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## The fungal natural product class of the sorbicillinoids: structures, bioactivities, biosynthesis, and synthesis†

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Covering 1948 up to October 2024

Sorbicillinoids are a growing class of natural products (NPs) that stem from a variety of fungi including members of the orders *Hypocreales* and *Eurotiales*. This compound class is unique in its combination of structural complexity and pharmaceutically relevant biological activities. The majority of the sorbicillinoids, which are named after the common hexaketide precursor sorbicillin, exhibit anti-inflammatory, antimicrobial, cytotoxic, phytotoxic, and other selective enzyme inhibitory activities. Over the last eight decades, more than 170 sorbicillinoids, many with strong pharmaceutical potential, have been isolated and described in the literature. This review aims to provide an overview of the structural diversity, biosynthetic pathways, and synthetic studies of this exceptional NP class.

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## 1. Introduction

The first representative of this class of natural products (NPs), sorbicillin (**1**), was initially isolated and characterized in 1948 by Cram from *Penicillium notatum*.<sup>1,2</sup> Three decades later, more complex bicyclic compounds were found in the culture medium of *Verticillium intertextum* and *Trichoderma longibrachiatum*.<sup>3–5</sup> Since then, a large number of new analogues were isolated, and structurally elucidated from marine and terrestrial sources.<sup>6–8</sup> It is important to note that the taxonomy of the fungi has been reclassified in the literature since initial sorbicillinoid isolation publications (e.g. *Clonostachys rosea* instead of *Verticillium intertextum*). Throughout this article, the original references are used to avoid confusion. One major characteristic of this class of NPs is their broad range of biological activities. Due to their anti-inflammatory, antimicrobial, cytotoxic, phytotoxic, and enzyme inhibitory properties, sorbicillinoids are potential candidates for pharmaceutical and agrochemical applications. The combination of stereochemically complex three-dimensional architectures and highly promising biological properties makes sorbicillinoids a special class of NPs.

Since the last comprehensive review of Harned in 2011, multiple further sorbicillinoid NPs were isolated and significant progress has been made in understanding sorbicillinoid biosynthesis.<sup>6</sup> In this review, we first focus on the full biosynthetic pathway accessing the sorbicillinoids NP class. Second,

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an overview of the structural diversity ranging from monomeric to polymeric and hybrid sorbicillinoids will be given. Finally, synthetic approaches and chemo-enzymatic strategies to assemble these NPs will be presented.

## 2. Biosynthesis

In the course of the isolation of the initial monomeric and dimeric sorbicillinoids, the first biosynthetic hypotheses were suggested by Dreiding and Rast.<sup>4,9</sup> They determined that sorbicillin (**1**) is the key biosynthetic precursor of all compounds isolated up to that point. Further hypotheses<sup>10–12</sup> led to biosynthetic investigations using stable-isotope labelling experiments employing [1-<sup>13</sup>C] and [1,2-<sup>13</sup>C<sub>2</sub>] labeled sodium acetate.<sup>13</sup> This work firmly established the hexaketide origin of the sorbicillinoids and the exact labelling pattern, pointing at the polyketide origin of this NP family.<sup>14,15</sup> The exact mechanism of assembly of sorbicillin (**1**) was finally uncovered by in-depth biosynthetic investigation and functional analysis of the sorbicillinoid biosynthetic gene cluster from *Penicillium chrysogenum* by the Cox lab,<sup>16</sup> including the mechanism of the crucial oxidative dearomatization of **1** to sorbicillinol (**2**). The BGC (Fig. 1A) contains two iterative polyketide synthases (iPKS), SorbA and SorbB, that build up sorbicillin (**1**). The non-reducing (NR) iPKS SorbA (*orf4*) consists of a starter-unit acyl transferase (SAT),  $\beta$ -ketoacylsynthase (KS), acyl transferase (AT), product template (PT), acyl carrier protein (ACP), C-methyl transferase (CMeT), and reductive release (Red) domain. Open reading frame 5 (*orf5*) encodes the highly reducing (HR) iPKS SorbB comprising KS, AT, dehydratase (DH), CMeT,  $\beta$ -ketoacylreductase (KR), enoylreductase (ER), and ACP domains. SorbA fuses three C<sub>2</sub> units with reduction by the KR and DH domains

after each elongation step to provide the SorbA-bound sorbyl side chain (Fig. 1B). This is further extended with three additional C<sub>2</sub> units accompanied with two C-methylation events to deliver a SorbB-bound hexaketide precursor. The latter is released by action of the reductive domain (Red) as an intermediate aldehyde that undergoes a Knoevenagel cyclization to give sorbicillin (**1**).

In addition to the PKS machinery, there are two FAD-dependent monooxygenases (FMO) located in the BGC, which fulfill different tasks. Oxidative dearomatization of sorbicillin (**1**) to sorbicillinol (**2**) is carried out by the FMO SorbC, which uses FAD and NADH or NADPH as cofactors.<sup>16</sup> The conversion into sorbicillinol (**2**) occurs with perfect regio- and stereo-control. For the production of higher oxidised monomeric sorbicillinoids, such as epoxysorbicillinol (**3**) and oxosorbicillinol (**4**), it already became apparent at an early stage that a further oxidising enzyme is required.<sup>17</sup> Two decades later, it was confirmed by the Cox lab that the FMO Sor(b)D catalyses exactly these reactions.<sup>15,18,19</sup> Furthermore, the flavin-dependent monooxygenase Sor(b)D is supposed to additionally be involved in the intermolecular reactions to sorbicillinoid dimers.<sup>18,19</sup> Oxosorbicillinol (**4**) can also be considered as precursor of the recently isolated nitrogen-containing aminosorbicillinol (**5**).<sup>20</sup> Their interconversion could be catalysed by an aminotransferase. However, this hypothesis has not yet been experimentally validated.

The diversity of sorbicillinoids is further extended by two derivatisations of the sorbyl side chain: reduction to 2'-3'-dihydro analogues and hydroxylation to 6'-hydroxy derivatives. In rare cases, a combination of both functionalisations was observed.<sup>21</sup> Biosynthetically, the formation of the corresponding 2'-3'-dihydro species is likely caused by a reduction step



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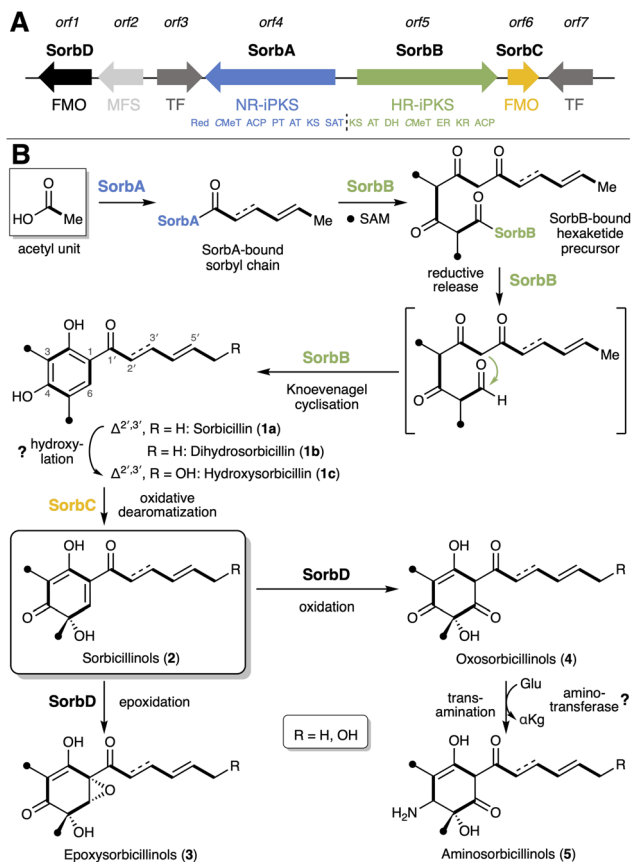


Fig. 1 (A) Sorbicillinoid biosynthetic gene cluster in *P. chrysogenum*. (B) Biosynthesis of monomeric sorbicillinoids.

during sorbyl side chain biosynthesis by the ER domain within the SorbA PKS machinery. The exact timing of this transformation and the enzyme involved in the hydroxylation of the sorbyl chain has not yet been described in the literature. The isolation of hydroxysorbicillin (**1c**) and the fact that heterologously expressed monooxygenase SorbC is able to oxidise **1c** to hydroxysorbicillinol suggest that the hydroxylation might take place at the level of sorbicillin (**1a**).<sup>21–23</sup> However, a hydroxylation of the sorbicillinoids at the end of the biosynthetic pathway leading to a specific compound cannot be excluded.

The biosynthesis of sorbicillinols (**2**) provides the key building blocks of all known sorbicillinoids. In addition to the monomeric representatives, there is a high tendency within the sorbicillinoid NP class to form dimeric derivatives. The reactivity-based mechanisms for the generation of these dimers will be explained in detail in Section 3.2.

### 3. Structural overview

In general, only those compounds that contain the, in part heavily modified, carbon skeleton of sorbicillin (**1a**) are designated as sorbicillinoids. The NP class can be divided into monomeric, dimeric, polymeric (so far only trimeric), and hybrid sorbicillinoids. Monomeric to trimeric representatives are exclusively composed of sorbicillin (**1a**) or closely related

precursors, whereas hybrid ones contain additional structural elements. In the following sections, the different subclasses of sorbicillinoids are described and special aspects of these compounds, such as structural scaffolds, biological activities, and biosynthetic mechanisms, are highlighted.

In order to provide a comprehensive knowledge base to the field, the ESI† contains a fact sheet for each currently known sorbicillinoid that includes: full structure, fungal producer, known bioactivities, biosynthesis, previous total syntheses, and full analytical data (e.g., melting point, thin layer chromatography, specific optical rotation, NMR, IR, and HRMS data, as far as available).

#### 3.1. Monomeric sorbicillinoids

The monomeric sorbicillinoids include three main groups: sorbicillin (**1a**) and its derivatives with alterations at the sorbyl side chain (Fig. 2A), sorbicillinol (**2a**) and congeners including higher oxidised analogues (Fig. 2B), and the vertinolides (Fig. 3). In addition, there are many other monomeric compounds that can be classified as sorbicillinoids in a broader context due to similar structural characteristics and/or co-isolation in sorbicillinoid-forming fungi. These types of compounds are described in Section 3.4.

**3.1.1. Sorbicillin(ol) analogues.** The founding member of these NPs is sorbicillin (Fig. 2A, **1a**). As mentioned above, sorbicillin (**1a**) not only provides the name for these NPs, but also acts as the key building block for the formation of all other sorbicillinoids. Since the first isolation of sorbicillin (**1a**) from

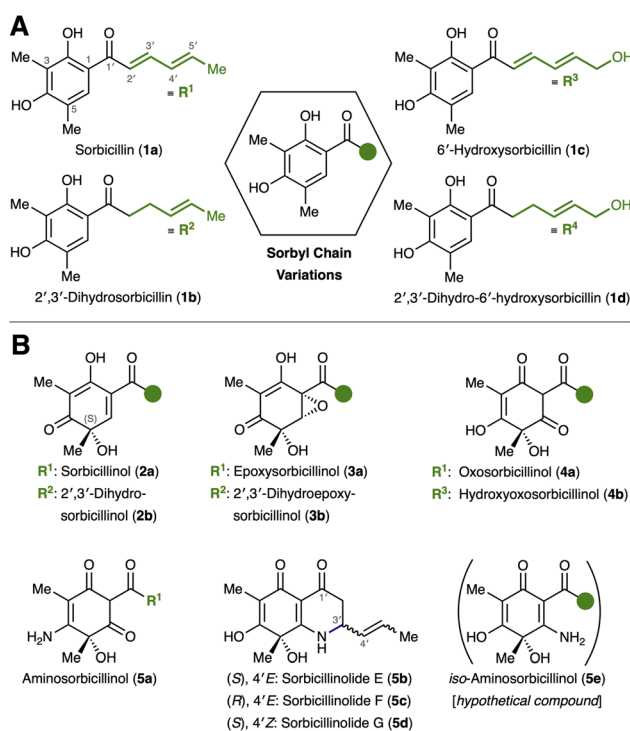


Fig. 2 (A) Structures of sorbicillins **1** and definition of different sorbyl side chain variations (shown in green). (B) Overview of structures of sorbicillinols **2** and derivatives **3–5**.



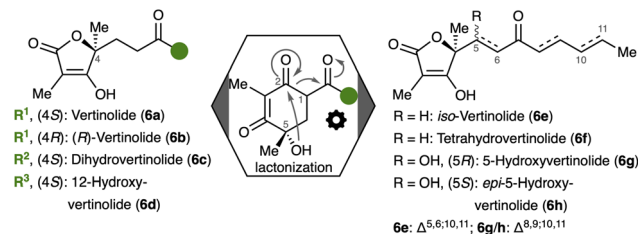


Fig. 3 Structures of the monomeric subclass of vertinolides.

*Penicillium notatum* in 1948,<sup>1,2</sup> the compound has been found in numerous other fungi, most commonly in diverse *Verticillium intertextum*,<sup>3,9</sup> *Penicillium*,<sup>16,24</sup> and *Trichoderma* species.<sup>5,25,26</sup> Notably, sorbicillin (**1a**) exhibits multiple biological activities, such as cytotoxicity against cancer cell lines,<sup>26–28</sup> antioxidative,<sup>25</sup> and antibacterial<sup>29</sup> properties. In addition, other monomeric analogues can be derived from the structural framework of sorbicillin (**1a**) by derivatisation of the sorbyl side chain (see Fig. 2A, green highlighting). For instance, an additional reductive step is required to generate 2',3'-dihydrosorbicillin (**1b**, see Chapter 2).<sup>3,9</sup> Hydroxylation gives access to 6'-hydroxysorbicillin (**1c**) and the combination of both functionalisations leads to 2',3'-dihydro-6'-hydroxysorbicillin (**1d**).<sup>21</sup> Like sorbicillin (**1a**), derivatives **1b–c** possess cytotoxic and anti-inflammatory properties.<sup>21,30</sup> The modification of the sorbyl side chain is an important factor increasing the number of different sorbicillinoids. For ease of reference, these derivations are always shown in green below and are labelled R<sup>1</sup> to R<sup>12</sup>.

Oxidative dearomatization of the sorbicillins **1a–d** at C5 by SorbC gives rise to the sorbicillinols 2–5 (Fig. 2B), thereby installing the required reactivity for downstream formation of all complex sorbicillinoids (for more details, see Section 3.2). Sorbicillinol (**2a**) was first proposed as a starting material for dimeric sorbicillinoids by Dreiding and co-workers in 1983.<sup>3</sup> Two decades later, the structure of the unstable sorbicillinol (**2a**) was elucidated by trapping experiments.<sup>10</sup> In the same manner as sorbicillin (**1a**), sorbicillinol (**2a**) was also identified in its reduced form, dehydrosorbicillinol (**2b**), in *Penicillium chrysogenum*.<sup>16</sup> In addition to sorbicillinol (**2a**), even higher oxidised sorbicillinols 3–5 were isolated. This includes the epoxysorbicillinols **3a–b**,<sup>17,31</sup> oxosorbicillinols **4a–b**,<sup>32–34</sup> and aminosorbicillinol (**5a**).<sup>20</sup> Besides aminosorbicillinol (**5a**), further nitrogen-containing monomers, sorbicillinoids E–F (**5b–d**) were isolated from the deep-sea derived fungus *Penicillium rubens* F54.<sup>35</sup> Retrobiosynthetically, these compounds **5b–d** can be formed by a Michael addition of the amino group to the  $\Delta^{2',3'}$  double bond of the sorbyl side chain (dark blue bond). This would require the hypothetical iso-aminosorbicillinol (**5e**) as a precursor for sorbicillinoids E–F (**5b–d**). Among the sorbicillinols, only the oxosorbicillinols **4a–b** have significant reported biological activities. Oxosorbicillinol (**4a**) and hydroxyoxosorbicillinol (**4b**) both exhibit antioxidative properties due to 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity for **4a** and the inhibition of lipoxygenases and cyclooxygenases for **4b**.<sup>32,34</sup>

**3.1.2. Vertinolides.** The vertinolides (Fig. 3) are another subclass of the monomeric sorbicillinoids. Biosynthetically, these derive of a tautomeric form of sorbicillinol (**2a**, C2 carbonyl). The alcohol at the stereogenic center at position C5 initiates a lactonisation to the C2 carbonyl, followed by a *retro*-Claisen to remove the strained oxabicyclo (C1–C2 bond cleavage, see hexagon, Fig. 3).<sup>12</sup> This cascade leads to vertinolide (**6a**) and its (*R*)-enantiomer **6b**.<sup>3,9,36,37</sup> Analogous to sorbicillins and sorbicillinols, there are a number of other vertinolides with a modified sorbyl side chain: dihydrovertinolide<sup>38,39</sup> (**6c**) with reduced side chain, 12-hydroxyvertinolide<sup>40</sup> (**6d**) including a hydroxylation at the sorbyl terminus, iso-vertinolide<sup>41</sup> (**6e**) as a regioisomer of **6a**, biosynthetically fully reduced tetrahydrovertinolide<sup>42</sup> (**6f**), as well as another hydroxylated 5-hydroxyvertinolide (**6g**)<sup>5</sup> and its epimer **6h**.<sup>12</sup> Within the vertinolides, the bioactive compounds are dihydrovertinolide (**6c**), displaying phytotoxicity against lettuce seedlings, and 12-hydroxyvertinolide (**6d**), bearing antibiotic activity against *S. aureus*, vancomycin-resistant *E. faecalis*, *B. subtilis*, *P. aeruginosa*, and *K. pneumoniae* (MIC = 1.60–64.0  $\mu\text{g mL}^{-1}$ ).<sup>38,40</sup>

### 3.2. Oligomeric sorbicillinoids

The formation of oligomeric sorbicillinoids is based on the high reactivity of sorbicillinol (**2a**). On the one hand, its cyclic dienone functionality (*s-cis* conformation) together with the double bonds within the sorbyl side are prone to Diels–Alder reactions, carrying both, a dienophile and an enophile. On the other hand, the cyclic dienone displays a Michael acceptor and donor, allowing classical Michael-addition chemistry. Diels–Alder reactions generate the typical bicyclo[2.2.2]octane scaffold of sorbicillinoids, whereas the Michael reactivity leads to cage-like sorbicillinoids. This inherent reactivity of sorbicillinols explains the formation of all complex sorbicillinoids and hybrid sorbicillinoids (Section 3.3) known to date, which will be discussed in detail in the following chapters.

**3.2.1. Dimeric Diels–Alder-type sorbicillinoids.** There are two different categories of Diels–Alder-type dimers. First, the [4+2] cycloaddition between two sorbicillinol units *via* their enone core functionalities results in the formation of dimers such as bisorbicillinol (**7a**) and bisvertinoquinol (**7b**) (see Fig. 4, top hexagon). In 1998, bisorbicillinol (**7a**) was first isolated by Abe and co-workers from a *Trichoderma* species.<sup>25,43</sup> Bisvertinoquinol (**7b**) with reduced sorbyl side chains was found in *Verticillium intertextum* more than a decade earlier.<sup>3,9</sup> Both dimers **7a** and **7b** exhibit weak antibacterial activity.<sup>44</sup> Moreover, bisorbicillinol (**7a**) displays DPPH radical scavenging activity (ED<sub>50</sub> = 31.4  $\mu\text{M}$ ) and inhibits the Lyn tyrosine kinase making it a new candidate as anti-allergic agent.<sup>43,45</sup> The bisorbutenolides (**8a–f**) are derived of bisorbicillinol (**7a**) following a nucleophilic acyl substitution (see ESI, Section 2†).<sup>11,13,25</sup> In recent years, bisorbutenolide (**8a**), which is also named bislongiquinolide or trichotetronine, was isolated from over 15 different fungi.<sup>46–51</sup> Bisorbutenolide (**8a**) is a notable example of a sorbicillinoid possessing cytotoxicity against various human cancer cell lines (IC<sub>50</sub> = 3.00–22.0  $\mu\text{M}$ ).<sup>26,50</sup> Besides, the reduced di- to octahydro derivatives (**8b–e**) show siderophore activity influencing the iron





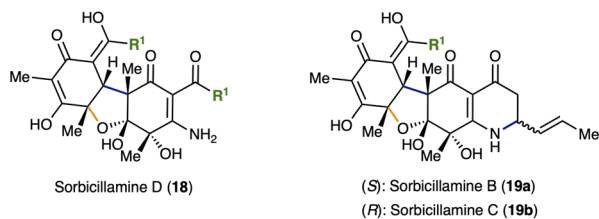


Fig. 6 Nitrogen-containing Michael-type dimers.

ketalisation between sorbicillinols (2) and oxosorbicillinols (4). Bioactivity screens revealed that the bisvertinolones 14 display cytotoxicity ( $IC_{50} = 0.50\text{--}100\ \mu\text{M}$ ) against leukemic cell lines (HL60, P388) and human lung cancer cells (A549).<sup>26,51,60</sup>

The structurally most sophisticated representatives of the sorbicillinoids are the trichodimerols (15). Their cage-like core is derived of two Michael reactions and ketalizations between two sorbicillinol units (2). Trichodimerol (15a) was isolated from *Trichoderma longibrachiatum*<sup>5</sup> and further fungal species, like *Penicillium chrysogenum*<sup>57,61–63</sup> and *Trichoderma*.<sup>47–49,64,65</sup> Compound 15a is known for the inhibition of bacterial endotoxin-induced production of tumor necrosis factor (TNF- $\alpha$ ), which plays diverse roles in cellular mechanisms like cell survival, proliferation, differentiation, and death.<sup>62</sup> Concerning bioactivities, trichodimerols (15a–f) are typical examples among the sorbicillinoids. Their broad bioactive spectra is ranging from cytotoxicity (15b:  $IC_{50} = 2.10\text{--}33.0\ \mu\text{M}$ , 15c:  $IC_{50} = 4.30\text{--}8.80\ \mu\text{M}$ , 15d:  $IC_{50} = 5.10\text{--}13.7\ \mu\text{M}$ ; against A549, HCT116, Hs683, OE21, U373 cell lines)<sup>50,56,66</sup> over antibacterial (15c: MIC =  $128\ \mu\text{g mL}^{-1}$  against *S. aureus* sp.)<sup>29</sup> and antifungal properties (15e:  $IC_{50} = 69.1\ \mu\text{M}$  against *C. neoformans*)<sup>67</sup> to 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity (15f:  $ED_{50} = 42.4\ \mu\text{M}$ ).<sup>43</sup> Bisorbibetanone (16), which was found in *Trichoderma* sp. USF-2690 by Abe and co-workers, features a slightly different Michael-addition pattern (see Fig. 5) compared to the trichodimerols (15: C1'–4, 16: C2'–4) and displays DPPH-radical scavenging activity ( $ED_{50} = 62.5\ \mu\text{M}$ ).<sup>33,68</sup> Comparable to the trichobisvertinolols D (13), the trichodimerols can undergo a further Michael addition within the sorbyl side chain leading to the ustisorbicillinol (17a, 17b) and trichodimer ethers (17c–e).<sup>57,58,69</sup>

The sorbicillamines B–D (18, 19a, 19b) represent one of the first nitrogen-containing sorbicillinoids (see Fig. 6).<sup>70</sup> The retrobiosynthetic basis of these compounds lies in an isomeric precursor 5e of the recently isolated aminosorbicillinol (5a).<sup>20</sup> Sorbicillamine D (18) is the amine analogue of the bisvertinolones (14) using the aminated precursor instead of oxosorbicillinol (4a) in the cyclization reactions. Subsequent Michael addition of the amine to the sorbyl chain results in sorbicillamines B (19a) and C (19a), as isolated from *Penicillium* sp. F23-2. The sorbicillamines (18, 19a, 19b) show cytotoxic properties against various cancer cells (BEL7402, HCT 116, HEK293, HeLa;  $IC_{50} > 10.0\ \mu\text{M}$ ).<sup>70</sup>

**3.2.3. Trimeric sorbicillinoids.** Up to date, only seven trimeric sorbicillinoids (see Fig. 7) were isolated, from *Acremonium citrinum* SS-g13, *Penicillium chrysogenum* 581F1, and

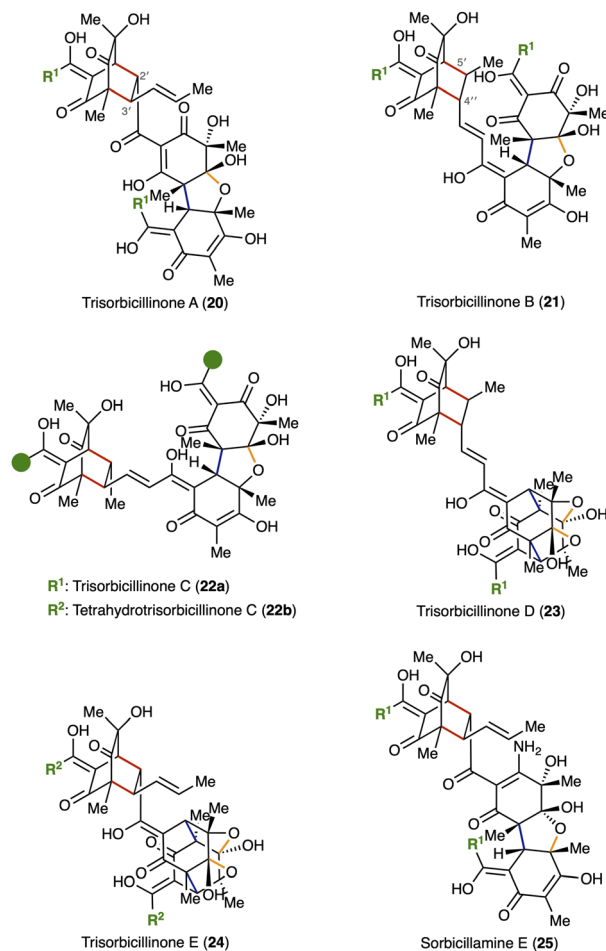


Fig. 7 Examples of trimeric sorbicillinoids.

*Phialocephala* sp. FL30r.<sup>71–73</sup> In the trimeric compounds, all previously discussed reactivities ([4+2], Michael addition, ketalization) of the monomers and dimers are combined. The biosynthesis of trisorbicillinone A (20) can be explained by two different pathways.<sup>71</sup> First, a Michael addition using sorbicillinol (2a) and oxosorbicillinol (11a) would lead to trimer 20. The second pathway is described by *endo*-selective Diels–Alder cycloaddition between sorbicillinol (2a) as enophile and bisvertinolone (14a) as dienophile. The latter also represents the biosynthetic pathway to trisorbicillinone B (21) and C (22) using the same building blocks (2a, 14a).<sup>74</sup> However, starting with trisorbicillinone B (21), there are deviations with regards to selectivity (*endo/exo*, regioselectivity) when compared to the sorbicillinoids presented so far. Up to this point, the Diels–Alder reaction was exclusively *endo*-selective and typically used the  $\Delta^{2',3'}$  double bond in the sorbyl side chain or the dienone core as the dienophile. In the case of trisorbicillinone B (21), the *endo*-selectivity is maintained, but the regioselectivity of the dienophile is changed towards the  $\Delta^{4'',5''}$  double bond. Trisorbicillinone C (22a) and the corresponding tetrahydro analogue 22b are based on an *exo*-selective [4+2] cycloaddition using the same  $\Delta^{4'',5''}$  double bond as dienophile. Trisorbicillinone D (23) and E (24) are both derived of sorbicillinol (2a) and



trichodimerol (**15a**).<sup>74</sup> The Diels–Alder adduct **23** is sharing the selectivity (*endo*,  $\Delta^{4'',5''}$  dienophile) with trisorbicillinone B (**21**), whereas trimer **24** shows the same connectivity (*endo*,  $\Delta^{2',3'}$  dienophile) as trisorbicillinone A (**20**). The change in selectivities can probably be explained by the size of the compounds and the associated greater steric demand: the larger the dienophile in the [4+2] reaction, the more likely it is that the *exo*-variant will occur in addition to the *endo*-type main product. This behavior can also be observed within hybrid sorbicillinoids (Section 3.3). Another trimeric sorbicillinoid belongs to the class of nitrogen-containing sorbicillamines (Fig. 6). Sorbicillamine E (**25**), which was isolated from *Penicillium* sp. F23-2 together with the other sorbicillamines **18** and **19** is structurally highly similar to trisorbicillinone A (**20**).<sup>70</sup> The only difference is the amino functionality in the bisvertinolone scaffold (**14**) indicating that sorbicillamine D (**18**) is needed as a dienophile in the corresponding Diels–Alder reaction.

The trimeric sorbicillinoids are another notable example of sorbicillinoids with cytotoxicity against various cancer cell lines (**20–23**: HL60, K562, P388;  $IC_{50}$  = 3.14–88.2  $\mu$ M).<sup>70,71,74</sup> Furthermore, tetrahydrotrisorbicillinone C (**22b**) displays high affinities in targeting diabetes and cancer related proteins (GLP-1R:  $K_d$  = 16.2 nM, EF2K:  $K_d$  = 74.6 nM).<sup>72</sup>

### 3.3. Hybrid sorbicillinoids

Sorbicillinoids derived of reactions between monomeric sorbicillinoids (Section 3.1) and an additional non-sorbicillinoid building block are categorised as hybrid sorbicillinoids. Like the dimeric sorbicillinoids (Section 3.2), hybrid sorbicillinoids can be divided into Diels–Alder-type (Section 3.3.1) and Michael-type sorbicillinoids (Section 3.3.2).

**3.3.1. Diels–Alder-type hybrid sorbicillinoids.** The more than 30 hybrid Diels–Alder-type sorbicillinoids that were isolated so far are retrobiosynthetically all based on sorbicillinols **2** as enophiles. The structural diversity is introduced by the different dienophiles. Below, the hybrid sorbicillinoids will be categorised based on the necessary biosynthetic dienophiles (cyclic, terminal, and complex olefins).

An example of the incorporation of cyclic olefins is sorbicillamine A (**26**), in which a pyrrole species acts as a dienophile (see Fig. 8).<sup>70</sup> Other nitrogen-containing dienophiles such as lactams and cyclic ureas are leading to trichisorbicillin A (**27**) and sorbicillinoid urea **28a**, respectively.<sup>21,75</sup> In 2023, the first sorbicillinoid glycoside, paeciureallin (**28b**), was isolated from the rhizospheric soil-derived fungus *Paecilomyces* sp. KMU21009.<sup>76</sup> Paeciureallin (**28b**) contains a N-ribofuranose functionality, which is based on the *N*-glycosidation of urea **28a** with  $\beta$ -D-ribose ( $\beta$ -D-Rib). The glycoside **28b** exhibits cytotoxicity against colorectal cancer (SW480,  $IC_{50}$  = 32.0  $\mu$ M) and adenocarcinomic cell lines (A549,  $IC_{50}$  = 34.4  $\mu$ M).<sup>76</sup> Nature also integrates oxygenated cyclic olefins, such as lactones and furanes. One of the simplest representatives is ustisorbicillinol E (**29**), which was isolated from the causal pathogen of rice false smut, fungus *Ustilaginoidea virens* UV8b, by Zhou and co-workers.<sup>58</sup> Together with the antibacterial rezishanone A (**30**), both compounds are derived of an unsaturated lactone.<sup>44,77</sup> A

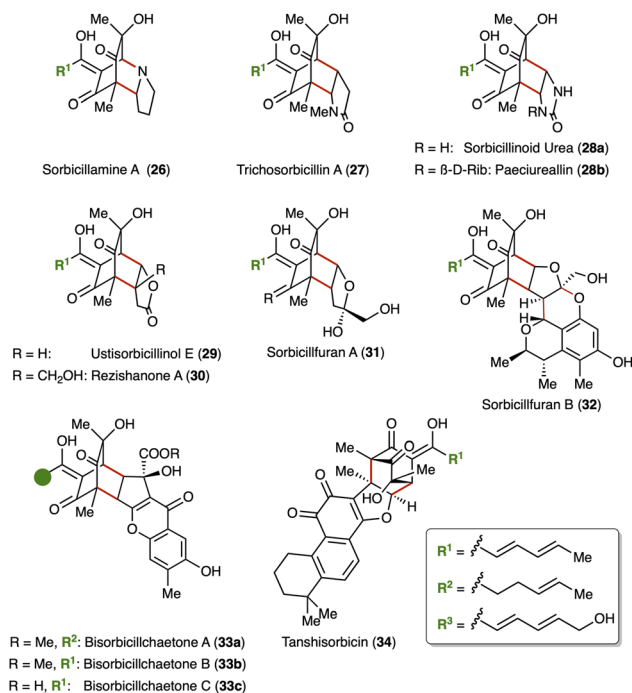


Fig. 8 Hybrid Diels–Alder-type sorbicillinoids based on cyclic alkenes as dienophiles.

tetrahydrofuran derivative provides the dienophilic precursor for the sorbicillfurans A (**31**) and B (**32**).<sup>78</sup> The saturated five-membered ring in **31** is obtained upon epoxidation (and epoxide opening) of the Diels–Alder product between sorbicillinol (**2a**) and furan-2-ylmethanol (see ESI, Section 2†). Sorbicillfuran A (**31**) was evaluated for cytotoxicity against human renal cancer (ACHN, OS-RC2, 786O) and leukemia cell lines (HL60, K562, MOLT4). Notably, hybrid sorbicillinoid **31** only showed a cytotoxic effect against HL60 cells ( $IC_{50}$  = 9.60  $\mu$ M) after further derivatisation *via* an oxo-Diels–Alder reaction with citrinin leading to sorbicillfuran B (**32**).<sup>78</sup> This might reveal that the citrinin moiety contributes significantly to the cytotoxicity. In recent years, other complex, furane-based, hybrid sorbicillinoids such as bisorbicillchaetones A–C (**33a–c**) and tanshisorbicin (**34**) were isolated from *Trichoderma* and *Penicillium* species.<sup>20,79,80</sup> The anti-inflammatory bisorbicillchaetones (**33a**:  $IC_{50}$  = 80.3  $\mu$ M, **33b**:  $IC_{50}$  = 38.4  $\mu$ M), which inhibit nitric oxide production, differ in their sorbyl chain reduction and carboxylic acid protection (methyl ester for **33a**, **33b**; free acid for **33c**). Tanshisorbicin (**34**) displays antibacterial properties against *Bacillus* species and *Staphylococcus aureus* ( $IC_{50}$  = 16.0–125  $\mu$ M). Structurally, it is derived of the diterpenoid tanshinone IIA.<sup>79</sup>

There are also multiple sorbicillinoids that appear to be derived of a Diels–Alder reaction in which a terminal olefin served as dienophile (see Fig. 9). This includes, for example, the remaining representatives of the rezishanones.<sup>81,82</sup> The biosynthesis of the rezishanones B–D (**35a–c**) would be based on vinyl ethers as dienophiles. Since vinyl ether have never been isolated from natural sources, an artificial origin (like contaminations in the organic solvent used for extraction) cannot be excluded. In



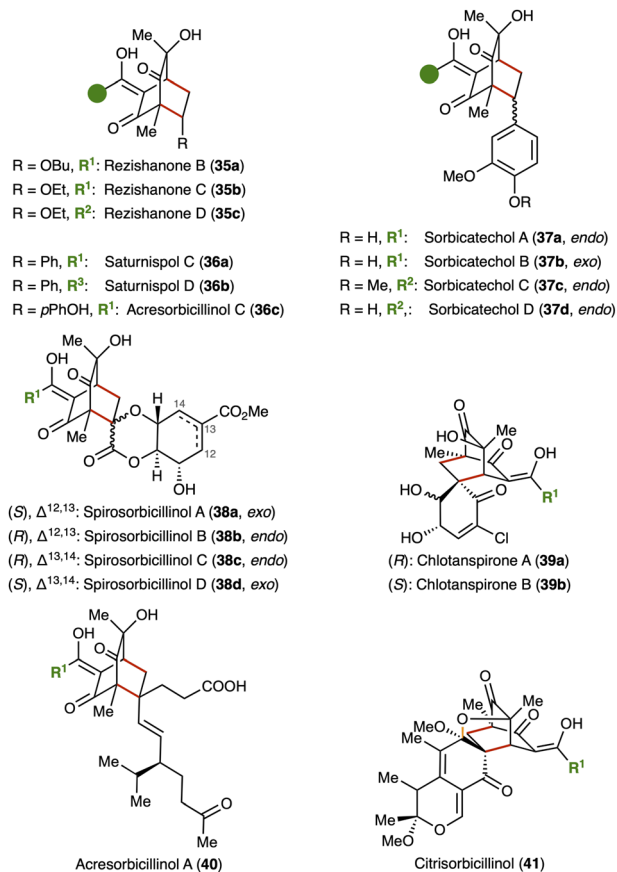


Fig. 9 Hybrid Diels–Alder-type sorbicillinoids based on terminal alkenes as dienophiles.

bioactivity screens, rezishanones B–D (**35a–c**) displayed weak antibacterial activity against *S. aureus* and *B. subtilis* as well as cytotoxicity against murine leukemic lymphoblasts L5178y (**35b**: IC<sub>50</sub> > 10.0 mg mL<sup>-1</sup>).<sup>24,44</sup> The saturnispols,<sup>40</sup> acresorbicillinol B,<sup>67</sup> and sorbicatchols<sup>83,84</sup> (**36a–c**, **37a–d**) are structurally related to rezishanones. Instead of vinyl ethers, various substituted styrenes now act as dienophiles. Presumably due to the increased size of the dienophiles, the *exo*-Diels–Alder product (*cf.* Section 3.2.3) is also obtained, here in the form of sorbicatchol B (**37b**). In addition to antibacterial properties similar to those of rezishanones (**35a–c**), this group of sorbicillinoids (**36a–c**, **37a–d**) exhibits antiviral (H1N1, **37a**: IC<sub>50</sub> = 85.0 μM, **37b**: IC<sub>50</sub> = 113 μM)<sup>83</sup> and cytotoxic effects (**37d**: HT29 cells).<sup>84</sup> It should be emphasized that further evaluation of the antiviral activity against influenza A virus has revealed cytotoxicity against the host cell system, rather than true antiviral effects.<sup>85</sup> The spirosorbicillinols A–D (**38a–d**) were first isolated from *Trichoderma* sp. USF-4860 by Hirota and co-workers.<sup>86</sup> Their uniqueness lies in the fact that they are composed of two NPs: sorbicillinol (**2a**) and scytolide. The spirosorbicillinols only differ in their Diels–Alder selectivity (*exo*: **38a**, **38d**; *endo*: **38b**, **38c**) and the double bond isomer of scytolide (Δ<sup>12,13</sup>: **38a**, **38b**; Δ<sup>13,14</sup>: **38c**, **38d**) used.<sup>31,77,86</sup> A structurally and biomedically highly interesting subgroup of the hybrid sorbicillinoids are the chlotanspirones (**39a**, **39b**), which are among the only halogen-

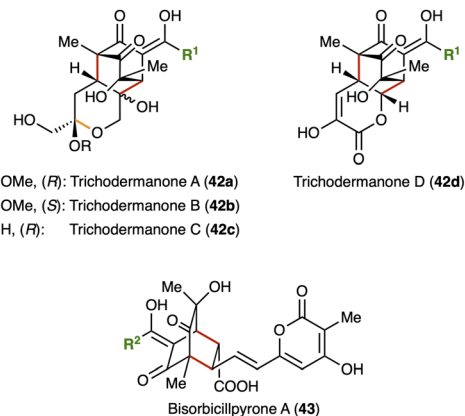


Fig. 10 Hybrid Diels–Alder-type sorbicillinoids based on *cis*-/*trans*-disubstituted, acyclic alkenes as dienophiles.

containing sorbicillinoids. Chlotanspirones A (**39a**) and B (**39b**) possess cytotoxic activities against leukemic (HL60, **39a**: IC<sub>50</sub> = 9.20 μM, **39b**: IC<sub>50</sub> = 37.8 μM) and adenocarcinoma cell lines (A549, **39a**: IC<sub>50</sub> = 39.7 μM).<sup>87</sup> Acresorbicillinol A (**40**) is named after the fungal source: *Acremonium chrysogenum* C10.<sup>67</sup> This compound was isolated in recent years together with citrisorbicillinol (**41**).<sup>88</sup> Similarly, the name of compound **41** is again based on the fungal source (*Penicillium citrinum* ZY-2) and furthermore on the employed dienophile, a citrinin derivative.

Some hybrid Diels–Alder-type sorbicillinoids are not derived of cyclic or terminal alkenes (see Fig. 10). These comprise the trichodermanones A–D (**42a–d**) and bisorbicillipyron A (**43**). At first glance, trichodermanones **42a–d** are expected to incorporate cyclic dienophiles. However, the six-membered ring with ether/ester functionality is generated after [4+2] cycloaddition by ketalisation (orange bond) or lactonisation. Accordingly, (*Z*)-5-oxohex-2-enoic acid serves as the dienophile for the trichodermanones A–D (**42a–d**).<sup>48</sup> Further biosynthetic steps, like hydroxylation, methylation, and redox processes, lead to the final compounds (ESI, Fig. S5†). The trichodermanones **42a–c** are another example of sorbicillinoids showing DPPH-radical scavenging activities.<sup>48</sup> Besides, bisorbicillipyron A (**43**) isolated from *Penicillium* species, inhibits α-glycosidase (IC<sub>50</sub> = 156 μM) making it a potential antihyperglycemic agent.<sup>41,80</sup> Bisorbicillipyron A (**43**) is derived of sorbicillinol (**2a**) and a further PKS-derived metabolite, trichopyrone.

**3.3.2. Michael-type hybrid sorbicillinoids.** On the mechanistic basis of the Michael-type dimers presented so far (Fig. 5), it is also possible to deduce a number of hybrid sorbicillinoids (see Fig. 11A). The bisvertinol analogues JBIR-59 (**44a**) and JBIR-124 (**44b**) were isolated from the marine-derived fungus *Penicillium citrinum*.<sup>51,89</sup> Retrobiosynthetically, these compounds are based on dimethylorcin and duro-*p*-hydroquinone as Michael donors instead of sorbicillinol (**2a**). Ustilobisorbicillinol A (**45**) displays the same Michael addition pattern as bisorbicillipyron (**16**) using phenanthrenedione as reaction partner.<sup>90</sup> Sorbicillinoid **45** isolated from *Ustilagoidea virens* is reported to have an influence on cell-cycle progression within gastric cancer cell line BGC823.<sup>90</sup> Despite the major structural differences, sorbiterrines **46a**, **46b** also belong to the Michael-type hybrid



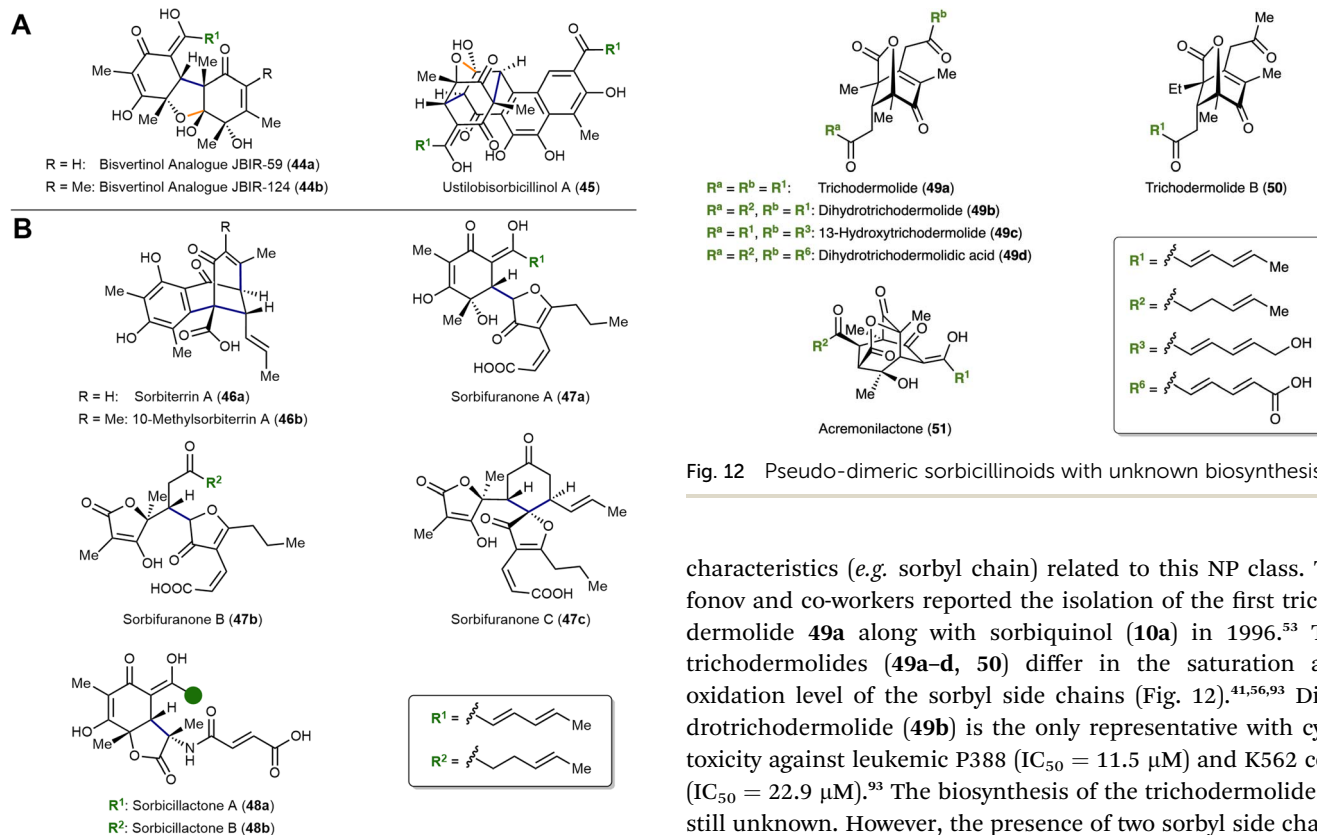


Fig. 12 Pseudo-dimeric sorbicillinoids with unknown biosynthesis.

Fig. 11 Hybrid Michael-type sorbicillinoids based on previously introduced dimers (A) and new scaffolds (B).

sorbicillinoids (Fig. 11B).<sup>41</sup> Their biosynthetic pathway is based on 3,5-dioxohexanoic acid as Michael donor and includes three Michael reactions (ESI, Fig. S6†).<sup>91</sup> Sorbiterrin A (**46a**) acts as a cholinesterase inhibitor ( $IC_{50} = 25.0 \mu\text{g mL}^{-1}$ ) making it interesting for the treatment of Alzheimer and dementia symptoms.<sup>91</sup> In 2010, Bringmann and co-workers isolated the sorbifuranones **47a–c** from *Penicillium* strains in Mediterranean sponges.<sup>92</sup> The sorbicillinoids **47a–c** incorporate a furanone unit, which, *via* a Michael addition to sorbicillinol (**2a**), initially results in sorbifuranone A **47a**. Sorbifuranone A (**47a**) or its dehydroform likely serve as biosynthetic precursors to sorbifuranones B (**47b**) and C (**47c**). Similar to the vertinolides **6**, lactonisation followed by *retro*-Claisen leads to sorbifuranone B (**47b**). The spiro-compound **47c** is based on a further Michael addition to the sorbyl side chain, generating the cyclohexanone ring. Another set of compounds isolated by the Bringmann lab five years earlier are the sorbicillactones A and B (**48a**, **48b**).<sup>24</sup> These compounds are likely derived of a Michael reaction formally using alanine and a C<sub>4</sub>-fumaryl unit. Sorbicillactone A (**48a**) possesses anti-HIV activity by inhibition of the expression of viral proteins, as well as cytotoxicity against L5178y.

### 3.4. Other related structures

In addition to the approximately 120 sorbicillinoids, there are also numerous compounds with similar structural

characteristics (*e.g.* sorbyl chain) related to this NP class. Trifonov and co-workers reported the isolation of the first trichodermolide **49a** along with sorbiquinol (**10a**) in 1996.<sup>53</sup> The trichodermolides (**49a–d**, **50**) differ in the saturation and oxidation level of the sorbyl side chains (Fig. 12).<sup>41,56,93</sup> Dihydrotrichodermolide (**49b**) is the only representative with cytotoxicity against leukemic P388 ( $IC_{50} = 11.5 \mu\text{M}$ ) and K562 cells ( $IC_{50} = 22.9 \mu\text{M}$ ).<sup>93</sup> The biosynthesis of the trichodermolides is still unknown. However, the presence of two sorbyl side chains suggests a dimerization of two sorbicillin units (see Section 3.2). Retrobiosynthetic analyses indicate that the trichodermolides are based on a single sorbicillinol unit. The second sorbyl side chain is probably introduced by incorporation of another PKS-based metabolite. The same applies to acremonilactone (**51**), which was isolated from *Acremonium* sp. AN-13.<sup>29</sup>

Structural homologues to the vertinolides **6a–h** (Fig. 3) were found in the recently isolated subfamily of anti-neuroinflammatory sorbicillinolides **52**, **53** (Fig. 13A).<sup>35</sup> Sorbicillinolides A–D (**52**, **53**) are based on an oxidative dearomatization of sorbicillins **1a** and **1b** at position C3 instead of C5 (for sorbicillinol, **2a**). This oxidation pattern is reminiscent of the azaphilones and tropolones, which will be briefly discussed later in Section 4.2.2.<sup>94,95</sup> Sorbicillinolide J (**54**) is derived of 2',3'-

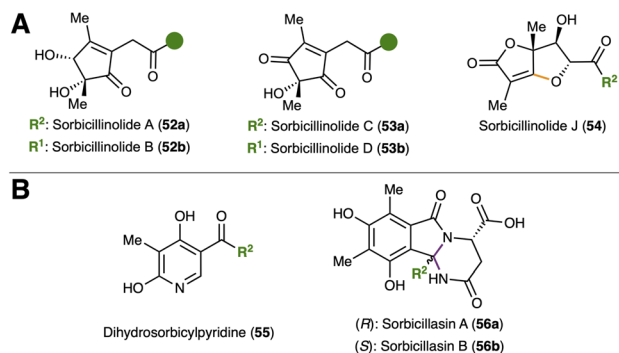


Fig. 13 (A) Monomeric derivatives related to vertinolides. (B) Nitrogen-containing monomeric analogues.



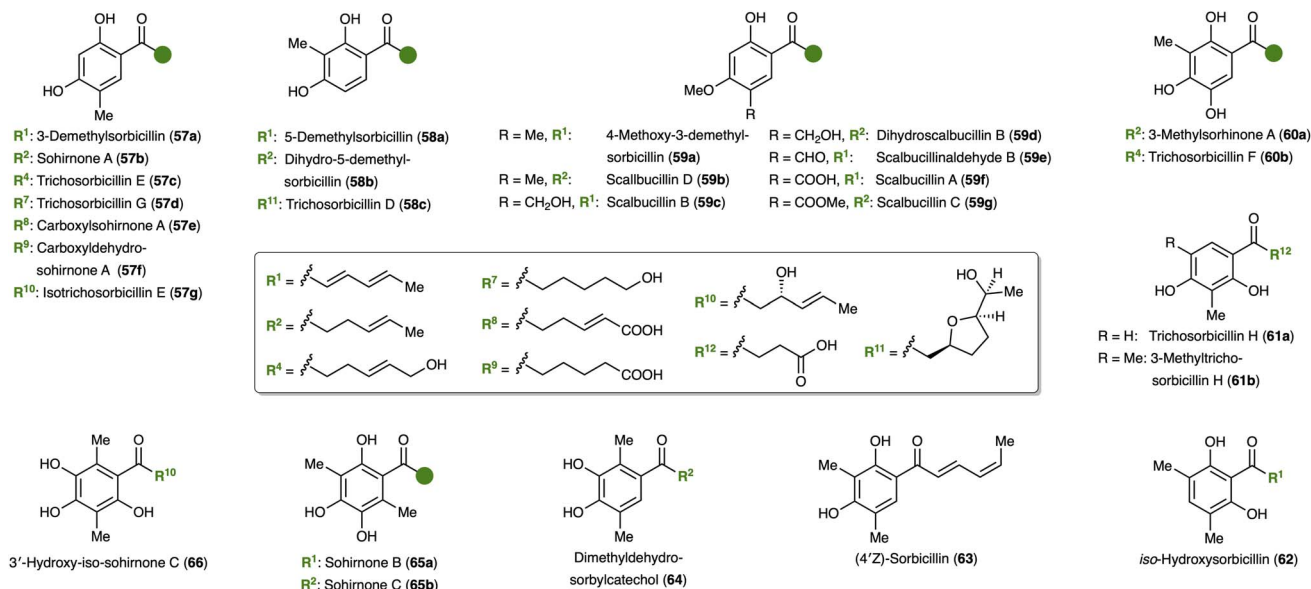


Fig. 14 Monomeric phenols related to sorbicillin.

dihydroepoxysorbicillinol (3b) performing the same lactonization as seen for the vertinolides 6a–h (ESI, Fig. S7W†).

Nitrogen-containing related compounds (see Fig. 13B) include sorbicillinoid-type pyridine 55 and sorbicillasins (56a, 56b).<sup>57,96</sup> The latter were isolated from *Phialocephala* sp. FL30r and could be based on oxidated 2',3'-dihydrosorbicillin (1b) and L-asparagine. The tricyclic system is built by *via* subsequent imination.

The largest group of monomeric related structures are phenols (Fig. 14). These differ in the number of substituents, substitution patterns (catechols, resorcinols), sorbyl chain variations (R<sup>1</sup> to R<sup>12</sup>) and comprise the subfamilies of scalbuicillins,<sup>97,98</sup> sohirmones,<sup>26,44,65</sup> and trichosorbicillin.<sup>21</sup> The phenols 57–66 possess many different types of bioactivities, including anti-inflammatory activity due to inhibition of nitric oxide production (mainly for trichosorbicillins: 57c, 57g, 60b, 61a, 61b),<sup>21</sup> antibacterial activity against various *S. aureus* strains (57b, 60a, 62, 64),<sup>29,41,44,99</sup> and cytotoxicity against leukemic cell lines (57e, 57f, 58b, 62).<sup>65,93,100</sup>

Derived from phenols, other metabolites, such as quinones (67–71), pyranones (72, 73), and chromanones (74, 75), are found in sorbicillinoid-forming fungi (Fig. 15, detailed structures are given in the ESI†). Information on these structural classes and further details on the phenols can be found in the ESI (Section 1.4).†

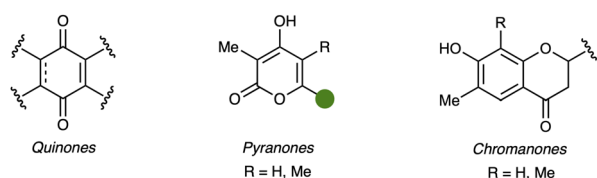


Fig. 15 Overview of core scaffolds and functionalities of further related compounds 67–75 (detailed structures are given in the ESI†).

## 4. Synthesis of the sorbicillinoids

The unique biological activities and three-dimensional architectures of sorbicillinoids have led to high interest in their synthetic access. Over the years, K. C. Nicolaou and E. J. Corey independently reported purely chemical approaches to generate dimeric sorbicillinoids.<sup>101–103</sup> Since 2014, chemo-enzymatic strategies applying the heterologously expressed monooxygenase SorbC have been established.<sup>16</sup> In the upcoming sections, we aim to provide a brief overview on previous synthetic work (Section 4.1) followed by in-depth discussion of the straightforward chemo-enzymatic approaches using the biosynthetic key enzyme SorbC (Section 4.2). Detailed information on all total syntheses can be found in the ESI (Section 3).†

### 4.1. Chemical total synthesis

Efficient synthetic access to sorbicillin (1a) is an important basis of total syntheses of sorbicillinoids. In 1954, Kuhn and Staab reported the first synthesis of sorbicillin (1a).<sup>104</sup> After the synthesis of dimethylresorcinol (77), sorbicillin (1a) can be obtained upon formation of a chlorinated acetophenone followed by addition of crotonaldehyde. Due to the low overall yield of less than 1%, further strategies were developed. The key step in all recent approaches is a Friedel–Crafts acylation using different Lewis acids (Grignard,<sup>105</sup> boron trifluoride,<sup>103</sup> aluminium chloride<sup>59</sup>) to introduce the sorbyl side chain (Fig. 16A). Aluminium chloride in combination with sorbyl chloride yields 1a in up to 61% yield.<sup>59</sup>

The first total syntheses of dimeric sorbicillinoids (Fig. 16B) were independently reported by the groups of Corey and Nicolaou.<sup>101,103</sup> Both groups used a biomimetic approach (Fig. 16B, reaction 1) in which the oxidative dearomatization of sorbicillin (1a) with Pb(OAc)<sub>4</sub> was the key step in the formation of acetate rac-79. Acetyl deprotection under basic conditions directly led to



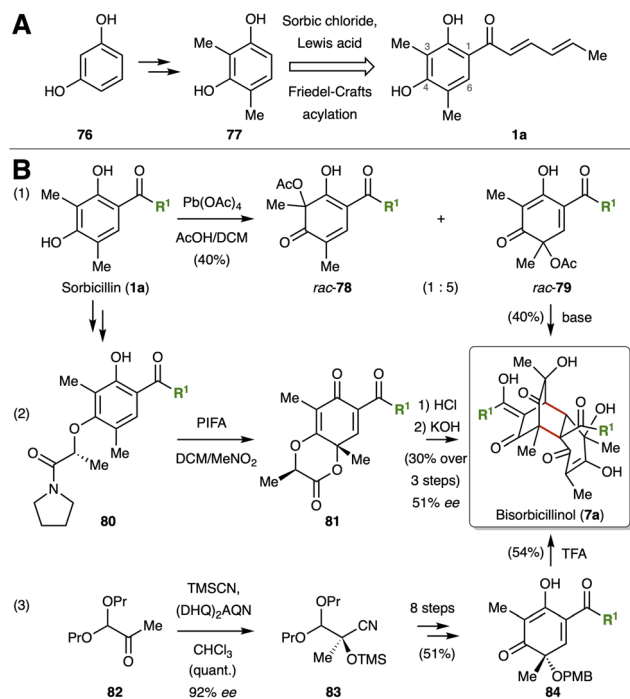


Fig. 16 Overview of different total syntheses accessing sorbicillin (A) as well as sorbicillinol and dimers (B).

the formation of bisorbicillinol (7a) and trichodimerol (15a). However, the overall yields were low due to the lack of regio- (see 78) and stereocontrol in the oxidative dearomatization reaction. Improvements were introduced by Pettus *et al.* using a chiral pyrrolidine tether (80) and a hypervalent iodine species in the oxidative dearomatization (Fig. 16B, reaction 2).<sup>106</sup> Bisorbicillinol (7a) was obtained upon hydrolysis of intermediate 81 in 51% enantiomeric excess (ee). Further progress was achieved by Deng *et al.* synthesizing sorbicillinol synthon 84 in 92% ee starting with an enantioselective cyanosilylation of 82 to give 83 (Fig. 16B, reaction 3).<sup>107</sup> Compared to the previous strategies, this represents a fundamentally different approach, in which the stereocenter is selectively introduced in the first step. The required sorbicillinol core structure is assembled in eight further steps to deliver 84, and upon PMB-removal, bisorbicillinol (7a) is formed. Access to Diels–Alder-type dimer 7a readily enabled the formation of bisorbibutenolide (8a) and bisorbicillinolide (9) after treatment of 7a with KHMDS or MeOH.<sup>103,107</sup> Overall, all these previous total syntheses either lack stereoselectivity or require long linear synthetic sequences.

Another strategy that uses hypervalent iodine reagents for oxidation is found in the biomimetic synthesis of sorbiterrin A (46a).<sup>108</sup> After Michael addition of 4-hydroxypyron to acetoxy sorbicillinol 79, the [3.3.1] ring system was generated by a silver nanoparticle catalyzed bridged aldol condensation (ESI, Fig. S13†).

Further total syntheses were reported for monomeric sorbicillinoids epoxysorbicillinol (3a) and vertinolide (6a). Epoxysorbicillinol (3a) was synthesized with a similar pyrrolidine tether as for bisorbicillinol (7a).<sup>106</sup> Notably, the use of twice the

amount of (bis(trifluoroacetoxy)iodo)benzene (PIFA) achieved the introduction of the characteristic epoxide functionality. Another strategy based on diethyl methylmalonate used a novel 1,3-dipolar cycloaddition between an  $\alpha$ -diazo ketone and a propiolate ester to generate monomer 3a over 13 steps.<sup>109</sup> To date, a total of six approaches leading to the monomeric sorbicillinoid 6a were introduced (ESI, Section 3).<sup>†</sup> The main difference is the implementation of stereo information using asymmetric reactions,<sup>110</sup> cinchonine resolution,<sup>111</sup> or enantio-pure building blocks ((*R*)-phenylethylamine,<sup>112</sup> (*R*)-lactic acid<sup>113</sup>). In analogy to Corey's short synthetic route, Takabe and co-workers developed a four step, racemic synthesis of vertinolide (6a) representing the fastest access to monomer 6a.<sup>114</sup>

In summary, the synthesis of monomeric sorbicillinoids is significantly more efficient in terms of stereocontrol and overall yield compared to more complex, dimeric NPs of this class. Thereby, the key problem is the regio- and stereoselective formation of the reactive intermediate, sorbicillinol (2a), through oxidative dearomatization of sorbicillin (1a). The optimization of this step would be the basis for a better access to complex sorbicillinoids.

#### 4.2. Chemo-enzymatic approaches

As outlined in Section 4.1, the regio- and stereoselective oxidative dearomatization of sorbicillin (1a) into sorbicillinol (2a) represents a challenging key step in the synthesis of sorbicillinoids. Pioneering research by Cox and co-workers revealed that this transformation is biosynthetically catalysed by the flavin-dependent monooxygenase SorbC in exceptional regio- and stereoselectivity.<sup>16</sup> The successful cloning of the encoding gene SorbC from *Penicillium chrysogenum* and its heterologous expression in *Escherichia coli* provided an excellent basis for the development of chemo-enzymatic approaches towards sorbicillinoids. In general, chemo-enzymatic strategies constitute a beneficial alternative to classical total synthesis by combining traditional organic-synthetic methods with biocatalysis. In this section, we introduce examples of applied chemo-enzymatic syntheses and display how this approach can be used for the formation of artificial sorbicillinoids.

**4.2.1. Basic principles and examples.** As a flavin-dependent monooxygenase, SorbC requires a cofactor (electron donor) to balance the redox system.<sup>115</sup> In most of the chemo-enzymatic approaches, nicotinamide adenine dinucleotide (NADH) or the corresponding phosphate NADPH is used in stoichiometric amounts for this purpose (Fig. 17A). Catalytic use of the cofactor can be achieved by addition of a regenerating system (*e.g.*, glucose-6-phosphate dehydrogenase).<sup>116,117</sup> Due to stability and the solubility of the enzyme(s) and cofactors, the reaction is typically carried out in aqueous phosphate buffer (50 mM, pH 8.0).<sup>16</sup> Further organic co-solvents can be added for the improvement of the solubility of the substrate 1a. Besides, the addition of co-solvents also has a significant influence on the formation of dimeric sorbicillinoids, which will be discussed later.<sup>59</sup> The mechanism of FMOs includes the attachment of molecular oxygen to the enzyme complex to generate the active hydroperoxyflavin species.<sup>118</sup> Accordingly, SorbC-catalysed



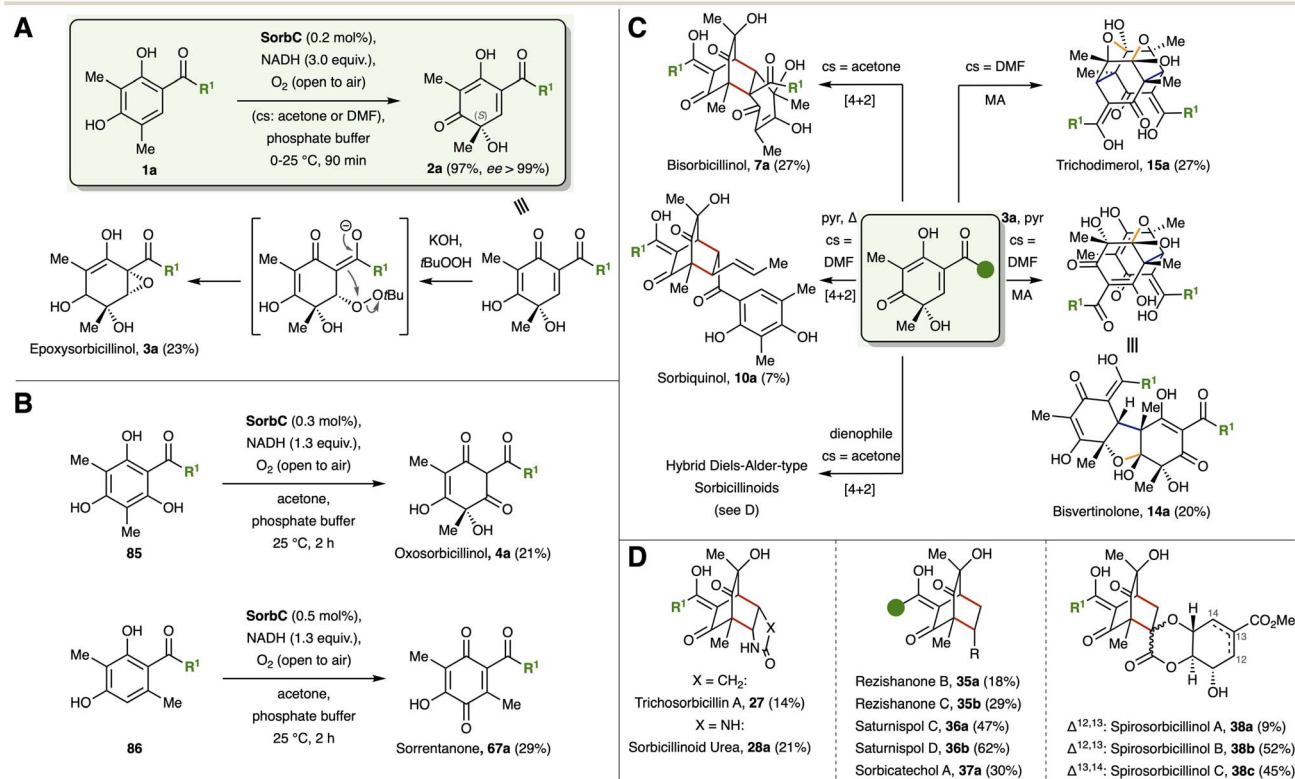


Fig. 17 Examples of the chemo-enzymatic synthesis of sorbicillinoids ((A and B) monomers, (C and D) dimers and hybrid sorbicillinoids) cs: co-solvent.

reactions are always performed aerobically to ensure sufficient oxygen saturation. Taking all these aspects into account, sorbicillinol (**2a**) can be synthesised biocatalytically from sorbicillin (**1a**) in almost quantitative yield (97%) and with perfect regio- and stereocontrol (ee > 99%).<sup>59</sup>

The straightforward access to sorbicillinol (**2a**) can be used to generate further monomeric sorbicillinoids. Direct addition of potassium hydroxide and *tert*-butylhydroperoxide to the sorbicillinol-containing reaction batch enabled the stereo-selective formation of epoxysorbicillinol (**3a**) in 23% yield (Fig. 17A).<sup>119</sup> Furthermore, the same enzymatic system can be used together with other substrates (see Fig. 17B), such as 6-hydroxysorbicillin (**85**) and 2,5-dimethylsorbicillin (**86**). Slight adjustment of the catalytic loading and reaction time thereby leads to oxosorbicillinol (**4a**) and sorrentanone (**67a**) in yields of 21% and 29%, respectively.<sup>119</sup> Biosynthetically, the generation of these monomers might indicate that the FMO, SorbC, accepts a broad range of substrates, which provides further insight into the biosynthesis of the NP class.

Despite the reactivity towards dimerization described in Section 4.1, sorbicillinol (**2a**) is relatively stable in aqueous solution. Only after quenching of the aqueous reaction with larger quantities of organic solvent (usually dichloromethane), the formation of dimeric sorbicillinoids is observed.<sup>59,119</sup> The tendency to form Diels–Alder or Michael addition type dimers can be controlled *via* the co-solvent employed (see Fig. 17C). Acetone as a co-solvent favours the formation of bisorbicillinol

(**7a**, 27%), whereas dimethylformamide (DMF) promotes the slower generation of alternative dimerization products, such as trichodimerol (**15a**, 27%; along **7a**, 20%). The addition of pyridine to the enzymatic set up together with DMF and heat resulted in the synthesis of sorbiquinol (**10a**, 7%). Bisvertinolone (**14a**) can be obtained in a DMF-promoted Michael addition (20% yield) upon combination of sorbicillinol (**2a**) and epoxysorbicillinol (**3a**).

In addition to dimeric sorbicillinoids, hybrid sorbicillinoids can also be synthesised by incorporating external dienophiles and Michael donors (Fig. 17C and D). The use of unsaturated pyrroles and imidazoles allows the synthesis of trichosorbicillin A (**27**, 14%) and sorbicillinoid urea (**28a**, 21%).<sup>23,117</sup> Rezishanones B (**35a**, 18%) and C (**35b**, 29%), saturnispol C (**36a**, 47%) and D (**36b**, 62%), as well as sorbicatechol A (**37a**, 30%) are formed by reaction with vinyl-type dienophiles.<sup>23,85,119</sup> The synthesis of saturnispol D (**36b**) should be emphasised, as 6'-hydroxysorbicillin (**1c**) was used as a substrate instead of sorbicillin (**1a**). The direct conversion of 6'-hydroxysorbicillin (**1c**) by SorbC once again illustrates the broad substrate tolerance of the monooxygenase. The more complex examples within the hybrid sorbicillinoids include the spirosorbicillinols A–C (**38a–c**) and sorbicillactone A (**48a**). The spirosorbicillinols A–C (**38a–c**) require the synthetically challenging (epi-)scytolide as dienophile reaction partner, which is itself a NP. In our previous work, we presented four different scytolide isomers, which were produced over 7–11 synthetic steps in yields between 2% and 49%.<sup>120</sup>



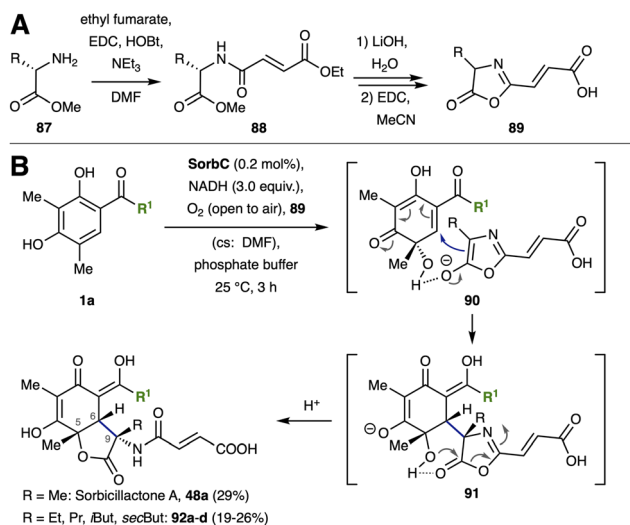


Fig. 18 (A) Synthesis of azlactones. (B) Chemo-enzymatic synthesis of sorbicillactones.

Spirosorbicillinol A (**38a**) and its *endo*-analogue, spiro-sorbicillinol B (**38b**) were synthesized in one pot from scytolide. Notably, the [4+2] cycloaddition led to *endo*-**38b** in higher yield (52%) compared to the *exo*-product **38a** (9%). Spirosorbicillinol C (**38c**) was generated analogously using episcytolide (45%). The chemo-enzymatic synthesis of the Michael-type hybrid sorbicillinoid, sorbicillactone A (**48a**), is based on a fumarylazlactone building block **89** serving as Michael donor (Fig. 18A).<sup>121</sup> The synthesis of the fumarylazlactone was initiated by an amide coupling between alanine precursor **87** and ethyl fumarate to give **88**. Azlactone **89** was finally obtained after saponification of intermediate **88** by ring closure. Convergent application of **89** in the chemo-enzymatic one-pot formation of sorbicillinol (**2a**) directly furnished sorbicillactone A (**48a**, R=Me) in 26% yield with perfect stereocontrol at all three established, contiguous stereocenters. From the mechanistic side, a coordination of the C5 alcohol to the enolate form of **89** was proposed as starting point (see **90**). Thus, Michael addition and subsequent lactonization (see **91**) led to the final NP, sorbicillactone A (**48a**). The strategy was further extended to introduce different C9 analogs (of **48a**) by applying derivatives of the fumarylazlactone. Compared to the also reported chemical synthesis of **48a** using PIFA, several advantages of the chemo-enzymatic strategy are immediately apparent.<sup>121</sup> When comparing the relative performances of both approaches from the same starting point (2-methylresorcinol), the number of reaction steps is reduced (chemical: 12, chemo-enzymatic: 4) in addition to improved yield (chemical: 0.13%, chemo-enzymatic: 29%) and stereoselectivity (chemical: none, chemo-enzymatic: ee > 99%).<sup>122</sup> This observation applies not only to sorbicillactone A (**48a**) but to all chemo-enzymatically produced sorbicillinoids, making this strategy the currently best approach for the synthesis of sorbicillinoids.

**4.2.2. Overcoming nature's limitations.** The successful enzymatic conversion of various sorbicillin derivatives (**1a-c**, **85**, **86**) indicated a broader substrate tolerance of the

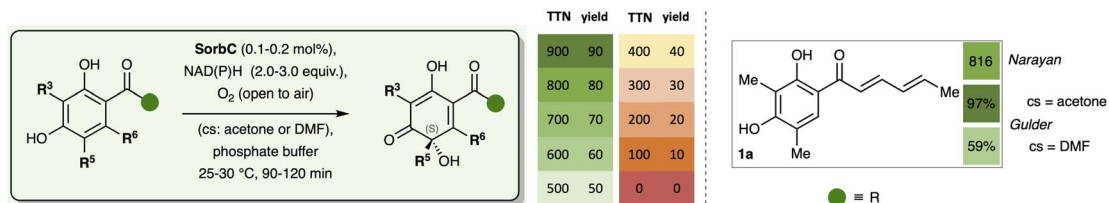
monooxygenase SorbC. In fact, several studies by Narayan and co-workers, as well as by our group, revealed a broader substrate spectrum beyond the known natural analogues (Fig. 19A).<sup>23,117</sup> Based on the natural occurring sorbicillins **1a-d**, the sorbyl side chain offered the greatest potential for artificial variations. Interestingly, derivative **93a** with completely removed sorbyl side chain (R = H) is also converted to the corresponding sorbicillinol by the FMO SorbC (TTN = 371), but with lower turnover compared to sorbicillin (**1a**, TTN = 816). On average, the saturated homologues **93b-f** with increasing alkyl chain length (R = Me, *n*Pr, *n*Bu, *i*Bu, *n*Pent) showed a lower conversion (average yield of 16%) than the natural substrate **1a** (83%). The same applied to the partially unsaturated derivatives **93g** (R = *trans*-crotyl, 29%), **93h** (R = 1-pentene, 37%), dihydrosorbicillin (**1b**, R = 3-pentene, 31%), and hydroxysorbicillin (**1c**, R = hydroxysorbyl, 61%). An improvement in enzymatic conversion is observed when the alkyl chain length at position R<sup>3</sup> is modified. The ethylated derivatives **88a** (R = *n*Bu, R<sup>3</sup> = Et, TTN = 858) and **88b** (R = sorbyl, R<sup>3</sup> = Et, average yield = 66%) displayed the same activity as sorbicillin (**1a**, TTN = 816, average yield = 78%). Further extensions of the alkyl side chain (**88c**: R<sup>3</sup> = *n*Pr, **88d**: R<sup>3</sup> = *n*Bu, **88e**: R<sup>3</sup> = *n*Pent) did not lead to an increase of the corresponding enzymatic conversion (average yield = 59%). Derivatization of position R<sup>5</sup> resulted in a higher turnover rate for **89a** (R = *n*Bu, R<sup>5</sup> = Et, TTN = 919), whereas the sorbyl-based analogues **89b** (R<sup>5</sup> = Et) and **89c** (R<sup>5</sup> = *n*Pr) had an average conversion of 29%. Combination of R<sup>3</sup>- and R<sup>5</sup>-substitution in form of double-ethylated sorbicillin **96** (R<sup>3</sup> = R<sup>5</sup> = Et) enabled an enzymatic conversion of 78%. Expansion of the substitution pattern to position R<sup>6</sup> led to derivatives **97a-c** (**97a**: R<sup>6</sup> = Me, **97b**: R<sup>6</sup> = Et, **97c**: R<sup>6</sup> = *n*Pr). The best enzymatic conversion within the R<sup>6</sup>-modified derivatives was observed for **97b** (84%). The analysis of two substrates **98a** (R = *iso*Bu, R<sup>5</sup> = H, R<sup>6</sup> = Me) and **98b** (R = *n*Bu, R<sup>5</sup> = H, R<sup>6</sup> = Me), which rather belong to the substrate spectrum of the related FMO TropB, showed turnover numbers of 331 and 479. Overall, modifications within R<sup>3</sup> seem to be better accepted than those at R<sup>5</sup> and R<sup>6</sup> with a general catalytic cutoff for substitutions larger than *n*-butyl.

The above-mentioned substrates were used in the one pot chemo-enzymatic synthesis of unknown sorbicillinoids (Fig. 19B). Various bisorbicillinols **99**, **100a-e** and saturnispol C analogues **101a-e** were obtained using acetone as co-solvent.<sup>23</sup> The use of hydroxysorbicillin (**1c**) gave access to dihydroxybisorbicillinol (**99**) in 41% yield. In this case, DMF was used as cosolvent instead due to the higher polarity of the substrate. Particularly noteworthy is the [4+2] cycloaddition using 3-ethyl derivative **88b**, enabling the formation of the corresponding bisorbicillinol **100c** in increased yield (61%, natural **7a**: 27%). A possible explanation might be the stronger donor activity of the ethyl group compared to the methyl group in sorbicillinol (**2a**). Switching to DMF as a co-solvent allowed the formation of trichobisvertinol D analogue **102** in 27% yield.

In addition to the diversification of the substrate, the additional structural elements (dienophile, Michael donor) can also be exchanged within the hybrid sorbicillinoids. One example of this is the demethylated species of trichosorbicillin A (**103**,



A



|  |                  |  |                   |  |                  |  |                   |
|--|------------------|--|-------------------|--|------------------|--|-------------------|
|  | 371<br>nd<br>nd  |  | 383<br>0%<br>0%   |  | 646<br>9%<br>15% |  | 889<br>14%<br>30% |
|  | nd<br>17%<br>26% |  | 656<br>23%<br>26% |  | nd<br>32%<br>25% |  | nd<br>34%<br>40%  |
|  | nd<br>22%<br>40% |  | nd<br>61%<br>nd   |  | 858<br>nd<br>nd  |  | nd<br>79%<br>60%  |
|  | nd<br>74%<br>56% |  | nd<br>61%<br>50%  |  | nd<br>63%<br>48% |  | 919<br>nd<br>nd   |
|  | nd<br>38%<br>34% |  | nd<br>14%<br>30%  |  | nd<br>83%<br>73% |  | nd<br>28%<br>30%  |
|  | nd<br>79%<br>86% |  | nd<br>29%<br>22%  |  | 331<br>nd<br>nd  |  | 479<br>nd<br>nd   |

B

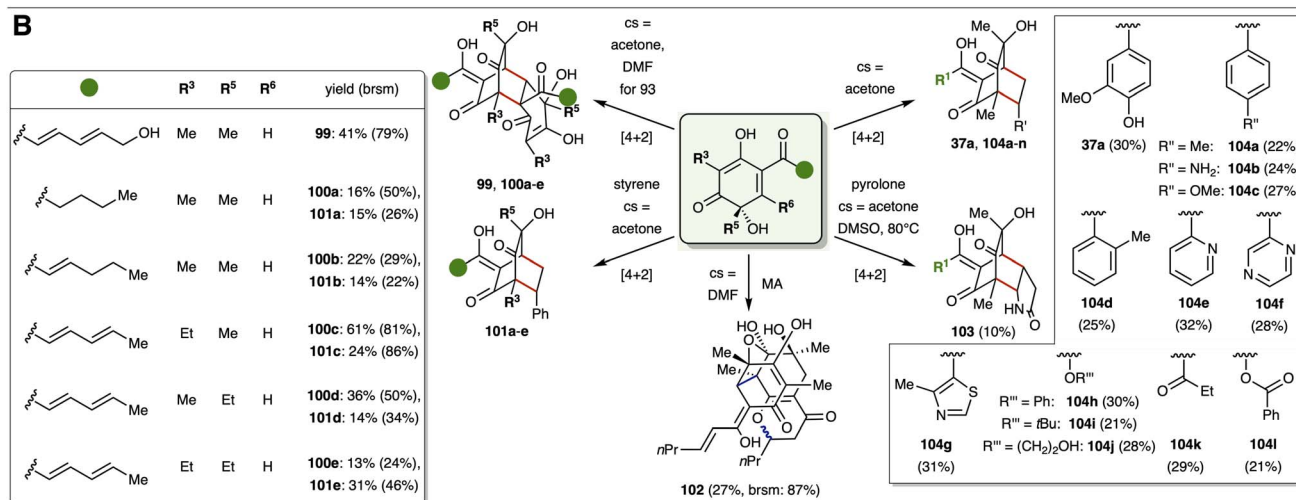


Fig. 19 (A) Promiscuity profile of the monoxygenase SorbC. Reaction conditions by Narayan:<sup>117</sup> 2.5 mM substrate, 2.5 μM SorbC, 1.0 mM NADP<sup>+</sup>, 5.0 mM glucose-6-phosphate (G6P), 1.0 U ml<sup>-1</sup> glucose-6-phosphate dehydrogenase (G6PDH), 50 mM potassium phosphate buffer, pH 8.0, 30 °C, 1 h. TTN: total turnover numbers (complete conversion: TTN = 1000, dark green). Reaction conditions by Gulder:<sup>23</sup> 2.0 mM substrate, 20 μM SorbC, 4.0 mM NADH, 50 mM potassium phosphate buffer, pH 8.0, 17% co-solvent v/v (acetone or DMF), 25 °C, 1.5 h. Yield of 90% and higher are shown in dark green. nd: not determined. (B) Formation of artificial new-to-science sorbicillinoids. brsm: based on reisolated starting material.



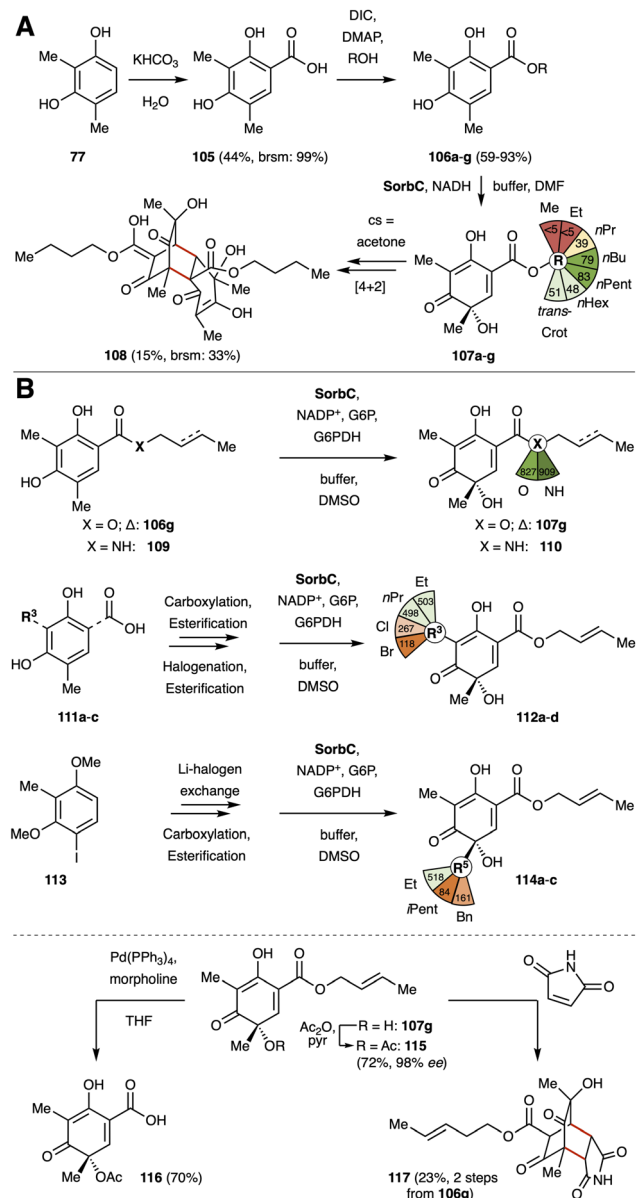


Fig. 20 Ester mimicking strategies by our group (A) and Narayan (B). Pie chart(s) in (A) shows enzymatic conversion in percentage yield, whereas in (B), the total turnover numbers are given.

Fig. 19B), which was synthesized in analogous fashion to tri-chosorbicillin A (27) using 1,5-dihydro-2H-pyrrol-2-one (10% yield).<sup>23</sup> Sorbicatchols can be derivatised in the same manner using comparatively simpler, and in some cases commercially available, dienophiles. In previous work of our group, a library of sorbicatchol structural analogues was established by using diverse substituted vinyls, including phenyls, heterocycles, ethers, and carbonyls (37a, 104a–I).<sup>85</sup> The sorbicatchol derivatives 104a–I were obtained in yields between 21 and 31% and were submitted to anti-HIV assays based on the reported antiviral activity of natural sorbicatchol A (37a, IC<sub>50</sub> = 85  $\mu\text{M}$ ).<sup>83</sup> However, in-depth analysis of their bioactivity against the influenza A virus displayed cytotoxicity against the host cell system instead of having a true antiviral effect. Nevertheless,

this work illustrates the potential of the one-pot chemo-enzymatic approach for the synthesis of focussed compound libraries to obtain structure activity relationship data.

Another approach for broadening the substrate spectrum of the monooxygenase SorbC lies in the introduction of dummy groups. This strategy is based on mimicking the sorbyl structure within the natural substrate 1a. The big advantage here is the flexibility for downstream late stage modification after removal of the dummy group. This principle was developed in parallel by our group (Fig. 20A) as well as Narayan and co-workers (Fig. 20B).<sup>123,124</sup> Starting with the same dimethylated resorcinol 77, which was used in the total synthesis of sorbicillin (1a, Fig. 16A), carboxylic acid 105 can be obtained by treatment with potassium bicarbonate. Esterification with multiple alcohols enabled access to the sorbyl mimicking ester analogues 106a–g in yields of 59–93%. HPLC analysis of their enzymatic conversion to sorbicillinols 107a–g revealed the requirement for an alkyl chain length of at least three carbon atoms. The optimum is reached with *n*-pentyl (107e, 83%) and *n*-butyl species (107d, 79%). The latter has already been successfully dimerised into the corresponding bisorbicillinol 108 (15%, brsm: 33%). In contrast, the work of Narayan and co-workers<sup>124</sup> focused on the use of crotyl esters (106g, TTN of 827) and amides (109, TTN of 909), which showed higher activities in their studies (Fig. 20B). The exploration of the tolerance of modifications in position R<sup>3</sup> (112a–d) and R<sup>5</sup> (114a–c) indicated the same trends as observed for the sorbyl analogues 94a–e and 95a–c (see Fig. 19A). Deprotection of the acetylated crotyl ester 115 to the free acid 116 can be performed under reductive conditions in presence of a palladium catalyst. Furthermore, crotyl ester bearing sorbicillinol 107g can be transformed into a hybrid sorbicillinoid by addition of external dienophiles, such as pyrrole-2,5-dione. This allowed the synthesis of pyrrolidine 117 in 23% yield. Subsequent deprotection of the crotyl ester 117 or basic saponification of bisorbicillinol 108 would enable the generation of artificial sorbicillinoids bearing a directly modifiable free acid moiety. Further linkage with suitable probes combined with target docking studies would allow the in-depth analysis of the mode of action of all current members of the sorbicillinoid family.

Besides derivatization of the substrates, the flavoprotein monooxygenase used can also be exchanged.<sup>125</sup> Biocatalytic transformations by Narayan *et al.* using the FMOs TropB or AzaH lead to the structurally related compound classes of tropolones and azaphilones (see Fig. 21A, 118–120).<sup>117,126,127</sup> Their biosyntheses are based on oxidative dearomatisation reactions comparable to those of the sorbicillinoids, but with a different site and stereo selectivity (C3/C5). Similar to SorbC, the enzymes were successfully used in chemo-enzymatic approaches (Fig. 21B), for example in the synthesis of stipitatic aldehyde 123 or trichoflectin (126). Screening experiments by Cox and co-workers revealed a broad substrate scope of the monooxygenase TropC, similar to SorbC.<sup>128</sup> In another interesting study by Narayan *et al.*, identification of the AzaH FMO homologue AfD enabled the formation of the corresponding (*S*)-trichoflectin (126) with excellent enantioselectivity.<sup>129</sup> In addition to the identification of natural enzyme homologues, selective



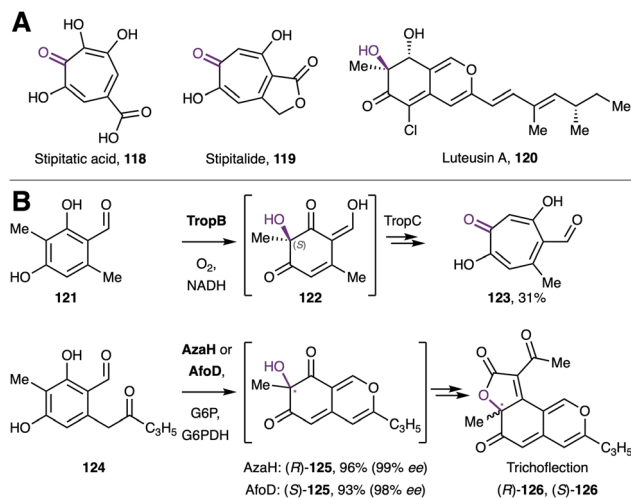


Fig. 21 (A) Representatives of the structurally related tropolones and azaphilones. (B) FMO diversity in chemo-enzymatic syntheses.

protein engineering would enable the generation of highly specific enzymes for the targeted control of regio- and stereoselectivity. This represents an exciting strategy and its use within the sorbicillinoids would open up further derivatisation possibilities.

Overall, the biocatalytic transformation with ester substrate mimics and the variation of the flavin-dependant monooxygenase demonstrates the impact of substrate and protein alteration in the expansion of the utility of enzymes in synthetic procedures.

## 5. Conclusions and outlook

Over the last eight decades, the NP class of the sorbicillinoids has grown steadily and up to now includes around 120 natural representatives and over 60 related and new-to-science analogs. Their molecular architectures and manifold biological activities have attracted the interest of NP researchers including synthetic organic chemists, biochemists, and molecular biologists. Since the last comprehensive review of Harned in 2011,<sup>6</sup> significant progress was made in understanding sorbicillinoid biosynthetic assembly by the Cox laboratory and also concerning efficient synthetic access towards this NP family. Only few open questions remain, including the enzymatic process behind sorbyl side-chain hydroxylation or the installation of the amino groups in some sorbicillinoids. The chemo-enzymatic approaches presently offer the shortest access combined with the highest overall synthetic yields and nearly perfect stereocontrol. For the first time, sufficient amounts of synthetic natural and artificially modified sorbicillinoids are thus available to enable structure-activity studies and in-depth investigations into molecular modes of action leading to the diverse bioactivities within the sorbicillinoid NP class. However, there is still enormous potential to improve chemo-enzymatic access, in particular concerning the yields in the reactions capturing the reactive sorbicillinol intermediate (**2**), which are currently in most cases around 30% of the desired product, owing to the high reactivity

of **2** and hence the occurrence of undesired side-reactions. Strategies to tame this inherent reactivity of **2** and to guide downstream functionalization reactions to exclusively furnish the desired products are thus needed. Furthermore, approaches that allow enzyme recovery and regeneration would be highly beneficial. In addition to the identification of further natural SorbC homologues with different product selectivity or substrate scope, the development of SorbC homologues with altered properties by protein engineering has the potential to significantly expand the accessible structural and functional chemical space.

On the purely chemical side, the Holy Grail of sorbicillinoid total synthesis lies in the development of an oxidising agent that would allow for a regio- and stereoselective dearomatisation of sorbicillin (**1a**) to sorbicillinol (**2a**), with similar overall efficiency when compared to the SorbC-catalyzed enzymatic transformation. However, previous strategies using, *e.g.*, asymmetric hypervalent iodine reagent have highlighted the many challenges in developing such a reagent.<sup>130</sup> All in all, the combination of new developments with the already existing synthetic and biocatalytic toolkit will offer enormous potential for the assembly of sorbicillinoid structural libraries with high potential in diverse medical applications.

## 6. Author contributions

The review was conceptualised by TMM and TAMG. TMM prepared the initial draft and figures. Both authors contributed to the reviewing and editing process of the manuscript.

## 7. Conflicts of interest

There are no conflicts to declare.

## 8. Acknowledgements

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