Supporting information

Structure-Based Design of Potent Pan-YTHDF Ligands

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Table of Contents

Supplementary Figures S3

- **S1** : YTHDF2 TR-FRET dose-response curves.
- **S2** : YTHDF2 FP dose-response curves.
- **S3** : YTHDC2 FP dose-response curves.
- **S4** : DSF dose-response results.
- **S5** : TR-FRET selectivity measurements of compounds 10, 20, 32, 33 and 36.
- **S6** : Expression of differentiation markers in THP-1 cells treated with compound **31**.
- **S7** : Growth inhibition of THP-1 dose-response curves.
- **S8** : Growth inhibition of MOLM-13 dose-response curves.
- **S9** : Controls for TNF experiments.

Supplementary Tables S12

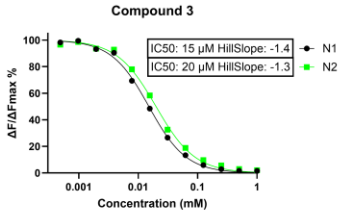
- **S1** : X-ray data collection and refinement statistics of the six YTHDF2 complexes.

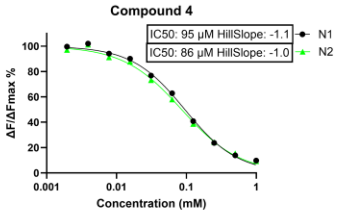
^{1}H and ^{13}C NMR traces of compounds **4**, **6-15**, **17-37**, **31b** and **55** S14

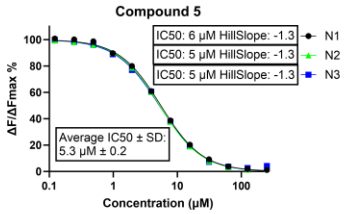
HPLC traces of compounds **4**, **6-15**, **17-37**, **31b** and **55** S47

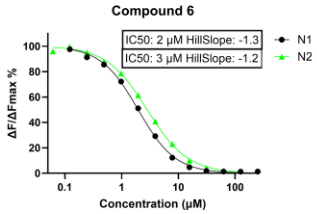
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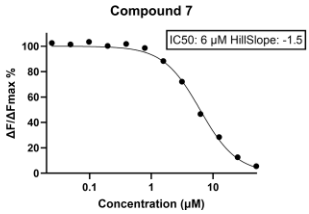
Supplementary figures

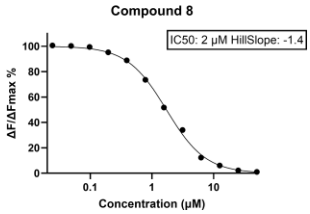


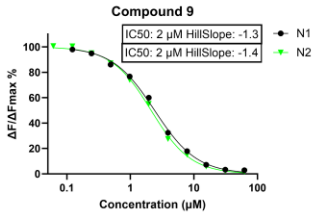


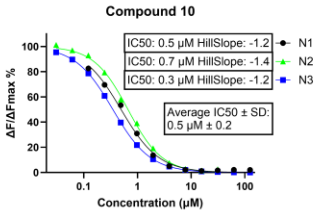


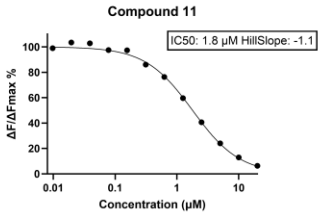


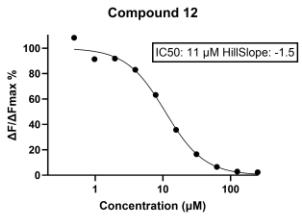


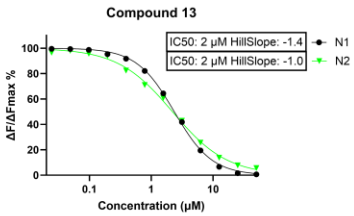


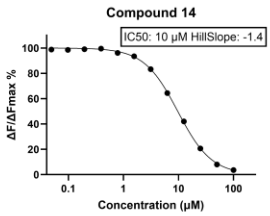


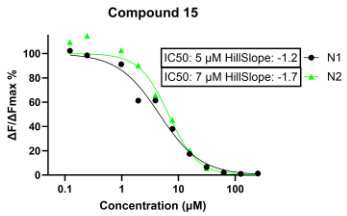


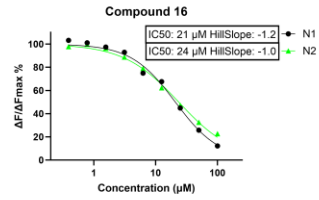


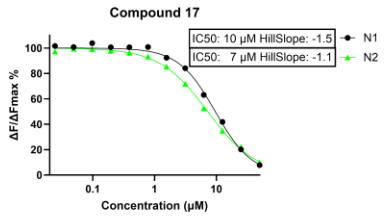


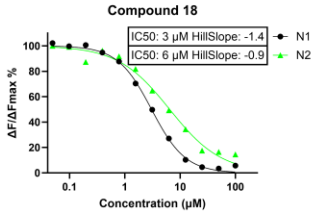


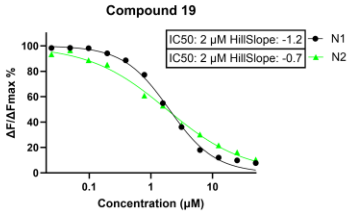


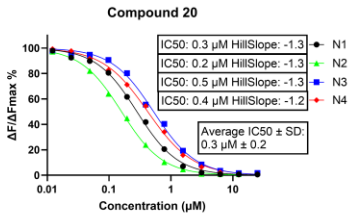












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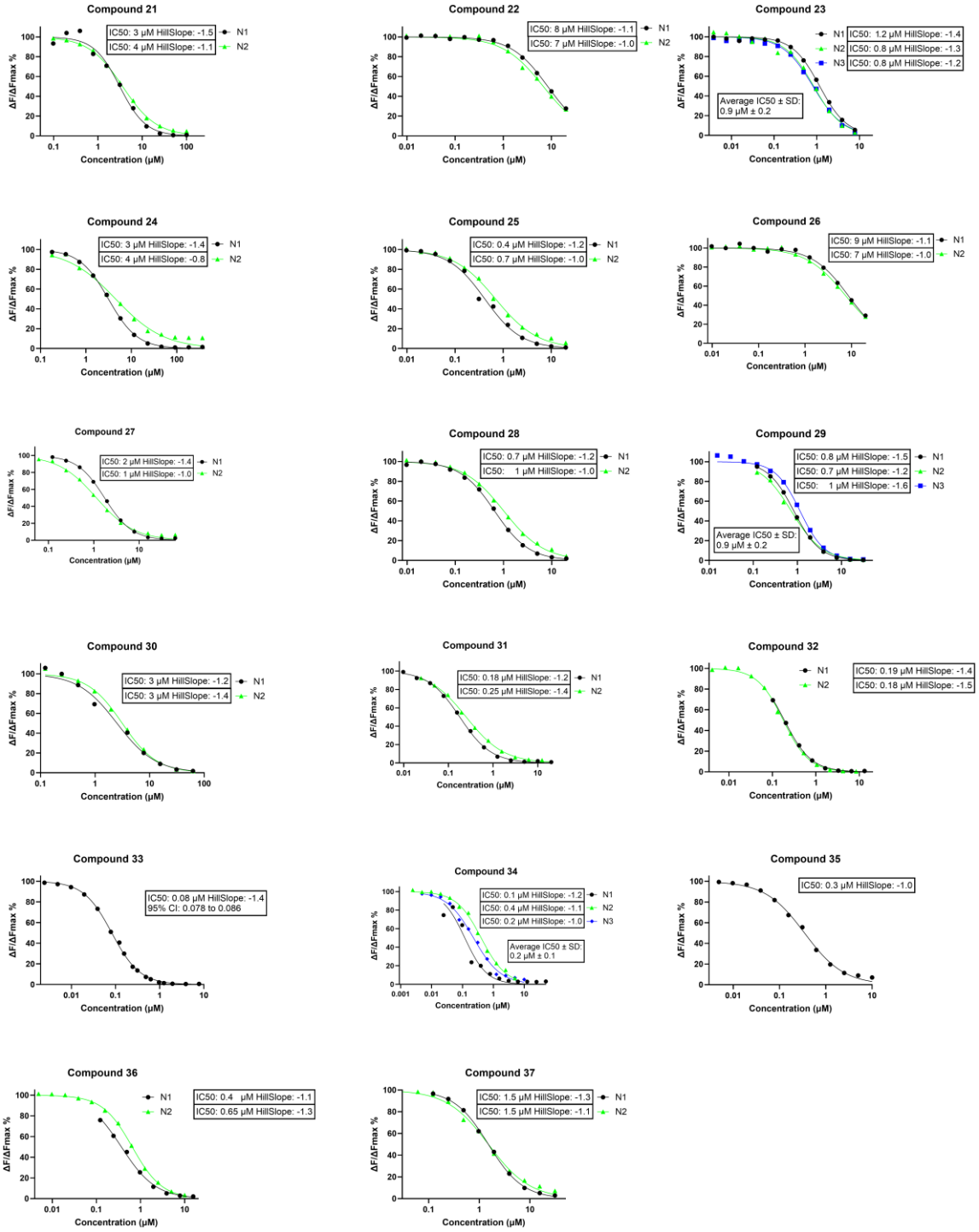


Figure S1. YTHDF2 TR-FRET dose–response curves. Each curve represents one biological replicate, and each biological replicate is the average of two technical replicates; the IC_{50} and Hill slope of each individual fit are reported in the corresponding panel. The final IC_{50} value (main-text tables) is calculated as the average of the IC_{50} values obtained from the individual curves; for compounds measured in biological triplicate (N = 3, and N = 4 for compound **20**) it is reported as the mean ± standard deviation. For compound **33**, one of the three biological replicates gave an incomplete curve; its IC_{50} was therefore obtained from a single average fit of the pooled complete-replicate data (95% confidence interval shown) rather than by averaging the individual curve fits, and no standard deviation is reported.

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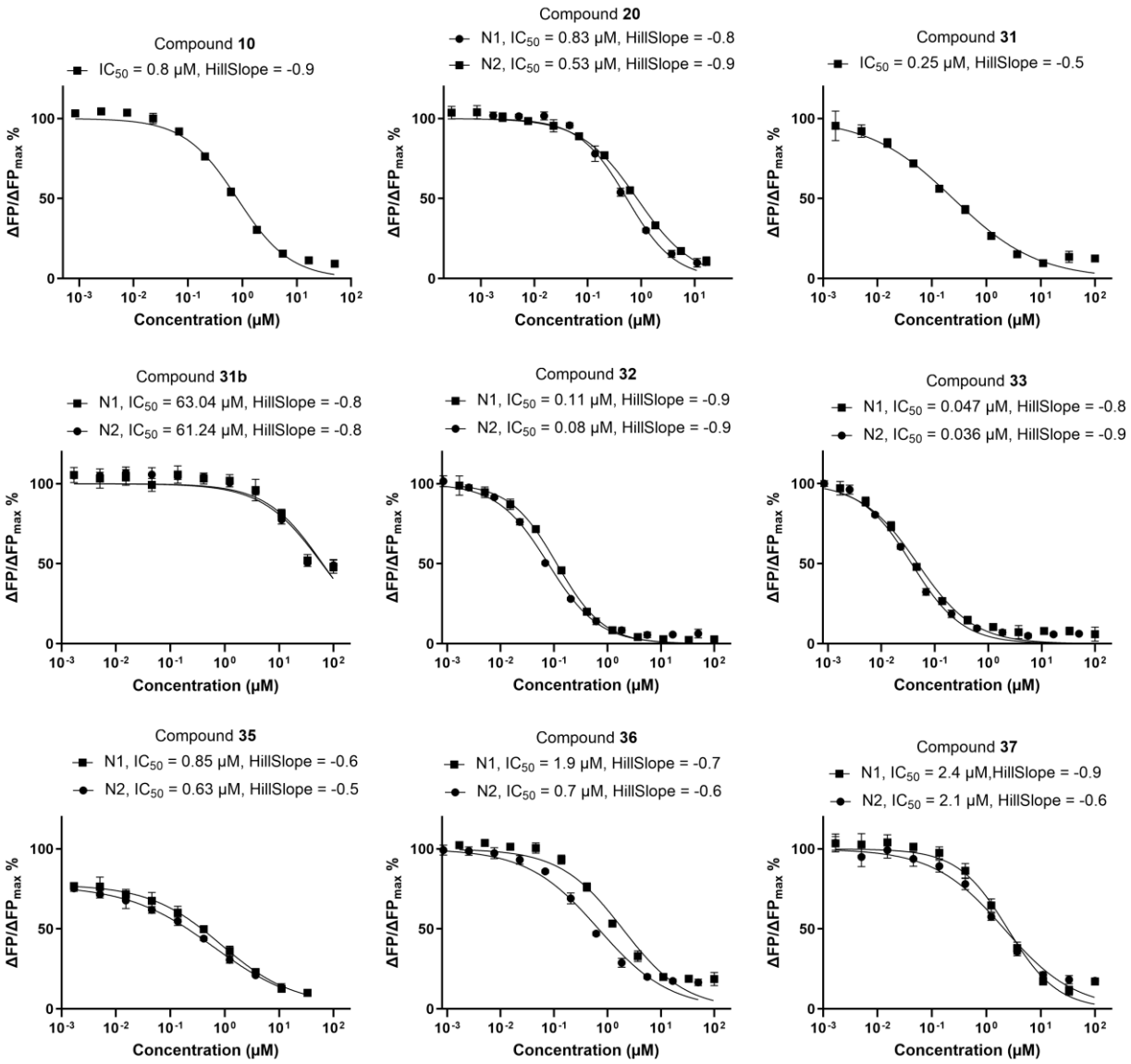


Figure S2. YTHDF2 FP dose-response curves. Biological duplicates are shown as separate curves with standard deviation. Each biological replicate is the average of four technical replicates.

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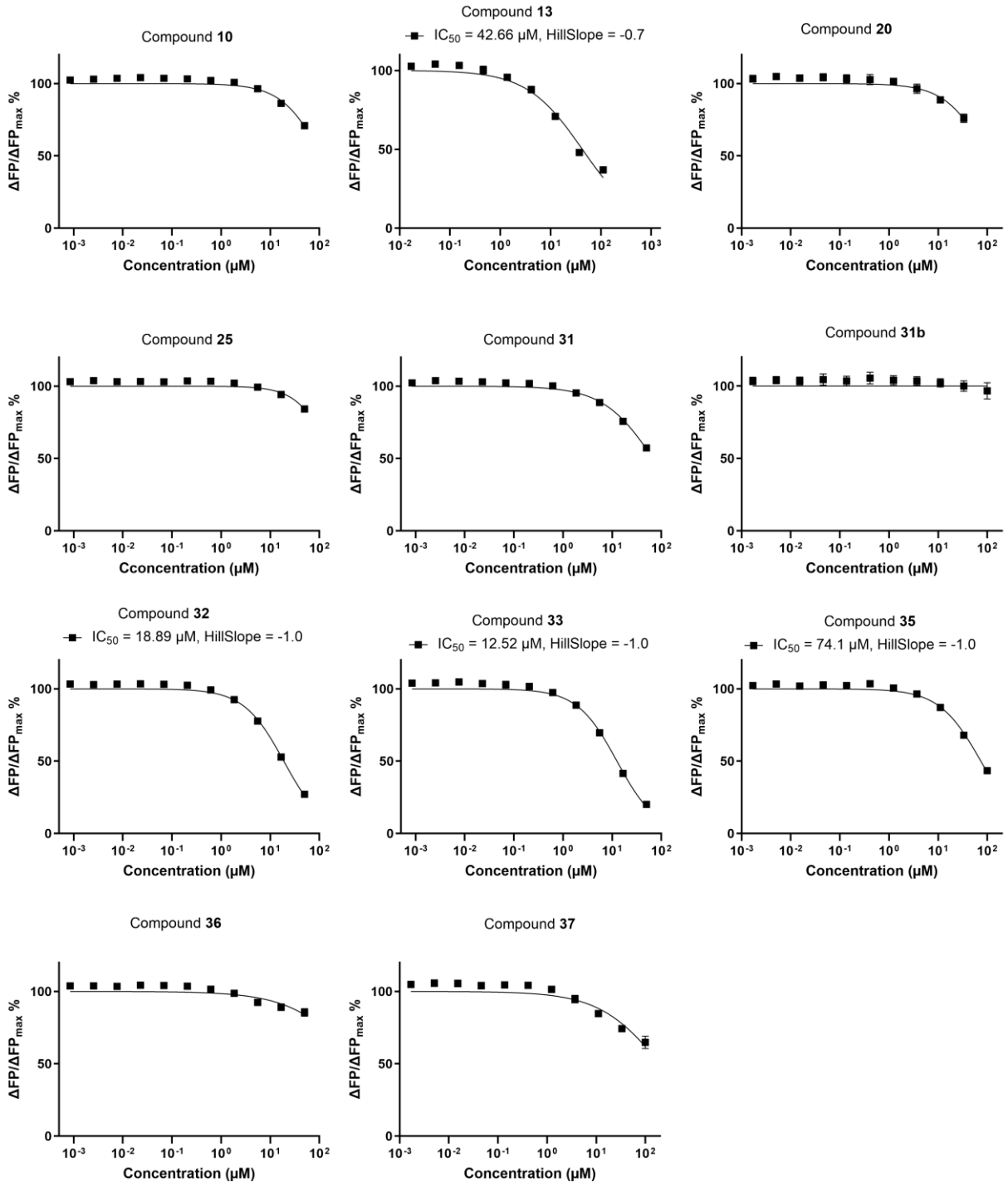


Figure S3. YTHDC2 FP dose-response curves. Each biological replicate is the average of four technical replicates.

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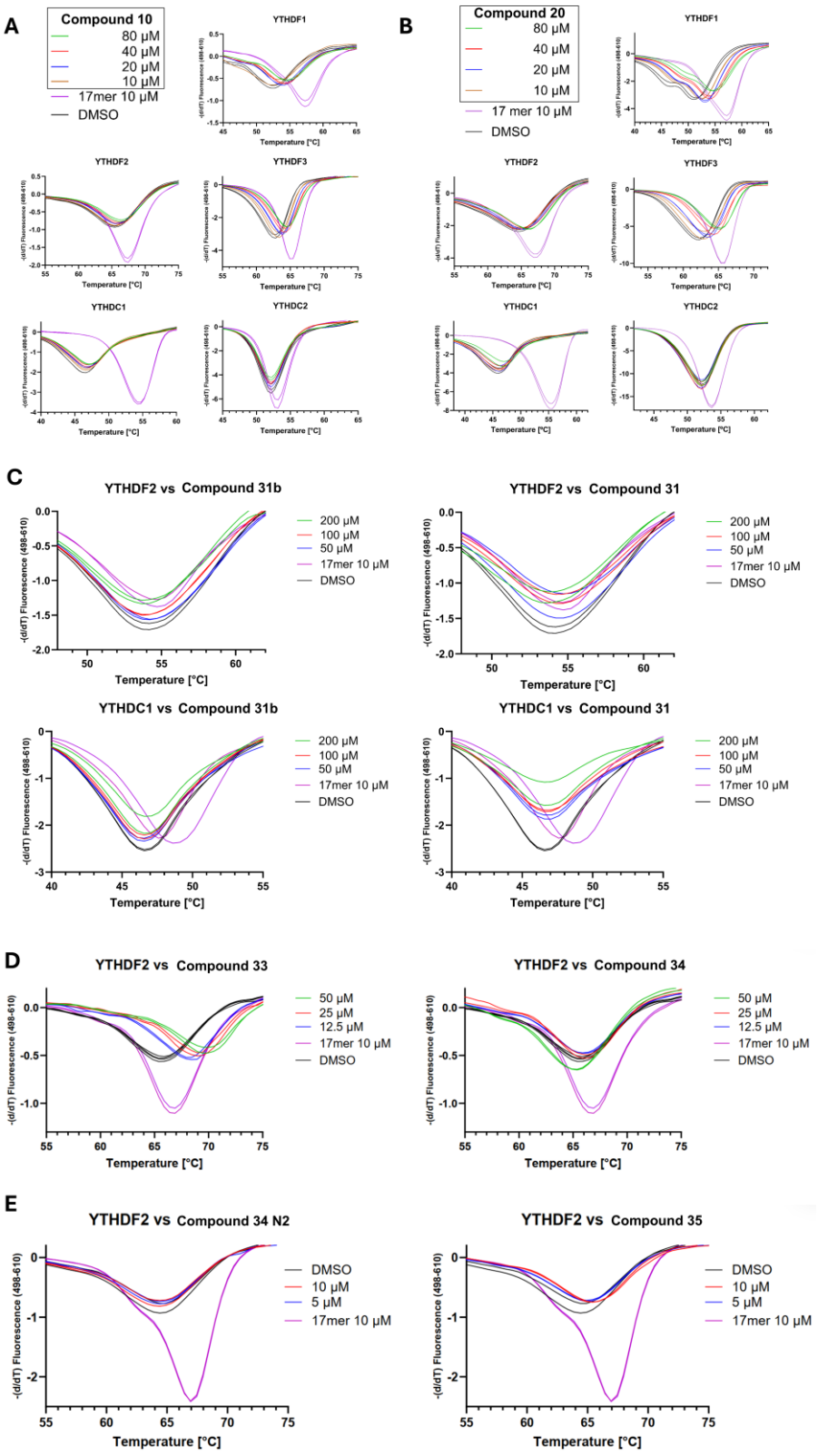


Figure S4. DSF dose-response results of compound **10** (**A**), **20** (**B**) and YTHDF1, YTHDF2, YTHDF3, YTHDC1, YTHDC2; of **31b** and **31** (**C**) and YTHDF2, YTHDC1; of **33** and **34** (**D**), and of **34** N2 and **35** (**E**) and YTHDF2. A 17mer containing the m^{6}A-reader consensus sequence GGm6ACU is included as a control and to show the extent of thermal stabilization in the presence of a natural substrate.

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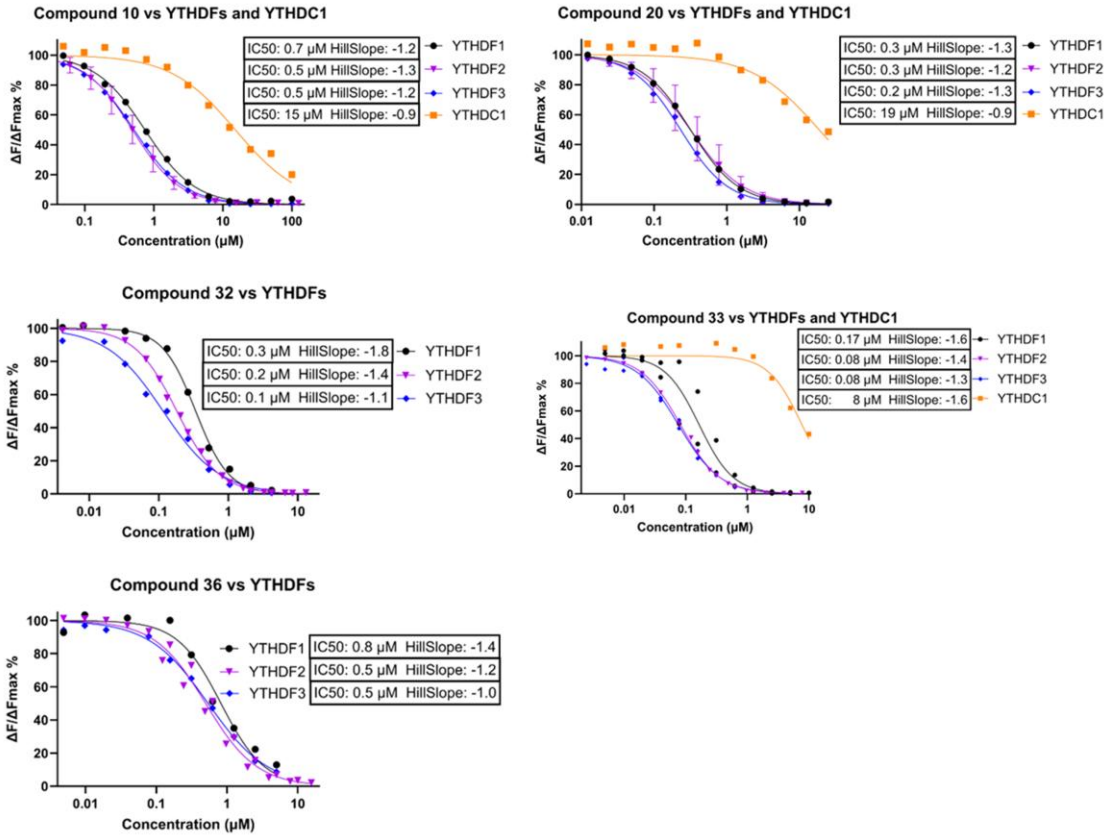


Figure S5. TR-FRET selectivity measurements of compounds 10, 20, 32, 33 and 36 against YTHDF1, YTHDF2 and YTHDF3, and against YTHDC1 for compounds 10, 20 and 33. For clarity, a single fit is shown per protein, so the YTHDF2 IC_{50} shown here derives from global fitting and may differ from the main-text table values.

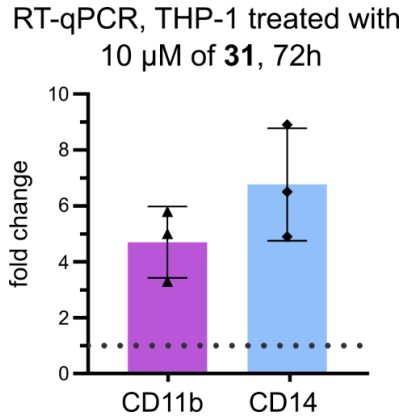
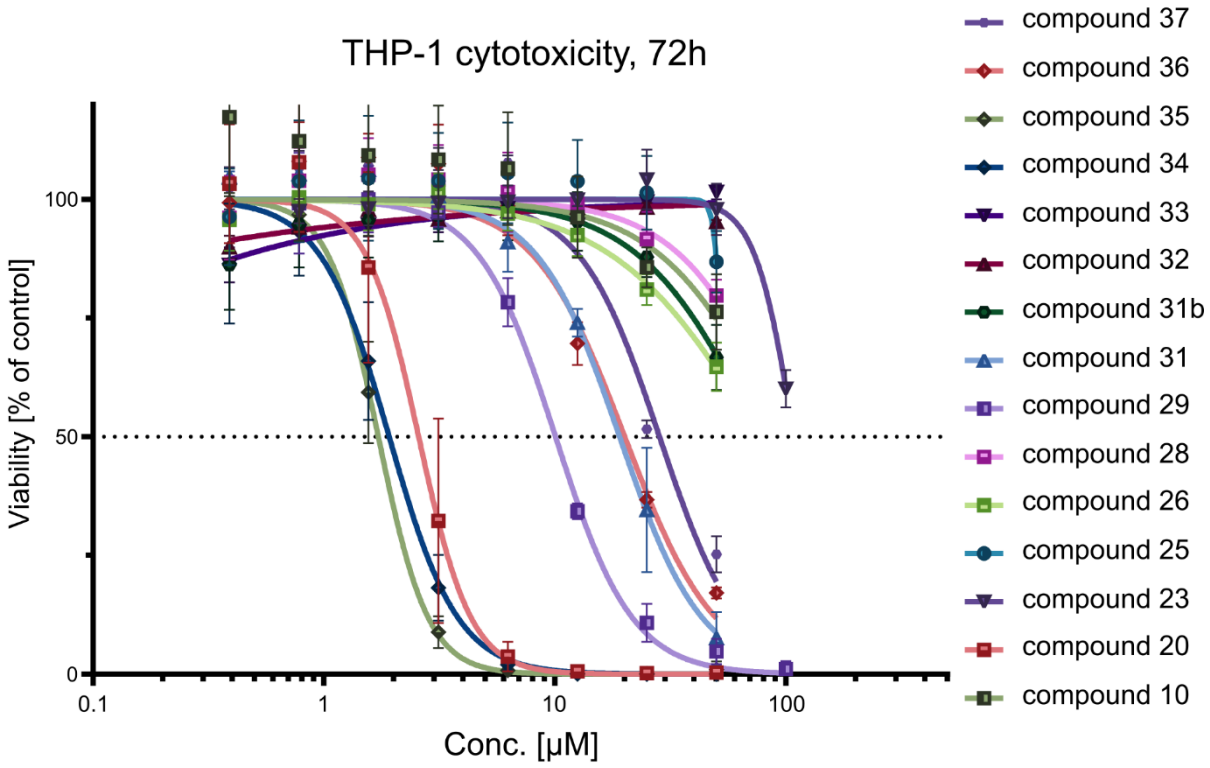


Figure S6. Expression of differentiation markers in THP-1 cells treated with compound **31**. RT-qPCR analysis of CD11b and CD14 mRNA expression upon treatment with 10 μM of **31** for 72 h. Gene expression levels are shown as fold change normalized to 18S rRNA and DMSO control (dotted line at 1.0), mean ± SD, N = 3.

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	compound 10	compound 20	compound 23	compound 25
GI50	>50	2.6±0.2	>50	>50
HillSlope	-1.67	-3.83	-5.14	-24.5

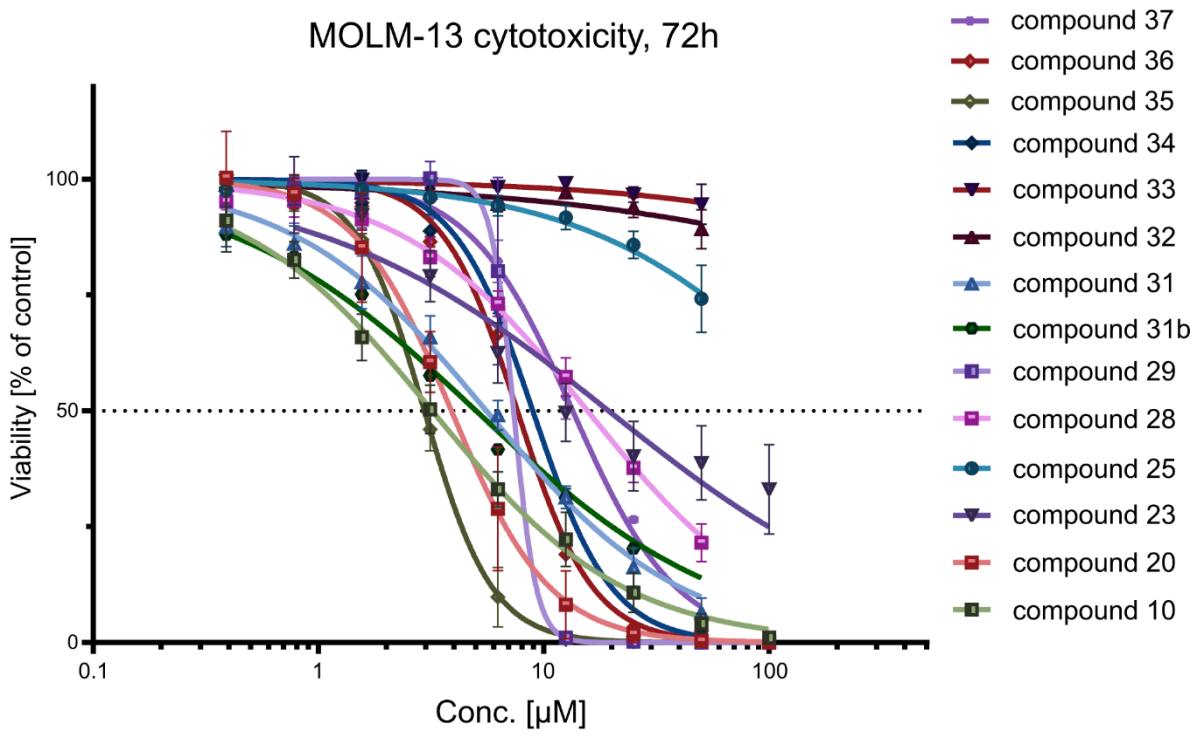
	compound 26	compound 28	compound 29	compound 31
GI50	>50	>50	10.1±0.3	19±0.7
HillSlope	-1.37	-1.87	-2.61	-2.43

	compound 31b	compound 32	compound 33	compound 34
GI50	>50	>50	>50	1.9±0.1
HillSlope	-1.67	0.437	0.613	-2.92

	compound 35	compound 36	compound 37
GI50	1.7±0.03	20±1.1	28.3±1.4
HillSlope	-4.02	-2.20	-2.50

Figure S7. Growth inhibition of THP-1 dose-response curves. Cell viability is expressed as a percentage relative to DMSO control, after 72 h of treatment.

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	compound 10	compound 20	compound 23	compound 25
GI50	3.2±0.2	3.9±0.3	19±2.5	>50
HillSlope	-1.02	-1.99	-0.67	-0.83

	compound 28	compound 29	compound 31	compound 31b
GI50	15.6±0.5	7.4±0.4	5.6±0.2	5±0.3
HillSlope	-1.04	-8.64	-1.01	-0.79

	compound 32	compound 33	compound 34	compound 35
GI50	>50	>50	8.9±0.1	3±0.1
HillSlope	-0.45	-0.62	-2.49	-2.88

	compound 36	compound 37
GI50	7.6±0.2	13.4±0.5
HillSlope	-2.60	-1.89

Figure S8. Growth inhibition of MOLM-13 dose-response curves. Cell viability is expressed as a percentage relative to DMSO control, after 72 h of treatment.

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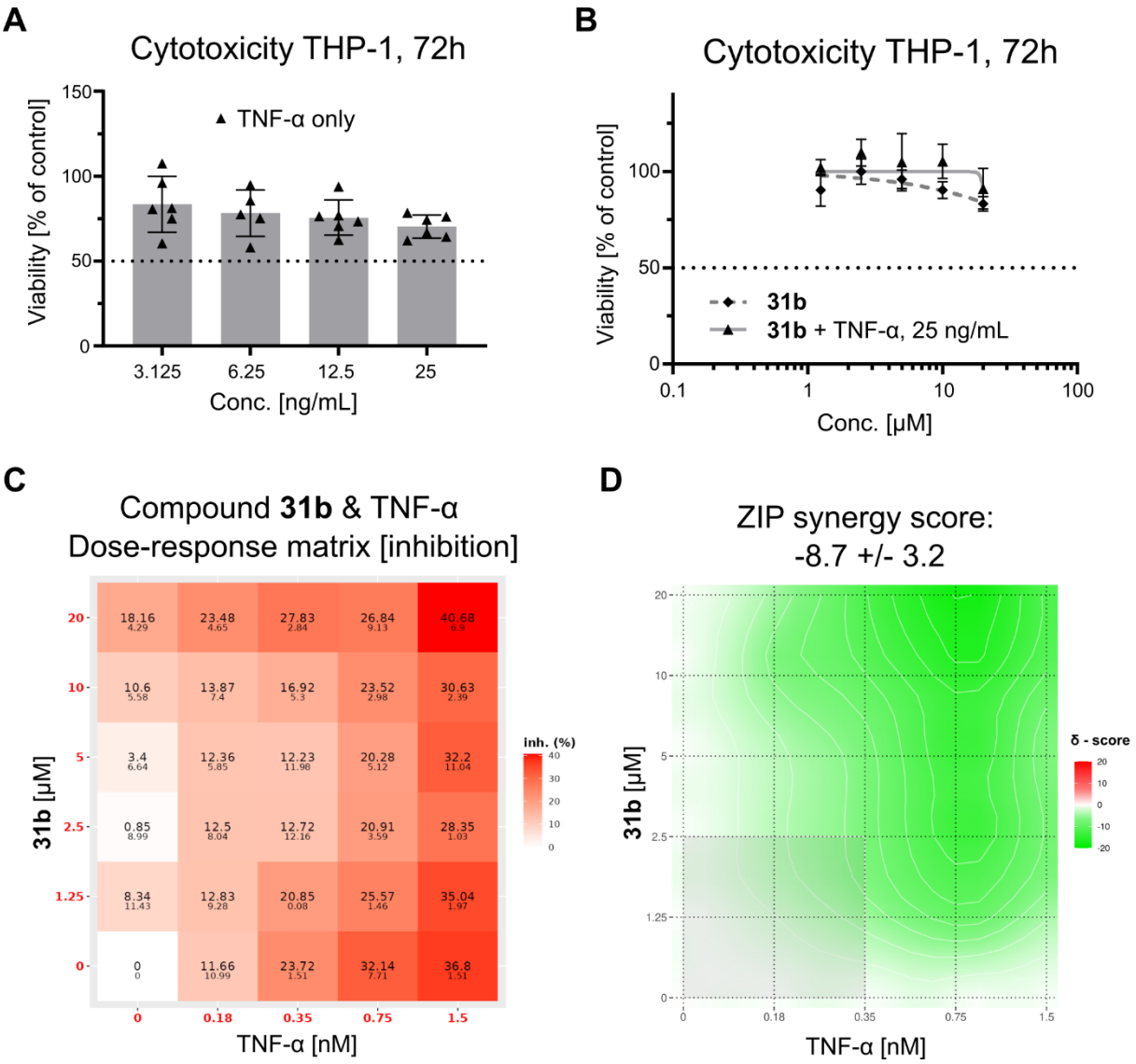


Figure S9. Additional controls for the co-treatment experiment with TNF-α and YTHDFs inhibitor. **A)** Cytotoxic effect of different concentrations of TNF-α alone in THP-1 cells, after 72 h of treatment. **B)** Growth inhibition effects of **31b** (the negative control compound) alone and in combination with TNF-α (25 ng/mL) after 72 h of treatment. Cell viability is expressed as a percentage relative to DMSO control for **31b** alone, and relative to DMSO + TNF-α (25 ng/mL) for the combination, N = 3. **C)** Dose-response inhibition matrix for combined treatment of **31b** (0–20 µM) and TNF-α (0–1.5 nM) in THP-1 cells. Inhibition percentages are normalized to untreated controls (**31b** 0 µM, TNF-α 0 nM), N = 3. **D)** The ZIP synergy score derived from the dose-response matrix data.

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Supplementary Tables

Table S1. X-ray data collection and refinement statistics for the six YTHDF2 complex crystal structures (compounds **3**, **4**, **5**, **20**, **22** and **24**), generated with phenix.table_one from the deposited coordinate and reflection files together with the unmerged intensities, over the resolution range of the deposited data. Numbers in parentheses refer to the highest-resolution shell.

	3 (32RD)	**4 (32SH)**	**5 (32SA)**	**20 (32TH)**	**22 (32SJ)**	**24 (32QY)**
Data collection						
Resolution range	43.87 - 1.555 (1.58 - 1.55)	44.1 - 1.61 (1.64 - 1.61)	40.56 - 1.525 (1.55 - 1.52)	37.53 - 1.675 (1.78 - 1.68)	44.27 - 2.2 (2.32 - 2.2)	40.28 - 1.836 (1.89 - 1.84)
Space group	P 65	P 65	P 65	P 21 21 21	P 65	P 65
Unit cell	80.38 80.38 113 90 90 120	80.848 80.848 113.57 90 90 120	81.12 81.12 113.21 90 90 120	43.796 46.295 72.845 90 90 90	81.486 81.486 113.692 90 90 120	80.554 80.554 113.864 90 90 120
Total reflections	521835 (15050)	559734 (30467)	475836 (18295)	186512 (28424)	312936 (40261)	740406 (53001)
Unique reflections	59135 (2626)	54348 (2860)	64028 (2680)	17454 (2851)	20919 (2670)	36303 (2805)
Multiplicity	8.8 (5.7)	10.3 (10.7)	7.4 (6.8)	10.7 (10.0)	15.0 (15.1)	20.4 (18.9)
Completeness (%)	99.67 (93.44)	99.99 (99.97)	99.81 (96.57)	99.81 (99.76)	96.80 (88.11)	99.98 (100.00)
Mean I/sigma(I)	19.87 (1.74)	16.49 (1.26)	22.54 (1.41)	9.90 (2.37)	11.60 (1.93)	20.60 (2.89)
Wilson B-factor	25.04	27.82	26.97	17.76	36.15	31.98
R-merge	0.04713 (1.034)	0.06344 (1.546)	0.03323 (1.152)	0.1371 (0.5378)	0.1746 (1.289)	0.08759 (0.954)
R-meas	0.04999 (1.139)	0.06685 (1.625)	0.03577 (1.247)	0.1439 (0.5662)	0.1808 (1.334)	0.08984 (0.9803)
R-pim	0.01653 (0.4657)	0.02097 (0.497)	0.01308 (0.4703)	0.04299 (0.1744)	0.0464 (0.3412)	0.0199 (0.2248)
CC1/2	1 (0.632)	0.999 (0.559)	1 (0.659)	0.997 (0.913)	0.997 (0.809)	0.998 (0.872)
CC*	1 (0.88)	1 (0.847)	1 (0.891)	0.999 (0.977)	0.999 (0.946)	0.999 (0.965)
Refinement						
Reflections used in refinement	59120 (2633)	54342 (2867)	64007 (2676)	17583 (2864)	21049 (2727)	36476 (2790)
Reflections used for R-free	2958 (132)	2690 (143)	3201 (134)	871 (147)	1047 (161)	1855 (147)
R-work	0.1980 (0.2686)	0.2029 (0.2874)	0.1986 (0.3123)	0.1818 (0.2380)	0.1906 (0.2285)	0.1879 (0.2718)
R-free	0.2236 (0.2769)	0.2202 (0.2944)	0.2210 (0.3248)	0.2006 (0.3215)	0.2454 (0.2812)	0.2136 (0.3279)
Number of non-hydrogen atoms	2686	2692	2788	1404	2718	2749
macromolecules	2436	2412	2427	1224	2404	2434
ligands	72	79	68	60	133	80
solvent	178	201	293	120	181	235
Protein residues	299	301	299	146	300	302
RMS(bonds)	0.006	0.006	0.006	0.006	0.006	0.007

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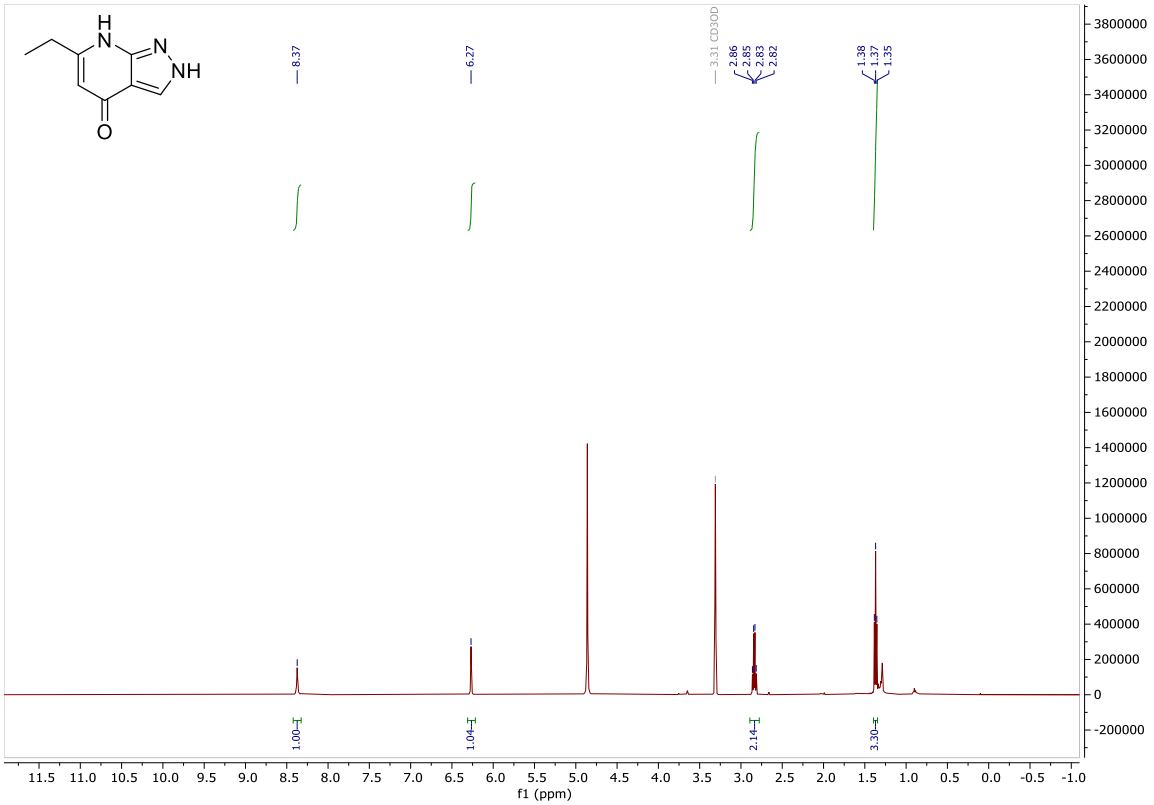
RMS(angles)	0.76	0.75	0.73	0.72	0.80	0.76
Ramachandran favored (%)	97.97	98.65	97.95	98.59	97.97	98.66
Ramachandran allowed (%)	2.03	1.35	2.05	1.41	2.03	1.34
Ramachandran outliers (%)	0.00	0.00	0.00	0.00	0.00	0.00
Rotamer outliers (%)	0.00	0.41	0.40	2.33	0.41	0.00
Clashscore	2.06	2.31	1.88	4.39	2.70	2.90
Average B-factor	28.43	32.11	32.65	22.59	38.07	35.69
macromolecules	27.91	31.50	31.48	21.48	37.35	34.68
ligands	38.36	37.98	41.15	30.87	46.60	47.89
solvent	31.57	37.07	40.38	29.72	41.34	41.98

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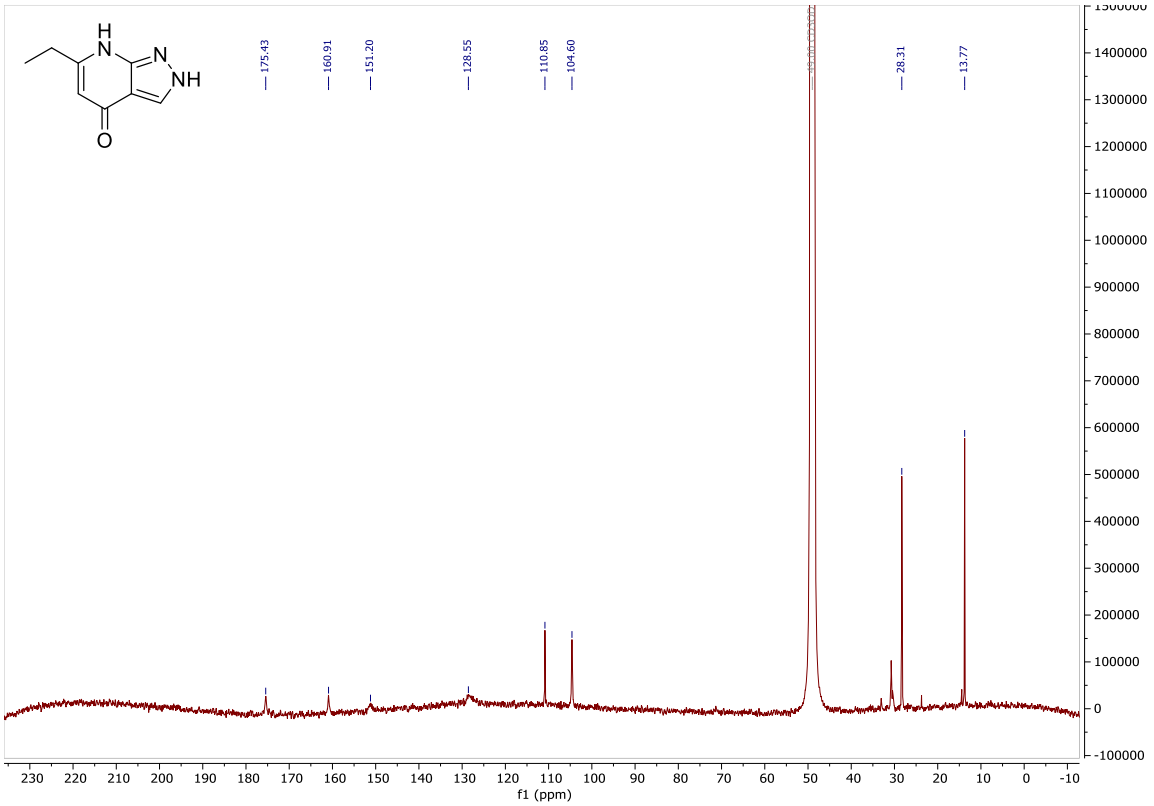
^{1}H and ^{13}C NMR traces of compounds 4, 6-15, 17-37, 31b and 55

6-ethyl-2,7-dihydro-4H-pyrazolo[3,4-b]pyridin-4-one (4)

^{1}H NMR (500 MHz, MeOD)



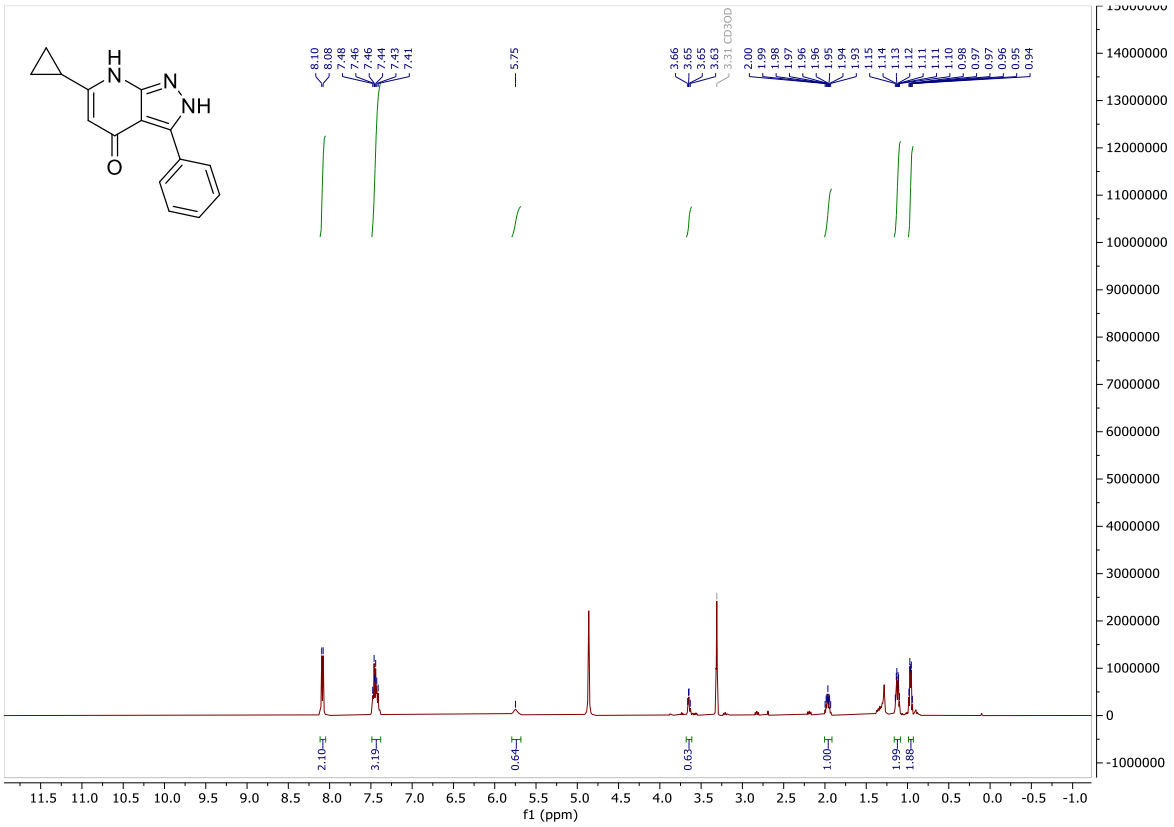
^{13}C NMR (126 MHz, MeOD)



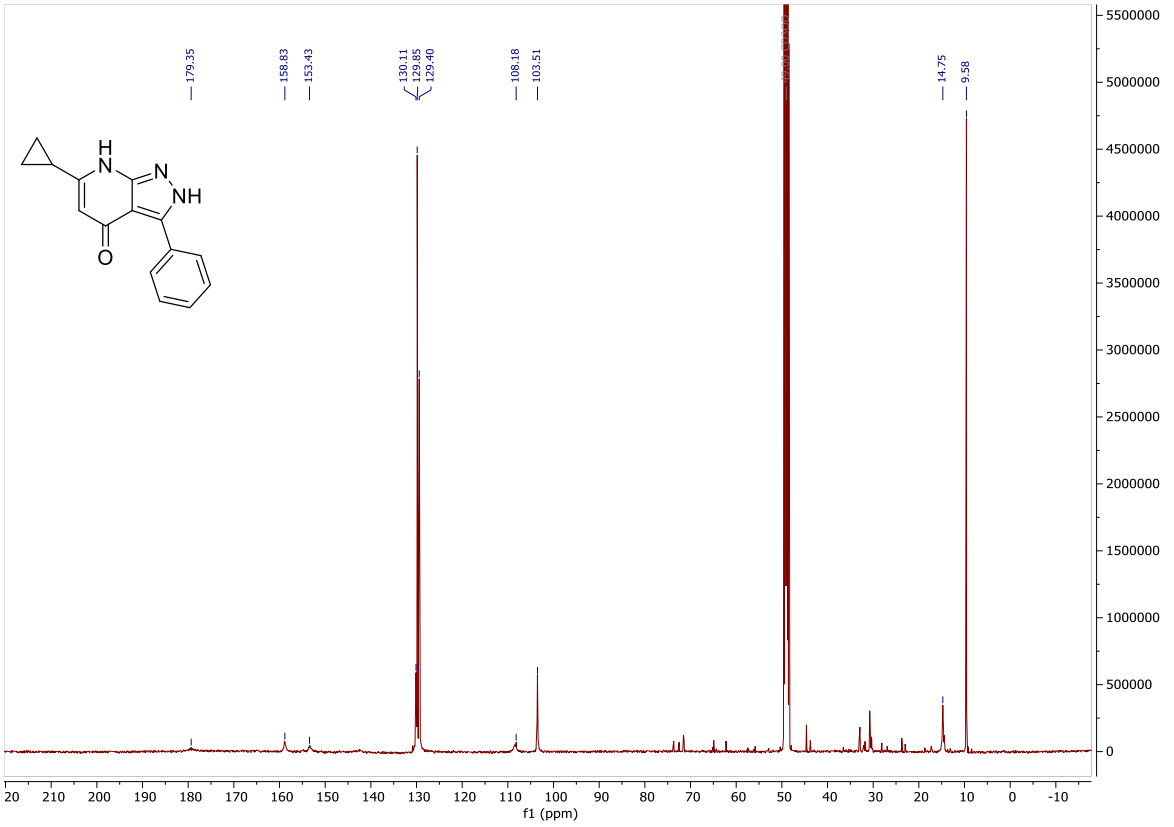
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6-cyclopropyl-3-phenyl-2,7-dihydro-4H-pyrazolo[3,4-b]pyridin-4-one (6)

^{1}H NMR (400 MHz, MeOD)



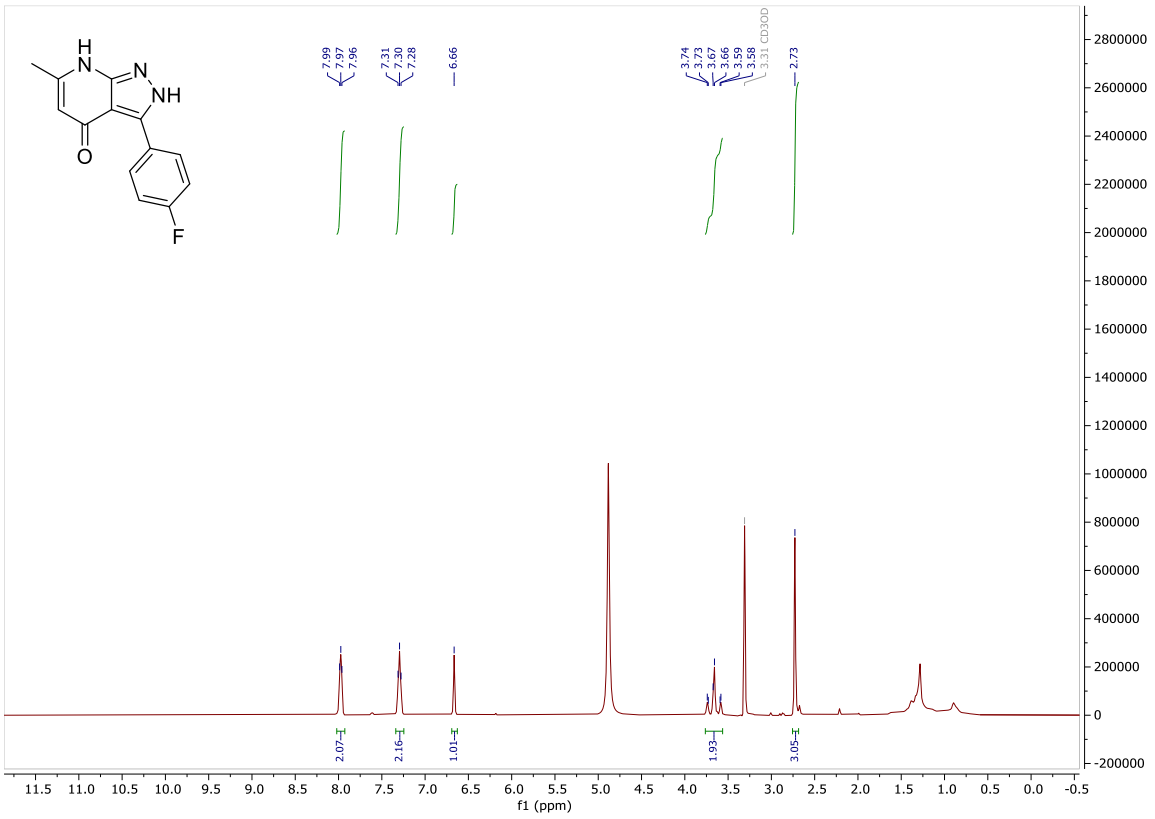
^{13}C NMR (126 MHz, MeOD)



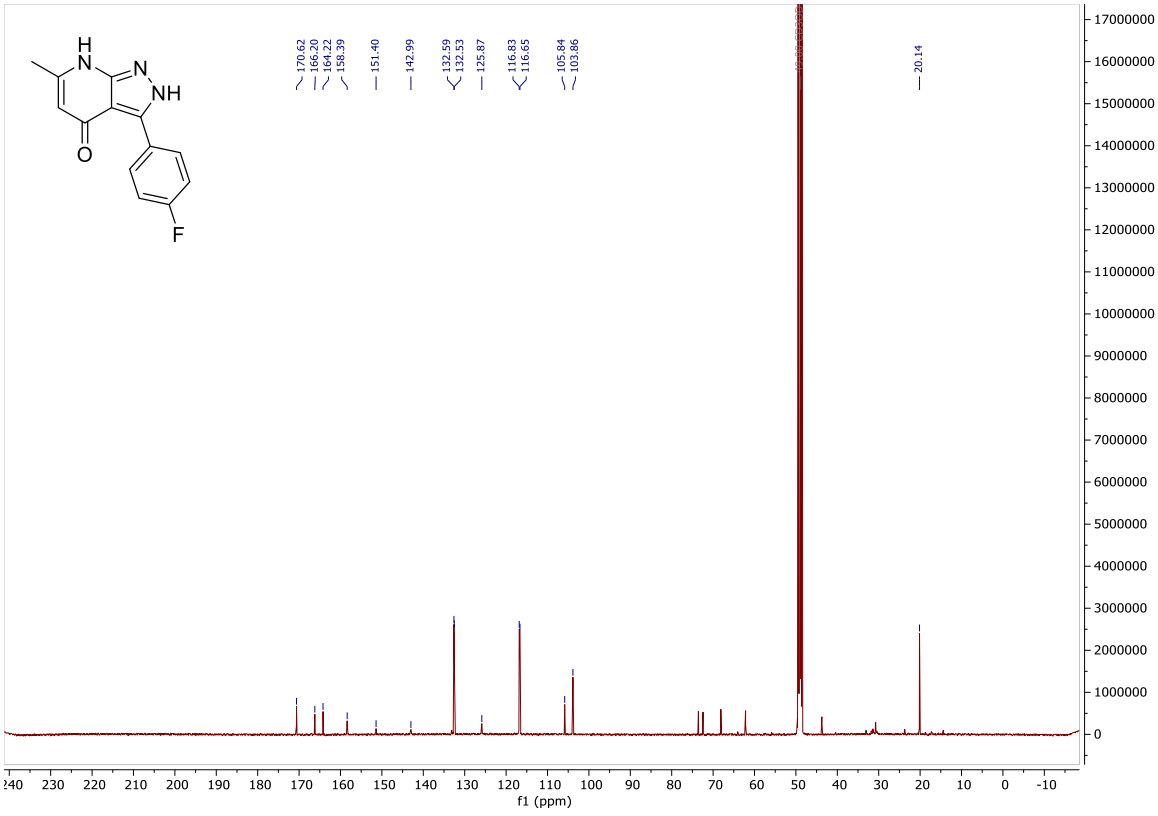
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3-(4-fluorophenyl)-6-methyl-2,7-dihydro-4H-pyrazolo[3,4-b]pyridin-4-one (7)

^{1}H NMR (500 MHz, MeOD)



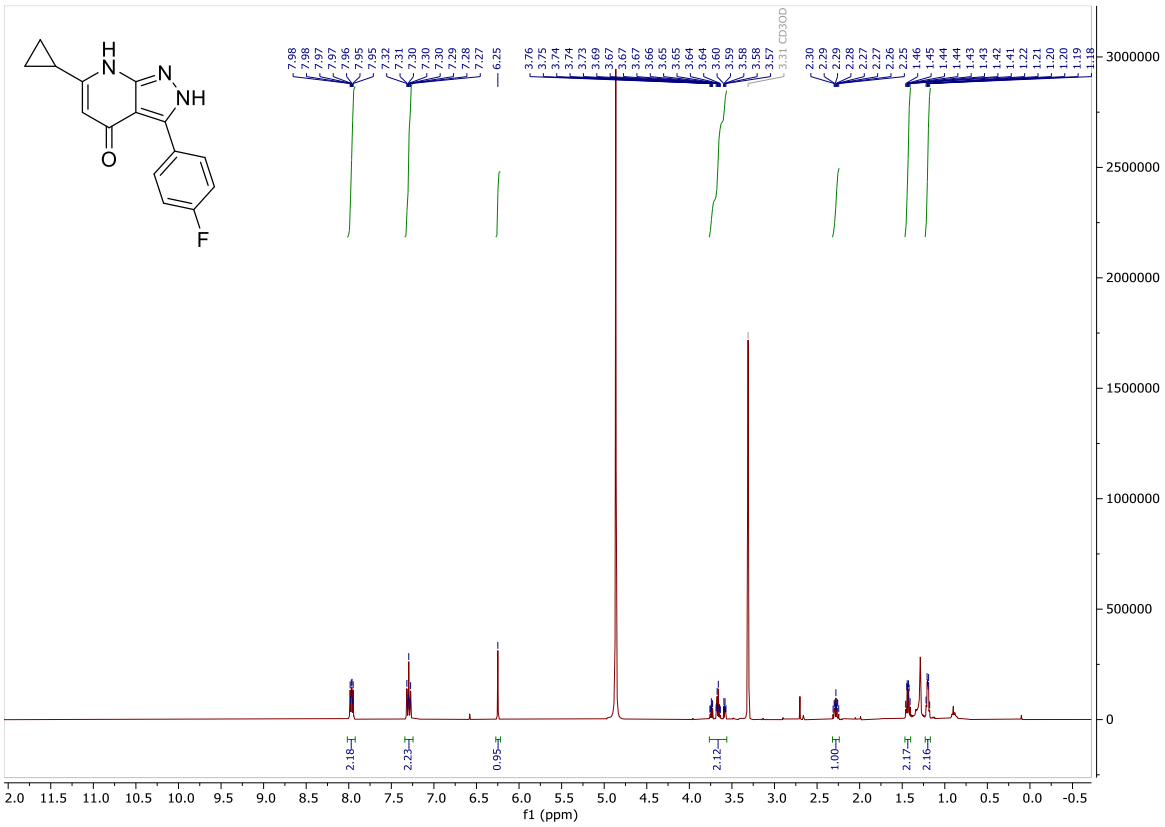
^{13}C NMR (126 MHz, MeOD)



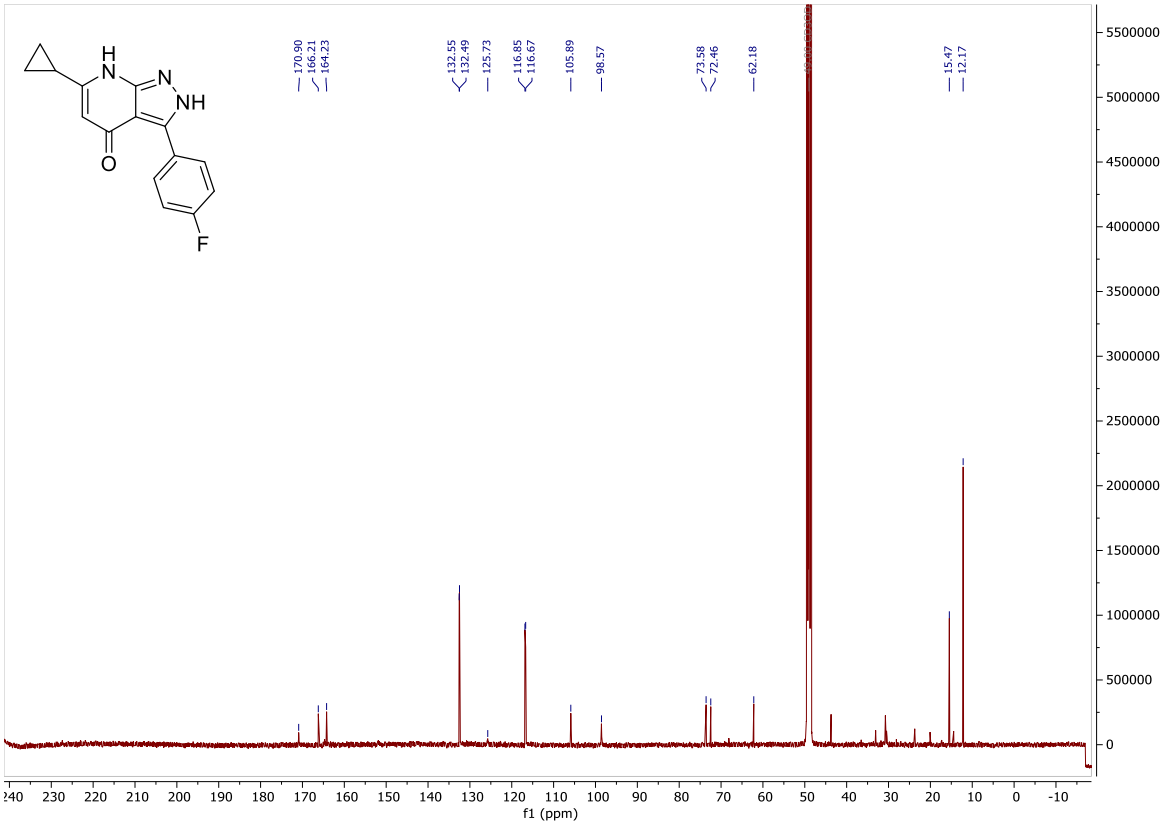
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6-cyclopropyl-3-(4-fluorophenyl)-2,7-dihydro-4H-pyrazolo[3,4-b]pyridin-4-one (8)

^{1}H NMR (400 MHz, MeOD)



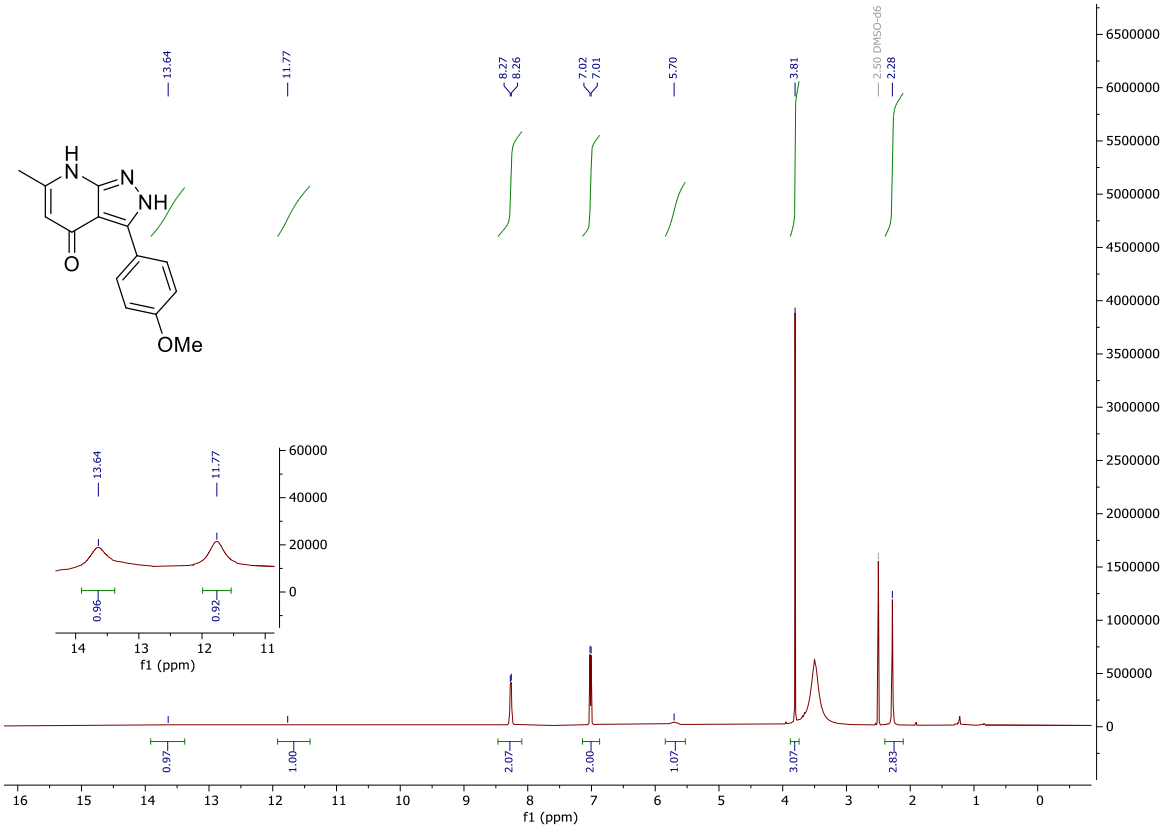
^{13}C NMR (126 MHz, MeOD)



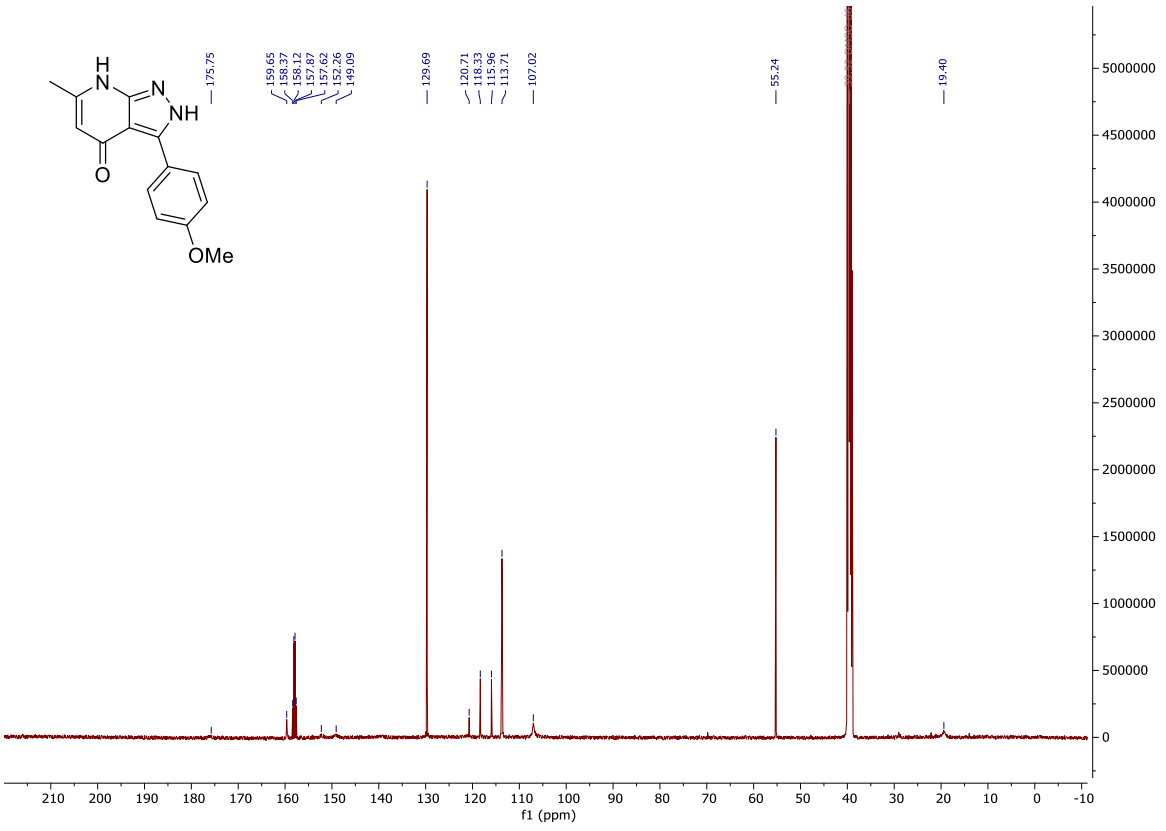
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3-(4-methoxyphenyl)-6-methyl-2,7-dihydro-4H-pyrazolo[3,4-b]pyridin-4-one (9)

^{1}H NMR (500 MHz, DMSO)



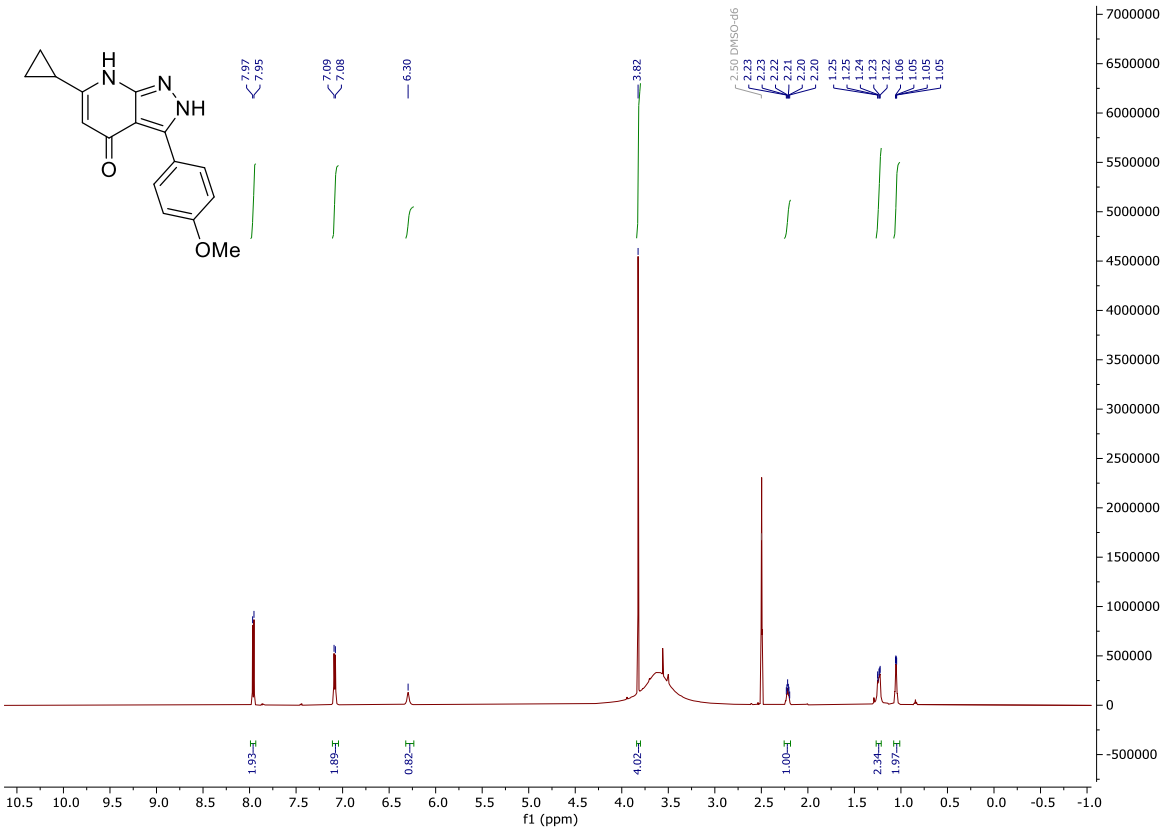
^{13}C NMR (126 MHz, DMSO)



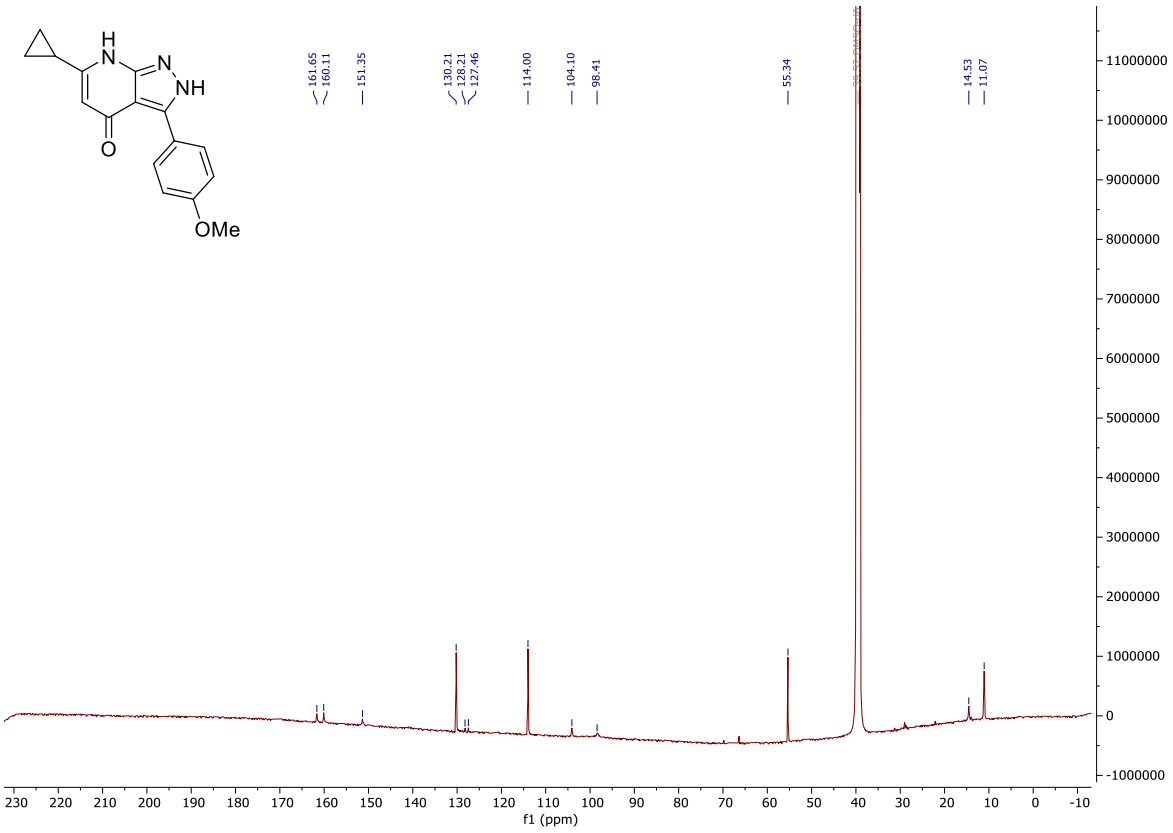
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6-cyclopropyl-3-(4-methoxyphenyl)-2,7-dihydro-4H-pyrazolo[3,4-b]pyridin-4-one (10)

^{1}H NMR (600 MHz, DMSO)



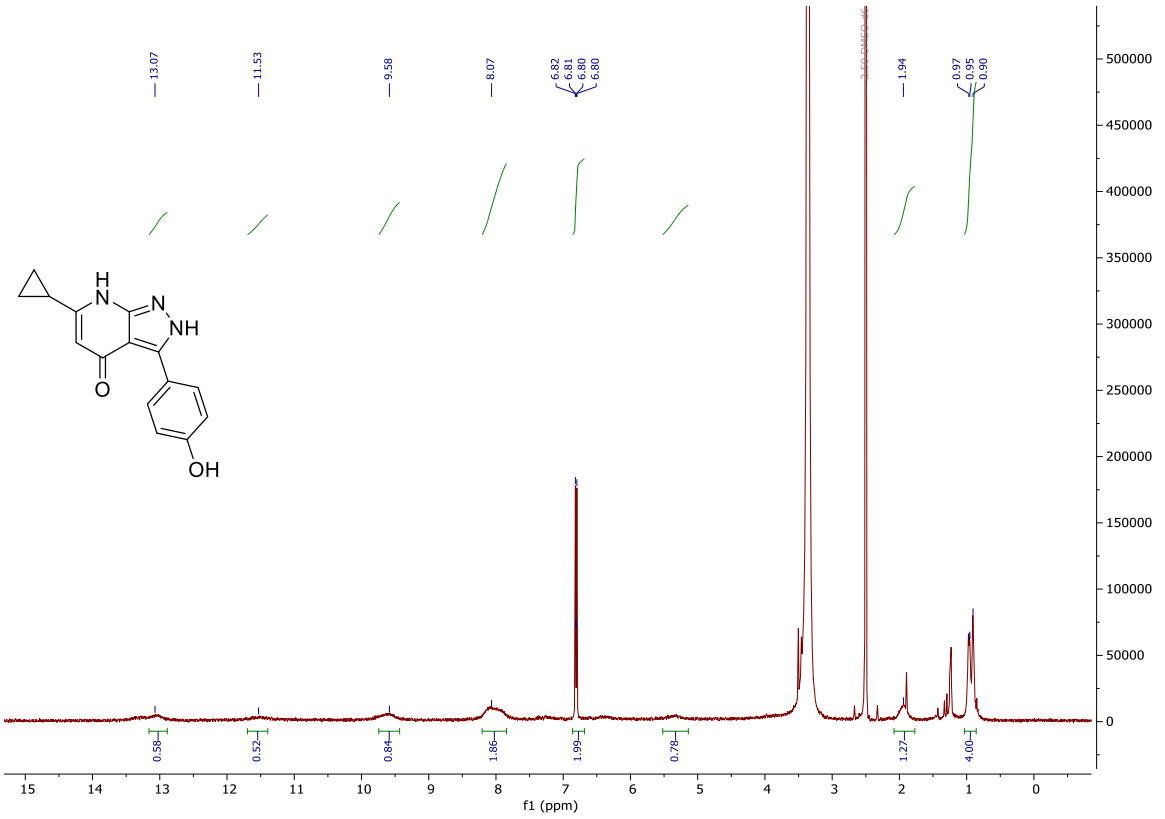
^{13}C NMR (151 MHz, DMSO)



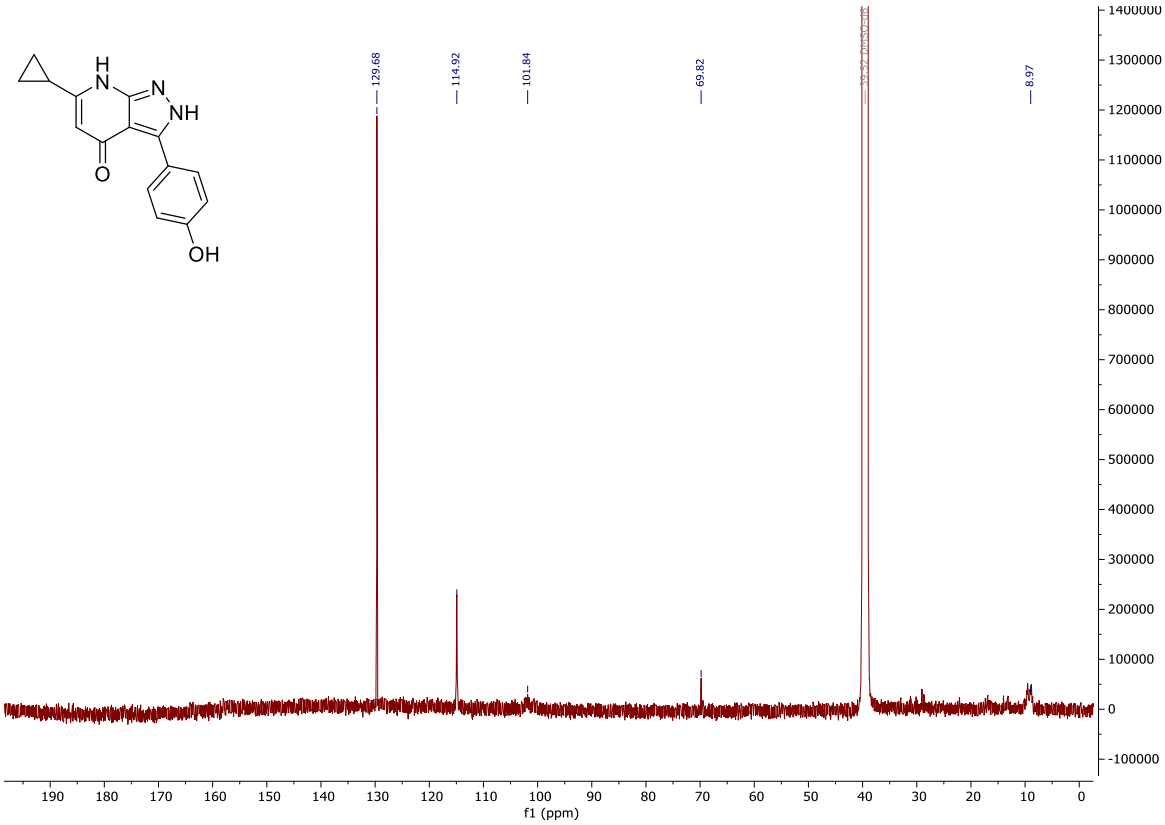
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6-cyclopropyl-3-(4-hydroxyphenyl)-2,7-dihydro-4H-pyrazolo[3,4-b]pyridin-4-one (11)

^{1}H NMR (400 MHz, DMSO)



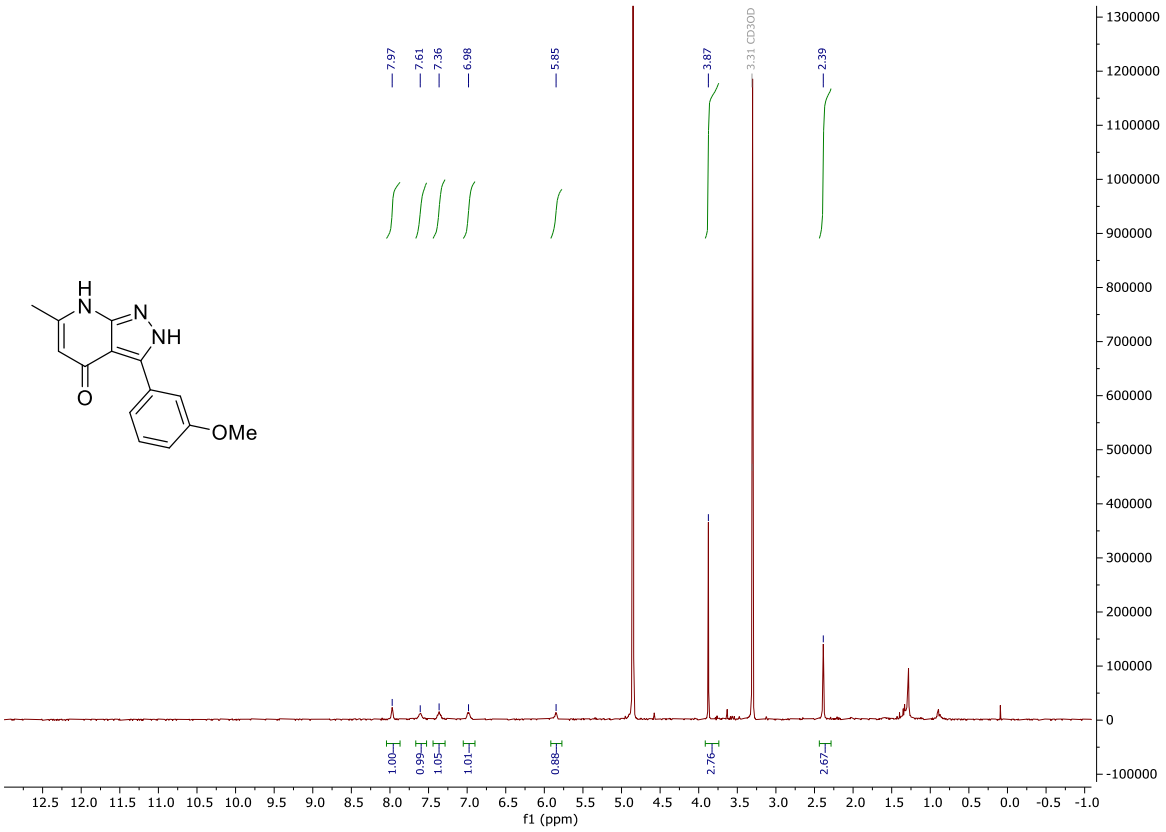
^{13}C NMR (126 MHz, DMSO)



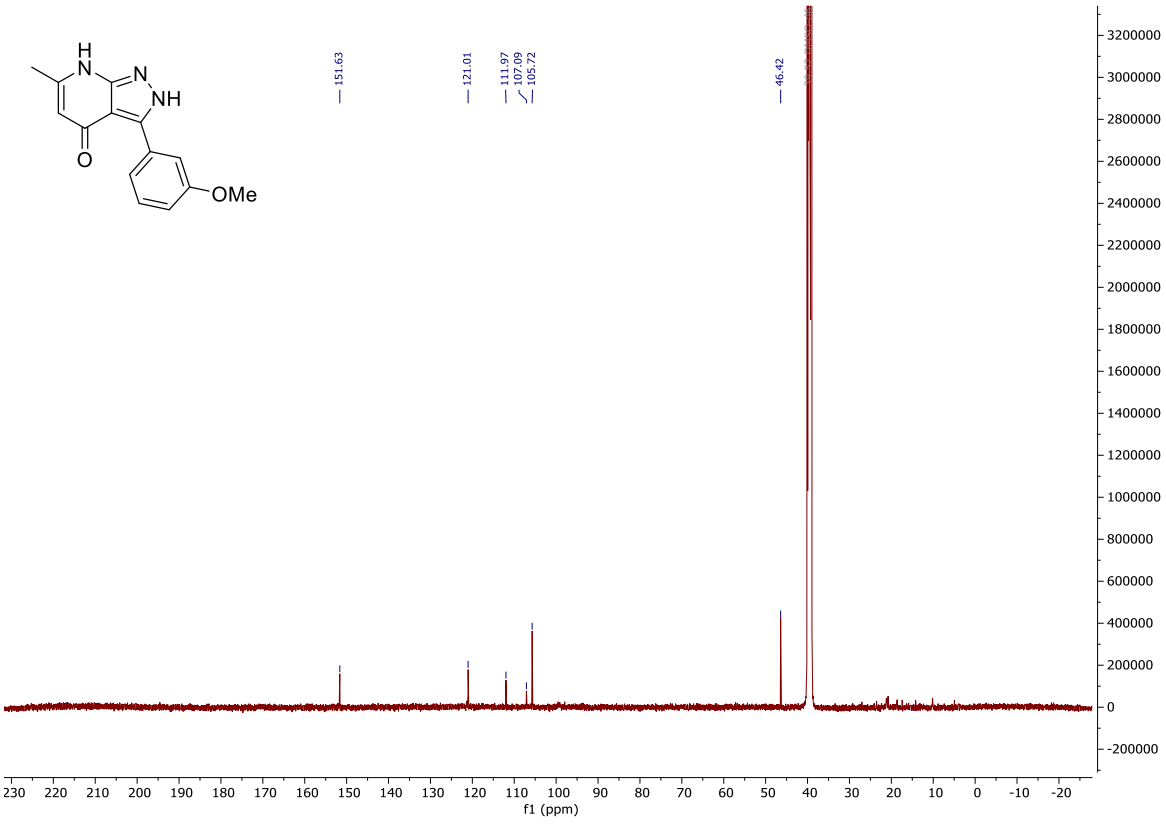
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3-(3-methoxyphenyl)-6-methyl-2,7-dihydro-4H-pyrazolo[3,4-b]pyridin-4-one (12)

^{1}H NMR (400 MHz, MeOD)



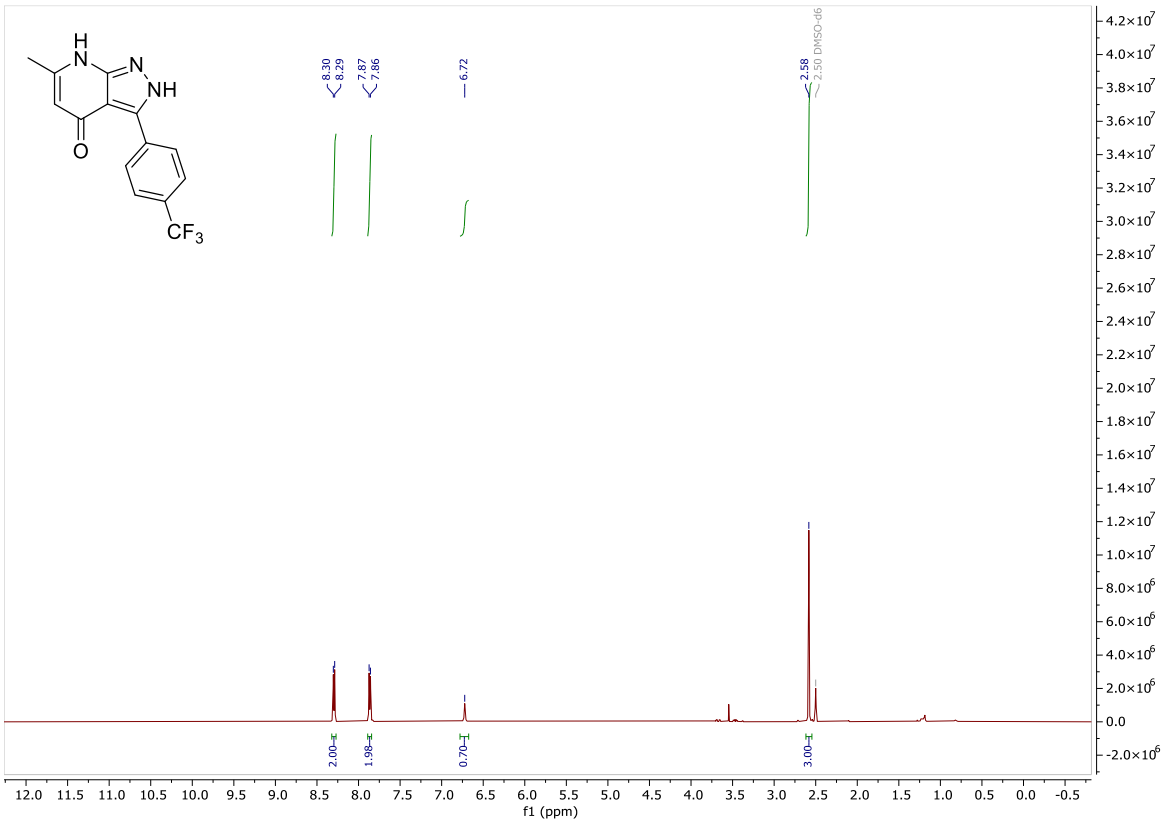
^{13}C NMR (126 MHz, DMSO)



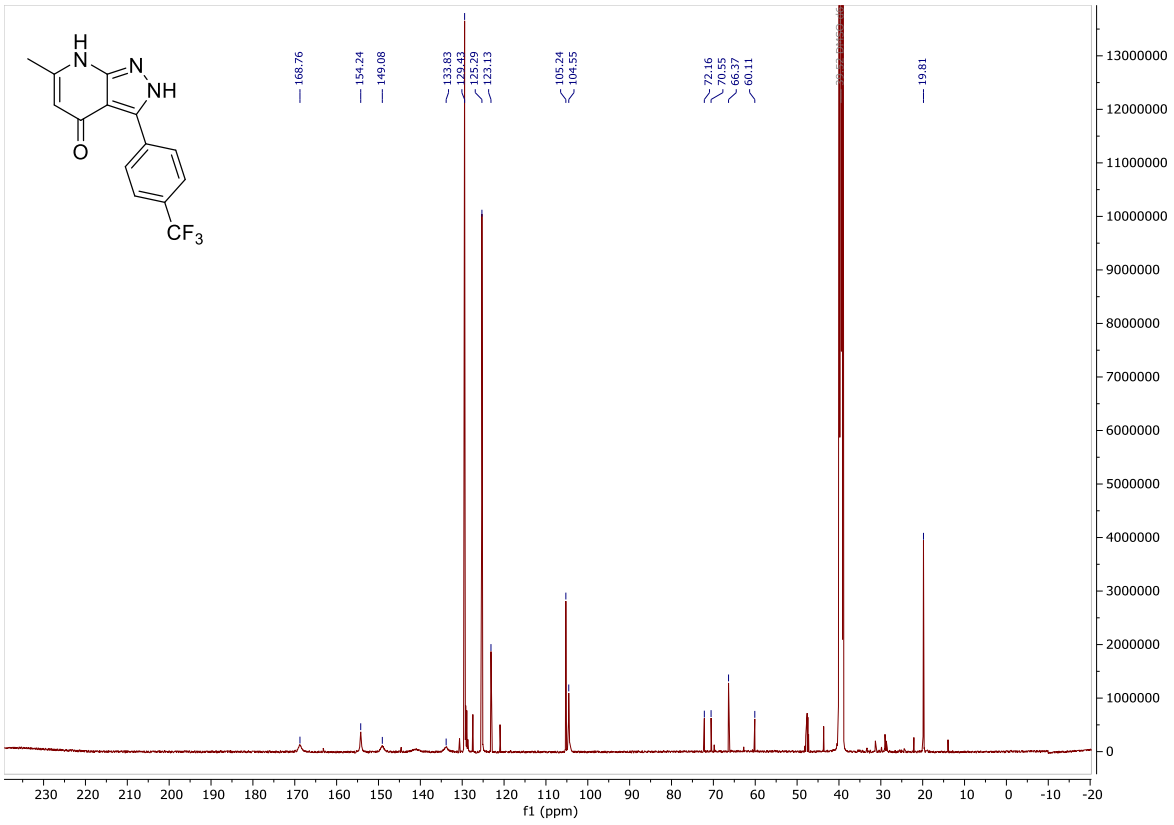
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6-methyl-3-(4-(trifluoromethyl)phenyl)-2,7-dihydro-4H-pyrazolo[3,4-b]pyridin-4-one (13)

^{1}H NMR (500 MHz, DMSO)



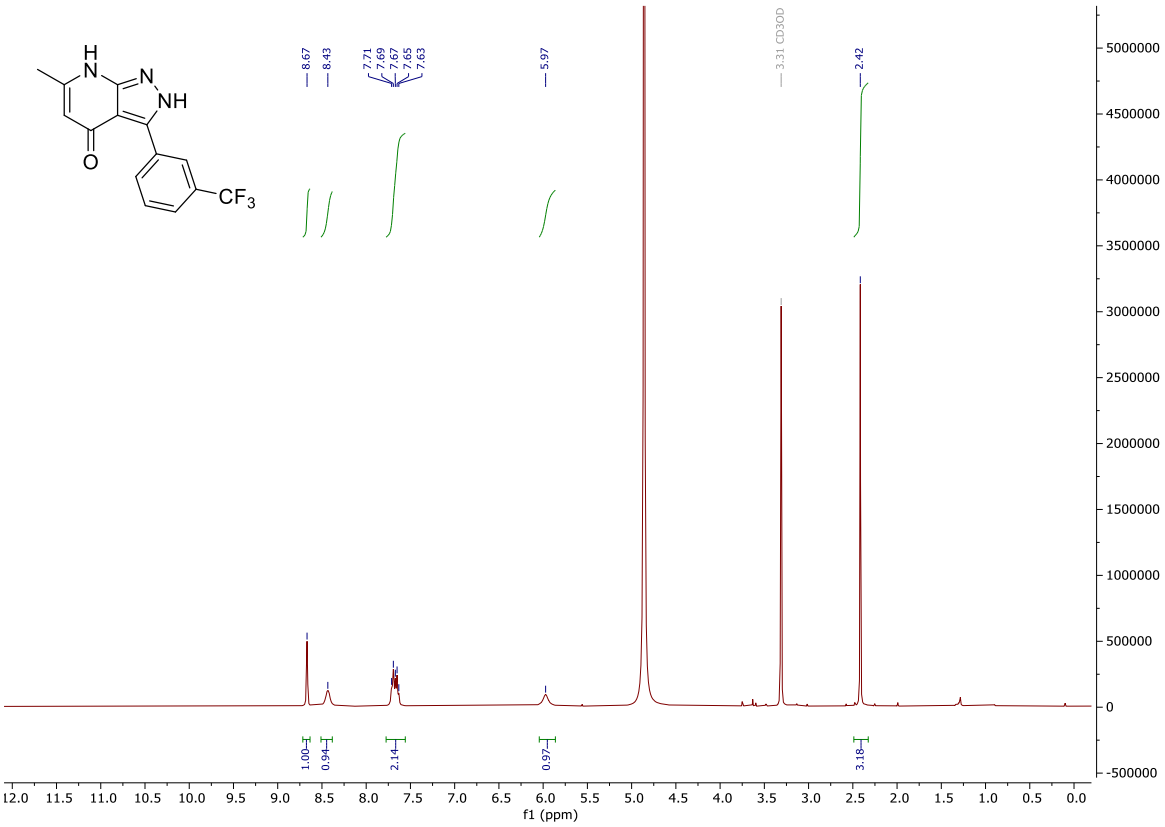
^{13}C NMR (126 MHz, DMSO)



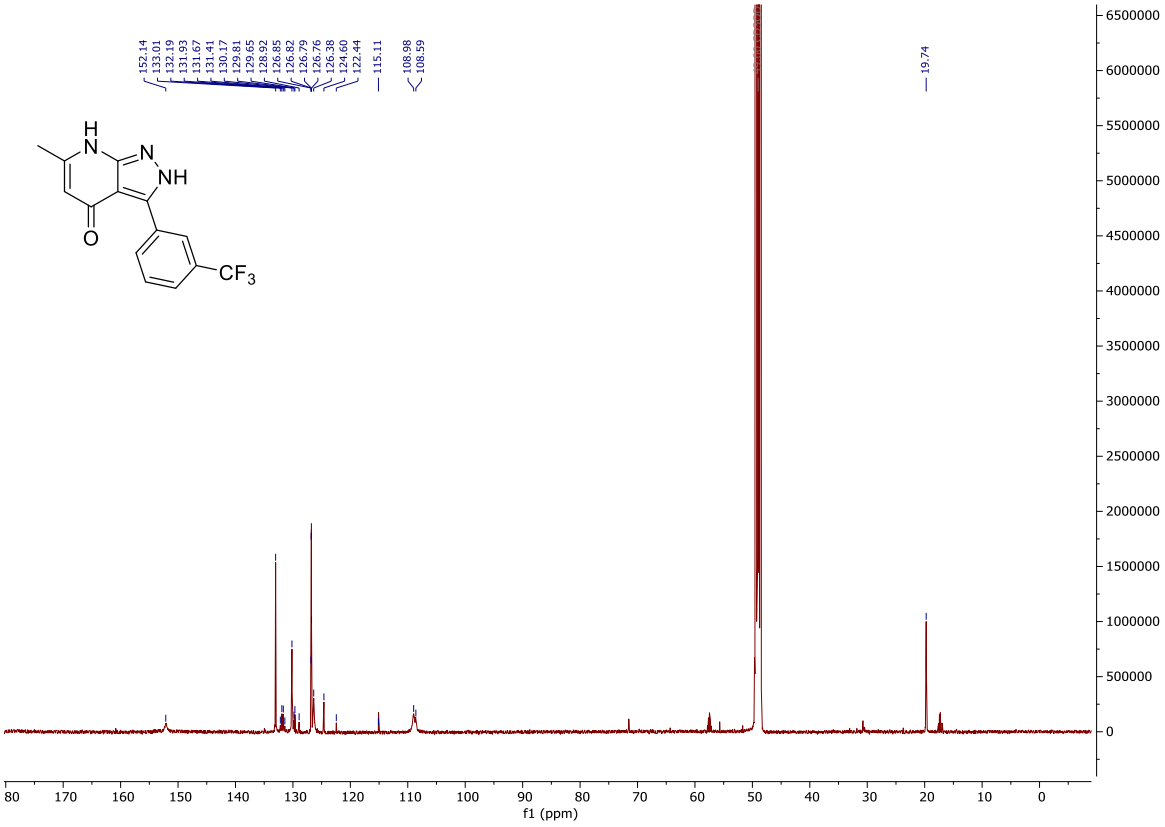
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6-methyl-3-(3-(trifluoromethyl)phenyl)-2,7-dihydro-4H-pyrazolo[3,4-b]pyridin-4-one (14)

^{1}H NMR (400 MHz, MeOD)



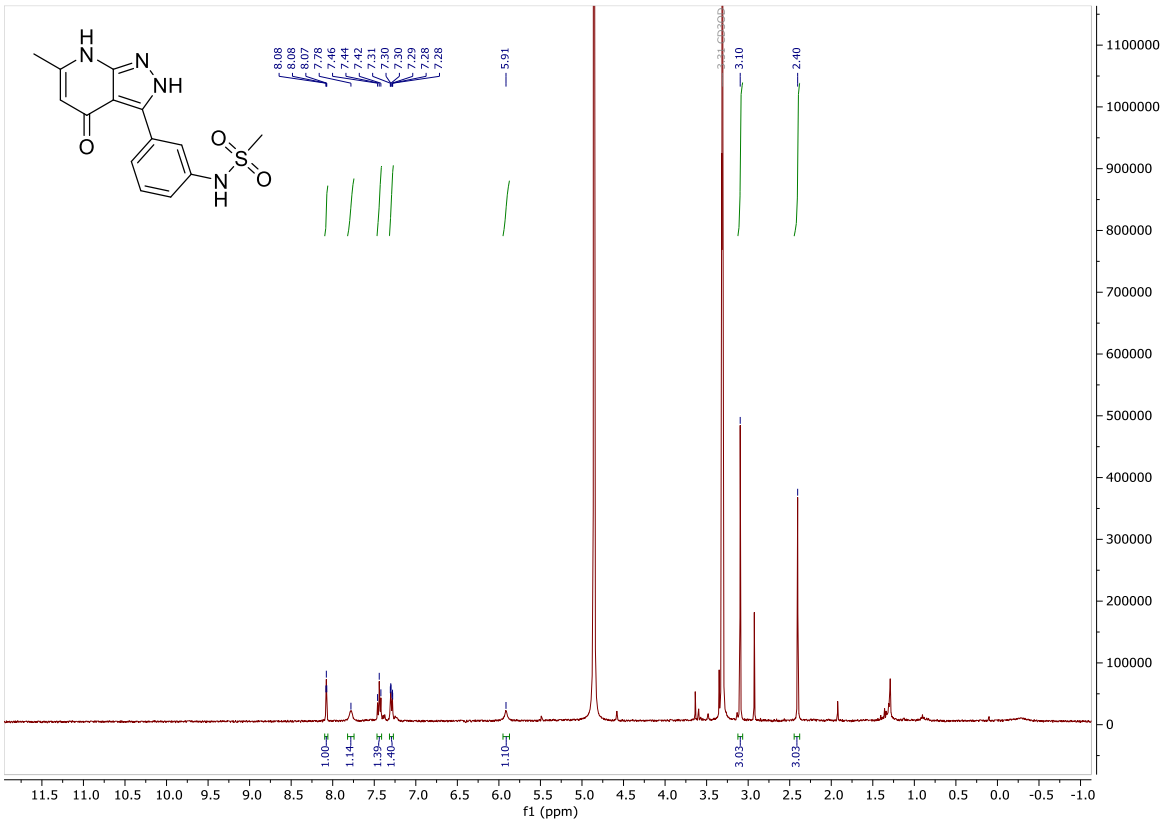
^{13}C NMR (126 MHz, MeOD)



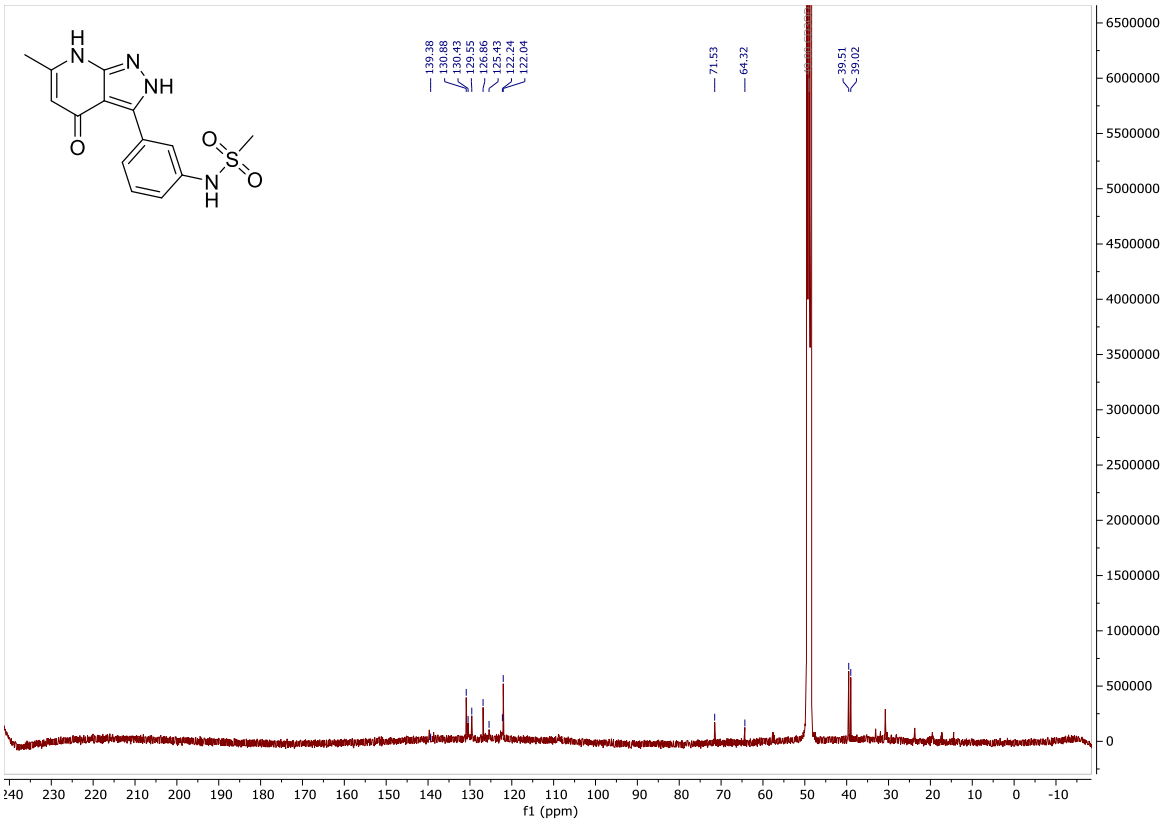
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N-(3-(6-methyl-4-oxo-4,7-dihydro-2H-pyrazolo[3,4-b]pyridin-3-yl)phenyl)methanesulfonamide (15)

^{1}H NMR (400 MHz, MeOD)



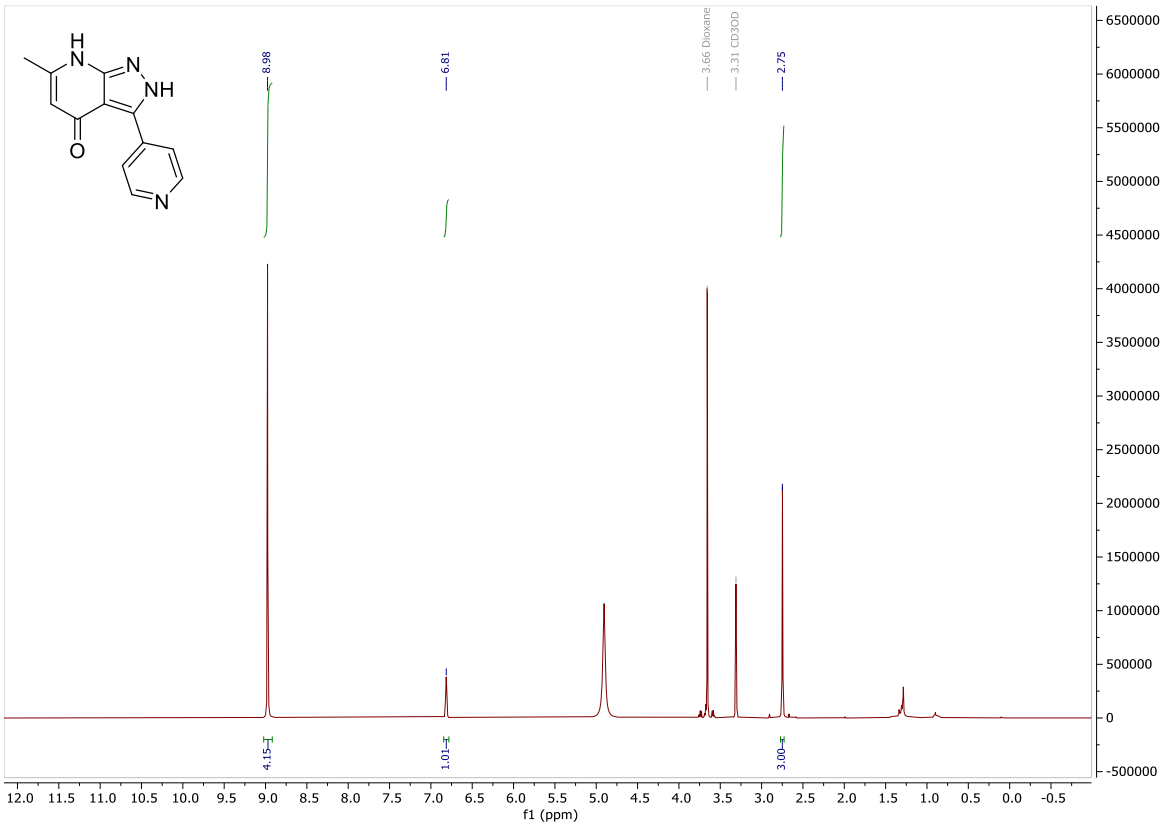
^{13}C NMR (126 MHz, MeOD)



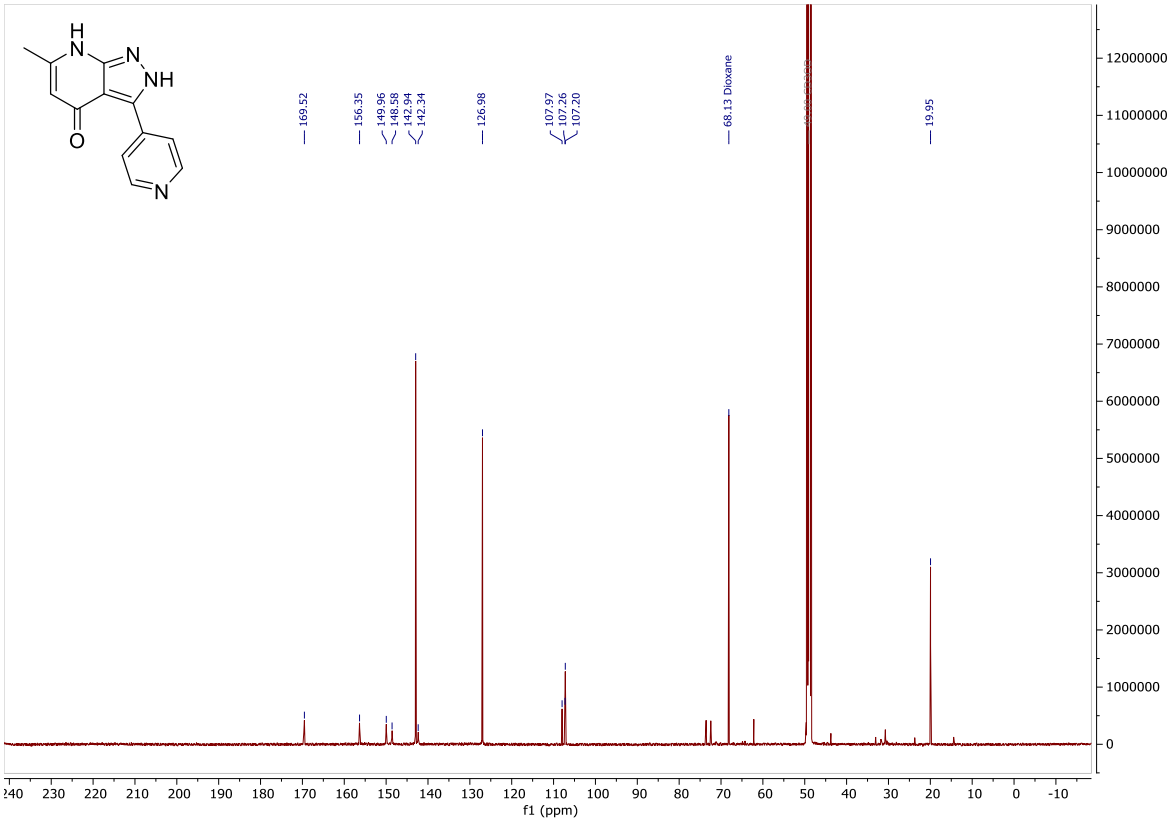
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6-methyl-3-(pyridin-4-yl)-2,7-dihydro-4H-pyrazolo[3,4-b]pyridin-4-one (17)

^{1}H NMR (400 MHz, MeOD)



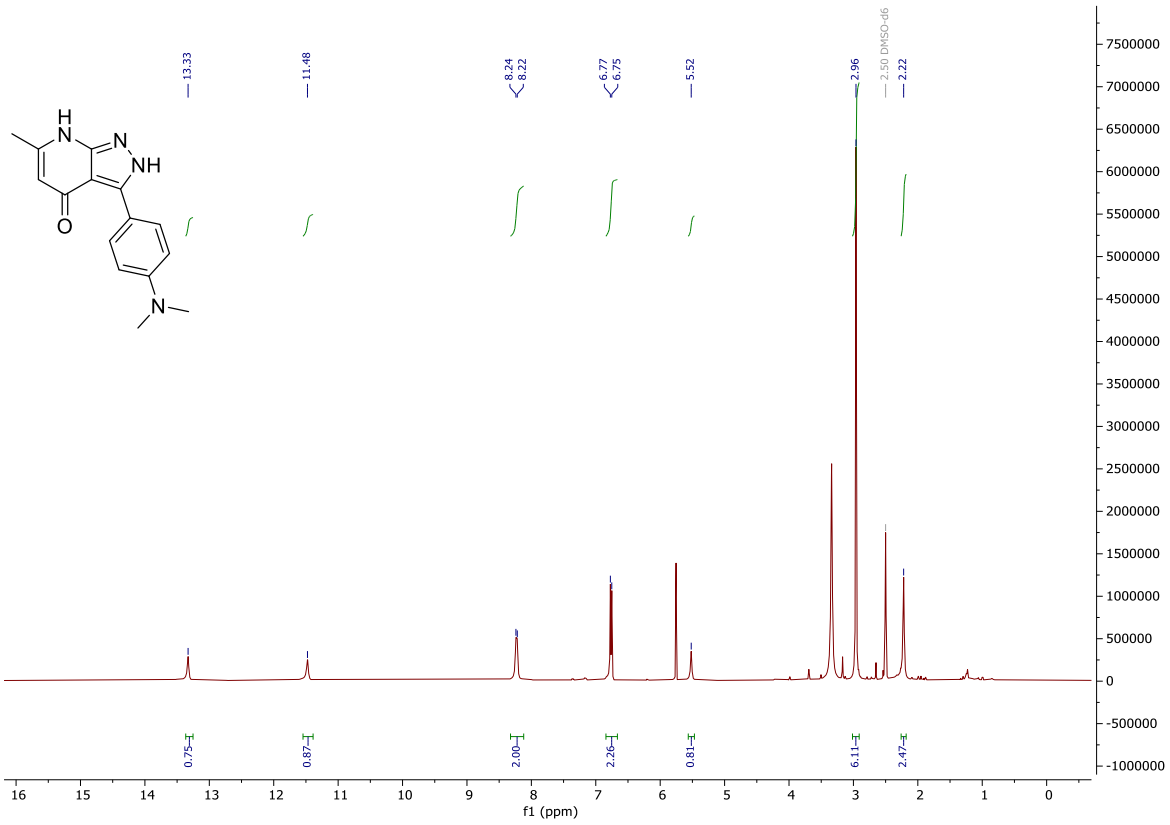
^{13}C NMR (126 MHz, MeOD)



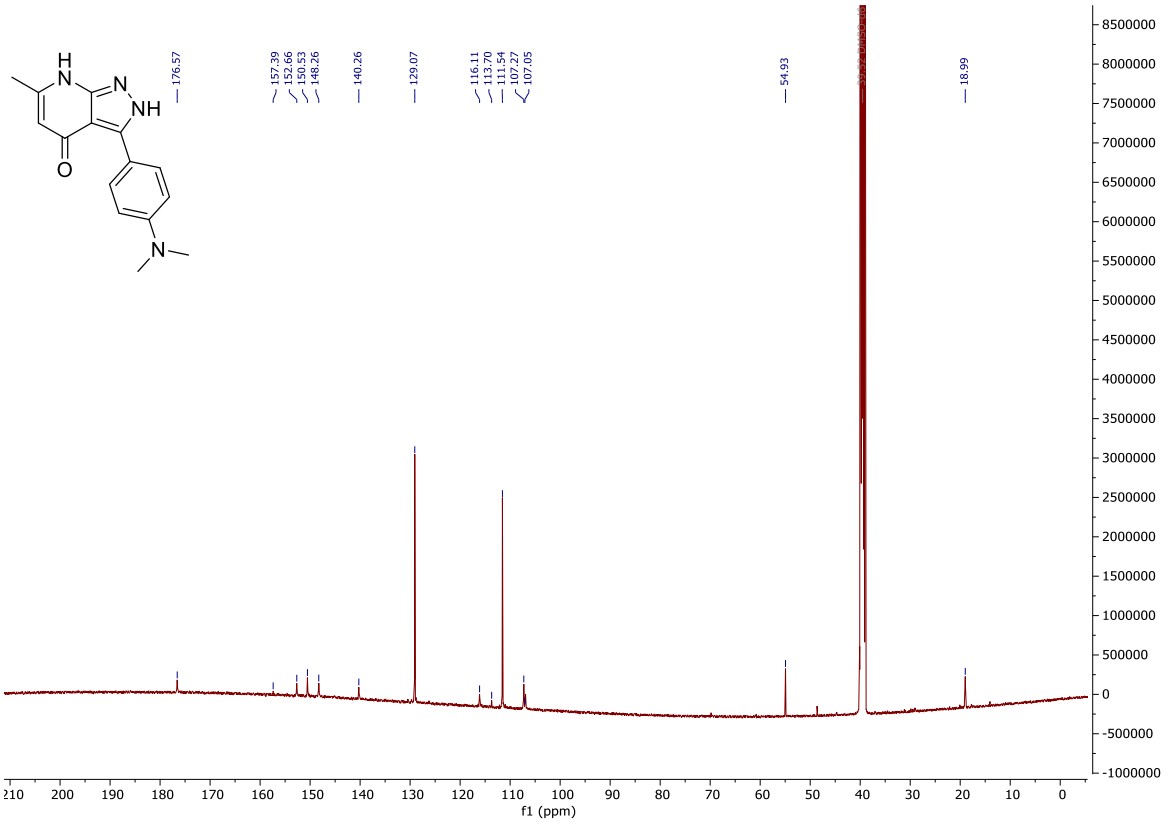
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3-(4-(dimethylamino)phenyl)-6-methyl-2,7-dihydro-4H-pyrazolo[3,4-b]pyridin-4-one (18)

^{1}H NMR (400 MHz, DMSO)



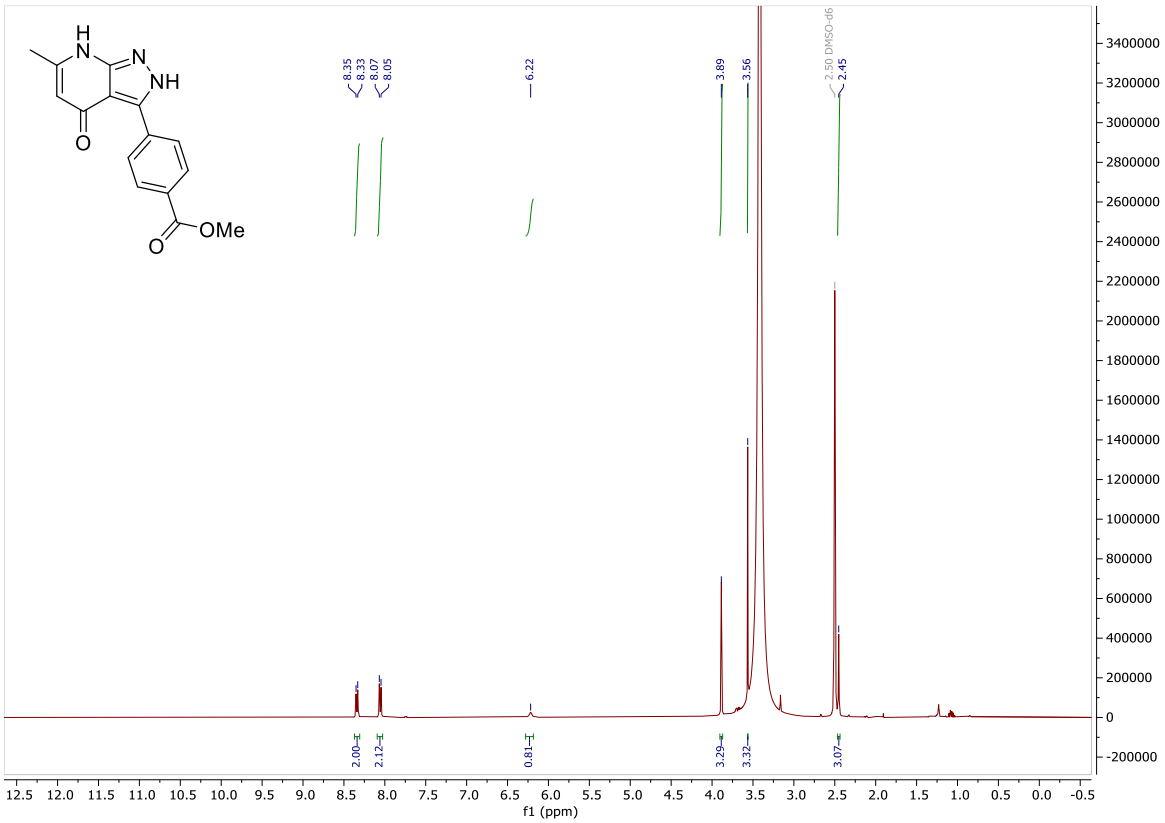
^{13}C NMR (126 MHz, DMSO)



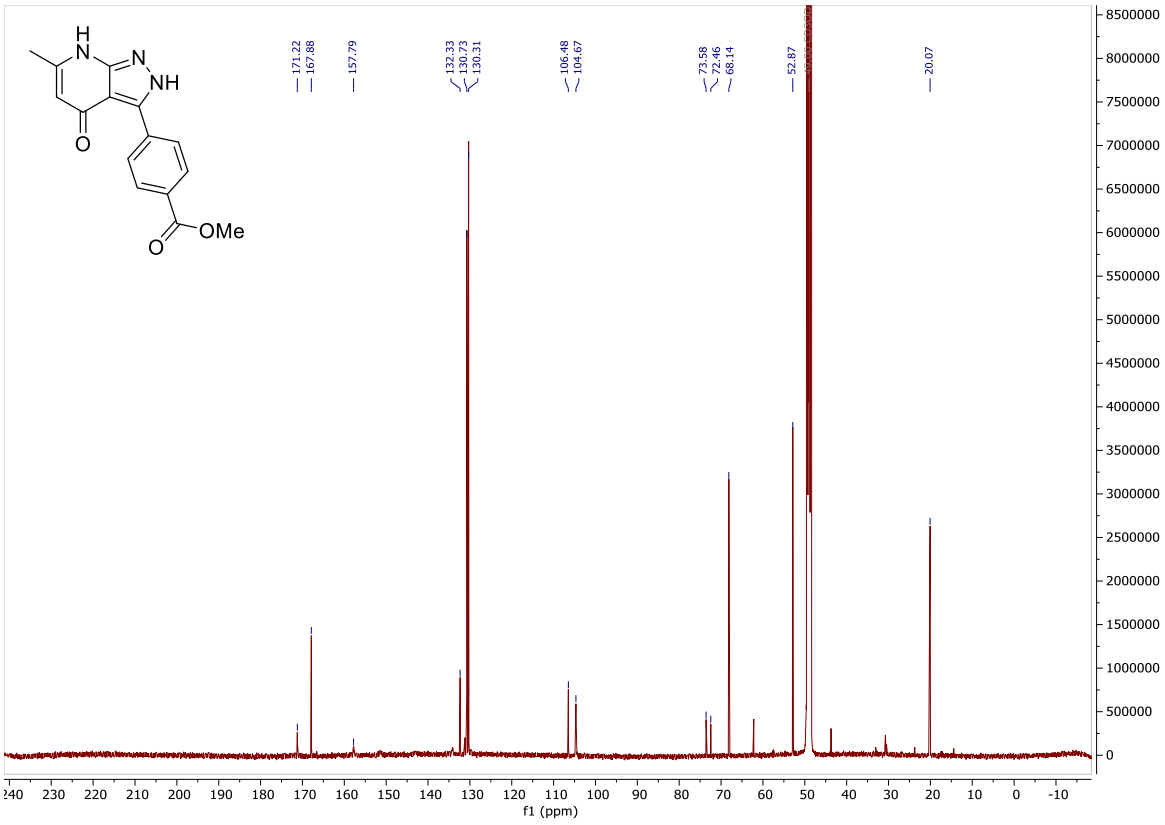
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methyl 4-(6-methyl-4-oxo-4,7-dihydro-2H-pyrazolo[3,4-b]pyridin-3-yl)benzoate (19)

^{1}H NMR (400 MHz, DMSO)



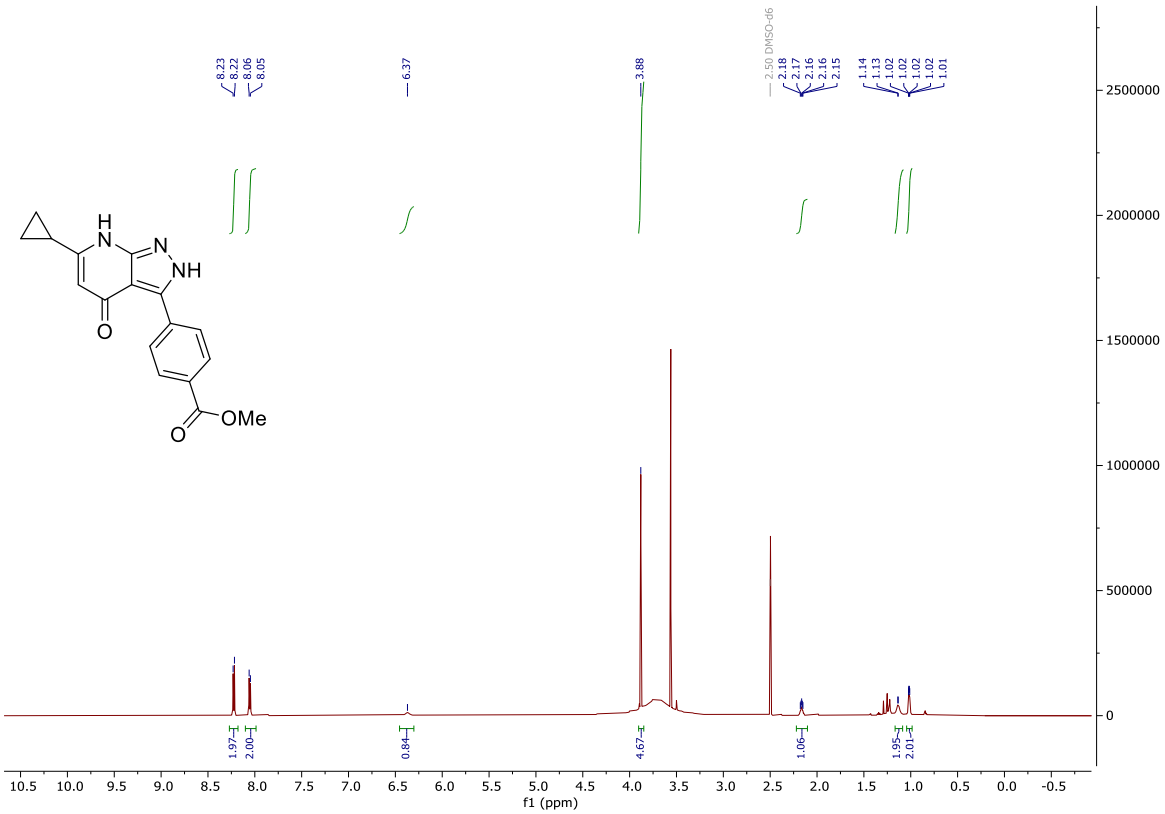
^{13}C NMR (126 MHz, MeOD)



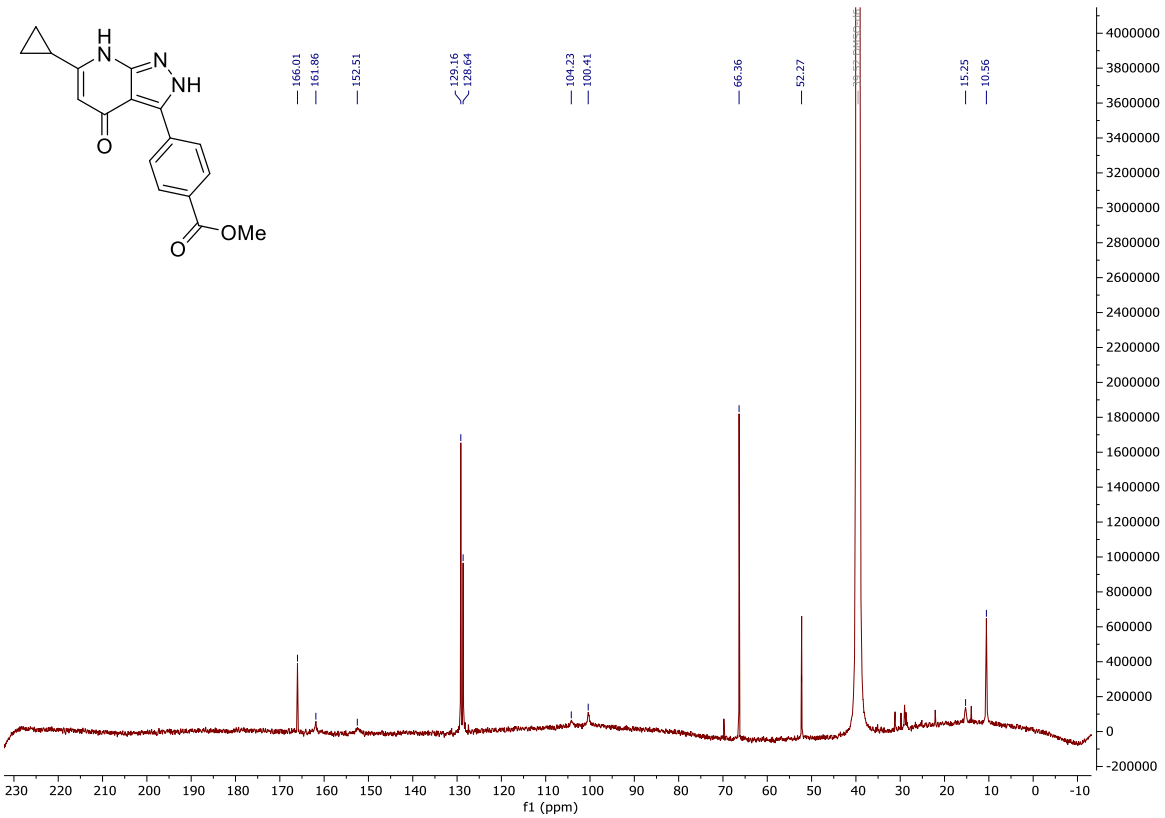
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methyl 4-(6-cyclopropyl-4-oxo-4,7-dihydro-2H-pyrazolo[3,4-b]pyridin-3-yl)benzoate (20)

^{1}H NMR (600 MHz, DMSO)



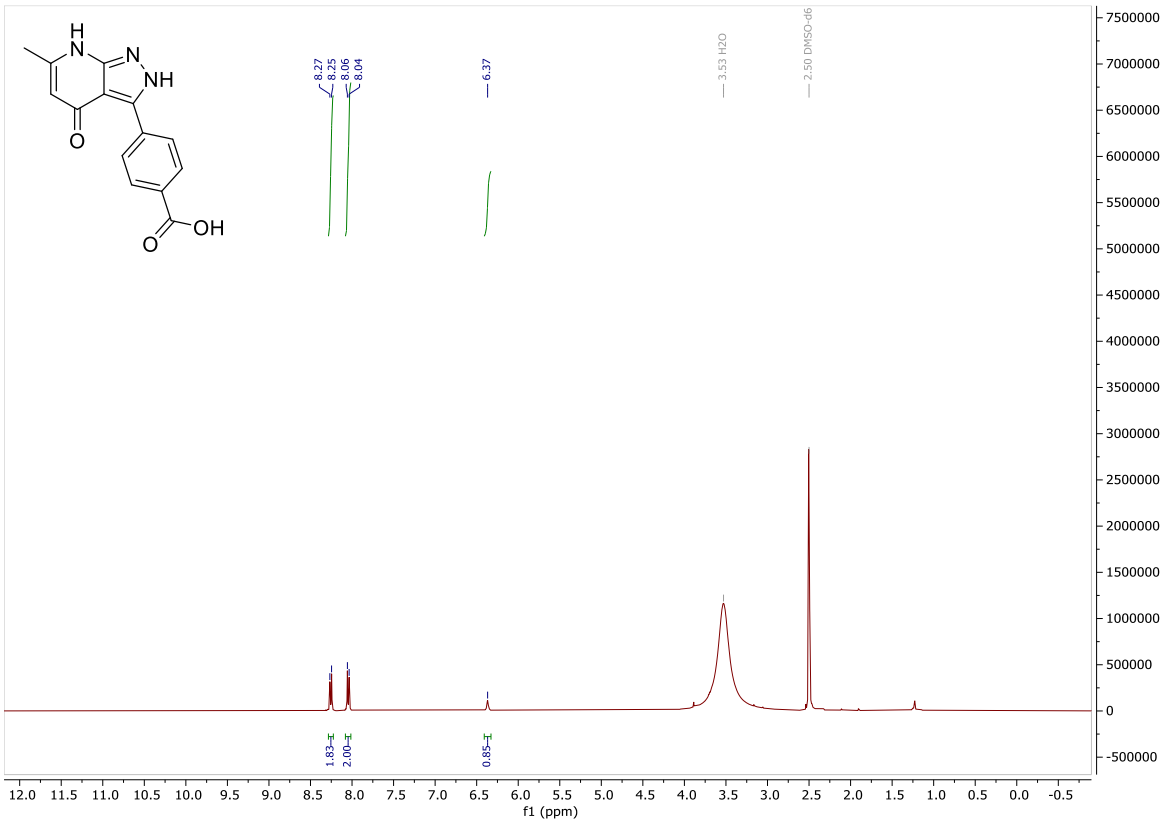
^{13}C NMR (151 MHz, DMSO)



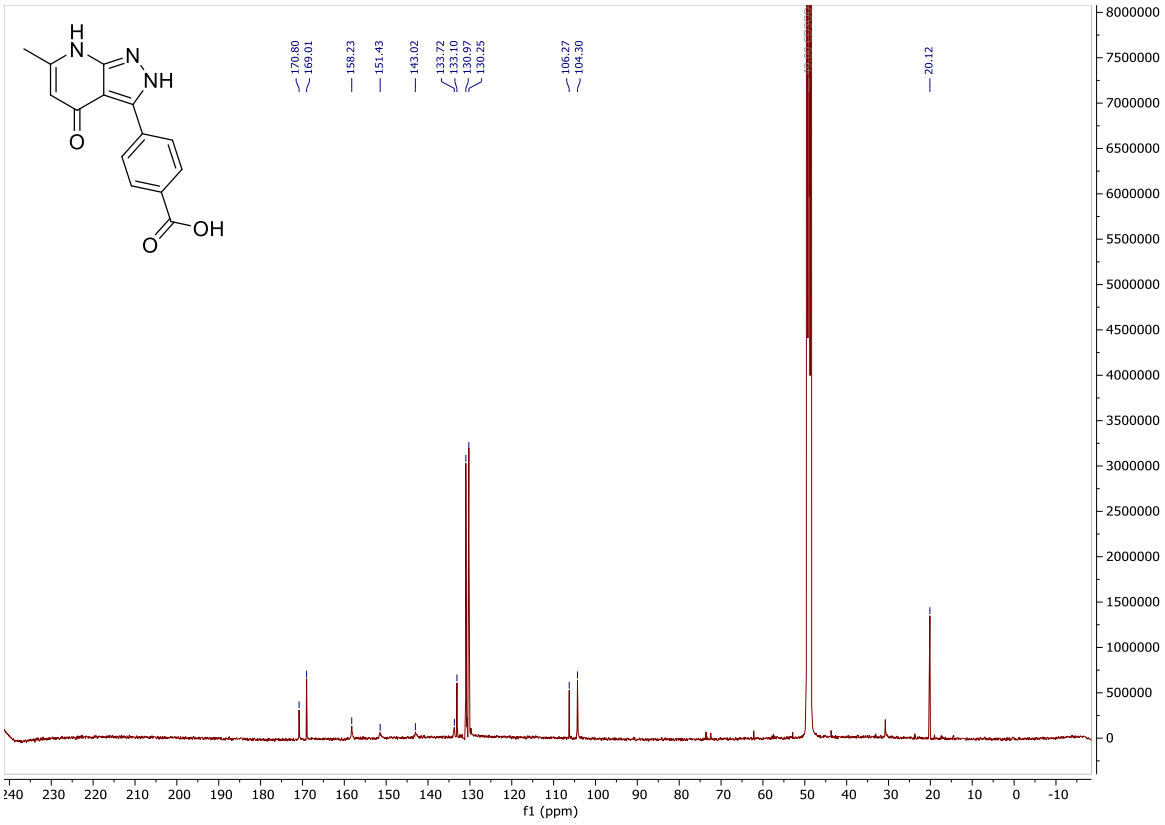
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4-(6-methyl-4-oxo-4,7-dihydro-2H-pyrazolo[3,4-b]pyridin-3-yl)benzoic acid (21)

^{1}H NMR (400 MHz, DMSO)



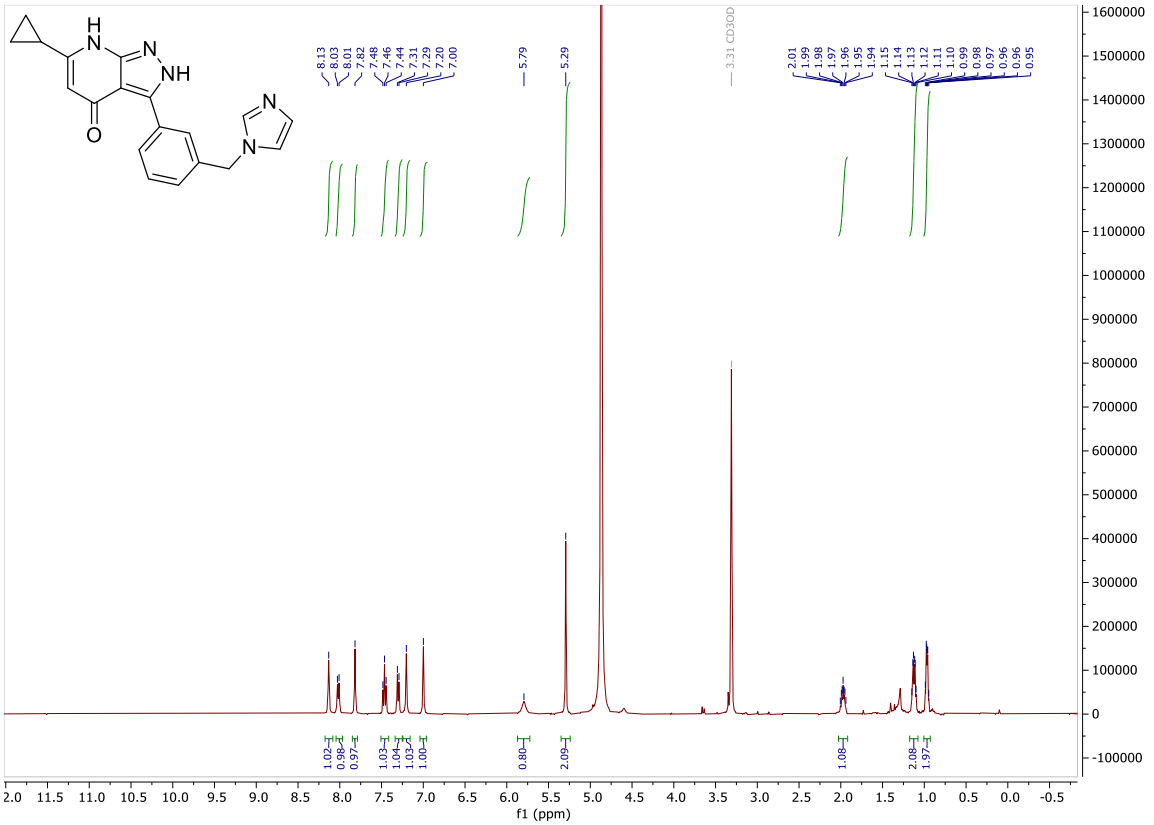
^{13}C NMR (126 MHz, MeOD)



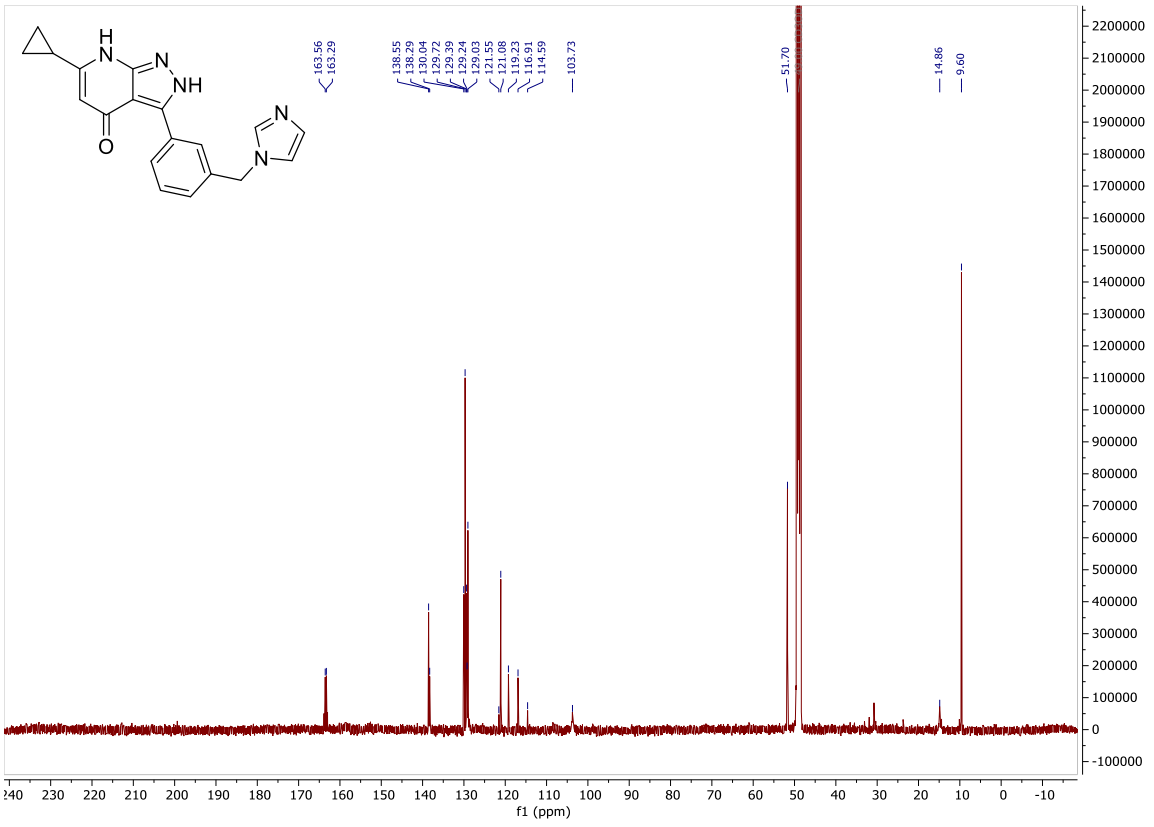
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3-(3-((1H-imidazol-1-yl)methyl)phenyl)-6-cyclopropyl-2,7-dihydro-4H-pyrazolo[3,4-b]pyridin-4-one (22)

^{1}H NMR (400 MHz, MeOD)



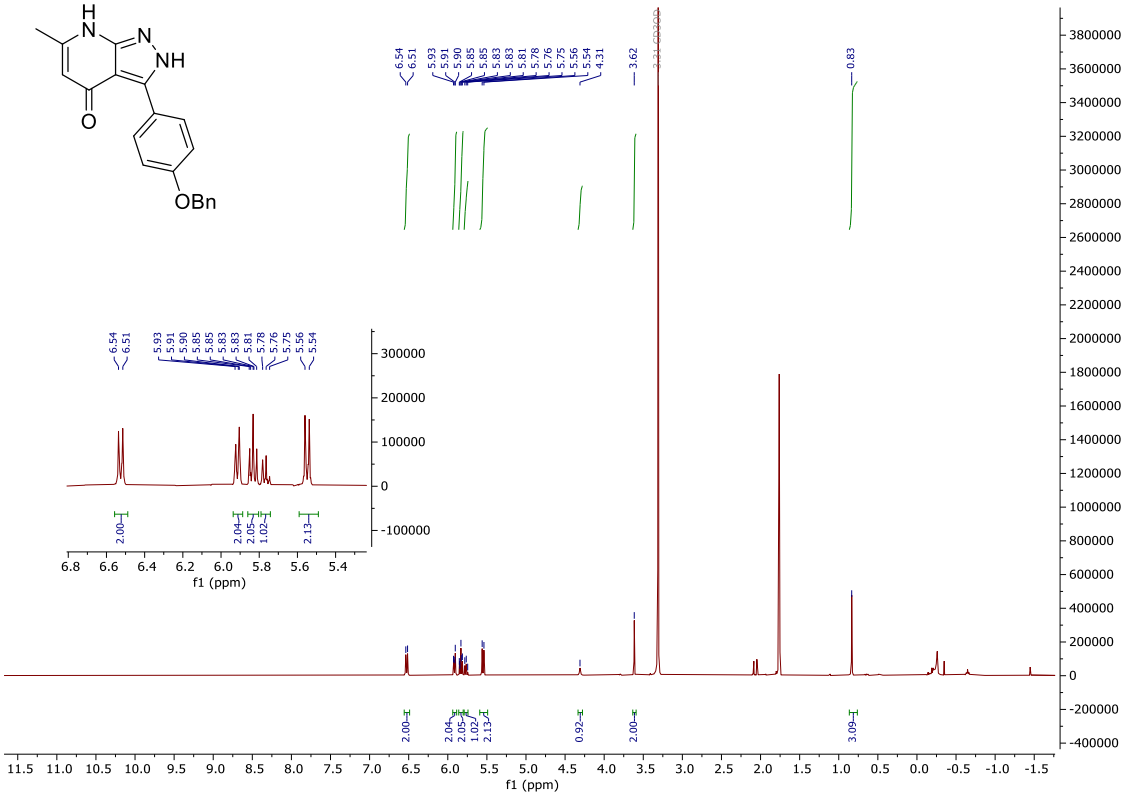
^{13}C NMR (126 MHz, MeOD)



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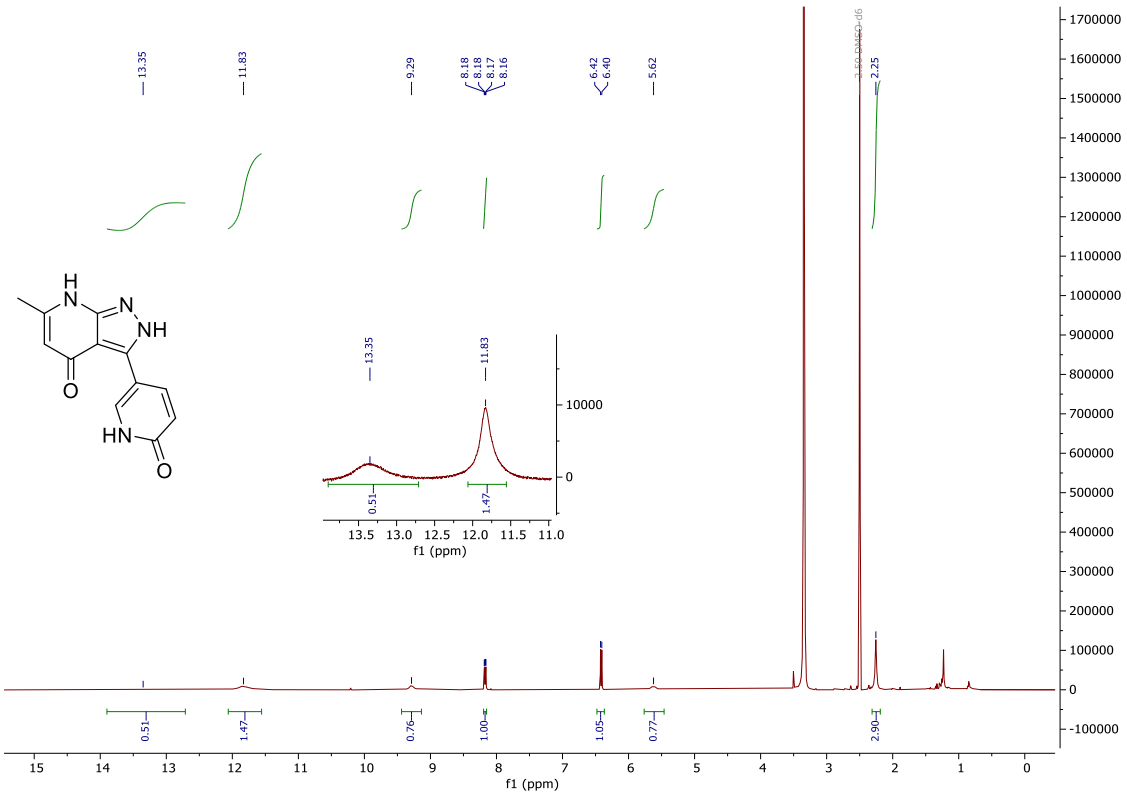
3-(4-(benzyloxy)phenyl)-6-methyl-2,7-dihydro-4H-pyrazolo[3,4-b]pyridin-4-one (23)

^{1}H NMR (400 MHz, MeOD)



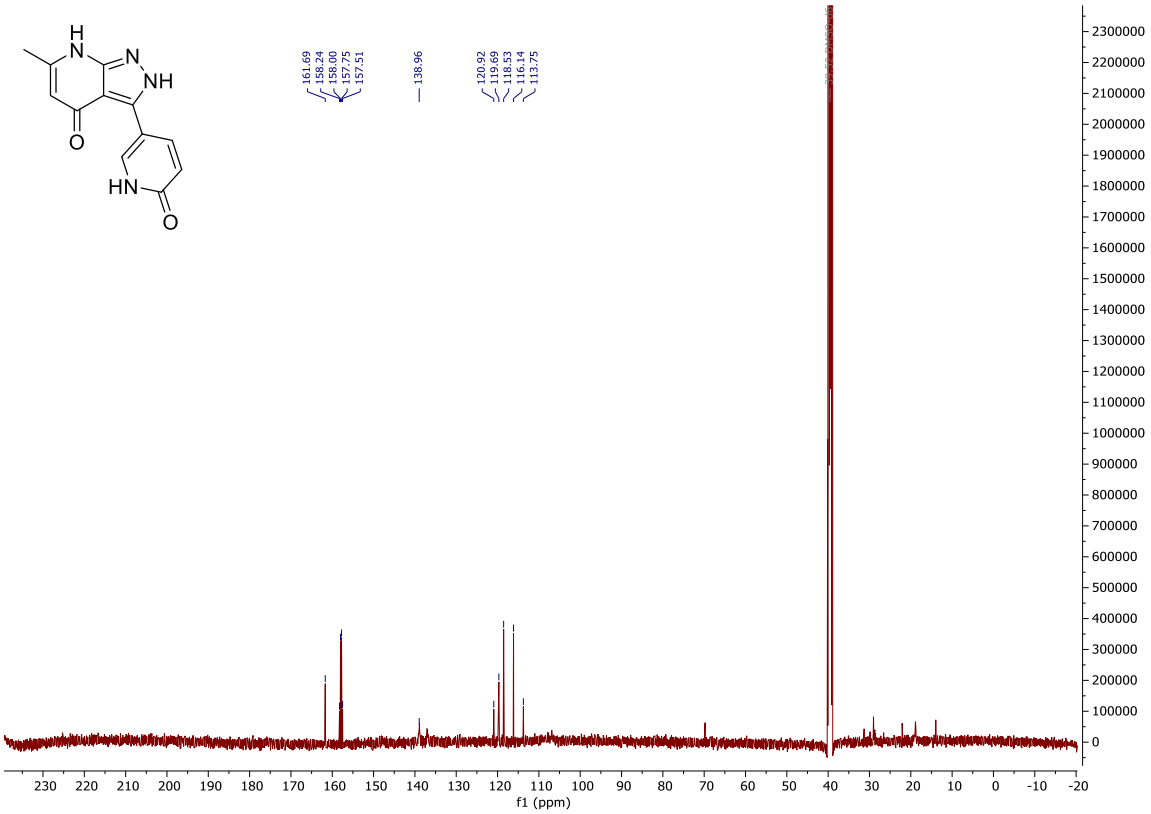
6-methyl-3-(6-oxo-1,6-dihydropyridin-3-yl)-2,7-dihydro-4H-pyrazolo[3,4-b]pyridin-4-one (24)

^{1}H NMR (500 MHz, DMSO)



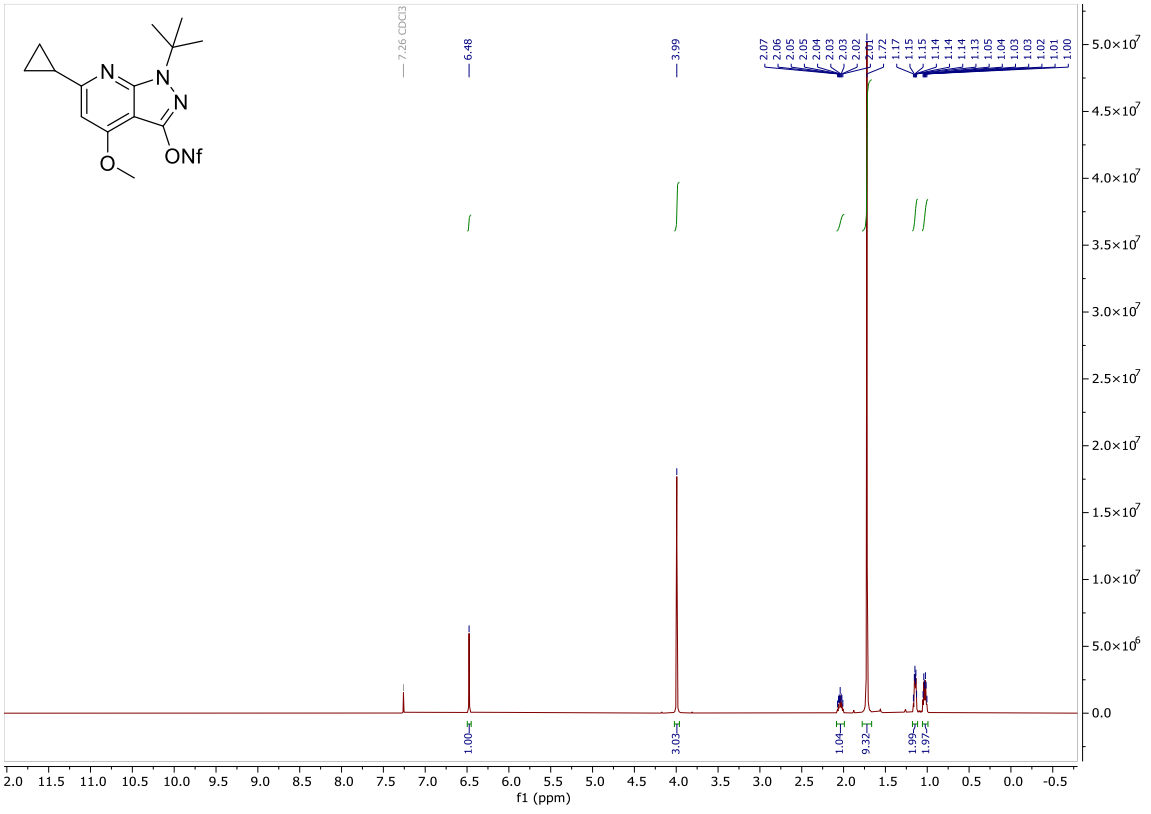
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^{13}C NMR (126 MHz, DMSO)



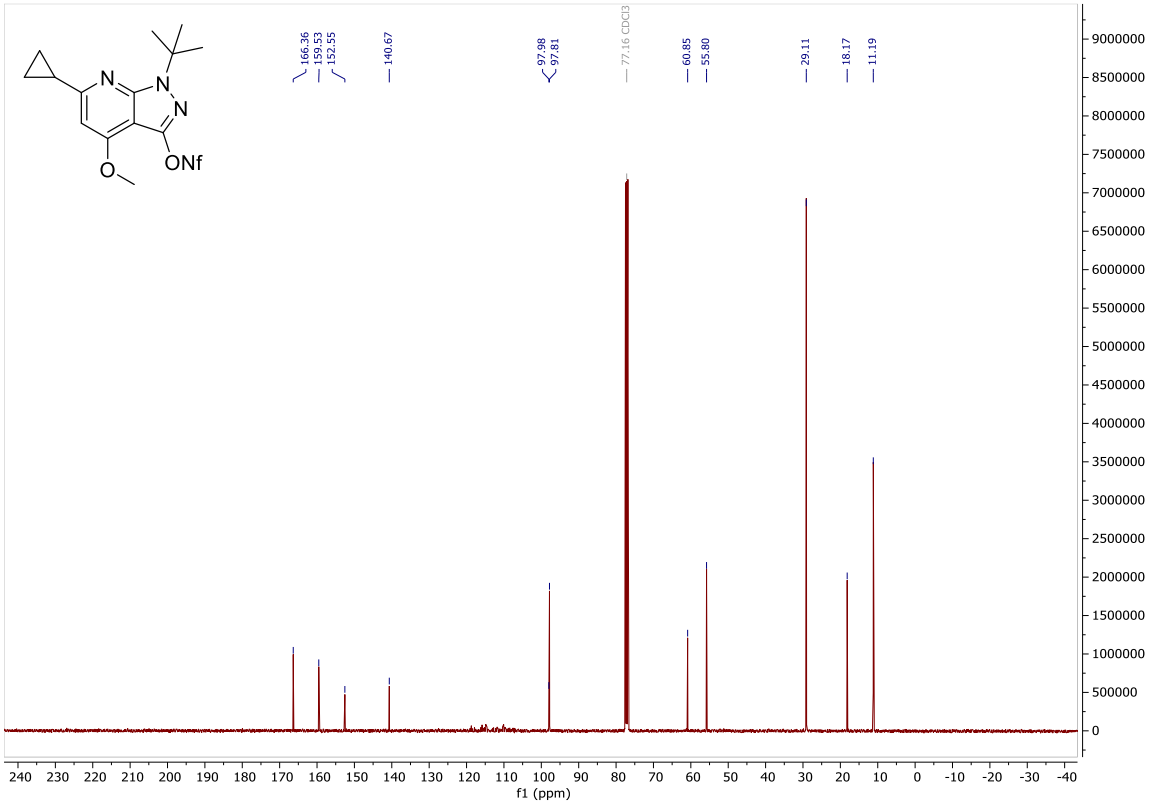
1-(tert-butyl)-6-cyclopropyl-4-methoxy-1H-pyrazolo[3,4-b]pyridin-3-yl 1,1,2,2,3,3,4,4,4-nonafluorobutane-1-sulfonate (55)

^{1}H NMR (400 MHz, $CDCl_3$)



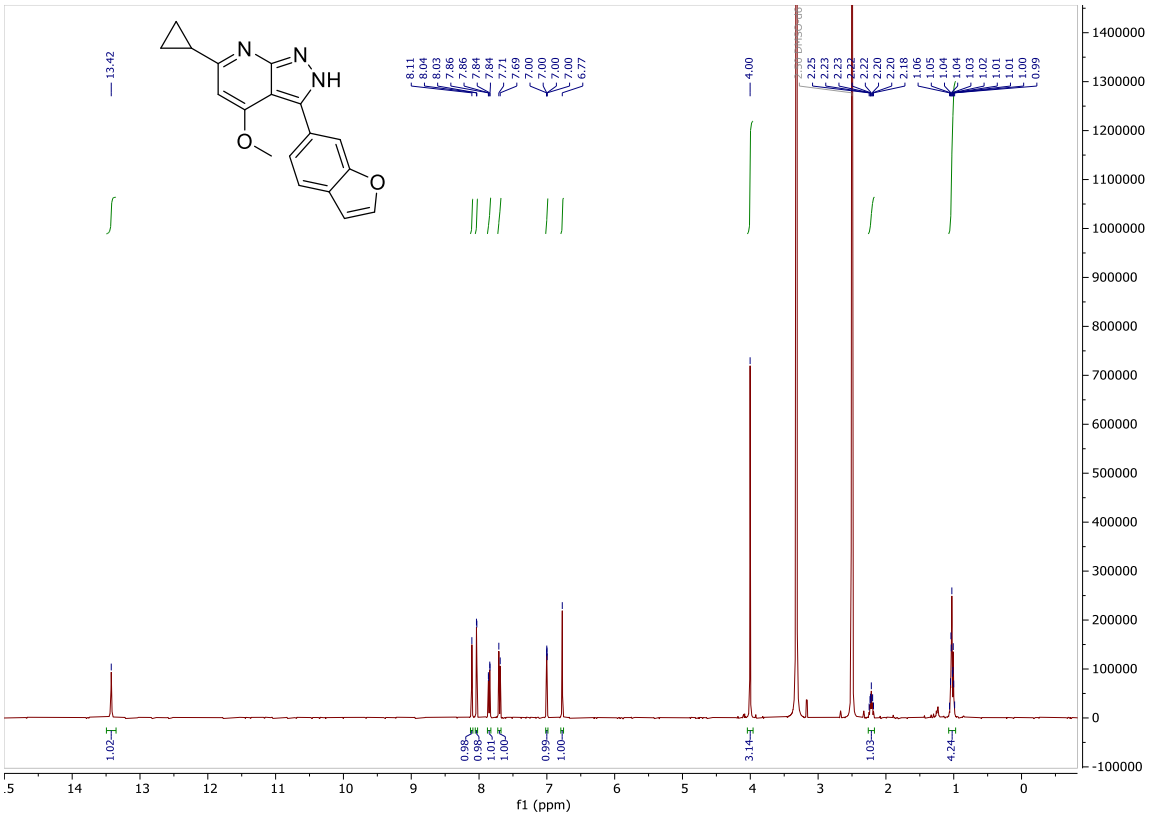
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^{13}C NMR (101 MHz, $CDCl_3$)



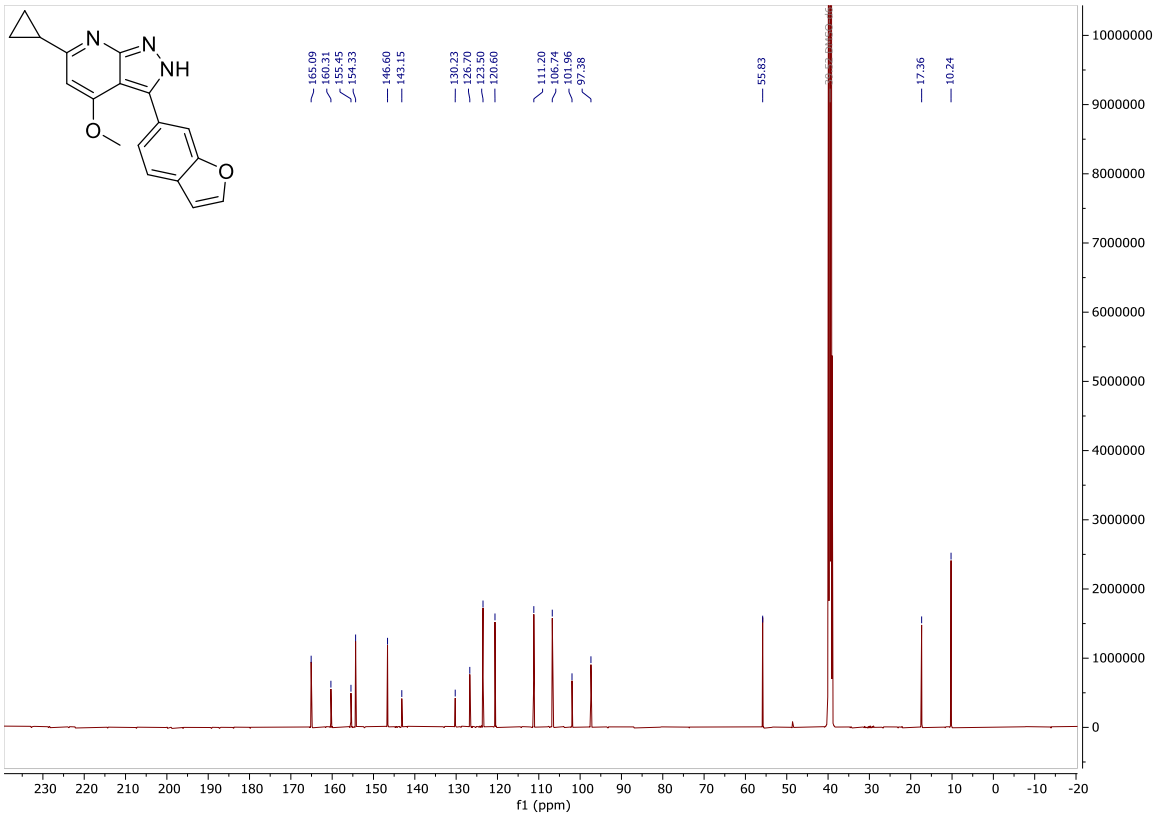
3-(benzofuran-6-yl)-6-cyclopropyl-4-methoxy-2H-pyrazolo[3,4-b]pyridine (31b)

^{1}H NMR (400 MHz, DMSO)



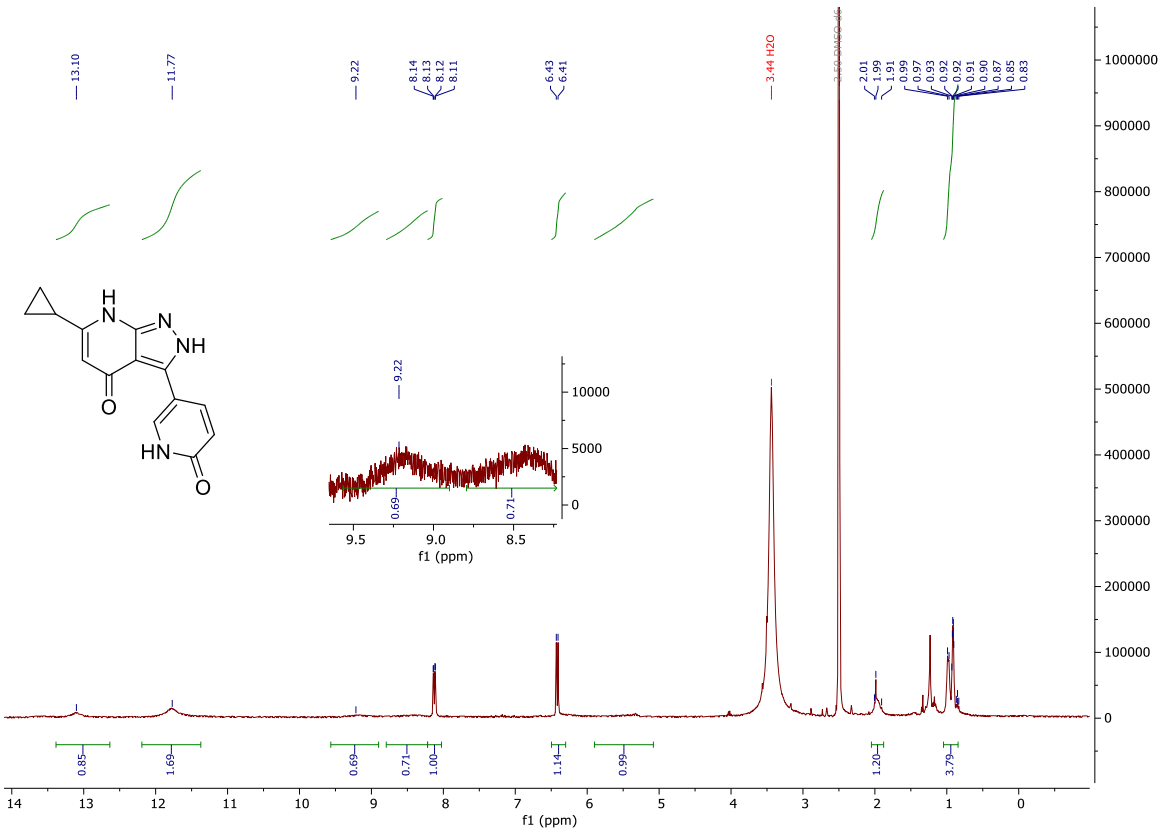
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^{13}C NMR (126 MHz, DMSO)



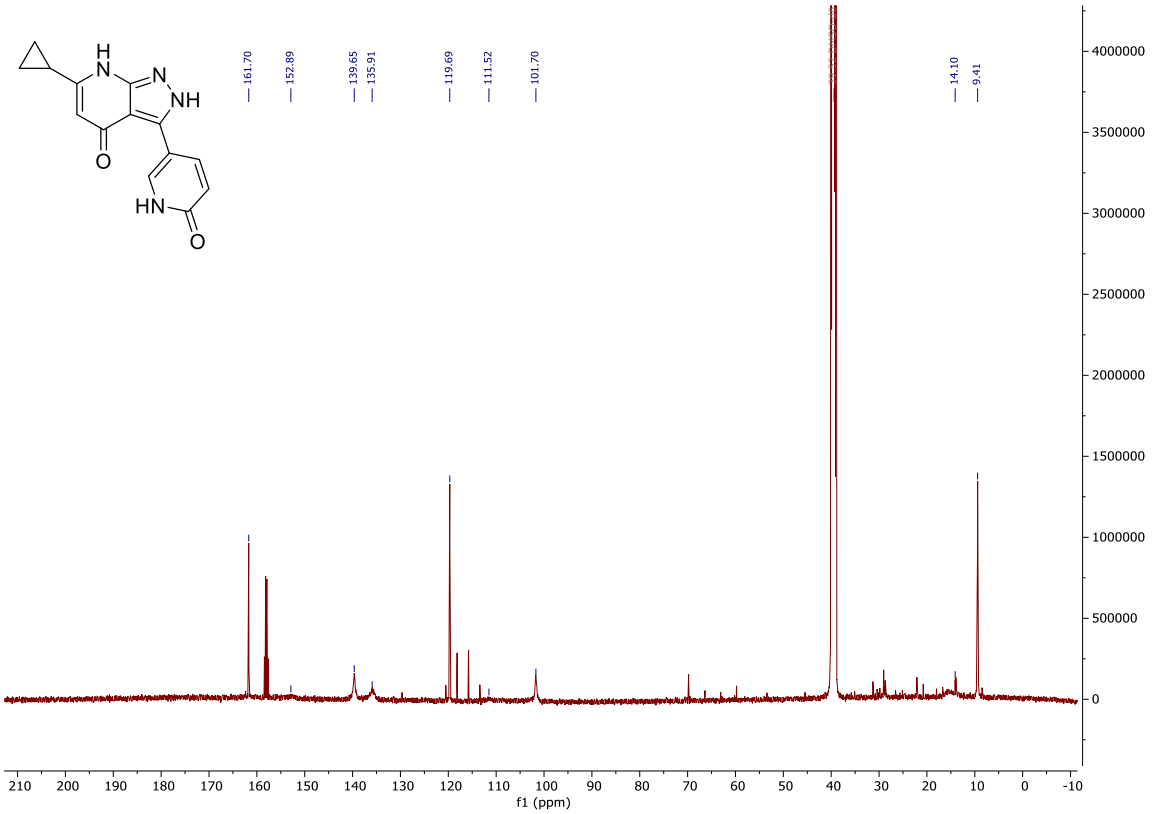
6-cyclopropyl-3-(6-oxo-1,6-dihydropyridin-3-yl)-2,7-dihydro-4H-pyrazolo[3,4-b]pyridin-4-one (25)

^{1}H NMR (400 MHz, DMSO)



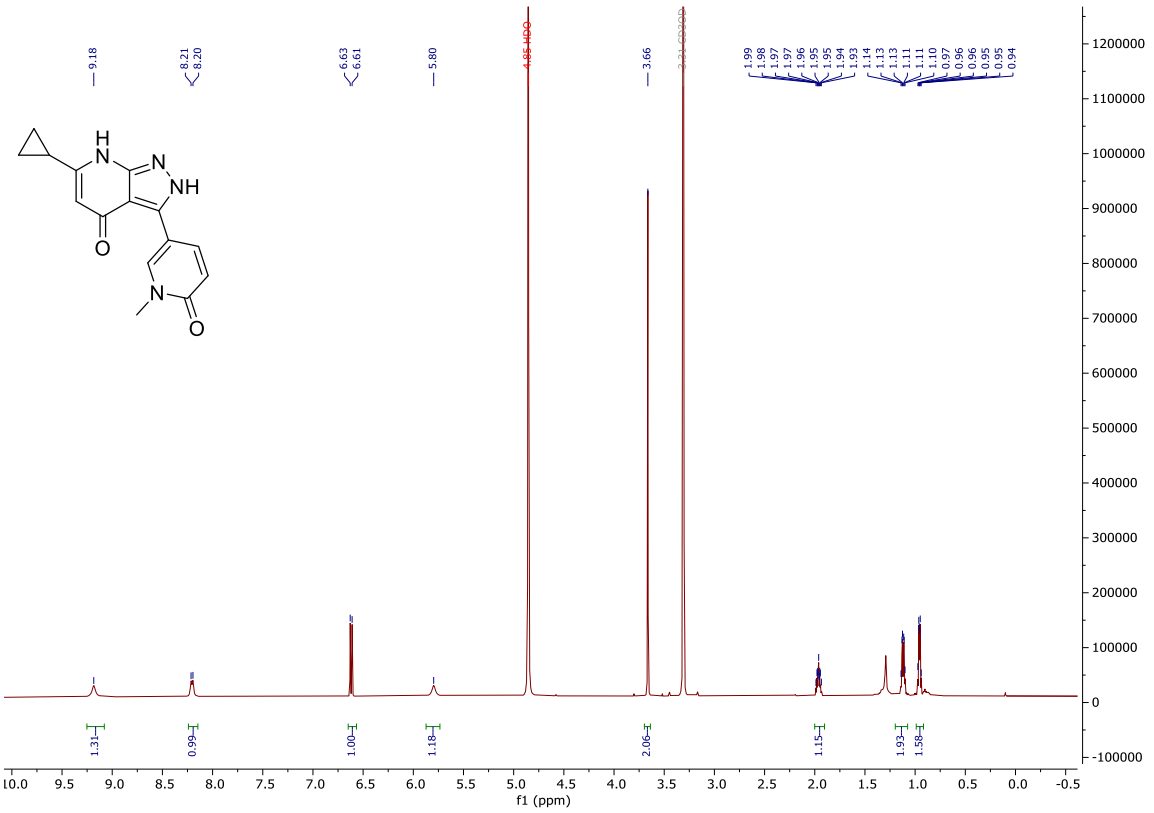
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^{13}C NMR (126 MHz, DMSO)



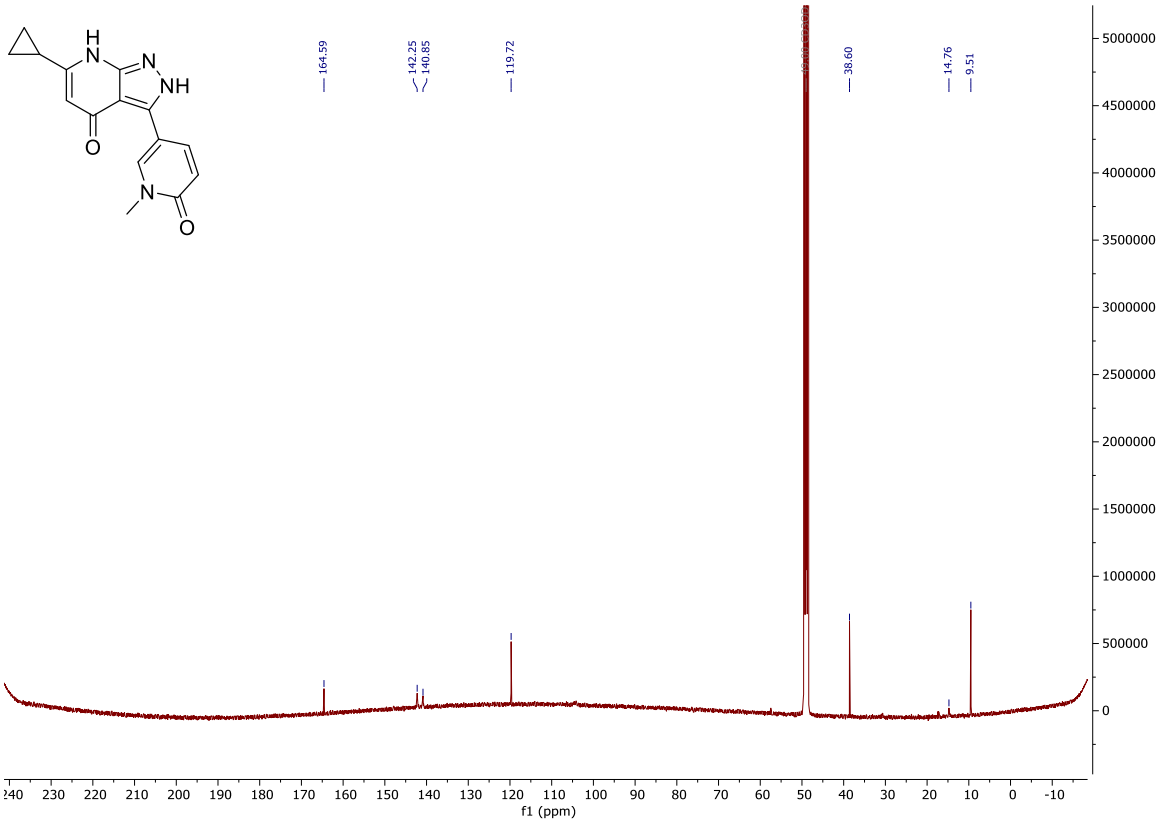
6-cyclopropyl-3-(1-methyl-6-oxo-1,6-dihydropyridin-3-yl)-2,7-dihydro-4H-pyrazolo[3,4-b]pyridin-4-one (26)

^{1}H NMR (500 MHz, MeOD)



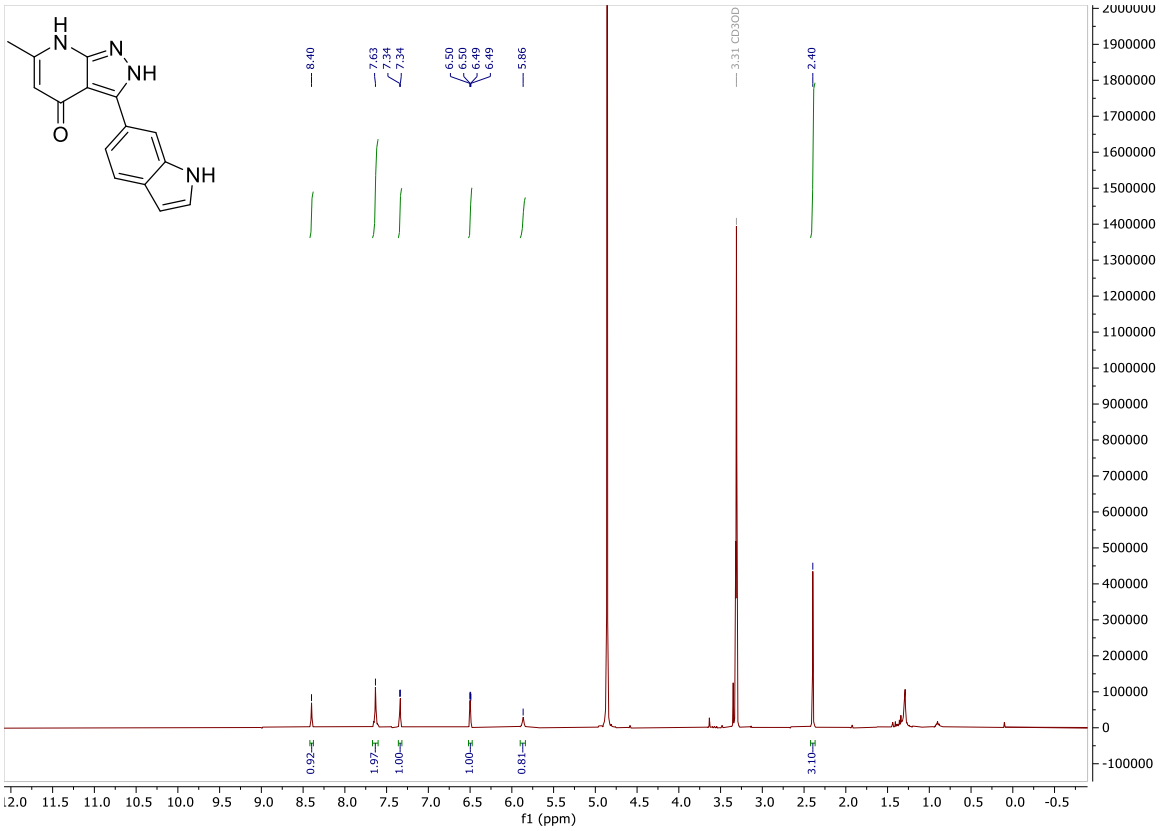
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^{13}C NMR (126 MHz, MeOD)



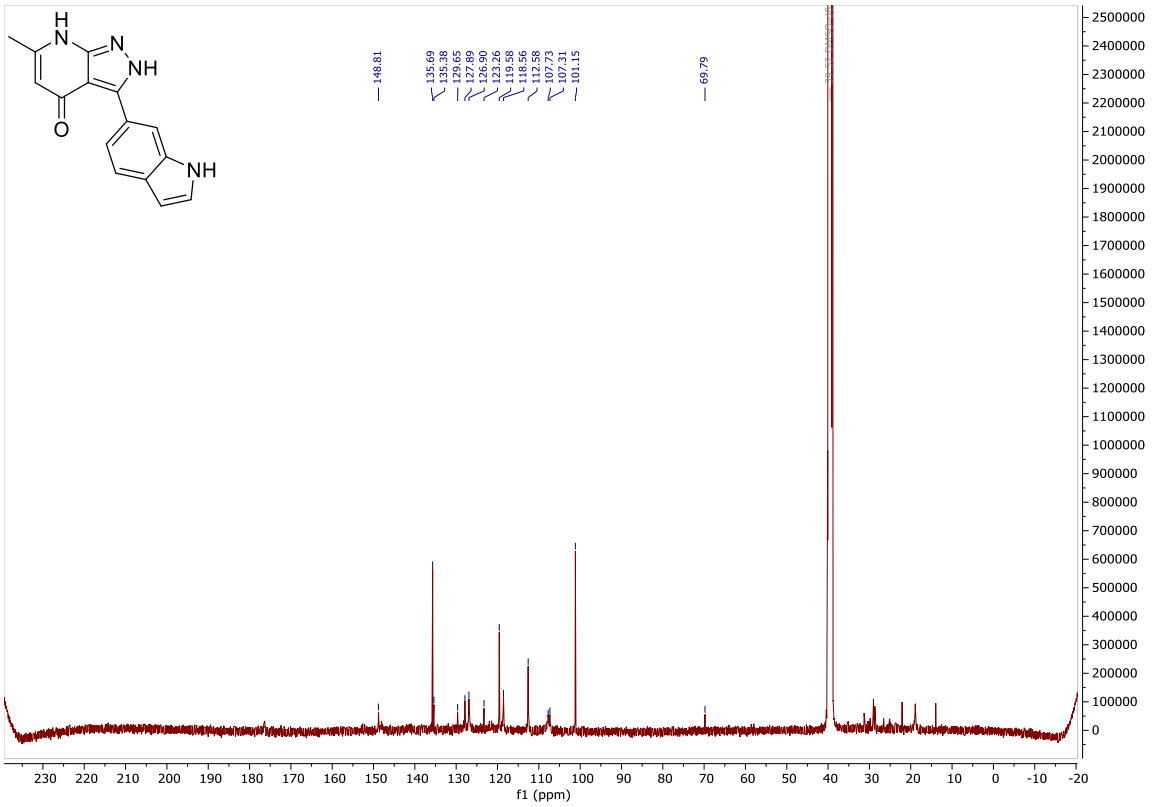
3-(1H-indol-6-yl)-6-methyl-2,7-dihydro-4H-pyrazolo[3,4-b]pyridin-4-one (27)

^{1}H NMR (400 MHz, MeOD)



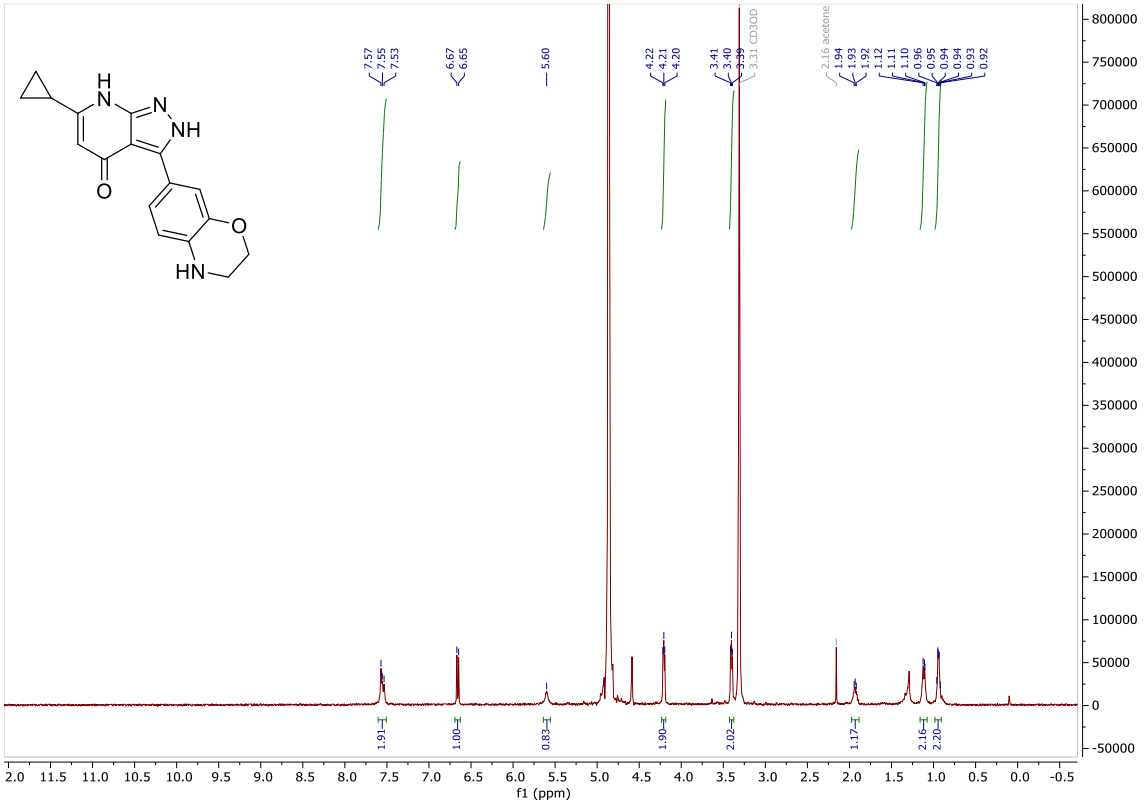
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^{13}C NMR (126 MHz, DMSO)



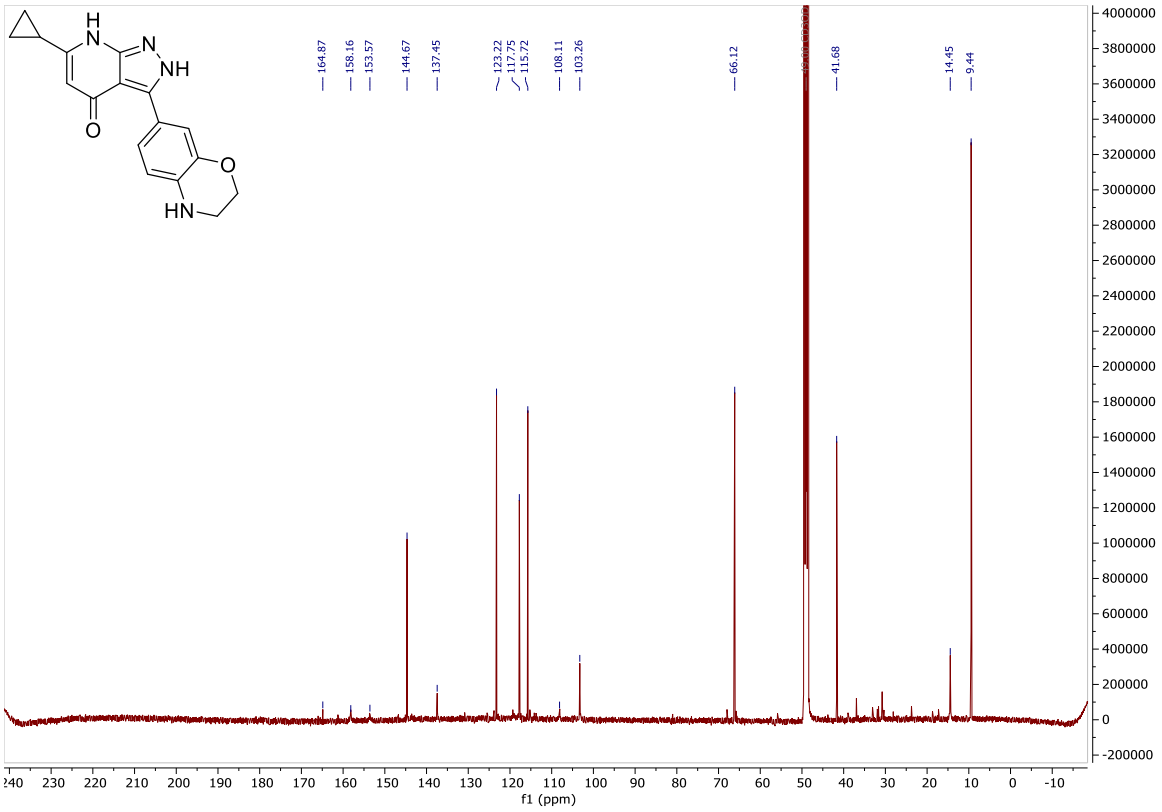
6-cyclopropyl-3-(3,4-dihydro-2H-benzo[b][1,4]oxazin-7-yl)-2,7-dihydro-4H-pyrazolo[3,4-b]pyridin-4-one (28)

^{1}H NMR (400 MHz, MeOD)



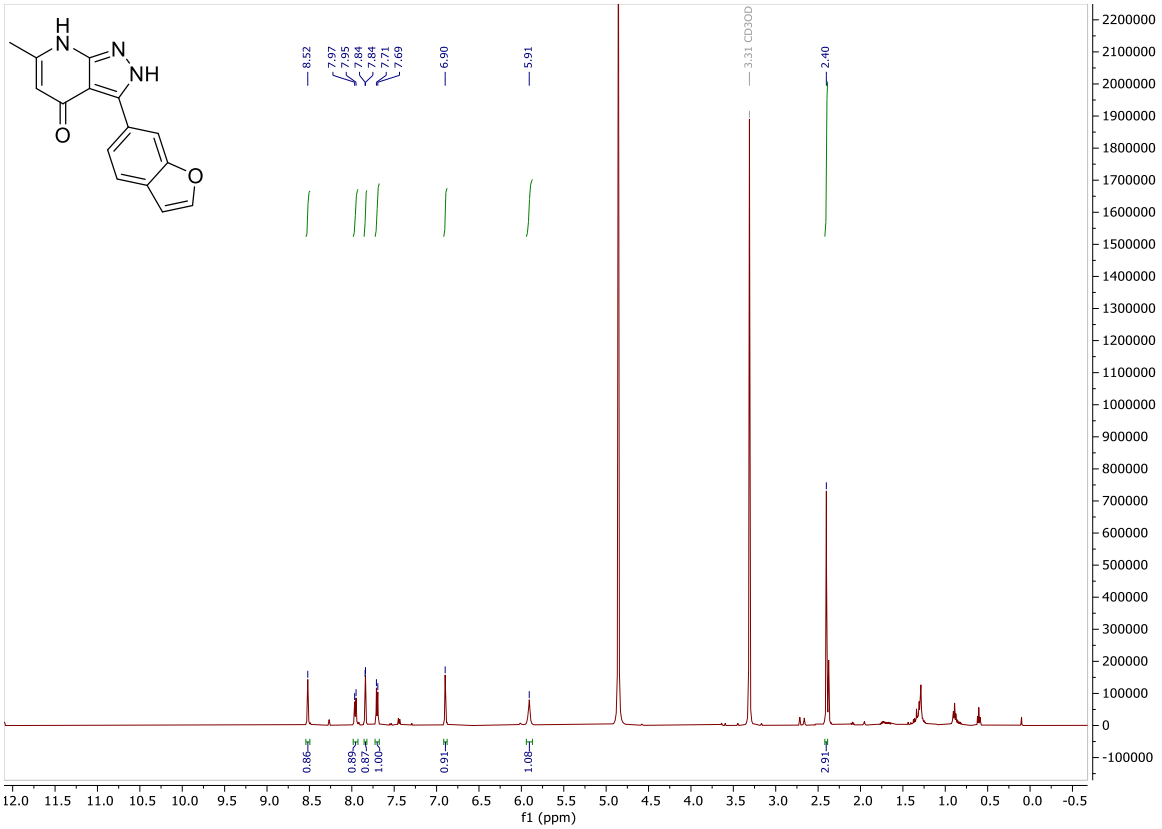
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^{13}C NMR (126 MHz, MeOD)



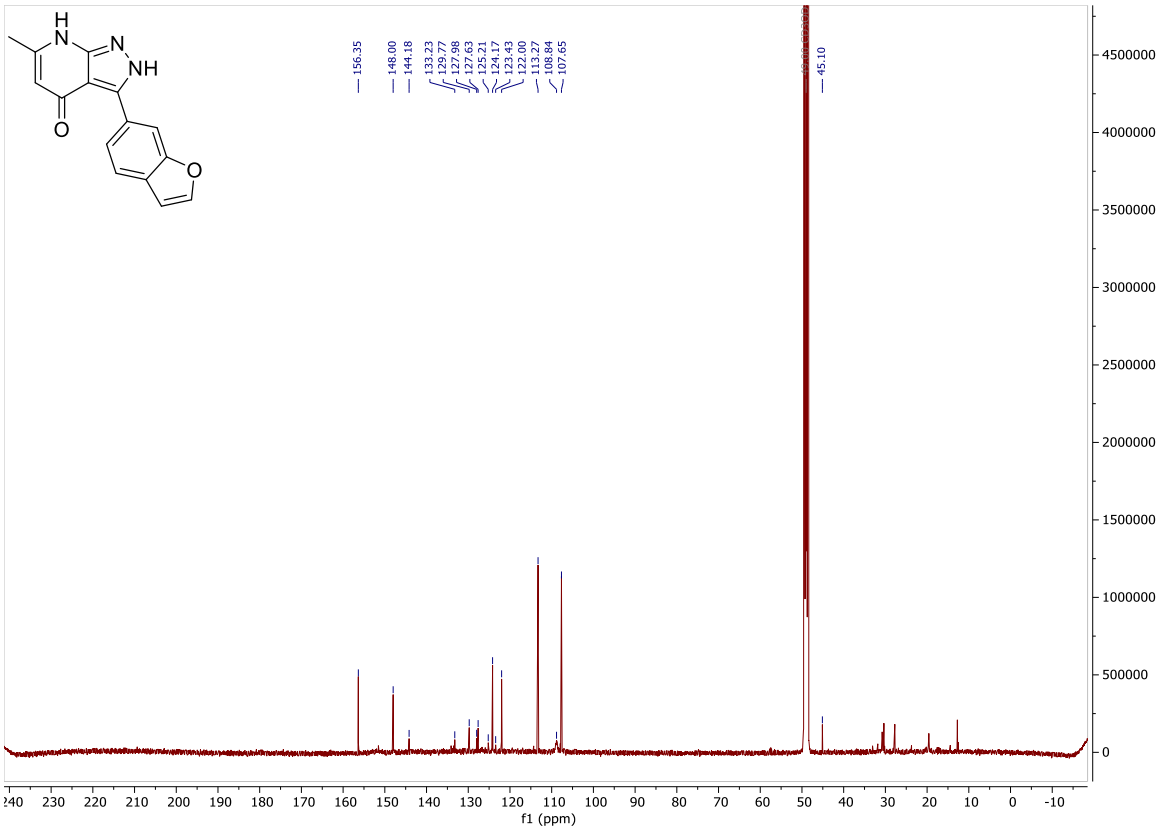
3-(benzofuran-6-yl)-6-methyl-2,7-dihydro-4H-pyrazolo[3,4-b]pyridin-4-one (29)

^{1}H NMR (500 MHz, MeOD)



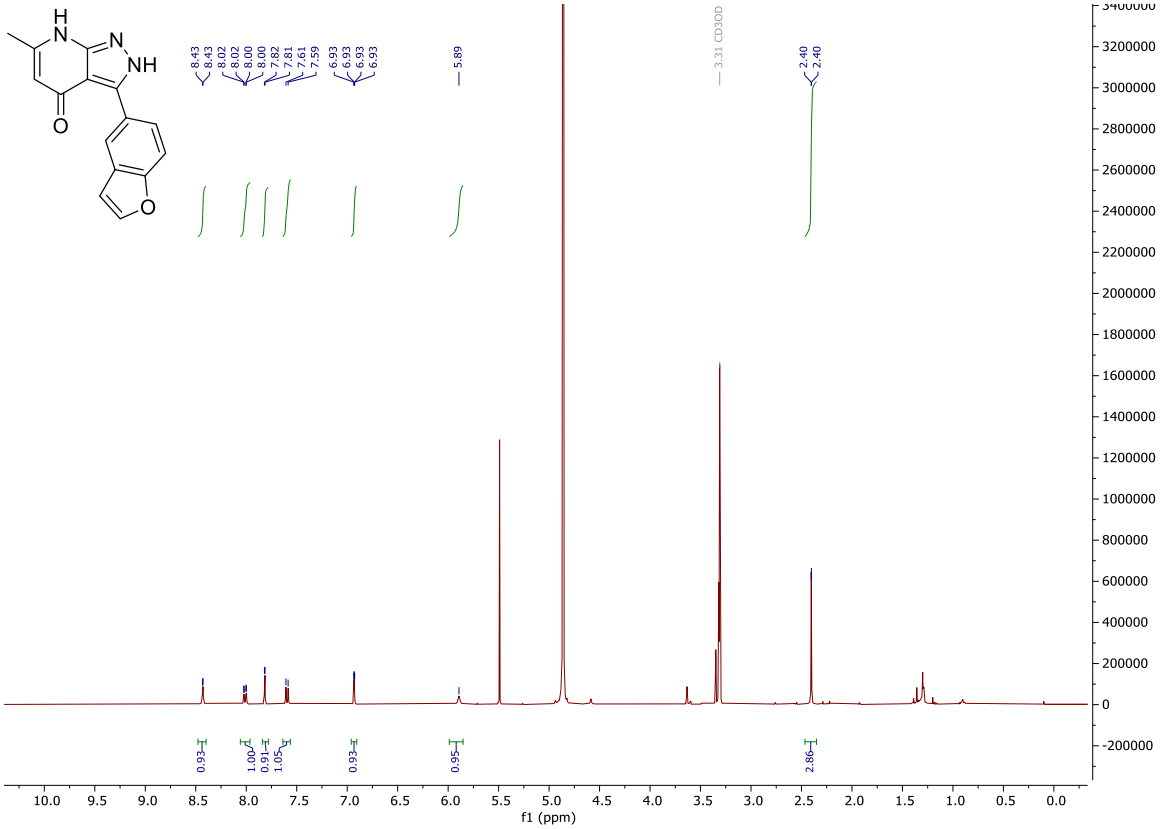
Downloaded from pubs.acs.org/jmcmar/article-supplement/5462685/pdf/jmdc02977_si_002/ by guest on 06 November 2026

^{13}C NMR (126 MHz, MeOD)



3-(benzofuran-5-yl)-6-methyl-2,7-dihydro-4H-pyrazolo[3,4-b]pyridin-4-one (30)

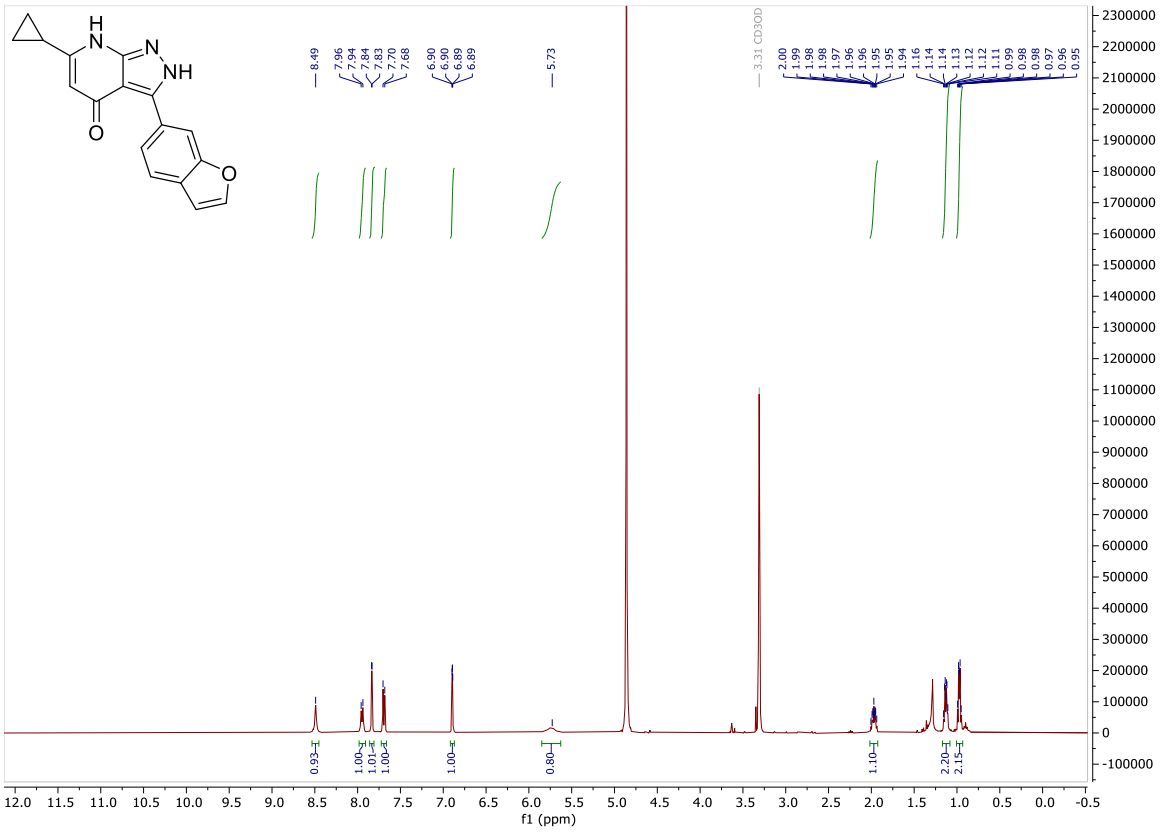
^{1}H NMR (400 MHz, MeOD)



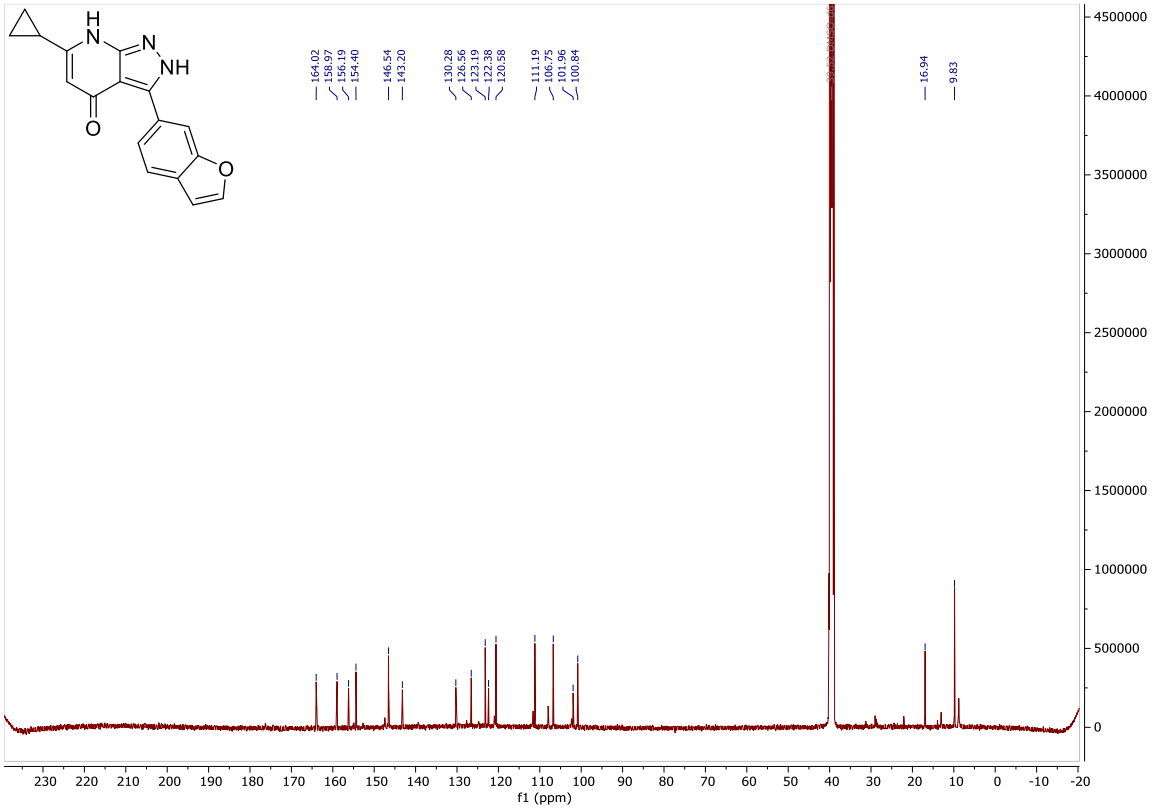
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3-(benzofuran-6-yl)-6-cyclopropyl-2,7-dihydro-4H-pyrazolo[3,4-b]pyridin-4-one (31)

^{1}H NMR (400 MHz, MeOD)



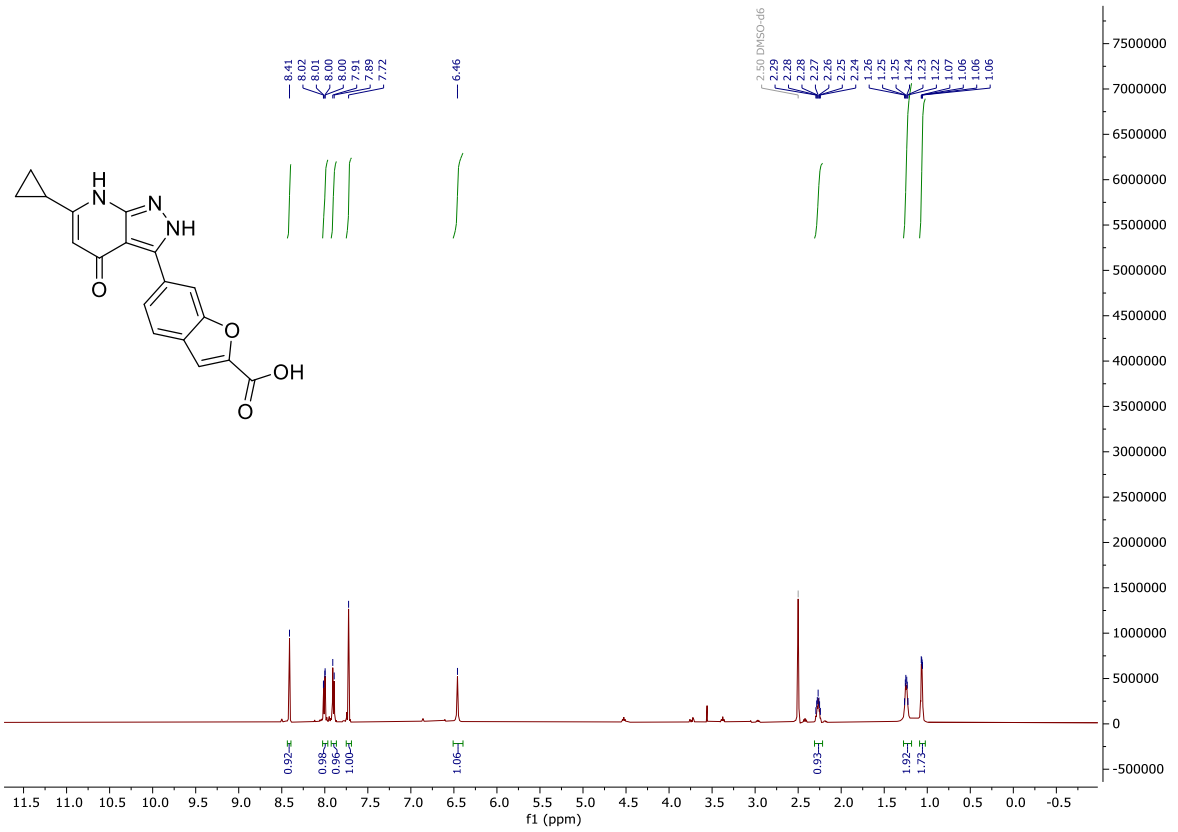
^{13}C NMR (126 MHz, DMSO)



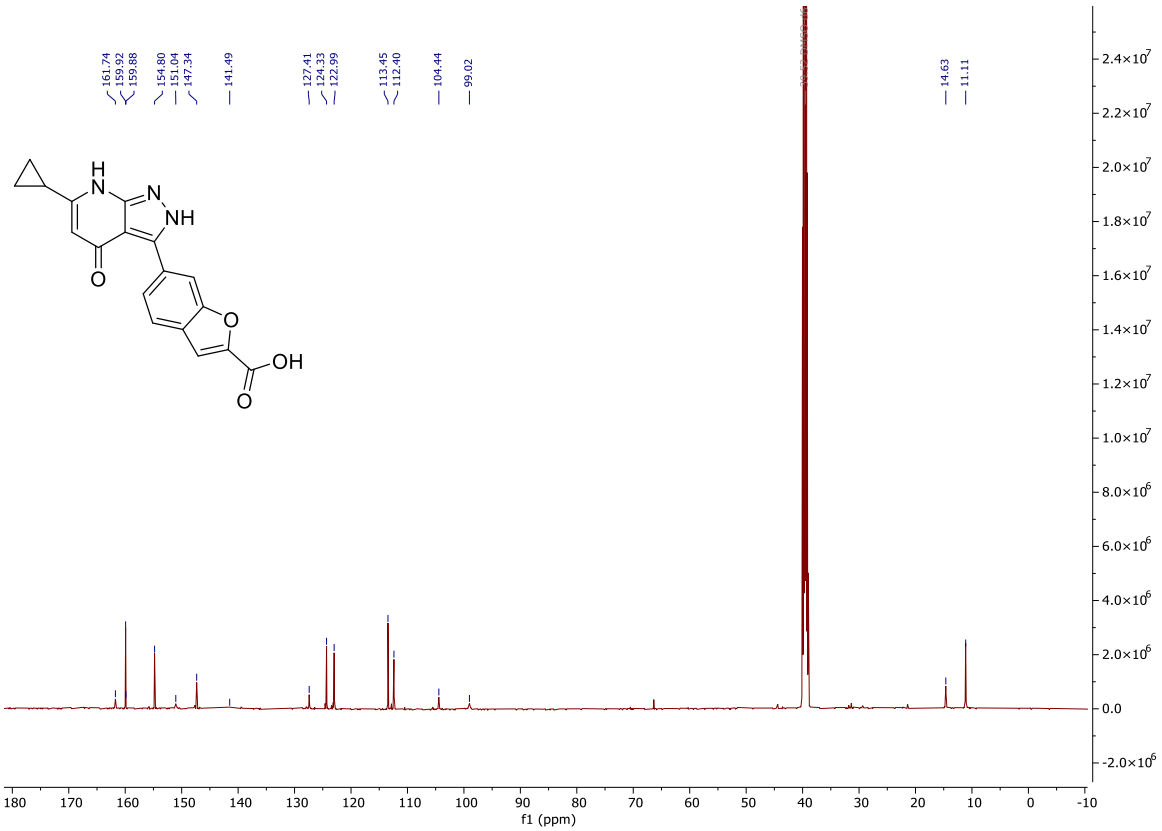
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6-(6-cyclopropyl-4-oxo-4,7-dihydro-2H-pyrazolo[3,4-b]pyridin-3-yl)benzofuran-2-carboxylic acid (32)

^{1}H NMR (500 MHz, DMSO)



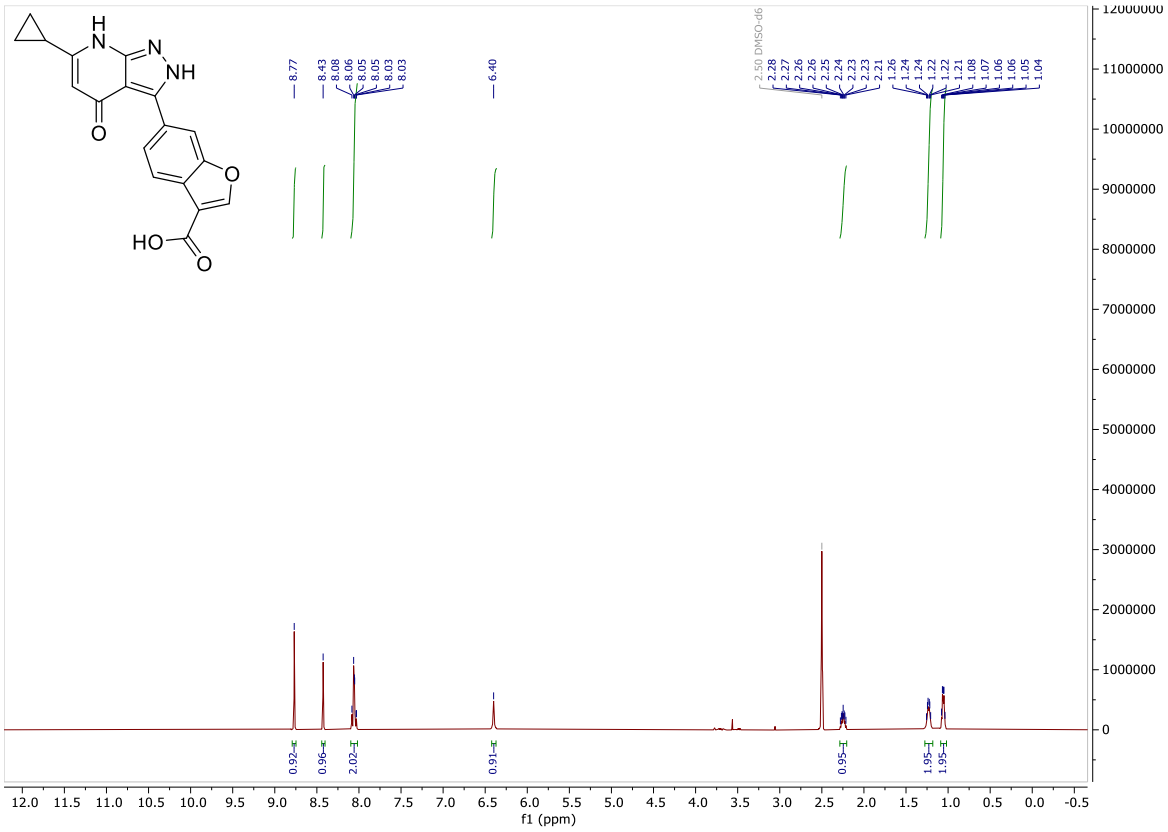
^{13}C NMR (126 MHz, DMSO)



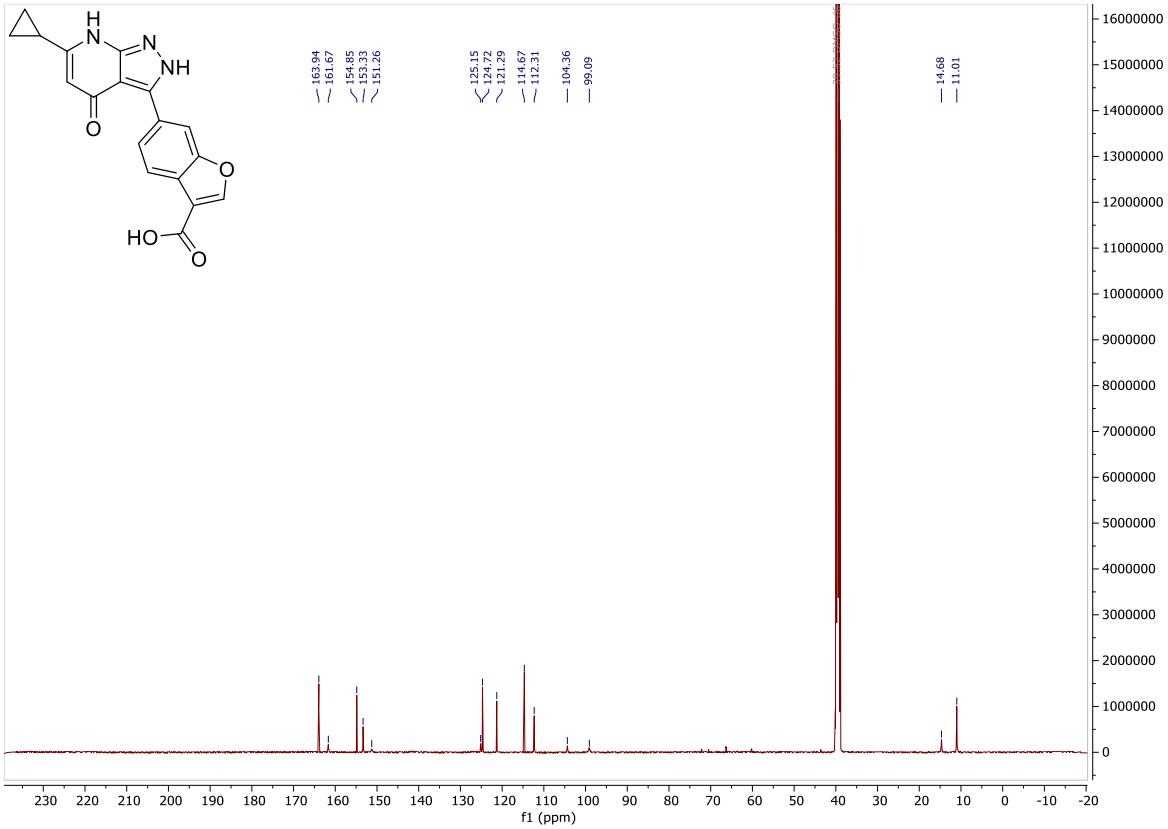
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6-(6-cyclopropyl-4-oxo-4,7-dihydro-2H-pyrazolo[3,4-b]pyridin-3-yl)benzofuran-3-carboxylic acid (33)

^{1}H NMR (400 MHz, DMSO)



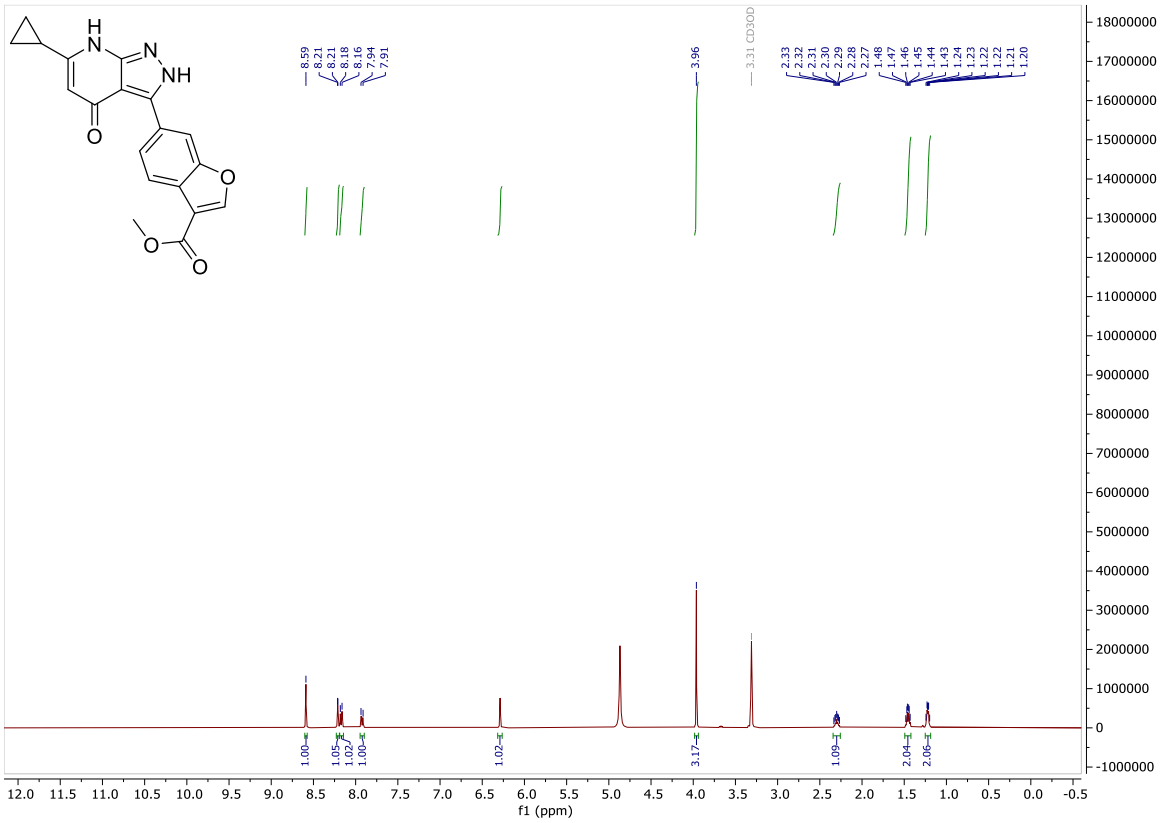
^{13}C NMR (126 MHz, DMSO)



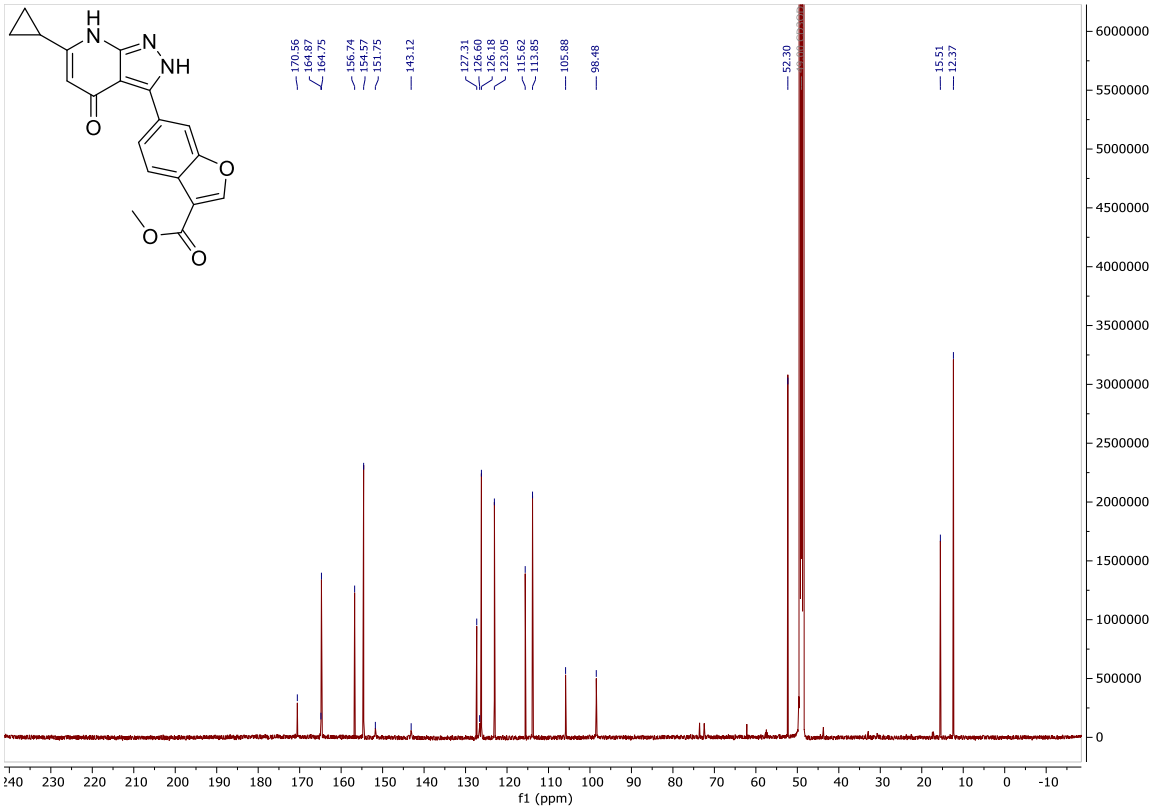
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Methyl 6-(6-cyclopropyl-4-oxo-4,7-dihydro-2H-pyrazolo[3,4-b]pyridin-3-yl)benzofuran-3-carboxylate (34)

^{1}H NMR (400 MHz, MeOD)



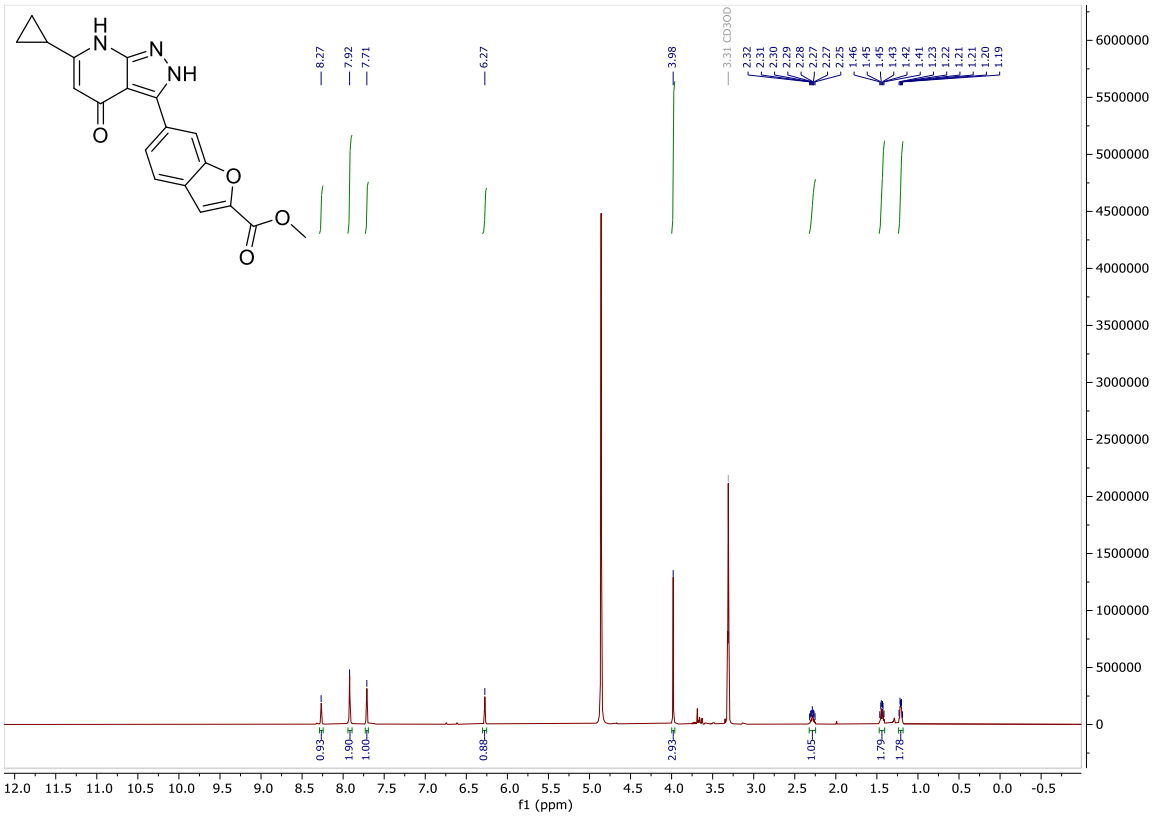
^{13}C NMR (126 MHz, MeOD)



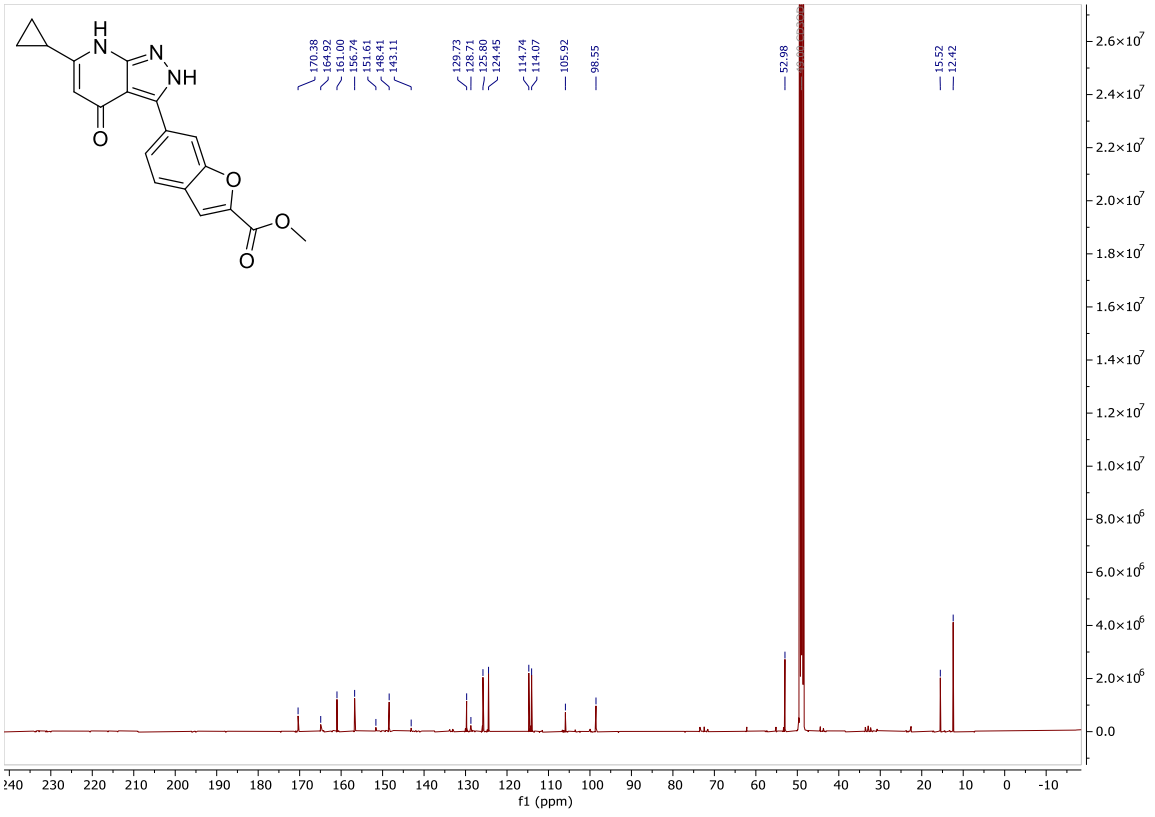
Downloaded from pubs.acs.org/jmcmar/article-supplement/5462685/pdf/jmdc02977_si_002/ by guest on 06 November 2026

Methyl 6-(6-cyclopropyl-4-oxo-4,7-dihydro-2H-pyrazolo[3,4-b]pyridin-3-yl)benzofuran-2-carboxylate (35)

^{1}H NMR (400 MHz, MeOD)



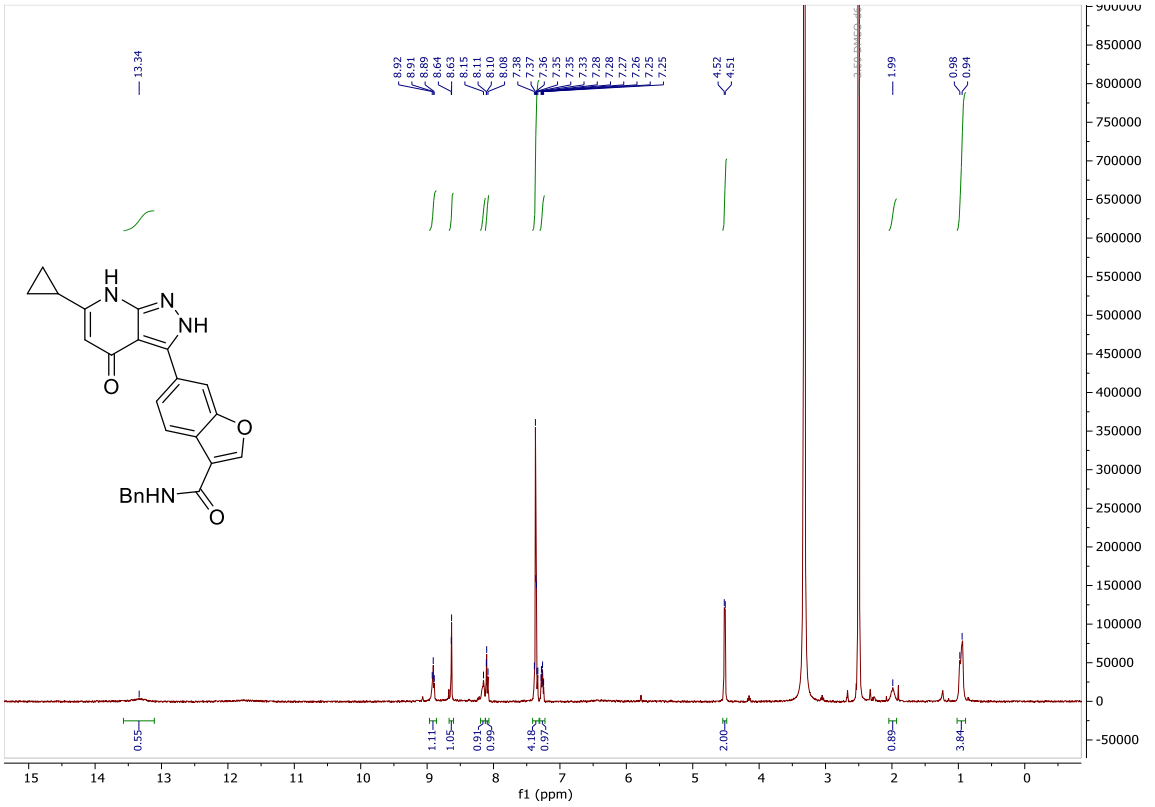
^{13}C NMR (126 MHz, MeOD)



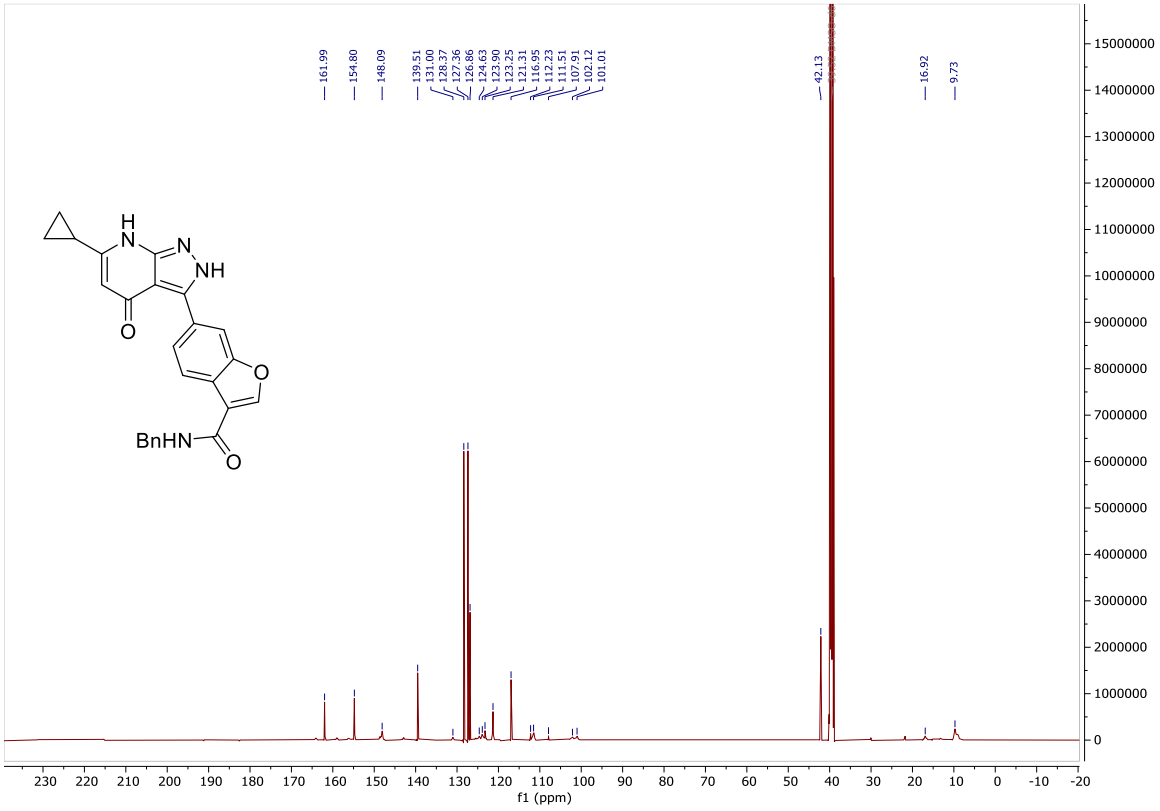
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N-benzyl-6-(6-cyclopropyl-4-oxo-4,7-dihydro-2H-pyrazolo[3,4-b]pyridin-3-yl)benzofuran-3-carboxamide (36)

^{1}H NMR (400 MHz, DMSO)



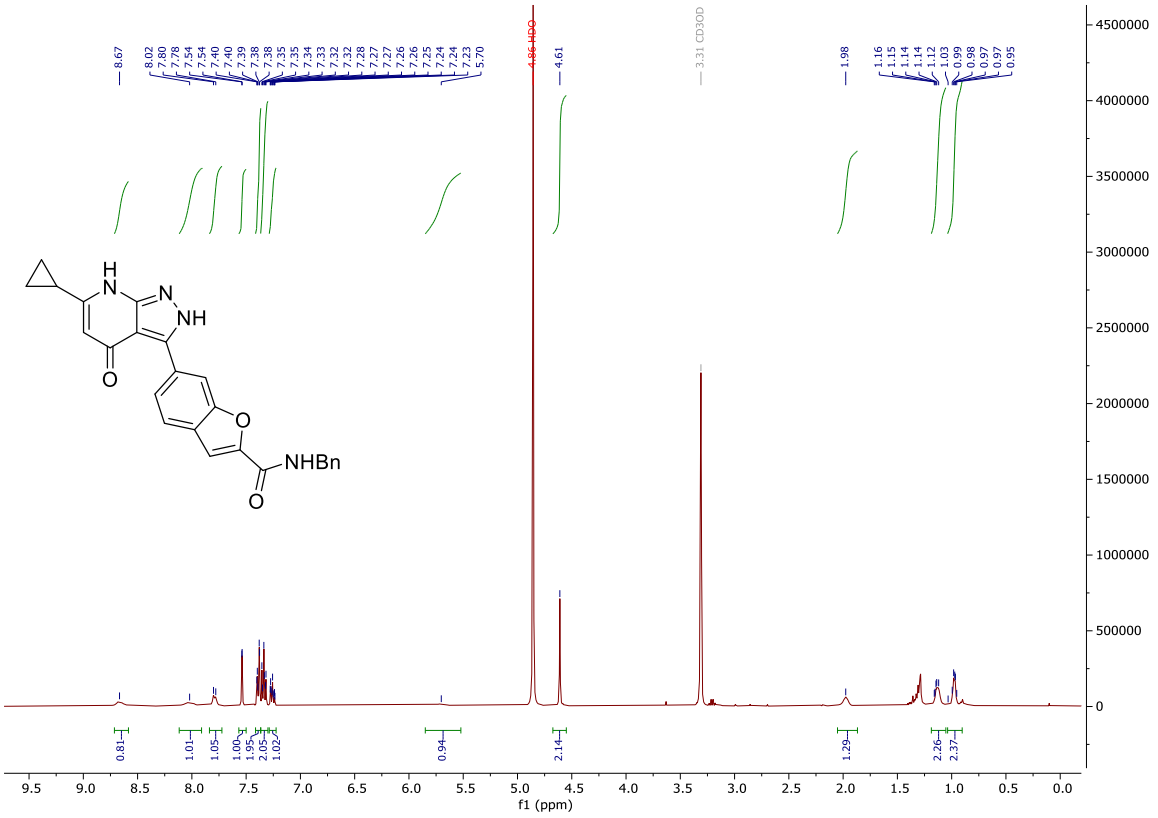
^{13}C NMR (126 MHz, DMSO)



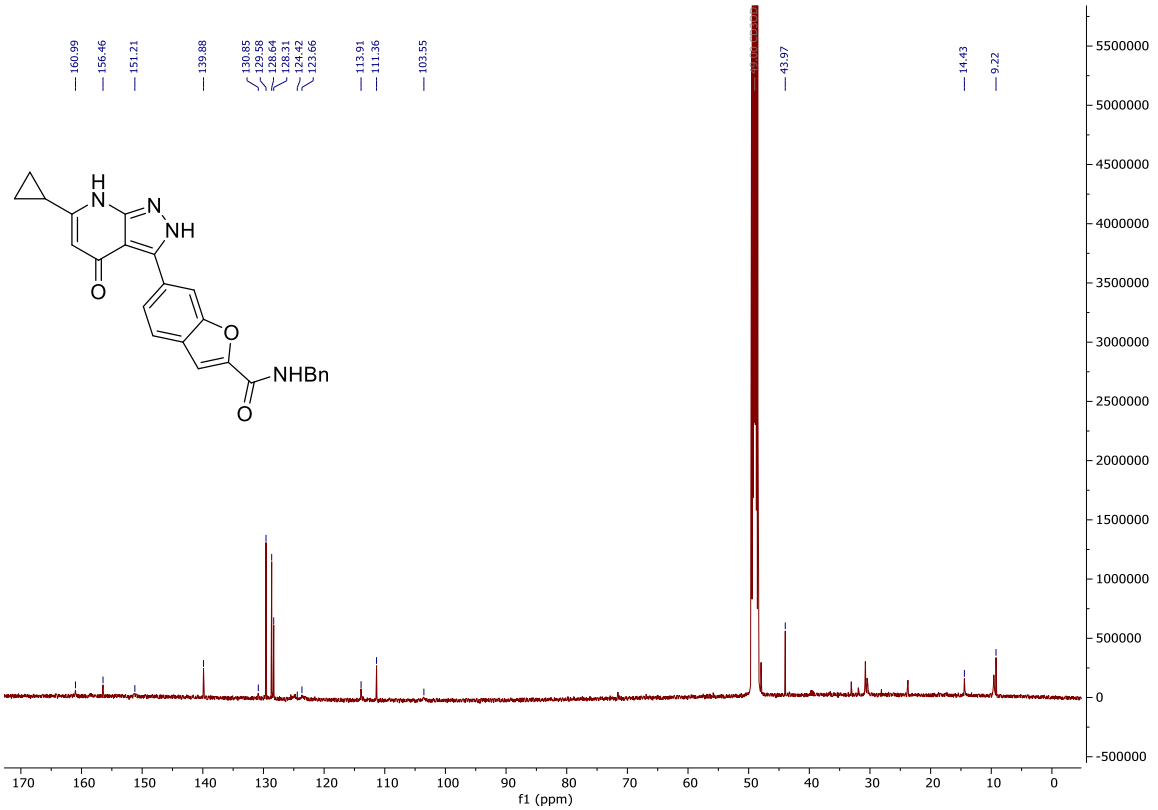
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N-benzyl-6-(6-cyclopropyl-4-oxo-4,7-dihydro-2H-pyrazolo[3,4-b]pyridin-3-yl)benzofuran-2-carboxamide (37)

^{1}H NMR (400 MHz, MeOD)



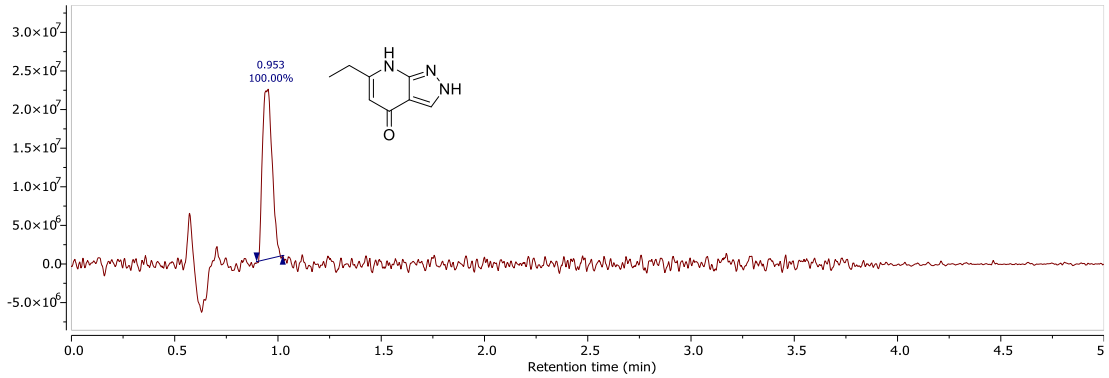
^{13}C NMR (126 MHz, MeOD)



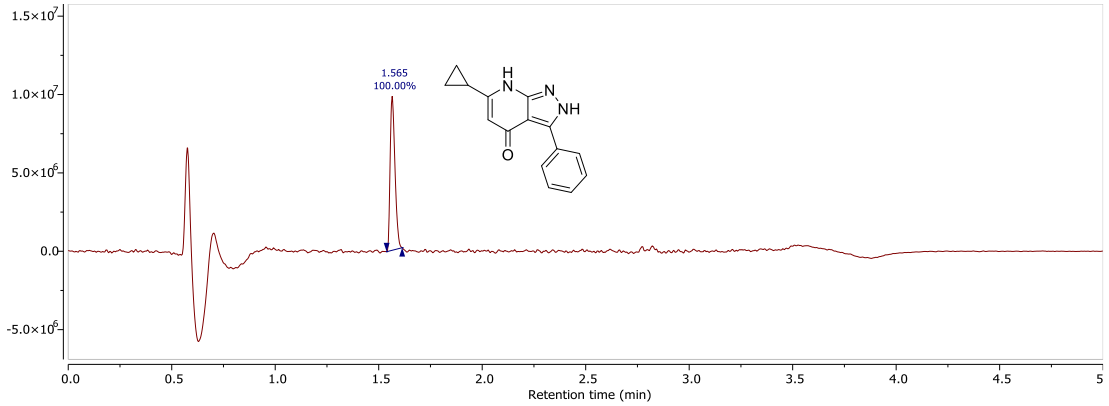
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HPLC traces of compounds 4, 6-15, 17-37, 31b and 55

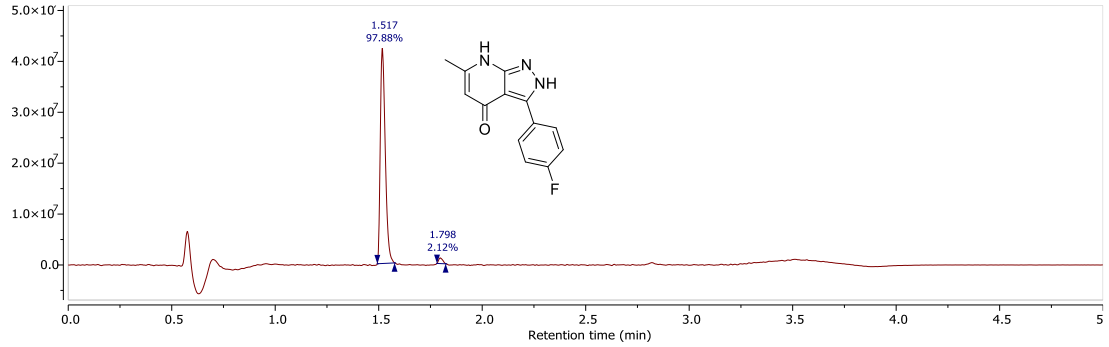
6-ethyl-2,7-dihydro-4H-pyrazolo[3,4-b]pyridin-4-one (4)



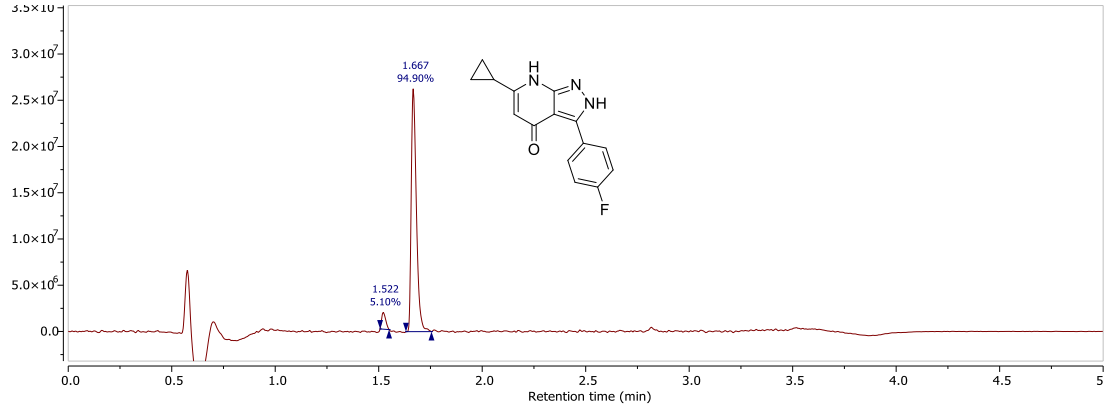
6-cyclopropyl-3-phenyl-2,7-dihydro-4H-pyrazolo[3,4-b]pyridin-4-one (6)



3-(4-fluorophenyl)-6-methyl-2,7-dihydro-4H-pyrazolo[3,4-b]pyridin-4-one (7)

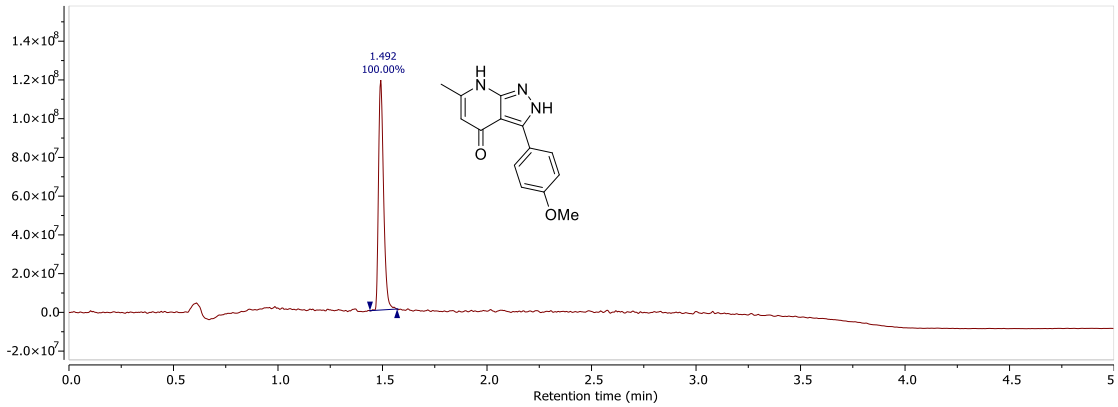


6-cyclopropyl-3-(4-fluorophenyl)-2,7-dihydro-4H-pyrazolo[3,4-b]pyridin-4-one (8)

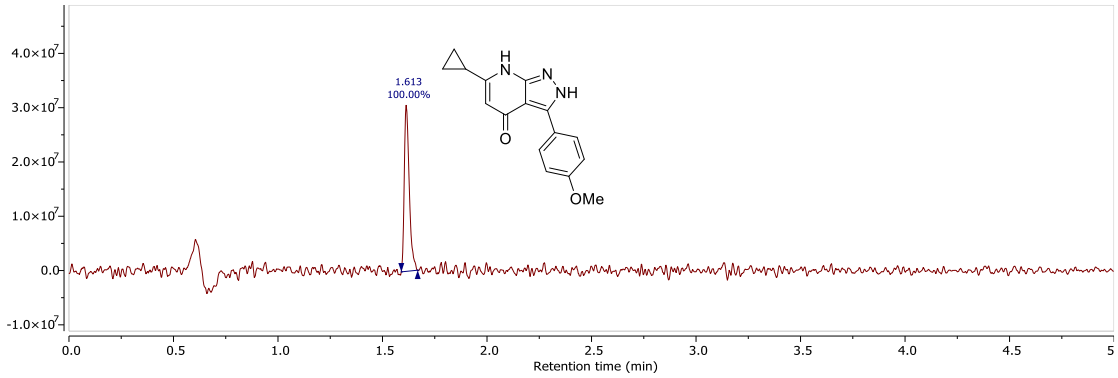


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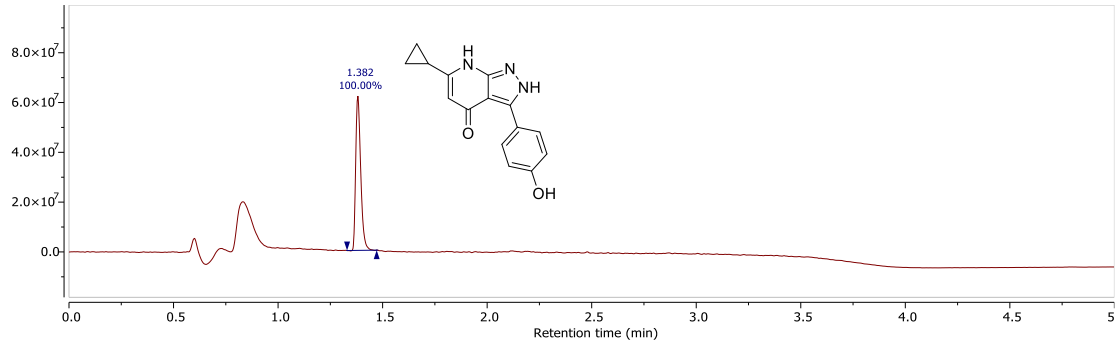
3-(4-methoxyphenyl)-6-methyl-2,7-dihydro-4H-pyrazolo[3,4-b]pyridin-4-one (9)



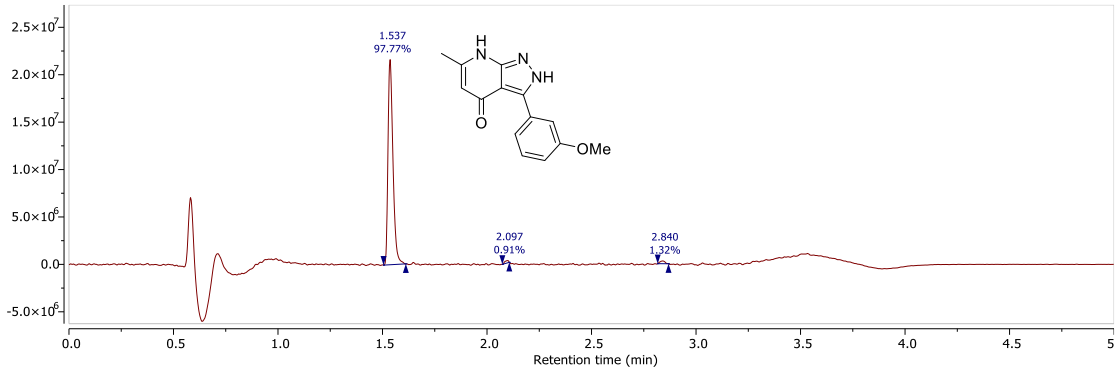
6-cyclopropyl-3-(4-methoxyphenyl)-2,7-dihydro-4H-pyrazolo[3,4-b]pyridin-4-one (10)



6-cyclopropyl-3-(4-hydroxyphenyl)-2,7-dihydro-4H-pyrazolo[3,4-b]pyridin-4-one (11)

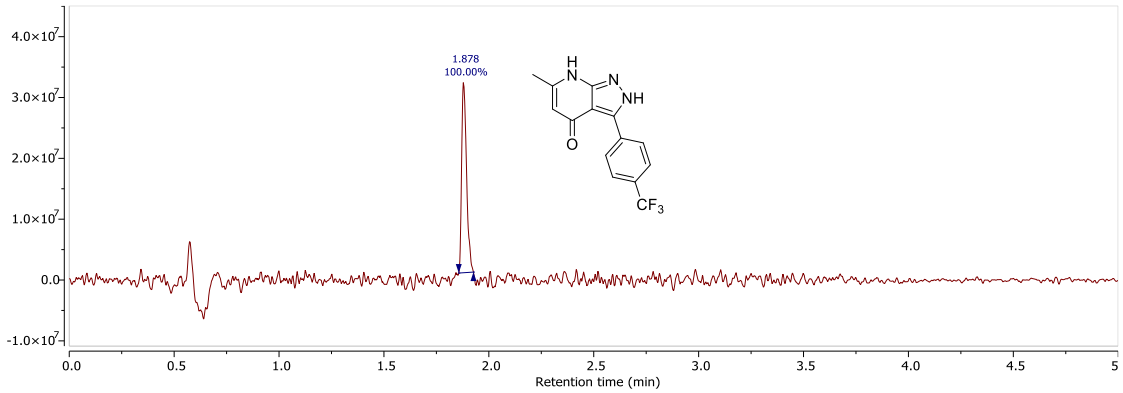


3-(3-methoxyphenyl)-6-methyl-2,7-dihydro-4H-pyrazolo[3,4-b]pyridin-4-one (12)

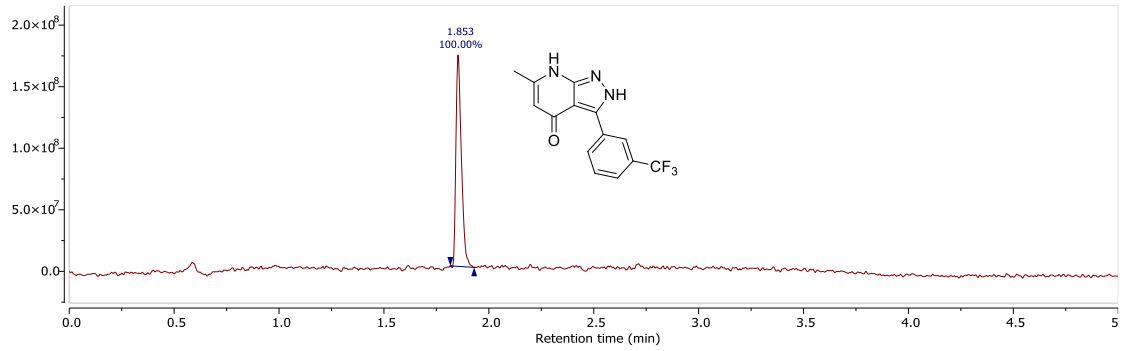


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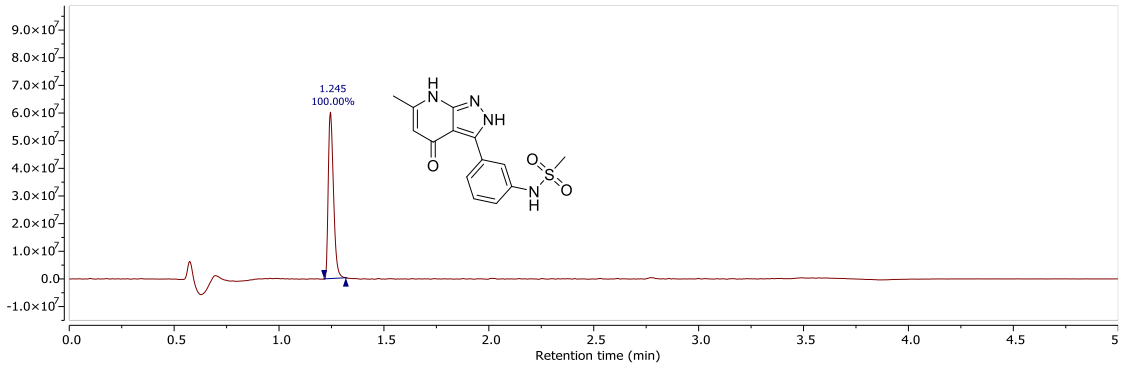
6-methyl-3-(4-(trifluoromethyl)phenyl)-2,7-dihydro-4H-pyrazolo[3,4-b]pyridin-4-one (13)



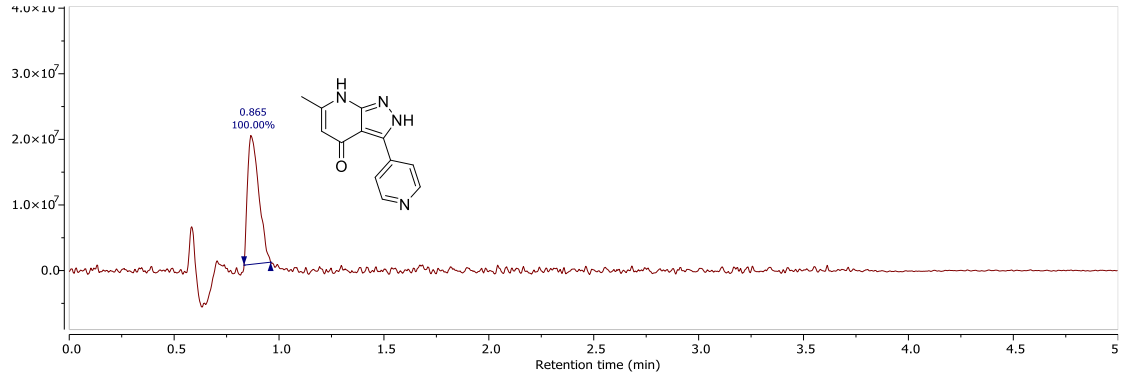
6-methyl-3-(3-(trifluoromethyl)phenyl)-2,7-dihydro-4H-pyrazolo[3,4-b]pyridin-4-one (14)



N-(3-(6-methyl-4-oxo-4,7-dihydro-2H-pyrazolo[3,4-b]pyridin-3-yl)phenyl)methanesulfonamide (15)

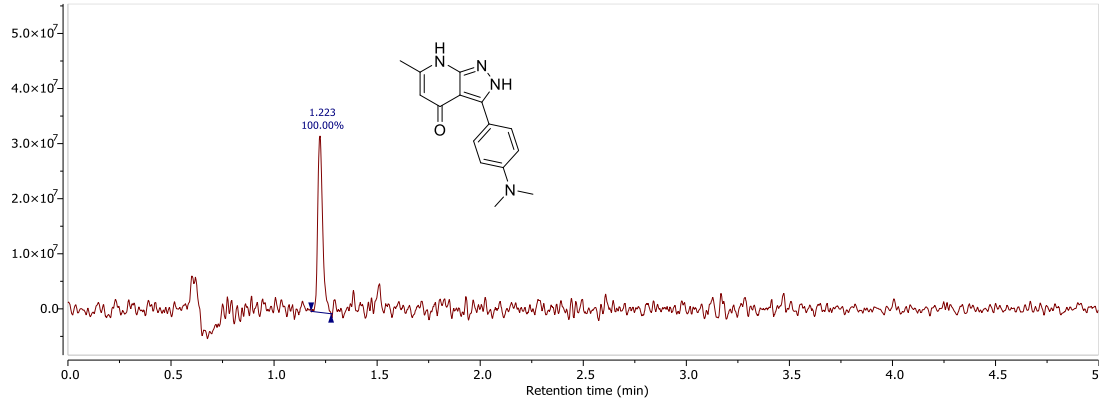


6-methyl-3-(pyridin-4-yl)-2,7-dihydro-4H-pyrazolo[3,4-b]pyridin-4-one (17)

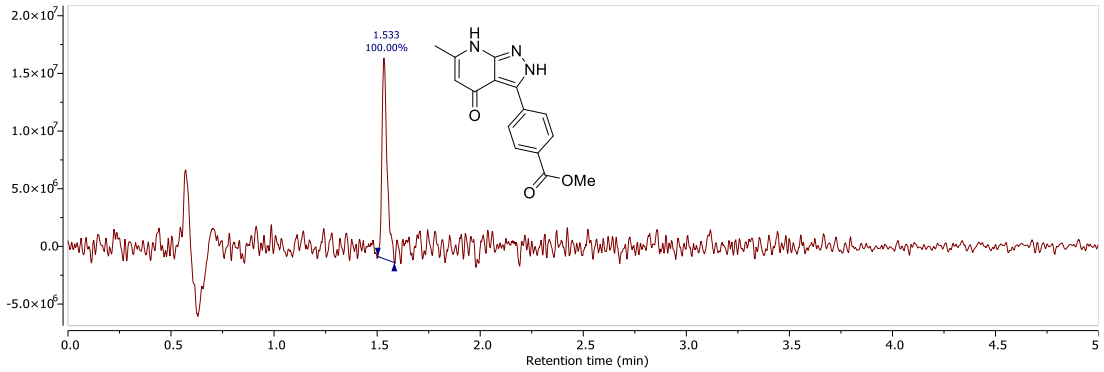


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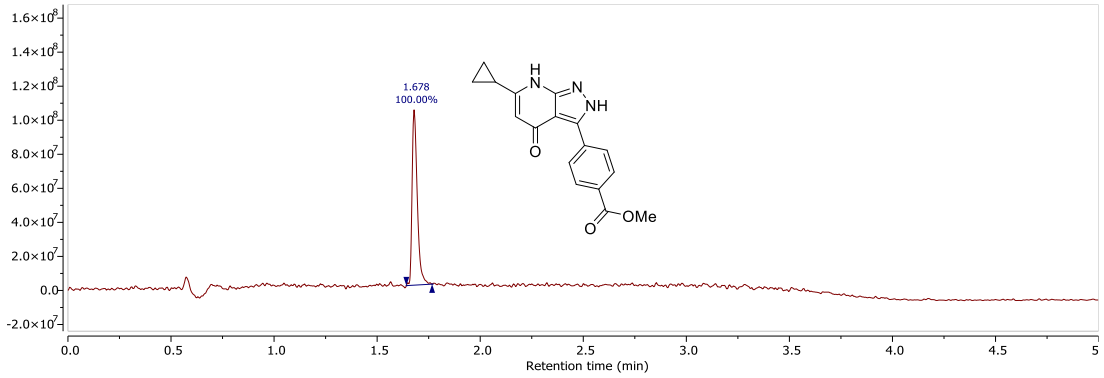
3-(4-(dimethylamino)phenyl)-6-methyl-2,7-dihydro-4H-pyrazolo[3,4-b]pyridin-4-one (18)



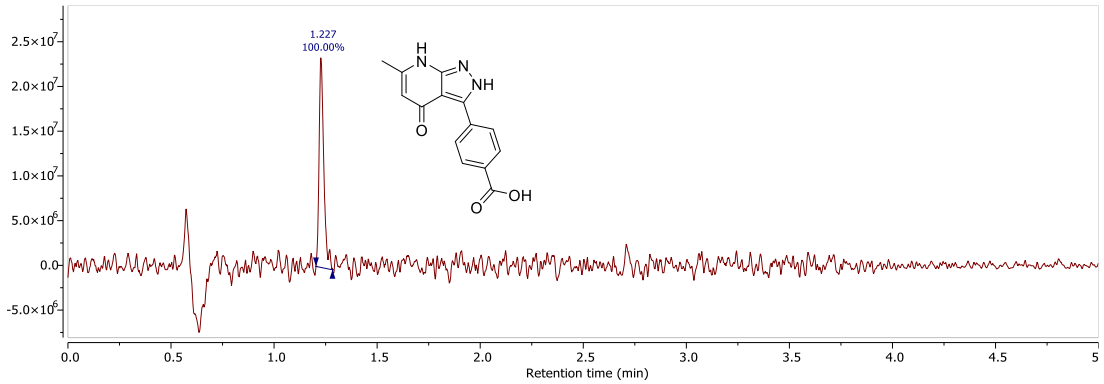
methyl 4-(6-methyl-4-oxo-4,7-dihydro-2H-pyrazolo[3,4-b]pyridin-3-yl)benzoate (19)



methyl 4-(6-cyclopropyl-4-oxo-4,7-dihydro-2H-pyrazolo[3,4-b]pyridin-3-yl)benzoate (20)

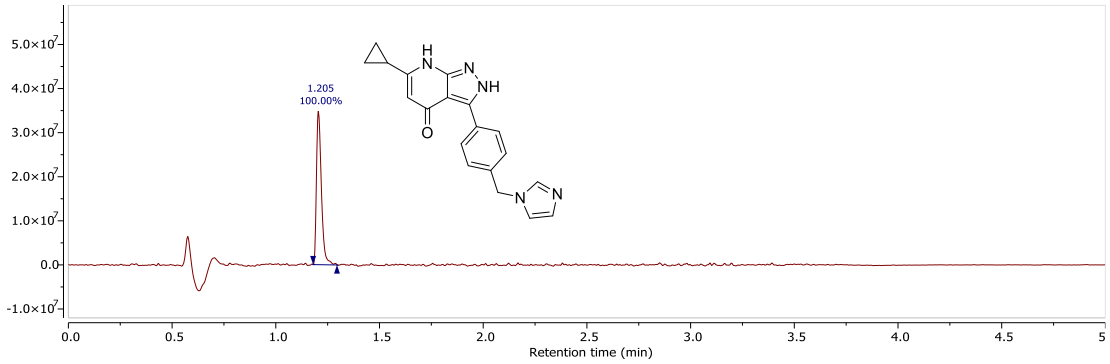


4-(6-methyl-4-oxo-4,7-dihydro-2H-pyrazolo[3,4-b]pyridin-3-yl)benzoic acid (21)

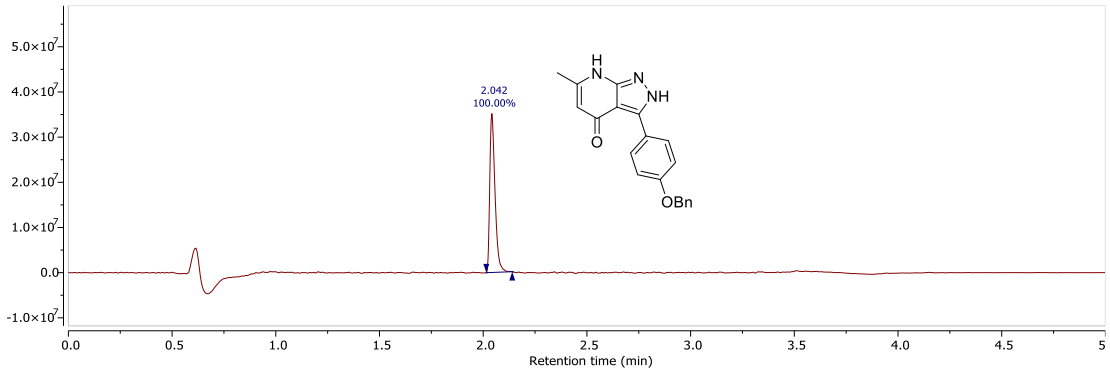


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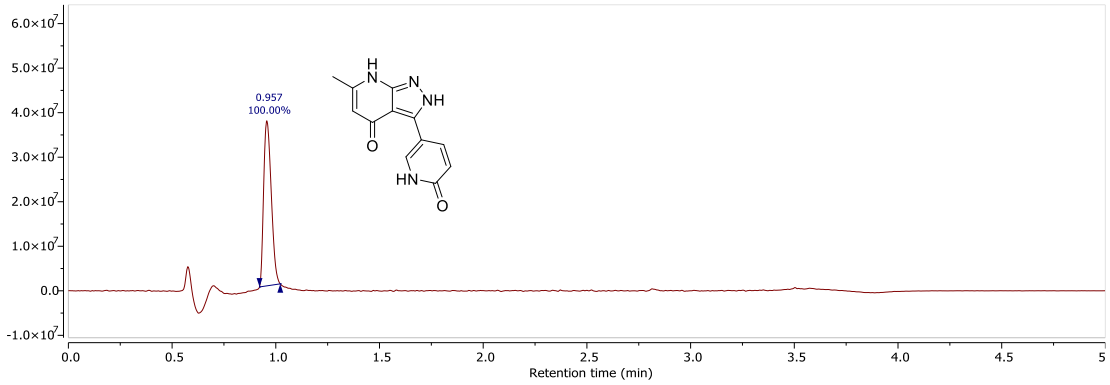
3-(3-((1H-imidazol-1-yl)methyl)phenyl)-6-cyclopropyl-2,7-dihydro-4H-pyrazolo[3,4-b]pyridin-4-one (22)



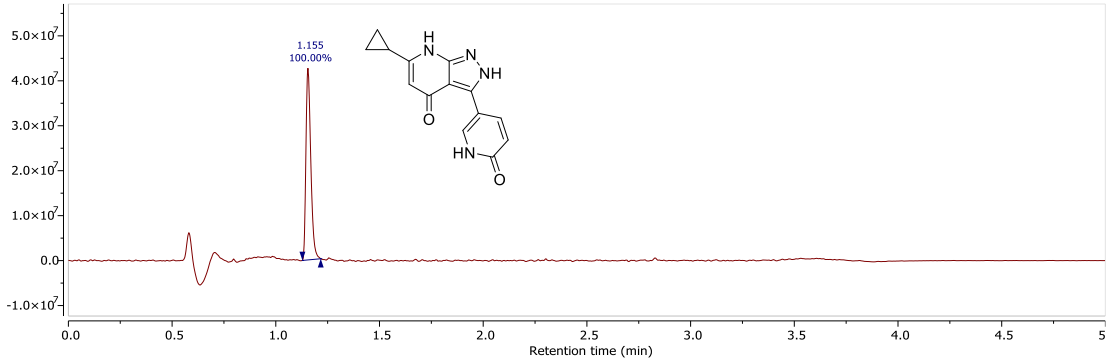
3-(4-(benzyloxy)phenyl)-6-methyl-2,7-dihydro-4H-pyrazolo[3,4-b]pyridin-4-one (23)



6-methyl-3-(6-oxo-1,6-dihydropyridin-3-yl)-2,7-dihydro-4H-pyrazolo[3,4-b]pyridin-4-one (24)

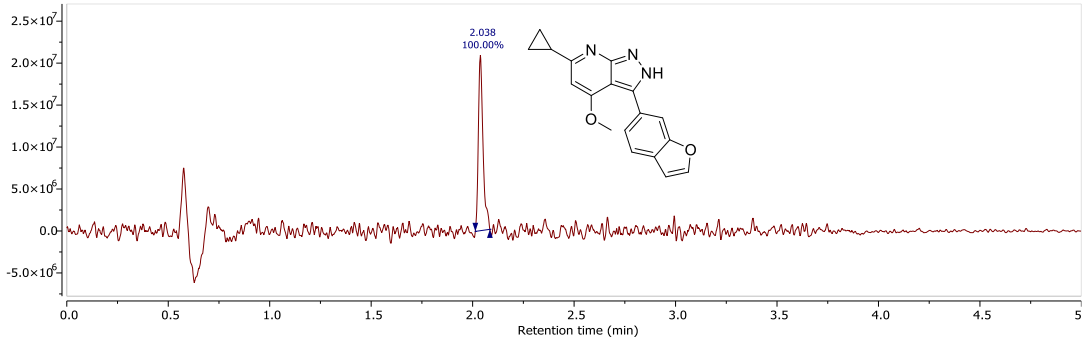


6-cyclopropyl-3-(6-oxo-1,6-dihydropyridin-3-yl)-2,7-dihydro-4H-pyrazolo[3,4-b]pyridin-4-one (25)

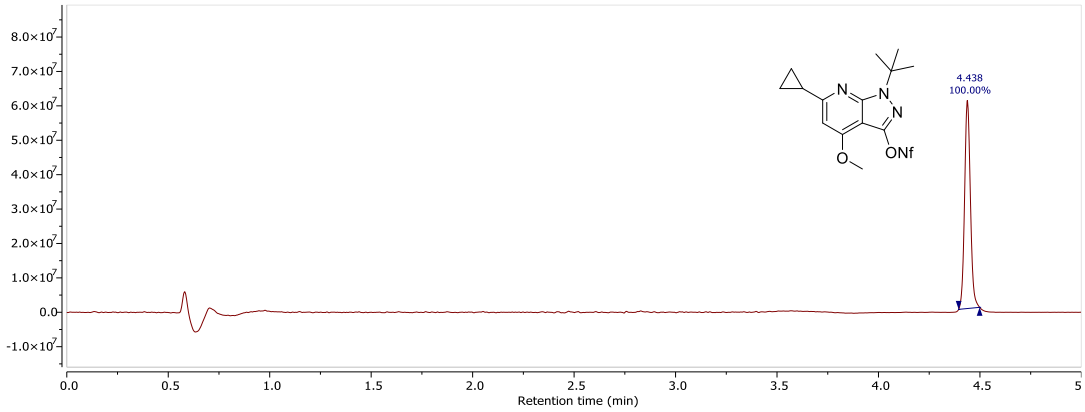


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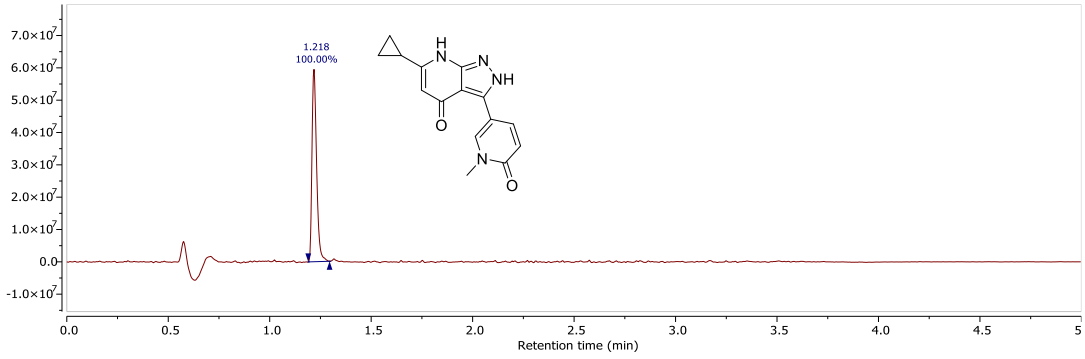
3-(benzofuran-6-yl)-6-cyclopropyl-4-methoxy-2H-pyrazolo[3,4-b]pyridine (31b)



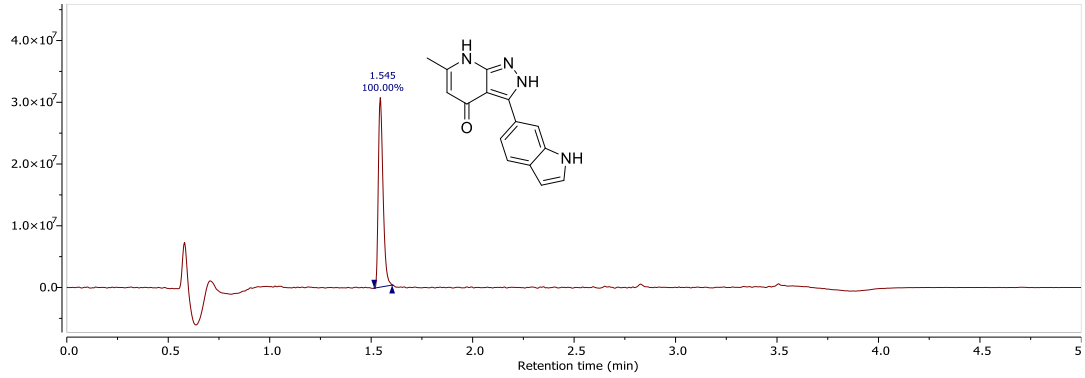
1-(tert-butyl)-6-cyclopropyl-4-methoxy-1H-pyrazolo[3,4-b]pyridin-3-yl 1,1,2,2,3,3,4,4,4-nonafluorobutane-1-sulfonate (55)



6-cyclopropyl-3-(1-methyl-6-oxo-1,6-dihydropyridin-3-yl)-2,7-dihydro-4H-pyrazolo[3,4-b]pyridin-4-one (26)

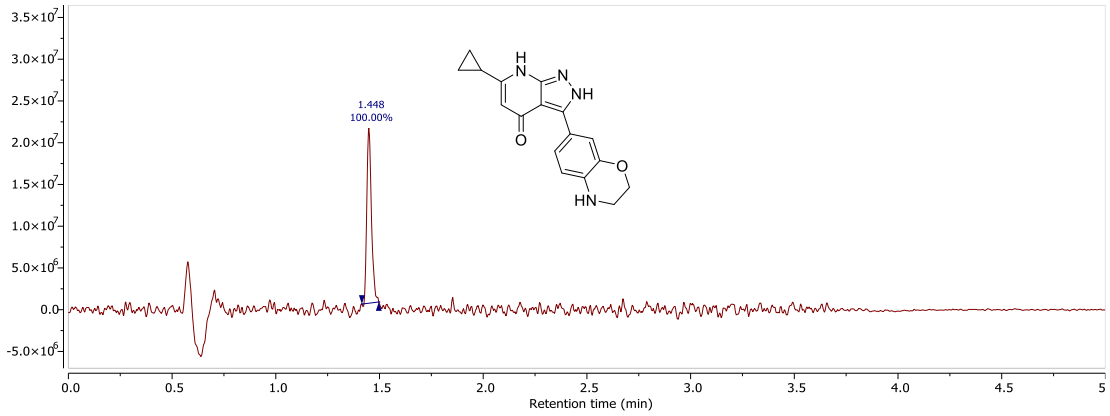


3-(1H-indol-6-yl)-6-methyl-2,7-dihydro-4H-pyrazolo[3,4-b]pyridin-4-one (27)

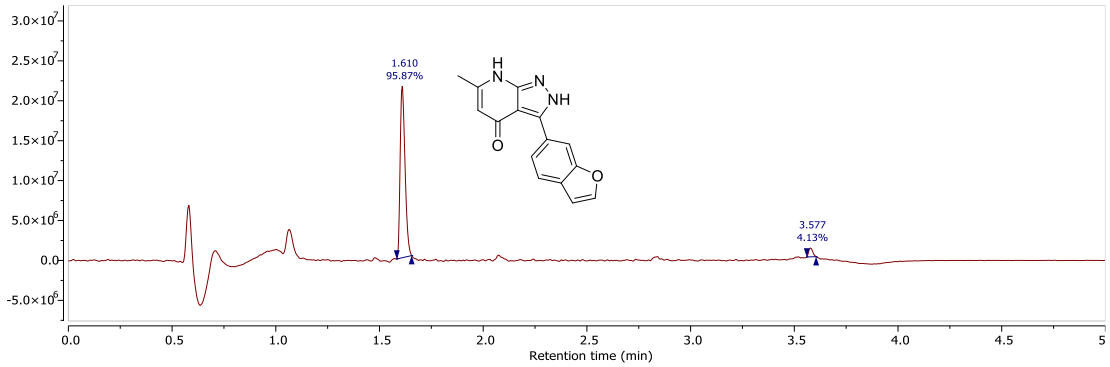


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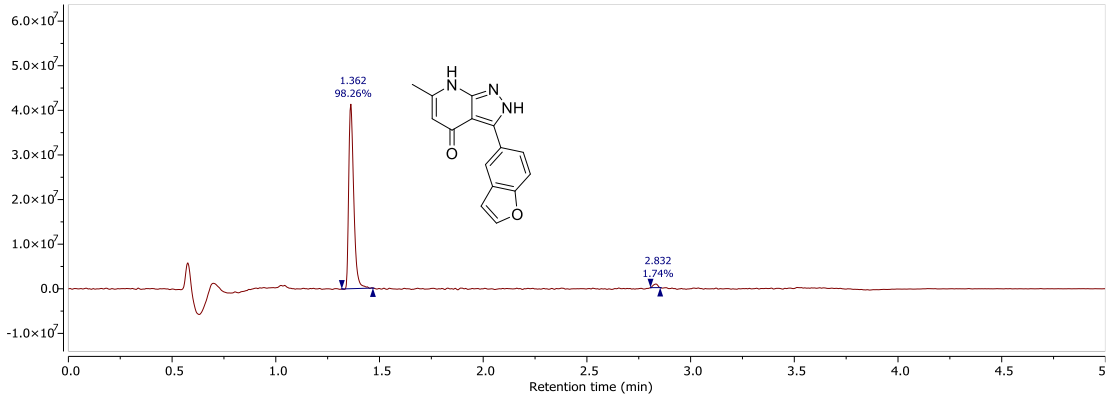
6-cyclopropyl-3-(3,4-dihydro-2H-benzo[b][1,4]oxazin-7-yl)-2,7-dihydro-4H-pyrazolo[3,4-b]pyridin-4-one (28)



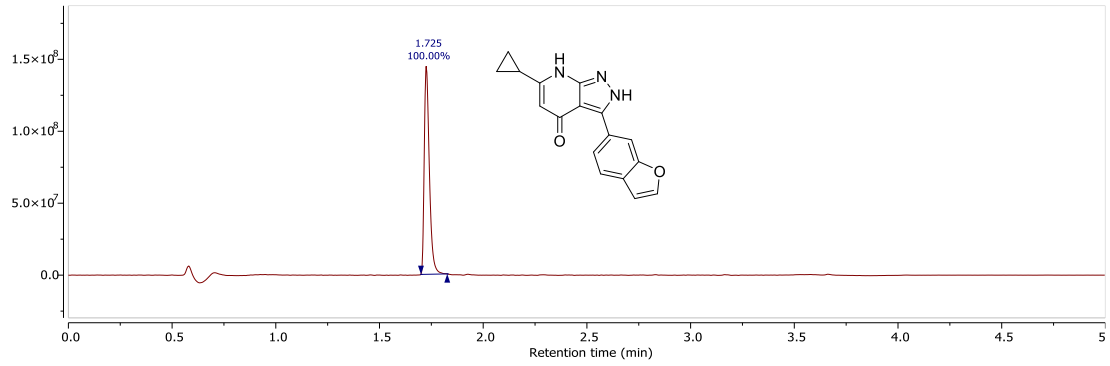
3-(benzofuran-6-yl)-6-methyl-2,7-dihydro-4H-pyrazolo[3,4-b]pyridin-4-one (29)



3-(benzofuran-5-yl)-6-methyl-2,7-dihydro-4H-pyrazolo[3,4-b]pyridin-4-one (30)

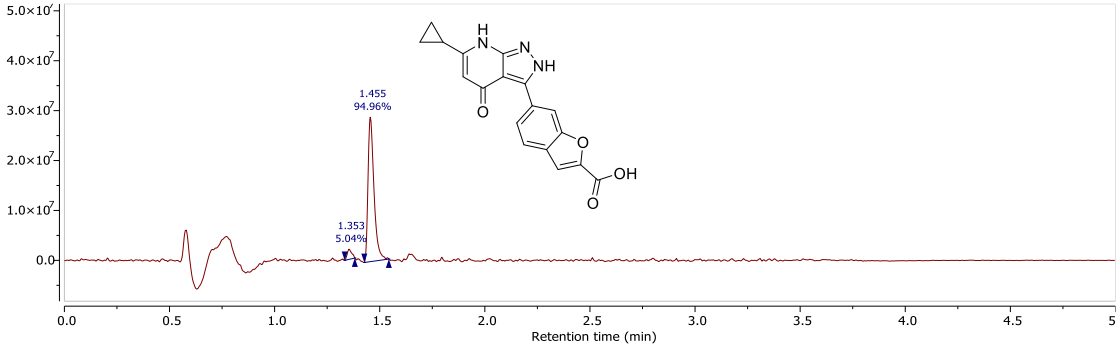


3-(benzofuran-6-yl)-6-cyclopropyl-2,7-dihydro-4H-pyrazolo[3,4-b]pyridin-4-one (31)

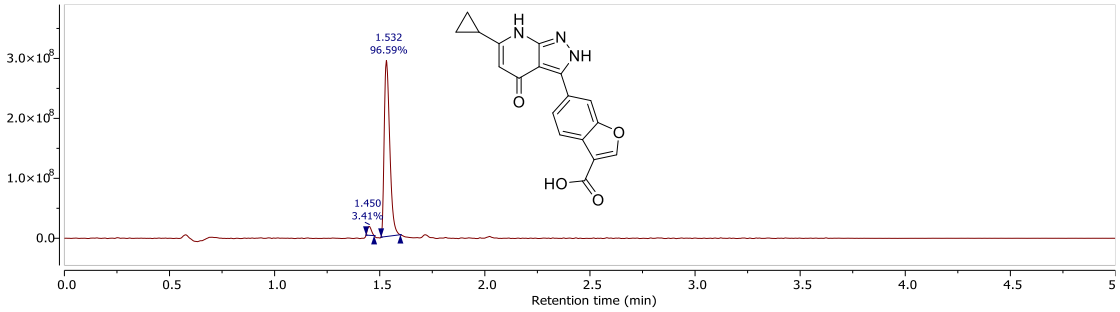


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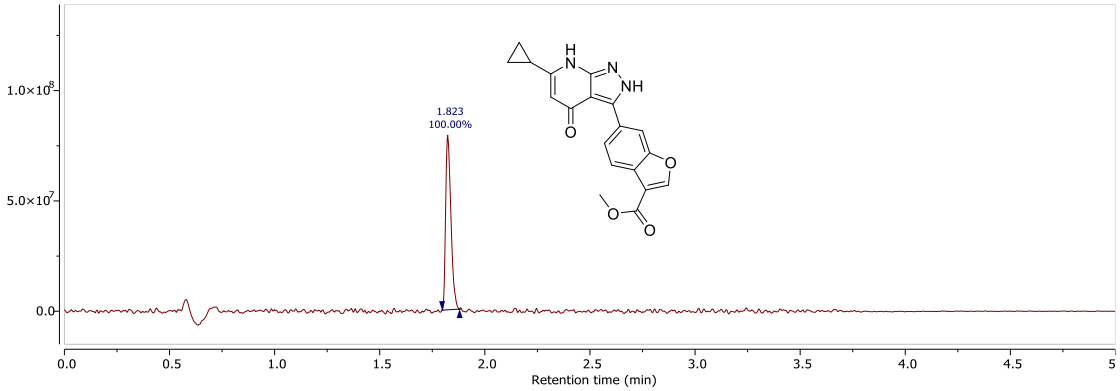
6-(6-cyclopropyl-4-oxo-4,7-dihydro-2H-pyrazolo[3,4-b]pyridin-3-yl)benzofuran-2-carboxylic acid (32)



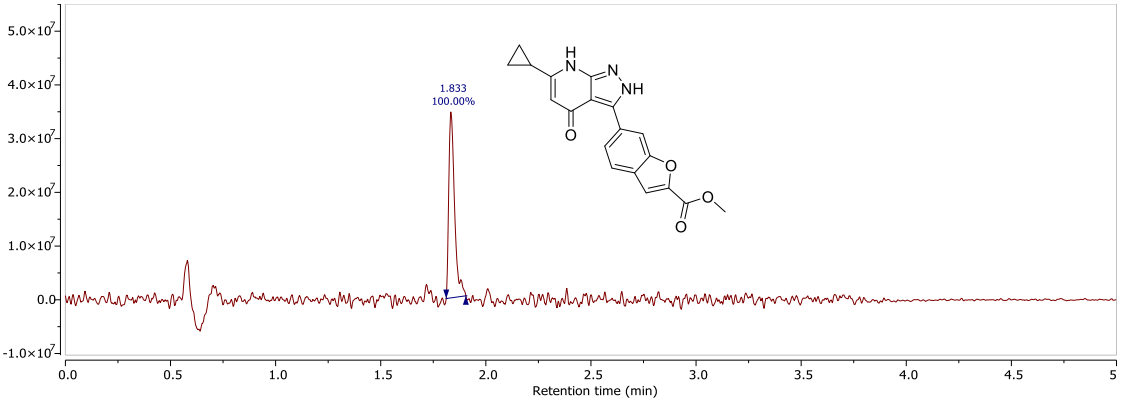
6-(6-cyclopropyl-4-oxo-4,7-dihydro-2H-pyrazolo[3,4-b]pyridin-3-yl)benzofuran-3-carboxylic acid (33)



Methyl 6-(6-cyclopropyl-4-oxo-4,7-dihydro-2H-pyrazolo[3,4-b]pyridin-3-yl)benzofuran-3-carboxylate (34)

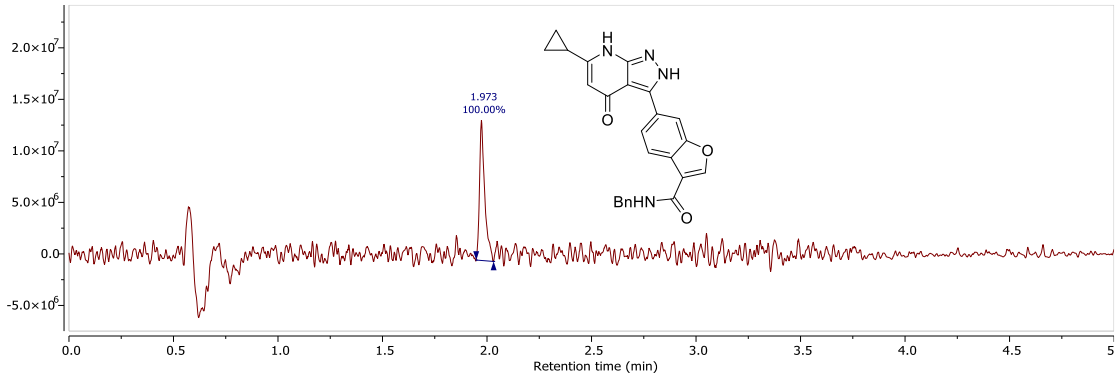


Methyl 6-(6-cyclopropyl-4-oxo-4,7-dihydro-2H-pyrazolo[3,4-b]pyridin-3-yl)benzofuran-2-carboxylate (35)

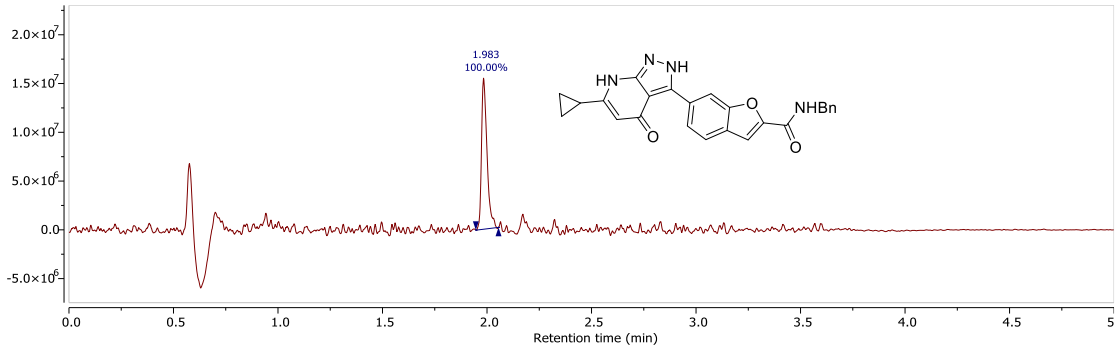


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N-benzyl-6-(6-cyclopropyl-4-oxo-4,7-dihydro-2H-pyrazolo[3,4-b]pyridin-3-yl)benzofuran-3-carboxamide (36)



N-benzyl-6-(6-cyclopropyl-4-oxo-4,7-dihydro-2H-pyrazolo[3,4-b]pyridin-3-yl)benzofuran-2-carboxamide (37)



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