A Review of Coumarin Derivatives in Pharmacotherapy of Breast Cancer

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Abstract

The coumarin (benzopyran-2-one, or chromen-2-one) ring system, present in natural products (such as the anticoagulant warfarin) that display interesting pharmacological properties, has intrigued chemists and medicinal chemists for decades to explore the natural coumarins or synthetic analogs for their applicability as drugs. Many molecules based on the coumarin ring system have been synthesized utilizing innovative synthetic techniques. The diversity oriented synthetic routes have led to interesting derivatives including the furanocoumarins, pyranocoumarins, and coumarin sulfamates (COUMATES), which have been found to be useful in photochemotherapy, antitumor and anti-HIV therapy, and as stimulants for central nervous system, antibacterials, anti-inflammatory, anti-coagulants, and dyes. Of particular interest in breast cancer chemotherapy, some coumarins and their active metabolite 7-hydroxycoumarin analogs have shown sulfatase and aromatase inhibitory activities. Coumarin based selective estrogen receptor modulators (SERMs) and coumarin-estrogen conjugates have also been described as potential antibreast cancer agents. Since breast cancer is the second leading cause of death in American women behind lung cancer, there is a strong impetus to identify potential new drug treatments for breast cancer. Therefore, the objective of this review is to focus on important coumarin analogs with antibreast cancer activities, highlight their mechanisms of action and structure-activity relationships on selected receptors in breast tissues, and the different methods that have been applied in the construction of these pharmacologically important coumarin analogs.

Keywords

Coumarin; breast cancer; sulfatase inhibitor; aromatase inhibitor

1. INTRODUCTION

Coumarin (1,2-Benzopyrone or 2*H*-1-benzopyran-2-one, or phenylpropanoids, **1**) and its derivatives (coumarins) are widely distributed throughout nature and many exhibit useful and diverse biological activities [1, 2]. Coumarins occur as secondary metabolites in the seeds, roots and leaves of many plant species, notably in high concentration in the tonka

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bean and thus the name comes from a French word, *coumarou*, for the tonka bean. Their function is far from clear, although suggestions include plant growth regulations, fungistasis, bacteriostasis and, even, waste products [3]. Some naturally occurring coumarin derivatives include warfarin (**2**), umbelliferone (7-hydroxycoumarin, **3**), aesculetin (6,7-dihydroxycoumarin, **4**), herniarin (7-methoxycoumarin, **5**), psoralen (**6**) and imperatorin (**7**). Now the diversity of coumarin derivatives, both natural and synthetic, has grown and are thus divided into several subclasses. Most reviews classify coumarins according to whether particular compounds are simple coumarins (e.g. coumarin, **1** and limettin, **8**), linear furanocoumarins (e.g. imperatorin, 7 and isopimpinellin, **9**), angular furanocoumarins (e.g. angelicin, **10**), linear pyranocoumarins (e.g. xanthyletin, **11**) or angular pyranocoumarins (e.g. seselin, **12**) [4]. Murray *et al.* [5], however, used a biogenetic approach based upon the number of nuclear oxygen atoms in classifying coumarin-containing compounds.

Coumarin derivatives have been found to have numerous therapeutic applications including photochemotherapy, antitumor and anti-HIV therapy [6, 7], and as central nervous system (CNS) stimulants [8], antibacterials [9, 10] antiinflammatory [11], anti-coagulants [12] and dyes [13]. In addition, coumarins are known to be lipid lowering agents with moderate triglyceride lowering activity [14]. Furthermore, hydroxycoumarins, powerful chain-breaking antioxidants and can prevent free radical injury by scavenging reactive oxygen species [15]. Some of the coumarin derivatives formerly used as fixative and flavoring agents, are now regulated as food adulterants by the Food and Drug Administration (FDA) in the United States due to their adverse effects such as mild nausea, diarrhea, and hepatotoxicity when used in certain amounts [16–19]. Although currently marketed in several European countries, coumarin type drugs such as coumarin (**1**), used for the treatment of lymphoedema, has not been approved for therapeutic purposes in the United States, due to their hepatotoxicity. However, recent discovery of coumarins having weak estrogenic activity resulted in the use of such derivatives as therapeutic agents in preventing the emergence of menopause related diseases, such as osteoporosis, increased risk for cardiovascular event / disease and cognitive deficiencies [20].

The pattern of substitutions on the basic chemical structure is said to influence both the coumarin's pharmacological and biochemical properties, including the therapeutic applications, and can beneficially affect toxicity (Table 1) [21–23]. For example, introduction of a methoxy group at the 7-position and a 3-methyl-2-butenyl group at the 8-position of osthole (**13**) led to a strong reduction of plasma alkaline tranferase (ALT) level in hepatitis and inhibition of caspase-3 activation [24]. Some coumarins display cytostatic properties (growth-inhibitory) while others have cytotoxic activities [25]. For example, coumarin and its active metabolite, 7-hydroxycoumarin, demonstrated growth-inhibitory cytostatic activity in human cancer cell lines, such as A549 (lung), ACHN (renal), H727 (lung), MCF-7 (breast) and HL-60 (leukemia), and have also been reported to demonstrate activity against prostate cancer, malignant melanoma, and metastatic renal cell carcinoma in clinical trials [26–29]. Furthermore, the substituted benzopyranobenzothiazinones expressed estrogenic activity on MCF-7 breast carcinoma cells [30]. It has been demonstrated that the incorporation of a catechol to the basic structure of coumarin would increase cytotoxic activity in tumor cell lines [31]. Naturally occurring coumarins (NOCs), (e.g. **3** and **8**) induce mouse skin tumor initiation in a well-established model of multistage carcinogenesis [32–36].

Among the diverse biological activities of coumarins, the most intriguing is the notable effect of some of the coumarins against breast cancer. Studies have shown that compound 3 as well as 4-hydroxycoumarin inhibited cell proliferation in a gastric carcinoma cell line [37]. An *in vitro* proliferation assay of the mechanism of action of coumarins on the growth and metabolism of human tumor cells e.g. MCF-7 and A549 confirmed that coumarin itself

is not responsible for the observed *in vivo* effects, but it is a prodrug of other active metabolites [38]. Previous studies have indicated that the ortho-dihydroxycoumarins (e.g. **4**) or meta-dihydroxycoumarins possessed more potent cytotoxicity in human tumor cell lines than any of the mono-hydroxycoumarins [39,40].

2. COUMARINS AND BREAST CANCER

Breast cancer is a major cause of mortality in western countries and it has been reported that about one-third of postmenopausal breast cancer patients have hormone-dependent tumors involving the stimulation of estrogen receptor [41]. Treatment as well as prevention has been the focus of much laboratory work and clinical trials over the past 30 years. Clinical studies focusing on the use of therapeutic agents that prevent the synthesis and action of estrogens (ER antagonists) are known to be very successful in the treatment of breast cancer [42]. The current strategy thus involves the development of ER antagonists as a new approach for the treatment of postmenopausal women with hormone-dependent breast tumors. The high levels of estrogen as a result of its *in situ* synthesis are associated with the growth of tumors in endocrine-dependent tissues. Estrogens are formed exclusively in peripheral tissues, and there are two pathways associated with their synthesis in such tissues, the aromatase and sulfatase pathways (Fig. 2).

The aromatase pathway involves the conversion of androgen precursor, androstenedione, secreted mainly by the adrenal cortex, to estrone by the aromatase (AR) enzyme complex, while the estrone sulfatase pathways (E1-STS) involves the conversion of estrone formed *via* the aromatase route to estrone sulfate (E1S) by the sulfotransferase enzymes [44]. In the breast tumors, activity of the latter enzyme is higher than that of the former, resulting in poor prognosis [45–48]. The E1-STS pathway is considered the major source leading to estrogen formation, causing low response rate in ER+ breast tumor patients to highly potent AR inhibitor [49–52]. Furthermore, studies have shown that endocrine therapy involving the inhibition of enzymes within the steroid biosynthetic cascade may be one route to controlling the disease. This approach has led to the development of novel coumarins as STS [53] as well as AR inhibitors [54].

2.1. Sulfatase Inhibitor

As illustrated in Fig. (2), the cleavage of sulfated steroid hormone precursors, e.g. estrone sulfate, to the active hormones by STS represents the first step in the local production of estrogen and androgens. Therefore, the inhibition of this enzyme (STS), which should decrease the biosynthesis of active hormones, has been a new therapeutic option in the treatment for hormone-dependent diseases [55–58] such as breast, endometrial and prostate cancers, acne and androgenic alopecia [53, 59–61]. Since STS catalyzes the hydrolysis of sulfate monoester bonds in a range of physiological substrates, the incorporation of a sulfamate ester group linked to an aryl ring was considered to be key strategy in the development of potent STS inhibitors [42, 62–65]. Furthermore, attempt to identify non-steroidal STS inhibitors led to the development of various bicyclic and tricyclic coumarin sulfamates, which are active both *in vitro* and *in vivo* (Fig. 3) [66–70]. A number of potential STS inhibitors are still in preclinical phases of development with only 667 COUMATE (**30**) [71] set to enter clinical trials for the treatment of hormone-dependent breast cancer in postmenopausal women.

Chemically, bicyclic and tricyclic COUMATES (**23–37**) and their close relatives the 676 OXEPINs (e.g. 38) are aryl sulfamates (Ar-SO$_2$NH$_2$), which are irreversible inhibitors of STS. The crystal structures of the soluble enzymes arylsulfatase A (ASA) and arylsulfatase B (ASB) [72, 73] have provided an insight in the mechanism through which sulfamate group irreversibly inactivates steroid STS. The mechanisms for irreversibly inhibiting STS activity

by COUMATES and its congeners (Fig. 4–6) involve: i) the L-C -formylglycine (FGly), ii) the aldehyde hydrate (gem-diol residue), and iii) random specific or nonspecific sulfamoylation of amino acid residues in the active site [62, 63, 74–78]. Random specific or nonspecific sulfamoylation is proposed to occur *via* two mechanisms – a direct nucleophilic attack by the amino acid residue at the sulfur atom of, for example, compound 30 and elimination of sulfamic acid by an E1cB mechanism, assisted by the extended conjugation present in the coumarin ring [63,79].

Structure-activity relationship (SAR) studies of the "locking effect" of the lactone ring structure of the COU-MATE by Ahmed *et al.* confirmed that conformational restriction of the conjugated C=C bond plays an important role in the potency inhibitory activity displayed by coumarinbased compounds, in addition to the overall size of the inhibitors [80]. The pKa value of compound 30 is 9.1 in methanol/water (1:1), making it possible to exist in monoanionic form under physiological condition [63]. Compound 30 (Fig. 3) is a non-steroidal irreversible STS inhibitor and is the most potent of a series of tricyclic COUMATE developed with annulated carbocylic ring systems at positions 3 and 4 [41, 61, 68] with a very acceptable toxicological profile. It has an equipotent *in vivo* STS activity compared to estrone-3-*O*-sulfamate (EMATE), a potent, active sitedirected, irreversible steroidal STS inhibitor [81]. Moreover, Compound **30** is reported to be active both *in vitro* and *in vivo* [67] in causing significant regression of E1S-stimulated tumor growth without any sign for estrogenic effect [82].

SAR studies involving bicyclic COUMATES revealed that compound **25** (Fig. 3) displays stronger binding affinity for the enzyme active site *via* a hydrophobic interaction provided by the methyl groups at the 3- and 4- positions, thereby mimicking the A/B ring of EMATE, [81, 82]. Bilban *et al.* [83] demonstrated that the oxygen functionality substitution at position 7 of the coumarin core structure also mimics the A/B ring of EMATE. Previous computer-modelling study has shown that the seven-membered ring (third ring) of COUMATE (**30**) could not be described as closely mimicking the C/D ring regions of EMATE, attributed to its chair conformation form which is similar to the cycloheptene ring structure (Fig. 7) [63, 84]. However, recent finding indicated that the third ring appears to be predominately in the boat conformation rather than previously proposed chair conformation based on temperature factors (B-factors) and electron density map results [79]. With the advent of this new finding regarding the conformation of the 7-membered ring on compound **30** it can now be explained that its higher potency (IC_{50} value of 8 nM and K_i value of 40 nM) than EMATE (IC_{50} = 25 nM and K_i = 670 nM) is attributed to the tendency to mimic the steroidal CD-ring; perhaps, better hydrophobic interactions due to favorable binding to the active site of the enzyme are in play [61, 85, 86].

Benzocoumarin sulfamates (e.g. **39** and **40**), another group of aryl sulfamates closely related to compound **30**, mimics the ABC-ring of the steroidal skeleton. Although less active than EMATE, these sulfamates show high inhibitory potency due to strong binding of the benzocoumarin core structure to the enzyme. Removal or disruption of the coumarin ring conjugation results in lower potency [42, 69, 87] due to the resulting higher pKa value for the parent phenol. The extended conjugation present in the coumarin ring structure assists in the S-O-Ar bond breakage during E1-STS catalyzed sulfamoylation by improving the leaving ability of the coumarin compound as a result of lower pKa value of the phenol [63]. On the contrary, an extension of the coumarin conjugation core structure and the relocation of the sulfamate group to the 6-position of the ring resulted in lower potency exhibited by the COUMATE analogs [42].

2.2. Aromatase Inhibitor

Aromatase inhibitors (AIs) include drugs that are currently used for the treatment of hormone-dependent breast cancer, which involves blocking the estrogen action on tumor cells by preventing the biosynthesis of estrogen [88]. AI prevents breast cancer *via* reduction of cell proliferation, which involves reduction of estrogen level and thus prevention of the formation of genotoxic metabolites of estrogen. The genotoxic estrogen metabolites include i) catechol estrogens, which bind covalently to DNA and induce mutations that initiate cancer; ii) 2-hydroxyl-estradiol, which forms stable DNA adduct; and iii) 4-hydroxy-estradiol, which is a potential carcinogenic metabolite forming depurinating estrogen-DNA adducts with guanine base that are unstable and are rapidly lost from the DNA [89–91]. Clinical trial results have shown AIs to be of superior efficacy to tamoxifen as anti-estrogenic compounds with more favorable toxicity profiles [92–94]. In postmenopausal women, AIs have the potential to suppress circulating estrogen levels by approximately >96.7–98.9% and also abrogate autocrine and paracrine estrogen production by peri-tumoral stromal cells located in both primary and metastatic sites of the tumor [95–101]. The FDA has approved a number of AIs, e.g., anastrozole (Arimidex), exemestana (Femara) and letrozole (Aromasin), as first-line agents for the treatment of postmenopausal women with hormone receptor positive breast cancer [102–106]. In postmenopausal women, clinical results have shown that AIs used only as monotherapy are very effective in treating estrogen-dependent and aromatase-mediated diseases including breast cancer [107]. However, in premenopausal women there is an incomplete blockade of estrogen synthesis resulting in a reflux rise in gonadotrophin level, which in turn can stimulate ovarian aromatase and overcome the estrogen suppression [108].

As discussed in previous section, several coumarin derivatives have been reported to be steroid STS inhibitors and evaluated for breast cancer therapy [53, 109]; however, clear evidence of AIs as antibreast cancer agent has not been demonstrated yet. Studies have shown benzopyranone substrates such as 4-benzyl-3-(4'-chlorophenyl)-7-methoxy-coumarin (**41**) to be a more potent competitive AI than several known AIs such as aminoglutethimide with respect to the androgen substrate. The specific interaction of compound **41** with the AR shows a further reduction by several mutations in binding at the active site region of AR. In addition, compound **41** suppressed the proliferation of AR and ER positive MCF-7 breast cancer cells. It is neither cytotoxic at concentrations up to 40 μM nor an inhibitor of steroid 5 -reductase and also is not a ligand of ERs, ER and ER , or androgen receptor. Thus demonstrating that coumarin derivatives, which are potent inhibitors of aromatase, may not be cytotoxic yet can be useful in the suppression of AR and ER-positive breast tumors.

The SAR studies of (**41**) revealed that coumarin rings mimic the A and B rings, while the 3-(4-chlorophenyl) group 2670 mimics the D ring of the androgen (**42**) (Fig. 8) [54]. The methyl group at the C-19 of the androgen substrate, where the first and second hydroxylation reactions take place, points toward the heme group of AR [110]. The spatial orientation of the 4-benzyl group of the coumarin showed that it is aligned very closely to the methyl group at the C-19 position of the substrate. This behavior is attributed to the bending of the benzyl group (through the methylene group), resulting in overlay of the ring on top of the 3-(4-chlorophenyl) group. Furthermore, the 7-methoxyl group of coumarin (**41**) also aligns very closely with the C-3 keto oxygen of the substrate. These groups- 3-(4-chlorophenyl), 7-methoxyl and 4-benzyl- share the same physicochemical feature that is being hydrogen bond donor groups and are superimposable. However, result has demonstrated that the replacement of the 7-methoxyl group with 7-hydroxyl group on the coumarin significantly decreased the inhibitory activity against AR. A recent study has demonstrated that the presence of an electron-withdrawing group in the phenyl group, e.g. 3-(4-chlorophenyl) group, increase activity as a result of such group alignment that mimics the

C-17 keto oxygen of estrone [54]. Thus it is strongly suggested that 7-methoxyl, 3-(4-chlorophenyl) and 4-benzyl functional groups are very critical for the anti-AR activity of the coumarin derivative [54, 111, 112]. For example, the IC_{50} value of 4-benzyl-3-(4-chlorophenyl)-7-methoxycoumarin is 80 nM and the replacements of any of the three mentioned functional groups resulted to reduction in potency, e.g. 4-benzyl-3-(4'-chlorophenyl)-7-hydroxycoumarin (IC_{50} 300 nM). A proposed pharmacopore hypothesis indicated that the methoxy group might act as hydrogen bonding acceptor depending on its spatial position relative to the nitrogen atom coordinating the iron (II) [112], although there are varieties of possible binding modes, which may differ from those of the unsubstituted coumarin derivatives. The carbonyl group of the coumarin lactone ring is another structural determinant for the observed coumarins' AR inhibitory activity. Also, a favorable interaction at the 7-position of the coumarin ring is due to the formation of a hydrogen bond by the 7-oxygen atom and the lipophilic or - stacking interaction of the phenoxy or benzyloxy group.

2.3. Coumarin-based SERMs

Selective estrogen receptor modulators (SERMs) are a new category of therapeutic agents that are used for the prevention and in the treatment of diseases such as osteoporosis and uterus and breast cancers [113, 114]. They are known to have high affinity for ER, but no specific affinity for any other steroid hormone receptors. In addition, SERMs are known to stimulate estrogenic actions (ER agonist) in tissues, such as the bone, liver, and cardiovascular system but block estrogen action at other sites (ER antagonist) where stimulation is considered undesirable, such as the breast and uterus [115–119]. This agonistic or antagonistic activity causes different conformational changes of the receptors particularly at the helix 12 [119–121], resulting in activation (transactivation) or repression (transrepression) of the estrogen target genes [120]. Examples of drugs classified as SERMs are: estrogen metabolites, clomiphene, tamoxifen, toremifene, idoxifene and droloxifene [121–123]. Tamoxifen is the most widely used hormonal therapy for breast cancer today.

Compound **43** (Fig. 9) belongs to a new category of SERM called benzopyranone molecules or coumarin-based SERM, producing similar effects as other SERMs indicated above [124, 125]. However, it possesses a different structural feature from those of an ideal SERM such as tamoxifen and thus may induce a different conformational change to the ER, resulting in different cofactor recruitment [126–128]. Furthermore, compound **43** does not activate genes through estrogen response element (ERE). It is known to bind with high affinity through ER , effectively antagonize estrogen action in ER –expressing breast cancer cells by inhibiting IL-6 and GM-CSF gene expression and thus functions as potent antiestrogen in both *in vitro* and *in vivo* models of breast cancer [125, 129]. Previous studies have demonstrated that compound **43** acts as an antiestrogen in the breast and potently inhibits estrogen-dependent MCF-7 proliferation with similar IC_{50} value to tamoxifen [129]. Furthermore, compound 43 is more potent than estrogen, behaved as an estrogen agonist in the bone cells *in vivo*, as effective as raloxifene in decreasing the formation of osteoclast-like cells and partially protected animals against ovariectomy-induced osteopenia [124]. *In vitro* U2OS/IL-6 and MCF-7 assays revealed that i) movement of the basic amine side chain on the phenyl group at C-4 to the C-3 in the 4-phenyl of the benzopyranone series provided a significant improvement in antiproliferative activity; ii) manipulation of the side chain was effective in mimicking the hinge substituent found in the benzopyranone 4-benzyl series and iii) an extension of the side-chain length improved receptor-binding affinity (Fig. 10) [130]. Tetracylic benzopyranone ring system such as benzopyranobenzopyran (**44**, n=l), benzopyranobenzoxa-pane (**45**, n=2) and benzopyranobenzoxacane (**46**, n=3) are excellent in mimicking natural ligands as estrogen receptor modulators [131]. These molecules differ

from ideal SERMs in that they do not contain the basic side-chain group critical for providing selective tissue antagonist properties as seen with tamoxifen and raloxifene [132].

Study aimed at the development of newer SERMs revealed that 3-substituted coumarin (**47**) [133] and coumestrol (**48**) [134] possess estrogenic activity. Compound **48**, a plant derived tetracyclic coumarin, bears similar chemical features as estrogen attributed to its apparently crude mimicry of the steroid skeleton, thus making it possible for the coumarin moiety of the molecule to mimic the A/B ring of estrogen. SAR studies of the structure and relative binding affinities (RBAs) of various 3-substituted coumarins revealed that substituents at positions 3 and 7 resulted to an increase in RBA for the ERa [135–137]. In comparison to estradiol, 3-phenyl-4-ethyl-7-hydroxycoumarins and 3-(4-hydroxy-phenyl)-4,7-dihydroxycoumarin showed weak RBA and lack of selectivity toward both ERs. Furthermore, substitution with a second phenyl group at position 4 resulted in 3,4-diphenyl-7-hydroxycoumarin showing an increase in RBA to both ERs but with more selectivity for ER than ER [138].

2.4. Coumarin-Estrogen Conjugates

There is an over-expressed ER in breast tumor cell in the earlier stage and during hormonal treatment [139, 140]. The non-selectivity and acute toxicity of many antitumor agents have been the major deterrent in their usage for treating human cancer [141]. Among the current cancer therapy focusing on the improvement of drug selectivity, conjugation of cytotoxic drug components to a carrier with selectivity toward the tumor tissues has proven to be an effective strategy in the development of efficient antitumor drugs with high therapeutic indices [142–147]. Studies have shown that coupling of cytotoxic agent with steroid hormones results in improvement of antitumor activity and in the target selectivity of the conjugate as the result of sufficient binding to the ER, allowing selective accumulation of the conjugates in ER-rich cells [148–152]. During the past decades, the application of bioconjugates (i.e. biomolecules bearing unnatural organic structures) in molecular and cell biology has significantly increased [148].

We have recently extended this novel concept of bioconjugation involving 3-substituted coumarins and estradiol (**49–51**) (Fig. 11) to show antiproliferative activity in NCI-7 human breast cancer cell lines. Comparisons of the GI_{50} values among the conjugates showed that conjugate **49** has the highest antiproliferation activity against MDA-MB-435 breast cancer cell lines while conjugates (**50–51**) displayed the highest antiproliferative activities against MDA-MB-231/ATCC. As far as the distinction between noninvasive and invasive breast cancer cell lines, overall conjugate **50** appears to be active against both types while conjugate **49** has the least inhibitory activities against noninvasive MDA-MB-231/ATCC and NCI/ADR-RES cell lines among the conjugates. Moreover, conjugate **49** was surprisingly inactive against the estrogen receptor enriched MCF-7. In general, it was shown that cytotoxicity occurred at around 100 μM for all of the conjugates. It was also observed that conjugate **50** displayed the most cytostatic properties based upon TGI values being less than LC_{50} values. The detailed results will be published elsewhere and is available on request from the author.

3. SYNTHESIS OF COUMARIN DERIVATIVES WITH ANTICANCER ACTIVITY

Coumarins have been the subject of extensive studies because of their interesting biological activities and have, in fact, been used as therapeutic agents for the treatment of various diseases including breast cancer. There are various methods for preparing coumarin derivatives; these include the classic Pechmann [153],Claisen [154, 155],Perkin [156], Knoevenagel [157], and Wittig [158] reactions, to mention but a few. Recently, Musa [159] explored the intramolecular Baylis-Hillman reaction reported by Drewes *et al.* [160] as an

alternative route to the synthesis of substituted coumarins, which has been further explored in the synthesis of coumarin-estrogen conjugates as anticancer agents. There is, nevertheless, a continuing interest in the synthesis of these important coumarin systems. Of particular interest to this review, the synthesis of only those coumarins with anticancer potentials, namely the coumarin sulfamates, benzopyrano- benzopyran ring systems, coumarin 3-carboxamides, coumarin 3-sulfonamides and some others that have been outlined here.

3.1. Coumarin Sulfamates

The Pechmann reaction (Scheme 1), widely used method for preparing coumarins (**54**) in good yield, involves reacting phenol (**52**) with -oxo ester (**53**) in the presence of either homogeneous acid catalysts e.g. sulfuric [154, 161], phosphoric and trifluoroacetic acids [162], and Lewis acids, or heterogeneous catalysts e.g. Nafion-H, zeolite-HBEA and other solid acids [163]. The reaction is considered to involve the following steps: (i) addition across the double bond of the enolic form of the -keto ester; (ii) ring closure; and (iii) dehydration [164].

Previous studies demonstrating the estrogenicity of coumestrol (**48**) led to other coumarin ring systems being explored as an alternative phenolic-based non-steroidal sulfamate compound. Wool *et al.* reported the synthesis of bicyclic and tricylic coumarin sulfamates, e.g. COUMATE (**24**) (Fig. 3), a non-estrogenic active site-directed E1-STS inhibitor [43, 69, 70] *via* the Pechmann reaction between resorcinol and either of the corresponding L-ketoester (**55–57**) in presence of an equimolar mixture of trifluoroacetic acid and concentrated sulfuric acid as the condensing agent to afford the coumarins (**59–61**) in > 60% yield [165, 166] (Scheme 2). The lowest yield (25–33%) was obtained for tricyclic coumarin (**61**, n= 6), which was attributed to the ring size of its starting 8-membered cyclic L-ketoester.

3.2. Benzopyranobenzopyran Ring Systems

Perkin reaction (Scheme 3) [156], involves heating an *O*-hydroxybenzaldehyde (**65**) with acetic anhydride in the presence of sodium acetate at a high temperature (*ca.* 200°C) to afford a *traws*-cinnamic acid, followed by irradiation or treatment with iodine and then cyclization to afford the coumarin (**66**). This reaction is also carried out by reacting acetic anhydride and salicylaldehyde in the presence of trimeth-ylamine as the base catalyst [167]. However, the obvious advantage is that the formation of isomeric chromones is not possible, as is the case with the Pechmann reaction [168].

Efforts to synthesize the unsymmetrical tetracyclic benzopyranobenzopyran ring system (**74**), novel selective estrogen receptor modulators (SERMs) [169–171], by Kanojia *et al.* led to the synthesis of 4-methyl-3,7-substituted coumarins (**69**) as the key intermediates, *via* base-catalyzed Perkin condensation reaction in moderate to good yield (Scheme 4) [170]. These were furnished by reacting a mixture of 2-hydroxyacetophenones (**67**) and 2,4-dimethoxyphenylacetic acid (**68**) in the presence of triethylamine in refluxing acetic anhydride. The next step, which is known as the key step is said to involve the conversion of the 4-methyl functional group of coumarin (**69**) to the 4-halogenated methyl moiety, followed by ring closure to give the benzopyranobenzopyran compounds.

3.3. Coumarin 3-Carboxamides

Various coumarins (**76**) have been prepared *via* Knoevenagel condensation reaction (Scheme 5) by reacting 2-hydroxybenzaldehydes (**65**) with activated methylene com-pounds (**75**) in the presence of an amine (*e.g* piperidine) [168]. Two different mechanisms have been proposed for Knoevenagel reaction [172]: 1) formation of an imine or iminium

salt, followed by reaction with the enolate of the active methylene compound, then elimination of the amine and intramolecular ring closure to give the coumarin, ii) attack by the carbanion formed from the deprotonation of the active methylene compound by the amine, on the carbonyl group to give the intermediate follow by proton transfer, and ring-closure *via* acyl substitution, and finally dehydration to give the coumarin.

Intrigued by the recent discovery demonstrating that coumarin-3-carboxamide (**81**) inhibits the growth of cancer cell lines by up-regulating the activity of c-Jun NH_2 terminal kinase 1 (JNK1) [173], Reddy *et al.* explored two novel routes to synthesize it *via* Knoevenagel condensation reaction. These synthetic routes involved: i) the condensation of 3-anilino-3-oxopropionic acid (**80**) with substituted salicylaldehydes (**65**) in glacial acetic acid in the presence of a catalytic amount of benzylamine, to afford (**81**) in 60–80% isolated yield [174, 175], and ii) condensation of methyl 3-anilino-3-oxopropionates (**79**) with substituted salicylaldehydes (**65**) in the presence of piperidine in ethanol to give coumarin (**81**) in 68–88% isolated yield (Scheme 6) [174].

3.4. Coumarin 3-Sulfonamides

Some coumarin-3-sulfonamides have shown to be potent inhibitors of various cancer cell lines, which are used in the treatment of diseases arising from abnormal cell growth and proliferation [176, 177]. Previously, coumarin-3-sulfonamides was synthesized *via* the condensation of coumarin-3-sulfonyl chloride with different aromatic amines, which posed some limitations as the result of the difficulty of introducing a variety of substituents on the aromatic rings, thus making it impossible to create a library of new broadly un-substituted coumarin-3-arylsulfonamides [178–181]. Most recently, Reddy *et al.* [173] reported the synthesis of coumarin-3-sulfonamides (**84**) in their investigation involving the antitumor activity of novel compounds bearing coumarin and sulfonamide entities *via* Knoevenagel condensation reaction. This reaction involved reacting methyl-2-chloro-sulfonyl acetate with aromatic amines to afford intermediates (**82**), which were then hydrolyzed to anilinosulfonylacetic acids (**83**) and finally, condensation with substituted salicylaldehydes (**65**) in the presence of glacial acetic acid and a catalytic amount of benzylamine afforded coumarin-3-sulfonamides (**84**) in quantitative yields. An alternative route for the synthesis of (**84**) was also reported that involved the condensation of methyl anilinosulfonylacetates (**82**) with substituted salicylaldehydes (**65**) in the presence of piperidine and ethanol (Scheme 6) [173].

3.5. Miscellaneous

These methods involved the formation of isolable intermediate followed by cyclization using either acid or coupling agents to afford the coumarin derivatives. For example, McKe *et al.* [182] reported the synthesis of coumarin-based benzopyranone compounds and their salts used for treating bone-resorption disease, cancer, arthritis *via* the following steps: i) the reaction of 3-methoxyphenol (**86**) and 4-hydroxyphenylacetic acid (**85**) in the presence of boron trifluoride in diethyl etherate (Fries reaction) to give (**87**) in 40–50% yield; ii) coupling reaction using 1,1'-carbonyl-diimidazole to afford the desired coumarin (**89**) in 10–90% yield; iii) the introduction of the amino basic side-chain to yield coumarin-7-methyl ether (**90**) in 30–70% yield; and iv) demethylation of 90 using HBr to afford 60–75% of the desired coumarin (**91**) (Scheme 7).

Recent investigation by our research group involving the antitumor activity of coumarin derivatives revealed that the conjugation of coumarin substrate to 17 -estradiol, afforded coumarin-estrogen conjugates, which demonstrate growth inhibitory activities in various breast cancer cell lines. Using the generality of the methodology outlined by Drewes *et al.*, [183], which was further explored and reported [159], various 3-substituted coumarin

quaternary salts were prepared *via* the intramolecular Baylis-Hillmann reaction using DABCO as a catalyst and then coupled to 17 -estradiol and estrone oxime to afford coumarin-estrogen conjugates (**49–51**, Scheme 8).

4. CONCLUSIONS

The widespread distribution and numerous bioactivities of coumarins have intrigued scientists for decades to conduct research involving this ring system. This review has outlined such research approaches before focusing on the coumarins with antibreast cancer activity. Although coumarins have vast pharmacological significance, their antibreast cancer potential have been the main focus of this review. A number of innovative synthetic techniques, including the classic Pechmann, Claisen, Perkin, Knoevenagel, and Wittig reactions, have been described in the synthesis of coumarin. With accessibility of such synthetic methods, scientists were able to synthesize numerous analogs with antibreast cancer activity with diverse mechanisms of action, among which aromatase and sulfatase inhibitions were the most notable. However, the coumarin-based SERMs and most recently coumarin-estrogen conjugates have also shown to be reasonable approaches in the development of antibreast cancer agents. Although most natural coumarins exhibited major limitations due to hepatotoxicity, molecular manipulations afforded relatively safer analogs with higher potency and thus better therapeutic index. Some of these derivatives are in clinical trial, which might emerge as new therapeutic option for the treatment of breast cancer. Interestingly, in all these approaches coumarins have been viewed to be antiestrogenic due to the structural similarity with natural female sex hormone estrogen, which is believed to stimulate breast cancer growth.

Acknowledgments

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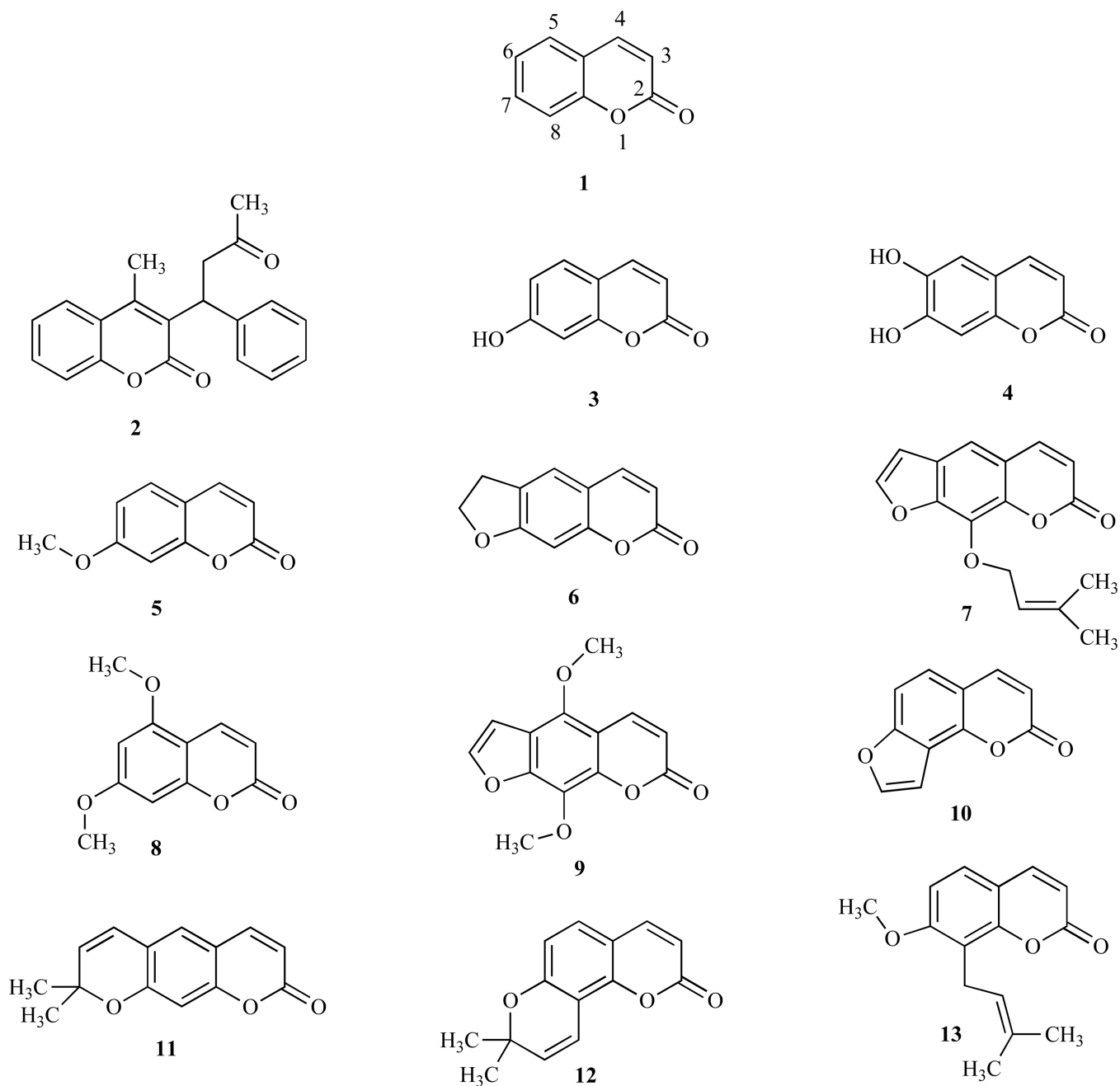
Fig. (1).
Structures of some naturally occurring coumarins.
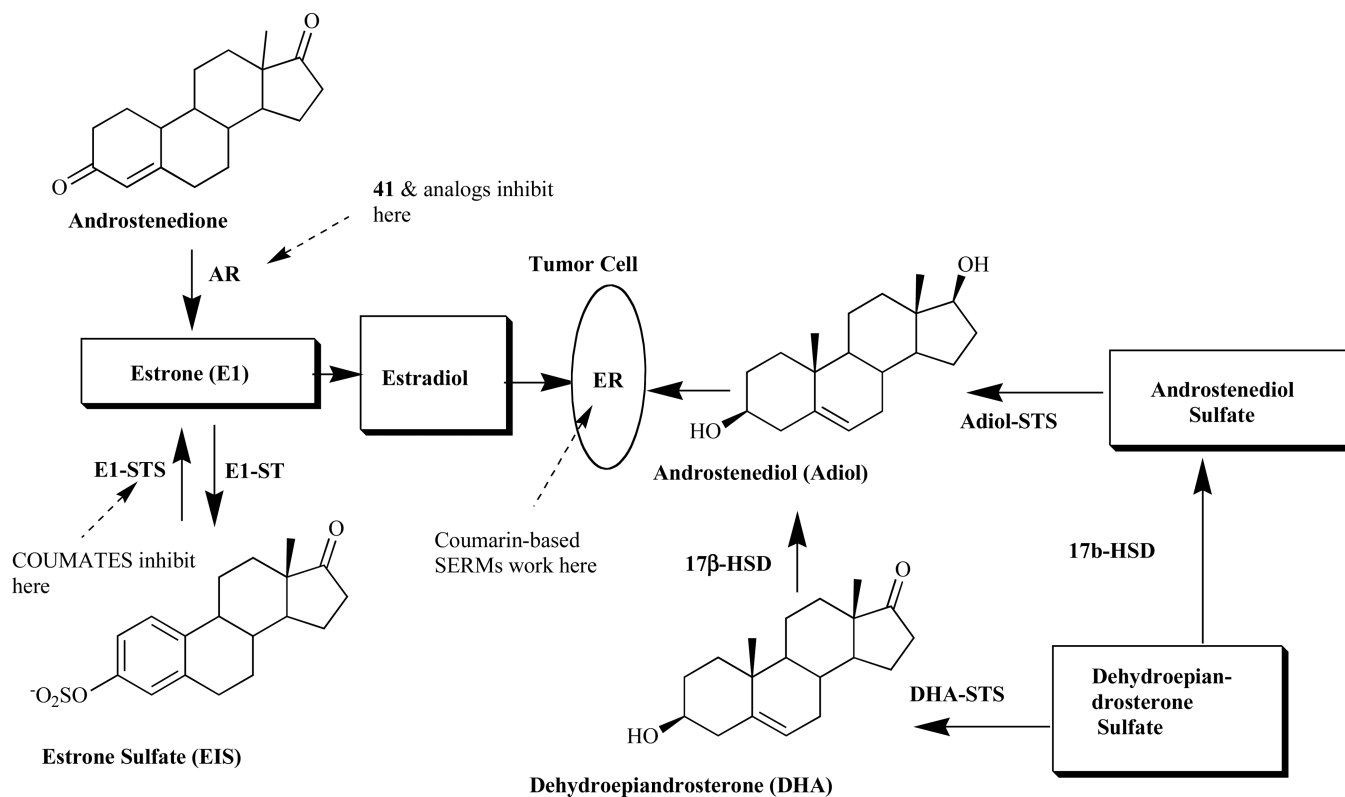
Fig. (2).
Estrogenic steroid origins showing the sites of action of coumarins AR, aromatase; ST, sulfotransferase; STS, sulfatase; 17(-HSD, 17 -hydroxysteroid dehydrogenase; ER, estrogen receptor) [43].

Bicyclic Coumarin Sulfamates
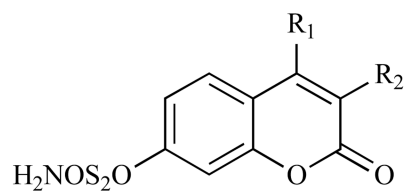
23, $R_1 = H$, $R_2 = H$
24, $R_1 = H$, $R_2 = CH_3$ (COUMATE)
25, $R_1 = CH_3$, $R_2 = CH_3$
26, $R_1 = CH_3CH_2$, $R_2 = CH_3$
27, $R_1 = CH_3CH_2CH_2$, $R_2 = CH_3$

Tricyclic Coumarin Sulfamates
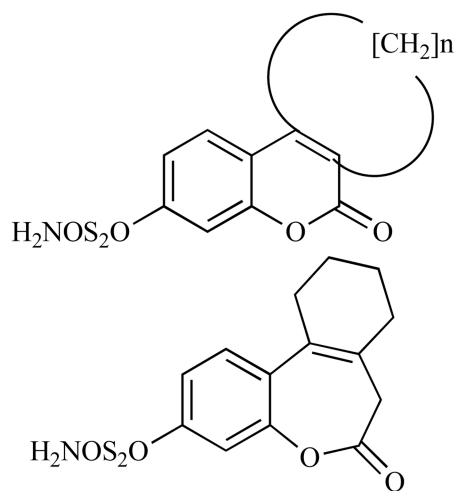
28, n = 3,   665 COUMATE   **33,** n = 8,   6610 COUMATE
29, n = 4,   666 COUMATE   **34,** n = 9,   6611 COUMATE
30, n = 5,   667 COUMATE   **35,** n = 10 6612 COUMATE
31, n = 6,   668 COUMATE   **36,** n = 11 6613 COUMATE
32, n = 7,   669 COUMATE   **37,** n = 12 6615 COUMATE

38, 676 OXEPIN

Fig. (3).
Structures of coumarin sulfamates and tricyclic coumarin sulfamates.
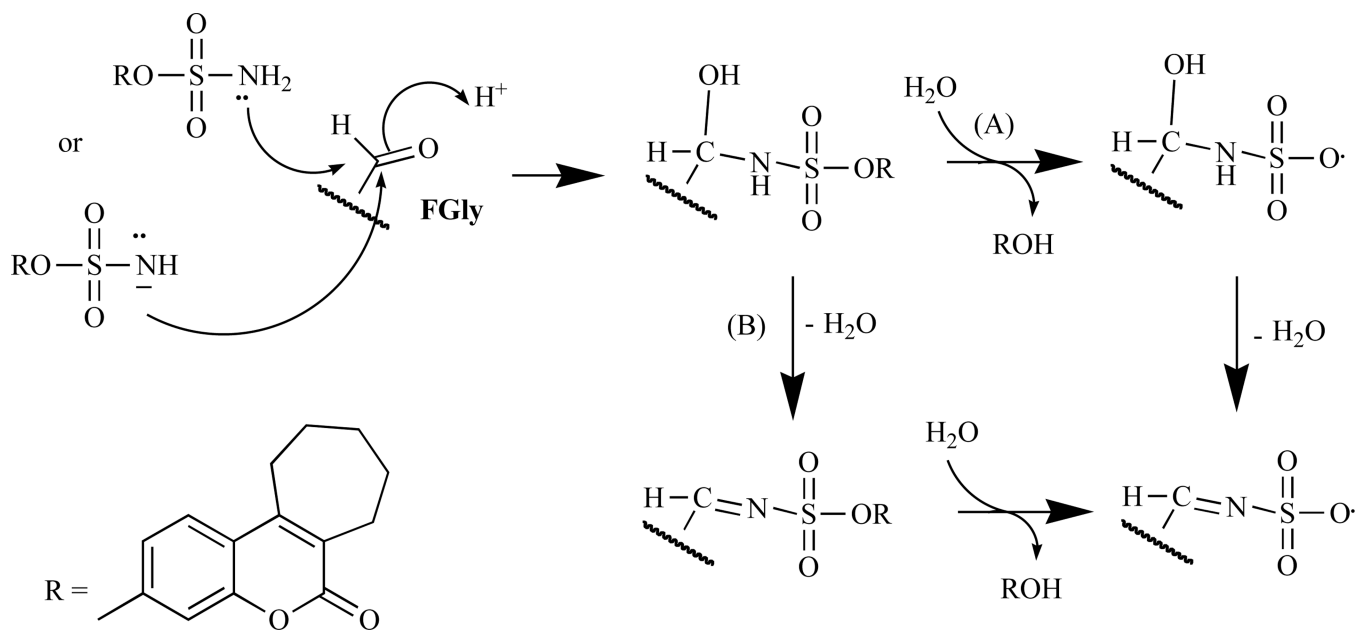
Fig. (4).
The proposed mechanism of STS inhibition by (**30**) involving Fgly in the enzyme active site [63].
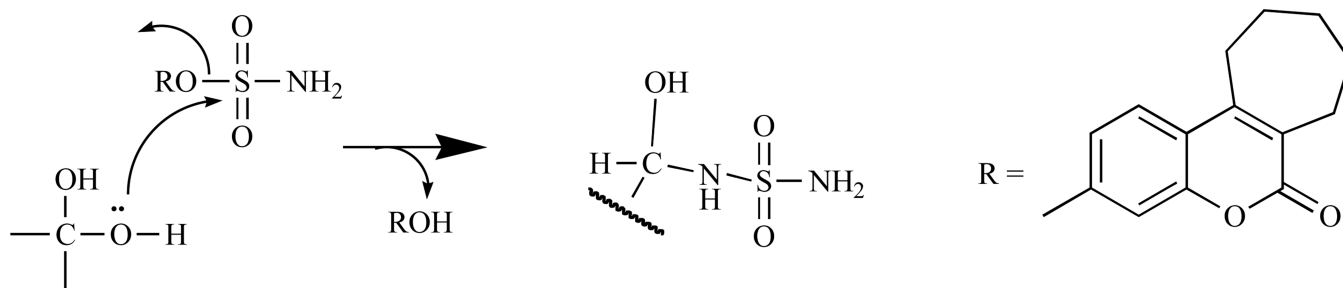
Fig. (5).
Proposed mechanism of STS inhibition by (**30**) involving the aldehyde hydrate in the enzyme active site [63].
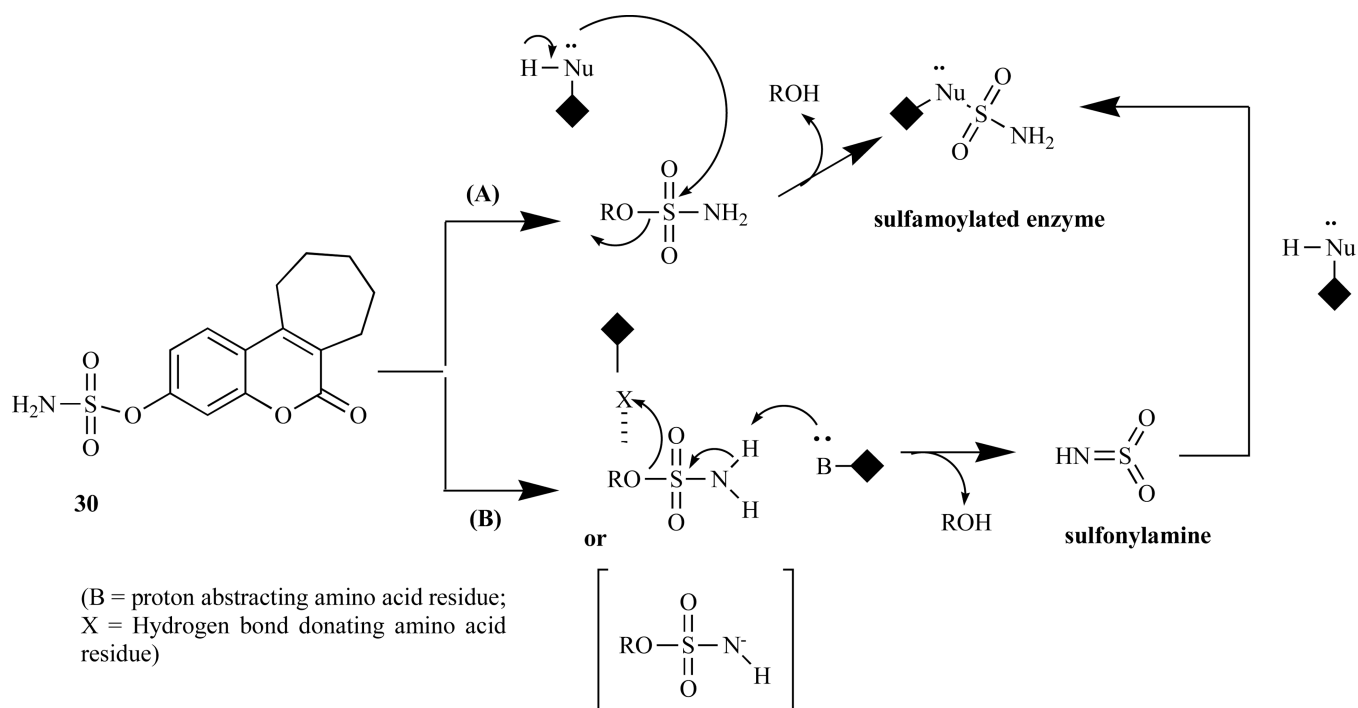
Fig. (6).

The proposed random specific or non-specific sulfamoylation by (**30**). Path A: a direct nucleophilic attack by the amino acid residue at the sulfur atom. Path B: elimination of sulfamic acid by an E1cB mechanism [63].
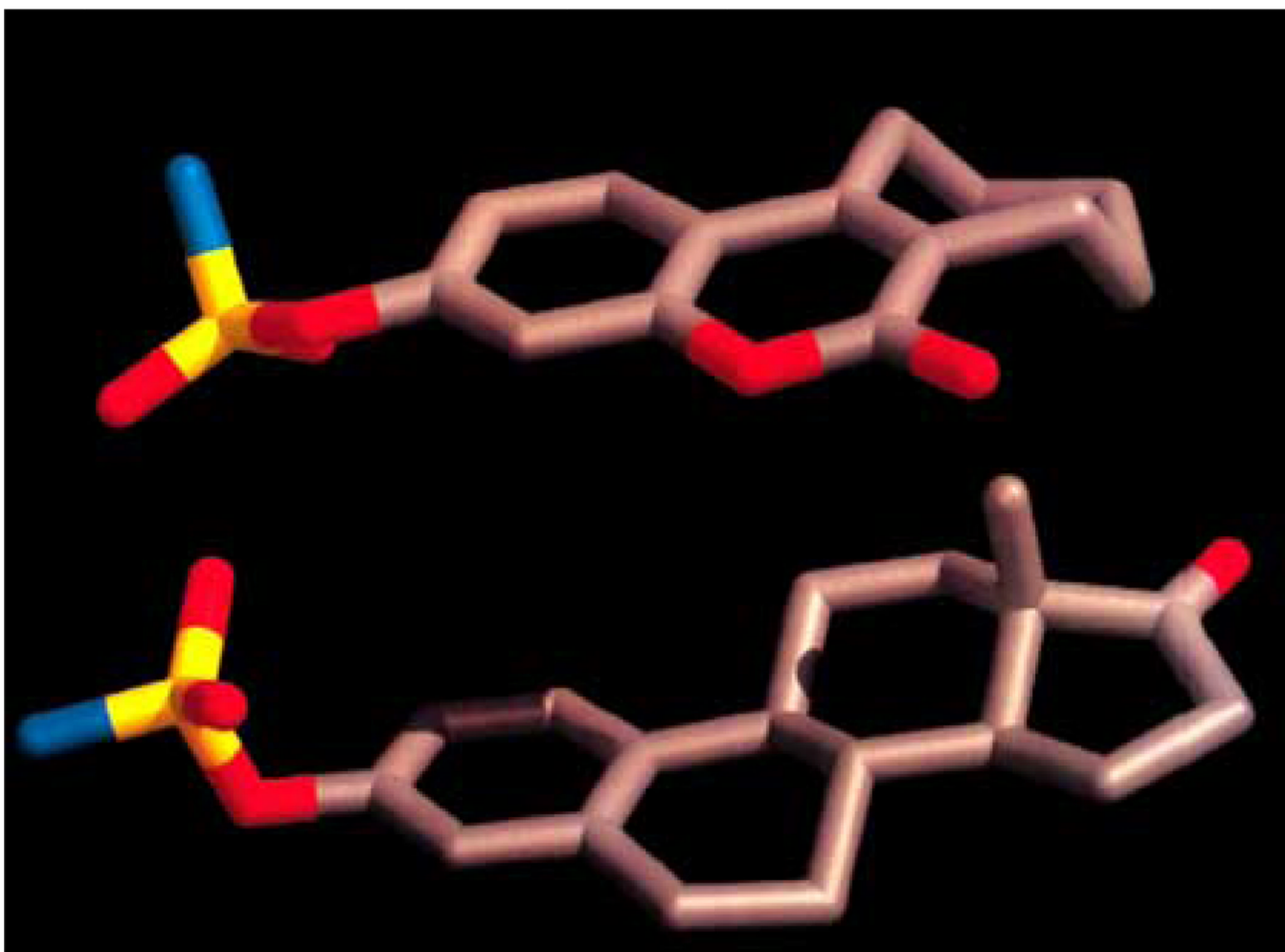
Fig. (7).
Molecular modelling of 667 COUMATE (top) and EMATE (bottom) (adapted from [63] with permission).

41

Androstenedione **(42)**
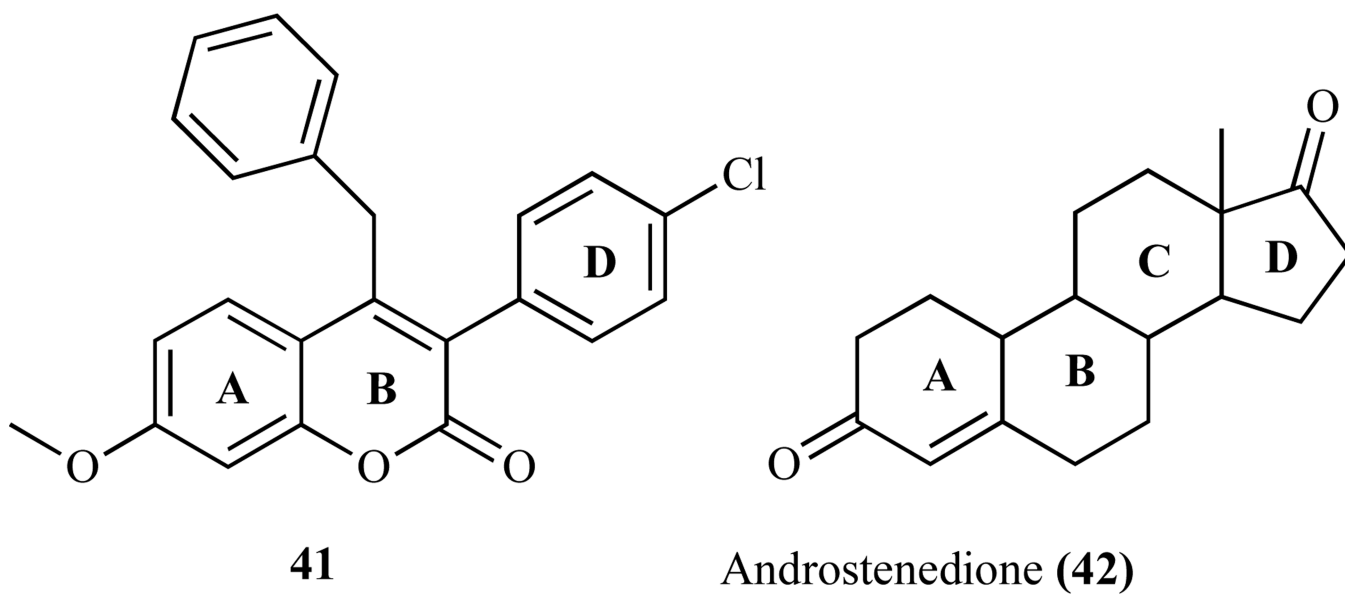
Fig. (8).
Proposed pharmacophores of AR inhibiting coumarins [54].
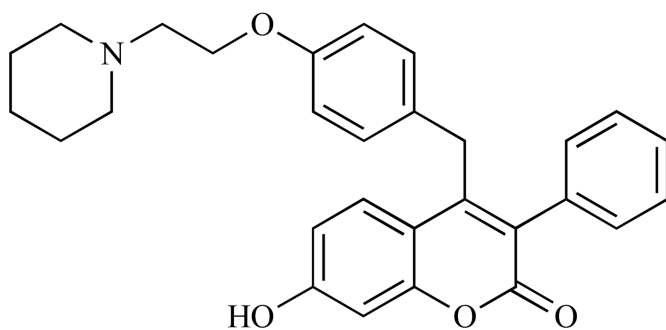
(43), SP500263
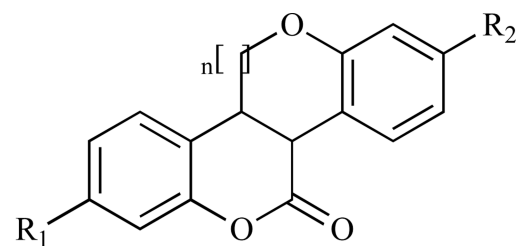
44 (n =1, R_1 = R_2 = OH)
45 (n =2, R_1 = R_2 = OH)
46 (n =3, R_1 = R_2 = OH)
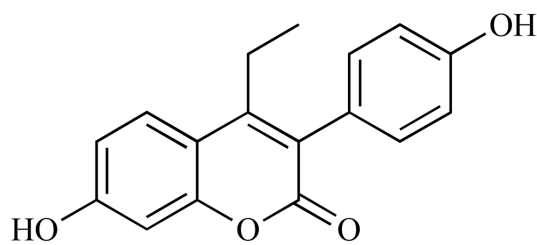
(47)
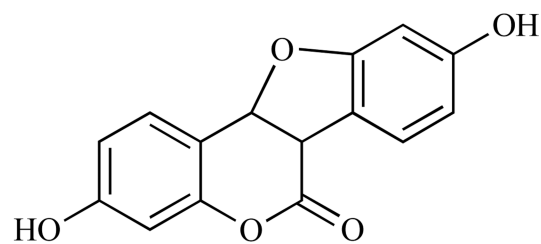
(48), coumestrol

Fig. (9).
Structures of coumarin-based SERMs.
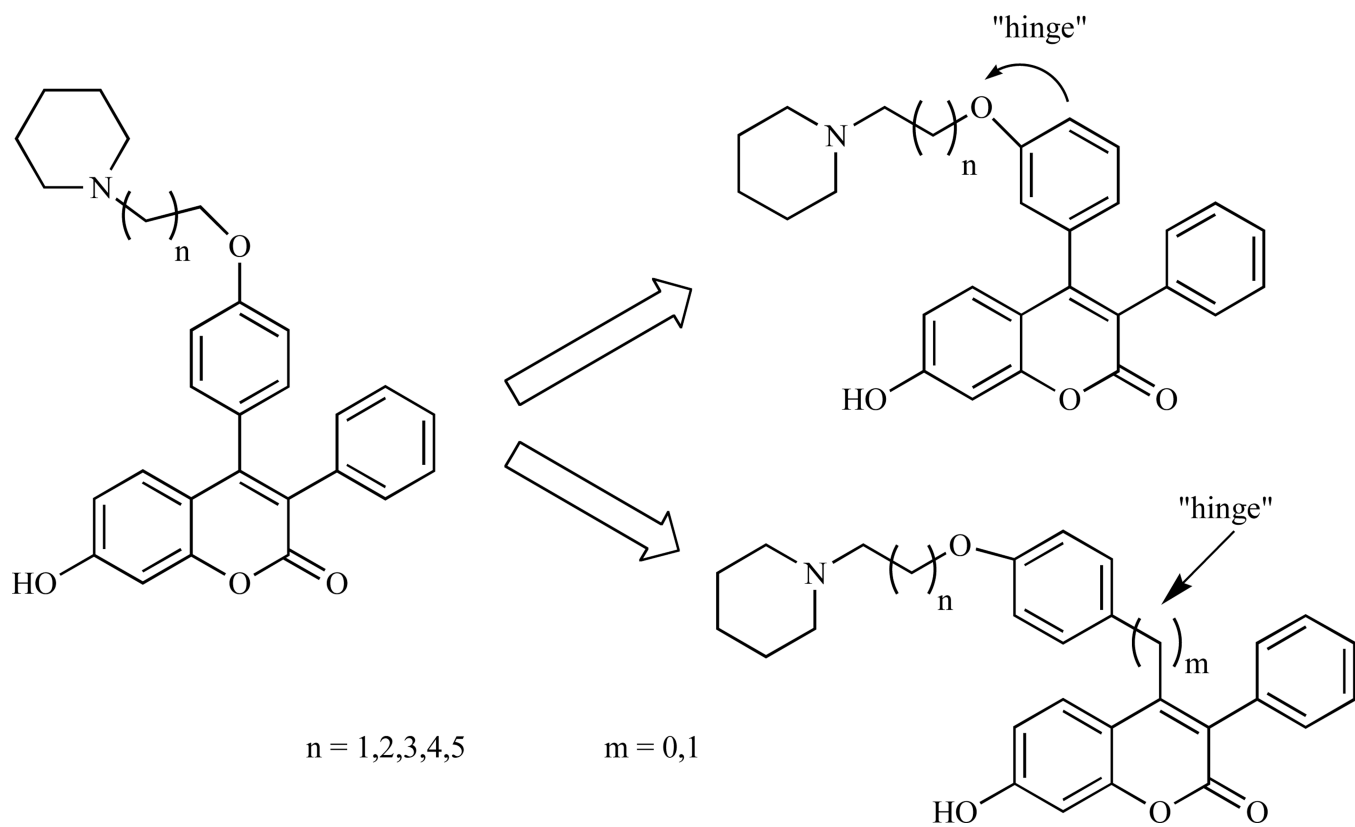
Fig. (10).
SARs of the benzopyranone series (**43**) incorporating the critical hinge feature 130].
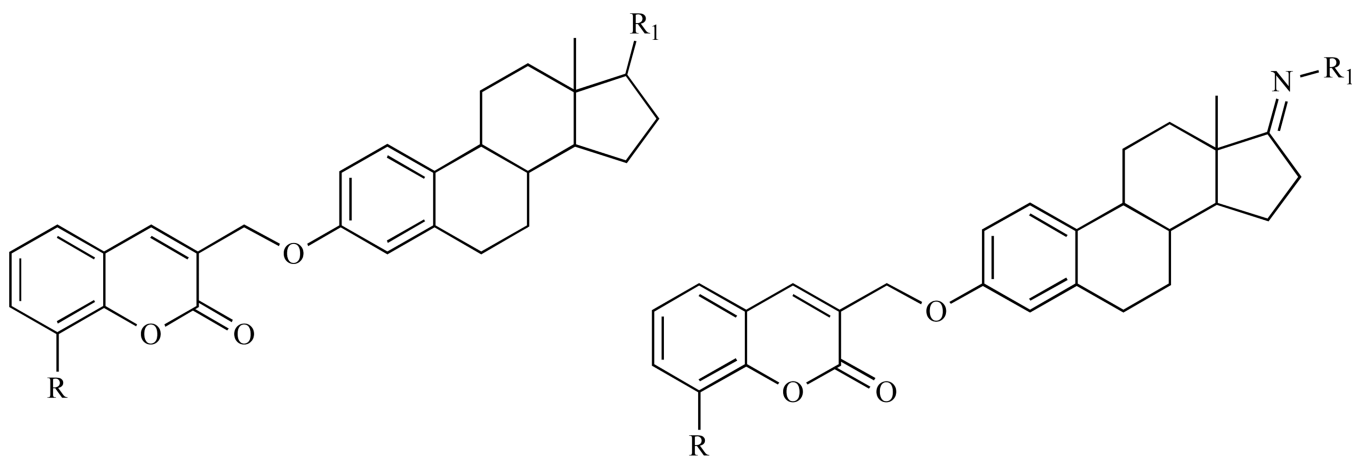
49, R = H, R$_1$ = -OH

50, R = OCH$_3$, R$_1$ = -OH

51, R = OCH$_3$, R$_1$ = -OH

Fig. (11).
Structures of coumarin-estradiol conjugates.

52 + **53** $\xrightarrow[\text{toluene, reflux (0-96\%)}]{\text{H}^+ \text{ or } \text{k-10 or KSF}}$ **54**
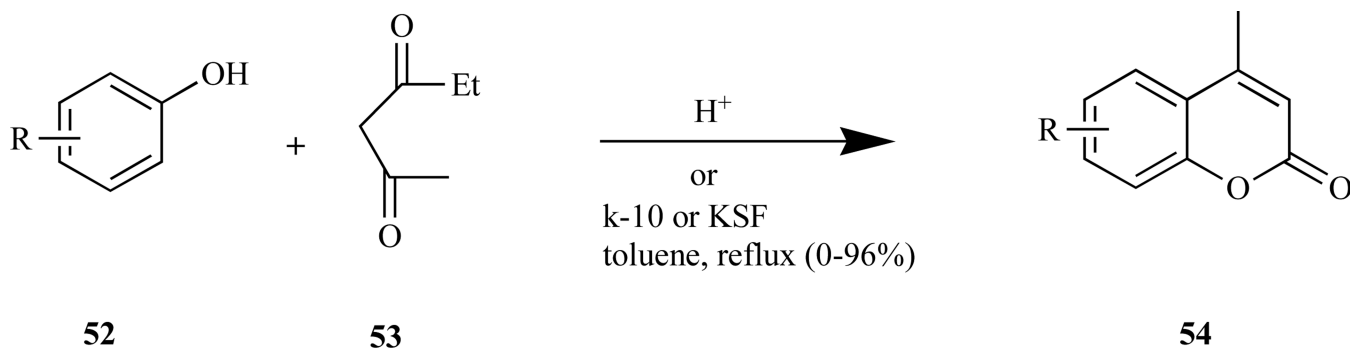
Scheme 1.
Pechmann reaction.
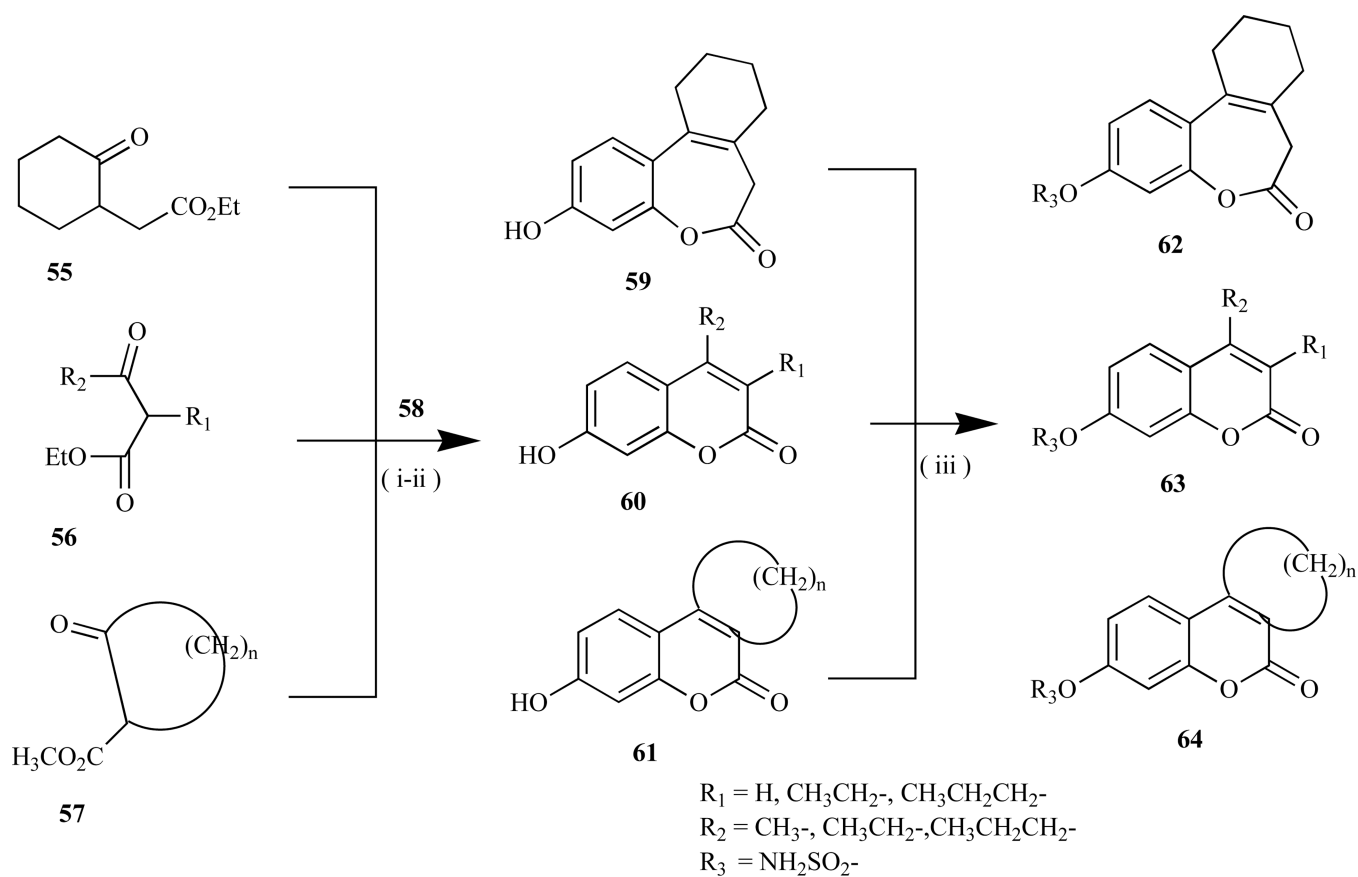
Scheme 2.
Reagents and conditions: (i) resorcinol (**58**), CF_3COOH/conc. H_2SO_4 (1:1), 0°C; (ii) NaH/DMF, H_2NSO_2Cl; (iii) K_2CO_3/$(Bu)_4N^+Cl^-.xH_2O$/H_2O/$CHCl_3$, r.t.

R_1 = H, CH_3CH_2-, $CH_3CH_2CH_2$-
R_2 = CH_3-, CH_3CH_2-,$CH_3CH_2CH_2$-
R_3 = NH_2SO_2-
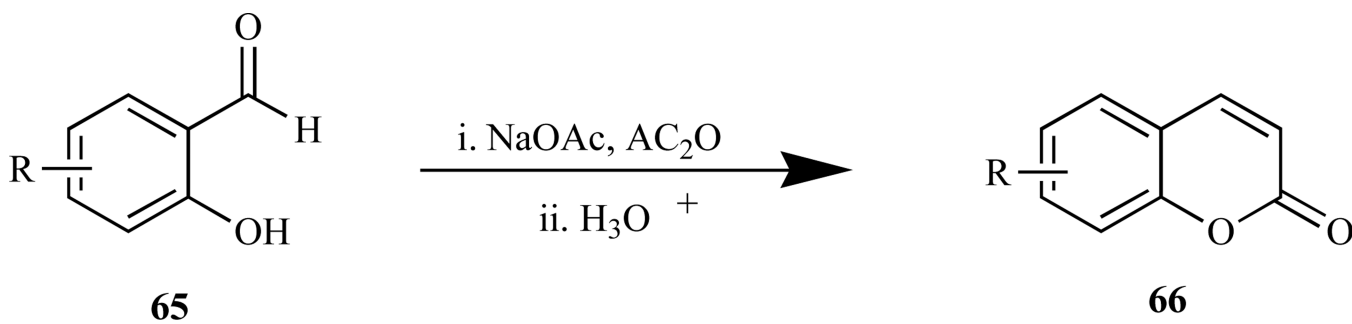
Scheme 3.
Perkin reaction.
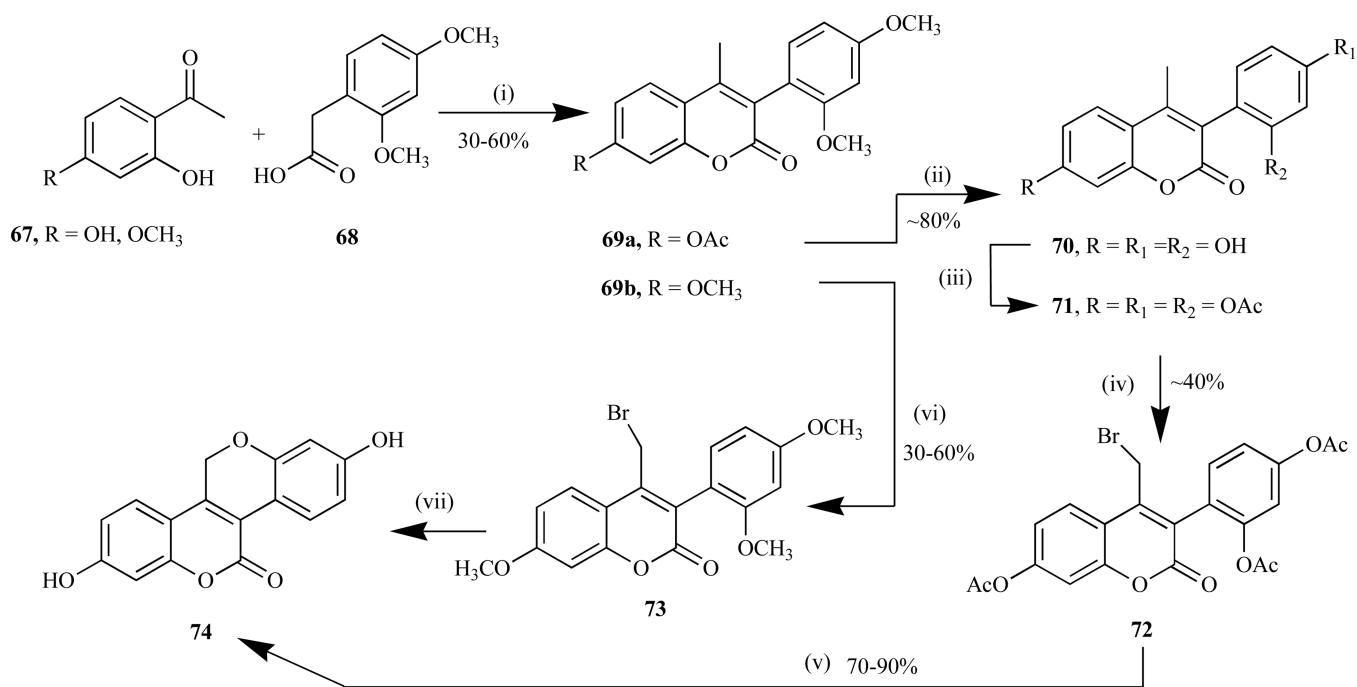
Scheme 4.
Reagents and conditions: (i) Et$_3$N, Ac$_2$O/reflux; ii) pyridine, HC1/200°C; iii) Ac$_2$O/ pyridine; iv) NBS /benzoyl peroxide, CCl$_4$/hv; v) K$_2$CO$_3$/MeOH/acetone; vi) LiHMDS/ THF/−32°C, then NBS/THF/78°C; vii) BBr$_3$/ DCM, then NaOH/H$^+$.

$$R_1 = CO_2H, CO_2Et, CONH_2, CN$$

$$R_2 = CO_2H, CO_2Et, CN$$
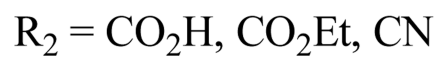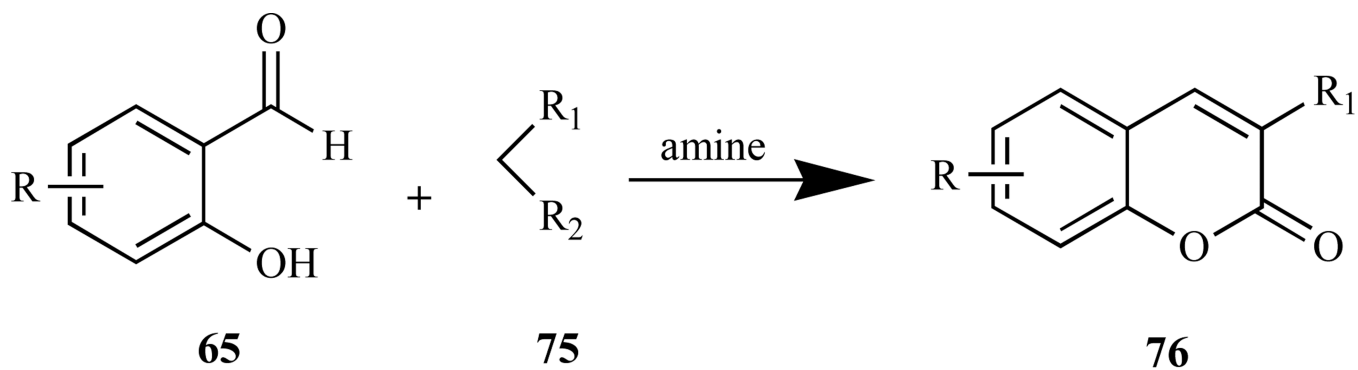
Scheme 5.
Knoevenagel reaction.
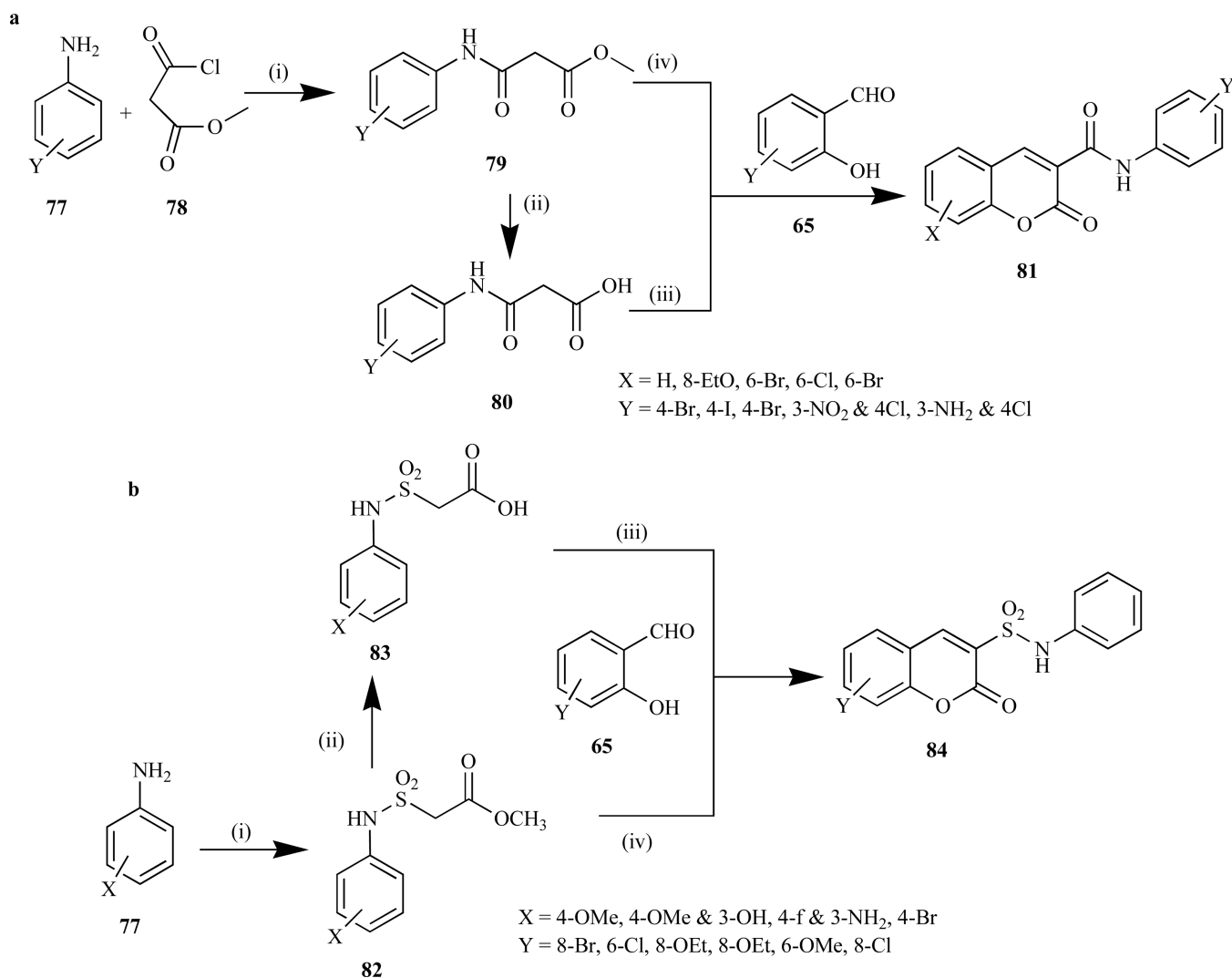
Scheme 6.
Reagents and conditions: i) Et_3N, CH_2Cl_2, 0°C to rt; ii) 10% NaOH, HCl; iii) $PhCH_2NH_2$, HOAc, reflux, iv) piperidine, ethanol, reflux 1 h.
b. Reagents and conditions: (i) $ClSO_2CH_2COOCH_3$, triethylamine/ THF, N2, rt, 3 h; (ii) 10% NaOH in H_2O; (iii) CH_3COOH, $C_6H_5CH_2NH_2$; (iv) piperidine, ethanol, reflux, 5 min.
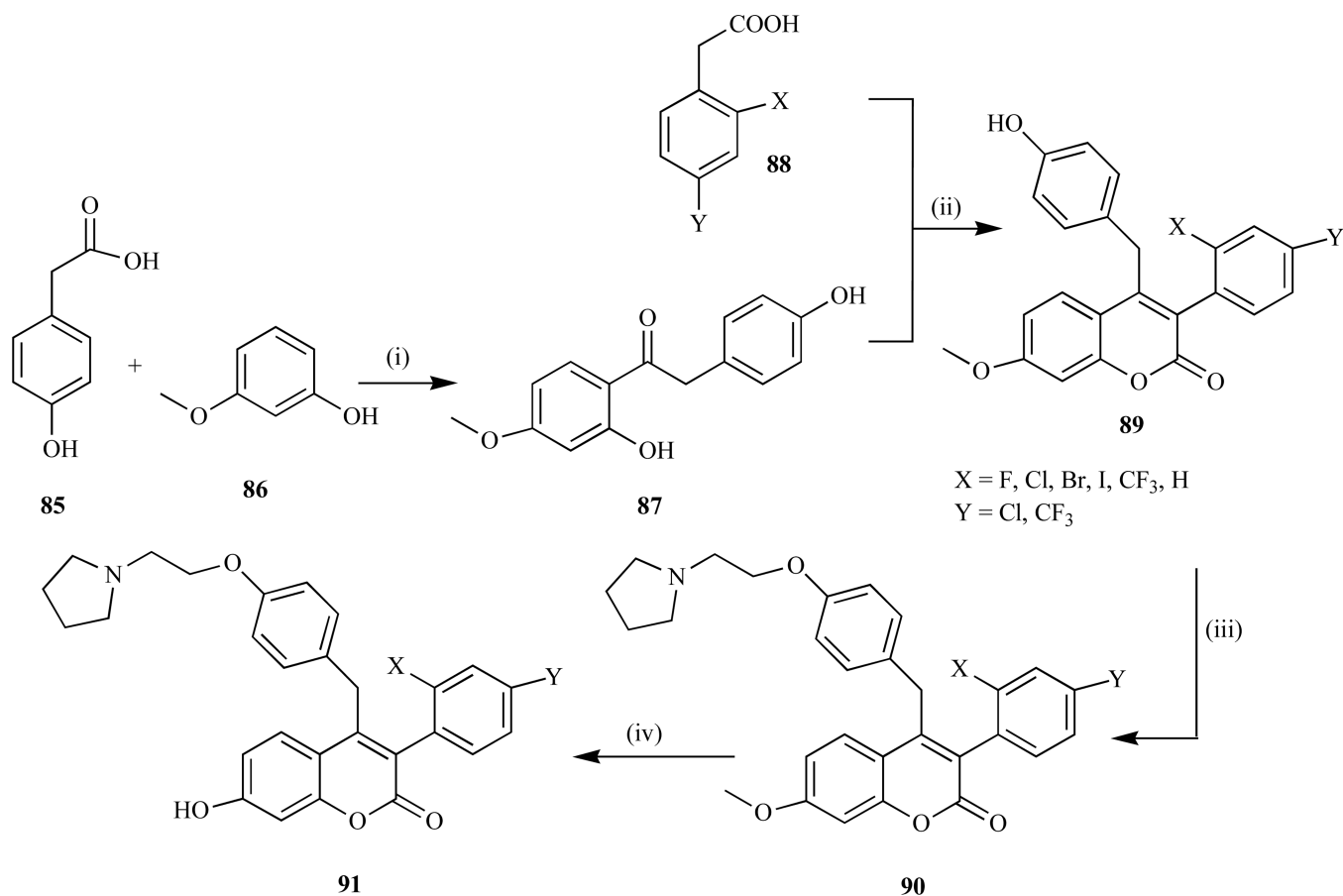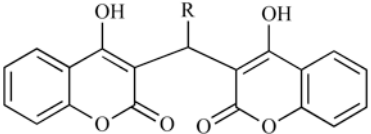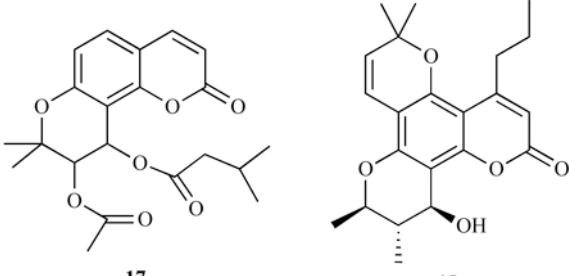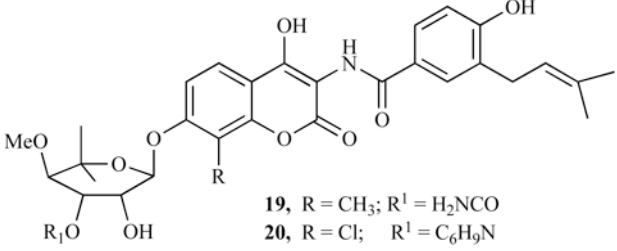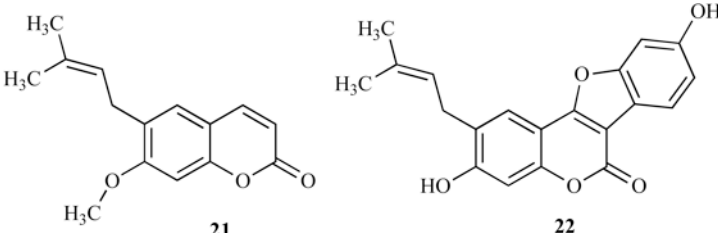
Scheme 7.
Reagents and conditions: i) BF3 diethyletherate (Fries reaction); ii) CDI, K_2CO_3, DMAP, 10–90%; iii) K_2CO_3, l-(2-chloroethyl)pyrrolidine, 30–75%; iv) 30–50% HBr, AcOH.
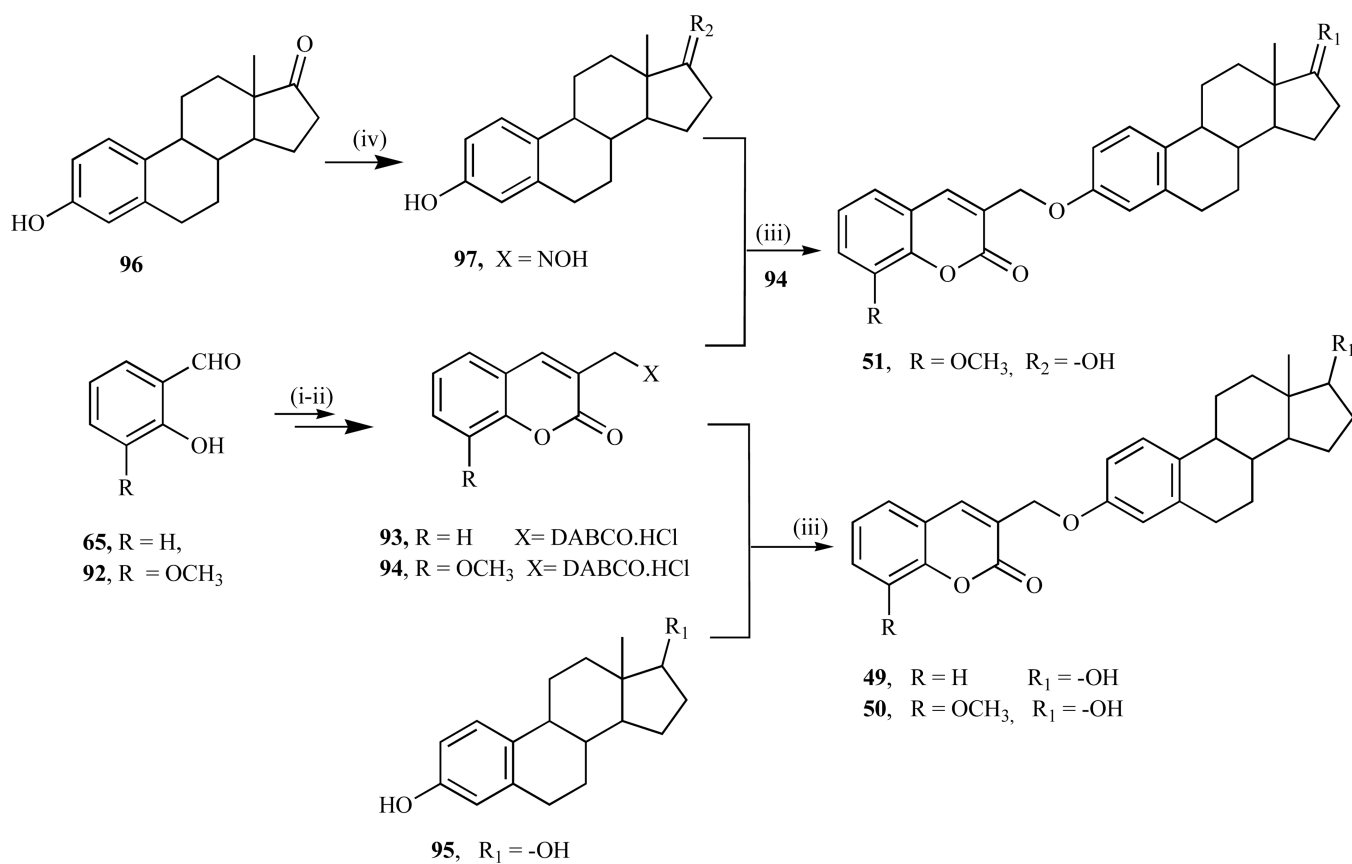
Scheme 8.
Reagents: i) Acryloychloride, NaH, reflux; ii) DABCO, CH_2C12, rt, iii) K_2CO_3, CH_3CN, reflux, overnight, iv) NH_2OH, THF, reflux.

Table 1

Some Biologically Active Coumarins

Examples	Biological Properties
14, R = H **15**, R = CH$_3$ **16**, R = CO$_2$Et	Anticoagulant
17 **18**	Anti-HIV (Reverse transcriptase inhibitors)
19, R = CH$_3$; R^1 = H$_2$NCO **20**, R = Cl; R^1 = C$_6$H$_9$N	Antibiotic and Antibacterial (DNA gyrase inhibitors)
21 **22**	Anticancer (Breast cancer, Stomach cancer, Colon cancer and Renal cancer)