Chapter 2

Synthesis, Characterization and Anti-Cancer Activity of Indole Derivative

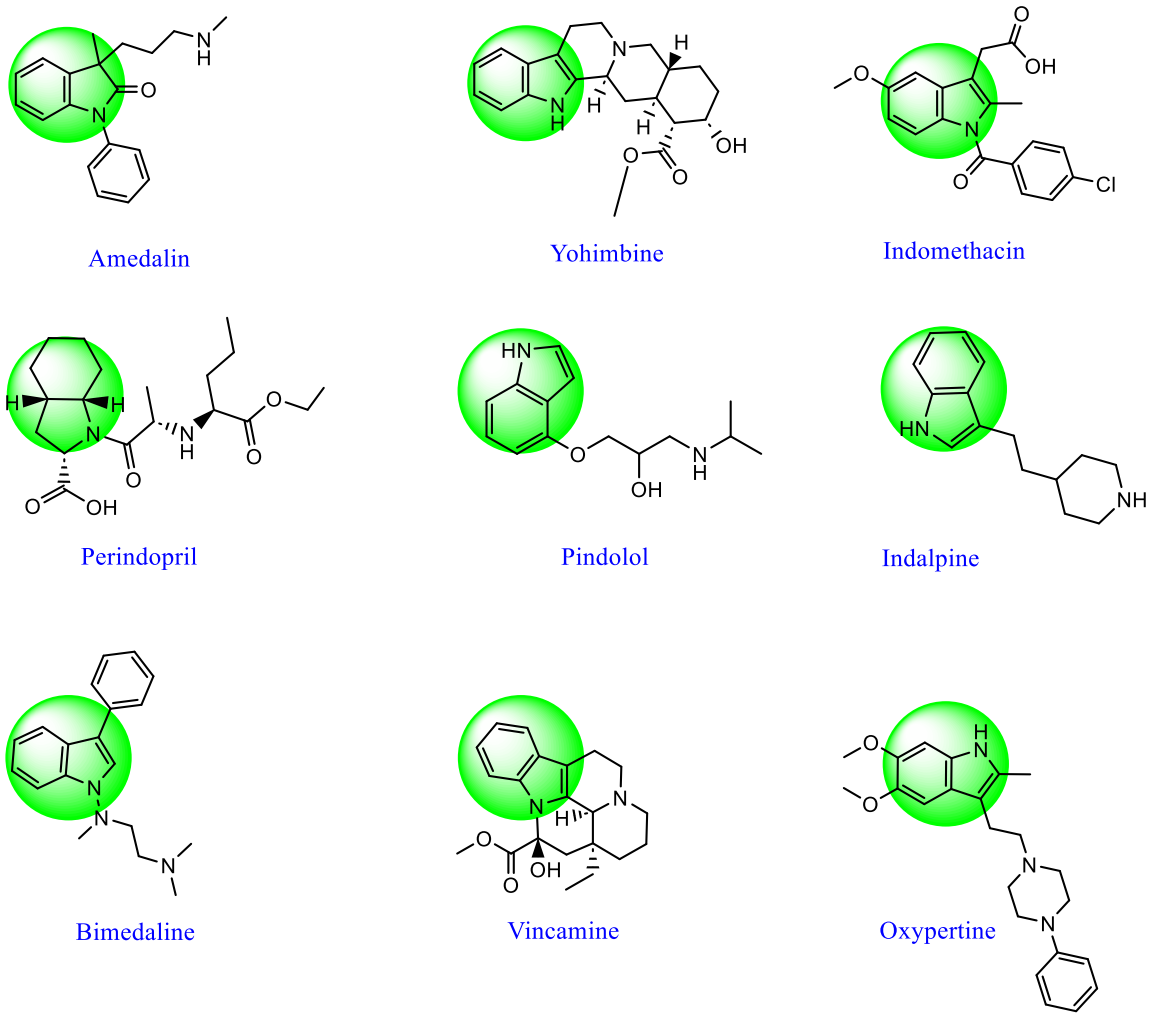
2.1. Introduction

Cancer is a significant global health concern that contributes significantly to global death rates. Novel therapeutic techniques are necessary to address problems including drug resistance and unfavorable side effects.[23] Natural products (NPs) provide a promising path because of their diverse molecular structures and their medical advantages, although their complex synthesis presents a barrier to their widespread use.[24] Furthermore, the anticancer potential of organic compounds especially those containing nitrogen heterocycles is a subject of active investigation. Despite advances, improving the selectivity and toxicity of cancer medicines remains imperative.[25]

Indole is an important molecule in drug discovery, pharmacology and chemistry because of its various chemical versatility and biological functions. It was first discovered in the indigo plant and is a vital component of many different synthetic and natural molecule.[26] The special structure of indole, fused by pyrrole and benzene rings, allows for the synthesis of new compounds in medicinal chemistry, providing numerous treatment pathways for treating various illnesses.[27] Due to the wide range of biological activities exhibited by indole derivative from CNS modulation to anticancer effects many studies in both academia and the pharmaceutical sector have been conducted on them. Recent advances in the synthesis and evaluation of indole derivatives, using methodologies such as combinatorial chemistry and rational drug design, show promise for generating molecules with improved pharmacological characteristics and lower toxicity. The importance of indole as a fundamental scaffold in medicinal chemistry is highlighted in this work, along with several promising pathways for further study and drug development. Indole derivatives have garnered significant global interest because of their diverse array of biological activities, including anti-cancer,[28] anti-diabetic,[29] anti-tubercular,[30] antimicrobial,[31] antifungal,[32] anti-HIV,[33] anti-inflammatory,[34] anticonvulsant,[35] anti-

hypertensive,[36] anti-malarial,[37] anti-leishmanial,[38] anti-hepatitis,[39] antioxidant,[40] and analgesic.[41]

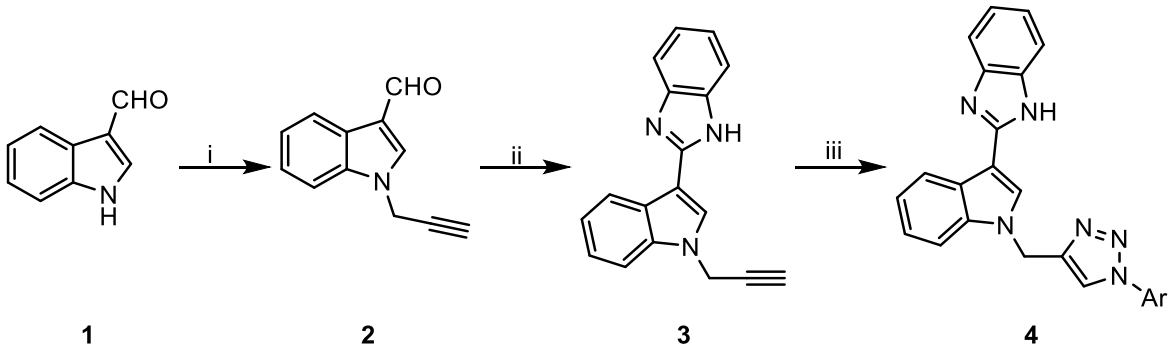
Pyrazole is a flexible chemical molecule having a five-membered ring structure consisting of three carbon and two nitrogen atoms.[42] It is frequently utilized in medicinal chemistry because of its numerous pharmacological properties, which include anticancer,[43] anti-inflammatory,[44] anticonvulsant,[45] antimicrobial,[46] antimalarial,[47] antibacterial,[48] antiviral[49] and analgesic activities.[50] Pyrazole derivatives are fundamental building blocks in drug discovery and development, offering structural variability and synthetic accessibility for synthesizing new molecules with therapeutic potential.



Scheme 1. Several bioactive indoles

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

Ashok *et al*[51] describe a method for synthesizing indole-benzimidazole based 1, 2, 3-triazole hybrids **4** using microwave assistance in three steps. This involved synthesizing indole-3-carboxaldehyde derivative **2** and indole-benzimidazole derivatives **3** from 1H-indole 3-carbaldehyde **1** as a precursor. Microwave irradiation was utilized to facilitate a click reaction, resulting in the synthesis of the desired compounds **4** from compound 3 **(Scheme 2.1).**

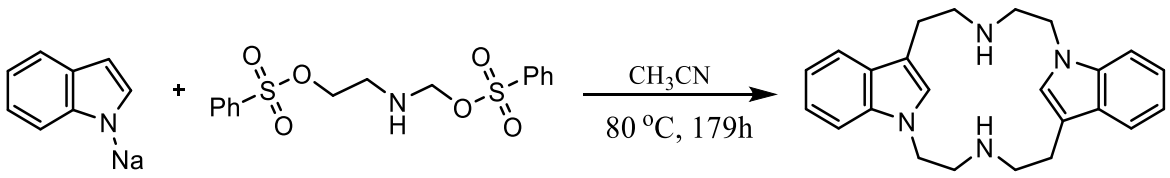


1 **2** **3** **4**

i = Propargl bromide, K_2CO_3, dry acetone, reflux, 4h
ii = o-phenylenediamine (OPDA), CH_3COOH, $CHCl_3$, reflux, 6h
iii = Ar-N_3, CuI, DMF/H_2O (1:3), 80 ^oC, 12 h or MWI, 180 W, 8 min

Scheme 2.1

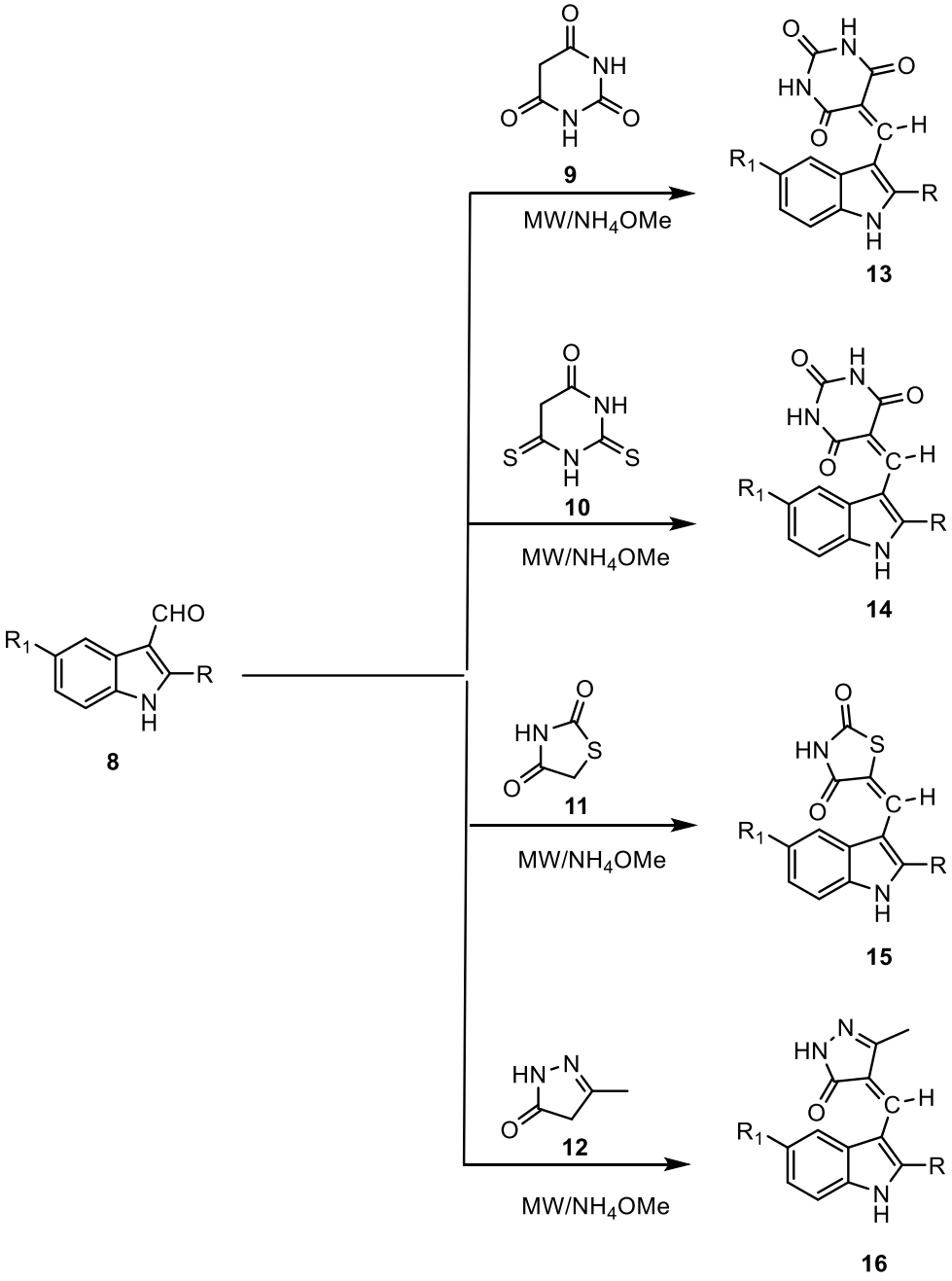
F. Essa *et al*[52], have described a novel method for synthesizing indole derivatives **7**. This method involves using Dibenzenesulphonyl diethyl amine as a starting material, which acts as an alkylating agent. In the presence of sodium and absolute ethanol, the reaction between indole **5** and dibenzenesulphonyl diethyl amine **6** produced seven unexpected products. Five of these products were isolated using flash chromatography. Some molecule shows great compatibility with ampicillin in terms of antibacterial activity **(Scheme 2.2).**



5 **6** **7**

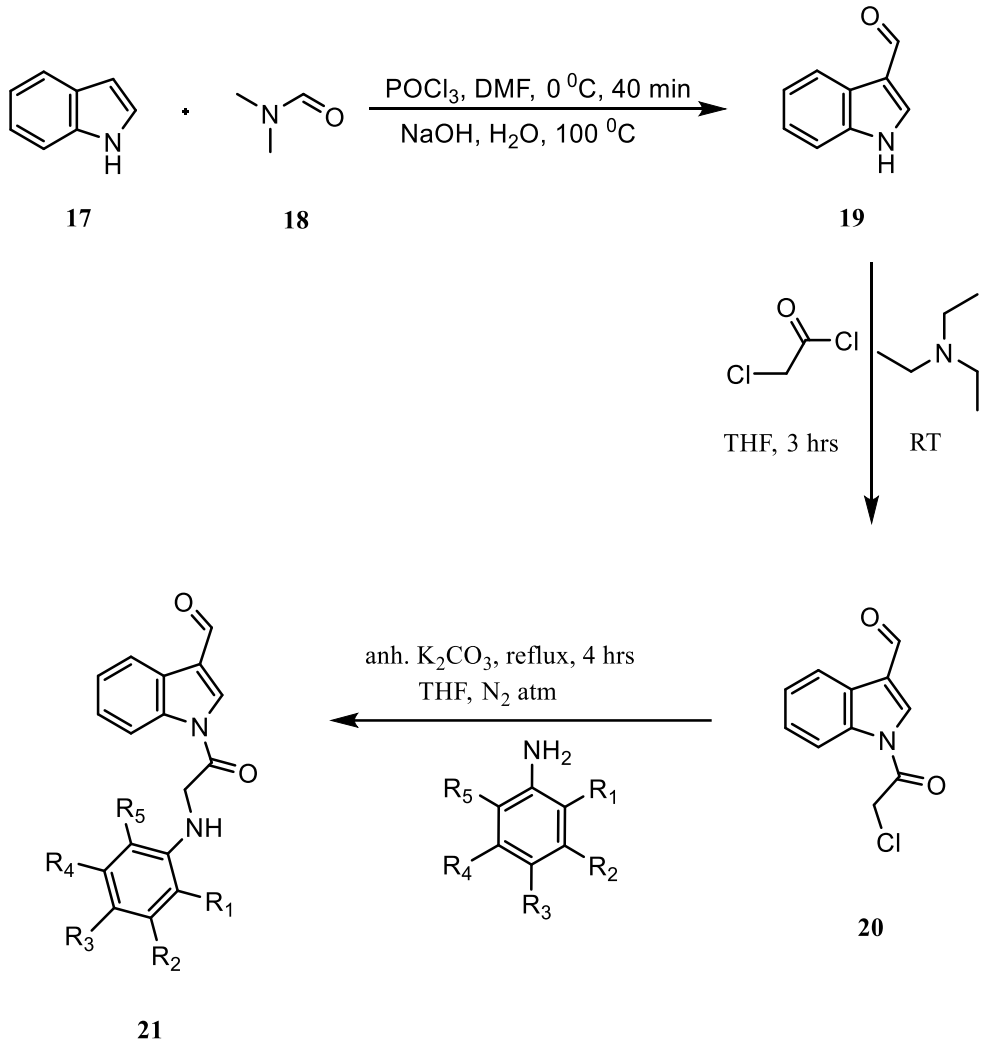
Scheme 2.2

Biradar *et al*[53] *reported the synthesis of new 2,5-disubstituted indole derivative using an environmentally friendly solvent-free approach*. The synthesis of 2,5-disubstituted indole analogues **13-16** is demonstrated via microwave-assisted Knoevenagel condensation involving 2,5-disubstituted indole-3-carboxaldehydes **8** and different active methylene compounds **9-12**, using NH4OAc as the catalyst **(Scheme 2.3).**



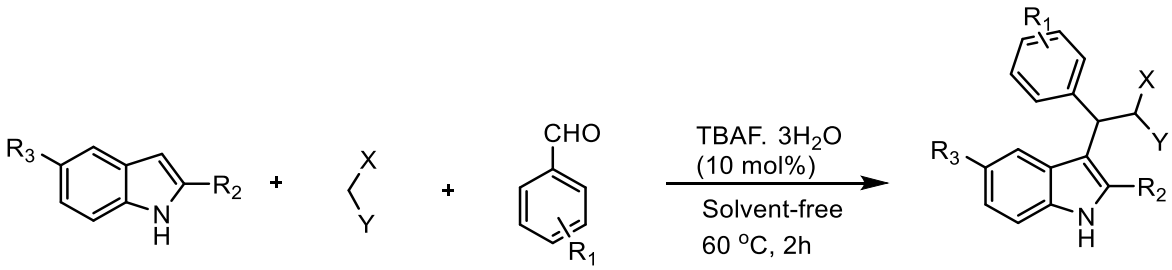
Scheme 2.3

N. Naik *et al*[54] *described a new indole derivative* ***21*** *that was synthesized by combining indole-3-carboxaldehyde* ***20*** *with various aryl amines.* The antioxidant potential of the synthesized molecules was investigated to identify the most effective analogues using two laboratory tests: the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay and the microsomal lipid peroxidation (LPO) inhibition assay. The compounds exhibited different levels of activity, with some showing higher antioxidant activity compared to other similar compounds. Additionally, these compounds were even more active than the standard antioxidant, butylated hydroxy anisole (BHA) **(Scheme 2.4).**



Scheme 2.4

Singh *et al*[55] utilized TBAF as a catalyst in three-component reactions to produce 3-indole derivatives **25** with a yield of 72%. The synthesis of 3-indole derivatives **25** without the use of solvents was successfully demonstrated in **(Scheme 2.5)**. To initiate the reaction, a catalyst of TBAF (10 mol%) was employed, along with indoles **22**, active methylene compounds **23**, and benzaldehydes **24**. Excellent yields (up to 97%) were achieved in the synthesis of 3-indole derivatives **25** under optimal conditions.



22 **23** **24** **25**

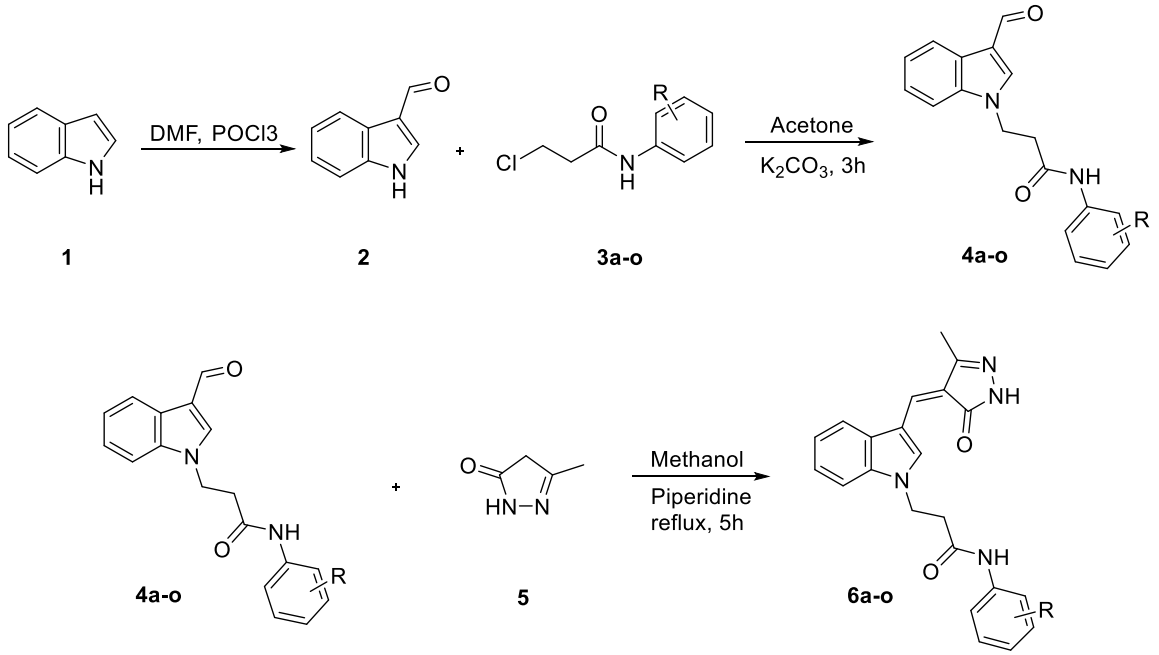
X, Y = CN, COOEt, COMe

Scheme 2.5

2.2. Result and Discussion

Our research elucidates the synthesis process for several heterocyclic molecules, as well as the discovery of a new anticancer drug. Comprehensive information is given regarding 15 recently synthesized compounds, all of which have indole as a key structural component. The molecular profiles of molecules **6a–o** were carefully investigated using analytical methods such as ^{1}H NMR, ^{13}C NMR, mass spectroscopy, and FTIR spectrum analysis. **Scheme 1** depicts the stepwise synthesis of novel indole derivatives.

The Vilsmeier-Haack reaction was used to initiate the process by formylation of indole. Following this, several *N*-phenyl propanamide derivatives were reacted with the formylated indole to form molecules **4a-o**, which were formed with good yields. The final step involves the interaction of the formylated indole derivatives **4a-o** with the active methylene group present in the pyrazole **5**, aided by a small quantity of piperidine in methanol, providing the desired product **6a-o** as indicated in **Scheme 1.**



A structural assignment has been determined for novel compounds based on elemental studies and spectral data. We present many physical properties and the elemental analysis information of these novel compounds, including ^{1}H NMR, ^{13}C NMR mass, and IR spectra.

The ^{1}H NMR study of the final molecules revealed that the singlet of two NH group seen between 9.82–11.08 ppm and singlet peaks of methyl proton seen at 2.25 ppm. CH_2 protons appeared as triplets between 2.97–4.71 ppm .The aromatic region was seen between 7.03–8.17 ppm. Each compound's mass spectra displayed a molecular ion, confirming its molecular weight. The mass spectra showed a molecular ion peak at 372 that corresponded to the molecular formula $C_{22}H_{20}N_4O_2$.

To enhance the experimental condition for the creation of novel compounds **6a–o**, we employed various solvent such as EtOH, IPA and methanol with different bases, including piperidinium acetate, triethylamine and piperidine. Through this study, we find that utilizing piperidine together with methanol led to an accelerated reaction between **4a–o** and **5**, as a result of a more rapid process and a favorable yield of indole derivative **6a–o.**

2.2.1. Optimizing reaction condition

Table 1: Optimization of the reaction conditions

Entry	Solvent	Base[a]	Temp. (C)	Time	Yield (%) [b]	Purification
1	No solvent	–	100	2h	–	–
2	H_2O	–	reflux	2h	–	–
3	H_2O	Piperidine	reflux	2h	–	–
4	IPA	Piperidinium acetate	reflux	2h	71	Yes
5	IPA	TEA	reflux	2h	68	Yes
6	IPA	Piperidine	reflux	2h	77	Yes
7	EtOH	Piperidinium acetate	reflux	2h	79	Yes
8	EtOH	TEA	reflux	2h	71	Yes
9	EtOH	Piperidine	reflux	2h	87	No
10	MeOH	Piperidinium acetate	reflux	2h	82	Yes
11	MeOH	TEA	reflux	2h	78	Yes
12	MeOH	Piperidine	reflux	2h	93	No

[a] Amount of base was 1 equivalent

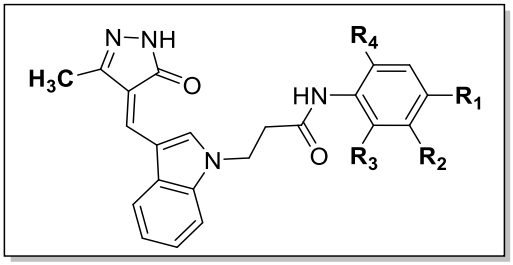
[b] Yield is given for isolated product without purification

The initial experiment was carried out at 100 °C without using any base, but unfortunately, no product was generated **(Table 1, entry 1)**. Similarly, employing water as a solvent in the absence of a base at reflux temperature produced no product (entry 2). Attempting to improve the reaction, we added piperidine and refluxed the mixture for 2 hours, but no product was produced (entry 3). Furthermore, using piperidinium acetate as the base and executing the reaction in IPA at reflux for 2 hours resulted in a 71% product yield (entry

4). Under identical conditions, TEA and piperidine as bases produced 68% and 77% yields, respectively (entries 5 and 6). Shifting to various solvents, utilizing piperidinium acetate, TEA, and piperidine as bases in EtOH under reflux, yielded 79%, 71%, and an outstanding 87%, respectively (entries 7,8 and 9). Remarkably, 82% of the product was produced when piperidinium acetate was used as the base in MeOH under reflux (entry 10). Morever, utilizing methanol with triethylamine yielded a respectable 78% yield (entry 11), whereas an impressive 93% yield was achieved utilizing piperidine as the base with the reaction mixture stirred at reflux temperature for 2 hours (entry 12). These findings demonstrate the efficacy of combining piperidine and methanol, resulting in good product yields and faster reaction kinetics. **Table 1** shows that our methods produced novel indole compounds successfully using these optimized reaction conditions.

2.2.2. Physicochemical Properties

Table 2. Physicochemical characteristics of the novel indole derivatives **6a-o**



Entry	R_1	R_2	R_3	R_4	Yield (%)	Melting point (°C)
6a	OCH_3	H	H	H	87	230-232
6b	Br	H	H	H	82	232-234
6c	Cl	H	H	H	78	210-212
6d	H	H	CH_3	CH_3	78	235-236
6e	H	Cl	H	H	82	240-242
6f	H	H	H	F	80	232-234
6g	H	H	H	CH_3	75	212-213
6h	CH_3	H	H	CH_3	72	206-208
6i	H	H	H	H	65	240-242
6j	Br	H	Br	Br	84	236-238
6k	NO_2	H	Cl	H	81	229-231
6l	CH_3	H	H	H	79	217-219
6m	Cl	H	H	Cl	75	231-231
6n	NO_2	H	H	H	71	224-226
6o	H	NO_2	H	H	79	228-230

2.2.3. Anti-cancer screening

The screening of novel synthesized indole derivatives for their anticancer activity encompassed a range of human cancer cell lines, comprising CNS cancer, non-small cell lung cancer, ovarian cancer, renal cancer, melanoma, colon cancer, Prostate Cancer, Breast Cancer and leukemia.

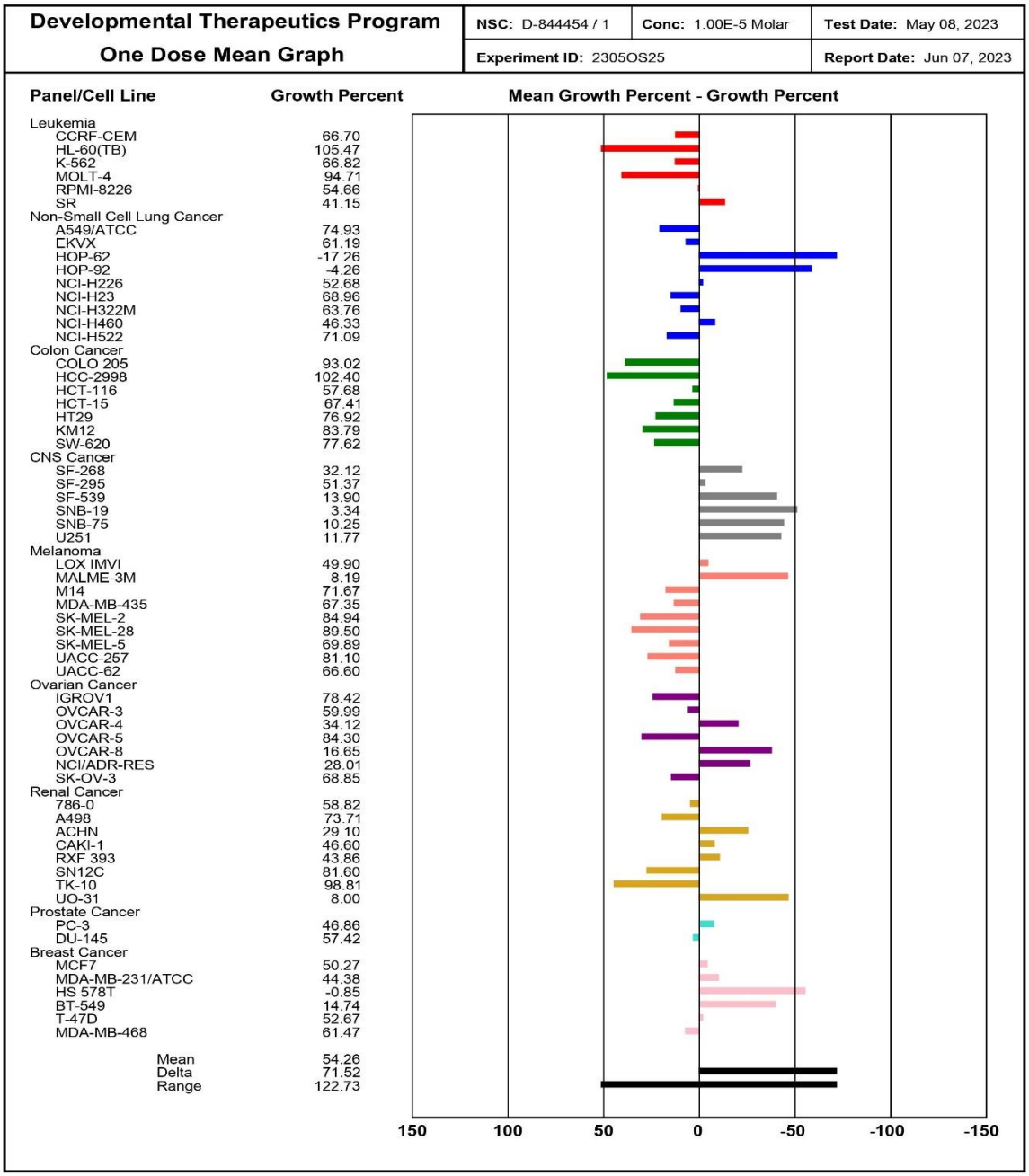


Figure 2. Anti-cancer activity of molecule **6b** as a mean graph plot of GI_{50} values against NCI-60 cell line panels

These cell lines were further split to increase screening efficiency. The NCI used a total of 60 distinct sub-cell lines to assess a single drug for enhanced antitumor potential. We submitted 10 compounds, all the synthesized compound (6a, 6b, 6c, 6d, 6e, 6f, 6g, 6h, 6i and 6j) were chosen for their single-dose response study in the screening for anticancer properties. More specifically, compounds 6b with the bromine group showed significant action. Compound 6b demonstrated strong cytotoxicity against NSCL Cancer, CNS Cancer, Melanoma, Ovarian Cancer, Renal Cancer and Breast cancer panels, and the one-dose response graph for anticancer activity showed higher GI50 values against specific cell lines. Based on the one-dose response graph for anticancer activity, it is evident that compound 6b exhibit higher GI50 values against certain cell lines including NSCL Cancer (HOP-62; GI50= -17.26, HOP-92; GI50= -4.26), CNS Cancer (SNB-19; GI50= 3.34, SNB-75; GI50= 10.25, U251; GI50= 11.77), Melanoma (MALME-3M; GI50= 8.19), Ovarian Cancer (OVCAR-8; GI50= 16.65), Renal Cancer (UO-31; GI50= 8.00), Breast Cancer (HS 578T; GI50= -0.85, BT-549; GI50= 14.74), compared to others (**Figure 2**). **Table 3** provides an overview of the anticancer activity of the chosen indole derivatives.

Table 3: Anticancer activity (GI_{50} Values in µM) for compounds 6a-6j in NCI-60 cell line

Cell line	**Growth of Cells (%)[3] 10^{-5} M**									
Compound	**6a**	**6b**	**6c**	**6d**	**6e**	**6f**	**6g**	**6h**	**6i**	**6j**
Leukemia										
CCRF-CEM	71.45	66.70	72.74	67.38	91.64	66.45	66.24	65.58	78.75	100.60
HL-60(TB)	107.23	105.47	112.42	81.25	101.82	94.71	79.00	96.47	104.71	101.21
K-562	105.25	66.82	73.98	95.39	96.52	81.76	87.30	97.43	86.34	106.86
MOLT-4	88.81	94.71	91.44	85.57	108.17	70.15	78.60	58.09	74.89	101.97
RPMI-8226	63.96	54.66	53.76	61.09	69.60	65.49	67.76	65.37	68.95	92.85
SR	67.70	41.15	50.36	41.82	101.24	39.09	53.32	49.47	51.65	-
NSCL Cancer										
A549/ATCC	96.26	74.93	84.75	99.88	76.70	99.06	94.24	103.11	103.92	94.79
EKVX	96.93	61.19	74.94	93.64	77.19	98.13	93.14	98.16	92.41	67.27
HOP-62	112.05	**-17.26**	29.92	89.52	16.64	96.40	93.49	103.29	101.21	110.65
HOP-92	90.45	**-4.26**	30.38	79.80	36.84	80.40	72.81	78.63	74.96	112.50
NCI-H226	95.08	52.68	72.80	108.16	34.67	101.66	98.25	94.06	93.44	76.49
NCI-H23	95.90	68.96	81.40	89.80	69.10	90.74	86.76	94.06	91.41	92.85
NCI-H322M	108.72	63.76	88.39	98.65	75.75	109.86	95.21	97.01	100.02	-
NCI-H460	111.53	46.33	60.48	106.50	50.24	90.05	104.84	113.19	109.04	99.39
NCI-H522	94.38	71.09	82.14	92.04	71.40	89.50	87.02	96.49	92.40	88.95
Colon Cancer										
COLO 205	119.59	93.02	99.09	96.46	97.57	105.62	103.43	106.50	111.29	119.01
HCC-2998	114.08	102.40	117.77	114.95	93.09	115.70	110.46	107.95	107.69	100.08
HCT-116	90.89	57.68	70.50	90.64	57.90	77.85	87.19	89.61	87.62	96.03

HCT-15	81.43	67.41	77.68	79.53	86.31	59.29	80.94	89.22	81.50	87.90
HT29	104.38	76.92	89.21	92.11	74.22	86.23	93.70	93.26	98.53	90.59
KM12	91.85	83.79	88.09	86.93	81.87	80.36	90.84	102.18	102.59	99.61
SW-620	106.76	77.6	78.34	86.17	92.82	92.91	88.13	104.40	111.80	98.49
CNS Cancer										
SF-268	101.18	32.12	60.66	108.65	44.47	88.37	88.14	100.82	95.97	88.39
SF-295	91.67	51.37	74.02	96.66	45.07	92.52	94.27	95.07	88.27	91.81
SF-539	90.55	13.90	43.50	90.55	38.43	90.38	90.22	99.31	99.27	86.42
SNB-19	94.25	**3.34**	31.17	97.16	32.80	94.03	93.83	93.37	94.99	93.53
SNB-75	112.79	**10.25**	114.09	101.45	76.16	84.40	79.62	112.14	111.83	-
U251	100.34	**11.77**	37.39	92.76	42.25	83.59	90.63	100.50	96.51	98.94
Melanoma										
LOX IMVI	91.68	49.90	71.82	83.06	61.22	70.26	76.19	89.22	85.13	79.14
MALME-3M	100.61	**8.19**	47.05	87.61	44.26	83.80	90.17	94.09	86.58	-
M14	97.99	71.67	83.30	81.85	89.34	85.89	82.23	88.39	97.24	97.98
MDA-MB-435	89.64	67.35	81.36	67.40	79.54	26.20	61.95	79.20	70.30	101.83
SK-MEL-2	102.31	84.94	94.23	104.61	90.75	101.62	97.46	96.96	99.17	98.76
SK-MEL-28	104.19	89.50	97.02	105.78	97.64	117.99	111.96	103.22	100.28	105.20
SK-MEL-5	88.97	69.89	78.09	90.15	79.87	83.78	89.29	94.08	93.99	95.25
UACC-257	99.09	81.10	94.58	96.81	89.53	98.06	93.17	106.02	103.23	102.28
UACC-62	86.69	66.60	80.21	83.55	70.28	78.71	72.95	77.29	77.75	78.15
Ovarian Cancer										
IGROV1	112.73	78.42	92.85	107.39	82.55	97.74	89.26	87.51	82.93	-
OVCAR-3	112.59	59.99	104.10	118.57	73.20	77.47	78.92	108.54	110.11	105.52
OVCAR-4	91.61	34.12	51.52	97.22	39.55	93.07	92.62	101.92	99.12	94.33
OVCAR-5	101.36	84.30	90.95	118.11	99.23	121.45	105.04	97.44	92.56	86.66
OVCAR-8	91.62	**16.65**	40.90	92.29	37.67	97.21	92.76	97.94	91.36	102.65
NCI/ADR-RES	87.36	28.01	55.19	92.32	58.35	79.45	80.88	89.35	90.40	93.28
SK-OV-3	100.59	68.85	88.57	89.47	59.57	86.41	84.65	97.26	95.71	97.65
Renal Cancer										
786-0	98.43	58.82	85.07	106.67	59.29	104.07	100.86	104.26	100.16	98.13
A498	117.15	73.71	101.95	108.61	94.79	111.38	107.54	127.28	142.18	109.28
ACHN	96.76	29.10	67.34	99.98	32.83	90.92	98.96	95.58	95.33	94.86
CAKI-1	84.78	46.60	66.16	74.12	41.33	68.05	67.85	79.58	72.90	72.58
RXF 393	96.31	43.86	70.27	102.27	42.12	91.16	94.72	99.74	97.03	91.43
SN12C	102.42	81.60	93.13	107.00	77.90	102.85	93.78	86.78	86.83	88.60
TK-10	101.25	98.81	105.39	105.25	100.67	104.77	107.51	105.68	110.83	103.00
UO-31	79.72	**8.00**	47.58	68.64	50.40	68.51	55.28	64.06	57.92	-
Prostate Cancer										
PC-3	82.94	46.86	70.92	82.93	51.94	78.62	75.98	76.61	70.40	89.05
DU-145	102.72	57.42	83.03	100.31	64.00	109.48	98.53	115.05	119.99	106.05
Breast Cancer										
MCF7	67.68	50.27	58.95	64.79	59.32	66.93	67.34	79.45	76.96	80.78
MDA-MB-231	80.52	44.38	66.15	105.08	71.17	81.91	79.01	85.52	80.30	86.19
HS 578T	92.38	**-0.85**	24.59	89.36	26.27	86.08	83.21	95.16	93.46	81.12
BT-549	72.23	**14.74**	37.91	83.89	62.99	88.21	91.60	114.86	127.28	88.41
T-47D	85.09	52.67	57.46	97.77	63.87	80.92	85.28	81.92	74.26	92.32
MDA-MB-468	102.29	61.47	75.69	106.45	71.13	110.71	97.38	108.92	107.55	88.02
Mean	95.45	54.26	73.41	92.40	67.72	87.81	87.56	93.89	93.35	94.55
Delta	31.49	71.52	48.82	50.58	51.08	61.61	34.24	44.42	41.70	27.28

Range	55.63	122.73	93.18	76.75	91.53	95.25	58.64	77.81	90.53	51.74

2.3. Conclusion

A series of novel (*Z*)-*N*-aryl-3-(3-((3-methyl-5-oxo-1,5-dihydro-4H-pyrazol-4-ylidene)methyl)-1H-indol-1-yl)propanamide has been synthesized starting from Vilsmeier-Haack reaction. The Vilsmeier-Haack reaction was used to initiate the process by formylation of indole. Following this, several *N*-phenyl propanamide derivatives were reacted with the formylated indole to form molecules 4a-j, which were formed with good yields. The final step involves the interaction of the formylated indole derivatives 4a-o having the methylene group that is active in the pyrazole 5, aided by a small quantity of piperidine in methanol, providing the desired product 6a-o as indicated in **Scheme 1**. A variety of human cancer cell lines were used in the screening process for new synthetic indole derivatives to determine their anticancer potential, comprising ovarian cancer, CNS cancer, non-small cell lung cancer, colon cancer, renal cancer, melanoma, these cell lines underwent further, Breast Cancer and Prostate Cancer. These cell lines underwent further split to increase screening efficiency. The NCI used a total of 60 distinct sub-cell lines to assess a single drug for enhanced antitumor potential. We submitted 10 compounds, all the synthesized compound (6a, 6b, 6c, 6d, 6e, 6f, 6g, 6h, 6i and 6j) were chosen for their single-dose response study in the screening for anticancer properties.

2.4. Experimental Section

Melting points were determined by utilizing an electrothermal device with open capillaries and are uncorrected. The silica gel G60 F254 precoated plates (0.2 mm thickness, Merck) were used for thin-layer chromatography. UV light with wavelengths of 254 nm and 365 nm was used to provide visualization, or iodine vapor might be used instead. DMSO-d6 was used as the solvent to obtain proton (^{1}H) spectra on a Bruker AVANCE III spectrometer running at 400 MHz, chemical shifts are expressed in δ parts per million (ppm) with relation to the internal standard, Tetramethylsilane (TMS). Utilizing a direct intake probe on a Shimadzu GCMS QP2010 Ultra mass spectrometer, mass spectra were achieved. All compounds were purchased from Molychem, SRL, Spectrochem, Merk, Sigma Aldrich, Loba, and CDH and used as received without further purification.

- **General synthesis of 3-chloro-*N*-arylpropanamide (3a-o)**

The mixture of different substituted anilines (20 mmol), 3-chloropropanoyl chloride (20 mmol), and a catalytic quantity of K_2CO_3 in acetone was agitated at room temperature for about an hour. The progress of the reaction was observed by TLC. After the reaction was completed, the solvent was evaporated, and the solid or oil produced was crystallized from methanol, providing pure product **3a-o**.

- **General synthesis of 2-methyl-1*H*-indole-3-carbaldehyde (2)**

A three-necked round flask was filled with anhydrous DMF (12.92 mmol) and slowly filled with phosphorus oxychloride (2.05 mmol). This process was done at 0°C. The mixture was stirred for forty minutes at 0°C. While keeping the temperature below 10°C, a mixture containing the proper amount of 2-methyl indole (1.86 mmol) in 1 mL of DMF was gradually added. At this temperature mixture was agitated for 40 minutes, then for another 40 minutes at 35°C. The flask was filled with crushed ice, then dropwise added solution of sodium hydroxide (23.27 mmol dissolved in 2.5 mL of water). After rapidly stirring the solution during the addition, it was heated to 100°C for 30 minutes before being allowed to cool down to room temperature. Ethyl acetate (3×30 mL) was used to extract the product after the mixture had been diluted with 100 mL of water. The organic layers were

combined, allowed to dry with sodium sulfate in anhydrous state, filtered then concentrated at low pressure.

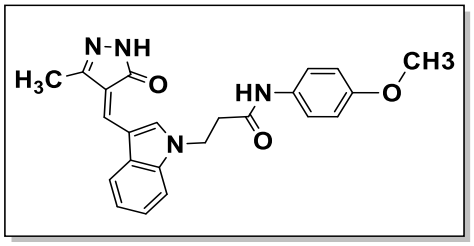
❖ **General synthesis of 3-(3-formyl-1*H*-indol-1-yl)-*N*-phenylpropanamide (4a-o)**

Formylated indole (20 mmol) and a various 3-chloro-N-phenylpropanamide (24 mmol) were dissolved in 20 ml of DMF, and then the mixture was refluxed in the presence of K2CO3 (30 mmol) until TLC indicated that the formylated indole had been completely consumed. Once the indole reaction mixture had been consumed completely, it was poured into crushed ice and neutralized with dilute HCl solution to produce the precipitates of 4a-j, which were vacuum filtered and utilized without further purification.

❖ **General synthesis of (*Z*)-*N*-(arylphenyl)-3-(3-((3-methyl-5-oxo-1,5-dihydro-4*H*-pyrazol-4-ylidene)methyl)-1*H*-indol-1-yl)propanamide 6a-o.**

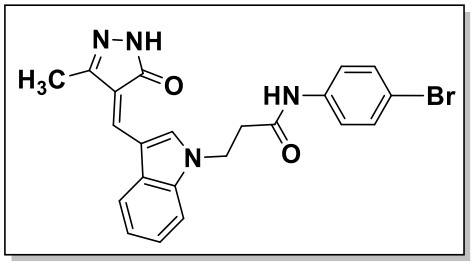
A solution containing the molecule 5a-j (20 mmol), 5-methyl-2,4-dihydro-3H-pyrazol-3-one (20 mmol), and a catalytic amount of piperidine in methanol underwent reflux for approximately 2 hours. TLC was used to monitor the reaction's progress. After completion, the solvent was evaporated under vacuum, and the resultant solid was purified by crystallization in methanol, generating the required pure product.

(*Z*)-*N*-(4-methoxyphenyl)-3-(3-((3-methyl-5-oxo-1,5-dihydro-4*H*-pyrazol-4-ylidene)methyl)-1*H*-indol-1-yl)propanamide 6a.



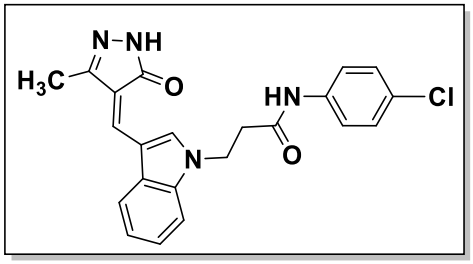
Yield 87%, mp 230-232 °C. IR spectrum, *v*, cm^{-1}: 3309.96 (-NH), 2831.60 (CH_3), 1674.27 (C=O). ^{1}H NMR spectrum, *δ*, ppm: 2.25 s (3H, *J* = 6.4,-CH3), 2.92 t (3H, *J* = 6.4 , -CH2), 3.71 s (3H, -CH3), 4.67 t (2H, *J* = 6.4, -CH2), 6.85 dd (2H, *J* = 8 ,Ar-H), 7.35 m (2H, Ar-H), 7.43 dd (4H, *J* = 8 ,Ar-H), 7.77 d (1H, *J* = 8 ,Ar-H), 7.898 s (1H, -CH), 8.15 d (1H, *J* = 8 ,Ar-H), 9.816 s (1H, -CH), 10.167 s (1H, -NH), 11.05 s (1H, -NH). ^{13}C NMR (101 MHz, DMSO) δ 167.67, 166.24, 155.19, 149.35, 139.18, 136.13, 134.27, 131.96, 128.62, 123.28, 122.02, 120.78, 119.10, 118.73, 113.76, 111.36, 111.28, 55.09, 43.13, 36.32, 12.98. Found, %: C, 68.71; H, 5.57; N, 13.88. $C_{23}H_{22}N_4O_3$. Calculated, %: C, 68.64; H, 5.51; N, 13.92; O. *M* 402.

(*Z*)-*N*-(4-bromophenyl)-3-(3-((3-methyl-5-oxo-1,5-dihydro-4*H*-pyrazol-4-ylidene)methyl)-1*H*-indol-1-yl)propanamide 6b.



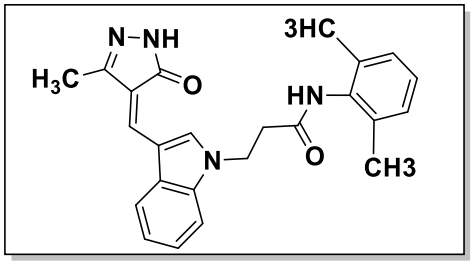
Yield 82%, mp 232-234 °C. IR spectrum, *v*, cm^{-1}: 3361.18 (-NH), 2849.93 (CH_3), 1683.24 (C=O). ^{1}H NMR spectrum, *δ*, ppm: 2.25 s (3H, -CH3), 2.98 t (2H, *J* = 6.4, -CH2), 4.68 t (2H, *J* = 6, -CH2), 7.29 m (2H, *J* = 7.2 ,Ar-H), 7.52 dd (4H, *J* = 8.4 ,Ar-H), 7.74 d (1H, *J* = 8 ,Ar-H), 7.87 s (1H, -CH), 8.13 d (1H, *J* = 7.6 ,Ar-H), 9.82 s (1H, -CH), 10.16 s (1H, -NH), 11.05 s (1H, -NH). ^{13}C NMR (101 MHz, DMSO) δ 168.95, 166.74, 149.87, 139.65, 138.66, 136.61, 134.76, 131.97, 129.11, 123.79, 122.53, 121.61, 119.62, 119.24, 115.34, 111.81, 43.40, 36.92, 13.48. Found, %: C, 58.47; H, 4.29; N, 12.37. $C_{22}H_{19}BrN_4O_2$. Calculated, %: C, 58.55; H, 4.24; N, 12.41. *M* 451.

(*Z*)-*N*-(4-chlorophenyl)-3-(3-((3-methyl-5-oxo-1,5-dihydro-4*H*-pyrazol-4-ylidene)methyl)-1*H*-indol-1-yl)propanamide 6c.



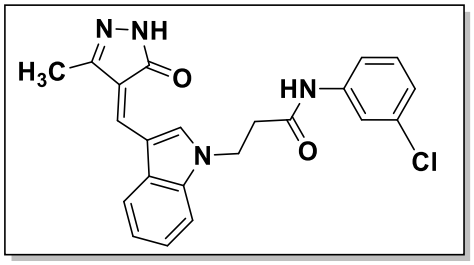
Yield 78%, mp 210-212 °C. IR spectrum, *v*, cm^{-1}: 3302.24 (-NH), 2847.03 (CH_3), 1681.98 (C=O). Found, %: C, 64.89; H, 4.63; N, 13.81. $C_{22}H_{19}ClN_4O_2$. Calculated, %: C, 64.95; H, 4.71; N, 13.77. *M* 406.

(*Z*)-*N*-(2,6-dimethylphenyl)-3-(3-((3-methyl-5-oxo-1,5-dihydro-4*H*-pyrazol-4-ylidene)methyl)-1*H*-indol-1-yl)propanamide 6d.



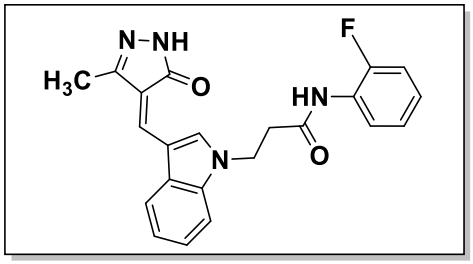
Yield 78%, mp 235-236°C. IR spectrum, *v*, cm^{-1}: 3271.38 (-NH), 2877.89 (CH_3), 1674.27 (C=O). Found, %: C, 71.91; H, 6.11; N, 13.93. $C_{24}H_{24}N_4O_2$. Calculated, %: C, 71.98; H, 6.04; N, 13.99. *M* 400.

(*Z*)-*N*-(3-chlorophenyl)-3-(3-((3-methyl-5-oxo-1,5-dihydro-4*H*-pyrazol-4-ylidene)methyl)-1*H*-indol-1-yl)propanamide 6e.



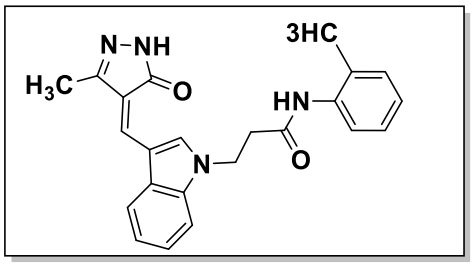
Yield 82%, mp 240-242 °C. IR spectrum, *v*, cm^{-1}: 3309.96(-NH), 2862.46 (CH_3), 1674.27 (C=O). ^{1}H NMR spectrum, *δ*, ppm: 2.25 s (3H, -CH3), 2.99 t (2H, *J* = 6.8 , -CH2), 4.69 t (2H, *J* = 6.4 , -CH2), 7.09 d (1H, *J* = 6.8 ,Ar-H), 7.38 m (4H, Ar-H), 7.75 m (2H, Ar-H), 7.88 s (1H, -CH), 8.14 d (1H, *J* = 7.6, Ar-H), 9.82 s (1H, -CH), 10.22 s (1H, -NH), 11.05 s (1H, -NH). ^{13}C NMR (101 MHz, DMSO) δ 169.18, 166.73, 149.85, 140.70, 139.63, 136.61, 134.75, 133.50, 130.87, 129.11, 123.79, 123.50, 122.53, 119.65, 119.25, 119.20, 118.04, 111.82, 43.36, 13.48. Found, %: C, 64.99; H, 4.77; N, 13.69. $C_{22}H_{19}ClN_4O_2$. Calculated, %: C, 64.95; H, 4.71; N, 13.77. *M* 406.

(*Z*)-*N*-(2-fluorophenyl)-3-(3-((3-methyl-5-oxo-1,5-dihydro-4*H*-pyrazol-4-ylidene)methyl)-1*H*-indol-1-yl)propanamide 6f.



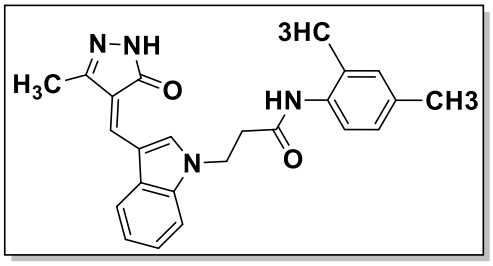
Yield 80%, mp 232-234 °C. IR spectrum, *v*, cm^{-1}: 3286.81 (-NH), 2854.74 (CH_3), 1651.12 (C=O). ^1H NMR spectrum, *δ*, ppm: 2.25 s (3H, -CH3), 3.09 t (2H, *J* = 6.8, -CH2), 4.71 t (2H, *J* = 6.4 , -CH2), 7.21 m (2H, Ar-H), 7.24 m (1H, -CH), 7.38 m (2H, Ar-H), 7.93 m (2H, Ar-H), 7.76 d (1H, *J* = 8 ,Ar-H), 8.17 d (1H, *J* = 7.6,Ar-H), 9.85 s (1H, -CH), 9.91 s (1H, -NH), 11.08 s (1H, -NH). ^{13}C NMR (101 MHz, DMSO) δ 169.31, 166.74, 149.86, 139.73, 136.63, 134.78, 129.12, 126.37, 125.76, 124.71, 124.64, 123.77, 122.51, 119.60, 119.21, 115.97, 115.78, 111.88, 111.80, 43.59, 36.51, 13.48. Found, %: C, 67.77; H, 4.86; N, 14.44. $C_{22}H_{19}FN_4O_2$. Calculated, %: C, 67.68; H, 4.91; N, 14.35. *M* 390.

(*Z*)-3-(3-((3-methyl-5-oxo-1,5-dihydro-4*H*-pyrazol-4-ylidene)methyl)-1*H*-indol-1-yl)-*N*-(o-tolyl)propanamide 6g.



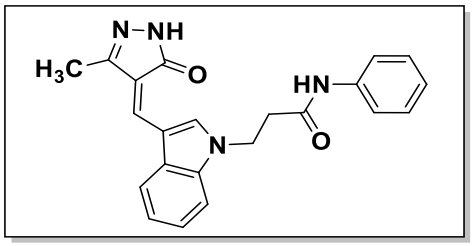
Yield 75%, mp 212-213 °C. IR spectrum, *v*, cm^{-1}: (-NH), (CH_3), (C=O). Found, %: C, 71.41; H, 5.68; N, 14.57. $C_{23}H_{22}N_4O_2$. Calculated, %: C, 71.48; H, 5.74; N, 14.50. *M* 386.

(*Z*)-*N*-(2,4-dimethylphenyl)-3-(3-((3-methyl-5-oxo-1,5-dihydro-4*H*-pyrazol-4-ylidene)methyl)-1*H*-indol-1-yl)propanamide 6h.



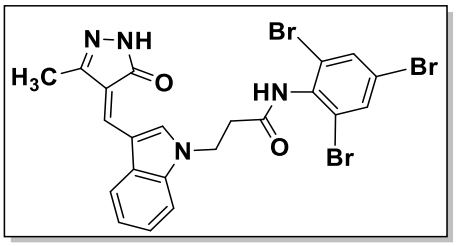
Yield 72%, mp 206-208 °C. IR spectrum, *v*, cm^{-1}: 3271.38 (-NH), (CH_3), (C=O). Found, %: C, 71.91; H, 6.13; N, 13.93. $C_{24}H_{24}N_4O_2$. Calculated, %: C, 71.98; H, 6.04; N, 13.99. *M* 400.

(*Z*)-3-(3-((3-methyl-5-oxo-1,5-dihydro-4*H*-pyrazol-4-ylidene)methyl)-1*H*-indol-1-yl)-*N*-phenylpropanamide 6i.



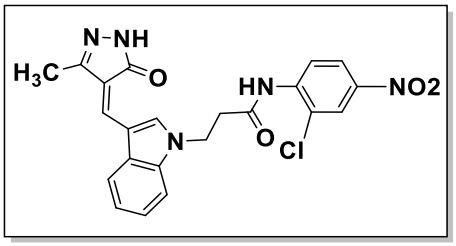
Yield 65%, mp 240-242 °C. IR spectrum, *v*, cm^{-1}: 2854.74 (CH_3), 1674.27 (C=O). ^{1}H NMR spectrum, *δ*, ppm: 2.25 s (3H, *J* = 6.4,-CH3), 2.97 t (3H, -CH2), 4.68 t (2H, -CH2), 7.03 s (1H, -CH), 7.32 m (4H, *J* = 8 ,Ar-H), 7.53 dd (2H, *J* = 8 ,Ar-H), 7.75 dd (2H, Ar-H), 8.13 s (1H, -CH), 8.15 d (1H, *J* = 8 ,Ar-H), 9.82 s (1H, -NH), 11.04 s (1H, -NH). Found, %: C, 70.89; H, 5.48; N, 15.12. $C_{22}H_{20}N_4O_2$. Calculated, %: C, 70.95; H, 5.41; N, 15.04. *M* 372.

(*Z*)-3-(3-((3-methyl-5-oxo-1,5-dihydro-4*H*-pyrazol-4-ylidene)methyl)-1*H*-indol-1-yl)-*N*-(2,4,6-tribromophenyl)propanamide 6j.



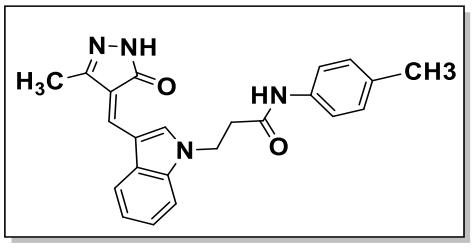
Yield 84%, mp 236-238 °C. IR spectrum, *v*, cm^{-1}: 2856.58 (CH_3), 1677.05 (C=O). Found, %: C, 43.44; H, 2.75; N, 9.25. $C_{22}H_{17}Br_3N_4O_2$. Calculated, %: C, 43.38; H, 2.81; N, 9.20. *M* 609.

(*Z*)-*N*-(2-chloro-4-nitrophenyl)-3-(3-((3-methyl-5-oxo-1,5-dihydro-4*H*-pyrazol-4-ylidene)methyl)-1*H*-indol-1-yl)propanamide 6k.



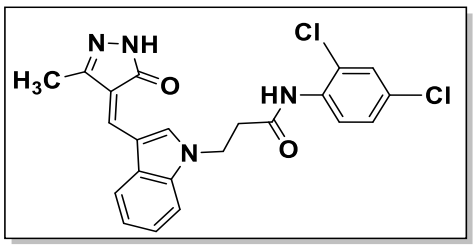
Yield 81%, mp 229-231 °C. IR spectrum, *v*, cm^{-1}: 3272.67 (-NH), 2879.28 (CH_3), 1677.18 (C=O). Found, %: C, 58.55; H, 4.09; N, 15.41. $C_{22}H_{18}ClN_5O_4$. Calculated, %: C, 58.48; H, 4.02; N, 15.50. *M* 451.

(*Z*)-3-(3-((3-methyl-5-oxo-1,5-dihydro-4*H*-pyrazol-4-ylidene)methyl)-1*H*-indol-1-yl)-*N*-(p-tolyl)propanamide 6l.



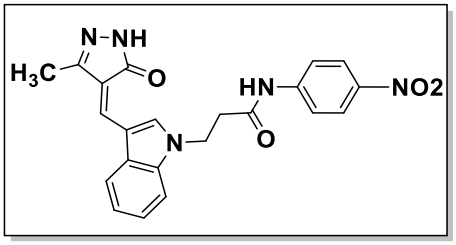
Yield 79%, mp 217-219 °C. IR spectrum, *v*, cm^{-1}: 3307.11 (-NH), 2833.27 (CH_3), 1675.55 (C=O). Found, %: C, 71.41; H, 5.79; N, 14.55. $C_{23}H_{22}N_4O_2$. Calculated, %: C, 71.48; H, 5.74; N, 14.50. *M* 386.

(*Z*)-*N*-(2,4-dichlorophenyl)-3-(3-((3-methyl-5-oxo-1,5-dihydro-4*H*-pyrazol-4-ylidene)methyl)-1*H*-indol-1-yl)propanamide 6m.



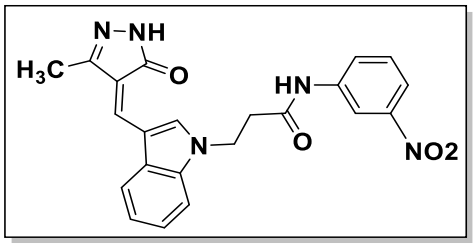
Yield 75%, mp 231-231 °C. IR spectrum, *v*, cm^{-1}: 3275.85 (-NH), 2860.25 (CH_3), 1677.58 (C=O). Found, %: C, 59.97; H, 4.19; N, 12.76. $C_{22}H_{18}Cl_2N_4O_2$. Calculated, %: C, 59.88; H, 4.11; N, 12.70. *M* 441.

(*Z*)-3-(3-((3-methyl-5-oxo-1,5-dihydro-4*H*-pyrazol-4-ylidene)methyl)-1*H*-indol-1-yl)-*N*-(4-nitrophenyl)propanamide 6n.



Yield 71%, mp 224-226 °C. IR spectrum, *v*, cm^{-1}: 3301.99 (-NH), 2844.78 (CH_3), 1679.82 (C=O). Found, %: C, 63.34; H, 4.67; N, 16.71. $C_{22}H_{19}N_5O_4$. Calculated, %: C, 63.30; H, 4.59; N, 16.78. *M* 417.

(*Z*)-3-(3-((3-methyl-5-oxo-1,5-dihydro-4*H*-pyrazol-4-ylidene)methyl)-1*H*-indol-1-yl)-*N*-(3-nitrophenyl)propanamide 6o.



Yield 79%, mp 228-230 °C. IR spectrum, *v*, cm^{-1}: 3307.61 (-NH), 2860.92 (CH_3), 1675.45 (C=O). Found, %: C, 63.38; H, 4.64; N, 16.73. $C_{22}H_{19}N_5O_4$. Calculated, %: C, 63.30; H, 4.59; N, 16.78. *M* 417.

2.4.1. Protocol for the Anti-cancer Screening

The novel synthesized molecules (6a-6j) underwent assessment for their potential anticancer activity through the National Cancer Institute (NCI/NIH), USA. This method is used to assess the effectiveness of anticancer medicines and includes 60 human cell lines that are generated from nine various types of cancer. The new indole molecules were subjected to anticancer screening using a minimum of five doses and 10-fold dilutions ranging from 0.01 to 100 µM, on these different cell lines. After a 48-hour exposure to the molecules, growth and cell viability were measured utilizing the sulforhodamine B (SRB) protein assay, which involved the application of a 0.4% (w/v) sulforhodamine B solution in 1% acetic acid (100 µl). Following a 48-hour exposure to the compounds, cell viability and growth were measured using the sulforhodamine B (SRB) protein assay, which involved the application of a 0.4% (w/v) sulforhodamine B solution in 1% acetic acid (100 µl). The screening process is detailed at https://dtp.cancer.gov/compsub/news.xhtml.

2.5. Spectral data

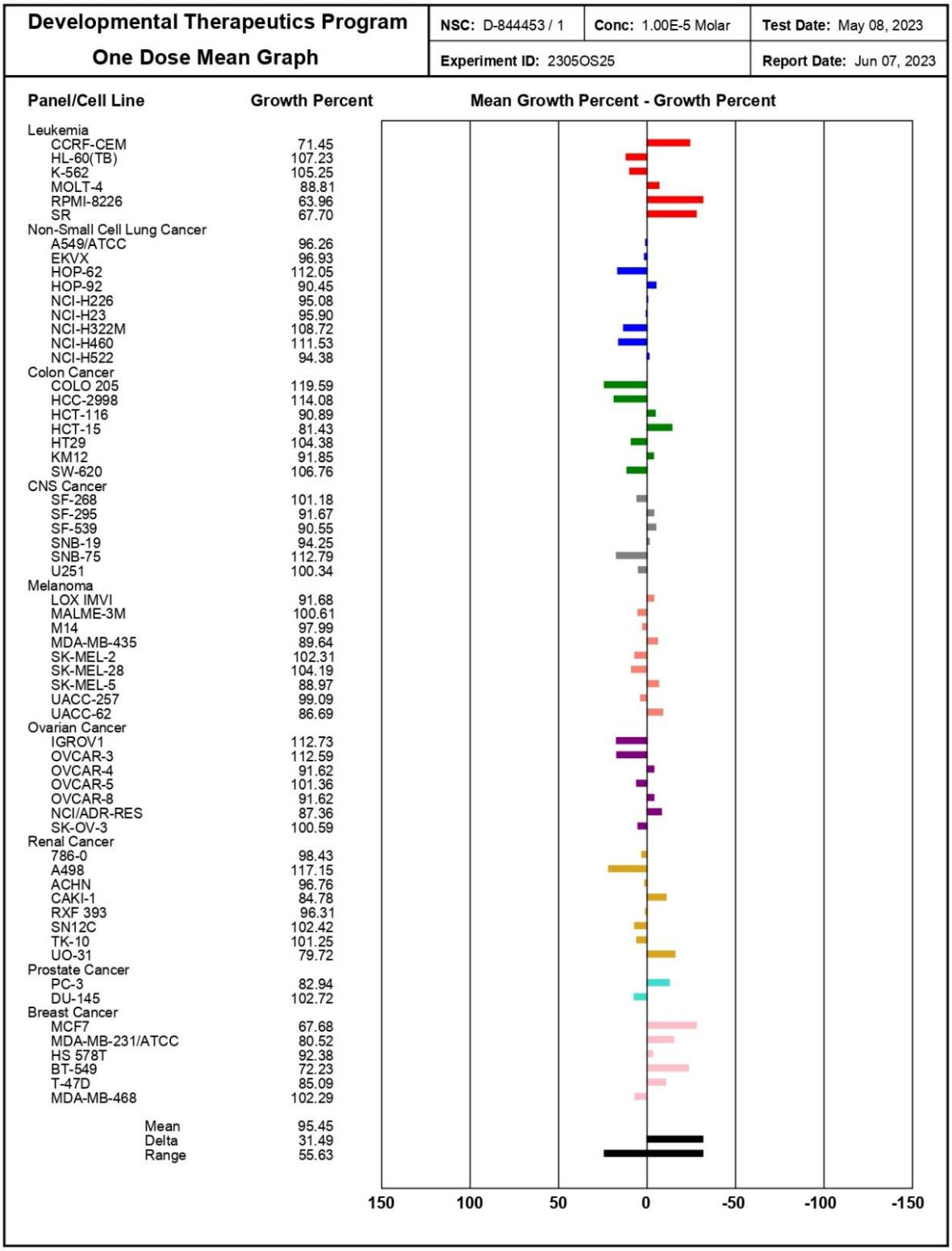


Fig. 1: Representative single dose data of compound **IPP-1**

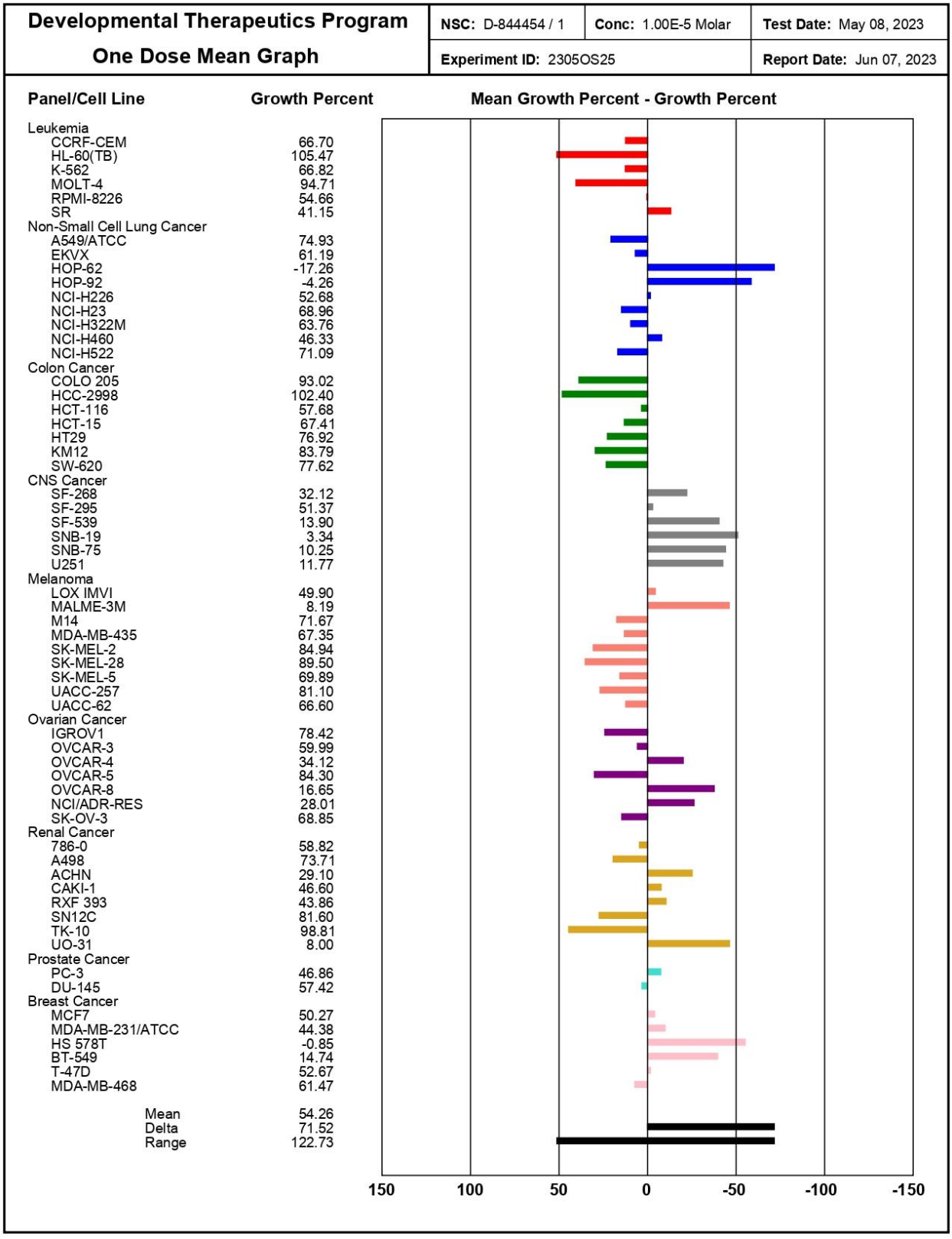


Fig. 2: Representative single dose data of compound **IPP-2**

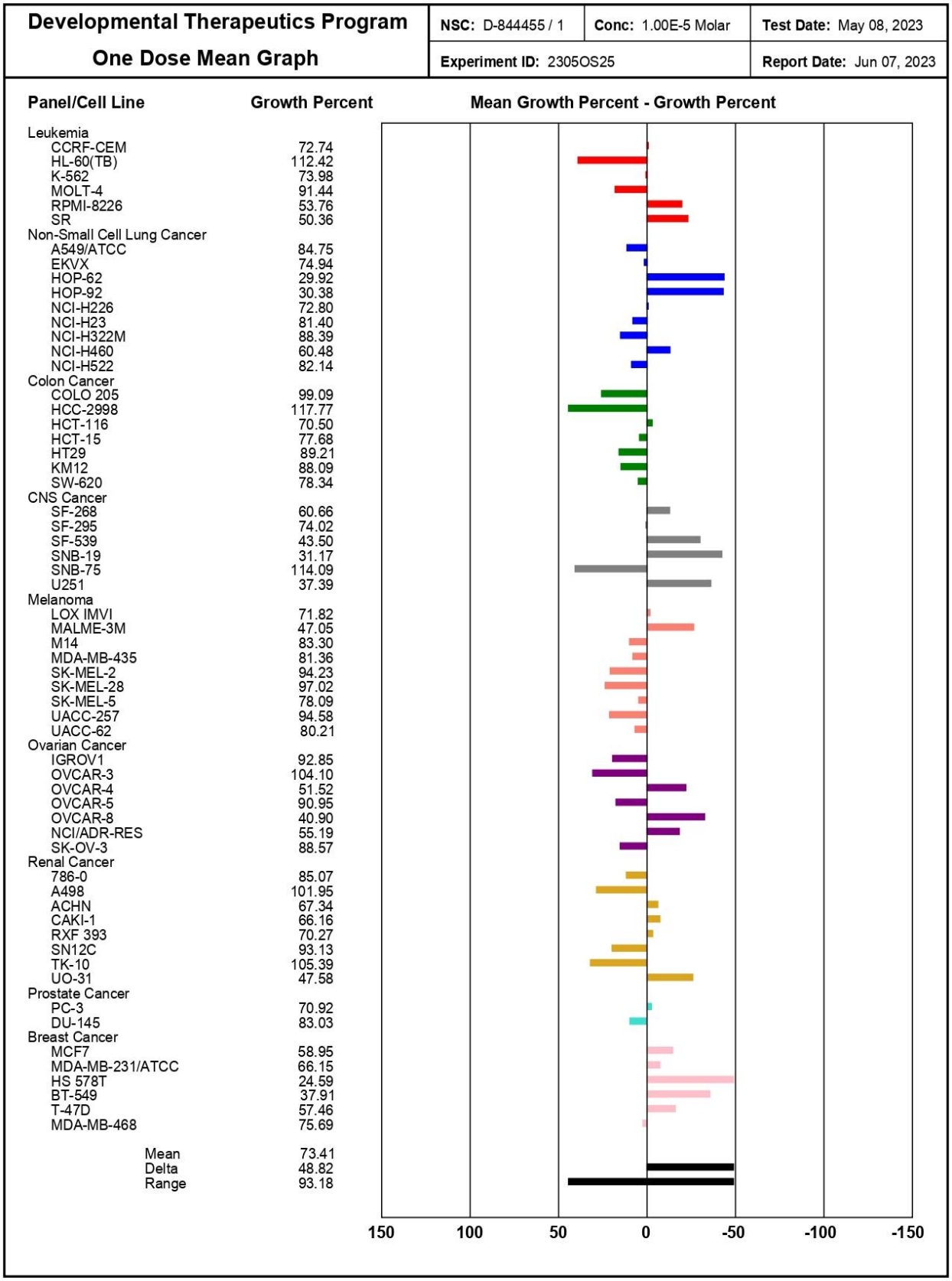


Fig. 3: Representative single dose data of compound **IPP-3**

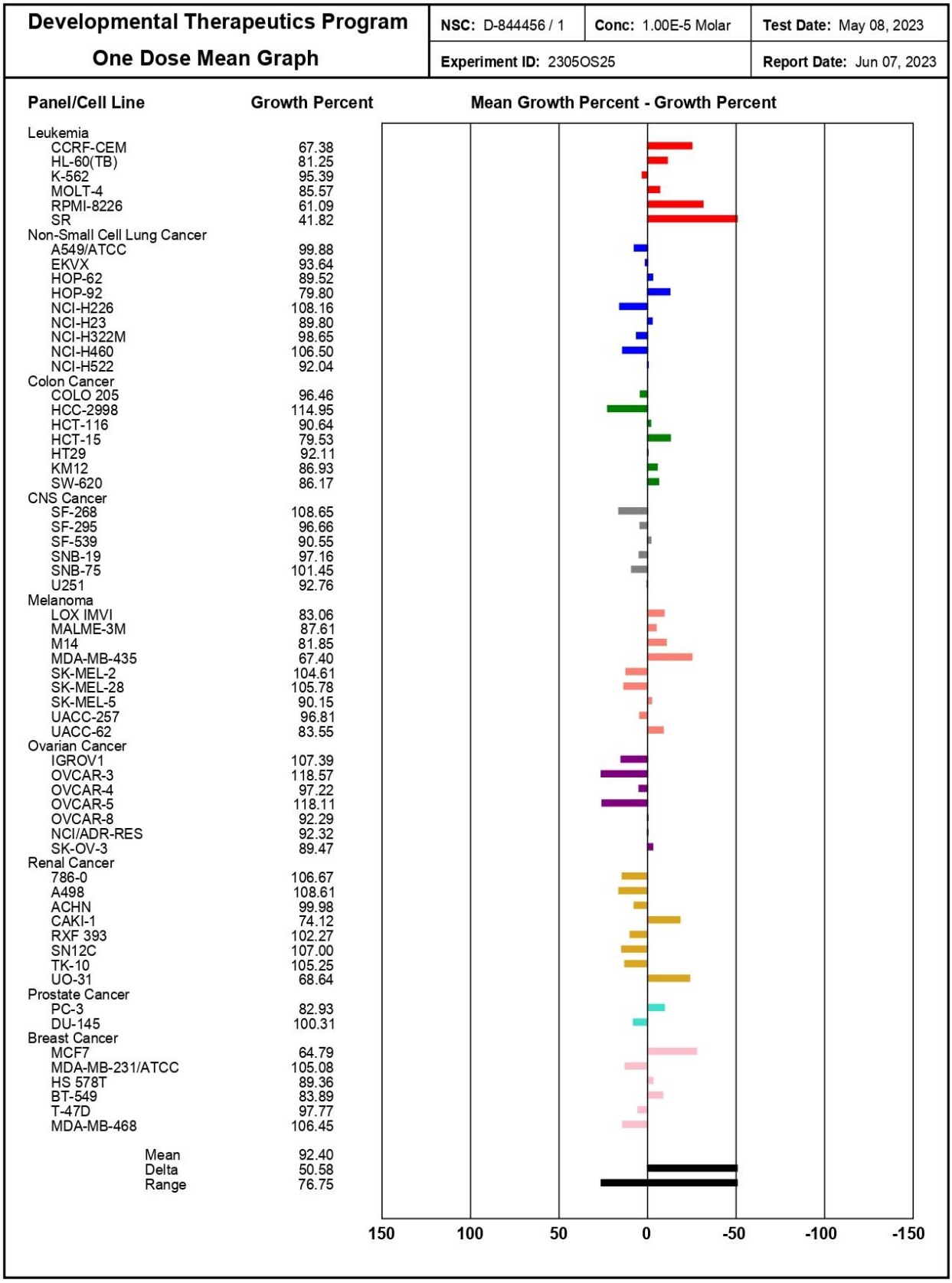


Fig. 4: Representative single dose data of compound **IPP-4**

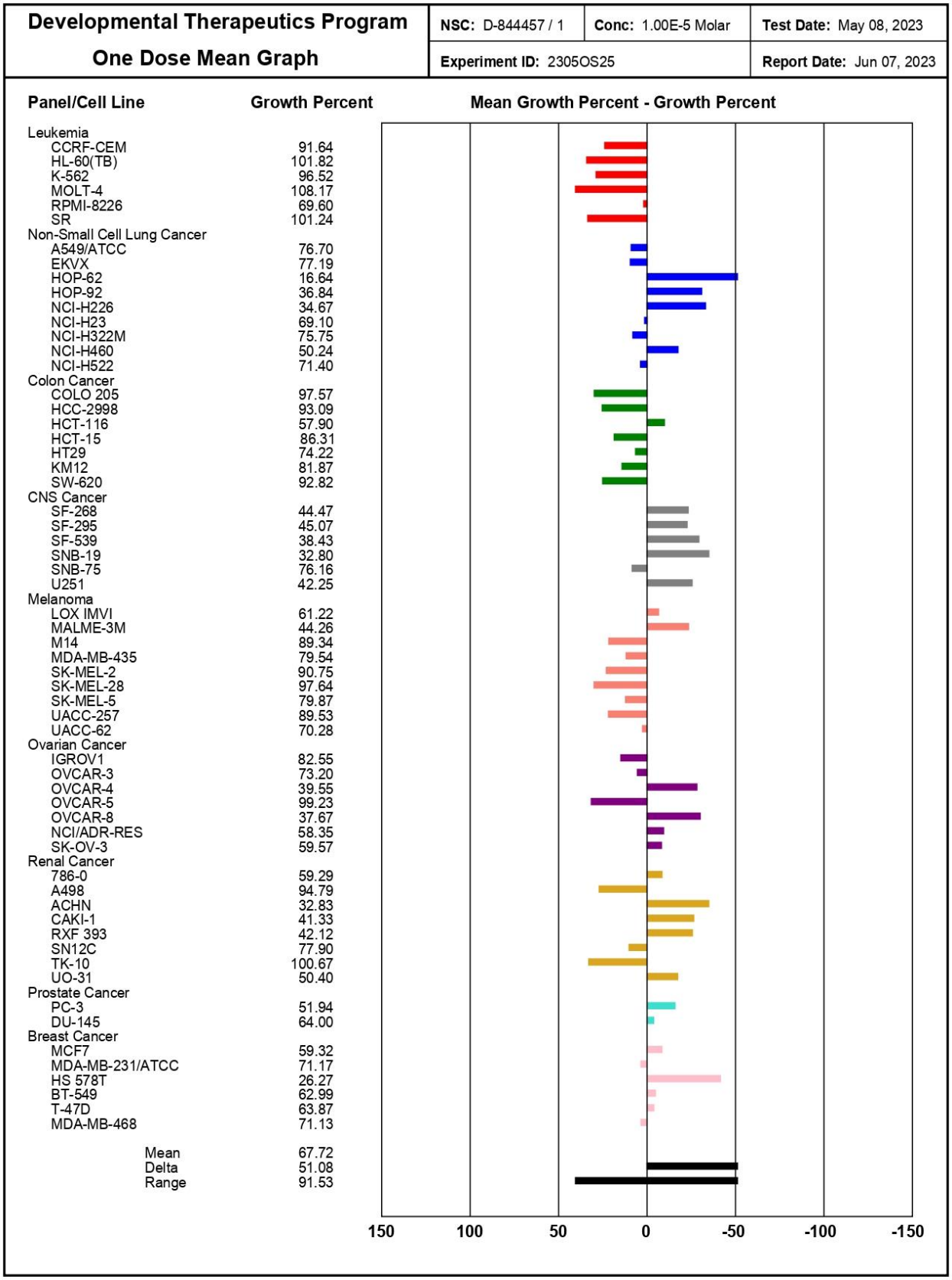


Fig. 5: Representative single dose data of compound **IPP-5**

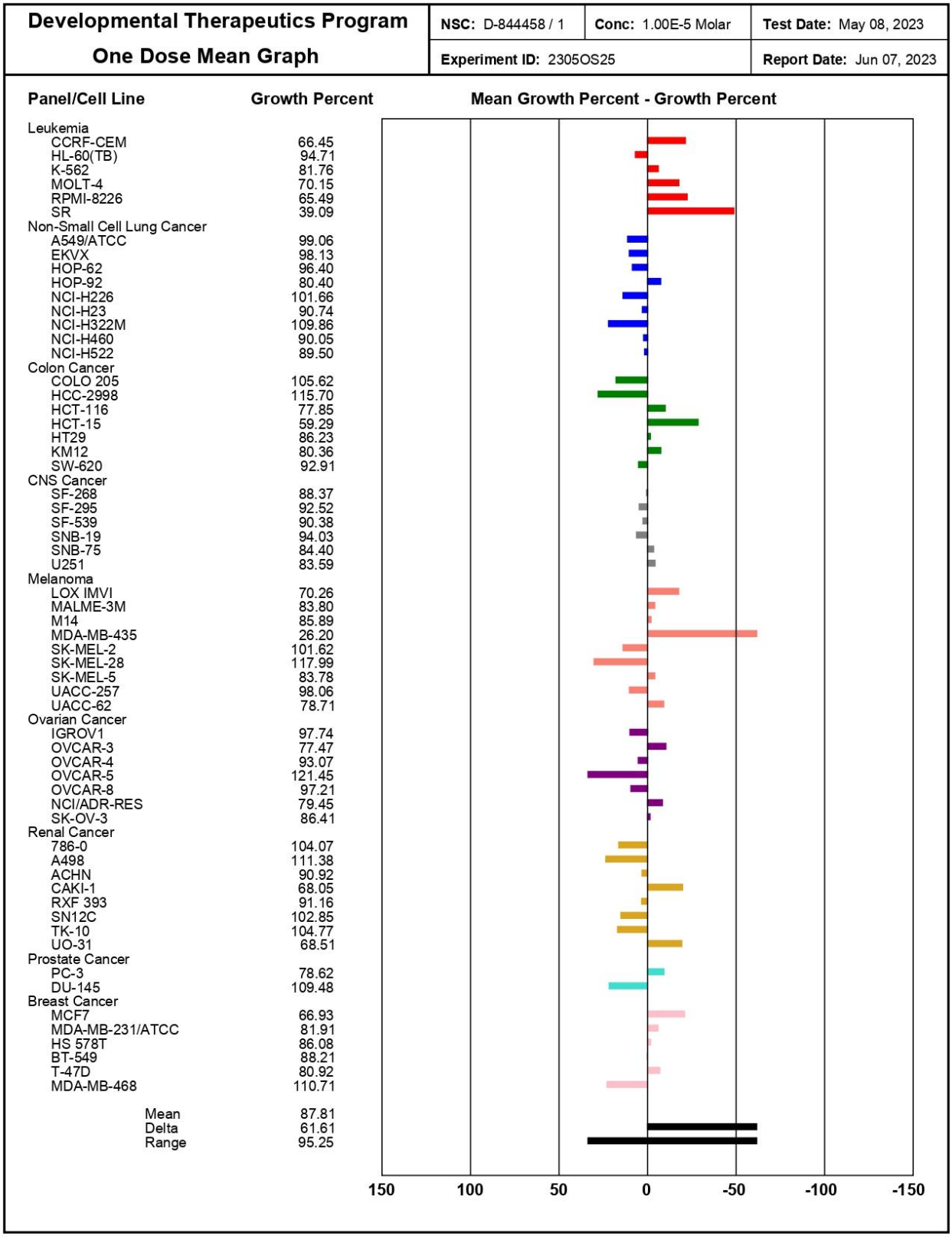


Fig. 6: Representative single dose data of compound **IPP-6**

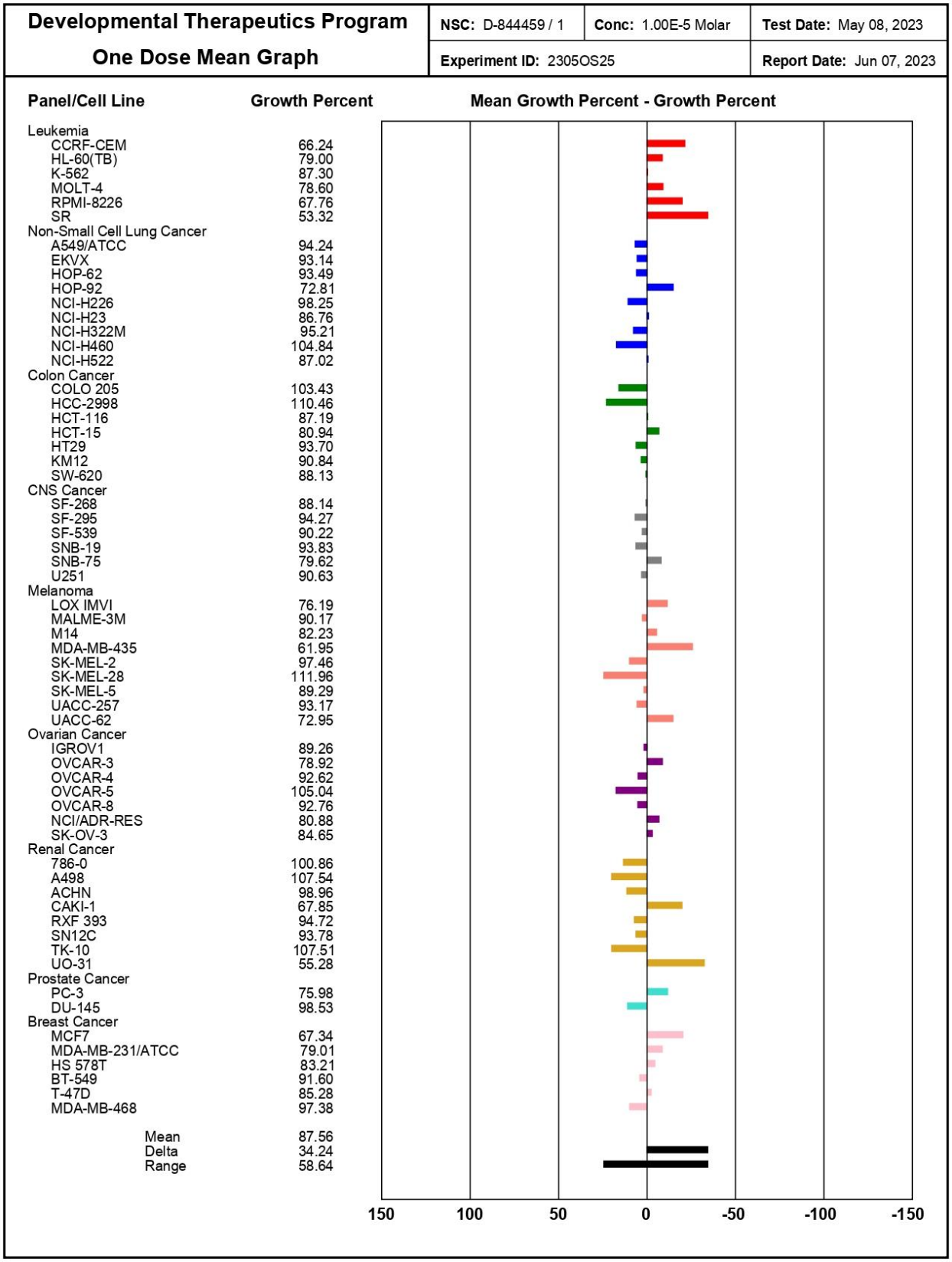


Fig. 7: Representative single dose data of compound **IPP-7**

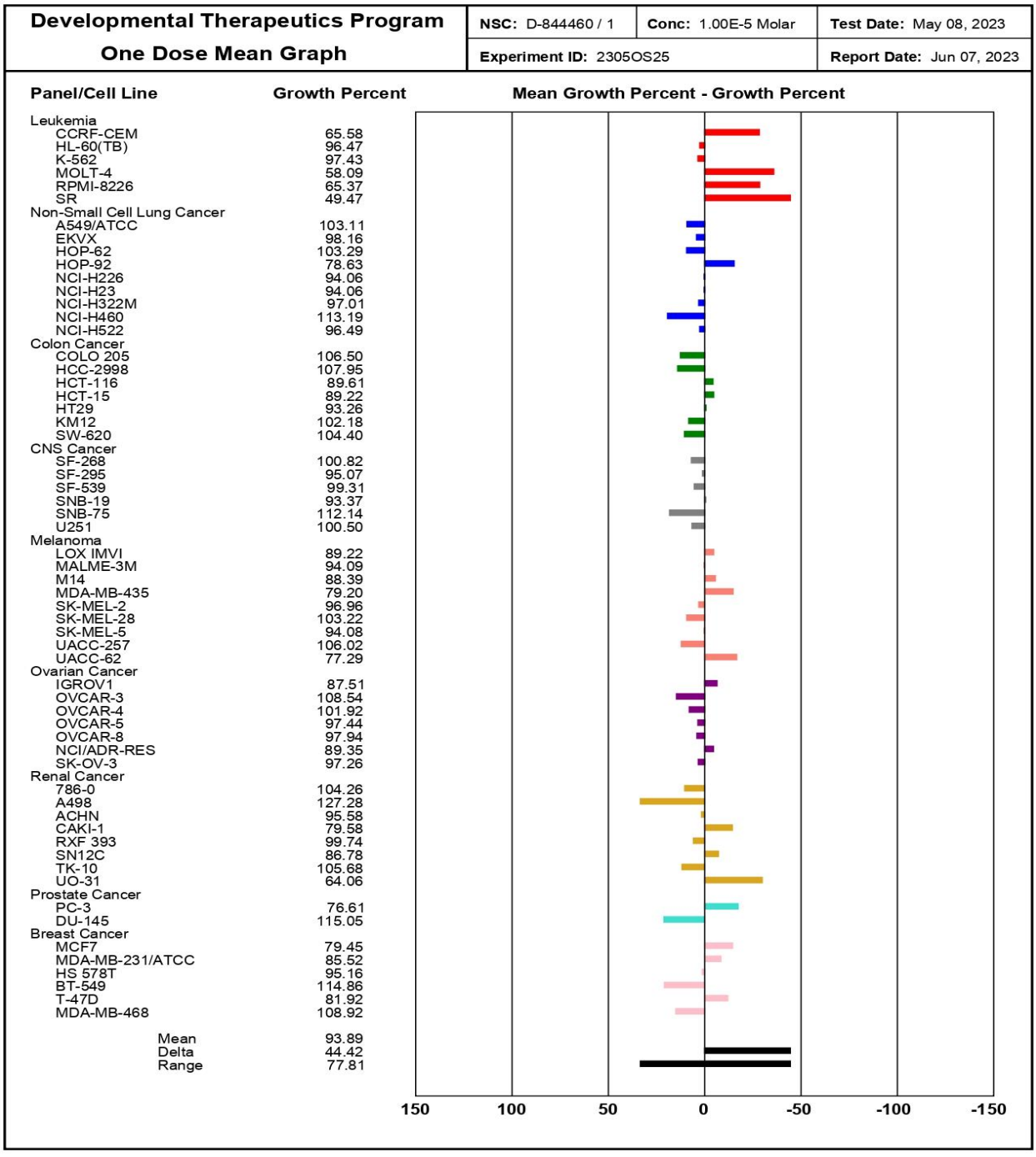


Fig. 8: Representative single dose data of compound **IPP-8**

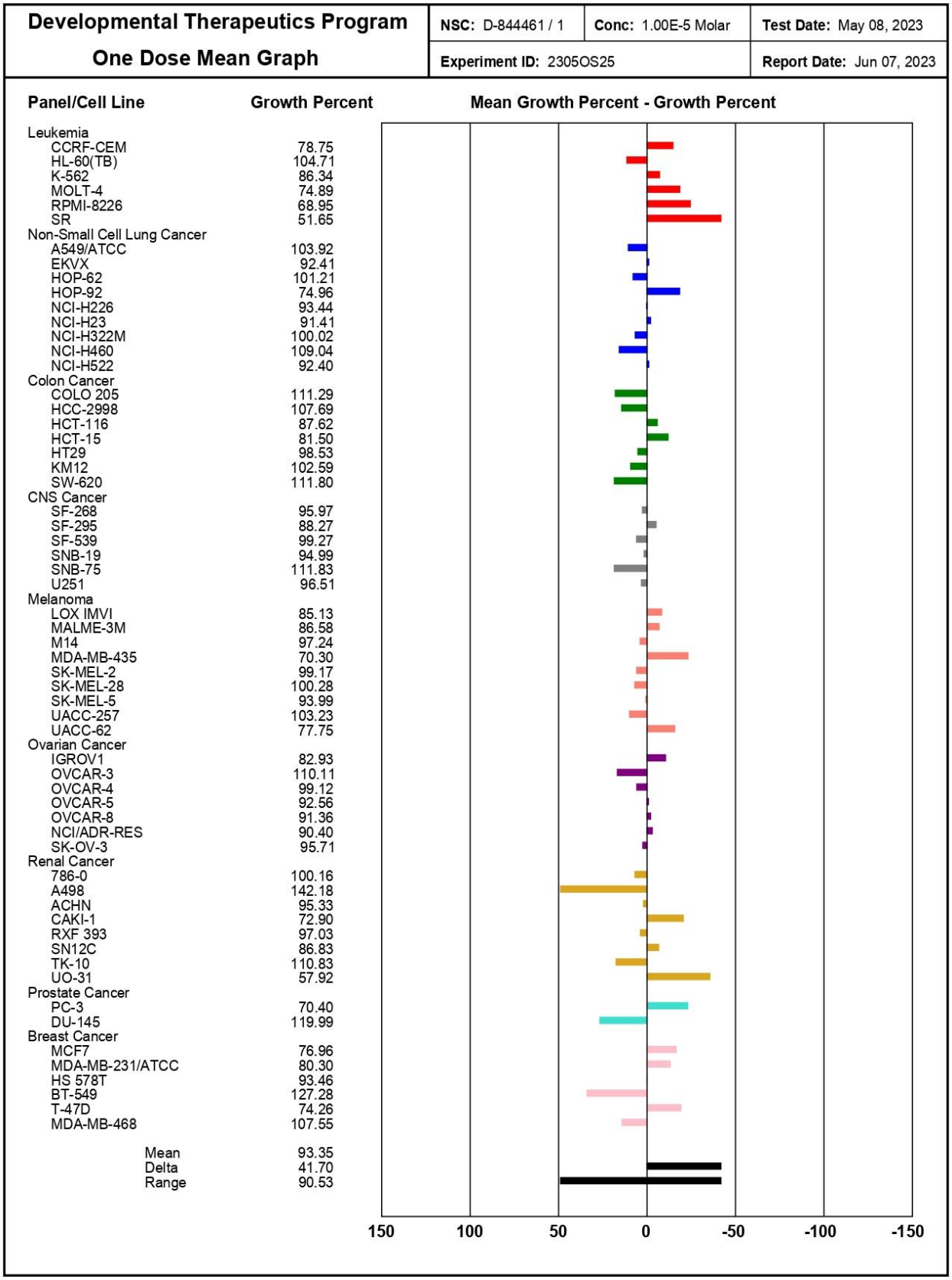


Fig. 9: Representative single dose data of compound **IPP-9**

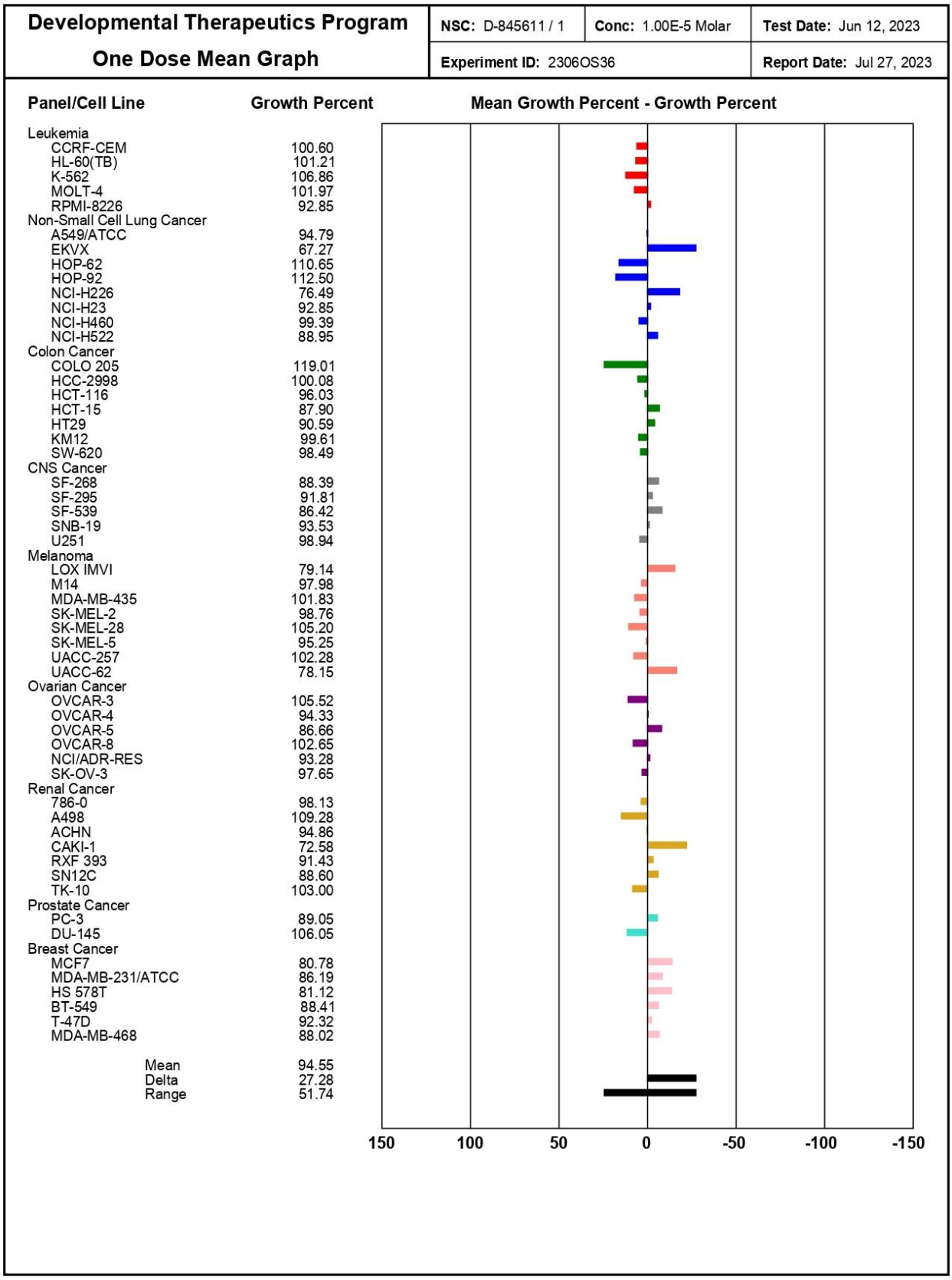


Fig. 10: Representative single dose data of compound **IPP-10**

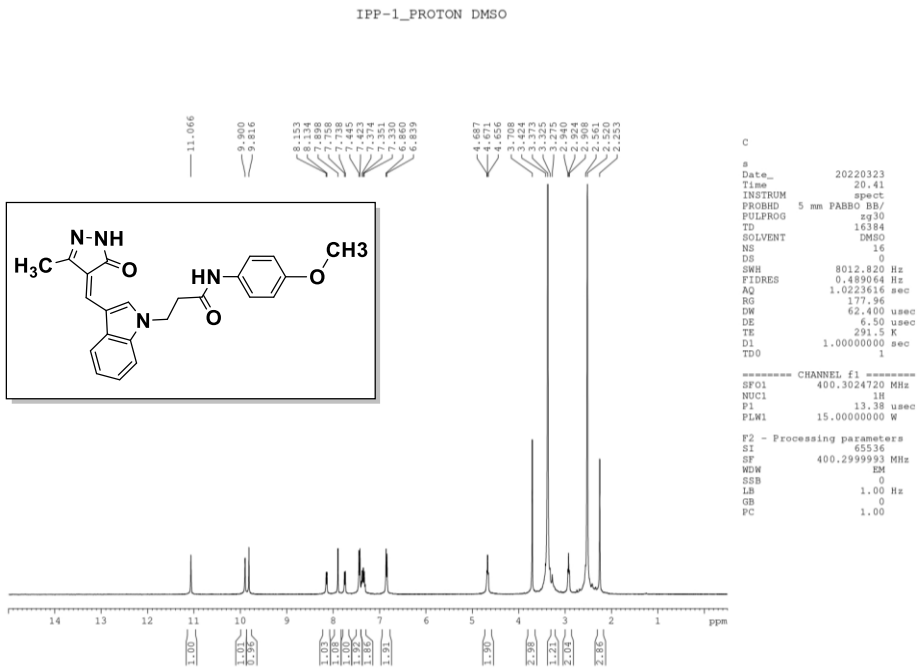


Fig. 11: Representative ^{1}H NMR spectrum of compound **IPP-1**

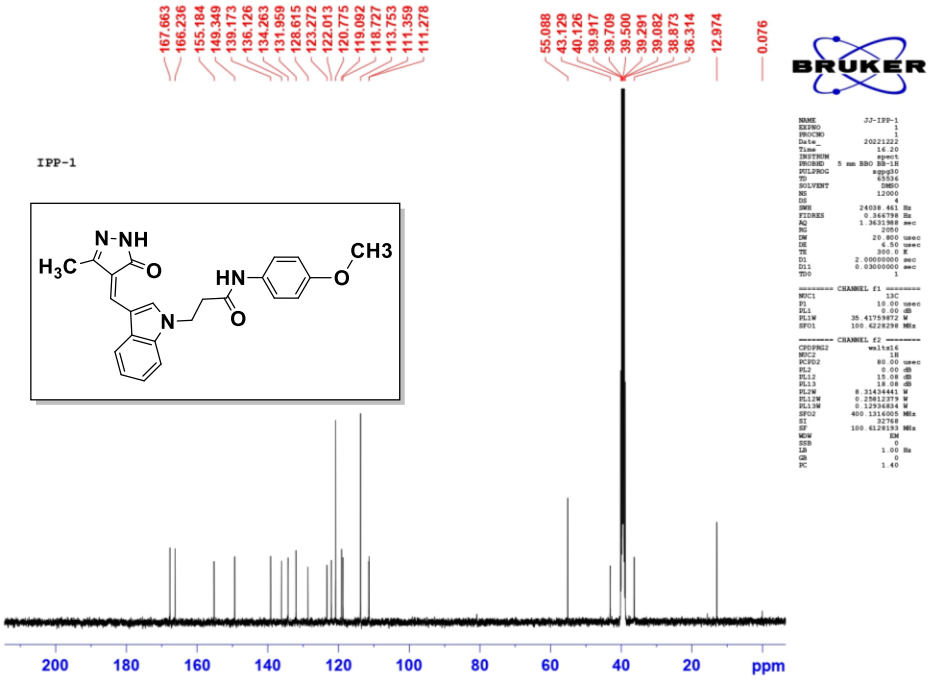
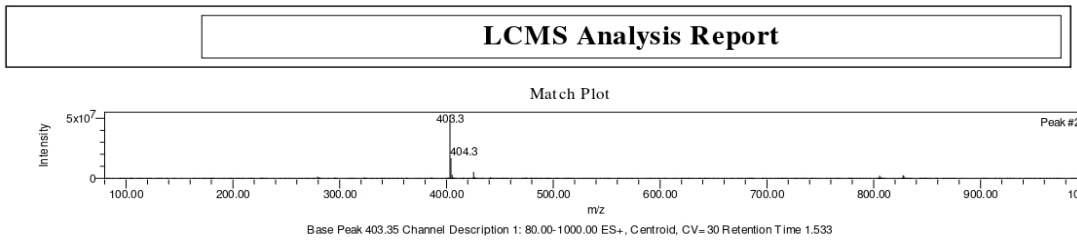
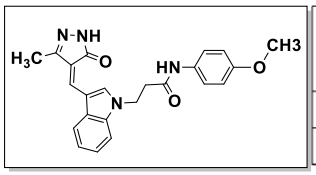


Fig. 12: Representative ^{13}C NMR spectrum of compound **IPP-1**





	Retention Time (min)	Base Peak (m/z)	Height (µV)	Area (µV*sec)	% Area	Channel	Channel Name
1	1.387		544	441	0.73	PDA Spectrum	254.0nm
2	1.512		85611	60283	99.27	PDA Spectrum	254.0nm

Fig. 13: Representative LCMS data of compound **IPP-1**

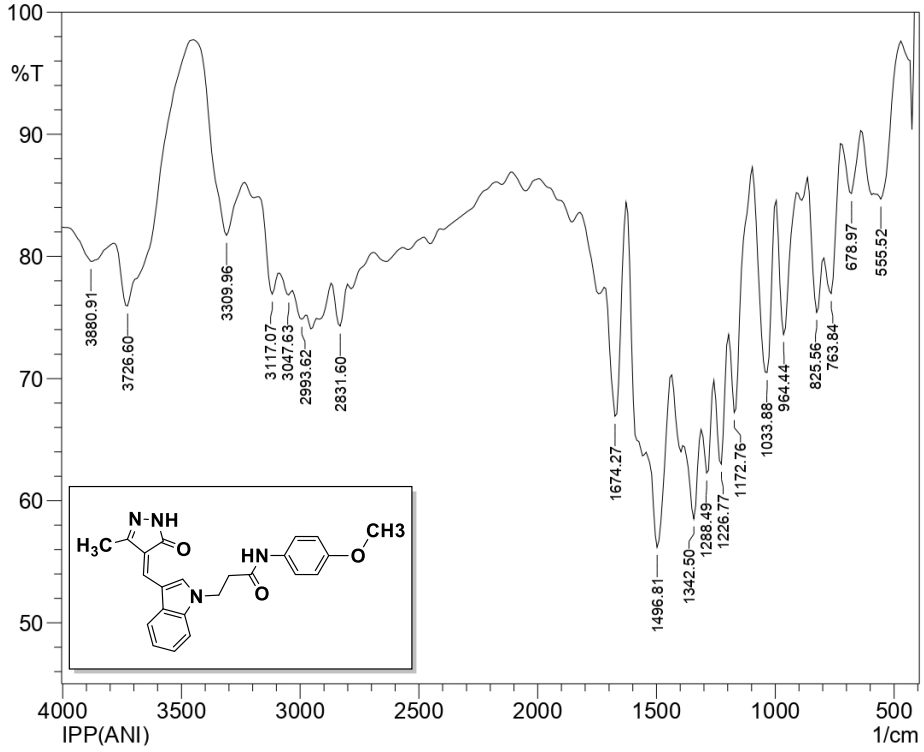


Fig. 14: Representative IR spectrum of compound **IPP-1**

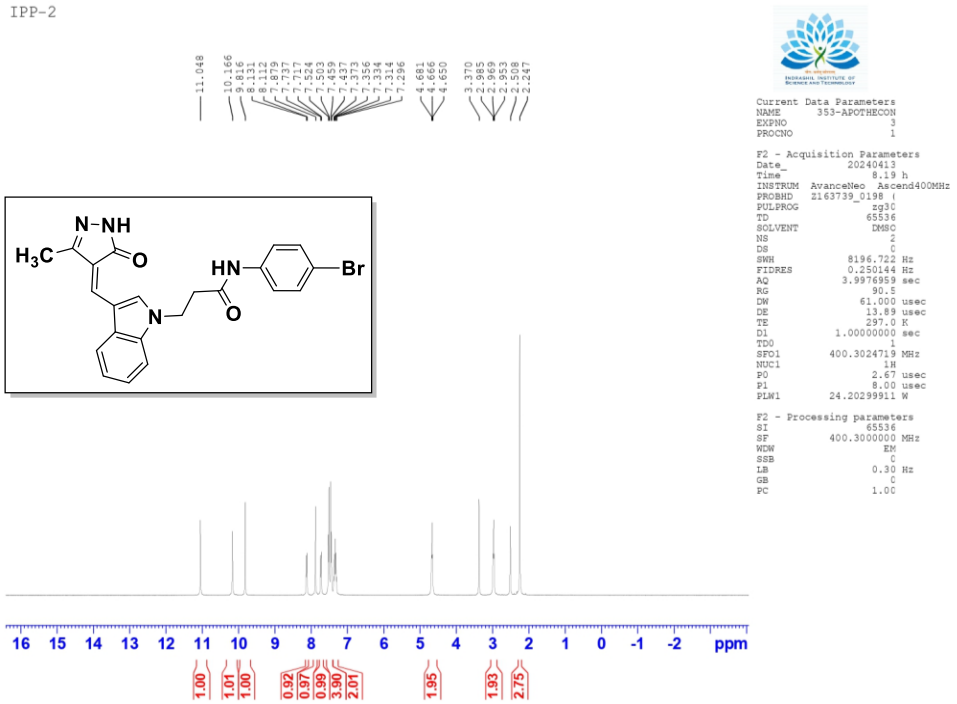


Fig. 15: Representative ^{1}H NMR spectrum of compound **IPP-2**

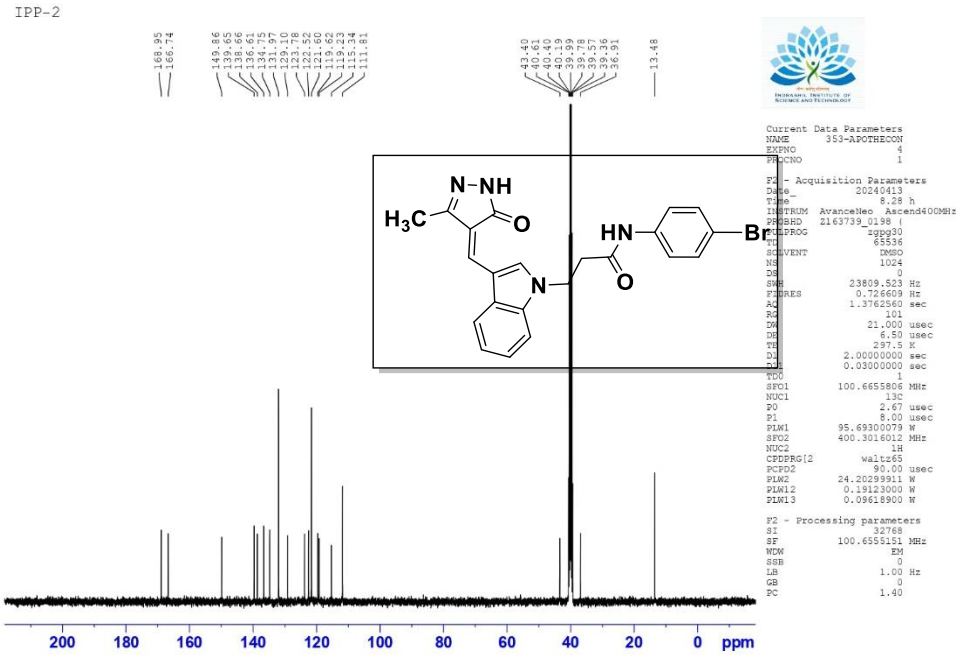


Fig. 16: Representative ^{13}C NMR spectrum of compound **IPP-2**

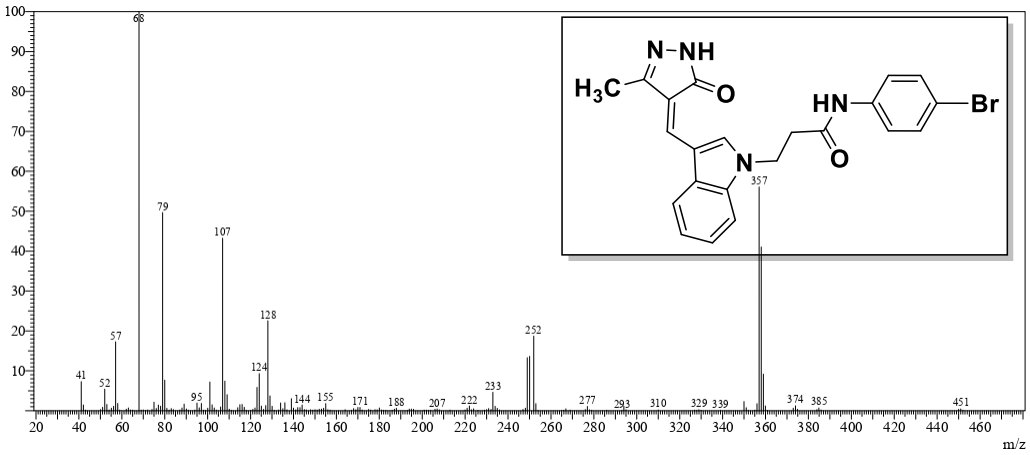


Fig. 17: Representative mass spectrum of compound **IPP-2**

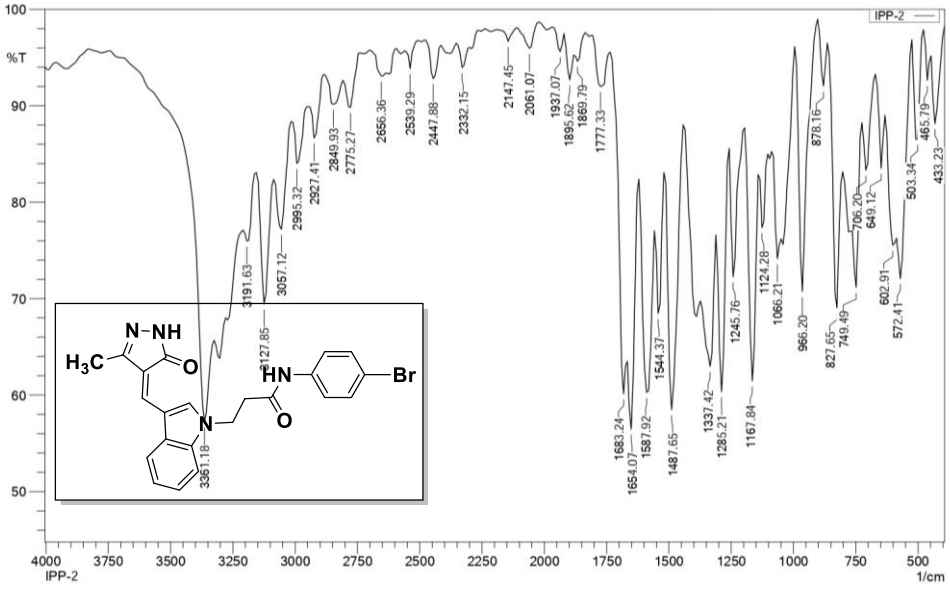


Fig. 18: Representative IR spectrum of compound **IPP-2**

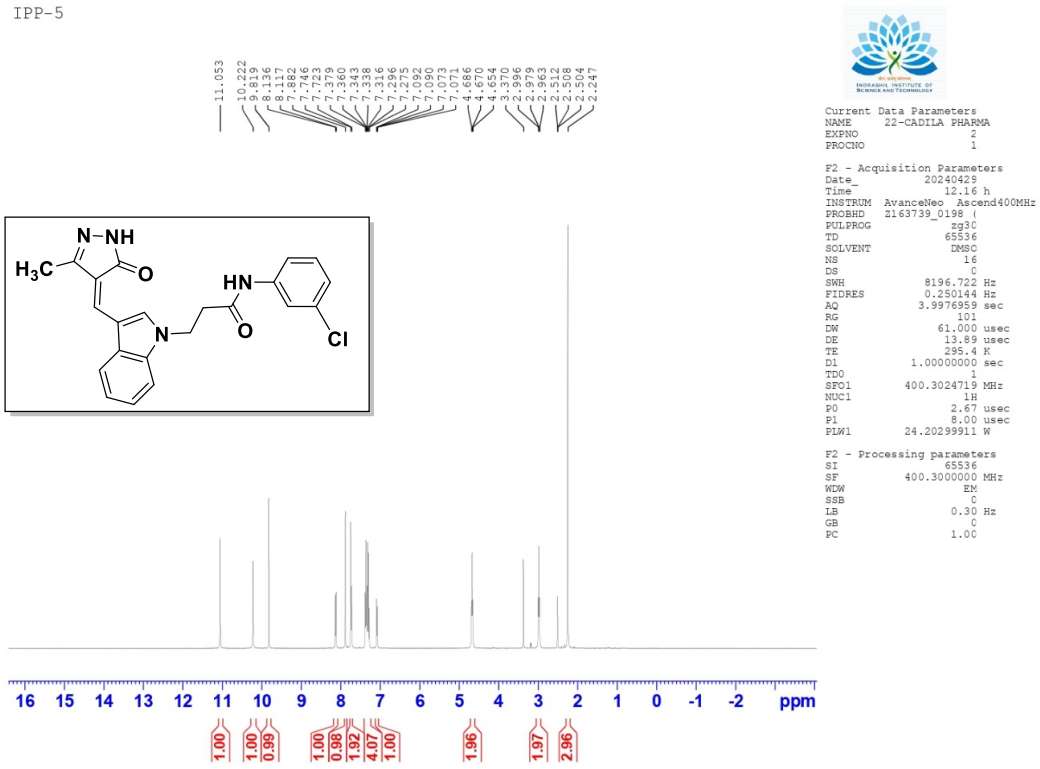


Fig. 19: Representative ^{1}H NMR spectrum of compound **IPP-5**

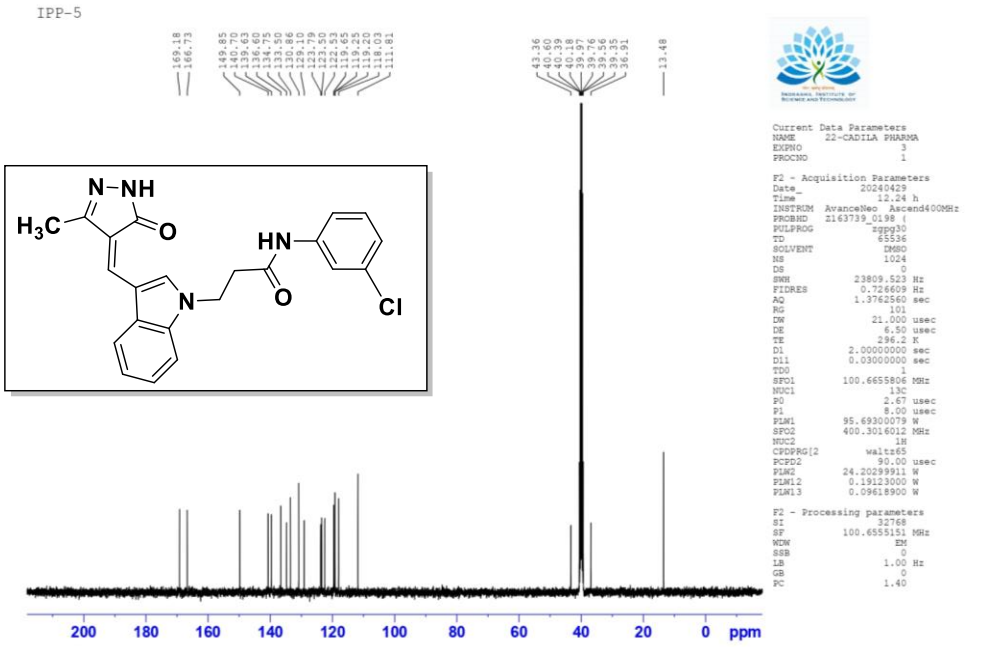


Fig. 20: Representative ^{13}C NMR spectrum of compound **IPP-5**

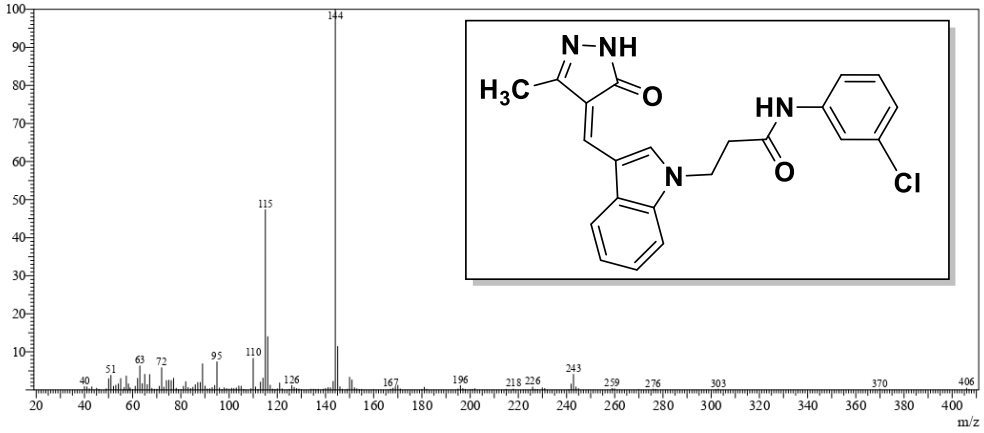


Fig. 21: Representative mass spectrum of compound **IPP-5**

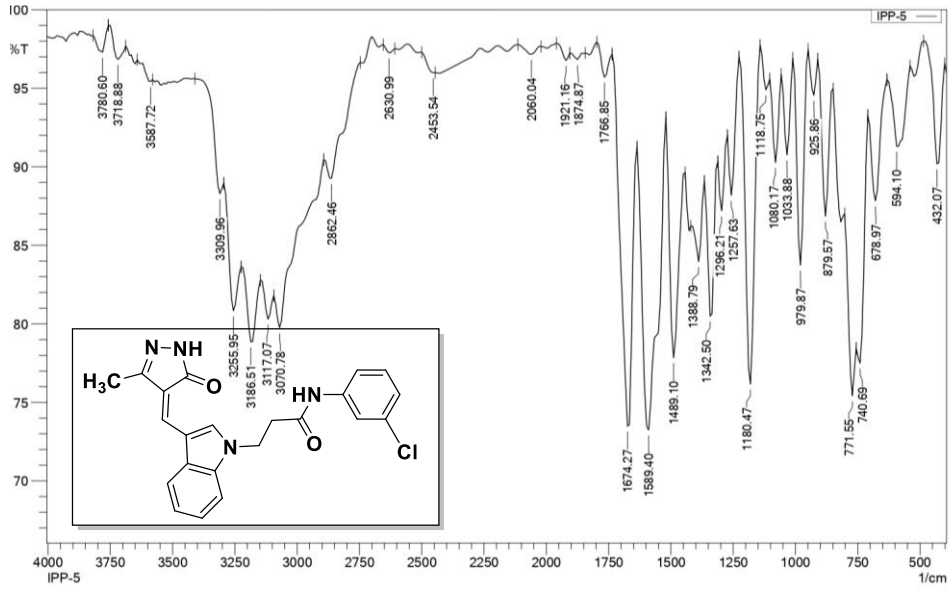


Fig. 22: Representative IR spectrum of compound **IPP-5**

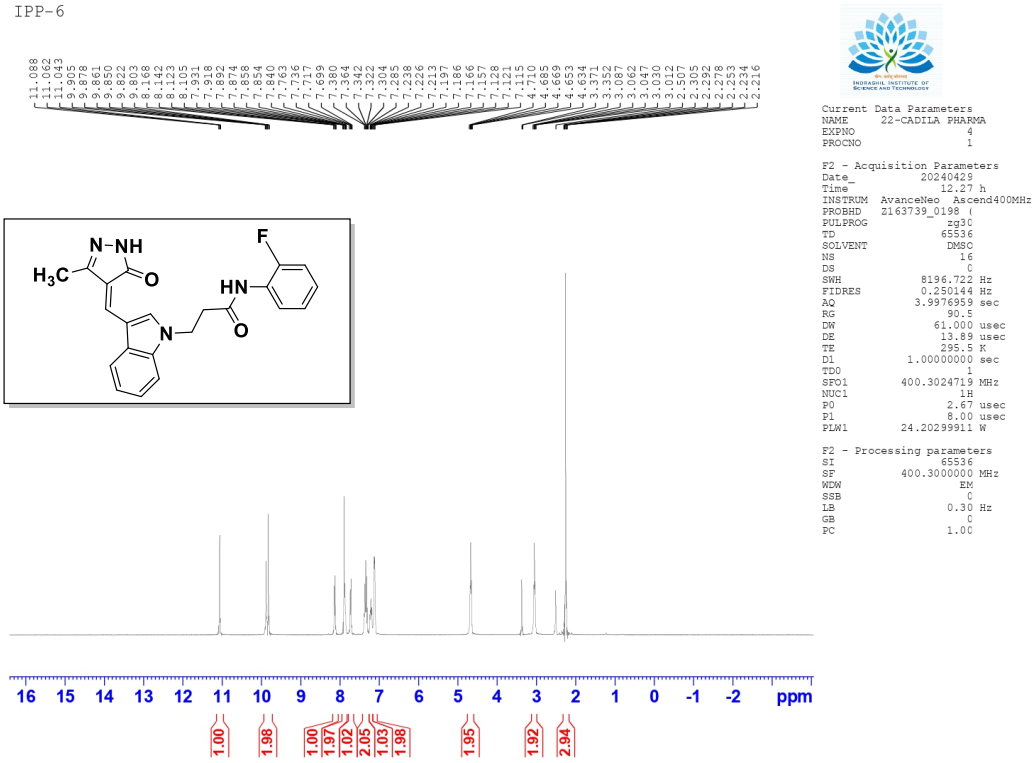


Fig. 23: Representative ^{1}H NMR spectrum of compound **IPP-6**

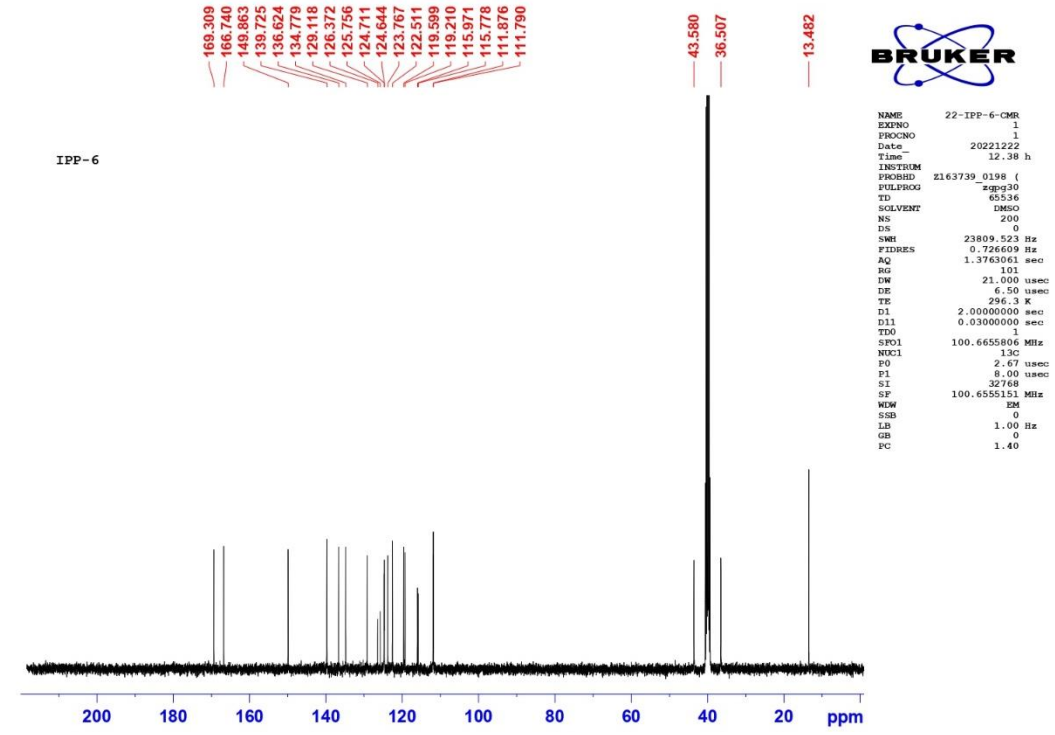
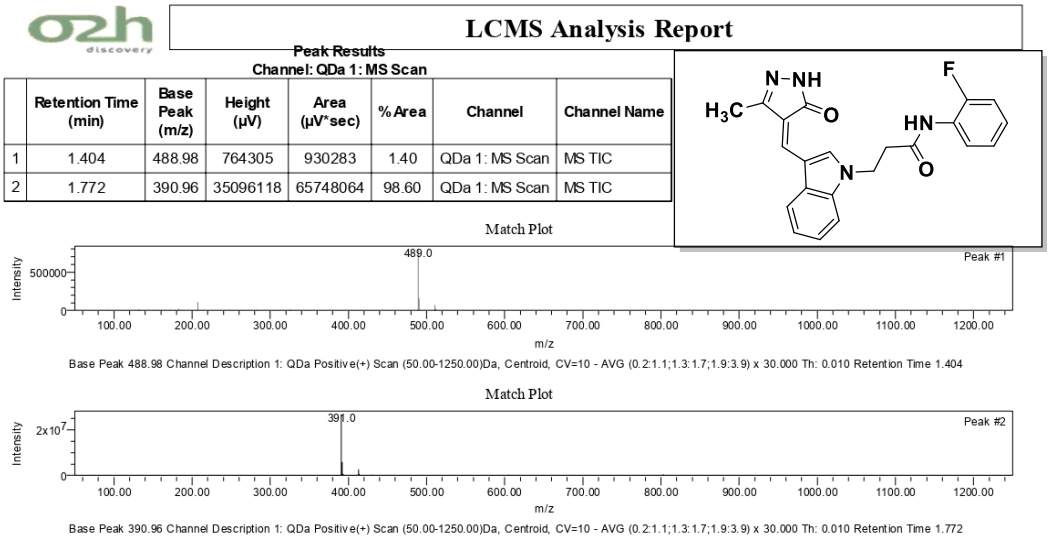


Fig. 24: Representative ^{13}C NMR spectrum of compound **IPP-6**



	Retention Time (min)	Base Peak (m/z)	Height (µV)	Area (µV*sec)	% Area	Channel	Channel Name
1	1.404	488.98	764305	930283	1.40	QDa 1: MS Scan	MS TIC
2	1.772	390.96	35096118	65748064	98.60	QDa 1: MS Scan	MS TIC

Base Peak 488.98 Channel Description 1: QDa Positive(+) Scan (50.00-1250.00)Da, Centroid, CV=10 - AVG (0.2:1.1;1.3:1.7;1.9:3.9) x 30.000 Th: 0.010 Retention Time 1.404

Base Peak 390.96 Channel Description 1: QDa Positive(+) Scan (50.00-1250.00)Da, Centroid, CV=10 - AVG (0.2:1.1;1.3:1.7;1.9:3.9) x 30.000 Th: 0.010 Retention Time 1.772

Fig. 25: Representative LCMS data of compound **IPP-6**

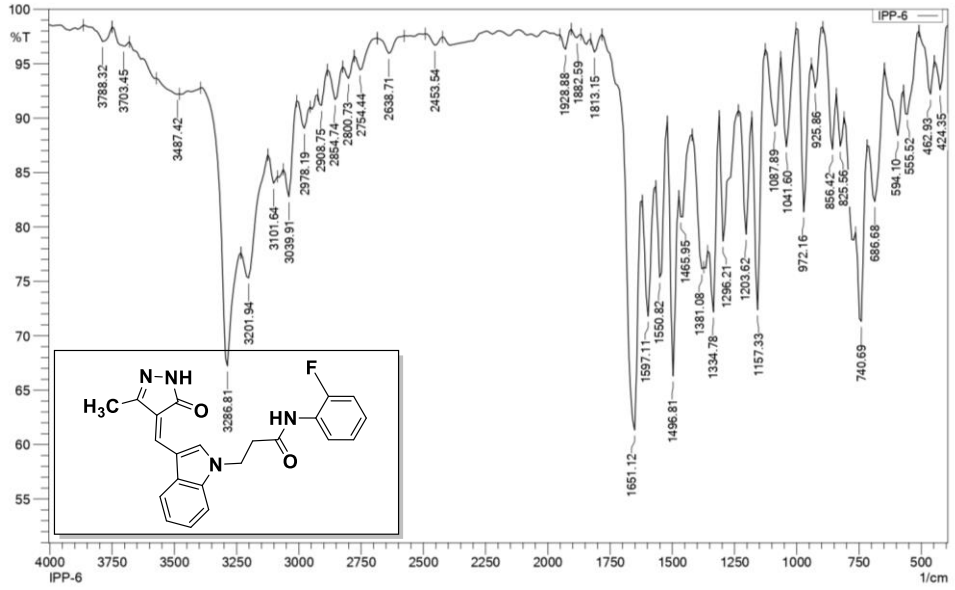


Fig. 26: Representative IR spectrum of compound **IPP-6**

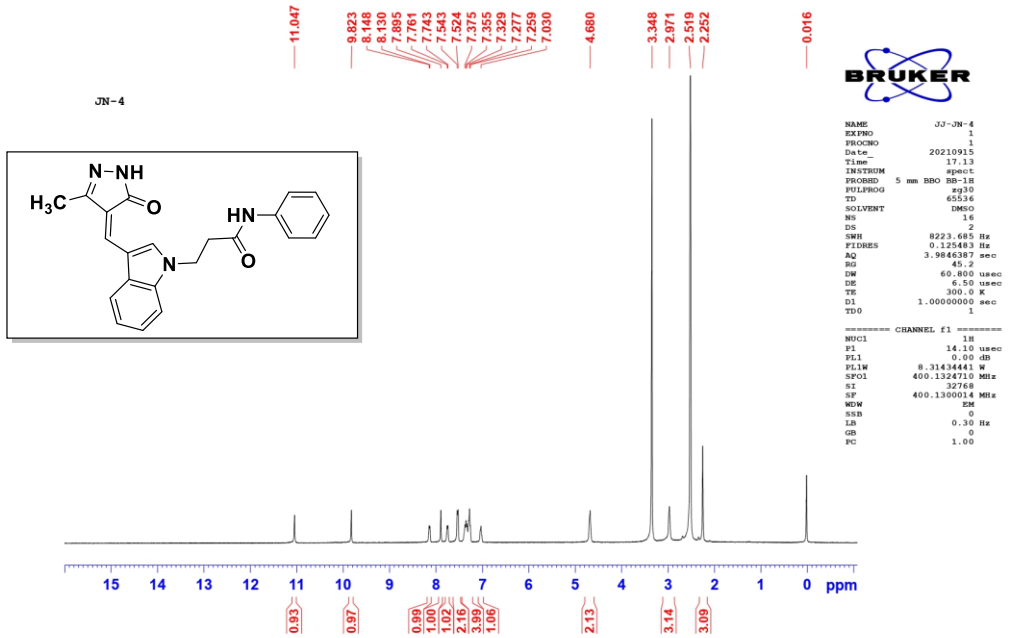


Fig. 27: Representative ^{1}H NMR spectrum of compound **IPP-9**

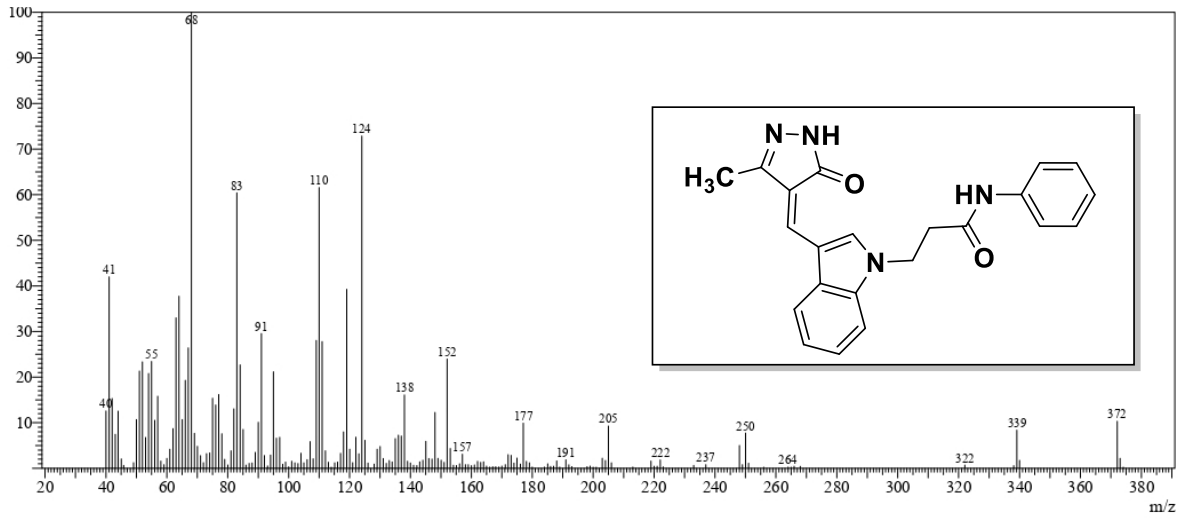


Fig. 28: Representative mass spectrum of compound **IPP-9**

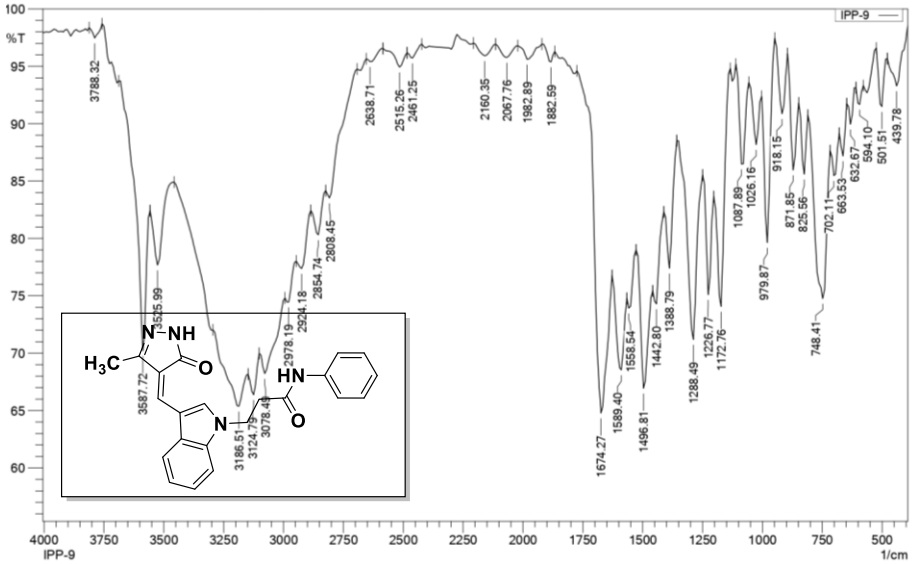


Fig. 29: Representative IR spectrum of compound **IPP-9**

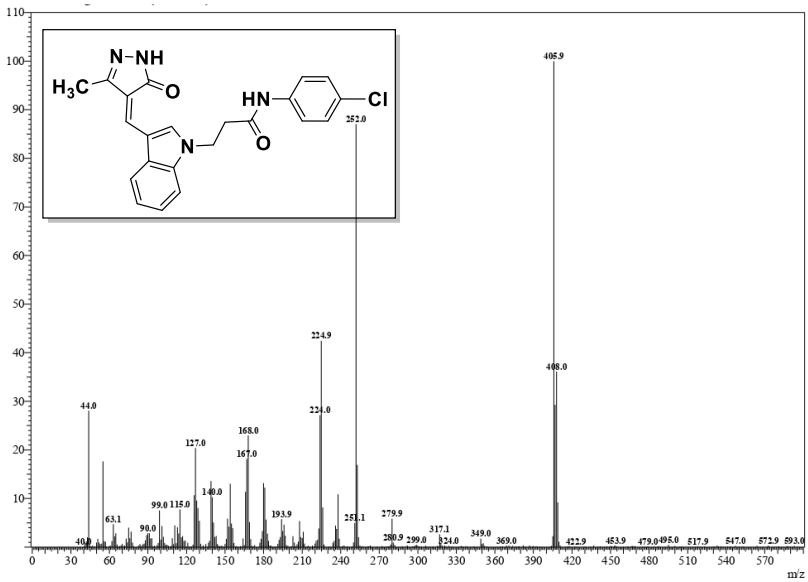


Fig. 30: Representative mass spectrum of compound **IPP-3**

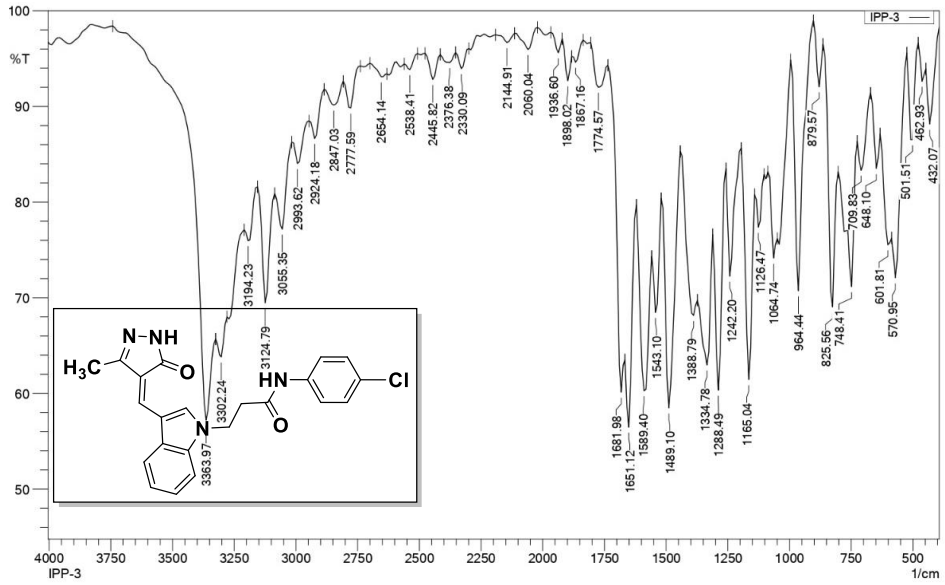


Fig. 31: Representative IR spectrum of compound **IPP-3**

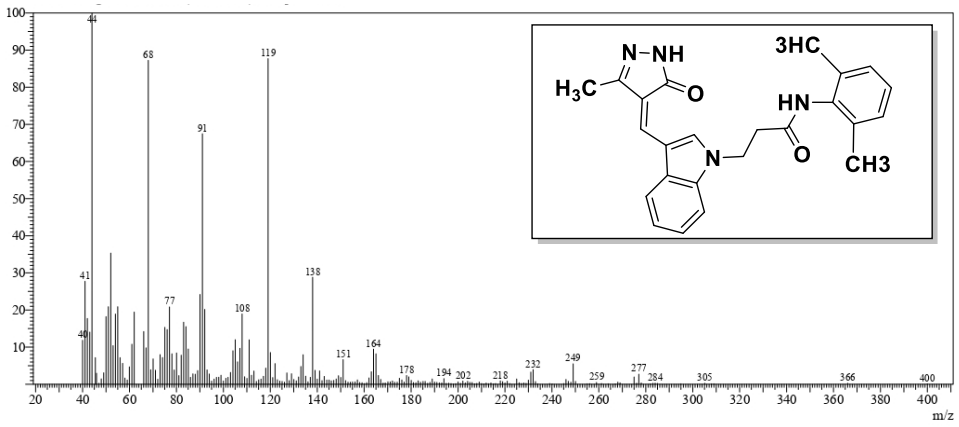


Fig. 32: Representative mass spectrum of compound **IPP-4**

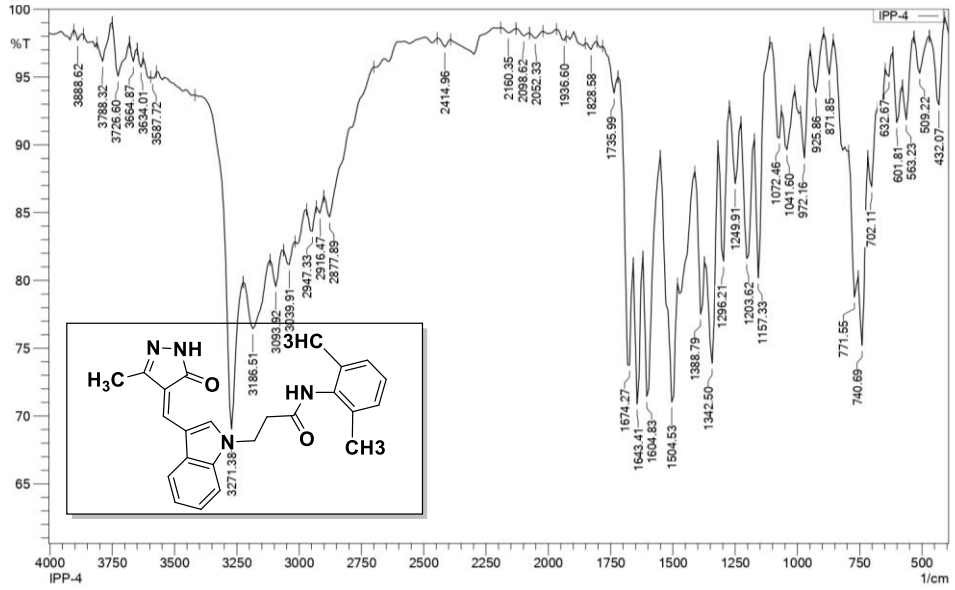


Fig. 33: Representative IR spectrum of compound **IPP-4**

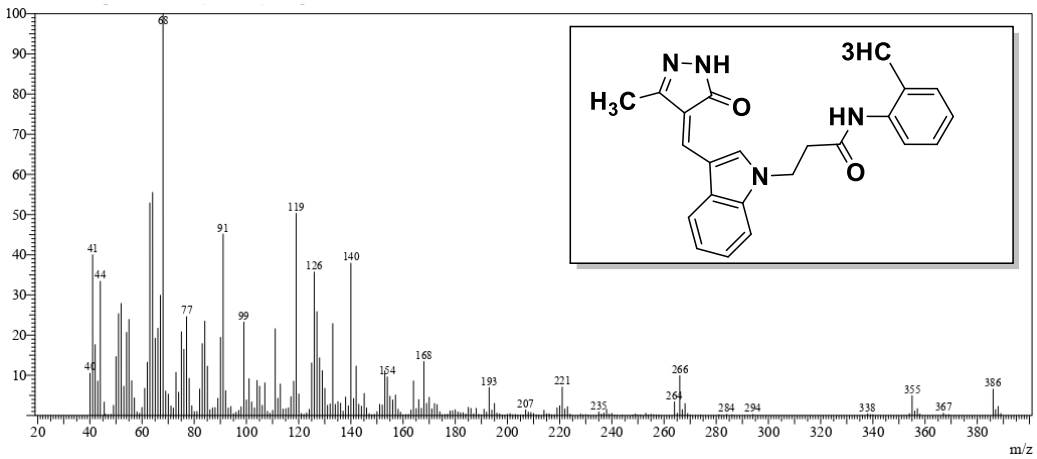


Fig. 34: Representative mass spectrum of compound **IPP-7**

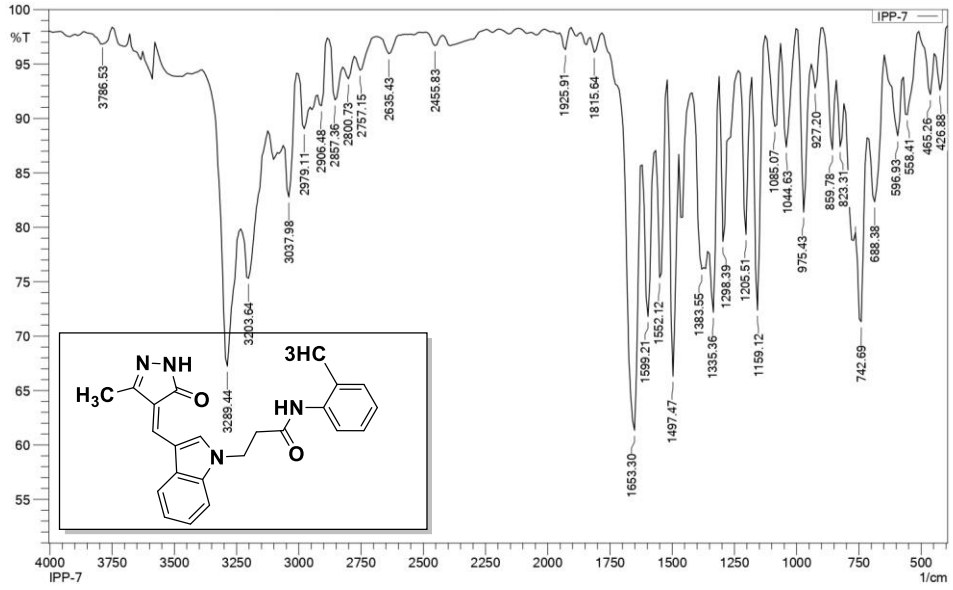


Fig. 35: Representative IR spectrum of compound **IPP-7**

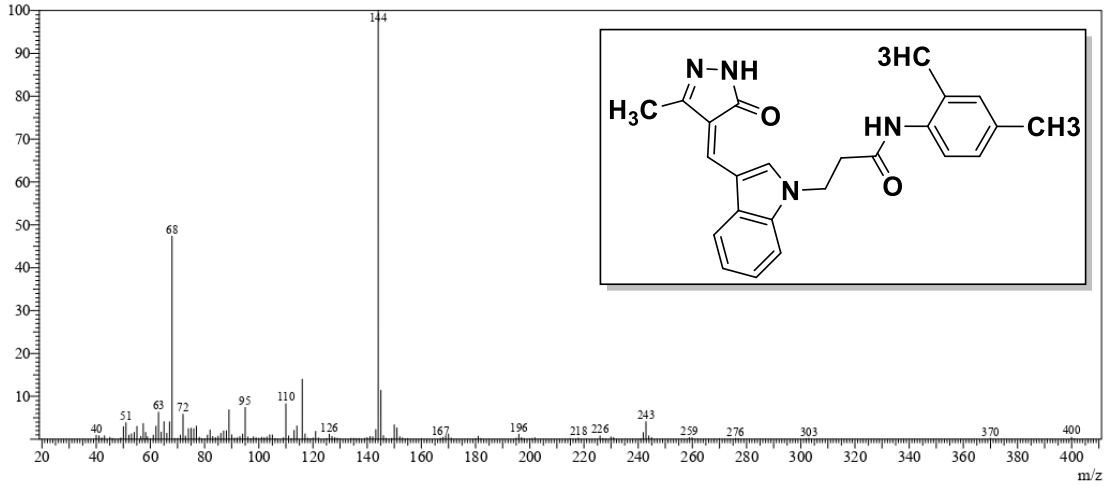


Fig. 36: Representative mass spectrum of compound **IPP-8**

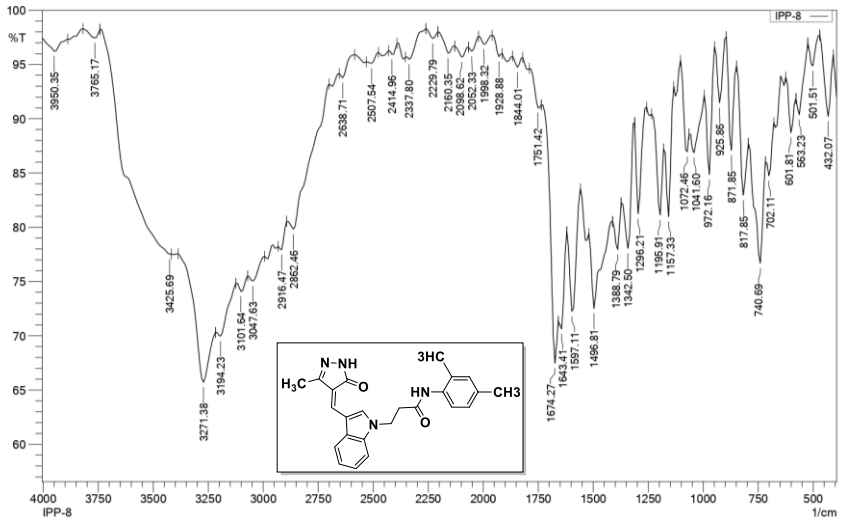


Fig. 37: Representative IR spectrum of compound **IPP-8**

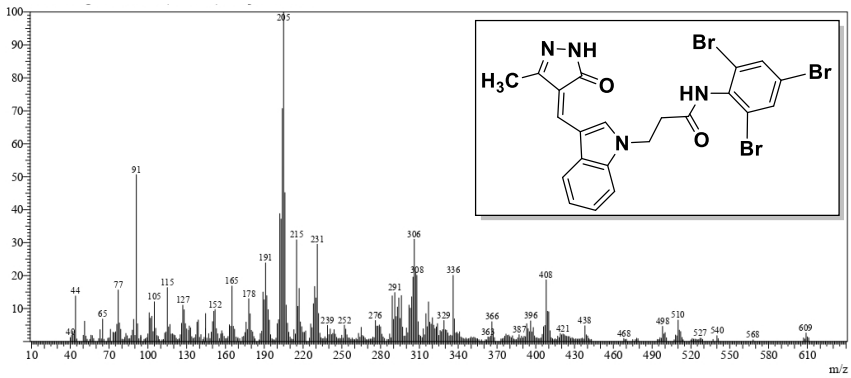


Fig. 38: Representative mass spectrum of compound **IPP-10**

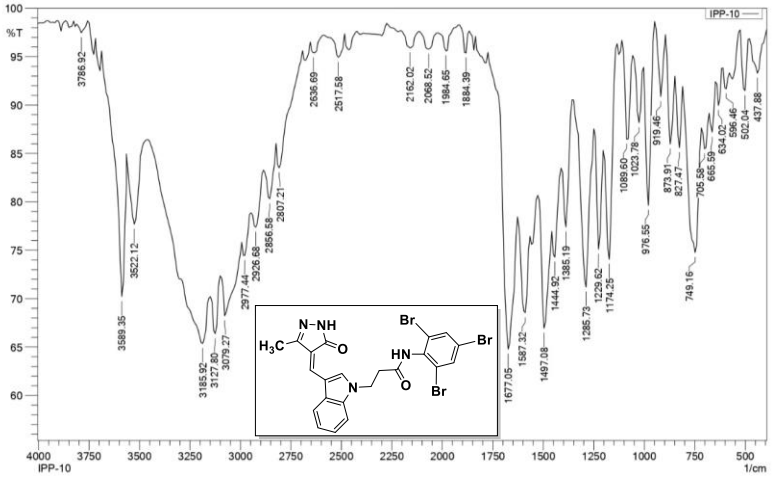


Fig. 39: Representative IR spectrum of compound **IPP-10**

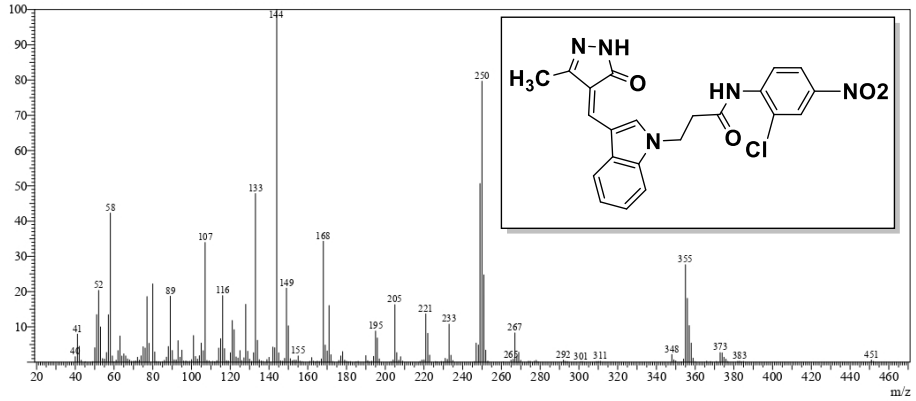


Fig. 40: Representative mass spectrum of compound **IPP-11**

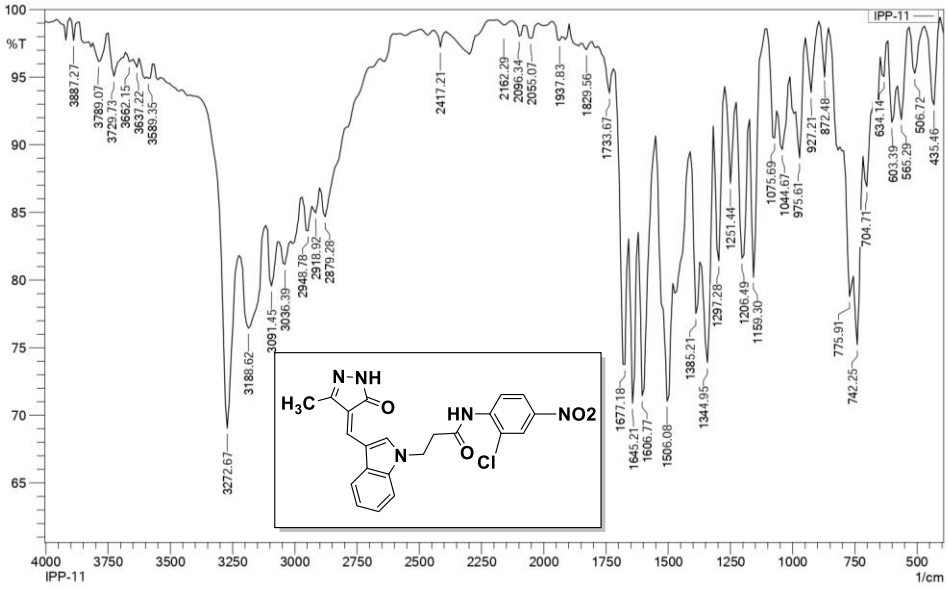


Fig. 41: Representative IR spectrum of compound **IPP-11**

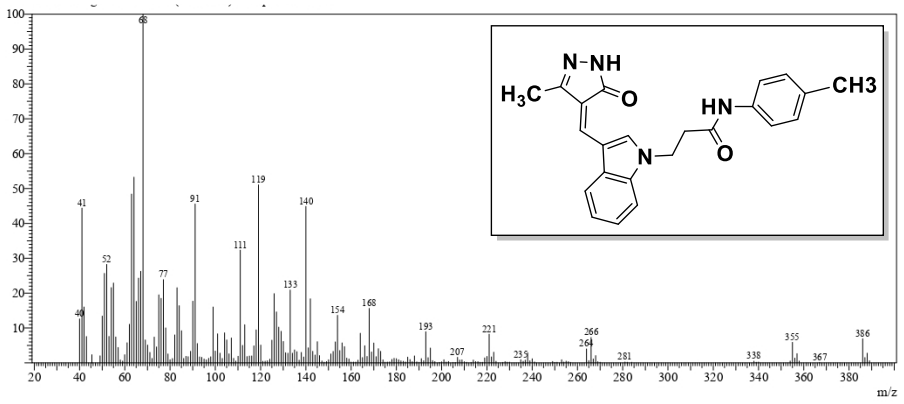


Fig. 42: Representative mass spectrum of compound **IPP-12**

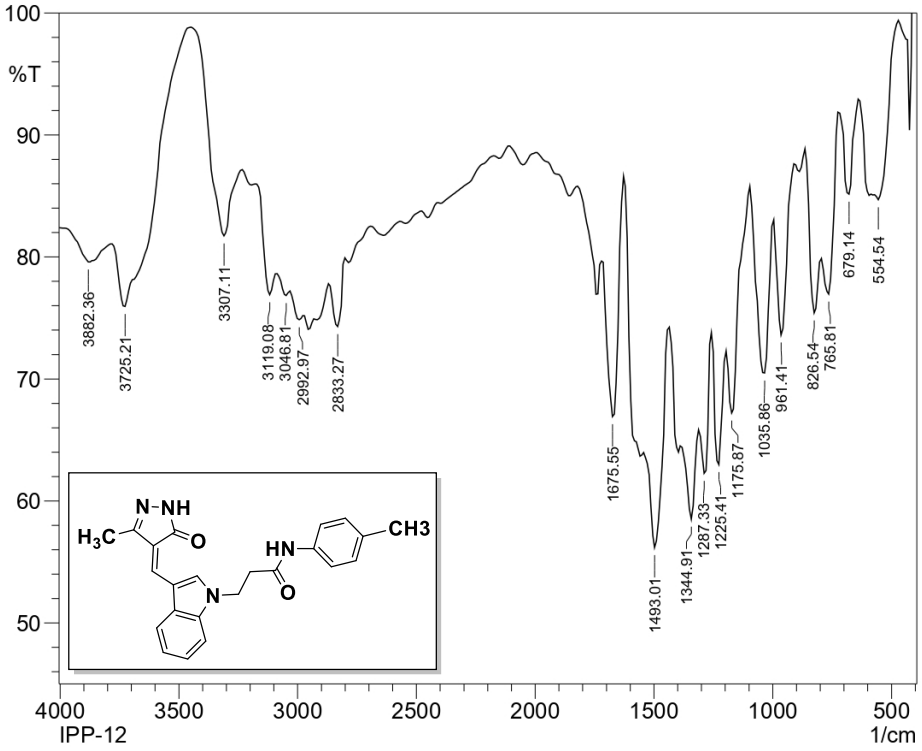


Fig. 43: Representative IR spectrum of compound **IPP-12**

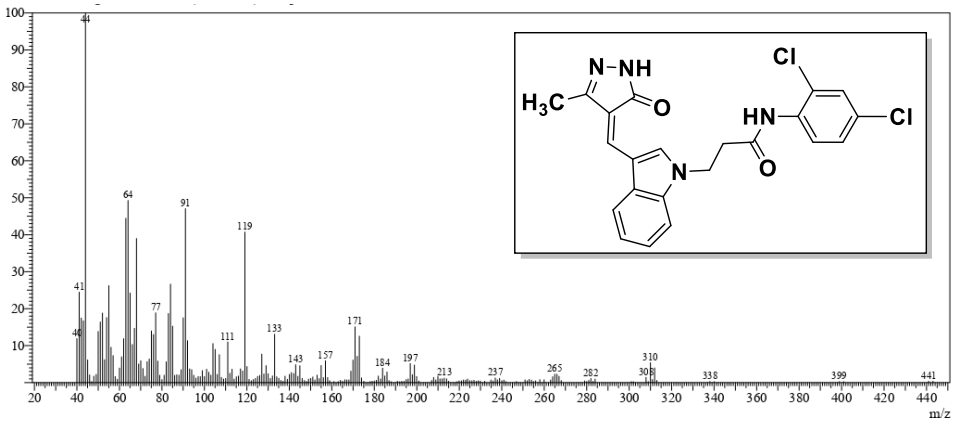


Fig. 44: Representative mass spectrum of compound **IPP-13**

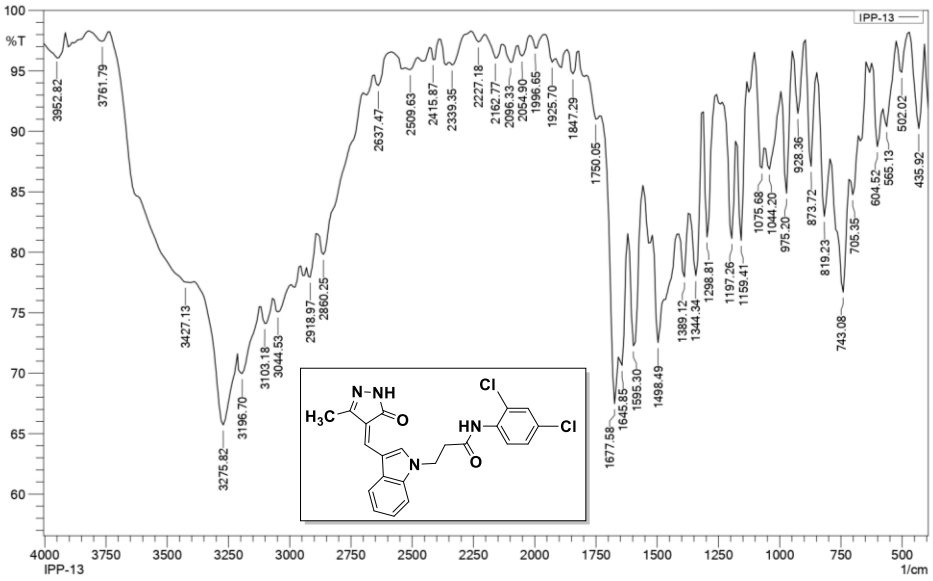


Fig. 45: Representative IR spectrum of compound **IPP-13**

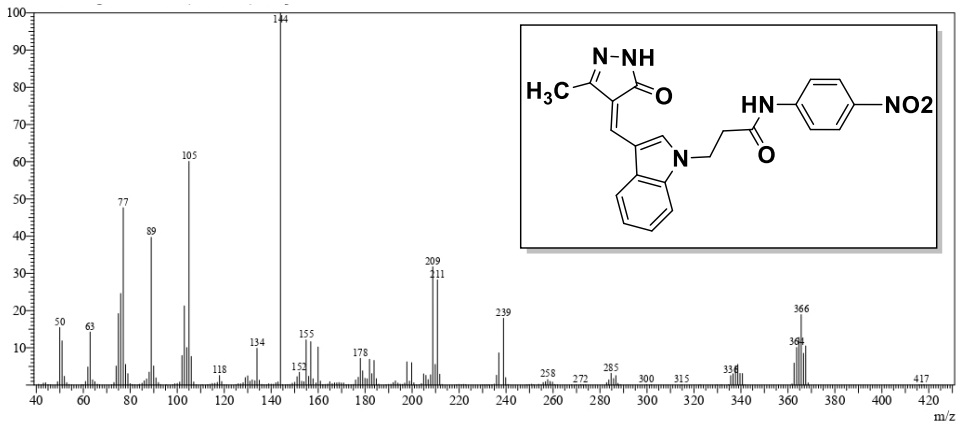


Fig. 46: Representative mass spectrum of compound **IPP-14**

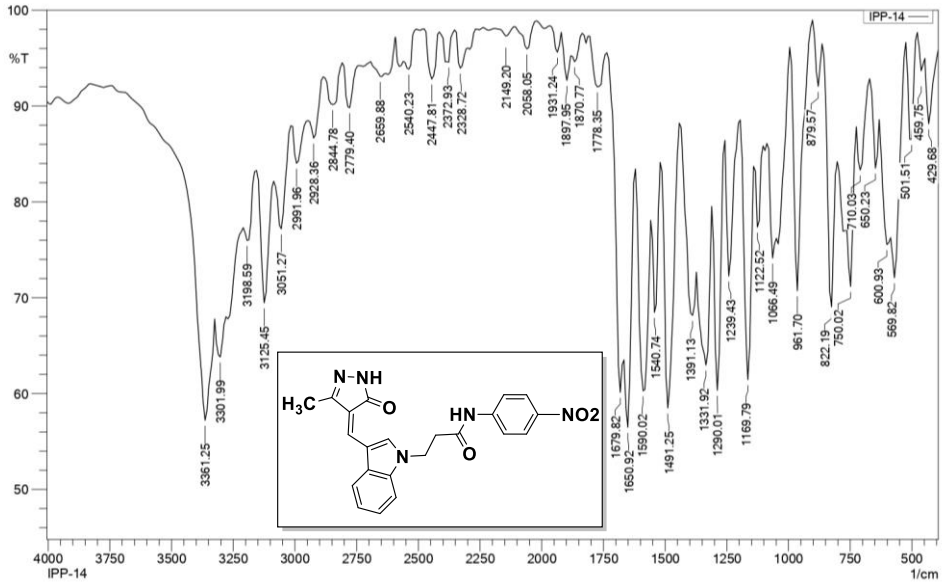


Fig. 47: Representative IR spectrum of compound **IPP-14**

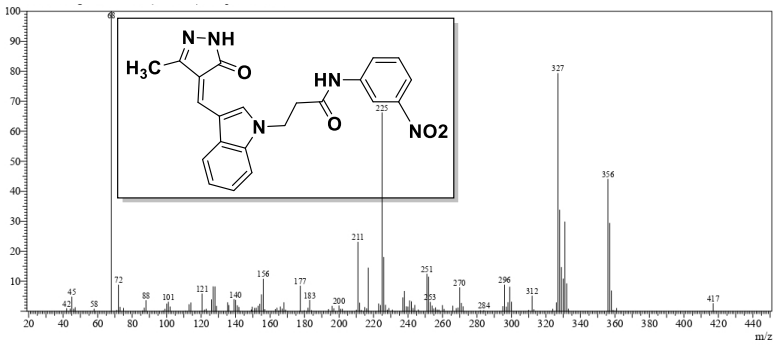


Fig. 48: Representative mass spectrum of compound **IPP-15**

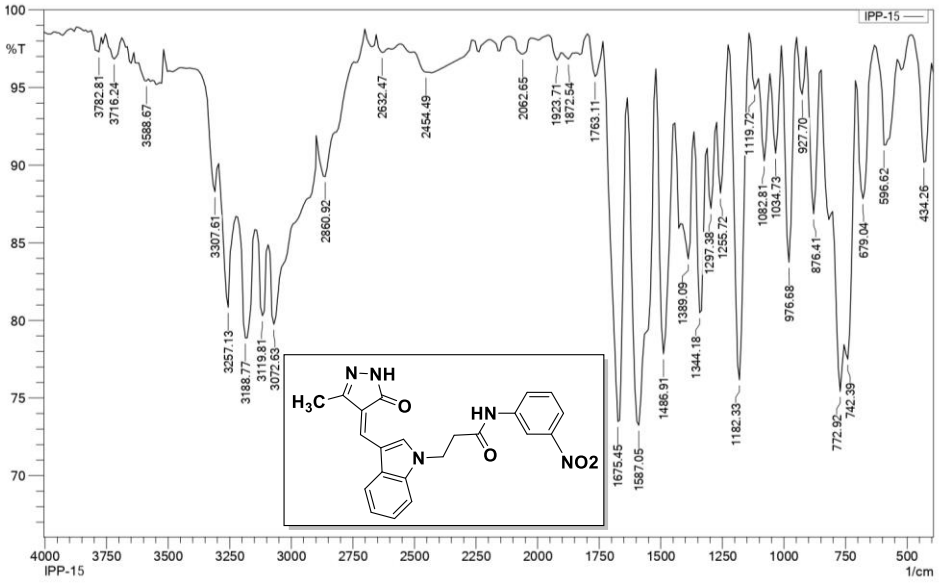


Fig. 49: Representative IR spectrum of compound **IPP-15**