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Total Synthesis, Biological Evaluation, and Target Identification of Rare *Abies* Sesquiterpenoids

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Abstract

Abiespiroside A (**1**), beshanzuenone C (**2**), and beshanzuenone D (**3**) belong to the *Abies* sesquiterpenoid family. Beshanzuenones C (**2**) and D (**3**) are isolated from the critically endangered Chinese fir tree species *Abies beshanzuensis* and demonstrated weak inhibiting activity against protein tyrosine phosphatase 1B (PTP1B). We describe herein the first total syntheses of these *Abies* sesquiterpenoids relying on the sustainable and inexpensive chiral pool molecule (+)-carvone. The syntheses feature a palladium-catalyzed hydrocarbonylative lactonization to install the 6,6-fused bicyclic ring system and a Dreiding-Schmidt reaction to build the oxaspirolactone moiety of these target molecules. Our chemical total syntheses of these *Abies* sesquiterpenoids have enabled (i) the validation of beshanzuenone C's weak PTP1B inhibiting potency, (ii) identification of new synthetic analogs with promising and selective protein tyrosine phosphatase SHP2 inhibiting potency, and (iii) preparation of azide-tagged probe molecules for target identification via a chemoproteomic approach. The latter has resulted in the identification and evaluation of DNA polymerase epsilon subunit 3 (POLE3) as one of the novel cellular targets of these *Abies* sesquiterpenoids and their analogs. More importantly, via POLE3 inactivation by probe molecule **29** and knockdown experiment, we further demonstrated that targeting POLE3 with small molecules may be a novel strategy for chemosensitization to DNA damaging drugs such as etoposide in cancer.

Graphical Abstract

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ASSOCIATED CONTENT © Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.8b07652.

Experimental procedures, compound characterization, and ESI-MS spectra (PDF)

Author Contributions

Notes

The authors declare no competing financial interest.

INTRODUCTION

Natural products have always held a privileged position as valuable sources and inspirations for new drug development.¹ They also often serve as important probe molecules and lead to the identification of new disease targets.² When the natural products are not readily accessible via natural isolation or biosynthesis, total synthesis has served as an effective approach to supply sufficient material for the corresponding biological studies and therapeutic development.³ The established synthetic approach can also be used to prepare chemical probe molecules to better understand the mode of actions and generate synthetic analogs or libraries to identify new lead compounds with improved function and physicochemical properties for the corresponding drug discovery efforts.⁴ Our recent total synthesis efforts of three rare *Abies* sesquiterpenoids have led us into such a journey of discovering new synthetic analogs with potent biological functions and new disease targets which may offer a new strategy for cancer treatment. The related studies are reported here.

Our ongoing efforts in developing novel catalytic carbonylation reactions⁵ for complex and bioactive natural product synthesis⁶ inspired endeavors to develop efficient and general syntheses of a family of *Abies* sesquiterpenoids represented by abiespiroside A (**1**, Figure 1A), beshanzuenone C (**2**), and beshanzuenone D (**3**). Abiespiroside A was isolated from the Chinese fir tree species *Abies delavayi* and was reported to possess inhibitory potency against the production of nitric oxide, a therapeutic effect for inflammatory diseases such as arthritis.⁷ Beshanzuenones C and D were isolated from the shed trunk barks of the critically endangered Chinese fir tree species *Abies beshanzuensis*.⁸ Seven such trees were first discovered in 1963 but only three currently remain in the world, distributed on the summit of Baishanzu Mountain in the Zhejiang Province of China.⁹ The other four were lost due to irrational transplant and climate change. The Species Survival Communication of the International Union for Conservation of Nature and Natural Resources regarded the *Abies beshanzuensis* as one of the 12 critically endangered plant species in the world.¹⁰

Both beshanzuenones C and D were reported to inhibit protein tyrosine phosphatase 1B (PTP1B), a key target for the treatment of type-II diabetes and obesity with IC₅₀ values of 59.7 and 40.4 μ M, respectively.⁸ Since rare and endangered plants have been shown to be superior sources for drug compounds compared to other botanical sources,¹¹ we wondered whether these natural products possess any other biological activity and what their potential cellular targets might be. Due to the critically endangered status of these trees, it is not practical to isolate them in large scale from the plants. Moreover, probe compounds with proper tags are needed for identifying their potential cellular targets. Thus, an efficient and flexible approach toward these natural products and their analogs is necessary.

Structurally, these natural products share a common 6/6/5 ring system featuring an oxaspirolactone motif. We recently developed a palladium-catalyzed carbonylative spirolactonization of hydroxycyclopropanols to access a variety of oxaspirolactones (Figure 1B).¹² We envisioned the conversion of hydroxycyclopropanol **9** to oxaspirolactone **10** containing the desired 6/6/5 ring system using our carbonylative spirolactonization method (Figure 1C). Compound **10** could serve as a platform to synthesize the selected *Abies* sesquiterpenoids and their analogs for biological evaluations and target identification. Retrosynthetically, hydroxycyclopropanol **9** could be synthesized from bicyclic lactone **8** using the Kulinkovich reaction. We envisioned a hydrocarbonylative lactonization of the simple alkenediol **7**, accessed from the chiral pool molecule (+)-carvone, to install the 6-membered lactone.

Herein, we report the details of our total syntheses of abiespiroside A, beshanzuenones C and D, and their analogs including azide-tagged chemical probe molecules for target identification. These syntheses enabled us to evaluate their protein tyrosine phosphatase inhibiting potency and discover new lead compounds as potent inhibitors of protein tyrosine phosphatase SHP2, a known oncogenic driver. Moreover, target identification using a classical chemoproteomic method with the azide-tagged probes has revealed DNA polymerase epsilon subunit 3 (POLE3) as their potential cellular target.

RESULTS

Total Synthesis.

Our synthesis began with the conversion of (+)-carvone (**6**) to hydroxyl ketone **11**¹³ via a Rubottom oxidation (Figure 2). The requisite stereoselective reduction of α -hydroxy ketone **11** to *anti*-diol **12** proved to be nontrivial. Using the existing α -hydroxyl group to deliver a hydride source from the same face with reagents such as NaBH(OAc)₃, Me₄NBH(OAc)₃, and NaBH₃CN failed, giving low conversion (<10%) and the *cis*-diol as the major product. The use of LiAlH₄ in Et₂O gave 100% conversion, but a 54:46 mixture of the *trans/cis* isomer. Interestingly, while the use of NaBH₄ in AcOH gave <10% conversion and *cis*-diol as the major product, simply switching the AcOH solvent to THF improved the conversion to 100% and gave an 86:14 mixture of *trans/cis* isomers in 74% isolated yield. The reduction is highly efficient on a large scale, yielding more than 15 g of the desired *trans*-diol **12** in 96% overall yield and 5:1 diastereomeric ratio.

We then moved on to the Pd-catalyzed hydrocarbonylative lactonization to synthesize bicyclic lactone **13** (Table 1 and Figure 2). In 1976, Knifton reported a series of highly active ligand-stabilized palladium(II)-group 4B metal halide catalysts such as PdCl₂(PPh₃)₂-SnCl₂ for regioselective carbonylation of α olefins to synthesize linear carboxylic acid esters.¹⁴ These dimetallic catalyst systems were later studied in detail by Kalck and others and expanded to the synthesis of lactones.¹⁵ Substrate **12** presents significant challenges when applied to the hydrocarbonylative lactonization. It contains an unprotected *trans*-1,2-diol and an endocyclic olefin in addition to the isopropenyl group, both of which increase the reaction complexity and could generate side reaction pathways such as carbonate formation and/or Wacker-type cyclization. We started with a combination of 0.01 equiv of PdCl₂(PPh₃)₂ and 0.02 equiv of hydrated SnCl₂ (Table 1, entry 1) under 7 atm of carbon monoxide in toluene

at 80 °C, but no desired product was obtained. Increasing the $\text{PdCl}_2(\text{PPh}_3)_2$ loading to 0.1 equiv afforded bicyclic lactone products **13** and **13'** in 50% yield as a 2:1 mixture of inseparable diastereomers. Kalck et al. showed that the introduction of chiral bidentate DIOP ligand improved the diastereoselectivity. We therefore switched PPh_3 to (+)-DIOP and found that the lactone products **13** and **13'** were produced as a 4:1 mixture of diastereomers in 30% yield at 65 °C (entry 3.) With a 1:1 ratio of PdCl_2 and (+)-DIOP, higher temperature (80 °C) was detrimental to the carbonylation reaction (entry 4), but lower temperature (50 °C) gave increased reaction yield after 60 h (57%, entry 5). These results indicated that the current palladium catalyst system was not stable at 80 °C, but slow for catalyzing the reaction at 50 °C. Therefore, we considered the possibility of adding more (+)-DIOP to help stabilize the palladium catalyst at higher temperature. Interestingly, addition of another 0.1 equiv of (+)-DIOP to the reaction mixture inhibited the carbonylation at 65 °C (entry 7), but was able to facilitate the production of the lactone product in 57% yield (d.r. 4:1) at 97 °C in 24 h (entry 8). It was hypothesized that a more stable, but unreactive palladium complex was formed with the addition of another 0.1 equiv of (+)-DIOP, and this complex was converted to an active palladium species at higher temperature (97 °C). The absolute configuration of the chiral DIOP ligand did not affect the diastereoselectivity (entry 9) as the use of (—)-DIOP ligand gave the same result. Changing the reaction solvent from toluene to dichloroethane (DCE, entry 6) gave poor results. Increasing the pressure of carbon monoxide helped improve the overall yield (entry 10 and 11). Under 20 atm of carbon monoxide, an overall 81% yield (7.4 g) of **13** and **13'** were obtained (d.r. 4:1). The diastereomers were separated after the secondary alcohol was protected as TBS ether (**13** → **14**, Figure 2).

On the basis of the previous studies and our investigations, a proposed reaction mechanism is shown in Figure 3. The initial PdCl_2L_2 would first react with SnCl_2 to produce the highly electron-withdrawing $[\text{SnCl}_3]^-$ ligand^{15e} and the palladium complex $\text{PdCl}(\text{SnCl}_3)\text{L}_2$ (**16**). The latter would undergo an allylic C—H chlorination with **12** to presumably form allyl chloride byproduct **18** and generate the active $\text{PdH}(\text{SnCl}_3)\text{L}_2$ (**17**) species. The formation of allyl chlorides via a hydride abstraction process has been confirmed by Kalck and co-workers.^{15a,b} Homoallylic alcohol coordination followed by migratory insertion would afford more stable alkyl palladium species **20**, which under carbon monoxide atmosphere would undergo carbon monoxide migratory insertion to form acyl palladium species **21**. Lactonization would produce major isomer **13** and regenerate the catalytically active $\text{PdH}(\text{SnCl}_3)\text{L}_2$ (**17**) species. The stereochemical outcome is consistent to the hydrocarbonylative lactonization of isopulegol from the studies of Kalck and co-workers.^{15f} In both cases, the asymmetric induction is controlled by the substrate itself and the steric bulkiness of the DIOP ligand, but not the absolute chirality of the ligand. When limonene was used as a substrate in the Kalck studies, an almost 1:1 mixture of stereoisomers was produced. These results indicate that the secondary alcohols of both **12** and isopulegol are interacting with the palladium center (cf. **19**, **20**, and **21**). An axial delivery of the hydride is expected to result more stable intermediates **20** and **21** with the methyl group occupies the pseudoequatorial position, which eventually lead to the formation of **13** as the major isomer.

With bicyclic lactone **14** prepared in sufficient quantity, the next step was to synthesize hydroxycyclopropanol substrate **9** (R = H or TBS) for the carbonylative spirocyclization. This seemingly straightforward transformation turned out to be challenging. None of the Kulinkovich reaction conditions¹⁷ we explored (EtMgBr, Ti(O ν Pr)₄, THF or MeMgBr, EtMgBr, Ti(OiPr)₄, THF or Cp₂ZrCl₂, EtMgBr, Ti(OiPr)₄, THF) gave any desired cyclopropanol product from either starting material **14** or the free alcohol **13**. The only identifiable product we obtained was the hemiketal product from 1,2- addition of EtMgBr to the lactone carbonyl carbon.

The unsuccessful attempt toward hydroxycyclopropanol **9** prompted us to explore different strategies to introduce the oxaspirolactone moiety. We envisioned a Dreiding—Schmidt reaction¹⁷ between lactone **14** and methyl 2-(bromomethyl)- acrylate to synthesize oxaspirolactone **22** containing an exo methylene group, which would need to be isomerized into the γ -butyrolactone ring. Upon the treatment of lactone **14** with the metal/zinc reagent derived from methyl 2- (bromomethyl)acrylate and zinc metal, the Dreiding—Schmidt reaction proceeded smoothly to give the desired product **22** in 65% yield (3.5 g) with over 20:1 diastereoselectivity at the spirocyclic center. The stereochemistry of the spirocyclic carbon center is controlled by anomeric effect to produce dominant isomer **22** with the carboxylate group orients in axial position.^{12a} Having installed the α -methylene oxaspirolactone, the next step was to isomerize the exo methylene double bond into the thermodynamically more stable internal alkene. After screening several conditions, we found that both DBU and Ru₃(CO)₁₂ were capable of carrying out this transformation. While DBU only achieved at most 50% conversion, Ru₃(CO)₁₂ was able to completely isomerize the double bond.¹⁸ A subsequent TBS removal provided product **23** in 83% yield.

To complete the total synthesis of abiespiroside A, we needed to introduce the glucose moiety with a β -glycosidic linkage. We found that treating compound **23** with tetraacetylated thioglycoside **24**¹⁹ using the conditions described by Davis et al.²⁰ effected a β -selective glycosidation. Following methanolysis of the acetyl groups, abiespiroside A was isolated in 75% yield from **23** (2 steps). To convert compound **23** to beshanzuenones C and D, an allylic alcohol oxidation and an allylic C—H oxidation were needed. The treatment of **23** with an excess of selenium dioxide (5.0 equiv) gave four different oxidation products which were separated by flash chromatography and characterized. The major product was identified as beshanzuenone D and isolated in 50% yield, while beshanzuenone C was isolated in 10% yield. Two other oxidation products were also isolated: **25** containing a β -hydroxy aldehyde (26% yield) and **27** containing a 1,3-diol (5% yield).

Probe Synthesis.

Since the reported PTP1B inhibiting potency of beshanzuenones C and D are weak, we were also interested in identifying other cellular targets which might potentially interact with these *Abies* sesquiterpenoids. Structurally, abiespiroside A and beshanzuenones C and D contain an α,β -unsaturated γ -butyrolactone, and beshanzue-nones C and D have an additional cyclohexenone moiety. Both of these electrophilic α,β -unsaturated carbonyl systems could potentially function as Michael acceptors to interact with cellular nucleophiles via covalent interactions.²¹ With this in mind, we therefore decided to prepare

azide-tagged cellular probes for quantitative proteomics analysis (Figure 4) and designed both **27** and **32** as the potential probe molecules. Probe **27** is a direct derivative of beshanzuenone C (**2**) and was synthesized from beshanzuenone C (**2**) via a sequence of mesylate formation and azide nucleophilic replacement in 40% yield. Probe **32** has a α -methylene- γ -butyrolactone moiety, which is more reactive than the α,β -unsaturated γ -butyrolactone of **27** and should interact with more potential cellular nucleophiles and form a covalent bond faster.²² Meanwhile, it still contains the 6/6/5 tricyclic core of beshanzuenone C so the structural perturbation is relatively small. Therefore, **32** could serve as a more reactive surrogate if **27** could not fish out any meaningful cellular targets. Azide-tagged analog **32** was prepared from intermediate **22**. After fluoride-assisted TBS removal and allylic oxidation with SeO₂, products **29**, **30**, and **31** were isolated in 56%, 22%, and 6% yield, respectively. Compound **31** was then converted to **32** in 35% yield via the mesylate formation and azide nucleophilic replacement sequence.

Protein Tyrosine Phosphatase Inhibiting Potency.

After completing the synthesis, we evaluated the inhibiting potency of abiespiroside A, beshanzuenones C, and D, and a few analogs including **14**, **22**, **23**, **25**, **26**, **28–32**, and acetylated abiespiroside A (**1-Ac**) against protein tyrosine phosphatases (PTPs) PTP1B and SHP2. PTP1B is a key target for the treatment of type-II diabetes and obesity²³ and SHP2 overexpression and mutations have been associated with a variety of human cancers.²⁴ Under comparable assay conditions, our synthetic beshanzuenone C (**2**) showed potency against PTP1B with an IC₅₀ value of 41.1 μ M (Table 2), which is similar to the previously reported result (IC₅₀ = 59.7 μ M).⁸ Moreover, synthetic beshanzuenone C (**2**) also inhibited SHP2 with an IC₅₀ value of 48.2 μ M (Table 2). While natural beshanzuenone D was reported to inhibit PTP1B with a 40.4 μ M IC₅₀ value, in our hands, the synthetic beshanzuenone D (**3**) exhibited no inhibiting potency against PTP1B or SHP2 even at 500 μ M compound concentration. Interestingly, abiespiroside A (**1**) also displays inhibitory potency against PTP1B and SHP2, albeit at about 2-fold lower potency than that of **2**, whereas acetylation of abiespiroside A (**1-Ac**) eliminated the PTP inhibitory potency. Compounds **14** and **25** inhibited both PTP1B and SHP2 with similar potency but **25** is three times more potent than **14** on both enzymes. Strikingly, compounds **26**, **29**, **30** and **31** showed up to 10-fold preference for SHP2 over PTP1B (Table 2). In fact, compound **30** turned out to be the most potent compound among all of the tested analogs, with an IC₅₀ value of 3.3 μ M for SHP2. Additionally, azide-tagged probe molecule **32** showed weak inhibition against both PTP1B and SHP2 with 100 μ M and 38 μ M IC₅₀ values respectively.

The PTPs share a common catalytic mechanism that utilizes a highly reactive nucleophilic Cys residue.²⁵ Given the presence of electrophilic α,β -unsaturated carbonyl systems within abiespiroside A, beshanzuenones C and D, and their synthetic analogs, we speculated that they could potentially function as covalent PTP inhibitors through Michael addition reaction with the PTP active site Cys. In support of this hypothesis, the apparent IC₅₀ values of compounds **2**, **3**, **26**, **29**, and **30** decreased several folds when they were preincubated with SHP2 for 20 min (data not shown). To determine whether compound **30** can indeed function as a covalent SHP2 inhibitor, we examined for its effect on SHP2 activity using para-nitrophenyl phosphate (*p*NPP) as a substrate.²⁶ Compound **30** inactivated SHP2 in a time-

and concentration-dependent first order process (Figure 5A). Analysis of the pseudo-first-order rate constant as a function of inhibitor concentration yielded a second-order rate constant of $7.1 \pm 0.4 \times 10^3 \text{ M}^{-1} \text{ min}^{-1}$ for compound **30**-mediated SHP2 inactivation (Figure 5B). Although cryptotanshinone was reported as an irreversible inhibitor of SHP2, its potency against SHP2 is 7-fold lower than that of **30**, and it also inhibits several other PTPs including PTP1B with the similar IC_{50} values.²⁷ Thus, compound **30** represents the first covalent inhibitor with selectivity for SHP2.

Target Identification.

Due to their electrophilic nature and potential to form covalent bond with nucleophilic proteins, we next sought to explore other possible cellular targets of these *Abies* sesquiterpenoids and their derivatives using the azide probes **27** and **32**. MDA-MB-231 cell lysates were treated with **32** or **27** for 1 h at different concentrations and the probe-treated lysates were then subjected to click chemistry using tetramethylrhodamine alkyne. The lysates were then separated by SDS-PAGE and the fluorescently labeled protein bands were visualized using in-gel fluorescence scanning (Figures 6A and S1 of the Supporting Information, SI). As expected, we observed much stronger labeling with the chemically more reactive α -methylene probe **32**. Interestingly, the proteome labeling pattern with probes **32** and **27** was very similar, meaning that repositioning of the double bond improved the electrophilicity but did not significantly affect the selectivity. We further confirmed that probe **32** binds to cysteines, as pretreatment of the lysates with the cysteine-reactive reagent JW-RF-001²⁸ completely abolished the labeling (Figures 6B and S1). Moreover, we tested whether **32** binds to proteins reversibly by posttreating with JW-RF-001 an irreversibly binding cysteine-reactive probe. Indeed, post-treatment with JW-RF-001 completely competed the MDA-MB-231 cell lysate labeling by **32** (Figure S2). Overall, these results indicate that **32** binds to cysteines in a covalent reversible manner.

We next treated live MDA-MB-231 cells with $30 \mu\text{M}$ **29** or DMSO as control. The cells were then lysed and treated with the azide probe **32**. The probe-labeled proteomes were then tagged via click chemistry with biotin alkyne, enriched over streptavidin beads, digested, and analyzed by LC-MS/MS. Using label-free quantification (LFQ),²⁹ we identified two proteins with competition >50% as potential targets of compound **29** (Figure 6C, Table S1). The best competed target in the list, POLE3, is a scarcely characterized histone-fold protein that represents the accessory subunit of the DNA polymerase ϵ (Pol ϵ). We decided to focus on the compound **29**-POLE3 interaction due to the reported role of Pol ϵ in the nucleotide excision repair (NER) and base excision repair (BER) pathways.³⁰ Thus, targeting POLE3 with small molecules may potentially be a novel strategy for chemo-sensitization in cancer. We performed an additional target ID experiment in HeLa cells and confirmed that compound **29** engages POLE3 also in this cell line (Table S2).

To verify that POLE3 is indeed a physical target of **29**, we overexpressed human POLE3-GFP in 293T cells and fluorescently labeled it with the azide probe **32** followed by gel separation and fluorescence scanning. This labeling was competed by **29** in a concentration-dependent manner ($\text{IC}_{50} = 59 \mu\text{M}$ Figure 7A and S4). Comparably efficient competition was also observed with the structurally closely related α -methylene compound **31** ($\text{IC}_{50} = 69$

μM ; Figures S3 and S4). Furthermore, POLE3 labeling was also competed by Beshanzuene C, albeit less potently ($\text{IC}_{50} = 91 \mu\text{M}$; Figures S3 and S4). Finally, the compounds **28** and **30**, reduced ketone derivatives of **29** and **31**, respectively, did not show any noticeable affinity to POLE3 even at $300 \mu\text{M}$ concentration (Figures S3 and S4). This result indicates that both electrophilic sites (the α,β -unsaturated enone and α -methylene- γ -butyrolactone) of **29** are critical for POLE3 binding. This case represents one of the rare examples where two electrophilic sites are required for effective target protein interaction.³¹ As POLE3 contains only one cysteine, we mutated C51 to serine and confirmed that the mutant POLE3 is not labeled by the probe **32** (Figure 7B). Moreover, we confirmed the C51 as the binding site for **29** via a targeted IAA-competitive proteomics experiment. Here, DMSO or $30 \mu\text{M}$ **29**-treated MDA-MB-231 cells were lysed and treated with IAA alkyne as a cysteine-reactive enrichment probe.³² Probe-labeled peptides were then enriched and released using our previously reported catch-and-release strategy. This strategy allows detection and quantification of individual cysteine sites rather than whole proteins. Detailed inspection of the POLE3 MS² spectra unambiguously revealed the carbamidomethyl modification on the C51 residue on one of the POLE3 tryptic peptides and this peptide had 2-fold higher MS1 intensity in the DMSO treated sample (Figure S5).

To understand whether binding of **29** to C51 of POLE3 is productive and leads to the loss of function of this protein, we knocked down POLE3 in HeLa cells using siRNA and compared by global proteomics the protein expression profile in these cells versus cells treated with compound **29** or DMSO as control. We found 224 overexpressed and 216 down-regulated proteins in POLE3 KD cells versus cells treated with DMSO (Table S3). Moreover, we observed a significant overlap in up- and downregulated proteins between the POLE3 KD cells and the cells after 6 h treatment with **29**, thus indicating that binding by **29** indeed leads to the loss of function of POLE3 (Figure 7C). Interestingly, we found several proteins involved in DNA repair that were upregulated in both POLE3 KD and **29**-treated cells, such as DNA ligase 3,³³ GEMIN2,³⁴ PEA15,³⁵ and GIT2.³⁶ Possibly as a compensatory effect, we also observed 4-fold upregulation of POLE, another subunit of DNA polymerase ϵ . These results suggest that POLE3 may indeed be involved in endogenous DNA repair pathways, although clearly deeper biological insight is required to decipher the mechanistic role of POLE3 in these critical processes.

Due to its potential involvement in DNA repair pathways, we sought to investigate the effect of POLE3 knockdown or inactivation by **29** in combination with a DNA damaging agent such as etoposide. Etoposide is a clinically applied drug to treat numerous types of cancer. It forms a ternary complex with DNA and topoisomerase II to eventually cause DNA strand breakage. Briefly, HeLa cells were treated or not with POLE3 siRNA and then subsequently exposed to treatment by $30 \mu\text{M}$ etoposide, $30 \mu\text{M}$ **29**, or $30 \mu\text{M}$ etoposide combined with $30 \mu\text{M}$ **29**. Induced DNA damage was measured by confocal microscopy using γH2AX staining as marker. Treatment with **29** alone did not lead to an increase in γH2AX signal intensity. However, intriguingly, we observed significantly stronger γH2AX signal in cells treated with etoposide and **29** versus etoposide only treated cells (Figures 8, S6, and S7). Likewise, untreated POLE3 KD cells did not show increased DNA damage, but adding etoposide again caused stronger γH2AX signal in comparison to the etoposide-treated wild-

type cells. Furthermore, combined treatment of the POLE3 KD cells with **29** and etoposide showed no statistically significant difference in γ H2AX signal compared to etoposide treatment of HeLa POLE3 KD cells or combined treatment of WT HeLa cells with **29** and etoposide. Collectively, these results demonstrate that targeting POLE3 with small molecules may indeed be a novel strategy for chemosensitization to DNA damaging drugs in cancer.

CONCLUSIONS

In summary, we report here the total syntheses and biological investigations of three *Abies* sesquiterpenoids and their analogs. The synthesis features a palladium-catalyzed hydrocarbonylative lactonization and a Dreiding-Schmidt reaction to build the 6/6/5 tricyclic ring system of the target molecules. Our efficient synthesis has enabled further biological evaluations of these *Abies* sesquiterpenoids. We found that a number of *Abies* sesquiterpenoids exhibit PTP inhibitory activity. Importantly, we identified compound **30** as the first covalent inhibitor with selectivity for SHP2. Since SHP2 is a known oncogenic driver and also a central signaling node in diverse regulatory pathways, further structure—activity optimization may lead to novel *Abies* sesquiterpenoid derivatives for SHP2 targeted anticancer therapies. In addition to their PTP inhibiting activities, we have identified and confirmed POLE3 as one of their cellular targets using a classical chemoproteomic approach. Furthermore, we have demonstrated that *Abies* sesquiterpenoid analogs inactivate POLE3 in cells, and this leads to chemosensitization to a DNA damaging agent, etoposide. Further studies aimed at understanding the involvement of POLE3 in this process as well as preparation of novel *Abies* sesquiterpenoid derivatives as more potent POLE3 inactivators are currently underway and will be reported in due course.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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A. Selected *Abies* sesquiterpenoids

B. Our reported Pd-catalyzed carbonylative spirocyclization

C. Synthetic Plan

Figure 1.
Background and synthetic plan.

Figure 2.

Total syntheses of abiespiroside A, beshanzuenones C and D, and analogs.

Figure 3.
Proposed catalytic cycle.

A. Synthesis of **27** B. Synthesis of **32**

Figure 4.
Azide-tagged probe molecule synthesis.

Figure 5.

Kinetics of SHP2 inactivation by compound **30**. (A) Time and concentration dependence of SHP2 inactivation by **30**. The experimental points are represented by various symbols and the line connecting them is the fitted line to a first-order exponential equation to yield a pseudo-first order rate constant k_{obs} . Compound **30** concentrations were as follows: closed circle, 11.75 μM ; closed triangle, 26.25 μM ; closed diamond, 39.5 μM ; open circle, 59.25 μM ; open square, 88.75 μM ; open triangle, 133.25 μM ; and open diamond, 200 μM ; (B) Concentration dependence of the pseudo-first-order rate constants k_{obs} for compound **30**.

mediated SHP2 inactivation. The points are experimental, and the line connecting them is the fitted straight line by linear regression.

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Figure 6. Identification of cellular targets of *Abies* sesquiterpenoid analogs. (A) Concentration-dependent labeling of MDA-MB-231 lysates with the azide probes **32** and **27**. (B) Competition of the proteome labeling by **32** using the general cysteine-reactive probe JW-RF-001. (C) Potential cellular targets of **29** in MDA-MB-231 cells identified by competitive proteomic profiling using 30 μ M of the azide probe **32** (normalized values $\pm$ SD, $n = 6$).

Figure 7.

Validation of POLE3 as a direct target of analog **29**. (A) Labeling of POLE3-GFP overexpressed in 293T cells using the azide probe **32** and concentration-dependent competition of the labeling with the analog **29**. The gel band corresponding to the overexpressed POLE3-GFP is indicated with a red arrow. (B) Labeling of the overexpressed WT and C51S mutant POLE3-GFP. (C) Venn diagrams showing common up- and downregulated proteins in HeLa cells induced by POLE3 KD or treatment with **30** μM **29**.

Figure 8.

Confocal fluorescence microscopy of γ H2AX staining as marker for DNA strand breakage. HeLa WT or POLE3 KD cells were treated with DMSO, **29**, etoposide, or etoposide in combination with analog **29** for 6 h. Scale bars indicate 30 μ m.

Table 1.

Reaction Condition Optimization

entry	reaction conditions (equiv)	yield ^a (d.r.)
1	PdCl ₂ (PPh ₃) ₂ (0.01), SnCl ₂ (0.02), PPh ₃ (0.02), CO (7 atm), tol., 80 °C, 24 h	NR
2	PdCl ₂ (PPh ₃) ₂ (0.1), SnCl ₂ (0.2), PPh ₃ (0.2), CO (7 atm), tol., 80 °C, 24 h	50% (2:1)
3	PdCl ₂ (+)-DIOP (0.1), SnCl ₂ (0.2), CO (7 atm), tol., 65 °C, 24 h	30% (4:1)
4	PdCl ₂ (+)-DIOP (0.1), SnCl ₂ (0.2), CO (7 atm), tol., 80 °C, 60 h	complex
5	PdCl ₂ (+)-DIOP (0.1), SnCl ₂ (0.2), CO (7 atm), tol., 50 °C, 60 h	57% (4:1)
6	PdCl ₂ (+)-DIOP (0.1), SnCl ₂ (0.2), CO (7 atm), DCE, 50 °C, 24 h	complex
7	PdCl ₂ (+)-DIOP (0.1), SnCl ₂ (0.2), (+)-DIOP (0.1), CO (7 atm), tol., 65 °C, 24 h	NR
8	PdCl ₂ (+)-DIOP (0.1), SnCl ₂ (0.2), (+)-DIOP (0.1), CO (7 atm), tol., 97 °C, 24 h	57% (4:1)
9	PdCl ₂ (-)-DIOP (0.1), SnCl ₂ (0.25), (-)-DIOP (0.1), CO (7 atm), tol., 97 °C, 24 h	55% (4:1)
10	PdCl ₂ (-)-DIOP (0.1), SnCl ₂ (0.25), (-)-DIOP (0.1), CO (11 atm), tol., 97 °C, 24 h	79% (4:1)
11	PdCl ₂ (+)-DIOP (0.1), SnCl ₂ (0.25), (+)-DIOP (0.1), CO (20 atm), tol., 97 °C, 24 h	81% (4:1)

^a Isolated reaction yields. NR: No Reaction.

Table 2.

PTP Inhibiting Potency

cmpd #	IC ₅₀ for PTP1B, μ M	IC ₅₀ for SHP2, μ M
1	91.3 $\pm$ 6.5	90.5 $\pm$ 7.0
1-Ac	>500	>500
2	41.1 $\pm$ 3.1	48.2 $\pm$ 1.6
3	>500	>500
14	193 $\pm$ 32	179 $\pm$ 21
22	>500	>500
23	>500	>500
25	38.6 $\pm$ 1.7	50.2 $\pm$ 1.9
26	>200	56 $\pm$ 15
28	>200	>200
29	>200	44.9 $\pm$ 9.6
30	34.7 $\pm$ 2.1	3.3 $\pm$ 0.1
31	>200	33.3 $\pm$ 0.9
32	100.9 $\pm$ 15	38.3 $\pm$ 1.9