Pharmacophore-Directed Retrosynthesis Applied to Ophiobolin A: Simplified Bicyclic Derivatives Displaying Anticancer Activity

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ABSTRACT: Pharmacophore-directed retrosynthesis applied to ophiobolin A led to bicyclic derivatives that were synthesized and display anticancer activity. Key features of the ultimate defensive synthetic strategy include a Michael addition/facially selective protonation sequence to set the critical C6 stereocenter and a ring-closing metathesis to form the cyclooctene. Cytotoxicity assays toward a breast cancer cell line (MDA-MB-231) confirm the anticipated importance of structural complexity for selectivity (vs MCF10A cells) while C3 variations modulate stability.
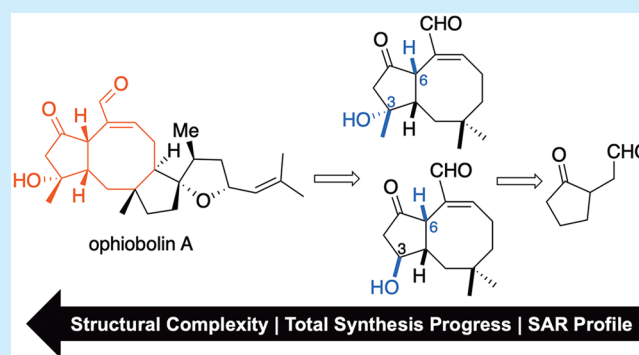
The ophiobolins are fungal-derived sesterterpenoids consisting of over 50 members,[1,2] with ophiobolin A (**1**, OpA, Figure 1) being the first isolated and also one of the
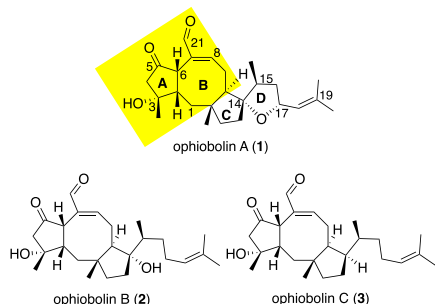

Figure 1. Structure of selected ophiobolins (A–C) and proposed minimal pharmacophore (yellow).

most bioactive congeners (Figure 1).[3−5] This family of natural products is drawing increasing interest[2,6,7] not only because of their high potency against various cancer cell lines[1,8] (e.g., chronic lymphocytic leukemia (CLL), IC$_{50}$ = 1 nM;[8] P335, IC$_{50}$ = 63 nM;[6] glioblastoma Hs683, IC$_{50}$ = 620 nM[9,10]) for OpA but also for their unique cellular effects and selectivity observed. For example, both OpA (**1**) and B[11] (**2**) show selectivity toward cancer stem cells (CSCs) by significantly reducing the CD44/CD24-positive population in an MDA-MB-231 cell line.[12] Moreover, the ability of certain ophiobolins to induce paraptosis[7,9,13] or autophagy[14] makes these natural products valuable leads toward apoptosis-resistant cancer cells. Our interest in the ophiobolins stems from their complex

structures, unique bioactivities and unknown cellular targets, and was further enhanced by our finding that OpA, but not 3-deoxy OpA, specifically induces cell death in stem-like cells with epithelial-mesenchymal transition (EMT) properties.[15]

Several structure−activity relationship (SAR) studies of the anticancer effects of ophiobolins, derived mainly from derivatization of OpA or congeners, led us to apply our recently described pharmacophore-directed retrosynthesis (PDR) strategy[16,17] to elaborate on SAR information with respect to effects on stem-like cancer cells. In particular, the anticancer effects of various ophiobolins[1,2,6] and synthetic derivatives,[10] in conjunction with our own work,[15] point to the importance of the A/B-rings as an initial minimal pharmacophore consisting of a 1,4-keto unsaturated aldehyde moiety, the C5/C6-relative stereochemistry, the C3-hydroxyl, and syn-fused cyclooctene (Figure 1, highlighted in yellow). The potential reactivity of the 1,4-ketoaldehyde to primary amines including cellular protein lysines through a potential Paal−Knorr pyrrole reaction has been reported.[10,18] Isolation of a phosphatidylethanolamine adduct from HEK293T cells treated with OpA supports this notion.[19] The natural C6-stereo-chemistry appears vital for the observed bioactivity across multiple cell lines with significant loss of activity observed for

the C6-epimer of OpA.[1,2] Molecular modeling studies[8] suggest one rationale for significant loss of bioactivity of *6-epi-ophiobolin* congeners, namely significant alteration of the eight-membered B-ring conformation. This altered conformation changes the orientation of the C21 aldehyde, thus impacting any potential Paal−Knorr condensation with the ketoaldehyde and also possibly impacting potential Michael additions to the unsaturated aldehyde.[8] Therefore, we incorporate the cyclooctene into our current proposed pharmacophore (Figure 1). Efforts toward a broader, deepseated SAR profile of the ophiobolins is precluded by structural convergence of the natural ophiobolins. Thus, we envisioned that application of PDR would enable a greater dissection of minimal structural requirements for anticancer activity through synthesis of simplified derivatives of OpA that retain structural features known to be critical for activity. Our initial PDR-guided studies toward this goal are described herein.

To date, three completed total syntheses of various ophiobolin and derivatives have been reported including the synthesis of OpA by the Nakada group who employed a late-stage ring closing metathesis (RCM) to form the 8-membered B-ring[20,21] and ophiobolin C by the Kishi group who employed a Nozaki−Hiyama−Kishi coupling to generate the C-ring.[22] Recently, Maimone reported an elegant 9-step radical cascade process to fashion the BC-ring system of (−)-6-*epi*-OpN,[23] and more recently a 15-step synthesis of (+)-6-*epi*-OpA employing a similar synthetic strategy.[24]

Herein, we report Stage I and an initial Stage II study of our PDR approach toward OpA with the goal of gaining further SAR information for this complex sesterterpene. To address questions for the C6-stereochemistry, the C3-hydroxy group, and required ring systems for bioactivity, we applied PDR to arrive at simplified monocyclic (e.g., known ketoaldehyde (±)-11[25]) and bicyclic (e.g., (±)-10) derivatives (Figure 2), all bearing our current hypothesized *minimal* pharmacophore (Figure 2, yellow highlight).

Our synthetic strategy placed an emphasis on installing the critical C6-stereocenter while retaining flexibility at certain positions such as the C3-hydroxyl bearing center to invert stereochemistry or alter substitution. For synthesis of the challenging 8-membered B-ring, we relied on the strategy of
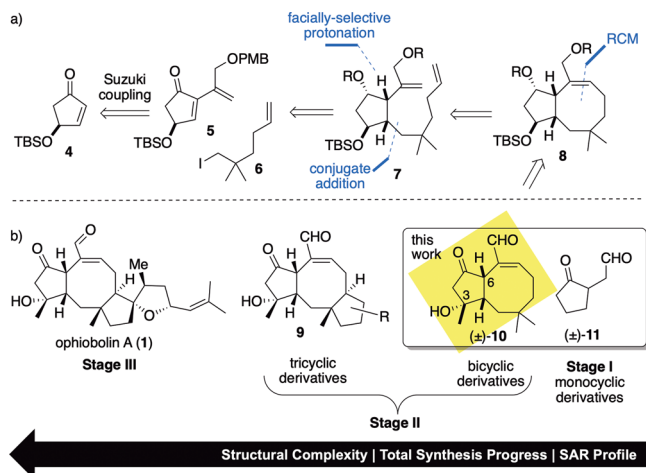

Figure 2. (a) Pharmacophore-directed retrosynthesis applied to OpA. (b) Staged syntheses of OpA derivatives including monocyclic (±)-11 and bicyclic (±)-10 derivatives bearing minimal pharmacophore described herein.

Nakada employing an RCM reaction.[20,21] Michael addition of a cuprate derived from iodide 6 to cyclopentenone 5 to form the C1−C4 bond followed by facially selective protonation would enable control of the key C6-stereocenter. A protected secondary alcohol at C3 would enable variations in substitution and also stereochemistry at this center. The described synthetic strategy sets the stage for more elaborate tricyclic congeners or the natural product itself. Importantly, application of PDR enables the exploration of key strategic bond disconnections that typically would only be considered model studies in a total synthesis effort; however, these studies can be utilized to access simplified derivatives and provide important SAR information as described herein.

We initiated our synthetic efforts by studying the conjugate addition of cuprate reagents derived from known neopentyl iodide 12a[26] to enone (±)-5, to mimic the eventual cyclopentyl cuprate that would be required for tricyclic analogs (Scheme 1). However, several problems quickly surfaced

Scheme 1. Development of the Conjugate Addition Sequence Leading to Enol Acetate 16
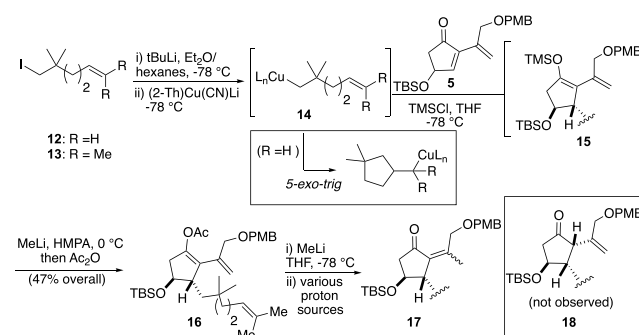

including the anticipated low reactivity of the neopentyl cuprate reagent which could be overcome by forming the more reactive higher order organocuprate using the method of Lipshutz.[27] Unfortunately, the resulting cuprate was prone to 5-exo-trig cyclization (see inset, Scheme 1),[26,28] likely accelerated by the Thorpe−Ingold (*gem*-dimethyl) effect. This side reaction is unavoidable even at −78 °C unless the olefin is trisubstituted, directing us toward a defensive strategy[29] and the use of iodide 13[30] bearing a pendant trisubstituted alkene known to lower the rate of intramolecular cyclization.[31] Lithium−halogen exchange[32] followed by addition of (2-thienyl)Cu(CN)Li[27] converted iodide 13 into a reactive organocuprate species leading to conjugate addition without detectable 5-exo-trig cyclization at −78 °C (Scheme 1). Use of TMSCl[33,34] was also beneficial for this conjugate addition but led to a TMS enol ether that was too labile for isolation. This problem was circumvented by treating the crude, intermediate silyl enol ether (±)-15 directly with MeLi followed by addition of Ac2O. This led to *in situ* conversion to the more stable and readily purified enol acetate (±)-16.[35] Overall, this process entailed a one-pot conjugate addition/double-enolate trapping for the key C1−C5 bond construction. However, enol acetate (±)-16 failed to undergo the subsequent desired RCM reaction[36−39] (not shown) to deliver the targeted cyclooctene despite trying a variety of conditions.

We anticipated that the *syn*-substituted dienyl cyclopentane (±)-18 would undergo the RCM reaction more readily. Toward this end, we next attempted to introduce the C6 stereocenter prior to the RCM through a facially selective

protonation of a derived enolate following treatment of the enol acetate with MeLi. However, enolate generation and protonation led to complete isomerization of the alkene to the more conjugated position leading to tetrasubstituted alkene (±)-17 rather than the desired *syn*-substituted cyclopentane (±)-18. To preclude this olefin isomerization, we decided to again employ a defensive strategy[29] by refining the structure of the substrates without altering the overall synthetic plan. After extensive exploration, we settled on introduction of a phenyl ring at C8 (OpA numbering) to preclude C7−C8 olefin isomerization (cf. enone (±)-26, Scheme 2) during installation

Scheme 2. Refined Scalable Synthesis of the Refined Enone Substrate 26
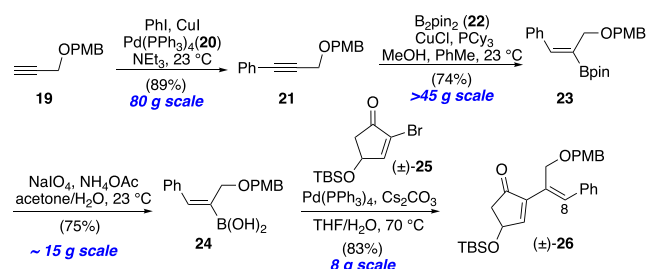
of the critical C6 stereocenter (for further description of this defensive strategy see Scheme S1 in the Supporting Information (SI)). Our revised synthesis began by developing an efficient route to cyclopentenone (±)-26 (Scheme 3).

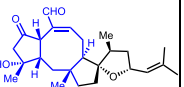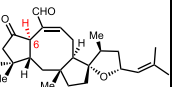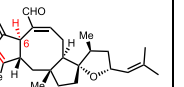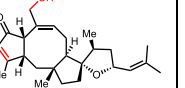## Scheme 3. Synthesis of Bicyclic OpA Derivatives (±)-38 and (±)-10
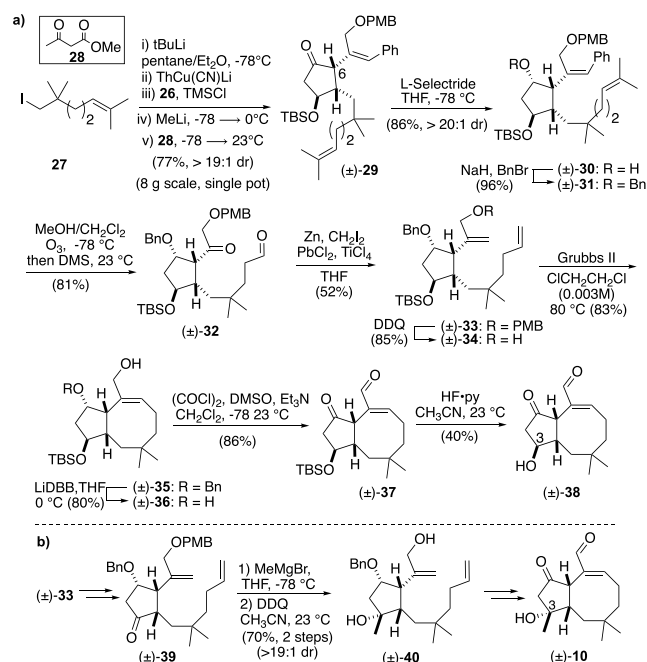
Sonogashira coupling[40] of PMB-protected propargyl alcohol 19 with iodobenzene followed by regioselective hydroboration with pinacol diborane,[41] both on multidecagram scale, delivered vinyl borane 23. Hydrolysis to the more reactive boronic acid 24,[42] followed by Suzuki−Miyaura coupling[43−45] with racemic, known cyclopentenyl bromide (±)-25,[46] gave enone (±)-26 on a multigram scale in 83% yield.

Enone (±)-26 was then subjected to the one-pot cuprate addition silylation sequence developed previously; however, a direct protonation of the intermediate enolate was also investigated to eliminate a step (Scheme 3). Following the same procedure developed previously for the conjugate addition−silylation/desilylation sequence beginning with iodide 27, a kinetic protonation delivered a single diastereomer of the trisubstituted cyclopentanone (±)-29 upon quenching with methyl acetoacetate (28) as the proton source.[47] This sequence forged the required C1−C5 bond and also sets the C6 stereocenter with high diastereoselectivity. As expected, minimal olefin isomerization was observed due to the presence of the conjugated phenyl ring. This complex Michael addition/ kinetic protonation sequence affording cyclopentanone (±)-29 was scalable (up to 8 g scale) and proceeded with high diastereoselectivity (>19:1) in 77% yield. Diastereoselective reduction of the ketone with L-selectride gave alcohol (±)-30 (>19:1, dr) and protection as the benzyl ether gave cyclopentane (±)-31. At this stage, the refined substrate served us well to prevent problematic issues encountered previously. However, in order to enable the proposed RCM reaction, terminal monosubstituted alkenes are required for optimal reactivity and efficiency which we verified through extensive experimentation.[48] Thus, benzyl deprotection followed by ozonolysis revealed 1,8-ketoaldehyde (±)-32. While direct cyclization of ketoaldehydes by a McMurry reaction[49,50] to form 8-membered rings have been reported,[51] several attempts were unsuccessful. Thus, toward applying RCM for cyclooctene formation with a substrate related to Nakada's, we converted the ketoaldehyde (±)-32 to the bis- terminal alkene (±)-33. However, this bis-olefination required extensive optimization, as the ketone was found to be inert to many common methylenation conditions.[52,53] After extensive studies, we were delighted to find that conditions developed by Takai[54−57] delivered the desired bis-alkene (±)-33 in 52% yield. Following PMB ether deprotection to further reduce steric bulk around one alkene following the precedent of Nakada,[20,21] RCM of bis-olefin (±)-34 successfully established the 5/8 bicyclic core.[58−60] Benzyl ether deprotection with Freeman's reagent,[61,62] Swern oxidation,[63,64] and TBS-ether deprotection provided the first bicyclic OpA derivative (±)-38 which was best purified on Fluorosil. This bicyclic OpA derivative shares the entire A/B-ring system with the natural product OpA, differing only by having an epimeric, secondary alcohol at C3.

Toward gaining more information regarding the importance of the C3-teriary alcohol, we also targeted derivative (±)-10 bearing a tertiary alcohol with natural configuration at C3 (Scheme 3b). While use of a later stage, common intermediate such as benzyl ether (±)-35 would appear prudent, attempts to selectively manipulate the C3 alcohol required extensive protecting group manipulations or led to extensive *β*-elimination. We therefore opted to begin with alcohol (±)-33 toward C3-derivatives, and an abbreviated synthetic scheme is shown (Scheme 3b; for the detailed synthetic sequence, see Scheme S2 in SI). Methyl Grignard addition to the derived ketone (±)-39 proceeded diastereoselectively (dr >20:1) to deliver tertiary alcohol (±)-40. Subsequent synthetic steps toward this bicyclic derivative followed a similar strategy to that described above for OpA derivative (±)-38; however, the final step, a bis-oxidation step to afford the ketoaldehyde, was found to be quite challenging, so it is described briefly here. This bis-oxidation was plagued by C6-epimerization or

Table 1. Cell Viability Data of Synthesized, Simplified Analogs of Ophiobolin A and Natural Congeners[a]

OpA & derivatives / Cell lines (IC$_{50}$, μM)	(±)-11	(±)-38	(±)-10	(+)-ophiobolin A	(+)-6-epi-ophiobolin A	(+)-3-anhydro-6-epi-ophiobolin A	(+)-ophiobolin I
MCF10A	197 ± 14.4[b]	13.4 ± 3.10	6.36 ± 1.99	0.198 ± 0.076	1.05 ± 0.076	1.74 ± 0.614	4.16 ± 0.763
MDA-MB-231	44.2 ± 16.1[b]	11.0 ± 0.935	7.09 ± 2.20	0.069 ± 0.024	0.80 ± 0.28	0.552 ± 0.124	4.94 ± 0.921

[a]IC$_{50}$ values are denoted in mean ± standard deviation of at least 3 independent replicates. [b]Ketoaldehyde (±)-11 was unstable in DMSO, and thus a freshly prepared sample was required to preclude inconsistencies observed when using stored DMSO stock solutions.

C3-dehydration through β-elimination under a variety of oxidative conditions screened. Ultimately, we found that Hünig's base in lieu of the more commonly employed Et$_3$N was key to a clean Swern bis-oxidation but still afforded a moderate yield (33%). Furthermore, it should be noted that the resulting ketoaldehyde (±)-10, while stable once purified, was quite sensitive to many stationary phases for purification. Silica gel deactivated with Et$_3$N, basic alumina, and Fluorosil all resulted in extensive decomposition. Use of reversed-phase semiprep HPLC was uniquely successful in enabling purification of OpA derivative (±)-10. This is in stark contrast to the stability observed for the C3-secondary alcohol-bearing derivative (±)-38 and points to the importance of the C3-substitution pattern for stability of OpA derivatives. While OpA itself is more stable to silica gel than (±)-38 and (±)-10, it is also prone to C6 epimerization and C3 dehydration under acidic or basic conditions leading to significant loss in bioactivity.[1,2,6]

We then assayed the cytotoxicity of the racemic, simplified OpA derivatives synthesized toward a breast cancer cell line (MDA-MB-231) and a noncancerous cell line (MCF-10A), to probe selectivity, if any, in comparison to OpA and a few naturally occurring OpA congeners (Table 1). While significantly less potent than OpA, simplified bicyclic OpA derivatives (±)-10 and (±)-38 bearing the proposed pharmacophore displayed cytotoxicity (~7 and 11 μM, respectively) against MDA-MB-231 cells. The activity of the highly simplified and unstable ketoaldehyde (±)-11 was unsurprisingly less active (~44 μM). The selectivity observed with OpA between a cancer vs noncancer cell line (~69 vs 200 nM, ~3-fold difference) is erased with the structurally simplified bicyclic derivatives (±)-10 and (±)-38. The similar potency of these simplified bicyclic derivatives is noteworthy and suggests that the C3-stereochemistry as either a secondary or tertiary alcohol has little effect on bioactivity. Overall, the described SAR data with racemic bicyclic derivatives of OpA suggest that cytotoxicity and selectivity increase in parallel with increasing complexity as expected.

A few closely related congeners of OpA were also assayed. Direct comparison of OpA and (+)-6-epi-OpA suggests the importance of the C6 relative stereochemistry for cytotoxicity toward this breast cancer cell line leading to an ~12-fold drop in activity (~69 nM vs ~0.8 μM, Table 1). Other structural changes also impact cytotoxicity including loss of the C3-hydroxyl through β-elimination in conjunction with epimerization at C6 leading to an 8-fold drop in activity (cf. (+)-3-anhydro-6-epi-OpA). Retaining the C6-stereochemistry but dehydration in combination with reduction of the aldehyde to the primary alcohol as found in (+)-OpI leads to a significant loss in cytotoxicity (~10-fold drop vs OpA) with insignificant

selectivity between the cancer vs noncancer cell line, ~4.9 vs 4.2 μM, respectively.

In summary, we completed the first stage and initiated studies of the second stage of a PDR approach toward OpA to access bicyclic analogs bearing the AB-ring system. To circumvent synthetic challenges including an inherent 5-exo-trig cyclization of a neopentyl alkyllithium and an undesired olefin isomerization, we adopted a defensive strategy by careful refinement of intermediates that ultimately enabled an RCM to form the required cyclooctene ring and maintain the critical C6-stereochemistry. Cell viability assays toward MDA-MB-231 breast cancer cells confirm our hypothesis that significant cytotoxicity observed for the ophiobolins originates from structural features of the A/B-ring system. In addition to the SAR studies of the ophiobolins, the work described represents model studies of key reactions envisioned toward more elaborate OpA derivatives, a useful byproduct of the PDR approach. This study further highlights the advantage of PDR to access SAR information at the early stages of a total synthesis effort and inform the design of more elaborate derivatives in the later stages.

◼ ASSOCIATED CONTENT

🅂🅸 Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.orglett.0c02938.

Experimental procedures and compound characterization data (^{1}H and ^{13}C and select 2D (NOESY, HMBC, HSQC) NMR spectra) for all new compounds reported (PDF)

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