Chapter 2

Literature Review

CHAPTER 2: LITERATURE REVIEW

- **Sanduza *et al.*** have designed and synthesized a series of coumarin-uracil hybrid molecules. The compounds were tested against a panel of six human cancer cell lines namely Colo-205, MCF-7, A-549,PA-1, PC-3 and Hela cells by Sulforhodamine B assay. The results indicated that the hybrid molecules can specifically inhibit the MCF-7 cancer cell proliferation amongst which compound **1** (Figure 2.1) was found to be most potent hybrid (GI_{50}= 1.55mM) with fluorine atom as R with two carbon chain length between triazole and coumarin moieties. Cell cycle analysis revealed that compound **1** significantly arrest the G2/M phase to inhibit proliferation of MCF-7 cells. Due to its mitotic arrest, compound **1** was further analyzed to predict its various binding interactions within the active site of tubulin, which revealed its best binding pattern within the vinblastine binding site. Overall studies revealed some interesting features of synthetics to be active which stated that, the compounds with electronegative atom on R and compounds with two carbon chain lengths between triazole and coumarin showed best results (Sanduza *et al.*, 2020).

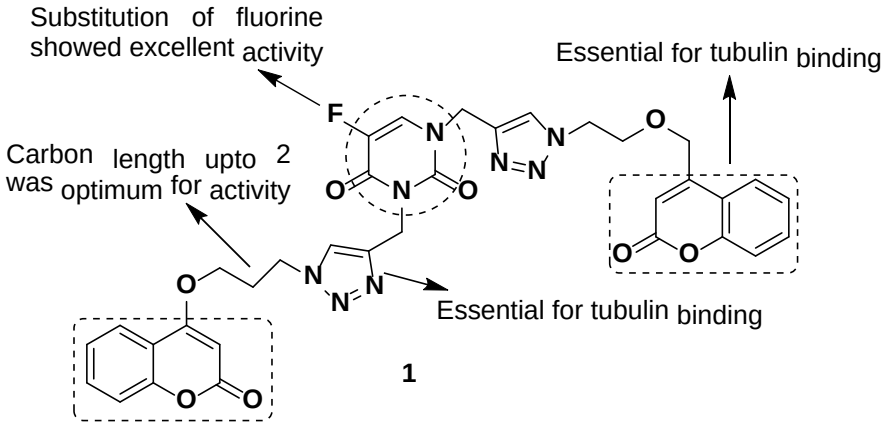


Figure 2.1: SAR of coumarin-uracil hybrids

- **Yamaguchi *et al.*** have reported a series of coumarin-pyridine and diethylamino bis-coumarin hybrids as potential aromatase inhibitors. The synthesized compounds were evaluated for the aromatase inhibitory potentials by comparing them with the well established aromatase inhibitor exemestane. It was worth noting that substitution patterns and substituents' position

presented a wide variation in inhibitory potentials. In both types of hybrid compounds, the attachment through 7th position of coumarin scaffold produced most potent compounds. Also, the attachment pattern of pyridyl moiety varied the potency. It was found that when it was attached through its 4th position **2**, the activity was maximum (IC_{50} = 30.3 nM) as compared to the other positions. In the case of bis-coumarin hybrids, diethylamino substitution at 7th position **3** revealed maximum potency (IC_{50} = 28.2 nM) as compared to other substituents (Figure 2.2). The diethylamino group produced 20 times greater inhibition in comparison to methoxy substituents **4.** Some derivatives revealed equivalent inhibition to exemestane ((IC_{50} = 42.5 nM). These molecules may act as new substrates for aromatase with high specificity (Yamaguchi *et al.*, 2017).

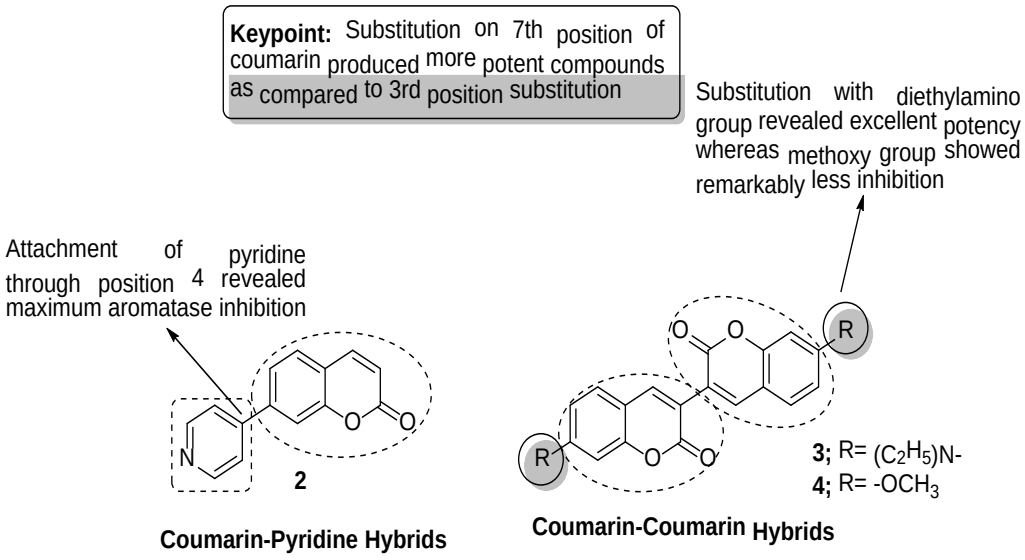


Figure 2.2: SAR of coumarin-pyridine & coumarin-coumarin hybrids

- **Dasko *et al.*** have synthesized a series of 3-(4-aminophenyl)- coumarin-7-O-sulfamate hybrids as steroid sulphatase inhibitors. The synthesized compounds were then evaluated for human placental STS inhibitory potentials for cytotoxicity against estrogen receptor-positive MCF7, T47D cell lines, and negative MDA-MB-231, SkBr3 cancer cell lines. The enzyme inhibition assay revealed that substitution on different positions of phenyl ring B remarkably influenced the inhibitory potentials. The compounds with fluoro **5** and trifluoro-methane **6** substitutions revealed excellent inhibition with IC_{50}=0.18 µM as compared to the reference compound coumarin-7- O-sulfamate

(IC_{50}=1.38 µM). These outcomes were strongly justified by docking results against aromatase, where the molecules were well occupied inside the binding pocket with a good number of hydrogen bonding and hydrophobic interactions. The most potent compounds were found moderately cytotoxic against MCF7 and T47D, which was 2-3 times lower than the reference drug tamoxifen. Here, the compound with CF3 substitution on the 1st and 4th position of phenyl ring B was most potent (Figure 2.3). Also, these compounds were not selective towards estrogen-dependent cells (Dasko *et al.,* 2020).

- **Hng *et al.*** prepared coumarin-benzylamino hybrids as potent STS inhibitors to develop therapeutic candidates against breast cancer. They replaced the carbonyl group of amide and just incorporated the methoxy substituents instead of fluoro at 1^{st} and 2^{nd} positions of phenyl ring B and significant improvement in activity was observed (IC_{50}=0.13 µM). It was also worth noting that an increase in the length of linking alkyl chain (n) led to a remarkable decrease in inhibitory potentials. The most potent compound with dimethoxy substituent **7** also displayed significant cytotoxicity against MCF7 cell lines but was 13 times lower that the reference compound STX64. The methoxy substitution on para position was not necessary for inhibition (Hng *et al.*, 2020). The essential aspects related to SAR have been outlined in Figure 2.3.

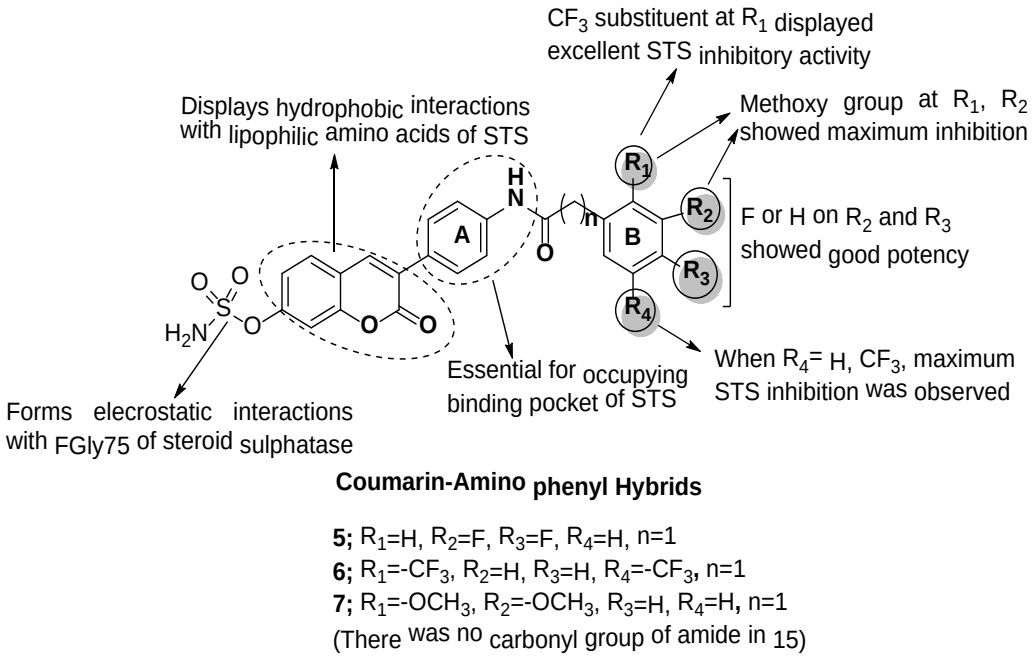


Figure 2.3: Aspects related to SAR of coumarin-aminophenyl hybrids

- **Ding *et al.*** have prepared coumarin-N-hydroxycinnamide hybrids as potential histone deacetylase inhibitors. The prepared compounds were also investigated for antiproliferative activity against MCF-7, HepG2, HeLa and HCT-116 cell lines. The synthesized compounds showed moderate to excellent antiproliferative activity and HDAC inhibition. It was worth notable that various substitutions on the 6^{th},7^{th} and 8^{th} positions of coumarin moiety produced great diversity in the activity. Compounds with methoxy substitution at 7^{th} position **8** showed excellent HDAC inhibition with IC_{50}= 0.32µM in comparison to the reference drug suberanilohydroxamic acid (SAHA) with IC_{50}= 0.48µM. The compounds bearing ethoxy **9** and propyloxy **10** substituents at the same position also displayed good HDAC inhibition as well as antiproliferative activity. The substitution of halogens on 6^{th} and 8^{th} positions of coumarin remarkably reduced the activity upto manyfolds. Further the potent compounds were subjected to molecular docking against HDAC and were completely fitted inside the binding pocket. Aryl ring linker displayed hydrophobic interactions, whereas –NH and –OH groups of hydroxycinnamide segment displayed hydrogen bondings (Figure 2.4). The coumarin scaffold showed arene-cation type interactions (Ding et al., 2020).

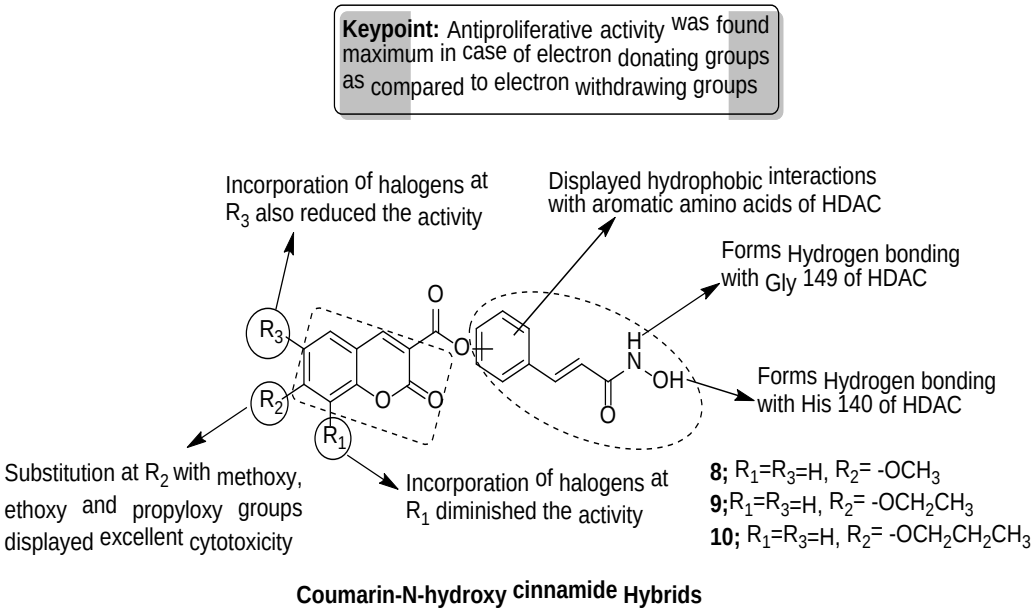


Figure 2.4: SAR of coumarin-n-hydroxy cinnamide hybrids

- **Xu *et al.*** have reported coumarin-pyrimidine hybrids **11** as potent CDK9 inhibitors with high selectivity (160- to 8300-fold) over CDK1/2/3/4/5/6/7/8/19. In these molecules, substituted coumarin moiety was found to bind at flexible hinge/αD region of CDK9 i.e a key target site for this kinase inhibition. SAR studies revealed that the di-substitution is preferred over phenyl ring attached to pyrimidine moiety. On replacement of halogen atom with electron donation or a less electronegative atom the CDK9 inhibition activity was lost. This effect was thought due to the alteration of electron density which ultimately disrupts the hydrogen-bond interaction with Cys106 in the hinge region. Replacement of morpholino moiety with 4-methylpiperazin-1-yl, 4-ethylpiperazin-1-yl or piperidin-1-yl does not affect the cytotoxic and CDK9 inhibitory potential significantly (Xu *et al.*, 2020). Similarly, replacement of acetyl moiety with a 3-propionyl also gives the same inhibitory activity (Figure 2.5).

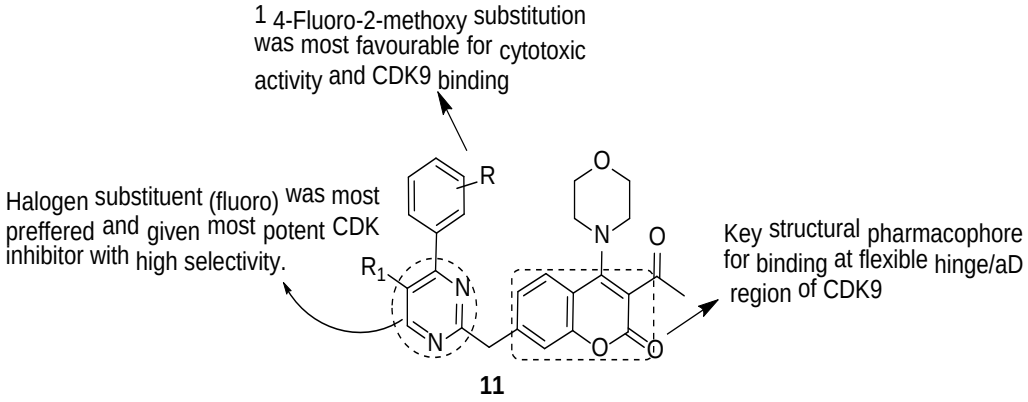


Figure 2.5: SAR studies of coumarin-pyrimidine hybrids

- **Fayed *et al.*** have recently reported coumarin-pyridine hybrids as promising anti-cancer agents. The cytotoxic activity of the hybrids was evaluated against breast (MCF7), colon (HCT116), hepatocellular (HepG2) and lung cancer (A549) cell lines. All the hybrids displayed moderate to excellent activity against all the cell lines. Compounds bearing amide group, cyano group and fusion of pyrimidinone moiety on pyridine **12, 13** displayed excellent cytotoxic potentials with IC_{50} =1.1-2.4 μM in comparison to the reference drug fluoro-uracil (IC_{50} =7.1-8.2 μM). The fusion of pyrimidinone moiety remarkably increased the capsase-3 activity and apoptosis rate, whereas the fusion of pyridine with five-membered heterocycles reduced the activity. The outcomes were further evidenced by docking studies carried out against caspase-3. The potent compounds were well fitted inside the binding pocket with high docking scores. The significant interactions were arene-arene, arene-cation and hydrogen bonding (Figure 2.6). Coumarin moiety displayed arene-arene type interactions, whereas phenyl ring substituted on pyridine showed arene-cation type interactions. In the case of pyrimidinone fused hybrid, additional two hydrogen bonds were observed corresponding to the oxygen atom and nitrogen atom (Fayed *et al.*, 2019).

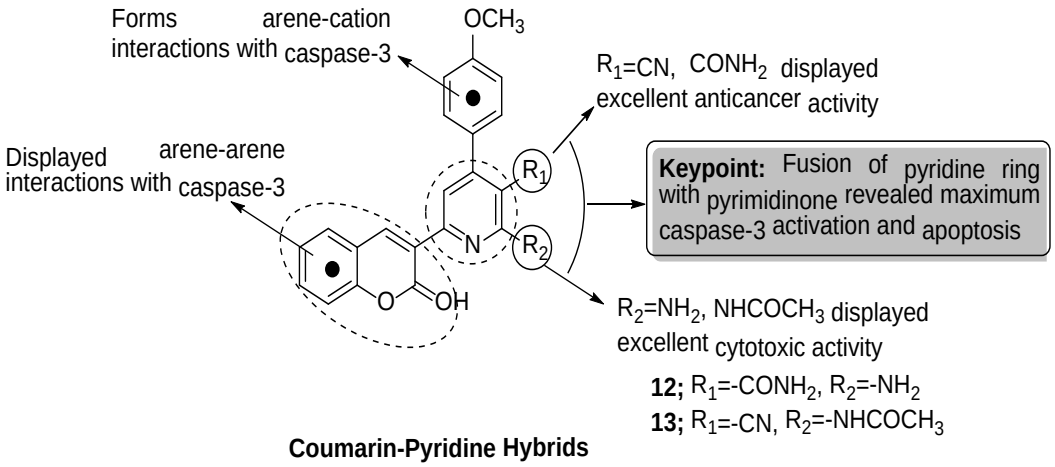


Figure 2.6: SAR of coumarin-pyridine hybrids

- **Mohamed *et al.*** prepared coumarin-thiazole-pyrazole hybrids and evaluated for cytotoxic and apoptosis induction potentials. The prepared hybrids were assessed against various cancer cell lines, including breast MCF7, lung A549, prostate PC3, liver HepG2 and normal melanocyte HFB4. 4-chlorophenyl **14** and 4-methyl phenyl diazenyl derivative **15** were found to be the most potent compounds among the series with an IC_{50} value of 5.41-6.1 µM against MCF7 cell lines in comparison to the reference drug doxorubicin (IC_{50}= 6.73 µM). 5-methylthiazolidinone derivatives of coumarin revealed comparatively low cytotoxicity (Figure 2.7). The most potent compound was further evaluated for caspase activation potentials. The apoptosis induction in MCF7 cell lines occurs due to activation of caspase-7 due to lack of caspase-3. The treatment of MCF7 cell lines with the most potent hybrid resulted in 4.4 fold elevation in the concentration of active caspase-7 and 11 fold increase in the level of inducer caspase-9 (Mohamed *et al.*, 2019). Further QSAR studies and *in silico* ADMET studies suggested that the developed hybrids can be promising therapeutic candidates against cancer.

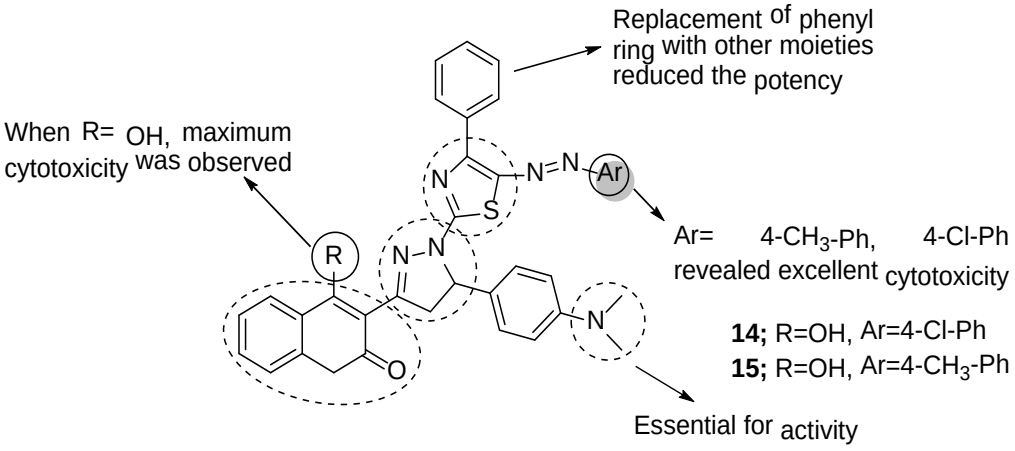


Figure 2.7: SAR of coumarin-thiazole-pyrazole hybrids

- **Sandal *et al.*** reported the synthesis of coumarin substituted benzimidazolium salts **16** and evaluated their cytotoxic activity against prostate and ovarian cancer cells (Figure 2.8). Cytotoxicities of all compounds were tested by [3-(4,5-dimethylthiazole)-2-yl]-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay against human prostate (PC-3) and ovarian (A2780) cancer cells. All compounds performed significant cytotoxicities at 100 μM against both cancer cell lines. Moreover, some compounds performed significant activities at 1μM against both cancer cell lines and the obtained results suggest that this type of compounds are promising candidates for the treatment of human prostate and ovarian cancers (Sandal *et al.,* 2019).

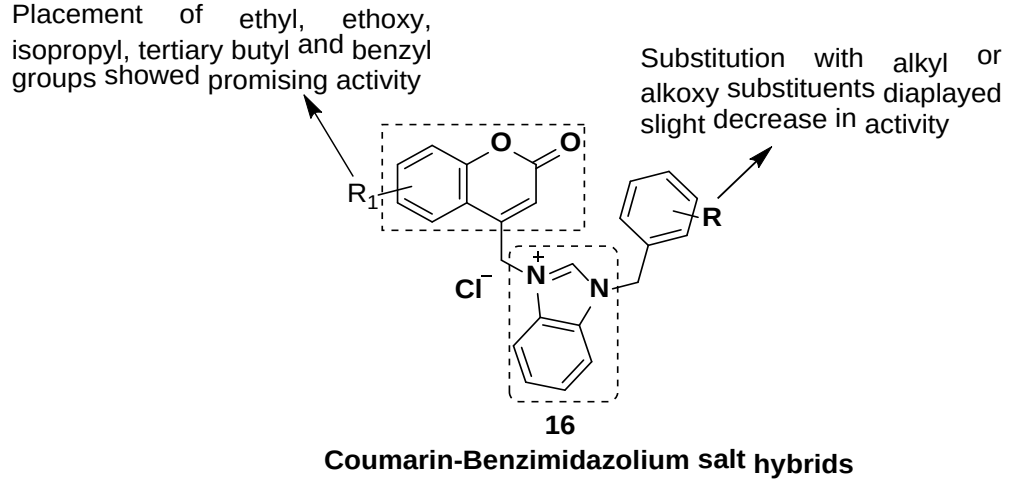


Figure 2.8: Coumarin-benzimidazolium salt hybrids

- **Amin *et al.*** synthesized a novel series of coumarin-thiadiazole hybrids in order to design new therapeutic agents. The synthesized compounds revealed a

broad spectrum of activities. The compounds were screened for analysing the DNA intercalation capacity and anti-cancer activity against 60 cell lines. DNA intercalation capacities were determined through K_{SV} (Stern Volmer Constant) values and synthesized compounds showed good to moderate activities. The anti-breast cancer activity was evaluated against MCF7, MDA/MB-231, HS578T, BT549 and T47D cell lines and the potency was expressed in terms of percent of mean growth of the treated cells. SAR studies predicted that amino substituted compound **17** and thioureidyl substituted compound **18** showed good activity (Amin *et al.,* 2018) (Figure 2.9). The mode of action proposed on the basis of above findings was through intercalation of DNA templates.

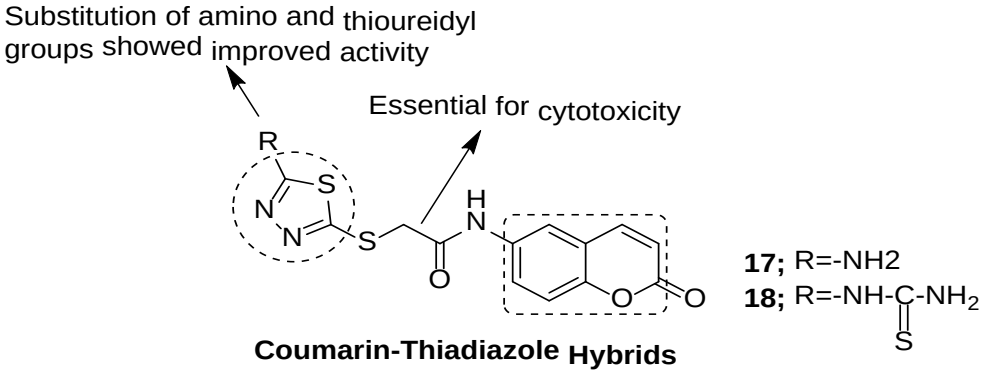
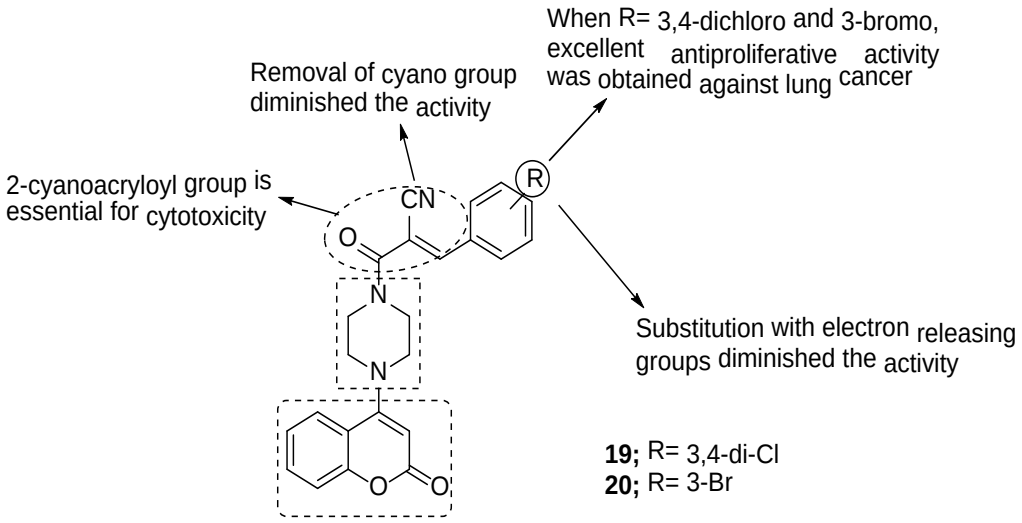


Figure 2.9: SAR studies of coumarin-thiadiazole hybrids

- **Zhang *et al.*** synthesized a series of coumarin-piperazine hybrids as antiproliferative agents and apoptosis inducers. The synthesized compounds were further evaluated for cytotoxic activity against a variety of cancer cell lines (A549, H157, HepG2, MCF7 and MG63). The results revealed that 2-cyanoacryloyl component was essential for significant activity. It was also evident that compounds having electron-donating groups like -Me, -NMe_2, -OEt etc. displayed inferior activity against all the cell lines. But it was notable that halogen substituents showed significant antiproliferative activity, suggesting that halogen substitution have a promising effect on activity. The compounds having 3,4-dichloro **19** and 3-bromo **20** substitution were found most potent among the series with IC_{50} values of 6.26 and 7.28 μM respectively, which was slightly lower than the reference drug doxorubicin (Figure 2.10). Further, the most potent compound evaluated for its apoptosis potentials in MG63 cell lines and results indicated that the compound had

produced significant apoptosis marked by nuclear fragmentation, chromatin damage, cell shrinkage etc. Further the mechanism of apoptosis in MG63 cell lines was studied through PCR and western blot which revealed that the expression of Bcl-2 gene was decreased, which suppresses apoptosis whereas the expression of Bax was increased. Also, the levels of caspase 3, 8 and 9 were found elevated which were the clear indicators of apoptosis (Zhang *et al.*, 2018).



Coumarin-Piperazine Hybrids

Figure 2.10: SAR of coumarin-piperazine hybrids

- **Lingaraju *et al.*** reported isoxazole tethered coumarin derivatives **21** (Figure 2.11) as promising anti-cancer agent. The synthesized compounds were evaluated for their cytotoxicity against human melanoma (UACC 903) and fibroblast (FF2441) cancer and normal cells, respectively. The synthesized compounds showed promising activity against cancer cells with IC_{50} values ranging from 4.5 μM to 10.5 μM. SAR studies revealed that subtitution pattern at phenyl ring substituted to isoxazoline plays important role in cytotoxicity aginst UACC 903 cancer cells. Electron withdrawing halogen substituents at ortho while electron releasing (methoxy) substituents at meta and para positions are favourable for good cytotoxic activity. These compounds were also found to possess little toxicity to normal cells (Lingaraju *et al.*, 2018). On change of substitution pattern to 3,4-dimethoxy at phenyl ring removed the toxicity to normal cells and increased selectivity toward cancer cells. This

most active compound was found to possess good *in vitro* activity too, and increased the life span in treated mice significantly.

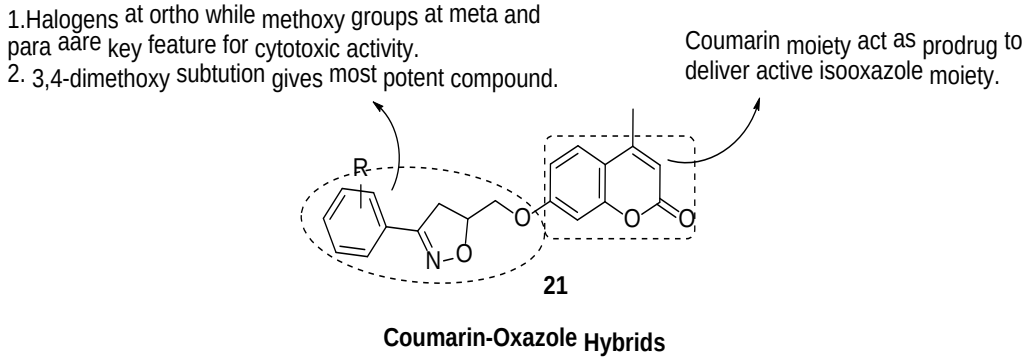


Figure 2.11: SAR studies of isoxazole tethered coumarin derivatives

- **Kahveci *et al.*** reported synthesis of coumarin-triazole hybrids and screened them for anti-tumour activity against breast cancer utilizing BT20 cell lines by taking cisplatin as reference drug. The compounds with maximum activity have been depicted in Figure 2.12. SAR studies predicted that bromobenzyl substitution on triazole ring **22** and diethylamine substitution on coumarin nucleus **23** produced compounds with excellent activity, whereas dichloro substituted hybrids displayed moderate activity (Kagveci *et al.*, 2017).

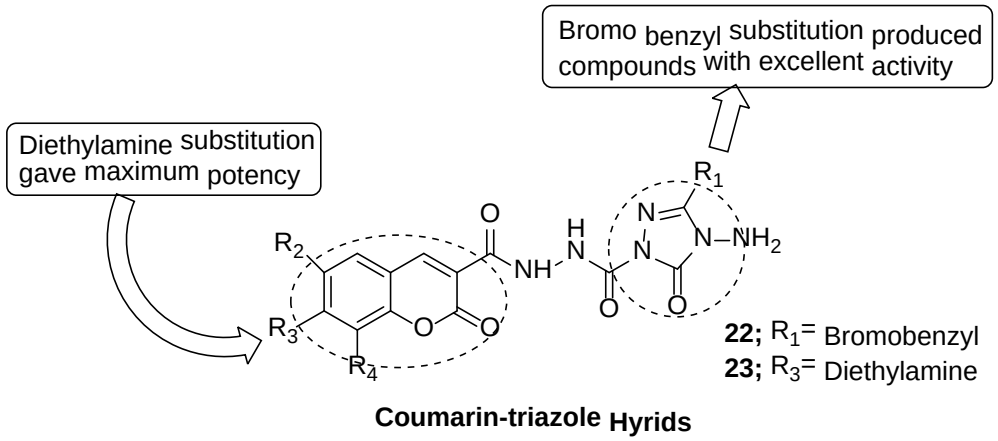


Figure 2.12: Coumarin-triazole hybrid compounds

- **Hussain *et al.*** reported a series of benzocoumarin-stilbene hybrids prepared by Horner-Wadsworth-Emmons (HWE) reaction. The synthesized compounds were screened further for anticancer activity against breast carcinoma by utilizing MDA-MB-231 and mouse 4T1 cell lines. Compound **24** was found most potent which showed excellent activity which was equal to the reference

drug resveratrol (IC_{50} 12µM). SAR studies suggested that increase in electron donating species (dimethoxy substitution) on the aromatic ring increased the potency significantly (Figure 2.13). Mechanistic studies of compound **24** revealed that it binds and inhibits human DNA ligase I enzyme which is responsible for reducing the growth of tumour and arrest of cell cycle progression at S and G2/M phase (Hussain *et al.*, 2017). The compound **24** was also investigated for *in vivo* anti-tumor activity in a dose dependent manner. The dose was administered through oral gavage continuously for 32 days and there was a significant reduction in tumor size. There was no marked change in in body weight during the treatment period which is indicator of non-toxic nature of the compounds.

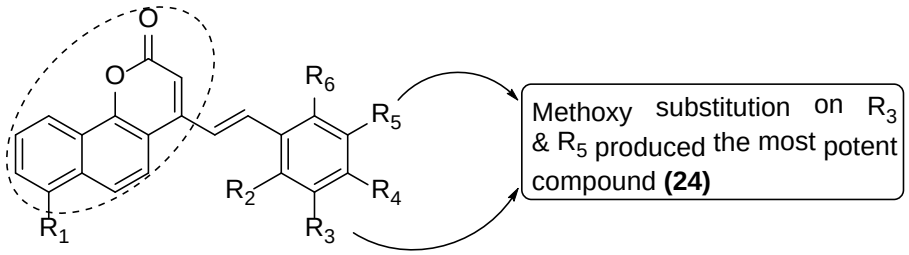


Figure 2.13: Benzocoumarin-stilbene hybrid with maximum potency

- **Kamath *et al.*** reported synthesis of hybrid molecules by combining coumarin scaffold with indole and thiadiazole moieties. The synthesized compounds were further screened for cytotoxic activity in MCF7 cell lines against breast adenocarcinoma. It was observed that the compound **25** showed maximum cytotoxic activity as presented in Figure 2.14. From the results, it was evident that one, two and three methylene spacers placed between indole and thiadiazole ring have direct impact on the cytotoxix efficiency of hybrid molecules. It was found that when spacer linker was propyl between indole and thiadiazole, excellent potency was obtained. Further investigations to explain mode of action were carried out on compound **25** which showed induction of apoptosis through activation of caspases. Wound healing assay of compound **25** displayed impairment of motility of MCF7 cell lines which displays anti-metastatic potency of this (Kamath *et al.*, 2017).

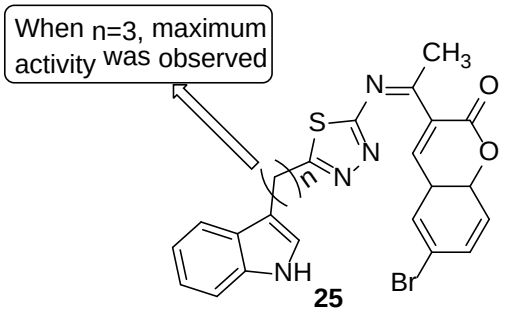


Figure 2.14: Indole-coumarin-thiadiazole hybrids with maximum potency

- **Morsy *et al.*** reported a series of coumarin pyrimidine hybrid molecules through a methylene thio linker (Figure 2.15). The synthesized molecules were screened for activity against breast cancer against MCF7 cell lines utilizing 5-fluoro uracil as reference. Compounds revealed strong cytotoxic activity with MIC values between 6.9±0.38 µg/mL-10.9±0.97µg/mL respectively. On the basis of procured data the SAR can be explained as on substituting pyrimidine motif with hydrazine **26** or piperazine **27**, significant amount of activity is produced. But upon replacement of these groups with aniline or morpholine there is a big drop in activity which confirms that to obtain sufficient amount of potency the compound must bear hydrogen bond donor like moiety. The most potent compound **26** was subjected to docking studies against the cyclin dependent kinase-2 (CDK2). CDKs play a vital role in multiplication and division of cells hence it is a suitable target for drug candidates against variety of cancers. Docking studies of **26** revealed that the target compound gets fit into the cavity and occupies the same pocket of the active site and forms a hydrogen bond with the amino acid Leu83. On the basis of these studies the proposed anti-proliferative action of these compounds was via suppression of CDK2 activity (Morsy *et al.*, 2017).

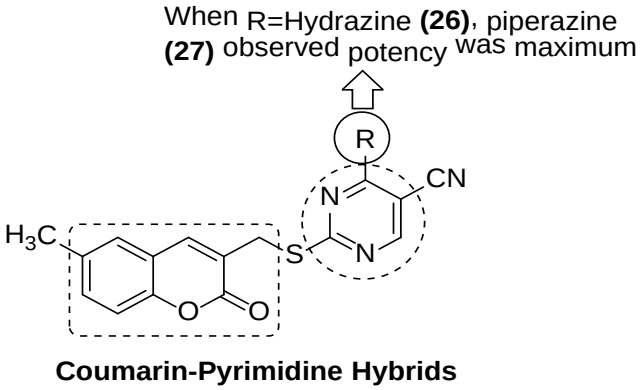
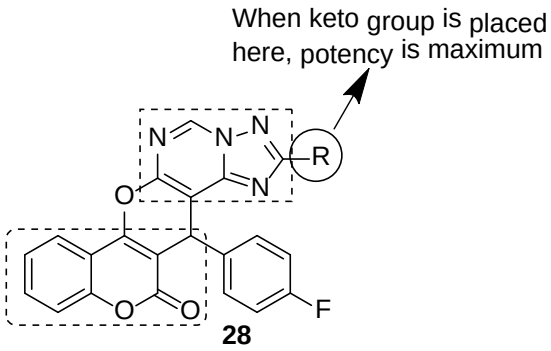


Figure 2.15: Most potent coumarin-pyrimidine hybrids

- **Batran *et al.*** synthesized new hybrids containing coumarin and triazolo-[1-5c] pyrimidine scaffolds. The synthesized compounds were further evaluated for anti-proliferative activity against breast cancer on human MCF-7 cell lines and VEGFR-2 (vascular endothelial growth factor receptor-2) inhibitory potential. Compounds showed strong to moderate activity on MCF7 cell lines with best MIC values of 19.80±2.20, 11.00±1.37, 7.90±0.88 µg/mL respectively, whereas compound **28** revealed good inhibitory activity for VEGFR-2 at a concentration of 117.00±12.80 ng/ml. SAR suggested that triazolone derivative **28** exhibits excellent potency, whereas replacement of it with $–CH_3$ or H causes slight decrease in potency (Figure 2.16). Compound **28** was further subjected to docking to determine the receptor binding pattern against VEGFR-2. The interactions observed were H-bonding, arene-arene, arene-cation and hydrophobic with different amino acids. Carbonyl oxygen formed hydrogen bond with Lys868 at a distance of 2.26Å, fluorophenyl moiety showed arene-cation interaction with Lys868 and hydrophobic interaction with Leu889 whereas chromene nucleus formed arene-arene interaction with Phe 1047. The proposed mode of action was through suppression of VEGFR-2 activity (Batran *et al.*, 2017)



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Coumarin-Triazolo [1,5-c] Pyrimidine Hybrids

Figure 2.16: Most potent coumarin-triazolo pyrimidine hybrids

- **Ghorab *et al.*** designed and synthesized coumarin-sulphonamide hybrid molecules and evaluated them for potency against breast cancer as well as aromatase inhibition. The cytotoxic activity was evaluated against breast cancer cell lines T47D, where the compounds revealed moderate to excellent activity (IC_{50}=108.9-8.8 μM) in comparison to the reference drug doxorubicin with IC_{50}=9.8 μM. Further to investigate the mechanism of action the compounds were also evaluated for aromatase inhibitory potentials. It was worth notable that substitution at the terminal sulphonamide group with six-membered heterocycles displayed greater inhibition as compared to substitution with five-membered heterocycles. It was observed that the methoxy **29** dimethoxy **30** substituted pyrimidinyl group displayed maximum aromatse inhibition (81%) (Figure 2.17). *In silico* investigation through molecular docking studies against aromatse also justified the *in vitro* outcomes. The synthesized molecules were utterly fit into the binding pocket of aromatase and revealed good hydrogen bonds and hydrophobic interactions. Methoxy, dimethoxy substitution led to improvement in the molecules' lipophilic character and aided to the potency (Ghorab *et al.*, 2016).

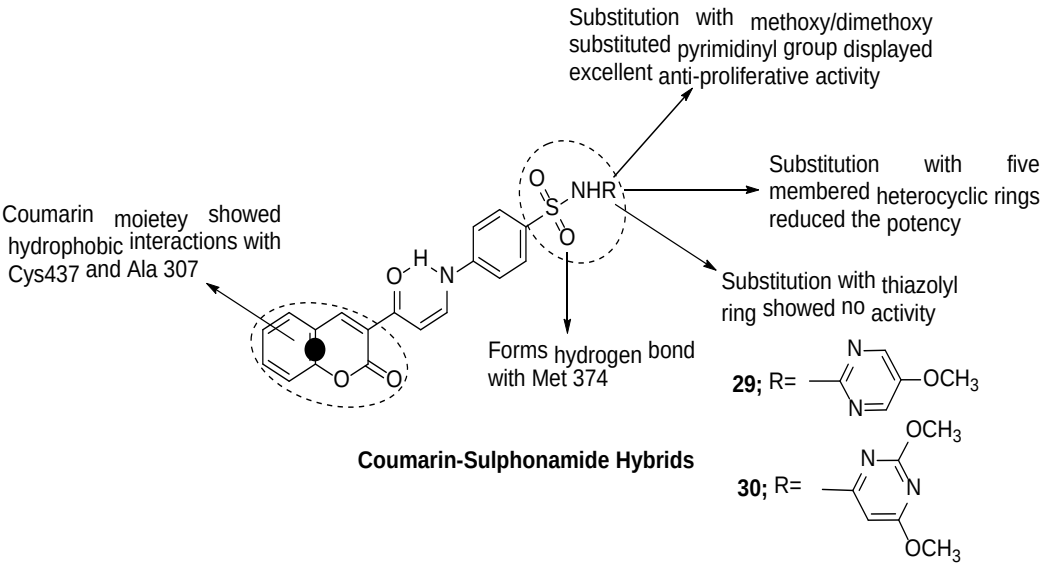


Figure 2.17: SAR of coumarin-sulphonamide hybrids as aromatase inhibitors

- **Ma *et al.*** reported multi-subtituted coumarin derivatives (Figure 2.18) having pyridine scaffold in structure as potential PI3K inhibitors (Ma *et al.*, 2016). The synthesized coumarin derivatives showed good *in vitro* inhibitory activities in MTT assay against K562, Hela, A549 and MCF-7 cancer cells with some of them possessing potency better than BENC-511. Compounds with halogen substituents such as chloro **31** and bromo groups **32** were found to have more potent PI3K inhibitory activity with affinity toward PI3Kα/β/δ. Further SAR studies revealed that di-subtitution at phenyl ring is more preffered over mono substitution.

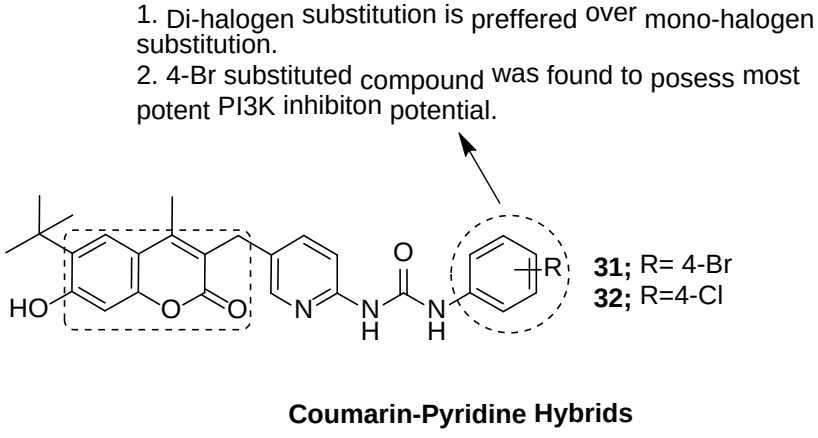


Figure 2.18: SAR studies of pyridine-coumarin hybrids as PI3K inhibitors

- **Lu *et al.*** reported C4 (Figure 2.19) pyrimidine substituted coumarin derivatives with potent anti-cancer activities against various cell lines. The 6-methyl-4-substituted coumarin-pyrimidine hybrids **33** were found to possess

promising anti-cancer activity against HepG2 and MCF-7 cell lines. The presence of hydrogen bond donors such as hydrazyl or piperazinyl groups at R position of pyrimidine is favoured while its replacement with an aniline group reduced activity. The docking studies revealed the binding mode of ligands in which carbonyl group of coumarin was found to favour hydrogen binding with Leu83 amino acid residue of cyclin dependent kinase 2 (CDK2) (Lu *et al.,* 2016). This is key interaction for CDK2 inhibition in anti-cancer activity.

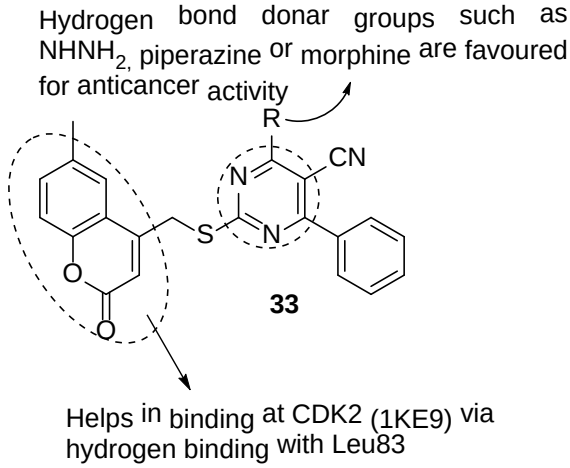
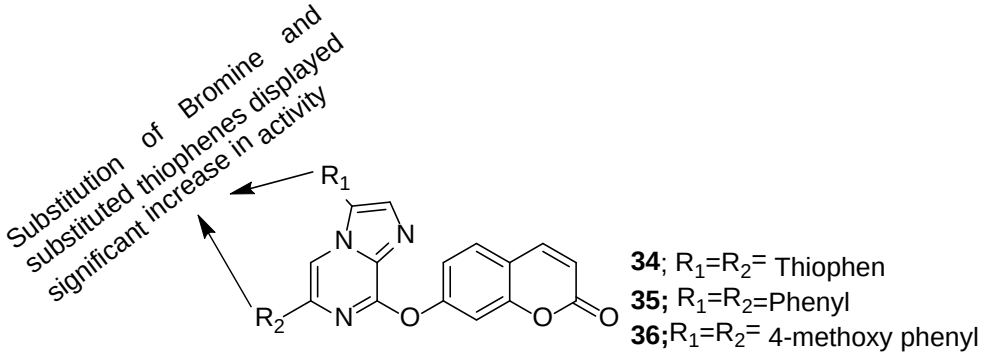


Figure 2.19: SAR studies of C4 substituted coumarin derivatives as CDK inhibitor

- **Goel *et al.*** reported synthesis of hybrid molecules by combining two biologically active heterocyclic moieties imidazo [1,2-a] pyrazine and coumarin (Figure 2.20). The synthesized compounds were further screened for activity against breast cancer by evaluating percentage growth inhibition on MCF7, MDA-MB231, HS5787, BT549, T47D and MDA-MB468 cell lines utilizing 6-fluoro uracil (5-FU) as standard drug. Compound presented good spectrum of growth inhibition against the various cell lines. Structural activity relationship concluded that substitution of two thiophen rings on 3^{rd} and 6^{th} position **34** slightly increased the activity whereas placing of phenyl rings **35** and 4-methoxy phenyl rings **36** on the same positions increased the activity in higher amounts. Simple Bromo substitution on these positions also showed good percentage inhibition. Molecular docking studies of compound **36** were carried on B-Raf kinase protein which is involved in sending signals inside cells to direct cell growth. Compound **36** showed H-bonding interaction of N7

atom of pyrazine ring with M517 amino acid residue at a distance of 2.17 Å, N1 and N4 atoms also formed hydrogen bonds with G518 amino acid residue with distances of 2.72 and 1.75 Å respectively. Oxygen atom of 4-methoxy group showed H-bond interaction with R781 amino acid residue at a distance of 2.85 Å. Carbonyl group of coumarin forms H-bonding interaction with Q530 amino acid residue at a distance 2.31 Å. So from the binding pattern of compound **36** with its target the proposed mode of action of synthesized compounds is through inhibition of B-Raf kinase function (Goel *et al.*, 2015).



Coumarin-Imidazo [1,2-a] Pyrazine Hybrids

Figure 2.20: Most potent coumarin-imidazo [1,2-a] pyrazine hybrids

- **Kamath *et al.*** utilized the molecular hybridization approach for the designing of newer coumarin-indole hybrids and carried out docking studies with apoptosis inducer gene Bcl-2. The synthesized compounds were further screened for dose dependent cytotoxic effect against human breast adenocarcinoma (MCF7) cell lines by taking microtubule interfering drug vincristine as the reference drug. Compound **37** and **38** revealed best potency with IC_{50} values of 7.4 and 5.5 µM respectively. Cell cycle analysis of MCF7 cells after treatment of compound **37** revealed that it causes cell cycle arrest at G_2/M phase which ensured that the compound works through introducing apoptosis. SAR studies demonstrate that presence of Br substituent on the coumarin scaffold of the coumarin-indole aldehyde series, showed maximum potency (Compound **37**), whereas substitution of Cl atom at the same position of the coumarin-indole carboxylic acid series produced compound with maximum activity **38** (Figure 2.21). It can be concluded that Br may enhance the liphophilic character of the molecule to facilitate easy passage through membranes. Beyond this, bromine generates a more reactive alkylating

reactive intermediate as compared to other halogens which is advantageous in long term therapy. Docking studies of compound **37** on Bcl-2 gene predicted that there is excellent affinity of the compound with the target and it showed affinity with Gln 118 (O), Tyr 108 (OH), Arg 129 (NH1), His 120 (NH), His (O) protein residues at a distance of 3.70, 2.36, 3.62, 2.58 and 3.60 Å respectively (Kamath *et al.,* 2015).

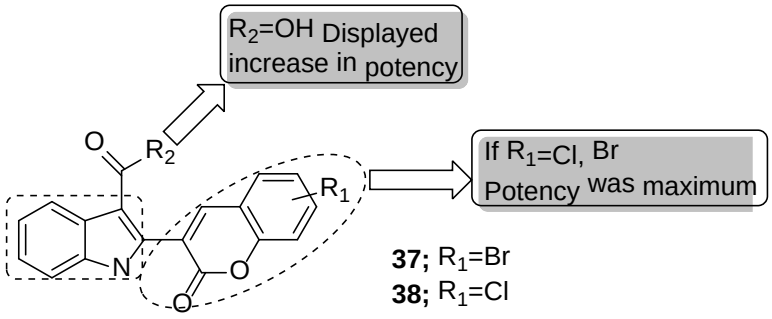


Figure 2.21: Coumarin-indole hybrids with maximum potency

- **Ballazhi *et al.*** have reported hybrid molecules of coumarin with isoxazole and thiazole **39** as potent agents against breast-cancer**.** The results demonstrated dose- and time-dependent activity, with the most potent molecules having a thiazole moiety, without or with additional methyl group(s) attached to the carbon(s) at position(s) 5 and/or 4 in the thiazole ring. These molecules possessed significantly higher potency against both test cell lines (SCP1833 & SCP4175) as compared to 4-hydroxycoumarin (4-HC) (Ballazhi *et al.,* 2015). Compounds showed >2 to 4 fold increase in anti-breast cancer activity. Structural activity relationship (SAR) described that the most potent compounds bear a thiazole moiety attached to coumarin scaffold through a hydrazinylidene linker at position 3 with methyl group substitutions on the thiazole ring (Figure 2.22). When thiazole moiety is replaced by isoxazole, the activity is remarkably less. This report is helpful in designing newer molecules by modifying 4-hydroxy coumarin.

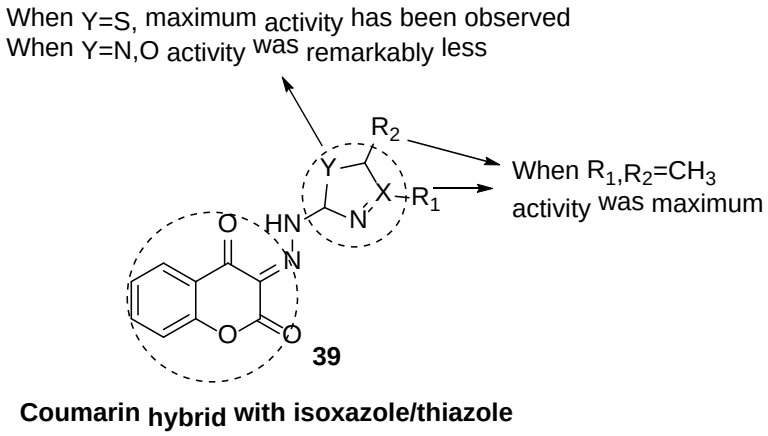


Figure 2.22: Coumarin hybrids with isoxazole/thiazole with best activity

- **Liu *et al.*** reported the synthesis of hybrid molecules by combining coumarin scaffold with phenylsulfonylfuroxan moiety. The synthesized compounds were evaluated for cytotoxic activity against breast cancer utilizing normal MDA/MB-231 and gemcitabine resistant MDA/MB-231/gem cell lines. The compound **40** was found most potent with IC_{50} values 0.15 and 0.14μM for both the cell lines respectively. The incorporation of oxygen atom in linker chain showed prominent activity. The length of the spacers connecting the NO donors displayed direct effect on antitumor activity. Compound **40** with C-2 spacer displayed excellent potency (Figure 2.23). Further investigation on compound **40** revealed that it was a significant inhibitor of tubule formation and angiogenesis. The mode of action of these hybrids was proposed through induction of apoptosis caused by release of NO. Western blot analysis of compound **40** depicted that it has ability to down regulate the anti-apoptotic gene Bcl-2 in a dose dependent manner (Liu *et al.*, 2014).

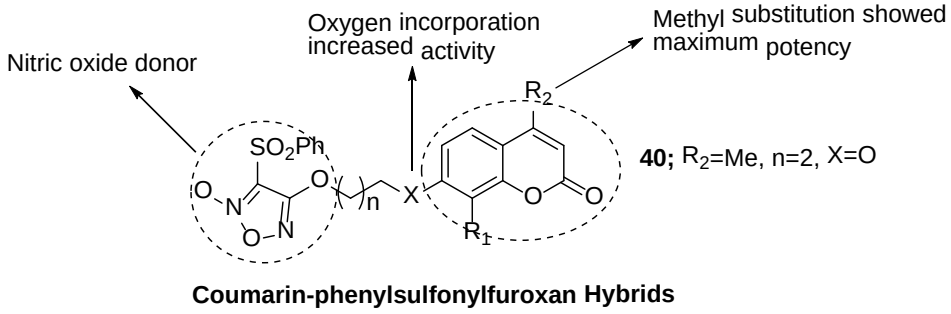


Figure 2.23: Coumarin-phenylsulfonylfuroxan hybrid with maximum potency

- **Sashidhara *et al.*** have discovered a novel class of coumarin-monastrol hybrid compounds as novel anti-breast cancer agents which selectively induce

apoptosis in both primary and metastatic breast cancer cell lines **(**MCF7 and T47D) using tamoxifen (TAM) and epirubicin as reference drugs. The structures of the best compounds have been shown in the Figure 2.24. It was observed that increase in methoxy substitution on phenyl ring and tertiary butyl substitution on the coumarin scaffold **41** showed high potency against MCF 7 and T 47D cell lines. Along with this substitution of pyrimidine with =S and =O showed significant activity against MCF7 cell lines. Along with this Caspase-3 activation studies and cell cycle analysis on compound 4**1** were also carried out to understand the mode of action. The activation of Caspase-3 is an indicator of induction of apoptosis. Compound **41** increased the activity of Caspase-3 and flow cytometric analysis revealed cell cycle arrest of MCF7 cell lines as G1 phase due to induction of apoptosis (Sashidhara *et al.*, 2013).

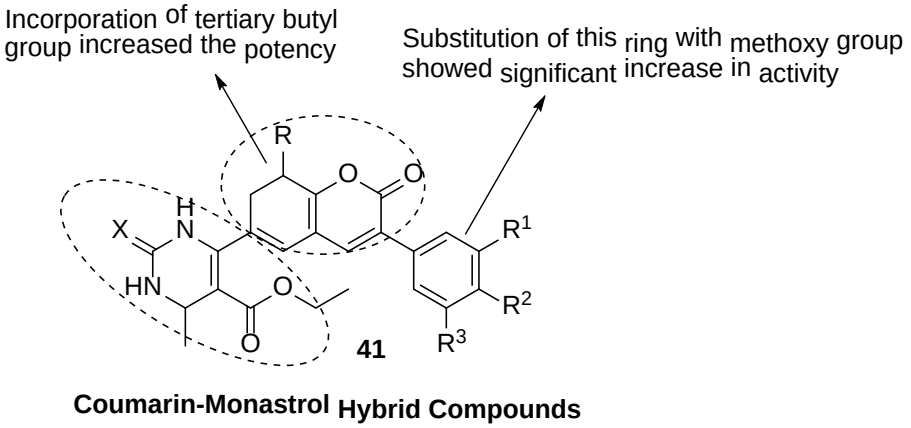


Figure 2.24: Coumarin-monastrol hybrid compounds against breast cancer

- **Chen *et al.*** have designed and synthesized novel triphenylethylene-coumarin hybrid derivatives containing different types of amino side chains and were characterized for anti-proliferative against breast cancer MCF-7 cell lines by taking tamoxifen and cisplatin as reference drugs. The most potent compound **42** and its SAR have been depicted in Figure 2.25. It was observed that number of amino alkyl chain on 3,4-diphenyl coumarin was directly influencing the anti-proliferative activity of the compounds and 2 side chains were most suitable for it. Presence of weaker basic amino groups demonstrated significant elevation in activity. Compound with open chain amino group **42** revealed excellent potency with IC_{50} value of 7.90±0.82 µM. Along with DNA binding properties of these compounds were also evaluated

which expressed that DNA might be a potential targets for anti-tumour activity of such compounds (Chen *et al.*, 2013).

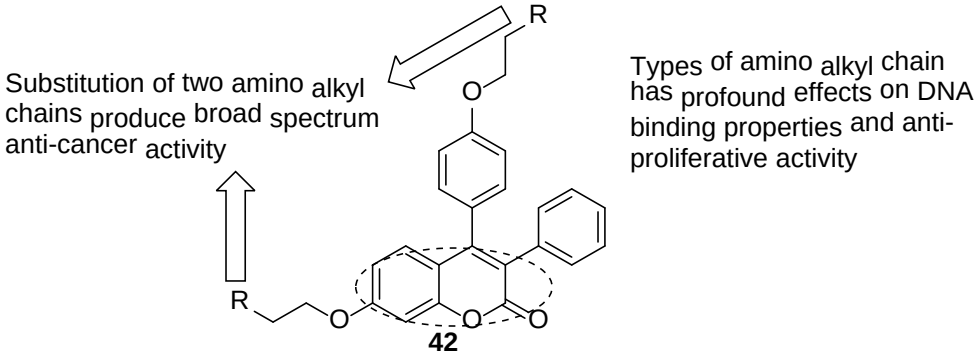


Figure 2.25: Coumarin-triphenylethylene hybrids with maximum potency

- **Paul *et al.*** have developed hybrid compounds by combining coumarin scaffold to the benzimidazole nucleus without any spacer or linker (Figure 2.26). All the previous reported hybrids were containing a spacer between two moieties. The synthesized compounds were further screened for evaluation of anti-breast cancer activity by utilizing MCF7, MDA-MB-231, HS578T, BT-549 and T-47D cell lines by taking 5-flourouracil as a reference drug. Compounds **43** with bromide substitution and **44** with ethanolamine showed maximum growth inhibition. To investigate the possible mode of action, the most potent compound **44** was subjected to molecular docking studies on Topoisomerase II which is responsible for DNA replication, Ribonucleotide reductase (RNR) and dihydrofolate reductase (DHFR). It showed five hydrogen bonding interactions with different amino acid residues of Topoisomerase II. RNR is responsible for synthesis of raw materials for DNA replication. Docking of compound **44** within pocket of RNR, four hydrogen bonding interactions were observed within a short distance (less than 4 Å). Similarly in the pocket of DHFR, three hydrogen-bonding interactions were expressed. Therefore probable mode of action to exhibit might be through suppression of the activity of these three enzymes (Paul *et al.*, 2013).

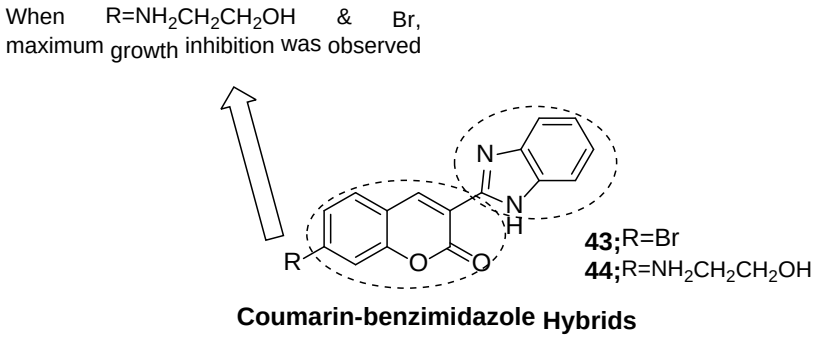


Figure 2.26: Coumarin-benzimidazole hybrids with maximum potency

- **Chand *et al.*** has prepared a series of coumarin-cinnamoyl/pyrano **45** derivatives by reacting coumarin scaffold with various aromatic aldehydes. The synthesized compounds were further screened for Src kinase inhibitory activity. Src kinase is a key biomarker for the breast cancer. The most potent cinnamoyl-coumarin and pyrano-coumarin derivatives have been depicted in Figure 2.27. The drug staurosporine was taken as reference drug and the compounds displayed moderate inhibitory activity in comparision to it. It was found through SAR studies that disubstitution on the phenyl ring of cinnamoyl group increases the anti-proliferative activity (Chand *et al.*, 2013).

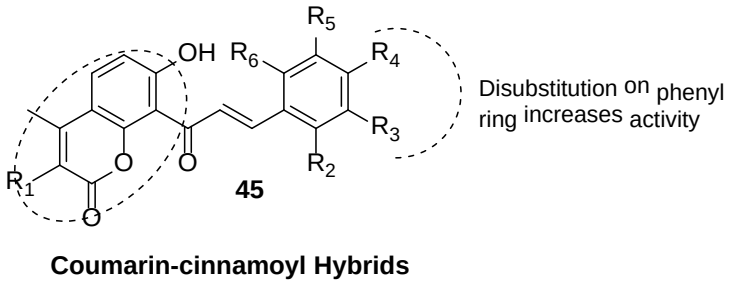


Figure 2.27: Coumarin-cinnamoyl hybrids with maximum potency

- **Kini *et al.*** reported a series of coumarin-benzothiazole hybrids against breast cancer. The synthesized compounds were further evaluated for anti-breast cancer activity on MCF7 cell lines by determining percentage growth inhibition and IC_{50} values. Compounds **46** and **47** showed 78.68 and 79.40% growth inhibition with IC_{50} values of 50.00 and 59.80 µM/ml respectively. From the activity results it was analysed that substitution of acetoxy group on 3rd position of coumarin phenyl ring and methyl sulphide on 2nd position produced compounds with maximum activity (Kini *et al.*, 2012) (Figure 2.28).

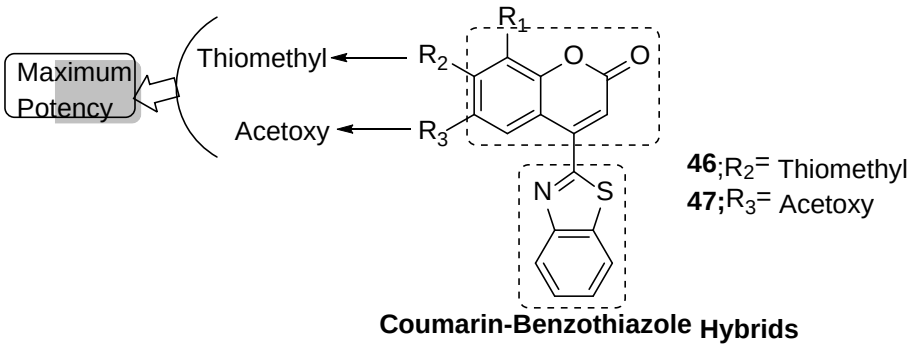


Figure 2.28: Most potent coumarin-benzothiazole Hybrids

- **Patel *et al.*** have synthesized a series of coumarinyl chalcone hybrids **48** bearing different substitutions at ring B of chalcone. Their anti-proliferative activities were evaluated against three different breast cancer cell lines (MDA-MB231, MDA-MB468 and MCF7) and one non-cancer breast epithelial cell line (184B5) by SRB-based spectrophotometry. The best potent compounds have been depicted in Figure 2.29 (Patel *et al.,* 2011). The SAR studies revealed that substitution of two chloro groups on alternating positions of phenyl ring showed moderate activity, whereas substitution of three methoxy groups on first three positions of phenyl ring showed sharp increase in activity against all the cell lines. So, it is evident that the activity of the reported compounds depends upon pattern of substitution and position of substituents as well and the reported compounds can be utilized as new leads for further drug development.

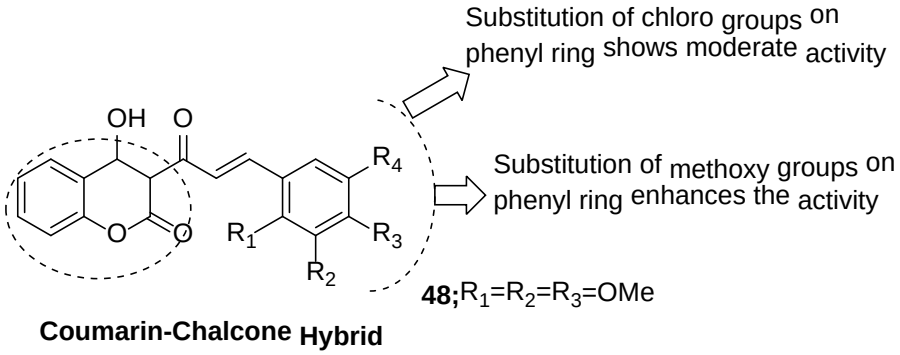


Figure 2.29: Most potent coumarin-chalcone hybrid compounds

- **Stefanchi *et al.*** prepared newer compounds by fusing 7-substituted coumarins and imidazole scaffold. The synthesized compounds were further screened for aromatase inhibitory activity. The compounds showed excellent

aromatase (CYP19) inhibitory activity in nanomolar range. Compounds with maximum potency are depicted in Figure 2.30. The compounds displayed excellent CYP19 inhibitory activity within IC_{50} range of 0.15-0.04 µM. It was evident that there was a direct effect of the nature and position of the substituent on the inhibitory activity. The compound7-(3,4-difluorophenoxy)-4-imidazolylmethyl **49** was the most potent compound with IC_{50} of 0.047 µM. In order to predict binding pattern of the synthesized compounds with the target compounds were docked in the pocket of human aromatase (AR). The predictions revealed that when coumarin scaffold carries an imidazole group on 4th position and a phenoxy group on 7th position, the affinity of compounds gets enhanced. The extent of affinity was regulated by distance between Nitrogen of imidazole ring and oxygen of lactone ring (Stefanchi *et al.*, 2011).

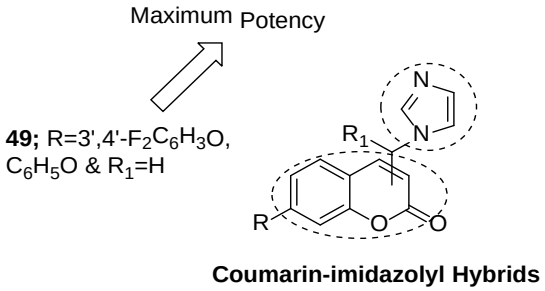


Figure 2.30: Coumarin-imidazolyl hybrids with maximum potency

- **Xiao *et al.*** reported synthesis of some coumarin-stilbene hybrids called as 3-arylcoumarins by simple Perkin reaction. The synthesized compounds were further screened for in vitro anti breast cancer activity on MCF7 and MCF7/ADR cell lines. The SAR studies depicted that 3-arylcoumarins bearing 7,8-dihydroxy substitution or diacetyloxy groups possessed the maximum cytotoxic activity. The compounds with maximum potency **50-51** have been presented in Figure 2.31 (Xiao *et al.*, 2011).

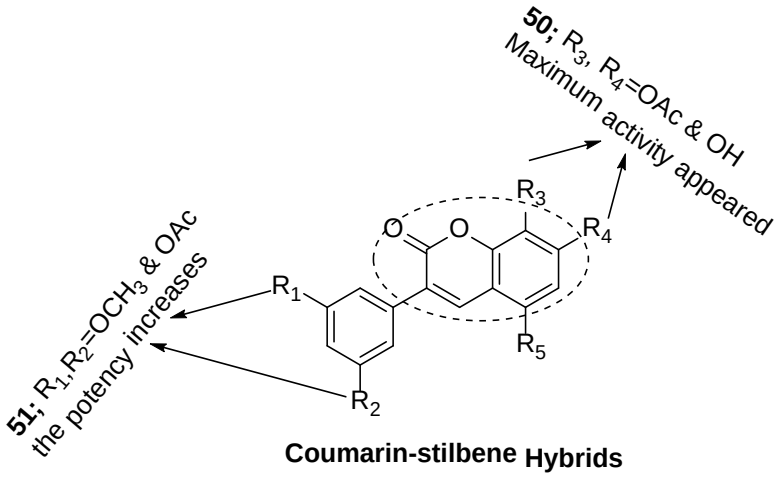


Figure 2.31: Coumarin-stilbene hybrids with maximum potency