Lactones: Generic Inhibitors of Enzymes?

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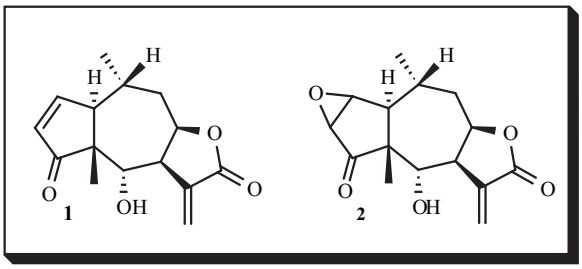
Abstract: The ability to affect eukaryotic and prokaryotic cellular growth, signaling and differentiation is a continuing focus in the pharmaceutical industry. The fundamental ability to affect these cellular processes is inherent in lactones. Lactones, which are ubiquitous in nature, reflect a broad phylogenetic diversity indicative of their ability to act as simple alkylating compounds, with their *in situ* activities falling into one of two categories, i.e., protect or conquer. Medically, their utility as pharmaceutical agents range from that of antimicrobial to anti-neoplastic agent depending on the functional groups attached.

INTRODUCTION

Although less well known than the lactams, as structurally simple alkylating agents, lactones can also function as enzyme inhibitors. This structure-function relationship appears to be highly conserved phylogenetically and taxonomically since lactones are produced by organisms in all five Kingdoms (Monera, Protista, Plantae, Fungi, Animalia) [1a-g]. Lactones have a wide range of functions, ranging from signaling molecules in bacteria (homoserine lactones) to inhibitors of NF-κB, the transcription regulator in mammalian cells. Overall, their basic functionality is to promote the survival of the organisms that produce them. For fungi and bacteria production of lactones containing compounds can eliminate the competition for an ecological niche, thus ensuring an adequate food supply. Those organisms that are pathogens appear to take this fight for survival a step further. In obligate pathogens, lactones acting as enzyme inhibitors appear to promote successful parasitism since they are capable of blocking the host defense machinery, by inhibiting NF-κB and nitric oxide generation [1h]. Plants appear to use lactones to prevent being eaten and to avoid biofouling by bacteria [1i-l], while members of Animalia use lactones to regulate their biochemical processes. Industry in its search for alternative novel antimicrobials is now looking to make use of lactone's anti-biofouling capabilities for industrial production applications. The function of lactones as immune system regulators and controllers of mammalian cell growth is also being exploited in the development of both anticancer drugs and cell cycle inhibitors. The focus of this review is to describe the major classes of lactones that have a demonstrated functionality as inhibitors of both mammalian and prokaryotic cells.

DNA POLYMERASE INHIBITORY ACTIVITY

Eukaryotic cells contain at least six types (α, β,γ, δ, ε and ζ) of DNA polymerases [2]. DNA polymerases α and β are required for nuclear DNA replication, DNA polymerase γ participates in mitochondrial DNA replication, DNA polymerases β and ε have been implicated to be involved in DNA repair, but there are also reports suggesting that these enzymes are related to recombination and DNA replication, respectively. DNA polymerase ζ may be also involved in the repair process. The goal for development of selective inhibitors is to aid in the elucidation of DNA polymerase functions and provide potential therapeutic aid in the treatment of cancer and HIV-1. While *in vitro* compounds having only a cyclopentenone ring, such as lactone **1** or an epoxicyclopentanone system **2,** may only marginally inhibit DNA polymerase, *in vivo,* compound **1**, was shown to be an effective inhibitor of nuclei DNA polymerase activity in Ehrlich ascites tumor cells [3], probably due to the supposedly exposed sulfhydril groups of the alpha and gamma DNA polymerase enzymes [4, 5] that can be alkylated. Furthermore, sesquiterpene lactones containing two of these moieties were demonstrated to be more active as inhibitors of DNA polymerase, as compared to the similar compounds having only one of them [3].



Other lactones, which include the terpenoids, exhibit activity against DNA polymerases. Sakaguchi and colleagues recently reported the isolation of three terpenoids, **3**, **4**, **5** from the fruiting body of the basidiomycete *Ganoderma lucidum*, a medicinal mushroom used in traditional Chinese medicine for cancer chemotherapy. Of the three terpenoids isolated, **4** contains a lactone ring at position 17 [6]. Both lucidenic acid O, **4** and lactone **5** appear to be selective inhibitors of mammalian DNA polymerases *in vitro* with steroid **5**, demonstrated to be a dose dependent inhibitor for DNA polymerase α, (IC_{50} of 84μM). The inhibition of DNA polymerase α by **3** and **4** was also dose dependent, with an IC_{50} 35 and 42 μM, respectively, while for DNA polymerase β the IC_{50} was 72 and 99μM, respectively. In addition, to their ability to inhibit DNA dependent-DNA polymerases, both **3** and **4** also exhibited activity against RNA-dependent DNA polymerase, a.k.a. reverse

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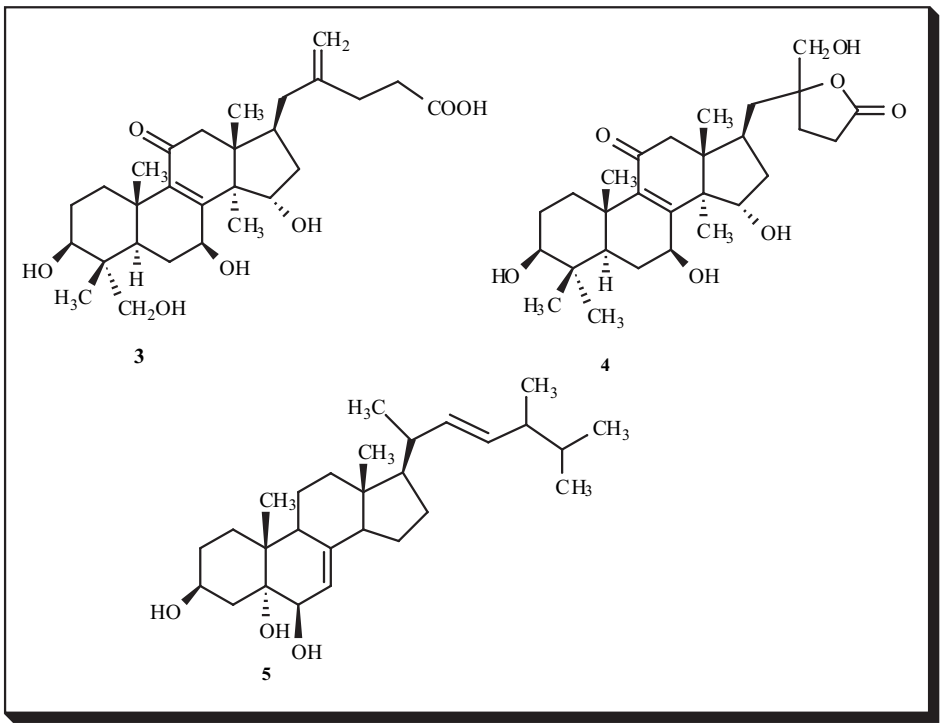
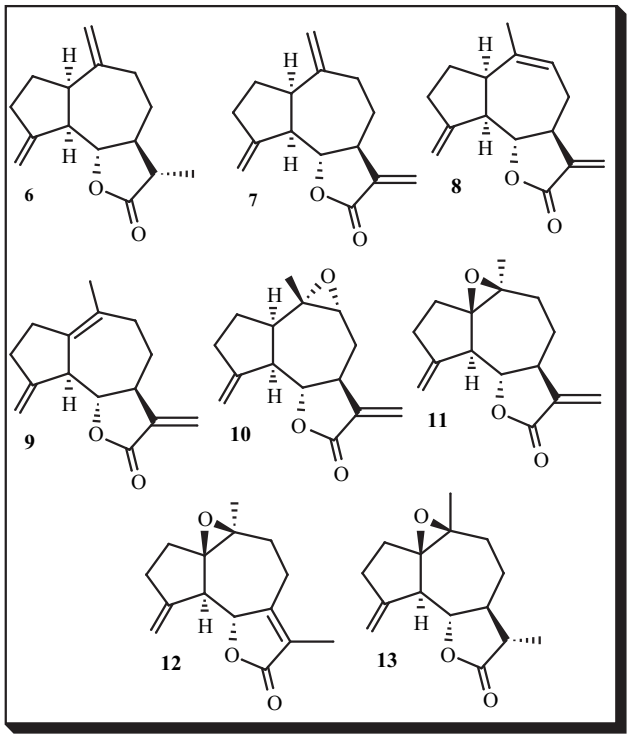


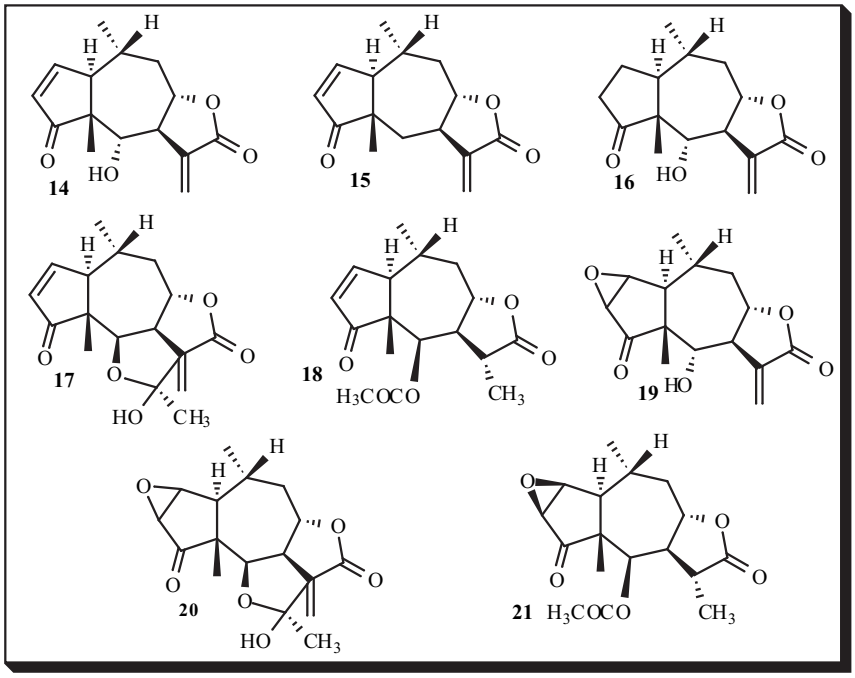
transcriptase. This additional activity resulted in inhibition of the human immunodeficiency virus type 1 (HIV-1) reverse transcriptase at an IC_{50} of 67 and 69μM, respectively. Further investigation into the spectrum of anti-polymerase activities of these compounds may provide a new direction for anti-retroviral therapy.



REGULATION OF NF-κB ACTIVITY

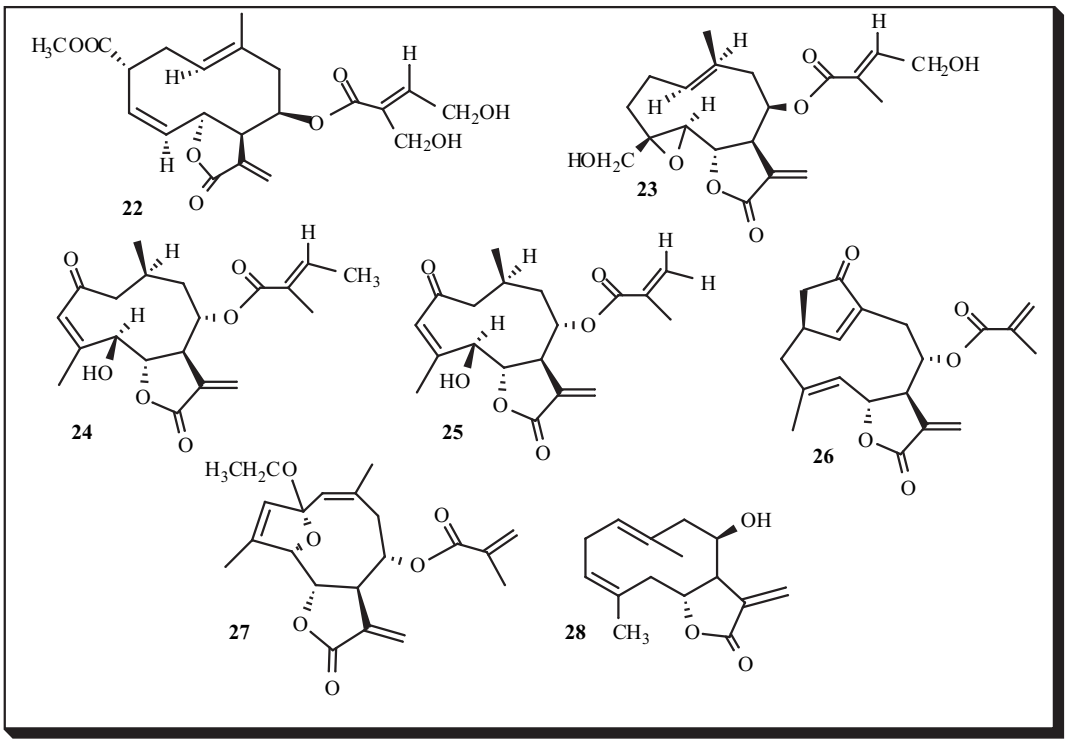
The ability to regulate NF-κB can have a multivariant effect on immune function and cellular survival. As a transcription factor that enhances the production of inflammatory mediators, NF-κB is now understood to be an essential element of the immune system, controlling immune and inflammatory responses, lymphoid organ structure, apoptosis, stress response, development of hematopoietic cells and neoplasms. NK-κB regulates the transcription of inflammatory cytokines, such as IL-1, IL-2, IL-6, IL-8 and TNF-α, as well as genes encoding cyclooxygenase-II, nitric oxide synthase, hematopoetic growth factors, growth factor receptors, immunoreceptors, and cell adhesion molecules [7h-i].In addition, this transcription factor is essential for the life cycle of various viruses, including HTLV, the etiologic agent for Adult T Cell Leukemia and HIV, the cause of AIDS, which use it for their own replication.

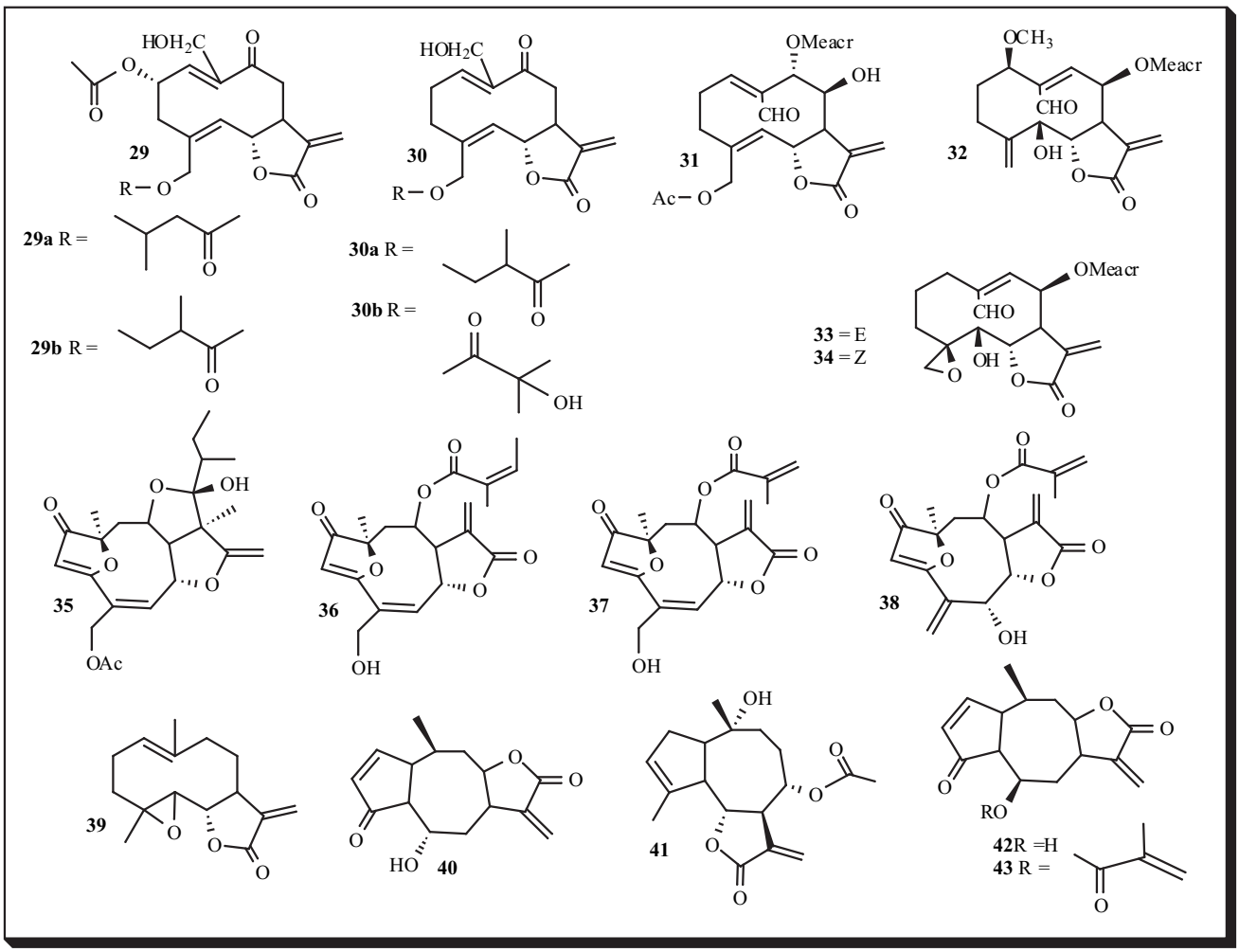
Considerable effort has gone into preparation of synthetic compounds having the α-methylene-γ-lactone moiety as regulators of NF-κB [8a, b]. Some of these efforts were directed toward synthesis of the naturally occurring guanolides, e.g. mokko lactone **6**, dehydrocostus lactone **7**, eremantin **8** and compounds related to these three core structure derivatives [9]. It was found that the overall effect of compounds having an α-methylene-γ-lactone moiety, such as **7**, **8, 9**, **10**, **11**, and **12** was to significantly inhibit the cytotoxic activity of T cytotoxic lymphocytes (CTL), as well as the induction of intercellular adhesion molecule-1 (ICAM-1) which results in a down-regulation of immune response. In fact, the α-methylene-γ-lactone **11** has shown a 14-fold higher activity than the corresponding endo-unsaturated γ-lactone **12** and 50-fold higher activity than the corresponding saturated α-methylene-γ-lactone **13**. The extent of immunoinhibitory activity has been correlated to the location of the double bond in the B-ring and by the introduction of the epoxide ring at the C-9 and C-1(10) positions. Thus, the epoxide **10** possesses an 8-fold higher activity than that of the corresponding unsaturated



compound, eremantin **8**. The most active compound obtained in this work was the α-methylene-γ-lactone derivative **10**. It also appears that the possible target of inhibition of ICAM-1 induction may be different from that resulting in the inhibition of the killing function of CTL cells. This anti-CTL activity appears to have potential in the treatment of several inflammatory and degenerative central nervous system diseases, such as multiple sclerosis (MS), virus-induced inflammatory brain diseases and paraneoplastic neurological disorders as well as other autoimmune disorders such as type 1 diabetes, and ulcerative colitis [10a, b].

Studies determining how sesquiterpene lactones, which inhibit the transcription factor NF-κB at μM concentrations, exert their anti-inflammatory effect have shown that these structures modulate inflammatory proces including oxidative phosphorylation, platelet aggregation, histamine and serotonin release [7b-f]. Sesquiterpene lactones, isolated from numerous species of *Compositae,* posses a wide variety of biological activities, including anti-inflammatory effects [7a]. Sesquiterpene lactones containing an α-methylene-γ-lactone moiety are potent inhibitors of carrageenan-induced edema and chronic adjuvant-induced arthritis in rodents [7b]. The pseudoguanolides **14-16** and germacranolides **22**-**28** at

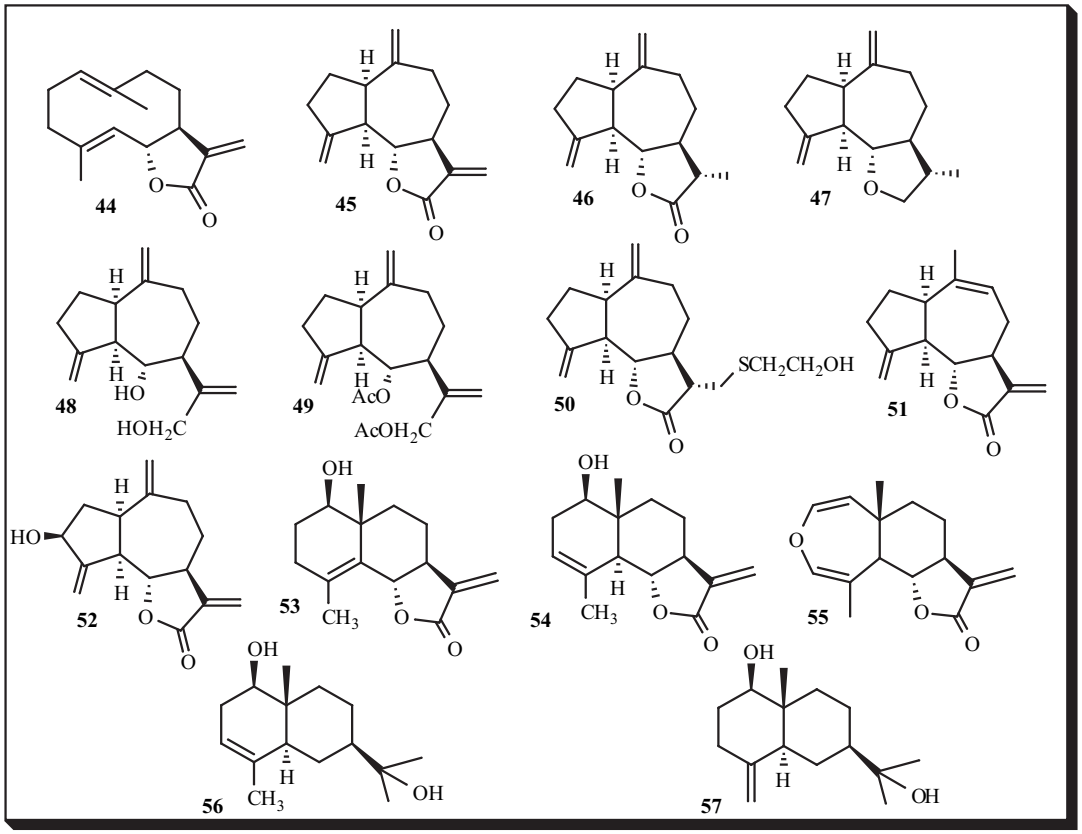




2.5mg/kg significantly suppressed inflammation in rats. In a carrageenan-induced edema screen, the α-methylene-γ-lactone moiety was shown to play a major role in the sequiterpene lactones anti-inflammatory activity. An epoxycyclopentanone system contributed to the anti-inflammatory activity of compounds **19**-**21** against the induced edema. In a chronic adjuvant-induced arthritic screen, not only did the α-methylene-γ-lactone and α-epoxycyclopentanone system contribute significantly to the anti-arthritic activity, but the β-unsubstituted cyclopentanone ring in **17** and **18** also instilled anti-arthritic activity in the sesquiterpene lactones.

Sesquiterpene lactones appear to inhibit NF-kB by selectively alkylating its p65 subunit probably by reacting with cysteine residues. Merfort's group proposed a mechanism of action of the sesquterpene lactones based on their study of inhibition of NF-kB by 28 lactones from different structural classes. The most potent group of compounds – **29**-**31**, **32**-**34**, **35**-**38**, and **39**-**43**, comprise molecules of different skeletal types, namely pseudoguanolides –**40**, **42**, and **43**, guanolides, **41**, germacranolides **29** and **39**, heliangolides **35**-**38**, melampolides **30, 31** and 4,5-dehydrogermacranolides **32**-**34**. With three exceptions –**35**, **30**, and **41**, these potent inhibitors posses at least one further α,β- or α,β, γ, δ-unsaturated carbonyl group in addition to α-methylene-γ-lactone group, and may thus be considered bifunctional Michael acceptors. Some compounds, which possess additional α, β-unsaturated acyl side chain may even be considered trifunctional.

Two of the compounds, which also posses more than one potentially alkylating group, have intermediate activity. Based on computer modeling [7d], Merfort and co-workers proposed a molecular mechanism of action which is able to explain the high inhibitory activity with bifunctional Michael acceptor capability. An associated single bifunctional sesquiterpene molecule can alkylate the cysteine residue (Cys 38) in DNA binding loop 1 (L1) and a further cysteine (Cys 120) in the nearby E' region. This cross-link alters the position of tyrosine 36 and additional amino acids in such a way that their specific interactions with the DNA become impossible. Further investigation of four germacranolide sesquiterpenes which differ in skeleton and the number of reactive centers **29**, **33**, **35** and **30b**, was performed to determine their effect on production of proinflammatory cytokines (interleukin 1-β, (IL-1β), Il-6, and tumor necrosis factor-α (TNF-α), as well as mouse lymphocyte proliferation in response to the mitogens concanavalin A (Con A) and lipopolysaccharide (LPS) [7f]. Compounds **29** and **33** which possess an α-methylene-γ-lactone moiety and a conjugated carbonyl group induced a half-maximal inhibition of cytokine synthesis in adherent mouse peritoneal exudate cells at μM concentrations, while an isobutyryloxy derivative of **30b** which contains only an

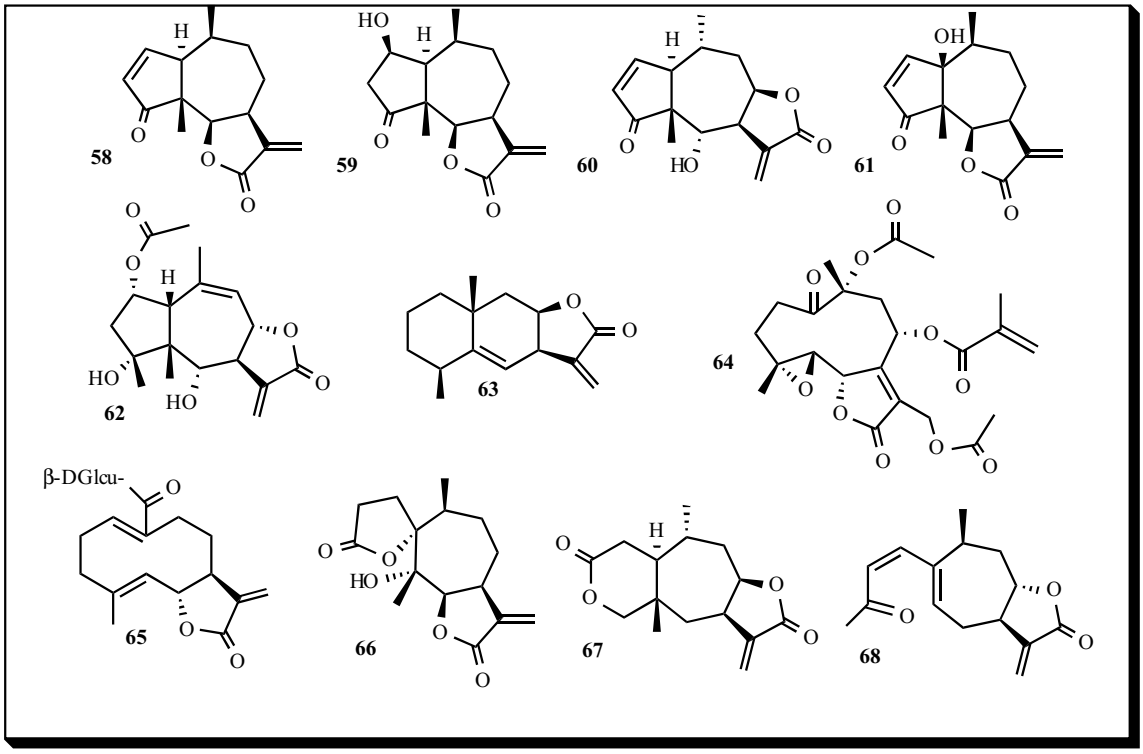


α-methylene-γ-lactone residue was less active. Compound **35**, which carries only a conjugated keto group, displayed potency similar to those of the bifunctional compounds **29** and **31**. All four lactones suppressed proliferation of murine lymphocytes at μM concentrations. Inhibitory concentrations correlated well with those necessary for inhibition of NF-κB. Therefore, it was assumed that NF-κB may be involved in the suppressive effect of sesquiterpene lactones on cytokine production and lymphocyte proliferation.

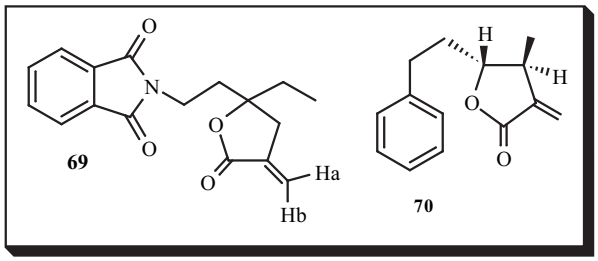
Results suggest that, as one of their mechanisms of action, sesquiterpene lactones may also induce heat shock protein (HSP 72) thereby preventing NF-κB activation followed by iNOS induction [11] were obtained with sesquiterpene lactones in methanolic extracts from the leaves of *Laurus nobilis* (bay leaf), used in European folk medicine and shown to inhibit NO production in lipopolysaccharide (LPS)-activated mouse peritoneal macrophages [11]. Of the fourteen isolated from the active methanolic fraction, as determined by bioassay-guided separation, seven lactones **44**-**55** inhibited LPS-induced NO production at the μM range. α-Methylene-γ-butyrolactone also showed inhibitory activity, while compounds such as **56**-**57** lacking the α-methyle-γ-butyrolactone showed little activity. These results indicate that this moiety is important for the activity. Furthermore, lactones **51** and **52** inhibited inducible iNOS induction in accordance with induction of HSP 72.

Another study on the structural requirements for the sesquiterpene lactones regarding their capability to inhibit iNOS-dependent NO synthesis, examined lactones **58**-**68** for their influence on nitrite accumulation in cell culture supernatants of LPS-induced RAW 264.7 macrophages [12]. Except for compound **65,** all lactones showed a dose-dependent inhibition of nitrite accumulation at the μM range. High activity seemed to be dependent on the α-methylene-γ-lactone functionality. Another objective of this study was to determine whether the structural requirements of sesquiterpene lactones for these activities differ from those needed to inhibit iNOS-dependent NO synthesis. Interestingly, compounds almost equally effective in inhibiting nitrite accumulation did not show the same cytotoxic potential, and most sesquiterpene lactones inhibited nitrite accumulation at concentrations where inhibition of NF-κB activation was not significant. These results suggest that different biological activities of sesquiterpene lactones have different structural requirements [12].

Another platform for the development of small molecules with anti-NF-κB activity, but directed towards its role in neoplasms, is preparation of synthetic compounds containing an α-methylene-γ-lactone group connected to an aromatic system, which have also been shown to act as Michael acceptors. An α-methylene-γ-lactone derivative of phtalamide **69** was prepared [13] based on reports that the phthalimido moiety had served as the carrier molecule for different anti-tumor functionalities. Additionally, since phthalimido derivatives containing the α-methylene-γ-lactone may have synergistic activity, a library of compounds having different substituents in the aromatic part was synthesized. For example, compound **69** exhibited marginal to moderate *in vivo* activity against Ehrlich ascites carcinoma (EAC) and sarcoma-180 (S-180), respectively, and significant *in vitro* cytotoxicity against SF-268, a human CNS tumor cell line. The combination of an aromatic moiety and α-methylene-γ-lactone moiety, (compound **70**)



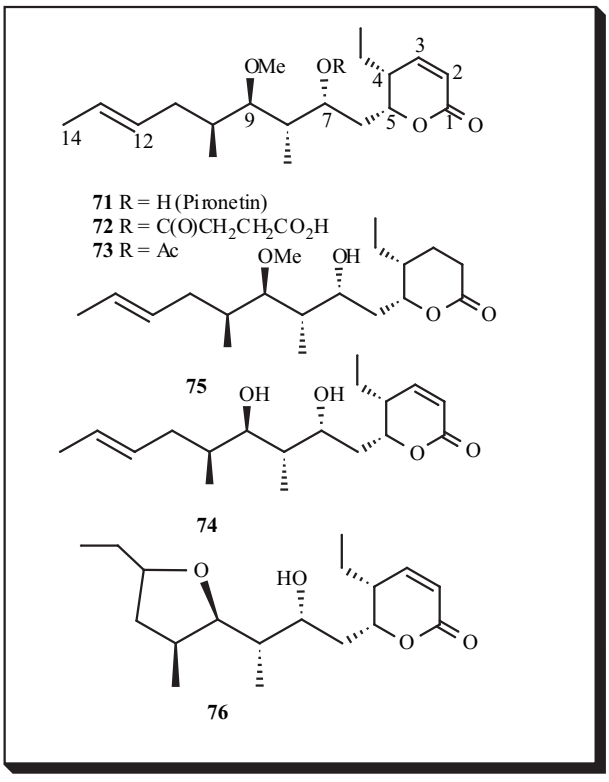
was shown to induce apoptosis in the HL-60 cell line, and has the capacity to inhibit mitogen-induced proliferation of murine splenocytes and peripheral blood mononuclear cells (PBMC) with no apparent toxic side effects [14]. These findings are very similar to those found for the thiol alkylating agent imexon, a cyanohydride derivative that induces apoptosis in multiple myeloma cells, whereas normal lymphocytes are less sensitive [14]. Further definition of the molecular steps and individual components involved in NF-kB activation *via* specific receptors has and will continue to provide potential targets for therapeutic approaches intended to inhibit NF-kB activity in various inflammatory, neoplastic and viral diseases.

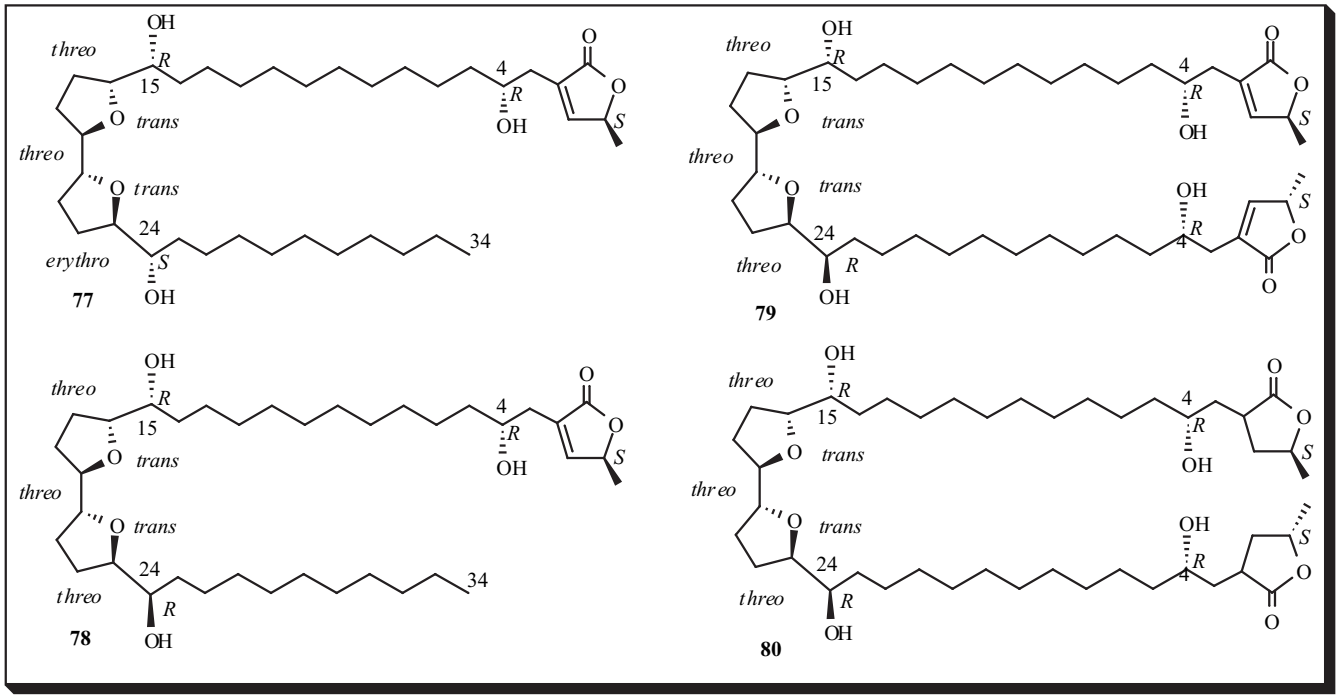


INHIBITION OF MICROTUBULE ASSEMBLY

The ability of compounds to inhibit microtubule assembly has been correlated with anti-neoplastic activity. Pirotenin **71** [15a, b] and demethylpirotenin **74** [15c] not only regulate plant growth but have immunosuppressive and antitumor activity [15d]. Investigation of the mechanism of anti-tumor activity, demonstrated that **71** and **74** are potent inhibitors of tubulin assembly [15e]. Compound **71** directly binds to tubulin and inhibits the tubulin assembly in a vinblastine-like manner. Interestingly, although its effective cytotoxic dose is slightly higher than that of vinblastine, its K_d value is 10-fold lower, indicating that the affinity of pirotenin to tubulin is stronger than that of vinblastine. However, **71**'s membrane permeability appears to be less than that of vinblastine.

Compounds **71** and **74** are unique structures having only one pyran residue and an alkyl chain. The structures are simpler compared to other M-phase inhibitors. Based on structure-function studies, the features needed for anti-tumor



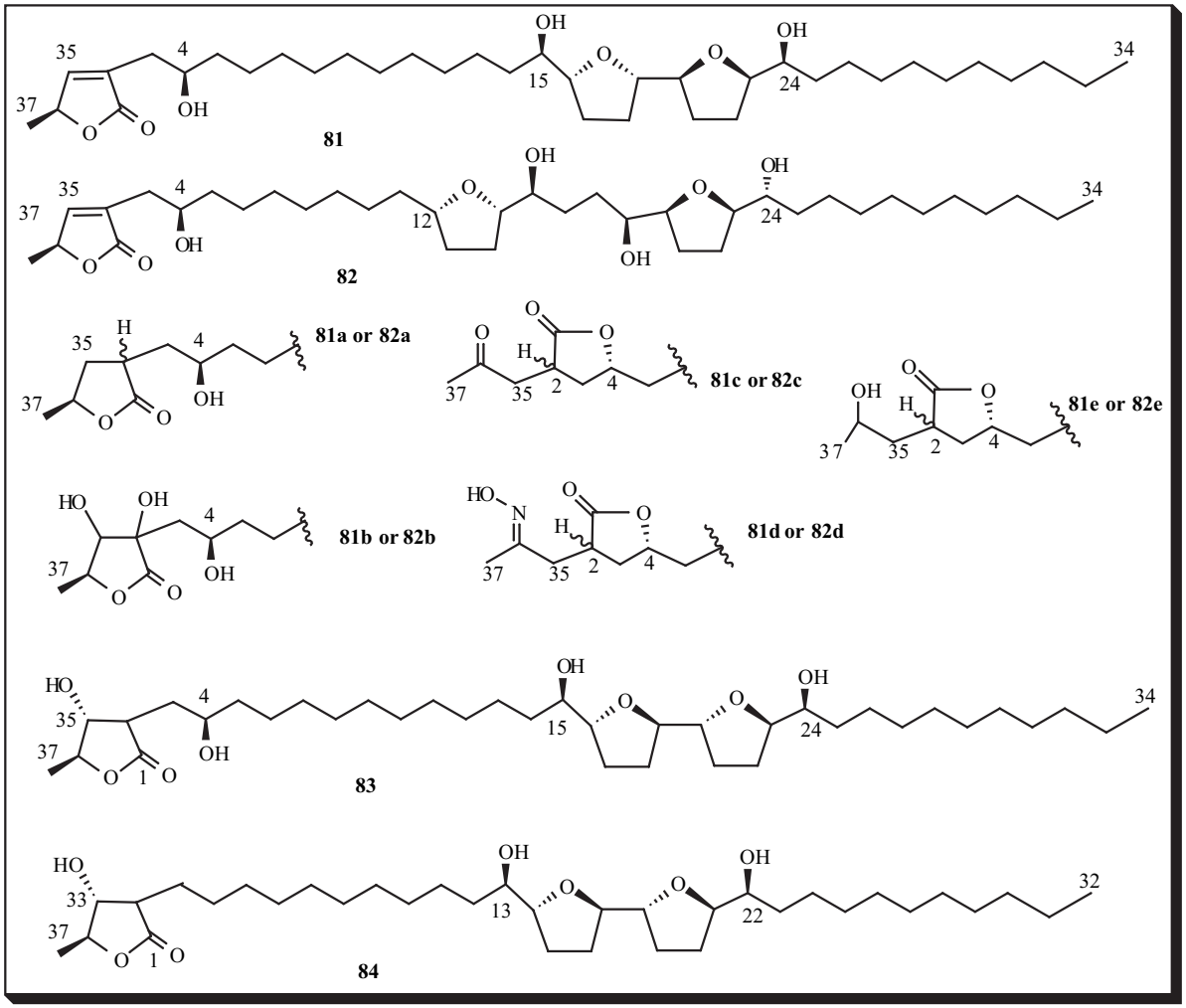


activity appeared to be an – α,β-unsaturated lactone, while chirality of the hydroxyl group at the 7-position, and the terminal portion of the alkyl chain are important for inhibition of tubulin assembly [16]. For example, esterification of the 7-OH group as in **72** and **73** resulted in a 40- to 100-times decrease in *in situ* activity. Further, saturation of the lactone moiety also led to a dramatic decrease in the activity of compound **75**. Recently, it was reported that lactone-containing drugs bind to their target biomolecules covalently. Based on this, it appears that the α,β-unsaturated lactone may be necessary for tubulin assembly inhibition through covalent binding. In addition, replacement of the branched alkyl chain with furan-type ring in compound **76**, also results in a decline in activity; suggesting that the structure of the terminal portion of the alkyl chain is essential for inhibition of microtubule assembly.

MITOCHONDRIAL NADH:UBIQUINONE OXIDO-REDUCTASE (COMPLEX 1) INHIBITION

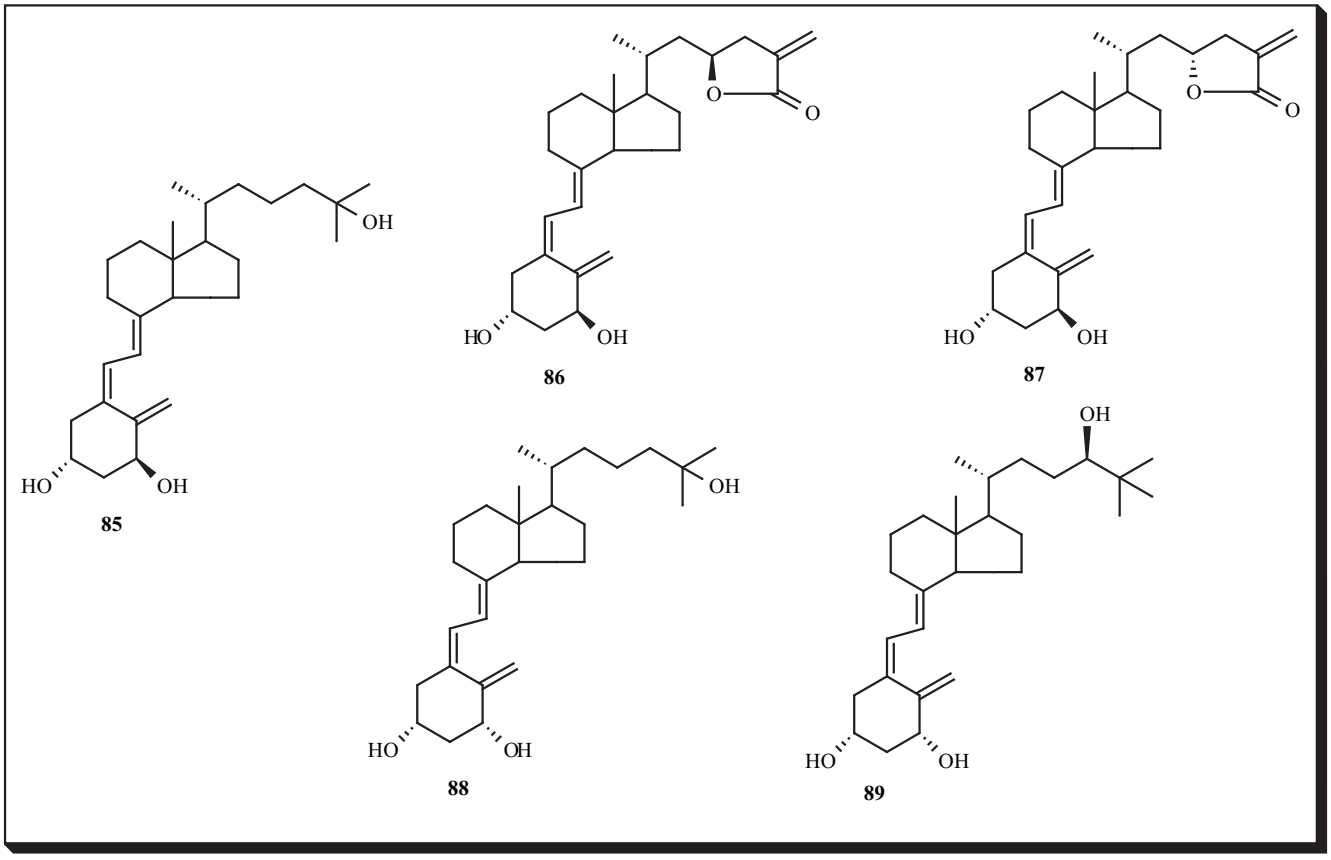
The proton-pumping NADH:ubiquinone oxidoreductase, also called complex I, is the first of the respiratory complexes providing the proton motive force which is essential for the synthesis of ATP. Certain natural acetogenins are extremely competent inhibitors of complex I. Many of the acetogenins have diverse biological activities, such as cytotoxicity, anti-tumor, anti-malarial, pesticidal and anti-feedant activities [17a,b,c.d]. Acetogenins are characterized by two functional units: a hydroxylated tetrahydrofuran (THF) unit and α,β-unsaturated-γ-lactone moieties, separated by a long alkyl spacer. A large number of natural acetogenins (also called acetogenins of Annonaceae (ACG) have been isolated from several genera of the plant family *Annonaceae*. Acetogenin's inhibitory effect(s) on mitochondrial complex I are important to note for the following reasons: (a) the diverse biological activities are believed to be due to this effect [17a, 17d, 17e-g]; and (b) some of the compounds, such as bullatacin, **77**, are the most potent inhibitors of this enzyme identified to date [17e, 17g, 17h]. However, it is difficult to structurally compare acetogenins with ordinary complex I inhibitors, such as pierecidin A and rotenone, even though the acetogenins act at the terminal electron transfer step of the complex, in a manner similar to that reported for other complex I inhibitors [17h, 17i]. Several important structural features of acetogenins were identified through structure-activity relationship (SAR) studies using both naturally occurring acetogenins [17e] and compounds obtained synthetically. [18a,b,c,d]. Based on the SAR studies, McLaughlin and colleagues [19a,b] proposed that in a membrane environment, the active conformation of these inhibitors has two particular characteristics. First, the THF ring(s), with flanking hydroxy groups, resides near the glycerol backbone of phosphatidylcholine where it acts as a hydrophilic anchor at the membrane surface. Secondly, the γ-lactone ring interacts directly with the target site of complex I (probably at the ubiquinone reductive site) [17h] by lateral diffusion into the interior of the mitochondrial membrane. Data that seems to support the necessity of unsaturation in the natural lactone ring [19a,c] is the observation of a significant decrease (about 10^{-6} fold) in the cytotoxicity of bullatacin **77**, against carcinoma cells after saturation of the double bond in the α,β-unsaturated-γ-lactone ring.

Based on the work of Miyoshi and colleagues who delineated the components of natural acetogenins essential for mitochondrial complex I inhibition, simplification of synthetic protocols should be greatly facilitated, allowing for increased ease in the preparation of structural analogs such as **78**-**80** [20]. Briefly, they showed that there is a high degree of tolerance in the active site of the enzyme toward several structural features in the acetogenins. This indicates that neither the number of THF rings nor the stereochemistry surrounding the THF ring moiety are crucial structural



factors [17e,17d, 19d]. Furthermore, the hydrogen-bond donating ability of the OH groups is favorable, but not crucial for the inhibitor to have a potent effect. In contrast, some of the synthetic compounds which have a saturated γ-lactone ring also demonstrate good inhibitory properties; an observation difficult to explain, since natural acetogenins contain an unsaturated γ-lactone ring, which was assumed to be the sole reactive moiety interacting directly with the binding site of complex I. However, as has been pointed by Miyoshi in a recent review, [17j] essential structural factors of complex I inhibitors that drastically affect the inhibitory potency are not necessarily obvious, perhaps due to the large cavity-like inhibitor binding domain of the enzyme, in contrast to complex III inhibitors. What is imperative for activity as an effective inhibitor of the mitochondrial complex I, is the presence of a THF ring and a γ-lactone ring either linked directly, or linked by an alkyl spacer. Further studies on the design and synthesis of wide structurally modified acetogenins might lead to simplified, yet potent inhibitors of the mitochondrial complex I.

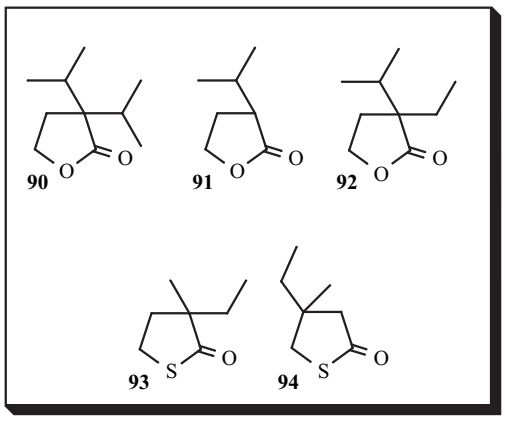
The relevance of the γ-lactone moiety itself for potent complex I inhibition was determined by Cortes *et al*. through their preparation of two series of semisynthetic ACG. One series of compounds was related to rolliniastatin-I, **81**, and the second series to cherimolin-I, **82**, with modifications only in the terminal α,β-unsaturated-γ-lactone moiety and the close 4-hydroxy group of the alkyl chain spacer [21a]. The results showed that all rolliniastatin-I derivatives, such as **81a**, **81e** were more potent than the natural compound **81**. The authors speculated that this increase in activity could be due to the shorter distance between the THF system and the terminal γ-lactone in accordance with previous results obtained for rollimembrin. Compound **81e** demonstrated the highest potency found to date for a complex I inhibitor. It appears that the presence of hydroxyl groups in the core of the lactone correlate with the potency of these compounds. Confirming the relevance of the hydroxyl group placed in the terminal saturated γ-lactone moiety, especially at a proper length from the THF system, are compounds laherradurin **83** and itrabin **84** which exhibit high levels of activity as complex I inhibitors. However, a different THF system and the consequent different length of the alkyl spacer between this and the terminal lactone moiety also appear to play a role in the diminished enzyme inhibition of derivatives **82a**, **82c** and **82d,** which were more potent than **82**, but less potent than rolliniastatin-I derivatives [21a, b]. Cortes' *et al*. studies on semisynthetic derivatives of ACG [21b] also indicated that the terminal lactone of the long and flexible ACG structure contributes significantly to tight binding to the enzyme and potent inhibitory activity, findings which should promote optimal design of antitumor drugs.



NUCLEAR VITAMIN D RECEPTOR INHIBITION

1α,25-Dihydroxyvitamin D_3 (1α,25-$(OH)_2D_3$) **85** regulates a wide variety of biological functions. Its fundamental activities include stimulation of intestinal calcium absorption and increase of bone calcium mobilization [22a, b]. Recently, other functions for 1α,25-$(OH)_2D_3$ have been reported, such as inhibition of cell proliferation, induction of cell differentiation, [22c], modulation of immunological responses, [18d], stimulation of insulin secretion [22e, f] and neurobiological functions [22g, h]. It is believed that 1α,25-$(OH)_2D_3$ mediates biological responses as a consequence of its interaction with a nuclear vitamin D receptor (VDR) to regulate gene transcription [22i, j] and with a putative membrane receptor resulting in rapid non-genomic effects [22k] including the opening of voltage-gated calcium and chloride channels [22l] and activation of mitogen-activated protein kinase [22m].

Over the years, a considerable number of vitamin D analogs have been synthesized which have helped clarify vitamin D's mode of action, and direct the development of therapeutically useful compounds. Recently, it has been found that two 1α,25-dihydroxyvitamin D_3-26,23-lactone analogs, (23S)-25-dehydro-1α-hydroxyvitamin D3-26,23 lactone (TEI-9647) **86** and (23R)-25-dehydro-1α-hydroxyvitamin D3-26,23-lactone (TEI-9648) **87,** inhibit human leukemia cell HL-60 differentiation induced by 1α,25-$(OH)_2D_3$ [23a]. These results strongly suggest that **86** and **87** might be antagonists of VDR/VDRE-mediated genomic actions of 1α,25-$(OH)_2D_3$, since HL-60 cell differentiation initiated by 1α,25-$(OH)_2D_3$ is believed to occur through a VDR/VDRE-mediated pathway. Several other 1α,25-dihydroxyvitamin D_3-26,23-lactone analogs were synthesized [23b] to determine whether these compounds could also antagonize 1α,25-$(OH)_2D_3$**,** but via the alternate non-genomic receptor pathway. Analogs TEI-9647, **86**, TEI-9648, **87** suppressed 1α,25-$(OH)_2D_3$-mediated HL-60 cell differentiation, but not NB4 cell differentiation, a model used to study non-genomic actions and signaling pathways [23c]. However, 1β-25-$(OH)_2D_3$, **88** and 1β-24R-hydroxyvitamin D3, (1β-24R-$(OH)_2D_3$), **89**, did suppress 1α,25-$(OH)_2D_3$ - mediated NB4 cell differentiation. These results indicate that **88** and **89** may be the first antagonists specific for 1α,25-$(OH)_2D_3$ -mediated genomic actions. It is still uncertain which factors will be imperative for non-genomic actions; however, it seems that both the orientation of C-1 and the side-chain structure may be important since both would affect affinity for the putative receptors, or signal transduction.



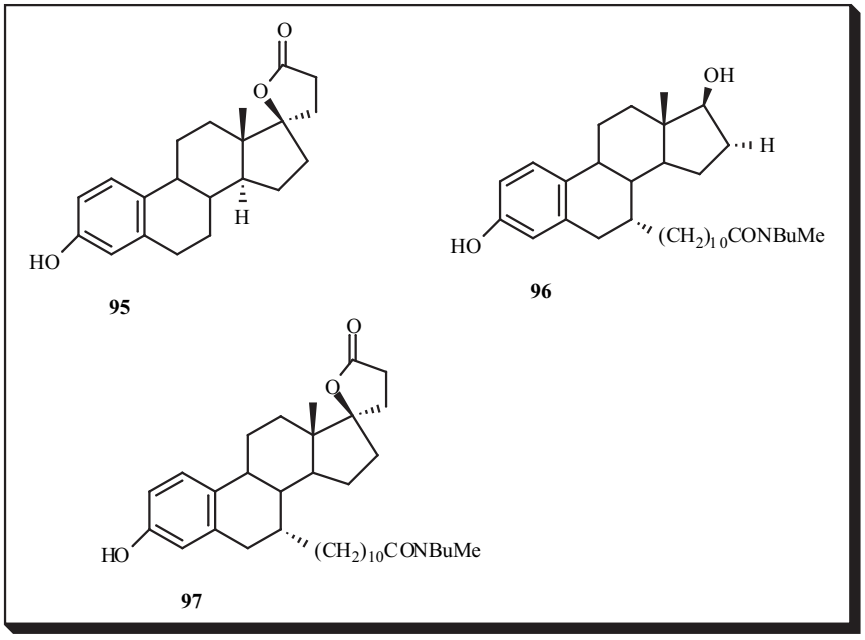
$GABA_a$ REGULATORS

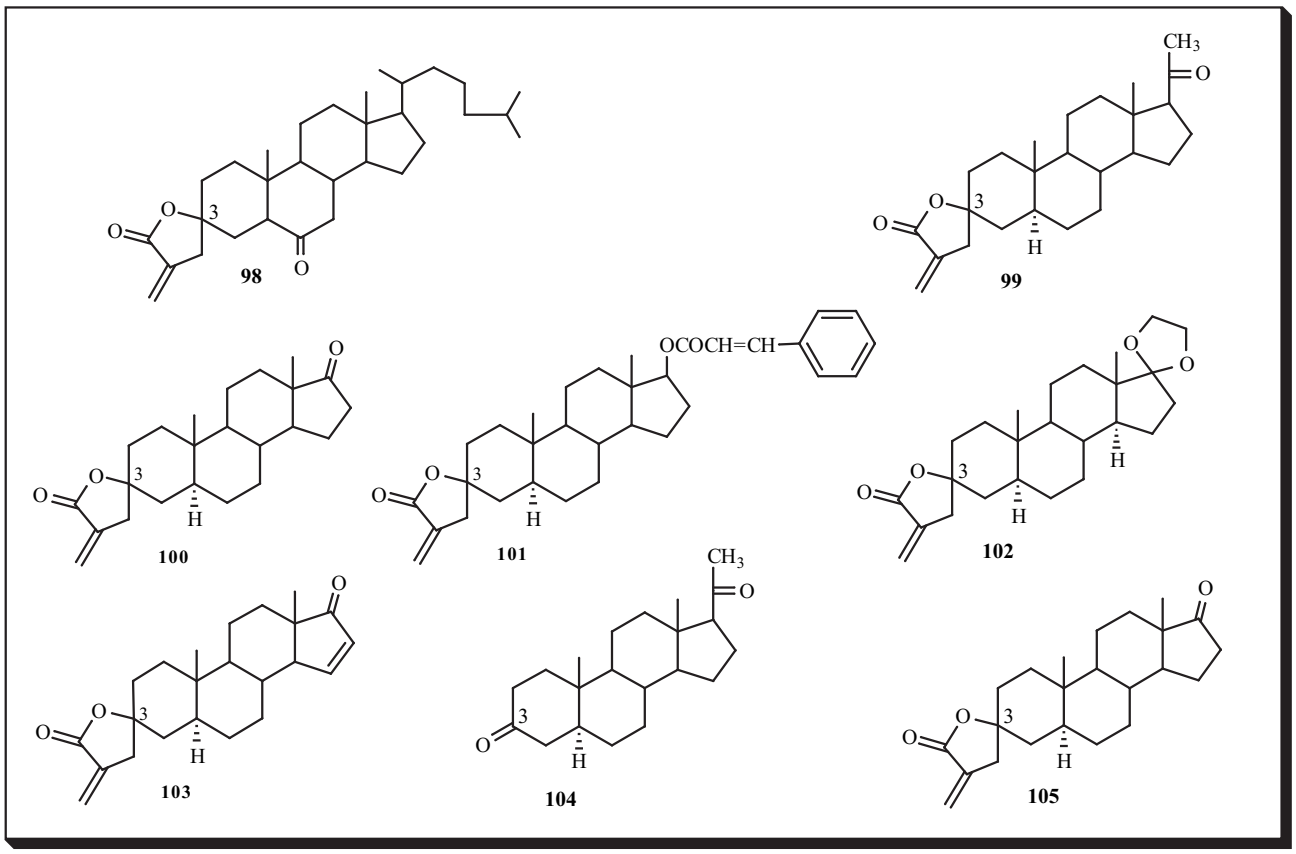
The γ-aminobutyric acid - $GABA_a$ receptor/ionophore complex represents a family of ligand-gated ion channels that account for the majority of rapid inhibitory synaptic transmissions in the mammalian brain [24a]. Alkyl-substituted γ-butyrolactones (GBLs) such as **90**-**92** and γ-thiobutyrolactones such as **93**,**94** have been described to exhibit convulsant or anticonvulsant activity, depending on the alkyl substituents [24b]. Unfortunately, the distinction between the biologic activities of α-and β-substituted lactones is not clear-cut. Some compounds can both block and augment GABA α currents, but with different time courses. Thus α,α-diisopropyl-GBL (α-GBL) **90** potentiates exogenous GABA currents but diminishes GABA-mediated inhibitory post-synaptic currents, leading to the conclusion that compound **90** can inhibit and potentiate GABA currents with kinetically different time courses. Another lactone β-ethyl,β-methyl-γ-thiobutyrolactone **94**, also has dual action, with inhibition predominating at low concentrations and potentiation predominating in high concentrations. Based on this study, two distinct GBL modulatory sites on the GABAa receptor, i.e. an inhibitory "picrotoxin" site and an enhancing "lactone site" have been proposed.

CELLULAR STEROIDAL REGULATION/INHIBITION

Intracellular formation and degradation of androgens and estrogens play important roles in regulation of cell function and proliferation. The enzyme 17β-hydroxysteroid dehydrogenase (17β-HSD) regulates intracellular levels of androgens and estrogens. 17β-HSD is broadly distributed in human tissues, found both in the classic steroidogenic tissues [25a, b, c], and in peripheral intracrine tissues [25d-l]. Five types of 17β-HSD have been identified in humans. Type 1 [25a, 26a] and Type 3 [26b] are substrate specific, catalyzing almost exclusively the transformation of estrone to estradiol and 4-androstenedione to testosterone, respectively. In contrast, Type 2 17β-HSD, [26c] is the only isoform of the enzyme that has a broad spectrum of activity including transformation of both androgenic, C19-steroids and estrogenic C18-steroids, as well as the interconversion of DHEA, delta4-dione and E_1 into delta5-diol, T and E_2, respectively. In an effort to reduce the levels of active steroids, Poirier and co-workers, who worked towards developing potent inhibitors of 17β-HSD, reported that steroidal spiro-γ-lactones inhibit 17β-HSD activity [26d]. Analysis of the inhibitory properties of spiro-γ-lactone analogs containing a steroidal C-18 and C-19 nucleus showed that the ones containing a C-18 nucleus were more potent inhibitors than C-19 nucleus analogs, with the best inhibition of 17β-HSD activity in human placenta microsomes (which catalyze interconversion of androgens to estrogens) obtained with phenolic spirolactone **95**. Furthermore, preincubation of 17β-HSD with compound **95** did not inactivate 17β-HSD activity, indicating that its inhibition may be reversible. However, lactone **95** appears selective for microsomal 17β-HSD activity, exhibiting inhibitory activity only for cytosolic 17β-HSD [25a, b, c]. Thus, this man-made lactone (**95**) proved to be the first inhibitor of human microsomal 17β-HSD. Unfortunately, therapeutic use was limited due to their agonistic estrogenic activity. However, adding a lactone at the 17 position of an anti-estrogenic nucleus, such as **96** (a long methyl butyl alkanamide side chains at position 7α posses anti-estrogenic activity) results in a compound (**97**) that exhibits selective inhibition of type 2 17β-HSD enzyme, without residual estrogenic activity [26e]. The selective inhibition by **97** was demonstrated by the absence of inhibitory activity toward type 1 17β-HSD (cytosolic fraction of the human placenta) and by a lack of detectable androgenic activity.

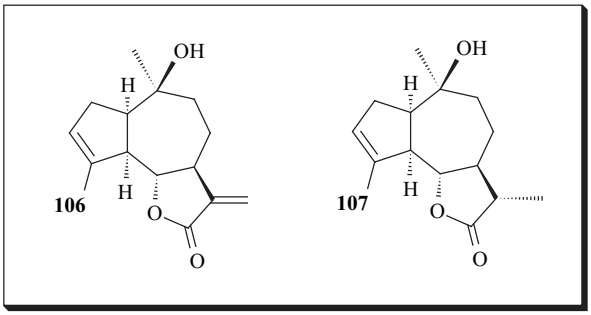
Early (ca. 1970) clinical trials tested both - α-methylene-γ-lactone and steroidal alkylating agents, such as phenesterin [27, b, c] , estradiol mustard [27d, e, f] and some homoaza steroid mustards [27g] for *in vivo* anti-tumor activity [27h]. In 1975 Lee and co-workers [27a] suggested combining an α-methylene-γ-lactone alkylating moiety with a steroidal or hormonal carrier as a means of obtaining chemotherapeutic





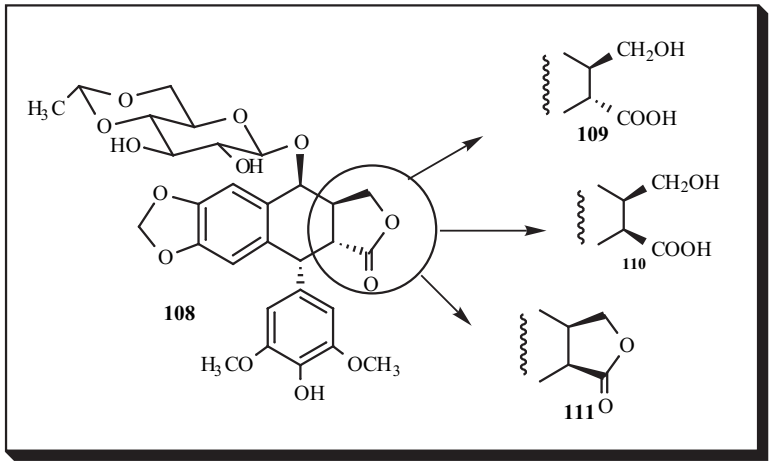
agents which might be specific for steroid-dependent tumors. The initial steroid - α-methylene-γ-lactone combinations, e.g. **98-103** showed approximately equal *in vitro* activity against human epidermoid carcinoma of the larynx (H.Ep-2). However, steroids lacking the α-methylene-γ-lactone ring were essentially inactive except for compound **104**. Enhanced cytotoxicity was not evident in bifunctional derivatives, such as **103**, nor monofunctional derivatives, such as **101**. Compounds **98-103** and **105** were cytotoxic for Walker 256 ascites carcinoma, while compound **105** demonstrated additional activity against Ehrlich ascites carcinoma.

Compounds that inhibit aromatase activity (e.g. aminoglutethimide) are used in the treatment of breast cancer [28a]. This is a promising therapeutic approach since the biosynthesis of estrogens is catalyzed by the enzyme complex aromatase. A group of sesquiterpene lactones has been shown to inhibit aromatase activity and also exert cytotoxicity. The many biological activities of sesquiterpene lactones are associated with the presence of an α-methylene-γ-lactone moiety, and when present in the molecule, by the α, β-unsaturated cyclopentenone ring. These chemical groups are considered powerful alkylating agents by a Michael type addition of a suitable nucleophile; however, this alkylating activity appears to be nonspecific, leading to inhibition of a large number of enzymes or factors involved in key biological processes [28b]. In order to test the hypothesis that the α-methylene-γ-lactone moiety of sesquiterpene lactones (cytotoxic activity) is not essential for their aromatase inhibitory activity, a reduction of the α-methylene moiety of compound **106** leading to its 11,13-dihydroderivative **107** was done [28c]. The results showed that chemical reduction of the exocyclic double bond of lactone **106** does not affect the capacity of the compound to inhibit aromatase activity, but does eliminate the cytotoxic activity. This finding may open the way for further modifications designed to enhance the activity of these active natural compounds.



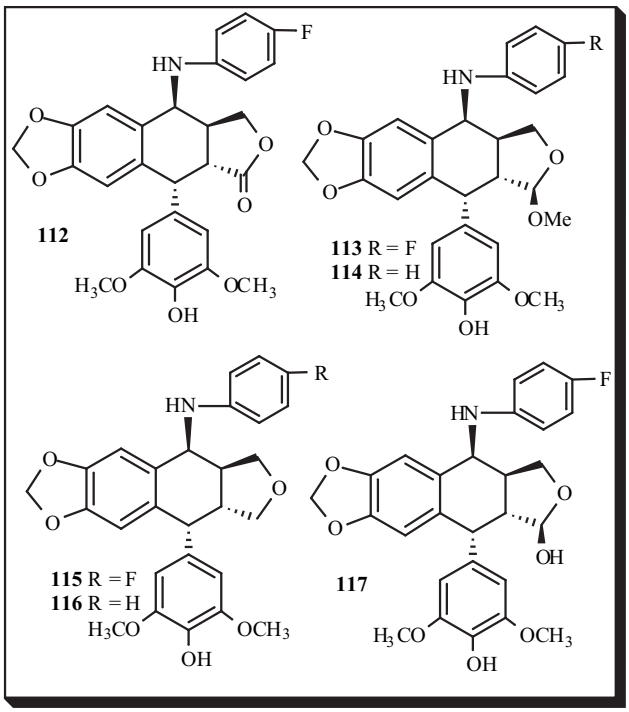
HUMAN DNA TOPOISOMERASE II INHIBITORS

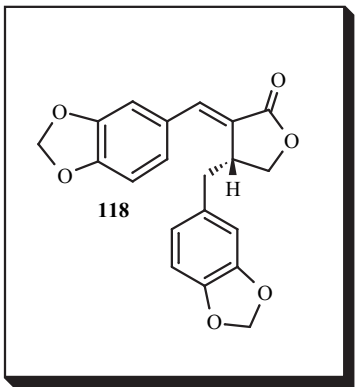
DNA topoisomerases are ubiquitous essential nuclear enzymes that control topological interconversions of DNA. The type II enzyme is required for DNA replication, chromosome segregation, and maintenance of chromosome structure. Regulation of type II enzyme expression occurs at multiple stages in the cell cycle and by a multitude of signaling factors. Topoisomerase II is also the cellular target for a variety of clinically relevant anti-tumor drugs. Etoposide (VP-16) **108** is a drug used in the treatment of small-cell lung cancer, testicular carcinoma, leukemia, lymphoma, and Kaposi's sarcoma [29a, b]. One of the problems which hinders its clinical efficacy is metabolic



deactivation [29 c-f]. The major metabolites of **108** are the corresponding *trans*- and *cis*-hydroxy acids **109** and **110**, respectively, formed by hydrolysis of the lactone ring, and the cis-picro lactone isomer **111**, which forms upon epimerization at the lactone ring.

Unfortunately, none of these metabolites exhibit biological activity [29g]. Towards development of derivatives of **108** that cannot be readily inactivated, a series of compound **112** derivatives**,** i.e., **113-117** were prepared, since **112** exhibits 7 times less toxicity *in vivo* than **108**. *In vitro* screening (Topo II inhibition assay) indicated that compounds **115**, **116**, and **117** have activity comparable to etoposide **108**, while compounds **113** and **114** are less active. In fact, compounds **115** and **116** exhibited a higher level of activity than **108** in the protein-linked DNA complex formation assay. The other three compounds (**113, 114, 117**) were less active than **108**. Further testing revealed that in the KB-ATCC cell toxicity assay all the compounds have higher ID_{50} values than **108**. These data indicate that there is a lack of correlation between the ability of the compounds to cause protein-linked DNA breaks and their cytotoxicity. This suggests that in addition to etoposide's inhibition of the catalytic activity of Topo II, other mechanisms of action may also be involved in the cytotoxicity observed with this class of compounds. Furthermore, although the γ-cyclic ethers **115** and **116** had the highest activity of the derivatives tested, they were less active than the parent compound **112**, a finding which indicates that alternation of the lactone moiety reduces activity, and confirms that the lactone moiety is essential for cellular activity.





INHIBITORS OF TUMOR NECROSIS FACTOR-α PRODUCTION (TNF-α) AND T CELL PROLIFERATION

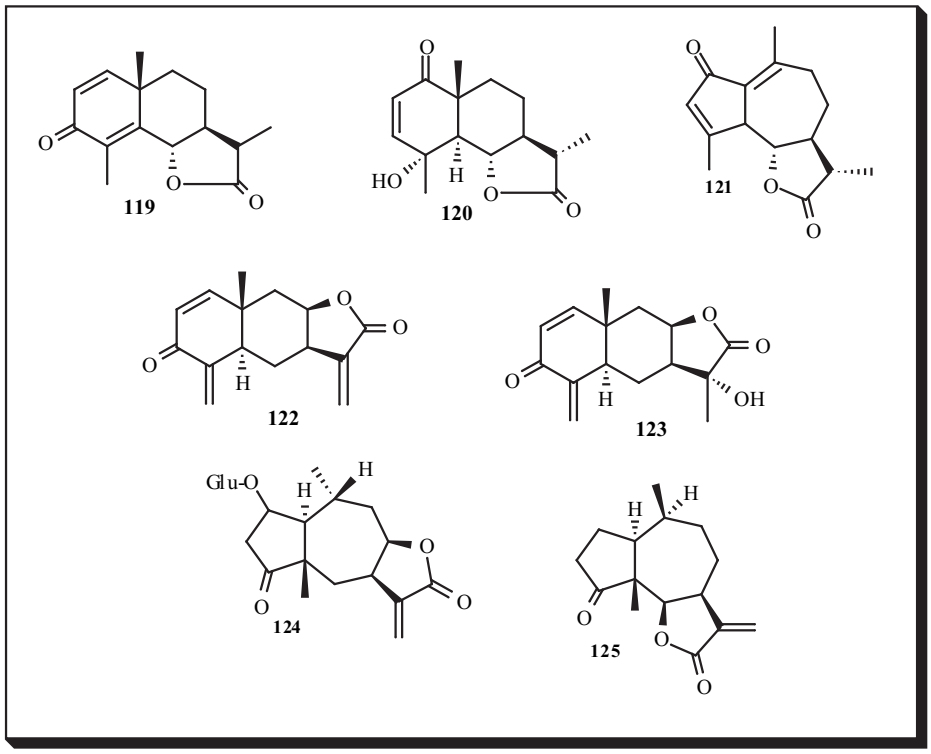
TNF-α is one of the most important proinflammatory cytokines. Normally produced early in the inflammatory process, [30a] TNF-α also plays a major roll as a mediator of the inflammatory cascades associated with the immunopathology of septic shock, as well as autoimmune diseases including asthma, and rheumatoid arthritis. Lignan compounds have been shown *in vitro* to significantly inhibit TNF-α production, in the absence of a cytotoxic effect, in LPS-stimulated murine macrophages [30b, c]. Lignan compounds constitute a large and diverse group of phenylpropanoid metabolites of plant origin. They are divided into several different types, such as the dibenzyl butyrolactones (arctigenin and savinin), furofurans (pinoresinol and eudesmin) and dibenzylbutanes

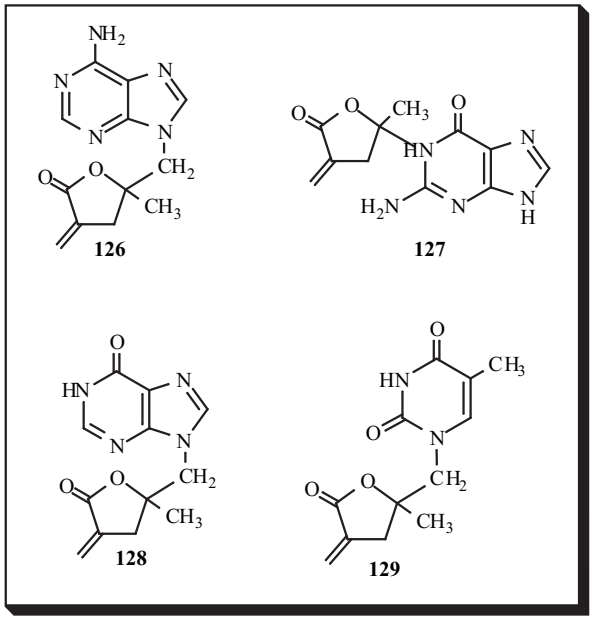
(secoisolariciresinol). In a recent study [30d], savinin **118**, isolated from *Pterocarpus santalinus* (red sandalwood, a medicinal leguminous tree used in the middle eastern part of Asia and India) strongly suppressed both TNF-α production and T cell proliferation. It was suggested that the butyrolactone ring on the C-9' position in dibenzylbutyrolactone-type lignans may be the functional moiety in inhibition of TNF-α production and T cell proliferation, but additional work is needed to delineate their mechanism of action.

INHIBITORS OF DNA REPLICATION

By the 1970's the discovery and structural elucidation of numerous sesquiterpene lactones, isolated from different plant families, provided the opportunity for the screening of these compounds as new therapeutic agents. Most of the medicinal investigations on sesquiterpene lactones concentrated on santonin **119**, a santolide, and its derivatives as antihelmintic and ascaricidal agents [31]. As the result of resources directed in the 1970's towards identifying tumor inhibitors from both natural sources and synthetic production under a program supported by the Cancer Chemotherapy National Service Center, at the National Cancer Institute, many sesquiterpene lactones bearing α-methylene-γ-lactone moiety exhibiting antitumor or cytotoxic activity were isolated. An extensive survey by Lee, and co-workers [32a] on nearly 100 sesquiterpene lactones from the plant family *Compositae* provided insight into the structure-activity relationship of this class of compounds as cytotoxic agents. Results obtained with three different mammalian cell lines showed that the presence of an α,β-unsaturated carbonyl system was the most immediate factor responsible for cell cytotoxic effects. The authors concluded that regardless of structural type (some of which are shown below) their basic activity is principally due to introduction of an α-methylene-γ-lactone moiety into the molecule. Hydrogenation of the conjugated α-methylene-γ-lactone moiety as in α-santonin **119**, vulgarin **120**, deacetoxymatricarin **121**, eliminates their activity. The enzyme-alkylating ability of an α-methylene-γ-lactone moiety is well known [33a- e]. Comparison of the cytotoxic activity of encelin **122**, and farinosin **123**, showed that they are about equally active, suggesting that the principal active center is the α,β-unsaturated ketone, in which the methylene group is exocyclic, since it can act in the same way as the α-methylene-γ-lactone. This hypothesis led to the conclusion that a system which can be engaged in Michael-type addition reactions from biological nucleophiles is necessary for activity, regardless of whether the α,β-unsaturated system is a lactone or a ketone. The compound, from the study performed by Lee and co-workers, that exhibited the highest cytotoxic activity, helenalin **1**, has in addition to the α-methylene-γ-lactone moiety, an α,β-unsaturated ketone system. Therefore, helenalin possesses two alkylating functions in its molecule. The high level of cytotoxicity associated with lactone **1** was suggested to result from the inhibition of DNA synthesis by interstrand cross-linking of the DNA helix [33e-g]. In addition, this study revealed the influence a carbohydrate moiety has on cytotoxicity, i.e., an increase in cytotoxicity in paucin **124,** as compared to damsin **125**, presumably occurring via carbohydrate-assisted transport of the former.

Evaluation of numerous other sesquiterpene lactones for the ability to inhibit tumor growth, respiration and nucleic acid synthesis [3] confirmed the necessity of having an α,β-unsaturated moiety in the structures to serve as a Michael-type acceptor for a given biological nucleophile [34a,b]. Examples of acceptable biological nucleophiles include sulfur-containing nucleophiles, e.g. the cytochrome cofactors

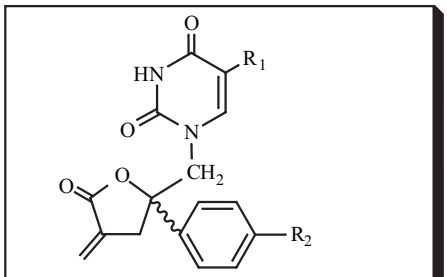




of the electron transport chain, which contain iron-sulfur centers or sulfide ions as components of their structure, and a number of Krebs cycle dehydrogenases which contain sulfhydryl groups in their molecules [4]. Mechanistically, for the sesquiterpene lactones and synthetic compounds possessing the α-methylene-γ-lactone moiety, there appears to be a strong correlation between their antitumor activity and their ability to suppress basal and coupled oxidative phosphorylation in certain tumor lines [34c-e]. However, the ability of these compounds to inhibit aerobic respiratory processes appears to exceed their ability to inhibit tumor cell growth. The latter phenomenon may be due to alkylation by the α,β-unsaturated systems of the sulfur-containing nucleophiles listed above, therefore reducing activity and state 4 respiration [3].

α-Methylene-γ-lactone containing pyrimidines and purines exhibit *in vivo* antineoplastic and cytotoxic activity by interacting directly with DNA, or with a drug incorporated into the DNA, resulting in DNA fragmentation as demonstrated by a DNA strand scission study [32b, c]. α-Methylene-γ-lactone nucleosides **126**-**129** all demonstrate activity against murine L1210 lymphoid leukemia cells. Of these compounds, the adenine derivative **126** had good activity against L1210 lymphoid leukemia growth [32d]. This multiple interaction indicates that α-methylene-γ-lactone purine **126** may not be a specific inhibitor of DNA polymerase α catalytic function.

Evaluation of the antineoplastic activity of several α-methylene-γ-(4-substituted phenyl)-γ-butyrolactone bearing thymine, uracil, and 5-bromouracil **130-132** with the substituent at the *p*-position on the phenyl ring indicated strong growth inhibitory activity against leukemia cell lines with the bromouracil derivative **132** (R_1=Br, R_2=Ph), the most potent inhibitor [32f].

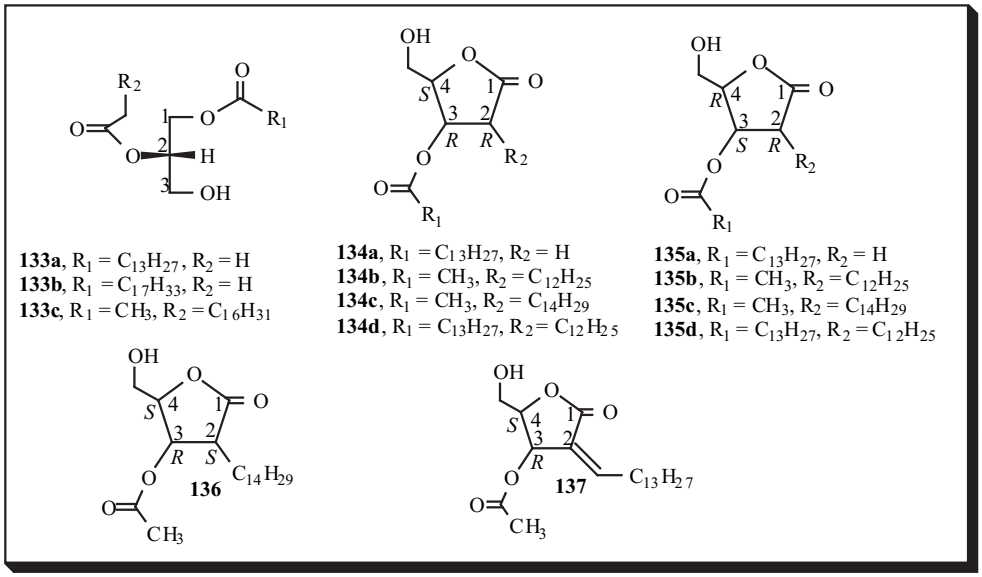


130 R_1 = Me, R_2 = Ph, F, Cl, Br, Me, NO_2
131 R_1 = H, R_2 = Ph, F, Cl, Br, Me, NO_2
132 R_1 = Br, R_2 = Ph, F, Cl, Br, Me, NO_2

ENZYME INHIBITORS

1) Inhibitors of Signal Transduction Pathways

Signal transduction cascades in cells can lead to the formation of diacylglycerol (DAG) [35a, b]. DAG and its analogs, the phorbol ester tumor promoters, act as second messengers activating protein kinase C (PK-C) enzymes which catalyze the phosphorylation of proteins that control cell processes, including cell cycling, cell differentiation, and oncogenic expression [35a-c]. It is hypothesized that the "rigid" structure of the phorbol esters is responsible for their



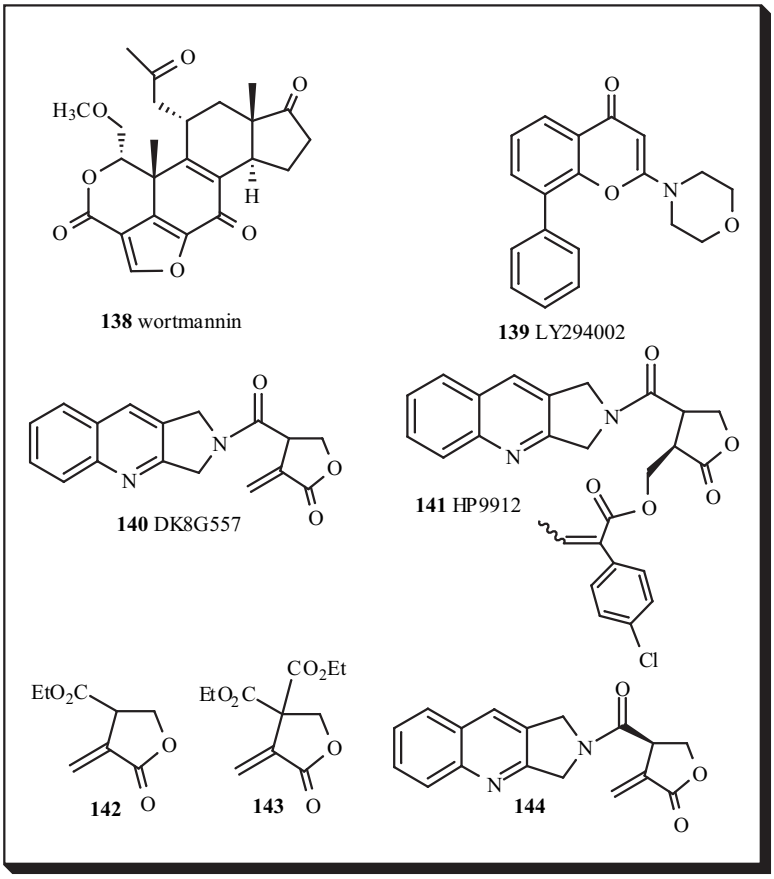
133a, $R_1 = C_{13}H_{27}$, R_2 = H
133b, $R_1 = C_{17}H_{33}$, R_2 = H
133c, $R_1 = CH_3$, $R_2 = C_{16}H_{31}$

134a, $R_1 = C_{13}H_{27}$, R_2 = H
134b, $R_1 = CH_3$, $R_2 = C_{12}H_{25}$
134c, $R_1 = CH_3$, $R_2 = C_{14}H_{29}$
134d, $R_1 = C_{13}H_{27}$, $R_2 = C_{12}H_{25}$

135a, $R_1 = C_{13}H_{27}$, R_2 = H
135b, $R_1 = CH_3$, $R_2 = C_{12}H_{25}$
135c, $R_1 = CH_3$, $R_2 = C_{14}H_{29}$
135d, $R_1 = C_{13}H_{27}$, $R_2 = C_{12}H_{25}$

136

137



superior ability to activate PK-C [35d]. Building on this hypothesis, Marquez, V. E. *et al.* synthesized several lactones (**134** and **135)** via connecting the acetyl moiety to the sn-1 carbon in glycerol-1-myristate-2-acetate **133a**, with the goal to test whether increasing compound rigidity would increase PK-C binding affinity. The compounds were evaluated for their ability to inhibit [20-^{3}H] phorbol-12,13-dibutyrate (PDBU) binding to either a PK-C mixture [35e] or to purified PK-Ca [35f]. Glycerol-1-myristate-2-acetate **133a** was one of the simplest DAG molecules capable of binding and activating PK-C, indicating that restricting the glycerol backbone within a γ-lactone structure has important impact on the PK-C binding. If the lactone mimics a *S*-1-O-long acyl-2-O-acetate analogue, such as **133a** and **133b** – the enzyme has only a modest preference for the stereochemically equivalent 3R,4S-lactone **134a** (**134a** vs **135a**).

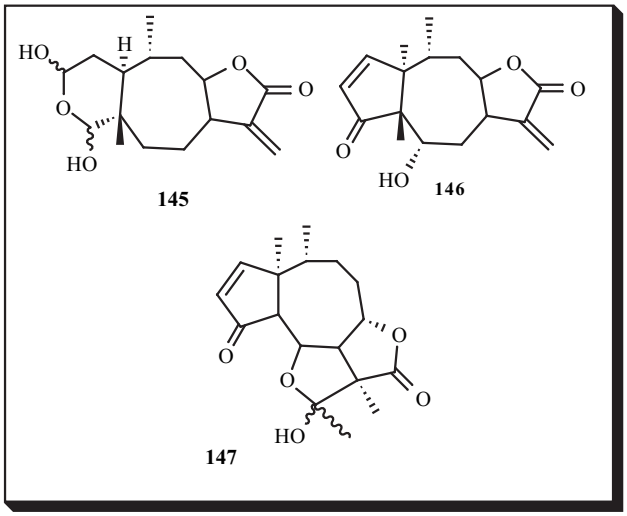
If the lactone mimics a *S*-1-O-acetyl-2-O-long acyl analogue, such as **133c** – the enzyme has a strict preference for the stereochemically equivalent 3R,4S-lactone **134b** (**134b** vs **135b**). In addition, the latter mimics produced compounds with higher binding affinities for PK-C. In all of the lactones described so far the C-2 alkyl chain was synthesized as having an *R* configuration. A subsequent investigation showed that compounds with either *R* or *S* stereochemistry at C-2 **136** have nearly identical potencies [35g]. In addition, despite the three-fold increase in binding, the difference between *Z*- and *E*-geometries of the 2-alkylidene lactones, such as **137**, also appears to be small.

Several pyrrolo-quinolone γ-lactones have been found to be novel inhibitors for two members of PIS-kinase related kinase (PIKK) family, Ataxia-Telangiectasia-*mutated* (ATM) protein and the mammalian target of rapamycin (mTOR). In mammalian cells, the PIKK family consists of ATM protein, mTOR [36a] , Ataxia-Telangiectasia-*mutated* and Rad3-related (ATR) protein [36b], and DNA-dependent protein kinase (DNA-PK) [36c], which differ from phosphatidylinositol 3-kinase (PI3-kinase) in the sense that they do not posses lipid kinase activity, but are serine threonine protein kinases [36d]. Both ATM and mTOR proteins are associated with development of cancer. The finding that wortmannin inhibits both enzymes served as a starting point for the development of small molecule inhibitors for these proteins. Wortmannin **138**, a fungal metabolite, has been identified as a potent irreversible inhibitor of PI3-kinase [36e, f]. The mechanism by which wortmannin inhibits PI3-kinase has been recently confirmed as an irreversible modification of the primary amine, Lys-833, in the enzyme active site. Similarly, wortmannin has been shown to inhibit other members of PIKK family, which is likely to result from inactivation of the PI3-kinase-related catalytic domains in these enzymes [36c]. Another competitive inhibitor LY294002, **139,** which occupies the ATP-binding site of PI3-kinase, was reported to inhibit both PisK [36f] and DNA PK [36g]. Inhibitors **138** and **139** are valuable tools in studying the biological roles of members of the PIKK family in intracellular signaling events. However, their significant toxicity and narrow therapeutic indices has limited their development as drug candidates. Peng and co-workers recently reported on the synthesis and

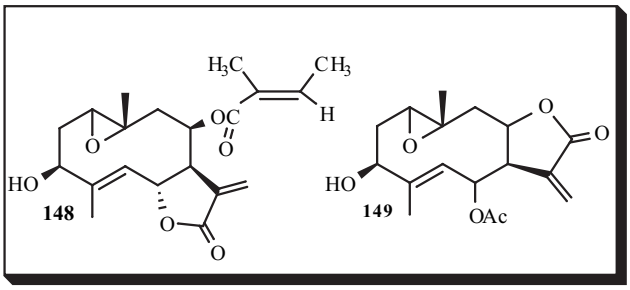
biological evaluation of novel pyrollo-quinolone derivatives, e.g. such as DK8G557, **140** and HP9912, **141,** as potent irreversible inhibitors for the ATM and mTOR protein kinases [36h]. Initial SAR indicated that an electrophilic exocyclic double bond conjugated to the carbonyl group of the γ-lactone ring as in compound **140** is crucial for ATM inhibitory activity. Simple lactones, such as compounds **142** and **143,** also exhibited potent and selective irreversible inhibitory activity for ATM over mTOR, with a reversed selective preference and higher selectivity ratio as compared to wortmannin. This lower activity of **142** and **143** towards mTOR suggests that in addition to a Michael acceptor, inhibition of mTOR may require a more extended enzyme-inhibitor interaction than that of ATM. The best mTOR inhibitor, HP9912, **141,** showed selectivity for mTOR over ATM with the same selective preference as that of wortmannin, but with a higher selectivity ratio [36h].

2) Inhibition of Macrophage Adenylate Cyclase and Inducible Nitric Oxide Synthase (iNOS)

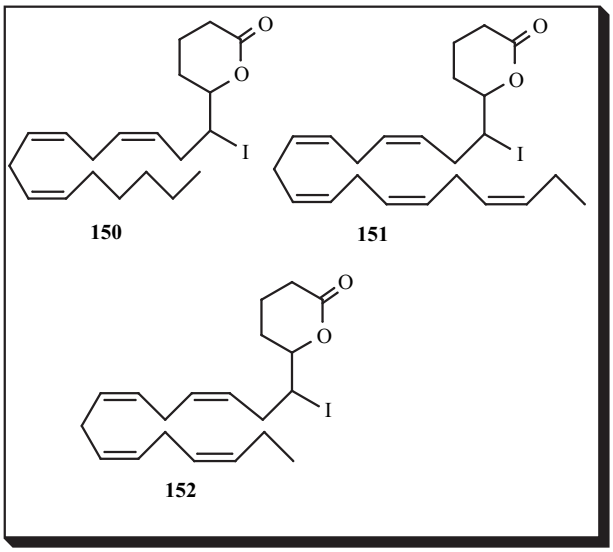
Inhibition of murine macrophage adenylate cyclase activity by natural sesquiterpene lactones isolated from toxic forage plants has been correlated with the presence of the α-methylene-γ-lactone moiety as in hymenovin **145** and helenalin **146** [37a]. Tenulin, **147**, a sesquiterpene lactone that does not contain this reactive moiety caused minimal inhibition of the enzyme. Inhibition of the enzymatic capability of adenylate cyclase by alkylation of its thiol groups by sesquiterpene lactones could disrupt the cellular transmission of external signals to the internal regulatory proteins. Disruption of the production of cAMP, due to deactivation of adenylate cyclase by the sesquiterpene lactones, could lead to negative alteration of the capability of the cell to adequately respond to the needs of the organism. Since livestock poisoning due to grazing on toxic forage plants has been a major obstacle to animal production in many areas of the world [37b-d], these data lead to the conclusion that the α-methylene-γ-lactone moiety of sesquiterpene lactones have a significant role in the toxicity of these poisonous plants. Since this moiety can also act to inhibit plant growth (see below) investigation into how the plants detoxify the compounds may prove useful in animal management.



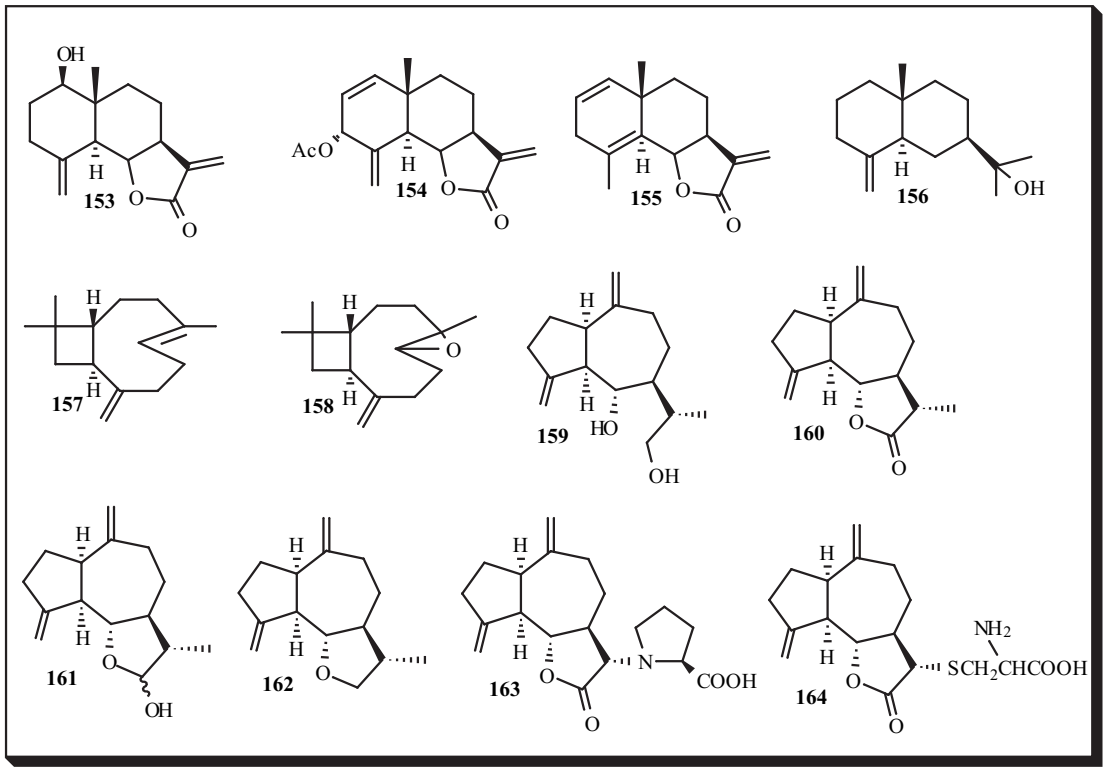
The α-methylene-γ-lactone moiety has also been demonstrated to be essential for the activity of sesquiterpene lactones as plant growth inhibitors [38a-d]. The inhibitory activities of these compounds were assayed on rice seedlings and *Avena coleoptile* sections. In the latter bioassay, the inhibitory activities of the synthetic α-methylene-γ-lactones were comparable to those of heliangine **148** and pyrethrosin **149**, the natural inhibitors possessing α-methylene-γ-lactone moiety.



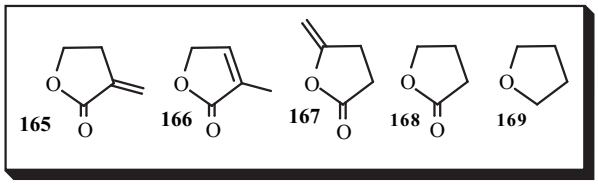
Since the discovery of the role of local growth factors in thyroid tissue, it is now well accepted that there are at least two pathways that regulate thyroid cell growth: a thyrotropin (TSH)- and cyclic adenosine-3',5'-monophosphate (cAMP)-dependent pathway and a cAMP independent pathway. Which of these pathways is triggered in goiter development *in vivo* has not been fully clarified [39a-d]. δ-Iodolactone (δ-IL) **150**, an iodinated derivative of arachidonic acid, has been shown to be synthesized in thyroid tissue and to inhibit thyroid cell proliferation. It is discussed as a potential mediator of the autoregulatory pathway of iodide in cAMP and TSH-dependent growth. Dugrillion and Gatner observed that iodide and δ-IL inhibited epidermal growth factor (EGF)- and 12-O-tetradecanoylphorbol-13 acetate (TPA)-induced proliferation in a dose dependent manner. The growth inhibitory effect was restricted to δ-lactones such as **151** and **152**; γ-lactones did not show an inhibitory effect. Thus, δ-IL appears to be a specific inhibitory mediator of iodide on growth factor-induced thyroid cell proliferation [39e].



Nitric oxide (NO) plays a role in physiological and pathological processes such as vasodilation, nonspecific host defense, ischemia reperfusion injury and chronic



inflammation. NO is produced by the oxidation of L-arginine by a NO synthase (NOS). Inducible NOS (iNOS) is both part of innate host defense against pathogens and involved in pathological processes. Inducible NO production occurs in response to pro-inflammatory agents such as interleukin-1β, tumor necrosis factor-α and lipopolysaccharide (LPS) in various cell types including macrophages, endothelial cells and smooth muscle cells. Inhibition of iNOS enzyme activity or its induction may have therapeutic effects in various types of inflammation [40a].

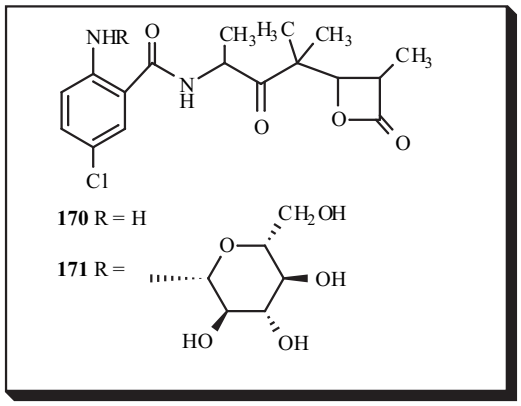


In addition to their ability to inhibit NO production, bay leaf extracts have also been used in folk medicine to prevent alcoholic disorders. Matsuda's group also investigated the structural requirements of sesquiterpene lactones from *Laurus nobilis* to inhibit alcohol absorption [41a]. Several sesquiterpene lactones were chosen, through a bioassay-guided separation [41d], using inhibitory activity on blood ethanol evaluation in oral ethanol-loaded rat **44**, **45**, **153-158**, as well as synthesized reduction products **159-162** and amino acid adducts **163**, **164** of the α-methylene-γ-butyrolactone **165** and its related compounds **166-169**. The results indicated that the γ--butyrolactone or γ--butyrolactol moiety having α-methylene or α-methyl group are essential for the inhibitory activity on ethanol absorption.

Since **44**, **45** and **165** showed no significant effect on glucose absorption, these sesquiterpenes appeared to selectively inhibit ethanol absorption; the acute toxicities of lactones **44** and **45** in a single oral administration were found to be lower than that of compound **165**.

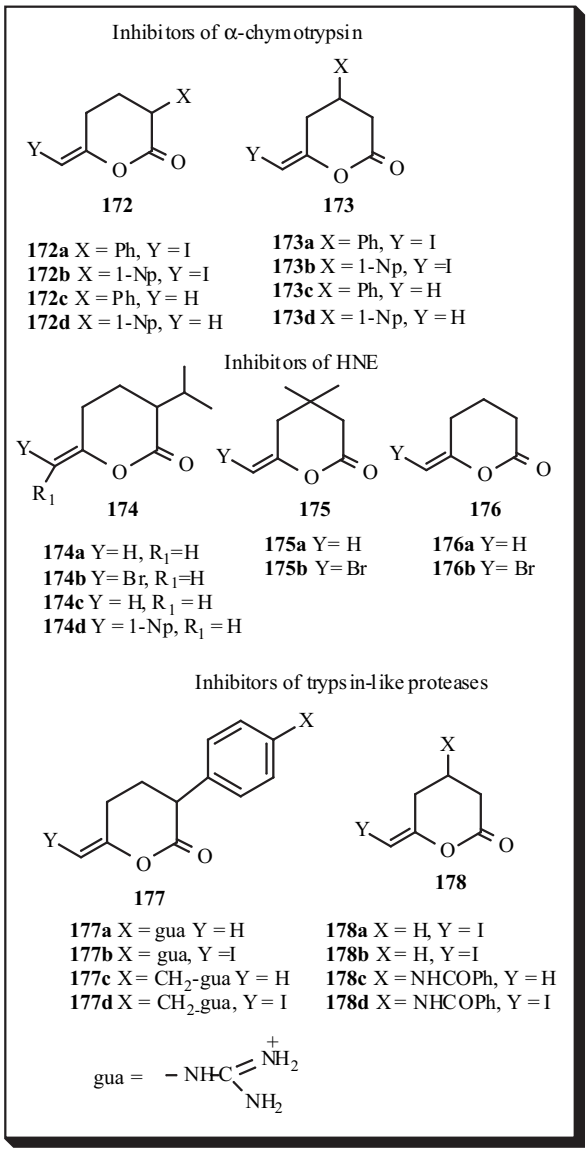
3) Inhibitors of Serine Carboxypeptidase and Trypsin-Like Serine Protease

Belactins A **170** and B **171**, inhibitors of serine carboxypeptidase, were discovered in the fermentation broth of *Saccharopolyspora sp*. MK-19-42F6 [42a]. These compounds do not inhibit elastase or lipase at 100μg/ml but have more specific inhibitory activity towards carboxypeptidase Y (CP-Y) as compared with other β-lactone containing inhibitors, such as ebelactones A, B and esterastin. The results from the inhibitory characterizations of the belactin **170** and its derivatives (β-lactones **170)** have shown that the β-lactone moiety is essential for activity [42b].



Serine proteases with trypsin-like specificity are most frequently involved in physiological regulation. Most of the

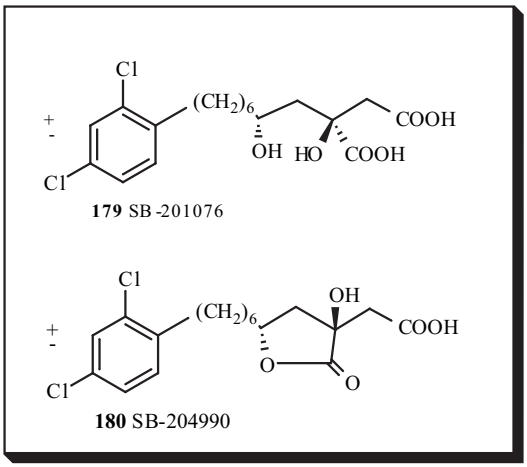
dozen enzymes involved in the coagulation of blood exhibit trypsin-like selectivity, but with much greater specificity than trypsin itself. Therefore, specific inhibitors of blood clotting enzymes are of therapeutic potential as anticoagulants [43a]. Thus, valero mimic enol lactones, as powerful inhibitors of α-chymotrypsin, human neutrophil elastase (HNE), and guanidine-aryl substituted enol lactones inhibition of trypsin-like enzymes (trypsin, plasmin, urokinase, and thrombin) have been accomplished [43b].



Enol lactones appear to inhibit the enzyme by initially acylating the active site serine residue. Protio enol lactones can act as substrate inhibitors, i.e., transient inactivators that form very stable acyl-enzyme complexes. With halo enol lactones, formation of the acyl-enzyme intermediate leads to the formation of a halomethyl ketone group, which can alkylate a suitably positioned active-site residue and become permanently attached to the enzyme. α- and β-aryl (phenyl and naphthyl) substituted lactones **172** and **173** proved to be potent inhibitors of α-chymotrypsin, **174-176** of HNE and **177-178** of trypsin-like enzymes. These studies demonstrated that an α-substitution on valero enol lactones is a good system for the design of potent suicide substrates. This also reiterates the similarity in tertiary structure in the active site of these enzymes. In general, the guanidinophenyl-substituted enol lactones, such as **177c** and **178d** showed a preference for inactivating trypsin-like enzymes over α-chymotrypsin and HNE [43c].

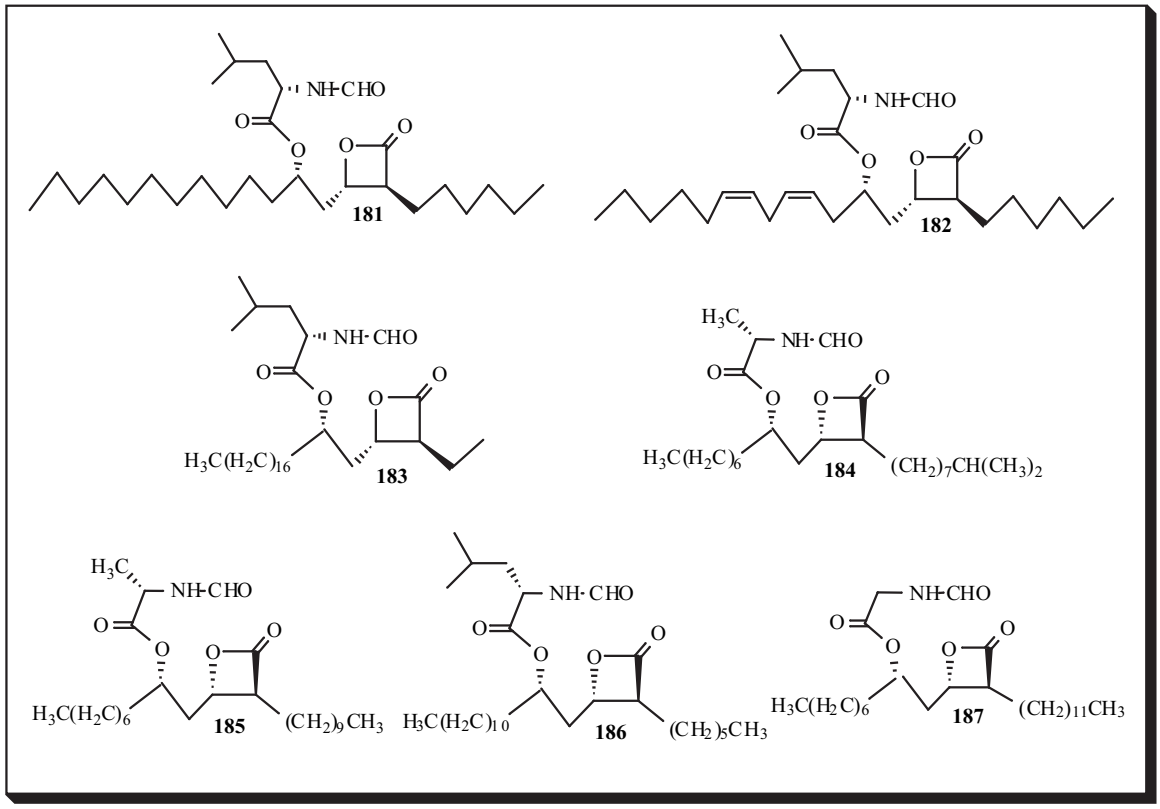
4) ATP Citrate-Lyase Inhibitors

ATP citrate (pro-S)-lyase is a cytosolic enzyme that catalyzes the cleavage of citrate into acetyl-CoA and oxaloacetate [44a-c]. In non-ruminating mammals this enzyme is abundantly expressed in lipogenic tissues, such as liver and adipose tissue [44d], where it has an important role in supplying acetyl-CoA for both cholesterogenesis and lipogenesis. Studies with (-)-hydroxycitrate, a potent inhibitor of ATP citrate-lyase [44a], demonstrated that inhibition of this enzyme leads to a decrease in the synthesis of both cholesterol and fatty acids [44e-i], and an increase in low-density lipoprotein (LDL) receptor activity [44j]. Decreases in plasma cholesterol and plasma triglyceride levels were also measured in rats treated with (-)-hydroxycitrate, suggesting a potential utility of ATP citrate-lyase inhibitors as hypolipidaemic agents [44i]. However, hydroxycitrate is not well absorbed, making its therapeutic use difficult. Because of this, researchers from SmithKline Beecham laboratories developed a series of potent inhibitors, such as SB-201076, **179**, and its prodrug SB-204990 **180.** The latter was determined to be the most potent inhibitor synthesized capable of inducing hypocholesterolemia and hypotriglyceridaemia when given orally to rats and dogs [44k, l]. The pharmacology of compound **180** is consistent with the hypothesis that the inhibition of this enzyme is a feasible target for hyperlipidemic intervention and that this compound, or related analogue, may have potential for developing new anti-hyperlipidemic agents.

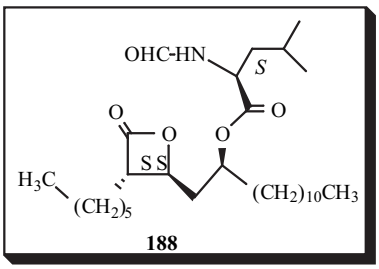


5) Pancreatic Lipase and Cytosolic Phospholipase A2 Inhibitors

Pancreatic lipase is a serine esterase, belonging to the family of serine hydrolases. Serine hydrolases encompass a diverse cadre of enzymes such as trypsin, thrombin, beta-lactamases, lipases, and cetylcholinesterase [45a-e]. Pancreatic lipase (PL) is inhibited by tetrahydrolipstatin (THL) **181**, which contains a β-lactone that is opened by the catalytic serine; the alcohol leaving group prohibits deacylation by blocking the pathway of incoming water



molecule and thus inactivates the enzyme. THL, a hydrogenated derivative of lipstatin **182,** was originally isolated from *Streptomyces toxytricini*, and along with analog **183** are potent inhibitors of human PL [45f-m]. Several years after the discovery of lipstatin and THL, Nippon Roche Research Center reported that panclicins, such as **184**-**187**, are novel pancreatic lipase inhibitors which contain a β-lactone moiety, and are structurally related to β-lactone esterase inhibitors of microbial origin, such as lipstatin, valilactone [45n], ebelactones [45o], and esterasin [45p,q].



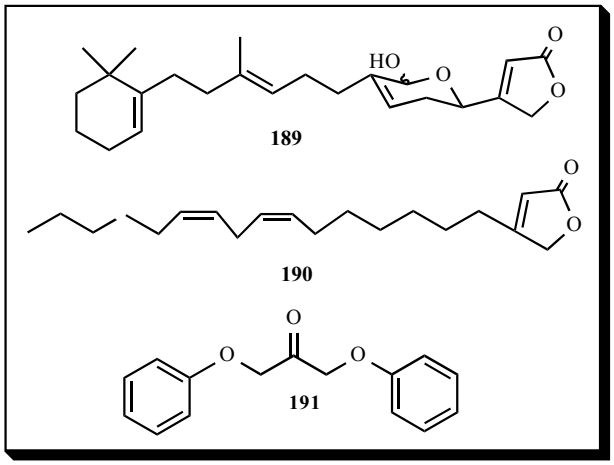
It was this research that led to the production of the drug Orlistat **188**. Its pharmacological activity is associated with partial inhibition of hydrolysis of triglycerides due to its binding to the gastric and pancreatic lipases [45r].

6) Phospholipase A2 Inhibitors

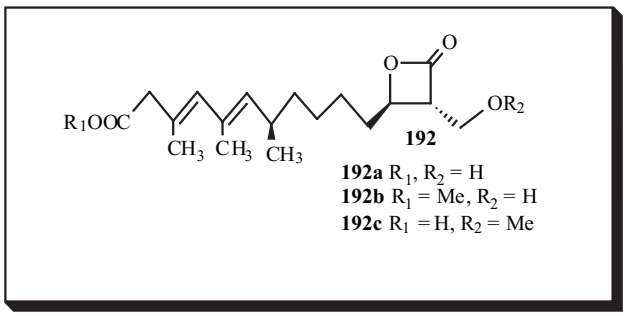
Phospholipases A2 (PLA2s) are a class of esterases that cleave the sn-2 ester bond of the membrane phopsholipids in both a regiospecific and stereoselective manner [46a]. PLA2 is generally accepted as the enzyme responsible for the cellular production of the arachidonic acid from its esterified glycerophospholipid form [46b]. Inhibition of the action of PLA2 has the potential to block the cellular production of a wide variety of proinflammatory lipid mediators, including prostaglandins and leukotrienes, and has been the subject of intense study by the pharmaceutical industry [46c-e]. It is difficult, however, to find a drug candidate inhibitor of PLA2 due to the enzyme's stability, its lack of specificity for the fatty acid cleaved from the sn-2 position of phospholipids, and the high concentration of Ca^{2+} required for activation [46f]. Amongst the reported inhibitors, manoalide **189** has been shown to be a potent inhibitor of cobra venom phospholipase A2 [46f] and bee venom phospholipase A2 [46h]. Manoalide **189** is an anonsteroidal sesquiterpenoid isolated from the sponge *Luffariella variabilis* [46i]. In order to establish which moieties are necessary for the inhibitory activity of compound **189**, Dennis and co-workers synthesized a manolide analog containing the α,β-unsaturated γ-lactone ring **190**. Their observation on the inhibitory behavior of compounds **189** and **190** suggested that the opening of the lactone ring appears to be required for inhibition by manoalide. Compound **189** is very potent irreversible inhibitor, while its analog **190** is only a moderately strong reversible one. Thus, compound **190** has shown that the γ-lactone ring by itself is not sufficient to produce all of the manoalide **189** inhibitory properties. Recently, researchers from Astra Zeneca demonstrated that the 1,3-disubstituted propan-2-one **191** skeleton can serve as a unique serine trap for a series of novel inhibitors of the PLA2 [46j].

7) Hydroxymethylglutaryl Coenzyme A Synthase (HMG-CoA) Inhibitors

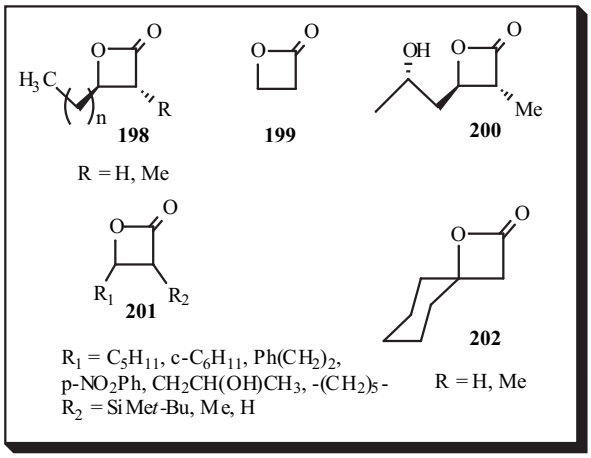
By the early 1990's the discovery and biological activity of "statins" generated great interest in identifying other



natural product inhibitors of the enzymes in the cholesterol biosynthetic pathway. Lovastatin, a natural product from *Aspergillus terreus* [47a], together with fluvastatin is in clinical usage as inhibitors of HMG-CoA reductase [47b, c]. Related compounds such as simvastatin and pravastatin, which have also been shown to lower plasma cholesterol, have lead to identifying natural products which inhibit the steps immediately before HMG-CoA reductase. Compounds such as tryine carbonate (L-660,631), from *Actynomycete* inhibited liver cytoplasmic acetoacetyl-CoA thiolase and β-lactone **192** inhibited HMG-CoA synthase [47d]. The enzyme HMG-CoA synthase catalyses the formal aldol condensation of 1 mol of acetyl-coenzyme A (AcCoA) and 1 mol of acetocetyl-coenzyme A (AcAcCoA). The product of the reaction, 3*S*-HMG-CoA synthase, is the universal precursor of terpenes and steroids [47e]. Thus, inhibitors of this pathway could also act as potent antifungal agents, since fungi posses an active sterol biosynthetic machinery [47f].



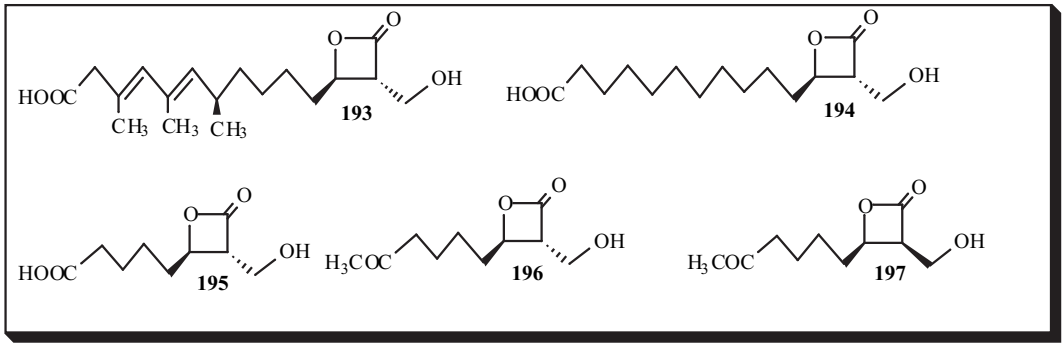
A naturally occurring β-lactone 1233A **193** isolated from *Cephalosporin sp*.[47g], *Scopulariopsis sp*. [47h], and *Fusarium sp*. [47i] was used as the basis for development of analogs **194-197** with HMG-CoA synthase inhibitory activity [1233A].



What was observed was that opening of the β-lactone ring results in a complete loss of inhibitory activity, while saturation of the alkyl chain leads to 50% reduction in activity compared to **193**. Acylation of the hydroxyl residue also significantly reduced enzyme inhibition. In addition, the *trans*-stereochemistry of the β-lactone ring as well as the length of the side chain was also an important factor affecting inhibitory activity. The feature of the molecule that was not crucial was the replacement of the acid with a methyl ester. The results of extensive SAR investigations [47k, l] established that the lactone ring is the only structural component required for irreversible inhibition, and suggests that the ring substituents play a role solely in guiding the inhibitor into the enzyme active site. The role of substituents at C3 and C4 of the oxetan-2-one ring was determined with a series of β-lactones with either hydrogen or methyl at C-3 and alkyl chains of increasing length at C4, as well as several β-lactones containing variations on these substitution patterns **198-202** [47m]. Both 4-alkyl- and 3-methyl-4-alkyl-oxetan-2-ones exhibited a logical trend in inhibition of HMG-CoA synthase with potencies that are dependent on the length of the C4 substituent; a chain of 10-11 carbons gave maximal inhibition. Overall these results can be explained by hydrophobic interactions.

CONCLUSION

The basic lactone ring structure has been demonstrated to exhibit a high degree of utility as an effective alkylating agent. Dependent on the attached groups, its ability to engage in Michael-type addition reactions has been



demonstrated to allow this nearly ubquitous molecule to act as either a positive or negative signaling molecule, and enzyme inhibitor, regardless of the phylogenetic origins of the organism impacted (both eukaryotic and prokaryotic). The relative ease of transformation of this ring bodes well for the their use as inhibitors in a variety of systems. The potential for this group of inhibitors to be added to the armamentarium of both antimicrobials and anti-neoplastic drugs is high, but more work must be done elucidating the structure-function relationships, as well as resistance development, before they come into broad clinical usage. This being said, there is the potential that lactones may one day replace lactams as the basis for chemotherapy.

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