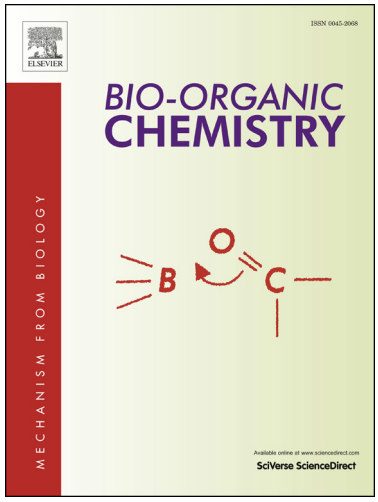


Some new indole-coumarin hybrids; Synthesis, anticancer and Bcl-2 docking studies

Pooja R Kamath, Dhanya Sunil, Abdul Ajees, K.S.R. Pai, Shubhankar Das

PII: S0045-2068(15)30025-0
DOI: http://dx.doi.org/10.1016/j.bioorg.2015.10.001
Reference: YBIOO 1848

To appear in: *Bioorganic Chemistry*

Received Date: 24 June 2015
Revised Date: 1 October 2015
Accepted Date: 5 October 2015

Please cite this article as: P.R. Kamath, D. Sunil, A. Ajees, K.S.R. Pai, S. Das, Some new indole-coumarin hybrids; Synthesis, anticancer and Bcl-2 docking studies, *Bioorganic Chemistry* (2015), doi: http://dx.doi.org/10.1016/j.bioorg.2015.10.001

Some new indole-coumarin hybrids; Synthesis, anticancer and Bcl-2 docking studies

Pooja R Kamath[a], Dhanya Sunil[a*], Abdul Ajees[b], KSR Pai[c] and Shubhankar Das[d]

[*a]Department of Chemistry, Manipal Institute of Technology, Manipal University, 576 104, India.

dhanyadss3@gmail.com

[b]Department of Atomic and Molecular Physics, Manipal University, 576 104, India.

[c]Department of Pharmacology, Manipal College of Pharmaceutical Sciences, Manipal University, 576 104, India.

[d]Department of Radiation Biology & Toxicology, School of Life Sciences, Manipal University, 576 104, India.

Abstract

Hybrid molecules have attracted attention for their improved biological activity, selectivity and lesser side effects profile, distinct from their individual components. In the quest for novel anticancer drug entities, three series of indole-coumarin hybrids - 3-(1-benzyl-1*H*-indol-2-yl)-2*H*-chromen-2-ones, 2-(2-oxo-2*H*-chromen-3-yl)-1*H*-indole-3-carbaldehydes and 2-(2-oxo-2*H*-chromen-3-yl)-1*H*-indole-3-carboxylic acids were synthesized. All the synthesized compounds were characterized by spectral techniques like IR, ^{1}HNMR, ^{13}CNMR, mass spectrometry and elemental analysis. *In silico* docking studies of synthesized molecules with apoptosis related gene Bcl-2 that is recognized to play an important role in tumerogenesis were carried out. Dose-dependent cytotoxic effect of the compounds in human breast adenocarcinoma (MCF-7) and normal cell lines were assessed using MTT assay and compared with that of the standard marketed drug, Vincristine. Compound **4c** had a highly lipophilic bromine substituent capable of forming halogen bond and was identified as a potent molecule both in docking as well as cytotoxicity studies. Flow cytometric cell cycle analysis of **4c** exhibited apoptotic mode of cell death due to cell cycle arrest in G2/M phase. Structure activity relationship of these hybrid molecules was also studied to determine the effect of steric and electronic properties of the substituents on cell viability.

Keywords: Apoptosis. Cytotoxicity. Indole-coumarin. Bcl-2

* Author for correspondence
Dhanya Sunil
Department of Chemistry,
Manipal Institute of Technology,
Manipal-576104, India.
Tel. +91 820 2924412; Email: dhanyadss3@gmail.com

1. Introduction

Breast cancer is the most common cause of cancer deaths in females. The tumour cells will no longer be sensitive to the signals within the tissue which regulate cellular differentiation, survival, proliferation and cell death. This will result in accumulation of the cells within the tissue, causing local damage and inflammation [1]. Amongst many therapeutic strategies, chemotherapy produces significant clinical responses. However, these chemotherapeutic agents have a narrow therapeutic index, exhibit non-specificity and high-systemic toxicity. Target-selective chemotherapeutics that could reduce off-target binding thereby ensuing side effects remains as a major challenge.

Heterocyclic compounds exhibit diverse biological activity owing to their unique ability to mimic the structure of peptides and to bind reversibly to proteins. Amidst them, heterocycles with polycyclic ring system possess distinct rigid geometry displaying high functional specialization by orienting its substituents in three dimensional spaces, there by attracting great interest [2]. Indole is a bicyclic heterocyclic scaffold that find applications in medical therapy due to a variety of valuable biologic activities such as antiviral [3, 4], anti-inflammatory [5, 6], anti-hyperlipidemic, antihypoglycemic, anti-hypertensive, anti-asthmatic, anti HIV [7], antidepressant [8] and notably anticancer [9-15] activity. Few marketed anticancer indole derivatives are vincristine, vinblastine, vinorelbine, vindesine, mitraphylline, cediranib and apaziquone [2]. Coumarin is a bicyclic heterocycle belonging to benzopyran-2-one family which is constantly drawing interest due to its versatile pharmacological activities such as anti-HIV [16], anti-alzheimer's [17], antimalarial [18] and antioxidant [19]. The cytotoxic potential of coumarin derivatives are also extensively studied [20-23].

A new approach of developing hybrid molecules by combination of different pharmacophores may end up with compounds having interesting biological profile. Hybrid molecules can be designed to target simultaneously two different isoenzymes or a receptor and an enzyme to produce potent synergestic effects or as dual acting drugs. Hence a single molecule having more than one pharmacophore which results in a hybrid multifunctional entity, where each individual pharmacophore exhibits different modes of action could be more useful in the treatment of cancer. As indole and coumarin scaffolds have exhibited favorable response as anticancer agents, these pharmacophores were subjected to structural modifications, to synthesize hybrid molecules and to investigate their anticancer potency which has been a core interesting strategy for the researchers.

Vincristine, an antitumor drug bearing indole moiety hinders microtubule polymerization, causes G2/M arrest and induces Bcl-2 hyperphosphorylation and apoptosis in MCF-7 cells [24, 25]. In accord with our program to discover potent and selective antagonists that target Bcl-2, indole-coumarin hybrid based small-molecules were designed and synthesized; molecular modeling and docking studies were carried out and subsequently the promising molecules were biochemically tested for their anticancer potencies.

2. Materials and methods

2.1. Chemistry

The chemicals used for synthesis and anticancer assays were commercially procured from various chemical units viz. E. Merck India Ltd, Sigma Aldrich, Himedia and Spectrochem Chemicals Pvt. Ltd. The progress of reactions and the purity of the synthesized compounds were monitored by thin layer chromatography using pre coated aluminium sheets with Aluchrosep silica Gel 60/ UV_{254} and spots were visualized in UV chamber. Melting points were determined by open capillary method and are uncorrected. IR spectra in KBr pellets were recorded in Schimadzu FTIR 8400S spectrophotometer. The ^{1}H NMR and ^{13}C NMR were recorded in a Bruker 300 MHz instrument in DMSO/ $CDCl_3$ using tetramethylsilane as internal standard. Mass spectra were recorded using an Agilent 6510 series mass spectrometer. The elemental analysis was done in Flash thermo 1112 series CHN analyser.

Substituted 3-acetyl-coumarin-2-ones (**1a-d)** were synthesised by Knoevenagel reaction between different substituted salicylaldehydes and ethyl acetoacetate in presence of catalytic amount of piperidine [26]. Fischer indole synthesis of **1a-d** in presence of Eaton's reagent gave substituted 3-(1*H*-Indol-2-yl)-chromen-2-ones (**2a-d)** [27]. Compounds **2a-d** were allowed to undergo benzylation and Vilsmeyer-Haack formylation to yield substituted 3-(1-benzyl-1*H*-indol-2-yl)-2*H*-chromen-2-ones (**3a-d)** [28] and 2-(2-oxo-2*H*-chromen-3-yl)-1*H*-indole-3-carbaldehydes (**4a-d)** [29]. The oxidation of **4a-d** in presence of potassium permanganate afforded to give 2-(2-Oxo-2*H*-chromen-3-yl)-1*H*-indole-3-carboxylic acids (**5a-d)** [30]. The synthetic scheme for the preparation of indole-coumarin hybrids is presented in scheme-1.

Scheme-1

2.2. In-silico molecular modelling and docking studies

Vincristine acts as a wedge at the interface of two tubulin molecules and thereby inhibits microtubule polymerisation. As a consequence, formation of the mitotic spindle of dividing cells cannot occur, which leads to activation of c-Jun NH2-terminal Kinase (JNK), phosphorylation of Bcl-2, followed by G2/M arrest and finally apoptosis [31]. In order to understand binding and inhibition of the synthesized hybrid molecules with Bcl-2 and thereby sensitize cancer cells to apoptosis, *in silico* docking studies were performed.

The Bcl-2 human sequence (GI: 231632) was obtained from NCBI (www.ncbi.nlm.nih.gov) and the target structure was selected using protein sequence similarity search (www.ebi.ack.u/pdbe). Crystal structure of Bcl-2 in complex with a BAX BH3 peptide (PDB ID: 2XA0) was selected due to 100 % sequence identity with minimum E-value of 2.8e-98 as a target. The Bcl-2 protein is monomeric in it stoichiometry and is present as a dimer with two chains A and B. Each monomeric unit has a ligand peptide BAX bound to the protein. The chain A of structure of Bcl-2 PDB ID 2XA0 was selected for docking and the bound peptide was removed from active site of the receptor. The active site was identified using residue information from PDBsum (http://www.ebi.ac.uk/pdbsum/).

Structures of the compounds were sketched using MarvinSketch (www.chemaxon.com) and ligand molecules were converted from 2D to 3D using Open Babel software [32]. Docking study was performed using AutoDock Vina [33] and input files necessary for AutoDock program were prepared using AutoDock Tools [34]. The size of the grid box in AutoDock Vina was kept as 25 x 25 x 20 for X, Y, Z and the default setting was kept for energy range. The automated program yielded nine possible conformations with distinguished binding energy for each ligand output. The final model was selected based on the binding affinity as well as molecular contacts. The molecular contacts were calculated using the program CONTACT available in CCP4 suite [35]. Docked complexes were analysed and figures were rendered using PyMOL (www.pymol.org).

2.3. Anticancer studies

The studies to evaluate the anticancer potential of synthesized compounds were carried out in human breast adenocarcinoma (MCF-7) cells purchased from National Cancer Centre for Cell Science, Pune, India. The cells were cultured in Dulbecco's modified Eagles medium (DMEM) supplemented with 10 % fetal bovine serum (FBS) at 37 °C in 5 % CO_2 atmosphere.

2.3.1. MTT assay

MTT - based colorimetric cell viability assay is a sensitive *in vitro* assay for measuring cell proliferation or cytotoxicity. Exponentially growing cells were harvested from T-25 tissue culture flask and a stock cell suspension of 1×10^5 cells / mL was prepared. A sterile 96-well flat bottom tissue culture plate was seeded with 5×10^3 cells in 0.1 mL of MEM medium containing 10 % FBS and allowed to attach for 24 h. The cells were treated with different concentration of test compounds (50-400 µM) in triplicates and incubated for 48 h. The control group cells were treated with medium containing 0.1 % DMSO. MTT reagent (30 µL, 4 mg/mL) was added in to the wells and incubated for 4 h at 37 °C. Medium containing MTT was removed and the formazan crystals formed in each well were dissolved in 100 µL of DMSO. The absorbance was measured by an ELISA plate reader at 540 nm [36, 37].

2.3.2. Flow cytometry

Propidium iodide (PI) flow cytometric assay can be used to identify apoptotic cells which are characterized by DNA fragmentation and consequently the loss of nuclear DNA content. MCF-7 cells (2×10^6 cells/mL) were seeded in 6-well plates, treated with 0.5 µM Vincristine and 5 µM test compound. Cells were harvested after 48 h, washed twice with ice-cold PBS and were fixed with 70 % ice-cold ethanol. After centrifugation, 100 mg/L RNase and 5 g/L PI were added to the cells, and were stained in the dark for 30 min. Detection of cell cycle distribution was then performed on a BD Accuri™ C6 flow cytometer and related software was used to analyse the data [38].

3. Results and discussion

*3.1. 2-(6-Chloro-2-oxo-2H-chromen-3-yl)-1H-indole-3-carbaldehyde **(4b)** and 2-(6-Bromo-2-oxo-2H-chromen-3-yl)-1H-indole-3-carbaldehyde **(4c)** as potential Bcl-2 antagonists*

In parallel with biochemical studies, *in silico* docking was performed to examine the structure of Bcl-2 and its interaction with all the synthesized compounds. In the analysis, all four 2-(2-oxo-2*H*-chromen-3-yl)-1*H*-indole-3-carbaldehydes molecules are well fit into the active site of Bcl-2 as depicted in Fig 1. The Bcl-2 residues that are proposed to interact with the four ligand molecules are represented in Fig 2. The corresponding affinity scores and the molecular contacts are summarized in Table 1.

Table 1 Ligand molecules and Bcl-2 docking affinities and residues predicted *in silico* docking to be with in 4.0Å of each other.

Compound	Affinity* (kcal/mol)	Molecular Contacts**		
		Ligand atoms	BCL-2 protein atoms	Distance (in 4.0Å)
4a	-7.7	O12	Gln118 O	3.95
		O12	Met115 O	3.22
		O12	Leu119 N	3.82
		O13	Tyr108 OH	2.63
		O22	Gln118 NE2	2.59
		O22	Tyr108 OH	2.30
4b	-6.7	O12	Gln118 NE2	2.52
		O12	Tyr108 OH	2.42
		Cl	Gln118 O	3.79
		Cl	Arg129 NH1	2.74
		Cl	His120 N	2.50
		Cl	His120 O	3.46
4c	-6.4	O12	Gln118 NE2	2.67
		O12	Tyr108 OH	2.36
		Br	Gln118 O	3.70
		Br	Arg129 NH1	2.62
		Br	His120 HN	2.58
		Br	His120 O	3.60
4d	-6.3	O12	Gln118 NE2	2.34
		O12	Tyr108 OH	1.94
		O13	Gln118 NE2	3.11
		O22	Tyr108 OH	2.37
		O22	Gln118 NE2	3.24

	O23	Gln118 O	3.31

*Results obtained from Autodock vina.

**Results obtained from CONTACT program of CCP4 suite.

Over all, four Bcl-2 amino acids Tyr108, Met115, Gln118 and Leu119 are predicated to be within 4.0 Å of molecule **4a**. Similarly compound **4b** and **4c** are observed to interact with Tyr108, Gln118, His120 and Arg129 and compound **4d** with Tyr108 and Gln118. The Bcl-2 amino acids Tyr108 and Gln118 are the common residues that interacted with all four compounds and it is interesting to notice that Gln118 makes strong interactions with the peptide BAX [39]. Halogen atoms are always attractive and play a major role in biological molecules [40, 41, 7]. In our case, the molecules **4b** and **4c** contains Cl and Br atoms and are well fit with in the cavity of Bcl-2 protein as compared to **4a** and **4d** (Fig2). Moreover, the molecules **4b** and **4c** interact with two positively charged amino acids His120 and Arg129 of Bcl-2. This type of interaction is not observed with either molecule **4a** or **4d**. As both halogen (chlorine and bromine) atoms have strong interactions with Bcl-2 protein, these molecules may act as good candidates for further biochemical studies.

Fig.1

Fig.2

*3.2. 2-(6-Bromo-2-oxo-2H-chromen-3-yl)-1H-indole-3-carbaldehyde **(4c)** induces cytotoxicity and shows selectivity in MCF-7 cells*

The cytotoxicity induced by the new indole-coumarin hybrids was determined using MTT assay by analysing dose-response inhibition curves (Graph Pad prism, 4). Cell proliferation was estimated by MTT reduction by live cells when treated with compounds **(2a-d), (3a-d), (4a-d)** and **(5a-d)** on MCF-7 and normal kidney (VERO) cells. The marketed anticancer drug vincristine was used as the standard drug. The results of MTT assay in terms of their IC_{50} values are summarized in Table 2. Neither the substituted 3-(1*H*-Indol-2-yl)-chromen-2-ones nor 3-(1-benzyl-1*H*-indol-2-yl)-chromen-2-ones were active against the two cell lines. Compounds **4c, 5b** and **5c** were found to be potent against MCF-7 cell lines. The selectivity index of **4c**, **5b** and **5c** for MCF-7 cells was very high which indicated the high selectivity for these molecules towards MCF-7 cells than to normal cells.

Table 2 *In vitro* cytotoxicity data (IC_{50}, µM) for compounds (2a-d), (3a-d), (4a-d) and (5a-d)

Compound	IC_{50} (µM) MCF-7	IC_{50} (µM) Vero	Compound	IC_{50} (µM) MCF-7	IC_{50} (µM) Vero
Vincristine	0.3	0.9	4a	73.2	>100
2a	>100	>100	4b	>100	9.9
2b	>100	>100	4c	7.4	>100

2c	>100	>100	4d	48.1	>100
2d	>100	>100	5a	>100	87.5
3a	>100	>100	5b	5.5	15.4
3b	>100	>100	5c	13.5	>100
3c	>100	>100	5d	>100	22.0
3d	>100	>100			

The halogen substituted aldehyde and acid showed better activity compared to unsubstituted and hydroxy substituted compounds. The standard drug vincristine exhibited an IC_{50} value of 0.3 μM and selectivity index of 3. Compound **4c** was selected for studying the mechanism of cytotoxicity and cell death because of its inhibitory effect with low IC_{50} value in MCF-7 cells and its high selectivity index when compared to vincristine.

*3.3. 2-(6-Bromo-2-oxo-2H-chromen-3-yl)-1H-indole-3-carbaldehyde **(4c)** affects the cell cycle in G_2/M phase*

As **4c** could effectively inhibit cancer cell growth *in vitro*, this inhibitory activity may be attributable to its ability to interfere with the cell cycle. The cell cycle distribution on treatment with **4c** was measured by fluorescence activated flow cytometric cell sorting analysis by PI-labelled cells. The histograms obtained in cell cycle analysis studies are presented in Fig 3. The standard drug vincristine is found to induce cell cycle arrest at the G_2/M phase by promoting microtubule depolymerization in MCF-7 cell lines. The histogram of vincristine treated cells was observed on similar lines during our cell cycle analysis studies confirming the previously published data. Interestingly, a change in the normal cell cycle pattern leading to a cell cycle arrest at the G_2/M phase was observed in MCF-7 cells upon incubation with **4c**. Fig 3 shows that 64 % of the cell population was in the G2/M phase when treated with vincristine, and 46 % on incubation with **4c**. Accumulation of apoptotic cells (21 %) was observed in subG1 phase of the histogram. In contrast, the histogram of control DMSO treated cells exibited a standard cell cycle pattern with G1 and G2/M peaks seperated by S peak and a cell population of only 15 % in G2/M phase. The Sub G1 peak that constitutes mostly dead cells was not prominent. Thus, these results suggest that the inhibitory effect of **4c** on MCF-7 cells are due to a G2/M arrest of the cell cycle which can lead to apoptosis.

Fig. 3

3.4. Structure activity relationship studies

The broad variety of the synthesized compounds was designed in order to gain insight into the influence of halogen and hydroxyl groups on the coumarin nucleus and also benzyl, aldehyde and acid groups on the indole moiety of the hybrid molecules on their anticancer activity. In the present study, N- benzylation of the indole nucleus is found to have no effect on anticancer activity. Benzylation might have deactivated the weakly basic amino group of the indole ring. The presence of an aldehydic or acid group was found to increase the cytotoxic potential as well as the selectivity index of the compounds towards MCF-7 cells. The carboxylic group is often highly ionized in physiological pH and cannot cross the biological membranes or is subjected to

rapid clearance from the body due to its high hydrophilicity. To compensate the loss in lipophilicity of an active compound, it is usually necessary to attach a halogen atom at an appropriate place. Aldehyde **4c** and acids **5b** and **5c** with halogen substituents in the coumarin ring exhibited better anticancer potential than unsubstituted or hydroxyl substituted aldehydes and acids, where there is a proper balance between hydrophilic and lipophilic nature of the molecule. The introduction of a hydroxyl group in a molecule changes the partition coefficient toward more hydrophilicity.

The presence of non-covalent halogen bonds has a potential significance because of their distinct interaction with ligand recognition, binding site, molecular folding and in assembly of proteins and nucleic acids. Halogens are believed to polarize with covalently attached atom to generate electropositive centres, thus acting as a lewis acid to pair with oxygen and nitrogen that is similar to hydrogen bonds, both in strength and directionality and hence they are termed as charge transfer bonds. As a result, the bromine interaction in **4c**, depending on the geometry of the halogen bond is estimated to be stronger than the analogous hydrogen bond in this environment. Drug candidates that are substituted with bromine atoms (induces an important lipophilic contribution even larger than that of a methyl group) are interestingly found to increase the lipophilicity of the molecule which in turn helps in easy penetration of drug through the lipid membranes and tissues thus enhancing the intercellular drug delivery. It also increases protein-ligand stability there by contributing to the binding affinity and selectivity of the molecule [42]. It is also found that halogen bond containing molecules are involved in the inhibition of several classes of protein kinases like MEK, CDK1 and CDK2 [41].

The better activity of molecule **4c** was also analysed using steric and electronic effects caused by bromine atom. The obstruction of the hybrid molecule by means of bromine substitution can also impose certain conformations for certain functions. The bulky bromine atoms prevent the free rotation and maintain the planes of the aromatic rings. The steric effects of halogens are ascribed to their inductive electron-attracting properties which are maximal for chlorine and bromine. The mesomeric donor effect of bromine is usually not considered in biological media [43]. The better anticancer activity of compound **4c** as opposed to **4b** could be attributed to higher lipophilic nature of bromine when compared to chlorine.

4. Conclusion

In the present study, three new series of indole-coumarin hybrid derivatives having different halogen and hydroxyl substitution on coumarin ring and benzyl, aldehyde and acid substituents on indole nucleus have been synthesised and their biologic activities are described. Bromine substituted aldehyde and acid displayed good *in vitro* cytotoxic activity against MCF-7 cells. 2-(6-Bromo-2-oxo-2*H*-chromen-3-yl)-1*H*-indole-3-carbaldehyde (**4c**) was found to display dose dependent cytotoxicity and high selectivity towards MCF-7 cells when compared to standard microtubule-interfering chemotherapeutic agent, vincristine. Cell cycle analysis of MCF-7 cells on treatment with **4c** revealed cell cycle arrest in the G2/M phase confirming its action via the apoptotic pathway. From the current investigation, the structure activity relationships of the indole-coumarin hybrid aldehyde series

demonstrate that bromine substituent in the coumarin pharmacophore showed increased antitumor activity. Presence of bromine might have contributed to the lipophilic nature of molecule and hence favour the passage of biomembranes. Bromine, when usually incorporated into a molecule generates a reactive alkylating intermediate more easily when compared to fluorine or chlorine and benefits in long term treatment. The results of the biochemical assays carried out were in complete agreement with the *in silico* docking studies done in order to predict the possible anticancer activity of the three series of indole-coumarin hybrids. The docking studies have provided excellent information about the binding affinity of molecules and have helped to understand the weak force of interactions. Due to the high incidence of undesirable side effects induced by the majority of current anticancer drugs and by considering the high selectivity index value when compared to vincristine, compound **4c** appears as promising and interesting lead for the design of improved selective therapeutic agents to fight cancer.

5. Experimental

*5.1. General procedure for the synthesis of substituted 3-(1H-Indol-2-yl)-chromen-2-ones **(2a-d)***

Phenyl hydrazine (0.001 mol, 0.098 g) and substituted 3-acetyl-chromen-2-ones **(1a-d)** (0.001 mol) in ethanol with catalytic quantity of glacial acetic acid were refluxed for 6 h to yield respective phenyl hydrazones. Further, substituted phenyl hydrazones (0.004 mol) was heated for 30 - 60 min at 90-95 °C with a mixture of methane sulfonic acid (0.015 mol, 0.97 mL) and phosphorus pentoxide (0.003 mol, 0.85 g) resulting in the formation of target compound which was recrystallized using dimethylformamide

*5.2. General procedure for the synthesis of substituted 3-(1-Benzyl-1H-indol-2-yl)-chromen-2-one **(3a-d)***

Powdered potassium carbonate (0.011 mol, 1.52 g) was added to a solution of **2a-d** (0.002 mol) in DMF and was stirred at r.t. for 30 min. Benzyl chloride (0.002 mol, 0.23 mL) was added, mixture stirred vigorously and refluxed for 6 h. The reaction mixture was cooled and poured in to ethanol water mixture (1:10). The precipitated N-benzylated indole-coumarin derivative was filtered, washed with water and recrystallized using ethanol-water mixture.

*5.2.1. 3-(1-Benzyl-1H-indol-2-yl)-chromen-2-one (**3a**)*

Brown solid (44 %); m.p. 138-140 °C; R_f. 0.68; IR (KBr) [cm^{-1}]: 3030 (Ar. C-H *str.*), 2915 (methyl C-H *asym. str.*), 2845 (methyl C-H *sym. str.*), 1735 (lactone C=O *str.*), 1510 (Ar. C=C *str.*), 1203 (Ar. C-O-C *str.*), 1361 (C-N *str.*); ^{1}H NMR (400 MHz, DMSO-d_6) [ppm]: δ 4.58 (s, 2H, CH_2), δ 6.88 (s, 1H, indole 3-H), δ 6.94 (m, 1H, phenyl 4-H), δ 7.00-7.15 (d, 2H, phenyl 2-H and 6-H), δ 7.25 (d, 1H, coumarin 8-H), δ 7.42-7.45 (t, 2H, phenyl 3-H and 5-H), δ 7.50 (d, 1H, indole 7-H), δ 7.55 (d, 1H, indole 4-H), δ 7.71-7.73 (m, 2H, indole 5-H and 6-H), δ 7.84-7.90 (m, 3H, coumarin 5-H, 6-H and 7-H), δ 8.45 (s, 1H, coumarin 4-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ = 55.82, 105.23, 112.11, 118.72, 120.03, 120.14, 120.25, 121.26, 121.68, 121.76, 123.48, 128.34, 129.36, 129.87, 130.41, 131.25, 131.62, 134.11, 134.18, 134.92, 137.71, 138.14, 151.63, 158.52; MS (m/z): 351 (M^+); Anal. Calcd. for $C_{24}H_{17}NO_2$: C, 82.03; H, 4.88; N, 3.99; found: C, 82.19; H, 4.90; N, 4.00.

*5.2.2. 3-(1-Benzyl-1H-indol-2-yl)-6-chloro-chromen-2-one (**3b**)*

Brown solid (29 %); m.p. 258-260 °C; R_f. 0.85; IR (KBr) [cm^{-1}]: 3067 (Ar. C-H *str.*), 2928 (methyl C-H *asym. str.*), 2850 (methyl C-H *sym. str.*), 1704 (lactone C=O *str.*), 1515 (Ar. C=C *str.*), 1245 (Ar. C-O-C *str.*), 1150 (C-N *str.*), 798 (C-Cl *str.*); ^{1}H NMR (400 MHz, DMSO-d_6) [ppm]: δ 4.59 (s, 2H, CH_2), δ 6.87 (s, 1H, indole 3-H), δ 6.95 (m, 1H, phenyl 4-H), δ 7.01-7.15 (d, 2H, phenyl 2-H and 6-H), δ 7.26 (d, 1H, coumarin 8-H), δ 7.43-7.48 (t, 2H, phenyl 3-H and 5-H), δ 7.52 (d, 1H, indole 7-H), δ 7.59 (d, 1H, indole 4-H), δ 7.71-7.74 (m, 2H, indole 5-H and 6-H), δ 7.81 (d, 1H, coumarin 7-H), δ 7.89 (s, 1H, coumarin 5-H), δ 8.45 (s, 1H, coumarin 4-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ = 55.85, 105.21, 112.15, 118.70, 120.00, 120.13, 120.20, 121.22, 121.65, 121.75, 123.46, 128.34, 129.31, 129.85, 130.47, 131.19, 131.58, 134.00, 134.15, 134.97, 137.75, 138.13, 151.62, 158.58; MS (m/z): 385 (M^+); Anal. Calcd. for $C_{24}H_{16}ClNO_2$: C, 74.71; H, 4.18; N, 3.63; found: C, 74.88; H, 4.19; N, 3.64.

*5.2.3. 3-(1-Benzyl-1H-indol-2-yl)-6-bromo-chromen-2-one (**3c**)*

Brown solid (40 %); m.p. 220-224 °C; R_f. 0.58; IR (KBr) [cm^{-1}]: 3031 (Ar. C-H *str.*), 2923 (methyl C-H *asym. str.*), 2854 (methyl C-H *sym. str.*), 1709 (lactone C=O *str.*), 1519 (Ar. C=C *str.*), 1243 (Ar. C-O-C *str.*), 1151 (C-N *str.*), 806 (C-Br *str.*); ^{1}H NMR (400 MHz, DMSO-d_6) [ppm]: δ 4.60 (s, 2H, CH_2), δ 6.90 (s, 1H, indole 3-H), δ 6.98 (m, 1H, phenyl 4-H), δ 7.03-7.17 (d, 2H, phenyl 2-H and 6-H), δ 7.31 (d, 1H, coumarin 8-H), δ 7.43-7.48 (t, 2H, phenyl 3-H and 5-H), δ 7.54 (d, 1H, indole 7-H), δ 7.59 (d, 1H, indole 4-H), δ 7.72-7.75 (m, 2H, indole 5-H and 6-H), δ 7.85 (d, 1H, coumarin 7-H), δ 7.95 (s, 1H, coumarin 5-H), δ 8.51 (s, 1H, coumarin 4-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ = 55.86, 105.23, 112.14, 118.71, 120.01, 120.15, 120.23, 121.24, 121.69, 121.77, 123.47, 128.35, 129.34, 129.86, 130.48, 131.22, 131.60, 134.10, 134.19, 134.99, 137.76, 138.15, 151.66, 158.58; MS (m/z): 430 (M^+); Anal. Calcd. for $C_{24}H_{16}BrNO_2$: C, 66.99; H, 3.75; N, 3.26; found: C, 67.20; H, 3.77; N, 3.27.

*5.2.4. 3-(1-Benzyl-1H-indol-2-yl)-7-hydroxy-chromen-2-one (**3d**)*

Dark brown solid (19 %); m.p. 140-142 °C; R_f. 0.85; IR (KBr) [cm^{-1}]: 3375 (OH *str.*), 3058 (Ar. C-H *str.*), 2920 (methyl C-H *asym. str.*), 2851 (methyl C-H *sym. str.*), 1720 (lactone C=O *str.*), 1523 (Ar. C=C *str.*), 1254 (Ar. C-O-C *str.*), 1211 (C-N *str.*); ^{1}H NMR (400 MHz, DMSO-d_6) [ppm]: δ 4.76 (s, 2H, CH_2), δ 5.12 (s, 1H, OH), δ 6.14 (s, 1H, indole 3-H), δ 6.70 (s, 1H, coumarin 8-H), δ 6.87 (d, 1H, coumarin 6-H), δ 6.92 (d, 2H, phenyl 2-H and 6-H), δ 7.01 (m, 1H, phenyl 4-H), δ 7.08-7.02 (t, 2H, phenyl 3H and 5H), δ 7.10 (d, 1H, indole 7-H), δ 7.18-7.23 (m, 2H, indole 5-H and 6-H), δ 7.43 (d, 1H, indole 4-H), δ 7.69 (d, 1H, coumarin 5-H), δ 8.51 (s, 1H, coumarin 4-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ = 55.80, 105.21, 112.11, 114.48, 118.70, 120.01, 120.09, 120.12, 120.15, 121.18, 121.63, 121.71, 128.31, 129.75, 129.57, 131.31, 131.62, 134.21, 134.15, 134.92, 137.71, 138.24, 156.63, 158.53; MS (m/z): 367 (M^+); Anal. Calcd. for $C_{24}H_{17}NO_3$: C, 78.46; H, 4.66; N, 3.81; found: C, 78.65; H, 4.67; N, 3.82.

*5.3 General procedure for the synthesis of substituted 2-(2-oxo-2H-chromen-3-yl)-1H-indole-3-carbaldehyde **(4a-d)***

Phosphorous oxychloride (0.038 mol, 3.5 mL) was added drop wise to dimethyl formamide (0.051 mol, 4.1 mL) maintained at -10 – 0 °C and stirred for 30 min. **2a-d** (0.0001 mol) dissolved in minimum quantity of DMF was added part by part to the reaction mixture maintained at 0-10 °C, stirred for 30 min, warmed at 60-70 °C for 5 h

and poured in to crushed ice. The *pH* was adjusted to 7.5-8 by addition of sodium hydroxide solution and extracted using ethyl acetate. The crude aldehyde so obtained was recrystallized using ethanol: DMF mixture.

5.3.1. 2-(2-Oxo-2H-chromen-3-yl)-1H-indole-3-carbaldehyde ***(4a)***

Dark brown solid (87.12 %); decomposes at 200 °C; R_f. 0.28; IR (KBr) [cm^{-1}]: 3310 (N-H *str*.), 3108 (Ar. C-H *str*.), 1728 (lactone C=O *str*.), 1650 (C=O *str*.), 1499 (Ar. C=C *str*.), 1357 (Ar. C-O-C *str*.); ^{1}H NMR (400 MHz, DMSO-d_6) [ppm]: δ 7.02 (m, 1H, indole 6-H), δ 7.21 (t, 1H, indole 5-H), δ 7.35 (d, 1H, indole 7-H), δ 7.50 (d, 1H, indole 4-H), δ 7.66 (d, 1H, coumarin 8-H), δ 7.93-7.96 (m, 2H, coumarin 6-H and 7-H), δ 8.09 (d, 1H, coumarin 5-H), δ 8.48 (s, 1H, coumarin 4-H), δ 9.85 (s, 1H, CHO), δ 9.92 (s, 1H, indole N-H); ^{13}C NMR (100 MHz, DMSO-d6) δ = 105.24, 112.17, 120.02, 120.13, 120.25, 121.26, 121.69, 121.78, 123.48, 128.45, 129.59, 129.89, 130.59, 134.94, 138.18, 151.67, 162.05, 190.05; MS (m/z): 289 (M^+); Anal. Calcd. for $C_{18}H_{11}NO_3$: C, 74.73; H, 3.83; N, 4.84; found: C, 74.98; H, 3.85; N, 4.85.

5.3.2. 2-(6-Chloro-2-oxo-2H-chromen-3-yl)-1H-indole-3-carbaldehyde ***(4b)***

Dark brown solid (78.12 %); decomposes at 218 °C; R_f. 0.82; IR (KBr) [cm^{-1}]: 3325 (N-H *str*.), 3109 (Ar. C-H *str*.), 1700 (lactone C=O *str*.), 1650 (C=O *str*.), 1550 (Ar. C=C *str*.), 1374 (Ar. C-O-C *str*.), 750 (C-Cl *str*.); ^{1}H-NMR (400 MHz, DMSO-d_6) [ppm]: δ 7.04 (t, 1H, indole 6-H), δ 7.21 (t, 1H, indole 5-H), δ 7.35 (d, 1H, indole 7-H), δ 7.50 (d, 1H, indole 4-H), δ 7.68 (d, 1H, coumarin 8-H), δ 7.95 (d, 1H, coumarin 7-H), δ 8.10 (s, 1H, coumarin 5-H), δ 8.50 (s, 1H, coumarin 4-H), δ 9.86 (s, 1H, CHO), δ 9.94 (s, 1H, indole N-H); ^{13}C NMR (100 MHz, DMSO-d6) δ = 105.20, 112.13, 120.00, 120.11, 120.20, 121.25, 121.68, 121.77, 123.48, 128.34, 129.32, 130.00, 130.47, 134.99, 138.15, 151.62, 162.02, 190.00; MS (m/z): 323 (M^+); Anal. Calcd. for $C_{18}H_{10}ClNO_3$; C, 66.78; H, 3.11; N, 4.33; found: C, 66.96; H, 3.13; N, 4.34.

5.3.3. 2-(6-Bromo-2-oxo-2H-chromen-3-yl)-1H-indole-3-carbaldehyde ***(4c)***

Dark brown solid (82 %); m.p. 230-232 °C; R_f. 0.1; IR (KBr) [cm^{-1}]: 3305 (N-H *str*.), 3100 (Ar. C-H *str*.), 1724 (lactone C=O *str*.), 1643 (C=O *str*.), 1548 (Ar. C=C *str*.), 1388 (Ar. C-O-C *str*.), 813 (C-Br *str*.); ^{1}H-NMR (400 MHz, DMSO-d_6) [ppm]: δ 7.06 (m, 1H, indole 6-H), δ 7.22 (t, 1H, indole 5-H), δ 7.35 (d, 1H, indole 7-H), δ 7.50 (d, 1H, indole 4-H), δ 7.70 (d, 1H, coumarin 8-H), δ 7.97 (d, 1H, coumarin 7-H), δ 8.13 (s, 1H, coumarin 5-H), δ 8.52 (s, 1H, coumarin 4-H), δ 9.86 (s, 1H, CHO), δ 9.94 (s, 1H, indole N-H); ^{13}C NMR (100 MHz, DMSO-d6) δ = 105.23, 112.15, 120.01, 120.14, 120.23, 121.24, 121.68, 121.77, 123.47, 128.35, 129.33, 129.86, 130.48, 134.98, 138.16, 151.66, 162.01, 190.03; MS (m/z): 368 (M^+); Anal. Calcd. for $C_{18}H_{10}BrNO_3$: C, 58.72; H, 2.74; N, 3.80; found: C, 58.87; H, 2.75; N, 3.81.

5.3.4. 2-(7-Hydroxy-2-oxo-2H-chromen-3-yl)-1H-indole-3-carbaldehyde ***(4d)***

Orange solid (76 %); m.p. 210-212 °C; R_f. 0.5; IR (KBr) [cm^{-1}]: 3600 (O-H *str*.), 3352 (N-H *str*.), 3080 (Ar. C-H *str*.), 1724 (lactone C=O *str*.), 1623 (C=O *str*.), 1546 (Ar. C=C *str*.), 1261 (Ar. C-O-C *str*.); ^{1}H-NMR (400 MHz, DMSO-d_6) [ppm]: δ 5.33 (s, 1H, O-H), δ 7.01 (m, 1H, indole 6-H), δ 7.19 (t, 1H, indole 5-H), δ 7.33 (d, 1H, indole 7-H), δ 7.56 (d, 1H, indole 4-H),δ 6.69 (d, 1H, coumarin 6-H), δ 6.85 (s, 1H, coumarin 8-H), δ 7.43 (d, 1H, coumarin 5-H), δ 8.18 (s, 1H, coumarin 4-H), δ 9.89 (s, 1H, CHO), δ 9.96 (s, 1H, indole N-H); ^{13}C NMR (100 MHz, DMSO-d6) δ = 105.24, 112.14, 120.00, 120.13, 120.22, 121.21, 121.67, 121.76, 123.48,

128.31, 129.39, 129.85, 130.41, 134.99, 138.16, 156.63, 162.09, 190.01; MS (m/z): 305 (M^+); Anal. Calcd. for $C_{18}H_{11}NO_4$: C, 70.82; H, 3.63; N, 4.59; found: C, 71.10; H, 3.65; N, 4.60.

5.4. *General procedure for the synthesis of substituted 2-(2-oxo-2H-chromen-3-yl)-1H-indole-3-carboxylic acids* ***(5a-d)***

Potassium permanganate (0.001 mol, 0.158 g) solution was added to 0.001 mol of **4a-d** dissolved in 10 ml pyridine and stirred at r.t for 3 h. 50 mL 1% sodium hydroxide solution was added to the reaction mixture and further stirred for 2 h at 50 °C, cooled, filtered and washed with water. The filtrate was acidified with 6N HCl to pH 4, precipitate was filtered, washed with water and recrystallized using glacial acetic acid

5.4.1. *2-(2-Oxo-2H-chromen-3-yl)-1H-indole-3-carboxylic acid* ***(5a)***

Straw yellow solid (30 %); decomposes at 180 °C; R_f. 0.3; IR (KBr) [cm^{-1}]: 3390 (COOH *str.*), 3350 (N-H *str.*), 3018 (Ar. C-H *str.*), 1714 (lactone C=O *str.*), 1630 (C=O *str.*), 1590 (Ar. C=C *str.*), 1356 (Ar. C-O-C *str.*); ^{1}H NMR (400 MHz, DMSO-d_6) [ppm]: δ 7.28-7.30 (m, 2H, coumarin 7-H and 6-H), δ 7.57-7.54 (m, 2H, indole 5-H and 6-H), δ 7.87 (d, 1H, indole 4-H), δ 8.10-8.12 (d, 1H, indole 7-H), δ 8.20-8.23 (d, 2H, coumarin 5-H and 8-H), δ 8.49 (s, 1H, coumarin 4-H), δ 9.98 (s, 1H, indole N-H), δ 12.42 (s, 1H, COOH); ^{13}C NMR (100 MHz, DMSO-d6) δ = 112.04, 120.05, 120.19, 120.28, 121.26, 121.67, 123.48, 125.09, 128.35, 129.38, 129.87, 130.50, 131.26, 137.75, 138.18, 151.69, 158.56, 170.18; MS (m/z): 305 (M^+); Anal. Calcd. for $C_{18}H_{11}NO_4$: C, 70.82; H, 3.63; N, 4.59; found: C, 71.03; H, 3.64; N, 4.60.

5.4.2. *2-(6-Chloro-2-oxo-2H-chromen-3-yl)-1H-indole-3-carboxylic acid* ***(5b)***

Straw yellow solid (50 %); decomposes at 163 °C; R_f. 0.9; IR (KBr) [cm^{-1}]: 3400 (COOH *str.*), 3300 (N-H *str.*), 3036 (Ar. C-H *str.*), 1720 (lactone C=O *str.*), 1632 (C=O *str.*), 1492 (Ar. C=C *str.*), 1357 (Ar. C-O-C *str.*), 785 (C-Cl *str.*); ^{1}H NMR (400 MHz, DMSO-d_6) [ppm]: δ 7.30-7.32 (d, 2H, coumarin 7-H and 8-H), δ 7.57-7.54 (m, 2H, indole 5-H and 6-H), δ 7.87 (d, 1H, indole 4-H), δ 8.21 (d, 1H, indole 7-H), δ 8.20 (s, 1H, coumarin 5-H), δ 8.45 (s, 1H, coumarin 4-H), δ 9.97 (s, 1H, indole N-H), δ 12.41 (s, 1H, COOH); ^{13}C NMR (100 MHz, DMSO-d6) δ = 112.14, 120.00, 120.12, 120.21, 121.23, 121.68, 123.49, 125.02, 128.36, 129.34, 129.85, 130.49, 131.21, 137.75, 138.15, 151.63, 158.57, 170.00; MS (m/z): 339 (M^+); Anal. Calcd. for $C_{18}H_{10}ClNO_4$: C, 63.64; H, 2.97; N, 4.12; found: C, 63.89; H, 2.99; N, 4.13.

5.4.3. *2-(6-Bromo-2-oxo-2H-chromen-3-yl)-1H-indole-3-carboxylic acid* ***(5c)***

Straw yellow solid (45 %); m.p. 220-222 °C; R_f. 0.4; IR (KBr) [cm^{-1}]: 3402 (COOH *str.*), 3379 (N-H *str.*), 3048 (Ar. C-H *str.*), 1724 (lactone C=O *str.*), 1643 (C=O *str.*), 1525 (Ar. C=C *str.*), 1388 (Ar. C-O-C *str.*), 810 (C-Br *str.*); ^{1}H NMR (400 MHz, DMSO-d_6) [ppm]: δ 7.31-7.35 (d, 2H, coumarin 7-H and 8-H), δ 7.52-7.57 (m, 2H, indole 5-H and 6-H), δ 7.87 (d, 1H, indole 4-H), δ 8.21 (d, 1H, indole 7-H), δ 8.23 (s, 1H, coumarin 5-H), δ 8.49 (s, 1H, coumarin 4-H), δ 9.98 (s, 1H, indole N-H), δ 12.42 (s, 1H, COOH); ^{13}C NMR (100 MHz, DMSO-d6) δ = 112.15, 120.01, 120.14, 120.23, 121.24, 121.69, 123.47, 125.03, 128.34, 129.35, 129.86, 130.48, 131.23, 137.76, 138.16, 151.65, 158.58, 170.01; MS (m/z): 384 (M^+); Anal. Calcd. for $C_{18}H_{10}BrNO_4$: C, 56.27; H, 2.62; N, 3.65; found: C, 56.40; H, 2.63; N, 3.66.

5.4.4. 2-(7-Hydroxy-2-oxo-H-chromen-3-yl)-1H-indole-3-carboxylic acid ***(5d)***

Straw yellow solid (23.8 %); m.p. 198-200 °C; R_f. 0.8; IR (KBr) [cm^{-1}]: 3550 (COOH *str*.), 3440 (O-H *str*.), 3320 (N-H *str*.), 3108 (Ar. C-H *str*.), 1718 (lactone C=O *str*.), 1640 (C=O *str*.), 1560 (Ar. C=C *str*.), 1380 (Ar. C-O-C *str*.); ^{1}H NMR (400 MHz, DMSO-d_6) [ppm]: δ 5.38 (s, 1H, O-H), δ 6.70 (s, 1H, coumarin 8-H), δ 6.87 (d, 1H, coumarin 6-H), δ 7.40-7.10 (d, 1H, coumarin 5-H), δ 7.55-7.51 (m, 2H, indole 5-H and 6-H), δ 7.86 (d, 1H, indole 4-H), δ 8.18 (d, 1H, indole 7-H), δ 8.20 (s, 1H, coumarin 4-H), δ 9.96 (s, 1H, indole N-H), δ 12.40 (s, 1H, COOH); ^{13}C NMR (100 MHz, DMSO-d6) δ = 112.05, 120.04, 120.18, 120.20, 121.23, 121.61, 123.47, 125.00, 128.31, 129.35, 129.84, 130.47, 131.25, 137.73, 138.17, 151.66, 158.57, 170.10; MS (m/z): 321 (M^+); Anal. Calcd. for $C_{18}H_{11}NO_5$: C, 67.29; H, 3.45; N, 4.36; found: C, 67.54; H, 3.47; N, 4.37.

References

1. P. Porter, Westernizing women's risks? Breast cancer in lower income countries, N. Engl. J. Med. 358 (2008) 213-216. doi: 10.1056/NEJMp0708307
2. N.K. Kaushik, N. Kaushik, P. Attri, N. Kumar, C.H. Kim, A.K. Verma, E.H. Choi, Biomedical importance of indoles, Molecules. 18 (2013) 6620-6662. doi: 10.3390/molecules18066620
3. P.L. Beaulieu, P.C. Anderson, R. Bethell, M. Bös, Y. Bousquet, C. Brochu, M.G. Cordingley, G. Fazal, M. Garneau, J.R. Gillard, S. Kawai, M. Marquis, G. McKercher, M.A. Poupart, T. Stammers, B. Thavonekham, D. Wernic, J. Duan, G. Kukolj, Discovery of BI 207524, an indole diamide NS5B thumb pocket 1 inhibitor with improved potency for the potential treatment of chronic hepatitis C virus infection, J. Med. Chem. 57 (2014) 10130-10143. doi: 10.1021/jm501532z
4. M. Zhang, Q. Chen, G. Yang, A review on recent developments of indole-containing antiviral agents, Eur. J. Med. Chem. 89C (2015) 421-441. doi: 10.1016/j.ejmech.2014.10.065
5. A. Ozdemir, M.D. Altıntop, G.T. Zitouni, G.A. Çiftçi, İ. Ertorun, Ö. Alataş, Z.A. Kaplancıklı, Synthesis and evaluation of new indole-based chalcones as potential antiinflammatory agents, Eur. J. Med. Chem. 89 (2015) 304-309. doi: 10.1016/j.ejmech.2014.10.056
6. Q.V. Vo, C. Trenerry, S. Rochfort, A.B. Hughes, Synthesis and anti-inflammatory activity of indole glucosinolates, Bioorg. Med. Chem. 22 (2014) 856-864. doi: 10.1016/j.bmc.2013.12.003
7. V. Famiglini, G.L. Regina, A. Coluccia, S. Pelliccia, A. Brancale, G. Maga, E. Crespan, R. Badia, E. Riveira-Muñoz, J.A. Esté, R. Ferretti, R. Cirilli, C. Zamperini, M. Botta, D. Schols, V. Limongelli, B. Agostino, E. Novellino, R. Silvestri, Indolylarylsulfones carrying a heterocyclic tail as very potent and broad spectrum HIV 1 non-nucleoside reverse transcriptase inhibitors, J. Med. Chem. 57 (2014) 9945-9967. doi: 10.1021/jm5011622
8. N.T. Tzvetkov, S. Hinz, P. Kuppers, G. Marcus, M.E. Christa, Indazole- and indole-5-carboxamides: selective and reversible monoamine oxidase B inhibitors with subnanomolar potency, J. Med. Chem. 57 (2014) 6679-6703. doi: 10.1021/jm500729a
9. Shaveta, P. Singh, Structural optimization of indole based compounds for highly promising anti-cancer activities: structure activity relationship studies and identification of lead molecules, Eur. J. Med. Chem. 74 (2014) 440-450. doi: 10.1016/j.ejmech.2013.12.047

10. G. Wang, C. Li, L. He, X. Yan, N. Ding, J. Ye, Design, synthesis and biological evaluation of a series of pyrano chalcone derivatives containing indole moiety as novel anti-tubulin agents, Bioorg. Med. Chem. 22 (2014) 2060-2079. doi: 10.1016/j.bmc.2014.02.028
11. S.H. Zhuang, Y.C. Lin, L.C. Chou, M.H. Hsu, H.Y. Lin, C.H. Huang, J.C. Lien, S.C. Kuo, L.J. Huang, Synthesis and anticancer activity of 2,4-disubstituted furo[3,2-b]indole derivatives, Eur. J. Med. Chem. 66 (2013) 466-479. doi: 10.1016/j.ejmech.2013.06.012
12. V.J. Raja, K.H. Lim, C.O. Leong, T. Kam, D.T. Bradshaw, Novel antitumour indole alkaloid, Jerantinine A, evokes potent G2/M cell cycle arrest targeting microtubules, Invest. New Drugs. 32 (2014) 838-850. doi: 10.1007/s10637-014-0126-1
13. H.E. Dweedar, H. Mahrous, H.S. Ibrahim, H.A. Abdel-aziz, Analogue-based design, synthesis and biological evaluation of anticancer agents, Eur. J. Med. Chem. 78 (2014) 275-280. doi: 10.1016/j.ejmech.2014.03.058
14. R. Gali, J. Banothu, M. Porika, R. Velpula, S. Hnamte, R. Bavantula, S.A. Busi, Indolylmethylene benzo[h]thiazolo[2,3-b]quinazolinones: synthesis, characterization and evaluation of anticancer and antimicrobial activities, Bioorg. Med. Chem. Lett. 24 (2014) 4239-4242. doi: 10.1016/j.bmcl. 2014.07.030
15. S.G. Alp, M. Mumcuoglu, C. Andac, E. Dayanc, R. Cetin-Atalay, E. Buyukbingol, Synthesis, anticancer activities and molecular modeling studies of novel indole retinoid derivatives, Eur. J. Med. Chem. 58 (2012) 346-354. doi: 10.1016/j.ejmech.2012.10.013
16. T.O. Olomola, R. Klein, N. Mautsa, Y. Sayed, Corrigendum to Synthesis and evaluation of coumarin derivatives as potential dual-action HIV-1 protease and reverse transcriptase inhibitors , Bioorg. Med. Chem. 21 (2013) 1964-1971. doi: 10.1016/j.bmc.2013.08.003
17. P. Anand, B. Singh, N. Singh, A review on coumarins as acetylcholinesterase inhibitors for Alzheimer's disease, Bioorg. Med. Chem. 20 (2012) 1175-1180. doi: 10.1016/j.bmc.2011.12.042
18. R. Pingaew, A. Saekee, P. Mandi, N. chanin, P. Supaluk, R. Somsak, Synthesis , biological evaluation and molecular docking of novel chalcone coumarin hybrids as anticancer and antimalarial agents, Eur. J. Med. Chem. 85 (2014) 65-76. doi: 10.1016/j.ejmech.2014.07.087
19. M. Roussaki, K. Zelianaios, E. Kavetsou, S. Hamilakis, D. Hadjipavlou-Litina, C. Kontogiorgis, T. Liargkova, A. Detsi, Structural modifications of coumarin derivatives : Determination of antioxidant and lipoxygenase (LOX) inhibitory activity, Bioorg. Med. Chem. 22 (2014) 6586-6594. doi: 10.1016/j.bmc.2014.10.008
20. T. Nasr, S. Bondock, M. Youns, Anticancer activity of new coumarins substituted hydrazide-hydrazone derivatives, Eur. J. Med. Chem. 9 (2014) 539-548. doi: 10.1016/j.ejmech.2014.02.026
21. S, Sandhu, Y. Bansal, O. Silakari, G. Bansal, Coumarin hybrids as novel therapeutic agents, Bioorg. Med. Chem. 22 (2014) 3806-3814. doi: 10.1016/j.bmc.2014.05.032
22. K. Paul, S. Bindal, V. Luxami, Synthesis of new conjugated coumarin-benzimidazole hybrids and their anticancer activity, Bioorg. Med. Chem. Lett. 23 (2013) 3667–3372. doi: 10.1016/j.bmcl.2012.12.071
23. K.V. Sashidhara, A. Kumar, M. Kumar, J. Sarkar, S. Sinha, Synthesis and in vitro evaluation of novel coumarin-chalcone hybrids as potential anticancer agents, Bioorg. Med. Chem. Lett. 20 (2010) 7205-7211. doi: 10.1016/j.bmcl.2010.10.116

24. P. Casado, P. Zuazua-Villar, E. del Valle, C. Martínez-Campa1, P.S. Lazo, S. Ramos, Vincristine regulates the phosphorylation of the antiapoptotic protein HSP27 in breast cancer cells, Cancer Lett. 247 (2007) 273-282. doi: 10.1016/j.canlet.2006.05.005
25. R.K. Srivastava, A.R. Srivastava, S.J. Korsmeyer, M. Nesterova, Y.S. Cho-Chung, D.L. Longo, Involvement of microtubules in the regulation of Bcl2 phosphorylation and apoptosis through cyclic AMP-dependent protein kinase, Mol. Cell Biol. 18 (1998) 3509-3517.
26. S. Khode, V. Maddi, P. Aragade, M, Palkar, P.K. Ronad, S. Mamledesai, A.H. Thippeswamy, D. Satyanarayana, Synthesis and pharmacological evaluation of a novel series of 5-(substituted)aryl-3-(3-coumarinyl)-1-phenyl-2-pyrazolines as novel anti-inflammatory and analgesic agents, Eur. J. Med. Chem. 44 (2009) 1682-1688. doi: 10.1016/j.ejmech.2008.09.020
27. A.D. Billimoria, M.P. Cava, Chemistry of Indolo [1,2c]quinazoline: An Approach to the Marine Alkaloid Hinckdentine, J. Org. Chem. 545 (1994) 6777-6782.
28. B.S. Furniss, A.J. Hannaford, P.W.G. Smith, A.R. Tatchell, Vogel's textbook of practical organic chemistry. Dorling Kindersley (India) Pvt. Ltd, Delhi, 2009.
29. M. Jha, M. Edmunds, K. Lund, A. Ryan, A new route to the versatile synthesis of thiopyrano [2,3-b:6,5-b0]diindoles via 2-(alkylthio)-indole-3-carbaldehyde, Tetrahedron Lett. 55 (2014) 5691-5694. doi: 10.1016/j.tetlet.2014.08.100
30. M.A.H. Zahran, A.M. Ibrahim, Synthesis and cellular cytotoxicities of new N-substituted indole-3-carbaldehyde and their indolylchalcones, J. Chem. Sci. 121 (2009) 455-462. doi: 10.1007/s12039-009-0054-3
31. B. Gigant, C. Wang, R.B.G. Ravelli, F. Roussi, M.O. Steinmetz, P.A. Curmi, A. Sobel, M. Knossow, Structural basis for the regulation of tubulin by vinblastine, Nature. 435 (2005) 519–522. doi: 10.1038/nature03566
32. N.M.O. Boyle, M. Banck, C.A. James, C. Morley, T. Vandermeersch, G.R. Hutchison, Open Babel: An open chemical toolbox, J. Cheminform. 3 (2011) 33. doi: 10.1186/1758-2946-3-33
33. O. Trott, A.J. Olson, AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading, J. Comput. Chem. 31 (2011) 455-461. doi: 10.1002/jcc.21334
34. G.M. Morris, R. Huey, W. Lindstrom, M.F. Sanner, R.K. Belew, D.S. Goodsell, A.J. Olson, AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility, J. Comput. Chem. 30 (2009) 2785-2791. doi: 10.1002/jcc.21256
35. M.D. Winn, C. Charles, K.D. Cowtan, D. Kevin, D.J. Eleanor, E. Paul, E.R. Phil, K.M. Ronan, K.B. Eugene, L.G.W. Andrew, M. Airlie, M.J. Stuart, M.N. Garib, P.S. Navraj, P.A. Elizabeth, P.R. Harold, R.J. Randy, V. Alexei, W.S. Keith, Overview of the CCP 4 suite and current developments research papers, Acta Crystall. D. 67 (2011) 235-242. doi: 10.1107/S0907444910045749
36. A.M. Isloor, D. Sunil, P. Shetty, M. Shridhar, K.S.R. Pai, M. Naseer, Synthesis, characterization, anticancer, and antioxidant activity of some new thiazolidin-4-ones in MCF-7 cells, Med. Chem. Res. 22 (2013) 758-767. doi: 10.1007/s00044-012-0071-5

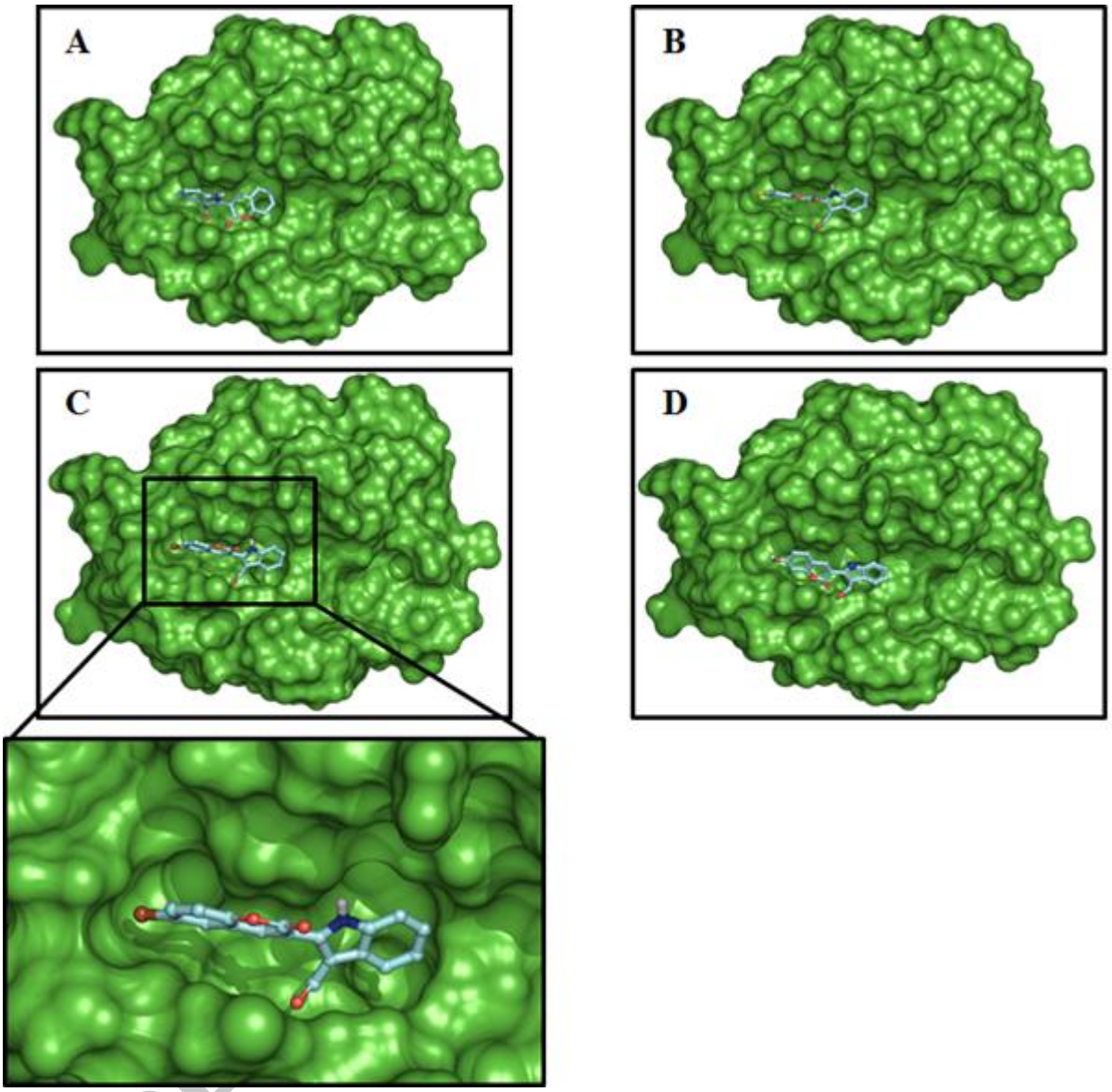
37. C. Kavitha, M. Nambiar, A.C.S. Kumar, B. Choudhary, K. Muniyappa, K.S. Rangappa, S.C. Raghavan, Novel derivatives of spirohydantoin induce growth inhibition followed by apoptosis in leukemia cells, Biochem. Pharmacol. 77 (2009) 348-363. doi: 10.1016/j.bcp.2008.10.018
38. A.L. Blajeski, V.A. Phan, T.J. Kottke, S.H. Kaufmann, G1 and G2 cell-cycle arrest following microtubule depolymerization in human breast cancer cells, J. Clin. Invest. 110 (2002) 91-99. doi: 10.1172/JCI200213275
39. B. Ku, C. Liang, J.U. Jung, B. Oh, Evidence that inhibition of BAX activation by BCL-2 involves its tight and preferential interaction with the BH3 domain of BAX, Nat. Publ. Gr. 21 (2011) 627-641. doi: 10.1038/cr.2010.149
40. A.R. Voth, F.A. Hays, P.S. Ho, Directing macromolecular conformation through halogen bonds, Proc. Natl. Acad. Sci. USA 104 (2011) 10-15.
41. M.R. Scholfield, C.M.V. Zanden, M. Carter, P.S. Ho, Halogen bonding (X-bonding): a biological perspective, Protein Sci. 22 (2013) 139-152. doi: 10.1002/pro.2201
42. P. Auffinger, F.A. Hays, E. Westhof, P.S. Ho, Halogen bonds in biological molecules, Proc. Natl. Acad. Sci. USA 101 (2004) 16789-16794. doi: 10.1073/pnas.0407607101
43. C.G. Wermuth, The practice of medicinal chemistry. Elsevier (India), Delhi, (2009).

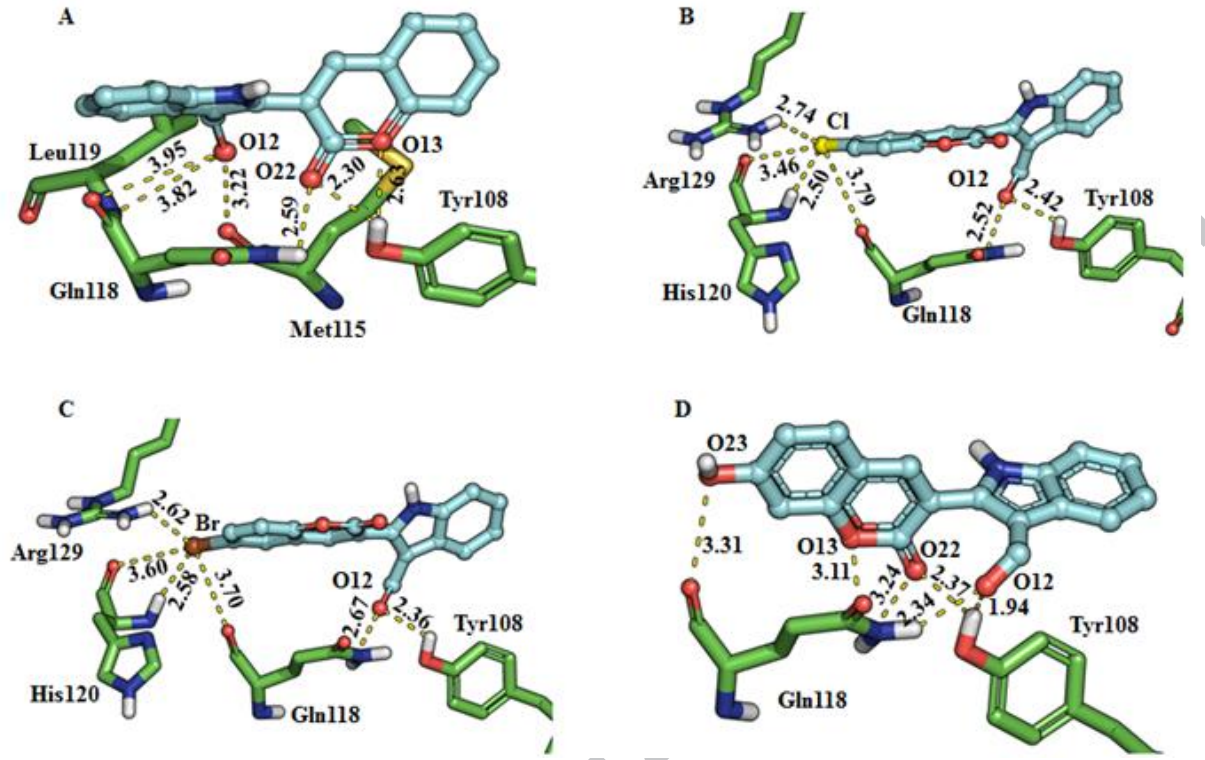
Scheme 1. Protocol for the synthesis of different substituted indole-coumarin derivatives.

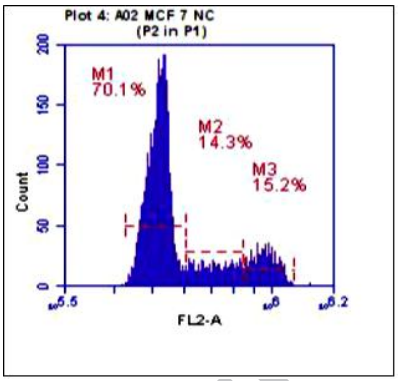
Fig.1. Bcl-2 docking: Docking results from Autodock vina is shown for compounds **4a** (A), **4b** (B), **4c** (C) and **4d** (D). Bcl-2 is shown as surface and coloured green. Ligand molecules **4a-d** shown as ball and stick model are coloured cyan (carbon), red (oxygen), blue (nitrogen), white (hydrogen), yellow (chloride) and brown (bromine). The inset shows the close view of binding pocket of Bcl-2 docked with compound **4c**.

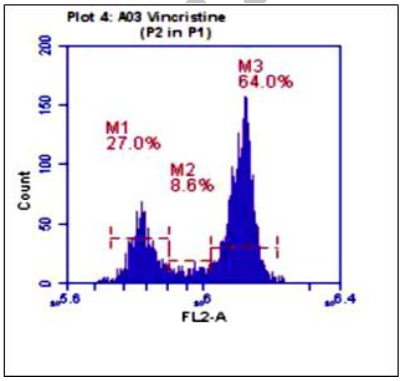
Fig. 2. Bcl-2 molecular interactions: Bcl-2 protein amino acids interacting with compounds **4a** (A), **4b** (B), **4c** (C) and **4d** (D) is shown in ball and stick model. Bcl-2 amino acids carbons are coloured green and ligand carbons are shown in cyan color. For the clarity purpose only polar hydrogens are shown and remaining residues are coloured as Figure 1. Interactions are shown in dotted line and distances (Å) between amino acids and corresponding ligand atoms are marked.

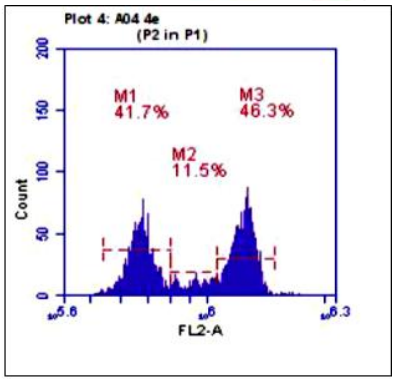
Fig. 3. Cell cycle analysis: Normal cell cycle pattern observed in control. G2/M phase cell cycle arrest seen on treatment with vincristine and compound **4c**

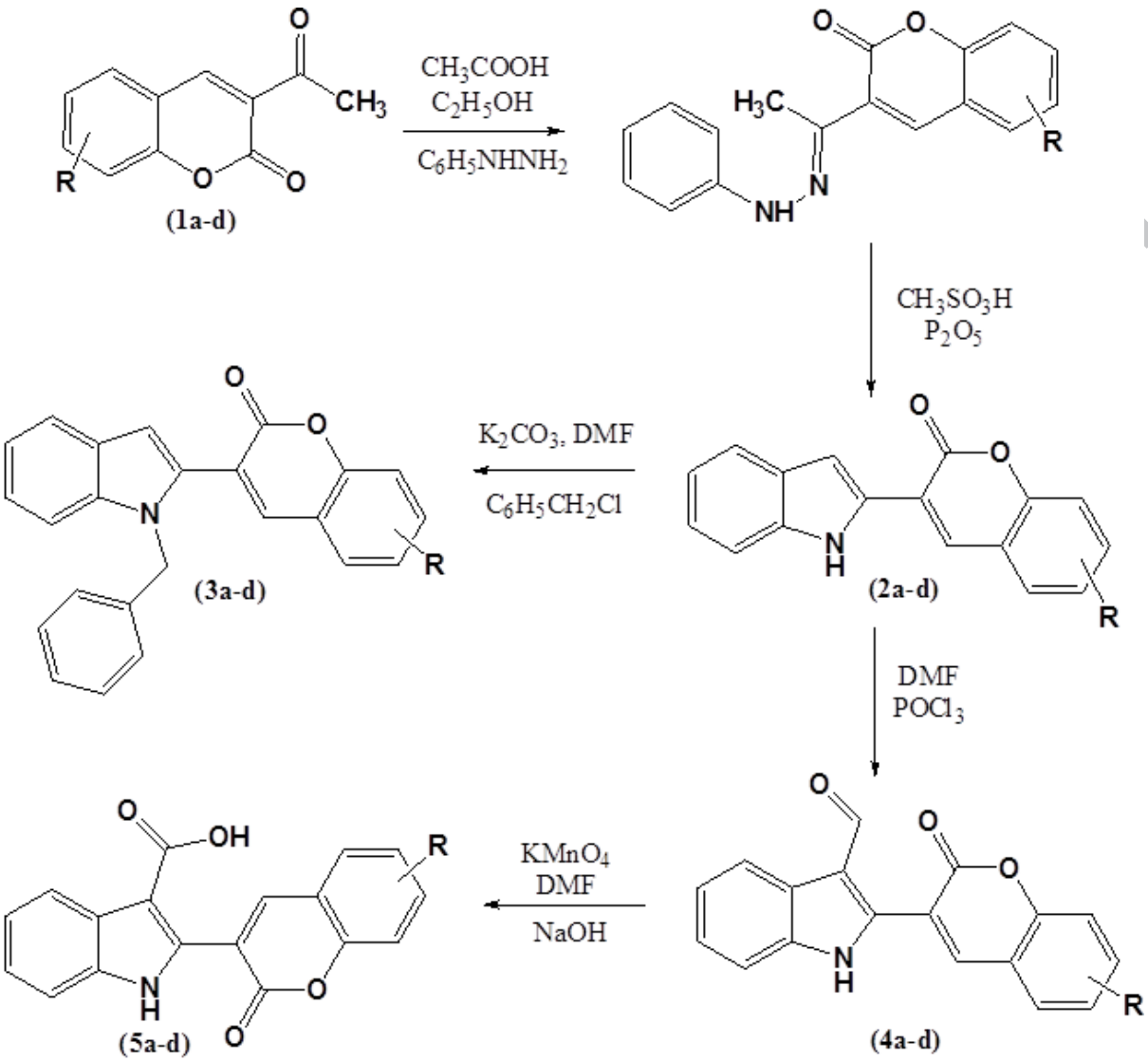




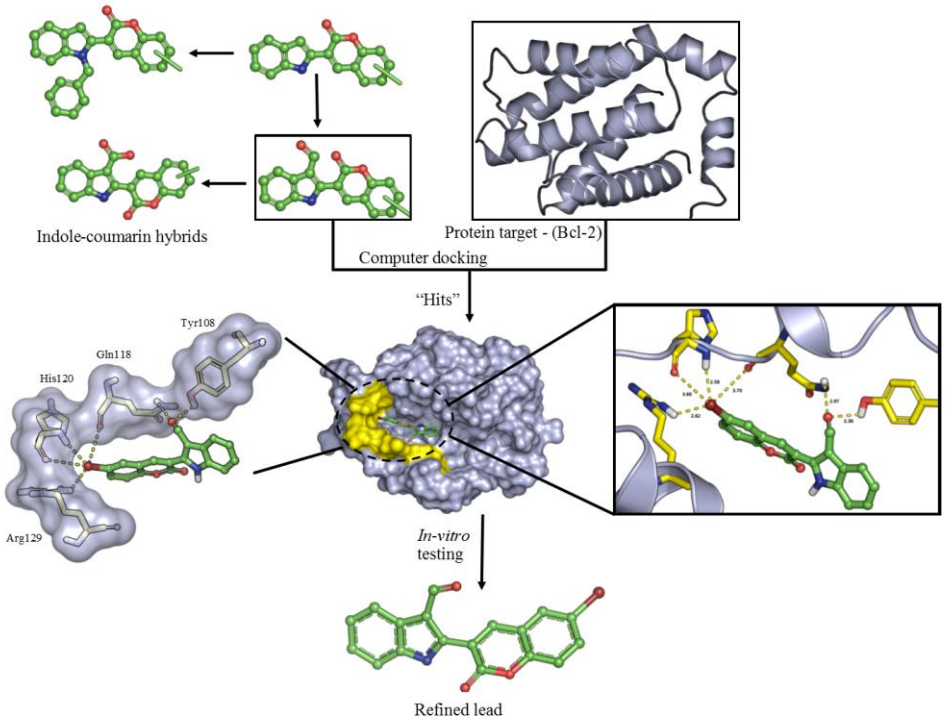








Where, R= H, Cl, Br, OH



Highlights

- Synthesis of novel indole-coumarin hybrids mediated by eaton's reagent is proposed.
- A new antimitotic agent, **4c** is identified with high selectivity to MCF7 cells.
- Presence of bromine atom in coumarin ring is found to enhance anticancer activity in SAR studies.
- Inhibition of apoptosis related protein Bcl-2 is observed in *in silico* docking studies.