Synthesis of Indole-Benzothiazole-Acridone Hybrids for Anticancer Activity

A

Thesis submitted

In the partial fulfilment of the requirement for degree

MASTERS OF SCIENCE

IN

CHEMISTRY

Submitted By

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(301502007)

UNDER THE SUPERVISION OF

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THAPAR UNIVERSITY,

PATIALA-147004

2017

CERTIFICATE

This is to certify that the project entitled "**Synthesis of Indole-Benzothiazole-Acridone Hybrids for Anticancer activity**" being submitted by **Ms. Bhavya Khurana,** in the partial fulfilment of requirement for the award of the degree of Masters of Science in the School of Chemistry and Biochemistry, Thapar University, Patiala, is a bonafide work carried under the supervision of **Dr. Kamaldeep Paul** and no part of this project has been submitted for award of any other degree in this or any other university.

(BHAVYA KHURANA)

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

(Dr. Kamaldeep Paul)
Associate Professor (Supervisor),
School of Chemistry and Biochemistry,
Thapar University, Patiala

SELF DECLARATION

The work embodied in the project entitled **"Synthesis of Indole-Benzothiazole-Acridone Hybrids for Anticancer activity"** has been done by me in the partial fulfilment of requirement for the award of degree of **Masters of Science in Chemistry,** submitted in the **School of Chemistry and Biochemistry, Thapar University, 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 University, Patiala. All the ideas and references have been duly acknowledged.

Date: 13/7/2017

Place: Patiala

Bhavya Khurana

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

(Dr. Kamaldeep Paul)
Associate Professor (Supervisor),
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Bhavya Khurana

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1. INTRODUCTION

Cancer is one of the major causes of mortality worldwide and is characterized by an uncontrollable cell division and growth and thus destroys healthy body tissues.[1] A normal human cell divides and grows to form new cells and when cells are damaged or aged, they died, and new cells replace them. But cancer affects this orderly process and these cells become abnormal and old or damaged cells survive instead of being eliminated, and new cells form when they are not needed.[2] These excessive cells can divide uncontrollably and may form massive growth called "Tumours". These tumours can undergo a process called Metastasis and thus causes a life threatening condition until eliminated from body as they can grow and interfere with physiological functions of body.

Approximately 5,75,000 people die every year due to cancer and about millions of people have been diagnosed with recurrence of cancer per year in the US. Large amount of wealth is spent as medical costs every year. African Americans are infected with cancer more than people of any other race or ethnicity due to genetic predisposition. World Health Organization (WHO) reports suggest the occurrence of cancer among people and continuously rising by about 70 - 80% over years. In India, a woman dies every 8 minute due to untreatable cancer especially cervical cancer and breast cancer.[3-4]

Cancers can be prevented by some behavioural practices such as choosing not to smoke, drink alcohol or consumption of tobacco. These significantly lower the risk of cancers such as lung, throat, mouth, and liver cancers. There are many options available for treatments of cancer. These types of treatments are based upon its advancement overall. A combination of treatments such as surgery with chemotherapy, radiation therapy, immunotherapy, targeted therapy, or hormone therapy are available. A very comprehensive study and research is being carried out in order to control the outbreak of cancer at genetic level. But as the history signifies the failed attempts, the success at a clinical level is very low.[5] A number of drugs has been optimized on molecular level and their activity was studies against the potent cancer cell lines. But there is need to develop much effective drugs which provide a relief at an early diagnostic stage.

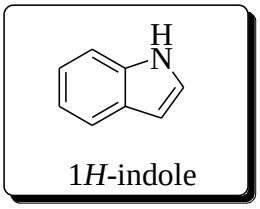
The majority of heterocycle compounds are used as a potent pharmacophore in manufacturing of drugs against several malignancies and thus they are being investigated for their potential activity against several diseases including cancer.

2. REVIEW OF LITERATURE

Advent of cancer conditions has been cured by a number of drugs designed with heterocyclic moieties in their nucleus. Majority of heterocyclic compounds such as indole, thiazole, acridone, acridine, naphthalimide, benzimidazole etc. have found a potent use in medicinal and pharmaceutical applications. These heterocyclic moieties are frequently synthesised to target against a number of commonly occurring pathological conditions. Applications of these heterocyclic cores in oncology have received an efficient response towards the targets. Thus research is being carried out to synthesize efficient and effective hybridised moieties as drug candidate and check their anticancer activity.

2.1. Indole and its biological activity

Compounds of indole are naturally occurring and most commonly found in the family of cruciferiae, members of which are broccoli, cauliflower, cabbage, and brussels sprouts. It is frequently used as an intermediate in production of dyes and perfume fixative and also is a key moiety in many pharmaceuticals, alkaloids and hormones. Indole is a bicyclic compound containing six-membered benzene ring, fused with five-membered pyrrole ring.[6]



1*H*-indole

The indole nucleus is a biologically and physiologically potent pharmacophore in pharmaceutical chemistry. This potency of indole has made it a versatile heterocycle that possess wide range of biological activities such as anti-HIV, anti-cancer, antimicrobial, antidepressant, antidiabetics, antihypertensive etc. (**Figure-1**).[7] Various derivatives of indole exhibited cytotoxicity against a panel of mammalian cancer cell lines and were toxic to microorganisms, thus, considered as effective mutagens and carcinogens.[7]

Hong *et al*. has been synthesized flouro and cyano derivatives of diindolylmethanes **(1)** and were tested against cell lines of breast cancer (MCF7), lung cancer (NCI–H460) and CNS cancer (SF-268). Compounds 5,5′-dicyano-3,3′-methanediyl-bis-indole and 5,5′-difluoro-3,3′-methanediyl-bis-indole were tested against these cell lines. Flouro derivative reduced the growth of cancer cell lines NCI-H460, MCF7, and SF-268 to 0%, 1% and 2% respectively,

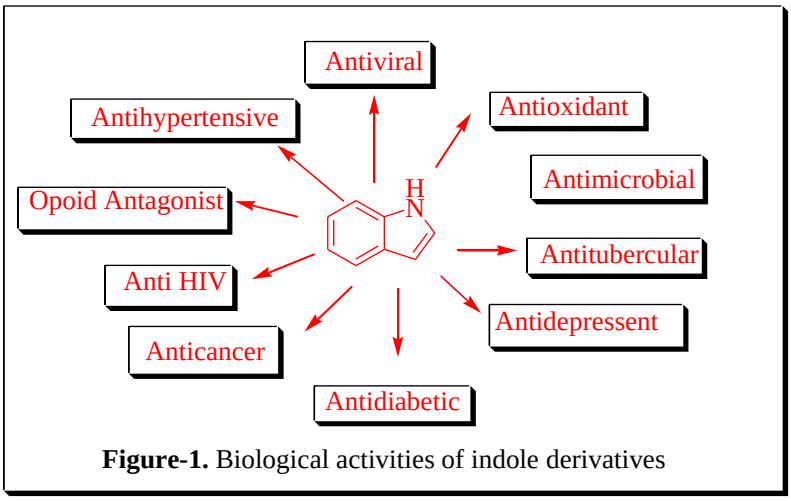
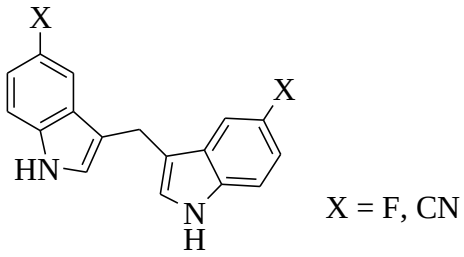


Figure-1. Biological activities of indole derivatives

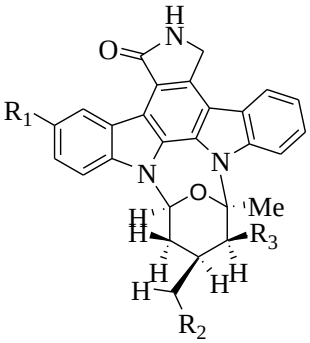
at concentration of about 1.10–4.00 mol/L, whereas the cyano derivative reduced the growth to respective 4%, 1% and 9%, at concentration of about 5.10–5.00 mol/L. Their potential cytotoxicity against cancer cell lines made these derivatives as important chemotherapeutic agents.[8-9]



X = F, CN

(1)

Schupp and co-workers have reported the activity of indolocarbazole and their derivatives



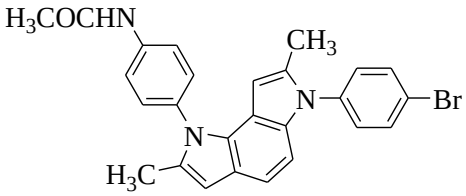
(2)

a: R_1 = H, R_2 = CH_3, R_3 = OCH_3
b: R_1 = OH, R_2 = CH_3, R_3 = OH
c: R_1 = H, R_2 = H, R_3 = OCH_3
d: R_1 = OH, R_2 = CH_3, R_3 = OCH_3
e: R_1 = H, R_2 = CH_3, R_3 = OH

such as staurosporine **(2)** which worked as inhibitors of macromolecule synthesis and cell explosion. Staurosporine D was cytotoxic against human monocytic cell lines (MONO-MAC-6) and also inhibited the synthesis of DNA. The IC_{50} values of staurosporines **2a**, **2d**

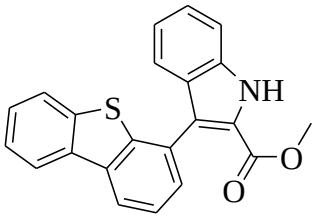
and **2e** for inhibiting monocytic cell lines were found to be 0.13, 0.24, and 0.34 µg/mL, respectively while those of staurosporine **2b** and **2c** were greater than 100 µg/mL.[10]

Derivatives of pyrrolo[2,3-*e*]indole **(3)** have been synthesised by Garcia and co-workers. These compounds were evaluated for possible *in-vitro* cytotoxic activity. Compound **3** (IC_{50} (µg/mL) = less than 1000) was found to be the most active in the series and showed the best result in prostate cancer cell lines.[11]



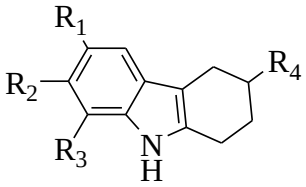
(3)

Queiroz and co-workers have worked on heteroaryl indoles *viz.*, phenylbenzothienoindole which showed a significant inhibitory activity against human cancer cell lines such as CNS cancer, breast adenocarcinoma, and small cell lung cancer. Methyl 3-(dibenzothien-4-yl) indole-2-carboxylate **(4)**, a derivative has shown a potent activity as it inhibited cancer cell lines MCF-7, HeLa and HEK293 with GI_{50} values ranging from 11 to 17 µM.[12]

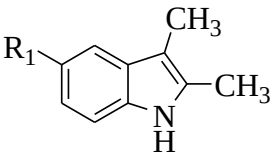


(4)

Kumar *et al*. have synthesized various derivatives of indole such as tetrahydrocarbazoles **(5)** and 2,3-dimethyl-indoles **(6)**. Anticancer activities of these compounds were checked against six tumour cell lines *viz.*, lung carcinoma (GIII) (Calu1), kidney adenocarcinoma (ACHN), normal breast epithelium (MCF10A), non-small cell lung carcinoma (H460), colon cancer cell (HCT116) and pancreas carcinoma (Panc1). 2,3-Dimethylindole (R_1 = H, F) exhibited anticancer activity against cell lines of pancreas carcinoma and lung carcinoma with IC_{50} values ranging from 0.027 to 0.032 µmol/L. Tetrahydrocarbazole derivatives showed a significant activity against lung cancer cell line with IC_{50} value of 0.025 µmol/L.[13]



(5)

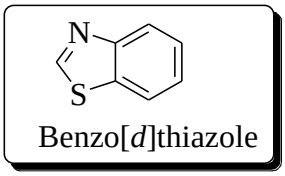


(6)

R_1 = H, F, CH_3, OCH_3
R_2 = R_3 = H, F
R_4 = H, CH_3, Ph, PhCN

2.2. Benzothiazole and its biological activity

Benzothiazole is a bicyclic moiety having a benzene ring fused with a thiazole ring. Benzothiazoles are present in a number of natural occurring compounds and useful for various biological activities *viz.*, antimicrobial, anticonvulsant, antitubercular, anti-inflammatory, anticancer etc. (**Figure-2**).[14] Benzothiazole moiety has also been identified as an intermediate of dyes such as thioflavin.



Benzothiazole has a promised pharmocophoric profile against a number of ailments which makes this moiety as an interesting molecule for designing a number of medicinal derivatives.

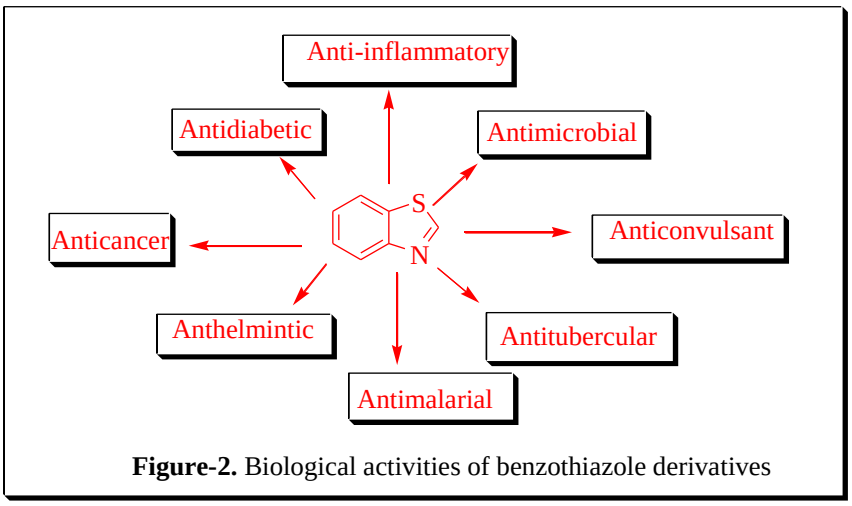
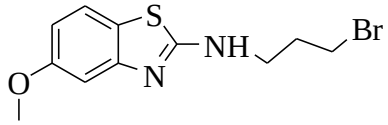


Figure-2. Biological activities of benzothiazole derivatives

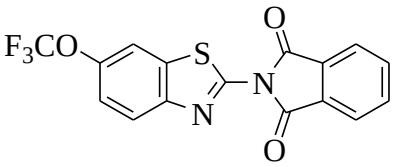
Benzothiazole derivatives have been accessed to show desired anticancer activity against various cell lines. *N*-Alkylbromo-benzothiazoles have been evaluated as a potent anticancer moiety. These compounds have shown significant cytotoxic activity. (3-Bromo-propyl)-(6-

methoxy-benzothiazol-2-yl)amine **(7)** has shown most promising anticancer activity against the prostate cancer cell lines (PC-3) with IC_{50} value of 0.006 µM, leukaemia cancer cell lines (THP-1) with IC_{50} value of 0.003 µM and colon carcinoma cell lines (Caco-2) with IC_{50} value of 0.09 µM, respectively.[15]



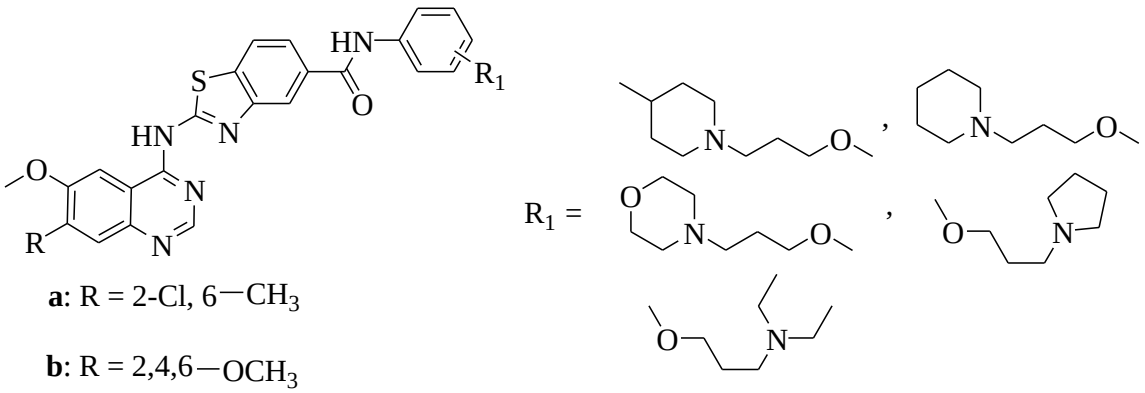
(7)

Phthalic imide derivatives of benzothiazole (**8**) exhibited *in vitro* cytotoxic activity on human cancer cell lines such as human hepatoma (SKHep1), Burkitt's lymphoma (CA46) and the chronic myelogenous leukaemia (K562). Benzothiazole containing phthalimide activated caspase dependent pathway to induce apoptosis in cell lines SKHep1 and CA46 at a concentration of 110 µM (40µg/ml) with IC_{50} value of 0.069 µM and 0.071 µM, respectively.[16]



(8)

The compound, 4-benzothiazole-amino-quinazolines (**9**) was studied for their cytotoxic activity against various cancer cell lines. Compounds **9a** and **9b** have potent cytotoxicity against chronic leukaemia myeloid cell line and displayed Src/ABI kinase inhibitory activity.[17]

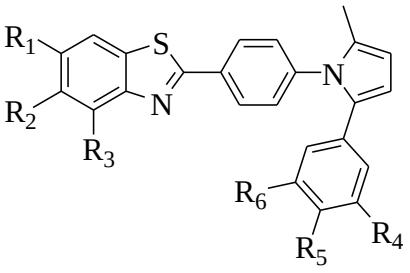


a: R = 2-Cl, 6—CH_3

b: R = 2,4,6—OCH_3

(9)

Kamal *et al*. synthesized conjugates based upon benzothiazole and pyrrole moieties (**10**) and checked for potent cytotoxicity against breast cancer cell lines. Compounds **10a** and **10b** induced cell death in breast cancer cell lines (MCF-7). Compound **10a** also showed antitumor activity against protein kinases *viz.*, MEK1, ERK1/2, p38MAPK and VEGF by inhibition of the succinate receptor (GPR91). [18]

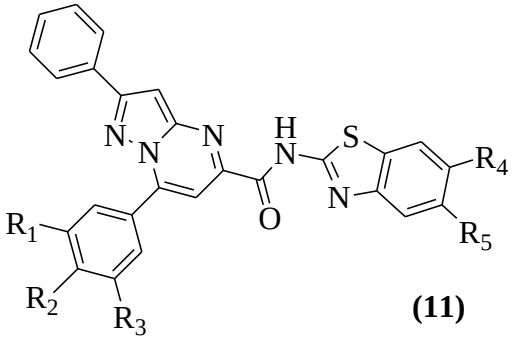


a: $R_1 = R_3 = H$, $R_2 = -OCH_3$, $R_4 = R_5 = R_6 = -OCH_3$

b: $R_1 = R_2 = R_3 = -OCH_3$, $R_4 = H$, $R_5 = -OCH_3$

(10)

Substituted pyrazolo[1,5-*a*]pyrimidine carboxylic acid on the 2nd position of amino benzothiazole moiety (**11**) through an amide functional group has also been synthesized by Kamal *et al.*[19] and evaluated against various cancer cell lines. Compounds **11a** and **11b** have appreciable anticancer activity as they showed reduction in Cdk1 expression level.



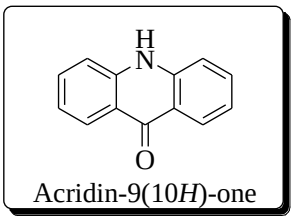
a: $R_1 = R_3 = R_4 = R_5 = H$, $R_2 = F$

b: $R_1 = R_2 = R_3 = -OCH_3$, $R_4 = R_5 = H$

(11)

2.3. Acridone and its biological activity

Derivatives of acridone occur naturally in plants and a number of marine organisms.[20-21] Acridone is a heterocyclic structure and is well characterised due to its unique chemical and physical properties as well as biological activities (**Figure-3**). Industrially, it is used for the manufacturing of pigments and dyes.



Acridin-9(10*H*)-one

Acridone derivatives have been chemically synthesized and are tested for their antitumour ac

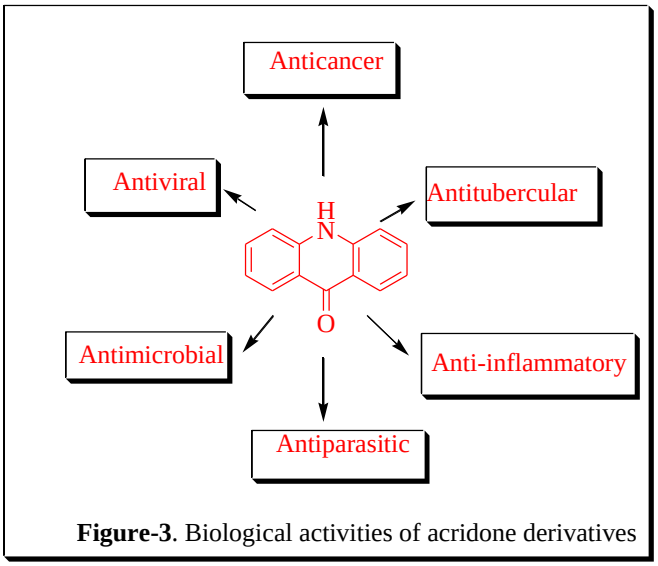


Figure-3. Biological activities of acridone derivatives

-tivities against various cancer cell lines. The unique property of acridone having a heterocyclic, tricyclic and planar structure that allows acridone moeity to intercalate in between the base pairs of DNA, a double stranded structure.[22-23] The enzymes effecting structure and functioning of DNA such as topoisomerase, telomerase, cyclin-dependent kinases[24] have found to be deactivated by acridone moiety. They thus act as potential anticancer drug and can be applied in pharmaceutics.[25]

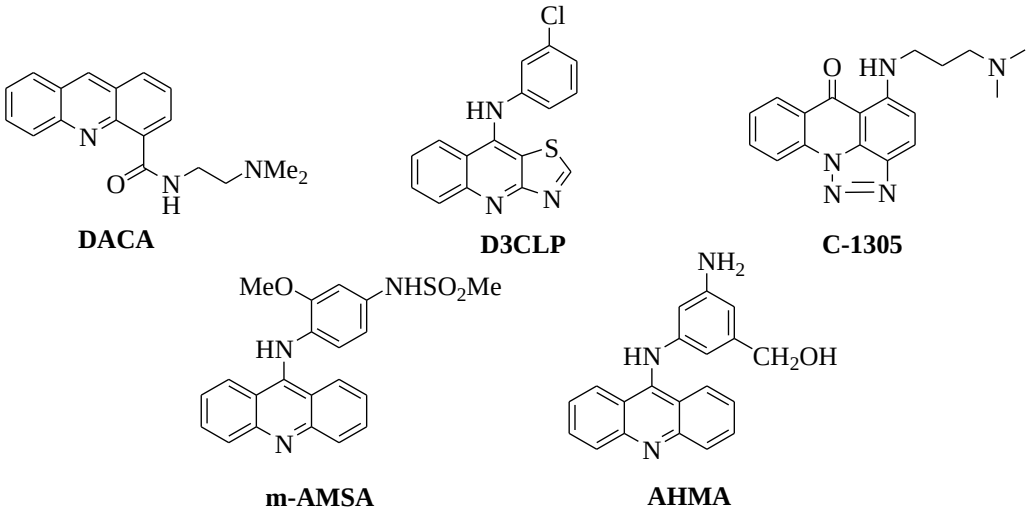
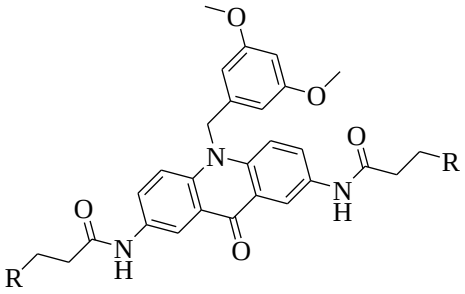


Figure 4. Acridine/Acridone derivatives

Analogues *N*-(2-(dimethylaminoethyl),acridine-4-carboxamide (DACA), triazolo acridone (C-1305), and amsacrine (*m*-AMSA), bearing acridine/acridone moieties have a potential anticancer property and have been clinically assessed. Molecules, *m*-AMSA, D3CLP and AHMA (**Figure-4**), amongst all the derivatives exhibited a clinical efficacy towards

inhibition of topoisomerase enzyme. Derivatives of *m*-AMSA have been clinically assessed for stronger anti-cancer properties and the least harmful to all side effects.[26-27]

Gao and co-workers, have been synthesized derivatives of 10-(3,5-dimethoxy)benzyl-9(10*H*)-acridone (**12**). Highest activity against leukaemia CCRF-CEM cells was exhibited by compound **12a** (IC_{50} = 0.003 µM), having dimethyl amine residue. Compounds **12b** and **12c**, bearing diethyl amine and diethanol amine moieties have lower inhibitory effect against cancer cell lines. Compound **12a** had a lower toxicity against human mast cell leukaemia (293T) cell lines (IC_{50} = > 0.1 µM).[28]



(12)

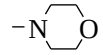
a: R = $(CH_3)_2N$

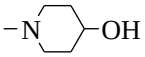
b: R = $(CH_3CH_2)N$

c: R = $(OHCH_3CH_2)_2N$

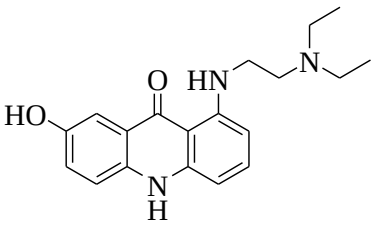
d: R = 

e: R = 

f: R = 

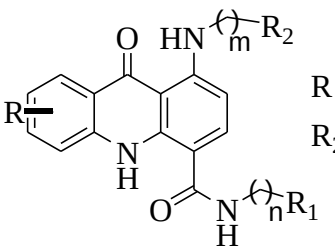
g: R = 

Singh and group synthesized acridone derivatives as potential leads to anticancer drugs. These amino derivatives of acridone were tested for antiproliferative activity. One of the derivative of imidazoacridinone (**13**) with IC_{50} values ranging from 0.50 to 4.10 µM, worked through inhibition of DNA-Topo II complex.[29-30]



(13)

Zhang and group carried out nucleophilic substitution with amides to the acridone scaffold (**14**) and tested *in-vitro* against potential cancer cell lines. This compound exhibited the best anticancer activity with IC_{50} values of 0.0016 and 0.0032 µM against human melanoma (A375) and human liver (HepG-2) cancer cell lines, respectively.[31]

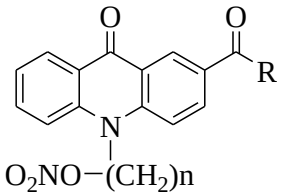


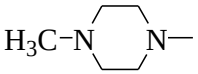
R = 5-CH_3, R_1 = $N(CH_3)_2$

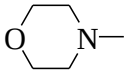
R_2 = 4-pyridyl, m = 1, n = 2

(14)

Prasad *et al*. synthesized acridone carboxamide derivatives (**15**) having potent antitumor activity. Compounds with NO donor moiety (**15a**–**d**) were found to have cytotoxic activity against human breast cancer (MCF7) cell lines with IC_{50} values ranging from 0.008 µM to 0.027 µM. Derivatives **15b–d** were tested for colorectal cancer cell lines (WiDrand, SW1398, and LS174T) and they showed potent, selective inhibition (IC_{50} = 0.017–0.11 µM against SW1398 cell lines, IC_{50} = 0.028–0.191 µM against WiDrand cell lines and IC_{50} = 0.031–0.182 µM against LS174T cell lines).[32]



a, **c** R = 

b, **d** R = 

a, b: n = 3

c, d: n = 4

(15)

2.4. Molecular hybridization

The hybridization of molecules is one of the best strategies of designing new potent molecules by combination of ligands or prototypes which have been recognized as effective pharmacophoric sub-entities known. Biochemical derivatives have been synthesised by the sufficient fusion of these sub-entities, thus, leading to the formation of a new hybrid unity maintaining important characteristics of the original molecules.[33] Application of this technique has resulted in synthesis of modified compounds having desirable selectivity profile, modified modes of action and reduced side effects. This strategy has thus been utilised for designing new drugs presenting analgesic, anti-inflammatory, platelet anti-aggregating, anti-infectious, anticancer, cardio- and neuroactive properties.[34]

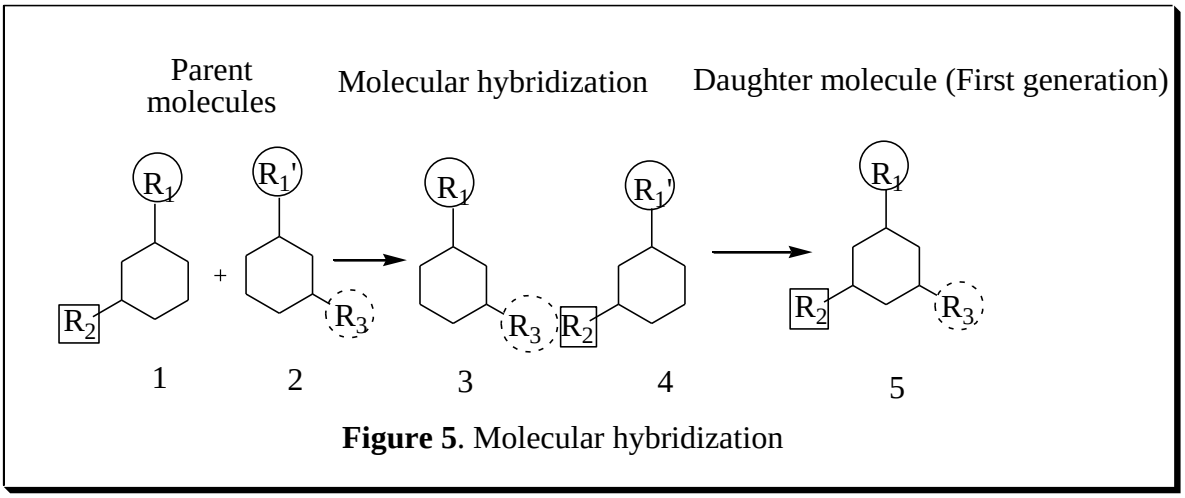
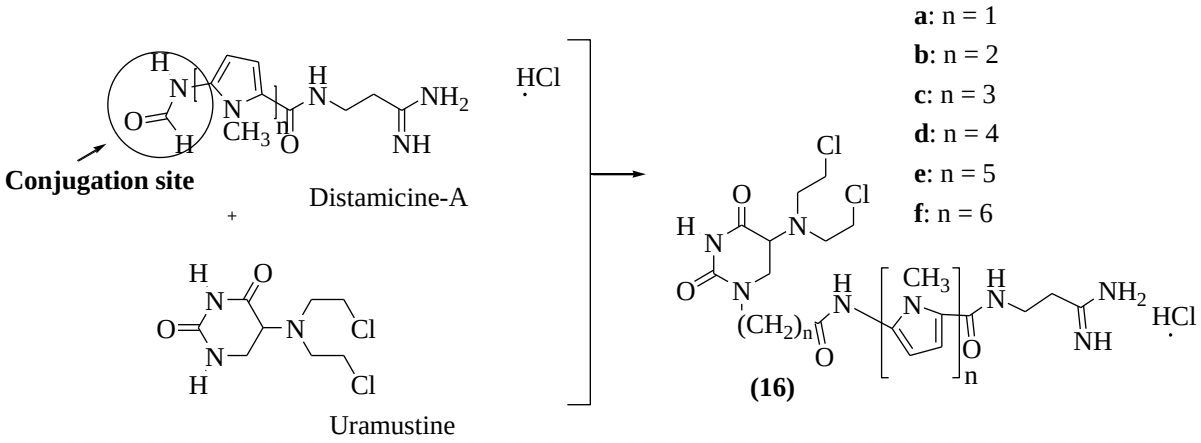


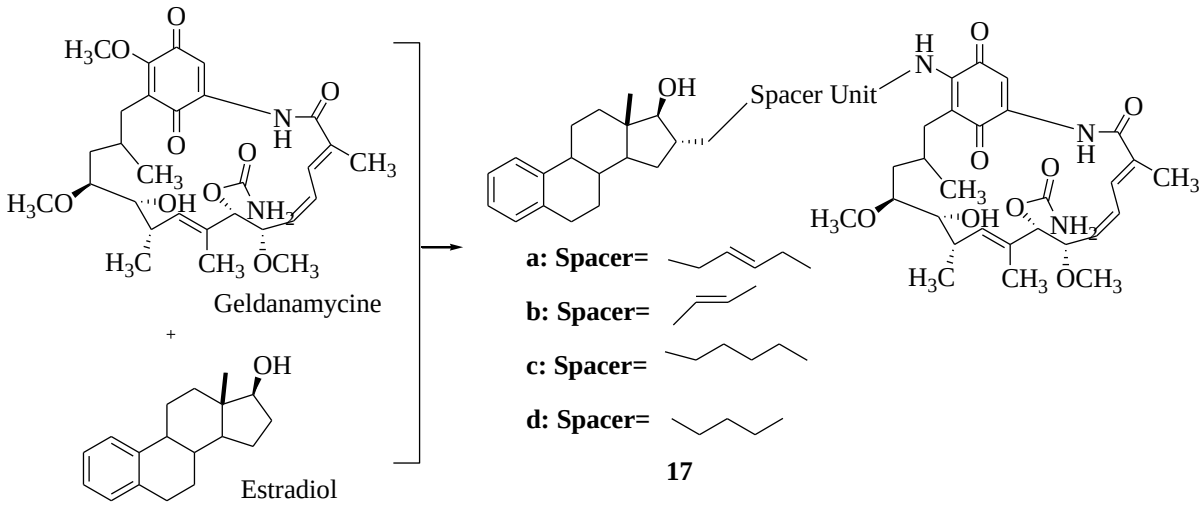
Figure 5. Molecular hybridization

Baraldi *et al*. synthesized a number of hybrid active compounds (**16**). One of these has combination of distamicine-A and uramustine, which are strong DNA alkylating agents and thus can be used to synthesize anti-tumoral hybrid agents. Thus, six (**16a-f**) new hybrid compounds were formed by conjugation of the essential pharmacophoric entities of distamicine-A and uramustine. These hybrids had superior anticancer activity than distamicine-A (IC_{50} > 100 μM) and uramustine (IC_{50} = 5.1 μM), tested individually against leukemia cell line in humans (K562).[35]



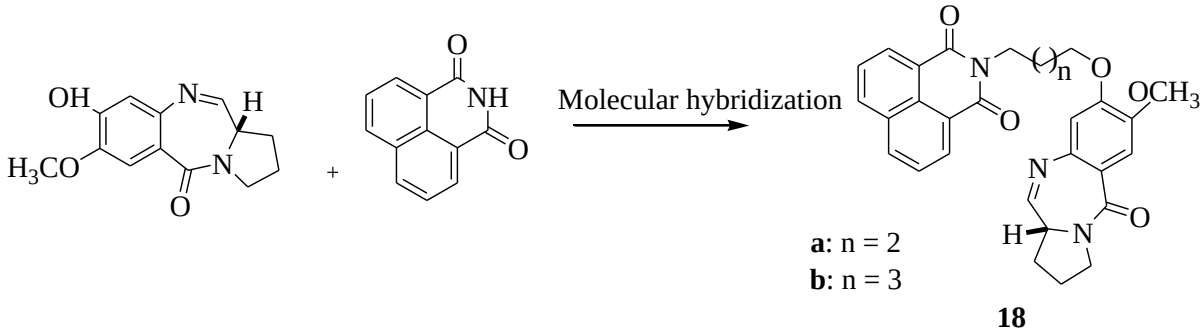
Kuduk and co-workers synthesized geldanamycine (GDM)-estradiol (ED) hybrids (**17**), in order to obtain molecules which can cause the degradation of estradiol receptors (ER) and the transaminase enzyme against the HER2 (human epidermal growth factor receptor 2) membrane, having an expression in numerous types of breast cancer. Geldanamycine acts as kinase inhibitor and degradation by binding to the cavity of heat shock protein (Hsp90),[36] causing restoration of protein structures leading to conformational modulation. The hybridisation between the molecules, estradiol and geldanamycine led to formation of new

potent ligands having activity against cancer cell lines. New compounds had spacer unit linking estradiol and GDM at their pharmocophoric moiety which significantly affects anticancer property of the compound. Compound **17a** was found to be most active of the series with IC_{50} values of 0.1 μM and 0.08 μM against HER2 and ER, respectively, while compound **17b** showed efficient activity against HER2, comparable to compound **17a** and a low activity against breast cancer ER cell line with IC_{50} value of 0.22 μM. Compounds **17c** and **17d** which have saturated spacer units, showed lower activity against cancer cell lines.[36-37]



17

Kamal *et al.* synthesized naphthalimide – poly(ADP-ribose) hybrids **18** and evaluated against anti-tumoral activity towards various cell lines. The activity of the molecule has been depended on spacer n, between the two moieties. Compound **18a** (n = 2) had a cytotoxic activity against colon and renal cancer cell lines with log LC_{50} values of -4.34 and -4.57,



18

respectively while compound **18b** (n = 3) showed cytotoxic activity over colon and melanoma cancer cell lines with log LC_{50} values of -4.41 and -4.43, respectively. These

molecules intercalated between the DNA strands and thus seem to be potent anticancer drug.[38]

3. RESEARCH GAPS AND OBJECTIVES

Literature survey suggests that individual moieties such as indole, benzothiazole and acridone showed potent anticancer properties but lack an efficient activity values against the human cancer cell lines. Few reports have been given in literature using the combination of three biological active pharmacophores for molecular hybridized compounds. Here, we have adopted technique of drug hybridisation to synthesize a new hybridized by the combination of three moieties *viz.*, indole, benzothiazole and acridone as drug candidate. These hybridized compounds will be used for anticancer activity against appropriate cancer cell lines.

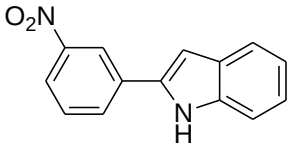
4. EXPERIMENTAL

4.1. General chemistry

All reactions were carried out in oven-dried glasswares. Commercial grade solvents were used without further purification and were supplied by Loba, Spectrochemicals and Aldrich. Melting points were determined in open capillaries and were uncorrected. Jeol-ECS 400 MHz and 100 MHz NMR spectrometer was used for recording ^{1}H and ^{13}C NMR spectra, respectively using $CDCl_3$ as solvent. The chemical shifts were expressed in parts per million with TMS as an internal reference and *J* values are given in Hz. Reactions were monitored by thin layer chromatography (TLC) using plates coated with silica gel HF-254 and column chromatography was performed with silica gel 60-120 mesh. Hexane/ethyl acetate was the adopted solvent system. Microwave irradiation reactions were carried out in CEM-Discover 99105 microwave synthesizer.

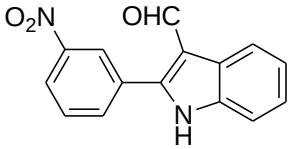
4.2. Procedure for the synthesis of 3-(3-(benzo[*d*]thiazol-2-yl)-1*H*-indol-2-yl)-10-(2-(cyclohexylamino)acetyl)acridin-9(10*H*)-one (10)

4.2.1. Synthesis of 2-(3-nitrophenyl)-1*H*-indole (3)



To phenyl hydrazine (10 gm, 92 mmol), 3-nitroacetophenone (15.3 gm, 92 mmol) and 30-40 ml of acetic acid were added in a 250 ml round bottom flask. The reaction mixture was stirred constantly at room temperature for about 30 minutes until precipitation occurs. The product formation was confirmed by TLC. The obtained precipitate was filtered off, and dried. Yield: 89% (crude); colour: orange-yellow.

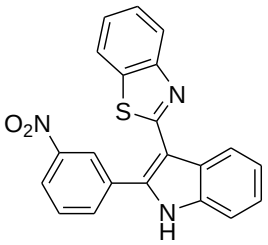
4.2.2. Synthesis of 2-(3-nitrophenyl)-1*H*-indole-3-carbaldehyde (4)



To 2-(3-nitrophenyl)-1*H*-indole (15 gm, 108 mmol), obtained from the first step taken in a 250 ml round bottom flask, dimethylformamide was added until the solid dissolved completely. $POCl_3$ was added dropwise carefully to the solution and stirred at room temperature for 1 hour. The product formation was confirmed by TLC. On addition of water, precipitates were obtained, filtered off the precipitates and dried. Yield: 74%; colour: grey; m. pt.: 200 °C - 203 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 10.09 (s, 1H, CHO), 8.86 (t, *J* = 1.84 Hz, 1H, ArH), 8.59 (s, 1H, NH), 8.30-8.34 (m, 1H, ArH), 7.82 (d, *J* = 7.80 Hz, 2H, ArH), 7.68 (t, *J* =

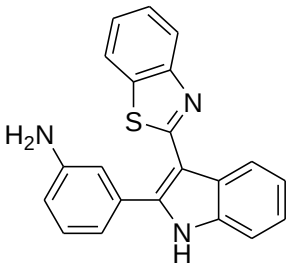
8.24 Hz, 1H, ArH), 7.55 (t, *J* = 7.80 Hz, 2H, ArH), 7.42-7.46 (m, 1H, ArH); ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 183.5 (CHO), 151.2, 148.3, 138.6, 134.7, 133.5, 133.0, 129.8, 129.5, 128.3, 123.8, 123.7, 122.6, 119.6 (ArC).

4.2.3. Synthesis of 2-(2-(3-nitrophenyl)-1*H*-indol-3-yl)benzo[*d*]thiazole (5)



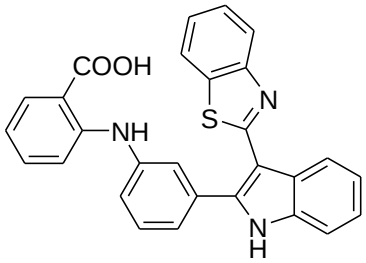
2-(3-nitrophenyl)-1*H*-indole-3-carbaldehyde (10 gm, 37 mmol) was taken in a 250 ml round bottom flask, 2-aminothiophenol (4.7 gm, 37 mmol) and nitrobenzene (20 - 30 ml) were added and the reaction mixture was heated at 70 °C for about 2 hours. The product formation was confirmed by TLC. The obtained precipitate was filtered off, and dried. Yield: 71%; colour: brown; m.pt.: 230 - 234 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 9.00 (s, 1H, ArH), 8.58 (s, 1H, NH), 8.32 (d, *J* = 7.32 Hz, 1H, ArH), 8.25 (d, *J* = 6.84 Hz, 1H, ArH), 8.04 (d, *J* = 7.32 Hz, 1H, ArH), 7.85 (d, *J* = 6.88 Hz, 3H, ArH), 7.64 (t, *J* = 7.76 Hz, 1H, ArH), 7.52-7.55 (m, 3H, ArH), 7.40 (d, *J* = 6.40 Hz, 1H, ArH); ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 158.9, 153.4, 149.1, 148.1, 139.0, 135.3, 134.6, 133.8, 129.6, 129.1, 127.7, 125.3, 126.4, 124.6, 123.5, 123.4, 122.9, 121.3, 119.4, 117.2 (ArC).

4.2.4. Synthesis of 3-(3-(benzo[*d*]thiazol-2-yl)-1*H*-indol-2-yl)benzenamine (6)



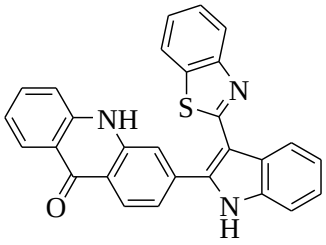
Tetrahydrofuran (20 ml) was taken in a 250 ml round bottom flask and 30 ml of water was added. 2-(2-(3-Nitrophenyl)-1*H*-indol-3-yl)benzo[*d*]thiazole (8 gm, 21 mmol), sodium dithionate (2.3 gm, 107 mmol) and excess of ammonia solution were added to the reaction mixture. The reaction was carried out for 2 hours at room temperature. The product was confirmed by TLC. THF was evaporated and washed the obtained solid with diethyl ether. Yield: 61% (crude); colour: yellow.

4.2.5. Synthesis of 2-(3-(3-(benzo[*d*]thiazol-2-yl)-1*H*-indol-2-yl)phenylamino)benzoic acid (7)



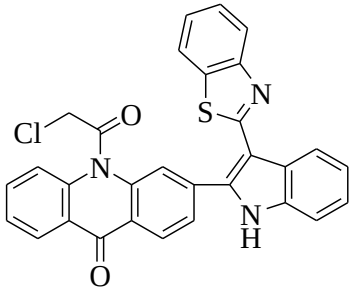
To 3-(3-(benzo[*d*]thiazol-2-yl)-1*H*-indol-2-yl) benzenamine (5 gm, 14 mmol), 2-chlorobenzoic acid (6.8 gm, 44 mmol), potassium carbonate (0.2 gm, 14 mmol) and a catalyst CuO (0.025 gm, 3 mmol) were added in a 100 ml round bottom flask. The reaction mixture was heated at 70 °C for 2 hours in the presence of DMF. The product formation was confirmed by TLC. The obtained precipitate was filtered off, and dried. Yield: 83% (crude); colour: white.

4.2.6. Synthesis of 3-(3-(benzo[*d*]thiazol-2-yl)-1*H*-indol-2-yl)acridin-9(10*H*)-one (8)



To 2-(3-(3-(benzo[*d*]thiazol-2-yl)-1*H*-indol-2-yl) phenylamino)benzoic acid in a round bottom flask, excess of concentrated sulphuric acid was added drop-wise at 0 °C. The reaction mixture was then stirred for 30 minutes at room temperature. The product formation was confirmed by TLC. Water was added; obtained precipitate was filtered off, and dried. Yield: 72% (crude); colour: white.

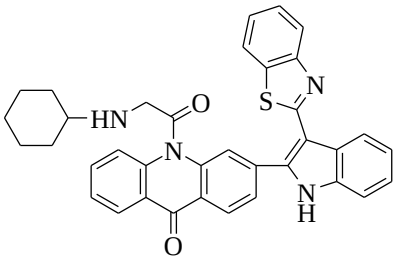
4.2.7 Synthesis of 3-(3-(benzo[*d*]thiazol-2-yl)-1*H*-indol-2-yl)-10-(2 chloroacetyl)acridin- 9(10*H*)-one (9)



To 3-(3-(benzo[*d*]thiazol-2-yl)-1*H*-indol-2-yl)acridin-9(10*H*)-one (1 gm, 2.25 mmol), triethylamine (0.33 gm, 4.5 mmol) and chloroacetyl chloride (0.72 gm, 4.5 mmol) were added in a 100 ml round bottom flask containing 10 ml acetonitrile. The reaction mixture was stirred at room temperature for 30 minutes. The pure product was separated by column chromatography using hexane:ethylacetate as eluents. Yield: 74%; colour: white; m.pt.: > 300 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.58 (s, 1H, NH), 7.98 (d, *J* = 8.24 Hz, 1H, ArH), 7.91 (dd, ^{2}J = 8.00 Hz, ^{3}J =1.20 Hz, 1H, ArH), 7.88 (d, *J* = 7.80 Hz, 1H, ArH), 7.82 - 7.84 (m, 2H, ArH), 7.81 (d, *J* = 2.32 Hz, 1H, ArH), 7.58 (d, *J* = 8.24 Hz, 2H, ArH), 7.46 - 7.54 (m, 2H, ArH), 7.42 - 7.45 (m, 1H, ArH), 7.34 - 7.38 (m, 2H, ArH),

7.29 - 7.32 (m, 1H, ArH), 7.02 (d, *J* = 7.80 Hz, 1H, ArH), 4.82 (s, 2H, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz); δ (ppm) 167.9, 165.7, 159.5, 153.1, 150.2, 141.0, 139.3, 139.0, 134.8, 133.8, 133.7, 132.4, 129.6, 129.4, 129.3, 128.8, 128.6, 127.4, 126.2, 126.1, 125.0, 124.5, 123.6, 122.7, 121.3, 119.4, 117.0 (ArC), 67.7 (CH_2).

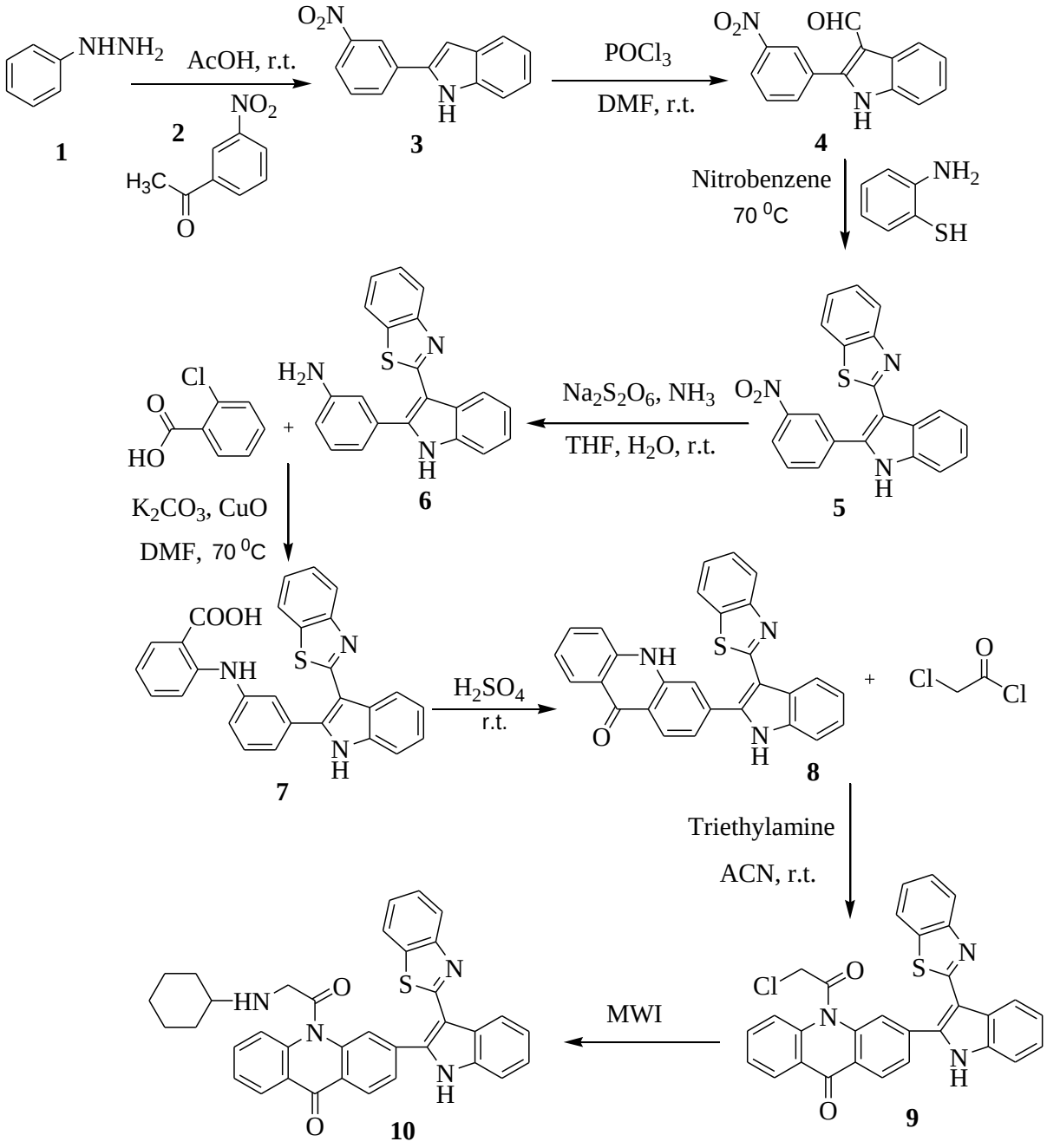
4.2.8 Synthesis of 3-(3-(benzo[*d*]thiazol-2-yl)-1*H*-indol-2-yl)-10-(2-(cyclohexylamino)acetyl)acridin-9(10*H*)-one (10)



3-(3-(Benzo[*d*]thiazol-2-yl)-1*H*-indol-2-yl)-10-(2-chloroacetyl)acridin-9(10*H*)-one (0.1 gm, 1.92 mmol) was added to cyclohexylamine (0.019 gm, 1.92 mmol) in ethanol (30ml) in a 50 ml round bottom flask. The reaction mixture was reacted under microwave irradiation for 10 minutes. The solvent was evaporated using rota-evaporator and column chromatography was done to separate the pure compound. Yield: 45%; colour: white; m.pt.: > 300 °C; ^1H NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.57 (s, 1H, NH), 8.01 (d, *J* = 5.60 Hz, 2H, ArH), 7.79 - 7.86 (m, 3H, ArH), 7.67 (d, *J* = 6.80 Hz, 1H, ArH), 7.47 - 7.54 (m, 4H, ArH), 7.38 (t, *J* = 7.8 Hz, 2H, ArH), 7.31 - 7.33 (m, 2H, ArH), 7.21 (s, 1H, ArH), 4.10 - 4.30 (m, 2H, CH_2), 3.70 - 3.90 (m, 2H, CH, NH), 1.73 - 1.95 (m, 2H, CH_2), 1.60 - 1.69 (m, 2H, CH_2) 1.25 – 1.42 (m, 4H, CH_2), 1.10 - 1.21 (m, 2H, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 139.1, 137.6, 133.9, 130.9, 130.5, 129.6, 129.3, 128.9, 128.7, 128.5, 128.1, 127.5, 126.3, 125.1, 122.8, 121.3, 119.4, 117.0 (ArC), 62.0 (CH), 48.7, 33.0, 32.7, 29.6, 25.4, 24.7 (CH_2).

5. RESULTS AND DISCUSSION

Using commercially available phenylhydrazine (**1**) and 3-nitroacetophenone (**2**) as initial substrates, benzothiazol-indole-acridone hybridized product **10** was synthesized according to the **scheme 1**. Treatment of phenylhydrazine (**1**) with 3-nitroacetophenone (**2**) in the presence of acetic acid at room temperature for 30 minutes, gave orange-yellow coloured solid of 2-(3-nitrophenyl)-1*H*-indole (**3**) in 89% yield. Compound **3** was used further without purification and reacted with DMF and $POCl_3$ in Vilsmeier-Haack reaction at room temperature for 1 hour, gave grey coloured solid of 2-(3-nitrophenyl)-1*H*-indole-3-carbaldehyde (**4**) in 74 % yield.



Scheme-1. Synthesis of 3-(3-(benzo[*d*]thiazol-2-yl)-1*H*-indol-2-yl)-10-(2(cyclohexylamino)acetyl)acridin-9(10*H*)-one (**10**)

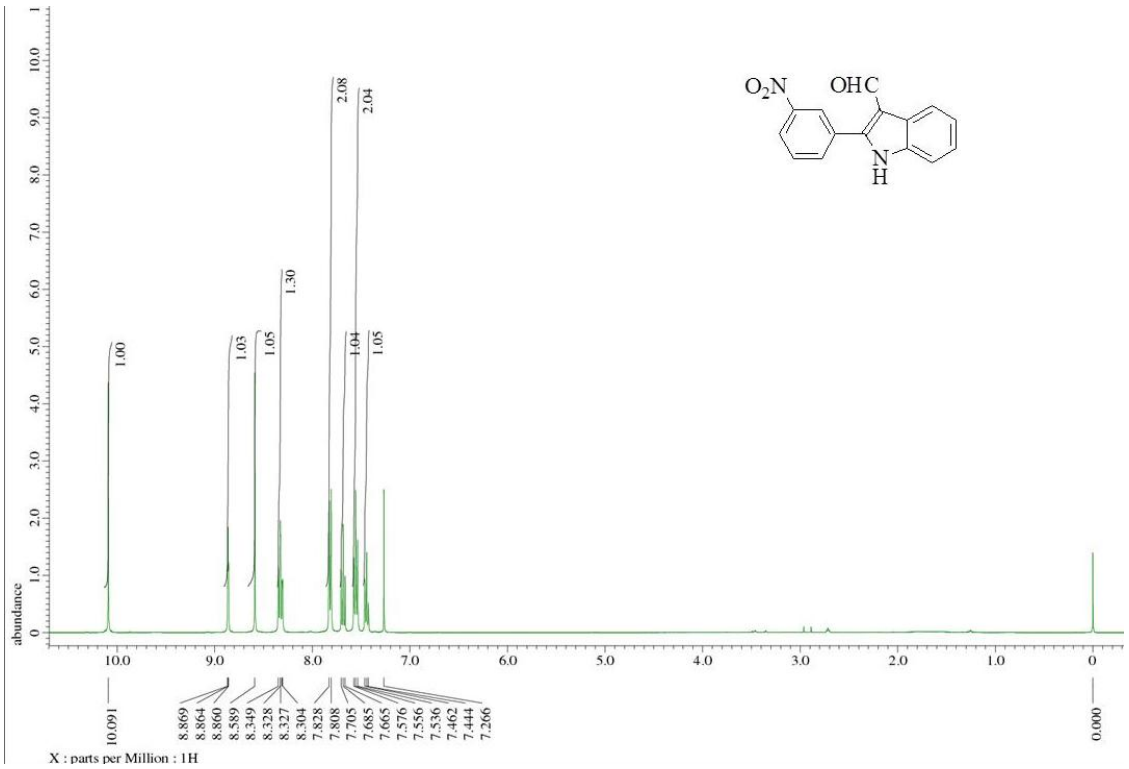


Figure-6. ^{1}H NMR spectrum of 2-(3-nitrophenyl)-1*H*-indole-3-carbadehyde **(4)**

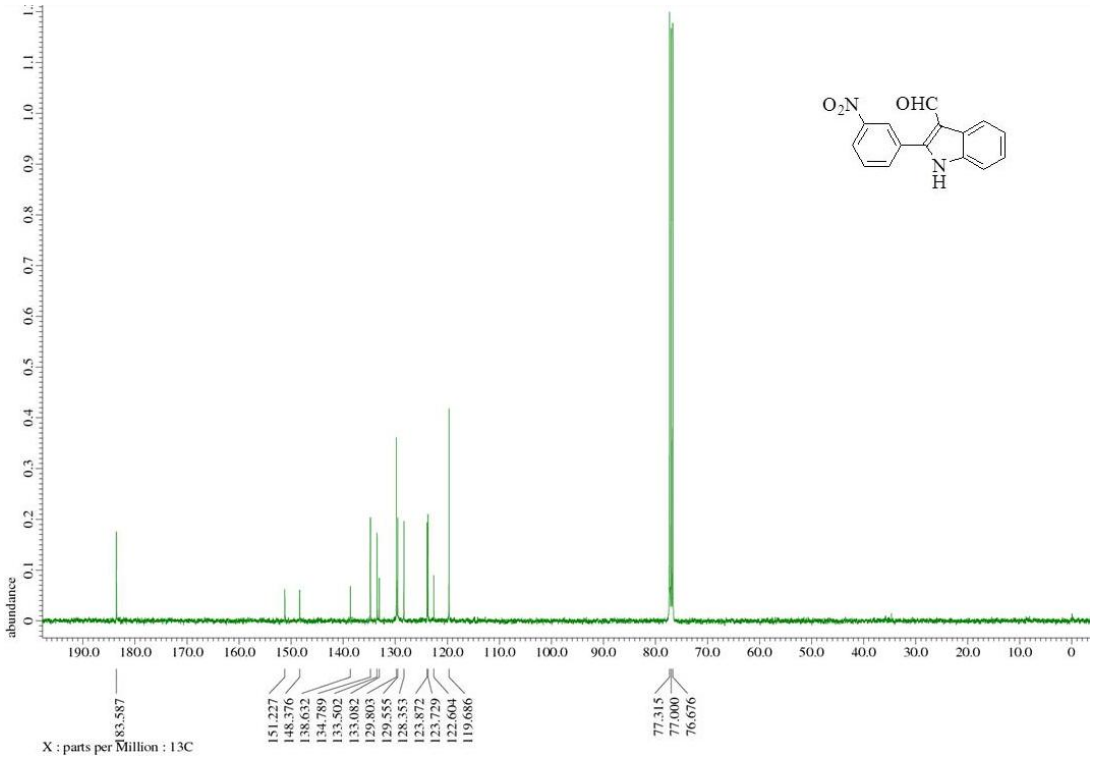


Figure-7. ^{13}C NMR spectrum of 2-(3-nitrophenyl)-1*H*-indole-3-carbadehyde **(4)**

Carbaldehyde derivative of indole **4** was well characterized by ^{1}H and ^{13}C NMR spectrometry (**Figures 6** and **7**). ^{1}H NMR spectrum of compound **4** showed singlet at δ 10.09 ppm corresponding to CHO proton. Aromatic protons of 2-(3-nitrophenyl)-1*H*-indole-3-carbaldehyde (**4**) showed wide range of splitting pattern ranging from δ 7.42 - 8.86 ppm corresponding to eight protons. ^{13}C NMR spectrum showed the distinctive –CHO peak at δ 183.5 ppm. Appearance of new signals at δ 10.09 and 183.5 in respective ^{1}H and ^{13}C NMR spectrum confirmed the formation of 2-(3-nitrophenyl)-1*H*-indole-3-carbaldehyde (**4**).

Compound **4** was then treated with 1.0 equivalent of 2-aminothiophenol in the presence of nitrobenzene at 70 °C for 2 hours, gave brown coloured solid of 2-(2-(3-nitrophenyl)-1*H*-indol-3-yl)benzo[*d*]thiazole (**5**) in 71% yield.

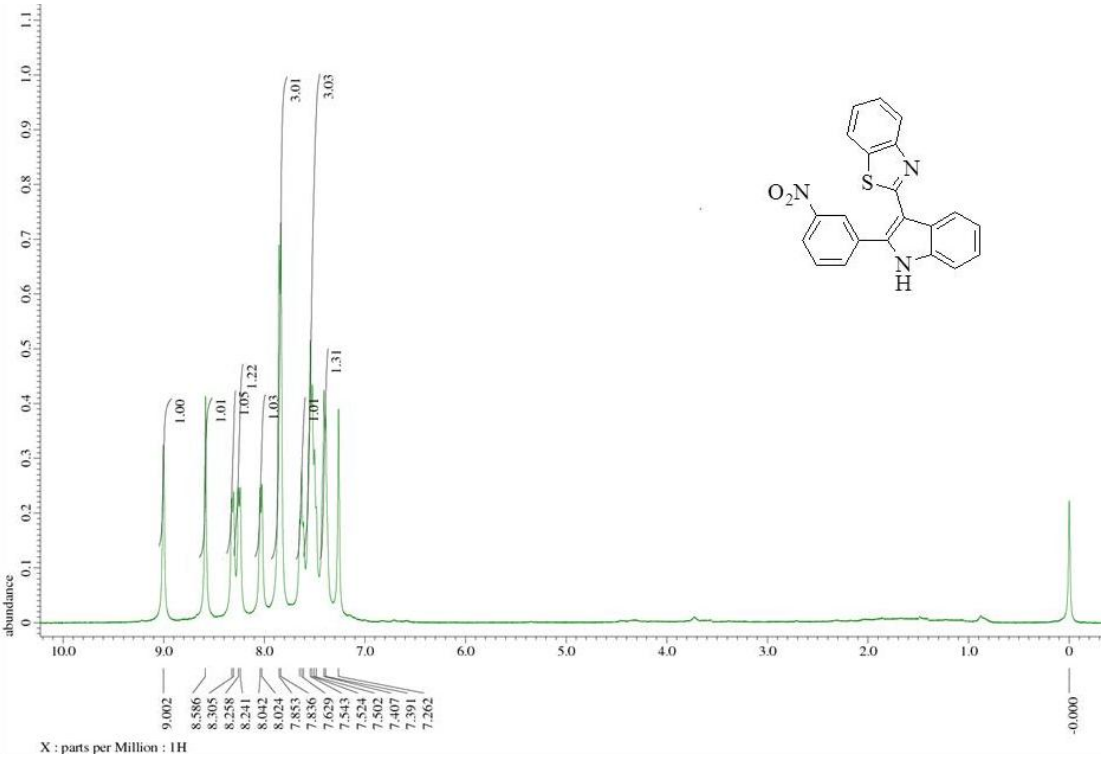


Figure-8. ^{1}H NMR spectrum of 2-(2-(3-nitrophenyl)-1*H*-indol-3-yl)benzo[*d*]thiazole **(5)**

^{1}H NMR spectrum of compound **5** showed one singlet at δ 9.00 corresponding to one aromatic proton. Another aromatic protons of 2-(2-(3-nitrophenyl)-1*H*-indol-3-yl)benzo[*d*]thiazole showed wide range of splitting pattern ranging from δ 7.40 – 8.32 ppm corresponding to twelve protons. Disappearance of peak at δ 10.09 ppm of CHO proton and appearance of another four protons in aromatic region, confirmed the formation of compound **5**.

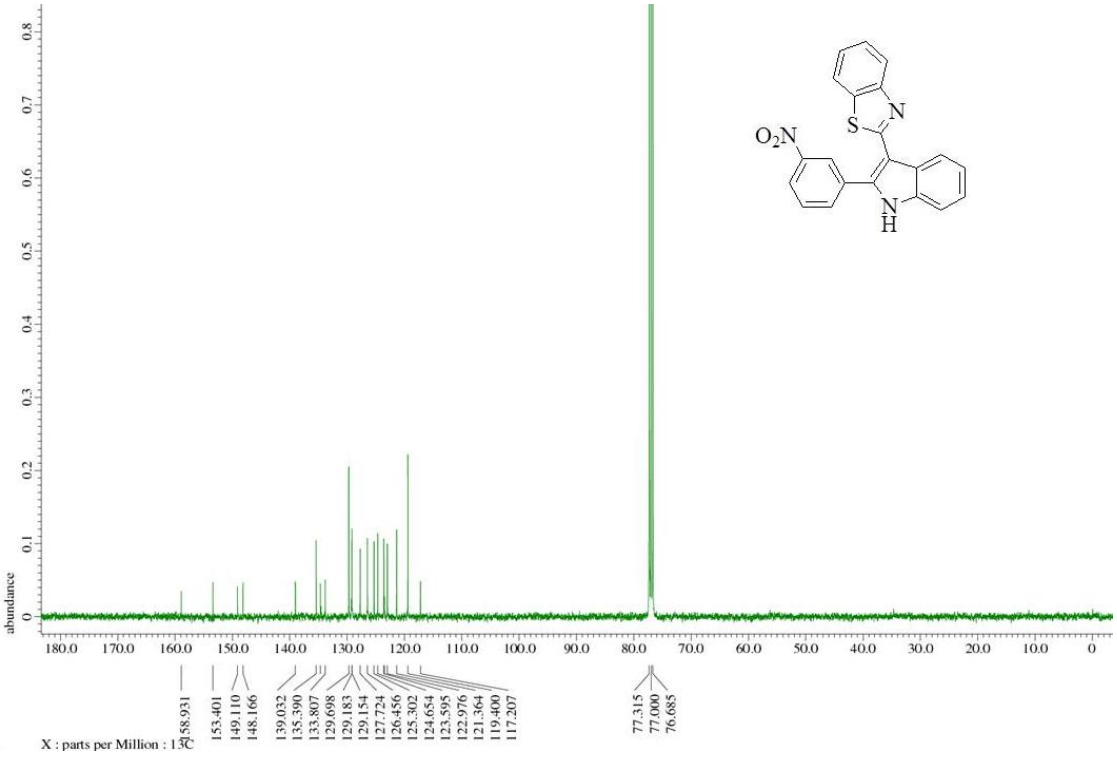


Figure-9. ^{13}C NMR spectrum of 2-(2-(3-nitrophenyl)-1*H*-indol-3-yl)benzo[*d*]thiazole **(5)**

^{13}C NMR spectrum showed the signals at δ 158.9, 153.4, 149.1, 148.1, 139.0, 135.3, 134.6, 133.8, 129.6, 129.1, 127.7, 126.4, 125.3, 124.6, 123.5, 123.4, 122.9, 121.3, 119.4, and 117.2 of aromatic carbons. So, ^{1}H and ^{13}C NMR spectral analysis confirmed the formation of 2-(2-(3-nitrophenyl)-1*H*-indol-3-yl)benzo[*d*]thiazole **(5)** (**Figures 8** and **9**). Reduction of compound **5** was done with sodium dithionate in THF/H_2O and excess of ammonia solution at room temperature for 2 hours, gave yellow coloured solid of 3-(3-(benzo[*d*]thiazol-2-yl)-1*H*-indol-2-yl) benzenamine (**6**) in 61% yield. Compound **6** was used further without any purification. Compound **6** was then treated with 2-chlorobenzoic acid in the presence of potassium carbonate in DMF and CuO (catalyst) at 70 °C for 2 hours, gave white solid of 2-(3-(3-(benzo[*d*]thiazol-2-yl)-1*H*-indol-2-yl) phenylamino benzoic acid (**7**). The crude of **7** was directly used for the next step without any further purification. Compound **7** was treated with excess of concentrated H_2SO_4 at room temperature for 30 minutes and then poured into cold water to give white solid of 3-(3-(benzo[*d*]thiazol-2-yl)-1*H*-indol-2-yl)acridin-9(10*H*)-one (**8**) in 72% yield. Compound **8** was then treated with 2 equivalents of chloroacetyl chloride in the presense of triethylamine and acetonitrile at room temperature for 30 minutes, gave white solid of 3-(3-(benzo[*d*]thiazol-2-yl)-1*H*-indol-2-yl)-10-(2-chloroacetyl)acridin-9(10*H*)-one (**9**) in 74% yield.

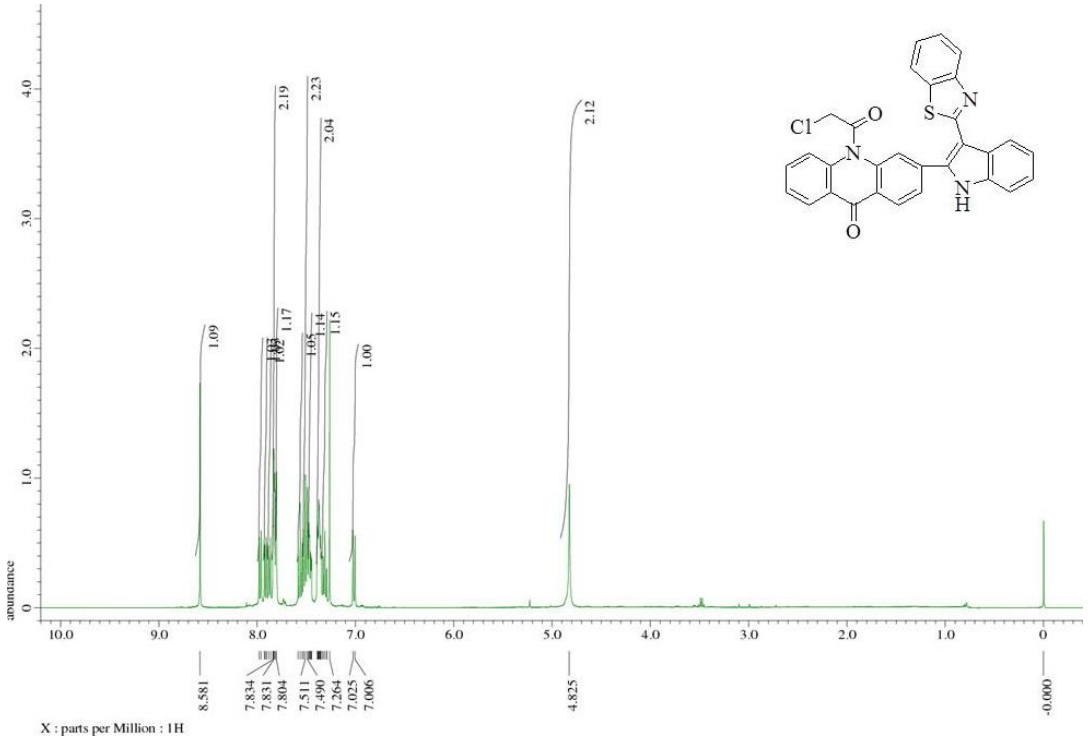


Figure-10. ^{1}H NMR spectrum of 3-(3-(benzo[*d*]thiazol-2-yl)-1*H*-indol-2-yl)-10-(2-chloroacetyl)acridin-9(10*H*)-one **(9)**

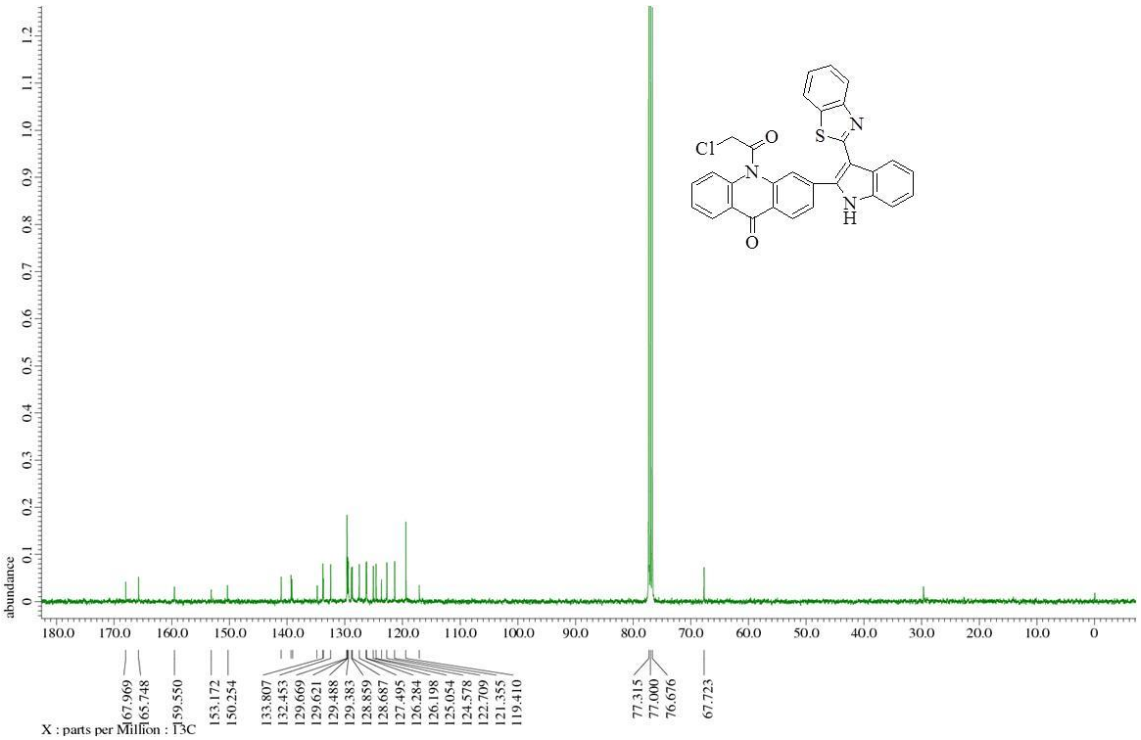


Figure-11. ^{13}C NMR spectrum of 3-(3-(benzo[*d*]thiazol-2-yl)-1*H*-indol-2-yl)-10-(2-chloroacetyl)acridin-9(10*H*)-one **(9)**

^{1}H NMR spectrum of compound **9** showed one singlet at δ 8.58 ppm corresponding to NH group. Aromatic protons of compound **9** showed wide range of splitting pattern ranging from δ 7.02-7.98 ppm corresponding to fifteen protons. The spectrum also gave a singlet at δ 4.82 ppm of two protons of –CH_2 group. ^{13}C NMR spectrum showed the signals at δ 167.9, 165.7, 159.5, 153.1, 150.2, 141.0, 139.3, 139.0, 134.8, 133.8, 133.7, 132.4, 129.6, 129.4, 129.3, 128.8, 128.6, 127.4, 126.2, 126.1, 125.0,124.5, 123.6, 122.7, 121.3, 119.4 and 117.0 of aromatic carbons and signal at δ 67.7 corresponding to CH_2. Appearance of new signals at δ 4.82 in ^{1}H NMR and at δ 67.7 in ^{13}C NMR confirmed the formation of 3-(3-(benzo[*d*]thiazol-2-yl)-1*H*-indol-2-yl)-10-(2-chloroacetyl)acridin-9(10*H*)-one (**9**) (**Figures 10** and **11**).

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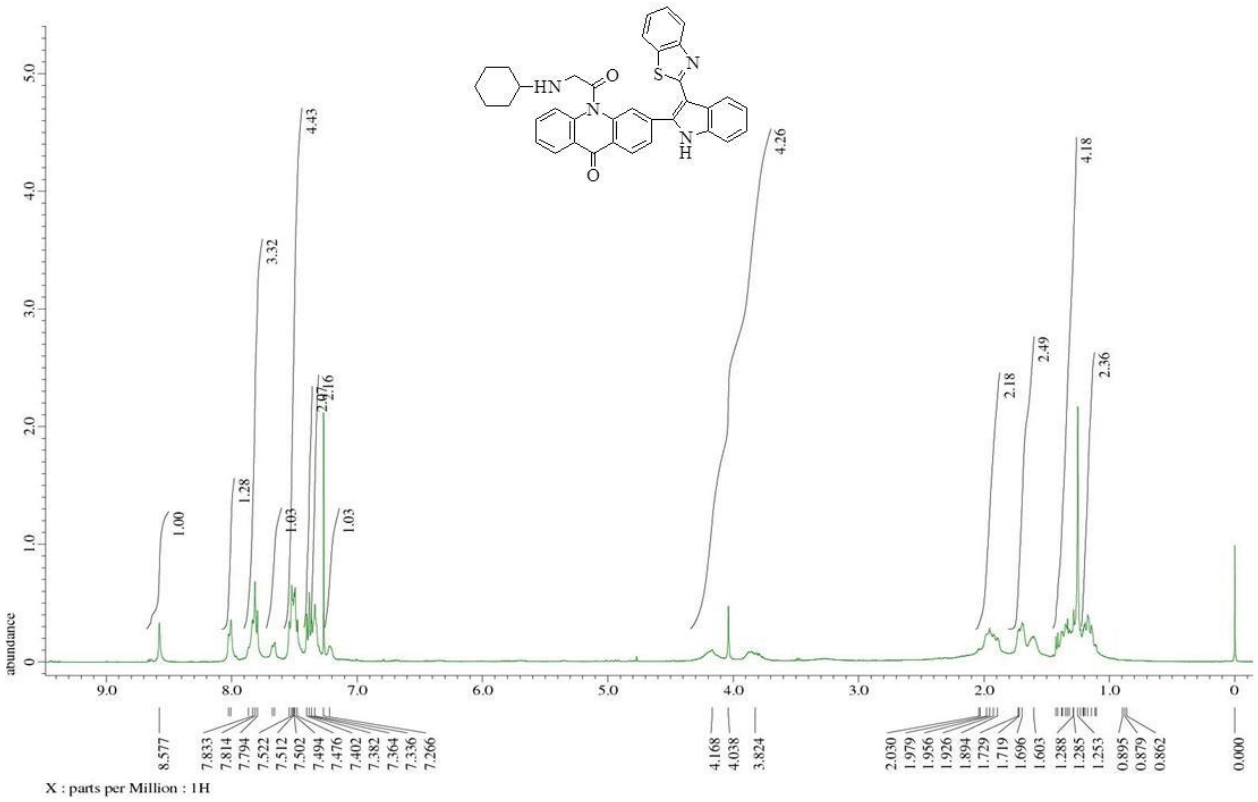

Figure-12. ^{1}H NMR spectrum of 3-(3-(benzo[*d*]thiazol-2-yl)-1*H*-indol-2-yl)-10-(2-(cyclohexylamino)acetyl)acridin-9(10*H*)-one **(10)**

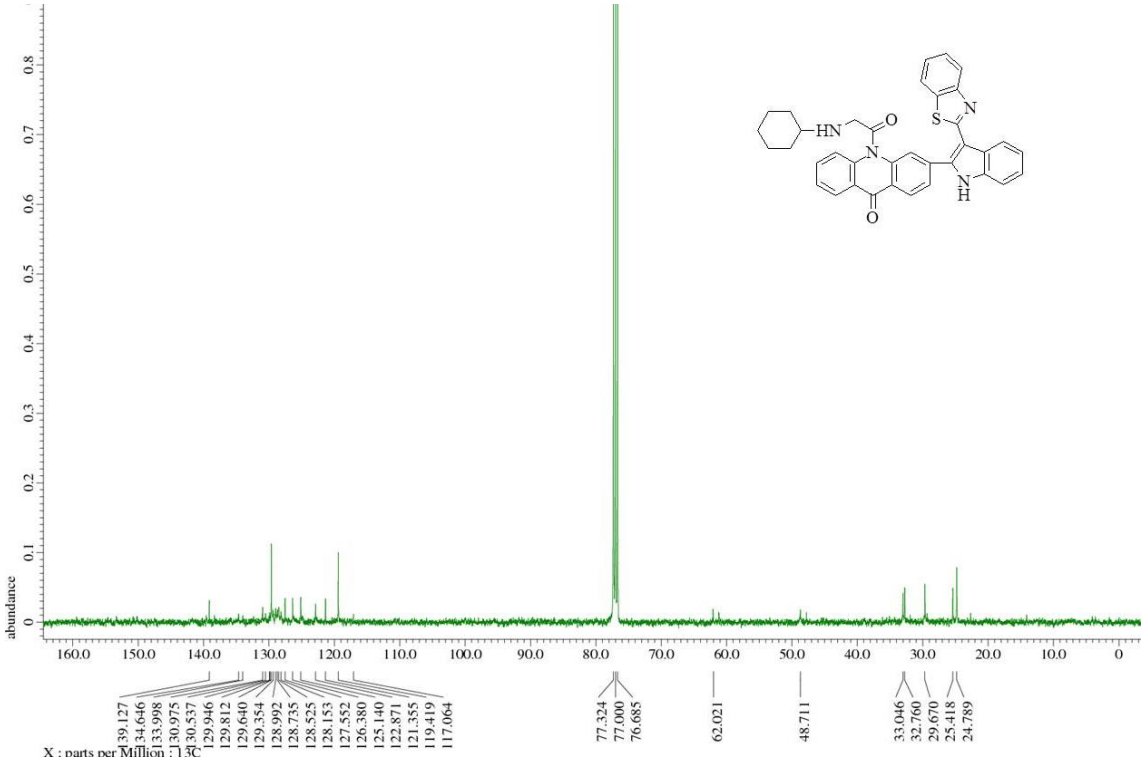

Figure-13. ^{13}C NMR spectrum of 3-(3-(benzo[*d*]thiazol-2-yl)-1*H*-indol-2-yl)-10-(2-(cyclohexylamino)acetyl)acridin-9(10*H*)-one **(10)**

Compound **9** was finally treated with one equivalent of cyclohexylamine in ethanol for 10 minutes under microwave irradiation and after work up, gave final derivative **10** in 45 % yield. Column chromatography was done to further purify this compound using hexane-ethyl acetate solvent system. ^{1}H NMR spectrum of compound **10** showed aromatic protons of 3-(3-(benzo[*d*]thiazol-2-yl)-1*H*-indol-2-yl)-10-(2-(cyclohexylamino)acetyl)acridin-9(10*H*)-one in a wide range of splitting pattern ranging from δ 7.21-8.01 ppm corresponding to fifteen protons, one multiplet of two protons at δ 4.20 due to CH_2, one multiplet of two protons at δ 3.80 due to CH and NH and four multiplets at δ 1.10 – 1.95 due to ten protons of cyclohexane ring. ^{13}C NMR spectrum showed the signals at δ 139.1, 137.6, 133.9, 130.9, 130.5, 129.6, 129.3, 128.9, 128.7, 128.5, 128.1, 127.5, 126.3, 125.1, 122.8, 121.3, 119.4, 117.0 of aromatic carbons, signals at δ 62.0 of CH and signals at δ 48.7, 33.0, 32.7, 29.6, 25.4 and 24.7 corresponding to CH_2. Thus, shifting of signal of CH_2 and appearance of new signals at aliphatic region confirmed the formation of 3-(3-(benzo[*d*]thiazol-2-yl)-1*H*-indol-2-yl)-10-(2-(cyclohexylamino)acetyl)acridin-9(10*H*)-one **(10)** (**Figures 12** and **13**). Intermediates **8** and **9**, and final compound **10** have been submitted to National Cancer Institute (NCI), NIH, Bethesda, USA for 60 human cancer lines studies.

6. CONCLUSIONS

- Intermediates such as 2-(3-nitrophenyl)-1*H*-indole-3-carbadehyde (**4**), 2-(2-(3-nitrophenyl)-1*H*-indol-3-yl)benzo[*d*]thiazole (**5**), 2-(3-(benzo[*d*]thiazol-2-yl)-1*H*-indol-2-yl)-10-(2-chloroacetyl)acridin-9(10*H*)-one (**9**), etc. were synthesized in moderate to good yields.
- Final compound 3-(3-(benzo[*d*]thiazol-2-yl)-1*H*-indol-2-yl)-10-(2-(cyclohexylamino) acetyl)acridin-9(10*H*)-one (**10**) was synthesized in good yield. These compounds were well characterized by ^{1}H and ^{13}C NMR spectrometry.
- The final compound will further be used for its biological activity as anti-cancer agents as well as DNA interaction.