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Chemoenzymatic Total Synthesis of Sorbicatechol Structural Analogues and Evaluation of Their Antiviral Potential

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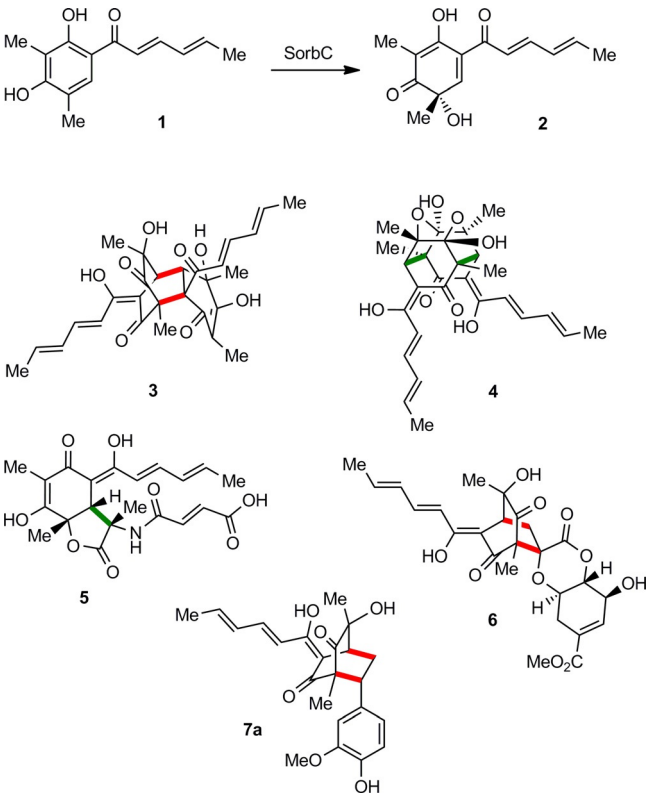
Sorbicillinoids are fungal polyketides characterized by highly complex and diverse molecular structures, with considerable stereochemical intricacy combined with a high degree of oxygenation. Many sorbicillinoids possess promising biological activities. An interesting member of this natural product family is sorbicatechol A, which is reported to have antiviral activity, particularly against influenza A virus (H1N1). Through a straightforward, one-pot chemoenzymatic approach with recently developed oxidoreductase SorbC, the characteristic bicyclo[2.2.2]octane core of sorbicatechol is structurally diversified by variation of its natural 2-methoxyphenol substituent. This facilitates the preparation of a focused library of structural analogues bearing substituted aromatic systems, alkanes, heterocycles, and ethers. Fast access to this structural diversity provides an opportunity to explore the antiviral potential of the sorbicatechol family.

Sorbicillinoids are a large polyketide natural product family consisting of more than 50 members.[1] They can be isolated from a diverse set of marine and terrestrial fungi and can be categorized into four different groups, according to their molecular structures: monomeric, dimeric, trimeric, and further functionalized sorbicillinoids. Biosynthetically, all sorbicillinoids are derived from stereo- and regioselective oxidative dearomatization of sorbicillin (**1**) to the highly reactive sorbicillinol (**2**) by the oxidoreductase SorbC (Scheme 1).[2] The di-/trimeric sorbicillinoids result from subsequent di-/trimerization of **2** with **1** or **2** by Michael addition or Diels–Alder cycloaddition due to the inherent respective reactivities of **2**, leading to beautiful molecular architectures, such as bisorbicillinol (**3**, resulting from dimerization through a Diels–Alder reaction)[3] or trichodimerol (**4**, resulting from double Michael addition/ketalization;[4] Scheme 1). Further functionalized sorbicillinoids are likewise formed by these transformations, but instead of a simple dime-



Scheme 1. Top: Oxidative dearomatization of **1** to **2**. Bottom: Examples of dimeric sorbicillinoid natural products **3** and **4**, and of further functionalized congeners formed by Michael addition (green bonds), for example, sorbicillactone A (**5**), or Diels–Alder cycloaddition chemistry (red bonds), for example, spirosorbicillinol A (**6**) and sorbicatechol A (**7a**).

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rization, these transformations involve non-sorbicillinoid nucleophiles or dienophiles. Structurally and biomedically interesting examples of this class of sorbicillinoids include **5**,[5] **6**,[6] and **7a** (Scheme 1).[7]

The biological activities of the functionalized sorbicillinoids are as diverse as their structures. Compound **5** is active against murine leukemic lymphoblast cell line L5178y, with an IC_{50} of 2.2 mg mL^{-1}, and protects human T cells from HIV-1 over a concentration range of 0.3 and 3.0 mg mL^{-1}.[5] Compound **6** shows 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity;[6] this property is also typical for dimeric sorbicillinoids, such as **3**.[3] Although currently far less studied, compared with the above-mentioned compounds, compound **7a** was reported to exhibit promising antiviral activity against H1N1.[7] With an IC_{50} value of 85 μM, compound **7a** shows identical potential to that of the antiviral drug ribavirin (IC_{50} of 84 μM).

Because the structural variability of any given type of natural sorbicillinoid core is rather limited and the isolation of these compounds from the complex metabolic matrix of the fungal producer strains is tedious, synthetic strategies are required to facilitate biomedical studies on these fascinating molecules. Although a number of elegant total synthetic routes towards dimeric sorbicillinoids, such as **3** and **4**, do exist, most of these are not stereoselective and/or rather lengthy.[8] In the case of the sorbicatechol core structure, only a single total synthetic approach exists. This led to the synthesis of *ent*-rezishanone C, with an ethoxy substituent instead of the 2-methoxyphenol substituent in **7a**. Overall, the synthetic route contains >20 individual steps, with a combined yield of less than 3%;[9] this clearly provides evidence of the need for improved synthetic strategies towards the sorbicillinoids. To this end, we have recently developed chemoenzymatic approaches for the efficient one-pot synthesis of dimeric sorbicillinoids, such as **3** and **4**, utilizing synthetically readily available **1**, which is oxidatively dearomatized by employing SorbC to give **2** enantioselectively. Compound **2** can be dimerized in a controlled manner, depending on the organic cosolvent employed during the biocatalytic reaction.[10] This methodology was further extended to enable functionalization of **2** with external nucleophiles, for example, leading to the first stereoselective total synthesis of epoxysorbicillinol, and dienophiles, yielding the first synthetic access to rezishanones and **7a**.[11] We herein present the application of this chemoenzymatic toolkit, featuring a facile intramolecular Diels–Alder reaction, for the straightforward preparation of a focused library of sorbicatechol-type structural analogues to enable the evaluation of their antiviral potential.

The required synthetic starting material, **1**, for the biocatalytic reaction was prepared in three-steps starting from 2-methylresorcinol, following our published procedure.[11] In a regio- and stereoselective oxidative dearomatization reaction with SorbC, compound **1** was transformed into **2**, which was the reactive precursor for all sorbicatechol derivatives. This oxidative dearomatization reaction proceeds with perfect stereocontrol, exclusively delivering the desired product, (*S*)-**2**.[10] Quenching of the reaction solution containing **2** with a diverse set of dienophiles readily delivered the target compounds in yields ranging from 21 to 32% (Scheme 2), irrespective of the substitution of the ene function. This is in the range of typical product yields for all sorbicillinoids following this chemoenzymatic strategy, and can be explained by unreacted starting material **1** and the formation of dimeric side products, particularly **3**, which cannot be fully suppressed due to the intrinsically high reactivity of **2**. Besides the synthesis of **7a**, sets of styrenes substituted at the aromatic portion (**9a–e**), vinyl ethers (**9f–k**), ethyl vinyl ketone (**9l**), and heteroaromatic building blocks (**9m–o**) were employed to give the respective sorbicatechol analogues **7a–o**. All compounds were isolated by means of semipreparative HPLC to give pure and stereochemically defined material (Figure S33 in the Supporting Information).

In the original isolation paper, the absolute configuration of the biologically more active *endo* Diels–Alder product, **7a**, and its likewise isolated, minor *exo* congener was thoroughly investigated through a combination of DFT calculations, 2D NOESY measurements, and analysis of experimental versus calculated circular dichroism (CD) spectra; thus allowing for an unambiguous assignment of the absolute configuration of both compounds.[7] A comparison of the NMR spectroscopy data of compound **7a** produced by our approach with the data reported for the respective *endo* and *exo* products clearly revealed that our material corresponded to the *endo* product. This can most convincingly be deduced from chemical shift analysis of the ^{13}C NMR spectroscopy data, most importantly at C2 (**7a**: δ = 198.0 ppm, reported for *endo*: δ = 197.9 ppm, reported for *exo*: δ = 199.7 ppm), C3 (δ = 112.2, 112.0, 110.5 ppm), C7 (δ = 47.8, 47.8, 49.9 ppm), C8 (δ = 31.5, 31.4, 30.2 ppm), C16 (δ = 110.4, 110.2, 111.2 ppm), and C5-methyl (δ = 24.4, 24.3, 25.2 ppm), as well as from differences in ^{1}H NMR chemical shifts, particularly at H8 (δ = 1.84 and 3.05, 1.84 and 3.05, 2.25 and 2.57 ppm), H16 (δ = 6.44, 6.45, 6.71 ppm), and H20 (δ = 24.4, 24.3, 25.2 ppm). In addition, NOESY interactions between the protons of C1-methyl at the sorbicillin-derived core structure with H16 and C17-OMe can only be seen in the *exo* analogue,[7] and are consequently also absent in our product **7a** (Figure S4); this is in agreement with the above *endo* assignment. Most importantly, the large chemical shift difference of the geminal

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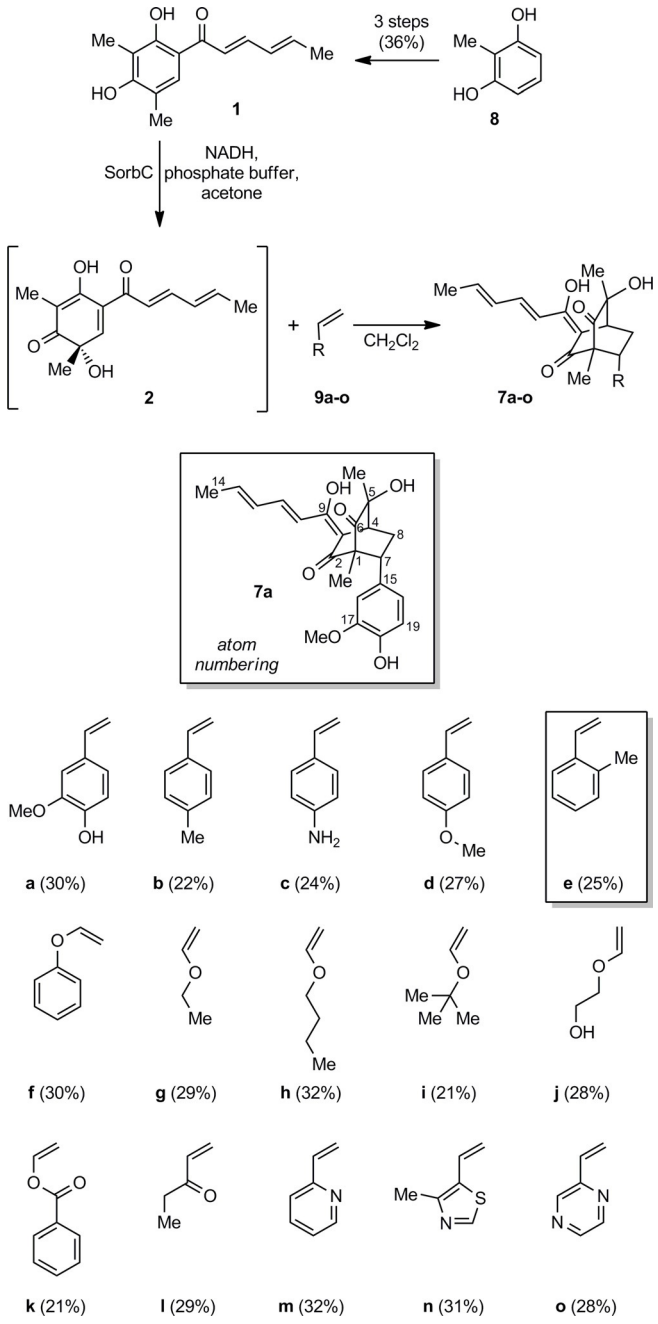

Scheme 2. Synthesis of **7a** and 14 structural analogues (**7b**–**o**). Yields for each analogue are given in parentheses. NAD^+: nicotinamide adenine dinucleotide.

protons attached to C8, which are typical for the *endo* product **7a** (difference 1.21 ppm), compared with the *exo* product (0.32), can also be found in all other compounds presented herein (**7b**: 1.15; **7c**: 1.23, **7d**: 1.16, **7e**: 1.24, **7f**: 1.22, **7g**: 1.10, **7h**: 1.09, **7i**: 1.17, **7j**: 1.12, **7k**: 1.42, **7l**: 1.46, **7m**: 1.53, **7n**: 1,45, **7o**: 0.89); thus corroborating that all products are *endo*. The regioselectivity of the Diels–Alder cycloadditions can generally be explained by the directing effect of the C1-methyl group (see numbering of **7a** in Scheme 2, *ortho* rule).

Because of the reported antiviral activity of **7a** against H1N1, a set of the prepared sorbicatechol analogues, **7a**–**o**, were evaluated for their antiviral activity against influenza A virus (IAV). Initial tests were performed by using the *Gaussia* luciferase reporter virus.[12] The abundance of *Gaussia* luciferase activity that accumulates in the supernatant of cells infected with this virus can be used as a proxy for virus replication. A resazurine conversion assay was also performed to assess the cytotoxic effect of the compounds on the cells.

Unfortunately, the tested compounds exhibited cytotoxicity to the viral host cells at concentrations below the observation of antiviral effects. This effect thus not only made it impossible to assess any antiviral effects against IAV, but also raised the question of whether the initially reported antiviral activity of **7a**[7] was a true antiviral effect or rather the result of the cytotoxicity of the compound to the host cells. To further broaden our antiviral screening, we thus decided to expand from only testing *Orthomyxoviridae* (IAV) to *Retroviridae* by including tests against HIV-1. This system was also chosen due to the significant need for finding compounds active against HIV. Despite the fact that current anti-HIV drugs can minimize virus replication and thereby prevent the outbreak of AIDS, a series of severe problems remain, including the rapid emergence of resistant viruses, high virus variability, high costs, and adverse side effects.[13] As a test system for anti-HIV activity, the EASY-HIT assay[14] was performed; this is based on the reporter cell line LC5-RIC, which contains a stably integrated fluorescent reporter gene. Upon HIV infection, these cells express the fluorescent marker dsRed, which can be directly connected to the anti-HIV activity of a compound when reduced during treatment.[14] We used the resazurin conversion assay, which is based on the enzymatic reduction of resazurin,[15] to check for any signs of negative effects on the vitality and metabolism of the LC5-RIC cells that could appear during the course of the EASY-HIT assay. Of the 15 sorbicatechol analogues evaluated for anti-HIV properties, 6 compounds showed activities beyond their respective cytotoxicity values, as determined by the viability assay.[15] Interestingly, all active compounds were either derived from a styrene (**9a**–**e**) or equipped with an aromatic portion in close proximity to the ene functionality in the substrate, as in phenyl vinyl ether **9f**. No other compounds exhibited a measurable activity, including all additional ethers and all heteroaromatic systems, as well as the benzoylated derivate **7k**, with only one additional keto function between the alkene and aromatic moiety, when compared with **7f**. These results strongly indicate that an aromatic portion in close proximity to the bicyclo[2.2.2]octane core structure of the sorbicatechols is required for activity. The selectivity indices (equaling the CC_{50}/IC_{50}) for the active compounds are mostly in the range of 1.37 to 1.72 (Table 1). In this range, cytotoxic effects on the host cells might indeed influence the antiviral activity values. For the *ortho*-methyl substituted analogue, **7e**, however, a significant increase in activity can be observed, along with a slight decrease of cytotoxicity. Overall, this leads to a selectivity index of 3.49 for **7e**. Interestingly, a simple repositioning of the methyl substituent from the *para* (as in **7b**) to the *ortho* position led to a twofold increase in activity.

In summary, we have synthesized 15 sorbicatechol derivatives **7a**–**o** by application of a chemoenzymatic synthesis of **2**

Table 1. Anti-HIV activity given as IC_{50} values and cytotoxicity determined as CC_{50} in the viability assay.[15] The selectivity index is calculated from CC_{50}/IC_{50}. Emtricitabine (2′,3′-dideoxy-5-fluoro-3′-thiacytidine, FTC) was used as the HIV inhibition control.

Compound	Anti-HIV activity IC_{50} [μM]	Viability assay CC_{50} [μM]	Selectivity index (CC_{50}/IC_{50})
7a	65.9±4.68	102*	1.55
7b	68.8±6.97	105.2*	1.53
7c	75.9±7.37	125.2*	1.65
7d	62.7±10.08	108.4*	1.72
7e	32.2±2.52	112.3*	3.49
7f	76.6±4.28	105.2*	1.37
FTC	0.7±0.22	>100.0	>140

followed by quenching with a diverse set of dienophiles. Evaluation of their activity against IAV has revealed their cytotoxicity against the host cell system, rather than a true antiviral effect. Screening of the compound library against HIV-1 showed that aromatic substitution, as the bicyclo[2.2.2]octane substructure, was required to obtain antiviral activity, although it remained weak. The strongest activity was obtained with **7e**, with an IC_{50} value of approximately 32 μM and a selectivity index of 3.49. The activity is thus about 50 times lower than that of the commercial virostatic drug emtricitabine (FTC), with a >40-fold decrease in selectivity index. In contrast to previous reports, our findings did not reveal antiviral activity of the sorbicatechols over a range that would be promising for further antiviral lead optimization.

Experimental Section

Experimental details: The synthesis of sorbicatechol derivatives followed a two-phase procedure, which is described here for the production of the most active derivative, **7e**, as an example. First, compound **1** was dissolved in acetone and added to phosphate buffer (50 mM, pH 8) with the enzyme SorbC. The reaction started upon the addition of NADH and was incubated for 6 h at room temperature. Second, produced **2** was extracted with organic solvent and *o*-methylstyrene (**9e**) was added. After slow evaporation of the organic solvent under reduced pressure, to increase the concentration of the dienophile slowly over time, the crude product was purified by means of preparative HPLC.

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Conflict of Interest

The authors declare no conflict of interest.

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