Medicinal Attributes of Lignans Extracted from *Piper Cubeba*: Current Developments
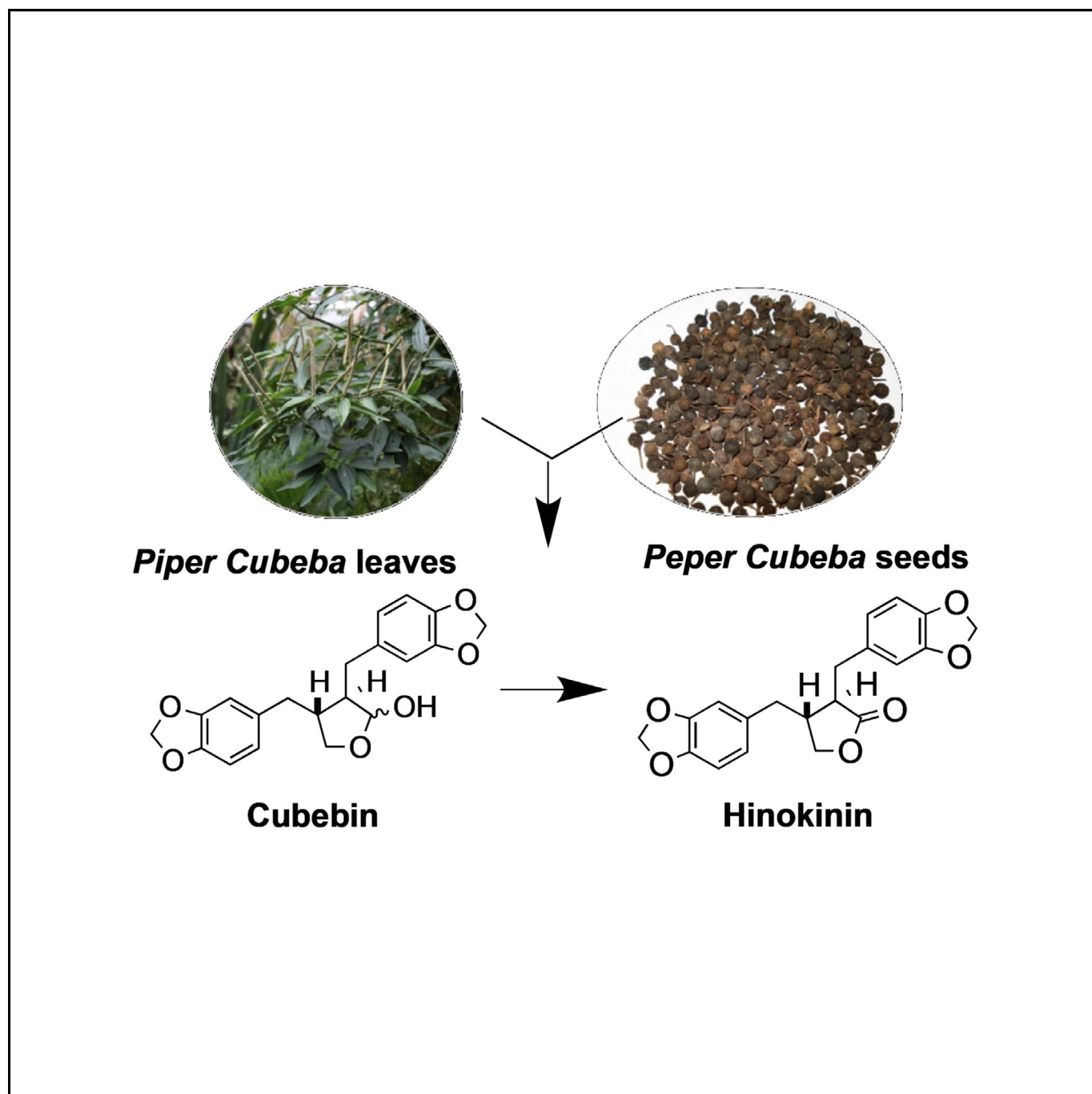
Regiane Godoy de Lima,[a, b] Maria Teresa Barros,*[a] and Rosangela da Silva Laurentiz[b, c]

Piper Cubeba leaves

Peper Cubeba seeds

Cubebin

Hinokinin

Lignans are a large class of natural products that have been isolated from many plants. They reveal diverse biological activities, especially antiviral and antitumor properties. From *Piper cubeba*, lignans of several classes can be isolated from the roots, rhizomes, stems, leaves, seeds, and fruits. Among its various chemical constituents, (−)-cubebin and (−)-hinokinin are found in significant quantities. Although they have been known for some time, during the last few decades their biological properties have been studied by several research groups.

The cubebins have been identified as a lactol monomer and dimers as a mixture of diastereoisomers. Recently, their structural characterization and the synthesis of the possible structures have led to the correction of some earlier structural proposals. This review describes the more recent developments in the study of the medicinal attributes of cubebin and hinokinin extracted from *Piper cubeba* and the synthesis and biological testing of some analogues.

1. Introduction

Piper cubeba is an important plant belonging to the Piperaceae family, commonly known as cubeb or Java pepper, and it is cultivated for its fruit and essential oils. It is grown in Indonesia, mainly in the Java and Sumatra islands, and in southern India.[1] *Piper cubeba* is used as a spice in many countries and is economically important.[1, 2]

The bioactive compounds from *Piper cubeba* have been of great value in the discovery of new therapeutic agents to treat ailments associated with inflammatory problems, renal disorders, gonorrhea, syphilis, abdominal pain, enteritis, and asthma.[3, 4]

2. Lignans in General

Analysis of the literature indicates that 24 lignans have so far been reported from extracts of *P. cubeba*.[1, 3, 5–7] Lignans are secondary metabolites produced by plants that have a wide range of biological properties which are intrinsically related to their stereochemistry.[5, 6, 8, 9] This variety is related to the diversity of carbon skeletons that these classes of natural products present in plants.[10] The denomination of the lignan class of compounds was created in 1936 by Haworth to describe a group of dimeric phenylpropanoids and, therefore, originated from a wide variety of lignans. There are eight subclasses of lignans, including dibenzylbutane, dibenzylbutyrolactol, dibenzylbutyrolactone, furan, furofuran, aryltetralin, arylnaphthalene, and dibenzocyclooctadiene (Figure 1).[10–12]
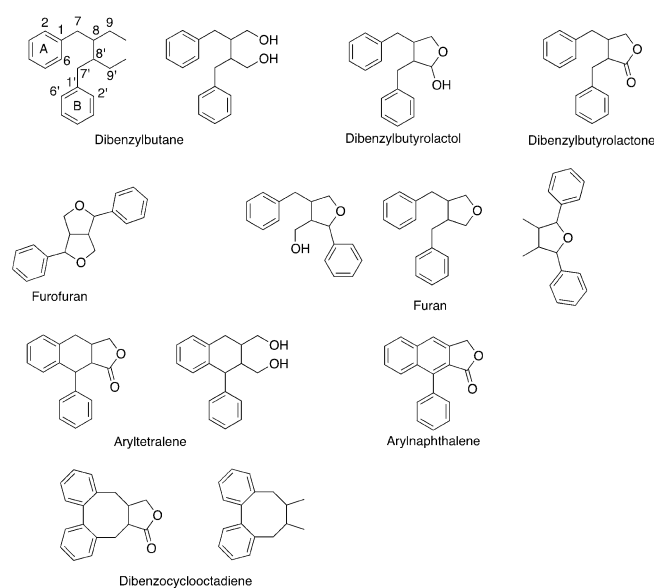

Figure 1. General classes of lignans.[10]

Lignans are present mainly in plants but can also be found in mammals and are produced by the action of intestinal bacteria from food lignans (matairesinol, secoisolariciresinol, pinoresinol, sesamin, lariciresinol, syringaresinol, 7-hydroxytatairesinol, and artigenina) found in flax seeds, fruits, and vegetables.[13–18] In *Linum*, *Anthriscus*, and *Podophyllum* plants, matairesinol is also converted into hinokinin, yatein, or podophyllotoxin through multiple biosynthetic pathways, even though all relevant enzymes have not yet been identified.[19]

Enterolactone and enterodiol have been cited as providing protection against cardiovascular disease, diabetes, and cancer.[18] These and other lignans also have antioxidant activity[20] and reduce oxidative stress. Lignans with anticancer activity behave in metabolic pathways, however, through the induction of cell death by apoptosis.[21–24]

Of the *Piper cubeba* lignans, several classes can be isolated from roots, rhizomes, stems, leaves, seeds (Figure 2), and

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Maria Teresa Barros received her Ph.D. degree in organic chemistry from the University of Paris-Sud (Doctorat d'État) in 1981. Her doctoral studies involved methodological improvements for the application of pentavalent bismuth derivatives in organic synthesis working under the supervision of Professor Sir Derek Barton. She worked as a postdoctoral researcher at the Chemistry Department, University of California at Berkeley studying organic bridged uranocenes with Professor Andrew Streitwieser and at the Lawrence Berkeley Laboratory as a visiting scholar. Currently, she is Associate Professor with habilitation in the Chemistry Department of the Faculty of Sciences and Technology of the New University of Lisbon and is a researcher of REQUIMTE/LAQV at the same institution. Her research covers a broad range of topics in synthetic bioorganic chemistry, especially the synthesis of large and small molecules with potential biological activity. Her research group is also involved in the development of new strategies for drug delivery.

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Figure 2. *Piper cubeba* seeds.[29]

fruits.[1,25] Recent studies aimed at investigating the biological activities of crude fruit extracts[26,27] have been related to biological properties discovered in lignans.[28]

Some interesting biological activities of lignans extracted from *P. cubeba* have been reported, and these lignans could potentially be developed as new drugs.[1,6,25,29] Thus, the aim of this review is to describe the medicinal attributes and current developments of the structurally similar lignans hinokinin and cubebin, which possess a broad range of biological activities and are major components of the fruits of *P. cubeba*.[1]

3. (−)-Cubebin

An important dibenzylbutyrolactone lignan is (−)-cubebin (**1**); the lactol structure and stereochemistry of cubebin (Figure 3), a constituent of cubeb fruits (*Piper cubeba*), was elucidated by Batterbee.[30] (−)-Cubebin (**1**) has been isolated from a crude hexane extract of the leaves of *Zanthoxylum naranjillo*.[31] The *Piper nigrum* crude ethanol extract presents phytochemical compounds,[32] although the crude ethanol extract of *P. cubeba* has higher antioxidant activity than the extracts from other *Piper* species.[27] (−)-Cubebin is a major component of *Piper cubeba* seeds.[33] During the last decades, interesting new biological properties of this compound have been discovered.
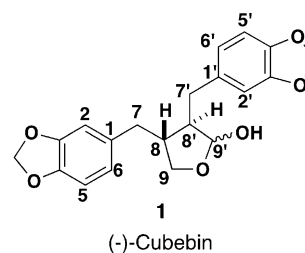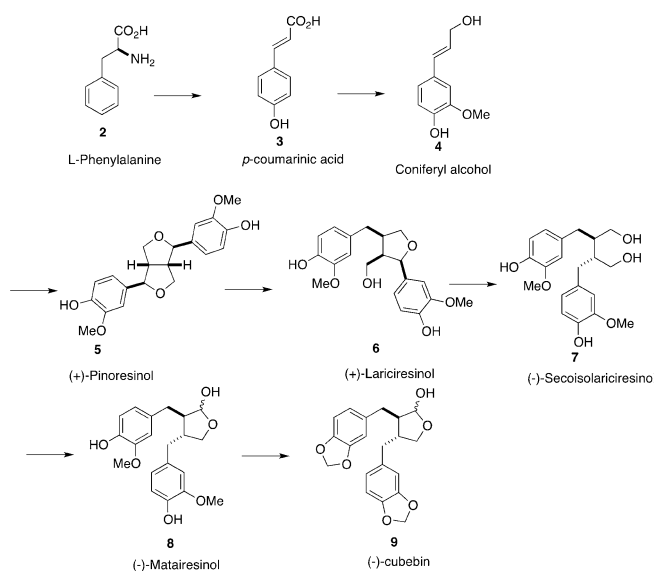

Figure 3. (−)-Cubebin structure as a mixture of epimers at C-9′.[33]

3.1. Synthesis

3.1.1. Biosynthetic Pathways

In 2017, Pissurno and Laurentiz proposed biosynthetic pathways for lignans containing oxygen atoms at the C-9 and C-9′

positions (Scheme 1). The biosynthesis of these lignans is initiated by enantioselective dimerization of two units of coniferyl alcohol (**4**) to provide pinoresinol (**5**), which is reduced to lariciresinol (**6**) to produce secoisolariciresinol (**7**). Compound **7** is then oxidized by secoisolariciresinol dehydrogenase to produce matairesinol (**8**). Cubebin (**9**) is obtained through condensation of the hydroxy and methoxy groups on the aromatic rings thus to produce the methylenedioxy group.[11]

Scheme 2. Semisynthetic derivatives of (−)-cubebin. Reagents and conditions: a) Ac$_2$O, pyridine, RT, 24 h; b) NaH, BnBr, THF, RT, 24 h; c) PCC, CH$_2$Cl$_2$, RT, 12 h; d) HNO$_3$, 2 h, −10 °C.[8, 35]

Scheme 1. Proposed biosynthetic pathways for cubebin.[11, 74]

Scheme 3. Compounds **15 a–i** as amide derivatives of (−)-hinokinin.[2]
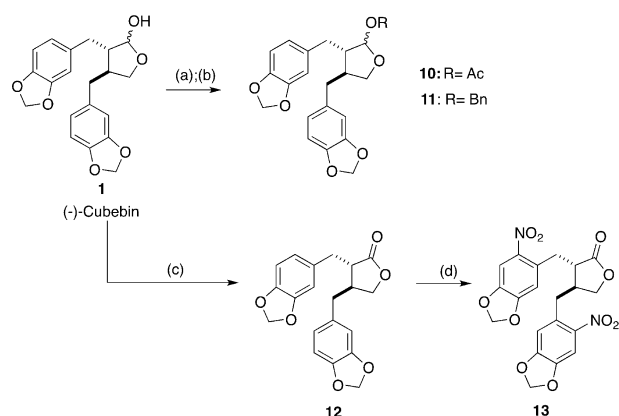
3.1.2. Structure Determination and Chemical Synthesis

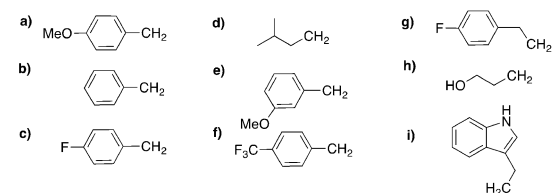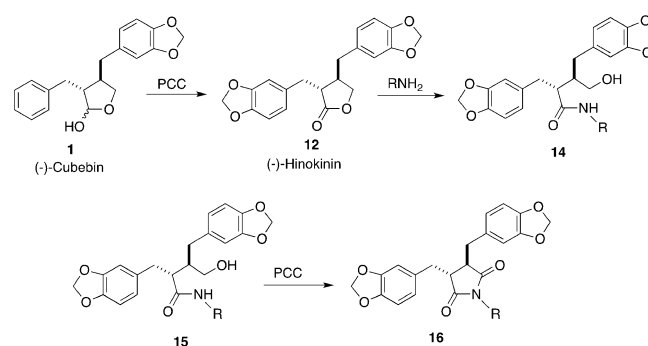#### 3.1.2.1. Semisynthetic Derivatives of (−)-Cubebin

Semisynthetic derivatives of (−)-cubebin (**1**) have been obtained by transformation of natural compound **1** by using the reagents and conditions indicated in Scheme 2.[2, 8, 29, 30, 34–36]

In 2005, De Souza et al. developed novel therapeutic compounds for the treatment of Chagas disease and studied their biological activity against the amastigote forms of lignans **1**, **10**, **11**, and **12** (Scheme 2).[35] Of the (−)-cubebin derivatives tested, the most promising was (−)-hinokinin (**12**).[35]

In 2016, Rajalekshmi et al.[2] isolated a lignan lactol then known as (−)-cubebin from *Piper cubeba* seeds and prepared several derivatives by modifying the lactol ring to obtain different types of functionalities. The authors tested their compounds for in vitro anticancer activity against six human cancer cell lines (i.e. A549, K562, SiHa, KB, HCT116, and HT29) by using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. In that study, cell death occurred through necrosis or apoptosis, and morphological analysis was performed and two cell lines (A549 and KB) were selected. Comparison with literature values obtained for podophyllotoxin and etoposide that were then used as chemotherapeutic agent showed that these lignans possessed promising in vitro anticancer activity. In the study, the cells were treated with (−)-hinokinin and amide derivatives **15** (Scheme 3), which

showed better results than the parent compounds, cubebin/hinokinin, in several of the tested cell lines.[2]

3.1.2.2. Assignment of Structure and Absolute Stereochemistry of (−)-Cubebin and Some Bicubebins

In 1969, the first synthesis of cubebin was described[30] on the basis of the structure and absolute stereochemistry proposed by Haworth,[37] Mameli,[38] and Lin.[39]

Generally, cubebin, whether obtained from a natural source or by synthesis, has been assumed to be a mixture of two 8,8′-*trans*-lactol epimers at C-9 (Figure 4)[6, 40–45] with the relative configuration at C-9 not defined.[33, 35]

In 2006, De Pascoli et al. published very informative work[46] concerning the configurational analysis of cubebins and characterization of the first bicubebin. Complex mixtures of cubebins obtained from an ethanol extract of the tubercula of
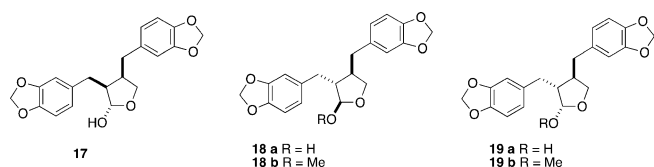
Figure 4. Proposed cubebin structures: mixture of two 8,8′-isomers and epimers at C-9′.

A. pubescens and an acetone extract from the roots of *A. lagesiana* were subjected to preparative chromatography followed by semipreparative HPLC to obtain four different compounds. Their structures were determined by spectroscopic methods, including ^{1}H NMR, ^{13}C NMR, UV, and IR spectroscopy. On the basis of previous reports on cubebin,[6,40,41,47] the structure of *cis*-cubebin (**1**) was suggested (Figure 4) after careful analysis of ^{1}H–^{1}H COSY, gHMQC, and gHMBC data, and precise assignment of all of the hydrogen and carbon atoms in the basic structure [(8S,8′R,9S)-cubebin (**1**)] were made.

Comparing the spectral data obtained for compounds **18** and **19** with those of **17** it was suggested that the mixture also contained (8R,8′R,9R)-cubebin (α-cubebin or epicubebin) (**18a**) and (8R,8′R,9S)-cubebin (**19a**). Curiously, it was noticed that the anomeric hydrogen atoms of **18a** and **19a** showed the same chemical shift at $\delta = 5.15$ ppm but had different coupling constants (d, $J = 1.5$ and 4.5 Hz), whereas the anomeric carbon atoms showed signals at $\delta = 103.3$ and 98.8 ppm for **18a** and **19a**, respectively. Also, ^{1}H–^{1}H COSY experiments showed strong correlations between all four carbinolic hydrogen atoms (dH-90: 4.03, 3.93, 3.73, and 3.50). On the basis of these data the authors suggested two hypotheses: there were two epimers (at C-9 and/or C-8) in equilibrium and the cubebin species existed in equilibrium due to the fact that lactols can undergo ring opening and closing in solution.

In another approach to try to confirm the relative configurations and the absolute configurations of compounds **17**, **18**, and **19**, the authors transformed these compounds into hinokinins that had already been reported.[30,48] The results confirmed that anomers **18a** and **19a** had the same configuration [i.e. (8R,8′R)] at the same chiral centers.

Finally, another compound was isolated and its structure was elucidated by using ^{1}H NMR and ^{13}C NMR spectroscopy, HMQC, and ESI-MS experiments by comparing all of the NMR spectroscopy data obtained for this compound with those for **18** and **19**, in addition to the data obtained by 1D NOESY experiments. On the basis of the results, the stereochemistry was assigned as the dimer (8R,8′R,8″R,8‴R,9R,9″S)-cubebin (**20**) (named bicubebin A), and this compound was the first bicubebin described. Compound **20** could be formed through dehydration of two cubebin units (Figure 5).

More recently, Davidson et al.[49] explored the synthesis of cubebin, as outlined in Scheme 4. Stereoselective allylation of the enolate of piperonyl acetic acid derivative **21** having an Evans chiral auxiliary afforded compound **22**. This was dihydroxylated to form **23** that cyclized to form lactone **24**. Reduction to the triol and oxidative cleavage produced the hydroxy aldehyde that spontaneously cyclized to form the correspond-
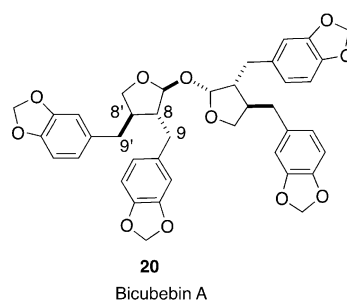
Figure 5. Structure of bicubebin A.[45]
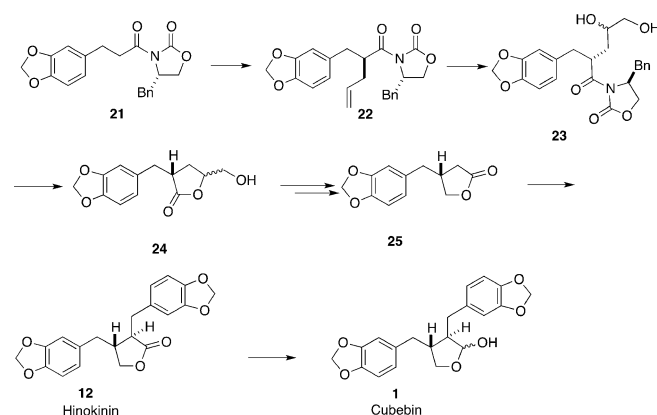
Scheme 4. Summary of the synthesis of (−)-cubebin.[49]

ing lactol. Mild oxidation produced lactone **25** with the carbonyl positioned such that stereoselective transalkylation of its enolate with piperonyl bromide produced hinokinin (**12**), which was reduced to cubebin (**1**) with diisobutylaluminum hydride (DIBAL-H).

A dehydration reaction using copper reagents produced the dimers through an acetal linkage (Figure 6).

The unsymmetrical dimer (−)-bicubebin A (**20**) was previously described by De Pascoli et al.[46] but the other two symmetrical bicubebins, that is, bicubebin B (**26**) and bicubebin C (**27**), had not previously been reported. Comparison of the spectro-
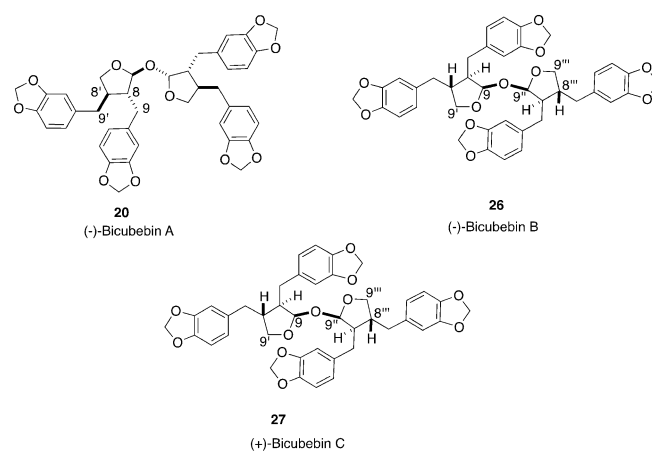

Figure 6. Bicubebins obtained by dimerization of (−)-cubebin.[48]

scopic data of synthetic optically pure bicubebin A with those described in the literature[46] confirmed the absolute stereochemistry as (8R,9S,8′R,8′′R,9′′R,8′′′R).

Further, the authors compared the analytical data for (−)-bicubebin B (**26**) with those reported for (−)-*cis*-cubebin (**17**), which had been isolated along with (−)-bicubebin A, and they found that the ^{1}H NMR and ^{13}C NMR spectroscopy data and optical rotation values overlapped.

Taking these results together, Davidson et al.[49] believed that the structure proposed in the literature as *cis*-cubebin was in fact a symmetrical dimer and not the lactol reported until then. To confirm this interpretation, racemic *cis*-cubebin was prepared (Scheme 5).
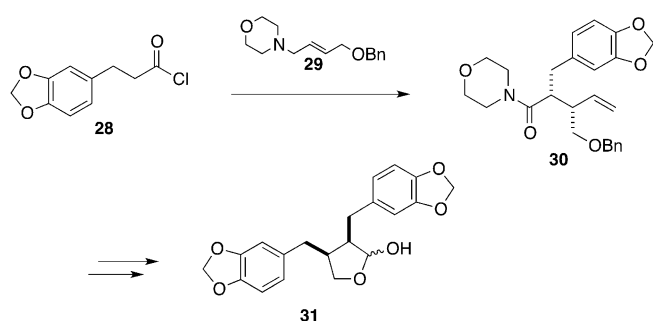
Scheme 5. Synthesis of racemic *cis*-cubebin.

The synthesis of racemic supposed *cis*-cubebin (Scheme 5) started from picolinylacetyl chloride (**28**), which was treated with allyl amine **29** in an acid-catalyzed acyl–Claisen rearrangement to form morpholinide **30** stereoselectively. Having all of the stereochemistry defined, simple transformations produced *cis*-cubebin (**31**) as a mixture of ketols.

In addition, the spectroscopic data for compound **31** obtained through this stereoselective synthesis were very different from those described in the literature for *cis*-cubebin by De Pascoli et al.[46] Thus, this result confirmed that the structure described in the literature until then as *cis*-cubebin was not lactol **17** (Figure 4), but a dimer (−)-[bicubebin B] (**26**) (Figure 6).

3.2. Biological Activities of (−)-Cubebin

Although (−)-cubebin has been known for over 100 years, during the last decades this molecule has attracted the attention of several research groups because of the interesting biological properties attributed to the extract of the fruits of *P. cubeba*, in which it occurs to the extent of 3%.[1]

3.2.1. Cytotoxic Activity

The cytotoxicity of cubebin has been investigated by several authors against different cancer lines: A549 (human lung adenocarcinoma), KB (human nasopharyngeal carcinoma), K562 (human chronic myeloid leukemia), SiHa (human cervical carcinoma), and HCT116 (human colon carcinoma) and HT29

(human adenocarcionoma).[2] (−)–Cubebin possesses very good activity against the A549, K562, and KB cell lines, with median inhibitory concentration (IC$_{50}$) values below 9 μM, but it is less effective against other cell lines.[2]

In human colon adenocarcinoma cells (HT29) treated with (−)-cubebin[33] at concentrations up to 28 μM, cytotoxicity, mutagenicity, apoptotic cell death, and enhanced growth were not observed. (−)-Cubebin was cytotoxic at 280 μM and decreased cell viability by approximately 50% after treatment for 24 h. Lower concentrations caused no apparent damaging effects, but caution is needed upon using (−)-cubebin at high concentrations.[33]

3.2.2. Anti-inflammatory Activity

Inflammation can be caused by cancer development, and for this reason, nowadays, the anti-inflammatory activity of natural compounds is broadly studied.[34] (−)-Cubebin, isolated from the crude hexane extract of the leaves of *Z. Naranjillo*,[31] showed significant anti-inflammatory activity in paw edema induced by carrageenan in rats; it showed 53% inhibition of edema. In the evaluation of analgesic activity by using a 0.6% acetic acid induced abdominal contortion model, (−)-cubebin presented 50% inhibition of animal contortions relative to the control group.[31]

In 2013, Perazzo et al. studied the anti-inflammatory effect of *P. cubeba L.* seed extract (PCE) and its fractions through in vivo assays on rat paw carrageenan-induced edema, and the median effective dose (ED$_{50}$) and on-ear edema induced by croton oil were investigated.[3] This study showed that all fractions of PCE obtained with different organic solvents showed different degrees of inhibition of carrageenan-induced edema. Moreover, the methylene chloride fraction showed the best activity, which indicated that the active compounds were concentrated in this fraction. These compounds were probably cubebins that were previously described as anti-inflammatory agents.[3, 31]

In 2013, Mukhija and Sundriyal reported that the lignan isolated from the crude hexane extract of the leaves of *Zanthoxylum naranjillo* showed significant anti-inflammatory activity in paw edema induced by carrageenan in rats but did not provide a significant reduction in cell migration for the acute carrageenan-induced inflammatory reaction in the peritoneal cavity of rats. Furthermore, this lignan significantly reduced edema induced by prostaglandin PGE2 and the number of writhings (amount of discomfort) induced by both acetic acid and PGI2 in mice. The mechanism of action of (−)-cubebin is similar to that observed for most nonsteroidal drugs.[50]

3.2.3. Antiparasitic Activity

(−)-Cubebin showed interesting activity against *Trypanosoma cruzi*, the parasite responsible for Chagas disease, a neglected protozoan disease that affects some 10 million people in Latin America.[7, 34, 35, 51, 52]

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In 2005, De Souza et al. reported the trypanocidal activity of (−)-cubebin and its semisynthetic derivatives against free amastigote forms of *T. cruzi*.[35]

In 2010, Esperandim et al.[34] evaluated the trypanocidal activity of cubebins and hinokinins in vivo during the chronic Chagas disease phase. In that study, Albino BALB/c mice were divided into groups according to the drug administration type [oral (p.o.) and intraperitoneal (i.p.)] and dosage (20 and 50 mg kg^{-1}). The groups included a negative control, which was separated (inoculated with trypomastigotes) and treated with the solvent used to prepare the solutions; a positive group, which was treated with benznidazole; and an negative group. In all cases, treatment was initiated 90 days after infection and parasitism reduction was assessed by β-galactosidase quantification. Relative to the group treated with benznidazole, the group treated with lignans resulted in a greater reduction in parasitism in all organs evaluated. Oral treatment showed more effective results. The data suggested that cubebins and their analogues could be considered as potential compounds for the development of new drugs against Chagas disease.[34]

In 2013, Esperadim et al. conducted an in vivo study to verify cubebin activity against *T. cruzi*. In this study, BALB/c mice were inoculated with 2×10^4 trypomastigote forms 48 h before treatment. The mice were divided into six groups during the acute phase of *T. cruzi* infection: negative control (5% i.p. injection with 5% DMSO, 2.5% Tween, 5% ethanol), positive control (benznidazole, 20 and 50 mg kg^{-1} p.o.), and cubebin (20 and 50 mg kg^{-1} p.o.). The animals with acute parasitaemia were investigated by morphometric tissue analysis. There was a significant parasitaemia reduction in animals treated with cubebin compared to the negative control.[53]

3.2.4. Antitumor Activity

Carcinogenesis is a complex process that occurs by the interaction of a carcinogenic agent (or oncogenic agent) with genes that changes cell characteristics and results in the loss of control of cell division and culminates in the uncontrolled growth of neoplastic cells.[6]

In 2015, Graidist et al. determined the cytotoxic effect of crude *P. cubeba* extracts on normal fibroblast (L929), normal breast (MCF-12A), and three breast cancer cell lines (i.e. MCF-7, MDA-MB-468, and MDA-MB 231). Fraction C was further separated into seven fractions, CA to CG. The ^{1}H NMR spectrum showed that fraction CE consisted mainly of long-chain hydrocarbons.[54] Jiménez-Arellanes et al. recently reported a study on the action of (−)-cubebin extracted from *Aristolochia elegans Rhizomes* against Mycberculosis H37Rv. An encouraging minimum inhibitory concentration of 50 µg mL^{-1} was found, and the authors concluded that this compound could be used as a lead for the synthesis of derivatives with greater antimicrobial action.[55]

3.2.6. Erectile Dysfunction Activity

Erectile dysfunction (ED) is defined as the inability to achieve or maintain an erection for satisfactory sex. Prevalence of ED increases with age, and it is most commonly associated with poor cardiovascular health, psychosocial factors, hormonal disorders, recreational drug abuse, and adverse effects from prescribed medications.[56]

Carvalho et al. investigated the effect produced by (−)-cubebin from the dried seeds of *P. cubeba* on the contractility and relaxation of rat aortic rings with phenylephrine to evaluate the possible mechanism involved. It was suggested that (−)-cubebin elicited endothelium-dependent and endothelium-independent vascular relaxation in rat aorta mediated by the nitric oxide (NO)/cyclic guanosine monophosphate (cGMP) signaling pathway without prostanoid participation.[57]

3.2.7. Antibacterial Activity

In 2016, Rezende et al. studied the antibacterial activity of (−)-cubebin and its semisynthetic derivatives. Evaluation of the antibacterial activity was performed by using the broth microdilution technique to determine the minimum inhibitory concentration and the minimum bactericidal concentration against *Porphyromonas gingivalis*, *Prevotella nigrescens*, *Actinomyces naeslundii*, *Bacteroides fragilis*, and *Fusobacterium nucleatum*. In this study, it was possible to obtain relevant data concerning the relationship between structure and antimicrobial activity for (−)-cubebin and other lignan derivatives against microorganisms that cause root-canal infections. This study allowed determination of which substituent groups were important to maintain or increase the antimicrobial activity of these compounds.[29]

3.3. Drug-Delivery Studies

In 2012, Saraiva et al. began a study on the encapsulation of the lignan cubebin in microparticulate polymers, as poly(D,L-lactide-co-glycolide), for drug delivery. The microparticles were able to sustain the release of the drug for a considerable period of time; this allowed for a reduction in the required frequency of administration, which increased patient compliance, avoided plasmatic fluctuations, decreased side effects, and facilitated dosage administration.[58]

4. Hinokinin

(−)-Hinokinin, a dibenzylbutyrolactone lignan (Figure 7), is the oxidized form of the cubebin lactols and is found to accumulate in significant quantities in extracts of *P. cubeba*. Furthermore, (−)-hinokinin has been reported to exhibit potent biological activities such as trypanosomicidal activity,[7,35,52,59] anti-inflammatory activity,[12,31,50,60] and analgesic effects.[3,7,61]

4.1. Synthetic Approaches Described

4.1.1. Biosynthetic Pathways

In 2008, Bayindir et al. isolated (−)-hinokinin from callus cultures of *Linum corymbulosum* and proposed two possible hypothetical pathways for the biosynthesis of (−)-hinokinin starting from (+)-pinoresinol (Scheme 3).[10,62]
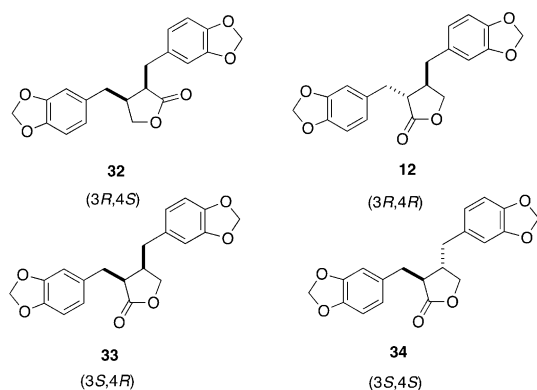
Figure 7. Possible chemical structures of hinokinin. Enantiomer pairs for *cis*-**32**/**33** and *trans*-hinokinin (**12**/**34**).[10] (−)-Hinokinin (**12**)[36] and (+)-Hinokinin (**34**).[65]

In the first pathway, (+)-pinoresinol is reduced to (−)-secoi-solariciresinol by a pinoresinollariciresinol reductase (PLR). Then, (−)-matairesinol is formed by secoisolariciresinol dehydrogenase, and (−)-hinokinin is synthesized by the formation of the methylenedioxy bridges.[62]

In the second pathway, the methylenedioxy bridges are formed directly on (+)-pinoresinol by piperitol–sesamin synthase to give (+)-sesamin and may be converted into (−)-dihydrocubebin and hinokinin. By the isolation of the PLR, (−)-hinokinin could be formed by a secoisolariciresinol dehydrogenase-like enzyme.[62,63]

4.1.2. Chemical Synthesis

4.1.2.1. Total Synthesis of (−)-Hinokinin

In 2015, Zhou et al. outlined the total synthesis of the lignan (−)-hinokinin (**12**) in eight steps. The synthesis is based on a three-step cascade reaction involving a highly stereoselective Michael addition, anion-oxidative hydroxylation, and oxygen-anion cyclization to construct the pivotal butyrolactonimidate (Scheme 6).[64]

The carbanion of optically pure sulfoxide **35** reacted with unsaturated malonate **36** in a highly enantio- and diastereoselective manner to form the essential stereochemical centers. Oxidation of the malonate species permitted formation of iminolactone **37**. Acidolysis by using trifluoroacetic acid (TFA) afforded lactone **38**. Monodecarboxylation followed by surprisingly chemoselective reduction of the remaining ester group afforded primary alcohol **39**, which underwent free-radical-mediated reduction of the carbonyl group by using a metal catalyst to give **40**. Oxidation of **40** with the use of pyridinium chlorochromate (PCC) gave the target product, (−)-hinokinin.

4.1.2.2. Synthesis of (−)-Hinokinin by Oxidation of (−)-Cubebin

In 2008, Andrade et al. obtained excellent results upon using catalysts based on Fe–porphyrins (Scheme 7) and different re-oxidants. The conversion of (−)-cubebin into (−)-hinokinin was
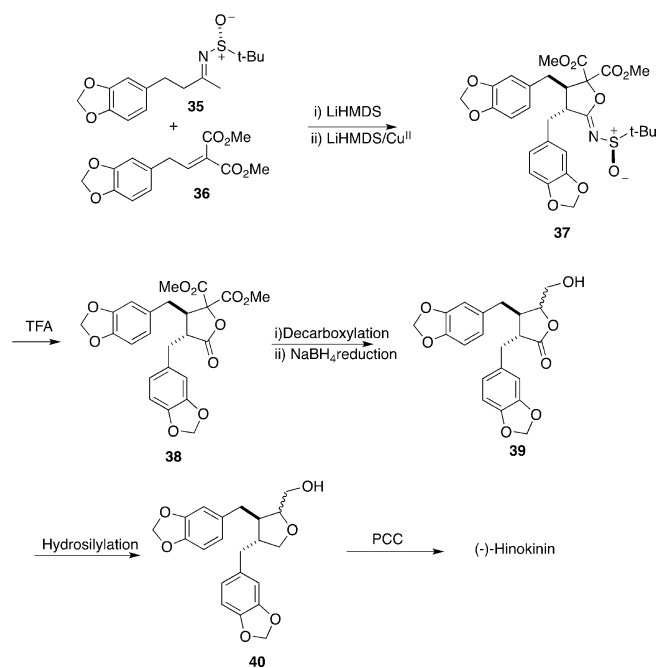
Scheme 6. Synthesis of (−)-hinokinin.[63] LiHMDS = lithium hexamethyldisilazide.
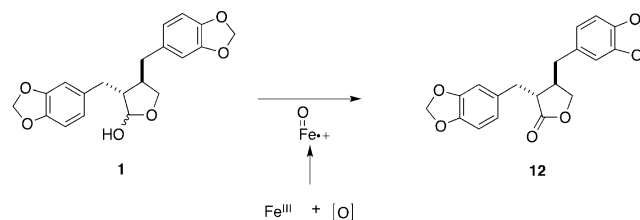
Scheme 7. Preparation of (−)-hinokinin (**12**) by oxidation of (−)-cubebin (**1**) catalyzed by Fe–porphyrin.[36]

of the order of 100 %. This oxidation of (−)-cubebin gave better results than oxidation by more toxic heavy-metal-based oxidants such as PCC.[36]

4.1.2.3. Biotransformation of (−)-Cubebin into (−)-Hinokinin

In 2017, Arruda et al. reported studies on the biotransformation of (−)-cubebin by the filamentous fungi *Aspergillus terreus* and *Aspergillus niger* is an efficient bioprocess to obtain (−)-hinokinin (**12**) and (−)-parabenzlactone (**40**) (Figure 8).[65] The advantage was the use of reagents and/or solvents that were less toxic and/or less polluting than those used in the synthesis with PCC. The biotransformation of (−)-cubebin by two *Aspergillus* species was performed, and a validated and reliable reverse-phase HPLC analytical method was developed to quantify the products in fungal extracts.[65]

4.1.2.4. Asymmetric Synthesis of (+)-Hinokinin

In 2007, Enders and Milovanovic published the first asymmetric synthesis of (+)-hinokinin (**34**) by using asymmetric nucleophil-
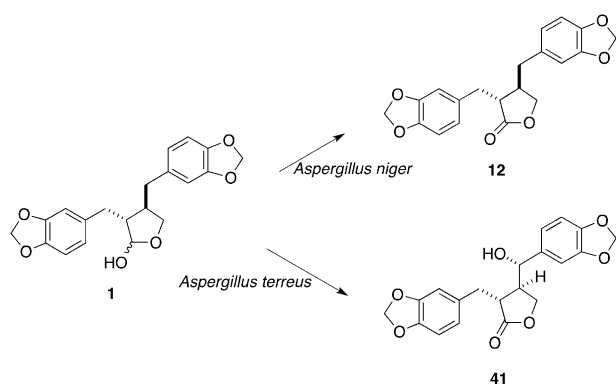
Figure 8. Biotransformation of (−)-cubebin (**1**) into (−)-hinokinin (**12**) and (7′s)-parabenzolactone (**41**).[64]

ic acylation methodology. Using the methodology indicated in Scheme 8, aminonitrile (S,S,R,R,S)-**42** was obtained in quantitative yield with high diastereomeric purity, from which it was possible to provide enantiopure *trans*-2,3-disubstituted γ-butyrolactone (S,S)-**45** with excellent asymmetric induction. Reduction of the ketone to the corresponding alcohol as an epimeric mixture and then hydrogenolysis afforded (+)-hinokinin (**34**) with excellent stereoisomeric purity.[66]
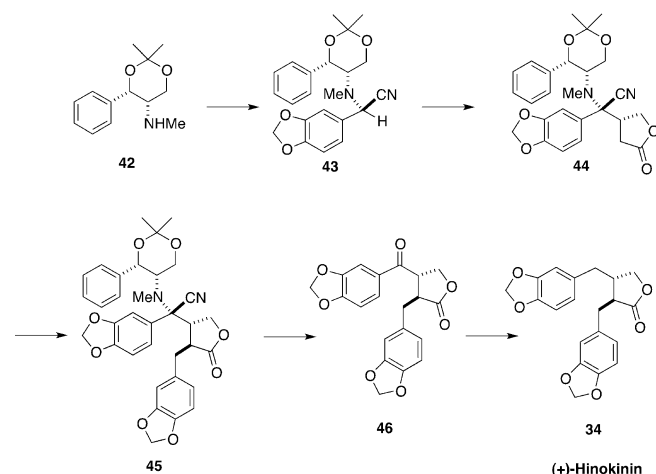
Scheme 8. Asymmetric synthesis of (+)-hinokinin.[65]

4.2. Biological Activities

4.2.1. Cytotoxic Activity

The cytotoxicity of (−)-hinokinin has been investigated against different cancer lines by several authors. Compared to (−)-cubebin, (−)-hinokinin presents better results.[2,67,68]

In 2010, Pusztai et al. isolated (−)-hinokinin (**12**) from the stem bark of the African tree *Pycnanthus angolensis (Welw.) Warb. (Myristicaceae)* and evaluated its potential as a cancer chemopreventative agent by its ability to inhibit HCMV early (IE) antigen expression in human lung adenocarcinoma (A549) cells.[67] Human *cytomegalovirus* (HCMV) was used in a modified

in vitro model to evaluate the potential antitumor-promoting effects of this lignan on human lung cancer cells. Concentrations of $40.72\ \mu g\,mL^{-1}$ caused 50% inhibition of growth (ID_{50}) of A549 lung cancer cells. Significant results were obtained with hinokinin to reduce the IE gene expression of HCMV in a dose-dependent manner.[67]

4.2.2. Anti-inflammatory Activity

In 2013, Desai et al. isolated (−)-hinokinin from the extracts of *Aristolochia indica L.* by using an efficient preparative HPLC method,[69] and it was tested for its anti-inflammatory potential. The authors reported the anti-inflammatory effects of hinokinin against IL-6 [$IC_{50} = (20.5 \pm 0.5)\ \mu M$] and TNFα [$IC_{50} = (77.5 \pm 27.5)\ \mu M$]. Both IL-6 and TNFα are key regulators of inflammation and are implicated in several diseases such as rheumatoid arthritis and colitis.[69]

In 2017, Lima et al. isolated (−)-cubebin from the seeds of *Piper cubeba* and obtained (−)-hinokinin by oxidation with PCC. Using paw edema as the experimental model and different chemical mediators (prostaglandin and dextran), it was observed that both derivatives were active in comparison with both negative (5% Tween 80 in saline) and positive (indomethacin) controls. The reduction in prostaglandin-induced edema with (−)-hinokinin was 59.2%.[12]

4.2.3. Antitumor Activity

In 2016, Cunha et al. evaluated the ability of (−)-hinokinin (**12**) to modulate the antiproliferative effects of doxorubicin (DOX) in tumoral (MCF-7 and SKBR-3) and normal (MCF-10 A) breast cell lines.[68] Treatment with (−)-hinokinin did not affect cellular proliferation or contribute to the antiproliferative effects of doxorubicin in MCF-10A cells. After 24 and 48 h of treatment with (−)-hinokinin, MCF-7 and SKBR-3 were accumulated in G2/M, and if combined with doxorubicin, (−)-hinokinin contributed to the antiproliferative effects of this chemotherapeutic by modulating the cyclin-dependent kinase inhibitor. The clinical implications of the selectiveness of (−)-hinokinin if associated with subcytotoxic concentrations of DOX may be further investigated to reduce the side effects.[68]

4.2.4. Antiparasitic Activity

(−)-Hinokinin (**12**), in later years, has been studied as an interesting antitripanosomal compound.[52,53,58,70,71] In 2005, De Souza et al. initiated a study by using hinokinin in vitro against free amastigote forms of the Y strain of *T. cruzi*. The results were encouraging, as an IC_{50} value of 0.7 μM was measured compared to an IC_{50} value of 0.8 μM for benznidazole.[35]

In 2013, Esperandim et al. reported studies on infections by *Trypanosoma cruzi*. This study has already been referenced in Section 3.2.3. for the lignan (−)-cubebin.[53] The authors described a study criterion in which animals with acute parasitemia were investigated by tissue morphometric analysis. A significant parasitemia reduction was observed in the groups of animals treated with (−)-cubebin (**1**) or (−)-hinokin (**12**) by oral

administration compared to the negative control. For the spleen, a perimeter value of 10.90 µm ($p < 0.05$) was obtained for mice treated with (−)-hinokinin (20 mg kg^{-1}), whereas untreated infected animals showed a perimeter of 11.76 µm. For the liver, perimeter values of 18.61 µm ($p < 0.001$) were achieved for mice treated with (−)-hinokinin at 20 mg kg^{-1}, whereas a perimeter of 18.54 µm was obtained for untreated infected animals. Cytotoxicity assays demonstrated that (−)-hinokinin did not display toxicity. Therefore, both (−)-cubebin and (−)-hinokinin are promising therapeutic agents and could be used in future clinical studies for the treatment of Chagas disease.[53]

4.2.5. Antimycobacterial Activity

In 2010, Silva et al. prepared (−)-hinokinin and evaluated its antimycobacterial activity against *Mycobacterium tuberculosis* (ATCC 27294), *M. kansasii* (ATCC 12478), and *M. avium* (ATCC 15769).[72] (−)-Hinokinin was moderately active against *M. tuberculosis*, with a minimum inhibitory concentration of 62.5 µg mL^{-1}. These are promising results that are important in the search for biologically active natural products, as they highlight the fact that new approaches for the prevention, treatment, and cure of tuberculosis are extremely important.[72,73]

4.3. Drug-Delivery Studies

In 2010, Saraiva et al. loaded (−)-hinokinin into PLGA microparticles for the treatment of Chagas disease.[59] PLGA polymers have generated great interest due to their excellent biocompatibility and biodegradability. In this study, the microparticles formed presented a narrow size distribution and a mean diameter of 0.862 µm with a polydispersity index of 0.072 nm. The encapsulation efficiency of (−)-hinokinin-loaded microparticles with a drug-to-polymer weight ratio of 1:10 was (72.46 ± 2.92)%.[59]

In 2013, Timple et al. examined the lignan (−)-hinokinin with several different human neurotransmitter transporters for potential use in drug therapy.[74] Using in vitro pharmacological assays, (−)-hinokinin selectively inhibited the human dopamine and norepinephrine transporters in a noncompetitive manner, possibly mediated by binding to a novel site within the transporters, and displayed low affinity for the serotonin transporter.[74]

5. Conclusions

Piper cubeba has been widely studied by several research groups. It is a genus that can be found in all continents within several species. The main compounds isolated from two species have shown potential to become new molecules in their natural and semisynthetic forms for therapeutic applications. Researchers have been studying cubebin and its derivatives obtained by semisynthesis and have found great therapeutic perspectives. Many variations of the aromatic ring substituents

of cubebin are possible, and these could be tailored according to the desired biological activity.

Cubebin (1) has proven in vivo efficacy in most of the activities described in this article. Hinokinin is a substance that has demonstrated high analgesic, anti-inflammatory, antimutagenic, chemopreventive and antitumoral activities; it has proven activity against trypanosoma cruzi and antibacterial activity against oral pathogens. Hinokinin certainly deserves attention and has great therapeutic potential. Considering the patents on the use of cubebin and the conduction of nonclinical and clinical trials, in a few years it should certainly be possible to have a new drug based upon these natural products studies.

Recently, the total syntheses of (−)-*cis*-cubebin, (−)-bicubebin A (**20**), (−)-bicubebin B (**26**), and (+)-bicubebin C (**27**) have confirmed their structures and absolute stereochemistry. The proposed structure of an isolated natural product ascribed to the *cis*-cubebin structure has been shown to be incorrect and is in fact (−)-bicubebin B.

In summary, the following absolute stereochemistries have been assigned: (−)-bicubebin A (**20**) as (8*R*,9*S*,8′*R*,8″*R*,9″*R*,8″′*R*), (−)-bicubebin B (or *cis*-cubebin) (**26**) as (8*R*,9*S*,8′*R*, 8″*R*,9″*R*,8″′*R*), (+)-bicubebin C (**27**) as (8*R*,9*R*,8′*R*,8″*R*,9″*R*,8″′*R*), and (−)-hinokinin (**12**) as (3*R*,4*R*).

Conflict of Interest

The authors declare no conflict of interest.

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