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# Antioxidant Hybrid Compounds: A Promising Therapeutic Intervention in Oxidative Stress Induced Diseases

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**Abstract:** Reactive oxygen species/nitrogen species (ROS/RNS) are major causative agents of oxidative stress related diseases such as neurodegenerative, cancer, cardiovascular, and inflammation via intracellular signal transduction pathways. Synthetic modification of antioxidants and development of hybrid compounds by conjugation or integration of two or more moiety opened a new era in development of antioxidant based therapeutics. In this review, our attention is focused on structural, chemical and biochemical feature of free radicals, description of mechanistic modulation in signaling pathways by antioxidants and establishment of relationship between structural and biological accepts of antioxidant hybrid systems (1,2-dithiolone, α,β-unsaturated carbonyl, cinnamate based hybrids and miscellaneous hybrids).

**Keywords:** Antioxidant hybrid compound, reactive oxygen species, structure-activity relationship.

## INTRODUCTION

Reactive species (RS), including reactive oxygen (ROS) and reactive nitrogen species (RNS), are constantly generated during normal oxidative metabolism in aerobic organisms and in response to environmental stimuli. These are small molecules or ions formed by the incomplete reduction of oxygen, and include free radicals such as superoxide anion ($O_2^{\bullet-}$), hydroxyl radical ($OH^{\bullet}$), peroxyl radical ($RO_2^{\bullet}$), and alkoxyl radical ($RO^{\bullet}$) as well as non-radical species that are oxidizing agents and/or easily converted into radicals, such as hydrogen peroxide ($H_2O_2$), hypochlorous acid (HOCl), ozone ($O_3$), and singlet oxygen ($^1O_2$) [1]. ROS are generated by exogenous source (UV light, ionizing radiation and inflammatory cytokine) and endogenous source such as mitochondria [2], xanthine oxidase [3], neutrophils, eosinophils, macrophages, CP450 [4], microsomes and peroxisomes [5]. Several other factors have also been investigated to elicit production of ROS [6]. Furthermore, some of the transition metals such as iron, copper, chromium, cobalt, vanadium, cadmium, arsenic, and nickel have been investigated to play an important role in the generation of free radicals via Fenton chemistry [7-9]. Under physiological conditions, intricate defense systems composed of antioxidant enzyme systems (superoxide dismutase, catalase, and glutathione peroxidase) and non-enzymatic antioxidants such as glutathione (GSH), vitamins (A, E and C), melatonin, uric acid, lipoic acid, carotenoids, and polyphenols (flavonoids, curcumin, resveratrol and others), impart balance between generation and neutralization of reactive oxygen species [10, 11]. Inadequate antioxidant defense systems, improper intake of dietary antioxidant supplements and excessive generation of ROS, leads to so called "oxidative stress" (OS). The RS can also interact and damage cellular macromolecules such as nucleic acid, protein and lipids [12-17].

Cysteine residues and protein bound metals, including heme iron are the primary targets of ROS, as it readily (reversibly or irreversibly) oxidized to a disulfide bond (–SSR), sulfenic acid (–SOH), sulfinic acid ($–SO_2H$) or sulfonic acid ($–SO_3H$) [18, 19]. Cysteine residue containing signaling enzymes and proteins include phospholipase C [20, 21], phospholipase $A_2$ [22, 23] and phospholipase D [24]. Ion channels [25, 26], including calcium channels [27], have been proposed as potential target for ROS. Further, signaling mechanisms that respond to changes in the thiol/disulfide redox state, such as Src family kinase [28, 29], MAPKs [30, 31], activator protein-1 (AP-1) [32] and nuclear factor-κB (NF-κB) [33] transcription factors can also be targets.

Apart from deleterious nature, ROS play central role in intracellular signal transduction pathways for variety of pathophysiological cellular responses and pathophysiology of various diseases such as acute respiratory distress syndrome [34], aging [35], Alzheimer [36, 37], atherosclerosis [38], cancer [39-41], cardiovascular diseases [42, 43], diabetes [44], inflammation [45], inflammatory joint diseases [46], neurological diseases [47], obesity [48, 49], Parkinson [50], pulmonary fibrosis [51], rheumatoid arthritis [52], and vascular diseases [53, 54].

Since, endogenous antioxidant defense system is not always completely effective and there is continuous exposure to various environmental factors hence, there is always a need to search new drug candidates to counter oxidative damage. In this quest, co-administration of antioxidant enzyme

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system and non-enzymatic antioxidant or their integration or covalently attachment with existing drug candidates played a pivotal role. A number of fruits, vegetables such as citrus fruits, berries, apple, grapes, onion, beans and their fermented products have been abundant source of potential antioxidants and their uses on regular basis may counteract ROS and decrease rate of oxidative stress induced diseases such as neurodegeneration, cardiovascular diseases, cancer and many more [55-66]. In this context, antioxidant hybrid approach may provide possibilities for generating a diverse array of new types of molecules as promising therapeutic agents in oxidative stress induced diseases.

## BIOLOGICAL EFFECTS OF ANTIOXIDANTS AND THEIR MECHANISM

A variety of enzymatic and non-enzymatic antioxidants have been investigated from natural or unnatural origins with potential role in the biological systems. Mainly following three mechanism of action have been proposed of antioxidants against RS induced oxidative damage: - (i) modulation of signaling pathways that mediated gene regulation in response to ROS, (ii) quenching of free radicals, (iii) direct chemical interaction of the antioxidant with signaling enzymes and transcription factors [67]. The mechanism of some of the important antioxidants is discussed here. The α-tocopherol is an important antioxidant which plays a vital role in various inflammations and it directly binds with phospholipase $A_2$ ($PLA_2$) [68]. At the same time, it leads to modulation of NADPH oxidase, protein kinase C (PKC), protein kinase B (PKB) via inhibition of subunit assembly [69-72]. The trans retinoic acid regulates the physiology of cells differentiation and apoptosis via direct binding with protein kinase C (PKCα and PKCβ) [73, 74]. Similarly, ascorbic acid potentiates enzymatic degradation of hypoxia inducible factor-1 (HIF-1α) via proline and asparagine hydroxylase [75]. Curcumin is another important antioxidant which has attracted various research groups in recent times and it has been observed that it modulates PKC, NF-κB and AP-1 by direct binding [76, 77], and by inhibition of IκBα proteasome [78, 79] and Fos-Jun-DNA complex formation [80]. Resveratrol modulate c-Src, PK (by direct binding) [81], NF-κB (by inhibition of the pathway and/or of IκBα proteasome degradation) [82, 83], and AP-1 by the alteration in its composition [84]. Inhibition of NADP oxidase subunit translocation by epigallocatechin gallate imparts its mast stabilizing and antiallergic activity [85]. Flavonoids regulate RTK, MAPKs, PI3K/Akt by direction action [86-89], and modulation of NF-κB (by inhibition of the pathway and/or of IκBα proteasome degradation) [90]. Anti-inflammatory effects of kaempferol and quercetin are exerted by direct binding with vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), endothelial cell selectin (E-selectin), inducible NO synthase (iNOS) and cyclo-oxygenase-2 (COX-2) [91]. GSH plays its crucial role against oxidative damage via S-glutathionylation of PKCs [92], MAPKs [93], IKKβ, p65, NF-κB [94, 95] and p53 [96]. Apart from these molecular targets there is another important pathway which plays an important role in oxidative stress. Nrf2 is a nuclear transcription factor that controls the expression and coordinates induction of a battery of defensive genes encoding detoxifying enzymes and antioxidant proteins. This mechanism is of critical importance for cellular protection and cell survival. Nrf2 is retained in the cytoplasm by an inhibitor (INrf2) which functions as an adapter for Cul3/Rbx1-mediated degradation of Nrf2. In response to oxidative/ electrophilic stress, Nrf2 is switched on and then off by distinct early and delayed mechanisms. Oxidative/ electrophilic modification of INrf2 cysteine 151 and/or protein kinase C phosphorylation of Nrf2 serine 40 results in the escape or release of Nrf2 from INrf2. Nrf2 is stabilized and translocates to the nucleus, forms heterodimers with unknown proteins, and binds the antioxidant response element, which leads to the coordinated activation of gene expression. The switching on and off of Nrf2 protects cells against free radical damage, prevents apoptosis, and promotes cell survival. Recently, various antioxidants such as curcumin, caffeic acid, resveratrol, lipoic acid, catechin and their derivatives result in up-regulation of Nrf2 in various *in vitro* and *in vivo* models [97-115] and Nrf2 have been found as an important activator of phase II antioxidant genes.

## ANTIOXIDANT HYBRIDS APPROACH

Hybrid systems are construction of different molecular entities from natural or unnatural origin to transform or augment different entities or to generate a molecule with bifunctional feature with new properties. This design of antioxidant enzymes or non-enzymatic antioxidant conjugation with other bioactive molecules/ drug used in oxidative stress induced diseases might be helpful to increase the potencies and diversity of drug candidates/ molecules [116].

### Hybrids based on 1, 2-dithiolone Moiety

Alpha-lipoic acid (ALA), also known as 1, 2-dithiolane-3-pentanoic acid, is a natural antioxidant that scavenges reactive oxygen species (ROS) and regenerates or recycles endogenous antioxidants, and exists as R- and S-enantiomeric forms. However, only R-LA is conjugated to conserved lysine residues in an amide linkage of the mitochondrial multi-enzyme complexes that catalyze the oxidative decarboxylation of α-keto acids (e.g. pyruvate dehydrogenase, 2-oxo-glutarate dehydrogenase, and transketolase) and glycine cleavage, thus plays a critical role in mitochondrial energy metabolism. ALA **1** is readily taken up and reduced in cells and tissues to dihydrolipoic acid **2** (DHLA), and exert oxidative protection in both intracellular and extracellular environments (Fig. **1**). Furthermore, they have also been involved in regeneration of other antioxidants (vitamin C and vitamin E) via redox coupling and increase intracellular glutathione levels [117-119]. Thiol functionality of glutathione has been proposed for major contributor to oxidative defense in brain, but glutathione cannot be directly administered whereas, α-LA can be administered directly. *In vitro*, animal, and preliminary human studies indicate that alpha-lipoate may be effective in numerous neurodegenerative disorders [120].

#### *(a) Hybrids with 1, 2-dithiolone Moiety as Neuroprotective Agent*

In an effort to design potential neuroprotective agents with 1, 2-dithiolone scaffold, Koufaki M. and co-workers

conjugated ALA **1** with catechol **3** and were screened on glutamate-challenged hippocampus HT22 cells [121]. Interestingly, neuroprotective potential were increased on bioisosteric replacement of the amide group with heteroaromatic rings such as triazole, 1, 2, 4-oxadiazole, 1, 3, 4-oxadiazole, tetrazole or thiazole **4a-e** in comparison to parent ALA (Fig. **2**). Similarly, bioisosteric replacement of amide functionality with heteroaromatic ring in LA-chroman conjugates **5** were done to observe the influence in oxidative stress-induced cell death of glutamate-challenged HT22 hippocampus neurons by Koufaki M. and co-workers [122]. The results showed that in case of **4d** ($EC_{50}$ 2.99±0.14 μM) and **5**, the effect of housing a hetero aromatic ring and free phenolic moiety in one molecule gives synergistic effect rather than additive (Fig. **2**).

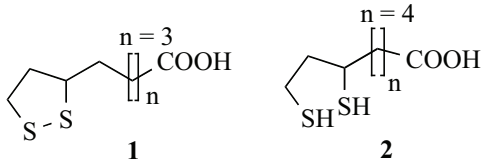



**Fig.** (**1**)**.** Structures of ALA (**1**) and dihydrolipoic acid (**2**).

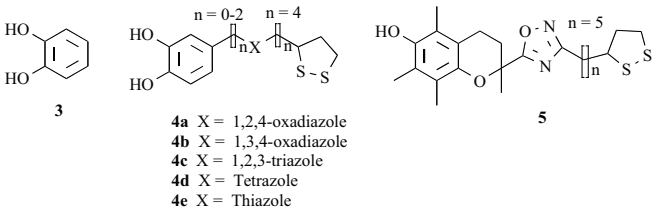



**Fig.** (**2**)**.** Chemical structures of ALA and catechol (**3**) hybrids (**4a-4e and 5**).

Cholinesterase enzyme (Acetylcholinesterase, AChE and Butyrylcholinesterase, BuChE), are key regulators of acetylcholine level in synaptic region, and have significant contribution in many neurodegenerative disorder especially in Alzheimer's disease (ADs). Recognizing the importance of polyphenolic moiety in many biologically active natural/ synthetic products, and their well establish neuroprotective ability, Woo, Y.J. *et al*. have crafted LA-polyphenolic hybrids and concluded that cinnamate based polyphenolic compounds were potential cholinesterase inhibitor, which might be due to the presence of α,β-unsaturated carbonyl moiety [123]. Acetylated caffeic acid conjugate **6** was ~800 fold selective inhibitor of BuChE ($IC_{50}$ = 0.5±0.2 μM and $K_i$ = 1.52±0.18 μM) over AChE (Fig. **3**). In another report, Decker, M. *et al.* designed [2, 1-b] quinazolinimines **7** and α-LA **1** hybrids connected through varying length of methylene spacer (n = 2-6) [124] (Fig. **4**) and it was observed that spacer chain length is proportional to selectivity towards BuChE. The hybrid bearing octamethylene spacer **8** exhibit ~10 fold and ~1000 fold more potent AChE and BChE inhibitory activity in contrast to parent quinazolinimines, respectively.

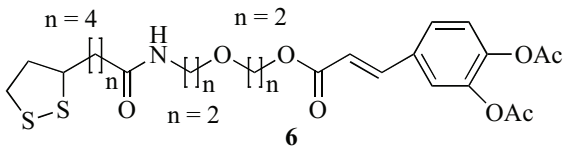



**Fig.** (**3**)**.** Structure of ALA - acetylated caffeic acid hybrid (**6**).

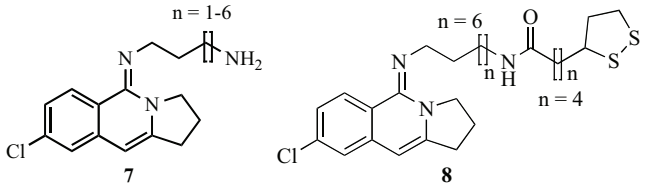



**Fig.** (**4**)**.** Chemical structures of quinazolinimines (**7**) and ALA - quinazolinimines hybrid (**8**).

### *(b) Hybrids with 1, 2-dithiolone Moiety as Cardioprotective Agent*

Coronary artery occlusion results from the deposition of fatty materials or damage to vasculature endothelial lining, leading to myocardium infraction. In general, treatment of acute myocardial ischemia involves the use of either thrombolytic agents or percutaneous transluminal coronary balloon angioplasty (PTCA), which effectively restores blood flow to the myocardium and reduce overall mortality. However, these therapies do not protect the heart from the damage caused by ROS, produced upon the readmission of oxygenated blood into the ischemic myocardium (reperfusion). Furthermore, it was found that oxygen free radicals react with the phospholipid components of the myocardium and affecting the selective permeability of cell membranes, thus resulting in the development of life threatening ventricular arrhythmias and/or fibrillation. On the other hand, experimental findings support the hypothesis that lipid peroxidation inhibitors such as vitamin E protect the myocardium from I/R injury [125]. In order to explore the role of lipoic acid on cardiovascular damage, Koufaki, M. *et al.* conjugate ALA **1** with trolox 9 **(**6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), an analogue of vitamin E **10**, and evaluated for the lipid peroxidation inhibition and antioxidant capacities (measured as contents of malondialdehyde) [126] (Fig. **5**). Lipoic acid substitution with amide functionality at C-2 and C-5 position by methylene spacer **11a-b** and **12-15**, were assessed as essential feature in order to total suppression of reperfusion arrhythmias while, compounds with direct attachment on C-4 position **13** and trolox/lipoic acid mixture reduced the arrhythmia score by 63.5% and 53%, respectively. Furthermore, antioxidant and inhibitory lipid peroxidation capacity of hybrid compounds were comparable to trolox/ lipoic acid mixture.

### *(c) Hybrids with 1, 2-dithiolone Moiety as Radioprotective Agent*

Melatonin (*N*-acetyl-5-methoxytryptamin) **16**, a pineal gland hormone which regulates circadian rhythms, critically controls the sleep-wake cycle [127] and also has the capacity

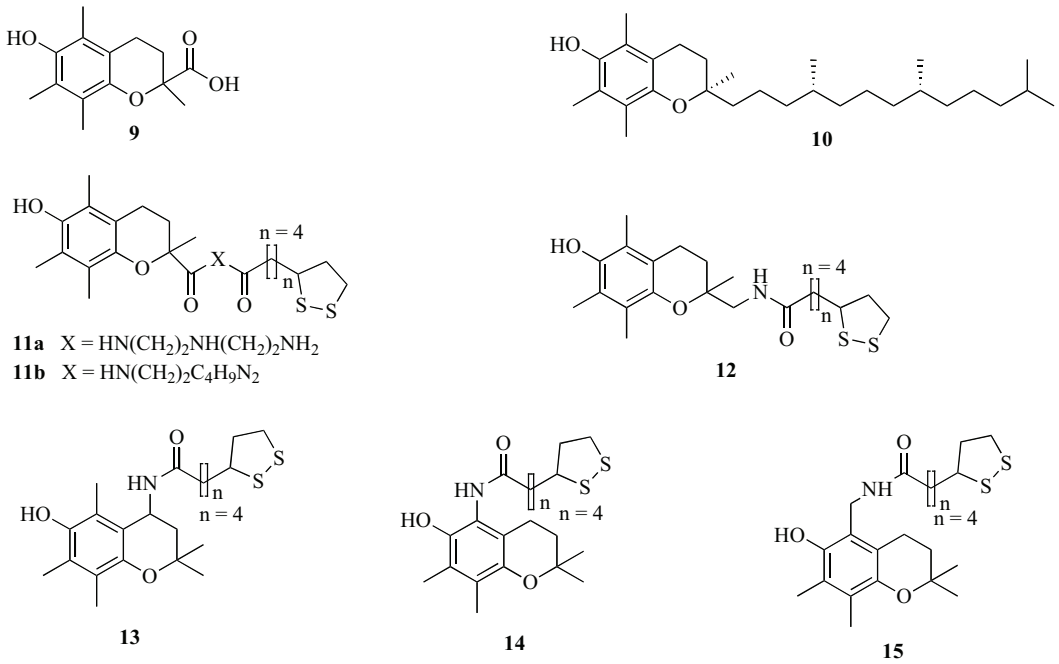
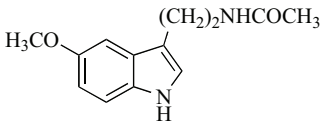
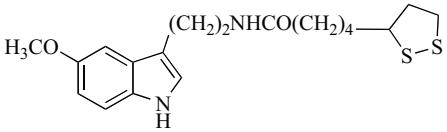



**Fig. (5).** Structures of trolox (**9**), vitamin E-trolox hybrid (**10**) and trolox-lipoic acid hybrids (**11a-11b, 12, 13, 14** and **15**).

to protect nuclear and mitochondrial DNA from oxidative damage [128]. Furthermore, significant application in cancer, immune disorders, cardiovascular diseases, depression, seasonal affective disorder (SAD), circadian rhythm sleep disorders, and sexual dysfunction make it interesting biomolecule. The radioprotective activity of melatonin was increased by conjugating 1, 2-dithiolane moiety (ALA) with melatonin to form new hybrid molecule melatoninolipoamide **17** [129] (Fig. **6**). Pulse radiolysis induced one-electron oxidation and reduction of **17** showed that the melatonin moiety in the conjugate is more susceptible for the oxidation whereas, the lipoic acid moiety for the reduction.




**16**




**17**

**Fig. (6).** Structures of melatonin (**16**), melatoninolipoamide (**17**).

### *(d) Hybrids with 1, 2-dithiolone Moiety as Anti-Inflammatory Agent*

In recent years, role of antioxidants are well established in combating inflammatory disorders. There are many hetero aromatic ring containing compounds from natural and synthetic origin, which have been shown potential inflammation inhibitory activity [130-135]. In view of fact that, hetero aromatic ring conjugates with lipoic acid via amide linkage might give potential drug candidates, number of molecules like quinolinone-3-aminoamides **18** [136] and coumarin-3-aminoamides **19** [137] were conjugated with lipoic acid and tested for their inhibitory ability to lipoxygenase (LOX %, 0.1 mM) and Carrageenan rat paw edema (CPE %, 0.01 mmol/Kg body weight) (Fig. **7**). The results indicated that compounds with aromatic diamine (especially 1, 2-phenylene diamine) were more potent in comparison to aliphatic diamine while, vice versa in case of %LOX inhibition. Lipoxygenase inhibitory capacities of quinolinone-3-aminoamides-lipoic acid hybrid **20** were observed higher (100%) in comparison to corresponding parent amino amides while, vice versa in case of CPE inhibition. In case of CPE inhibition %, coumarin-3-aminoamides-lipoic acid hybrid **21** was observed more potential (73%) in comparison to corresponding parent amino amides while, vice versa in case of %LOX inhibition.

### Hybrids based on α,β-Unsaturated Carbonyl Moiety

α,β-unsaturated carbonyl moiety bearing compounds comprise a wide group of naturally occurring compounds like coumarin (benz-α-pirone) **22**, flavones **23**, chalcone **24**, and curcumin **25** (Fig. **8**). A remarkable array of biological activities and low toxicity of these compounds give them a special place in nature. The mode of biological action of this class of compounds has long been believed to be due to their interaction with thiol groups of enzymes via Michael addition at ketovinyl double bond [138]. The SAR studies showed that electron withdrawing (EW) group is favorable because it increases the electrophilicity of the C-β bond and thus facilitate the nucleophilic attack of the cellular thiol groups and opposite is true for the electron donating (ED)

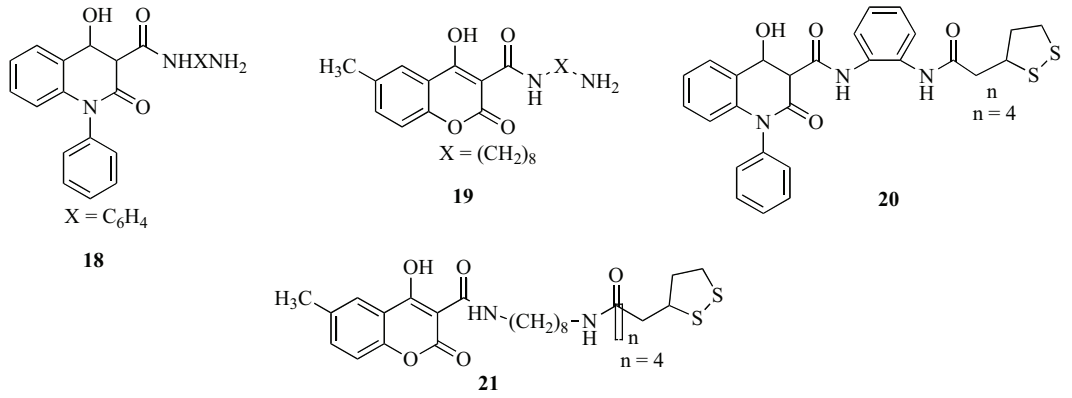



**Fig. (7).** Structures of quinolinone-3-aminoamides (**18**), coumarin-3-aminoamides (**19**), quinolinone-3-aminoamides-lipoic acid hybrid (**20**) and coumarin-3-aminoamides-lipoic acid hybrid (**21**).

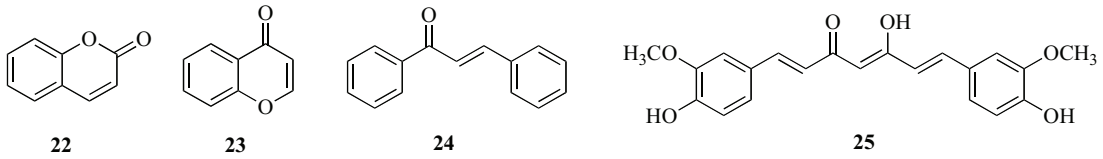



**Fig. (8).** Structures of coumarin (**22**), flavone (**23**), chalcone (**24**), and curcumin (**25**).

groups. Coumarin (1, 2-benzopirone) is one of the important class of α,β-unsaturated carbonyl group and several natural and synthetic coumarins have been found to exhibit variety of pharmacological activities like anti-HIV, anticoagulant, antibacterial, antioxidant, anti-inflammatory, and fluorescent labeling [139-146].

### *(a) Hybrids with α,β-Unsaturated Carbonyl Moiety as Anticancer Agent*

Among the diverse biological activities of coumarin, the most intriguing bioactivity is the effect against breast cancer [147-150]. Tamoxifen **26** is a selective estrogen receptor modulator (SERM) and used thoroughly in breast cancer for more than three decades [151]. The side effects and drug resistance are major concerns of tamoxifen [152-154]. Recently, 667 COUMATE **27** and neo-tanshinlactone **28** are in phase I clinical trials with increase in 10-fold potency and 20-fold selectivity in comparison to Tamoxifen **27** [155-157]. In order to explore role of α,β-carbonyl moiety in cancer, Sashidhara, K.V. *et al.* synthesized a series of coumarin-chalcone hybrids and evaluated their cytotoxic potential against KB (Oral squamous cell carcinoma), C33A (cervical carcinoma), MCF-7 (Breast adenocarcinoma), A549 (lung) and normal mouse embryo fibroblast [158]. Overview of the results proposed that a chalcone-coumarin hybrid with electron withdrawing group **29** was more potent in comparison to parent coumarin (Fig. **9**).

The structure activity relationship (SAR) studies of various anticancer agents auspicate that methoxy group is one of important functional group responsible for cytotoxic potency and it was backed by significant role of resveratrol (3,5,4'-trihydroxy-trans-stilbene) **30**, and 3,4,5,4'-tetramethoxystilbene (DMU-212, **31**) in various types of cancer. In this context, Belluti, F. *et al.* have inserted substituted *trans*-vinylbenzene moiety on coumarin backbone and screened for antiproliferative activity against lung carcinoma H460, squamous cell carcinoma A431 and melanoma JR8 [159]. The 3,5-dimethoxy- **32a** and 3,5-dimethylstilbin **32b** substitution on 7-methoxycoumarin scaffolds at C-4 position was found to exert optimum inhibitory action against H460 (0.45±0.09 μM), A431 (3.44 μM) and JR8 (3.2, 3.5 μM) (Fig. **10**).

Estrogens (oestrogen) are primary female sex hormone, which stimulate the proliferation of normal and malignant cells *via* induction of nucleic acid synthesis and activation of growth regulatory genes namely ERα and ERβ. Steroidal framework of estradiol (E2) provide site of attachment of variety of substituents such as cytotoxic moieties, radioisotopes, dietary antioxidants, affinity and photo affinity-labeling groups, of which several E2 conjugates have advanced as synthetic ligands for targeting the ER [160-162]. In this direction, flavones and coumarins have been conjugated with ER to obtain hybrid molecules **33**, **34** and it leads to enhancement of potency and selectivity towards ERα in comparison to estradiol [163, 164] (Fig. **11**).

Aqueous solubility (log W) is an important consideration in formulation and development phase of drug candidates, as most of drugs are orally administered and is likely to hamper the bioavailability [165]. Various approaches have been employed to improve aqueous solubility such as salt formation, amino acid conjugate and prodrug approach. Paclitaxel, potential mitotic inhibitors, have been used in chemotherapy of patient suffering from lung, ovarian, breast cancer, and advanced forms of Kaposi's sarcoma [166]. The complex structural feature of paclitaxel renders its hydrophobicity, allergic reaction and precipitation on aqueous dilution. Therefore, it is administered intravenously

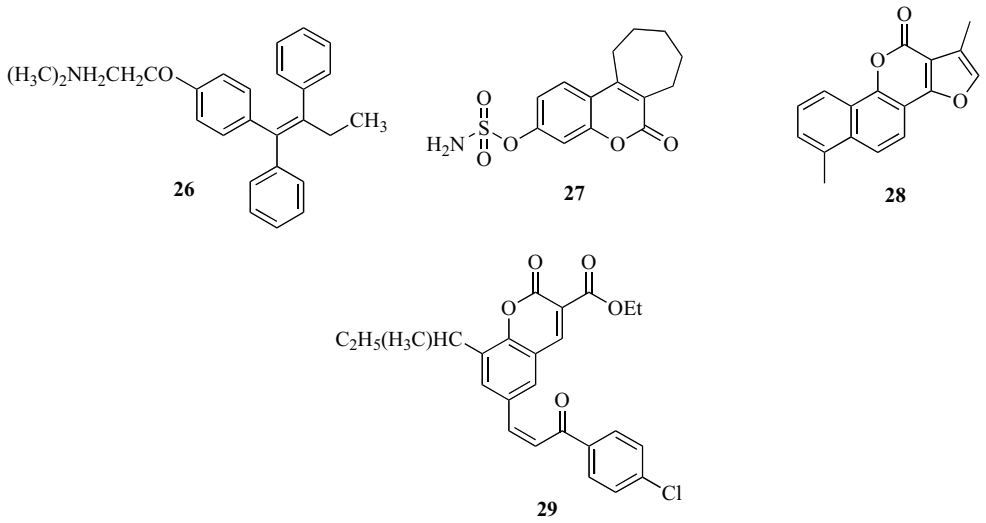


**Fig. (9).** Structures of tamoxifen (**26**), coumate (**27**), neo-tanshinlactone (**28**) and chalcone-coumarin hybrid (**29**).

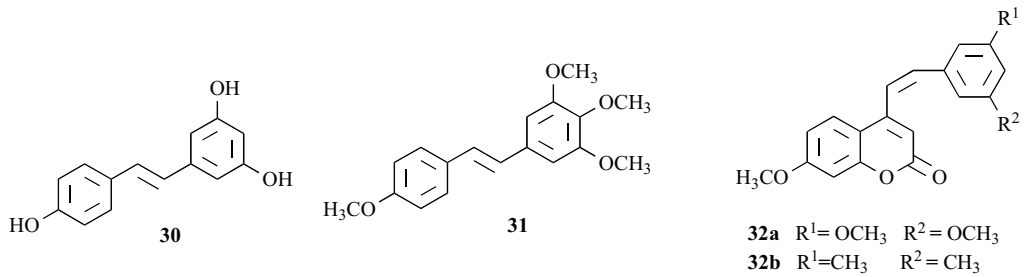


**Fig. (10).** Structures of resveratrol (**30**), 3, 4, 5, 4'-tetramethoxystilbene (DMU-212, **31**) and coumarin analogues with 3, 5-dimethoxy- **32a** and 3, 5-dimethylstilbin **32b** substitution on 7-methoxycoumarin scaffolds.

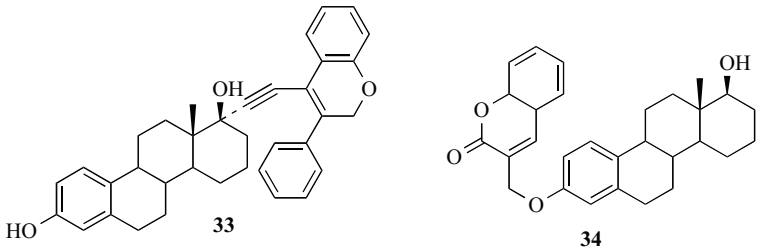
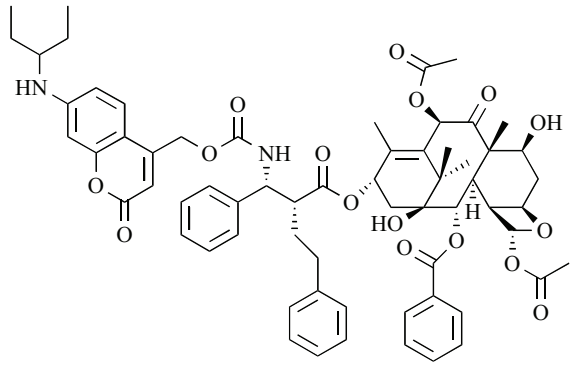


**Fig. (11).** Structures of estradiol- flavones and coumarins hybrids (**33, 34**).

with non-aqueous vehicle containing detergent like Cremophor EL [167]. Paclitaxel also have serious side effects such as unusual bruising or bleeding, pain/redness/ swelling at the injection site, fever, chills, cough, sore throat, difficulty in swallowing, dizziness, shortness of breath, severe exhaustion, skin rash, facial flushing, female infertility by ovarian damage [168], which enforce for further structural modification or develop new delivery systems to reduce its toxicity. In this direction, Noguchi, M. *et al.* prepared photoliable 7-N,N-diethylamino-4-hydroxymethyl coumarin (DECM) hybrid of paclitaxel **35** to improve water solubility along with enhancement of target specificity [169] (Fig. **12**).

Fluorescent labeling involves covalent attachment of drug with antibodies, protein, amino acid, and peptide which are used as specific probes for detection of a particular target via fluorescence microscopy, flow cytometer or some other fluorescence reading instrument [170]. Commonly used fluorescent dyes are fluorescein, rhodamine, alexa fluors, dylight fluors, ATTO dyes (labeling of DNA, RNA and protein), BODIPY dyes (4,4-difluoro-4-bora-3a,4a-diaza-s-indacene), and 6-FAM phosphoramidite. The presence of chromophore, α,β-unsaturated carbonyl moiety in coumarin and structure resemblance with fluorescent dyes imparts significant fluorescent characteristic to them. There are several examples such as 7-diethylaminocoumarin succinimidyl ester (DEAC-SE) **36**, 7-amino-4-methyl coumarin-3-acetic acid **37** (AMCA) [171], sulfosuccinimidyl-2(7-azido-4-methycoumarin-3-acetamido)-ethyl-1,3′-dithiopropionate (SAED) **38** [172], 7-hydroxycoumarinyl-3-glyoxal **39** (HOCGO), 7-(dimethylamino)coumarinyl-3-glyoxal (DMACGO) **40** [173],

and 4-bromometyl-7-methoxy-coumarin **41** (BrMMC) [174] which shows wild applicability of coumarin and similar moieties in the biological fields. Recently in 2008, Wells, G. *et al.* observed supportive role of 7-diethylaminocoumarin in nuclear penetration on conjugation with sequence-selective DNA-targeting agents pyrrolo [2, 1-c] [1, 4] benzodiazepine (PBD) **42** via varying length of the spacer [175] (Fig. **13**).



**35**

**Fig. (12).** Structure of 7-N, N-diethylamino-4-hydroxymethyl coumarin hybrid of paclitaxel (**35**).

### *(b) Hybrids with α,β-Unsaturated Carbonyl Moiety as Cardioprotective Agent*

Limited success of antiarrhythmic drugs in suppression of reperfusion arrhythmia and sudden cardiac death is due to the associated increased risk of proarrhythmia and lack of selectivity towards ion channels, which enforce to find out new drug candidates. The vitamin E has been shown protective role in myocardium from ischemia/ reperfusion (I/R) injury, but hydrophobicity impedes it to gain access to the intracellular compartment. In this context, Koufaki M. *et al.* have conjugated α-tocopherol (Vitamin E, **10**) with class I antiarrhythmics namely procainamide **43** and lidocaine **44** in order to obtain bifunctional antiarrhythmic antioxidants [176]. The phytyl chain of vitamin E was replaced with different alkyl chain of one, six or twelve carbon atoms. Among procainamide (**45a-c**, **46a-c**) only C-2 methyl substituted analogue **45a** was able to decrease in premature beats (5±2 and 7±3.5 at 100 and 30 mM, respectively), in comparison to procainamide (5±3 and 6±2.5 at 100 and 30 mM, respectively). Similarly, only **45a** was able to increase in QRS intervals which were comparable to that of procainamide (**45a**: 60±6, 54±8 ms at 100 and 30 mM, respectively; procainamide: 66±10, 58±5 ms at 100 and 30 mM, respectively). While both lidocaine analogues (**47a-b**) showed potential to decrease premature beats (**47a**: 7±2 and 8±2.1 at 100 and 30 mM, respectively; **47b**: 6±2.5 and 6±3 at 100 and 30 mM, respectively) which was slightly less than lidocaine (4±3 and 5±3 at 100 and 30 mM, respectively). The hybrids **47a** and **47b** showed 100% inhibition of lipid peroxidation at 10 μM. At the same time, QRS intervals were comparable to parent drug lidocaine. Substitution at C-5 position with methylene spacer between amino amide groups is key feature of these hybrids against reperfusion arrhythmias (Fig. **14**).

### *(c) Hybrids with α,β-Unsaturated Carbonyl Moiety as Antimicrobial Agent*

Hepatitis C virus (HCV), the major etiological agent of the non-A non-B hepatitis, was identified at the molecular level at the end of the 1980s [177]. Presently, it is estimated that HCV infects more than 170 million people worldwide and thus represents a viral pandemic that is about five times more widespread than infection by the human immunodeficiency virus (HIV) [178]. RNA-dependent RNA polymerase (RdRP) is essential for viral replication and no functional equivalent in uninfected mammalian cells make validated drug target to block HCV replication with negligible associated toxicity [179]. Currently three classes of inhibitors are used namely; nucleoside analogues, non-nucleoside inhibitors (NNIs) and pyrophosphate inhibitors. Non-nucleoside inhibitors include structurally heterogeneous compounds having benzimidazole **48, 49** [180, 181] and benzothiadiazine derivative **50** [182] (Fig. **15**). In order to

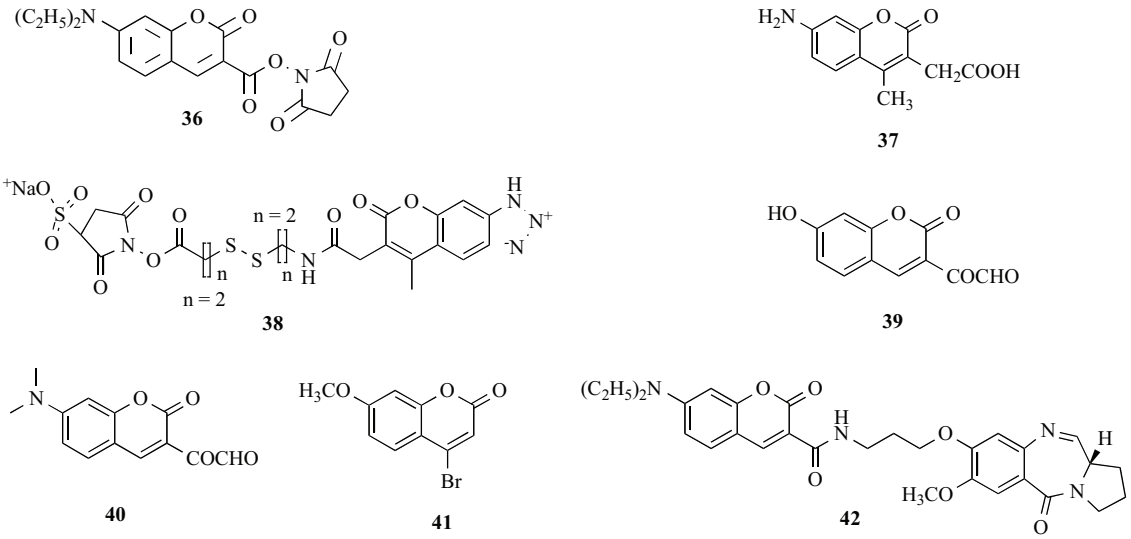


**Fig. (13).** Structures of coumarin based fluorescent compounds (**36-42**).

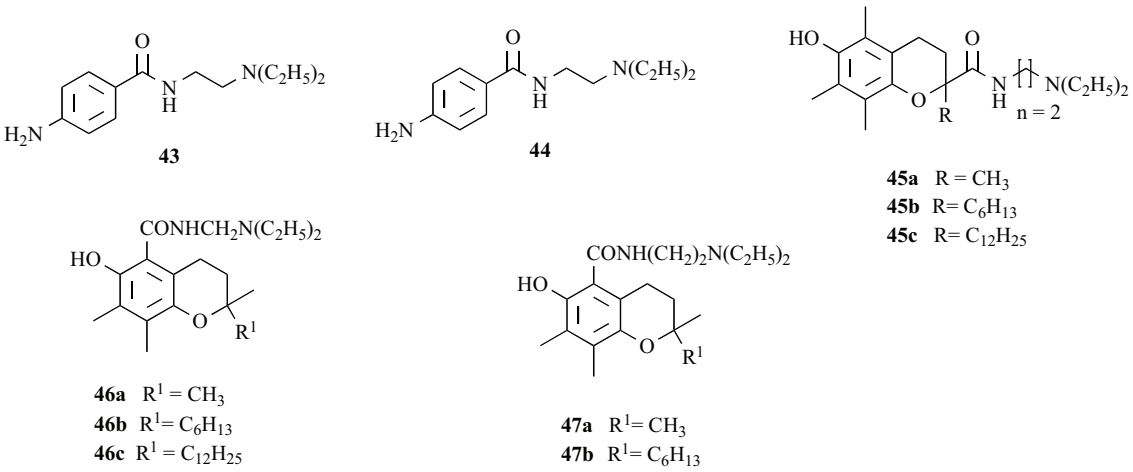



**Fig. (14).** Structures of procainamide (**43**), lidocaine (**44**) and their antioxidant hybrids (**45a-45c, 46a-46c and 47a-47b**).

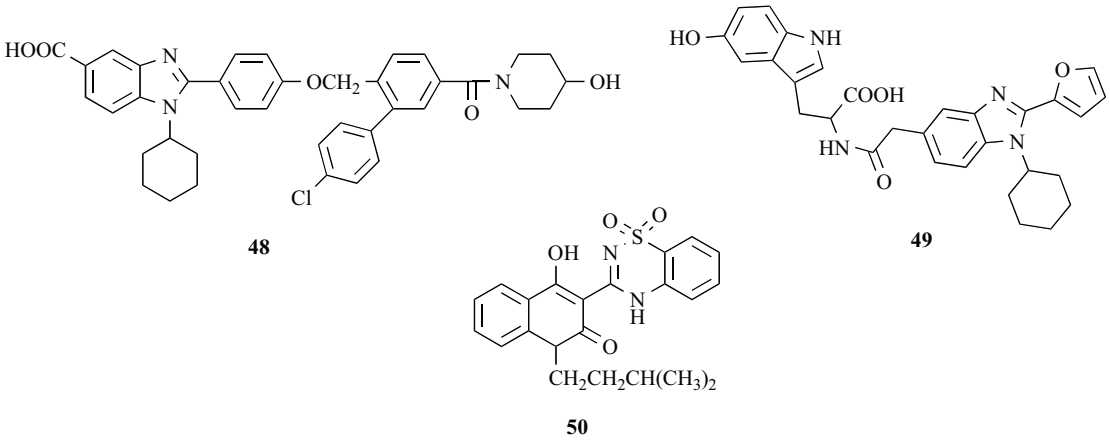
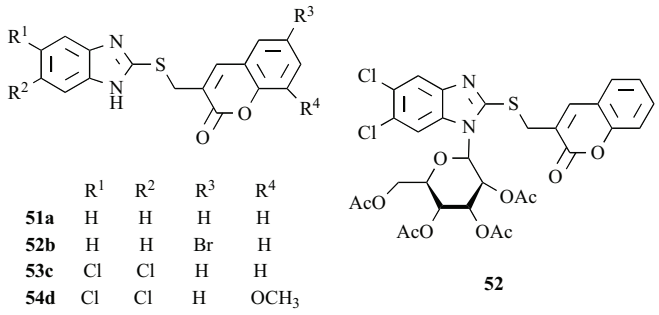



**Fig. (15).** Non-nucleoside inhibitors having benzimidazole **48, 49** and benzothiadiazine moieties **50**.

optimize structure of benzimidazole based RNA-dependent RNA polymerase (RdRP) inhibitors against hepatitis C virus, Hwua, J.R. *et al.* synthesized a series of benzimidazole-coumarin conjugates by one-flask methods and evaluated against hepatitis C virus [183]. The SAR analysis concluded that (a) introduction of a Br group on the coumarin ring (e.g., **51b** *versus* **51a**) enhanced HCV inhibition by 6.7-fold and also the selectivity by 2.9-fold; (b) introduction of an -OMe group on the coumarin ring (e.g., **51d** *versus* **51c**) further improved the antiviral activity; (c) enhancement of the selectivity resulting from substitution in the coumarin nucleus (cf. **51d**, **51a**, and **51c**) followed the order -OMe <H <Br; (d) incorporation of β-D-glucose peracetate moiety into the benzimidazole-coumarin conjugate (e.g., **51c** *versus* **52**) resulted in a 4.8-fold increase in anti-HCV activity (Fig. **16**).

### *(d) Hybrids with α,β-Unsaturated Carbonyl Moiety as Radical Quenching Agent*

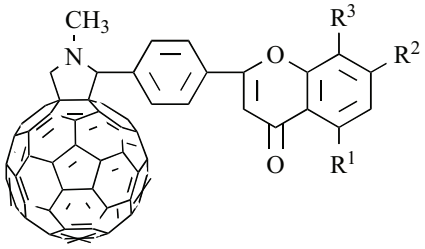
Fullerene also known as buckminsterfullerene is composed of $C_{60}$ carbon exists in the form of a hollow sphere, ellipsoids, or tube. The ability of $C_{60}$ and its derivatives to scavenge a large number of radicals per molecule [184, 185] makes them potential drug candidates in number of oxidative stress induced disorder, including cardiovascular [186, 187] and neurodegenerative diseases [188, 189]. Recently, researcher have succeeded in conjugation of fullerenes with number of radical scavenging agents such as flavonoids **53**, **54** [190], and quercetin **55, 56** [191]. In further advancement, Enes, R.F. *et al.* integrated 3, 5-di-tert-butyl-4-hydroxyphenyl groups (BHT) with $C_{60}$-flavonoid conjugate **57-59** [192]. These hybrids **57-59** showed synergistic free radical scavenging ability (Fig. **17**).



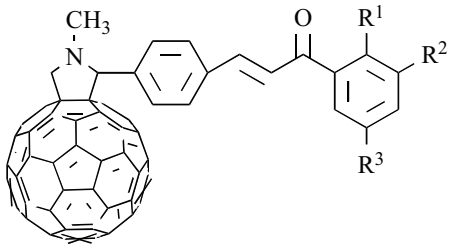
| | $R^1$ | $R^2$ | $R^3$ | $R^4$ |
|---|---|---|---|---|
| **51a** | H | H | H | H |
| **52b** | H | H | Br | H |
| **53c** | Cl | Cl | H | H |
| **54d** | Cl | Cl | H | $OCH_3$ |



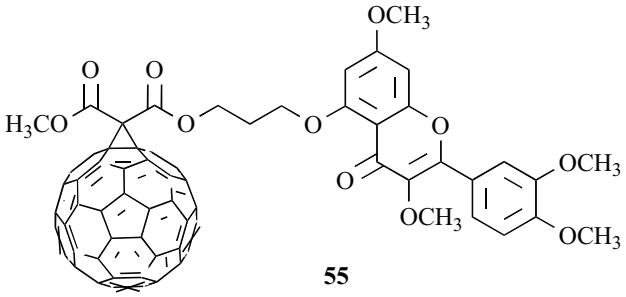
**Fig. (16).** Structures of benzimidazole-coumarin conjugates (**51a-51d** and **52**).



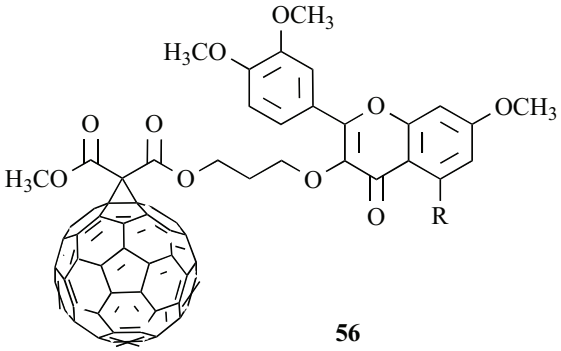
| | $R^1$ | $R^2$ | $R^3$ |
|---|---|---|---|
| **53a** | H | H | H |
| **53b** | Bn | H | H |
| **53c** | H | OBn | OBn |



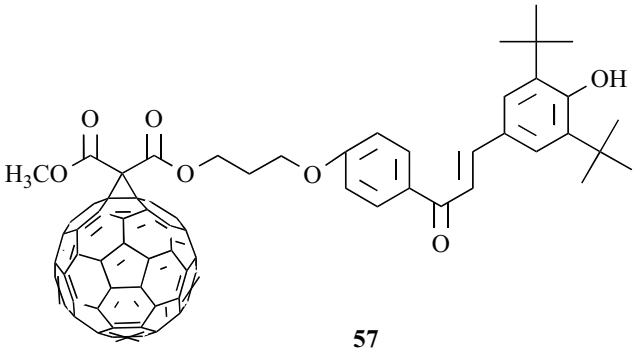
| | $R^1$ | $R^2$ | $R^3$ |
|---|---|---|---|
| **54a** | OH | H | H |
| **54b** | OBn | OBn | OBn |



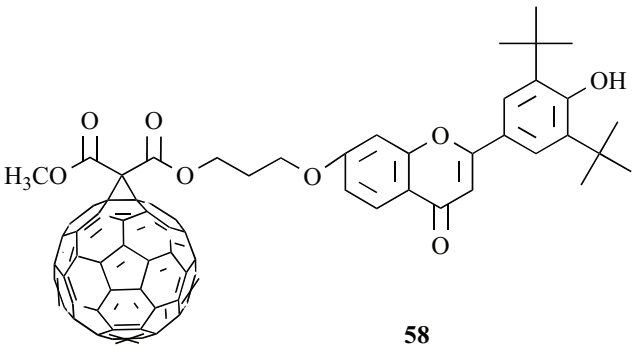
**55**



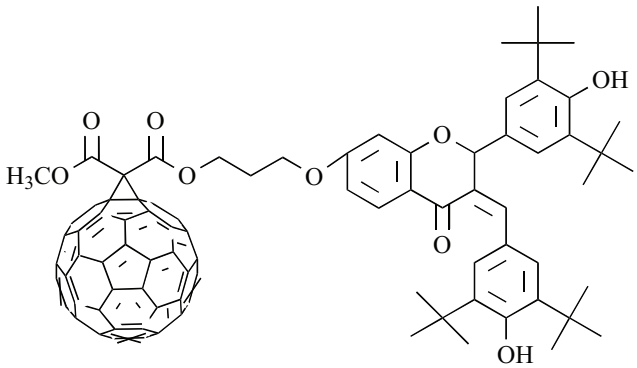
**56**



**57**



**58**



**59**

**Fig. (17).** Structures of fullerene-flavonoid conjugates (**53a-53c, 54a-54b, 55-59**).

## Hybrids based on Cinnamate Moiety

### *(a) Hybrids with Cinnamate Moiety as Neuroprotective Agent*

Presence of olefinic bond with carbonyl functionality in cinnamate based compounds such as cinnamic acid, ferulic acid, and synapic acid, impart wide range of biological applications. Tacrine is one of potential drug candidates used in neurodegenerative disorder like ADs. Further, structural optimization of tacrine **60** was done by conjugating with ferulic acid **61** via varying length of methylene spacer by Fang, L. *et al*. [193]. It was postulated that conjugation with octamethylene spacer **62** exhibited a reversible and non-competitive inhibition to acetylcholinesterase (AChE) whereas, reversible and competitive inhibitory activity was observed to butyrylcholinesterase (BChE) with $IC_{50}$ (nM) 9.6±2.1 and 12.7±2.6 respectively.

(Fig. **18**), Calpain is $Ca^{+2}$-activated cysteine protease typically associated with cellular necrosis. One of the major causes of neurodegenerative disorder is ROS mediated activation of calpain, which is involved in various neurological disorders such as stroke, Parkinson's disease and Alzheimer [194-198]. Lee, K.S. *et al.* has synthesized chromone carboxamide **63** and has shown potential calpain inhibitory activity [199]. To elucidate structural requirements for μ-calpain inhibition, Yoo, Y.J. *et al*. synthesized acyclic variants of chromone ring in **63** by conjugating cinnamoyl functionality on α-position [200]. The potential role of acyclic variants of chromone carboxamide **64** against neurodegeneration and increasing role of cinnamate based compounds as neuroprotective agents, a series of cinnamoyl ketoamides were synthesized with varying substitution at α-position of ketoamides group. Calpain inhibitory activity was increased in order of propyl ≡ isopropyl > ethyl ≡ butyl as alkyl substituent at α-position of ketoamides group and hydroxyl group on aromatic ring system. Compound **65** showed most potent inhibitory activity ($IC_{50}$ = 0.13 μM) against m-calpain and its potency was 4-fold higher than that of acyclic variant **64** ($IC_{50}$ = 0.52 mM) and 2-fold lower than that of parent compound **63** ($IC_{50}$ = 0.07 mM) (Fig. **19**).

### *(b) Hybrids with Cinnamate moiety as Cardioprotective Agent*

Angiotensin converting enzyme (ACE) inhibitors are most widely used antihypertensive agents. Selective inhibition of Angiotensin-II (AT-II) by Sartans with absence of side effects (coughing) makes it superior over other ACE inhibitor. Garcia, G. *et al.* have synthesized polyphenolic compounds conjugate with losartan **66** [201]. Integration of phenolic functionalities on losartan improves 4-8 fold antioxidant capacity than losartan. Hybrids were less efficient to oppose binding of radiolabelled AT-II to receptor than losartan except **67a** (41%) and **67b** (40%) which exhibited equivalent potential as losartan (47%). The 3-(3,4-dihydroxyphenyl) propionic acid was potential candidate among all integrated polyphenolic compounds and its structural resemblance with cinnamate based compounds revealed out that structural optimization of losartan with cinnamate based compounds might improve its basic properties as an AT-II receptor blocker (Fig. **20**).

### *(c) Hybrids with Cinnamate Moiety as Anticancer/Anti-Inflammatory Agent*

Nomura, E. *et al.* have synthesized gallic acid-ferulic acid ester and investigated their inhibitory effects on 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced Epstein-Barr virus (EBV) activation and superoxide ($O_2^-$) generation

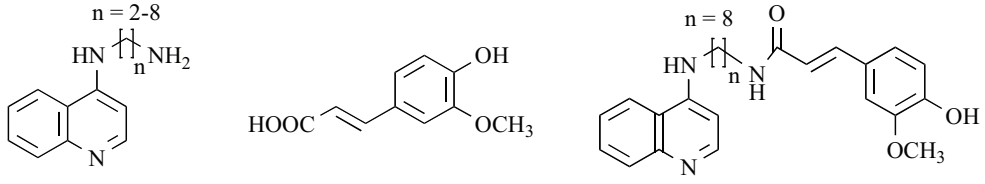



**Fig. (18).** Structures of tacrine (**60**), ferulic acid (**61**) and tacrine-ferulic acid hybrid (**62**).

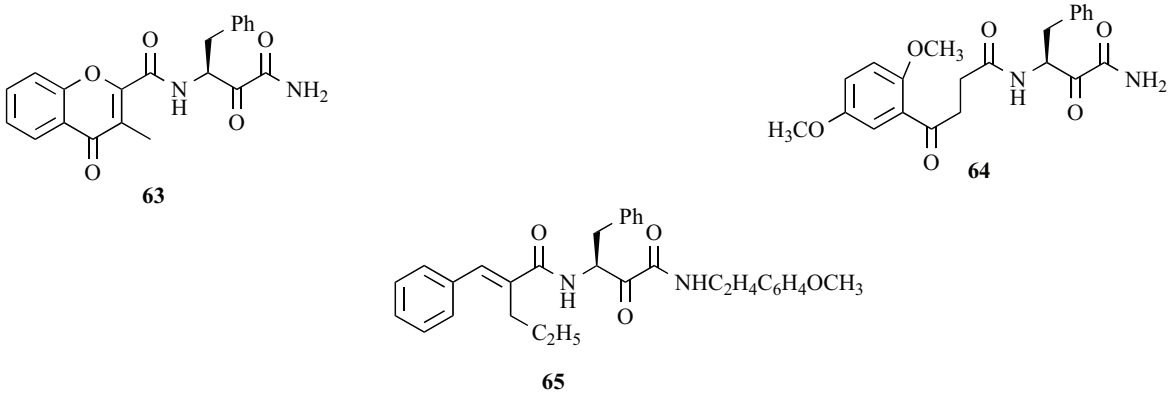
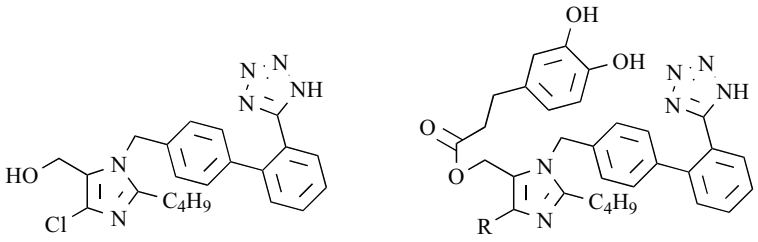
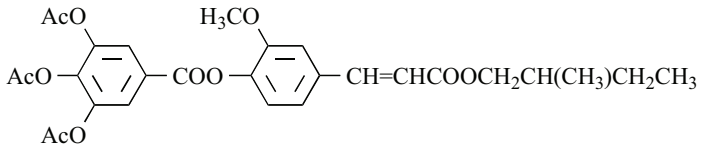



**Fig. (19).** Structures of various chromone-carboxamide hybrids (**63-65**).

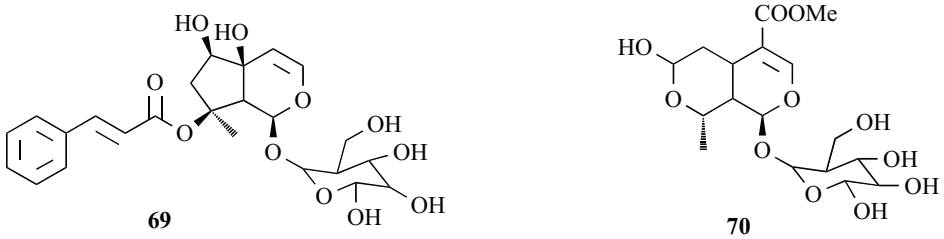


**66** **67a R = Cl** **67b R = H**

**Fig. (20).** Structures of losartan (**66**) and 3-(3,4-Dihydroxyphenyl) propionic acid-losartan hybrids (**67a-67b**).



**68**



**69** **70**

**Fig. (21).** Structures of gallic acid-ferulic acid ester (**68**), harpagoside (**69**) and morroniside (**70**).

and **68** was observed promising chemopreventive agent [202]. Harpagoside **69**, naturally occurring phenylpropanoids-conjugated iridoide glycoside have potential anti-inflammatory agent and structural characterization revealed that cinnamate moiety might be responsible for the activity. Takeda, Y. *et al.* conjugated cinnamate moiety on morroniside **70** (iridoide glycoside) and showed potential TNF-α induced E-selectin expression inhibition ($IC_{50}$ = 49.3μM) over harpagoside ($IC_{50}$ = 88.2μM) [203] (Fig. **21**).

### Miscellaneous Hybrids

Various natural and synthetic polymers such as gelatin, albumin, cellulose, poly (2-hydroxyethyl methacrylate), chitosan, and polyethylene glycol (PEG) have wide range of pharmaceutical and biomedical applications, due to their biocompatibility, biodegradation, non toxicity, and non immunogenicity. Some of polymeric products, especially medical equipment and food packaging are sterilized by radiation, which results in potential risk of degradation (chain scission and/or cross linking, resulting in discoloration, cracking of the surface, stiffening, and loss of mechanical properties) [204]. Furthermore, protein composite polymers (i.e. gelatin, pectin) and poly (2-hydroxyethyl methacrylate (important constituents of contact lens) are liable to oxidative damage. Various strategies had been employed to protect these biomolecules from oxidative damage [205-207]. Currently, grafting of antioxidant moiety on polymeric side chain has been used to overcome oxidative damage. Researcher have synthesized various polymeric-antioxidant combination like PEG-lipoic acid conjugates 7**1** [208], gallic acid-gelatin, catechin-gelatin [209], poly (2-hydroxyethyl methacrylate)-quercetin [210], catechin-alginate **72**, catechin-inulin **73** [211] and chitosan-gallic acid **74** [212] via grafting and other technique. These hybrids were synthesized with an aim to improve physical, chemical and biological properties of polymers. The improvement in radical scavenging capacity of antioxidant polymer in comparison to parent polymer indicated that covalent attachment of antioxidant moieties with polymer might improve their oxidative resistance and introduce new features for specific applications in pharmaceutical, cosmetic and food industry (Fig. **22**).

## STRUCTURE-ACTIVITY RELATIONSHIP (SAR) ANALYSIS OF ANTIOXIDANT HYBRID COMPOUNDS

From above mentioned detailed investigation, it was concluded that many antioxidant hybrid compounds have been synthesized and observed significant and to moderate range of biological action in comparisons to parent compounds. Some key structure activity relationship features are as under:-

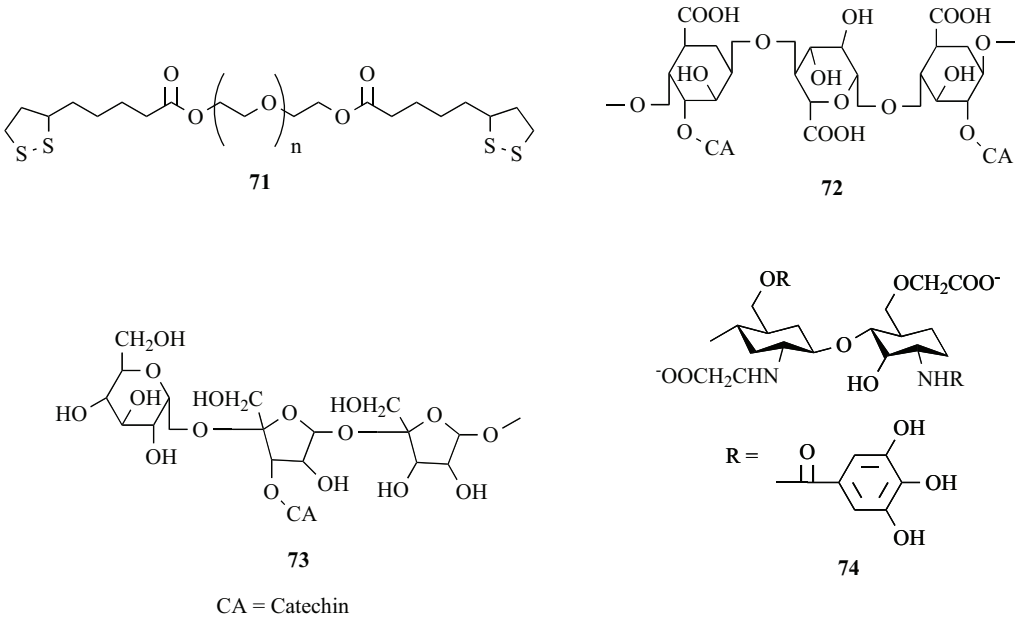



**Fig. (22).** Structures of PEG-lipoic acid conjugate (**71**), catechin-alginate (**72**) catechin-inulin (**73**) and chitosan-gallic acid (**74**).

1) The presence of 1, 2-dithiolone functionality is important for neuroprotective action.
2) Numbers of free phenolic functionality are proportional to their radical quenching capacity.
3) Insertion of α,β-unsaturated carbonyl moiety in compounds impart wide range of biological application. The presence of electron withdrawing groups impart electrophilicity of C-β carbon which results in enhance Michel interaction with cellular/enzymatic thiol groups.
4) Potential role of cinnamate based compounds in neuroprotection, antimicrobial and inflammation again showing the role of double bonds and carbonyl functionality in bioactivity.

## CONCLUSIONS

Natural products play an important role in the development of drugs, especially for the treatment of infections and cancer, as well as immunosuppressive compounds. However, the number of bioactive natural products is limited, whereas millions of hybrids by combinations of different natural products can be prepared. This new approach seems to be very promising in the development of leads for both medicinal and agrochemical applications, as the biological activity of several new hybrids exceeds that of the parent compounds. The advantage of this concept over a combinatorial chemistry approach is the high diversity and the inherent biological activity of the hybrids. The possibilities of generating hybrid systems of creating molecular diversity through either domain integration or covalent connection of two or more diver's entities are almost unlimited. Artificial natural product hybrids have not yet been used as drugs, as this idea is quite new, but several novel compounds of this type developed in the last few years show promising biological activity.

Recent success of antioxidant hybrid compounds as promising therapeutic intervention in oxidative stress induced diseases will continue to engage the attention of organic chemist. From recent research it can be inferred that hybrid compounds have shown significant to moderate level of activities in comparisons to parent compounds and there is need of further optimization of pharmacophore to make these compounds as drug like molecules.

## CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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