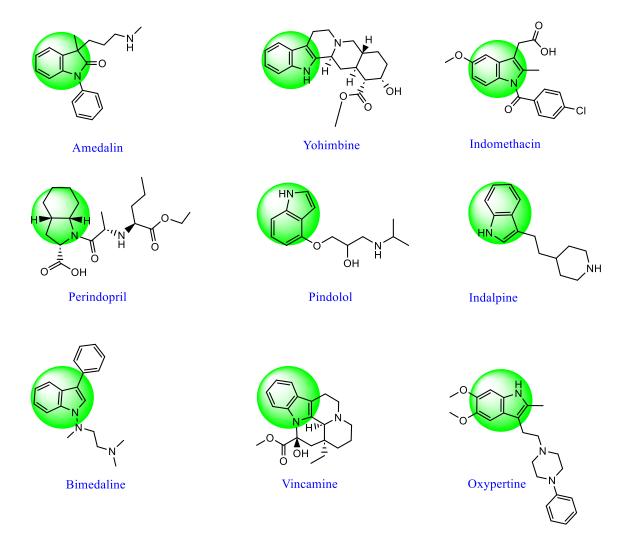
## Chapter 2 Synthesis, Characterization and Anti-Cancer Activity of Indole Derivative

## **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.<sup>23</sup> 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.<sup>24</sup> 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.<sup>25</sup>

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.<sup>26</sup> 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.<sup>27</sup> 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 including anti-cancer,<sup>28</sup> anti-diabetic,<sup>29</sup> anti-tubercular,<sup>30</sup> biological activities, antimicrobial,<sup>31</sup> antifungal,<sup>32</sup> anti-HIV,<sup>33</sup> anti-inflammatory,<sup>34</sup> anticonvulsant,<sup>35</sup> antihypertensive,<sup>36</sup> anti-malarial,<sup>37</sup> anti-leishmanial,<sup>38</sup> anti-hepatitis,<sup>39</sup> antioxidant,<sup>40</sup> and analgesic.<sup>41</sup>

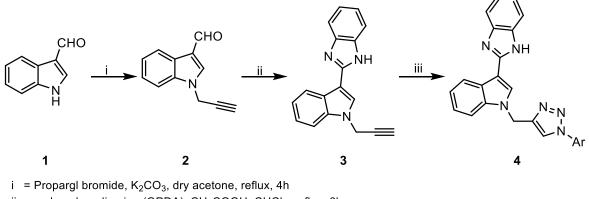
Pyrazole is a flexible chemical molecule having a five-membered ring structure consisting of three carbon and two nitrogen atoms.<sup>42</sup> It is frequently utilized in medicinal chemistry because of its numerous pharmacological properties, which include anticancer,<sup>43</sup> antiinflammatory,<sup>44</sup> anticonvulsant,<sup>45</sup> antimicrobial,<sup>46</sup> antimalarial,<sup>47</sup> antibacterial,<sup>48</sup> antiviral<sup>49</sup> and analgesic activities.<sup>50</sup> 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*<sup>51</sup> describe a method for synthesizing indole-benzimidazole based 1, 2, 3triazole hybrids **4** using microwave assistance in three steps. This involved synthesizing indole-3-carboxaldehyde derivative **2** and indole-benzimidazole derivatives **3** from 1Hindole 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).

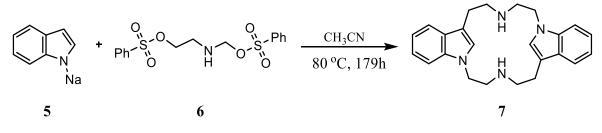


ii = o-phenylenediamine (OPDA), CH<sub>3</sub>COOH, CHCl<sub>3</sub>, reflux, 6h

iii = Ar-N<sub>3</sub>, Cul, DMF/H<sub>2</sub>O (1:3), 80 °C, 12 h or MWI, 180 W, 8 min

#### Scheme 2.1

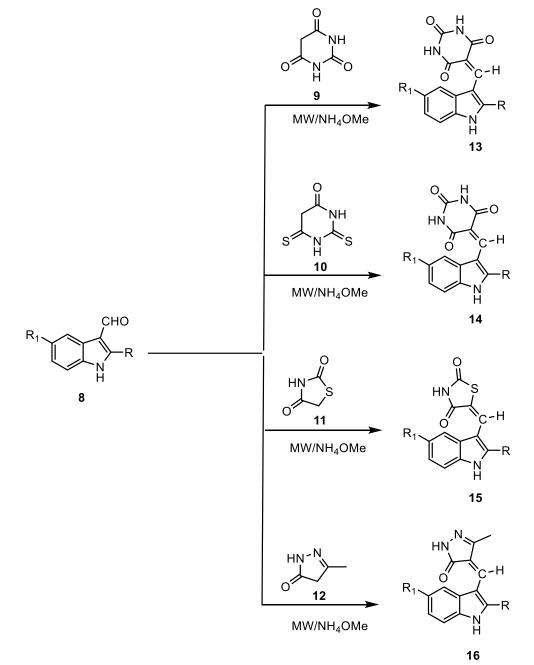
F. Essa *et al*<sup>52</sup>, have described a novel method for synthesizing indole derivatives **7**. This method involves using Dibenzenesulphonyl diethyl amine as a starting material, which acts as an alkylating agent. In the presence of sodium and absolute ethanol, the reaction between indole **5** 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**).





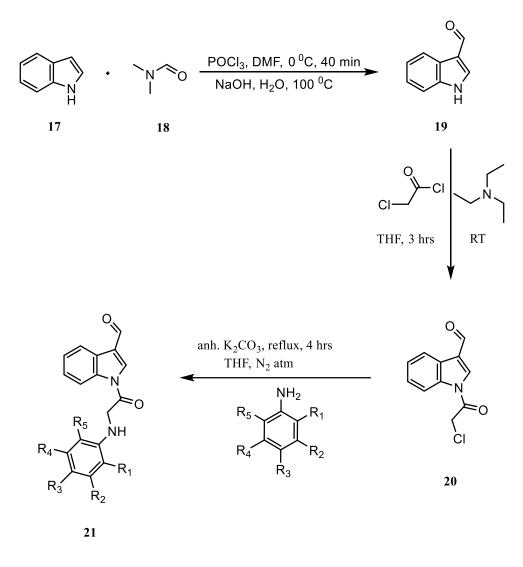
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Biradar *et al*<sup>53</sup> *reported the synthesis of new 2,5-disubstituted indole derivative using an environmentally friendly solvent-free approach.* The synthesis of 2,5-disubstituted indole analogues **13-16** is demonstrated via microwave-assisted 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**).



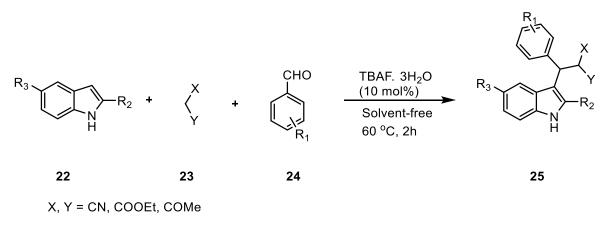
Scheme 2.3

N. Naik *et al*<sup>54</sup> *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*<sup>55</sup> 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.

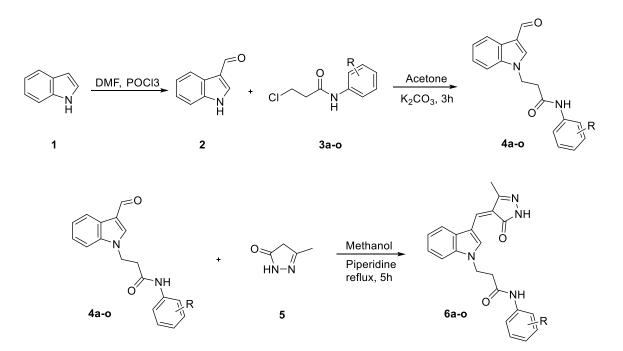


Scheme 2.5

## 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 <sup>1</sup>H NMR, <sup>13</sup>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 <sup>1</sup>H NMR, <sup>13</sup>C NMR mass, and IR spectra.

The <sup>1</sup>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<sub>2</sub> 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

Entry	Solvent	Base <sup>a</sup>	Temp. (C)	Time	Yield (%) <sup>b</sup>	Purification
1	No solvent	_	100	2h	_	_
2	H <sub>2</sub> O	—	reflux	2h		—
3	H <sub>2</sub> O	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

**Table 1:** Optimization of the reaction conditions

<sup>a</sup> Amount of base was 1 equivalent

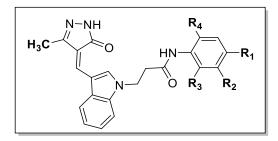
<sup>b</sup> Yield is given for isolated product without purification

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	<b>R</b> 1	<b>R</b> 2	<b>R</b> 3	<b>R</b> 4	Yield	Melting
					(%)	point (°C)
6a	OCH <sub>3</sub>	Η	Н	Η	87	230-232
6b	Br	Η	Н	Н	82	232-234
6c	Cl	Η	Н	Η	78	210-212
6d	Η	Η	CH <sub>3</sub>	CH <sub>3</sub>	78	235-236
6e	Η	Cl	Н	Н	82	240-242
6f	Η	Η	Н	F	80	232-234
6g	Η	Η	Н	CH <sub>3</sub>	75	212-213
6h	CH <sub>3</sub>	Η	Н	CH <sub>3</sub>	72	206-208
6i	Η	Η	Н	Η	65	240-242
6j	Br	Η	Br	Br	84	236-238
6k	NO <sub>2</sub>	Η	Cl	Η	81	229-231
61	CH <sub>3</sub>	Η	Н	Η	79	217-219
6m	Cl	Η	Н	Cl	75	231-231
6n	NO <sub>2</sub>	Η	Н	Η	71	224-226
60	Н	NO <sub>2</sub>	Н	Η	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.

Developmental men	apeutics Program	NSC: D-844454 / 1	Test Date: May 08, 2023		
One Dose Mea	an Graph	Experiment ID: 2305	0825	Report Date: Jun 07, 2023	
Panel/Cell Line	Growth Percent	Mean Growth	Percent - Growth Perc	cent	
Leukemia CCRF-CEM	66.70		_		
HL-60(TB)	66.70 105.47				
K-562	66.82		_		
MOLT-4	94.71				
RPMI-8226	54.66				
SR Non-Small Cell Lung Cancer	41.15		_		
A549/ATCC	74.93				
A549/ATCC EKVX	61.19		-		
HOP-62	-17.26				
HOP-92	-4.26				
NCI-H226 NCI-H23	52.68 68.96				
NCI-H322M	63.76				
NCI-H460	46.33				
NCI-H522	71.09				
Colon Cancer					
COLO 205 HCC-2998	93.02				
HCC-2998 HCT-116	102.40 57.68				
HCT-15	67.41				
HCT-15 HT29	76.92				
KM12	83.79				
SW-620	77.62				
CNS Cancer	32.12				
SF-268 SF-295	51.37		-		
SF-539	13.90				
SNB-19	3.34 10.25				
SNB-75					
U251 Melanoma	11.77				
LOX IMVI	49.90		-		
MALME-3M	8.19				
M14	71.67				
MDA-MB-435 SK-MEL-2	67.35 84.94				
SK-MEL-28	89.50				
SK-MEL-28 SK-MEL-5	69.89				
UACC-257 UACC-62	81.10				
Ovarian Cancer	66.60				
IGROV1	78.42				
OVCAR-3	59.99				
OVCAR-4	34.12				
OVCAR-5 OVCAR-8	84.30 16.65				
NCI/ADR-RES	28.01				
SK-OV-3	68.85				
Renal Cancer					
786-0 A498	58.82 73.71				
ACHN	29.10				
CAKI-1	46.60				
RXF 393 SN12C	43.86				
SN12C	81.60				
TK-10 UO-31	98.81 8.00				
Prostate Cancer					
PC-3	46.86		<b>—</b>		
DU-145	57.42		•		
Breast Cancer MCF7	50.27				
MDA-MB-231/ATCC	44.38				
HS 578T BT-549	-0.85				
BT-549	14.74				
T-47D MDA-MB-468	52.67 61.47				
MDA-WD-+00	01.47				
Mean	54.26				
Delta	71.52 122.73				
Range	122.13				
	150	100 50	0 -50	-100 -150	

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

Atmiya University, Rajkot, Gujarat, India

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.

Cell line				Grow	th of Ce	lls (%) <sup>3</sup>	10 <sup>-5</sup> 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

Table 3: Anticancer activity (GI<sub>50</sub> Values in µM) for compounds 6a-6j in NCI-60 cell line

LICT 15	81.43	67.41	77.68	79.53	86.31	59.29	80.94	89.22	81.50	87.90
HCT-15										
HT29	104.38 91.85	76.92 83.79	89.21 88.09	92.11 86.93	74.22 81.87	86.23 80.36	93.70 90.84	93.26 102.18	98.53 102.59	90.59 99.61
KM12	106.76									
SW-620	100.70	77.6	78.34	86.17	92.82	92.91	88.13	104.40	111.80	98.49
SE 269	101.18	32.12	60.66	CNS C 108.65	<b>ancer</b> 44.47	88.37	88.14	100.82	95.97	88.39
SF-268	91.67	51.37	74.02	96.66	45.07	92.52	94.27	95.07	88.27	91.81
SF-295 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-19 SNB-75	112.79	10.25	114.09	101.45	76.16	84.40	79.62	112.14	111.83	-
U251	100.34	10.23	37.39	92.76	42.25	83.59	90.63	100.50	96.51	98.94
0231	100.54	11.77	51.55	L	_	05.57	90.05	100.50	70.51	70.74
LOX IMVI	91.68	49.90	71.82	Melan 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	-
MALME-3M 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	101.01	97.64	117.99	111.96	103.22	100.28	105.20
SK-MEL-28	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
UACC-02	00.03	00100		Ovarian		/ 01/ 1	, 21, 0	///=>	,,,,,,,	, 0.10
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
			I	Renal C	Cancer	1				
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

series of (Z)-N-aryl-3-(3-((3-methyl-5-oxo-1,5-dihydro-4H-pyrazol-4-Α novel 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-i, 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 singledose 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 (<sup>1</sup>H) spectra on a Bruker AVANCE III spectrometer running at 400 MHz, chemical shifts are expressed in  $\delta$  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**.

#### **Ceneral 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.

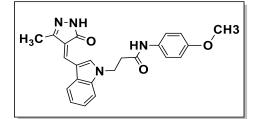
♦ General synthesis of 3-(3-formyl-1*H*-indol-1-yl)-*N*-phenylpropanamide (4a-o) Formylated indole (20 mmol) and a various 3-chloro-N-phenylpropanamide (24 mmol) were dissolved in 20 ml of DMF, and then the mixture was refluxed in the presence of K2CO3 (30 mmol) until TLC indicated that the formylated 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 4aj, which were vacuum filtered and utilized without further purification.

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

A solution containing the molecule 5a-j (20 mmol), 5-methyl-2,4-dihydro-3H-pyrazol-3one (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.

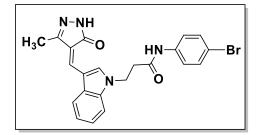
#### (Z)-N-(4-methoxyphenyl)-3-(3-((3-methyl-5-oxo-1,5-dihydro-4H-pyrazol-4-

ylidene)methyl)-1*H*-indol-1-yl)propanamide 6a.



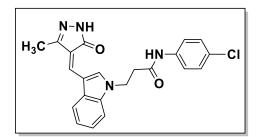
Yield 87%, mp 230-232 °C. IR spectrum, *v*, cm<sup>-1</sup>: 3309.96 (-NH), 2831.60 (CH<sub>3</sub>), 1674.27 (C=O). <sup>1</sup>H NMR spectrum,  $\delta$ , ppm: 2.25 s (3H, *J* = 6.4, -CH3), 2.92 t (3H, *J* = 6.4, -CH2), 3.71 s (3H, -CH3), 4.67 t (2H, *J* = 6.4, -CH2), 6.85 dd (2H, *J* = 8, Ar-H), 7.35 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). <sup>13</sup>C NMR (101 MHz, DMSO)  $\delta$  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<sub>23</sub>H<sub>22</sub>N<sub>4</sub>O<sub>3</sub>. 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.



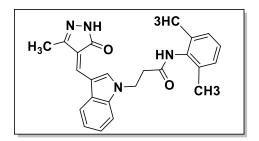
Yield 82%, mp 232-234 °C. IR spectrum, *v*, cm<sup>-1</sup>: 3361.18 (-NH), 2849.93 (CH<sub>3</sub>), 1683.24 (C=O). <sup>1</sup>H NMR spectrum,  $\delta$ , ppm: 2.25 s (3H, -CH3), 2.98 t (2H, *J* = 6.4, -CH2), 4.68 t (2H, *J* = 6, -CH2), 7.29 m (2H, *J* = 7.2 ,Ar-H), 7.52 dd (4H, *J* = 8.4 ,Ar-H), 7.74 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). <sup>13</sup>C NMR (101 MHz, DMSO)  $\delta$  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<sub>22</sub>H<sub>19</sub>BrN<sub>4</sub>O<sub>2</sub>. 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.



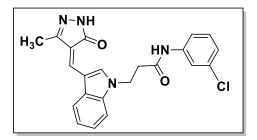
Yield 78%, mp 210-212 °C. IR spectrum, *v*, cm<sup>-1</sup>: 3302.24 (-NH), 2847.03 (CH<sub>3</sub>), 1681.98 (C=O). Found, %: C, 64.89; H, 4.63; N, 13.81. C<sub>22</sub>H<sub>19</sub>ClN<sub>4</sub>O<sub>2</sub>. Calculated, %: C, 64.95; H, 4.71; N, 13.77. *M* 406.

(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.



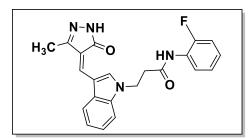
Yield 78%, mp 235-236°C. IR spectrum, *v*, cm<sup>-1</sup>: 3271.38 (-NH), 2877.89 (CH<sub>3</sub>), 1674.27 (C=O). Found, %: C, 71.91; H, 6.11; N, 13.93. C<sub>24</sub>H<sub>24</sub>N<sub>4</sub>O<sub>2</sub>. Calculated, %: C, 71.98; H, 6.04; N, 13.99. *M* 400.

(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.



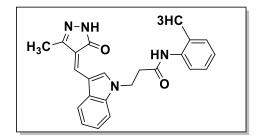
Yield 82%, mp 240-242 °C. IR spectrum, *v*, cm<sup>-1</sup>: 3309.96(-NH), 2862.46 (CH<sub>3</sub>), 1674.27 (C=O). <sup>1</sup>H NMR spectrum,  $\delta$ , ppm: 2.25 s (3H, -CH3), 2.99 t (2H, *J* = 6.8, -CH2), 4.69 t (2H, *J* = 6.4, -CH2), 7.09 d (1H, *J* = 6.8, Ar-H), 7.38 m (4H, Ar-H), 7.75 m (2H, 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). <sup>13</sup>C NMR (101 MHz, DMSO)  $\delta$  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<sub>22</sub>H<sub>19</sub>ClN4O<sub>2</sub>. 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.



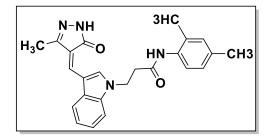
Yield 80%, mp 232-234 °C. IR spectrum, *v*, cm<sup>-1</sup>: 3286.81 (-NH), 2854.74 (CH<sub>3</sub>), 1651.12 (C=O). <sup>1</sup>H NMR spectrum,  $\delta$ , ppm: 2.25 s (3H, -CH3), 3.09 t (2H, *J* = 6.8, -CH2), 4.71 t (2H, *J* = 6.4, -CH2), 7.21 m (2H, Ar-H), 7.24 m (1H, -CH), 7.38 m (2H, Ar-H), 7.93 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). <sup>13</sup>C NMR (101 MHz, DMSO)  $\delta$  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<sub>22</sub>H<sub>19</sub>FN<sub>4</sub>O<sub>2</sub>. 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.



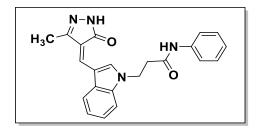
Yield 75%, mp 212-213 °C. IR spectrum, *v*, cm<sup>-1</sup>: (-NH), (CH<sub>3</sub>), (C=O). Found, %: C, 71.41; H, 5.68; N, 14.57. C<sub>23</sub>H<sub>22</sub>N<sub>4</sub>O<sub>2</sub>. Calculated, %: C, 71.48; H, 5.74; N, 14.50. *M* 386.

## (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.



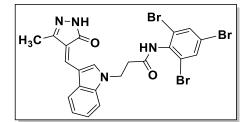
Yield 72%, mp 206-208 °C. IR spectrum, *v*, cm<sup>-1</sup>: 3271.38 (-NH), (CH<sub>3</sub>), (C=O). Found, %: C, 71.91; H, 6.13; N, 13.93. C<sub>24</sub>H<sub>24</sub>N<sub>4</sub>O<sub>2</sub>. Calculated, %: C, 71.98; H, 6.04; N, 13.99. *M* 400.

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



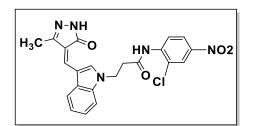
Yield 65%, mp 240-242 °C. IR spectrum, *v*, cm<sup>-1</sup>: 2854.74 (CH<sub>3</sub>), 1674.27 (C=O). <sup>1</sup>H NMR spectrum,  $\delta$ , ppm: 2.25 s (3H, *J* = 6.4, -CH<sub>3</sub>), 2.97 t (3H, -CH<sub>2</sub>), 4.68 t (2H, -CH<sub>2</sub>), 7.03 s (1H, -CH), 7.32 m (4H, *J* = 8 ,Ar-H), 7.53 dd (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<sub>22</sub>H<sub>20</sub>N<sub>4</sub>O<sub>2</sub>. 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.



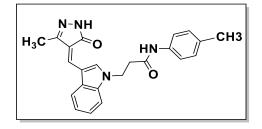
Yield 84%, mp 236-238 °C. IR spectrum, *v*, cm<sup>-1</sup>: 2856.58 (CH<sub>3</sub>), 1677.05 (C=O). Found, %: C, 43.44; H, 2.75; N, 9.25. C<sub>22</sub>H<sub>17</sub>Br<sub>3</sub>N<sub>4</sub>O<sub>2</sub>. Calculated, %: C, 43.38; H, 2.81; N, 9.20. *M* 609.

(*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.



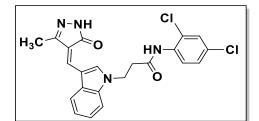
Yield 81%, mp 229-231 °C. IR spectrum, *v*, cm<sup>-1</sup>: 3272.67 (-NH), 2879.28 (CH<sub>3</sub>), 1677.18 (C=O). Found, %: C, 58.55; H, 4.09; N, 15.41. C<sub>22</sub>H<sub>18</sub>ClN<sub>5</sub>O<sub>4</sub>. Calculated, %: C, 58.48; H, 4.02; N, 15.50. *M* 451.

## (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.



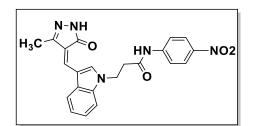
Yield 79%, mp 217-219 °C. IR spectrum, *v*, cm<sup>-1</sup>: 3307.11 (-NH), 2833.27 (CH<sub>3</sub>), 1675.55 (C=O). Found, %: C, 71.41; H, 5.79; N, 14.55. C<sub>23</sub>H<sub>22</sub>N<sub>4</sub>O<sub>2</sub>. Calculated, %: C, 71.48; H, 5.74; N, 14.50. *M* 386.

(*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.



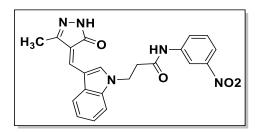
Yield 75%, mp 231-231 °C. IR spectrum, *v*, cm<sup>-1</sup>: 3275.85 (-NH), 2860.25 (CH<sub>3</sub>), 1677.58 (C=O). Found, %: C, 59.97; H, 4.19; N, 12.76. C<sub>22</sub>H<sub>18</sub>Cl<sub>2</sub>N<sub>4</sub>O<sub>2</sub>. Calculated, %: C, 59.88; H, 4.11; N, 12.70. *M* 441.

## (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<sup>-1</sup>: 3301.99 (-NH), 2844.78 (CH<sub>3</sub>), 1679.82 (C=O). Found, %: C, 63.34; H, 4.67; N, 16.71. C<sub>22</sub>H<sub>19</sub>N<sub>5</sub>O<sub>4</sub>. 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 60.



Yield 79%, mp 228-230 °C. IR spectrum, *v*, cm<sup>-1</sup>: 3307.61 (-NH), 2860.92 (CH<sub>3</sub>), 1675.45 (C=O). Found, %: C, 63.38; H, 4.64; N, 16.73. C<sub>22</sub>H<sub>19</sub>N<sub>5</sub>O<sub>4</sub>. 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  $\mu$ M, 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  $\mu$ I). 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 I (100  $\mu$ I). The screening process is detailed at https://dtp.cancer.gov/compsub/news.xhtml.

## 2.5. Spectral data

<b>Developmental Thera</b>	apeutics Program	NSC: D-844453/1	Conc: 1.00E-5 Molar	Test Date: May 08, 2023	
One Dose Mea	an Graph	Experiment ID: 2305	OS25	Report Date: Jun 07, 2023	
Panel/Cell Line	Growth Percent	Mean Growth	Percent - Growth Perc	cent	
Panel/Cell Line Leukemia CCRF-CEM HL-60(TB) K-562 MOLT-4 RPMI-8226 SR Non-Small Cell Lung Cancer A549/ATCC EKVX HOP-62 HOP-92 NCI-H226 NCI-H226 NCI-H227 NCI-H322M NCI-H323 NCI-H323 NCI-H323 NCI-H323 NCI-H323 NCI-H323 NCI-H323 NCI-H323 NCI-SC SC-257 UACC-257 UACC-257 UACC-62 Ovarian Cancer IGROV1 OVCAR-3 OVCAR-4 OVCAR-5 OVCAR-8 NCI/ADR-RES SK-OV-3	Growth Percent           71.45           107.23           105.25           88.81           63.96           67.70           96.26           96.93           112.05           90.45           95.90           108.72           111.53           94.38           119.59           114.08           90.89           81.43           104.38           91.85           106.76           101.18           91.67           90.55           94.25           112.79           100.34           91.68           100.61           97.99           89.64           102.31           104.19           88.97           99.09           86.69           112.73           112.59           91.62           101.36           91.62           101.36           91.62           101.36           91.62           101.36           91.62 <th>Mean Growth</th> <th>Percent - Growth Perc</th> <th>sent</th>	Mean Growth	Percent - Growth Perc	sent	
Renal Cancer 786-0 A498 ACHN CAKL-1 RXF 393 SN12C TK-10 UO-31 Prostate Cancer PC-3 DU-145 Breast Cancer MCF7 MDA-MB-231/ATCC HS 578T BT-549 T-47D MDA-MB-468 Mean Delta Range	98.43 117.15 96.76 84.78 96.31 102.42 101.25 79.72 82.94 102.72 67.68 80.52 92.38 72.23 85.09 102.29 95.45 31.49 55.63				
	150	100 50	0 -50	-100 -150	

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

Developmental Ther	apeutics Program	NSC: D-844454 / 1	Conc: 1.00E-5 Molar	Test Date: May 08, 2023
One Dose Mea	an Graph	Experiment ID: 2305	OS25	Report Date: Jun 07, 2023
Panel/Cell Line	Growth Percent	Mean Growth	Percent - Growth Perc	cent
Leukemia	00.70		_	
CCRF-CEM HL-60(TB)	66.70 105.47			
K-562	66.82		_	
MOLT-4	94.71			
RPMI-8226	54.66			
SR	41.15		_	
Non-Small Cell Lung Cancer A549/ATCC	74.02			
A549/ATCC	74.93 61.19			
EKVX HOP-62	-17.26			
HOP-92	-4.26			
NCI-H226	52.68			
NCI-H23	68.96			
NCI-H322M NCI-H460	63.76 46.33			
NCI-H522	71.09			
Colon Cancer				
COLO 205	93.02			
HCC-2998	102.40			
HCT-116 HCT-15	57.68 67.41			
HT29	76.92			
KM12	83.79			
SW-620	77.62			
CNS Cancer	22.12			
SF-268 SF-295	32.12 51.37			
SF-539	13.90			
SNB-19	3.34			
SNB-75	10.25		1	
U251	11.77			
Melanoma LOX IMVI	49.90			
MALME-3M	8.19			
M14	71.67			
MDA-MB-435	67.35			
SK-MEL-2 SK-MEL-28	84.94 89.50			
SK-MEL-5	69.89			
UACC-257	81.10			
UACC-62	66.60		_	
Ovarian Cancer	70.40			
IGROV1 OVCAR-3	78.42 59.99			
OVCAR-4	34.12			
OVCAR-5	84.30			
OVCAR-8	16.65			
NCI/ADR-RES SK-OV-3	28.01 68.85			
Renal Cancer	60.00			
786-0	58.82			
A498	73.71			
ACHN CAKI-1	29.10 46.60			
RXF 393	43.86			
SN12C	81.60			
TK-10	98.81			
UO-31 Brostate Cancer	8.00			
Prostate Cancer PC-3	46.86			
DU-145	57.42			
Breast Cancer	And and a second second			
MCF7	50.27			
MDA-MB-231/ATCC HS 578T	44.38 -0.85			
BT-549	14.74			
T-47D	52.67			
MDA-MB-468	61.47		-	
Mean	54.26			
Delta	71.52			
Range	122.73			
	150	100 50	0 -50	-100 -150
	150	100 00	J -50	-150

#### Synthesis, Characterization and Biological Activity of Heterocyclic Compounds

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

	apeutics Program	NSC: D-844455/1	Conc: 1.00E-5 Molar	Test Date: May 08, 2023	
One Dose Mea	an Graph	Experiment ID: 2305	OS25	Report Date: Jun 07, 2023	
Panel/Cell Line	Growth Percent	Mean Growth	Percent - Growth Perc	cent	
eukemia CCRF-CEM	72 74				
HL-60(TB)	72.74 112.42				
K-562	73.98				
MOLT-4	91.44				
RPMI-8226	53.76				
SR	50.36		_		
Ion-Small Cell Lung Cancer A549/ATCC	84.75				
EKVX	74.94				
HOP-62	29.92				
HOP-92	30.38				
NCI-H226	72.80		1		
NCI-H23	81.40				
NCI-H322M NCI-H460	88.39 60.48				
NCI-H522	82.14				
olon Cancer	1231434200000				
COLO 205	99.09				
HCC-2998	117.77				
HCT-116 HCT-15	70.50 77.68				
HT29	89.21				
KM12	88.09				
SW-620	78.34		-		
NS Cancer					
SF-268	60.66				
SF-295 SF-539	74.02 43.50				
SNB-19	31.17		8		
SNB-75	114.09				
U251	37.39				
lelanoma	71.00				
LOX IMVI MALME-3M	71.82 47.05				
MALME-SM M14	83.30				
MDA-MB-435	81.36		-		
SK-MEL-2	94.23				
SK-MEL-28	97.02				
SK-MEL-5	78.09				
UACC-257 UACC-62	94.58 80.21				
ovarian Cancer	50.21				
IGROV1	92.85				
OVCAR-3	104.10				
OVCAR-4 OVCAR-5	51.52 90.95				
OVCAR-5 OVCAR-8	40.90				
NCI/ADR-RES	55.19				
SK-OV-3	88.57				
enal Cancer	05.07				
786-0 A498	85.07 101.95				
ACHN	67.34				
CAKI-1	66.16		-		
RXF 393	70.27				
SN12C	93.13				
TK-10 UO-31	105.39 47.58				
rostate Cancer	47.50				
PC-3	70.92				
DU-145	83.03				
reast Cancer	50.05				
MCF7 MDA-MB-231/ATCC	58.95 66.15				
HS 578T	24.59				
BT-549	37.91				
T-47D	57.46				
MDA-MB-468	75.69				
Mean	73.41				
Delta	48.82				
Range	93.18				
940 (A 2007 <b>-</b> 010 7	4.252.052.055×.				
	150	100 50	0 -50	-100 -150	

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

Developmental mer	apeutics Program	NSC: D-844456 / 1	Conc: 1.00E-5 Molar	Test Date: May 08, 2023
One Dose Me	an Graph	Experiment ID: 2305	OS25	Report Date: Jun 07, 20
Panel/Cell Line	Growth Percent	Mean Growth I	Percent - Growth Perc	cent
eukemia	07.00			
CCRF-CEM HL-60(TB)	67.38 81.25			
K-562	95.39			
MOLT-4	85.57		<b>1</b>	
RPMI-8226	61.09			
SR	41.82			
Ion-Small Cell Lung Cancer	11.02		1.5	
A549/ATCC	99.88		-	
EKVX	93.64			
HOP-62	89.52			
HOP-92	79.80			
NCI-H226	108.16			
NCI-H23	89.80			
NCI-H322M	98.65			
NCI-H460	106.50			
NCI-H522	92.04			
olon Cancer COLO 205	96.46			
HCC-2998	114.95			
HCT-116	90.64			
HCT-15	79.53			
HT29	92.11			
KM12	86.93		-	
SW-620	86.17		-	
NS Cancer				
SF-268	108.65		in the second se	
SF-295	96.66			
SF-539	90.55			
SNB-19	97.16		_	
SNB-75	101.45			
U251 Ielanoma	92.76			
LOX IMVI	83.06			
MALME-3M	83.06 87.61			
M14	81.85			
MDA-MB-435	67.40			
SK-MEL-2	104.61			
SK-MEL-28	105.78			
SK-MEL-5	90.15			
UACC-257	96.81			
UACC-62	83.55			
Varian Cancer IGROV1	107.39			
OVCAR-3	118.57			
OVCAR-4	97.22			
OVCAR-5	118.11			
OVCAR-8	92.29			
NCI/ADR-RES	92.32			
SK-OV-3	89.47		•	
enal Cancer	n			
786-0	106.67			
A498	108.61			
ACHN CAKI-1	99.98 74.12			
RXF 393	102.27			
SN12C	102.27			
TK-10	105.25			
UO-31	68.64			
rostate Cancer	100 Suboren III.			
PC-3	82.93			
DU-145	100.31			
reast Cancer	C4 70			
MCF7	64.79		15 Martin Color	
MDA-MB-231/ATCC HS 578T	105.08 89.36			
BT-549	83.89			
T-47D	97.77			
MDA-MB-468	106.45			
Mean Delta	92.40 50.58			
Range	76.75			
	150	100 50	0 -50	-100 -15

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

Jevelopmental Ther	apeutics Program	NSC: D-844457 / 1	Conc: 1.00E-5 Molar	Test Date: May 08,	2023
One Dose Me	an Graph	Experiment ID: 2305	OS25	Report Date: Jun 07, 2023	
Panel/Cell Line	Growth Percent	Mean Growth	Percent - Growth Perc	cent	
eukemia	01.64				
CCRF-CEM HL-60(TB)	91.64 101.82				
K-562	96.52				
MOLT-4	108.17				
RPMI-8226	69.60				
SR	101.24				
on-Small Cell Lung Cancer	- 40.4 210-1979 (2010) NO				
A549/ATCC	76.70				
EKVX	77.19				
HOP-62	16.64				
HOP-92	36.84				
NCI-H226	34.67				
NCI-H23 NCI-H322M	69.10 75.75				
NCI-H460	50.24				
NCI-H522	71.40				
olon Cancer	11.40				
COLO 205	97.57				
HCC-2998	93.09				
HCT-116	57.90		-		
HCT-15	86.31				
HT29	74.22				
KM12	81.87				
SW-620	92.82				
NS Cancer	44.47				
SF-268 SF-295	44.47 45.07				
SF-295 SF-539	38.43				
SNB-19	32.80				
SNB-75	76.16		-		
U251	42.25				
elanoma					
LOX IMVI	61.22		-		
MALME-3M	44.26				
M14	89.34				
MDA-MB-435	79.54				
SK-MEL-2 SK-MEL-28	90.75 97.64				
SK-MEL-28	79.87				
UACC-257	89.53				
UACC-62	70.28				
Varian Cancer					
IGROV1	82.55				
OVCAR-3	73.20		-		
OVCAR-4	39.55				
OVCAR-5	99.23				
OVCAR-8	37.67				
NCI/ADR-RES	58.35				
SK-OV-3 enal Cancer	59.57				
786-0	59.29				
A498	94.79				
ACHN	32.83				
CAKI-1	41.33				
RXF 393	42.12				
SN12C	77.90				
TK-10	100.67				
UO-31	50.40				
rostate Cancer PC-3	51.94				
DU-145	64.00				
reast Cancer	04.00				
MCF7	59.32		-		
MDA-MB-231/ATCC	71.17				
HS 578T	26.27				
BT-549	62.99				
T-47D	63.87				
MDA-MB-468	71.13				
M	67.70				
Mean Delta	67.72 51.08				
Range	91.53				
range	01.00				
	450	400 50	0 -50	400	-150
	150	100 50	0 -30	-100	-150

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

Developmental Ther	apeutics Program	NSC: D-844458/1	Conc: 1.00E-5 Molar	Test Date: May 08, 2023	
One Dose Mea	an Graph	Experiment ID: 2305	OS25	Report Date: Jun 07, 2023	
Panel/Cell Line	Growth Percent	Mean Growth	Percent - Growth Per	cent	
Leukemia CCRF-CEM HL-60(TB) K-562 MOIT-4 RPMI-8226 SR Non-Small Cell Lung Cancer A549/ATCC EKVX HOP-62 HOP-92 NCI-H23 NCI-H322M NCI-H32 SK-MEL-28 SK-MEL-28 SK-MEL-28 SK-MEL-30 UACC-62 Ovarian Cancer IGROV1 OVCAR-3 OVCAR-4 OVCAR-4 OVCAR-5 OVCAR-8 NCI/ADR-RES SK-OV-3 Renal Cancer 786-0 A498 ACHN CAKI-1 RXF 393 SN12C TK-10 UO-31 Prostate Cancer PC-3 DU-145 Breast Cancer MCF7 MDA-MB-231/ATCC HS 578T BT-549 T-470 MDA-MB-468 Mean Delta	66.45         94.71         81.76         70.15         65.49         39.09         99.06         98.13         96.40         80.40         101.66         90.74         109.86         90.05         99.06         90.74         109.86         90.74         109.86         90.77         15.70         77.85         59.29         86.23         80.36         92.91         88.37         92.52         90.38         94.03         84.40         83.59         70.26         83.80         85.89         26.20         101.62         117.99         83.78         98.06         78.71         97.74         77.47         79.45         86.41         104.07         111.38         90.92         88.51         104.77         68.51<				
Range	95.25				
	150	100 50	0 -50	-100 -150	

#### Synthesis, Characterization and Biological Activity of Heterocyclic Compounds

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

Jevelopmental Ther	apeutics Program	NSC: D-844459/1	Conc: 1.00E-5 Molar	Test Date: May 08, 2023	
One Dose Me	an Graph	Experiment ID: 2305	OS25	Report Date: Jun 07, 2023	
Panel/Cell Line	Growth Percent	Mean Growth	Percent - Growth Perc	cent	
eukemia	~~~~				
	66.24 79.00				
HL-60(TB) K-562	87.30				
MOLT-4	78.60		_		
RPMI-8226	67.76				
SR	53.32				
Ion-Small Cell Lung Cancer	00.02				
A549/ATCC	94.24				
EKVX	93.14		-		
HOP-62	93.49		-		
HOP-92	72.81				
NCI-H226	98.25				
NCI-H23	86.76				
NCI-H322M	95.21				
NCI-H460 NCI-H522	104.84 87.02				
	87.02				
olon Cancer COLO 205	103.43				
HCC-2998	110.46				
HCT-116	87.19				
HCT-15	80.94				
HT29	93.70				
KM12	90.84		•		
SW-620	88.13		1 1		
NS Cancer	00.44				
SF-268	88.14				
SF-295 SF-539	94.27 90.22				
SNB-19	93.83				
SNB-75	79.62				
U251	90.63				
lelanoma					
LOX IMVI	76.19		-		
MALME-3M	90.17				
M14	82.23		-		
MDA-MB-435	61.95				
SK-MEL-2	97.46				
SK-MEL-28 SK-MEL-5	111.96 89.29				
UACC-257	93.17		<b>_</b>		
UACC-62	72.95				
Ovarian Cancer	. 2.00				
IGROV1	89.26				
OVCAR-3	78.92		-		
OVCAR-4	92.62				
OVCAR-5	105.04				
OVCAR-8	92.76				
NCI/ADR-RES	80.88				
SK-OV-3 enal Cancer	84.65		ГІ		
786-0	100.86				
A498	107.54				
ACHN	98,96		-		
CAKI-1	67.85				
RXF 393	94.72				
SN12C	93.78				
TK-10 UO-31	107.51				
rostate Cancer	55.28				
PC-3	75.98				
DU-145	98.53				
reast Cancer	60.0.1999/0000000000000000000000000000000				
MCF7	67.34				
MDA-MB-231/ATCC	79.01				
HS 578T	83.21				
BT-549	91.60				
T-47D MDA-MB-468	85.28				
WDA-WB-468	97.38				
Mean	87.56				
Delta Range	34.24 58.64				
Range	00.04				
	150	100 50	0 -50	-100 -150	
	100	100 :00	U -3U	-100 -130	

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

One Dose Mea	an Graph	Experiment ID: 2305OS25		Report Date: Jun 07, 20
anel/Cell Line	Growth Percent	Mean Growth	Percent - Growth Perc	cent
eukemia CCRF-CEM	65 59			
HL-60(TB)	65.58 96.47			
K-562	97.43			
MOLT-4	58.09			
RPMI-8226	65.37			
SR	49.47			
Ion-Small Cell Lung Cancer A549/ATCC	103.11			
EKVX	98.16			
HOP-62	103.29		_	
HOP-92	78.63			
NCI-H226	94.06			
NCI-H23	94.06			
NCI-H322M NCI-H460	97.01			
NCI-H480	113.19 96.49			
colon Cancer	30.45			
COLO 205	106.50			
HCC-2998	107.95			
HCT-116	89.61			
HCT-15	89.22 93.26		-	
HT29	93.26			
KM12 SW-620	102.18 104.40			
NS Cancer	104.40			
NS Cancer SF-268 SF-295	100.82		-	
SF-295	95.07		4 1	
SF-539	99.31			
SNB-19	93.37			
SNB-75	112.14			
U251 Ielanoma	100.50			
LOX IMVI	89.22		<b>–</b> I	
MALME-3M	94.09			
M14	88.39		-	
MDA-MB-435	79.20			
SK-MEL-2	96.96		_	
SK-MEL-28 SK-MEL-5	103.22 94.08			
UACC-257	106.02			
UACC-62	77.29			
varian Cancer				
IGROV1	87.51			
OVCAR-3	108.54			
OVCAR-4	101.92 97.44			
OVCAR-5 OVCAR-8	97.44 97.94			
NCI/ADR-RES	89.35			
SK-OV-3	97.26			
enal Cancer	4 CB-22 + CD-22 + CD-20			
786-0	104.26			
A498	127.28			
ACHN CAKI-1	95.58 79.58			
RXF 393	99.74			
SN12C	86.78			
TK-10	105.68			
UO-31	64.06			
rostate Cancer	70.01			
PC-3 DU-145	76.61			
reast Cancer	115.05			
MCF7	79.45			
MDA-MB-231/ATCC	85.52		-	
HS 578T BT-549	95.16			
BI-549	114.86			
T-47D MDA-MB-468	81.92 108.92			
WDA-WD-400	100.92			
Mean	93.89			
Delta	44.42 77.81			
Range	77.81			
	150	100 50	0 -50	-100 -15

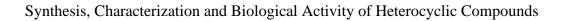
Fig. 8: Representative single dose data of compound IPP-8

	apeutics Program	NSC: D-844461/1		Test Date: May 08, 2023	
One Dose Mea	an Graph	Experiment ID: 2305OS25		Report Date: Jun 07, 202	
Panel/Cell Line	Growth Percent	Mean Growth Percent - Growth Perc		cent	
eukemia CCRF-CEM	79.7E				
HL-60(TB)	78.75 104.71		_		
K-562	86.34				
MOLT-4	74.89				
RPMI-8226	68.95				
SR	51.65				
Ion-Small Cell Lung Cancer					
A549/ATCC	103.92				
EKVX	92.41				
HOP-62	101.21				
HOP-92	74.96 93.44				
NCI-H226 NCI-H23	91.41				
NCI-H322M	100.02				
NCI-H460	109.04				
NCI-H522	92.40				
olon Cancer					
COLO 205	111.29				
HCC-2998	107.69				
HCT-116	87.62				
HCT-15 HT29	81.50 98.53				
KM12	102.59				
SW-620	111.80				
NS Cancer					
SF-268	95.97				
SF-295	88.27				
SF-539	99.27				
SNB-19	94.99				
SNB-75 U251	111.83 96.51				
lelanoma	30.01		1		
LOX IMVI	85.13		-		
MALME-3M	86.58		-		
M14	97.24				
MDA-MB-435	70.30				
SK-MEL-2	99.17 100.28				
SK-MEL-28 SK-MEL-5	93.99				
UACC-257	103.23				
UACC-62	77.75				
varian Cancer	4 (* 12 G) (* 20 G) (* 20 G)				
IGROV1	82.93				
OVCAR-3	110.11				
OVCAR-4	99.12				
OVCAR-5	92.56 91.36				
OVCAR-8 NCI/ADR-RES	90.40				
SK-OV-3	95.71				
enal Cancer	55.7 1				
786-0	100.16		-		
A498	142.18				
ACHN	95.33				
CAKI-1 RXF 393	72.90 97.03				
SN12C	97.03 86.83		1		
TK-10	110.83				
UO-31	57.92				
rostate Cancer			0.000		
PC-3	70.40				
DU-145	119.99				
reast Cancer MCF7	76.96				
MDA-MB-231/ATCC	80.30				
HS 578T	93.46				
BT-549	127.28				
T-47D	74.26				
MDA-MB-468	107.55				
Mean	93.35				
Delta	41.70				
Range	90.53				
	150	100 50	0 -50	-100 -150	

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

Developmental The	rapeutics Program	NSC: D-845611/1	Conc: 1.00E-5 Molar	Test Date: Jun 12, 20
One Dose Me	One Dose Mean Graph		Experiment ID: 2306OS36	
Panel/Cell Line	Growth Percent	Mean Growth	n Percent - Growth Pe	rcent
eukemia CCRF-CEM	100.60			
HL-60(TB)	101.21		-	
K-562	106.86		_	
MOLT-4	101.97		-	
RPMI-8226 Ion-Small Cell Lung Cancer	92.85			
A549/ATCC	94.79			
EKVX	67.27			
HOP-62	110.65			
HOP-92	112.50			
NCI-H226 NCI-H23	76.49 92.85			
NCI-H460	99.39		<b>_</b>	
NCI-H522	88.95			
olon Cancer				
COLO 205	119.01			
HCC-2998 HCT-116	100.08 96.03			
HCT-15	87.90			
HT29	90.59		•	
KM12	99.61			
SW-620 NS Cancer	98.49			
SF-268	88.39			
SF-295	91.81		•	
SF-539	86.42		-	
SNB-19	93.53			
U251 elanoma	98.94			
LOX IMVI	79.14			
M14	97.98		-	
MDA-MB-435	101.83			
SK-MEL-2 SK-MEL-28	98.76 105.20		_	
SK-MEL-5	95.25			
UACC-257	102.28		-	
UACC-62 varian Cancer	78.15		_	
OVCAR-3	105.52		_	
OVCAR-4	94.33			
OVCAR-5	86.66			
OVCAR-8 NCI/ADR-RES	102.65			
SK-OV-3	93.28 97.65			
enal Cancer				
786-0	98.13			
A498 ACHN	109.28 94.86		_	
CAKI-1	72.58			
RXF 393	91.43			
SN12C	88.60			
TK-10 rostate Cancer	103.00			
PC-3	89.05			
DU-145	106.05			
reast Cancer	00.70			
MCF7 MDA-MB-231/ATCC	80.78 86.19			
HS 578T	81.12			
BT-549	88.41			
T-47D	92.32			
MDA-MB-468	88.02			
Mean	94.55			
Delta Range	27.28 51.74			
92594				
	150	100 50	0 -5	0 -100 -1

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



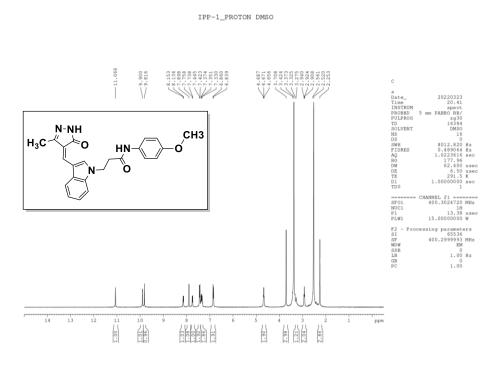


Fig. 11: Representative <sup>1</sup>H NMR spectrum of compound IPP-1

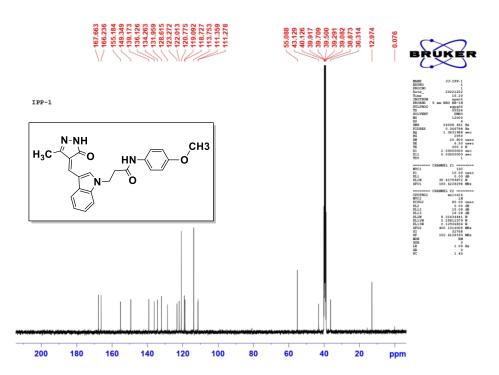
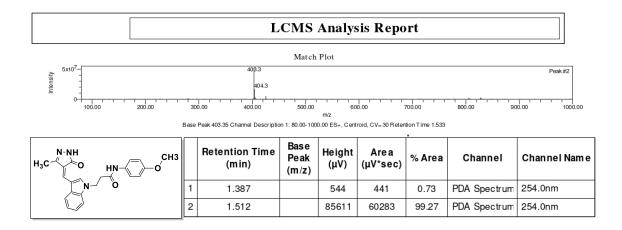


Fig. 12: Representative <sup>13</sup>C NMR spectrum of compound IPP-1



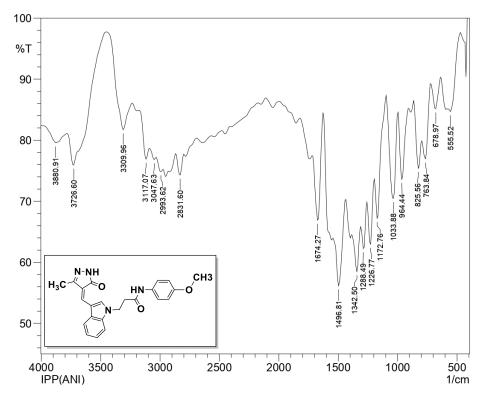


Fig. 13: Representative LCMS data of compound IPP-1

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

#### Synthesis, Characterization and Biological Activity of Heterocyclic Compounds

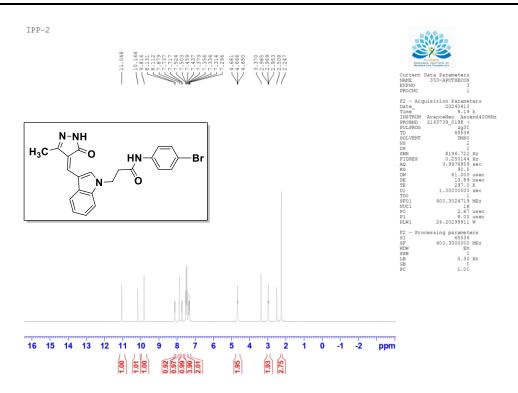


Fig. 15: Representative <sup>1</sup>H NMR spectrum of compound IPP-2

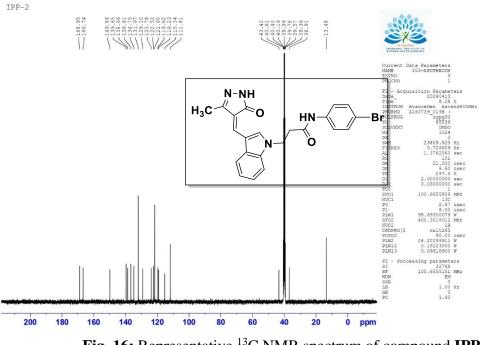
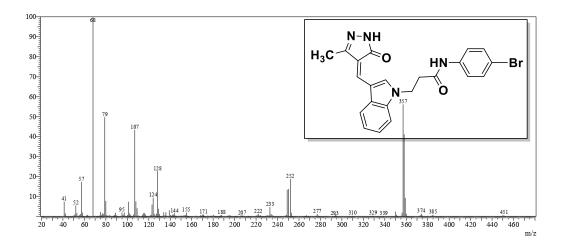


Fig. 16: Representative <sup>13</sup>C NMR spectrum of compound IPP-2





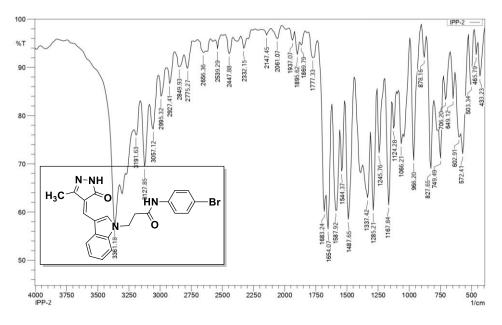


Fig. 18: Representative IR spectrum of compound IPP-2

## Synthesis, Characterization and Biological Activity of Heterocyclic Compounds

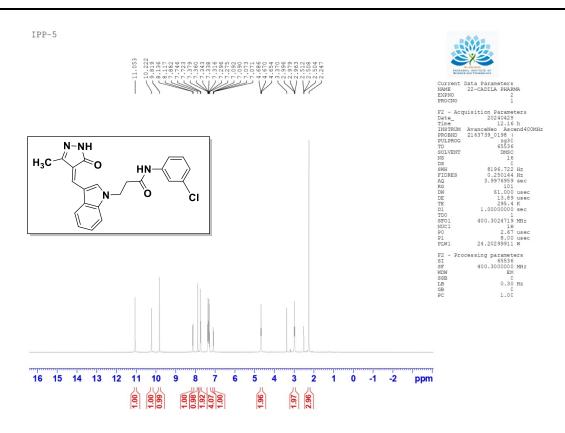


Fig. 19: Representative <sup>1</sup>H NMR spectrum of compound IPP-5

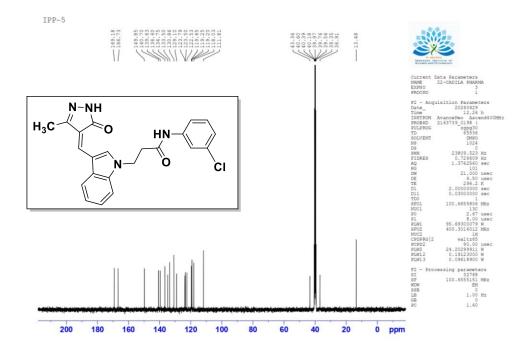


Fig. 20: Representative <sup>13</sup>C NMR spectrum of compound IPP-5

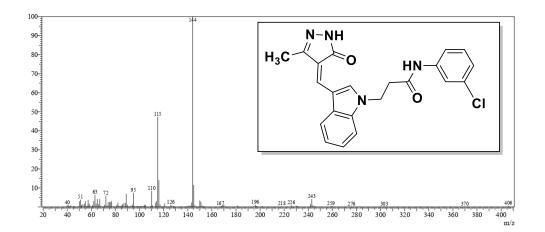


Fig. 21: Representative mass spectrum of compound IPP-5

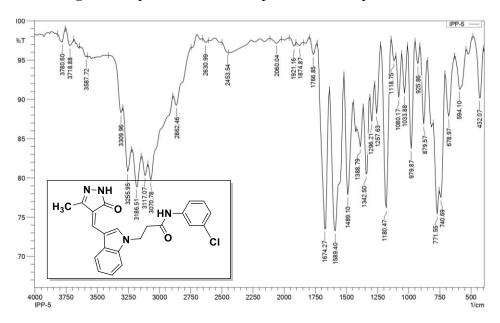
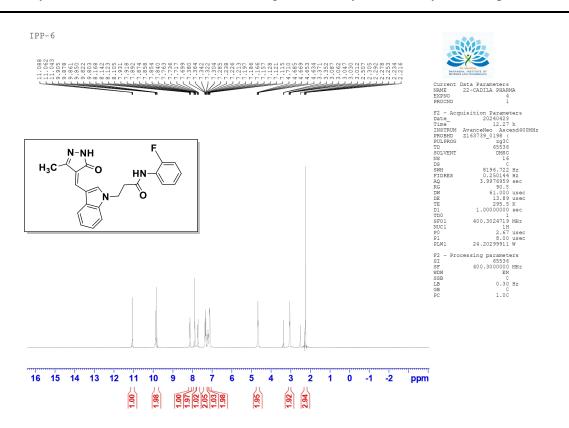


Fig. 22: Representative IR spectrum of compound IPP-5



## Synthesis, Characterization and Biological Activity of Heterocyclic Compounds

Fig. 23: Representative <sup>1</sup>H NMR spectrum of compound IPP-6

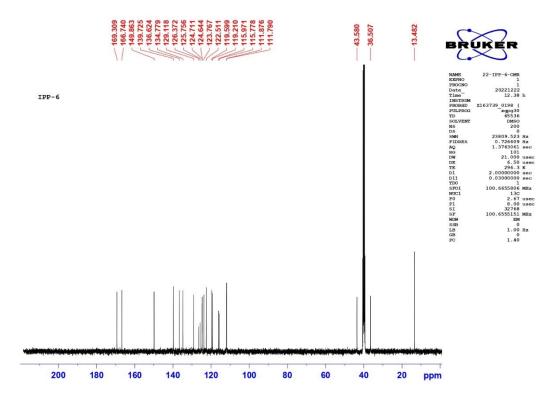
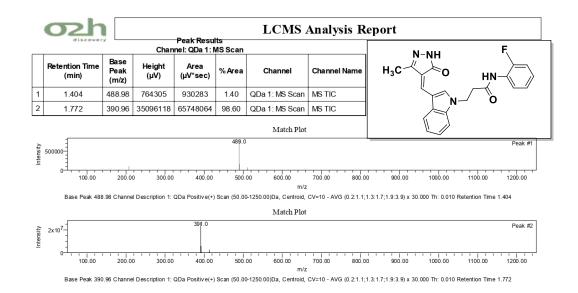


Fig. 24: Representative <sup>13</sup>C NMR spectrum of compound IPP-6



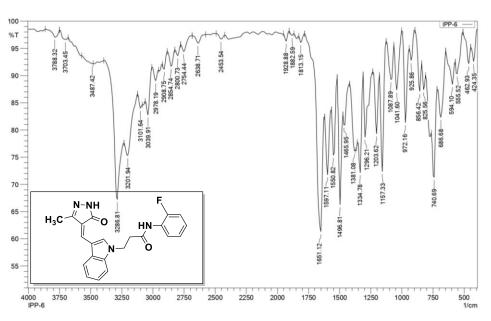


Fig. 25: Representative LCMS data of compound IPP-6

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

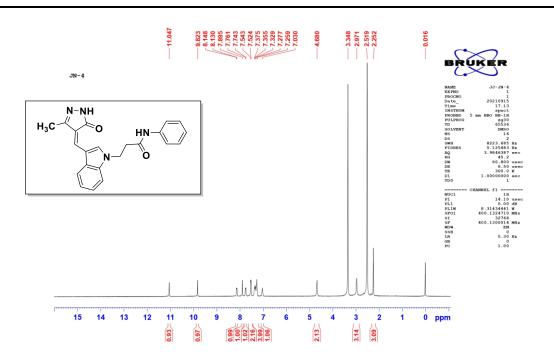


Fig. 27: Representative <sup>1</sup>H NMR spectrum of compound IPP-9

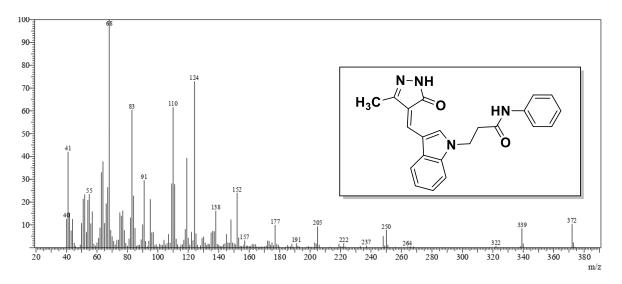


Fig. 28: Representative mass spectrum of compound IPP-9

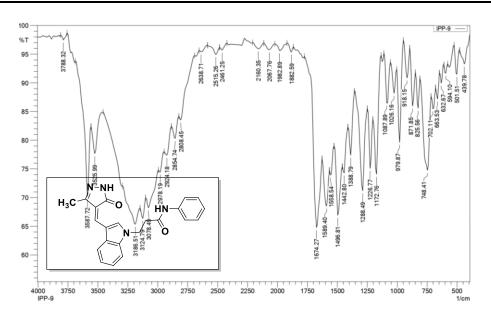


Fig. 29: Representative IR spectrum of compound IPP-9

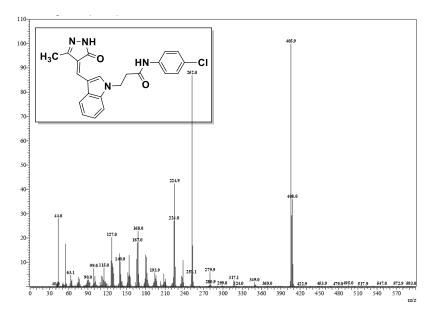


Fig. 30: Representative mass spectrum of compound IPP-3

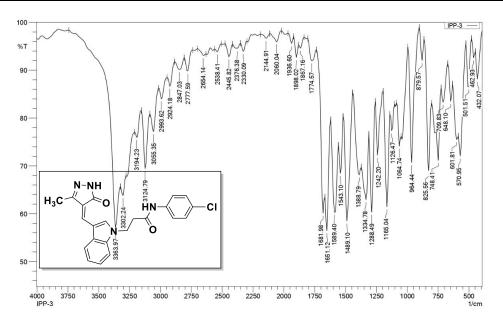


Fig. 31: Representative IR spectrum of compound IPP-3

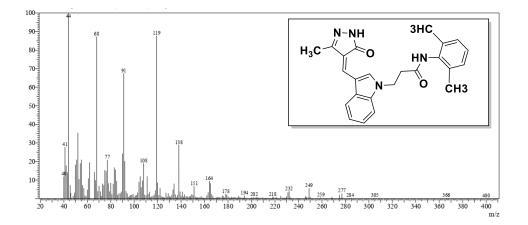


Fig. 32: Representative mass spectrum of compound IPP-4

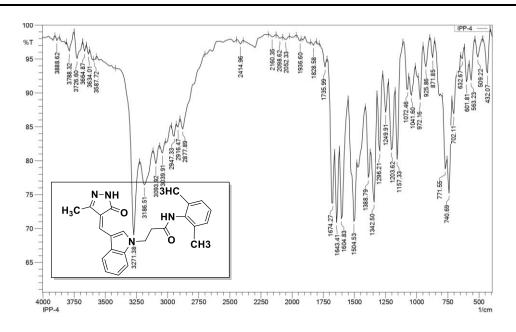


Fig. 33: Representative IR spectrum of compound IPP-4

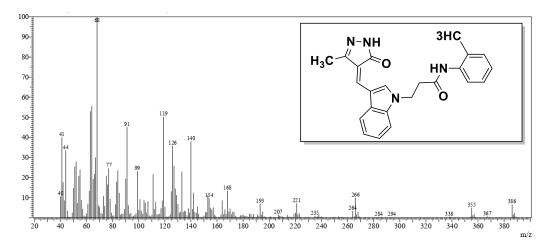


Fig. 34: Representative mass spectrum of compound IPP-7

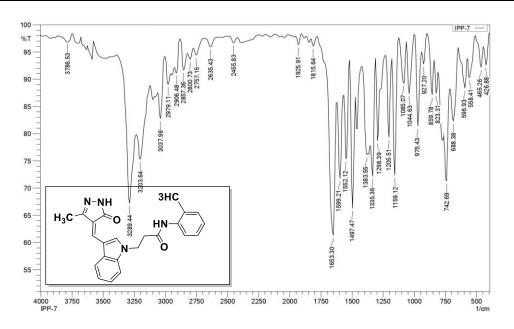


Fig. 35: Representative IR spectrum of compound IPP-7

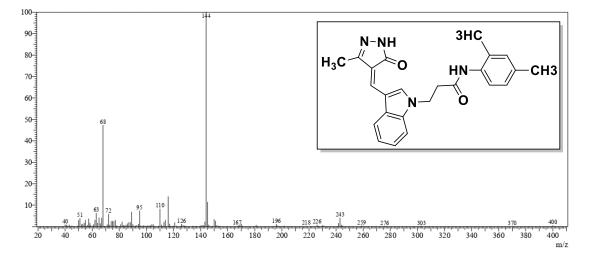


Fig. 36: Representative mass spectrum of compound IPP-8

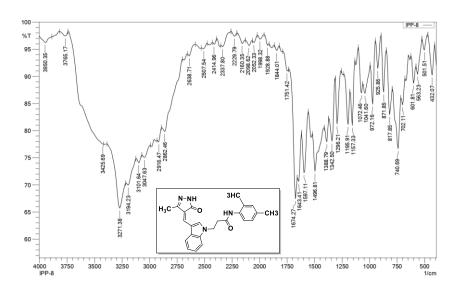


Fig. 37: Representative IR spectrum of compound IPP-8

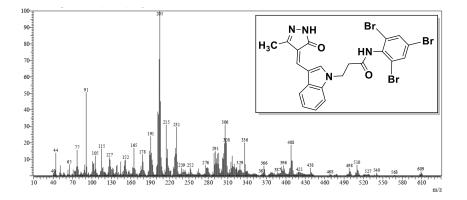


Fig. 38: Representative mass spectrum of compound IPP-10

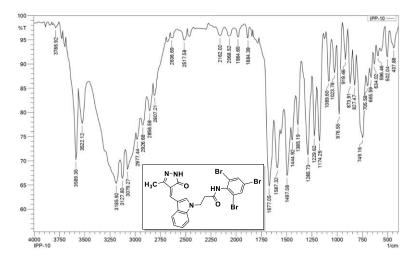


Fig. 39: Representative IR spectrum of compound IPP-10

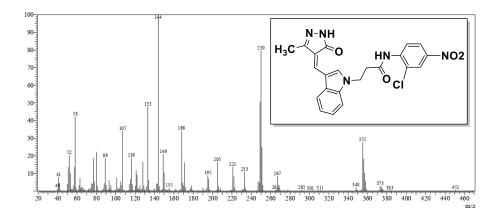


Fig. 40: Representative mass spectrum of compound IPP-11

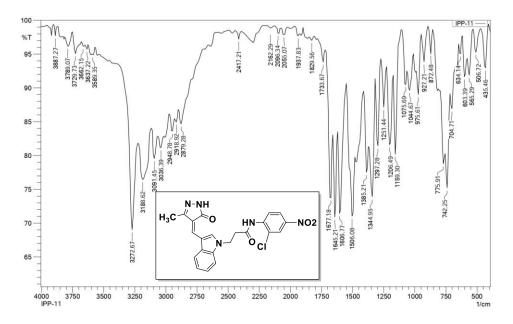
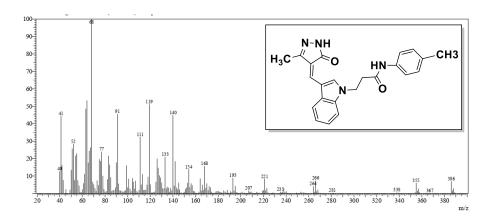


Fig. 41: Representative IR spectrum of compound IPP-11





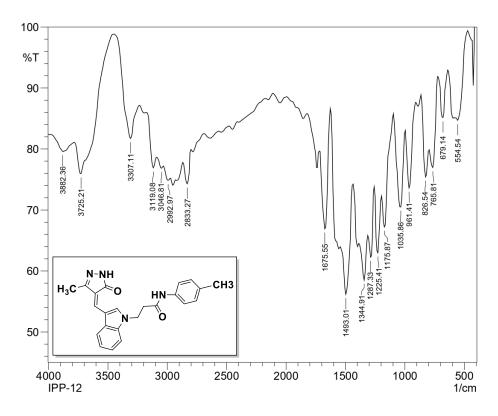
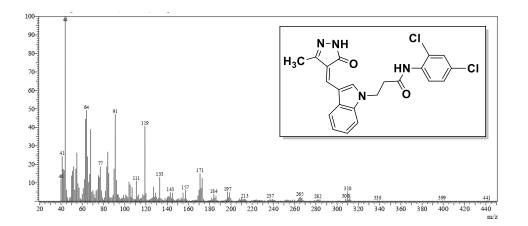
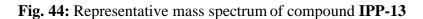


Fig. 43: Representative IR spectrum of compound IPP-12





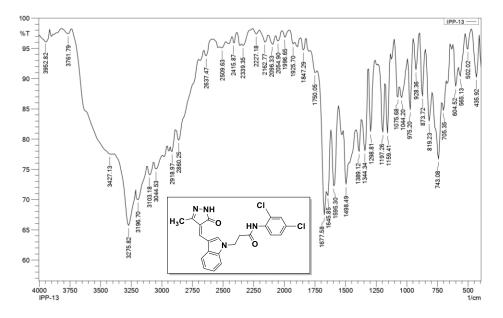


Fig. 45: Representative IR spectrum of compound IPP-13

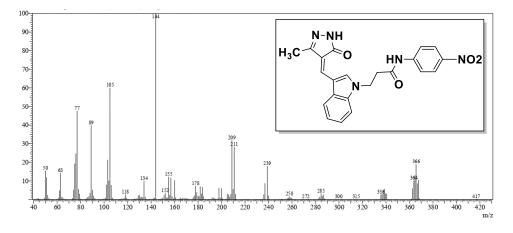


Fig. 46: Representative mass spectrum of compound IPP-14

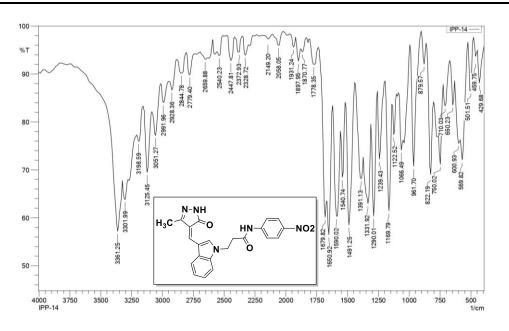


Fig. 47: Representative IR spectrum of compound IPP-14

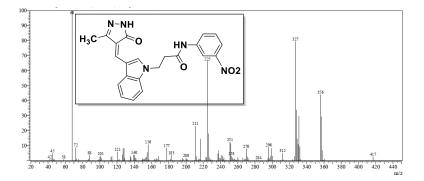


Fig. 48: Representative mass spectrum of compound IPP-15

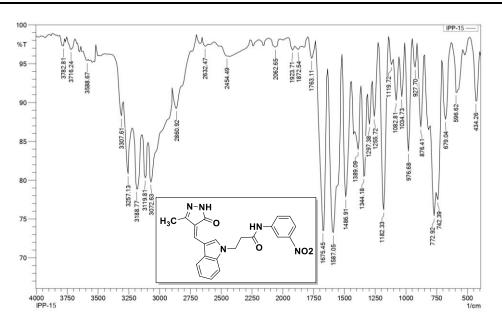


Fig. 49: Representative IR spectrum of compound IPP-15