14.02 Eight-Membered Rings With One Oxygen Atom

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14.02.1 Introduction

This chapter appears as a complement of the material and information previously published in Comprehensive Heterocyclic Chemistry II (CHEC-II) and CHEC-III, providing a privileged point of entry to the eight-membered ring oxygen heterocycle systems chemistry.

Throughout the last decade, the numerous reviews on the synthesis of naturally occurring derivatives have highlighted the importance of this heterocycle moiety. Reviews describing the synthesis of brevetoxins (PbTxs) and ciguatoxins (CTXs), on the most recent developments on the synthesis of maitotoxin (MTX) and CTX, on the synthesis of brevetoxin A (PbTx-1) on the 1990s and on the isolation and synthesis of heliannuols, namely A, G, H, K, and L have appeared. Nicolaou reviewed their work on the total synthesis of several natural compounds, including the synthesis of PbTx-1, which subunits B and G were obtained through lactonization and dithioketal cyclization reactions, respectively, and of brevetoxin B (PbTx-2), which subunit H was synthesized via hydroxyl dithioketal cyclization reaction. The synthesis of subunit H of PbTx-2 proposed by Nicolaou was also reviewed by T. Nakata.

The importance of specific reagents, namely SmI₂-promoted cyclizations applied to the synthesis of some cyclic ethers of PbTx-2 and oxiranyl anions as building blocks in the construction of CDEFGHJ-ring system of yessotoxin (YTX) and adriatoxin have been disclosed.

H. Oguri studied the absolute configuration of CTX using an approach which combined partial structure synthesis, microscale chemical transformations, and the CD exciton chirality method. In addition, the partial structures of CTXs were synthesized as haptens for the preparation of anti-ciguatoxin antibodies.

Reviews dedicated to toxic naturally occurring seafood contaminants have appeared, including the biosynthesis of PbTxs and YTX, biosynthesis of PbTxs and CTXs, monitoring and toxicity assessment of PbTxs in molluscan shellfish, inflammatory and immune effects of PbTxs and CTXs.

A critical overview on the chemistry, toxicity, distribution and fate of CTX derivatives has appeared. Friedman and a large group of collaborators of different scientific areas published in 2017 an update review on the effects of exposure to CTXs on human health, diagnosis, human pathophysiology of Ciguatera Fish Poisoning (CFP), treatment, detection of CTXs in fish, epidemiology of the illness, global dimensions, prevention, future directions, and recommendations not only for clinicians but also for patients. In the same year, reviews on the mechanism of action of CTXs and PbTxs, both activators of the same target, voltage-gated Na⁺ channels.

Comprehensive Heterocyclic Chemistry IV (2022)
and on the quantification of five CTXs [ciguatoxin-1B (CTX-1B), 52-epi-54-deoxyciguatoxin-1B (52-epi-54-deoxyCTX-1B), ciguatoxin-3C (CTX-3C), 51-hydroxyciguatoxin-3C (51-hydroxyCTX-3C) and ciguatoxin-4A (CTX-4A)] (see structures in Section 14.02.3.1) in the Pacific area using Quantitative NMR Spectroscopy have appeared.

Details on the origin, structure and clinical symptoms of YTX and its analogs can be seen in a brief review. In addition to these topics, the metabolism, biosynthetic origin, toxicological properties, potential risks to human health and advances in detection methods of YTXs have been overviewed.

Until now, specific names have been given to the natural compounds bearing an O-8-membered heterocycle without referring the ring itself. As recommended by IUPAC, a proper nomenclature is associated to this heterocycles. Thus, eight-membered rings with one oxygen atom are named as oxocanes (fully saturated), oxocenes (one double bond) or oxocines (more than one double bond in the cyclic system). When a benzene is fused to the eight-membered ring, benzoxocines are formed.

14.02.2 Theoretical models

Berry et al. reported an unusual long-range Perlin effect in the conformationally constrained oxocane I. They assigned this high Perlin effect to the proposed boat like conformation of the structure, which was proved by NMR (NOE effects and J analysis) and that agreed with the solved crystal structure and with the DFT-calculated, energy minimized, and gas-phase structure. They also reported the largest (1)\textsuperscript{\textit{C}-H} Perlin effect. Salome and Tormena revisited this long-range Perlin effect by theoretical calculations and experimental results, based on their high resolution NMR data, and proved that the difference between (1)\textsuperscript{\textit{C}-Hax} and (1)\textsuperscript{\textit{C}-Heq} (used to calculate Perlin effect) actually comes from the increase in the (1)\textsuperscript{\textit{C}-Hax} and not from the decrease in the (1)\textsuperscript{\textit{C}-Heq}. Thus, the reported largest Perlin effect are not real.
14.02.3 Experimental structural methods

14.02.3.1 Natural products

Many marine natural products, including marine toxins, possess complex and unusual chemical structures containing at least one 8-O-membered ring. Brevetoxins (PbTxs) are one of the most popular examples of toxins, produced by marine dinoflagellates belonging to the genus *Karenia*. These neurotoxins are associated with the harmful algal blooms and responsible for the deaths of several marine organisms or for their accumulation in the marine food chain with consequent intoxication in human consumers, known as neurotoxic shellfish poisoning (NSP). In the previous chapter of CHC-III dedicated to 8-O-membered rings, the identification and characterization of numerous brevetoxins has been assigned, due to the intense and challenging marine discovery programs back in the 1980s but also to the development of the analytical techniques for their structural assignment.

In 2007, Twiner et al. extracted from *K. brevis* of Texas Gulf coast using a C18 solid-phase extraction disc (SPEC) methodology, a series of brevetoxins and made their quantification by four independent methods: receptor binding assay (RBA), radioimmunoassay (RIA), neuroblastoma (N2A) cytotoxicity assay, and liquid chromatography/mass spectrometry (LC/MS). In addition, LC/MS analysis also provided the identification of several individual congeners (*PbTx*-1 (also known as brevetoxin A) 2, *PbTx*-2 (also known as brevetoxin B) 3, *PbTx*-3 4, *PbTx*-7 5, and *PbTx*-9 6) and of their hydrolyzed products (resulting from the lactone A ring opening).

Studies on the conformational analysis of a model structure for the trans-fused FGH ether rings in *PbTx*-1 2, involving several classical force field-solvent combinations provided ensembles containing 30–60 structures with the central G-ring, the ring that plays an important role in the ion channel active binding site, adopting the crown, twist-crown, boat-chair, and boat conformations. Moreover, quantum mechanical calculations showed that twist-crown and boat-chair conformers were typically lowest in energy, in agreement with other reports describing the need for the 8-membered G-ring of *PbTx*-1 2 to adopt the boat-chair conformation in order to bind to the ion channel active site. Other calculations including solvation indicated that solvent does not play a major role in the conformational preferences.

*PbTx*-2 3 was isolated for the first time from New Zealand and Delaware strains of *K. papilionacea*, being produced 25 times more in the former strain than in the latter. The toxin production also increased in response to hypoosmotic stress, as previously reported in *K. brevis*. The structural assignment of *PbTx*-2 3 was carried out by mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy. Indeed, a strong overlap was observed in 1H NMR spectra of the New Zealand extract with the *PbTx*-2 3 standard, namely in the signals corresponding to the aldehyde proton, the exomethylene protons and to the protons on the unsaturated bonds in the A and H-rings.

Two novel marine ladder-frame polyethers named brevisulcatic acid-1 (BSX-1 7, *C*49*H*72*O*16, [M + H] + calcd 917.4899) and brevisulcatic acid-4 (BSX-4 8, *C*49*H*70*O*15, [M + H] + calcd 899.4793) were isolated from red tide dinoflagellate *K. brevisulcata* in New Zealand. These molecules are similar to *PbTx*-1 2 (*C*49*H*70*O*13) and their structural elucidation was obtained after detailed analysis of low-temperature NMR spectra and high-energy MALDI TOF MS/MS spectra. The β-hydroxy-γ-methylene valeric acid side chain and the ether ring skeleton of both brevisulcatic acids are common to both acids, but BSX-4 8, has a γ-lactone as the 5-membered A-ring while BSX-1 7 is the seco acid analog. BSX-4 8 has structural and bioactivity similarities to *PbTx*-1 2.
Ciguatoxins (CTXs) are synthesized by microalgae of the dinoflagellate genus Gambierdiscus and composed by a ladder shaped structure made by 13 or 14 ether rings. Soliño and Costa suggested a classification of this class of compounds according to the differences in the number and substitution of the rings as well as their regional occurrence: Pacific Ocean, Caribbean Sea and Indian Ocean.\(^\text{18}\)

Pacific CTXs possess 13 rings with main differences in E ring, lack of the side-chain substituent and can be separated into CTX-I (CTX1B type) and CTX-II (CTX3C type). Caribbean and Indian CTXs have a structure similar to CTX-II with an extra fused ring. Humans of isolated island communities which depends mainly on fishing for their food supply can eat fish containing these naturally occurring CTXs and develop Ciguatera Fish Poisoning (CFP) illness. It is characterized by gastrointestinal, neurologic, and cardiovascular symptoms.\(^\text{18}\)

Using high-performance liquid chromatography tandem mass spectrometry (LC-MS/MS), the identification and quantification of 13 CTXs (CTX-1B 9, 52-epi-54-deoxyCTX-1B 10, 54-deoxyCTX-1B 11, CTX-4A 12, CTX-4B 13, M-seco-CTX-4A/B 14, CTX-3C 15, 40-epi-CTX-3C 16, 51-hydroxyCTX-3C 17, 2-hydroxyCTX-3C 18, 2,3-dihydroxyCTX-3C 19, M-seco-CTX-3C 20 and M-seco-CTX-3C methyl acetal 21) in fish flesh collected at different locations of Japan were accomplished. Regional differences were also possible to be made: ciguatoxin-1B-type were found in snappers and groupers from Okinawa, ciguatoxin-3C-type were found in a spotted knifejaw, Oplegnathus punctatus, from Miyazaki and both types of toxins were found in a red snapper, Lutjanus bohar, from Minamitorishima (Marcus) Island. In addition, 10 CTXs (compounds 10–17, 20, 21) were identified in the dinoflagellate Gambierdiscus toxicus collected in French Polynesia. This technique revealed high sensitivity, specificity, and rapidity in monitoring CTXs in these type of specimens.\(^\text{31}\)

Using quantitative LC-MS/MS, it was possible to estimate the production of CTX (18.2 pg per cell) in G. polynesiensis CAWD212, isolated from samples of seawater of Rarotonga, Cook Islands.\(^\text{32}\)

Maitotoxin (MTX-1, 22) is the largest non-polymeric natural compound known so far. It is a polycyclic ether isolated from the marine dinoflagellate G. toxicus, and similarly to CTXs, that are responsible for CFP illness. A critical review on the biosynthesis of

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marine polyether ladders, points out some mechanistic hypotheses for the contiguous rings formation that questioned the well-established structure of maitotoxin. Meanwhile, based on NMR studies, the conformation of maitotoxin and yessotoxin (described later), mode of action and their interactions in biomembranes, have been briefly reviewed by Murata et al.

Using quantitative LC-MS/MS, it was possible to estimate the production of MTX (8.3 pg per cell) in G. australis CAWD149 isolated from samples of seawater of Rarotonga, Cook Islands. Moreover, the production of MTX-3 23, a MTX analog, was also monitored in three species of Gambierdiscus: G. pacificus CAWD213, G. australis CAWD149 and 216. LC-MS analysis of G. cf.
yasumotoi CAWD210 isolated from the Bay of Islands, Northland, New Zealand revealed the production of MTX-3 but not of MTX-1 or CTXs by this species. Years later, the isolation of two Gambierdiscus species, G. australis and a previously unknown Gambierdiscus species named CAWD242, collected from North Meyer Island, Kermadec Islands, was accomplished. The production of MTX-1 in 10 of the isolates from G. australis ranged from detectable to 36.6 pg per cell while the presence of MTX-3 was detected in all isolates of G. australis. In addition, for the Gambierdiscus species CAWD242 was detected the production of MTX-3 but not of MTX-1. The structural identification of MTX-3 (initially reported as 44-methylgambierone) was assigned, by comparison of its NMR and MS data with those of gambierone, for the first time in 2019 by Boente-Juncal et al. from G. belizeanus collected in the Caribbean Sea and by Murray et al. from G. australis collected at Raoul Island (Rangitahua/Kermadec Islands).

A new MTX analog named as MTX-4, isolated from two Canary Islands G. excentricus strains VGO791 and VGO792, was reported by Pisapia et al. in 2017, which HRMS/MS spectra analysis allowed their identification (accurate mono-isotopic mass of 3292.4860 Da, as free acid form). In addition, the authors screened the identification and quantification of four MTX analogs (MTX-1, putative MTX-2, MTX-3 and MTX-4) in the extracts of two strains of Fukuyoa ruetzleri and 42 Gambierdiscus strains using LC-IRMS (low resolution mass spectrometry)/MS analysis. To note that MTX-4 was detected and quantified (13.0–72.8 pg MTX equiv. cell⁻¹) in all seven strains of G. excentricus analyzed, independently of their origin (Brazil, Canary Islands, and Caribbean) but not detected in any other species under study. Unfortunately, the structural elucidation of MTX-4 was not provided by the authors.

Very recently, MTX and two MTX-like compounds from Caribbean strains of Gambierdiscus spp were isolated by liquid chromatography: one from G. belezianus, isolate CCMP 399 from St. Barthelemy Island, Greater Antilles, Caribbean Sea; and the second from both Gambierdiscus ribotype-2, isolate CCMP 1655 from Martinique, Lesser Antilles, Caribbean Sea, and Gambierdiscus sp., isolate SIU 350 from Virgin Gorda, British Virgin Islands, Caribbean Sea. Besides MTX, HRMS analysis suggested that the first unknown compound is a desulfo-MTX congener and the second one is a didehydro-demethyl-desulfo-MTX congener. Furthermore, the authors used the Advanced Chemistry Development program, “ACD/C+H NMR Predictors and Data Base,” to predict the ¹³C chemical shifts of some carbons.

In 2013, Watanabe et al. identified a novel epoxy polyether compound named gambieroxide, isolated from G. toxicus (GTP2 strain) collected at Papeete, Tahiti, French Polynesia. The assignment of this structure was based on the analysis of NMR and MS spectroscopic data and comprises 12 contiguous trans-fused ether rings, a sulfate ester group, and an acyclic unsaturated side chain.

Yessotoxin (YTX, 26) is a disulfated polyether compound with a characteristic ladder-shape formed by 11 adjacent ether rings of different sizes, with two sulfate ethers in one terminus of the chain and an acyclic unsaturated side chain constituted by nine carbons in the opposite side. This compound was firstly isolated in 1987 from the scallop Pecten yessoensis and since then, several metabolites have been found in shellfish from throughout the world. Various YTX analogs have also been isolated from three dinoflagellate algae Protoceratium reticulatum, Lingulodinium polyedrum, and Gonyaulax spinihyla.

In 2006 a new YTX analog, 45,46,47-trinorhomoyessotoxin 27, was isolated from cultures of P. reticulatum collected at Yamada Bay, Iwate in Japan. YTX and a novel yessotoxin analog (furanoyessotoxin 28) containing a furan ring in the side chain have been isolated (by filtration followed by solid-phase extraction) and purified from a large-scale culture (226 L) of P. reticulatum strain CAWD129 from the western coast of South Island, New Zealand. All these structures have been established by 1D and 2D NMR experiments and MS studies.
From the GG1AM strain of P. reticulatum collected from phytoplankton cultures at the Centro Oceanográfico de Vigo, YTX, glycoyessotoxin (G-YTXA) and a novel polyether named adriatoxin-B were isolated. The latest compound is a C$_{13}$ terminal truncated YTX analogous metabolite and was characterized using a combination of NMR spectroscopy experiments and conformational analysis. Very recently, Rajotte et al. identified YTX and the analog (24R)-24-hydroxyyessotoxin from a Namibian isolate of Gonyaulax spinifera. The configuration at C-24 of 31 was based on the correlations observed in the ROESY NMR experiment. Thus, the signal of H-24 correlated with the signals of H-22, H-25a and H-26a. In addition, on the a-face, the signal of H-28a correlated with the signals of H-22a and H-26a; on the b-face, the signal of H-25b correlated with the signals of 23-Meb and H-27b.

The analysis of ethyl acetate extract obtained from the marine organism Laurencia obtusa, collected in Corsica, France, allowed the identification of the known microcladallene A. Chromatographic fractionations of the extract were made and spectroscopic analysis (NMR and MS experiments) supported the assignment of this compound. The assignment of structure of ansellone B was a challenging task. Wang et al. isolated from the marine sponge Phorbas sp. collected in Gageodo Island, southwestern Korea this sesterterpenoid and proposed a molecular formula established by the high-resolution FABMS of C$_{29}$H$_{40}$O$_6$ (calcd for [M + Na]$^+$ C$_{29}$H$_{40}$O$_6$Na, 507.2723). However, Daoust et al., collected specimens of Phorbas sp. at Ansell Point in Howe Sound, British Columbia and identified ansellone B as a tetracyclic ether bearing an eight-membered ring. The high-resolution ESIMS was consistent with a molecular formula of C$_{27}$H$_{40}$O$_5$ (calcd for [M + Na]$^+$ C$_{27}$H$_{40}$O$_5$Na, 467.2773) and the structure fully characterized by extensive NMR studies.

Ansellone C was identified as a novel sesterpenene isolated from the marine sponge Clathria gombawuiensis collected from Korean waters. NMR studies confirmed a tetracyclic compound containing an eight-membered oxocane moiety and the relative configuration was assigned by NOESY experiments as 5S, 6R, 8R, 9R, 10S, 14R and 19R. Despite the presence of a secondary 6-hydroxyl group, the attempts to determine its absolute configuration by Mosher’s method were unsuccessful.

The novel amaroxocane A, a dimeric sulfated sterol bridged by an oxocane moiety, was isolated from Phorbas amaranthus collected on shallow coral reefs off Key Largo, Florida. Full assignment of all protons and carbons was accomplished after extensive analysis of NMR spectra and the molecular formula proposed by HRESI-TOF/MS was C$_{55}$H$_{85}$Na$_2$O$_{17}$S$_3$ ([M–Na]$^+$ calcd for 1159.4744). The relative configuration of the different heterocyclic rings was possible due to the interpretation of the NOESY spectra and of the coupling constant data. The absolute configuration of the different heterocyclic rings in amaroxocane A was...
based on the natural (R)-configuration of C-20, assumed based on the biogenetic principles, and also on the NOESY data. However, the absence of scalar coupling between H-20 and H-22 (dihedral angle of 90 degrees) was compensated by the NOESY correlations observed between H-17 and H-23 eq and of H-16ax and H-22.

Five new C15 eight-membered cyclic ethers 36–40 along with the acetylenic chloro diol 41 were isolated from a 3:1 mixture of dichloromethane:methanol extract of the red alga *Laurencia glandulifera*, collected at Crete Island in South Greece. The assignment of all protons and carbon resonances were established by 1D and 2D NMR studies. The coupling constant \( J = 10.8 \text{ Hz} \) between H-3 and H-4 as well as the chemical shift of the acetylenic proton (3.11–3.13 ppm) indicated the (Z)-configuration of the C3=C4 double bond. The relative stereochemistry was assigned on the basis of NOESY experiments as: (3Z,7R,9S,10R,12S,13R)-10-acetoxy-6-chloro-9,12-dibromolauthisa-3-en-1-yne 36; (3Z,7R,9R,10R,13R)-9,10-diacetoxy-6-chlorolauthisa-3,11-dien-1-yne 37; (3Z,7R,9R,10R,13R)-10-acetoxy-9-hydroxy-6-chlorolauthisa-3,11-dien-1-yne 38; (3Z,7R,9R,10R,13R)-9-acetoxy-10-hydroxy-6-chlorolauthisa-3,11-dien-1-yne 39; (3Z,7R,9R,10R,13R)-10-acetoxy-6-chloro-9-bromolauthisa-3,11-dien-1-yne 40 and (3Z,7R,9R,10R,13R)-6-chlorolauthisa-3,11-dien-1-yne-9,10-diol 41.

A new cyclic ether assigned as methyl (3S,4R,5S,6Z)-3-[R]-hex-2-yl]-4-methyl-3,4,5,8-tetrahydro-2H-oxocine-5-carboxylate 42 was isolated from a 1:1 mixture of ethyl acetate:methanol extract of the red seaweed *Kappaphycus alvarezi*. Their structural elucidation and relative stereochemistry was based on the detailed analysis of NMR spectroscopy and MS data.

A non-marine natural compound bearing an eight-O-membered ring isolated in the past decade was heliannuol A 43, obtained from dried leaves of sunflower (*Helianthus annuus* L. var. Arianna) and extracted with pure supercritical CO2 or in combination with high-pressure techniques. This structure was elucidated based on a detailed interpretation of 1D and 2D NMR spectra.
The genus Physalis belongs to the Solanaceae family and includes about 120 species widely distributed around tropical and subtropical regions of the world. Some of these species are used as food as well as folk medicine for the treatment of several illnesses such as malaria, hepatitis, hyperglycemia, rheumatism, asthma, among others. There was a series of seco-steroid compounds named physalins that have been isolated from this plant family, since the firstly isolation of physalin A in 1969. In this article, we will only present the characterization of novel physalins reported after 2006, since the other derivatives have been characterized by NMR and MS techniques in the previous CHC chapters. Nevertheless, many of them have been isolated from different species and geographic regions for their biological evaluation, namely physalins B, D, G, H, I, J and U from *Physalis angulata* L. plant collected in Brazil, Republic of China or Republic of Congo.

For physalins B and F, crystallization of the dichloromethane extract from acetone allowed the structural determination of both compounds by X-ray crystallography. From the whole *Physalis angulata* L. plant collected in Tainan Hsien, Taiwan, Republic of China a new physalin, physalin W, was isolated and fully characterized by a combination of 1D and 2D NMR and MS studies. The relative configuration assignments were based on NOESY correlations and coupling constants data.

A novel physalin, 25-hydroxylisophysalin B, were isolated from whole plant of *Physalis angulata* collected in Hainan Province, China. The structure of such compound was based on the analysis of their spectroscopic data.

The known 6α-chloro-5β-hydroxy-5,6-dihydropysalin B and the new 5α-ethoxy-6β-hydroxy-5,6-dihydropysalin B were obtained from the whole *Physalis angulata* L. plant collected in Guangdong, Republic of China, and their structures assigned through MS, IR, NMR spectroscopy analyses and X-ray crystallography. The structural elucidation of novel physalin X was accomplished by spectral analysis of NMR and MS data and the configuration was based on NOESY experiments which showed correlations of 5-OH with H-9, 6-OH with H-19, 13-OH with H-9/21, and of H-22 with 28, giving α-orientations for 5-OH and 13-OH and β-orientations for 6-OH and H-22. The absolute configuration was deduced as 5R, 6R, 8R, 9S, 10R, 13S, 14R, 16R, 17R, 20S, 22R, 24S from the X-ray structure of physalin P, the skeletal structure of physalins, and their biosynthetic background.
Similarly, to Physalis angulata L. plant, a number of known physalins have been isolated mainly from the calyces of P. alkekengi var. Franchetii for pharmacological purposes. Examples are physalins A, B, D, E, F, G, J, L, O, and X, which structures were elucidated on the basis of NMR and MS studies and confirmed by comparison with a reference sample and spectroscopic data already published. More details on the ethnomedical, phytochemical and pharmacological properties of Physalis alkekengi L. var. Franchetii (Mast.) Makino can be seen in the review of Li et al. published in 2018.

A study reported the quantification of physalin D in methanolic extracts of fruit (mature and immature) and calyx of P. alkekengi collected from wild plants growing in the Mures district, Romania. The quantification was determined by RP-HPLC-UV method, being their content in a relation of immature calyx > mature calyx > immature fruits > mature fruits. Qiu et al. isolated from P. alkekengi var. Franchetii calyces hydroalcoholic extract two new physalins, physalin Y and Z, along with the already known 3α-hydroxy-2,3-dihydro-4,7-didehydrophysalin B. From the same type of extracts, Li et al. isolated three new physalins which structures were elucidated by UV, MS, 1D and 2D NMR spectroscopy as 5α,6β-dihydroxy-25,27-dihydro-7-deoxyphysalin A, 5α,6β-dihydroxyphysalin R, and 3α-hydroxy-2-hydrophysalin A. Two years later, from the fruits of P. alkekengi var. Franchetii (Mast.) Makino collected in Qiqihar City, China, was identified a novel physalin, 5α,6α-dihydroxyphysalin D. This compound is an epimer of physalin D, and the authors named it as physalin D_1. Their structural assignment was based on 1D and 2D NMR, IR and MS spectroscopic data. Thus, the main differences in 13C NMR spectra of physalin D and D_1 are the changes of the chemical shift of C-4, C-19 and C-11 due to the space field effect of the 6α (equatorial) hydroxy group in physalin D instead of the 6β (axial) hydroxy group in physalin D. The equatorial orientation of 6-OH in physalin D_1 was further confirmed by NOESY correlations of H-6 with 19-Me and of H-6 with H-28.
More recently, seven new physalins (7β-methoxylisophysalin B 68, 7β-methoxylphysalin C 69, physalin V 70, physalin VII 71, isophysalin I 72, 7β-ethoxylisophysalin C 73 and 3β-ethoxyl-2,3-dihydro-4,7-didehydrophysalin B 74) were also isolated from a Chinese *P. alkekengi* var. *Franchetti* calyces hydroalcoholic extract and identified by NMR and MS studies, along with 18 already known physalins, elucidated by comparison of the literature spectral data. A novel and efficient HPTLC (High Performance Thin-Layer Chromatography)–MS/(MS) methodology was developed for the first time for the analysis of physalins in crude plant extracts of different parts of *P. alkekengi* L. collected in Slovenia and harvested at different stages of maturity. This method proved to be more selective, sensitive and with higher resolution in the separation of physalin L standard and its impurity (2,3,25,27-tetrahydrophysalin A). More examples of physalins were collected from other plants of the genera *Physalis* and *Withania*. Thus, physalin F was isolated from *P. minima* L. whole plant collected in Arau-Perlis, Malaysia, while from that collected in Guangdong, Republic of China, five derivatives was isolated using a ultra-performance liquid chromatographic (UHPLC) method: isophysalin B 75, physalin G 49, physalin D 46, physalin I 51 and 5α,6β-dihydroxyphysalin R 65. Physalins A 44, B 45, D 46, F 48 and H 50 were also isolated from the extracts of the aerial parts of *P. dimericata* D. Don while from the leaves of *Witheringia solanacea* were isolated physalins B, D, and F. All structures were elucidated based on ultraviolet spectrophotometry, NMR and MS studies and confirmed by comparison with spectroscopic data already published.

14.02.4 Reactivity of substituents attached to ring carbon atoms

Several hydrogenation reactions of benzoxocines 76–78 over Pd/C have been reported to prepare benzoxocanes 79–81. One of them discloses the preparation of the natural compound helianane 82 (Scheme 1). Asymmetric total synthesis of trilobacin 87 was achieved through a multistep strategy starting from glycolate oxazolidinone 83 and undecanal. Initially, it involves a 13-step sequence for the formation of the 2,2′-cis-oxocene 85 that includes a ring-closing metathesis promoted by 1st generation Grubbs catalyst [bis(tricyclohexylphosphine)benzylidene ruthenium (IV) dichloride] (Grubbs I, 84). Then, additional 5-steps including an organoselenium-mediated oxonium ion formation/SiO₂-promoted fragmentation to form erythro-bis(2,2′)-tetrahydrofuran moiety 86 are required for the total synthesis of 87, in a 14% overall yield (Scheme 2).
In order to construct the PET probe with improved tumor-to-background ratios in neurotensin receptor (NTR) imaging, a diastereomeric mixture of trans-5-oxocene reacted with triethylene glycol di(p-toluenesulfonate), followed by treatment with $^{18}$F-TBAF to provide $^{18}$F-labeled trans-5-oxocene, which after mixing a few seconds with tetrazine-peptide conjugate, afforded the desired probe (Scheme 3). The computational guided synthesis of this probe was previously reported in seven steps from the commercially available glycidol and involves Sakurai allylation, olefin metathesis and flow enabled photoisomerization as key steps. Wallace et al. also demonstrated the potential use of trans-5-oxocene in studies where hydrophilicity and bioconjugation are required. Thus they showed the rapid and quantitative inverse electron-demand Diels-Alder (IED-DA) reaction of trans-5-oxocene...
with a water-soluble tetrazine derivative and with a green fluorescence protein encoded with an unnatural tetrazine containing amino acid.  

Stereoselective synthesis of (+)-cis-laúthisan 95 is accomplished in a three-step approach starting from eight-membered lactone 92 (the synthesis of 92 is described in Section 14.02.6.3). It involves saturation of the oxocene ring and conversion to thionolactone 93 by using Lawesson reagent, treatment with ethyl lithium followed by iodomethane to form intermediate 94 and finally reductive removal of sulfur in the presence of tributyltin hydride and a catalytic amount of 2,2′-azobis(2-methylpropionitrile) (AIBN) in refluxing toluene (Scheme 4).  

The synthesis of oxocene Holmes intermediate 102, which is involved in the formal synthesis of natural (+)-laurencin 103 can be achieved through a multistep transformation of bridged cyclic ether 96 (the synthesis of 96 is described in Section 14.02.6.2). It starts by the removal of the sulfur bridge via Raney nickel reduction in the presence of acetate buffer (pH = 4.6); decarboxylation of the oxocane formed 97 via microwave heating in aqueous DMF to afford ketone 98; reducing with L-selectride to form a 2:1 epimeric mixture of alcohols 99; formation of xanthate 100 by reaction with NaH, CS₂ and MeI; elimination reaction carried out by microwave heating in O-dichlorobenzene giving a 9:1 mixture of oxocene regioisomers (C₅=C₆ and C₆=C₇) 101; and finally acetate cleavage to provide Holmes intermediate 102 (Scheme 5).  

### 14.02.5 Ring syntheses classified by number of ring atoms in each component

#### 14.02.5.1 Hydroxydithioketal cyclization

The hydroxydithioketal cyclization approach was applied in the construction of the oxocene ring in some natural fused polycyclic ethers isolated from marine sources. Examples are the formation of the F-ring in the total synthesis of PbTx 1 2 (Scheme 6) and of the B'-ring in the convergent synthesis of the ent-ZA'B'C'D'-ring system of MTX-1 22 (Scheme 7).
14.02.5.2 Ring-closing metathesis (RCM)

A wide range of natural products bearing an oxocane or oxocene unit were synthesized through RCM approach to obtain their eight-membered heterocycle precursor namely in the synthesis of subunit B of PbTx-1, subunits B and F of PbTx-1, subunit H of PbTx-2, subunit B' of MTX-1, subunit G of YTX, subunit E of CTX-3C. oxocane unit in the total...
synthesis of natural (3E and 3Z)-bromofucincs 104, (3E and 3Z)-chlorofucincs 105,113 (+)-helianane 82,114-116 (+)-5-chloro-7,11-helianane 106,115 helianuol A 43,117-120 helianuol G 107,117 helianuol H 108,117 helianuol K 109,117-120 (+)-laurencin 103,121,122 (+)-laurencin 110,123,124 (+)-cis-lauthisan 95,125,126 (+)-trans-lauthisan 95,126 microcladallene B 111,127 (+)-pannosallene 112128 and also the lactone ring of the natural octalactins A 113 and B 114128.

Scheme 7

Eight-Membered Rings With One Oxygen Atom
In addition, RCM and other different strategies for the total synthesis of the eight-membered core of heliannuols A 43, K 109 and L 115 have been reviewed by Chen et al. 129 Another important aspect to refer is that Shishido group has assigned in 2005 benzoxocane structures for heliannuols G 107 and H 115 and in 2006 revised them to substituted dihydrobenzofurans 107a and 108a, respectively, with an external trans-olefin intact. 130 Using Grubbs I catalyst 84, 2-(but-3-en-1-yl)-1-(prop-2-en-1-yloxy)benzenes in dichloromethane at room temperature under argon atmosphere gave 5,6-dihydro-2H-benzo[6]oxocines 131; while under refluxing conditions and nitrogen atmosphere 7-(but-3-en-1-yloxy)-8-(prop-2-en-1-yloxy)-2-(prop-2-en-1-yl) pyran in argon atmosphere afforded pyran-fused oxocene. 132 Treating hex-5-en-1-yl prop-2-en-1-yl ether 116 with Grubbs I catalyst 84 in refluxing dichloromethane provides only dimer 117 as a single isomer. However, using 2nd generation Grubbs catalyst (Grubbs II, 118) in refluxing toluene, RCM occurs to give oxocene 119. Using these conditions, dimer 117 can be converted in oxocene 119. Additionally, treatment of ether 116 with Grubbs II catalyst 118 followed by treatment with sodium boron hydride, a tandem RCM-isomerization took place to give oxocene 120 (Scheme 8). 134 Biswas et al. also reported that Grubbs I catalyst 84 was not able to promote RCM of 2-(but-3-en-1-yl)-1-(prop-2-en-1-yloxy)benzene derivatives but Grubbs II catalyst 118 provided benzo [6]oxocines in good yields. These precursors were used in the synthesis of sesquiterpenes helianane 82, heliannuol A 43 and heliannuol K 109. 135

The natural oxocene terpene arenaran A 122 was obtained from the reaction of 1,1,3-trimethyl-2-(3-methylbut-3-en-1-yl)-3-[(3-methylbut-2-en-1-yl)oxy]cyclohexane 121 with Grubbs II catalyst 118 in refluxing dichloromethane under an argon atmosphere for 3 h. Subsequent epoxidation with MCPBA (m-chloroperoxybenzoic acid) leads to arenaran B 123 (Scheme 9). 136 Using a catalytic amount of Grubbs II catalyst 118 in refluxing dichloromethane, a couple of 2-allyl-3-(pent-4-en-1-yloxy)pyran derivatives furnished pyran-fused oxocenes. 137

The diastereoselectivity of RCM of penta-1,4-dien-3-yl ether derivative 124 depended on the ruthenium catalyst used. The reaction occurred for a prolonged reaction time (22 h) and high catalyst loading (30 mol%) for consumption of the starting material. Thus, precursor 124 in the presence of Grubbs I 84 gave rise to trans-125 as the major product (cis/trans 33:67). In contrast, treatment of 124 with Grubbs II 118 or 126 afforded cis-125 with good diastereoselectivity (118: cis/trans 79:21; 126: cis/trans 82:18). Moreover, no diastereoselectivity was found in the RCM of 124 with catalyst 127 (Scheme 10). 138

Under nitrogen atmosphere, RCM of 2,4-dimethylquinoline dienes 128 and 129 in toluene occurred efficiently to produce benzoxocenes 130 and 131 in the presence of Grubbs I 84 or II 118 catalysts, respectively, without deactivation of the basic quinoline nitrogen (Scheme 11). 139

Scheme 8

84 (5 mol%)\[CH_2Cl_2, reflux\] 3 days (27%)

84 (5 mol%)\[PhMe, reflux\] (61%)

1. 118 (5 mol%)\[PhMe, reflux\] (90%)

2. NaBH_4, reflux

Scheme 9

MCPBA = m-chloroperbenzoic acid
Two-directional double RCM was used to synthesize bis-oxocine-fused naphthalene \( 133 \) starting from 2,6-bis(prop-2-en-1-yloxy)-1,7-bis(but-3-en-1-yloxy)naphthalene \( 132 \) in the presence of Grubbs I catalyst \( 84 \) in dichloromethane at room temperature under nitrogen atmosphere (Scheme 12).\(^{140}\)

RCM was also used to prepare eight-membered lactones. Protected ester \( 134 \) undergo RCM in the presence of Grubbs I catalyst \( 84 \) at room temperature overnight, to give the unsaturated lactone \( 135 \) in quantitative yield. Meanwhile, RCM of unprotected derivative \( 136 \) was only possible with Grubbs II catalyst \( 118 \) leading to unsaturated lactone \( 137 \). However, the best conditions for the cyclization process of triene \( 138 \) was using Grubbs II \( 118 \) as catalyst and Ti(OiPr)\(_4\) as cocatalyst in refluxing dichloromethane producing unsaturated lactone \( 139 \) (Scheme 13).\(^{141}\)

14.02.5.3 Haloetherification of unsaturated alcohols

A single study reported iodine-promoted \( \textit{8-endo} \)-oxacyclizations of several dienyl alcohols \( 140 \) carried out in the presence of sodium bicarbonate in THF to provide iodinated oxocenes \( 141 \) in moderate yields (Scheme 14).\(^{142}\)

14.02.5.4 Titanocene-promoted cyclizations

There was a single report studying the radical cyclization of epoxides using titanocene (III) chloride, as the radical source, to construct eight-membered ring ethers. Thus, treating propargyl ether epoxides \( 142 \) with Cp\(_2\)TiCl (prepared in situ from commercially available Cp\(_2\)TiCl\(_3\) and activated zinc dust) in THF under argon gave mainly (5,6-dihydro-2\(H\)-benzo[b]oxocin-5-yl) methanols \( 143 \) along with a minor amount of the reduced product \( 144 \). Using the naphthyl analog \( 145 \), only the corresponding naphthyl ether \( 146 \) was obtained. Moreover, the reductive opening of allyl epoxide \( 147 \) afforded (3,4,5,6-tetrahydro-2\(H\)-benzo[b]oxocin-3-yl) methanol \( 148 \) along with the reduced product \( 149 \) (Scheme 15).\(^{143}\)
14.02.5.5 Palladium-promoted cyclizations

Regioselective intramolecular allylic alkylation of derivative 150 mediated by a palladium(0) catalyst led to the cyclization to an oxocene ring 151 along with small amounts of six-membered cyclic ether 152 [Scheme 16]. This strategy was applied in the asymmetric total synthesis of (+)-(3E)-pinnatifidenyne 153. A similar approach was used in the asymmetric total synthesis of (+)-intricenyne 154.
Intramolecular Heck reaction of 1-bromo-2-[(2-vinylaryloxy)methyl]benzene derivatives occurred via 8-endo-trig cyclization in the presence of palladium(II) acetate as catalyst, potassium acetate as base, tetrabutylammonium bromide (TBAB) as additive in dry DMF under nitrogen atmosphere to form eight-membered dibenzoxocine compounds. Using similar starting benzene derivatives, cyclization took place in the presence of tetrakis(triphenylphosphine)palladium(0), trimethylamine in refluxing acetonitrile for 24 h, to afford mainly via exo-trig mode of cyclization the seven-membered ring derivatives and also 8-endo-trig derivatives, as minor products. Using palladium(II) acetate as catalyst, potassium acetate and TBAB as additive in DMF, only the endo products were obtained (Scheme 17).

A series of alkylidendibenzo[c,e]oxocines were prepared via Heck-Suzuki tandem reaction of 1-bromo-2-[(alkynyl)benzyloxy]methyl benzene with phenyl boronic acid, Pd2(dba)3•CHCl3 (3 mol%) and Xantphos in a 5:1 mixture of DMF:water for 2.5 h. Further derivatives arise from regioselective reductive Heck 8-endo-dig cyclization reaction of the same substrates carried out in the presence of Ni(PPh3)2Cl2 (10 mol%), HCOONa (3 equiv.) at 90 °C in a 4:1 mixture of DMF:water for 4 h.

Intramolecular Buchwald-Hartwig etherification of bromoalcohol catalyzed by Pd(dba)2 with Q-Phos and sodium t-butoxide provided benzoxocine in very low yield along with debrominated as major product (Scheme 19). The synthesis of the eight-membered ring of natural (+)-laurendecumallene B involved palladium-catalyzed cyclization of cyclic propargyl carbonate using Pd2(dba)3 and dppf in the presence of 3 equiv. of water in DMF. This protocol was applied to the synthesis of the precursor of the referred natural product (Scheme 20). Oxidative cyclization of β-citronellol carried out under palladium(II) acetate and hydrogen peroxide catalysis in acetonitrile for 4 h afforded (Z)-4,8,8-trimethyl-3,4,5,8-tetrahydro-2H-oxocine with high selectivity and conversion (ca. 79% and 83%, respectively).

Palladium-mediated cyclizative coupling reaction of γ,δ-oligodiols 173 with allyl bromide or 2,3-dibromoprop-1-ene in DMF at room temperature produced β-lactam-fused oxocines 174 in moderate to good yields. It involves 8-endo cyclization by attack of the primary hydroxy group to the terminal allene carbon. In addition, oxybromination of γ,δ-oligodiols 175 with lithium bromide promoted by a dual catalytic system of palladium(II) and copper(I) in acetonitrile gave brominated β-lactam-fused oxocines (Scheme 21). These reactions occurred in a chemo-, regio-, and diastereoselective way.

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**Scheme 16**

Intramolecular Heck reaction of 1-bromo-2-[(2-vinylaryloxy)methyl]benzene derivatives occurred via 8-endo-trig cyclization in the presence of palladium(II) acetate as catalyst, potassium acetate as base, tetrabutylammonium bromide (TBAB) as additive in dry DMF under nitrogen atmosphere to form eight-membered dibenzoxocine compounds. Using similar starting benzene derivatives, cyclization took place in the presence of tetrakis(triphenylphosphine)palladium(0), trimethylamine in refluxing acetonitrile for 24 h, to afford mainly via exo-trig mode of cyclization the seven-membered ring derivatives and also 8-endo-trig derivatives, as minor products. Using palladium(II) acetate as catalyst, potassium acetate and TBAB as additive in DMF, only the endo products were obtained (Scheme 17).

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**Scheme 17**

Intramolecular Heck reaction of 1-bromo-2-[(2-vinylaryloxy)methyl]benzene derivatives occurred via 8-endo-trig cyclization in the presence of palladium(II) acetate as catalyst, potassium acetate as base, tetrabutylammonium bromide (TBAB) as additive in dry DMF under nitrogen atmosphere to form eight-membered dibenzoxocine compounds. Using similar starting benzene derivatives, cyclization took place in the presence of tetrakis(triphenylphosphine)palladium(0), trimethylamine in refluxing acetonitrile for 24 h, to afford mainly via exo-trig mode of cyclization the seven-membered ring derivatives and also 8-endo-trig derivatives, as minor products. Using palladium(II) acetate as catalyst, potassium acetate and TBAB as additive in DMF, only the endo products were obtained (Scheme 17).

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**Scheme 18**

Intramolecular Heck reaction of 1-bromo-2-[(2-vinylaryloxy)methyl]benzene derivatives occurred via 8-endo-trig cyclization in the presence of palladium(II) acetate as catalyst, potassium acetate as base, tetrabutylammonium bromide (TBAB) as additive in dry DMF under nitrogen atmosphere to form eight-membered dibenzoxocine compounds. Using similar starting benzene derivatives, cyclization took place in the presence of tetrakis(triphenylphosphine)palladium(0), trimethylamine in refluxing acetonitrile for 24 h, to afford mainly via exo-trig mode of cyclization the seven-membered ring derivatives and also 8-endo-trig derivatives, as minor products. Using palladium(II) acetate as catalyst, potassium acetate and TBAB as additive in DMF, only the endo products were obtained (Scheme 17).

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**Scheme 19**

Intramolecular Buchwald-Hartwig etherification of bromoalcohol catalyzed by Pd(dba)2 with Q-Phos and sodium t-butoxide provided benzoxocine in very low yield along with debrominated as major product (Scheme 19). The synthesis of the eight-membered ring of natural (+)-laurendecumallene B involved palladium-catalyzed cyclization of cyclic propargyl carbonate using Pd2(dba)3 and dppf in the presence of 3 equiv. of water in DMF. This protocol was applied to the synthesis of the precursor of the referred natural product (Scheme 20). Oxidative cyclization of β-citronellol carried out under palladium(II) acetate and hydrogen peroxide catalysis in acetonitrile for 4 h afforded (Z)-4,8,8-trimethyl-3,4,5,8-tetrahydro-2H-oxocine with high selectivity and conversion (ca. 79% and 83%, respectively).

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**Scheme 20**

Palladium-mediated cyclizative coupling reaction of γ,δ-oligodiols 173 with allyl bromide or 2,3-dibromoprop-1-ene in DMF at room temperature produced β-lactam-fused oxocines 174 in moderate to good yields. It involves 8-endo cyclization by attack of the primary hydroxy group to the terminal allene carbon. In addition, oxybromination of γ,δ-oligodiols 175 with lithium bromide promoted by a dual catalytic system of palladium(II) and copper(I) in acetonitrile gave brominated β-lactam-fused oxocines (Scheme 21). These reactions occurred in a chemo-, regio-, and diastereoselective way.
14.02.5.6 Lactonization

The synthesis of the lactone core of natural (−)-octalactin A 113 was accomplished by two different approaches starting from α-diazoketone 177: (1) treatment with silver benzoate and 4-(dimethylamino)pyridine (DMAP) in dilute tetrahydrofuran to give lactone 178 in 26% yield; (2) photolytic Wolff rearrangement in aqueous tetrahydrofuran provided seco-acid 179 which suffered lactonization, after treatment with benzoic anhydride (Scheme 22).154

An efficient lactone formation reaction was developed through the use of 2-methyl-6-nitrobenzoic anhydride (MNBA) as a coupling reagent and nucleophilic catalysts, namely acyl-transfer catalysts such as DMAP, 4-pyrrolidinylpyridine and 4-(dimethylamino)pyridine N-oxide (DMAPNO).155 Octalactin B 113 was synthesized from the commercially available methyl 3-butenoate and isobutyraldehyde, being the lactonization reaction one of the key-steps. Thus, the eight-membered ring 181 was obtained from the reaction of seco-acid 180 with MNBA and DMAP in toluene at room temperature (Scheme 23).156 This reaction
conditions were also applied for the synthesis of the lactone core of astakolactin 182. Meanwhile, 3 years later, the same group revised the structure of astakolactin to a pyran derivative 182a. A series of eight-membered lactones 188 were accomplished via [6 + 2] cyclization reaction of (1,6)-amphoteric systems, 2-(oxetan-3-yl)arylaldehydes 186, with siloxy alkynes 187 mediated by triflimide (HNTf₂) in dichloromethane at room temperature (Scheme 25). Oxidative lactonization of 1,7-diol 189 carried out in the presence of a catalytic amount of TEMPO and Ph(ΟAc)₂ afforded eight-membered lactone 190. This strategy was applied in a concise total synthesis of (+)-isolaurepan 191 (Scheme 26).

14.02.5.7 Intramolecular cyclization methods

Anodic oxidation of 3,4-dimethoxystilbene bearing O-substituted nucleophilic groups (OH or OSiMe₃) 192 involved both direct and crossover intramolecular cation-nucleophile reactions to give fused bisbenzopyran 193, bisbenzofurans 194 and 195 and the unexpected bridged oxocine 196 (Scheme 27).

Total synthesis of natural sesquiterpenoid ansellone B 33b was accomplished in a 23 steps starting from (+)-sclareolide 197 and (S)-carvone 198. After regioselective protection of the secondary alcohol 199 and regioselective epoxidation substitution in the presence of dimethyldioxirane (DMDO), unstable product 200 was formed which suffered 8-exo-trig cyclization promoted by PPTS to give the epoxide rearranged side product 201 along with small amounts of the desired oxocane 202. Finally, oxidation of oxocane 202 mediated by 2-iodoxybenzoic acid (IBX) and desilylation by TBAF furnished ansellone B 33b (Scheme 28).

Parent oxocane was obtained in 65% yield from intramolecular dehydration of hepta-1,7-diol promoted by CuBr₂ (2 mol%) at 190 °C for 3 h, under argon atmosphere. Intramolecular [4 + 2] cycloaddition reaction of tertiary benzyl alcohols 203 in the presence of N,N-diisopropylethylamine (DIPEA), 2,4,6-trichlorobenzoyl chloride and DMAP in benzene for the construction of the macrolactone 184 and subsequent removal of the protecting group in acid conditions (Scheme 24). Yamaguchi lactonization was the key step in the stereoselective synthesis of the eight-membered lactone (+)-cephalosporolide D 185. It involved the reaction of acid 183 in the presence of N,N-diisopropylethylamine (DIPEA), 2,4,6-trichlorobenzoyl chloride and DMAP in benzene for the construction of the macrolactone 184 and subsequent removal of the protecting group in acid conditions (Scheme 24).
presence of equimolar amount of toluenesulfonic acid hydrate provided adducts 204 in good to excellent yields (Scheme 29). This strategy was used to the first total synthesis of (+)-heliol 205.

Gold(I)-catalyzed intramolecular dehydrative alkoxylation of o-hydroxy allylic alcohol 206 led to the formation of α,α'-cis-oxocene 207, which were applied in the formal synthesis of (+)-laurencin 103 (Scheme 30).

The synthesis of oxocene unit 209 applied in the total synthesis of (+)-lauthisan 95 was achieved through endo-cyclization reaction of 1-(5-hydroxyhept-1-yl)-3-penty-1-phenylsulfonylellene 208 carried out in the presence of potassium t-butoxide in t-butanol (Scheme 31).
**Scheme 28**

A: DMAP, Ac₂O, pyridine, 25° C, 12 h  
B: DMDO, CH₂Cl₂, 0° C  
C: PPTS, CH₂Cl₂, 25° C, 1 h  
D: IBX, DMSO, 25° C, 2 h  
E: TBAF, dry THF, 25° C, 12 h

**Scheme 29**

TsOH = toluenesulfonic acid

**Scheme 30**

DCE = 1,2-dichloroethane

without NaHCO₃ 35%, >20:1 dr

**Scheme 31**

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It is through stereoselective intramolecular amide enolate alkylation reaction that (E)-allylic bromide 210 cyclize to give the corresponding oxocenes 211, used in the asymmetric total synthesis of (+)-bermudenynol 212 (Scheme 32). This type of transformation was also applied in the asymmetric total synthesis of (+)-(3Z)-laureatin 213, (+)-(3Z)-laurenyne 214, (+)-(3Z)-isolaureatin 215, and (−)-laurefucin 216.

The synthesis of 3-benzo[d]oxocine 218 was achieved through regioselective osmium-catalyzed 8-endo heterocyclization of 3-(2-ethynylphenyl)propan-1-ol 217 in pyridine at 130 °C for 4 h, in moderate yield (40%) (Scheme 33, path a). Further derivatives 220 were obtained from similar substrates 219 under the presence of low loading of a ruthenium complex 221 (Scheme 33, path b).

Regioselective reductive cyclization of alkynyl diol 222 using stoichiometric amount of Hg(OTf)_2 and triethylsilane prompted pyran-fused oxocane 223, via 8-endo oxacyclization process (Scheme 34). The structural elucidation of the corresponding acetate derivative 224 was possible through NMR spectroscopy, including COSY, HMBC, HMQC and NOESY correlations of the protons at the C-O-C portion of the oxocane ring.

(R)-9-Hydroxypentadecan-3-one (R)-225 underwent a diastereoselective reductive cyclization mediated by Et₃SiH and TMSOTf in dichloromethane to furnish exclusively the cyclic eight-membered ether (−)-cis-lauhisian 95. Similarly, (S)-9-hydroxypentadecan-4-one (S)-225 produced (+)-isolaurepan 191 (Scheme 35). Interestingly, using a similar strategy in the synthesis of (±)-cis-lauhisian 95 starting from (±)-9-hydroxypentadecan-3-one (±)-225, a mixture of (±)-cis-lauhisian 95 and...
diastereomeric diols \((\pm)-226\) were obtained. Starting from \((R)-13\)-hydroxypentadecan-7-one \((R)-227\), reductive cyclization provided a mixture of \((-\text{cis})\)-lauthisan 95 and diastereomeric diols \(228\) (Scheme 35).\(^{177,178}\)

Olefin-ester cyclization reaction of ester 229, in a multistep strategy, gave a mixture of the eight-membered ring 230 in 40% yields and the ether 231 in 56% yield (Scheme 36). This synthetic approach was used in the synthesis of the FGHI-ring system of YTX 26 and adriatoxin 30.\(^{179}\)

A couple of oxocanes 235 and 236 bearing an exocyclic methylene unit was synthesized via cycloisomerization reaction of 1,8-oxadienes 233 and 234 under catalysis of NHCl-Ni complexes, prepared from Ni(cod)\([\text{bis(cyclooctadienyl)nickel(0)}]\) and [1,3-bis(2,6-diisopropylphenyl)imidazol-2-ylidene] (IPr) or [1,3-bis(2,6-diisopropylphenyl)-imidazolidin-2-ylidene] (SIPr), 1-octene, triethylamine, \(p\)-anisaldehyde and triethylsilyl trifluoromethanesulfonate (TESOTf) in toluene under nitrogen and anhydrous atmosphere (Scheme 37).\(^{180}\)

A wide range of methyl 7H-dibenzo[6,8]oxocine-6-carboxylates 240 were obtained from a multistep synthesis involving: (i) - solvent-free Morita-Baylis-Hillman (MBH) reaction of 2-phenoxybenzaldehydes 237 with methyl acrylate in the presence of 1,4-diazabicyclo[2,2,2]octane (DABCO) and triethanolamine at room temperature; (ii) treatment of MBH adducts 238 with \(N\)-bromosuccinimide/dimethyl sulfide (NBS/DMS) in dichloromethane to produce MBH allyl bromides 239; (iii) Friedel-Crafts cyclization with aluminum chloride in dichloromethane at reflux temperature for 10 min to 2 h (Scheme 38).\(^{181}\)

One-pot, two-step protocol was applied to the synthesis of benzene-fused eight-membered ketones starting from inactivated terminal alkynes. It involves gold-catalyzed additions of protonated pyridine \(N\)-oxide to the alkyne triple bonds, generating \(N\)-alkenoxypyridinium salts, and oxidative cyclization under heating conditions. Thus, using 2-arylethyl propargyl ethers 241 as substrate, the expected ketones 242 were obtained. With naphthyl substrates 243–244, cyclization can occur in adjacent positions of the aromatic ring giving a mixture of isomers 245–248. With \((\text{pent-4-yn-1-yloxy})\)benzene 249, ketone 250 was obtained (Scheme 39).\(^{182}\)
Eight-Membered Rings With One Oxygen Atom

Scheme 36

80\% 

Scheme 37

Ni-iPr: 1) Ni(cod)$_2$ [bis-(cyclooctadienyl)nickel(0)], [1,3-bis(2,6-diisopropylphenyl)-imidazol-2-ylidene] (iPr), PhMe, N$_2$, rt, 1 h; 2) 1-octene, triethylamine, p-anisaldehyde and triethylsilyl trifluoromethanesulfonate (TESOTf), rt, 45 min

Ni-SiPr: 1) Ni(cod)$_2$ [bis-(cyclooctadienyl)nickel(0)], [1,3-bis(2,6-diisopropylphenyl)-imidazolidin-2-ylidene] (SiPr), PhMe, N$_2$, rt, 1 h; 2) 1-octene, triethylamine, p-anisaldehyde and triethylsilyl trifluoromethanesulfonate (TESOTf), rt, 45 min.

Scheme 38

DABCO = 1,4-diazabicyclo[2.2.2]octane
NBS = N-bromosuccinimide
DMS = dimethyl sulfide

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14.02.5.8 Domino/cascade reactions

Several examples of Lewis acids were applied as cyclization promoters for the diastereoselective synthesis of oxocene derivatives. Thus, intermolecular Prins-type cyclization reaction of 1-alkyl-5-methylhex-5-en-1-ols 252 with aromatic aldehydes mediated by TMSOTf provided a range of *endo*-oxocenes 253 as major products along with small amounts of the *exo*-derivatives 254 (Scheme 40). Using the same starting materials 252 in the presence of a combination of *p*-toluenesulfonic acid (*p*-TsOH) (30 mol%) and scandium or dysprosium(III) triflates (10 mol%) afforded a mixture of tetrahydropyrans 254 and few examples of the corresponding oxocene derivatives 253 (Scheme 40).

Few examples of benzoxocenes 257 are obtained from aza-ene/Prins cyclization reaction of *O*-aminoaryl alkenols 256 with electron-deficient aryl aldehydes promoted by TMSOTf in DCE (Scheme 41).

Scheme 39

Scheme 40
Allylsilyl alcohols ($R_2 = H$) \(258\) undergo tandem Sakurai-Prins cyclization with aldehydes in the presence of TMSOTf to give mainly dioxaspiroundecane \(259\) while in the presence of \(\text{BF}_3\cdot\text{OEt}_2\) direct silyl-Prins cyclization took place to afford selectively oxocanes \(260\). Using substituted allylsilyl alcohols ($R_2 \neq H$) \(258\) in the presence of TMSOTf, similar oxocanes \(261\) were obtained (Scheme 42).\(^{186}\)

A series of \((2Z,7Z)-6\text{-oxo-5,6-dihydro-4H-oxocine-3-carbonitriles}\) were obtained in moderate to good yields through DABCO-mediated \([4 + 4]\) domino annulation reactions of ynones with \(\alpha\)-cyano-\(\alpha,\beta\)-unsaturated ketones carried out in a 2:1 mixture of ethylene glycol:toluene at 60°C.\(^{187}\) Several \((Z)-1-(3\text{-hydroxy-3,4,7,8-tetrahydro-2H-oxocin-3-yl})\)ethan-1-one derivatives \(263\) were prompted from cascade reaction of enynyl esters \(262\) in the presence of \(\text{PPh}_3\text{AuCl/AgSbF}_6\) in dichloromethane at room temperature, involving enynyl ester isomerization and intramolecular \([3 + 2]\) cyclization reactions (Scheme 43).\(^{188}\)

An oxacene \(\text{Co}_3(\text{CO})_6\)-acetylenic complex \(268\) was prepared by a multistep strategy involving: (1) intermolecular Nicholas reaction of cobalt complex \(264\) with homoallylic alcohol \(265\) to obtain unsaturated branched linear ether \(266\), (2) RCM to form the cobalt complex cyclic ether \(267\), and (3) isomerization mediated by montmorillonite K-10. In addition, cleavage of the TMS protecting group with TBAF and subsequent catalytic hydrogenation provided the natural (+)-cis-lauhartian \(95\) (Scheme 44).\(^{121,125,126}\) This protocol was also extended to the synthesis of \((+)-\text{trans-lauhartian} 95\) and to the eight-membered core of the natural derivative (+)-laurencin \(103\).\(^{121}\)

Other cobalt complexes \(270\) and \(272\) were involved in the construction of the eight-membered I-ring in the synthesis of HIJ-fragment\(^\text{189}\) and HIJKLM-fragment\(^\text{190}\) of natural CTX-1B\(^\text{9}\). Thus, the cyclization of acetylene cobalt complexes \(269\) and \(271\) occurs via intramolecular \(1,4\)-hydroxy addition carried out in the presence of \(\text{BF}_3\cdot\text{OEt}_2\) in dichloromethane (Scheme 45). Diastereoselective Brook rearrangement-mediated \([5 + 4]\) annulation reaction of acryloylsilane \(273\) with \(\text{6-oxacyclohepten-2-one derivative} 274\) and subsequent oxidation (using Davis’ oxaziridine) of the enolate formed provided \(\alpha\)-hydroxyketone \(275\), which suffered oxidative cleavage of the two-atom internal tether to give \(\text{trans-\(\alpha,\alpha’\)-disubstituted oxocene} 276\) (Scheme 46). This strategy was applied in the formal synthesis of \((+)-\text{laurallene} 110\)\(^{191,192}\) and \((+)-\text{prelauratin} 277.\(^{192}\)
A wide range of 2-phenylpyrazolo[3,4-b]-4,5-dihydrooxocin-4-ones were synthesized via DBU-mediated [4 + 4] domino annihilation reactions of ynones with 4-benzylidene-2-phenylpyrazol-3-ones in dichloromethane at room temperature for 10–120 min.\textsuperscript{193} Few examples of oxocine-annulated heterocycles arise from sulfanyl radical addition and intramolecular 8-endo radical cyclization reactions of enynes 278 with 2 equiv. of thiophenol and the radical initiator AIBN in refluxing dry t-butanol (Scheme 47).\textsuperscript{194}
Treating (6S,7R)-epoxide 280 with two equiv. of NBS under high dilution conditions in water led to (±)-hexahydrolauroxanyne 281 as a bicyclic medium-ring ether, dibromoepoxides 282, several polar compounds and recovered of some starting epoxide 280 (Scheme 48). The reaction occurs via intramolecular bromonium ion assisted epoxide ring-opening (IBIAERO) reaction and subsequent bromoetherification of the remaining unsaturation.\(^{195}\)

Cascade reaction of 2-[(1-methyl-4-oxocyclohexa-2,5-dien-1-yl)oxy]acetaldehyde 283 with 2 equiv. of anilines in dichloromethane furnished highly functionalized 2-oxabicyclo[3.3.1]non-7-en-6-ones 284 (Scheme 49). It involves: (1) condensation of the first equiv. of the amine with aldehyde to form an imine, (2) aza-Michael addition of the second equiv. of the amine, (3) Mannich transannular reaction completed the cyclization process to give the oxocane unit.\(^{196}\)

14.02.6 Ring syntheses by transformation of another ring

14.02.6.1 Ring expansions of one atom

BF\(_3\)•OEt\(_2\)-mediated ring expansion reaction of ketone 285 with TMS-diazomethane at –80 °C followed by to mild acid treatment afforded mainly the eight-membered ketone derivative 286, along with the regioisomer ketone 287 and spiroepoxide 288.

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Expansion reaction of ketones 289 and 291 required higher temperature (−40 °C) to achieve the corresponding ketones 290 and 292 (Scheme 50). This methodology was involved in the synthesis of the CDEFGHIJ-ring system of YTX 26.\textsuperscript{197,198}

A study reported the benzoxepane ring enlargement, applied in the synthesis of sesquiterpenes heliannuols A 43 and K 109. Thus, benzoxopinenones 293 reacted with diazomethane in presence of a catalytic amount of palladium(II) acetate to give cyclopropyl ketones 294, subsequent catalytic hydrogenation afforded benzoxocanones 295, which can suffer demethylation (R\textsubscript{1} = OMe, R\textsubscript{2} = Me) promoted by in situ generated iodotrimethyl silane to give heliannuol K 109. Reduction of 109 with sodium borohydride in methanol afforded heliannuol A 43 (Scheme 51).\textsuperscript{199} These transformations were also used for the synthesis of other natural products, namely the bisabolene sesquiterpene elvirol 298, after reduction of benzoxocanones 295 to benzoxocanols 296, subsequent transformation into the thionocarbonate 297 and final fragmentation of the eight-membered ring (Scheme 51).\textsuperscript{199}

\begin{tikzpicture}
  \node (a) at (0,0) {H_2SO_4, \text{HCl} \rightarrow 286 (53\%)};
  \node (b) at (0,-1.5) {BBr\textsubscript{3} \rightarrow 287 (5\%) + 288 (16\%)};
  \node (c) at (0,-3) {\text{NaOH, } \text{H}_2\text{O} \rightarrow 289 (53\%)};
  \node (d) at (0,-4.5) {\text{PPTS, } \text{CH}_2\text{Cl}_2/\text{MeOH, rt then 60°C} \rightarrow 290 (38\%)};
  \node (e) at (0,-6) {\text{TsCl, } \text{EtN\textsuperscript{+}}/\text{PhH} \rightarrow 291 (38\%)};
  \node (f) at (0,-7.5) {\text{TsCl, } \text{MeOH} \rightarrow 292 (38\%)};

  \draw[->,thick] (a) -- (b);
  \draw[->,thick] (b) -- (c);
  \draw[->,thick] (c) -- (d);
  \draw[->,thick] (d) -- (e);
  \draw[->,thick] (e) -- (f);

  \node at (0,-8.5) {A: 1. TMSCH\textsubscript{2}N\textsubscript{2}, BF\textsubscript{3}, OEt\textsubscript{2}, mol. sieves 4 Å, CH\textsubscript{2}Cl\textsubscript{2}, -80°C \rightarrow 291 (38\%)};
  \node at (0,-9) {2. PPTS, CH\textsubscript{2}Cl\textsubscript{2}/MeOH, rt then 60°C}

  \node at (0,-9.5) {B: 1. TMSCH\textsubscript{2}N\textsubscript{2}, BF\textsubscript{3}, OEt\textsubscript{2}, mol. sieves 4 Å, CH\textsubscript{2}Cl\textsubscript{2}, -40°C \rightarrow 291 (38\%)};
  \node at (0,-10) {2. PPTS, CH\textsubscript{2}Cl\textsubscript{2}/MeOH, rt then 60°C}
\end{tikzpicture}
14.02.6.2 Ring expansions of two atoms

4-[2-(t-Butyldiphenylsilyloxy)methyl]cyclopropyl-6-vinyl-[1,3]-dioxan-2-one 299 underwent Holmes-Claisen rearrangement in hot toluene to afford lactone 300, intermediate in the total synthesis of natural solandelactones E 301 and F 302 (Scheme 52).²⁰⁰

Few examples of sulfur bridged cyclic ethers 304 were prompted via Stevens [1,2]-shift of sulfonium ylides derived from six-membered mixed-monothioacetal precursors 303, carried out in the presence of a catalytic amount of Cu(hfacac)₂ and using toluene or dichloromethane as solvent (Scheme 53). This strategy was applied to the synthesis of Holmes intermediate 102, involved in the formal synthesis of natural (+)-laurencin 103.⁹⁸

14.02.6.3 Ring expansions of three or more atoms

Regio- and enantiospecific domino reaction of camphor-derived bis(spiroepoxide)-substituted 1-norbornyl triflate 306 in refluxing aqueous ethanol buffered with trimethylamine for 30 days furnished cyclobutane-fused eight-membered cyclic ether 307 (Scheme 54). It involves (1) the formed 1-norbornyl cation undergo an epoxide-based pinacol-type rearrangement to give an hydroxyl epoxide; (2) hydroxy-epoxide cyclization to generate tricyclic β-hydroxy ketone, (3) retro-aldol condensation reaction to form a bicyclic enol and finally keto-enol equilibration to the more-stable trans-fused tautomer.²⁰¹

Polycyclic cyclohexadienone 309, obtained through oxidative dearomatization of bicyclic phenol 308 in the presence of PhI(OAc)₂ and potassium carbonate in trifluoroethanol, underwent ring-expansion in the presence of Tf₂O and DTBMP in

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**Scheme 51**

**Scheme 52**
dichloromethane to provide eight-membered derivative 310 (Scheme 55). Low yields were obtained (<25%) when TsOH or Cu [BF₄]₂ were used as ring-expansion promoters.²⁰²

It is through a ring formation-fragmentation-expansion cascade reactions that aldehydes bearing a tetrahydrofuran ring 311a,b reacted with ethyl diazoacetate in the presence of a catalytic amount of anhydrous SnCl₂ in dichloromethane at room temperature to provide β-keto esters 312a,b as major products along with small amounts of oxocenes 313a,b as single diastereomers (Scheme 56).²⁰³

Various 8-membered bromoethers 315, 317, 319 and 321 were regio- and stereocontrolled prepared through a ring-expanding bromoetherification protocol, using 1-(3-alkoxy-5-methylfuran-2-yl)hex-3-enes 314 and 316 or 1-(3-OBoc-5-methylfuran-2-yl)pent-2-enes 318 and 320 and bromodiethylsulfonium bromopentachloroantimonate (BDSB) in nitromethane (Scheme 57).²⁰⁴

This methodology was used in the racemic formal total syntheses of laurefucin 216 and (E- and Z-)pinnatifidenyne 153²⁰⁵ and in the concise asymmetric total syntheses of microcladallenes A 32 and B 111, desepilaurallene 322, laurallene 110, and prelaureatin 277.²⁰⁶
Eight-Membered Rings With One Oxygen Atom

Scheme 56

\[
\begin{align*}
\text{311a} + \text{311b} & \xrightarrow{\text{SnCl}_2, \text{Et}_3\text{SiH}, \text{CH}_2\text{Cl}_2, 23^\circ \text{C}} \text{312a} + \text{312b} \\
\text{313a} & \xrightarrow{\text{SnCl}_2, \text{Et}_3\text{SiH}, \text{CH}_2\text{Cl}_2, 23^\circ \text{C}} \text{313b}
\end{align*}
\]

\[
\begin{align*}
\text{312a} & (70\%) \\
\text{312b} & (69\%) \\
\text{313a} & (25\%) \\
\text{313b} & (22\%)
\end{align*}
\]

Scheme 57

\[
\begin{align*}
\text{314} & \xrightarrow{\text{BDSB, MeNO}_2, -25^\circ \text{C}, 5-10 \text{ min}} \text{315} \\
\text{316} & \xrightarrow{\text{BDSB, MeNO}_2, -25^\circ \text{C}, 5-10 \text{ min}} \text{317} \\
\text{318} & \xrightarrow{\text{BDSB, MeNO}_2, -25^\circ \text{C}, 20 \text{ min}} \text{319} \\
\text{320} & \xrightarrow{\text{BDSB, MeNO}_2, -25^\circ \text{C}, 20 \text{ min}} \text{321}
\end{align*}
\]

BDSB = bromodiethylsulfonyl bromopentachloroantimonate

322 Desipiliaurilene
Eight-membered lactones 325 were synthesized in moderate yields from the reaction of β-silyl ketone enolates 323 with MeLi in THF, subsequent treatment with epoxides and BF₃–OEt₂ in THF to yield the desired hemiketals 324 and finally, addition of Phil(OAc)₂ and iodine in dichloromethane (Scheme 58). One of these lactones was applied in the stereoselective total synthesis of (+)-cis-lauthisan 95, previously described in Section 14.02.4.⁹⁷

### 14.02.7 Syntheses of particular classes of compounds and critical comparison of the various routes available

As described along this chapter, a huge number of publications have emerged during the last decade focusing the eight-O-membered heterocyclic chemistry. Among the impressive number of methodologies developed, we can mention those dedicated to the synthesis of several marine natural products with unusual and complex structures such as PbTxs, MTX, CIXs and YTXs as well as some natural derivatives extracted from terrestrial origin, namely, heliannuols, psyllins, lauthisan and related derivatives. In fact, seven different routes have been established concerning the total synthesis of natural oxocene derivative lauthisan.

RCM was the most popular approach to construct the eight-membered ring and used as the key reaction in the synthesis of many types of natural compounds, or their precursors. Several reactions promoted by palladium catalysts also deserved special attention in the synthesis of several natural eight-membered oxacycles. Regio- and diastereoselectivity were achieved in the three-carbon ring-enlargement of tetrahydrofuran derivatives assisted by metals such as tin and antimony.

### 14.02.8 Important compounds and applications

The pharmacological importance of eight-O-membered heterocycles among the scientific community is demonstrated by the large number of reviews and original papers related to this topic, published every year.

Extensive studies have been devoted to the toxicology of dinoflagellate toxins such as PbTxs, MTX, CIXs and YTXs, with environmental and human impact. Their molecular mechanisms of action were compiled and described in several reviews (see Section 14.02.1), which can be complemented with the information provided in CHEC III. Here, it is our intention to give a brief presentation on the main therapeutic applications of natural eight-O-membered heterocyclic compounds, providing some of the most relevant results. It is noteworthy to mention that no synthetic derivative was evaluated in any biological assay.

Ansellone C 34 showed moderate antitumor activity against A549 (non-small cell lung cancer) and K562 (human leukemia) cell lines with LC₅₀ values of 3.9 and 4.5 μM, respectively, when compared with the positive control doxorubicin (LC₅₀ of 1.1 and 1.5 μM, respectively). No antibacterial activity was observed against Staphylococcus aureus and Proteus hauseri.⁴⁹

Antistaphylococcal activity against a panel of multidrug and methicillin-resistant S. aureus was tested for eight-membered cyclic ethers 36–41. Derivatives 36 and 40 did not show any activity at 128 mg/mL, although the remaining compounds exhibited significant antistaphylococcal activity, with minimum inhibitory concentrations (MICs) in the range of 8–256 mg/mL. Derivative 37 was the most active one (MIC 8–16 mg/mL) probably due to the presence of the two acetyl groups that improves its cellular bioavailability by being more lipophilic.⁵¹

The antioxidant activity of methyl 3,4,5,8-tetrahydro-2H-oxocine-5-carboxylate 42 were evaluated and compared to α-tocopherol and the synthetic antioxidants butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA). Similar 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2’-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical scavenging activities were observed for compound 42, BHA and BHT but slightly higher than α-tocopherol. For the anti-inflammatory assays, compound 42 exhibited higher inhibitory activity against cyclooxygenase-1 (COX-1, IC₅₀ 0.92 mg/mL) than cyclooxygenase-2 (COX-2, IC₅₀ 1.95 mg/mL) isoform. No significant differences were recorded for the in vivo 5-lipoxigenase (5-LOX) inhibitory activity of derivative 42 (IC₅₀ 0.95 mg/mL) and the control ibuprofen (IC₅₀ 0.93 mg/mL).⁵²

Some studies have been carried out on the isolation of sunflower constituents with biological activity, with special emphasis on phytotoxicity, an indication for the possible allelopathic activity of sunflower. Heliannuol A 43 have been tested in the etiolated wheat coleoptile bioassay and showed high inhibitory activity on coleoptile elongation at 10⁻³ M, with a value of 85% (very close to the value of the commercial herbicide logran, 93%), and of 50% at 3 × 10⁻³ M concentration, but the activity decreased significantly with dilution. Derivative 43 was also selected for the evaluation of phytotoxicity on tomato seed growth, in terms of percentage inhibition of germination and shoot and root length, revealing strong inhibitory effects, with activities similar to that of the herbicide logran.⁵³,⁵⁴

A great number of seco-steroids named psyllins are widely used in folk medicine in many tropical countries around the world. Its known pharmacological applications are antioxidant, anti-inflammatory, antimicrobial, antitumor, sedative, among others. Herein, we will describe the therapeutic potential of the eight-O-membered psyllins evaluated during the last decade.
Immunomodulatory activities of physalins B 45, F 48 and G 49 in lymphocyte proliferation, cytokine production and in transplantation were studied. The addition of physalins B 45, F 48 and G 49 to concanavalin A-activated spleocyte cultures induced a concentration-dependent inhibition of proliferation. Physalin B 45 also inhibited IL-2 production by concanavalin A-activated spleen cells. Rejection of allogeneic heterotopic heart transplant was delayed in mice treated with physalins B 45, F 48 or G 49.56

The immunosuppressive effect of physalin H 50 on T cells both in vitro and in vivo were also evaluated. The results point out that the immunosuppressive activity might be attributed to the suppression of T cell activation and proliferation, the modulation of Th1/Th2 cytokine balance and the induction of HO-1 in T cells.56

The activity of physalin F 48 was investigated in mice models of collagen-induced arthritis and allergic airway inflammation. Oral treatment caused a marked decrease in paw edema and joint inflammation while no effects were observed in mice with allergic airway inflammation. In addition, a different inhibitory mechanism is proposed for physalin F 48 when compared with that of glucocorticoids.58

Topic anti-inflammatory potential of physalin E 47 was demonstrated in acute and chronic models of dermatitis induced in mouse ear.59

The antimalarial activity of physalins B 45, D 46, F 48 and G 49 was evaluated using in vitro assays against Plasmodium falciparum. Although these physalins exhibited antimalarial activity, lower cytotoxicity was recorded when compared to the standard antimalarial drug, mefloquine.60

The inhibitory effects of some physalins on nitric oxide (NO) production induced by lipopolysaccharide (LPS) in macrophages were tested. Physalin X 58 exhibited a significant inhibitory effect against NO production with an IC50 value of 68.50 ± 1.51 μM (positive control hydrocortisone, IC50 58.79 ± 3.32 μM).70 Another study indicated that physalin F 48 possessed the strongest effect, with an IC50 of 0.33 ± 0.17 μM. Physalins A 44, B 45, and O 60 also showed strong inhibition with IC50 ranging from 0.84 to 17.11 μM. Moderate activities were observed for physalins Y 61 and Z 62 and 3β-hydroxy-2,3-dihydro-4,7-didehydrophysalin B 63, which were close to hydrocortisone (IC50 64.34 ± 7.49 μM).71 Other papers reported the anti-inflammatory potential of physalins A 44, G 49, L 59, O 6074 and of physalins B 45 and F 48.70

The anti-leishmanial activity of physalins B 45, D 46, F 48 in vitro and in vivo in models of cutaneous leishmaniasis was evaluated. For the in vitro assays, physalins B 45 and F 48 were able to reduce the percentage of leishmania-infected macrophages and the intracellular parasite number at concentrations non-cytoxic to macrophages. For the in vivo assays, physalin F 48 reduced the lesion size, the parasite load and histopathological alterations in the infected mice.63

An interesting study was undertaken to evaluate a new pharmacological property of physalins B 45, D 46, F 48 and G 49 in inflammatory and centrally mediated pain tests in mice. The results suggested that physalins present antinociceptive properties associated with central but not anti-inflammatory events.70

Several in vitro and in vivo antitumor activity studies were performed for physalins. Thus, physalin B 45 and D 46 displayed considerable cytotoxicity against several cancer cell lines [B-16 (murine skin), HCT-8 (human colon), PC3 (human prostate), MDA-MB-435, MDA-MB-231 and MCF-7 (human breast), K562, CEM and HL-60 (human leukaemia)], showing IC50 values of 0.58–15.18 μg/mL for physalin B 45, and 0.28–2.43 μg/mL for physalin D 46. The antitumor activity of both compounds was confirmed in vivo using mice bearing sarcoma 180 tumor cells.61 Physalins B 45, D 46, F 48, J 52, U 53 and W 54 were tested against a panel of human cancer cell lines [A549, ZR751 (estrogen receptor positive breast cancer), A431 (EGFR overexpressing skin cancer), LNCAP (AR-dependent prostate cancer), HCT-8, PC-3, KB (nasopharyngeal carcinoma), KB-VIN (vincristine-resistant KB subline)]. The results showed that physalins B 45, D 46, F 48 displayed strong cytotoxicity against most of the cell lines with IC50 values lower than 3 μg/mL. However, physalin B 45 showed moderate activity against LNCAP and A549, and physalin D 46 against KB-VIN tumor cell lines. In addition, physalin W 54 was only slightly active against 1A9 and physalin J 52 only against A431 cell lines. Physalin U 53 showed modest activity against almost all human tumor cell line panel.84 Other paper reported that physalin B 45 showed the highest cytotoxic activities against HeLa (human cervical), SMMC-7721 (human hepatoma) and HL-60 tumor cell lines while physalins A 44, D 46, D 51, L 58 and X 58 show moderate cytotoxic activities.85 Physalins B 45, F 48 and J 52 were evaluated for cytotoxicity against human HL-60, SMMC-7721 (hepatoma cell line), A-549, MCF-7 and SW-480 (colon cancer cell line) and displayed potent cytotoxicity (IC50 < 5 μM) while physalin V 70 exhibited a modest cytotoxicity.85 High cytotoxicity was observed for physalin H 50 against HXT-116 (human colorectal carcinoma) and NCI-H460 (human non-small cell lung cancer) cells but weak activity for physalins A 44, B 45, D 46 and F 48.48

Physalins A 44 and B 45 significantly inhibited the growth of two androgen-independent cell lines CWR22Rv1 and C42B, induced apoptosis and decreased androgen receptor expression.83 Other studies referred that physalin A 44 also induced cytotoxic effects in human melanoma A375-S2 cells73 and human fibrosarcoma HT1080 cells72 and the selective cytotoxicity of physalin B 45 for human melanoma A375 cells67 and HXT-116 cells,89 of physalin F 48 for human breast T-47D carcinoma56 and of 7β-ethoxylisophysalin C 73 for PC-3 cancer cell line.84

The phase II enzyme quinone reductase plays an important role in anticancer, detoxification pathways and antioxidant defense. From the tested compounds, physalin H 50 exhibited strong quinone reductase inhibitory activity while physalin G 49, 5β-hydroxy-6α-chloro-5,6-dihydrophysalin B 56 and 5α-ethoxy-6β-hydroxy-5,6-dihydrophysalin B 57 showed weak inhibitory activity.68 The inhibitory effects on quinone reductase activity by physalin A 4479 and isophysalin B 2572 were also reported. Physalin D 46 showed low antioxidant potential in the DPPH radical and thioarbitric acid (TBA) assays. Low antibacterial and antifungal activity was also recorded for physalin D 46.12 Further antibacterial assays indicated that physalins B 45 and J 52 showed high antibacterial activities against Bacillus subtilis and Escherichia coli.91
Acknowledgments

The authors are grateful to the Foundation for Science and Technology (FCT, Portugal) and FEDER under Programme PT2020 for financial support to Research Units CIMO (UID/AGR/00690/2019) and QOPNA (UID/QUI/00062/2019), co-financed by (FCT/MCTES).

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