Strategies for the synthesis of brevipolides

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Abstract

In recent years fifteen 5,6-dihydro-α-pyrene derivatives, bearing either a distinctive cyclopropane or furan ring and named brevipolides A–O (1–15), have been isolated from the invasive plant *Hyptis brevipes* Poit. Their fascinating structural features, and the potent biological activities, including cytotoxicity against an array of human cancer cell lines and inhibition of the chemokine receptor CCR5, make them attractive synthetic targets. This review article highlights the recent synthetic methodologies and briefly summarizes their biological activities.

Introduction

*Hyptis brevipes* Poit. is an invasive plant species belonging to the mint family *Lamiaceae* [1-4]. This plant has been reported to originate from tropical America but is now distributed broadly in other tropical areas of the world [1-5]. This herbaceous weed plant is often encountered in wastelands, plantation crops, orchards, forest verges, and grows abundantly in the fallow ground [1,3]. Hence, it is a potential threat to the crops and natural vegetation [1]. The extract of this suffruticose plant has long been used in folk medicines to treat headaches (Panama) and diarrhea (Paraguay) [6], for protection after giving birth (Panama, Indonesia, Malaysia) [6-9], against worms for newborn infants (Indonesia) [6], for prevention and treatment of different types of cancer (Indonesia) [10], asthma and malaria (southern Sahara) [2,3], and to combat intestinal parasites (Bolivia) [5]. In addition, this plant has also been used as a natural pesticide, particularly in cereal conservations, and to repel mosquitoes (southern Sahara) [2,3]. Biological activity investigations of the plant extract revealed various results, including toxicity to brine shrimp [9], DNA intercalation as well as antibacterial and fungal [4,9], and strong insecticidal activity against the 3rd instar larva of the cotton leaf worm *Spodoptera littoralis* (Biosd.) [7]. The essential oil extracted from the leaf of *Hyptis brevipes* Poit., furthermore, exhibited free radical scavenging and potential antitumor activities [2]. The ethnomedicinal background and preliminary biological studies triggered researchers to further examine the chemical constituents of the plant.
In 2009, Kinghorn and co-workers reported the first study to determine bioactive chemicals in *Hyptis brevipes* Poit. and isolated six new 5,6-dihydro-α-pyrones 1–6 along with other known compounds, including a 5,6-dihydro-α-pyrene derivative 7 [11], from the whole plant collected in Tawangmangu village, Indonesia (Figure 1) [4]. These six new compounds, 1–6, were given the trivial names of brevipolides A–F, respectively, and the absolute configuration was determined by analysis of data obtained from their CD spectra and by Mosher’s ester formation, as C6*R, C1’S, C2’S, and C4’S. The C6’ stereocenter at that time could not be established due to a rapid epimerization during cinamate hydrolysis. Later, in 2013 Pereda-Miranda and co-workers isolated ten compounds, namely brevipolides A–J (1–10), from the aerial part of *Hyptis brevipes* Poit. collected in Mexico [12]. The C6’S configuration was then determined by X-ray crystallographic data of the hydrogenated brevipolide derivative. It is interesting to note that all the brevipolides A–J (1–10) pose the conserved stereocenters and bear a cyclopropyl unit in the core structure, which are in agreement with the prior structural assignment of compounds 7–9, previously identified as unnamed inhibitors for the chemokine receptor 5 (CCR5), isolated from the Peruvian plant *Lippia alva* in 2004 [11]. Further, in 2017 Pereda-Miranda and co-workers isolated five more new 5,6-dihydro-α-pyrene derivatives, namely brevipolides K–O (11–15), from the same plant [1]. The structures have been determined by a comprehensive combination of quantum mechanical calculations and experimental spectroscopic analysis of their NMR and ECD data, to have a unique tetrahydrofuran ring instead of the cyclopropane functionality. The absolute configuration of these five compounds were evaluated and all conserved as C6R, C1’S, C2’R, C5’S, and C6’S.

Most brevipolide members exhibited cytotoxicity against various targets, including human colon, breast, laryngeal, cervix, prostate, and nasopharyngeal cancer cell lines with $ED_{50}$ and $IC_{50}$ values ranged in micromolar order [1,4,12]. One member showed activity in an enzyme-based ELISA NF-kB assay. Upon the mitochondrial transmembrane potential assay, three members demonstrated $ED_{50}$ values in the nanomolar level [4]. Moreover, three of the members were identified as inhibitors of the chemokine receptor CCR5 [11]. Therefore, they are potential agents for treating human immunodeficiency virus (HIV).

The newly discovered brevipolides with interesting structural features in conjunction with promising biological activities have prompted the researchers to conduct synthetic studies. To date, there are six reported works in the literature for the syntheses of brevipolides, with the following details: two reports present the unsuccessful syntheses of brevipolide H [13,14] and four reports cover the successful syntheses of brevipolide H [15] and its enantiomer [16], brevipolide M [17], and brevipolide N [17,18], respectively.

To the best of our knowledge, this work presents the first review on the synthetic strategies to obtain brevipolides H, M, and N, aiming to achieve a deeper understanding of this area. It

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**Figure 1:** Structures of brevipolides A–O (1–15).
also provides a summary of the biological activities of brevipolides. This present review is chronologically organized, encompassing all synthetic works published since 2014.

Review
Syntheses
Kumaraswamy’s approach to brevipolide H (8)
In 2014, Kumaraswamy and co-workers initiated the first attempt to synthesize brevipolide H (8, Scheme 1) [14]. In the retrosynthesis, the target compound 8 is achieved via esterification of the β-hydroxycyclopropyl intermediate 16 with the commercially available (E)-p-methoxycinnamic acid (17). The vital intermediate 16 is expected from cyclopropyl epoxy alcohol 18, which in turn can be prepared from allylic alcohol 19 via the Furukawa-modified Simmons–Smith cyclopropanation and VO-mediated epoxidation. Acetylfuran (20) is chosen as the six-carbon precursor for the synthesis.

The forward synthesis transformed 2-acetylfuran (20) to its corresponding alcohol 21 through an asymmetric transfer hydrogenation catalyzed by a ruthenium complex (0.5 mol %) in 98% yield with 95% ee (Scheme 2). The azeotropic mixture of HCO₂H/Et₃N 5:2 was employed as the hydrogen source. Following protection of the alcohol moiety with PMBCl, ether 22 was realized in 93% yield. Afterwards, this species was transformed into the γ-keto α,β-unsaturated aldehyde 23 through an NBS-assisted furan oxidation procedure in moderate yield (65%). The keto functionality was reduced in a stereo-selective manner adopting Luche conditions to provide diol 24 (dr 97:3), which after masking the primary alcohol moiety as a silyl ether, allowed isolation of the desired product 25 in 86% yield. This intermediate possessed the correct chirality on the free secondary alcohol to influence the stereo-outcome for the later cyclopropanation step. Thus, treatment of 25 with diethyl-zinc and diiodomethane delivered the expected syn-cyclopropyl carbinol 26 as the major diastereomer (dr 95:5) in 90% yield. After the protection of the secondary alcohol as MOM ether, the primary alcohol was liberated using TBAF to give compound 27 in 97% yield over two steps. The alcohol group in 27 was then oxidized to the corresponding aldehyde under Swern conditions and subsequently subjected to a Wittig reaction with a two-carbon phosphonium ylide reagent. The desired α,β-unsaturated ester 28 was then isolated in 80% yield over two steps. Reduction of the ester provided allylic alcohol 29 (92%) ready for later epoxidation. After considerable optimizations, the authors found that the dropwise addition of TBHP to 29 in refluxing benzene solution containing a catalytic amount of VO(acac)₂ afforded the desired epoxide 30 after one hour in 85% yield (dr 10:1). This species was next converted to the terminal carbonate derivative 31 to transform the epoxy functionality to a vicinal diol through a two-step manipulation involving protection of the terminal alcohol as Boc derivative followed by BF₃·Et₂O-promoted intramolecular oxacyclization. After TBS protection, intermediate 32 was collected in 86% yield from epoxide 30. Basic methanolysis of the cyclic carbonate followed by treatment with NaH and N-tosylimidazole then afforded terminal epoxide 34 in 97% yield. Unfortunately, attempts to open the epoxide using vinyl Grignard reagent followed by esterification with acrylic acid (35) proved to be inefficient due to low reproducibility and poor isolation of product 36.

The strategy was altered by changing the terminal epoxide 34 to an allylic alcohol (95%) utilizing dimethyl sulfonium methyldide followed by esterification with 3-butenioic acid (37) under Steglich conditions (Scheme 3). The resulting product 38 was isolated in 86% yield. A subsequent ring-closing metathesis reaction and DBU-assisted double bond migration then
furnished the anticipated structure 39. The PMB functionality was removed using DDQ to form alcohol 40. At this point, the stereogenic center at the C6’ carbon required an inversion to match the target molecule. Thus, a standard Mitsunobu procedure followed by basic methanolysis were conducted. The desired inverted product 16, however, did not form. The authors hypothesized a prospective possibility to obtain ent-21 from precursor 20 by utilizing an antipode ligand in a Noyori reduction. As the continuation, intermediate 40 was coupled with (E)-p-methoxycinnamic acid (17) under Steglich conditions and treated with a Lewis acid to remove the MOM protection giving ester 41. Oxidation of the secondary alcohol of this intermediate to its keto derivative was problematic and gave no desired product 42 after considerable experimentations. Eventually, the isolation of 43 marked the end of the synthetic study after treatment of 41 with HF·pyridine, which is the reduced form of 6’-epi-brevipolide H. Kumaraswamy and co-workers also performed a bioassay study for compounds 41 and 43 and found a higher cytotoxicity for the latter derivative against the MFC-7 cancer cell line.

Hou’s strategy to ent-brevipolide H (ent-8)
Hou and co-workers, in 2014, demonstrated an efficient approach to synthesize brevipolide H (8), but unexpectedly ended up with the isolation of its enantiomer (ent-8) [16]. Common to the most of the reported retrosynthetic analyses of brevipolide,
Scheme 3: Attempt to synthesize brevipolide H (8) by Kumaraswamy (continued).

Compound 8 is disconnected at the cinnamate ester bond giving β-hydroxy cyclopropyl intermediate 44 and (E)-p-methoxycinnamic acid (17) (Scheme 4). The β-hydroxy moiety in 44 can be installed via Sharpless dihydroxylation of the silyl enol ether derived from ketone 45. The 5,6-dihydro-α,β-pyrone group in ketone 45 is envisaged from protected diol 46 by the sequence of Mitsunobu esterification, ring-closing metathesis, and base-promoted double bond migration. The cyclopropyl functionality in 46 can be assembled from the reaction of sulfur ylide and the α,β-unsaturated ketone 47, which in turn can be realized from the cross metathesis between commercially available ethyl vinyl ketone (48) and the C2-symmetrical diene-diol 49.

The synthesis commenced with the monoprotection of the known diene-diol ent-49 as TBS ether affording compound 50 in 76% yield, which served as an excellent strategy to direct the later cross metathesis reaction to one site of the olefin over another (Scheme 5). Thus, the desired α,β-unsaturated ester 51 was afforded in 78% yield as an (E)-isomer exclusively after reaction with excess ethyl vinyl ketone (48) in the presence of a catalytic amount of Grubbs II catalyst and Cul. The free secondary alcohol in 51 required a protection prior to the next transformation. For this purpose, MEMCl was chosen instead of the previously arranged MOMCl, as the MEM functionality was found to have a better impact on the stereoselectivity of the later cyclopropanation reaction. Thus, the MEM ether 52 was obtained in 95% yield and subjected to in situ prepared dimethyl sulfoxonium methylide at low temperature to affect the Michael-initiated ring closure cyclopropanation at the more electrophilic olefin. The α-keto cyclopropyl intermediate 53 was formed in 79% yield with a dr value of >20:1. Reducing the proportion of DMF to 5% with respect to THF in the reaction mixture was hypothesized as the key factor for maximizing the anti-addition of the sulfoxonium ylide to 52. Hou highlighted that the good diastereoselectivity control for the sulfoxonium ylide addition to acyclic α,β-unsaturated substrates such as 52 observed in their work represented the first example in literature. Hereupon, deprotonation of 53 over LiHMDS followed by addition of TBSOTf at low temperature successfully formed the (Z)-silyl enol ether 54. Application of the Sharpless asymmetric dihydroxylation, promoted by AD-mix-β, gave the expected β-(R)hydroxy cyclopropyl product 55 in 84% yield with moderate diastereoselectivity (dr = 2). The formation of ester 56 was achieved through reaction of 55 with the pre-activated acid 17 with N,N'-diisopropylcarbodiimide (DIC) and DMAP. Removal of the TBS protection with a fluoride source and succeeding Mitsunobu inversion with 3-butenolic acid (37) went smoothly to give ester 57. This intermediate accommodated all the centers of the stereochemical brevipolides but in the mirror image of the natural configuration. After sequential ring-closing metathesis, base-promoted olefin migration, and MEM removal, the ent-brevipolide H (ent-8) was obtained. This compound showed inhibition activity in the preliminary assay against the cell proliferation of the human hormone-refractory prostate cancer cell line (PC-3).
Scheme 4: Retrosynthetic analysis of brevipolide H (8) by Hou.

Scheme 5: Synthesis ent-brevipolide H (ent-8) by Hou.
Mohapatra’s strategy to brevipolide H (8)

As part of the interest in synthesizing biologically active cyclopropane-containing natural products, Mohapatra and co-workers in 2015 started their attempt to prepare brevipolide H (8) [13]. The retrosynthesis started with envisaging a common 12-carbon intermediate 59 which can be derived from unsaturated ketone 60 (Scheme 6). The cyclopropyl moiety in 60 can be realized from allylic alcohol 61 via the Furukawa’s modified Simmons–Smith cyclopropanation protocol. Eventually, trans-crotonaldehyde (62) is selected as the precursor for this study.

The study began with the enantioselective epoxidation of trans-crotonaldehyde (62) under Jørgensen conditions using organocatalyst 63, followed by a two-carbon homologation to obtain α,β-unsaturated epoxy ester 64 in 78% yield over two steps (dr 95:5, de 93:7) (Scheme 7). Then, the epoxide ring was opened regioselectively by p-methoxybenzyl alcohol utilizing a palladium(0) catalyst to afford the secondary allylic alcohol 65 in 96% yield. The free alcohol group was protected as tert-butyldimethylsilyl (TBS) ether 66 (95%) and the ester group was reduced to the primary alcohol 67 (95%). After protection as tert-butyldiphenylsilyl (TBDPS) ether 68 (98%), a Simmons–Smith cyclopropanation was attempted yet no desired product was obtained. Hence, the PMB-protecting group was first removed and the cyclopropyl product 69 was successfully attained in 97% yield with very high diastereoselectivity (dr 99:1). The free secondary alcohol group was re-protected as MOM ether 70 in 96% yield. After removal of the TBDPS group, the resulting free primary alcohol was oxidized under Dess–Martin conditions followed by Grignard reaction with vinylmagnesium bromide. The allylic alcohol products 71 and 72 were obtained as a diastereomeric mixture in 86% yield with poor stereoselectivity.

To gain more of the desired diastereomer 71, the mixture of 71 and 72 was subjected to a two-step procedure involving a Dess–Martin oxidation followed by stereoselective reduction (Scheme 8). Among a selection of reagents, Mohapatra found that lithium tri-tert-butoxylaluminum hydride in ethanol at low temperature furnished 71 as a single diastereoisomer in 94% yield. The allylic alcohol moiety was protected as TBDPS ether 73 (92%) and oxidatively cleaved following Jin’s one step dihydroxylation–oxidation protocol using a NaIO4/(cat.) OsO4 system. Allylation of the resulting aldehyde 74 was best performed under Brown’s protocol at low temperature utilizing a chiral allyl reagent prepared from allylmagnesium bromide and (+)-B-chloro-diisopinocampheylborane. By this route, the alcohol product 75 was isolated in 81% yield (dr 85:15). Esterification of this molecule with acryloyl chloride (76) went smoothly providing the diene 77 (85%), which, after the sequential ring-closing metathesis with Grubbs I catalyst and TBS removal, gave the 5,6-dihydro-α-pyrone 78 in 71% yield over two steps. The stereocenter at the C6’ carbon demanded an inversion to fit the natural form. The standard Mitsunobu inver-
**Scheme 7:** Attempt to synthesize brevipolide H (8) by Mohapatra.

**Scheme 8:** Attempt to synthesize brevipolide H (8) by Mohapatra (continued). (+)-(IPC)_2-BCl = (+)-B-chloro-disopinocampheylborane.
sion protocol, unfortunately, failed to reach the expected transformation. The isolation of 78 ended their attempt to synthesize brevipolide H.

**Hou’s strategy to brevipolide H (8)**
Two years after the successful synthesis of ent-brevipolide H (ent-8), in 2016, Hou and co-workers reported the first total synthesis of natural brevipolide H (8) [15]. The retrosynthesis was initiated by disconnection of the cinnamate ester bond to give intermediate 79. The 5,6-dihydro-α-pyrone moiety is obtained via ring-closing metathesis reaction of acrylate ester 80, which in turn can be proposed from allylic alcohol 81 via Furukawa’s modified Simmons–Smith cyclopropanation (Scheme 9). The species 81 can be constructed through the sequential epoxide opening and esterification of compound 82. This molecule is expected to be available from cross metathesis of olefins 83 and 84. These two intermediates can then be readily prepared from optically active oxirane 85 and its enantiomer which can be derived through the Sharpless epoxidation of penta-1,4-dien-3-ol (86).

In the synthesis, desymmetrization of 86 was achieved under Sharpless epoxidation conditions employing a t-BuOOH/(+)-DIPT/Ti(O-iPr)4 system to give epoxide 85 (63%), which was subsequently protected as PMB ether 84 in 86% yield (Scheme 10). In parallel, the same precursor 86 was subjected to another Sharpless epoxidation using (−)-DIPT affording ent-85 in 65% yield. A series of functional group transformations involving hydroxy group protection, reduction of the epoxide, protection of the resultant free alcohol as TBS ether, and removal of the acetal protection afforded the expected allylic alcohol 83. Accordingly, cross-metathesis reaction between 83 and 84 was successfully achieved utilizing Grubbs II catalyst to give the adduct 82 in 76% yield. The E/Z ratio for this compound was determined from the 1H NMR spectral analysis as >20:1. After protection of the secondary alcohol as MEM ether 88 (83%), the epoxide ring was opened with vinylmagnesium bromide to give the allylic alcohol 89 (92%). The acrylate ester 90 was smoothly obtained from this molecule in 95% yield and deprotected using TMSBr to give the allylic alcohol 81. Subjection of this compound to a system containing Et2Zn/CH3I2 successfully furnished the expected syn adduct in 65% yield as a single diastereomer. Oxidation of this molecule under Swern conditions proceeded smoothly giving ketone 91 in 94% yield, which was highlighted as an important observation as the same transformation using the similar compound was reported to be unsuccessful [14]. Ring-closing metathesis of this compound installed the 5,6-dihydro-α-pyrone moiety, and TBS removal followed by esterification with 4-methoxycinnamic acid provided compound 92. After removal of the PMB group using DDQ, the natural brevipolide H (8) was successfully achieved.

![Scheme 9: Retrosynthetic analysis of brevipolide H (8) by Hou.](image-url)
Sabitha’s strategy to brevipolide M (13)

Sabitha and Raju demonstrated the first total synthesis of brevipolide M (13) in 2017 [17]. In the retrosynthesis, compound 93 is hypothesized from the Mitsunobu inversion at the C6’ stereocenter with (E)-p-methoxycinnamic acid (17) and ring-closing metathesis of tetrahydrofuran 93 (Scheme 11). The olefin moieties can be installed via stereoselective allylation and cinnamic acid esterification of 94, which is derived from symmetrical alcohol 95. The right-hand side portion of this molecule can be constructed by epoxidation of the allylic alcohol derived from α,β-unsaturated ester 96 and the cyclization to form the furan ring is then expected to occur concurrently. (−)-Diethyl tartrate (98) was eventually selected as the precursor to construct the unsaturated ester 96 after a double two-carbon homologation.

In the reverse order, the synthesis of 13 began with the conversion of diethyl tartrate 98 into the PMB-protected α,β-unsaturated ester 97 in four steps adopting the literature procedure (Scheme 12). The chemoselective reduction of the olefin in 97 was furnished in 88% yield applying NiCl₂·6H₂O/NaBH₄ as reagents in methanol followed by reduction of the ester part with DIBAL-H providing the primary alcohol 99 (90%). Oxidation of this moiety with IBX to its corresponding aldehyde served as a substrate for the two-carbon homologation via Wittig reaction giving ester 96 in 80% yield over the two steps. After reduction of the ester group to its primary alcohol counterpart, the Sharpless epoxidation protocol was applied to the double bond using (−)-DET to afford chiral oxirane 100 in 85% yield. The free primary alcohol was then protected as tosyl ester 101 and treated with p-TSA to induce intramolecular cycliza-
tion. The anticipated furan 95 was successfully isolated in 85% accompanied with the desired stereochemistry inversion at the C5’ carbon. Treatment with excess NaH furnished a terminal epoxide derivative which after the sequential treatment with benzyl bromide and reduction with LiAlH₄ afforded alcohol 102 (81% yield from 95). The free secondary alcohol was then protected as TBS ether 94 (90%) and the PMB ether was cleaved to liberate the primary alcohol. After being oxidized with IBX, the aldehyde 103 was isolated in 68% yield over two steps. Application of the asymmetric Brown’s allylation afforded 104 in 80% yield (dr 95:5) that was readily esterified to its cinnamate ester derivative in 80% yield. The TBS protecting group was removed under acidic conditions to give secondary alcohol 93 (85%). Afterwards, the 5,6-dihydro-α-pyrone moiety was constructed through cross-metathesis protocol and the stereochemistry at C6’ was inverted with (E)-p-methoxycinnamic acid (17) via a Mitsunobu esterification. The resulting product 106 contained all the correct stereochemistry, which after removal of the benzyl protection, provided the target molecule brevipolide M (13) as a colorless oil.

**Sabitha’s strategy to brevipolide M and N (13, 14)**

Following the previous success, Sabitha and Raju reported another approach to synthesize brevipolide M (13) which was shorter and more efficient than the former strategy [18]. Furthermore, this improved strategy was applied to achieve the first total synthesis of brevipolide N (14) by utilizing a different acid counterpart in the late esterification step. In the retrosynthesis, compound 107 is conceived as the branching intermediate to access both brevipolides M and N (Scheme 13).

The synthesis commenced with the reaction between aldehyde 111 and phosphonate ester 112 using Ba(OH)₂·8H₂O to provide the unsaturated ketone 110 in 85% yield (Scheme 14). Application of a tandem 1,4/1,2-reduction to this compound under Noyori conditions gave the expected diastereomer 113 as the major product in 90% yield (dr 96:4). After protection of the resulting free secondary alcohol as the tosyl ester 114 (88%), this molecule was treated with an acid to remove the isopropylidene protection and induce intramolecular cyclization to the 2’,5’-
Scheme 12: Synthesis of brevipolide M (13) by Sabitha.

Scheme 12: Synthesis of brevipolide M (13) by Sabitha.

Syn-furan 109 in 80% yield. This triol was subjected to excess sodium hydride and tosylating agent, and the mixture was allowed to react for 90 minutes, after which benzyl bromide was added to furnish the terminal epoxide 115 in 85% from 109. Then, the epoxide ring was opened with deprotonated propargylic ether 116. Global removal of the PMB functionality with DDQ gave triol 117. The partial reduction of the triple bond in 117 to the (Z)-olefin derivative was achieved using Lindlar catalyst and subsequent oxidation of the primary alcohol with the TEMPO/BAIB system facilitated the formation of pyrone 107. Initially, Sabitha and Raju intended to synthesize brevipolides K and L by esterification of this furanyl alcohol 107 with (Z)-4-OMe- and (Z)-4-OPMB-cinnamic acid 118 and 119, respectively. Unexpectedly, olefin isomerization occurred which led to the formation of brevipolides M (13) and N (15) after treatment with titanium tetrachloride in ≈85% yield each.

Biological activities

The extracts of Hypits brevipes Poit. have been utilized by humanity across the world in tropical regions ranging from folk medicines [2,3,5-10] to pest management [2,3]. These traditional applications stimulated researchers to conduct biological studies with these plant extracts. A variety of bioactivities were then evidenced, such as antifungal, antibacterial [4,9], insecticidal [7], and radical scavenging [2]. Interestingly, the extracts
Scheme 13: Retrosynthetic analysis of brevipolides M (13) and N (14) by Sabitha.

Scheme 14: Synthesis of brevipolides M (13) and N (14) by Sabitha.
also exhibited promising DNA intercalating agents [9] and antitumor activity [2]. The latter became more obvious when the chemical constituents of *Hyptis brevipes* Poit., the brevipolides A–F (1–6), were isolated for the first time by Kinghorn and co-workers in 2009 [4]. These compounds demonstrated cytotoxicity against various human cancer cells. Additionally, the research group led by Pereda-Miranda later isolated more members of brevipolide (1–15) from the same plant during 2013–2017 and further confirmed the cytotoxic activity of these natural products [1,12].

The biological activity data of brevipolides A–O (1–15) and their analogs from literature has been summarized in Tables 1–3. Table 1 presents the cytotoxicity data of compounds 1–15 and their analogs, if existing, against a range of cancer cell lines.

A cytotoxicity study against Lu1 human lung cancer cells was conducted for brevipolides A–G (1–7) by Kinghorn and co-workers but none of the compounds showed significant activity (Table 1, entries 1–7) [4]. These seven compounds were also evaluated against HT-29 human colon carcinoma cells and only three of them were potent (Table 1, entries 1, 2, and 6) with the lowest ED$_{50}$ value of 5.8 μM obtained for brevipolide A (1) (Table 1, entry 1). Similar cytotoxicity evaluation was performed for brevipolides G–O (7–15) against HCT-15 human colon cancer cells (Table 1, entries 7–15), and relatively high ED$_{50}$ values were obtained with the lowest number of 14.0 μM observed for brevipolide I (9) (Table 1 entry 9) [1,12]. Moving forward, cytotoxicity examination against MCF-7 human breast carcinoma cells was done for all the brevipolide members (Table 1, entries 1–15) [1,4,12]. Brevipolides A–H (1–8) were evaluated in vivo (Table 1, entries 1–8), and the highest cytotoxicity against MCF-7 was obtained for brevipolide G (7) with ED$_{50}$ values ranging between 3.6–5.1 μM (Table 1, entry 7). The remaining members of brevipolides were evaluated in vitro against MCF-7 cells (Table 1, entries 9–15), and the best result was obtained for brevipolide J (10) with an IC$_{50}$ value of 8.4 μM (Table 1, entry 10). Interestingly, brevipolide analogue 43, which is the reduced form of 6‘-epi-brevipolide H, showed even better in vitro cytotoxicity with an IC$_{50}$ value of as low as 7.0 μM (Table 1, entry 18) [14]. Also an evaluation on Hep-2 human laryngeal epidermoid cancer cells was conducted for brevipolides G–O (7–15) (Table 1, entries 7–15), and the lowest IC$_{50}$ value (8.8 μM) was observed for brevipolide G (7) (Table 1, entry 7) [1,12]. These nine compounds were also evaluated against HeLa, PC-3, and KB human cervix, prostate, and nasopharyngeal cancer cells, respectively, with the lowest IC$_{50}$

| entry | compounds | ED$_{50}$ or IC$_{50}$ (μM) | reference |
|-------|------------|-----------------------------|-----------|
|       |            | Lu1 | HT-29 | HCT-15 | MCF-7 | Hep-2 | HeLa | PC-3 | KB |
| 1     | 1          | NS  | 5.8$^b$ | NT | >10$^b$ | NT | NT | NT | NT | NT | [4] |
| 2     | 2          | NS  | 6.1$^b$ | NT | 6.1$^b$ | NT | NT | NT | NT | NT | [4] |
| 3     | 3          | NS  | NS | NT | >10$^b$ | NT | NT | NT | NT | NT | [4] |
| 4     | 4          | NS  | NS | NT | >10$^b$ | NT | NT | NT | NT | NT | [4] |
| 5     | 5          | NS  | NS | NT | >10$^b$ | NT | NT | NT | NT | NT | [4] |
| 6     | 6          | NS  | 7.5$^b$ | NT | 6.7$^b$ | NT | NT | NT | NT | NT | [4] |
| 7     | 7          | NS  | NS | 21.7$^c$ | 3.6–5.1$^b$ | 8.8$^c$ | 0.18$^c$ | 13.2$^c$ | 0.8$^c$ | [4,11,12] |
| 8     | 8          | NT  | NT | 17.0$^c$ | 5.2$^b$ | 20.7$^c$ | 14.5$^c$ | 13.5$^c$ | 5.0$^c$ | [11,12] |
| 9     | 9          | NT  | NT | 14.0$^c$ | 17.7$^c$ | 15.7$^c$ | 11.0$^c$ | 11.7$^c$ | 4.7$^c$ | [11,12] |
| 10    | 10         | NT  | NT | 25.7$^c$ | 8.4$^c$ | 14.6$^c$ | 5.5$^c$ | 13.9$^c$ | 2.9$^c$ | [12] |
| 11    | 11         | NT  | NT | >24.8$^c$ | >24.8$^c$ | >24.8$^c$ | >10.4$^c$ | 24.8$^c$ | 23.6$^c$ | [1] |
| 12    | 12         | NT  | NT | >25.7$^c$ | 23.9$^c$ | 19.8$^c$ | 8.5$^c$ | 22.7$^c$ | 19.3$^c$ | [1] |
| 13    | 13         | NT  | NT | >24.8$^c$ | >24.8$^c$ | 14.2$^c$ | 14.9$^c$ | 18.6$^c$ | 4.2$^c$ | [1] |
| 14    | 14         | NT  | NT | >25.7$^c$ | >25.7$^c$ | >25.7$^c$ | 24.4$^c$ | >25.7$^c$ | 9.3$^c$ | [1] |
| 15    | 15         | NT  | NT | >24.7$^c$ | >24.7$^c$ | 23.2$^c$ | 17.1$^c$ | >24.7$^c$ | 12.1$^c$ | [1] |
| 16    | ent-8      | NT  | NT | NT | NT | NT | NT | NT | NT | [16] |
| 17    | 17         | NT  | NT | NT | NT | NT | NT | NT | NT | [14] |
| 18    | 43         | NT  | NT | NT | 7.0$^c$ | NT | NT | NT | NT | [14] |

Abbreviations: Lu1 = lung carcinoma; HT-29 = colon carcinoma; HCT-15 = colon carcinoma; MCF-7 = breast carcinoma; Hep-2 = laryngeal epidermoid carcinoma; HeLa = cervix carcinoma; PC-3 = prostate carcinoma; KB = nasopharyngeal carcinoma; NS = not significant; NT = not tested. ED$_{50}$. IC$_{50}$. 

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Table 2 summarizes the results of other important biological evaluations for brevipolides A–I (1–9). The mitochondrial transmembrane potential assay was investigated for brevipolides A–G (1–7) (Table 2, entries 1–7). A very low ED\textsubscript{50} value of 8.5 nM was obtained for brevipolide C (3) (Table 2, entry 3) [4], making it a very potent lead compound for anticancer candidates related to mitochondrial dysfunction effect. A reduction in the mitochondrial membrane potential (MMP) could also indicate apoptosis [20]. These seven compounds, 1–7, were also evaluated for enzyme-based ELISA NF-κB and proteasome inhibition assays (Table 2, entries 1–7), but only brevipolide G (7) and brevipolide C (3) showed significant activities with ED\textsubscript{50} values of 15.3 and 38.0 μM, respectively (Table 2, entries 7 and 3) [4]. Lastly, brevipolides G–I (7–9) were found to inhibit the CCR5 receptor signaling as measured by a calcium mobilization assay with IC\textsubscript{50} values of 15.5, 13.7, and 18.0 μM, respectively, which make them potential agents for treating HIV disease (Table 2, entries 7–9) [11].

Table 3: IC\textsubscript{50} (mM) values of 3, 7, 8, and 10 (brevipolides C, G, H, and J) against protozoa parasites and HeLa cells.

| entry | compounds | IC\textsubscript{50} (mM) values |
|-------|-----------|------------------|
|       | 3 | 7 | 8 | 10 |
| 1 | Lma | 202.0 ± 8.0 | 62.1 ± 23.3 | 18.7 ± 6.7 | 24.0 ± 7.2 |
| 2 | Lae | >248.5 | 121.6 ± 16.6 | 17.5 ± 3.5 | 41.5 ± 1.0 |
| 3 | M2904 | 182.4 ± 67.1 | 155.5 ± 32.1 | 12.5 ± 1.5 | 120.3 ± 36.3 |
| 4 | LbG | 167.2 ± 59.1 | >258.8 | 32.5 ± 15.0 | 146.5 ± 35.1 |
| 5 | Llan | >248.5 | 155.5 ± 18.1 | 23.5 ± 1.0 | 64.8 ± 26.4 |
| 6 | T.c | >248.5 | >258.8 | 50.4 ± 8.7 | 149.8 ± 17.3 |
| 7 | P.I | 54.4 ± 18.6 | 56.9 ± 9.8 | 34.0 ± 16.0 | 34.6 ± 13.2 |
| 8 | G.I | >248.5 | 111.3 ± 36.2 | 76.7 ± 10.0 | 43.2 ± 0.7 |
| 9 | HeLa | 47.2 ± 7.7 | 82.8 ± 4.7 | 94.9 ± 4.0 | 70.8 ± 9.4 |

Abbreviations: Lma: Leishmania amazonensis; Lae: L. aethiopica; M2904: L. braziliensis; LbG: L. braziliensis native; Llan: L. lainsoni, native; T.c: Trypanosoma cruzi; P.I: Plasmodium falciparum; G.I: Giardia lamblia; HeLa: human cervix carcinoma.
multiple Leishmania strains, Trypanosoma cruzi, Plasmodium falciparum, and Giardia lamblia (Table 3, entries 1–8). Except for G. lamblia, brevipolide H (8) showed the best antiparasitic activity against all the tested human intestine parasites with IC\textsubscript{50} values of 12.5–50.4 mM. Thus, this compound has great potential as a lead structure in parasite research. A cytotoxicity study of these four compounds against HeLa human cervix cancer cells was also performed, and the results showed values spanning between 47.2–70.8 mM (Table 3, entry 9). However, these results were considerably much lower than those obtained by the Kinghorn [4] and Pereda-Miranda [12] research groups, which lied in a micromolar order (see Table 1, entries 3, 7, 8, and 10). This prominent difference might arise from the differing methods used.

In the same year, Borges and co-workers performed an ovicidal activity evaluation against Haemonchus placei eggs using the extract of the Hyptis brevipes plant [21]. In tropical areas, H. placei is particularly harmful to cattle, causing hypoproteinemias, anemia, and anorexia in the host animals [22]. The extract showed hatchability inhibition with ED\textsubscript{50} and EC\textsubscript{50} values of 3.34 and 5.71 mg/mL, respectively. A complete inhibition was achieved at the concentration of 25 mg/mL of the H. brevipes extract. Analysis of data combination from ovicidal activity, mass spectrometry, and metabolomics using HPLC-diode array detector-MS (HPLC-DAD-MS), partial least squares regression analysis (PLS-DA), and a correlation map (univariate correlation analyses) enabled the prediction of compounds that have a positive correlation with biological activity. This analysis attributed two detected [M + H]\textsuperscript{+} signals to dihydrobrevipolides C and F. Nevertheless, further structural identification was not performed.

**Conclusion**

The characteristic structural features of 5,6-dihydro-α-pyron derivatives isolated from Hyptis brevipes Poit., i.e., the brevipolides A–O, parallel with their appealing biological activity, in addition to the fact that they were produced in a small quantity from nature, make these natural products highly relevant when considering total synthesis. Since the first attempt to prepare brevipolide H by Kumaraswamy in 2014, several model, formal, and total syntheses of brevipolides have been performed, adding more understanding to the chemical and biological aspects of these compounds. In particular, in this review, the main strategies for the synthesis of brevipolides involve: 1) olefin metathesis; 2) asymmetric dihydroxylation and epoxidation; 3) asymmetric hydrogenation; 4) Horner–Wadsworth–Emmons olefination; and 5) cyclopropanation, which are summarized in Table 4, including the overall yields and the number of steps required.

| synthesis                     | products                        | key transformations                                                                 | overall yield | number of steps |
|-------------------------------|---------------------------------|-------------------------------------------------------------------------------------|---------------|-----------------|
| Kumaraswamy [14], 2014        | reduced 6′-epi-brevipolide H (43)| C4-C5: ring-closing metathesis C5-C6: vinyl Grignard addition C1′-OH: VO-catalyzed epoxidation C6′-OH: catalytic asymmetric transfer hydrogenation cyclopropane ring: Furukawa’s modified Simmons–Smith | 2.5% from 20   | 23              |
| Hou [16], 2014                | ent-brevipolide H (ent-8)       | C4-C5: ring-closing metathesis C2′-C4′: cross metathesis C6′-OH: Sharpless asymmetric dihydroxylation cyclopropane ring: Michael-initiated ring closure | 10% from ent-49 | 11              |
| Mohapatra [13], 2015          | C1-C12 fragment of reduced 6′-epi-brevipolide H (77) | C3-C4: ring-closing metathesis C5-C6: Brown asymmetric allylation C1′-OH: anti-selective reduction C6′-OH: Jørgensen epoxidation cyclopropane ring: Furukawa’s modified Simmons–Smith | 12.5% from 62  | 18              |
| Hou [15], 2016                | brevipolide H (8)               | C3-C4: ring-closing metathesis C5-C6: vinyl Grignard addition C6′-OH: Sharpless epoxidation C2′-C4′: cross metathesis C6′-OH: Sharpless epoxidation cyclopropane ring: Furukawa’s modified Simmons–Smith | 3.5% from 85   | 15              |
Table 4: Summary of the syntheses described in this review. (continued)

| References                  | Synthesis Description                                                                 | Conversion Efficiency |
|-----------------------------|--------------------------------------------------------------------------------------|-----------------------|
| Sabitha [17], 2017          | brevipolide M (13) C3=C4: ring-closing metathesis C5-C6: Brown asymmetric allylation  |
|                            | C3´-C4´: Horner–Wadsworth–Emmons olefination C5’-C6’: Horner–Wadsworth–Emmons olefination |
|                            | C5’-OH: Sharpless epoxidation C6’-OH: Sharpless epoxidation, Mitsuonbu inversion        |
|                            | furan ring: acid-catalyzed cyclization                                                | 5.8% from 97 17       |
| Sabitha [18], 2018          | brevipolide M (13) and N (14) C3=C4: Lindlar reduction C5-C6: alkyn addition         |
|                            | C3´-C4´: Horner–Wadsworth–Emmons olefination C5’-OH: Noyori reduction                 |
|                            | furan ring: acid-catalyzed cyclization                                                | 20.2% from 111 11     |

This work is expected to provide useful information for researchers to design new synthetic methodologies for reproducing these natural products and their analogues as well as to develop new pharmaceuticals from them.

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