Electronic Supplementary Material (ESI) for RSC Advances.
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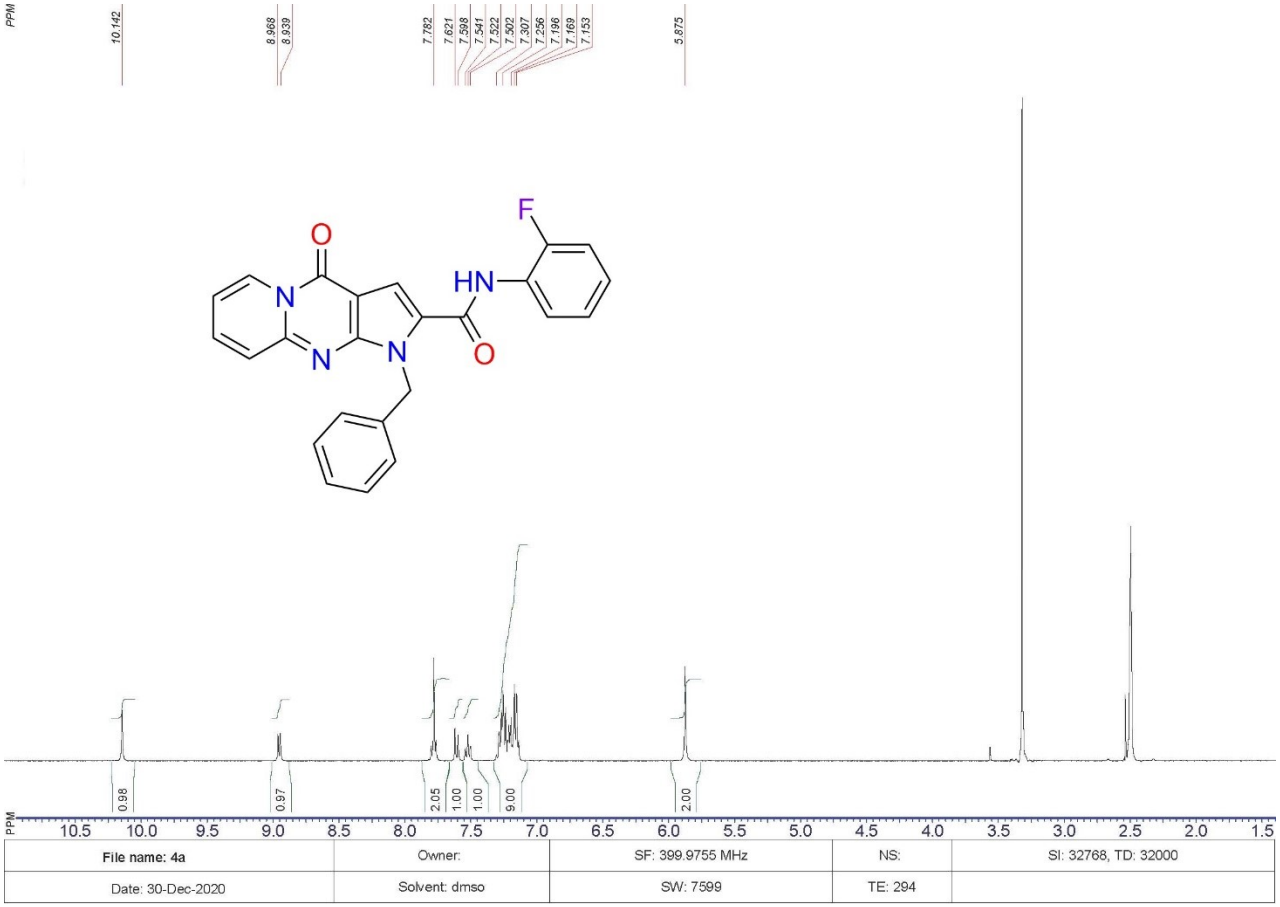
In vitro and computational investigations of novel synthetic carboxamide-linked pyridopyrrolopyrimidines with potent activity as SARS-CoV-2-M^{Pro} inhibitors

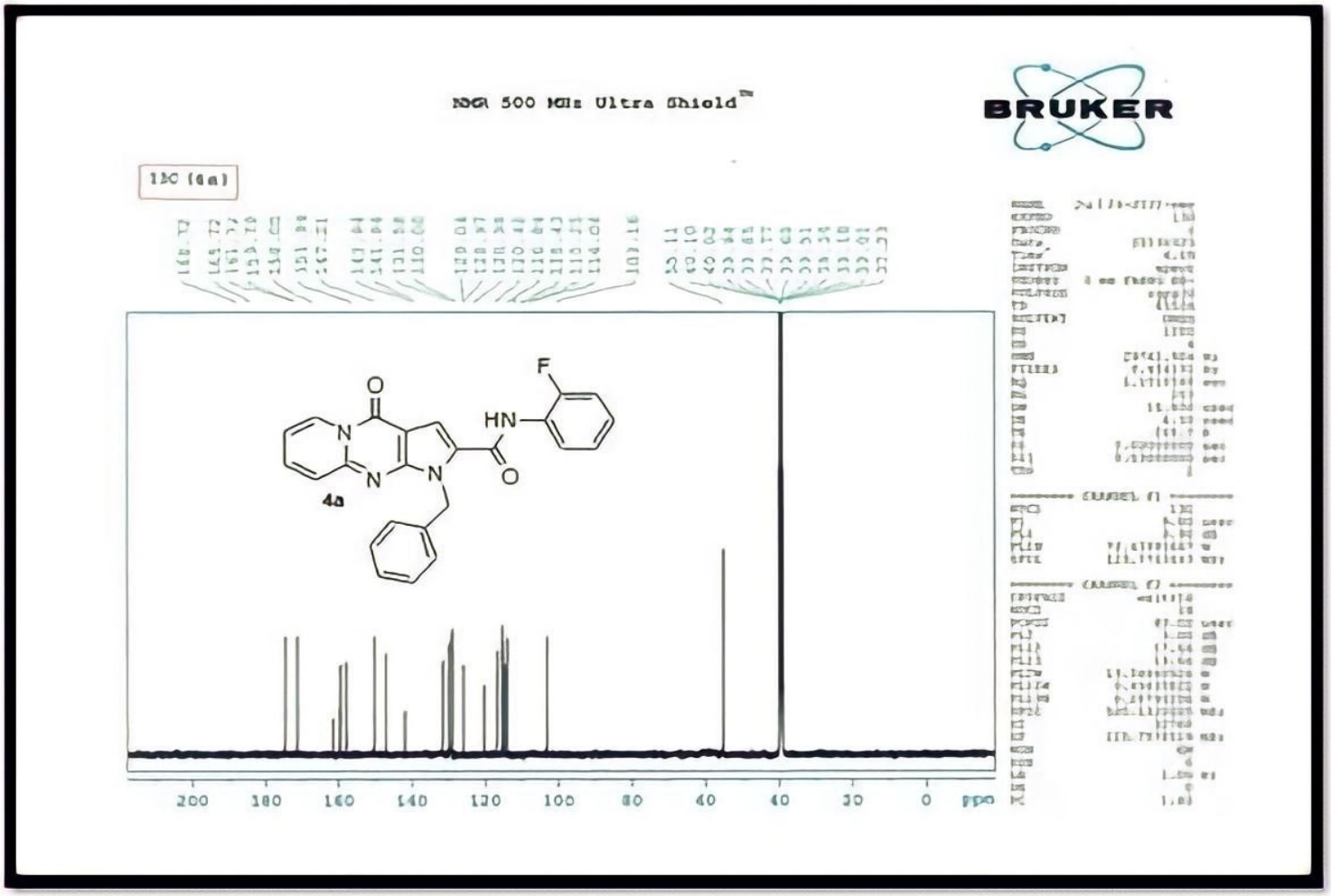
Supporting Information

Contents

1 ^{1}H NMR Spectral Data of New Compounds

1.1 Spectral data of 21

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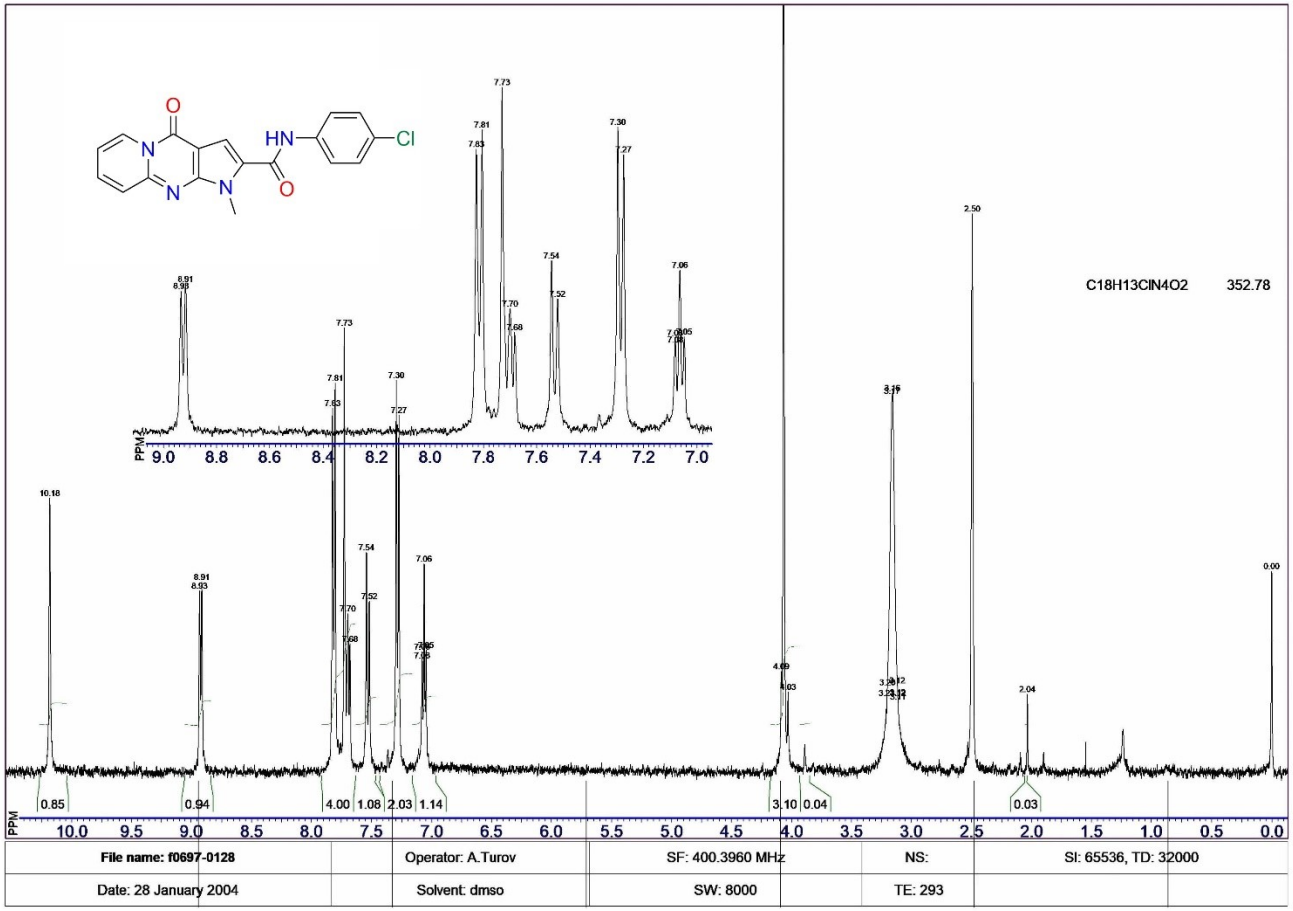
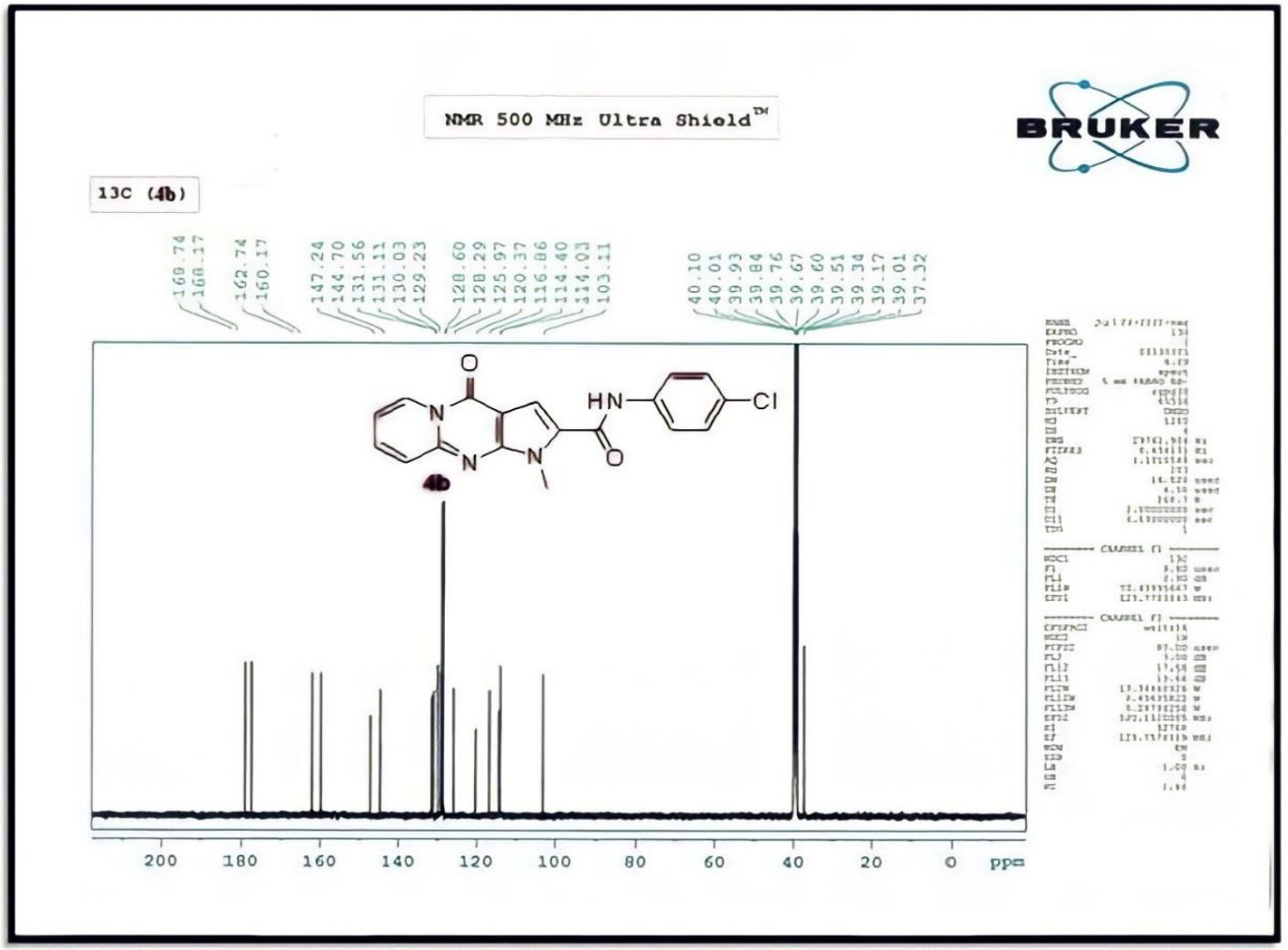
13C (6a)
O
N
N
HN
F
O
4a
200
180
160
140
120
100
80
40
60
20
0
ppm

1.2 Spectral data of 22




NMR 500 MHz Ultra Shield™
BRUKER
13C (4b)
169.74
168.17
162.74
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144.70
131.56
131.11
130.03
129.23
120.60
120.29
125.97
120.37
116.86
114.40
114.03
103.11
40.10
40.01
39.93
39.84
39.76
39.67
39.60
39.51
39.34
39.17
39.01
37.32
O
N
N
N
HN
Cl
O
4b
200
180
160
140
120
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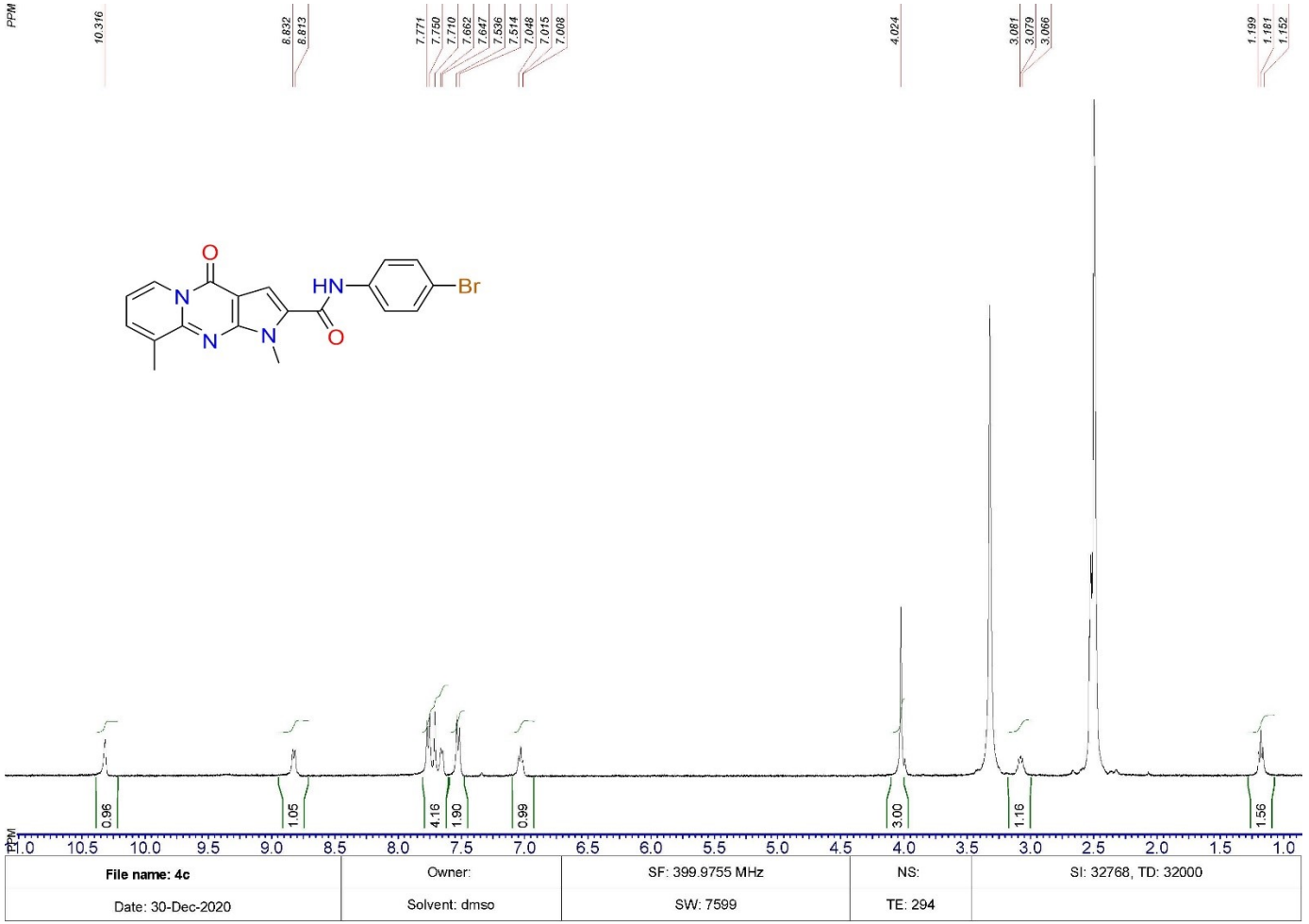
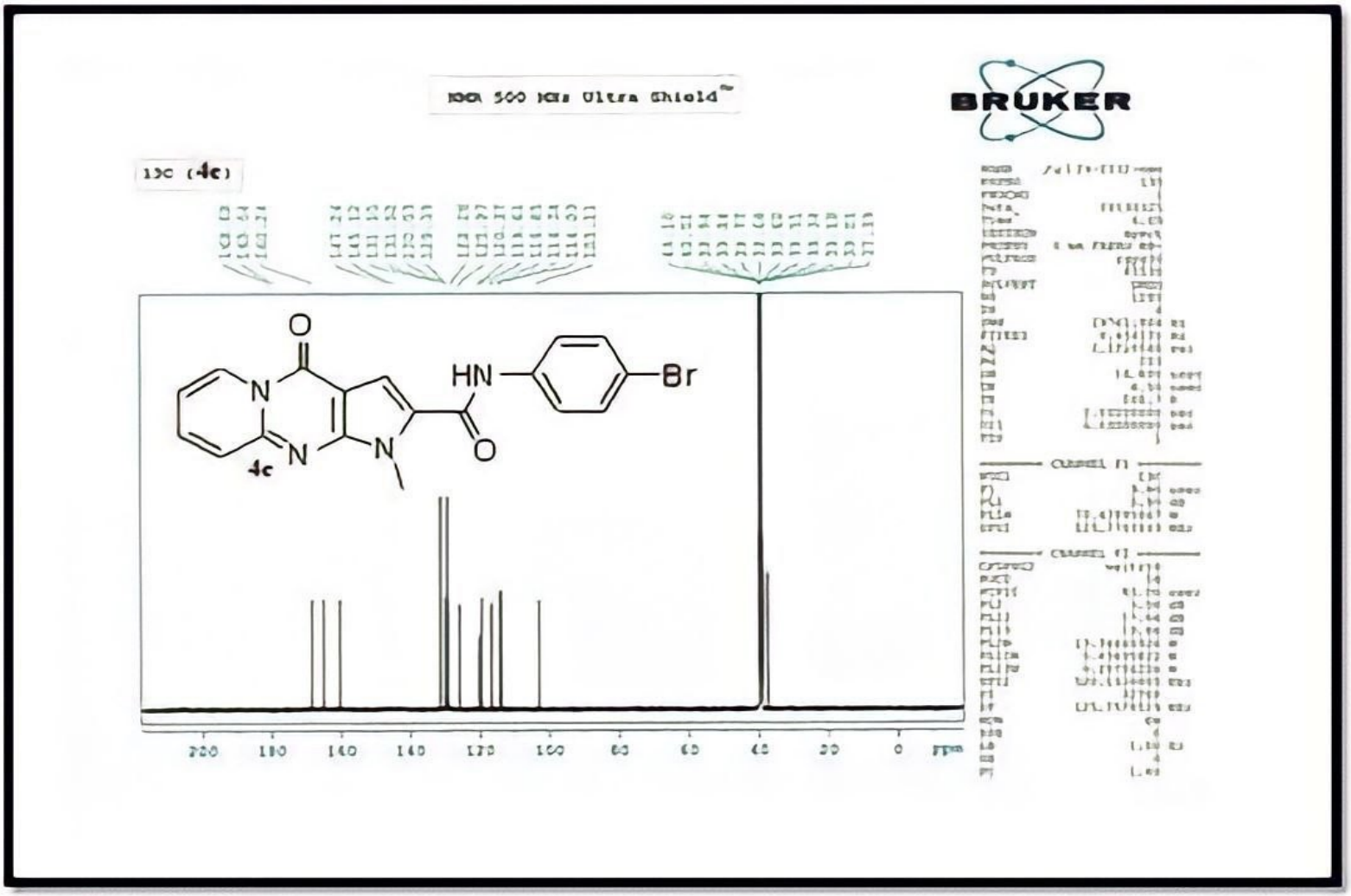
80
60
40
20
0
ppm

1.3 Spectral data of 23




BRUKER
13C (4c)
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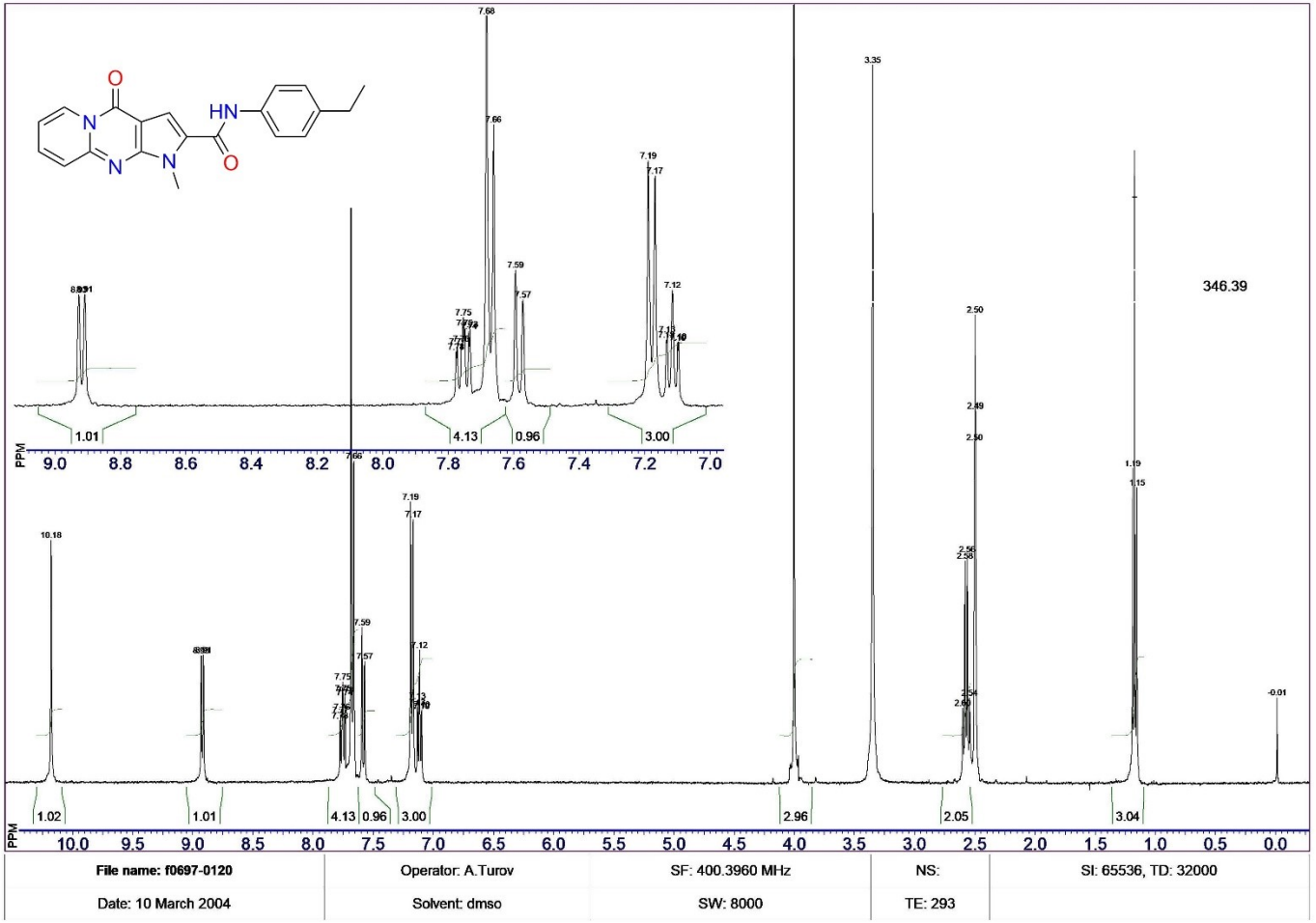
HN
Br
N
N
N
O
4c

1.4 Spectral data of 24



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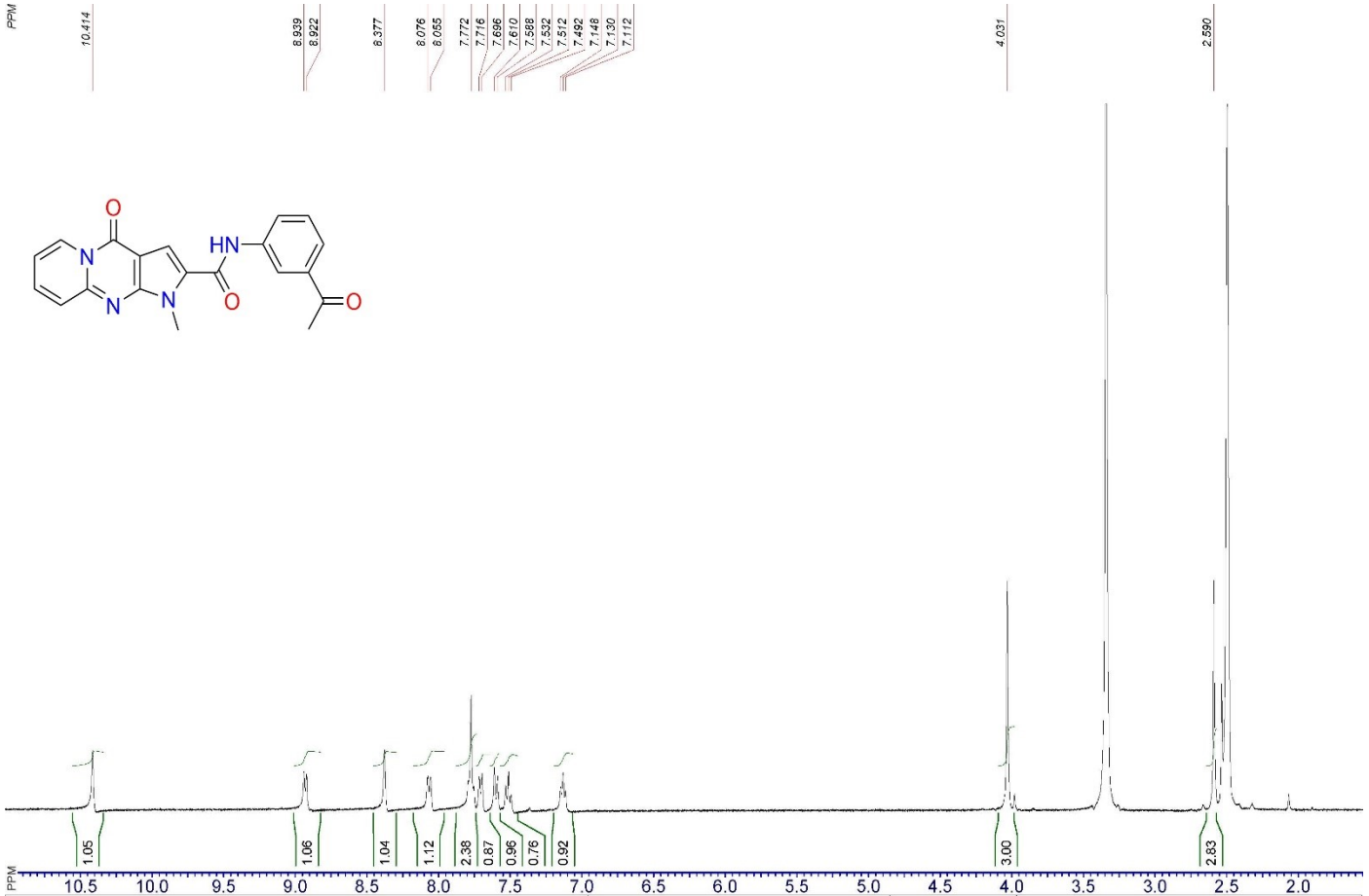

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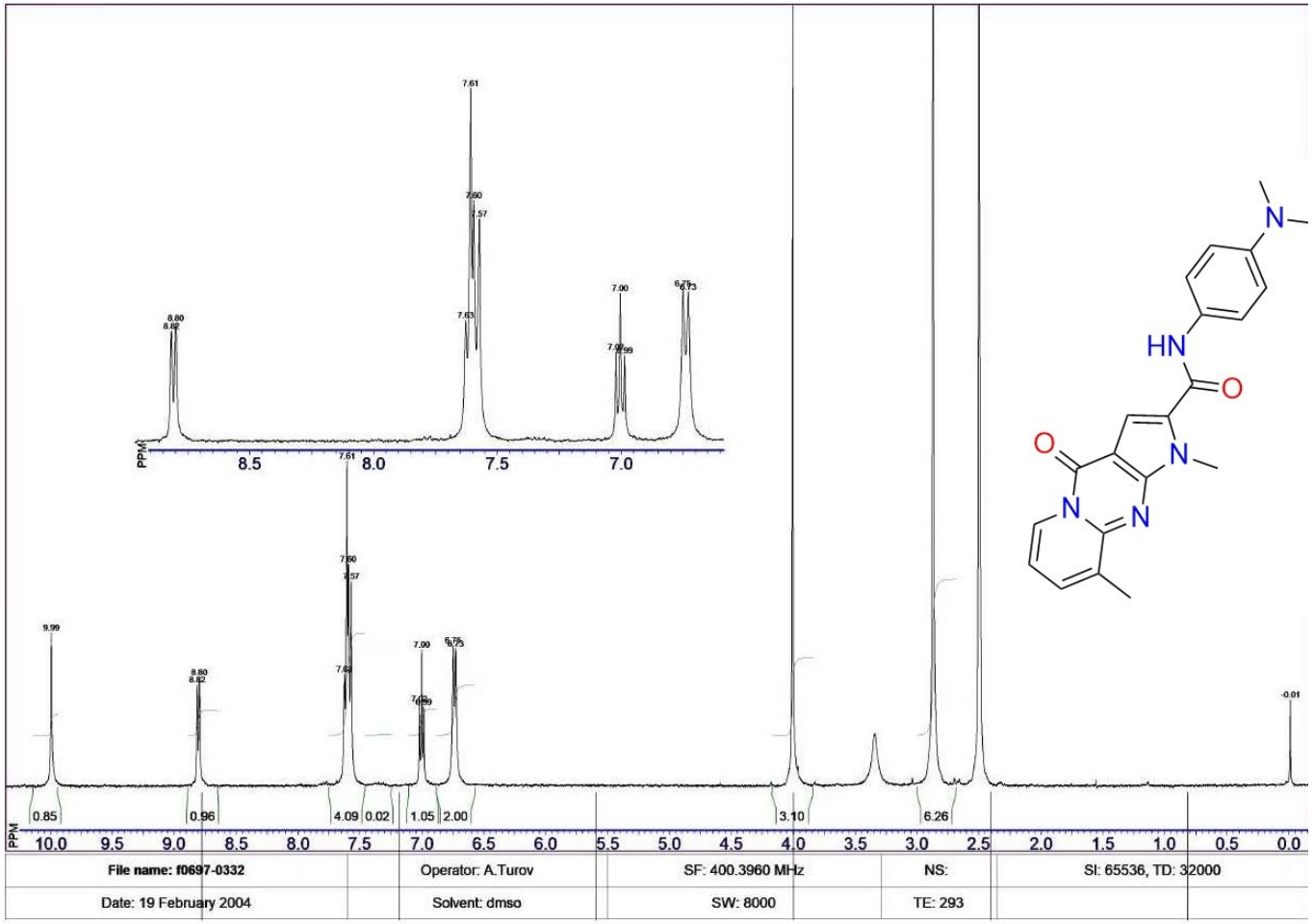
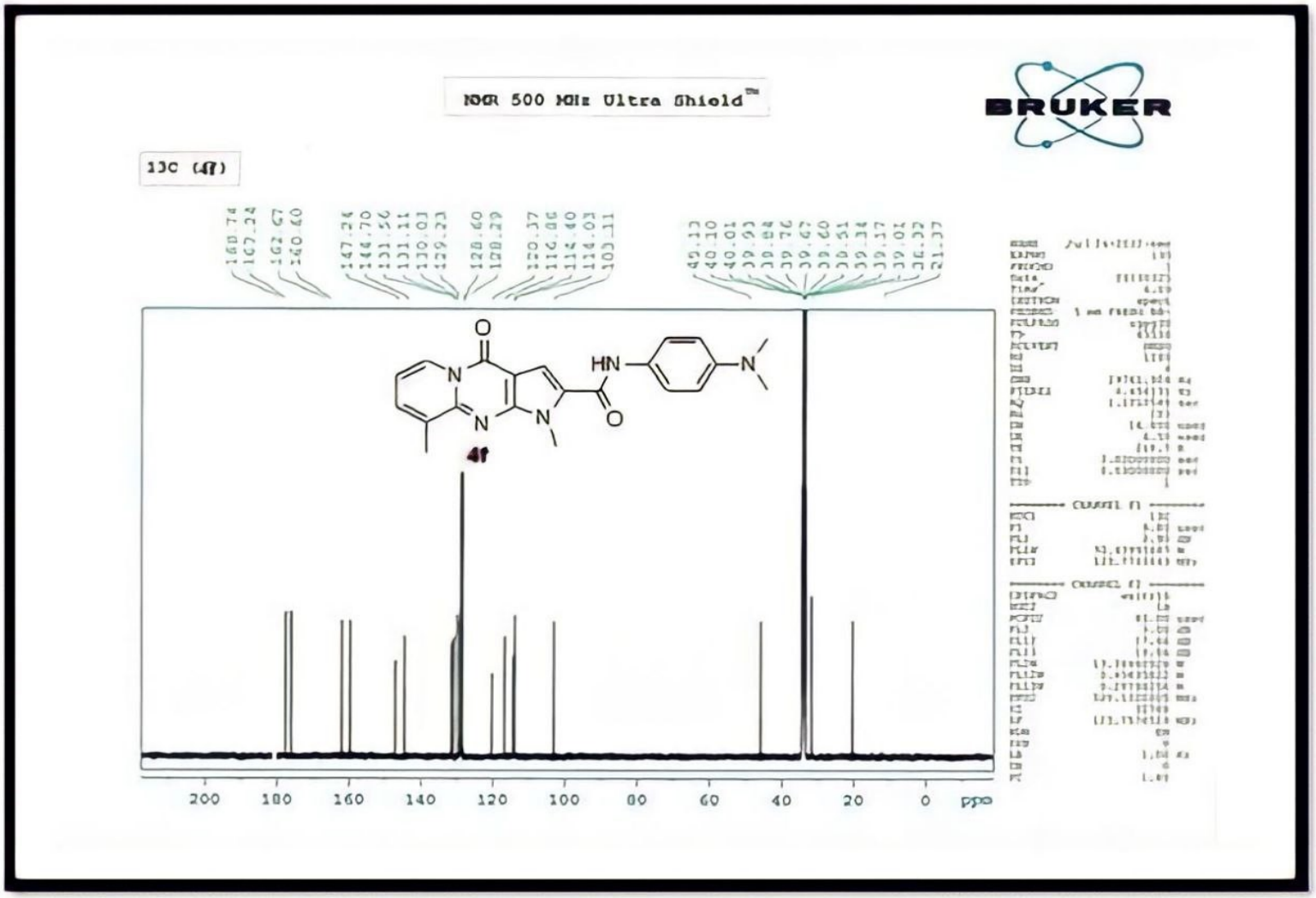
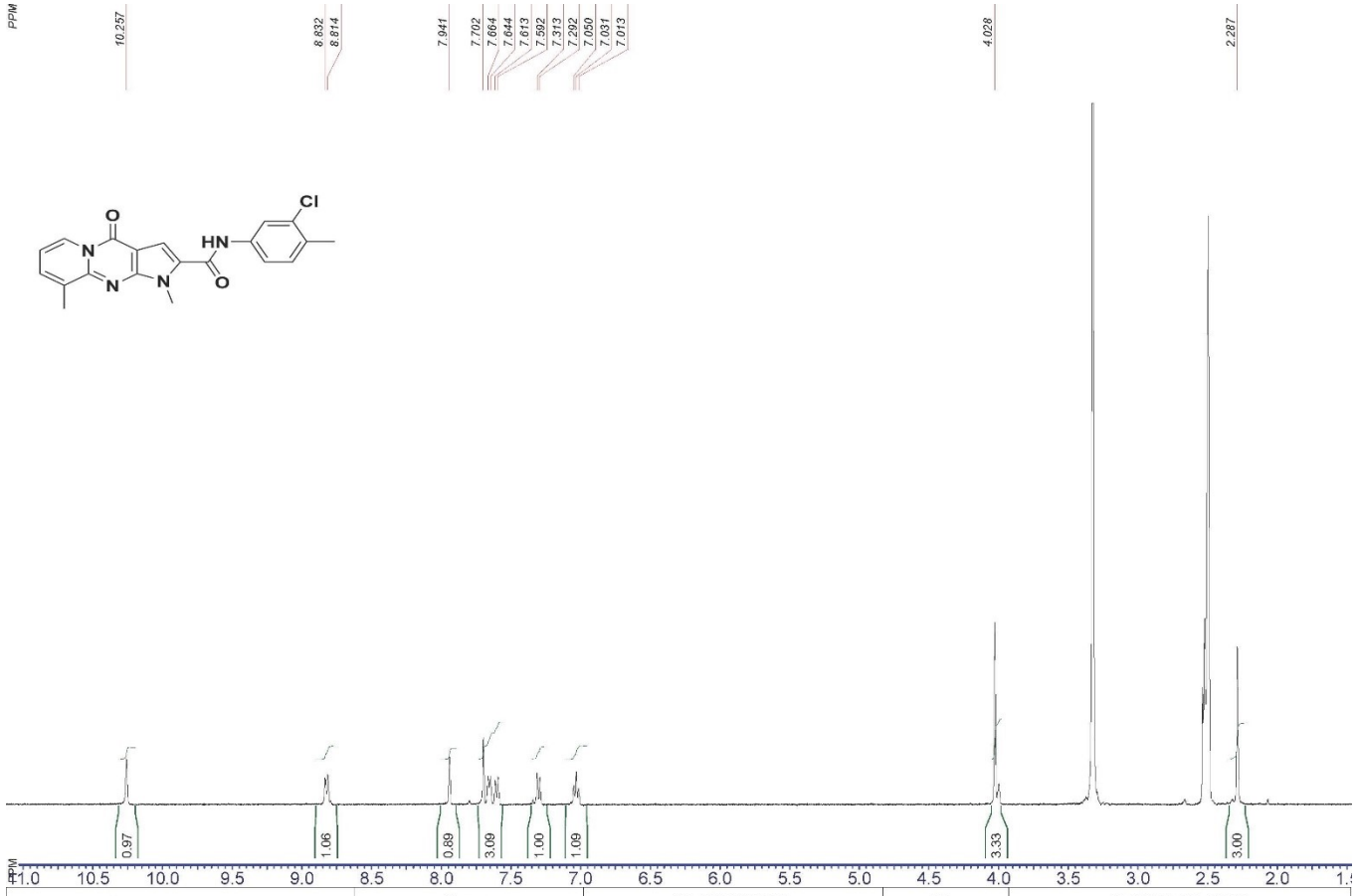
1.6 Spectral data of 26




BRUKER
200
180
160
140
120
100
80
60
40
20
0
ppm

1.7 Spectral data of 27



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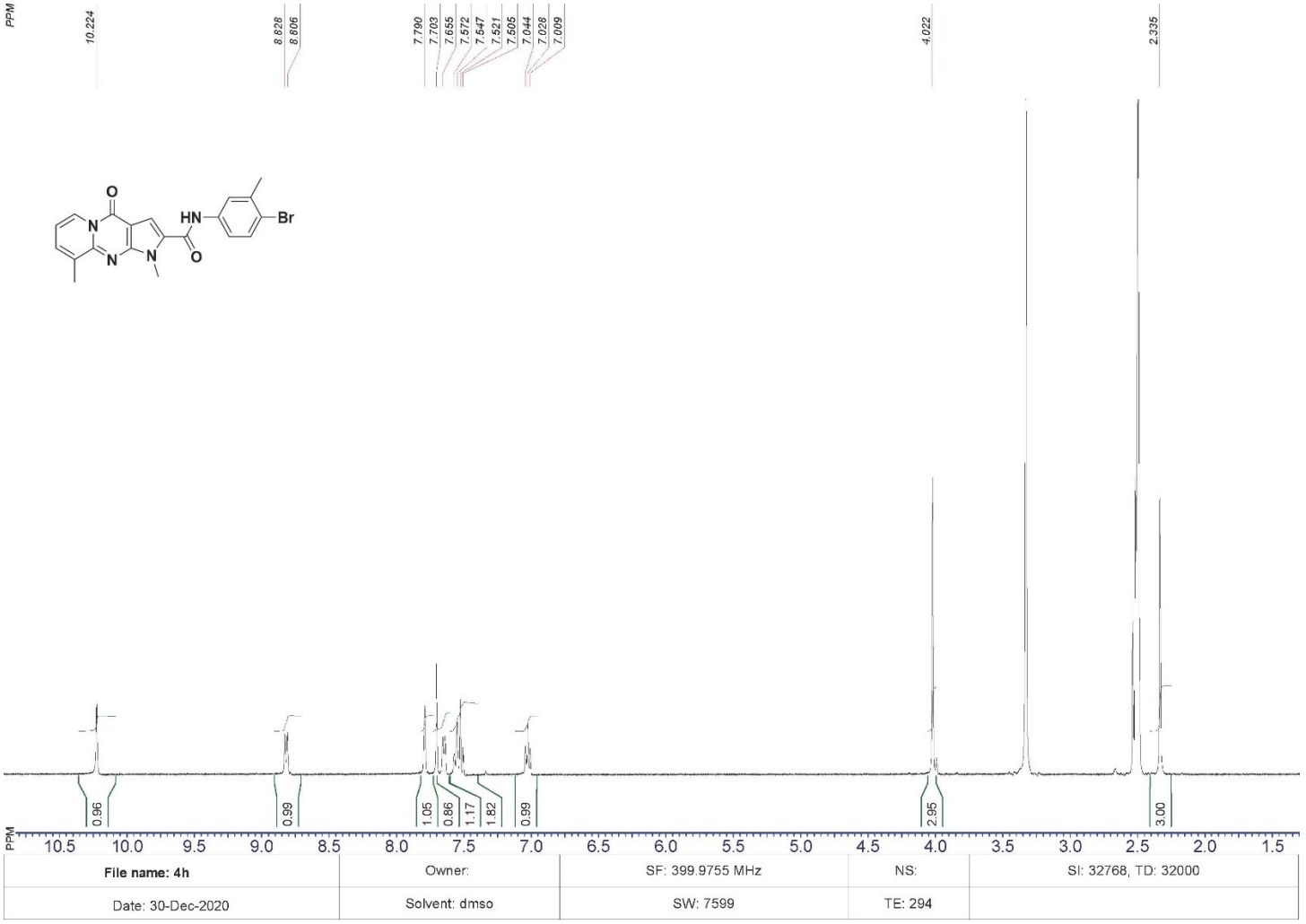
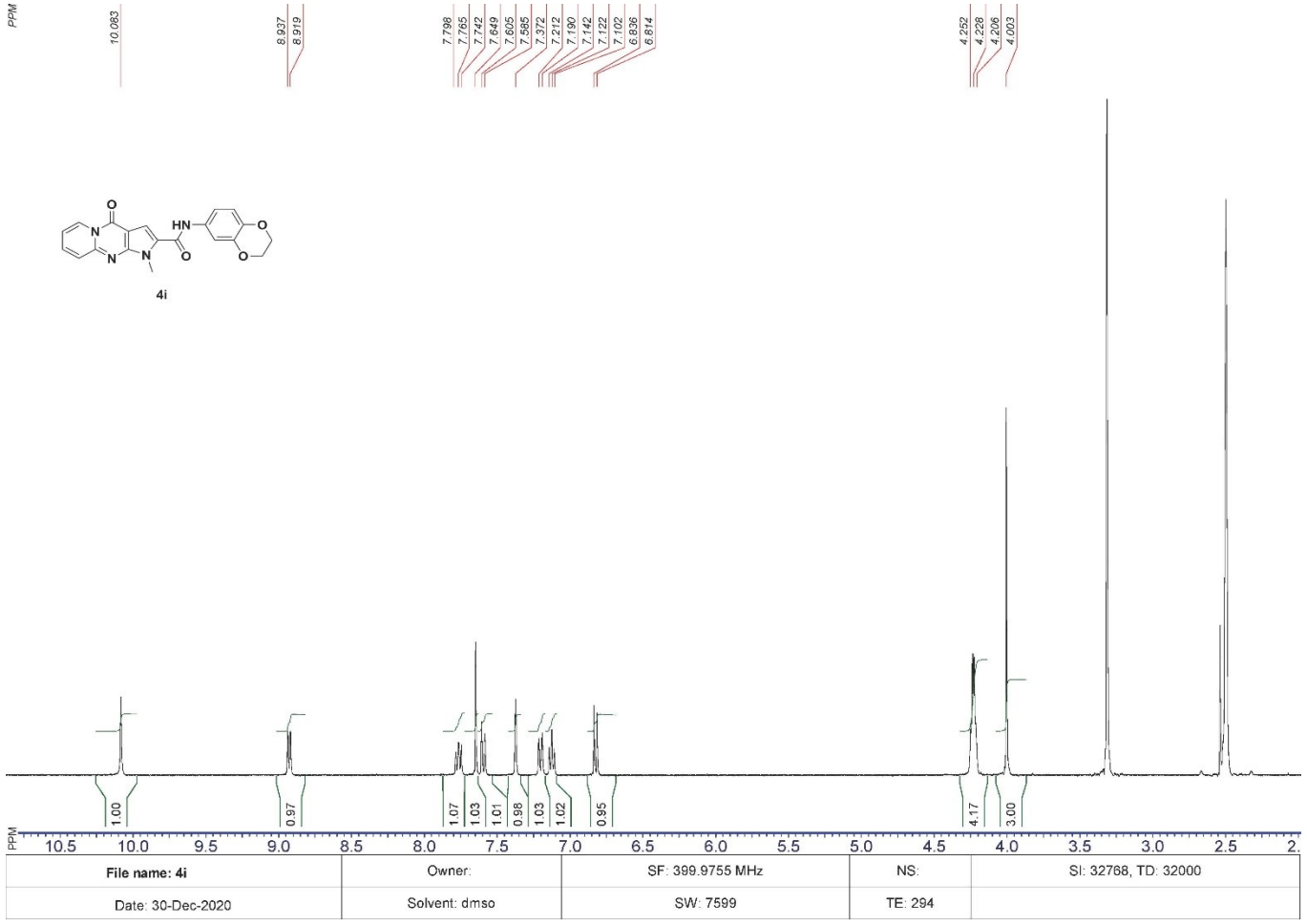


1.9 Spectral data of 29



2 Raw Data of Results of Biological Evaluation Studies

2.1 COV19 Antiviral report (EC_{50})

Researcher	: Dr.Hany Emary email: jan_25_misr@yahoo.com **mob.** 00966534391522
Assay	: Human coronavirus (COV 19) Antiviral assay
Samples	: 10 sample.
Cell lines	: Vero

Antiviral report_EC50

s		Human COVID-19 Antiviral activity			SE
	code	ID	MW	EC50 uM	
1	A1	17	333.35	22.1	1.012
2	A2	21	412.42	0.0138	0.00198
3	A3	22	352.77	0.1093	0.01105
4	A4	23	411.25	0.0216	0.00656
5	A5	24	346.36	0.0762	0.00973
6	A6	25	360.37	0.1066	0.00115
7	A7	26	375.42	0.0376	0.00423
8	A8	27	380.83	0.1035	0.00344
9	A9	28	425.28	0.108	0.00304
10	A10	29	376.37	0.0519	0.00512
11	Remdesivir	Rem	602.6	0.0099	0.00175

s	Human COVID-19							
	sample		viral Titer			Average inh	reduction	log diff
	code	conc uM	CT	IU/ml	Log	%	%	
A1	17	**10**	27.68	**40837.44764**	4.611058591	65.84874	**24.47277063**	**-1.494101931**
		1	26.41	**98717.42845**	4.994393834	62.33552	**18.19389816**	**-1.110766688**
		0.1	25.69	**162823.7493**	5.211717751	57.26799	**14.63422244**	**-0.893442771**
		0.01	23.75	**627025.1669**	5.797284972	51.88122	**5.042873957**	**-0.30787555**
		0.001	22.93	**1108645.675**	6.044792767	11.81672	**0.988798817**	**-0.060367755**
A2	21	**10**	26.76	**77401.65783**	4.888750263	96.56116	**19.92429609**	**-1.216410259**
		1	25.61	**172133.2834**	5.235864853	92.29183	**14.23870291**	**-0.869295669**
		0.1	23.95	**545653.8422**	5.736917218	87.20714	**6.031672757**	**-0.368243304**
		0.01	22.81	**1205073.177**	6.08101342	50.76645	**0.395519527**	**-0.024147102**
		0.001	22.79	**1221940.883**	6.087050195	12.32199	**0.296639653**	**-0.018110327**

A3	25	**10**	27.55	**44698.98189**	4.650297631	96.35706	**23.83005141**	**-1.454862891**
		1	26.73	**79032.44649**	4.897805426	93.65733	**19.77597627**	**-1.207355096**
		0.1	24.85	**291918.9721**	5.465262321	77.04097	**10.48126742**	**-0.639898201**
		0.01	23.56	**715540.6967**	5.85463434	43.80263	**4.103515069**	**-0.250526182**
		0.001	22.91	**1124163.661**	6.050829543	11.6221	**0.889918927**	**-0.054330979**
A4	23	**10**	26.94	**68299.77664**	4.834419283	94.40136	**20.81421503**	**-1.270741239**
		1	25.38	**201970.421**	5.305287771	84.24793	**13.10158428**	**-0.799872751**
		0.1	24.15	**474842.3688**	5.676549463	62.60013	**7.020471574**	**-0.428611059**
		0.01	23.07	**1005854.907**	6.002535339	21.05225	**1.68095798**	**-0.102625183**
		0.001	22.76	**1247686.163**	6.096105359	2.147868	**0.148319818**	**-0.009055163**

A5	24	**10**	27.05	**63272.79485**	4.801217018	95.02447	**21.35805438**	**-1.303943504**
		1	25.95	**135906.3252**	5.13323967	89.31063	**15.91966089**	**-0.971920852**
		0.1	23.78	**614086.8665**	5.788229809	51.52506	**5.191193775**	**-0.316930713**
		0.01	22.82	**1196726.842**	6.077995032	6.100991	**0.444959472**	**-0.02716549**

		0.001	22.79	**1221940.883**	6.087050195	4.084029	**0.296639653**	**-0.018110327**
A6	22	**10**	26.64	**84133.87959**	4.924970916	91.75463	**19.3310168**	**-1.180189606**
		1	25.85	**145687.9214**	5.163423547	74.74431	**15.42526149**	**-0.941736975**
		0.1	24.51	**369732.3952**	5.567887504	37.66246	**8.800309444**	**-0.537273018**
		0.01	23.27	**875321.4759**	5.942167584	8.001796	**2.669756797**	**-0.162992938**
		0.001	22.92	**1116377.705**	6.047811155	5.408329	**0.939358872**	**-0.057349367**
A7	26	**10**	26.82	**74240.33729**	4.870639936	94.15448	**20.22093574**	**-1.234520586**
		1	25.05	**254035.5908**	5.404894566	80.03899	**11.47006624**	**-0.700265956**
		0.1	24.19	**461823.3762**	5.664475912	63.59745	**7.218231337**	**-0.44068461**
		0.01	22.84	**1180207.192**	6.071958257	7.298603	**0.543839345**	**-0.033202265**
		0.001	22.77	**1239044.69**	6.093086971	2.505814	**0.197759763**	**-0.012073551**
A8	27	**10**	27.01	**65056.48119**	4.813290569	94.75049	**21.16029461**	**-1.291869953**
		1	25.71	**160576.1258**	5.205680975	87.26222	**14.73310233**	**-0.899479547**

		0.1	24.34	**416102.2803**	5.619200096	67.35924	**7.959830446**	**-0.485960426**
		0.01	23.16	**944865.1895**	5.975369849	25.78157	**2.125917452**	**-0.129790673**
		0.001	22.85	**1172033.079**	6.068939869	8.114086	**0.59327929**	**-0.036220653**

A9	29	**10**	26.38	**100797.3227**	**5.003448997**	91.75463	**18.04557834**	**-1.101711525**
		1	24.71	**321750.8845**	**5.507519749**	74.74431	**9.789108261**	**-0.597640773**
		0.1	23.41	**794163.9256**	**5.899910156**	37.66246	**3.36191596**	**-0.205250366**
		0.01	22.85	**1172033.079**	**6.068939869**	8.001796	**0.59327929**	**-0.036220653**
		0.001	22.81	**1205073.177**	**6.08101342**	5.408329	**0.395519527**	**-0.024147102**

A10	28	**10**	26.38	**100797.3227**	**5.003448997**	91.87169	**18.04557834**	**-1.101711525**
		1	24.71	**321750.8845**	**5.507519749**	74.72702	**9.789108261**	**-0.597640773**
		0.1	23.41	**794163.9256**	**5.899910156**	37.80133	**3.36191596**	**-0.205250366**
		0.01	22.85	**1172033.079**	**6.068939869**	8.19156	**0.59327929**	**-0.036220653**
		0.001	22.81	**1205073.177**	**6.08101342**	5.506479	**0.395519527**	**-0.024147102**

ref	**Remdesivir**	**10**	28.36	**25457.05872**	4.405808224	97.9982	**27.8346866**	**-1.699352298**
		1	27.11	**60688.5403**	4.783106692	95.20432	**21.65469401**	**-1.32205383**
		0.1	26.44	**96680.45161**	4.98533867	64.68271	**18.342218**	**-1.119821852**
		0.01	23.84	**589005.6799**	5.770119483	53.7732	**5.487833412**	**-0.335041039**
		0.001	22.96	1085769.415	6.035737604	14.79105	**1.137118635**	**-0.069422918**

***	**pos.cont**	---	22.81	**1273973.874**	6.105160522	**0**	**0**	**0**

Crude data of biology (readout of triplicate assay)

ID		Reading1	reading2	reading3	Average %inh	**EC50**
21	**10**	96.79448	96.11	96.779	96.56116	
	1	92.2115	92.553	92.111	92.29183	
	0.1	87.21922	87.20333	87.19887	87.20714	
	0.01	50.78195	50.59887	50.91854	50.76645	
	0.001	12.97736	11.9899	11.9987	12.32199	

ID		Reading1	reading2	reading3	Average %inh	**EC50**
25	**10**	96.491374	96.365214	96.214587	96.35706	
	1	93.796384	93.521478	93.654128	93.65733	
	0.1	77.085953	77.021458	77.015487	77.04097	
	0.01	43.833958	43.852452	43.721485	43.80263	
	0.001	11.759285	11.654125	11.452879	11.6221	

ID		Reading1	reading2	reading3	Average %inh	**EC50**
23	**10**	94.63884	94.2111154	94.3541267	94.40136	
	1	84.146424	83.9452154	84.652143	84.24793	

	0.1	62.727464	62.251463	62.8214561	62.60013	
	0.01	21.045876	21.1254621	20.9854123	21.05225	
	0.001	2.0634419	2.2546981	2.1254651	2.147868	

ID		Reading1	reading2	reading3	Average %inh	**EC50**
24	**10**	95.033431	94.9857621	95.0542181	95.02447	
	1	89.332095	89.2852164	89.3145824	89.31063	
	0.1	51.797531	51.5621482	51.2154897	51.52506	
	0.01	6.0634706	6.0852136	6.1542895	6.100991	
	0.001	4.084306	4.0952148	4.0725648	4.084029	

ID		Reading1	reading2	reading3	Average %inh	**EC50**
26	**10**	94.172538	94.185422	94.105487	94.15448	
	1	80.059592	80.035217	80.022148	80.03899	
	0.1	63.749384	63.521481	63.521485	63.59745	
	0.01	7.3601731	7.2154801	7.3201548	7.298603	
	0.001	2.7417504	2.521584	2.2541065	2.505814	

ID		Reading1	reading2	reading3	Average %inh	EC50
27	**10**	94.893421	94.8452121	94.5128413	94.75049	
	1	87.39565	87.3951248	86.9958789	87.26222	
	0.1	67.338241	67.214598	67.5248796	67.35924	
	0.01	25.833237	25.652148	25.859314	25.78157	
	0.001	8.0017964	8.2150601	8.1254004	8.114086	

ID		Reading1	reading2	reading3	Average %inh	EC50
28	**10**	92.08796	91.98583	91.541281	91.87169	
	1	74.744311	74.541287	74.895476	74.72702	
	0.1	37.662464	37.7541203	37.987412	37.80133	
	0.01	8.0017964	8.0250187	8.547866	8.19156	
	0.001	5.4083289	5.236587	5.874521	5.506479	

ID		Reading1	reading2	reading3	Average %inh	EC50
29	**10**	91.08796	92.08796	92.08796	91.75463	
	1	74.744311	74.744311	74.744311	74.74431	
	0.1	37.662464	37.662464	37.662464	37.66246	

	0.01	8.0017964	8.0017964	8.0017964	8.001796	
	0.001	5.4083289	5.4083289	5.4083289	5.408329	

ID		Reading1	reading2	reading3	Average %inh	**EC50**
22	**10**	91.08796	92.08796	92.08796	91.75463	
	1	74.744311	74.744311	74.744311	74.74431	
	0.1	37.662464	37.662464	37.662464	37.66246	
	0.01	8.0017964	8.0017964	8.0017964	8.001796	
	0.001	5.4083289	5.4083289	5.4083289	5.408329	

ID		Reading1	reading2	reading3	Average %inh	**EC50**
17	**10**	65.89448	65.66448	65.987254	65.84874	
	1	62.25122	62.50122	62.254126	62.33552	
	0.1	57.21922	57.21954	57.365214	57.26799	

	0.01	51.78195	51.8741501	51.987562	51.88122	
	0.001	11.97736	11.874595	11.598214	11.81672	

ID		Reading1	reading2	reading3	Average %inh	**EC50**
REM	**10**	97.9982	98.00176	98.005413	97.987421	
	1	95.20432	95.236281	95.138281	95.238412	
	0.1	64.68271	92.411112	92.421548	9.2154777	
	0.01	53.7732	53.766267	53.768741	53.784592	
	0.001	14.79105	14.773023	14.754129	14.845987	

* This table of data was extracted from triplicate experiments and EC50 values were calculated using the equation

Sample	Code	Conc	% inh.	Linear equation	EC50
A1	**17**	**1**	65.89448	**y = 11.83x + 34.0295**	22.1
		0	62.25122		
		-1	57.21922		
		-2	51.78195		
		-3	11.97736		
A1	**21**	**1**	96.79448	**y = 20.91x + 88.915**	0.01377
		0	92.25122		
		-1	87.21922		
		-2	50.78195		
		-3	12.97736		
		1	93.92439	**y = 26.076x + 75.491**	0.10661
		0	86.48848		
		-1	57.16915		
		-2	5.408329		

		-3	4.084306

		1	96.49137		
		0	93.79638		
		-1	77.08595	y = 21.943x + 86.536	0.021625
		-2	43.83396		
A3	23	-3	11.75929		

		1	94.63884		
		0	84.14642		
		-1	62.72746	y = 24.825x + 77.75	0.076239
		-2	21.04588		
A5	24	-3	2.063442		

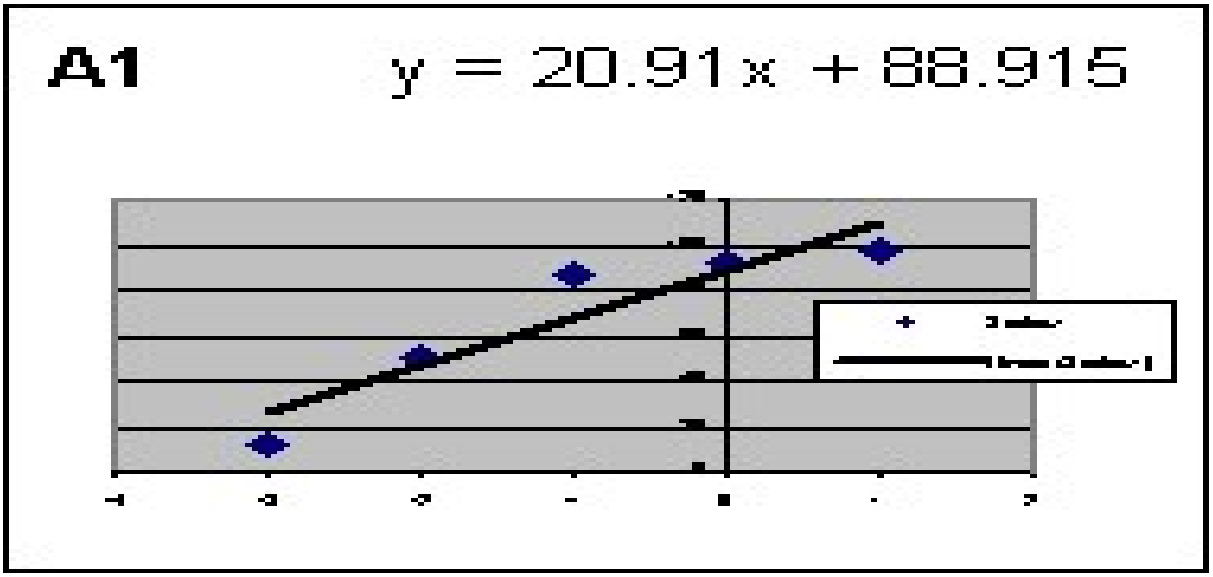
		1	95.03343		
		0	89.3321		
		-1	51.79753	y = 26.517x + 75.77	0.109318
		-2	6.063471		
A6	22	-3	4.084306		

		1	93.39595

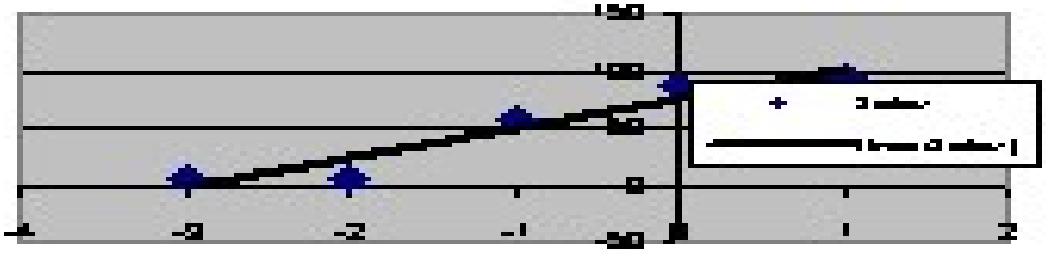
		0	88.56429	**y = 21.932x + 81.252**	
		-1	70.97802		
		-2	31.29204		
		-3	12.37044		
		1	94.17254		
		0	80.05959	**y = 25.536x + 75.173**	
		-1	63.74938		0.103511
		-2	7.360173		
A8	**27**	**-3**	2.74175		
		1	94.89342		
		0	87.39565		
		-1	67.33824		0.051959
		-2	25.83324	**y = 23.535x + 80.227**	
A9	**29**	**-3**	8.001796		
		1	92.08796		
		0	74.74431	**y = 24.01x + 67.591**	
		-1	37.66246		0.1085075

		-2	8.001796
		-3	5.408329

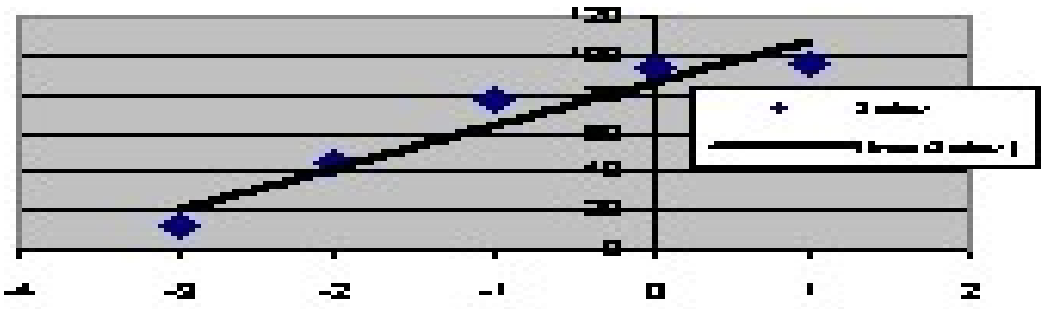
		1	98.00176		
		0	95.23628		
		-1	92.41111	**Y = 20.78*X + 86.07**	0.00591
		-2	53.76627		
ref	**Remdesivir**	**-3**	14.77302		



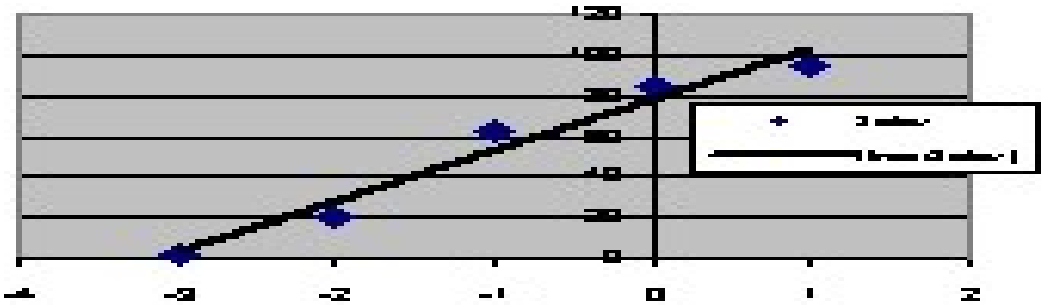
A2 $y = 26.076x + 75.491$



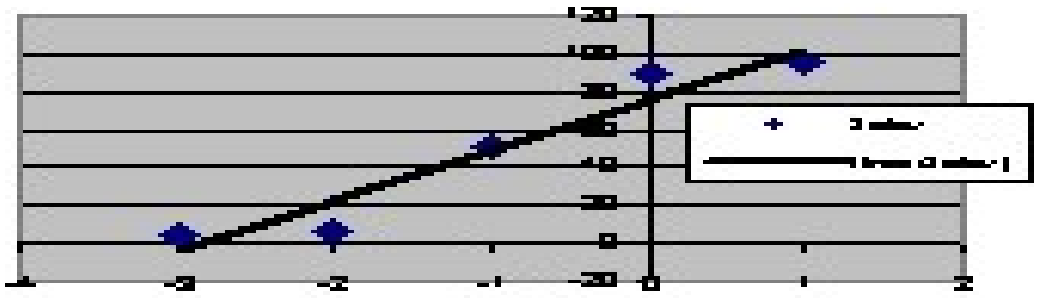
A3 $y = 21.943x + 86.536$



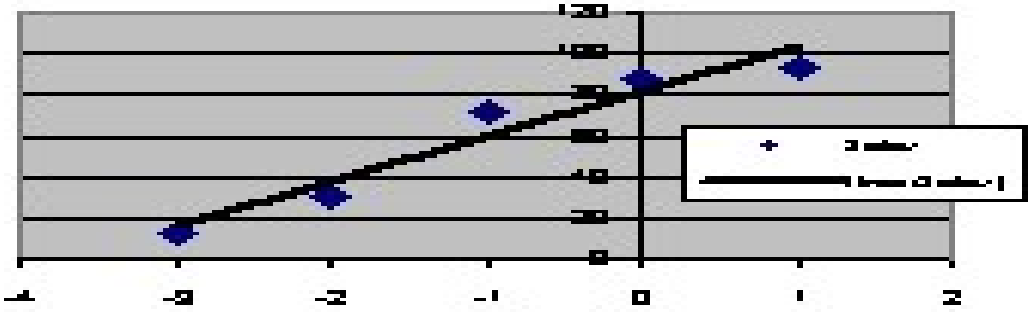
A5 $y = 24.825x + 77.75$



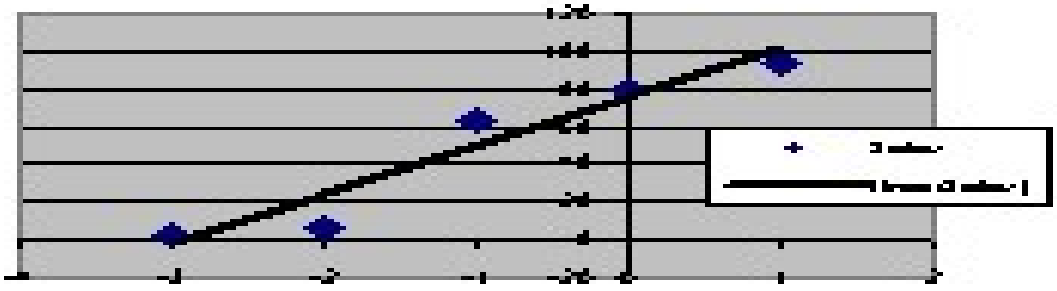
A6 $y = 26.517x + 75.779$



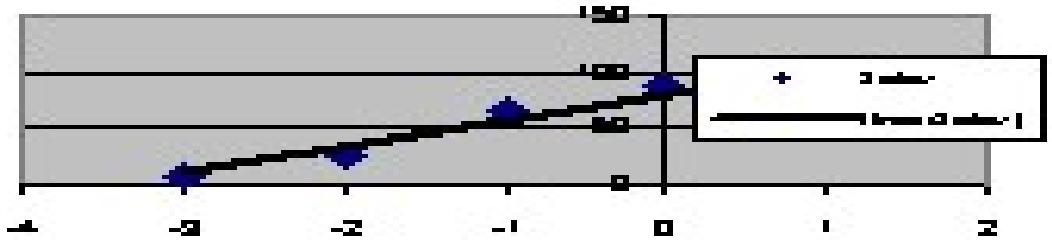
A7 $y = 21.932x + 81.252$



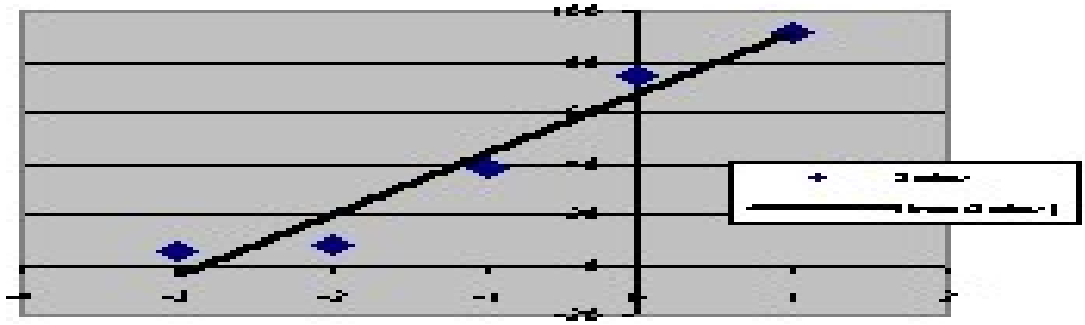
A8 $y = 25.556x + 75.173$



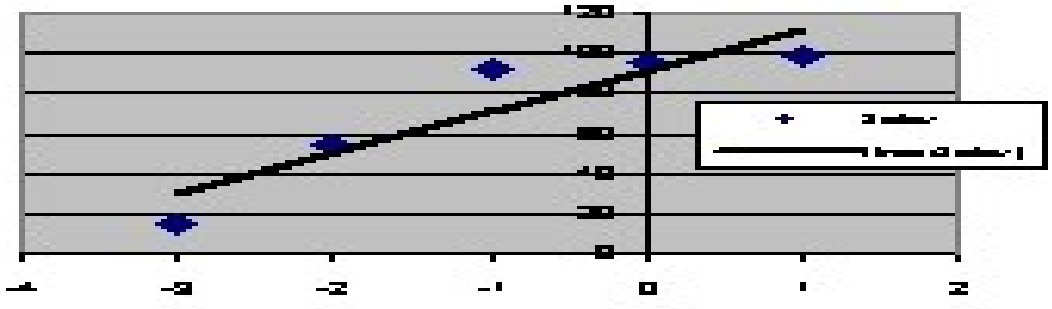
A9 $y = 23.535x + 80.227$



ST $y = 24.01x + 67.591$



Remdesivir $y = 20.793x + 91.63$



Quantitation Report

Experiment Information

Run Name	2021-12-08 (1)
Run Start	08/12/2021 06:12:15 PM
Run Finish	08/12/2021 08:46:29 PM
Operator	NE
Notes	
Run On Software Version	Rotor-Gene 1.7.87
Run Signature	The Run Signature is valid.
Gain Green	5.
Gain Yellow	5.

Quantitation data

Legend:

NEG (NTC) - Sample cancelled due to NTC Threshold.

NEG (R. Eff) - Sample cancelled as efficiency less than reaction efficiency threshold.

This report generated by Rotor-Gene 6000 Series Software 1.7 (Build 87)
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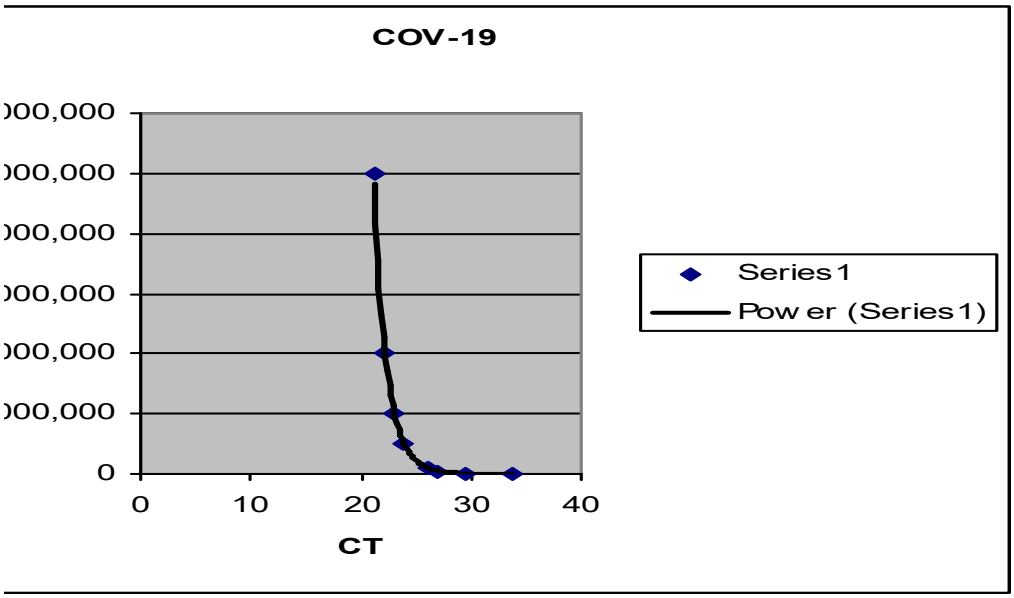
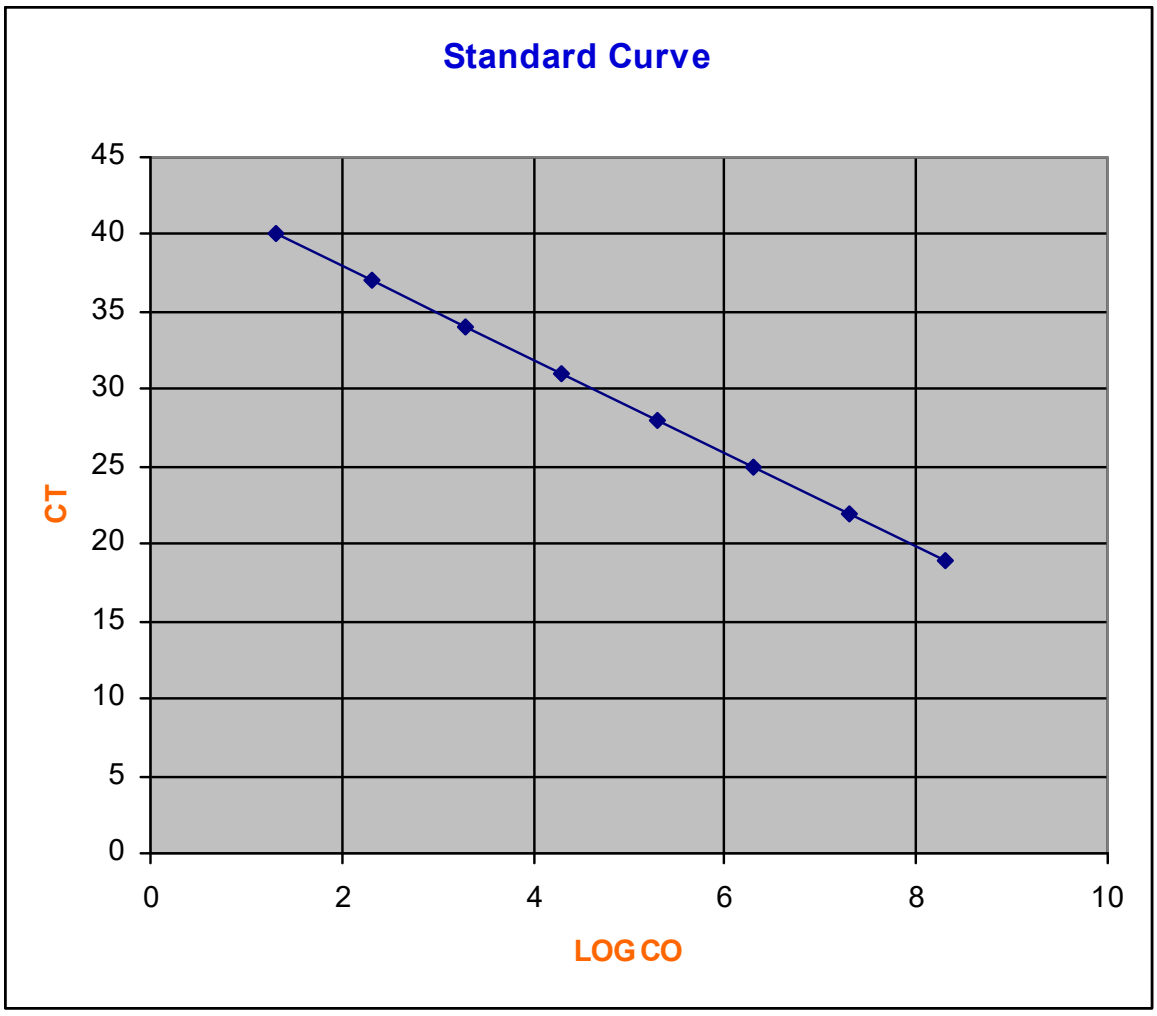
ISO 9001:2000 (Reg. No. QEC21313)



CT	V.Titer
21.18	5,000,000
22.26	2,000,000
23.07	1,000,000
23.91	500,000
26.04	100,000
26.98	50,000
29.44	10,000
33.62	1,000

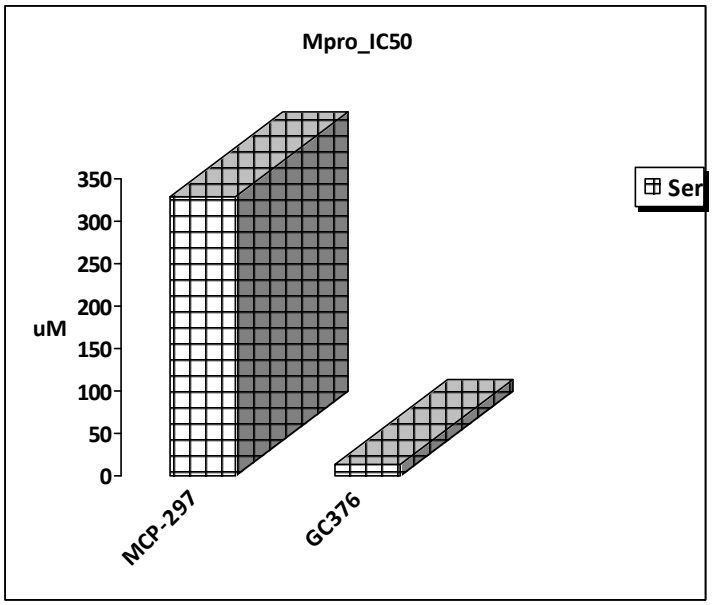

Standard Curve
45
40
35
30
25
20
15
10
5
0
CT
0
2
4
6
8
10
LOG CO

2.2 SARS-CoV-2-M^{Pro} inhibition assay(GC376)

Researcher	: Dr.Hany Emary email: jan_25_misr@yahoo.com *mob.* +966534391522
Assay	: COV-3CL protease assay
Samples	: 01 compounds.
Cell line	: ---
Reference	: ---

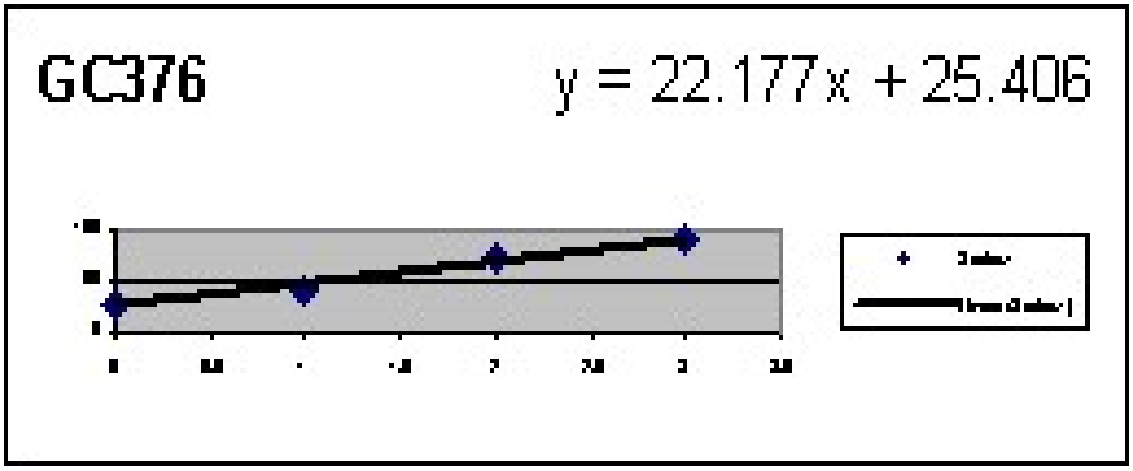
Lab Report I

ser	Compound		Results	
	code	MW g/mol	COV-3CL protease IC50 uM	SD ±
***	GC376	507.53	12.85	0.74



Detailed results

code	IC50	conc.uM	log conc	%inh	T2	T1	ΔT	RFU2	RFU1	ΔRFU	slope	K.Activity
GC376		1000	3	91	30	0	30	8.63	0	8.63	3.3333	10.3561
		100	2	73	30	0	30	26.54	0	26.54	3.3333	31.8483
		10	1	42	30	0	30	58.11	0	58.11	3.3333	69.7327
		1	0	28	30	0	30	72.03	0	72.03	3.3333	86.4369
EC				0	30	0	30	100	0	100	3.3333	120

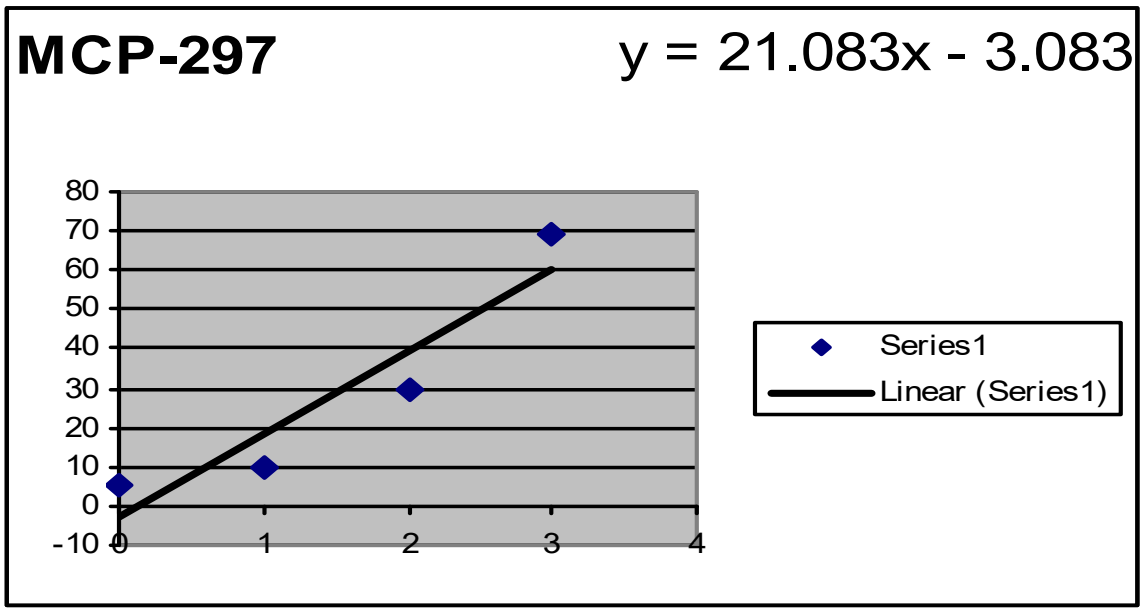


2.3 SARS-CoV-2-M^{Pro} inhibition assay (Lopinavir)

Researcher	: Dr.Hany Emary email: jan_25_misr@yahoo.com *mob.* +966534391522
Assay	: COV-3CL protease assay
Samples	: 01 compounds.
Cell line	: ---
Reference	: ---

Lab Report I

ser	Compound		Results	
	code	MW g/mol	COV-3CL protease IC50 uM	SD ±
***	**Lopinavir**	720.94	82.17	7.66



Detailed results

Mpro												
code	IC50	conc	conc	%inh	T2	T1	ΔT	RFU2	RFU1	ΔRFU	slope	K.Activity
Lopinavir		1000	3	69	30	0	30	31.18	0	31.18	3.3333	37.4164
		100	2	30	30	0	30	70.02	0	70.02	3.3333	84.0248
		10	1	10	30	0	30	89.75	0	89.75	3.3333	107.701
		1	0	5.1	30	0	30	94.88	0	94.88	3.3333	113.857
EC				0	30	0	30	100	0	100	3.3333	120

2.4 SARS-CoV-2-M^{Pro} inhibition assay of new compounds

Researcher	: Dr.Hany Emary email: jan_25_misr@yahoo.com *mob.* +966534391522
Assay	: COV-3CL protease assay
Samples	: 10 compounds.
Cell line	: ---
Reference	: ---

Lab Report I

ser	Compound		Results	
	code	MW g/mol	COV-3CL protease IC50 uM	SD ±
1	17	333.11	329.5	15.5
2	21	412.42	25.36	2.4
3	22	352.78	236.5	4.29
4	23	360.37	65.92	12.3
5	24	346.39	82.17	15
6	25	411.26	5.426	21.3
7	26	376.37	407.5	3.44
8	27	380.83	165.2	16.7
9	28	425.29	45.96	8.62
10	29	375.43	3.22	2.83

***	Lopinavir	720.94	82.17	7.66
***	GC376	507.53	12.85	0.74

Detailed results

		Equation										
Mpro												
code	IC50	conc	conc	%inh	T2	T1	ΔT	RFU2	RFU1	ΔRFU	slope	K.Activity
29 Y=20.494x+39.507		1000	3	96	30	0	30	31.03	0	31.03	3.3333	37.2364
		100	2	71	30	0	30	69.22	0	69.22	3.3333	83.0648
		10	1	42	30	0	30	83.46	0	83.46	3.3333	100.153
		1	0	31	30	0	30	92.72	0	92.72	3.3333	111.265
EC				0	30	0	30	100	0	100	3.3333	120
code	IC50	conc.uM	log conc	%inh	T2	T1	ΔT	RFU2	RFU1	ΔRFU	slope	K.Activity
24 Y=21.541x+8.7551		1000	3	76	30	0	30	23.62	0	23.62	3.3333	28.3443
		100	2	49	30	0	30	51.44	0	51.44	3.3333	61.7286
		10	1	28	30	0	30	72.15	0	72.15	3.3333	86.5809
		1	0	11	30	0	30	88.5	0	88.5	3.3333	106.225

					0			2		2		
					3							
EC				0	0	0	30	100	0	100	3.3333	120
	[redacted]		[redacted]		[redacted]		[redacted]		[redacted]	[redacted]		[redacted]
code	IC50	conc.uM	log conc	%inh	T2	T1	ΔT	RFU2	RFU1	ΔRFU	slope	K.Activity
22												
Y=21.211x-0.35		1000	3	72	3 0	0	30	28.31	0	28.31	3.3333	33.9723
		100	2	33	3 0	0	30	67.03	0	67.03	3.3333	80.4368
		10	1	14	3 0	0	30	86.15	0	86.15	3.3333	103.381
		1	0	7.4	3 0	0	30	92.64	0	92.64	3.3333	111.169
EC				0	3 0	0	30	100	0	100	3.3333	120
	[redacted]		[redacted]		[redacted]		[redacted]		[redacted]	[redacted]		[redacted]
code	IC50	conc.uM	log conc	%inh	T2	T1	ΔT	RFU2	RFU1	ΔRFU	slope	K.Activity
23												
Y=21.829x+10.293		1000	3	80	3 0	0	30	19.83	0	19.83	3.3333	23.7962
		100	2	50	3 0	0	30	49.51	0	49.51	3.3333	59.4126
		10	1	26	3 0	0	30	74.12	0	74.12	3.3333	88.9449
		1	0	16	3 0	0	30	84.39	0	84.39	3.3333	101.269
EC				0	3 0	0	30	100	0	100	3.3333	120
	[redacted]		[redacted]		[redacted]		[redacted]		[redacted]	[redacted]		[redacted]

code	IC50	conc.uM	log conc	%inh	T2	T1	ΔT	RFU2	RFU1	ΔRFU	slope	K.Activity
26												
Y=23.948x-12.50		1000	3	64	30	0	30	36.29	0	36.29	3.3333	43.5484
		100	2	33	30	0	30	66.91	0	66.91	3.3333	80.2928
		10	1	8.7	30	0	30	91.34	0	91.34	3.3333	109.609
		1	0	3.5	30	0	30	96.46	0	96.46	3.3333	115.753
EC				0	30	0	30	100	0	100	3.3333	120
	[redacted]		[redacted]		[redacted]		[redacted]		[redacted]	[redacted]		[redacted]

code	IC50	conc.uM	log conc	%inh	T2	T1	ΔT	RFU2	RFU1	ΔRFU	slope	K.Activity
17												
Y=22.785x-7.349		1000	3	62	30	0	30	16.26	0	16.26	3.3333	19.5122
		100	2	33	30	0	30	46.33	0	46.33	3.3333	55.5966
		10	1	18	30	0	30	72.51	0	72.51	3.3333	87.0129
		1	0	10	30	0	30	87.36	0	87.36	3.3333	104.833
EC				0	30	0	30	100	0	100	3.3333	120
	[redacted]		[redacted]		[redacted]		[redacted]		[redacted]	[redacted]		[redacted]

code	IC50	conc.uM	log conc	%inh	T2	T1	ΔT	RFU2	RFU1	ΔRFU	slope	K.Activity
21												
Y=21.525x+19.649		1000	3	71	30	0	30	28.92	0	28.92	3.3333	34.7043

					30			73.22		73.22		
		100	2	27	0	0	30		0		3.3333	87.8649
					30			89.61		89.61		
		10	1	10	0	0	30		0		3.3333	107.533
					30			97.13		97.13		
		1	0	2.9	0	0	30		0		3.3333	116.557
					30							
EC				0	0	0	30	100	0	100	3.3333	120

code	IC50	conc.uM	log conc	%inh	T2	T1	ΔT	RFU2	RFU1	ΔRFU	slope	K.Activity
27												
Y=20.858x+3.736		1000	3	75	30	0	30	24.51	0	24.51	3.3333	29.4123
					30			63.27		63.27		
		100	2	37	0	0	30		0		3.3333	75.9248
					30			84.13		84.13		
		10	1	16	0	0	30		0		3.3333	100.957
					30			93.56		93.56		
		1	0	6.4	0	0	30		0		3.3333	112.273
					30							
EC				0	0	0	30	100	0	100	3.3333	120

code	IC50	conc.uM	log conc	%inh	T2	T1	ΔT	RFU2	RFU1	ΔRFU	slope	K.Activity
28												
Y=21.665x+14.036		1000	3	82	30	0	30	17.94	0	17.94	3.3333	21.5282
					30			41.46		41.46		
		100	2	59	0	0	30		0		3.3333	49.7525
					30			69.85		69.85		
		10	1	30	0	0	30		0		3.3333	83.8208

		1	0	14	30	0	30	85.82	0	85.82	3.3333	102.985
EC				0	30	0	30	100	0	100	3.3333	120
code	IC50	conc.uM	log conc	%inh	T2	T1	ΔT	RFU2	RFU1	ΔRFU	slope	K.Activity
21 **Y=21.525x+19.649**		1000	3	91	30	0	30	29.46	0	29.46	3.3333	35.3524
		100	2	77	30	0	30	65.12	0	65.12	3.3333	78.1448
		10	1	42	30	0	30	91.02	0	91.02	3.3333	109.225
		1	0	22	30	0	30	96.44	0	96.44	3.3333	115.729
EC				0	30	0	30	100	0	100	3.3333	120
code	IC50	conc.uM	log conc	%inh	T2	T1	ΔT	RFU2	RFU1	ΔRFU	slope	K.Activity
Lopinavir **Y=22.687x+0.846**		1000	3	76	30	0	30	23.72	0	23.72	3.3333	28.4643
		100	2	37	30	0	30	62.54	0	62.54	3.3333	75.0488
		10	1	19	30	0	30	81.06	0	81.06	3.3333	97.273
		1	0	6.8	30	0	30	93.17	0	93.17	3.3333	111.805
EC				0	30	0	30	100	0	100	3.3333	120

code	IC50	conc.uM	log conc	%inh	T2	T1	ΔT	RFU2	RFU1	ΔRFU	slope	K.Activity
GC376 **Y=22.177x+25.406**		1000	3	91	30	0	30	8.63	0	8.63	3.3333	10.3561
		100	2	73	30	0	30	26.54	0	26.54	3.3333	31.8483
		10	1	42	30	0	30	58.11	0	58.11	3.3333	69.7327
		1	0	28	30	0	30	72.03	0	72.03	3.3333	86.4369
EC				0	30	0	30	100	0	100	3.3333	120

2.5 Vero cells Cytotoxicity Assay

Researcher	: Dr.Hany Emary email: jan_25_misr@yahoo.com mob. +966534391522
Assay	: MTT cytotoxicity assay
Samples	: 9 compounds.
Cell lines	: ----

Lab REPORT I

* Cytotoxicity results

Ser	Sample		Cytotoxicity IC50 uM	SD ±
	code	MW g/mol	Vero	
1	ST	333.35	201.55	5.47
2	21	412.42	48.8	3.72
3	22	352.77	193	14.7
4	23	411.25	47.6	3.62
5	24	346.36	21.6	1.64
6	25	360.37	121	9.21
7	26	375.42	87.3	6.65
8	27	380.83	64.4	4.91

9	28	425.28	**139**	10.6
10	29	376.37	**151**	11.5
***	Remdesivir	602.6	**43.1**	3.28

Detailed Results

researcher	assay	Date	cells
Dr.Hany Emary	MTT	04-Sep	

	Blank	CC	Sample No. 17/Vero					Sample No. 21/Vero				
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	B	C	100uM	25uM	6.3uM	1.6uM	0.4uM	100uM	25uM	6.3uM	1.6uM	0.4uM
B	B	C	100uM	25uM	6.3uM	1.6uM	0.4uM	100uM	25uM	6.3uM	1.6uM	0.4uM
C	B	C	100uM	25uM	6.3uM	1.6uM	0.4uM	100uM	25uM	6.3uM	1.6uM	0.4uM

ROBONIK P2000 eia reader

Wave length:

560 450 nm

Reference: 630 nm

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.001	0.535	0.242	0.313	0.364	0.418	0.463	0.202	0.267	0.321	0.374	0.419
B	0.001	0.529	0.253	0.319	0.354	0.424	0.467	0.189	0.263	0.319	0.379	0.415
C	0.001	0.541	0.258	0.305	0.358	0.425	0.458	0.208	0.265	0.332	0.373	0.424
mean	0.0004	0.535	0.251	0.312	0.3587	0.4223	0.4627	0.1997	0.265	0.324	0.3753	0.4193
%		`	46.916	58.38	67.04	78.941	86.48	37.321	49.533	60.561	70.156	78.38

17/Vero 21/Vero

log conc.	% viability
2	46.916
1.398	58.38
0.796	67.04
0.193	78.941
-0.41	86.48

log conc.	% viability
2	37.321
1.3979	49.533
0.7959	60.561
0.1931	70.156
-0.4089	78.38

IC50=

IC50=

[redacted]

	Blank	CC	Sample No. 22/Vero					Sample No. 23/Vero				
	1	2	3	4	5	6	7	8	9	10	11	12
A	B	C	100uM	25uM	6.3uM	1.6uM	0.4uM	100uM	25uM	6.3uM	1.6uM	0.4uM
B	B	C	100uM	25uM	6.3uM	1.6uM	0.4uM	100uM	25uM	6.3uM	1.6uM	0.4uM
C	B	C	100uM	25uM	6.3uM	1.6uM	0.4uM	100uM	25uM	6.3uM	1.6uM	0.4uM

ROBONIK P2000 eia reader

Wave length:

560 450 nm

Reference: 630 nm

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.001	0.492	0.275	0.341	0.389	0.428	0.469	0.257	0.318	0.362	0.424	0.461
B	0.001	0.518	0.262	0.338	0.382	0.435	0.467	0.263	0.326	0.354	0.435	0.474
C	0.001	0.526	0.268	0.329	0.391	0.439	0.465	0.266	0.323	0.357	0.438	0.481
mean	0.001	0.512	0.2683	0.336	0.3873	0.434	0.467	0.262	0.3223	0.3577	0.4323	0.472

% viability			52.409	65.63	75.651	84.766	91.211	51.172	62.956	69.857	84.44	92.188

22/Vero

log conc.	% viability
2	52.409
1.398	65.625
0.796	75.651
0.193	84.766
-0.41	91.211

IC50=

23/Vero

log conc.	% viability
2	51.172
1.3979	62.956
0.7959	69.857
0.1931	84.44
-0.4089	92.188

IC50=

[redacted]

	Blank	**CC**	**Sample No. 24/Vero**					**Sample No. 25/Vero**				
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	B	C	100uM	25uM	6.3uM	1.6uM	0.4uM	100uM	25uM	6.3uM	1.6uM	0.4uM
B	B	C	100uM	25uM	6.3uM	1.6uM	0.4uM	100uM	25uM	6.3uM	1.6uM	0.4uM
C	B	C	100uM	25uM	6.3uM	1.6uM	0.4uM	100uM	25uM	6.3uM	1.6uM	0.4uM

ROBONIK P2000 eia reader

Wave length:

560 450 nm

Reference: 630 nm

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	0.001	0.545	0.281	0.334	0.383	0.431	0.482	0.234	0.296	0.346	0.419	0.464
B	0.003	0.527	0.277	0.326	0.379	0.442	0.477	0.228	0.294	0.344	0.427	0.458

C	0.001	0.493	0.267	0.329	0.387	0.445	0.496	0.226	0.286	0.347	0.423	0.537
mean	0.0017	0.522	0.275	0.33	0.383	0.4393	0.485	0.2293	0.292	0.3457	0.423	0.4863
% viability			52.716	63.19	73.419	84.217	92.971	43.962	55.974	66.262	81.086	93.227

24/Vero

2	52.716
1.398	63.195
0.796	73.419
0.193	84.217
-0.41	92.971

25/Vero

2	43.962
1.3979	55.974
0.7959	66.262
0.1931	81.086
-0.4089	93.227

IC50=

IC50=

[redacted]

	Blank	CC	Sample No. 26/Vero					Sample No. 27/Vero				
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	B	C	100uM	25uM	6.3uM	1.6uM	0.4uM	100uM	25uM	6.3uM	1.6uM	0.4uM
B	B	C	100uM	25uM	6.3uM	1.6uM	0.4uM	100uM	25uM	6.3uM	1.6uM	0.4uM
C	B	C	100uM	25uM	6.3uM	1.6uM	0.4uM	100uM	25uM	6.3uM	1.6uM	0.4uM

ROBONIK P2000 eia reader

Wave length:

560 450 nm

Reference: 630 nm

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.001	0.472	0.225	0.267	0.324	0.379	0.432	0.219	0.264	0.297	0.356	0.431

B	0.001	0.449	0.221	0.265	0.319	0.382	0.461	0.221	0.269	0.305	0.364	0.416
C	0.001	0.461	0.232	0.269	0.316	0.404	0.454	0.223	0.255	0.318	0.362	0.422
mean	0.001	0.461	0.226	0.267	0.3197	0.3883	0.449	0.221	0.2627	0.3067	0.3607	0.423
% viability			49.059	57.96	69.392	84.298	97.467	47.974	57.019	66.57	78.292	91.823

26/Vero

log conc.	% viability
2	49.059
1.398	57.959
0.796	69.392
0.193	84.298
-0.41	97.467

IC50=

27/Vero

log conc.	% viability
2	47.974
1.3979	57.019
0.7959	66.57
0.1931	78.292
-0.4089	91.823

IC50=

[redacted]

	Blank	**CC**	**Sample No.**			**28/Vero**		**Sample No.**		**29/Vero**		
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	B	C	100uM	25uM	6.3uM	1.6uM	0.4uM	100uM	25uM	6.3uM	1.6uM	0.4uM
B	B	C	100uM	25uM	6.3uM	1.6uM	0.4uM	100uM	25uM	6.3uM	1.6uM	0.4uM
C	B	C	100uM	25uM	6.3uM	1.6uM	0.4uM	100uM	25uM	6.3uM	1.6uM	0.4uM

ROBONIK P2000 eia reader

Wave length:

560 450 nm

Reference: 630 nm

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.001	0.551	0.281	0.334	0.383	0.431	0.482	0.234	0.296	0.346	0.419	0.468
B	0.003	0.526	0.277	0.326	0.379	0.442	0.477	0.228	0.294	0.344	0.427	0.471
C	0.001	0.507	0.267	0.329	0.387	0.445	0.496	0.226	0.286	0.347	0.423	0.453
mean	0.0017	0.528	0.275	0.33	0.383	0.4393	0.485	0.2293	0.292	0.3457	0.423	0.464
% viability			52.083	62.44	72.538	83.207	91.856	43.434	55.303	65.467	80.114	87.879

28/Vero

2	52.083
1.398	62.437
0.796	72.538
0.193	83.207
-0.41	91.856

29/Vero

2	43.434
1.3979	55.303
0.7959	65.467
0.1931	80.114
-0.4089	87.879

3 Docking studies

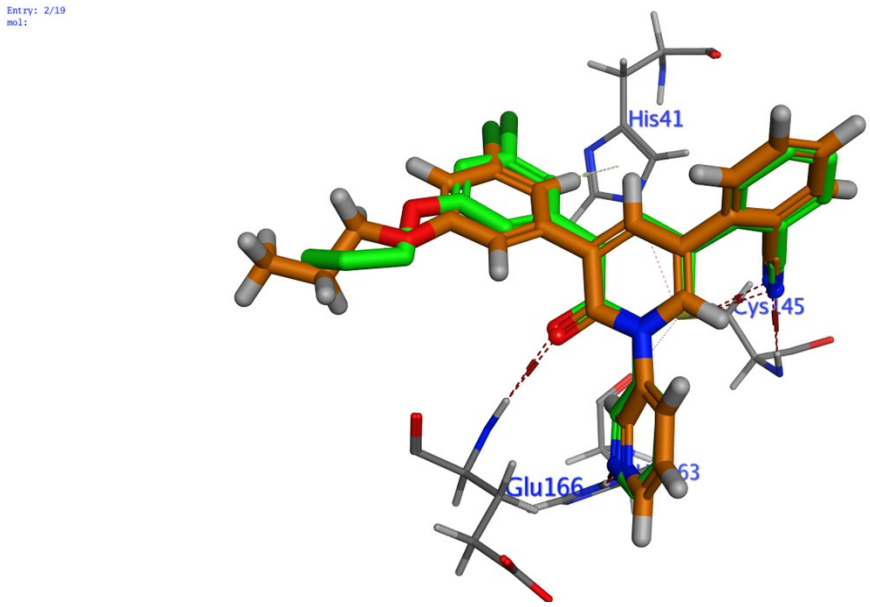


Figure S1: Superimposition of the re-docked XF1 conformer (golden) over that of the co-crystalized one (green) with RMSD value of 1.49