Synthesis and Characterization of Indole and benzimidazole Conjugates as Anticancer Agents

A
Thesis submitted in partial
Fulfillment of the requirement of the degree of

Masters of Science in Chemistry

Submitted by
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July, 2019

Certificate

This is to certify that the thesis entitled "**Synthesis and Characterization of Indole and benzimidazole Conjugates as Anticancer Agents**" which is submitted by Ms. Rohini in partial fulfillment of the requirement for the award of the Degree of Masters of Science in Chemistry in the School of Chemistry and Biochemistry, Thapar Institute of Engineering and Technology, Patiala, is a record of candidate's own independent and original research work carried out by her under my supervision and guidance. The material embodied in this thesis has not been submitted in part or full to any other University or Institute for the award of any other degree.

Rohini

(Rohini)

This is to certify above statement made by student concerned is correct and true to the best of my knowledge.

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Candidate's Declaration

I, hereby declare that the work presented in the thesis entitled **"Synthesis and Characterization of Indole and benzimidazole Conjugates as Anticancer Agents"** fulfillment of the requirement for the award of the Degree of **Masters of Science in Chemistry, School of Chemistry and Biochemsitry , Thapar institute of engineering and technology, Patiala** is an authentic record of my own work carried out under the supervision of **Dr. Kamaldeep Paul,** Associate Professor, and **Dr. Satnam Singh**, Professor, School of Chemistry & Biochemistry, THAPAR INSTITUTE OF ENGINEERING AND TECHNOLOGY, Patiala, India. The matter embodied in this thesis has not been submitted in part or full to any other university or institute for the award of any degree in India or abroad.

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ACKNOWLEDGEMENT

Firstly, I would like to express my heartfelt gratitude towards the supreme almighty for keeping me in hollow of his hand, providing me nourishment and comfort during the course of my research.

I am thankful to **Dr. Amjad Ali**, Associate Professor and Head, School of Chemistry and Bio-Chemistry for giving me this opportunity to work and allowed me to use various facilities in the department.

I am highly gratified for the inspiration and guidance of **Dr. Kamaldeep Paul,** Associate Professor and **Dr. Satnam Singh**, Professor. We had a pragmatic discussion over my interest and lab specification after that we decided the topic for my project work. From basic amenities to guidance he was a great help throughout my research. He was always available there for listening my quires and cleared my all doubts in a very nice and interesting way. I am beyond thankful for their guidance and support

My Heart-warming thanks to **Iqubal Singh**, SRF for his diligent presence throughout my research. He listened to me and taught me how to work in lab in a very soothing way. I am pleased that he corrected me whenever I committed any mistake during this project work. Special thanks to him for showing believe in me for my doing this project. His friendly nature helped me to complete my project smoothly. Our interaction has proved to be of great assistance.

I have had a very constructive journey working with Phd scholars **Gulshan Kumar, Ruhi Mehta, Richa Bansal, Sudesh Rani, Dinesh singla, Aastha Palta, Rekha thakur, Swati Rana, Rohitash** and research associate **Dr. Rohini Verma** and **Nandan Sarkar** and thanks to them for helping me during my research work.

This work would not have been possible without my lab mates **Saurabh Gupta, Princedeep kaur, Anchal Sharma, Geetika Bansal, Deepika Sharma** and **Diksha Bansal**.

Most of the results described in the thesis would have not been possible without the help of laboratories at institutes like, **SAI** labs, Thapar Institute of Engineering and Technology, Patiala, **SAIF**, Punjab University and **IIT** Ropar

In the presence of this project, there has been an important role of my father Mr. **Jai parkash Gupta,** my mother Mrs. **Yashoda Gupta,** and my siblings Mr. **Ashu Gupta**, Ms. **Shruti Gupta,s** and I am thankful to them who have showered unconditional love on me, encouraged and supported in every aspect.

I am also grateful to the Thapar Institute of Engineering and Technology for the provision of food and accommodation. This hospitality made my stay memorable.

Date: 15-7-19

Rohini

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ABSTRACT

Cancer is a life-threatening disease that can invade anywhere in the body tissues. Abnormal growth/division of cells cause cancer. When a cell is individually damaged and cannot repair itself, it frequently endures a so called programme that is cell death or apoptosis. Optimisation of drugs is done at the molecular level in order to produce an improved version of anticancer drug. Indole exhibits immense biological activity and has always attracted researchers to design target-based compounds possessing indole moiety as anticancer agents and become one of the important building blocks of many drug development and research centers. In this work, we have synthesized indole and benzimidazole based conjugates followed by Suzuki coupling with various boronic acids in good to moderate yields. The synthesized compounds were characterized by ^{1}H NMR spectroscopic technique. These compounds will further be used to evaluate their anticancer activity.

TABLE OF CONTENT

1. INTRODUCTION

Cancer is a life-threatening disease that can invade anywhere in the body tissues. Abnormal growth/division of cells cause cancer. Changes in DNA sequence (DNA mutation) results in slow progression of this deadly disease. Selective round of mutation and expansion results in formation of tumour cells. These tumour cells further grow, expand and spread to other parts of body causing metastasis. Even after advance research and methodologies available, chemists and biologists are not able to resolve this deadliest disease despite doing number of research on it. There is no sure short formula available till date to cure cancer and it still remains one of the biggest threats to human health. Thus it is a biggest task or dream of a medicinal chemist to design or develop such chemotherapeutic drug that completely cures cancer without any side effects. Cancer mainly considered as a lifestyle related disease. There are several environmental factors like ozone hole, exposure to sun rays (10%) cause melanoma; tobacco (20-25%) and alcohol consumption also responsible for throat and nasal cancer. Heredity (genes) also plays a vital role to decide whether the person is cancer prone or not. Prolonged exposure to some substances is also one of the important causes of cancer like exposure to benzene causes leukaemia, obesity, lack of physical activity and pollution also contribute to cancer. According to a survey in india, there are approximately 18.1 million recent cases and 9.6 million deaths reported due to cancer in 2018. Out of which lung cancer is most commonly found in both male and female causing 18.4% of total deaths due to cancer, while colorectal (6.1%) and female breast, prostate cancer (7.1%), stomach (8.2%), colorectal (9.2%) and liver cancer (8.2%) for deaths due to cancer. There are several ways for cure of cancer viz. chemotherapy, radiation therapy, hormonal therapy and surgery, etc. and their use depends upon the type, location and grade of cancer. No single medicine has yet been discovered despite innumerable attempts to completely eradicate the disease. This urgency of new and better drug arises due to less selectivity and resistance created by body to conventional drugs system.[1] Chemotherapy is well-known, accepted and most reliable technique used to cure cancer with continuous increased demand of new highly effective, safer, less toxic and more selective drugs. Though it is a tedious process immediate recovery can be seen through reports because of its quick action. But there are some downsides also. There is blockage of drug binding to target side because of chances of some undesirable mutation or resistance created by target proteins or certain side effects left after chemotherapy. As patients are subjected to radiation therapy where cancer cells are killed and disease is cured. But along with cancer cells some normal cells also get killed. In order to

develop selective killing and less cytotoxic drugs, molecular hybridization technique may give a new effective compound. Optimisation of drugs is done at the molecular level in order to produce an improved version of anticancer drug. This approach is helpful in formulating the drugs of selective killing and less cytotoxic. Indole exhibits immense biological activity and wide distribution in nature. Indole derivatives always attracted researchers to design target-based compounds possessing indole moiety as anticancer agentsand become one of the important building blocks of many drug development and research centres.[2,3] The vinca alkaloids (vincristine[4] and vinblastine[5]) obtained from ***Catharanthusroseus*** were the major turning point in the discovery of indole based anticancer agents.

2. REVIEW OF LITERATURE

Indole is an important nitrogen containing heterocyclic ring with benzene attached to pyrole ring at α and β positions and become core structure of many drugs due to its privileged structure and broad spectrum activities like antiviral, anti-inflammatory, anticancer, antimicrobial and antifungal activities. Vincristine and vinblastine having indole moiety are antitumor agents being recognized since in 1965 and is characterized as tubulin polymerization inhibitor. This can cure Hodgkin lymphoma and non-Hodgkin lymphoma cancer. Numerous efforts are being going on to develop novel indole based drugs of better biological activity and lesser cytotoxicity.

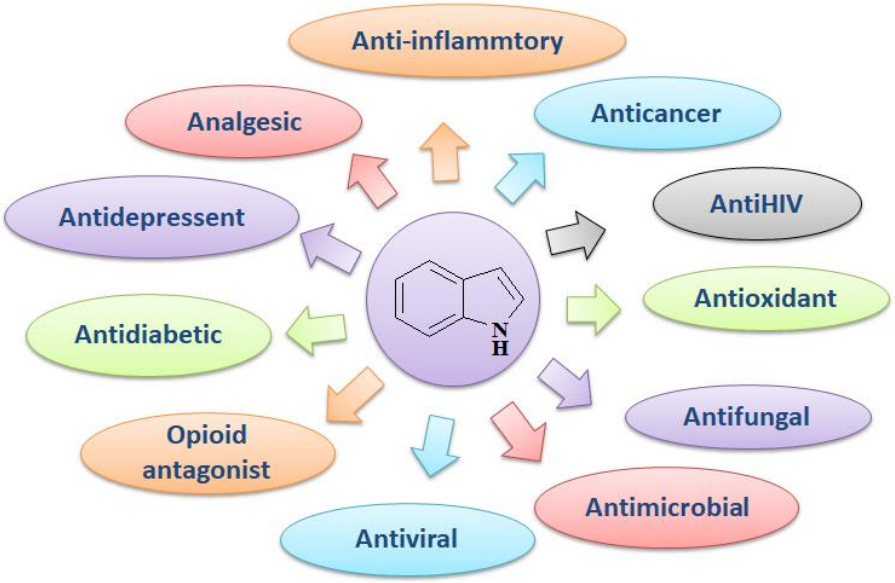
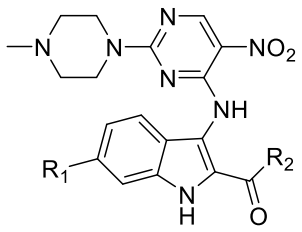


Figure 1. Biological activities of indole derivatives

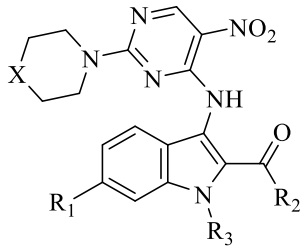
Hu *et al.* worked on new indole-pyrimidine conjugates having piperazine moiety **(1)** and tested their antitumor activity. Among all compounds, **1d** showed highest potency or cytotoxicity towards MDA-MB-231, MCF-7 and A549 malignant cell lines having IC_{50} values in the range 5.01 - 14.36 μM.[6]



1a-g

	R_1	R_2
1a	H	4-FC_6H_4
1b	H	4-BrC_6H_4
1c	H	4-PhC_6H_4
1d	Cl	C_6H_4
1e	Cl	4-ClC_6H_4
1f	Cl	4-$MeOC_6H_4$
1g	Cl	

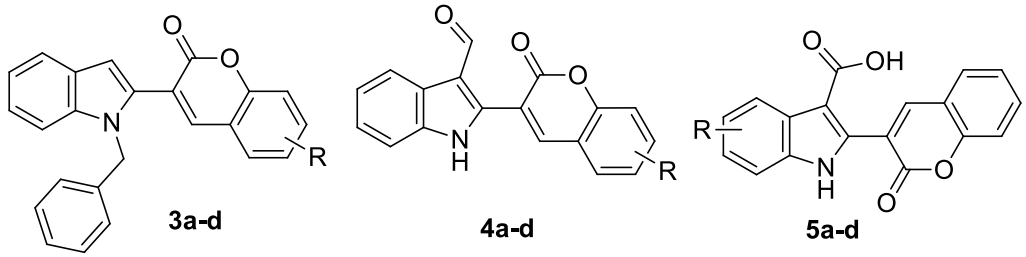
Diao and co-workers synthesized indole-pyrimidine based hybrids bearing thiomorpholine or morpholine moieties **(2)** and their antiproliferative properties were tested towards MCF-7, HCT-116, MDA-MB-231 and HeLa human cancer cell line. It was observed that compound **2e** caused inhibition of 42% of tubulin polymerization at 10μM concentration and have most excellent activity having IC_{50} values of 9.48 μM, 0.29μM, 4.04μM, for HCT-116, MCF-7 and HeLa cell lines. Among all analogues, **2d** showed highest inhibition activity towards HeLa cancer cells with IC_{50} value of 2.51μM.[7]



2a-j

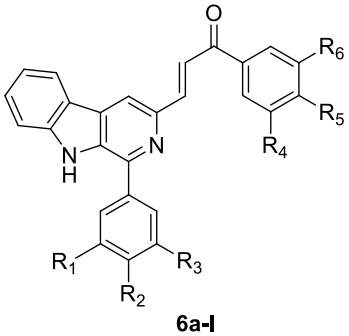
	R_1	R_2	R_3	X		R_1	R_2	R_3	X
2a	H	C_6H_5	H	O	**2f**	H		H	O
2b	H	4-BrC_6H_4	H	O	**2g**	H	4-$MeOC_6H_4$	CO_2Et	O
2c	H	4-$MeOC_6H_4$	H	O	**2h**	Cl	C_6H_5	H	O
2d	H	3-BrC_6H_4	H	O	**2i**	H	C_6H_5	H	S
2e	H	4-PhC_6H_4	H	O	**2j**	H	4-$MeOC_6H_4$	H	S

Kamath and co-workers synthesised indole and coumarin based chromenones **(3)**, carbaldehydes **(4)** and carboxylic acids (**5).** It has been observed that bromine substituted derivative **4c** showed highest antiproliferative towards MCF-7 human breast tumour cell lines.[8]



R: **a** = H; **b** = Cl; **c** = Br; **d** = OH

Shankaraiah and co-workers prepared β-carboline analogue associated with chalcone conjugates **(6)** and studied their anticancer activity and binding affinity with DNA. Most of the hybrids showed highest inhibitory effect towards A-549 cell lines having IC_{50} values lesser than 10μM. Among all the synthesised compounds, **6b** exhibited high antagonistic activity towards certain cell lines having IC_{50} value lower than 50 μM.[9]

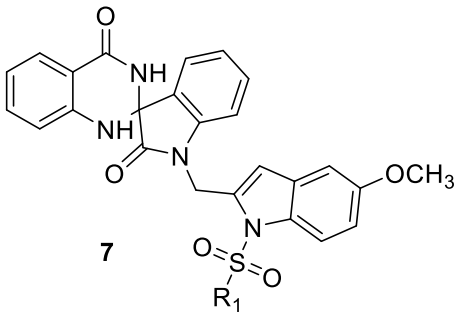


6a-l

	R_1	R_2	R_3	R_4	R_5	R_6
6a	OCH_3	OCH_3	OCH_3	H	OCH_3	H
6b	OCH_3	OCH_3	OCH_3	OCH_3	OCH_3	OCH_3
6c	OCH_3	OCH_3	OCH_3	H	F	H
6d	OCH_3	OCH_3	OCH_3	H	NO_2	H
6e	OCH_3	OCH_3	OCH_3	H	NH_2	H
6f	OCH_3	OCH_3	OCH_3	H	OH	H
6g	H	F	H	H	OCH_3	H
6h	H	F	H	OCH_3	OCH_3	OCH_3
6i	H	F	H	H	F	H
6j	H	F	H	H	NO_2	H
6k	H	H	H	H	NH_2	H
6l	H	OCH_3	OCH_3	H	CH_3	H

Rambabu *et al.* had prepared *N*-indolylmethyl quinazoline derivatives (**7**) which exhibited antiproliferative activities. Results of the primary screening of compound revealed that substitution of indole at C-5 position played an important role in Sir 2 protein inhibition. The

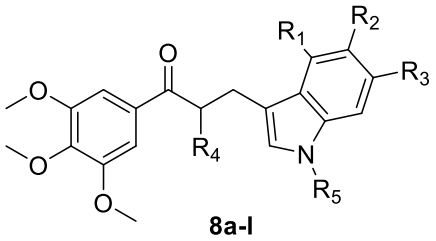
compounds having *N*-sulfonyl-5-methoxy moiety showed excellent inhibitory activity at 50 μM concentration.[10]



7

R_1 = Me or Ph

Yan *et al.* synthesised highly selective indole-chalcone conjugates and checked their antiproliferative activity. Among all synthesized derivatives of propane-1-one based indole, compound **8k** exhibited highest inhibition with IC_{50} values between 3-9 μM.[11]



8a-l

8a $R_1 = OCH_3$ $R_2 = R_3 = R_4 = R_5 = H$

8b $R_1 = OCH_3$ $R_2 = R_3 = R_4 = H$, $R_5 = CH_3$

8c $R_1 = OCH_3$ $R_2 = R_3 = R_5 = H$, $R_4 = CH_3$

8d $R_1 = OCH_3$ $R_2 = R_3 = H$, $R_4 = R_5 = CH_3$

8e $R_2 = OCH_3$ $R_1 = R_3 = R_4 = R_5 = H$

8f $R_2 = OCH_3$ $R_1 = R_3 = R_4 = H$, $R_5 = CH_3$

8g $R_2 = OCH_3$ $R_1 = R_3 = R_5 = H$, $R_4 = CH_3$

8h $R_2 = OCH_3$ $R_1 = R_3 = H$, $R_4 = R_5 = CH_3$

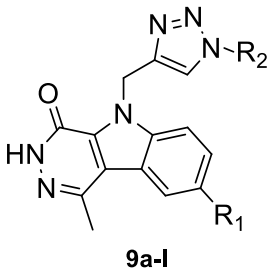
8i $R_3 = OCH_3$ $R_1 = R_2 = R_4 = R_5 = H$

8j $R_3 = OCH_3$ $R_1 = R_2 = R_4 = H$, $R_5 = CH_3$

8k $R_3 = OCH_3$ $R_1 = R_2 = R_5 = H$, $R_4 = CH_3$

8l $R_3 = OCH_3$ $R_1 = R_2 = H$, $R_4 = R_5 = CH_3$

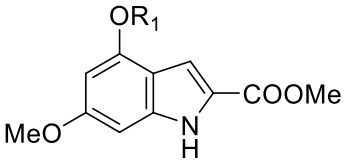
Panthaur *et al*. have synthesized pyridazino[4,5-*b*]indole analogues bearing phenacyl-substituted aryl, alkyl and 1,2,3-triazolylmethyl and checked their antitumour activity towards four cell lines *viz*; U-87 (human primary glioblastoma), IMR-32 (human neuroblastoma), MDA-MB-231 and MCF 7. All compounds exhibited growth inhibition with IC_{50} values lesser than 1μM towards various cancer cell lines. While derivatives **9i** with IC_{50} values of 0.04 μM and **9k** with IC_{50} values of 0.07 μM showed highest growth inhibition towards human neuroblastoma (IMR-32) cancer cell line, respectively.[12]



9a-l

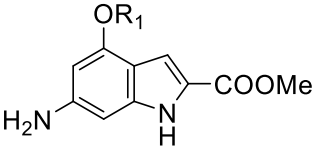
	R_1	R_2
9a	H	Cyclopentyl
9b	H	Cyclohexyl
9c	H	4-Methylphenacyl
9d	H	4-Methoxyphenacyl
9e	F	Benzyl
9f	F	4-Cyanobenzyl
9g	F	4-Nitrobenzyl
9h	F	4-Fluorobenzyl
9i	F	Cyclohexyl
9j	F	Cyclopentyl
9k	F	2-Fluorobenzyl
9l	F	4-Methylphenacyl

Ji *et al*. prepared pyroquinoline quinone based indole-2-carboxylate analogues and evaluated their anticancer activity towards HepG2, A549, and MCF7 cell lines. The derivatives **10** and **11e** showed highest potency having IC_{50} values varying between 3.78 ± 0.58 to 24.08 ± 1.76 μM concentration.[13]



10

R_1 = Alkoxy



11a-e

11a R_1 = Benzyl

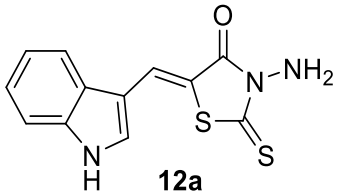
11b R_1 = *p*-Fluoro benzyl

11c R_1 = Allyl

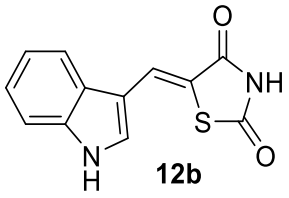
11d R_1 = Isobutyl

11e R_1 = Cyclohexylmethyl

Lafayette *et al*. synthesized novel series of indole analogues having thiazolidine moieties and checked their binding affinity with DNA, antitopoisomerase I and antitumor activity. The indole derivative **(12a)** displyed antitumor activity towards HL60 and K562, and determined value of DNA binding constat of 5.69×10^4 M^{-1}. On the other hand compound **12b** exhibited higest antitumor activity towards T47D cell line having IC_{50} value of 1.93μM.[14]

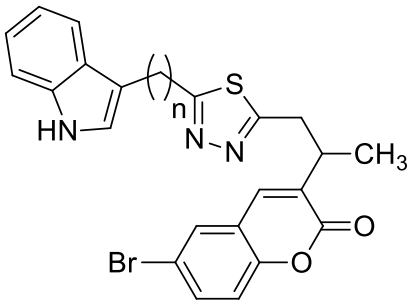


12a



12b

Kamath *et al.* synthesized three hybrids of thiadiazole moiety by linking indole and thiadiazole rings **(13)** through spacers of varying length. One of the derivative **13c** exhibited highest antiproliferative activity against breast adenocarcinoma (MCF-7) cells.[15]



n

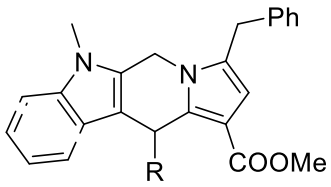
13a = 1

13b = 2

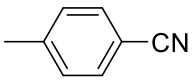
13c = 3

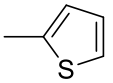
13 a-c

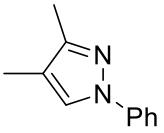
Nagesh *et al.* synthesized three different dihydroindolizinoindole analogues. Among these compounds, compound having 4-cyanophenyl group **(14)** showed highly selective interaction, highest affinity, and maximum stability towards G-quadruplex DNA and showed excellent inhibitory activity towards cell proliferation of cancer cell.[16]



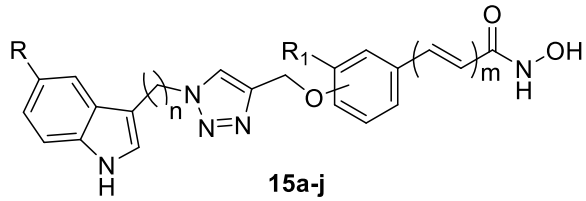
14a-c

R - **14a** 

14b 

14c 

Cai *et al*. worked on novel conjugates of indole moiety with *N*-hydroxyarylamide via substituted triazole **(15a-j).** Synthesised derivatives were checked for anticancer activity towards carcinoma cells. It was observed that compound **15g** exhibited most efficient activity and selectivity for HDAC8 than SAHA and HDAC1 over HDAC6.[17]

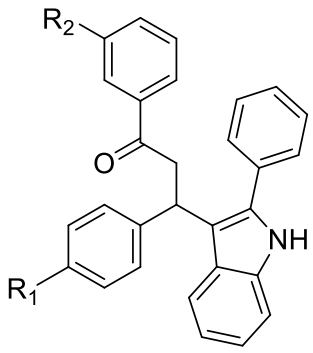


15a-j

	m	n	R	R_1		m	n	R	R_1
15a	0	1	H	H	**15f**	1	1	OMe	OMe
15b	0	1	H	OMe	**15g**	1	2	H	H
15c	1	1	H	OMe	**15h**	1	2	H	OMe
15d	1	1	H	H	**15i**	1	2	H	H
15e	0	2	H	H	**15j**	0	1	OMe	H

Patel *et al*. synthesized 3-(3-oxoaryl) indole derivatives (**16a-i**) and evaluated against towards human breast cancer MCF7 and murine melanoma B16F10 cell lines. Derivatives **16e** and **16i**

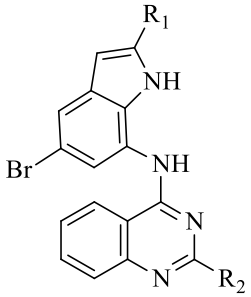
exhibited potent anticancer activity against B16F10 as well as MCF7 tumour cell having IC_{50} values of 10 μM - 15 μM.[18]



16a-i

	R_1	R_2		R_1	R_2
16a	H	H	**16f**	NO_2	H
16b	Br	H	**16g**	OCH_3	H
16c	F	H	**16h**	CH_3	H
16d	Cl	H	**16i**	H	Cl
16e	CF_3	H			

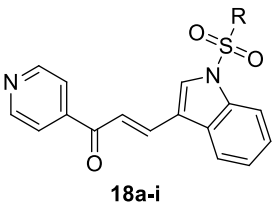
Mphahlele and co-workers worked on indole-aminoquinazoline conjugates and tested all the compounds against various cancer cells. Compound **17b** having IC_{50} values of 12.67 μM, 17.65 μM, **17f** having IC_{50} values of 9.16 μM, 19.42 μM and **17g** with IC_{50} values of 16.45, 12.20 μM showed highest activity against Caco-2 (epithelial colorectal adenocarcinoma) and C3A (hepatocellular carcinoma) cells, respectively.[19]



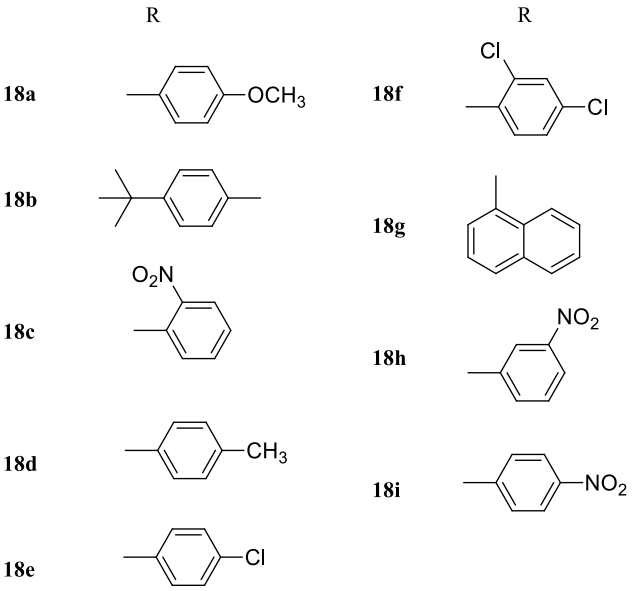
17a-h

	R_1	R_2
17a	C_6H_5	C_6H_5
17b	4-FC_6H_4	C_6H_5
17c	3-ClC_6H_4	C_6H_5
17d	4-$CH_3OC_6H_4$	C_6H_5
17e	C_6H_5	4-FC_6H_4
17f	4-FC_6H_4	4-FC_6H_4
17g	3-ClC_6H_4	4-FC_6H_4
17h	4-$CH_3OC_6H_4$	4-FC_6H_4

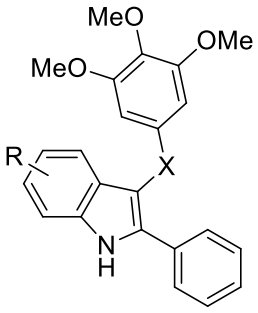
Mudasir and co-workers worked on a series of sulfonamide analogues of chalcone (**18**) based on pyridyl-indole as carbonic anhydrase inhibitor and antitumour agent. The results revealed that compounds **18b, 18f** and **18i** were observed to have excellent binding affinity and showed excellent anticancer activity against MCF-7 and HepG-2 human cancer cells.[20]



18a-i



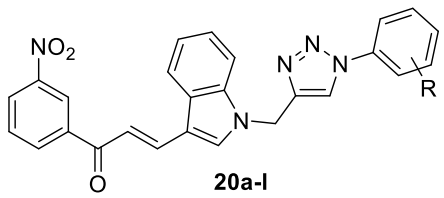
Regina and co-workers worked on a series of 2-phenylindole analogues (**19**) as potent anticancer agents containing 3, 4, 5-trimethoxyphenyl moiety linked with substituted indole via carbonyl group, methylene or sulphur. Compounds **19e** and **19h** exhibited highest inhibition at nanomolar concentration against numerous cancer cell lines like U343, MDA-MB-231, A-549, MDA-MB-436, T98G, NB4, MDA-MB-468, NCI-H1975 and MV4−11 cell lines.[21]



19a-k

	R	X		R	X
19a	4-Br	S	**19g**	F	C=O
19b	4-Br	C=O	**19h**	6,7-Cl_2	S
19c	4-Cl	S	**19i**	5-Br	S
19d	4-Cl	C=O	**19j**	5-Br	C=O
19e	6-MeO	S	**19k**	5-Br	CH_2
19f	F	S			

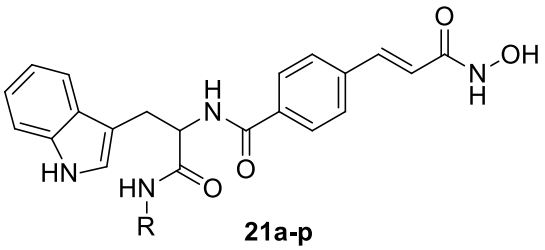
Babita *et al.* synthesized triazole based indole-chalcone analogues **(20)** and checked their antiproliferative, and binding properties with DNA. Among all the synthesised derivatives, compound **20b** exhibited highest inhibitiory effect against the growth of SW620 and human tumour cell lines (SiHa) having IC_{50} values of 48.96 and 67.99 μg mL^{-1}, respectively.[22]



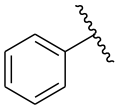
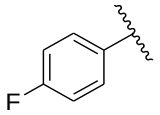
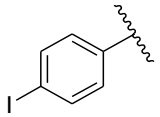
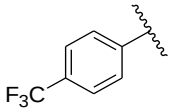
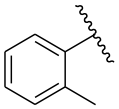
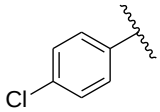
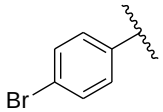
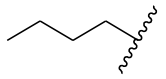
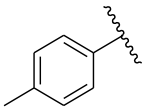
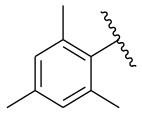
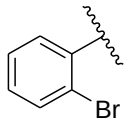
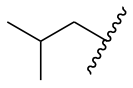
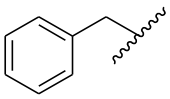
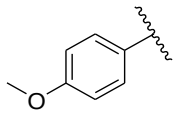
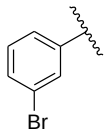
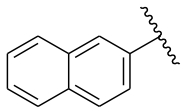
20a-l

	R		R
20a	H	**20g**	*p*-NO_2
20b	*o*-Cl	**20h**	*o*-F
20c	*m*-Cl	**20i**	*m*-F
20d	*p*-Cl	**20j**	*p*-F
20e	*o*-NO_2	**20k**	*o*-CH_3
20f	*m*-NO_2	**20l**	*m*-CH_3

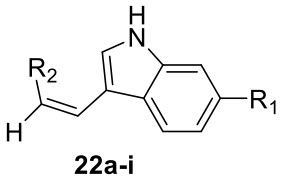
Zhang *et al*. synthesized *N*-hydroxycinnamamide-based derivatives as histone deacetylases inhibitors (**21**) having various aromatic ring and aliphatic linker for effective treatment of cancer. Biological evaluations established that compounds **21h, 21i, 21j, 21l,** and **21p** exhibited highest inhibition of histone deacetylase and antitumor activity towards few of human cancer cells [PC-3, SK-N-BE(2), HeLa] than known inhibitor SAHA.[23]



21a-p

	R		R		R		R
21a		**21e**		**21i**		**21m**	
21b		**21f**		**21j**		**21n**	
21c		**21g**		**21k**		**21o**	
21d		**21h**		**21l**		**21p**	

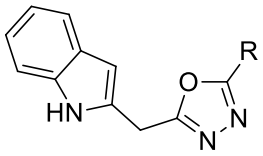
Dolusic *et al.* synthesized substituted 3-(2-(ethenyl)indoles (**22**) as indoleamine 2,3 dioxygenase inhibitor and checked antitumor activity. Compounds **22f** and **22i** exhibited highest inhibitory effect towards TDO having IC_{50} values of 2.0 and 3.0 μM, respectively. [24]



22a-i

	R_1	R_2		R_1	R_2
22a	H	pyrid-3-yl	**22f**	6-F	tetrazole
22b	6-F	pyrid-3-yl	**22g**	6-F	3-F-phenyl
22c	H	pyrid-2-yl	**22h**	6-F	3-Cl-phenyl
22d	6-F	pyrid-2-yl	**22i**	6-F	COOH
22e	H	CH_3			

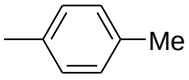
Anouar and co-workes prepared and tested indole and oxadiazole based conjugates (**23**) for inbition of β-glucuronidase enzyme. Majority of compounds exhibited very good inhibitory activity having IC_{50} values ranging from 0.9 ± 0.01 to 46.4 ± 0.9 μM when compared with the positive control D-saccharic acid 1,4-lactone (IC_{50} = 48.1 ± 1.2 μM) and compound **23d** was proved to be effective derivative having IC_{50} value of 0.9±0.01 μM.[25]



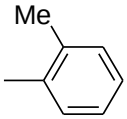
23a-e

R

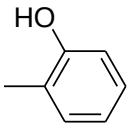
23a



23b

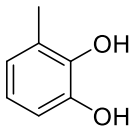


23c

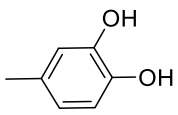


R

23d



23e



3. RESEARCH GAPS AND OBJECTIVES

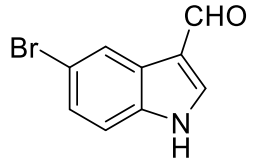
Heterocyclic aromatic compound serves as an important pharmacophore in many drugs, for example, indole moiety, exhibits potent anticancer properties. Efficiency of indole moiety can be enhanced by hybridization with other bioactive molecule such as benzimidazole which is rarely reported in literature. By hybridization, new and more effective hybrid compounds based on indole and benzimidazole moeities would be synthesized. These novel compounds would further be characterized using NMR spectroscopy technique.

4. EXPERIMENTAL

4.1 Material and Methods

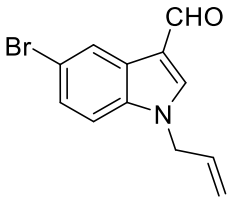
All the used solvents and chemicals were of commercial grade and pure. These were purchased from Loba, Spectrochemicals, Aldrich etc. Melting points (^oC) were recorded on Digital Melting Point apparatus and were uncorrected. ^1H NMR spectra was recorded using Bruker 400 MHz NMR spectrophotomter in $CDCl_3$ solvent. The chemical shifts were indicated as ppm using tetramethylsilane as an internal standard and *J* values were calculated in Hz. Silica gel HF-254 coated plates were used for monitoring the reactions. Mesh size 60-120 Silica gel was used to accomplish column chromatography. Hexane:ethyl acetate was the accepted solvent system for TLC and coloumn chromatopgraphy.

4.2. Synthesis of 5-bromo-1*H*-indole-3-carbaldehyde (2):



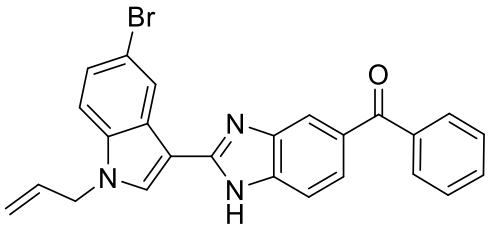
5-Bromo-1*H*-carbaldehyde (**2**) was synthesised by Vilsmiyer-Hack reaction of 5-bromo indole (3 g, 1.5mmol) using $POCl_3$ (1 ml) and DMF (2 ml) at room temperature. Reaction progress was monitored by TLC. Reaction mixture was added into crushed ice and *p*H was maintained at 7 using aqueous solution of NaOH. Precipitate was filtered and washed with water. Yield: 95%; colour: white; m.pt.: 51-54 ^oC

4.3. Synthesis of 1-allyl-5-bromo-1*H*-indole-3-carbaldehyde (3):



Allylbromide (2g, 1.6 mmol) and compound **2** (1.28 g, 0.8 mmol) along with aq. KOH were taken in acetone in a well dried round bottom flask. Reaction mixture was allowed to stir at room temperature for 1 h. Completion of reaction was confirmed by TLC. Solvent of reaction mixture was rota evaporated under pressure and crude was precipitated out by adding water to reaction mixture. Precipitate formed was filtered off to get the pure product. Yield: 87%; colour: brown; m.pt.: 59-61 ^oC

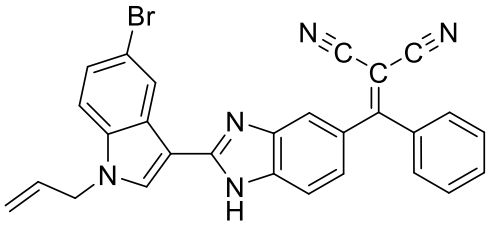
4.4. Synthesis of [2-(1-allyl-5-bromo-1*H*-indol-3-yl)-1*H*-benzoimidazol-5-yl]-phenyl-methanone (5):



Mixture of compound **3** (1.6 g, 0.6 mmol) and 3,4-diaminobenzophenone (**4**) (2 g, 0.9 mmol) in nitrobenzene was heated for 4 h at 80 ºC. Reaction monitoring was done with the help of TLC. Hexane was added to the reaction mixture on completion of reaction and filtered off to get the crude product.Yield: 68%; colour: brownish yellow; m.pt.: 153-156 ºC

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.44 (s, 1H, ArH), 8.05 (s, 1H, ArH), 7.87 (s, 1H, ArH), 7.81 (d, *J* = 7.72 Hz, 2H, ArH), 7.75 (d, *J* = 8.28 Hz, 1H, ArH), 7.57 (d, *J* = 7.44 Hz, 1H, ArH), 7.48 (t, *J* = 7.44 Hz, 3H, ArH), 7.37 (d, *J* = 8.96 Hz, 1H, ArH), 7.21 (d, *J* = 8.80 Hz, 1H, ArH), 5.98-5.89 (m, 1H, CH-allyl), 5.25 (d, *J* = 10.12 Hz, 1H, CH-allyl), 5.13 (d, *J* = 17.12 Hz, 1H, CH-allyl), 4.70 (d, *J* = 2.92 Hz, 2H, N-CH_2-allyl).

4.5. Synthesis of 2{[2-(1-allyl-5-bromo-1*H*-indol-3-yl)-1*H*-benzoimidazol-5-yl]-phenyl-methylene}-malonitrile (6):



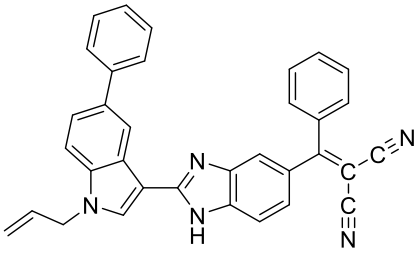
Mixture of compound **5** (2.0 g, 0.4 mmol) and malononitrile (5.7 g, 19 mmol) was refluxed in acetic anhydride at 110 ºC for about 15 h. After the reaction completion as confirmed by TLC, solvent was distilled off by rotator evaporator. Water was added to the reaction mixture and product was filtered off. Column chromatography was used to purify the product using hexane: ethyl acetate as eluents. Yield: 61%; colour: yellow; m.pt.: 168-171 ºC.

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.39 (s, 1H, ArH), 7.76 (s, 1H, ArH), 7.71 (s, 1H, ArH), 7.59-7.52 (m, 2H, ArH), 7.49-7.42 (m, 4H, ArH), 7.37 (d, *J* = 8.76 Hz, 1H, ArH), 7.27 (s, 1H, ArH), 7.21 (d, *J* = 8.64 Hz, 1H, ArH), 5.99-5.89 (m, 1H, CH-allyl), 5.27 (d, *J* = 10.24 Hz, 1H, CH-allyl), 5.14 (d, *J* = 16.92 Hz, 1H, CH-allyl), 4.70 (d, *J* = 5.12 Hz, 2H, N-CH_2-allyl).

4.6. Synthesis of 2-((2-(1-allyl-5-aryl-1*H*-indol-3-yl)-1*H*-benzo[*d*]imidazol-5-yl)(phenyl)methylene)malononitrile (7a-b):

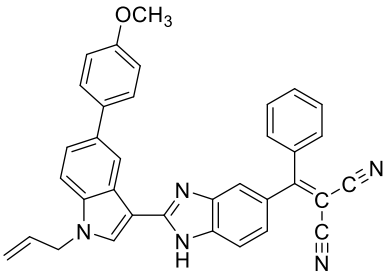
Intermediate (**6**) (100 mg, 0.19 mmol), substituted phenyl boronic acid, potassium carbonate (28 mg, 0.19 mmol) and $Pd(PPh_3)_4$(5 mol%) were added in well dried round bottom flask (50 ml) having 10 ml of $CH_3CN:H_2O$ (9:1). Reaction mixture was refluxed for 12-15 h. On reaction completion, solvent was evaporated using rotary evaporator and crude product was extracted using chloroform. Column Chromatography was used to purify the product using hexane: ethyl acetate as eluents.

4.6.1. Spectral data of 7a:



Yield: 72%; colour: yellow; m.pt.: 191-193 ºC; ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.46 (s, 1H, ArH), 8.09 (s, 1H, ArH), 7.84-7.77 (m, 6H, ArH), 7.70-7.65 (m, 2H, ArH), 7.59-7.56 (m, 2H, ArH), 7.50 (t, *J* = 7.72 Hz, 3H, ArH), 7.40-7.38 (m, 1H, ArH), 7.26 (s, 1H, ArH), 6.02-5.93 (m, 1H, CH-allyl), 5.28 (d, *J* = 10.40 Hz, 1H, CH-allyl), 5.15 (d, *J* = 17.28 Hz, 1H, CH-allyl), 4.75 (d, *J* = 5.32 Hz, 2H, N-CH_2-allyl).

4.6.2. Spectral data of 7b:



Yield: 72%; colour: yellow; m.pt.: 191-193 ºC; ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.72 (s, 1H, ArH), 8.21 (s, 1H, ArH), 8.17 (s, 1H, ArH), 7.95 (s, 1H, ArH), 7.78 (d, 2H, *J*= 5.84 Hz, ArH), 7.67-7.62 (m, 5H, ArH), 7.57-7.49 (m, 4H, ArH), 7.38 (d, 1H, *J*=6.96, ArH), 6.12-6.05 (m, 1H, CH-allyl), 5.28 (d, *J* = 7.84 Hz, 1H, CH-allyl), 5.20 (d, *J* = 13.72 Hz, 1H, CH-allyl), 4.95 (d, *J* = 4.24 Hz, 2H, N-CH_2-allyl), 3.32 (S (merged with DMSO), 3H, OCH_3)

5. RESULTS AND DISCUSSION

5-Bromo-1*H*-indole-3-carbaldehyde **(2)** was synthesized with Villsmiyer-Hack reaction by the treatment of 5-bromo indole **(1)** with $POCl_3$ and DMF at room temperature for 30 min. After work up with ice and NaOH, white coloured solid product was obtained in 95% yield. Compound **2** was further allowed to react with allyl bromide in acetone in the presence of aquaous KOH at room temperature for about 1 h gave brown colour solid of **3** in 87% yield. Compound **3** was further treated with 3,4 diaminobenzophenone (**4**) in nitrobenzene at 80 ºC for 4 h gave compound **5** as yellow coloured solid with 68% yield. Compound **5** was characterized by ^{1}H NMR spectroscopy. ^{1}H NMR spectrum of **5** showed broad range of

splitting pattern between δ 8.44–7.21 ppm corresponding to twelve aromatic protons. The characteristic peaks of allyl group (one proton showed muliplet at δ 5.98-5.89 ppm corresponding to CH of allyl, two doublets of two protons at δ 5.24 and 5.17 ppm corresponding to CH_2 of allyl and two protons showed doublet at δ 4.70 ppm corresponding to N-CH_2 of allyl) in aliphatic region confirmed the formation of compound **5 (Figure - 2)**.

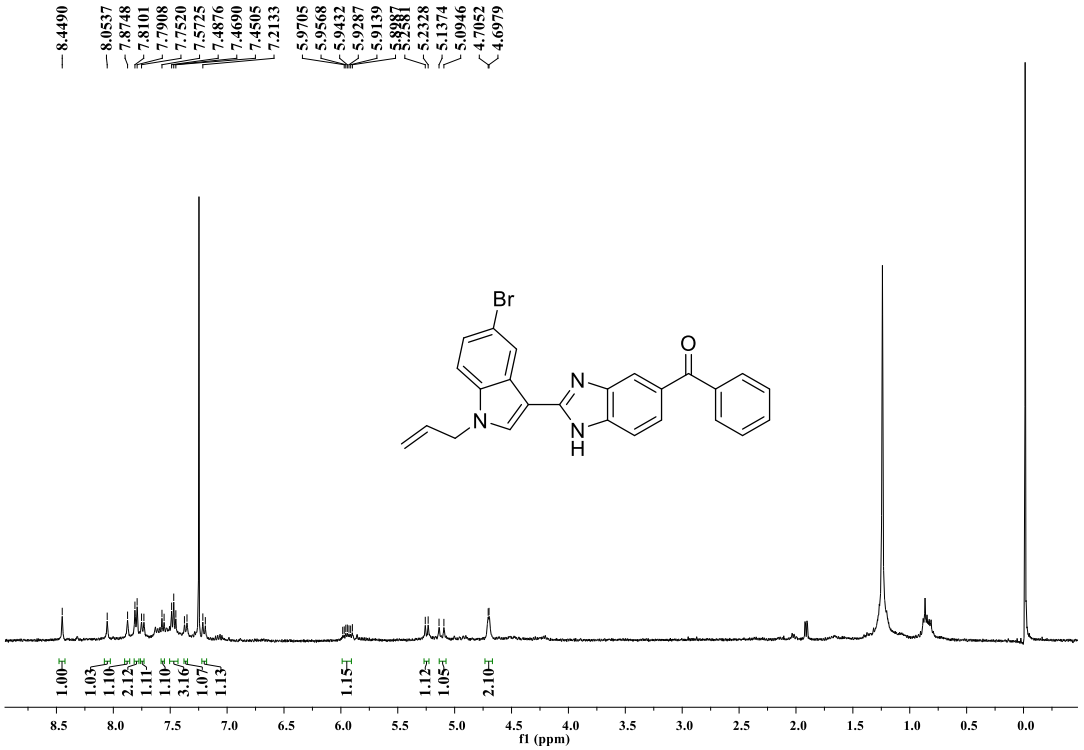


Figure 2. ^{1}H NMR spectrum of compound 5

Compound **5** was than reacted with malononitrile in the presence of acetic anhydride at 110 ^oC for 15 h, excess solvent was distilled off under reduced pressure and water was added to the reaction mixture to give solid yellow coloured intermediate **6** with 61% yield. ^{1}H NMR spectrum of **6** showed broad range of splitting pattern as it was observed in compound **5** ranging from δ 8.39 –7.21 ppm corresponding to twelve aromatic protons and characteristic peaks of allyl group (one proton showed multiplet at δ 5.99-5.89 ppm corresponding to CH of allyl, two protons showed two doublets at δ 5.27 and 5.14 ppm corresponding to CH_2 of allyl and two protons showed doublet at δ 4.70 ppm corresponding to N-CH_2 of allyl) in aliphatic region **(Figure – 3)**. Compound **6** showed same splitting pattern as compound **5** but shifting in peak positions and change in spots at TLC confirmed the formation of compound **6.**

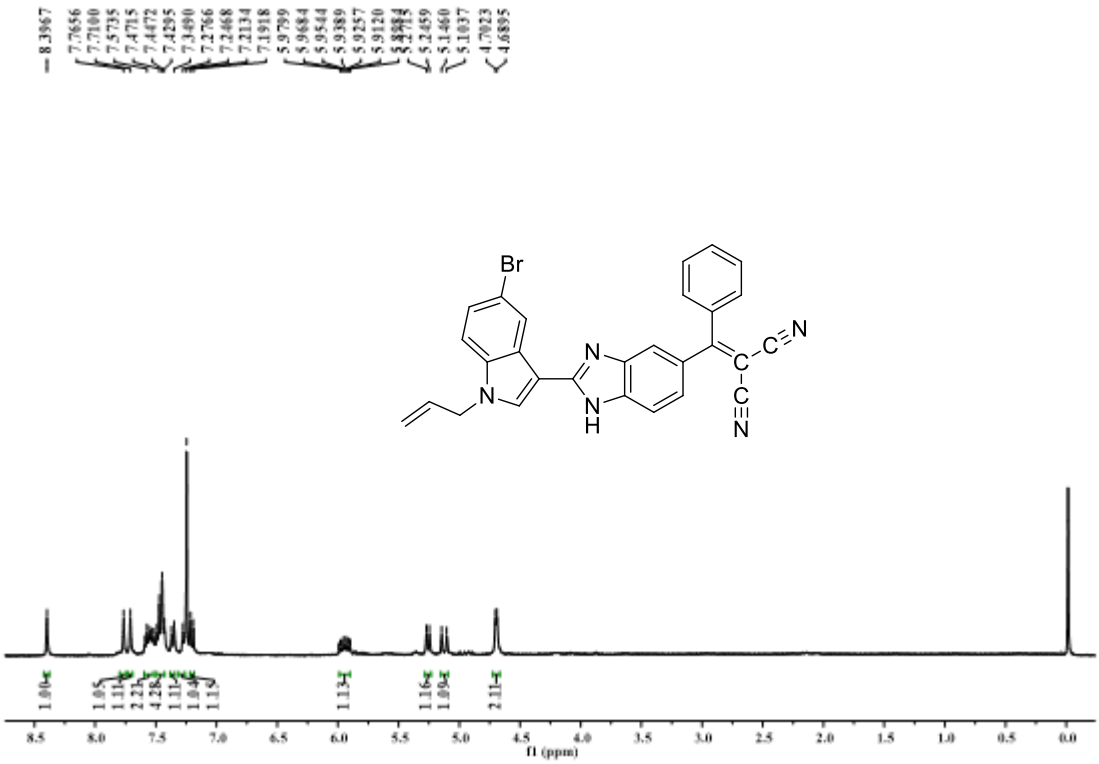
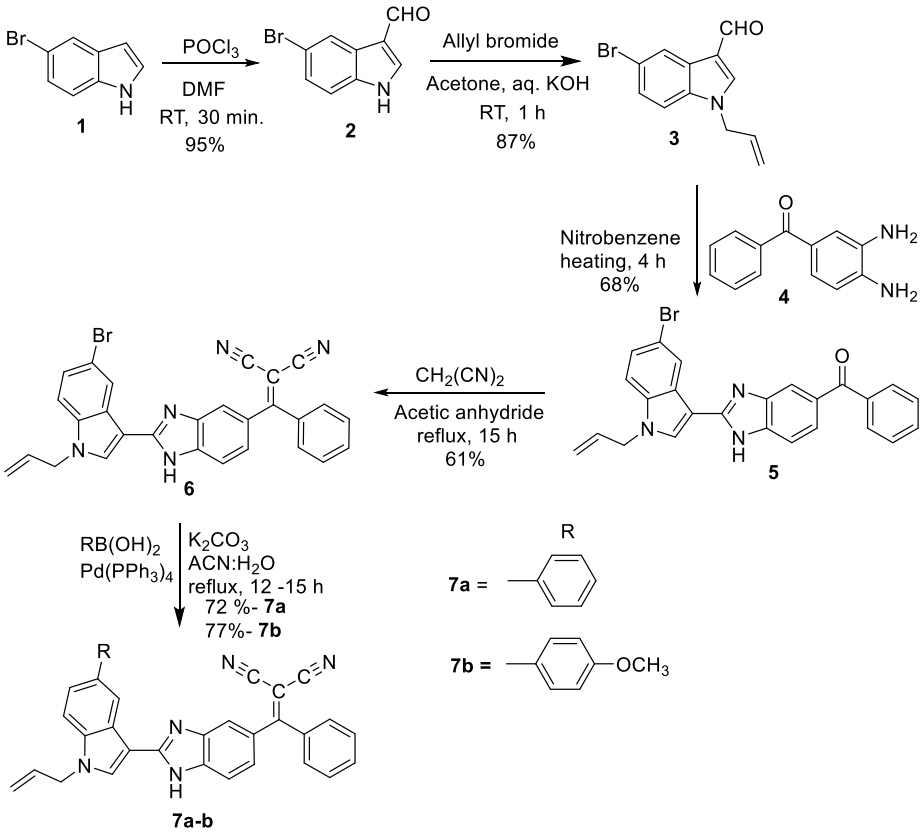


Figure 3.^{1}H NMR spectrum of compound 6



Scheme 1. Synthesis of 2-((2-(1-allyl-5-aryl-1*H*-indol-3-yl)-1*H*-benzo[*d*]imidazol-5-yl)(phenyl)methylene)malononitrile

Compound **6** was further used for derivaitization by Suzuki-Miyaura couplings with phenyl boronic acids and 4- methoxy phenylboronic acid in the present of potassium carbonate, and tetra kis(triphenylphosphine) palladium [$Pd(PPh_3)_4$] in ACN:H_2O (9:1) (Suzuki coupling) to give compounds **7a** and **7b** in 72% and 77% yields, respectively. These two compounds were characterized by proton NMR spectroscopy **(Figures - 4, 5).**

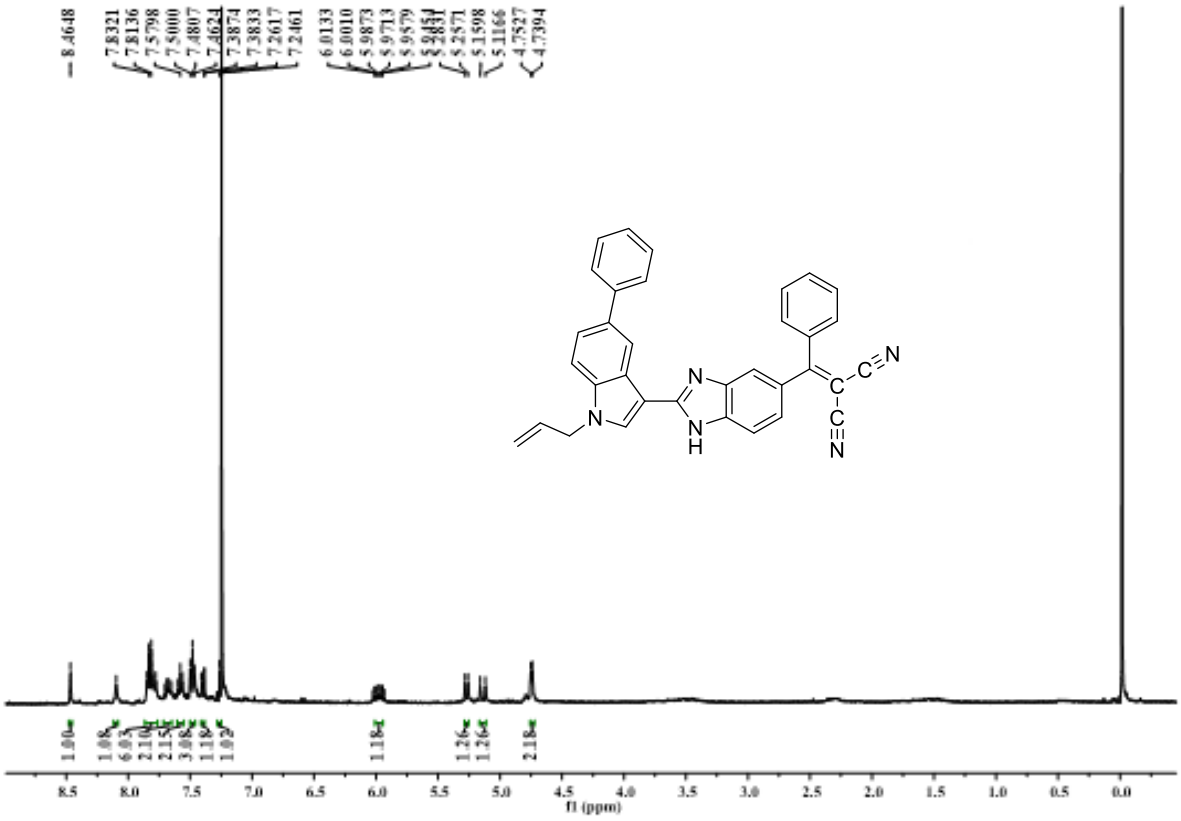


Figure 4. ^{1}H NMR spectrum of compound 7a

^{1}H NMR spectrum of **7a** showed same splitting pattern like compound **6** ranging from δ 8.46–7.26 ppm that corresponds to seventeen aromatic protons. Five additional aromatic protons of phenyl ring due to Suzuki coupling of phenyl boronic acid are appeared in aromatic region. The characteristic peaks of allyl group (one proton showed muliplet at δ 6.0-5.93 ppm corresponding to CH of allyl, two doublets of two protons at δ 5.28 and 5.15 ppm corresponding to CH_2 of allyl and two protons showed doublet at δ 4.75 ppm corresponding to N-CH_2 of allyl) in aliphatic region confirmed the formation of compound **7a** (**Figure 4**).

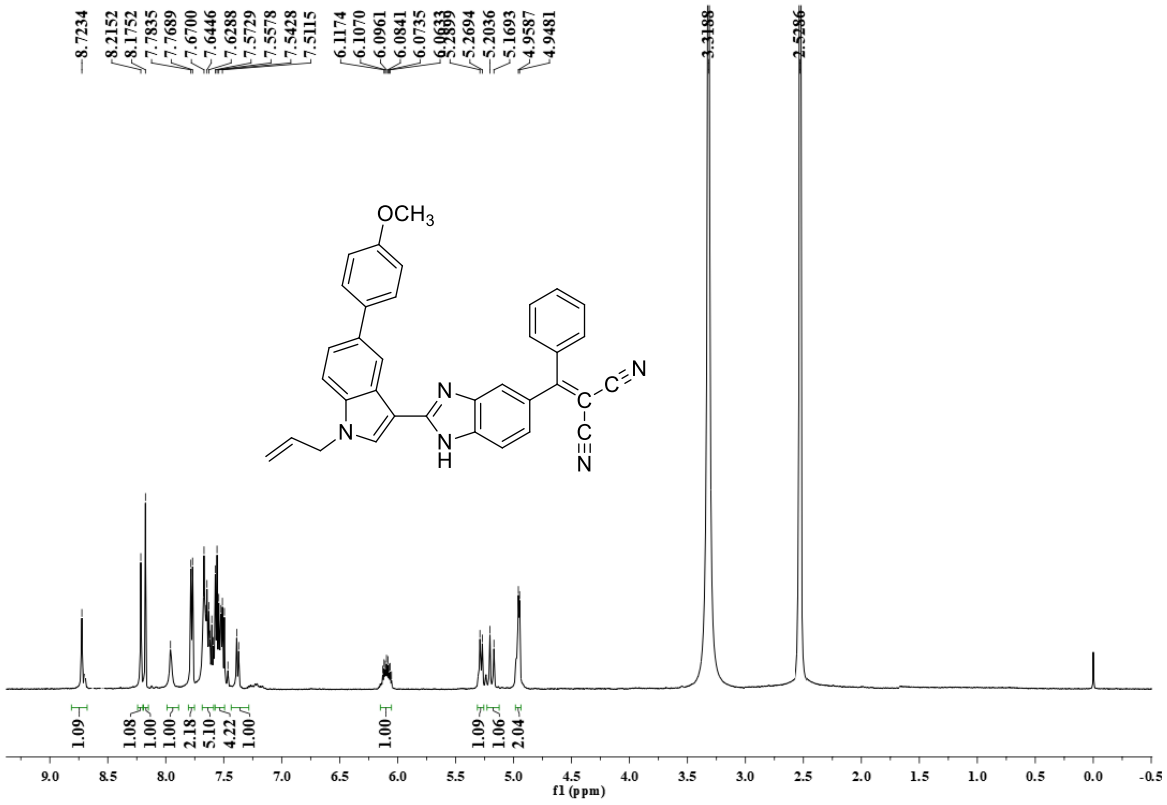


Figure 5. ^{1}H NMR spectrum of compound 7b

^{1}H NMR spectrum of **7b** showed same splitting pattern ranging from δ 8.72–7.38 ppm that corresponds to sixteen aromatic protons. Four additional aromatic protons appeared due to 4-methoxy phenyl boronic acid confirmed the formation of compound **7b**. Additionally, the characteristic peaks of allyl group (one proton showed muliplet at δ 6.12-6.05 ppm corresponding to CH of allyl, two doublets of two protons at δ 5.28 and 5.20 ppm corresponding to CH_2 of allyl and two protons showed doublet at δ 4.95 ppm corresponding to N-CH_2 of allyl) in aliphatic region confirmed the formation of compound **7b** (**Figure 5**).

6. CONCLUSION:

- Intermediates such as **2, 3, 5** and **6** were prepared in moderate to good yields.
- Our targeted molecules **7a** and **7b** were synthesised in moderate yield and were characterized by ^{1}H NMR spectrometry. Further synthesis and characterization of indole based derivatives are in progress.
- The compounds that were synthesized will be used for studying their biological activities as anti-cancer agents.

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