Synthesis of Molecular Hybridization Based Aromatic linked Naphthalimide-Benzimidazole Conjugates

A

Thesis submitted

In the partial fulfillment of the requirement for the degree of

MASTERS OF SCIENCE

IN

CHEMISTRY

Submitted By

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(301602002)

UNDER THE SUPERVISION

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(Associate Professor)

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THAPAR INSTITUTE OF ENGINEERING & TECHNOLOGY,

PATIALA-147004

2018

CERTIFICATE

This is to certify that the thesis entitled **"Synthesis of Molecular Hybridization Based Aromatic linked Naphthalimide-Benzimidazole Conjugates"** submitted by **Ms. Akanksha Chhikara** in the partial fulfillment of the requirements for the degree of **Master of Science in Chemistry** from **Thapar Institute of Engineering & Technology, Patiala** is a bonafied piece of work carried out under the guidance and supervision of **Dr. Kamaldeep Paul**, Associate Professor, School of Chemistry and Biochemistry, **Thapar Institute of Engineering & Technology**, Patiala and no part of this project has been submitted for award of any other degree in this or any other university.

Akanksha

(AKANKSHA)

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

Kamaldeep Paul.

(Dr. Kamaldeep Paul)

Associate Professor (Supervisor),

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Patiala

SELF DECLARATION

The work embodied in the project entitled **"Synthesis of Molecular Hybridization Based Aromatic linked Naphthalimide-Benzimidazole Conjugates"** has been done by me in the partial fulfillment of requirement for the award of degree of **Masters of Science in Chemistry,** submitted in the **School of Chemistry and Biochemistry, Thapar Institute of Engineering & Technology, Patiala,** is an authentic record of my own carried out under the supervision and guidance of **Dr. Kamaldeep Paul,** Associate Professor, School of Chemistry and Biochemistry, Thapar Institute of Engineering & Technology, Patiala. All the ideas and references have been duly acknowledged.

Date: 30/06/2018

Akanksha

Place: Patiala

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

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TABLE OF CONTENT

ABSTRACT

Cancer is the biggest problem of health, fear throughout the world. Number of deaths due to cancer is increasing day by day worldwide, especially in the developing countries. Thus, there is a great need to create precisely new anticancer candidate that can bind to various biological targets effectively. Here, we have synthesized aromatic linked naphthalimide-benzimidazole conjugates using molecular hybridization methodology. These compounds are characterized by ^{1}H and ^{13}C NMR spectroscopy. These compounds will further be used for the evaluation for anti-proliferative activity as well as DNA interaction studies.

1. INTRODUCTION

Cancer is the biggest problem of health, fear throughout the world. Number of deaths due to cancer is increasing day by day worldwide, especially in the developing countries. Most efficacious technique for cancer treatment is chemotherapy. Chemotherapy is the most suitable way of treatment of cancer till date, but due to number of side effects and development of resistance for chemotherapeutic candidates, the use of chemotherapeutic is limited.[1] Thus, there is a great need to create precisely new anticancer candidate that can bind to various biological targets effectively. With its roots lies in medicinal chemistry and organics synthesis, heterocyclic compounds are presented as foundational part of chemistry. In addition to carbon and hydrogen, cyclic compounds with two different elements in its ring structure such as oxygen, nitrogen and sulfur, constitute the most important substituent.[2]

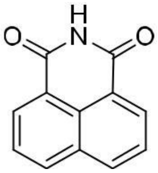
Most of the marketed drugs are composed of heterocycles as their common structural unit. The presence of heterocycles with biologically and pharmacologically interested properties in wide range of compounds is evident on its importance in the field of medicinal chemistry. Indeed, new medication drugs and molecular structures are documented every year that can enhance the quality of life care and make the humankind free from most of the regular diseases.[3]

Naphthalimide is a π deficient aromatic, flat tri-cyclic ring system that recently gained advancement in the cancer research because of its ability to bind with the target compound (DNA).[4] The planar structure of naphthalimide is responsible for DNA intercalation that disturb the cellular events of DNA binding by inserting itself within DNA base-pairs and thus effect the enzyme activity (TOPO II etc.).[5,6] Recently, sulfonated derivatives of 1,8-naphthalimide have been identified as antiviral activity against human immunodeficiency virus, HIV-1.[7] In case of benzimidazole ring system, it also has been evaluated as a noteworthy heterocyclic ring system, that shows a broad range of biological activites.[8,9]

2. REVIEW OF LITERATURE

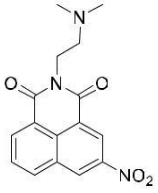
2.1 Activities of naphthalimide derivatives

Naphthalimides (1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione) is one of the backbone of organic and medicinal chemistry. The antitumor activity of this moiety is found in many bioactive compounds due to their DNA-intercalating properties and is present naturally.[10] The feature that brought much consideration is to formation of potent mono naphthalimides and bisnaphthalimide derivatives for better antitumor activities, because of having flat aromatic structure of naphthalimide ring.[11]

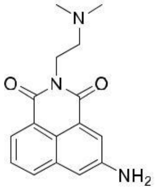


1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione

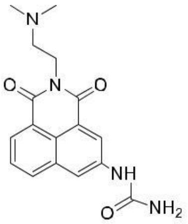
The modification of naphthalimide derivatives at different positions have been improved the anticancer activity and also showed less lethal effects. Amonafide has been the first naphthalimide derivative that reached in clinical trial and exhibits significant antiproliferative efficacy for solid tumors.[12]



1.Mitonafide



2.Amonafide



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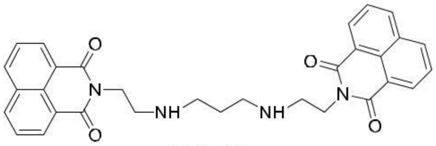

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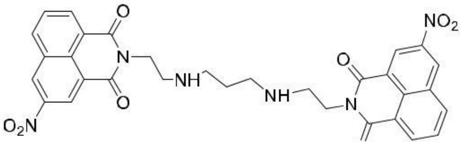


5.Bisnafide

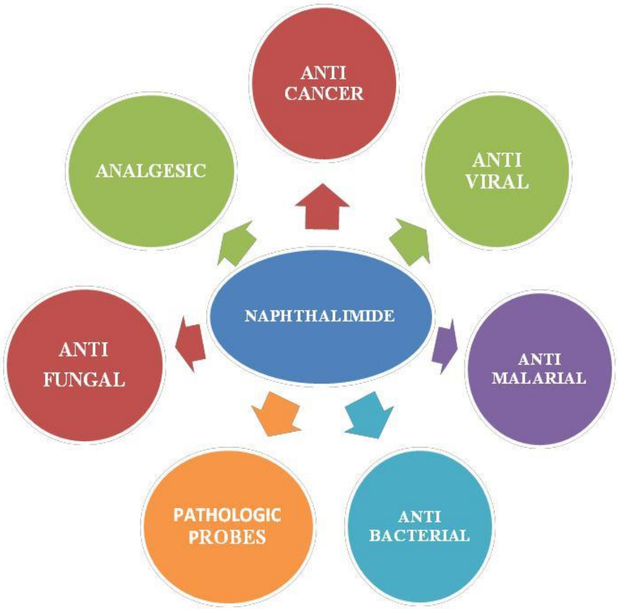
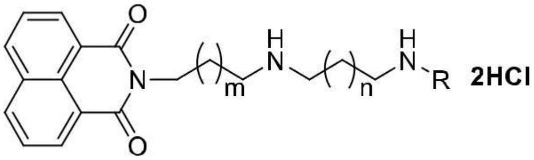


Figure1. Biological activities of naphthalimide derivatives

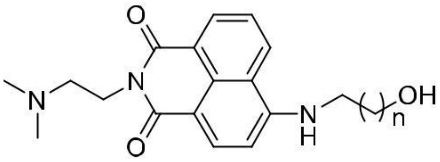
Huang *et al*. worked on various naphthalimide-polyamines. All derivatives were evaluated for antitumor efficacy for various cancer cell lines. Compound **6g** displayed anticancer activity with IC_{50} values of 6.49 ± 2.37 µM (MCF-70), 8.65 ± 1.27 µM (MDA-MB-231), 6.76 ± 1.59 µM (HT-29), 8.31 ± 0.58 µM (H22) and 7.44 ± 0.14 µM (CT-26).[13]



6a-g

	m	n	R
6a	1	1	C_2H_5
6b	2	2	C_2H_5
6c	1	1	C_3H_7
6d	1	1	cyclopropyl
6e	2	1	cyclopropyl
6f	2	2	cyclopropyl
6g	2	1	cyclohexyl

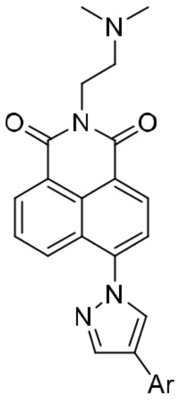
Wang *et al*. have worked on a 2-(2-(dimethylamino)ethyl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (**7**). These derivatives have been investigated for their antitumor efficacy against various cancer cell lines and mechanism aspects of these activities were studied with Ct-DNA using circular dichroism, fluorescence and UV–Vis spectroscopies. Compounds **7c** and **7**e showed anti-proliferative activities for Bel-7402 cancer cell line having IC_{50} values of 5.57 and 9.17 µM, respectively.[14]



7a-e

	n
7a	1
7b	2
7c	3
7d	4
7e	5

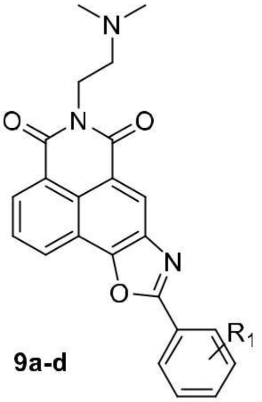
Li *et al*. prepared various hybrids of pyrazole and naphthalimide (**8**). Based on *in vitro* evalution of antitumor activity, all synthesized derivatives showed anti-proliferative activity for MCF-7, Hela and A549 cancer cell lines. Derivative **8i** has been found to be active against MCF-7, Hela and A549 cell lines with IC_{50} values of 0.51 µM, 3.09 µM and 5.14 µM, respectively and having 3.3-folds, 2.2-folds, and 2.5-folds lesser IC_{50} values than that of amonafide. Compound **8e** having cytotoxicity for A549 cells (IC_{50} = 5.09 µM), was 2.6-folds higher than that of amonafide.[15]



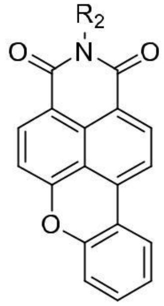
8a-l

	Ar		Ar
8a	C_6H_5	**8h**	3,4-$(CH_3O)_2C_6H_3$
8b	4-FC_6H_4	**8i**	3,4,5-$(CH_3O)_3C_6H_2$
8c	4-ClC_6H_4	**8j**	4-$CF_3C_6H_4$
8d	4-BrC_6H_4	**8k**	3,5-$(CF_3)_2C_6H_3$
8e	4-$OCH_3C_6H_4$	**8l**	H
8f	4-$CH_3OC_6H_4$		
8g	3-$OCH_3C_6H_4$		

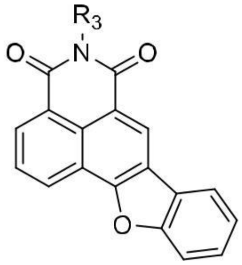
Tan *et*.al. have worked on three different series of naphthalimide with oxo-heterocyclic moiety (**9a-d**, **10a-b**, **11a-b**). Derivatives **9b** and **9c** showed high antiproliferative activity. Compound **9d** has been 7.6 folds more potent than **9b** against A549 cancer cell lines whereas **11a** and **11b** showed less antitumor efficacy for A549 having IC_{50} values of 98.26 µM and 28.95 µM, respectively.[16]



9a-d



10a-b



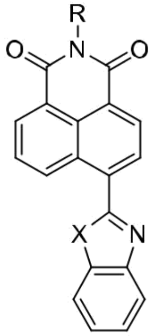
11a-b

9a R_1 = H
9b R_1 = *p*-OMe
9c R_1 = *p*-NO_2
9d R_1 = *o*-OMe

10a $R_2 = CH_2CH_2N(CH_3)_2$
10b $R_2 = CH_2CH_2CH_2CH_3$

11a $R_3 = CH_2CH_2CH_2N(CH_3)_2$
11b $R_3 = CH_2CH_2CH_2CH_3$

Lu *et al.* have designed and synthesized a variety of naphthalimide derivatives with benzazole substitution (**12**) and evaluated for anticancer activity. No cytotoxic activity has been found in compounds **12a-d** at the concentrations of 0.01 to 40 µM. Compounds **12e** and **12f** showed anticancer activity with IC_{50} values of 7.8 ± 0.05 µM and 4.5 ± 0.06 µM, respectively.[17]

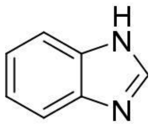


12a-f

	R	X
12a	*n*-Bu	O
12b	*n*-Bu	S
12c	CH_2CH_2OH	O
12d	CH_2CH_2OH	S
12e	$CH_2CH_2N(CH_3)_2$	O
12f	$CH_2CH_2N(CH_3)_2$	S

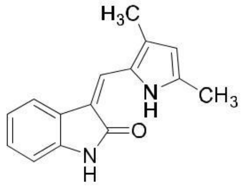
2.2 Activities of benzimidazole derivatives

Benzimidazole is an important moiety for many bioactive derivatives. It exhibits many pharmacological activities such as antihypertensive, anti-HIV, anti-plasmodic, antihistaminic, antibacterial, antiulcer, cardiotonic and antifungal (**Figure 2**). These are a foundation for a number of derivatives that are important contents of biologically important molecules.[18]



1*H*-benzo[*d*]imidazole

Benzimidazole derivatives have gained interest *in vitro* anticancer agents. High levels of cytotoxicity was shown by benzimidazole derivatives for various cancer cell lines (**13-16**).[19]



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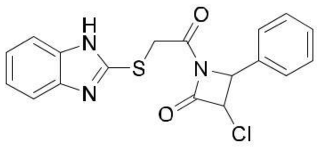

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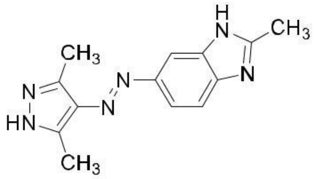

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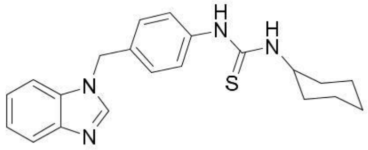

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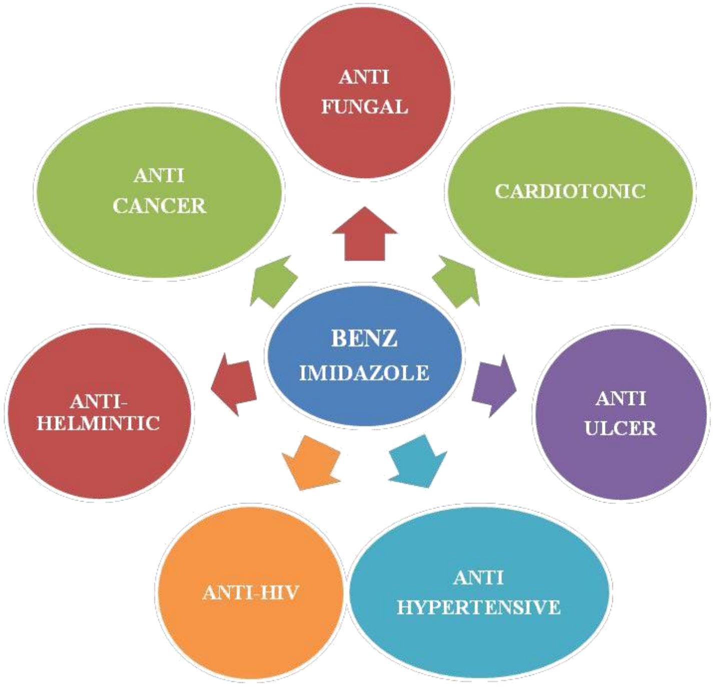
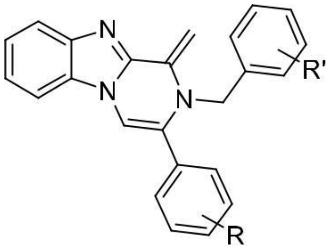


Figure 2. Biological activities of benzimidazole derivatives

Demirayak *et al.* prepared a series of fused benzimidazole derivatives (**17a–r**). Derivatives **17c** and **17n** were shown potent anticancer activity with percentages growth inhibition of 24.53% and 22.62%, respectively at concentration level of 10 µM. Compounds **17c** and **17n** showed $\log_{10}GI_{50}$ values of -6.32 and -5.87, respectively at five dose concentration level (10^{-4} - 10^{-8} µM).[20]

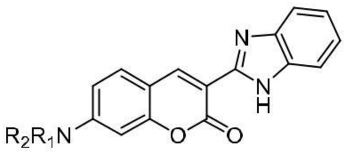


17a-t

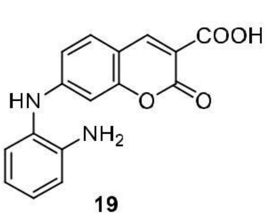
	R	R'		R	R'
17a	H	H	**17k**	3-Cl	H
		3-OCH_3	**17**	3-Cl	3-OCH_3
17c	H	3-Cl	**17 m**	3-Cl	3-Cl
		4-OCH_3	**17n**	3-Cl	4-OCH_3
17e	H	4-Cl	**17 o**	3-Cl	4-Cl
17f	4-OCH_3	H	**17** p	4-Cl	H
17g	4-OCH_3	3-OCH_3	**17q**	4-Cl	3-OCH_3
17h	4-OCH_3	3-Cl	**17 r**	4-Cl	3-Cl
17i	4-OCH_3	4-OCH_3			
17j	4-OCH_3	4-Cl			

Paul *et. al.* worked on various coumarin–benzimidazole hybrids and evaluated for antitumor efficacy against various cancer cell lines. Derivative **18** displayed highest anticancer activity with > 50% cell growth inhibition. Better antitumor efficacy for leukemia, colon and breast human cancer cell lines have been shown by compounds **19** and **20a-h**.[21]

18



20a-h

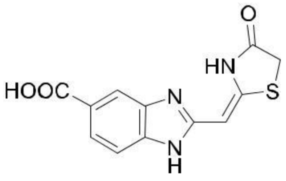


19

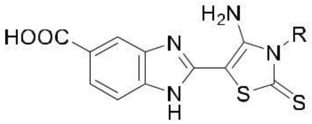
	NR_1R_2
20a	ethylenediamine
20b	ethanolamine
20c	*n*-butylamine
20d	cyclohexylamine
20e	4-fluoroaniline
20f	morpholine
20g	methylpiperazine
20h	2-aminoethylmorpholine

.

Hanan *et al*. prepared various 2-substituted benzimidazoles and all prepared derivatives were investigated for antiproliferative efficacy. These compounds were found active against HEPG2 (liver cancer), MCF7 (breast cancer) as well as HCT 116 (colon cancer) cell lines, having IC_{50} values less than 10 µM. Compound **21** displayed antitumor activity against HEPG2, MCF7 and HCT 116 cell lines with IC_{50} values of 0.55 ± 0.05, 3.51 ± 0.50 and 4.23 ± 0.04 µM, respectively.[22]

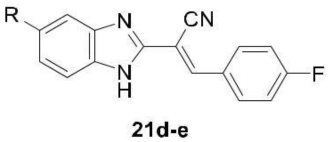


21a

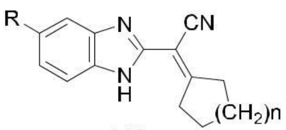


21b-c

	R
21b	$CH_2C_6H_5$
21c	C_6H_5



21d-e

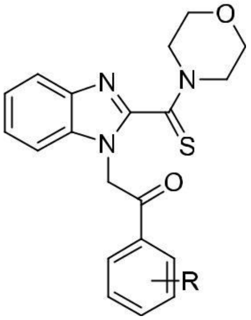


21f-g

	R
21d	Cl
21e	COOH

	R	n
21f	Cl	1
21g	COOH	2

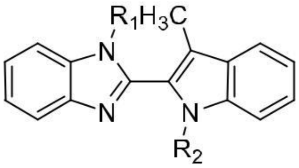
Yurttas *et. al*. prepared a variety of substituted benzimidazole derivatives (**22**). All the synthesized compounds (**22a-p)** revealed the maximum cytotoxic activities for MCF-7 cells. Compounds **22f-i** along with **22p** expressed more than 50% growth inhibition against MCF-7 cancer cells.[23]



22

	R		R
22a	H	**22i**	4-Cl
22b	3-OCH_3	**22j**	4-F
22c	3-Cl	**22k**	2,4-diCH_3
22d	3-F	**22l**	3,4-diOCH_3
22e	3-NO_2	**22m**	2,4-diCl
22f	4-CH_3	**22n**	2,5-diCl
22g	4-OCH_3	**22o**	3,4-diCl
22h	4-Br	**22p**	3,4-diF

Kishore *et al.* prepared a variety of 2-(3-methylindolyl)benzimidazole derivatives **23**. All the synthesized compounds were examined against various cancer cell lines for their anticancer efficacy. Derivatives **23a-c** and **23h** showed greater anti-proliferative efficacy for all tested cancer cell lines. Compound **23a** exhibited highest anticancer activity against COLO 205, DU145, HepG2 and MDA-MB-231 cancer cell lines with IC_{50} values of 10.3 ± 0.13, 12.3 ± 0.12, 14.4 ± 0.22 and 16.1 ± 0.41 µM, respectively.[24]

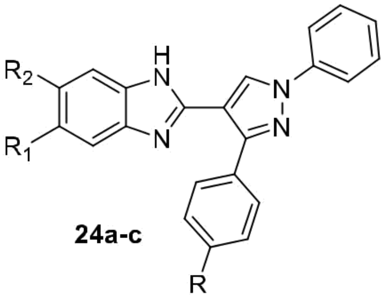


23

	R_1	R_2
23a	H	H
23b	CH_3	H
23c	C_2H_5	H
23d	CH_3	CH_3
23e	CH_3	CH_2Ph
23f	CH_2CH_3	CH_3
23g	CH_2CH_3	CH_2CH_3
23h	CH_2CH_3	CH_2Ph

2.3. Molecular hybridization

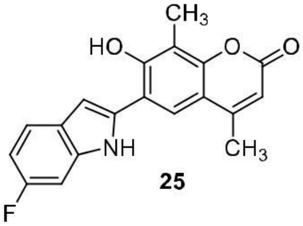
Reddy *et al*. have prepared and characterized a variety of hybrids of benzimidazole and pyrazole moieties **24**. These hybrids were evaluated against antitumor activity for A549, MCF-7, and HeLa cancer cell lines. Hybrids **24a-c** showed highest anticancer activity with IC_{50} value in the range of 0.83 to 1.81 µM. Derivative **24a**, showed the maximum efficacy with IC_{50} value of 0.83 µM for MCF-7 cell lines.[25]



24a-c

	R	R_1	R_2
24a	H	H	Br
24b	F	F	H
24c	Cl	Cl	H

Galayev *et al.* prepared a variety of hybrids of coumarins with indole **25** and pyrazole **26a-c** as second pharmacophores and these hybrids were examined for their anti-proliferative activity for the NCI-60 cancer cell lines. Hybrid of coumarin and indole **25**, showed highest anti-proliferative activity against leukemia as well as melanoma cancer cell lines with GI50 value of 3.08 µM and TGI value of 9.71 µM.[26]



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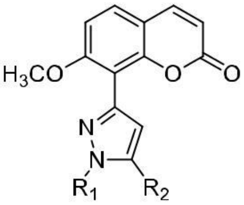
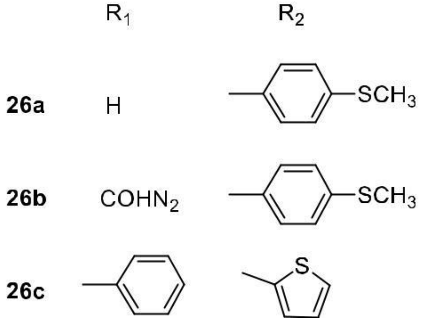
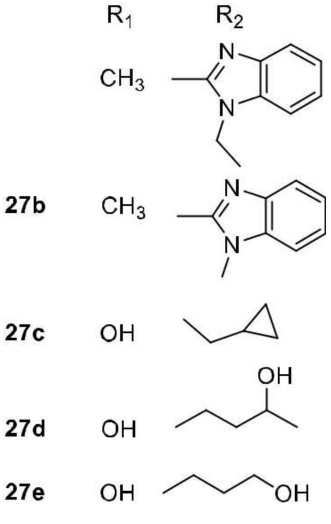


26a-c

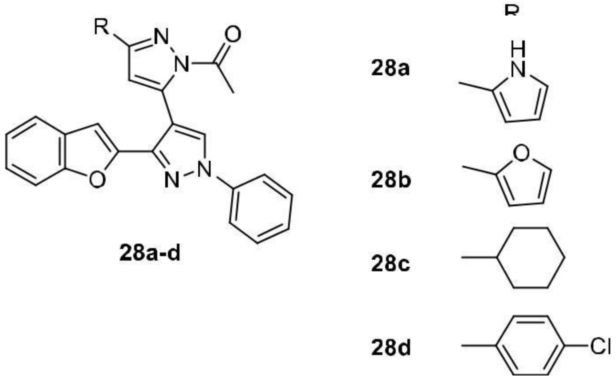


Kraljevic *et al*. prepared a series of hybrids of coumarin and triazole moieties. All the prepared hybrids were examined for their anti-tumor potential against A-549 (lung) with HeLa (cervical) cancer cell lines and showed IC50 < 30 µM. Hybrid **27a** showed maximum anti-proliferative potential having IC50 value of 0.90 µM for HepG2 (liver) cancer cell line.[27]

27a-e



Karim *et al*. have prepared novel hybrids of pyazole with benzofuran moiety (**28**) and all the prepared hybrids were examined for anti-proliferative efficacy for the NCI 60 cancer cell lines. Derivative **28a** was found to be most active anticancer agent with a range of GI_{50} values of 1.00-2.71 µM, displayed highest growth inhibition against in all the evaluated cancer cell lines. [28]



3. RESEARCH GAPS AND OBJECTIVES

Literature survey suggests that individual moieties such as naphthalimide and benzimidazole showed significant anticancer property against most of the cancer cell lines and their hybrids with other heterocyclic moieties showed highest activity than individual moiety. The hybrids of naphthalamide and benzimdazole are rarely known. So, here our objective is to synthesize and characterization of hybrid derivatives of naphthalimide with benzimidazole moieties with conjugated aromatic linker between these two pharmacophores. These compounds will also be examined *in vitro* for 60 human cancer cell lines.

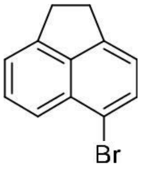
4. EXPERIMENTAL

4.1 General chemistry

All reactions are performed in oven-dried glass wares. Without further purification commercial grade solvents were used and supplied by Lobachem, Spectrochem and Sigma Aldrich. Melting points of synthesized compounds were investigated in an open capillary. Jeol-ECS 400 MHz and 100 MHz NMR spectrometer was used for recording ^{1}H and ^{13}C NMR spectra, respectively using $CDCl_3$ and DMSO-d_6 as solvents. The chemical shifts of derivatives were shown in parts per million taking TMS as reference and all the values of coupling constant (*J*) are expressed in Hz. Thin layer chromatography has been used to observed the reactions using plates coated with silica gel HF-254. All synthesized compounds were purified by column chromatography using silica gel 60-120 mesh. Ethyl acetate and hexane were used as eluents.

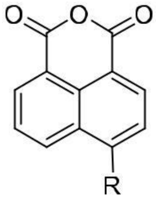
4.2 Synthesis of naphthalimide-benzimidazole derivatives

4.2.1 Synthesis of 5-bromoacenaphthene (2):



5-Bromoacenaphthene (**2**) was synthesized by suspending *N*-bromosuccinimide (1.15 g, 6.46 mmol) in dimethyl formamide (10 ml) followed by transferring into the solution of acenaphthene (**1**) (1 g, 6.40 mmol) in DMF. The reaction mixture was stirred thoroughly at room temperature for 2 hrs and the progress of reaction was monitored by TLC. After reaction got completed, 100 ml water was added to the reaction mixture. Solid product was filtered and dried. The crude was recrystallized for ethanol to get pure solid. Yield = 85%; light yellow solid; m.pt. = 54-56 ºC; (lit[29] m.pt =55-56 ºC).

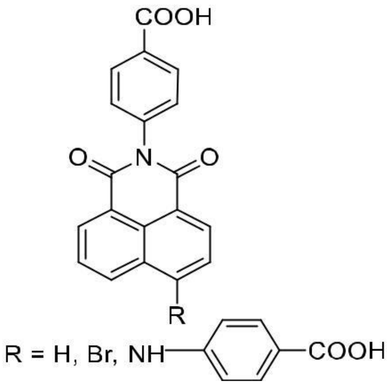
4.2.2 Typical procedure for the synthesis of 1,8-naphthalic anhydride (3a-b):



R = H, Br

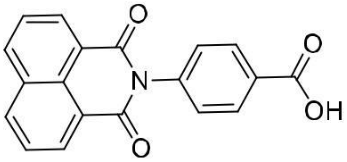
Acenaphthene (**1**) (1 g, 6.49 mmol) or 5-bromoacenaphthene (**2**) (1 g, 4.3 mmol) was dissolved in glacial acetic acid and then oxidized by adding sodium dichromate (3.8 g, 12.9 mmol). The reaction mixture was refluxed for 2.5 hrs. Completion of reaction was monitored by thin layer chromatography. After the completion of reaction, the solvent was removed under vacuum. The chromium salt was removed from the residue with boiling water and the obtained solid crude product formed, was recrystallized from glacial acetic acid to give pure compound 1*H*,3*H*-benzo[*de*]isochromene-1,3-dione (**3a**), Yield = 74%; yellow solid; m.pt. = 261-263 ºC; (lit[30] m.pt =262-264 ºC) / 6-bromo-1*H*,3*H*-benzo[*de*]isochromene-1,3-dione (**3b**), Yield = 78%; white solid; m.pt. = 266-269 ºC; (lit[29] m.pt = 268-269 ºC).

4.2.3 Typical procedure for the synthesis of 5a-c:



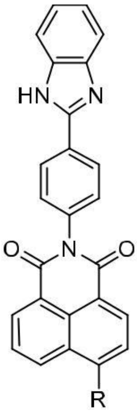
1,8-Naphthalic anhydride **(3a-b)** and 4-amino benzoic acid (**4)** were dissolved in DMF and heated at 100 ºC for 2 hrs. After completion of reaction (monitored by TLC), 50 ml water was added to the reaction mixture. The precipitates formed were filtered off and washed with water. The product was purified by column chromatography using hexane: ethyl acetate (80:20) as eluents to yield 65-68%.

4.2.3.1 Spectral data of 4-(1,3-dioxo-1*H*-benzo[*de*]isoquinolin-2(3*H*)-yl)benzoic acid (5a):



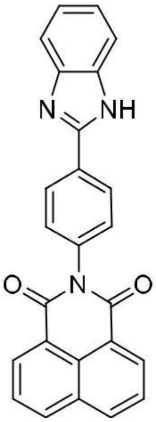
Yield = 68%; pale yellow solid; m.pt. = 244-247 ºC. ^{1}H NMR (DMSO-d_6 400 MHz): (ppm) 8.51 (dd, ^{2}J = 5.48 Hz, ^{3}J = 0.92 Hz, 2H, ArH), 8.49 (d, J = 3.64 Hz, 2H, ArH), 8.08 (d, J = 8.24 Hz, 2H, ArH), 7.91 (t, J = 7.32 Hz, 2H, ArH), 7.54 (d, J = 8.24 Hz, 2H, ArH).

4.2.4 Typical procedure for synthesis of compound 7a-c:



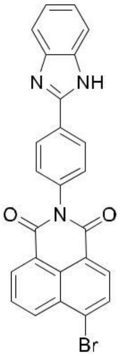
Compound **5a-c** and benzene-1,2-diamine (**6**) were added in an oven dried RBF and heated at 100 ºC in PPA for 2 hrs. Progress of reaction was checked using TLC. When both starting material got consumed, 100 ml water was added to the reaction mixture. Reaction mixture was basifed using aq. ammonia solution. Solid precipitates were separated out, filtered and washed with excess of water. Pure product was obtained by column chromatography using ethyl acetate and hexane as eluents.

4.2.4.1 Spectral data of 2-(4-(1*H*-benzo[d]imidazol-2-yl)phenyl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (7a):



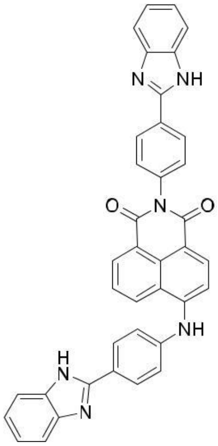
Yield = 72%; dark yellow solid; m.pt. = 269-271 ^{o}C, ^{1}H NMR (CDCl$_3$, 400 MHz): (ppm) 8.81 (dd, ^{2}J = 7.32 Hz, ^{3}J = 0.92 Hz, 1H, ArH), 8.76 (dd, ^{2}J = 7.36 Hz, ^{3}J = 0.92 Hz, 1H, ArH), 8.58-8.53 (m, 2H, ArH), 8.26-8.21 (m, 2H, ArH), 8.12 (d, *J* = 8.28 Hz, 1H, ArH), 7.89-7.86 (m, 1H, ArH), 7.81-7.73 (m, 4H, ArH), 7.50-7.45 (m, 2H, ArH); ^{13}C NMR (CDCl$_3$, 100 MHz): δ (ppm) 163.8, 160.6 (CO), 149.2, 143.6, 135.2, 134.6, 132.1, 131.8, 131.7, 131.6, (ppm) 8.79 (d, *J* = 7.36 Hz, 1H, ArH), 8.75-8.67 (m, 1H, ArH), 8.62 (d, *J* = 8.24 Hz, 1H, ArH), 8.57-8.54 (m, 2H, ArH), 8.34 (d, *J* = 7.76 Hz, 1H, ArH), 131.0, 127.3, 127.0, 126.8, 125.7, 125.3, 123.0, 120.5, 119.9, 115.8 (ArC).

4.2.4.2 Spectral data of 2-(4-(1*H*-benzo[*d*]imidazol-2-yl)phenyl)-6-bromo-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (7b):



7.88-7.80 (m, 2H, ArH), 7.69 (t, *J* = 7.76 Hz, 1H, ArH), 7.48-7.41 (m, 3H, ArH), 7.13 (d, *J* = 8.24 Hz, 1H, ArH).

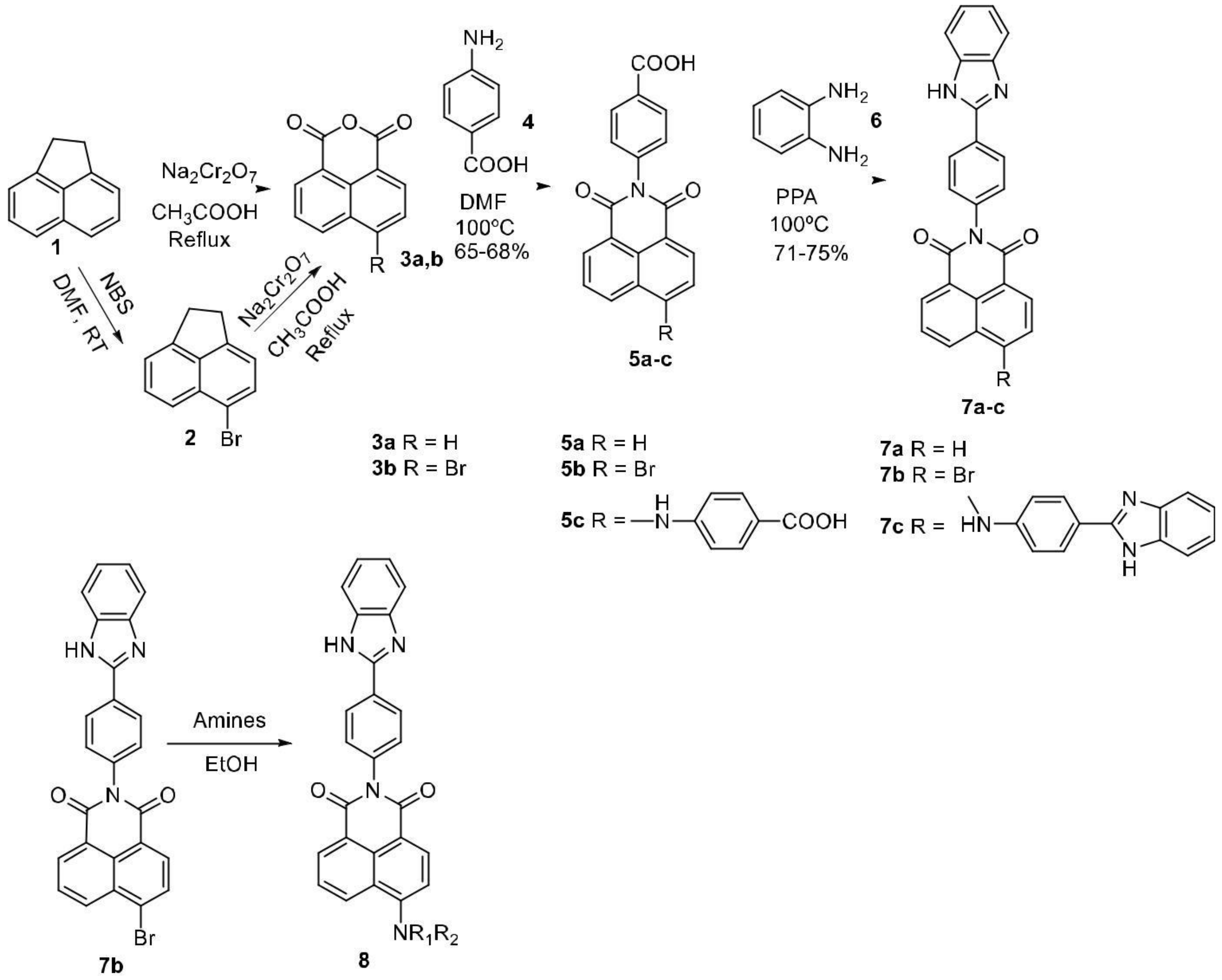
4.2.4.3 Spectral data of 2-(4-(1*H*-benzo[*d*]imidazol-2-yl)phenyl)-6-((4-(1*H*-benzo[*d*]imidazol-2-yl)phenyl)amino)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (7c):



Yield = 71%; yellow solid; m.pt. = 298-301 ^{o}C; ^{1}H NMR (CDCl$_3$, 400 MHz): (ppm) 8.86-8.80 (m, 2H, ArH), 8.66 (d, *J* = 7.36 Hz, 1H, ArH), 8.63 (d, *J* = 7.80 Hz, 1H, ArH), 8.57 (d, *J* = 7.76 Hz, 1H, ArH), 8.52-8.48 (m, 4H, ArH), 8.10 (d, *J* = 7.80 Hz, 1H, ArH), 8.07 (d, *J* = 8.28 Hz, 1H, ArH), 7.91-7.85 (m, 5H, ArH), 7.49-7.47 (m, 5H, ArH); ^{13}C NMR (CDCl$_3$, 100 MHz): δ (ppm) 143.5, 134.6, 132.4, 131.7, 131.6, 131.5, 131.2, 128.5, 128.1, 128.0, 127.8, 127.0, 126.0, 125.7, 123.6, 121.1, 120.1, 120.0, 115.8 (ArC).

5. RESULTS AND DISCUSSION

Using commercially available acenaphthene as an initial substrate, naphthalimide derivatives **(7a, 7b** and **7c)** were synthesized according to the **Scheme 1**. Acenaphthene **(1)** was treated with *N*-bromosuccinimide in DMF at room temperature for 2 hrs, followed by recrystallization from ethanol, gave light yellow solid of 5-bromoacenaphthene (**2**) in 85% yield (m.pt.: = 54-56 ^{O}C).



Scheme1. Synthesis of naphthalimide derivatives

Under refluxing conditions, oxidation of the acenaphthene (**1**) and bromoacenaphthene (**2**) was done with sodium dichromate in acetic acid for 2.5 hrs, gave 1,8-naphthalic anhydride**.** These crude products were purified by recrystallization from glacial acetic acid to give pure products **(3a-b)**. Heating the compound **3a-b** with 4-amino benzoic acid (**4)** in DMF at 100 ^{O}C for 2 hrs gave 4-(1,3-dioxo-1*H*-benzo[*de*]isoquinolin-2(3*H*)-yl)benzoic acid (**5a-c**). The precipitates formed were filtered off and washed with water. The product was purified by column chromatography using hexane: ethyl acetate (80:20) as eluents to give **5a-c** in 65-68% yields. Compound **5a** was well characterized by ^{1}H NMR spectroscopy. ^{1}H NMR spectrum of compound **5a** showed the splitting pattern of aromatic protons over a wide range of δ 8.51-7.54 ppm corresponding to ten protons. ^{1}H NMR spectral analysis showed increment of four protons of benzoic acid in aromatic region confirmed the formation of 4-(1,3-dioxo-1*H*-benzo[*de*]isoquinolin-2(3*H*)-yl)benzoic acid (**5a**) **(Figure 3).**

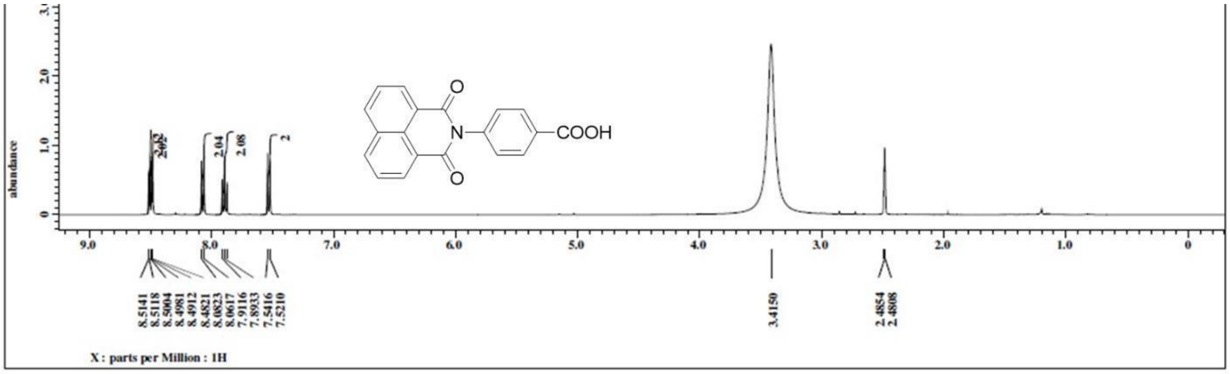


Figure 3 ^{1}H NMR spectrum of 4-(1,3-dioxo-1*H*-benzo[*de*]isoquinolin-2(3*H*)-yl)benzoic acid

Compounds **5b** and **5c** were not separated and used without purification. These compounds were further used for the substitution reaction with benzene-1,2-diamine for the hybridization of naphthalimide and benzimidazole. Derivative **5a-c** was treated with benzene-1,2–diamine (**6**) in the presence of PPA for 2 hrs at 100 ºC to give naphthalimide-benzimidazole conjugates **7a-c** in 71-75% yield. Column chromatography was used to purify these compounds in a hexane-ethyl acetate solvent system. These compounds were then characterized by ^{1}H and ^{13}C NMR spectroscopy.

^{1}H NMR spectrum of compound **7a** showed wide range of splitting pattern ranging from δ 8.81-7.45 ppm corresponding to fourteen protons. ^{1}H NMR spectral analysis showed increment of four protons of benzimidazole in aromatic region. ^{13}C NMR spectrum showed the signals at 163.8, 160.6 of two carbonyl carbons,and 149.2, 143.6, 135.2, 134.6, 132.1, 131.8, 131.7, 131.6, 131.0, 127.3, 127.0, 126.8, 126.8, 125.7, 125.3, 123.0, 120.5, 119.9 and 115.8 of aromatic carbons. So, ^{1}H NMR and ^{13}C NMR spectra confirmed the formation of 2-(4-(1*H*-benzo[*d*]imidazol-2-yl)phenyl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (**7a**) (**Figures 4** and **5)**.

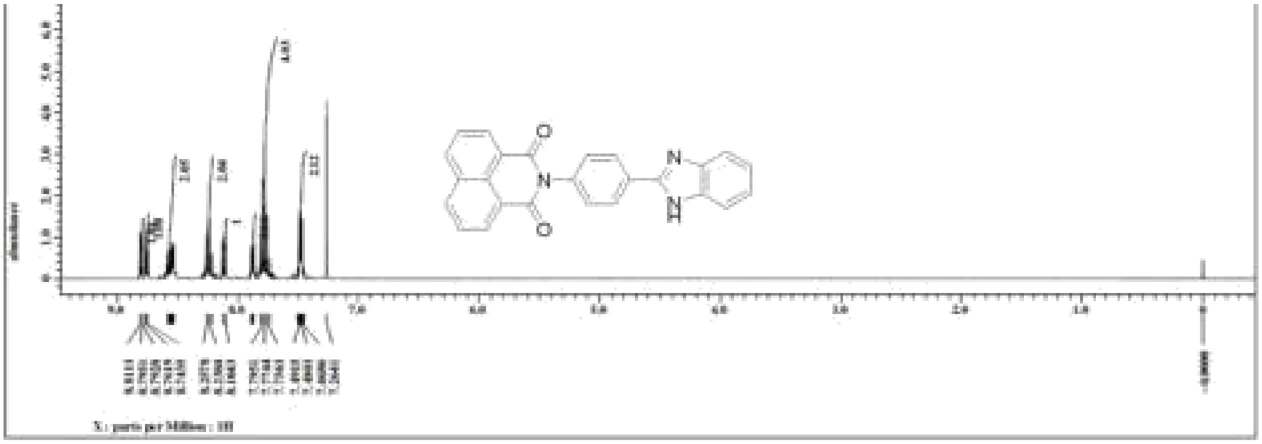


Figure 4 ^{1}H NMR spectrum of 2-(4-(1*H*-benzo[*d*]imidazol-2-yl)phenyl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione **(7a)**

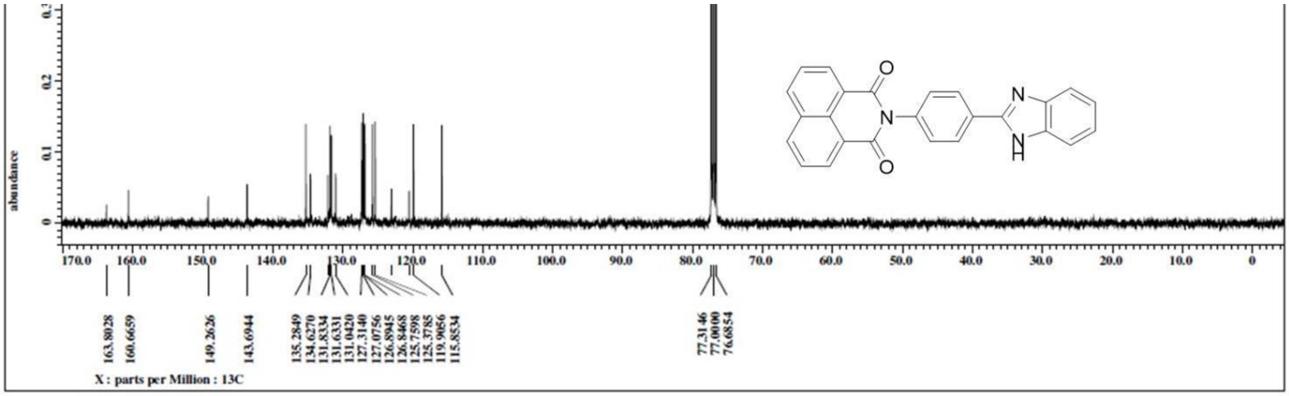


Figure 5 ^{13}C NMR spectrum of 2-(4-(1*H*-benzo[*d*]imidazol-2-yl)phenyl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione

^{1}H NMR spectrum of compound **7b** showed wide range of splitting pattern ranging from δ 8.79-7.13 ppm corresponding to thirteen protons. ^{1}H NMR spectral analysis showed increment of four protons of benzimidazole in aromatic region. ^{1}H NMR spectrum confirmed the formation of 2-(4-(1*H*-benzo[*d*]imidazol-2-yl)phenyl)-6-bromo-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (**7b**) (**Figure 6)**.

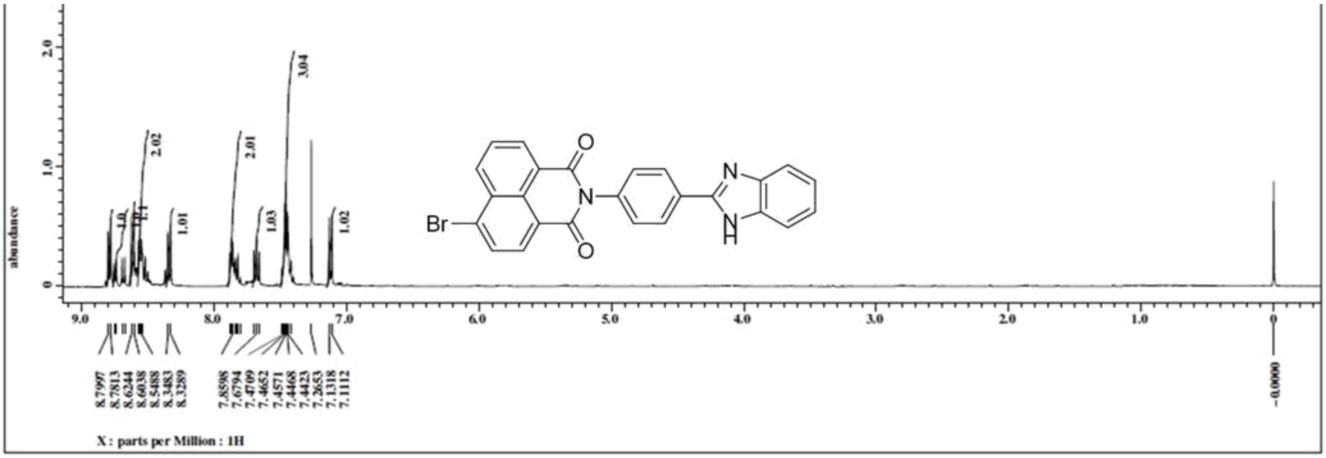


Figure 6 ^{1}H NMR spectrum of 2-(4-(1*H*-benzo[*d*]imidazol-2-yl)phenyl)-6-bromo-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (**7b**)

Similarly, ^{1}H NMR spectrum of compound **7c** showed wide range of splitting pattern ranging from δ 8.86-7.47 ppm corresponding to twenty one protons. ^{1}H NMR spectral analysis showed increment of eight protons of two benzimidazole moieties in aromatic region. ^{13}C NMR spectrum showed the signals at δ 143.5, 134.6, 132.4, 131.7, 131.6, 131.5, 131.2, 128.5, 128.1, 128.0, 127.8, 127.0, 126.0, 125.7, 123.6, 121.1, 120.1, 120.0 and 115.8 of aromatic carbons. So, ^{1}H NMR and ^{13}C NMR spectral analysis confirmed the formation of 2-(4-(1*H*-benzo[*d*]imidazol-2-yl)phenyl)-6-((4-(1*H*-benzo[*d*]imidazol-2-yl)phenyl)amino)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (**7c**) **(Figures 7** and **8**). Compound **7b** will be further used for the substitution reactions with primary and secondary amines as well as suzuki reactions at C-5 position. These compounds will be evaluated to human cancer cell lines and DNA interactions for anti-proliferative activities.

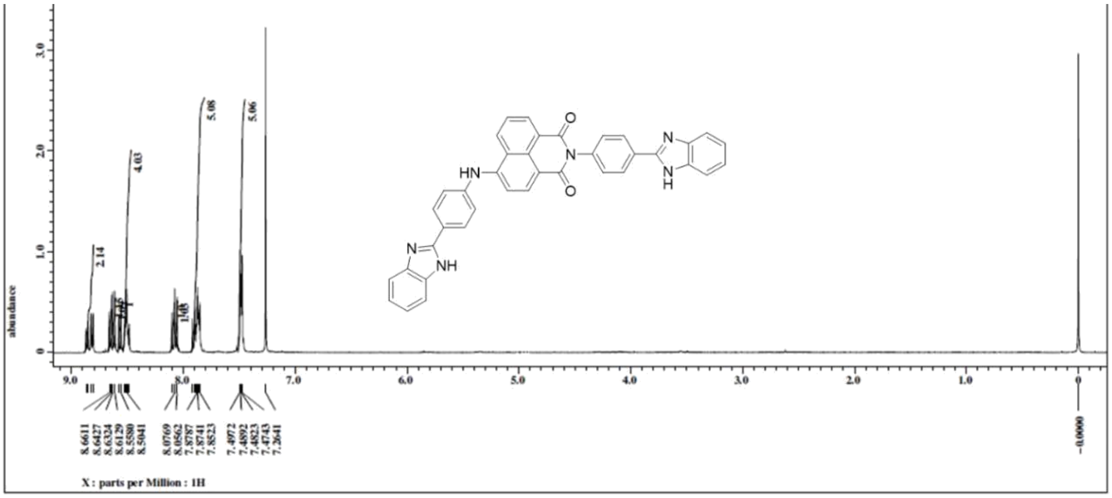


Figure 7 ^{1}H NMR spectrum of 2-(4-(1*H*-benzo[*d*]imidazol-2-yl)phenyl)-6-((4-(1*H*-benzo[*d*]imidazol-2-yl)phenyl)amino)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (**7c**)

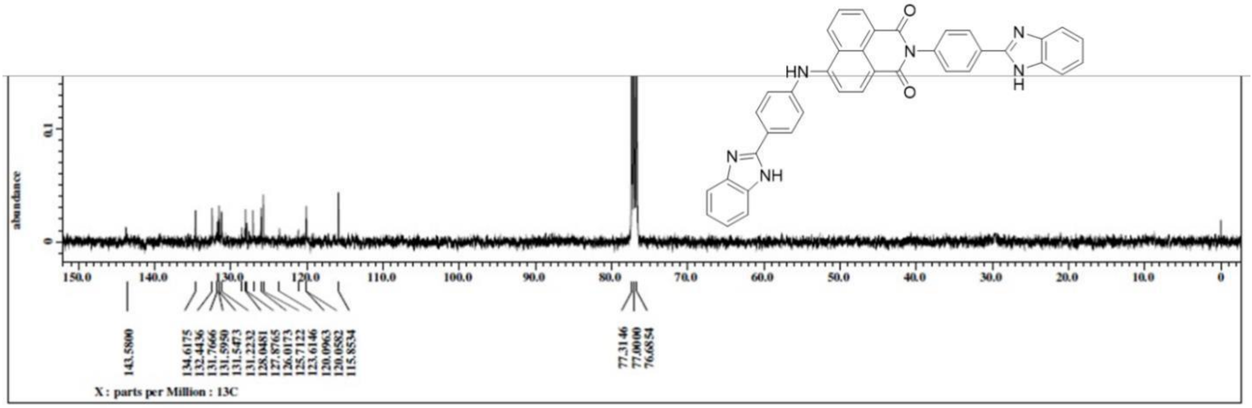


Figure 8 ^{13}C NMR spectrum of 2-(4-(1*H*-benzo[*d*]imidazol-2-yl)phenyl)-6-((4-(1*H*-benzo[*d*]imidazol-2-yl)phenyl)amino)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (**7c**)

6. CONCLUSIONS

- Two monobenzimidazole based naphthalimide derivatives 2-(4-(1*H*-benzo[*d*]imidazol-2-yl)phenyl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (**7a**) and 2-(4-(1*H*-benzo[*d*]imidazol-2-yl)phenyl)-6-bromo-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (**7b**) were synthesized in good yields and characterized by ^{1}H and ^{13}C NMR spectroscopy.
- Bisbenzimidazole derivative of naphthalimide 2-(4-(1*H*-benzo[*d*]imidazol-2-yl)phenyl)-6-((4-(1*H*-benzo[*d*]imidazol-2-yl)phenyl)amino)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (**7c**) was also synthesized in good yield and well characterized by ^{1}H and ^{13}C NMR spectroscopy.

- The final compounds will further be used for their biological activity as anti-cancer agents as well as for DNA intercalation.

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