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# Construction of a meroterpenoid-like compound collection by precursor-assisted biosynthesis†

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Natural products (NPs) and their derivatives play a pivotal role in drug discovery due to their complexity and diversity. The strategies to rapidly generate NP-like compounds offer unique opportunities to access bioactive compounds. Here we present a new approach, precursor-assisted biosynthesis (PAB), for the creation of NP-like compounds by combination of artificial supplementation of common precursors and divergent post-modifications of precursor-deficient fungi. This method was applied to construct a meroterpenoid-like compound collection containing 43 compounds with diverse molecular scaffolds. Extensive bioactive screening of the collection revealed novel STING (stimulator of interferon genes) inhibitors, cytotoxic and antifungal compounds. This result indicates that PAB is an effective methodology for producing compound collections for the purpose of drug discovery.



## Introduction

Natural products (NPs) and their derivatives remain a rich source of inspiration in the discovery and development of potential drugs and biological probes.[1] The scaffolds of NPs represent the biologically relevant and privileged fractions of chemical space because they have been naturally selected in evolution to specifically interact with biological macromolecules.[2] It has been suggested that NP-like compound collections are enriched in biochemical and biological activity.[3] Thus, the construction of NPs inspired compound collections and their evaluation is a highly promising strategy for the identification of unique bioactive compounds. Based on this reasoning, a number of chemical synthesis strategies have been developed to rapidly generate NP-like compounds including diversity-oriented synthesis (DOS),[4] biology-oriented synthesis (BIOS),[5] diversity-enhanced extracts[6] and the complexity-to-diversity (CtD) strategy.[7] However, the synthetic accessibility and efficiency of these strategies may face challenges associated with their structural complexity.

Biosynthetic machineries usually make use of divergent pathways to produce a variety of structurally complex and diverse NPs from only a handful of fundamental building blocks. Large numbers of NP-like compounds have been generated by various combinations of chemical and biosynthetic approaches.[8,9] Unfortunately, most of them possess similar scaffolds with original natural products. Since skeletal diversity is a major issue associated with the development of a high-value compound library for screening of promising drug leads and biological probes, a general strategy for the creation of NP-like compounds with diverse molecular scaffolds needs to be formulated.

The common biosynthetic precursors can be divergently modified to produce distinct skeletons in different organisms and therefore show significant potential to increase the skeletal diversity of NPs. During the evolution of secondary metabolism, the emergence of enzymes responsible for the biosynthesis of common precursors and post modifications sometimes happened separately.[10] Besides, non-specific enzymes could modify natural products and their biosynthetic precursors to increase the chemical diversity.[11] Thus, although some organisms possess post modification enzymes, the deficiency of the ability to synthesize the common precursors precludes the production of NPs. Artificial supplementation of the precursors to these organisms may lead to diverse NP-like compounds. We therefore focused on the development of a precursor-assisted biosynthesis (PAB) that combines artificial supplementation of common precursors and divergent post-modifications of precursor-deficient fungi to produce a large number of NP-like compounds.

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Meroterpenoids are natural products of mixed biosynthetic origin that are partially derived from terpenoids.[12] Particularly, meroterpenoids derived from the shikimate and terpenoid pathway produced by plants, mushrooms and marine invertebrates display high scaffold diversity and significant bioactivity.[13–16] Therefore, constructing a diverse compound collection based on shikimate derived meroterpenoids would increase the likelihood of discovering new drug candidates.[17] Intriguingly, a large number of members with diverse scaffolds of this family were biosynthesized from only several simple common precursors, prenylhydroquinone (**p1a**), geranylhydroquinone (**p1b**) and farnesylhydroquinone (**p1c**) by divergent pathways (Fig. 1). For example, a biogenetic hypothesis for representative members is outlined in Fig. 1, where the prenylation and oxidative decarboxylation of 4-hydroxybenzoate (4-HBA) lead to the formation of common precursors **p1a–c**. Divergent modification including oxidation, cyclization and dehydration of **p1a–c** directly generates a vast chemical diversity and produces panepoxydone, shikonin, clavilactone A, conicol, longithorone B, pleurotin and rossinone B.[13–16] Biosynthesis of structurally distinct meroterpenoids from common precursors showed the huge potential of post-modification to create chemical diversity.

In this paper, we reported the construction of a meroterpenoid-like compound collection containing 43 compounds with twelve distinct scaffolds by feeding chemical synthesized common precursors, **p1a–c**, and their analogues, **p2–5**, of meroterpenoids to five precursor-deficient basidiomycete fungi which do not produce meroterpenoids under normal lab conditions. Among these compounds, STING (stimulator of interferon genes) inhibitors, cytotoxic and antifungal compounds were obtained through extensive bioactive screening.

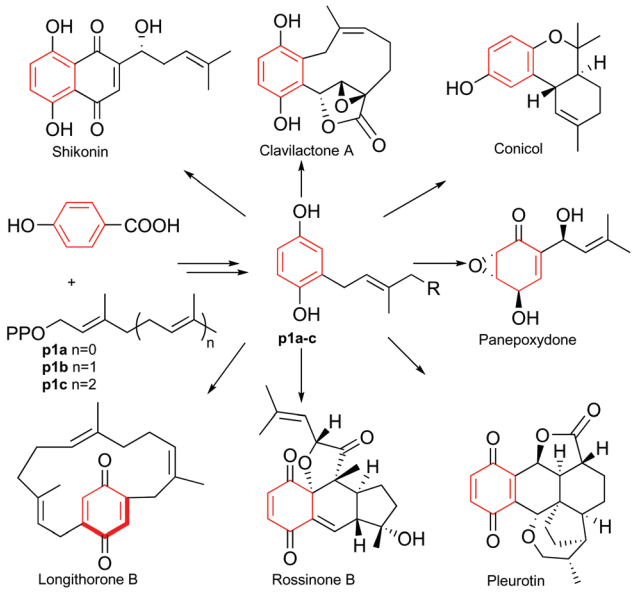


**Fig. 1** Proposed divergent biosynthetic pathways of shikimate derived meroterpenoids.

## Results and discussion

Prenylhydroquinone (**p1a**) is a common precursor of meroterpenoids with diverse scaffolds. To test whether this common precursor could be used for the generation of meroterpenoid-like compounds by PAB, **p1a** was synthesized and fed to the culture of five precursor-deficient basidiomycete fungi *Agrocybe pediades*, *Marasmius graminum*, *Stereum histurum*, *Psathyrella candolleana* and *Trametes hirsute* on the seventh day after inoculation, respectively. After an additional two days of culture, the culture broth was harvested and extracted with ethyl acetate, and the extracts were analyzed by HPLC. Notably, some new peaks were observed in the HPLC profiles of the crude extracts of **p1a** supplemented culture of all five strains compared with controls (Fig. S1–S5†). To elucidate the structures of the transformed products, **p1a** was supplemented in the scaled-up culture of these fungi. Systematic purification of their ethyl acetate extracts by a combination of column chromatography over silica gel and Sephadex LH-20 and semi-preparative reversed-phase HPLC afforded eight (**1–8**), two (**9–10**), two (**7–8**), two (**7–8**) and three (**1**, **11–12**) meroterpenoid-like compounds from these fungi, respectively (Fig. 2). Their structures were elucidated on the basis of extensive spectroscopic analysis (ESI-MS, HR-ESI-MS, and 1D and 2D NMR) (Tables S1–S3†) and by comparison of their NMR data with those reported in the literature. The absolute configuration of **1** was deduced by comparing its specific rotation, −43.5 (*c* 0.23, MeOH), with that of (*S*)-4-hydroxy-3-methyl-2-cyclohexen-1-one, −35.2 (*c* 1.0, $CHCl_3$)[18] and speciosin K, −14.5 (*c* 0.13, $CHCl_3$).[19] The relative configurations of **2–3** were deduced from $^1$H NMR coupling constants (Table S1†) and their absolute configurations were assigned by octant rule analysis of the Cotton effects observed in the CD spectra (Fig. S23 and S24†). The relative configuration of **10** was determined by single-crystal X-ray diffraction analysis (Fig. S16 and Table S15†). The β-xylose unit in **11–12** was elucidated based on the $^1$H NMR coupling constants (Table S3†).[20] Compounds **1–3** and **10–12** are new compounds, while **4–6**, **7**, **8** and **9** were isolated previously from *Hexagonia crinigera*,[21] *Acremonium sp.*,[22] *Aplidium californicum*,[23] and *M. graminum*,[24] respectively. Compounds **1–6** are assumed to be formed by successive reduction of prenylquinone (A) as shown in Fig. 3. Oxidation of **p1a** followed by reduction of the C5–C6 double bond and the C1 ketone generates **1** and its enantiomer **1a**. Further reduction of **1a** at the C2–C3 double bond afforded **2–3** and subsequent reduction of **2–3** at the C4 ketone led to the formation of **4–6**. Compounds **7–8** seem to be formed by epoxidation of **p1a** followed by cyclization and dehydration.[25] Compound **9** is assumed to be produced by 6π electrocyclization followed by dehydration of intermediate D, while compound **10** seems to be formed by radical addition of intermediate C.[26] The formation of **11–12** may be catalyzed by a beta-xylosidase.[27]

Meromonoterpenoids derived from geranylhydroquinone (**p1b**) display greater chemical diversity than **p1a** derived meroterpenoids, owing to the geranyl offering more modification sites. To obtain meromonoterpenoid-like compounds, we syn-

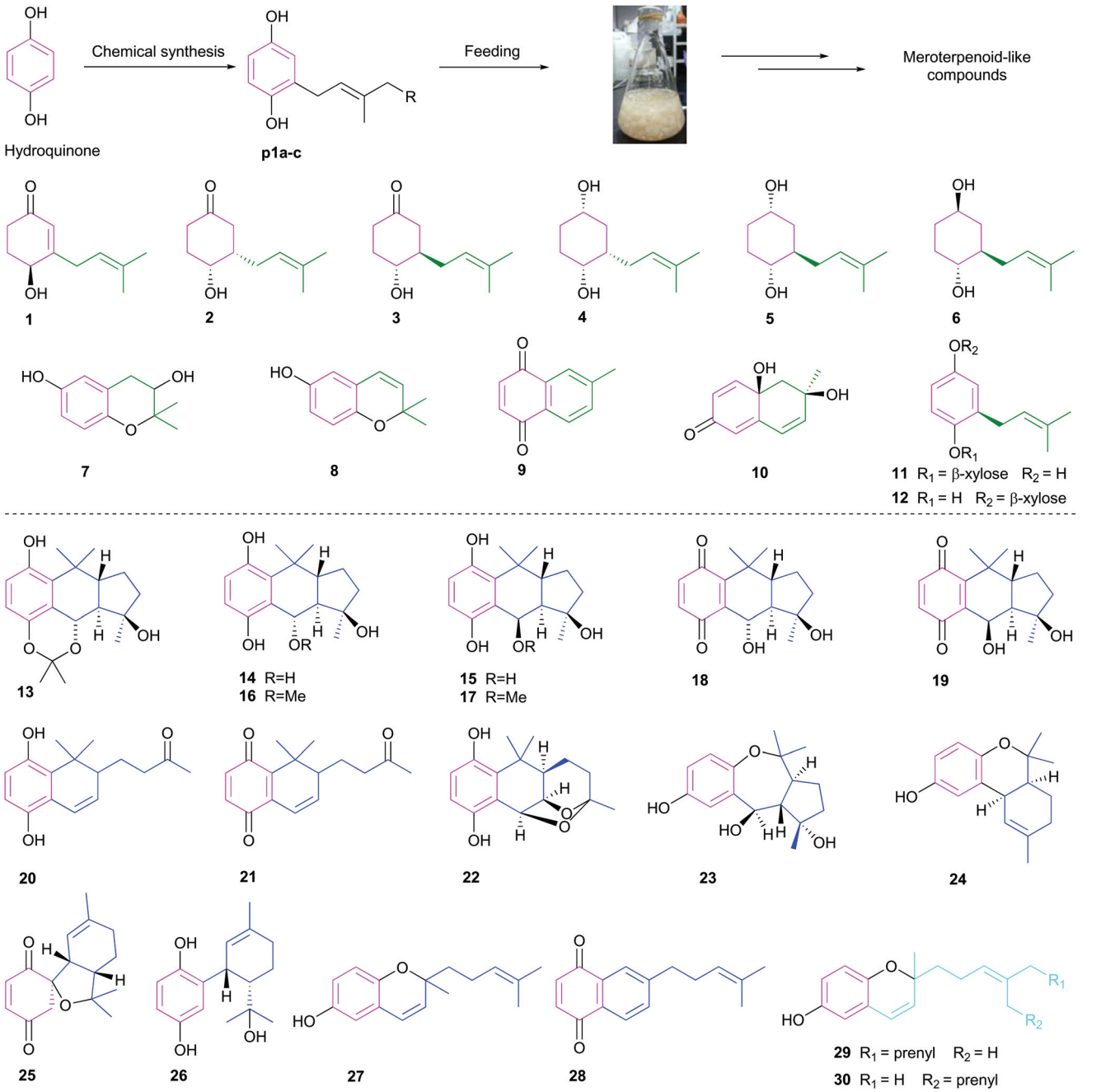


**Fig. 2** Meroterpenoid-like compounds isolated from **p1a–c** supplemented culture of precursor-deficient fungi.

thesized **p1b** and fed it to the culture of *A. pediades*, *M. graminum*, *S. histurum*, *P. candolleana* and *T. hirsute* as described for **p1a**. HPLC analysis of the extracts indicated obvious new peaks emerged from the crude extracts of **p1b** supplemented culture of all five strains compared with controls (Fig. S6–S10†). The crude extracts derived from the **p1b** supplemented culture of five strains were separated by repeated column chromatography and semi-preparative reversed-phase HPLC. Eleven meroterpenoid-like compounds (**13–17**, **20–21**, **23–25** and **27**) were isolated from the extracts of **p1b** supplemented culture of *A. pediades* (Fig. 2), whereas nine (**13–19**, **22** and **28**), three (**24–26**), nine (**13–17**, **21–23** and **25**) and two (**20–21**) compounds were isolated from those of *M. graminum*, *S. histurum*, *P. candolleana* and *T. hirsute*, respectively (Fig. 2). The structures including the relative stereochemistry of these compounds were established based on NMR spectroscopy and single-crystal X-ray diffraction analysis (Fig. S17–S20, Tables S4–S8 and S16–S19†). The specific rotation, +4.2 (*c* 0.95, $CHCl_3$) and the absence of CD Cotton effects indicated that **13** is likely a racemate which was further confirmed by chiral HPLC analysis and single-crystal X-ray data (Fig. S17 and S21†). Considering the same biosynthesis

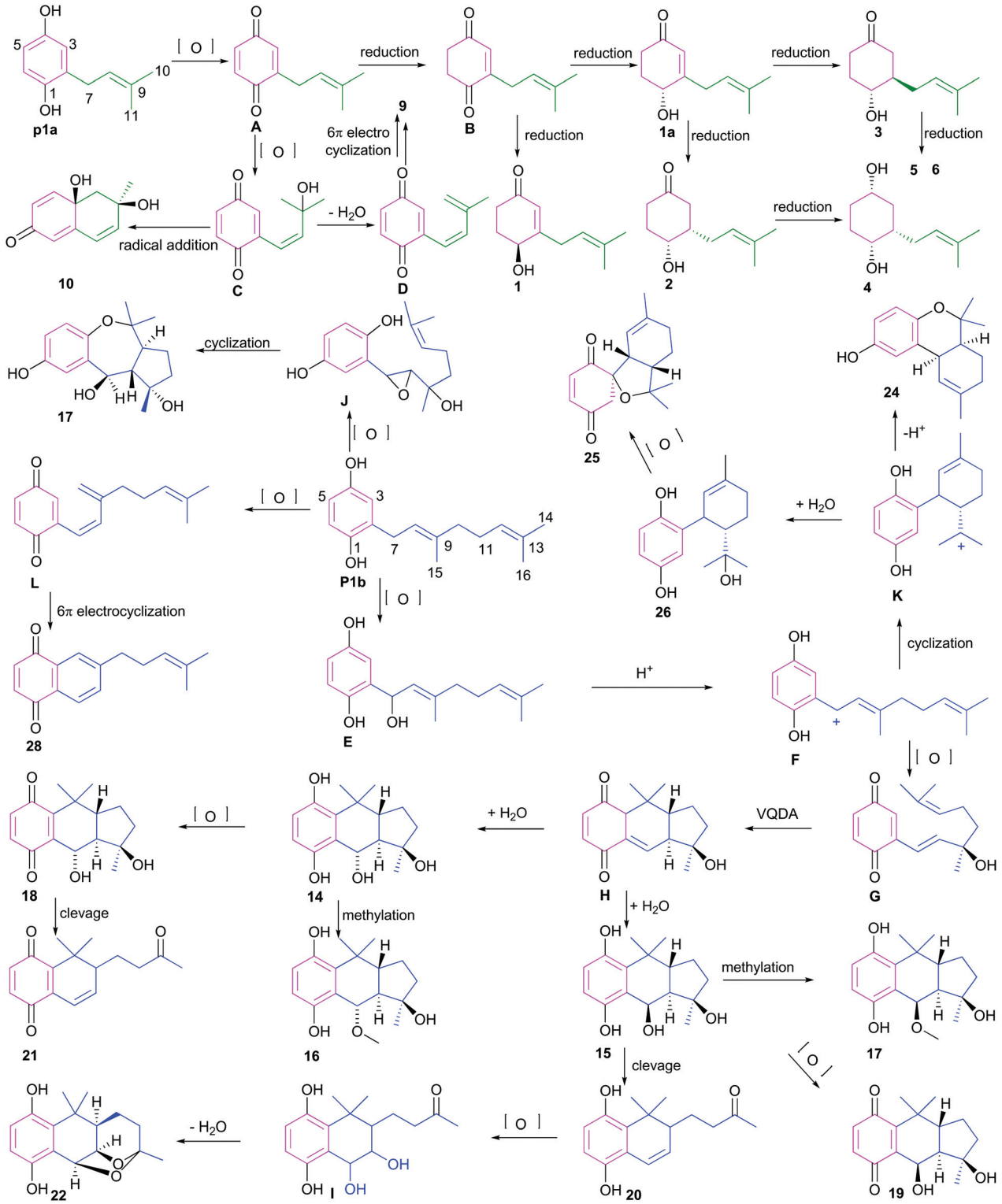


Fig. 3 Plausible biosynthetic pathways for the meroterpenoid-like compounds obtained by PAB.

pathway as well as their specific rotation and CD spectra, compounds **14–22** were also deduced to be racemate. Compounds **13–19** have a rare linearly fused 6,6,5-tricyclic carbon skeleton, which seems to be formed by intramolecular vinyl quinone Diels–Alder reactions (VQDA) of intermediate G. Several meroterpenoids containing this rare 6,6,5-ring system, pycnanthuquinones A–C and rossinone B, have been reported and the biomimetic synthesis of (−)-pycnanthuquinone C was accom-

 

plished by the Trauner group in 2010.[28] The fact that **13–22** are racemate accords with proposed nonenzymatic biosynthetic pathways as shown in Fig. 3. Hydroxylation of **p1b** at C-7 followed by rearrangement of hydroxyl and oxidation generates the vinyl quinone G. The intramolecular VQDA reaction and subsequent addition of water at C-7 afforded **14** and **15**. Methylation of C-7 hydroxyl could yield **16–17**. On the other hand, aerobic oxidation of **14–15** afforded **18–19**. Compounds **20–22** are assumed to be produced by cleavage of the C-8/C-9 carbon bond of **14–15** depicted in Fig. 3. Compound **23** possesses a rare benzo[*b*]cyclopenta[*e*]oxepine ring system, which was assumed to be formed by 8,12 cyclization of J. The optical rotation for **23** was −2.7 (*c* 0.10, MeOH), as well as the CD spectrum showed no Cotton effect indicating that **23** was also a racemic mixture, which was further confirmed by chiral HPLC analysis (Fig. S22†). Epiconicol (**24**), conidione (**25**), conitriol (**26**) and cordiachromene A (**27**) were isolated previously from Ascidian *Aplidium conicum* and **24–26** were likely formed by 7,12 cyclization of F.[29] Compound **28** was reported as a synthetic compound and seems to be formed by 6π electrocyclization followed by dehydration of intermediate L.[30]

To obtain merosesquiterpenoid-like compounds, **p1c** (*Z*, *E* mixture) was synthesized and fed to the culture of *A. pediades*, *M. graminum*, *S. histurum*, *P. candolleana* and *T. hirsute* as described for **p1a**. Unfortunately, new peaks were observed only in the crude extracts of **p1c** supplemented culture of *S. histurum* (Fig. S11–S15†). After a 10 L scale culture, (*E*)-dictyochromenol (**29**) and (*Z*)-dictyochromenol (**30**) were isolated as transformed products of **p1c** (Fig. 2). Dictyochromenol was reported previously as an antifeedant with potent activity comparable to that of zonarol and zonarone from *Dictyopteris undulata*.[31] Compounds **29–30** seem to be formed by oxidative cyclization of **p1c**.[25]

The production of compounds **1–30** from common precursors **p1a–c** showed the huge potential of precursor-deficient basidiomycete fungi to create the chemical diversity of meroterpenoids by divergent modification. To further expand the chemical diversity of meroterpenoid-like compounds, analogues, **p2–5**, of **p1b** were synthesized as described in supporting materials and fed to the culture of *A. pediades*. As a result, four (**31–34**), three (**35–37**), two (**38–39**) and four (**40–43**) meroterpenoid-like compounds were isolated as the transformed

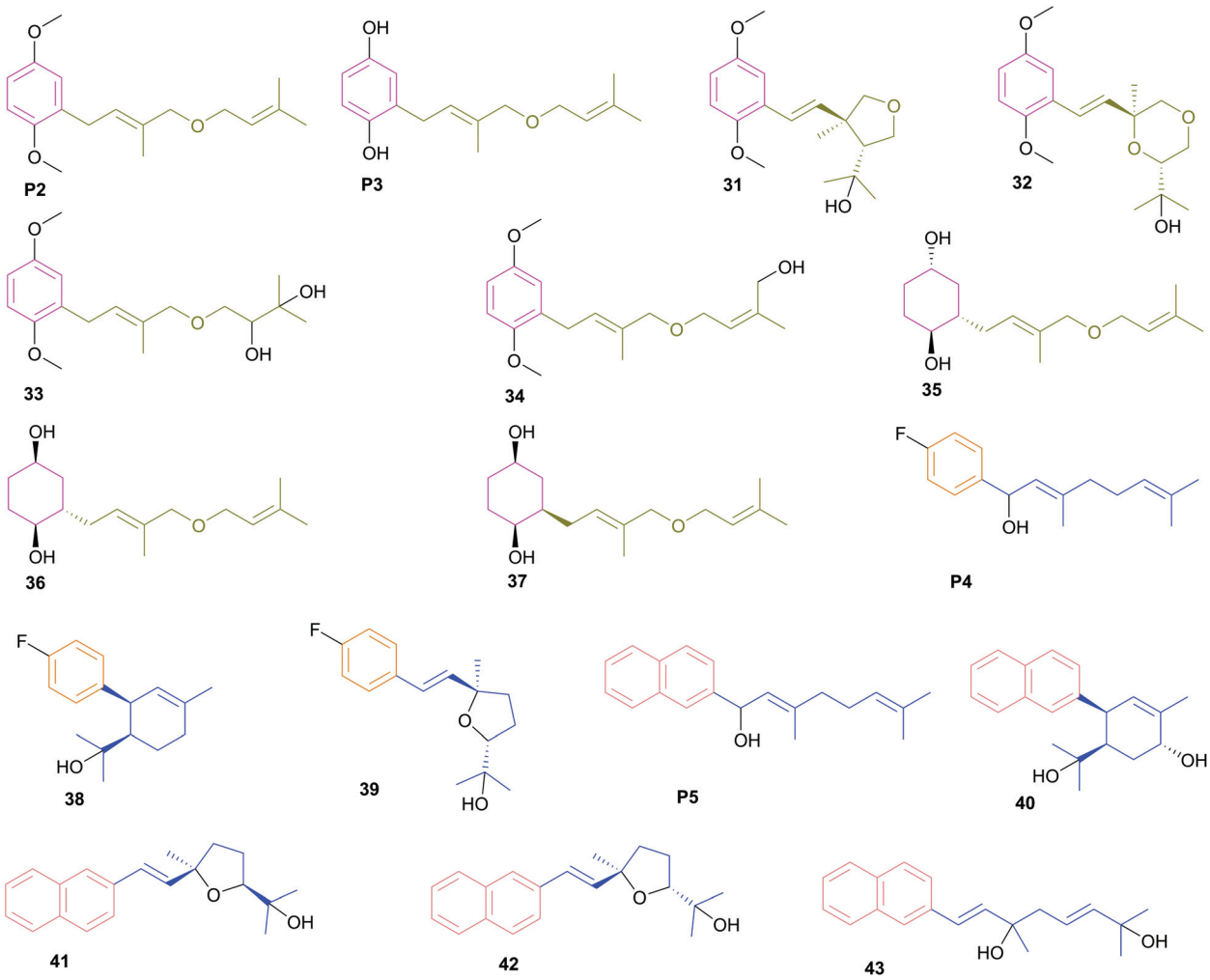


**Fig. 4** Meroterpenoid-like compounds isolated from **p2–5** supplemented culture of *A. pediades*.

products of **p2**, **p3**, **p4** and **p5**, respectively (Fig. 4). The structures including the relative configurations of these compounds were established based on NMR spectroscopy (Tables S9–S14 and Fig. S122–S197†). Compounds **31**, **38** and **40** are assumed to be formed by carbocation-initiated cyclization. Compounds **32**, **39** and **41–42** seem to be formed by intramolecular dehydration. The formation of compounds **35–37** is similar to that of **4–6**.

To verify the usefulness of the collection of meroterpenoid-like compounds for drug discovery and to discover new bioactive compounds, the collection was extensively screened for biological activities. As a result, compounds **16–19** showed potent inhibition activity against STING (stimulator of interferon genes) with $IC_{50}$ values of 50.1, 41.6, 66.2 and 32.3 μM (Fig. S25†), compound **25** showed cytotoxicity against NCI-H226 with the $IC_{50}$ value of 14.8 μM, and compound **40** showed moderate inhibition of growth of *Rhizoctonia solani* (42.3%) at 10 μM. The cGAS-STING signaling is essential for innate immunity. Chronic activation of the STING pathway has been found to be involved in autoimmune diseases as interferon (IFN) overproduction. Considering the central role of STING in IFN induction, developing inhibitors that target STING could be a promising way to treat several autoimmune diseases.[32] Therefore, compounds **16–19** could be seed compounds for treating autoimmune diseases.

## Conclusions

In summary, we constructed a collection of meroterpenoid-like compounds by the combination of artificial supplementation of common precursors and divergent post modifications of precursor-deficient fungi. The collection contains 30 new meroterpenoid-like compounds and 13 natural meroterpenoids isolated previously from fungi and marine invertebrates. The production of natural meroterpenoids by PAB confirms that some precursor-deficient fungi possess post modification enzymes for the biosynthesis of NPs and provides new insight into the biosynthesis of meroterpenoids. After extensive bioactive screening of the collection, compounds **16–19** were identified as inhibitors of STING, **25** was identified as a cytotoxic compound and **40** was identified as an antifungal compound. This fact indicates that PAB is an effective methodology for constructing compound collections for screening biologically active compounds. To expand the chemical diversity of NP-like compound collections, more common biosynthetic precursors and organisms will be used in PAB.

## Conflicts of interest

The authors declare no competing financial interest.

## Acknowledgements

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