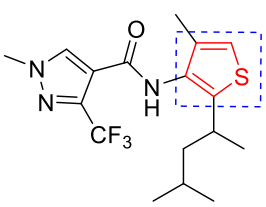
Chapter 1

Synthesis, Characterization and Anti-Cancer Activity of Thiophene Derivative

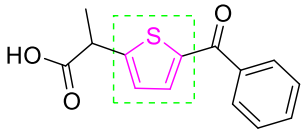
1.1. Introduction

One of the most common diseases worldwide and the leading cause of death is cancer. Therefore, there is a constant need for the design and synthesis of novel anti-cancer agents[1]. According to the World Health Organization, there will be approximately 10 million cancer-related deaths in 2020. Breast (2.26 million cases), Lung (2.21 million cases), Colon and rectum (1.93 million cases), Prostate (1.41 million cases), Skin (1.20 million cases), and Stomach (1.09 million cases) were the most common in 2020[2]. A significant challenge for the medical and scientific communities is the creation of drugs, treatments, and care for an improved and more effective cancer therapy. The most prevalent cancer in women among worldwide is breast cancer, which is harmful to the general public's health[3]. Therefore, it is essential to create medicines that are more effective and have fewer side effects. Due to their various pharmacological and biological characteristics, fused heterocycles, such as thiophene scaffolds, have drawn significant attention and have been identified as anti-cancer agents. It has been discovered that many thiophene compounds with various substitutions, such as mono-, di-, and tri-substitution, are effective anti-cancer drugs[4]. Several chemical processes have been reported for the synthesis of thiophene, and they have all been used to produce different substituted thiophene derivatives. Karl Gewald found that the one-pot reaction of a ketone with an activated nitrile, elemental sulphur, and morpholine as a base produces the most useful and established method for synthesized thiophene with a high degree of functionality[5]. The five-membered heterocyclic thiophene is significant in organic chemistry. There are numerous applications for thiophene derivatives in pharmaceuticals, dyes, and agrochemicals. Thiophene derivative are still being studied in terms of their biological effects due to their diverse modes of action. Thiophene derivatives have higher safety and specificity profiles as a result of their varied synthesis processes. Due to improvements in their synthetic pathways, thiophene's availability, stability, and simplicity of structure have attracted a lot of interest. Thiophene also exhibits a number of other pharmacological properties, including anti-

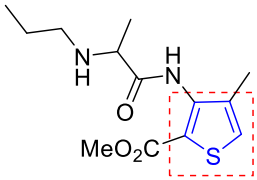
bacterial[6], anticancer[7], anti-fungal[8], analgesic[9], antimicrobial[10], antidiabatic[11], anti proliferative[12], antiulcer[13], antitumor[14], antidepressant[15], anti-oxidant[16], anti-inflammatory effect[17].



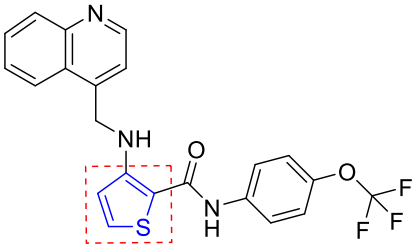
Penthiopyrad
Fungicide



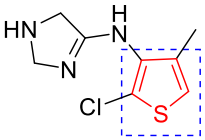
Tiaprofenic acid
Anti-inflammatory



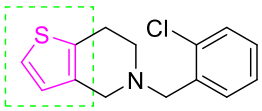
Articaine
Local anesthetic



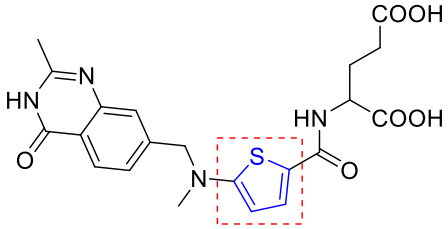
OSi-930
Anticancer



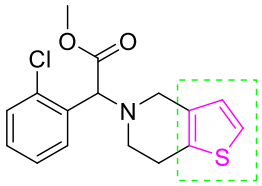
Tiamenidine
Antihypertension



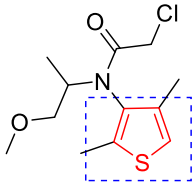
Ticlopidine
Antiplatelet



Raltitrex
Anticancer



Clopidogrel
Anti-platelet

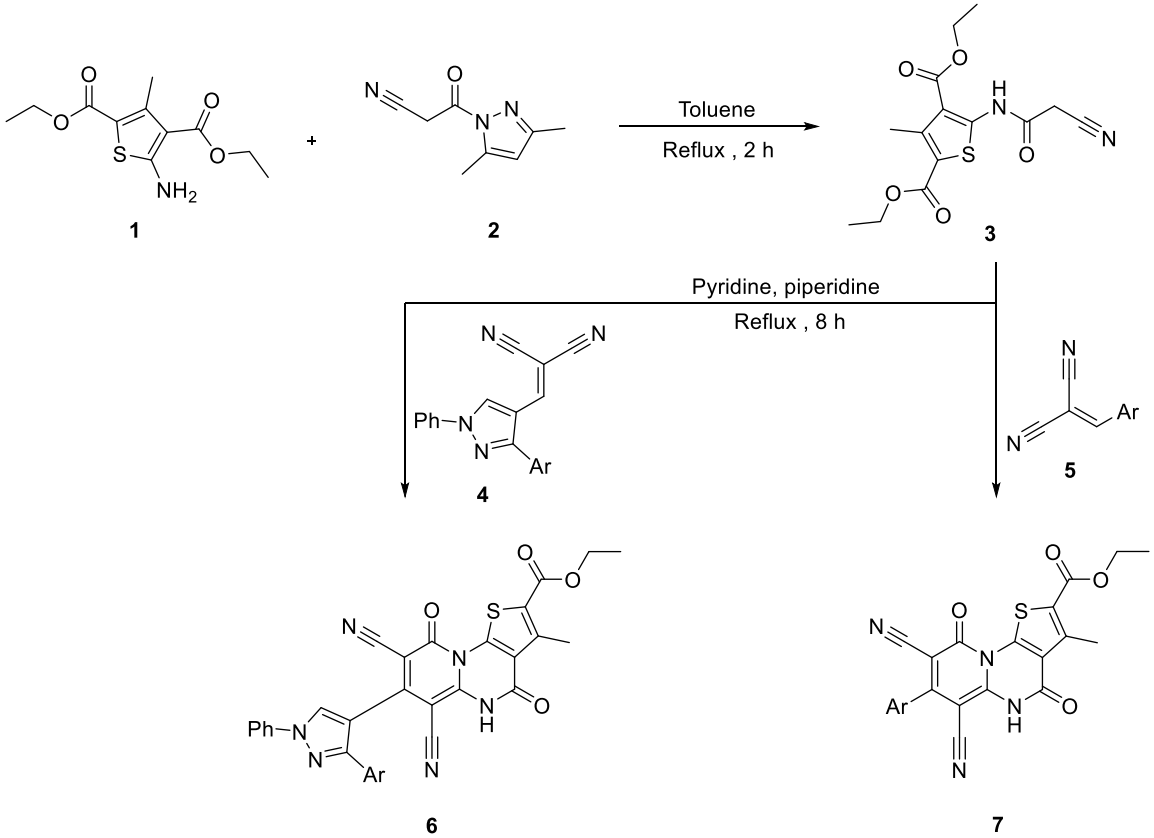


Dimethenamide
Herbicide agent

Scheme 1. Several bioactive thiophene

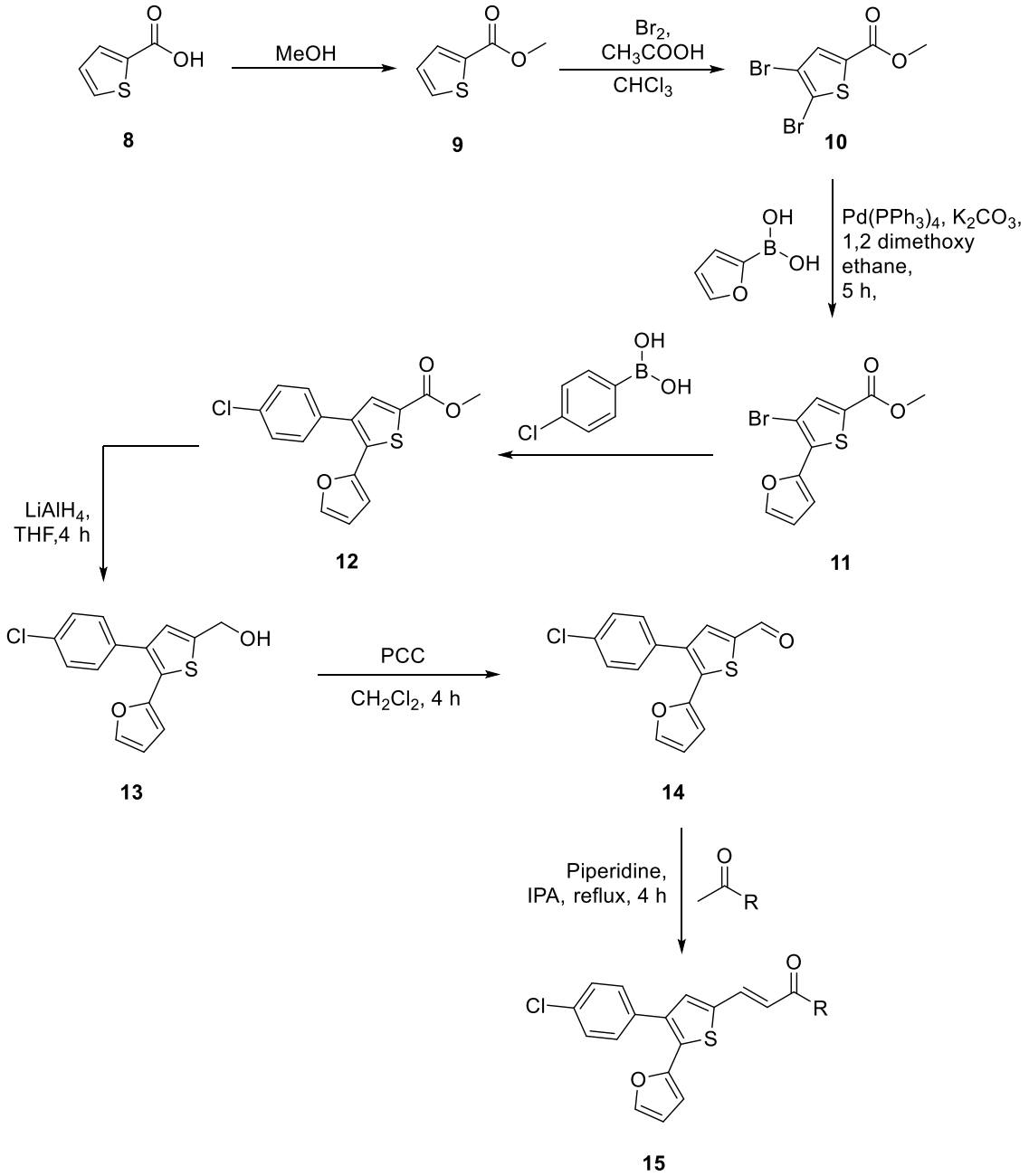
1.1.1. Synthetic methods for substituted thiophene scaffold and its biological significance

A. Abdelmoniem *et al*[18] have successfully synthesized the reaction of compound diethyl 5-amino-3-methylthiophene-2,4- dicarboxylate **1** with 3-(3,5-dimethyl-1H-pyrazol-1-yl)-3-oxopropanenitrile **2** in toluene under reflux conditions, resulting in the formation of afforded diethyl 5-(2-cyanoacetamido)-3-methylthiophene-2,4-dicarboxylate **3**. It was observed that afforded molecule **3** possesses two nucleophilic positions capable of initiating addition to the activated double bond reagent, compound **4**. Consequently, the reaction between compound **3** and cinnamonitriles **4** yielded product **6**. Additionally, when compound afforded molecule **3** was reacted with pyrazolidine malononitriles **5**, the desired target products **7** were obtained. The synthesized compounds underwent screening to assess their antibacterial activity against five bacterial strains. The results revealed that these compounds exhibited moderate to good inhibition zones, indicating their potential as antibacterial agents **(Scheme 1.1).**



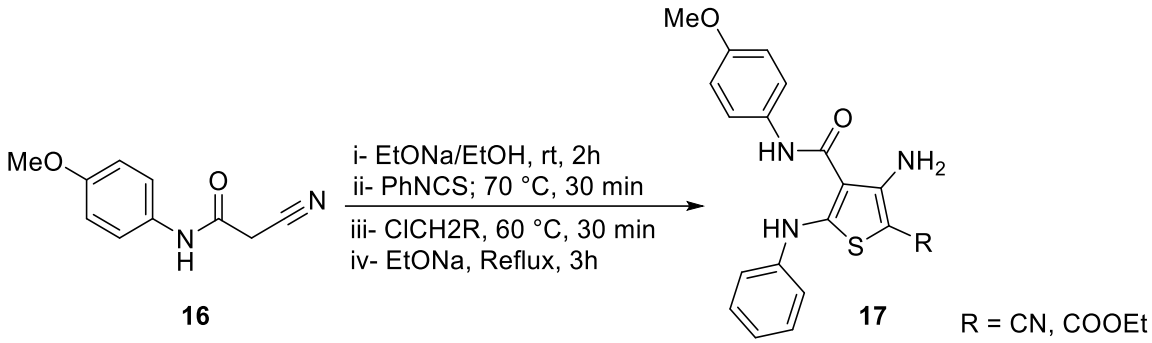
Scheme 1.1

N. Daddukuri *et al*[19] describe new thiophene linked chalcone hybrids. Using the Suzuki reaction, the key intermediate thiophene-2-carbaldehyde has been synthesized by coupling the furan and phenyl ring. In order to synthesize the chalcone core, the new scaffolds have been synthesized through a base-catalyzed condensation reaction. In selected cancer cell lines, including prostate cancer (PC-3), lung (A549), Human breast (MCF-7) cell lines, all 15 compounds of these thiophene-chalcone derivatives has been screened **(Scheme 1.2)**.



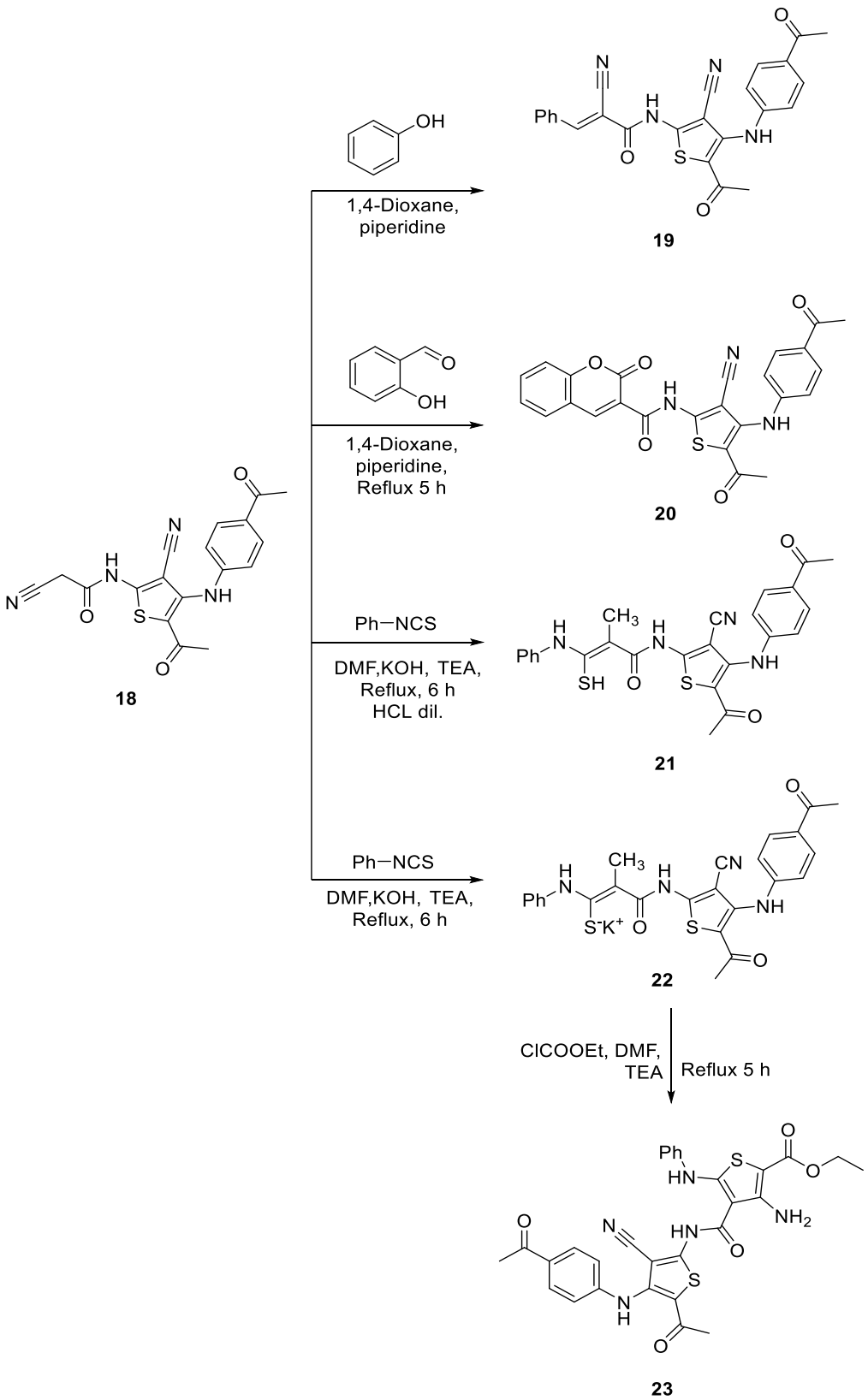
Scheme 1.2

M. Abdelhameid and co-workers[20] demonstrated novel thiophene derivative **17**. The reaction mixture was stirred at room temperature while ethanolic sodium ethoxide solution was slowly added drop by drop to a stirred solution of anilide in absolute ethanol. After 2 hours, phenyl isothiocyanate was added, and the mixture was heated at 70 C for 30 minutes, then cooled to 25 C. Following the addition of the α-chloromethylene derivative, the temperature was raised to 60 C and maintained for another 30 minutes. Sodium ethoxide was then added, and the heating process was continued for an additional 3 hours at reflux temperature. The reaction mixture was cooled to room temperature, poured into ice-cold water, separated, dried, and recrystallized from ethanol to obtain the desired compound **17** **(Scheme 1.3)**.



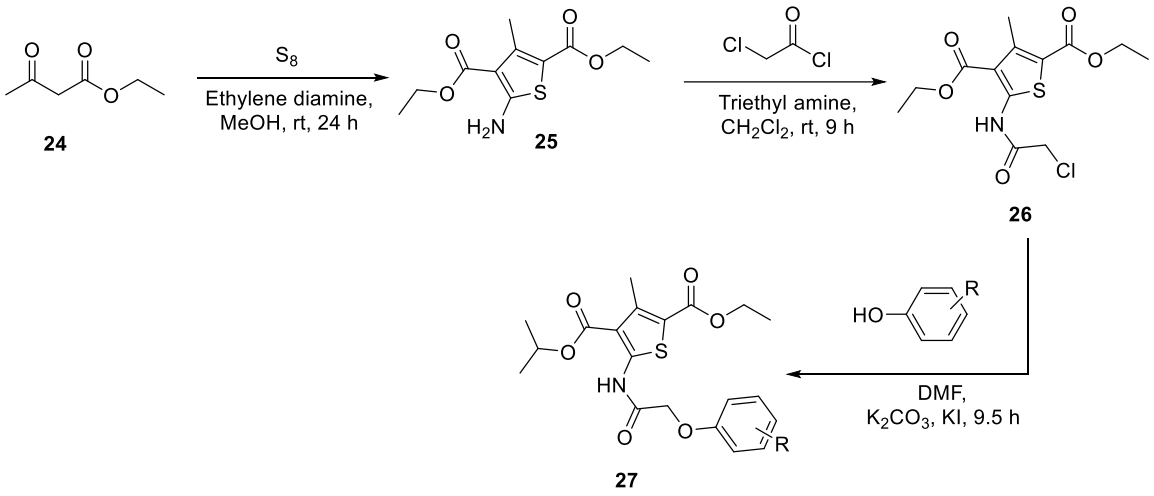
Scheme 1.3

M. Khalifa *et al*[21] have successfully synthesized novel thiophene derivative. The addition of benzaldehyde to the hot mixture of cyanoacteamide derivative **18** in 1,4-dioxane and piperidine followed by refluxing for 5 hours to synthesized molecule **19**. Similarly, the reaction of cyanoacteamide **18** in 1,4-dioxane with piperidine catalyst, followed by the addition of salicylaldehyde and refluxing for 5 hours, and further refluxing with conc. HCl, leads to the formation of the intended product **20**. Furthermore, the cyanoacteamide **18** derivative is effectively refluxed with PhNCS in DMF and KOH for 6 hours, followed by neutralization using an acidified ice-water bath to formed molecule **21**. Lastly, when a mixture of thienyl derivative and ethylchloroacetate in DMF containing TEA is heated under reflux for 5 hours, the desired product 23 is achieved **(Scheme 1.4)**.


18
1,4-Dioxane, piperidine
19
1,4-Dioxane, piperidine, Reflux 5 h
20
Ph—NCS
DMF,KOH, TEA, Reflux, 6 h
HCL dil.
21
Ph—NCS
DMF,KOH, TEA, Reflux, 6 h
22
ClCOOEt, DMF, TEA
Reflux 5 h
23

Scheme 1.4

The synthesis of thiophene derivatives **27** was described by Zhong *et al*[22]. Thiophene molecule **25** was created at room temperature from the gewald reactions of ethylene diamine, ethyl aceto acetate **24** and sulphur in methanol at room temperature. Molecule **26** was created by adding the chloroacetyl chloride to molecule **25**. Additionally, different substituted aromatic phenol were attached to it in DMF containing potassium iodide and potassium carbonate to synthesize a series of molecules known **27.** The neuraminidase activity of the synthesized molecules was tested and results indicated positive progress **(Scheme 1.5)**.



Scheme 1.5

1.2. Result and discussion

In this study, we present a discovery of fifteen newly synthesized molecules with thiophene as their main structural component, aimed at identifying innovative anti-cancer agents and synthesizing diverse heterocyclic compounds. The elucidation of compounds **6a-o** was conducted by thoroughly analyzing their spectroscopic data, including ^{1}H-NMR, ^{13}C-NMR and mass spectroscopy. Starting compound ethyl 2-amino-4-methyl-5-(arylcarbamoyl)thiophene-3-carboxylate were obtained by known literature method via Gewald's reaction which involves one-pot reaction of a ketone with an activated nitrile and elemental sulfur in the presence of morpholine as base according to method describe in literature. Next compound 4 was reacted with 2-cyano-3,3-bis(methylthio)-N-arylacrylamide 5 and potassium carbonate in DMF to produce thiophene derivatives that are novel and highly functionalized.

Table 1 Presents the elemental analysis information as well as some physical characteristics of these novel compounds which displayed ^{1}H NMR spectra analysis of these novel substances, we also examined their behavior in both CDCl3 and DMSO-d6. Compounds 6a-o were found to exhibit distinct characteristics. The proton signals corresponding to the CH_3 group of the ester appeared at t 1.33-1.44 ppm. While SCH_3 was observed at s 2.53-2.58 ppm as a singlet. Ester methylene protons were observed at q 4.34-4.49 ppm (CH_2), and the aromatic region ranged between 6.92-7.70 ppm. Furthermore, proton singlets of three NH groups were detected between s 9.55-13.67 ppm. Each compound's mass spectra displayed a molecular ion, confirming its molecular weight. The mass spectra showed a molecular ion peak at 585 m/z that corresponded to the molecular formula $C_{27}H_{25}ClN_4O_5S_2$.

1.2.1. Optimizing of the reaction conditions.

Table 1: Optimization of the reaction conditions.

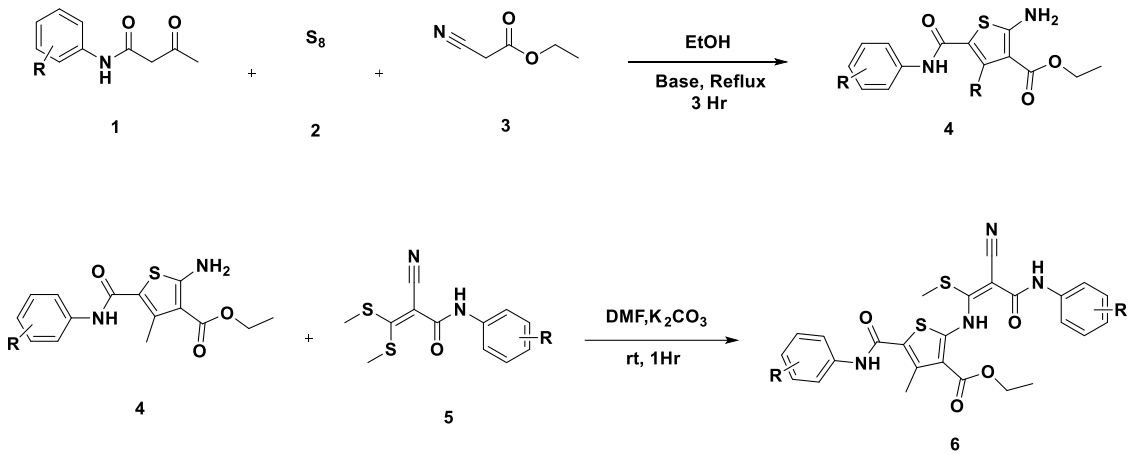
Entry	Solvent	Base	Temp. (C)	Yield (%)	Purification
1	No solvent	-	rt	-	-
2	H_2O	-	rt	-	-
3	H_2O	K_2CO_3	rt	-	-
4	THF	K_2CO_3	rt	39	Yes
5	THF	Et_3N	rt	33	Yes
6	MECN	K_2CO_3	rt	47	Yes
7	MECN	Et_3N	rt	42	Yes

8	Acetone	K_2CO_3	rt	81	Yes
9	Acetone	Et_3N	rt	83	Yes
10	MeOH	K_2CO_3	rt	55	Yes
11	MeOH	Et_3N	rt	48	Yes
12	EtOH	K_2CO_3	rt	56	Yes
13	EtOH	Et_3N	rt	47	Yes
14	DMF	Et_3N	rt	87	Yes
15	DMF	K_2CO_3	rt	91	No

To improve the testing conditions for synthesizing molecules 6a-o, various solvents such as tetrahydrofuran, ethanol, acetone, IPA, DMF and methanol were used in conjunction with different bases such as triethylamine and piperidine. After conducting several experiments, it was found that the use of potassium carbonate with DMF resulted in a faster reaction between ethyl 2-amino-4-methyl-5-(arylcarbamoyl)thiophene-3-carboxylate and 2-cyano-3,3-bis(methylthio)-N-arylacrylamide, producing a desirable yield of thiophene derivatives ethyl (*E*)-2-((2-cyano-1-(methylthio)-3-oxo-3-(arylamino)prop-1-en-1-yl)amino)-4-methyl-5-(arylcarbamoyl)thiophene-3-carboxylate **6a-o.**

Initially, the reaction was attempted without any solvent or catalyst at room temperature, but no product was formed **(Table 1, entry 1)**. Further experiments were conducted to optimize the reaction conditions. Water was used as a solvent with no base at room temperature **(entry 2),** followed by using water as a solvent with potassium carbonate as a base **(entry 3),** but no product was obtained. Subsequently, different combinations of solvents and bases were tried. Tetrahydrofuran with potassium carbonate as a base yielded a 39% product yield **(entry 4)** and a 33% yield when triethylamine was used as the base **(entry 5).** The reaction was then carried out with acetonitrile as a solvent and potassium carbonate as a base, resulting in a 47% product yield **(entry 6)** and a 42% yield when triethylamine was used as the base **(entry 7).** Acetone as the solvent and potassium carbonate as the base resulted in an 81% yield **(entry 8),** and 83% yield was obtained when triethylamine was used as the base **(entry 9).** Methanol was used as the solvent with potassium carbonate as the base, resulting in a product yield of 55% **(entry 10),** and a 48% yield was obtained when triethylamine was used as the base **(entry 11).** Ethyl alcohol as a solvent and potassium carbonate as a base led to a 56% yield **(entry 12)** and 47% yield with triethylamine as the base **(entry 13).** Finally, DMF was used with triethylamine, and a yield of 87% was obtained **(entry 14).** The highest yield of 91% was achieved when

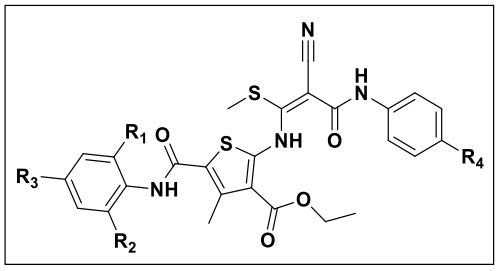
potassium carbonate was used as the base and the reaction mixture was stirred at room temperature for 1 hour **(entry 15).** These results suggest that using potassium carbonate with DMF is the optimal condition for synthesizing thiazole derivatives as it yields a high product yield and the reaction proceeds rapidly.



Scheme 1. Reagents and conditions

1.2.2. Physicochemical Properties

Table 2: Novel thiazole derivative's physicochemical characteristeristics.



Entry	R_1	R_2	R_3	R_4	Molecular Formula	Molecular Weight	Yield (%)	MP (°C)
JOOET-2	H	H	OCH_3	Cl	$C_{26}H_{22}ClFN_4O_4S_2$	573.05	88	275-277
JOOET-3	H	H	OCH_3	CH_3	$C_{27}H_{25}ClN_4O_5S_2$	584.10	81	255-257
JOOET-5	H	H	OCH_3	F	$C_{28}H_{228}N_4O_5S_2$	564.68	72	292-294
JOOET-6	H	H	CH_3	CH_3	$C_{27}H_{25}N_4O_4S_2$	533.13	83	287-289
JOOET-7	CH_3	CH_3	H	CH_3	$C_{27}H_{25}FN_4O_4S_2$	552.64	85	277-279
JOOET-8	CH_3	CH_3	H	Br	$C_{26}H_{22}FN_4O_5S_2$	553.60	82	285-286
JOOET-9	CH_3	H	CH_3	Br	$C_{29}H_{30}N_4O_4S_2$	562.70	85	291-293
JOOET-10	H	H	F	Br	$C_{28}H_{27}BrN_4O_4S_2$	627.57	74	269-271
JOOET-11	H	H	Cl	Br	$C_{28}H_{27}BrN_4O_4S_2$	627.57	92	208-210
JOOET-12	H	H	OCH_3	Br	$C_{26}H_{22}BrFN_4O_4S_2$	617.51	93	206-208
JOOET-13	H	H	CH_3	Br	$C_{26}H_{22}BrClN_4O_4S_2$	633.96	89	224-226
JOOET-14	H	H	Cl	F	$C_{27}H_{25}BrN_4O_5S_2$	629.54	90	216-218
JOOET-15	H	H	OCH_3	Cl	$C_{27}H_{25}BrN_4O_4S_2$	629.54	92	216-218

The molecular docking of the synthesized compounds **6a-o** was further studied to ascertain their binding affinities. The results might be significant for the creation of effective and harmless anticancer compounds.

1.2.3. Anticancer activity of synthesized molecules

Screening of anticancer activity at a Single Dose (10 µM)

The National Cancer Institute (NCI) chose synthesized molecules for in vitro anticancer testing in accordance with the Drug Evaluation Branch methodology in Bethesda, Maryland, USA (http://www.dtp.nci.nih.gov). The primary in vitro anticancer study consisted of giving a single dosage to all NCI 60 cell lines representing nine tumor subpanels: breast, prostate, renal, ovarian, CNS, colon, lung, melanoma, and leukemia. The molecules were introduced at 10^{-5} M concentrations and incubated for 48 hours. Sulforhodamine B (SRB), a protein binding dye, was used to identify the endpoint. The results for all molecule were shown as a graphical depiction of the average percentage of growth inhibition in treated cells versus untreated control cells. Furthermore, these findings were summarized in Table X. The results of growth inhibition for the two most potent molecules, 3 and 12, are listed in Table 1. An analysis of thiophene analogs 1 - 15 that had been examined revealed a wide range of anticancer efficacy. Notably, Molecule **3** exhibited significant activity, with a mean value of -17.01. Specifically, Molecule **3** demonstrated lethal effects/complete cell death against 39 cell lines, including Leukemia (HL-60, MOLT-4, SR), NSCL Cancer (EKVX, HOP-62, HOP-92, NCI-H226, NCI-H23, NCI-H460, NCI-H522), Colon Cancer (COLO 205, HCC-2998, HCT-116, HCT-15, KM12), CNS Cancer (SF-295, SF-539, U251), Melanoma (LOX IMVI, MALME-3M, M14, MDA-MB-435, SK-MEL-2, SK-MEL-28, SK-MEL-5, UACC-62), Ovarian Cancer (OVCAR-3, OVCAR-5, SK-OV-3), Renal Cancer (A498, CAKI-1, RXF 393) and Breast Cancer (MCF7, MDA-MB-231/ATCC, HS 578T, BT-549, T-47D, MDA-MB-468), with growth percentages ranging from -2.03% to -77.60%. Additionally, high effectiveness against 21 tested cell lines, including Leukemia (K-562, RPMI-8226), NSCL Cancer (A549/ATCC, NCI-H322M), Colon Cancer (HT29, SW-620), CNS Cancer (SF-268, SNB-19, SNB-75), Melanoma (UACC-257), Ovarian Cancer (IGROV1, OVCAR-4, OVCAR-8), Renal

Cancer (786-0, ACHN, SN12C, TK-10, UO-31), Prostate Cancer (PC-3, DU-145), with growth percentages ranging from 0.62% to 21.59%.

Notably, Molecule **12** exhibited significant activity, with a mean value of -30.30. Molecule 12 demonstrated lethal effects/complete cell death against 46 cell lines, including Leukemia (CCRF-CEM, HL-60, RPMI-8226), NSCL Cancer (A549/ATCC, EKVX, HOP-62, HOP-92, NCI-H226, NCI-H23, NCI-H322M, NCI-H522), Colon Cancer (COLO 205, HCC-2998, HCT-116, HCT-15, HT29, KM12), CNS Cancer (SF-268, SF-295, SF-539, SNB-19, SNB-75, U251), Melanoma (LOX IMVI, MALME-3M, M14, MDA-MB-435, SK-MEL-2, SK-MEL-28, SK-MEL-5, UACC-257, UACC-62), Ovarian Cancer (OVCAR-3, OVCAR-8, NCI/ADR-RES, SK-OV-3), Renal Cancer (786-0, A498, CAKI-1, RXF 393, UO-31), Prostate Cancer (PC-3), Breast Cancer (MDA-MB-231/ATCC, HS 578T, BT-549, MDA-MB-468), with growth percentages ranging from -0.30% to -89.96%. Additionally, high effectiveness against 13 tested cell lines, including Leukemia (K-562, MOLT-4), NSCL Cancer (NCI-H460), Colon Cancer (SW-620), Ovarian Cancer (IGROV1, OVCAR-4, OVCAR-5), Renal Cancer (ACHN, SN12C, TK-10), Prostate Cancer (DU-145), Breast Cancer (MCF7, T-47D), with growth percentages ranging from 0.63% to 15.39%.

Notably, Molecule **2, 9, 10, 11, 13** and **14** Demonstrate Complete cell death and high effectiveness against most of the cell lines with a mean value of -16.02, -4.21, -14.56, -6.78, -13.60 and -11.09, respectively. Additionally, molecule **5, 6, 7 and 8** showed complete cell death, high effectiveness and moderate cell growth inhibition against most of the tested cell lines with a mean value of 18.71, 19.42, 10.74 and 10.12, respectively (**Table 1**).

Table 3: Anticancer activity (GI_{50} Values in µM) for all compounds in NCI-60 cell line.

Cell line	**Growth of Cells (%)[3] 10^{-5} M**												
Compound	**2**	**3**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
Leukemia													
CCRF-CEM	-12.03	17.73	29.79	4.54	23.31	10.96	10.38	14.15	12.15	-13.01	-12.23	-19.97	86.43
HL-60(TB)	26.36	-18.11	31.54	1.71	7.28	15.50	-21.36	-7.96	-3.47	-0.30	5.01	-13.50	92.59
K-562	2.88	10.55	27.58	12.00	11.23	15.13	4.30	6.94	6.69	5.07	7.79	9.98	91.81
MOLT-4	13.28	-2.49	26.94	22.71	14.73	8.91	10.65	14.45	7.86	6.66	5.68	4.92	95.17
RPMI-8226	0.26	4.30	14.82	-8.02	4.04	4.27	-25.25	-10.64	-19.90	-20.25	-17.91	-16.98	93.58
SR	-32.60	-13.63	9.67	-	5.36	0.42	-	-	-	-	-	-	-
NSCL Cancer													
A549/ATCC	6.59	9.79	34.85	29.71	23.35	26.63	10.79	4.77	11.80	-18.45	4.23	8.16	96.39
EKVX	-2.97	-5.91	27.53	23.05	21.49	23.77	1.02	1.28	3.96	-8.21	-4.15	6.19	102.01
HOP-62	-57.44	-64.24	18.34	39.44	-10.76	-11.94	0.04	-39.92	-18.73	-65.28	-41.55	-31.50	96.31
HOP-92	-25.67	-24.91	-10.86	-10.73	-9.40	-5.93	-23.27	-30.89	-30.15	-49.75	-37.89	-23.50	85.25
NCI-H226	-6.79	-10.88	23.24	18.90	38.37	26.48	-4.55	-1.13	2.88	-15.18	-4.73	5.35	104.96
NCI-H23	-19.62	-5.17	17.64	10.09	5.31	12.28	-12.09	-16.08	-24.37	-50.95	-33.69	-20.09	99.80
NCI-H322M	1.30	9.52	46.15	29.52	30.49	29.68	15.30	9.09	16.31	-4.29	18.99	15.17	87.66
NCI-H460	-17.16	-15.21	9.48	6.43	4.76	4.18	13.42	7.79	11.64	0.90	2.20	-4.71	107.48
NCI-H522	-25.62	-22.92	7.51	11.32	-2.40	-5.88	-31.61	-56.82	-38.56	-57.90	-39.80	-29.16	96.07
Colon Cancer													
COLO 205	-63.77	-77.60	26.42	35.52	-13.72	-24.05	-12.57	-76.71	-55.32	-81.83	-65.13	-58.14	117.54
HCC-2998	-43.35	-63.44	26.31	24.67	19.81	27.24	-7.11	-43.83	-39.40	-71.90	-65.71	-61.32	119.25
HCT-116	-43.41	-34.08	24.52	13.63	12.63	12.45	-9.53	-22.19	-5.57	-33.87	-20.77	-1.98	96.57
HCT-15	-30.64	-63.59	12.89	12.92	12.92	14.23	-14.41	-49.10	-41.11	-52.73	-23.84	-44.68	104.98
HT29	6.43	3.11	37.52	22.60	20.85	21.69	9.88	-14.52	-5.85	-17.77	2.19	2.77	111.79
KM12	-33.16	-52.65	23.29	13.75	16.32	12.13	-1.62	-52.69	-11.61	-64.44	-33.28	-73.64	96.72
SW-620	7.53	9.62	32.93	35.42	24.62	24.93	18.65	11.37	18.98	3.97	10.54	12.79	99.05
CNS Cancer													
SF-268	5.75	7.65	14.07	36.66	15.20	20.84	31.53	54.86	46.47	-4.24	16.00	22.77	94.87
SF-295	-57.98	-70.46	10.68	0.46	1.79	5.41	-50.64	-57.81	-37.32	-68.78	-51.30	-55.02	103.51
SF-539	-60.87	-68.57	23.51	32.07	24.74	28.91	7.87	-10.21	-9.62	-84.54	-47.96	-31.37	102.64
SNB-19	32.07	21.59	22.10	17.77	22.44	19.81	7.06	0.16	9.44	-5.80	12.44	12.85	91.08
SNB-75	-1.47	2.81	27.13	40.18	19.60	16.31	18.47	4.85	19.33	-29.04	-4.79	-19.79	82.36
U251	-42.74	-30.84	27.40	11.60	19.88	21.30	-23.38	-62.57	-51.44	-79.03	-44.95	-41.18	93.04

Melanoma													
LOX IMVI	-57.42	-40.55	42.47	26.06	36.56	39.72	-44.79	-74.63	-71.91	-89.96	-68.03	-59.69	103.76
MALME-3M	-24.03	-12.30	20.02	3.16	6.73	10.90	-19.40	-10.67	-16.42	-24.42	-15.77	-4.53	94.69
M14	0.33	-22.66	25.86	26.58	20.43	18.22	-22.12	-43.68	-18.82	-60.64	-1.47	-29.47	99.94
MDA-MB-435	-40.35	-42.00	-3.62	7.20	8.78	6.59	2.56	-24.73	-8.31	-65.85	-29.46	-28.02	93.70
SK-MEL-2	-66.45	-70.71	-41.73	-8.63	-50.92	-54.07	-71.69	-61.20	-51.80	-85.10	-72.88	-71.93	102.51
SK-MEL-28	7.30	-20.13	15.29	36.02	18.61	19.67	23.16	19.51	26.97	-12.92	16.33	0.22	113.64
SK-MEL-5	-61.62	-72.58	-43.12	-31.23	-51.72	-51.58	-87.52	-75.41	-75.86	-87.13	-76.49	-79.92	101.93
UACC-257	-2.77	9.77	17.78	13.73	1.58	2.31	-4.08	2.30	7.17	-2.54	-0.31	-0.63	95.25
UACC-62	-45.68	-58.57	8.55	11.83	12.79	1.52	-58.01	-67.43	-42.97	-81.56	-44.74	-52.09	94.68
Ovarian Cancer													
IGROV1	11.87	15.39	35.57	30.28	28.53	25.66	14.22	5.78	26.66	7.21	22.73	17.79	106.47
OVCAR-3	-28.56	-34.29	-0.88	12.27	-6.87	-16.33	-28.66	-10.36	12.25	-45.62	9.52	1.08	115.04
OVCAR-4	9.66	15.91	24.69	28.88	19.21	15.73	20.89	20.49	23.73	15.39	14.33	18.65	95.38
OVCAR-5	1.49	-9.53	41.47	79.04	41.15	40.12	52.30	20.02	33.04	4.81	27.90	43.99	131.63
OVCAR-8	10.52	16.24	38.51	33.12	21.01	20.59	6.91	6.22	8.95	-34.03	-41.17	5.71	101.58
NCI/ADR/RES				23.90			11.64	4.07	3.59	-25.95	-8.29	-2.37	104.77
SK-OV-3	37.81	-51.44	-24.78	19.76	-39.07	-39.51	-18.88	-24.11	-19.76	-36.52	-22.00	-20.85	98.80
Renal Cancer													
786-0	3.42	11.32	25.20	31.83	21.10	26.01	7.48	-5.54	4.59	-25.89	3.59	10.42	100.47
A498	-9.97	-5.52	30.01	27.99	13.69	4.07	-10.38	-19.79	-4.46	-39.11	-22.65	-14.27	105.83
ACHN	4.84	7.09	28.32	20.09	16.44	18.60	7.94	6.48	11.38	3.03	8.60	13.13	103.90
CAKI-1	-11.02	-2.03	19.47	33.46	3.81	7.97	0.64	-29.68	-10.20	-14.53	-9.26	1.82	83.27
RXF 393	-30.31	-21.38	-5.36	38.92	-5.73	-3.88	8.96	-12.56	-1.99	-49.81	-7.09	2.22	101.43
SN12C	9.33	11.62	23.17	15.22	16.06	12.60	10.16	9.35	15.49	6.02	14.31	14.26	94.90
TK-10	7.50	14.30	35.82	28.20	17.56	20.96	12.07	-4.95	9.25	4.39	11.69	14.63	101.06
UO-31	5.89	12.27	21.69	13.53	14.96	13.91	2.54	-17.50	-12.93	-69.10	-7.82	12.13	86.02
Prostate Cancer													
PC-3	-4.90	0.62	21.30	13.89	8.58	11.16	4.05	-5.45	2.11	-5.64	2.51	2.06	98.43
DU-145	17.24	20.84	35.04	28.23	23.15	23.15	25.92	35.25	28.67	2.21	19.45	21.13	97.70
Breast Cancer													
MCF7	4.69	-2.69	23.63	16.01	17.11	23.26	8.18	6.77	6.43	3.60	4.75	5.90	100.22
MDA-MB-231	-50.33	-35.69	50.22	42.16	60.13	47.77	9.35	-2.97	-2.41	-16.71	-11.12	1.09	96.31
HS 578T	-10.34	-3.28	33.66	-0.50	16.14	20.85	-9.30	-19.86	-18.09	-27.73	-15.98	-12.78	95.36
BT-549	-31.53	-34.66	12.98	26.52	13.49	11.85	0.59	-21.71	-6.17	-34.53	-20.10	-16.54	105.58
T-47D	-25.92	-31.89	-28.67	12.10	-31.26	-32.49	9.92	7.73	5.00	0.63	-2.03	4.08	101.20

MDA-MB-468	-25.15	-19.14	-1.91	-1.93	-2.58	-3.80	-14.30	-19.10	-13.82	-18.90	-16.87	-5.66	108.58
Mean	-16.02	-17.01	18.71	19.42	10.74	10.12	-4.21	-14.56	-6.78	-30.30	-13.60	-11.09	99.71
Delta	50.43	60.59	61.83	50.65	62.46	64.19	83.31	62.15	69.08	59.66	62.89	68.83	17.35
Range	98.52	99.19	93.34	110.2	111.85	101.84	139.82	131.57	122.33	105.35	104.3	123.91	49.27

1.2.4. *In vitro* Anticancer Screening at 5 dose Full NCI 60 cell Panel

The outcomes of the single-dose screening demonstrated that twelve molecules, including 2 (D-839235/1), 3 (D-839236/1), 5 (D-8392337/1), 6 (D-842065/1), 7 (D-839238/1) 8 (D-839239/1), 9 (D-842066/1), 10 (D-842067/1), 11 (D-842068/1), 12 (D-842069/1), 13 (D-842070/1) and 14 (D-842071/1) showed greater activity in initial experiments conducted on sixty human cancer cell lines. As a result, these molecules were evaluated further using a range of sixty cancer cell lines. To achieve this, five different concentrations (100 μM, 10 μM, 1 μM, 0.1 μM, and 0.01 μM) were tested using 10-fold dilutions. The findings of the five-dose screening for all eleven compounds are shown according to the response parameters GI50 (the molar concentration needed to prevent 50% of cancer cell lines from growing) and LC50 (the molar concentration needed to kill 50% of the cells) for each tested cell line. A molecule's selectivity criteria is expressed as the ratio that results from dividing the mean inhibitory dose (MID) of the entire panel (in μM) by the MID of each individual subpanel (also in μM). Moderate selectivity is indicated by ratios between 3 and 6, and strong selectivity is shown by ratios more than 6 toward the particular cell line. Compounds failing to match either of these criteria are characterized as nonselective. From the results presented in **Table 2**, it is obvious that molecule 11 displayed significant anticancer effects across the majority of the examined cell lines, representing nine different subpanels. Molecule 12 showed significant effects against most examined cell lines, with GI50 values ranging from 1.81 to 2.84 μM. According to the selectivity criterion, compound 12 demonstrated broad-spectrum anticancer efficacy against the nine subpanels examined, with selectivity ratios ranging from 0.78 to 1.23 at the GI50 level. The results above indicate that thiophene derivatives exhibited outstanding cytotoxic effects against multiple cancer cell lines, with GI50 values for several of them falling below the low <1.0 μM range. It indicates that further derivatization of molecules with similar structures could be useful in the development of even more effective and selective anticancer drugs.

Table 4: *In vitro* Anticancer Screening at 5 dose for JOOET-12.

JOOET-12	GI50	Subpanel MID[b]	Selectivity ratio (MID[a]/MID[b])	TGI	LC50
Leukemia					
CCRF-CEM	2.02	2.09	1.07	1.06	> 100
HL-60(TB)	2.38			6.65	5.65
K-562	1.95			9.62	> 100
MOLT-4	2.41			8.73	6.69
RPMI-8226	1.72			6.93	> 100
SR					
NSCL Cancer					
A549/ATCC	2.36	2.06	1.08	7.73	3.00
EKVX	1.88			5.71	2.51
HOP-62	1.80			3.94	8.62
HOP-92	1.26			3.97	1.54
NCI-H226	2.04			5.68	3.55
NCI-H23	1.70			4.10	9.90
NCI-H322M	3.03			1.17	4.37
NCI-H460	2.71			7.55	4.27
NCI-H522	1.80			4.88	1.76
Colon Cancer					
COLO 205	2.14	2.19	1.01	4.56	9.74
HCC-2998	1.65			3.63	8.00
HCT-116	2.14			5.79	2.17
HCT-15	1.44			3.50	8.51
HT29	2.33			5.50	1.91
KM12	2.03			4.61	1.13
SW-620	3.63			1.24	4.15
CNS Cancer					
SF-268	2.19	2.02	1.10	7.25	3.30
SF-295	1.50			3.12	6.52
SF-539	1.98			4.49	1.11
SNB-19	2.43			1.05	4.01
SNB-75	2.38			6.41	2.38
U251	1.63			3.68	8.32
Melanoma					
LOX IMVI	1.72	2.07	1.07	3.31	6.38
MALME-3M	2.50			8.99	4.07
M14	2.14			5.00	1.59
MDA-MB-435	2.20			6.01	2.17
SK-MEL-2	1.63			3.35	6.89
SK-MEL-28	2.92			1.49	7.00
SK-MEL-5	1.33			2.89	6.27
UACC-257	2.12			6.98	2.90
UACC-62					
Ovarian Cancer					
IGROV1	3.67	2.84	0.78	2.72	> 100
OVCAR-3	2.26			5.66	1.90
OVCAR-4	2.73			1.20	6.48
OVCAR-5	4.54			1.54	5.76
OVCAR-8	2.13			5.55	2.32

	2.26			5.79	2.43
SK-OV-3	2.31			6.47	> 100
Renal Cancer					
786-0	2.62	2.46	0.91	8.53	3.69
A498	2.88			7.29	2.83
ACHN	3.48			1.51	8.21
CAKI-1	1.76			3.84	8.39
RXF 393	1.67			4.73	1.71
SN12C	2.57			1.40	4.59
TK-10	2.88			1.19	3.94
UO-31	1.81			6.21	2.62
Prostate Cancer					
PC-3	2.38	2.80	0.79	1.02	4.46
DU-145	3.23			1.08	4.14
Breast Cancer					
MCF7	2.26	1.81	1.23	7.89	3.15
MDA-MB-231/ATCC	2.08			4.93	1.93
HS 578T	1.73			5.62	> 100
BT-549	1.77			4.24	1.07
T-47D					
MDA-MB-468	1.22			4.98	2.28
MID[a] 127.33/57 = 2.23					

1.3. Conclusion

A series of novel Ethyl (Z)-5-(arylcarbamoyl)-2-((2-cyano-3-((4-fluorophenyl)amino)-1-(methylthio)-3-oxoprop-1-en-1-yl)amino)-4-methylthiophene-3-carboxylate has been produced beginning with Gewald's reaction. ethyl 2-amino-4-methyl-5-(arylcarbamoyl)thiophene-3-carboxylate 6a-o were obtained by known literature method via Gewald's reaction which involves one-pot reaction of a ketone with an activated nitrile and elemental sulfur in the presence of morpholine as base according to method describe in literature. Next compound 4 was reacted with 2-cyano-3,3-bis(methylthio)-N-arylacrylamide 5 and potassium carbonate in DMF to produce thiophene derivatives that are novel and highly functionalized. The National Cancer Institute (NCI) chose synthesized molecules for in vitro anticancer testing. The primary in vitro anticancer study consisted of giving a single dosage to all NCI 60 cell lines that correspond to nine subpanels of tumors: breast, CNS, ovarian, prostate, renal, colon, lung, melanoma, and leukemia. After displaying strong anti-cancer activity in initial screening against all the cell lines, 12 substance was chosen for five- dose assay**.**

1.4. Experimental Section

An electrothermal equipment, in conjunction with an open capillary, was utilized for the determination of melting points, and these values remained consistent without the need for any adjustments. Thin-layer chromatography was carried out on Merck-supplied silica-gel 60 F254 precoat plates. Molecules were visualized using UV light at wavelengths of 365 and 264 nm, as well as iodine vapor. ^{1}H and ^{13}C NMR spectra were obtained in DMSO-d6 conditions utilizing a Bruker AVANCE III (400 MHz) spectrometer, with chemical shifts expressed in δ ppm with respect to Tetramethylsilane (TMS) as the internal standard. Mass spectra were acquired utilizing a direct inlet probe paired with a Shimadzu GCMS QP2010 Ultra mass spectrometer. All of the chemicals were utilized without further purification and were obtained from reputable sources such as Spectrochem, Combi-Blocks BLD pharm, SDFCL, Loba, TCI, CDH, Avra and Sigma-Aldrich.

- **General procedure for synthesis of ethyl 2-amino-4-methyl-5-(phenylcarbamoyl)thiophene-3-carboxylate (4).**

Ethyl cyanoacetate (1 mmol), sulphur (1 mmol), and substituted acetoacetanilide (1 mmol) were refluxed in EtOH for three hours in the presence of morpholine (1 mmol). After completion of the reaction resulting mixture was chilled and kept in refrigerator overnight. The separated product was filtered, washing with a small amount of EtOH then dried in air. White crystal of the substance was produced by recrystallization from ethanol.

- **General procedure for synthesis of 2-cyano-3,3-bis(methylthio)-*N*-phenylacrylamide (5)**

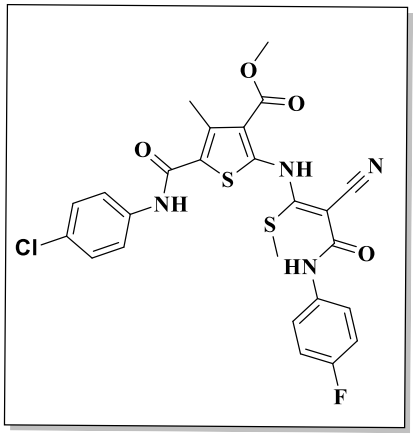
Anhydrous potassium carbonate (1 mmol) was added to a solution of acetoacetanilide (1 mmol) in DMF. After stirring the reaction mixture for 30-45 minutes at room temperature, carbon disulfide (1 mmol) was added at 0-5 C and stirring continued for a further two hours. Then, dimethyl sulphate (1 mmol) was added in little amounts at 0-5^{o} C over the period of 30-45 minutes, and stirring the reaction mixture for a further 5 hours at room temperature. The resulting dark red solution was poured into crushed ice, the separated

product was filtered and washed with water until produce a free red colored washing filtrate. To obtain the pure product, the solid product was crystallized from ethanol.

- **General procedure for synthesis of ethyl (*E*)-2-((2-cyano-1-(methylthio)-3-oxo-3-(phenylamino)prop-1-en-1-yl)amino)-4-methyl-5-(phenylcarbamoyl)thiophene-3-carboxylate (6)**

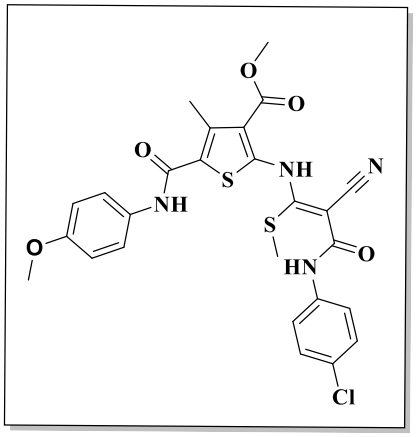
Anhydrous potassium carbonate (1 mmol) was agitated at room temperature for one hour with a combination of **3a** (1 mmol) and **4a** (1 mmol) in 15 mL of DMF. After completion of the reaction, the suspension had been added to the ice-cold water. The final product was filter and repeatedly washed in cold water and purified by recrystallization from DMF to give yellow colour compounds (**6a-o**).

Ethyl (*Z*)-5-((4-chlorophenyl)carbamoyl)-2-((2-cyano-3-((4-fluorophenyl)amino)-1-(methylthio)-3-oxoprop-1-en-1-yl)amino)-4-methylthiophene-3-carboxylate (JOOET-2)



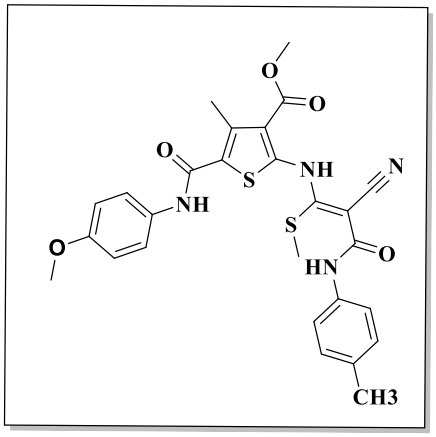
Yield 92%, mp 205-207 °C. ^{1}H NMR spectrum, δ, ppm: 13.67 (s, 1H), 7.59 – 7.48 (m, 4H),7.37 (d, J = 8.6 Hz, 2H), 7.07 (d, J = 8.5 Hz, 2H), 4.49 (q, J = 7.1 Hz, 2H),2.58 (s, 3H), 1.44 (t, J = 7.1 Hz, 3H), 1.28 (s, 3H). M 573.

Ethyl (*Z*)-2-((3-((4-chlorophenyl)amino)-2-cyano-1-(methylthio)-3-oxoprop-1-en-1-yl)amino)-5-((4-methoxyphenyl)carbamoyl)-4-methylthiophene-3-carboxylate (JOOET-3).



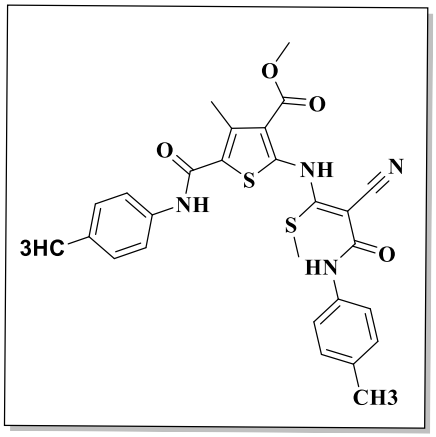
Yield 96%, mp 208-210 °C. ^{1}H NMR spectrum, *δ*, ppm: 12.98 (s, 1H), 10.00 (s, 2H), 7.63 (d, 2H), 7.56 (d, 2H), 7.40 (d, *J* = 8.6 Hz, 2H), 6.93 (d, *J* = 8.5 Hz, 2H), 4.34 (q, *J* = 7.2 Hz, 2H), 3.75 (s, 3H), 2.55 (s, 3H), 1.33 (t, *J* = 7.2 Hz, 3H). *M* 585.

Ethyl (*E*)-2-((2-cyano-1-(methylthio)-3-oxo-3-(p-tolylamino)prop-1-en-1-yl)amino)-5-((4-methoxyphenyl)carbamoyl)-4-methylthiophene-3-carboxylate (JOOET-5)



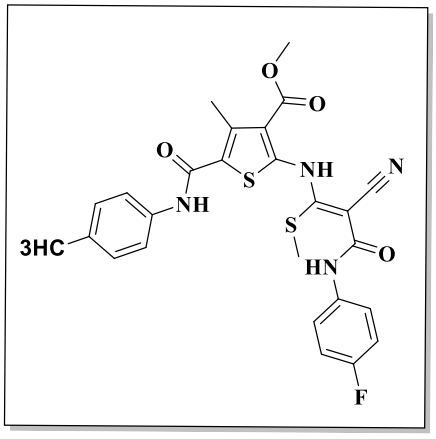
Yield 94 %, mp 214-216 °C. ^{1}H NMR spectrum, δ, ppm: 13.09 (s, 1H), 10.05 (s, 1H), 9.82 (s, 1H), 7.56 (d, *J* = 8.8 Hz, 2H), 7.47 (d, *J* = 8 Hz, 2H), 7.14 (d, *J* = 8.4 Hz, 2H), 6.92 (d, *J* = 9.2 Hz, 2H), 4.35 (q, *J* = 7.2 Hz, 2H), 3.74 (s, 3H), 2.53 (s, 9H), 1.33 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR spectrum, *δ*, ppm: 163.26, 163.09, 162.54, 160.54, 156.26, 148.59, 137.95, 135.70, 133.95, 132.08, 129.47, 126.53, 122.43, 121.85, 119.79, 117.88, 114.30, 87.37, 61.47, 55.66, 40.66, 40.45, 20.97, 18.45, 16.22, 14.48. *M* 564.

Ethyl (*E*)-2-((2-cyano-1-(methylthio)-3-oxo-3-(p-tolylamino)prop-1-en-1-yl)amino)-4-methyl-5-(p-tolylcarbamoyl)thiophene-3-carboxylate (JOOET-6)



Yield 95%, mp 212-214 °C. ^{1}H NMR spectrum, *δ*, ppm: 13.09 (s, 1H), 10.09 (s, 1H), 9.81 (s, 1H), 7.54 (d, *J* = 8.0 Hz, 2H), 7.47 (d, *J* = 8.0 Hz, 2H), 7.15 (m, *J* = 6.4 Hz, 4H), 4.35 (q, *J* = 7.1 Hz, 2H), 2.54 (s, 3H), 2.28 (s, 6H), 1.33 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR spectrum, *δ*, ppm: 163.29, 163.01, 162.53, 160.78, 148.66, 138.15, 136.43, 135.55, 134.05, 133.56, 129.52, 129.47, 126.30, 121.85, 120.86, 119.69, 117.86, 87.34, 61.50, 20.93, 18.44, 16.20, 14.43. *M* 548.

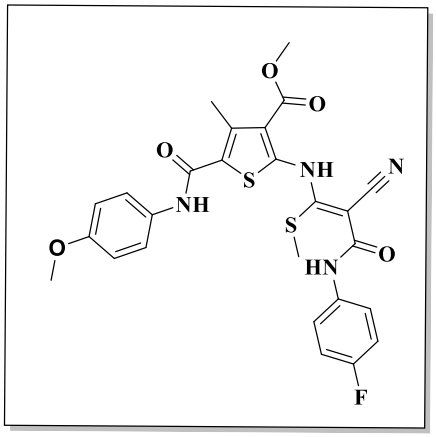
Ethyl (*E*)-2-((2-cyano-3-((4-fluorophenyl)amino)-1-(methylthio)-3-oxoprop-1-en-1-yl)amino)-4-methyl-5-(p-tolylcarbamoyl)thiophene-3-carboxylate (JOOET-7)



Yield 94%, mp 220-222 °C. ^{1}H NMR spectrum, δ, ppm: 13.08 (s, 1H), 10.11 (s, 1H), 9.97 (s, 1H), 7.62 – 7.53 (m, 4H), 7.21 – 7.14 (m, 4H), 4.34 (q, *J* = 7.2 Hz, 2H), 2.74 (s, 3H), 2.54 (s, 3H), 2.28 (s, 3H), 1.33 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR spectrum, δ, ppm: 163.29,

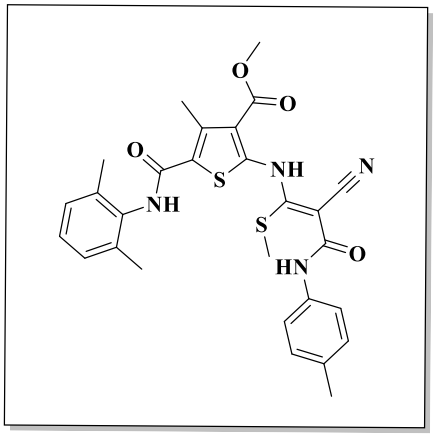
162.89, 162.78, 160.71, 158.04, 148.62, 138.05, 136.53, 134.57, 133.50, 129.56, 126.61, 123.97, 123.89, 120.77, 119.89, 117.82, 115.79, 115.56, 87.04, 61.48, 36.24, 31.23, 20.96, 18.46, 16.23, 14.47. M552 *m*/*z*

Ethyl (*E*)-2-((2-cyano-3-((4-fluorophenyl)amino)-1-(methylthio)-3-oxoprop-1-en-1-yl)amino)-5-((4-methoxyphenyl)carbamoyl)-4-methylthiophene-3-carboxylate (JOOET-8)



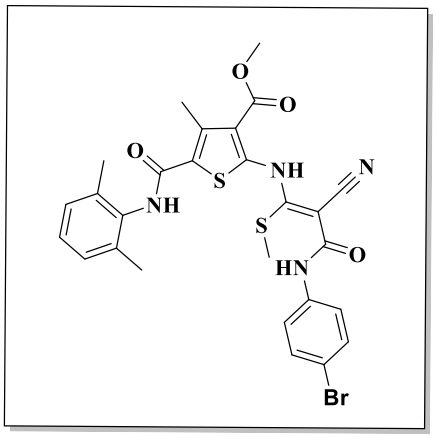
Yield 89%, mp 212-214 °C. ^{1}H NMR spectrum, δ, ppm: 13.07 (s, 1H), 10.06 (s, 1H), 9.97 (s, 1H), 7.62 – 7.55 (m, 4H), 7.19 (d, *J* = 8.8 Hz, 2H), 6.92 (d, *J* = 9.2 Hz, 2H), 4.34 (q, *J* = 7.2 Hz, 2H), 3.74 (s, 3H), 2.54 (s, 3H), 1.33 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR spectrum, δ, ppm: 163.29, 162.92, 160.53, 158.04, 156.26, 148.51, 137.91, 134.58, 132.07, 126.65, 123.98, 123.90, 122.44, 119.93, 117.83, 115.80, 115.57, 114.30, 86.99, 61.48, 55.66, 18.46, 16.23, 14.48. *M* 568.

Ethyl (*E*)-2-((2-cyano-1-(methylthio)-3-oxo-3-(p-tolylamino)prop-1-en-1-yl)amino)-5-((2,6-dimethylphenyl)carbamoyl)-4-methylthiophene-3-carboxylate (JOOET-9)



Yield 85%, mp 204-206°C. ^{1}H NMR spectrum, δ, ppm: 13.07 (s, 1H), 9.83 (s, 1H), 9.56 (s, 1H), 7.47 (d, *J* = 8.4 Hz, 2H), 7.16 – 7.11 (m, 5H), 4.35 (q, *J* = 7.2 Hz, 2H), 2.74 (s, 6H), 2.55 (s, 3H), 2.28 (s, 6H), 1.34 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR spectrum, δ, ppm: 163.27, 163.09, 162.68, 160.86, 148.47, 138.11, 135.97, 135.72, 135.31, 133.94, 129.47, 128.29, 127.42, 125.96, 121.84, 120.02, 117.91, 87.29, 61.46, 40.66, 40.45, 20.98, 18.70, 18.46, 16.13, 14.48. *M* 562.

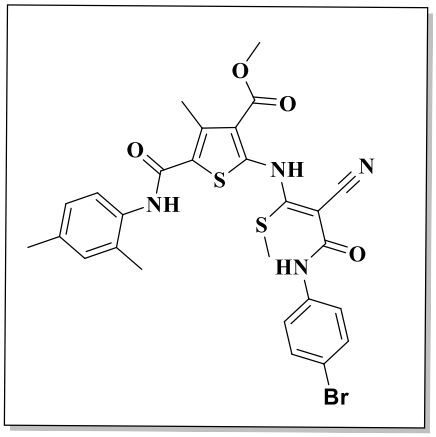
Ethyl (*Z*)-2-((3-((4-bromophenyl)amino)-2-cyano-1-(methylthio)-3-oxoprop-1-en-1-yl)amino)-5-((2,6-dimethylphenyl)carbamoyl)-4-methylthiophene-3-carboxylate (JOOET-10)



Yield 96%, mp 216-218 °C. ^{1}H NMR spectrum, δ, ppm: 12.97 (s, 1H), 10.04 (s, 1H), 9.55 (s, 1H), 7.56 (q, *J* = 8.8 Hz, 3H), 7.14 (s, 4H), 4.36 (q, *J* = 7.2 Hz, 2H), 2.58 (s, 3H), 2.56 (s, 3H), 2.20 (s, 6H), 1.33 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR spectrum, δ, ppm: 162.77, 160.31,

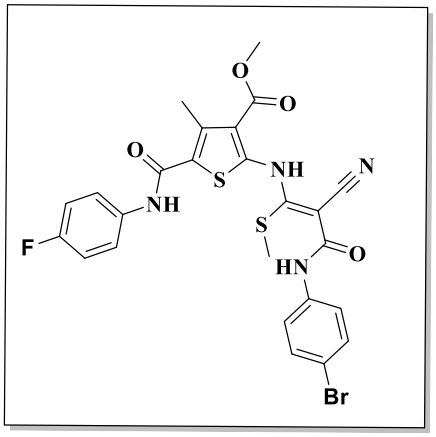
137.52, 135.42, 134.75, 131.34, 127.73, 126.88, 123.14, 119.76, 60.93, 18.12, 17.92, 15.55, 13.93. *M* 626.

Ethyl (*E*)-2-((3-((4-bromophenyl)amino)-2-cyano-1-(methylthio)-3-oxoprop-1-en-1-yl)amino)-5-((2,4-dimethylphenyl)carbamoyl)-4-methylthiophene-3-carboxylate (JOOET-11)



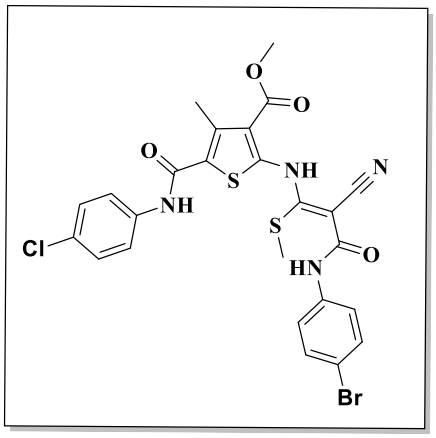
Yield 92%, mp 208-210 °C. ^{1}H NMR spectrum, δ, ppm: 13.00 (s, 1H), 10.04 (s, 1H), 9.64 (s, 1H), 7.59 – 7.52 (m, 4H), 7.22 (d, *J* = 8.0 Hz, 1H), 7.08 – 7.00 (m, 2H), 4.35 (q, *J* = 7.2 Hz, 2H), 2.56 (s, 3H), 2.54 (s, 3H), 2.27 (s, 3H), 2.19 (s, 3H), 1.33 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR spectrum, δ, ppm: 163.31, 163.26, 161.01, 148.54, 138.12, 137.77, 135.74, 133.87, 133.58, 131.89, 131.40, 127.07, 126.47, 123.70, 120.18, 117.80, 116.68, 86.94, 61.50, 40.66, 40.45, 21.01, 18.48, 16.18, 14.49. *M* 626.

Ethyl (*E*)-2-((3-((4-bromophenyl)amino)-2-cyano-1-(methylthio)-3-oxoprop-1-en-1-yl)amino)-5-((4-fluorophenyl)carbamoyl)-4-methylthiophene-3-carboxylate. (JOOET-12)



Yield 93%, mp 206- °C. ^{1}H NMR spectrum, δ, ppm: 13.03 (s, 1H), 10.24 (s, 1H), 10.04 (s, 1H), 7.70 – 7.65 (m, 2H), 7.59 – 7.52 (m, 4H), 7.20 (t, *J* = 6.8 Hz, 2H), 4.35 (q, *J* = 7.2 Hz, 2H), 2.55 (s, 3H), 1.33 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR spectrum, δ, ppm: 163.28, 163.15, 160.84, 160.13, 157.74, 148.72, 138.40, 137.76, 135.41, 135.38, 131.89, 126.28, 123.71, 122.72, 122.64, 120.02, 117.76, 116.69, 115.91, 115.69, 87.12, 61.53, 18.51, 16.25, 14.49. *M* 616.

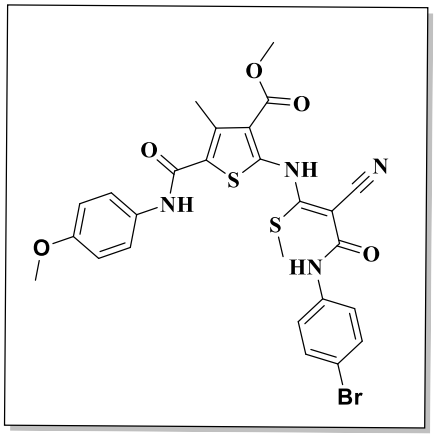
Ethyl (*E*)-2-((3-((4-bromophenyl)amino)-2-cyano-1-(methylthio)-3-oxoprop-1-en-1-yl)amino)-5-((4-chlorophenyl)carbamoyl)-4-methylthiophene-3-carboxylate. (JOOET-13)



Yield 89%, mp 224-226 °C. ^{1}H NMR spectrum, δ, ppm: 13.04 (s, 1H), 10.31 (s, 1H), 10.04 (s, 1H), 7.68 (d, *J* = 7.2 Hz, 2H), 7.59 – 7.51 (m, 4H), 7.42 (d, *J* = 8.9 Hz, 2H), 4.35 (q, *J* = 7.2 Hz, 2H), 2.55 (s, 3H), 1.33 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR spectrum, δ, ppm: 163.27,

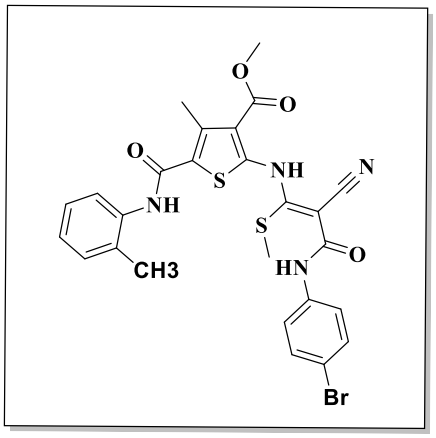
163.10, 160.96, 148.87, 138.66, 138.02, 137.75, 131.89, 129.11, 128.09, 126.12, 123.71, 122.29, 119.98, 116.70, 87.21, 61.54, 40.67, 40.46, 18.51, 16.28, 14.49. *M* 631.

Ethyl (*E*)-2-((3-((4-bromophenyl)amino)-2-cyano-1-(methylthio)-3-oxoprop-1-en-1-yl)amino)-5-((4-methoxyphenyl)carbamoyl)-4-methylthiophene-3-carboxylate. (JOOET-14)



Yield 90%, mp 216-218 °C. ^{1}H NMR spectrum, δ, ppm: 13.02 (s, 1H), 10.05 (s, 1H), 10.03 (s, 1H), 7.59 – 7.52 (m, 6H), 6.92 (d, *J* = 9.2 Hz, 2H), 4.35 (q, *J* = 7.2 Hz, 2H), 3.75 (s, 3H), 2.55 (s, 3H), 1.33 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR spectrum, δ, ppm: 163.32, 163.21, 160.52, 156.27, 148.46, 137.91, 137.76, 132.07, 131.89, 126.74, 123.71, 122.44, 120.05, 117.79, 116.68, 114.31, 86.96, 61.50, 55.67, 40.67, 40.46, 18.48, 16.22, 14.49. *M* 628.

Ethyl (*E*)-2-((3-((4-bromophenyl)amino)-2-cyano-1-(methylthio)-3-oxoprop-1-en-1-yl)amino)-4-methyl-5-(o-tolylcarbamoyl)thiophene-3-carboxylate. (JOOET-15)



Yield 92%, mp 216-218 °C. ^{1}H NMR spectrum, δ, ppm: 13.00 (s, 1H), 10.03 (s, 1H), 9.71 (s, 1H), 7.59 – 7.54 (m, 4H), 7.37 (d, *J* = 7.2 Hz, 1H), 7.28 – 7.15 (m, 3H), 4.35 (q, *J* = 7.2 Hz, 2H), 2.58 (s, 3H), 2.55 (s, 3H), 2.24 (s, 3H), 1.34 (t, *J* = 7.2 Hz, 3H). *M* 612.

1.4.1. Anticancer activity

NCI anticancer screening.

The NCI anticancer screening approach is extensively described elsewhere (http://www.dtp.nci.nih.gov) In brief, the primary anticancer experiment used about sixty human tumor cell lines obtained from nine neoplastic illnesses, in accordance with the guidelines provided by the Drug Evaluation Branch at the National Cancer Institute in Bethesda, USA. The molecules under investigation were added to the cultures at a single concentration of 10^{-5} M, and they were left to incubate for 48 hours. Endpoint evaluations were carried out with SRB, a protein-binding dye. Each molecule's results were expressed as a percentage of treated cells' growth inhibition relative to untreated controls, measured by spectrophotometry against controls that had not been exposed to the test agents. Using doses ranging from 10-4 to 10-8 M, the most active molecule was tested further for growth inhibitory and/or cytotoxic effects across the whole panel of about 60 human tumor cell lines. A continuous drug exposure regimen of 48 hours was followed, and SRB protein tests were used to measure cell survival or growth. The percentage growth was calculated at different drug concentration levels utilizing seven absorbance measurements: initial time (Tz), control growth without drug (C), and test growth with drug at five concentration levels (Ti). In concentrations in which Ti exceeded Tz, the percentage growth inhibition was [(Ti - Tz)/(C - Tz)] × 100, while in concentrations where Ti was less than Tz, it was [(Ti - Tz)/Tz] × 100. For each molecule, three dose-response parameters were calculated. The GI50, which represents the concentration producing a 50% decrease in net protein growth relative to control, was calculated as [(Ti - Tz)/(C - Tz)] × 100 = 50. TGI, the concentration that causes complete growth inhibition, was discovered where Ti equaled Tz. The LC50, which indicates a fifty percent reduction for measured protein in comparison to the beginning and indicating cell loss post-treatment, was determined from [(Ti - Tz)/Tz] × 100 = -50. These values were calculated if the activity level was met; otherwise, these were expressed as falling or falling under the tested concentration range. Log TGI, log GI50, and log LC50 were then determined as the average of the logarithms of the respective TGI, GI50 and LC50 values. Lower value suggest that cell lines are more sensitive. molecules were considered active if their log GI50 values were -4 or less.

1.5. Spectral Data

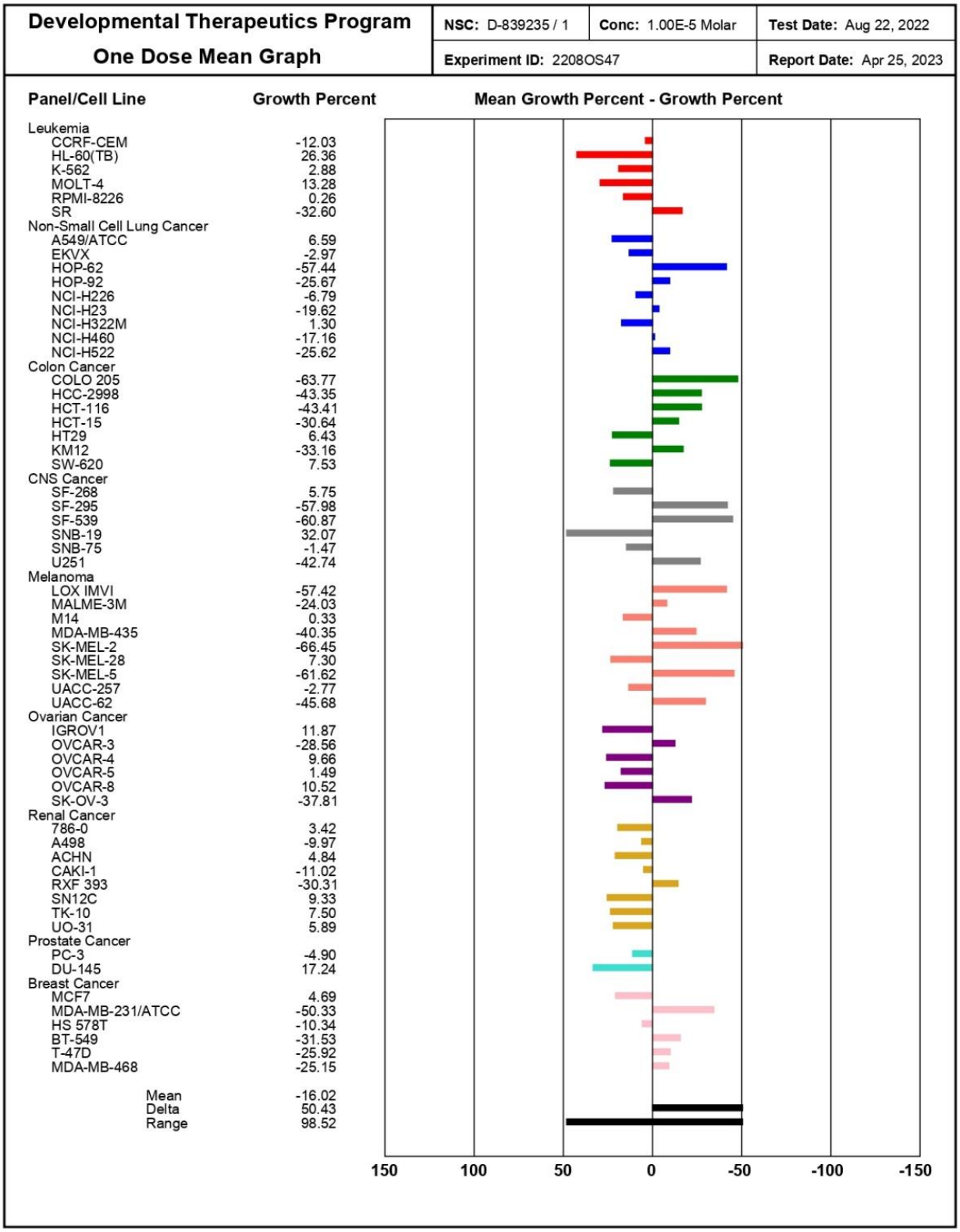


Fig. 1: Representative single dose data of compound **JOOET-2**

National Cancer Institute Developmental Therapeutics Program
In-Vitro Testing Results

NSC : D - 839235 / 1	Experiment ID : 2301NS84	Test Type : 08	Units : Molar
Report Date : February 24, 2023	Test Date : January 09, 2023	QNS :	MC :
COMI : JOOET-2	Stain Reagent : SRB Dual-Pass Related	SSPL : 1COF	

			Log10 Concentration												
	Time		Mean Optical Densities					Percent Growth							
Panel/Cell Line	Zero	Ctrl	-8.0	-7.0	-6.0	-5.0	-4.0	-8.0	-7.0	-6.0	-5.0	-4.0	GI50	TGI	LC50
Leukemia															
CCRF-CEM	0.782	3.005	3.102	3.070	2.703	0.716	0.523	104	103	86	-9	-33	2.42E-6	8.14E-6	> 1.00E-4
HL-60(TB)	0.654	2.533	2.378	2.327	2.295	0.401	0.316	92	89	87	-39	-52	1.98E-6	4.93E-6	7.34E-5
K-562	0.221	2.064	1.986	1.941	1.431	0.316	0.194	96	93	66	5	-12	1.81E-6	1.96E-5	> 1.00E-4
MOLT-4	0.694	2.665	2.620	2.467	2.153	0.460	0.309	98	90	74	-34	-55	1.67E-6	4.86E-6	5.60E-5
RPMI-8226	0.673	2.623	2.590	2.479	1.937	0.550	0.581	98	93	65	-18	-14	1.51E-6	6.03E-6	> 1.00E-4
SR	0.633	2.406	2.356	2.352	2.213	0.377	0.394	97	97	89	-41	-38	2.00E-6	4.87E-6	> 1.00E-4
Non-Small Cell Lung Cancer															
A549/ATCC	0.485	2.345	2.291	2.275	2.130	0.753	0.120	97	96	88	14	-75	3.30E-6	1.45E-5	5.23E-5
EKVX	0.587	1.842	1.722	1.692	1.641	0.537	0.018	90	88	84	-9	-97	2.33E-6	8.09E-6	2.94E-5
HOP-62	0.883	2.500	2.500	2.352	2.363	1.020	0.365	100	91	92	8	-59	3.16E-6	1.34E-5	7.43E-5
HOP-92	1.154	1.742	1.703	1.613	1.463	0.849	0.254	93	78	53	-26	-78	1.08E-6	4.63E-6	2.86E-5
NCI-H23	0.727	2.336	2.340	2.300	2.133	0.618	0.043	100	98	87	-15	-94	2.32E-6	7.14E-6	2.77E-5
NCI-H322M	0.767	2.084	1.990	1.916	1.826	0.866	-0.007	93	87	80	7	-100	2.61E-6	1.17E-5	3.43E-5
NCI-H460	0.363	3.197	3.157	3.163	2.990	0.342	0.059	99	99	93	-6	-84	2.71E-6	8.73E-6	3.69E-5
NCI-H522	1.386	3.119	3.018	3.024	2.781	1.232	0.269	94	95	81	-11	-81	2.15E-6	7.56E-6	3.63E-5
Colon Cancer															
COLO 205	0.574	2.113	2.094	2.116	2.157	0.623	0.108	99	100	103	3	-81	3.39E-6	1.09E-5	4.27E-5
HCC-2998	0.728	2.731	2.659	2.649	2.500	0.618	-0.004	96	96	88	-15	-100	2.35E-6	7.15E-6	2.58E-5
HCT-116	0.293	2.525	2.584	2.507	2.224	0.199	0.066	103	99	86	-32	-77	2.03E-6	5.36E-6	2.48E-5
HCT-15	0.307	2.382	2.303	2.301	1.949	0.210	-0.006	96	96	79	-32	-100	1.83E-6	5.17E-6	1.85E-5
HT29	0.288	1.792	1.805	1.815	1.731	0.550	0.127	101	102	96	17	-56	3.85E-6	1.73E-5	8.31E-5
KM12	0.887	3.241	3.251	3.253	3.086	0.839	0.112	100	100	93	-5	-87	2.75E-6	8.82E-6	3.50E-5
SW-620	0.435	2.984	2.920	2.937	2.801	0.783	0.144	97	98	93	14	-67	3.47E-6	1.48E-5	6.17E-5
CNS Cancer															
SF-268	1.146	2.825	2.793	2.671	2.431	1.255	0.336	98	91	77	6	-71	2.39E-6	1.21E-5	5.39E-5
SF-295	1.076	3.121	3.053	2.993	2.627	0.490	0.003	97	94	76	-55	-100	1.58E-6	3.82E-6	9.23E-6
SF-539	0.890	2.654	2.636	2.567	2.521	0.716	-0.003	99	95	92	-20	-100	2.39E-6	6.69E-6	2.39E-5
SNB-19	0.656	2.152	2.042	1.999	1.820	0.808	0.038	93	90	78	10	-94	2.57E-6	1.25E-5	3.77E-5
SNB-75	2.600	3.391	3.301	3.264	3.262	2.452	0.436	89	84	84	-6	-83	2.38E-6	8.63E-6	3.73E-5
U251	0.363	1.855	1.812	1.692	1.590	0.444	0.352	97	89	82	5	-3	2.63E-6	4.28E-5	> 1.00E-4
Melanoma															
LOX IMVI	0.184	1.424	1.439	1.367	1.348	0.179	0.004	101	95	94	-3	-98	2.85E-6	9.37E-6	3.14E-5
MALME-3M	0.868	1.637	1.543	1.492	1.410	0.639	0.080	88	81	71	-26	-91	1.63E-6	5.34E-6	2.33E-5
M14	0.441	1.523	1.535	1.496	1.538	0.493	0.063	101	97	101	5	-86	3.40E-6	1.13E-5	4.03E-5
MDA-MB-435	0.880	3.188	3.145	3.148	3.046	0.622	0.132	98	98	94	-29	-85	2.27E-6	5.78E-6	2.35E-5
SK-MEL-2	1.529	2.414	2.462	2.408	2.297	0.640	0.355	105	99	87	-58	-77	1.79E-6	3.97E-6	8.79E-6
SK-MEL-28	0.872	2.386	2.406	2.383	2.195	1.029	0.023	101	100	87	10	-97	3.06E-6	1.25E-5	3.63E-5
SK-MEL-5	0.867	3.033	2.929	2.818	2.484	0.104	-0.013	95	90	75	-88	-100	1.42E-6	2.88E-6	5.84E-6
UACC-257	1.619	2.907	2.812	2.745	2.595	1.489	0.722	93	87	76	-8	-55	2.03E-6	8.01E-6	7.69E-5
UACC-62	1.122	2.945	2.834	2.776	2.440	0.557	0.013	94	91	72	-50	-99	1.52E-6	3.88E-6	9.93E-6
Ovarian Cancer															
IGROV1	0.927	2.786	2.806	2.765	2.539	1.087	0.106	101	99	87	9	-89	2.95E-6	1.23E-5	4.01E-5
OVCAR-3	0.716	2.087	2.166	2.107	1.904	0.572	0.075	106	101	87	-20	-90	2.20E-6	6.48E-6	2.70E-5
OVCAR-4	1.361	2.845	2.836	2.780	2.668	1.432	0.927	99	96	88	5	-32	2.86E-6	1.35E-5	> 1.00E-4
OVCAR-5	0.575	1.457	1.385	1.357	1.289	0.638	-0.007	92	89	81	7	-100	2.62E-6	1.17E-5	3.41E-5
OVCAR-8	0.668	2.776	2.771	2.689	2.523	0.854	0.136	100	96	88	9	-80	3.02E-6	1.26E-5	4.62E-5
NCI/ADR-RES	0.498	1.855	1.868	1.826	1.679	0.503	0.068	101	98	87	0	-86	2.68E-6	1.01E-5	3.81E-5
SK-OV-3	1.214	2.400	2.451	2.327	2.177	1.180	0.159	104	94	81	-3	-87	2.35E-6	9.25E-6	3.64E-5
Renal Cancer															
786-0	0.820	2.711	2.771	2.697	2.573	0.825	0.090	103	99	93	0	-89	2.90E-6	1.01E-5	3.65E-5
A498	1.533	2.444	2.384	2.351	2.272	1.375	0.104	93	90	81	-10	-93	2.19E-6	7.71E-6	3.01E-5
ACHN	0.363	1.695	1.701	1.706	1.539	0.391	-0.012	100	101	88	2	-100	2.78E-6	1.05E-5	3.24E-5
CAKI-1	0.765	2.356	2.206	2.145	1.985	0.631	0.041	91	87	77	-18	-95	1.92E-6	6.52E-6	2.64E-5
RXF 393	0.564	1.358	1.323	1.315	1.244	0.635	0.038	96	95	86	9	-93	2.92E-6	1.22E-5	3.77E-5
SN12C	1.363	3.346	3.291	3.269	2.926	1.356	0.142	97	96	79	0	-90	2.31E-6	9.84E-6	3.59E-5
TK-10	1.345	2.520	2.448	2.418	2.392	1.562	0.729	94	91	89	18	-46	3.58E-6	1.94E-5	> 1.00E-4
UO-31	0.691	2.685	2.532	2.421	2.030	0.772	-0.012	92	87	67	4	-100	1.87E-6	1.09E-5	3.31E-5
Prostate Cancer															
PC-3	0.589	1.940	1.879	1.824	1.744	0.579	0.182	95	91	86	-2	-69	2.55E-6	9.54E-6	5.19E-5
DU-145	0.594	2.338	2.387	2.341	2.133	0.852	0.153	103	100	88	15	-74	3.32E-6	1.47E-5	5.34E-5
Breast Cancer															
MCF7	0.363	2.242	2.109	2.039	1.908	0.403	0.052	93	89	82	2	-86	2.52E-6	1.06E-5	3.92E-5
MDA-MB-231/ATCC	0.656	1.689	1.664	1.637	1.550	0.545	0.172	98	95	87	-17	-74	2.25E-6	6.85E-6	3.81E-5
HS 578T	1.367	2.534	2.473	2.374	2.267	1.269	1.193	95	86	77	-7	-13	2.10E-6	8.21E-6	> 1.00E-4
BT-549	1.522	2.827	2.895	2.871	2.769	1.098	0.083	105	103	96	-28	-95	2.34E-6	5.94E-6	2.15E-5
T-47D	1.035	2.527	2.445	2.331	2.285	1.205	1.008	94	87	84	11	-3	2.92E-6	6.46E-5	> 1.00E-4
MDA-MB-468	0.749	1.792	1.776	1.705	1.586	0.543	0.164	99	92	80	-28	-78	1.91E-6	5.55E-6	2.78E-5

Fig. 2: Representative five dose data of compound **JOOET-2**

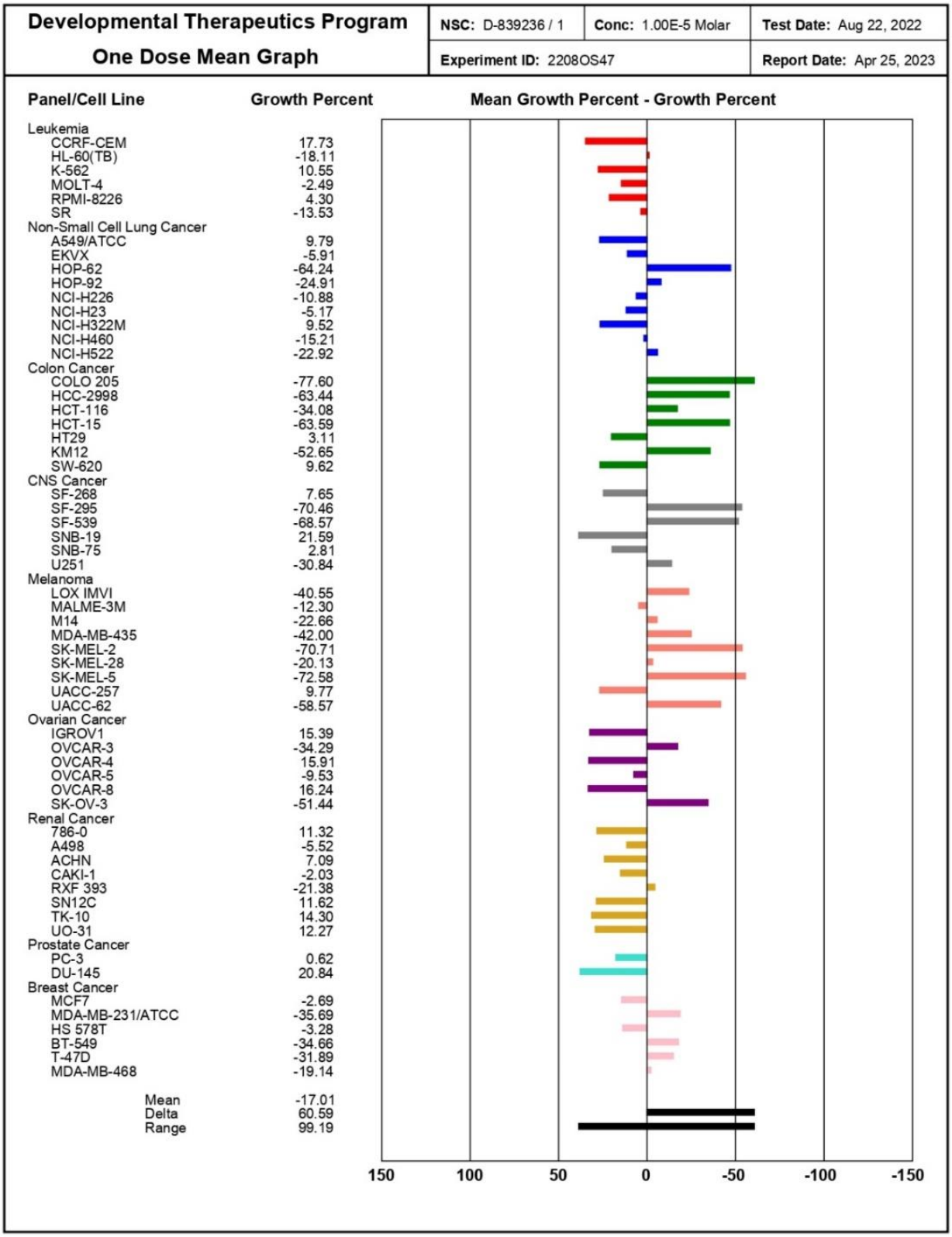


Fig. 3: Representative single dose data of compound **JOOET-3**

National Cancer Institute Developmental Therapeutics Program
In-Vitro Testing Results

NSC : D - 839236 / 1	Experiment ID : 2301NS84	Test Type : 08	Units : Molar
Report Date : February 24, 2023	Test Date : January 09, 2023	QNS :	MC :
COMI : JOOET-3	Stain Reagent : SRB Dual-Pass Related	SSPL : 1COF	

		Log10 Concentration												
	Time	Mean Optical Densities						Percent Growth						
Panel/Cell Line	Zero	Ctrl	-8.0	-7.0	-6.0	-5.0	-4.0	-8.0	-7.0	-6.0	-5.0	-4.0	GI50	TGI	LC50
Leukemia															
CCRF-CEM	0.782	2.872	2.882	2.860	2.557	0.706	0.566	100	99	85	-10	-28	2.34E-6	7.89E-6	> 1.00E-4
HL-60(TB)	0.654	2.520	2.288	2.114	2.201	0.429	0.450	88	78	83	-34	-31	1.91E-6	5.09E-6	> 1.00E-4
K-562	0.221	2.097	2.019	1.964	1.613	0.418	0.366	96	93	74	11	8	2.40E-6	> 1.00E-4	> 1.00E-4
MOLT-4	0.694	2.504	2.486	2.513	2.204	0.508	0.404	99	100	83	-27	-42	2.01E-6	5.71E-6	> 1.00E-4
RPMI-8226	0.673	2.573	2.535	2.424	1.915	0.486	0.538	98	92	65	-28	-20	1.46E-6	5.03E-6	> 1.00E-4
SR	0.633	2.268	2.214	2.120	2.150	0.422	0.493	97	91	93	-33	-22	2.18E-6	5.44E-6	> 1.00E-4
Non-Small Cell Lung Cancer															
A549/ATCC	0.485	2.405	2.330	2.284	2.184	0.802	0.328	96	94	88	17	-32	3.43E-6	2.18E-5	> 1.00E-4
EKVX	0.587	1.848	1.711	1.700	1.682	0.563	0.012	89	88	87	-4	-98	2.54E-6	9.02E-6	3.08E-5
HOP-62	0.883	2.574	2.519	2.482	2.487	1.314	0.264	97	95	95	25	-70	4.43E-6	1.85E-5	6.16E-5
HOP-92	1.154	1.765	1.766	1.716	1.542	0.922	0.312	100	92	63	-20	-73	1.45E-6	5.75E-6	3.67E-5
NCI-H23	0.727	2.363	2.361	2.333	2.216	0.635	0.024	100	98	91	-13	-97	2.49E-6	7.54E-6	2.78E-5
NCI-H322M	0.767	2.071	2.012	1.972	1.920	0.863	-0.008	95	92	88	7	-100	2.98E-6	1.17E-5	3.42E-5
NCI-H460	0.363	3.152	3.192	3.200	2.989	0.346	0.040	101	102	94	-5	-89	2.80E-6	8.97E-6	3.45E-5
NCI-H522	1.386	3.145	3.081	3.062	2.894	1.083	0.070	96	95	86	-22	-95	2.15E-6	6.26E-6	2.42E-5
Colon Cancer															
COLO 205	0.574	2.079	2.131	2.209	2.188	0.648	0.182	103	109	107	5	-68	3.62E-6	1.17E-5	5.61E-5
HCC-2998	0.728	2.711	2.574	2.645	2.633	0.565	0.003	93	97	96	-22	-100	2.45E-6	6.46E-6	2.27E-5
HCT-116	0.293	2.625	2.652	2.613	2.376	0.245	0.056	101	100	89	-17	-81	2.35E-6	6.98E-6	3.30E-5
HCT-15	0.307	2.328	2.231	2.216	2.005	0.162	-0.001	95	94	84	-47	-100	1.82E-6	4.37E-6	1.13E-5
HT29	0.288	1.774	1.760	1.826	1.777	0.390	0.010	99	104	100	7	-97	3.45E-6	1.17E-5	3.55E-5
KM12	0.887	3.239	3.244	3.238	3.111	0.616	0.028	100	100	95	-31	-97	2.27E-6	5.70E-6	1.96E-5
SW-620	0.435	2.930	2.988	2.894	2.887	0.702	0.049	102	99	98	11	-89	3.56E-6	1.28E-5	4.08E-5
CNS Cancer															
SF-268	1.146	2.907	2.787	2.712	2.681	1.312	0.153	93	89	87	9	-87	3.00E-6	1.25E-5	4.15E-5
SF-295	1.076	3.119	2.957	2.914	2.709	0.403	0.001	92	90	80	-63	-100	1.62E-6	3.64E-6	8.16E-6
SF-539	0.890	2.717	2.701	2.597	2.626	0.747	-0.002	99	93	95	-16	-100	2.54E-6	7.16E-6	2.53E-5
SNB-19	0.656	2.054	2.016	1.960	1.886	0.772	0.001	97	93	88	8	-100	3.00E-6	1.19E-5	3.46E-5
SNB-75	2.600	3.354	3.284	3.267	3.268	2.503	0.233	91	88	89	-4	-91	2.62E-6	9.11E-6	3.39E-5
U251	0.363	1.828	1.922	1.785	1.786	0.469	0.284	106	97	97	7	-22	3.34E-6	1.78E-5	> 1.00E-4
Melanoma															
LOX IMVI	0.184	1.401	1.399	1.375	1.317	0.193	0.009	100	98	93	1	-95	2.93E-6	1.02E-5	3.37E-5
MALME-3M	0.868	1.652	1.580	1.497	1.461	0.665	0.061	91	80	76	-23	-93	1.81E-6	5.80E-6	2.41E-5
M14	0.441	1.619	1.589	1.605	1.627	0.407	0.045	97	99	101	-8	-90	2.93E-6	8.47E-6	3.26E-5
MDA-MB-435	0.880	3.185	3.138	3.122	2.998	0.503	0.019	98	97	92	-43	-98	2.05E-6	4.81E-6	1.35E-5
SK-MEL-2	1.529	2.443	2.425	2.373	2.291	0.397	0.063	98	92	83	-74	-96	1.63E-6	3.39E-6	7.04E-6
SK-MEL-28	0.872	2.349	2.360	2.309	2.194	1.010	0.002	101	97	90	9	-100	3.11E-6	1.22E-5	3.50E-5
SK-MEL-5	0.867	3.045	2.930	2.943	2.574	0.064	-0.006	95	95	78	-93	-100	1.47E-6	2.87E-6	5.63E-6
UACC-257	1.619	2.975	2.897	2.859	2.735	1.520	0.607	94	91	82	-6	-63	2.32E-6	8.53E-6	5.99E-5
UACC-62	1.122	2.936	2.852	2.827	2.611	0.456	-0.003	95	94	82	-59	-100	1.69E-6	3.80E-6	8.58E-6
Ovarian Cancer															
IGROV1	0.927	2.804	2.845	2.801	2.710	0.997	0.134	102	100	95	4	-86	3.11E-6	1.10E-5	4.00E-5
OVCAR-3	0.716	2.101	2.191	2.190	2.073	0.467	-0.007	106	106	98	-35	-100	2.30E-6	5.47E-6	1.71E-5
OVCAR-4	1.361	2.847	2.792	2.774	2.632	1.402	0.806	96	95	86	3	-41	2.69E-6	1.16E-5	> 1.00E-4
OVCAR-5	0.575	1.566	1.525	1.474	1.360	0.661	-0.007	96	91	79	9	-100	2.60E-6	1.20E-5	3.47E-5
OVCAR-8	0.668	2.798	2.817	2.788	2.665	0.958	0.215	101	99	94	14	-68	3.51E-6	1.47E-5	6.04E-5
NCI/ADR-RES	0.498	1.881	1.905	1.860	1.742	0.539	0.031	102	98	90	3	-94	2.88E-6	1.07E-5	3.53E-5
SK-OV-3	1.214	2.443	2.367	2.382	2.282	1.189	0.285	94	95	87	-2	-77	2.60E-6	9.48E-6	4.40E-5
Renal Cancer															
786-0	0.820	2.773	2.799	2.758	2.798	0.990	0.135	101	99	101	9	-84	3.58E-6	1.24E-5	4.33E-5
A498	1.533	2.422	2.400	2.406	2.283	1.377	0.041	98	98	84	-10	-97	2.31E-6	7.80E-6	2.86E-5
ACHN	0.363	1.661	1.641	1.711	1.579	0.393	-0.004	98	104	94	2	-100	3.01E-6	1.05E-5	3.24E-5
CAKI-1	0.765	2.263	2.103	2.079	1.951	0.614	0.010	89	88	79	-20	-99	1.97E-6	6.32E-6	2.42E-5
RXF 393	0.564	1.348	1.311	1.332	1.286	0.687	0.032	95	98	92	16	-94	3.55E-6	1.39E-5	3.95E-5
SN12C	1.363	3.349	3.277	3.274	3.078	1.280	0.054	96	96	86	-6	-96	2.47E-6	8.59E-6	3.08E-5
TK-10	1.345	2.441	2.351	2.333	2.290	1.503	0.166	92	90	86	14	-88	3.19E-6	1.38E-5	4.27E-5
UO-31	0.691	2.687	2.522	2.479	2.232	0.811	-0.004	92	90	77	6	-100	2.41E-6	1.14E-5	3.37E-5
Prostate Cancer															
PC-3	0.589	2.142	2.091	2.003	1.918	0.642	0.250	97	91	86	3	-58	2.71E-6	1.14E-5	7.52E-5
DU-145	0.594	2.298	2.297	2.273	2.168	0.813	0.084	100	99	92	13	-86	3.41E-6	1.35E-5	4.33E-5
Breast Cancer															
MCF7	0.363	2.165	2.006	1.952	1.895	0.407	0.019	91	88	85	2	-95	2.65E-6	1.06E-5	3.46E-5
MDA-MB-231/ATCC	0.656	1.642	1.591	1.595	1.572	0.540	0.102	95	95	93	-18	-85	2.44E-6	6.91E-6	3.04E-5
HS 578T	1.367	2.495	2.385	2.335	2.281	1.235	1.007	90	86	81	-10	-26	2.20E-6	7.83E-6	> 1.00E-4
BT-549	1.522	2.933	2.982	2.931	2.824	1.259	0.156	103	100	92	-17	-90	2.43E-6	6.95E-6	2.83E-5
T-47D	1.035	2.638	2.587	2.487	2.324	1.258	1.062	97	91	80	14	2	2.87E-6	> 1.00E-4	> 1.00E-4
MDA-MB-468	0.749	1.845	1.782	1.746	1.627	0.582	0.116	94	91	80	-22	-85	1.97E-6	6.05E-6	2.78E-5

Fig. 4: Representative five dose data of compound **JOOET-3**

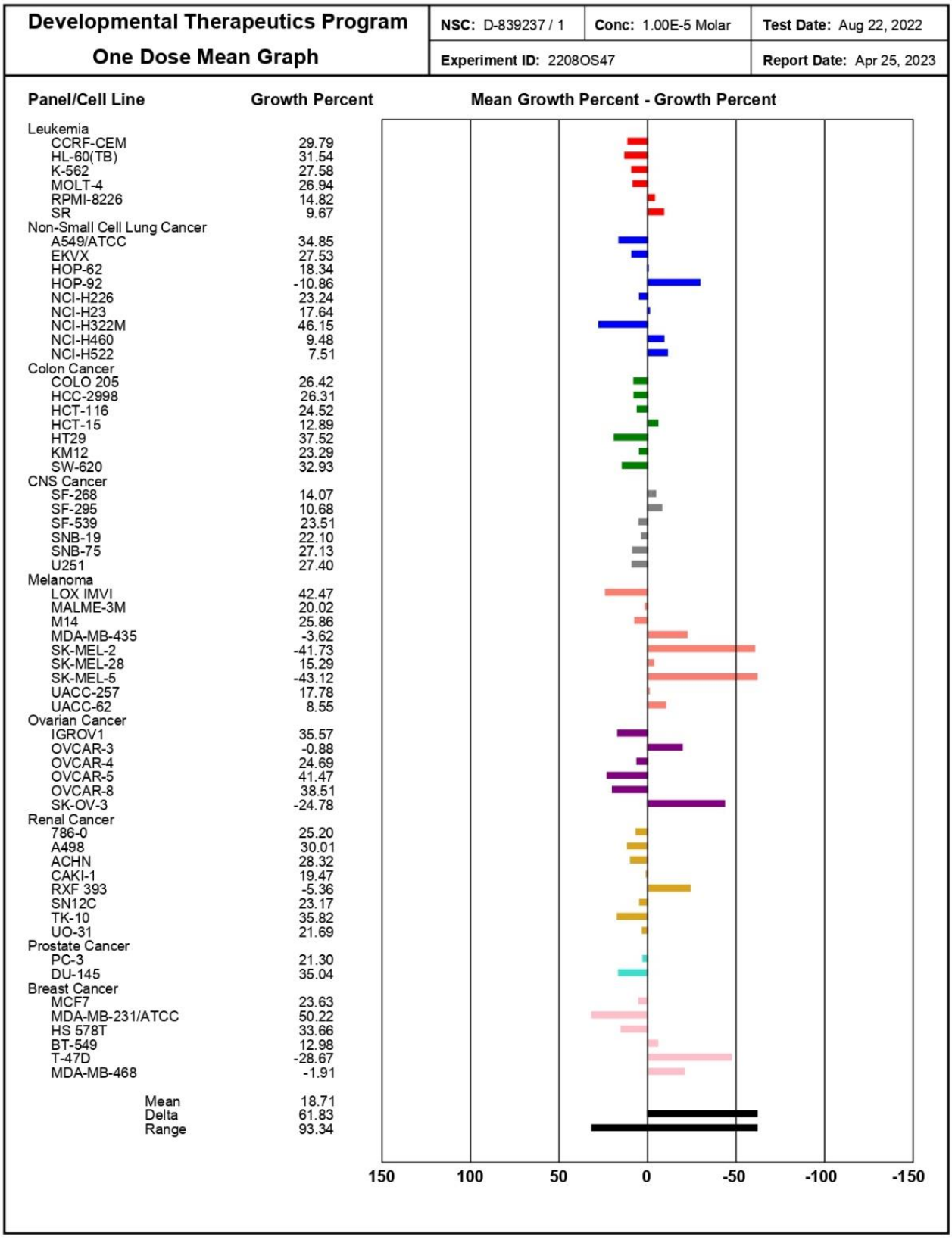


Fig. 5: Representative single dose data of compound **JOOET-5**

National Cancer Institute Developmental Therapeutics Program
In-Vitro Testing Results

NSC : D - 839237 / 1	Experiment ID : 2301NS84	Test Type : 08	Units : Molar
Report Date : February 24, 2023	Test Date : January 09, 2023	QNS :	MC :
COMI : JOOET-5	Stain Reagent : SRB Dual-Pass Related	SSPL : 1COF	

			Log10 Concentration												
	Time		Mean Optical Densities					Percent Growth							
Panel/Cell Line	Zero	Ctrl	-8.0	-7.0	-6.0	-5.0	-4.0	-8.0	-7.0	-6.0	-5.0	-4.0	GI50	TGI	LC50
Leukemia															
CCRF-CEM	0.782	2.872	2.825	2.856	2.731	0.841	0.553	98	99	93	3	-29	3.01E-6	1.22E-5	> 1.00E-4
HL-60(TB)	0.654	2.520	2.346	2.109	1.981	0.497	0.343	91	78	71	-24	-48	1.67E-6	5.59E-6	> 1.00E-4
K-562	0.221	2.097	1.967	2.002	1.786	0.392	0.206	93	95	83	9	-7	2.82E-6	3.67E-5	> 1.00E-4
MOLT-4	0.694	2.504	2.362	2.400	2.209	0.644	0.345	92	94	84	-7	-50	2.35E-6	8.33E-6	9.85E-5
RPMI-8226	0.673	2.573	2.594	2.624	2.215	0.615	0.471	101	103	81	-9	-30	2.22E-6	8.02E-6	> 1.00E-4
SR	0.633	2.268	2.249	2.358	2.020	0.431	0.338	99	105	85	-32	-47	1.99E-6	5.32E-6	> 1.00E-4
Non-Small Cell Lung Cancer															
A549/ATCC	0.485	2.405	2.208	2.272	2.126	0.823	0.153	90	93	85	18	-68	3.33E-6	1.60E-5	6.10E-5
EKVX	0.587	1.848	1.681	1.739	1.675	0.671	0.070	87	91	86	7	-88	2.86E-6	1.18E-5	3.96E-5
HOP-62	0.883	2.574	2.377	2.378	2.349	1.288	0.230	88	88	87	24	-74	3.84E-6	1.76E-5	5.69E-5
HOP-92	1.154	1.765	1.697	1.746	1.588	0.927	0.222	89	97	71	-20	-81	1.70E-6	6.07E-6	3.14E-5
NCI-H23	0.727	2.363	2.337	2.414	2.142	0.613	0.109	98	103	86	-16	-85	2.28E-6	7.02E-6	3.12E-5
NCI-H322M	0.767	2.071	2.055	2.034	1.931	0.968	0.020	99	97	89	15	-97	3.40E-6	1.37E-5	3.80E-5
NCI-H460	0.363	3.152	3.148	3.192	2.930	0.400	0.013	100	101	92	1	-96	2.91E-6	1.03E-5	3.35E-5
NCI-H522	1.386	3.145	3.049	3.096	3.002	1.192	0.131	95	97	92	-14	-91	2.49E-6	7.38E-6	2.95E-5
Colon Cancer															
COLO 205	0.574	2.079	2.066	2.102	2.102	0.841	0.163	99	102	102	18	-72	4.12E-6	1.58E-5	5.73E-5
HCC-2998	0.728	2.711	2.631	2.735	2.672	0.771	0.003	96	101	98	2	-100	3.17E-6	1.05E-5	3.25E-5
HCT-116	0.293	2.625	2.612	2.627	2.448	0.515	0.035	99	100	92	10	-88	3.25E-6	1.25E-5	4.06E-5
HCT-15	0.307	2.328	2.188	2.205	2.088	0.360	-0.007	93	94	88	3	-100	2.79E-6	1.06E-5	3.26E-5
HT29	0.288	1.774	1.708	1.892	1.884	0.496	0.015	96	108	107	14	-95	4.12E-6	1.34E-5	3.87E-5
KM12	0.887	3.239	3.231	3.240	3.112	0.797	0.068	100	100	95	-10	-92	2.67E-6	8.00E-6	3.05E-5
SW-620	0.435	2.930	2.875	2.888	2.750	0.933	0.006	98	98	93	20	-99	3.87E-6	1.47E-5	3.89E-5
CNS Cancer															
SF-268	1.146	2.907	2.718	2.730	2.529	1.250	0.079	89	90	78	6	-93	2.47E-6	1.15E-5	3.67E-5
SF-295	1.076	3.119	2.907	2.960	2.805	0.904	-0.004	90	92	85	-16	-100	2.21E-6	6.93E-6	2.54E-5
SF-539	0.890	2.717	2.699	2.699	2.562	0.959	-0.009	99	99	92	4	-100	2.97E-6	1.09E-5	3.30E-5
SNB-19	0.656	2.054	2.000	2.034	1.862	0.818	0.027	96	99	86	12	-96	3.06E-6	1.28E-5	3.74E-5
SNB-75	2.600	3.354	3.257	3.304	3.223	2.126	0.991	87	93	83	-18	-62	2.10E-6	6.59E-6	5.34E-5
U251	0.363	1.828	1.774	1.779	1.701	0.599	0.105	96	97	91	16	-71	3.54E-6	1.53E-5	5.72E-5
Melanoma															
LOX IMVI	0.184	1.401	1.367	1.415	1.297	0.458	-0.009	97	101	91	22	-100	3.99E-6	1.53E-5	3.91E-5
MALME-3M	0.868	1.652	1.546	1.549	1.442	0.685	0.073	86	87	73	-21	-92	1.76E-6	5.98E-6	2.57E-5
M14	0.441	1.619	1.559	1.576	1.442	0.639	0.047	95	96	85	17	-89	3.26E-6	1.44E-5	4.25E-5
MDA-MB-435	0.880	3.185	3.122	3.130	2.934	0.473	0.022	97	98	89	-46	-98	1.94E-6	4.55E-6	1.18E-5
SK-MEL-2	1.529	2.443	2.325	2.381	2.272	0.589	0.063	87	93	81	-61	-96	1.66E-6	3.71E-6	8.31E-6
SK-MEL-28	0.872	2.349	2.420	2.402	2.175	1.005	0.136	105	104	88	9	-84	3.04E-6	1.25E-5	4.28E-5
SK-MEL-5	0.867	3.045	2.914	2.953	2.671	0.181	-0.006	94	96	83	-79	-100	1.59E-6	3.25E-6	6.61E-6
UACC-257	1.619	2.975	2.841	2.878	2.730	1.380	0.511	90	93	82	-15	-68	2.14E-6	7.04E-6	4.53E-5
UACC-62	1.122	2.936	2.917	2.827	2.581	0.685	0.101	99	94	80	-39	-91	1.80E-6	4.72E-6	1.63E-5
Ovarian Cancer															
IGROV1	0.927	2.804	2.813	2.834	2.763	0.969	0.162	100	102	98	2	-83	3.16E-6	1.06E-5	4.13E-5
OVCAR-3	0.716	2.101	2.026	2.165	1.956	0.389	-0.009	95	105	89	-46	-100	1.96E-6	4.59E-6	1.20E-5
OVCAR-4	1.361	2.847	2.720	2.746	2.640	1.366	0.401	91	93	86	0	-71	2.63E-6	1.01E-5	5.13E-5
OVCAR-5	0.575	1.566	1.589	1.500	1.360	1.045	0.097	102	93	79	47	-83	8.27E-6	2.31E-5	5.57E-5
OVCAR-8	0.668	2.798	2.719	2.824	2.644	1.101	0.126	96	101	93	20	-81	3.89E-6	1.59E-5	4.93E-5
NCI/ADR-RES	0.498	1.881	1.863	1.935	1.724	0.656	0.070	99	104	89	11	-86	3.16E-6	1.31E-5	4.27E-5
SK-OV-3	1.214	2.443	2.274	2.397	2.174	1.147	0.343	86	96	78	-6	-72	2.17E-6	8.59E-6	4.70E-5
Renal Cancer															
786-0	0.820	2.773	2.715	2.817	2.711	1.170	0.059	97	102	97	18	-93	3.92E-6	1.45E-5	4.11E-5
A498	1.533	2.422	2.265	2.339	2.307	1.594	0.441	82	91	87	7	-71	2.89E-6	1.22E-5	5.34E-5
ACHN	0.363	1.661	1.706	1.765	1.541	0.507	-0.015	103	108	91	11	-100	3.25E-6	1.26E-5	3.55E-5
CAKI-1	0.765	2.263	2.047	2.094	1.917	0.929	-0.009	86	89	77	11	-100	2.56E-6	1.26E-5	3.54E-5
RXF 393	0.564	1.348	1.321	1.336	1.278	0.715	0.010	96	98	91	19	-98	3.72E-6	1.46E-5	3.88E-5
SN12C	1.363	3.349	3.309	3.307	3.140	1.291	0.171	98	98	89	-5	-87	2.61E-6	8.79E-6	3.50E-5
TK-10	1.345	2.441	2.309	2.354	2.385	1.377	0.172	88	92	95	3	-87	3.08E-6	1.08E-5	3.86E-5
UO-31	0.691	2.687	2.531	2.540	2.288	0.778	-0.002	92	93	80	4	-100	2.49E-6	1.10E-5	3.32E-5
Prostate Cancer															
PC-3	0.589	2.142	2.059	2.024	1.906	0.727	0.139	95	92	85	9	-76	2.87E-6	1.27E-5	4.89E-5
DU-145	0.594	2.298	2.319	2.334	2.095	0.762	-0.007	101	102	88	10	-100	3.07E-6	1.23E-5	3.51E-5
Breast Cancer															
MCF7	0.363	2.165	2.003	2.126	1.920	0.493	-0.010	91	98	86	7	-100	2.88E-6	1.17E-5	3.42E-5
MDA-MB-231/ATCC	0.656	1.642	1.586	1.625	1.511	0.850	0.029	94	98	87	20	-96	3.53E-6	1.48E-5	4.02E-5
HS 578T	1.367	2.495	2.324	2.417	2.275	1.223	0.902	85	93	81	-11	-34	2.16E-6	7.66E-6	> 1.00E-4
BT-549	1.522	2.933	2.946	2.968	2.888	1.313	0.270	101	102	97	-14	-82	2.65E-6	7.51E-6	3.38E-5
T-47D	1.035	2.638	2.381	2.418	2.419	1.140	0.830	84	86	86	7	-20	2.86E-6	1.77E-5	> 1.00E-4
MDA-MB-468	0.749	1.845	1.772	1.779	1.664	0.689	0.027	93	94	83	-8	-96	2.32E-6	8.16E-6	2.98E-5

Fig. 6: Representative five dose data of compound **JOOET-5**

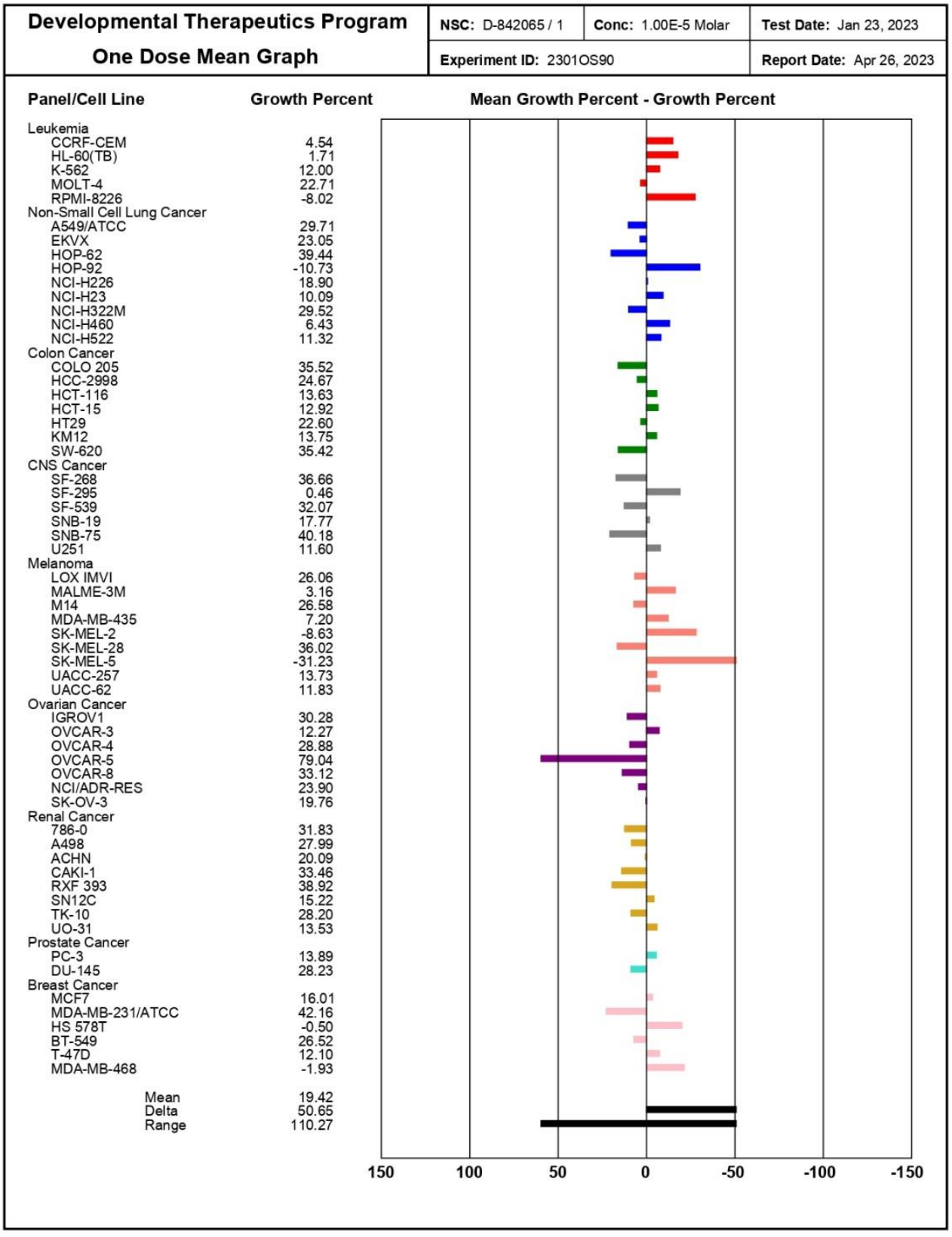


Fig. 7: Representative single dose data of compound **JOOET-6**

National Cancer Institute Developmental Therapeutics Program
In-Vitro Testing Results

NSC : D - 842065 / 1	Experiment ID : 2306NS37	Test Type : 08	Units : Molar
Report Date : July 28, 2023	Test Date : June 20, 2023	QNS :	MC :
COMI : JOOET-6	Stain Reagent : SRB Dual-Pass Related	SSPL : 1COF	

		Log10 Concentration												
	Time	Mean Optical Densities					Percent Growth							
Panel/Cell Line	Zero	Ctrl	-8.0	-7.0	-6.0	-5.0	-4.0	-8.0	-7.0	-6.0	-5.0	-4.0	GI50	TGI	LC50
Leukemia															
CCRF-CEM	0.475	1.770	1.861	1.765	1.706	0.771	0.455	107	100	95	23	-4	4.20E-6	6.99E-5	> 1.00E-4
HL-60(TB)	0.528	2.019	1.943	1.877	1.839	0.990	0.402	95	90	88	31	-24	4.63E-6	3.66E-5	> 1.00E-4
K-562	0.122	0.758	0.716	0.790	0.687	0.246	0.080	93	105	89	19	-34	3.62E-6	2.29E-5	> 1.00E-4
MOLT-4	0.570	2.379	2.378	2.301	2.271	1.097	0.540	100	96	94	29	-5	4.77E-6	7.03E-5	> 1.00E-4
RPMI-8226	0.580	2.171	2.226	2.105	2.118	0.779	0.473	103	96	97	12	-19	3.58E-6	2.53E-5	> 1.00E-4
Non-Small Cell Lung Cancer															
A549/ATCC	0.300	1.789	1.716	1.749	1.676	0.836	0.324	95	97	92	36	2	5.65E-6	> 1.00E-4	> 1.00E-4
EKVX	0.616	2.017	1.972	1.935	1.925	0.930	0.577	97	94	93	22	-6	4.09E-6	6.02E-5	> 1.00E-4
HOP-62	0.743	2.464	2.291	2.172	2.239	1.825	0.653	90	83	87	63	-12	1.48E-5	6.88E-5	> 1.00E-4
HOP-92	1.120	1.798	1.753	1.734	1.675	1.187	0.791	93	91	82	10	-29	2.77E-6	1.78E-5	> 1.00E-4
NCI-H226	0.673	1.395	1.402	1.346	1.322	0.975	0.569	101	93	90	42	-15	6.77E-6	5.37E-5	> 1.00E-4
NCI-H23	0.610	1.775	1.684	1.678	1.655	0.763	0.535	92	92	90	13	-12	3.30E-6	3.27E-5	> 1.00E-4
NCI-H322M	0.732	2.204	2.226	2.132	2.197	1.794	1.055	102	95	100	72	22	2.76E-5	> 1.00E-4	> 1.00E-4
NCI-H460	0.237	2.139	2.222	2.191	2.102	0.860	0.216	104	103	98	33	-9	5.44E-6	6.07E-5	> 1.00E-4
NCI-H522	0.702	2.110	1.990	1.954	1.905	0.993	0.609	91	89	85	21	-13	3.52E-6	4.06E-5	> 1.00E-4
Colon Cancer															
COLO 205	0.489	2.143	2.124	2.173	2.270	1.746	0.749	99	102	108	76	16	2.70E-5	> 1.00E-4	> 1.00E-4
HCC-2998	0.669	2.130	1.987	2.068	2.082	1.078	0.785	90	96	97	28	8	4.79E-6	> 1.00E-4	> 1.00E-4
HCT-116	0.233	2.064	2.003	1.950	2.049	0.765	0.201	97	94	99	29	-14	5.03E-6	4.74E-5	> 1.00E-4
HCT-15	0.260	1.841	1.775	1.718	1.659	0.540	0.217	96	92	89	18	-17	3.50E-6	3.26E-5	> 1.00E-4
HT29	0.212	1.380	1.427	1.415	1.519	0.905	0.298	104	103	112	59	7	1.51E-5	> 1.00E-4	> 1.00E-4
KM12	0.453	1.412	1.420	1.459	1.398	0.781	0.267	101	105	99	34	-41	5.68E-6	2.84E-5	> 1.00E-4
SW-620	0.244	1.700	1.744	1.821	1.622	1.086	0.433	103	108	95	58	13	1.49E-5	> 1.00E-4	> 1.00E-4
CNS Cancer															
SF-268	0.779	2.249	2.221	2.254	2.154	1.611	0.875	98	100	94	57	6	1.35E-5	> 1.00E-4	> 1.00E-4
SF-295	0.957	2.762	2.690	2.725	2.608	1.411	0.660	96	98	91	25	-31	4.22E-6	2.80E-5	> 1.00E-4
SF-539	0.789	2.464	2.589	2.623	2.500	1.976	0.490	107	109	102	71	-38	1.56E-5	4.48E-5	> 1.00E-4
SNB-19	0.726	2.612	2.530	2.517	2.529	1.465	0.739	96	95	96	39	1	6.43E-6	> 1.00E-4	> 1.00E-4
SNB-75	1.022	2.074	2.036	2.047	1.929	1.646	0.776	96	97	86	59	-24	1.29E-5	5.14E-5	> 1.00E-4
U251	0.319	1.671	1.662	1.625	1.596	0.760	0.207	99	97	94	33	-35	5.24E-6	3.02E-5	> 1.00E-4
Melanoma															
LOX IMVI	0.248	1.835	1.756	1.735	1.727	0.914	0.315	95	94	93	42	4	6.97E-6	> 1.00E-4	> 1.00E-4
MALME-3M	0.517	1.233	1.256	1.252	1.272	0.917	0.724	103	103	105	56	29	1.64E-5	> 1.00E-4	> 1.00E-4
M14	0.493	2.114	2.074	2.091	2.039	1.303	0.888	98	99	95	50	24	9.98E-6	> 1.00E-4	> 1.00E-4
MDA-MB-435	0.588	2.069	2.027	1.915	1.912	1.384	0.677	97	90	89	54	6	1.20E-5	> 1.00E-4	> 1.00E-4
SK-MEL-2	1.303	3.122	3.113	3.078	3.019	1.402	1.076	99	98	94	5	-17	3.15E-6	1.73E-5	> 1.00E-4
SK-MEL-28	0.607	1.962	1.905	1.898	1.890	1.079	0.848	96	95	95	35	18	5.58E-6	> 1.00E-4	> 1.00E-4
SK-MEL-5	0.723	2.586	2.502	2.455	2.380	0.612	0.523	95	93	89	-15	-28	2.36E-6	7.13E-6	> 1.00E-4
UACC-257	1.026	2.501	2.431	2.378	2.364	1.533	1.200	95	92	91	34	12	5.28E-6	> 1.00E-4	> 1.00E-4
Ovarian Cancer															
IGROV1	0.360	1.735	1.903	1.954	2.058	1.431	0.676	112	116	123	78	23	3.22E-5	> 1.00E-4	> 1.00E-4
OVCAR-3	0.545	2.249	2.325	2.307	2.204	1.315	0.631	104	103	97	45	5	8.09E-6	> 1.00E-4	> 1.00E-4
OVCAR-4	0.758	1.752	1.745	1.838	1.698	1.249	0.884	99	109	95	49	13	9.68E-6	> 1.00E-4	> 1.00E-4
OVCAR-5	0.626	1.856	1.843	1.800	1.765	1.560	1.156	99	95	93	76	43	6.16E-5	> 1.00E-4	> 1.00E-4
OVCAR-8	0.398	1.883	1.862	1.882	1.865	1.150	0.407	99	100	99	51	1	1.03E-5	> 1.00E-4	> 1.00E-4
NCI/ADR-RES	0.415	1.363	1.351	1.344	1.319	0.932	0.497	99	98	95	55	9	1.26E-5	> 1.00E-4	> 1.00E-4
SK-OV-3	0.802	2.251	2.170	2.117	2.228	1.467	0.986	94	91	98	46	13	8.36E-6	> 1.00E-4	> 1.00E-4
Renal Cancer															
786-0	0.585	2.338	2.259	2.228	2.256	1.571	0.837	95	94	95	56	14	1.41E-5	> 1.00E-4	> 1.00E-4
A498	1.476	2.292	2.329	2.550	2.237	2.017	1.446	105	132	93	66	-2	1.73E-5	9.33E-5	> 1.00E-4
ACHN	0.438	2.021	2.050	1.958	2.027	1.030	0.580	102	96	100	37	9	6.30E-6	> 1.00E-4	> 1.00E-4
CAKI-1	0.528	2.340	2.273	2.238	2.229	1.287	0.573	96	94	94	42	2	6.97E-6	> 1.00E-4	> 1.00E-4
RXF 393	0.851	1.575	1.618	1.549	1.510	1.143	0.687	106	96	91	40	-19	6.44E-6	4.75E-5	> 1.00E-4
SN12C	0.597	2.555	2.445	2.475	2.372	1.186	0.900	94	96	91	30	15	4.69E-6	> 1.00E-4	> 1.00E-4
TK-10	1.236	2.811	2.738	2.750	2.727	1.876	1.426	95	96	95	41	12	6.71E-6	> 1.00E-4	> 1.00E-4
UO-31	0.406	1.524	1.400	1.413	1.368	0.958	0.496	89	90	86	49	8	9.63E-6	> 1.00E-4	> 1.00E-4
Prostate Cancer															
PC-3	0.555	1.973	1.944	1.914	1.854	0.878	0.586	98	96	92	23	2	4.02E-6	> 1.00E-4	> 1.00E-4
DU-145	0.333	1.276	1.343	1.286	1.273	0.878	0.461	107	101	100	58	14	1.50E-5	> 1.00E-4	> 1.00E-4
Breast Cancer															
MCF7	0.333	1.754	1.666	1.629	1.547	0.744	0.314	94	91	85	29	-6	4.23E-6	6.79E-5	> 1.00E-4
MDA-MB-231/ATCC	0.687	1.643	1.695	1.711	1.812	1.268	0.796	105	107	118	61	11	1.65E-5	> 1.00E-4	> 1.00E-4
HS 578T	1.315	2.398	2.316	2.300	2.273	1.852	1.080	92	91	88	50	-18	9.74E-6	5.43E-5	> 1.00E-4
BT-549	1.105	2.200	2.092	2.007	1.946	1.381	0.773	90	82	77	25	-30	3.31E-6	2.86E-5	> 1.00E-4
MDA-MB-468	0.681	1.073	1.058	1.038	1.002	0.688	0.467	96	91	82	2	-31	2.49E-6	1.12E-5	> 1.00E-4

Fig. 8: Representative five dose data of compound **JOOET-6**

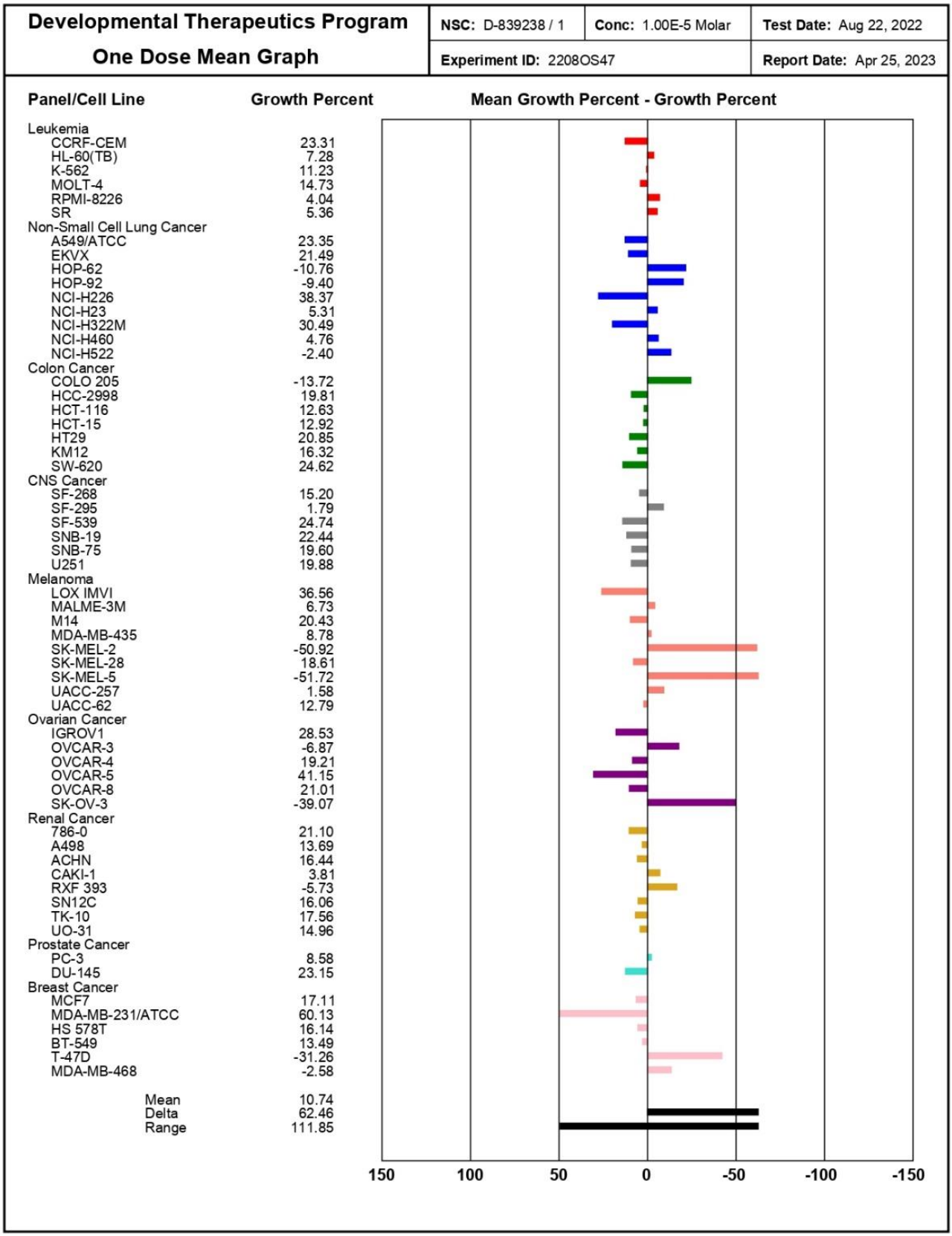


Fig. 9: Representative single dose data of compound **JOOET-7**

National Cancer Institute Developmental Therapeutics Program
In-Vitro Testing Results

NSC : D - 839238 / 1	Experiment ID : 2301NS84	Test Type : 08	Units : Molar
Report Date : February 24, 2023	Test Date : January 09, 2023	QNS :	MC :
COMI : JOOET-7	Stain Reagent : SRB Dual-Pass Related	SSPL : 1COF	

			Log10 Concentration											
	Time		Mean Optical Densities					Percent Growth						
Panel/Cell Line	Zero	Ctrl	-8.0	-7.0	-6.0	-5.0	-4.0	-8.0	-7.0	-6.0	-5.0	-4.0	GI50	TGI	LC50
Leukemia															
CCRF-CEM	0.782	2.968	2.920	2.951	2.742	0.740	0.438	98	99	90	-5	-44	2.62E-6	8.78E-6	> 1.00E-4
HL-60(TB)	0.654	2.245	2.104	1.983	1.993	0.442	0.521	91	84	84	-32	-20	1.96E-6	5.27E-6	> 1.00E-4
K-562	0.221	1.966	1.809	1.819	1.715	0.342	0.308	91	92	86	7	5	2.83E-6	> 1.00E-4	> 1.00E-4
MOLT-4	0.694	2.390	2.299	2.325	2.141	0.581	0.335	95	96	85	-16	-52	2.23E-6	6.91E-6	8.94E-5
RPMI-8226	0.673	2.380	2.297	2.199	1.959	0.481	0.503	95	89	75	-29	-25	1.75E-6	5.31E-6	> 1.00E-4
SR	0.633	2.257	2.131	2.123	2.025	0.510	0.498	92	92	86	-20	-21	2.19E-6	6.53E-6	> 1.00E-4
Non-Small Cell Lung Cancer															
A549/ATCC	0.485	2.338	2.266	2.393	2.346	0.997	0.173	96	103	100	28	-64	4.93E-6	2.00E-5	6.99E-5
EKVX	0.587	1.840	1.708	1.709	1.668	0.659	0.035	89	90	86	6	-94	2.82E-6	1.14E-5	3.62E-5
HOP-62	0.883	2.520	2.393	2.379	2.272	1.384	0.383	92	91	85	31	-57	4.39E-6	2.24E-5	8.40E-5
HOP-92	1.154	1.771	1.677	1.645	1.509	0.891	0.156	85	80	58	-23	-86	1.24E-6	5.20E-6	2.67E-5
NCI-H23	0.727	2.323	2.320	2.279	2.213	0.629	0.013	100	97	93	-14	-98	2.54E-6	7.46E-6	2.69E-5
NCI-H322M	0.767	2.032	1.959	1.902	1.838	0.916	0.003	94	90	85	12	-100	2.99E-6	1.27E-5	3.58E-5
NCI-H460	0.363	3.095	3.141	3.106	2.997	0.391	0.007	102	100	96	1	-98	3.07E-6	1.02E-5	3.27E-5
NCI-H522	1.386	3.100	3.022	2.988	2.933	1.259	0.080	95	93	90	-9	-94	2.54E-6	8.08E-6	3.02E-5
Colon Cancer															
COLO 205	0.574	1.987	2.096	2.141	2.091	0.877	0.195	108	111	107	21	-66	4.65E-6	1.76E-5	6.56E-5
HCC-2998	0.728	2.541	2.472	2.573	2.494	0.866	-0.012	96	102	97	8	-100	3.37E-6	1.18E-5	3.43E-5
HCT-116	0.293	2.704	2.708	2.608	2.530	0.410	0.064	100	96	93	5	-78	3.07E-6	1.14E-5	4.58E-5
HCT-15	0.307	2.275	2.260	2.142	2.123	0.428	-0.002	99	93	92	6	-100	3.09E-6	1.14E-5	3.38E-5
HT29	0.288	1.724	1.744	1.709	1.763	0.503	0.057	101	99	103	15	-80	3.99E-6	1.44E-5	4.82E-5
KM12	0.887	3.227	3.225	3.240	3.182	1.084	0.012	100	101	98	8	-99	3.44E-6	1.20E-5	3.51E-5
SW-620	0.435	2.910	2.927	2.856	2.825	0.873	0.012	101	98	97	18	-97	3.89E-6	1.42E-5	3.88E-5
CNS Cancer															
SF-268	1.146	2.847	2.733	2.645	2.472	1.267	0.262	93	88	78	7	-77	2.48E-6	1.21E-5	4.76E-5
SF-295	1.076	3.097	2.961	2.874	2.746	0.947	0.016	93	89	83	-12	-99	2.21E-6	7.46E-6	2.75E-5
SF-539	0.890	2.665	2.578	2.483	2.546	1.226	-0.011	95	90	93	19	-100	3.82E-6	1.44E-5	3.80E-5
SNB-19	0.656	2.057	1.942	1.941	1.796	0.807	0.008	92	92	81	11	-99	2.78E-6	1.25E-5	3.59E-5
SNB-75	2.600	3.364	3.289	3.293	3.260	2.419	0.953	90	91	86	-7	-63	2.45E-6	8.42E-6	5.79E-5
U251	0.363	1.919	1.898	1.785	1.587	0.756	0.252	99	91	79	25	-31	3.44E-6	2.83E-5	> 1.00E-4
Melanoma															
LOX IMVI	0.184	1.375	1.345	1.323	1.311	0.430	-0.008	98	96	95	21	-100	4.01E-6	1.48E-5	3.85E-5
MALME-3M	0.868	1.623	1.534	1.483	1.434	0.648	0.035	88	81	75	-25	-96	1.77E-6	5.58E-6	2.23E-5
M14	0.441	1.644	1.666	1.617	1.603	0.694	0.046	102	98	97	21	-90	4.13E-6	1.55E-5	4.38E-5
MDA-MB-435	0.880	3.145	3.136	3.113	3.050	0.871	0.069	100	99	96	-1	-92	2.97E-6	9.75E-6	3.44E-5
SK-MEL-2	1.529	2.370	2.324	2.287	2.235	0.634	0.048	95	90	84	-59	-97	1.73E-6	3.88E-6	8.71E-6
SK-MEL-28	0.872	2.291	2.276	2.230	2.180	1.063	0.058	99	96	92	13	-93	3.43E-6	1.34E-5	3.93E-5
SK-MEL-5	0.867	3.027	2.898	2.852	2.738	0.124	-0.015	94	92	87	-86	-100	1.63E-6	3.18E-6	6.20E-6
UACC-257	1.619	2.944	2.886	2.982	2.758	1.487	0.465	96	103	86	-8	-71	2.41E-6	8.19E-6	4.60E-5
UACC-62	1.122	2.864	2.733	2.720	2.534	0.932	0.005	93	92	81	-17	-100	2.07E-6	6.71E-6	2.51E-5
Ovarian Cancer															
IGROV1	0.927	2.760	2.810	2.653	2.585	1.084	0.126	103	94	90	9	-86	3.12E-6	1.23E-5	4.14E-5
OVCAR-3	0.716	2.060	2.174	2.064	1.968	0.498	-0.009	109	100	93	-30	-100	2.23E-6	5.67E-6	1.91E-5
OVCAR-4	1.361	2.782	2.752	2.732	2.635	1.404	0.831	98	96	90	3	-39	2.87E-6	1.18E-5	> 1.00E-4
OVCAR-5	0.575	1.370	1.325	1.302	1.288	0.983	-0.006	94	91	90	51	-100	1.02E-5	2.18E-5	4.67E-5
OVCAR-8	0.668	2.777	2.769	2.781	2.609	0.928	0.226	100	100	92	12	-66	3.37E-6	1.43E-5	6.21E-5
NCI/ADR-RES	0.498	1.811	1.827	1.788	1.717	0.632	0.075	101	98	93	10	-85	3.30E-6	1.28E-5	4.29E-5
SK-OV-3	1.214	2.336	2.328	2.253	2.370	1.287	0.389	99	93	103	7	-68	3.54E-6	1.22E-5	5.74E-5
Renal Cancer															
786-0	0.820	2.912	2.848	2.824	2.704	1.111	0.135	97	96	90	14	-84	3.36E-6	1.39E-5	4.53E-5
A498	1.533	2.348	2.325	2.305	2.189	1.488	0.159	97	95	80	-3	-90	2.32E-6	9.22E-6	3.49E-5
ACHN	0.363	1.635	1.604	1.566	1.559	0.431	-0.010	98	95	94	5	-100	3.14E-6	1.12E-5	3.35E-5
CAKI-1	0.765	2.271	2.119	2.091	1.923	0.830	-0.002	90	88	77	4	-100	2.35E-6	1.10E-5	3.32E-5
RXF 393	0.564	1.320	1.293	1.302	1.258	0.715	0.031	96	98	92	20	-95	3.82E-6	1.49E-5	4.08E-5
SN12C	1.363	3.313	3.281	3.248	3.114	1.281	0.159	98	97	90	-6	-88	2.60E-6	8.65E-6	3.42E-5
TK-10	1.345	2.412	2.345	2.274	2.244	1.404	0.404	94	87	84	5	-70	2.72E-6	1.18E-5	5.43E-5
UO-31	0.691	2.680	2.494	2.428	2.193	0.781	-0.010	91	87	76	5	-100	2.29E-6	1.10E-5	3.32E-5
Prostate Cancer															
PC-3	0.589	1.934	1.873	1.809	1.770	0.609	0.197	95	91	88	1	-67	2.74E-6	1.05E-5	5.71E-5
DU-145	0.594	2.234	2.316	2.234	2.132	0.766	0.243	105	100	94	10	-59	3.36E-6	1.42E-5	7.40E-5
Breast Cancer															
MCF7	0.363	2.135	2.005	1.957	1.883	0.453	0.109	93	90	86	5	-70	2.77E-6	1.17E-5	5.42E-5
MDA-MB-231/ATCC	0.656	1.615	1.604	1.562	1.538	0.850	0.143	99	94	92	20	-78	3.84E-6	1.60E-5	5.16E-5
HS 578T	1.367	2.462	2.369	2.300	2.244	1.386	0.738	92	85	80	2	-46	2.42E-6	1.09E-5	> 1.00E-4
BT-549	1.522	2.926	2.889	2.891	2.921	1.436	0.170	97	97	100	-6	-89	2.96E-6	8.84E-6	3.41E-5
T-47D	1.035	2.600	2.516	2.469	2.368	1.292	1.014	95	92	85	16	-2	3.25E-6	7.76E-5	> 1.00E-4
MDA-MB-468	0.749	1.757	1.725	1.656	1.627	0.631	0.140	97	90	87	-16	-81	2.30E-6	7.03E-6	3.33E-5

Fig. 10: Representative five dose data of compound **JOOET-7**

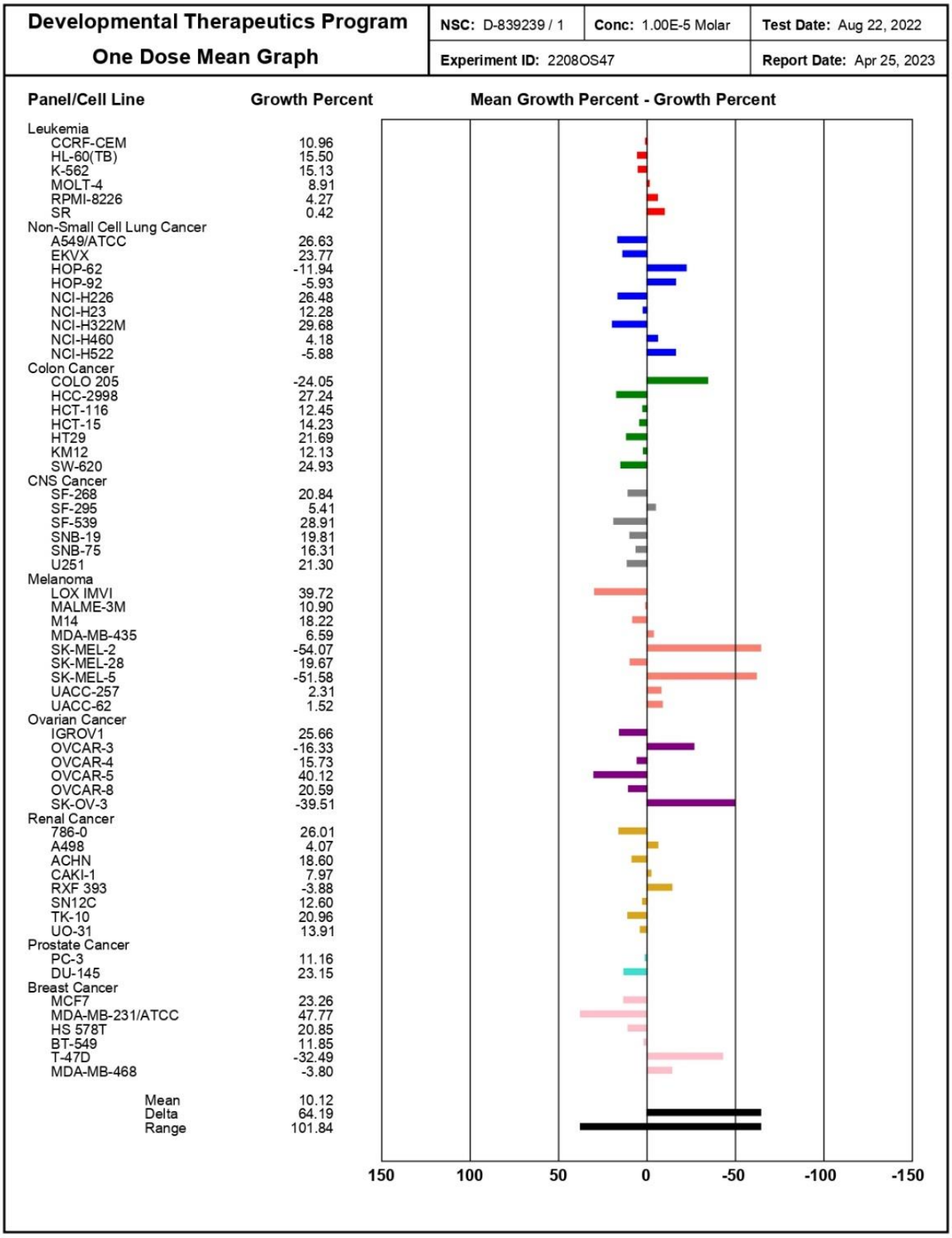


Fig. 11: Representative single dose data of compound **JOOET-8**

National Cancer Institute Developmental Therapeutics Program
In-Vitro Testing Results

NSC : D - 839239 / 1	Experiment ID : 2301NS84	Test Type : 08	Units : Molar
Report Date : February 24, 2023	Test Date : January 09, 2023	QNS :	MC :
COMI : JOOET-8	Stain Reagent : SRB Dual-Pass Related	SSPL : 1COF	

		Log10 Concentration													
	Time	Mean Optical Densities						Percent Growth							
Panel/Cell Line	Zero	Ctrl	-8.0	-7.0	-6.0	-5.0	-4.0	-8.0	-7.0	-6.0	-5.0	-4.0	GI50	TGI	LC50
Leukemia															
CCRF-CEM	0.782	2.968	2.921	3.005	2.683	0.774	0.474	98	102	87	-1	-39	2.63E-6	9.72E-6	> 1.00E-4
HL-60(TB)	0.654	2.245	2.063	2.077	2.104	0.433	0.420	89	89	91	-34	-36	2.13E-6	5.36E-6	> 1.00E-4
K-562	0.221	1.966	1.880	1.918	1.659	0.337	0.266	95	97	82	7	3	2.68E-6	> 1.00E-4	> 1.00E-4
MOLT-4	0.694	2.390	2.331	2.330	2.000	0.555	0.393	97	96	77	-20	-43	1.90E-6	6.22E-6	> 1.00E-4
RPMI-8226	0.673	2.380	2.451	2.462	1.939	0.491	0.572	104	105	74	-27	-15	1.73E-6	5.40E-6	> 1.00E-4
SR	0.633	2.257	2.257	2.248	2.110	0.504	0.418	100	99	91	-20	-34	2.33E-6	6.55E-6	> 1.00E-4
Non-Small Cell Lung Cancer															
A549/ATCC	0.485	2.338	2.112	2.236	2.145	0.792	0.462	88	94	90	17	-5	3.48E-6	5.99E-5	> 1.00E-4
EKVX	0.587	1.840	1.679	1.725	1.677	0.627	0.072	87	91	87	3	-88	2.76E-6	1.08E-5	3.84E-5
HOP-62	0.883	2.520	2.326	2.404	2.270	1.193	0.413	88	93	85	19	-53	3.37E-6	1.83E-5	9.02E-5
HOP-92	1.154	1.771	1.686	1.675	1.547	0.908	0.481	86	85	64	-21	-58	1.45E-6	5.61E-6	5.96E-5
NCI-H23	0.727	2.323	2.299	2.306	2.115	0.599	0.032	98	99	87	-18	-96	2.26E-6	6.79E-6	2.60E-5
NCI-H322M	0.767	2.032	1.974	2.036	1.894	0.940	0.008	95	100	89	14	-99	3.30E-6	1.32E-5	3.67E-5
NCI-H460	0.363	3.095	3.148	3.155	2.930	0.393	0.055	102	102	94	1	-85	2.97E-6	1.03E-5	3.92E-5
NCI-H522	1.386	3.100	2.996	2.999	2.880	1.158	0.097	94	94	87	-16	-93	2.28E-6	6.94E-6	2.74E-5
Colon Cancer															
COLO 205	0.574	1.987	2.000	2.063	2.053	0.709	0.320	101	105	105	10	-44	3.76E-6	1.51E-5	> 1.00E-4
HCC-2998	0.728	2.541	2.500	2.616	2.631	0.782	0.002	98	104	105	3	-100	3.46E-6	1.07E-5	3.28E-5
HCT-116	0.293	2.704	2.620	2.707	2.464	0.422	0.061	97	100	90	5	-79	2.97E-6	1.16E-5	4.50E-5
HCT-15	0.307	2.275	2.156	2.206	2.052	0.389	0.005	94	96	89	4	-99	2.87E-6	1.10E-5	3.37E-5
HT29	0.288	1.724	1.704	1.763	1.723	0.453	0.026	99	103	100	11	-91	3.67E-6	1.29E-5	3.97E-5
KM12	0.887	3.227	3.215	3.224	3.020	0.943	0.018	99	100	91	2	-98	2.91E-6	1.06E-5	3.33E-5
SW-620	0.435	2.910	2.853	2.834	2.735	0.872	0.069	98	97	93	18	-84	3.72E-6	1.49E-5	4.61E-5
CNS Cancer															
SF-268	1.146	2.847	2.731	2.703	2.437	1.223	0.296	93	92	76	5	-74	2.31E-6	1.14E-5	4.93E-5
SF-295	1.076	3.097	2.904	2.939	2.577	0.919	0.027	90	92	74	-15	-98	1.87E-6	6.85E-6	2.67E-5
SF-539	0.890	2.665	2.605	2.604	2.486	1.223	0.017	97	97	90	19	-98	3.64E-6	1.45E-5	3.87E-5
SNB-19	0.656	2.057	1.968	2.013	1.830	0.802	0.030	94	97	84	10	-95	2.89E-6	1.25E-5	3.72E-5
SNB-75	2.600	3.364	3.254	3.275	3.251	2.348	0.048	86	88	85	-10	-98	2.35E-6	7.90E-6	2.86E-5
U251	0.363	1.919	1.783	1.764	1.618	0.575	0.088	91	90	81	14	-76	2.87E-6	1.42E-5	5.14E-5
Melanoma															
LOX IMVI	0.184	1.375	1.297	1.340	1.258	0.354	0.021	93	97	90	14	-89	3.38E-6	1.38E-5	4.21E-5
MALME-3M	0.868	1.623	1.515	1.528	1.422	0.628	0.248	86	87	73	-28	-71	1.70E-6	5.32E-6	3.23E-5
M14	0.441	1.644	1.634	1.635	1.495	0.658	0.136	99	99	88	18	-69	3.47E-6	1.61E-5	6.02E-5
MDA-MB-435	0.880	3.145	3.138	3.131	2.971	0.826	0.131	100	99	92	-6	-85	2.69E-6	8.66E-6	3.59E-5
SK-MEL-2	1.529	2.370	2.285	2.310	2.221	0.572	0.055	90	93	82	-63	-96	1.67E-6	3.70E-6	8.19E-6
SK-MEL-28	0.872	2.291	2.330	2.405	2.122	1.065	0.072	103	108	88	14	-92	3.24E-6	1.35E-5	4.01E-5
SK-MEL-5	0.867	3.027	2.865	2.916	2.605	0.082	0.009	92	95	80	-91	-99	1.51E-6	2.95E-6	5.79E-6
UACC-257	1.619	2.944	2.836	2.877	2.651	1.380	0.631	92	95	78	-15	-61	2.00E-6	6.93E-6	5.77E-5
UACC-62	1.122	2.864	2.773	2.793	2.517	0.773	0.041	95	96	80	-31	-96	1.86E-6	5.25E-6	1.95E-5
Ovarian Cancer															
IGROV1	0.927	2.760	2.777	2.812	2.632	0.959	0.354	101	103	93	2	-62	2.96E-6	1.06E-5	6.51E-5
OVCAR-3	0.716	2.060	2.078	2.109	1.851	0.384	0.001	101	104	84	-46	-100	1.83E-6	4.42E-6	1.17E-5
OVCAR-4	1.361	2.782	2.714	2.731	2.535	1.324	1.020	95	96	83	-3	-25	2.41E-6	9.29E-6	> 1.00E-4
OVCAR-5	0.575	1.370	1.371	1.358	1.328	0.974	0.022	100	98	95	50	-96	1.00E-5	2.20E-5	4.83E-5
OVCAR-8	0.668	2.777	2.693	2.760	2.605	0.975	0.239	96	99	92	15	-64	3.48E-6	1.53E-5	6.59E-5
NCI/ADR-RES	0.498	1.811	1.795	1.824	1.657	0.600	0.166	99	101	88	8	-67	2.99E-6	1.27E-5	5.96E-5
SK-OV-3	1.214	2.336	2.279	2.390	2.254	1.148	0.740	95	105	93	-5	-39	2.72E-6	8.80E-6	> 1.00E-4
Renal Cancer															
786-0	0.820	2.912	2.885	2.879	2.750	1.197	0.313	99	98	92	18	-62	3.71E-6	1.68E-5	7.10E-5
A498	1.533	2.348	2.260	2.284	2.208	1.410	0.023	89	92	83	-8	-98	2.30E-6	8.15E-6	2.91E-5
ACHN	0.363	1.635	1.691	1.656	1.486	0.416	0.035	104	102	88	4	-90	2.85E-6	1.11E-5	3.73E-5
CAKI-1	0.765	2.271	2.101	2.070	1.893	0.837	0.007	89	87	75	5	-99	2.26E-6	1.11E-5	3.37E-5
RXF 393	0.564	1.320	1.294	1.313	1.257	0.734	0.073	97	99	92	23	-87	4.00E-6	1.60E-5	4.59E-5
SN12C	1.363	3.313	3.252	3.245	3.069	1.287	0.422	97	96	87	-6	-69	2.53E-6	8.70E-6	5.01E-5
TK-10	1.345	2.412	2.249	2.335	2.360	1.415	0.179	85	93	95	7	-87	3.23E-6	1.18E-5	4.04E-5
UO-31	0.691	2.680	2.494	2.532	2.174	0.745	0.034	91	93	75	3	-95	2.20E-6	1.07E-5	3.46E-5
Prostate Cancer															
PC-3	0.589	1.934	1.843	1.950	1.773	0.662	0.410	93	101	88	5	-30	2.89E-6	1.42E-5	> 1.00E-4
DU-145	0.594	2.234	2.294	2.275	2.028	0.686	-0.002	104	103	87	6	-100	2.87E-6	1.13E-5	3.36E-5
Breast Cancer															
MCF7	0.363	2.135	2.017	1.962	1.848	0.453	0.077	93	90	84	5	-79	2.69E-6	1.15E-5	4.54E-5
MDA-MB-231/ATCC	0.656	1.615	1.601	1.638	1.524	0.842	0.330	99	102	90	19	-50	3.71E-6	1.91E-5	> 1.00E-4
HS 578T	1.367	2.462	2.284	2.305	2.190	1.307	1.243	84	86	75	-4	-9	2.07E-6	8.80E-6	> 1.00E-4
BT-549	1.522	2.926	3.003	3.004	2.855	1.417	0.204	105	106	95	-7	-87	2.76E-6	8.55E-6	3.47E-5
T-47D	1.035	2.600	2.386	2.351	2.311	1.115	0.929	86	84	82	5	-10	2.58E-6	2.15E-5	> 1.00E-4
MDA-MB-468	0.749	1.757	1.671	1.725	1.567	0.623	0.163	92	97	81	-17	-78	2.08E-6	6.73E-6	3.46E-5

Fig. 12: Representative five dose data of compound **JOOET-8**

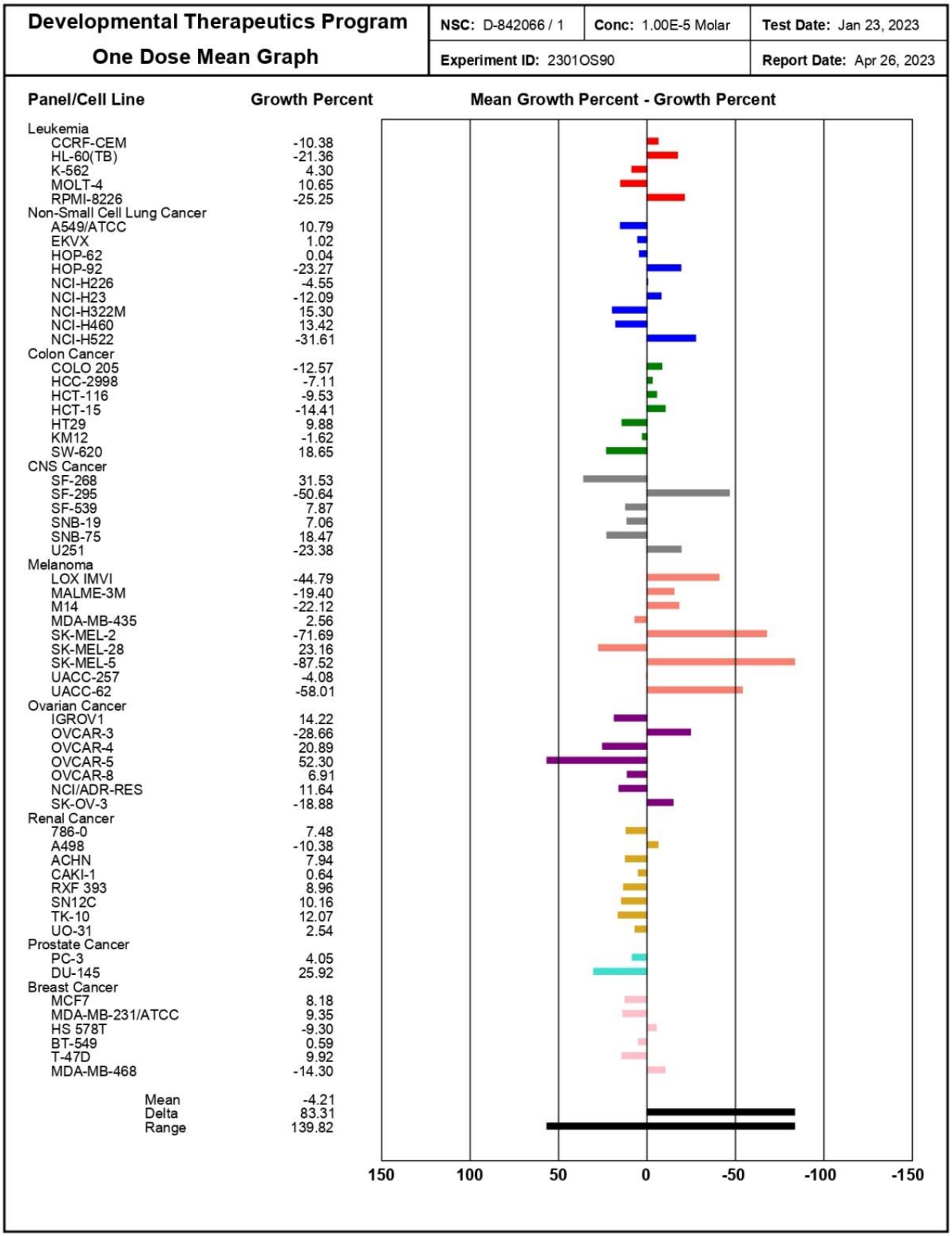


Fig. 13: Representative single dose data of compound **JOOET-9**

National Cancer Institute Developmental Therapeutics Program
In-Vitro Testing Results

NSC : D - 842066 / 1	Experiment ID : 2306NS37	Test Type : 08	Units : Molar
Report Date : July 28, 2023	Test Date : June 20, 2023	QNS :	MC :
COMI : JOOET-9	Stain Reagent : SRB Dual-Pass Related	SSPL : 1COF	

		Log10 Concentration													
	Time	Mean Optical Densities						Percent Growth							
Panel/Cell Line	Zero	Ctrl	-8.0	-7.0	-6.0	-5.0	-4.0	-8.0	-7.0	-6.0	-5.0	-4.0	GI50	TGI	LC50
Leukemia															
CCRF-CEM	0.475	1.770	1.722	1.735	1.466	0.496	0.331	96	97	76	2	-30	2.26E-6	1.12E-5	> 1.00E-4
HL-60(TB)	0.528	2.019	1.892	1.890	1.648	0.395	0.356	91	91	75	-25	-33	1.78E-6	5.60E-6	> 1.00E-4
K-562	0.122	0.758	0.745	0.756	0.592	0.114	0.084	98	100	74	-7	-31	1.97E-6	8.20E-6	> 1.00E-4
MOLT-4	0.570	2.379	2.281	2.286	1.841	0.550	0.448	95	95	70	-4	-21	1.88E-6	8.96E-6	> 1.00E-4
RPMI-8226	0.580	2.171	2.172	2.185	1.627	0.462	0.490	100	101	66	-20	-16	1.52E-6	5.79E-6	> 1.00E-4
Non-Small Cell Lung Cancer															
A549/ATCC	0.300	1.789	1.731	1.734	1.497	0.411	0.025	96	96	80	7	-92	2.61E-6	1.19E-5	3.80E-5
EKVX	0.616	2.017	1.906	1.972	1.781	0.532	0.203	92	97	83	-14	-67	2.20E-6	7.23E-6	4.80E-5
HOP-62	0.743	2.464	2.312	2.353	2.093	1.017	0.158	91	94	78	16	-79	2.85E-6	1.47E-5	4.96E-5
HOP-92	1.120	1.798	1.710	1.702	1.479	0.876	0.296	87	86	53	-22	-74	1.09E-6	5.11E-6	3.50E-5
NCI-H226	0.673	1.395	1.394	1.394	1.265	0.610	0.124	100	100	82	-9	-82	2.24E-6	7.89E-6	3.65E-5
NCI-H23	0.610	1.775	1.759	1.723	1.536	0.362	0.071	99	96	80	-41	-88	1.76E-6	4.58E-6	1.56E-5
NCI-H322M	0.732	2.204	2.082	2.111	1.872	0.896	0.056	92	94	77	11	-92	2.59E-6	1.28E-5	3.90E-5
NCI-H460	0.237	2.139	2.118	2.186	1.902	0.250	0.029	99	102	88	1	-88	2.70E-6	1.02E-5	3.73E-5
NCI-H522	0.702	2.110	1.980	1.979	1.791	0.566	0.078	91	91	77	-19	-89	1.92E-6	6.30E-6	2.76E-5
Colon Cancer															
COLO 205	0.489	2.143	2.240	2.299	2.076	0.624	0.153	106	109	96	8	-69	3.34E-6	1.28E-5	5.71E-5
HCC-2998	0.669	2.130	2.004	2.057	1.983	0.536	-0.003	91	95	90	-20	-100	2.31E-6	6.59E-6	2.38E-5
HCT-116	0.233	2.064	1.938	1.978	1.771	0.283	0.128	93	95	84	3	-45	2.62E-6	1.14E-5	> 1.00E-4
HCT-15	0.260	1.841	1.750	1.801	1.487	0.255	0.004	94	97	78	-2	-99	2.23E-6	9.46E-6	3.14E-5
HT29	0.212	1.380	1.481	1.575	1.316	0.342	0.023	109	117	94	11	-89	3.42E-6	1.29E-5	4.07E-5
KM12	0.453	1.412	1.389	1.444	1.265	0.279	-0.001	98	103	85	-39	-100	1.91E-6	4.87E-6	1.54E-5
SW-620	0.244	1.700	1.707	1.684	1.613	0.529	0.051	100	99	94	20	-79	3.90E-6	1.58E-5	5.05E-5
CNS Cancer															
SF-268	0.779	2.249	2.079	2.140	2.008	0.983	0.244	88	93	84	14	-69	3.03E-6	1.47E-5	5.94E-5
SF-295	0.957	2.762	2.670	2.733	2.423	0.563	0.055	95	98	81	-41	-94	1.80E-6	4.61E-6	1.47E-5
SF-539	0.789	2.464	2.444	2.427	2.248	0.979	0.018	99	98	87	11	-98	3.09E-6	1.27E-5	3.65E-5
SNB-19	0.726	2.612	2.552	2.556	2.078	0.913	0.067	97	97	72	10	-91	2.25E-6	1.25E-5	3.94E-5
SNB-75	1.022	2.074	1.959	2.052	1.942	1.243	0.067	89	98	87	21	-93	3.66E-6	1.53E-5	4.17E-5
U251	0.319	1.671	1.593	1.631	1.388	0.375	-0.002	94	97	79	4	-100	2.44E-6	1.10E-5	3.31E-5
Melanoma															
LOX IMVI	0.248	1.835	1.724	1.766	1.509	0.146	.	93	96	79	-41	-100	1.75E-6	4.55E-6	1.41E-5
MALME-3M	0.517	1.233	1.184	1.250	1.176	0.543	0.067	93	102	92	4	-87	2.98E-6	1.09E-5	3.90E-5
M14	0.493	2.114	2.103	2.125	2.016	0.673	0.235	99	101	94	11	-52	3.39E-6	1.50E-5	9.19E-5
MDA-MB-435	0.588	2.069	2.002	2.006	1.893	0.692	0.052	95	96	88	7	-91	2.95E-6	1.18E-5	3.81E-5
SK-MEL-2	1.303	3.122	3.082	3.137	2.977	0.355	0.144	98	101	92	-73	-89	1.80E-6	3.62E-6	7.28E-6
SK-MEL-28	0.607	1.962	1.965	2.073	1.919	0.829	0.070	100	108	97	16	-89	3.82E-6	1.43E-5	4.29E-5
SK-MEL-5	0.723	2.586	2.480	2.513	2.128	0.185	0.009	94	96	75	-74	-99	1.48E-6	3.18E-6	6.87E-6
UACC-257	1.026	2.501	2.419	2.412	2.313	0.948	0.073	94	94	87	-8	-93	2.47E-6	8.31E-6	3.14E-5
Ovarian Cancer															
IGROV1	0.360	1.735	1.711	1.941	1.567	0.496	0.249	98	115	88	10	-31	3.05E-6	1.75E-5	> 1.00E-4
OVCAR-3	0.545	2.249	2.210	2.285	2.048	0.719	0.031	98	102	88	10	-94	3.09E-6	1.25E-5	3.76E-5
OVCAR-4	0.758	1.752	1.695	1.775	1.638	0.885	0.570	94	102	89	13	-25	3.22E-6	2.18E-5	> 1.00E-4
OVCAR-5	0.626	1.856	2.038	1.878	1.817	0.979	0.090	115	102	97	29	-86	4.87E-6	1.78E-5	4.87E-5
OVCAR-8	0.398	1.883	1.831	1.880	1.671	0.581	0.030	96	100	86	12	-93	3.06E-6	1.31E-5	3.93E-5
NCI/ADR-RES	0.415	1.363	1.385	1.389	1.258	0.453	0.110	102	103	89	4	-74	2.87E-6	1.13E-5	4.96E-5
SK-OV-3	0.802	2.251	2.122	2.023	1.926	1.017	0.378	91	84	78	15	-53	2.75E-6	1.66E-5	9.07E-5
Renal Cancer															
786-0	0.585	2.338	2.288	2.388	2.230	0.981	0.232	97	103	94	23	-60	4.12E-6	1.87E-5	7.49E-5
A498	1.476	2.292	2.225	2.242	2.104	1.442	0.038	92	94	77	-2	-97	2.19E-6	9.35E-6	3.17E-5
ACHN	0.438	2.021	2.034	2.158	1.790	0.567	0.116	101	109	85	8	-74	2.87E-6	1.26E-5	5.14E-5
CAKI-1	0.528	2.340	2.309	2.319	2.099	0.687	0.059	98	99	87	9	-89	2.96E-6	1.23E-5	4.00E-5
RXF 393	0.851	1.575	1.550	1.545	1.489	0.849	0.058	97	96	88	0	-93	2.70E-6	9.92E-6	3.43E-5
SN12C	0.597	2.555	2.350	2.419	2.138	0.727	0.126	90	93	79	7	-79	2.50E-6	1.20E-5	4.59E-5
TK-10	1.236	2.811	2.704	2.710	2.578	1.321	0.070	93	94	85	5	-94	2.76E-6	1.13E-5	3.59E-5
UO-31	0.406	1.524	1.418	1.378	1.067	0.427	-0.002	91	87	59	2	-100	1.44E-6	1.04E-5	3.23E-5
Prostate Cancer															
PC-3	0.555	1.973	1.960	2.010	1.742	0.620	0.310	99	103	84	5	-44	2.67E-6	1.24E-5	> 1.00E-4
DU-145	0.333	1.276	1.290	1.302	1.207	0.467	0.012	101	103	93	14	-97	3.50E-6	1.34E-5	3.80E-5
Breast Cancer															
MCF7	0.333	1.754	1.628	1.687	1.660	0.351	0.046	91	95	93	1	-86	2.96E-6	1.03E-5	3.86E-5
MDA-MB-231/ATCC	0.687	1.643	1.612	1.688	1.517	0.680	0.277	97	105	87	-1	-60	2.63E-6	9.74E-6	6.84E-5
HS 578T	1.315	2.398	2.158	2.235	2.224	1.209	1.098	78	85	84	-8	-17	2.34E-6	8.17E-6	> 1.00E-4
BT-549	1.105	2.200	2.097	2.151	2.061	1.013	0.421	91	96	87	-8	-62	2.45E-6	8.18E-6	5.98E-5
MDA-MB-468	0.681	1.073	1.045	1.063	0.950	0.541	0.273	93	97	68	-21	-60	1.61E-6	5.87E-6	5.58E-5

Fig. 14: Representative five dose data of compound **JOOET-9**

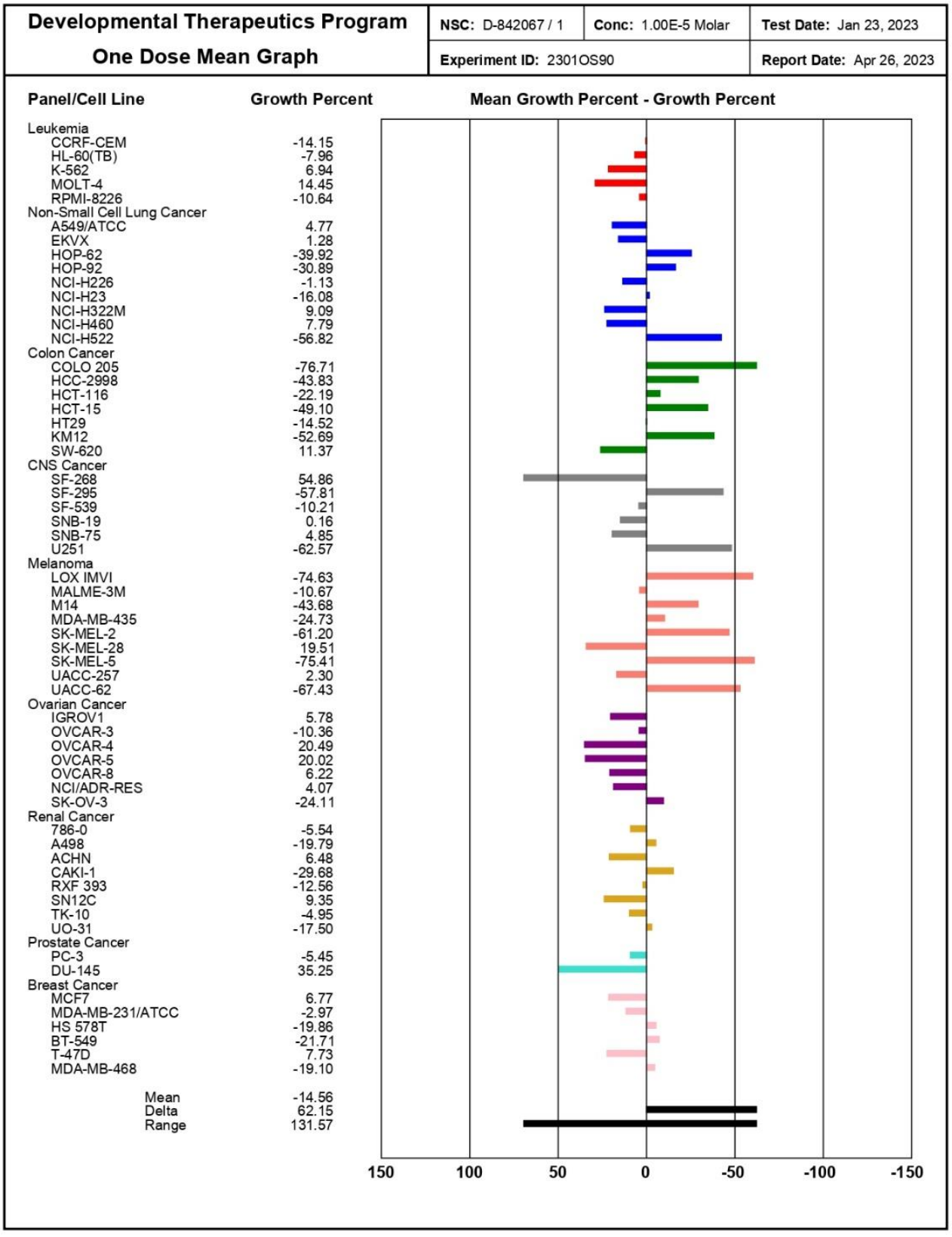


Fig. 15: Representative single dose data of compound **JOOET-10**

National Cancer Institute Developmental Therapeutics Program
In-Vitro Testing Results

NSC : D - 842067 / 1	Experiment ID : 2306NS37	Test Type : 08	Units : Molar
Report Date : July 28, 2023	Test Date : June 20, 2023	QNS :	MC :
COMI : JOOET-10	Stain Reagent : SRB Dual-Pass Related	SSPL : 1COF	

		Log10 Concentration												
	Time	Mean Optical Densities					Percent Growth							
Panel/Cell Line	Zero	Ctrl	-8.0	-7.0	-6.0	-5.0	-4.0	-8.0	-7.0	-6.0	-5.0	-4.0	GI50	TGI	LC50
Leukemia															
CCRF-CEM	0.475	1.810	1.911	1.757	1.531	0.506	0.394	108	96	79	2	-17	2.39E-6	1.32E-5	> 1.00E-4
HL-60(TB)	0.528	2.098	2.038	2.052	1.980	0.459	0.414	96	97	92	-13	-22	2.52E-6	7.51E-6	> 1.00E-4
K-562	0.122	0.852	0.804	0.820	0.683	0.165	0.115	93	96	77	6	-6	2.39E-6	3.07E-5	> 1.00E-4
MOLT-4	0.570	2.420	2.529	2.469	2.383	0.676	0.751	106	103	98	6	10	3.31E-6	> 1.00E-4	> 1.00E-4
RPMI-8226	0.580	2.163	2.240	2.172	1.761	0.471	0.600	105	101	75	-19	1	1.83E-6	.	> 1.00E-4
Non-Small Cell Lung Cancer															
A549/ATCC	0.300	1.788	1.673	1.720	1.600	0.410	0.011	92	95	87	7	-96	2.93E-6	1.18E-5	3.57E-5
EKVX	0.616	2.035	1.981	1.929	1.776	0.525	0.118	96	93	82	-15	-81	2.13E-6	7.03E-6	3.41E-5
HOP-62	0.743	2.245	2.113	2.152	2.134	0.626	0.219	91	94	93	-16	-71	2.47E-6	7.15E-6	4.22E-5
HOP-92	1.120	1.795	1.726	1.652	1.524	0.844	0.433	90	79	60	-25	-61	1.31E-6	5.11E-6	4.90E-5
NCI-H226	0.673	1.410	1.345	1.345	1.233	0.592	0.183	91	91	76	-12	-73	1.97E-6	7.30E-6	4.21E-5
NCI-H23	0.610	1.785	1.731	1.722	1.642	0.432	0.080	95	95	88	-29	-87	2.11E-6	5.63E-6	2.29E-5
NCI-H322M	0.732	2.098	1.988	2.054	1.881	0.944	0.029	92	97	84	15	-96	3.14E-6	1.38E-5	3.87E-5
NCI-H460	0.237	2.039	2.119	2.207	2.304	0.320	0.074	104	109	115	5	-69	3.87E-6	1.15E-5	5.52E-5
NCI-H522	0.702	2.124	2.050	2.005	1.922	0.492	0.102	95	92	86	-30	-85	2.04E-6	5.51E-6	2.30E-5
Colon Cancer															
COLO 205	0.489	2.100	2.214	2.281	2.213	0.487	0.337	107	111	107	0	-31	3.39E-6	9.89E-6	> 1.00E-4
HCC-2998	0.669	2.185	2.159	2.121	1.967	0.417	0.009	98	96	86	-38	-99	1.94E-6	4.94E-6	1.59E-5
HCT-116	0.233	1.867	1.773	1.818	1.724	0.149	0.023	94	97	91	-36	-90	2.10E-6	5.19E-6	1.80E-5
HCT-15	0.260	1.793	1.733	1.678	1.549	0.202	0.010	96	92	84	-22	-96	2.09E-6	6.17E-6	2.37E-5
HT29	0.212	1.394	1.570	1.592	1.475	0.308	0.045	115	117	107	8	-79	3.76E-6	1.24E-5	4.64E-5
KM12	0.453	1.401	1.414	1.416	1.326	0.278	0.012	101	102	92	-39	-97	2.10E-6	5.06E-6	1.56E-5
SW-620	0.244	1.626	1.691	1.753	1.758	0.450	0.055	105	109	110	15	-77	4.26E-6	1.45E-5	5.04E-5
CNS Cancer															
SF-268	0.779	2.177	2.119	2.156	2.028	0.985	0.280	96	98	89	15	-64	3.36E-6	1.54E-5	6.62E-5
SF-295	0.957	2.777	2.725	2.655	2.459	0.294	0.112	97	93	83	-69	-88	1.64E-6	3.50E-6	7.46E-6
SF-539	0.789	2.592	2.595	2.640	2.439	0.819	0.365	100	103	91	2	-54	2.90E-6	1.07E-5	8.56E-5
SNB-19	0.726	2.565	2.504	2.490	2.164	0.826	0.128	97	96	78	5	-82	2.44E-6	1.15E-5	4.28E-5
SNB-75	1.022	2.115	2.183	2.103	1.938	1.237	0.175	106	99	84	20	-83	3.36E-6	1.55E-5	4.77E-5
U251	0.319	1.711	1.666	1.652	1.404	0.284	0.011	97	96	78	-11	-97	2.06E-6	7.53E-6	2.85E-5
Melanoma															
LOX IMVI	0.248	1.811	1.729	1.636	1.586	0.037	0.012	95	89	86	-85	-95	1.62E-6	3.17E-6	6.23E-6
MALME-3M	0.517	1.164	1.175	1.187	1.247	0.522	0.057	102	104	113	1	-89	3.64E-6	1.02E-5	3.67E-5
M14	0.493	2.076	2.024	2.007	2.016	0.429	0.087	97	96	96	-13	-82	2.65E-6	7.59E-6	3.41E-5
MDA-MB-435	0.588	2.049	1.989	1.954	1.916	0.490	0.016	96	94	91	-17	-97	2.40E-6	7.00E-6	2.59E-5
SK-MEL-2	1.303	3.119	3.097	3.083	3.012	0.385	0.241	99	98	94	-70	-82	1.85E-6	3.73E-6	7.51E-6
SK-MEL-28	0.607	1.865	1.829	1.778	1.827	0.935	0.166	97	93	97	26	-73	4.59E-6	1.84E-5	5.89E-5
SK-MEL-5	0.723	2.582	2.517	2.474	2.188	0.084	0.003	97	94	79	-88	-100	1.49E-6	2.96E-6	5.89E-6
UACC-257	1.026	2.493	2.407	2.377	2.354	0.937	0.084	94	92	90	-9	-92	2.56E-6	8.18E-6	3.14E-5
Ovarian Cancer															
IGROV1	0.360	1.663	1.901	1.975	1.577	0.734	0.161	118	124	93	29	-55	4.69E-6	2.20E-5	8.65E-5
OVCAR-3	0.545	2.230	2.167	2.148	2.192	0.607	0.044	96	95	98	4	-92	3.22E-6	1.09E-5	3.64E-5
OVCAR-4	0.758	1.806	1.856	1.847	1.737	0.957	0.378	105	104	93	19	-50	3.83E-6	1.88E-5	9.93E-5
OVCAR-5	0.626	1.779	1.673	1.774	1.766	0.796	0.281	91	100	99	15	-55	3.81E-6	1.63E-5	8.45E-5
OVCAR-8	0.398	1.897	1.886	1.844	1.686	0.490	0.029	99	96	86	6	-93	2.82E-6	1.15E-5	3.70E-5
NCI/ADR-RES	0.415	1.382	1.386	1.342	1.308	0.405	0.058	100	96	92	-2	-86	2.80E-6	9.43E-6	3.70E-5
SK-OV-3	0.802	2.152	2.278	2.232	2.160	0.974	0.084	109	106	101	13	-90	3.77E-6	1.33E-5	4.10E-5
Renal Cancer															
786-0	0.585	2.407	2.278	2.271	2.289	0.856	0.058	93	93	94	15	-90	3.58E-6	1.39E-5	4.15E-5
A498	1.476	2.295	2.290	2.361	2.195	1.365	0.025	99	108	88	-8	-98	2.49E-6	8.34E-6	2.94E-5
ACHN	0.438	2.036	2.148	2.118	1.952	0.613	0.097	107	105	95	11	-78	3.42E-6	1.33E-5	4.86E-5
CAKI-1	0.528	2.335	2.270	2.253	2.233	0.559	0.100	96	95	94	2	-81	3.01E-6	1.05E-5	4.21E-5
RXF 393	0.851	1.604	1.617	1.590	1.462	0.854	0.088	102	98	81	0	-90	2.43E-6	1.01E-5	3.62E-5
SN12C	0.597	2.499	2.568	2.727	2.422	0.930	0.306	104	112	96	17	-49	3.85E-6	1.84E-5	> 1.00E-4
TK-10	1.236	2.874	2.749	2.788	2.684	1.386	0.037	92	95	88	9	-97	3.05E-6	1.22E-5	3.61E-5
UO-31	0.406	1.437	1.293	1.350	1.067	0.362	0.001	86	91	64	-11	-100	1.54E-6	7.17E-6	2.76E-5
Prostate Cancer															
PC-3	0.555	1.945	1.998	1.972	1.752	0.589	0.273	104	102	86	2	-51	2.70E-6	1.11E-5	9.62E-5
DU-145	0.333	1.308	1.329	1.354	1.245	0.513	0.008	102	105	94	18	-98	3.80E-6	1.44E-5	3.89E-5
Breast Cancer															
MCF7	0.333	1.795	1.654	1.620	1.488	0.341	0.034	90	88	79	1	-90	2.34E-6	1.01E-5	3.63E-5
MDA-MB-231/ATCC	0.687	1.657	1.729	1.698	1.676	0.490	0.354	107	104	102	-29	-48	2.50E-6	6.02E-6	> 1.00E-4
HS 578T	1.315	2.391	2.304	2.303	2.269	1.160	1.124	92	92	89	-12	-15	2.43E-6	7.63E-6	> 1.00E-4
BT-549	1.105	2.050	1.943	1.923	1.818	0.706	0.277	89	87	75	-36	-75	1.69E-6	4.75E-6	2.28E-5
MDA-MB-468	0.681	1.072	1.081	1.047	0.958	0.547	0.161	102	94	71	-20	-76	1.70E-6	6.05E-6	3.42E-5

Fig. 16: Representative five dose data of compound **JOOET-10**

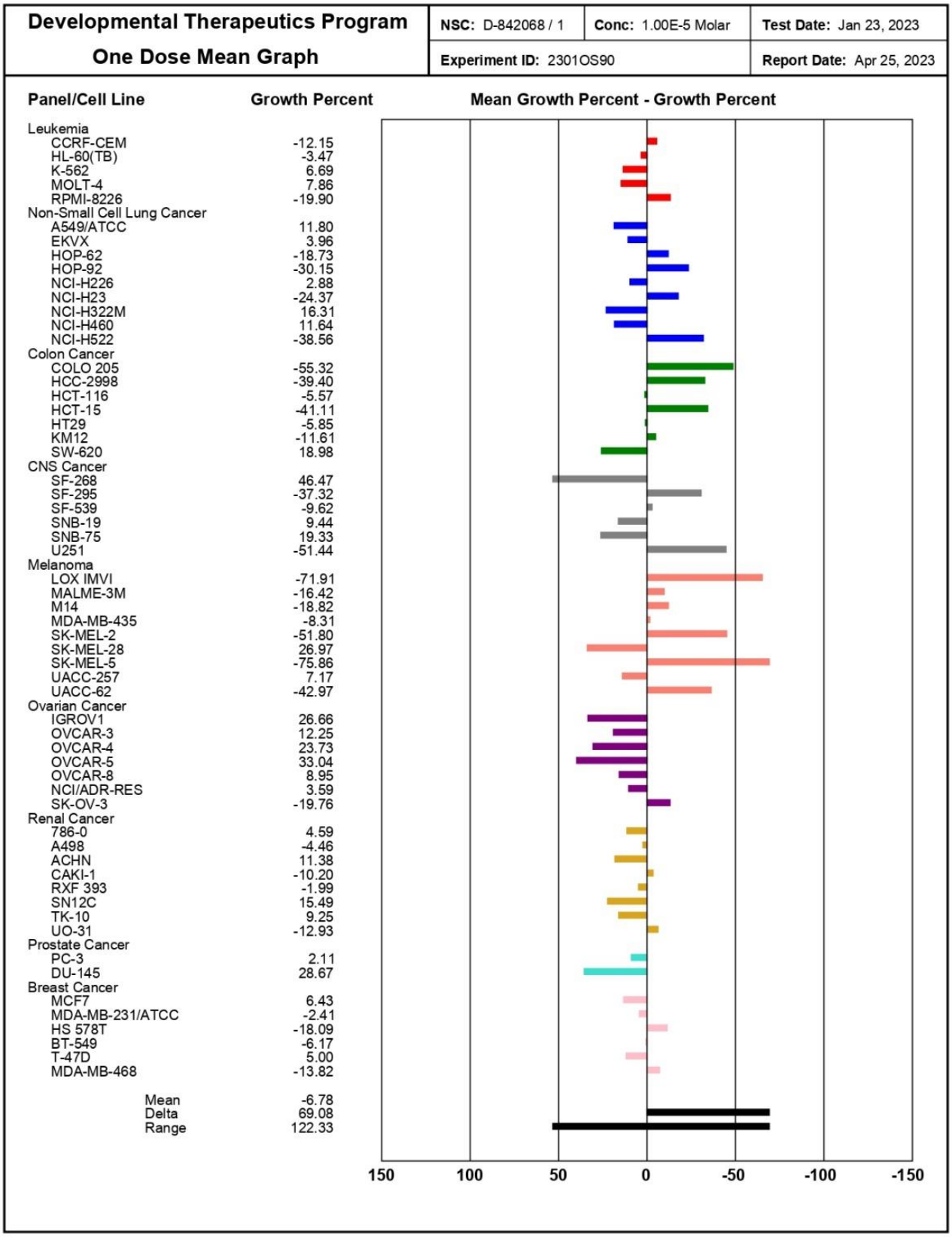


Fig.17: Representative single dose data of compound **JOOET-11**

National Cancer Institute Developmental Therapeutics Program
In-Vitro Testing Results

NSC : D - 842068 / 1	Experiment ID : 2306NS37	Test Type : 08	Units : Molar
Report Date : July 28, 2023	Test Date : June 20, 2023	QNS :	MC :
COMI : JOOET-11	Stain Reagent : SRB Dual-Pass Related	SSPL : 1COF	

		Log10 Concentration												
	Time	Mean Optical Densities					Percent Growth							
Panel/Cell Line	Zero	Ctrl	-8.0	-7.0	-6.0	-5.0	-4.0	-8.0	-7.0	-6.0	-5.0	-4.0	GI50	TGI	LC50
Leukemia															
CCRF-CEM	0.475	1.810	1.798	1.774	1.422	0.451	0.292	99	97	71	-5	-39	1.88E-6	8.58E-6	> 1.00E-4
HL-60(TB)	0.528	2.098	1.936	2.048	1.970	0.461	0.353	90	97	92	-13	-33	2.51E-6	7.56E-6	> 1.00E-4
K-562	0.122	0.852	0.759	0.808	0.561	0.128	0.071	87	94	60	1	-42	1.48E-6	1.04E-5	> 1.00E-4
MOLT-4	0.570	2.420	2.456	2.410	2.039	0.509	0.409	102	99	79	-11	-28	2.12E-6	7.61E-6	> 1.00E-4
RPMI-8226	0.580	2.163	2.144	2.128	1.618	0.416	0.395	99	98	66	-28	-32	1.46E-6	4.99E-6	> 1.00E-4
Non-Small Cell Lung Cancer															
A549/ATCC	0.300	1.788	1.689	1.736	1.550	0.378	0.014	93	96	84	5	-95	2.70E-6	1.13E-5	3.54E-5
EKVX	0.616	2.035	1.900	1.994	1.755	0.484	0.169	90	97	80	-21	-73	1.98E-6	6.16E-6	3.61E-5
HOP-62	0.743	2.245	2.269	2.220	2.202	0.690	0.111	102	98	97	-7	-85	2.83E-6	8.53E-6	3.55E-5
HOP-92	1.120	1.795	1.738	1.717	1.573	0.794	0.243	91	88	67	-29	-78	1.51E-6	4.98E-6	2.66E-5
NCI-H226	0.673	1.410	1.333	1.377	1.260	0.518	0.135	90	95	80	-23	-80	1.94E-6	5.96E-6	2.97E-5
NCI-H23	0.610	1.785	1.741	1.722	1.525	0.329	0.028	96	95	78	-46	-95	1.68E-6	4.24E-6	1.20E-5
NCI-H322M	0.732	2.098	2.036	1.985	1.819	0.806	0.071	95	92	80	5	-90	2.51E-6	1.14E-5	3.79E-5
NCI-H460	0.237	2.039	2.036	2.063	1.891	0.225	0.024	100	101	92	-5	-90	2.70E-6	8.82E-6	3.38E-5
NCI-H522	0.702	2.124	1.983	1.995	1.736	0.568	0.042	90	91	73	-19	-94	1.77E-6	6.19E-6	2.58E-5
Colon Cancer															
COLO 205	0.489	2.100	2.183	2.232	2.019	0.305	0.233	105	108	95	-38	-52	2.18E-6	5.20E-6	6.81E-5
HCC-2998	0.669	2.185	1.903	2.128	2.056	0.262	0.007	81	96	92	-61	-99	1.87E-6	3.99E-6	8.49E-6
HCT-116	0.233	1.867	1.876	1.839	1.684	0.167	0.072	101	98	89	-28	-69	2.14E-6	5.73E-6	3.40E-5
HCT-15	0.260	1.793	1.723	1.741	1.478	0.125	0.015	95	97	79	-52	-94	1.67E-6	4.02E-6	9.64E-6
HT29	0.212	1.394	1.429	1.547	1.315	0.240	0.017	103	113	93	2	-92	2.99E-6	1.06E-5	3.59E-5
KM12	0.453	1.401	1.398	1.450	1.266	0.346	0.013	100	105	86	-24	-97	2.12E-6	6.07E-6	2.28E-5
SW-620	0.244	1.626	1.602	1.598	1.474	0.381	0.047	98	98	89	10	-81	3.11E-6	1.28E-5	4.56E-5
CNS Cancer															
SF-268	0.779	2.177	2.067	2.116	1.921	0.877	0.151	92	96	82	7	-81	2.66E-6	1.20E-5	4.47E-5
SF-295	0.957	2.777	2.669	2.715	2.496	0.233	0.046	94	97	85	-76	-95	1.64E-6	3.37E-6	6.92E-6
SF-539	0.789	2.592	2.435	2.509	2.467	0.554	0.061	91	95	93	-30	-92	2.24E-6	5.72E-6	2.10E-5
SNB-19	0.726	2.565	2.546	2.525	2.205	0.845	0.196	99	98	80	6	-73	2.58E-6	1.21E-5	5.13E-5
SNB-75	1.022	2.115	1.954	1.952	1.833	1.107	0.091	85	85	74	8	-91	2.31E-6	1.20E-5	3.84E-5
U251	0.319	1.711	1.635	1.651	1.364	0.233	0.002	95	96	75	-27	-99	1.76E-6	5.44E-6	2.08E-5
Melanoma															
LOX IMVI	0.248	1.811	1.722	1.789	1.656	0.019	-0.007	94	99	90	-93	-100	1.66E-6	3.11E-6	5.85E-6
MALME-3M	0.517	1.164	1.065	1.095	1.029	0.423	0.116	85	89	79	-18	-78	1.99E-6	6.50E-6	3.43E-5
M14	0.493	2.076	2.014	2.051	1.942	0.486	0.257	96	98	92	-2	-48	2.79E-6	9.63E-6	> 1.00E-4
MDA-MB-435	0.588	2.049	1.805	1.846	1.770	0.457	0.032	83	86	81	-22	-95	1.99E-6	6.08E-6	2.42E-5
SK-MEL-2	1.303	3.119	3.048	3.117	2.761	0.512	0.169	96	100	80	-61	-87	1.64E-6	3.71E-6	8.40E-6
SK-MEL-28	0.607	1.865	1.883	2.017	1.641	0.743	0.106	101	112	82	11	-83	2.82E-6	1.30E-5	4.48E-5
SK-MEL-5	0.723	2.582	2.438	2.556	2.116	0.108	0.015	92	99	75	-85	-98	1.43E-6	2.94E-6	6.03E-6
UACC-257	1.026	2.493	2.378	2.384	2.190	0.873	0.784	92	93	79	-15	-24	2.05E-6	6.94E-6	> 1.00E-4
Ovarian Cancer															
IGROV1	0.360	1.663	1.747	1.710	1.549	0.591	0.179	106	104	91	18	-50	3.64E-6	1.82E-5	9.91E-5
OVCAR-3	0.545	2.230	2.166	2.249	1.972	0.482	0.047	96	101	85	-12	-91	2.29E-6	7.58E-6	3.03E-5
OVCAR-4	0.758	1.806	1.711	1.784	1.646	0.859	0.768	91	98	85	10	1	2.90E-6	> 1.00E-4	> 1.00E-4
OVCAR-5	0.626	1.779	2.009	1.789	1.687	0.653	0.146	120	101	92	2	-77	2.94E-6	1.07E-5	4.59E-5
OVCAR-8	0.398	1.897	1.898	1.898	1.737	0.433	0.005	100	100	89	2	-99	2.83E-6	1.05E-5	3.29E-5
NCI/ADR-RES	0.415	1.382	1.359	1.390	1.280	0.337	0.015	98	101	89	-19	-97	2.31E-6	6.70E-6	2.52E-5
SK-OV-3	0.802	2.152	2.093	2.151	1.969	0.938	0.370	96	100	86	10	-54	3.00E-6	1.44E-5	8.68E-5
Renal Cancer															
786-0	0.585	2.407	2.277	2.311	2.311	0.949	0.270	93	95	95	20	-54	3.97E-6	1.86E-5	8.85E-5
A498	1.476	2.295	2.213	2.268	2.195	1.315	0.071	90	97	88	-11	-95	2.41E-6	7.75E-6	2.91E-5
ACHN	0.438	2.036	2.063	2.080	1.833	0.595	0.296	102	103	87	10	-33	3.03E-6	1.70E-5	> 1.00E-4
CAKI-1	0.528	2.335	2.290	2.311	2.055	0.496	0.176	97	99	84	-6	-67	2.40E-6	8.55E-6	5.30E-5
RXF 393	0.851	1.604	1.562	1.580	1.451	0.764	0.081	94	97	80	-10	-91	2.14E-6	7.70E-6	3.13E-5
SN12C	0.597	2.499	2.392	2.553	2.206	0.719	0.083	94	103	85	6	-86	2.77E-6	1.17E-5	4.07E-5
TK-10	1.236	2.874	2.766	2.792	2.627	1.305	0.683	93	95	85	4	-45	2.71E-6	1.22E-5	> 1.00E-4
UO-31	0.406	1.437	1.320	1.282	1.140	0.384	-0.002	89	85	71	-5	-100	1.89E-6	8.50E-6	2.96E-5
Prostate Cancer															
PC-3	0.555	1.945	1.940	2.029	1.725	0.562	0.168	100	106	84	1	-70	2.56E-6	1.02E-5	5.24E-5
DU-145	0.333	1.308	1.303	1.329	1.245	0.521	0.391	100	102	94	19	6	3.86E-6	> 1.00E-4	> 1.00E-4
Breast Cancer															
MCF7	0.333	1.795	1.655	1.722	1.637	0.313	0.252	90	95	89	-6	-24	2.58E-6	8.62E-6	> 1.00E-4
MDA-MB-231/ATCC	0.687	1.657	1.735	1.691	1.541	0.422	0.438	108	103	88	-39	-36	2.00E-6	4.96E-6	> 1.00E-4
HS 578T	1.315	2.391	2.221	2.289	2.135	1.111	0.938	84	90	76	-16	-29	1.93E-6	6.77E-6	> 1.00E-4
BT-549	1.105	2.050	1.960	2.199	2.078	0.765	0.497	90	116	103	-31	-55	2.49E-6	5.88E-6	6.20E-5
MDA-MB-468	0.681	1.072	1.035	1.052	0.935	0.522	0.338	91	95	65	-23	-50	1.48E-6	5.44E-6	9.63E-5

Fig. 18: Representative five dose data of compound **JOOET-11**

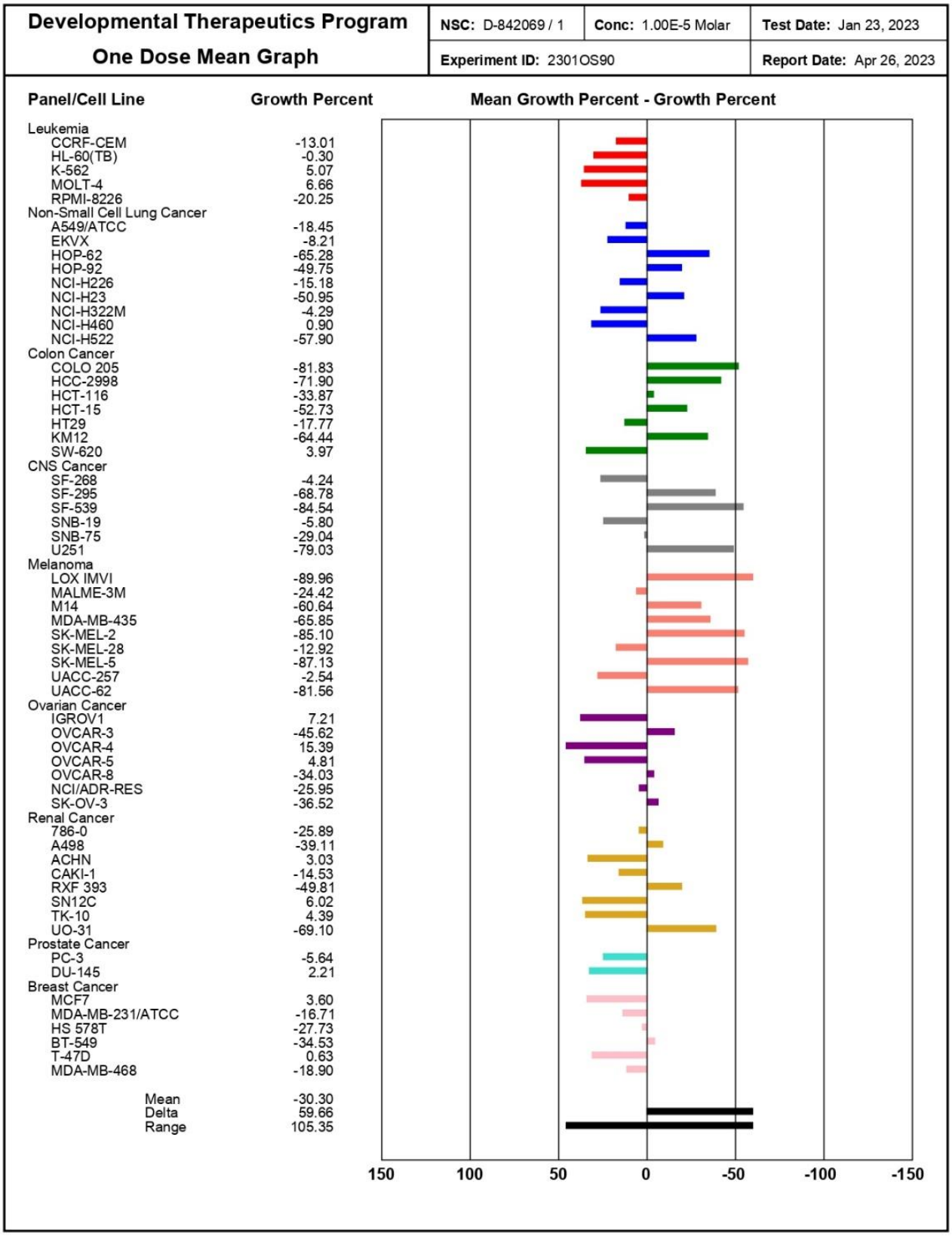


Fig. 19: Representative single dose data of compound **JOOET-12**

National Cancer Institute Developmental Therapeutics Program
In-Vitro Testing Results

NSC : D - 842069 / 1	Experiment ID : 2306NS37	Test Type : 08	Units : Molar
Report Date : July 28, 2023	Test Date : June 20, 2023	QNS :	MC :
COMI : JOOET-12	Stain Reagent : SRB Dual-Pass Related	SSPL : 1COF	

			Log10 Concentration												
	Time		Mean Optical Densities					Percent Growth							
Panel/Cell Line	Zero	Ctrl	-8.0	-7.0	-6.0	-5.0	-4.0	-8.0	-7.0	-6.0	-5.0	-4.0	GI50	TGI	LC50
Leukemia															
CCRF-CEM	0.475	1.768	1.773	1.737	1.401	0.485	0.336	100	98	72	1	-29	2.02E-6	1.06E-5	> 1.00E-4
HL-60(TB)	0.528	1.962	1.834	1.838	1.852	0.423	0.212	91	91	92	-20	-60	2.38E-6	6.65E-6	5.65E-5
K-562	0.122	0.717	0.758	0.706	0.545	0.121	0.066	107	98	71	-1	-46	1.95E-6	9.62E-6	> 1.00E-4
MOLT-4	0.570	2.182	2.234	2.181	1.925	0.540	0.231	103	100	84	-5	-59	2.41E-6	8.73E-6	6.69E-5
RPMI-8226	0.580	2.170	2.244	2.178	1.687	0.504	0.343	105	100	70	-13	-41	1.72E-6	6.93E-6	> 1.00E-4
Non-Small Cell Lung Cancer															
A549/ATCC	0.300	1.715	1.672	1.629	1.518	0.268	0.022	97	94	86	-11	-93	2.36E-6	7.73E-6	3.00E-5
EKVX	0.616	1.979	1.937	1.849	1.685	0.461	0.079	97	90	78	-25	-87	1.88E-6	5.71E-6	2.51E-5
HOP-62	0.743	2.269	2.073	2.112	2.078	0.301	0.024	87	90	87	-59	-97	1.80E-6	3.94E-6	8.62E-6
HOP-92	1.120	1.783	1.765	1.719	1.518	0.671	0.082	97	90	60	-40	-93	1.26E-6	3.97E-6	1.54E-5
NCI-H226	0.673	1.392	1.329	1.365	1.282	0.487	0.214	91	96	85	-28	-68	2.04E-6	5.68E-6	3.55E-5
NCI-H23	0.610	1.770	1.715	1.699	1.540	0.302	0.013	95	94	80	-51	-98	1.70E-6	4.10E-6	9.90E-6
NCI-H322M	0.732	2.104	2.219	2.145	1.980	0.814	0.136	108	103	91	6	-81	3.03E-6	1.17E-5	4.37E-5
NCI-H460	0.237	2.040	2.175	2.143	2.018	0.205	0.068	107	106	99	-14	-71	2.71E-6	7.55E-6	4.27E-5
NCI-H522	0.702	2.045	1.976	1.923	1.767	0.451	0.044	95	91	79	-36	-94	1.80E-6	4.88E-6	1.76E-5
Colon Cancer															
COLO 205	0.489	2.038	1.971	2.015	2.040	0.236	0.142	96	98	100	-52	-71	2.14E-6	4.56E-6	9.74E-6
HCC-2998	0.669	2.175	2.019	2.105	1.897	0.240	-0.003	90	95	82	-64	-100	1.65E-6	3.63E-6	8.00E-6
HCT-116	0.233	2.010	1.922	1.994	1.803	0.169	0.013	95	99	88	-27	-95	2.14E-6	5.79E-6	2.17E-5
HCT-15	0.260	1.842	1.755	1.665	1.373	0.107	0.003	94	89	70	-59	-99	1.44E-6	3.50E-6	8.51E-6
HT29	0.212	1.347	1.380	1.460	1.337	0.139	0.023	103	110	99	-35	-89	2.33E-6	5.50E-6	1.91E-5
KM12	0.453	1.329	1.360	1.262	1.271	0.239	0.006	103	92	93	-47	-99	2.03E-6	4.61E-6	1.13E-5
SW-620	0.244	1.534	1.766	1.618	1.561	0.361	0.033	118	107	102	9	-86	3.63E-6	1.24E-5	4.15E-5
CNS Cancer															
SF-268	0.779	2.112	2.128	2.004	1.881	0.675	0.125	101	92	83	-13	-84	2.19E-6	7.25E-6	3.30E-5
SF-295	0.957	2.766	2.707	2.637	2.356	0.201	0.004	97	93	77	-79	-100	1.50E-6	3.12E-6	6.52E-6
SF-539	0.789	2.596	2.568	2.552	2.445	0.404	0.190	98	98	92	-49	-76	1.98E-6	4.49E-6	1.11E-5
SNB-19	0.726	2.523	2.428	2.421	2.168	0.760	0.115	95	94	80	2	-84	2.43E-6	1.05E-5	4.01E-5
SNB-75	1.022	1.973	1.930	1.952	1.915	0.793	0.046	95	98	94	-22	-96	2.38E-6	6.41E-6	2.38E-5
U251	0.319	1.678	1.610	1.582	1.404	0.124	0.022	95	93	80	-61	-93	1.63E-6	3.68E-6	8.32E-6
Melanoma															
LOX IMVI	0.248	1.787	1.701	1.697	1.654	0.039	-0.004	94	94	91	-84	-100	1.72E-6	3.31E-6	6.38E-6
MALME-3M	0.517	1.221	1.222	1.193	1.122	0.496	0.107	100	96	86	-4	-79	2.50E-6	8.99E-6	4.07E-5
M14	0.493	2.057	2.005	2.016	1.978	0.292	0.069	97	97	95	-41	-86	2.14E-6	5.00E-6	1.59E-5
MDA-MB-435	0.588	2.058	2.024	2.019	1.898	0.439	0.008	98	97	89	-25	-99	2.20E-6	6.01E-6	2.17E-5
SK-MEL-2	1.303	3.050	3.050	3.048	2.765	0.316	0.044	100	100	84	-76	-97	1.63E-6	3.35E-6	6.89E-6
SK-MEL-28	0.607	1.968	1.910	1.756	1.728	0.783	0.234	96	84	82	13	-62	2.92E-6	1.49E-5	7.00E-5
SK-MEL-5	0.723	2.553	2.461	2.416	1.977	0.144	-0.002	95	93	69	-80	-100	1.33E-6	2.89E-6	6.27E-6
UACC-257	1.026	2.453	2.373	2.344	2.190	0.872	0.097	94	92	82	-15	-91	2.12E-6	6.98E-6	2.90E-5
Ovarian Cancer															
IGROV1	0.360	1.758	1.745	2.004	1.703	0.564	0.292	99	118	96	15	-19	3.67E-6	2.72E-5	> 1.00E-4
OVCAR-3	0.545	2.168	2.093	2.131	2.080	0.376	0.006	95	98	95	-31	-99	2.26E-6	5.66E-6	1.90E-5
OVCAR-4	0.758	1.702	1.715	1.749	1.555	0.809	0.282	101	105	84	5	-63	2.73E-6	1.20E-5	6.48E-5
OVCAR-5	0.626	1.874	2.030	1.934	2.055	0.830	0.182	112	105	114	16	-71	4.54E-6	1.54E-5	5.76E-5
OVCAR-8	0.398	1.887	1.904	1.821	1.728	0.276	0.017	101	96	89	-31	-96	2.13E-6	5.55E-6	1.98E-5
NCI/ADR-RES	0.415	1.360	1.371	1.349	1.297	0.295	0.056	101	99	93	-29	-87	2.26E-6	5.79E-6	2.32E-5
SK-OV-3	0.802	1.967	1.926	1.940	1.858	0.633	0.033	97	98	91	-21	-96	2.31E-6	6.47E-6	2.43E-5
Renal Cancer															
786-0	0.585	2.319	2.321	2.122	2.160	0.546	0.099	100	89	91	-7	-83	2.62E-6	8.53E-6	3.69E-5
A498	1.476	2.186	2.228	2.237	2.234	1.226	0.148	106	107	107	-17	-90	2.88E-6	7.29E-6	2.83E-5
ACHN	0.438	2.053	2.071	2.127	1.965	0.636	0.194	101	105	95	12	-56	3.48E-6	1.51E-5	8.21E-5
CAKI-1	0.528	2.248	2.166	2.156	2.009	0.205	0.024	95	95	86	-61	-95	1.76E-6	3.84E-6	8.39E-6
RXF 393	0.851	1.536	1.567	1.494	1.363	0.545	0.032	104	94	75	-36	-96	1.67E-6	4.73E-6	1.71E-5
SN12C	0.597	2.553	2.369	2.394	2.064	0.873	0.103	91	92	75	14	-83	2.57E-6	1.40E-5	4.59E-5
TK-10	1.236	2.844	2.737	2.745	2.621	1.355	0.136	93	94	86	7	-89	2.88E-6	1.19E-5	3.94E-5
UO-31	0.406	1.544	1.391	1.391	1.251	0.328	0.030	87	86	74	-19	-93	1.81E-6	6.21E-6	2.62E-5
Prostate Cancer															
PC-3	0.555	1.967	1.911	1.901	1.679	0.567	0.126	96	95	80	1	-77	2.38E-6	1.02E-5	4.46E-5
DU-145	0.333	1.240	1.307	1.338	1.229	0.361	0.057	107	111	99	3	-83	3.23E-6	1.08E-5	4.14E-5
Breast Cancer															
MCF7	0.333	1.769	1.639	1.637	1.518	0.302	0.031	91	91	83	-9	-91	2.26E-6	7.89E-6	3.15E-5
MDA-MB-231/ATCC	0.687	1.658	1.773	1.668	1.583	0.407	0.186	112	101	92	-41	-73	2.08E-6	4.93E-6	1.93E-5
HS 578T	1.315	2.317	2.247	2.177	2.051	0.993	0.819	93	86	73	-25	-38	1.73E-6	5.62E-6	> 1.00E-4
BT-549	1.105	2.062	1.969	1.941	1.895	0.565	0.135	90	87	82	-49	-88	1.77E-6	4.24E-6	1.07E-5
MDA-MB-468	0.681	1.050	1.020	0.991	0.892	0.512	0.034	92	84	57	-25	-95	1.22E-6	4.98E-6	2.28E-5

Fig. 20: Representative five dose data of compound **JOOET-12**

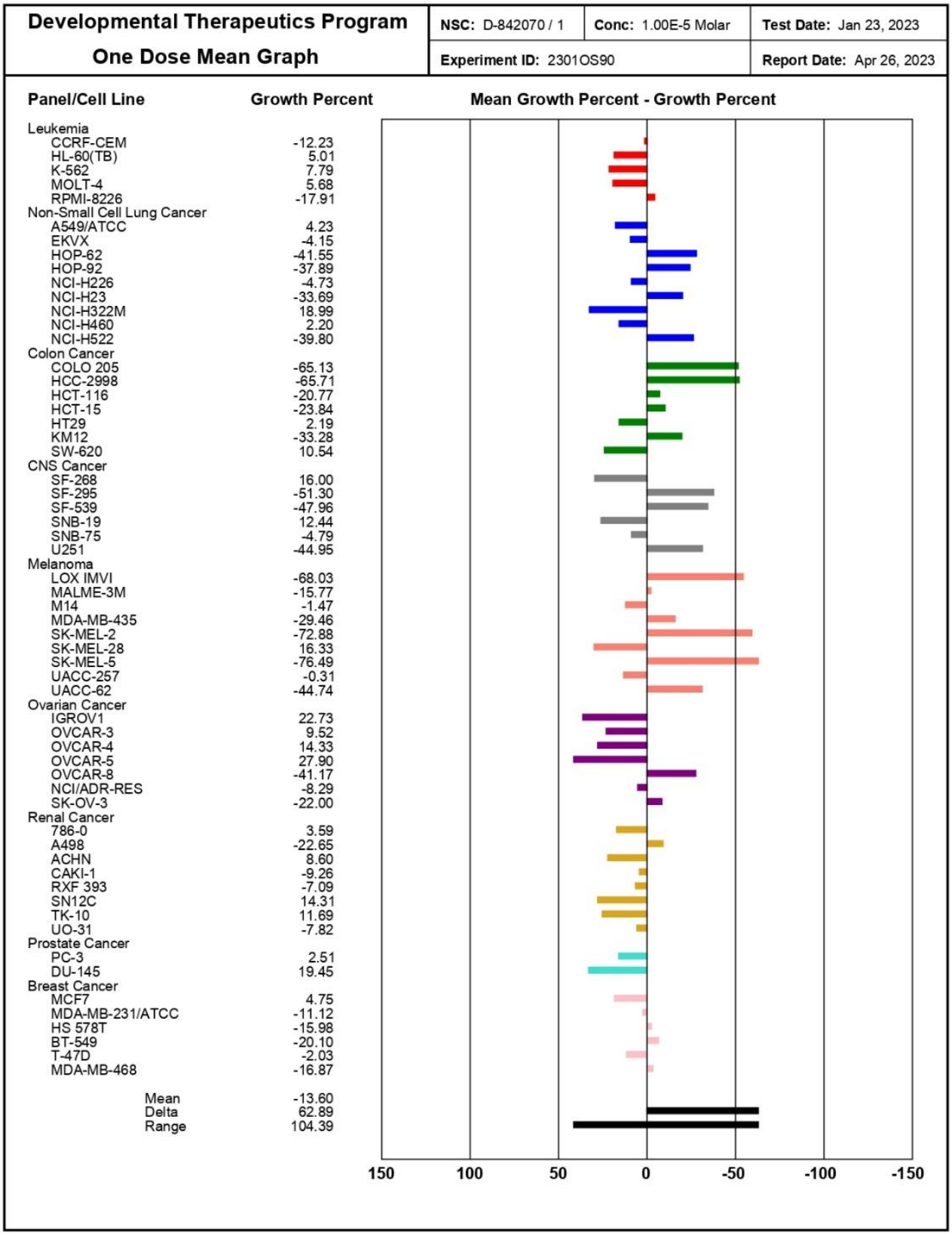


Fig. 21: Representative single dose data of compound **JOOET-13**

National Cancer Institute Developmental Therapeutics Program
In-Vitro Testing Results

NSC : D - 842070 / 1	Experiment ID : 2306NS37	Test Type : 08	Units : Molar
Report Date : July 28, 2023	Test Date : June 20, 2023	QNS :	MC :
COMI : JOOET-13	Stain Reagent : SRB Dual-Pass Related	SSPL : 1COF	

		Log10 Concentration												
	Time	Mean Optical Densities						Percent Growth						
Panel/Cell Line	Zero	Ctrl	-8.0	-7.0	-6.0	-5.0	-4.0	-8.0	-7.0	-6.0	-5.0	-4.0	GI50	TGI	LC50
Leukemia															
CCRF-CEM	0.475	1.768	1.787	1.847	1.473	0.420	0.286	101	106	77	-12	-40	2.02E-6	7.40E-6	> 1.00E-4
HL-60(TB)	0.528	1.962	1.888	1.901	1.797	0.413	0.332	95	96	88	-22	-37	2.23E-6	6.34E-6	> 1.00E-4
K-562	0.122	0.717	0.750	0.714	0.573	0.131	0.078	106	99	76	1	-36	2.22E-6	1.09E-5	> 1.00E-4
MOLT-4	0.570	2.182	2.148	2.287	1.938	0.446	0.388	98	107	85	-22	-32	2.12E-6	6.24E-6	> 1.00E-4
RPMI-8226	0.580	2.170	2.169	2.121	1.697	0.445	0.464	100	97	70	-23	-20	1.65E-6	5.64E-6	> 1.00E-4
Non-Small Cell Lung Cancer															
A549/ATCC	0.300	1.715	1.662	1.771	1.617	0.360	0.026	96	104	93	4	-91	3.05E-6	1.11E-5	3.69E-5
EKVX	0.616	1.979	1.843	1.825	1.561	0.486	0.166	90	89	69	-21	-73	1.64E-6	5.83E-6	3.59E-5
HOP-62	0.743	2.269	2.139	2.081	2.000	0.698	0.217	91	88	82	-6	-71	2.32E-6	8.54E-6	4.77E-5
HOP-92	1.120	1.783	1.733	1.726	1.569	0.760	0.232	92	91	68	-32	-79	1.50E-6	4.76E-6	2.39E-5
NCI-H226	0.673	1.392	1.355	1.343	1.265	0.527	0.260	95	93	82	-22	-61	2.05E-6	6.19E-6	5.17E-5
NCI-H23	0.610	1.770	1.721	1.692	1.516	0.344	0.048	96	93	78	-44	-92	1.70E-6	4.38E-6	1.35E-5
NCI-H322M	0.732	2.104	1.986	2.047	1.853	0.846	0.042	91	96	82	8	-94	2.70E-6	1.20E-5	3.70E-5
NCI-H460	0.237	2.040	2.063	2.008	1.972	0.218	0.031	101	98	96	-8	-87	2.77E-6	8.34E-6	3.38E-5
NCI-H522	0.702	2.045	1.935	2.028	1.813	0.587	0.071	92	99	83	-16	-90	2.14E-6	6.82E-6	2.86E-5
Colon Cancer															
COLO 205	0.489	2.038	2.024	1.979	1.919	0.262	0.062	99	96	92	-47	-87	2.02E-6	4.62E-6	1.22E-5
HCC-2998	0.669	2.175	1.941	2.117	2.055	0.350	0.003	84	96	92	-48	-100	2.00E-6	4.56E-6	1.11E-5
HCT-116	0.233	2.010	1.826	1.901	1.722	0.213	0.082	90	94	84	-9	-65	2.32E-6	8.07E-6	5.45E-5
HCT-15	0.260	1.842	1.730	1.738	1.500	0.185	0.008	93	93	78	-29	-97	1.84E-6	5.38E-6	2.05E-5
HT29	0.212	1.347	1.373	1.428	1.353	0.229	0.020	102	107	101	1	-91	3.24E-6	1.04E-5	3.61E-5
KM12	0.453	1.329	1.347	1.386	1.230	0.392	0.002	102	106	89	-14	-100	2.39E-6	7.36E-6	2.65E-5
SW-620	0.244	1.534	1.500	1.537	1.508	0.362	0.066	97	100	98	9	-73	3.47E-6	1.29E-5	5.25E-5
CNS Cancer															
SF-268	0.779	2.112	2.011	2.095	1.946	0.848	0.175	92	99	87	5	-78	2.85E-6	1.15E-5	4.65E-5
SF-295	0.957	2.766	2.639	2.693	2.525	0.393	0.051	93	96	87	-59	-95	1.78E-6	3.93E-6	8.68E-6
SF-539	0.789	2.596	2.599	2.458	2.365	0.595	0.095	100	92	87	-25	-88	2.15E-6	6.02E-6	2.51E-5
SNB-19	0.726	2.523	2.355	2.416	2.284	0.882	0.144	91	94	87	9	-80	2.95E-6	1.25E-5	4.57E-5
SNB-75	1.022	1.973	1.878	1.896	1.717	0.918	0.191	90	92	73	-10	-81	1.89E-6	7.54E-6	3.63E-5
U251	0.319	1.678	1.574	1.626	1.494	0.318	0.030	92	96	86	0	-91	2.63E-6	9.88E-6	3.54E-5
Melanoma															
LOX IMVI	0.248	1.787	1.688	1.730	1.658	0.117	.	94	96	92	-53	-100	1.94E-6	4.30E-6	9.53E-6
MALME-3M	0.517	1.221	1.131	1.136	1.075	0.480	0.090	87	88	79	-7	-83	2.18E-6	8.24E-6	3.69E-5
M14	0.493	2.057	2.043	2.017	1.952	0.718	0.154	99	97	93	14	-69	3.53E-6	1.49E-5	5.95E-5
MDA-MB-435	0.588	2.058	1.922	1.953	1.852	0.428	0.014	91	93	86	-27	-98	2.08E-6	5.74E-6	2.10E-5
SK-MEL-2	1.303	3.050	3.072	3.045	2.806	0.540	0.080	101	100	86	-59	-94	1.77E-6	3.93E-6	8.72E-6
SK-MEL-28	0.607	1.968	1.972	1.993	1.848	0.762	0.195	100	102	91	11	-68	3.28E-6	1.39E-5	5.94E-5
SK-MEL-5	0.723	2.553	2.380	2.526	2.216	0.201	0.016	91	99	82	-72	-98	1.60E-6	3.39E-6	7.17E-6
UACC-257	1.026	2.453	2.352	2.404	2.278	0.936	0.270	93	97	88	-9	-74	2.46E-6	8.11E-6	4.31E-5
Ovarian Cancer															
IGROV1	0.360	1.758	1.712	1.771	1.468	0.560	0.153	97	101	79	14	-58	2.82E-6	1.58E-5	7.83E-5
OVCAR-3	0.545	2.168	2.159	2.170	2.052	0.441	0.025	99	100	93	-19	-96	2.41E-6	6.75E-6	2.54E-5
OVCAR-4	0.758	1.702	1.659	1.695	1.595	0.804	0.473	95	99	89	5	-38	2.89E-6	1.30E-5	> 1.00E-4
OVCAR-5	0.626	1.874	2.225	2.107	1.830	0.696	0.240	128	119	96	6	-62	3.25E-6	1.21E-5	6.71E-5
OVCAR-8	0.398	1.887	1.858	1.901	1.777	0.437	0.021	98	101	93	3	-95	2.98E-6	1.06E-5	3.47E-5
NCI/ADR-RES	0.415	1.360	1.392	1.406	1.331	0.319	0.045	103	105	97	-23	-89	2.46E-6	6.40E-6	2.55E-5
SK-OV-3	0.802	1.967	1.907	1.861	1.696	0.870	0.156	95	91	77	6	-81	2.38E-6	1.17E-5	4.43E-5
Renal Cancer															
786-0	0.585	2.319	2.371	2.378	2.366	0.933	0.263	103	103	103	20	-55	4.34E-6	1.85E-5	8.57E-5
A498	1.476	2.186	2.120	2.223	2.263	1.228	0.399	91	105	111	-17	-73	3.00E-6	7.39E-6	3.90E-5
ACHN	0.438	2.053	2.005	2.081	1.752	0.619	0.121	97	102	81	11	-72	2.80E-6	1.36E-5	5.40E-5
CAKI-1	0.528	2.248	2.245	2.250	2.014	0.430	0.128	100	100	86	-19	-76	2.22E-6	6.66E-6	3.55E-5
RXF 393	0.851	1.536	1.484	1.479	1.425	0.671	0.095	92	92	84	-21	-89	2.10E-6	6.28E-6	2.67E-5
SN12C	0.597	2.553	2.456	2.448	2.119	0.667	0.116	95	95	78	4	-81	2.37E-6	1.10E-5	4.33E-5
TK-10	1.236	2.844	2.721	2.834	2.901	1.401	0.397	92	99	104	10	-68	3.75E-6	1.35E-5	5.90E-5
UO-31	0.406	1.544	1.325	1.326	1.142	0.416	0.017	81	81	65	1	-96	1.70E-6	1.02E-5	3.36E-5
Prostate Cancer															
PC-3	0.555	1.967	1.887	1.946	1.738	0.581	0.148	94	99	84	2	-73	2.58E-6	1.06E-5	4.89E-5
DU-145	0.333	1.240	1.238	1.284	1.217	0.487	0.102	100	105	97	17	-69	3.88E-6	1.57E-5	5.96E-5
Breast Cancer															
MCF7	0.333	1.769	1.598	1.633	1.576	0.298	0.137	88	91	87	-11	-59	2.38E-6	7.79E-6	6.52E-5
MDA-MB-231/ATCC	0.687	1.658	1.599	1.576	1.470	0.429	0.308	94	92	81	-38	-55	1.82E-6	4.81E-6	5.09E-5
HS 578T	1.315	2.317	2.088	2.154	2.057	1.055	0.882	77	84	74	-20	-33	1.81E-6	6.15E-6	> 1.00E-4
BT-549	1.105	2.062	2.017	2.139	2.085	0.854	0.351	95	108	102	-23	-68	2.62E-6	6.58E-6	3.97E-5
MDA-MB-468	0.681	1.050	1.030	1.030	0.950	0.509	0.127	95	94	73	-25	-81	1.71E-6	5.53E-6	2.76E-5

Fig. 22: Representative five dose data of compound **JOOET-13**

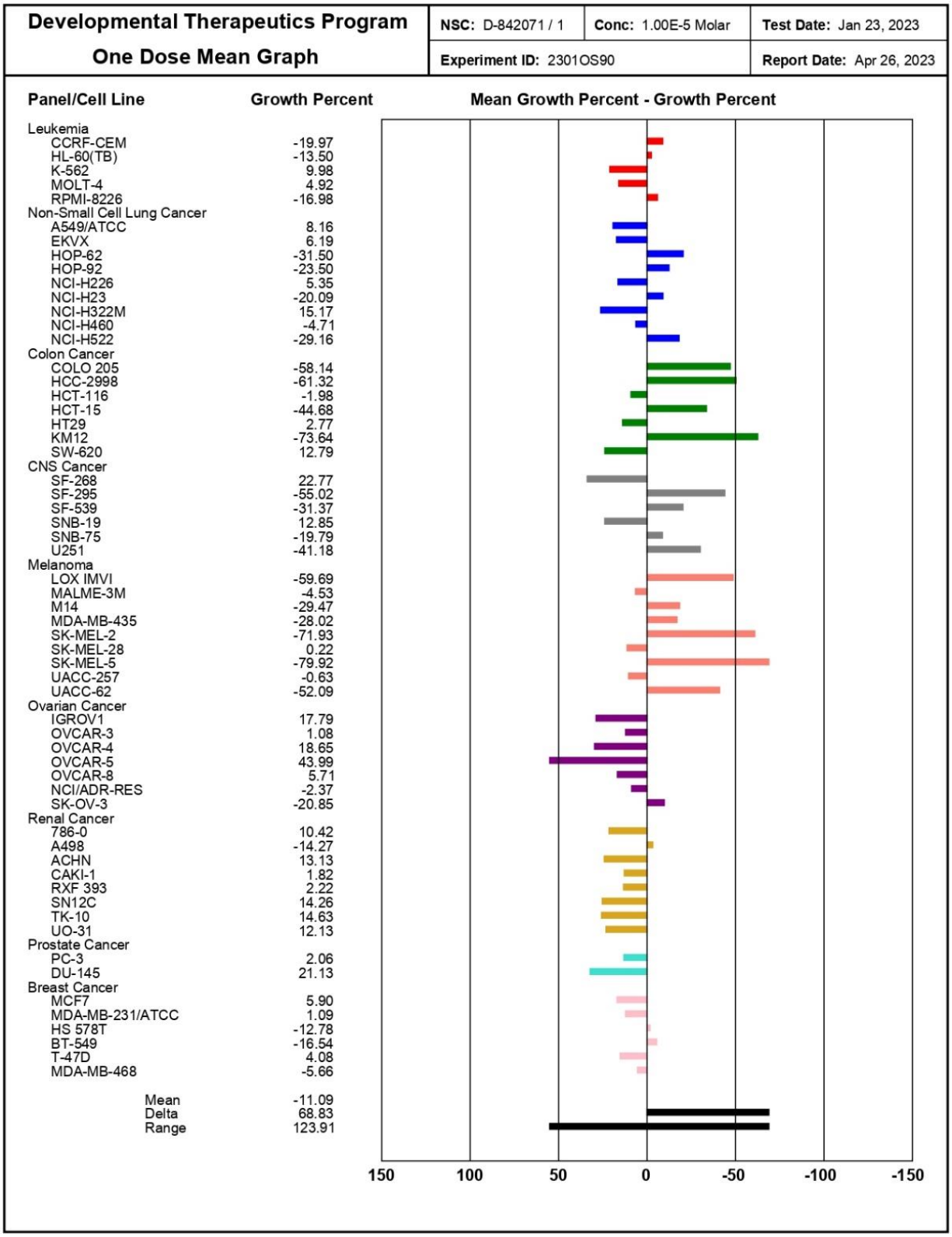


Fig. 23: Representative single dose data of compound **JOOET-14**

National Cancer Institute Developmental Therapeutics Program
In-Vitro Testing Results

NSC : D - 842071 / 1	Experiment ID : 2306NS37	Test Type : 08	Units : Molar
Report Date : July 28, 2023	Test Date : June 20, 2023	QNS :	MC :
COMI : JOOET-14	Stain Reagent : SRB Dual-Pass Related	SSPL : 1COF	

		Log10 Concentration												
	Time	Mean Optical Densities						Percent Growth						
Panel/Cell Line	Zero	Ctrl	-8.0	-7.0	-6.0	-5.0	-4.0	-8.0	-7.0	-6.0	-5.0	-4.0	GI50	TGI	LC50
Leukemia															
CCRF-CEM	0.475	1.660	1.737	1.628	1.420	0.444	0.331	106	97	80	-7	-30	2.21E-6	8.40E-6	> 1.00E-4
HL-60(TB)	0.528	1.943	1.865	1.895	1.957	0.413	0.407	95	97	101	-22	-23	2.60E-6	6.65E-6	> 1.00E-4
K-562	0.122	0.780	0.773	0.743	0.616	0.148	0.106	99	94	75	4	-13	2.25E-6	1.70E-5	> 1.00E-4
MOLT-4	0.570	2.208	2.198	2.208	1.962	0.545	0.449	99	100	85	-4	-21	2.46E-6	8.93E-6	> 1.00E-4
RPMI-8226	0.580	2.126	2.192	2.126	1.744	0.463	0.502	104	100	75	-20	-13	1.84E-6	6.14E-6	> 1.00E-4
Non-Small Cell Lung Cancer															
A549/ATCC	0.300	1.782	1.667	1.750	1.608	0.431	0.017	92	98	88	9	-95	3.03E-6	1.22E-5	3.71E-5
EKVX	0.616	2.014	1.952	1.848	1.754	0.544	0.147	96	88	81	-12	-76	2.17E-6	7.49E-6	3.93E-5
HOP-62	0.743	2.294	2.351	2.187	2.301	0.802	0.103	104	93	100	4	-86	3.33E-6	1.10E-5	3.96E-5
HOP-92	1.120	1.764	1.707	1.703	1.586	0.904	0.272	91	91	72	-19	-76	1.75E-6	6.16E-6	3.50E-5
NCI-H226	0.673	1.364	1.373	1.311	1.301	0.635	0.133	101	92	91	-6	-80	2.65E-6	8.73E-6	3.92E-5
NCI-H23	0.610	1.788	1.700	1.694	1.554	0.467	0.030	93	92	80	-24	-95	1.95E-6	5.93E-6	2.34E-5
NCI-H322M	0.732	2.116	1.972	2.125	2.099	1.013	0.032	90	101	99	20	-96	4.18E-6	1.50E-5	4.04E-5
NCI-H460	0.237	2.064	2.199	2.127	2.004	0.256	0.020	107	103	97	1	-92	3.08E-6	1.03E-5	3.56E-5
NCI-H522	0.702	2.113	2.047	2.039	1.900	0.647	0.120	95	95	85	-8	-83	2.38E-6	8.22E-6	3.64E-5
Colon Cancer															
COLO 205	0.489	2.149	2.108	2.239	2.224	0.378	0.121	98	105	105	-23	-75	2.68E-6	6.62E-6	3.30E-5
HCC-2998	0.669	2.137	2.054	2.042	2.044	0.436	-0.002	94	93	94	-35	-100	2.19E-6	5.36E-6	1.71E-5
HCT-116	0.233	2.166	2.081	2.209	1.886	0.266	0.037	96	102	86	2	-84	2.65E-6	1.05E-5	3.99E-5
HCT-15	0.260	1.829	1.685	1.672	1.506	0.158	0.001	91	90	79	-39	-100	1.77E-6	4.67E-6	1.51E-5
HT29	0.212	1.441	1.466	1.444	1.457	0.312	0.041	102	100	101	8	-81	3.55E-6	1.23E-5	4.50E-5
KM12	0.453	1.414	1.401	1.408	1.319	0.367	0.004	99	99	90	-19	-99	2.33E-6	6.70E-6	2.44E-5
SW-620	0.244	1.663	1.625	1.630	1.613	0.439	0.032	97	98	97	14	-87	3.65E-6	1.37E-5	4.29E-5
CNS Cancer															
SF-268	0.779	2.202	2.229	2.145	1.974	1.033	0.146	102	96	84	18	-81	3.26E-6	1.51E-5	4.84E-5
SF-295	0.957	2.774	2.769	2.672	2.553	0.316	0.014	100	94	88	-67	-99	1.76E-6	3.69E-6	7.77E-6
SF-539	0.789	2.621	2.491	2.494	2.469	0.677	0.172	93	93	92	-14	-78	2.48E-6	7.34E-6	3.63E-5
SNB-19	0.726	2.658	2.541	2.491	2.432	1.095	0.114	94	91	88	19	-84	3.58E-6	1.53E-5	4.66E-5
SNB-75	1.022	2.095	2.139	2.052	1.953	1.145	0.110	104	96	87	11	-89	3.07E-6	1.30E-5	4.08E-5
U251	0.319	1.714	1.670	1.713	1.575	0.360	0.016	97	100	90	3	-95	2.88E-6	1.07E-5	3.46E-5
Melanoma															
LOX IMVI	0.248	1.788	1.719	1.656	1.657	0.168	-0.008	96	91	91	-32	-100	2.16E-6	5.47E-6	1.82E-5
MALME-3M	0.517	1.221	1.200	1.177	1.178	0.569	0.080	97	94	94	7	-85	3.22E-6	1.20E-5	4.20E-5
M14	0.493	2.065	1.987	1.969	1.980	0.470	0.096	95	94	95	-5	-81	2.81E-6	8.97E-6	3.96E-5
MDA-MB-435	0.588	2.143	2.049	1.992	1.905	0.579	0.031	94	90	85	-2	-95	2.52E-6	9.60E-6	3.31E-5
SK-MEL-2	1.303	3.116	3.093	3.074	2.877	0.439	0.098	99	98	87	-66	-93	1.74E-6	3.69E-6	7.82E-6
SK-MEL-28	0.607	1.862	1.832	1.759	1.786	0.918	0.186	98	92	94	25	-69	4.32E-6	1.83E-5	6.23E-5
SK-MEL-5	0.723	2.579	2.513	2.439	2.137	0.118	0.004	96	92	76	-84	-100	1.46E-6	3.00E-6	6.16E-6
UACC-257	1.026	2.456	2.420	2.373	2.270	0.915	0.190	97	94	87	-11	-81	2.39E-6	7.74E-6	3.58E-5
Ovarian Cancer															
IGROV1	0.360	1.731	1.795	1.820	1.682	0.732	0.235	105	106	96	27	-35	4.67E-6	2.74E-5	> 1.00E-4
OVCAR-3	0.545	2.314	2.197	2.150	2.118	0.481	0.015	93	91	89	-12	-97	2.44E-6	7.64E-6	2.80E-5
OVCAR-4	0.758	1.806	1.783	1.958	1.710	0.982	0.574	98	115	91	21	-24	3.87E-6	2.94E-5	> 1.00E-4
OVCAR-5	0.626	1.873	1.771	1.754	1.821	0.891	0.139	92	90	96	21	-78	4.12E-6	1.64E-5	5.24E-5
OVCAR-8	0.398	1.868	1.877	1.912	1.770	0.506	0.014	101	103	93	7	-97	3.19E-6	1.18E-5	3.56E-5
NCI/ADR-RES	0.415	1.350	1.322	1.331	1.290	0.393	0.014	97	98	94	-5	-97	2.76E-6	8.84E-6	3.08E-5
SK-OV-3	0.802	2.186	2.116	1.968	2.045	0.787	0.152	95	84	90	-2	-81	2.72E-6	9.54E-6	4.05E-5
Renal Cancer															
786-0	0.585	2.424	2.341	2.226	2.413	1.028	0.083	95	89	99	24	-86	4.53E-6	1.66E-5	4.72E-5
A498	1.476	2.240	2.266	2.306	2.239	1.364	0.288	103	109	100	-8	-80	2.91E-6	8.50E-6	3.82E-5
ACHN	0.438	2.039	2.073	2.059	1.933	0.679	0.103	102	101	93	15	-76	3.58E-6	1.46E-5	5.14E-5
CAKI-1	0.528	2.345	2.264	2.288	2.195	0.423	0.046	96	97	92	-20	-91	2.36E-6	6.62E-6	2.63E-5
RXF 393	0.851	1.572	1.606	1.545	1.454	0.831	0.062	105	96	84	-2	-93	2.46E-6	9.39E-6	3.37E-5
SN12C	0.597	2.560	2.503	2.574	2.304	0.834	0.224	97	101	87	12	-62	3.12E-6	1.45E-5	6.80E-5
TK-10	1.236	2.903	2.828	2.814	2.719	1.577	0.332	96	95	89	20	-73	3.70E-6	1.65E-5	5.65E-5
UO-31	0.406	1.495	1.316	1.287	1.223	0.485	0.008	84	81	75	7	-98	2.34E-6	1.17E-5	3.50E-5
Prostate Cancer															
PC-3	0.555	1.919	1.865	1.873	1.689	0.590	0.215	96	97	83	3	-61	2.58E-6	1.10E-5	6.66E-5
DU-145	0.333	1.304	1.372	1.336	1.303	0.575	0.109	107	103	100	25	-67	4.63E-6	1.86E-5	6.48E-5
Breast Cancer															
MCF7	0.333	1.826	1.729	1.621	1.515	0.338	0.052	94	86	79	0	-84	2.34E-6	1.01E-5	3.93E-5
MDA-MB-231/ATCC	0.687	1.779	1.803	1.672	1.788	0.571	0.296	102	90	101	-17	-57	2.70E-6	7.19E-6	6.72E-5
HS 578T	1.315	2.383	2.270	2.257	2.158	1.195	0.984	89	88	79	-9	-25	2.13E-6	7.87E-6	> 1.00E-4
BT-549	1.105	2.086	1.993	1.940	1.922	0.785	0.192	91	85	83	-29	-83	1.98E-6	5.52E-6	2.46E-5
MDA-MB-468	0.681	1.058	1.055	1.018	0.951	0.555	0.106	99	89	72	-19	-85	1.74E-6	6.23E-6	3.00E-5

Fig. 24: Representative five dose data of compound **JOOET-14**

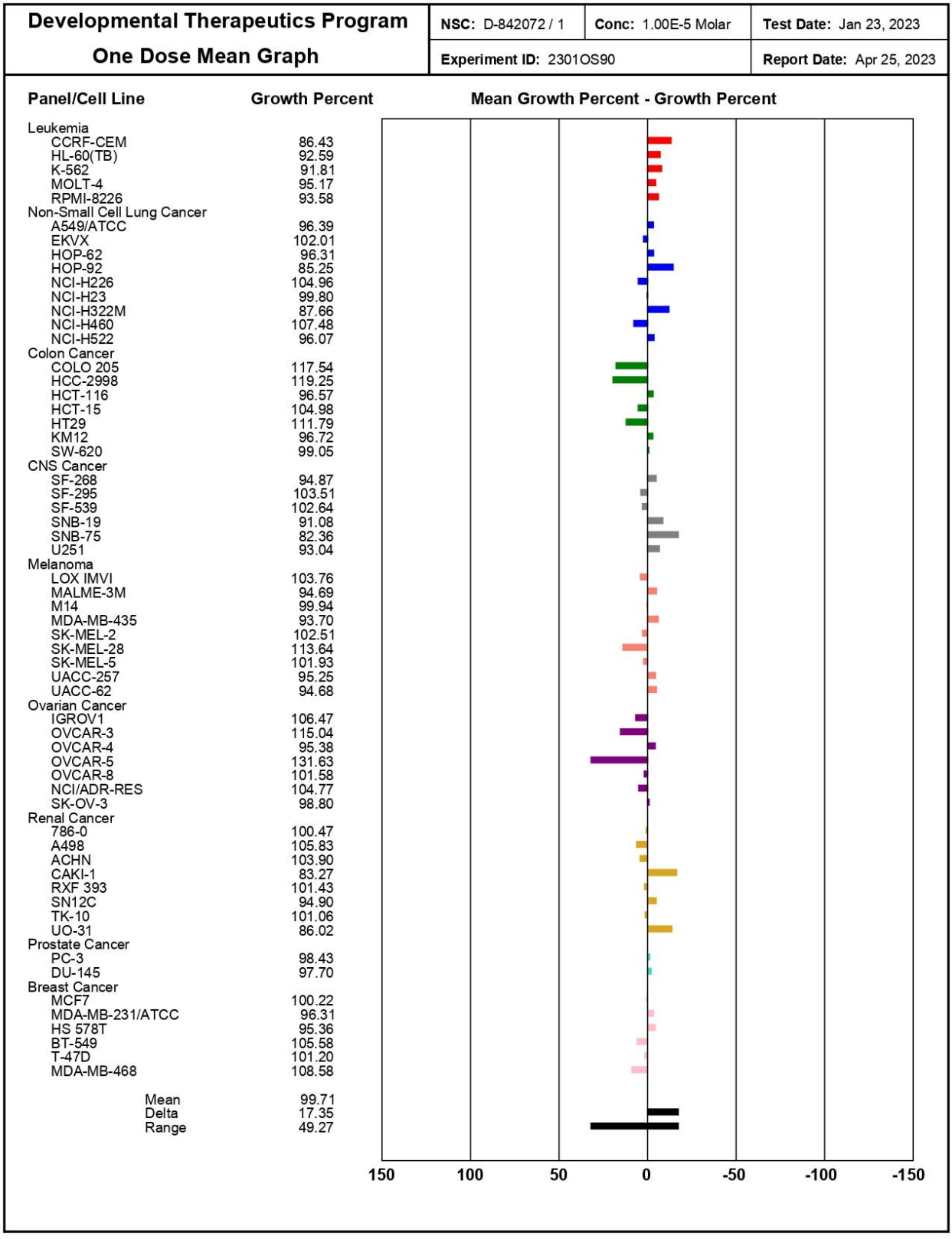


Fig. 25: Representative single dose data of compound **JOOET-15**

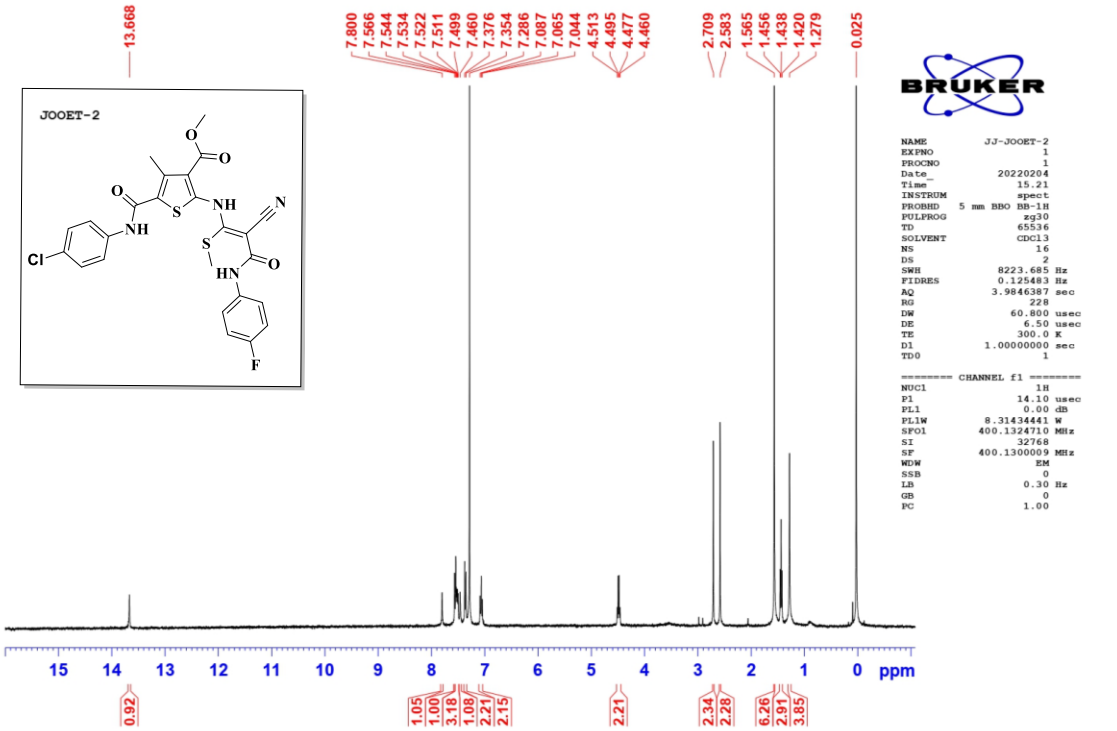


Fig. 26: Representative ^{1}H NMR spectrum of compound **JOOET-2**

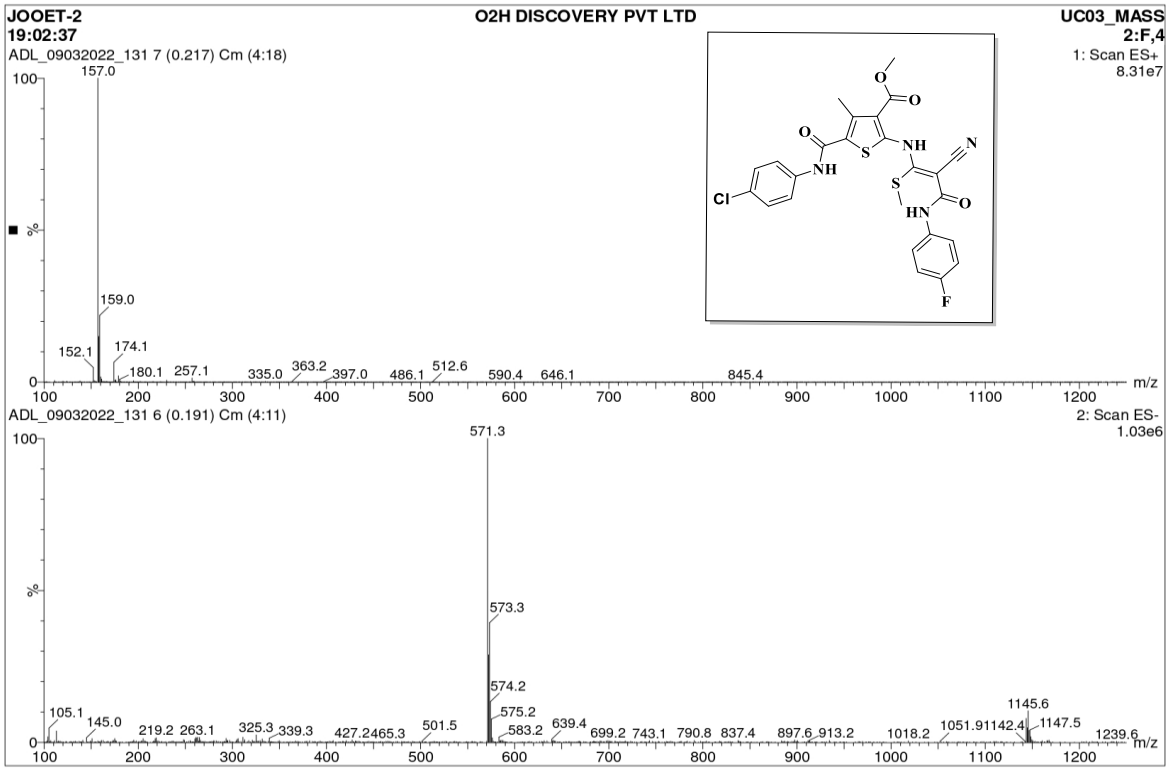


Fig. 27: Representative mass spectrum of compound **JOOET-2**

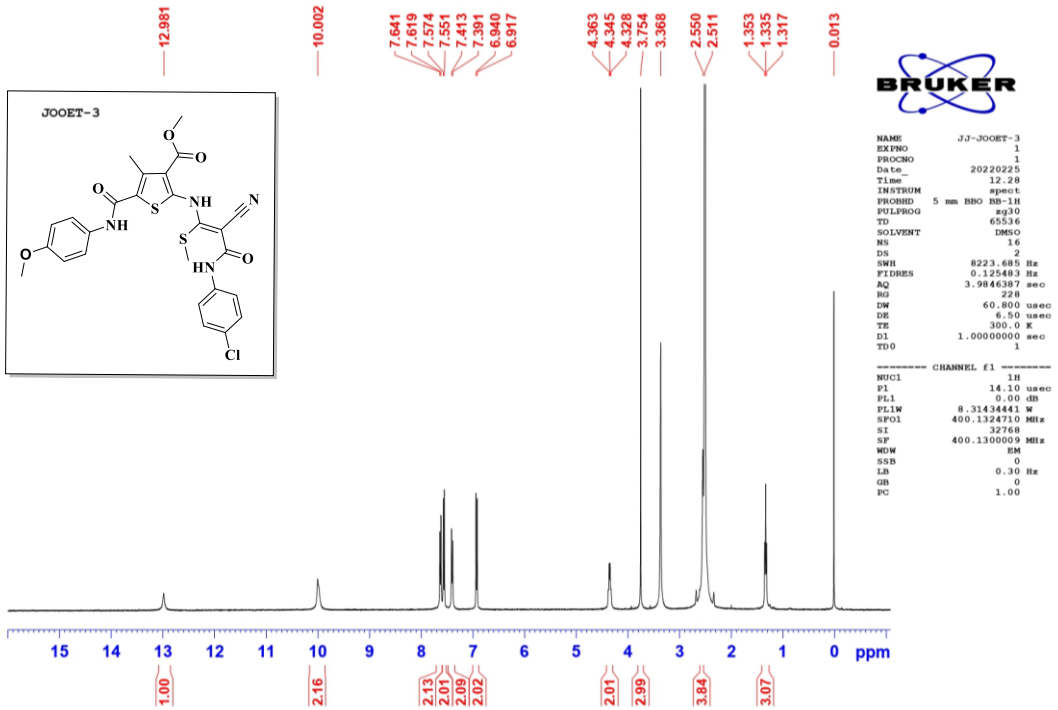


Fig. 28: Representative ^{1}H NMR spectrum of compound **JOOET-3**

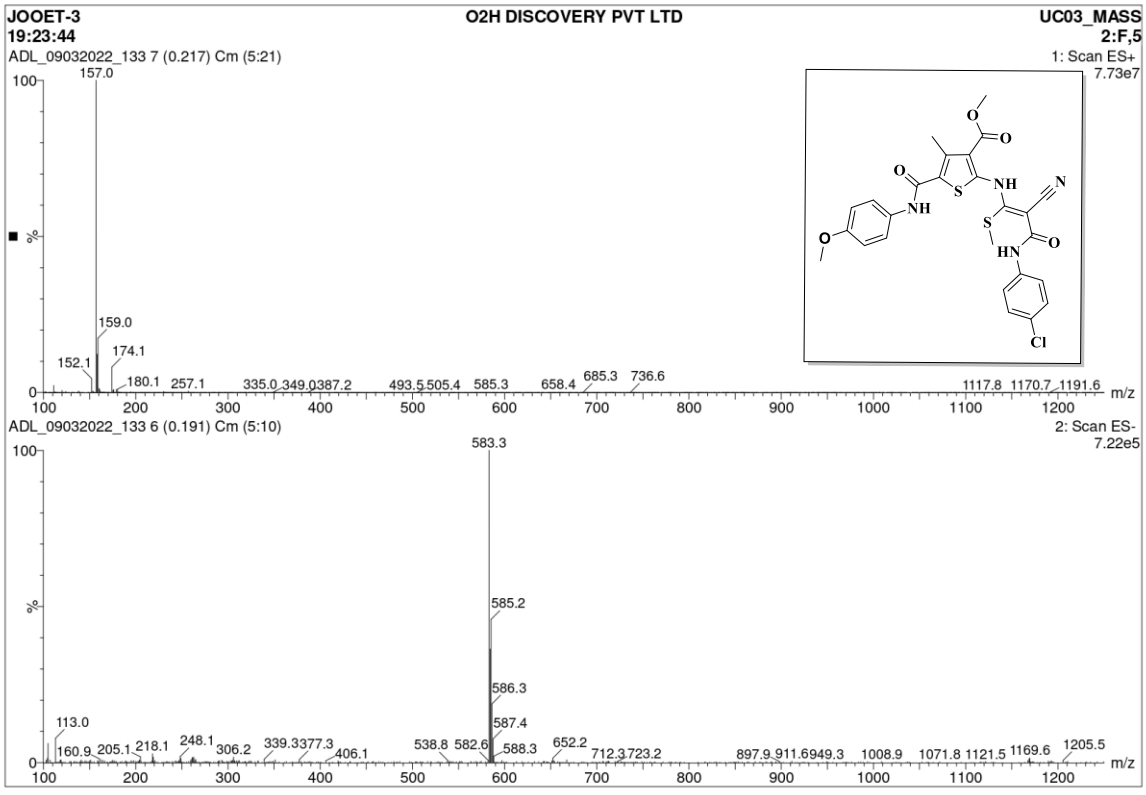


Fig. 29: Representative mass spectrum of compound **JOOET-3**

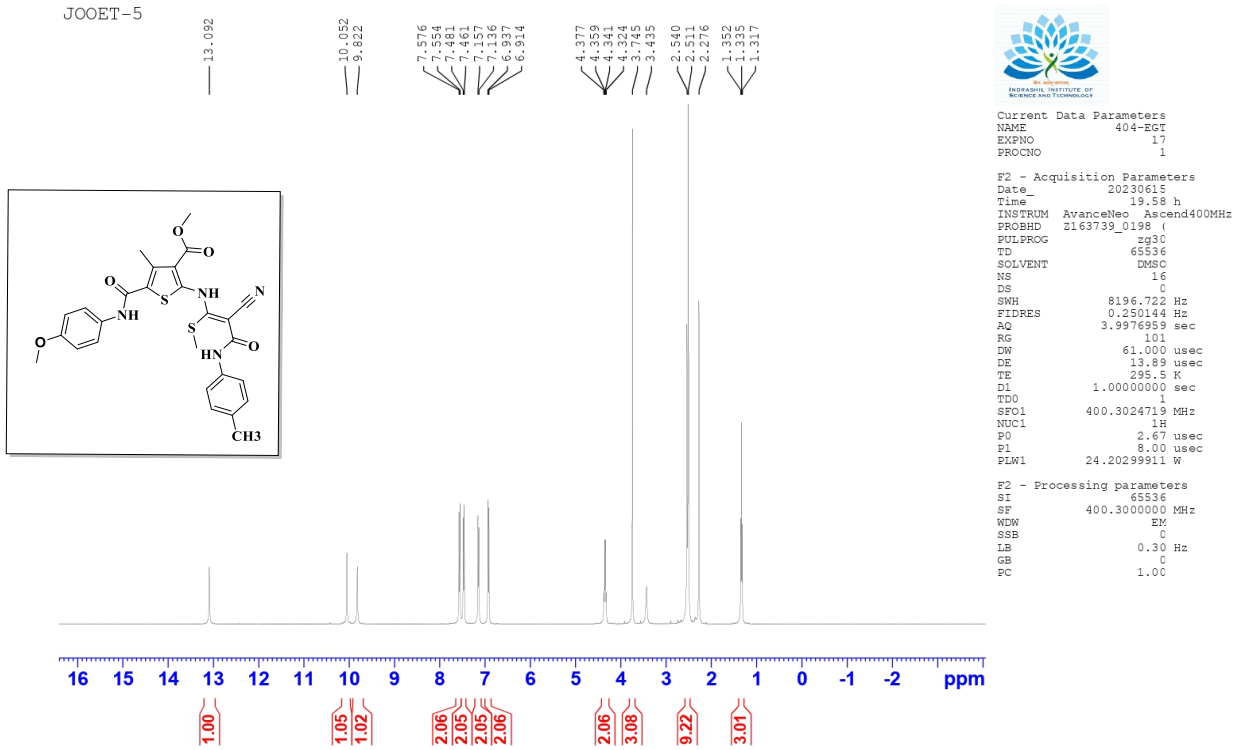


Fig. 30: Representative ^{1}H NMR spectrum of compound **JOOET-5**

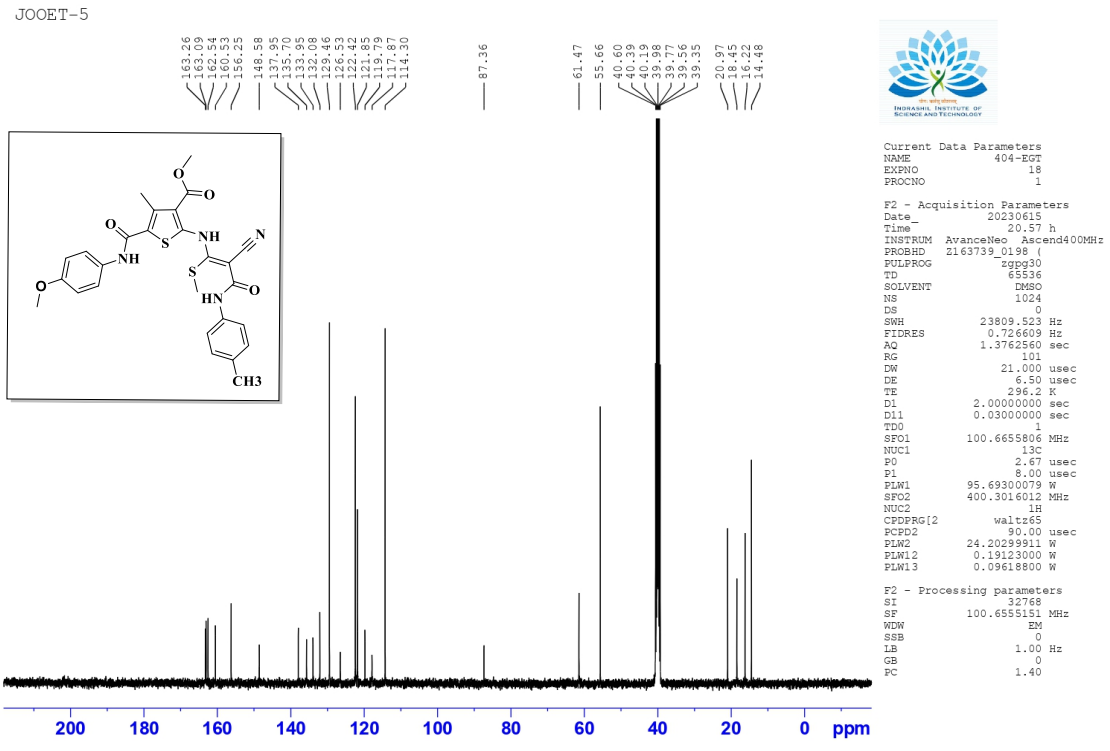


Fig. 31: Representative ^{13}C NMR spectrum of compound **JOOET-5**

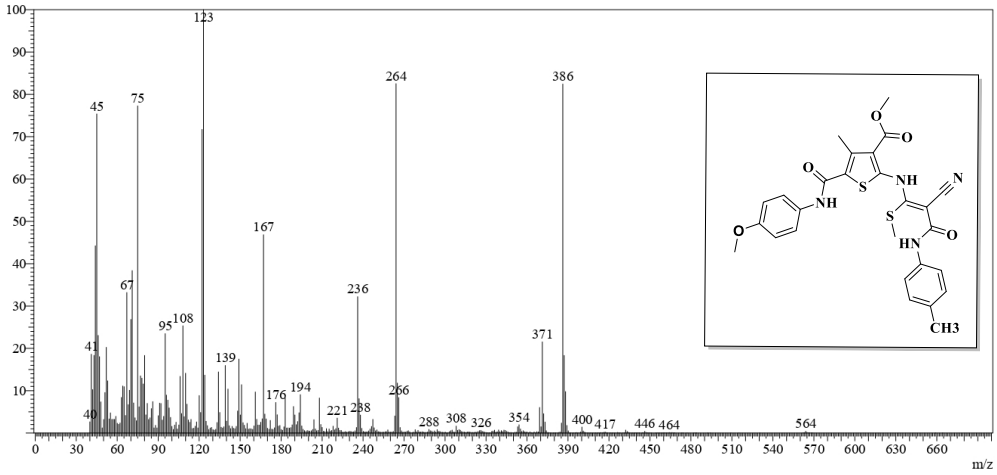


Fig. 32: Representative mass spectrum of compound **JOOET-5**

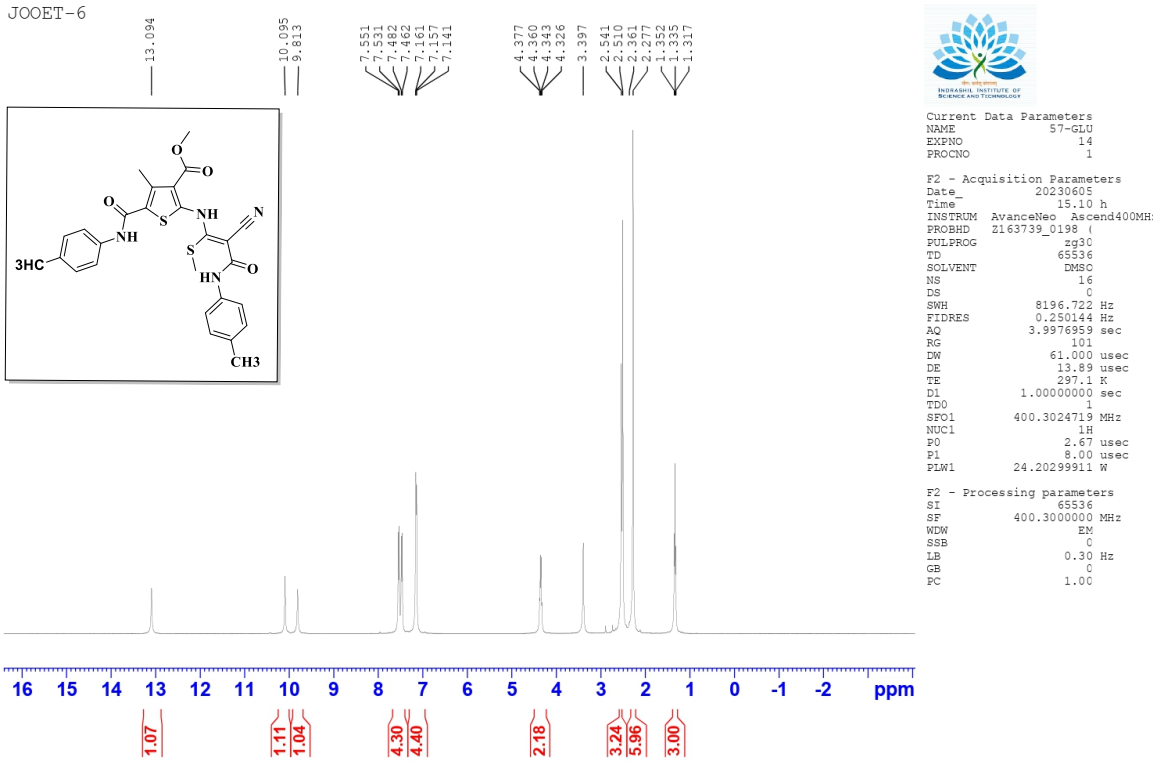


Fig. 33: Representative ^{1}H NMR spectrum of compound **JOOET-6**

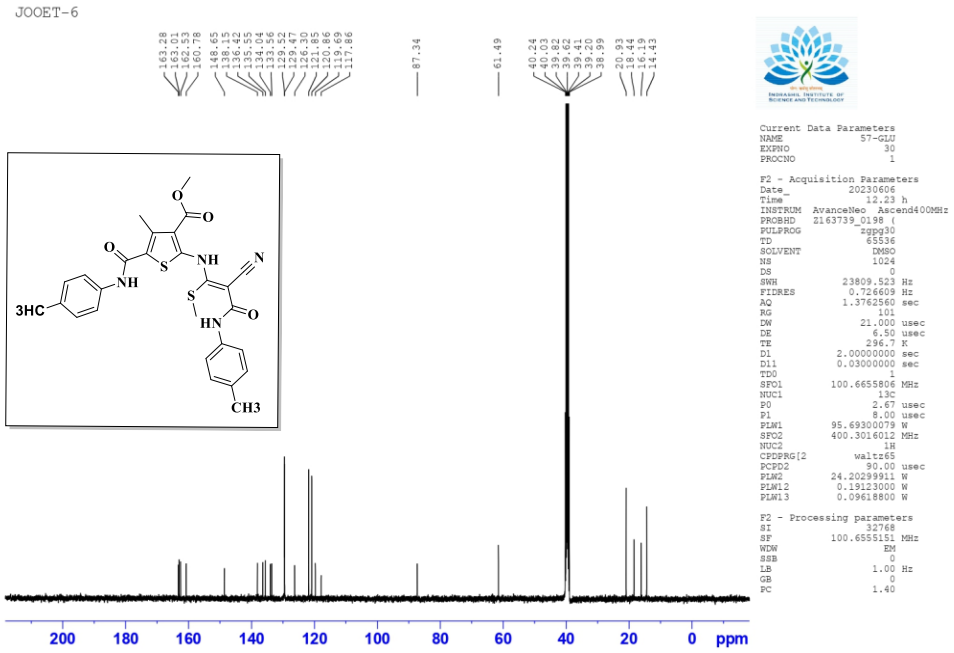


Fig. 34: Representative ^{13}C NMR spectrum of compound **JOOET-6**

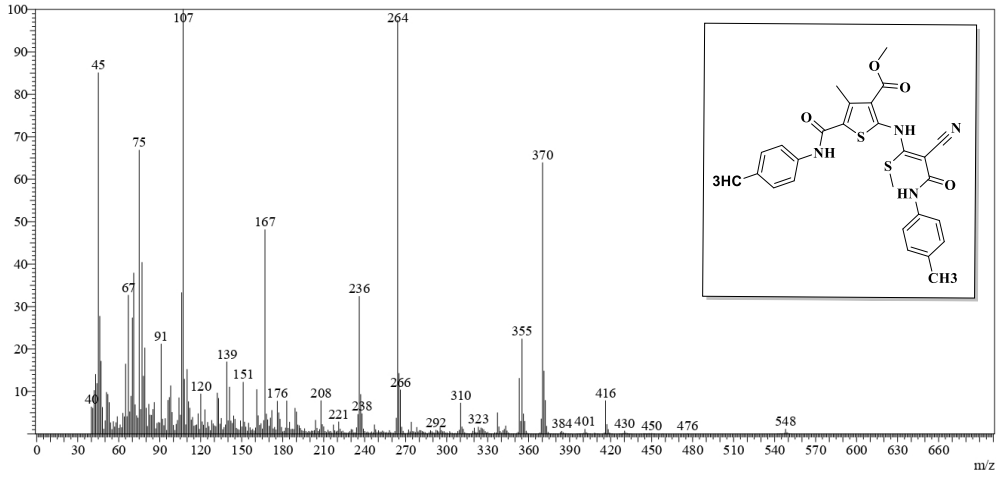


Fig. 35: Representative mass spectrum of compound **JOOET-6**

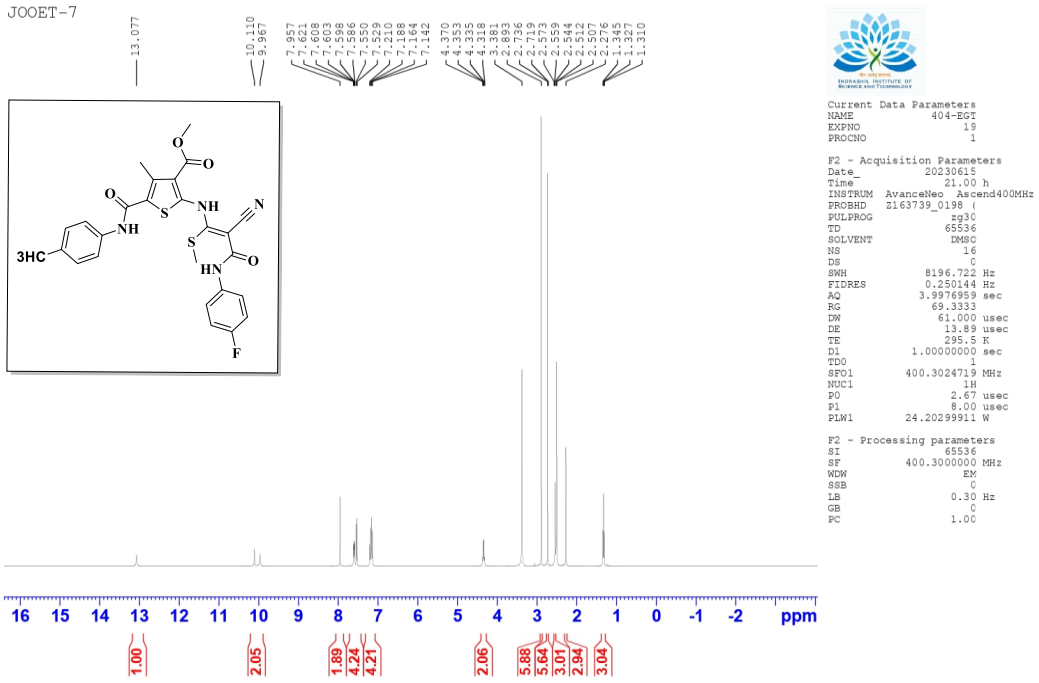


Fig. 36: Representative ^{1}H NMR spectrum of compound **JOOET-7**

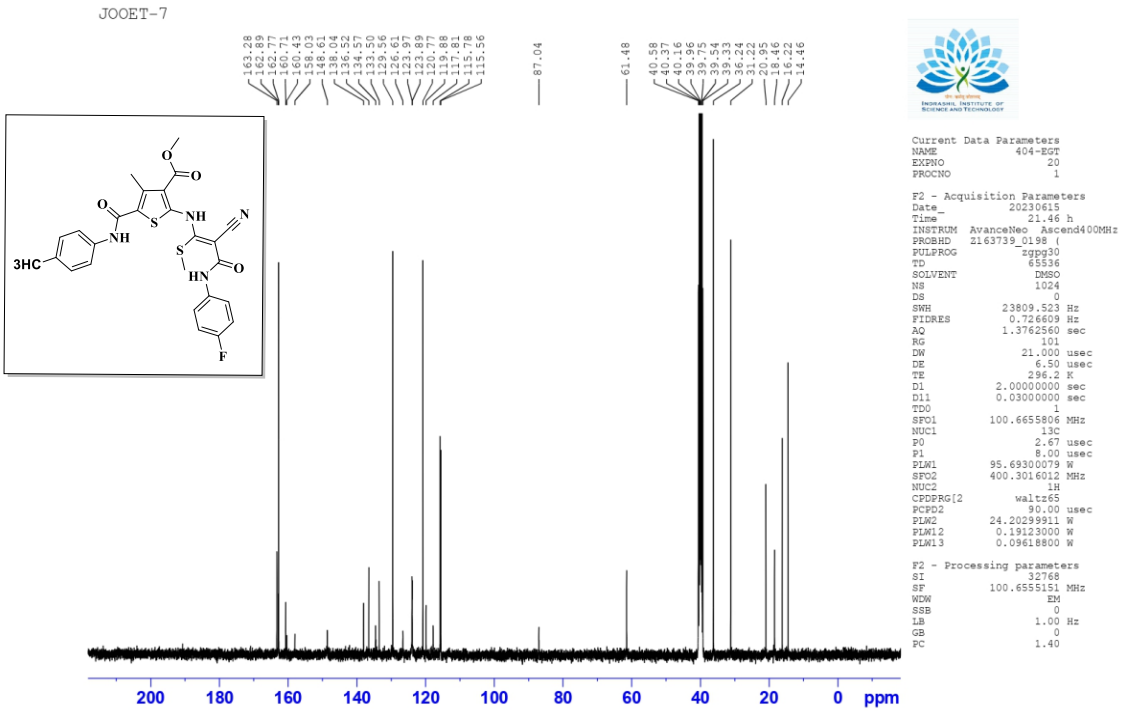


Fig. 37: Representative ^{13}C NMR spectrum of compound **JOOET-7**

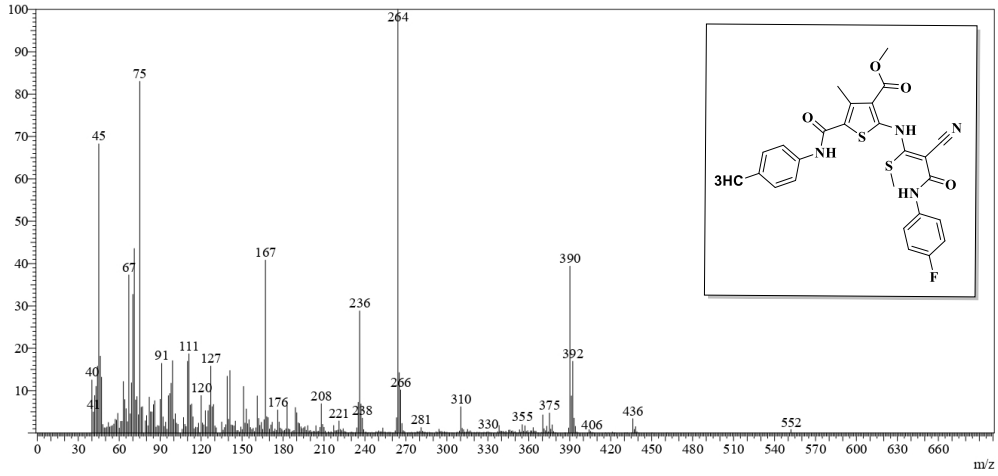


Fig. 38: Representative mass spectrum of compound **JOOET-7**

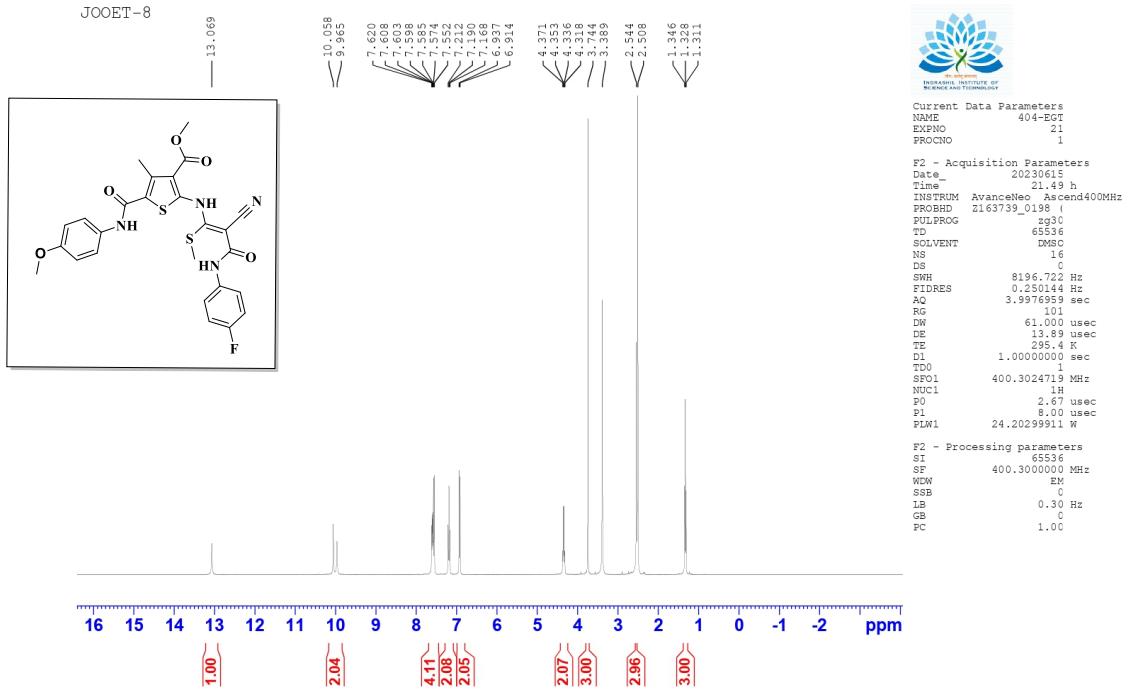


Fig. 39: Representative ^{1}H NMR spectrum of compound **JOOET-8**

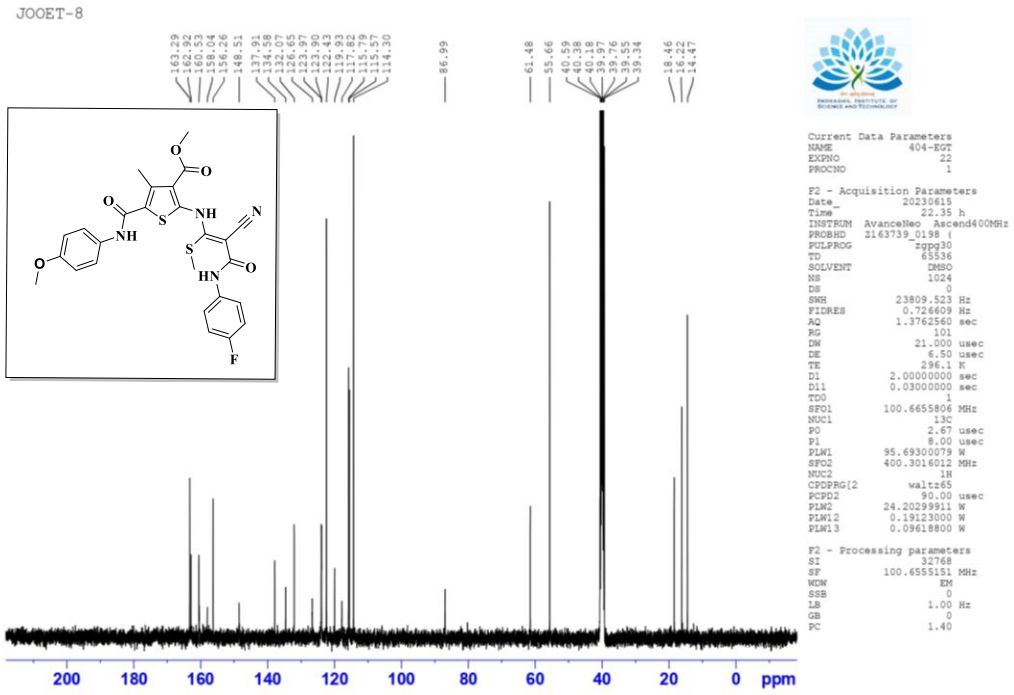


Fig. 40: Representative ^{13}C NMR spectrum of compound **JOOET-8**

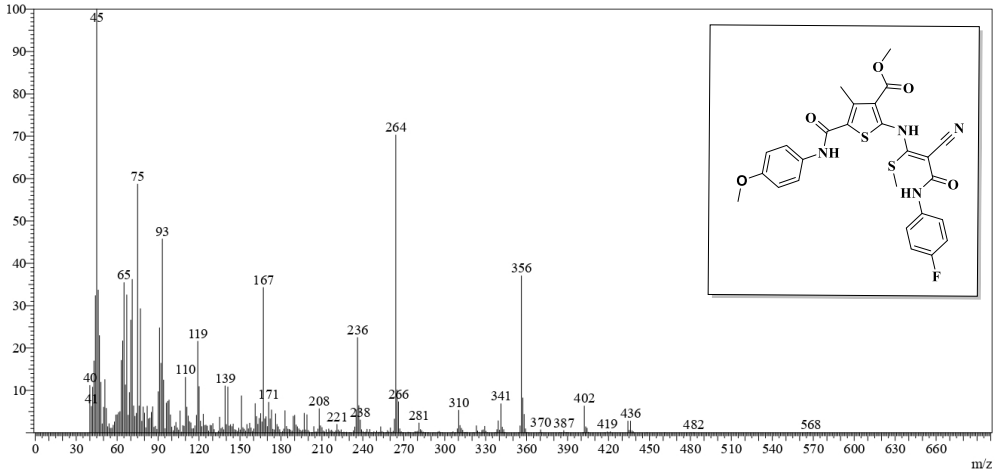


Fig. 41: Representative mass spectrum of compound **JOOET-8**

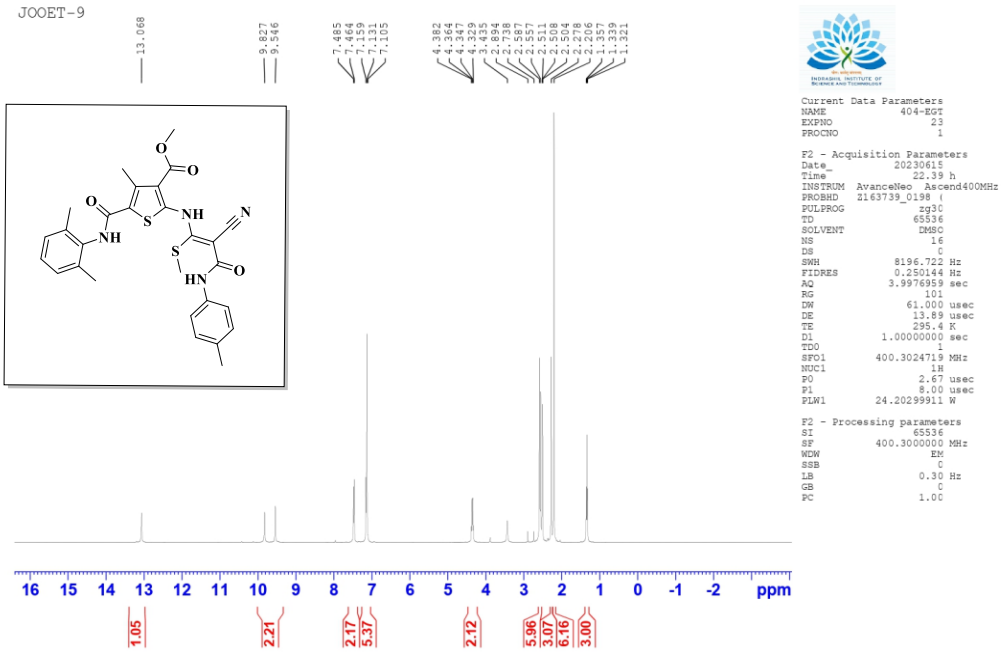


Fig. 42: Representative ^{1}H NMR spectrum of compound **JOOET-9**

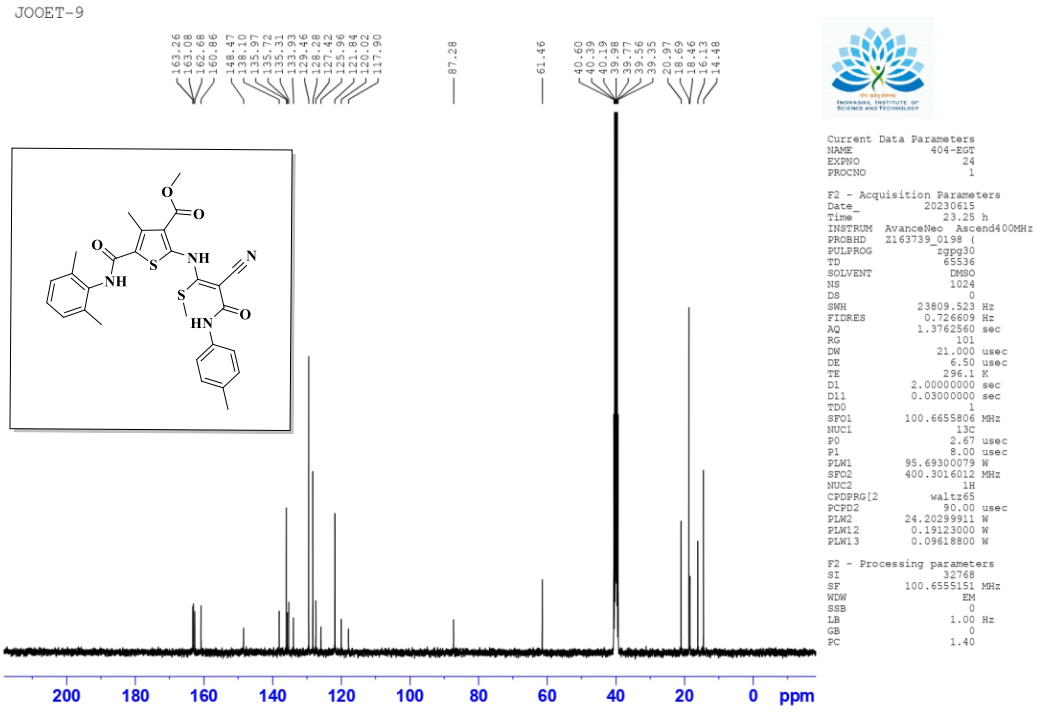


Fig. 43: Representative ^{13}C NMR spectrum of compound **JOOET-9**

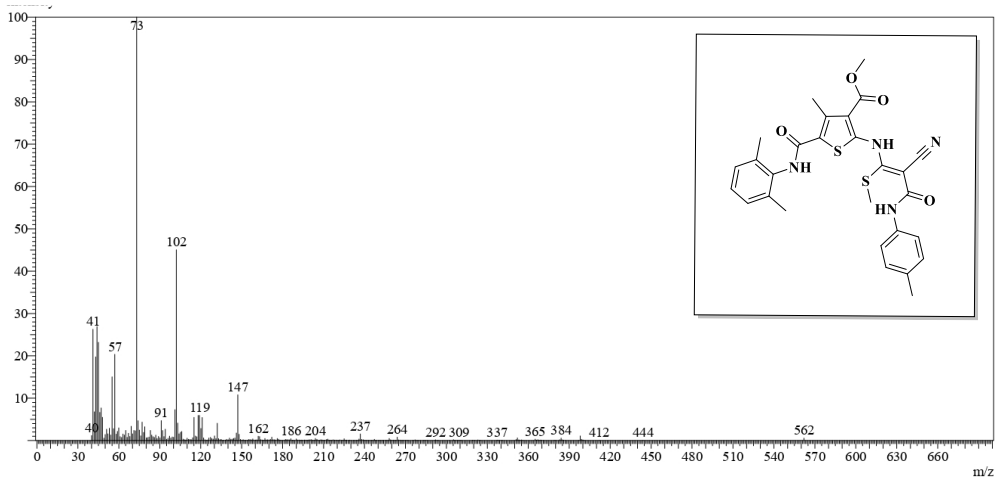


Fig. 44: Representative mass spectrum of compound **JOOET-9**

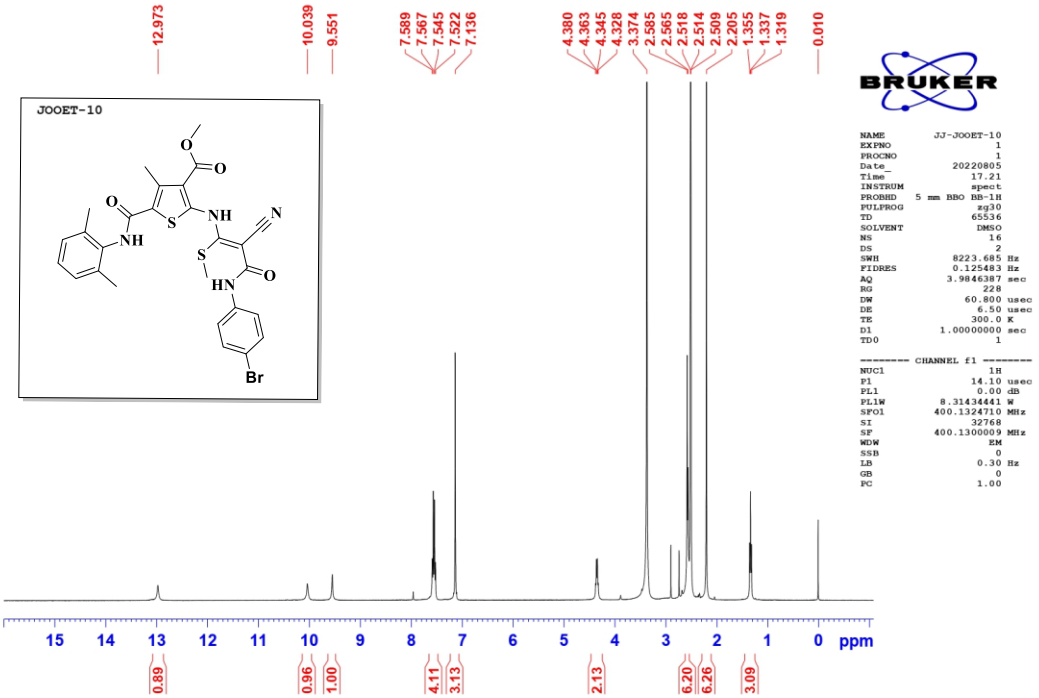


Fig. 45: Representative ^{1}H NMR spectrum of compound **JOOET-10**

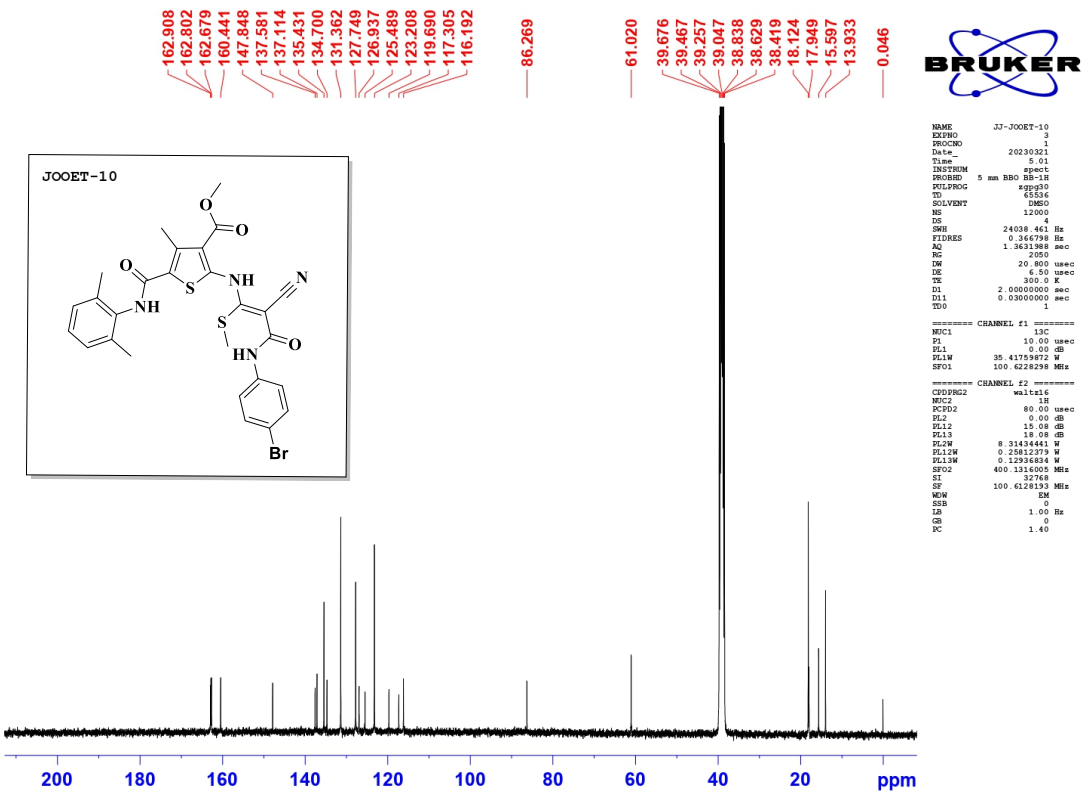


Fig. 46: Representative ^{13}C NMR spectrum of compound **JOOET-10**

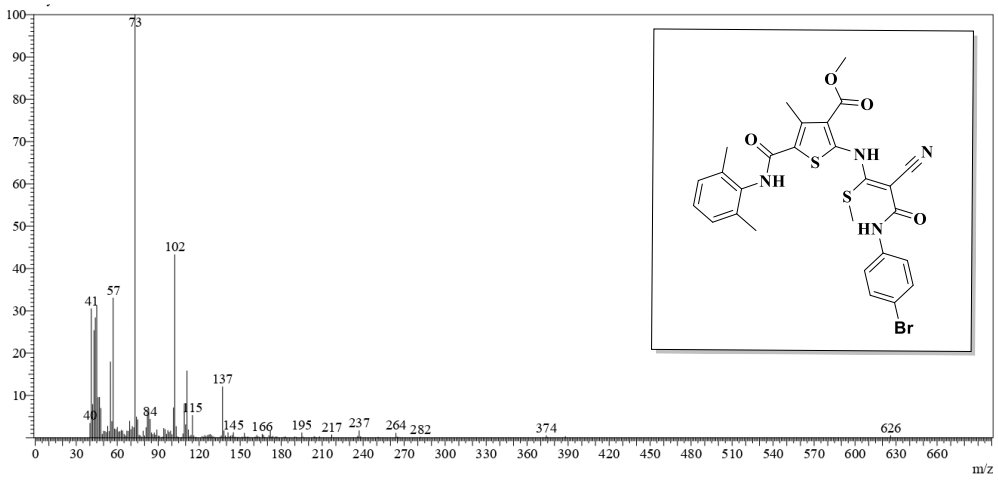


Fig. 47: Representative mass spectrum of compound **JOOET-10**

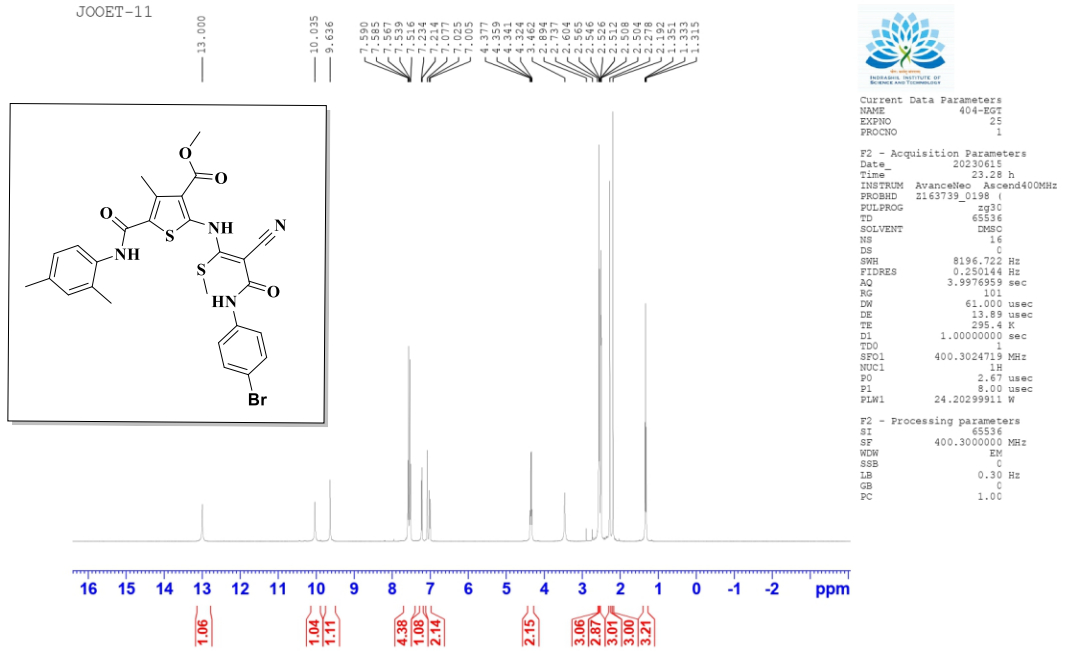


Fig. 48: Representative ^{1}H NMR spectrum of compound **JOOET-11**

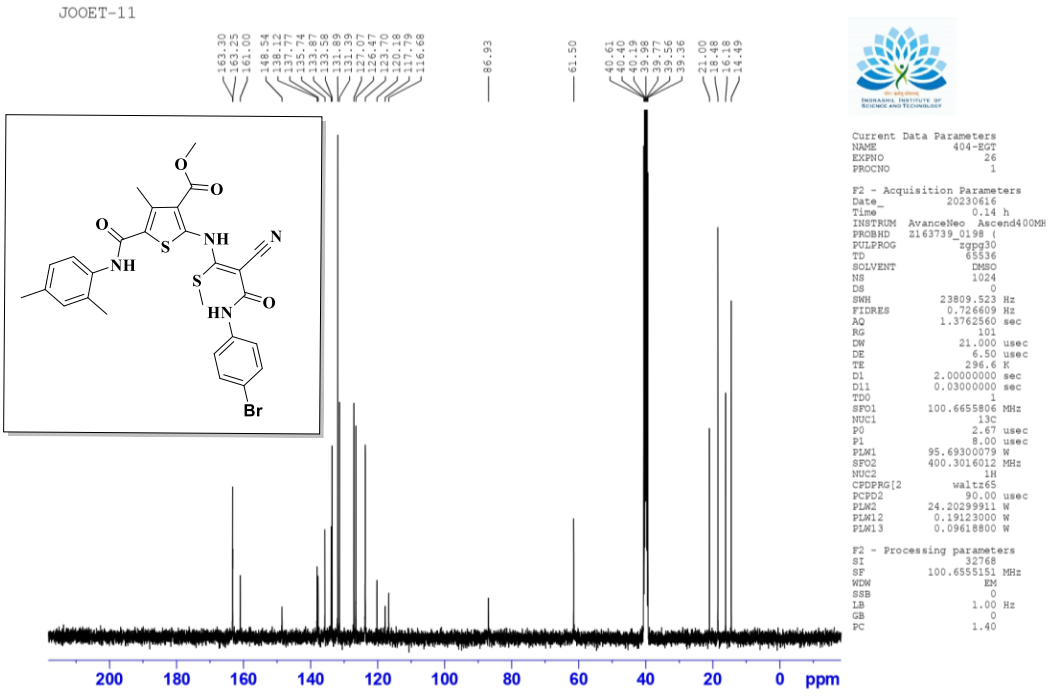


Fig. 49: Representative ^{13}C NMR spectrum of compound **JOOET-11**

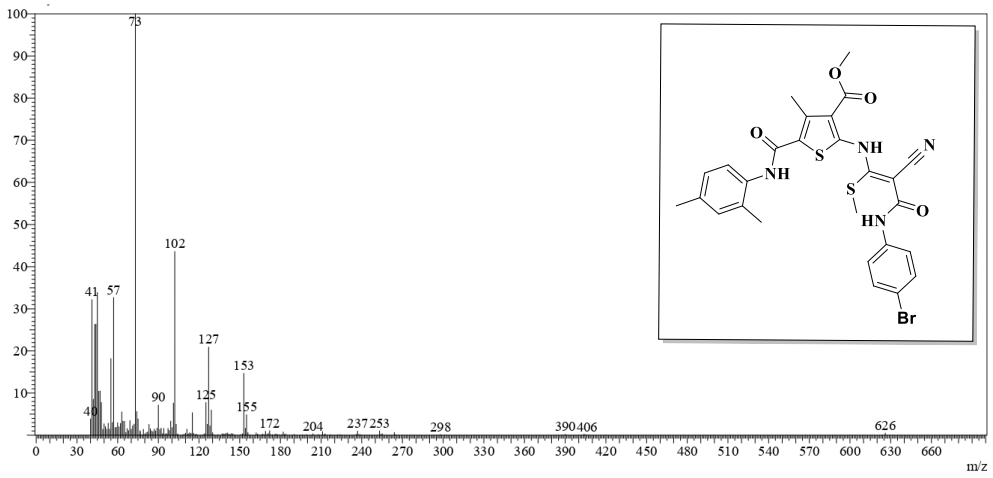


Fig. 50: Representative mass spectrum of compound **JOOET-11**

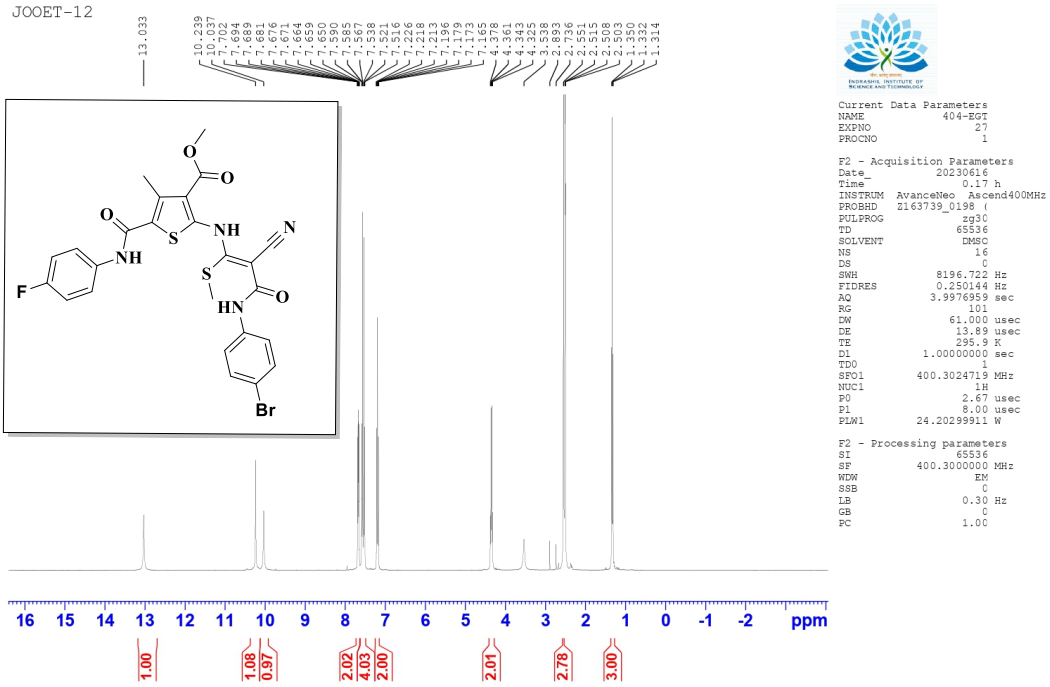


Fig. 51: Representative ^{1}H NMR spectrum of compound **JOOET-12**

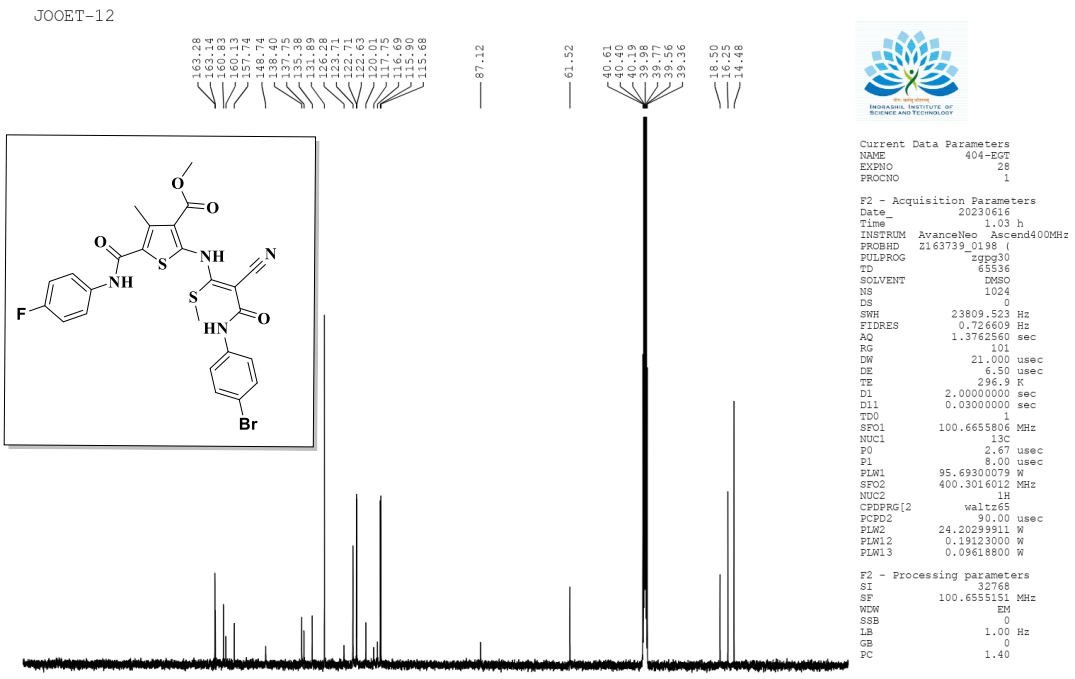


Fig. 52: Representative ^{13}C NMR spectrum of compound **JOOET-12**

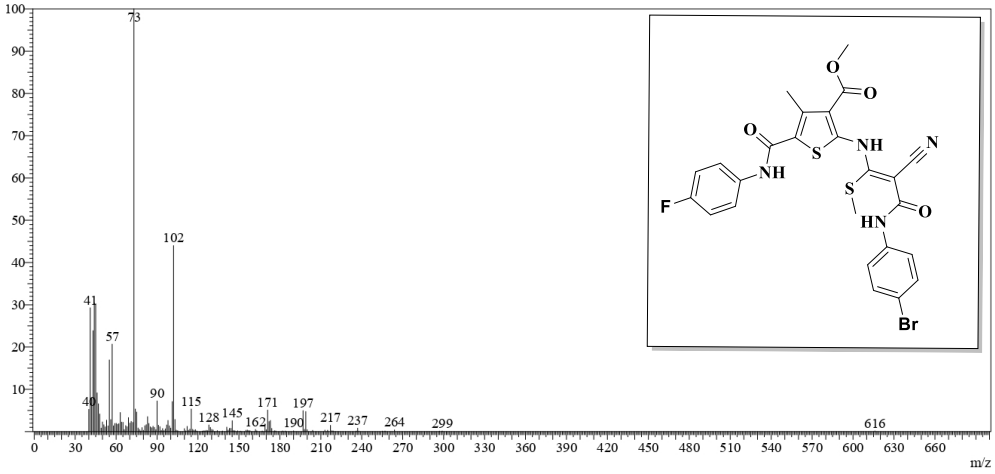


Fig. 53: Representative mass spectrum of compound **JOOET-12**

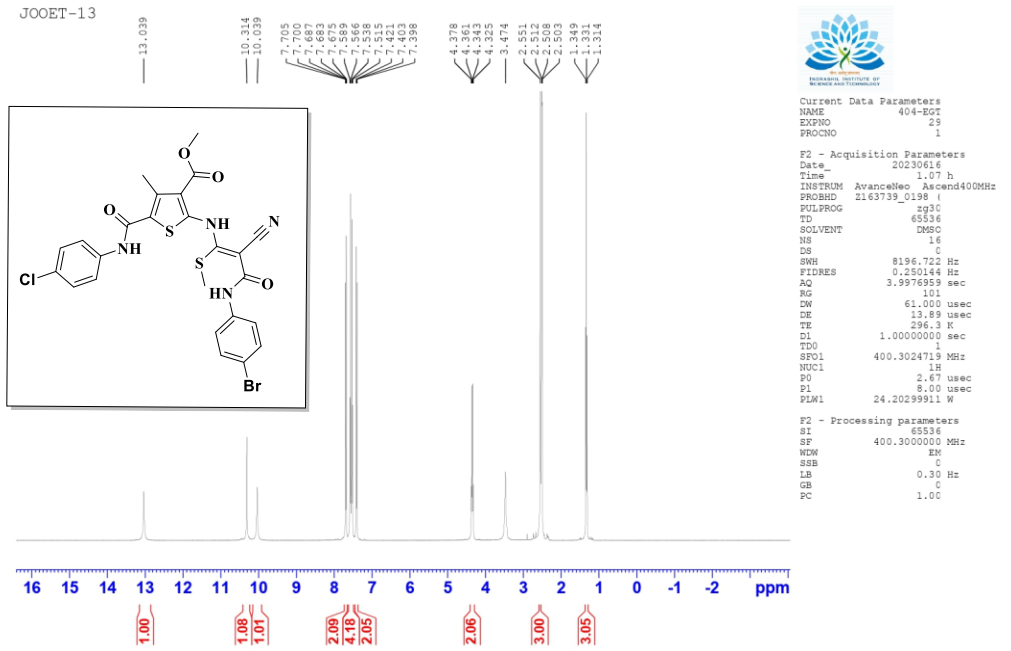


Fig. 54: Representative ^{1}H NMR spectrum of compound **JOOET-13**

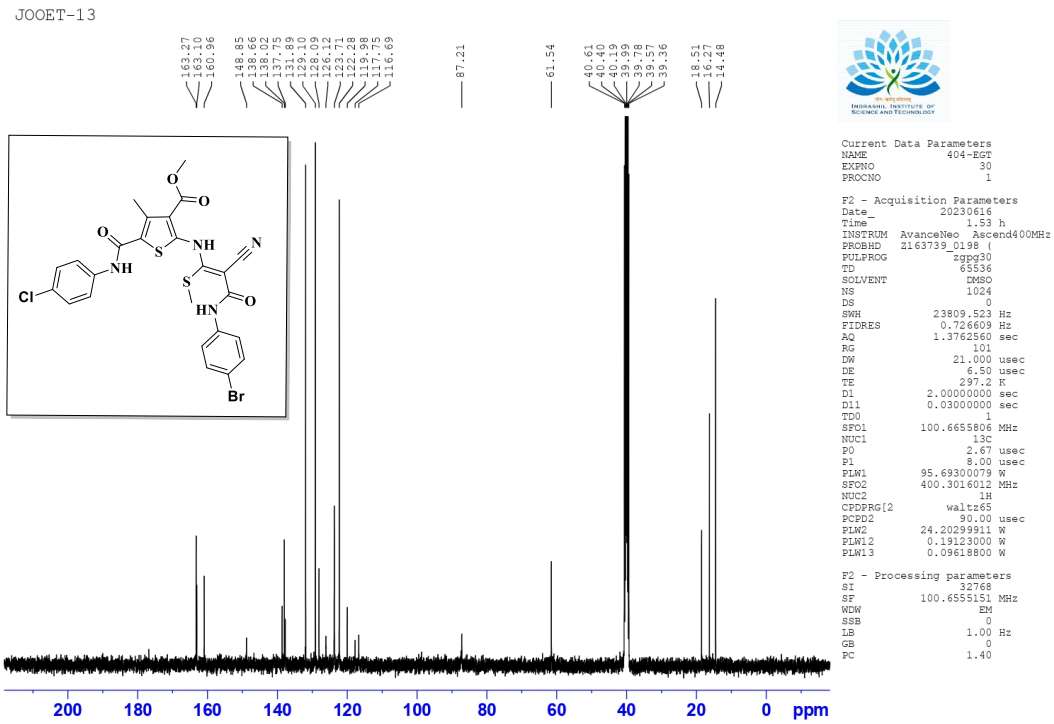


Fig. 55: Representative ^{13}C NMR spectrum of compound **JOOET-13**

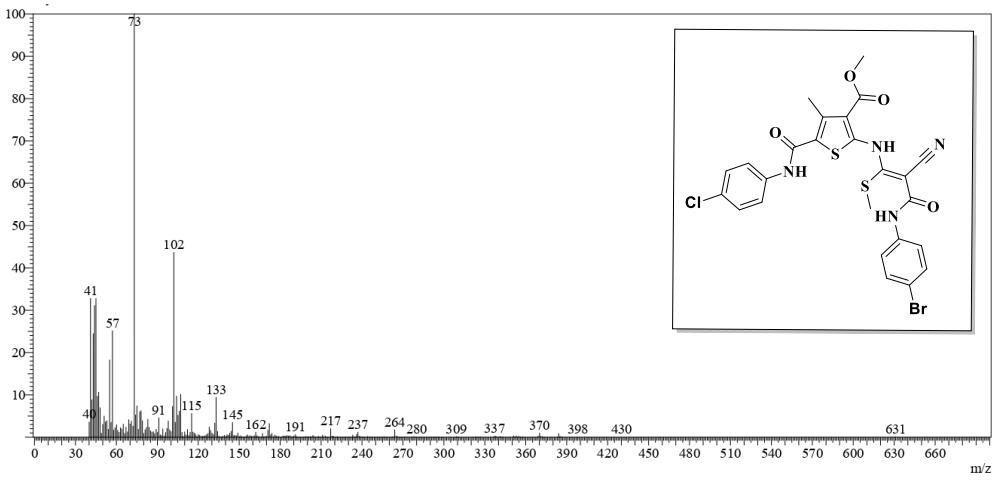


Fig. 56: Representative mass spectrum of compound **JOOET-13**

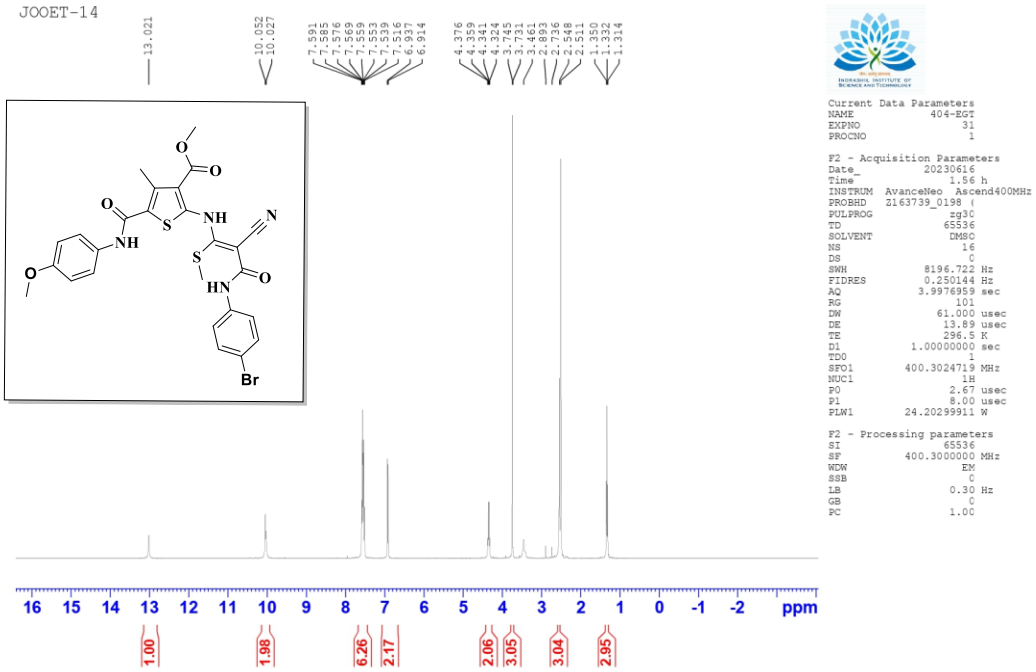


Fig. 57: Representative ^{1}H NMR spectrum of compound **JOOET-14**

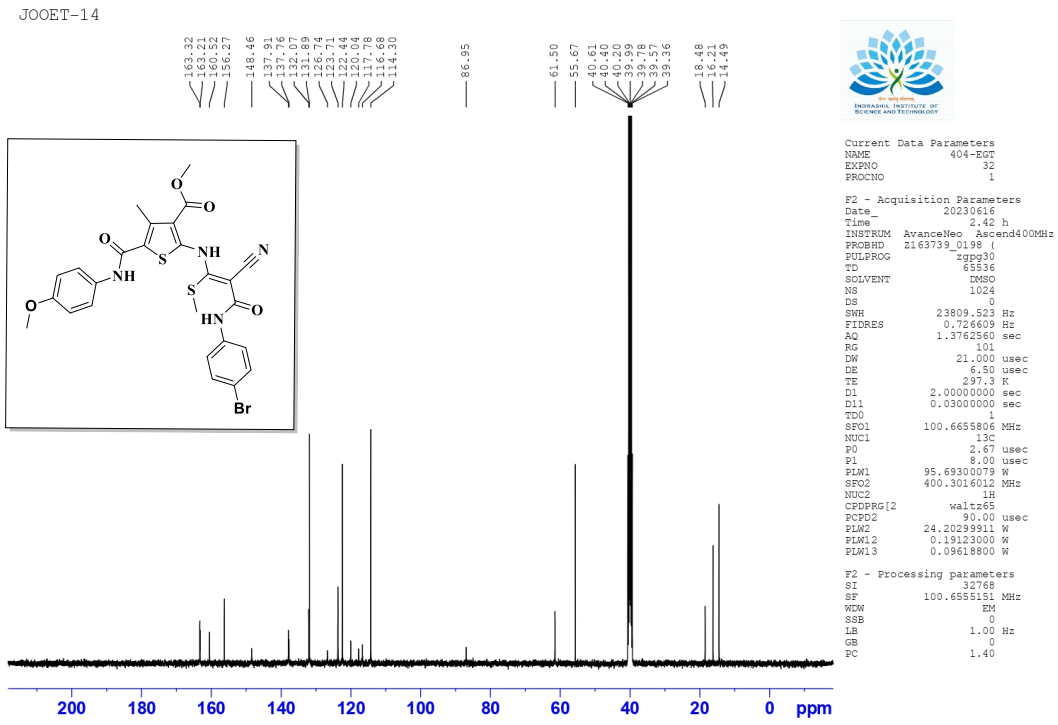


Fig. 58: Representative ^{13}C NMR spectrum of compound **JOOET-14**

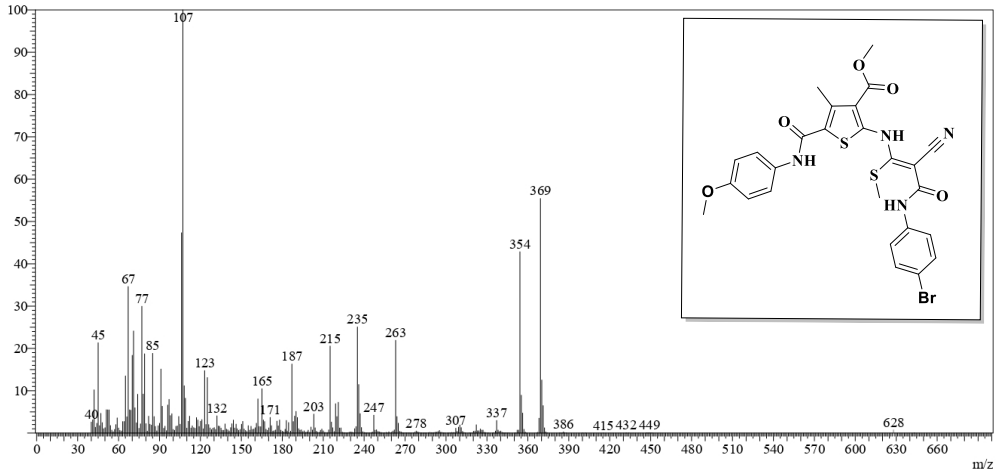


Fig. 59: Representative mass spectrum of compound **JOOET-14**

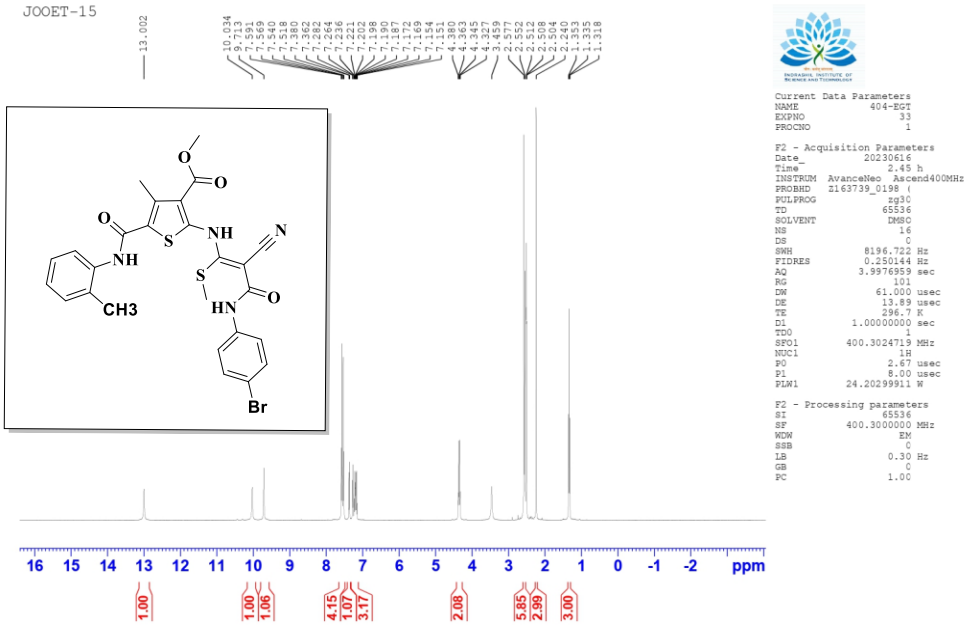


Fig. 60: Representative ^{1}H NMR spectrum of compound **JOOET-15**

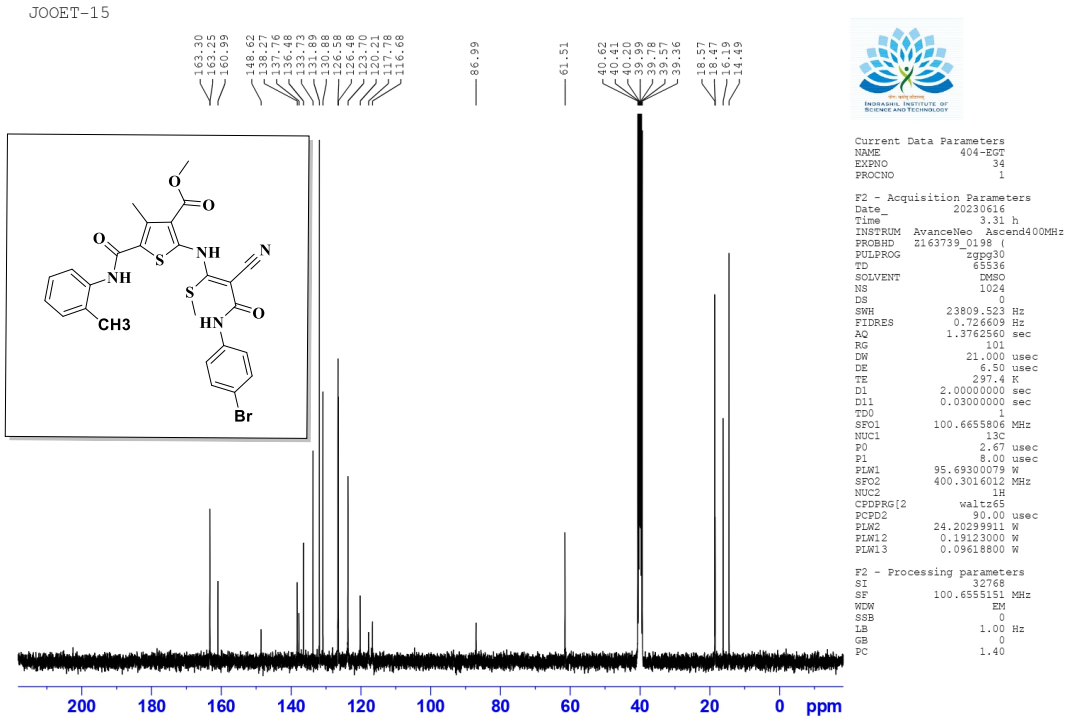


Fig. 61: Representative ^{13}C NMR spectrum of compound **JOOET-15**

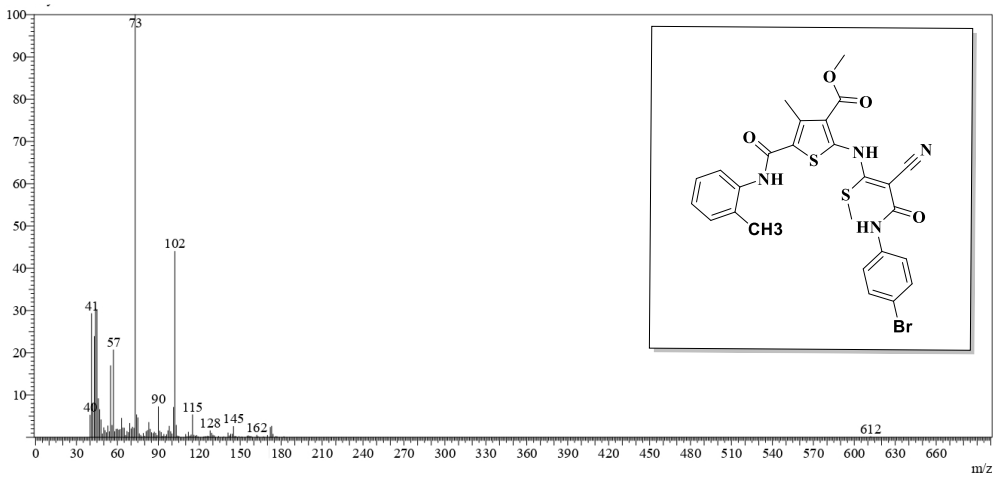


Fig. 62: Representative mass spectrum of compound **JOOET-15**