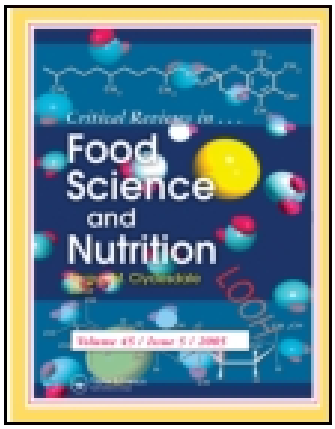

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An Overview on Chemical Aspects and Potential Health Benefits of Limonoids and Their Derivatives

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An Overview on Chemical Aspects and Potential Health Benefits of Limonoids and Their Derivatives

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Limonoids are heavily oxygenated, modified triterpenes dominant in Meliaceae and Rutaceae plant families. The term 'limonoid' is derived from limonin, which was first identified as the bitter constituent of Citrus seeds in 1841. This group of secondary metabolites exhibits a wide range of biological properties, including anticancer, antibacterial, antifungal, antimalarial, and antiviral activities. Significant progress on the role of limonoids as promising candidates for cancer chemoprevention and/or therapy has been achieved in particular in recent years. The aim of this review article is to discuss the recent developments on limonoids chemical aspects and biological activities with the relationship between structure and activity, supporting the new possibilities for the medicinal and/or nutraceutical use of these compounds.

Keywords Limonoids, *citrus*, anticancer, antimicrobial, antioxidant, anti-inflammatory

INTRODUCTION

Limonoids are a class of secondary metabolites confined to the families of Rutaceae and Meliaceae, and less frequently to Cneoraceae and Simaroubaceae, which represent chemotaxonomic markers.

They are heavily oxygenated, modified triterpenes that have been demonstrated to possess a wide range of biological properties, including anticancer, antibacterial, antifungal, antimalarial, and antiviral activities. Significant progress on the role of limonoids as promising candidates for cancer chemoprevention and/or therapy has been achieved in recent years.

Several review articles have been covered limonoids properties (Manners et al., 2003; Roy and Saraf, 2006; Manners 2007; Patil et al, 2009b). The potential role of these secondary metabolites as antineoplastic agents is the main subject of two other reviews (Jacob et al., 2000; Ejaz et al., 2006). A particular attention was addressed to the distribution, bioavailability, and bioactivity of *Citrus* limonoids. In fact, in the past years, there has been a renewed interest in studying and quantifying constituents of fruits and vegetables for their potential health functionality against various disorders such as diabetes, cancer, and cardiovascular and neurodegenerative diseases (Kaur and Kapoor, 2001).

This review article aimed to report the recent results on the bioactivity of naturally occurring, and synthetic and semisynthetic limonoids, with particular emphasis on their anticancer and antimicrobial properties. The mechanism of action and the structure–activity relationships (SAR) were also discussed.

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GENERAL CHARACTERISTICS, SOURCES, AND BIOAVAILABILITY OF LIMONOIDS

Limonoids are triterpene derivatives from a precursor with a 4,4,8-trimethyl-17-furanylsteroid skeleton found in plant families such as Rutaceae, in particular in *Citrus* species, and Meliaceae (see Fig. 1).

Citrus limonoids are composed of two main nucleus structures. The structure of limonin (**1**) exemplifies the first general nucleus, which consists of five rings. The second limonoid structure, such as nomilin (**2**), consists of four rings designated as A, B, C, and D. The structural variations of limonoids found in Rutaceae are less than in Meliaceae and are generally limited to the modification of A and B rings. The limonoids found in Meliaceae are more complex, with very high degree of oxidation and rearrangement exhibited in the parent limonoid structure.

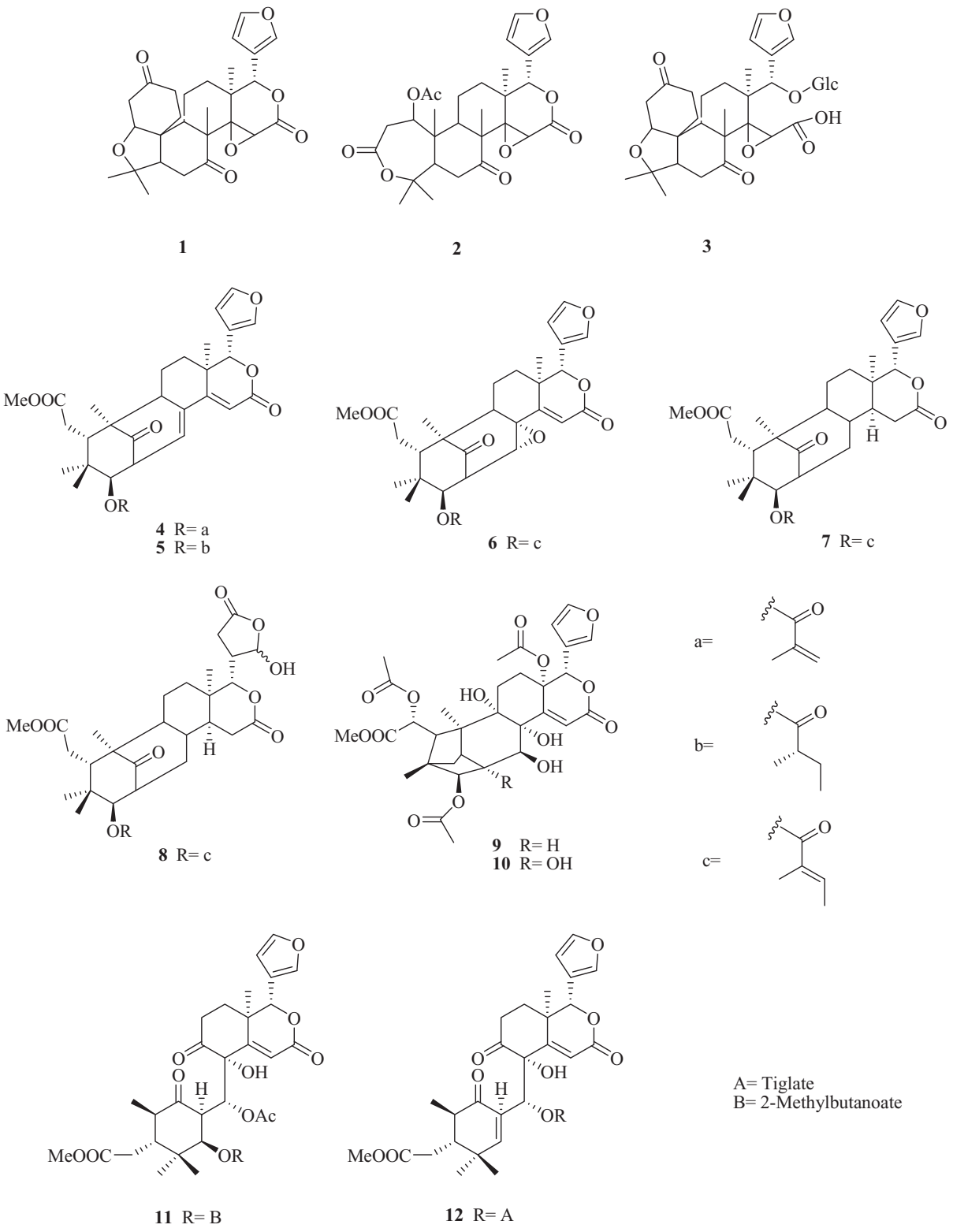
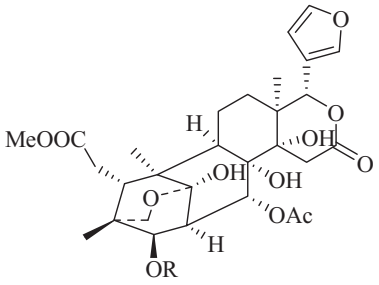
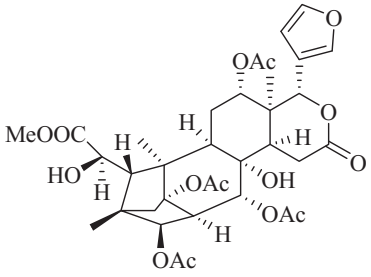


Figure 1 Different liminoids with their chemical structures (Continued).



13 R= A



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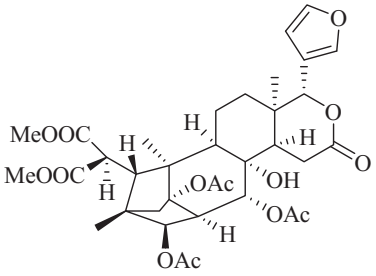

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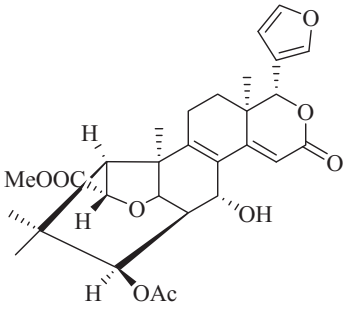

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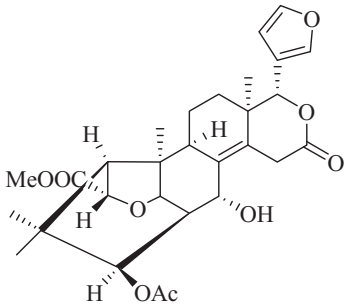

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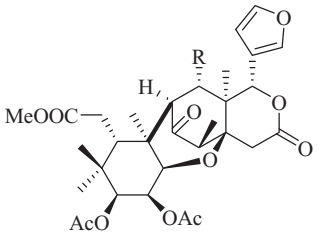
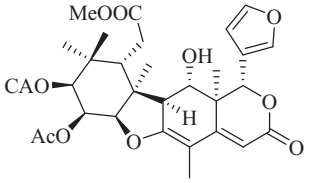


18 R= OAc
19 R= H



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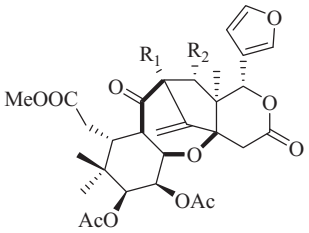
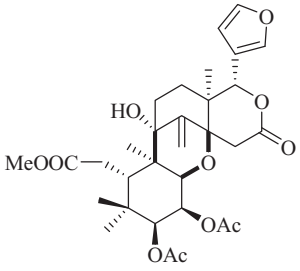


21 R_1= H, R_2= OAc
22 R_1= OH, R_2= H



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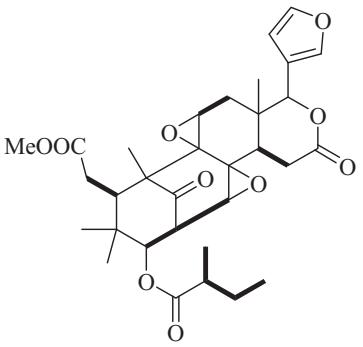

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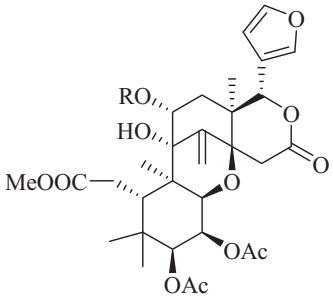
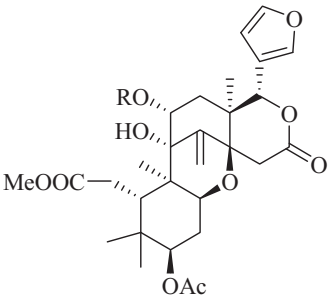
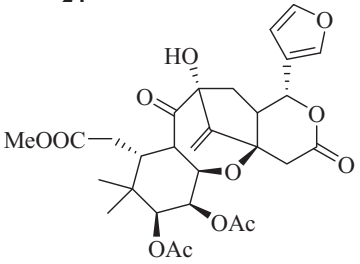


25 R= H
29 R= Ac



26 R= H
27 R= Ac



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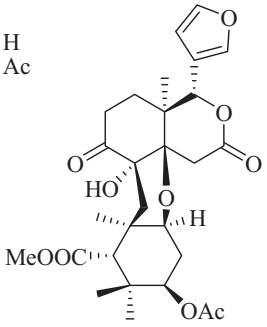

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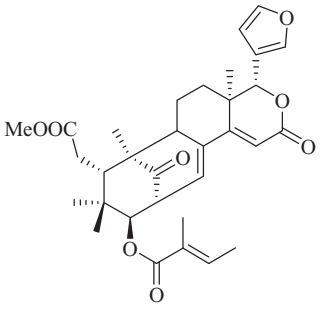

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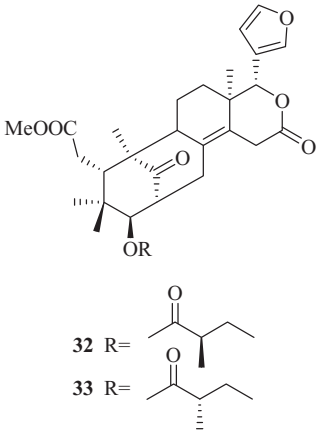
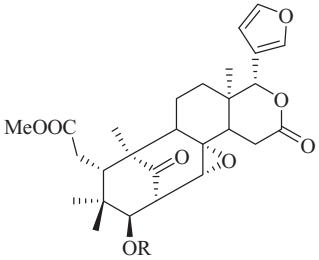
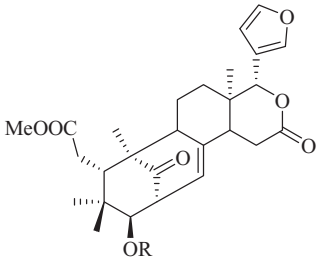


32 R=
33 R=



34 R=
35 R=



36 R=
37 R=

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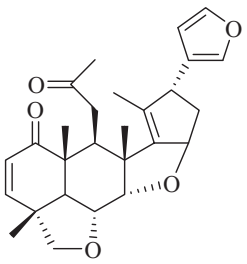

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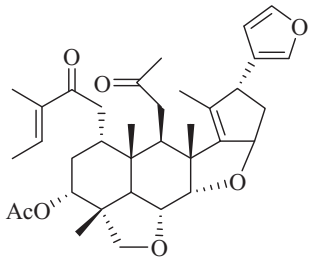

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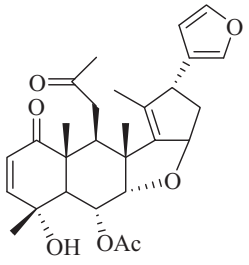

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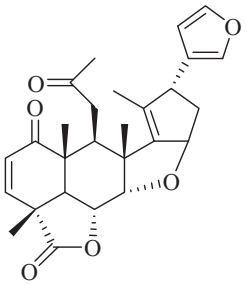

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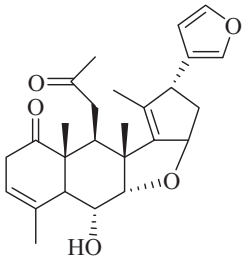

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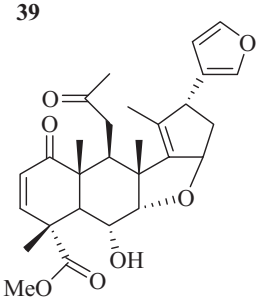

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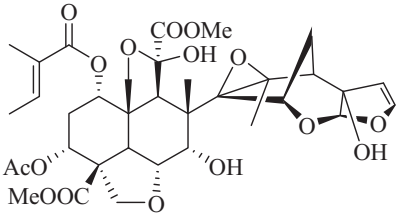

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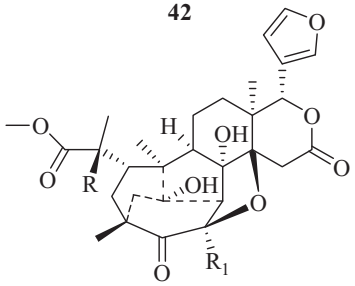
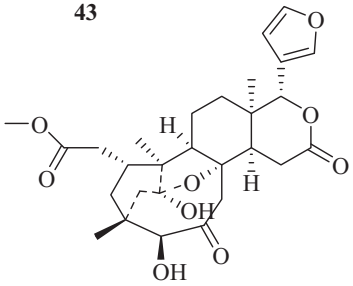


45 R= R_1= H
46 R= R_1= OH



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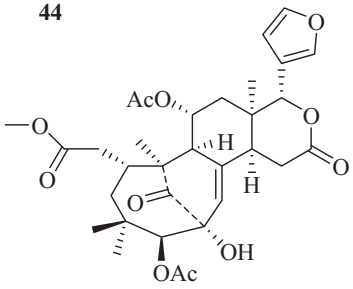

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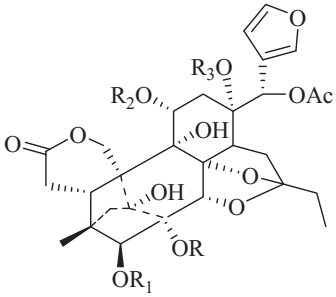
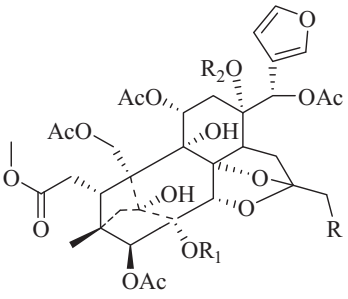
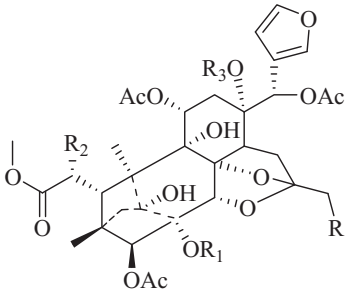


49 R= H, R_1=R_2=R_3= Ac
50 R= R_1=R_2= Ac, R_3= a
51 R= R_1=R_2= Ac, R_3= b
52 R=R_1= H, R_2=R_3= Ac
53 R=R_1= Ac, R_2=R_3= H
54 R= H, R_1= Ac, R_2=R_3= H

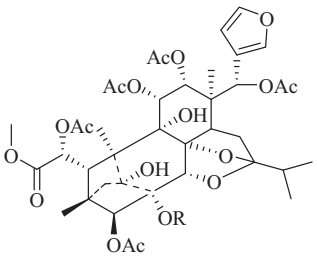


55 R= H, R_1=R_2= Ac
56 R= Me, R_1=H, R_2= Ac
57 R= Me, R_1=R_2= Ac
58 R= Me, R_1= Ac, R_2= a
59 R= Me, R_1= Ac, R_2= b

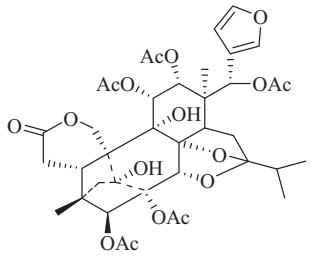


60 R= H, R_2= OH, R_1=R_3= Ac
61 R= Me, R_1=R_2=R_3= Ac
62 R= Me, R_1=R_2= H, R_3= Ac
63 R=R_1=R_2= H, R_3= Ac
64 R=H, R_1= Ac, R_2=R_3= H

Figure 1 (Continued)



65 R= H
66 R= Ac



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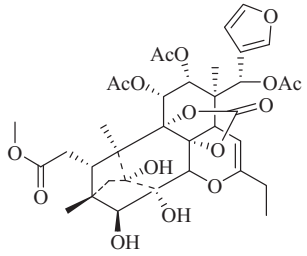

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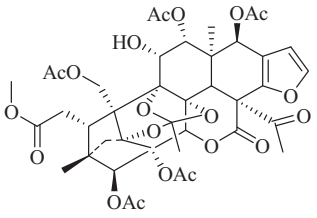

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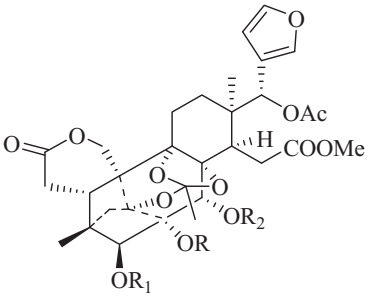
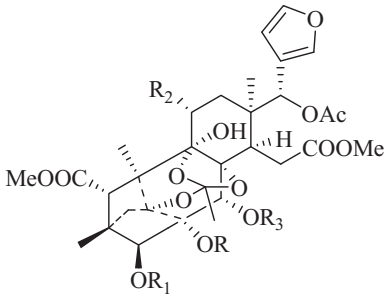
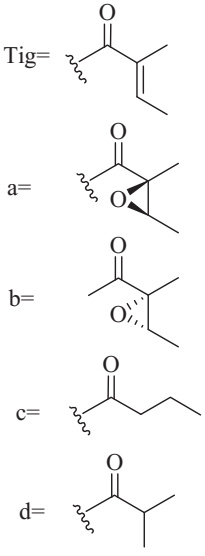
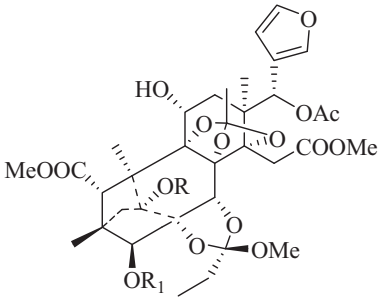


70 R=R_2= Ac, R_1= a
71 R=R_2= Ac, R_1= b
72 R=Ac, R_1= a, R_2= c
73 R= H, R_1= a, R_2= c
74 R= Ac, R_1= Tig, R_2= c
75 R= H, R_1= Tig, R_2= d

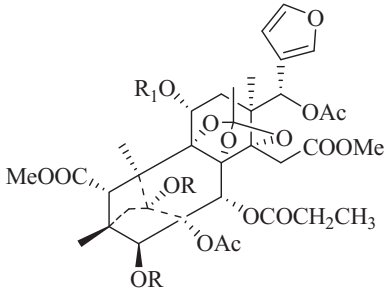


76 R= Ac, R_1= a, R_2= OH, R_3= c
77 R=R_3= Ac, R_1= a, R_2= OH
78 R= Ac, R_1= a, R_2= OAc, R_3= c
79 R= Ac, R_1= Tig, R_2= OAc, R_3= c
80 R=R_2= H, R_1= a, R_3= c



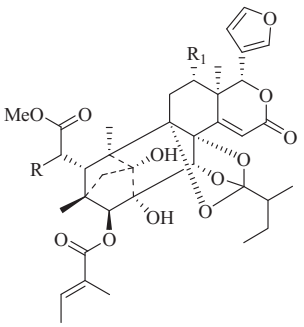


81 R= H, R_1= a
82 R= Ac, R_1= a
83 R= Ac, R_1= Tig

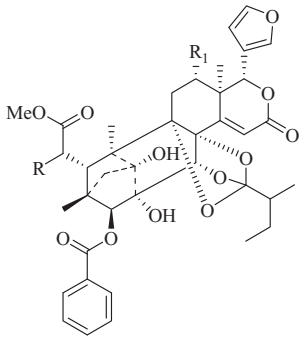


84 R= a, R_1= H
85 R= a, R_1= Ac

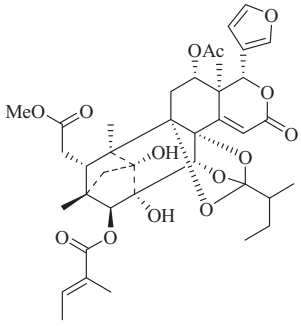
Figure 1 (Continued)



86 R=OAc, R_1= H
88 R=H, R_1= OAc



87 R=OAc, R_1= H
89 R=H, R_1= OAc



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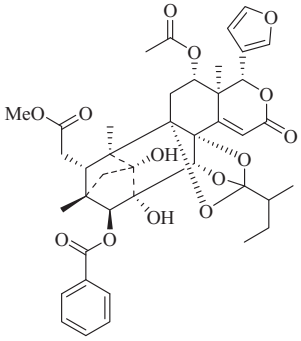

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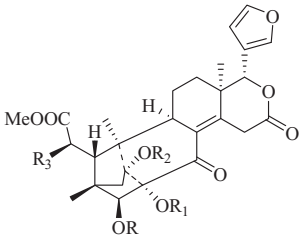
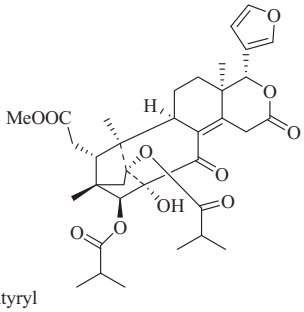
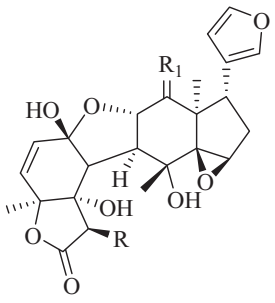


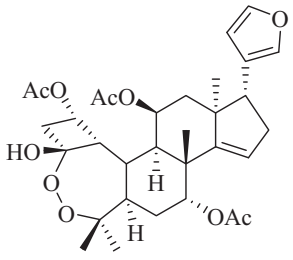


98

92 R=R_2= isobutyryl, R_1=R_3= H
93 R= 2S-methylbutyryl, R_1=R_3= H, R_2= isobutyryl
94 R= isobutyryl, R_1=R_3= H, R_2= 2S-methylbutyryl
95 R= 2S-methylbutyryl, R_1=H, R_2= isobutyryl, R_3= OH
96 R= isobutyryl, R_1=H, R_2= 2S-methylbutyryl, R_3= OH
97 R=R_1= isobutyryl, R_2=R_3= H



99 R= OAc, R_1= α-OH
100 R= OAc, R_1= O
101 R= OH, R_1= α-OH



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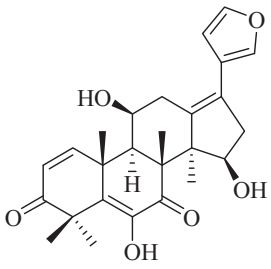

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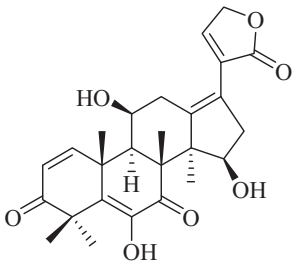

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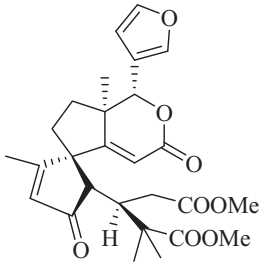
Figure 1 (Continued)



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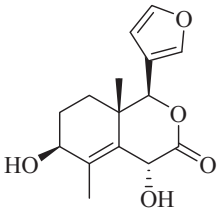

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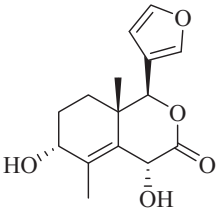

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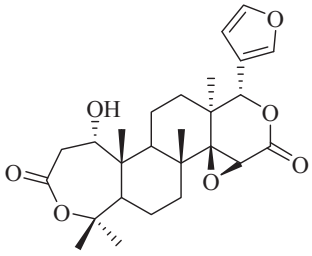

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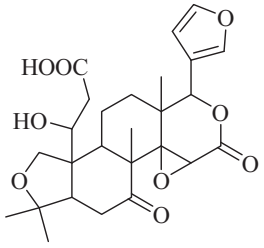

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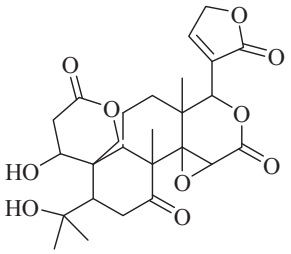

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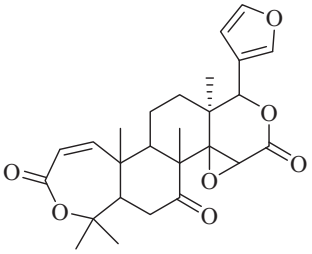

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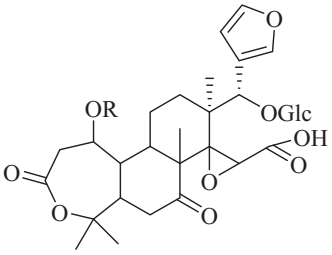
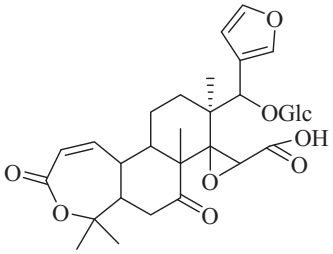


112 R= Ac
113 R= H



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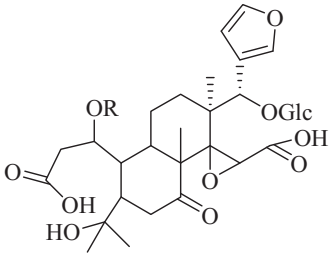
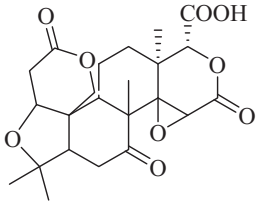


115 R= Ac
116 R= H



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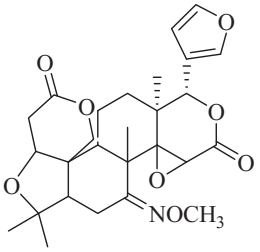

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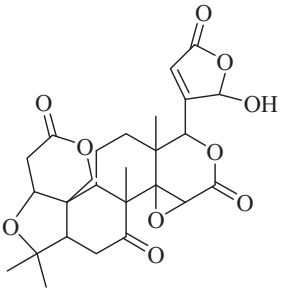

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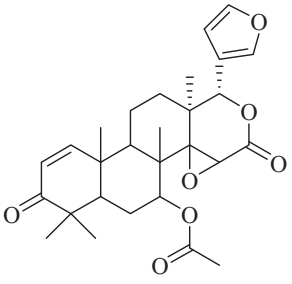

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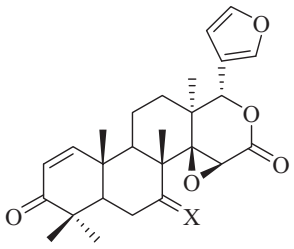
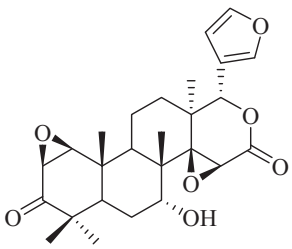
Figure 1 (Continued)



121 X= H, OMe
122 X= H, OEt
123 X= H, OPr
124 X= H, OBn
125 X= H,
126 X= H,
127 X=O



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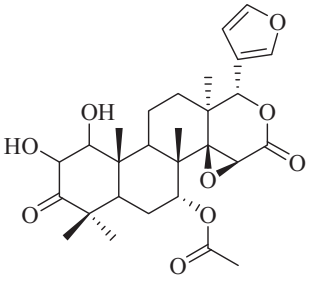

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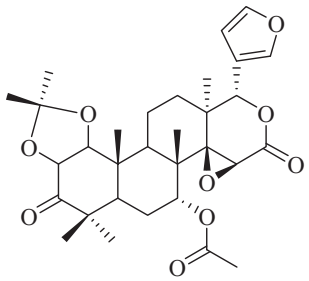

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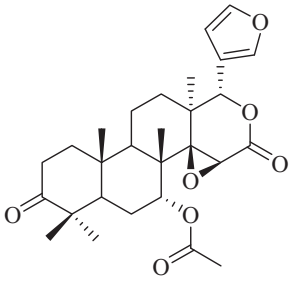

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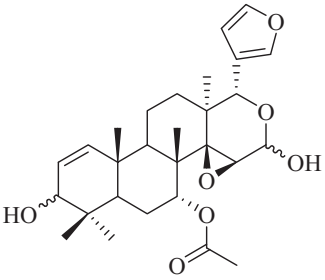

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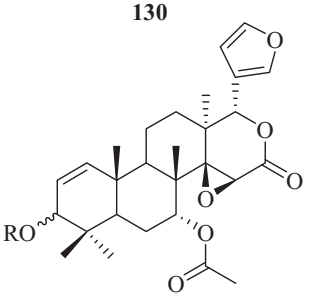
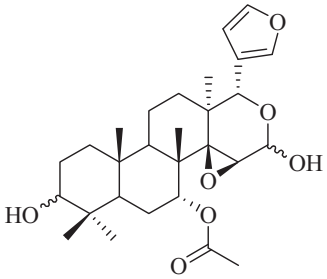


133 R= H
134 R= Ac



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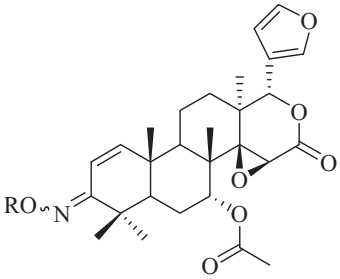
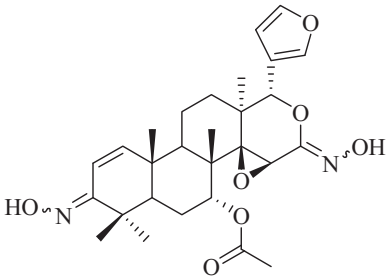


136 R= Me
137 R= Et
138 R= Bn



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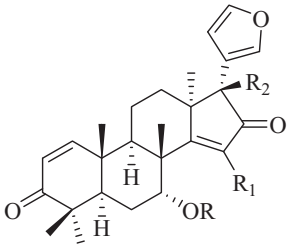
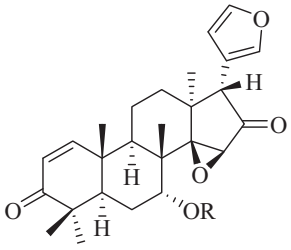
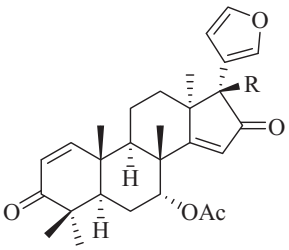


140 R= Ac, R_1=R_2= H
141 R= Bz, R_1=R_2= H
142 R= Ac, R_1= OH, R_2= H
143 R= Ac, R_1= H, R_2= OH
144 R= Bz, R_1= H, R_2= OH



145 R= Ac
146 R= Bz



147 R= H
148 R= OH

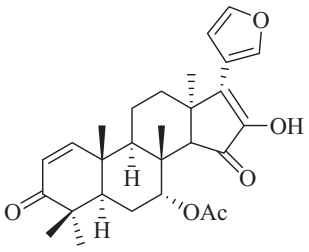


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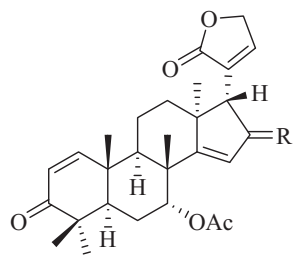
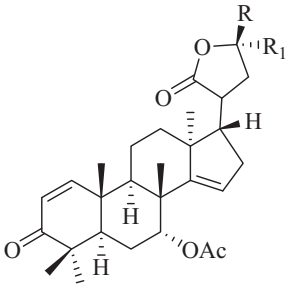
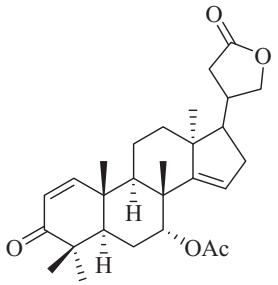
Figure 1 (Continued)



150 R= O
151 R= H_2



152 R= H, R_1= OMe
153 R= OMe, R_1= H



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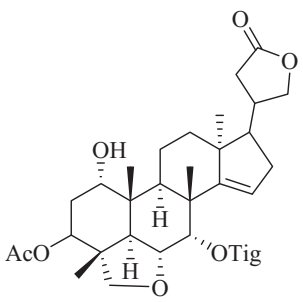

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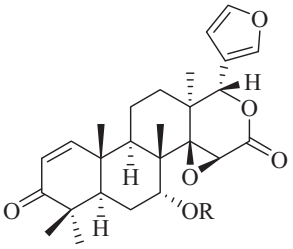
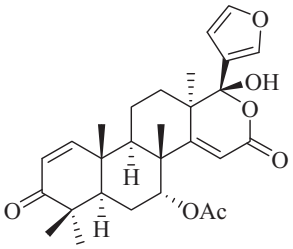


156 R= Ac
157 R= H
158 R= Bz



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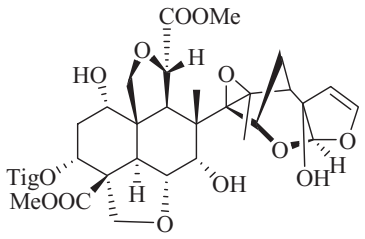

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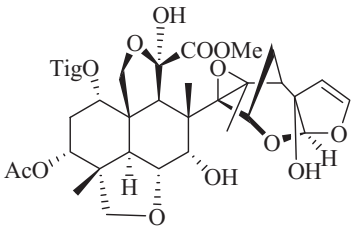

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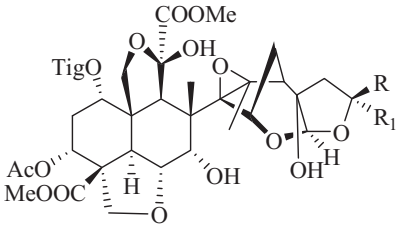
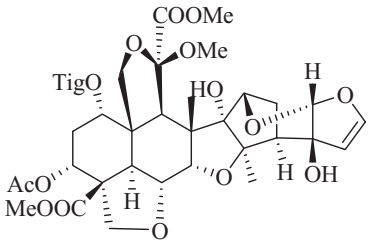


162 R= OMe, R_1= H
163 R= H, R_1= OMe



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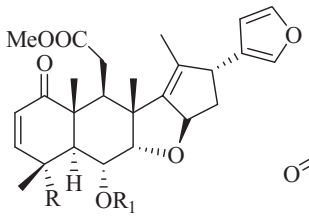
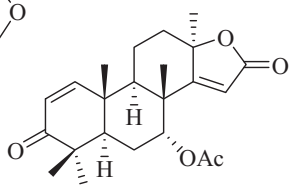


165 R= COOMe, R_1= Ac
166 R= COOMe, R_1= H
167 R= OH, R_1= Ac



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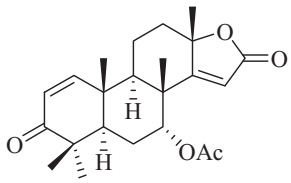

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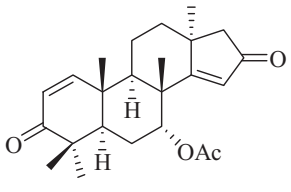

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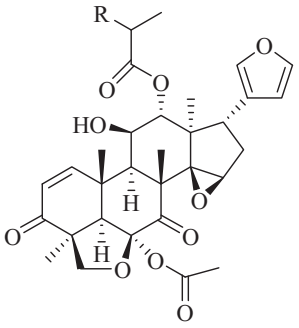
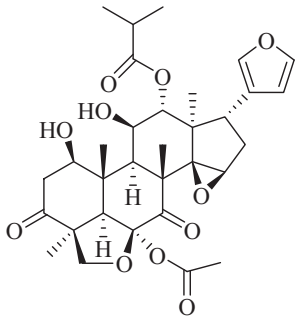


171 R= CH_3
172 R= CH_2CH_3



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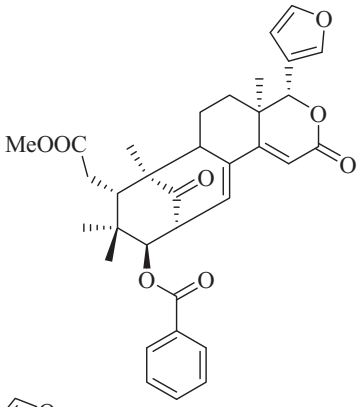

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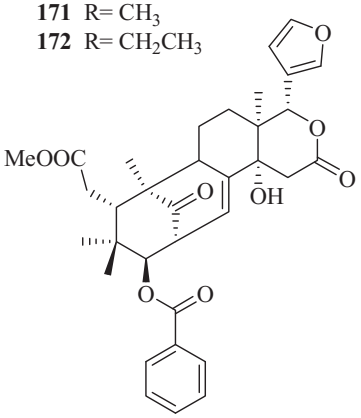

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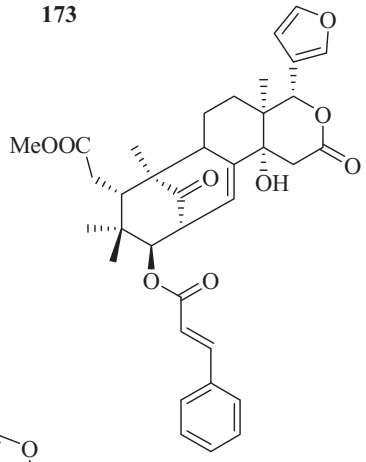

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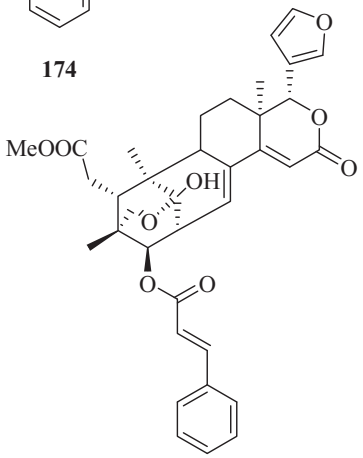

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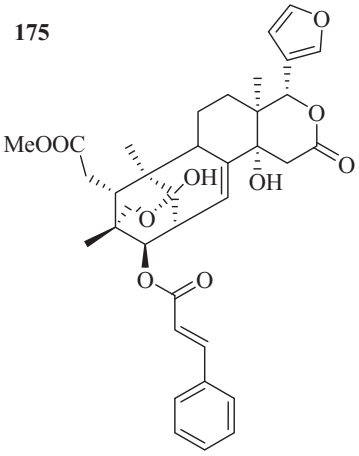

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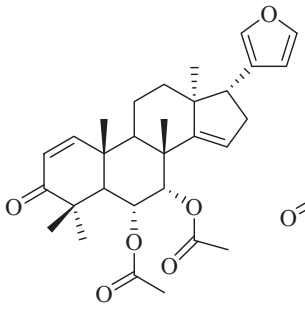

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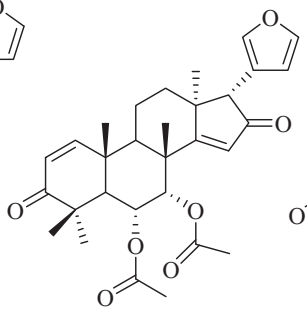

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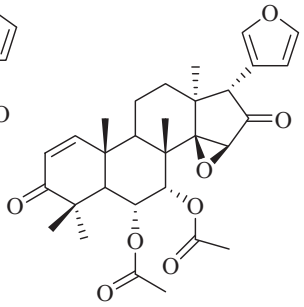

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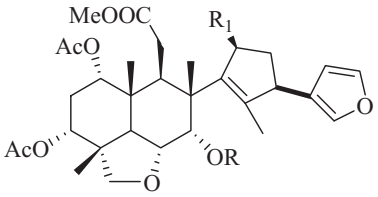
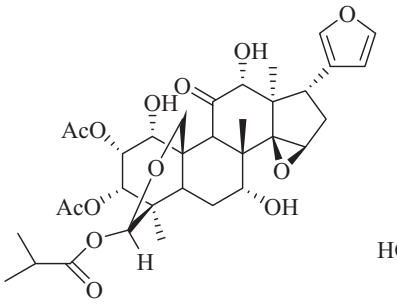


182 R= Bz, R_1= OMe
183 R= Tig, R_1= OMe
184 R= Tig, R_1= OH

Figure 1 (Continued)



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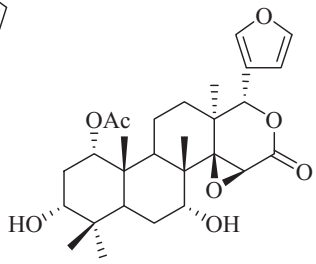

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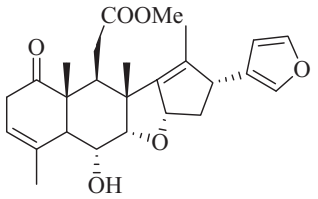

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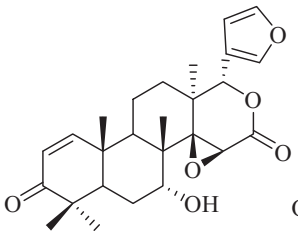

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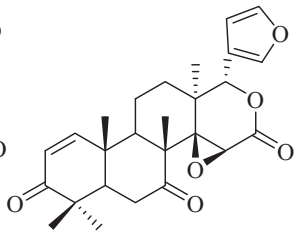

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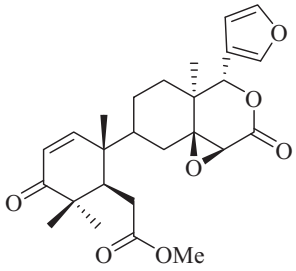

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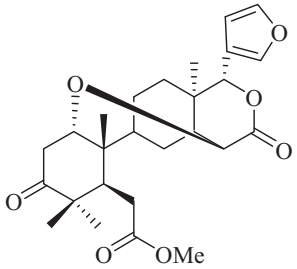

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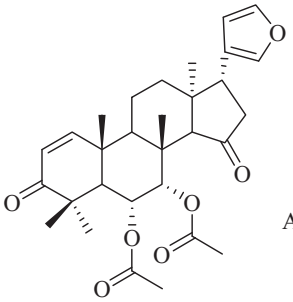

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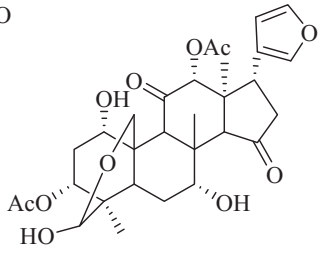

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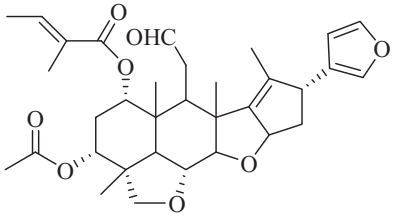

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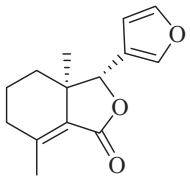

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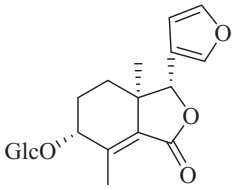

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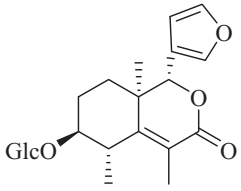

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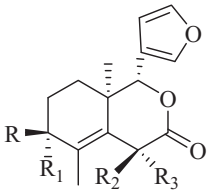
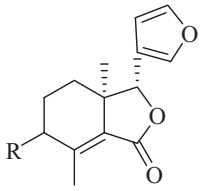
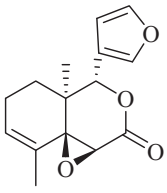


198 R= R_3= OH, R_1=R_2= H
199 R= R_3= H, R_1=R_2= OH



200 R= O
201 R= β-OH



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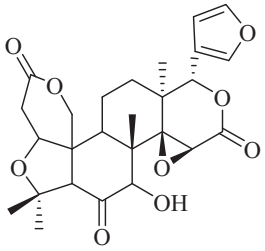

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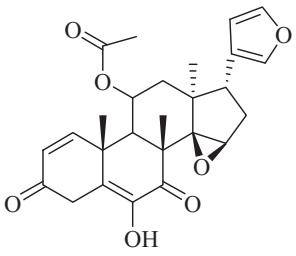

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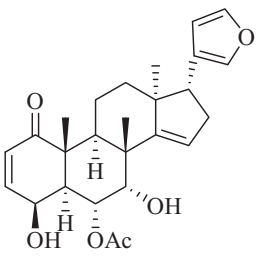

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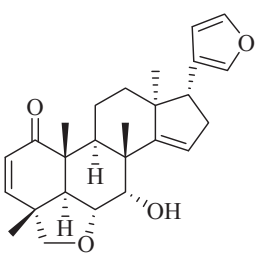

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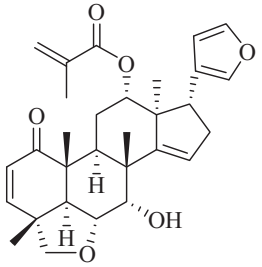

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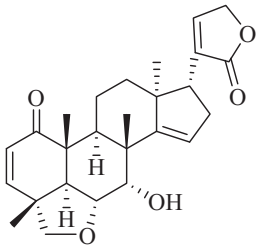

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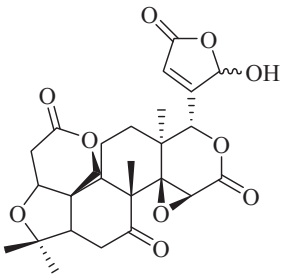

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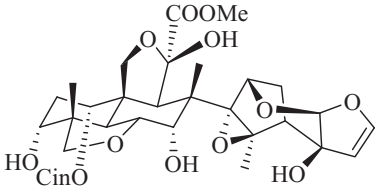

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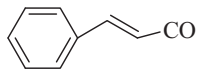
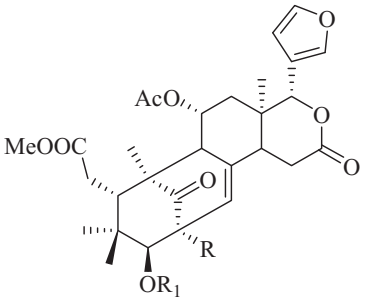
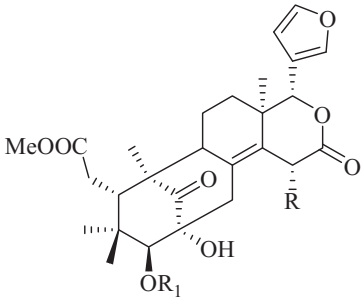
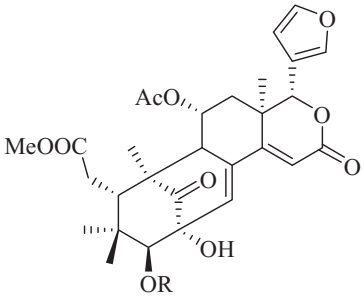
Cin= 



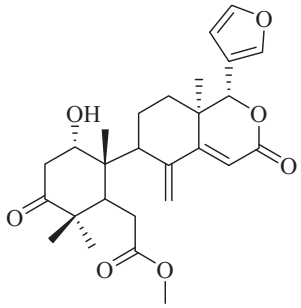
211 R= H, R_1= a
212 R= H, R_1= Ac
213 R= R_1 = H
214 R= OAc, R_1= Ac



215 R= H, R_1= a
216 R= H, R_1= b
217 R= OH, R_1= Tig
218 R= OH, R_1= Ac



219 R= a
220 R= Ac
221 R= Tig



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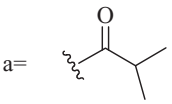
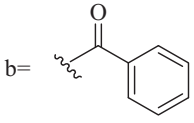
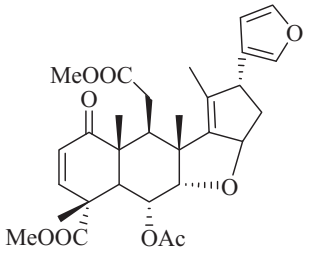
a= 

b= 



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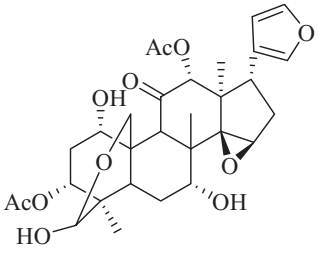

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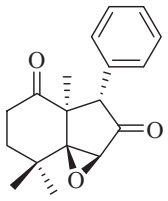

225

Figure 1 (Continued)

Until now, more then 50 limonoids aglycones and glucosides have been isolated from different *Citrus* species, including *C. aurantium*, *C. limon*, *C. reticulata*, *C. sudachi*, *C. unshiu*, *C. paradisi*, *C. sinensis*, *C. jambhiri*, and *C. pyriformis*, and are one of the most healthful components of the human diet for their health-promoting and disease-preventing properties.

Citrus limonoids appear in large amounts in *Citrus* juices and tissues as water-soluble limonoid glucosides or in seeds as water-insoluble limonoid aglycones. For most *Citrus* species, limonin (**1**), followed by nomilin (**2**), is the most abundant aglycone, whereas the most representative glucoside is limonin glucoside (**3**).

Citrus limonoids were considered a major problem for the *Citrus* juice industry as they cause delayed bitterness of the juices at room temperature, thus lowering the quality and value of the commercial juice. Limonoids bitterness occurs gradually after juice processing (Mayer and Beverly, 1968). However, at present, these bitter compounds have gained significant importance because of their role in inhibition of several chronic diseases since it is possible to detect limonoids in human plasma after *Citrus* ingestion (Tian et al., 2007).

In particular, recent studies have showed that limonin (**1**) was detected in human plasma after ingestion of limonin glucoside (**3**), indicating that **3** was probably hydrolyzed in the intestine and absorbed in the aglycone form (Manners et al., 2003). It is possible that *Escherichia coli* and *Candida albicans* present in the lower gastrointestinal tract hydrolyzed glucoside to release limonin (**1**) and glucose.

However, the fact that limonin (**1**) was not detected in the medium of all samples argues against the assumption that limonin glucoside (**3**) was hydrolyzed into limonin and glucose, but immediately limonin was converted into other metabolites. The human bioavailability study by Manners et al. (2003) showed that nanomolar levels of limonoids detected in the plasma after the administration of up to 2 g of limonin glucoside (**3**). This bioavailability study indicates a low-level absorption and nontoxic effects of these bioactive compounds.

In addition to *Citrus* species, several limonoids have been identified in plants from Meliaceae family. Among them, *Azadirachta indica* contain more than hundred different limonoids and their derivatives (Roy and Saraf, 2006). Other important sources of limonoids in Meliaceae family are *Cedrela* sp., *Khaya* sp., *Melia azedarach*, *Sandoricum koetjape*, *Swietenia mahogany*, *Trichilia* sp., and *Turraea* sp.

NATURALLY OCCURRING LIMONOIDS

In the last years, more than 100 limonoids have been isolated and characterized. Recently, Kuroyanagi et al. (2008) isolated limonin (**1**) and nomilin (**2**), which are the most abundant *Citrus* limonoids, from the molasses of tangerine orange (*C. unshiu*, Rutaceae) as a waste product in the course of production of tangerine orange juice. Seven new limonoids named granatumins A-G (**4**–**10**) were isolated from the seeds of an Indian mangrove (*Xylocarpus granatum*, Meliaceae) (Li et al., 2009a). From the seeds of the same plant, granaxylocarpins A-E (**11**–**15**) were previously isolated (Yin et al., 2007b). Granaxylocarpins A (**11**) and B (**12**) are mexicanolide-type limonoids with a 9,10-seco skeleton, and granaxylocarpin C (**13**) possesses an 8α,30α-epoxy ring and a rare 1,29-oxygen bridge.

Two limonoids, namely khayalenoids A (**16**) and B (**17**), with an unprecedented 8-oxa-tricyclo[4.3.2.02,7]undecane motif in the nortriterpenoid core, were isolated from the stems of *Khaya senegalensis* (Meliaceae) (Yuan et al., 2009). From *Cipadessa cinerascens* (Meliaceae), cipadesins A-C (**18–20**) and cipadesins D-F (**21–23**) were isolated (Yuan et al., 2005; Yuan et al., 2007). From the same plant, cineracipadesins A-F (**24**–**29**) and cipadonoid A (**30**) were also identified (Fang et al., 2008; Fang et al., 2009).

Previously, five mexicanolide-type tetranortriterpenoids, tigloylseneganolide A (**31**), 2′R-methylbutanoylproceranolide (**32**), 2′Smethylbutanoylproceranolide (3) (**33**), 2′R-cipadesin A (**34**), and 2′R-cipadesin (**36**), as well as the known 2′S-epimers of **34** and **36** (**35** and **37**), were isolated from the seeds of *C. baccifera* (Meliaceae) (Gan et al., 2007).

Recently, an investigation was conducted on the extracts from the stem, leaves, and roots of a graft of *A. indica* on *M. azedarach* (Meliaceae) rootstock to search for limonoids (Forim et al., 2010). A dichloromethane-soluble fraction of the methanol extract from the stem afforded the limonoids 28-deoxonimbolide (**38**), salannin (**39**), and 6-*O*-acetylnimbandiol (**40**). Compound **38** was previously found in leaves, **39** in seeds, and **39** and **40** in the oil from *A. indica* seeds (Kigodi et al., 1989; Akhila and Rani, 1999), but not in the stem.

Salannin (**39**) was also found in the root of *M. azedarach* (Srivastava and Gupta, 1985). Thus, the finding of **39** in this study suggests that it could have been translocated from the *M. azedarach* rootstock to the *A. indica* graft, and it might be the biosynthetic intermediate to **38** and **40**. The synthesis of the two latter compounds may be directly related to the graft. However, it is clear that a great deal of work is needed to establish whether metabolites formed de novo have a significant role in the biochemical adaptation of plant growing on a second one, such as *M. azedarach* rootstock. The *n*-hexane and dichloromethane extracts from leaves were combined and after successive chromatographic separations afforded the limonoid nimbolide (**41**) and predominantly a mixture of limonoids isolated previously from the leaves of *A. indica*, which were not separated. The dichloromethane-soluble fraction of methanol extract from leaves gave the limonoids, **38**, 6-deacetylnimbinene (**42**), and 6-deacetylnimbin (**43**). Azadirachtin (**44**) is present in all parts of the fruits, stem, flowers, and roots, but is absent in the leaves.

1-*O*-deacetyl-6-deoxykhayanolide E (**45**), 1-*O*-deacetyl-2α-hydroxykhayanolide E (**46**), 3-acetyl-khayalactone (**47**), and 11a-acetoxy-2α-hydroxy-6-deoxy-destigloylswietenine acetate (**48**) were isolated from the stems of *K. ivorensis* (Meliaceae), a source of one of the most popular traditional African medicines, mainly distributed throughout Angola, Cameroon, and Nigeria (Zhang et al., 2009).

From the stem bark of *Chukrasia tabularis* var. v*elutina* (Meliaceae), sixteen 16-norphragmalin limonoids, chuktabularins E-T (**49**–**64**), were isolated (Luo et al., 2010). These compounds possess a biosynthetically extended propionyl or acetyl group at C-15 and a characteristic ketal moiety between the limonoid skeleton and the acyl substituent at C-15. From the same plant, three 16-norphragmalin limonoids, namely chukvelutins A-C (**65**–**67**), were also isolated (Luo et al., 2009). These compounds are characterized by 16-norphragmalin limonoid skeletons featured with a characteristic ketal moiety between the phragmalin skeleton and a biosynthetically extended isobutyryl group at C-15, forming a characteristic 2,7-dioxabicyclo[2.2.1]heptane moiety. The limonoids chuktabrin A (**68**), featuring the unique motifs of a 1,3-dioxolan-2-one and a 3,4-dihydro-2H-pyran and chuktabrin B (**69**), possessing a polycyclic skeleton, were previously isolated from *C. tabularis* (Meliaceae) (Zhang et al., 2008).

Sixteen D-ring-opened phragmalin limonoid orthoesters, swietenitins A-M (**70**–**75**, **77**, **79**, **81**–**85**) 2-acetoxyswietenialide D (**76**), 2,11-diacetoxyswietenialide D (**78**), and 11-deoxyswietenialide D (**80**), were isolated from the twigs of *Swietenia macrophylla* (Meliaceae), a timber tree that grows in tropical areas of Asia, such as India, Malaysia, and southern mainland China, and which has traditional applications in the treatment of hypertension (Lin et al., 2009a).

Previously, the hexane extract from leaves of *S. macrophylla* afforded six phragmalins with a 8,9,30-ortho-ester unit, namely 6-*O*-acetylswietephragmin E (**86**), 3β-*O*-destigloyl-3β-*O*-benzoyl-6-*O*-acetylswietephragmin E (**87**), 12α-acetoxyswietephragmin C (**88**), 3β-*O*-destigloyl-3β-*O*-benzoyl-12α-acetoxyswietephragmin C (**89**), 12α-acetoxyswietephragmin D (**90**), and 3β-*O*-destigloyl-3β-*O*-benzoyl-12α-acetoxyswietephragmin D (**91**) (da Silva et al., 2008). Seven phragmalins with a C-30 carbonyl moiety, named moluccensins A-G (**92–98**), among which moluccensins A–F, possessing a $\Delta^{8,14}$ double bond, and moluccensin G, containing conjugated $\Delta^{8,9}$ and $\Delta^{14,15}$ double bonds, were isolated from the seeds of an Indian mangrove, *X. moluccensis* (Meliaceae) (Li et al., 2009b). *Toona ciliata* Roem. var. *ciliata* (Meliaceae), used in Chinese folk medicine, is a timber tree mainly growing in the tropical areas of Asia. From its leaves and twigs, three norlimonoids (**99**–**101**) were isolated (Chen et al., 2009). Walsuronoids A-C (**102**–**104**) were isolated from the leaves and twigs of *Walsura robusta* (Meliaceae), an arbor tree mainly growing in Yunnan and Guangdong provinces of China (Yin et al., 2007a). Walsuronoid A (**102**) is a limonoid thar features an unprecedented A-seco limonoid skeleton incorporating a 3,4-peroxide bridge, while walsuronoids B (**103**) and C (**104**) possess the rare 18(13→14)-*abeo*-limonoid skeleton. The limonoid guyanin (**105**) was obtained by high-speed countercurrent chromatography from the leaves of *Hortia oreadica* (Rutaceae) (Severino et al., 2009).

Chemical investigation of the roots of *Dictamnus radicis* cortex (Rutaceae), a traditional folk herb used in the Chinese medicine, led to the isolation of isodictamdiol (**106**) and dictamdiol (**107**), the first 5S/9S-type degraded limonoids (Zhao et al., 2008).

BIOLOGICAL PROPERTIES

Anticancer Activity

A large number of studies demonstrated that nonnutritive dietary bioactive compounds derived from fruits and vegetables showed antiproliferative activities through different mechanisms of action.

Among them, limonoids have attracted the scientists' interest. A recent study (El-Readi et al., 2010) investigated the P-gp reversal activities of limonin (**1**) and deacetylnomilin (**108**), isolated from *C. jambhiri* and *C. pyriformis* (Rutaceae) in human leukemia cells (CEM/ADR5000), and their potential cytotoxicity against this cell line, its parental cell line CCRF-CEM (Adriamycin-sensitive human leukemia cell line, no expression of P-gp) and Caco-2 (human colon adenocarcinoma cell line), which is used as a model for intestinal epithelial cells with a relatively high expression of *P-gp*/*MDR1* gene. P-gp is an ATP-binding cassette (ABC) transporter that pumps out lipophilic agents from cells that have entered them by free diffusion. Overexpression of multidrug resistance proteins has been shown to cause cross-resistance to many chemotherapeutic agents. A strategy to reverse multidrug resistance is to inhibit the activity of these transporters by co-administration of transport inhibitors and anticancer agents. The inhibition of P-gp and other ABC transporters can increase the intracellular concentration of cytotoxic drugs. Uptake and/or efflux of isotopically labeled drugs or rhodamine 123 (Rho123) is used frequently for a functional P-gp assay in tumor cells. Both limonoids **1** and **108** significantly reduced P-gp efflux in drug-resistant human leukemia cells (CEM/ADR5000) at nontoxic concentrations (0.32–32 μM). When tested in doxorubicin-treated Caco-2 cells, these compounds substantially reverse doxorubicin resistance and restore doxorubicin cytotoxicity. Limonin (**1**), which minimally inhibited cell growth in all cells tested (IC_{50} was 519.77 μM in Caco-2 cells, 284.77 μM in CEM/ADR5000, and 159.44 μM in CCRF-CEM cells), was the most active compound and significantly enhanced doxorubicin cytotoxicity in the CEM/ADR5000 cell line.

Potential cancer preventive constituents of sour orange (*C. aurantium*, Rutaceae) were isolated and identified as isolimonic acid (**109**) and ichanexic acid (**110**) (Kim et al., 2009a). Both compounds exhibited differential inhibition at various concentrations. Significant arrest of cell growth by **109** was noticed within 24 hours of treatment on the HT-29 colon cancer cells at a concentration as low as 5.0 μM and by **110** at 10.0 μM. Isolimonic acid (**109**) and ichanexic acid (**110**) exerted nearly four- to five-fold increase in the counts of G2/M stage cells at 5 μM, indicating a potential role in the cell cycle arrest.

Limonin (**1**), nomilin (**2**), deacetylnomilin (**108**), and obacunone (**111**), and their glucosides (**3**, **112**–**114**) were tested

for the potential effects against two human cancer cell lines, SH-SY5Y neuroblastoma and Caco-2 colonic adenocarcinoma (Kim et al., 2009b). Neuroblastoma cells were more sensitive than colon carcinoma cells. Although micromolar levels of both aglycones and glucosides arrested cell growth, biochemical and morphological data showed that the glucosides induced a more rapid cell death. Aglycone toxicity was dose-dependent but below the killing potential of glucosides. This observation correlated with a slower rate of induction of caspase 3/7 activity by aglycones. The mechanism of limonoid action underpinning apoptosis induction, cell cycle arrest, and aneuploidy is not known. Among the possible candidates are the *N-myc* and *c-myc* oncogenes. Both oncogenes have been shown to play pivotal roles in DNA synthesis and the maintenance of ploidy levels in neuroblastoma. Previously, Poulose et al. (2005) demonstrated apoptosis as a primary cause of neuroblastoma cell death from limonin and obacunone glucosides (**3** and **114**). In this study, limonin (**1**) and obacunone (**111**) induced cell death similar to their respective glucosides but at a significantly slower pace and never to the same extent. Caspase induction was also achieved at a lower level of limonin glucoside (**3**) and obacunone glucoside (**114**) than nomilinic acid glucoside (**115**) and deacetylnomilinic acid glucoside (**116**). A careful inspection of the structures shows that **114** and **2** share the same structural features as **115** and **116** with the exception of a sealed A ring with no carboxyl group in the molecules of **3** and **114**. According to Miller et al. (1992), who speculated that the A ring is more decisive in the biological activity of limonoid glucosides, this study suggested that apoptosis-inducing potential may be associated with the A-ring configuration.

Exposure to environmental chemicals has often been considered as the major risk factor for several cancers. These exposures can result in the generation of reactive oxygen and nitrogen species, which have been implicated as causative agents in an array of inflammatory and degenerative diseases, including many types of cancers. In humans, many of these chemicals are precarcinogens and are activated to carcinogenic and mutagenic substances by cytochrome P450 oxidoreductase enzymes (CYPs) during metabolic and detoxification processes. Overexpression of CYP isoenzymes has been specifically implicated in the onset of cancers of lung, breast, colon, and prostate. In-vitro studies with phytochemicals revealed a reduction in activities involved in the generation of carcinogens through partial inhibition of these enzymes, which may represent a novel mechanism in the anticarcinogenesis strategy. On the basis of these considerations, Poulose et al. (2007) evaluated the effects of limonoid aglycones and glucosides on the activity of human CYP isoenzymes such as CYP1A2, CYP1B1, CYP19, and CYP3A4. In this study, partial-to-high inhibition of CYPs was observed in dose-dependent assays. Significant reductions in enzyme activities were observed with purified compounds above 2 μM. Kinetic analyses indicated that limonin glucoside (**3**) inhibited CYP19 competitively, whereas nomilinic acid glucoside (**115**) inhibited it noncompetitively. Nomilinic acid glucoside (**115**) was the most potent limonoid, with an overall IC_{50} < 10 μM for all the enzymes tested. The variation in IC_{50} values for different limonoids can be ascribed to the affinity of substrates or limonoids toward various enzymes in the formation of enzyme–substrate or enzyme–inhibitor complexes. The multiple open rings and two reactive carboxylic acid and hydroxyl groups on the acidic limonoid glycoside could be factors for better efficacy. More recently, limonin (**1**) and its glucoside (**3**) have demonstrated cytotoxic activity also against the pancreatic cancer cell line Panc-28, with IC_{50} values of 89.3 and 31.7 μg/mL, respectively (Patil et al., 2009a). The chemopreventive ability of **1** was evaluated in different studies (Vanamala et al., 2006; Lin et al., 2009b; Perez et al., 2009). In particular, Vanamala et al. (2006) evaluated the potential protective effect of **1** against azoxymethane (AOM)-induced aberrant crypt foci (ACF) by suppressing proliferation and elevating apoptosis through anti-inflammatory activities in Sprague–Dawley rats. Rats were injected with saline or AOM during the third and fourth week, and colons were resected for evaluation of ACF, proliferation, apoptosis, and cyclooxygenase (COX)-2 and inducible nitric oxide synthase (iNOS) protein levels. Experimental diets had no effect on the variables measured in saline-injected rats. However, in AOM-injected rats, the experimental diets suppressed aberrant crypt and high-multiplicity ACF (HMACF) formation and the proliferative index compared with the control diet.

Limonin (**1**) suppressed HMACF formation per centimeter and expansion of the proliferative zone that occurred in the AOM-injected rats consuming the control diet. All diets elevated the apoptotic index in AOM-injected rats, compared with the control diet; however, the greatest enhancement was seen with limonin. Moreover, limonin diets suppressed elevation of both iNOS and COX-2 levels observed in AOM-injected rats consuming the control diet. Thus, lower levels of iNOS and COX-2 are associated with suppression of proliferation and upregulation of apoptosis, which may have contributed to a decrease in the number of HMACF in rats provided with limonin. Limonin (**1**) did not showed chemopreventive effects on the drug efflux transporters P-glycoprotein (ABCB1) and multidrug resistance protein 1 (MRP1, ABCC1) investigated using P-glycoprotein overexpressing human carcinoma KB-C2 cells and human *MRP1* gene-transfected KB/MRP cells (Nabekura et al., 2008).

Recently, limonin (**1**), together with limonin glucoside (**3**), deacetylnomilic acid glucoside (DNAG) (**116**), defuran limonin (**117**), and limonin 7-methoxime (**118**), was analyzed for the induction of phase II detoxification enzymes, glutathione *S*-transferase (GST) and quinone reductase (QR) (Perez et al., 2009). DNAG (67%) showed the highest induction of GST activity against 1-chloro-2,4-dinitrobenzene (CDNB) in lung homogenates, followed by **118** (32%) in treated liver homogenates. Interestingly, limonin-7-methoxime showed the highest GST activity (270%) in liver against 4-nitroquinoline 1-oxide (4NQO), while the same compound in the stomach induced GST by 51% compared with the control. Modified compounds showed very significant GST activity in the liver against 4NQO. Defuran limonin (**117**) is similar in structure to limonin except for the absence of the furan ring. Limonin glucoside differs from limonin

only by the presence of a glucose moiety at the C-17 position. Deacetyl nomilinic acid glucoside (**116**) differs from limonin glucoside by the presence of the A ring. The presence of the furan moiety is thought to be responsible for the induction of GST activity. Another phase II enzyme, QR, was significantly induced by limonin-7-methoxime by 65% and 32% in liver and lung homogenates, respectively.

Defuran limonin (**117**) induced QR in lung homogenates by 45%. The differential induction potential of certain limonoids to induce GST activity was further attributed to different structural components of the limonoid nucleus. It was suggested that an intact A ring is required for antineoplastic effects, such as those present in nomilin (**2**) (Lam et al., 1982). It is possible that modification to the B ring of the limonoid nucleus may also alter the induction of GST activity. The D-ring of the limonoid nucleus has a furan ring attached to its third position. A previous study demonstrated that the furan moiety plays a role in the induction of GST activity (Lam et al., 1989).

Azadirachtin (**44**), isolated from seed kernels, and nimbolide (**41**), present in leaves and flowers, are recognized as the most potent neem limonoids that exhibit cytotoxic effects against various cancer cell lines such as N1E-115 neuroblastoma (mouse), 143B.TX$^-$ osteosarcoma (human) and Sf9 (insect), HT-29, SW-620 (colon) and HOP-62, A-549 (lung), PC-3 (prostate), OVCAR-5 (ovary), HL-60, U937, THP1, and B16 (Cohen et al., 1996; Roy et al., 2007; Sastry et al., 2006). The chemopreventive potential of azadirachtin (**44**) and nimbolide (**41**) was evaluated with in-vitro antioxidant assays and in-vivo inhibitory effects on 7,12-dimethylbenz[a]anthracene (DMBA)-induced hamster buccal pouch (HBP) carcinogenesis. Both **41** and **44** exhibited concentration-dependent antiradical scavenging activity and reductive potential. Administration of both limonoids inhibited the development of DMBA-induced HBP carcinomas by influencing multiple mechanisms, including prevention of procarcinogen activation and oxidative DNA damage, upregulation of antioxidant and carcinogen detoxification enzymes, and inhibition of tumor invasion and angiogenesis. On a comparative basis, nimbolide (**41**) was found to be a more potent antioxidant and chemopreventive agent (Priyadarsini et al., 2009).

Nimbolide (**41**) was also evaluated for its inhibition of the growth of human choriocarcinoma (BeWo) cells accompanied by downregulation of proliferating cell nuclear antigen (PCNA), a marker of cell proliferation and induction of apoptosis as revealed by nuclear morphology (Harish Kumar et al., 2009). In particular, nimbolide (**41**) exerted cytotoxic effects on BeWo cells in a dose- and time-dependent manner, with IC_{50} values of 2.01 and 1.19 μM for 7 and 24 hours, respectively. The relative chemopreventive potential of **41** and **44** was also studied in the HBP carcinogenesis model by analyzing the expression of PCNA, $p21^{waf1}$, cyclin D1, glutathione S-transferase pi (GST-P), NF-κB, inhibitor of κB (IκB), p53, Fas, Bcl-2, Bax, Bid, Apaf-1, cytochrome C, survivin, caspases-3, -6, -8, and -9, and poly(ADPribose) polymerase (PARP) by reverse transciptase-polymerase chain reaction (RT-PCR), immunohistochemical, and Western blot analyses (Harish Kumar et al., 2010). Downregulation of cyclin D1, GST-P, and PCNA with enhanced expression of p21waf1 and p53 by **44** and **41** suggests regulatory effects on cell cycle progression. Previously, nimbolide (**41**) demonstrated to exert antiproliferative effects in cancer cell lines by modulating cyclins, CDK inhibitors, and p53 (Roy and Saraf, 2006; Roy et al., 2007). Several proteins that are involved in the regulation of cell survival and cell proliferation also play critical roles in apoptosis by influencing the Bcl-2 family proteins and caspases. PCNA, a cofactor for DNA polymerase δ, which plays a central role in the cell cycle, also blocks apoptosis by inhibition of Gadd45 and MyD118, negative regulators of growth (Moldovan et al., 2007). Activation of wild-type p53 exerts its antitumoral effects through activation or inactivation of Bcl-2 family proteins. In particular, targeting NF-κB, which plays a vital role in cell proliferation, differentiation, apoptosis, inflammation, stress response, and several signal transduction pathways, is considered a novel preventive and therapeutic strategy against human cancers. Although nuclear translocation is required for activation of NF-κB, only 10–20% of the activated Rel-A is detected in the nucleus, whereas 80–90% is located in the cytoplasm. The higher expression of p50 in the nucleus relative to the cytosol in DMBA painted animals indicates that NF-κB undergoes activation via the canonical pathway, leading to the dissociation of p50-p65 subunits, with subsequent nuclear translocation and activation of downstream target genes involved in carcinogenesis. Thus, inhibition of PCNA and nuclear translocation of NF-κB with upregulation of IκB and p53 by **41** and **44** may be key factors for suppressing the growth of HBP carcinomas and inducing apoptosis. In the present study, we provide compelling evidence to show that azadirachtin (**44**) and nimbolide (**41**) transduce apoptosis by both the mitochondrial and the death receptor pathway. In addition, both the limonoids also activated initiator and effector caspases and enforced nuclear localization of surviving, enabling increased susceptibility to intrinsic apoptosis. The intracellular localization of survivin is crucial for its antiapoptotic function, while cytosolic localization enables interaction with caspases and Smac/DIABLO, thereby blocking apoptosis, and nuclear localization favours apoptosis by increasing p53 and Bax levels. Upregulation of wild-type p53 in turn has been reported to ablate the antiapoptotic activity of survivin and induce a number of proapoptotic factors, including Bax and caspases.

Limonexic acid (**119**), tested for the potential activity on human colon cancer cell (HT-29) proliferation and apoptosis, exhibited significant inhibition and caused four- to five-fold increases in the counts of G2/M stage cells at 50 μM, indicating a potential role in cell cycle arrest (Jayaprakasha et al., 2010).

Another limonoid that demonstrated antiproliferative activity was gedunin (**120**), isolated from *Xylocarpus granatum* (Meliaceae) (Uddin et al., 2007). Compound **120** showed cytotoxic activity against Caco-2 colon cancer cell line, with an IC_{50} value of 16.83 μM. Moreover, gedunin (**120**) recently showed anticancer activity via inhibition of the 90-kDa heat shock protein (Hsp90) folding machinery to induce the degradation of Hsp90-dependent client proteins similar to other Hsp90 inhibitors. In

an effort to further probe the mechanism of action, 19 semisynthetic derivatives (**121**–**139**) of this limonoid were evaluated for their antiproliferative activity against MCF-7 and SKBr3 cells, alongside geldanamycin, which is a previously reported Hsp90 inhibitor that binds to the *N*-terminal ATP-binding pocket (Brandt et al., 2008). None of the derivatives are more active than the natural product. However, some important preliminary structure–activity relationships have been identified. Substituents at the 7-position are placed in an environment that appears to be sensitive to the effects of steric bulk that deviates from the native ligand. From compounds **121** through **125**, a pronounced decrease in antiproliferative activity resulted in a decrease or increase in the size of the substituent from an ethyl ether (**122**) to either a methyl ether or an *n*-propyl ether (**121** and **123**) and complete loss in activity was produced by incorporation of the benzyl ether, as evidenced by compound **124**. A complete loss in activity was also noted upon incorporation of the propionate ester, as in **125**. The *n*-propyl ether in **123** may retain activity because of its less rigid nature compared with the propionate ester **125**. Also, preliminary molecular modeling results suggest that the carbonyl oxygen present on the propionate is tilted out of alignment with the carbonyl oxygen of **125**, suggesting that this moiety, when present, is important for binding. It also appears that the binding site for substituents at the 7-position is not sensitive to a change in the electronic nature of the substituent.

Compound **126**, which incorporates a carbamate as a no bulky hydrogen bond acceptor/donor, exhibited an IC_{50} value almost identical to that of the natural product. The most active analogue found in the 7-position series is **127**, the 7-oxo derivative, further demonstrating the tolerance of this binding site to small, hydrogen bond acceptors rather than small hydrophobic moieties, as in **121**. These results supported two reasonable conclusions in regard to the effect of differing substituents at the 7-position: (a) the binding site complementary to substituents at this position may provide a hydrogen bond donor that resides in a shallow pocket. This may explain why hydrogen bond acceptors exhibit the most potent antiproliferative activities, while decreasing or increasing steric bulk from that possessed by the native ligand without the presence of a hydrogen bond acceptor decreases activity. (b) Substituents placed at the 7-position of the molecule are positioned in an area critical to the overall conformation of the molecule, thereby exhibiting significant impact on binding. The antiproliferative activity of the α,β-unsaturated ketone series also provides key information regarding the importance of this functional group.

Compounds lacking the 1,2-olefin of gedunin (**120**) showed IC_{50} values greater than 100 μM (i.e., **129**–**131** and **135**). These data might suggest that the α,β-unsaturated ketone is acting as a Michael acceptor; however, antiproliferative activity of related compounds in this series suggest otherwise. For example, compound **132**, which contains the reduced ketone and lactone moieties, retains antiproliferative activity. Although **132** exhibits activity 5- to 10-fold less potent than the natural product, evidence suggests that the α,β-unsaturated ketone is not acting as a Michael acceptor, as a far less potent compound would be expected. Support of this reasoning is substantiated with compound **133**, which resulted from chemoselective reduction of the α,β-unsaturated ketone. Compound **133** showed antiproliferative activity five times less potent than gedunin (**120**) in both MCF-7 and SKBr3 cells. Compound **136** also retained some activity and is only 3–4 times less potent than the natural product and incorporates an electron-rich oxime, which serves as a poor Michael acceptor. Compounds **134**, **136**–**138**, and **139** also provided valuable insight into the binding site of the α,β-unsaturated ketone. This binding pocket appears sensitive to steric bulk and/or intolerant to hydrophobic moieties. Compound **137**, the acetylated version of **136**, exhibits no activity. Compound **137**, the *O*-methyloxime, also manifested no antiproliferative activity. This is interesting because aside from the additional projection of steric bulk and the increase in hydrophobicity from the methyl group on the oxime ether, **136** appeared to include the requirements for successful binding at other positions of the molecule, including the α,β-olefin, a hydrogen bond acceptor at the 3-position, and the appropriately fitting substituent at the 7-position. Neither **137** nor **139** exhibited antiproliferative activity that also parallels this trend. In order to demonstrate the retention of Hsp90 inhibition despite structural manipulation, the most active compounds in this series were evaluated to determine their ability to induce Hsp90-dependent client protein degradation. Both compounds **126** and **127** demonstrated client protein degradation similar to **121** and geldanamycin, which further supports that their activity stems from modulation of the Hsp90 molecular chaperone.

From the seed extract of *A. indica*, 31 nortriterpenoids, including 28 limonoids (**140**–**167**) and three degraded limonoids (**168**–**170**) were isolated and were tested to examine the inhibitory effect on EBV-EA activation induced by TPA as a preliminary evaluation of the potential antitumor-promoting effects (Akihisa et al., 2009). Of the tested compounds, molecules **145**, **149**, **160**–**164**, **168**, and **170** (318–398 mol ratio/32 pmol TPA) exhibited more potent inhibitory effects than the others. Since the inhibitory effects on EBV-EA induction have been demonstrated to correlate with those against tumor promotion in vivo, these compounds may be considered potential antitumor promoters. Among them, compound **160**, azadirachtin B, tested for its antitumor-initiating activity in the two-stage carcinogenesis of mouse skin tumor, induced by peroxynitrite as an initiator and TPA as a promoter, exhibited marked inhibitory activity.

Bioassay-guided fractionation of an ethanol extract of a *Malleastrum* sp. (Meliaceae) afforded three limonoids characterized by a rare tetranortriterpenoid skeleton, malleastrones A–C (**171**–**173**) (Murphy et al., 2008). Compounds **171** and **172**, tested against MDA-MB-435 breast cancer cells, HT-29 colon cancer cells, H522-T1 nonsmall cell cancer cells, and U937 histiocytic lymphoma cells, displayed significant antiproliferative activity and exhibited IC_{50} values ranging from 0.19 to 0.63 μM (Table 1). It is apparent that saturation at the 1-position of this particular limonoid skeleton results in a complete loss of its antiproliferative properties and emphasizes the importance of the

Table 1 Antiproliferative data of malleastrones A–C

Compound	IC_{50} (μM)				
	A2780	MDA-MB-435	HT-29	H522-TI	U937
Malleastrone A (**171**)	0.49	0.41	0.24	0.24	0.20
Malleastrone B (**172**)	0.63	0.34	0.22	0.23	0.19
Malleastrone C (**173**)	18	ND	ND	ND	ND

A ring composition. Overall, compounds **171** and **172** appeared to exhibit general antiproliferative activity toward tumor cells, and so no further exploration of their bioactive potential was carried out.

Previously, five limonoids, namely erythrocarpines A–E (**174**–**178**), were isolated from the bark of *Chisocheton erythrocarpus* (Meliaceae) and tested for their potential cytotoxicity against P388 murine leukemia cells (Awang et al., 2007). These compounds are A, B, and D-seco heptacyclic limonoids having mexicanolide-type skeleton with either benzoyl or cinnamoyl group as side chains at C-3. All of them showed cytotoxicity with IC_{50} values ranging from 2.0 μg/mL to 16.0 μg/mL. Erythrocarpines A (**174**) and B (**175**) with a benzoyl side chain exhibited more potent activities (IC_{50} values of 2.0 μg/mL and 6.0 μg/mL, respectively) than erythrocarpines C (**176**)-E (**178**) with a cinnamoyl side chain (IC_{50} values of 9.9–16.0 μg/mL).

The cytotoxic activity of dysobinin (**179**), azadiradione (**140**), mahonin (**180**), epoxyazadiradione (**145**), and 6α-acetoxyepoxyaza-diradione (**181**), isolated from the seeds of *Chisocheton siamensis* (Meliaceae), was also demonstrated (Maneerat et al., 2008). All limonoids showed inhibitory effects against NCI-H187, KB, and MCF-7 cancer cell lines except **181**, which was found to be inactive with all three cancer cell lines. Dysobinin (**179**) exhibited strong activity against NCI-H187, KB, and MCF-7 cancer cell lines, with IC_{50} values of 3.17, 1.67, and 2.15 μg/mL, respectively. Interestingly, the structural differences of all limonoids are only at rings B and D. Both of the diacetate groups on ring B and the double bond on ring D are crucially important for the cytotoxic activity.

From the fruits of *M. azedarach*, three *C*-seco limonoids—15-*O*-deacetyl-15-*O*-methylnimbolidin A (**182**), 15-*O*-deacetyl-15-*O*-methylnimbolidin B (**183**) and 15-*O*-deacetylnimbolidin B (**184**)—and one tetracyclic limonoids, such as 12-*O*-deacetyltrichilin H (**185**), were isolated (Zhou et al., 2005). Compounds **184** and **185** exhibited significant cytotoxic activity (IC_{50} values of 0.10 and 0.48 μM, respectively), whereas compounds **182** and **183** showed weak cytotoxicity in the range of IC_{50} 30–40 μM. The strong cytotoxicity of **185** is anticipated because most tetracyclic sendanin- and trichilin-type limonoids with a 14,15-epoxide ring and a C-19/C-29 acetal bridge exhibit strong cytotoxicity against P388 cells (less than IC_{50} 0.1 μg/mL). Nevertheless, compound **184**, a *C*-seco limonoid, also exhibited an interesting activity (Ahn et al., 1994; Itokawa et al., 1995; Takeya et al., 1996).

The limonoid 3α,7α-dideacetylkhivorin (**186**), isolated from the methanol extract of *K. senegalensis* (Meliaceae), showed significant growth inhibitory activities against MCF-7, SiHa, and Caco-2 cells, with IC_{50} values in the range of 0.07–0.14 μM (Zhang et al., 2007).

Extensive studies on the semisynthetic modifications of limonoids have been performed particularly on the photooxidations, photolysis, oxidation of the furan ring, acetylation, and enone reduction. Novel limonoids were obtained also by the cleavage of the C-ring and were tested for their cytotoxicity using the brine shrimp lethality bioassay (BSLB) method (Genupur et al., 2006). Among substrates, 6-desacetylnimbinene (**187**) and salannin (**39**) were more potent than nimbolide (**41**), considered as the reference standard, as it is established to be the most potent cytotoxic limonoid among several other limonoids of neem. The isomeric products exhibited better bioefficacy than their corresponding substrates except 6-desacetylnimbinene (**187**). The examination of the cytotoxic data provides a structure activity correlate, which may be summarized as follows. The novel isomers prove to be more potent than the naturally available limonoids. Theoretical hydrophobicity constant showed a positive correlation, while the change in the chromatographic hydrophobicity constant for the substrates and the products did not follow any pattern. While the position of the oxygen is important rather than the oxidation state, hydrogen bonding may play a vital role in the bioactivity, as is evinced from the trend exhibited by the active compounds in the O–O diad ranges between 4.5 and 5.5 Å, which emphasizes the significance of the orientation of the furan ring in enhancing the activity.

Antioxidant and Anti-inflammatory Activity

A variety of in-vitro models were used to measure the antioxidant activity of limonin (**1**) and its glucoside (**3**). Both limonoids inhibited < 7% using the β-carotene-linoleate model system. A percentage of inhibition of 0.5% and 0.25% for **1** and **3**, respectively, was observed using the 1,1-diphenyl-2-picryl hydrazyl (DPPH) assay. In the superoxide model, limonoids inhibited the production of superoxide radicals by 5–10% as compared with the negative control incubations. Moreover, limonin aglycone (**1**), but not its glucoside (**3**), prevented the copper-initiated accumulation of conjugated diene fatty acid oxidation products in hamster LDL, increasing lag time to 345 minutes (three-fold) compared with the control. The found weak antioxidant activity could be explained by a fewer hydroxyl group in the structure of these highly oxygenated triterpenoids and as a consequence of poor aqueous solubility (Yu et al., 2005). Previous studies reported the greater antioxidant activity of *Citrus* limonoids, with particular reference to limonin (**1**) and nomilinic acid glucoside (**115**) (Patil et al., 2004; Poulose et al., 2005; Sun et al., 2005; Yu et al., 2005).

Carapa guianensis is a member of the Meliaceae family widely used in folk medicine in Brazil and other countries encompassing the Amazon rainforest. From the seed of this species, 6α-acetoxygedunin (**188**), 7-deacetoxy-7-oxogedunin (**189**), andirobin (**190**), gedunin (**120**), methyl angolensate

(**191**), and 6α-acetoxyepoxyazadiradione (**192**) were isolated (Pereira et al., 1999). A fraction of all tetraterpenoids, namely TNTP, was screened in a murine model of experimental arthritis induced by zymosan. Zymosan-induced knee joint thickness was due to protein extravasation into the articular space, a phenomenon that was inhibited by TNTP oral pretreatment, starting at the dose of 100 mg/kg. It is noteworthy that the extent of inhibition provided by TNTP was similar to that of commercial corticosteroid dexamethasone, the reference inhibitor used (Penido et al., 2006). The same research also demonstrated that the oral pretreatment with TNTP was able to inhibit vascular permeability induced by bradykinin, platelet-activating factor (PAF), and histamine, as well as PGE2 generation during the allergic response (Penido et al., 2005). A possible antiedematogenic effect of TNTP might be exerted through the inhibition of these mediators. Moreover, the oral administration of TNTP was also able to significantly inhibit zymosan-induced neutrophil migration into the inflammatory site, as observed in synovial washes as well as in histological slices. The inhibition of neutrophil mobilization by TNTP was previously observed by us in the mice pleural cavity after antigenic challenge, a phenomenon shown to be related to the increase in CXCL8/IL-8 levels. The injection of zymosan induced the release of IL-1β in articular tissues at the initial phase of the inflammatory response, and in particular with edema formation and neutrophil influx at early times, and to a high extent in cartilage destruction via suppression of proteoglycan synthesis at later time points. The fact that TNTP pretreatment was able to inhibit the synthesis of IL-1β suggests that it is one of the mechanisms by which TNTP incites its anti-inflammatory effects on acute arthritis, mainly due to edema reduction at the acute response. However, the effect of TNTP at later time points after zymosan subministration remains to be investigated. In addition, TNTP pretreatment inhibited TNF-α generation, in addition to IL-1β, and might contribute to the inhibition of zymosan-induced increase in knee joint thickness, even though the blockade of other mediators is probably also important for this effect. All these evidences suggested that *C. guianensis* tetraterponoids-rich fraction blockade of TNF-α, IL-1β, and CXCL8/IL-8 synthesis via inhibition of NF-kB signaling pathway at the level of translocation into the nucleus.

From the fruit of *M. toosendan*, isotoosendanin (**193**) and 1-*O*-tigloyl-1-*O*-debenzoylohchinal (**194**) were isolated and evaluated for their anti-inflammatory and analgesic activities. The two limonoids showed a marked inhibition in both acetic acid-induced vascular permeability and λ-carrageenan-induced paw edema in mice tests (Xie et al., 2008).

Carrageenan-induced hind paw edema in mice is a biphasic event. As previously mentioned, the early phase of the inflammation is due to the release of histamine, serotonin, and similar substances; the later phase is associated with the activation of kinin-like substances. The results of the carrageenan experiment indicated that the extract and two limonoids functioned in blocking histamine and serotonins mediators. For the antinociceptive activity, the results of **193** and **194** showed inhibitory effects on the writhing response induced by acetic acid, but no obvious effect was observed in the hot-plate test, which suggested that the two compounds acted like nonsteroidal anti-inflammatory drugs, such as indomethacin, and that the analgesic effect may attribute to its anti-inflammatory action.

The effects, especially the analgesic activity, of the two compounds were weaker than those of the *M. toosendan* extract, which suggested that there were other bioactive constituents existing in extract that may act in positive synergistic interaction.

Recently, Kim et al. (2009a) reported the effect of dietary limonin (**1**) against the state of chronic inflammation. Inflammation is an imperative host defense response involving both innate and adaptative immune system. With respect to the adaptive immune response, $CD4^+$ T cells regulate inflammatory responses in part by clonal expansion into effector T helper cell subsets with distinct cytokine profiles. The evidence of the effect of **1** on downregulation of i/nOS and COX-2, which are regulated in part by NF-κB, as shown by Vanamala et al. (2006), suggests that limonin (**1**) may be a putative NF-κB inhibitor and therefore capable of modulating $CD4^+$ T-cell function and can address the research on the effect that this limonoid has on on NF-κB-dependent $CD4^+$ T-cell proliferation. For this purpose, DO11.10 transgenic mice were fed diets containing 10.02% Lim combined with either (ω-6) PUFA [5% corn oil (CO)] or (ω-3) PUFA [4% fish oil (FO), 11% CO] for 2 weeks. The limonoid suppressed NF-κB p65 nuclear translocation in activated $CD4^+$ T cells. In contrast, activator protein-1 (c-Jun) and nuclear factor of activated T cells c1 were not affected. Interestingly, dietary combination with FO enhanced the suppressive effects of **1** with respect to $CD4^+$ T-cell proliferation in response to anti-CD3/28 mAb. These results suggest that this combination chemotherapy (FO + limonin) may favorably modulate $CD4^+$ T-cell-mediated inflammation.

Fraxinellone (**195**), a product of limonoids' natural degradation, was investigated for its anti-inflammatory potential by Kim et al. (2009b). The effect of fraxinellone (**195**), isolated from *D. dasycarpus* (Meliaceae), was evaluated in lipopolysaccharide (LPS)-treated RAW 264.7 macrophages. This limonoid was found to inhibit LPS-induced nitric oxide (NO) and prostaglandin E_2 (PGE2) production, and to reduce the LPS-induced expressions of iNOS and COX-2 at the mRNA and protein levels in a dose-dependent manner. Results of Kim et al. (2009a) against NF-k B were confirmed also for fraxinellone (**195**), which significantly attenuated LPS-induced DNA binding activity and the transcription activity of NF-κB. This inactivation was mediated by phosphorylation of inhibitory kappa B-α (Ik B-α) and the subsequent translocation of p65 to the nucleus. Compound (**195**) also suppressed the Ik B kinase (IKK) activity and the phosphorylation of extracellular signal-related kinase (ERK1/2), whereas the phosphorylation of Jun N-terminal kinase (JNK1/2) and p38 was unaffected. These results suggest that the anti-inflammatory properties of **195** are related to the downregulation of the expression of proinflammatory inducible enzymes iNOS and COX-2 due to NF-kB inhibition through the negative regulation of IKK and ERK1/2 phosphorylation in RAW 264.7 cells.

Azadiradione (**140**), epoxyazadiradione (**145**), 17-epi-17-hydroxyazadiradione (**148**), 7-acetyl-16,17-dehydro-16-hydroxyneotrichilenone (**149**), 7-deacetylgedunin (**157**), nimolicinol (**159**), and azadirachtin B (**160**) isolated from *A. indica* were evaluated for their potential anti-inflammatory activity against TPA-induced inflammation in vivo. All of the compounds exhibited marked bioactivity compared with the commercial drug indomethacin (Akihisa et al., 2009).

Neuroprotective Activity

From the methanol extract of *D. dasycarpus* (Meliaceae) root bark, limonoids 9α-hydroxyfraxinellone-9-*O*-β-D-glucoside (**196**), dictamnusine (**197**), dictamdiol A (**198**), and dictamdiol B (**199**), together with eight known compounds, dictamdiol (**107**), fraxinellone (**195**), fraxinellonone (**200**), 9β-hydroxyfraxinellone (**201**), calodendrolide (**202**), obacunone (**111**), limonin (**1**), and rutaevin (**203**), were isolated. Compounds **1**, **111**, **195**, **197**, **198**, and **202** showed significant neuroprotective activity against glutamate-induced neurotoxicity in primary cultures of rat cortical cells at a concentration of 0.1 μM (Yoon et al., 2008). Obacunone (**111**), limonin (**1**), fraxinellone (**195**), and calodendrolide (**202**) effectively inhibited calcium influx and overproduction of cellular NO and reactive oxygen species accompanied by glutamate-induced neurotoxicity. In addition, these compounds significantly preserved the mitochondrial membrane potential and activities of antioxidative enzymes. Interestingly, the pretreatment of the limonoids appeared to be more effective for the protection of cortical cells against glutamate toxicity than when administer in post-treatment. In other words, these compounds were neuroprotective when they were administered during the phase of injury through a mechanism that involves the expression of neuroprotective proteins. Acting by preserving the antioxidant defense system, these compounds might offer potential drug development candidates for various neurodegenerative diseases involving glutamate (Yoon et al., 2010). At concentrations of 100–150 μM, obacunone (**111**) showed potent neuroprotective effects on glutamate-induced neurotoxicity and induced the expression of heme oxygenase (HO)-1 in mouse hippocampal HT22 cells. In addition, **111** increased p38 MAPK phosphorylation and induced HO-1 expression via the p38 MAPK pathway. These results suggest that obacunone increases cellular resistance to glutamate-induced oxidative injury in mouse hippocampal HT22 cells, presumably through the p38 MAPK pathway-dependent HO-1 expression (Jeong et al., 2010).

Antiplasmodial, Antiviral, and Antimicrobial Activities

Malaria is one of the major parasitic diseases in the tropical and subtropical regions of the world, and its etiological agents are protozoans of the genus *Plasmodium*. Several classes of natural products, including limonoids, were studied for the treatment of malaria.

Table 2 Antimalarial activities (IC_{50}) of limonoids isolated from *K. anthotheca*

Limonoid	[^{3}H]-hypoxanthine uptake (μM)	Parasite development (μM)
Anthothecol (**204**)	1.4	0.17
Gedunin (**120**)	3.1	0.14
Limonin (**1**)	>10	2.7
Obacunone (**111**)	>10	1.6

Four naturally occurring limonoids—limonin (**1**), obacunone (**111**), anthothecol (**204**), and gedunin (**120**)—were isolated from *K. anthotheca* and tested for their potential antimalarial activity against *P. falciparum* (Table 2) (Lee et al., 2008). Both **204** and **120** showed potent antimalarial activity against parasites, with IC_{50} values of 1.4 and 3.1 μM, respectively, using an assay based on inhibition of [^{3}H]-hypoxanthine uptake. All isolated limonoids influenced parasite development. In fact, the result of an assay of development that measures the formation of new ring-stage parasites after 48 hours of incubation with anthothecol (**204**), gedunin (**120**), limonin (**1**), and obacunone (**111**) exhibited antimalarial activity, with IC_{50} values ranging from 2.7 to 0.14 mM.

From *Esenbeckia febrifuga* (Rutaceae), a plant traditionally used to treat malaria in the Brazilian Amazon region, rutaevin (**203**) was isolated. This limonoid tested in vitro for antiplasmodial activity proved inactive (IC_{50} $\geq$100 μg/mL) (Dolabela et al., 2008).

The ceramicines A–D (**205**–**208**) have been isolated from the bark of *Chisocheton ceramicus*. Ceramicine B (**206**) exerted a strong antiplasmodial activity against *P. falciparum* (IC_{50} 0.23 μg/mL), whereas ceramicines C (**207**) and D (**208**) exhibited a moderate activity, with IC_{50} values of 2.38 and 2.15 μg/mL, respectively. Interestingly, ceramicine A (**205**), lacking a tetrahydrofuran ring at C-4 to C-6 and C-28, had weak activity (IC_{50} 44.22 μg/mL). Compounds **206**–**208** showed a weak cytotoxic activity on P388 cells, with IC_{50} values ranging from 5.5 to 27 μg/mL for **206** and **208**, respectively (Mohamad et al., 2008; Mohamad et al., 2009).

An increased impact on malaria burden may be achieved through the development of improved transmission-blocking formulations, including molecules complementing the gametocytocidal effects of artemisinin derivatives and/or acting on *Plasmodium* stages developing in the vector. The tetranortriterpenoid azadirachtin (**44**), obtained from the seeds of *A. indica*, inhibited *P. falciparum* and *P. berghei* exflagellation process, inducing an interruption of the endomitotic divisions and the formation of rigid extensions on axonemes, thus preventing their motility (Jones et al., 1994).

Lucantoni et al. (2010) reported the in-vivo transmission-blocking activity of an azadirachtin-enriched neem seed extract NeemAzal®. This commercial extract, containing azadirachtin (**44**) as the main component, completely blocked the development of *P. berghei* in *Anopheles stephensi* mosquitoes fed

on gametocytaemic mice treated with the extract at 50 mg/kg body weight. The treated mosquitoes did not produce oocysts and were thus unable to infect healthy mice. The examination of slides prepared from the midgut content of experimental mosquitoes showed that the extract activity was directed to the early sporogonic stages. Altogether, these results indicate that NeemAzal® interferes in the assembly of cytoskeletal microtubules, which is a fundamental process in gametogenesis and the formation of ookinete.

The tetranortriterpenoid 1-cinnamoyl-3,11-dihydroxymeliacarpin (**209**) isolated from *Melia azedarach* reduced both vesicular stomatitis virus (VSV) and HSV-1 infectivity. This action was exerted by blocking VSV entry and the intracellular transport of VSV-G protein, confining it to the Golgi apparatus, by pre- or posttreatment, respectively (Barquero et al., 2004). In order to clarify the mechanism of action, the effect of 1-cinnamoyl-3,11-dihydroxymeliacarpin (**209**) on NF-κB signaling pathway was evaluated. It is well known that many host cellular factors are necessary for a successful viral infection. For HSV-1 entry, viral glycoprotein synthesis is completely dependent upon cellular membrane trafficking. For this reason, these specific cellular functions essential for virus replication have attracted growing interest as targets for antiviral medication. In this sense, triggering of NF-κB activation is particularly relevant during HSV-1 infection because it harbors several consensus-binding sites for NF-κB in tits promoters and this factor is utilized by HSV-1 to enhance its replication (Santoro et al., 2003). The analyzed limonoid is able to impede NF-κB activation in HSV-1-infected conjunctival cells and leads to the accumulation of p65 NF-κB subunit in the cytoplasm of uninfected treated Vero cells. Successively, Bueno et al. (2009) concluded that **209** inhibited NF-κB translocation to the nucleus, leading to a decrease in IL-6 production. Besides, **209** seemed to modulate IL-6 and TNF-α responses in macrophages, whether they were infected with HSV-1 or stimulated with LPS. However, this limonoid did not affect NF-κB activation in these cells, suggesting that an alternative NF-κB cell signaling pathway would be involved in the modulation of cytokine production. Regarding the sequestration of p65 by 1-cinnamoyl-3,11-dihydroxymeliacarpin (**209**), this activity could be due to an inhibition of either the enzymatic activity of the IKK complex or due to the ubiquitination process. For the above-mentioned reason, 1-cinnamoyl-3,11-dihydroxymeliacarpin (**209**) is a pleiotropic agent that not only inhibits the multiplication of DNA and RNA viruses by the same mechanism of action but also modulates the NF-κB signaling pathway with an immuno-modulation action. Limonin (**1**) and a mixture of limonexic acid (**119**) and isolimonexic acid (**210**) were isolated from the branches and the leaves of *Glycosmis parva*. The three limonoids proved inactive against HSV-1 and HSV-2 infection at the maximum concentration tested (100 μg/mL) (Chansriniyom et al., 2009). Recently, nomilin (**2**) demonstrated to completely inhibit HTLV-1 tax/rex expression at 10 μg/mL (Balestrieri et al., 2011). Limonin (**1**) was also able to inhibit HTLV-1 tax/rex expression but at a concentration of 5 μg/mL.

Eleven mexicanolide-type limonoids—swietmanins A–I (**211**–**214**, **217–221**), 2-hydroxy-3-*O*-isobutyrylproceranolide (**215**), and 2-hydroxy-3-*O*-benzoylproceranolide (**216**)—and a new andirobin-type limonoid, swietmanin J (**222**), were isolated from the fruits of *S. mahagoni* (Meliaceae) (Yoon et al., 2010).

These limonoids were tested against 11 microbes, including seven bacteria and four fungi. 2-hydroxy-3-*O*-isobutyrylproceranolide (**215**) exhibited activity against *Micrococcus luteus*, with an MIC (minimal inhibitory concentration) value of 50. *Xylella fastidiosa* is a Gram-negative bacteria that colonizes the xylem of plants, causing diseases in several economically important crops such as sweet orange tree, and thus leading to a great economic damage to the industry that produces orange juice for the international market. The most abundant limonoid limonin (**1**) found in roots and shoots of *C. sinensis* (Rutaceae) exerted an interesting activity against *X. fastidiosa*, with an MIC value of 3.4×10^3 μM (Ribeiro et al., 2008).

Miscellaneous

In orchidectomized male rats, *Citrus* consumption enhanced bone density and improved bone strength by an average of 4% in comparison with the control group (Mandadi et al., 2009). In particular, crude extract and limonin (**1**) significantly preserved the concentration of calcium in the femur and in the fifth lumbar to the level of the sham control group. The pronounced effect on bone density might have been related to the increased rate of bone formation, as evident from elevated plasma IGF-1 concentration, rather than to the decreased rate of bone resorption. Moreover, the study underlines that rats fed on a diet supplemented with crude extract tended to have a higher plasma antioxidant status than those fed on diets with just bioactive compounds (Kroyer, 1986; Jung et al., 2003). The possible explanation was that these compounds may act in a synergistic way.

Related to the problem of malaria was the study of Howard et al. (2009), which reported the effect of *A. indica* limonoids-rich extract as a larvicide and growth disruptor of *Anopheles gambiae* s.s. High-performance liquid chromatography of aqueous extracts showed the presence of nimbin (**223**) and salannin (**39**). *Botulinum* neurotoxins (BoNT/A) are the etiological agents responsible for botulism, a disease characterized by peripheral neuromuscular blockade and a characteristic flaccid paralysis in humans.

The major limonoid found in *M. toosendan* (Meliaceae), used as an antibotulinum agent in Chinese medicine, is toosendanin (**224**). This limonoid has antihelmintic action through a multiple mode, including damage to midgut tissues and inhibition of esterases, cytochrome P450-aldrin epoxidase, and proteinase activities (Zhang et al., 2007). From a recent study (Nakai et al., 2009), a new synthetic strategy is put forth, allowing access to

a 4-acetoxy CD fragment analogue (**225**) of toosendanin (**224**), which was achieved from mesityl oxide and acetylacetone in 14 steps. The molecule provided no efficacy in vivo. However, **224** effectively inhibits the biological activity of BoNT/A in neuronal cells at concentrations of 200 nM, and partial inhibition can be observed with concentrations as low as 8 nM. Mechanistically, toosendanin's inhibition is due to prevention of transduction of the BoNT LC through the heavy-chain channel. Intriguing questions as to the molecular architecture of toosendanin (**224**) as related to its antibotulinum properties have addressed research on the synthesis of toosendanin's unusual AB ring, containing a unique bridged hemiacetal.

Toosendanin AB-ring and 7-epi AB ring were obtained and tested for BoNT/A1 inhibitory activity. Interestingly, only **224** at a concentration of 200 μM completely inhibited BoNT/A1 activity, while its derivatives, tested in primary neuronal cells, were unable to exert activity (Nakai et al., 2010).

CONCLUDING REMARKS

Limonoids comprise a group of natural products limited in their distribution but particularly abundant in *Citrus* species that demonstrated to possess a large number of biological properties, including anticancer, antioxidant, anti-inflammatory, neuroprotective, and antimicrobial activities.

Citrus limonoids are considered a problem for the *Citrus* juice industry as they cause delayed bitterness of the juices at room temperature, thus lowering the quality and value of the commercial juice. For this reason, limonoids obtained by the debittering process are used to remove or mitigate the bitterness of fruits and juice and can be used in pharmaceutical and/or nutraceutical industry and not only as waste generated in juice processing.

In the last years, more than 100 limonoids have been isolated and characterized.

Table 3 In-vivo limonoids bioactivity

Limonoid	Effect	Animal	Reference
Isolated compound			
Limonin (**1**)	Anticarcinogenic	Sprague–Dawley rats	Vanamala et al. (2006)
	Chemopreventive	FemaleA/JOlaHsd 8-to 9-week-old mice	Perez et al. (2009)
	Anti-inflammatory and chemopreventive	TCR transgenic DO11.10 Rag2$^{-/-}$ mice	Kim et al. (2009)
	Improvement bone quality and plasma antioxidant activity	Orchidectomized rats	Mandadi et al. (2009)
Nomilin (**2**)	Antioxidant	Female ICR/Ha mice	Lam et al., (1989)
Limonin glucoside (**3**)	Chemopreventive	FemaleA/JOlaHsd 8- to 9-week-old mice	Perez et al. (2009)
Nimbolide (**41**)	Anticarcinogenic	Hamster	Priyadarsini et al. (2009)
	Antiapoptotic	Hamster	Harish Kumar et al. (2010)
Azadirachtin (**44**)	Anticarcinogenic	Hamster	Priyadarsini et al. (2009)
	Antiapoptotic	Hamster	Harish Kumar et al. (2010)
Obacunone (**111**)	Antioxidant	Female ICR/Ha mice	Lam et al. (1989)
Deacetylnomilic acid glucoside (**116**)	Chemopreventive	FemaleA/JOlaHsd 8- to 9-week-old mice	Perez et al. (2009)
Azadiradione (**140**)	Anti-inflammatory	ICR mice	Akihisa et al. (2009)
Epoxyazadiradione (**145**)	Anti-inflammatory	ICR mice	Akihisa et al. (2009)
17-Epi-17-hydroxyazadiradione (**148**)	Anti-inflammatory	ICR mice	Akihisa et al., (2009)
7-Acetyl-16,17-dehydro-16-hydroxyneotrichilenone (**149**)	Anti-inflammatory	ICR mice	Akihisa et al. (2009)
7-Deacetylgedunin (**157**)	Anti-inflammatory	ICR mice	Akihisa et al. (2009)
Nimolicinol (**159**)	Anti-inflammatory	ICR mice	Akihisa et al. (2009)
Azadirachtin B (**160**)	Anti-inflammatory	ICR mice	Akihisa et al. (2009)
Isotoosendanin (**195**)	Anti-inflammatory	ICR mice	Xie et al. (2008)
1-O-Tigloyl-1-O-debenzoylohchinal (**196**)	Anti-inflammatory	ICR mice	Xie et al. (2008)
Extract and/or fraction			
C. guianensis tetranortriterpenoids fraction (**TNTP**)	Anti-inflammatory	Swiss and C57/Bl10 mice mice	Penido et al. (2005); Penido et al. (2006)
	Antihyperalgesic	Wistar rats	Penido et al. (2005)
Azadirachtin-enriched neem seed extract (**NeemAzal®**)	Antimalarial	BALB/c mice	Lucantoni et al. (2010)
A. indica limonoids-rich extract	Antifeedant	*Anopheles gambiae* s.s. mosquitoes	Howard et al. (2009)

TCR = T-cell receptor, ICR = imprinting control region.

Different in-vivo (Table 3) and in-vitro studies have provided evidence supporting the hypothesis that limonoids are effective cytotoxic and apoptosis-promoting agents, and incorporation of enriched fractions of these compounds in the diet may serve to prevent several cancer types. Moreover, the ability of these compounds to induce the activity of detoxifying phase II enzymes makes them valuable bioactive compounds in the quest to prevent cancer and oxidation-related diseases and thus deserves more in-depth research in order to improve human health. Nevertheless, perusal of the literature revealed that till now no human studies have been performed.

ABBREVIATIONS

ABTS = 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACF = aberrant crypt foci
AOC = antioxidant capacity
AOM = azoxymethane
BHT = butylated hydroxytoluene
BoNT/A = botulinum neurotoxins
BSLB = brine shrimp lethality bioassay
CDNB = 1-chloro-2,4-dinitrobenzene
COX-2 = cyclooxygenase 2
CYPs = cytochrome P450 oxidoreductase enzymes
DPPH = 1,1-diphenyl-2-picryl hydrazyl
EBV-EA = Epstein-Barr virus early antigen
ERK1/2 = extracellular-signal related kinase
GST = glutathione *S*-transferase
HBP = hamster buccal pouch
HSV 1 = virus herpes simplex type 1
HSV 2 = virus herpes simplex type 2
HTLV-1 = human T-cell leukemia/lymphoma virus type 1
IC_{50} = 50% inhibitory concentration
ID_{50} = 50% inhibitory dose
IGF-1 = insuline-like growth factor
IKK = Ik B kinase
iNOS = inducible nitric oxide synthase
JNK1/2 = jun *N*-terminal kinase
LDL = low-density lipoprotein
LPS = lipopolysaccharide
MIC = minimum inhibitory concentration
NF-κB = nuclear factor-kappa B
NO = nitric oxide
4NQO = 4-nitroquinoline 1-oxide
ORAC = oxygen radical absorbance capacity
PAF = platelet activating factor
PCNA = proliferating cell nuclear antigen
PGE2 = prostaglandin E2
QR = quinone reductase
TEAC = trolox-equivalent antioxidant capacity
TNTP = tetraterpenoids fraction
TPA = 12-*O*-tetradecanoylphorbol-13-acetate
VSV = vesicular stomatitis virus.

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