Chapter 3

Experimental

CHAPTER 3: EXPERIMENTAL (MATERIAL AND METHODS)

3.1 Chemicals and Equipments

All the chemicals and starting materials (substituted o-phenylene diamines, substituted salicyaldehydes, substituted benzaldehydes and substituted 7-amino coumarin) utilized in this work were procured from Loba Chemie Pvt. Ltd., Sigma Aldrich and S.D. fine Chem Ltd. All the solvents used were of AR grade and all were redistilled before use. To monitor the progress of the reaction, silica gel 60 F254 aluminium sheets were used which were procured from E. Merck India Ltd. A mixture of pure hexane, ethylacetate and toluene was used as mobile phase solvent for TLC. Whattman No.1 filter paper was used for the filtration operations. The uncorrected melting points of the synthesized compounds were determined through Bruchi B-540 melting point apparatus in open capillaries. The TLC spots were visualized under UV light or iodine chamber. For reflux purpose, JSGW heating mantle was utilized and for drying purpose, ILMVAC rotary evaporator was used. The IR spectra of synthesized compounds were recorded on FT-IR spectroscope using potassium bromide pressed pellet technique. ^{1}H and ^{13}C NMR spectra were recorded on Bruker Avance-II spectrometer at 400 MHz and 100 MHz in DMSO-d6 and $CDCl_3$ solvents. The compound tetramethylsilane (($\delta = 0$) was used as the internal standard. The mass spectra were recorded on MicrOTOF-Q II Bruker Daltonics mass spectrometer.

3.2 Softwares

3.2.1 MOE Software

Molecular operating environment (MOE) software version 2019.0102 was used to carry out the docking studies including protein preparation, ligand preparation, energy minimization and validation of docking protocol.

3.2.2 swissADME

An online tool swissADME was used to predict the drug like properties of the designed compounds and to determine whether the compounds are following the Lipinski's rule of five or not.

3.2.3 preADMET

The ADME properties of the designed compounds were predicted using preADMET software. The compounds were evaluated for determination of absorption through HIA (human intestinal absorption), Caco-2 cell, MDCK (Maden Darby Canine Kidney), BBB, and plasma protein binding.

3.2.4 PreADME and PROTOX

Two online softwares PreADME and Protox were used to predict the toxicity of designed compounds. PreADME predicts toxicity of compounds on carcino rats, carcino mice models, and Human Ether Related Gene factor (hERG). Protox calculates the minimum lethal dose of the compounds on the basis of interactions of the compounds with various targets.

3.3 Molecular Docking Studies

Three series of 90 coumarin-quinoxaline, 90 coumarin-dihydropyrimidinone and 75 coumarin-dihydropyridine hybrid molecules were designed by making different substitutions on different positions. The designed molecules were further subjected to molecular docking against aromatase and human epidermal growth factor receptor 2 (HER-2). Various steps involved in docking process are as follows:

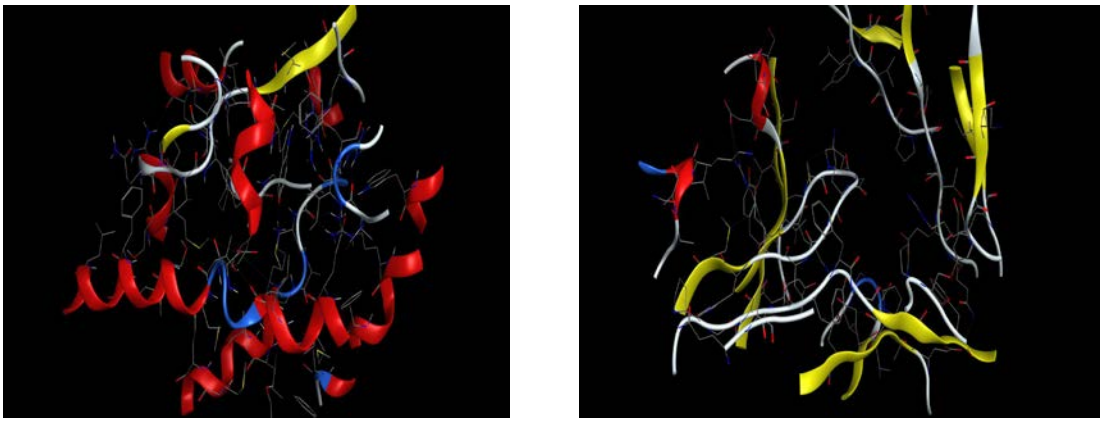
3.3.1 Preparation of Protein

The 3D crystal structures of aromatase (PDB Id: 3S7S) and HER2 (PDB Id: 3WSQ) were procured from RCSB-PDB (http:/www.rcsb.org/pdb) in pdb format. The protein cavity was prepared by using Molecular Operating Environment (MOE) version 2019.0102 by selecting the ligand nearby area up to 10Å. The sequence of steps involved addition of hydrogens, deleting the internal ligands, deletion of waters/cofactors and isolation of atoms. 3D structures of prepared cavities of both proteins have been depicted in Figure 3.1.

3.3.2 Preparation of Ligand

The 2D structures of designed compounds were drawn in Chemdraw Professional 15.0 software and were saved as mol files. The 90 ligands were designed and subjected to energy minimization in MOE software by choosing MMFF94x forcefield, Austin model 1 with gradient value of 0.0001 kcal/mol. The energy

minimized ligands were saved as tripos mol2 files and converted into mdb.mol files.



(a) **(b)**

Figure 3.1: (a) Cavity of aromatase (PDB Id: 3S7S) (b) Cavity of HER2 (PDB Id: 3WSQ)

3.3.3 Docking of designed ligands and validation

Docking of energy minimized ligands was carried out on aromatase and HER2 using MOE 2019.0102 software. Various sequence of steps involved selection of receptor, selection of site, selection of ligand file and run. The docking protocol was validated by reproducing the confirmation of internal ligand and root mean square deviation value was determined by considering confirmation of internal ligand and re-docked confirmation. The docking scores of all the ligands were determined against both the PDB Ids. Among them, top twelve from each series with best scores for both the targets were selected which were further analysed for determination of various types of interactions with the receptor amino acids in the best orientation and lowest energy state. Various types of interactions at particular distances were determined and compared with standard marketed drugs. The interaction poses and best orientation 3D poses were saved in JPEG format.

3.4 *In silico* drug likeliness prediction

Drug likeliness studies are significant to predict 'drug like' behaviour of the designed compounds and also helpful in predicting the bio-availability on the basis of physicochemical and structural properties. A compound can behave like a drug if it follows Lipinski's Rule of Five (Lipinski *et al.*, 2012). It describes that

for a compound to act as a potential therapeutic candidate, it must obey the five parameters: (a) Number of hydrogen bond donors should not be more than 5 (b) Number of hydrogen bond acceptors should not be more than 10 (c) Molecular mass should be less than 500 daltons (d) Number of rotatable bonds should not be less than 10 (e) Log P (octanol-water partition coefficient) should not be greater than 5 (Ertl *et al.,* 2000). Drug likeliness properties were determined using swissADME software.

3.5 *In silico* ADME prediction

To exert maximum therapeutic action, a drug candidate must reach at its target site in sufficient concentration. Therefore the preliminary idea about ADME properties of designed molecules is very much helpful in drug development. The ADME properties of the designed compounds were predicted using preADMET software. The compounds were evaluated for determination of absorption through HIA (human intestinal absorption), Caco-2 cell, MDCK (Maden Darby Canine Kidney), BBB (blood brain barrier) and plasma protein binding (https://preadmet.bmdrc.kr/, 2020).

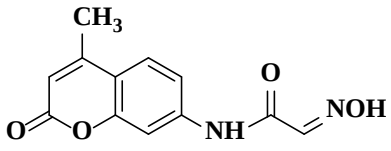
3.6 *In silico* toxicity prediction

Toxicity prediction studies are very much helpful in predicting the toxicity of designed compounds on normal cells and tissues. These are also significant to determine the safe or toxic doses of a particular compound. These *in silico* predictions reduce the number of experimental animals, are cost effective and time saving. Two online softwares PreADME and Protox were used to predict the toxicity of designed compounds. PreADME (https://preadmet.bmdrc.kr/, 2020) predicts toxicity of compounds on carcino rats, carcino mice models and Human Ether Related Gene factor (hERG).This hERG is associated with fatal cardiotoxicity (Drwal *et al.,* 2014). Protox calculates the minimum lethal dose of the compounds on the basis of interactions of the compounds with various targets (http://tox.charite.de/tox, 2020).

3.7 Synthesis of coumarin-quinoxaline derivatives (RB13-14, RB16-19, RB36-37, RB86-89)

3.7.1 Synthesis of (Z)-2-(hydroxyimino)-*N*-(2-oxo-2*H*-chromen-7-yl)acetamide derivatives (Intermediate)

A mixture of chloral hydrate (0.05 mol) and sodium sulphate (0.1 mol) was taken in a round bottomed flask and 150 ml of water was added. 0.01 mol of substituted 7-amino coumarin **1** in 50 ml of water was added to it. Further concentrated hydrochloric acid was added to dissolve the substituted coumarin and finally a solution of 0.05 mol of hydroxylamine hydrochloride, in 50 ml of water was mixed and stirred. The flask was heated under reflux for 40- 45 minutes. The crystals of product **2** get separated on cooling which were further filtered and air dried. The structure of the intermediates was confirmed with the help of IR spectroscopy and characterization of representative intermediate has been depicted in Figure 3.2.



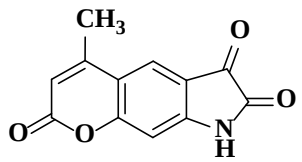
Mol. Formula: $C_{12}H_{10}N_2O_4$ **Molecular Weight:** 246.06
Yield:: 62.5% **Melting Point:** 130-132 ^oC
FTIR(KBr) cm^{-1}: 3287(-NH), 2937(-CH),3250(-OH), 1639(-C=O)

Figure 3.2: Characterization of Intermediate

3.7.2 Synthesis of substituted isatins (pyrano[3,2-f]indole-2,6,7(8*H*)-triones)

30 ml of concentrated Sulphuric acid was warmed at 50^{o}C in RBF fitted with an efficient mechanical stirrer. To this 0.05 mol of compound **2** was added by maintaining a temperature between 60-70^{o}C. After addition of compound **2**, the solution was heated at 80^{o}C and kept at this temperature for about 10 minutes. Then the reaction mixture was allowed to cool to room temperature and poured upon crushed ice. After standing for about one and half hour, the substituted isatin **3** is filtered with suction, washed several times with cold water to remove the sulphuric acid, and then dried in air (Klein *et al.*, 2013). The structure of the intermediates was confirmed with the help of IR

spectroscopy and characterization of representative isatin intermediate has been depicted in Figure 3.3.



Mol. Formula: $C_{12}H_7NO_4$ **Molecular Weight:** 229.2
Yield:: 68.5% **Melting Point:** 190-192 ^oC
FTIR(KBr) cm^{-1}: 3187(-NH), 3085(-CH), 1623(-CN), 1757(-C=O)

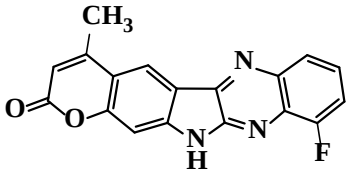
Figure 3.3: Characterization of isatin intermediate

3.7.3 Synthesis of coumarin-quinoxaline Hybrids (pyrano[3',2':5,6]indolo[2,3-b]quinoxalin-2(12H)-ones)

A solution of 0.1 mol of substituted o-phenylenediamine **4** and 0.1 mol of isatin analogs **3** was heated overnight under reflux in 0.25 mol of glacial acetic acid. After cooling to room temperature the reaction solvent was evaporated to half of its original volume. For most compounds the crude products **5 (RB series)** get precipitated immediately. In the cases in which no precipitate appeared, the reaction mixture was poured into 25 ml of water (Ivashchenko *et al.,* 1981; Shibinskaya *et al.,* 2010). The resulting precipitate was filtered off, washed with ether, and purified by crystallization from methanol-acetic acid.

***10-fluoro-4-methylpyrano[3',2':5,6]indolo[2,3-b]quinoxalin-2(12H)-one*(RB13)**

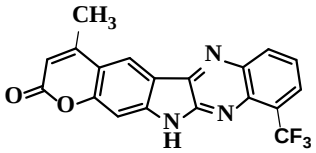
A solution of 0.1 mol of substituted 4-fuoro-o-phenylenediamine and 0.1 mol of isatin analog (4-methylpyrano[3,2-f]indole-2,6,7(8H)-trione) was heated overnight under reflux in 0.25 mol of glacial acetic acid. After cooling to room temperature the reaction solvent was evaporated to half of its original volume. The crude products get precipitated immediately. The resulting precipitate was filtered off, washed with ether, and purified by crystallization from methanol-acetic acid.



Light brown crystals, ***Rf*** : 0.72, **M.P:** 288-290º C, **Yield:** 78%	
FT-IR (KBr) cm^{-1}:	3357(-NH), 3085(-CH), 1623(-CN), 1757(-C=O), 1670(C=N), 1026(-CF)
^{1}H NMR(400 MHz, $CDCl_3$-d_6):*δ*	13.05 (s, 1H, NH), 7.83-7.67 (m, 3H, Ar-CH), 7.29 (t, 1H, Ar-CH), 7.17 (s, 1H, Ar-CH),6.17 (s, 1H, Pyrone), 2.87 (s, 3H, CH_3)
^{13}C NMR (100 MHz, $CDCl_3$-d_6):*δ*	161.6, 160.8, 152.8, 144.7, 144.5, 142.6, 141.7, 135.7, 129.6, 128.3, 125.3, 121.9, 118.6, 116.7, 113.5, 112.6, 102.3, 20.7
HRMS (micro TOF-QII, MS, ESI):	m/z $[M+H]^+$ Calculated for $C_{18}H_{10}FN_3O_2$ 320.0791, Obsd.: 320.0798.

***4-methyl-10-(trifluoromethyl)pyrano[3',2':5,6]indolo[2,3-b]quinoxalin-2(12H)-one* (RB14)**

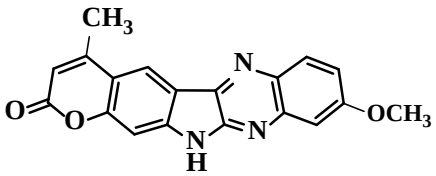
A solution of 0.1 mol g of substituted 3-(trifluoromethyl)benzene-1,2-diamine and 0.1 mol g of isatin analog (4-methylpyrano[3,2-f]indole-2,6,7(8H)-trione) was heated overnight under reflux in 0.25 of glacial acetic acid. After cooling to room temperature the reaction solvent was evaporated to half of its original volume. The crude products get precipitated immediately. The resulting precipitate was filtered off, washed with ether, and purified by crystallization from methanol-acetic acid.



Brown crystals, ***Rf*** : 0.78, **M.P:** 268-270º C, **Yield:** 68%	
FT-IR (KBr) cm^{-1}:	3362(-NH), 3065(-CH), 1633(-CN), 1752(-C=O), 1667(C=N), 1088(-CF)
^{1}H NMR(400 MHz, $CDCl_3$-d_6):*δ*	12.85 (s, 1H, NH), 7.87-7.84 (dd, 2H, Ar-CH, J=7.5, 1.5 Hz), 7.61-7.58 (t, 2H, Ar-CH), 7.27 (s, 1H, Ar-CH), 6.27 (s, 1H, Pyrone), 2.85 (s, 3H, CH_3)
^{13}C NMR (100 MHz, $CDCl_3$-d_6):*δ*	161.2, 152.4, 145.8, 145.5, 144.6, 141.6, 139.7, 135.7, 134.9, 130.5, 129.6, 127.3, 124.3, 121.3, 118.2, 116.6, 112.8, 102.3, 19.8
HRMS (micro TOF-QII, MS, ESI):	m/z $[M+H]^+$ Calculated for $C_{19}H_{10}F_3N_3O_2$ 370.0759, Obsd.: 370.0698.

***9-methoxy-4-methylpyrano[3',2':5,6]indolo[2,3-b]quinoxalin-2(12H)-one* (RB16)**

A solution of 0.1 mol of substituted 4-methoxybenzene-1,2-diamine and 0.1 mol of isatin analog (4-methylpyrano[3,2-f]indole-2,6,7(8H)-trione) was heated overnight under reflux in 0.25 mol of glacial acetic acid. After cooling to room temperature the reaction solvent was evaporated to half of its original volume. The contents were then poured into ice cold water with constant stirring leading to conversion into solid precipitate. The resulting precipitate was filtered off, washed with ether, and purified by crystallization from methanol-acetic acid.

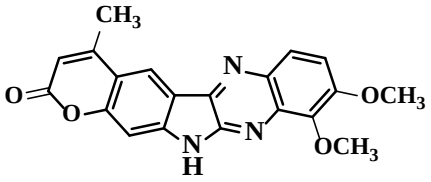


Orange crystals, ***Rf*** : 0.68, **M.P:** 298-301º C, **Yield:** 72%	
FT-IR (KBr) cm^{-1}:	3371(-NH), 3096(-CH), 1673(-CN), 1748(-C=O), 1667(C=N), 1250(-CO)
^{1}H NMR(400 MHz, $CDCl_3$-d_6):*δ*	12.54 (s, 1H, NH), 7.57-7.63 (m, 3H, Ar-CH), 7.30 (d, 1H, Ar-CH, J=7.5 Hz), 7.21 (s, 1H, Ar-CH), 6.23 (s, 1H, Pyrone), 2.53 (s, 3H, CH_3), 3.91 (s, 3H, OCH_3)
^{13}C NMR (100 MHz, $CDCl_3$-d_6):*δ*	161.2, 160.48, 154.8, 152.6, 144.8, 141.5, 138.6, 137.6, 135.7, 130.5, 127.6, 121.3, 118.6, 116.2, 112.7, 103.3, 102.5, 55.8, 19.4
HRMS (micro TOF-QII, MS, ESI):	m/z $[M+H]^+$ Calculated for $C_{19}H_{13}N_3O_3$ 332.0990, Obsd.: 332.0927.

***9,10-dimethoxy-4-methylpyrano[3',2':5,6]indolo[2,3-b]quinoxalin-2(12H)-one* (RB17)**

A solution of 0.1 mol of substituted 3,4-dimethoxybenzene-1,2-diamine and 0.1 mol of isatin analog (4-methylpyrano[3,2-f]indole-2,6,7(8H)-trione) was heated overnight under reflux in 0.25 mol of glacial acetic acid. After cooling to room temperature the reaction solvent was evaporated to half of its original volume. The contents were then poured into ice cold water with constant stirring leading to conversion into solid

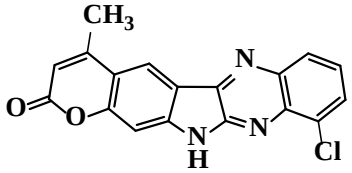
precipitate. The resulting precipitate was filtered off, washed with ether, and purified by crystallization from methanol-acetic acid.



Orange crystals, ***Rf*** : 0.61, **M.P:** 298-301º C, **Yield:** 66%	
FT-IR (KBr) cm^{-1}:	3371(-NH), 3026(-CH), 1671(-CN), 1758(-C=O), 1677(C=N), 1250(-CO)
^{1}H NMR(400 MHz, CDCl$_3$-d$_6$):*δ*	12.64 (s, 1H, NH), 7.63 (s, 1H, Ar-CH), 7.35-7.31 (dd, 2H, Ar-CH, J= 11.5, 3.5 Hz), 7.21 (s, 1H, Ar-CH), 6.31 (s, 1H, Pyrone), 2.49 (s, 3H, CH_3), 3.81 (s, 6H, OCH_3)
^{13}C NMR (100 MHz, CDCl$_3$-d$_6$):*δ*	160.48, 152.8, 143.6, 141.8, 141.5, 139.6, 138.6, 135.7, 134.5, 132.6, 122.9, 122.3, 121.6, 118.2, 116.7, 112.3, 102.5, 60.8, 55.8, 20.04
HRMS (micro TOF-QII, MS, ESI):	m/z $[M+H]^+$ Calculated for $C_{20}H_{15}N_3O_4$ 362.1091, Obsd.: 362.1027.

10-chloro-4-methylpyrano[3',2':5,6]indolo[2,3-b]quinoxalin-2(12H)-one **(RB18)**

A solution of 0.1 mol of substituted 4-chloro-o-phenylenediamine and 0.1 mol of isatin analog (4-methylpyrano[3,2-f]indole-2,6,7(8H)-trione) was heated overnight under reflux in 0.25 mol of glacial acetic acid. After cooling to room temperature the reaction solvent was evaporated to half of its original volume. The crude products get precipitated immediately. The resulting precipitate was filtered off, washed with ether, and purified by crystallization from methanol-acetic acid.

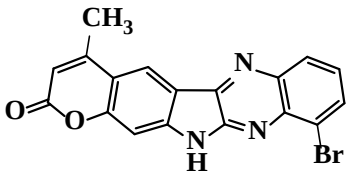


Brown crystals, ***Rf*** : 0.72, **M.P:** 278-280º C, **Yield:** 78%	
FT-IR (KBr) cm^{-1}:	3357(-NH), 3085(-CH), 1623(-CN), 1757(-

	C=O), 1670(C=N), 670(-CCl)
^{1}H NMR(400 MHz, $CDCl_3$-d_6):*δ*	13.05 (s, 1H, NH), 7.73-7.61 (m, 3H, Ar-CH), 7.21 (s, 1H, Ar-CH), 7.17 (d, 1H, Ar-CH, J=8.2 Hz), 6.27 (s, 1H, Pyrone), 2.67 (s, 3H, CH_3)
^{13}C NMR (100 MHz, $CDCl_3$-d_6):*δ*	160.8, 152.8, 144.7, 144.5, 143.5, 142.6, 141.7, 135.7, 129.6, 128.3, 125.3, 121.9, 118.6, 116.7, 113.5, 112.6, 102.3, 20.7
HRMS (micro TOF-QII, MS, ESI):	m/z $[M+H]^+$ Calculated for $C_{18}H_{10}ClN_3O_2$ 336.0432, Obsd.: 336.0398.

10-bromo-4-methylpyrano[3',2':5,6]indolo[2,3-b]quinoxalin-2(12H)-one **(RB19)**

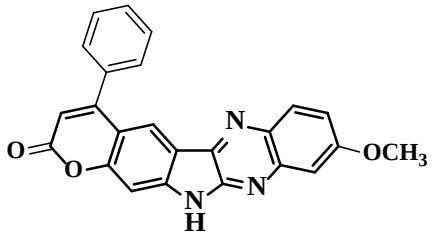
A solution of 0.1 mol of substituted 4-bromo-o-phenylenediamine and 0.1 mol of isatin analog (4-methylpyrano[3,2-f]indole-2,6,7(8H)-trione) was heated overnight under reflux in 0.25 mol of glacial acetic acid. After cooling to room temperature the reaction solvent was evaporated to half of its original volume. The crude products get precipitated immediately. The resulting precipitate was filtered off, washed with ether, and purified by crystallization from methanol-acetic acid.



Dark Brown crystals, ***Rf*** : 0.69, **M.P:** 271-273º C, **Yield:** 76%	
FT-IR (KBr) cm^{-1}:	3357(-NH), 3085(-CH), 1623(-CN), 1757(-C=O), 1670(C=N), 626(-CBr)
^{1}H NMR(400 MHz, $CDCl_3$-d_6):*δ*	12.67 (s, 1H, NH), 7.81 (d, 1H, Ar-CH, J=7.5 Hz), 7.67 (d, 1H, Ar-CH, J= 8.5 Hz), 7.57-7.51 (m, 2H, Ar-CH), 7.21 (s, 1H, Ar-CH), 6.23 (s, 1H, Pyrone), 2.57 (s, 3H, CH_3)
^{13}C NMR (100 MHz, $CDCl_3$-d_6):*δ*	160.8, 152.8, 142.7, 142.1, 141.7, 141.2, 135.7, 130.7, 129.6, 128.3, 127.3, 121.7, 118.6, 116.7, 113.5, 112.6, 102.3, 20.3
HRMS (micro TOF-QII, MS, ESI):	m/z $[M+H]^+$ Calculated for $C_{18}H_{10}BrN_3O_2$ 379.9927, Obsd.: 379.9988.

***9-methoxy-4-phenylpyrano[3',2':5,6]indolo[2,3-b]quinoxalin-2(12H)-one* (RB36)**

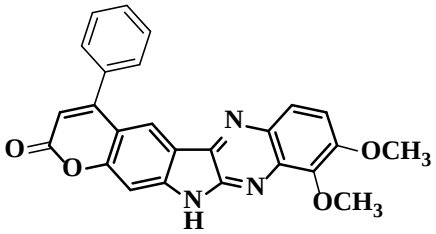
A solution of 0.1 mol of substituted 3-methoxybenzene-1,2-diamine and 0.1 mol of isatin analog 4-phenylpyrano[3,2-f]indole-2,6,7(8H)-trione was heated overnight under reflux in 0.25 mol of glacial acetic acid. After cooling to room temperature the reaction solvent was evaporated to half of its original volume. The crude products get precipitated immediately. The resulting precipitate was filtered off, washed with ether, and purified by crystallization from methanol-acetic acid.



Buff colored crystals, ***Rf*** : 0.62, **M.P:** 281-283º C, **Yield:** 56%	
FT-IR (KBr) cm^{-1}:	3371(-NH), 3026(-CH), 1671(-CN), 1758(-C=O), 1677(C=N), 1250(-CO)
^{1}H NMR(400 MHz, $CDCl_3$-d_6):*δ*	12.57 (s, 1H, NH), 7.67-7.61 (t, 3H, Ar-CH), 7.41-7.37 (m, 6H, Ar-CH), 7.21 (s, 1H, Ar-CH), 6.27 (s, 1H, Pyrone), 3.87 (s, 3H, OCH_3)
^{13}C NMR (100 MHz, $CDCl_3$-d_6):*δ*	161.3, 160.8, 155.7, 154.8, 144.8, 141.7, 139.1, 138.4, 137.7, 135.8, 135.4, 130.7, 128.6, 128.4, 128.3, 128.6, 127.6, 121.7, 118.6, 116.8, 112.6, 103.5, 102.3, 55.3
HRMS (micro TOF-QII, MS, ESI):	m/z $[M+H]^+$ Calculated for $C_{24}H_{15}N_3O_3$ 394.1147, Obsd.: 394.1188.

***9,10-dimethoxy-4-phenylpyrano[3',2':5,6]indolo[2,3-b]quinoxalin-2(12H)-one* (RB37)**

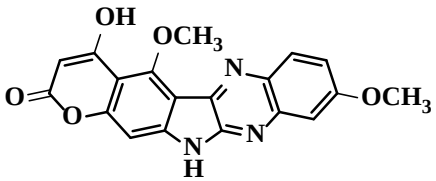
A solution of 0.1 mol of substituted 3,4-dimethoxybenzene-1,2-diamine and 0.1 mol of isatin analog 4-phenylpyrano[3,2-f]indole-2,6,7(8H)-trione was heated overnight under reflux in 0.25 mol of glacial acetic acid. After cooling to room temperature the reaction solvent was evaporated to half of its original volume. The crude products get precipitated immediately. The resulting precipitate was filtered off, washed with ether, and purified by crystallization from methanol-acetic acid.



Dark yellow crystals, ***Rf*** : 0.67, **M.P:** 278-281º C, **Yield:** 58%	
FT-IR (KBr) cm^{-1}:	3371(-NH), 3026(-CH), 1671(-CN), 1758(-C=O), 1677(C=N), 1250(-CO)
^{1}H NMR(400 MHz, CDCl$_3$-d$_6$):*δ*	12.48 (s, 1H, NH), 7.67 (s, 1H, Ar-CH), 7.37-7.29 (m, 7H, Ar-CH), 7.21 (s, 1H, Ar-CH), 6.27 (s, 1H, Pyrone), 3.81 (s, 6H, OCH_3)
^{13}C NMR (100 MHz, CDCl$_3$-d$_6$):*δ*	160.8, 155.7, 143.8, 141.9, 141.3, 139.1, 138.4, 137.7, 135.8, 135.4, 130.7, 128.6, 128.4, 128.3, 128.6, 127.6, 122.3, 121.7, 118.6, 116.8, 112.6, 103.5, 102.3, 60.8, 55.3
HRMS (micro TOF-QII, MS, ESI):	m/z $[M+H]^+$ Calculated for $C_{25}H_{17}N_3O_4$ 424.1253, Obsd.: 424.1281.

***4-hydroxy-5,9-dimethoxypyrano[3',2':5,6]indolo[2,3-b]quinoxalin-2(12H)-one* (RB86)**

A solution of 0.1 mol of 3-methoxybenzene-1,2-diamine and 0.1 mol of isatin analog 4-hydroxy-5-methoxypyrano[3,2-f]indole-2,6,7(8H)-trione was heated overnight under reflux in 0.25 mol of glacial acetic acid. After cooling to room temperature the reaction solvent was evaporated to half of its original volume. The mixture was then poured into ice cold water with constant stirring leading to formation of precipitates. The resulting precipitate was filtered off, washed with ether, and purified by crystallization from methanol-acetic acid.

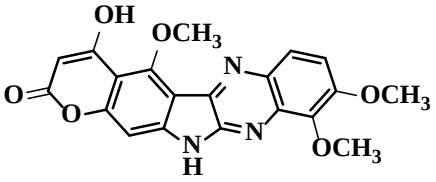


Dull green crystals, ***Rf*** : 0.57, **M.P:** 308-310º C, **Yield:** 62%	
FT-IR (KBr) cm^{-1}:	3540(-OH), 3371(-NH), 3026(-CH), 1671(-CN), 1758(-C=O), 1677(C=N), 1250(-CO)

^{1}H NMR(400 MHz, $CDCl_3$-d_6):*δ*	14.98 (s, 1H, OH), 10.08 (s, 1H, NH), 7.69-7.67 (d, 2H, Ar-CH, J=8 Hz), 6.77 (d, 1H, Ar-CH, 6.5 Hz), 6.44 (s, 1H, Ar-CH), 6.27 (s, 1H, Pyrone), 3.87-3.83 (d, 6H, OCH_3, 16 Hz)
^{13}C NMR (100 MHz, $CDCl_3$-d_6):*δ*	167.7, 162.4, 159.6, 154.5, 152.6, 148.5, 142.8, 140.7, 138.2, 136.6, 130.6, 128.6, 112.5, 108.8, 103.2, 94.5, 91.2, 61.3, 50.7
HRMS (micro TOF-QII, MS, ESI):	m/z $[M+H]^+$ Calculated for $C_{19}H_{13}N_3O_5$ 364.0889, Obsd.: 364.1045.

***4-hydroxy-5,9,10-trimethoxypyrano[3',2':5,6]indolo[2,3-b]quinoxalin-2(12H)-one* (RB87)**

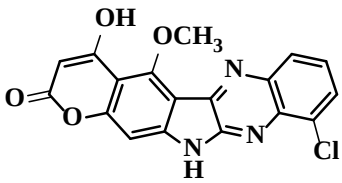
A solution of 0.1 mol of 3,4-dimethoxybenzene-1,2-diamine and 0.1 mol of isatin analog 4-hydroxy-5-methoxypyrano[3,2-f]indole-2,6,7(8H)-trione was heated overnight under reflux in 0.25 mol of glacial acetic acid. After cooling to room temperature the reaction solvent was evaporated to half of its original volume. The mixture was then poured into ice cold water with constant stirring leading to formation of precipitates. The resulting precipitate was filtered off, washed with ether, and purified by crystallization from methanol-acetic acid.



Dull green crystals, ***Rf*** : 0.59, **M.P:** 308-310º C, **Yield:** 65%	
FT-IR (KBr) cm^{-1}:	3548(-OH), 3371(-NH), 3026(-CH), 1671(-CN), 1758(-C=O), 1677(C=N), 1250(-CO)
^{1}H NMR(400 MHz, $CDCl_3$-d_6):*δ*	14.08 (s, 1H, OH), 12.74 (s, 1H, NH), 7.33-7.29 (m, 2H, Ar-CH), 7.14 (s, 1H, Ar-CH), 6.47 (s, 1H, Pyrone), 3.87 (s, 9H, OCH_3)
^{13}C NMR (100 MHz, $CDCl_3$-d_6):*δ*	167.7, 162.4, 161.9, 154.6, 152.6, 144.5, 142.8, 139.4, 137.6, 136.2, 130.6, 127.8, 112.8, 108.8, 103.2, 94.5, 91.2, 61.3, 60.8, 56.2
HRMS (micro TOF-QII, MS, ESI):	m/z $[M+H]^+$ Calculated for $C_{20}H_{15}N_3O_6$ 394.0994, Obsd.: 394.0981.

***10-chloro-4-hydroxy-5-methoxypyrano[3',2':5,6]indolo[2,3-b]quinoxalin-2(12H)-one* (RB88)**

A solution of 0.1 mol of 4-chloro-o-phenylenediamine and 0.1 mol of isatin analog 4-hydroxy-5-methoxypyrano[3,2-f]indole-2,6,7(8H)-trione was heated overnight under reflux in 0.25 mol of glacial acetic acid. After cooling to room temperature the reaction solvent was evaporated to half of its original volume. The mixture was then poured into ice cold water with constant stirring leading to formation of precipitates. The resulting precipitate was filtered off, washed with ether, and purified by crystallization from methanol-acetic acid.

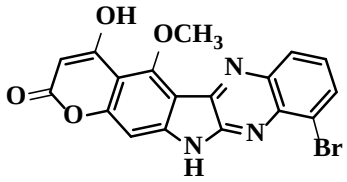


Light brown crystals, ***Rf*** : 0.69, **M.P:** 265-267º C, **Yield:** 68%	
FT-IR (KBr) cm^{-1}:	3558(-OH), 3371(-NH), 3026(-CH), 1671(-CN), 1758(-C=O), 1677(C=N), 1250(-CO), 691(-CCl)
^{1}H NMR(400 MHz, $CDCl_3$-d_6):*δ*	15.28 (s, 1H, OH), 12.54 (s, 1H, NH), 7.63-7.59 (m, 2H, Ar-CH), 7.14-7.11 (m, 2H, Ar-CH), 6.37 (s, 1H, Pyrone), 3.83 (s, 3H, OCH_3)
^{13}C NMR (100 MHz, $CDCl_3$-d_6):*δ*	167.7, 162.4, 152.6, 144.5, 144.2, 143.7, 142.8, 140.4, 136.6, 133.2, 131.6, 129.8, 128.8, 109.8, 106.2, 94.5, 91.2, 61.3
HRMS (micro TOF-QII, MS, ESI):	m/z $[M+H]^+$ Calculated for $C_{18}H_{10}ClN_3O_4$ 368.0393, Obsd.: 368.0381.

***10-bromo-4-hydroxy-5-methoxypyrano[3',2':5,6]indolo[2,3-b]quinoxalin-2(12H)-one* (RB89)**

A solution of 0.1 mol of 4-bromo-o-phenylenediamine and 0.1 mol of isatin analog 4-hydroxy-5-methoxypyrano[3,2-f]indole-2,6,7(8H)-trione was heated overnight under reflux in 0.25 mol of glacial acetic acid. After cooling to room temperature the reaction solvent was evaporated to half of its original volume. The mixture was then poured into ice cold water with constant stirring leading to formation of precipitates.

The resulting precipitate was filtered off, washed with ether, and purified by crystallization from methanol-acetic acid.



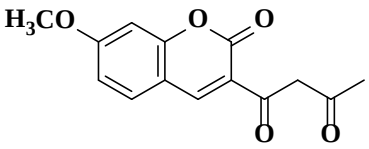
Brown crystals, ***Rf*** : 0.69, **M.P:** 266-268º C, **Yield:** 72%	
FT-IR (KBr) cm^{-1}:	3526(-OH), 3371(-NH), 3026(-CH), 1671(-CN), 1758(-C=O), 1677(C=N), 1250(-CO), 616(-CBr)
^{1}H NMR(400 MHz, CDCl$_3$-d$_6$):*δ*	15.28 (s, 1H, OH), 12.54 (s, 1H, NH), 7.83-7.73 (dd, 2H, Ar-CH, J=7.5, 2.5 Hz), 7.55 (t, 1H, Ar-CH), 6.27 (s, 1H, Pyrone), 7.04 (s, Ar-CH), 3.87 (s, 3H, OCH_3)
^{13}C NMR (100 MHz, CDCl$_3$-d$_6$):*δ*	167.2, 162.1, 150.6, 144.5, 144.2, 143.5, 141.8, 140.4, 136.6, 133.2, 131.6, 129.8, 128.8, 109.8, 105.2, 93.5, 91.7, 61.5
HRMS (micro TOF-QII, MS, ESI):	m/z $[M+H]^+$ Calculated for $C_{18}H_{10}BrN_3O_4$ 411.9888, Obsd.: 411.9876.

3.8 Synthesis of coumarin-dihydropyrimidinone derivatives (CD6, CD8-10, CD19-20, CD28, CD32, CD42-44, CD49)

3.8.1 Synthesis of substituted 3-acetoacetyl coumarins 4 (1-(2-oxo-2H- chromen-3-yl)butane-1,3-dione)

0.07 mol of substituted salicyaldehyde **6** was mixed with 0.07 mol of ethylacetoacetate **7**; the mixture was basified with piperidine and stirred under cold conditions for 2 hours. The mixture was neutralized by addition of 1M HCl solution to obtain compound substituted 3-acetyl coumarins **8**. Compound **8** (0.04 mol) was further dissolved in ethyl acetate (15 ml) **9** and stirred under cold conditions. Further solution of potassium tertiary butoxide (0.04 mol) in toluene was introduced dropwise to the mixture with continuous stirring. After two hours potassium salt of 3-acetoacetyl coumarin separates out and the mixture was kept in ice box overnight. The solid was filtered by aid of ether, dissolved in cold water and acidified with acetic acid. The crude 3-acetoacetyl coumarin **10** was

again filtered, air dried and recrystallized from ethanol. The structure of this intermediate was confirmed with the help of IR spectroscopy and 1H-NMR (Figure 3.4).



Mol. Formula: $C_{14}H_{12}O_5$ **Molecular Weight:** 260.0685
Yield: 45% **Melting Point:** 152-154^oC
FTIR(KBr)cm^{-1}: 2937(-CH), 1751(-C=O), 1257(-CO)
^{1}H NMR(400 MHz, $CDCl_3$-d_6): 8.45(s, 1H, pyrone), 7.68(d, 1H, Ar-CH), 7.01-7.04(t, 2H, Ar-CH), 4.10(s, 2H, CH_2), 3.81(s, 3H, OCH_3), 2.42(s, 3H, CH_3)

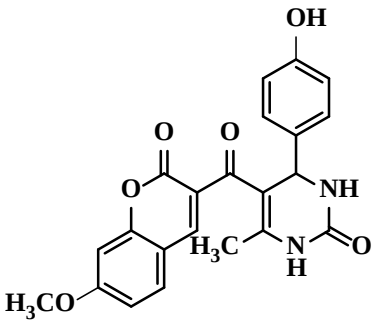
Figure 3.4: Characterization of 3-acetoacetyl coumarin derivative

3.8.2 General procedure for synthesis of coumarin-dihydropyrimidinone hybrids (6-methyl-5-(2-oxo-2H-chromene-3-carbonyl)-4-phenyl-3,4 dihydropyrimidin-2(1H)-ones)

0.07 mol of compound **10** was mixed with 0.05 mol of substituted benzaldehydes **11** and 0.2 mol of urea and refluxed in the presence of acetonitrile and sulphuric acid for 4 hours. The oily liquid was then poured in water and target compounds **12 (CD series)** were precipitated out as solid mass. It was filtered, washed with cold water and recrystallized from ethanol.

4-(4-hydroxyphenyl)-5-(7-methoxy-2-oxo-2H-chromene-3-carbonyl)-6-methyl-3,4-dihydropyrimidin-2(1H)-one **(CD6)**

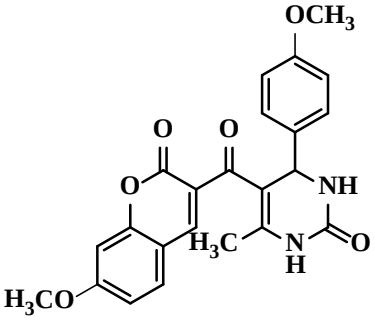
0.07 mol of 7-methoxy-3-acetoacetyl coumarin was mixed with 0.05 mol of 4-hydroxy benzaldehyde and 0.2 mol of urea and refluxed in the presence of acetonitrile and sulphuric acid for 4 hours. The oily liquid was then poured in water and target compounds were precipitated out as solid mass. It was filtered, washed with cold water and recrystallized from ethanol.



Pale yellow crystals, ***Rf*** : 0.72, **M.P:** 268-270º C, **Yield:** 66%	
FT-IR (KBr) cm^{-1}:	3526(-OH), 3371(-NH), 3026(-CH), 1671(-CN), 1758(-C=O), 1677(C=N), 1250(-CO)
^{1}H NMR(400 MHz, CDCl$_3$-d$_6$):*δ*	9.08-9.05 (d, 2H, OH & NH, J= 12Hz), 8.52 (s, 1H, pyrone), 7.62 (d, 1H, Ar-CH, J=6.5 Hz), 7.52 (s, 1H, NH), 6.97-6.93 (m, 4H, Ar-CH), 6.68(d, 2H, Ar-CH), 5.18 (s, 1H, CH), 3.78 (s, 3H, OCH_3), 2.32(s, 3H, CH_3)
^{13}C NMR (100 MHz, CDCl$_3$-d$_6$):*δ*	199.2, 160.4, 159.7, 156.6, 154.8, 154.2, 150.2, 147.6, 135.8, 134.4, 129.8, 126.2, 126.1, 118.3, 115.4, 115.3, 111.6, 110.4, 100.5, 55.6, 51.2, 17.8
HRMS (micro TOF-QII, MS, ESI):	m/z $[M+H]^+$ Calculated for $C_{22}H_{18}N_2O_6$ 407.1198, Obsd.: 407.1186.

5-(7-methoxy-2-oxo-2H-chromene-3-carbonyl)-4-(4-methoxyphenyl)-6-methyl-3,4-dihydropyrimidin-2(1H)-one **(CD8)**

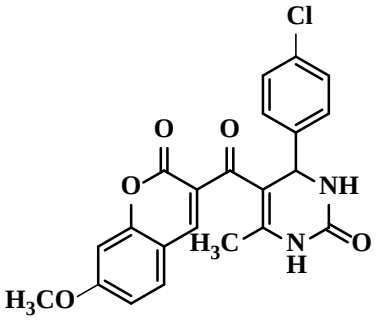
0.07 mol of 7-methoxy-3-acetoacetyl coumarin was mixed with 0.05 mol of 4-methoxy benzaldehyde and 0.2 mol of urea and refluxed in the presence of acetonitrile and sulphuric acid for 4 hours. The oily liquid was then poured in water and target compounds were precipitated out as solid mass. It was filtered, washed with cold water and recrystallized from ethanol.



Yellow crystals, ***Rf*** : 0.78, **M.P:** 286-288º C, **Yield:** 72%	
FT-IR (KBr) cm^{-1}:	3561(-OH), 3341(-NH), 3046(-CH), 1673(-CN), 1742(-C=O), 1673(C=N), 1256(-CO)
^{1}H NMR(400 MHz, CDCl$_3$-d$_6$):*δ*	8.97 (s, 1H, NH), 8.57 (s, 1H, pyrone), 7.59 (d, 1H, Ar-CH, J=6.5 Hz), 7.58 (s, 1H, NH), 7.16 (d, 2H, Ar-CH, 2Hz), 7.04-7.01 (t, 2H, Ar-CH), 6.67(d, 2H, Ar-CH), 5.12 (s, 1H, CH), 3.81-3.78 (d, 6H, OCH_3), 2.42(s, 3H, CH_3)
^{13}C NMR (100 MHz, CDCl$_3$-d$_6$):*δ*	199.7, 160.1, 159.3, 156.2, 154.9, 154.3, 150.4, 147.3, 135.5, 134.4, 129.8, 126.2, 126.1, 118.3, 115.4, 115.3, 111.6, 110.4, 100.5, 55.8, 55.8, 51.4, 17.2
HRMS (micro TOF-QII, MS, ESI):	m/z $[M+H]^+$ Calculated for $C_{23}H_{20}N_2O_6$ 421.1355, Obsd.: 421.1386.

4-(4-chlorophenyl)-5-(7-methoxy-2-oxo-2H-chromene-3-carbonyl)-6-methyl-3,4-dihydropyrimidin-2(1H)-one **(CD9)**

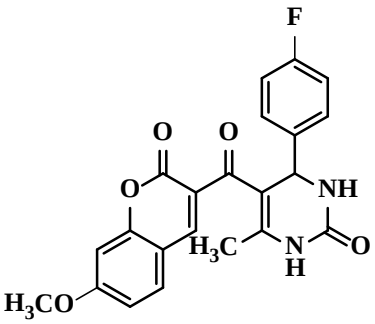
0.07 mol of 7-methoxy-3-acetoacetyl coumarin was mixed with 0.05 mol of 4-chloro benzaldehyde and 0.2 mol of urea and refluxed in the presence of acetonitrile and sulphuric acid for 4 hours. The oily liquid was then poured in water and target compounds were precipitated out as solid mass. It was filtered, washed with cold water and recrystallized from ethanol.



Off white crystals, ***Rf*** : 0.70, **M.P:** 258-261º C, **Yield:** 78%	
FT-IR (KBr) cm^{-1}:	3341(-NH), 3046(-CH), 1673(-CN), 1742(-C=O), 1673(C=N), 1256(-CO), 691(-CCl)
^{1}H NMR(400 MHz, CDCl$_3$-d$_6$):*δ*	9.07 (s, 1H, NH), 8.67 (s, 1H, pyrone), 7.69 (d, 1H, Ar-CH, J=6.5 Hz), 7.58 (s, 1H, NH), 7.38-7.33 (m, 4H, Ar-CH), 7.07-7.03 (t, 2H, Ar-CH), 5.13 (s, 1H, CH), 3.84 (s, 3H, OCH_3), 2.52(s, 3H, CH_3)
^{13}C NMR (100 MHz, CDCl$_3$-d$_6$):*δ*	198.7, 160.3, 159.6, 154.9, 154.2, 154.3, 150.4, 141.3, 134.5, 132.4, 129.8, 128.2, 128.1, 126.3, 126.2, 118.3, 111.6, 110.4, 100.5, 55.8, 51.8, 17.2
HRMS (micro TOF-QII, MS, ESI):	m/z $[M+H]^+$ Calculated for $C_{22}H_{17}ClN_2O_5$ 425.0860, Obsd.: 425.0876.

4-(4-fluorophenyl)-5-(7-methoxy-2-oxo-2H-chromene-3-carbonyl)-6-methyl-3,4-dihydropyrimidin-2(1H)-one **(CD10)**

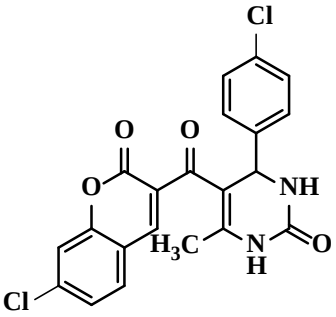
0.07 mol of 7-methoxy-3-acetoacetyl coumarin was mixed with 0.05 mol of 4-fluoro benzaldehyde and 0.2 mol of urea and refluxed in the presence of acetonitrile and sulphuric acid for 4 hours. The oily liquid was then poured in water and target compounds were precipitated out as solid mass. It was filtered, washed with cold water and recrystallized from ethanol.



Dull yellow crystals, ***Rf*** : 0.62, **M.P:** 242-244º C, **Yield:** 80%	
FT-IR (KBr) cm^{-1}:	3371(-NH), 3026(-CH), 1643(-CN), 1748(-C=O), 1671(C=N), 1256(-CO), 1026(-CF)
^{1}H NMR(400 MHz, $CDCl_3$-d_6):*δ*	9.03 (s, 1H, NH), 8.52 (s, 1H, pyrone), 7.59 (d, 1H, Ar-CH, J=6.5 Hz), 7.48 (s, 1H, NH), 7.35-7.31 (m, 4H, Ar-CH), 7.07-7.03 (t, 2H, Ar-CH), 5.13 (s, 1H, CH), 3.84 (s, 3H, OCH_3), 2.52(s, 3H, CH_3)
^{13}C NMR (100 MHz, $CDCl_3$-d_6):*δ*	199.3, 160.9, 159.1, 154.6, 154.8, 154.3, 150.4, 141.3, 134.5, 132.4, 129.8, 128.2, 128.1, 126.3, 126.2, 118.3, 111.6, 110.4, 100.5, 55.7, 51.3, 17.2
HRMS (micro TOF-QII, MS, ESI):	m/z $[M+H]^+$ Calculated for $C_{22}H_{17}FN_2O_5$ 409.1155, Obsd.: 409.1167.

5-(7-chloro-2-oxo-2H-chromene-3-carbonyl)-4-(4-chlorophenyl)-6-methyl-3,4-dihydropyrimidin-2(1H)-one **(CD19)**

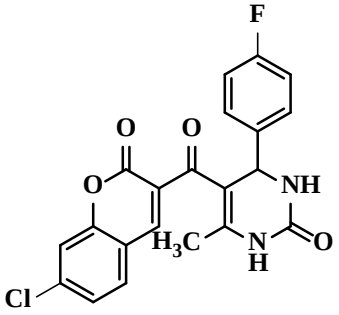
0.07 mol of 7-chloro-3-acetoacetyl coumarin was mixed with 0.05 mol of 4-chloro benzaldehyde and 0.2 mol of urea and refluxed in the presence of acetonitrile and sulphuric acid for 4 hours. The oily liquid was then poured in water and target compounds were precipitated out as solid mass. It was filtered, washed with cold water and recrystallized from ethanol.



Light brown crystals, ***Rf*** : 0.67, **M.P:** 229-233º C, **Yield:** 56%	
FT-IR (KBr) cm^{-1}:	3371(-NH), 3026(-CH), 1643(-CN), 1748(-C=O), 1671(C=N), 1256(-CO), 730(-CCl)
^{1}H NMR(400 MHz, CDCl$_3$-d$_6$):*δ*	9.08 (s, 1H, NH), 8.57 (s, 1H, pyrone), 7.68 (d, 1H, Ar-CH, J=6.5 Hz), 7.48 (s, 1H, NH), 7.38-7.29 (m, 6H, Ar-CH), 5.13 (s, 1H, CH), 2.57(s, 3H, CH_3)
^{13}C NMR (100 MHz, CDCl$_3$-d$_6$):*δ*	199.9, 159.3, 154.6, 154.3, 150.2, 147.8, 141.3, 135.7, 134.5, 132.4, 129.8, 128.2, 128.1, 126.3, 126.2, 119.2, 118.3, 117.6, 116.2, 51.3, 17.2
HRMS (micro TOF-QII, MS, ESI):	m/z $[M+H]^+$ Calculated for $C_{21}H_{14}Cl_2N_2O_4$ 429.0364, Obsd.: 429.0377.

5-(7-chloro-2-oxo-2H-chromene-3-carbonyl)-4-(4-fluorophenyl)-6-methyl-3,4-dihydropyrimidin-2(1H)-one **(CD20)**

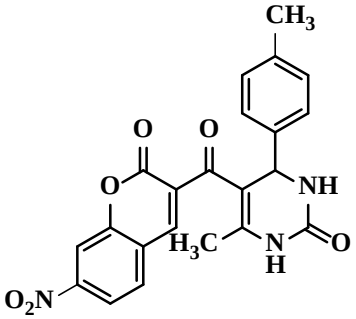
0.07 mol of 7-chloro-3-acetoacetyl coumarin was mixed with 0.05 mol of 4-fluoro benzaldehyde and 0.2 mol of urea and refluxed in the presence of acetonitrile and sulphuric acid for 4 hours. The oily liquid was then poured in water and target compounds were precipitated out as solid mass. It was filtered, washed with cold water and recrystallized from ethanol.



Light green crystals, ***Rf*** : 0.67, **M.P:** 229-233º C, **Yield:** 56%	
FT-IR (KBr) cm^{-1}:	3371(-NH), 3026(-CH), 1643(-CN), 1748(-C=O), 1671(C=N), 1256(-CO), 680(-CCl), 1082(-CF)
^{1}H NMR(400 MHz, $CDCl_3$-d_6):*δ*	9.02 (s, 1H, NH), 8.67 (s, 1H, pyrone), 7.61 (d, 1H, Ar-CH, J=6.5 Hz), 7.48 (s, 1H, NH), 7.26-7.19 (m, 6H, Ar-CH), 5.13 (s, 1H, CH), 2.57(s, 3H, CH_3)
^{13}C NMR (100 MHz, $CDCl_3$-d_6):*δ*	199.1, 159.7, 154.7, 154.3, 150.2, 147.8, 141.3, 135.7, 134.5, 132.4, 129.8, 128.2, 128.1, 126.3, 126.2, 119.2, 118.3, 117.6, 116.2, 51.5, 17.8
HRMS (micro TOF-QII, MS, ESI):	m/z $[M+H]^+$ Calculated for $C_{21}H_{14}ClFN_2O_4$ 413.0660, Obsd.: 413.0677.

6-methyl-5-(7-nitro-2-oxo-2H-chromene-3-carbonyl)-4-(p-tolyl)-3,4-dihydropyrimidin-2(1H)-one **(CD28)**

0.07 mol of 7-nitro-3-acetoacetyl coumarin was mixed with 0.05 mol of 4-methyl benzaldehyde and 0.02 mol of urea and refluxed in the presence of acetonitrile and sulphuric acid for 4 hours. The oily liquid was then poured in water and target compounds were precipitated out as solid mass. It was filtered, washed with cold water and recrystallized from ethanol.

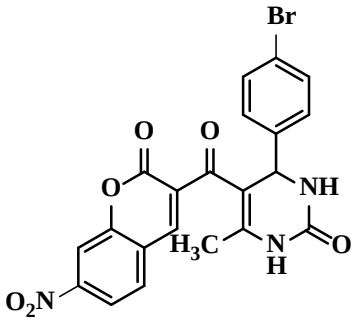


Cream colored crystals, ***Rf*** : 0.77, **M.P:** 245-246º C, **Yield:** 58%	
FT-IR (KBr) cm^{-1}:	3371(-NH), 3026(-CH), 1643(-CN), 1748(-C=O), 1671(C=N), 1256(-CO), 1358 & 1570(-NO_2)
^{1}H NMR(400 MHz, $CDCl_3$-d_6):*δ*	9.09 (s, 1H, NH), 8.56 (s, 1H, pyrone), 8.29-

	8.21 (m, 3H, Ar-CH), 7.51 (s, 1H, NH), 7.30 (d, 2H, Ar-CH, J=8 Hz), 7.11(d, 2H, Ar-CH, 4.5 Hz), 5.13 (s, 1H, CH), 2.28-2.12(d, 6H, CH_3, J=12Hz)
^{13}C NMR (100 MHz, $CDCl_3$-d_6):δ	197.7, 159.6, 154.5, 154.3, 148.5, 145.8, 140.7, 136.6, 134.5, 132.4, 129.8, 126.2, 126.1, 121.3, 121.3, 119.6, 117.3, 112.6, 109.2, 50.5, 22.7, 17.7
HRMS (micro TOF-QII, MS, ESI):	m/z $[M+H]^+$ Calculated for $C_{22}H_{17}N_3O_6$ 420.1088, Obsd.: 420.1062.

4-(4-bromophenyl)-6-methyl-5-(7-nitro-2-oxo-2H-chromene-3-carbonyl)-3,4-dihydropyrimidin-2(1H)-one **(CD32)**

0.07 mol of 7-nitro-3-acetoacetyl coumarin was mixed with 0.05 mol of 4-bromo benzaldehyde and 0.2 mol of urea and refluxed in the presence of acetonitrile and sulphuric acid for 4 hours. The oily liquid was then poured in water and target compounds were precipitated out as solid mass. It was filtered, washed with cold water and recrystallized from ethanol.

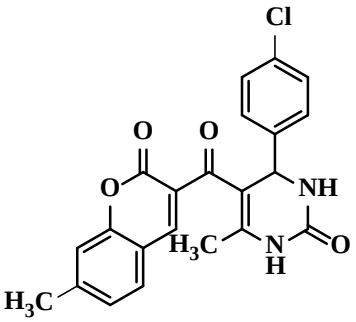


Dull brown crystals, ***Rf*** : 0.69, **M.P:** 263-265º C, **Yield:** 76%	
FT-IR (KBr) cm^{-1}:	3371(-NH), 3026(-CH), 1643(-CN), 1748(-C=O), 1671(C=N), 1256(-CO), 1358 & 1570(-NO_2), 595(-CBr)
^{1}H NMR(400 MHz, $CDCl_3$-d_6):δ	9.02 (s, 1H, NH), 8.67 (s, 1H, pyrone), 8.26-8.21 (m, 3H, Ar-CH), 7.83 (d, 2H, Ar-CH, J=6.5 Hz), 7.49 (s, 1H, NH), 7.17(d, 2H, Ar-CH, J=1.5 Hz), 5.13 (s, 1H, CH), 2.57(s, 3H, CH_3)
^{13}C NMR (100 MHz, $CDCl_3$-d_6):δ	199.3, 159.7, 154.5, 154.2, 150.1, 147.7,

	147.3, 142.7, 134.5, 131.6, 131.2, 129.5, 129.2, 129.2, 124.1, 121.3, 120.2, 118.6, 112.2, 51.5, 17.8
HRMS (micro TOF-QII, MS, ESI):	m/z $[M+H]^+$ Calculated for $C_{21}H_{14}BrN_3O_6$ 484.0066, Obsd.: 484.0100.

4-(4-chlorophenyl)-6-methyl-5-(7-methyl-2-oxo-2H-chromene-3-carbonyl)-3,4-dihydropyrimidin-2(1H)-one **(CD42)**

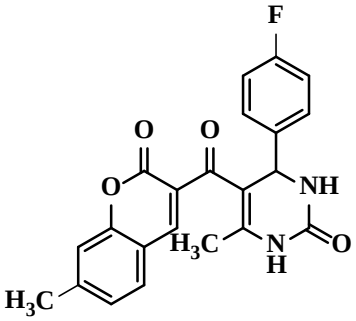
0.07 mol of 7-methyl-3-acetoacetyl coumarin was mixed with 0.05 mol of 4-chloro benzaldehyde and 0.2 mol of urea and refluxed in the presence of acetonitrile and sulphuric acid for 4 hours. The oily liquid was then poured in water and target compounds were precipitated out as solid mass. It was filtered, washed with cold water and recrystallized from ethanol.



Dull yellow crystals, ***Rf*** : 0.72, **M.P:** 265-267º C, **Yield:** 74%	
FT-IR (KBr) cm^{-1}:	3371(-NH), 3026(-CH), 1643(-CN), 1748(-C=O), 1671(C=N), 1256(-CO), 695(-CCl)
^{1}H NMR(400 MHz, $CDCl_3$-d_6):*δ*	9.02 (s, 1H, NH), 8.57 (s, 1H, pyrone), 7.83 (d, 1H, Ar-CH, J=6.5 Hz), 7.49 (s, 1H, NH), 7.23-7.19 (m, 4H, Ar-CH), 7.11-7.09 (t, 2H, Ar-CH), 5.13 (s, 1H, CH), 2.27-2.25 (d, 6H, CH_3, J=8 Hz)
^{13}C NMR (100 MHz, $CDCl_3$-d_6):*δ*	199.1, 159.4, 154.8, 154.4, 150.2, 147.2, 143.1, 141.4, 134.2, 132.3, 128.6, 128.6, 128.0, 126.1, 126.1, 125.7, 118.1, 117.3, 115.2, 51.5, 21.8, 17.8
HRMS (micro TOF-QII, MS, ESI):	m/z $[M+H]^+$ Calculated for $C_{22}H_{17}ClN_2O_4$ 409.0877, Obsd.: 409.0910.

4-(4-fluorophenyl)-6-methyl-5-(7-methyl-2-oxo-2H-chromene-3-carbonyl)-3,4-dihydropyrimidin-2(1H)-one **(CD43)**

0.07 mol of 7-methyl-3-acetoacetyl coumarin was mixed with 0.05 mol of 4-fluoro benzaldehyde and 0.2 mol of urea and refluxed in the presence of acetonitrile and sulphuric acid for 4 hours. The oily liquid was then poured in water and target compounds were precipitated out as solid mass. It was filtered, washed with cold water and recrystallized from ethanol.

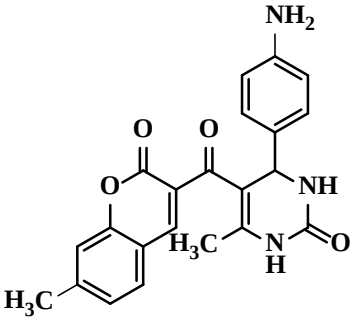


Light green crystals, ***Rf*** : 0.68, **M.P:** 241-243º C, **Yield:** 78%	
FT-IR (KBr) cm^{-1}:	3371(-NH), 3026(-CH), 1643(-CN), 1748(-C=O), 1671(C=N), 1256(-CO), 1057(-CF)
^{1}H NMR(400 MHz, $CDCl_3$-d_6):*δ*	9.06 (s, 1H, NH), 8.52 (s, 1H, pyrone), 7.73 (d, 1H, Ar-CH, J=6.5 Hz), 7.56 (s, 1H, NH), 7.23-7.08 (m, 6H, Ar-CH), 5.13 (s, 1H, CH), 2.27-2.25 (d, 6H, CH_3, J=8 Hz)
^{13}C NMR (100 MHz, $CDCl_3$-d_6):*δ*	199.1, 160.4, 159.4, 154.7, 154.3, 150.4, 147.5, 143.1, 138.4, 134.2, 128.6, 128.6, 128.0, 125.1, 118.1, 117.3, 115.7, 115.3, 115.2, 51.5, 21.8, 17.8
HRMS (micro TOF-QII, MS, ESI):	m/z $[M+H]^+$ Calculated for $C_{22}H_{17}FN_2O_4$ 393.1172, Obsd.: 393.1162.

4-(4-aminophenyl)-6-methyl-5-(7-methyl-2-oxo-2H-chromene-3-carbonyl)-3,4-dihydropyrimidin-2(1H)-one **(CD44)**

0.07 mol of 7-methyl-3-acetoacetyl coumarin was mixed with 0.06 mol of 4-amino benzaldehyde and 0.2 mol of urea and refluxed in the presence of acetonitrile and sulphuric acid for 4 hours. The oily liquid was then poured in

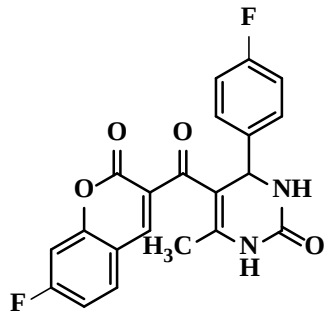
water and target compounds were precipitated out as solid mass. It was filtered, washed with cold water and recrystallized from ethanol.



Cream colored crystals, ***Rf*** : 0.72, **M.P:** 230-232º C, **Yield:** 62%	
FT-IR (KBr) cm^{-1}:	3371(-NH), 3026(-CH), 1643(-CN), 1748(-C=O), 1671(C=N), 1256(-CO), 1057(-CF)
^{1}H NMR(400 MHz, CDCl$_3$-d$_6$):*δ*	9.06 (s, 1H, NH), 8.52 (s, 1H, pyrone), 7.73 (d, 1H, Ar-CH, J=6.5 Hz), 7.56 (s, 1H, NH), 7.10-7.08 (d, 2H, Ar-CH, J=8 Hz), 6.78 (d, 2H, Ar-CH, J=2.5 Hz), 6.48 (d, 2H, Ar-CH, J=2.5 Hz), 5.17 (s, 1H, CH), 4.81 (s, 2H, NH_2), 2.27-2.24 (d, 6H, CH_3, J=8 Hz)
^{13}C NMR (100 MHz, CDCl$_3$-d$_6$):*δ*	199.0, 159.4, 154.7, 154.3, 150.4, 147.5, 146.3 143.1, 138.4, 134.2, 128.6, 126.0, 126.0, 125.1, 118.1, 117.3, 115.7, 115.1, 115.1, 51.7, 21.3, 17.7
HRMS (micro TOF-QII, MS, ESI):	m/z $[M+H]^+$ Calculated for $C_{22}H_{19}N_3O_4$ 390.1376, Obsd.: 390.1367.

5-(7-fluoro-2-oxo-2H-chromene-3-carbonyl)-4-(4-fluorophenyl)-6-methyl-3,4-dihydropyrimidin-2(1H)-one **(CD49)**

0.07 mol of 7-fluoro-3-acetoacetyl coumarin was mixed with 0.05 mol of 4-fluoro benzaldehyde and 0.2 mol of urea and refluxed in the presence of acetonitrile and sulphuric acid for 4 hours. The oily liquid was then poured in water and target compounds were precipitated out as solid mass. It was filtered, washed with cold water and recrystallized from ethanol.



Greenish crystals, ***Rf*** : 0.66, **M.P:** 252-254º C, **Yield:** 63%	
FT-IR (KBr) cm^{-1}:	3371(-NH), 3026(-CH), 1643(-CN), 1748(-C=O), 1671(C=N), 1256(-CO), 1057(-CF)
^{1}H NMR(400 MHz, CDCl$_3$-d$_6$):*δ*	9.06 (s, 1H, NH), 8.52 (s, 1H, pyrone), 7.73 (d, 1H, Ar-CH, J=6.5 Hz), 7.56 (s, 1H, NH), 7.26-7.18 (m, 4H, Ar-CH), 6.98-6.93 (t, 2H, Ar-CH), 5.13 (s, 1H, CH), 2.47 (s, 3H, CH_3)
^{13}C NMR (100 MHz, CDCl$_3$-d$_6$):*δ*	199.3, 162.4, 160.7, 159.4, 154.7, 151.3, 150.6, 147.2, 138.0, 134.6, 128.5, 128.5, 128.4, 118.4, 115.3, 115.3, 113.7, 112.2, 109.2, 51.2, 17.7
HRMS (micro TOF-QII, MS, ESI):	m/z $[M+H]^+$ Calculated for $C_{21}H_{14}F_2N_2O_4$ 397.0922, Obsd.: 397.0955.

3.9 Synthesis of coumarin-dihydropyridine derivatives ((DP12, DP18-20, DP26-28, DP32, DP56, DP58, DP61, DP63)

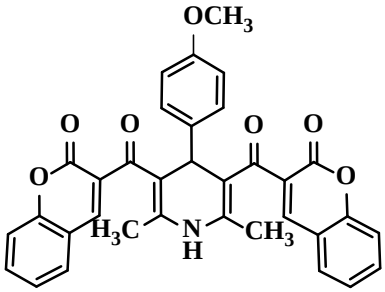
3.9.1 General procedure

A mixture of substituted 3-acetoacetyl Coumarins **10** (0.02 mol), substituted benzaldehydes **11** (0.01 mol) and ammonium acetate **12** (0.01 mol) was refluxed in the presence of ethanol overnight. The reaction progress was monitored through TLC and upon completion of reaction the mixture was cooled to room temperature followed by pouring into ice cold water. The products **13 (DP series)** gets separated out as solid which was further recrystallized from ethanol.

3,3'-(4-(4-methoxyphenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarbonyl)bis(2H-chromen-2-one) **(DP12)**

A mixture of 3-acetoacetyl coumarin (0.02 mol), 4-methoxy benzaldehyde (0.01 mol) and ammonium acetate (0.01 mol) was refluxed in the presence of

ethanol overnight. The reaction progress was monitored through TLC and upon completion of reaction the mixture was cooled to room temperature followed by pouring into ice cold water. The product gets separated out as solid which was further recrystallized from ethanol.

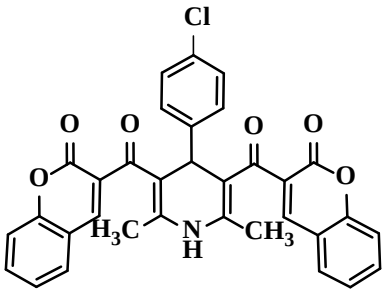


Light brown crystals, ***Rf*** : 0.76, **M.P:** 286-288º C, **Yield:** 66%	
FT-IR (KBr) cm^{-1}:	3371(-NH), 3026(-CH), 1643(-CN), 1748(-C=O), 1671(C=N), 1256(-CO)
^{1}H NMR(400 MHz, $CDCl_3$-d_6):*δ*	8.76 (s, 1H, NH), 8.57 (s, 2H, pyrone), 7.83 (d, 2H, Ar-CH, J=1.5 Hz), 7.66 (t, 2H, Ar-CH), 7.46-7.41 (m, 4H, Ar-CH), 7.15 (d, 2H, Ar-CH, J=2 Hz), 6.81 (d, 2H, Ar-CH, J=2.5 Hz), 4.83 (s, 1H, CH), 3.81 (s, 3H, OCH_3), 2.37 (s, 6H, CH_3)
^{13}C NMR (100 MHz, $CDCl_3$-d_6):*δ*	199.1, 199.1, 159.4, 159.4, 158.4, 158.4, 157.6, 153.0, 153.0, 147.3, 147.3, 136.7, 134.2, 134.2, 130.2, 130.2, 128.2, 128.2, 127.3, 127.3, 125.4, 125.4, 118.2, 118.2, 116.7, 116.7, 114.3, 114.3, 112.2, 112.2, 55.8, 40.1, 19.3, 19.3
HRMS (micro TOF-QII, MS, ESI):	m/z $[M+H]^+$ Calculated for $C_{34}H_{25}NO_7$ 560.1631, Obsd.: 560.1665.

3,3'-(4-(4-chlorophenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarbonyl)bis(2H-chromen-2-one) **(DP18)**

A mixture of 3-acetoacetyl coumarin (0.02 mol), 4-chloro benzaldehyde (0.01 mol) and ammonium acetate (0.01 mol) was refluxed in the presence of ethanol overnight. The reaction progress was monitored through TLC and upon completion of reaction the mixture was cooled to room temperature

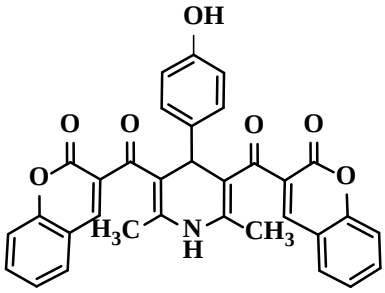
followed by pouring into ice cold water. The product gets separated out as solid which was further recrystallized from ethanol.



Off white crystals, ***Rf*** : 0.68, **M.P:** 292-294º C, **Yield:** 82%	
FT-IR (KBr) cm^{-1}:	3371(-NH), 3026(-CH), 1643(-CN), 1748(-C=O), 1671(C=N), 1256(-CO), 691(-CCl)
^{1}H NMR(400 MHz, CDCl$_3$-d$_6$):*δ*	8.71 (s, 1H, NH), 8.52 (s, 2H, pyrone), 7.81 (d, 2H, Ar-CH, J=2.5 Hz), 7.66 (t, 2H, Ar-CH), 7.48-7.43 (m, 4H, Ar-CH), 7.15-7.11 (m, 4H, Ar-CH), 4.83 (s, 1H, CH), 2.37 (s, 6H, CH_3)
^{13}C NMR (100 MHz, CDCl$_3$-d$_6$):*δ*	199.2, 199.2, 159.7, 159.7, 158.4, 158.4, 157.6, 153.0, 153.0, 147.3, 147.3, 136.7, 134.2, 134.2, 130.2, 130.2, 128.2, 128.2, 127.3, 127.3, 125.4, 125.4, 118.2, 118.2, 116.7, 116.7, 114.3, 114.3, 112.6, 112.6, 40.1, 19.1, 19.1
HRMS (micro TOF-QII, MS, ESI):	m/z $[M+H]^+$ Calculated for $C_{33}H_{22}ClNO_6$ 564.1136, Obsd.: 564.1123.

3,3'-(4-(4-hydroxyphenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarbonyl)bis(2H-chromen-2-one) **(DP19)**

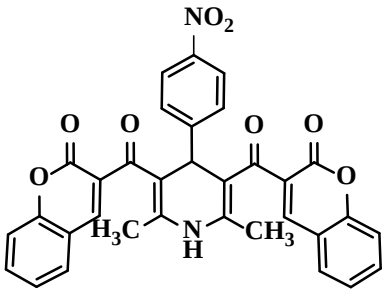
A mixture of 3-acetoacetyl coumarin (0.02 mol), 4-hydroxy benzaldehyde (0.01 mol) and ammonium acetate (0.01 mol) was refluxed in the presence of ethanol overnight. The reaction progress was monitored through TLC and upon completion of reaction the mixture was cooled to room temperature followed by pouring into ice cold water. The product gets separated out as solid which was further recrystallized from ethanol.



Off white crystals, ***Rf*** : 0.69, **M.P:** 268-271º C, **Yield:** 45%	
FT-IR (KBr) cm^{-1}:	3572(-OH), 3371(-NH), 3026(-CH), 1643(-CN), 1748(-C=O), 1671(C=N), 1256(-CO)
^{1}H NMR(400 MHz, $CDCl_3$-d_6):*δ*	9.12 (s, 1H, OH), 8.71 (s, 1H, NH), 8.52 (s, 2H, pyrone), 7.87 (d, 2H, Ar-CH, J=2.5 Hz), 7.62 (t, 2H, Ar-CH), 7.43-7.38 (m, 4H, Ar-CH), 6.98 (d, 2H, Ar-CH, J= 2Hz), 6.63 (d, 2H, Ar-CH, J=2.5 Hz), 4.83 (s, 1H, CH), 2.27 (s, 6H, CH_3)
^{13}C NMR (100 MHz, $CDCl_3$-d_6):*δ*	199.0, 199.0, 159.4, 159.4, 158.6, 158.6, 155.6, 153.0, 153.0, 147.4, 147.4, 135.7, 134.1, 134.1, 130.2, 130.2, 128.3, 128.3, 127.3, 127.3, 125.4, 125.4, 118.2, 118.2, 116.7, 116.7, 114.3, 114.3, 112.6, 112.6, 40.1, 19.1, 19.1
HRMS (micro TOF-QII, MS, ESI):	m/z $[M+H]^+$ Calculated for $C_{33}H_{23}NO_7$ 546.1475, Obsd.: 546.1508.

3,3'-(2,6-dimethyl-4-(4-nitrophenyl)-1,4-dihydropyridine-3,5-dicarbonyl)bis(2H-chromen-2-one) **(DP20)**

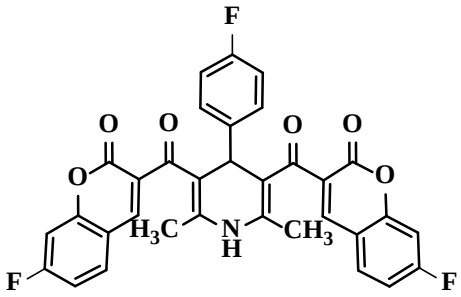
A mixture of 3-acetoacetyl coumarin (0.02 mol), 4-nitro benzaldehyde (0.01 mol) and ammonium acetate (0.01 mol) was refluxed in the presence of ethanol overnight. The reaction progress was monitored through TLC and upon completion of reaction the mixture was cooled to room temperature followed by pouring into ice cold water. The product gets separated out as solid which was further recrystallized from ethanol.



Dark yellow crystals, ***Rf*** : 0.67, **M.P:** 256-258º C, **Yield:** 54%	
FT-IR (KBr) cm^{-1}:	3371(-NH), 3026(-CH), 1643(-CN), 1748(-C=O), 1671(C=N), 1256(-CO), 1351 & 1572(-NO_2)
^{1}H NMR(400 MHz, $CDCl_3$-d_6):*δ*	8.77 (s, 1H, NH), 8.54 (s, 2H, pyrone), 8.13 (d, 2H, Ar-CH, J=2.5 Hz), 7.88 (t, 2H, Ar-CH), 7.63-7.58 (m, 4H, Ar-CH), 7.48-7.43 (m, 4H, Ar-CH), 4.83 (s, 1H, CH), 2.27 (s, 6H, CH_3)
^{13}C NMR (100 MHz, $CDCl_3$-d_6):*δ*	199.0, 199.0, 159.4, 159.4, 158.6, 158.6, 153.0, 153.0, 152.4, 147.4, 147.4, 135.7, 134.1, 134.1, 130.2, 130.2, 128.3, 128.3, 127.3, 127.3, 125.4, 125.4, 118.2, 118.2, 116.7, 116.7, 114.3, 114.3, 112.6, 112.6, 40.1, 19.1, 19.1
HRMS (micro TOF-QII, MS, ESI):	m/z $[M+H]^+$ Calculated for $C_{33}H_{22}N_2O_8$ 575.1376, Obsd.: 575.1408.

3,3'-(4-(4-fluorophenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarbonyl)bis(7-fluoro-2H-chromen-2-one) **(DP26)**

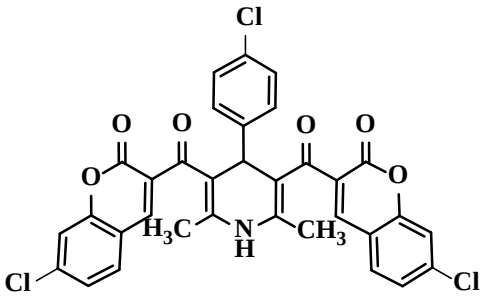
A mixture of 7-fluoro-3-acetoacetyl coumarin (0.02 mol), 4-fluoro benzaldehyde (0.01 mol) and ammonium acetate (0.01 mol) was refluxed in the presence of ethanol overnight. The reaction progress was monitored through TLC and upon completion of reaction the mixture was cooled to room temperature followed by pouring into ice cold water. The product gets separated out as solid which was further recrystallized from ethanol.



Greenish crystals, ***Rf*** : 0.69, **M.P:** 246-249º C, **Yield:** 66%	
FT-IR (KBr) cm^{-1}:	3371(-NH), 3026(-CH), 1643(-CN), 1748(-C=O), 1671(C=N), 1256(-CO), 1052(-CF)
^{1}H NMR(400 MHz, $CDCl_3$-d_6):*δ*	8.67 (s, 1H, NH), 8.61 (s, 2H, pyrone), 7.83 (t, 2H, Ar-CH), 7.42 (t, 2H, Ar-CH), 7.16 (t, 2H, Ar-CH), 6.88-6.43 (m, 4H, Ar-CH), 4.73 (s, 1H, CH), 2.29 (s, 6H, CH_3)
^{13}C NMR (100 MHz, $CDCl_3$-d_6):*δ*	199.0, 199.0, 162.5, 162.5, 159.9, 159.4, 159.4 158.6, 158.6, 151.2, 151.2, 147.4, 147.4, 140.7, 134.1, 134.1, 130.2, 130.2, 128.3, 128.3, 115.4, 115.4, 113.2, 113.2, 112.7, 112.7, 112.6, 112.6, 109.3, 109.3, 40.1, 19.1, 19.1
HRMS (micro TOF-QII, MS, ESI):	m/z $[M+H]^+$ Calculated for $C_{33}H_{20}F_3NO_6$ 584.1243, Obsd.: 584.1208.

3,3'-(4-(4-chlorophenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarbonyl)bis(7-chloro-2H-chromen-2-one) **(DP27)**

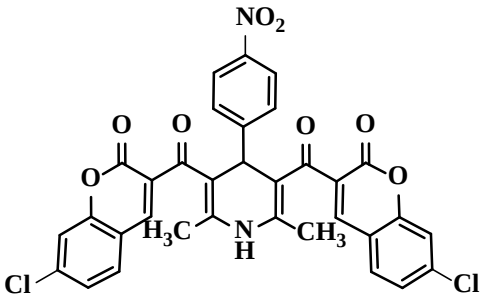
A mixture of 7-chloro-3-acetoacetyl coumarin (0.02 mol), 4-chloro benzaldehyde (0.01 mol) and ammonium acetate (0.01 mol) was refluxed in the presence of ethanol overnight. The reaction progress was monitored through TLC and upon completion of reaction the mixture was cooled to room temperature followed by pouring into ice cold water. The product gets separated out as solid which was further recrystallized from ethanol.



Off white crystals, ***Rf*** : 0.76, **M.P:** 276-278º C, **Yield:** 72%	
FT-IR (KBr) cm^{-1}:	3371(-NH), 3026(-CH), 1643(-CN), 1748(-C=O), 1671(C=N), 1256(-CO), 652(-CCl)
^{1}H NMR(400 MHz, $CDCl_3$-d_6):*δ*	8.67 (s, 1H, NH), 8.51 (s, 2H, pyrone), 7.83 (d, 2H, Ar-CH, J=2.5 Hz), 7.28-6.23 (m, 8H, Ar-CH), 4.71 (s, 1H, CH), 2.29 (s, 6H, CH_3)
^{13}C NMR (100 MHz, $CDCl_3$-d_6):*δ*	199.0, 199.0, 159.4, 159.4 158.6, 158.6, 154.2, 154.2, 147.4, 147.4, 142.7, 135.2, 135.2, 134.1, 134.1, 131.3, 130.2, 130.2, 129.4, 129.4, 128.3, 128.3, 125.4, 125.4, 119.2, 119.2, 116.7, 116.7, 112.6, 112.6, 40.1, 19.1, 19.1
HRMS (micro TOF-QII, MS, ESI):	m/z $[M+H]^+$ Calculated for $C_{33}H_{20}Cl_3NO_6$ 632.0356, Obsd.: 632.0390.

3,3'-(2,6-dimethyl-4-(4-nitrophenyl)-1,4-dihydropyridine-3,5-dicarbonyl)bis(7-chloro-2H-chromen-2-one) **(DP28)**

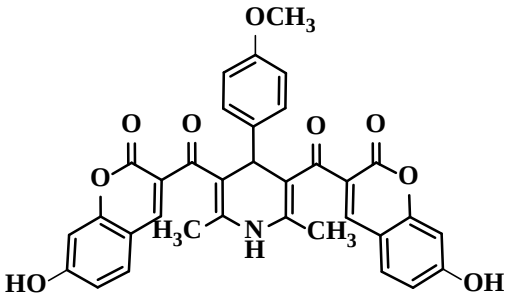
A mixture of 7-chloro-3-acetoacetyl coumarin (0.02 mol), 4-nitro benzaldehyde (0.01 mol) and ammonium acetate (0.01 mol) was refluxed in the presence of ethanol overnight. The reaction progress was monitored through TLC and upon completion of reaction the mixture was cooled to room temperature followed by pouring into ice cold water. The product gets separated out as solid which was further recrystallized from ethanol.



Yellow crystals, ***Rf*** : 0.68, **M.P:** 255-257º C, **Yield:** 78%	
FT-IR (KBr) cm^{-1}:	3371(-NH), 3026(-CH), 1643(-CN), 1748(-C=O), 1671(C=N), 1256(-CO), 652(-CCl)
^{1}H NMR(400 MHz, CDCl$_3$-d$_6$):*δ*	8.67 (s, 1H, NH), 8.51 (s, 2H, pyrone), 8.13 (d, 2H, Ar-CH, J= 6.5 Hz), 7.73 (d, 2H, Ar-CH, J=8 Hz), 7.43 (d, 2H, Ar-CH, J=2 Hz), 7.28-7.23 (m, 4H, Ar-CH), 4.71 (s, 1H, CH), 2.29 (s, 6H, CH_3)
^{13}C NMR (100 MHz, CDCl$_3$-d$_6$):*δ*	199.0, 199.0, 159.4, 159.4, 157.6, 157.6, 154.2, 154.2, 150.2, 147.4, 147.4, 144.7, 135.2, 135.2, 134.1, 134.1, 130.2, 130.2, 129.4, 129.4, 128.3, 128.3, 125.4, 125.4, 119.2, 119.2, 116.7, 116.7, 112.6, 112.6, 40.1, 19.1, 19.1
HRMS (micro TOF-QII, MS, ESI):	m/z $[M+H]^+$ Calculated for $C_{33}H_{20}Cl_2N_2O_8$ 643.0597, Obsd.: 643.0630.

3,3'-(4-(4-methoxyphenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarbonyl)bis(7-hydroxy-2H-chromen-2-one) **(DP32)**

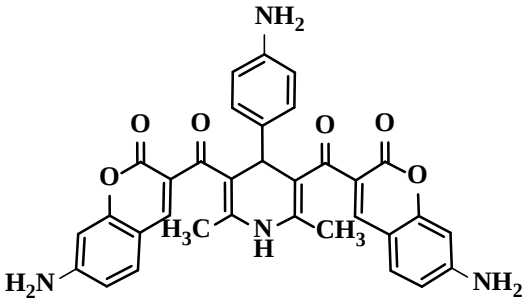
A mixture of 7-hydroxy-3-acetoacetyl coumarin (0.02 mol), 4-methoxy benzaldehyde (0.01 mol) and ammonium acetate (0.01 mol) was refluxed in the presence of ethanol overnight. The reaction progress was monitored through TLC and upon completion of reaction the mixture was cooled to room temperature followed by pouring into ice cold water. The product gets separated out as solid which was further recrystallized from ethanol.



White crystals, ***Rf*** : 0.66, **M.P:** 273-275º C, **Yield:** 66%	
FT-IR (KBr) cm^{-1}:	3572(-OH), 3371(-NH), 3026(-CH), 1643(-CN), 1748(-C=O), 1671(C=N), 1256(-CO)
^{1}H NMR(400 MHz, $CDCl_3$-d_6):*δ*	10.76 (s, 2H, OH), 8.66 (s, 1H, NH), 8.56 (s, 2H, pyrone), 7.58 (d, 2H, Ar-CH, J=4.5 Hz), 7.38 (d, 2H, Ar-CH, J= 4.5 Hz), 7.21 (d, 2H, Ar-CH, J= 4Hz), 6.76-6.69 (m, 4H, Ar-CH), 4.82 (s, 1H, CH), 3.88 (s, 3H, OCH_3), 2.28 (s, 6H, CH_3)
^{13}C NMR (100 MHz, $CDCl_3$-d_6):*δ*	197.7, 197.7, 166.5, 166.1, 161.6, 161.6, 158.2, 158.2, 148.5, 148.5, 147.12, 138.6, 138.6, 130.8, 130.3, 130.2, 130.1, 117.8, 114.4, 114.4, 112.2, 112.2, 112.2, 112.2, 110.2, 110.2, 102.7, 102.7, 54.2, 40.2, 19.1, 19.1
HRMS (micro TOF-QII, MS, ESI):	m/z $[M+H]^+$ Calculated for $C_{34}H_{25}NO_9$ 592.1529, Obsd.: 592.1686.

3,3'-(4-(4-aminophenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarbonyl)bis(7-amino-2H-chromen-2-one) **(DP56)**

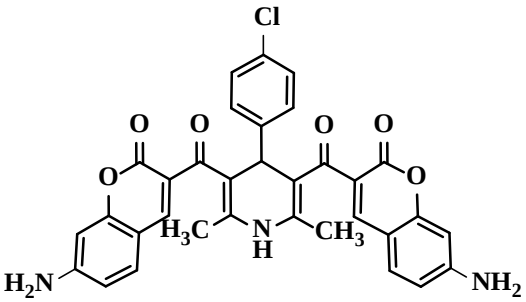
A mixture of 7-amino-3-acetoacetyl coumarin (0.02 mol), 4-amino benzaldehyde (0.01 mol) and ammonium acetate (0.01 mol) was refluxed in the presence of ethanol overnight. The reaction progress was monitored through TLC and upon completion of reaction the mixture was cooled to room temperature followed by pouring into ice cold water. The product gets separated out as solid which was further recrystallized from ethanol.



Pale yellow crystals, ***Rf*** : 0.72, **M.P:** 275-277° C, **Yield:** 70%	
FT-IR (KBr) cm^{-1}:	3442(-NH_2), 3371(-NH), 3026(-CH), 1643(-CN), 1748(-C=O), 1671(C=N), 1256(-CO)
^{1}H NMR(400 MHz, $CDCl_3$-d_6):*δ*	8.67 (s, 1H, NH), 8.51 (s, 2H, pyrone), 7.41 (d, 2H, Ar-CH, J=4.5 Hz), 6.93 (d, 2H, Ar-CH, J= 4Hz), 6.53 (d, 2H, Ar-CH, J= 4.5 Hz), 6.48-6.43 (m, 4H, Ar-CH), 5.96 (s, 4H, NH_2), 4.91 (s, 2H, NH_2), 4.78 (s, 1H, CH), 2.29 (s, 6H, CH_3)
^{13}C NMR (100 MHz, $CDCl_3$-d_6):*δ*	199.0, 199.0, 159.4, 159.4, 158.6, 158.6, 156.2, 156.2, 157.2, 157.2, 147.4, 147.7, 145.4, 136.2, 134.1, 134.1, 129.5, 129.5, 115.1, 115.1, 114.4, 114.4, 112.3, 112.3, 111.1, 111.1, 110.2, 110.2, 100.5, 100.5, 40.1, 19.1, 19.1
HRMS (micro TOF-QII, MS, ESI):	m/z $[M+H]^+$ Calculated for $C_{33}H_{26}N_4O_6$ 575.1852, Obsd.: 575.1875.

3,3'-(4-(4-chlorophenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarbonyl)bis(7-amino-2H-chromen-2-one) **(DP58)**

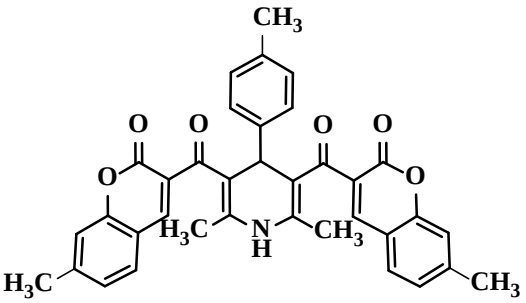
A mixture of 7-amino-3-acetoacetyl coumarin (0.02 mol), 4-chloro benzaldehyde (0.01 mol) and ammonium acetate (0.01 mol) was refluxed in the presence of ethanol overnight. The reaction progress was monitored through TLC and upon completion of reaction the mixture was cooled to room temperature followed by pouring into ice cold water. The product gets separated out as solid which was further recrystallized from ethanol.



Pale yellow crystals, ***Rf*** : 0.70, **M.P:** 251-253º C, **Yield:** 71%	
FT-IR (KBr) cm^{-1}:	3442(-NH_2), 3371(-NH), 3026(-CH), 1643(-CN), 1748(-C=O), 1671(C=N), 1256(-CO), 681(-CCl)
^{1}H NMR(400 MHz, $CDCl_3$-d_6):*δ*	8.67 (s, 1H, NH), 8.51 (s, 2H, pyrone), 7.43 (d, 2H, Ar-CH), J= 6 Hz, 7.23-7.17 (m, 4H, Ar-CH), 6.59 (d, 2H, Ar-CH), 6.42 (d, 2H, Ar-CH, J= 4.5 Hz), 5.97 (s, 4H, NH_2), 4.78 (s, 1H, CH), 2.29 (s, 6H, CH_3)
^{13}C NMR (100 MHz, $CDCl_3$-d_6):*δ*	199.0, 199.0, 159.4, 159.4, 158.6, 158.6, 156.2, 156.2, 157.2, 157.2, 147.4, 147.7, 142.4, 134.1, 134.1, 131.3, 130.5, 130.5, 129.5, 129.5, 128.3, 128.3, 112.3, 112.3, 110.1, 110.1, 108.2, 108.2, 100.5, 100.5, 40.1, 19.1, 19.1
HRMS (micro TOF-QII, MS, ESI):	m/z $[M+H]^+$ Calculated for $C_{33}H_{24}ClN_3O_6$ 594.1354, Obsd.: 594.1394.

3,3'-(2,6-dimethyl-4-(p-tolyl)-1,4-dihydropyridine-3,5-dicarbonyl)bis(7-methyl-2H-chromen-2-one) **(DP61)**

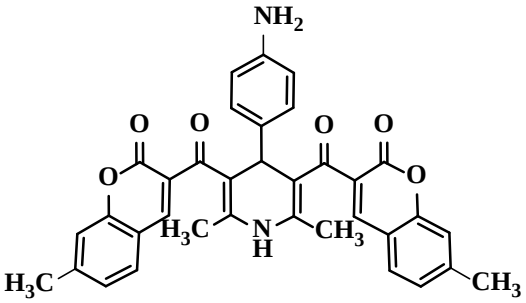
A mixture of 7-methyl-3-acetoacetyl coumarin (0.02 mol), 4-methyl benzaldehyde (0.01 mol) and ammonium acetate (0.01 mol) was refluxed in the presence of ethanol overnight. The reaction progress was monitored through TLC and upon completion of reaction the mixture was cooled to room temperature followed by pouring into ice cold water. The product gets separated out as solid which was further recrystallized from ethanol.



Brown solid, ***Rf*** : 0.67, **M.P:** 240-242º C, **Yield:** 87%	
FT-IR (KBr) cm^{-1}:	3371(-NH), 3026(-CH), 1643(-CN), 1748(-C=O), 1671(C=N), 1256(-CO)
^{1}H NMR(400 MHz, $CDCl_3$-d_6):*δ*	8.67 (s, 1H, NH), 8.51 (s, 2H, pyrone), 7.73 (d, 2H, Ar-CH, J= 6.6 Hz), 7.13-7.11 (m, 6H, Ar-CH), 6.91 (d, 2H, Ar-CH, J= 4.5 Hz), 4.78 (s, 1H, CH), 2.39 (d, 12H, CH_3, J= 8 Hz), 2.27 (s, 3H, CH_3)
^{13}C NMR (100 MHz, $CDCl_3$-d_6):*δ*	199.0, 199.0, 159.4, 159.4, 158.6, 158.6, 156.2, 156.2, 157.2, 157.2, 147.4, 147.7, 141.4, 135.1, 134.1, 134.3, 130.5, 130.5, 129.5, 129.5, 128.3, 128.3, 112.3, 112.3, 110.1, 110.1, 108.2, 108.2, 100.5, 100.5, 40.1, 21.3, 21.3, 21.3, 19.1, 19.1
HRMS (micro TOF-QII, MS, ESI):	m/z $[M+H]^+$ Calculated for $C_{36}H_{29}NO_6$ 572.1995, Obsd.: 572.2028.

3,3'-(4-(4-aminophenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarbonyl)bis(7-methyl-2H-chromen-2-one) **(DP63)**

A mixture of 7-methyl-3-acetoacetyl coumarin (0.02 mol), 4-methyl benzaldehyde (0.01 mol) and ammonium acetate (0.01 mol) was refluxed in the presence of ethanol overnight. The reaction progress was monitored through TLC and upon completion of reaction the mixture was cooled to room temperature followed by pouring into ice cold water. The product gets separated out as solid which was further recrystallized from ethanol.



Brown solid, ***Rf*** : 0.74, **M.P:** 262-264° C, **Yield:** 74%	
FT-IR (KBr) cm^{-1}:	3487(-NH_2), 3371(-NH), 3026(-CH), 1643(-CN), 1748(-C=O), 1671(C=N), 1256(-CO)
^{1}H NMR(400 MHz, $CDCl_3$-d_6):*δ*	8.69 (s, 1H, NH), 8.54 (s, 2H, pyrone), 7.79 (d, 2H, Ar-CH, J=2.5 Hz), 7.13-7.10 (m, 4H, Ar-CH), 6.98 (d, 2H, Ar-CH, J= 8.5 Hz), 6.48 (d, 2H, Ar-CH, J= 8 Hz), 4.91 (s, 2H, NH_2), 4.77 (s, 1H, CH), 2.27 (d, 12H, CH_3, J= 8 Hz)
^{13}C NMR (100 MHz, $CDCl_3$-d_6):*δ*	199.2, 199.2, 159.4, 159.4, 158.2, 158.2, 154.4, 154.2, 147.2, 147.2, 145.4, 143.4, 143.4, 134.4, 134.3, 134.3, 128.5, 128.5, 125.5, 125.5, 117.3, 117.3, 115.3, 115.3, 115.3, 115.3, 112.6, 112.6, 111.7, 111.7, 40.1, 21.3, 21.3, 19.1, 19.1
HRMS (micro TOF-QII, MS, ESI):	m/z $[M+H]^+$ Calculated for $C_{35}H_{28}N_2O_6$ 573.1947, Obsd.: 573.1981.

3.10 Anticancer activity

3.10.1 MTT assay

The anticancer potentials of all the synthesized compounds were evaluated using the MTT assay. This is a type of colorimetric analytical assay which has been widely adopted to assess the cell viability. This assay is based upon the potentials of certain enzymes (NADPH dependent oxidoreductases) to convert the tetrazolium dye compound MTT into an insoluble purple colored compound formazan by virtue of reduction (Mosmann *et al.,* 1983). The synthesized compounds from all the three series were evaluated against breast cancer cell lines (MCF7 & T47D), lung cancer cell lines (A549) and liver cancer cell lines (HepG2). Exemestane and trastuzumab

were used as reference drugs against breast cancer cell lines whereas doxorubicin was used against lung and liver cancer cell lines.

MCF7: MCF7 stands for Michigan Cancer Foundation-7. MCF7 cells are employed for *in vitro* anticancer evaluation of compounds against breast cancer. These cell lines retain the ideal characteristics of the mammary epithelium. These are capable of processing estrogen as Estradiol through estrogen receptors in the cytoplasm. Therefore it is regarded as estrogen receptor positive cell line. These cell lines are widely used in research works on estrogen receptor positive breast cancer cell. Along with this wide variety of subclones are also developed which represent various classes of estrogen receptor positive tumors with different expression extents of receptor. MCF7 cells are long adherent cells with a size of 20-25µ (*Comsa et al.,* 2015).

T47D: These are the human ductal breast cancer cell lines similar to the epithelium. These are commonly employed in research experimentation involving hormonal expression of tumor cells. These cells were originated from pleural effusion of a ductal cancer of the mammary gland. These differ from other breast cancer cell lines by the fact that their progesterone receptors do not require Estradiol for regulation.

A549: These are the widely used human non-small lung cancer cell lines. These consist of hypotriploid alveolar basal epithelial cells. A549 cell lines have squamous arrangement and these cause diffusion of electrolytes/water across the lung alveoli. When these are grown *in vitro* in a culture media, these show a monolayer along with the surface of culture flask. These cells are widely used in lung cancer models for the design and development of therapeutic candidates against lung cancer.

HepG2: HepG2 cell lines are the human Caucasian hepatocyte cancer cell lines which are widely employed for the anti liver cancer potentials of therapeutic agents and other research works. These are epithelial cells in nature and present a stable phenotype and have unlimited life span. The special feature of these cells includes secretion of various significant plasma proteins like albumin, fibrinogen, macroglobins, transferring and plasminiogens.

Cytotoxic effect to the cells was measured by the extent to which MTT reduction has been occurred upon treatment with the synthesized coumarin derivatives. The precultured cancer cells were incorporated to a 96-well plate and were kept

undisturbed for 24 hours. Each well was loaded with a cell concentration of 10000 cells per well and were allowed to stick. Then each compound was treated as a replicate of five and culturing was allowed to occur for a time period of 48 to 78 hours. The MTT dye (20 μL) was added to each well at a concentration of 5mg/mL and allowed to culture for next four hours. After the culturing period, the supernatant was removed and DMSO was added to each well. Followed by this, incubation was carried out for half an hour at 37°C and the absorbance was recorded at 570nm using a microplate reader. Each measurement was repeated in a triplicate set.

To measure the cell viability, the 96-well plate was then seeded with a cell concentration of 10^5 cells in each well followed by incubation for 6-8 hours in RPMI-1640 medium. After washing with PBS, the cells were treated with MTT dye at a concentration of 0.5mg/mL prepared in PBS medium. The plates were left undisturbed for a time period of 30 minutes to 4 hours at 37°C until there appeared purple crystals when observed under a microscope. After appearance of purple color, 200 μL of DMSO was added which acted as a solubilizing medium after the removal of MTT solution. Then the absorbance of the samples was measures through a microplate reader at a wavelength of 570 nm.

The following equation was used to calculate the percentage cell viability.

$\%\ \text{viable cells} = (abs_{sample} - abs_{blank}) / (abs_{control} - abs_{blank}) * 100$

Where abs_{sample} = Absorbance of sample solution

abs_{blank} = Absorbance of blank solution

$abs_{control}$ = Absorbance of control solution

All the graphs generation and statistical treatment was carried out using GraphPad Prism 7.04 software.

3.10.2 Normal cell toxicity

Normal cell toxicity evaluation is a crucial part of drug development process in the modern therapeutics. These tests provide insights about a new molecule's pharmacological attributes by majorly targeting on its tolerability and toxic effects on normal cells/tissues. Therefore these studies are associated with assurance of safety of the prepared molecules.

Most potent compounds among the three series were also evaluated for *in vitro* toxicity assessment on non-tumorigenic human normal lung fibroblast cell lines WI-38 to investigate the potential safety of the coumarin hybrids towards the normal cells. The results were expressed as IC_{50} values, and selectivity index was calculated.

WI-38: WI-38 is the diploid human cell line consisting of fibroblasts derived from lung tissues. These form mutilayered membrabnes when these are held for long times and body temperature along with time to time pH adjustments. These are widely used in the preparation of viral vaccines as well as to study the cytotoxic effects of newer drugs on the normal body cells (Olshansky *et al.*, 2017).