Supporting information for

5,6-Dihydroxypyrimidine Scaffold to Target HIV-1 Nucleocapsid Protein

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[#]This work is dedicated to the beloved memory of Prof. Maurizio Botta (August 2, 2019) and Steven Harper (June 30, 2019)

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Molecular modelling for compounds 4, 25 and 20

Molecular docking was carried out with the FRED docking program (OpenEye)[1,2] using the highest accuracy while all other parameters were kept at their default value. The NMR structure of the NC in complex with a small molecule inhibitor (PDB 2M3Z) was used as a receptor[3]. The binding site was defined as the protein region occupied by the inhibitor above the side chain of Trp37, and was prepared with the make_receptor program (OpenEye). Ligands conformational analysis was carried out with OMEGA (OpenEye)[4,5] by storing up to 600 conformers of each ligand. The final ligand score was assigned through a rescoring procedure with XSCORE[6].

Figure S1. Predicted binding mode of 4 (a), 25 (b), and 20 (c) within the hydrophobic pocket of the NC. The protein is shown as cyan cartoon. Residues within 5 Å from the ligands are showed as lines, and are labeled. NC inhibitors are showed as yellow sticks. H-bond interactions are highlighted by black dashed lines.

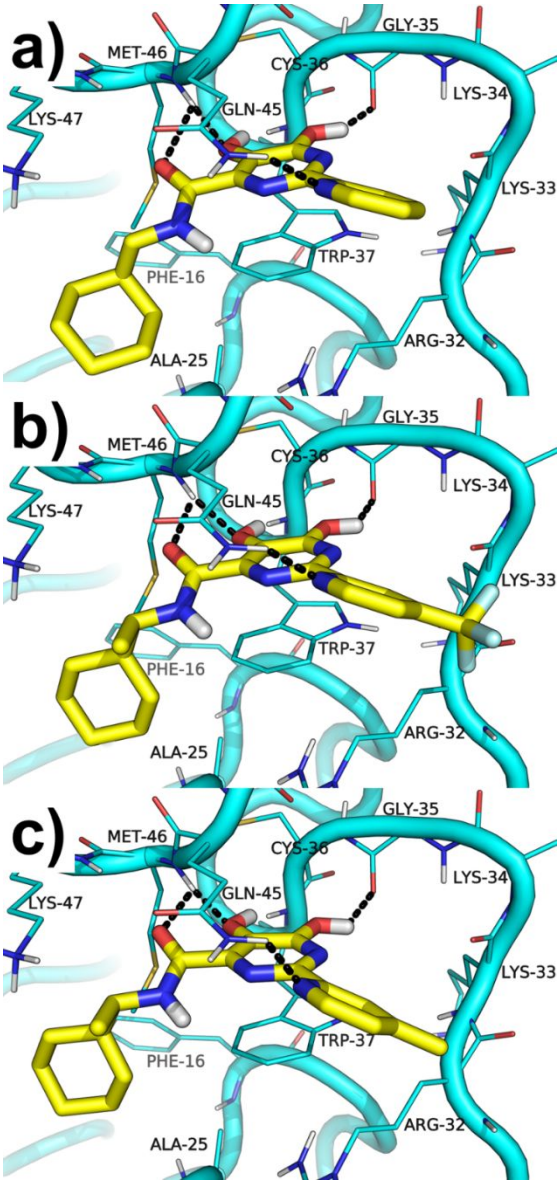


Figure S2. Over-imposition between the NMR-based binding mode of the reference NC inhibitor identified by Goudreau et al[3]and the predicted binding mode of compound **4** (a), **25** (b), and **20** (c). The reference NC inhibitor is shown as green sticks, and its H-bond network is highlighted by green dashed lines. Compounds studied in this work as showed as yellow sticks, and their H-bond networks are highlighted by black dashed lines. Residues contacted by NC inhibitors are highlighted by an orange sphere (contact atom) and are labeled.

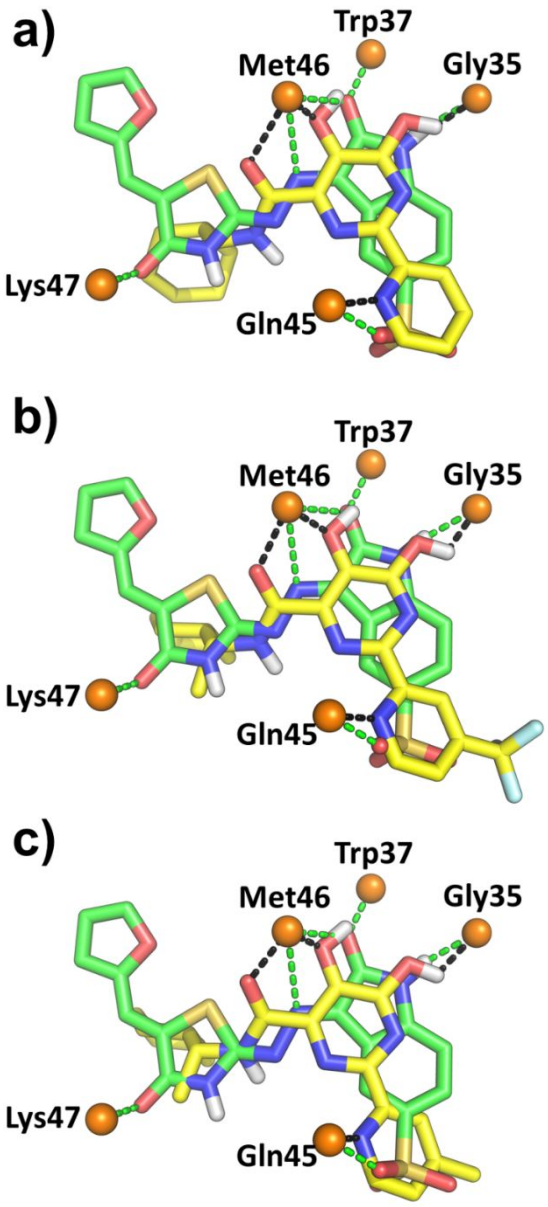
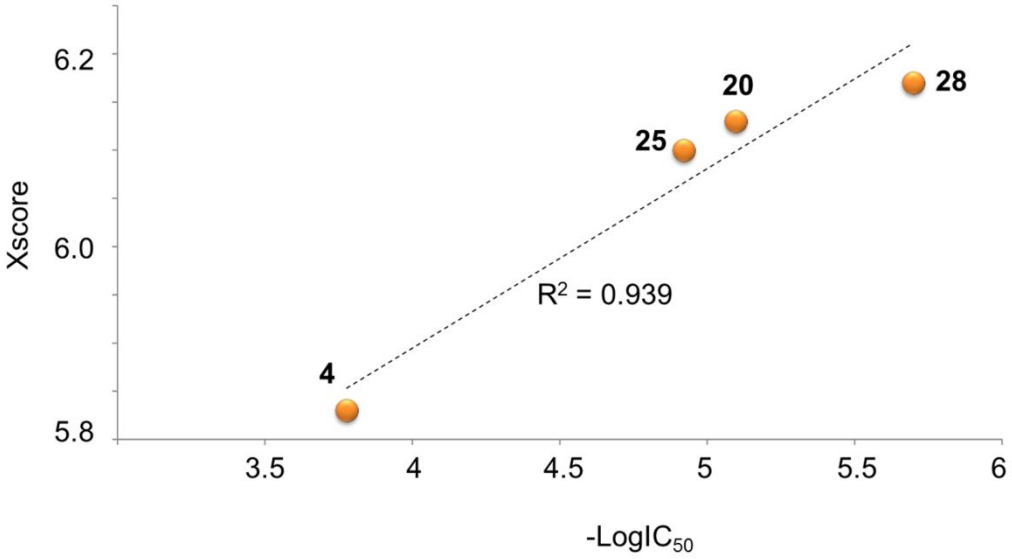


Figure S3. Correlation between predicted ligand scores and the experimental $-\log IC_{50}$. The linear regression tendency is shown as dashed line, and is highlighted by the R^2 value.



NC inhibition assay

The Alexa488-5'-cTAR-3'-Dabcyl oligonucleotide labelled by Alexa 488 at its 5' end and 4-(4′-dimethylaminophenylazo)benzoic acid (Dabcyl) at its 3' end was synthesized and purified by IBA GmbH Nucleic Acids Product Supply (Göttingen, Germany). An extinction coefficient at 260 nm of 573 240 M^{-1} cm^{-1} was used to determine its concentration. The NC(11–55) peptide was synthesized by solid-phase peptide synthesis on a 433A synthesizer (ABI, Foster City, CA) as previously described[7]. The peptide concentration was determined by using an extinction coefficient of 5700 M^{-1} cm^{-1} at 280 nm. To obtain the zinc-bound protein, 2.5 mol equiv of $ZnSO_4$ were added, and pH was raised to its final value by adding buffer.

To screen the compounds synthesized or bought in this project, we used an assay in which we tested their ability to inhibit the NC-induced destabilization of Alexa488-5'-cTAR-3'-Dabcyl. All experiments were performed in 96-wells black polystyrene CORNING (3686) plates with non-binding surface at 20 °C in 25 mM Tris-HCl (pH 7.5), 30 mM NaCl, and 0.2 mM $MgCl_2$. Before addition of a compound, the doubly labeled cTAR DNA (0.1 µM) was preincubated with 1 µM NC(11–55) (molar ratio NC/cTAR = 10:1) for 15 min at 20°C. In the first screening round, all compounds were tested at two final concentrations, 10 µM and 100 µM. DMSO was fixed at 1% v/v in all wells. After addition of compound, the mixture was incubated for 15 min at 20°C before the reading. The fluorescence signal was recorded at 20 °C with a plate reader Xenius (SAFAS Monaco). Excitation and emission wavelengths were 480 and 520 nm. In the 96-well plates, we used 18 control

wells: 3 wells with buffer only, 6 wells with doubly labelled cTAR 0.1 µM, 6 wells with doubly labelled cTAR 0.1 µM + NC(11-55) 1 µM, and 3 wells with doubly labelled cTAR/NC mixture + 1 mM EDTA as a positive control (as EDTA ejects zinc, it fully prevents the NC-induced destabilization). The intrinsic fluorescence of the tested compounds was checked by recording their fluorescence at 100 µM in the same plate. Each experiment was performed at least in duplicate. The percentage of inhibition for each concentration of inhibitor (Inh) was calculated using:

$$\%\ inh = \frac{I_{(cTAR+NC)} - I_{(cTAR+NC+Inh)}}{I_{(cTAR+NC)} - I_{(cTAR)}} \times 100,$$

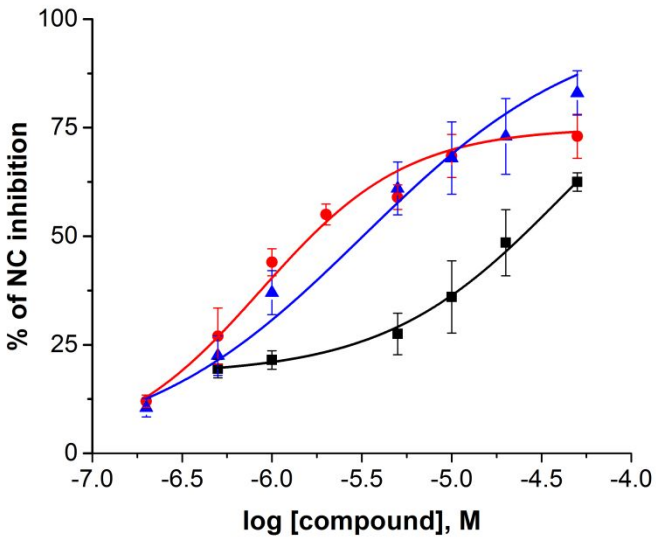
where $I_{(cTAR)}$, $I_{(cTAR+\ NC)}$ and $I_{(cTAR+\ NC+\ Inh)}$ correspond to the fluorescence intensity of the doubly labelled cTAR alone, in the presence of NC(11-55), and in the presence of both NC(11-55) and tested compound, respectively.

For the active compounds identified in the first screening round, a titration was performed by adding increasing concentrations of compound to the preformed complex of 1 µM NC(11–55) with 0.1 µM Alexa488-5'-cTAR-3'-Dabcyl. The IC50 values were determined by fitting the plots of their percentage of inhibition against their concentration (C) to a modified version of the dose-response equation[8,9]:

$$\%\ inh = A_1 + \frac{(A_2 - A_1)}{1 + 10^{(\log(IC_{50}) - \log(C)) \times p)}}$$

where, A1 and A2 are the percentage of inhibition in the absence and with saturating concentrations of the active compound, respectively. IC_{50} represents the half maximal inhibitory concentration, and p denotes the Hill coefficient.

Figure S4. Inhibition of NC-induced cTAR Alexa488/Dabcyl destabilization by compound **27** (black line), compound **28** (red line) and compound **29** (blue line).



Cell-based determination of antiviral activity

The antiviral activity of dihydroxypyrimidine carboxyamides was evaluated by measuring their IC_{50} values against the HIV-1 wild-type reference strain NL4-3 in a TZM-bl cell line based phenotypic assay. TZM-bl cells are characterized by luciferase and β-galactosidase reporter genes integrated in the cell genome under the control of an HIV-1 LTR promoter. The expression of reporter genes is regulated by the viral Tat protein, which is produced following transcription of the integrated provirus. Considering the potential involvement of NC viral protein in both early and late events of HIV-1 life cycle, we adopted a two round infection assay, named BiCycle Assay, consisting in a first cycle of replication in the T-cell derived MT-2 cell line followed by an additional round of replication in TZM-bl cells. MT-2 cells were seeded at a concentration of 50,000 cells/well in a 96-well plate and infected with the viruses at multiplicity of infection (M.O.I.) 0.03 in the presence of five-fold dilutions of the compounds. After 48-72 h, 50 µL of supernatant from each well, containing the virus produced in the first round of infection, were used to infect TZM-bl cells seeded in a 96-plate well at a concentration of 30,000 cells/well. Two days later, cells were lysed by adding 40 µL of Glo Lysis Buffer (Promega) in each well for 5 min, then 40 µL of Bright-Glo Luciferase Reagent (Promega) were added to each well for relative luminescence units (RLU) counting using a Glo-Max Multi Detection System (Promega). RLU values from each well were used to calculate the IC_{50} value of each compound using the GraphPad v6.0 software.

Determination of cytotoxicity of the compounds

Peripheral blood mononuclear cells obtained from healthy donors were stimulated with Phytohemagglutinin (Sigma) and Interleukin-2 (Miltenyi Biotec) and treated with different concentrations of the NC inhibitors. After 7 days, cytotoxicity was measured through MTS assay (Promega).

MTS assay is a colorimetric assay for assessing cell viability. NAD(P)H-dependent cellular oxidoreductase enzymes reflect the number of viable cells present in the cell culture. These enzymes are capable of reducing the tetrazolium dye MTS into formazan. The absorbance of this colored solution was quantified through and ELISA reader (Infinite F50, Tecan).

Viability was expressed as percentage of alive cells compared to the untreated control, indicated as 100%. CC_{50}, Cytotoxic Concentration 50% (concentration of compound that kills 50% of cells) was calculated by interpolating the dose-response curve.

In-vitro metabolic profile for selected dihydroxypyrimidine carboxamide analogs

Assessments of the pharmacological properties of selected dihydroxypyrimine carboxamide analogs was performed using a set of *in vitro* analysis.

The metabolic stability was assessed both in plasma and hepatocytes. Compounds were diluted into plasma or into the cell suspension from 3 mM stock solution to give a 3 µM concentration (0.1% DMSO) and incubated for a predetermined time period at 37°C. Aliquots were removed at pre-defined time points, quenched with acetonitrile plus 0.1% formic acid and analyzed by LC/MS/MS, as reported below. The peak area for the parent compound relative to an internal standard, at each time point, was compared to the time zero sample in order to assess the amount of compound remaining. The elimination constant k was calculated by plotting mean disappearance values on a semi-logarithmic scale and fitting with a best fit linear regression.

The half-life (t1/2) hours was derived using the following Equation 1:

Equation 1: $t1/2 = \ln 2/(-k)$

For those compounds which half-life could not be calculated, data were reported as : <0.16 or > than the last point considered in the assay (plasma) or as : <0.5 or >4, (hepatocytes).

For each compound tested a suitable liquid chromatography high resolution mass spectrometry (LC-HRMS) method was developed. The linearity of MS response between a standard curve from 6 to 600 nM in human and mouse plasma and hepatocyte extracts was evaluated. After drying under N_2, samples and calibration standards were resuspended with 0.1% formic acid in H_2O/ACN 70/30 containing 150 nM of labetalol or warfarin (internal standard) and directly injected into LC-MS system.

CYP450 inhibition profile was performed by measuring the % metabolism of a luminogenic substrate in the presence and in the absence of a test compound. (P450-Glo™ Assays, Promega). Known inhibitors of each CYP450 were included as positive controls. Data were expressed as % inhibition of selected metabolites formation for each CYP450 enzyme (1A2, 2D6, 3A4). IC_{50} values were determined through nonlinear regression curve fitting analysis, with the software program XLfit 5.2.0.0 (IDBS LtD).

The hERG (human Ether-a-go-go-Related Gene) inhibition assay used employs a membrane fraction containing hERG channel protein (Invitrogen's Predictor™ hERG Fluorescence Polarization Assay) and a high-affinity red fluorescent hERG channel ligand, or "tracer" (Predictor™ hERG Tracer Red), in a fluorescence polarization (FP)-based format. Compound dilutions were made in 100% DMSO starting from 10 mM stock to have 3 mM in DMSO. The highest concentration of 3 mM was used to do a titration curves (10 points, 1:3 dilution). All concentration points were tested in duplicates. The

positive control (compound E-4031) was used also starting at 30 μM concentration, as recommended by the manufacturer. After incubation for 4 hours at room temperature, the fluorescence polarization was measured. The results were expressed as % of inhibition and IC_{50} values were determined as reported for the CYP450 inhibition assay.

Pharmacokinetic studies

Definitions and abbreviations

PK: pharmacokinetic

IV: intravenous

PO: oral

NCE: new chemical entity

WS-CS: Working Solution – Calibration Standards

WS-QC: Working Solution – Quality Controls

IS: Internal Standard

LC-MS/MS: Liquid Chromatography – tandem mass spectrometry

Study design

The goal of these studies was to evaluate plasma concentrations and pharmacokinetic parameters in C57BL/6 male mice following single intravenous and oral administration at 2 and 5mg/kg, respectively. The vehicle used was 10% (2-Hydroxypropyl)-β-cyclodextrin in citrate buffer 50mM, pH 5.5.

In life procedure:		
PK study	**IV**	**PO**
Species	C57BL/6 Mouse Male	C57BL/6 Mouse Male
Number of animals	15	15
Dose Volume Administration	2 mg/kg-5mL/kg (fed animals)	5 mg/kg-10mL/kg (fasted animals)
Time points	0, 0.083, 0.25, 0.5, 1, 4, 8, 24 h	0, 0.016, 0.5, 1, 2, 4, 6, 8, 24 h
Anticoaugulant	Lithium heparin	Lithium heparin

Reagents, blank matrices and stock solutions

All reagents are stored at room temperature unless otherwise specified. Blank plasma is stored at -20°C. Stock solutions are stored at -20°C unless otherwise specified. Internal Standard was dissolved

in the reconstitution solvent, which is the solvent used for sample reconstitution, at a fixed concentration of 25 ng/mL.

Solvents and reagents	Supplier
Acetonitrile (MeCN), LC-MS grade	Fluka
Water (H_2O), LC-MS grade	Fluka
Formic Acid (FA), LC-MS grade	Fluka
2-Propanol, LC-MS grade	Fluka
Acetone	Merck
Dimethyl Sulfoxide (DMSO)	Sigma
(2-Hydroxypropyl)-β-cyclodextrin	Sigma

Blank Matrix	Species/Strain/Gender	Supplier
Plasma	mouse/C57BL/6N/male	CliniSciences

Internal Standard	Stock Solution Concentration (mg/mL)	Solvent
Warfarin (sigma)	1.0	DMSO

Equipment

- Eppendorf 1.5 ml tubes
- Eppendorf 5 ml tube
- Eppendorf 10-200 µl tips
- Porvair 96-deepwell plates, 2 ml
- Starlab 12-Channel Reservoir
- Reservoir for reconstitution solvent with internal standard (IS)
- Reservoir for Acetonitrile (MeCN)
- Hamilton tips 50µL (1 x 96 tips assembly)
- Hamilton tips 300µL (2 x 96 tips assembly)
- Hamilton tips 1000µL (1 x 96 tips assembly)
- Hamilton tip support

Apparatus

- Freezer: Liebherr Comfort (-20 °C) - N.INV. 13010
- Freezer: Platinum 500, AS Biomedical Division (-80 °C) - N.INV. 20455

- Liquid Handler Workstation: Hamilton Microlab STAR Plus – SN 2190 IRBM19705
- Centrifuge: Thermo Scientific Heraeus Multifuge X3R - N.INV. 16CES-00065
- Analytical balance: Mettler Toledo XS205 - N.INV. 19709
- LC-MS/MS systems:
  - Waters UPLC Acquity system
  - Acquity Sample Manager
  - Acquity Binary Solvent Manager
  - AB Sciex API4000 mass spectrometer

Matrix collection

In life part is performed by IRBM LAR according to the administration routes and study design. After blood collection, centrifugation was performed within max. 15' from collection, at 4°C using Heraeus Multifuge®, set at 2200 x g for 10 minutes. After centrifugation, plasma is transferred to Micronic® tubes appropriately labeled and stored immediately at -80°C.

Analytical method development

LC-MS/MS analytical method is developed on ABSciex triple quadrupole (API4000) mass spectrometer coupled to a Waters UPLC system. A fine tuning of the compound parameters (DP, EP, CE, CXP) is done by infusion. The parameters are reported in table below:

Entry	**Parent (m/z)**	**Product (m/z)**	**DP (eV)**	**EP (eV)**	**CE (eV)**	**CXP (eV)**
27	373.4	246.0	90	11	31	6
	373.4	218.0	90	11	41	6
28	377.2	249.8	90	10	37	14
	377.2	266.8	90	10	29	15
29	393.3	266.0	90	10	36	15
	393.3	238.0	90	10	43	14
Warfarin (IS)	309.0	163.2	48	13	20	10

Chromatographic conditions

Tables below reported the final chromatographic condition for the analytes **27**, **28**, **29**:

Compound ID	**27**
Instrument	Waters Acquity UPLC
Column	Atlantis dC18 2.1x30 mm
Injection volume	2 µL

Column temperature	45 °C	
Mobile phase	A) 0.1 % formic acid in H_2O B) 0.1% formic acid in MeCN	
Flow (mL/min)	1.00	
Gradient Profile	Time (min)	% Eluent B
	0.50	2
	1.20	90
	1.40	95
	1.41	100
	1.90	100
	2.00	2
	3.00	2

Compound ID	**28**	
Instrument	Waters Acquity UPLC	
Column	Waters, Acquity BEH C18, 1.7µm, 2.1x50 mm	
Injection volume	2 µL	
Column temperature	40 °C	
Mobile phase	A) 0.1 % formic acid in 7 mM Ammonium Formate B) 0.1% formic acid in MeCN	
Flow (mL/min)	0.600	
Gradient Profile	Time (min)	% Eluent B
	0.20	20
	1.80	100
	2.80	100
	2.81	20
	3.50	20

Compound ID	**29**	
Instrument	Waters Acquity UPLC	
Column	Acquity BEH C18 1.7µm 2.1x50 mm	
Injection volume	2 µL	
Column temperature	40 °C	
Mobile phase	A) 0.1 % formic acid in 10 mM Ammonium Formate B) 0.1% formic acid in MeCN	
Flow (mL/min)	0.600	
Gradient Profile	Time (min)	% Eluent B
	Initial	30
	1.50	100
	2.50	100
	2.51	30
	3.50	30

Calibration curve, quality controls and study samples

Calibration standards (CS), Quality Controls (QC) and Study Samples preparation are performed using the automated Liquid Handling Hamilton STAR Plus.

Calibration standards and quality controls preparation

The table below shows the generic scheme of calibration standards and quality controls preparation and the extraction procedure used for the analysis of plasma study samples. The calibration curve and quality controls ranges used for the quantitation of each compound in the study samples depends on compound linearity and response.

Calibration Curve			
100µL of stock solution (1mg/mL) + 400µL DMSO → 200µg/mL (WS-CS)			
ID	**Conc. in DMSO (ng/mL)**	**Dilution scheme**	**Final conc. in 50uL plasma (ng/mL)**
A0	20000	50µL of WS-CS + 450µL DMSO	10000
A1	10000	200µL of A0 + 200µL DMSO	5000
B1	5000	200µL of A1 + 200µL DMSO	2500
C1	2500	200µL of B1 + 200µL DMSO	1250
D1	1250	200µL of C1 + 200µL DMSO	625
E1	312	200µL of D1 + 600µL DMSO	156
F1	78	200µL of E1 + 600µL DMSO	39
G1	19.5	200µL of F1 + 600µL DMSO	9.76
H1	9.76	200µL of G1 + 200µL DMSO	4.88
I1	4.88	200µL of H1 + 200µL DMSO	2.44
J1	2.44	200µL of I1 + 200µL DMSO	1.22
K1	1.22	200µL of J1 + 200µL DMSO	0.61
L1	0.61	200µL of K1 + 200µL DMSO	0.305
Quality Controls			
100µL of stock solution (1mg/mL) + 400µL DMSO → 200µg/mL (WS-QC)			
ID	**Conc. in DMSO (ng/mL)**	**Dilution scheme**	**Final conc. in 50uL plasma (ng/mL)**
A0	20000	50µL of WS-QC + 450µL DMSO	10000
A2	10000	100µL of A0 + 100µL DMSO	5000
B2	2000	100µL of A2 + 400µL DMSO	1000
C2	200	50µL of B2 + 450µL DMSO	100
D2	20	50µL of C2 + 450µL DMSO	10
E2	10	200µL of D2 + 200µL DMSO	5
F2	4	100µL of E2 + 150µL DMSO	2
Sample Extraction			
CC & QC			
50µL blank plasma + 25µL DMSO solution + 200µL MeCN			
SAMPLES			

50µL sample + 25µL DMSO + 200µL MeCN
Vortex and Centrifuge for 15min @ 4500rpm
Dry 50µL of supernatant and reconstitute with 100µL of H_2O/MeCN 90/10 containing Warfarin as IS (50 ng/mL)

The calibration standards were prepared in the same matrix as the matrix of the intended study samples by spiking the blank matrix with known concentrations of the analyte.
The QC samples were spiked independently from the calibration standards, using separately prepared stock solutions. The QC samples are analysed against the calibration curve, and the obtained concentrations are compared with the nominal value.

Plasma study samples preparation

Plasma sample preparation is based on protein precipitation with acetonitrile (Matrix: organic solvent ratio = 1:4).

Scheme and volumes:

1. 50 µL of plasma are spiked with 25 µL of spiking solutions (or with blank DMSO in the case of study samples)
2. 200 µL of ACN are added to all STD, QCs and unknown study samples
3. Samples are centrifuged15 min, 4 °C, 4000 rpm
4. 200 µL of supernatant are transferred in a clean plate
5. Evaporate with N2 to dryness (25 °C)
6. Redissolve in the appropriate volume of reconstitution solvent containing ISs
7. Sonicate in water bath (15 min), vortex (15 min), spin (1 min)
8. Inject into LC-MS/MS analysis system for analysis

- Matrix blank is prepared via the addition of the same volume of extraction solvent into the control matrix that does not contain IS
- Control blank is prepared via the addition of the same volume of extraction solvent into the control matrix with IS. Control blanks are injected prior to the calibration curve to minimize any system carryover and after the calibration curve to assess carryover

Data analysis and pharmacokinetic parameters

Analyst software 1.6.2 is used to run samples, integrate peaks and generate quantitation file, which is exported as .txt file and subsequently imported in Watson LIMS (Thermo Fisher). The data imported in Watson LIMS are used to perform standard regression, data analysis and pharmacokinetic calculations.

Pharmacokinetic analysis for studies with serial bleeds from the same animal

The pharmacokinetic parameters are calculated on each animal and an average of individual pharmacokinetic parameters are then obtained. By default, and unless otherwise specified, pharmacokinetic parameters are calculated by non-compartmental analysis using Watson LIMS.

Pharmacokinetic Results compound 27

The compound showed medium clearance (33 mL/min/Kg), corresponding to c.a. 37% of the hepatic blood flow, and a low volume of distribution (0.8 L/kg). Mean C_{max} (0.7 µM) was observed at 0.4 hours after oral administration.The oral bioavailability, determined with respect to IV dose, was low (circa 20 %). The half-life, determined by CL and Vd, was 1.4 hours.The presence of conjugated metabolites was observed during analysis in both IV and PO plasma samples, probably due to glucuronides.

ID	$T_{1/2}$ (hours)	V_{dss} (L/kg)	CL (mL/min/Kg)	C_{max} (µM)	T_{max} (hours)	$F_{(0-t)}$ (%)	Formulation
27	1.4	0.8	33.1	0.7	0.42	19.2	10% HP-β-Cyclodextrin

Pharmacokinetic Results compound 28

The compound showed medium clearance (37.1 mL/min/Kg), corresponding to c.a. 45% of the hepatic blood flow, and medium volume of distribution (1.2 L/Kg). Mean Cmax (2.7 µM) was observed at 0.16 hours, indicating a quick absorption phase after oral administration. The oral bioavailability was medium (40%) and a good correlation between calculated and extrapolated bioavailability was observed. The presence of conjugated metabolites was observed during analysis in both IV and PO plasma samples, probably due to glucuronides.

ID	T1/2 (hours)	Vdss (L/kg)	CL (mL/min/Kg)	Cmax (µM)	Tmax (hours)	F (0-t) (%)	Formulation
28	3.05	1.2	37.1	2.7	0.16	40.4	10% HP-β-Cyclodextrin

Pharmacokinetic Results compound 29

The compound showed medium clearance (30.2 mL/min/Kg) corresponding to c.a. 35% of the hepatic blood flow and medium volume of distribution (0.8 L/Kg). Mean Cmax (2.6 μM) was observed at 0.16 hours, indicating a quick absorption phase after oral administration. The oral bioavailability was low (26.3%) and a good correlation between calculated and extrapolated was observed.

ID	$T_{1/2}$ (hours)	V_{dss} (L/kg)	CL (mL/min/Kg)	C_{max} (μM)	T_{max} (hours)	$F_{(0-t)}$ (%)	Formulation
29	1.1	0.8	30.2	2.6	0.2	26.3	40% HP-β-Cyclodextrin

Synthesis

General experimental details

Unless otherwise stated, all reagents were obtained from commercial sources and were used as received without further purification. UPLC-MS analysis was conducted on a Waters UPLC system with both Diode Array detection and Electrospray (+'ve and -'ve ion) MS detection. The stationary phase was a Waters Acquity UPLC BEH C18 1.7um (2.1x50mm) column. The mobile phase comprised H_2O containing 0.1% formic acid (A) and MeCN containing 0.1% formic acid (B) in the following linear gradients:

- **Method A:** 90% A (0.1 min), 90%-0% A (2.5 min), 0% A (0.3 min), 0-90% A (0.1 min) with a flow rate 0.5 mL/min.
- **Method B:** isocratic 45% A (3.0 min) with a flow rate 0.5 mL/min.

Reverse phase (C18) column chromatography was carried out using as mobile phase H**2**O containing 0.1% of trifluoroacetic acid and MeCN containing 0.1 % trifluoroacetic acid. High resolution molecular ion determinations (HRMS) were performed using a Dionex Ultimate 3000 RS UHPLC system coupled to an Orbitrap™ Q-Exactive high-resolution mass spectrometer (Thermo Scientific) operating in ESI positive full scan (m/z range 100e-1000 at resolution 140,000 FWHM at 200 m/z). Mass error was within 2.2 ppm accuracy.

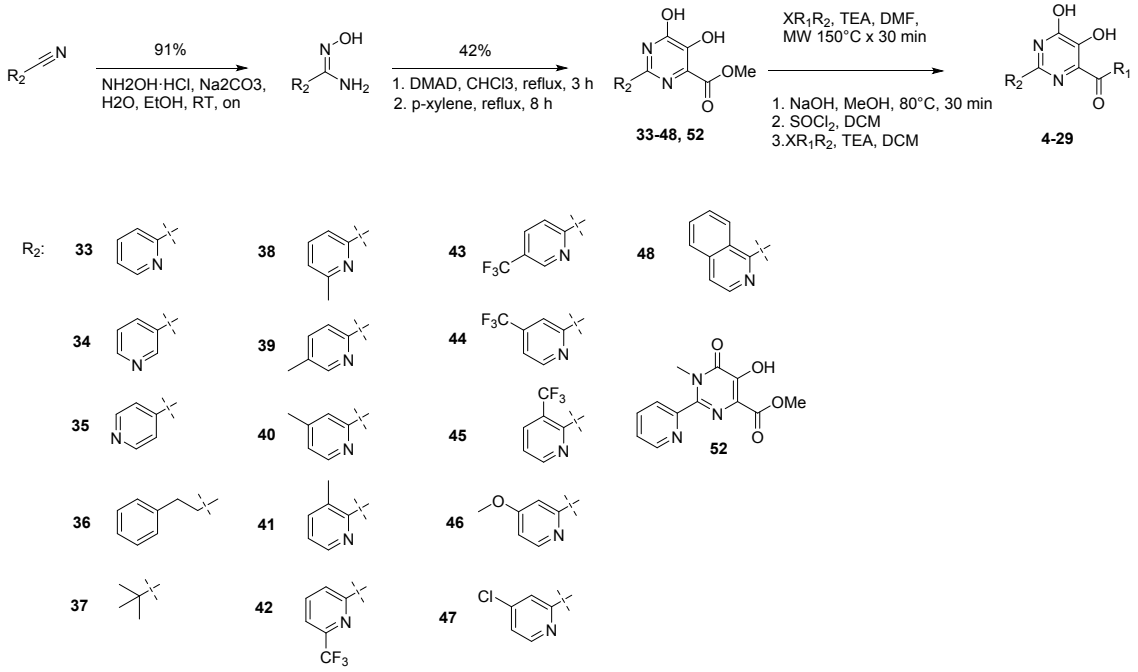
Purity of final compounds were ≥95%. ^{1}H and 13C NMR spectra were recorded on Bruker AV400 and AV600 spectrometers. NMR spectra were recorded at 400 or 600 MHz for ^{1}H-NMR and at 125 MHz for ^{13}C-NMR. ^{13}C-NMR signals were assigned through ^{1}H-^{13}C HSQC and HMBC spectra. Chemical shift (δ) are reported in parts per million relatives to TMS using CDCl3 as a solvent or

impurity of the solvent using DMSO-d6. Coupling costants (J) are reported in Hertz (Hz). Multiplicities are reported as singlet (s), broad (br), doublet (d), doublet of doublets (dd), doublet of doublets of doublets (ddd), triplet (t), doublet of triplet (dt) or multiplet (m). Unless indicated, spectra were acquired at 300 K. Temperatures are expressed in degrees Celsius (°C) and are uncorrected.

Abbreviations

HPLC: High Performance Liquid Chromatography
LRMS: Low Resolution Mass Spectrometry
HRMS: High Resolution Mass Spectrometry
ES+: Electrospray Positive Ionisation
DMAD: Dimethyl acetylenedicarbonate
*t*R: Retention time
min: minutes
DCM: Dichloromethane
EtOAc: Ethyl acetate
DMF: Dimethylformamide
DMSO: Dimethylsulfoxide
Eq: equivalent
TFA: Trifluoroacetic acid
Pd/C: Palladium on Carbon
DIPEA: N,N-Diisopropylethylamine
HBTU: N,N,N′,N′-Tetramethyl-O-(1H-benzotriazol-1-yl)uronium hexafluorophosphate, O-(Benzotriazol-1-yl)-N,N,N′,N′-tetramethyluronium hexafluorophosphate

2-Substituted 5,6-dihydroxypyrimidine-4-carboxamide



Scheme 1. Synthetic procedure

The general procedure[10] for the synthesis of 2-subtituted methyl 5,6-dihydroxypyrimidine-4-carboxamide derivatives is reported in the *Scheme 1*. The synthesis starts with the conversion of commercially available nitrile into amidoximes. The adduct formed reacting the amidoximes with dimethyl acetylenedicarboxylate (DMAD), is than cyclized in thermal conditions to afford the 2-subtituted methyl 5,6-dihydroxypyrimidine-4- carboxylate intermediates (General procedure A) used as such in the next steps. Following the amides are installed to afford the desired 2-subtituted methyl 5,6-dihydroxypyrimidine-4-carboxamide (General procedure B).

General procedure A: synthesis of 2-subtituted methyl 5,6-dihydroxypyrimidine-4- carboxylate 33-48 and 52. Nitriles (1.0 eq) were suspended in water and ethanol and treated with sodium carbonate (1.6 eq) and hydroxylamine hydrochloride (3.0 eq). After stirring overnight at room temperature the precipitate formed was isolated by filtration. The amidoxime was suspended in chloroform, treated with DMAD (1.1 eq) and refluxed for the appropriate time (3-16 h, UPLC monitoring). After cooling, the solvent was removed and the residue was refluxed in *p*-xylene to yield the desired 2-substituted pyrimidine methyl ester. In most cases the esters precipitated from the reaction mixture and were isolated by filtration and used as such in the next step.

Methyl 5,6-dihydroxy-2-(pyridin-2-yl)pyrimidine-4-carboxylate (33)

The compound was obtained following the general procedure A using picolinonitrile as starting material. Pale brown solid, yield 42%. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 12.60 (bs, 1H), 10.78 (bs,

1H), 8.69 (ddd, *J* = 4.7 Hz, *J* = 1.6 Hz, *J* = 1.0 Hz, 1H), 8.22-8.19 (m, 1H), 8.00 (ddd, *J* = 7.7 Hz, *J* = 7.7 Hz, *J* = 1.7 Hz, 1H), 7.57 (ddd, *J* = 7.6 Hz, *J* = 4.8 Hz, *J* = 1.2 Hz, 1H), 3.87 (s, 3H); UPLC *t*R 0.93 min (method A); LRMS (ES^+) *m/z* calculated for $C_{11}H_9N_3O_4$ 247.06, found 248 $(M+H)^+$.

Methyl 5,6-dihydroxy-2-(pyridin-3-yl)pyrimidine-4-carboxylate (34)

The compound was obtained following the general procedure A using nicotinonitrile as starting material. Dark brown solid, yield 10%. UPLC *t*R 1.23 min (method A); LRMS (ES^+) *m/z* calculated for $C_{11}H_9N_3O_4$ 247.06, found 248 $(M+H)^+$.

Methyl 5,6-dihydroxy-2-(pyridin-4-yl)pyrimidine-4-carboxylate (35)

The compound was obtained following the general procedure A using isonicotinonitrile as starting material. Yellow solid, yield 10%. UPLC *t*R 1.09 min (method A); LRMS (ES^+) *m/z* calculated for $C_{11}H_9N_3O_4$ 247.06, found 248 $(M+H)^+$.

Methyl 5,6-dihydroxy-2-phenethylpyrimidine-4-carboxylate (36)

The compound was obtained following the general procedure A using 3-phenylpropanenitrile as starting material. Dark brown solid, yield 10%. UPLC *t*R 1.23 min (method A); LRMS (ES^+) *m/z* calculated for $C_{14}H_{14}N_2O_4$ 274.1, found 275 $(M+H)^+$.

Methyl 2-(tert-butyl)-5,6-dihydroxypyrimidine-4-carboxylate (37)

The compound was obtained following the general procedure A using pivalonitrile as starting material. Pale brown solid, yield 10%. UPLC *t*R 1.05 min (method A);; LRMS (ES^+) *m/z* calculated for $C_{10}H_{14}N_2O_4$ 226.20, found 227 $(M+H)^+$.

Methyl 5,6-dihydroxy-2-(6-methylpyridin-2-yl)pyrimidine-4-carboxylate (38)

The compound was obtained following the general procedure A using 6-methylpicolinonitrile as starting material. Pale brown solid, yield 35%. UPLC *t*R 1.16 min (method A); LRMS (ES^+) *m/z* calculated for $C_{12}H_{11}N_3O_4$ 261.08, found 262 $(M+H)^+$.

Methyl 5,6-dihydroxy-2-(5-methylpyridin-2-yl)pyrimidine-4-carboxylate (39)

The compound was obtained following the general procedure A using 5-methylpicolinonitrile and as starting material. Brown solid, yield 35%. UPLC *t*R 1.13 min (method A); LRMS (ES^+) *m/z* calculated for $C_{12}H_{11}N_3O_4$ 261.08, found 262 $(M+H)^+$.

Methyl 5,6-dihydroxy-2-(4-methylpyridin-2-yl)pyrimidine-4-carboxylate (40)

The compound was obtained following the general procedure A using 4-methylpicolinonitrile as starting material. Beige solid, yield 30%. UPLC *t*R 1.21 min (method A); LRMS (ES^+) *m/z* calculated for $C_{12}H_{11}N_3O_4$ 261.08, found 262 $(M+H)^+$.

Methyl 5,6-dihydroxy-2-(3-methylpyridin-2-yl)pyrimidine-4-carboxylate (41)

The compound was obtained following the general procedure A using 3-methylpicolinonitrile as starting material. Pale brown solid, yield 38%. UPLC *t*R 1.11 min (method A); LRMS (ES^+) *m/z* calculated for $C_{12}H_{11}N_3O_4$ 261.0.8, found 284 $(M+Na)^+$.

Methyl 5,6-dihydroxy-2-(6-(trifluoromethyl)pyridin-2-yl)pyrimidine-4-carboxylate (42)

The compound was obtained following the general procedure A using 6-(trifluoromethyl)picolinonitrile as starting material. Brown solid, yield 28%. UPLC *t*R 1.33 min (method A); LRMS (ES^+) *m/z* calculated for $C_{12}H_8F_3N_3O_4$ 315.05, found 316 $(M+H)^+$.

Methyl 5,6-dihydroxy-2-(5-(trifluoromethyl)pyridin-2-yl)pyrimidine-4-carboxylate (43)

The compound was obtained following the general procedure A using methyl 5-(trifluoromethyl)picolinonitrile as starting material. Beige solid, yield 27%. UPLC *t*R 1.42 min (method A); LRMS (ES^+) *m/z* calculated for $C_{12}H_8F_3N_3O_4$ 315.05, found 316 $(M+H)^+$.

Methyl 5,6-dihydroxy-2-(4-(trifluoromethyl)pyridin-2-yl)pyrimidine-4-carboxylate (44)

The compound was obtained following the general procedure A using 4-(trifluoromethyl)picolinonitrile as starting material. Brown solid, yield 45%. UPLC *t*R 1.34 min (method A); LRMS (ES^+) *m/z* calculated for $C_{12}H_8F_3N_3O_4$ 315.05, found 316 $(M+H)^+$.

Methyl 5,6-dihydroxy-2-(3-(trifluoromethyl)pyridin-2-yl)pyrimidine-4-carboxylate (45)

The compound was obtained following the general procedure A using 3-(trifluoromethyl)picolinonitrile as starting material. Brown solid, yield 76%. UPLC *t*R 1.12 min (method A); LRMS (ES^+) *m/z* calculated for $C_{12}H_8F_3N_3O_4$ 315.05, found 316 $(M+H)^+$.

Methyl 5,6-dihydroxy-2-(4-methoxypyridin-2-yl)pyrimidine-4-carboxylate (46)

The compound was obtained following the general procedure A using 4-methoxypicolinonitrile as starting material. Beige solid, yield 48%. UPLC *t*R 0.96 min (method A); LRMS (ES^+) *m/z* calculated for $C_{12}H_{11}N_3O_5$ 277.07, found 278 $(M+H)^+$.

Methyl 2-(4-chloropyridin-2-yl)-5,6-dihydroxypyrimidine-4-carboxylate (47)

The compound was obtained following the general procedure A using 4-chloropicolinonitrile as starting material. Brown solid, yield 51%. UPLC *t*R 1.21 min (method A); LRMS (ES^+) *m/z* calculated for $C_{11}H_8ClN_3O_4$ 281.02, found 282 $(M+H)^+$.

Methyl 5,6-dihydroxy-2-(isoquinolin-1-yl)pyrimidine-4-carboxylate (48)

The compound was obtained following the general procedure A using isoquinoline-1-carbonitrile as starting material. Beige solid, yield 40%. UPLC *t*R 1.32 min (method A); LRMS (ES^+) *m/z* calculated for $C_{15}H_{11}N_3O_4$ 297.27, found 298 $(M+H)^+$.

Methyl 5-hydroxy-1-methyl-6-oxo-2-(pyridin-2-yl)-1,6-dihydropyrimidine-4-carboxylate (52)
The compound was obtained following the general procedure A using picolinonitrile and N-methyl-hydroxylamine hydrochloride as starting materials. Brown solid, 10% yield. UPLC *t*R 0.87 min (method A); LRMS (ES^+) *m/z* calculated for $C_{12}H_{11}N_3O_4$ 261.07, found 262 $(M+H)^+$.

General procedure B: synthesis of the amide analogs 4-29. 2-subtituted methyl 5,6-dihydroxypyrimidine-4- carboxylate (1.0 eq) was dissolved in DMF (0.2 M) then the amine (1.0 eq) and TEA (2.0 eq) were added and the mixture was stirred at 150 °C for 30 min under microwave irradiation. The reaction mixture was evaporated *in vacuo* and the crude was purified by automated RP-HPLC or reverse phase chromatography (C_{18}) using 0-100% gradient of acetonitrile in water.

***N*-(cyclohexylmethyl)-5,6-dihydroxy-2-(pyridin-2-yl)pyrimidine-4-carboxamide (4)**
The compound was obtained following the general procedure B using methyl 5,6-dihydroxy-2-(pyridin-2-yl)pyrimidine-4-carboxylate (**33**) and cyclohexylmethanamine as starting materials. White solid trifluoroacetate salt, yield 4%.^{1}H-NMR (400 MHz, DMSO-d_6) δ ppm 13.04 (bs, 1H), 12.29 (bs, 1H), 9.17 (t, *J* = 6.5 Hz, 1H), 8.72-8.68 (m, 2H), 8.05 (ddd, *J* = 7.7 Hz, *J* = 7.7 Hz, *J* = 1.8 Hz, 1H), 7.61-7.58 (m, 1H), 3.21-3.18 (m, 2H), 1.72-1.57 (m, 6H), 1.25-1.10 (m, 3H), 1.01-0.91 (m, 2H); UPLC *t*R 1.71 min (method A), 0.97 min (method B); LRMS (ES^+) *m/z* calculated for $C_{17}H_{20}N_4O_3$ 328.15, found 329 $(M+H)^+$; HRMS (ES^+) *m/z* calculated for $C_{17}H_{20}N_4O_3$ $(M+H)^+$ 329.1608, found 329.1604.

N-(cyclohexylmethyl)-5,6-dihydroxy-2-phenylpyrimidine-4-carboxamide (5)
The compound was obtained following the general procedure B using commercial methyl 5,6-dihydroxy-2-phenylpyrimidine-4-carboxylate and cyclohexylmethanamine as starting materials. Pale brown solid, yield 8%. ^{1}H-NMR (400 MHz, DMSO-d_6) δ ppm 12.90 (br s, 1 H), 12.80 (br s, 1 H), 9.02 (br t, *J* = 1.0 Hz, 1 H), 8.24 (d, *J* = 6.8 Hz, 2 H), 7.58-7.46 (m, 3 H), 3.19 (t, *J* = 6.6 Hz, 2 H), 1.69 (m, *J* = 10.7 Hz, 4 H), 1.65-1.56 (m, 2 H), 1.28-1.08 (m, 3 H), 1.08-0.85 (m, 2 H); UPLC tR 2.0 min (method A), 1.16 min (method B); LRMS (ES^+) *m/z* calculated for $C_{18}H_{21}N_3O_3$ 327.16, found 328 $(M+H)^+$; HRMS (ES^+) *m/z* calculated for $C_{18}H_{21}N_3O_3$ $(M+H)^+$ 328.1656, found 328.1654.

***N*-(cyclohexylmethyl)-5,6-dihydroxy-2-(pyridin-3-yl)pyrimidine-4-carboxamide (6)**
The compound was obtained following the general procedure B using methyl 5,6-dihydroxy-2-(pyridin-3-yl)pyrimidine-4-carboxylate (**34**) and cyclohexylmethanamine as starting materials. Beige solid trifluoroacetate salt, yield 10%. ^{1}H-NMR (400 MHz, DMSO- d_6) δ ppm13.09 (bs, 1H), 12.90 (bs, 1H), 9.46 (s, 1H), 9.15-9.12 (m, 1H), 8.72 (d, *J* = 4.0 Hz, 1H), 8.61 (d, *J* = 7.9 Hz, 1H), 7.58 (dd, *J* = 7.9 Hz, *J* = 4.0 Hz, 1H), 3.20-3.16 (m, 2H), 1.70-1.62 (m, 6H), 1.23-1.13 (m, 3H), 0.99-0.91 (m,

2H); UPLC *t*R 1.62 min (method A), 1.71 min (method C); LRMS (ES^+) *m/z* calculated for $C_{17}H_{20}N_4O_3$ 328.15, found 329 $(M+H)^+$; HRMS (ES^+) *m/z* calculated for $C_{17}H_{20}N_4O_3$ $(M+H)^+$ 329.1608, found 329.1613.

***N*-(cyclohexylmethyl)-5,6-dihydroxy-2-(pyridin-4-yl)pyrimidine-4-carboxamide (7)**

The compound was obtained following the general procedure B using methyl 5,6-dihydroxy-2-(pyridin-4-yl)pyrimidine-4-carboxylate (**35**) and cyclohexylmethanamine as starting materials. Yellow solid trifluoroacetate salt, yield 13%. ^{1}H-NMR (400 MHz, DMSO-d_6) δ ppm 13.18 (bs, 1H), 13.06 (bs, 1H), 9.18-9.15 (m, 1H), 8.80 (d, J = 5.6 Hz, 2H), 8.33 (d, J = 5.6 Hz, 2H), 3.20 (t, J = 6.6 Hz, 2H), 1.71-1.61 (m, 6H), 1.24-1.16 (m, 3H), 1.00-0.92 (m, 2H); UPLC *t*R 1.55 min (method A), 1.52 min (method C); LRMS (ES^+) *m/z* calculated for $C_{17}H_{20}N_4O_3$ 328.15, found 329 $(M+H)^+$; HRMS (ES^+) *m/z* calculated for $C_{17}H_{20}N_4O_3$ $(M+H)^+$ 329.1608, found 329.1611.

N-(cyclohexylmethyl)-5,6-dihydroxy-2-phenethylpyrimidine-4-carboxamide (8)

The compound was obtained following the general procedure B using methyl 5,6-dihydroxy-2-phenethylpyrimidine-4-carboxylate (**36**) and cyclohexylmethanamine as starting materials. Pink solid, yield 6%. ^{1}H-NMR (400 MHz, DMSO-d_6,) δ ppm 12.73-12.46 (br m, 2H), 8.69 (br t, J = 5.92 Hz, 1H), 7.32-7.16 (m, 5H), 3.20-3.09 (m, 2H), 3.03 (t, J = 7.78 Hz, 2H), 2.80 (t, J = 7.78 Hz, 2H), 1.73-1.53 (m, 6H), 1.26-1.15 (m, 2H), 1.13-1.04 (m, 1H), 0.97-0.89 (m, 2H); UPLC *t*R 2.13 min (method A), 1.11 min (method D); LRMS (ES^+) *m/z* calculated for $C_{20}H_{25}N_3O_3$ 355.20, found 356 $(M+H)^+$; HRMS (ES^+) *m/z* calculated for $C_{20}H_{25}N_3O_3$ $(M+H)^+$ 356.1969, found 356.1966.

N-(cyclohexylmethyl)-5,6-dihydroxy-2-(1-phenylethyl)pyrimidine-4-carboxamide (9)

The compound was obtained following the general procedure B using commercial methyl 5,6-dihydroxy-2-(1-phenylethyl)pyrimidine-4-carboxylate and cyclohexylmethanamine as starting materials. White solid, yield 8%. ^{1}H-NMR (400 MHz, DMSO-d_6) δ ppm 12.66 (br s, 2H), 8.75 (br t, J = 6.03 Hz, 1H), 7.42-7.35 (m, 2H), 7.35-7.20 (m, 3H), 4.15-3.87 (m, 1H), 3.26-3.08 (m, 2H), 1.74-1.52 (m, 9H), 1.27-1.05 (m, 3H), 1.04-0.84 (m, 2H); UPLC *t*R 2.16 min (method A), 1.52 min (method B); LRMS (ES^+) *m/z* calculated for $C_{20}H_{25}N_3O_3$ 355.19, found 356 $(M+H)^+$; HRMS (ES^+) *m/z* calculated for $C_{20}H_{25}N_3O_3$ $(M+H)^+$ 356.1969, found 356.1973.

2-(tert-butyl)-N-(cyclohexylmethyl)-5,6-dihydroxypyrimidine-4-carboxamide (10)

The compound was obtained following the general procedure B using commercial methyl 2-(tert-butyl)-5,6-dihydroxypyrimidine-4-carboxylate (**37**) and cyclohexylmethanamine as starting materials. White solid, yield 25%. ^{1}H-NMR (400 MHz, DMSO-d_6) δ ppm 12.47 (s, 1H), 12.36 (br s, 1H), 8.53 (br t, J = 6.14 Hz, 1H), 3.33-3.12 (m, 2H), 1.78-1.56 (m, 6H), 1.14-1.37 (m, 12H), 1.09-0.89 (m, 2H); UPLC *t*R 2.02 min (method A), 1.55 min (method B); LRMS (ES^+) *m/z* calculated for

$C_{16}H_{25}N_3O_3$ 307.12, found 308 $(M+H)^+$; HRMS (ES^+) *m/z* calculated for $C_{16}H_{25}N_3O_3$ (M+H)+ 308.1969, found 356.1972.

N-benzyl-5,6-dihydroxy-2-(pyridin-2-yl)pyrimidine-4-carboxamide (11)

The compound was obtained following the general procedure B using methyl 5,6-dihydroxy-2-(pyridin-2-yl)pyrimidine-4-carboxylate (**33**) and phenylmethanamine as starting materials. White solid, yield 20%. ^{1}H-NMR (400 MHz, DMSO-d_6) δ ppm 12.80 (br s, 1 H), 12.32 (br s, 1H), 9.76 (br t, *J* = 1.00 Hz, 1H), 8.78-8.63 (m, 2H), 8.08-7.96 (m, 1H), 7.64-7.49 (m, 1H), 7.41-7.31 (m, 4H), 7.30-7.23 (m, 1H), 4.57 (d, *J* = 1.00 Hz, 2H); UPLC *t*R 2.00 min (method A), 1.60 min (method C); LRMS (ES^+) *m/z* calculated for $C_{17}H_{14}N_4O_3$ 322.11, found 323 $(M+H)^+$.

N-ethyl-5,6-dihydroxy-2-(pyridin-2-yl)pyrimidine-4-carboxamide (12)

The compound was obtained following the general procedure B using methyl 5,6-dihydroxy-2-(pyridin-2-yl)pyrimidine-4-carboxylate (**33**) and ethanamine as starting materials. Brown solid, yield 23%. ^{1}H-NMR (400 MHz, DMSO-d_6) δ ppm 13.02 (br s, 1H), 12.30 (br s, 1H), 9.23 (m, 1H), 8.76-8.65 (m, 2H), 8.05 (dt, *J* = 7.73, 1.64 Hz, 1H), 7.60 (t, *J* = 6.15 Hz, 1H), 2.33 (m, 2H), 1.19 (t, *J* = 7.13 Hz, 3H); UPLC *t*R 1.20 min (method A), 0.70 min (method C); LRMS (ES^+) *m/z* calculated for $C_{12}H_{12}N_4O_3$ 260.09, found 260 $(M+H)^+$; HRMS (ES^+) *m/z* calculated for $C_{12}H_{12}N_4O_3$ $(M+H)^+$ 261.0982, found 261.0986.

N-(cyclohexylmethyl)-5,6-dihydroxy-N-methyl-2-(pyridin-2-yl)pyrimidine-4-carboxamide (13)

The compound was obtained following the general procedure B using methyl 5,6-dihydroxy-2-(pyridin-2-yl)pyrimidine-4-carboxylate (**33**) and 1-cyclohexyl-N-methylmethanamine as starting materials. Dark brown solid, yield 7%. ^{1}H-NMR (400 MHz, DMSO-d_6) δ ppm 12.42 (br s, 1H), 10.26 (br s, 1H), 8.70 (br d, *J* = 4.82 Hz, 1H), 8.23-8.11 (m, 1H), 8.05-7.92 (m, 1H), 7.57 (dd, *J* = 6.91, 5.15 Hz, 1H), 3.32-3.21 (m, 3H), 3.10 (br d, *J* = 7.02 Hz, 2H), 1.79-1.51 (m, 5H), 1.28-0.90 (m, 5H), 0.85-0.70 (m, 1H); UPLC *t*R 1.61 min (method A), 1.77 min (method C); LRMS (ES^+) *m/z* calculated for $C_{18}H_{22}N_4O_3$ 342.17, found 365 $(M+Na)^+$.

N-cyclohexyl-5,6-dihydroxy-2-(pyridin-2-yl)pyrimidine-4-carboxamide (14)

The compound was obtained following the general procedure B using methyl 5,6-dihydroxy-2-(pyridin-2-yl)pyrimidine-4-carboxylate (**33**) and cyclohexanamine as starting materials. Pale yellow solid, yield 28%. ^{1}H-NMR (400 MHz, DMSO-d_6) δ ppm 13.07 (bs, 1H), 12.29 (bs, 1H), 8.89-8.61 (m, 3H), 8.05 (td, *J* = 7.78, 1.75 Hz, 1H), 7.60 (ddd, *J* = 7.45, 4.82, 1.10 Hz, 1H), 1.90-1.45 (m, 7H), 1.42-1.08 (m, 4H); UPLC *t*R 1.94 min (method A), 0.80 min (method B); LRMS (ES^+) *m/z* calculated for $C_{16}H_{18}N_4O_3$ 314.14, found 337 $(M+Na)^+$; HRMS (ES^+) *m/z* calculated for $C_{16}H_{18}N_4O_3$ $(M+H)^+$ 315.1452, found 315.1447.

***N*-(2-cyclohexylethyl)-5,6-dihydroxy-2-(pyridin-2-yl)pyrimidine-4-carboxamide (15)**

The compound was obtained following the general procedure B using methyl 5,6-dihydroxy-2-(pyridin-2-yl)pyrimidine-4-carboxylate (**33**) and 2-cyclohexylethan-1-amine as starting materials. Pale brown solid, yield 9%. ^{1}H-NMR (400 MHz, DMSO-d_6) δ ppm 12.95 (bs, 1H), 12.28 (bs, 1H), 9.17 (t, J = 6.2 Hz, 1H), 8.71-8.68 (m, 2H), 8.05 (ddd, J = 7.5, 7.5, 1.9 Hz, 1H), 7.59 (ddd, J = 7.6, 4.7, 1.2 Hz, 1H), 3.39-3.34 (m, 2H), 1.77-1.58 (m, 5H), 1.51-1.46 (m, 2H), 1.35-1.09 (m, 4H), 0.98-0.88 (m, 2H); UPLC *t*R 2.07 min (method A), 1.19 min (method B); LRMS (ES$^+$) *m/z* calculated for $C_{18}H_{22}N_4O_3$ 342.17, found 343 (M+H)$^+$; HRMS (ES$^+$) *m/z* calculated for $C_{16}H_{22}N_4O_3$ (M+H)+ 343.1765, found 343.1759.

***(R)*-*N*-(1-cyclohexylethyl)-5,6-dihydroxy-2-(pyridin-2-yl)pyrimidine-4-carboxamide (16)**

The compound was obtained following the general procedure B using methyl 5,6-dihydroxy-2-(pyridin-2-yl)pyrimidine-4-carboxylate (**33**) and *(1R)*-1-cyclohexylethanamine as starting materials. Off white solid, yield 21%. ^{1}H-NMR (400 MHz, DMSO-d_6) δ ppm 13.10 (bs, 1H), 12.26 (bs, 1H), 8.73-8.66 (m, 3H), 8.06 (ddd, J = 7.7, 7.7, 1.8 Hz, 1H), 7.59 (ddd, J = 7.6, 4.8, 1.2 Hz, 1H), 3.91-3.79 (m, 1H), 1.82-1.51 (m, 6H), 1.23-0.90 (m, 8H); UPLC *t*R 2.03 min (method A), 1.20 min (method B); LRMS (ES$^+$) *m/z* calculated for $C_{18}H_{22}N_4O_3$ 342.17, found 343 (M+H)$^+$; HRMS (ES$^+$) *m/z* calculated for $C_{18}H_{22}N_4O_3$ (M+H)$^+$ 343.1765, found 343.1763.

***(S)*-*N*-(1-cyclohexylethyl)-5,6-dihydroxy-2-(pyridin-2-yl)pyrimidine-4-carboxamide (17)**

The compound was obtained following the general procedure B using methyl 5,6-dihydroxy-2-(pyridin-2-yl)pyrimidine-4-carboxylate (**33**) and *(1S)*-1-cyclohexylethanamine as starting materials. Pale yellow solid, yield 23%. ^{1}H-NMR (400 MHz, DMSO-d_6) δ ppm 13.11 (bs, 1H), 12.28 (bs, 1H), 8.73-8.65 (m, 3H), 8.06 (ddd, J = 7.7, 7.7, 1.8 Hz, 1H), 7.60 (ddd, J = 7.6, 4.8, 1.2 Hz, 1H), 3.91-3.82 (m, 1H), 1.82-1.50 (m, 6H), 1.26-0.89 (m, 8H);); UPLC *t*R 2.05 min (method A), 1.19 min (method B); LRMS (ES$^+$) *m/z* calculated for $C_{18}H_{22}N_4O_3$ 342.17, found 343 (M+H)$^+$; HRMS (ES$^+$) *m/z* calculated for $C_{18}H_{22}N_4O_3$ (M+H)$^+$ 343.1765, found 343.1768.

***(R)*-N-(1-cyclohexylethyl)-5,6-dihydroxy-2-(6-methylpyridin-2-yl)pyrimidine-4-carboxamide (18)**

The compound was obtained following the general procedure B using methyl 5,6-dihydroxy-2-(6-methylpyridin-2-yl)pyrimidine-4-carboxylate (**38**) and *(1R)*-1-cyclohexylethanamine as starting materials. Pale yellow solid, yield 17%. ^{1}H-NMR (400 MHz, DMSO-d_6) δ ppm 13.09 (br s, 1H), 12.06 (br s, 1H), 8.66 (br d, J = 9.21 Hz, 1H), 8.51 (d, J = 7.67 Hz, 1H), 7.93 (t, J = 7.78 Hz, 1H), 7.45 (d, J = 7.67 Hz, 1H), 3.92-3.65 (m, 1H), 2.59 (s, 3H), 1.83-1.66 (m, 4H), 1.65-1.50 (m, 2H), 1.22 (d, J = 6.80 Hz, 4H), 1.19-1.07 (m, 2H), 1.06-0.87 (m, 2H); UPLC *t*R 2.23 min (method A), 1.62 min

(method B); LRMS (ES^+) *m/z* calculated for $C_{19}H_{24}N_4O_3$ 356.19, found 357 $(M+H)^+$; HRMS (ES^+) *m/z* calculated for $C_{19}H_{24}N_4O_3$ $(M+H)^+$ 357.1921, found 357.1924.

***(R)*-N-(1-cyclohexylethyl)-5,6-dihydroxy-2-(5-methylpyridin-2-yl)pyrimidine-4-carboxamide (19)**

The compound was obtained following the general procedure B using methyl 5,6-dihydroxy-2-(5-methylpyridin-2-yl)pyrimidine-4-carboxylate (**39**) and *(1R)*-1-cyclohexylethan-1-amine as starting materials. Brown solid trifluoroacetate salt, yield 17%. ^{1}H-NMR (400 MHz, DMSO-d_6) δ ppm 13.06 (s, 1H), 12.19 (br s, 1H), 8.70-8.60 (m, 2H), 8.53 (s, 1H), 7.87 (dd, *J* = 1.4, 8.0 Hz, 1H), 3.90-3.81 (m, 1H), 1.82-1.71 (m, 5H), 1.75-155 (m, 3H), 1.29-1.11 (m, 6H); 1.13-0.92 (m, 3H); UPLC *t*R 2.19 min (method A), 1.55 min (method B); LRMS (ES^+) *m/z* calculated for $C_{19}H_{24}N_4O_3$ 356.19, found 357 $(M+H)^+$; HRMS (ES^+) *m/z* calculated for $C_{19}H_{24}N_4O_3$ $(M+H)^+$ 357.1921, found 357.1925.

***(R)*-*N*-(1-cyclohexylethyl)-5,6-dihydroxy-2-(4-methylpyridin-2-yl)pyrimidine-4-carboxamide (20)**

The compound was obtained following the general procedure B using methyl 5,6-dihydroxy-2-(4-methylpyridin-2-yl)pyrimidine-4-carboxylate (**40**) and *(1R)*-1-cyclohexylethanamine as starting materials. Pale yellow solid trifluoroacetate salt, yield 16%. ^{1}H-NMR (400 MHz, DMSO-d_6) δ ppm 13.09 (bs, 1H), 12.25 (bs, 1H), 8.60-8.57 (m, 2H), 8.46 (s, 1H), 7.47 (d, *J* = 4.4 Hz, 1H), 3.92-3.82 (m, 1H), 2.52-2.51 (m, 3H), 1.84-1.69 (m, 4H), 1.64-1.55 (m, 2H), 1.27-1.12 (m, 6H), 1.05-0.92 (m, 2H); UPLC *t*R 2.27 min (method A), 1.50 min (method B); LRMS (ES^+) *m/z* calculated for $C_{19}H_{24}N_4O_3$ 356.19, found 357 $(M+H)^+$; HRMS (ES^+) *m/z* calculated for $C_{19}H_{24}N_4O_3$ $(M+H)^+$ 357.1921, found 357.1924.

***(S)*-N-(1-cyclohexylethyl)-5,6-dihydroxy-2-(4-methylpyridin-2-yl)pyrimidine-4-carboxamide (21)**

The compound was obtained following the general procedure B using methyl 5,6-dihydroxy-2-(4-methylpyridin-2-yl)pyrimidine-4-carboxylate (**40**) and *(1S)*-1-cyclohexylethanamine as starting materials. Pale yellow solid trifluoroacetate salt, yield 11%. ^{1}H-NMR (400 MHz, DMSO-d_6) δ ppm 13.11 (s, 1H), 12.19 (s, 1H), 8.61-8.53 (m, 2H), 8.45 (s, 1 H), 7.45 (d, *J* =4 .38 Hz, 1H), 3.91-3.81 (m, 1H), 2.49-2.47 (m, 3H), 1.84-1.67 (m, 4H), 1.65-1.55 (m, 2H), 1.29-1.11 (m, 6H), 1.08-0.87 (m, 2H); UPLC *t*R 2.08 min (method A), 1.51 min (method B); LRMS (ES^+) *m/z* calculated for $C_{19}H_{24}N_4O_3$ 356.19, found 357 $(M+H)^+$; HRMS (ES^+) *m/z* calculated for $C_{19}H_{24}N_4O_3$ $(M+H)^+$ 357.1921, found 357.1924.

***(R)*-N-(1-cyclohexylethyl)-5,6-dihydroxy-2-(3-methylpyridin-2-yl)pyrimidine-4-carboxamide (22)**

The compound was obtained following the general procedure B using methyl 5,6-dihydroxy-2-(3-methylpyridin-2-yl)pyrimidine-4-carboxylate (**41**) and *(1R)*-1-cyclohexylethan-1-amine as starting materials. Off white solid, yield 11%. ^{1}H-NMR (400 MHz, DMSO-d_6) δ ppm 12.33-12.85 (m, 2H), 8.54 (dd, J = 4.60, 0.88 Hz, 1H), 8.17 (br d, J = 9.40 Hz, 1H), 7.92-7.76 (m, 1H), 7.46 (dd, J = 1.00 Hz, 1H), 3.84 (m, 1H), 2.58 (s, 3H), 1.79-1.44 (m, 6 H), 1.26-1.05 (m, 6H), 1.05-0.87 (m, 2H); UPLC *t*R 2.37 min (method A), 1.58 min (method B); LRMS (ES$^+$) *m/z* calculated for $C_{19}H_{24}N_4O_3$ 356.19, found 379 $(M+Na)^+$; HRMS (ES$^+$) *m/z* calculated for $C_{19}H_{24}N_4O_3$ $(M+H)^+$ 357.1921, found 357.1924.

***(R)*-N-(1-cyclohexylethyl)-5,6-dihydroxy-2-(6-(trifluoromethyl)pyridin-2-yl)pyrimidine-4-carboxamide (23)**

The compound was obtained following the general procedure B methyl 5,6-dihydroxy-2-(6-(trifluoromethyl)pyridin-2-yl)pyrimidine-4-carboxylate (**42**) and *(1R)*-1-cyclohexylethan-1-amine as starting materials. Beige solid trifluoroacetate salt, yield 19%. ^{1}H-NMR (400 MHz, DMSO-d_6) δ ppm 13.24 (bs, 1H), 12.48 (bs, 1H), 8.98 (d, J = 8.1 Hz, 1H), 8.71 (br d, J = 9.2 Hz, 1H), 8.34 (t, J = 7.9 Hz, 1H), 8.08 (d, J = 7.7 Hz, 1H), 3.94-3.83 (m, 1H), 1.83-1.68 (m, 4H), 1.63-151 (m, 2H), 1.24-1.11 (m, 6H), 1.03-0.90 (m, 2H). UPLC *t*R 2.21 min (method A), 1.82 min (method B); LRMS (ES$^+$) *m/z* calculated for $C_{19}H_{21}F_3N_4O_3$ 410.16, found 411 $(M+H)^+$; HRMS (ES$^+$) *m/z* calculated for $C_{19}H_{21}F_3N_4O_3$ $(M+H)^+$ 411.1639, found 411.1641.

***(R)*-N-(1-cyclohexylethyl)-5,6-dihydroxy-2-(5-(trifluoromethyl)pyridin-2-yl)pyrimidine-4-carboxamide (24)**

The compound was obtained following the general procedure B using methyl 5,6-dihydroxy-2-(5-(trifluoromethyl)pyridin-2-yl)pyrimidine-4-carboxylate (**43**) and *(1R)*-1-cyclohexylethan-1-amine as starting materials. White solid trifluoroacetate salt, yield 22%. ^{1}H-NMR (400 MHz, DMSO-d_6) δ ppm 13.29 (br s, 1H), 12.59 (br s, 1H), 9.01-9.11 (m, 1H), 8.94 (d, J = 8.3 Hz, 1H), 8.76 (br d, J=9.2 Hz, 1H), 8.47 (dd, J = 1.8, 8.6 Hz, 1H), 3.81-3.95 (m, 1H), 1.78-1.47 (m, 6H), 1.04-0.86 (m, 8H); UPLC *t*R 2.28 min (method A), 2.06 min (method B); LRMS (ES$^+$) *m/z* calculated for $C_{19}H_{21}F_3N_4O_3$ 410.16, found 411 $(M+H)^+$; HRMS (ES$^+$) *m/z* calculated for $C_{19}H_{21}F_3N_4O_3$ $(M+H)^+$ 411.1639, found 411.1640.

***(R)-N*-(1-cyclohexylethyl)-5,6-dihydroxy-2-(4-(trifluoromethyl)pyridin-2-yl)pyrimidine-4-carboxamide (25)**

The compound was obtained following the general procedure B using Methyl 5,6-dihydroxy-2-(4-(trifluoromethyl)pyridin-2-yl)pyrimidine-4-carboxylate (**44**) and *(1R)-1*-cyclohexylethanamine as

starting materials. Pale brown solid, yield 27%. ^{1}H-NMR (400 MHz, DMSO-d_6) δ ppm 13.24 (bs, 1H), 12.58 (bs, 1H), 8.96 (d, *J* = 4.9 Hz, 1H), 8.84 (bs, 1H), 8.76-8.70 (m, 1H), 7.96 (d, *J* = 3.3 Hz, 1H), 3.89-3.79 (m, 1H), 1.82-1.54 (m, 6H), 1.25-0.92 (m, 8H); ^{19}F-NMR (400 MHz, DMSO-d_6) δ ppm -62.92; UPLC *t*R 2.42 min (method A), 2.08 min (method B); LRMS (ES$^+$) *m/z* calculated for $C_{19}H_{21}F_3N_4O_3$ 410.16, found 411 (M+H)$^+$. HRMS (ES$^+$) *m/z* calculated for $C_{19}H_{21}F_3N_4O_3$ (M+H)$^+$ 411.1639, found 411.1633.

***(R)-N*-(1-cyclohexylethyl)-5,6-dihydroxy-2-(3-(trifluoromethyl)pyridin-2-yl)pyrimidine-4-carboxamide (26)**

The compound was obtained following the general procedure B using methyl 5,6-dihydroxy-2-(3-(trifluoromethyl)pyridin-2-yl)pyrimidine-4-carboxylate (**45**) and *(1R)*-1-cyclohexylethanamine as starting materials. Beige solid trifluoroacetate salt, yield 9%. ^{1}H-NMR (400 MHz, DMSO-d_6) δ ppm 13.20 (bs, 1H), 12.65 (s, 1H), 8.90 (d, *J* = 4.3 Hz, 1H), 8.37 (d, *J* = 8.0 Hz, 1H), 7.89 (d, *J* = 9.2 Hz, 1H), 7.77 (dd, *J* = 8.0, 4.3 Hz, 1H), 3.77-3.75 (m, 1H), 1.63-1.61 (m, 4H), 1.55-1.52 (m, 1H), 1.40-1.32 (m, 1H), 1.17-0.99 (m, 6H), 0.93-0.79 (m, 2H); UPLC *t*R 2.38 min (method A), 1.77 min (method B); LRMS (ES$^+$) *m/z* calculated for $C_{19}H_{21}F_3N_4O_3$ 410.16, found 411 (M+H)$^+$; HRMS (ES$^+$) *m/z* calculated for $C_{19}H_{21}F_3N_4O_3$ (M+H)$^+$ 411.1639, found 411.1641.

***(R)-N*-(1-cyclohexylethyl)-5,6-dihydroxy-2-(4-methoxypyridin-2-yl)pyrimidine-4-carboxamide (27)**

The compound was obtained following the general procedure B using methyl 5,6-dihydroxy-2-(4-methoxypyridin-2-yl)pyrimidine-4-carboxylate (**46**) and *(1R)-1*-cyclohexylethanamine as starting materials. Off white solid yield 32%. ^{1}H-NMR (400 MHz, DMSO-d_6) δ ppm 13.09 (s, 1H), 12.17 (s, 1H), 8.68 (d, *J* = 9.2 Hz, 1H), 8.54 (d, *J* = 5.6, 1H), 8.24 (d, *J* = 2.2 Hz, 1H), 7.21 (dd, J = 2.2, 5.6 Hz, 1H), 3.96 (s, 3H), 3.88 - 3.82 (m, 1H), 1.81-1.53 (m, 6H), 1.24 - 0.92 (m, 8H). UPLC *t*R 2.20 min (method A), 1.27 min (method B); LRMS (ES$^+$) *m/z* calculated for $C_{19}H_{24}N_4O_3$ 372.18, found 373 (M+H)$^+$; HRMS (ES$^+$) *m/z* calculated for $C_{19}H_{24}N_4O_3$ (M+H)$^+$ 373.1870, found 373.1863.

***(R)*-2-(4-chloropyridin-2-yl)-*N*-(1-cyclohexylethyl)-5,6-dihydroxypyrimidine-4-carboxamide (28)**

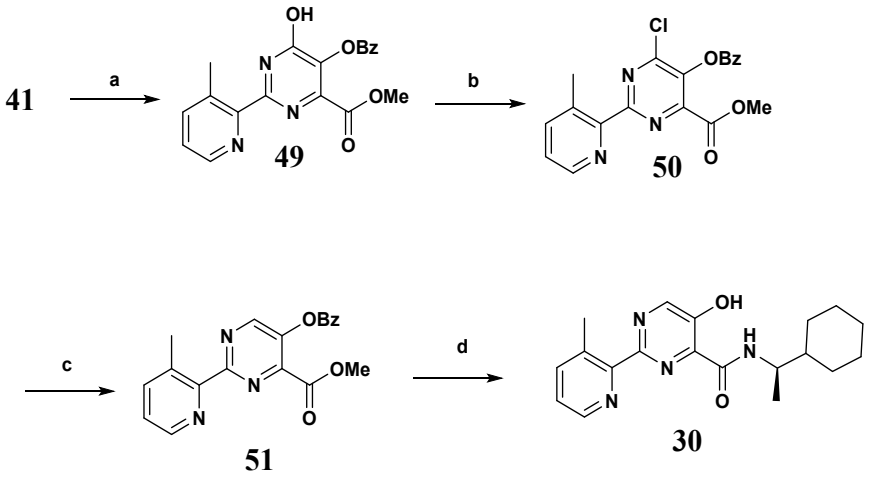
The compound was obtained following the general procedure B using Methyl 2-(4-chloropyridin-2-yl)-5,6-dihydroxypyrimidine-4-carboxylate (**47**) and *(R)-1*-cyclohexylethanamine as starting materials. Pale brown solid, yield 41%. ^{1}H-NMR (400 MHz, DMSO-d_6) δ ppm 13.30 (bs, 1H), 12.38 (bs, 1H), 8.81 (s, 1H), 8.77 (d, *J* = 8.8 Hz, 1H), 8.65 (d, *J* = 5.3 Hz, 1H), 7.73 (dd, *J* = 5.3, 2.0 Hz, 1H), 3.90-3.81 (m, 1H), 1.83-1.54 (m, 6H), 1.25-1.11 (m, 6H), 1.02-0.89 (m, 2H); UPLC *t*R 2.17 min

(method A), 1.81 min (method B); LRMS (ES^+) *m/z* calculated for $C_{18}H_{21}ClN_4O_3$ 376.14, found 377 $(M+H)^+$; HRMS (ES^+) *m/z* calculated for $C_{18}H_{21}ClN_4O_3$ $(M+H)^+$ 377.1375, found 377.1370.

***(R)-N*-(1-cyclohexylethyl)-5,6-dihydroxy-2-(isoquinolin-1-yl)pyrimidine-4-carboxamide (29)**

The compound was obtained following the general procedure B using Methyl 5,6-dihydroxy-2-(isoquinolin-1-yl)pyrimidine-4-carboxylate **48** and *(1R)-1*-cyclohexylethanamine as starting materials. Pale yellow solid, yield 52%. ^{1}H-NMR (400 MHz, DMSO-d_6, 300 K) δ ppm 12.96 (bs, 1H), 12.80 (s, 1H), 8.68 (bs, 1H), 8.63 (d, *J* = 5.6 Hz, 1H), 8.24 (bs, 1H), 8.10 (d, *J* = 8.2, 1H), 8.04 (d, *J* = 5.6, 1H), 7.87 (ddd, *J* = 8.2, 6.8, 1.2 Hz, 1H), 7.69 (ddd, *J* = 8.3, 7.0, 1.0 Hz, 1H), 3.88-3.79 (m, 1H), 1.77-1.60 (m, 5H), 1.51-1.44 (m, 1H), 1.23-0.91 (m, 8H). UPLC *t*R 2.32 min (method A), 2.06 min (method B); LRMS (ES^+) *m/z* calculated for $C_{22}H_{24}N_4O_3$ 392.46, found 393 $(M+H)^+$; HRMS (ES^+) *m/z* calculated for $C_{22}H_{24}N_4O_3$ $(M+H)^+$ 393.1921, found 393.1915.

Synthesis of *(R)*-N-(1-cyclohexylethyl)-5-hydroxy-2-(3-methylpyridin-2-yl)pyrimidine-4-carboxamide (30)



Scheme 2: Reagents and conditions: a) BzCl, Py, DCM, 57%; b) $POCl_3$, 21%; c) cyclohexa-1,4-diene, Pd/C, EtOAc, 67%; d) *(R)-1*-cyclohexylethanamine, TEA, DMF, 150°C x 30 min under microwave irradiation, 10%.

Methyl 5-(benzoyloxy)-6-hydroxy-2-(3-methylpyridin-2-yl)pyrimidine-4-carboxylate (**49**)

Benzoyl chloride (2.02 mL, 17.23 mmol) was added dropwise to a solution of Methyl 5,6-dihydroxy-2-(3-methylpyridin-2-yl)pyrimidine-4-carboxylate **41** (3.0 g, 11.48 mmol) was dissolved in DCM: Pyridine (70 mL, ratio 6:1) and stirring continued for 1 hour at room temperature. The reaction was than diluted with DCM and organic phase washed with saturated solution of $NaHCO_3$ and brine and dried over Na_2SO_4. After filtration, the solvent was removed under vacuum to afford the titled compound as dark solid used as such in the following step (3.4 gr, 57% yield). UPLC *t*R 1.71 min (method A); LRMS (ES^+) *m/z* calculated for $C_{19}H_{15}N_3O_5$ 365.10, found 366 $(M+H)^+$.

Methyl 5-(benzoyloxy)-6-chloro-2-(3-methylpyridin-2-yl)pyrimidine-4-carboxylate (**50**)

Compound **49** (3.4 g, 9.31 mmol) was dissolved in phosphoryl chloride (26.7mL) and the mixture heated at reflux for 3h. Volatiles were evaporated and resulting crude was purified by chromatography on silica gel (100g) eluting with DCM / AcOEt to afford the titled compound (768 mg, 21% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.60 (br dd, *J* = 1.00 Hz, 1 H), 8.23 (m, *J* = 1.00 Hz, 2 H), 7.82 - 7.93 (m, 2 H), 7.65 - 7.76 (m, 2 H), 7.47 (dd, *J* = 1.00 Hz, 1 H), 3.84 (s, 3 H), 2.48 (s, 3 H).

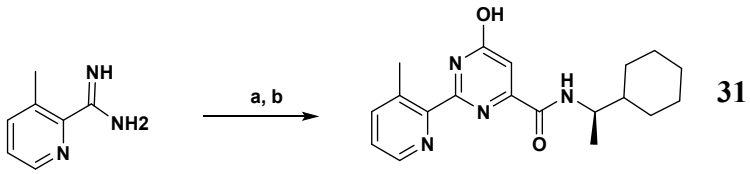
Methyl 5-(benzoyloxy)-2-(3-methylpyridin-2-yl)pyrimidine-4-carboxylate (**51**)

Compound **50** (399.64 mg, 1.04 mmol) was dissolved in ethyl acetate (26 mL), cyclohexa-1,4-diene (10.24 mL, 108.62 mmol) and Pd/C (731.38 mg) were added. The mixture was stirred at reflux for 3h. Pd/C was filtered off and washed with ethyl acetate. The filtrate was evaporated to the titled compound (244 mg, 67% yield). UPLC *t*R 1.53 min (method A); LRMS (ES$^+$) *m/z* calculated for $C_{19}H_{15}N_3O_4$ 349.35, found 350 $(M+H)^+$.

(R)-N-(1-cyclohexylethyl)-5-hydroxy-2-(3-methylpyridin-2-yl)pyrimidine-4-carboxamide **(30)**

Compound **50** was reacted with *(R)-1*-cyclohexylethanamine according to the general procedure B to afford the titled compound **30** as pale brown solid, yield 10%. ^{1}H-NMR (400 MHz, DMSO-d_6) δ ppm 12.28 (br s, 1 H), 9.09 - 9.37 (m, 1 H), 8.59 - 8.83 (m, 2 H), 7.81 - 8.16 (m, 1 H), 7.48 - 7.64 (m, 1 H), 3.32 (m, 1 H), 2.46 (s, 3H), 1.51 - 1.83 (m, 6 H), 1.00 - 1.29 (m, 6 H), 0.56 - 0.97 (m, 2 H); UPLC *t*R 1.93 min (method A), 0.97 min (method B); LRMS (ES$^+$) *m/z* calculated for $C_{19}H_{24}N_4O_2$ 340.19, found 341 $(M+H)^+$.

Synthesis of (R)-N-(1-cyclohexylethyl)-6-hydroxy-2-(3-methylpyridin-2-yl)pyrimidine-4-carboxamide (31)

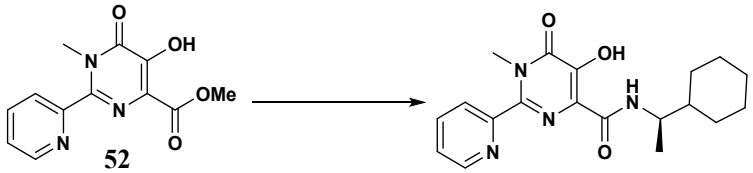


Scheme 3: Reagents and conditions: a) sodium (Z)-1,4-diethoxy-1,4-dioxobut-2-en-2-olate, EtOH, NaOH, H_2O; b) *(R)-1*-cyclohexylethanamine, HBTU, DIPEA, DMF, 34%.

Sodium (Z)-1,4-diethoxy-1,4-dioxobut-2-en-2-olate (1.47 g, 6.99 mmol) was suspended in ethanol (61.86 mL). After 5 min of stirring a solution of sodium hydroxide (0.28 g, 6.99 mmol) in 2 mL of water was added. The suspension was stirred at RT for 15 min to give a clear solution. To this was added a solution of 3-methylpicolinimidamide hydrochloride (0.50 gr, 2.91 mmol) in water (5.00 mL), giving a solution with pH=11. Next, the reaction was stirred at 70 °C for 2 h. Additional NaOH was added during the heating period to maintain the pH between 11 and 12. After cooling to 0 °C, a solution of HCl (6N) was added until pH=1. The resulting white solid was

collected, washed with water, and dried to give 466 mg of 6-hydroxy-2-(3-methylpyridin-2-yl)pyrimidine-4-carboxylic acid used as such in the next step. The latter (200.0 mg, 0.870 mmol) was dissolved in DMF (2 mL) and treated with DIPEA (0.30 mL) and HBTU (360.85 mg, 0.95 mmol). After stirring at RT for 1hour volatiles were evaporated and residue purified by automated RP-HPLC eluting with H_2O + 0.1% TFA and MeCN + 0.1% TFA. The titled compound was obtained as white solid, 34% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 12.71 (br s, 1H), 8.58 (br d, J = 1.00 Hz, 1H), 8.08 (d, J = 1.00 Hz, 1H), 7.90 (d, J = 1.00 Hz, 1H), 7.55 (m, 1H), 6.86 (s, 1H), 3.85-3.77 (m, 1H), 2.60 (s, 3H), 1.77-1.57 (m, 5H), 1.52-1.41 (m, 1H), 1.26-1.04 (m, 6H), 1.04-0.84 (m, 2H); UPLC *t*R 1.85 min (method A), 0.99 min (method B); LRMS (ES$^+$) *m/z* calculated for $C_{19}H_{24}N_4O_2$ 340.19, found 341 (M+H)$^+$; HRMS (ES$^+$) m/z calculated for $C_{19}H_{24}N_4O_2$ (M+H)+ 340.1899, found 340.1975.

Synthesis of (R)-N-(1-cyclohexylethyl)-5-hydroxy-1-methyl-6-oxo-2-(pyridin-2-yl)-1,6-dihydropyrimidine-4-carboxamide (32)



The compound was obtained following the general procedure B using methyl 5-hydroxy-1-methyl-6-oxo-2-(pyridin-2-yl)-1,6-dihydropyrimidine-4-carboxylate **52** and *(R)-1*-cyclohexylethanamine as starting materials. Pale yellow solid, yield 52%. ^{1}H-NMR (400 MHz, DMSO-d_6) δ ppm 12.86 (br s, 1 H), 8.70 (m, 1 H), 8.46-8.32 (m, 1 H), 8.14-7.99 (m, 1H), 7.99-7.86 (m, 1H), 7.67-7.46 (m, 1H), 3.94-3.67 (m, 1H), 3.44 (s, 3H), 1.81-1.37 (m, 5H), 1.30-0.71 (m, 8H); UPLC *t*R 1.21 min (method A), 1.07 min (method B); LRMS (ES$^+$) *m/z* calculated for $C_{19}H_{24}N_4O_3$ 356.18, found 357 (M+H)$^+$; HRMS (ES$^+$) *m/z* calculated for $C_{19}H_{24}N_4O_3$ 357.1921, found 357.1923 (M+H)$^+$.

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