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**Supplementary material**

# **Development of New Meridianin/Leucettine-Derived Hybrid Small Molecules as Nanomolar Multi-Kinase Inhibitors with Antitumor Activity**

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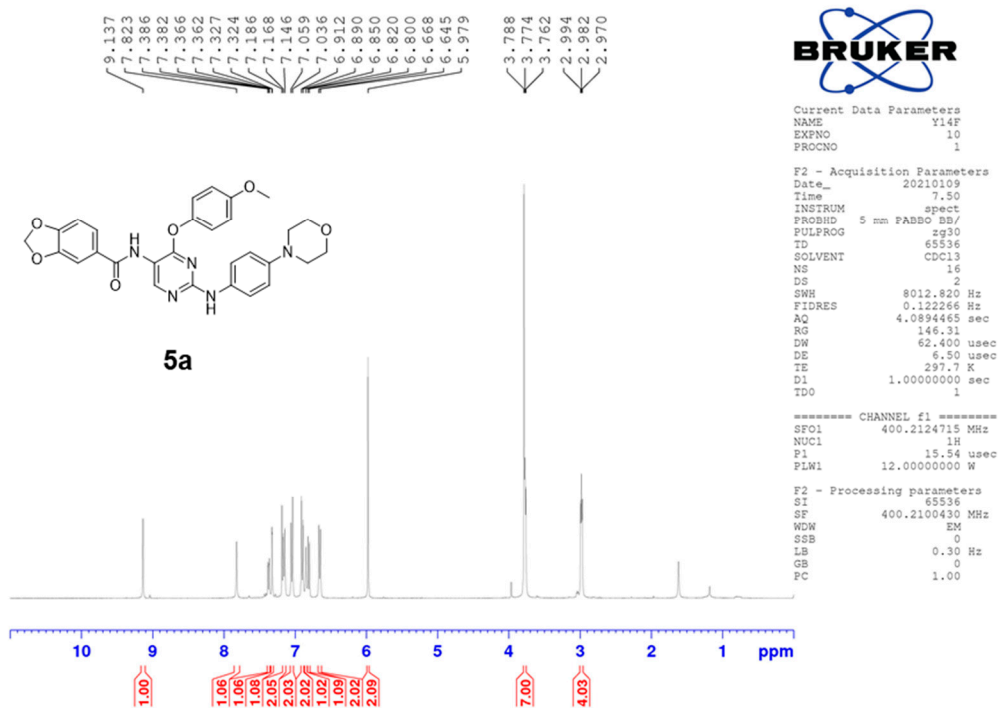
## Contents

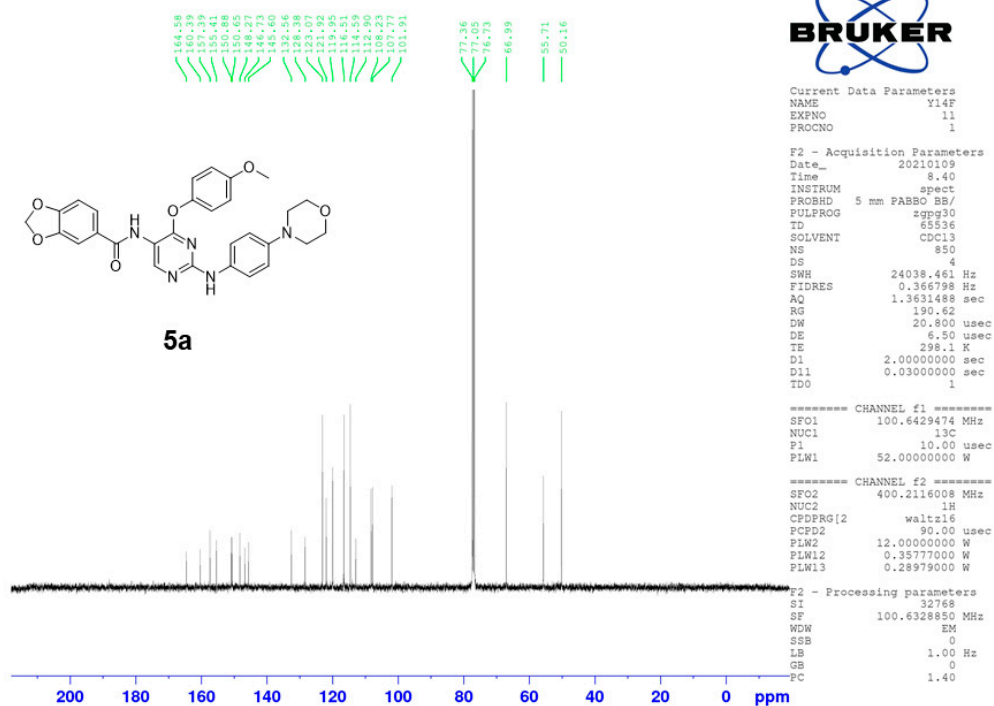
- 1. General methods and instruments (Chemistry)**
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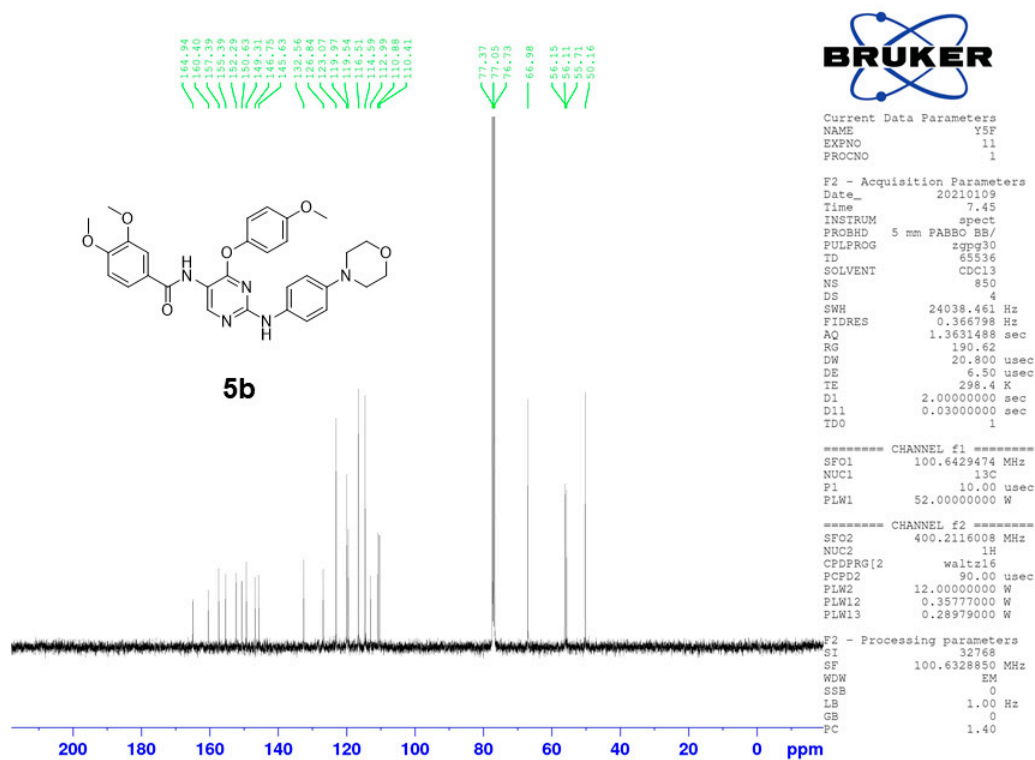
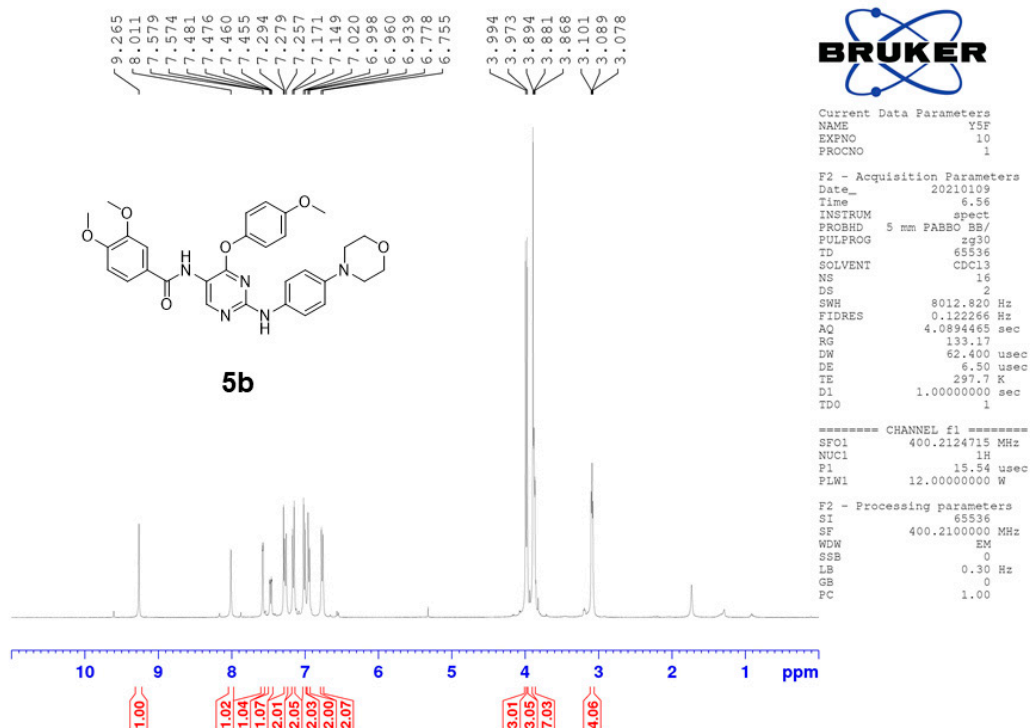
## 1. General methods and instruments (Chemistry)

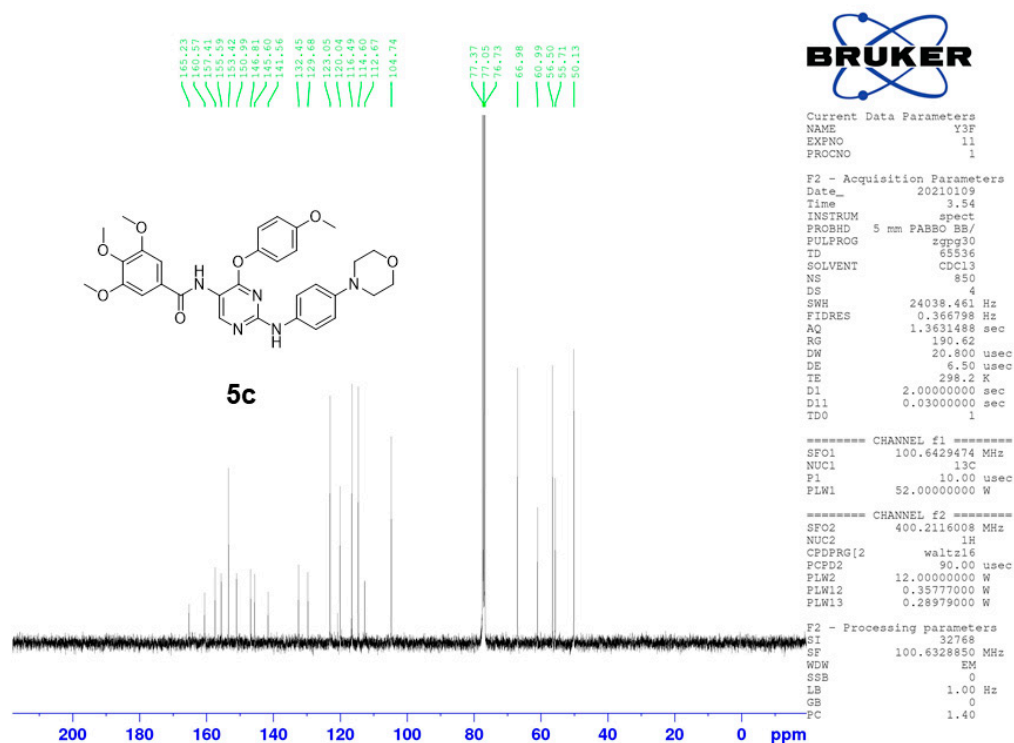
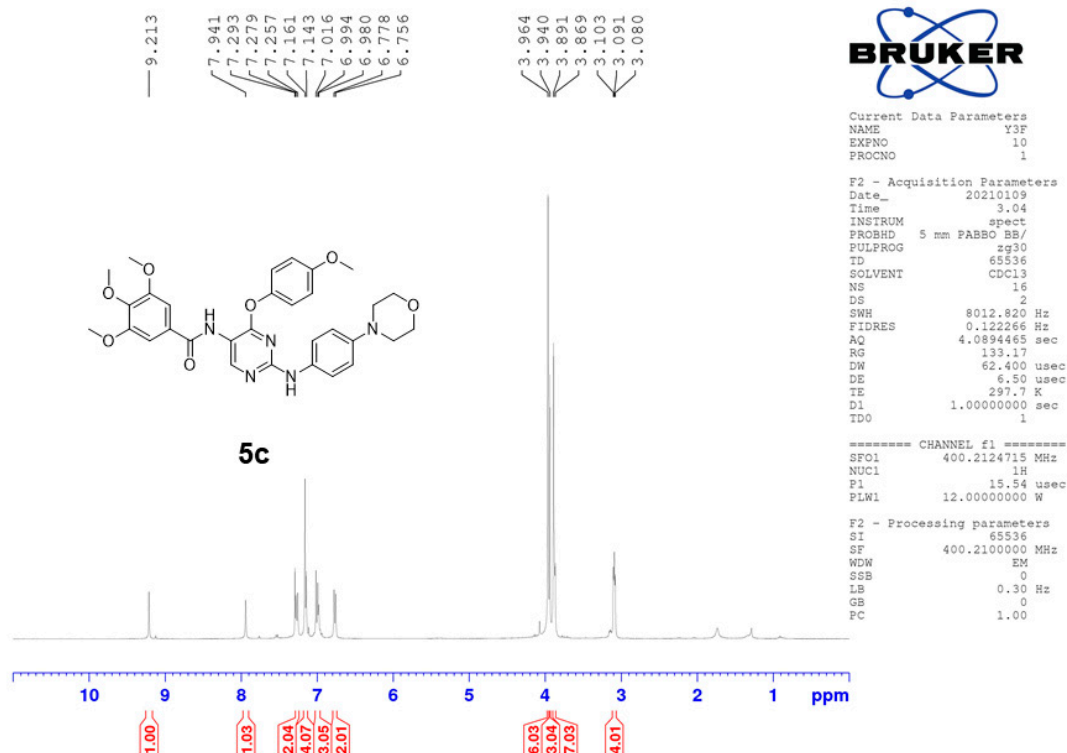
The NMR spectra were obtained on Bruker Avance 400.  $^1\text{H}$  NMR spectra were referenced to tetramethylsilane ( $\delta = 0.00$  ppm) as an internal standard and are reported as follows: chemical shift, multiplicity (br = broad, s = singlet, d = doublet, t = triplet, dd = doublet of doublet, m = multiplet). Column chromatography was performed on Merck Silica Gel 60 (230–400 mesh) and eluting solvents for all these chromatographic methods are noted as appropriated-mixed solvent with given volume-to-volume ratios. TLC was carried out using glass sheets pre-coated with silica gel 60 F<sub>254</sub> purchased by Merk. The purity of samples was determined by analytical HPLC using a Water ACQUITY UPLC (CORTECS™) with C18 column (2.1 mm x 100 mm; 1.6  $\mu\text{m}$ ) at temperature 40 °C. HPLC data were recorded using parameters as follows: 0.1% formic acid in water and 0.1% formic acid in methanol and flow rate of 0.3 mL/min. High-resolution spectra were performed on Waters ACQUITY UPLC BEH C18 1.7 $\mu$ –Q-TOF SYNAPT G2-Si High-Definition Mass Spectrometry.

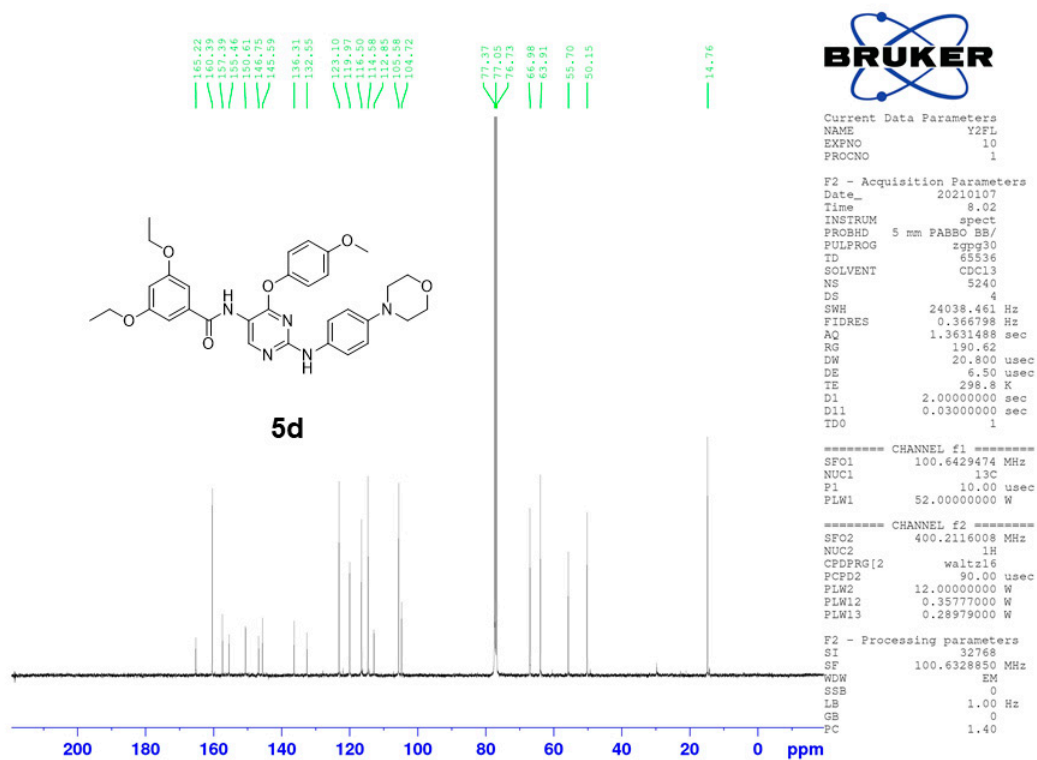
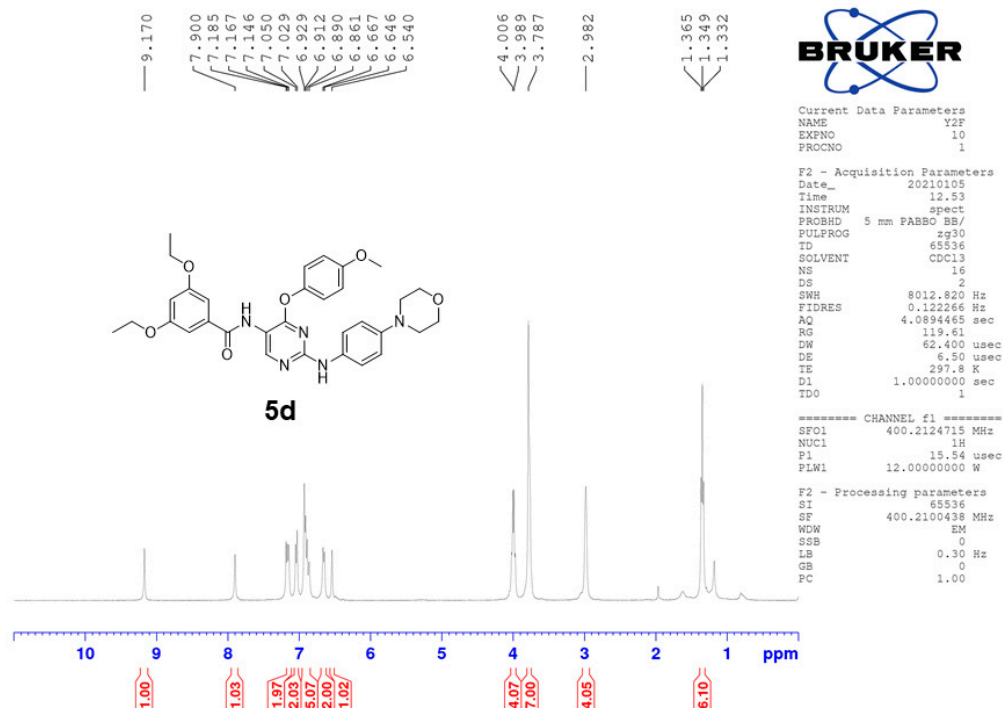
## 2. $^1\text{H}$ NMR and $^{13}\text{C}$ NMR data of compounds 5a–g

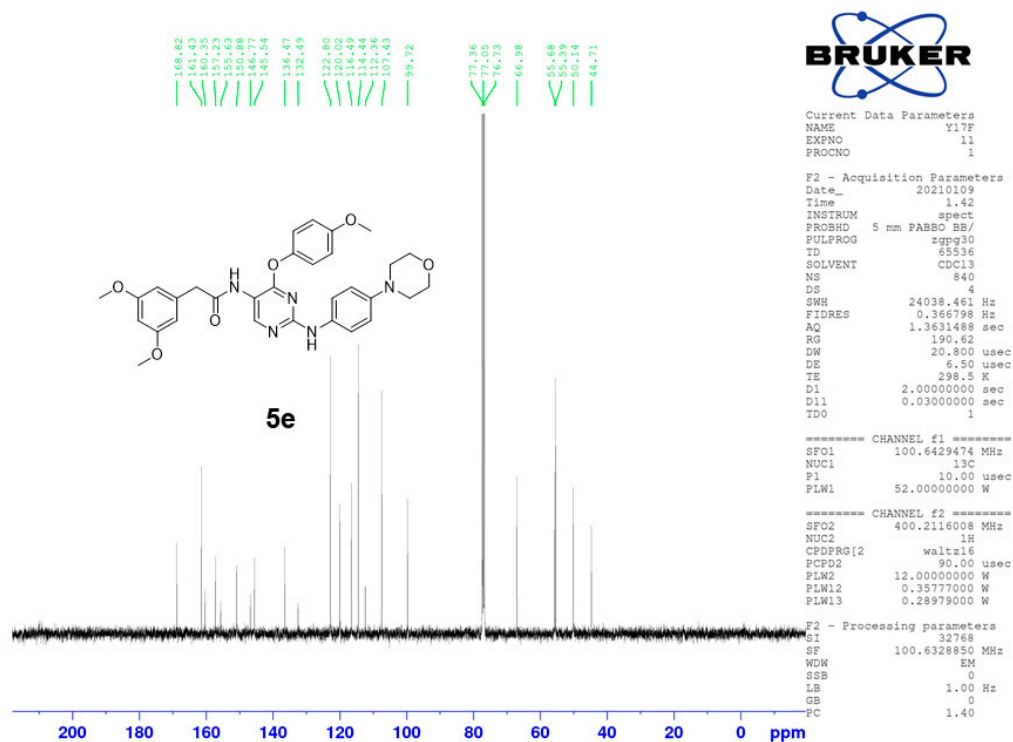
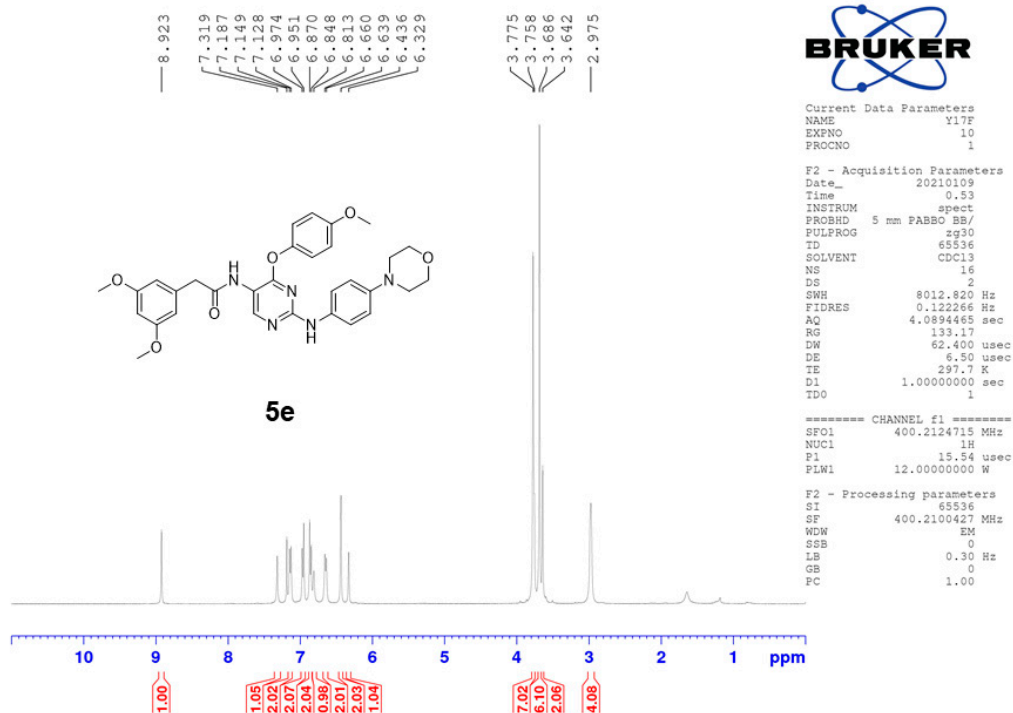




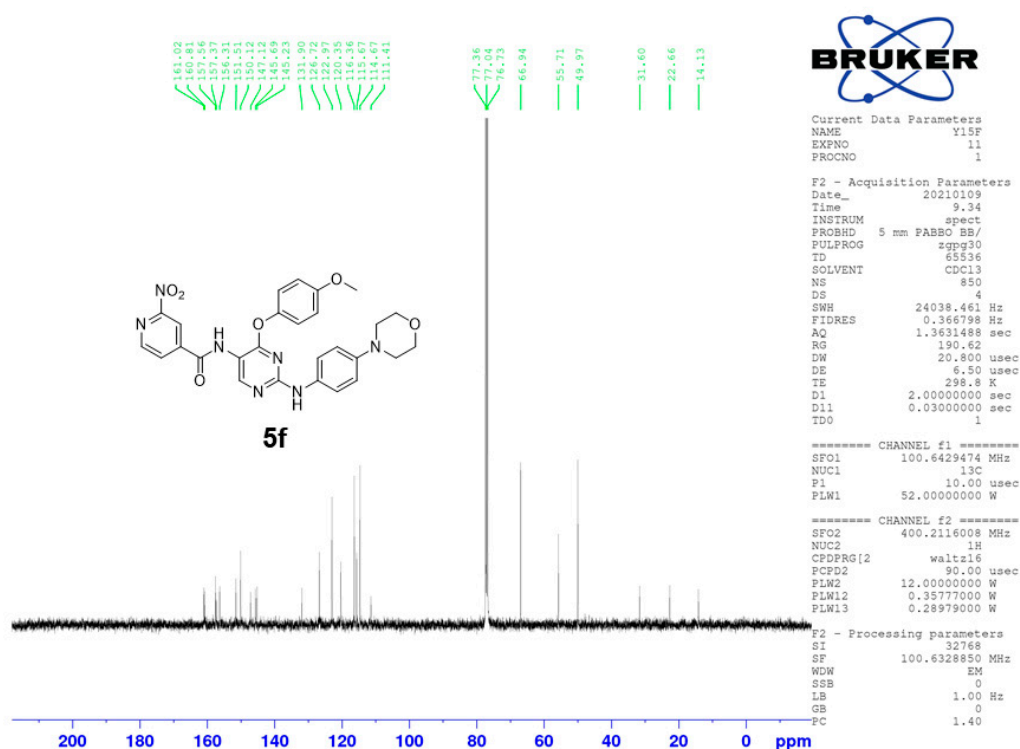
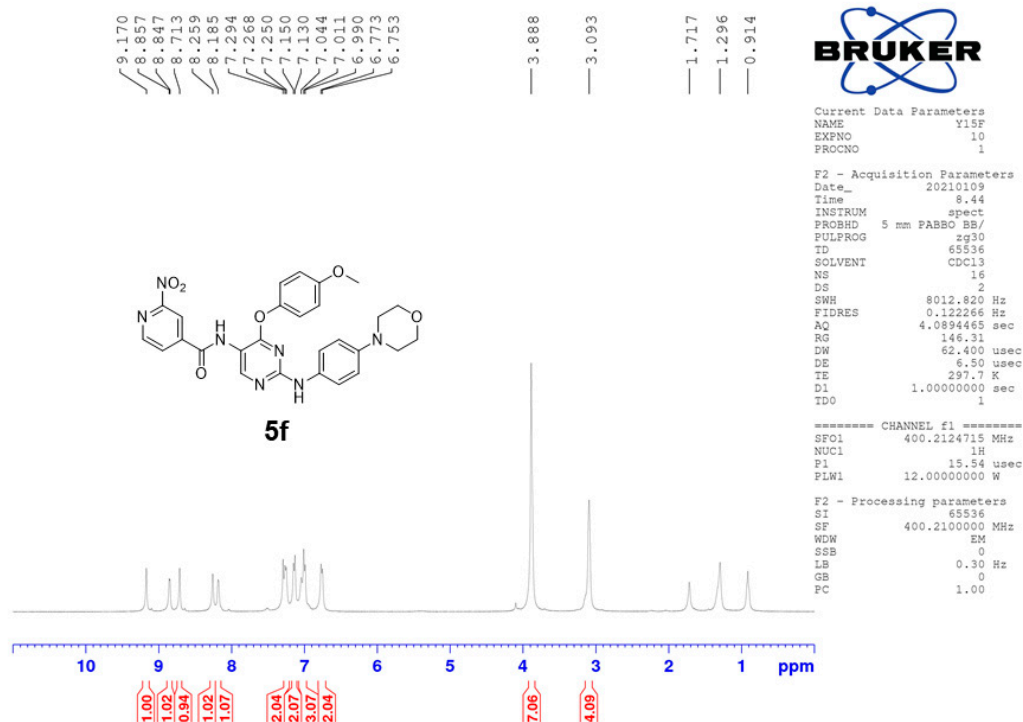


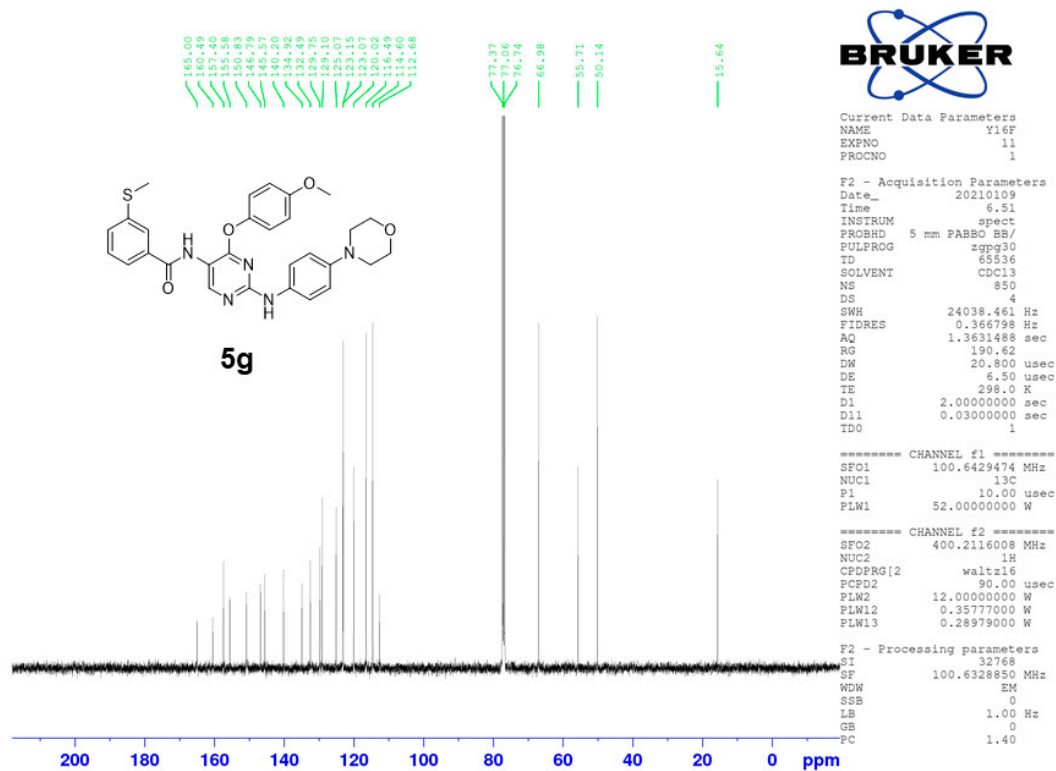
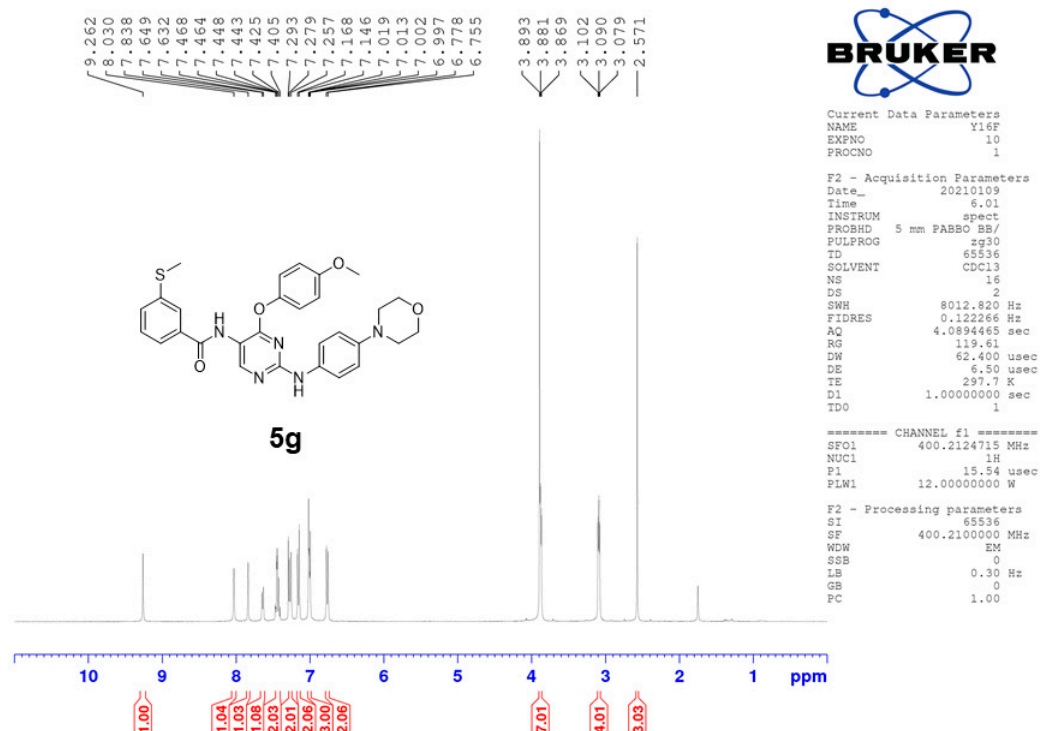








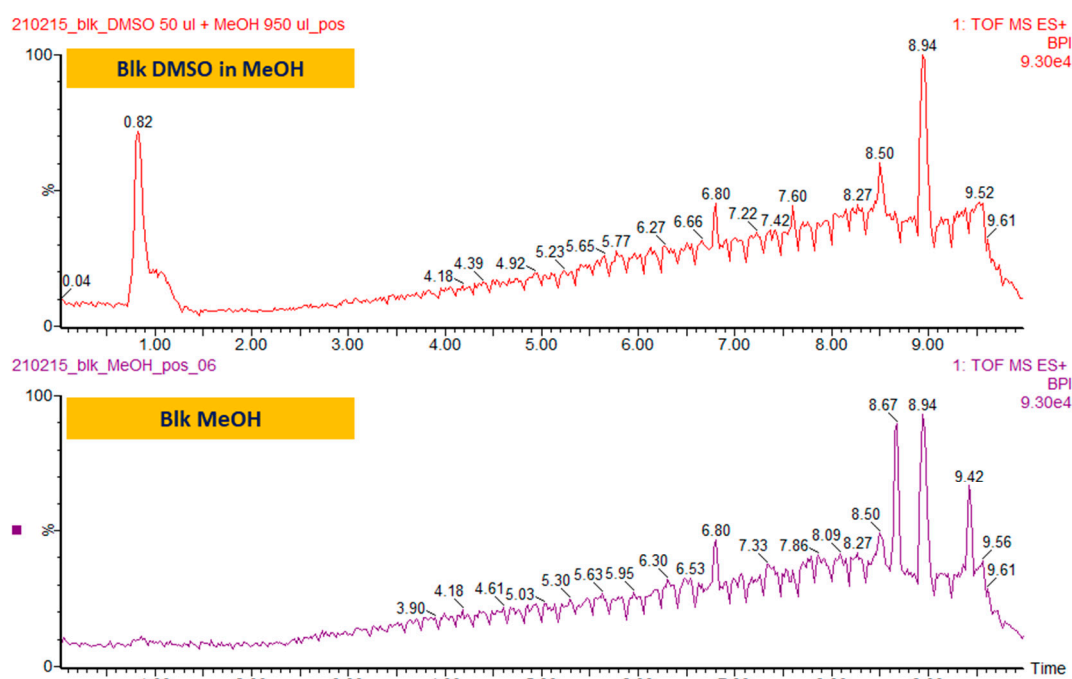




### 3. LC and MS conditions

LC Condition (Waters® ACQUITY™ UPLC)			
Column	CORTECS® UPLC® C18 1.6 µm (2.1x100 mm)		
	Temperature : 40 °C		
Mobile phase	A : 0.1% formic acid in water, v/v		
	B : 0.1% formic acid in methanol, v/v		
Gradient	Time (min)	A (%)	B (%)
	0.0	99.0	1.0
	1.0	99.0	1.0
	7.0	1.0	99.0
	8.5	1.0	99.0
	9.0	99.0	1.0
	10.0	99.0	1.0
Flow rate	0.3 ml/min		
Injection volume	5 µl		
MS Condition (SYNAPT™ G2)			
Ionization Mode	ESI+		
Capillary	+3.1 kv		
Sampling cone	40 V		
Extraction cone	4.0		
Source	120 °C		
Desolvation	350 °C		
Cone gas	100 L/h		
Desolvation gas	800 L/h		

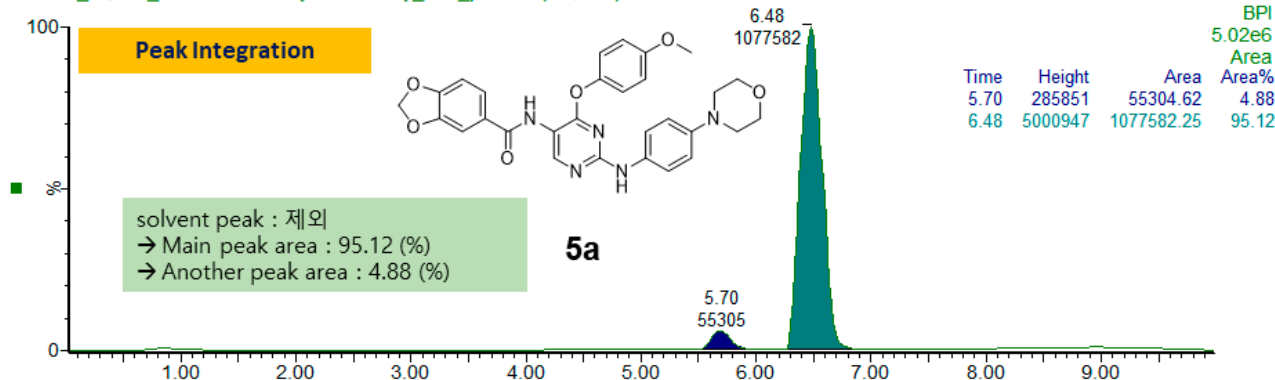
#### Chromatogram\_blank



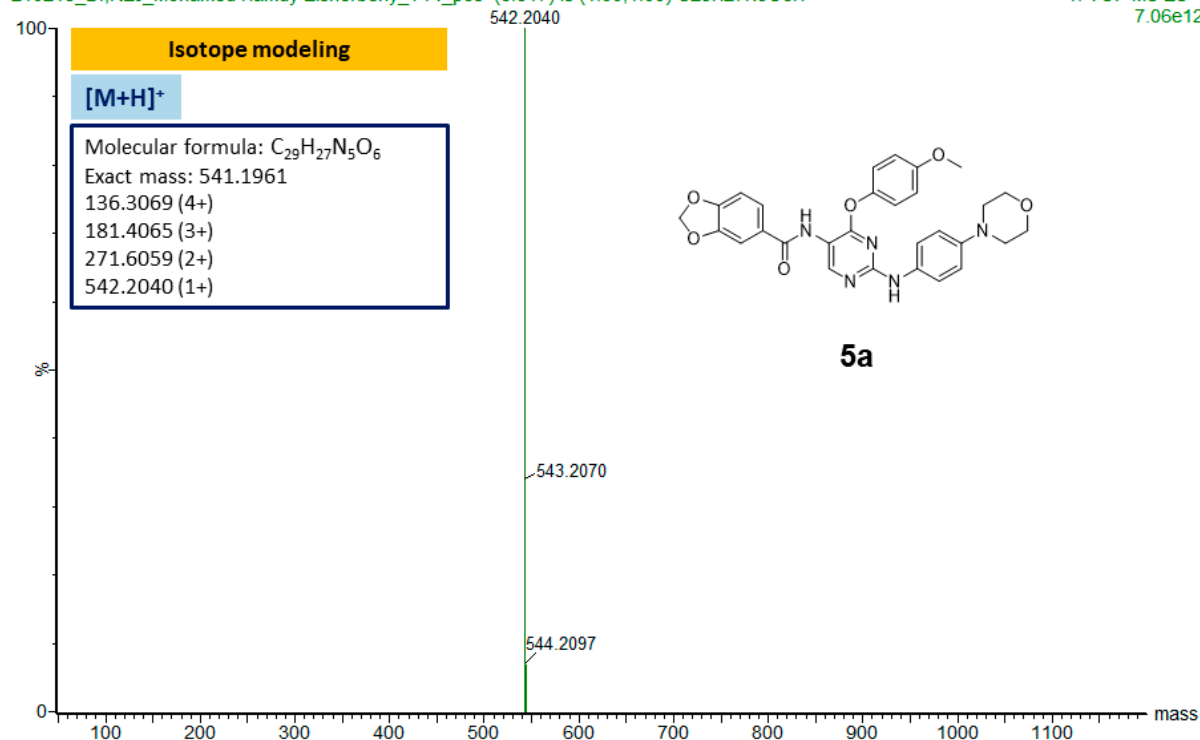
#### 4. Representative purity and HRMS data of compounds 5a–g

##### Peak area(%)\_ Y14

210215\_Dr.REJ\_Mohamed Hamdy Elsherbeny\_Y14\_pos Sm (Mn, 2x3)



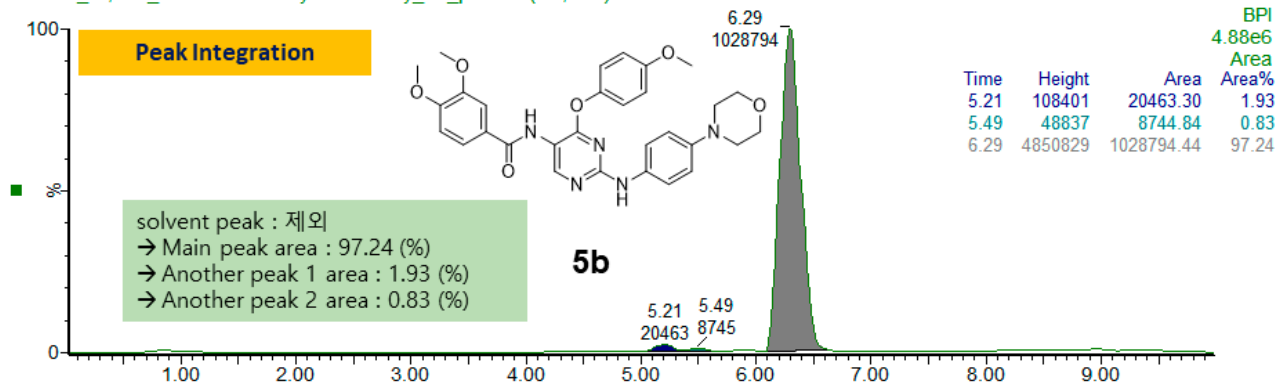
##### MS Spectrum\_ Isotope modeling\_Y14

210215\_Dr.REJ\_Mohamed Hamdy Elsherbeny\_Y14\_pos (6.517) Is (1.00,1.00) C<sub>29</sub>H<sub>27</sub>N<sub>5</sub>O<sub>6</sub>H1: TOF MS ES+  
7.06e12

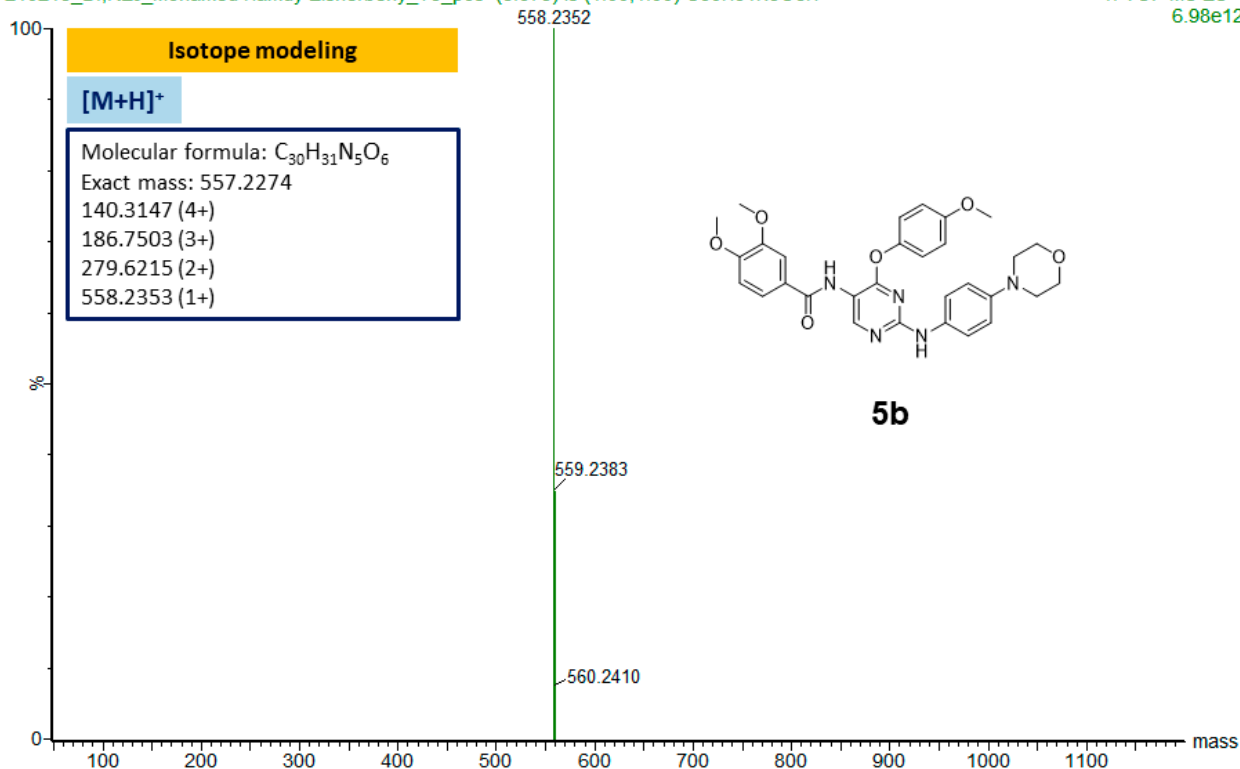
## Peak area(%)\_Y5

210215\_Dr,REJ\_Mohamed Hamdy Elsherbeny\_Y5\_pos Sm (Mn, 2x3)

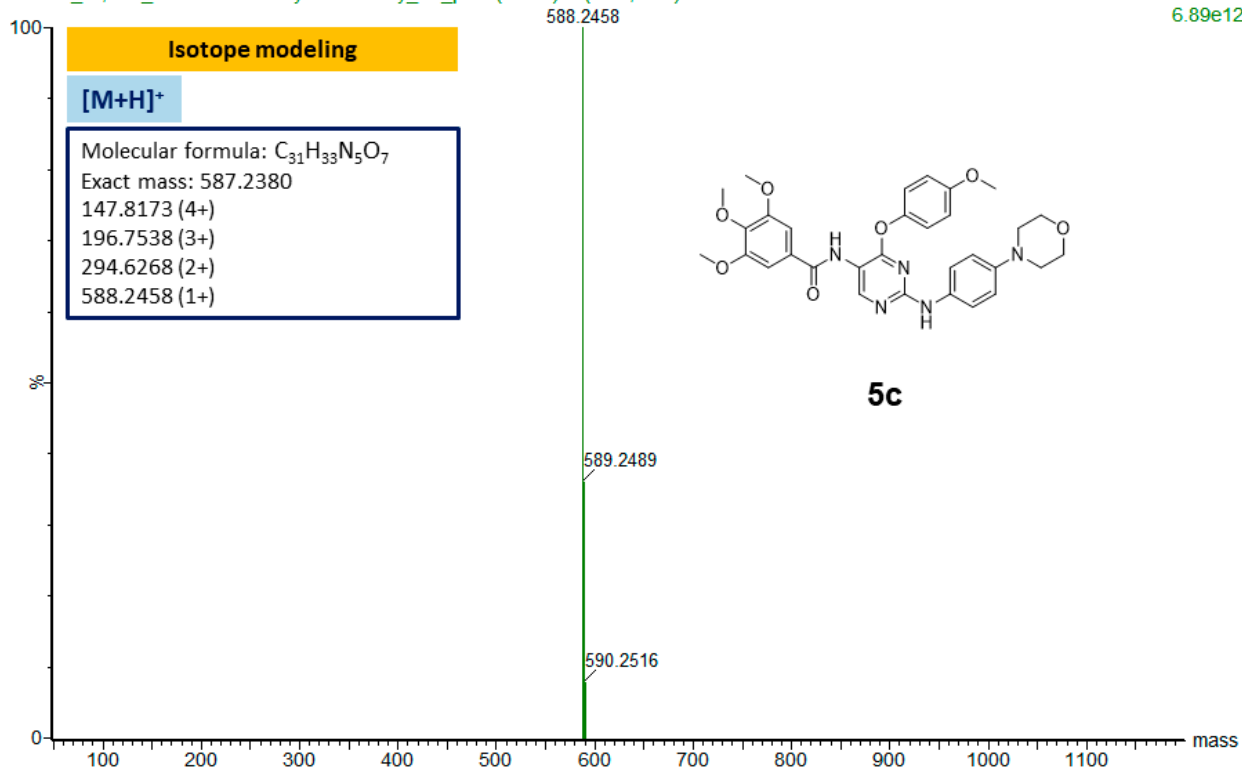
1: TOF MS ES+



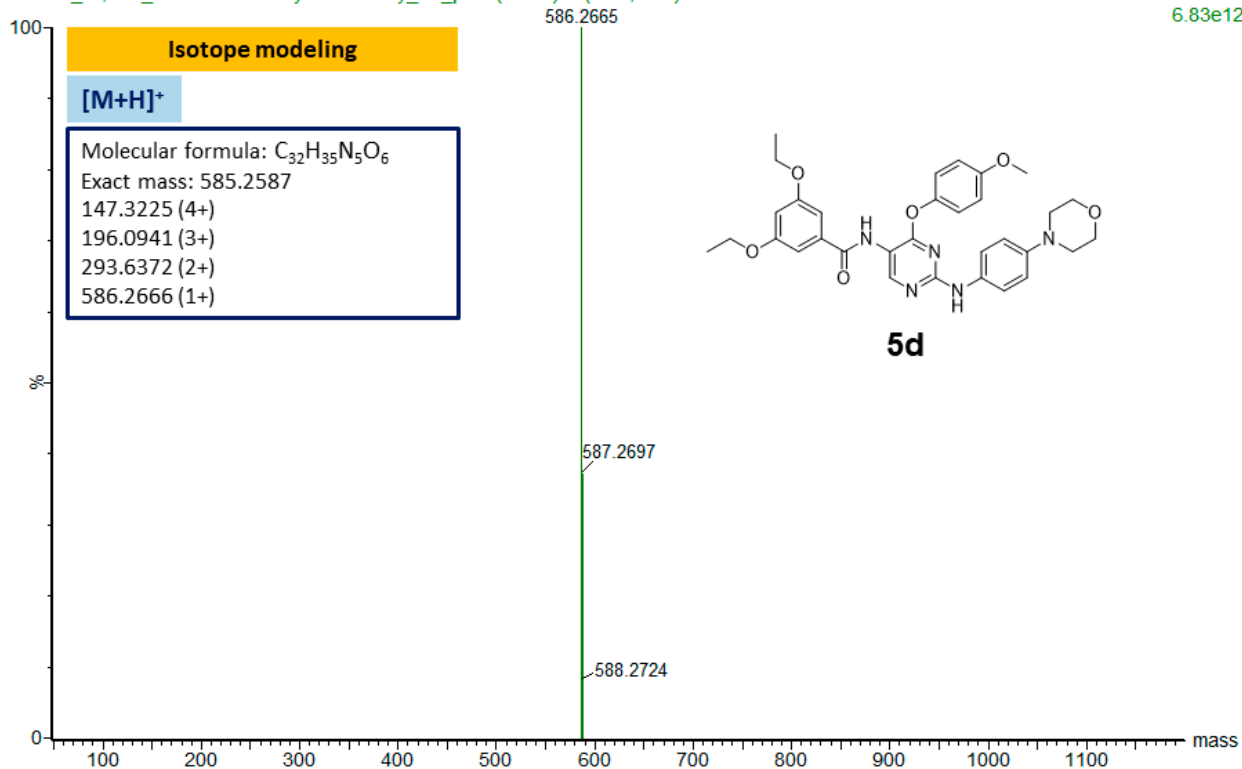
## MS Spectrum\_ Isotope modeling\_Y5

210215\_Dr,REJ\_Mohamed Hamdy Elsherbeny\_Y5\_pos (6.375) Is (1.00,1.00) C<sub>30</sub>H<sub>31</sub>N<sub>5</sub>O<sub>6</sub>H1: TOF MS ES+  
6.98e12

## MS Spectrum\_ Isotope modeling\_Y3

210215\_Dr.REJ\_Mohamed Hamdy Elsherbeny\_Y3\_pos (6.412) Is (1.00,1.00) C<sub>31</sub>H<sub>33</sub>N<sub>5</sub>O<sub>7</sub>H1: TOF MS ES+  
6.89e12

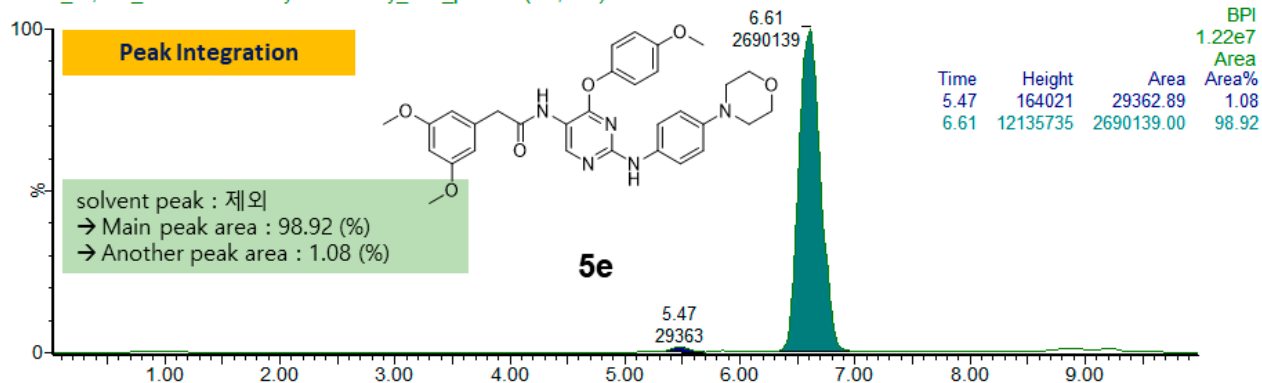
## MS Spectrum\_ Isotope modeling\_Y2

210215\_Dr.REJ\_Mohamed Hamdy Elsherbeny\_Y2\_pos (7.065) Is (1.00,1.00) C<sub>32</sub>H<sub>35</sub>N<sub>5</sub>O<sub>6</sub>H1: TOF MS ES+  
6.83e12

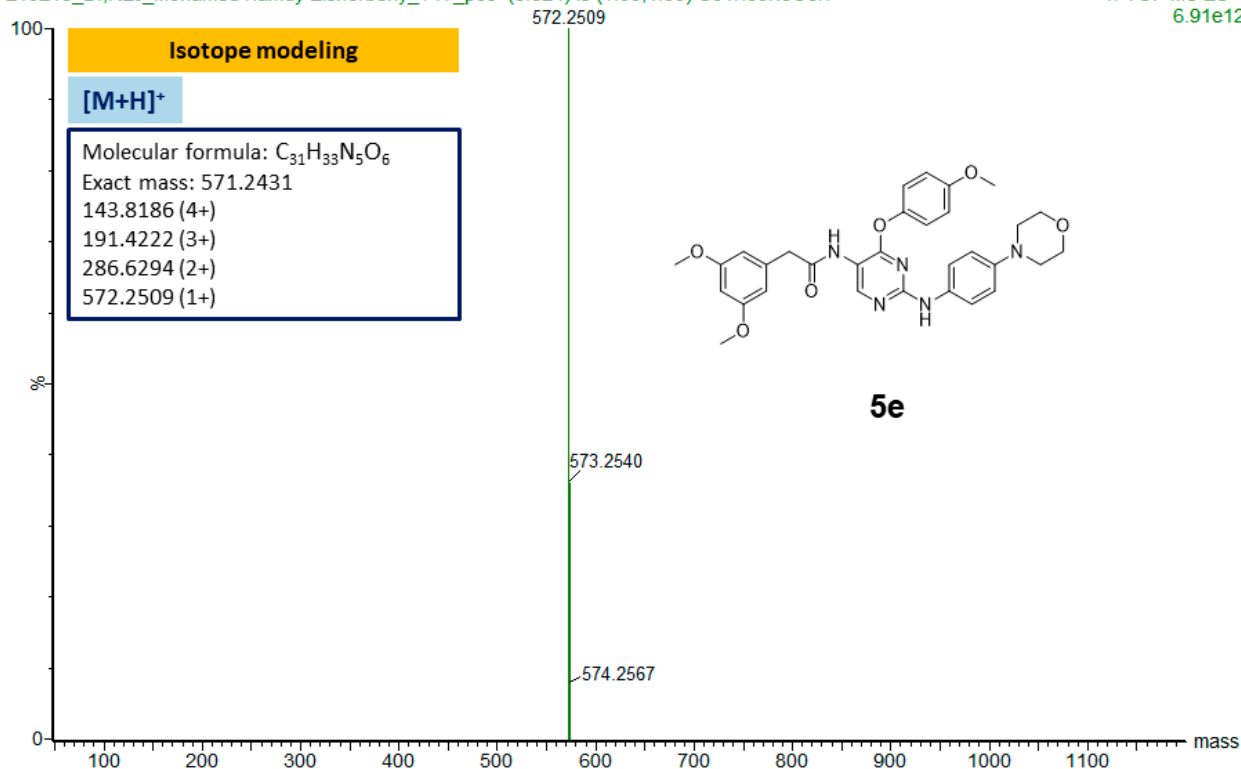
## Peak area(%)\_ Y17

210215\_Dr,REJ\_Mohamed Hamdy Elsherbeny\_Y17\_pos Sm (Mn, 2x3)

1: TOF MS ES+

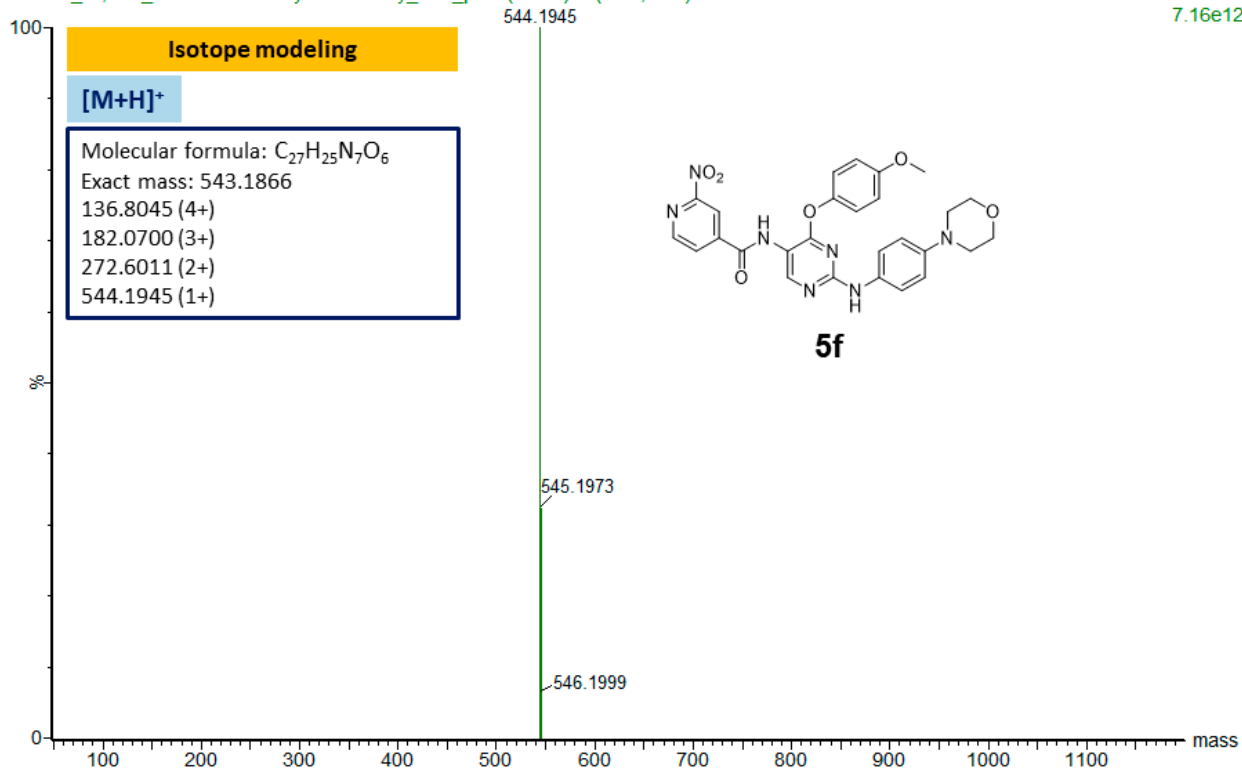


## MS Spectrum\_ Isotope modeling\_Y17

210215\_Dr,REJ\_Mohamed Hamdy Elsherbeny\_Y17\_pos (6.624) Is (1.00,1.00) C<sub>31</sub>H<sub>33</sub>N<sub>5</sub>O<sub>6</sub>H1: TOF MS ES+  
6.91e12



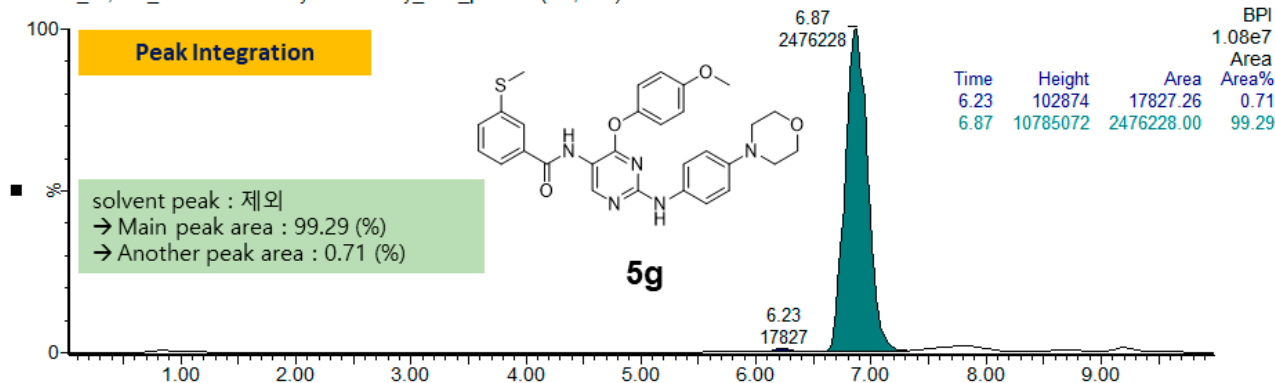
## MS Spectrum\_ Isotope modeling\_Y15

210215\_Dr.REJ\_Mohamed Hamdy Elsherbeny\_Y15\_pos (6.340) Is (1.00,1.00) C<sub>27</sub>H<sub>25</sub>N<sub>7</sub>O<sub>6</sub>H1: TOF MS ES+  
7.16e12

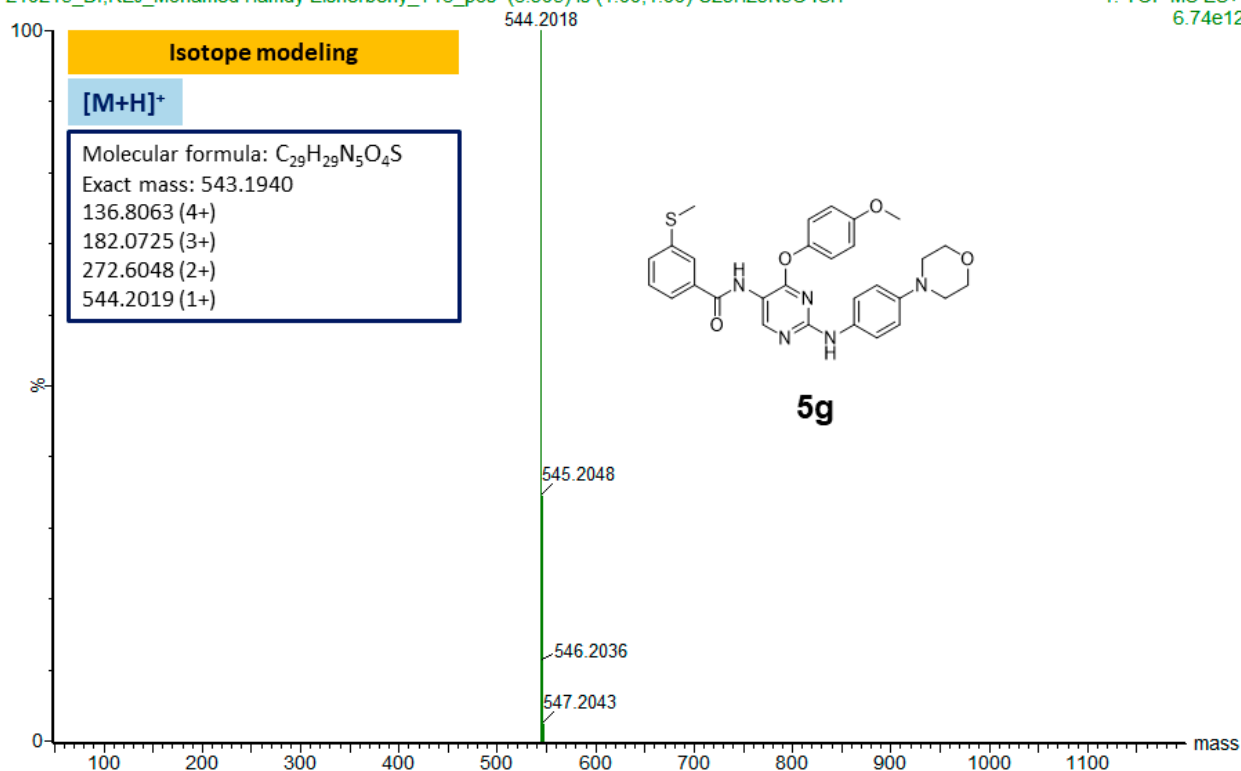
## Peak area(%)\_ Y16

210215\_Dr,REJ\_Mohamed Hamdy Elsherbeny\_Y16\_pos Sm (Mn, 2x3)

1: TOF MS ES+



## MS Spectrum\_ Isotope modeling\_ Y16

210215\_Dr,REJ\_Mohamed Hamdy Elsherbeny\_Y16\_pos (6.905) Is (1.00,1.00) C<sub>29</sub>H<sub>29</sub>N<sub>5</sub>O<sub>4</sub>SH1: TOF MS ES+  
6.74e12

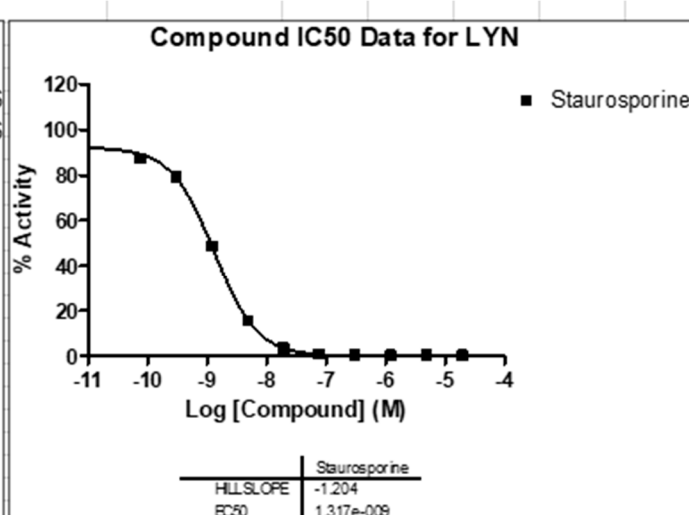
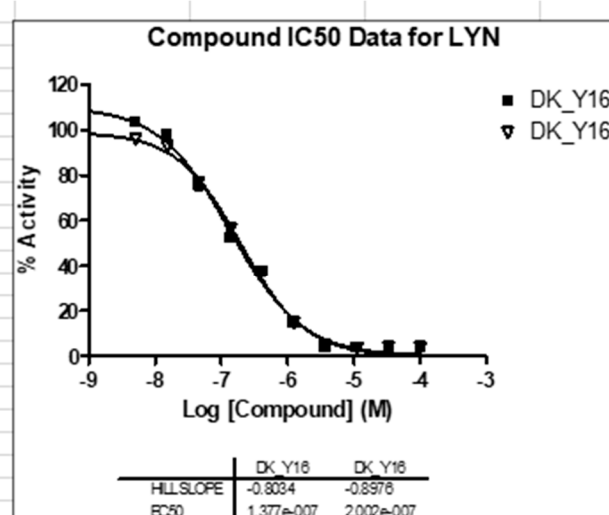
## 5. Protocol of Kinase assay

Kinase Profiling is 10 dose IC<sub>50</sub> singlet assay. Kinases inhibitory activities were assessed by the HotSpot assay platform, which contained specific kinase/substrate pairs along with required cofactors. Base reaction buffer: 20 mM Hepes (pH 7.5), 10 mM MgCl<sub>2</sub>, 1 mM EGTA, 0.02% Brij35, 0.02 mg/ml BSA, 0.1 mM Na<sub>3</sub>VO<sub>4</sub>, 2 mM DTT, 1% DMSO. Testing compounds were dissolved in 100% DMSO to specific concentration. The serial dilution was conducted by Integra Viaflo Assist in DMSO. The reaction mixture containing the examined compound and <sup>33</sup>P-ATP was incubated at room temperature for 2h and radioactivity was detected by filter-binding method. Kinase activity data were expressed as the percent remaining kinase activity in test samples compared to vehicle (dimethyl sulfoxide) reactions. IC<sub>50</sub> values and curve fits were obtained using Prism (GraphPad Software).

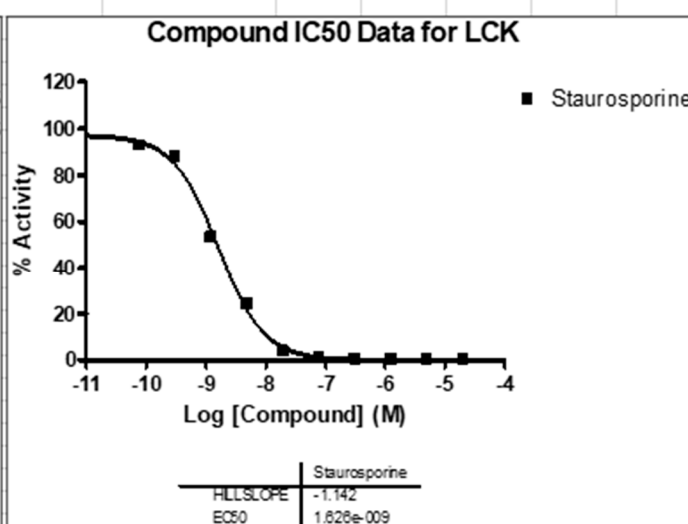
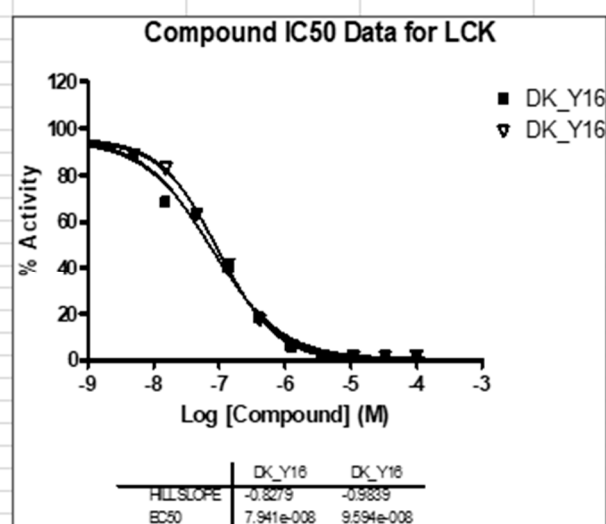
## 6. Biology raw data for IC<sub>50</sub> determination

Paper ID	Cpd ID	NSC Number
<b>5d</b>	DK_Y2	827664
<b>5c</b>	DK_Y3	827665
<b>5b</b>	DK_Y5	827666
<b>5a</b>	DK_Y14	827667
<b>5f</b>	DK_Y15	827668
<b>5g</b>	DK_Y16	827669
<b>5e</b>	DK_Y17	827670

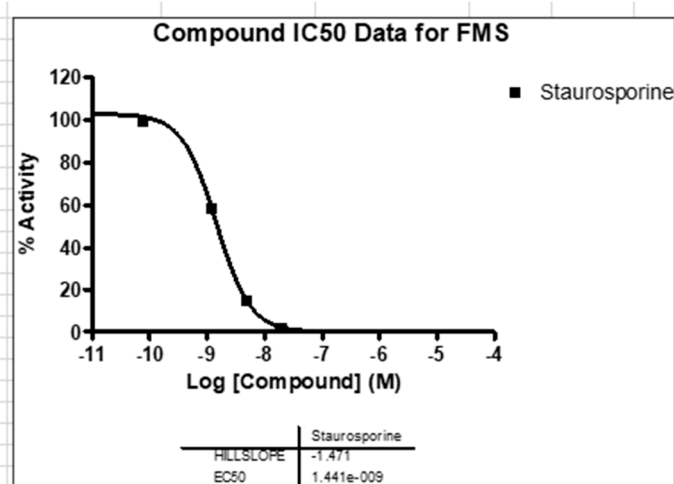
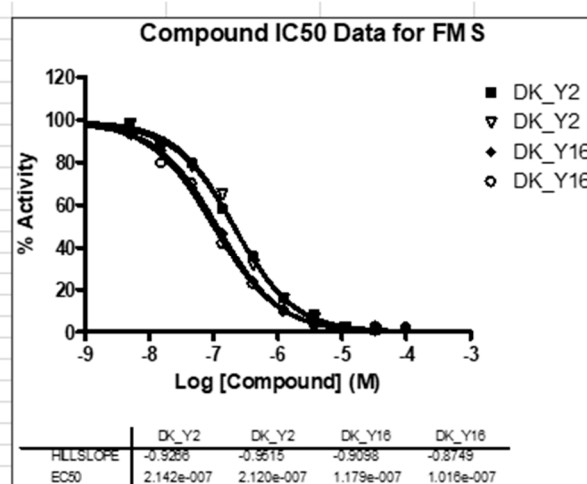
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1	Kinase Profiling Report for:			LYN		
2						
3	Raw data					
4		Conc. (M)	DK_Y16	DK_Y16	Staurosporine	Stauro Conc. (M)
5		1.00E-04	102058	97795	457	2.00E-05
6		3.33E-05	91411	96509	4781	5.00E-06
7		1.11E-05	99636	88012	2031	1.25E-06
8		3.70E-06	107790	123234	5061	3.13E-07
9		1.23E-06	402276	397973	17915	7.81E-08
10		4.12E-07	1012948	1008099	85789	1.95E-08
11		1.37E-07	1424580	1521179	415780	4.88E-09
12		4.57E-08	2039036	2069042	1312529	1.22E-09
13		1.52E-08	2645282	2501772	2138576	3.05E-10
14		5.08E-09	2797609	2601868	2360674	7.63E-11
15		DMSO	2948480	2640293	2536190	DMSO
16						
17						
18	%Activity					
19		Conc. (M)	DK_Y16	DK_Y16	Staurosporine	Stauro Conc. (M)
20		1.00E-04	3.77	3.61	0.02	2.00E-05
21		3.33E-05	3.38	3.56	0.18	5.00E-06
22		1.11E-05	3.68	3.25	0.08	1.25E-06
23		3.70E-06	3.98	4.55	0.19	3.13E-07
24		1.23E-06	14.85	14.69	0.66	7.81E-08
25		4.12E-07	37.40	37.22	3.17	1.95E-08
26		1.37E-07	52.60	56.17	15.35	4.88E-09
27		4.57E-08	75.29	76.40	48.46	1.22E-09
28		1.52E-08	97.67	92.37	78.96	3.05E-10
29		5.08E-09	103.30	96.07	87.16	7.63E-11
30		DMSO	108.87	97.49	93.64	DMSO
31						
32		HILLSLOPE	-0.80	-0.90	-1.20	
33		IC50 (M)	1.38E-07	2.00E-07	1.32E-09	
34						
35						



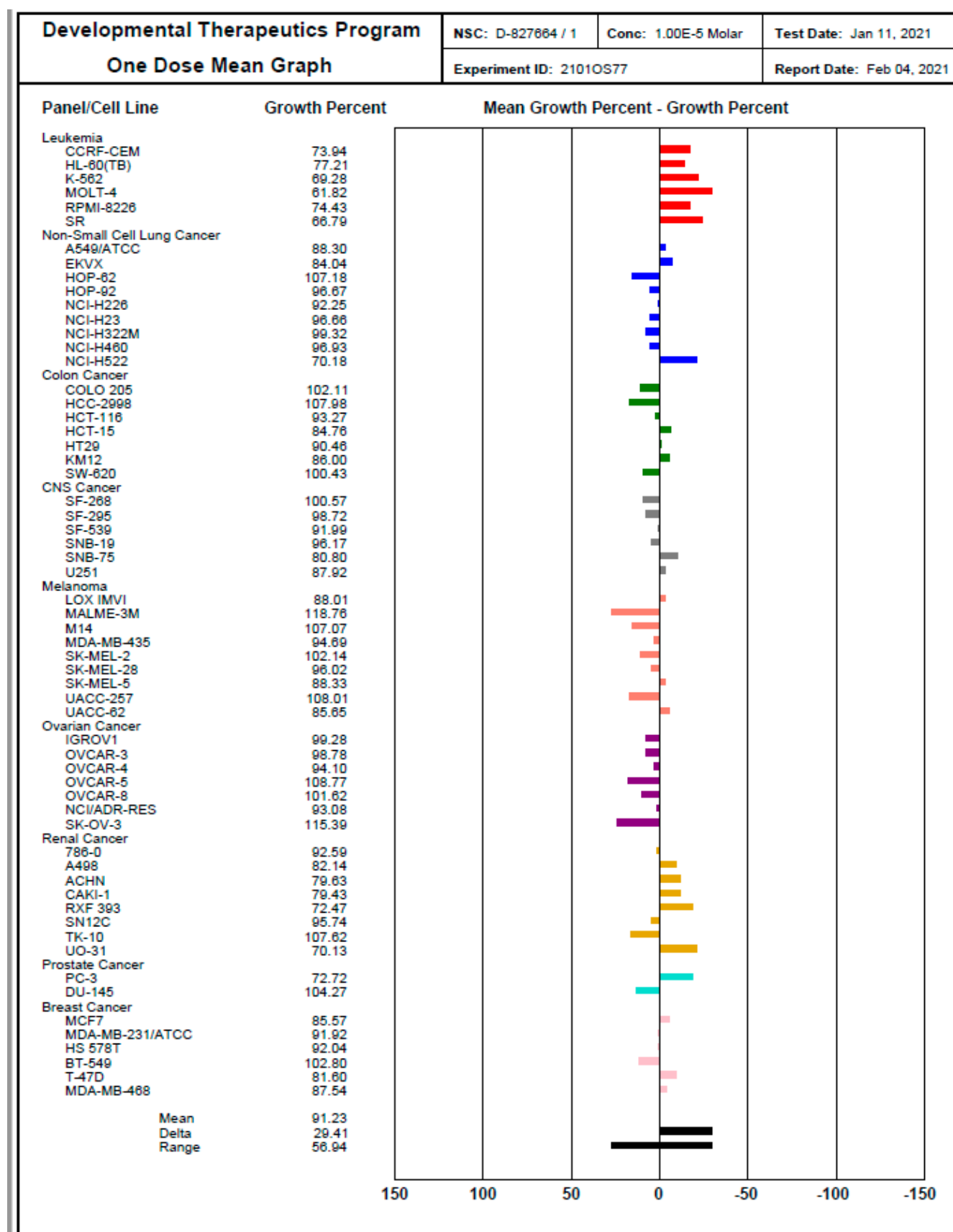
	A	B	C	D	E	F
1	Kinase Profiling Report for:			LCK		
2						
3	Raw data					
4		Conc. (M)	DK_Y16	DK_Y16	Staurosporine	Stauro Conc. (M)
5		1.00E-04	23600	28153	2798	2.00E-05
6		3.33E-05	25579	22567	2536	5.00E-06
7		1.11E-05	24308	24444	3537	1.25E-06
8		3.70E-06	24200	25454	6391	3.13E-07
9		1.23E-06	87291	97081	16456	7.81E-08
10		4.12E-07	283755	269570	62683	1.95E-08
11		1.37E-07	625889	650022	379559	4.88E-09
12		4.57E-08	984306	991895	835742	1.22E-09
13		1.52E-08	1067683	1298284	1382806	3.05E-10
14		5.08E-09	1392250	1385928	1452961	7.63E-11
15		DMSO	1517343	1516956	1515635	DMSO
16						
17						
18	%Activity					
19		Conc. (M)	DK_Y16	DK_Y16	Staurosporine	Stauro Conc. (M)
20		1.00E-04	1.50	1.79	0.18	2.00E-05
21		3.33E-05	1.63	1.44	0.16	5.00E-06
22		1.11E-05	1.55	1.56	0.23	1.25E-06
23		3.70E-06	1.54	1.62	0.41	3.13E-07
24		1.23E-06	5.56	6.19	1.05	7.81E-08
25		4.12E-07	18.08	17.18	3.99	1.95E-08
26		1.37E-07	39.89	41.43	24.19	4.88E-09
27		4.57E-08	62.73	63.21	53.26	1.22E-09
28		1.52E-08	68.04	82.74	88.12	3.05E-10
29		5.08E-09	88.73	88.32	92.60	7.63E-11
30		DMSO	96.70	96.67	96.53	DMSO
31						
32		HILLSLOPE	-0.83	-0.98	-1.14	
33		IC50 (M)	7.94E-08	9.59E-08	1.63E-09	
34						
35						

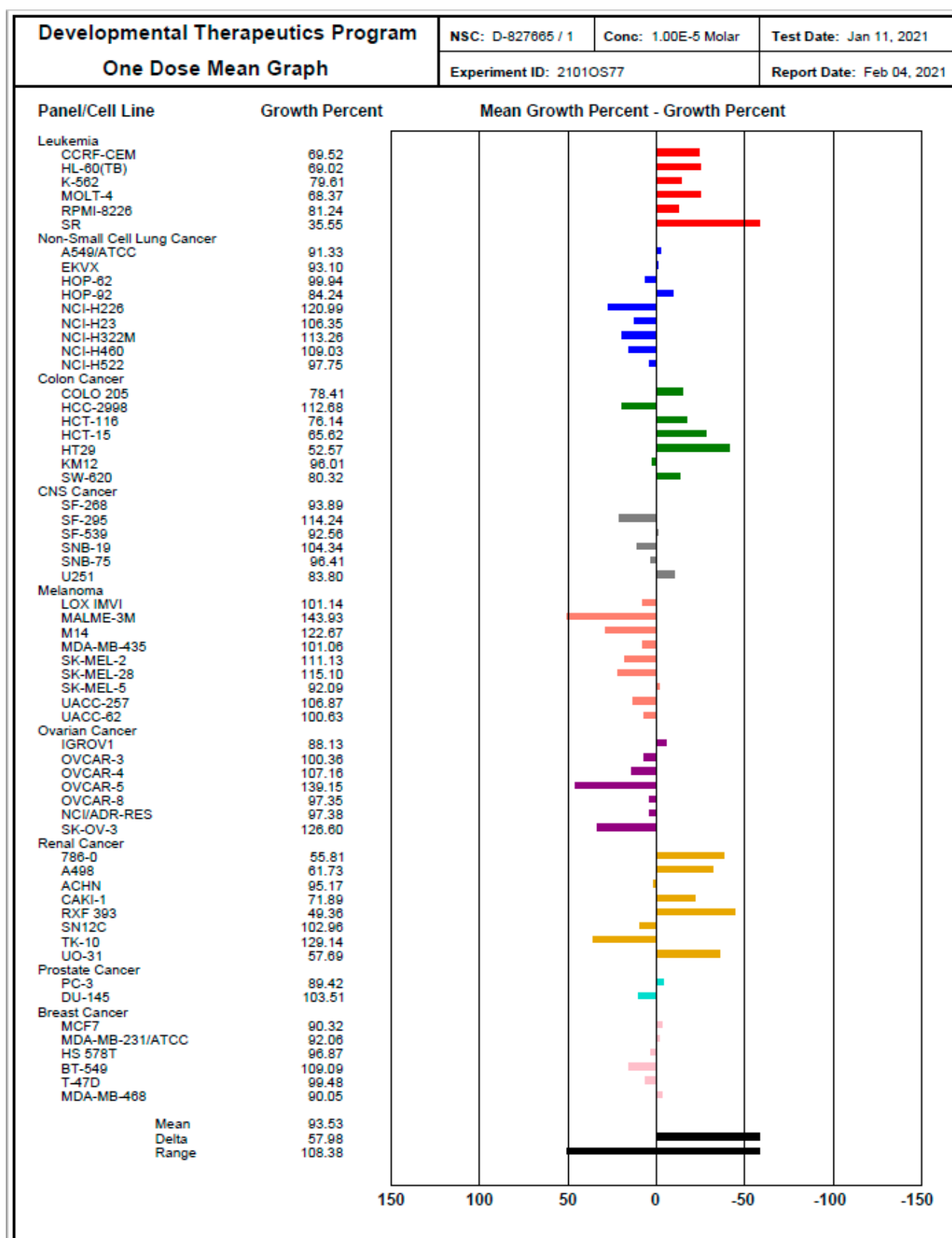


	A	B	C	D	E	F	G	H
1	Kinase Profiling Report for:			FMS				
2								
3	Raw data							
4		Conc.(M)	DK_Y2	DK_Y2	DK_Y16	DK_Y16	Staurosporine	Stauro Conc.(M)
5		1.00E-04	-97	-1909	9816	9452	-1707	2.00E-05
6		3.33E-05	2760	4094	12057	11554	-7936	5.00E-06
7		1.11E-05	10980	9617	7998	7906	-3557	1.25E-06
8		3.70E-06	39265	31468	15729	18380	-3529	3.13E-07
9		1.23E-06	76846	77042	49210	48785	-3941	7.81E-08
10		4.12E-07	177528	156344	119524	112724	7057	1.95E-08
11		1.37E-07	289596	324465	233088	208611	72113	4.88E-09
12		4.57E-08	395925	391901	341069	349825	289324	1.22E-09
13		1.52E-08	446517	436856	426845	397685	186	3.05E-10
14		5.08E-09	491433	487793	464322	466912	493584	7.63E-11
15		DMSO	478093	504603	491922	498472	525118	DMSO
16								
17								
18	%Activity						Value excluded from curve fit	
19		Conc.(M)	DK_Y2	DK_Y2	DK_Y16	DK_Y16	Staurosporine	Stauro Conc.(M)
20		1.00E-04	-0.02	-0.38	1.96	1.89	-0.34	2.00E-05
21		3.33E-05	0.55	0.82	2.41	2.31	-1.59	5.00E-06
22		1.11E-05	2.20	1.92	1.60	1.58	-0.71	1.25E-06
23		3.70E-06	7.86	6.30	3.15	3.68	-0.71	3.13E-07
24		1.23E-06	15.38	15.42	9.85	9.76	-0.79	7.81E-08
25		4.12E-07	35.53	31.29	23.92	22.56	1.41	1.95E-08
26		1.37E-07	57.96	64.94	46.65	41.75	14.43	4.88E-09
27		4.57E-08	79.24	78.44	68.26	70.02	57.91	1.22E-09
28		1.52E-08	89.37	87.43	85.43	79.59	0.04	3.05E-10
29		5.08E-09	98.36	97.63	92.93	93.45	98.79	7.63E-11
30		DMSO	95.69	100.99	98.45	99.77	105.10	DMSO
31								
32		HILLSLOPE	-0.93	-0.95	-0.91	-0.87	-1.47	
33		IC50 (M)	2.14E-07	2.12E-07	1.18E-07	1.02E-07	1.44E-09	
34								

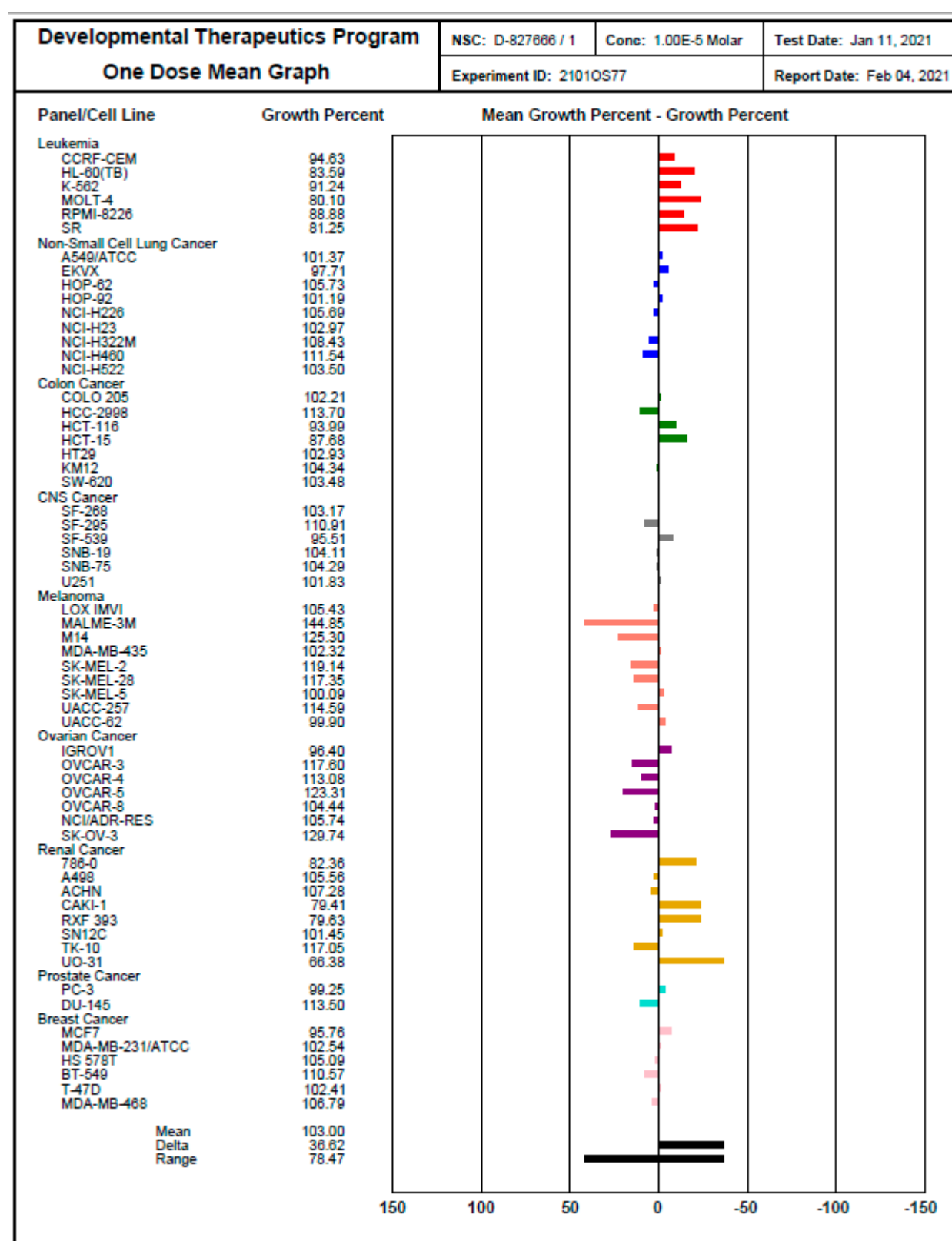


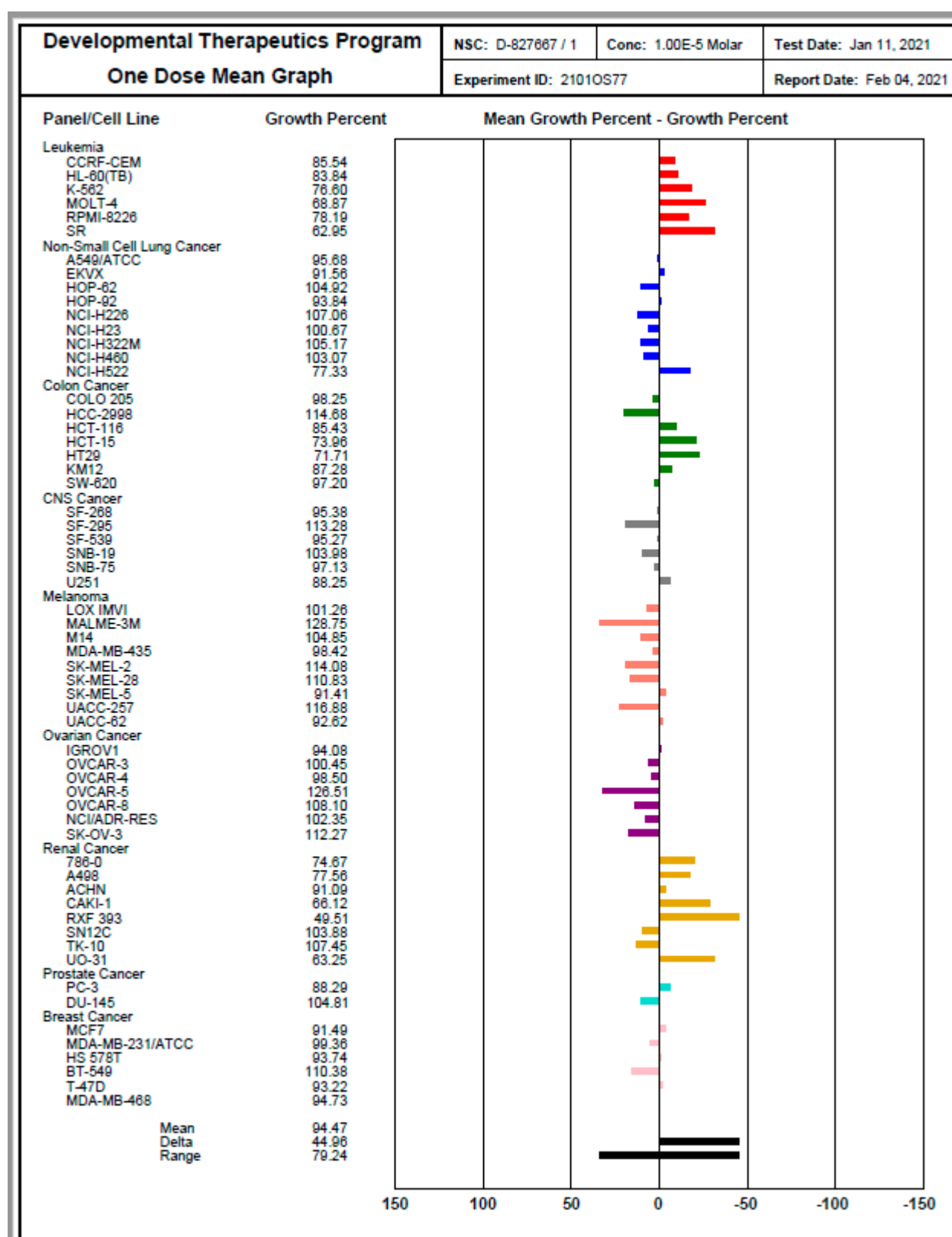
## 7. Antitumor data sheets carried out at NCI, USA.

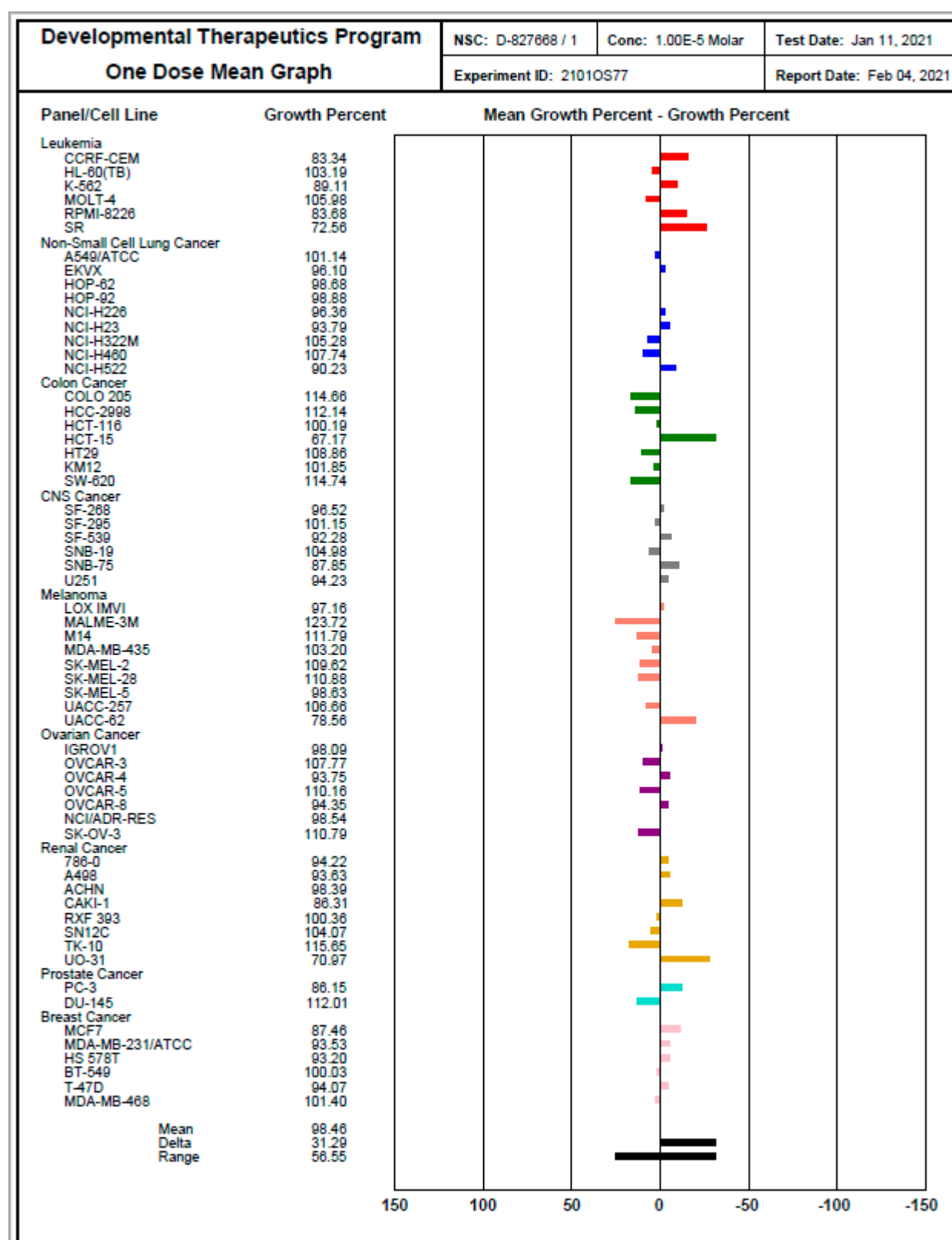


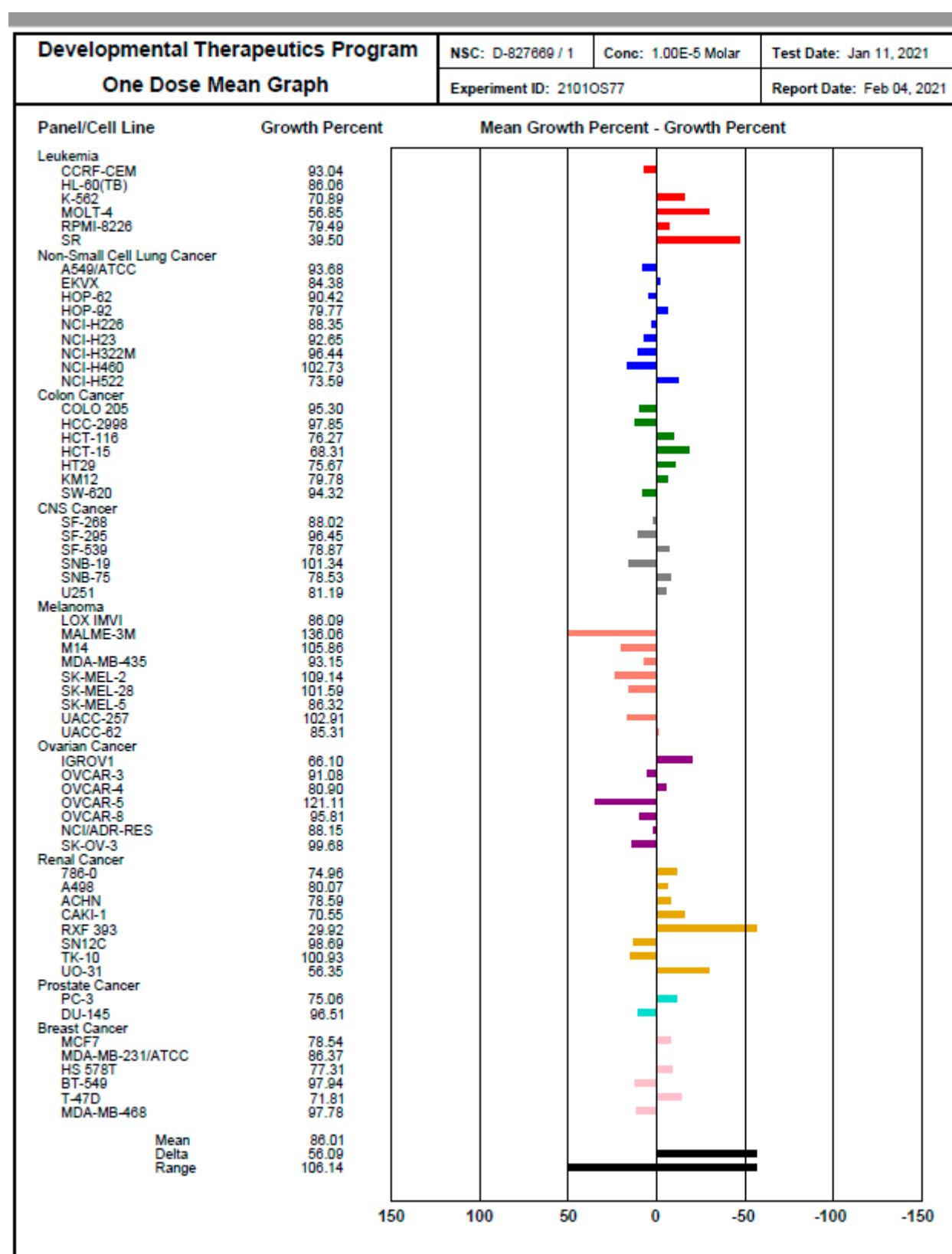


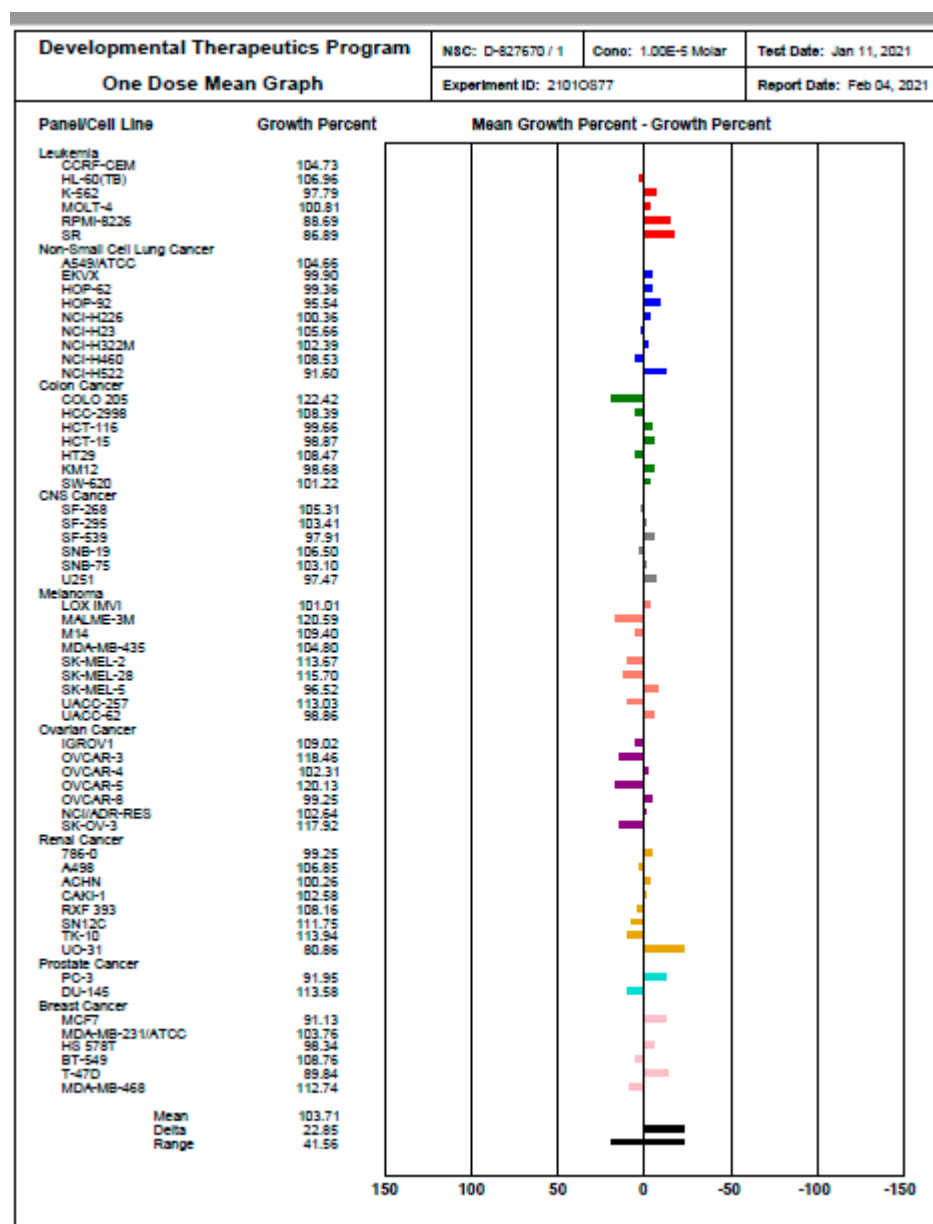












## 8. ADME (SwissADME)

Calculations were performed by the SwissADME web service <http://www.swissadme.ch> developed by the Molecular Modeling Group of the Swiss Institute of Bioinformatics that gives free access to a pool of fast yet robust predictive models for small molecules pharmacokinetic properties. (Sci. Rep. 2017, 7, 42717).