Caged Garcinia Xanthones: Development Since 1937

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Caged xanthones, characterized by a unique 4-oxa-tricyclo[4.3.1.0³,7]dec-2-one scaffold, are a special class of bioactive components mainly derived from the Garcinia genus (Guttiferae family). Around 100 compounds from this family have been reported to date and most of them have potent antitumor activity, with gambogic acid being the best representative. During the past decades, inspired by the unusual caged skeleton and remarkable bioactivity, scientists from various fields have shown increasing interest on these promising natural products. In this review, the plant resources, structural characteristics, total synthesis, biological activity and mechanisms of action, structure activity relationship, and anticancer drug development of these caged xanthones are described.

Keywords: Guttiferae, Garcinia, gamboge, caged xanthone, cytotoxicity, anticancer activity.

1. INTRODUCTION

Caged Garcinia xanthones, a special group of polyphenylated xanthones that is naturally found in Garcinia plants (Guttiferae family), have generated increasing research interest among phytochemical, pharmacological, synthetic, and biological communities in recent years due to their strong bioactivities and unique 4-oxa-tricyclo[4.3.1.0³,7]dec-2-one scaffold, in which a highly substituted tetrahydrofuran core with three quaternary carbon centers is featured.

The first report of caged Garcinia xanthones was that on morellin from G. morella in 1937 [1]. Despite 25 years of synthetic efforts and spectral analysis, only a partial structure of morellin was deduced [2]. The complete structure of morellin was first elucidated by a polyphenylated and caged xanthone by an X-ray crystallography of its p-bromobenzenesulphonyl ester in 1963 [3]. With this breakthrough, the structures of a series of caged Garcinia xanthones were clearly characterized [2]. Continuous efforts were made to achieve the total synthesis of this kind of caged xanthones [4-7]. It was also found that most of these xanthones showed interesting bioactivities, especially cytotoxicities against various cancer cells [8-11]. Gambogic acid (GA), extracted from the resin of Garcinia hanburyi, is the best representative [12-15]. GA exhibited potent cytotoxic effect against cancer cell lines in culture with LD₅₀ below 1 μM [12,16]. In vitro and in vivo pharmacological studies demonstrated both that GA could efficiently inhibit the growth of a broad panel of human cancer cells (e.g., lung carcinoma and hepatoma) and prevent cancer metastasis and angiogenesis [17-21]. Furthermore, GA can be well tolerated as reported in different animal tests, suggesting that there might be a therapeutic window at which only cancer cells but not the normal cells are killed [12,15,22]. This caged xanthone, as a candidate intravenous antitumor drug, has been approved by the Chinese State Food and Drug Administration to enter phase II clinical trial [23]. The mechanism of GA’s anticancer activities seems very complex, which attracts the increasing interest of pharmacologists worldwide. Multiple mechanisms have been proposed, such as apoptosis induction, cell cycle arrest, telomerase inhibition and anti-angiogenesis activity [24]. Recent studies have further demonstrated that GA could inhibit NF-κB signaling pathway and induce apoptosis through its interaction with the transferrin receptor and acti-
vation of T lymphocyte [25,26]. Other molecular targets of GA were also reported including bcl-2 family [27], survivin [28], topoisomerase IIα [29], p53/mdm2 [30], and stathmin 1 [31]. Following the R&D success of GA as a new anticancer drug, caged *Garcinia* xanthones will become a research focus in chemistry, natural medicine and other related fields.

The isolation, characterization, and chemistry of pigments from *Garcinia* species were reviewed by Prof. Venkataraman in 1973, but only 9 natural caged xanthones were mentioned [2]. We herein present the first comprehensive review focusing solely on caged *Garcinia* xanthones with an emphasis on their natural resource, synthesis, biological activities, mechanism of action, and structure-activity relationship. The literatures cited in this review article are primarily from 1937 to 2008.

2. NATURALLY OCCURRING CAGED XANTHONES

As shown in Table 1, a total of 98 caged xanthones (1-98) from natural sources have been reported to date, almost all of them are from *Garcinia* plants. Although the genus of *Garcinia* has around 450 members and most of them contain prenylated xanthones [32-40], caged xanthones mainly occur in five *Garcinia* plants, namely *G. morella*, *G. hanburyi*, *G. bracteata*, *G. gaudichaudii*, and *G. scortechinii*. These plants are widely distributed in Southeast Asia, India, and the southern part of China [40].

### Table 1. Reported Naturally Occurring Caged Xanthones (up to March, 2008)

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<td>C33H38O8</td>
<td>G. bracteata</td>
<td>[80]</td>
</tr>
<tr>
<td>88</td>
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<td>C33H38O8</td>
<td>G. bracteata</td>
<td>[80]</td>
</tr>
<tr>
<td>89</td>
<td>7-Hydroxyforbesione</td>
<td>C33H38O8</td>
<td>G. cantleyana</td>
<td>[81]</td>
</tr>
<tr>
<td>90</td>
<td>Cantleyanone A</td>
<td>C33H38O8</td>
<td>G. cantleyana</td>
<td>[81]</td>
</tr>
<tr>
<td>91</td>
<td>Cantleyanone B</td>
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<td>G. cantleyana</td>
<td>[81]</td>
</tr>
<tr>
<td>92</td>
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<td>[81]</td>
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<td>G. cantleyana</td>
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<tr>
<td>94</td>
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<td>G. urophylla</td>
<td>[82]</td>
</tr>
<tr>
<td>96</td>
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<td>C33H38O8</td>
<td>Cratoxylum cochinchinense</td>
<td>[85, 86]</td>
</tr>
<tr>
<td>97</td>
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<td>C33H38O8</td>
<td>Cratoxylum cochinchinense</td>
<td>[85]</td>
</tr>
<tr>
<td>98</td>
<td>4-Deprenylbractatin</td>
<td>C33H38O8</td>
<td>Cratoxylum cochinchinense</td>
<td>[11, 86]</td>
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</tbody>
</table>

**G. morella**

The first caged xanthone named morrelin (1) was obtained by crystallization from an alcoholic extract of the pericarp of the seeds of *G. morella* [1]. Although this compound crystallizes readily in large and beautiful orange-yellow needles, homogeneous and crystalline derivatives for X-ray analysis are difficult to obtain. Early chemical work led to erroneous conclusions concerning the molecular formula as well as the number of hydroxyl and carbonyl groups.
The molecular formula of morrelin was initially assigned as C$_{30}$H$_{34}$O$_{6}$ in 1937 [1,41], and was subsequently revised to C$_{33}$H$_{36}$O$_{7}$ with only the partial structure established [42-44]. For a long time, the structure of morellin remained undetermined, serving as a challenge for synthetic chemists. In 1961, the structure of morellin was determined by X-ray crystallographic study of the $p$-bromobenzenesulphonyl ester, which formed large needles and had an orthorhombic unit.
The name gamboge is more popular than G. hanburyi. It is generally accepted that GA is the major component of gamboge, the resin of G. hanburyi. However, the resin of G. morella is also often wrongly called “gamboges” [2,53]. This traces back to the denomination of G. hanburyi. The plant that generates gamboge was initially named *Garcinia morella* var. *pedicellata* by Dr. Hanbury. Later, a younger botanist, Dr. Hooker, thought that it is distinctly different from the *Garcinia morella* and should be regarded as new rank of species and therefore re-named it as *Garcinia hanburyi* [54]. Despite the change in plant name, the resin of G. morella continued to be called “gamboge”, leading to much confusion [53]. According to the published literatures [2,52,55-57], GA is absent in *G. morella* while morellin (1) has seldom been found in *G. hanburyi*. These are two *Garcinia* species which have similar but definitely different chemical profiles. Therefore, the plant that produces GA should be *G. hanburyi*, not *G. morella*.

**G. hanburyi**

The name gamboge is more popular than *G. hanburyi*, since most research work have been focused on the resin and not the aerial part. The earliest evidence of the use of gamboge was in 8th century East Asia, where it was used as a fresh yellow watercolor pigment [58]. Evidence of yellow ink made from gamboge exists on black (khoi) paper in Thailand and on a 12th century scroll depiction of The Tale of The Genji [58]. In recent centuries, gamboge has also been used as a folk medicine in the treatment of chronic dermatitis, hemorrhoids, and bedsore. Due to its potent cytotoxicities against a wide variety of cancer cell lines, many pharmaceutical studies have been conducted on its antitumor activities in the past decades [12, 19, 59-61]. As the major component of gamboges, gambogic acid (GA) is not only the main coloring matter but also the source of the bioactivity. For a long time, GA was thought to be an inseparable mixture of C-2 epimers. In 2002, the crystal of the pyridine salt of R-gambogic acid was first obtained and analyzed by X-ray diffraction [62-63]. The S-epimer was recently isolated using chromatographic methods [64,65], which led to the separation of three pairs of epimers, namely gambogic acid (11) and epigambogic acid (12), isogambogic acid (13) and episo-gambogic acid (14), 30-hydroxygambogic acid (15) and 30-hydroxyepigambogic acid (16) [56,66]. A total of 30 caged xanthones (11-40) from the resin and fruits of *G. hanburyi* that have been reported to date (Fig. (1)) [9,67-71].

Besides the aforementioned species, only three other *Garcinia* plants, namely *G. scortechinii*, *G. gaudichaudii*, and *G. bracteata*, have been found to be abundant in caged xanthones. Most of the caged xanthones from *G. scortechinii*, namely scortechinones A-T (41-60, Fig. 2), are bridgehead-methoxylated, tetraprenylated and have a C-7 bridgehead methoxyl group and a 2,2,3-trimethylhydrofuran ring between C-3 and C-4 [72-75]. 26 new caged
xanthones (Fig. 3), gaudichaudiones A-J (61-70),
gaudichaudic acids A-I (71-79), and 7-isoprenylnorrellic acid (80), were found in the leaf and bark extract of G. gaudichaudii [10,76-79]. Among them, gaudichaudic acids G-I, contain an unusual toluene-fused dimethylpyran ring.

Three of the eight caged xanthones obtained from the EtOAc extract of the leaves of G. bracteata (Fig. (4)), namely 1-O-methylneobractatin (86), neoisobractatins A and B (87 and 88), represent a novel type of caged xanthones [11,80]. In their structures, the ketone group at C6 and the quaternary carbon C5 exchanged positions, still keeping a similar caged bridge core.

From a chemotaxonomic standpoint, the Garcinia genus can be divided into two groups. The first group includes species that produce caged xanthones, such as G. hanburyi and G. morella, and the other group includes those generating classical xanthones. However, in G. cantleyana [81], G. urophylla [82], G. lateriflora [83], and G. forbesii [84], a few caged xanthones (31, 89-95, Fig. (5)) were isolated in small amounts along with normal xanthones, suggesting that these species fall between the two typical groups.

Caged xanthones can rarely be found outside the Garcinia genus. Cratoxylum cochichinense and Dasymaschalon sootepense are the two exceptions from which only four caged xanthones (83, 96-98) were isolated (Fig. (5)) [85-87].

3. STRUCTURAL AND SPECTRAL CHARACTERISTICS OF CAGED XANTHONES

3.1. Structural Characteristics of Caged Xanthones

The chemical structures of most caged xanthones feature a unique 4-oxa-tricyclo[4.3.1.0^{3,7}]dec-8-en-2-one scaffold built into a common xanthone backbone. This motif is further customized by substitutions on the aromatic residue and peripheral oxidations to produce a variety of structural families as mentioned above. On the other hand, neobractatins (86-88) [11,80], with an isomerized, neo caged bridgecore, represent another interesting branch of caged xanthones. Unfortunately, the absolute stereochemistry of the majority of caged xanthones remains undetermined. There are two kinds of structural drawings of the caged skeleton in the literatures, e. g. 11a and 11b, which are mirror images of each other (Fig. (6)). These two enantiomers are undistinguishable
either by single crystal X-ray diffraction or by common spectroscopic means. The CD spectrum has not been reported for any caged xanthones. Therefore, it is still unknown whether these xanthones exist as one of the enantiomers or as a mixture of enantiomers.

3.2. Spectral Characteristics of Caged Xanthones

3.2.1. UV, IR, and Optical Rotation Spectra

Most of the caged xanthones, being fresh yellow, show strong absorption at 360-366 nm in the UV spectra, making it easy to distinguish them from other natural products. Their IR spectra often display the absorption band at 3400-3500 (OH), 1738-1745 (an unconjugated carbonyl group), 1685-1700 (an α,β-unsaturated carbonyl group), and 1635-1640 cm⁻¹ (a chelated ortho-hydroxyl carbonyl group). The optical rotation spectra of most natural caged xanthones exhibit negative values while those of synthesized products have never been reported.

3.2.2. Mass Spectra

The mass spectra of morellin and its analogues were first studied by Yemul and Rao in 1974 [88]. Mass spectrometry coupled with high-performance liquid chromatography has been used to identify the metabolites of caged xanthones in vivo in rat bile [89]. Apart from fragmentations commonly due to the 3,3-dimethylallyl groups [90], morellin-like caged xanthones show characteristic fragmentations in EI mass
spectra. The typical cleavage is the opening of the bicyclic(2.2.2)octenone ring system by a retro-Diels-Alder reaction (Fig. 7). The γ-pyrone ring can also undergo retro-Diels-Alder fragmentation. These two modes of cleavage indicated the different substituents on the main skeleton of morellin [88].

Using UPLC-MS/MS/MS in positive ESI mode, fragmentation mechanisms of protonated molecular ions, [M+H]+, were studied extensively in 12 caged xanthones [91]. The Retro-Diels-Alder (RDA) reaction occurred in the CID processes and produced the characteristic fragment ions as shown in Fig. 8. Elimination of 2-methylbut-3-en-2-ol (86 Da) by transferring neighbor hydrogen (H-26) to the oxygen or eliminating 2,2’-dimethyl-2H-oxete (84 Da) could be observed. Due to the electronic effect of both the xanthone oxygen (O-15) and the C-8 carbonyl group, this RDA reaction was different from that in EI mode. By comparing the actual MS/MS/MS data with the predicted fragment ions of standard samples, and using the computation methods to rationalize the observed MS data, UPLC-QTOF–MS/MS/MS allows rapid and accurate identification of caged xanthones, including some cis- and trans-isomers [91].

3.2.3. Nuclear Magnetic Resonance (NMR) Spectroscopy

In the 1H NMR spectra (usually measured in CDCl3) of caged xanthones, three groups of signals can be observed, besides the chelated ortho-carbonyl hydroxyl proton commonly at δ 12.5-13.0. The first group includes signals of olefinic protons at δ 4.50-7.50, assignable to H-10, which disappears in morellin (7)-like xanthones; ortho-coupled H-3/H-4, which is absent in those with open ring A such as gambogenic acid (17); and the olefinic protons of isoprenyl substitutions. The second group consists of signals at δ 2.00-3.50 due to the methylene and methine protons of the caged scaffold and isoprenyl groups. The last group mainly shows signals of methyl groups at δ 1.00-2.00. The 13C NMR spectra provide clear confirmation of the 1H NMR spectra analysis, especially the existence of the aldehyde and ketone groups, the isoprenyls, and the oxygenated functions. Neo-caged xanthones (86-88) exhibit typical signals that are attributable to the sequence =CH-CH-CH-CH2 in the 1H and 13C NMR spectra [11,80].

The NMR technique is powerful in establishing the planar structures but often fails in differentiating epimers, which always present identical 1D and 2D NMR spectra, e.g. gambogic acid and epigambogic acid (11 and 12) [62], neoisobractatins A and B (87 and 88) [80]. X-ray diffraction is able to differentiate epimers but fine crystals are not easily attainable, therefore some pyridine salt and p-bromobenzenesulphonyl ester were prepared for crystallization [3,63].

4. BIOSYNTHESIS OF CAGED XANTHONES

From the biosynthetic point of view, these caged xanthones are thought to be derived from a common benzophenone intermediate of a mixed shikimate-acetate pathway that has undergone plant-specific prenylations, rearrangements, and/or oxidation reactions [46,92]. An elegant proposal for the biosynthesis of the caged scaffold in which a Claisen rearrangement was followed by an intramolecular Diels-Alder reaction was put forward by Quillinan and Scheinmann over 30 years ago [93]. This biosynthetic hypothesis was successfully tested on 5,6-bis(allyloxy)-1-hydroxy-9H-xanthen-9-one (99). As shown in Fig. 9, heating 99 in boiling decalin induced the Claisen rearrangement and subse-

Fig. (6). Chemical structures of two enantiomers 11a and 11b.

Fig. (7). Typical cleavage of the bicycle(2.2.2)octenone ring system by a Retro-Diels-Alder reaction.
Subsequently, the Diels-Alder addition, to produce the elusive caged structure [93]. The Claisen reaction was regarded as nonregioselective due to concomitant allylation at both C-5 and C-6 centers [4,5,94-97], creating intermediates 100 and 101, respectively [93]. These intermediates then generated an isomeric mixture of 102 and 103, representing the regular caged scaffold, and the so-called neo scaffold, respectively [4,5,94-97]. The isolation of neobractatins (86-88) from G. bracteata provided strong support for this hypothesis.

5. PROGRESS ON THE SYNTHETIC CHEMISTRY OF CAGED XANTHONES

5.1. Synthesis of Bridged Tricyclic Core

The first step of accomplishing the total synthesis of the intriguing caged molecules is the synthesis of the bridged tricyclic core of 4-oxatricyclo[4.3.1.03,7]decan-2-one. Several different intramolecular Diels-Alder strategies have been reported to do this, including Rao’s approaches (Fig. 10),
Wessely Oxidation/Diels-Alder approach, and biomimetic Claisen rearrangement/Diels-Alder approach (Fig. 11).

5.1.1. Rao’s Approaches

For the construction of the oxatricyclo[4.3.1.0\textsubscript{3,7}]decananes, the intramolecular Diels-Alder protocol was primarily established using dienophiles and 3,4-dihydoxanthene [98]. To develop more efficient methods for the conversion of intramolecular Diels-Alder adducts \(104\) into \(105\), two routes which involve halocyclisation and oxidative addition were investigated by Rao’s team, as illustrated in Fig. (10) [99,100]. Since the halocyclisation method resulted in mixtures, better yields of the target molecules were obtained by the second method.

5.1.2. Wessely Oxidation/Diels-Alder Approach

This approach was initiated by Yates and involved a tandem Wessely oxidation/intramolecular Diels-Alder cycladdition protocol that led to a tricyclic lactone (Fig. 11) [101]. As reviewed by Theodorakis [94], this protocol gave rise to isomeric structures, and its application to the synthesis of the caged xanthones should await some fine-tuning, especially as related to the electronic effects of the substituents of the aromatic ring. Very recent efforts based on this synthetic strategy succeeded in accessing the caged core within two steps [102].

5.1.3. Biomimetic Claisen Rearrangement/Diels-Alder approach

The proposed Claisen rearrangement/intramolecular Diels-Alder cascade was successfully developed into a useful strategy for the construction of the intriguing 4-oxatricyclo[4.3.1.0\textsubscript{3,7}]decan-2-one ring system [4,94,95,97]. Quantum mechanical calculations demonstrated that the Claisen rearrangement is reversible and relatively unselective, while the Diels-Alder reaction determines the product ratio and favors the formation of the five-membered ring [96]. It was proposed that differences in resonance stabilization of the oxygen and the carbonyl groups of the products could lead to selectivity [103].

5.2. Total Synthesis of Caged Garcinia Xanthones

5.2.1. Total Synthesis of 1-O-Methylforbesione

In addition to supporting the proposed biogenetic origin of these caged structures, the biomimetic tandem Claisen/Diels-Alder cascades succeeded in the first total synthesis of 1-O-methylforbesione (106) [4], which opened a facile entry into the complex molecular architecture. At the first step, the precursor \(107\) was prepared starting from the lithium derivative of \(108\) and aldehyde \(109\), as illustrated in Fig. (12). Upon heating prenylated xanthone \(107\) in DMF at 120 °C for 20 min, the expected compound \(106\) was indeed obtained as the major product (63% yield), presumably by the anticipated double Claisen rearrangement followed by an intramolecular Diels-Alder reaction. Its isomers \(116\) (2% yield), \(117\) (<1% yield), and \(118\) (26% yield), were also produced as outlined in Fig. (12). The success of the Claisen rearrangement/intramolecular Diels-Alder cascade reaction in the construction of caged xanthone further demonstrated the value of “biomimetically” inspired synthetic strategies toward natural products. Total synthesis of more caged \textit{Garcinia} xanthones, such as gambogin (33) and 1-O-
methyllateriflorone, was subsequently completed in the same manner [6,7,104,105].

5.2.2. Unified Synthesis of Caged Xanthones

A unified strategy was designed as a general synthetic approach to the caged *Garcinia* xanthones, central to which is a tandem Claisen/Diels-Alder/Claisen rearrangement of a suitably substituted xanthone precursor. Forbesione (31) is constructed first and then served as a template to produce its analogues, including desoxygaudichaudione A, desoxymorellin, and gambogin [5,103,106]. A study on the timing of this reaction cascade using high-temperature $^1$H NMR showed that the C-ring Claisen/Diels-Alder reaction was activated before the A-ring Claisen rearrangement [5].

5.2.3. Regio-Selectivity Study for the Synthesis of Caged Xanthones

Every functional group in the structure of xanthone precursor could affect the site selectivity of Claisen rearrangements [5]. The carbonyl group, the xanthone oxygen, and
Caged xanthones isolated from *Garcinia* were only reported very recently [68]. Twelve medicine for a long time, the antiviral activity of single resins have been applied in the treatment of herpes as folk assay; seven of them including 8,8a-epoxymorellic acid (HSV), HSV-1 and HSV-2 desoxymorellin (30), hanburin (33), gambogic acid (11), hanburin (30), forbesione (31), and dihydroisomorellin (10), exhibited anti-HIV-1 activity [68].

### 6.1. Anti-Viral Activity

The resin of *G. morella*, at the concentration of 10^{-10} g/L, exhibited direct inhibitory effect on herpes simplex virus (HSV) in *vitro* [107]. Although *Garcinia* resins have been applied in the treatment of herpes as folk medicine for a long time, the antiviral activity of single caged xanthone was only reported very recently [68]. Twelve caged xanthones isolated from *G. hanburyi* were examined for their anti-HIV-1 activities in the reverse transcriptase assay; seven of them including 8,8a-epoxymorellic acid (38), desoxymorellin (3), morellic acid (4), gambogic acid (11), hanburin (30), forbesione (31), and dihydroisomorellin (10), exhibited anti-HIV-1 activity [68].

### 6.2. Anti-Bacterial Activity

Gamboge, the total gambogic acids from *G. hanburyi*, showed strong inhibitory effect against *Propionibacterium acnes* and *Staphylococcus aureus* with MIC being 0.005 and 0.05 mg/ml, respectively [108].

All scortechinones isolated from *G. scortechinii* were tested for their anti-bacterial activity [72]. Among them, scortechinone B, the major component in all studied parts of this plant, displayed potent anti-bacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA) strain at a MIC value of 2 μg/mL. Some structure-activity relationships on its anti-bacterial activity were established. Both the C-2 prenyl substituent and the terminal carboxyl group of the C-5 substituent significantly affect the anti-bacterial activity, while the configuration of C-15 and the geometry of the C21/C22 double bond slightly affect the activity [72]. Partial cleavage of the caged unit could diminish the anti-bacterial effect [72].

### 6.3. Neurotrophic Activity

Gambogic amide is a potent agonist for the transmembrane tyrosine kinase TrKA receptor and triggers the signaling cascade activation, which plays a critical role in neuronal plasticity, survival and neurite outgrowth [109]. It has been demonstrated to specifically bind to the cytoplasmic juxtamembrane domain of the TrKA receptor, trigger receptor dimerization and activate Akt and MAPKs. In addition, it strongly prevents glutamate-induced neuronal cell death and provokes prominent neurite outgrowth *in vitro*. The administration of gambogic amide could thus substantially diminish kainic acid-triggered neuronal cell death and decreases infarct volume in animal model. Therefore, gambogic amide might not only establish a powerful platform for dissection of the TrKA receptor but also provide effective treatments for neurodegenerative diseases and stroke [109].

### 6.4. Anti-Cancer Activity

Most caged xanthones showed potent cytotoxic effects against a variety of cancer cell lines. Gaudichaudiones 61-67 and gaudichaudic acids 71-73 and 75 isolated from *G. gaudichaudii* were broadly cytotoxic against a panel of cancer cell lines with ED_{50} values mostly in the range of 0.5-8.0 μg/mL. The aldehydes show in general stronger cytotoxicity than the acids [77]. In addition, gaudichaudione H (68) exhibited cytotoxic effects on breast cancer MCF-7 cells, human prostate cancer DU-145 cells, and lung cancer NCI-H460 cells with ED_{50} values ranging from 6.6 μM to 7.6 μM [82]. Gaudichaudione A (61) activated caspase-3 and induced the apoptosis of Jurkat human leukemic cells. It has similar cytotoxicity against parental murine leukemic P388 and P388/DOX-resistant cells, but is less toxic towards normal human Chang liver cells [110]. Significant cytotoxic effects on human ovarian cancer CaOV-3 cells, human cervical cancer HeLa cells, human breast cancer MDA-MB-231 cells, and human breast cancer MCF-7 cells were demonstrated by cantleyanones (90-93), 7-hydroxyforbesione (89), and deoxygaudichaudione A (27) from *G. cantleyana*, with ED_{50} values in the range of 0.2-17.2 μg/mL [81]. Bractatin and its derivatives showed strong cytotoxicity against oral cavity cancer KB cells, with ED_{50} values of 0.2-1.5 μg/mL. In this assay, two enantiomers exhibited the same activity, suggesting that the cytotoxic effect was not specific [11].

Among these cytotoxic xanthones, the most studied compound is gambogic acid, which has shown promising anticancer effects *in vitro* and *in vivo* against a variety of cancer cell lines, such as human breast cancer T47D cells [16], human hepatoma SMMC-7721 cells [14,60], human leukemia HL-60 and K562 cells [13], human gastric carcinoma BGC-823, MGC-803 and SGC-7901 cells [12,111-114], human lung carcinoma SPC-A1 cells [19], glioblastoma cells [21], pancreatic cancer cell line PC-3 [115], etc.

Chemoresistance is a major obstacle to the success of cancer chemotherapy. Recently, it has been reported that gambogic acid can overcome the resistance to docetaxel in docetaxel-resistant gastric cancer BGC-823/Doc cells [28]. Treating these BGC-823/Doc cells with gambogic acid at its non-toxic concentrations (0.05-0.2 μM) significantly increased the cytotoxicity of docetaxel. Cell cycle analysis indicated that gambogic acid treatment potentiated docetaxel-induced G2/M arrest. Analysis of anti-apoptotic gene revealed that gambogic acid alone or in combination with docetaxel significantly downregulated the mRNA expression of survivin. These observations indicated that gambogic acid is a potent inhibitor of survivin [28]. This might be related to its effect as a non-substrate of multi-drug resistance (MDR) transporter P-glycoprotein [59,64]. MDR in cancer cells is a significant factor for the failure of chemotherapy in many
patients, since MDR transporters contribute significantly to the pharmacokinetic disposition of anticancer drugs [116].

GA was reported to selectively induce apoptosis of human hepatoma SMMC-7721 cells, but has a relatively weak effect on human normal embryonic hepatic L02 cells [18]. The study on the drug distribution in cultured cells and tumor-bearing mice showed that SMMC-7721 cells have a higher level of GA binding activity than L02 cells [18]. GA has a longer retention time in grafted tumor than in liver, kidney, or other organs. Its significant apoptosis inducing effects as well as its higher distribution and longer retention time in tumor cells all contribute to its selective anticancer activity [18]. These results suggested that GA might be an effective anticancer drug candidate with low toxicity to normal cells.

The synergistic effect of gambogoric acid with 5-fluorouracil was also studied on BGC-823 human gastric carcinoma [117]. Although 5-fluorouracil is one of the most popularly used drug in the chemotherapy of gastrointestinal cancers, its single-agent treatment response rate is only 21% [118]. In vitro and in vivo studies showed that the anticancer activity of 5-fluorouracil combined with GA was much stronger than that of GA or 5-fluorouracil alone. It was proposed that GA enhances 5-fluorouracil-induced apoptosis by regulating gene expressions of 5-fluorouracil’s metabolic enzymes, such as thymidine synthetase, dihydropyrimidine dehydrogenase, and orotate phosphoribosyltransferase [117].

6.5. Mechanisms of Anti-Cancer Activity

Continuous efforts have been made to reveal the anti-cancer mechanism of gambogoric acid. The first mechanism study suggests that gambogoric acid mediates its apoptotic effect by binding to the human transferrin receptor (hTFR) [25]. It was subsequently reported that gambogoric acid potentiated TNF-induced apoptosis in human leukemia cancer cells by inhibiting the nuclear factor-kappaB (NF-κB) activity via the transferrin receptor [119].

Using different cancer cell lines, several reports collectively suggested that the potent anticancer activity of gambogoric acid might be attributed to its ability to repress telomerase activity through two different approaches. The first one is to reduce the mRNA expression of human telomerase reverse transcriptase (hTERT) by reducing the level of transcription activator, c-Myc [14,19,113]. The second pathway is to posttranslationally downregulate the activity of hTERT by inhibiting the phosphorylation of Akt [111].

GA can also arrest cells in the G2/M phase [112]. Using BGC-823 human gastric carcinoma cells as a model, it was found that GA induces the reduction of cyclin-dependent kinase (CDK)-activating kinase activity and thereby causes the inactivation of CDC2/p34 kinase and the irreversible G2/M phase cell-cycle arrest of BGC-823 cells [112]. The cell cycle arrest and apoptosis induced by GA in human breast carcinoma MCF-7 cells might be attributed to the depolymerization of the microtubule and the phosphorylation of c-Jun N-terminal kinase-1 (JNK-1) and p38 [120].

The antiangiogenic effect of GA has also been elucidated [15,114,121]. These investigations showed that gambogoric acid “significantly inhibits human umbilical vascular endothelial cell (HUVEC) proliferation, migration, invasion, tube formation, and microvessel growth” either by “suppressing activations of vascular endothelial growth factor receptor 2 and its downstream protein kinases, such as c-Src, focal adhesion kinase, and AKT” or by “suppressing vascular endothelial growth factor-induced tyrosine phosphorylation of KDR/Flik-1” [15,114,121].

In a recent study [31], proteomic approach was used to reveal the target proteins of gambogoric acid (GA) and gambogenic acid (GEA). Both xanthones inhibited hepatocellular carcinoma (HCC) cell growth in a dose-dependant manner. Twenty differentially expressed proteins were identified and the four most distinctly expressed proteins were further validated by Western blotting. The expression of cyclin-dependent kinase 4 inhibitor A (P16-INK4A) and guanine nucleotide-binding protein beta subunit 1 (Gβ) was upregulated by both xanthones, whilst the expression of 14-3-3 protein sigma (14-3-3 σ) and stathmin 1 (STMN1) was downregulated. The expression of STMN1 was found to be positively correlated with HCC cell proliferation and migration [122]. Further studies have shown that the overexpression of STMN1 reversed the inhibitory effect of GA and GEA on HCC cell growth, while small interfering RNAs targeting stathmin 1 enhanced the sensitivity of HCC cells to GA and GEA, suggesting that STMN1 is the major target and that its downregulation is partly the underlying therapeutic mechanism of GA and GEA in HCC.

Therefore, GA might be a pleiotropic anti-cancer agent that induces cytotoxicity through multiple mechanisms. Recent studies demonstrated the cytotoxic effect of GA through an iron-independent and hTFR-independent mechanism [123]. GA can also increase the generation of ROS (reactive oxygen species), which induce the oxidation of sulfydryl groups in NF-κB [123], inhibiting its DNA-binding activity [124-126]. It was suggested that GA not only inhibits NF-κB translocation to the nucleus but also block NF-κB activity by abolishing its DNA-binding activity. Therefore, oxidative stress is considered as part of the mechanism of action of this pleiotropic drug [122]. Another study found that GA exerts its antiproliferative effect by inhibiting the catalytic activity of topoisomerase IIα, which binds to its ATPase domain [29]. The effects of GA on the expression of Bcl-2, NF-κB, procaspase-3, p17 and p20 were confirmed in a study of GA induced apoptosis of Jurkat T cells, in which the death inducer-obliterator-1 (DIO-1) was revealed as a new molecular target [127].

7. DEVELOPMENT OF GAMBOGIC ACID AS POTENTIAL NEW ANTI-CANCER DRUG

7.1. Toxicity and Safety

Gambose, the total gambogoric acids, is traditionally listed as a “toxic” herbal medicine due to its potent effect to cause diarrhea and bellyache, and has been put under strict safety control in China [128]. However, information concerning the potential side effects and toxicity is surprisingly limited [129]. The acute and chronic toxicity of GA were recently studied using albino mice and Beagle dogs as model animals [22]. The LD_{50} (i.v.) was found to be 45-96 mg/kg [22]. Since GA is not a substrate of multi-drug resistance transporters [64,116], it is mainly distributed in the liver, lung,
and kidney [130,131], and so these are generally the locations where toxicity occurs in experimental animals [22]. Chronic toxicity tests, in which the rats were treated with high doses (120 mg/kg) of GA for a long period of time, confirmed the damage of kidney and liver by GA [132]. An innocuous dose determined in dogs was 4 mg/kg administered once a day for a total of 13 weeks. This dose was approximately 9.6 (body weight) or 5.1 (body surface area) times higher than that recommended for human trials (25 mg/60kg, every other day) [22].

The effect of GA on the hemopoietic and immune functions was also evaluated in experimental animals. Nakano et al. [133], demonstrated that GA (i.v., 2, 4 or 8 mg/kg) did not significantly affect the count of peripheral leucocyte in herpes infected mice. Neither the count of hemolysin in blood serum nor phagocytotic function of macrophage in mice was affected by this compound. When GA (6, 3, 1.5 mg/kg) was injected in rats, no dramatic effect was observed on the count of peripheral leucocyte and marrow karyote or on the coefficient of thymus gland and spleen in rat. It has been confirmed that there is an effective concentration range where GA can specifically induce apoptosis in malignant cells without inducing toxicity in normal cells or experimental animals [22,122,131,132].

7.2. Clinical Trials

Gamboge has been traditionally used in China for the treatment of skin carcinoma [134], and suppurrative dermopathy [135]. It was reported that gamboges is very effective at treating acne [108], palpirus dermatitis [136], HSV induced herpes zoster [137,138], and genital herpes [107].

Gamboge has been used internally as an anti-cancer chemotherapeutic agent. Since 1973, Chinese scientists of the Anticancer Gamboge Research Team have conducted clinical trials and a series studies on the chemotherapy, anticancer activities, and toxicity of gamboge. In 1982, the results were briefly summarized in a short report [139]. In the clinical trial on gamboge’s anticancer activity, three forms of preparation (injection, tablet, and ointment) were tested on 125 patients who were diagnosed and classified into six different groups, including carcinoma of breast, malignant lymphomas, etc. It was reported that gamboge showed therapeutic effects with the effective rate ranging from 33.3% to 84.2% [139].

More than 20 years later, the injectable form of GA, the major component of gamboges, was approved for clinical trial in China. Based on the information disclosed by SFDA (State Food and Drugs Administration, China), GA has entered the phase II clinical test. In the report about its human tolerability [23], the maximal tolerated dose (MTD) and dose-limiting toxicity (DLT) of intravenously injected GA in patients with cancer were evaluated. By a modified Fibonacci series, fifteen patients in five groups (3 each) were injected with a single-dose starting at 10 mg/m² and then at 20, 35, 55 and 70 mg/m². The dose escalation to the next level was continuous until DLT was reached. Sixteen additional patients were injected with successive doses (10, 25, 35, 45 and 60 mg/m²), respectively. Safety and efficacy were examined one month after treatment. As a result, the DLT incidence rate of 33% occurred in the patients injected at 70 mg/m² level, with MTD = 55 mg/m². The patients with increasing dose showed adverse effects including pain, nausea/vomiting, and an increase of transaminase. Finally, the regimen of GA in Phase II clinical trial was augmented to 45 mg/m², qd × 5 or qod × 5, with a repeated cycle of every 3–4 weeks [23].

In addition to the injection, the preparation, physical properties, and stability of gambogic acid-loaded micelles based on chitosan derivatives were also investigated [140].

7.3. Structure Modification and Structure Activity Relationship (SAR)

To find analogues with more potent therapeutic effect and lower toxicity, modifications to different functional groups of GA were made, which generated many derivatives [120-131] as illustrated in Fig. (13) [16,141,142]. SAR studies, as measured by the caspase activation screening combined with traditional growth inhibition assay, disclosed that the α,β-unsaturated ketone group plays an important role in the biological activity [25], while the 6-hydroxy and 30-carboxy groups can tolerate a variety of modifications [16]. The importance of the α,β-unsaturated ketone group was confirmed by a stability test of GA in which the addition of an alcohol group occurred at the double bond, producing gambogic acids (22 and 23) with significantly decreased cytotoxicity [143]. More examples can be found in the well-known ent-kaurane diterpenoids from Isodon herbs, such as oridonin, poncindin, and eriocalyxin B [144]. Most of them contain a α,β-unsaturated carbonyl group and have strong cytotoxicity. Compounds without this moiety are always inactive [144]. It is popularly accepted that the α,β-unsaturated carbonyl group contributes to the cytotoxic effects of small molecules. Compounds bearing the α,β-unsaturated carbonyl group induce apoptosis through similar approaches, such as generation of ROS and inhibition of NF-kB and telomerase activity [145-147].

Apart from the α,β-unsaturated carbonyl group, the isoprenyl substituents are also believed to be important for sustaining activity. Through simplifying the complicated skeleton of GA, two derivatives of chromone and xanthone were synthesized and examined for their cytotoxicity against several cancer cell lines by MTT method. The results indicated that the appropriate introduction of prenyl group to the molecule could increase the bioactivity and that the polypropenyl-constructed bridgecore in GA is necessary for its antitumor activity [148]. Similar conclusion was also made in subsequent investigations on prenylated Garcinia xanthones [149]. A new effort to determine the minimum active structure of GA for its apoptosis inducing activity yielded six simplified molecules as shown in Fig. (14) [105]. The caged compounds (135-138), synthesized from xanthone 132 and 2-phenylchromene-4-one 133, possessed apoptosis inducing and cell growth inhibiting activities comparable to that of GA. However, 139 and 140, based on benzophenone 134, were inactive, suggesting the contribution of the caged bridgecore.

In another SAR study, GA was modified to produce five derivatives including 123, 124, ethyl gambogate, 33-chlorogambogelic acid and 33,37-dichloro-gambogelic acid and their cytotoxic effects against SMMC-7721 cells were
compared [150,151]. The results showed that 33-chlorogambogellic acid and 33,37-dichloro-gambogellic acid are 100 times more potent than other artifacts and even over twice more potent than GA, suggesting that forming more bridgecores and adding chlorine atoms could greatly promote the cytotoxicity of caged xanthones.

The SAR study with T17 cells and hippocampal neurons indicated that the carboxyl group could tolerate different modifications [109]. Therefore, this side chain might not be critical for binding biological targets. Reduction of the unsaturated prenyl side chains or C-9/C-10,12-unsaturated ketone completely abolishes the protective effect. Therefore, the spatial structure of prenyl side chains and unsaturated double bonds in the caged polycyclic skeleton are essential for the neurotrophic effects [109].

A recent SAR study of GA derivatives showed that modification of the double bond and methyl groups of prenyl substituents could enhance the antitumor activity [152]. As illustrated in Fig. (15), 11 oxidized derivatives were synthesized using GA as the parent compound. Their inhibition effects were compared against several cancer cell lines. Compounds 141-143 have one or two epoxy groups on the prenyl substituents and were the most effective in all cell lines, including A549, Bel7402, HT-09, BGC-823, SKOV3 cells. Other modifications provided better selectivity towards certain cell lines. 148 and 149, a pair of isomers with two hydroxyl substituents in opposite stereochemistry, exhibited clear differences in the inhibition of all cell lines, suggesting that stereochemistry is always important for bioactivity [152].
7.4. Neo-gambogic Acid and Gambogenic Acid

Neo-gambogic acid (19) was believed to be the second major bioactive ingredient of gamboge. Compared to gambogic acid, neo-gambogic acid showed more potent inhibitory effect against L1210 in mice. It also exhibited significant anticancer activity against Ehrlich ascites carcinoma, P388 leukemia, ARS ascites carcinoma, Lewis lung carcinoma, La795 carcinoma and other solid tumors in mice [153,154]. As newly reported, it inhibits the proliferation of more cancer cell lines including human colon carcinoma HCT-8 cell, human hepatoma Bel-7402 cell, human gastric carcinoma BGC-823 cell, human non-small cell lung cancer A549 cell and human ovarian cancer A2780 cell. The ED₅₀
ranges from 1.75 to 3 μM. Inhibition on the tumor growth of nude mice transplanted with A549 cell was also observed when were intravenously treated with neo-gambogic acid at the doses of 8, 16, 32 mg/kg [155].

However, its chemical structure is still questionable. First of all, there is only one publication on its isolation and structure elucidation and all subsequent phytochemical investigations have not identified the structure [67]. Second, quantitative and qualitative analysis on the chemical profile of gamboge using different HPLC methods also failed to prove its existence [55-57]. Third, its chemical structure was elucidated based on limited spectral data. The reported NMR data is consistent with that of gambogenic acid (17), the second major component of gamboge. The study of its structure characterization is so limited that it was even reported as a new compound in a recent study [156]. Based on the above facts and analysis, it is believed that the chemical structure of the so called “neo-gambogic acid” may be wrongly elucidated and should be that of gambogenic acid (17). A further comparison of HPLC and NMR data between neogambogic acid and gambogenic acid is therefore strongly recommended. If confirmed, all the reports regarding the bioactivities of neo-gambogic acid would actually be that of gambogenic acid and would make gambogenic acid an alternative to the hard-to-separate gambogic acid mixture. This idea was supported by a recent report of the bioassay guided identification of apoptosis inducers from gamboge in which gambogenic acid was discovered as a lead compound whose activity is comparable to that of GA [157].

8. CONCLUDING REMARKS

A unique caged skeleton, 4-oxa-tricyclo[4.3.1.03,7]dec-2-one, is naturally biosynthesized and occurs in only around 100 xanthones of nine Garcinia plants. Not only the synthesis of the bridged tricyclic core, but also the total synthesis of caged xanthones was performed through several different intermolecular and intramolecular approaches. More importantly, these caged xanthones exhibited a variety of potent bioactivities, especially anticancer activity. As such, gambogic acid, the most studied compound, has been developed into an anticancer drug. However, the mechanism of action is so complicated that it seems to involve every known mechanism. As suggested by SAR studies, the caged core is responsible for the bioactivities. With its unusual caged skeleton, remarkable bioactivities, and the undetermined mechanism of action, this special group of natural products attract increasing attention among scientists from various fields.

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