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Rational Approaches, Design Strategies, Structure Activity Relationship and Mechanistic Insights for Therapeutic Coumarin Hybrids

Harbinder Singh[1], **Jatinder Vir Singh**[1], **Kavita Bhagat**[1], **Harmandeep Kaur Gulati**[1], **Mohit Sanduja**[2], **Nitish Kumar**[1], **Nihar Kinarivala**[*,3], **Sahil Sharma**[*,1,3]

[1]Department of Pharmaceutical Sciences, Guru Nanak Dev University, Amritsar-143005, Punjab, India

[2]School of Pharmaceutical Sciences, MVN University, Palwal-121105, Haryana, India

[3]Program in Chemical Biology, Sloan Kettering Institute, New York, NY-10065, USA

Abstract

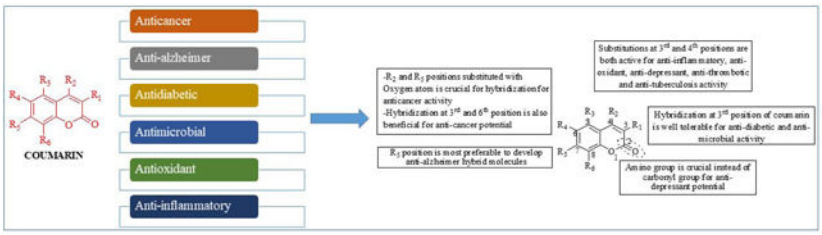
Hybrid molecules, furnished by combining two or more pharmacophores is an emerging concept in the field of medicinal chemistry and drug discovery that has attracted substantial traction in the past few years. Naturally occurring scaffolds such as coumarins display a wide spectrum of pharmacological activity including anticancer, antibiotic, antidiabetic and others, by acting on multiple targets. In this view, various coumarin-based hybrids possessing diverse medicinal attributes were synthesized in the last five years by conjugating coumarin moiety with other therapeutic pharmacophores. The current review summarizes the recent development (2014 and onwards) of these pharmacologically active coumarin hybrids and demonstrates rationale behind their design, structure-activity relationships (SAR) and mechanistic studies performed on these hybrid molecules. This review will be beneficial for medicinal chemist and chemical biologist, and in general to the drug discovery community and will facilitate the synthesis and development of novel, potent coumarin hybrid molecules serving as lead molecules for the treatment of complex disorders.

Graphical Abstract

[*]Corresponding authors: 1) Dr. Sahil Sharma, Department of Pharmaceutical Sciences, Guru Nanak Dev University, Amritsar-143005, Punjab, India, ss.gq2009@gmail.com, 2) Dr. Nihar Kinarivala, Program in Chemical Biology, Sloan Kettering Institute, New York, NY-10065, USA, kinarivn@mskcc.org.

Conflict of interest

The authors declare no conflict of interest.



Keywords

coumarin hybrids; design strategies; structure-activity relationship; mechanistic insights; anticancer; antimicrobial; antidiabetic; antioxidant; anti-Alzheimer's; anti-inflammatory

1. Introduction

Since prehistoric times, natural products have played a significant role as traditional medicines for the therapeutic treatment of various diseases. Natural products have also played a critical role as lead molecules for drug discovery [1]. The synthetic and semi-synthetic derivatives of natural products have been found to exhibit various biological activities. It has been established that the heterocyclic compounds containing oxygen atom play an important role in designing novel molecular architectures for medical use [2]. Among oxygen-containing heterocyclic compounds, the chemistry of coumarin (2*H*-chromen-2-one) has been deeply explored owing to its wide range of biological activities. The parent coumarin was first isolated in 1820 by Vogel from Tonka beans [3]. Recently, about 150 species of coumarin have been found in 30 diverse plant families, such as Clusiaceae, Guttiferae, Rutaceae, Oleaceae, Umbelliferae [4]. Its diverse pharmacological activities like anti-bacterial [5], anticoagulant [6], anti-HIV [7,8], antioxidant [9], antitubercular [10], antihypertensive [11], anticonvulsant [12], anti-fungal [13], antihyperglycemic and anticancer [14] brought this class forefront. Some of the coumarin derivatives in clinical use are depicted in Figure 1A [15–17]. Its therapeutic applications depend upon the nature and position of substituents present on the basic nucleus. (Figure 1B)

Coumarin derivatives follow a number of complex pathways to target various diseases. The detailed description is shown in Figure 2. To target cancer, coumarin derivatives directly or indirectly inhibit the process of tubulin polymerization, mitosis, and DNA replication by inhibiting various enzymes like protein kinases, sulphatases, aromatase, caspases and heat shock proteins [18–20]. Coumarin derivatives block the catalytic activity of cholinesterase enzymes (acetylcholinesterase and butyrylcholinesterase) thereby retaining the levels of acetylcholine in the brain and act as anti-Alzheimer's agents. Coumarin derivatives were also developed as monoamine oxidase enzyme type B (MAO-B) inhibitors demonstrating neuroprotective activity [21,22]. α-amylase and α-glucosidase are other primary targeted enzymes of coumarin derivatives, the inhibition of which treats hyperglycemia [23,24]. Coumarin derivatives were also reported as inhibitors of fatty acid degradation protein D32 (FadD32) for the treatment of tuberculosis [25]. Several reports demonstrate the anti-

inflammatory potential of these derivatives by targeting cyclooxygenase and lipoxygenase enzymes [26–29].

Recent trends in the field of medicinal chemistry suggest a change from the traditional “one molecule – one target – one disease” approach. Most disorders arise from a complex interplay of proteins, which is difficult to treat with single targeting agents. Targeting multiple pathways simultaneously in complex disorders such as cancer, CNS disorders and others is thus a promising approach. Vorinostat, a potent histone deacetylase inhibitor (HDAC inhibitor) was approved by the FDA in 2006 for the treatment of cutaneous T-cell lymphoma. CUDC-907, a hybrid molecule of vorinostat and GDC-0941 (a P13K kinase inhibitor) [30] is more efficient for the treatment of cancer both in *in vitro* and *in vivo* models without showing any systemic toxicity and resistance. This dual HDAC/PI3K inhibitor recently has passed the Phase I trial for the treatment of lymphoma or multiple myeloma and advanced solid tumours [31] and entered in Phase II trials (Figure 3A) [32–37]. Ziprasidone, ladostigil and duloxetine for multifactorial CNS diseases, and lapatinib and sunitinib for treatment of cancers are the other examples of prominent hybrid molecules (Figure 3B).

Design and synthesis of these hybrids are typically performed using molecular hybridization techniques by identifying pharmacophoric sub-units in the molecular skeleton of two or more known biologically active derivatives. The recent interest among researchers in the discovery of hybrids that can concomitantly address more than one biological target is increasing day by day [38–40]. Additionally, hybrid molecules provide new dimensions by lowering the risk of drug-drug interactions and minimizing drug resistance [41].

Coumarin-based molecular hybrids are developed by attaching the coumarin to pharmacologically active fragments with or without any tethering agents (Figures 4–7). Efforts have been conducted in the past to review coumarin derived hybrid molecules possessing pharmacological activity. This review sheds light on the recent work (2014 & onwards) conducted using coumarin hybrids active against various diseases. This compilation presents the *in vitro* as well as *in vivo* data of reported coumarin-based hybrids. The review also demonstrates some strategies for the design, structure-activity relationships (SAR) along with the mechanistic insights and the key interactions of the designed hybrids within their specific targets by computational studies. This compilation may help medicinal chemists to develop novel coumarin hybrids with varied therapeutic attributes.

2. Coumarin-based hybrids as anticancer agents:

A major affliction worldwide is a multifactorial disease, cancer, responsible for an estimated 9.6 million deaths in 2018 [42]. Multiple protein and enzymes are dysregulated in this devasting disease-causing difficulties for treatment with one target-based therapeutics. The initiation and progression of this complex disease depends on numerous receptors or signaling pathways indicating that multi-targeted therapy could have prominent efficacy as compared to solo-targeting therapies [43–48]. This can be achieved by using hybridization technique. In continuous efforts to fight against this tremendous problem, various research groups have developed coumarin-based hybrid molecules which are described below.

Keeping in view the success of isatin moiety as a potential anticancer agent [49], Singh *et al.* in 2017 rationally design and synthesized a series of novel isatin-coumarin hybrid molecules by conjoining through triazole ring. All the synthesized compounds were assessed to check their anticancer potential against four human cancer cell lines (Leukemia cancer cells: THP-1, Colon cancer cells: COLO-205, Colon cancer cells: HCT-116 and Prostate cancer cells: PC-3). Compounds were found active against three human cancer cells lines except for colon cancer cells (PC-3). Three compounds **1**, **2** and **3** were found most active against leukaemia cancer cells. Careful examination of biological data with the structural features of compounds revealed that the substitution on the isatin moiety and length of the linker between isatin and triazole remarkably influenced the anticancer activity. Unsubstituted isatin (R = H) with two carbon chain (n = 1) was found to be crucial for activity. Additionally, electron withdrawing groups on isatin were well tolerable for anticancer activity as compared to electron donating groups. Because of the anticancer potential of isatin and coumarin derivatives is attributed to tubulin inhibition, tubulin inhibition was evaluated for most potent compounds by using western blotting analysis amongst which compound **1** showed best tubulin inhibition with the IC_{50} value of 1.06 μM (potent than combretastatin IC_{50} = 1.2 μM), which was further confirmed through confocal microscopy. It indicates that the anticancer potential of the synthesized hybrids is through tubulin inhibition. Possible molecular mechanism of binding within the tubulin active site of compound **1** was further revealed by using molecular modelling studies which exposed the binding pattern of the compound within the tubulin enzyme (Figure 8) [50].

The avoidance of apoptosis is a leading hallmark of cancer and prompting apoptosis in specific cancer cells is one of the convenient and extensively used approaches for the development of anticancer chemotherapeutics. Kamath *et al.* designed and synthesized indole-coumarin hybrids tethered through thiadiazole moiety with alkyl spacers of varying lengths and evaluated them *in vitro* as anticancer chemotherapeutics. Hybrid **4** linking via a three methylene spacer specifically inhibit breast adenocarcinoma (MCF-7) cells and Vero cells with the IC_{50} values of 8.01 and 116.02 μM, respectively both by the induction of apoptosis and by preventing metastasis (confirmed through wound healing assay) in a dose-dependent manner. Bcl-2, an anti-apoptotic protein, is overexpressed in a range of cancers. Computational docking studies revealed that compound **4** has the ability to inhibit the antiapoptotic Bcl-2 protein by acting as a small molecule BH3 mimetic (Figure 9) [51].

Estrogen receptor alpha plays an important role in breast cancer cell proliferation and various estrogen receptor modulator have been developed, but tumour develops resistance against these agents rapidly. Therefore, Mokale *et al.* designed and synthesized a series of hybrid molecules containing coumarin and chalcones and were evaluated for anti-proliferative activity both *in vitro* and *in vivo* methods. SAR revealed compound **5** as a hit. Administration of **5** (5 mg/kg, P.O.) showed a protective effect on mammary tumorigenesis in *N*-methyl nitrosourea (MNU) induced carcinoma female rat carcinoma model. Later on, compound **5** was docked and revealed that interactions were similar as that of standard compound (Raloxifene & Tamoxifen) with ER-α receptor and stabilized by various electrostatic interactions, thus concluding that **5** can be taken as lead and could be optimized further for better potency (Figure 10) [52].

Falcon *et al.* synthesized naphthoquinone-coumarin molecular hybrids and evaluated their activity as topoisomerase II (Topo-II) inhibitors. Results demonstrate that all the synthesized compounds were capable of inhibiting the *h*TopoIIα-mediated relaxation of negatively supercoiled pRYG in the micromolar range (between 10 to 30 μM). ATPase inhibitory assay revealed that compound **6** showed higher inhibition among the synthesized compounds. These synthesized conjugates, possessing a chiral carbon and were obtained as racemic mixtures. Therefore, the binding interaction of these molecules into the ATP pocket of Topoisomerase II of both enantiomers was examined with the molecular docking studies. Results of the docking studies revealed that compounds were actively fit into the ATP-binding site and formed cation-π interactions between the aromatic ring of naphthoquinone moiety for *S*-enantiomers, while in *R*-enantiomers similar type of interactions were observed with the aromatic ring of coumarin moiety. Additionally, hydrogen bonding and hydrophobic interactions increase their effectiveness as topoisomerase II catalytic inhibitors (Figure 11) [53].

Elshemy *et al.* designed and synthesized new coumarin hybrids in which 8-methoxy coumarin scaffold was conjoined with various bioactive pharmacophores viz. chalcone, acrylohydrazide and pyridine. The anticancer activity was checked against two cancer cell lines (HEPG2 and K562) and toxicity against fibroblast cells (WI-38). Results indicated that chalcone related hybrids active as anticancer agents and compound **7** and **8** were the most potent in this series with IC_{50} values 0.65 μM and 0.82 μM, respectively against HEPG2 cells. Coumarin-acrylohydrazides series compounds were active as well; compound **9** demonstrated IC_{50} of 0.49 μM against leukaemia cancer cell line (K562) while compounds **10** and **11** demonstrated IC_{50} of 0.77 μM and 0.93 μM, respectively against HEPG2 cells. Coumarin-pyridine hybrids were found inactive while substituted pyridine derivatives showed valuable anticancer results but were lesser potent than former two series of hybrid molecules. SAR revealed that coumarin-chalcone and acrylohydrazide compounds comprising of 4-methoxy (at R_2 position) substitution were most potent against HEPG2 cell line (compounds **7** & **10**), followed by di-methoxy substitutions (compounds **8** & **11**). Whereas, trimethoxy substitution on the phenyl ring (at R_1 to R_3) was found crucial against leukaemia cancer cells (compound **9**). It was demonstrated that all the coumarin hybrids increase caspase-3 and caspase-9 protein levels along with the downregulation of Bcl-2 and up-regulation of Bax protein indicating activation of apoptotic signals, as a consequence of arrest in G2/M phase (Figure 12) [54].

Kraljevic *et al.* intellectualized the new technique of molecular hybridization by merging 7-hydroxycoumarin (umbelliferone) or its 7-methyl analogue and 1,2,3-triazole entity with the aim to develop anticancer agents. The effect of these synthesized hybrids was also assessed on the regulation of specific lipid metabolic pathways which were facilitated by 5-lipoxygenase (5-LO), sphingosine kinase 1 and acid ceramidase, enzymes that control major aspects of cancer cell behaviour. Compounds containing phenyl- and 4-(*p*-alkylphenyl)-substituted 1,2,3-triazoles showed activity against lung adenocarcinoma (A549; IC_{50} = 8.87–32.34 μM) Incorporation of benzo-fused heterocycles such as benzothiazole, 5-iodoindole and benzimidazole, was responsible for the improvement in inhibitory activity. 7-methylcoumarin analogues such as compound **12** exhibited higher cytotoxicity against

HepG2 cells (IC_{50} = 0.90 μM). The Prediction of Activity Spectra for Substances (PASS) revealed that compounds exhibited high cytotoxic potential for leukotriene synthesis with Pa values of and 0.49 [55–58]. Compound **12** in HepG2 cells could be associated with its ability to inhibit 5-LO and acid ceramidase activities that may, in turn, lead to accumulation of pro-apoptotic lipids arachidonic acid and ceramide, respectively leading to apoptosis (Figure 13) [59].

In 2015, Singh *et al.* Rationally designed and synthesized triazole tethered C_5-curcuminoid coumarin bifunctional hybrids by using click chemistry approach and tested for their *in vitro* cytotoxicity against four human cancer cell lines (THP-1, COLO-205, HCT-116 and PC-3). Results of the inhibitory activity suggest that compounds **13**, **14** and **15** exhibited cytotoxicity against THP-1, COLO-205, HCT-116 with IC_{50} values range from 0.82–4.68 μM, 2.34–6.78 μM, 4.48–9.95 μM respectively. The hybrid molecules were inactive against the prostate cancer cell line (PC-3). SAR revealed that methoxy substituted phenyl ring (Ring A) remarkably enhances the cytotoxic activity (compounds **13** and **14**). The spacer length of two carbons between the coumarin and triazole moiety was found crucial for anticancer activity. The placement of naphthyl ring as Ring X acts as a surrogate for dimethoxy substituted phenyl ring. This set of compounds exert their cytotoxic potential through tubulin inhibition and compound **13** was found to be most promising in this series, exhibiting IC_{50} value of 1.55 μM against tubulin. Molecular docking studies compound **13** indicated that it completely blocks the catalytic assembly of tubulin by adhering at the interface of β1/α2 subunits (Figure 14) [60, 61].

Keeping in view the imperative role of iminothiazole in various biological activities [62], Ibrar *et al.* reported a new series of bis-coumarin–iminothiazole hybrid molecules and evaluated for alkaline phosphatase inhibition and anticancer activity. Alkaline phosphatase inhibition assay revealed that **16** was the most potent compound among the series with an IC_{50} value of 1.38 μM. Synthesized series also evaluated against kidney fibroblast (BHK-21) and lung carcinoma (H-157) cell lines at four different concentrations (100, 10, 1.0 and 0.1 μM). Results showed that compounds **17** and **18** were the most active analogue in this series. All synthesized compounds were found to follow the Lipinski Rule of five and Veber's Ro3. Various types of the binding interactions of compound **18** within the active site of the alkaline phosphatase enzyme were streamlined by using docking studies. The rigid and bulky structure favoured maximum exposure of the ligand to various amino acids of the active site (Figure 15A) [63].

Kamath *et al.* designed and synthesized new indole-coumarin hybrid molecules. The hit compound, **19** exhibited inhibitory activity against MCF-7 and Vero cancer cell lines with the IC_{50} values of 7.4 μM and 100 μM by arresting the cell cycle at the G2/M phase. In order to understand the other possible mechanism to act as anticancer agent, binding behaviour of **19** was analyzed by using molecular docking studies within the catalytic active site of Bcl-2 protein (Figure 15B) [64].

Keeping in mind the various therapeutic potential of heterocyclic moieties in pharmaceuticals, Goel *et al.* synthesized a new series of boronic acid-based imidazo[1,2-*a*]pyrazine-coumarin hybrids and screened for their *in vitro* anticancer activities against a

panel of 60 human cancer cell lines (NCI-60). At 10 μM concentration, compounds **20, 21** and **22** were found excellent molecules against small lung cancer cell line. SAR indicated that C3- and C6-positions of imidazo[1,2-*a*]pyrazine considerably influenced the anticancer activity. The anticancer activity slightly increased with the substitution of 2-thiophene-3-boronic acid rings at C3 and C6 positions, as demonstrated by compound **20**. Compound **22** containing a *para* boronic acid showed broad-spectrum anticancer activity with remarkable selectivity against healthy cells (Figure 16) [65].

Inspired form various biological attributes of fluorine-containing organic compounds [66] and pyrimidine-based drug compounds [67], Hosamani *et al.* synthesized coumarin-pyrimidine based fluorinated compounds under microwave-irradiation in high yields and examined against two human cancer cell lines viz. A-549 (human lung carcinoma) and MDA-MB-231 (human adenocarcinoma mammary gland). *In vitro* results concluded that para substitution enhanced activity to deliver hits **23** and **24**. SAR revealed that the halogenated compounds (at R) showed higher anticancer activity. It was also noteworthy that both the compounds cleave the DNA completely which could be a possible mechanism for their anticancer activity (Figure 17) [68].

Pingaew *et al.* synthesized eleven novel chalcone-coumarin hybrids linked via 1,2,3-triazole using azide/alkyne dipolar cycloaddition reactions (click chemistry). Hybrid molecules were evaluated for their cytotoxic activity against four cancer cell lines (human bile duct epithelial cells: HuCCA-1, Hepatic cancer cells: HepG2, Lung cancer cells: A549 and Lymphoblastic T-cells: MOLT-3). The hybrid **27** was shown to be the most potent in this series (IC_{50} = 4.26 μM, HepG2), but toxic towards Vero cells while compound **26** displayed IC_{50} = 8.18 μM (HepG2) and was non-toxic to Vero cells. SAR investigation of the compounds revealed that the cytotoxicity depends on the substitution pattern of substituents on both rings A and B as well as coumaryl moieties. Compound **26** simultaneously was also found to bind with both α and β subunits of the tubulin, the probable mechanism for the anticancer potential of these hybrid molecules. This study provides novel chalcone-coumarin hybrids as potential lead molecules for further structural optimization (Figure 18) [69].

Tan *et al.* Rationally designed and synthesized novel dimers of triphenylethylene-coumarin hybrids with an amide side chain. The dimeric compounds **29** and **30** linked by the malonic acid, showed a broad-spectrum and good anti-proliferative activity against four tumour cell lines (MCF-7, A549, K562 and Hela) with approximate IC_{50} values of 10 μM, and low cytotoxicity in osteoblast. SARs suggested that the length of the linker (dicarboxylic acids) had profound effects on their anti-proliferative activities and DNA binding properties. UV–vis, fluorescence, and circular dichroism (CD) spectroscopies and thermal denaturation exhibited that compounds **30** and **31** had significant interactions with circulating tumor-DNA(Ct-DNA) by the intercalative mode of binding. Compound **30**, possessing the malonic amide, had the highest binding constant, which suggest that the short length of the linker (n = 1) is beneficial to bind with DNA (Figure 19A) [70].

Ye *et al.*, inspired by the significant activities of coumarins against lysine-specific demethylase enzymes (LSD1), designed and synthesized two series of coumarin–1,2,3-triazole dithiocarbamate hybrids and evaluated their *in vitro* inhibitory activity. LSD1 is a

member of the monoamine oxidase (MAO) family, which shows homology with MAO-A and B. It has been reported that LSD 1 removes the methyl group from mono-, di-methylated Lys4 and Lys9 of histone H3 (H3K4 and H3K9) through flavin adenine dinucleotide (FAD) dependent enzymatic oxidation [71]. LSD1 also demethylates p53, DNA methyltransferase, E2F transcription factor 1 (E2F1) and regulate their cellular functions, therefore downregulation of LSD1 can inhibit cancer progression [72]. Initial work carried out by Liu and co-workers synthesized and evaluated a series of 1,2,3-triazole–dithiocarbamate hybrids for anticancer potential (Figure 19B). The compounds showed excellent activity and it has been reported that tert-butyloxycarbonyl group attached to the piperazine ring and only one carbon length between the phenyl ring and triazole ring were crucial for their anticancer activity (Figure 19B). Keeping intact the previous molecular architecture, coumarin moiety was introduced to develop a new series of hybrid molecules. Several compounds displayed activity against LSD1 in micromolar or submicromolar range. Compounds **32** and **33** showed potent and reversible inhibition against LSD1 with the IC_{50} values of 0.67 and 0.39 μM, respectively, without showing inhibition against MAO A and B. SAR studies revealed that substituents on coumarin moiety greatly influenced the LSD1 inhibitory activity. Substituted 7^{th} position of coumarin moiety increased activity. The further mechanistic investigation revealed that compound upregulated the expression of H3K4me1, H3K4me2 and H3K9me2 (Figure 19B) [73].

Coumarin derivatives suppress cancer cell proliferation by arresting the cell cycle in G0/G1, G2/M phases [74]. Literature survey revealed that the hydrazide-hydrazone moiety plays a role as an antitumor agent due to their ability to inhibit dihydrofolate reductase (DHFR) enzyme [75,76]. It was also reported that furan, thiophene, pyrrole and isatin derivatives possess biological activity [76]. Based on these findings, novel hybrid compounds having coumarin hydrazide-hydrazone backbone were designed and synthesized by Nasr and coworkers. The library was evaluated against human drug-resistant pancreatic carcinoma (Panc-1) cells and drug-sensitive (hepatic carcinoma; Hep-G2 and leukaemia; CCRF) cell lines. Bromocoumarin based hybrids (**34**-**37**) were found to be the most active antitumor agent against drug-resistant pancreatic carcinoma cells among this series of analogues. To get further insights to understand the mechanism of compounds to act as anticancer agents, cell cycle analysis and gene regulation was analyzed. Mechanistically, hybrid molecules activate caspases 3/7 and induced the expression of cell cycle arrest at the G2/M phase in resistant Panc-1 cells. Moreover, microarray-based gene expression analysis indicated the up-regulation of CDKN1A, DDIT4, GDF-15 and down-regulation of CDC2, CDC20, CDK2 genes, genes involved in apoptosis, cell cycle regulation and tumour suppression (Figure 20) [77].

3. Coumarin-based hybrids as anti-Alzheimer's agents:

There are four major pathophysiological causes of Alzheimer's disease: 1) aggregation of β-amyloids, 2) formation of tau proteins, 3) degradation of neurotransmitter (Acetylcholine) in the brain and 4) oxidative stress. The proteolysis of amyloid precursor proteins by β-secretase and γ-secretase generates β-amyloids (Aβ). The elevated levels of acetylcholinesterase (AChE) enzyme which breakdowns acetylcholine in the brain is also responsible for aggravation of Alzheimer's disease [78–81]. Being a complex disorder, a

multi-target directed ligand (MTDL) may be beneficial in reducing the rate of progression of this disease.

Lan *et al.* designed and synthesized a novel series of coumarin-*N*-benzyl pyridinium hybrids. The inhibition of compounds against AChE, MAO-B and β-amyloid aggregate ion was checked. Results concluded that all the compounds effectively inhibited the AChE and MAO-B enzymes and identified lead molecule **38** which displayed micromolar to submicromolar activity. SAR revealed that the AChE inhibitory activity greatly influenced by different substitutions on the benzyl group. The electron donating groups (like $–CH_3$) on the benzyl ring is crucial for AChE inhibition. Enzyme kinetic studies and molecular docking validated that compound **38** was a mixed-type inhibitor, binding to both catalytic active site (CAS) and peripheral active site (PAS) of AChE & blocking the active site of MAO-B. In addition, **38** compound also penetrated the BBB, an essential requirement for a molecule targeting the central nervous system (Figure 21) [82].

Pargyline is an irreversible selective MAO-B inhibitor and plays an important role in neuroprotection [83]. Based on these findings novel coumarin-pargyline hybrids were designed and evaluated to inhibit AChE, MAO-B and β-amyloid aggregation. The lead from this project, compound **39** exhibited inhibitory activities against monoaminoxidase with an IC_{50} value of 3.275 μM and 0.027 μM against MAO-A and MAO-B, respectively. About fifty percent reduction in β-amyloid aggregation was observed with compound **39** at 25 μM. SAR indicated that the nature and locality in the phenoxy ring of synthesized hybrids significantly influenced the β-amyloid aggregation inhibition. Hybrids with three to four carbon chain length were found most active against self-induced β-amyloid aggregation. PAMPA-assay (parallel artificial membrane permeation assay) revealed that compound **39** would be excellent molecule to cross the blood-brain barrier with no significant toxicity which demonstrates that compound **39** was an effective and promising candidate for AD therapy. Computational studies were performed to predict their binding modes in the active pocket of *h*MAO-B and illustrate that the size of compound **39** with a four-carbon spacer is better suited to completely fit the binding cavity compared to one & two carbon spacers (Figure 22) [84].

Previously Xie *et al.* synthesized series of multi-target compounds using tacrine (well-known ChE inhibitor) and -coumarin (MAO inhibitor) scaffolds [85], and identified compound **40** which showed inhibitory activity against AChE and BuChE and also exhibited selective inhibition for MAO-B. But compound **40** suffered from major drawback-severe hepatotoxicity due to the presence of tacrine moiety which hindered its further development. Based on these findings, Xie *et al.* designed and synthesized a new series of donepezil-coumarin hybrids as multi-target compounds for the treatment of AD. For the synthesis of these hybrids, tacrine moiety was replaced with an *N*-benzylpiperidine moiety of donepezil because donepezil was more potent and showed no hepatotoxicity [86–88] compared to tacrine. The *N*-benzylpiperidine moiety exhibited the same mechanism of inhibiting ChEs through binding to the catalytic anionic site (CAS) of ChEs whereas the alkyl chain between coumarin and *N*-benzylpiperidine moiety was also retained in the donepezil-coumarin hybrids. After the identification of optimal length of the linker compounds were synthesized and were subjected to their biological evaluation against *ee*AChE (from electric eel) and

*eq*BuChE (from equine serum). Tacrine and Donepezil were used as reference compounds. Results of the inhibitory activity indicated that compound **41** exhibited IC_{50} = 4.42 μM against *ee*AChE and IC_{50} = 5.34 μM against *eq*BuChE. Among the series of 3,4-cyclohexane-fused coumarin, compound **42** showed potent inhibitory activity with the IC_{50} value of 0.87 μM and 0.93 μM against *ee*AChE and *eq*BuChE, respectively. The influence of inhibition was further assessed by replacing oxygen atom linked at 7^{th}-position of coumarin moiety with carbon atom and the results showed that compound **43** having a two-carbon linker (m = 2) between amino group and *N*-benzyl piperidine moiety was more potent against human ChEs (IC_{50} = 0.067 μM for *h*AChE and IC_{50} = 3.45 μM for *h*BuChE). The investigational compound **44** demonstrated IC_{50} of 1.93 μM against MAO-B. The kinetic studies revealed that compound **42** was a mixed type inhibitor of both AChE and MAO-B. Various types of binding interactions of compound **42** within the active sites of AChE and MAO-B were also rationalized by using molecular modelling studies which revealed that the compound was stabilized within the active sites by various electrostatic interactions that were responsible for the inhibition of the enzyme. PAMPA-assay confirmed the BBB penetrability of compound **42** and it was noteworthy that no toxicity was observed with **42** on SH-SY5Y neuroblastoma cells when tested via *in vitro* toxicity assay (Figure 23) [89].

Singla *et al.* synthesized series of novel hybrids by linking coumarin moiety with various substituted heterocyclic amines with the help of an appropriate spacer. The library was tested for their cognition improving ability, AChE inhibitory potency and antioxidant activity. The influence of these synthesized hybrids on biochemical markers of oxidative stress- lipid peroxidation, superoxide dismutase, and plasma nitrite, were also evaluated and identified compound **45** as a hit. SAR revealed that the potency of AChE inhibition was mainly influenced by the catalytic site interacting moieties attached at the end of the linker, as well as on the length of the alkyl chain- three methylene units which were found optimal for AChE inhibition and compounds containing four methylene units, lead to a reduction in binding affinity towards AChE enzyme. While compounds containing carbamate linkage did not affect the binding affinity towards receptor sites, it was observed that compounds having same alkyl chain length but a variation of the functionality at the end of the linker led to a great change in their inhibitory potency. Conformational and electronic changes caused by the replacement of the phenyl ring present at piperazine with any other heterocyclic ring affects the interaction with the AChE enzyme and thereby, affecting its potency. Introduction of the *o*-fluorophenyl group showed higher activity than *p*-fluoro substituents because fluoro group at *ortho*-position might favour the π-π stacking interaction via the rotation of the phenyl ring. Limited space around the nitrogen atom leading to a decrease in conformational flexibility associated with the presence of a piperazine ring might be the possible reason for enhanced potency of **45**. Furthermore, disubstituted compounds showed remarkably decrease in activity due to the steric hindrance which prevented proper alignment of the molecule within the enzyme. Molecular docking of compound **45** indicated that it interacts with all the crucial amino acids (Figure 24) present at the CAS, mid-gorge and PAS of *Tc*AChE which ultimately results in increased inhibition of AChE enzyme (Figure 24) [90].

Hamulakova *et.al.* designed and synthesized novel conjugates of tacrine-7-hydroxycoumarin connected by a linker of different lengths as multifunctional cholinesterase inhibitors.

Among the targeted compounds, **46** displayed acetylcholinesterase inhibitory activity with an IC_{50} value of 38 nM while compound **47** showed butyrylcholinesterase inhibition with the IC_{50} value of 63.5 nM. SAR studies indicate that with an increase in the length of the linker, AChE inhibitory activity remarkably decreased while β-amyloid aggregation inhibition increased, as shown by compound **48** (82% at 1 μM concentration) confirmed through Thioflavin T assay. Thioflavin T is a fluorescent dye which upon binding with β-amyloids generates a fluorescent product. Due to extensive metal (copper, iron)-induced oxidative stress in Alzheimer's disease, they also evaluated the hybrid molecules to check their antioxidant activity. It was concluded that chelation of free copper(II) ions by tacrine-coumarin hybrids **48** and **49** suppressed the formation of reactive hydroxyl radicals. This was further confirmed by *in vitro* DNA damage protection experiments which suggested that compound **49** upon coordination to free copper ion prevents the decomposition of hydrogen peroxide decomposition catalyzed by Cu(II) (Figure 25) [91].

Leonardo *et. al.* synthesized a multi-target ligand series of 3,4-dimethylcoumarin-piperidine hybrid molecules and assessed them against ChEs and MAO enzymes. For hydrophilic/lipophilic balance, various modifications were done at 7^{th} carbon of coumarin and spacer length connecting the coumarin to piperidine moiety. *In vitro* studies gave the promising multitarget hit compound **50** exhibiting high *h*MAO B inhibitory activity (IC_{50} = 30 nM) and good MAO B/A selectivity (SI = 94) along with *ee*AChE inhibition (IC_{50} = 1.03 μM). The study revealed that flexibility of basic side chain at the 7^{th} position of coumarin and substitution on the piperidine ring greatly influenced the MAO-B inhibition while influenced AChE inhibition to a lesser extent (Figure 26) [92].

Inspired by the neuroprotective activity of tryptamine, Nasab *et. al.* synthesized a library of coumarin-tryptamine hybrids and evaluated them against both AChE and BuChE. Compound **51** containing a 4-fluorobenzyl group demonstrated sub-micromolar IC_{50} against AChE with the IC_{50} value of 0.16 μM. All synthesized compounds demonstrated moderate or no activity against BuChE. The SAR of these synthesized compounds concluded that unsubstituted compounds exhibited good inhibitory activity against AChE whereas 8-methoxy substitution on R_2 position of coumarin was responsible for the improvement in activity. On the other hand, O-alkylation or O-benzylation in the same position maintained the AChE inhibitory activity. It was observed that the elongation of O-alkylation mainly abolishes the activity. In O-benzyl derivatives (at R_2 position), fluoro substituent on benzyl moiety increased inhibitory activity. In particular, para fluoro substitutions increased activity in comparison to *ortho*- and *meta*-fluoro substitutions. The kinetic studies demonstrated that compound **51** showed a mixed type inhibition with their K_i value of 0.49 μM. Molecular modelling studies revealed that compound **51** was placed across the active site of the enzyme and also occupied both PAS and CAS of AChE enzyme (Figure 27) [93].

Xie *et al.* designed and synthesized a new series of tacrine-coumarin hybrids and evaluated the effect of linkers and substitutions on coumarin scaffold against cholinesterase enzymes. Piperazine linked compounds **52** and **53** exhibited good inhibitory activity against both the enzymes AChE and BuChE with IC_{50} values within the nanomolar range. Using these optimized linker lengths, another series of compounds were synthesized and evaluated against AChE, BuChE and MAO-B enzymes. Compound **54** showed IC_{50} values in the

nanomolar range against human AChE (16 nM), BuChE (112 nM) and MAO-B (240 nM). Hybrid **55**, a chloro substituted coumarin derivative was equipotent to compound **54**. Compounds **54** and **55** displayed *Pe* value greater than 4.2 in the PAMPA-BBB assay suggesting that compounds have the ability to cross the blood-brain barrier. Kinetic behaviour of compound **54** against AChE inhibition which indicated its mixed-type inhibition. Docking studies revealed that compound **54** simultaneously interacted with both the catalytic sites (PAS and CAS) of AChE. In addition, compound **54** was non-toxic to the human neuroblastoma cell line SH-SY5Y (Figure 28) [94,95].

Sun *et al.* designed and synthesized eighteen novel tacrine-coumarin hybrids using methylene chain as a linker and evaluated against primary Alzheimer's hallmarks AChE, BuChE, Aβ aggregation, and β-secretase inhibition. Inhibitors that simultaneously interact with multiple targets provide more effective candidates for neurodegenerative disorders including AD [96–99]. Compound **56** was identified as a potent dual-site AChE inhibitor with a K_i value of 16.7 nM, about 2-fold higher potency than tacrine. Compounds **57** and **58** revealed better activities against Aβ aggregation with IC_{50} values of ~5.0 μM and ~6.1 μM, while compounds **58**, **59** and **60** displayed moderate to high activities for all the tested targets, which indicated that they could be multifunctional lead candidates for AD therapy. SAR investigation revealed that the linker length influences the inhibitory activities of hybrid compounds and six carbon chain/n-hexyl seemed to the best suitable length for AChE inhibition. Molecular docking and molecular dynamics (MD) studies suggested that the tacrine part and coumarin part of the hybrids bind with the catalytic active site (CAS) and peripheral active site with the conserved binding mode (Figure 29) [100].

4. Coumarin-based hybrids as Antidiabetic agents

Diabetes mellitus is a metabolic disorder, which is characterized by hyperglycemia, and occurs due to insulin deficiency or resistance [101]. This dreadful disease can cause serious damage to many of the body's organs like eyes, heart, nerves, kidneys and blood vessels [102,103]. The enzymes that play an imperative role in hyperglycemia are, α-glucosidase, α-amylase and aldose reductase which catalyze the cleavage of glucose from disaccharides. The inhibitors of these enzymes can significantly reduce the absorption of glucose and ultimately can decrease the blood glucose level. Thus, these enzymes are an important target to control the disease condition of diabetic patients.

Ibrar *et al.* reported coumarin-thiazolidinediones hybrid molecules as novel glucosidase inhibitors based on the precedence that thiazolidinediones activate PPARϒ activation. *In vitro* screening of the molecules against α-glucosidase and maltase-glucoamylase revealed compounds (**61** and **62**) with submicromolar inhibitory activity. The enzyme kinetic studies suggested that both the compounds show the competitive mode of inhibition. SAR revealed that the change in position of the methoxy group in **61** from *ortho* to *meta* or *para* decreased activity. The inhibitory activity was greatly affected by the varied electronic environment on the *N*-substituted phenyl ring. Electronegative atom such as chlorine, at the *ortho*-position, was tolerated. Docking studies validated the binding of hits with α-glucosidase and maltase-glucoamylase (Figure 30A) [104].

Pan *et al*. analyzed the antidiabetic effect of novel hybrid molecules of natural flavonoids and coumarin. Hybrids of apigenin, chrysin, quercetin and luteolin were synthesized and glucose disposal activity was measured. SAR indicated that the presence of coumarin at the 8^{th} carbon atom of flavonoids (on chromone nucleus of flavone) is beneficial for activity. Hydroxyl groups of flavonoids were favourable for activity and the diminished activity was observed with the removal of these hydroxyl groups. Furthermore, compounds **63-66** promoted 2-NBDG uptake into 3T3-L1 which is generally responsible for the regulation of the glucose level in blood stream and could act as hit molecules for further development of antidiabetic agents (Figure 30B) [105].

In order to increase the potency of previously synthesized compounds **67** and **68**, Salar *et al.* designed and synthesized new series of 3-thiazolylcoumarin hybrids by incorporating three pharmacophores (Coumarin, thiazole, hydrazide) into a single entity. The library was evaluated for their *in vitro* α-glucosidase inhibitory activity and their results revealed that all compounds exhibited inhibitory activity within the range of IC_{50} = 0.12 ± 0.01 to 16.20 ± 0.23 μM. SAR suggested that 2*H*-chromen-2-one moiety, thiazole as well as arene rings were crucial for inhibitory activity. It was also found that compounds containing both electrons donating and electron withdrawing groups were well tolerated. Docking studies indicated that compound **69** interacted with three active site residues (Asn241, Arg312 and Phe300) and these interactions were mainly responsible for their biological activity. (Figure 31A) [106].

Various pharmacological therapeutics based on thiazole moiety inspired Wang *et al*. to design and synthesize new hybrid molecules by joining thiazole with coumarin as α-glucosidase inhibitors. Majority of the compounds of this library were active against enzyme when tested *in vitro*. Two compounds **70** and **71** were found to be most active among the series with the IC_{50} value of 6.24 μM and 8.23 μM, respectively. SAR revealed that electron withdrawing groups and *O*-hydroxy group present on the terminal phenyl ring is crucial for inhibitory activity. Lineweaver-Burk plot of most potent compound **70** indicated that it non-competitively inhibits the enzyme. Various types of binding interactions of compound **70** within the active site of enzyme were also streamlined by docking studies which are depicted in Figure 31B [107].

Inspired from the previous attempts towards the development of potent and selective aldose reductase (ALR2), and to control the diabetes mellitus (DM), Ibrar *et al.* synthesized novel coumarin-thiazole and coumarin-oxadiazole analogues. The inhibitory activity was evaluated against aldose reductase (ALR2) and the selectivity against aldehyde reductase (ALR1) was also determined. Most of the compounds were found selective inhibitors for ALR2 (**72-75**), amongst which **74** (IC_{50} = 0.11 μM) was the most potent. Molecular docking of compound **74** revealed its binding behaviour with various amino acid residues in the active sites (Figure 32) [108].

5. Coumarin-based hybrids as Antimicrobial agents

Microbial resistance has become a serious threat to the future of human health, majorly due to increased drug resistance. The recent focus on the development of anti-microbial agents

has failed to reach the expectations due to high risk of toxicity, insufficient anti-microbial activity as well as microbial resistance which led to a search for novel anti-microbial agents. Due to the multifactorial nature of microbial infections, the hybrid molecules could be beneficial to fight against these pathogens [109–118]. Following are some recent advancements made by various research groups in order to develop novel coumarin-based anti-microbial hybrid molecules.

Chavan *et al.* reported a novel series of coumarin-pyrimidine hybrid molecules and evaluated their anti-bacterial property by using the agar plate method. Among the synthesized compounds, hit **76** exhibited better inhibition (MIC = 2.5 μg/mL) against gram-positive *S. aureus* bacterial strain as compared to ciprofloxacin (MIC = 10 μg/mL). SAR revealed that electron donating groups present on coumarin moiety enhanced the activity. While for potent inhibition against *S. aureus,* 5,6-benzo-coumarin moiety is crucial. Moreover, due to the imperative role of dihydropteroate synthase (DHPS) enzyme in folate metabolism (a valuable target for anti-bacterial drugs), molecular modelling studies were performed with the compound **76**, within its active site which analyzed that four hydrogen bonds formed may be responsible for its potent anti-bacterial activity (Figure 33) [119].

Recently Mangasuli *et al.* reported novel series of coumarin-theophylline hybrids as anti-microbial agents which were evaluated against gram-positive bacteria (S. aureus) and gram-negative bacteria (E. coli, S. Typhi) as well as fungi (C. albicans). Compounds with electron donating substituents (**77** and **78**) increased activity. It was found that the methyl and methoxy substitution at C-6 position on coumarin moiety is well tolerable for activity. Halogen substituted coumarin hybrids were found moderately active against *Mycobacterium tuberculosis*. Binding behaviour of **77** within the active site of Mycobacterium tuberculosis enzyme (Enoyl-[acyl-carrier-protein] reductase [NADH]) was also analyzed by molecular modelling studies (Figure 34) [120].

Due to its innumerable therapeutic potential, Gupta *et. al.* generated a novel series of scaffolds containing conjugates of 4-hydroxy coumarin and phenyl glyoxal and evaluated the effect of this library of hybrid molecules on the spermicidal and anti-microbial profile. A total of 11 compounds were active in both spermicidal and antitrichomonal assays, out of which, **79** was the most potent. These compounds were further evaluated by cytotoxicity and safety studies and were found safe in the cytotoxicity assay (HeLa cell lines) and safety assay (Lactobacillus jenseii strains). It was revealed that on the addition of polar groups like $–OCH_3$ and –OH on 4^{th} position of the glyoxal phenyl ring, the activity enhanced while the addition of $–CH_3$ group on 2^{nd} and 3^{rd} position of the phenyl ring decreased activity (Figure 35A) [121].

Kraljevic *et al.* developed a series of 7-hydroxy coumarin-triazole molecular hybrids and accessed their anti-bacterial potential against Gram-positive bacteria including *S. aureus*, *E. faecalis*, vancomycin-resistant *E. faecium* (VRE) and Gram-negative bacteria including *P. aeurigonsa*, *E.*, *A. baumannii*, extended-spectrum β-lactamase *K. pneumoniae*. The results revealed that the compounds could not show a considerable effect on any of the Gram-positive as well as Gram-negative strains except *Enterococcus* species. Hybrid **80** (containing *p*-pentylphenyl), **81 (**containing 2-chloro-4-fluorobenzenesulfonamide) and **82**

(containing dithiocarbamate) were found active with high selectivity against *Enterococcus* species with the MIC values ranging from 8–64 μg/mL (Figure 35B) [59].

Inspired by vast biological activities of thiosemicarbazone, Vekariya *et al.* synthesized its hybrid molecules by combining with coumarin moiety and screened them for their anti-microbial activity. The screening was performed against two gram-positive bacterial strains (*S. aureus*, *S. pyogenes*), two gram-negative bacterial strains (*E. coli*, *P. aeruginosa*) and three fungal strains (*A. clavatus*, *C. albicans* and *A. niger*). The assay revealed hybrid molecule **83** as a hit which demonstrated broad-spectrum activity (Figure 36) [122].

Keeping in view the imperative role of iminothiazole in various biological activities [123–125], Ibrar *et al.* reported a new series of bis-coumarin-iminothiazole hybrid molecules and evaluated for antileishmanial activity. MTT assay revealed compound **84** as a potent hybrid which showed 70.4% inhibition at a concentration of 100 μM. SAR revealed that electron-rich (-OMe), as well as electron-poor (-Cl) groups, were tolerated which showed moderate inhibition of Leishmania major (Figure 36) [63].

Soraya *et al.* synthesized Ubiquicidine-peptide (UBI)-coumarin conjugates via copper(I)catalyzed azide-alkyne cycloaddition reaction (click chemistry). The conjugates were assessed for their *in vitro* anti-fungal activity against *Cryptococcus gattii* and *Cryptococcus neoformans* fungal strains. The results revealed that the analogue **85** exerted inhibitory potential against both the fungal strains with MIC values ranging from 0.04 to 0.18 μmol.mL^{-1}. Additionally, conjugate **85** (with UBI 31–38) efficiently inhibited the growth of a fluconazole-resistant strain of C. *gattii* (L27/01F) at a concentration of 0.09 μmol.mL^{-1}. The peptide-coumarin conjugate **85** was non-toxic to human cell line (lung fibroblast CCD-Lu). Compound **85** increases the number of ROS to exert anti-fungal activity (Figure 36) [126].

6. Coumarin-based hybrids as Antioxidants

Adopting a hybrid pharmacophore approach, Saeed *et al.* designed and synthesized new series of coumarinyl pyrazolinyl thioamide derivatives as jack bean urease inhibitors and free radical scavengers. To overcome the scientific problems in designing of novel drugs, fusing Coumarinyl, pyrazolinyl and thioamide as potential pharmacophoric aptitude serves as a structural template in drug designing. Compound **86** was found to be the most effective derivative in the series with IC_{50} = 0.358 ± 0.017μM. Kinetic studies revealed that compound **86** inhibits urease enzyme by non-competitive mode of inhibition. The ADMET properties result justified that these novel synthesized compounds showed significant antioxidant with little hepatotoxic and skin sensitive effects. Molecular docking studies were performed within the urease enzyme which revealed that derivative **86** directly interacts with Asp494 and Ala440 amino acid residues through hydrogen bonding (Figure 37) [127].

Niu *et al.* evaluated the antioxidant potential of coumarin-chalcone molecular hybrids using the 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\cdot+}$) assay. Hit compounds **87** and **88** with the IC_{50} values of 8.51 μM and 18.63 μM, respectively were identified (Figure 37) [128].

Karina Perez-Cruz *et al.* designed and synthesized new hybrid compounds inserting hydroxybenzoic acids with common coumarin scaffold and evaluated their antioxidant activity against reactive oxygen species (ROS) using oxygen radical absorbance capacity-fluorescein (ORAC-FL), electron spin resonance (ESR) spin trapping, quenching of superoxide anion, cellular antioxidant activity (CAA) and ferric reducing ability of plasma (FRAP assay). Additionally, bond dissociation energies (BDE) values were calculated in order to obtain information about the antioxidant capacity for HAT (transfer of Hydrogen atom) mechanisms. Compound **89** exhibit synergy phenomenon for FRAP and ORAC assays. The superoxide scavenging results revealed that compound **90** was the most active molecule of synthesized hybrid series with an AI_{50} of 64 μM. CAA assay results showed the activity of the new compounds is limited to those oxidative processes in lipophilic media. From the theoretical calculations (Fukui index and BDE values) the different reactive sites were found within the molecules where the oxidative process actually occur (Figure 37) [129].

Previously reports of the various research articles concluded that coumarin-chalcone hybrids act as inhibitors for free radical over-production in oxidative stress-related diseases but there were no kinetic data and mechanism was reported on these hybrids [130]. So, based on this, Mazzone *et al.* provide quantitative data as well as their antioxidant behaviour of previously identified two coumarin-chalcone hybrids, compounds **92** [131] and **93** [132]. Kinetic calculations of these two coumarin-chalcone hybrids were performed and the results indicated that the compound **92** was a stronger radical scavenger than **93**. Previously, it was also reported that the number and position of the hydroxyl substituents at catechol moiety of coumarin-chalcone hybrids directly related to their scavenging ability towards free radicals such as $O_2^{\bullet}$, $^{\bullet}$CH3, and $^{\bullet}$OH [130]. Different reaction mechanisms of these hybrids were also investigated, and it was found that the largest contributions to the overall peroxyl radical scavenging activity of the studied compounds are the H transfer in lipid media, and the sequential proton loss hydrogen atom transfer (SPLHAT) in aqueous solution. It was also concluded that both hybrids are expected to be more efficient for scavenging peroxyl radicals in lipid media as compared to Trolox which was used as a reference drug. Peroxyl radical scavenging ability was also studied and the results suggested that the activity of the hybrids increases with the polarity of the environment and the number of phenolic sites (Figure 37) [133].

7. Coumarin hybrids as Anti-inflammatory agents

Today, most commonly used anti-inflammatory agents are non-steroidal anti-inflammatory drugs (NSAIDs) that exhibit their anti-inflammatory potential by inhibiting the release of prostaglandins (a potent mediator of inflammation). The primary target of these agents is cyclooxygenase (COX) enzyme which exists in two isoforms, namely, the constitutive form COX-1 and the inducible form COX-2. COX-1 is abundantly present in the body tissues, while COX-2 is expressed during inflammation. The NSAIDs were found to inhibit both the isoforms of COX enzyme, which is associated with gastrointestinal side effects [134–137]. Therefore to overcome this drawback, it is of great significance to develop novel compounds with improved profiles.

Shen *et al.* clubbed the active pharmacophores of COX-2 inhibitors (Celecoxib and Valdecoxib) and 5-LOX enzyme inhibitor coumarin derivatives into a single molecular architecture by using a molecular hybridization technique. All the designed compounds were evaluated against COX-2 and 5-LOX enzyme which revealed **94** as a most potent hybrid molecule in this library with the IC_{50} values of 0.23 μM and 0.87 μM, respectively for both the enzymes. Biological results also revealed an established SAR which indicated that electron withdrawing groups on *para*-position exhibited good COX-2 inhibitory activity. More the electron withdrawing groups present on the substituted phenyl ring, more the inhibitory activity against COX-2. The compound **94** showed strong binding interactions within the active sites of both 5-LOX and COX-2 enzymes which stated that the compound strongly binds with the enzymes (Figure 38) [138].

Tak-1, a primary LPS receptor was reported to cause activation of NF-κB through various signalling pathways. Therefore, Tak-1 inhibition prevents the generation of various interleukins and TNF-α. Chen *et al.* designed and synthesized a series of arylpyrazoline-coumarin hybrids and evaluated the library for anti-inflammatory activity using carrageenan-induced paw oedema by evaluating LPS-induced IL-6 release. The results demonstrated that all the compounds possess inhibitory activity among which compound **95** was found to be most potent with the IC_{50} values of 16.83 μM against TNF-α and 14.18 μM against IL-6. Further study also demonstrated this compound also reduced the expression of iNOS (Figure 39) [139].

Arora *et al.* Rationally designed and synthesized two series of coumarin-benzimidazole hybrid molecules and assessed their anti-inflammatory activity. The two active moieties were conjoined with or without amide linkage. *In vitro* assay identified hits **96**, **97** and **98** which were further evaluated *in vivo* experiment using formalin-induced oedema rat model. SAR demonstrated that the compounds with electron withdrawing groups enhanced the activity profile, while compounds with electron donating groups diminished the activity. Series of compounds with amide linkage were found less active. It has been reported that NSAIDs are associated with gastric intolerance due to their acidic nature [140]. Thus, the gastric safety (gastric toxicity) profile of these most potent compounds was also evaluated which concluded that **97** and **98** could be used as clinical candidates due to their safety profile (Figure 39) [141].

8. Miscellaneous active coumarin-based hybrids

Niu H *et al.* utilized the multifarious coumarin molecule to develop a new hybrid with chalcones and fibrates to act as an agonist for PPAR-α (hyperlipidaemia) and PPAR-γ (Hyperglycaemia) with additional antioxidant activity. A novel series of molecules were synthesized and evaluated for PPAR-α/γ by transactivation assay using firefly luciferase reporter gene technology in HEK293 cells and antioxidant activity by ABTS evaluation method [142]. The results depicted that all compounds possess good agonistic activity for PPAR-α/γ and identified compound **88** as a hit for further development. Antioxidant activity revealed that compound **88** possesses free radical scavenging property. The SAR of the synthesized compounds confirmed that the C6 position of the benzopyran moiety was mandatory for agonistic activity and potency was improved by nitro group substitution.

Moreover, PPARα/γ agonistic activity was enhanced by the presence of a double bond on the benzopyran moiety but the absence of a double bond enhanced the antioxidant activity. The hit compound **88** was docked, which stated that the hybrid showed hydrophobic interactions with Cys275, Met330, Val332, Ile339 and Ile344 and these interactions were potentiated by coumarin nucleus. In addition, a hydrogen bond is formed between the nitro group and thr279 amino residue, further potentiating the activity of compound **88** (Figure 40) [128].

It has been reported that the coumarin-based protease inhibitors are associated with problems like lack of activity and poor pharmacokinetic properties within the *in vivo* model of thrombosis [143–147]. To overcome these drawbacks, Bauckaert *et al.* synthesized two series- A) containing the basic group (-NR_2) and B) oxygen-containing groups (-OH or -COOH) of new 3-carboxamide derivatives by carrying out the structural modification of lead compound **99** (3-phenylamide coumarin). All the synthetics were screened on FXIIa at a concentration of 50 μM to determine their inhibitory potency and it was found that all the compounds displayed above 85% inhibition. Among the hybrids, **100–102** showed potent inhibitory activity with IC_{50} of 5, 8 and 7 μM, respectively. Moreover, the binding interactions between the synthesized compounds and the target molecule were also analyzed to understand the SAR. From these results, it was observed that oxygen-based group exhibited higher activity than the amino group such as compounds **100**-**102**. The hit compounds were selective for FXIIa and did not demonstrate activity against thrombin, activated factor X (FXa), complex tissue factor/activated factor VII (TF/FVIIa) and plasma kallikrein at a concentration of 100 μM. Molecular docking studies of compound **100** indicated that π-alkyl interaction formed between halogens atom (bromine group) and indole of Trp35 from hydrophobic H1 pocket. This bromine atom also involved in two other hydrophobic interactions, one with Phe41 and other with Cys42 residues. Coumarin moiety formed a hydrogen bond with Gly193 whereas the hydroxyl group also showed hydrogen bond formation with Ser195. The phenyl and its chlorine atom both interact with Cys220 with π-alkyl and alkyl interactions. From the docking results, it was cleared that the H1 pocket was the typical feature of FXIIa which mainly responsible for the selectivity of compound **100** (Figure 41) [148].

Due to serious side effects associated with current drug therapy to treat depression [149], Sashidhara *et al.* reported coumarin-aminopyran based hybrid molecules and assessed them *in vivo* to check their ability to treat depression. The compounds were evaluated after IP administration of lead compounds in adult male Swiss albino mice using Forced Swimming Test (FST). In *in vivo* test Compound, **103** demonstrated a dose-dependent decrease in immobility, even at a low dose of 0.25 mg/kg. These results were also confirmed by using the Tail Suspension Test (TST). Furthermore, it was also demonstrated that the compound did not affect the locomotor activity when mice were treated with an effective dose of 0.5 and 1.0 mg/kg IP (Figure 42) [150].

9. Conclusion

This review demonstrates that hybridization of coumarin with various active pharmacophores plays an important role in increasing the pharmacological profile of the

novel molecules in the form of potency or pharmacokinetics or both. Hybrid molecules that can simultaneously engage more than one target will play a key role in developing small molecule therapeutics that can target complex disorders, where several key proteins are dysregulated [151]. Coumarin hybrids possessing anticancer activity or acting against neurodegenerative disorders such as Alzheimer's disease have been the major focus of drug discovery since 2014. This review highlights the key SAR studies performed on coumarin hybrids and identifies key structural features on the coumarin scaffold, where, substitutions can increase or decrease the activity of the hit molecules. Molecules displaying polypharmacology are either in clinical trials or have been approved for the treatment of complex multifactorial disorders. The next few decades of drug discovery research will reveal the fate of hybrid molecules including coumarin hybrids in terms of drug molecules to treat diseases.

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- Rational approaches towards the design of pharmacologically active coumarin based hybrids
- Structure-activity relationships and mechanistic insights for therapeutic coumarin hybrids
- Chemotherapeutic potential of coumarin based hybrids
- Coumarin hybrids as antioxidant and anti-Alzheimer's agents
- Antidiabetic and anti-inflammatory activity of coumarin based hybrids

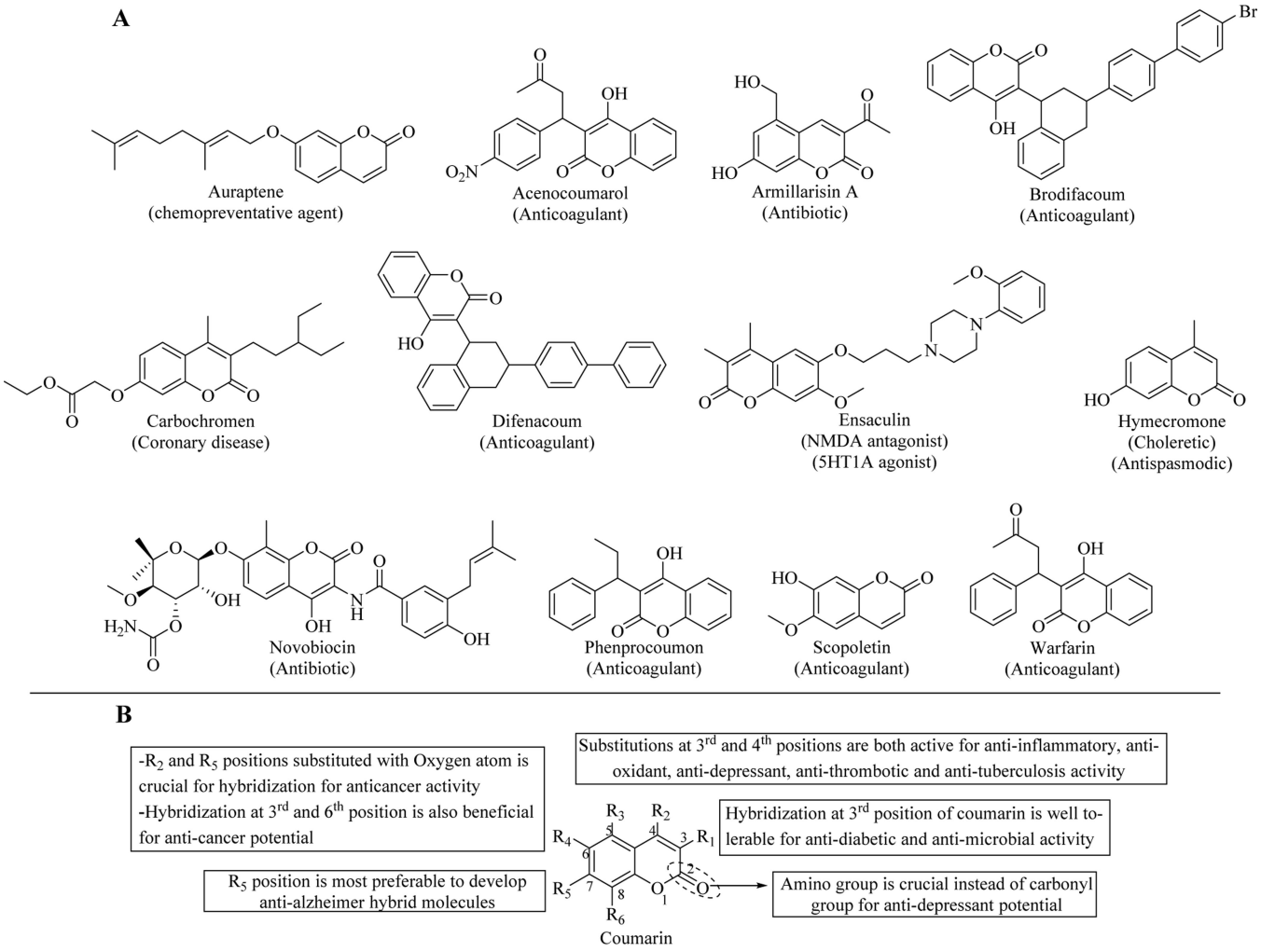


Figure 1.

(A) Marketed formulations of coumarin derivatives and their clinical uses; (B) General structure-activity relationship of coumarins

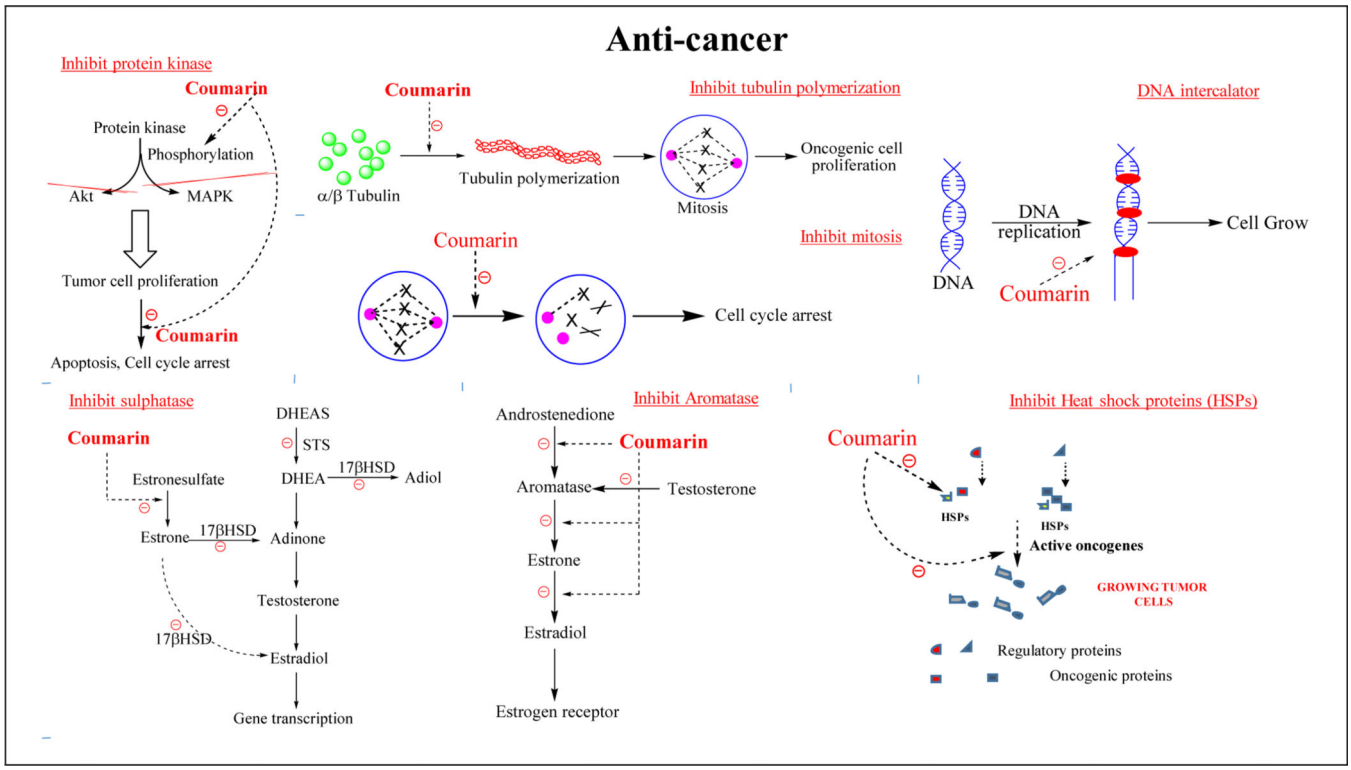


Figure 2a.

Mechanism of actions of coumarin derivatives against cancer

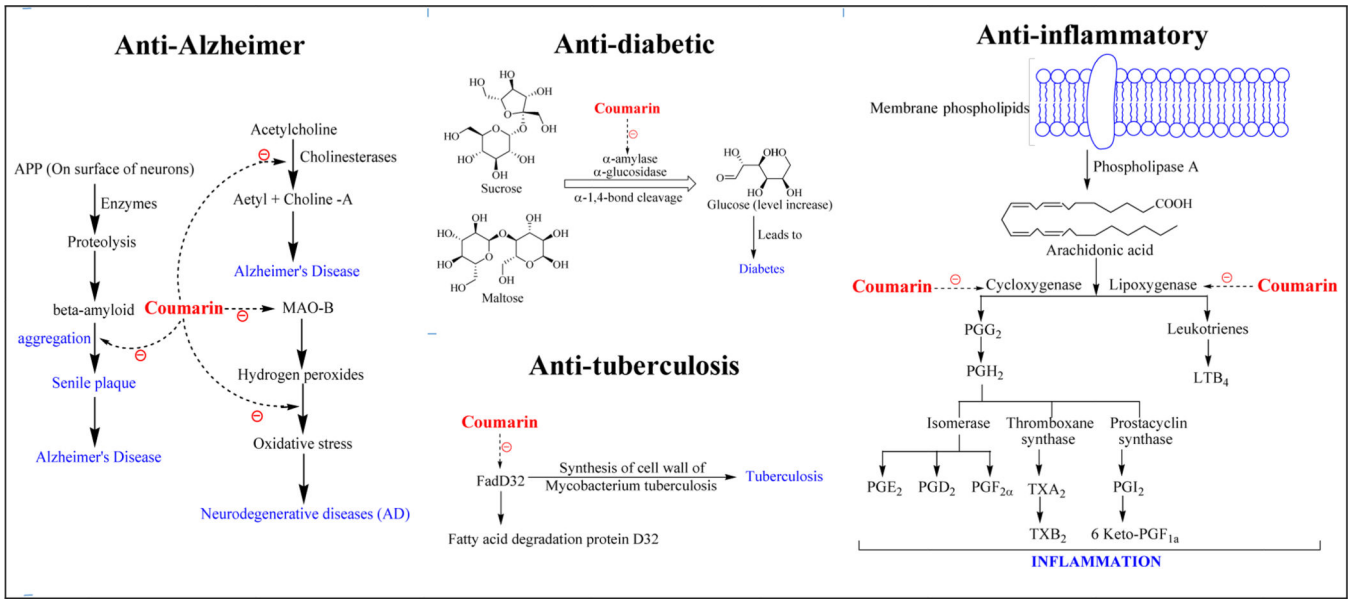


Figure 2b.

Mechanism of actions of coumarin derivatives against various diseases

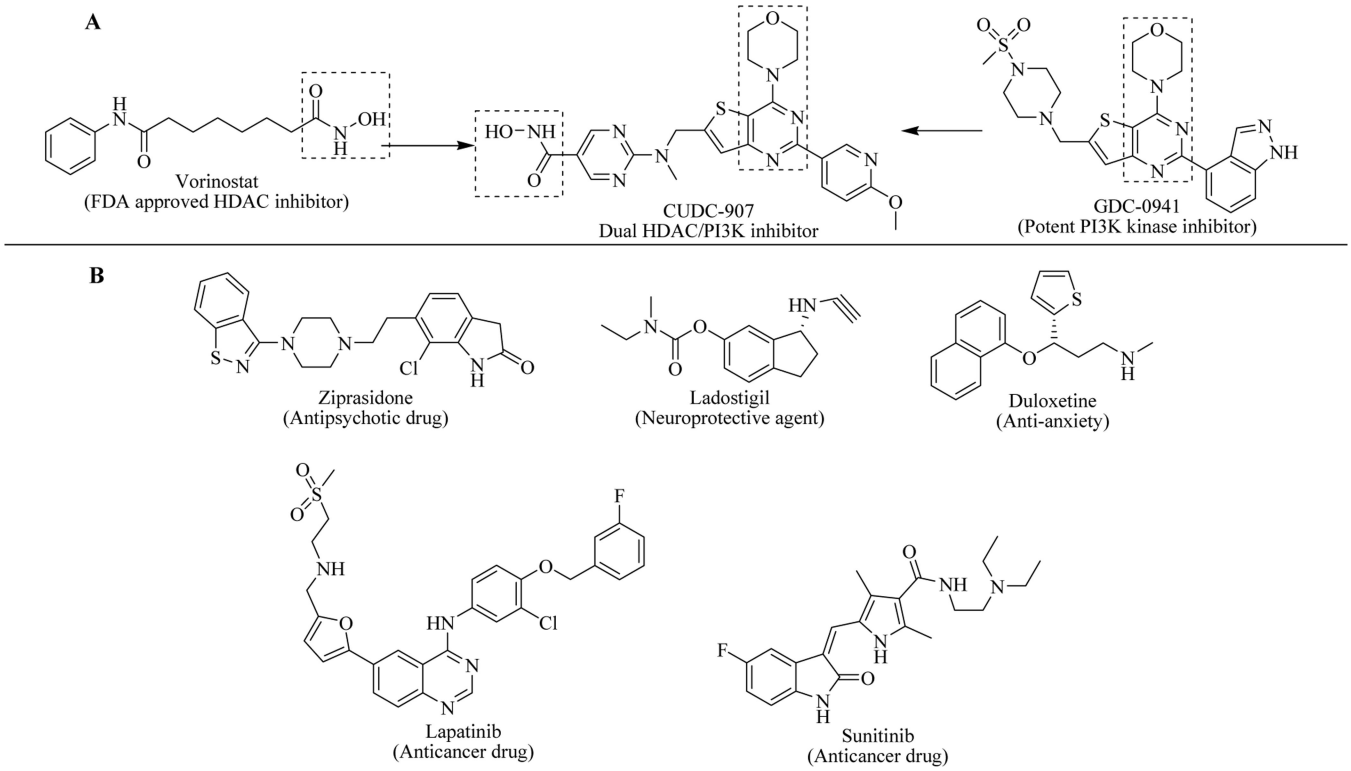


Figure 3.

(A) Hybrid molecule CUDC-907 in clinical trial; (B) Various hybrid molecules as drug candidates

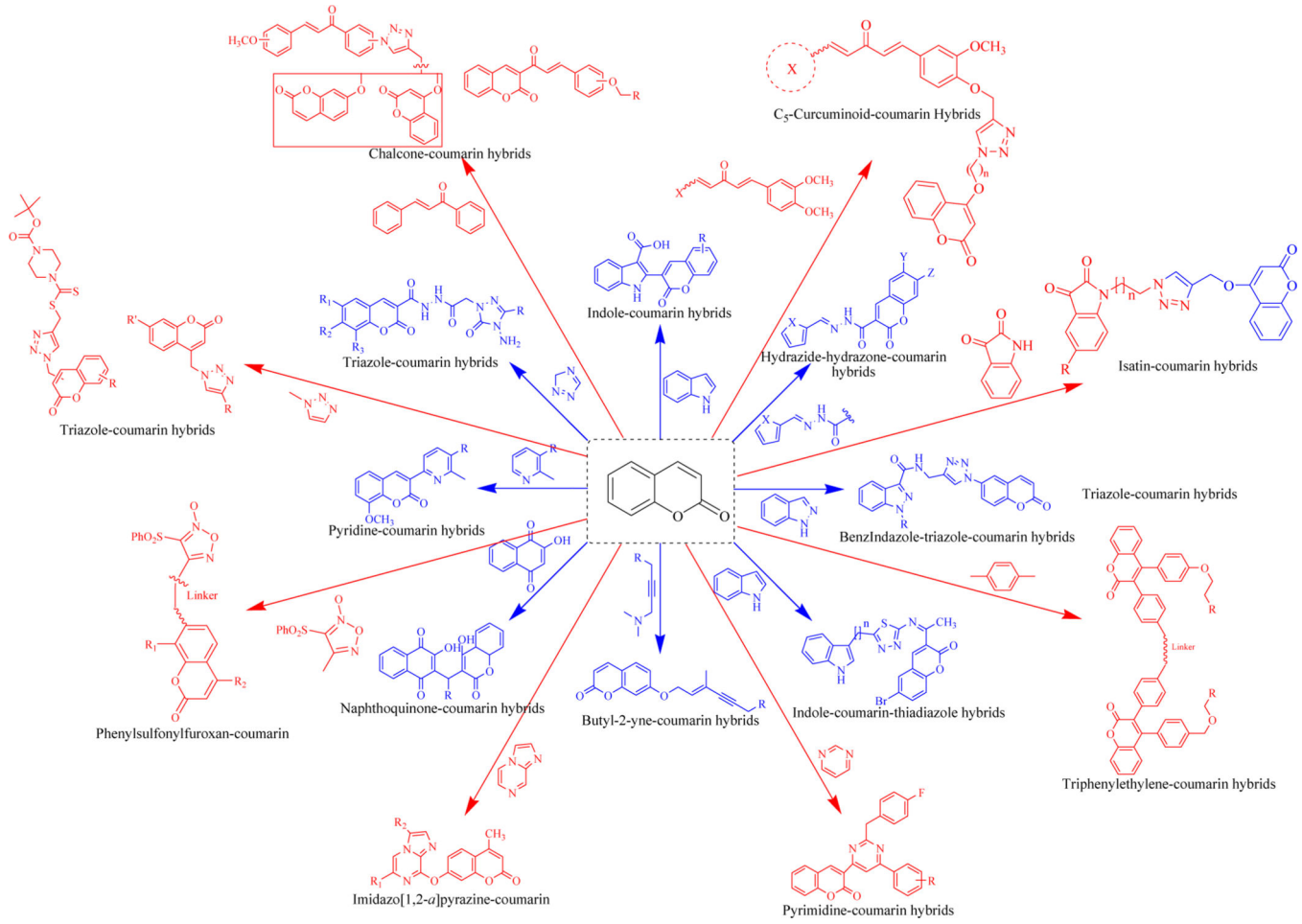


Figure 4.
Recently reported coumarin-based anticancer hybrid molecules

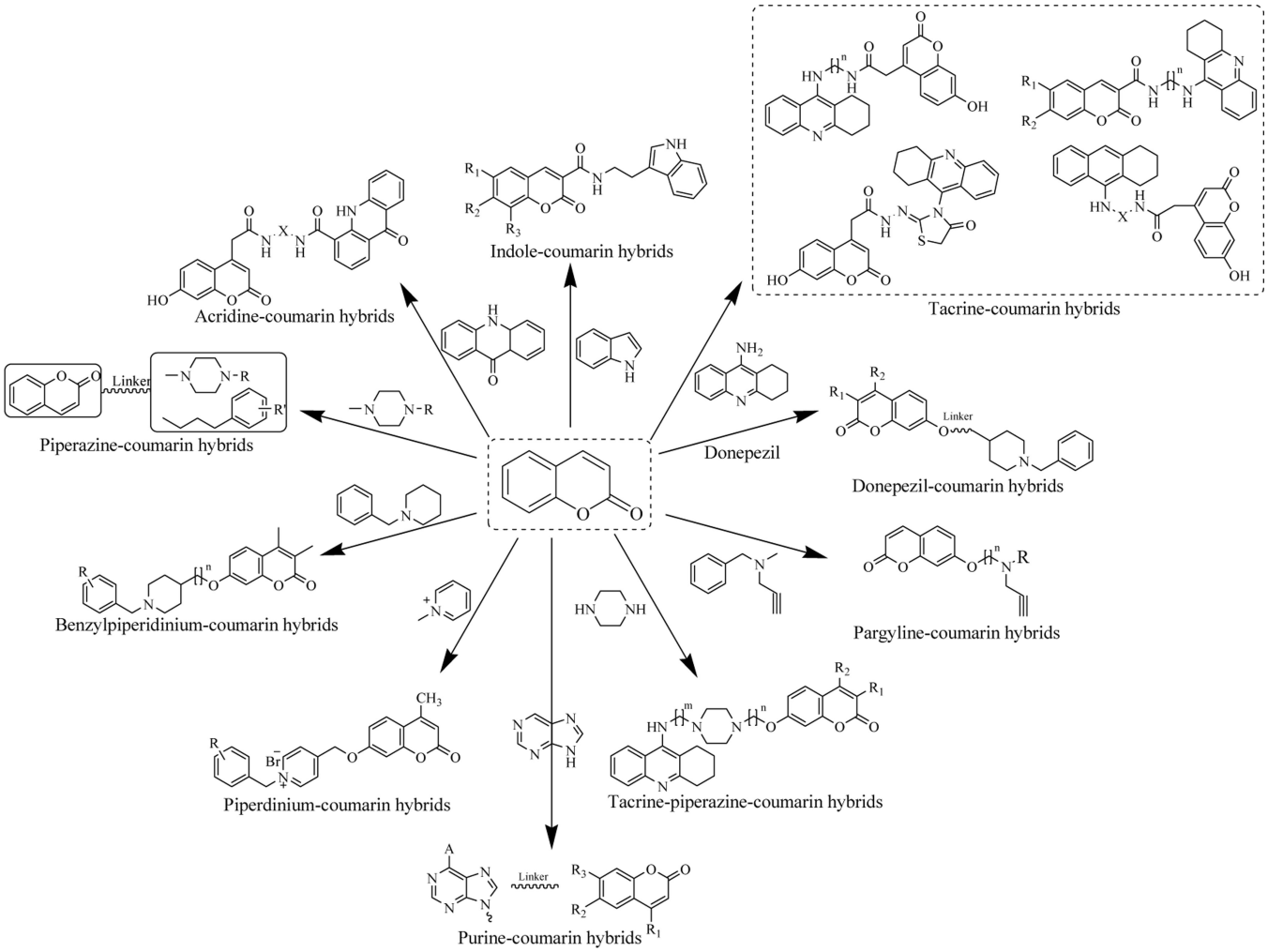


Figure 5.
Recently reported Coumarin based hybrid molecules having anti-Alzheimer's properties

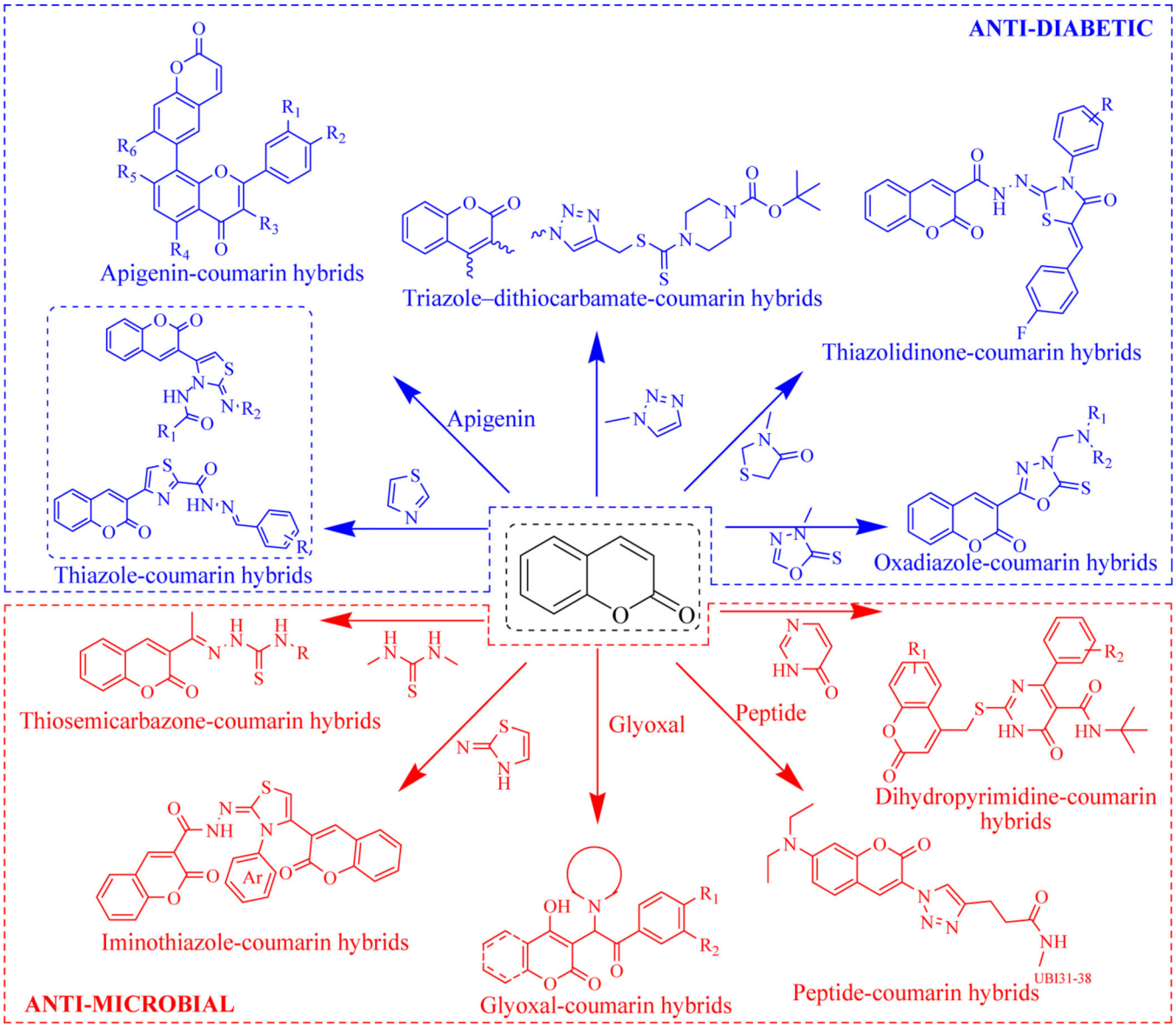


Figure 6.
Coumarin based antidiabetic and anti-microbial hybrid molecules

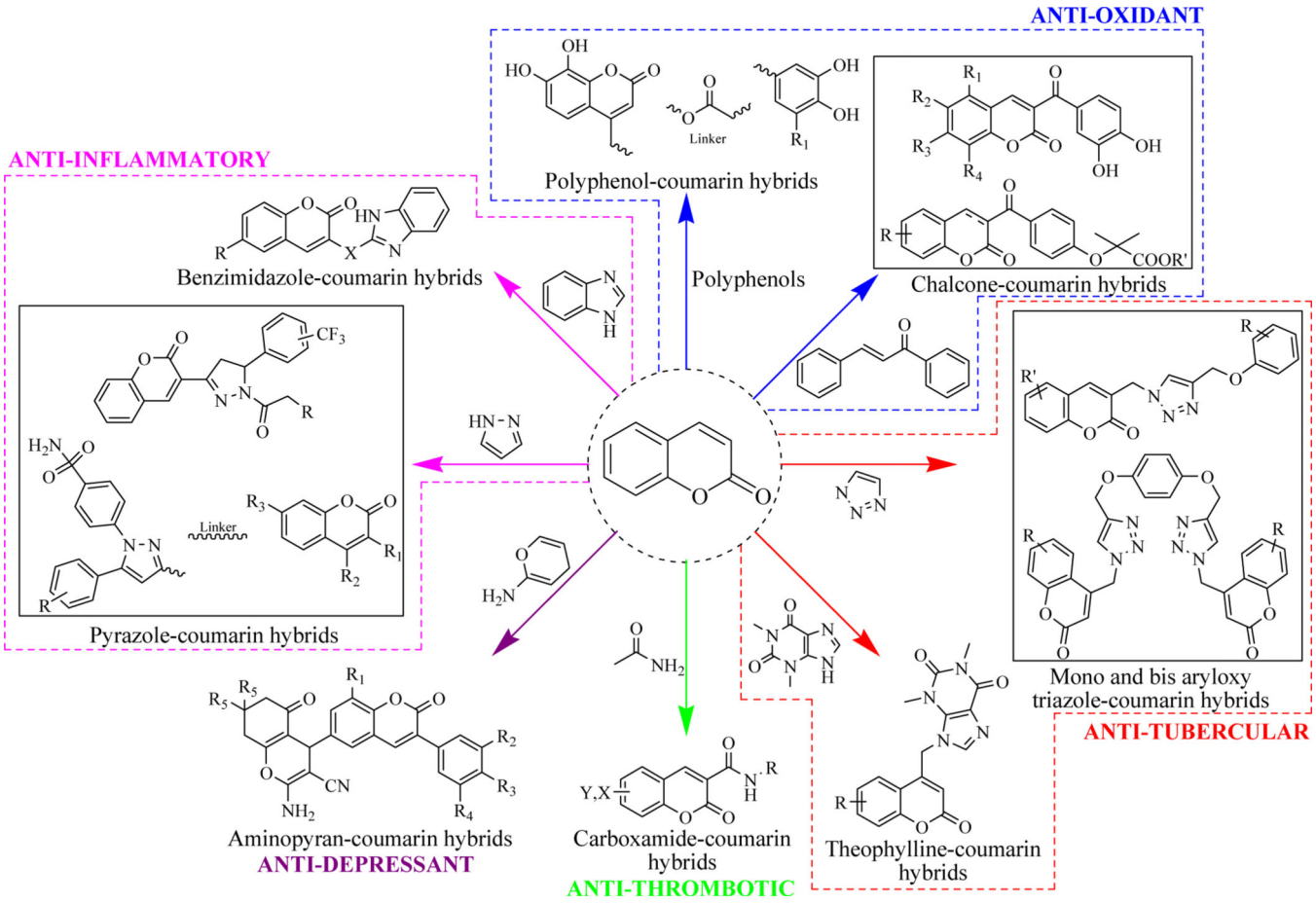


Figure 7.
Various coumarin-based bioactive hybrid molecules

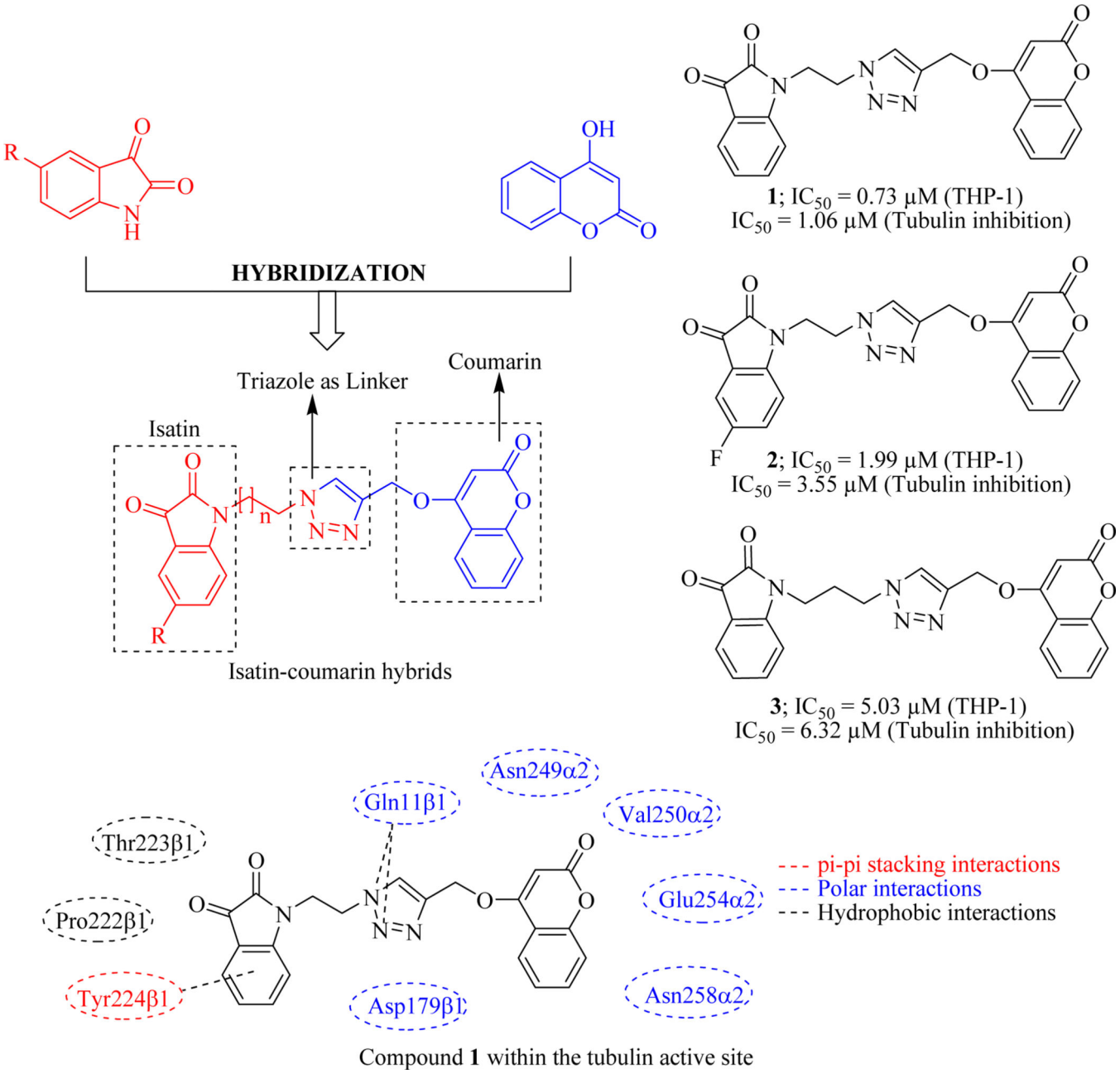


Figure 8.
Isatin-coumarin hybrids as tubulin inhibitors

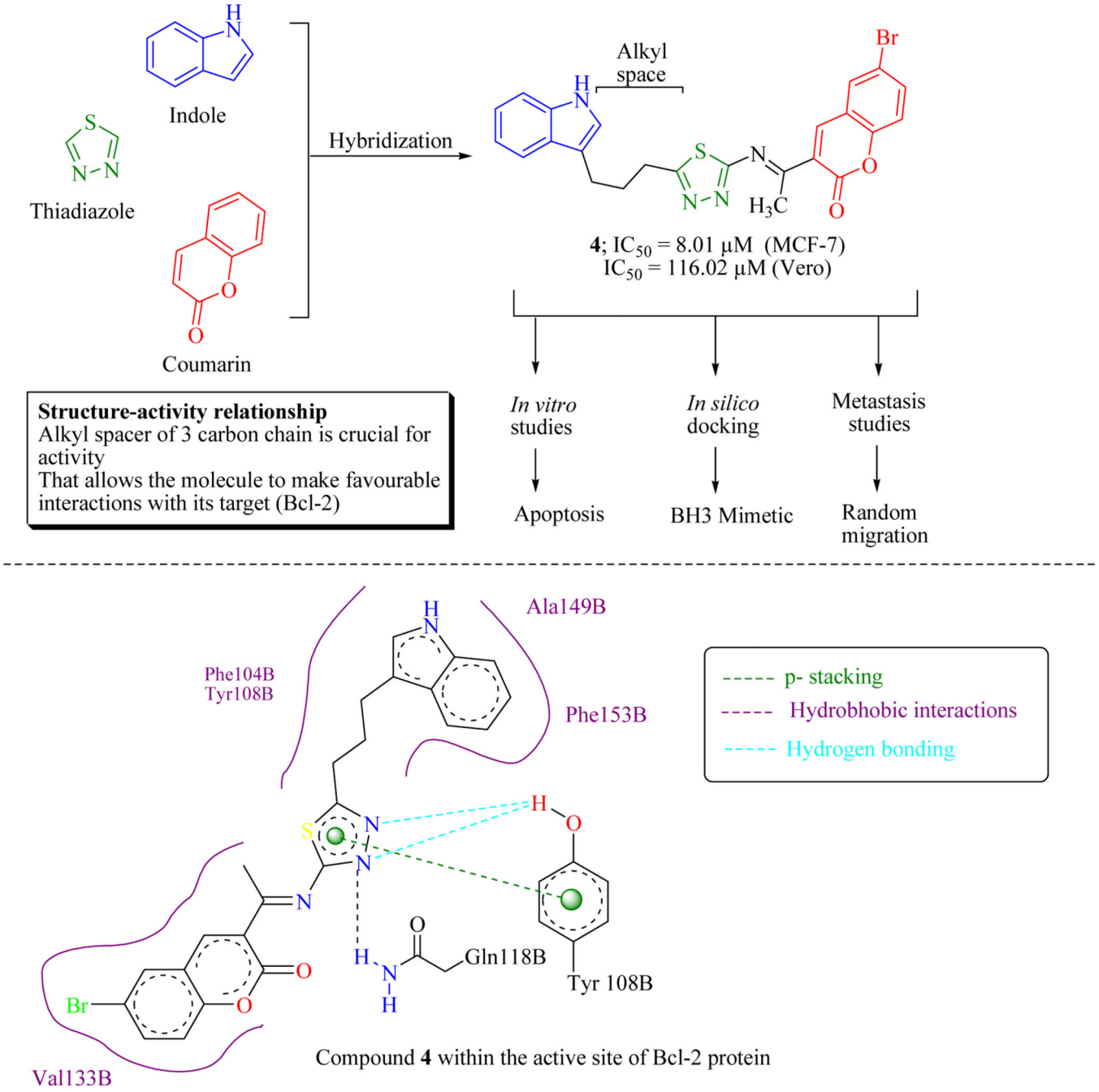


Figure 9.
Indole-coumarin hybrids as anticancer agents

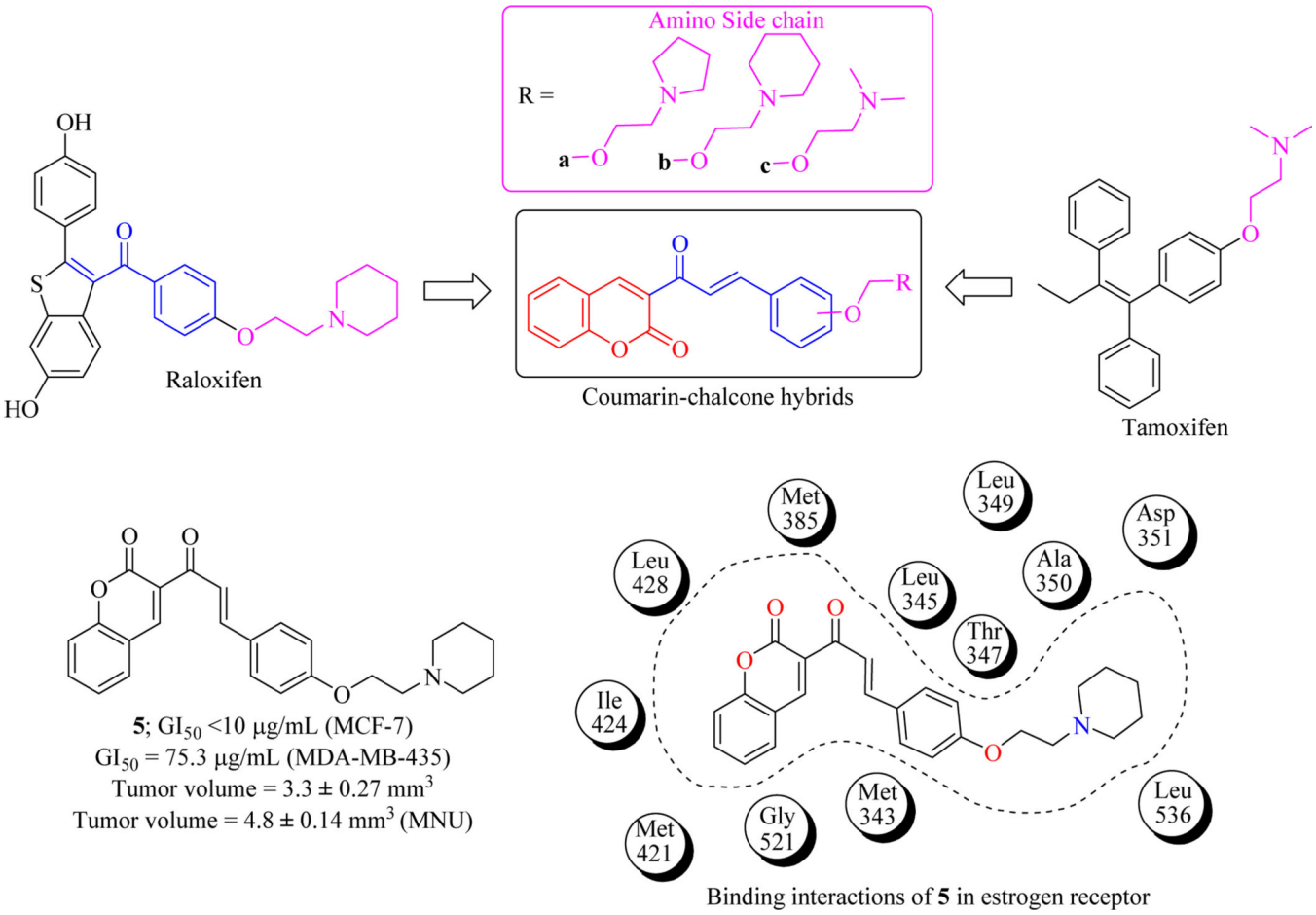


Figure 10.
Coumarin-chalcone hybrids as anticancer agents

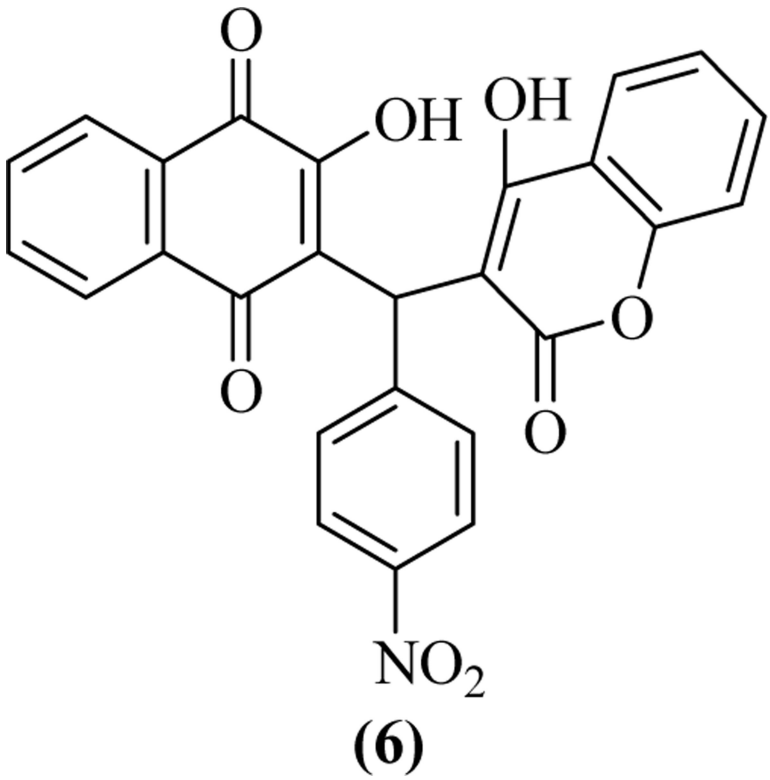


Figure 11.
Naphthoquinone-coumarin hybrid molecule topoisomerase II inhibitor

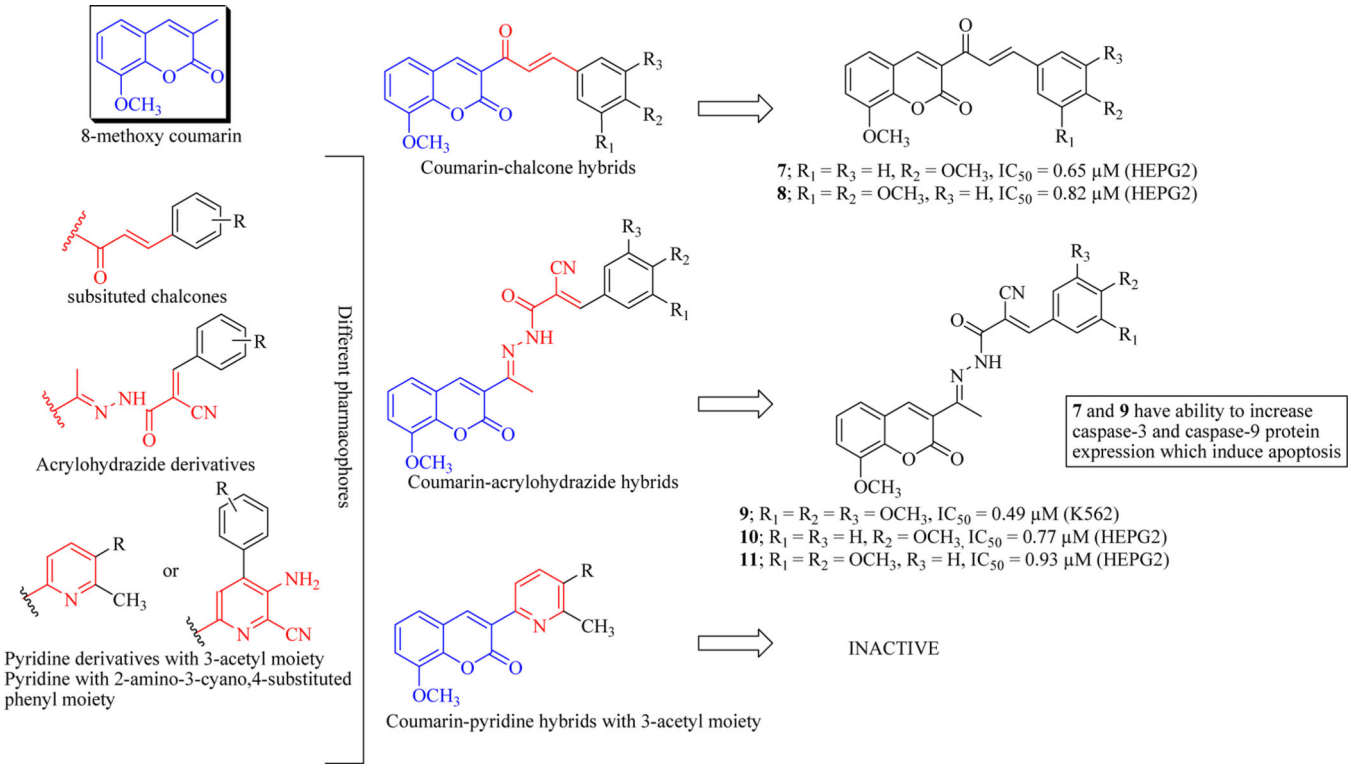


Figure 12.

Coumarin-chalcone/acrylohydrazide/pyridine hybrids as anticancer agents

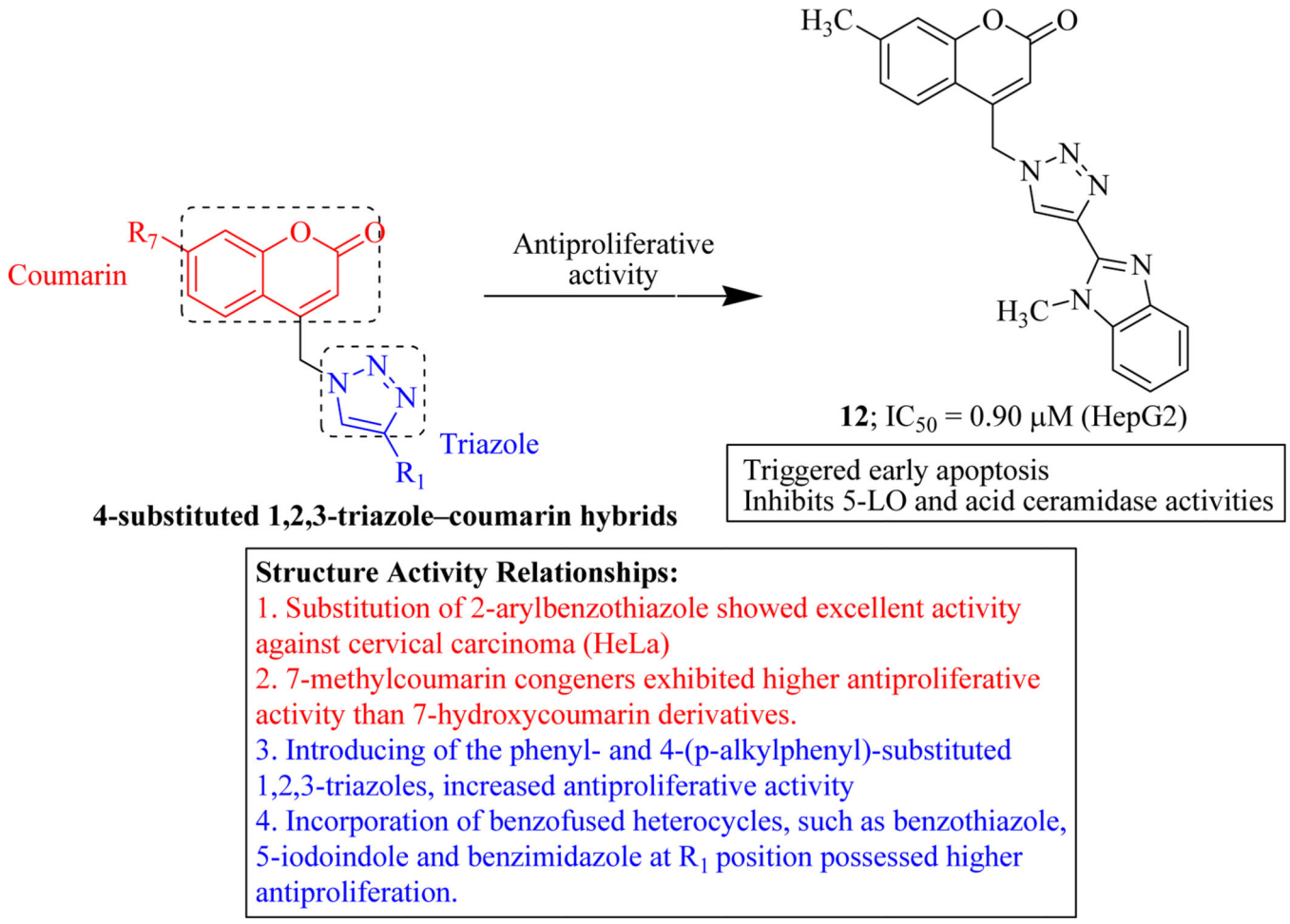


Figure 13.
Triazole-coumarin hybrids as 5-LO and ceramidase inhibitors

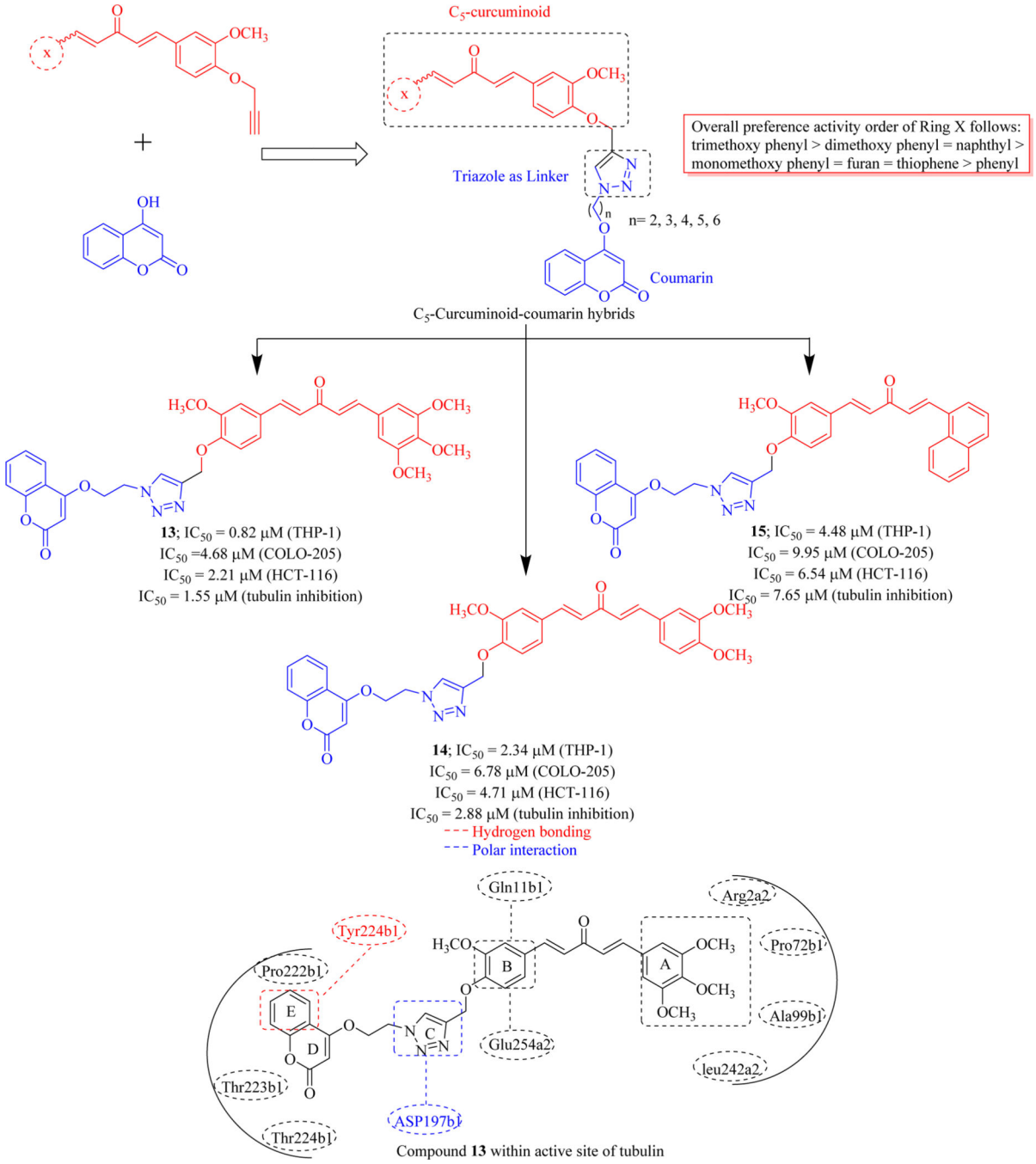
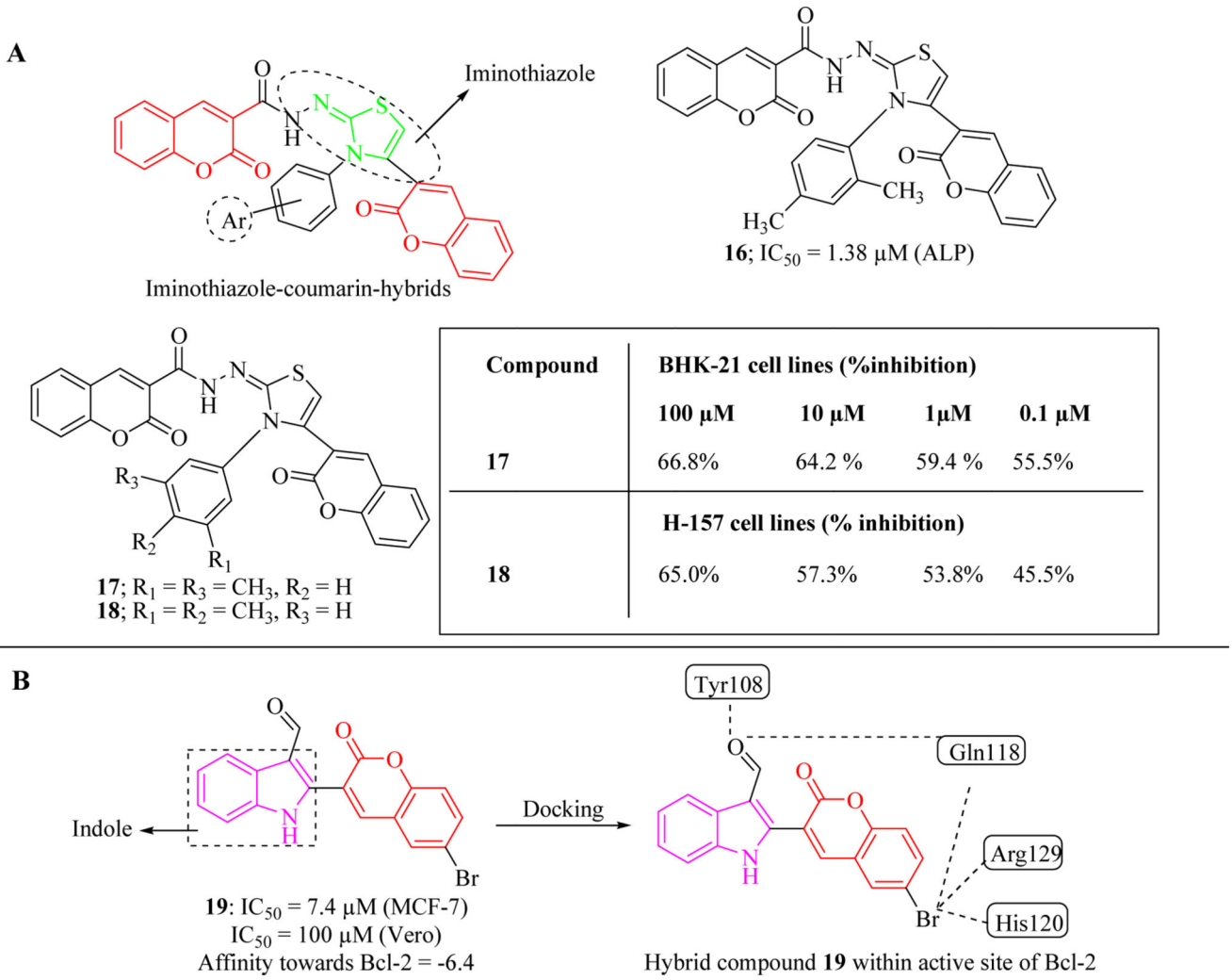


Figure 14.

C_5-curcuminoid-coumarin bifunctional hybrids as tubulin inhibitors



Compound	BHK-21 cell lines (%inhibition)			
	100 µM	10 µM	1µM	0.1 µM
17	66.8%	64.2 %	59.4 %	55.5%
	H-157 cell lines (% inhibition)			
18	65.0%	57.3%	53.8%	45.5%

Figure 15.

(A) bis-coumarin–iminothiazole hybrids as anticancer agents; (B) indole-coumarin hybrid molecules as anticancer agents

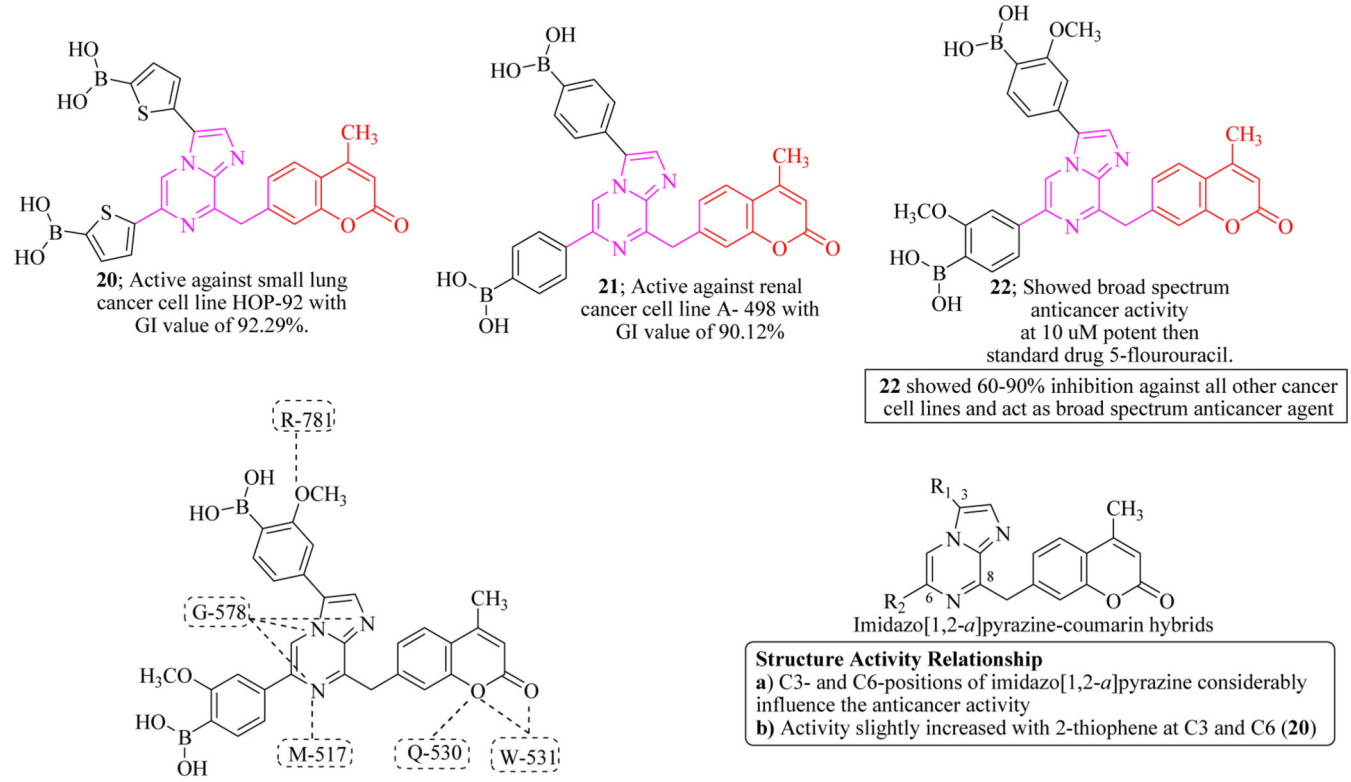


Figure 16.

Imidazo[1,2-*a*]pyrazine-coumarin hybrids as B-Raf Kinase inhibitors

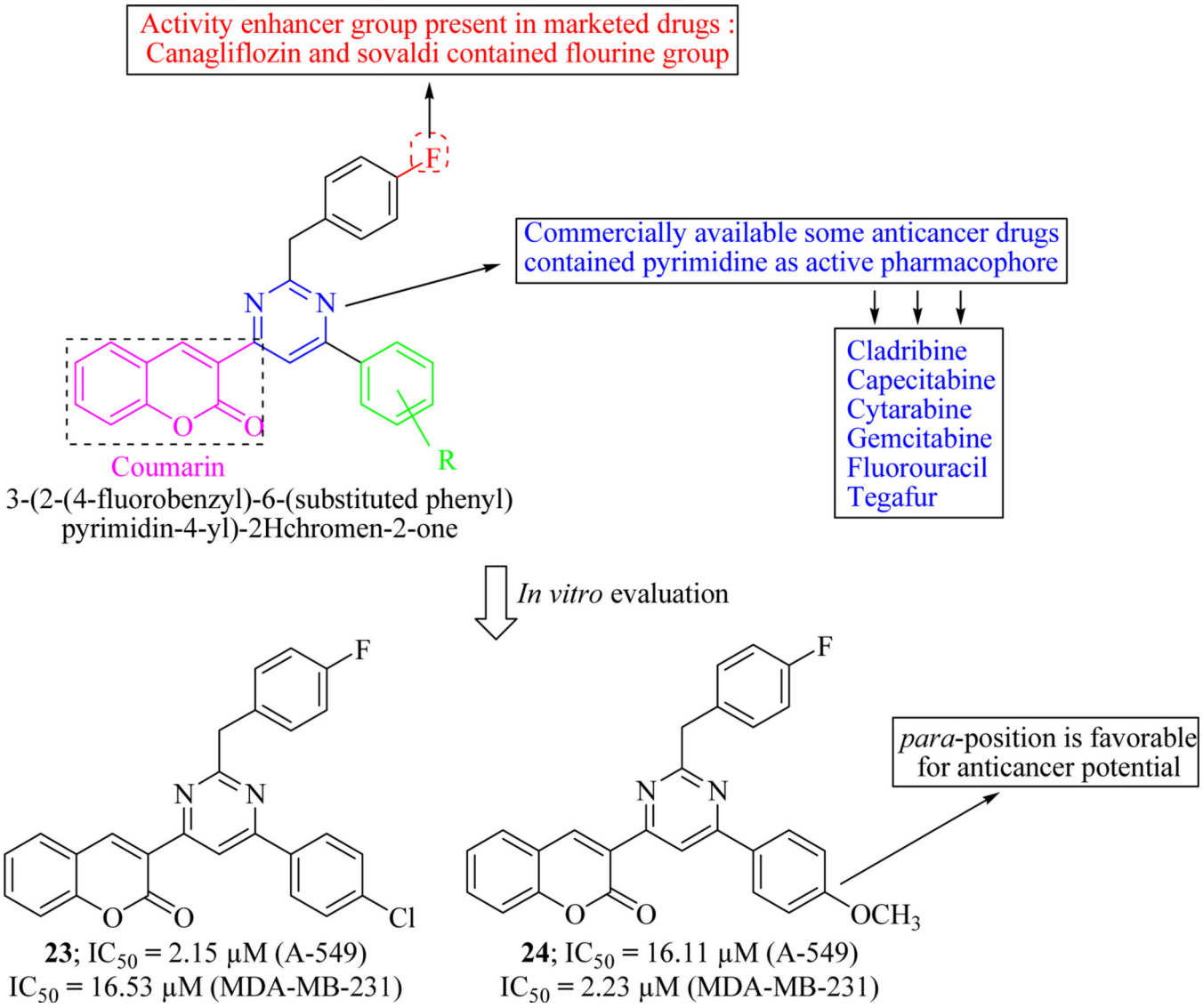


Figure 17.
Coumarin-pyrimidine hybrids as anticancer agents

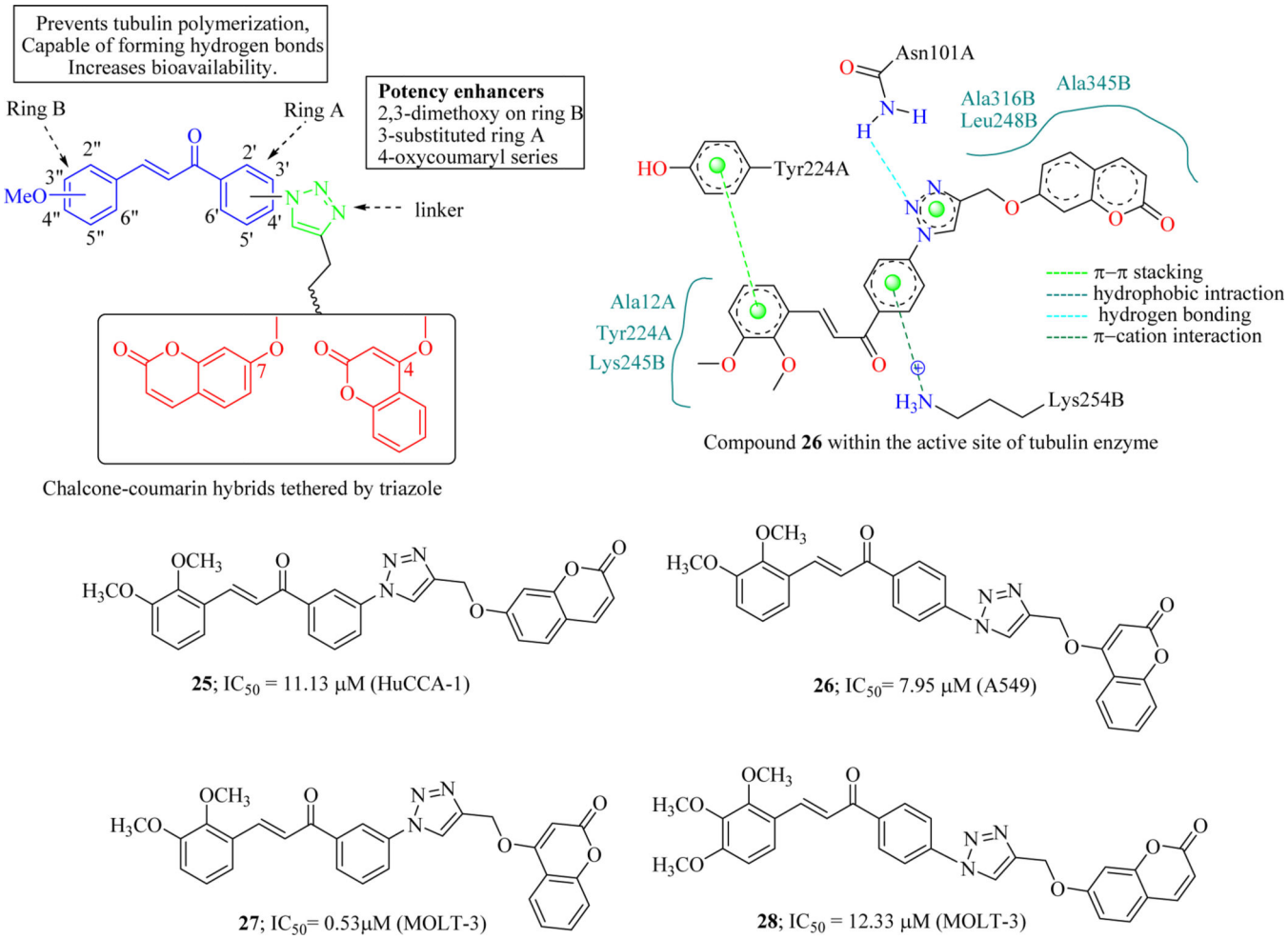


Figure 18.

Chalcone-coumarin hybrids linked via 1,2,3-triazole as tubulin inhibitors

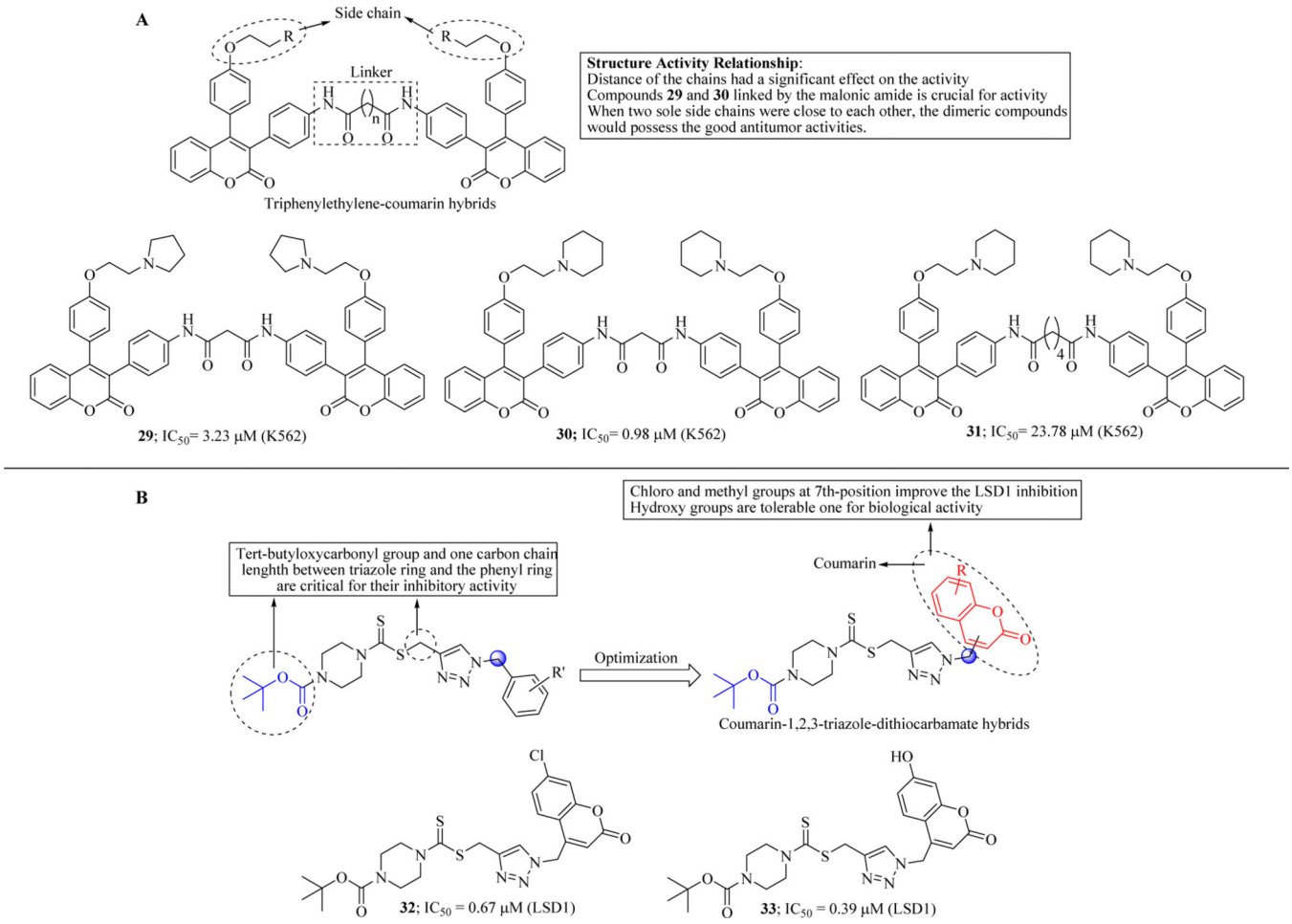


Figure 19.

(A) Triphenylethylene-coumarin hybrids active against leukemic cancer cell line; (B) Coumarin-1,2,3-triazole dithiocarbamate hybrids as LSD1 inhibitors

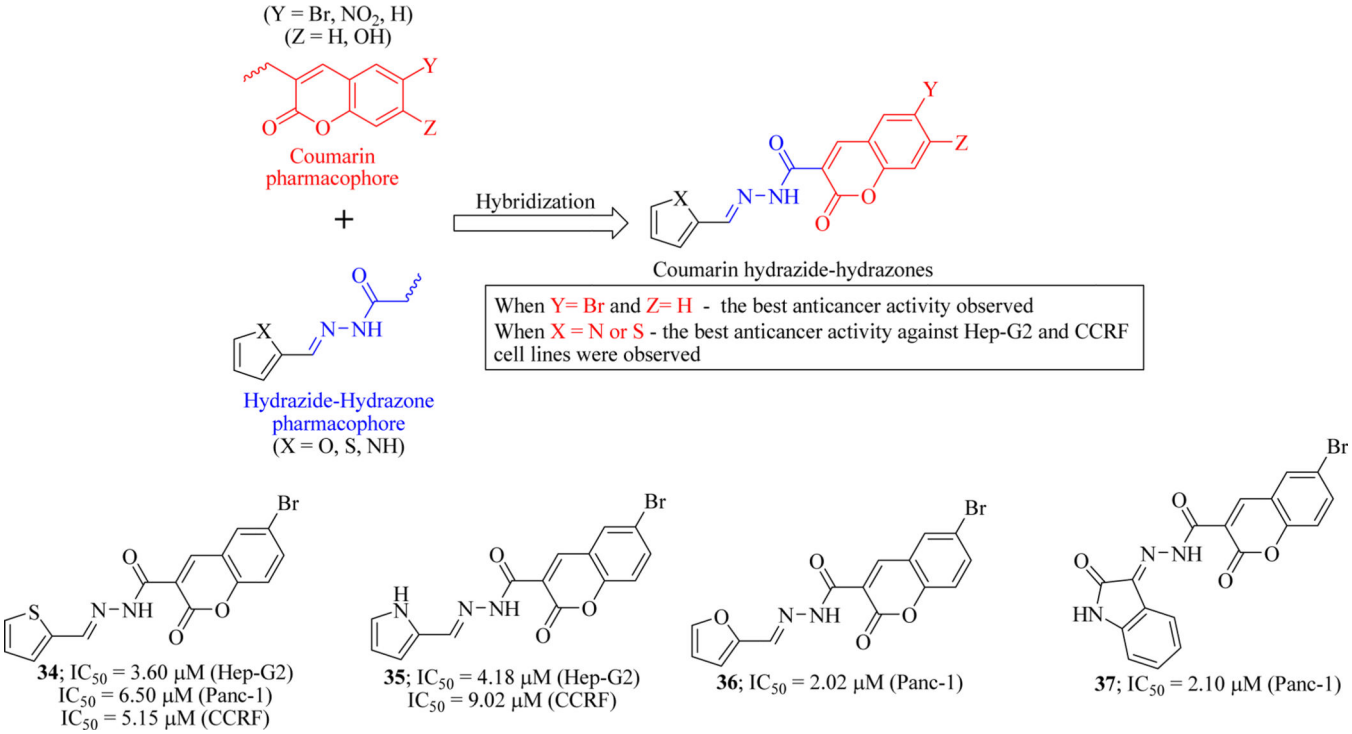


Figure 20.
Coumarin-hydrazide-hydrazone hybrids as anticancer agents

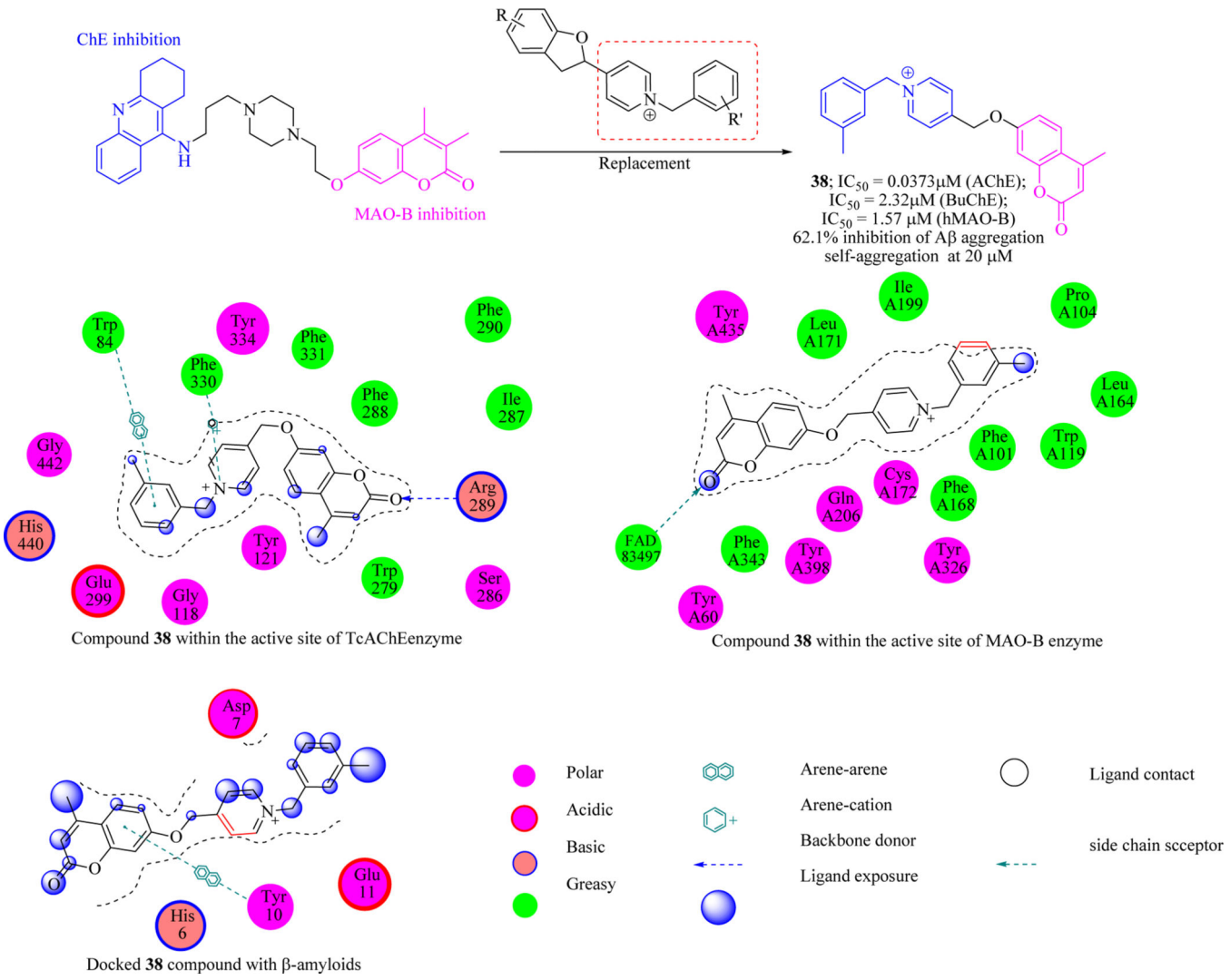


Figure 21.

Coumarin-*N*-benzylpyridinium hybrids as anti-Alzheimer's agents

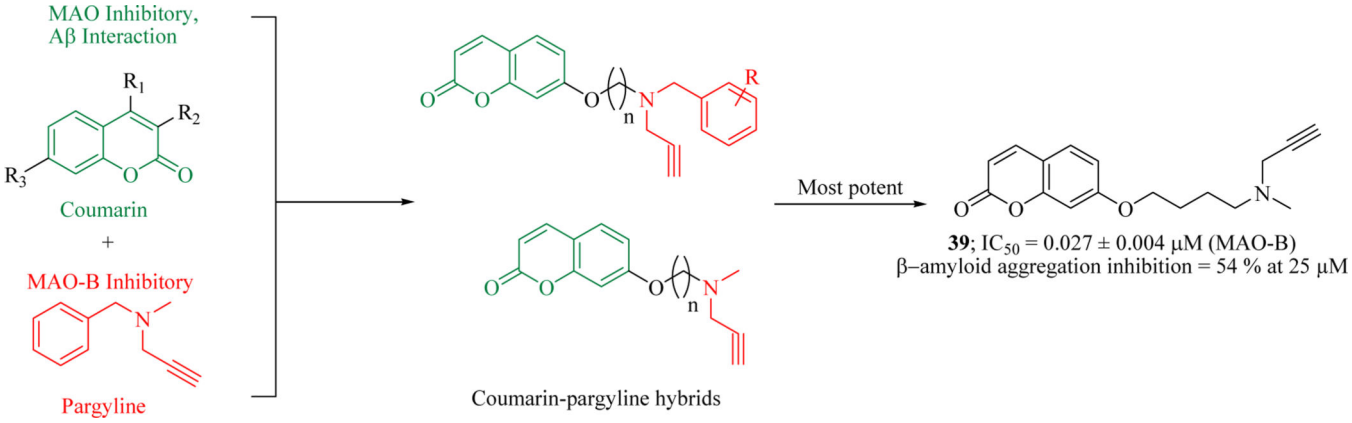


Figure 22.

Coumarin-pargyline hybrids as MAO-B inhibitors that prevent amyloid aggregation

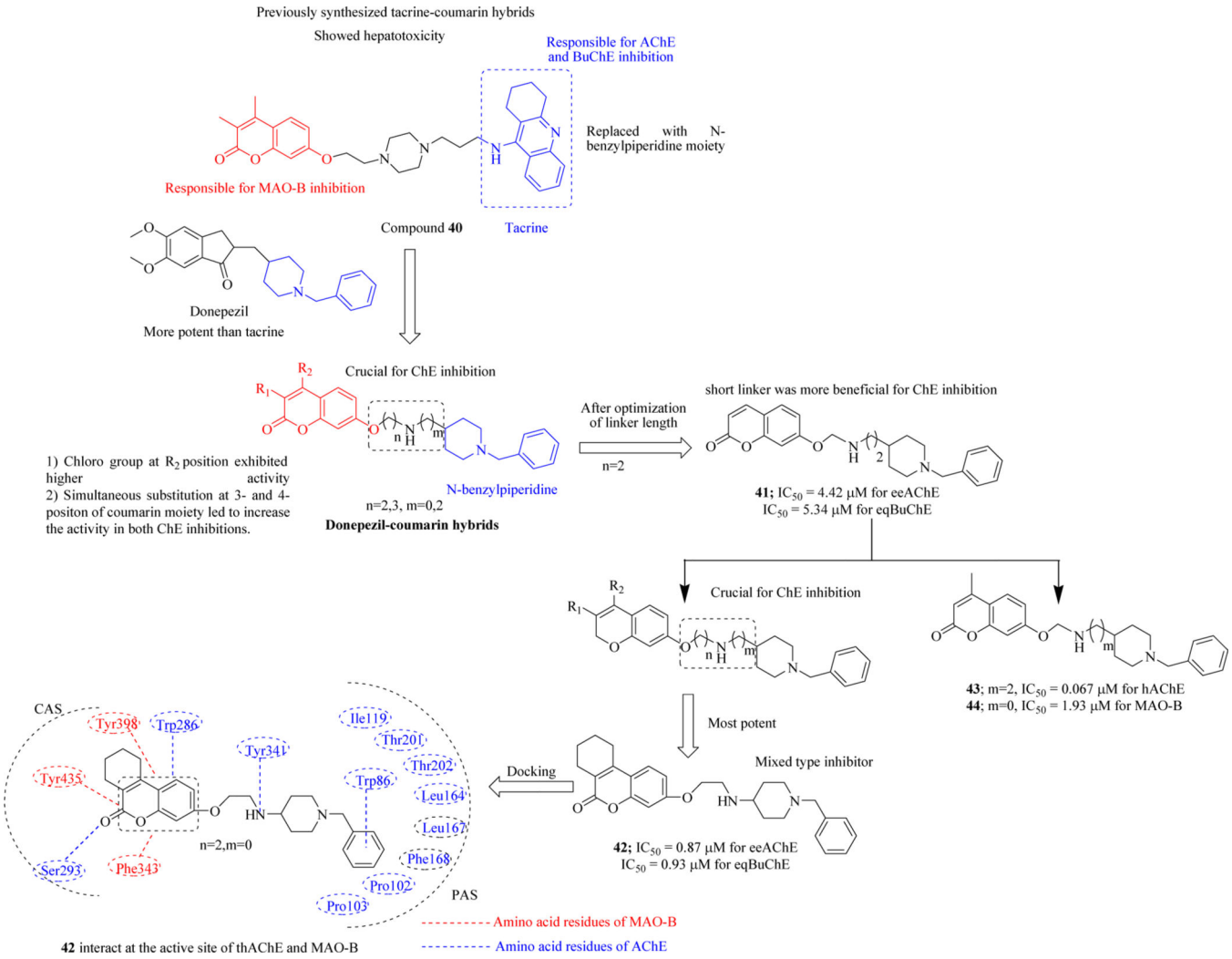


Figure 23.
Donepezil-coumarin hybrids as cholinesterase and MAO-B inhibitors

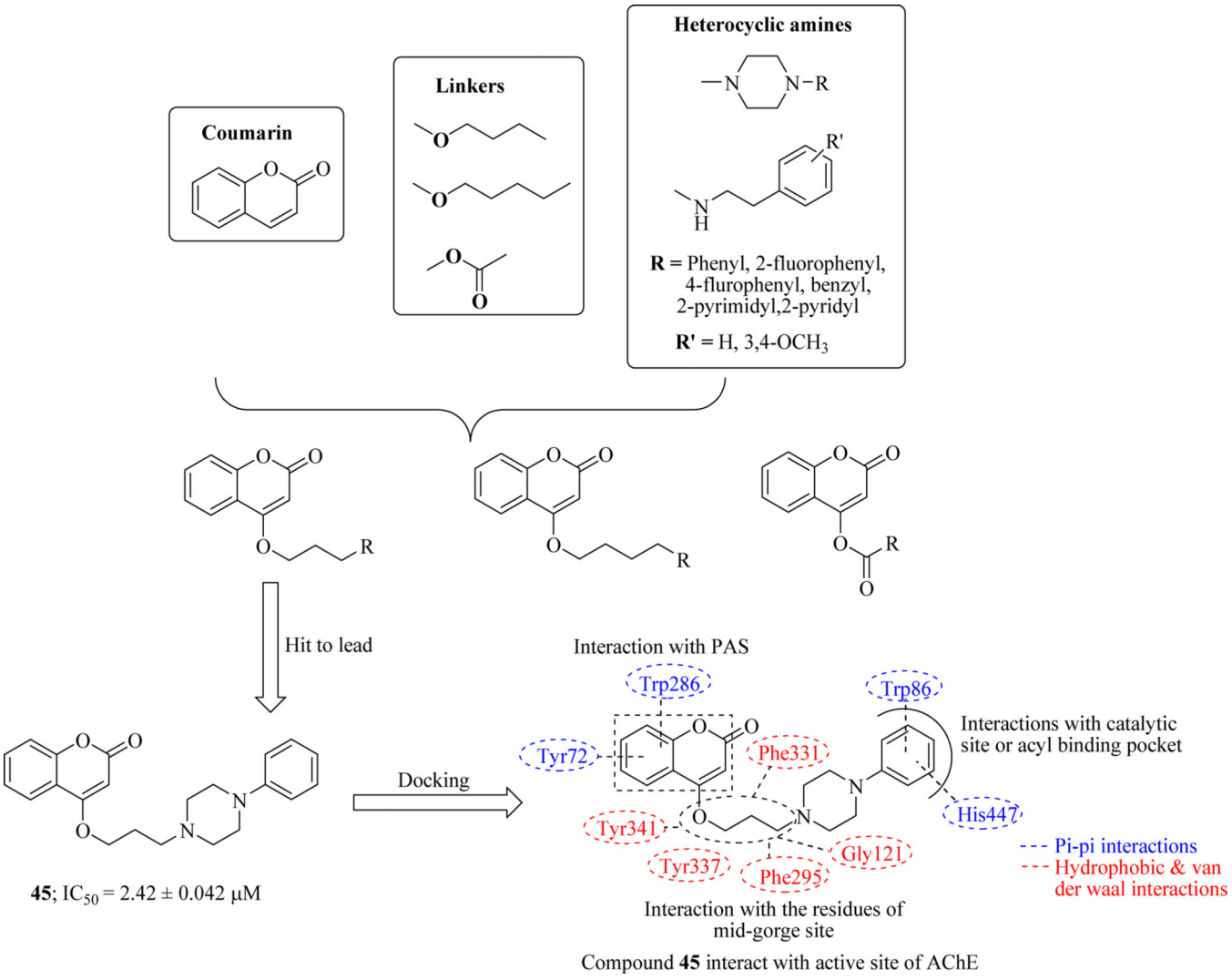


Figure 24.
Hybrid molecules of coumarin and various aromatic amines as AChE inhibitors

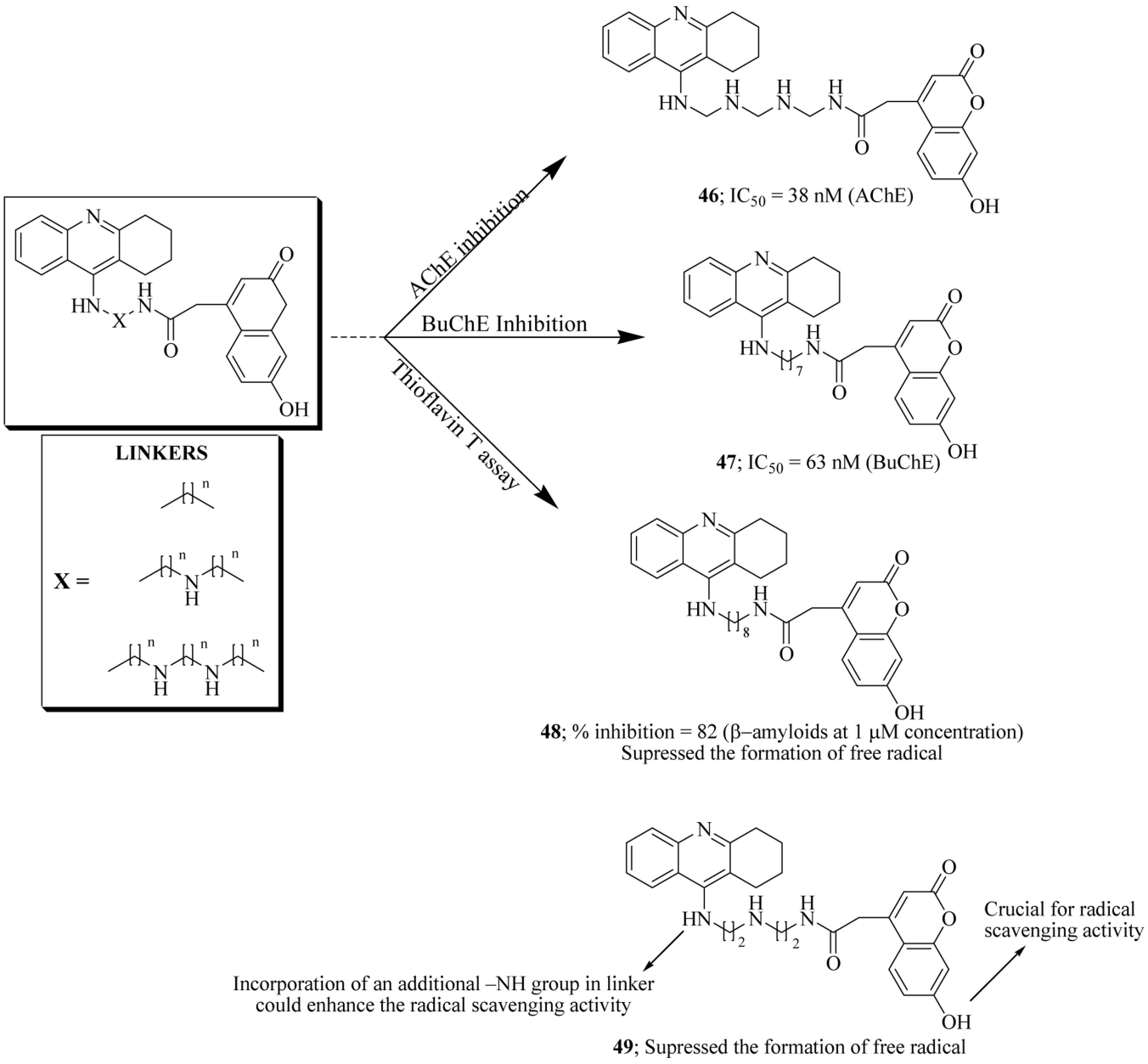
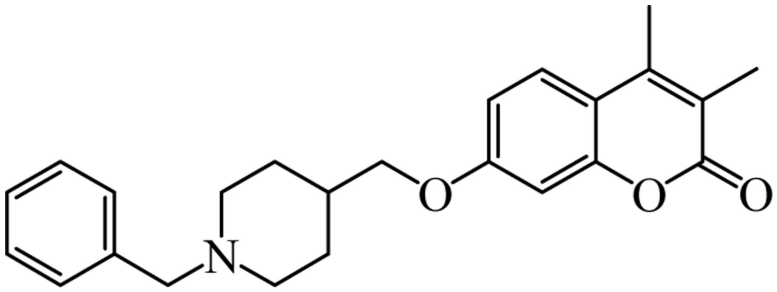


Figure 25.

Tacrine-coumarin hybrids as cholinesterase inhibitors



50; IC_{50} = 1.03 μM (*ee*AChE)
IC_{50} = 30 nM (*h*MAO-B)
Sparingly soluble in water
Neuroprotective

Figure 26.
3,4-dimethylcoumarin-piperidine hybrid as anti-Alzheimer's agents

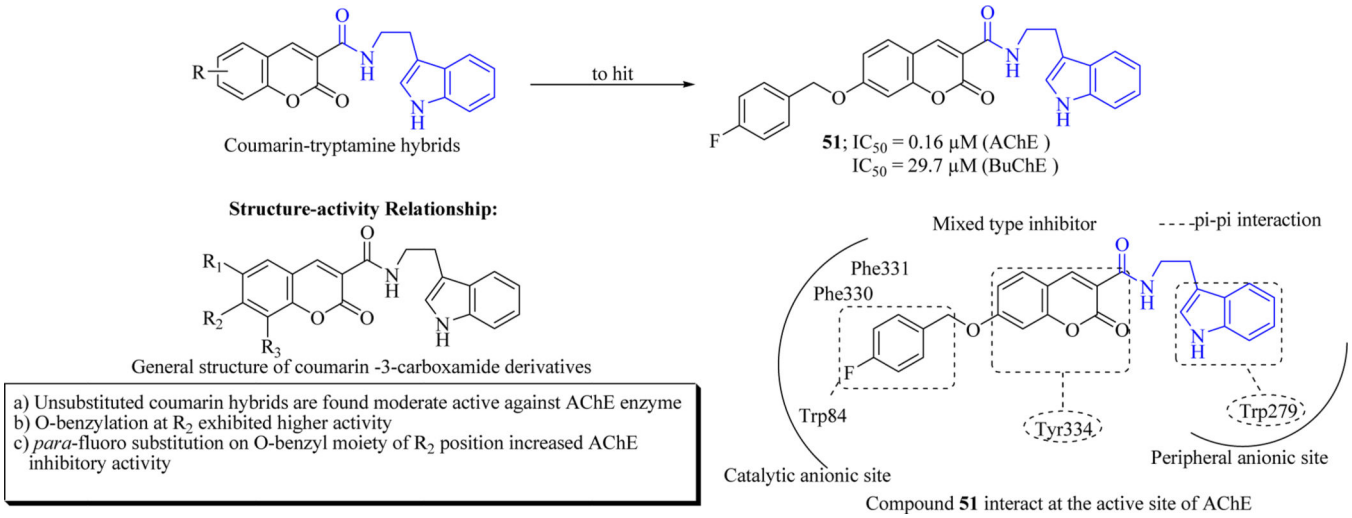
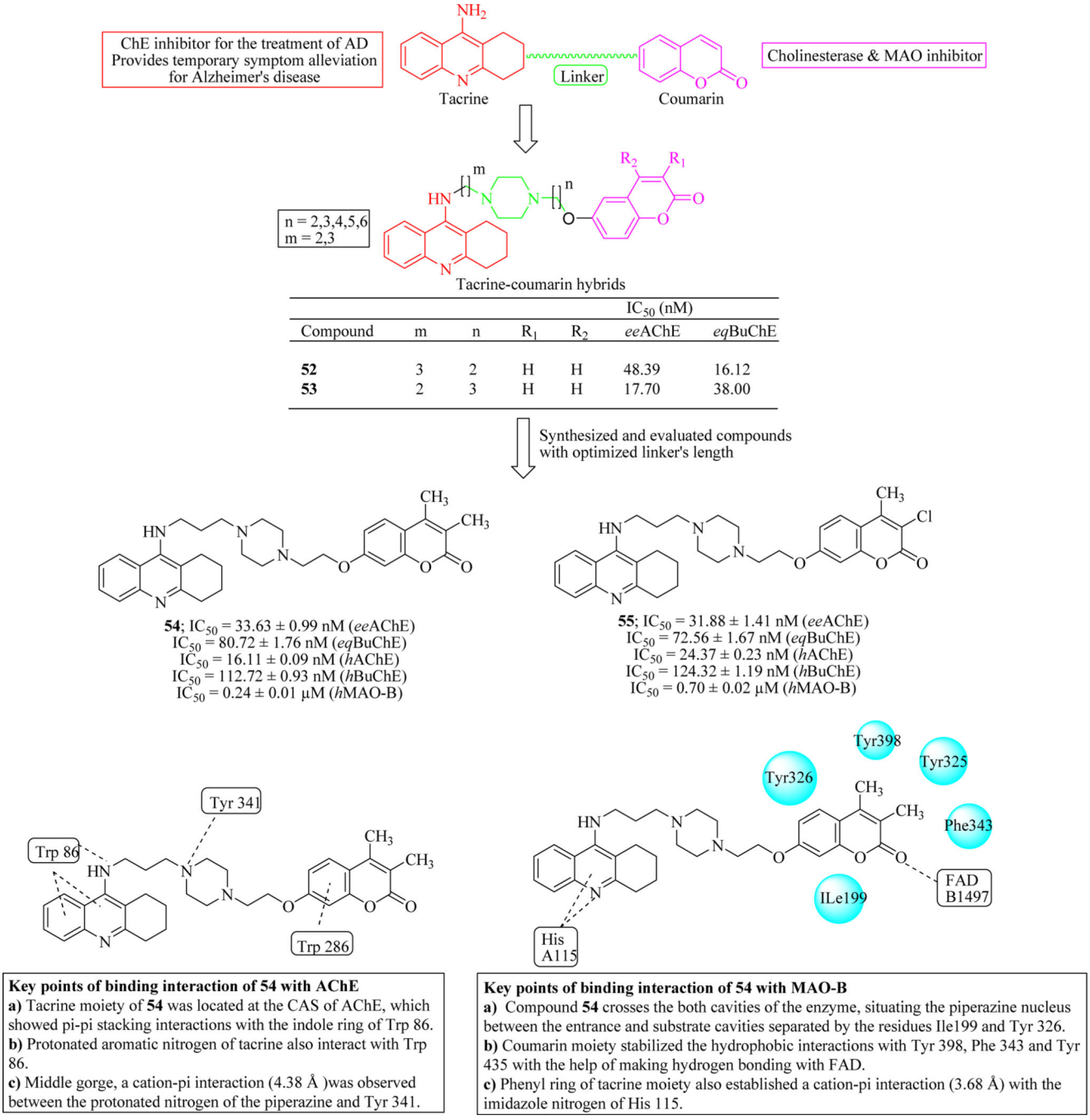


Figure 27.

Coumarin-tryptamine hybrids as cholinesterase inhibitors



					IC50 (nM)	
Compound	m	n	R_1	R_2	*ee*AChE	*eq*BuChE
52	3	2	H	H	48.39	16.12
53	2	3	H	H	17.70	38.00

Key points of binding interaction of 54 with AChE
a) Tacrine moiety of **54** was located at the CAS of AChE, which showed pi-pi stacking interactions with the indole ring of Trp 86.
b) Protonated aromatic nitrogen of tacrine also interact with Trp 86.
c) Middle gorge, a cation-pi interaction (4.38 Å)was observed between the protonated nitrogen of the piperazine and Tyr 341.

Key points of binding interaction of 54 with MAO-B
a) Compound **54** crosses the both cavities of the enzyme, situating the piperazine nucleus between the entrance and substrate cavities separated by the residues Ile199 and Tyr 326.
b) Coumarin moiety stabilized the hydrophobic interactions with Tyr 398, Phe 343 and Tyr 435 with the help of making hydrogen bonding with FAD.
c) Phenyl ring of tacrine moiety also established a cation-pi interaction (3.68 Å) with the imidazole nitrogen of His 115.

Figure 28.
Tacrine-coumarin hybrids tethered through piperazine as cholinesterase inhibitors

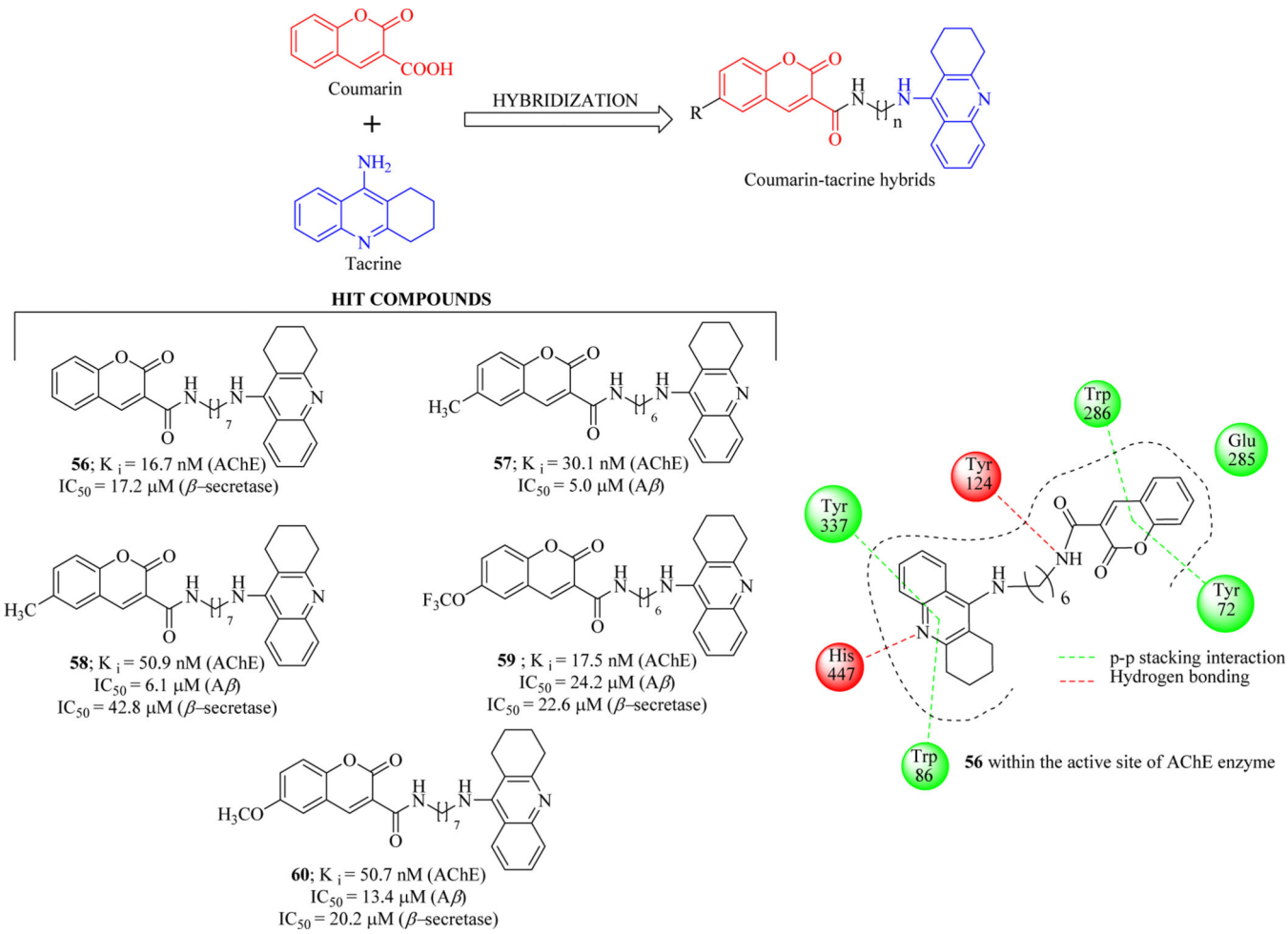


Figure 29.

Coumarin-tacrine hybrids using methylene chain linker as dual AChE/β-secretase inhibitors

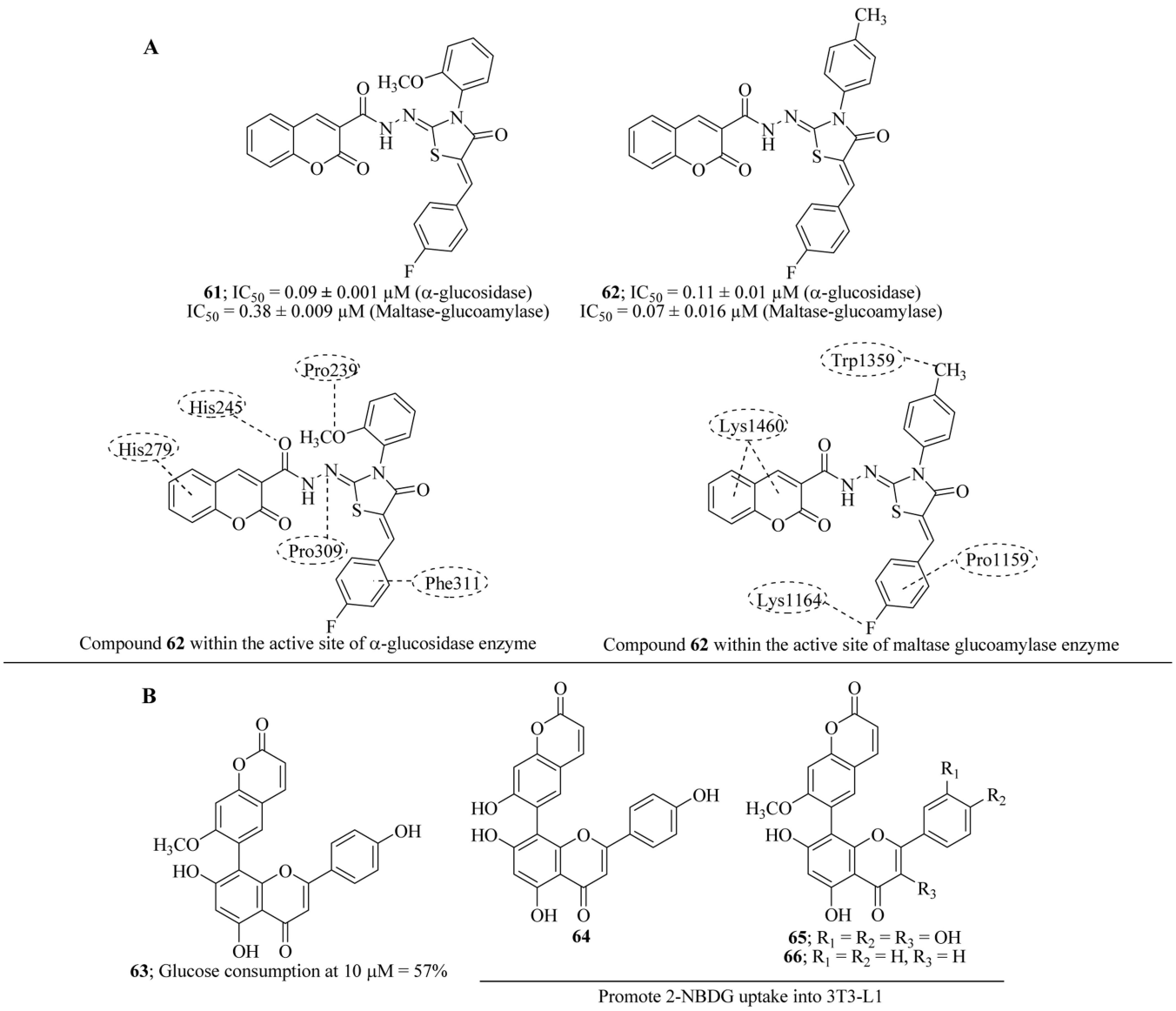


Figure 30.

(A) Coumarin-thiazolidinediones hybrid molecules as novel glucosidase inhibitors; (B) Flavonoid-coumarin hybrids as antidiabetic agents

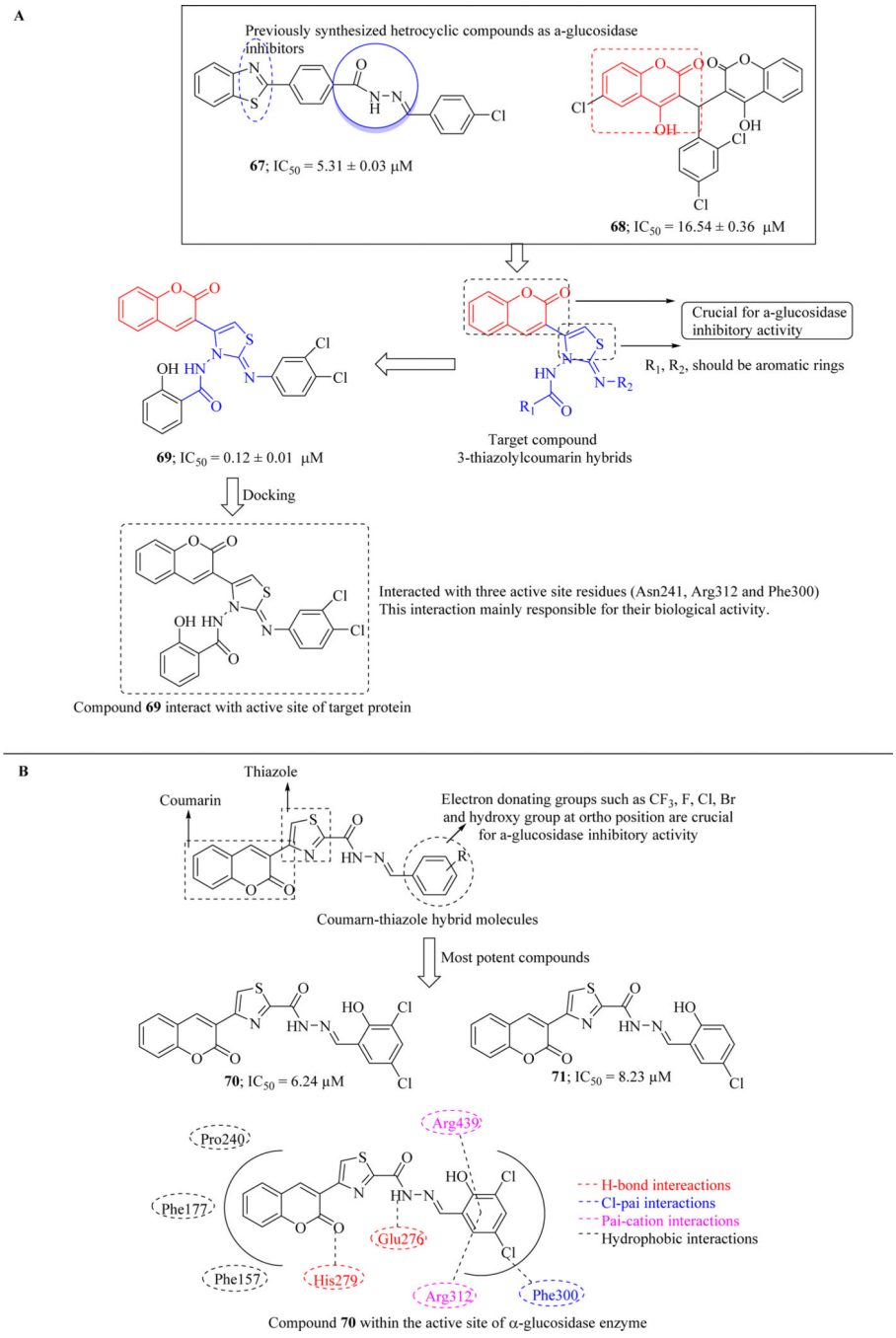


Figure 31.
Thiazole-coumarin hybrids as α-glucosidase inhibitors

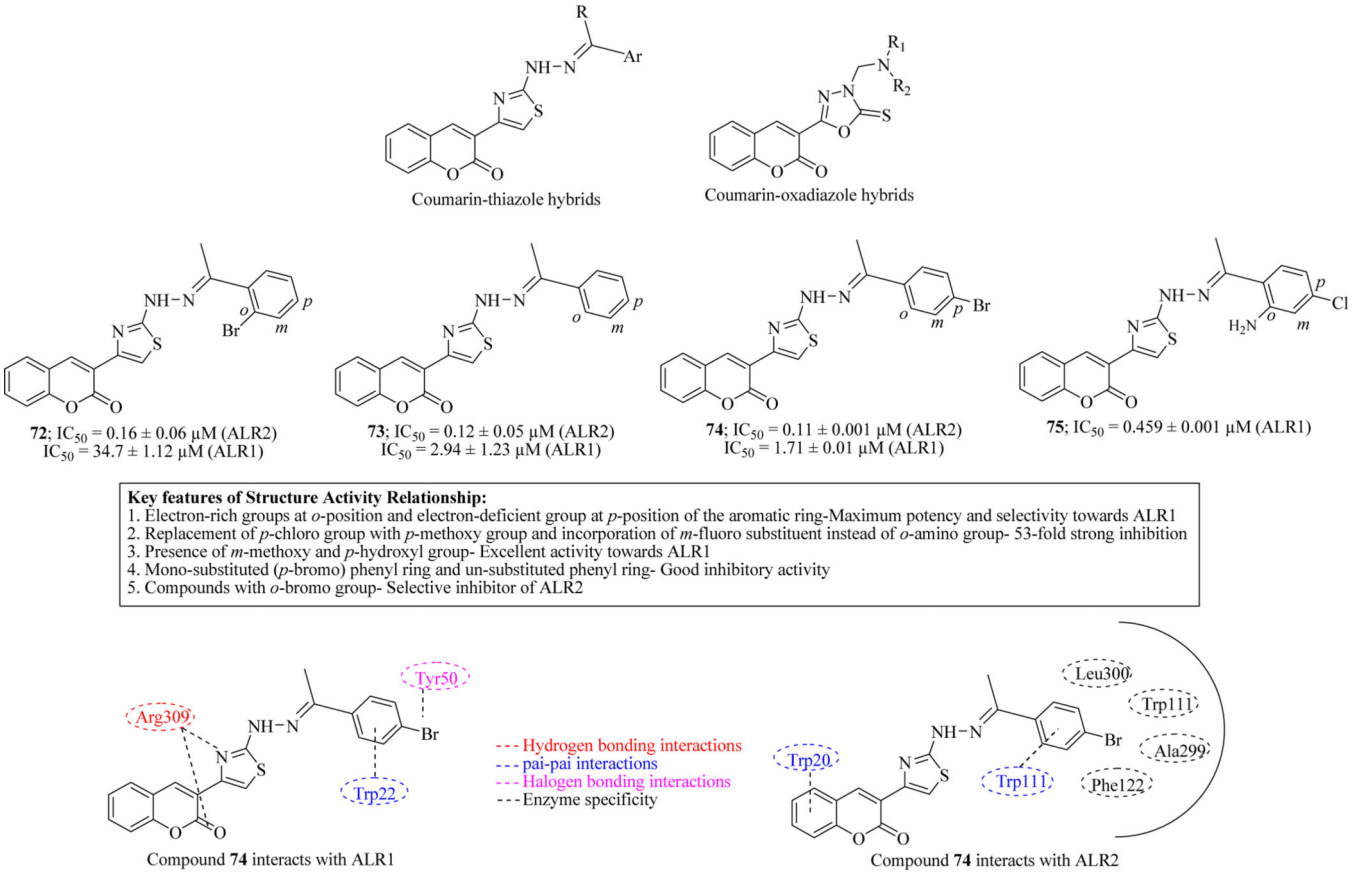
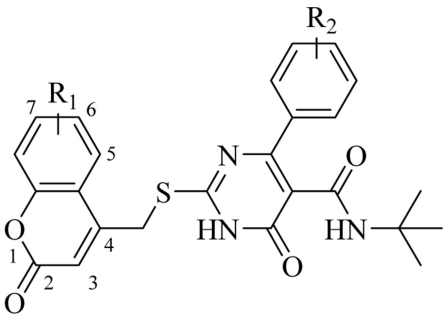


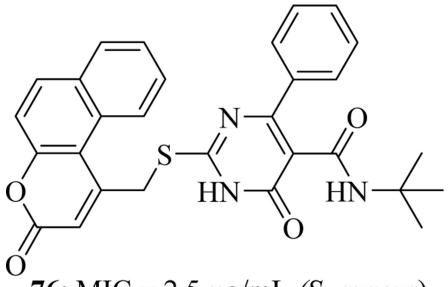
Figure 32.

Coumarin-thiazole and coumarin-oxadiazole hybrids as ALR inhibitors

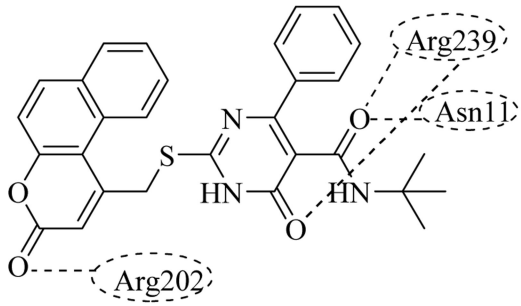


Coumarin-pyrimidine hybrids

Salient features of structure activity relationship:
1) Electron donation groups present on coumarin moiety contribute positive effect
2) Methyl group at 5th and 7th carbon atom on coumarin is tolerable for good inhibition
3) Activity decreasing in order at 6th carbon atom of coumarin: CH_3 > tert-butyl > OCH_3
4) 5,6-benzo substituted coumarin is crucial for *S. aureus* inhibition



76; MIC = 2.5 μg/mL *(S. aureus)*



Hydrogen bonding interactions of **76** within the active site of DHPS

Figure 33.
Coumarin-pyrimidine as anti-microbial agents

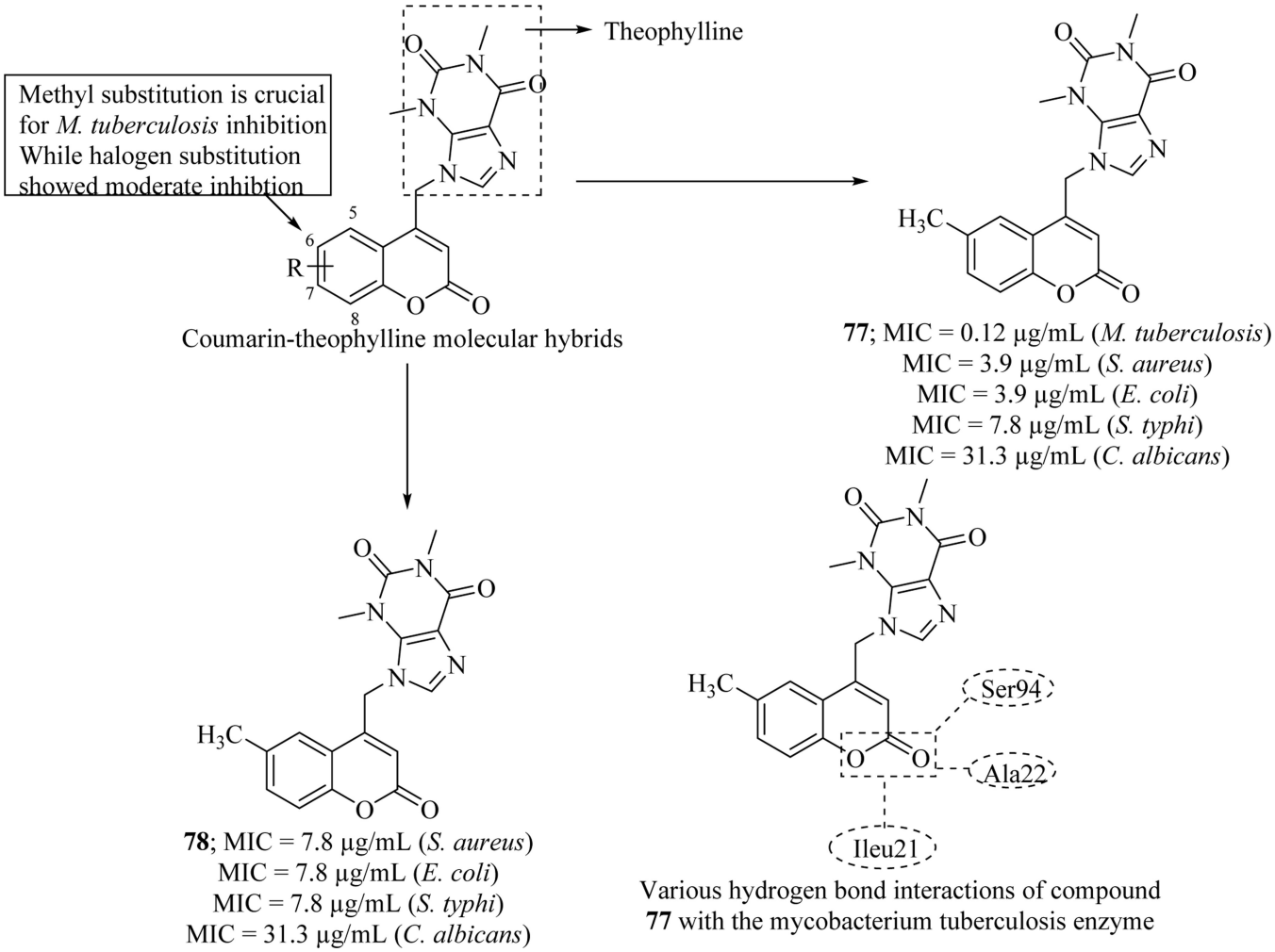


Figure 34.
Coumarin-theophylline hybrids as anti-microbial and antitubercular agents

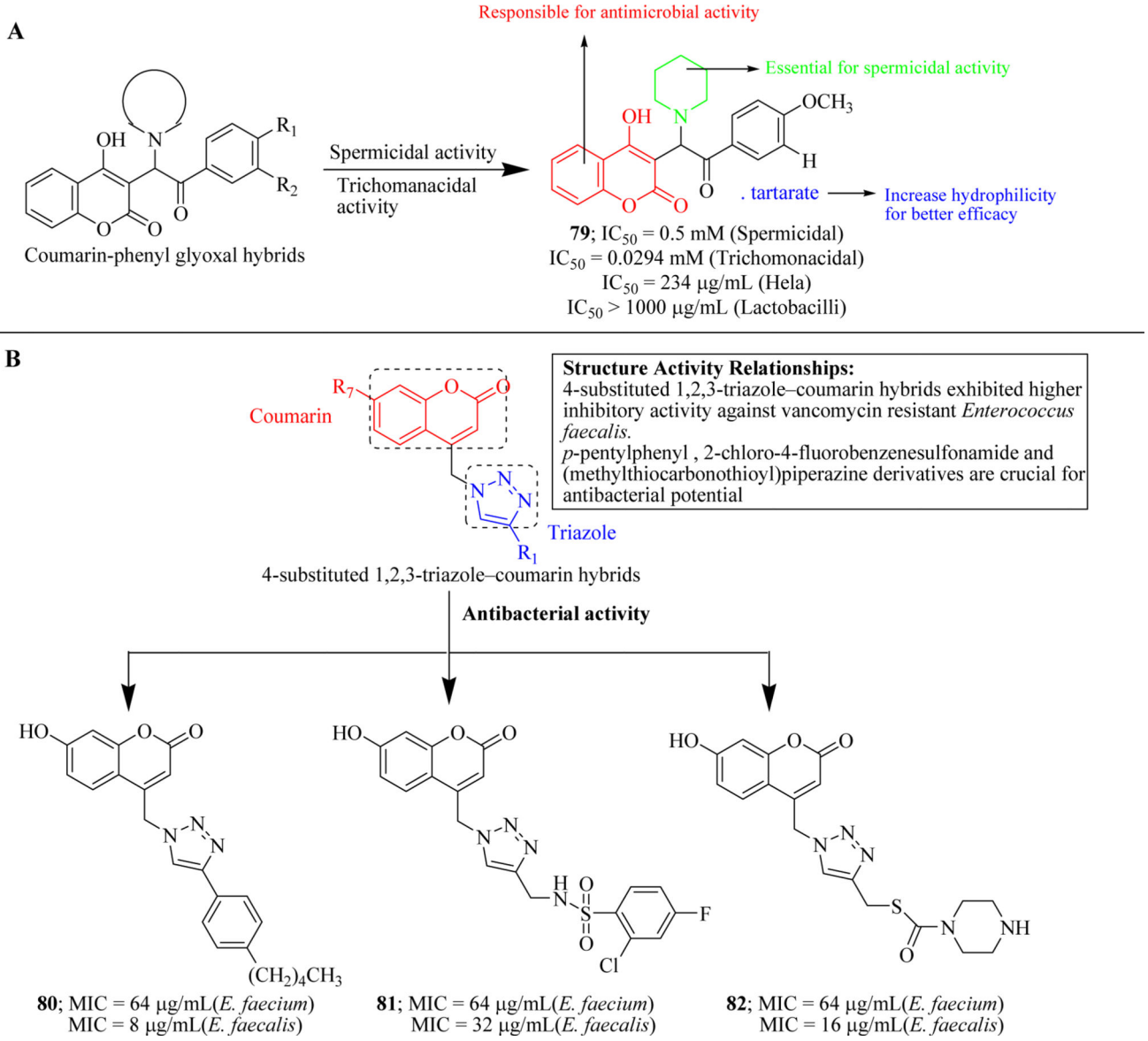
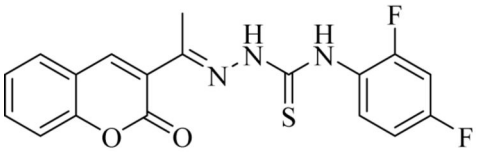
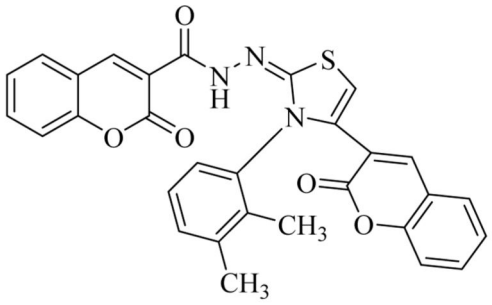


Figure 35.

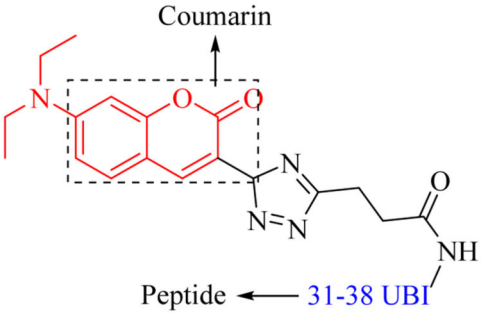
(A) Coumarin and phenyl glyoxal hybrids; (B) Triazole-coumarin hybrids as anti-microbial agents



83; MIC = 50 μg/mL (*E. coli*)
MIC = 100 μg/mL (*P.aeruginosa*)
MIC = 50 μg/mL (*S. aureus*)
MIC = 100 μg/mL (*A. clavatus*)
MIC = 100 μg/mL (*C. albicans*)
MIC = 100 μg/mL (*A. niger*)

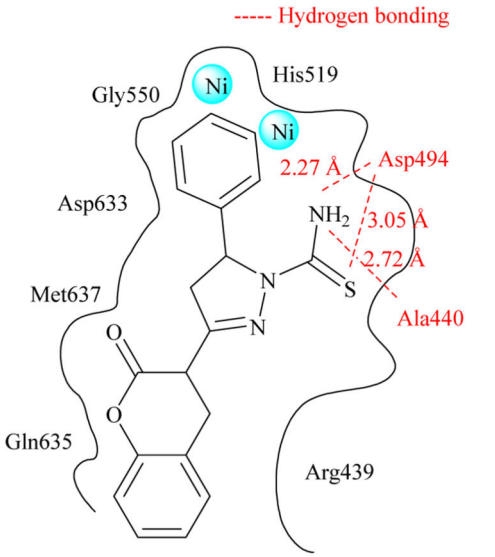


Compound	% inhibition at conentration			
	100 μM	**10 μM**	**1μM**	**0.1 μM**
84	70.4%	66.2%	60.7%	53.9%
Amphotericin B	79.8 %	76.3%	74.8%	69.9%



85; MIC = 0.04 to 0.18 μmol.ml $^{-1}$
Nontoxic (normal cell viability > 95%) up to 0.21 μg/mL

Figure 36.
Various coumarin hybrid molecules as anti-microbial agents



Compound **86** within the active site of urease enzyme

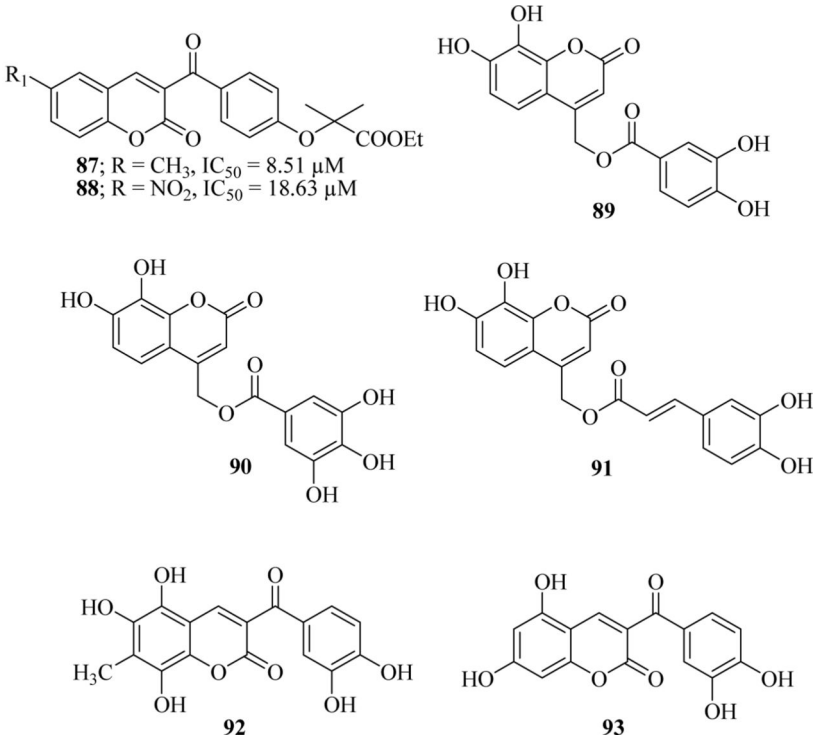


Figure 37.
Various coumarin hybrid molecules with antioxidant activity

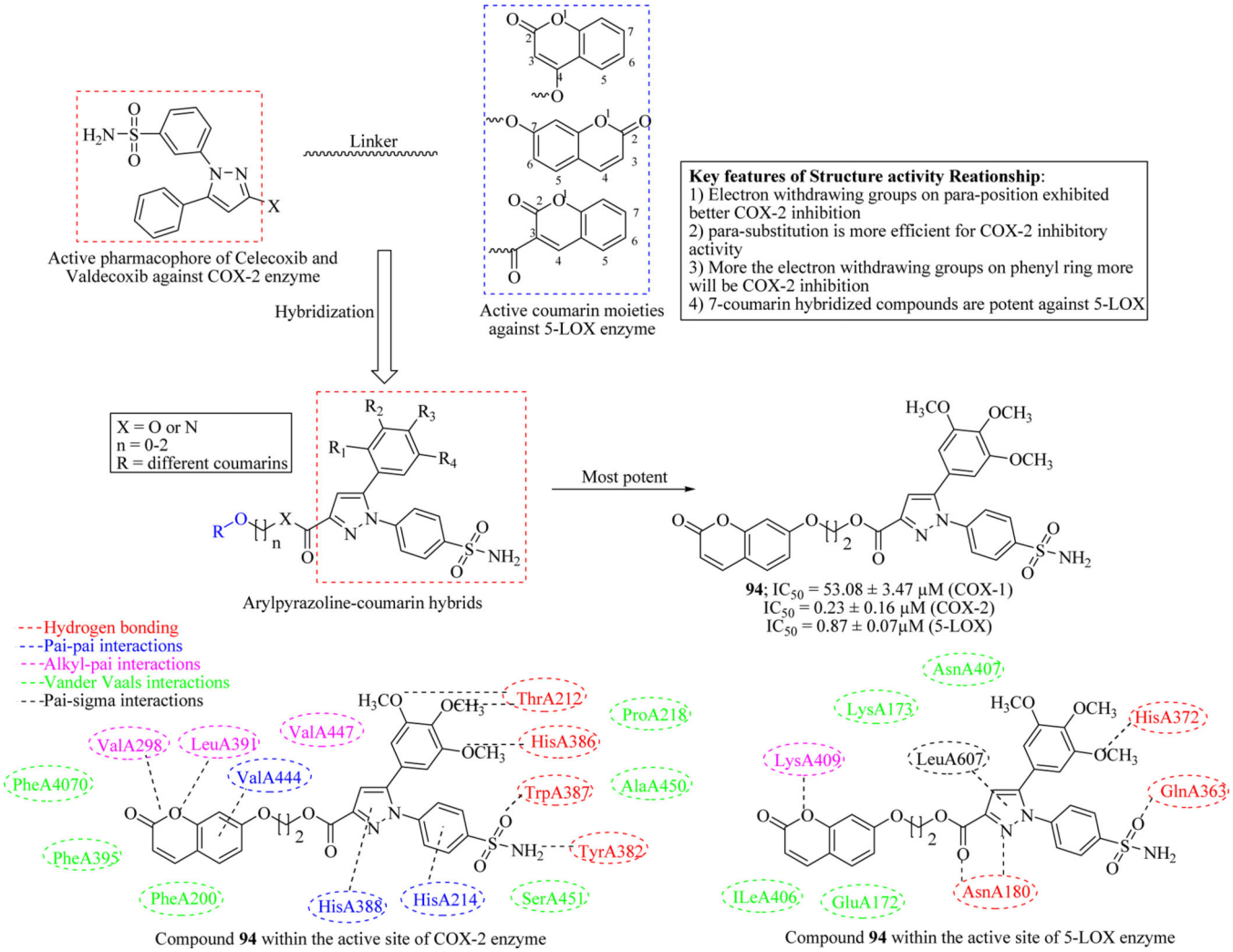
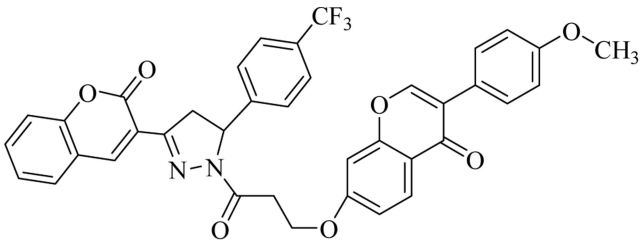
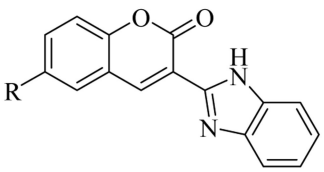


Figure 38.

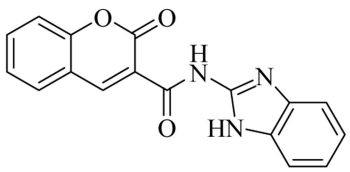
Arylpyrazoline-coumarin hybrids as anti-inflammatory agents



95; IC_{50} = 16.83 μM (TNF-α)
IC_{50} = 14.18 μM (IL-6)



96; R = Br, 45.45% inflammation inhibition in paw oedema of rats
97; R = Cl, 46.75% inflammation inhibition in paw oedema of rats



98; 42.85% inflammation inhibition in paw oedema of rats

Figure 39.
Various coumarin hybrid molecules as anti-inflammatory agents

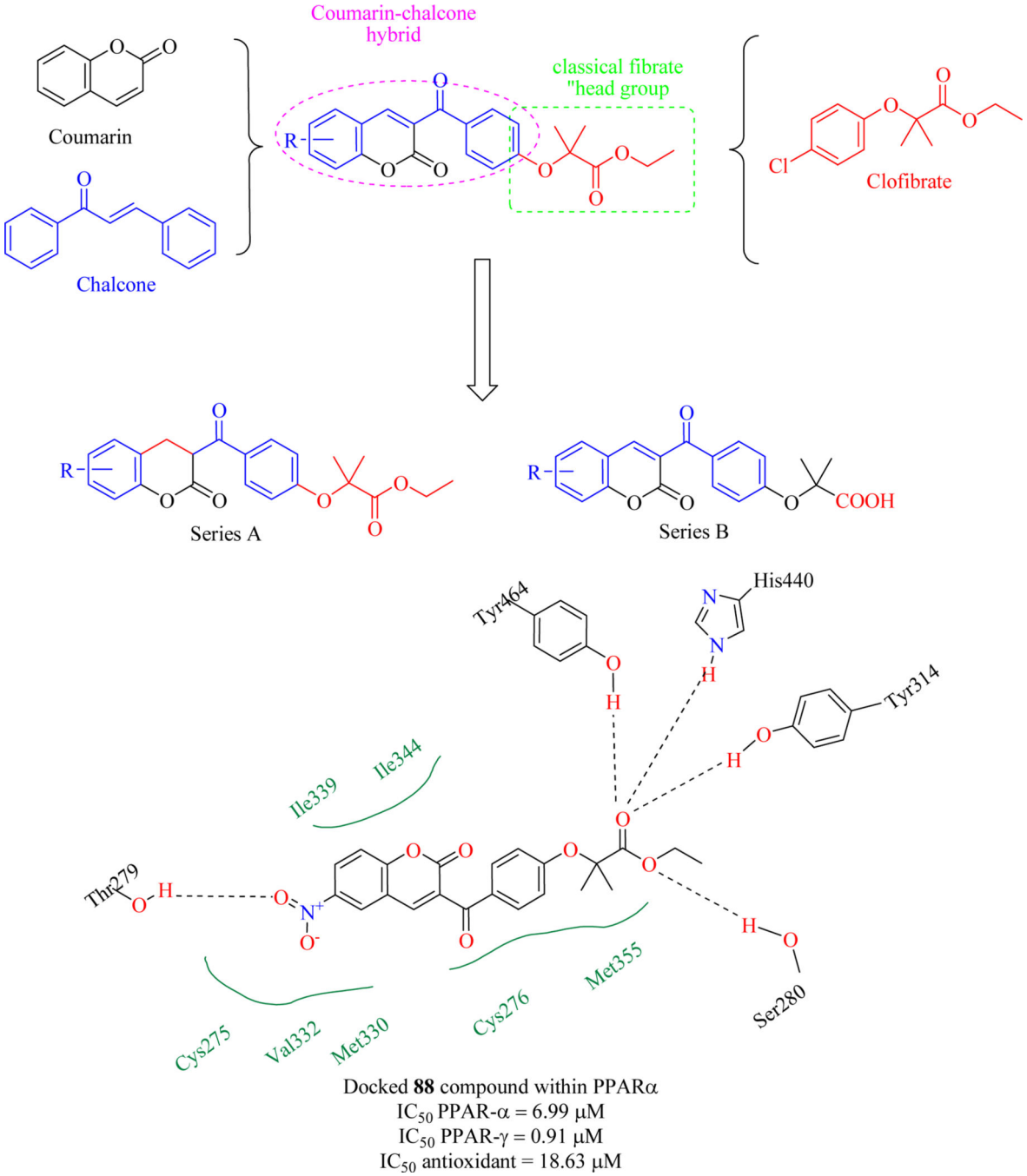


Figure 40.
Coumarin-chalcone hybrid as a blood coagulator agent with antioxidant property

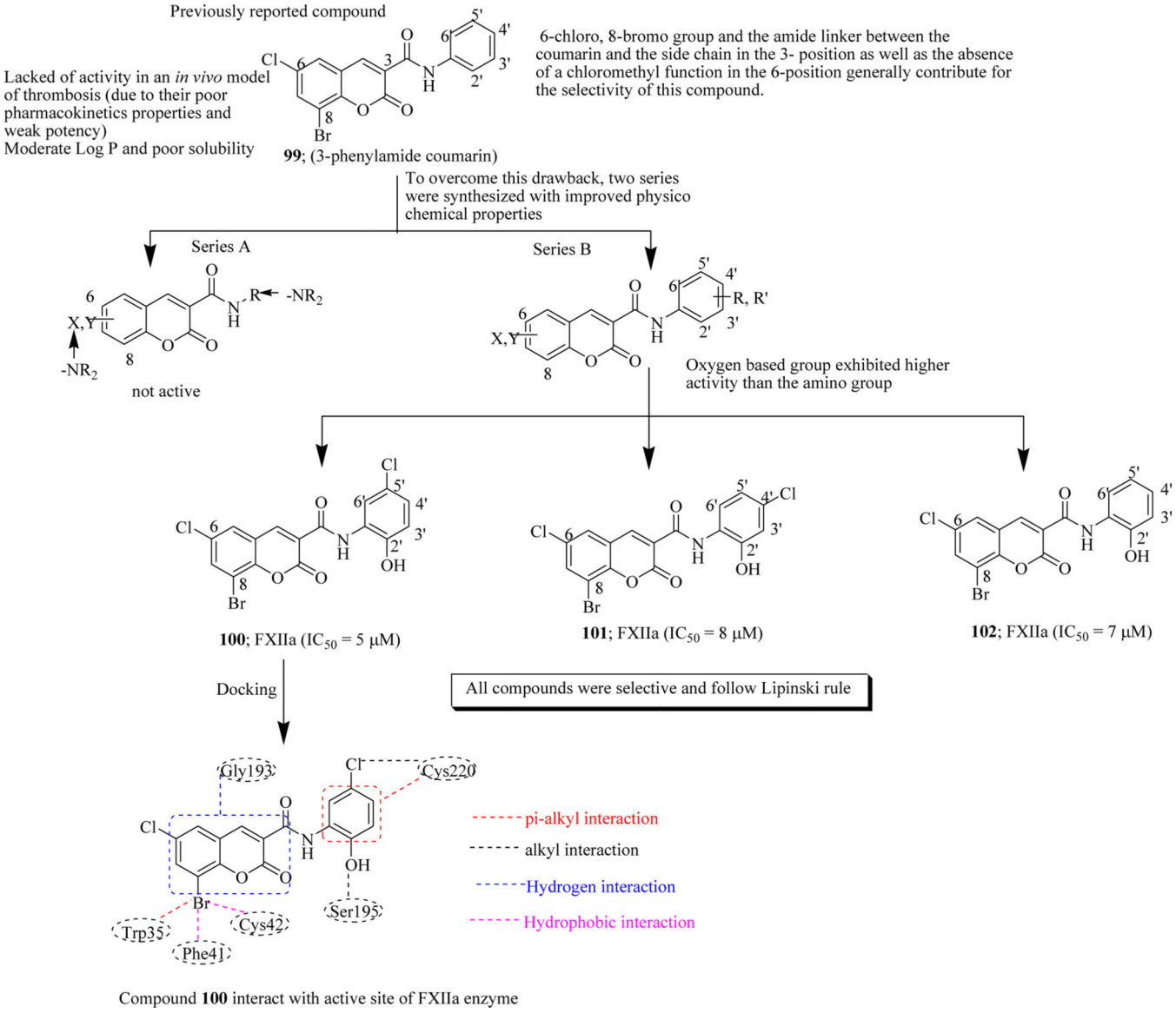


Figure 41.

Phenylamide-coumarins as FXIIa enzyme inhibitors

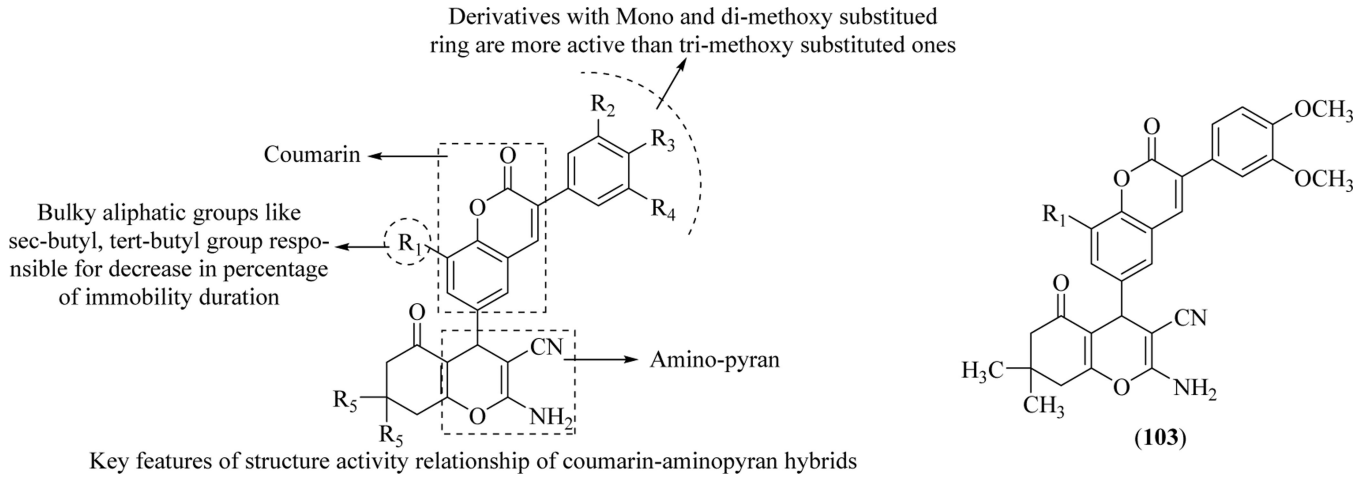


Figure 42.

Coumarin-aminopyran based hybrids as an antidepressant