Development of the telescoped continuous flow Pd-catalyzed aerobic alcohol oxidation/reductive amination in the synthesis of new phosphatidylinositide 3-kinase inhibitor (CPL302415).

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1. Materials and Methods

Solvents and chemicals were obtained from commercial suppliers and were used without any further purification unless otherwise noted. The {5-[2-(difluoromethyl)-2,3-dihydro-1,3-benzodiazol-1-yl]-7-(morpholin-4-yl)pyrazolo[1,5-a]pyrimidin-2-yl}methanol (**1**) was synthesized according to the procedure published by us. [1,] Oxygen was purchased from Air Product. The 20 % $Pd(OH)_2/C$ and 10% Pd/C both CatCart® 70 mm length and 30 mm length were purchased from ThalesNano.
Experiments were performed using a combined two Vapourtec easy-Medchem with four standard PFA tubular reactors (10 mL each, id=1mm) and Cube®Pro from ThalesNano. All tubes and mixers were bought from Vapourtec.
Pressure to the system was delivered to the system using a second Vapourtec SF-10 pump and back pressure regulator (BPR)
Vapourtec V-3 pump was used as a mass flow controller.

Main components utilized within a telescoped sequence	
Pump	Vapourtec V-3 Peristaltic pump
Mass flow meter	Vapourtec V-3
Mixer	Vapourtec Y-type mixer Material: PTFE i.d.= 1 mm <0.1mL
Reactors	Vapourtec PFA 10 mL coil reactor i.d=1mmVapourtec PFA 20 mL rapid mixing reactor i.d=3.2mmTemperature control: Vapourtec easy-Medchem
Back pressure regulator	Vapourtec SF-10 Adjustable pressure

Miscellaneous fittings	Vapourtec PFA tubes i.d.=1 mm
H-cube®Pro	ThalesNano H-cube®Pro
Büchi Heating Bath	B-491
VWR Ultrasonic Cleaner	USC-TH

1.1 Design of Experiments (DoE)

The DoE study and statistical analysis were performed by using the design of experiment tools of STATISTICA software (v.13.3). The experimental data were fitted by using multiple linear regression. The main and interaction effects were generated based on multivariate ANOVA. The statistical significance level was set up to 0.05. The goodness of fit of the models was expressed in regression coefficient R^2.

1.2 Structure analysis (NMR and MS)

^1H and ^{13}C NMR spectra were performed on JOEL JNMR-ECZR 600 MHz spectrometers with ^1H being observed at 600 MHz and ^{13}C at 151 MHz. Chemical shifts for ^1H and ^{13}C were reported in *d* (ppm) using the residual proton in a deuterated solvent. Mass spectra (Atmospheric Pressure Ionization Electrospray, API-ES) were obtained on Agilent 6130 LC/MSD spectrometer or Agilent 1290 UHPLC coupled with Agilent QTOF 6545 mass spectrometer.

1.3 UHPLC analytical method

Reaction in-process monitoring was conducted by the RP UHPLC method using the parameters listed in Table 1.3.1:

Table 1.3.1. Chromatographic conditions

Parameters	**Range**	
Instrumentation	Ultra-high performance liquid chromatograph equipped with an UV/DAD detector, autosampler, and column heater	
Column	Acquity UPLC CSH C18; 2.1 mm x 100 mm, 1.7 µm	
Mobile phases	Phase A: 0.1 % ortho-phosphoric acid in water Phase B: 0.1 % ortho-phosphoric acid in ACN	
Diluent	Methanol	
Flow	0.5 mL/min	
Run time	11 min	
Column temperature	30 °C	
Autosampler temperature	10 °C	
Injection volume	1 µL	
Detection Wavelength	254 nm	
Typical Retention Time	Alcohol **1b** about 4.1 min, **1b** about 4.7 min, Aldehyde **2** about 5.5 min, 2.3 min **3**	
Rinsing the column	After analysis rinse the column for 10 min using ACN : water (10:90, v/v) solution then during 10 min using ACN; store the column with ACN	
Gradient program		
Time, min	Mobile phase A, %	Mobile phase B, %
0.0	90.0	10.0
6.0	40.0	60.0
7.5	10.0	90.0
9.0	10.0	90.0
9.10	90.0	10.0
11.0	90.0	10.0

Reporting of results and calculations

System suitability:

- The resolution between the peaks of alcohol **1** as well as **3** and its nearest impurity must not be less than 1.5;
- Symmetry factor for alcohol **1** and **3** peaks: within the range from 0.8 to 1.5.

Evaluation of chromatograms

- Disregard peaks from the blank matrix and diluents;
- Disregard peaks less than 0.05%;

Calculations

The progress of the reaction (product aldehyde yield) was controlled based on the normalization procedure according to the following formula:

$$X = \frac{A_X \cdot CF \cdot 100\%}{\Sigma A_{Xi}},$$

where:

X – aldehyde product **2** percentage of in the chromatogram of the sample solution,

A_X – aldehyde **2** peak area in the chromatogram of the sample solution,

CF – correction factor of aldehyde **2** versus alcohol **1**

SA_{Xi} – the sum of the areas of all integrated peaks in the chromatogram of the sample solution.

The progress of the reaction (product **3** yield) was controlled based on the normalization procedure according to the following formula:

$$Y = \frac{A_Y \cdot CF \cdot 100\%}{\Sigma A_{Yi}}$$

where:

Y – **3** percentage of in the chromatogram of the sample solution,

A_Y – compound **2** peak area in the chromatogram of the sample solution,

CF1 – correction factor of aldehyde **2** versus **3**

CF2 – correction factor of alcohol **1** versus **3**

SA_{Yi} – the sum of the areas of all integrated peaks in the chromatogram of the sample solution.

1.4 Palladium content

Palladium content analysis was perfomed on Agilent Technologies ICP-MS 7900.

Blank:

7.5 mL of nitric acid, 1mL of hydrogen peroxide and 200 µL of internal standard (10 µ/mL) was transferred to reaction vial and left for 0.5h. The vial was placed into microvawe etching chamber and mineralization was conducted. After etching process the mixure was transferred to 100 mL volumetric flask and water was added ad 100 mL.

Sample detection of Pd only after reductive amination:

300 mg of the sample, 7.5 mL of nitricacid, 1 mL of hydrogen peroxide and 200 µL of internal standard (10 µ/mL) was transferred to reaction vial and left for 0.5h. The vial was placed into microvawe etching chamber and mineralization was conducted. After etching process the mixure was transferred to 100 mL volumetric flask and water was added ad 100 mL.

$$Z = \frac{1 - C}{m} x \frac{100}{nb} x \frac{1}{1000}$$

Z – Palladium content [ppm]

I - response factor for sample (element intensity/yttrium intensity)

C – intersection of the linearity curve

m – slope of linearity curve

nb – sample mass [g]

C	0.003219
m	0.04971

y=0.04971x+0.003219

105 Pd [He]

	CPS Pd	CPS Y	CPS Pd/CPS Y	nb	100/nb	Z [ppm]	Z average [ppm]
sample 1	1025.08	210709.8	0.00486489	0.30327	329.7392	0.010918	0.005
sample 2	660.04	204600.9	0.003225987	0.29976	333.6002	0.0000469	

Sample detection of Pd after combined oxidation/reductive amination:

100 mg of the sample, 7.5 mL of nitric acid, 1 mL of hydrogen peroxide and 200 µL of internal standard (10 µ/mL) was transferred to reaction vial and left for 0.5h. The vial was placed into microvawe etching chamber and mineralization was conducted. After etching process the mixure was transferred to 100 mL volumetric flask and water was added ad 100 mL.

$$Z = \frac{1 - C}{m} x \frac{100}{nb} x \frac{1}{1000}$$

Z – Palladium content [ppm]

I - response factor for sample (element intensity/yttrium intensity)

C – intersection of the linearity curve

m – slope of linearity curve

nb – sample mass [g]

C	0.002
m	0.1611

y=0.1611x+0.0020

105 Pd [He]

	CPS Pd	CPS Y	CPS Pd/CPS Y	nb	1000/nb	Z [ppm]	Z average [ppm]
sample 1	29140.65	46778.88	0.622944585	0.0989	10111.22	38.97275	38.847
sample 2	30308.05	48552.79	0.624228803	0.09975	10025.06	38.72056	
sample 3	30356.21	47915.63	0.633534611	0.09985	10015.02	39.26029	

1.5 Note of Caution

The mixture of O_2 with organic solvent vapours is extremely flammable! Pressurized equipment should be operated with care! Before conducting any experiments, an individual, careful safety assessment including reaction kinetics and explosive hazards should be carried out!

The H_2 is extremely flammable! Pressurized equipment should be operated with care! Before conducting any experiments, an individual, careful safety assessment including reaction kinetics and explosive hazards should be carried out!

The mixture of O_2 with H_2 is extremely flammable!! Before conducting any experiments, an individual, careful safety assessment including reaction kinetics and explosive hazards should be carried out!

2. Experimental Details

2.1 General procedure

Synthesis of compound 3 in batch reaction

I step

To the 4.5g (11.18 mmol) of alcohol **1b** was added 50 mL of butyl acetate and 50 mL of toluene. After heating to 70 °C alcohol dissolved. Then manganese (II) oxide activated 9.67g (111mmol) was added. The reaction mixture was heated at 120 °C for 1.5 h. The mixture was cooled, filtered through Cellite® (10 g), and washed with 20 mL of DCM. Filtrate was evaporated resulting in 3.1 g of aldehyde **2** as a yellow solid which was used for the next reaction without further purification.

II step

To the 3.1g (7.8 mmol) was added dry 30 mL DCM and 1.33 g (9.4 mmol). The mixture was stirred for 1 h at room temperature, and then 3.03 g (14.3 mmol) of sodium triacetoxyborohydride was added. The reaction mixture was stirred at room temperature overnight (~16 h). The reaction mixture was transferred to the extraction funnel. Water 30ml and sodium carbonate saturated solution was added 8ml. Phases were separated and the water phase was extracted with 2x20 mL DCM. Organic phases were combined, dried with sodium sulfate, and evaporated. Crude oil was macerated with 50 mL of MTBE. The precipitated white solid was filtered and dried which resulted in 3.39 g (6.46 mmol) compound **3**. The total yield after two steps was 57.8 %. The structure was consistent with data from the literature [1].

Synthesis of compound 3 by reductive amination in flow on Cube®Pro from ThalesNano tests:

The aldehyde **2** 220 mg (0.552 mmol) and 157 mg N-*tert*-butylpiperazine were dissolved in a mixture of toluene/EtOAc (4 mL/4 mL) in a 16 mL vial. The resulting mixture was stirred for 1.5 h at room temperature. Next, the solution was pumped by H-Cube®Pro fromThalesNano through a suitable fixed-bed catalyst encapsulated in the metal cartridge. The hydrogen gas was generated by H-Cube®Pro. The crude reaction mixture was analyzed by UHPLC. The structure was consistent with data from the literature [1].

Synthesis of compound 3 by reductive amination in flow on Cube®Pro from ThalesNano – gram -scale reaction:

The aldehyde **2** 4.63 g (11.6 mmol) and 3.3 g N-*tert*-butylpiperazine were dissolved in a mixture of toluene/EtOAc (84 mL/84 mL) in a 250 mL vial. The resulting mixture was stirred for 1.5 h at room temperature. Next, the solution was pumped by H-Cube®Pro fromThalesNano through a suitable fixed-bed catalyst encapsulated in the metal cartridge. The hydrogen gas was generated by H-Cube®Pro. The crude reaction mixture was evaporated. The remaining half-solid oil was recrystallized in 60 mL ethanol resulting in 5.27 g (10.0 mmol) of compound **3** as a white solid. The structure was consistent with data from the literature [1].

2.2 Telescoped sequence

Feed 1: Alcohol **1b** 1.0 g was dissolved in toluene/EtOAC (1:1) mixture (1.0 g/100 mL).

Feed 2: O_2 gas was introduced from a gas cylinder through the Vapourtec SF-10 pump.

Feed 3: Palladium acetate ($Pd(OAc)_2$) 114 mg was dissolved in 100 mL of toluene/EtOAC (1:1), then pyridine 52.1 uL was added using an automatic pipette. Upon addition of pyridine to palladium acetate solution (orange) brightened to yellow.

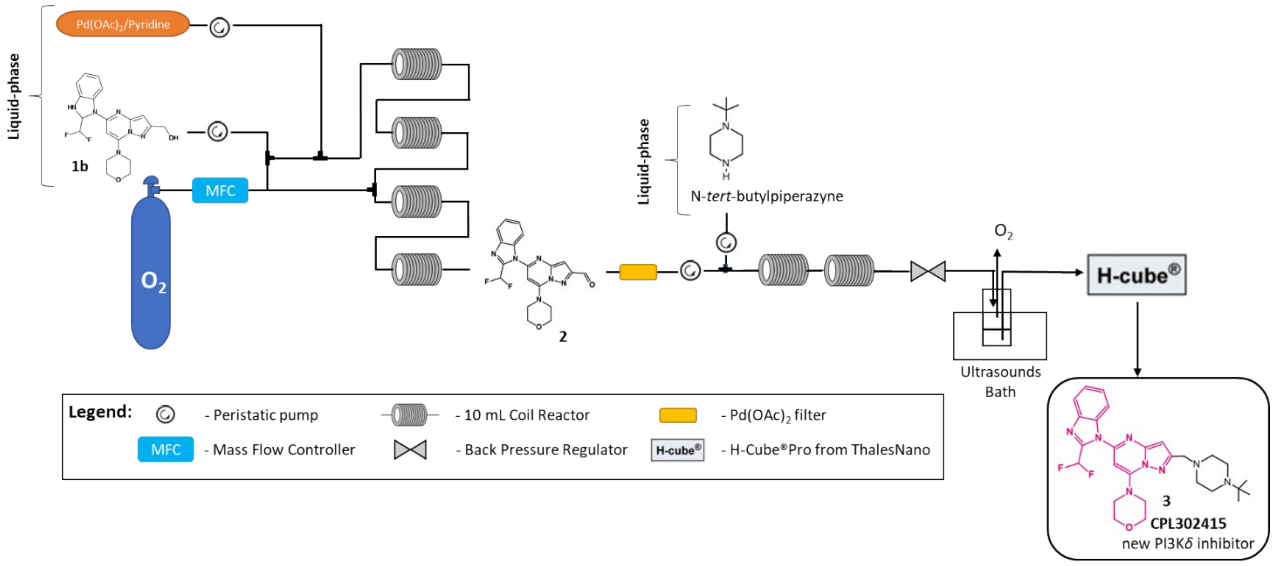
Feed 4: 0.71 g of N-*tert*-butylpiperazine was dissolved in 20 mL of toluene/EtOAC (1:1) - twice the required amount due to the need to fill the tubes.

Flow procedure: The solvent bottle of the Vapourtec easy-Medchem system was filled with toluene/EtOAC (1:1). For the reaction, 100 mL of each solution was used. Both reagents were pumped with 1 mL min^{-1} flow rate each, the oxygen was pumped with 0.1 mL min^{-1}, P_{O2}= 5 bar. Feed 1 was mixed with oxygen using the Y-shaped mixer, run through a 28 cm long tube to saturate it with gas, and later combined with Feed 2 also in the Y-shape mixer. The reaction was performed at 120°C first within two heated PFA tubular reactors. Next, in order to extend the reaction time, the reaction mixture feed was supplemented with oxygen and transferred into the additional two reactors as previously heated at 120°C. The temperature was set manually. After overall 20 min of oxidation reaction in the next step, Feed 4 is introduced to the reaction stream by a Y-shape mixer, and the solution of N-*tert*-butylpiperazine was pumped with 0.2 mL min^{-1}. Then the reaction mixture passed through two PFA tubular reactors (20 mL, id=3.2 mm) and one PFA tubular reactor (10 mL, id= 1 mm) at 50°C. The system pressure was maintained by a Vapourtec SF-10 pump as a back pressure regulator (BPR). Next, the solution was collected in the tank where it was sonificated in an ultrasound bath and purged with Ar stream. The collection began 68 min after the reaction start [20 min to go through the oxidation step + 22.73 min to go through Schiff base step (2.2 mL · min^{-1} through 50 mL) + 25 min to run the first 50 mL of Feed 1+ Feed 3].

The reaction mixture was collected for 50 min (the equivalent of 0.5 g (2.49 mmol) of alcohol **1b**) and was pumped by H-Cube®Pro apparatus from ThalesNano through a CatCart® 10 %Pd/C 70 mm length cartridge. The reaction conditions were set at T= 90 °C. P_{sys}=3 bar. The flow of H_2= 18 mL min^{-1}. Flow of reagents H_2= 0.7 mL min^{-1}. The hydrogen gas was generated by H-Cube®Pro. The crude reaction mixture was analyzed by UHPLC, collected, and evaporated. The remaining half-solid oil

was recrystallized in 5 mL ethanol resulting in 0.467 g (1.78 mmol) of compound **3** as a white solid. The structure was consistent with data from the literature [1].

Reaction conditions:
Oxydation step T= 120 °C. P_{O2}= 5 bar. P_{sys}=18 bar. V_{O2} = 0.1 mL min^{-1}. V of **1b** = 1.0 mL min^{-1}.
Formation imine step T= 50 °C. Flow of N-*tert*-butylpiperazine = 0.2 mL min^{-1}.
Amination reductive step T= 90 °C. P_{sys}=3 bar. Flow of H_2= 18 mL min^{-1}. Flow of reagents H_2= 0.7 mL min^{-1}.



Scheme S1. Telescoped flow Pd-catalysed aerobic alcohol oxidation/reductive amination of alcohol **1b** to CPL302415 (**3**).

Table S1. Additional conditions screened for amination reductive of **2** carried out at toluene/EtOAc (1:1) mixture. DoE Central Composite Design (CCD) model 2^(5).

Entry	T (°C)	Psys (bar)	V of reagents (mL/min)	V of H_2 (mL/min)	Eq. N-*tert*-butylpiperazine	% of **2**	% of **1**	% of **3**
1	70.0	1.0	0.3	6	1.2	1.60	73.41	0.00
2	70.0	1.0	0.3	6	4.0	0.00	25.49	71.45
3	70.0	1.0	0.3	48	1.2	0.00	67.92	4.54
4	70.0	1.0	0.3	48	4.0	0.00	27.62	66.88
5	70.0	1.0	2.1	6	1.2	0.00	52.62	23.38
6	70.0	1.0	2.1	6	4.0	2.14	47.11	44.38
7	70.0	1.0	2.1	48	1.2	0.00	55.21	39.09
8	70.0	1.0	2.1	48	4.0	1.67	51.11	43.36
9	70.0	5.0	0.3	6	1.2	0.00	44.92	36.00
10	70.0	5.0	0.3	6	4.0	0.00	25.06	65.10
11	70.0	5.0	0.3	48	1.2	0.00	44.81	33.64
12	70.0	5.0	0.3	48	4.0	0.00	23.51	61.27
13	70.0	5.0	2.1	6	1.2	0.00	55.77	28.76
14	70.0	5.0	2.1	6	4.0	1.74	51.17	43.78
15	70.0	5.0	2.1	48	1.2	0.00	55.28	28.93
16	70.0	5.0	2.1	48	4.0	1.14	49.52	43.60
17	100.0	1.0	0.3	6	1.2	0.00	10.30	69.32
18	100.0	1.0	0.3	6	4.0	1.25	20.57	48.20
19	100.0	1.0	0.3	48	1.2	0.00	10.44	42.49
20	100.0	1.0	0.3	48	4.0	0.00	14.88	31.81
21	100.0	1.0	2.1	6	1.2	1.18	40.36	41.75
22	100.0	1.0	2.1	6	4.0	0.00	42.94	34.72
23	100.0	1.0	2.1	48	1.2	0.00	35.16	30.33
24	100.0	1.0	2.1	48	4.0	0.00	39.95	36.72
24	100.0	5.0	0.3	6	1.2	0.00	11.02	28.95
26	100.0	5.0	0.3	6	4.0	0.00	10.09	41.60
27	100.0	5.0	0.3	48	1.2	0.00	8.69	33.31
28	100.0	5.0	0.3	48	4.0	0.00	13.64	17.79
29	100.0	5.0	2.1	6	1.2	0.00	42.74	34.80
30	100.0	5.0	2.1	6	4.0	0.00	43.28	46.30
31	100.0	5.0	2.1	48	1.2	0.00	35.07	29.25
32	100.0	5.0	2.1	48	4.0	0.00	42.10	46.74
33	70.0	3.0	1.2	27	2.6	0.00	44.10	50.04
34	100.0	3.0	1.2	27	2.6	0.00	31.07	54.29
35	85.0	1.0	1.2	27	2.6	0.00	35.86	55.17
36	85.0	5.0	1.2	27	2.6	0.00	39.65	51.03
37	85.0	3.0	0.3	27	2.6	0.00	14.62	69.58
38	85.0	3.0	2.1	27	2.6	0.00	46.60	48.94
39	85.0	3.0	1.2	6	2.6	0.00	38.52	56.58
40	85.0	3.0	1.2	48	2.6	0.00	36.31	58.14
41	85.0	3.0	1.2	27	1.2	0.00	33.01	57.11
42	85.0	3.0	1.2	27	4.0	0.00	38.92	54.28
43	85.0	3.0	1.2	27	2.6	0.00	38.42	56.38
44	85.0	3.0	1.2	27	2.6	0.00	34.29	60.88
45	85.0	3.0	1.2	27	2.6	0.00	36.36	56.55

[a] Standard reaction conditions appled on H-Cube®PRO: substrate = 220 mg (0.552 mmol) and N-*tert*-butylpiperazine were dissolved in mixture toluene/EtOAc (4mL/4mL) ; CatCart® 70mm long containing 10 % Pd/C 260±5 mg [b] % determined by UHPLC.

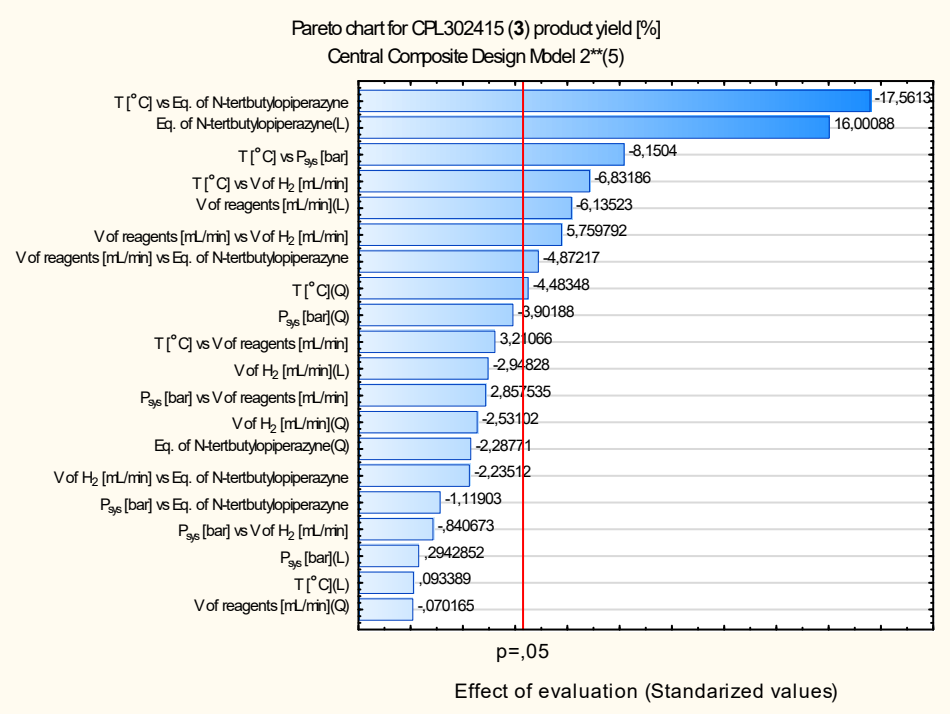
Table S2. Additional conditions screened for amination reductive of 2 carried out at toluene/EtOAc (1:1) mixture.

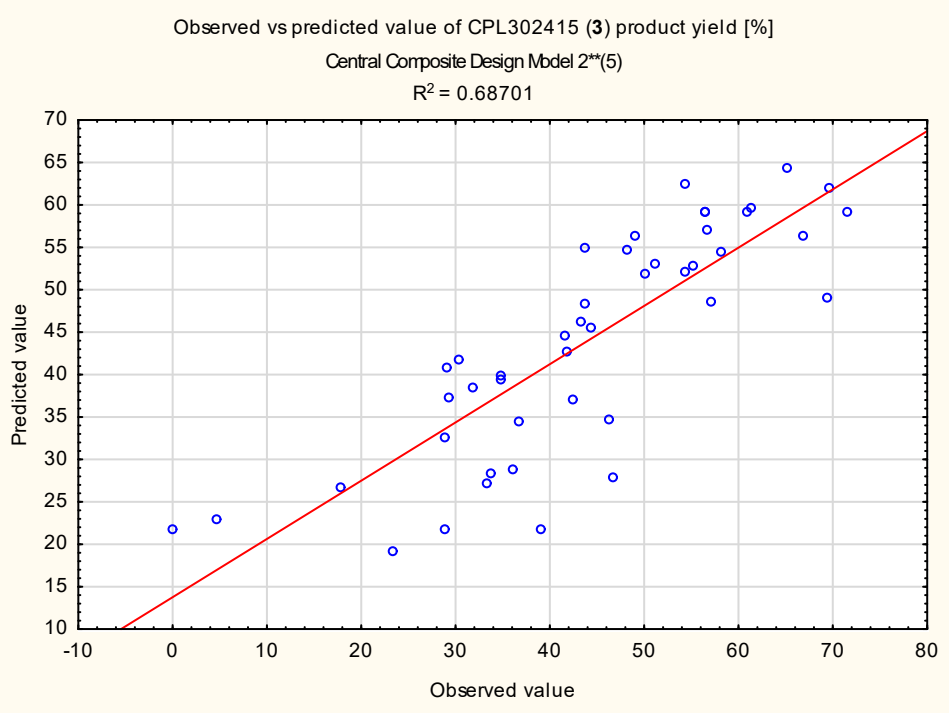
Entry	catalyst	Length of CatCart®	Psys (bar)	V of reagents (mL/min)	V of H_2 (mL/min)	% of **2**	% of **1**	% of **3**
1	10%Pd/C	70 mm	1	0.7	18	0	6.6	91.0
2	10%Pd/C	70 mm	3	0.7	18	0	4.9	93.1
3	10%Pd/C	70 mm	3	0.7	30	3.2	9.0	86.2
4	10%Pd/C	30 mm	3	0.3	18	0	8.5	79.8
5	10%Pd/C	30 mm	3	0.3	30	0	21.4	64.0
6	10%Pd/C	30 mm	3	0.3	42	0	15.9	73.1
7	10%Pd/C	30 mm	3	0.4	24	0	8.9	87.7
8	10%Pd/C	30 mm	3	0.5	18	0	6.8	85.5
9	10%Pd/C	30 mm	1	0.3	18	0	21.4	68.4
10	10%Pd/C	30 mm	3	0.5	30	0	15.9	73.1
11	10%Pd/C	30 mm	3	0.5	42	0	12.3	72.1
12	10%Pd/C	30 mm	3	0.7	42	0	20.4	70.9
13	10%Pd/C	30 mm	5	0.5	18	0	22.4	71.1
14	10%Pd/C	30 mm	5	0.5	18	0	13.9	79.6
15	20%$Pd(OH)_2$/C	30 mm	3	0.3	18	0	24.3	21.2
16	5%Pt/C	30 mm	3	0.3	18	0	13.9	79.6

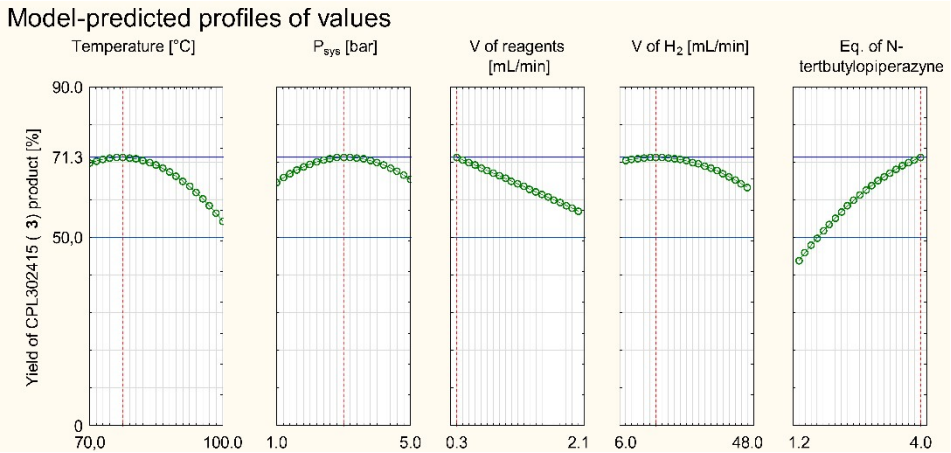
[a] Standard reaction conditions appled on H-Cube®PRO: substrate = 220 mg (0.552 mmol) and 2 eq. of N-*tert*-butylpiperazine were dissolved in mixture toluene/EtOAc (4mL/4mL) ; T = 90°C; [b] % determined by UHPLC.

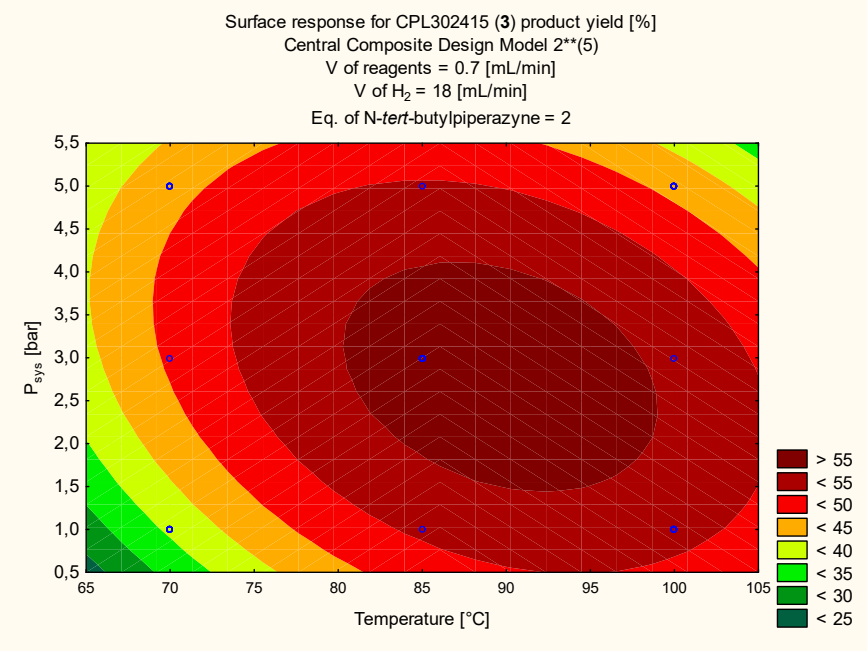
3. DoE Analysis

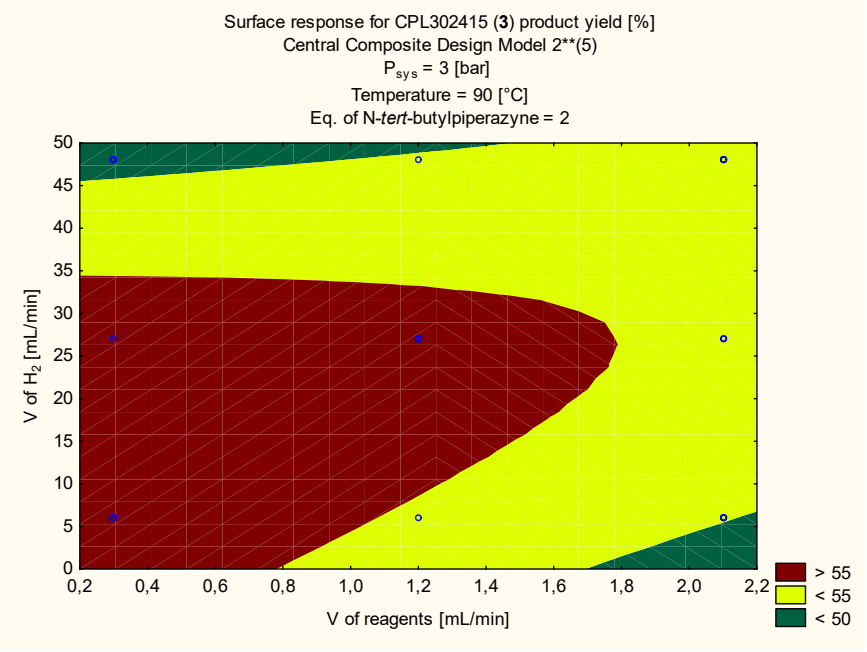
Figure S1. Summary of the DoE Central Composite Design (CCD) model 2^(5) for CPL302415 (**3**) product yield.

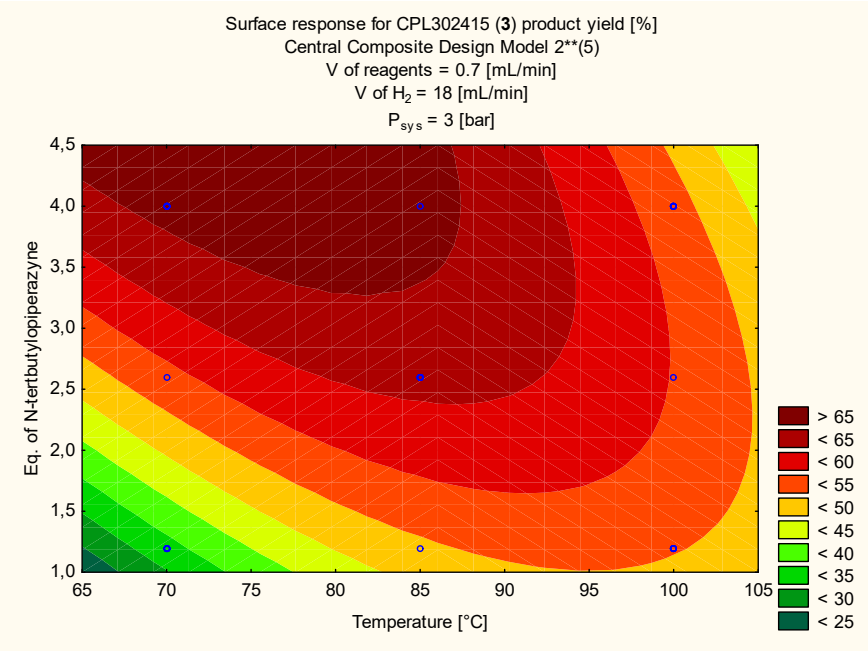







Surface response for CPL302415 (3) product yield [%]
Central Composite Design Model 2**(5)
V of reagents = 0.7 [mL/min]
V of H2 = 18 [mL/min]
Eq. of N-tert-butylpiperazyne = 2
Psys [bar]
Temperature [°C]
> 55
< 55
< 50
< 45
< 40
< 35
< 30
< 25


Surface response for CPL302415 (3) product yield [%]
Central Composite Design Model 2**(5)
Psys = 3 [bar]
Temperature = 90 [°C]
Eq. of N-tert-butylpiperazyne = 2
V of H2 [mL/min]
V of reagents [mL/min]
> 55
< 55
< 50


Surface response for CPL302415 (3) product yield [%]
Central Composite Design Model 2**(5)
V of reagents = 0.7 [mL/min]
V of H2 = 18 [mL/min]
Psys = 3 [bar]
Eq. of N-tertbutylopiperazyne
Temperature [°C]
> 65
< 65
< 60
< 55
< 50
< 45
< 40
< 35
< 30
< 25

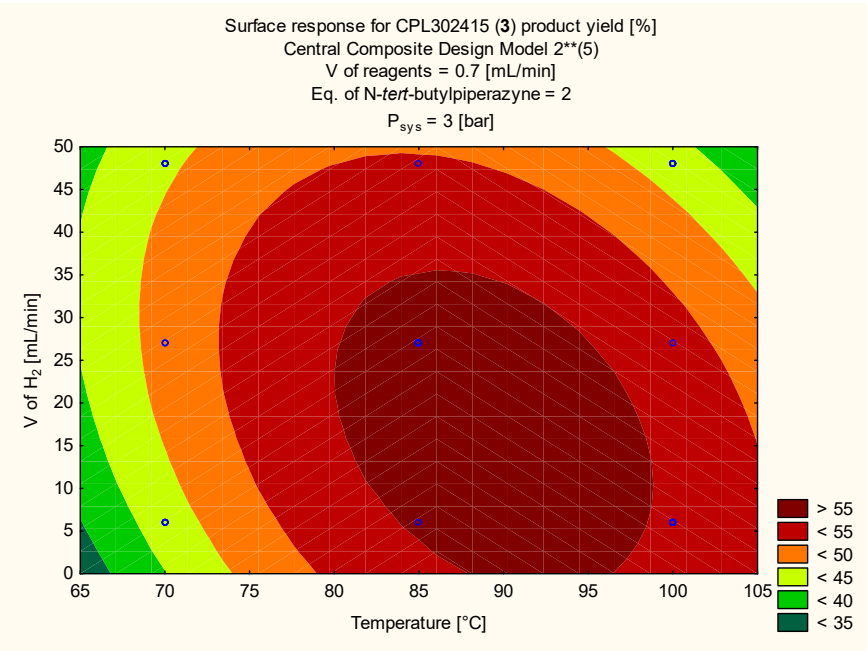
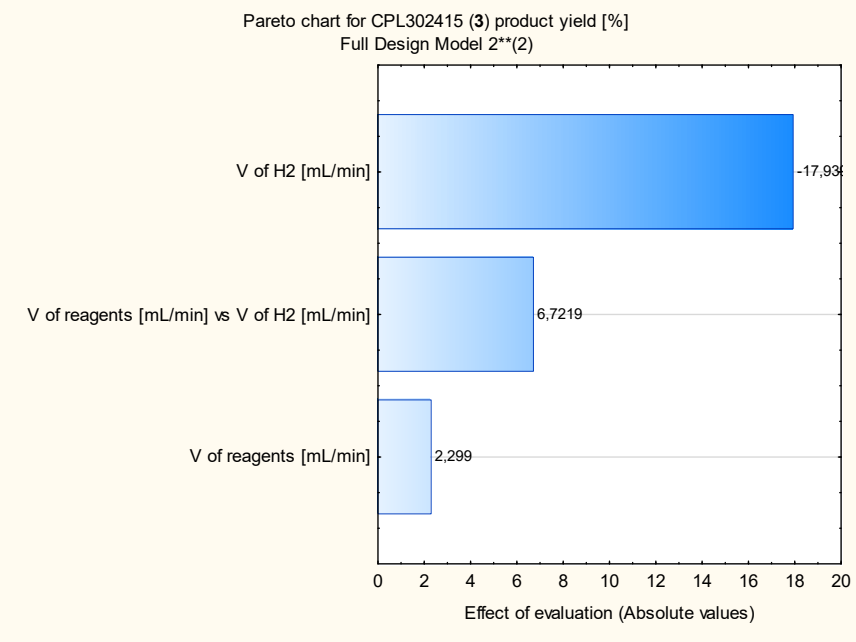
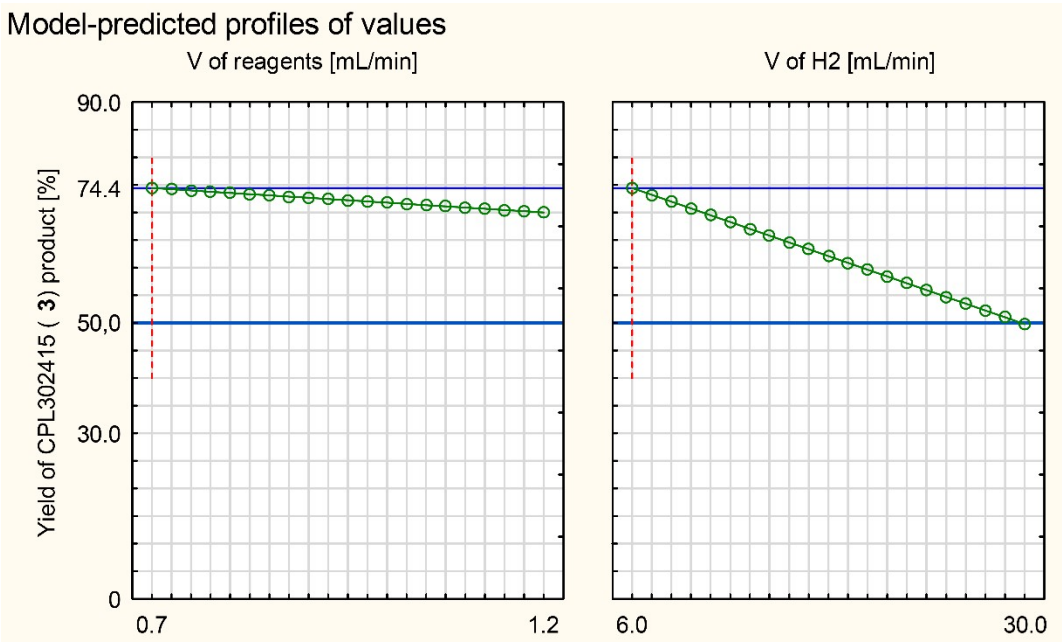

Surface response for CPL302415 (3) product yield [%]
Central Composite Design Model 2**(5)
V of reagents = 0.7 [mL/min]
Eq. of N-tert-butylpiperazyne = 2
Psys = 3 [bar]
V of H2 [mL/min]
Temperature [°C]
> 55
< 55
< 50
< 45
< 40
< 35

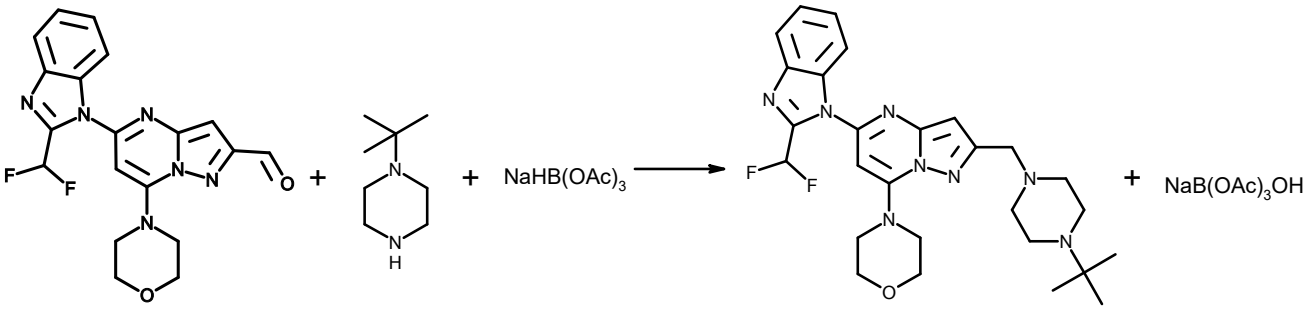
Figure S2. Summary of the DoE full design model 2^(2) for CPL302415 (**3**) product yield.





4. Green metrics calculations [3-5]

4.1 Reductive amination – batch



Reactants	MW(g/mol)	Stoich. Coeff. (SC)	Adjusted MW (g/mol)	mass used (g)
Aldehyde	398.37	1	398.37	3.1
$NaHB(OAc)_3$	211.94	1	211.94	3.03
t-BuPi	142.24	1	142.24	1.33
		SUM	752.55	7.46
Products	MW(g/mol)	Stoich. Coeff.	Adjusted MW (g/mol)	
$NaB(OAc)_3OH$	227.93	1	227.93	
CPL302415 (**3**)	524.62	1	524.62	
		SUM	752.55	
Reaction Solvents	MW(g/mol)	density (g/mL)	volume (mL)	mass used (g)
DCM	84.93	1.325	30	39.75
		SUM		39.75
Catalysts	MW(g/mol)	mass used (g)		
none	-			0
		SUM		0
Workup Materials		density (g/mL)	volume (mL)	mass used (g)
Water		1.0	30	30
DCM		1.325	30	39.75
$NaHCO_3$ (aq. sat. sol.)		1.1	8	8.8
		SUM		78.55

Purification Materials	density (g/mL)	volume (mL)	mass used (g)
MTBE	0.74	50	37.0
	SUM		37.0

TOTAL INPUT MASS (g)	162.76

Target Product	MW(g/mol)	moles collected	mass collected (g)	
CPL302415 (**3**)	524.62	0.00646	**MASS OF TARGET PRODUCT**	3.39
MASS RAW WASTE = TOTAL INPUT MASS - MASS OF TARGET PRODUCT				159.37

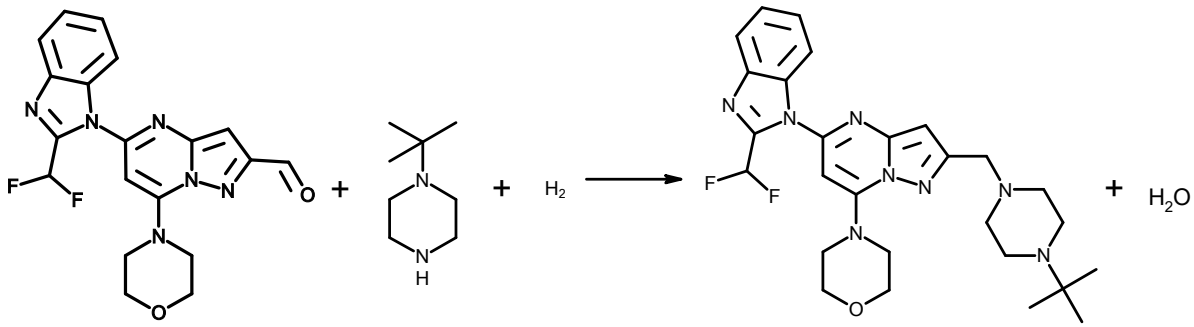
Recycling-Recovery of Materials	
Item	mass recovered (g)
MTBE	33.3
SUM	33.3

MASS ADJUSTED WASTE = MASS RAW WASTE - MASS RECOVERED MATERIALS	126.07
E-factor (E) = mass adjusted waste / mass product	37.19
Process Mass Intensity (PMI) = (total input mass - mass recovered materials) / mass product	38.19
Reaction Mass Efficiency (RME) = 100 * (mass product / (total input mass - mass recovered materials))	2.6%
Atom Economy (AE) = 100 * (MW product / sum of MWs of reactants)	69.7%
Yield (Y) = 100 * (moles product / moles limiting reagent) * (SC limiting reagent / SC Product)	83.0%

Actual Mass of Reagents	7.46
Stoichiometric Mass of Reagents	5.86
Stoichiometric Factor (SF)	1.27
Materials Recovery Parameter (MPR)	0.058

Parameter	**Actual**	**Ideal Limit**
AE	0.697	1
Rxn Yield	0.830	1
1/SF	0.785	1
MRP	0.058	1
RME	0.026	1

4.2 Reductive amination flow



CPL302415

Reactants	MW(g/mol)	Stoich. Coeff. (SC)	Adjusted MW (g/mol)	mass used (g)
Aldehyde	398.37	1	398.37	4.63
Hydrogen	2.02	1	2.02	0.362
t-BuPi	142.24	1	142.24	3.30
		SUM	542.63	8.292
Products	MW(g/mol)	Stoich. Coeff.	Adjusted MW (g/mol)	
Water	18.01	1	18.01	
CPL302415 (**3**)	524.62	1	524.62	
		SUM	542.63	
Reaction Solvents	MW(g/mol)	density (g/mL)	volume (mL)	mass used (g)
Toluene	92.14	0.867	84	72.828
Ethyl Acetate	88.11	0.902	84	75.768
		SUM		148.596
Catalysts	MW(g/mol)	mass used (g)		
10% Pd/C	Cartridge	0.156		
		SUM	0.156	
Workup Materials		mass used (g)		
none		0		
		SUM	0	

Purification Materials	density (g/mL)	volume (mL)	mass used (g)
Ethanol	0.789	60	47.34
	SUM		47.34

TOTAL INPUT MASS (g)	204.38

Target Product	MW(g/mol)	moles collected	mass collected (g)	
CPL302415 (**3**)	524.62	0,01005	**MASS OF TARGET PRODUCT**	5.27
MASS RAW WASTE = TOTAL INPUT MASS - MASS OF TARGET PRODUCT				199.11

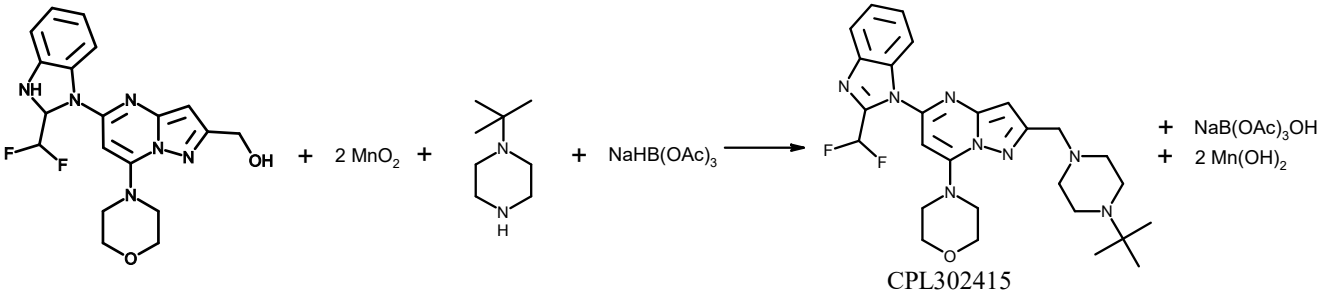
Recycling-Recovery of Materials	
Item	mass recovered (g)
Toluene	65.54
Ethyl Acetate	68.19
Ethanol	42.61
SUM	176.34

MASS ADJUSTED WASTE = MASS RAW WASTE - MASS RECOVERED MATERIALS	22.77
E-factor (E) = mass adjusted waste / mass product	4.321
Process Mass Intensity (PMI) = (total input mass - mass recovered materials) / mass product	5.321
Reaction Mass Efficiency (RME) = 100 * (mass product / (total input mass - mass recovered materials))	18.8%
Atom Economy (AE) = 100 * (MW product / sum of MWs of reactants)	96.7%
Yield (Y) = 100 * (moles product / moles limiting reagent) * (SC limiting reagent / SC Product)	86.4%

Actual Mass of Reagents	8.29
Stoichiometric Mass of Reagents	6.31
Stoichiometric Factor (SF)	1.31
Materials Recovery Parameter (MPR)	0.296

Parameter	**Actual**	**Ideal Limit**
AE	0.967	1
Rxn Yield	0.864	1
1/SF	0.761	1
MRP	0.296	1
RME	0.188	1

4.3 Two steps – batch



CPL302415

Reactants	MW(g/mol)	Stoich. Coeff. (SC)	Adjusted MW (g/mol)	mass used (g)
Alcohol	402.4	1	402.4	4.50
MnO2	86.94	2	173.88	9.67
t-BuPi	142.24	1	142.24	1.33
NaHB(OAc)3	211.94	1	211.94	3.03
		SUM	930.46	18.53
Products	MW(g/mol)	Stoich. Coeff.	Adjusted MW (g/mol)	
Mn(OH)2	88.95	2	177.9	
NaB(OAc)3OH	227.94	1	227.94	
CPL302415 (**3**)	524.62	1	524.62	
		SUM	930.46	
Reaction Solvents	MW(g/mol)	density (g/mL)	volume (mL)	mass used (g)
Toluene	92.14	0.867	50	43.35
Butyl acetate	116.16	0.882	50	44.1
DCM	84.93	1.325	30	39.75
		SUM		127.2
Catalysts	MW(g/mol)	mass used (g)		
none	-			0
		SUM		0
Workup Materials		density (g/mL)	volume (mL)	mass used (g)
Cellite®		-	-	10
Water		1.0	30	30
DCM		1.325	60	79.5
NaHCO3 (aq. sat. sol.)		1.1	8	8.8
		SUM		128.3

Purification Materials	density (g/mL)	volume (mL)	mass used (g)
MTBE	0.74	50	37.0
	SUM		37.0

TOTAL INPUT MASS (g)	311.03

Target Product	MW(g/mol)	moles collected	mass collected (g)	
CPL302415 (**3**)	524.62	0.00646	**MASS OF TARGET PRODUCT**	3.39
MASS RAW WASTE = TOTAL INPUT MASS - MASS OF TARGET PRODUCT				307.64

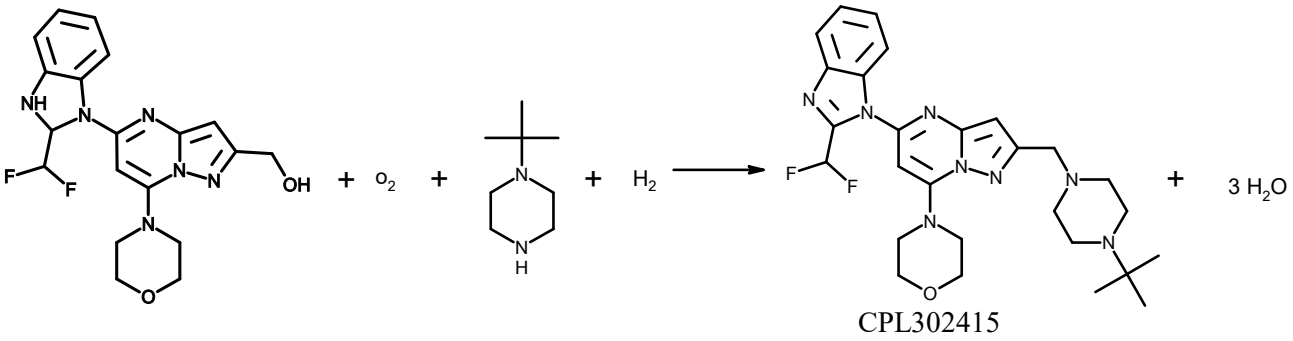
Recycling-Recovery of Materials	
Item	mass recovered (g)
MTBE	33.3
SUM	33.3

MASS ADJUSTED WASTE = MASS RAW WASTE - MASS RECOVERED MATERIALS	274.34
E-factor (E) = mass adjusted waste / mass product	80.93
Process Mass Intensity (PMI) = (total input mass - mass recovered materials) / mass product	81.93
Reaction Mass Efficiency (RME) = 100 * (mass product / (total input mass - mass recovered materials))	1.2%
Atom Economy (AE) = 100 * (MW product / sum of MWs of reactants)	56.4%
Yield (Y) = 100 * (moles product / moles limiting reagent) * (SC limiting reagent / SC Product)	57.8%

Actual Mass of Reagents	1 8.53
Stoichiometric Mass of Reagents	1 0.41
Stoichiometric Factor (SF)	1 .78
Materials Recovery Parameter (MPR)	0 .067

Parameter	**Actual**	**Ideal Limit**
AE	0.564	1
Rxn Yield	0.578	1
1/SF	0.562	1
MRP	0.067	1
RME	0.012	1

4.4 Telescopic sequence flow



CPL302415

Reactants	MW(g/mol)	Stoich. Coeff. (SC)	Adjusted MW (g/mol)	mass used (g)
Alcohol	402.4	1	402.40	0.5
Oxygen	32	1	32	0.04
Hydrogen	2.02	1	2,02	0.00255
t-BuPi	142.24	1	142.24	0.355
		SUM	578.66	0.898
Products	MW(g/mol)	Stoich. Coeff.	Adjusted MW (g/mol)	
Water	18.015	3	54.04	
CPL302415 (**3**)	524.62	1	524.62	
		SUM	578.66	
Reaction Solvents	MW(g/mol)	density (g/mL)	volume (mL)	mass used (g)
Toluene	92.14	0.867	50	43.35
Ethyl Acetate	88.11	0.902	50	45.1
		SUM		88.45
Catalysts	MW(g/mol)	mass used (g)		
Pd(OAc)2	224.51			0.057
Pyridine	79.1			0.0256
		SUM		0.0826
Workup Materials		mass used (g)		
Silica				0.5
		SUM		0.5

Purification Materials	density (g/mL)	volume (mL)	mass used (g)
Ethanol	0.789	5	3.945
	SUM		3.945

TOTAL INPUT MASS (g)	93.88

Target Product	MW(g/mol)	moles collected	mass collected (g)	
CPL302415 (**3**)	524.62	0.00089	**MASS OF TARGET PRODUCT**	0.467
MASS RAW WASTE = TOTAL INPUT MASS - MASS OF TARGET PRODUCT				93.41

Recycling-Recovery of Materials	
Item	mass recovered (g)
Toluene	39.02
Ethyl Acetate	40.59
Ethanol	3.55
SUM	83.16

MASS ADJUSTED WASTE = MASS RAW WASTE - MASS RECOVERED MATERIALS	10.25
E-factor (E) = mass adjusted waste / mass product	21.95
Process Mass Intensity (PMI) = (total input mass - mass recovered materials) / mass product	22.95
Reaction Mass Efficiency (RME) = 100 * (mass product / (total input mass - mass recovered materials))	4.4%
Atom Economy (AE) = 100 * (MW product / sum of MWs of reactants)	90.7%
Yield (Y) = 100 * (moles product / moles limiting reagent) * (SC limiting reagent / SC Product)	71.6%

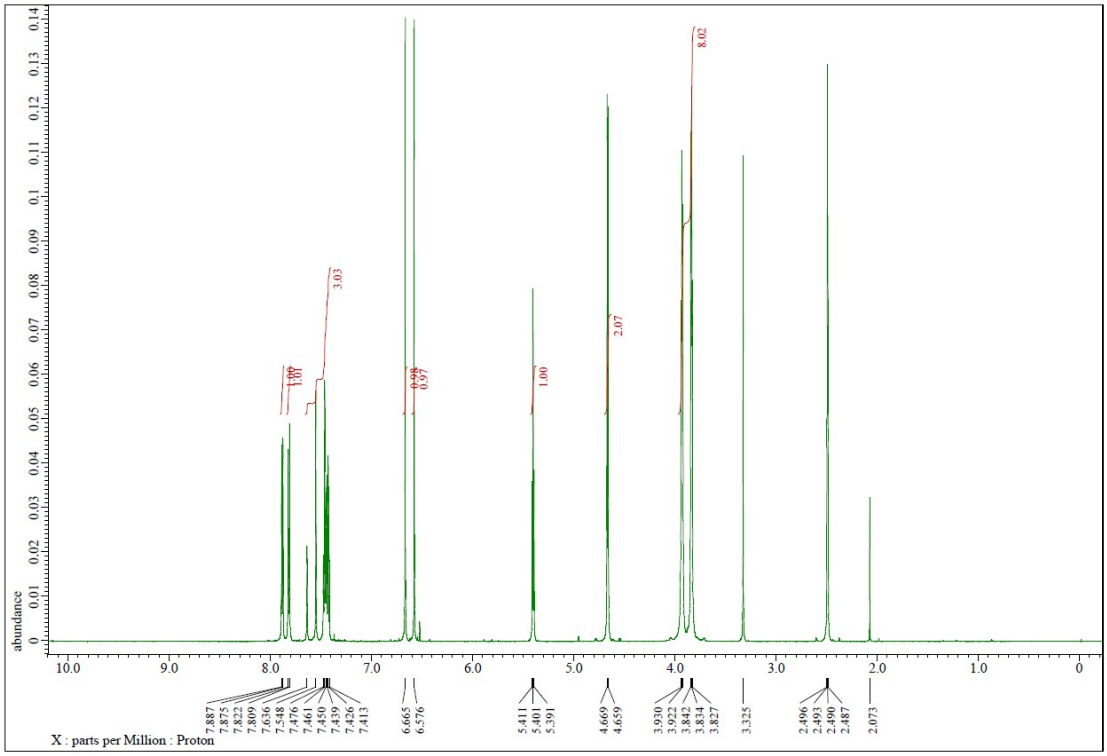
Actual Mass of Reagents	0.8976
Stoichiometric Mass of Reagents	0.7190
Stoichiometric Factor (SF)	1.248
Materials Recovery Parameter (MPR)	0.084

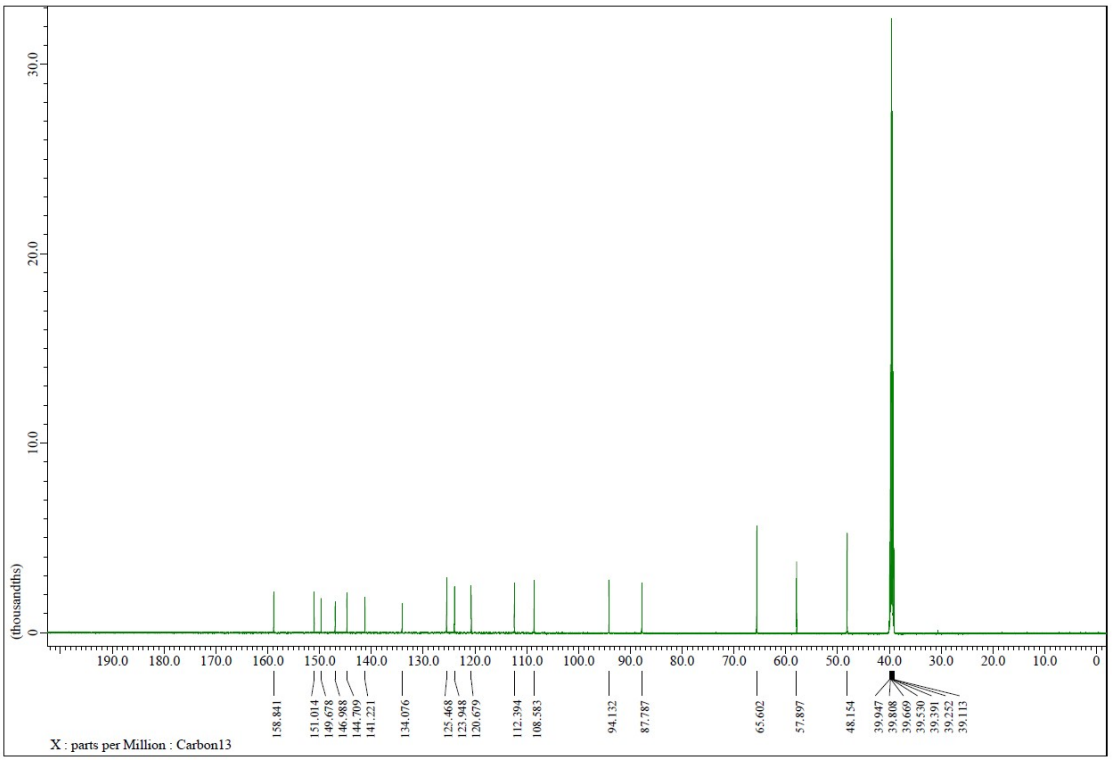
Parameter	**Actual**	**Ideal Limit**
AE	0.907	1
Rxn Yield	0.716	1
1/SF	0.801	1
MRP	0.084	1
RME	0.044	1

5. NMR spectrum

{5-[2-(difluoromethyl)-1H-1,3-benzodiazol-1-yl]-7-(morpholin-4-yl)pyrazolo[1,5-a]pyrimidin-2-yl}methanol (1)

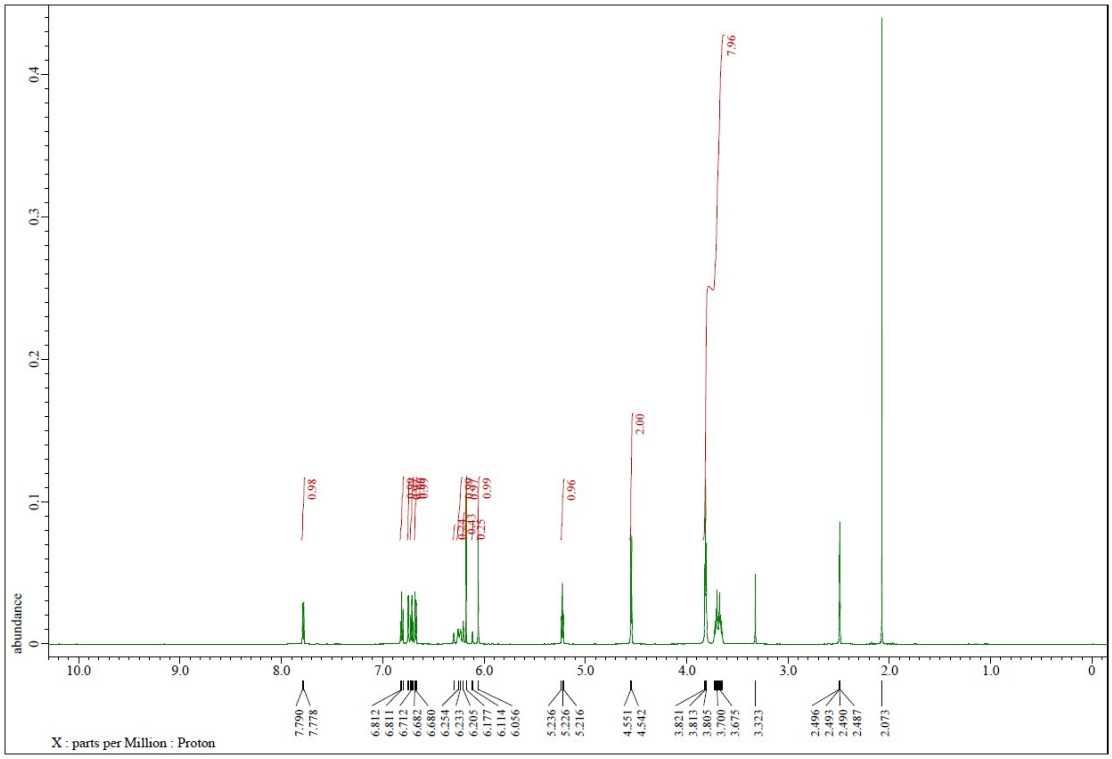
^{1}H-NMR (600 MHz, DMSO-D6) δ 7.88 (d, 1H, Ar-H), 7.82 (d, 1H, Ar-H), 7.64-7.46 (t, J = 52.8 Hz, 1H, CF_2H), 7.48-7.41(m, 2H, Ar-H) 6.67 (s, 1H, Ar-H), 6.58 (s, 1H, Ar-H), 5.40 (t, 1H, OH), 4.66 (d, 2H, CH_2), 3.94-3.83 (m, 8H, morph.); ^{13}C-NMR (151 MHz, DMSO-D6) δ 158.8, 151.0, 149.7, 147.0, 144.7, 141.2, 134.1, 125.5, 123.9, 120.7, 112.4, 108.6, 94.1, 87.8, 65.6, 57.9, 48.2
HRMS (ESI/MS): *m/z* calculated for $C_{19}H_{18}F_2N_6O_2$ $[M + H]^+$ 400.1459 found 400.1456.

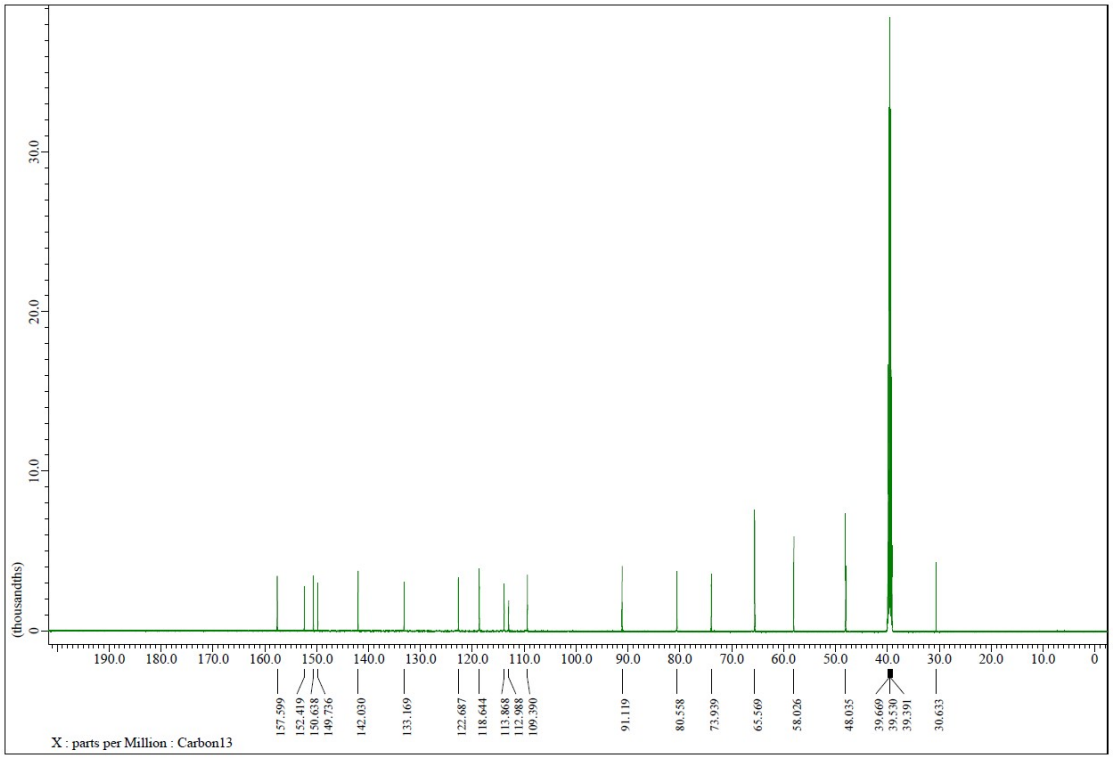




{5-[2-(difluoromethyl)-2.3-dihydro-*1H*-1.3-benzodiazol-1-yl]-7-(morpholin-4- yl)pyrazolo[1.5-a]pyrimidin-2-yl}methanol (1b)

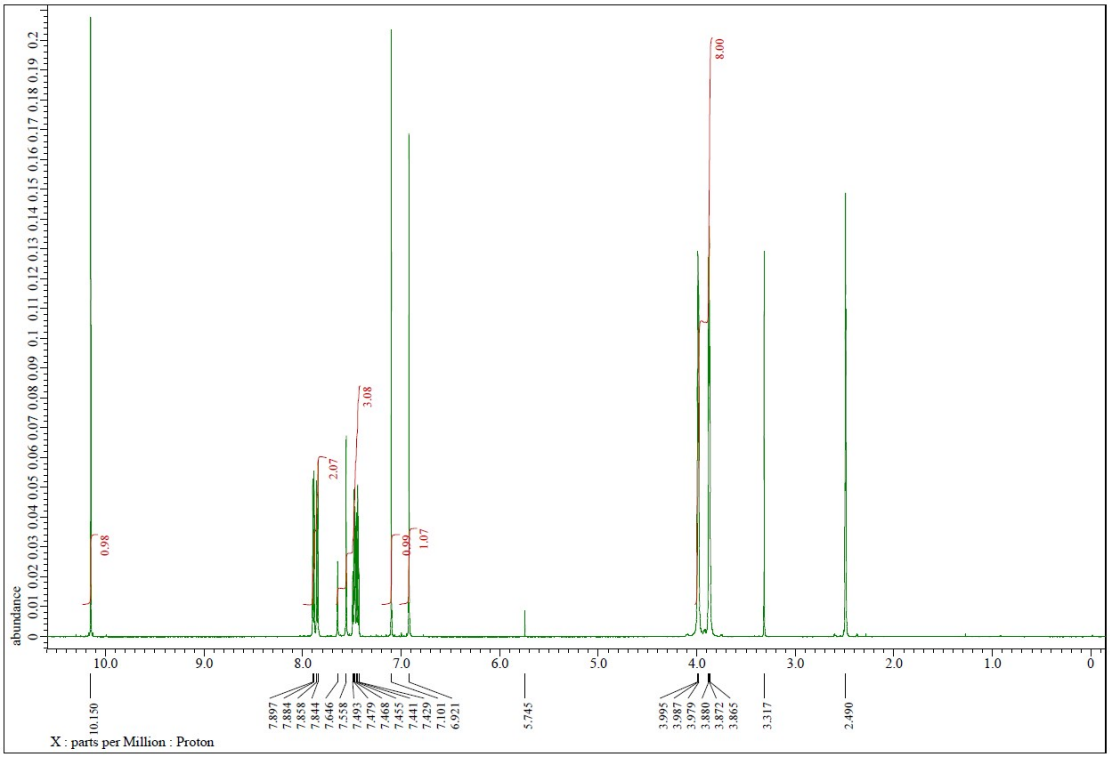
^{1}H-NMR (600 MHz. DMSO-D6) δ ppm: 7.78 (d, 1H, NH), 6.81 (td, 1H, Ar-H), 6.75 (d, 1H, Ar-H), 6.71 (td. J = 7.7, 1H, Ar-H), 6.67 (dd, 1H, Ar-H), 6.30-6.11(t, J = 54.6 Hz, 1H, CF_2H), 6.24 (d, 1H, CH), 6.18 (s, 1H, Ar-H), 6.06 (s, 1H, Ar-H), 5.23 (t, 1H, OH), 4.55 (d, 2H, CH_2), 3.82-3.65 (m. 8H, morph.); ^{13}C-NMR (151 MHz. DMSO-D6) δ ppm: 157.6, 152.4, 150.6, 149.7, 142.0, 133.2, 122.7, 118.6, 113.9, 113.0, 109.4, 91.1, 80.6, 73.9, 65.6, 58.0, 48.0, HRMS (ESI/MS): *m/z* calculated for $C_{19}H_{20}F_2N_6O_2$ [M + H]+ 402.1616 found 402.1613.

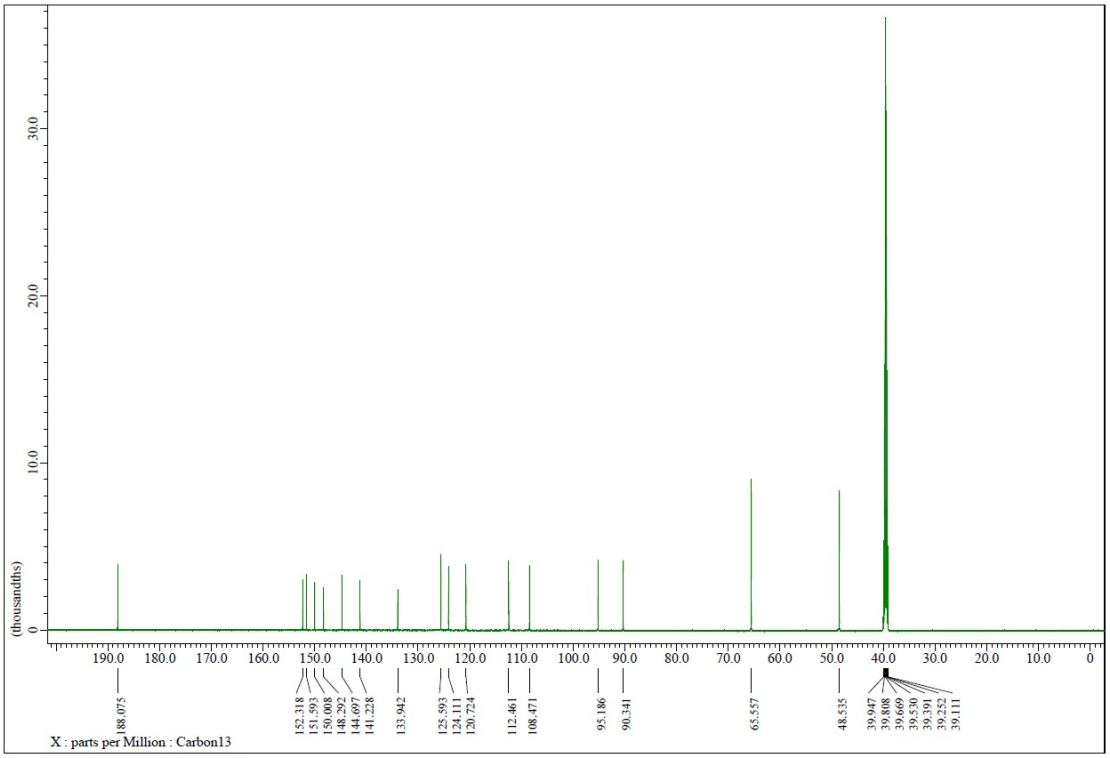




5-[2-(difluoromethyl)-*1H*-benzimidazol-1-yl]-7-(morpholin-4-yl)pyrazolo[1,5-a]pyrimidine-2-carbaldehyde (2)

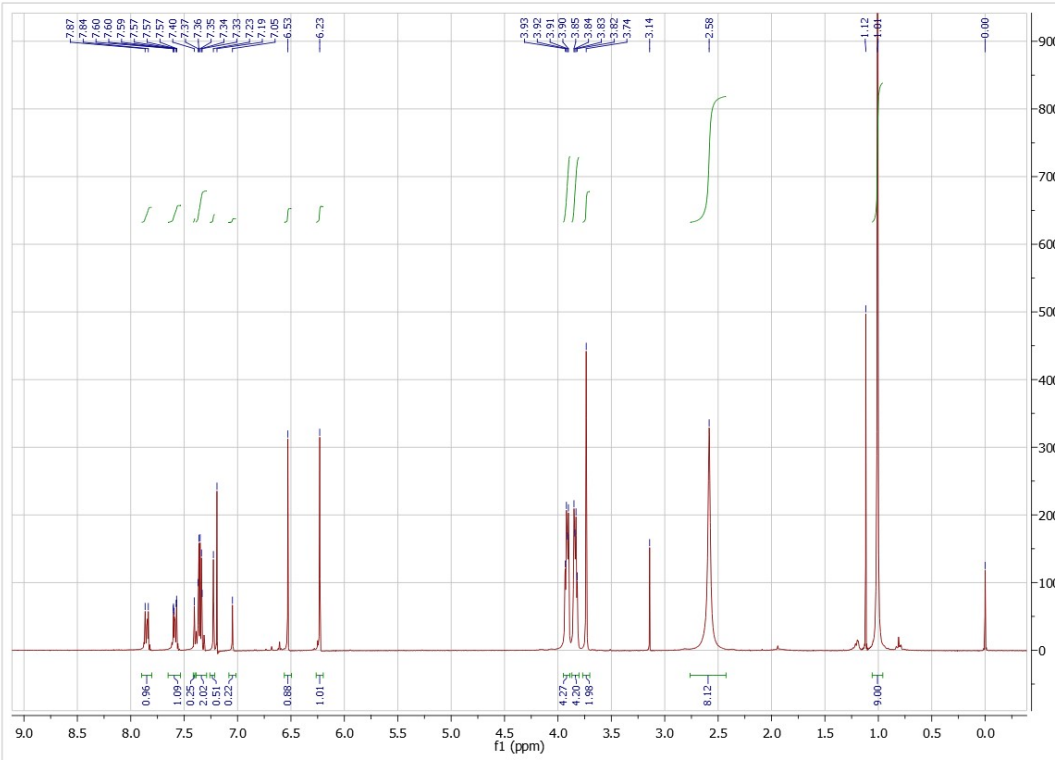
^{1}H-NMR (600 MHz, DMSO-D6) δ 10.15 (s, 1H, CHO), 7.87 (dd, 2H, Ar-H), 7.65- 7,47 (t, 1H; J = 52.8 Hz, CF_2H) 7,49-7.43 (m, 2H, Ar-H), 7.10 (s, 1H, Ar-H), 6.92 (s, 1H, Ar-H), 3.99-3.86 (m, 8H, morph); ^{13}C-NMR (151 MHz, DMSO-D6) δ 188.1, 152.3, 151.6, 150.0, 148.3, 144.7, 141.2, 133.9, 125.6, 124.1, 120.7, 112.5, 108.5, 95.2, 90.3, 65.6, 48.5; HRMS (ESI/MS): *m/z* calculated for $C_{19}H_{16}F_2N_6O_2$ $[M + H]^+$ 398.1303 found 398.1303.

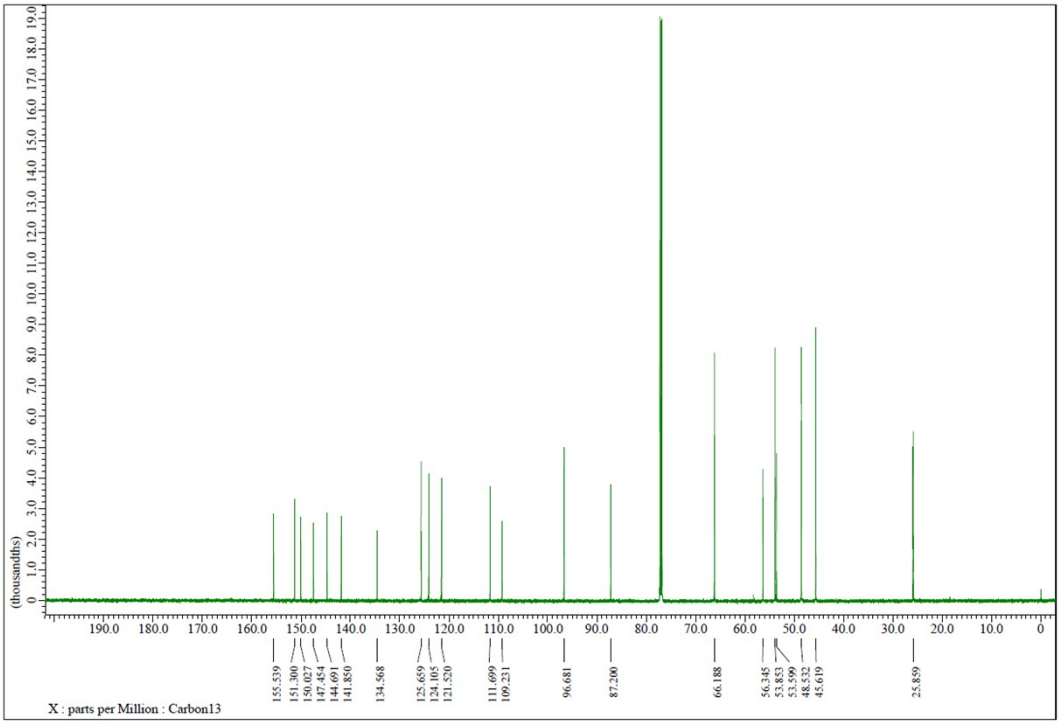




1-{2-[(4-tert-butylpiperazin-1-yl)methyl]-7-(morpholin-4-yl)pyrazolo[1,5-a]pyrimidin-5-yl}-2-(difluoromethyl)-1H-benzimidazole (3)

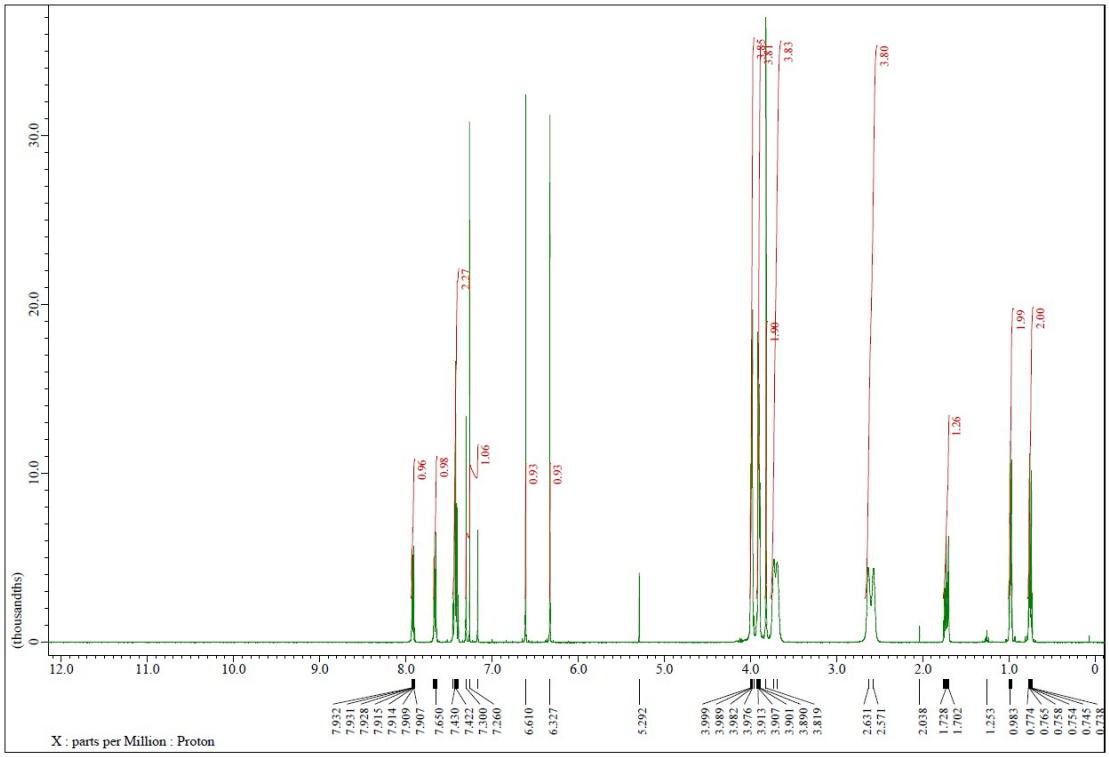
^{1}H-NMR (600 MHz, $CDCl_3$) δ 7.87–7.84 (m, 1H, Ar-H), 7.60–7.57 (m, 1H, Ar-H), 7.40–7.33 (m, 2H, Ar-H), 7.23 (t, J = 53.4 Hz, 1H, CHF_2), 6.53 (s, 1H, Ar-H), 6.23 (s, 1H, Ar-H), 3.93–3.90 (m, 4H, morph.), 3.85–3.82 (m, 4H, morph.), 3.74 (s, 2H, CH_2), 2.58 (s, 8H, piperazine), 1.01 (s, 9H, t-Bu). ^{13}C{^{1}H, ^{19}F}-NMR (151 MHz, $CDCl_3$) δ 155.4, 151.3, 150.0, 147.4, 144.7, 141.9, 134.6, 125.7, 124.0, 121.5, 111.7, 109.2 (CF_2), 96.7, 87.2, 66.2, 56.3, 53.9, 53.6, 48.5, 45.6, 25.9 (t-Bu.) HRMS (ESI/MS): m/z calculated for $C_{27}H_{34}F_2N_8O$ [M + H]+ 525.2896 found 525.2904.

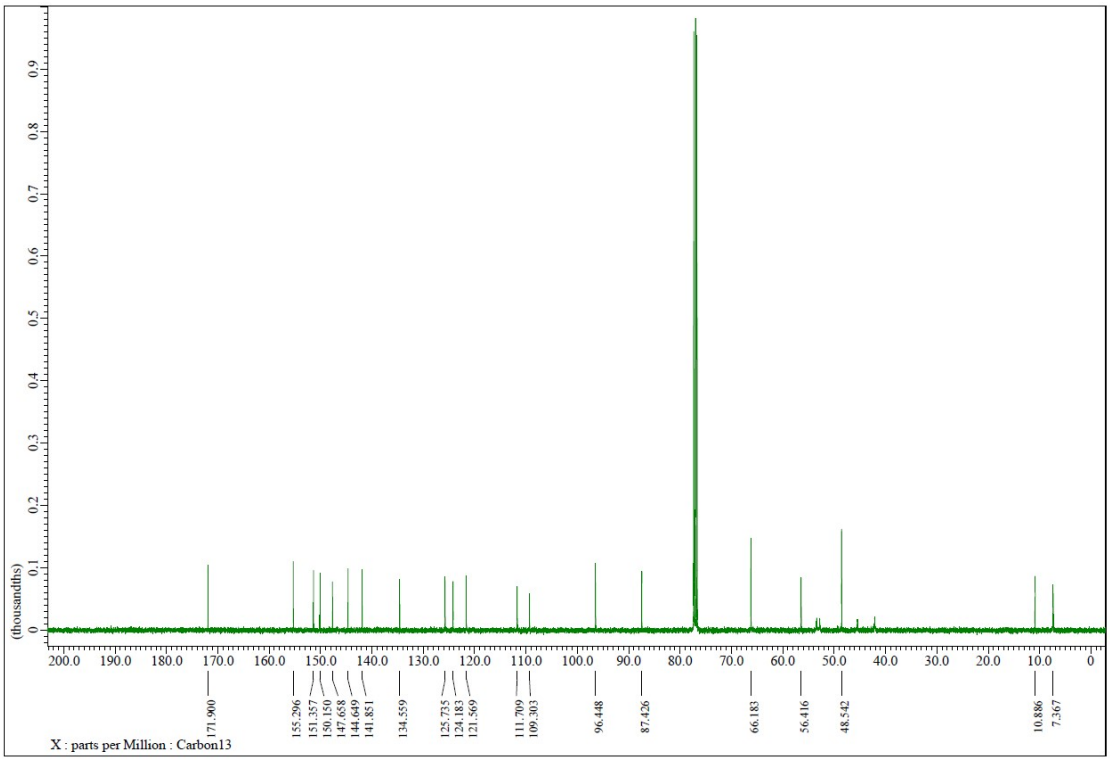




2-{2-[(4-cyclopropanecarbonylpiperazin-1-yl)methyl]-7-(morpholin-4-yl)pyrazolo[1,5-a]pyrimidin-5-yl}-2-(difluoromethyl)-1H-1,3-benzimidazole (4a)

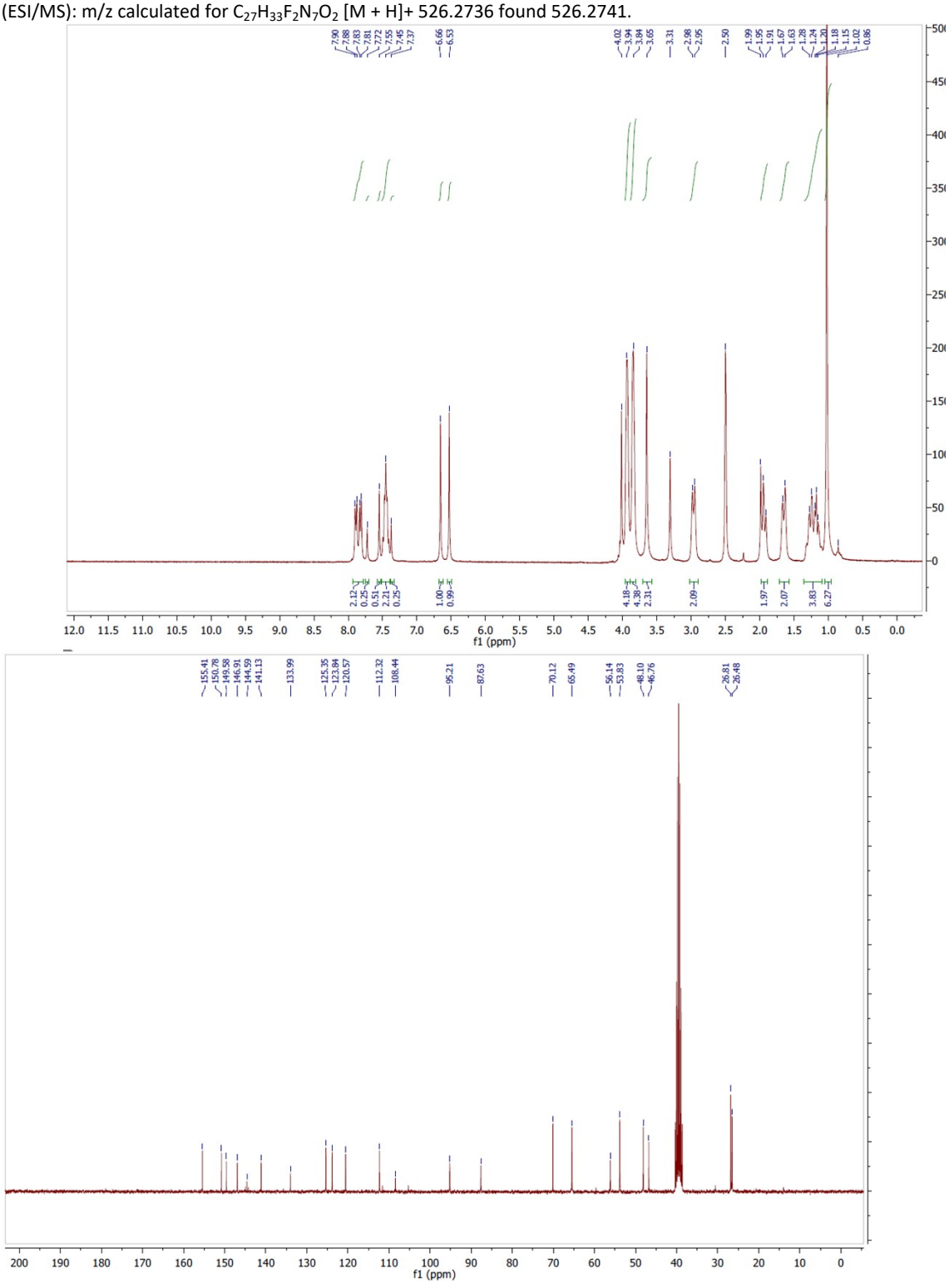
^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.93–7.90 (m, 1H, Ar-H),7.68–7.64 (m, 1H, Ar-H), 7.45–7.39 (m, 2H, Ar-H), 7.30 (t, J = 53.6 Hz, 1H, CHF_2), 6.61 (s, 1H, Ar-H), 6.32 (s, 1H, Ar-H), 4.00–3.96 (m, 4H, morph.), 3.92–3.89 (m, 4H, morph.), 3.82 (s, 2H, CH_2), 3.73–3.69 (m, 4H), 2.60 (d, J = 24.3 Hz, 4H, 2xCH_2), 1.76–1.69 (m, 1H, CH), 1.00–0.96 (m, 2H, CH_2), 0.77–0.73 (m, 2H, CH_2). ^{13}C{^{1}H, ^{19}F}-NMR (101 MHz, $CDCl_3$) δ 171.9, 155.3, 151.3, 150.1, 147.6, 144.7, 141.8, 134.5, 125.7, 124.2, 121.5, 111.7, 109.3 (CF_2), 96.5, 87.4, 66.1, 56.4, 48.5, 10.9, 7.3. HRMS (ESI/MS): m/z calculated for $C_{27}H_{30}F_2N_8O_2$ [M + H]+ 537.2532 found 537.2541.





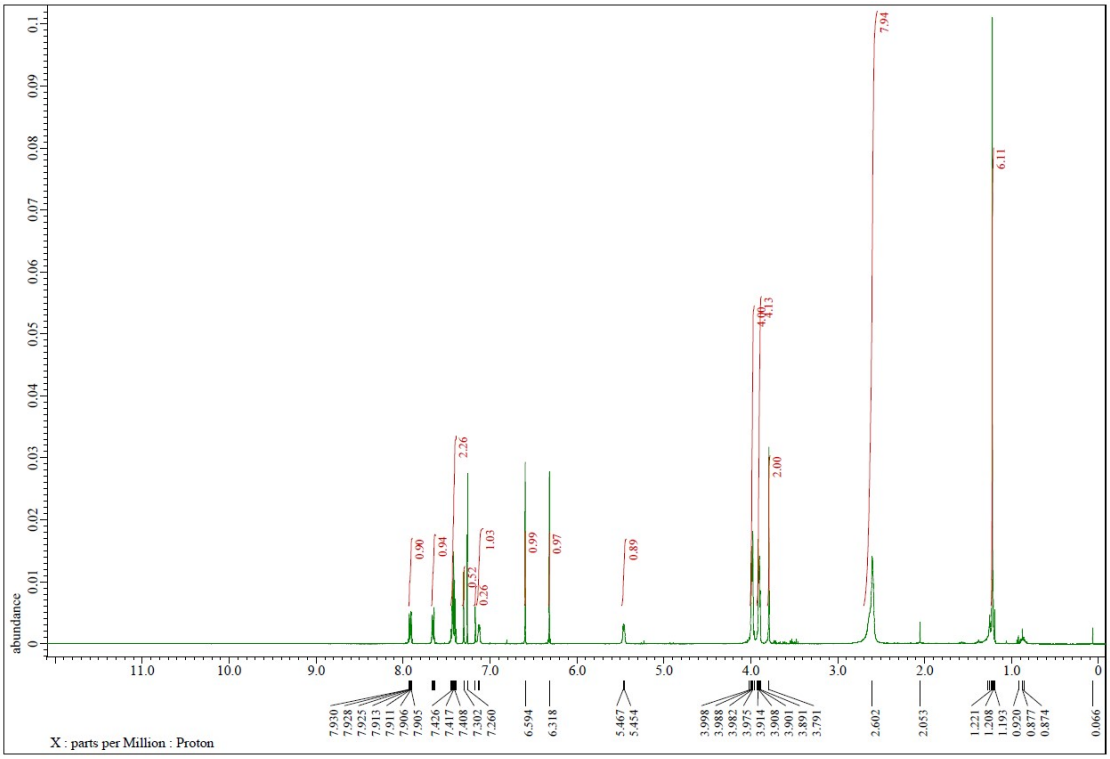
2-[1-({5-[2-(difluoromethyl)-1H-benzimidazol-1-yl]-7-(morpholin-4-yl)pyrazolo[1,5-a]pyrimidin-2-yl}methyl)piperidin-4-yl]propan-2-ol (4b)

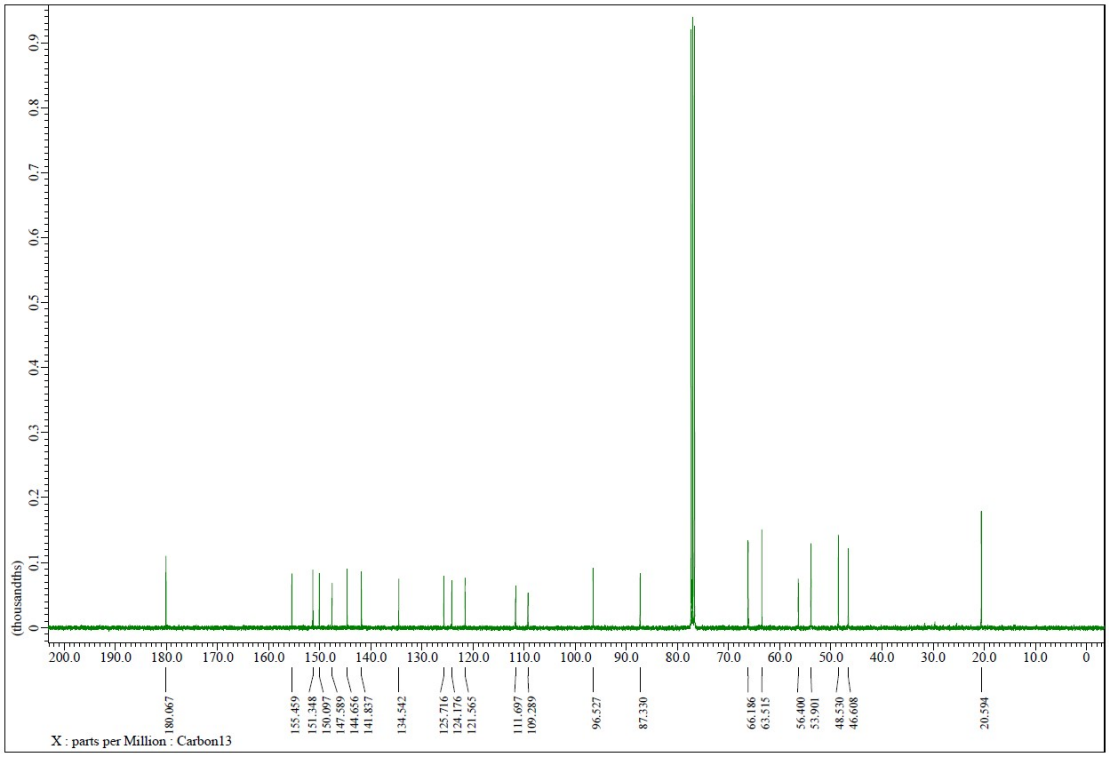
^{1}H-NMR (300 MHz, DMSO-D6) δ 7.85 (dd, J = 20.1, 7.3 Hz, 2H, Ar-H), 7.55 (t, J = 51 Hz, 1H, CHF_2) 7.51–7.40 (m, 2H, Ar-H), 6.66 (s, 1H, Ar-H), 6.53 (s, 1H, Ar-H), 3.94 (s, 4H, morph.), 3.84 (s, 4H, morph.), 3.65 (s, 2H, CH_2), 2.97 (d, J = 10.5 Hz, 2H, CH_2 pyrimidine), 1.93 (d, J = 10.8 Hz, 2H, CH_2 pyrimidine), 1.65 (d, J = 11.8 Hz, 2H, CH_2 pyrimidine), 1.36–1.10 (m, 3H, C-H; CH_2 pyrimidine), 1.02 (s, 6H, 2xCH_3). ^{13}C{^{1}H}-NMR (75 MHz, DMSO-D6) δ 155.4, 150.8, 149.6, 146.9, 144.6 (t, J = 47.5 Hz), 141.2, 134.0, 125.4, 123.9, 120.6, 112.4, 108.4 (t, J = 177.7 Hz), 95.2, 87.7, 70.2, 65.5, 56.2, 53.9, 48.1, 46.8, 26.8, 26.5. HRMS (ESI/MS): m/z calculated for $C_{27}H_{33}F_2N_7O_2$ [M + H]+ 526.2736 found 526.2741.



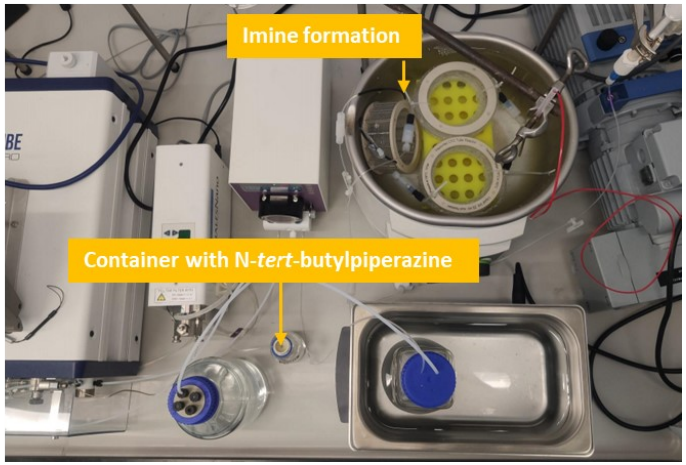
2-[4-({5-[2-(difluoromethyl)-1H-1,3-benzimidazol-1-yl]-7-(morpholin-4-yl)pyrazolo[1,5-a]pyrimidin-2-yl}methyl)piperazin-1-yl]-2-methylpropanamide (4c)

^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.93–7.90 (m, 1H, Ar-H), 7.67–7.63 (m, 1H, Ar-H), 7.45–7.39 (m, 2H, Ar-H), 7.23 (t, J = 53.6 Hz, 1H, CHF_2), 7.13 (d, J = 5.2 Hz, 1H, NH_2), 6.59 (s, 1H, Ar-H), 6.32 (s, 1H, Ar-H), 5.46 (d, J = 5.1 Hz, 1H, NH_2), 4.00–3.96 (m, 4H, morph.), 3.93–3.89 (m, 4H, morph.), 3.79 (s, 2H, CH_2), 2.60 (s, 8H, piperazine), 1.22 (s, 6H, 2xCH_3). ^{13}C{^{1}H, ^{19}F}-NMR (101 MHz, $CDCl_3$) δ 180.1, 155.5, 151.3, 150.1, 147.6, 144.7, 141.8, 134.5, 125.7, 124.2, 121.6, 111.7, 109.3 (CF_2), 96.5, 87.3, 66.2, 63.5, 56.4, 53.9, 48.5, 46.6, 20.6. HRMS (ESI/MS): m/z calculated for $C_{27}H_{33}F_2N_9O_2$ [M + H]+ 554.2798 found 554.2800.





6. Real photo of the flow equipment



7. References

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