Synthesis and *In vitro* Evaluation of Coumarin - Benzimidazole Hybrids as Anticancer Agents

Thesis submitted in partial fulfillment of the requirements for the award of degree of Master of Science

in

Chemistry

Submitted By

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THAPAR UNIVERSITY

PATIALA – 147004

July 2012

CERTIFICATE

This is to certify that the project entitled "Synthesis and *in vitro* evaluation of coumarin - benzimidazole hybrids as anticancer agents" being submitted by Ms. Shweta Bindal, Regn.No. 301002016 in the partial fulfillment of the requirements for the award of degree of Masters of Science in Chemistry, School of Chemistry and Biochemistry, Thapar University, Patiala, in a bonafied work carried out under our supervision and guidance. The report has not been submitted for the award of any other degree or certificate in this or any other university.

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Candidate's Declaration

I hereby declare that the work which is being presented in the dissertation entitled " Synthesis and *in vitro* evaluation of coumarin – benzimidazole hybrids as anticancer agents" in the partial fulfillment of the requirements for the award of the degree of Master of Science in Chemistry, School of Chemistry and Biochemistry, Thapar University, Patiala is an authentic record of my own work during a period of six months from January 2012 to July 2012, under the supervision of Dr. Kamaldeep Paul, Assistant Professor and Dr. Vijay Luxami, DST Young Scientist, School of Chemistry and Biochemistry, Thapar University, Patiala. The report has not been submitted for the award of any other degree or certificate in this or any other university.

Shweta 9/7/2012

Place: Patiala

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Date: 9/7/2012

This is to certify that the above statement given by the candidate is correct and true to the best of our knowledge.

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Shweta 9/7/2012

Shweta Bindal

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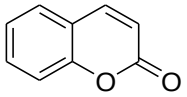
INTRODUCTION & REVIEW OF LITERATURE

1. Coumarin and its biological application

Coumarin owes their class name to 'Coumarou', the vernacular name of the tonka bean (*Dipteryx odorata*, Fabaceae), from which it was isolated in 1820. Coumarin is a fragrant chemical compound in the benzopyrone family, all of which consists of a benzene ring joined to a pyrone ring.



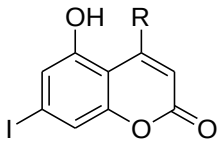
Coumarin {2H-Chromen-2-one}

Coumarin is found in many plants, notably in high concentration in the tonka bean (*Dipteryx odorata*), vanilla grass (*Anthoxanthum odoratum*), sweet woodruff (*Galium odoratum*), mullein (*Verbascum* spp.), sweet grass (*Hierochloe odorata*), cassia cinnamon (*Cinnamomum aromaticum*), melilot (*Melilotus* spp.), and sweet clover (*Fabaceae* spp.). Coumarin are also found at high levels in some essential oils, particularly cinnamon bark oil (7000 ppm), cassia leaf oil (up to 87,300 ppm) and lavender oil. Coumarin is also found in fruits (e.g. bilberry, cloudberry), green tea and other foods such as chicory. Coumarin has a sweet odor, readily recognised as the scent of newly-mown hay, and has been used in perfumes since 1882. It has been used as an aroma enhancer in pipe tobaccos and certain alcoholic drinks, although in general it is banned as a flavorant food additive, due to concerns about hepatotoxicity coumarin causes in animal models. Coumarin is moderately toxic to the liver and kidneys, with a "Median Lethal Dose" (LD_{50}) of 275 mg/kg. Although only somewhat dangerous to humans, coumarin is a potent rodenticide: Rats and other rodents metabolize it largely to 3,4-coumarin epoxide, a toxic compound that can cause internal hemorrhage and death. Humans metabolize it largely to 7-hydroxycoumarin, a compound of lower toxicity. The German Federal Institute for Risk Assessment has established a "tolerable daily intake" (TDI) of 0.1 mg coumarin per kg body weight. For example, a person weighing 60 kg (about 132 lbs) would have a TDI of approximately 6.0 mg of coumarin.

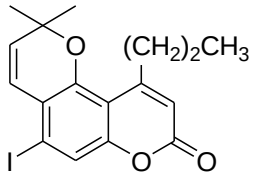
The diverse biological activities of natural and synthetic coumarin derivatives as anticoagulants and antithrombotics[1] are well known. Some of the coumarin derivatives are also reported as triplet sensitizers, anti-HIV,[2] anti-tuberculosis,[3] anti-angiogenesis,[4] antimicrobial,[5] anti-

hepatitis,[6] lipid-lowering agents and as antioxidants. They have also been found to inhibit lipid peroxidation and to possess vasorelaxant, anti-inflammatory,[7] anticancer[8,9] anti-influenza[10] and anti-alzheimer[11,12] activity. Many coumarin derivatives are also known as free radical scavengers and few naturally occurring coumarins have been found to exhibit cytotoxicity against a panel of mammalian cancer cell lines. They are frequently used as intermediates in the production of dyes and herbicides.

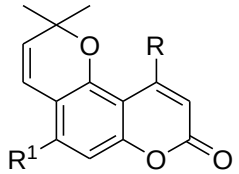
Cancer, a diverse group of diseases characterized by uncontrolled growth of abnormal cells, is a major worldwide problem. Cancer is projected as the primary cause of deaths in future. Breast cancer, a leading pandemic, affects women of all ages. In hormone dependent breast carcinoma, estrogens play a critical role, stimulating cancer cell proliferation. Currently, tamoxifen is most commonly used adjuvant drug for estrogen receptor (ER)-positive breast cancer, which competes with estrogen and downregulates estrogenic actions in breast cancer, however, it is less effective in ER-negative breast cancer, and its safety is also controversial. Therefore, there is a strong impetus to identify new anti-breast cancer agents with improved activity and reduced side effects. Compounds **1**-**6** have potent anti-breast cancer activity.



1 R = phenyl, (IC_{50} = 25.0 µM)
2 R = methyl, (IC_{50} = 18.3 µM)

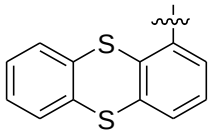


3 (IC_{50} = 13.7 µM)

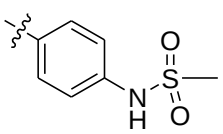


4 R = phenyl, R^1 = A, (IC_{50} = 7.2 µM)
5 R = phenyl, R^1 = B, (IC_{50} = 5.3 µM)
6 R = propyl, R^1 = B, (IC_{50} = 3.3µM)

R^1 =



A

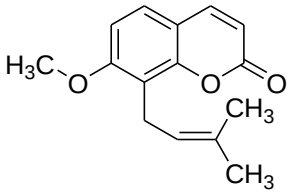


B

The antiproliferative activities of compounds **4**-**6** were better than those of **1**-**3**, suggesting that the incorporation of additional heterocyclic moieties viz. a viz thianthren and sulfamate moieties[13] in conjugation with the basic structure of hydroxycoumarin and pyranocoumarin could increase antiproliferative activity in breast cancer cell lines MCF-7.

Osthole – a coumarin derivative (**7**), an ingredient of Traditional Chinese Medicine (TCM) from natural product *Cnidium monnieri* (L.) *Cusson*, demonstrated some estrogenic activity by

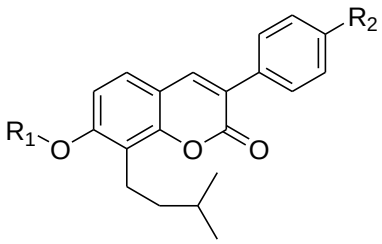
preventing the synthesis and action of estrogens (ER antagonists) which indicated that osthole has the potential to become breast cancer treatment reagents. A series of osthole derivatives[14] bearing aryl substituents at 3-position of coumarin, has been prepared and evaluated for their growth inhibitory activity against human breast cancer cell lines MCF-7 and MDA-MB-231.



Osthole **(7)**

MCF-7 (IC_{50} = 25.8 µM)

MDA-MB-231 (IC_{50} = 30.2 µM)

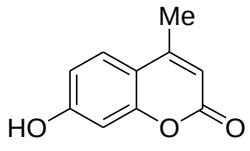


8 $R_1 = CH_3$, $R_2 = OCF_3$

9 $R_1 = CH_3$, $R_2 = Cl$

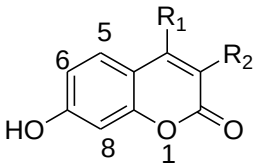
Compound **8** (MCF-7, IC_{50} = 0.24 µM and MDA-MB-231, IC_{50} = 0.31µM) was more potent as its cytotoxic activity improved to 100-fold as compared to parent **7**. Compound **9 (**MCF-7, IC_{50} = 1.27 µM and MDA-MB-231, IC_{50} = 5.23 µM) bearing *p*-chloro phenyl substituent at 3-position has 20-fold higher antitumor activity as compared to **7**.

Coumarin has also been used as 17β-hydroxysteroid dehydrogenase type 3 inhibitors (HSD 3 inhibitors) for the treatment of prostrate cancer. The androgens testosterone (T) and dihydrotestosterone (DHT) are hormones that play an important role in the development of prostate cancer. The regulation of androgen biosynthesis or its action on the androgen receptor is central to the management of prostate cancer.



10

HSD IC_{50} = 1.0 µM



11 $R_1 = CF_3$, $R_2 = CH_2Ph$, (HSD IC_{50} = 0.03µM)

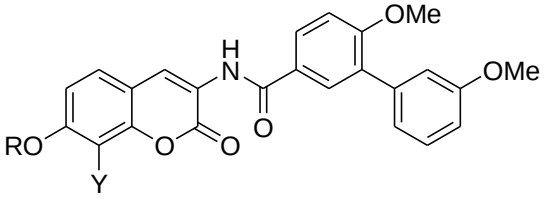
12 R_1 = Me, R_2 = Cl, (HSD IC_{50} = 0.10 µM)

13 R_1 = Me, $R_2 = CH_2Ph$, (HSD IC_{50} = 0.20 µM)

14 $R_1 = CH_2S$-6-Methyl-2-pyridyl, R_2 = H, (HSD IC_{50} = 0.0015 µM)

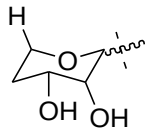
Introduction of an alkyl group at the 3-position of coumarin improved the activity.[15] 3-chloro derivative (**12**) was 10-fold more potent than **10**. Moreover, 4-trifluoromethyl analog (**11**) was found to be favorable and resulted in a seven fold increase in potency as compared to **13**. 6-Methyl-2-pyridyl derivative (**14**) was most potent 17β-HSD3 inhibitor among the other alkyl substituted derivatives.

The heat-shock protein 90 (Hsp90) molecular chaperone has emerged as a promising target for the treatment of cancer. Hsp90 serves to modulate multiple oncogenic pathways. There are more than 150 client proteins dependent upon Hsp90 for their folding and conformational maintenance, many of which contribute to cancer cell proliferation and growth.

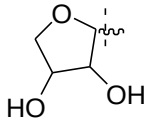


15-19

R =



A



B

15 R = A, Anomer = β, Y = CH_3, IC_{50} (SKBr3 = 6.2 µM, MCF-7 = 2.5 µM)

16 R = A, Anomer = α, Y = CH_3, IC_{50} (SKBr3 = 6.7 µM, MCF-7 = 42.5 µM)

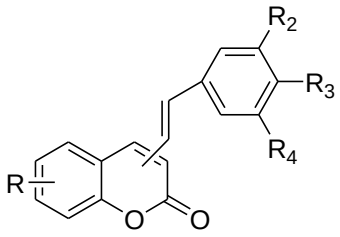
17 R = A, Anomer = β, Y = OCH_3, IC_{50} (SKBr3 = 1.4 µM, MCF-7 = 17.4 µM)

18 R = A, Anomer = α, Y = OCH_3, IC_{50} (SKBr3 = 11.1 µM, MCF-7 = 10.8 µM)

19 R = B, Anomer = β, Y = CH_3, IC_{50} (SKBr3 = 12.46 µM, MCF-7 = 37.17 µM)

Analogs containing six-membered pyranose moieties (scaffold A) (**15-18)** were found to be more active than that containg scaffold B (**19**). β epimer (**15** and **17)** exhibited better activity then its α counterparts (**16** and **18**). In these derivatives α and β anomer affects the biological activity of the compounds.[16]

Compounds **20-23** showed potent antiproliferative activity in human tumor cells[17] from different tumor cell types (lung carcinoma H460, squamous cell carcinoma A431 and melanoma JR8).



20-23

20 Insertion position = 4, R = H, $R_2 = OCH_3$, R_3 = H, $R_4 = OCH_3$, IC_{50} (H460 = 2.6 µM)

21 Insertion position = 4, R = 7-OCH_3, $R_2 = OCH_3$, R_3 = H, $R_4 = OCH_3$, IC_{50} (H460 = 0.45 µM, A431 = 3.4 µM, JR8 = 3.2 µM)

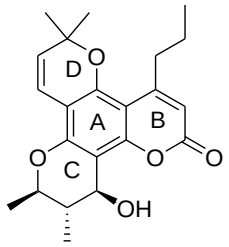
22 Insertion position = 3, R = 7-OCH_3, $R_2 = OCH_3$, R_3 = H, $R_4 = OCH_3$, IC_{50} (H460 = >10 µM)

23 Insertion position = 4, R = 7-OCH_3, $R_2 = CH_3$, R_3 = H, $R_4 = CH_3$, IC_{50} (H460 = 0.29 µM, A431 = 3.5 µM, JR8 = 3.5 µM)

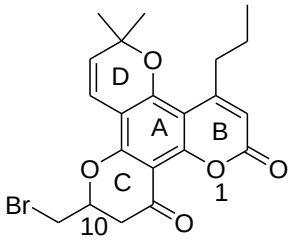
Compound **21** was 6-fold more active than **20**; this shows the importance of 7-methoxycoumarin nucleus as main backbone. Shifting the *trans*-3,5-dimethoxy-phenylvinyl moiety of derivative **21** from C-4 to C-3 position to yield compound **22**, shows a dramatic drop of potency, suggesting that the C-4 insertion position plays a pivotal role. Replacement of 3,5-dimethoxy groups on the *trans*- vinylbenzene moiety with methyl group leads to compound **23** which was even more potent than **21**.

Human immunodeficiency virus (HIV) is the cause of acquired immunodeficiency syndrome (AIDS). It was first reported in 1981. AIDS has spread rapidly through the human population worldwide. The development of chemotherapeutic strategies for AIDS has recently been considered one of the most challenging scientific projects. Highly active antiretroviral therapy (HAART) has been very effective in suppressing HIV load; these medications present several limitations such as the rapid emergence of drug resistant mutant strains due to the narrow range of chemical structure of the cocktail components. Because of the viral resistance and issues related to drug side effects, there remains a great need to discover novel antivirals, especially ones that function as non-nucleoside reverse transcriptase inhibitors (NNRTIs).

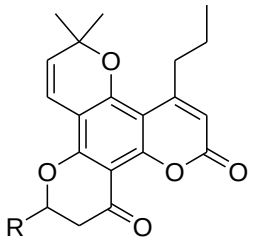
In 1992, Kashman reported (+)-calanolide A (**24**) isolated from tropical rain forest plant of the species Calophyllum lanigerum,[18] to be active against HIV-1, with an EC_{50} of 0.1µM.



24 (EC_{50} = 0.1 µM)



25 (EC_{50} = 0.0028 µM)



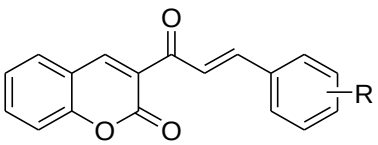
26 R = CH_2Cl (EC_{50} = 0.0074 µM)

27 R = CF_3 (EC_{50} = 14.9 µM)

(+)-Calanolide A (**24**) was previously described as the first natural product that inhibits HIV-1 reverse transcriptase. It inhibits reverse transcriptase by a mechanism involving at least two binding sites, which are distinguished as having competitive and uncompetitive components. (+)-Calanolide A is unique in inhibiting HIV-1 isolates carrying the viral reverse transcriptase Y181C amino acid mutation, which is associated with high level resistance to most current NNRTIs. Through SARs it was found that compound **25** having Br at position C-10 of ring C have significantly enhanced antiviral potency, thus further modifications were done on the C ring to find better anti-HIV activities.

Bromomethylation **(25)** and Chloromethylation (**26)** of 12-Oxo-calanolide A gave better results, this shows that penetration ability of cell membrane is the key factor because, fluorine atom is able to improve the binding ability of small molecule through the mimic of hydrogen atom, while chlorine atom could help a small molecule passing through a cell membrane with its hydrophobic property. Furthermore, the much stronger electron-withdrawing ability of CF_3 group than that of CH_2Br and CH_2Cl might be another reason that it significantly decreased the electronic interaction between **27** and HIV-1 reverse transcriptase.

Malaria, a major cause of morbidity and mortality is one of the foremost health and developmental challenges facing mankind. Following compounds **28-31** show significant antimalarial activity against the chloroquine-sensitive (3D7) strain and also against chloroquine-resistant (RKL9) field isolate of *P.falciparum.*



28-31

28 R = 3,4,5-TriCH_3O-, IC_{50}^{3D7} = 3.1 µM, IC_{50}^{RKL9} = 1.9 µM

29 R = 3-MeO-4H, IC_{50}^{3D7} = 11 µM, IC_{50}^{RKL9} = 4.9 µM

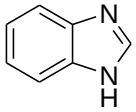
30 R = 3,4-DiMeO, IC_{50}^{3D7} = 26.7 µM, IC_{50}^{RKL9} = not tested

31 R = 2,3,4-TriMeO-, IC_{50}^{3D7} = 3.1 µM, IC_{50}^{RKL9} = 1.1 µM

The (E)-3-(3-(2,3,4-trimethoxyphenyl)-acryloyl)-2H-chromen-2-one (**31**) turned out to be the most potent analog of the series, showing IC_{50} of 3.1 µg/ml against chloroquine-sensitive (3D7) strain and IC_{50} of 1.1 µg/ml against chloroquine-resistant field isolate (RKL9) of Plasmodium falciparum.[19] The high activity of trimethoxy derivative (**28** and **31**) is due the influence of hydrophobicity and steric bulk on the ring. Among the disubstituted compounds, the monohydroxylated compound **29** is more active than dimethoxy derivative **30**.

2. Benzimidazole and its biological application

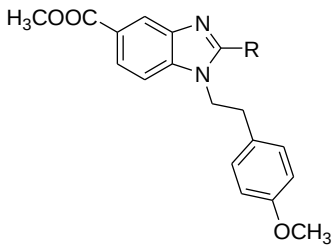
Benzimidazole is a heterocyclic aromatic organic compound. This bicyclic compound consists of the fusion of benzene and imidazole. The most prominent benzimidazole compound in nature is *N*-ribosyl-dimethylbenzimidazole, which serves as an axial ligand for cobalt in vitamin B_{12}.



Benzimidazole {1H-benzo[d]imidazole}

Benzimidazole moiety exists in many biologically active natural products and synthetic compounds. Substituted benzimidazole derivatives have found applications in diverse therapeutical areas including antimicrobial, antioxidant, anti-HIV,[20] antiprotozoal, anti-inflammatory and molluscicidal agents. Furtheremore, benzimidazoles showed anticancer activity against breast cancer, leukemia,[21] and colon cancer cell lines. Some of them also possess potent antiviral activities.

Among different types of cancers, leukemia is one of the major causes of cancer related deaths. Leukemia originates from hematopoietic stem cells or cells at different stages of myeloid or erythroid differentiation which spread throughout the body. Although, the success of clinical trials in identifying new agents and treatment modalities has been significant, current treatments suffer from many limitations such as side effect of the drugs and drug resistance. Hence, the identification of novel, efficient and less toxic anticancer agents remains an important and challenging task in cancer biology. The compound **32** induced maximum inhibition on human leukemic cell lines (K562 and CEM) with an IC_{50} value of 3 µM and 4 µM.



32-34

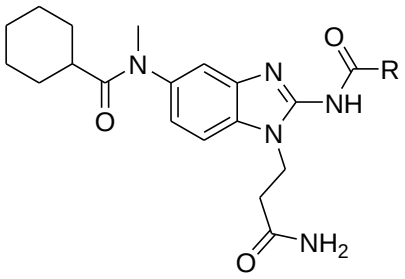
32 R = 4-fluro-3-nitrophenyl, IC_{50} (K562 = 3 µM, CEM = 4 µM)

33 R = 2-flurophenyl, IC_{50} (K562 = 56 µM, CEM = 55 µM)

34 R = 4-fluro-3-aminophenyl, IC_{50} (K562 = 78 µM, CEM = 75 µM)

Compound **32** having a fluoro at the *para* position and a nitro at *meta* position exhibit growth inhibitory activity at an IC_{50} value of 3µM (K562), whereas replacement of the same with only ortho fluoro group (**33**) and nitro group with amine group (**34**) decrease the activity by 19 and 25 fold.

IL-2 inducible T-cell kinase (Itk) is a key member of the Tec kinase family and a number of factors point to the importance of this kinase in immune disease. Deletion of Itk in mice results in reduced TCR-induced proliferation and reduced secretion of the cytokines IL-2, IL-4, IL-5, IL-10 and IFN-γ.[22]



35-43

35 R = 2-Thiophene, ItK IC_{50} = 0.04 µM

36 R = 3-Pyridyl, ItK IC_{50} = 0.005 µM

37 R = 2-Furyl, ItK IC_{50} = 0.01 µM

38 R = C_6H_4(*o*-CH_3), ItK IC_{50} = 3.3 µM

39 R = C_6H_4(*m*-CH_3), ItK IC_{50} = 0.05 µM

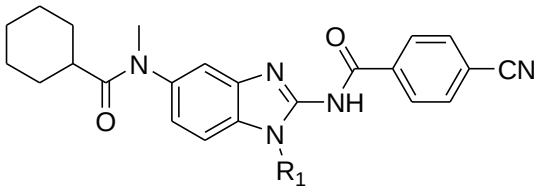
40 R = C_6H_4(*p*-CH_3), ItK IC_{50} = 0.03 µM

41 R = C_6H_4(*p*-Br), ItK IC_{50} = 0.003 µM

42 R = C_6H_4(*p*-OCH_3), ItK IC_{50} = 0.01 µM

43 R = C_6H_4(*p*-COOH), ItK IC_{50} = 4.5 µM

Replacement of R substituent from 2-thiophene (**35**) with 2-furyl (**37**) or 3-pyridyl (**36**) resulted in an improvement in activity against ItK. *ortho*- substitution (**38**) reduces while substitution at *meta* (**39**) and *para* - positions (**40**) increases the activity. Small electron-donating (**42**) and electron-withdrawing groups (**41**) show little difference in ItK inhibitory activity. Carboxylic acid substitution (**43**) at *para*-position was also not tolerated leading to a >100 fold loss in activity relative to compound **35.**



44-50

44 $R_1 = CH_3$, ItK IC_{50} = 0.27 µM

45 $R_1 = (CH_2)_3CH_3$, ItK IC_{50} = 0.01µM

46 $R_1 = (CH_2)_6CH_3$, ItK IC_{50} = 4.5 µM

47 $R_1 = CH_2CH_2C_6H_5$, ItK IC_{50} = 0.006 µM

48 $R_1 = CH_2CH_2C_6H_4(p\text{-}OCH_3)$, ItK IC_{50} = 0.08 µM

49 $R_1 = CH_2CH_2C_6H_4(o\text{-}CH_3)$, ItK IC_{50} = 0.6 µM

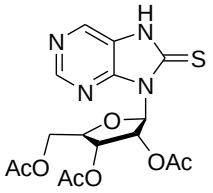
50 $R_1 = CH_2CH_2C_6H_3$(2,4-di-Cl), ItK IC_{50} = 2.0 µM

Substitution of the N-1 with linear alkyl group leads improvement in potency going from methyl (**44**) to a butyl (**45**) but extension of the chain to heptyl (**46**) results in 17-fold loss in activity relative to compound **44**. Presence of a phenethyl group (**47**) leads to improvement in potency, however substitution of phenyl ring (**48-50**) was not tolerated and leads to loss in activity.

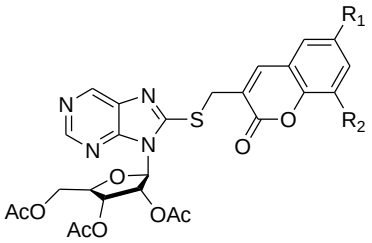
3. Coumarin - Benzimidazole hybrids and their biological application

Hybrid molecules are formed by the combination of two different moieties. The combination of features of two biological active moieties lead to more biological activity. As predicted from literature both coumarin and benzimidale moieties has a high potency for the biological activity, but recently a few examples where coumarin moiety has been based in conjugation with some heterocyclic moieties as anticancer, anti-HIV, anti-hepatitis[23] and anti-angiogenesis has been reported. Recently, a new scenario of synthesizing the hybrid compounds by combining the different heterocyclic moieties has been emerged.

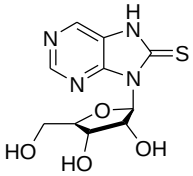
The attachment of a coumarin moiety to the purine ribofuranoside is the key to gain appealing anti-hepatitis C virus (anti-HCV) activity. Compound **51** and **53** does not show significant anti-HCV activity, but on attachment with coumarin moiety they exhibit potent HCV inhibition (**52** and **54**). On introducing various substitutents with electron-withdrawing (F, Cl, Br) and electron-donating (OMe) substitutents onto the coumarin moiety, the order of anti-HCV activity does not affect much (**52a, b, d, e** and **54a, c**).



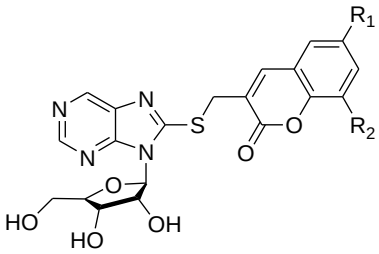
51



52



53

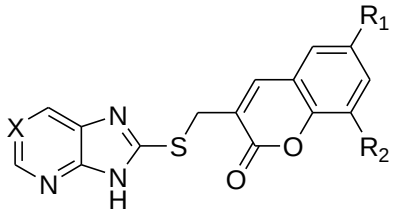


54

	a	b	c	d	e
R_1	H	F	Cl	Br	H
R_2	H	H	H	H	OMe

S.NO	EC_{50} (µM)
51	>122
52a	6.6
52b	6.1
52e	2.3
52d	4.9
53	>176
54a	5.5
54c	4.3

Following compounds **55-58** also exhibit potent anti-HCV activity.



55-58

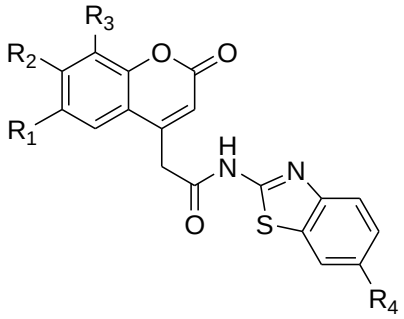
55 X = CH, R_1 = H, R_2 = H, HCV EC_{50} = 59 µM

56 X = CH, R_1 = H, R_2 = OMe, HCV EC_{50} = 11 µM

57 X = CH, R_1 = Br, R_2 = H, HCV EC_{50} = 6.8 µM

58 X = N, R_1 = H, R_2 = H, HCV EC_{50} = 2.0 µM

Replacement of a carbon atom with a nitrogen atom in the imidazopyridine nucleus of the conjugated coumarins increased the HCV inhibition by a factor of 29-fold (cf. **55** vs **58**). Placement of a Br- substituent onto the coumarin nucleus of its imidazopyridine conjugate (cf. **55** vs **57**) enhanced the HCV inhibition by a factor of 8.7-fold. Placement of an MeO- substituent on the coumarin nucleus of its imidazopyridine conjugate (cf. **55** vs **56**) improved the antiviral activity by a factor of 5.4-fold.



59-62

59 R_1 = H, R_2 = OH, R_3 = H, R_4 = NO_2, EC_{50} = 8 µg/mL

60 R_1 = H, R_2 = OH, R_3 = H, R_4 = Cl, EC_{50} = 7 µg/mL

61 R_1 = CH_3, R_2 = H, R_3 = CH_3, R_4 = NO_2, EC_{50} = 100 µg/mL

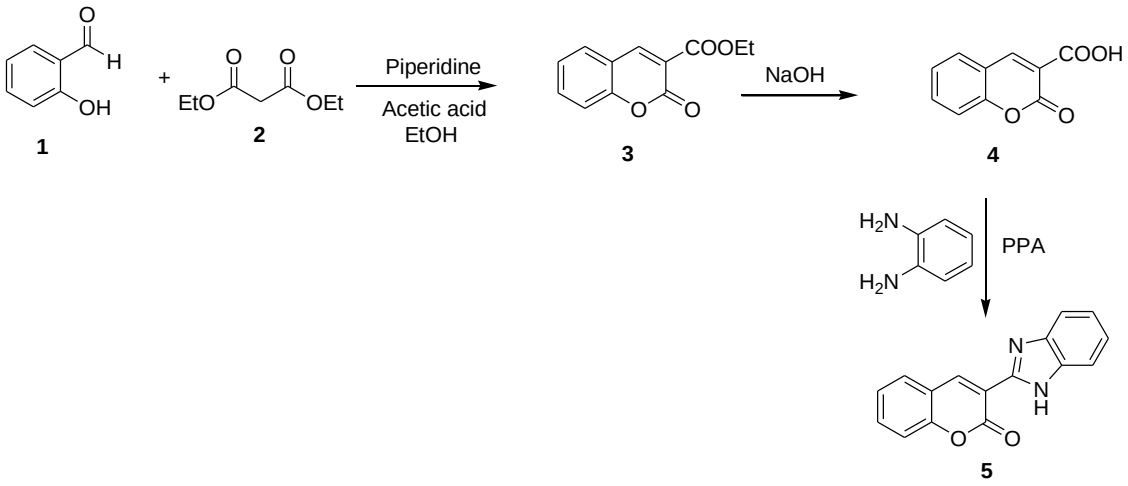
62 R_1 = H, R_2 = R_3 = -Benzo-, R_4 = OCH_3, EC_{50} = 100 µg/mL

On attachment of coumarin moiety with benzothiazole, acts as anti-HIV agents, electron-withdrawing substitutent on the coumarin moiety greatly affect the biological activity while substitution of electron-withdrawing or donating group at benzimidazole moiety does not affect the biological activity. OH substitution increases the potency (**59-60**), while methyl substitution at R_1 and R_3 lowers the activity **(61),** this shows that bulky groups at R_1 and R_3 are not favorable and this explains the inactivity of compound **62** having benzo ring attached to R_2 and R_3 position. Literature reports prove that coumarin moieties when combined with heterocyclic moieties exhibit potent biological activity, thus we designed hybrid molecules formed by the combination of some of the structural features of coumarin and heterocyclic moiety viz. benzimidazole.

RESULTS AND DISCUSSION

As coumarin moieties are used for anticancer, anti-HIV, antihepatitis, antimalarial, antiprotozoal agents. Similarly, benzimidazole moieties have also been used for anticancer, antiangiogenesis, anti-influenza, antiprotozoal drugs. Both coumarin and benzimidazole are biological active moieties. Their hybrid molecules by combining some of the structural features of both coumarin and benzimidazole moieties are expected to exhibit more potent biological properties. So, in the present research programme, differently substituted coumarin-benzimidazole hybrid moieties has been synthesized and evaluated for their *in vitro* anticancer activities (**Scheme 1-3**)

1. Synthesis of 3-(*1H*-benzo[d]imidazol-2-yl)-*2H*-chromen-2-one (5)

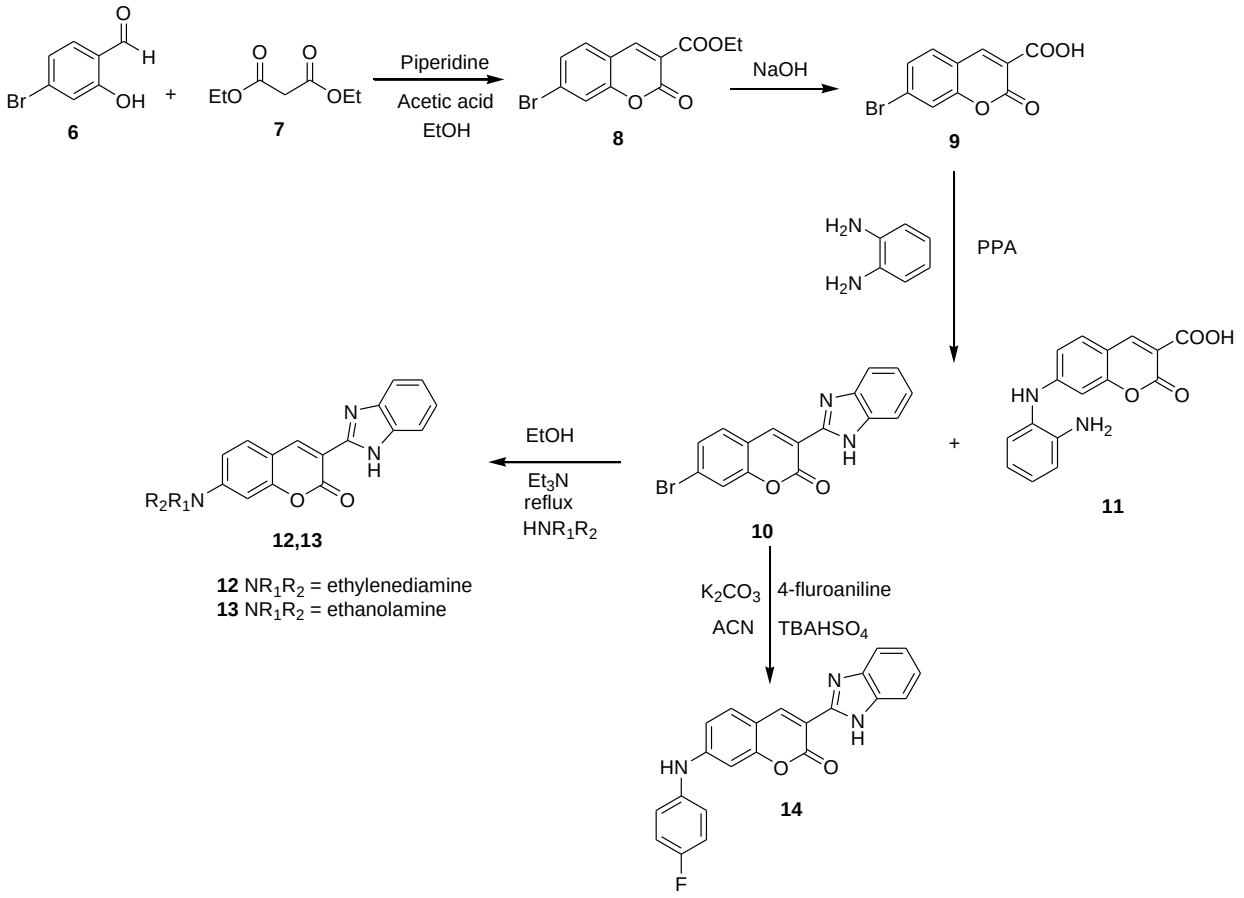


Scheme-1

3-(*1H*-benzo[d]imidazol-2-yl)-*2H*-chromen-2-one (**5**) was synthesized according to **scheme-1**. A solution of salicyaldehyde (**1**) and 2 eq. of diethylmalonate (**2**) in EtOH was refluxed with piperidine and glacial acetic acid for 3 hrs. After the completion of reaction (monitored by TLC), 100 ml of water was added and cooled to 0 °C. The crystalline solid washed with 50% cold ethanol. White fine powder ester of ethyl 2-oxo-*2H*-chromene-3-carboxylate (**3**) (yield = 88%); mp 82-84 °C (lit. value[24] 92-94 °C) was obtained. Ester (**3**) was refluxed with EtOH and 0.5% NaOH for 3 hrs. Acidification to pH 2.0 using concentrated hydrochloric acid and cooling to 0 °C gave acid, 2-oxo-*2H*-chromene-3-carboxylic acid (**4**) as white crystalline deposit (yield = 74%); mp 180-182 °C (lit. value[24] 189-192 °C). Acid (**4**) was refluxed with 1eq. of *o*-phenylenediamine in polyphosphoric acid (PPA) for 38-40 hrs. After the completion of reaction

(monitored by TLC), 50 ml water and ammonia were added until the reaction mixture becomes neutral, filtered and washed with water. Impure product was purified by column chromatography to get yellow fine powder of 3-(*1H*-benzo[d]imidazol-2-yl)-*2H*-chromen-2-one (**5**) (yield = 50%); mp > 250 °C. ^{1}H NMR spectrum of compound **5** shows 1H broad singlet at δ 12.49 for NH, 1H singlet at δ 9.12 for CH, 1H double doublet at δ 7.93 for aromatic-H, 3H multiplet at δ 7.67 for aromatic-H, 1H doublet at δ 7.46 for aromatic-H, 1H triplet at δ 7.40 for aromatic-H, and 2H multiplet at δ 7.21 for aromatic-H. NMR spectra confirmed the structure of 3-(*1H*-benzo[d]imidazol-2-yl)-*2H*-chromen-2-one.

2. Synthesis of 3-(*1H*-benzo[d]imidazol-2-yl)-*2H*-chromen-2-one analogs (12-14)



Scheme-2

3-(*1H*-benzo[d]imidazol-2-yl)-*2H*-chromen-2-one analogs (**12-14**) were synthesized by **scheme-2**. Bromosalicylaldehyde (**6**) and 2 eq. of diethylmalonate (**7**) in EtOH was refluxed with piperidine and glacial acetic acid for 3 hours. After the completion of reaction (monitored by TLC), 100 ml of water was added and cooled to 0 °C. The crude solid was filtered and washed

with 50% cold ethanol to get cream colored fine powder of ethyl 7-bromo-2-oxo-*2H*-chromene-3-carboxylate (**8**) (yield = 86%); mp 148-150 °C. Ester (**8**) was refluxed with EtOH and 0.5% NaOH for 2 hrs. Acidification to pH 2 using conc. HCl and cooling to 0 °C gave crystalline white powder of 7-bromo-2-oxo-*2H*-chromene-3-carboxylic acid (**9**) (yield = 63%); mp 178-180 °C. Acid (**9**) was refluxed with 1 eq. of *o*-phenylenediamine in polyphosphoric acid (PPA) for 38-40 hrs. After the completion of reaction (monitored by TLC), 50 ml water and ammonia were added until the reaction mixture becomes neutral, filtered and washed with water to obtain two products which were separated by column chromatography as florescent yellow powder of 3-(*1H*-benzo[d]imidazol-2-yl)-7-bromo-*2H*-chromen-2-one (**10**) (yield = 62 %); mp > 250 °C and dark yellow powder of 7-(2-aminophenylamino)-2-oxo-*2H*-chromene-3-carboxylic acid (**11**) (yield = 31%); mp > 250 °C. ^{1}H NMR spectrum of compound **10** shows 1H broad singlet at δ 12.52 for NH, 1H singlet at δ 9.11 for CH, 1H triplet at δ 8.20 for aromatic-H, 1H double doublet at δ 7.80 for aromatic-H, 2H multiplet at δ 7.69 for aromatic-H, 1H doublet at δ 7.45 for aromatic-H and 2H multiplet at δ 7.21 for aromatic-H. ^{13}C NMR spectrum shows peaks at δ 158.69, 152.16, 145.18, 142.81, 140.75, 134.95, 134.79, 131.27, 122.78, 122.11, 120.79, 118.47, 118.17, 117.67, 116.73, 112.75. NMR spectra confirmed the structure of 3-(*1H*-benzo[d]imidazol-2-yl)-7-bromo-*2H*-chromen-2-one (**10**).

^{1}H NMR spectrum of compound **11** shows 1H singlet at δ 10.19 for NH, 1H singlet at δ 8.95 for CH, 1H doublet at δ 8.14 for aromatic-H, 1H double doublet at δ 7.83 for aromatic-H, 1H double doublet at δ 7.53 for aromatic-H, 1H doublet at δ 7.43 for aromatic-H, 1H triplet at δ 6.98 for aromatic-H, 1H double doublet at δ 6.85 for aromatic-H, 1H triplet at δ 6.68 for aromatic-H and 2H singlet at δ 4.64 for NH_2. ^{13}C NMR spectrum shows peaks at δ 158.67, 152.05, 146.57, 145.01, 140.50, 134.89, 131.91, 130.99, 122.80, 122.11, 118.41, 117.98, 117.60, 117.21, 116.92, 112.60. NMR spectra confirmed the structure of 7-(2-aminophenylamino)-2-oxo-*2H*-chromene-3-carboxylic acid (**11**).

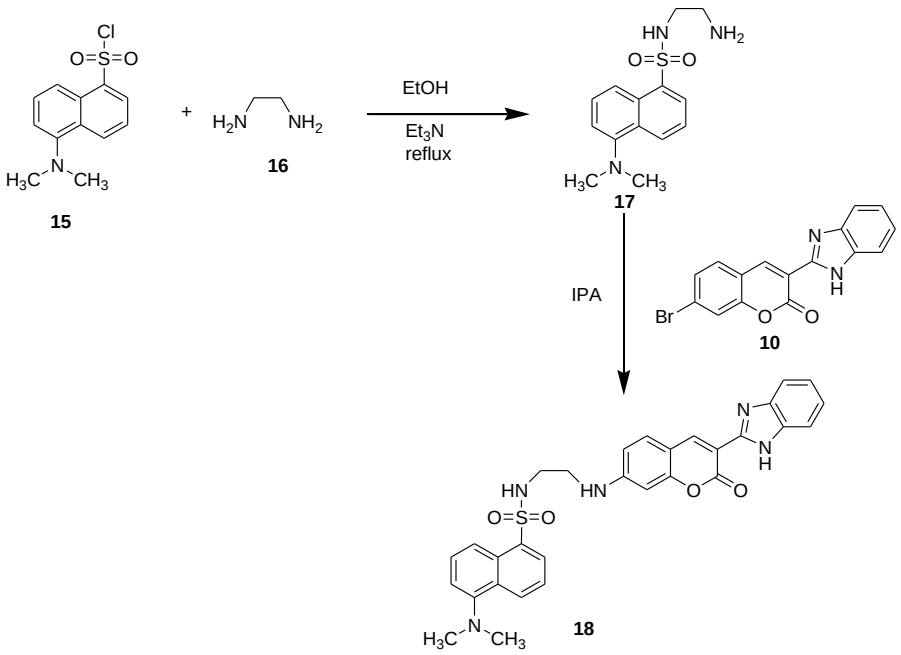
3-(*1H*-benzo[d]imidazol-2-yl)-7-bromo-*2H*-chromen-2-one (**10**) was reacted with primary aliphatic/aromatic amines to obtain compounds **12-14 (Scheme-2)**. To synthesize 7-(2-aminoethylamino)-3-(*1H*-benzo[d]imidazol-2-yl)-*2H*-chromen-2-one (**12)**, compound **10** was refluxed with excess of ethylenediamine using triethylamine as base for 6-8 hrs. After completion of the reaction (monitored by TLC), the reaction mixture was extracted with

chloroform, dried over anhydrous sodium sulphate, filtered and concentrated to get dark brown crystals (**12**) (yield = 77.41%); mp 138-140°C. ^{1}H NMR spectrum shows 1H singlet at δ 8.46 for CH, 1H singlet at δ 7.93 for aromatic-H, 1H singlet at δ 7.49 for aromatic-H, 1H singlet at δ 7.47 for NH, 2H multiplet at δ 7.39 for aromatic-H, 1H multiplet at δ 7.14 for aromatic-H, 2H multiplet at δ 6.82 for aromatic-H, 2H singlet at δ 3.82 for NH_2, 2H triplet at δ 3.73 for CH_2 and 2H triplet at δ 3.59 for CH_2. ^{13}C NMR spectrum shows peaks at δ 165.46, 159.75, 134.48, 133.29, 119.92, 118.64, 109.20, 58.72. NMR spectra confirmed the structure of 7-(2-aminoethylamino)-3-(*1H*-benzo[d]imidazol-2-yl)-*2H*-chromen-2-one (**12**).

For the synthesis of 3-(*1H*-benzo[d]imidazol-2-yl)-7-(2-hydroxyethylamino)-*2H*-chromen-2-one (**13**), compound **10** was refluxed with ethanolamine using triethylamine as base for 6-8 hrs. After completion of the reaction (monitored by TLC), the reaction mixture was extracted with chloroform, dried over anhydrous sodium sulphate, filtered and concentrated to get brown semisolid product (**13**) (yield = 79%). ^{1}H NMR spectrum shows 1H singlet at δ 8.89 for CH, 1H singlet at δ 7.99 for CH, 2H doublet at δ 7.50 for aromatic-H, 2H double doublet at δ 7.37 for aromatic-H, 1H multiplet at δ 7.13 for aromatic-H, 1H doublet at δ 6.80 for aromatic-H, 2H multiplet at δ 3.79 for CH_2, 1H singlet at δ 3.69 for NH, 2H multiplet at δ 3.58 for CH_2 and 1H singlet at δ 2.75 for OH. ^{13}C NMR spectrum shows peaks at δ 164.81, 160.27, 134.13, 133.03, 121.12, 119.80, 118.61, 108.69, 60.81, 60.33. NMR spectra confirmed the structure of 3-(*1H*-benzo[d]imidazol-2-yl)-7-(2-hydroxyethylamino)-*2H*-chromen-2-one (**13**).

To synthesize 3-(*1H*-benzo[d]imidazol-2-yl)-7-(4-fluorophenylamino)-*2H*-chromen-2-one (**14)**, compound **10** was refluxed with 4-fluoroaniline, 1.2eq. K_2CO_3 as base and $TBAHSO_4$ as catalyst in acetonitrile for 4-5 hrs. After completion of reaction (monitored by TLC), the reaction mixture was extracted with chloroform, dried over anhydrous sodium sulphate, filtered and concentrated to get impure product which was purified by column chromatography to get brown colored semisolid product **14**. (yield = 74%). ^{1}H NMR spectrum of this compound shows 1H singlet at δ 9.05 for CH, 1H doublet at δ 7.83 for aromatic-H, 1H double doublet at δ 7.74 for aromatic-H, 2H multiplet at δ 7.54 for aromatic-H, 3H multiplet at δ 7.38 for aromatic-H, 2H multiplet at δ 7.14 for aromatic-H, 2H multiplet at δ 7.06 for aromatic-H and 1H singlet at δ 6.51 for NH. NMR spectra confirmed the structure of 3-(*1H*-benzo[d]imidazol-2-yl)-7-(4-fluorophenylamino)-*2H*-chromen-2-one (**14**).

3. Synthesis of N-(2-3-(*1H*-benzo[d]imidazol-2-yl)-2-oxo-*2H*-chromen-7-ylamino) ethyl)-5-(dimethylamino)naphthalene-1-sulfonamide (18)



Scheme-3

N-(2-3-(*1H*-benzo[d]imidazol-2-yl)-2-oxo-*2H*-chromen-7-ylamino)ethyl)-5-(dimethylamino) naphthalene-1-sulfonamide (**18**) was synthesized as shown in **scheme-3.** Dansyl chloride (**15**) in EtOH was refluxed with excess of ethylenediamine (**16**) using triethylamine as base for 7-8 hrs. After completion of reaction (monitored by TLC), the reaction mixture was extracted with chloroform, dried over anhydrous sodium sulphate, filtered and concentrated to get orange crystals of N-(2-aminoethyl)-5-(dimethylamino)naphthalene-1-sulfonamide (**17**) (yield = 74%). This compound was used for next reaction without further purification. Compound **17** was refluxed with 3-(*1H*-benzo[d]imidazol-2-yl)-7-bromo-*2H*-chromen-2-one (**10**) in IPA for 7-8 hrs. Impure product was obtained and purified by column chromatography to get dark brown crystals of N-(2-3-(*1H*-benzo[d]imidazol-2-yl)-2-oxo-*2H*-chromen-7-ylamino)ethyl)-5-(dimethylamino) naphthalene-1-sulfonamide (**18**) (Yield = 60%); mp 170-175°C. ^{1}H NMR spectrum of compound **18** shows 2H doublet at δ 8.41 for aromatic-H, 2H doublet at δ 8.22 for aromatic-H, 1H singlet at δ 8.17 for aromatic-H, 2H doublet at δ 8.11 for aromatic-H, 1H multiplet at δ 7.53 for aromatic-H, 3H multiplet at δ 7.37 for aromatic-H, 2H doublet at δ 7.03 for aromatic-H, 2H singlets at δ 4.06 and δ 4.00 for 2xNH, 2H triplet at δ 3.97 for CH_2, 2H triplet at δ 2.71 for CH_2 and 6H

singlet at δ 2.67 for N-CH_3. NMR spectra confirmed the structure of N-(2-3-(*1H*-benzo[d]imidazol-2-yl)-2-oxo-*2H*-chromen-7-ylamino)ethyl)-5-(dimethylamino) naphthalene-1-sulfonamide (**18**).

4. Preliminary anti-tumor activities:

The compounds **10** and **12** were selected by National Cancer Institute, Bethesda, Maryland, USA on the basis of degree of the structure variation and computer modeling techniques for evaluation of their antineoplastic activity. The selected compounds were subjected to *in vitro* anticancer assay against tumor cells in a full panel of 60-cell lines taken from nine different organs (lung, colon, breast, ovary, blood, kidney, skin, prostrate and brain). The compounds were tested at a single dose concentration of 10 µM, and the percentages of growth inhibition over sixty tested cell lines were determined and the results were compiled **(Table-1).**

Preliminary *in vitro* antitumor screening was revealed that compound **10** and **12** show moderate inhibition for the most cancer cell lines. The percentages of inhibition for cancer cells were more than 20% in a number of the tested derivatives. Significant inhibition was observed for breast cancer (13.21-33.98%), prostate cancer (14.98-23.65%), CNS cancer (5.75-22.87%), leukemia (5.5-21.5%), non-small cell lung cancer (5.11-20.59%), colon cancer (5.36-20.58%) and renal cancer (5.33-22.67%).

Regarding the activity towards individual cell lines; Compound **10** showed selective potency against breast cancer cells MCF-7, MDA-MB-231/ATCC, HS 578T, T-47D with GI values of 29.9%, 26%, 25.08% and 28.42% respectively and compound **12** exhibited selective potency against MDA-MB-231/ATCC with GI value of 33.98%. Regarding prostate cancer, compound **10** showed selective potency against DU-145 with GI value of 23.65% and compound **12** exhibited selective potency against PC-3 with GI value of 20.28%. Compound **10** showed selective potency against renal cancer cells UO-31 with GI value of 22.67%. In addition, compound **10** proved active against CNS cancer cells SNB-75 with GI value of 22.87%. Compound **12** showed selective potency against colon cancer cells HCT-15 with GI value of 20.58%. Regarding non small cell lung cancer cells, compound **10** exhibited potency against HOP-92 and NCI-H522 with GI values of 20.11% and 20.59%. Compound **10** also exhibited selective potency against leukemia cells SR with GI value of 21.5%.

Table 1: Percentages growth inhibition of compounds **10** and **12** over the full panel of 60 tumor cell lines.

Cell line type	Cell line name	10	12
Leukemia	CCRF-CEM	-	18.09
	HL-60(TB)	-	19.92
	K-562	5.5	18.71
	MOLT-4	-	11.64
	RPMI-8226	9.75	-
	SR	**21.5**	13.76
Non-Small Cell Lung Cancer	A549/ATCC	-	-
	HOP-62	12.82	5.35
	HOP-92	**20.11**	17.34
	NCI-H226	5.11	12.62
	NCI-H23	15.53	9.34
	NCI-H322M	-	-
	NCI-H460	-	-
	NCI-H522	**20.59**	-
Colon Cancer	COLO 205	7.64	-
	HCC-2998	-	-
	HCT-116	5.36	6.13
	HCT-15	-	**20.58**
	HT29	-	-
	KM12	5.76	-
	SW-620	-	-
CNS Cancer	SF-268	-	7.73
	SF-295	8.68	-
	SF-539	11.57	5.75
	SNB-19	5.92	-
	SNB-75	**22.87**	14.9
	U251	-	5.11
Melanoma	LOX IMVI	12.21	15.01
	MALME-3M	-	-
	M14	-	-
	MDA-MB-435	-	-
	SK-MEL-2	-	-
	SK-MEL-28	-	6.31
	SK-MEL-5	8.32	6.5
	UACC-257	-	-
	UACC-62	7.33	12.6
Ovarian cancer	IGROV1	11.68	-
	OVCAR-3	-	-
	OVCAR-4	17.6	13.87
	OVCAR-5	-	-
	OVCAR-8	-	-
	NCI/ADR-RES	6.42	-
	SK-OV-3	-	-
Renal Cancer	786-0	-	-
	A498	-	5.33
	ACHN	-	-
	CAKI-1	-	-
	RXF 393	-	-
	SN 12C	-	7.82
	TK-10	-	-
	UO-31	**22.67**	18.25
Prostrate Cancer	PC-3	14.98	**20.28**
	DU-145	**23.65**	-
Breast Cancer	MCF7	**29.96**	19.11
	MDA-MB-231/ATCC	**26.00**	**33.98**
	HS 578T	**25.08**	8.37
	BT-549	13.21	15.09
	T-47D	**28.42**	17.76
	MDA-MB-468	13.81	5.1

-GI < 5%, compounds tested at one dose concentration (10 μM)

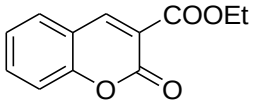
EXPERIMENTAL

All reactions were carried out in oven-dried glassware (120 °C). Bromosalicylaldehyde and Dansyl chloride were purchased from Sigma Aldrich and rest of the chemicals and solvents were purchased from Merck, Loba, SD Fine and Spectrochem. Melting points were obtained with Perfit India melting point apparatus and are uncorrected. Analytical thin layer chromatography (TLC) was performed on glass plates coated with silica gel purchased from Merck Inc. Purification was carried out by column chromatography using chloroform:methanol (80:20) as eluents and 60-120 mesh silica gel. ^{1}H NMR and ^{13}C NMR were obtained on a Bruker AC-400 (400 MHz) spectrometer with use of chloroform-*d* and dimethylsulfoxide-d_6 as solvents. Chemicals shifts were recorded in parts per million (ppm, δ) and were reported relative to the solvent peak or TMS. Multiplicities are recorded with the following abbreviations: s, singlet; d, doublet; dd, double doublet; t, triplet; q, quartet; m, multiplet; *J*, coupling constant (hertz).

General procedure for synthesis of compound 5

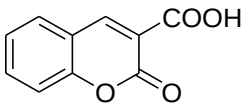
Ethyl 2-oxo-*2H*-chromene-3-carboxylate (3)

A solution of salicyaldehyde (5g, 40.94 mmol) and 2eq. of diethylmalonate (13.11 g, 81.85 mmol) in EtOH (60 ml) was treated with piperidine (0.5 ml), glacial acetic acid (2 drops) and refluxed for 3 hrs. After completion of reaction (monitored by TLC), 100 ml water was added to the reaction mixture and cooled to 0 °C. The crystalline solid was washed with 50% cold ethanol (20 mL). White fine powder of ethyl 2-oxo-*2H*-chromene-3-carboxylate (**3**) was obtained (7.9 g, 88%); mp 82-84 °C (lit. value[24] 92-94 °C).



2-Oxo-*2H*-chromene-3-carboxylic acid (4)

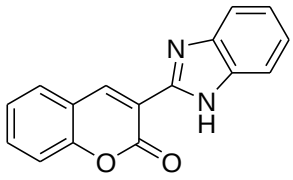
To a solution of ethyl 2-oxo-*2H*-chromene-3-carboxylate (**3**) (2 g, 9.16 mmol) in EtOH (40 ml), 200 ml 0.5% NaOH was added and refluxed for 3 hrs. Acidification to pH 2.0 using concentrated hydrochloric acid and cooling to 0 °C gave a white crystalline deposit of 2-Oxo-



2H-chromene-3-carboxylic acid (**4**) (1.3g, 74%); mp 180-182 °C (lit. value[24] 189-192 °C).

3-(*1H*-benzo[d]imidazol-2-yl)-*2H*-chromen-2-one (5)

2-Oxo-*2H*-chromene-3-carboxylic acid (**4**) (1 g, 5.2 mmol) was treated with 1eq. of

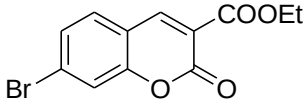


o-phenylenediamine (0.56 g, 5.2 mmol) in polyphosphoric acid (2-3 ml) and refluxed for 38-40 hrs. After completion of reaction (monitored by TLC), 50 ml water and ammonia was added until the reaction mixture becomes neutral, filtered and washed with water. Impure product was purified by column chromatography to obtain yellow colored fine powder of 3-(*1H*-benzo[d]imidazol-2-yl)-*2H*-chromen-2-one (**5**) (690 mg, 50%); mp > 250 °C; ^{1}H NMR (DMSO-d_6): δ 12.49 (bs, 1H, NH), 9.12 (s, 1H, CH), 7.93 (dd, 1H, ^{2}J = 6.28 Hz, ^{3}J = 1.48 Hz, ArH), 7.67 (m, 3H, ArH), 7.46 (d, 1H, J = 14.5 Hz, ArH), 7.40 (t, 1H, J = 7.5 Hz, ArH), 7.21 (m, 2H, ArH).

General procedures for the synthesis of compounds 12-14

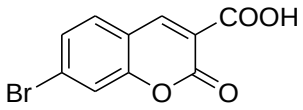
Ethyl 7-Bromo-2-oxo-*2H*-chromene-3-carboxylate (8)

A solution of bromosalicylaldehyde (2 g, 9.94 mmol) and 2 eq. of diethylmalonate (3.18 g, 19.8 mmol) in EtOH (60 ml) was refluxed using piperidine (0.5 ml) and glacial acetic acid (2 drops) for 3 hrs. After completion of reaction (monitored by TLC), 100 ml water was added to the reaction mixture and cooled to 0°C. The crystalline solid was filtered and washed with 50% cold ethanol (20 ml). Cream colored fine powder of Ethyl 7-Bromo-2-oxo-*2H*-chromene-3-carboxylate (**8**) was obtained (2.5 g, 86%); mp 148-150 °C.



7-Bromo-2-oxo-*2H*-chromene-3-carboxylic acid (9)

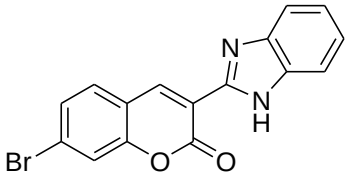
To a solution of Ethyl 7-Bromo-2-oxo-*2H*-chromene-3-carboxylate (**8**) (2 g, 6.7 mmol) in EtOH



(33.6 ml) was added 168.3 ml 0.5% NaOH and refluxed for 2 hrs. Acidification to pH 2 using concentrated hydrochloric acid and cooling to 0 °C gave a crystalline white fine powder of 7-Bromo-2-oxo-*2H*-chromene-3-carboxylic acid (**9**) (1.15 g, 63%); mp 178-180 °C.

3-(*1H*-benzo[d]imidazol-2-yl)-7-bromo-*2H*-chromen-2-one (10)

7-Bromo-2-oxo-*2H*-chromene-3-carboxylic acid (**9**) (1 g, 3.7 mmol) was refluxed with 1 eq. of

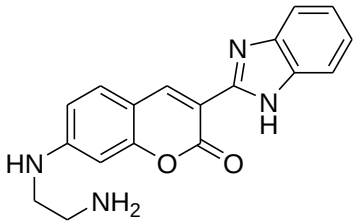


o-phenylenediamine (0.4 g, 3.7 mmol) in polyphosphoric acid (PPA), for 38-40 hrs. After completion of reaction (monitored by TLC), 50 ml water and ammonia were added until the reaction mixture becomes neutral, filtered and washed with water to obtain two products which were separated by column chromatography as light yellow powder of 3-(*1H*-benzo[d]imidazol-2-yl)-7-bromo-*2H*-chromen-2-one (**10**) and dark yellow powder of 7-(2-Aminophenylamino)-2-oxo-*2H*-chromene-3-carboxylic acid (**11**). For compound **10** (0.78 g, 62 %); mp > 250°C; ^{1}H NMR (DMSO-d_6): δ 12.52 (bs, 1H, NH), 9.11 (s, 1H, CH), 8.20 (t, 1H, J = 5.28 Hz, ArH), 7.80 (dd, 1H, ^{2}J = 6.44 Hz, ^{3}J = 2.36 Hz, ArH), 7.69 (m, 2H, ArH), 7.45 (d, 1H, J = 8.84 Hz, ArH), 7.21 (m, 2H, ArH); ^{13}C NMR (DMSO-d_6): δ 158.69, 152.16, 145.18, 142.81, 140.75, 134.95, 134.79, 131.27, 122.78, 122.11, 120.79, 118.47, 118.17, 117.67, 116.73, 112.75.

For compound **11** (0.35 g, 31%); mp > 250°C; ^{1}H NMR (DMSO-d_6): δ 10.19 (s, 1H, NH), 8.95 (s, 1H, CH), 8.14 (d, 1H, J = 2.32 Hz, ArH), 7.83 (dd, 1H, ^{2}J = 6.44 Hz, ^{3}J = 2.44 Hz, ArH), 7.53 (dd, 1H, ^{2}J = 6.64 Hz, ^{3}J = 1.24 Hz, ArH), 7.43 (d, 1H, J = 8.92 Hz, ArH), 6.98 (t, 1H, J = 7.28 Hz, ArH), 6.85 (dd, 1H, ^{2}J = 6.68 Hz, ^{3}J = 1.28 Hz, ArH), 6.68 (t, 1H, J = 7.28 Hz, ArH), 4.64 (s, 2H, NH_2), ^{13}C NMR (DMSO-d_6): δ 158.67, 152.05, 146.57, 145.01, 140.50, 134.89, 131.91, 130.99, 122.80, 122.11, 118.41, 117.98, 117.60, 117.21, 116.92, 112.60.

7-(2-Aminoethylamino)-3-(*1H*-benzo[d]imidazol-2-yl)-*2H*-chromen-2-one (12)

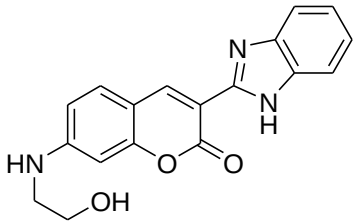
3-(*1H*-benzo[d]imidazol-2-yl)-7-bromo-*2H*-chromene-2-one (**10**) (100 mg, 0.29 mmol) in EtOH (5 ml) was treated with 10eq. of ethylenediamine (0.175 g, 2.9 mmol) and 2 drops of



triethylamine, refluxed for 6-8 hrs. After completion of the reaction (monitored by TLC), the reaction mixture was extracted with chloroform, dried over anhydrous sodium sulphate, filtered and concentrated to get dark brown colored crystals of 7-(2-Aminoethylamino)-3-(*1H*-benzo[d]imidazol-2-yl)-*2H*-chromen-2-one (**12**) (72 mg, 77.41%); mp 138-140°C; ^{1}H NMR **(**DMSO-d_6): δ 8.46 (s, 1H, CH), 7.93 (s, 1H, ArH), 7.49 (s, 1H, ArH), 7.47 (s, 1H, NH), 7.39 (m, 2H, ArH), 7.14 (m, 1H, ArH), 6.82 (m, 2H, ArH), 3.82 (s, 2H, NH_2), 3.73 (t, 2H, CH_2), 3.59 (t, 2H, CH_2); ^{13}C NMR (DMSO-d_6): δ 165.46, 159.75, 134.48, 133.29, 119.92, 118.64, 109.20, 58.72.

3-(*1H*-benzo[d]imidazol-2-yl)-7-(2-hydroxyethylamino)-*2H*-chromen-2-one (13)

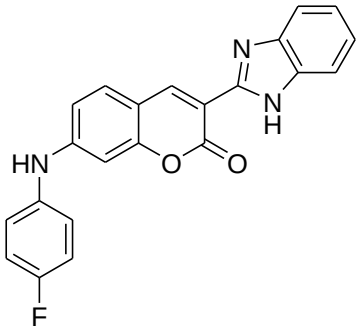
3-(*1H*-benzo[d]imidazol-2-yl)-7-bromo-*2H*-chromen-2-one(**10**) (100 mg, 0.29 mmol) in EtOH



(5 ml) was refluxed with 2 eq. of ethanolamine (0.035 g, 0.56 mmol) and 2 drops of triethylamine for 6-8 hrs. After completion of reaction (monitored by TLC), the reaction mixture was extracted with chloroform, dried over anhydrous sodium sulphate, filtered and concentrated to get brown colored semisolid product of 3-(*1H*-benzo[d]imidazol-2-yl)-7-(2-hydroxyethylamino)-*2H*-chromen-2-one (**13**). (75 mg, 79%); ^{1}H NMR ($CDCl_3$): δ 8.89 (s, 1H, CH), 7.99 (s, 1H, ArH), 7.50 (d, 2H, J = 6.6 Hz, ArH), 7.37 (dd, 2H, ^{2}J = 2.80 Hz, ^{3}J = 6.28 Hz, ArH), 7.13 (m, 1H, ArH), 6.80 (d, 1H, J = 8.8 Hz, ArH), 3.79 (m, 2H, CH_2), 3.69 (s, 1H, NH), 3.58 (m, 2H, CH_2), 2.75 (s, 1H, OH); ^{13}C NMR ($CDCl_3$**)**: δ 164.81, 160.27, 134.13, 133.03, 121.12, 119.80, 118.61, 108.69, 60.81, 60.33.

3-(*1H*-benzo[d]imidazol-2-yl)-7-(4-fluorophenylamino)-*2H*-chromen-2-one (14)

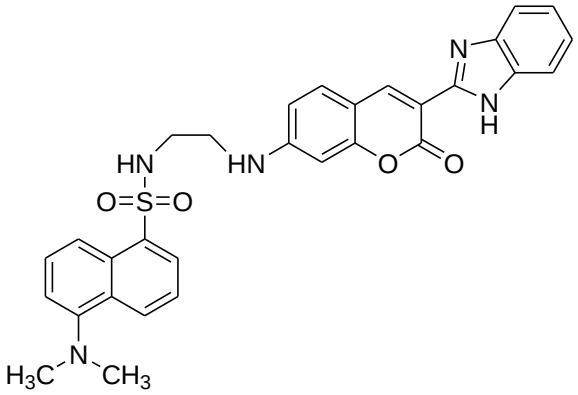
3-(*1H*-benzo[d]imidazol-2-yl)-7-bromo-*2H*-chromen-2-one (**10**) (100 mg, 0.29 mmol) was refluxed with 2eq. of 4-fluoroaniline (0.065 g, 0.58 mmol), 1.2eq. K_2CO_3 (0.096 g) and a pinch of catalyst $TBAHSO_4$ in acetonitrile (10 ml) for 4-5 hours. After completion of reaction



(monitored by TLC), the reaction mixture was extracted with chloroform, dried over anhydrous sodium sulphate, filtered and concentrated to get impure product and purified by column chromatography as brown colored semisolid product of 3-(*1H*-benzo[d]imidazol-2-yl)-7-(4-fluorophenylamino)-*2H*-chromen-2-one (**14**) (80 mg, 74%); ^{1}H NMR ($CDCl_3$): δ 9.05 (s, 1H, CH), 7.83 (d, 1H, *J* = 2.3 Hz, ArH), 7.74 (dd, 1H, ^{2}J = 6.52 Hz, ^{3}J = 2.28 Hz, ArH), 7.54 (m, 2H, ArH), 7.38 (m, 3H, ArH), 7.14 (m, 2H, ArH), 7.06 (m, 2H, ArH), 6.51 (s, 1H, NH).

N-(2-3-(*1H*-benzo[d]imidazol-2-yl)-2-oxo-*2H*-chromen-7-ylamino)ethyl)-5-(dimethylamino) naphthalene-1-sulfonamide (18)

Dansyl chloride {5-(dimethylamino)naphthalene-1-sulfonyl chloride} (100 mg, 0.37 mmol)



in EtOH (10 mL) was refluxed with 10eq. of ethylenediamine (0.23 g, 3.8 mmol) and Et_3N (2 drops) for 7-8 hrs. After completion of reaction (monitored by TLC), the reaction mixture was extracted with chloroform, dried over sodium sulphate, filtered and concentrated to get orange coloured crystals of N-(2-aminoethyl)-5-(dimethylamino)naphthalene-1-sulfonamide (**17**) (80 mg, 74%). Compound **17** (80 mg, 0.27 mmol) (without further purification) was refluxed with 3-(*1H*-benzo[d]imidazol-2-yl)-7-bromo-*2H*-chromen-2-one (**10**) (90 mg, 0.26 mmol) in IPA for 7-8 hrs. Impure product was obtained and purified by column chromatography to get dark brown crystals of N-(2-3-(*1H*-benzo[d]imidazol-2-yl)-2-oxo-*2H*-chromen-7-ylamino)ethyl)-5-(dimethylamino)naphthalene-1-sulfonamide (**18**) (90 mg, 60%); mp 170-175 °C; ^{1}H NMR ($CDCl_3$): δ 8.41 (d, 2H, *J* = 4.16 Hz, ArH), 8.22 (d, 2H, *J* = 8.68 Hz, ArH), 8.17 (s, 1H, ArH), 8.11 (d, 2H, *J* = 7.08 Hz, ArH), 7.53 (m, 1H, ArH), 7.37 (m, 3H, ArH), 7.03 (d, 2H, *J* = 12.63 Hz, ArH), 4.06 (s,1H, NH), 4.00 (s, 1H, NH), 3.97 (t, 2H, *J* = 8.68 Hz, CH_2), 2.71 (t, 2H, *J* = 8.68 Hz, CH_2), 2.67 (s, 6H, N-CH_3).

Antitumor methodology: The human tumor cell lines of the cancer screening panel were grown in RPMI 1640 medium containing 5% fetal bovine serum and 2.0 mM $_{L}$-glutamine. For a typical screening experiment, cells were inoculated into 96 well microtiter plates in 100 µL at plating densities ranging from 5,000 to 40,000 cells/well depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates were incubated at 37 °C, 5% CO_2, 95% air and 100% relative humidity for 24 h prior to addition of experimental drugs. After 24 h, two plates of each cell line were fixed *in situ* with TCA, to represent a measurement of the cell population for each cell line at the time of drug addition (Tz). Experimental drugs were solubilized in dimethyl sulfoxide at 400- fold the desired final maximum test concentration and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate was thawed and diluted to twice the desired final maximum test concentration with complete medium containing 50 µg/ml gentamicin. Aliquot of 100 µl of this drug dilution was added to the appropriate microtiter wells already containing 100 µl of medium, resulting in the required final drug concentrations. Following drug addition, the plates were incubated for an additional 48 h at 37 °C, 5% CO_2, 95% air, and 100% relative humidity. For adherent cells, the assay was terminated by the addition of cold TCA. Cells were fixed *in situ* by the gentle addition of 50 µl of cold 50% (w/v) TCA (final concentration, 10% TCA) and incubated for 60 min at 4 °C. The supernatant was discarded, and the plates were washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (100 µl) at 0.4% (w/v) in 1% acetic acid was added to each well, and plates were incubated for 10 min at room temperature. After staining, unbound dye was removed by washing five times with 1% acetic acid and the plates were air dried. Bound stain was subsequently solubilized with 10 mM trizma base, and the absorbance is read on an automated plate reader at a wavelength of 515 nm. For suspension cells, the methodology is the same except that the assay is terminated by fixing settled cells at the bottom of the wells by gently adding 50 µl of 80% TCA (final concentration, 16% TCA).

CONCLUSION

1. Novel hybrid compound 3-(*1H*-benzo[d]imidazol-2-yl)-7-bromo-*2H*-chromen-2-one (**10**) was synthesized in moderate yield and characterized by NMR experiments. Substitution was done with compound **10** to gave 7-(2-aminoethylamino)-3-(*1H*-benzo[d]imidazol-2-yl)-*2H*-chromen-2-one (**12**), 3-(*1H*-benzo[d]imidazol-2-yl)-7-(2-hydroxyethylamino)-*2H*-chromen-2-one (**13**) and 3-(*1H*-benzo[d]imidazol-2-yl)-7(4-fluorophenylamino)-*2H*-chromen-2-one (**14**) in high yield and characterized by NMR experiments.
2. N-(2-3(*1H*-benzo[d]imidazol-2-yl)-2-oxo-*2H*-chromen-7-ylamino)ethyl)-5-(dimethylamino)naphthalene-1-sulfonamide (**18**) was also syntheised in moderate yield and was confirmed by ^{1}H NMR spectrum.
3. 3-(*1H*-benzo[d]imidazol-2-yl)-7-bromo-*2H*-chromen-2-one (**10)** and 7-(2-aminoethylamino)-3-(*1H*-benzo[d]imidazol-2-yl)-*2H*-chromen-2-one (**12**) showed selectivity towards numerous cell lines belong to different tumor organs. Among all 60-cell lines for one dose concentration, both compounds are potent towards breast cancer cells.
4. These compounds will be useful as template for future development of more potent antitumor agents.

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