Total Syntheses and Biological Evaluation of ($\pm$)-Botryosphaeridione, ($\pm$)-Pleodendione, 4-*epi*-Periconianone B, and AnaloguesKishor L. Handore,[†] Prakash D. Jadhav,[†] Bibhabasu Hazra,[‡] Anirban Basu,^{*,‡} and D. Srinivasa Reddy^{*,†}[†]CSIR-National Chemical Laboratory, Dr. Homi Bhabha Road, Pune 411008, India[‡]National Brain Research Centre, NH-8, Manesar, Gurgaon, Haryana-122051, India

Supporting Information

ABSTRACT: The total syntheses of ($\pm$)-botryosphaeridione, ($\pm$)-pleodendione, ($\pm$)-hoaensieremodione, 4-*epi*-periconianone B, and their analogues have been accomplished for the first time. All the synthesized target compounds were screened in neural anti-inflammatory assays using LPS induced microglia cells (N9). Among them, compounds **1** and **21** were identified as potential lead compounds for further profiling.

KEYWORDS: Natural product, total synthesis, antineuroinflammatory agents, botryosphaeridione, pleodendione

The bioassay-guided fractionation of the ethyl acetate extract from the fermentation broth of the endophytic fungus *Periconia* sp. F-31, which was derived from the medicinal plant *Annona muricata*, resulted in the isolation of the natural products called botryosphaeridione **1**, periconianone A **2**, and periconianone B **3** by the research group led by Jungui Dai.¹ Compound **1** and another closely related eremophilane-type sesquiterpene pleodendione **4** were isolated previously.^{2,3} The structures and absolute configurations were established by extensive spectroscopic analyses, ECD calculations, and single-crystal X-ray diffraction as shown in Scheme 1. Compounds **1**–**3** were reported to have shown neural anti-inflammatory activity (mouse microglia BV2 cells) with impressive IC₅₀ values of 0.23, 0.15, and 0.38 μ M, respectively.¹ This interesting biological activity suggests that dihydro-, tetrahydro-naphthalene-2,6-dione scaffolds can be promising lead structures for the treatment of CNS disorders such as Parkinson's disease (PD) and Alzheimer's disease (AD).^{4,5} Therefore, we became interested in this novel chemotype and synthesized several compounds and tested their neural anti-inflammatory potential.

The retrosynthetic analysis for the target compounds is shown in Scheme 1. The target compounds and their closely related analogues could be prepared from the key dienone intermediate **5** through a series of steps viz. alkylation of the dienolate, epoxidation, allylic oxidation, and base-mediated ring opening of epoxide. The key intermediate enone **5** could be prepared from diene **6** and tiglic aldehyde **7** through a Diels–Alder chemistry,⁶ which is extensively practiced in our group, followed by a few functional group transformations including Wittig, Grignard, and ring-closing metathesis (RCM) reactions.

The present synthesis began with the aldehyde **8**, which was previously reported from our group⁷ through a sequence of BF₃·Et₂O-mediated Diels–Alder reaction,^{8,9} Wittig reaction, and DIBAL-H reduction. The aldehyde **8** on a Grignard

Scheme 1. Structures of Targets and Retrosynthetic Plan

reaction, using vinylmagnesium bromide, gave an alcohol that was subjected to RCM¹⁰ to obtain the *cis*-fused decalin, which was further oxidized to the desired enone **5** in overall 44% yield. The conjugated double bond in enone **5** was subjected to selective epoxidation (H₂O₂–NaOH) to produce **9** in 71% yield. Assigned stereochemistry of the epoxide in compound **9**

Received: June 13, 2015

Accepted: September 28, 2015

Published: September 28, 2015

was established by 2D-NMR analysis, and the corresponding key NOE correlations are shown in Scheme 2. Epoxide 9 was

Scheme 2. Synthesis of Botryosphaeridione

treated with NaOH in MeOH for 30 min to afford the methoxenone 10¹¹ in 84% yield. Enone 10 was subjected to selenium dioxide mediated oxidation to produce compound 11 in 67% yield. We anticipated that further allylic oxidation might take place in the same pot to have the desired compound 12, but we did not observe that transformation. The desired allylic oxidation was achieved using an additional step under the *t*-BuOOH-PDC conditions¹² to give compound 12. Toward the final target 1, the required epimerization of the methyl group at C-4 position in 12 was necessary, and it was accomplished by simply exposing it to K_2CO_3 in methanol solvent. A significant shift of the methyl signal (doublet) in the ^1H NMR spectrum from 1.34 to 0.92 ppm indicated the epimerization.

Finally, the deprotection of the enol group in 13 using BBr_3 ¹³ in CH_2Cl_2 at low temperature furnished the target compound (±)-botryosphaeridione (1) in 85% yield (Scheme 2). Such an introduction of a OH group on the α carbon of an enone is an interesting finding of this synthesis, and it can be applied to other compounds of interest. Very few methods are available in literature for the introduction of a hydroxy functionality on the α carbon of an enone moiety.^{14–17} All the spectral data for 1 (^1H NMR, ^{13}C NMR, and MS) were found to be identical to the values reported in the isolation paper.²

After the successful synthesis of botryosphaeridione 1, efforts were diverted to synthesize pleodendione 4, which contains tetrahydronaphthalene-2,6-dione skeleton. Toward this, enone double bond present in 5 was chemoselectively reduced using

Li/liq.NH₃ conditions to give the corresponding saturated ketone, which on treatment with IBX/DMSO¹⁸ resulted in dienone 14, where the double bond was regioselectively introduced in the desired position with the help of an extended conjugation (Scheme 3). Treatment of the enone 14 with

Scheme 3. Synthesis of Pleodendione

lithium diisopropylamide (LDA) and isopropyl iodide in THF gave the alkylated product 15 in low yields (40% brsm).¹⁹ Although, this reaction gave poor yields, the pleasing outcome was its high diastereoselectivity (>95%). Finally, the compound 15 was transformed to the target compound (±)-pleodendione 4 using allylic oxidation under the same conditions used in the synthesis of 1 (Scheme 2). All the spectral data of 4 were found to be identical with the literature values.³ Similarly, efforts were made to synthesize another interesting and structurally related sesquiterpenoid, periconianone B 3. Stereoselective alkylation of the lithium enolate of the enone 14 with ethyl-2-iodopropanoate proceeded smoothly, to give the desired ketoester 16 as a major diastereomer (>85%) with 68% isolated yield.²⁰ This result suggests that the methyl group underwent epimerization under the reaction conditions. The stereochemistry of the new chiral centers was confirmed by X-ray structure analysis of the corresponding carboxylic acid 17 (see Figure 1). Compound 16 was subjected to allylic oxidation to give compound 18 in 43% yield (Scheme 4).

Ester hydrolysis using LiOH in aqueous THF resulted in (±) periconianone B analogue 19 in 72% yield. However, the desired epimerization of C-4 methyl group in the present case was not successful despite a few efforts toward the target compound 3. A thorough 2D-NMR analysis of the compound 19 confirmed that the methyl group at 4 position was indeed in

Scheme 4. Synthesis of Periconianone B Analogue (19)

Figure 1. Comparisons of the coupling constants between H-7, H-11, and H-6 for the series of compounds **16**, **17**, **18**, **19**, and periconianone B.

β -orientation.²¹ Although compounds **12** and **18** are closely related structures, the success of C-4 epimerization in **12** could be explained by the formation of enolate with extended conjugation, which is not possible in the present case.

On a careful analysis of the coupling constants of H-7 with H-11 and H-6 in the natural periconianone B and the synthesized periconianone B analogue **19**, it was observed that they are identical (ddd 14.4, 4.8, 4.8 Hz), which prompted us to make further investigations. We found that these coupling constants were almost the same also for a series of compounds **16**, **17**, and **18** as listed in Figure 1.

Based on the single X-ray crystal structure analysis (CCDC 1056739), we knew that H-7 and H-11 are *cis* to each other. The very close match of the coupling constants of H-7 with H-11 and H-6 suggests that the stereochemistry of the C13 methyl group in periconianone B is in question, which needs further investigation. ¹H and ¹³C NMR data comparisons for all peaks are available in the Supporting Information (SI). In addition to the target compounds, a few close analogues were also synthesized around this skeleton, and all of them are compiled together in the Figure 2. Details related to the synthesis of these analogues are available in the SI.

While we were working on this project, isolation of another closely related natural product from the roots of *Drypetes hoensis*, called hoensieremodione, attracted our attention.²² We have attempted the synthesis of this new natural product by following similar chemistry as mentioned above. This synthesis, commenced with the alkylation of the dienone **14** with methyl iodoacetate, resulted in compound **26** with very high stereoselectivity and good yield.²⁰ Initial oxidation using DDQ produced compound **27**, followed by allylic oxidation using PDC/*t*-BuOOH, furnished the target compound hoensieremodione **28** (Scheme 5). All the spectral data is in complete agreement with the reported data.

Once we had all the synthesized compounds in hand, they were screened for their antineuroinflammatory potential in the presence of lipopolysaccharide (LPS)-induced inflammation using mouse microglia N9 cells since microglial activation in

Figure 2. Structures of the selected synthesized compounds.

Scheme 5. Synthesis of Hoensieremodione

response to invading pathogen or other stimuli in central nervous system contributes to neuroinflammation. First, all the compounds were evaluated for their inhibition of NO production in LPS stimulated N9 cells, and nitrite levels, a major metabolite of NO, were measured in culture media using Griess reagent.²³ In neuroinflammation, a large amount of NO is produced and in turn exaggerates the inflammatory response. Primary results indicated that almost all our evaluated compounds evidently decreased NO level in LPS treated cells as shown in Table 1. Based on the results, it is concluded that the compounds with the dihydronaphthalene-2,6-dione skeleton (such as **1**, **12**, **13**, **21**) gave most potent compounds from this series. The carbonyl functionality at the C3 position in this skeleton seems to be essential for the activity (see **11** and **14**). Introduction of an oxygen functionality on α -position of the enone enhanced the potency (**22** vs **13** and **21** vs **12**). The compound **23** with an epoxide moiety also showed good activity. From periconianone B series, the compounds with a side chain showed moderate potency, among them esters were relatively better compared to corresponding the acids (**16** vs **17** and **18** vs **19**).

Meanwhile, the cytotoxicity of these compounds in N9 cells were assessed by MTT assay,²⁴ and the compounds **12** and **23** were found to be toxic with $IC_{50} < 10 \mu M$ (Table 1). Compounds showing effective NO inhibitory activity with low cytotoxicity were further profiled based on the selectivity index

Table 1. Cytotoxicity and Inhibition Data of the Compounds on LPS Induced NO Generation in N9 Cells

compd	cytotoxicity (A)		NO inhibition (B)		selectivity index (A/B)
	IC ₅₀ (μM)	95% confidence interval (μM)	IC ₅₀ (μM)	95% confidence interval (μM)	
1	479.5	311–648.1	3.3	2–4.5	145.3
4	132.4	110.4–154.3	20.6	11.5–29.7	6.4
11	107.2	79.5–135	34.5	18.8–50.2	3.1
12	8.1	7.7–8.6	2.4	1.1–3.7	3.3
13	67.3	69–105.7	6.4	2.3–10.5	10.5
14	678.6	404.1–953.2	61.7	46.6–74	10.9
16	60.9	43.3–78.5	58.9	41.5–76.4	1.1
17	785.8	664.8–906.7	177	131–223.2	4.4
18	299.1	351–247.2	18	13.6–22.5	16.6
19	284.2	260.7–307.8	26.1	18–34.2	10.9
21	570.7	402.1–739.3	4.1	2.9–5.1	139.2
22	416.2	335.2–497	17.6	13.9–21.4	23.6
23	5.3	4.2–6.3	3.7	2.8–4.8	1.4
24	183.5	163.8–203.2	12.3	8.5–16	14.9
25	125.8	76.8–174.9	34.1	25.1–43.2	3.7

(cytotoxicity IC₅₀/NO inhibition IC₅₀). Although several compounds displayed a better NO inhibition than the well-known standard curcumin (IC₅₀, 13.6 μM), only compounds **1** and **21** were selected for further study since they exhibited superior selectivity indices (>100) among all the derivatives.

Next, the anti-inflammatory potency of the compounds **1** and **21** were determined by measuring the level of intracellular reactive oxygen species (ROS) in LPS treated N9 cells.²⁵ Generation of ROS in microglia is a key marker of neuroinflammation. As depicted in Figure 3, LPS increased the ROS by more than 2-fold as analyzed by measuring the mean fluorescence intensity (MFI) by fluorescence activated cell sorting (FACS), whereas intense reduction in ROS levels was observed in the case of treatment with both these compounds in a dose-dependent manner. LPS treatment can

Figure 3. Effects of the compounds on ROS level in LPS stimulated N9 cells. The graph and subsequent plot represent the MFI of ROS generation in the presence of compounds **1** (a) and **21** (b). Data represent mean $\pm$ SD of three independent experiments. * p < 0.05 and ** p < 0.01 in comparison to LPS-treated values.

induce neuroinflammation, resulting in the extreme production of numerous pro-inflammatory mediators including TNF- α , IFN- γ , MCP-1, and IL-6.²⁶ Hence, the antineuroinflammatory activity of the compounds **1** and **21** was attained by measuring the expression of these inflammatory mediators in LPS treated N9 cells by cytokine bead array.²⁷ It was found that both the compounds **1** and **21** significantly suppressed the inflammatory mediators dose-dependently with respect to the LPS-treated conditions (Figure 4).

Figure 4. Cytometric bead array (CBA) analysis of protein extract isolated from N9 cells treated with LPS along with the compounds. Dose-dependent addition of the compounds revealed substantial decrease in the levels of TNF- α (a), IL-6 (b), IFN- γ (c), and MCP-1 (d) compared to the LPS treated cells. Data represent mean $\pm$ SD of three independent experiments. * p < 0.05, ** p < 0.01, and *** p < 0.001 in comparison to LPS treated values.

In conclusion, we have accomplished the first syntheses of several compounds based on dihydro-, tetrahydro-naphthalene-2,6-dione skeleton, in particular, botrysphaeridione **1**, pleodendione **4**, 4-*epi*-periconianone **B 19**, and hoensieremodione **28**. The antineuroinflammatory effects of these compounds (except **28**) were evaluated using different assays in mouse microglia cells. Based on the potency, selectivity index, and modulation of the inflammatory markers, compounds **1** and **21** were identified as the potential leads for further optimization. All these compounds with new chemotype may be explored in other biological assays in due course.

■ ASSOCIATED CONTENT

● Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmchemlett.5b00241.

NMR spectra, 2D-NMR analysis, and detailed experimental procedures including biological assays (PDF)
Characterization data of CCDC 1056739 (CIF)

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Funding

CSIR, New Delhi for the support through CSC0108 (ORIGIN) and BSC0123 (GenCode) projects under XII Five Year Plan is acknowledged. K.L.H. thanks CSIR for the award of research fellowship. The part of the work done at A.B.'s laboratory at NBRC was supported by Tata Innovation Fellowship (BT/HRD/35/01/02/2014) from the Department of Biotechnology.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

Dr. Rajesh Gonnade and Mr. Sridhar Thorat of Center for Materials Characterization, CSIR-NCL, Pune for X-ray analysis, and Dr. Rajamohan of Central NMR Facility, CSIR-NCL, Pune for his help in NMR analysis are acknowledged.

ABBREVIATIONS

ECD, electronic circular dichroism; CNS, central nervous system; NOE, Nuclear Overhauser Effect; brsm, based on recovered starting material; NO, nitric oxide; TNF, Tumor necrosis factor; IFN, interferon; MCP, monocyte chemo-attractant protein; IL, interleukin

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