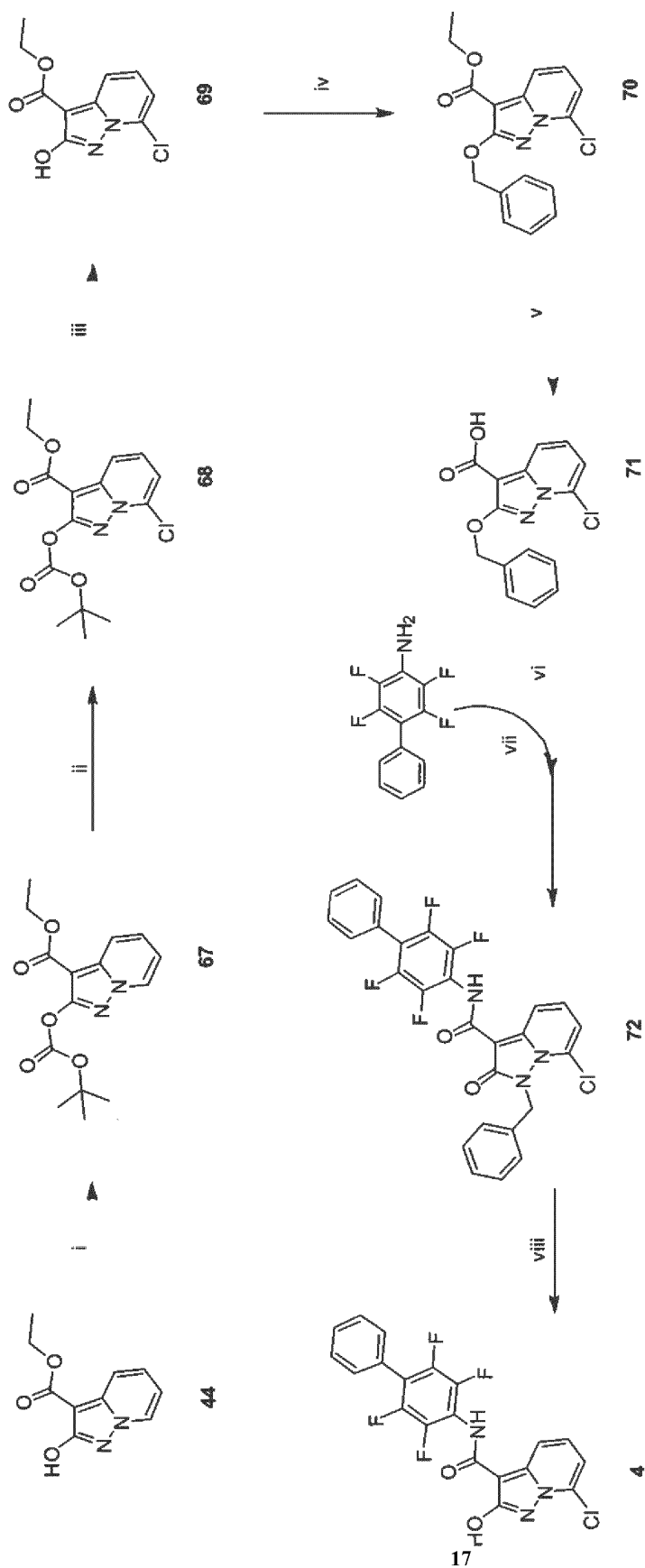
Synthesis schemes:



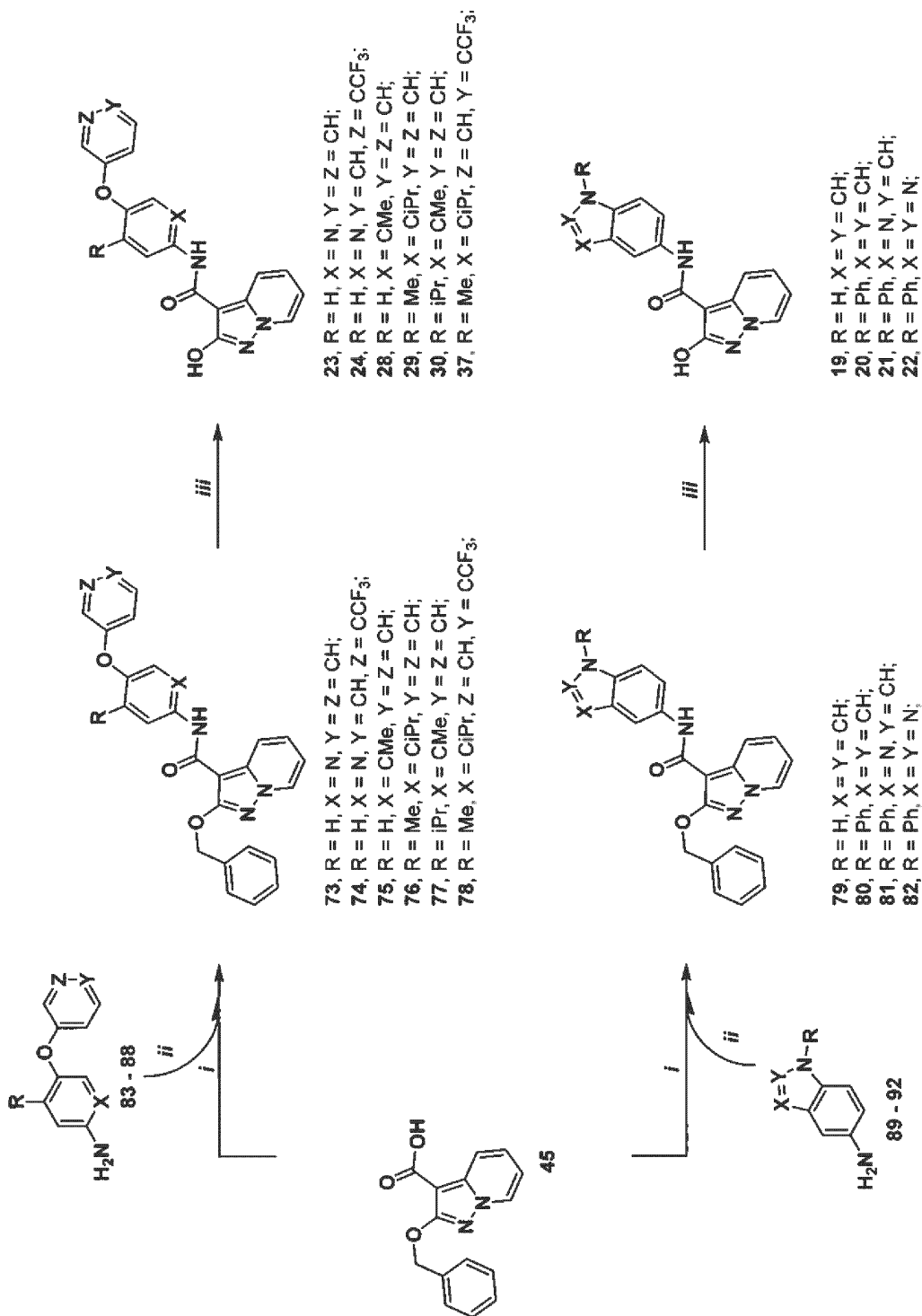
Scheme 1. Synthetic methodologies for the synthesis of compounds **1**, **5**, **10–13**: *i*) oxalyl chloride, dry DMF, dry THF; *ii*) AlMe₃, dry toluene, reflux; *iii*) H₂, Pd/C, 37 % w/w HCl, ethanol; *iv*) H₂, Pd/C, dry THF, 40 bar, 65 °C, SynthWAVE.



Scheme 2. Synthetic methodologies for the synthesis of compounds **6 - 9, 14 -17**: i) Cs_2CO_3 , 4-MeOBnBr, dry DMF; ii) 5M NaOH, ethanol, 75 °C; iii) nitrogen atmosphere, oxalyl chloride, dry DMF, dry THF; iv) AlMe_3 , dry toluene, reflux; v) a) $\text{Pd}(\text{Ph}_3)_4$, K_2CO_3 , dioxane/water (9:1 v/v), 1h r.t, b) corresponding boronic acid, reflux; vi) nitrogen atmosphere, morpholine, Cs_2CO_3 , $\text{Pd}(\text{OAc})_2$, BINAP, dry toluene, sealed tube, 110°C; vii) thioanisole, trifluoroacetic acid, 70 °C.



Scheme 3. Synthetic methodologies for the synthesis of compound **4**: i) Cs_2CO_3 , *tert*-butoxycarbonyl anhydride, dry THF, reflux; ii) a) nitrogen atmosphere, lithium hexamethyldisililazide (LiHMDS, 1.0 M, dry THF), $-78\text{ }^\circ\text{C}$, 1h, b) nitrogen atmosphere, hexachloroethane r.t.; iii); trifluoroacetic acid, dry dichloromethane, r.t.; iv) benzyl bromide, Cs_2CO_3 , dry DMF, r.t.; v) 6 M NaOH, ethanol abs, $75\text{ }^\circ\text{C}$; vi) nitrogen atmosphere, oxalyl chloride, dry DMF, dry THF; vii) AlMe_3 , dry toluene, reflux; viii) thioanisole, trifluoroacetic acid, $70\text{ }^\circ\text{C}$.



Scheme 4. Synthetic methodologies for the synthesis of compounds **19 - 24**, **28 - 30** and **37**: i) oxalyl chloride, dry DMF, dry THF, nitrogen atmosphere; ii) dry toluene, dry pyridine; iii) H₂, Pd/C, dry THF.

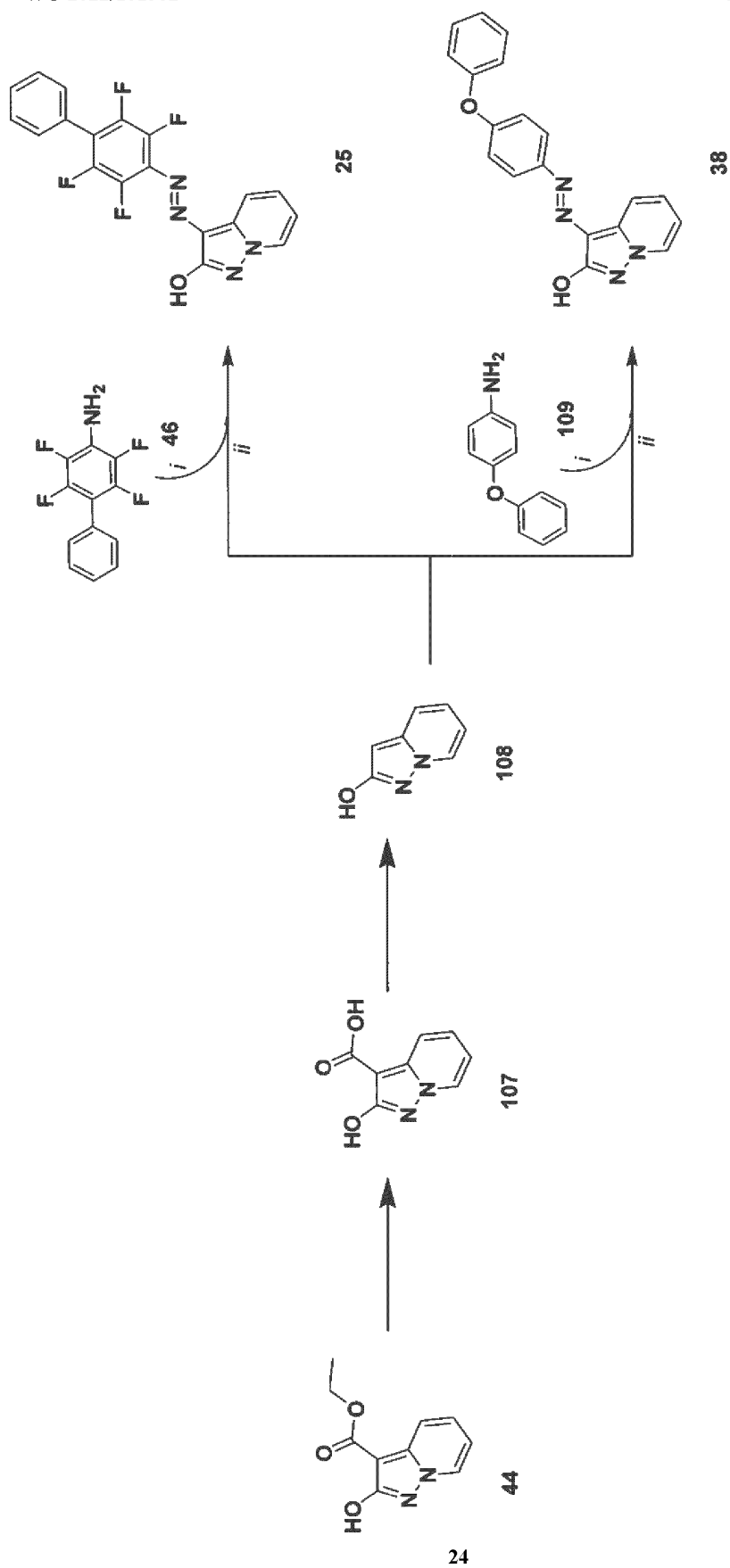


31. R = Me, R₁ = 2-propoxy, X = O, Y = CH;
32. R = Me, R₁ = cyclobutoxy, X = O, Y = CH;
33. R = Me, R₁ = 2-butoxy, X = O, Y = CH;
34. R = Me, R₁ = 2-penthoxy, X = O, Y = CH;
35. R = Me, R₁ = 3-penthoxy, X = O, Y = CH;
36. R = Me, R₁ = iPr, X = O, Y = N;

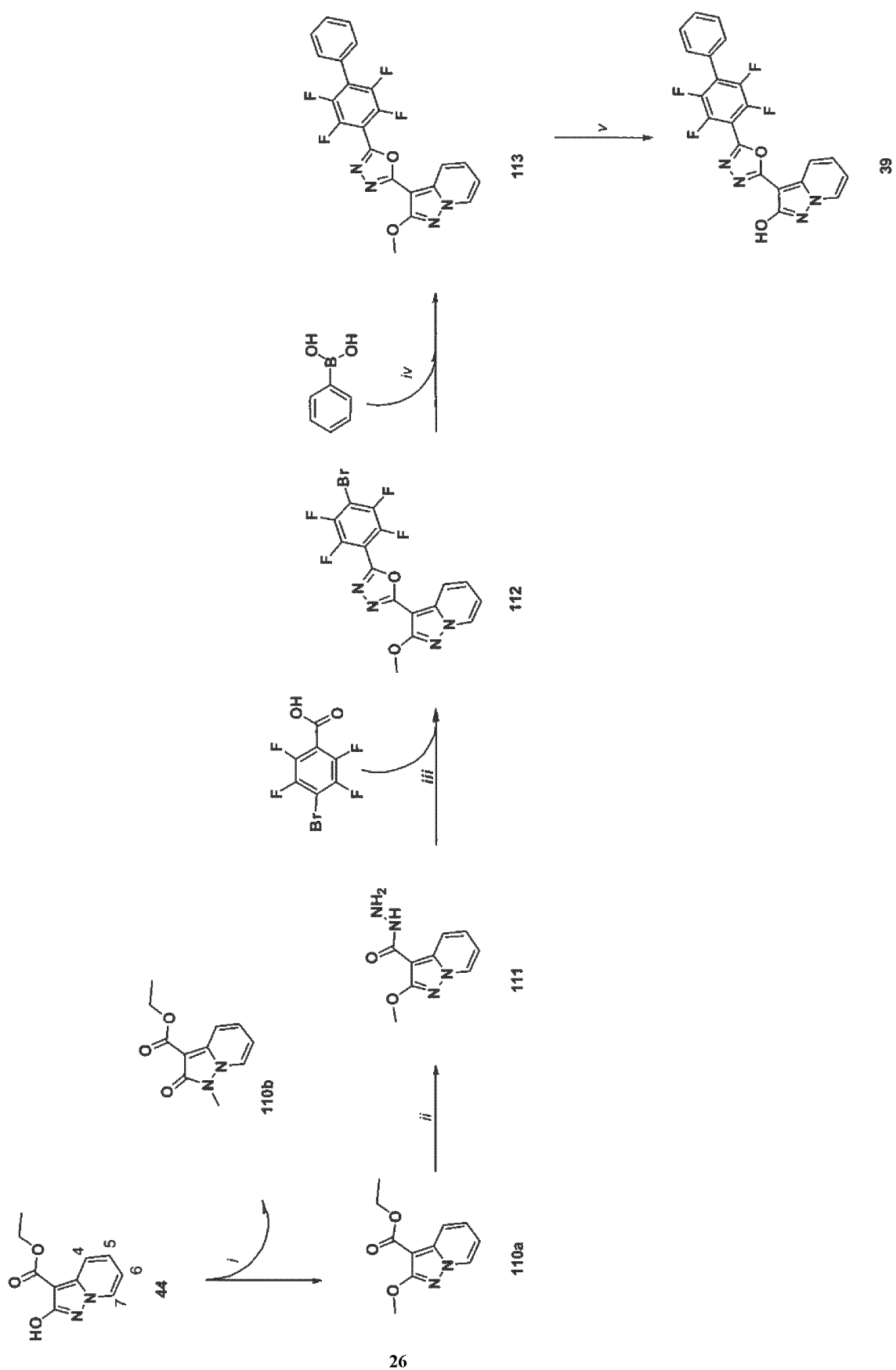
93. $R = R_1 = \text{Me}$, $X = \text{S}$, $Y = \text{N}$;
 94. $R = \text{Me}$, $R_1 = 2\text{-propoxy}$, $X = \text{O}$, $Y = \text{CH}$;
 95. $R = \text{Me}$, $R_1 = \text{cyclobutoxy}$, $X = \text{O}$, $Y = \text{CH}$;
 96. $R = \text{Me}$, $R_1 = 2\text{-butoxy}$, $X = \text{O}$, $Y = \text{CH}$;
 97. $R = \text{Me}$, $R_1 = 2\text{-pentoxy}$, $X = \text{O}$, $Y = \text{CH}$;
 98. $R = \text{Me}$, $R_1 = 3\text{-pentoxy}$, $X = \text{O}$, $Y = \text{CH}$;
 99. $R = \text{Me}$, $R_1 = \text{iPr}$, $X = \text{O}$, $Y = \text{N}$;

Scheme 5. Synthetic methodologies for the synthesis of compounds **26**, **31 - 36**: i) oxalyl chloride, dry DMF, dry

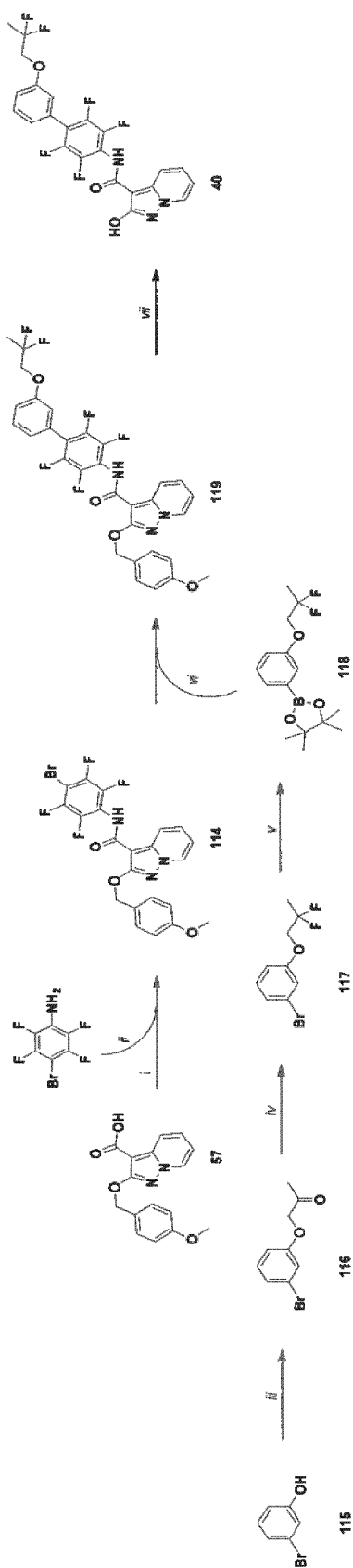
THF, nitrogen atmosphere; ii) dry toluene, dry pyridine;
iii) thioanisole, trifluoroacetic acid, 70°C.



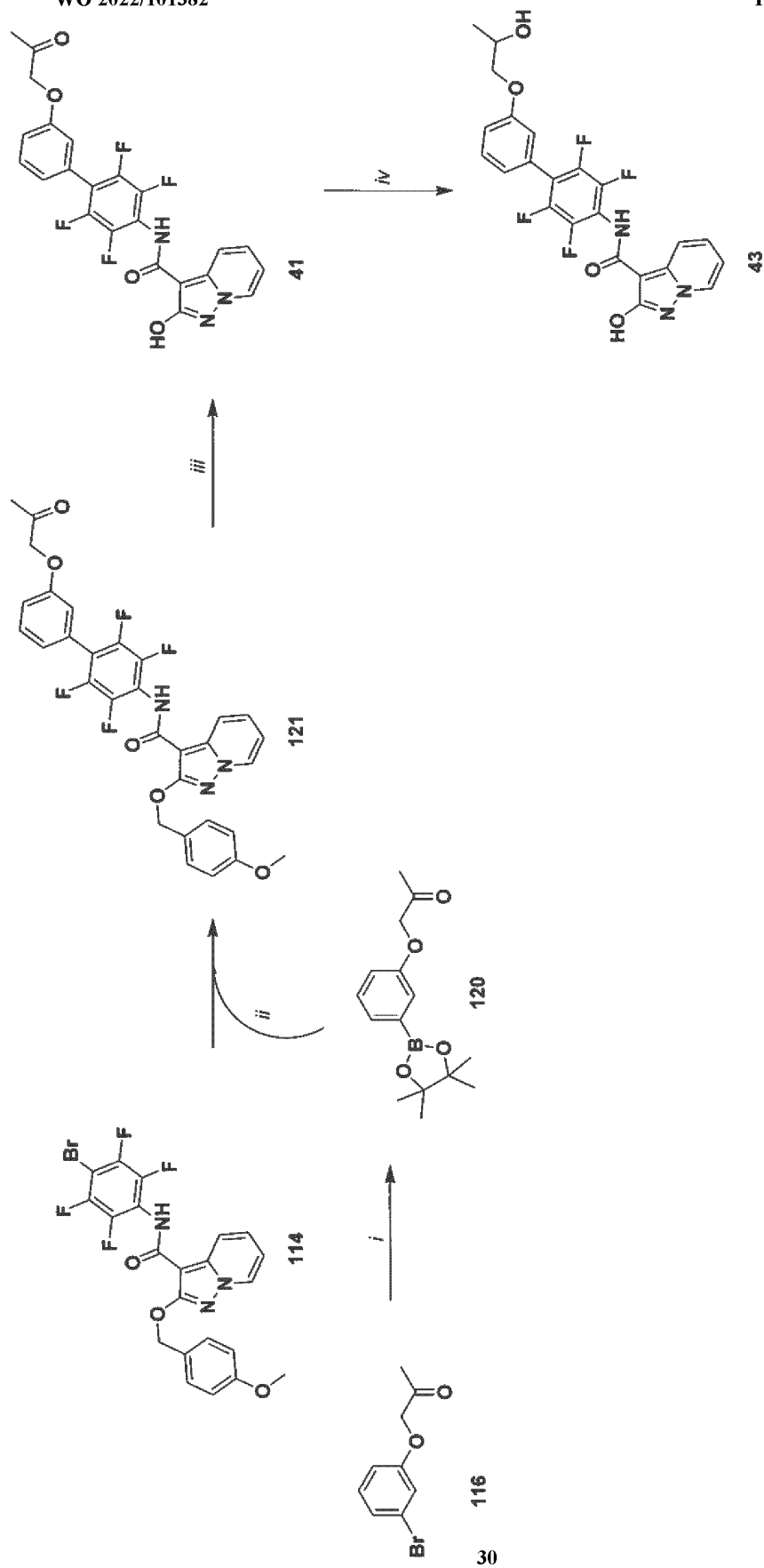
Scheme 6. Synthetic methodologies for the synthesis of compounds **25** and **38**: i) NaNO_2 , HNO_3 , water, 0°C , ii) water solution at pH 10 0°C .



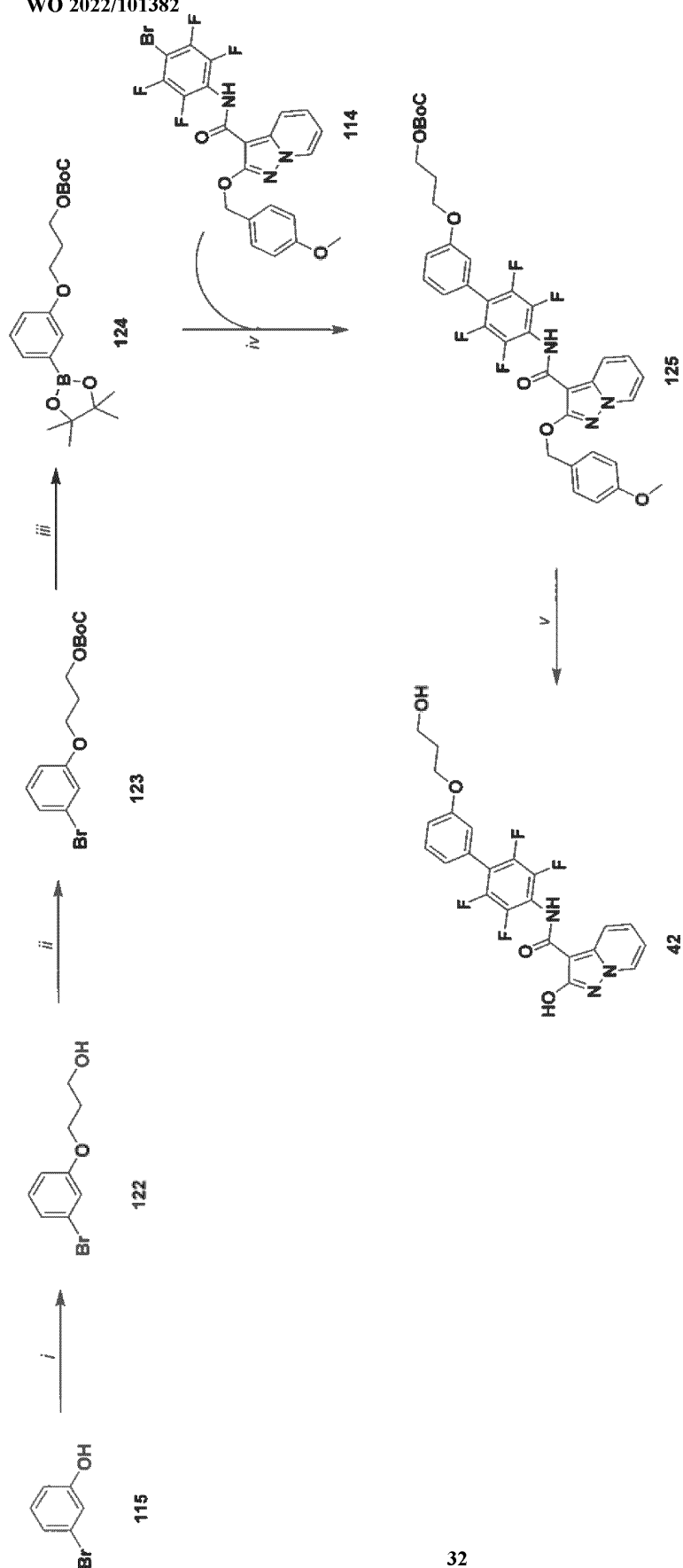
Scheme 7. Synthetic methodologies for the synthesis of compound **39**: *i*) CH₃I, Cs₂CO₃, dry DMF, rt; *ii*) Hydrazine monohydrate, EtOH, 80°C; *iii*) 4-Bromo-2,3,5,6-tetrafluorobenzoic, POCl₃, 50°C; *iv*) Pd(PPh₃)₄, K₂CO₃, phenylboronic acid, dioxane:H₂O 9/1 v/v, 90°C; *v*) BBr₃ 1M, dry DCM, 0°C.



Scheme 8. Synthetic methodologies for the synthesis of compound **40**: i) oxalyl chloride, dry DMF, dry THF, r.t.; ii) 4-bromo-2,3,5,6-tetrafluoroaniline, lithium bis(trimethylsilyl)amide (LiHMDS), 1M in THF, 70°C; iii) 1,8-diazabicyclo(5.4.0)undec-7-ene (DBU), dry DMF, chloroacetone, r.t.; iv) diethylaminosulfur trifluoride (DAST), DCM, r.t.; v) K₂CO₃, PdCl₂ (dppf), bis(pinacolato)diboron, dioxane, 100°C; vi) K₂CO₃, Pd(PPh₃)₄, dioxane / water (9:1 v/v), 90°C; vii) TFA, thioanisole, r.t.;



Scheme 9. Synthetic methodologies for the synthesis of compounds **41** and **43**: i) K_2CO_3 , $PdCl_2$ (dppf), bis(pinacolato)diboron, dioxane, $100^\circ C$; ii) K_2CO_3 , $Pd(PPh_3)_4$, dioxane / water (9:1 v/v), $90^\circ C$; iii) TFA, thioanisole, r.t., iv) $NaBH_4$, abs EtOH, r.t.;



Scheme 10. Synthetic methodologies for the synthesis of compound **42**: i) diisopropyl azodicarboxylate (DIAD), PPh_3 , 1,3-propandiol, dry THF, r.t.; ii) NaH, di-tert-

butyl dicarbonate, dry THF, r.t.; iii) K_2CO_3 , $PdCl_2$ (dppf), bis(pinacolato)diboron, dioxane, 100°C; iv) K_2CO_3 , $Pd(PPh_3)_4$, dioxane / water (9:1 v/v), 90°C; iii) TFA, thioanisole, r.t..

Results and discussion

Chemistry: synthesis of compounds 2 - 43

For the syntheses of compounds **5** and **10 - 13**, a chemical strategy already investigated to the lead compound **1** was used (see Scheme 1). The scheme starts from protected 2-hydroxypyrazolo[1,5-a]pyridine building block **45**, this latter obtained by **44** in two steps⁶. From **45**, the corresponding acyl chloride was obtained and used directly without further purification. Due to their poor reactivity with acyl chloride, each aniline (**46 - 50**) was converted into the more reactive dimethylaluminum amide, these latter were allowed to react with the above acyl chloride obtaining the desired amides **51 - 55** in 31 - 40 % yield range. Note how, during the coupling step the benzylic protecting group transposed from the exocyclic oxygen to the endocyclic N1 nitrogen the pyrazolo[1,5-a]pyridine system. During the synthesis of **1**,⁶ the removal of the benzyloxy moiety for **51** was always effected by the presence of a side reaction that lead to traces of the reduced **5**. On this occasion, **5** was obtained in 44 % yield applying stronger catalytic hydrogenation conditions (40-

bar) and using a SynthWAVE apparatus. To the opposite, to avoid such side reaction, compounds **52** - **55** were converted to desired target compounds **10** - **13** by applying room-pressure catalytic hydrogenation in presence of 37 % w/w HCl.

For the synthesis of compounds **6** - **9** and **14** - **17**, we designed a convergent synthetic approach with late-step Suzuki coupling of compound **58**, as common intermediate (Scheme 2). Also in this case the scheme started from 2-hydroxypyrazolo[1,5-a]pyridine **44**, that was protected with 4-methoxybenzyl bromide to afford the regioisomers **56a** and **56b** in ratio 61 and 27 % respectively (see SI for the spectroscopical characterization of the two regioisomers). The 4-methoxybenzyl protecting group can be easily removed in acidic conditions, applicable also to molecules containing sulphur atoms and pyridine ring, both known to poison metal catalyst during hydrogenation. Ester **56a** was then hydrolyzed under basic conditions to obtain the corresponding acid **57** (quantitative yield), which were then used for the preparation of the common intermediate **58**. Starting from acid **57**, the corresponding acyl chloride was obtained via treatment with oxalyl chloride that was used without any further purification in the reaction with the dimethylaluminum amide of 2,3,5,6-tetrafluoro-4-bromoaniline affording the desired amide **58** in the 55 % yield. Also in this case,

transposition of the benzylic protecting group from the exocyclic oxygen to the endocyclic N1 nitrogen the pyrazolo[1,5-a]pyridine system was observed. Compound **58** was used as commune building block for the desired compounds **59**, **60** - **66**. Firstly, by applying a *Buchwald-Hartwig* coupling⁷ conditions with morpholine, was obtained **59** (59 % yield), then by *Suzuki* reaction involving the corresponding boronic acids were obtained **60** - **66** (yield range: 70 - 94 %). Compounds **59** - **66** were then converted to the desired targets **6** - **9**, **14** - **17** by treatment with trifluoroacetic acid (TFA) in presence of thioanisole as scavenger.

For the synthesis of compound **4**, a dedicated synthetic scheme was applied (Scheme 3). In this case, the hydroxyl group of **44** was O-protected with Boc group to afford **67**. Using lithium hexamethyldisilylazide on **67**, the pyrazolo[1,5-a]pyridine moiety was selectively deprotonated on the 7 position; in the following, the quench of the lithium salt of **67** with hexachloroethane, employed as electrophile source of Cl⁺, afforded compound **68** in good yield⁸. In order to move on the reaction scheme and prepare the following coupling steps, the Boc group was ideally exchanged for a benzyl group. The Boc group was quantitatively removed under mild acid condition (TFA) obtaining the hydroxyazole **69** that was allowed to reach with benzyl bromide affording compound **70** (90 %

over two steps). It is worth to note that in this case, because of the presence of a chlorine in position 7, the endocyclic N1 isomer was obtained only in traces. The ester **70** was then hydrolyzed under basic conditions to obtain the corresponding acid **71** (quantitative yield) that was used for the preparation of the amide **72** using the above described conditions that involved the activation of the 2,3,5,6-tetrafluoro-4-phenylaniline as dimethylaluminum amide affording the desired amide **72** in the 38 % yield. Compound **72** was then converted to desired target **4** by treatment with TFA in presence of thioanisole as scavenger.

The synthetic strategy used to produce the 2-aryloxypyrazolo[1,5-a]pyridine building blocks **45** and **57**, which are useful in the syntheses of target compounds **19** - **24**, **26** and **28** - **37**, were achieved using procedures and conditions detailed in our previous publication ⁶ (Scheme 4). Starting from the acid **45**, compounds **73** - **82** were prepared, via a known procedure (see Scheme 4), the corresponding acyl chloride was obtained via treatment with oxalyl chloride and used directly after drying without further purification. On the opposite of our earlier work dedicated to *h*DHODH, the acyl chloride, was allowed to react directly with the corresponding aniline (**83** - **92**) previously synthesized. The desired amides **73** - **82** were obtained in the 50 - 92 % yield range. Moving

forward, compounds **73** - **82** were then converted to desired target compounds **19** - **24**, **28** - **30** and **37** by applying room-pressure catalytic hydrogenation. Starting from the acid **57**, compounds **93** - **99** were prepared, (see Scheme 5), the corresponding acyl chloride was obtained via treatment with oxalyl chloride and used directly after drying without further purification. The acyl chloride was allowed to react directly with the corresponding aniline (**100** - **106**) previously synthesized. The desired amides **93** - **99** were obtained in the 61 - 94 % yield range. Moving forward, compounds **93** - **99** were converted to the desired targets **26**, **31** - **36** by treatment with trifluoroacetic acid (TFA) in presence of thioanisole as scavenger. On the opposite of strategy applied in Scheme 1, the 4-methoxybenzyl protecting group was used, because it can be easily removed in acidic conditions, applicable also to molecules containing sulphur atoms and pyridine ring, both known to poison metal catalyst during hydrogenation in the last step.

The synthetic strategy used to produce compounds **25** and **38**, were achieved via a diazotization of 2,3,5,6-tetrafluoro-[1,1'-biphenyl]-4-amine and commercial 4-phenoxyaniline using sodium nitrite and nitric acid and its was coupling with **108** under basic condition (Scheme 6). Compound **108** can be assimilated to a phenol, and the C-atom with the highest electron density is usually the

preferred coupling position of diazonium salt. Due to the directing influence of hydroxyl groups in pyrazolo[1,5-*a*]pyridin-2-ol systems, coupling takes place at the 3-position.

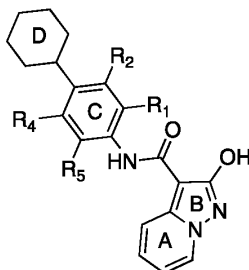
The synthetic strategy used to produce compound **39** (Scheme 7) started from 2-hydroxypyrazolo[1,5-*a*]pyridine **44**, that was protected with methyl iodine to afford the regioisomers **110a** and **110b** in ratio 77 and 13 % respectively. The methyl protecting group can be easily removed in acidic conditions. The regioisomer **110a** was allowed to react with hydrazine monohydrate to afford compound **111**. The reactions proceeded through cyclocondensation of **111** with 4-bromo-2,3,5,6-tetrafluorobenzoic acid to obtain compound **112**. This latter was functionalized via *Suzuki* reaction to obtain compound **113**. Moving forward, compound **113** was converted to the desired target **39** by treatment with BBr_3 .

For the synthesis of compounds **40** - **43** we designed a convergent synthetic approach with late-step Suzuki coupling of compound **114**, as common intermediate. Also in this case the scheme started from **57**, which was then used for the preparation of the common intermediate **114**. Starting from acid **57**, the corresponding acyl chloride was obtained via treatment with oxalyl chloride that was used without any further purification in the reaction with the lithium salt of 2,3,5,6-tetrafluoro-4-

bromoaniline affording the desired amide **114** in the 80 % yield. In this case, the transposition of the benzylic protecting group from the exocyclic oxygen to the endocyclic N1 nitrogen the pyrazolo[1,5-a]pyridine system was not observed and the yield was increased. Compound **57** was used as commune building block for the synthesis of desired compounds **119**, **121** and **125** by *Suzuki* reaction involving the corresponding boronic acid pinacol ester (referrer Schemes 8, 9 and 10 for the synthesis) Compounds **119**, **121** and **125** were then converted to the desired targets **40** - **42** by treatment with trifluoroacetic acid (TFA) in presence of thioanisole as scavenger. Finally compound **43** was obtained from compound **41** by reduction of ketone using NaBH₄.

hDHODH inhibitory activity and SAR. We evaluated the recombinant hDHODH inhibition activity of compounds **4** - **43** using three clinical-trial candidates (brequinar, BAY-2402234 and ASLAN003) and **1** as comparisons. While BAY-2402234 was purchased from a commercial source, brequinar was synthesized following known procedures. In order to complete the scenario and prepare the discussion of the following cell-based studies, LogD^{7.4}, solubility in PBS and protein binding were also measured for each compound.

SAR analysis of the pyridine ring (A ring) of the 2-hydroxypyrazolo[1,5-a]pyridine scaffold.



As reported in our previous publication,⁶ the interaction between **1** and the small lipophilic pocket created by Val134 and Val143 (subsite 4) was explored using Molecular Dynamics (MD) free energy perturbation (FEP),⁹ as a possible source of further modulation. Of the four sites on the A ring (positions 4 - 7), *in-silico* analyses suggested that position 7 is the most profitable for hydrogen substitution. Moreover, the study indicated that chlorine derivatives were generally preferred over methyl ones. Moving to experimental work (Table 1), taking into account the MD/FEP results, a derivative with a chlorine substituent in position 7 (**4**, IC₅₀ = 3.4 nM) was synthesized. Compared to the methyl analogue (**2**, IC₅₀ = 4.3 nM), the chlorine is better tolerated, leading to an analogue of **1** with comparable activity, but higher LogD^{7.4}. We therefore also considered a reduced 4,5,6,7-tetrahydropyrazolo[1,5-a]pyridine analogue, **5** (IC₅₀ = 5.8 nM), which gave a slight decrease in potency compared to **1**. While the A ring modulations did not result in increased inhibitory activity compared to **1**, higher LogDs^{7.4} were observed in all compounds, but were

unfortunately all associated with reduced solubility. Solubility in PBS was not measured, as the concentration of the soluble fraction was below the LOD value (6 μ M). In terms of protein binding, any significant improvement was observed.

Table 1: Enzymatic inhibitor activity of compounds **2** - **17**, brequinar, BAY-2402234, ASLAN003 and **1** as comparisons and relative LogD^{7.4}, solubility and protein binding. The effect of the compounds is expressed as IC₅₀ values. Limit of Detection (LOD): value 6 μ M. The "nd" notation indicates that the compound was not tested in that specific assay.

Compound	<i>h</i> DHODH ^a IC ₅₀ $\pm$ SE (nM)	Log D ^{7.4} $\pm$ SD ^c	Solubility (μ M) in PBS	Protein Binding (% bond)
<i>Brequinar</i>	1.8 $\pm$ 0.3	1.83 $\pm$ 0.02	229	98.83
BAY- 2402234	6.0 $\pm$ 0.6 (1.2 from lit ¹⁰)	2.7 ¹⁰	<1 ¹¹	90.1 ¹⁰
ASLAN003	35 ¹²	nd	nd	> 99 ¹²
1	1.2 $\pm$ 0.2	2.35 $\pm$ 0.02	12	99.10
2	4.3 $\pm$ 0.5	2.70 $\pm$ 0.02	< LOD	nd
3	35 $\pm$ 3	2.47 $\pm$ 0.09	< LOD	nd
4	3.4 $\pm$ 0.5	2.81 $\pm$ 0.13	< LOD	nd
5	5.8 $\pm$ 0.4	2.36 $\pm$ 0.02	< LOD	nd
6	90.9 $\pm$ 13.1	0.66 $\pm$ 0.08	438	nd
7	1.35 $\pm$ 0.45	nd	nd	nd
8	6.23 $\pm$ 0.63	0.98 $\pm$ 0.03	47.3	99.58
9	150 $\pm$ 15	1.84 $\pm$ 0.06	20.2	nd
10	17.7 $\pm$ 3.30	<i>Insoluble</i>	<LOD	nd
11	2.03 $\pm$ 0.44	2.09 $\pm$ 0.04	<LOD	99.96
12	71.8 $\pm$ 9.42	<i>Insoluble</i>	<LOD	nd

13	6.34 ± 0.63	2.69 ± 0.03	<LOD	99.94
14	2.78 ± 0.32	1.82 ± 0.09	55.3	nd
15	2.30 ± 0.33	3.27 ± 0.19	8.1	100
16	2.75 ± 0.31	2.46 ± 0.04	74.3	99.95
17	4.09 ± 0.62	3.28 ± 0.12	12.9	100

SAR analysis of the D ring of the biphenylic scaffold:

phenyl replacement/modulation. Moving to the D ring, we assigned the first four compounds (**6** – **9**) to the investigation of its possible isosteric replacement (Table 1). The incorporation of a morpholine substituent (**6**, IC₅₀ = 90.9 nM) was not well tolerated, as a phenyl ring, and resulted in around a 50-fold potency decrease compared to **1**. The introduction of heteroatoms that may interact with the lipophilic sub-pocket, composed of Pro69 and Leu68, may induce repulsive interactions, as the potency decrease is also observed for pyridine derivative **9** (Figure 1A). However, **6** was the most soluble of the series, showing almost twice the solubility of brequinar. The replacement of the D ring with classical bioisostere thiophen (**7**, IC₅₀ = 1.35 nM) retained the inhibitory profile. The optimal logD^{7.4} range for optimal drug absorption, via the phenomena of passive permeability or diffusion, is considered to be in the range between 1 and 3.¹³ In the case of hDHODH inhibitors, the literature indicates an optimal logD^{7.4} value superior to 2.50 reduce adsorption issue.¹¹ In terms of activity, the replacement of the phenyl position of **1** with a classic

isostere nitrogen, as in **8** and **9**, resulted in losses of activity (IC_{50} = 6.23 nM and 150 nM), as *meta* replacement is better tolerated. To better understand this result, **9** must be compared with **13** (IC_{50} = 6.34 nM) in which the -CF₃ in the *meta* position is still present, but the nitrogen is ideally removed. The two pyridine analogues, **8** and **9** display better solubility than **1**, four- and 1.5-times respectively. In terms of protein binding, any significant improvement was observed.

Moving on, we investigated the positions on the D ring that are suitable for substitution in compounds **10** - **17**. The binding mode of **1** and derivatives places the D ring next to the entrance of the ubiquinone binding pocket, exposing the *meta* position to an empty area of the binding site, on the border between the pocket and the vacuum. With **10** - **13**, we investigated the effect of lipophilic substitutions, such as F and CF₃, in the *para* (**10**, **12**) and *meta* (**11**, **13**) positions of the D ring. Analyzing the results (Table 1), it can be observed how *meta* replacement, **11** and **13** with IC_{50} = 2.03 nM and IC_{50} = 6.34 nM respectively, were better tolerated than the *para* isomers, **10** and **12** with IC_{50} = 17.7 nM and IC_{50} = 71.8 nM. While finding the activity of **11** to be in the same range as **1** is not surprising, as the fluorine is a classical proton bioisosteric replacement, this cannot be said for **13**, for which small lipophilic groups, such as -CF₃, are

well accepted. This replacement validated the predicted binding mode of **13**, in which a trifluoromethyl is placed in an empty area of the binding site. These modulations resulted in compounds being more lipophilic, as expected, but unfortunately, this property is associated with insolubility, and these values are largely below the reference limit of 6 μM . Focusing on substitution on the *meta* position, we obtained compound **14** ($\text{IC}_{50} = 2.78 \text{ nM}$), which is comparable to **1** itself in terms of potency, but characterized by better solubility (around five times), as the oxygen atom is able to form hydrogen bonds with water, and has a $\log D^{7.4}$ comparable with that of brequinar itself. By ideally modulating **14**, we introduced substitution to the phenolic oxygen, giving **15 – 17** (Table 1) and **40–43** (Table 2). This modulation resulted in an IC_{50} that is comparable to that of **1**, proving the predicted binding mode again, and was associated with an increase in $\log D^{7.4}$ for each compound. The most interesting compounds are **17**, **40–43** which are characterized by the introduction of a propoxy group. These are the most interesting compounds in the series here described, as they are comparable to the lead **1** in terms of potency, although the compound **17** showing similar solubility but higher $\log D^{7.4}$ (above the 2.5 threshold).

SAR analysis of the biphenylic scaffold. Although its potent inhibition of *h*DHODH evaluated *in vitro*, compound

1 solubility profile is far away from that of brequinar and the presence of the tetrafluorobiphenylic substituent is detrimental for its solubility. With the purpose to obtain an alternative scaffold that will provide hDHODH inhibition comparable to that of compound **1**, a new series of inhibitors was developed starting from lead structure of compound **18**⁶. Eighteen analogues (compound **19–24**, **26–37**) that lacked the biphenylic scaffold were therefore designed to explore novel possibilities. (Figure 2A and 2B) The inspiration was taken from Das et al.¹⁴ in which "brequinar-like" activity was replicated by analogues which included a substituted diarylether. The rationale behind the design of targets **26** rises from isosteres concept. Since compound **18** showed good interactions within the enzyme binding pocket, the general structure of the molecule was maintained, but to achieve a more polar compound, it has been decided to develop a target where ring D has been replaced by a more polar isostere: the pyridine ring with the oxygen bridge between ring C and D changed into a sulphur one. Targets **27 – 35** represent the proposing compounds after SAR studies on compound **18** in attempt to increase its binding affinity. The presence of additional ether substituent could explore in the binding site a region that has still remained almost unexploited. The investigation on the possibility of additional hydrophobic interactions with some lipophilic amino acidic residues located in *subsite*

1 conducted to the development of differently substituted diaryl ethers which might make the compounds gain in potency and selectivity for *h*DHODH. Table 2 shows the result of the SAR study directed to modulate **18**, which differs from **1** for the presence of an aryloxy moiety demanded to interact with *h*DHODH subsite 1.

The replacement of phenoxyphenyl moiety by the azotated moieties indole-5-yl, N-phenylindole-5-yl, N1-phenylbenzominidazol-5-yl or N1-phenylbenzotriazolyl (compounds **19–22** respectively) is detrimental for the activity as well as the replacement of one of the two phenyl by a pyridine ring (compound **23, 24, 26**). The removal of the methyl in orto position respect to the amide function (compound **27**) decrease ten time the activity while the removal of the methyl in meta position (compound **28**) is detrimental highlighting the importance of the small substituent in the meta position. In fact, the substitution of the methyl substituent in meta position by bigger chain as isopropyl (compound **30**) halve the activity. Moving to the orto position, huge substituents as isopropyl (compound **29**) increase the activity, while its replacement by different alkyloxy moieties cancel the activity (compounds **31–35**). Also in the phenoxyphenyl frame, a trifluoromethyl substituent in para position (compound **37**) decrease the activity.

SAR analysis of the "M-Q" and "Het" linkers. The replacement of the amide function by the diazo function maintains the activity only in the presence of the tetrafluorobiphenylic substituent as showed by the IC₅₀ of derivative **25** and **38** showing that the bioisosteric properties between amide and diazo linkers are present only in compound **25**. While the replacement of the amide function by 1,3,5-oxadiazole moieties is not beneficial for the activity as showed by the very low IC₅₀ of derivative **39** in comparison with compound **1**.

Table 2: Enzymatic inhibitor activity of compounds **18** - **43**, and relative LogD^{7.4} and solubility. The effect of the compounds is expressed as IC₅₀ value. Limit of Detection (LOD): value 6 μ M.

Compound	<i>h</i> DHODH ^a IC ₅₀ $\pm$ SE (nM)	Log D ^{7.4} $\pm$ SD ^c	Solubility (μ M) in PBS
18 ⁶	43 $\pm$ 5	nd	1.72 $\pm$ 0.03
19	> 10000	0.45 $\pm$ 0.02	nd
20	2757 $\pm$ 386	2.89 $\pm$ 0.05	nd
21	> 10000	2.55 $\pm$ 0.04	nd
22	7196 $\pm$ 1600	1.59 $\pm$ 0.03	nd
23	12000 $\pm$ 1000	nd	nd
24	> 10000	nd	nd
25	13 (n=2)	nd	
26	2209 $\pm$ 366	1.76 $\pm$ 0.07	nd
27 ⁶	480 $\pm$ 31	nd	nd

28	400 ± 60	2.46 ±0.05	nd
29	7.2 ± 0.9	3.18 ±0.09	1.2 ± 0.28
30	114 ± 11	2.91 ±0.07	0.98 ± 0.23
31	5201 ± 924	nd	nd
32	> 10000	nd	nd
33	3938 ± 797	nd	nd
34	2209 ± 346	nd	nd
35	6983 ± 1007	nd	nd
36	70 ± 11	2.51 ± 0.07	111.1 ± 0.35
37	18 ± 4	> 3	1.23 ± 0.09
38	Inactive at 1 microM	nd	nd
39	Inactive at 1 microM	nd	nd
40	1.48 ± 0.20	nd	nd
41	0.82 ± 0.10	nd	nd
42	3.29 ± 0.33	nd	nd
43	2.50 ± 0.20	nd	nd

Physicochemical properties

The determination of the main physicochemical properties that govern the ADME profile was carried out for all compounds by measuring their lipophilicity (logD_{7.4}) and solubility at physiological pH.

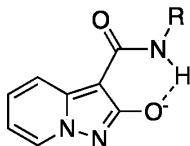
Thermodynamic solubility was determined using the equilibrium shake flask thermodynamic solubility method in phosphate saline buffer (PBS at pH = 7.4), chosen for its capability to simulate plasmatic pH and osmolarity.

Lipophilicity. The shake flask method was chosen to determine logD_{7.4}. Besides, lipophilicity was estimated

in silico, clogP has been calculated for each compound with the software Bio-Loom for Windows, version 1.5.

Summarizing the results, an optimization in terms of solubility was observed for compound **6** (438 μM), due to the depletion of the second aromatic ring and the insertion of a morpholine ring. This replacement gives to the molecule two important polar contributions: an amine and an ether. Also compounds **8** and **14** represent an effort to increase solubility, introducing a pyridine and a phenol ring instead of ring C. These compounds allow to move from 12 μM of the analogue compound **1** to 47 μM and 55 μM . Target **17**, with its propoxy chain, has almost the same low solubility (12.9 μM) of compound **1**, so it does not represent an effective improvement in this sense.

In general it is possible to say that all the measured solubility are far away from that of brequinar and this low solubility has a reasonable explication: at $\text{pH} = 7.4$, the hydroxy moiety of most compounds is completely deprotonated ($\text{pK}_a \sim 5-6$) and it has been hypothesized that an intramolecular reinforced hydrogen bond takes place between the amidic hydrogen and the deprotonated hydroxy group, consequently forming a stable six-term ring.



In this way, the amidic hydrogen would not be available to hydrogen-binding (HBD) to water's molecules and thus is not contributing for solubility. This hypothesis is confirmed by the crystallographic pose of that molecule in the enzyme, where the intramolecular HB is highlighted.

All the compounds have a good lipophilic-hydrophilic balance with optimal logD values for a favourable pharmacokinetic behaviour; the differences observed between clogP and measured logD7.4 agree with the large ionization of these compounds at physiological pH.

The goal for lipophilicity is a value between 1 and 3, that is an optimal value for absorption by passive diffusion permeability after oral dosing. Compounds **6**, **8** and **14** that are characterised by a morpholine, a pyridine and a phenol ring, have a very low lipophilicity, instead their solubilities are high; as result these molecules probably are not able to pass membranes. Compound **17** presents a propoxy substituent that could contribute to make it able to easily cross phospholipids bilayers. Even though this target seems to have a good attitude to reach the enzymatic target, it is very insoluble and therefore

difficult to use for in vitro tests. A good compromise appears to be compound **26** in which the presence of the pyridine allows to have a better solubility and, at the same time, the thioether a good lipophilic-hydrophilic balance.

Antiviral profile of selected compounds.

Compound 1 as a new potent BSAA candidate. To investigate the feasibility of targeting hDHODH activity to develop pan-coronavirus HTA, 13 newly designed hDHODH inhibitors (Table 3) were selected for antiviral activity against the prototypical human β -CoV, hCoV-OC43, by using FFRA in which test compounds were present before, during and after infection (full-treatment). As shown in Table 3, when tested at 0.1 μ M, six of hDHODH inhibitors (1, 9, 15, 16, 17, 40) were able to decrease hCoV-OC43 replication more than 50%. Notably, compound **1**, MEDS433, was the most effective among the tested hDHODH inhibitors, since it abrogated completely hCoV-OC43 replication. Compound **1** was therefore selected for further investigations. Having such an interesting preclinical hDHODH inhibitor in hand, we assayed **1** for antiviral activity against a large panel of human viruses.

Table 3. % Decrease of hCoV-OC43 replication in presence of inhibitor 0.1 μ M. HCT8 cells were pretreated and

treated vehicle (DMSO) or with 0.1 μ M of the different hDHODH inhibitors during infection with hCoV-OC43 (100 PFU/well), and throughout the experiment. At 72 h p.i., viral foci were immunostained and the mean foci number in treated culture compared to that of DMSO-treated and hCoV-OC43-infected control HCT8 cell monolayers.

Compound	% Decrease of hCoV-OC43 replication in presence of inhibitor 0.1 μ M
1	99.30
5	31.30
9	52.56
15	93.59
16	94.87
17	85.89
25	18.70
37	36.09
40	97.39

Herpes simplex virus. Incidence and severity of HSV infections have increased over the past decades due to the increasing number of immunocompromised patients, with genital herpes infection becoming one of the world's most prevalent sexually transmitted infections (STIs). In the absence of efficient preventive vaccines, the control of HSV infections, in particular genital herpes, thus remains a high-priority. These facts highlight the need to development of new anti-HSV agents, possibly endowed with a mechanism of action different from that of approved

nucleoside analogs (acyclovir, famcyclovir, valacyclovir) whose prolonged use may lead to treatment failures due to the development of antiviral-resistant strains. To this end, we tested **1** against HSV-1 and HSV-2 compared to *brequinar*. As shown in Table 4, **1** potently inhibited HSV-1 and HSV-2 replication (PRA, Vero cells) with EC₅₀ of 0.110 and 0.170 μ M, respectively. Noteworthy, the anti-HSV activity of **1** was about one order of magnitude more potent than that of *brequinar*, and even lower than that of the reference drug ACV (0.180 μ M). Further preliminary observations indicated that the anti-HSV activity of **1** was associated to an inhibition of viral DNA synthesis and Late protein expression, while Immediate-Early proteins were not significantly reduced, thus indicating that **1** targets a phase of HSV replication cycle prior to viral DNA replication, in agreement to its ability to inhibit hDHODH activity, thus causing pyrimidine depletion.

Table 4: Activity against the replication of different viruses of compound **1** and **17**, compared to brequinar, remdesivir and EIDD-1931, the drug released by pro-drug molnupiravir.

Compound	Virus	EC ₅₀ ^a (μ M)	CC ₅₀ ^b (μ M)	SI ^c
1	SARS-CoV-2	0.063	> 500	> 7,900

	hCoV-OC43	0.012	78.48	> 6,329
	a-hCoV-229E	0.022	104.8	> 4,763
	HSV-1	0.110	234	2,127
	HSV-2	0.170	234	1,376
	RSV	0.008	84	> 10,000
	Influenza A	0.120	12.2	102
17	SARS-CoV-2	Nd	nd	nd
	HSV-1	0.090	117	1300
	HSV-2	0.110	117	1063
	RSV	Nd	nd	nd
	Influenza A	Nd	nd	nd
<i>Brequinar</i>	SARS-CoV-2	0.200	-	-
	hCoV-OC43	0.0427		
	a-hCoV-229E	0.022		
	HSV-1	0.53	100	188
	HSV-2	1.77	100	56
	RSV	0.015	168	11,200
	Influenza A	0.76	5.0	6.6
<i>Remdesivir</i>	SARS-CoV-2 ¹⁵	0.77	> 100	> 129.87
	hCoV-OC43	0.147		
	a-hCoV-229E	0.0348		
<i>EIDD-1931</i>	SARS-CoV-2 ¹⁶	0.3	>10	33
	RSV ¹⁷	0.69		394

Respiratory viruses: *Respiratory viral infections* (RVI) are a global health concern in terms of morbidity and mortality. Influenza virus, respiratory syncytial virus and coronavirus are among the most common viruses causing lower respiratory tract infections.

Influenza virus. *Influenza viruses A and B* are widespread major human pathogens and responsible for seasonal epidemics and pandemics. Seasonal vaccines represent the

most effective measure to prevent and control Influenza infections. Treatment of Influenza infections may also benefit from two classes of licensed DAA drugs, such as *matrix protein inhibitors* and *neuraminidase inhibitors*. However, their use is severely restricted by selection of resistance strains. Thus, the development of alternative anti-Influenza compounds, both effective against antigenically different viruses and characterized by new mechanisms of action, is an urgent priority. **1** was therefore tested against a reference strains of Influenza A virus (A/Puerto Rico/8/34) and it showed a potent inhibitory activity (EC_{50} 0.120 μ M, PRA, MDCK cells, Table 4). Again, **1** performed better than *brequinar* with an EC_{50} value of more than 6 times lower.

Respiratory syncytial virus. *Respiratory syncytial virus* (RSV) is the most important cause of lower-respiratory tract infections in infants and young children, leading to severe bronchiolitis and pneumonia. Nevertheless, no vaccines are yet available and antiviral treatment are restricted to palivizumab for preventive treatment and ribavirin, a nucleoside purine analog, which is penalized by severe drawbacks. Thus, there is an urgent medical need to develop new compounds able to block RSV replication. With this in mind, we tested **1** vs RSV and a very potent antiviral activity (EC_{50} 0.008 μ M) (Table 3) was measured in HEp-2 cells associated with an effective

SI ($> 10,000$, CC_{50} $84 \mu\text{M}$), and with a profile slightly superior to that of *brequinar* (EC_{50} $0.015 \mu\text{M}$). Moreover, to verify whether the observed inhibition of RSV replication by **1** resulted from the blockage of pyrimidine biosynthesis, antiviral assays were repeated using uridine-supplemented culture medium. Since uridine can be converted to UMP via the salvage pathway (Figure 3), cells can bypass the requirement for the *de novo* pyrimidine biosynthesis. As shown in Figure 3, the uridine supplement abrogated the anti-RSV of **1**, thus confirming that the pyrimidine pathway is affected by **1** in RSV-infected cells. However, UMP from which all the other pyrimidines are derived, can be generated by both the salvage pathway and *de novo* biosynthesis. To distinguish between the two pathways, we examined the effects of addition of the substrate (Dihydroorotic acid, DHO) or product (Orotic acid, ORO) of *hDHODH* for their ability to restore RSV replication. Consistent with a pivotal role of *hDHODH* inhibition in the anti-RSV activity of **1**, the addition of ORO but not DHO reversed the inhibitory effect of **1** (Figure 3). Taken together, these preliminary observations suggest the specific targeting of *hDHODH* activity by **1** as the mechanism of the antiviral activity against RSV.

α - and β -coronavirus. Effective pan-CoVs antivirals can be highly valuable to fill the gap in the control of

emerging CoVs diseases given their rapid repositioning from one pandemic event to another. A significant concentration-dependent inhibition of hCoV-OC43 replication was then confirmed in HCT-8 cells treated with compound **1** (Table 4). The measured EC_{50} was $0.0124 \pm 0.0034 \mu\text{M}$. Compound **1** was also very effective against another hCoV, the prototypic β -hCoV-229E, whose replication in MRC5 fibroblasts was severely impaired (Table 4) with EC_{50} value of $0.022 \pm 0.003 \mu\text{M}$. The comparison with the reference drug RDV, used as a positive control for anti-hCoV antiviral activity, highlighted an anti-hCoV-229E potency of compound **1** comparable to that of RDV ($EC_{50} 0.0348 \pm 0.005 \mu\text{M}$), while the hDHODH inhibitor was much more effective than RDV ($EC_{50} 0.147 \pm 0.034 \mu\text{M}$) against hCoV-OC43. To the contrary, compound **1** was more effective than brequinar against hCoV-229E, as the EC_{50} of the latter was $0.0427 \pm 0.003 \mu\text{M}$, whereas against hCoV-OC43 the EC_{50} of brequinar ($0.022 \pm 0.003 \mu\text{M}$) was commensurate with that of compound **1**. Finally, the anti-hCoVs activity of compound **1** was not due to cytotoxicity of target cells themselves, since its cytotoxic concentration (CC_{50}) as determined in uninfected cells was $78.48 \pm 4.6 \mu\text{M}$ for HCT8 cells, and $104.80 \pm 19.75 \mu\text{M}$ for MRC5 fibroblasts, with a favorable Selective Index (SI) greater than 6,329 and 4,763 for hCoV-OC43 and hCoV-299E, respectively.

SARS-CoV-2. The emergence of COVID-19 as global public health emergency in the past few months prompted us to immediately investigate the sensitivity of SARS-CoV-2 to **1**. As shown in Table 4 and Figure 4, we measured a potent antiviral effect (EC_{50} 0.063 μ M) (PRA, Vero E6 cells) against the COVID-19 virus that was associated to a very safe profile ($SI > 7900$, $CC_{50} > 500$ μ M). Just to allocate these numbers, the anti-SARS-CoV-2 activity of **1** is one order of magnitude more potent than that of the *Best Available Therapy Remdesivir* (EC_{50} 0.77 μ M) and five time more potent than EIDD-1931, the drug released by pro-drug **Molnupiravir**.

Taken as a whole, these preliminary results sustained by a strong rational on the development of *h*DHODH inhibitors as BSAA, strongly validate the proposal to further develop **1** as an effective anti-SARS-CoV-2 agent and a potent and safe BSAA that could be very valuable also in future pandemic scenarios.

Conclusions

The present inventors identified a novel class of inhibitors that are based on hydroxyl-pyrazolo[1,5-*a*]pyridine, an unusual bioisostere of the carboxylic acid function. Compounds **1** and **17**, the most powerful *h*DHODH inhibitors yet discovered, show brequinar-like *h*DHODH potency levels *in vitro* and are superior in terms of

antiviral potency and selectivity blocking the viral replication at concentrations that are one log digit lower than those achieved in experiments with brequinar.

Since compounds **1** and **17** target a cellular gene product (DHODH), and it is unlikely that viruses can overcome the need for pyrimidine nucleotides, the likelihood that their therapeutic effect will be compromised by the development of viral resistance is low. This may be particularly important for RNA viruses whose high mutation frequency often promotes evasion of direct-acting antivirals.

The key to a successful COVID-19 treatment is to not only have a potent molecule, but to also have a dose that can be delivered safely and that will sustain exposure in the blood to inhibit viral replication or infection.

It is therefore apparent that compound **1** displays an optimal toxicity profile and highly selective on-target activity, making it an ideal candidate for further *in vivo* studies in SARS-CoV-2 models. Against other virus, compound **1** was found also effective being its EC₅₀ always below 170 nM

Materials and methods

Chemistry

General methods. All chemical reagents were obtained from commercial sources (Sigma Aldrich, Alfa Aesar, FluoroChem), and used without further purification. Thin-layer chromatography (TLC), was carried out to monitor reaction progress. Analytical grade solvents (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 (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 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 % and 99 % purity. Biological experiments were performed on compounds with a purity of at least 95 %. Purity was

checked using two UHPLC 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) and reverse-phase (RP) C18 Phenomenex column (2.1x100 mm, 1.7 μ m particle size). Compounds were dissolved in acetonitrile and injected through a 20 μ l loop. The mobile phase consisted of acetonitrile / water with 0.1 % trifluoroacetic acid (ratio between 60 / 40 and 40 / 60, 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 254 and 262 nm, referenced against a 360 nm wavelength. Melting points (m.p.), were measured on a 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. The IR spectra of solid compounds were recorded on FT-IR (PerkinElmer SPECTRUM BXII, KBr dispersions), using the diffuse reflectance apparatus DRIFT ACCY. MS spectra were performed on a Waters Micromass ZQ equipped with an ESCi source for electrospray ionization mass spectra. ¹H- and ¹³C-NMR spectra were performed on a JEOL ECZR600. The 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, l, m, n, o, p, q, r and s) according to figure in supporting info. Values marked with an asterisk (*, ** and ***) are interchangeable. Detailed ^{13}C spectra of tetrafluorinated biphenyl compounds (final compounds **4** – **17** and protected final compounds) have not been entirely reported due to their especially complicated patterns (attributable to the multiple couplings between fluorine and carbon atoms). For these spectra, only the ^{13}C signals caused by the heterocyclic substructure and non-aromatic carbons are assigned. For the final compounds **4** – **17**, HRMS spectra were recorded on an LTQ-Orbitrap XL Plus (Thermo Scientific, Bremen, Germany) mass spectrometer, equipped with an atmospheric pressure interface and an ESI ion source instrument. Compounds **44**, **45**^{6, 18} has been prepared according to previously-described.

Ethyl 2-((4-methoxybenzyl)oxy)pyrazolo[1,5-a]pyridine-3-carboxylate (56a) and *ethyl N-(4-methoxybenzyl)-2-oxo-pyrazolo[1,5-a]pyridine-3-carboxylate (56b)*. 4-Methoxybenzyl bromide (645 mg, 3.20 mmol, 1.10 eq) was added dropwise to a mixture of **44** (600 mg, 2.91 mmol) and Cs_2CO_3 (2.295 g, 7.04 mmol, 2.4 eq) in dry DMF (15 mL). The reaction mixture was stirred overnight at room temperature, then water (100 mL) was added. The mixture

was extracted with EtOAc (4 × 70 mL), the combined organic layer was dried under Na₂SO₄ and evaporated under reduced pressure to afford a yellow oil. This latter showed two spots on TLC (eluent: petroleum ether/EtOAc 60/40 v/v), ascribed to the two pyrazolo[1,5-a]-pyridine regioisomers. The mixture was separated using flash chromatography (eluent: petroleum ether/EtOAc 2/1 v/v, then dichloromethane/MeOH 95/5 v/v).

56a) First isomer eluted. White solid after trituration with hexane followed by water (111.3 – 112.5 °C). Yield 61 %. ¹H NMR (600 MHz, Chloroform-*d*) δ 1.40 (*t*, *J* = 7.1 Hz, 3H, -OCH₂CH₃); 3.81 (*s*, 3H, -OCH₃), 4.36 (*q*, *J* = 7.1 Hz, 2H, -OCH₂CH₃), 5.43 (*s*, 2H, -OCH₂Ar), 6.83 (*t*, 1H, *J* = 6.7 Hz, *H-b*), 6.91 (*d*, 2H, *J* = 8.6 Hz, *H-n*), 7.35 (*t*, 1H, *J* = 7.7 Hz, *H-c*), 7.48 (*d*, 2H, *J* = 8.5 Hz, *H-m*), 8.00 (*d*, 1H, *J* = 8.8 Hz, *H-d*), 8.29 (*d*, 1H, *J* = 6.8 Hz, *H-a*), ¹³C NMR (151 MHz, Chloroform-*d*) δ 14.7 (-OCH₂CH₃), 55.4 (-OCH₃), 59.7 (-OCH₂CH₃), 70.7 (-OCH₂Ar), 88.5 (*C-f*), 112.6 (*C-b*), 113.9 (*C-n*), 118.3 (*C-d*), 127.8 (*C-c*)*, 128.9 (*C-a*)*, 129.0 (*C-l*)*, 129.3 (*C-m*), 142.9 (*C-e*), 159.5 (*C-o*), 163.4 (*C-g*)*, 165.2 (*C-h*)*. MS (ES⁺): 327 (*M*+1).

56b) Second isomer eluted. White solid. (158.3 – 159.2 °C, from diisopropyl ether). Yield 27 %. ¹H NMR (600 MHz, DMSO-*d*₆) δ 1.28 (*t*, 3H, *J* = 7.1 Hz, -OCH₂CH₃); 3.69 (*s*, 3H, -OCH₃), 4.21 (*q*, 2H, *J* = 7.1 Hz, -OCH₂CH₃), 5.35 (*s*, 2H, -NCH₂Ar), 6.88 (*d*, 2H, *J* = 8.5 Hz, *H-n*), 6.96 (*t*, 1H,

$J = 6.8$ Hz, $H-b$), 7.19 (d , $2H$, $J = 8.4$ Hz, $H-m$), 7.58 (t , $1H$, $J = 8.0$ Hz, $H-c$), 7.91 (d , $1H$, $J = 8.8$ Hz, $H-d$), 8.45 (d , $1H$, $J = 6.8$ Hz, $H-a$); ^{13}C NMR (151 MHz, $DMSO-d_6$) δ 14.6 ($-OCH_2CH_3$), 43.2 ($-NCH_2Ar$), 55.1 ($-OCH_3$), 58.5 ($-OCH_2CH_3$), 83.5 ($C-f$), 112.4 ($C-b$), 114.3 ($C-n$), 116.3 ($C-d$), 125.3 ($C-a$), 125.7 ($C-l$), 128.8 ($C-m$), 132.4 ($C-c$), 142.8 ($C-e$), 159.0 ($C-o$), 160.0 ($C-h$)*, 163.2 ($C-g$)*; MS (ES+): 327 ($M+1$).

2-((4-Methoxybenzyl)oxy)pyrazolo[1,5-a]pyridine-3-carboxylic acid (57). 6 M NaOH (5.0 eq) was added to a solution of compound **56a** (785 mg, 2.40 mmol) in EtOH (20 mL). The mixture was stirred for 4 h at 75 °C, then neutralized with 6 M HCl and concentrated under reduced pressure. The mixture was cooled 0 °C then acidified with 2 M HCl until pH 2 was reached, obtaining a suspension. This latter was filtered to get **57** as a white solid ($162.8 - 163.9$ °C dec., from water). Yield 90 %. 1H NMR (600 MHz, $DMSO-d_6$) δ 3.76 (s , $3H$, $-OCH_3$), 5.34 (s , $2H$, $-OCH_2Ar$), 6.96 (d , $2H$, $J = 8.4$ Hz, $H-n$), 7.02 (t , $1H$, $J = 6.7$ Hz, $H-b$), 7.45 (d , $2H$, $J = 8.3$ Hz, $H-m$), 7.51 (t , $1H$, $J = 7.9$ Hz, $H-c$), 7.92 (d , $1H$, $J = 8.8$ Hz, $H-d$), 8.66 (d , $1H$, $J = 6.7$ Hz, $H-a$), 12.07 (s , $1H$, $-COOH$); ^{13}C NMR (151 MHz, $DMSO-d_6$) δ 55.1 ($-OCH_3$), 70.1 ($-OCH_2Ar$), 87.6 ($C-f$), 113.1 ($C-b$), 113.8 ($C-n$), 117.3 ($C-d$), 128.4 ($C-a$)*, 128.5 ($C-l$)*, 129.5 ($C-c$)*, 129.9 ($C-m$), 142.3 ($C-e$), 159.2 ($C-o$), 163.5 ($C-h$)*, 164.4 ($C-g$)*; MS (ES+): 299 ($M+1$).

N-(4-Bromo-2,3,5,6-tetrafluorophenyl)-2-((4-methoxybenzyl)oxy)pyrazolo[1,5-*a*]pyridine-3-carboxamide (**58**). Oxalyl chloride (0.54 mL, 6.30 mmol, 3.0 eq) and dry DMF (1 drop) were added to a cooled (0 °C) solution of **57** (630 mg, 2.10 mmol) in dry THF (15 mL) kept under a nitrogen atmosphere; the resulting mixture was stirred for 2 h at room temperature. In parallel a 2M solution of AlMe₃ in toluene (1.8 mL, 3.57 mmol, 1.7 eq) was added to a solution of 4-bromo-2,3,5,6-tetrafluoroaniline (769 mg, 3.15 mmol, 1.5 eq) in dry toluene (10 mL) under nitrogen atmosphere. The resulting suspension was stirred 3 h at room temperature. The solution of acyl chloride was then concentrated under reduced pressure, and the residue was dissolved in dry THF (10 mL, this step was repeated three times). The acyl chloride was dissolved in dry toluene (15 mL) and the solution was added to the above described suspension. The reaction mixture was stirred at 85 °C overnight, then cooled to room temperature, quenched with methanol, then evaporated. The residue was dissolved in EtOAc (80 mL), 0.5 M HCl (50 mL) was then added and layers resolved. The combined organic layers were washed with brine, dried and evaporated under reduced pressure. The crude material was purified using flash chromatography (eluent: petroleum ether/EtOAc/DCM 2/1/1 v/v/v) to afford the title compound as white solid (177.4 - 178.0 °C, triturated with diisopropyl ether). Yield 55 %. ¹H NMR (600 MHz, Chloroform-*d*) δ 3.79 (s, 3H,

-OCH₃), 5.41 (s, 2H, -NCH₂Ar), 6.77 (t, 1H, J = 6.9 Hz, H-b), 6.90 (d, 2H, J = 8.5 Hz, H-n), 7.21 (d, 2H, J = 8.5 Hz, H-m), 7.46 (t, 1H, J = 7.9 Hz, H-c), 7.75 (d, 1H, J = 6.9 Hz, H-a), 8.27 (t, 1H, J = 8.8 Hz, H-d), 9.98 (s, 1H, -NH); ¹³C NMR (151 MHz, Chloroform-d) δ 45.2 (-NCH₂Ar), 55.5 (-OCH₃), 87.1 (C-f), 96.4 (t, J = 22.6 Hz, C-s)*, 112.9 (C-b), 115.0 (C-n), 117.0 (t, J = 14.8 Hz, C-p)*, 118.3 (C-d), 123.1 (C-a), 124.2 (C-l), 128.6 (C-m), 131.8 (C-c), 142.5 (C-e), 142.8 (dd, J = 251.6, 14.9 Hz, (C-r)**), 145.2 (dd, J = 246.4, 14.2 Hz, (C-q)**), 160.0 (C-o***), 161.4 (C-h***), 162.2 (C-g***); MS (ES+): 524 / 526 (M+1).

1-(4-Methoxybenzyl)-2-oxo-N-(2,3,5,6-tetrafluoro-4-morpholinophenyl)-1,2-dihydropyrazolo[1,5-a]pyridine-3-carboxamide (59). To a solution of **58** (420 mg, 0.80 mmol, 1.00 eq) and morpholine (209 mg, 2.40 mmol, 3.00 eq) in toluene (30 mL) was added Cs₂CO₃ (782 mg, 2.4 mmol, 3.00 eq). After degasification with nitrogen for 10 min, Pd(OAc)₂ (18 mg, 0.08 mmol, 0.10 eq) and BINAP (100 mg, 0.16 mmol, 0.20 eq) were added, and mixture was degassed again for 5 min. The resulting suspension was heated at 110 °C in sealed flask under atmosphere of nitrogen. After 3.5 h, the heating was stopped, the mixture concentrated to reduced pressure then water was added. The resulting suspension was extracted with EtOAc (3 x 50 mL), the combined organic fractions collected, dried and

concentrated under reduced pressure. The crude was purified by flash chromatography (eluent: petroleum ether/ EtOAc/ DCM 1/1/1 v/v/v) affording a solid crude that was triturated with diisopropyl ether to obtain the title compound as a white solid (237.2 -237.5 °C dec). Yield: 59 %. ¹H NMR (600 MHz, Chloroform-d) δ 3.24 - 3.28 (m, 4H, -NCH₂CH₂O-), 3.79 (s, 3H, -OCH₃), 3.81 - 3.85 (m, 4H, -NCH₂CH₂O-), 5.40 (s, 2H, -NCH₂Ar), 6.74 (t, 1H, J = 6.9 Hz, H-b), 6.90 (d, 2H, J = 8.5 Hz, H-n), 7.21 (d, 2H, J = 8.5 Hz, H-m), 7.44 (t, 1H, J = 7.9 Hz, H-c), 7.73 (d, 1H, J = 6.9, Hz, H-a), 8.28 (d, 1H, J = 8.9 Hz, H-d), 9.75 (s, 1H, -NH); ¹³C NMR (151 MHz, Chloroform-d) δ 45.1 (-NCH₂Ar), 51.5 (-NCH₂CH₂O-), 55.5 (-OCH₃), 67.5 (-NCH₂CH₂O-), 87.2 (C-f), 111.3 (t, J = 15.5 Hz, C-p)*, 112.6 (C-b), 114.9 (C-n), 118.3 (C-d), 123.1 (C-a), 124.3 (C-l), 127.8 (t, J = 11.0 Hz, C-s), 128.6 (C-m), 131.6 (C-c), 142.5 (C-e), 143.2 (d, J = 248.2 Hz, C-q)**, 143.5 (d, J = 247.9, Hz, C-r)**, 160.0 (C-o)***, 162.0 (C-h)***, 162.1 (C-g)***; MS (ES+): 553 (M+Na).

General Procedure: the Suzuki reaction used for production of compounds 60 - 66. Pd(PPh₃)₄ (90 mg, 0.08 mmol, 0.20 eq) was added to a solution of **58** (200 mg, 0.38 mmol, 1.00 eq) and K₂CO₃ (158 mg, 1.14 mmol, 3.00 eq) in dioxane/water mixture (9:1 v/v). After stirring the resulting mixture under atmosphere of nitrogen for 1 h at r.t. the corresponding boronic acid (0.760 mmol, 2.0

eq) was added; the reaction mixture was then heated at reflux under atmosphere of nitrogen. After 2 h, an additional amount of boronic acid (0.38 mmol, 1.0 eq) was added, the reaction mixture was heated at reflux for another 2h before cooling to room temperature and concentrating under reduced pressure. The crude material was *taken-up* with water (100 mL) and the mixture was extracted with EtOAc (3 x 60 mL), the combined organic layers were dried over Na₂SO₄ and concentrated under reduced pressure. The crude was purified by flash chromatography (see below the conditions).

1-(4-Methoxybenzyl)-2-oxo-N-(2,3,5,6-tetrafluoro-4-(thiophen-2-yl)phenyl)-1,2-dihydropyrazolo[1,5-a]pyridine-3-carboxamide (60). The crude was purified by flash chromatography (eluent: *petroleum ether*/ EtOAc 1/1 v/v) obtaining a solid crude that was recrystallized from acetonitrile (8 mL) to obtain the title compound as white solid (197.4 -198.1 °C from acetonitrile). Yield: 72 %. ¹H NMR (600 MHz, Chloroform-d) δ 3.79 (*s*, 3H, -OCH₃), 5.41 (*s*, 2H, -NCH₂Ar), 6.76 (*t*, 1H, *J* = 6.6 Hz, *H-b*), 6.90 (*d*, 2H, *J* = 8.5 Hz, *H-n*), 7.16 - 7.20 (*m*, 1H, *aromatic proton*), 7.21 (*d*, 2H, *J* = 8.5 Hz, *H-m*), 7.45 (*t*, 1H, *J* = 7.7 Hz, *H-c*), 7.54 (*d*, 1H, *J* = 5.0 Hz, *aromatic proton*), 7.59 (*d*, 1H, *J* = 3.2 Hz, *aromatic proton*), 7.75 (*d*, 1H, *J* = 6.9 Hz, *H-a*), 8.28 (*d*, 1H, *J* = 8.8 Hz, *H-d*), 10.00 (*s*, 1H, -NH); ¹³C NMR (151 MHz, Chloroform-d) δ 45.2 (-

NCH₂Ar), 55.5 (-OCH₃), 87.2 (C-f), 112.3 (C-b), 115.0 (C-n), 115.7 (t, J = 16.2 Hz, C-p)*, 118.4 (C-d), 123.1 (C-a), 124.3 (C-l), 127.3 (thiophene carbon), 127.9 (thiophene carbon), 128.1 (t, J = 3.3 Hz, C-s)*, 128.6 (C-m), 128.5 (thiophene carbon), 130.0 (t, J = 5.3 Hz, thiophene carbon), 131.7 (C-c), 142.6 (C-e), 142.9 (dd, J = 248.0, 15.8 Hz, C-q)**, 144.0 (d, J = 247.0, Hz, C-r)**, 160.0 (C-o)***, 161.6 (C-h)***, 162.2 (C-g)***. MS (ES⁺): 528.2 (M+1).

1-(4-Methoxybenzyl)-2-oxo-N-(2,3,5,6-tetrafluoro-4-(pyridin-3-yl)phenyl)-1,2-dihydropyrazolo[1,5-a]pyridine-3-carboxamide (61). The crude was purified by flash chromatography (eluent: from petroleum ether/EtOAc 6/4 v/v to 3/7 v/v) obtaining a solid crude. This latter was then triturated with diisopropyl ether to obtain the title compound as a white solid (m.p. 202.6 - 203.8 °C from trituration with diisopropyl ether). Yield: 90 %. ¹H NMR (600 MHz, Chloroform-d) δ 3.78 (s, 3H, -OCH₃), 5.41 (s, 2H, -NCH₂Ar), 6.77 (t, 1H, J = 6.7 Hz, H-b), 6.89 (d, 2H, J = 8.5 Hz, H-n), 7.21 (d, 2H, J = 8.5 Hz, H-m), 7.43 - 7.49 (m, 2H, aromatic protons and H-c), 7.77 (d, 1H, J = 6.9 Hz, H-a), 7.84 (d, 1H, J = 7.8 Hz, aromatic proton), 8.27 (d, 1H, J = 8.8 Hz, H-d), 8.69 (d, 1H, J = 2.9 Hz, aromatic proton), 8.76 (s, 1H, aromatic proton), 10.06 (s, 1H, -NH); ¹³C NMR (151 MHz, Chloroform-d) δ 45.2 (-NCH₂Ar), 55.5 (-OCH₃), 87.1 (C-f), 112.9 (C-b), 114.0 (t,

$J = 16.9$ Hz, C-*p*)*, 115.0 (C-*n*), 117.4 (*t*, $J = 15.8$ Hz, C-*s*)*, 118.3 (C-*d*), 123.1 (C-*a*), 123.7 (pyridine carbon), 124.2 (C-*l*), 124.3 (pyridine carbon), 128.6 (C-*m*), 131.8 (C-*c*), 137.9 (pyridine carbon), 142.6 (C-*e*), 142.9 (*d*, $J = 252.7$ Hz, C-*q*)**, 144.2 (*d*, $J = 249.7$ Hz, C-*r*)**, 149.9 (pyridine carbon), 150.5 (pyridine carbon), 160.0 (C-*o****, 161.6 (C-*h****, 162.2 (C-*g****; MS (ES⁺): 523 (M+1).

1-(4-Methoxybenzyl)-2-oxo-N-(2,3,5,6-tetrafluoro-4-(2-(trifluoromethyl)pyridin-4-yl)phenyl)-1,2-dihydropyrazolo[1,5-*a*]pyridine-3-carboxamide (**62**). The crude was purified by flash chromatography (eluent: from petroleum ether/ EtOAc 6/4 v/v to 3/7 v/v) obtaining a solid crude. This latter was then triturated with diisopropyl ether and obtain the title compound as a pale yellow solid (m.p 192.7 - 193.9 °C from trituration with diisopropyl ether). Yield: 70 %. ¹H NMR (600 MHz, Chloroform-*d*) δ 3.79 (*s*, 3H, -OCH₃), 5.42 (*s*, 2H, -NCH₂Ar), 6.79 (*t*, 1H, $J = 6.7$ Hz, H-*b*), 6.90 (*d*, 2H, $J = 8.5$ Hz, H-*n*), 7.22 (*d*, 2H, $J = 8.5$ Hz, H-*m*), 7.48 (*t*, 1H, $J = 7.9$ Hz, H-*c*), 7.64 (*d*, 1H, $J = 4.9$ Hz, pyridine proton), 7.78 (*d*, 1H, $J = 6.9$ Hz, H-*a*), 7.84 (*s*, 1H, pyridine proton), 8.29 (*d*, 1H, $J = 8.8$ Hz, H-*d*), 8.88 (*d*, 1H, $J = 4.9$ Hz, pyridine proton), 10.15 (*s*, 1H, -NH); ¹³C NMR (151 MHz, Chloroform-*d*) δ 45.3 (-NCH₂Ar), 55.5 (-OCH₃), 87.1 (C-*f*), 113.0 (C-*b*), 113.2 (*t*, $J = 15.6$ Hz, C-

p)*, 115.0 (*C-n*), 118.4 (*C-d*), 118.9 (*t*, *J* = 14.7 Hz, *C-s*)*, 121.5 (*q*, *J* = 274.5 Hz, -CF₃), 121.7 (*q*, *J* = 2.1 Hz, pyridine carbon), 123.1 (*C-a*), 124.2 (*C-l*), 127.6 (pyridine carbon), 128.6 (*C-m*), 131.9 (*C-c*), 137.9 (pyridine carbon), 142.6 (*C-e*), 142.8 (*d*, *J* = 247.0 Hz, *C-q*)**, 144.2 (*d*, *J* = 245.4 Hz, *C-r*)**, 149.0 (*q*, *J* = 34.9 Hz, pyridine carbon), 150.6 (pyridine carbon), 160.1 (*C-o****, 161.4 (*C-h****, 162.3 (*C-g****; MS (ES⁺): 591.

1-(4-Methoxybenzyl)-2-oxo-*N*-(2,3,5,6-tetrafluoro-3'-hydroxy-[1,1'-biphenyl]-4-yl)-1,2-dihydropyrazolo[1,5-*a*]pyridine-3-carboxamide (**63**). The crude was purified by flash chromatography (eluent: petroleum ether/ EtOAc 1/2 v/v) obtaining a solid crude. This latter was then triturated with diisopropyl ether, in order to remove O=PPh₃O, obtaining the title compound as a white solid (236.9 -237.4 °C from diisopropyl ether). Yield: 79 %. ¹H NMR (600 MHz, DMSO-*d*₆) δ 3.71 (*s*, 3H, -OCH₃), 5.49 (*s*, 2H, -NCH₂Ar), 6.88 - 6.97 (*m*, 5H, aromatic protons and *H-n*), 7.07 (*t*, 1H, *J* = 7.1Hz, *H-b*), 7.28 (*d*, 2H, *J* = 8.3 Hz, *H-m*), 7.35 (*t*, 1H, *J* = 8.1 Hz, aromatic proton), 7.67 (*t*, 1H, *J* = 7.9 Hz, *H-c*), 8.03 (*d*, 1H, *J* = 8.8 Hz, *H-d*), 8.57 (*d*, 1H, *J* = 6.9 Hz, *H-a*), 9.78 (*br s*, 1H, -OH), 10.07 (*s*, 1H, -NH); ¹³C NMR (151 MHz, DMSO-*d*₆) δ 43.8 (-NCH₂Ar), 55.1 (-OCH₃), 85.5 (*C-f*), 113.2 (*C-b*), 114.4 (*C-n*), 116.2 (*C-d*), 116.3 (aromatic carbon), 116.6 (*t*, *J* = 14.6 Hz, *C-p*)*, 116.9 (2 carbon, *C-s* partially overlapped

with aromatic carbon)*, 120.7 (aromatic carbon), 125.3 (C-a), 125.4 (C-l), 127.7 (aromatic carbon), 128.8 (C-m), 129.9 (aromatic carbon), 132.9 (C-c), 141.4 (C-e), 142.3 (d, J = 248.9 Hz, C-q)**, 143.3 (d, J = 251.3 Hz, C-r)**, 157.5 (aromatic carbon), 159.1 (C-o)***, 160.7 (C-h)***, 161.7 (C-g)***; MS (ES+): 538 (M+1).

1-(4-Methoxybenzyl)-2-oxo-N-(2,3,5,6-tetrafluoro-3'-(trifluoromethoxy)-[1,1'-biphenyl]-4-yl)-1,2-dihydropyrazolo[1,5-a]pyridine-3-carboxamide (**64**) The crude was purified by flash chromatography (eluent: petroleum ether/ DCM 2 /1 v/v) obtaining a solid crude. This latter was then triturated with diisopropyl ether obtaining the title compound as a white solid (162.7 - 163.0 °C from diisopropyl ether). Yield: 82 %. ¹H NMR (600 MHz, Chloroform-d) δ 3.80 (s, 3H, -OCH₃), 5.42 (s, 2H, -NCH₂Ar), 6.77 (t, 1H, J = 6.9 Hz, H-b), 6.91 (d, 2H, J = 8.5 Hz, H-n), 7.22 (d, 2H, J = 8.5 Hz, H-m), 7.32 (d, 1H, J = 8.1 Hz, aromatic proton), 7.37 (s, 1H, aromatic proton), 7.43 (d, 1H, J = 7.7 Hz, aromatic proton), 7.47 (t, 1H, J = 7.9 Hz, H-c), 7.53 (t, 1H, J = 8.0 Hz, aromatic proton), 7.76 (d, 1H, J = 6.9 Hz, H-a), 8.31 (d, 1H, J = 8.8 Hz, H-d), 10.03 (s, 1H, -NH); ¹³C NMR (151 MHz, Chloroform-d) δ 45.2 (-NCH₂Ar), 55.4 (-OCH₃), 87.1 (C-f), 112.8 (C-b), 115.0 (C-n), 115.9 (t, J = 16.1 Hz, C-p)*, 117.0 (t, J = 15.6 Hz, C-s)*, 118.3 (C-d), 120.6 (q, J = 257.6 Hz, -OCF₃), 121.5 (aromatic carbon), 123.0

(aromatic carbon), 123.1 (C-a), 124.2 (C-l), 128.5 (C-m), 128.8 (aromatic carbon), 129.4 (aromatic carbon), 130.1 (aromatic carbon), 131.7 (C-c),, 142.5 (C-e), 142.8 (d, J = 247.5 Hz, C-q)**, 144.0 (d, J = 249.5 Hz, C-r)**, 149.3 (aromatic carbon), 160.0 (C-o)***, 161.6 (C-h)***, 162.2 (C-g)***; MS (ES+): 606.6, 628.6.

1-(4-Methoxybenzyl)-2-oxo-N-(2,3,5,6-tetrafluoro-3'-methoxy-[1,1'-biphenyl]-4-yl)-1,2-dihydropyrazolo[1,5-a]pyridine-3-carboxamide (65) The crude was purified by flash chromatography (eluent: petroleum ether/EtOAc/dichloromethane 1.5/1/2 v/v/v) obtaining the title compound as a beige solid (172.9 -173.8 °C from diisopropyl ether). Yield: 94 %. ¹H-NMR (600 MHz, Chloroform-d): δ 3.79 (s, 3H, -OCH₃), 3.85 (s, 3H, -OCH₃), 5.42 (s, 2H, -NCH₂Ar), 6.76 (t, 1H, J = 6.9 Hz, H-b), 6.90 (d, 2H, J = 8.5 Hz, H-n), 6.97 - 7.03 (m, 2H, aromatic protons), 7.06 (d, 1H, J = 7.5 Hz, aromatic proton), 7.22 (d, 2H, J = 8.5 Hz, H-m), 7.41 (t, 1H, J = 7.9 Hz, aromatic proton), 7.45 (t, 1H, J = 7.9 Hz, H-c), 7.75 (d, 1H, J = 6.9 Hz, H-a), 8.29 (d, 1H, J = 8.8 Hz, H-d), 9.99 (s, 1H, -NH); ¹³C-NMR (150 MHz, Chloroform-d): δ 45.2 (-NCH₂Ar), 55.5 (2 x -OCH₃), 87.2 (C-f), 112.3 (C-b), 114.9 (aromatic carbon), 115.0 (C-n), 115.8 (aromatic carbon), 116.3 (t, J = 15.0 Hz, C-p)*, 117.6 (t, J = 19.6 Hz, C-s)*, 118.4 (C-d), 122.7 (aromatic carbon), 123.1 (C-a), 124.3 (C-l), 128.6 (C-m), 128.8 (aromatic carbon),

129.7 (aromatic carbon), 131.7 (C-c), 142.6 (C-e), 142.9 (d, J = 248.6 Hz, C-q)**,, 144.2 (d, J = 244.2, Hz, C-r)**, 159.7 (aromatic carbon)***, 160.0 (C-o)***161.7 (C-h)****, 162.2 (C-g)****; MS (ES+): 552.5 (M+1).

1-(4-Methoxybenzyl)-2-oxo-N-(2,3,5,6-tetrafluoro-3'-propoxy-[1,1'-biphenyl]-4-yl)-1,2-dihydropyrazolo[1,5-a]pyridine-3-carboxamide (**66**) The crude was purified by flash chromatography (eluent: petroleum ether/ EtOAc 2/3 v/v) to obtain the title compound as a beige solid (168.8 -169.9 °C from diisopropyl ether). Yield: 86 %. ¹H NMR (600 MHz, Chloroform-d) δ 1.06 (t, 3H, J = 7.4 Hz, -OCH₂CH₂CH₃), 1.84 (h, 2H, J = 7.3 Hz, -OCH₂CH₂CH₃), 3.80 (s, 3H, -OCH₃), 3.96 (t, 2H, J = 6.6 Hz, -OCH₂CH₂CH₃), 5.42 (s, 2H, -NCH₂Ar), 6.76 (t, 1H, J = 7.0 Hz, H-b), 6.91 (d, 2H, J = 8.6 Hz, H-n), 6.99 (dd, 1H, J = 8.4, 2.2 Hz, aromatic proton), 7.00 (s, 1H, aromatic proton), 7.04 (d, 1H, J = 7.6 Hz, aromatic proton), 7.22 (d, 2H, J = 8.6 Hz, H-m), 7.39 (t, 1H, J = 7.9 Hz, aromatic proton), 7.46 (t, 1H, J = 7.9 Hz, H-c), 7.75 (d, 1H, J = 7.0 Hz, H-a), 8.31 (d, 1H, J = 8.8 Hz, H-d), 9.98 (s, 1H, -NH); ¹³C NMR (151 MHz, Chloroform-d) δ 10.6 (-OCH₂CH₂CH₃), 22.7 (-OCH₂CH₂CH₃), 45.2 (-NCH₂Ar), 55.5 (-OCH₃), 69.8 (-OCH₂CH₂CH₃), 87.2 (C-f), 112.8 (C-b), 115.0 (C-n), 115.5 (aromatic carbon), 116.2 (t, J = 15.6 Hz, C-p)*, 116.4 (aromatic carbon), 117.7 (t, J = 16.8 Hz, C-s)*, 118.3 (C-d), 122.5 (aromatic carbon), 123.1 (C-a), 124.3 (C-

1), 128.6 (C-m), 128.7 (aromatic carbon), 129.7 (aromatic carbon), 131.7 (C-c), 142.5 (C-e), 142.8 (dd, $J = 248.5$, 15.3 Hz, C-q)**, 144.2 (d, $J = 248.5$ Hz, C-r)**, 159.2 (aromatic carbon)***, 160.0 (C-o)***161.7 (C-h)****, 162.2 (C-g)****; MS (ES+): 580 (M+1).

General procedure for the synthesis of aniline 47–50.

$\text{Pd(PPh}_3)_4$ (90 mg, 0.08 mmol, 0.20 eq) was added to a solution of 4-bromo-2,3,5,6-tetrafluoroaniline (200 mg, 0.38 mmol, 1.00 eq) and K_2CO_3 (158 mg, 1.14 mmol, 3.00 eq) in 1,2-dimethoxyethane (35 mL). After stirring the resulting mixture under atmosphere of nitrogen for 1 h at r.t. the corresponding boronic acid (0.760 mmol, 2.0 eq) was added; the reaction mixture was then heated at reflux under atmosphere of nitrogen. After 2 h, an additional amount of boronic acid (0.38 mmol, 1.0 eq) was added, the reaction mixture was heated at reflux for another 2h before cooling to room temperature and concentrating under reduced pressure. The crude material was *taken-up* with water (100 mL) and the mixture was extracted with EtOAc (3 x 60 mL), the combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The crude was purified by flash chromatography (see below the conditions).

2,3,4',5,6-pentafluoro-[1,1'-biphenyl]-4-aniline (47).
Flash chromatography (eluent: petroleum ether / EtOAc

from 95:5 v/v). White solid (m.p. 134.1 – 134.7 °C from trituration with diisopropyl ether). Yield 74%. ¹H NMR (600 MHz, Chloroform-*d*): δ 4.05 (*s*, 2H, -NH₂), 7.11 – 7.17 (*m*, 2H, *aromatic protons*), 7.37 – 7.42 (*m*, 2H, *aromatic protons*); ¹³C NMR (151 MHz, CDCl₃): δ 107.3 (*t*, *J* = 16.8 Hz), 115.7 (*d*, *J* = 21.7 Hz), 124.0 (*d*, *J* = 1.7 Hz), 125.6 (*t*, *J* = 14.1 Hz), 132.2 (*d*, *J* = 7.7 Hz), 136.9 (*d*, *J* = 238.0 Hz), 144.1 (*d*, *J* = 243.5 Hz), 162.7 (*d*, *J* = 248.3 Hz). MS (ES-) 258 (M-1).

2,3,3',5,6-pentafluoro-[1,1'-biphenyl]-4-aniline (**48**). Flash chromatography (eluent: *petroleum ether* / EtOAc from 95:5 v/v). White solid (m.p. 108.8 – 109.7 °C from trituration with diisopropyl ether). Yield 90%. ¹H NMR (600 MHz, Chloroform-*d*): δ 4.08 (*s*, 2H, -NH₂), 7.10 (*t*, 1H, *J* = 8.4 Hz *aromatic proton*), 7.15 (*d*, 1H, *J* = 9.7 Hz, *aromatic protons*), 7.21 (*d*, 1H, *J* = 7.5 Hz, *aromatic proton*), 7.38 – 7.44 (*m*, 1H, *aromatic proton*); ¹³C NMR (151 MHz, CDCl₃): δ 107.0 (*t*, *J* = 17.6 Hz), 115.3 (*d*, *J* = 20.8 Hz), 117.5 (*d*, *J* = 22.3 Hz), 125.9 (*t*, *J* = 14.2 Hz), 126.2, 130.0 (*d*, *J* = 8.3 Hz), 130.1, 136.8 (*dd*, *J* = 238.4, 16.2 Hz), 144.1 (*d*, *J* = 243.9 Hz), 162.7 (*d*, *J* = 245.9 Hz). MS (ES-) 258 (M-1).

2,3,5,6-tetrafluoro-4'-(trifluoromethyl)-[1,1'-biphenyl]-4-aniline (**49**). Flash chromatography (eluent: *petroleum ether* / EtOAc from 95:5 v/v). White solid (m.p.

163.9 – 164.8 °C from trituration with diisopropyl ether). Yield 60%. ¹H NMR (600 MHz, Chloroform-d): δ 4.12 (s, 2H, -NH₂), 7.56 (d, 2H, J = 8.0 Hz, aromatic protons), 7.71 (d, 2H, J = 8.2 Hz, aromatic protons); ¹³C NMR (151 MHz, CDCl₃): δ 106.8 (t, J = 15.1 Hz), 124.1 (q, J = 271.7 Hz, -CF₃), 125.5 (q, J = 6.7 Hz), 126.4 (t, J = 3.8 Hz), 130.4 (q, J = 32.7 Hz), 130.8, 132.0, 136.9 (d, J = 238.7 Hz), 144.2 (d, J = 244.2 Hz). MS (ES-) 308 (M-1).

2,3,5,6-tetrafluoro-3'-(trifluoromethyl)-[1,1'-biphenyl]-4-aniline (50). Flash chromatography (eluent: petroleum ether / EtOAc from 95:5 v/v). Pale yellow solid (m.p. 46.6 – 47.0 °C from trituration with diisopropyl ether). Yield 92%. ¹H NMR (600 MHz, Chloroform-d): δ 4.11 (s, 2H, -NH₂), 7.55 – 7.60 (m, 1H, aromatic proton), 7.61 (d, 1H, J = 7.2 Hz, aromatic proton), 7.65 (d, 1H, J = 7.4 Hz, aromatic proton), 7.70 (s, 1H, aromatic proton); ¹³C NMR (151 MHz, CDCl₃): δ 106.7 (t, J = 16.6 Hz), 124.1 (q, J = 272.7 Hz, -CF₃), 125.2 (q, J = 3.8 Hz), 126.3 (t, J = 14.3 Hz), 127.3, 129.0, 129.1, 131.5 (q, J = 32.7 Hz), 133.8, 136.9 (dd, J = 239.4, 16.5 Hz), 144.2 (d, J = 244.2 Hz). MS (ES-) 308 (M-1).

General procedure for the synthesis of pyrazolo[1,5-a]pyridine related amides 52 – 55.

Oxalyl chloride (3.0 mmol) and dry DMF (1 drop) were added to a cooled (0 °C) solution of the O-protected

pyrazolo[1,5-a]pyridine acid (1.0 mmol) **45** in dry THF (20 mL), under a nitrogen atmosphere. The obtained solution was stirred at room temperature for 2 hours. The solution was then concentrated under reduced pressure and the residue dissolved in dry THF (10 mL, this step was repeated three times). The resulting acyl chloride was immediately used without any further purification and dissolve in 10 mL of dry toluene and transferred to the solution described later. Trimethylaluminium (2.0 M in hexane, 1.5 mmol), was added to a solution of the appropriate aniline (see supporting info for the synthesis, 1.5 mmol), in dry toluene (15 mL), under a nitrogen atmosphere. The resulting mixture was stirred for 2 hours at room temperature producing a brown suspension, then the solution of the previously described acyl chloride in dry toluene (30 mL) was quantitatively added. The mixture was heated overnight at 90 °C and then cooled to r.t. The reaction was quenched with 1M HCl. The layers were resolved, and the aqueous phase was exhaustively extracted using EtOAc. The combined organic layer was washed with 1M NaOH and brine, dried and the solvent was evaporated under reduced pressure. The crude product was purified by column chromatography.

1-Benzyl-2-oxo-N-(2,3,4',5,6-pentafluoro-[1,1'-biphenyl]-4-yl)-1,2-dihydropyrazolo[1,5-a]pyridine-3-carboxamide (52). *2,3,4',5,6-Pentafluoro-[1,1'-*

biphenyl]-4-aniline was used. flash chromatography (eluent: *petroleum ether* / EtOAc from 80:20 v/v to 50:50 v/v). Grey solid (m.p. 234.2 - 235.4 °C from trituration with diisopropyl ether). Yield 31%. ¹H NMR (600 MHz, Chloroform-*d*): δ 5.48 (*s*, 2H, -NCH₂Ph), 6.77 (*t*, 1H, *J* = 6.9 Hz, *H-b*), 7.19 (*t*, 2H, *J* = 8.6 Hz, *aromatic protons*), 7.28 (*d*, 2H, *J* = 7.4 Hz, *aromatic protons*), 7.32 -750 (*m*, 6H, *aromatic protons*), 7.73 (*d*, 1H, *J* = 6.9, *H-a*), 8.30 (*d*, 1H, *J* = 8.8, *H-d*), 9.98 (*s*, 1H, -NH); ¹³C NMR (151 MHz, Chloroform-*d*): δ 45.7 (-NCH₂Ph), 87.2 (*C-f*), 112.9 (*C-b*), 115.9 (*d*, *J* = 21.9 Hz, *aromatic carbon*), 116.4 (*t*, *J* = 17.1 Hz, *C-p*)*, 116.7 (*d*, *J* = 16.7 Hz, *C-s*)*, 118.4 (*C-d*), 123.0 (*C-a*), 123.5 (*aromatic carbon*), 127.1 (*aromatic carbon*), 129.0 (*aromatic carbon*), 129.7 (*aromatic carbon*), 131.8 (*C-c*), 132.8 (*d*, *J* = 8.4 Hz, *aromatic carbon*), 132.5 (*aromatic carbon*), 142.6 (*C-e*), 142.8 (*d*, *J* = 244.1 Hz, *C-q*)**, 144.2 (*d*, *J* = 250.7 Hz, *C-r*)**, 161.7 (*C-g****, 162.2 (*C-h****, 163.1 (*d*, *J* = 249.5 Hz, -CF); MS (ESI) 510 (*M*+1).

1-Benzyl-2-oxo-N-(2,3,3',5,6-pentafluoro-[1,1'-biphenyl]-4-yl)-1,2-dihydropyrazolo[1,5-a]pyridine-3-carboxamide (**53**). *2,3,3',5,6-Pentafluoro-[1,1'-biphenyl]-4-aniline* was used. flash chromatography (eluent: *petroleum ether* / EtOAc from 80:20 v/v to 50:50 v/v). Pale yellow solid (m.p. 195.4 - 196.3 °C from trituration with diisopropyl ether). Yield 40 %. ¹H NMR

(600 MHz, DMSO- d_6): δ 5.57 (s, 2H, $-NCH_2Ph$), 7.07 (t, 1H, $J = 6.9$ Hz, $H-b$), 7.29 – 7.45 (m, 7H, aromatic protons), 7.50 (d, 1H, $J = 9.5$ Hz, aromatic proton), 7.62 (dd, 1H, $J = 14.3, 7.7$ Hz, aromatic proton), 7.68 (t, 1H, $J = 7.9$ Hz, $H-c$), 8.05 (d, 1H, $J = 8.7$ Hz, $H-d$), 8.53 (d, 1H, $J = 7.0$ Hz, $H-a$), 10.12 (s, 1H, $-NH$); ^{13}C NMR (151 MHz, DMSO- d_6): δ 44.2 ($-NCH_2Ph$), 85.4 (C-f), 113.3 (C-b), 115.4 (t, $J = 17.3$ Hz, C-p)*, 116.2 (C-d), 116.4 (d, $J = 20.8$ Hz, aromatic carbon), 117.0 (C-s)*, 117.2 (d, $J = 23.1$ Hz, aromatic carbon), 125.2 (C-a), 126.5 (C-l), 127.2 (aromatic carbon), 128.2 (C-c), 128.8 (d, $J = 9.8$ Hz, aromatic carbon), 129.1 (aromatic carbon), 130.9 (d, $J = 8.4$ Hz, aromatic carbon), 132.9 (aromatic carbon), 133.7 (aromatic carbon), 141.4 (C-e), 142.2 (d, $J = 242.5$ Hz, C-q)**, 143.3 (d, $J = 245.8$ Hz, C-r)**, 144.1, 160.6 (C-g)***, 161.2 (C-h)***, 162.0 (d, $J = 244.3$ Hz, aromatic carbon); MS (ESI) 508 (M-1).

1-Benzyl-2-oxo-N-(2,3,5,6-tetrafluoro-4'-(trifluoromethyl)-[1,1'-biphenyl]-4-yl)-1,2-dihydropyrazolo[1,5-a]pyridine-3-carboxamide (54). 2,3,5,6-Tetrafluoro-4'-(trifluoromethyl)-[1,1'-biphenyl]-4-aniline was used; flash chromatography eluent: from petroleum ether/EtOAc 80:20 v/v to 50:50 v/v. Pale yellow solid (m.p. 262.2 – 263.5 °C from trituration with diisopropyl ether). Yield 41 %. 1H NMR (600 MHz, Chloroform- d): δ 5.49 (s, 2H, $-NCH_2Ph$), 6.78

(*t*, 1H, *J* = 6.8 Hz, *H-b*), 7.29 (*d*, 2H, *J* = 7.4 Hz, aromatic protons), 7.33 – 7.43 (*m*, 3H, aromatic protons), 7.48 (*t*, 1H, *J* = 7.9 Hz, *H-c*), 7.62 (*d*, 2H, *J* = 7.9 Hz, aromatic protons), 7.73 (*d*, 1H, *J* = 6.9 Hz, *H-a*), 7.76 (*d*, 2H, *J* = 8.1 Hz, aromatic protons), 8.31 (*d*, 1H, *J* = 8.8 Hz, *H-d*), 10.04 (*s*, 1H, –NH); ¹³C NMR (151 MHz, Chloroform-*d*): δ 45.7 (–NCH₂Ph), 87.2 (*C-f*), 112.9 (*C-b*), 116.1 (*t*, *J* = 16.5 Hz, *C-p*)*, 117.2 (*t*, *J* = 15.6 Hz, *C-s*)*, 118.4 (*C-d*), 123.0 (*C-a*), 124.0 (*q*, *J* = 272.5 Hz, –CF₃), 125.7 (*q*, *J* = 3.8 Hz, aromatic carbon), 127.1 (aromatic carbon), 129.0 (*C-c*), 129.7 (aromatic carbon), 130.8 (aromatic carbon), 131.1 (*q*, *J* = 32.8 Hz, aromatic carbon), 131.4 (aromatic carbon), 131.9 (aromatic carbon), 132.5 (aromatic carbon), 142.6 (*C-e*), 142.8 (*dd*, *J* = 251.8, 17.4 Hz, *C-q*)**, 144.1 (*dd*, *J* = 244.7, 15.4 Hz, *C-r*)**, 161.6 (*C-g****), 162.2 (*C-h****); MS (ESI) 560 (*M*+1).

1-Benzyl-2-oxo-N-(2,3,5,6-tetrafluoro-3'-(trifluoromethyl)-[1,1'-biphenyl]-4-yl)-1,2-dihydropyrazolo[1,5-*a*]pyridine-3-carboxamide (55). 2,3,5,6-Tetrafluoro-3'-(trifluoromethyl)-[1,1'-biphenyl]-4-aniline was used; flash chromatography eluent: from petroleum ether / EtOAc 80:20 v/v to 50:50 v/v. White solid (m.p. 190.9 – 191.8 °C from trituration with diisopropyl ether). Yield 40 %. ¹H NMR (600 MHz, Chloroform-*d*): δ 5.49 (*s*, 2H, –NCH₂Ph), 6.78 (*t*, 1H, *J* =

6.8 Hz, *H-b*), 7.29 (*d*, 2H, $J = 7.3$ Hz, aromatic protons), 7.33 – 7.42 (*m*, 3H, aromatic protons), 7.48 (*t*, 1H, $J = 7.9$ Hz, *H-c*), 7.64 (*t*, 1H, $J = 7.7$ Hz, aromatic proton), 7.68 (*d*, 1H, $J = 7.5$ Hz, aromatic proton), 7.72 (*t*, 2H, $J = 7.6$ Hz, aromatic protons), 7.76 (*s*, 1H, aromatic proton), 8.31 (*d*, 1H, $J = 8.8$, *H-d*), 10.04 (*s*, 1H, -NH); ^{13}C NMR (151 MHz, Chloroform-*d*): δ 45.7 (-NCH₂Ph), 87.2 (*C-f*), 112.9 (*C-b*), 116.4 (*t*, $J = 16.2$ Hz, *C-p*)*, 117.2 (*t*, $J = 15.2$ Hz, *C-s*)*, 118.5 (*C-d*), 123.0 (*C-a*), 124.0 (*q*, $J = 272.1$ Hz, -CF₃), 125.9 (*q*, $J = 3.9$ Hz, aromatic carbon), 127.1 (aromatic carbon), 127.2 (aromatic carbon), 128.5 (aromatic carbon), 129.0 (aromatic carbon), 129.3 (aromatic carbon), 129.7 (aromatic carbon), 131.3 (*q*, $J = 32.4$ Hz), 131.8 (*C-c*), 132.5 (aromatic carbon), 133.7 (aromatic carbon), 142.7 (*C-e*), 142.8 (*d*, $J = 252.0$ Hz, *C-q*)**, 144.2 (*d*, $J = 248.2$ Hz, *C-r*)**, 161.6 (*C-g****, 162.2 (*C-h****; MS (ESI) 560 (*M*+1).

General Procedure: removal of the 4-MeObenzyloxy moiety for the production of cpds 7 – 9, 15–17. Thioanisole (220 μL , 1.87 mmol, from 5.0 eq to 10 equivalent) was added to a solution of corresponding starting material (200 mg, 0.37 mmol, 1.0 eq) in TFA (3 mL). The mixture was heated at 70 °C for 2h then cooled to r.t.. The mixture was partially concentrated, and the crude was taken up with water obtaining a suspension that was filtered, the solid washed with additional amount of cold water. The

resulting solid was triturated with diisopropyl ether to afford the title compounds, often directly in pure form (see details above).

2-Hydroxy-N-(2,3,5,6-tetrafluoro-4-morpholinophenyl)pyrazolo[1,5-a]pyridine-3-carboxamide (**6**). Compound **59** (200 mg, 0.38 mmol, 1.0 eq) was dissolved in a solution of thioanisole (250 μ L, 2.26 mmol, 6.0 eq) in TFA (2 mL). The residue was triturated with hexane and diisopropyl ether, then purified by flash chromatography (eluent: *petroleum ether*/ DCM/ MeOH 5/4/0.4 v/v/v). The resulting crude solid was triturated with diisopropyl ether to afford the title compound as white solid (276.5–277.2 °C dec. from diisopropyl ether). Yield: 42%. ^1H NMR (600 MHz, DMSO- d_6) δ 3.18 – 3.25 (*m*, 4H, $-\text{NCH}_2\text{CH}_2\text{O}-$), 3.68 – 3.75 (*m*, 4H, $-\text{NCH}_2\text{CH}_2\text{O}-$), 7.0 (*t*, 1H, $J = 6.8$ Hz, *H-b*), 7.48 (*t*, 1H, $J = 7.8$ Hz, *H-c*), 7.94 (*d*, 1H, $J = 8.7$ Hz, *H-d*), 8.58 (*d*, 1H, $J = 6.7$ Hz, *H-a*), 8.76 (*s*, 1H, $-\text{NH}$), 12.83 (*v br s*, 1H, $-\text{OH}$); ^{13}C NMR (151 MHz, DMSO- d_6) δ 51.0 ($-\text{NCH}_2\text{CH}_2\text{O}-$), 66.7 ($-\text{NCH}_2\text{CH}_2\text{O}-$), 88.2 (*C-f*), 111.5 (*t*, $J = 15.1$ Hz, *C-p*)*, 113.1 (*C-b*), 116.8 (*C-d*), 127.5 (*t*, $J = 11.2$ Hz, *C-s*)*, 128.2 (*C-a*), 129.1 (*C-c*), 141.7 (*C-e*), 142.2 (*dd*, $J = 243.5, 6.2$ Hz, *C-q*)**, 143.4 (*dd*, $J = 244.6, 14.4$ Hz, *C-r*)***, 160.8 (*C-h*)***, 162.7 (*C-g*)***. MS (ES $^-$): 409 (*M*-1). ESI-HRMS (*m/z*): [*M*-H] $^-$ calcd for $\text{C}_{18}\text{H}_{13}\text{F}_4\text{N}_4\text{O}_3$, 409.0929; obsd, 409.0925.

2-Hydroxy-N-(2,3,5,6-tetrafluoro-4-(thiophen-2-yl)phenyl)pyrazolo[1,5-a]pyridine-3-carboxamide (**7**). The solid was crystallized three times from acetonitrile (20 mL) to obtain the title compound as a grey solid (278.4 - 279.9 °C dec. from acetonitrile). Yield: 23 %. ¹H NMR (600 MHz, DMSO-d₆) δ 7.03 (t, 1H, J = 6.6 Hz, H-b), 7.31 (t, 1H, J = 4.3 Hz, aromatic proton), 7.51 (t, 1H, J = 7.8 Hz, H-c), 7.64 (d, 1H, J = 2.8 Hz, aromatic proton), 7.92 (d, 1H, J = 5.0 Hz, aromatic proton), 7.97 (d, 1H, J = 8.8 Hz, H-d), 8.61 (d, 1H, J = 6.7 Hz, H-a), 8.95 (s, 1H, -NH), 12.90 (br s, 1H, -OH); ¹³C NMR (151 MHz, DMSO-d₆) δ 88.2 (C-f), 110.9 (t, J = 15.6 Hz, C-p)*, 113.8 (C-b), 17.03 (t, J = 16.5 Hz, C-s)*, 116.8 (C-d), 126.2 (thiophene carbon), 127.8 (thiophene carbon), 128.4 (C-a), 129.2 (thiophene carbon), 129.5 (C-c), 130.7 (t, J = 4.0 Hz, thiophene carbon), 141.8 (C-e), 143.0 (dd, J = 246.5, 14.3 Hz, C-q and C-r), 160.4 (C-h)***, 162.7 (C-g)**. MS (ES-): 406 (M-1). ESI-HRMS (m/z): [M-H]⁻ calcd for C₁₈H₈F₄N₃O₂S, 406.0279; obsd, 406.0275.

2-Hydroxy-N-(2,3,5,6-tetrafluoro-4-(pyridin-3-yl)phenyl)pyrazolo[1,5-a]pyridine-3-carboxamide (**8**). Pale yellow solid (m.p. 283.4 - 286.7 °C dec. from trituration with diisopropyl ether). Yield: 76%. ¹H NMR (600 MHz, DMSO-d₆) δ 6.98 (t, 1H, J = 6.7 Hz, H-b), 7.47 (t, 1H, J = 7.9 Hz, H-c), 7.61 (dd, 1H, J = 7.7, 5.0 Hz, aromatic proton), 7.95 (d, 1H, J = 8.6 Hz, H-d), 8.05 (d,

1H, J = 7.4 Hz, aromatic proton), 8.57 (d, 1H, J = 6.5 Hz, H-a), 8.71 (d, 1H, J = 4.1 Hz, aromatic proton), 8.78 (s, 1H, aromatic proton), 9.30 (br s, 1H, -NH); ¹³C NMR (151 MHz, DMSO-d₆) δ 88.3 (C-f), 112.9 (C-b), 113.8 (t, J = 17.4 Hz, C-p)*, 116.6 (C-d), 117.9 (t, J = 14.7 Hz, C-s)*, 123.3 (pyridine carbon), 124.0 (pyridine carbon), 128.0 (C-a), 128.9 (C-c), 137.9 (pyridine carbon), 141.7 (C-e), 142.6 (dd, J = 245.2, 15.1 Hz, C-q)**, 143.4 (dd, J = 245.7, 17.2 Hz, C-q)**, 150.2 (pyridine carbon), 150.3 (pyridine carbon), 160.6 (C-h)***, 163.9 (C-g)***. MS (ES+): 403 (M+H). ESI-HRMS (m/z): [M+H]⁺ calcd for C₁₉H₁₁F₄N₄O₂, 403.0813; obsd, 403.0810.

2-Hydroxy-N-(2,3,5,6-tetrafluoro-4-(2-(trifluoromethyl)pyridin-4-yl)phenyl)pyrazolo[1,5-a]pyridine-3-carboxamide (**9**). Pale yellow solid (m.p. 258.4 - 259.2 °C dec. from trituration with diisopropyl ether). Yield: 55%. ¹H NMR (600 MHz, DMSO-d₆) δ 7.01 (t, 1H, J = 6.6 Hz, H-b), 7.49 (t, 1H, J = 7.7 Hz, H-c), 7.96 (d, 1H, J = 8.6 Hz, H-d), 8.00 (d, 1H, J = 3.5 Hz, aromatic proton), 8.22 (s, 1H, aromatic proton), 8.59 (d, 1H, J = 6.6 Hz, H-a), 8.99 (d, J = 4.6 Hz, 1H, aromatic proton), 9.22 (br s, 1H, -NH); ¹³C NMR (151 MHz, DMSO-d₆) δ 88.2 (C-f), 113.1 (C-p* overlapped with C-b), 116.7 (C-d), 118.9 (t, J = 13.5 Hz, C-s)*, 121.5 (q, J = 274.6 Hz, -CF₃), 121.8 (pyridine carbon), 128.2 (C-a), 128.5 (C-c), 129.1 (q, J = 12.5 Hz, pyridine carbon), 137.3 (pyridine

carbon), 141.7 (C-e), 142.6 (d, $J = 246.8$ Hz, C-q)**,
143.3 (d, $J = 247.0$ Hz, C-r)**, 147.1 (q, $J = 34.1$ Hz,
pyridine carbon), 151.0 (pyridine carbon), 160.3 (C-
h)***, 163.4 (C-g)***. MS (ES+): 470 (M+H). ESI-HRMS
(m/z): [M+H]⁺ calcd for C₂₀H₁₀F₇N₄O₂, 471.0686; obsd,
471.0684.

2-Hydroxy-N-(2,3,5,6-tetrafluoro-3'-hydroxy-[1,1'-
biphenyl]-4-yl)pyrazolo[1,5-a]pyridine-3-carboxamide
(14). white solid (275.9 -276.4 °C dec. from diisopropyl
ether). Yield: 58%. ¹H NMR (600 MHz, DMSO-d₆) δ 6.84 -
6.99 (m, 3H, aromatic protons), 7.03 (t, 1H, $J = 6.8$ Hz,
H-b), 7.35 (t, 1H, $J = 8.2$ Hz, aromatic proton), 7.51 (t,
1H, $J = 7.8$ Hz, H-c), 7.98 (d, 1H, $J = 7.4$ Hz, H-d), 8.61
(d, 1H, $J = 4.5$ Hz, H-a), 8.90 (s, 1H, -Ar-OH), 9.78 (s,
1H, -NH), 12.86 (s, 1H, -OH); ¹³C NMR (151 MHz, DMSO-d₆)
 δ 88.2 (C-f), 113.3 (C-b), 114.7 (t, $J = 13.5$ Hz, C-p)*,
116.3 (C-d), 116.8 (aromatic carbon), 117.4 (t, $J = 16.3$
Hz, C-s)*, 120.7 (aromatic carbon), 127.6 (aromatic
carbon), 128.4 (C-a), 128.3 (aromatic carbon), 129.1 (C-
c), 129.9 (aromatic carbon), 141.7 (C-e), 142.7 (d, $J =$
248.4 Hz, C-q)**, 143.2 (d, $J = 244.8$ Hz, C-r)**, 157.5
(aromatic carbon), 160.4 (C-h)***, 162.6 (C-g)***; MS
(ES-): 416 (M-1). ESI-HRMS (m/z): [M+H]⁺ calcd for
C₂₀H₁₂F₄N₃O₃, 418.0809; obsd, 418.0807.

2-Hydroxy-N-(2,3,5,6-tetrafluoro-3'-(trifluoromethoxy)-
[1,1'-biphenyl]-4-yl)pyrazolo[1,5-a]pyridine-3-

carboxamide (**15**). The crude was dissolved in methanol, filtered from insoluble solid, partly evaporated and precipitated with addition of diisopropyl ether. Beige solid (228.1 -229.5 °C dec. from diisopropyl ether). Yield: 26%. ¹H NMR (600 MHz, DMSO-*d*₆) δ 7.03 (*t*, 1H, *J* = 6.7 Hz, *H*-*b*), 7.51 (*t*, 1H, *J* = 7.8 Hz, *H*-*c*), 7.55 (*d*, 1H, *J* = 8.0 Hz, aromatic proton), 7.60 - 7.67 (*m*, 2H, aromatic protons), 7.71 (*t*, 1H, *J* = 7.9 Hz, aromatic proton), 7.98 (*d*, 1H, *J* = 8.7 Hz, *H*-*d*), 8.61 (*d*, 1H, *J* = 6.7 Hz, *H*-*a*), 8.97 (*s*, 1H, -NH), 12.94 (*br s*, 1H, -OH); ¹³C NMR (151 MHz, DMSO-*d*₆) δ 88.2 (*C*-*f*), 113.2 (*C*-*b*), 115.5 (*t*, *J* = 17.2 Hz, *C*-*p*)*, 116.8 (*C*-*d*), 117.6 (*t*, *J* = 15.1 Hz, *C*-*s*)*, 120.1 (*q*, *J* = 257.0 Hz, -OCF₃), 122.1 (aromatic carbon), 123.0 (aromatic carbon), 128.4 (*C*-*a*), 128.8 (aromatic carbon), 129.2 (*C*-*c*), 129.5 (aromatic carbon), 130.9 (aromatic carbon), 141.7 (*C*-*e*), 142.7 (*dd*, *J* = 246.2, 13.6 Hz, *C*-*q*)**, 143.3 (*d*, *J* = 245.6 Hz, *C*-*r*)**, 148.4 (aromatic carbon), 160.3 (*C*-*h****), 162.7 (*C*-*g****); MS (ES⁻): 484 (*M*-1). ESI-HRMS (*m/z*): [*M*+*H*]⁺ calcd for C₂₁H₁₁F₇N₃O₃, 486.0683; obsd, 486.0681.

2-Hydroxy-*N*-(2,3,5,6-tetrafluoro-3'-methoxy-[1,1'-biphenyl]-4-yl)pyrazolo[1,5-*a*]pyridine-3-carboxamide (**16**). Residue was treated with diethyl ether and purified by flash chromatography (eluent: DCM / MeOH 97 / 3 v/v then petroleum ether/dichloromethane/methanol 5/4/0.6 v/v/v) to obtain the title compound as a white solid

(223.6 – 224.1 °C dec. from diisopropyl ether). Yield: 47 %. ¹H NMR (600 MHz, DMSO-*d*₆) δ 3.81 (*s*, 3H, -OCH₃), 7.02 (*t*, 1H, *J* = 6.6 Hz, *H-b*), 7.08 – 7.16 (*m*, 3H, aromatic protons), 7.45 – 7.54 (*m*, 2H, *H-c* and aromatic proton), 7.98 (*d*, 1H, *J* = 8.6 Hz, *H-d*), 8.61 (*d*, 1H, *J* = 6.6 Hz, *H-a*), 8.99 (*br s*, 1H, -NH); ¹³C-NMR (150 MHz, DMSO-*d*₆): δ 55.4 (-OCH₃), 88.2 (*C-f*), 113.2 (*C-b*), 115.0 (*C-d*), 115.8 (*aromatic carbon*), 116.8 (*aromatic carbon*), 117.0 (*t*, *J* = 16.1 Hz, *C-s* and *C-p*)*, 122.3 (*aromatic carbon*), 127.8 (*C-a*), 128.3 (*aromatic carbon*), 129.1 (*C-c*), 130.0 (*aromatic carbon*), 141.8 (*C-e*), 142.9 (*d*, *J* = 246.3 Hz, *C-q*)**, 143.4 (*d*, *J* = 252.5 Hz, *C-r*)**, 159.4 (*aromatic carbon*), 160.5 (*C-h*)****, 163.0 162.2 (*C-g*)****; MS (ES⁻): 430 (*M*-1), MS (ES⁺) 432 (*M*+1). ESI-HRMS (*m/z*): [*M*+H]⁺ calcd for C₂₁H₁₄F₄N₃O₃, 432.0966; obsd, 432.0969.

2-Hydroxy-N-(2,3,5,6-tetrafluoro-3'-propoxy-[1,1'-biphenyl]-4-yl)pyrazolo[1,5-*a*]pyridine-3-carboxamide (**17**). Beige solid (209.4 – 210.0 °C from diisopropyl ether). Yield: 69 %. ¹H NMR (600 MHz, DMSO-*d*₆) δ 0.98 (*t*, 3H, *J* = 7.2 Hz, -OCH₂CH₂CH₃), 1.68 – 1.81 (*m*, 2H, -OCH₂CH₂CH₃), 3.98 (*t*, 2H, *J* = 6.1 Hz, -OCH₂CH₂CH₃), 7.02 (*t*, 1H, *J* = 6.5 Hz, *H-b*), 7.05 – 7.14 (*m*, 3H, aromatic protons), 7.46 (*t*, 1H, *J* = 7.8 Hz, aromatic proton or *H-c*), 7.51 (*t*, 1H, *J* = 7.6 Hz, aromatic proton or *H-c*), 7.99 (*d*, 1H, *J* = 8.7 Hz, *H-d*), 8.61 (*d*, 1H, *J* = 6.5 Hz,

H-a), 8.91 (*s*, 1H, -NH), 12.86 (*s*, 1H, -OH); ^{13}C NMR (151 MHz, DMSO- d_6) δ 10.4 (-OCH₂CH₂CH₃), 22.0 (-OCH₂CH₂CH₃), 69.2 (-OCH₂CH₂CH₃), 88.2 (*C-f*), 113.3 (*C-b*), 115.5 (*C-d*), 116.2 (*aromatic carbon*), 116.8 (*aromatic carbon*), 116.9 - 117.7 (*m*, *C-s* and *C-p*), 122.2 (*aromatic carbon*), 127.8 (*aromatic carbon*), 128.4 (*C-a*), 129.2 (*C-c*), 129.9 (*aromatic carbon*), 141.7 (*C-e*), 142.8 (*dd*, *J* = 245.5, 21.8 Hz, *C-q*)**, 143.3 (*d*, *J* = 245.6 Hz, *C-r*)**, 158.8 (*aromatic carbon*)***, 160.4 (*C-h*)****, 162.6 (*C-g*)****; MS (ES⁻): 458 (*M*-1), MS (ES⁺): 460 (*M*+1). ESI-HRMS (*m/z*): [*M*+H]⁺ calcd for C₂₃H₁₈F₄N₃O₃, 460.1279; obsd, 460.1280.

2-Hydroxy-*N*-(2,3,5,6-tetrafluoro-[1,1'-biphenyl]-4-yl)-4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyridine-3-carboxamide (**5**). Palladium on carbon (Pd/C, 20% w / w) was added to a solution of compound **51**, (1.0 mmol) in dry THF (10 mL). The resulting mixture was stirred under a hydrogen atmosphere of 40 bar, at temperature of 65°C for 3 hours using Microwave SynthWAVE. The suspension was filtered through Celite and the cake was washed with methanol. The filtrate was concentrated under reduced pressure. The obtained solid was further purified by flash chromatography (eluent: dichloromethane / EtOAc / HCOOH 80:20:1 v/v/v). White solid (m.p. 270.9 - 272.9 °C dec, from diisopropyl ether). Yield 40% ^1H NMR (600 MHz, DMSO- d_6): δ 1.70-1.80 (*m*, 2H, *H-b*), 1.89-1.98 (*m*, 2H, *H-c*), 2.92 (*t*, 2H, *J*=6.1 Hz, *H-d*), 3.86 (*t*, 2H, *J*=5.8 Hz, *H-*

a), 7.49 – 7.55 (m, 5H, aromatic protons), 9.11 (s, 1H, -NH), 11.94 (v br s, 1H, -OH). Exchangeable proton signals overlapped with the water signal; ^{13}C NMR (151 MHz, DMSO- d_6): δ 18.5 (C-b), 22.1 (C-d), 22.9 (C-c), 46.5 (C-a), 95.5 (C-f), 116.8 (t, J = 14.3 Hz, C-s)*, 117.0 (t, J = 17.4 Hz, C-p)*, 126.7 (aromatic carbon), 128.9 (aromatic carbon), 129.4 (aromatic carbon), 130.1 (aromatic carbon), 141.8 (C-e) 143.2 (d, J = 248.4, 21.8 Hz, C-q)**, 144.3 (d, J = 244.0 Hz, C-r)**, 159.7 (C-g)***, 160.7 (C-h)***; MS (ES-) 404 (M-1). IR (KBr) ν (cm^{-1}): 3338, 2924, 2519, 1685, 1577, 1522, 1437, 1374, 1316, 1283, 1241, 1144, 992. ESI-HRMS (m/z): $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{20}\text{H}_{16}\text{F}_4\text{N}_3\text{O}_2$, 406.1173; obsd, 406,1170.

General hydrogenation procedure for the obtainment of target compounds 10 – 13.

Palladium on carbon (Pd/C, 6% w/w), was added to a solution of the appropriate amide (compounds 52 – 55, 1.0 mmol), in dry THF (15 mL), and HCl (1.0 mmol). The resulting mixture was vigorously stirred under a hydrogen atmosphere for 6 hours. The suspension was filtered through Celite then the cake was washed with methanol. The filtrate was concentrated under reduced pressure. When necessary, the obtained solid was further purified by flash chromatography (see details below).

2-Hydroxy-N-(2,3,4',5,6-pentafluoro-[1,1'-biphenyl]-4-yl)pyrazolo[1,5-a]pyridine-3-carboxamide (10). Obtained from **52**, flash chromatography (eluent: dichloromethane / EtOAc / HCOOH 80:20:1 v/v/v). White solid (m.p. 293.4 - 294.5 °C dec. from trituration with diisopropyl ether). Yield 75 %. ¹H NMR (600 MHz, DMSO-d₆): δ 7.03 (t, 1H, J = 6.7 Hz, H-b), 7.42 (t, 2H, J = 8.7 Hz, aromatic protons), 7.51 (t, 1H, J = 7.8 Hz, H-c), 7.57 - 7.71 (m, 2H, aromatic protons), 7.98 (d, 1H, J = 8.7, H-d), 8.62 (d, 1H, J = 6.7, H-a), 8.93 (s, 1H, -NH), 12.88 (br s, 1H, -OH); ¹³C NMR (151 MHz, DMSO-d₆): δ 88.2 (C-f), 113.3 (C-b), 116.0 (d, J = 22.0 Hz, aromatic carbon), 116.3 (t, J = 17.4 Hz, C-p)*, 116.8 (C-d), 117.1 (t, J = 14.1 Hz, C-s)*, 123.0 (aromatic carbon), 128.4 (C-a), 129.2 (C-c), 132.5 (d, J = 8.4 Hz, aromatic carbon), 141.7 (C-e), 142.8 (d, J = 246.4 Hz, C-q)**, 143.3 (d, J = 248.6 Hz, C-r)**, 160.4 (C-g)***, 162.6 (d, J = 247.1 Hz, -CF), 162.7 (C-h)***; MS (ESI) 420 (M+1). ESI-HRMS (m/z): [M+H]⁺ calcd for C₂₀H₁₁F₅N₃O₂, 420.0766; obsd, 420.0766.

2-Hydroxy-N-(2,3,3',5,6-pentafluoro-[1,1'-biphenyl]-4-yl)pyrazolo[1,5-a]pyridine-3-carboxamide (11). Obtained from **53**, flash chromatography eluent: dichloromethane/EtOAc / HCOOH 80:20:1 v/v/v. White solid (m.p. 255.4 - 256.2 °C dec. from trituration with diisopropyl ether). Yield 75 %. ¹H NMR (600 MHz, DMSO-d₆): δ 7.03 (t, 1H, J = 6.8 Hz, H-b), 7.33 - 7.56 (m, 4H,

aromatic protons), 7.62 (dd, 1H, $J = 14.3, 7.7$ Hz, aromatic proton), 7.99 (d, 1H, $J = 8.7$, H-d), 8.62 (d, 1H, $J = 6.8$, H-a), 8.94 (s, 1H, -NH), 12.83 (br s, 1H, -OH); ^{13}C NMR (151 MHz, CDCl_3): δ 88.2 (C-f), 113.3 (C-b), 115.9 (t, $J = 17.4$ Hz, C-p)*, 116.4 (d, $J = 20.9$ Hz, aromatic carbon), 116.9 (C-d), 117.2 (d, $J = 23.0$ Hz, aromatic carbon), 117.4 (t, $J = 14.8$ Hz, C-s)*, 126.5 (aromatic carbon), 128.4 (C-a), 128.7 (d, $J = 9.6$ Hz, aromatic carbon), 129.2 (C-c), 130.9 (d, $J = 8.3$ Hz, aromatic carbon), 141.7 (C-e), 142.8 (d, $J = 243.6$ Hz, C-q)**, 143.2 (d, $J = 244.6$ Hz, C-r)**, 160.4 (C-g)***, 162.0 (d, $J = 244.4$ Hz, -CF), 162.7 (C-h)***; MS (ESI) 418 (M-1). ESI-HRMS (m/z): $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{20}\text{H}_{11}\text{F}_5\text{N}_3\text{O}_2$, 420.0766; obsd, 420.0763.

2-Hydroxy-N-(2,3,5,6-tetrafluoro-4'-(trifluoromethyl)-[1,1'-biphenyl]-4-yl)pyrazolo[1,5-a]pyridine-3-carboxamide (**12**). Obtained from **54**, flash chromatography eluent: dichloromethane/EtOAc/HCOOH 80:20:1 v/v/v. White solid (m.p. 286.1 – 286.8 °C dec. from trituration with diisopropyl ether). Yield 95 %. ^1H NMR (600 MHz, $\text{DMSO}-d_6$): δ 7.03 (t, 1H, $J = 6.7$ Hz, H-b), 7.51 (t, 1H, $J = 7.8$ Hz, H-c), 7.83 (d, 2H, $J = 7.8$ Hz, aromatic protons), 7.94 (d, 2H, $J = 8.0$ Hz, aromatic protons), 7.98 (d, 1H, $J = 8.7$ Hz, H-d), 8.61 (d, 1H, $J = 6.7$ Hz, H-a), 9.00 (s, 1H, -NH), 12.92 (br s, 1H, -OH); ^{13}C NMR (151 MHz, $\text{DMSO}-d_6$): δ 88.2 (C-f), 113.3 (C-b), 115.7 (t, $J = 16.9$ Hz, C-

p)*, 116.8 (*C-d*), 117.8 (*t*, *J* = 13.1 Hz, *C-s*)*, 124.0 (*q*, *J* = 272.4 Hz, -CF₃), 125.8 (*q*, *J* = 3.3 Hz, aromatic carbon), 128.4 (*C-a*), 129.2 (*C-c*), 129.7 (*q*, *J* = 32.3 Hz, aromatic carbon), 131.0 (aromatic carbon), 131.2 (aromatic carbon), 141.7 (*C-e*), 142.7 (*dd*, *J* = 246.0, 14.9 Hz, *C-q*)**, 143.2 (*dd*, *J* = 244.9, 19.5 Hz, *C-r*)**, 160.3 (*C-g****, 162.7 (*C-h****; MS (ESI) 468 (M-1). ESI-HRMS (*m/z*): [M+H]⁺ calcd for C₂₁H₁₁F₇N₃O₂, 470.0734; obsd, 470.0731.

2-Hydroxy-N-(2,3,5,6-tetrafluoro-3'-(trifluoromethyl)-[1,1'-biphenyl]-4-yl)pyrazolo[1,5-a]pyridine-3-carboxamide (13). Obtained from **55**, flash chromatography eluent: dichloromethane/EtOAc/HCOOH 80:20:1 v/v/v. Pale pink solid (m.p. 220.3 – 220.7 °C dec. from trituration with diisopropyl ether). Yield 98 %. ¹H NMR (600 MHz, DMSO-d₆): δ 7.03 (*t*, 1H, *J* = 6.7 Hz, *H-b*), 7.51 (*t*, 1H, *J* = 7.8 Hz, *H-c*), 7.82 (*t*, 1H, *J* = 7.7 Hz, aromatic proton), 7.91 (*d*, 2H, *J* = 7.6 Hz, aromatic protons), 7.95 – 8.02 (*m*, 2H, aromatic proton and *H-d*), 8.62 (*d*, 1H, *J* = 6.7, *H-a*), 8.98 (*s*, 1H, -NH), 12.87 (*br s*, 1H, -OH); ¹³C NMR (151 MHz, DMSO-d₆): δ 88.2 (*C-f*), 113.3 (*C-b*), 115.6 (*t*, *J* = 17.0 Hz, *C-p*)*, 116.8 (*C-d*), 117.6 (*t*, *J* = 15.6 Hz, *C-s*)*, 123.9 (*q*, *J* = 271.9 Hz, -CF₃), 126.2 (aromatic carbon), 126.9 (aromatic carbon), 127.9 (aromatic carbon), 128.4 (*C-a*), 129.2 (*C-c*), 129.7 (*q*, *J* = 32.4 Hz, aromatic carbon), 130.1 (aromatic carbon), 134.4

(aromatic carbon), 141.7 (C-e), 143.8 (d, J = 246.0 Hz, C-q)**, 143.9 (d, J = 246.9 Hz, C-r)**, 160.3 (C-g)***, 162.7 (C-h)***; MS (ESI) 468 (M-1). ESI-HRMS (m/z): [M+H]⁺ calcd for C₂₁H₁₁F₇N₃O₂, 470.0734; obsd, 470.0735.

Ethyl 2-((tert-butoxycarbonyl)oxy)pyrazolo[1,5-a]pyridine-3-carboxylate (**67**). Cs₂CO₃ (2.86 g, 8.74 mmol) and tert-butoxycarbonyl anhydride (0.699 g, 3.2 mmol) were added to a solution of **44** (0.600 g, 2.91 mmol) in dry THF (25 mL). The reaction mixture was stirred under reflux overnight and allowed to reach room temperature. The solvent was concentrated under reduced pressure and the residue was dissolved in water (50 mL) and extracted with diethyl ether (3 x 50 mL). The combined organic layer was washed with brine, dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. The residue was purified by flash chromatography (eluent: petroleum ether/ethyl acetate 80:20 v/v) to afford the title compound as a white solid (m.p. 95.4 – 96.4 °C from trituration with diisopropyl ether). Yield 93%. ¹H NMR (600 MHz, Chloroform-d₃): δ 1.39 (t, 3H, J = 7.1 Hz, –OCH₂CH₃), 1.58 (s, 9H, –OC(CH₃)₃), 4.36 (q, 2H, J = 7.0 Hz, –OCH₂CH₃), 6.96 (t, 1H, J = 6.8 Hz, H-b), 7.42 (t, 1H, J = 7.9 Hz, H-c), 8.08 (d, 1H, J = 8.8 Hz, H-d), 8.38 (d, 1H, J = 6.6 Hz, H-a). ¹³C NMR (151 MHz, Chloroform-d₃): δ 14.6 (–OCH₂CH₃), 27.8 (–C(CH₃)₃), 60.2 (–OCH₂CH₃), 84.8 (C-f), 93.4 (–C(CH₃)₃), 114.1 (C-b), 119.2 (C-d),

128.0 (C-a), 129.3 (C-c), 142.3 (C-e), 150.2 (C-h), 158.2 (C-g)*, 162.1 (C-i)*. MS (ESI) 307 (M+1).

Ethyl 2-((tert-butoxycarbonyl)oxy)-7-chloropyrazolo[1,5-a]pyridine-3-carboxylate (68). LiHMDS (1.0 M THF solution: 0.980 mL, 0.98 mmol, 1.5 equiv.) was added dropwise to a solution of **67** (0.400 g, 0.654 mmol) in dry THF (10 mL) cooled to a -78°C. The mixture was stirred at -78°C for 1 h, then a solution of hexachloroethane (0.170 g, 0.72 mmol, 1.1 equiv.) in dry THF was added at -78°C and the reaction mixture was stirred for 15 minutes at room temperature. Subsequently, the reaction was quenched with aqueous saturated solution of NH₄Cl (100 mL). The water phase was extracted with dichloromethane (4 x 100 mL). The combined organic phases were dried over Na₂SO₄, filtered and evaporated to dryness under vacuum. The crude product was purified by flash chromatography (eluent: petroleum ether/ethyl acetate 80:20 v/v) to afford the title compound as a white solid (m.p. 104.6 - 106.0 °C from trituration with diisopropyl ether). Yield 81%. ¹H NMR (600 MHz, Chloroform-d₃): δ 1.40 (t, 3H, J = 7.1 Hz, -OCH₂CH₃), 1.58 (s, 9H, -OC(CH₃)₃), 4.37 (q, 2H, J = 7.2 Hz, -OCH₂CH₃), 7.11 (dd, 1H, J = 7.5, 1.1 Hz, H-b), 7.39 (dd, 1H, J = 8.9, 7.5 Hz, H-c), 8.09 (dd, 1H, J = 8.9, 1.2 Hz, H-d). ¹³C NMR (151 MHz, Chloroform-d₃): δ 14.6 (-OCH₂CH₃), 27.8 (-C(CH₃)₃), 60.5 (-OCH₂CH₃), 85.0 (C-f)₃, 95.2 (-C(CH₃)₃), 114.4 (C-b), 117.5 (C-d), 128.2 (C-

c), 131.0 (C-a), 143.9 (C-e), 150.0 (C-h), 158.0 (C-g)*, 161.8 (C-i)*. MS (ESI) 241 (M+1, -Boc).

Ethyl 7-chloro-2-hydroxypyrazolo[1,5-a]pyridine-3-carboxylate (**69**). Trifluoroacetic acid (10 mL) was added to a solution of **68** in dry dichloromethane (25 mL) and the reaction mixture was stirred at room temperature for 4 h. The mixture was quenched with water and the layers were separated. The aqueous solution was further extracted with dichloromethane (3 x 25 mL). The combined organic phases were dried over Na₂SO₄, filtered and evaporated to dryness under reduced pressure. The crude product was purified by flash chromatography (eluent: dichloromethane/methanol 98:2 v/v) to afford the title compound as a white solid (m.p. 134.0 – 135.8 °C from trituration with diisopropyl ether). Yield 94%. ¹H NMR (600 MHz, Chloroform-d₃): δ 1.44 (t, 3H, J = 7.2 Hz, -OCH₂CH₃), 4.44 (q, 2H, J = 7.2 Hz, -OCH₂CH₃), 7.02 (dd, 1H, J = 7.5, 1.2 Hz, H-b), 7.35 (dd, 1H, J = 8.7, 7.5 Hz, H-c), 7.72 (dd, 1H, J = 8.7, 1.2 Hz, H-d), 9.09 (s, 1H, -OH). ¹³C NMR (151 MHz, Chloroform-d₃): δ 14.6 (-OCH₂CH₃), 60.9 (-OCH₂CH₃), 87.9 (C-f), 113.6 (C-b), 115.5 (C-d), 128.4 (C-c), 131.3 (C-a), 142.0 (C-e), 166.0 (C-h)*, 166.9 (C-g)*. MS (ESI) 241 (M+1).

Ethyl 2-(benzyloxy)-7-chloropyrazolo[1,5-a]pyridine-3-carboxylate (**70**). Benzyl bromide (645 mg, 3.20 mmol, 1.10

eq) was added dropwise to a mixture of **69** (600 mg, 2.91 mmol) and Cs₂CO₃ (2.295 g, 7.04 mmol, 2.4 eq) in dry DMF (15 mL). The reaction mixture was stirred overnight at room temperature, then water (100 mL) was added. The mixture was extracted with EtOAc (4 × 70 mL), the combined organic layer was dried under Na₂SO₄ and evaporated under reduced pressure to get a yellow oil. The mixture was separated using flash chromatography (eluent: *petroleum ether*/EtOAc 6/4 v/v) to afford the title compound as a pale-yellow solid (m.p. 98.2 - 99.3 °C from trituration with diisopropyl ether). Yield 85% ¹H NMR (600 MHz, Chloroform-d₃): δ 1.41 (t, 3H, J = 7.1 Hz, -OCH₂CH₃), 4.38 (q, 2H, J = 7.1 Hz, -OCH₂CH₃), 5.58 (s, 2H, -OCH₂Ph), 6.96 (dd, 1H, J = 7.4, 1.2 Hz, H-b), 7.27 - 7.34 (m, 2H, H-c and aromatic proton), 7.39 (t, 2H, J = 7.5 Hz, aromatic protons), 7.59 (d, 2H, J = 7.4 Hz, aromatic protons), 7.99 (dd, 1H, J = 8.8, 1.2 Hz, H-d). ¹³C NMR (151 MHz, Chloroform-d₃): δ 14.6 (-OCH₂CH₃), 60.0 (-OCH₂CH₃), 71.1 (-OCH₂Ph), 90.1 (C-f), 112.8 (C-b), 116.5 (C-d), 127.8 (aromatic carbon), 127.9 (aromatic carbon), 128.0 (C-c), 128.5 (aromatic carbon), 130.6 (C-a), 136.7 (aromatic carbon), 144.6 (C-e), 163.1 (C-h)*, 164.8 (C-g)*. MS (ESI) 331 (M+1).

2-(Benzyloxy)-7-chloropyrazolo[1,5-a]pyridine-3-carboxylic acid (**71**). 6 M NaOH (5.0 eq) was added to a solution of compound **70** (785 mg, 2.40 mmol) in EtOH abs

(20 mL). The mixture was stirred for 4 h at 75 °C, then neutralized with 6 M HCl and concentrated under reduced pressure. The mixture was cooled 0 °C then acidified with 2 M HCl until pH 2 was reached, obtaining a suspension. This latter was filtered to get the title compound as a white solid (m.p. 178.4 - 179.8 °C dec. with gas developed, from trituration with diisopropyl ether). Yield 84 %. ¹H NMR (600 MHz, DMSO-d₆): δ 5.48 (s, 2H, -OCH₂Ph), 7.31 (dd, 1H, J = 7.5, 1.1 Hz, H-b), 7.35 (t, 1H, J = 7.4 Hz, aromatic proton), 7.41 (t, 2H, J = 7.4 Hz, aromatic protons), 7.52 (dd, 1H, J = 8.7, 7.6 Hz, H-c), 7.55 (d, 2H, J = 7.5 Hz, aromatic protons), 7.95 (dd, 1H, J = 8.9, 1.1 Hz, H-d), 12.34 (br s, 1H, -COOH). ¹³C NMR (151 MHz, DMSO-d₆): δ 70.5 (-OCH₂Ph), 89.4 (C-f), 113.3 (C-b), 116.1 (C-d), 127.9 (C-a), 128.1 (aromatic carbon), 128.4 (aromatic carbon), 128.9 (aromatic carbon), 129.4 (C-c), 136.4 (aromatic carbon), 144.1 (C-e), 163.3 (C-h)*, 164.0 (C-g)*. MS (ESI) 301 (M-1).

1-Benzyl-7-chloro-2-oxo-N-(2,3,5,6-tetrafluoro-[1,1'-biphenyl]-4-yl)-1,2-dihydropyrazolo[1,5-a]pyridine-3-carboxamide (72). Oxalyl chloride (3.0 mmol) and dry DMF (1 drop) were added to a cooled (0°C) solution of the **71** (1.0 mmol) 1-3, in dry THF (20 mL), under a nitrogen atmosphere. The obtained solution was stirred at room temperature for 2 hours. The solution was then concentrated under reduced pressure and the residue

dissolved in dry THF (10 mL, this step was repeated three times). The resulting acyl chloride was immediately used without any further purification and dissolve in 10 mL of dry toluene and transferred to the solution described later. Trimethylaluminium (2.0 M in hexane, 1.5 mmol), was added to a solution of 4-phenyl-2,3,5,6-tetrafluoroaniline (1.5 mmol) in dry toluene (15 mL), under a nitrogen atmosphere. The resulting mixture was stirred for 2 hours at room temperature producing a brown suspension, then the solution of the previously described acyl chloride in dry toluene (30 mL) was quantitatively added. The mixture was heated overnight at 90 °C and then cooled to r.t. The reaction was quenched with 1M HCl, the layers resolved, and the aqueous phase was exhaustively extracted using EtOAc. The combined organic layer was washed with 1M NaOH and brine, dried and the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography (eluent: from *petroleum ether*/EtOAc 8:2 v/v to *petroleum ether*/EtOAc 4:6 v/v) to afford the title compound as a white solid (m.p. 201.1 - 202.4 °C from trituration with diisopropyl ether). Yield 38 %.¹H NMR (600 MHz, Chloroform-d₃): δ 5.74 (s, 2H, -NCH₂Ph), 6.73 (dd, 1H, J = 7.5, 1.2 Hz, H-b), 7.06 (dd, 2H, J = 7.4, 1.7 Hz, aromatic protons), 7.22- 7.30 (m, 3H, aromatic protons), 7.35 (dd, 1H, J = 8.8, 7.6 Hz, H-c), 7.42 - 7.53 (m, 5H, aromatic protons), 8.28 (dd, 1H, J = 8.8, 1.2 Hz, H-d), 10.01 (s, 1H, -NH); ¹³C NMR (151

MHz, Chloroform- d_3): δ 52.6 (-NCH₂Ph), 89.2 (C-f), 114.6 (C-b), 115.9 (t, J = 16.3 Hz, C-p)*, 116.7 (C-d), 118.0 (t, J = 17.2 Hz, C-s)*, 127.2 (aromatic carbon), 127.6 (C-a), 128.6 (aromatic carbon), 128.7 (aromatic carbon), 129.1 (aromatic carbon), 129.2 (aromatic carbon), 130.4 (C-c), 130.6 (aromatic carbon), 133.7 (aromatic carbon), 133.8 (aromatic carbon), 142.8 (dd, J = 248.8, 15.1 Hz, C-q)**, 144.2 (d, J = 248.3 Hz, C-r)**, 150.3 (C-e), 161.2 (C-g)***, 167.5 (C-h)***. MS (ESI) 526 (M-1).

7-Chloro-2-hydroxy-N-(2,3,5,6-tetrafluoro-[1,1'-biphenyl]-4-yl)pyrazolo[1,5-a]pyridine-3-carboxamide

(4). Thioanisole (240 μ L, 1.90 mmol, 10.0 eq) was added to a solution of **72** (100 mg, 0.19 mmol, 1.0 eq) in TFA (2 mL). The mixture was heated at 70 °C for 3h then cooled to r.t.. The mixture was partially concentrated, and the crude was taken up with water obtaining a suspension that was filtered, the solid washed with additional amount of cold water. The resulting solid was triturated with diisopropyl ether to afford the title compounds, in pure form as a white solid (m.p. 259.3 – 260.4 °C dec. from trituration with diisopropyl ether). Yield 64%. ¹H NMR (600 MHz, DMSO- d_6): δ 7.32 (dd, 1H, J = 7.5, 1.1 Hz, H-b), 7.53 (dd, 1H, J = 8.7, 7.5 Hz, H-c), 7.53 – 7.60 (m, 5H, aromatic protons), 8.02 (dd, 1H, J = 8.8, 1.1 Hz, H-d), 8.99 (s, 1H, -NH), 13.33 (br s, 1H, -OH). ¹³C NMR (151 MHz, DMSO- d_6): δ 89.9 (C-f), 113.5 (C-b), 115.6 (C-d),

116.7 (t, J = 17.8 Hz, C-p)*, 117.4 (t, J = 17.7 Hz, C-s)*, 126.6 (C-a), 128.8 (aromatic carbon), 128.9 (aromatic carbon), 128.9 (C-c), 129.4 (aromatic carbon), 130.1 (aromatic carbon), 142.9 (d, J = 246.6 Hz, C-q)**, 143.2 (d, J = 241.3 Hz, C-r)***, 143.5 (C-e), 160.2 (C-g)***, 162.6 (C-h)***. MS (ESI) 436 (M+1). ESI-HRMS (m/z): [M+H]⁺ calcd for C₂₀H₁₁ClF₄N₃O₂, 436.0470; obsd, 436.0472.

General procedure for synthesis of pyrazolo[1,5-a]pyridine related amides (73 - 82). Oxalyl chloride (1.75 mL, 3.5 mmol) and dry DMF (7 µL) were added to a cooled (0° C) solution of **45** (1.0 mmol) in dry THF (15 mL) under a nitrogen atmosphere. The reaction mixture was stirred for 2 hours at room temperature under a nitrogen atmosphere, then concentrated under reduced pressure. The residue was dissolved in dry THF (10 mL) and the solution again concentrated, this step was repeated three times. A solution of the appropriate aniline (**83 - 92**, 1.00 mmol), dry pyridine (3.0 mmol) in dry toluene (5 mL) was added dropwise to the solution of above acyl chloride in dry toluene (15 mL) kept under nitrogen atmosphere. The resulting mixture was stirred at reflux overnight, then cooled to room temperature and only for compounds **75 - 78** quenched with 0.5 M HCl (25 mL). The layers were resolved, the aqueous phase was further extracted with EtOAc (3 x 50 mL), the combined organic layers were washed

with brine, dried over Na₂SO₄ and evaporated under reduced pressure. The crude material was purified using flash chromatography (details in each specific recipe).

2-Benzyloxy-N-(5-phenoxy-2-pyridinyl)pyrazolo[1,5-a]pyridine-3-carboxamide (73). Obtained from **45** using aniline **83**. Flash chromatography eluent: petroleum ether / EtOAc 80 / 20 v/v. White solid (m.p. 120.6 – 120.7 °C; from trituration with diisopropyl ether) Yield 87 %. ¹H NMR (600 MHz, Chloroform-d): δ 5.55 (s, 2H, -OCH₂Ph), 6.86 – 6.92 (m, 2H, aromatic proton and H-b), 7.10 (d, 2H, J = 7.9 Hz, aromatic protons), 7.17 (t, 1H, J = 7.4 Hz, H-c), 7.34 – 7.42 (m, 4H, aromatic protons), 7.45 (t, 2H, J = 7.3 Hz, aromatic protons), 7.53 (d, 2H, J = 7.2 Hz, aromatic protons), 8.01 (d, 1H, J = 2.6 Hz, aromatic proton), 8.25 – 8.30 (m, 2H, aromatic proton and H-d), 8.32 (d, 1H, J = 6.8 Hz, H-a), 8.64 (s, 1H, -NH). ¹³C NMR (151 MHz, Chloroform-d): δ 72.4 (-OCH₂Ph), 90.5 (C-f), 111.8, 113.1 (C-b), 118.8 (C-d), 120.7, 124.4, 127.9 (C-c), 128.3, 128.8 (C-a), 129.1, 129.2, 129.8, 131.4, 131.9, 135.6, 138.6, 143.0 (C-e), 155.0 (C-s)*, 159.6 (C-v)*, 161.4 (C-g)*, 162.3 (C-h)*. IR (KBr) ν (cm⁻¹): 3373, 3100, 3044, 2925, 1947, 1663, 1636, 1534, 1473, 1365, 1296, 1249, 1207, 1120, 1005. MS (ESI) 435 (M-1).

2-Benzyloxy-N-5-[3-(trifluoromethyl)phenoxy]pyridin-2-ylpyrazolo[1,5-a]pyridine-3-carboxamide (74). Obtained from **45** using aniline **84**. Flash chromatography eluent:

petroleum ether / EtOAc 70 / 30 v/v). White solid (m.p. 133.6 - 135.9 °C; from trituration with diisopropyl ether). Yield 50 %. ¹H NMR (600 MHz, *Chloroform-d*): δ 5.65 (*s*, 2H, -OCH₂Ph), 6.89 (*t*, 1H, *J* = 6.7 Hz, *H-b*), 7.14 (*dd*, 1H, *J* = 8.2, 1.9 Hz, *aromatic proton*), 7.23 (*s*, 1H, *aromatic proton*), 7.33 - 7.46 (*m*, 7H, *aromatic protons*), 7.60 (*d*, 2H, *J* = 7.3 Hz, *aromatic protons*), 8.12 (*d*, 1H, *J* = 2.8 Hz, *aromatic proton*), 8.29 (*d*, 1H, *J* = 8.9 Hz, *H-d*), 8.31 (*d*, 1H, *J* = 6.9 Hz, *H-a*), 8.42 (*d*, 1H, *J* = 9.0 Hz, *aromatic proton*), 9.40 (*s*, 1H, -NH), ¹³C-NMR (151 MHz, *Chloroform-d*): δ 72.0 (-OCH₂Ph), 90.7 (*C-f*), 113.1 (*C-b*), 114.6 (*q*, *J* = 3.8 Hz), 114.9, 118.8 (*C-d*), 119.8 (*q*, *J* = 3.8 Hz), 120.8, 123.8 (*q*, *J* = 272.0 Hz, -CF₃), 127.9, 128.0, 128.6, 128.8, 128.9, 129.7, 130.6, 132.5 (*q*, *J* = 32.8 Hz), 135.9, 140.3, 143.2 (*C-e*), 148.3, 148.9 (*C-s*)*, 158.2 (*C-v*)*, 161.2 (*C-g*)*, 162.4 (*C-h*)*. IR (KBr) ν (cm⁻¹): 3373, 3069, 2924, 2853, 1666, 1634, 1538, 1449, 1328, 1287, 1163, 1130, 1012. MS (ESI) 505 (M+1).

2-(Benzyloxy)-N-(2-methyl-4-phenoxyphenyl)pyrazolo[1,5-*a*]pyridine-3-carboxamide (**75**). Obtained from **45** using aniline **85**. Flash chromatography (eluent petroleum ether / EtOAc 85 / 15 v/v). Light yellow solid (m.p. 186.1 - 190.9 °C from diisopropyl ether). Yield 87 %. ¹H NMR (600 MHz, *Chloroform-d*): δ 1.83 (*s*, 3H, Ar-CH₃), 5.54 (*s*, 2H, -OCH₂Ph), 6.76 (*d*, *J* = 2.2 Hz, 1H, *H-t*), 6.85 - 6.91 (*m*,

2H, aromatic protons), 6.96 (d, 2H, $J = 8.1$, aromatic protons), 7.05 (t, 1H, $J = 7.3$ Hz, H-b), 7.30 (t, 2H, $J = 7.8$ Hz, aromatic protons), 7.34 - 7.45 (m, 4H, aromatic protons and H-c), 7.53 (d, 2H, $J = 6.3$ Hz, aromatic protons), 8.20 (d, 1H, $J = 8.8$ Hz, H-d), 8.29 - 8.37 (m, 2H, aromatic protons), 8.44 (s, 1H, -NH). ^{13}C NMR (151 MHz, Chloroform-d): δ 17.6 (Ar-CH₃), 72.6 (-OCH₂Ph), 91.1 (C-f), 112.9 (C-b), 117.5 (C-d), 118.3, 119.0, 121.1, 122.8, 123.0, 127.6, 128.6, 129.0, 129.2, 129.4, 129.5, 129.7, 132.7, 135.4, 143.1 (C-e), 152.6 (C-g)*, 158.1 (C-s)*, 161.2 (C-v)*, 162.3 (C-h)*; IR (KBr) ν (cm⁻¹): 3391, 3308, 3040, 2922, 2737, 1963, 1882, 1660, 1590, 1588, 1362, 1334, 1219, 1151, 1130, 1101; MS (ESI) 450 (M+1).

2-(Benzyloxy)-N-(2-isopropyl-5-methyl-4-phenoxyphenyl)pyrazolo[1,5-a]pyridine-3-carboxamide (76). Obtained from 45 using aniline 86. Flash chromatography eluent: petroleum ether / EtOAc 70 / 30 v/v). White solid (m.p. 166.2 - 167.7 °C; from trituration with diisopropyl ether). Yield 54 %. ^1H NMR (600 MHz, Chloroform-d): δ 0.90 (d, 6H, $J = 6.8$ Hz, CH(CH₃)₂), 2.16 (s, 3H, Ar-CH₃), 2.72 (hept, 1H, $J = 6.7$ Hz, -CH(CH₃)₂), 5.55 (s, 2H, -OCH₂Ph), 6.81 (s, 1H, H-t), 6.84 (d, 2H, $J = 8.1$ Hz, aromatic protons), 6.87 (t, 1H, $J = 6.9$ Hz, H-b), 6.98 (t, 1H, $J = 7.3$ Hz, aromatic proton), 7.22 - 7.28 (m, 2H, aromatic protons), 7.34 - 7.45 (m, 4H, aromatic protons), 7.52 (d, 2H, $J = 6.9$ Hz, aromatic

protons), 8.02 (s, 1H, H-q), 8.31 (d, 1H, J = 6.8 Hz, H-a), 8.35 (d, 1H, J = 8.8 Hz, H-d), 8.48 (s, 1H, -NH). ¹³C NMR (151 MHz, Chloroform-d): δ 16.1 (Ar-CH₃), 22.7 CH(CH₃)₂, 27.8 (-CH(CH₃)₂), 72.5 (-OCH₂Ph), 91.1 (C-f), 112.9 (C-b), 116.3, 117.9, 119.1 (C-d), 121.8, 126.4, 127.6 (C-c), 128.3, 128.6, 129.0 (C-a), 129.1, 129.2, 129.7, 131.5, 135.5, 138.4, 143.2 (C-e), 150.4 (C-s)*, 158.7 (C-v)*, 161.5 (C-h)**, 162.4 (C-g)**; IR (KBr) ν (cm⁻¹): 3398, 3040, 2963, 1652, 1636, 1528, 1490, 1445, 1402, 1368, 1289, 1220, 1181, 1147, 1127, 1044, 993; MS (ESI) 492 (M+1).

2-(Benzyloxy)-N-(5-isopropyl-2-methyl-4-phenoxyphenyl)pyrazolo[1,5-a]pyridine-3-carboxamide (**77**). Obtained from **45** using aniline **87**. Flash chromatography eluent: petroleum ether / EtOAc 60 / 40 v/v). White solid (m.p. 157.5 - 158.9 °C; from trituration with diisopropyl ether). Yield 92 %. ¹H NMR (600 MHz, Chloroform-d): δ 1.24 (d, 6H, J = 6.8 Hz, -CH(CH₃)₂), 1.77 (s, 3H, Ar-CH₃), 3.20 (hept, 1H, J = 6.8 Hz, -CH(CH₃)₂), 5.54 (s, 2H, -OCH₂Ph), 6.64 (s, 1H, H-t), 6.85 - 6.91 (m, 3H, aromatic protons and H-b), 7.01 (t, 1H, J = 7.3 Hz, H-c), 7.20 - 7.45 (m, 6H, aromatic protons), 7.52 (d, 2H, J = 6.4 Hz, aromatic protons), 8.29 (s, 1H, H-q), 8.32 (d, 1H, J = 6.8 Hz, H-a), 8.37 (d, 1H, J = 8.8 Hz, H-d), 8.47 (s, 1H, -NH). ¹³C NMR (151 MHz Chloroform-d): δ 17.1 (-CH(CH₃)₂), 23.2 (-CH(CH₃)₂), 27.4 (Ar-CH₃),

72.6 (–OCH₂Ph), 91.2 (C-*f*), 112.8 (C-*b*), 117.0, 119.1 (C-*d*), 120.2, 122.0, 122.1, 126.4, 127.5, 128.6, 128.9, 129.2, 129.4, 129.6, 133.6, 135.4, 138.8, 143.0 (C-*e*), 148.8 (C-*s*)*, 159.0 (C-*v*)*, 161.2 (C-*g*)**, 162.3 (C-*h*)**;
IR (KBr) ν (cm⁻¹): 3392, 3045, 2970, 1652, 1636, 1597, 1533, 1486, 1456, 1407, 1360, 1290, 1223, 1146, 1129, 1007, 911; MS (ESI) 492 (M+1).

2-(Benzyloxy)-N-(2-isopropyl-5-methyl-4-(4-(trifluoromethyl)phenoxy)phenyl)pyrazolo[1,5-*a*]pyridine-3-carboxamide (**78**). Obtained from **45**, using aniline **88**. Flash chromatography (eluent petroleum ether / EtOAc 85 / 15 v/v) to obtain a white solid (186.2 – 187.3 °C from diisopropyl ether). Yield 95 %. ¹H NMR (600 MHz Chloroform-*d*): δ 0.91 (*d*, 6H, *J* = 6.8 Hz, –CH(CH₃)₂), 2.14 (*s*, 3H, Ar-CH₃), 2.73 (*hept*, 1H, *J* = 6.8 Hz, –CH(CH₃)₂), 5.56 (*s*, 2H, –OCH₂Ph), 6.82 (*s*, 1H, *H-t*), 6.87 – 6.91 (*m*, 3H, aromatic protons and *H-b*), 7.36 – 7.46 (*m*, 4H, aromatic protons), 7.49 – 7.56 (*m*, 4H, aromatic protons), 8.10 (*s*, 1H, *H-q*), 8.33 (*d*, 1H, *J* = 6.9 Hz, *H-a*), 8.35 (*d*, 1H, *J* = 8.8 Hz, *H-d*), 8.53 (*s*, 1H, –NH). ¹³C NMR (151 MHz, Chloroform-*d*): δ 16.0 (Ar-CH₃), 22.7 (–CH(CH₃)₂), 27.8 (–CH(CH₃)₂), 72.6 (–OCH₂Ph), 91.0 (C-*f*), 112.9 (C-*b*), 115.9, 118.2 (C-*d*), 119.0, 123.8 (*q*, *J* = 33.2 Hz), 124.5 (*q*, *J* = 270.9, –CF₃), 126.4, 127.2 (*q*, *J* = 3.7 Hz), 127.7, 128.4, 128.7, 129.0, 129.2, 129.3, 132.3, 135.5, 138.5, 143.2 (C-*e*), 149.2 (C-*s*), 161.4 (C-

ν)*, 161.5 (C-g)*, 162.4 (C-h)*; IR (KBr) ν (cm⁻¹): 3403, 3084, 3043, 2976, 2891, 1655, 1638, 1613, 1578, 1543, 1511, 1501, 1477, 1446, 1401, 1360, 1330, 12090, 1240, 1215, 1148, 1120, 1064, 1042, 994; MS (ESI) 560 (M+1).

2-(Benzyloxy)-N-(1H-indol-5-yl)pyrazolo[1,5-a]pyridine-3-carboxamide (**79**). Obtained from **45** using aniline **89**. Flash chromatography eluent: petroleum ether / EtOAc 60 / 40 v/v). White solid (m.p. 133.6 – 135.9 °C; from trituration with diisopropyl ether). Yield 74 %. ¹H NMR (600 MHz, DMSO-d₆): δ 5.60 (s, 2H, -OCH₂Ph), 6.38 (s, 1H, aromatic proton), 7.03 (t, 1H, J = 6.9 Hz, H-b), 7.11 (dd, 1H, J = 8.6, 1.6 Hz, aromatic proton), 7.30 – 7.34 (m, 2H, aromatic protons), 7.41 (t, 1H, J = 7.4 Hz, H-c), 7.47 (t, 2H, J = 7.5 Hz, aromatic protons), 7.50 – 7.54 (m, 2H, aromatic protons), 7.65 (d, 2H, J = 7.4 Hz, aromatic protons), 7.88 (s, 1H, aromatic proton), 8.13 (d, 1H, J = 8.8 Hz, H-d), 8.69 (d, 1H, J = 6.8 Hz, H-a), 8.82 (s, 1H, -NH), 11.03 (s, 1H, -NH indole). ¹³C-NMR (151 MHz, DMSO-d₆): δ 71.5 (-OCH₂Ph), 90.4 (C-f), 101.1, 110.6, 111.4, 113.1 (C-b), 114.8, 117.5 (C-d), 126.1, 127.6, 128.1, 128.2, 128.5, 128.7, 129.4, 130.6, 132.8, 136.3, 141.9 (C-e), 160.0 (C-g)*, 161.6 (C-h)*. MS (ESI) 505 (M+1).

2-(Benzyloxy)-N-(1-phenyl-1H-indol-5-yl)pyrazolo[1,5-a]pyridine-3-carboxamide (**80**). Obtained from **45** using aniline **90**. Flash chromatography eluent: petroleum ether

/ EtOAc 60 / 40 v/v). White solid (m.p. 133.6 – 135.9 °C; from trituration with diisopropyl ether) Yield 74 %. ¹H NMR (600 MHz, DMSO-*d*₆): δ 5.60 (*s*, 2H, -OCH₂Ph), 6.38 (*s*, 1H, aromatic proton), 7.03 (*t*, 1H, *J* = 6.9 Hz, *H*-*b*), 7.11 (*dd*, 1H, *J* = 8.6, 1.6 Hz, aromatic proton), 7.30 – 7.34 (*m*, 2H, aromatic protons), 7.41 (*t*, 1H, *J* = 7.4 Hz, *H*-*c*), 7.47 (*t*, 2H, *J* = 7.5 Hz, aromatic protons), 7.50 – 7.54 (*m*, 2H, aromatic protons), 7.65 (*d*, 2H, *J* = 7.4 Hz, aromatic protons), 7.88 (*s*, 1H, aromatic proton), 8.13 (*d*, 1H, *J* = 8.8 Hz, *H*-*d*), 8.69 (*d*, 1H, *J* = 6.8 Hz, *H*-*a*), 8.82 (*s*, 1H, -NH). ¹³C-NMR (151 MHz, DMSO-*d*₆): δ 71.5 (-OCH₂Ph), 90.4 (*C*-*f*), 101.1, 110.6, 111.4, 113.1 (*C*-*b*), 114.8, 117.5 (*C*-*d*), 126.1, 127.6, 128.1, 128.2, 128.5, 128.7, 129.4, 130.6, 132.8, 136.3, 141.9 (*C*-*e*), 160.0 (*C*-*g*)*, 161.6 (*C*-*h*)*. MS (ESI) 505 (M+1).

2-(Benzyloxy)-N-(1-phenyl-1H-benzo[d]imidazol-5-yl)pyrazolo[1,5-*a*]pyridine-3-carboxamide (**81**). Obtained from **45** using aniline **91**. Flash chromatography eluent: DCM / MeOH 95 / 5 v/v). Pale pink solid (m.p. 133.6 – 135.9 °C; from trituration with diisopropyl ether) Yield 67 %. ¹H NMR (600 MHz, Chloroform-*d*): δ 5.29 (*s*, 1H, aromatic proton), 5.59 (*s*, 2H, -OCH₂Ph), 6.87 (*t*, 1H, *J* = 6.8 Hz, *H*-*b*), 7.35 – 7.60 (*m*, 11H, aromatic protons), 7.63 (*d*, 1H, *J* = 8.7 Hz, aromatic proton), 7.94 (*s*, 1H, aromatic proton), 8.10 (*s*, 1H, aromatic proton), 8.31 (*d*, 1H, *J* = 6.8 Hz, *H*-*a*), 8.35 (*d*, 1H, *J* = 8.8 Hz, *H*-*d*), 8.84

(s, 1H, -NH). ¹³C-NMR (151 MHz, Chloroform-d): δ 72.3 (-OCH₂Ph), 91.1 (C-f), 110.5, 111.4, 112.9 (C-b), 117.6, 119.0 (C-d), 124.0, 127.6, 128.1, 128.3, 128.7, 129.0, 129.1, 130.2, 130.4, 134.5, 135.9, 136.5, 142.9, 143.0 (C-e), 144.5, 161.4 (C-g)*, 162.3 (C-h)*. MS (ESI) 505 (M+1).

2-(Benzyloxy)-N-(1-phenyl-1H-benzo[d][1,2,3]triazol-5-yl)pyrazolo[1,5-a]pyridine-3-carboxamide (**82**). Obtained from **45** using aniline **92**. Flash chromatography eluent: DCM / MeOH 95 / 5 v/v). Pale pink solid (m.p. 133.6 - 135.9 °C; from trituration with diisopropyl ether) Yield 90 %. ¹H NMR (600 MHz, Chloroform-d): δ 5.61 (s, 2H, -OCH₂Ph), 7.06 (t, 1H, J = 6.8 Hz, H-b), 7.40 (t, 1H, J = 7.3 Hz, H-c), 7.47 (t, 2H, J = 7.5 Hz, aromatic protons), 7.53 - 7.72 (m, 7H, aromatic protons), 7.85 - 7.92 (m, 3H, aromatic protons), 8.12 (d, 1H, J = 8.8 Hz, H-d), 8.59 (s, 1H, aromatic proton), 8.70 (d, 1H, J = 6.8 Hz, H-a), 9.23 (s, 1H, -NH). ¹³C-NMR (151 MHz, Chloroform-d): δ 71.5 (-OCH₂Ph), 90.0 (C-f), 107.6, 111.3 (C-b), 113.5, 117.4 (C-d), 122.5, 128.1, 128.4, 128.5, 128.6, 128.7, 128.8, 129.6, 130.1, 135.8, 136.3, 136.4, 142.0 (C-e), 146.3, 160.6 (C-g)*, 161.9 (C-h)*. MS (ESI) 505 (M+1).

General hydrogenation procedure for target compounds **19** - **24**, **28** - **30**, and **37**. 10 % Palladium on carbon (Pd/C, 45 mg) was added to a solution of the appropriate amide (compounds **73** - **82**, 0.300 mmol) in dry THF (15 mL). The

resulting mixture was vigorously stirred under a hydrogen atmosphere for 3 hours. The suspension was filtered through Celite and the cake was washed with methanol. The filtrate was concentrated under reduced pressure. When necessary, the obtained solid was further purified by flash chromatography.

2-Hydroxy-N-(1H-indol-5-yl)pyrazolo[1,5-a]pyridine-3-carboxamide (19). Obtained from **79**. Flash chromatography eluent: dichloromethane / methanol 95 / 5 v/v). White solid (m.p. 133.6 - 135.9 °C; from trituration with diisopropyl ether). Yield 74 %. ¹H NMR (600 MHz, DMSO-*d*₆): δ 6.39 (s, 1H, aromatic proton), 6.95 (t, 1H, J = 6.7 Hz, H-b), 7.24 (dd, 1H, J = 8.6, 1.1 Hz, aromatic proton), 7.27 - 7.38 (m, 2H, aromatic protons), 7.44 (t, 1H, J = 7.8 Hz, H-c), 7.97 (d, 1H, J = 1.9 Hz, aromatic proton), 8.07 (d, 1H, J = 8.8 Hz, H-d), 8.55 (d, 1H, J = 6.8 Hz, H-a), 9.03 (s, 1H, -NH), 11.02 (s, 1H, -NH indole), 18.84 (v br s, 1H, -OH) . ¹³C-NMR (151 MHz, DMSO-*d*₆): δ 89.6 (C-f), 101.1, 110.6, 111.4, 112.5 (C-b), 115.0, 117.1 (C-d), 126.0, 127.3 (C-c), 127.7, 128.3, 130.8 (C-a), 132.7, 141.4 (C-e), 160.8 (C-g)*, 162.1 (C-h)*. MS (ESI) 293 (M+1) .

2-Hydroxy-N-(1-phenyl-1H-indol-5-yl)pyrazolo[1,5-a]pyridine-3-carboxamide (20). Obtained from **80**. Flash chromatography eluent: DCM / methanol 95 / 5 v/v). White solid (m.p. 133.6 - 135.9 °C; from trituration with

diisopropyl ether). Yield 90 %. ^1H NMR (600 MHz, $\text{DMSO}-d_6$): δ 6.68 (d, 1H, $J = 3.0$ Hz, aromatic proton), 6.96 (t, 1H, $J = 6.7$ Hz, H-b), 7.32 – 7.42 (m, 2H, aromatic protons), 7.45 (t, 1H, $J = 7.8$ Hz, H-c), 7.49 – 7.62 (m, 5H, aromatic protons), 7.65 (d, 1H, $J = 3.1$ Hz, aromatic proton), 8.08 (d, 1H, $J = 8.8$ Hz, H-d), 8.13 (s, 1H, aromatic proton), 8.56 (d, 1H, $J = 6.7$ Hz, H-a), 9.15 (s, 1H, -NH), 12.95 (s, 1H, -OH). ^{13}C -NMR (151 MHz, $\text{DMSO}-d_6$): δ 89.6 (C-f), 103.6, 110.5, 111.2, 112.6 (C-b), 115.9, 117.0 (C-d), 123.5, 126.3, 127.4 (C-c), 128.9, 129.0, 129.3 (C-a), 129.9, 131.7, 132.2, 139.2, 141.4 (C-e), 160.9, 162.2. MS (ESI) 369 (M+1).

2-Hydroxy-N-(1-phenyl-1H-benzo[d]imidazol-5-yl)pyrazolo[1,5-a]pyridine-3-carboxamide (**21**). Obtained from **81**. Flash chromatography eluent: DCM / MeOH 80 / 20 v/v). Pale pink solid (m.p. 133.6 – 135.9 °C; from trituration with diisopropyl ether). Yield 90 %. ^1H NMR (600 MHz, $\text{DMSO}-d_6$): δ 6.87 (t, 1H, $J = 6.5$ Hz, H-b), 7.37 (t, 1H, $J = 7.7$ Hz, H-c), 7.46 – 7.54 (m, 2H, aromatic protons), 7.57 (d, 1H, $J = 8.6$ Hz, aromatic proton), 7.63 (t, 2H, $J = 7.8$ Hz, aromatic protons), 7.69 (d, 2H, $J = 7.7$ Hz, aromatic protons), 8.00 (d, 1H, $J = 8.6$ Hz, H-d), 8.32 (s, 1H, aromatic proton), 8.48 (d, 1H, $J = 6.4$ Hz, H-a), 8.54 (s, 1H, aromatic proton), 9.99 (s, 1H, -NH). ^{13}C -NMR (151 MHz, $\text{DMSO}-d_6$): δ 89.6 (C-f), 109.8, 110.6, 111.8, 116.4 (C-b), 116.5, 123.4, 126.7, 127.6 (C-

c), 128.4, 128.9, 130.1 134.8, 136.1, 141.4 (C-e), 141.5, 143.6, 144.2, 161.9. MS (ESI) 368 (M-1).

2-Hydroxy-N-(1-phenyl-1H-benzo[d][1,2,3]triazol-5-yl)pyrazolo[1,5-a]pyridine-3-carboxamide (22). Obtained from **82**. Flash chromatography eluent: DCM / MeOH 95 / 5 v/v). Grey solid (m.p. 133.6 – 135.9 °C; from trituration with diisopropyl ether). Yield 72 %. ¹H NMR (600 MHz, DMSO-*d*₆): δ 6.85 (t, 1H, J = 6.6 Hz, H-b), 7.35 (t, 1H, J = 7.7 Hz, H-c), 7.58 (t, 1H, J = 7.5 Hz, aromatic proton), 7.70 (t, 2H, J = 7.9 Hz, aromatic protons), 7.78 (d, 1H, J = 8.1 Hz, aromatic proton), 7.84 – 7.91 (m, 3H, aromatic protons), 7.95 (d, 1H, J = 8.6 Hz, H-d), 8.44 (d, 1H, J = 6.6 Hz, H-a), 8.71 (s, 1H, aromatic proton), 10.53 (s, 1H, -NH). ¹³C-NMR (151 MHz, DMSO-*d*₆): δ 89.5 (C-f), 106.8, 111.1 (C-b), 111.7, 116.1 (C-d), 122.5, 122.7, 126.6, 128.0, 128.3, 128.7, 130.1, 136.5, 136.8, 137.6, 141.5 (C-e), 146.5, 162.5. MS (ESI) 369 (M-1).

2-Hydroxy-N-(5-phenoxy-2-pyridin-2-yl)pyrazolo[1,5-a]pyridine-3-carboxamide (23). Obtained from **73**, flash chromatography (eluent: DCM / methanol 90 / 10 v/v). White solid (m.p. 161.1 – 161.9 °C dec.; from trituration with diisopropyl ether). Yield 76 %. ¹H NMR (600 MHz, DMSO-*d*₆): δ 6.75 (t, 1H, J = 6.7 Hz, H-b), 6.97 (d, 1H, J = 8.7 Hz, aromatic proton), 7.08 (d, 2H, J = 8.0 Hz, aromatic protons), 7.16 (t, 1H, J = 7.4 Hz, aromatic proton), 7.26 (t, 1H, J = 7.8 Hz, aromatic proton) 7.39

(*t*, 2H, *J* = 7.8 Hz, aromatic protons), 7.83 (*d*, 1H, *J* = 8.7 Hz, *H-d*), 8.21 (*dd*, 1H, *J* = 8.8, 2.5 Hz, aromatic proton), 8.33 (*d*, 1H, *J* = 6.6 Hz, *H-a*), 8.48 (*d*, 1H, *J* = 2.2 Hz, aromatic proton), 10.78 (*s*, 1H, -NH). ¹³C NMR (151 MHz, DMSO-*d*₆): δ 89.3 (*C-f*), 111.1 (*C-b*), 111.7, 115.6 (*C-d*), 120.2, 123.9, 125.9 (*C-c*), 127.9, 129.7, 131.1 (*C-a*), 132.9, 137.8, 141.4 (*C-e*), 154.9, 157.6 (*C-g*), 162.8 (*C-h*). MS (ESI) 345 (*M*-1). IR (KBr) ν (cm⁻¹): 3061, 1653, 1636, 1534, 1476, 1379, 1248, 1205, 1124, 1023.

*2-Hydroxy-N-5-[3-(trifluoromethyl)phenoxy]pyridin-2-ylpyrazolo[1,5-*a*]pyridine-3-carboxamide (24)*. Obtained from **74**, flash chromatography (eluent: DCM / methanol 90 / 10 v/v). White solid (m.p. 241.2 - 242.0 °C dec.; from trituration with diisopropyl ether). Yield 45 %. ¹H NMR (600 MHz, DMSO-*d*₆): δ 6.59 (*t*, 1H, *J* = 6.7 Hz, *H-b*), 7.10 (*t*, 1H, *J* = 7.7 Hz, *H-c*), 7.26 - 7.34 (*m*, 2H, aromatic protons), 7.46 (*d*, 1H, *J* = 7.6 Hz, aromatic proton), 7.55 (*dd*, 1H, *J* = 9.0, 2.8 Hz, aromatic proton), 7.61 (*t*, 1H, *J* = 8.0 Hz, aromatic proton), 7.65 (*d*, 1H, *J* = 8.4 Hz, aromatic proton), 8.12 (*d*, 1H, *J* = 2.8 Hz, aromatic proton), 8.14 (*d*, 1H, *J* = 6.5 Hz, *H-a*), 8.40 (*d*, 1H, *J* = 9.0 Hz, *H-d*), 12.48 (*s*, 1H, -NH); ¹³C NMR (151 MHz, DMSO-*d*₆): δ 89.0 (*C-f*), 109.8, 113.6 (*q*, *J* = 4.0 Hz), 114.3, 119.4 (*q*, *J* = 3.4 Hz), 120.9, 123.8 (*q*, *J* = 272.0 Hz, -CF₃), 124.4, 127.0, 129.9, 130.7 (*q*, *J* = 32.1 Hz), 131.5, 140.1, 140.8, 141.7, 146.0, 150.7, 158.4, 163.9, 173.1.

MS (ESI) 413 (M-1). IR (KBr) ν (cm⁻¹): 3328, 2925, 1653, 1636, 1559, 1448, 1328, 1284, 1241, 1173, 1129, 1065.

2-Hydroxy-N-(2-methyl-4-(p-tolyloxy)phenyl)pyrazolo[1,5-a]pyridine-3-carboxamide (**28**). Obtained from **75**, flash chromatography (eluent: DCM / methanol 90 / 10 v / v). White solid (m.p. 238.3 - 239.9 °C from trituration with diisopropyl ether). Yield 87 %. ¹H NMR (600 MHz DMSO-d₆): δ 2.28 (s, 3H, Ar-CH₃), 6.88 (dd, 1H, J = 8.8, 2.6 Hz, H-r), 6.94 - 7.01 (m, 4H, aromatic protons), 7.09 (t, 1H, J = 7.3 Hz, H-b), 7.37 (t, 2H, J = 7.9 Hz, aromatic protons), 7.47 (t, 1H, J = 7.9, H-c), 8.06 (d, 1H, J = 8.8 Hz, H-q), 8.20 (d, 1H, J = 8.8 Hz, H-d), 8.57 (d, 1H, J = 6.8 Hz, H-a), 8.93 (s, 1H, -NH), 12.99 (v br s, 1H, -OH). ¹³C NMR (151 MHz, DMSO-d₆): δ 17.6 (Ar-CH₃), 89.5 (C-f), 112.9 (C-b), 117.0, 117.1 (C-d), 117.9, 121.0, 122.2, 122.9, 127.8 (C-c), 129.0 (C-a), 129.2, 130.0, 133.1, 141.5 (C-e), 151.6 (C-s), 157.5 (C-v), 160.8 (C-g)*, 162.1 (C-h)*; IR (KBr) ν (cm⁻¹): 3388, 3039, 2567, 1664, 1633, 1590, 1549, 1485, 1445, 1413, 1380, 1333, 1307, 1273, 1245, 1227, 1173, 1134; MS (ESI) 360 (M+1).

2-Hydroxy-N-(2-isopropyl-5-methyl-4-phenoxyphenyl)pyrazolo[1,5-a]pyridine-3-carboxamide (**29**). Obtained from **76**, flash chromatography (eluent: DCM / methanol 98 / 2 v/v). White solid (m.p. 244.2 - 247.9 °C dec.; from trituration with diisopropyl ether). Yield

91 %. ^1H NMR (600 MHz, $\text{DMSO}-d_6$): δ 1.17 (d, 6H, $J = 6.8$ Hz, $-\text{CH}(\text{CH}_3)_2$), 2.10 (s, 3H, $\text{Ar}-\text{CH}_3$), 3.10 (hept, 1H, $J = 6.8$ Hz, $-\text{CH}(\text{CH}_3)_2$), 6.85 (d, 2H, $J = 8.1$ Hz, aromatic protons), 6.89 (s, 1H, $H-t$), 6.98 (t, 1H, $J = 6.9$ Hz, $H-b$), 7.03 (t, 1H, $J = 7.3$ Hz, aromatic proton), 7.33 (t, 2H, $J = 7.9$ Hz, aromatic protons), 7.47 (t, 1H, $J = 7.9$ Hz, $H-c$), 8.00 (s, 1H, $H-q$), 8.06 (d, 1H, $J = 8.8$ Hz, $H-d$), 8.57 (d, 1H, $J = 6.8$ Hz, $H-a$), 8.98 (s, 1H, $-\text{NH}$), 12.95 (v br s, 1H, $-\text{OH}$). ^{13}C NMR (151 MHz, $\text{DMSO}-d_6$): δ 15.7 ($\text{Ar}-\text{CH}_3$), 22.6 ($-\text{CH}(\text{CH}_3)_2$), 27.4 ($-\text{CH}(\text{CH}_3)_2$), 89.4 ($C-f$), 112.9 ($C-b$), 116.0, 117.1 ($C-d$), 117.5, 122.0, 125.7, 127.0, 127.8 ($C-c$), 129.0 ($C-a$), 129.9, 131.9, 138.0, 141.5 ($C-e$), 149.5, 158.0 ($C-v$), 161.0 ($C-h$)*, 162.1 ($C-g$)*. IR (KBr) ν (cm^{-1}): 3400, 2964, 2579, 1661, 1637, 1547, 1492, 1446, 1404, 1332, 1228, 1185, 1130, 887; MS (ESI) 402 ($M+1$).

2-Hydroxy-N-(5-isopropyl-2-methyl-4-

phenoxyphenyl)pyrazolo[1,5-a]pyridine-3-carboxamide

(**30**). Obtained from **77**, flash chromatography (eluent: DCM / methanol 95 / 5 v/v). White solid (m.p. 273.9 – 276.5 °C dec.; from trituration with diisopropyl ether). Yield 70%. ^1H NMR (600 MHz, $\text{DMSO}-d_6$) δ 1.16 (d, 6H, $J = 6.8$ Hz, $-\text{CH}(\text{CH}_3)_2$), 2.23 (s, 3H, $\text{Ar}-\text{CH}_3$), 3.09 (hept, 1H, $J = 6.8$ Hz, $-\text{CH}(\text{CH}_3)_2$), 6.82 (s, 1H, $H-t$), 6.87 (d, 2H, $J = 8.0$ Hz, aromatic protons), 7.98 (t, 1H, $J = 6.6$ Hz, $H-b$), 7.03 (t, 1H, $J = 7.2$ Hz, aromatic proton), 7.33 (t, 2H,

$J = 7.7$ Hz, aromatic protons), 7.47 (t, 1H, $J = 7.8$ Hz, H-c), 8.10 (d, 1H, $J = 8.7$ Hz, H-d), 8.31 (s, 1H, H-q), 8.58 (d, 1H, $J = 6.6$ Hz, H-a), 8.98 (s, 1H, -NH), 13.01 (v br s, 1H, -OH). ^{13}C NMR (151 MHz, DMSO- d_6): δ 17.0 (-CH(CH $_3$) $_2$), 23.0 (-CH(CH $_3$) $_2$), 26.6 (Ar-CH $_3$), 89.5 (C-f), 112.9 8 (C-b), 116.4, 117.1 (C-d), 119.1, 122.1, 122.2, 126.2, 127.7 (C-c), 129.0 (C-a), 129.9, 134.2, 137.6, 141.5 (C-e), 147.5, 158.5, 160.8 (C-g)*, 162.1 (C-h)*. IR (KBr) ν (cm $^{-1}$): 3393, 2961, 2578, 1659, 1636, 1548, 1486, 1446, 1407, 1333, 1217, 1160, 1126, 1042, 978; MS (ESI) 402 (M+1).

2-Hydroxy-N-(2-isopropyl-5-methyl-4-(4-(trifluoromethyl)phenoxy)phenyl)pyrazolo[1,5-a]pyridine-3-carboxamide (**37**). Obtained from **78**, flash chromatography (eluent: DCM / methanol 95 / 5 v/v). White solid (m.p. 249.2 - 249.9 °C from trituration with diisopropyl ether). Yield 98 %. ^1H NMR (600 MHz, DMSO- d_6): δ 1.20 (d, 6H, $J = 6.7$ Hz, -CH(CH $_3$) $_2$), 2.08 (s, 3H, Ar-CH $_3$), 3.08 - 3.16 (m, 1H, -CH(CH $_3$) $_2$), 6.95 - 7.03 (m, 4H, aromatic protons, H-t and H-b), 7.48 (t, 1H $J = 7.8$ Hz, H-c), 7.70 (d, 2H $J = 8.6$ Hz, aromatic protons), 8.07 (d, 1H, $J = 8.8$ Hz, H-d), 8.10 (s, 1H, H-q), 8.58 (d, 1H, $J = 6.8$ Hz, H-a), 9.05 (s, 1H, -NH), 13.01 (v br s, 1H, -OH). ^{13}C NMR (151 MHz, DMSO- d_6): δ 15.6 (Ar-CH $_3$), 22.6 (CH(CH $_3$) $_2$), 27.4 (CH(CH $_3$) $_2$), 89.4 (C-f), 112.9 (C-b), 115.9, 117.0 (C-d), 118.2, 122.4 (q, $J = 33.1$ Hz), 124.4

(*q*, *J* = 271.1 Hz, $-CF_3$), 125.5, 127.1, 127.5 (*q*, *J* = 3.7 Hz), 127.8 (C-c), 129.0 (C-a), 132.8, 138.1, 141.6 (C-e), 148.1 (C-s), 161.0 (C-v), 161.1 (C-g), 162.1 (C-h); IR (KBr) ν (cm⁻¹): 3402, 2948, 2576, 1665, 1640, 1615, 1550, 1515, 1482, 1446, 1404, 1334, 1250, 1214, 1183, 1156, 114, 1103; MS (ESI) 470 (M+1).

N-(2,5-dimethyl-4-(pyridin-4-ylthio)phenyl)-2-((4-methoxybenzyl)oxy)pyrazolo[1,5-*a*]pyridine-3-carboxamide (**93**). Oxalyl chloride (201 μ L, 2.35 mmol, 3.6 eq.) and dry DMF (7 μ L) were added to a cooled (0° C) solution of **57** (0.783 mmol, 1.2 eq.) in dry THF (15 mL), under a nitrogen atmosphere. The reaction mixture was stirred for 2 hours at room temperature under a nitrogen atmosphere. The solution was concentrated under reduced pressure and the residue was dissolved in dry THF (10 mL), this step was repeated three times. A solution of aniline **100** (0.652 mmol, 1 eq.) and dry pyridine (2.347 mmol, 3.6 eq.) in dry toluene (5 mL) was added to the solution of acyl chloride under nitrogen atmosphere. Due to the partial insolubility of **100** in dry toluene, 5 mL of dry THF were added. The resulting mixture was stirred at room temperature for 12 h then at 70°C overnight. The mixture was then quenched with 0.5M HCl (25 mL). The layers were resolved, the aqueous phase was further extracted with EtOAc (3 x 50 mL), the combined organic layer was dried and evaporated under reduced pressure. The crude material

was purified using flash chromatography (eluent: from petroleum ether / EtOAc 70:30 v/v to DCM / methanol 80:20 v/v) to afford the title compound as a brown solid (melting point: missing). Yield 80 %. ¹H-NMR (600 MHz, Chloroform-d): δ 1.77 (s, 3H, Ar-CH₃), 2.34 (s, 3H, Ar-CH₃), 3.84 (s, 3H, -OCH₃), 5.48 (s, 2H, -OCH₂Ph), 6.81 (d, 2H, J = 5.7 Hz, aromatic proton), 6.90 (t, 1H, J = 6.9 Hz, H-b), 6.94 (d, 2H, J = 8.4 Hz, H-n), 7.24 (s, 1H, aromatic protons), 7.40 (t, 1H, J = 7.9 Hz, H-c), 7.47 (d, 2H, J = 8.4 Hz, aromatic protons), 8.28 (d, 2H, J = 5.5 Hz, aromatic protons); 8.31 – 8.36 (m, 2H, aromatic protons), 8.51 (s, 1H, aromatic proton), 8.62 (s, 1H, -NH); ¹³C-NMR (151 MHz Chloroform-d): δ 16.8 (Ar-CH₃), 20.7 (Ar-CH₃), 55.5 (-OCH₂Ph), 72.6 (-OCH₂Ph), 91.1 (C-f), 113.1 (C-b), 114.3, 118.9 (C-d), 120.1, 121.0, 122.5, 125.3, 127.4, 127.9 (C-a), 128.8 (C-c), 131.4, 138.5, 139.4, 141.9, 143.1 (C-e), 149.4, 150.9, 160.5, 161.4, 162.4; MS (ESI) 511 (M+1).

*General procedure for synthesis of pyrazolo[1,5-*a*]pyridine related amides (94 – 99).* Oxalyl chloride (1.75 mL, 1.5 mmol) and dry DMF (10 μ L) were added to a cooled (0° C) solution of **57** (1.2 mmol) in dry THF (15 mL), kept under a nitrogen atmosphere. The reaction mixture was stirred for 2 hours at room temperature under a nitrogen atmosphere. The solution was concentrated under reduced pressure and the residue was dissolved in

dry THF (10 mL), this step was repeated three times. To a solution of the appropriate aniline (**101** - **106**, 1.00 mmol) and dry pyridine (3.6 mmol) in dry toluene (5 mL), was added to the solution of the above acyl chloride in dry toluene (10 mL), kept under nitrogen atmosphere. The resulting mixture was stirred at reflux overnight, then quenched with 0.5M HCl (25 mL). The layers were resolved, the aqueous phase was further extracted with ethyl acetate (3 x 50 mL), and the combined organic layer was dried and evaporated under reduced pressure. The crude material was purified using flash chromatography.

2-(Benzyloxy)-N-(2-isopropoxy-5-methyl-4-phenoxyphenyl)pyrazolo[1,5-a]pyridine-3-carboxamide (**94**). Obtained from **57**, using aniline **101**. The crude product was purified by flash chromatography (eluent: from petroleum ether/EtOAc 85:15 v/v) to afford the title compound as a sticky solid. Yield 90 %. ¹H NMR (600 MHz, *Chloroform-d*): δ 1.12 (d, 6H, J = 6.1 Hz, -CH(CH₃)₂), 2.17 (s, 3H, Ar-CH₃), 4.36 (h, 1H, J = 6.1 Hz, -CH(CH₃)₂), 5.67 (s, 2H, -OCH₂Ph), 6.55 (s, 1H, H-t), 6.83 (t, 1H, J = 6.8 Hz, H-b), 6.88 (d, 2H, J = 8.1 Hz, H-n), 7.0 (t, 1H, J = 7.3 Hz, aromatic proton), 7.25 - 7.41 (m, 6H, aromatic protons), 7.53 (d, 2H, J = 7.5 Hz, aromatic protons), 8.26 (d, 1H, J = 6.8 Hz, H-a), 8.35 (d, 1H, J = 8.9 Hz, H-d), 8.48 (s, 1H, H-q), 9.20 (s, 1H, -NH); ¹³C NMR (151 MHz, *Chloroform-d*): δ 15.8 (Ar-CH₃), 21.9 (-CH(CH₃)₂),

71.5 ($-\text{CH}(\text{CH}_3)_2$), 71.6 ($-\text{OCH}_2\text{Ph}$), 91.6 (C-f), 106.7, 112.7 (C-b), 116.2, 119.0 (C-d), 121.8, 122.5, 122.9, 126.3, 127.4, 127.9, 128.4, 128.6, 128.8, 129.7, 136.4, 143.2 (C-e), 145.7, 148.6, 158.8, 161.3, 162.3. MS (ESI) 538 (M-1).

N-(2-Cyclobutoxy-5-methyl-4-phenoxyphenyl)-2-((4-methoxybenzyl)oxy)pyrazolo[1,5-*a*]pyridine-3-carboxamide (**95**). Obtained from **57** using aniline **102**. The crude product was purified by flash chromatography (eluent: petroleum ether / EtOAc 85:15 v/v) to afford the title compound as a sticky solid. Yield 79 %. ^1H NMR (600 MHz, *Chloroform-d*): δ 1.46 – 1.56 (*m*, 1H, cyclobutoxy proton), 1.59 – 1.68 (*m*, 1H, cyclobutoxy proton), 1.80 – 1.91 (*m*, 2H, cyclobutoxy proton), 2.15 (*s*, 3H, Ar- CH_3), 2.18 – 2.25 (*m*, 2H, cyclobutoxy proton), 3.80 (*s*, 3H, $-\text{OCH}_3$), 4.46 (*p*, 1H, $J = 7.1$ Hz, cyclobutoxy proton), 5.58 (*s* 2H, $-\text{OCH}_2\text{Ph}$), 6.38 (*s*, 1H, *H-t*), 6.84 (*t*, 1H, $J = 6.9$ Hz, *H-b*), 6.87 (*d*, 2H, $J = 8.0$ Hz, aromatic protons), 6.91 (*d*, 2H, $J = 8.4$ Hz, *H-n*), 7.00 (*t*, 1H, $J = 7.3$ Hz, aromatic protons), 7.25 – 7.30 (*m*, 2H, aromatic protons), 7.35 (*t*, 1H, $J = 7.9$ Hz, *H-c*), 7.49 (*d*, 2H, $J = 8.4$ Hz, *H-m*), 8.28 (*d*, 1H, $J = 6.8$ Hz, *H-a*), 8.34 (*d*, 1H, $J = 8.8$ Hz, *H-d*), 8.44 (*s*, 1H, *H-q*), 9.16 (*s*, 1H, $-\text{NH}$); ^{13}C NMR (151 MHz, *Chloroform-d*): δ 13.1, 15.8, 30.5, 55.4, 71.6, 72.2 ($-\text{OCH}_2\text{Ph}$), 91.6 (C-f), 105.6, 112.7 (C-b), 114.1, 116.3, 118.9 (C-d), 121.9, 122.3, 122.8, 125.3, 127.4, 128.4,

128.6, 129.7, 129.8, 143.1 (C-e), 145.4, 148.6, 158.6, 159.8, 161.3, 162.4. MS (ESI) 550 (M+1).

N-(2-(*Sec*-butoxy)-5-methyl-4-phenoxyphenyl)-2-((4-methoxybenzyl)oxy)pyrazolo[1,5-*a*]pyridine-3-carboxamide (**96**). Obtained from **57**, using aniline **103**. The crude product was purified by flash chromatography (eluent: petroleum ether / EtOAc 85:15 v/v) to afford the title compound as a sticky solid. Yield 94 %. ¹H NMR (600 MHz, *Chloroform-d*): δ 0.85 (t, 3H, J = 7.5 Hz, -CHCH₂CH₃), 1.12 (d, 3H, J = 6.1 Hz, -CH₃CHCH₂CH₃), 1.33 - 1.60 (m, 2H, -CH₃CHCH₂CH₃), 2.16 (s, 3H, Ar-CH₃), 3.79 (s, 3H, -OCH₃), 4.12 (h, 1H, J = 6.1 Hz, -CH₃CHCH₂CH₃), 5.56 (d, 1H, J = 12.1 Hz, -OCH₂Ph), 5.59 (d, 1H, J = 12.1 Hz, -OCH₂Ph), 6.54 (s, 1H, *H-t*), 6.83 (t, 1H, J = 6.8 Hz, *H-b*), 6.86 - 6.92 (m, 4H, aromatic protons), 7.0 (t, 1H, J = 7.3 Hz, aromatic proton), 7.25 - 7.31 (m, 2H, aromatic protons), 7.34 (t, 1H, J = 7.9 Hz, *H-c*), 7.47 (d, 2H, J = 8.5 Hz, *H-m*), 8.27 (d, 1H, J = 6.8 Hz, *H-a*), 8.34 (d, 1H, J = 8.9 Hz, *H-d*), 8.46 (s, 1H, *H-q*), 9.17 (s, 1H, -NH); ¹³C NMR (151 MHz, *Chloroform-d*): δ 10.1 (-CH₃CHCH₂CH₃) 15.8 (Ar-CH₃), 19.3 (-CH₃CHCH₂CH₃), 29.1 (-CH₃CHCH₂CH₃), 55.4 (-OCH₃), 71.5 (-CH₃CHCH₂CH₃), 76.8 (-OCH₂Ph), 91.6 (C-f), 106.7, 112.6 (C-b), 114.1, 116.2, 119.0 (C-d), 121.8, 122.4, 123.0, 126.3, 127.3, 128.4, 128.6, 129.7, 129.9, 143.2 (C-e), 146.0, 148.6, 158.8, 159.8, 161.4, 162.3. MS (ESI) 552 (M+1).

2-((4-Methoxybenzyl)oxy)-N-(5-methyl-2-(pentan-2-yloxy)-4-phenoxyphenyl)pyrazolo[1,5-a]pyridine-3-carboxamide (**97**). Obtained from **57**, using aniline **104**. The crude product was purified by flash chromatography (eluent: petroleum ether/EtOAc 85:15 v/v) to afford the title compound as a sticky solid. Yield 89%. ¹H NMR (600 MHz, Chloroform-d): δ 0.82 (t, 3H, J = 6.9 Hz, -CH₂CH₂CH₃), 1.11 (d, 2H, J = 6.0 Hz, -CH₃CHCH₂CH₂CH₃) 1.21 - 1.40 (m, 3H, --CH₃CHCH₂CH₂CH₃), 1.47 - 1.57 (m, 1H, -CH₃CHCH₂CH₂CH₃), 2.16 (s, 3H, Ar-CH₃), 3.79 (s, 3H, -OCH₃), 4.16 - 4.23 (m, 1H, -CH₃CHCH₂CH₂CH₃), 5.56 (d, 1H, J = 12.1 Hz, -OCH₂Ph), 5.60 (d, 1H, J = 12.1 Hz, -OCH₂Ph), 6.54 (s, 1H, H-t), 6.83 (t, 1H, J = 6.7 Hz, H-b), 6.85 - 6.92 (m, 4H, aromatic protons), 7.0 (t, 1H, J = 7.3 Hz, aromatic proton), 7.24 - 7.31 (m, 2H, aromatic protons), 7.34 (t, 1H, J = 7.9 Hz, H-c), 7.47 (d, 2H, J = 8.5 Hz, H-m), 8.27 (d, 1H, J = 6.8 Hz, H-a), 8.34 (d, 1H, J = 8.8 Hz, H-d), 8.47 (s, 1H, H-q), 9.17 (s, 1H, -NH); ¹³C NMR (151 MHz, Chloroform-d): δ 14.1 (-CH₃CHCH₂CH₂CH₃) 15.8 (Ar-CH₃), 18.9 (-CH₃CHCH₂CH₂CH₃), 19.8 (-CH₃CHCH₂CH₂CH₃), 38.4 (-CH₃CHCH₂CH₂CH₃), 55.4 (-OCH₃), 71.5 (-OCH₂Ph), 75.3 (-CH₃CHCH₂CH₂CH₃), 91.6 (C-f), 106.6, 112.6 (C-b), 114.1, 116.2, 118.9 (C-d), 121.8, 122.4, 122.9, 126.3, 127.3, 128.4, 128.6, 129.7, 129.8, 143.2 (C-e), 146.0, 148.6, 158.7, 159.8, 161.4, 162.3. MS (ESI) 566 (M+1).

2-((4-Methoxybenzyl)oxy)-N-(5-methyl-2-(pentan-3-yloxy)-4-phenoxyphenyl)pyrazolo[1,5-a]pyridine-3-carboxamide (**98**). Obtained from **57** using aniline **105**. The crude product was purified by flash chromatography (eluent: petroleum ether / EtOAc 85:15 v/v) to afford the title compound as a sticky solid (melting point from XXX). Yield 61 %. ^1H NMR (600 MHz, *Chloroform-d*): δ 0.84 (t, 6H, $J = 7.4$ Hz, $-\text{CHCH}_2\text{CH}_3$), 1.43 – 1.54 (m, 4H, $-\text{CHCH}_2\text{CH}_3$), 2.15 (s, 3H, Ar- CH_3), 3.79 (s, 3H, $-\text{OCH}_3$), 3.97 (p, 1H, $J = 5.8$ Hz, $-\text{CHCH}_2\text{CH}_3$), 5.58 (s, 2H, $-\text{OCH}_2\text{Ph}$), 6.54 (s, 1H, *H-t*), 6.83 (t, 1H, $J = 6.8$ Hz, *H-b*), 6.85 – 6.92 (m, 4H, aromatic protons), 7.0 (t, 1H, $J = 7.3$ Hz, aromatic proton), 7.25 – 7.31 (m, 2H, aromatic protons), 7.34 (t, 1H, $J = 7.9$ Hz, *H-c*), 7.46 (d, 2H, $J = 8.5$ Hz, *H-m*), 8.27 (d, 1H, $J = 6.8$ Hz, *H-a*), 8.34 (d, 1H, $J = 8.9$ Hz, *H-d*), 8.47 (s, 1H, *H-q*), 9.20 (s, 1H, $-\text{NH}$); ^{13}C NMR (151 MHz, *Chloroform-d*): δ 9.8 ($-\text{CHCH}_2\text{CH}_3$) 15.8 (Ar- CH_3), 26.3 ($-\text{CHCH}_2\text{CH}_3$), 55.4 ($-\text{OCH}_3$), 71.5 ($-\text{OCH}_2\text{Ph}$), 81.9 ($-\text{CHCH}_2\text{CH}_3$), 91.6 (*C-f*), 106.5, 112.6 (*C-b*), 114.1, 116.2, 119.0 (*C-d*), 121.8, 122.3, 122.9, 126.3, 127.3, 128.5, 128.6, 129.7, 129.8, 143.2 (*C-e*), 146.5, 148.5, 158.7, 159.8, 161.4, 162.3. MS (ESI) 566 ($\text{M}+1$).

N-(2-Isopropyl-5-methyl-4-(pyridin-4-yloxy)phenyl)-2-((4-methoxybenzyl)oxy)pyrazolo[1,5-a]pyridine-3-carboxamide (**99**). Oxalyl chloride (198 μL , 2.23 mmol, 3.6 eq.) and dry DMF (7 μL), were added to a cooled (0°C),

solution of **57** (0.743 mmol, 1.2 eq.), in dry THF (15 mL), under a nitrogen atmosphere. The reaction mixture was stirred for 2 hours at room temperature under a nitrogen atmosphere. The solution was concentrated under reduced pressure and the residue was dissolved in dry THF (10 mL, this step was repeated three times). The resulting acyl chloride was dissolved in dry toluene (10 mL). A solution of aniline **106** (0.619 mmol, 1 eq.), and dry pyridine (2.228 mmol, 3.6 eq.), in dry toluene (5 mL), was added to the solution of acyl chloride under nitrogen atmosphere. The resulting mixture was stirred at room temperature overnight. The mixture was quenched in water (80 mL) and partially concentrated under reduce pressure. The aqueous phase was extracted with EtOAc (3 x 50 mL). The combined organic layers were dried and evaporated under reduced pressure. The crude material was purified using flash chromatography (eluent: *petroleum ether*/EtOAc 50:50 v/v) to afford the title compound as a brown solid (melting point: missing). Yield 80 %. ¹H NMR (600 MHz, *Chloroform-d*): δ 0.94 (d, 6H, J = 6.7 Hz, CH(CH₃)₂), 2.11 (s, 3H, -CH₃), 2.70 (hept, 1H, J = 6.7 Hz, -CH(CH₃)₂), 3.82 (s, 3H, -OCH₃), 5.49 (s, 2H, -OCH₂Ph), 6.74 (d, 2H, J = 4.3 Hz, aromatic protons), 6.83 (s, 1H, aromatic proton), 6.88 (t, 1H, J = 6.8 Hz, H-b), 6.95 (d, 2H, J = 8.4 Hz, H-n), 7.38 (t, 1H, J = 7.9 Hz, H-c), 7.47 (d 2H, J = 8.4 Hz, H-m), 8.13 (s, 1H, aromatic proton), 8.31 - 8.36 (m, 2H, aromatic protons), 8.41 (d, 2H, J = 4.7 Hz,

aromatic protons), 8.55 (*s*, 1H, -NH). ¹³C NMR (151 MHz, Chloroform-*d*): δ 15.9 (Ar-CH₃), 22.6 (-CH(CH₃)₂), 27.8 (-CH(CH₃)₂), 55.5 (-OCH₃), 72.4 (-OCH₂Ph), 91.0 (*C-f*), 111.3, 112.9 (*C-b*), 114.3, 118.3, 119.0 (*C-d*), 126.1, 127.6, 127.7 (*C-a*), 128.3, 128.7 (*C-c*), 131.1, 132.9, 138.3, 143.2 (*C-e*), 148.1, 151.4, 160.4, 161.6, 162.5, 165.2. MS (ESI) 523 (M+1).

General procedure for synthesis of compounds 26, 31 – 36.

Thioanisole (10.0 eq.) was added to a solution of the respective protected amide **93 – 99** (1.0 eq.) in TFA (4 mL). The mixture was heated at 70 °C for 4h then cooled to r.t.. The mixture was partially concentrated, the crude was taken up with phosphate saline buffer pH = 5 obtaining a suspension that was filtered, the solid so obtained was triturated with hexane to afford the title compounds, in pure form.

*2-Hydroxy-N-(2,5-dimethyl-4-(pyridin-4-ylthio)phenyl)pyrazolo[1,5-*a*]pyridine-3-carboxamide (26).* White solid. Yield 80 %. ¹H-NMR (600 MHz, DMSO-*d*₆): δ 2.31 (*s*, 3H, Ar-CH₃), 2.32 (*s*, 3H, Ar-CH₃), 7.02 (*t*, 1H, *J* = 6.9 Hz, *H-b*), 7.28 (*d*, 2H, *J* = 6.1 Hz, aromatic protons), 7.48 – 7.56 (*m*, 2H, aromatic protons), 8.08 (*d*, 1H, *J* = 8.8 Hz, *H-d*); 8.50 (*d*, 2H, *J* = 5.0 Hz, aromatic protons), 8.58 – 8.65 (*m*, 2H, aromatic protons), 9.20 (*s*, 1H, -NH); ¹³C-NMR (151 MHz DMSO-*d*₆): δ 16.6 (Ar-CH₃), 20.2 (Ar-CH₃),

89.4 (C-f), 113.2 (C-b), 117.0, 118.1 (C-d), 120.8, 121.7, 125.5, 128.2 (C-a), 129.1 (C-c), 138.1, 140.2, 140.9, 141.6, 143.8 (C-e), 160.9, 162.2. MS (ESI) 389 (M-1).

2-Hydroxy-N-(2-isopropoxy-5-methyl-4-phenoxyphenyl)pyrazolo[1,5-a]pyridine-3-carboxamide (31). White solid. Yield 89 %. ¹H NMR (600 MHz, Chloroform-d): δ 1.40 (d, 6H, J = 6.0 Hz, -CH(CH₃)₂), 2.19 (s, 3H, Ar-CH₃), 4.51 (h, 1H, J = 6.0 Hz, -CH(CH₃)₂), 6.63 (s, 1H, H-t), 6.88 – 6.92 (m, 3H, H-b and aromatic protons), 7.0 (t, 1H, J = 7.3 Hz, aromatic proton), 7.30 (t, 2H, J = 7.9 Hz, aromatic protons), 7.43 (t, 1H, J = 7.9 Hz, H-c), 8.27 (d, 1H, J = 6.8 Hz, H-a), 8.35 (d, 1H, J = 8.8 Hz, H-d), 8.52 (s, 1H, H-q), 9.32 (s, 1H, -NH); ¹³C NMR (151 MHz, Chloroform-d): δ 15.9 (Ar-CH₃), 22.4 (-CH(CH₃)₂), 72.0 (-CH(CH₃)₂), 91.1 (C-f), 107.3, 113.2 (C-b), 116.3, 118.8 (C-d), 122.0, 122.5, 123.1, 126.6, 127.4, 128.2, 129.8, 136.4, 141.9 (C-e), 145.4, 148.7, 158.7, 161.4, 162.8. MS (ESI) 418 (M-1).

N-(2-cyclobutoxy-5-methyl-4-phenoxyphenyl)-2-hydroxypyrazolo[1,5-a]pyridine-3-carboxamide (32). White solid. Yield 75 %. ¹H NMR (600 MHz, DMSO-d₆): δ 1.55 – 1.65 (m, 1H, cyclobutoxy proton), 1.73 – 1.82 (m, 1H, cyclobutoxy proton), 2.00 – 2.14 (m, 2H, cyclobutoxy proton), 2.06 (s, 3H, Ar-CH₃), 2.30 – 2.39 (m, 2H,

cyclobutoxy proton), 4.70 (p, 1H, J = 7.0 Hz, cyclobutoxy proton), 6.53 (s, 1H, H-t), 6.85 (d, 2H, J = 8.1 Hz, aromatic proton), 6.98 (t, 1H, J = 6.8 Hz, H-b), 7.03 (t, 1H, J = 7.3 Hz, aromatic proton), 7.32 (t, 2H, J = 7.8 Hz, aromatic protons), 7.47 (t, 1H, J = 7.8 Hz, H-c), 8.09 (d, 1H, J = 8.7 Hz, H-d), 8.46 (s, 1H, H-q), 8.58 (d, 1H, J = 6.7 Hz, H-a), 9.62 (s, 1H, -NH), 12.85 (br s, 1H, -OH); ¹³C NMR (151 MHz, DMSO-d₆): δ 12.7, 15.5, 29.8, 71.8, 89.7 (C-f), 105.9, 112.8 (C-b), 115.9, 117.0 (C-d), 120.8, 121.0, 122.0, 125.8, 127.7, 129.0, 129.9, 141.5 (C-e), 144.3, 147.4, 158.0, 160.6, 162.0. MS (ESI) 430 (M+1).

N-(2-(*Sec*-butoxy)-5-methyl-4-phenoxyphenyl)-2-hydroxypyrazolo[1,5-*a*]pyridine-3-carboxamide (**33**). White solid. Yield 76 %. ¹H NMR (600 MHz, DMSO-d₆): δ 0.91 (t, 3H, J = 7.4 Hz, -CHCH₂CH₃), 1.22 (d, 3H, J = 6.0 Hz, -CH₃CHCH₂CH₃), 1.56 - 1.75 (m, 2H, -CH₃CHCH₂CH₃), 2.06 (s, 3H, Ar-CH₃), 4.36 (h, 1H, J = 5.8 Hz, -CH₃CHCH₂CH₃), 6.73 (s, 1H, H-t), 6.85 (d, 2H, J = 8.1 Hz, aromatic protons), 6.98 (t, 1H, J = 6.8 Hz, H-b), 7.02 (t, 1H, J = 7.3 Hz, aromatic proton), 7.32 (t, 2H, J = 7.9 Hz, aromatic protons), 7.34 (m, 1H, H-c), 8.09 (d, 1H, J = 8.8 Hz, H-d), 8.47 (s, 1H, H-q), 8.57 (d, 1H, J = 6.8 Hz, H-a), 9.63 (s, 1H, -NH), 12.79 (br s, 1H, -OH); ¹³C NMR (151 MHz, DMSO-d₆): δ 9.4 (-CH₃CHCH₂CH₃) 15.5 (Ar-CH₃), 18.9 (-CH₃CHCH₂CH₃), 28.4 (-CH₃CHCH₂CH₃), 76.1 (-CH₃CHCH₂CH₃), 89.7

(C-f), 107.0, 112.8 (C-b), 115.8, 117.1 (C-d), 120.9, 121.1, 121.9, 126.8, 127.7, 129.0, 129.9, 141.5 (C-e), 144.9, 147.4, 158.1, 160.6, 162.0. MS (ESI) 432 (M+1).

2-Hydroxy-N-(5-methyl-2-(pentan-2-yloxy)-4-phenoxyphenyl)pyrazolo[1,5-a]pyridine-3-carboxamide

(34). White solid. Yield 89%. ¹H NMR (600 MHz, DMSO-d₆): δ 0.84 (t, 3H, J = 7.3 Hz, -CH₂CH₂CH₃), 1.22 (d, 2H, J = 6.0 Hz, -CH₃CHCH₂CH₂CH₃), 1.27 - 1.44 (m, 2H, -CH₃CHCH₂CH₂CH₃), 1.47 - 1.57 (m, 1H, -CH₃CHCH₂CH₂CH₃), 1.63 - 1.74 (m, 1H, -CH₃CHCH₂CH₂CH₃), 2.06 (s, 3H, Ar-CH₃), 4.37 - 4.43 (m, 1H, -CH₃CHCH₂CH₂CH₃), 6.72 (s, 1H, H-t), 6.85 (d, 2H, J = 8.1 Hz, aromatic protons), 6.96 (t, 1H, J = 6.7 Hz, H-b), 7.02 (t, 1H, J = 7.3 Hz, aromatic proton), 7.32 (t, 1H, J = 7.8 Hz, aromatic proton), 7.45 (t, 1H, J = 7.8 Hz, H-c), 8.08 (d, 1H, J = 8.7 Hz, H-d), 8.47 (s, 1H, H-q), 8.55 (d, 1H, J = 6.7 Hz, H-a), 9.71 (s, 1H, -NH), 12.84 (s, 1H, -OH); ¹³C NMR (151 MHz, DMSO-d₆): δ 13.9 (-CH₃CHCH₂CH₂CH₃) 15.5 (Ar-CH₃), 18.1 (-CH₃CHCH₂CH₂CH₃), 19.4 (-CH₃CHCH₂CH₂CH₃), 37.8 (-CH₃CHCH₂CH₂CH₃), 74.9 (-CH₃CHCH₂CH₂CH₃), 89.7 (C-f), 106.9, 112.6 (C-b), 115.8, 116.9 (C-d), 120.9, 121.0, 121.9, 126.8, 127.5, 128.9, 129.9, 141.5 (C-e), 144.9, 147.4, 158.1, 160.7 MS (ESI) 446 (M+1).

2-Hydroxy-N-(5-methyl-2-(pentan-3-yloxy)-4-phenoxyphenyl)pyrazolo[1,5-a]pyridine-3-carboxamide

(35). White solid. Yield 30 %. ^1H NMR (600 MHz, $\text{DMSO}-d_6$): δ 0.96 (t, 6H, $J = 7.4$ Hz, $-\text{CHCH}_2\text{CH}_3$), 1.70 – 1.77 (m, 4H, $-\text{CHCH}_2\text{CH}_3$), 2.18 (s, 3H, $\text{Ar}-\text{CH}_3$), 4.12 (p, 1H, $J = 5.6$ Hz, $-\text{CHCH}_2\text{CH}_3$), 6.59 (s, 1H, $H-t$), 6.89 (d, 2H, $J = 8.1$ Hz, aromatic protons), 6.93 (t, 1H, $J = 6.7$ Hz, $H-b$), 7.02 (t, 1H, $J = 7.3$ Hz, aromatic proton), 7.30 (t, 2H, $J = 7.8$ Hz, aromatic protons), 7.47 (t, 1H, $J = 7.9$ Hz, $H-c$), 8.27 – 8.41 (m, 2H, $H-a$ and $H-d$), 8.49 (s, 1H, $H-q$), 9.25 (s, 1H, $-\text{NH}$); ^{13}C NMR (151 MHz, $\text{DMSO}-d_6$): δ 9.7 ($-\text{CHCH}_2\text{CH}_3$), 15.9 ($\text{Ar}-\text{CH}_3$), 26.2 ($-\text{CHCH}_2\text{CH}_3$), 81.7 ($-\text{CHCH}_2\text{CH}_3$), 91.0 ($C-f$), 106.5, 113.4 ($C-b$), 116.3, 118.6 ($C-d$), 122.0, 122.5, 122.6, 126.1, 127.6, 128.6, 129.8, 141.7 ($C-e$), 146.0, 148.9, 153.2, 158.7, 161.4. MS (ESI) 446 ($M+1$).

2-Hydroxy-*N*-(2-isopropyl-5-methyl-4-(pyridin-4-ylloxy)phenyl)pyrazolo[1,5-*a*]pyridine-3-carboxamide (36). White solid. Yield 74 %. ^1H NMR (600 MHz, $\text{DMSO}-d_6$): δ 1.22 (d, 6H, $J = 6.8$ Hz, $\text{CH}(\text{CH}_3)_2$), 2.01 (s, 3H, $-\text{CH}_3$), 3.14 (hept, 1H, $J = 6.8$ Hz, $-\text{CH}(\text{CH}_3)_2$), 6.96 – 7.04 (m, 1H, $H-b$), 7.09 – 7.16 (m, 3H, aromatic protons), 7.49 (t, 1H, $J = 7.9$ Hz, $H-c$), 8.07 (d, 1H, $J = 8.8$ Hz, $H-d$), 8.18 (s, 1H, aromatic protons), 8.59 (d, 1H, $J = 6.8$ Hz, $H-a$), 8.60 – 8.70 (m, 2H, aromatic protons), 9.10 (s, 1H, $-\text{NH}$). ^{13}C NMR (151 MHz, $\text{DMSO}-d_6$): δ 15.4 ($\text{Ar}-\text{CH}_3$), 22.5 ($-\text{CH}(\text{CH}_3)_2$), 27.4 ($-\text{CH}(\text{CH}_3)_2$), 89.4 ($C-f$), 112.3, 112.9 ($C-b$), 117.0, 118.3 ($C-d$), 125.3 ($C-a$), 127.1, 127.9 ($C-c$),

129.0, 133.7, 138.1, 141.6, (C-e), 146.6, 146.7, 147.7, 161.0, 162.1. MS (ESI) 403 (M+1)

2-Hydroxypyrazolo[1,5-a]pyridine-3-carboxylic acid (**107**). 5M NaOH solution (5 eq.) was added to a solution of **44** (1 g, 4.84 mmol) in EtOH (20 mL), the reaction mixture was stirred at reflux for 6h. Then the EtOH was evaporated under vacuum and the residue was taken up with distilled water (20 mL). The suspension was acidified with 6M HCl until pH = 2 in order to obtain the product as precipitate. The white solid was filtered and washed with distilled water until neutrality. Yield: 98 %.

Pyrazolo[1,5-a]pyridin-2-ol (**108**). A 6M NaOH solution (10 eq.) was added to a suspension of 2-hydroxypyrazolo[1,5-a]pyridine-3-carboxylic acid (**107**, 1.4 g, 7.86 mmol) in EtOH (25 mL). The reaction mixture was stirred and heated under reflux at 85°C until compound **107** was completely dissolved, then 12M HCl (2.38 mL) was added dropwise at 85°C. The reaction mixture was stirred for 20 minutes. The reaction was quenched in distilled water (30 mL) and extracted with EtOAc (3 x 15 mL). Organic phases were collected, washed with a saturated NaHCO₃ solution (3 x 20 mL), a saturated brine solution, dried over Na₂SO₄ and concentrated under reduced pressure. The latter was filtered via flash chromatography (eluent: petroleum ether/ethyl acetate 9/1 v/v). Yield: 90%.

2,3,5,6-tetrafluoro-[1,1'-biphenyl]-4-amine (**46**). K_2CO_3 (0.33 g, 3.0 eq.) and $Pd(PPh_3)_4$ (0.187 g, 0.2 eq.) were added to a solution of 4-Bromo-2,3,5,6-tetrafluoroaniline (0.2 g, 0.81 mmol) in dioxane/ H_2O (30 mL, 9/1 v/v) under inert atmosphere, and the reaction mixture was stirred for 1h. Then phenylboronic acid (0.296 g, 3.0 eq.) was added and the reaction mixture was stirred at 90°C overnight. The reaction was quenched in distilled water (30 mL) and extracted with EtOAc (3 x 15 mL). Organic layers were collected, washed with a saturated brine solution, dried over Na_2SO_4 and concentrated under reduced pressure. The latter was purified via flash chromatography (eluent: petroleum ether/ethyl acetate 95/5 v/v). Yield: 90%. 1H NMR (300 MHz, Chloroform- d): δ 4.04 (s, 2H, $-NH_2$), 7.35 – 7.51 (m, 5H, aromatic protons); ^{13}C NMR (75 MHz, Chloroform- d): δ 108.4 (t, $J = 17.1$ Hz), 125.5 (tt, $J = 13.8, 3.6$ Hz), 128.2 (t, $J = 2.2$ Hz), 128.4, 128.6, 130.5 (t, $J = 2.0$ Hz), 136.9 (d, $J = 238.4$ Hz), 144.2 (d, $J = 242.0$ Hz). MS (ESI) 242 (M+1).

(E)-3-((2,3,5,6-tetrafluoro-[1,1'-biphenyl]-4-yl)diazinyl)pyrazolo[1,5-a]pyridin-2-ol (**25**). A solution of $NaNO_2$ (51 mg, 0.74 mmol.) in water (5 mL) was added dropwise to a cooled (0 °C) solution of 2,3,5,6-tetrafluoro-[1,1'-biphenyl]-4-amine (**46**, 150 mg, 0.62 mmol) in nitric acid (1.55 mL); the resulting mixture was stirred for 10 minutes. The resulting solution was added

dropwise to a cooled solution of **108** (83 mg, 0.62 mmol) in water (5mL) at pH~10. The resulting mixture was stirred for 5 minutes, and an orange precipitate was observed. The precipitate was filtrated over Büchner to afford the title compound as orange solid (trituated with diisopropyl ether). Yield 48 %. ¹H NMR (600 MHz, DMSO-d₆): δ 7.27 (t, 1H, J = 6.5 Hz, H-b), 7.50 – 7.60 (m, 5H, aromatic protons), 7.80 (t, 1H, J = 7.7 Hz, H-c), 8.19 (d, 1H, J = 8.2 Hz, H-d), 8.75 (d, 1H, J = 6.6 Hz, H-a), ¹³C NMR (151 MHz, DMSO-d₆): δ 117.0, 117.2, 118.4, 127.4, 129.1, 129.3, 129.9, 130.7, 130.8, 130.9, 133.3, 133.4, 140.3 (d, J = 246.5 Hz, CF), 144.2 (d, J = 244.3 Hz, CF), 164.0; MS (ESI) 387 (M+1)

(E)-3-((4-phenoxyphenyl)diazinyl)pyrazolo[1,5-a]pyridin-2-ol (**38**). A solution of NaNO₂ (51 mg, 0.74 mmol, 1.2 eq.) in water (5 mL) was added dropwise to a cooled (0 °C) solution of 4-phenoxyaniline (**46**) (150 mg) in nitric acid (1.55 mL); the resulting mixture was stirred for 10 minutes. The resulting solution was added dropwise to a cooled solution of (**108**, 83 mg, 1 eq.) in water (5mL) at pH~10. The resulting mixture was stirred for 5 minutes, and a precipitate was observed. The precipitate was filtrated over Büchner to afford the title compound as orange solid (trituated with diisopropyl ether). Yield 60 %. ¹H NMR (600 MHz, DMSO-d₆): δ 7.04 – 7.13 (m, 4H, aromatic protons), 7.13 – 7.20

(m, 2H, aromatic proton, H-b), 7.42 (t, 2H, $J = 8.0$ Hz, aromatic protons), 7.63 (t, 1H, $J = 7.8$ Hz, H-c), 7.76 (d, 2H, $J = 8.8$ Hz, aromatic protons), 8.15 (d, 1H, $J = 8.5$ Hz, H-d), 8.61 (d, 1H, $J = 6.5$ Hz, H-a), ^{13}C NMR (151 MHz, DMSO- d_6): δ 116.2, 117.2, 117.7, 119.0, 119.0, 121.6, 123.8, 130.0, 130.2, 130.7, 132.8, 145.5, 156.39, 156.42, 164.5. MS (ESI) 331 (M+1)

Ethyl 2-methoxyppyrazolo[1,5a]pyridine-3-carboxylate (**110a**) and ethyl N-methyl-2-oxo-pyrazolo[1,5a]pyridine-3-carboxylate (**110b**) from (**44**). Cs_2CO_3 (3 eq.) was added to a solution of **44** (5.0 g, 24.24 mmol) in dry DMF (50 mL), and the resulting mixture was stirred for 30 minutes at room temperature before adding iodomethane (29.1 mmol, 1.81 mL) drop-wise to the mixture. The reaction was quenched with distilled water (300 mL) and extracted with ethyl acetate (6 x 70 mL). Organic phases were collected, washed with brine solution, dried over Na_2SO_4 , filtered and concentrated under reduced pressure to obtain a pale-yellow solid crude containing the two isomers. The latter were separated via flash chromatography (eluent: petroleum ether/ethyl acetate 6/4 v/v, then DCM/MeOH 95/5 v/v). Yield: 77% (**110a**) and 13% (**110b**).

(**110a**) m.p. 142.4 – 143.5°C; ^1H NMR (600 MHz, Chloroform- d): δ 1.40 (t, 3H, $J = 7.1$ Hz, $-\text{OCH}_2\text{CH}_3$), 4.12 (s, 3H- OCH_3), 4.37 (q, 2H, $J = 7.1$ Hz, $-\text{OCH}_2\text{CH}_3$), 6.83 (t, 1H $J = 6.8$ Hz, H-b), 7.35 (t, 1H, $J = 7.9$ Hz, H-c), 7.97

(d, 1H, $J = 8.9$ Hz, H-d), 8.28 (d, 1H, $J = 6.8$ Hz, H-a). ^{13}C NMR (151 MHz, Chloroform-d): δ 14.7 ($-\text{OCH}_2\text{CH}_3$), 57.0 ($-\text{OCH}_3$), 59.9 ($-\text{OCH}_2\text{CH}_3$), 88.1 (C-f), 112.6 (C-b), 118.4 (C-d), 127.9 (C-c)*, 128.9 (C-a)*, 142.9 (C-e), 163.3 (C-g)**, 166.0 (C-h)**. (ESI) 221 (M+1).

(110b) m.p. 234.4 – 235.8°C; ^1H NMR (600 MHz, Chloroform-d): δ 1.28 (t, 3H, $J = 7.1$ Hz, $-\text{OCH}_2\text{CH}_3$), 3.58 (s, 3H- OCH_3), 4.21 (q, 2H, $J = 7.1$ Hz, $-\text{OCH}_2\text{CH}_3$), 7.10 (t, 1H $J = 6.9$ Hz, H-b), 7.66 (t, 1H, $J = 7.9$ Hz, H-c), 7.90 (d, 1H, $J = 8.9$ Hz, H-d), 8.57 (d, 1H, $J = 6.8$ Hz, H-a). ^{13}C NMR (151 MHz, Chloroform-d): δ 15.5 ($-\text{OCH}_2\text{CH}_3$), 28.9 ($-\text{NCH}_3$), 59.3 ($-\text{OCH}_2\text{CH}_3$), 84.2 (C-f), 113.3 (C-b), 116.7 (C-d), 125.8 (C-c)*, 132.9 (C-a)*, 142.6 (C-e), 160.6 (C-g)**, 164.1 (C-h)**. (ESI) 221 (M+1).

2-Methoxypyrazolo[1,5-a]pyridine-3-carbohydrazide
(111). 20 eq. of Hydrazine monohydrate (0.273 g, 20 eq.) was added to a solution of **110a** (0.6 g, 2.73 mmol) in EtOH (3 mL) and the reaction mixture was stirred at reflux overnight. The reaction was cooled to room temperature and the solid was filtered and washed with distilled water to afford the title compound as white solid. Yield: 68.7%. ^1H NMR (600 MHz, Chloroform-d): δ 4.03 (s, 2H, $-\text{CONHNH}_2$), 4.13 (s, 3H, $-\text{OCH}_3$), 6.81 (t, 1H $J = 6.7$ Hz, H-b), 7.32 (t, 1H, $J = 7.9$ Hz, H-c), 7.87 (s, 1H, $-\text{CONHNH}_2$), 8.19 (d, 1H, $J = 8.9$ Hz, H-d), 8.24 (d, 1H, $J = 6.9$ Hz, H-a). ^{13}C NMR (151 MHz, Chloroform-d): δ 57.2 ($-\text{OCH}_3$),

88.8 (C-f), 112.6 (C-b), 118.5 (C-d), 127.5 (C-c)*, 128.6 (C-a)*, 142.7 (C-e), 163.2 (C-g)*, 164.6 (C-h)*. (ESI) 207 (M+1).

2-(4-Bromo-2,3,5,6-tetrafluorophenyl)-5-(2-methoxypyrazolo[1,5-a]pyridin-3-yl)-1,3,4-oxadiazole (**112**). Compound **111** (0.3 g, 1.52 mmol) and 4-Bromo-2,3,5,6-tetrafluorobenzoic acid (0.414 g, 1.52 mmol) were dissolved in POCl₃ (5 mL). The reaction mixture was stirred at 50°C for 4h. The mixture was concentrated under vacuum and the solid was taken up with distilled water (80 mL) and extracted with EtOAc (3 x 50 mL). Organic layers were collected, washed with a saturated brine solution, dried over Na₂SO₄ and concentrated under reduced pressure to afford a pale, yellow crude. The latter was purified via flash chromatography (eluent: petroleum ether/ethyl acetate 7/3 v/v) to afford the title compound as pale-yellow solid. Yield: 52%. ¹H NMR (600 MHz, Chloroform-d): δ 4.21 (s, 3H, -OCH₃), 6.92 (t, 1H, J = 6.9 Hz, H-b), 7.45 (m, 1H, H-c), 8.10 (d, 1H, J = 8.8 Hz, H-d), 8.36 (d, 1H, J = 6.8 Hz, H-a). ¹³C NMR (151 MHz, Chloroform-d): δ 57.3 (-OCH₃), 81.1 (C-f), 103.5 (t, J = 17.9 Hz), 105.1 (t, J = 14.4 Hz), 112.9 (C-b), 117.6 (C-d), 128.3 (C-c)*, 129.2 (C-a)*, 140.8 (C-e), 144.7 (d, J = 246.0 Hz), 145.6 (d, J = 249.7 Hz), 153.1, 161.2 (C-g), 164.2. (ESI) 443-445 (M+1).

2-(2-Methoxypyrazolo[1,5-a]pyridin-3-yl)-5-(2,3,5,6-tetrafluoro-[1,1'-biphenyl]-4-yl)-1,3,4-oxadiazole (**113**). K_2CO_3 (0.187 g, 3.0 eq) and $Pd(PPh_3)_4$ (0.053 g, 0.1 eq) was added to a solution of **112** (0.2 g, 0.45 mmol) in dioxane/ H_2O (30 mL, 9/1 v/v) under inert atmosphere. and stirred for 1h. Then, phenylboronic acid (0.165 g, 3.0 eq.) was added and the reaction mixture was stirred at 90°C overnight. The reaction mixture was quenched in distilled water (150 mL) and extracted with EtOAc (3 x 60 mL). Organic layers were collected, washed with a saturated brine solution, dried over Na_2SO_4 and concentrated under reduced pressure to afford a pale, yellow solid. Yield: 100%. ^{19}F NMR (565 MHz, $DMSO-d_6$): δ -137.74 (d, 2F, $J = 21.5$ Hz), -142.75 (d, 2F, $J = 30.2$ Hz). 1H NMR (600 MHz, $DMSO-d_6$): δ 4.12 (s, 3H, -OCH₃), 7.11 (t, 1H, $J = 6.8$ Hz, H-b), 7.55 - 7.62 (m, 6H, H-c and aromatic protons), 8.00 (d, 1H, $J = 8.8$ Hz, H-d), 8.78 (d, 1H, $J = 6.8$ Hz, H-a). ^{13}C NMR (151 MHz, $DMSO-d_6$): δ 57.1 (-OCH₃), 79.7 (C-f), 103.5, 113.5 (C-b), 116.4 (C-d), 126.1 (C-c)*, 127.3, 127.4, 128.9, 129.3 (C-a)*, 130.0, 134.1, 139.9 (C-e), 144.7 (d, $J = 246.0$ Hz), 145.6 (d, $J = 249.7$ Hz), 153.0, 160.0 (C-g), 163.3.

3-(5-(2,3,5,6-Tetrafluoro-[1,1'-biphenyl]-4-yl)-1,3,4-oxadiazol-2-yl)pyrazolo[1,5-a]pyridine-2-ol (**39**). BBr_3 1M solution in DCM (650 μ L, 0.69 mmol) was dissolved in dry DCM (5 mL) under inert atmosphere. The solution was cooled

at -10°C and compound **119** (0.12 g, 0.273 mmol) was added and the reaction mixture was stirred for 30 minutes at 0°C . The reaction was quenched in distilled water (150 mL) and extracted with DCM (3 x 60 mL). Organic layers were collected, washed with a saturated brine solution, dried over Na_2SO_4 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 90%. Degradation temperature: $272\text{--}273^{\circ}\text{C}$. ^1H NMR (600 MHz, DMSO-d_6): δ 7.06 (t, 1H, $J = 6.7$ Hz, H-b), 7.52–7.65 (m, 6H, H-c and aromatic protons), 7.98 (d, 1H, $J = 8.7$ Hz, H-d), 8.67 (d, 1H, $J = 6.7$ Hz, H-a), 12.05 (s, 1H, -OH). ^{13}C NMR (151 MHz, DMSO-d_6): δ 79.5 (C-f), 103.8 (t, $J = 12.9$ Hz), 113.2 (C-b), 116.1 (C-d), 122.6 (t, $J = 17.1$ Hz), 126.1 (C-c)*, 128.52, 128.92, 129.5, 129.9 (C-a)*, 130.0, 139.6 (C-e), 144.7 (d, $J = 246.0$ Hz), 145.6 (d, $J = 249.7$ Hz), 152.8, 160.6 (C-g), 162.8. (ESI) 427 (M+1).

N-(4-Bromo-2,3,5,6-tetrafluorophenyl)-2-((4-methoxybenzyl)oxy)pyrazolo[1,5-a]pyridine-3-carboxamide (**114**). Oxalyl chloride (811 mg, 6.39 mmol, 3.0 eq.) and dry DMF (10 mL) were added to a cooled (0°C) solution of **57** (635 mg, 2.13 mmol) in dry THF (49 mL) kept under a nitrogen atmosphere. The resulting mixture was stirred for 2 h at room temperature. In parallel, a 1 M solution of LiHMDS in THF (3.62 mL, 3.62 mmol, 1.7 eq.) was added

to a solution of 4-bromo- 2,3,5,6-tetrafluoroaniline (883 mg, 3.62 mmol, 1.7 eq.) in dry THF (10 mL) under a nitrogen atmosphere. The resulting suspension was stirred for 30 minutes at room temperature. The solution of acyl chloride was then concentrated under reduced pressure, and the residue was dissolved in dry THF (25 mL); this step was repeated three times to eliminate all gaseous residues). The acyl chloride was dissolved in dry THF (15 mL), and the solution was added to the solution described above. The reaction mixture was stirred at 70 °C overnight, then cooled to room temperature and evaporated. The residue was dissolved in EtOAc (40 mL), 0.5 M HCl (50 mL) was then added, and the layers were separated. The aqueous phase was extracted twice with EtOAc, and the combined organic layers were washed with brine, dried, and evaporated under reduced pressure. 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 (melting point= 157.9 - 161.9°C, triturated with diisopropyl ether). Yield: 78%. ¹H-NMR (600 MHz, chloroform-d) δ: 3.82 (s, 3H, -OCH₃), 5.50 (s, 2H, -OCH₂Ar), 6.91 (t, 1H, J = 6.9 Hz, H-b), 6.94 (d, 2H, J = 8.5 Hz, H-n), 7.41 (d, 1H, J = 7.8 Hz, H-c), 7.46 (d, 2H, J = 8.5 Hz, H-m), 8.21 (d, 1H, J = 8.8 Hz, H-d), 8.26 (s, 1H, -NH), 8.33 (d, 1H, J = 6.8 Hz, H-a). ¹³C NMR (151 MHz, chloroform-d) δ: 55.5 (-OCH₃), 72.3 (-OCH₂Ar), 89.8 (C-f), 94.6 (t, J = 22.5

Hz, C-s), 113.4 (C-b), 114.3 (C-n), 116.6 (t, $J = 14.6$ Hz, C-p), 118.9 (C-d), 127.6 (C-l), 128.4 (C-a)*, 128.8 (C-c)*, 130.3 (C-m), 142.6 (d, $J = 251.6$ Hz, C-r)**, 143.1 (C-e), 145.2 (d, $J = 246.5$ Hz, C-q)**, 160.19 (C-o)***, 160.24 (C-h)***, 162.9 (C-g)***. ^{19}F NMR (565 MHz, chloroform-d) -143.55 (d, 2F, $J = 19.6$ Hz, F-p), -133.93 (d, 2F, $J = 16.6$ Hz, F-r). MS (ES⁺): 524/ 526 (M + 1).

1-(3-Bromophenoxy)propan-2-one (**116**). 3-Chloroacetone (7.70 g, 83.2 mmol, 3.0 eq.) was added dropwise to a mixture of 3-bromophenol (4.80 g, 27.7 mmol) and DBU (16.9 g, 111 mmol, 4.0 eq.) in dry DMF (50 mL). The reaction mixture was stirred at room temperature overnight, then quenched in 2M HCl (250 mL). The mixture was extracted with EtOAc (3 × 200 mL). The combined organic layers were dried over Na₂SO₄ and concentrated under reduced pressure. The crude material was purified using flash chromatography (eluent: from petroleum ether/EtOAc 9/1 v/v to petroleum ether/EtOAc 8/2 v/v) to afford the title compound as a yellow oil. Yield 98%. ^1H NMR (600 MHz, chloroform-d) δ : 2.28 (s, 3H, -CH₃), 4.53 (s, 2H, -OCH₂), 6.82 (ddd, 1H, $J = 7.9, 2.5, 1.4$ Hz, aromatic proton), 7.04 - 7.06 (m, 1H, aromatic proton), 7.13 (dt, 1H, $J = 7.9, 1.5$ Hz, aromatic proton), 7.16 (d, 1H, $J = 7.9$ Hz, aromatic proton). ^{13}C NMR (151 MHz, chloroform-d) δ : 26.8 (-CH₃), 72.9 (-OCH₂), 113.6, 118.2,

123.1, 125.1, 130.9, 158.6, 204.9 ($-\text{OCH}_2\text{COCH}_3$). MS (ES^+): 227/229 ($\text{M} + 1$).

1-Bromo-3-(2,2-difluoropropoxy)benzene (**117**). DAST (1.40 g, 8.70 mmol, 5.0 eq.) was added dropwise to a solution of **116** (437 mg, 1.74 mmol) in dry DCM (20 mL) at 0°C. The reaction mixture was stirred at room temperature for two hours, then quenched in saturated solution of Na_2CO_3 (30 mL). The mixture was extracted with DCM (3 × 20 mL). The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The crude material was purified using flash chromatography (eluent: petroleum ether/EtOAc 8/2 v/v) to afford the title compound as a yellow oil. Yield: 78%. ^1H NMR (600 MHz, chloroform- d) δ : 1.77 (t, 3H, $J = 18.8$ Hz, $-\text{CH}_3$), 4.09 (t, 2H, $J = 11.3$ Hz, $-\text{OCH}_2$), 6.87 (ddd, 1H, $J = 7.8$, 2.4, 1.5 Hz, aromatic proton), 7.09 – 7.10 (m, 1H, aromatic proton), 7.15 (dt, 1H, $J = 7.9$, 1.5 Hz, aromatic proton); 7.17 (t, 1H, $J = 7.8$ Hz, aromatic proton). ^{13}C NMR (151 MHz, chloroform- d) δ : 21.0 (t, $J = 25.7$ Hz, $-\text{CH}_3$), 69.9 (t, $J = 34.8$ Hz, $-\text{OCH}_2$), 113.7, 118.3, 121.4 (t, $J = 239.3$ Hz, $-\text{CF}_2$), 123.0, 125.1, 130.9, 158.7. MS (ES^+): 183/ 185 ($\text{M} - 67$). Massa da rifare

3-(3-Bromophenoxy)propan-1-ol (**122**). PPh_3 (1.80 g, 6.87 mmol, 1.4 eq.) was added to a solution of the 3-bromophenol (1.34 g, 4.91 mmol, 1.0 eq.) and 1,3-propandiol (523 mg, 6.87 mmol, 1.4 eq.) in dry THF (20

mL). After stirring the resulting mixture under a nitrogen atmosphere at r.t. for 30 minutes, DIAD (1.19 g, 6.87 mmol, 1.4 eq.) was added dropwise. The reaction mixture was then stirred at r.t. for 2 days under a nitrogen atmosphere. The mixture was concentrated under reduced pressure and taken-up with 0.1 M NaOH (50 mL). The mixture was extracted with EtOAc (3 × 20 mL). The combined organic layers were dried over Na₂SO₄ and concentrated under reduced pressure. The crude product was purified by flash chromatography (eluent: petroleum ether/EtOAc 8/2 v/v and then 7/3 v/v). The white solid product was washed with hexane/diisopropyl ether 8/2 v/v and the supernatant liquid was filtrated and concentrated under vacuum obtaining a colourless oil (the procedure was repeated for 3 times). Yield: 69%. ¹H NMR (600 MHz, chloroform-d) δ: 1.83 (br s, -OCH₂CH₂CH₂OH), 2.00 – 2.06 (m, 2H, -OCH₂CH₂CH₂OH), 3.84 (t, 2H, J = 6.0 Hz, -OCH₂CH₂CH₂OH), 4.09 (t, 2H, J = 6.0 Hz, -OCH₂CH₂CH₂OH), 6.83 (ddd, 1H, J = 8.3, 2.3, 1.0 Hz, aromatic proton), 7.05–7.09 (m, 2H, aromatic protons), 7.13 (t, 1H, J = 7.9 Hz, aromatic proton); ¹³C NMR (151 MHz, chloroform-d) δ: 32.0 (-OCH₂CH₂CH₂OH), 60.2 (-OCH₂CH₂CH₂OH), 65.8 (-OCH₂CH₂CH₂OH), 113.6, 117.9, 122.9, 124.0, 130.7, 159.7; MS (ES⁺): 271/273 (M + 40).

3-(3-Bromophenoxy)propyl tert-butyl carbonate (**123**). A solution of di tert-butyl carbonate (827 mg, 4.26 mmol,

2.0 eq.) in dry THF (5 mL) was added dropwise to a mixture of **122** (705 mg, 2.13 mmol) and NaH (98.0 mg, 4.26 mmol, 2.0 eq.) in dry THF (10 mL). The reaction mixture was stirred at room temperature overnight, then concentrated under vacuum. The crude material was taken-up with water (50 mL) and the mixture was extracted with EtOAc (3 × 40 mL). The combined organic layers were dried over Na₂SO₄ and concentrated under reduced pressure. The crude material was purified using flash chromatography (eluent: petroleum ether/EtOAc 9/1 v/v) to afford the title compound as a yellow oil. Yield: 96%. ¹H NMR (600 MHz, chloroform-d) δ: 1.49 (s, 9H, -C(CH₃)₃), 2.10 – 2.16 (m, 2H, -OCH₂CH₂CH₂O-), 4.04 (t, 2H, J = 6.1 Hz, -OCH₂CH₂CH₂O-), 4.25 (t, 2H, J = 6.3 Hz, -OCH₂CH₂CH₂O-), 6.82 (ddd, 1H, J = 8.2, 2.4, 0.9 Hz, aromatic proton), 7.04 – 7.09 (m, 2H, aromatic protons), 7.13 (t, 1H, J = 8.0 Hz, aromatic proton). ¹³C NMR (151 MHz, chloroform-d) δ: 27.9 (-C(CH₃)₃), 28.7 (-OCH₂CH₂CH₂O-), 63.7 (-OCH₂CH₂CH₂O-)*, 64.6 (-OCH₂CH₂CH₂O-)*, 82.3, 85.3 (-C(CH₃)₃), 113.7, 117.9, 122.9, 124.1, 130.7, 146.9, 153.6, 159.7 (CO); MS (ES⁺): 353/355 (M + 23).

General Procedure: Synthesis of the Boron Pinacolato Compounds **117**, **120**, **124**. PdCl₂(dppf) (36.0 mg, 0.0495 mmol, 0.03 eq.) was added to a solution of the corresponding starting material (415 mg, 1.65 mmol) and K₂CO₃ (684 mg, 4.95 mmol, 3.0 eq.) in dioxane (15 mL).

After stirring the resulting mixture e under a nitrogen atmosphere for 1 h at r.t., bis(pinacolato)diboron (626 mg, 5.94 mmol 3.60 eq.) was added. The reaction mixture was then heated at reflux overnight under a nitrogen atmosphere. The mixture was cooled to room temperature and concentrated under reduced pressure. The crude material was taken-up with petroleum ether/ EtOAc 9/1 v/v (20 mL) and was purified using flash chromatography (see the conditions below).

2-(3-(2,2-Difluoropropoxy)phenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (**117**). The crude product was purified by flash chromatography (eluent: petroleum ether/ EtOAc 9/1 v/v), obtaining a pale-yellow oil. Yield: 97%. ¹H NMR (600 MHz, chloroform-d) δ: 1.35 (s, 12H, -OC(CH₃)₂), 1.77 (t, 3H, J= 18.8 Hz, -OCH₂CF₂CH₃), 4.14 (t, 2H, J = 11.4 Hz, -OCH₂CF₂CH₃), 7.04 (ddd, 1H, J = 8.2, 2.8, 1.0 Hz, aromatic proton), 7.29 - 7.35 (m, 2H, aromatic protons), 7.44 - 7.47 (m, 1H, aromatic proton); ¹³C NMR (151 MHz, chloroform-d) δ: 21.1 (t, J= 26.0 Hz, -OCH₂CF₂CH₃), 25.2 (-OC(CH₃)₂), 25.2 (-OC(CH₃)₂), 69.7 (t, J= 34.6 Hz, -OCH₂CF₂CH₃), 83.6 (-BOC(CH₃)₂C(CH₃)₂O), 84.1 (-B(OC(CH₃)₂C(CH₃)₂O), 118.3 (t, J= 109.9 Hz, OCH₂CF₂CH₃), 118.5, 119.7, 121.7, 128.3, 129.3, 157.5; MS (ES⁺): 299 (M +1).

1-(3-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)phenoxy)propan-2-one (**120**). The crude product was

filtered on silica gel (eluent: petroleum ether/ EtOAc 9/1 v/v), obtaining a yellow oil. Yield: 96%. ^1H NMR (600 MHz, chloroform- d) δ : 1.24 (s, 6H, $-\text{OC}(\text{CH}_3)_2$), 1.34 (s, 6H, $-\text{OC}(\text{CH}_3)_2$), 2.31 (s, 3H, $-\text{OCH}_2\text{COCH}_3$), 4.60 (s, 2H, $-\text{OCH}_2\text{COCH}_3$), 6.86 (dd, 1H, $J = 8.2, 2.5$ Hz, aromatic proton), 7.09 – 7.11 (m, 1H, aromatic proton), 7.21 (dd, 1H, $J = 7.6, 0.5$ Hz, aromatic proton), 7.36 (t, 1H, $J = 7.9$ Hz, aromatic proton); ^{13}C NMR (151 MHz, chloroform- d) δ : 25.0 ($-\text{OC}(\text{CH}_3)_2$), 26.8 ($-\text{OCH}_2\text{COCH}_3$), 73.2 ($-\text{OCH}_2\text{COCH}_3$), 84.1 ($-\text{OC}(\text{CH}_3)_2$), 113.5, 113.8, 118.2, 120.8, 128.3, 130.2, 158.2, 205.8 ($-\text{OCH}_2\text{COCH}_3$). MS (ES $^+$): 277 ($M + 1$).

tert-butyl (3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenoxy)propyl) carbonate (**124**). The crude product was filtered on silica gel (eluent: petroleum ether/ EtOAc 9/1 v/v), obtaining a colourless oil. Yield: 96%. ^1H NMR (600 MHz, chloroform- d) δ : 1.34 (s, 12H, $-\text{OC}(\text{CH}_3)_2$), 1.47 (s, 9H, $-\text{C}(\text{CH}_3)_3$), 2.10–2.14 (m, 2H, $-\text{OCH}_2\text{CH}_2\text{CH}_2\text{OC}-$), 4.08 (t, 2H, $J = 6.1$ Hz, $-\text{OCH}_2\text{CH}_2\text{CH}_2\text{OC}-$), 4.25 (t, 2H, $J = 6.3$ Hz, $-\text{OCH}_2\text{CH}_2\text{CH}_2\text{OC}-$), 6.98 (ddd, 1H, $J = 8.2, 2.7, 0.8$ Hz, aromatic proton), 7.27 (t, 1H, $J = 7.5$ Hz, aromatic protons), 7.32 (s, 1H, aromatic proton), 7.38 (d, 1H, $J = 6.5$ Hz, aromatic proton); ^{13}C NMR (151 MHz, chloroform- d) δ : 24.9 ($-\text{OC}(\text{CH}_3)_2$), 27.9 ($-\text{C}(\text{CH}_3)_3$), 28.9 ($-\text{OCH}_2\text{CH}_2\text{CH}_2\text{OC}-$), 64.0 ($-\text{OCH}_2\text{CH}_2\text{CH}_2\text{OC}-$), 64.2 ($-\text{OCH}_2\text{CH}_2\text{CH}_2\text{OC}-$), 82.1 ($-\text{C}(\text{CH}_3)_3$),

83.6, 83.9 ($-\text{OC}(\text{CH}_3)_2$), 114.6, 118.3, 119.7, 127.3, 129.0, 153.6, 158.3 ($-\text{COOC}(\text{CH}_3)_3$). MS (ES⁺): 401 (M + 40).

General Procedure for the synthesis of compounds **119**, **121** and **125**. Pd(PPh₃)₄ (63.8 mg, 0.0552 mmol, 0.20 eq.) was added to a solution of **114** (145 mg, 0.276 mmol) and K₂CO₃ (114 mg, 0.579 mmol, 3.00 eq.) in dioxane/water mixture (10 mL 9:1 v/v). After stirring the resulting mixture under a nitrogen atmosphere for 1 h at r.t., the corresponding boronic acid pinacol ester (262 mg, 0.579 mmol, 3.0 eq.) was added. The reaction mixture was then heated at reflux under a nitrogen atmosphere. After 5–6 h it was cooled to room temperature and concentrated under reduced pressure. The crude material was taken-up with water (50 mL) and the mixture was extracted with EtOAc (3 × 40 mL). The combined organic layers were dried over Na₂SO₄ and concentrated under reduced pressure. The crude product was purified by flash chromatography (see the conditions below).

N-(3'-(2,2-Difluoropropoxy)-2,3,5,6-tetrafluoro-[1,1'-biphenyl]-4-yl)-2-((4-methoxybenzyl)oxy)pyrazolo[1,5a]pyridine-3-carboxamide (**119**). The crude product was purified by flash chromatography (eluent: petroleum ether/ EtOAc 8/2 then 7:3 v/v) giving a solid. This solid was then triturated with diisopropyl ether, and the title compound was obtained as a yellow solid (melting point= 117.0 – 114.6°C). Yield: 79%. ¹H NMR (600 MHz, chloroform-

d) δ : 1.80 (t, 3H, $J = 6.9$ Hz, $-\text{OCH}_2\text{CF}_2\text{CH}_3$), 3.83 (s, 3H, $-\text{OCH}_3$), 4.15 (t, 2H, $J = 11.3$, Hz, $-\text{OCH}_2\text{CF}_2\text{CH}_3$), 5.52 (s, 2H, $-\text{CH}_2\text{Ar}$), 6.92 (td, 1H, $J = 6.9$, 1.2 Hz, H-b), 6.96 (d, 2H, $J = 8.6$ Hz, H-n), 7.02-7.05 (m, 2H, $J = 8.0$ Hz, aromatic proton and H-c), 7.10 (d, 1H, $J = 7.6$ Hz, aromatic proton), 7.42 (td, 2H, $J = 5.21$, 8.34, 5.2 Hz, aromatic protons), 7.48 (d, 2H, $J = 8.6$ Hz, H-m), 8.25 (d, 1H, $J = 8.8$ Hz, H-a), 8.32 (s, 1H, NH), 8.34 (d, 1H, $J = 6.9$ Hz, H-d); ^{13}C NMR (151 MHz, chloroform- d) δ : 21.1 (t, $J = 25.7$ Hz, $-\text{OCH}_2\text{CF}_2\text{CH}_3$), 55.4 ($-\text{OCH}_3$), 69.8 (t, $J = 35.0$ Hz, $-\text{OCH}_2\text{CF}_2\text{CH}_3$), 72.3 ($-\text{CH}_2\text{Ar}$), 89.9 (C-f), 113.3 (C-b), 114.3 (C-n), 115.5, 116.2 (t, $J = 14.6$ Hz, C-s)*, 116.7, 117.4 (t, $J = 17.1$ Hz, C-p)*, 118.8 (C-d), 121.5 (t, $J = 239.6$ Hz, $-\text{OCH}_2\text{CF}_2\text{CH}_3$), 123.8, 127.7, 128.3 (C-a), 128.8 (C-e), 128.9 (C-l), 129.9 (C-c), 130.3 (C-m), 142.6 (d, $J = 237.4$ C-r)**, 143.2, 144.13 (d, $J = 237.4$, C-q)**, 158.06 (C-o), 160.18 (C-h), 160.54, 162.96 (C-g); MS (ES+): 616 [M+1], 638 (M+22), 654 (M+39).

2-((4-Methoxybenzyl)oxy)-N-(2,3,5,6-tetrafluoro-3'-(2-oxopropoxy)-[1,1'-biphenyl]-4-yl)pyrazolo[1,5-a]pyridine-3-carboxamide (**121**). The crude product was purified by flash chromatography (eluent: petroleum ether/ EtOAc 8/2 then 7:3 v/v) giving a solid. This solid was then triturated with diisopropyl ether, and the title compound was obtained as a pale-yellow solid (melting point= 78.4 - 81.5°C). Yield: 70%. ^1H NMR (600 MHz,

chloroform-d) δ : 2.31 (s, 3H, $-\text{OCH}_2\text{COCH}_3$), 3.83 (s, 3H, $-\text{OCH}_3$), 4.58 (s, 2H, $-\text{OCH}_2\text{COCH}_3$), 5.52 (s, 2H, $-\text{CH}_2\text{Ar}$), 6.92 (td, 1H, $J = 7.0$ Hz, H-b), 6.93-6.98 (m, 4H, aromatic protons and H-n), 7.09 (d, 1H, $J = 7.6$ Hz, H-c), 7.40-7.43 (m, 1H, aromatic protons), 7.48 (d, 2H, $J = 7.6$ Hz, H-m), 7.48 (d, 2H, $J = 8.6$ Hz, H-n), 8.25 (d, 1H, $J = 8.8$ Hz, H-a), 8.31 (s, 1H, $-\text{NH}$), 8.34 (d, 1H, $J = 6.9$ Hz, H-d); ^{13}C NMR (151 MHz, chloroform-d) δ : 25.8 ($-\text{OCH}_2\text{COCH}_3$), 55.5 ($-\text{OCH}_3$), 72.3 ($-\text{OCH}_2\text{COCH}_3$), 73.2 ($-\text{CH}_2\text{Ar}$), 89.9 (C-f), 113.3 (C-b), 114.3 (C-n), 115.3, 116.1 (t, $J = 16.8$ Hz, C-s)*, 116.6, 117.3 (t, $J = 16.3$ Hz, C-p)*, 118.8 (C-d), 123.8, 127.7, 128.3 (C-a), 128.8 (C-e), 128.9 (C-l), 130.0 (C-c), 130.3 (C-m), 142.6 (d, $J = 233.2$ Hz, C-r)**, 143.2, 144.2 (d, $J = 221.7$ Hz, C-q)**, 157.9 (C-o), 160.17 (C-h), 160.55, 162.96 (C-g), 205.40 ($-\text{OCH}_2\text{COCH}_3$); MS (ES⁻): 592 (M-1).

Tert-butyl(3-((2',3',5',6'-tetrafluoro-4'-(2-((4-methoxybenzyl)oxy)pyrazolo[1,5-a]pyridine-3-carboxamido)-[1,1'-biphenyl]-3-yl)oxy)propyl) carbonate **(125)**. The crude product was purified by flash chromatography (eluent: petroleum ether/ EtOAc 75/25 v/v) giving a solid. This solid was then triturated with diisopropyl ether, and the title compound was obtained as a pale-yellow solid (melting point= 65.3 - 67.1°C). Yield: 67%. ^1H NMR (600 MHz, chloroform-d) δ : 21.48 (s, 9H, $-\text{C}(\text{CH}_3)_3$), 2.14-2.18 (m, 2H- $\text{OCH}_2\text{CH}_2\text{CH}_2\text{OCOC}(\text{CH}_3)_3$), 3.82

(s, 3H, -OCH₃), 4.09 (t, 2H, J=2.1 Hz, -OCH₂CH₂CH₂OCOC(CH₃)₃), 4.27 (t, 2H, J= 6.3 Hz, -OCH₂CH₂CH₂OCOC(CH₃)₃), 5.52 (s, 2H, -CH₂Ar), 6.91 (t, 1H, J= 6.91 Hz, H-c), 6.94-6.99 (m, 4H, H-m and aromatic protons), 7.02 (d, 1H, J= 7.5 Hz, aromatic proton), 7.37-7.42 (m, Hz, H-b and aromatic proton), 7.48 (d, 2H, J= 8.6 Hz, H-n), 8.25 (d, 1H, J= 8.8 Hz, H-a), 8.30 (s, 1H, -NH), 8.34 (d, 1H, J=6.9 Hz, H-d); ¹³C NMR (151 MHz, chloroform-d) δ: 27.9 (-C(CH₃)₃), 55.5 (-OCH₃), 63.9 (OCH₂CH₂CH₂OCOC(CH₃)₃), 64.5 (-OCH₂CH₂CH₂OCOC(CH₃)₃), 72.3 (-CH₂Ar), 75.2 (-OCH₂CH₂CH₂OCOC(CH₃)₃), 82.3 (-C(CH₃)₃), 90.0 (C-f), 113.3 (C-b), 114.3 (C-n), 115.4, 116.3 (C-s)*, 116.3, 115.86 C-p)*, 118.8 (C-d), 122.8, 127.7, 128.3 (C-a), 128.6 (C-l), 128.8 (C-e), 129.7 (C-c), 130.3 (C-m), 144.2 (d, J = 235.3 Hz, C-r)**, 145.5 (d, J = 221.4 Hz, C-q)**, 153.56, 158.9 (C-o), 160.2 (C-h), 160.6, 163.0 (C-g), 200.7 (-OCH₂CH₂CH₂OCOC(CH₃)₃). MS (ES-): 640 (M-58).

General Procedure: removal of the 4-methoxybenzyloxy moiety to give final compounds 40-42. Thioanisole (187 mg, 1.51 mmol, 10 eq.) was added to a solution of the corresponding starting material (94.8 mg, 0.151 mmol, 1.0 eq.) in TFA (2 mL). The mixture was stirred at room temperature for 30 min - 1 h. The mixture was partially concentrated, and the crude product was taken up with water (20 mL) and the mixture was extracted with EtOAc

(3 × 20 mL). The crude product was purified by flash chromatography (see the conditions below).

2-Hydroxy-N-(2,3,5,6-tetrafluoro-3'-(3-hydroxypropoxy)-[1,1'-biphenyl]-4-yl)pyrazolo[1,5-a]pyridine-3-carboxamide (**42**). The crude product was purified by flash chromatography (eluent: from DCM to DCM / MeOH 98/2 v/v) giving a solid. The solid underwent to Combiflash Rf 200 purification in reverse phase (eluent: from MeOH /water 35/75 v/v to water after thirteen volumes of column). The title was obtained as a white solid (melting point= X). Yield: 34%. ¹H NMR (600 MHz, DMSO-d₆) δ: 1.86-1.90 (m, 2H, -OCH₂CH₂CH₂OH), 3.57 (dd, 2H, J = 10.2, 5.4 Hz, -OCH₂CH₂CH₂OH), 4.09 (t, 2H, J = 6.3 Hz, -OCH₂CH₂CH₂OH), 4.57 (br t, 1H, J = 3.8, -OCH₂CH₂CH₂OH), 6.96 (t, 1H, J = 7.0 Hz, H-b), 7.08-7.11 (m, 3H, aromatic protons), 7.45-7.48 (m, 2H, H-c, aromatic proton), 7.91 (d, 1H, J = 7.0 Hz, H-d), 8.55 (d, 1H, J = 8.7 Hz, H-d), 9.37 (br s, 1H, -NH), 12.92 (br s, 1H, -OH); **NMR da rifare** MS (ES⁻): 474 (M-1).

N-(3'-(2,2-difluoropropoxy)-2,3,5,6-tetrafluoro-[1,1'-biphenyl]-4-yl)-2-hydroxypyrazolo[1,5-a]pyridine-3-carboxamide (**40**). The crude product was purified by flash chromatography (eluent: from DCM to DCM / EtOAc 95/5 v/v) giving a solid. This solid was then triturated with diisopropyl ether, and the title compound was obtained as a white solid (melting point= 229.6 - 231.7°C). Yield:

43%. MS (ES⁺): 494 [M-1]. ¹H NMR (600 MHz, DMSO-d₆) δ: 1.76 (t, 3H, J = 19.2 Hz, -OCH₂CF₂CH₃), 4.36 (t, 2H, J = 12.7 Hz, -OCH₂CF₂CH₃), 7.03 (t, 1H, J = 6.8 Hz, H-b), 7.17-7.20 (m, 2H, aromatic protons), 7.24 (s, 1H, aromatic proton), 7.50-7.52 (m, 2H, H-c and aromatic proton), 7.98 (d, 1H, J = 8.7 Hz, aromatic proton), 8.62 (d, 1H, J = 6.7 Hz, H-a), 8.94 (br s, 1H, -NH), 12.83 (br s, 1H, -OH); ¹³C NMR (151 MHz, DMSO-d₆) δ: 21.38 (t, J = 24.9 Hz, -OCH₂CF₂CH₃), 68.9 (t, J = 31.5 Hz, -OCH₂CF₂CH₃), 88.2 (C-f), 113.2 (C-b), 115.8, 116.5 (C-d), 116.8 (t, J = 10.6 Hz, C-s)*, 117.8 (C-p)*, 119.9 (t, J = 243.5 Hz, -OCH₂CF₂CH₃), 123.3, 128.0, 128.3, 129.1 (C-a), 130.1 (C-c), 141.6 (d, J = 269.4 Hz, C-q)**, 141.7 (C-e), 142.8 (d, J = 244.7 Hz, C-r)**, 152.6, 157.6 (C-h), 160.4, 170.6 (C-g); ¹⁹F NMR (565 MHz, DMSO-d₆) -145.3 (s, 2F, -OCH₂CF₂CH₃), -97.0 (d, 2F, J = 14.7 Hz), -96.9 (d, 2F, J = 15.0 Hz); MS (ES⁻): 494 [M-1].

2-Hydroxy-N-(2,3,5,6-tetrafluoro-3'-(2-oxopropoxy)-[1,1'-biphenyl]-4-yl)pyrazolo[1,5-a]pyridine-3-carboxamide (**41**). The crude product was purified by flash chromatography (eluent: from DCM to DCM/ MeOH 95/5 v/v) giving a solid. This solid was then triturated with diisopropyl ether, and the title compound was obtained as a white solid (melting point = 218.2 - 220.3°C). Yield: 43%. ¹H NMR (600 MHz, DMSO-d₆) δ: 2.17 (s, 3H, -OCH₂COCH₃), 4.90 (s, 2H, -OCH₂COCH₃), 7.02 (t, 1H, J = 6.6 Hz, H-b),

7.06 (d, 1H, $J = 8.5$ Hz, aromatic proton), 7.11–7.12 (m, 2H, aromatic protons), 7.46 (t, 1H, $J = 7.9$ Hz, H-c), 7.50 (t, 1H, $J = 7.8$ Hz, aromatic proton), 7.97 (d, 1H, $J = 8.7$ Hz, H-d), 8.60 (d, 1H, $J = 8.7$ Hz, H-a), 8.99 (s, 1H, -NH), 12.90 (br s, 1H, -OH); ^{13}C NMR (151 MHz, DMSO- d_6) δ : 26.2 (-OCH₂COCH₃), 72.1 (-OCH₂COCH₃), 88.2 (C-f), 113.2 (C-b), 115.5, 116.2 (C-d), 116.8, 116.9 (C-s)*, 118.1 (C-p)*, 122.7, 127.8, 128.3, 129.1 (C-a), 129.9 (C-c), 140.0 (d, $J = 246.7$ Hz, C-q)**, 141.7 (C-e), 143.80 (d, $J = 252.7$ Hz C-r)**, 157.9, 160.5 (C-h), 162.6 (C-g); 203.8 (-OCH₂COCH₃). MS (ES⁻): 472 [M-1].

2-Hydroxy-N-(2,3,5,6-tetrafluoro-3'-(2-hydroxypropoxy)-[1,1'-biphenyl]-4-yl)pyrazolo[1,5-a]pyridine-3-carboxamide (**43**). NaBH₄ (8.00 mg, 0.210 mmol, 2 equiv) (5 mL) was added to a mixture of **41** (50.0 mg, 0.105 mmol) in abs EtOH (5 mL). The reaction mixture was stirred at room temperature 1 h, then concentrated under vacuum. The crude material was taken-up with water (10 mL) and the mixture was extracted with EtOAc (3 × 10 mL). The combined organic layers were dried over Na₂SO₄ and concentrated under reduced pressure. The crude material was purified by trituration with diisopropyl ether, affording a white solid (melting point= 175.7 – 177.6 °C). Yield: 90%. ^1H NMR (600 MHz, DMSO- d_6) δ : 1.16 (d, 3H, $J = 6.2$ Hz, -OCH₂CH(OH)CH₃), 3.86 (td, 2H, $J = 9.2, 5.7$ Hz, -OCH₂CH(OH)CH₃), 3.94–4.05 (m, 1H, -

$\text{OCH}_2\text{CH}(\text{OH})\text{CH}_3$), 4.90 (s, 1H, $-\text{OCH}_2\text{CH}(\text{OH})\text{CH}_3$), 7.03 (t, 1H, $J = 6.6$ Hz, H-b), 7.09–7.12 (m, 3H, aromatic protons), 7.46 (t, 1H, $J = 7.9$ Hz, H-c), 7.51 (t, 1H, $J = 7.8$ Hz, aromatic proton), 7.98 (d, 1H, $J = 8.7$ Hz, H-d), 8.62 (d, 1H, $J = 6.7$ Hz, H-a), 8.92 (s, 1H, $-\text{NH}$), 12.86 (br s, 1H, acid $-\text{OH}$); ^{13}C NMR (151 MHz, DMSO- d_6) δ : 20.10 ($-\text{OCH}_2\text{CH}(\text{OH})\text{CH}_3$), 64.5 ($-\text{OCH}_2\text{CH}(\text{OH})\text{CH}_3$), 73.4 ($-\text{OCH}_2\text{CH}(\text{OH})\text{CH}_3$), 88.2 (C-f), 113.2 (C-b), 114.4, 115.6, 116.3 (C-d), 116.9 (C-s)*, 117.5 (C-p)*, 122.3, 127.8, 128.3, 129.2 (C-a), 130.0 (C-c), 141.68 (d, $J = 242.9$ Hz, C-q)**, 141.72 (C-e), 142.8 (d, $J = 25.3$ Hz, C-r)**, 158.7, 160.4 (C-h), 162.7 (C-g); MS (ES $^-$): 474 [M-1].

Protein expression and purification. BL21DE3 PyrD E. Coli cells were transformed using the plasmid construct pFN2A-hDHODH (kindly given by Department of Drug Science and Technology, University of Turin, Turin). The vector produces hDHODH as an N-terminal GST-fusion protein. Cells were grown at 37 °C in LB medium supplemented with 0.1 mM flavin mononucleotide (Cayman Chemical). After 20 h of growth, cells were induced with 0.8 mM isopropyl-D-thiogalactopyranoside at an OD₆₀₀ of 0.5–0.7 at 28 °C for an additional 6 h. A cell pellet from 250 mL of culture was lysed in 20 mL of PBS (50 mM Na₂HPO₄, 50 mM NaH₂PO₄, 500 mM NaCl), which had been supplemented with 24 mg of lysozyme and 0.2% v/v protease inhibitor cocktail, incubated for 30 min over ice, and disrupted by sonication

(total sonication time: 8 minutes with On/Off cycles of 10"/50"). Triton X-100 was added to the lysate, to a final concentration of 1%, before centrifugation at 14000g for 40 min at 4 °C. The clarified supernatant was incubated with DNase I for 30 min at room temperature, supplemented with 2 mM dithiothreitol (DTT), and filtered through a 0.45 µm syringe filter as previously described by Sainas *et al.*⁶. The GST-fused enzyme was purified from the bacterial lysate using affinity chromatography on immobilized glutathione-sepharose columns (GE- HiTrap Protein G HP 1ml). The GST tag was not cleaved for further analysis. All the reagents used in the protein expression and purification were supplied by Merck / Sigma-Aldrich, if not otherwise specified.

hDHODH inhibition assay. The enzymatic inhibition assay was optimized for being performed on a 96 well plate and to achieve higher throughput. For each well of the plate a total volume of 200 µL was used: 5 µL of purified GST-hDHODH; 60 µL of 2,6-dichloroindophenol (DCIP) 500 µM; 20 µL of coenzyme Q10 enzyme 100 µM; 20 µL of dihydroorotate (DHO) 500 µM; Tris-HCl pH8 up to a final volume of 200 µL. Inhibitory activity was assessed by monitoring the reduction of DCIP, which is associated with the oxidation of dihydroorotate as catalysed by the DHODH enzyme. The enzyme was pre-incubated for 5 min at 37 °C in Tris-HCl pH8 with coenzyme Q10, with DCIP (50

μM) and with the compounds to be tested used at different concentrations (final DMSO concentration 0.1% v/v). The reaction was initiated by the addition of DHO (500 μM), and the absorbance kinetic reduction was monitored at $\lambda = 650 \text{ nm}$ using a multi-plate reader (Tecan, M1000Pro). In order to assess the minimum and maximum absorbance values of the enzymatic reaction, a Min control value was obtained by measuring the absorbance without DHO. Similarly, a Max value was obtained by measuring the absorbance with DHO, but none inhibitor. A blank reduction calculation was also performed by measuring the absorbance values using 180 μL of Tris-HCl and 20 μL of coenzyme Q10. The Instrument was set to read the absorbance values every 10s for a total read time of 10 minutes at 37 °C. The initial rate was measured in the first 5 min ($\epsilon = 10\,400 \text{ M}^{-1} \text{ cm}^{-1}$) and an IC₅₀ value was calculated, ¹⁹using GraphPad Prism 7 software. Values are means $\pm$ SE of three independent experiments.

Statistical analysis. Statistical analyses were performed on Prism software, version 5.0 (GraphPad Software, San Diego, CA). Data are reported as means $\pm$ SD. Two-tail paired Student's t tests were calculated to assess the differences between mean values, and $P < 0.05$ was considered significant. For the determination of EC₅₀, a non-linear regression model was applied.

Preliminary ADME and chemophysical profiling

Solubility assay at pH 7.4. Solubility was assayed in Phosphate Buffered Saline (PBS: 12 mM with NaCl 137 mM and KCl 2.7 mM, pH 7.4). Each solid compound (1 mg) was added to 1 mL of PBS. The samples were shaken in an orbital shaker at 25 °C for 24 h. These suspensions were filtered through a PTFE 0.45 µm filter (VWR), and the solutions were chromatographically analyzed using a Perkin Elmer Ultra High Performance Liquid Chromatography (UHPLC) instrument, equipped with a reverse-phase (RP) C18 Phenomenex column (2.1x100 mm, 1.7 µm particle size). Gradient elution: the ratio of eluents A and B (0.1% trifluoroacetic acid in water and 0.1% trifluoroacetic acid in acetonitrile, respectively) changed linearly from 60% A-40% B to 0% A-100% B in 12 minutes, followed by 5 minutes in isocratic elution at 100% of eluent B and then 4 minutes in equilibration elution to reset the starting conditions. The flow rate was 0.5 mL/min. Standard injection volumes were either 2 or 4 µl for poorly soluble compounds. The detection system was a Perkin Elmer diode-array-detector. The wavelengths that were monitored for each compound were defined according to the compound's own absorption spectrum. Solubility, expressed as µM concentration of the saturated solution, was calculated via interpolation with external calibration curves that were obtained with solutions of each compound in acetonitrile.

Clog P and log D (pH 7.4). ClogP values were calculated using the Bio-Loom program for Windows, Version 1.5 (BioByte). The partition coefficients between n-octanol and PBS at pH 7.4 ($\log D^{7.4}$) were obtained using the shake-flask technique at room temperature. In the shake-flask experiments, 50 mM of phosphate buffered saline pH 7.4 was used as the aqueous phase. The organic (n-octanol) and aqueous phases were mutually saturated by shaking for 4 h. The compounds were solubilized in the buffered aqueous phase at the highest concentration compatible with solubility and appropriate amounts of n-octanol were added. The two phases were shaken for about 20 min, by which time the partitioning equilibrium of solutes had been reached, and then centrifuged (10000 rpm, 10 min). The concentration of the solutes was measured in the aqueous phase using a UV spectrophotometer (Varian Cary 50BIO); absorbance values (recorded for each compound at the wavelength of maximum absorption), were interpolated in calibration curves obtained using standard solutions of the compounds ($r^2 > 0.99$). Each log D value is an average of at least six measurements.

Protein binding in vitro. This was achieved via ultrafiltration using commercially available membrane systems (Centrifree ultrafiltration devices with ultracel YM-T membrane, Merck). A solution of the selected compound in DMSO was added to human serum (sterile-

filtered from human male AB plasma, Sigma-Aldrich), to give the final concentration of 50 μM with 2% of co-solvent. 1 mL of the solution obtained in the sample reservoir of the ultrafiltration device was gently shaken in an orbital shaker at 37 °C for 1 h. The tube was then centrifuged at 1000 x g for 15 min. The concentrations of the compounds in the ultrafiltrate and filtrate were determined using reverse-phase UHPLC and the chromatographic conditions were those described above with different injection volumes; 20 μL for ultrafiltrate samples and 2 μL for filtrate samples. The quantitation of the compounds in the filtrate and in ultrafiltrate was performed using two different calibration curves of compound standard solutions (linearity determined in concentration ranges of 0.5–25 μM with injection volume of 20 μL for ultrafiltrate and 10–100 μM with injection volume of 2 μL for filtrate ; $r^2 > 0.99$). The recovery of the ultrafiltration process was calculated in order to discover whether any compound was lost during ultrafiltration, in view of the limited solubility of tested compounds.

$$\text{Recovery} = 100 \times [(\text{vol.}_{\text{bound}} \times \text{conc}_{\text{bound}}) + (\text{vol.}_{\text{unbound}} \times \text{conc}_{\text{unbound}})] / \text{vol}_{\text{initial serum}} \times \text{conc}_{\text{initial}}$$

$\text{vol.}_{\text{bound}}$: calculated by dividing the weight of the bound fraction (difference between the weights of the sample reservoir after ultrafiltration and empty), by its

density (0.991 g/mL assessed by weighing five replicates of a known volume of the bound fraction).

vol._{unbound}: calculated by dividing the weight of the unbound fraction (difference between the weights of the ultrafiltrate cup after and before ultrafiltration), by its density (0.999 g/mL assessed by weighing five replicates of a known volume of the unbound fraction).

conc_{bound}: calculated using the RP-HPLC method.

conc_{unbound}: calculated using the RP-HPLC method (calibration with standard additions)

Average recovery was 90% for all tested compounds.

Virological Methods:

Herpes Simplex Virus type 1 and 2 (HSV-1/2)

Cells, culture conditions and viruses - African green monkey kidney cells (Vero) (ATCC CCL-81) were cultured in Dulbecco's Modified Eagle Medium (DMEM; Euroclone) supplemented with 10% fetal bovine serum (FBS; Euroclone), 2 mM L-glutamine, 1 mM sodium pyruvate, 100 U/ml penicillin, and 100 mg/ml streptomycin sulfate.

Clinical isolates of HSV-1 and HSV-2 sensitive to acyclovir were kindly provided by Dr. V. Ghisetti (Amedeo di Savoia Hospital, Turin, Italy). HSV-1 and HSV-2 were

propagated and titrated by plaque assay on Vero cells as previously described (Terlizzi et al., Antiviral Research 132, 154-164, 2016).

Antiviral Assay - To determine cell viability, Vero cells were exposed to increasing concentrations of **17**, **1** or Brequinar. After 3 days of incubation, the number of viable cells was determined using the Cell Titer Glo(R) Luminescent Cell Viability assay (Promega).

To evaluate the anti-HSV activity of **1**, **17** or Brequinar by plaque reduction assays (PRA), Vero cells were seeded in 24-well plates at a density of 70×10^3 cells. After 24 h, cells were treated with different concentrations of **17**, **1** or Brequinar 1 h prior to infection, and then infected with HSV-1 or HSV-2 (50 PFU/well). Following virus adsorption (2 h at 37 C), cultures were maintained in medium-containing 0.8% methylcellulose (Sigma) plus compounds. At 48 h post infection (h.p.i.), cells were fixed and stained by using 20% ethanol and 1% crystal violet. Plaques were microscopically counted, and the mean plaque counts for each concentration expressed as a percentage of the mean plaque count for the control virus. The number of plaques was plotted as a function of drug concentration; concentration producing 50% reduction in plaque formation (EC_{50}) was determined as described by Terlizzi et al. (Antiviral Research 132, 154-164, 2016).

Influenza Virus

Cells, culture conditions and viruses - Madin Darby Canine Kidney cells (MDCK, ATCC CCL-34TM) were propagated in DMEM supplemented with 10% fetal bovine serum (FBS; Euroclone), 2 mM L-glutamine, 1 mM sodium pyruvate, 100 U/ml penicillin, and 100 µg/ml streptomycin sulfate. Infections were performed in the presence of 1 µg/ml of trypsin TPCK treated from bovine pancreas (Sigma-Aldrich) and 0.14 % of Bovine Serum Albumin (Sigma-Aldrich). The influenza virus strains A/Puerto Rico/8/34 (IAV) (VR-1469) and B B/Lee/40 (IBV) (VR-101) were obtained from ATCC. IAV and IBV were cultured and titrated by plaque assay on MDCK cells as described by Luganini *et al.*, Front. Microbiol. 9:1826, 2018.

Antiviral Assays - Cytotoxicity of **1** and Brequinar was determined on MDCK cells after a 72 h of treatment by means of the Cell Titer Glo(R) Luminescent Cell Viability assay (Promega).

The antiviral activity of **1** and Brequinar was determined by PRA. To this end, MDCK cells were seeded in 24-well plates (3×10^5 cells/well) and after 24 h they were exposed 1 h prior to infection to increasing concentrations of **1** or Brequinar and then infected with IAV or IBV (40 PFU/well). After virus adsorption (1 h at 37 °C), cultures were incubated in medium containing 0.7

% Avicel (FMC BioPolymer) plus **1** or Brequinar. At 48 h post-infection (h p.i.), the cells were fixed with a solution of 4% formaldehyde in phosphate-buffered saline 1X (PBS) for 1 h at room temperature (RT) and stained with a solution of 1% crystal violet. The microscopic plaques count then allowed to define the concentration of either **1** produced 50 reduction in plaque formation (EC₅₀) (Luganini et al., Front. Microbiol. 9:1826, 2018).

Respiratory Syncytial Virus (RSV)

Cells, culture conditions and viruses - HEp-2 cells (ATCC CCL-23) were maintained in Dulbecco's Modified Eagle Medium (DMEM; Euroclone) supplemented with 10% fetal bovine serum (FBS; Euroclone), 2 mM L-glutamine, 1 mM sodium pyruvate, 100 U/ml penicillin, and 100 mg/ml streptomycin sulfate.

The Respiratory Syncytial Virus (RSV) strains A-Long (VR-26) and B-Washington (RSV-9320 VR-955) were obtained from ATCC and propagated and titrated on HEp-2 cells as described by Rameix-Welti et al. Nat. Commun. 5:5104, 2014.

Antiviral Assays - Cytotoxicity assays of **1** and Brequinar were performed on HEp-2 cells with the Cell Titer Glo(R) Luminescent Cell Viability assay (Promega) after 72 h of incubation with the compounds.

The antiviral activity of **1** and Brequinar was determined by PRA. Briefly, HEp-2 cells were seeded in 24-well plates (3×10^5 cells/well) and after 24 h they were treated with different concentrations of **1** or Brequinar 1 h prior to infection, and then infected with RSV A or B (50 PFU/well). Following virus adsorption (2 h at 37 °C), cultures were maintained in medium-containing 0.3% methylcellulose (Sigma) plus compounds. At 96 h post infection (h.p.i.), cells were fixed and stained by using 20% ethanol and 1% crystal violet. Plaques were microscopically counted, and the mean plaque counts for each concentration expressed as a percentage of the mean plaque count for the control virus. The plaque numbers were plotted against the compound concentrations and the EC₅₀ was determined as the compound concentration producing 50% reduction of the plaque numbers.

alpha- and beta-coronavirus. Human lung fibroblasts MRC5 (ATCC CCL-171), the human colorectal carcinoma HCT-8 (ATCC CCL-244), the human lung adenocarcinoma Calu-3 (ATCC HTB-55), and the African green monkey kidney Vero E6 (ATCC CRL-1586) cell lines were purchased from the American Type Culture Collection (ATCC), and maintained in Dulbecco's Modified Eagle Medium (DMEM; Euroclone) supplemented with 10% fetal bovine serum (FBS, Euroclone), 2 mM glutamine, 1 mM sodium pyruvate, 100

U/ml penicillin, and 100 µg/ml streptomycin sulfate (P/S, both from Euroclone).

hCoV-229E (ATCC VR-740) and hCoV-OC43 (ATCC VR-1558) were purchased from ATCC, and propagated and titrated in MRC5 and HCT-8 cells, respectively. SARS-CoV-2 (2019-nCoV/Italy-INMI1) was obtained from EVAg, and propagated and titrated in Vero E6 cells. SARS-CoV-2/01/human/2020/SWE was isolated on Vero E6 cells from a nasopharyngeal sample, cultivated and titrated as previously described [18].

Cytotoxicity assays

Cells were seeded in 96-well plates and after 24 h exposed to increasing concentrations of compounds or vehicle (DMSO), as control. After 72 h of incubation, the number of viable cells was determined using either the CellTiter-Glo Luminescent assay (Promega) according to the specifications of the manufacturer, or the MTT method [19].

Antiviral assays

To select the mini-library of hDHODH inhibitors, focus forming reduction assays (FFRAs) [20] were performed on HCT-8 cell monolayers treated with the vehicle (DMSO) for with 0.1 µM of the different compounds 1 h prior to and during infection with the hCoV-OC43 (100 PFU/well). At

72 h post-infection (p.i.) cell monolayers were fixed, and subjected to indirect immunoperoxidase staining with a mAb against the hCoV-OC43 N protein (clone.542-D7; Millipore) (diluted 1: 100). Viral foci were microscopically counted, and the mean counts for each drug concentration were expressed as a percentage of the mean plaque counts of control virus (DMSO). To determine the anti-hCoV-229E activity of MEDS433 or brequinar, MRC5 cell monolayers were treated with different concentrations of the compounds 1 h prior to and during infection with hCoV-229E (100 PFU/well). After 72 h p.i., cell viability was measured using CellTiter-Glo assay as a surrogate measurement of the viral cytopathic effect (CPE), as previously described [21]. To measure the anti-SARS-CoV-2 activity of MEDS433 or brequinar, virus yield reduction assay (VRA) was performed with Vero E6 or Calu-3 cells. Briefly, cell monolayers were treated with the vehicle or increasing concentrations of compounds 1h before and during infection with SARS-CoV-2 (50 or 100 PFU/well). At 48 h p.i., SARS-CoV-2 in cell super-natants was titrated by plaque assay on Vero E6 cells. Compounds concentrations producing 50 and 90% reductions in plaque formation (EC50) were determined as compared to control treatment (DMSO).

SARS-CoV-2

Cells, culture conditions and viruses - Vero E6 cells (ATCC CRL-1586) were maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, 1 mM sodium pyruvate, 100 U/ml penicillin, and 100 mg/ml streptomycin sulfate. SARS-CoV-2 virus strain 2019-nCoV/Italy-INMI1 was obtained from Istituto Nazionale Malattie Infettive "Lazzaro Spallanzani", and propagated and titrated on Vero E6 cells.

Antiviral assay - Cytotoxicity assays of **1** and Brequinar were performed on Vero E6 cells by means of the MTT assay after 72 h of incubation with the compounds.

The antiviral activity of **1** and Brequinar was determined by virus yield reduction assay (VRA). Briefly, Vero E6 cells were seeded in 24-well plates and after 24 h they were treated with different concentrations of **1** or Brequinar 1 h prior to infection, and then infected with SARS-CoV-2 (50 PFU/well). Following virus adsorption (2 h at 37 °C), cultures were maintained in medium-containing compounds. At 72 h post infection (h.p.i.), cell supernatants were harvested and their infectivity was titrated by plaque assay on Vero E6 cell monolayers. Plaques were microscopically counted at 72 h.p.i., and the mean plaque counts for each concentration expressed as a percentage of the mean plaque count for the control virus. The plaques numbers were plotted against the

compound concentrations and the EC₅₀ was determined as the compound concentration producing 50% reduction of SARS-CoV-2 infectivity.

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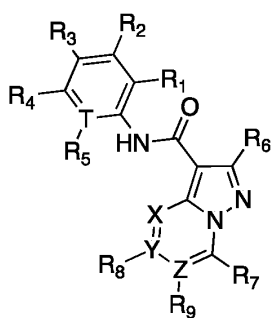
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CLAIMS

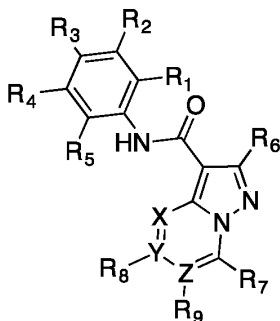
1. A compound for use in inhibiting virus replication or for use in the therapeutic treatment of viral infection in a subject, wherein the compound is selected from the group consisting of:

- compounds of Formula (I):



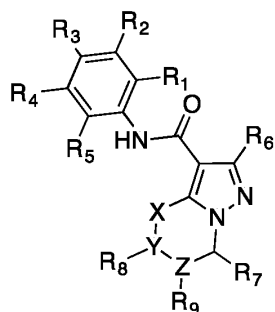
Formula (I)

- compounds of Formula (II):



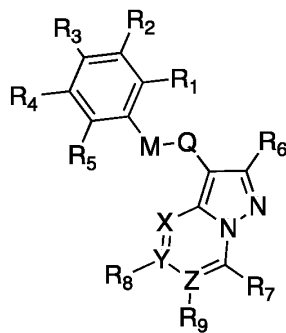
Formula (II)

- compounds of Formula (III):



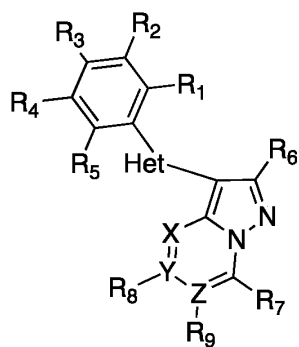
Formula (III)

- compounds of Formula (IV):



Formula (IV)

- compounds of Formula (V):



Formula (V)

wherein:

R₁, R₂, R₄ and R₅ are independently selected from a hydrogen atom, a halogen atom, an alkyl group, an alkoxy

group, a cycloalkyloxy group, an alkylthio group, a halo alkyl group, a halo alkyloxy group, a nitro group, a cyano group, and alkylamino group;

R₃ in Formulae (I), (II), (III), (IV) and (V) is independently selected from an optionally substituted phenyl group, heteroaryl group, pyridinyl group, piperidinyl group, phenoxy group, pyridinoxy group, piperidinyl group, phenylthio group, azinyl group, phenylsulfonyl group, phenylsulfinyl group, phenylsulfonylamino group, alkyl group, alkyloxy group, alkylthio group, halo alkyl group, and halo alkyloxy group;

R₇, R₈ and R₉ in Formulae (I), (II), (III), (IV) and (V) are independently selected from hydrogen atom, a halogen atom, a nitro group, a cyano group, a halo alkyl group, a thio alkyl group, an amino alkyl group, a alkyl group, and a hydroxy alkyl group;

R₆ in Formulae (I), (II), (III), (IV) and (V) is independently selected from a alkyloxy group, a halogen atom, an acyloxy group, a monophosphate group, a hydroxyl group, a thiol group, an amino group, or a salt thereof;

X, Y and Z in Formulae (I), (II), (III), (IV) and (V) are independently selected from a carbon atom, a nitrogen atom, an oxygen atom and a sulfur atom, with the proviso that when one of X, Y or Z is nitrogen, oxygen or sulfur, the other two of X, Y or Z are carbon atoms;

T in Formula (I) is a carbon atom or a nitrogen atom, with the proviso that when T is a nitrogen atom, R₅ in Formula (I) does not exist;

M in Formula (IV) is selected from a carbon sp² atom, a nitrogen sp³ atom, a nitrogen sp² atom, a carbonyl group and a sulfonyl group;

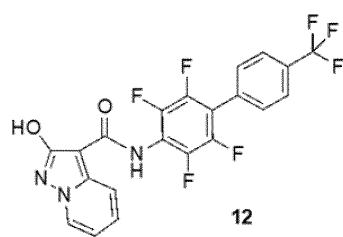
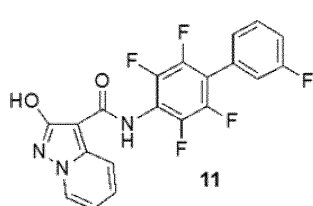
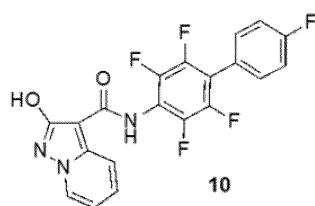
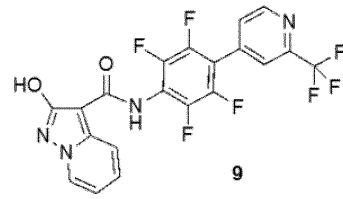
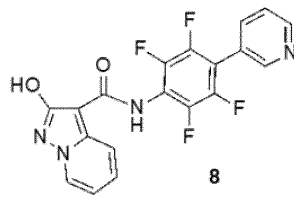
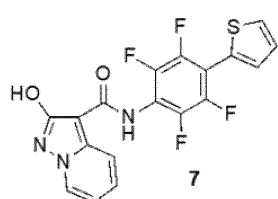
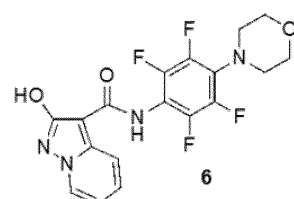
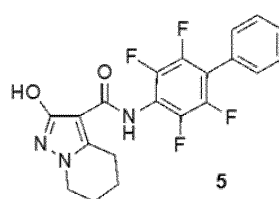
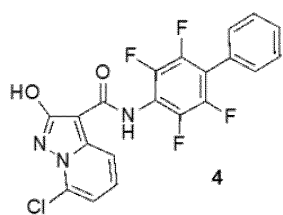
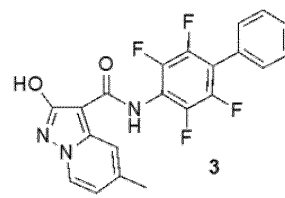
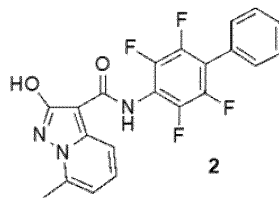
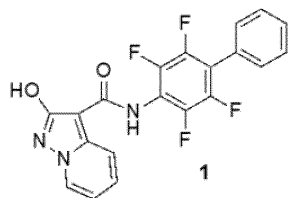
Q in Formula (IV) is selected from a carbon sp² atom, a carbonyl group, a thiocarbonyl group, a sulfonyl group, a polihalogenate-C₂-alkyl chain, a carbonylamino group, an aminocarbonyl group, a nitrogen sp² atom and a nitrogen sp³ atom,

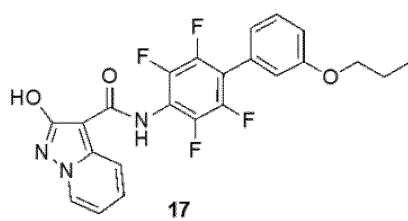
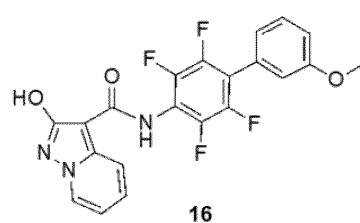
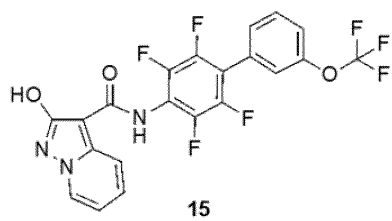
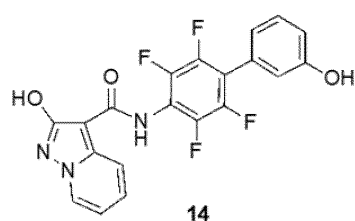
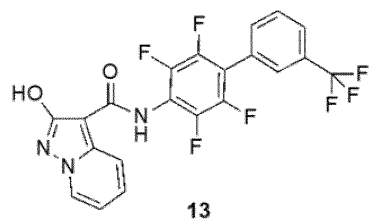
with the provisos that when M is a carbon sp² atom, Q is a carbon sp² atom; when M is a nitrogen sp³ atom, Q is selected from a carbonyl group, a thiocarbonyl group, a sulfonyl group, a polihalogenate-C₂-alkyl chain, a carbonylamino group, an aminocarbonyl group; when M is a nitrogen sp² atom, Q is a nitrogen sp² atom; when M is a carbonyl group or sulfonyl group, Q is a nitrogen sp³ atom;

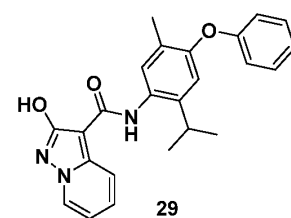
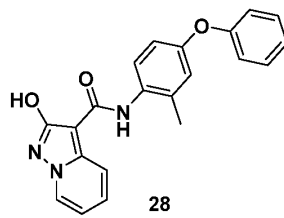
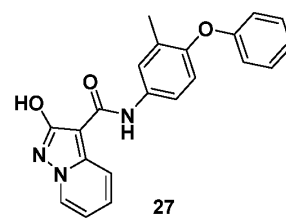
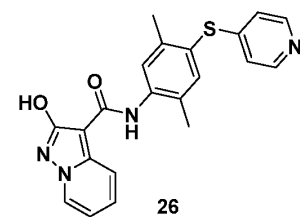
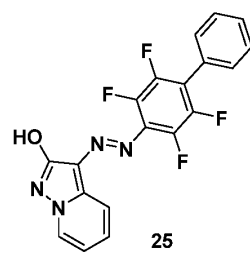
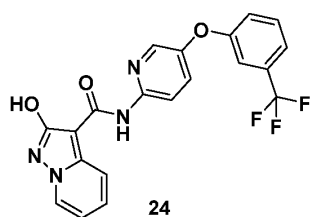
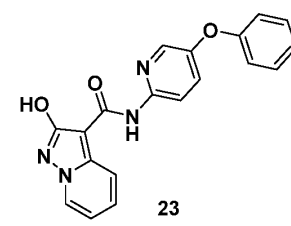
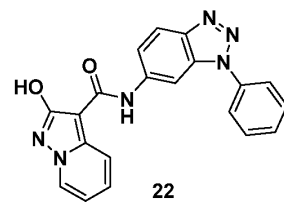
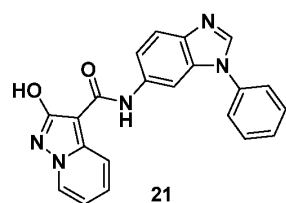
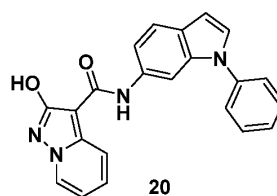
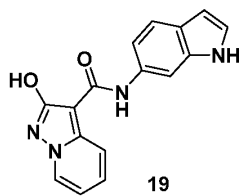
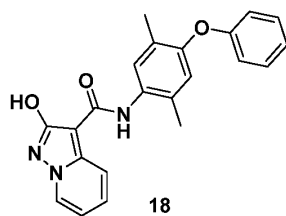
Het in Formula (V) is selected from azoles, such as an imidazole, a pyrazole, an oxazole, a thiazole, a triazole, an oxadiazole, a thiadiazole, a tetrazole.

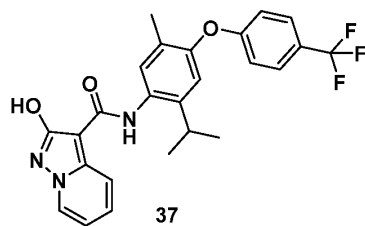
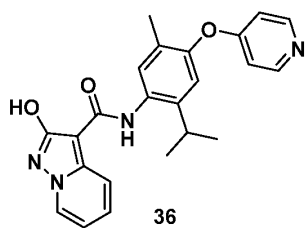
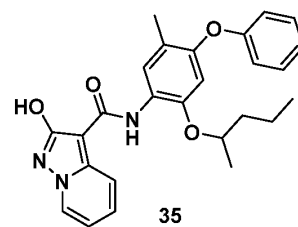
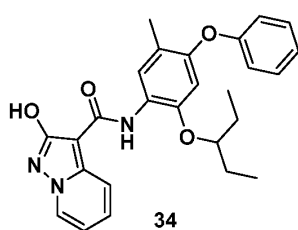
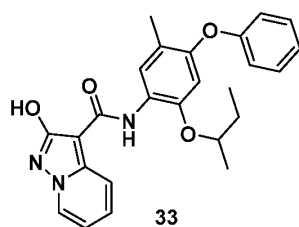
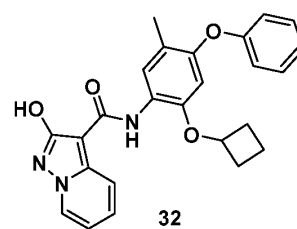
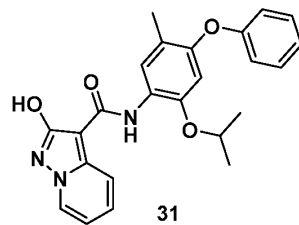
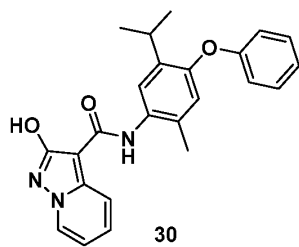
2. A compound for use according to claim 1, wherein the alkyls in the definitions of R₁, R₂, R₄ and R₅ are independently selected from the C₁-C₆ alkyls, preferably from the C₁-C₄ alkyls.

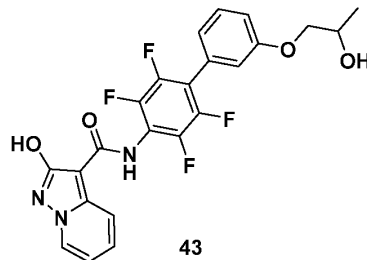
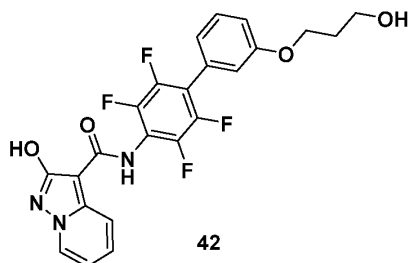
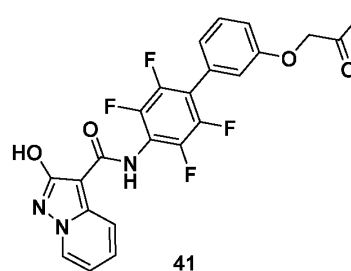
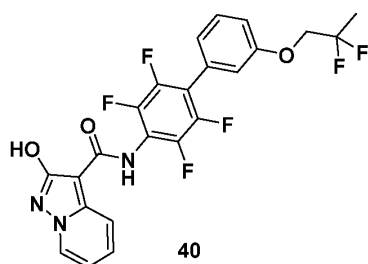
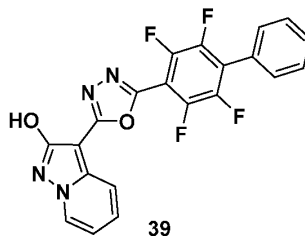
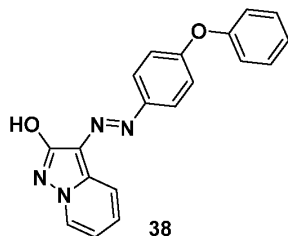
3. A compound for use according to claim or 2, wherein the alkyls in the definitions of R_3 are independently selected from the C1-C12 alkyls.
4. A compound for use according to any of claims 1 to 3, wherein the alkyls in the definitions of R_7 , R_8 and R_9 are independently selected from the C1-C6 alkyls, preferably from the C1-C4 alkyls.
5. A compound for use according to claim 1, which is a compound of Formula (I) wherein $X=Y=Z$ are sp^2 C atoms and R_9 is a proton.
6. A compound for use according to claim 1, which is a compound of Formula (II) in which $X=Y=Z$ are sp^3 C atoms and R_9 is a proton.
7. A compound for use according to any one of claims 1 to 6, wherein at least one of R_1 , R_2 , R_4 and R_5 is or contains a halogen atom, preferably a fluorine atom.
8. A compound for use according to any one of claims 1 to 7, wherein R_6 is OH or a monophosphate.
9. A compound for use according to claim 1, which is selected from the group consisting of:











10. A compound for use according to any one of claims 1 to 9, wherein the virus is a DNA or a RNA virus.

11. A compound for use according to any one of claims 1 to 9, wherein the virus is selected from the group consisting of *Herpesviridae*, *Orthomyxoviridae*, *Paramyxoviridae* and *Coronaviridae*.

12. A compound for use according to any one of claims 1 to 9, wherein the virus is selected from the group consisting of Herpes simplex virus 1 (HSV-1), Herpes simplex virus 2 (HSV-2), 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).

13. A pharmaceutical composition for use in inhibiting virus replication or in the treatment of a viral infection, the pharmaceutical composition comprising a compound as defined in any one of claims 1 to 9 and a pharmaceutically acceptable carrier, excipient and/or diluent.

14. The pharmaceutical composition for use according to claim 13, wherein the viral infection is caused by a DNA or RNA virus.

15. The pharmaceutical composition for use according to claim 13, wherein the viral infection is caused by a virus selected from the group consisting of *Herpesviridae*, *Orthomyxoviridae*, *Paramyxoviridae* and *Coronaviridae*, preferably from the group consisting of Herpes simplex virus 1 (HSV-1), Herpes simplex virus 2 (HSV-2), 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) .