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REVIEW

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New Synthetic Triterpenoids: Potent Agents for Prevention and Treatment of Tissue Injury Caused by Inflammatory and Oxidative Stress

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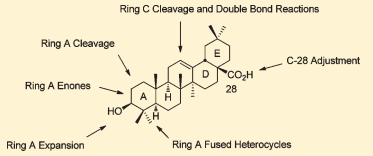
ABSTRACT: We review the original rationale for the development and the chemistry of a series of new synthetic oleanane triterpenoids (SO), based on oleanolic acid (**1**) as a starting material. Many of the new compounds that have been made, such as 2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oic acid ("CDDO", **8**), are highly potent (activities found at levels below 1 nM) anti-inflammatory agents, as measured by their ability to block the cellular synthesis of the enzyme inducible nitric oxide synthase (iNOS) in activated macrophages. Details of the organic synthesis of new SO and their chemical mechanisms of biological activity are reviewed, as is formation of biotin conjugates for investigation of protein targets. Finally, we give a brief summary of important biological activities of SO in many organ systems in numerous animal models. Clinical investigation of a new SO (methyl 2-cyano-3,12-dioxooleana-1,9(11)dien-28-oate, "CDDO-Me", bardoxolone methyl, **13**) is currently in progress.



INTRODUCTION

Cellular life arose in an extremely hostile chemical environment, with abundant electrophilic stress provided by reactive species of oxygen (ROS) and nitrogen (RNS) in the primitive biosphere. Mechanisms to protect our ancient genome from electrophilic damage, named "electrophile counterattack" by Talalay,[1] had high evolutionary survival value, and it is therefore not surprising that the genome evolved new mechanisms to protect itself from oxidative, nitrosative, or other mutagenic damage. Enzymes of DNA repair are perhaps the best known examples.

The ancient incorporation of mitochondria into cellular eukaryotic life allowed a great leap in cellular energetics, enabling organisms to become actively motile in their quest for nutrients. However, oxidative processes in the mitochondrion also have the capacity to generate reactive species of oxygen that can damage the genome, as well as proteins and lipids, in cells.[2] With the evolutionary development of the immune system, both macrophages and neutrophils arose; these cells generate reactive species of both oxygen and nitrogen in order to kill foreign pathogens.[3] As a result, as an undesirable side effect, the rest of the cells in the body as a whole could then suffer further damage from these reactive species. Thus, although ROS and RNS indeed have powerful ability to kill invading microorganisms, they also have potential for damaging cellular DNA, as well as cellular proteins and lipids. As a consequence, between the mitochondria found in essentially all animal cells that enable us to lead an active and thinking life and the cells of the immune system that are so essential to protect us from invading microorganisms, our bodies have great potential to damage themselves. Metabolic stress caused by ingestion of undesirable xenobiotics has added yet another layer of tissue damage.[4]

It is therefore not surprising that mechanisms evolved to protect the organism from both endogenous and exogenous damage to DNA and proteins, because of their survival value. The purine and pyrimidine bases of DNA are particularly vulnerable targets of reactive oxygen and nitrogen, but the highly reactive cysteine proteome of the cell also requires protection from oxidative or inflammatory stress.[5] Many of the phosphorylating kinases and dephosphorylating phosphatases of the cell have reactive cysteines at their catalytically active sites,[6] and these sites are particularly vulnerable to damage.

In this review we will not discuss enzymes or enzymatic mechanisms that repair DNA and drugs that interact with these enzymes. Instead, we will focus on a set of drugs, namely, synthetic oleanane triterpenoids (SO), that are highly potent agents for promoting the cellular control of reactive oxygen and nitrogen, as well as other chemical species that can damage both DNA and proteins. SO are potent agents for suppressing either the undesirable formation of both ROS and RNS or the deleterious electrophilic actions of both ROS and RNS. In experimental animal studies, SO can protect many organs of the body from such damage. Moreover, SO show great promise as practical therapeutic agents, especially with the recent clinical advances in the use of one such agent (methyl 2-cyano-3,12-dioxooleana-1,9(11)dien-28-oate, **13**; "CDDO-methyl ester" or "CDDO-Me"; its new generic name is "bardoxolone methyl") for treatment of a hitherto refractory serious disease, namely, chronic kidney disease (diabetic nephropathy). It is therefore appropriate to review the underlying assumptions, both biological and chemical, that relate to both the efficacy and safety of SO.

Our synthetic efforts began as an attempt to design new agents with potent anti-inflammatory activity. In addition to the

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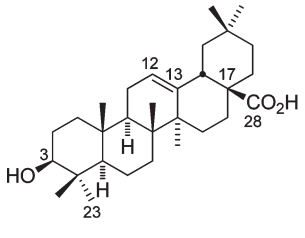
established classic role of inflammation as a causative factor in diseases such as rheumatoid arthritis or chronic lung and kidney disease, it was postulated more than 100 years ago that inflammation was an important process in the etiology of cancer,[7] and more recently there has been great interest in the role of inflammation in the genesis of both cardiovascular and neurodegenerative disease.[8] When we started this project in 1995, the naturally occurring triterpenoids oleanolic acid (**1**) and ursolic acid (**2**) were already known to have anticarcinogenic activity in experimental animals.[9,10] Both compounds were readily and cheaply available for further synthetic modifications. As quantitative assays for evaluating the efficacy of new molecules, we chose to measure their ability to block the cellular synthesis of an enzyme that plays a key role in the process of inflammation, namely, inducible nitric oxide synthase (iNOS).[11] This enzyme is responsible for high-level synthesis of NO from arginine, especially in macrophages, which play a critical role in inflammation throughout the body. The bioassays that we have used to evaluate the new synthetic compounds are easily performed, rapid, and quantitative, thus providing a good basis for establishing structure−activity relationships.

In the present review, we discuss the original chemical synthesis of 2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oic acid ("CDDO", **8**) and its related analogues and the chemistry of triterpenoid biogenesis from squalene and review some of the critical chemical parameters that are important for the selective action and safety of SO. Special emphasis will be placed on the rationale for synthetic chemical modification of a naturally occurring terpenoid scaffold, if we are to make safe and effective drugs. Important biological actions of the new SO and their potential clinical applications will be covered very briefly; this will be the subject of a subsequent review.

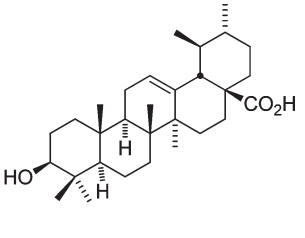
CHEMISTRY OF SYNTHETIC OLEANANE TRITERPENOIDS

The biological importance of the triterpenoid skeleton is almost without parallel among natural products. The annual reviews of triterpenoids in *Natural Product Reports* are ample testimony to the widespread distribution of these compounds in our natural world, including squalenes, lanostanes, fusidanes, dammaranes, euphanes, lupanes, oleananes, ursanes, hopanes, tetranortriterpenoids, quassinoids, and others.[12] Within each class are a dazzling array of structural diversity and a wide range of biological activity.

We chose to study the oleanane and ursane classes, typified by oleanolic acid (**1**) and ursolic acid (**2**), which are commercially available and occur in many natural sources.[13] Some of these representative triterpenoids are shown below.



(1) oleanolic acid

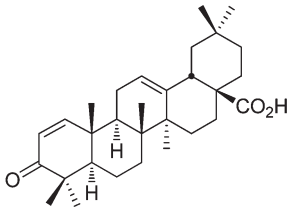


(2) ursolic acid

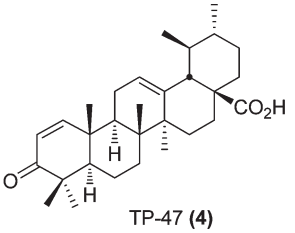
The extensive pharmacology of oleanolic acid (**1**) and ursolic acid (**2**) has been reviewed.[13−15] Although the biological activity of **1** and **2** is modest, oleanolic acid has been marketed in China as an oral drug for treating liver disorders in humans.[14] Moreover, both **1** and **2** are well recognized to possess anti-inflammatory and antitumor activities in animals. As the author of this 1995 review states, "more research is warranted to develop a therapy for patients".[14] Coincidently, it was 1995 when we began this project.

Our synthetic plan was to modify the three "active" portions of **1** and **2**, namely, the C-3 hydroxy, the ring C double bond (C-12−C-13), and the C-28 carboxylic acid. In addition, various ring-cleavage reactions were envisioned to lead to promising synthetic analogues for screening. This is summarized in Figure 1 for oleanolic acid.

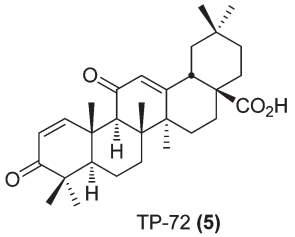
Following the synthesis and screening of some 70 oleanolic and ursolic acid derivatives, our first "hit" in the iNOS assay came when the A-ring enone **3** was prepared.[16] This compound was the 46th synthetic triterpenoid prepared in our laboratory ("TP-46") and was significantly active in the iNOS assay we used, with IC_{50} 6.0 μM. The corresponding ursolic acids, TP-47 (**4**) (IC_{50} 17.6 μM) and TP-81 (**6**), are uniformly less active. This prompted us to convert the C-ring to its corresponding enone. Indeed, compound TP-82 (**7**) with both rings similarly transformed displayed an increase in potency with an IC_{50} of 0.2 μM. Interestingly, the isomeric bis-enone TP-72 (**5**) is less active than TP-82 (**7**). With these encouraging and leading results, we considered that ring-A substitution at C-2 with an electron-withdrawing group would further activate ring A to a conjugate addition reaction (e.g., a thio- or aza-Michael reaction) since this mechanistic pathway seemed plausible for a biological mode of action. Indeed, TP-151 ("CDDO") (2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oic acid) (**8**), with a C-2 cyano group, markedly increased the activity by about 10 000-fold over that of TP-46 (**3**) and is approximately 400 000 times more potent than oleanolic acid in the iNOS assay.[11] The structure−activity profile is shown in Figure 2.



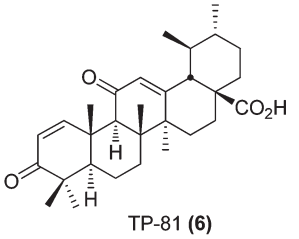
TP-46 (**3**)



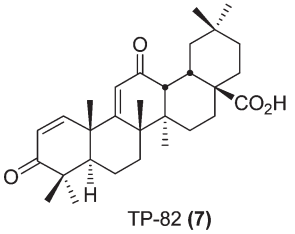
TP-47 (**4**)



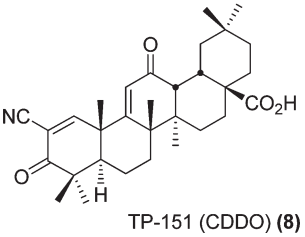
TP-72 (**5**)



TP-81 (**6**)



TP-82 (**7**)

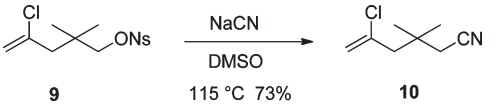


TP-151 (CDDO) (**8**)

Although the rigid scaffold of the oleanane−ursane structure is similar to that of a steroid, an important difference

is that the two functionalized carbons in OA and UA, C-3 and C-28, are of the neopentyl type [$(CH_3)_3CCH_2-$]. Thus, the geminal methyl groups on C-4 and the D−E ring fusion surrounding C-28 severely limit the reactivity of these positions to nucleophilic attack (e.g., S_N2 reactions). It is well known that neopentyl halides are markedly unreactive in S_N2 reactions (Table 1) due to the difficulty of approach by the nucleophile to the backside of the carbon−leaving group bond.

The lack of reactivity of neopentyl systems is illustrated dramatically by the conditions necessary to convert the neopentyl nosylate **9** to **10**: a 300-fold excess of sodium cyanide in DMSO at 115 °C for 12 h.[17] This fact would seem to limit side reactions at C-3 and C-28 with extraneous cellular nucleophiles.



9 **10**

Illustrative of the "protected" reactivity of C-3 and C-28 to nucleophilic attack is the fact that our attempts to displace the C-3 mesylate (**11**) prepared from OA under classic S_N2 conditions with sodium azide (DMF, 100 °C) resulted in E2 elimination to give **12** rather than substitution, and reaction of **11** with lithium bromide in boiling acetone led to recovered starting

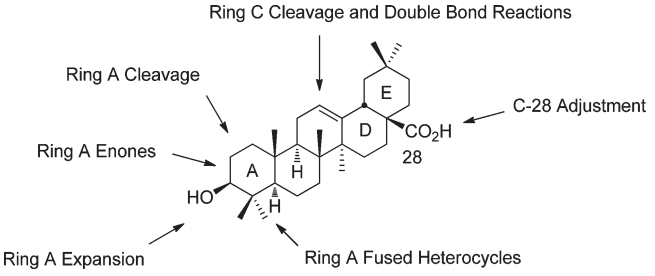


Figure 1. Proposed structural modifications of oleanolic acid.

Table 1. Average Relative Rates of Alkyl Halides with Nucleophiles[a]

alkyl	relative rate
methyl	30
ethyl	1.0
propyl	0.4
isopropyl	0.025
neopentyl	0.00001

[a] Compiled from several studies; see ref 17.

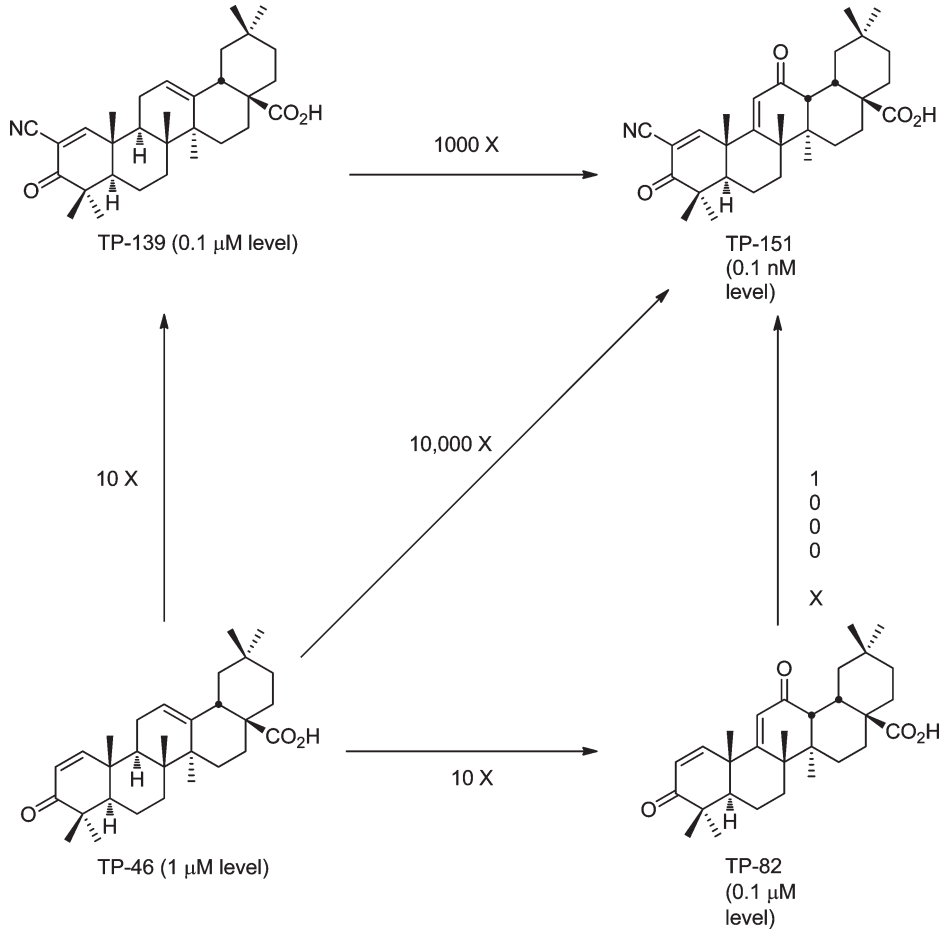


Figure 2. Summary of structure−activity relationships of lead compounds and CDDO (**8**).

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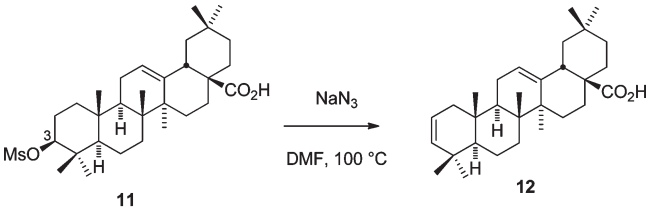
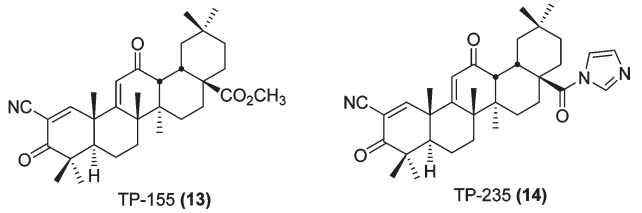
material. Likewise, hydrolysis of the C-28 methyl ester **13** (TP-155, CDDO-Me) to give CDDO (**8**) could not be accomplished under conventional saponification conditions, but required roundabout nucleophilic attack by lithium iodide on the methyl group (*vide infra*). Similarly, the extremely biologically active CDDO-Im (**14**) (TP-235) is relatively stable to *N*-acyl nucleophilic cleavage, unlike typical *N*-acylimidazoles, once again indicative of the neopentyl nature of C-28. The acid chloride of CDDO that was used to synthesize CDDO-Im (**14**) is remarkably stable to nucleophilic addition reactions compared to typical acid chlorides.[18]





Moreover, the high electrophilic reactivity of the A-ring cyanoenone (*vide infra*) is also reflected in the high reversibility of the thio-Michael adduct, which also precludes irreversible conjugate addition reactions (e.g., aza-Michael reactions) with cellular amine and other nucleophiles and presumed accompanying toxicity. We view the unique biological activity of CDDO and related triterpenoid cyanoenones as the result of a "dock and lock" process with a cysteine and/or other amino acid residues of target proteins. Indeed, as shown above, the enhanced activity of the oleanane scaffold over the ursane scaffold, where the only difference is the position of the two methyl groups in ring E, would suggest more favorable target docking for the former derivatives.

Synthesis of CDDO-Me (13) and CDDO (8). The synthesis of CDDO (**8**) features a two-stage modification of the C-ring and A-ring and is achieved in 11 steps from oleanolic acid (**1**), in an 29% overall yield (Scheme 1).[16,18] Initial regioselective esterification of the C-28 carboxylic acid using diazomethane followed by acylation of the C-3 hydroxy group afforded diester **15**. Epoxidation of **15** using hydrogen peroxide followed by *in situ* acid-mediated epoxide rearrangement gave the C-12 ketone **16**. A sequence of bromination/dehydrobromination of ketone **16** provided enone **17** and thus the desired C-ring elaboration. Alkaline hydrolysis released the C-3 hydroxy group, which was followed by Jones oxidation to give diketone **19**. Formylation of **19** with ethyl formate in the presence of sodium methoxide in benzene provided intermediate **20**. Isoxazole **21** was synthesized from **20** by condensation with hydroxylamine. Cleavage of isoxazole **21** using sodium methoxide gave nitrile **22**, predominantly as the enol tautomer shown. Oxidation with DDQ gave the corresponding bis-enone CDDO-Me (**13**) (TP-155). Final halogenolysis with lithium iodide in dimethylformamide afforded CDDO (**8**) (TP-151).

This synthesis has been further modified for large-scale commercial drug development, and it is now possible to produce CDDO-Me (bardoxolone methyl, **13**) in multiple kilogram amounts. The olive tree, both its fruit and its leaves, provides a widely available, commercially reasonable source of oleanolic acid (**1**).

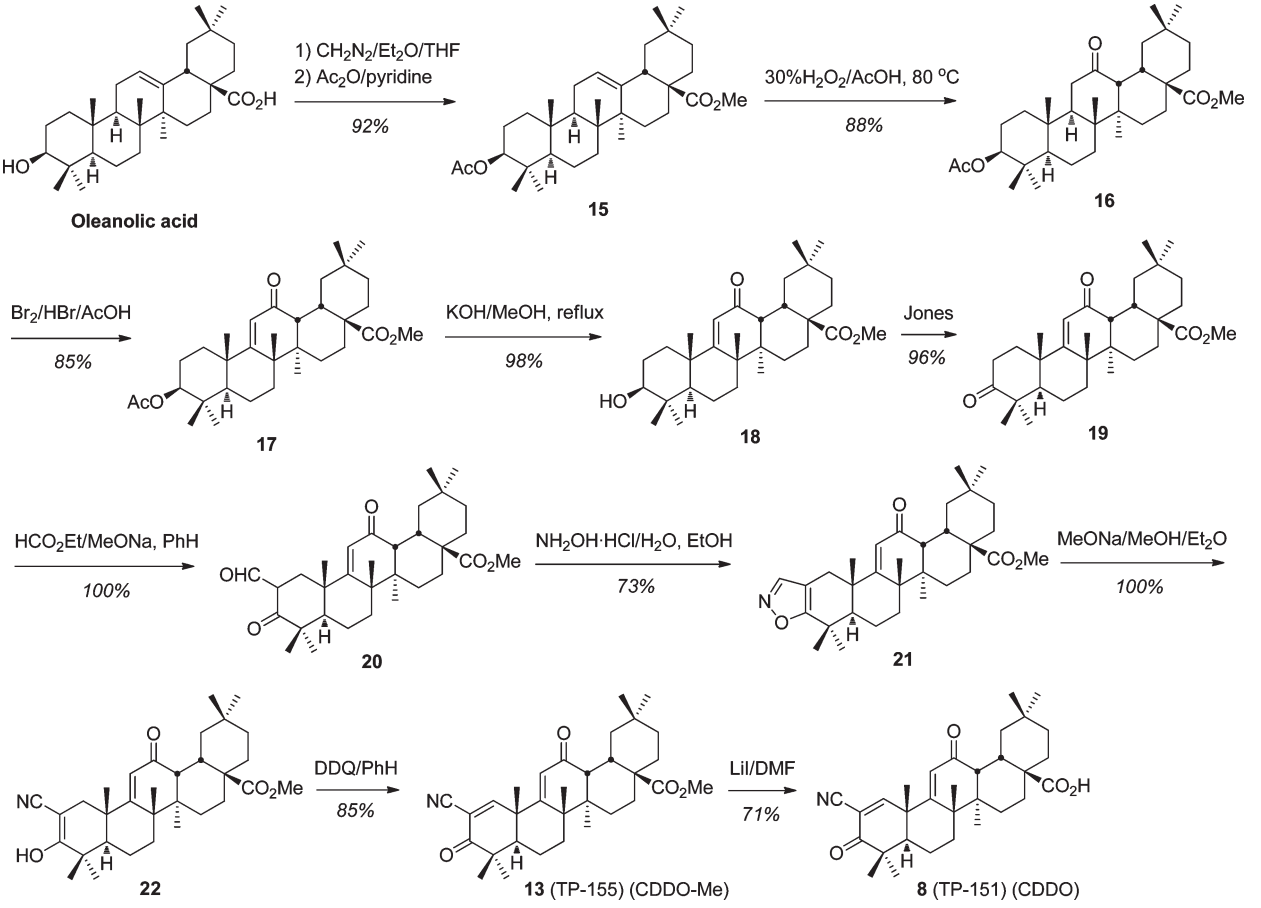
Biosynthesis of Olean-12-ene and Urs-12-ene Skeletons. As summarized in Scheme 2, the cyclization of 2,3-oxidosqualene (**24**) into sterols and other triterpenes is one of the most remarkable and fascinating biotransformations found in nature.[19−22] The construction of tetra- and pentacyclic carbon frameworks catalyzed by oxidosqualene cyclases (OSCs) establishes a number of asymmetric stereocenters [eight chiral centers in the cyclization of 2,3-oxidosqualene (**24**) to β-amyrin (**29**)] in a single biotransformation. The *chair*−*chair*−*chair*−*boat* conformation of **24** initiates the formation of β-amyrin and provides dammarenyl cation (**25**), which is followed by ring expansion to give the baccharenyl cation (**26**). Electrophilic addition of the tetracyclic baccharenyl cation (**26**) onto the terminal double bond generates the lupenyl cation (**27**) with the creation of a five-membered E-ring. The corresponding oleanyl secondary cation (**28**) is thus obtained from the lupenyl cation (**27**) through the E-ring expansion with the relief of ring strain by the generation of a six-membered E-ring, despite the normally unfavorable generation of a secondary cation from a tertiary carbocation. Finally, two sequential 1,2-hydride shifts followed by H-12α elimination yield β-amyrin (**29**) with a 6/6/6/6/6-fused ring system. Alternatively, a C-29 methyl shift indicated by pathway "b" generates the ursyl C-20 cation (**32**) from the oleanyl cation (**28**). Three sequential 1,2-hydride shifts followed by H-12α elimination afford α-amyrin (**31**), which can also be obtained from the bisnoroleanyl C-20 cation (**30**) through a sequential methyl shift and two hydride shifts followed by H-12α elimination.[21] Electrophilic addition of the baccharenyl cation (**26**) generates **30** with the creation of a six-membered E-ring and a secondary carbocation, rather than a five-membered E-ring and a tertiary carbocation.

CHEMICAL MECHANISMS OF BIOLOGICAL ACTIVITY

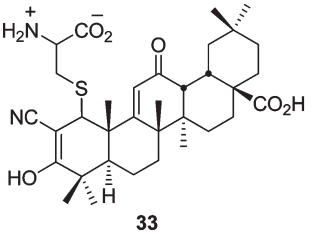
The construction of a 1-en-3-one moiety in the A-ring of oleanolic acid imparts tremendous biological activity compared to what is conveyed by the natural oleanane scaffold. Furthermore, the installation of certain electron-withdrawing groups at the C-2 position (e.g., carboxyl, nitrile) amplifies the potency of this triterpenoid.[18] As a result, we postulate that the biological mechanism of action involves nucleophilic attack (thio- or aza-Michael addition) of a thiol or other nucleophile to the C-1 position (Scheme 3). The significance of thio-Michael addition reactions in biological systems has been reported recently, including the fact that these reactions can be reversible.[23−26] Moreover, hetero-Michael reactions (e.g., thio-, aza-, and oxa-Michael reactions) are assuming enormous importance in organic synthesis.[27−29]

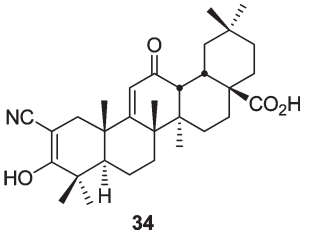
Surprisingly, our attempted reactions of CDDO (**8**) with various thiol and amine nucleophiles failed to afford isolable addition products.[30] Spectroscopic (UV−vis) studies of CDDO in the presence of either reduced glutathione (GSH) or dithiothreitol (DTT), as sulfur nucleophiles, revealed changes in the observed absorption peaks, indicating reactions between CDDO and GSH or DTT, but isolable adducts were not obtained.[30] These observations are supported by NMR studies that showed the expected reversible conjugate addition of sulfur nucleophiles to C-1 of CDDO to give products, such as **33**, wherein the C-1

Scheme 1. Synthesis of CDDO-Me (13) and CDDO (8)



vinyl proton is no longer present, similar to the NMR spectrum of comparison compound **34**.[31] Thus, temperature studies have revealed that the A-ring cyanoenone in CDDO is quite reactive to nucleophilic attack; however, these *in situ* formed adducts are readily reversible upon raising the temperature.[30]

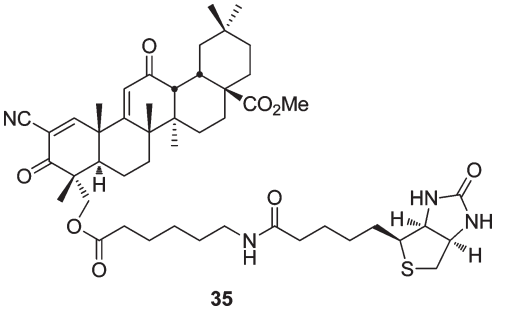




While an irreversible Michael acceptor might be more biologically potent, the reversible nature of CDDO should enhance its bioavailability to target proteins.[22] We consider that the A-ring in CDDO might be further activated toward conjugate addition by other interactions with a target protein *in vivo*. Studies designed to isolate intermediate CDDO-like adducts are currently underway in our laboratory.

BIOTIN CONJUGATES

In order to develop more potent CDDO analogues, we obviously need more information about the mechanism of action and we need to identify target proteins. The affinity chromatographic technique of biotinylation is an important molecular tactic for identifying target proteins.[32] After a biotinylated drug molecule docks with a target protein, the biotin moiety can be complexed with immobilized streptavidin and subsequently isolated and purified. Toward this goal we designed and synthesized the biotin−CDDO conjugate **35**, which was found to be only 3−10 times less potent than CDDO (**8**).[33] Multiple target proteins have now been identified with this biotinylated probe. Moreover, it has been used in a proteomics study (unpublished data) that has shown this compound can selectively bind to many different proteins in the cell with high affinity.



A GENERAL OVERVIEW OF STRUCTURE−ACTIVITY RELATIONSHIPS

Figure 3 summarizes the overall biological activity of hundreds of new SO we have synthesized, as measured in our iNOS assay for inhibition of induction of iNOS. It is important to emphasize that a

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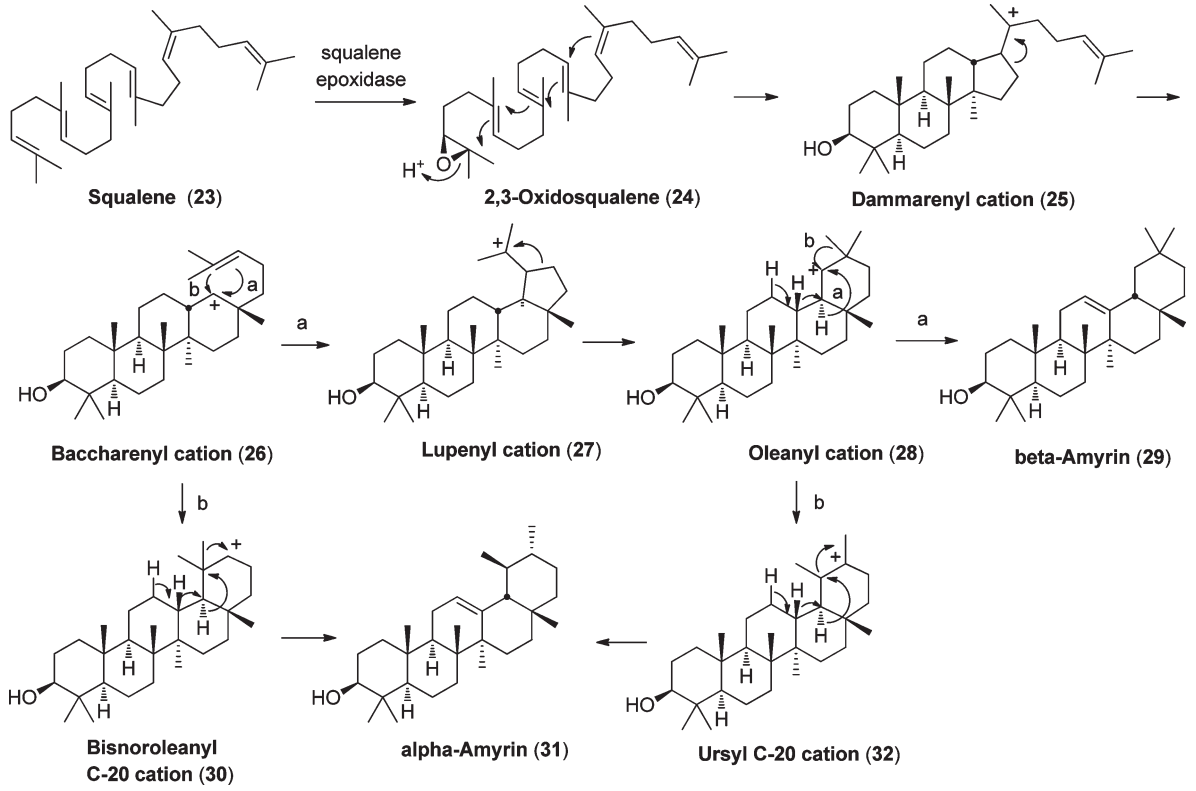
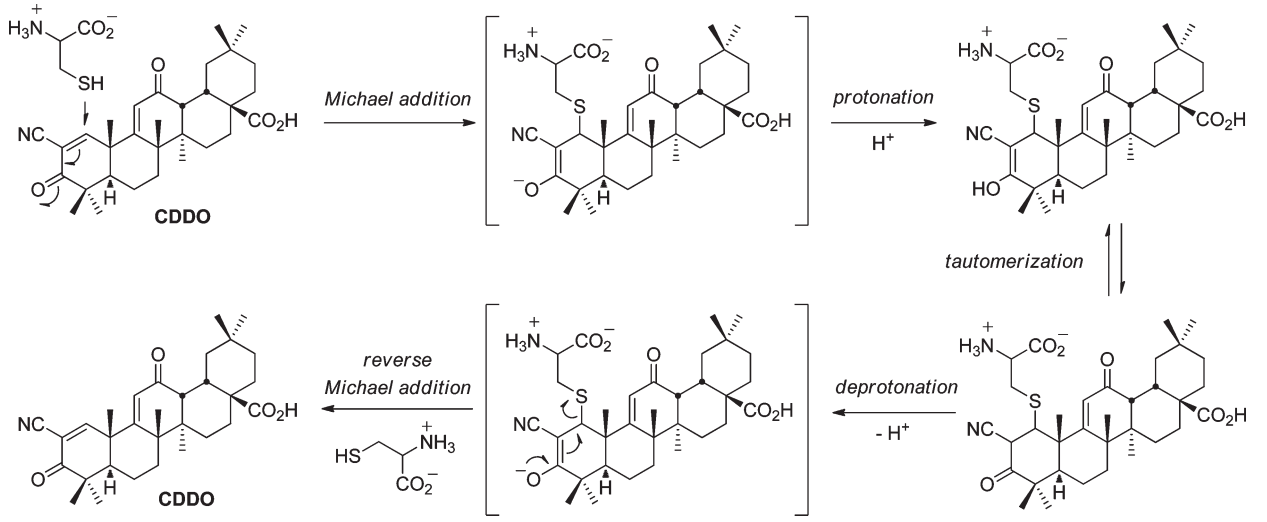
Scheme 2. Biosynthesis of the Oleanane and Ursane Skeletons



Scheme 3. Postulated Mechanism Involving a Thio-Michael Addition of Cysteine to CDDO (8)



totally different bioassay might yield a different SAR profile, but this has not yet been performed in a comprehensive manner. However, in general, measured activities in antiproliferative assays and apoptosis assays correlate with those found in the iNOS assay.

BIOLOGICAL ACTIVITIES OF SYNTHETIC OLEANANE TRITERPENOIDS

As noted in the Introduction, the original rationale for synthesis of new SO relied on their ability to suppress inflammation. This was first measured by their inhibition of the induction of the enzyme iNOS, whose expression is stimulated in cell cultures by various inflammatory cytokines, such as interferon gamma (IFN-γ), tumor necrosis factor-alpha (TNF-α), and interleukin-1 (IL-1). It should be emphasized that SO are not direct enzyme inhibitors themselves, but rather they block the ability of inflammatory cytokines to induce transcription of the respective iNOS gene.[11] In the original report of the biological activity of CDDO (**8**), it was shown that SO also can induce cell

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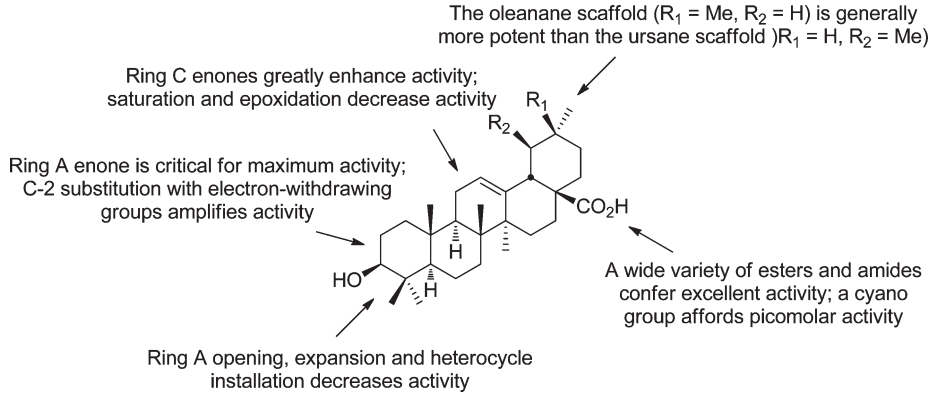

Figure 3. Triterpenoid structure−activity relationships.

differentiation and inhibit cell proliferation.[11] This was then followed by several observations that SO are potent agents for induction of apoptosis (programmed cell death, as opposed to nonspecific cytotoxicity)[34−36] and are also strongly cytoprotective against various forms of electrophilic attack.[37,38] The cytoprotective effects are believed to be mediated by binding of the SO to the inhibitory protein Keap1, which then releases its partner, Nrf2, to be an active transcription factor for expression of mRNA that codes for synthesis of a host of cytoprotective molecules. These molecules include enzymes that directly destroy ROS, as well as enzymes that synthesize small molecules such as glutathione that scavenge potentially destructive electrophiles. This action is known as the "phase 2 response" and has also been called electrophile counterattack.[1,39] These overall actions of SO were summarized in a definitive review written in 2007.[40]

Many protein targets of SO have already been identified. In almost all cases, it has been shown that the binding of the SO to its protein target involves an active cysteine residue in the target. The mechanism of such binding involves Michael addition, as has been discussed earlier in this review. Important protein targets include Keap1,[37,38] I-kappa-B kinase (IKK),[41,42] JAK1 (and one of its targets STAT3),[43−45] PTEN,[46] and proteins associated with the actin-cytoskeleton of the cell.[47,48] Proteomic studies, using the biotinylated triterpenoid described above, are currently ongoing in our laboratory for further elucidation of additional protein targets. It is important to recognize that binding of SO to cysteine residues in specific proteins is both context-dependent and dose-dependent. The nucleophilicity of the free SH group in cysteine in a protein is markedly influenced by neighboring amino acids. Moreover, the unique stereochemistry of the oleanane scaffold prevents SO from randomly alkylating protein targets, as has been discussed previously in this review. Thus, many of the SO that have been synthesized so far have extremely favorable therapeutic actions in animal studies, with minimal toxicity observed. In general, anti-inflammatory and cytoprotective responses to SO are favored by very low concentrations (low nanomolar) of drug, while higher doses (high nanomolar to low micromolar) of SO can even induce oxidative stress and apoptosis.[38,40]

Given the versatility of the SO, in terms of both their mechanism of action described above and their safety, it is not surprising that this class of agents has been extremely useful in prevention or treatment of many diseases in experimental animals, particularly those in which oxidative and inflammatory stress plays a key role in pathogenesis.[8] By now, there is a very large experimental literature documenting such activities. The following are some of the diseases in which SO have been used favorably in experimental animal models: several neurodegenerative diseases including Parkinson's disease,[49] Huntington's disease,[49,50] and Alzheimer's disease;[51] various inflammatory diseases of the lung including cystic fibrosis and emphysema induced by cigarette smoke;[52−55] inflammatory cardiovascular disease;[53,56] acute liver injury caused by carcinogens, hepatotoxic drugs such as acetaminophen, or other agents;[57−59] disease states characterized by hyperactivity of the immune system;[55−61] diseases of the retina and the uveal tract in the eye;[46,62] cancer, including both prevention and treatment;[57−68] kidney injury caused by toxic agents,[39,69] and most recently diabetes.[70]

This impressive list of activities in experimental animals makes it clear that clinical use in patients would be of major interest. Thus far, clinical investigations still are in their earliest stages, but it has already been shown that CDDO methyl ester (bardoxolone methyl, **13**) has yielded beneficial results in patients with advanced chronic kidney disease (CKD), many of whom suffer from diabetic nephropathy. Promising results have already been reported in phase 2 trials, and a large phase 3 study is planned. As is the case in many other diseases, the processes of oxidative and inflammatory stress are believed to play a major role in the pathogenesis of CKD, so there is a strong rationale for the use of SO for this indication.[71] Thus far, CDDO methyl ester has been very well tolerated in patients, and its clinical safety profile appears to be excellent. Again, these clinical findings suggest that the use of a triterpenoid scaffold has greatly diminished the potential for random alkylation that conceivably might occur with the use of a drug that acts by Michael addition.

CONCLUSIONS

Our very existence involves a trade-off between the requirement to use both reactive oxygen and nitrogen to provide energy for life and protection from death caused by infectious agents and the possibility that both reactive oxygen and nitrogen can also cause irreparable damage to the organism. Thus, life truly made a pact with the devil when it made a Faustian bargain and allowed the mitochondrial genome into eukaryotic cells and a second pact with the devil (another Faustian bargain) when it allowed the development of the immune system. Of course, the immune system can improve the quality of life, but it also can destroy the organism, if it is not controlled. Both ROS and RNS are used at extremely low concentrations within cells as essential mediators of intracellular communication.[72] At much higher concentrations, ROS and RNS produced by immune cells (macrophages,

neutrophils, and lymphocytes) can effectively destroy dangerous invading microorganisms. Thus, it is essential that the body tightly control the formation and activity of these molecules.

Indeed, research on oxidative, inflammatory, and metabolic stress is now at center stage in biomedical research, with the attendant development of new drugs to control such stress, which is a cause of so many chronic degenerative diseases, whether they be cardiovascular, pulmonary, arthritic, malignant, neurodegenerative, renal, or other. Enhancement of the physiological protective mechanisms that have evolved in our bodies over many millennia now offers unique opportunities to control the pathogenesis of such diseases; the synthetic triterpenoids offer such possibility. Because of the unique steric hindrance of the triterpenoid skeleton, triterpenoids provide a unique platform for drug development. Each triterpenoid scaffold that occurs in nature has its own stereochemistry of rings and exocyclic methyl groups that convey specific recognition of motifs in proteins in the cell. At the same time, this specific stereochemistry prevents random contact of the triterpenoid with all cellular proteins. Basic tetracyclic and pentacyclic triterpenoid scaffolds also have unique safety profiles, because they have been ingested safely by animals for millions of years. Here, we have reviewed the striking progress that had already been made with the new development of SO, but we have only scratched the surface. We can be optimistic that further advances will come that will provide relief from human physical suffering and mental anguish.

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DEDICATION

Dedicated to Dr. Koji Nakanishi of Columbia University for his pioneering work on bioactive natural products.

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REFERENCES

(1) Prestera, T.; Zhang, Y.; Spencer, S. R.; Wilczak, C. A.; Talalay, P. *Adv. Enzyme Regul.* **1993**, *33*, 281–296.

(2) Bereiter-Hahn, J.; Jendrach, M. *Int. Rev. Cell Mol. Biol.* **2010**, *284*, 1–65.

(3) Kraaij, M. D.; Savage, N. D.; van der Kooij, S. W.; Koekkoek, K.; Wang, J.; van den Berg, J. M.; Ottenhoff, T. H.; Kuijpers, T. W.; Holmdahl, R.; van Kooten, C.; Gelderman, K. A. *Proc. Natl. Acad. Sci. U. S. A.* **2010**, *107*, 17686–17691.

(4) Talalay, P.; Dinkova-Kostova, A. T.; Holtzclaw, W. D. *Adv. Enzyme Regul.* **2003**, *43*, 121–134.

(5) Marnett, L. J.; Riggins, J. N.; West, J. D. *J. Clin. Invest.* **2003**, *111*, 583–593.

(6) Paulsen, C. E.; Carroll, K. S. *ACS Chem. Biol.* **2010**, *5*, 47–62.

(7) Virchow, R. *Die Krankhaften Geschwulste*; August Hirschwald: Berlin, 1863−1867.

(8) Nathan, C. *Nature* **2002**, *420*, 846–852.

(9) Nishino, H.; Nishino, A.; Takayasu, J.; Hasegawa, T.; Iwashima, A.; Hirabayashi, K.; Iwata, S.; Shibata, S. *Cancer Res.* **1988**, *48*, 5210–5215.

(10) Huang, M. T.; Ho, C. T.; Wang, Z. Y.; Ferraro, T.; Lou, Y. R.; Stauber, K.; Ma, W.; Georgiadis, C.; Laskin, J. D.; Conney, A. H. *Cancer Res.* **1994**, *54*, 701–708.

(11) Suh, N.; Wang, Y.; Honda, T.; Gribble, G. W.; Dmitrovsky, E.; Hickey, W. F.; Maue, R. A.; Place, A. E.; Porter, D. M.; Spinella, M. J.; Williams, C. R.; Wu, G.; Dannenberg, A. J.; Flanders, K. C.; Letterio, J. J.; Mangelsdorf, D. J.; Nathan, C. F.; Nguyen, L.; Porter, W. W.; Ren, R. F.; Roberts, A. B.; Roche, N. S.; Subbaramaiah, K.; Sporn, M. B. *Cancer Res.* **1999**, *59*, 336–341.

(12) Connolly, J. D.; Hill, R. A. *Nat. Prod. Rep.* **2010**, *27*, 79–132, and previous reviews in this series.

(13) Ovesna, Z.; Vachalkova, A.; Horvathova, K.; Tothova, D. *Neoplasma* **2004**, *51*, 327–333.

(14) Liu, J. *J. Ethnopharmacol.* **1995**, *49*, 57–68.

(15) Liu, J. *J. Ethnopharmacol.* **2005**, *100*, 92–94.

(16) Honda, T.; Rounds, B. V.; Gribble, G. W.; Suh, N.; Wang, Y.; Sporn, M. B. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2711–2714.

(17) Chung, J. Y. L.; Reamer, R. A.; Reider, P. J. *Tetrahedron Lett.* **1992**, *33*, 4717–4720, and private communication from Dr. Chung. See also:Streitwieser, A., Jr. *Solvolytic Displacement Reactions*; McGraw-Hill: New York, 1962; p 13.

(18) Honda, T.; Honda, Y.; Favaloro, F. G., Jr.; Gribble, G. W.; Suh, N.; Place, A. E.; Rendi, M. H.; Sporn, M. B. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1027–1030.

(19) Nakanishi, K.; Goto, T.; Ito, S.; Natori, S.; Nozoe, S. *Natural Products Chemistry*; Kodansha Ltd., Toyko, University Science Books: Mill Valley, CA, 1983; Vol. 3, Chapter 3, pp 187−187.

(20) Kushiro, T.; Shibuya, M.; Ebizuka, Y. J. *J. Am. Chem. Soc.* **1999**, *121*, 1208–1216.

(21) Abe, I.; Sakano, Y.; Sodeyama, M.; Tanaka, H.; Noguchi, H.; Shibuya, M.; Ebizuka, Y. *J. Am. Chem. Soc.* **2004**, *126*, 6880–6881.

(22) Phillips, D. R.; Rasbery, J. M.; Bartel, B.; Matsuda, S. P. *Curr. Opin. Plant Biol.* **2006**, *9*, 305–314.

(23) Shi, B.; Greaney, M. F. *Chem. Commun.* **2005**, 886–888.

(24) Li, W. W.; Heinze, J.; Haehnel, W. *J. Am. Chem. Soc.* **2005**, *127*, 6140–6141.

(25) Steert, K.; El-Sayed, I.; Van der Veken, P.; Krishtal, A.; Van Alsenoy, C.; Westrop, G. D.; Mottram, J. C.; Coombs, G. H.; Augustyns, K.; Haemers, A. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 6563–6566.

(26) Dinkova-Kostova, A. T.; Massiah, M. A.; Bozak, R. E.; Hicks, R. J.; Talalay, P. *Proc. Natl. Acad. Sci. U. S. A.* **2001**, *98*, 3404–3409.

(27) For selected thio-Michael reactions, see: (a) Naidu, B. N.; Sorenson, M. E.; Bronson, J. J.; Pucci, M. J.; Clark, J. M.; Ueda, Y. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2069–2072. (b) Kumar, A.; Akanksha *Tetrahedron* **2007**, *63*, 11086–11092. (c) Chu, C.-M.; Gao, S.; Sastry, M. N. V.; Yao, C.-F. *Tetrahedron Lett.* **2005**, *46*, 4971–4974. (d) Karodia, N.; Liu, X.; Ludley, P.; Pletsas, D.; Stevenson, G. *Tetrahedron* **2006**, *62*, 11039–11043.

(28) For selected aza-Michael reactions, see: (a) Surendra, K.; Krishnaveni, N. S.; Sridhar, R.; Rao, K. R. *Tetrahedron Lett.* **2006**, *47*, 2125–2127. (b) Duan, Z.; Xuan, X.; Li, T.; Yang, C.; Wu, Y. *Tetrahedron Lett.* **2006**, *47*, 5433–5436. (c) Karodia, N.; Liu, X.; Ludley, P.; Pletsas, D.; Stevenson, G. *Tetrahedron* **2006**, *62*, 11039–11043.

(29) For selected oxa-Michael reactions, see: (a) Nising, C. F.; Bräse, S. *Chem. Soc. Rev.* **2008**, *37*, 1218–1228. (b) Karodia, N.; Liu, X.; Ludley, P.; Pletsas, D.; Stevenson, G. *Tetrahedron* **2006**, *62*, 11039–11043.

(30) Couch, R. D.; Browning, R. G.; Honda, T.; Gribble, G. W.; Wright, D. L.; Sporn, M. B.; Anderson, A. C. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2215–2219.
(31) Honda, T.; Gribble, G. W.; Suh, N.; Finlay, H. J.; Rounds, B. V.; Bore, L.; Favaloro, F. G., Jr.; Wang, Y.; Sporn, M. B. *J. Med. Chem.* **2000**, *43*, 1866–1877.
(32) For reviews, see: (a) Green, N. M. *Adv. Protein Chem.* **1975**, *29*, 85–133. (b) Billingsley, M. L.; Pennypacker, K. R.; Hoover, C. G.; Kincaid, R. L. *Biotechniques* **1987**, *5*, 22–31. (c) For the biotinylation of retinoids, see: Nesnas, N.; Rando, R. R.; Nakanishi, K. *Tetrahedron* **2002**, *58*, 6577–6584.
(33) Honda, T.; Janosik, T.; Honda, Y.; Han, J.; Liby, K. T.; Williams, C. R.; Couch, R. D.; Anderson, A. C.; Sporn, M. B.; Gribble, G. W. *J. Med. Chem.* **2004**, *47*, 4923–4932.
(34) Ikeda, T.; Sporn, M.; Honda, T.; Gribble, G. W.; Kufe, D. *Cancer Res.* **2003**, *63*, 5551–5558.
(35) Konopleva, M.; Tsao, T.; Ruvolo, P.; Stiouf, I.; Estrov, Z.; Leysath, C. E.; Zhao, S.; Harris, D.; Chang, S.; Jackson, C. E.; Munsell, M.; Suh, N.; Gribble, G.; Honda, T.; May, W. S.; Sporn, M. B.; Andreeff, M. *Blood* **2002**, *99*, 326–335.
(36) Ito, Y.; Pandey, P.; Place, A.; Sporn, M. B.; Gribble, G. W.; Honda, T.; Kharbanda, S.; Kufe, D. *Cell Growth Differ.* **2000**, *11*, 261–267.
(37) Dinkova-Kostova, A. T.; Liby, K. T.; Stephenson, K. K.; Holtzclaw, W. D.; Gao, X.; Suh, N.; Williams, C.; Risingsong, R.; Honda, T.; Gribble, G. W.; Sporn, M. B.; Talalay, P. *Proc. Natl. Acad. Sci. U. S. A.* **2005**, *102*, 4584–4589.
(38) Liby, K.; Hock, T.; Yore, M. M.; Suh, N.; Place, A. E.; Risingsong, R.; Williams, C. R.; Royce, D. B.; Honda, T.; Honda, Y.; Gribble, G. W.; Hill-Kapturczak, N.; Agarwal, A.; Sporn, M. B. *Cancer Res.* **2005**, *65*, 4789–4798.
(39) Tanaka, Y.; Aleksunes, L. M.; Goedken, M. J.; Chen, C.; Reisman, S. A.; Manautou, J. E.; Klaassen, C. D. *Toxicol. Appl. Pharmacol.* **2008**, *231*, 364–373.
(40) Liby, K. T.; Yore, M. M.; Sporn, M. B. *Nat. Rev. Cancer* **2007**, *7*, 357–369.
(41) Yore, M. M.; Liby, K. T.; Honda, T.; Gribble, G. W.; Sporn, M. B. *Mol. Cancer Ther.* **2006**, *5*, 3232–3239.
(42) Ahmad, R.; Raina, D.; Meyer, C.; Kharbanda, S.; Kufe, D. *J. Biol. Chem.* **2006**, *281*, 35764–35769.
(43) Liby, K.; Voong, N.; Williams, C. R.; Risingsong, R.; Royce, D. B.; Honda, T.; Gribble, G. W.; Sporn, M. B.; Letterio, J. J. *Clin. Cancer Res.* **2006**, *12*, 4288–4293.
(44) Ahmad, R.; Raina, D.; Meyer, C.; Kufe, D. *Cancer Res.* **2008**, *68*, 2920–2926.
(45) Liby, K.; Risingsong, R.; Royce, D. B.; Williams, C. R.; Yore, M. M.; Honda, T.; Gribble, G. W.; Lamph, W. W.; Vannini, N.; Sogno, I.; Albini, A.; Sporn, M. B. *Clin. Cancer Res.* **2008**, *14*, 4556–4563.
(46) Pitha-Rowe, I.; Liby, K.; Royce, D.; Sporn, M. *Invest. Ophthalmol. Vis. Sci.* **2009**, *50*, 5339–5347.
(47) To, C.; Kulkarni, S.; Pawson, T.; Honda, T.; Gribble, G. W.; Sporn, M. B.; Wrana, J. L.; Di Guglielmo, G. M. *J. Biol. Chem.* **2008**, *283*, 11700–11713.
(48) To, C.; Shilton, B. H.; Di Guglielmo, G. M. *J. Biol. Chem.* **2010**, *285*, 27944–27957.
(49) Yang, L.; Calingasan, N. Y.; Thomas, B.; Chaturvedi, R. K.; Kiaei, M.; Wille, E. J.; Liby, K. T.; Williams, C.; Royce, D.; Risingsong, R.; Musiek, E. S.; Morrow, J. D.; Sporn, M.; Beal, M. F. *PLoS One* **2009**, *4*, e5757.
(50) Stack, C.; Ho, D.; Wille, E.; Calingasan, N. Y.; Williams, C.; Liby, K.; Sporn, M.; Dumont, M.; Beal, M. F. *Free Radical Biol. Med.* **2010**, *49*, 147–158.
(51) Dumont, M.; Wille, E.; Calingasan, N. Y.; Tampellini, D.; Williams, C.; Gouras, G. K.; Liby, K.; Sporn, M.; Nathan, C.; Flint, B. M.; Lin, M. T. *J. Neurochem.* **2009**, *109*, 502–512.
(52) Nichols, D. P.; Ziady, A. G.; Shank, S. L.; Eastman, J. F.; Davis, P. B. *Am. J. Physiol. Lung Cell. Mol. Physiol.* **2009**, *297*, L828–L836.
(53) Sussan, T. E.; Rangasamy, T.; Blake, D. J.; Malhotra, D.; El Haddad, H.; Bedja, D.; Yates, M. S.; Kombairaju, P.; Yamamoto, M.; Liby, K. T.; Sporn, M. B.; Gabrielson, K. L.; Champion, H. C.; Tuder, R. M.; Kensler, T. W.; Biswal, S. *Proc. Natl. Acad. Sci. U. S. A.* **2009**, *106*, 250–255.
(54) Reddy, N. M.; Suryanaraya, V.; Yates, M. S.; Kleeberger, S. R.; Hassoun, P. M.; Yamamoto, M.; Liby, K. T.; Sporn, M. B.; Kensler, T. W.; Reddy, S. P. *Am. J. Respir. Crit. Care Med.* **2009**, *180*, 867–874.
(55) Segal, B. H.; Han, W.; Bushey, J. J.; Joo, M.; Bhatti, Z.; Feminella, J.; Dennis, C. G.; Vethanayagam, R. R.; Yull, F. E.; Capitano, M.; Wallace, P. K.; Minderman, H.; Christman, J. W.; Sporn, M. B.; Chan, J.; Vinh, D. C.; Holland, S. M.; Romani, L. R.; Gaffen, S. L.; Freeman, M. L.; Blackwell, T. S. *PLoS One* **2010**, *5*, e9631.
(56) Ichikawa, T.; Li, J.; Meyer, C. J.; Janicki, J. S.; Hannink, M.; Cui, T. *PLoS One* **2009**, *4*, e8391.
(57) Yates, M. S.; Kwak, M. K.; Egner, P. A.; Groopman, J. D.; Bodreddigari, S.; Sutter, T. R.; Baumgartner, K. J.; Roebuck, B. D.; Liby, K. T.; Yore, M. M.; Honda, T.; Gribble, G. W.; Sporn, M. B.; Kensler, T. W. *Cancer Res.* **2006**, *66*, 2488–2494.
(58) Osburn, W. O.; Yates, M. S.; Dolan, P. D.; Chen, S.; Liby, K. T.; Sporn, M. B.; Taguchi, K.; Yamamoto, M.; Kensler, T. W. *Toxicol. Sci.* **2008**, *104*, 218–227.
(59) Reisman, S. A.; Buckley, D. B.; Tanaka, Y.; Klaassen, C. D. *Toxicol. Appl. Pharmacol.* **2009**, *236*, 109–114.
(60) Sun, K.; Li, M.; Konopleva, M.; Konoplev, S.; Stephens, L. C.; Kornblau, S. M.; Frolova, O.; Wilkins, D. E.; Ma, W.; Welniak, L. A.; Andreeff, M.; Murphy, W. J. *Biol. Blood Marrow Transplant.* **2007**, *13*, 521–529.
(61) Thimmulappa, R. K.; Scollick, C.; Traore, K.; Yates, M.; Trush, M. A.; Liby, K. T.; Sporn, M. B.; Yamamoto, M.; Kensler, T. W.; Biswal, S. *Biochem. Biophys. Res. Commun.* **2006**, *351*, 883–889.
(62) Nagai, N.; Thimmulappa, R. K.; Cano, M.; Fujihara, M.; Izumi-Nagai, K.; Kong, X.; Sporn, M. B.; Kensler, T. W.; Biswal, S.; Handa, J. T. *Free Radical Biol. Med.* **2009**, *47*, 300–306.
(63) Liby, K.; Royce, D. B.; Williams, C. R.; Risingsong, R.; Yore, M. M.; Honda, T.; Gribble, G. W.; Dmitrovsky, E.; Sporn, T. A.; Sporn, M. B. *Cancer Res.* **2007**, *67*, 2414–2419.
(64) Ling, X.; Konopleva, M.; Zeng, Z.; Ruvolo, V.; Stephens, L. C.; Schober, W.; McQueen, T.; Dietrich, M.; Madden, T. L.; Andreeff, M. *Cancer Res.* **2007**, *67*, 4210–4218.
(65) Kress, C. L.; Konopleva, M.; Martinez-Garcia, V.; Krajewska, M.; Lefebvre, S.; Hyer, M. L.; McQueen, T.; Andreeff, M.; Reed, J. C.; Zapata, J. M. *PLoS One* **2007**, *2*, e559.
(66) Liby, K.; Risingsong, R.; Royce, D. B.; Williams, C. R.; Yore, M. M.; Honda, T.; Gribble, G. W.; Lamph, W. W.; Vannini, N.; Sogno, I.; Albini, A.; Sporn, M. B. *Clin. Cancer Res.* **2008**, *14*, 4556–4563.
(67) Nagaraj, S.; Youn, J. I.; Weber, H.; Iclozan, C.; Lu, L.; Cotter, M. J.; Meyer, C.; Becerra, C. R.; Fishman, M.; Antonia, S.; Sporn, M. B.; Liby, K. T.; Rawal, B.; Lee, J. H.; Gabrilovich, D. I. *Clin. Cancer Res.* **2010**, *16*, 1812–1823.
(68) Liby, K. T.; Royce, D. B.; Risingsong, R.; Williams, C. R.; Maitra, A.; Hruban, R. H.; Sporn, M. B. *Cancer Prev. Res.* **2010**, *3*, 1427–1434.
(69) Aleksunes, L. M.; Goedken, M. J.; Rockwell, C. E.; Thomale, J.; Manautou, J. E.; Klaassen, C. D. *J. Pharmacol. Exp. Ther.* **2010**, *335*, 2–12.
(70) Saha, P. K.; Reddy, V. T.; Konopleva, M.; Andreeff, M.; Chan, L. *J. Biol. Chem.* **2010**, *285*, 40581–40592.
(71) Forbes, J. M.; Coughlan, M. T.; Cooper, M. E.. *Diabetes* **2008**, *57*, 1446–1454.
(72) Nathan, C. *J. Clin. Invest.* **2003**, *111*, 769–778.