Biomolecular-chemical Screening: A Novel Screening Approach for the Discovery of Biologically Active Secondary Metabolites

III. New DNA-binding Metabolites

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Based on the chemical screening technique, biomolecular-chemical screening has been developed which makes use of two-dimensional TLC analysis of microbial extracts and combines thin-layer chromatography (RP-18) with binding studies towards DNA. In the first dimension the metabolites of the crude microbial extract are separated, and in the second dimension binding properties towards DNA are analysed. An initial screening program with 500 microbial extracts prepared by solid-phase extraction with XAD-16 resin resulted in 17 samples which contained metabolites with significant DNA-binding behavior. Fermentation, isolation and structural characterization led to already known metabolites [phenazine-1,6-dicarboxylate (1), phencomycin (2), 11-carboxy-menoxymycin B (3), soyasaponine I (4), and (8S)-3-(2-hydroxypropyl)-cyclohexanone (5)], as well as to new secondary metabolites. Fermentation of the producing organisms of the new DNA-binding metabolites, ent-8,8α-dihydro-ramulosin (6), (2R,4R)-4-hydroxy-2-(1,3-pentadienyl)-piperidine (7), (5R)-dihydro-5-pentyl-4′-methyl-4′-hydroxy-2(3H)-furanone (8), and seco-4,23-hydroxyoleane-12-en-22-one-3-carboxylic acid (9), as well as isolation, structural characterization, and physico-chemical properties are reported.

With the aim to develop a novel strategy based on the chemical screening technique, the so-called biomolecular-chemical screening has been established. Chemical screening allows to visualize a thorough picture of a microbial secondary metabolite pattern (metabolic fingerprint) by means of TLC in different solvent systems and various staining reagents. It was now integrated with a target-directed screening approach, the binding analysis of low molecular weight secondary metabolites towards DNA. In order to establish the method, binding of 470 pure natural products (mainly secondary metabolites) with random DNA was examined with the one-dimensional variant of the TLC method.

For binding studies of secondary metabolites out of crude extracts a modification of the biomolecular-chemical screening has been developed using two-dimensional TLC. In the first dimension the metabolites are chromatographically separated, and in the second dimension binding studies are performed similar to those with pure compounds. In this paper we present the results of the screening program and the structural elucidation of the newly obtained metabolites.

Screening

For our chemical screening routine, extracts from Streptomyces and Fungi imperfecti strains are prepared from the filtrate of 50 ml shaking cultures by solid-phase...
Table 1. Producing organisms, fermentation, yield and TLC-characteristics of the isolated metabolites.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Strain(^a)</th>
<th>Fermentation scale</th>
<th>Yield [mg/l]</th>
<th>RF in CHCl(_3)/MeOH, 9:1 (^b)</th>
<th>Detection</th>
<th>Molecular formula</th>
<th>MW</th>
<th>Appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Streptomyces</em> GT 41212</td>
<td>150 l</td>
<td>1.3</td>
<td>0.28</td>
<td>Green(^c)</td>
<td>C(_{14})H(_6)N(_2)O(_4)</td>
<td>268</td>
<td>Green crystals, amorph</td>
</tr>
<tr>
<td>2</td>
<td><em>Streptomyces</em> GT 41238</td>
<td>50 l</td>
<td>0.7</td>
<td>0.71</td>
<td>Yellow(^d)</td>
<td>C(_{15})H(_9)N(_2)O(_4)</td>
<td>282</td>
<td>Yellow crystals, amorph</td>
</tr>
<tr>
<td>3</td>
<td><em>Streptomyces</em> GT 51172</td>
<td>50 l</td>
<td>0.3</td>
<td>0.87</td>
<td>Purple(^e)</td>
<td>C(_{12})H(_2)NO(_3)</td>
<td>475</td>
<td>Purple crystals, amorph</td>
</tr>
<tr>
<td>4</td>
<td><em>Streptomyces</em> GT 51117</td>
<td>50 l</td>
<td>0.9</td>
<td>0.40</td>
<td>Orinin blue</td>
<td>C(_{10})H(_9)O(_3)</td>
<td>243</td>
<td>Colorless crystals, amorph</td>
</tr>
<tr>
<td>5</td>
<td><em>Fungi imperfecti</em> GT 46045</td>
<td>200 l</td>
<td>1.84</td>
<td>0.51</td>
<td>Anisaldehyde orange</td>
<td>C(_9)H(_8)O(_2)</td>
<td>156</td>
<td>Colorless crystals, amorph</td>
</tr>
<tr>
<td>6</td>
<td><em>Fungi imperfecti</em> GT 46045</td>
<td>200 l</td>
<td>1.20</td>
<td>0.61</td>
<td>Anisaldehyde pale yellow</td>
<td>C(_8)H(_7)O(_3)</td>
<td>164</td>
<td>Colorless oil</td>
</tr>
<tr>
<td>7</td>
<td><em>Streptomyces</em> GT 41006</td>
<td>150 l</td>
<td>58.7</td>
<td>0.20</td>
<td>Anisaldehyde purple</td>
<td>C(_8)H(_7)NO</td>
<td>167</td>
<td>Yellow crystals, amorph</td>
</tr>
<tr>
<td>8</td>
<td><em>Streptomyces</em> GT 61115</td>
<td>40 l</td>
<td>1.8</td>
<td>0.62</td>
<td>Anisaldehyde blue</td>
<td>C(_9)H(_8)O(_3)</td>
<td>186</td>
<td>Colorless oil</td>
</tr>
<tr>
<td>9</td>
<td><em>Streptomyces</em> GT 44003</td>
<td>40 l</td>
<td>5.5</td>
<td>0.83</td>
<td>Anisaldehyde turquoise</td>
<td>C(_5)H(_4)O(_5)</td>
<td>488</td>
<td>Colorless crystals, amorph</td>
</tr>
</tbody>
</table>

\(^{a}\) The strains have not been taxonomically characterized in detail;  
\(^{b}\) Data obtained with HPTLC silica gel plates;  
\(^{c}\) Color without staining;

Extraction (SPE) with XAD-16 resin. The metabolic fingerprint of each strain that is analysed by chromatography on silica gel TLC plates in two different solvent systems and with five staining reagents, allows to classify microbial isolates in: (i) non-producing organisms, (ii) organisms of limited productivity, and (iii) "talented" organisms with a broad spectrum of metabolites\(^2\).

For the biomolecular-chemical screening program we pre-selected 500 samples of strains which showed the biosynthesis of secondary metabolites (categories ii and iii) by regular TLC analysis. In a second step, these samples were examined for DNA-binding properties by making use of the 2D-TLC method of the biomolecular-chemical screening. Routinely, anisaldehyde-H\(_2\)SO\(_4\) was used as staining reagent. If no colorization was possible, we switched to orcinol-H\(_2\)SO\(_4\), or Ehrlich's reagent. Out of the 500 microbial extracts, 17 samples were identified which contained metabolites with moderate to strong DNA-binding properties (R\(_f^1\)/R\(_f^2\)<0.85). In addition, 38 extracts revealed metabolites for which weak DNA binding was observed (R\(_f^2\)/R\(_f^1\)>0.85). In order to isolate and characterize the compounds with high DNA-affinity eight strains (Table 1) were selected for further processing.

Fermentation and Isolation

On the basis of the results from the biomolecular-chemical screening seven *Streptomyces* isolates and one *Fungi imperfecti* isolates were subjected to large scale fermentation (40 to 200 liter) using the same cultivation media as in the screening routine. As the production of the metabolites in question could be pursued by TLC, the time for harvesting was chosen according to highest yields.

After harvesting, the culture filtrates of all strains were adsorbed on Amberlite XAD-16 resin, and after washing with water the metabolites were eluted with MeOH-H\(_2\)O (4:1). The oily concentrates obtained after evaporation of the solvents were subjected to subsequent column chromatography (e.g. silica gel, Sephadex LH-20, RP-18-silica gel on MPLC and HPLC) using suitable solvent systems adapted to each metabolite to be purified. The yields of the DNA-binding metabolites ranged from 0.3 to 58.7 mg/liter. Table 1 provides an overview on the different producing strains, fermentation data, yields, as well as the TLC-characteristics of the isolated metabolites.

The isolated pure compounds were characterized spectroscopically. The molecular formulae were determined by mass spectrometry and structures were elucidated by detailed analysis of the \(^1\)H-, \(^13\)C-, \(^1\)H\(^1\)H-, and \(^1\)H-\(^13\)C-shift correlation NMR-spectra and, where applicable, in comparison to literature data.
Knew Metabolites with DNA-binding Properties

Phenazine-1,6-dicarboxylate (1) and phencomycin (2): The green-colored DNA-binding metabolite 1 of *Streptomyces* sp. (strain GT 41212) as well as the yellow-colored component 2 of *Streptomyces* sp. (strain GT 41238) turned out to be known phenazines. A comparison with literature data proved the structures of phenazine-1,6-dicarboxylate (1) and phencomycin (2).

11-Carboxy-menoxymycin B (3): From *Streptomyces* sp. (strain GT 51172), a purple colored compound was isolated by silica gel-, gel permeation-, and RP-18 silica gel chromatography. Characteristic pH-dependent color changes (purple ($\lambda_{\text{max}} = 558$ nm) under basic conditions and yellow (418 nm) under acidic conditions) indicated a 5-hydroxy-1,4-naphthoquinone chromophor. On the basis of the spectroscopic data the isolated compound was assigned 11-carboxy-menoxymycin B (3).

Soyasaponine I (4): From *Streptomyces* sp. (strain GT 51117) soyasaponine I (4), most likely a biotransformation product from the culture medium, was recovered as DNA-binding compound.

(85)-3-(2-Hydroxypropyl)-cyclohexanone (5): From the culture broth of the fungus GT 46045 the substituted cyclohexanone derivative 5 could be isolated in 0.84 mg/liter yield. Although the compound was reported in 1964 as metabolite of *Petalotia ramulosa*, the missing NMR data are provided in the experimental section. The absolute configuration of the center of chirality in the propyl side chain (C-8) was determined to be 8S with Helmchen’s method via 2-(R)- and 2-(S)-\(\alpha\)-phenylbutyric acid derivatives of 5 and \(\text{H}^1\) NMR analysis. The center of chirality at C-3 was examined by CD-spectroscopy (5; $\lambda_{\text{max}}$ ($\theta$)=239 nm (+2,059)). Application of the octant rule resulted in a positive sign of the Cotton-effect for the n→
Fig. 2. Structural formulae of the new secondary metabolites [ent-8,8a-dihydro-ramulosin (6), (2R,4R)-4-hydroxy-2-(1,3-pentadienyl)-piperidine (7), dihydro-5-pentyl-4'-methyl-4'-hydroxy-2(3H)-furanone (8), and seco-4,23-hydroxyoleane-12-en-22-one-3-carboxylic acid (9)] discovered by biomolecular-chemical screening via their DNA-binding behavior.

ent-8,8a-Dihydro-ramulosin (6)

The colorless amorphous compound was isolated together with 5 as the stronger DNA-binding one. Based on both, the molecular formula C10H16O3 deduced from a HREI-mass spectrum (m/z= 184.21 13) and its NMR data, 6 was determined to be ent-8,8a-dihydro-ramulosin, formerly reported as metabolite of the endophyte Caroplea elegantula14). However, there is a disparity concerning the optical rotation value: [α]D = -244 (c 0.007, CHCl3) for the published compound vs. [α]D = +36.0 (c 0.8, CHCl3) in our compound 6. Identical 1H and 13C NMR spectra in CHCl3, as well as detailed analysis of the 1H-coupling pattern in d4-MeOH clearly indicated the same relative constitution for both compounds. Therefore, we assume 6 to be the enantiomer of the published compound despite the difference in magnitude of the optical rotation value. Taking into consideration the stereochemical assignment of the co-metabolite 5, this assumption is also supported by the similarity in 1H NMR-data of 5 and 6.

(2R,4R)-4-Hydroxy-2-(1,3-pentadienyl)-piperidine (7)

The culture broth of Streptomyces strain GT 41006 showed a striking DNA-binding metabolite which turned to purple after staining with anisaldehyde-H2SO4. The molecular formula C10H17NO resulted from a HREI-mass spectrum (m/z=167.1316). On the basis of detailed NMR studies, especially 3JHH-coupling patterns, the constitution of 7 turned out to be identical with a piperidine derivative 4-hydroxy-2-(1,3-pentadienyl)-piperidine which had been isolated previously from Streptomyces luteogriseus (strain FH-S 1307) after detection through our basic chemical screening approach14). However, the latter compound, as well as an earlier reported congener, SS20846A15 16), are both enantiomers of 7 (SS20846: [α]D = -15.2 (c 0.53, CHCl3)15), 7: [α]D = +10.8 (c 1.1, MeOH)). The absolute stereochemistry of SS20846A was proven to be S,S,4 by asymmetric total synthesis of the piperidine alkaloid16,17).

(5R)-Dihydro-5-pentyl-4'-methyl-4'-hydroxy-2(3H)-furanone (8)

After adsorption chromatography, followed by gel-permeation on Sephadex LH-20 (in methanol and ethyl acetate) and RP-18 silica gel chromatography on HPLC column, 70 mg of the DNA-binding metabolite were
isolated from a 40 liter cultivation of Streptomyces strain GT 61115. The IR spectrum revealed adsorption bands for a OH-group at $v=3470$ cm$^{-1}$ and for a $\gamma$-lactone moiety at 1770 cm$^{-1}$. The HREI-MS showed an ion at $m/z=171.1017$ ($C_9H_{15}O_3$) which according to HRFAB-MS analysis ($m/z=186.1279$ (M$^+$); $C_{10}H_{18}O_3$) was determined to be the M$^+$-CH$_3$ fragment. The $^1$H NMR spectrum indicated two methyl groups with an identical singlet signal ($\delta 1.19$); also all other proton signals were found in the aliphatic region ($\delta 1.50$ to 2.55). The $^{13}$C NMR spectrum exhibited the signals of ten carbon atoms, two methyl groups in the neighborhood of an oxygen atom with identical chemical shifts ($\delta 29.2$), five methylene groups, one methine group ($\delta 82.9$), as well as two quaternary carbon atoms at $\delta 71.2$ and 180.1 (lactone carbonyl). $^1$H/$^1$H-COSY- and HMBC- NMR analysis allowed to assign the constitution of the $\gamma$-lactone derivative depicted as 8 in Fig. 2. The stereochemistry at C-5 was postulated to be $5R$ based on comparison of the optical rotation values of 8 ($\alpha_D^0$ (MeOH) = +42.0 (c 1, MeOH)) with the structurally similar natural products dihydro-$5R$-octyl-2(3$H$)-furanone ($\alpha_D^0$ = +37.7 (c 1, MeOH)), dihydro-$5R$-pentyl-2(3$H$)-furanone ($\alpha_D^0$ = +47.5 (c 1.82, MeOH)), and dihydro-$5R$-hexyl-2(3$H$)-furanone ($\alpha_D^0$ = +42.5 (c 0.5, MeOH))$^{18}$.

Seco-4,23-hydroxyoleane-12-en-22-one-3-carboxylic Acid (9)

The isolated pure DNA-binding compound of Streptomyces sp. (strain GT 44003) was well soluble in MeOH, ethyl acetate, and CHCl$_3$, but only weakly soluble in H$_2$O. Due to the results from the HRFAB-mass spectra ($m/z=489.3613$) the molecular formula of 9 was found to be $C_{30}H_{48}O_5$. The molecular ion was also obtainable with DCI-MS and ESI-MS while HREI-MS gave only M$^+$-H$_2$O ($m/z=470.3399$). In addition, a conspicuous Retro-Diels-Alder fragmentation was observable with product peaks at 232.3 and 239.3 (Fig. 3).

The $^1$H NMR spectrum indicated seven methyl groups, one olefinic proton, two protons attached to a carbonyl moiety as well as 18 aliphatic protons. The $^{13}$C NMR spectra presented 29 carbon signals (DEPT: 10 CH$_2$-groups, eleven CH/CH$_3$-groups as well as 8 quaternary C-atoms). An additional carbon signal ($\delta 177.1$, s, C-3) was discovered via a HMBC experiment. From the $^1$H/$^1$H-COSY data only small fragments of the molecule were obtained which were separated by quaternary C-atoms (Fig. 4). From the HMBC-data a four-ring-system was unambiguously established pointing to an oleanane-type metabolite. In comparison to NMR data of oleanes reported in the literature$^{19,20}$ the constitution of the isolated compound was established to be the new seco-4,23-hydroxyoleane-12-en-22-one-3-carboxylic acid (9). Stereochemical analysis was done on the basis of NMR data, e.g. comparison of $^{13}$C data at the centers of chirality C-8 and C-14 of 9 with 4, as well as at C-17 with yunganogenin R$^{21}$. Between 28-CH$_3$ and 18-H a strong NOE was observed which proved a 18R-configuration as it is typical for oleanes. The assigned stereochemistry of C-9 resulted from proton coupling constants (equatorial position of 9-H due to a missing large axial-axial $^3J_{HH}$ coupling towards 11-H$_2$)$^{22}$. Because the NMR-spectroscopically deduced information, the stereochemistry of 9 appeared to be identical with that of other oleanes, and therefore we postulate C-10 to be $\alpha$-configured.

DNA-binding and Biological Activities

DNA-binding studies by one-dimensional TLC-analysis of the pure, isolated metabolites confirmed the initial screening results on DNA-binding (Table 2). The values of the $Rf/Rf_2$-ratio (chromatographic retention with and without DNA) are in the same range of magnitude (0.73–0.88) as those determined for 50 DNA-binding metabolites in our validation studies with 470 natural products$^4$. Additional confirmation for DNA interaction of the compounds was provided by comparative thermal analysis of random DNA and DNA-ligand complexes. Significant increases in DNA-melting temperature obtained with compounds 3, 4, 8 and 9 pointed to relevant DNA-interactions of these natural products. For 5 and 6, the weakest DNA-binders by TLC-analysis, and 7, $\Delta T_m$ was below the measurement error. 1 and 2 did not provide proper melting curves, however, they are known intercalators of DNA$^{23}$.

A compilation of published biological activities of the compounds themselves or of closely related analogous is
Fig. 4. $^3J_{CH}$-COSY NMR (a) and important $^3J_{CH}$ NMR (b) couplings of seco-4,23-hydroxyoleane-12-en-22-one-3-carboxylic acid (9).

Table 2. DNA-binding properties of the isolated compounds and biological activities.

<table>
<thead>
<tr>
<th>Compound</th>
<th>$R_f/R_f$ (a)</th>
<th>$\Delta T_m$ ($^\circ$C)(b)</th>
<th>Cytotoxicity</th>
<th>Biological activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.80</td>
<td>n.d.</td>
<td>antitumor23, antibiotic24, inhibition of xanthin-oxidase25</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.75</td>
<td>n.d.</td>
<td>antitumor26, antibacterial (gram-(+))27</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.73</td>
<td>1.5</td>
<td>antitumor28, intracellular peroxide formation29, antibacterial (gram-(+))30</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.83</td>
<td>0.9</td>
<td>antiviral29, antifungal30, inhibition of PI3-kinase31, ethnopharmacognosy: rheumatism32, ulcer33, pain34</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.89</td>
<td>0.2</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0.90</td>
<td>0.1</td>
<td>&gt;200</td>
<td>enantiomer: insecticidal19, anthelmintic19</td>
</tr>
<tr>
<td>7</td>
<td>0.84</td>
<td>0.4</td>
<td>&gt;200</td>
<td>enantiomer: antibacterial (gram-(+)), anticonvulsant16</td>
</tr>
<tr>
<td>8</td>
<td>0.88</td>
<td>0.9</td>
<td>&gt;200</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>0.83</td>
<td>0.8</td>
<td>12.5</td>
<td>antiviral19, inhibition of xanthine oxidase8, other oleanes: antiviral20, antitumor20, antiinflammatory27</td>
</tr>
</tbody>
</table>

Note: a) The $R_f/R_f$-ratios ($R_f$ without DNA; $R_f$ with DNA) were determined with pure substances using the 1D-TLC method3; b) Difference in DNA melting curves monitored at 260 nm3; c) HeLa cell line; d) 46 % inhibition at 800 ng/ml; 9: 35 % inhibition at 800 ng/ml; e) S. aureus (MET 10760): 20 mm zone of growth inhibition at 50 µg; B. subtilis ATCC 6633: 21 mm zone of growth inhibition at 50 µg; f) Coxsackievirus B3: 65 % inhibition at 25 µg/ml; 65 % inhibition at 12.5 µg/ml; 6 % inhibition at 6.25 µg/ml. n.d. not determinable.

Given in Table 2. Recent results obtained from our institute's internal primary biological profiling of compounds are also presented. In cytotoxicity and anti-proliferative testing on L-929, K562 and HeLa cell lines no general cellular toxicity was observed for the new compounds 6~9 (for results with HeLa cells see Table 2). Antibiotic properties of 1 have been correlated with RNA-interaction20. Therefore, it might be speculated that other biological activities, e.g. antifungal activity of 4 or insecticidal and anthelmintic activities of the enantiomer of 6 are also related to the DNA-binding property described for the first time for these compounds. Also, weak but
significant antiviral activity of 9, specifically against Coxsackie virus B3, might be regarded in this context.

**Discussion**

The concept of biomolecular-chemical screening was to integrate chemical screening by TLC-characterization of metabolites with a biologically relevant screening approach. Thus, we wanted to take advantage of the considerable rate of new metabolites resulting from the TLC-based chemical screening. The binding analysis right on the TLC plate was supposed to add biological information as an additional selection criteria in the search for natural products. This information can immediately be applied to the chromatographic isolation of the identified compound. In regular biological screening routines on complex extracts, time-consuming dereplication procedures are needed to identify active compounds. Due to the importance of DNA-binding in biochemical and biomedical research, there are many well-established analytical procedures for studying DNA-ligand interactions, most of them more precise than our TLC-based approach. However, it was our objective to make a simple detection of DNA-binding applicable to natural products screening from complex extracts.

In this study, our aim was not to focus on strong DNA-binding compounds as there are many known already, but to search for metabolites with moderate binding activity. Biological activities of such compounds as deduced from the literature or found through our own efforts on biological profiling are not necessarily to be correlated with the DNA-binding property. The broad spectrum of biological activities associated with the compounds described in our study shows that the initial selection marker of DNA-binding does not narrow the potential of these compounds down to mere toxic agents. Identification of compounds 1, 2 and 3 with literature known DNA-binding properties provides powerful proof for the general applicability of our method and the significance of TLC-detected DNA-binding. The broad structural variety of compounds that were identified by biomolecular-chemical screening indicate that the approach can be successfully applied as a novel selection tool for the screening for natural products from crude extracts.

**Experimental**

**General**

NMR spectra were measured on Bruker Avance DPX 300 (300 MHz) and Avance DRX 500 (500 MHz) instruments. The mass spectra were taken with Fisons VG Quattro (ESI-MS), and AMD Infactra (FAB/EI-MS). IR spectra in pressed KBr disks were recorded on Shimadzu, Modell IR 470, UV/VIS spectra on a Varian Cary 1E UV-visible spectrophotometer. Optical rotation values were recorded with a polarimeter from Perkin Elmer, and CD spectra with Jasco J 500 A, and Jasco 750. Analytical HPLC was performed on Hewlett Packard 1050 equipped with DAD-detector, and preparative scale HPLC with Gilson instruments (column: Licrosorb RP-18 (7 μm), 250/25). TLC was performed on silica gel 60 F254 (0.2 mm) from Merck (Darmstadt, Germany).

Salmon sperm DNA (Sigma; 2 mg/ml in water) was homogenized by sonication for 6 minutes (Labsionic U, Braun). DNA-fragments were between 300 and 3,000 base pairs in size, as revealed by agarose gel electrophoresis.

**TLC-screening**

Biomolecular-chemical screening was performed on silica gel plates (Merck, HPTLC-ready-to-use-plates, silica gel RP-18 WF254, on glass). Application of the substances and the homogenized DNA samples, chromatography on the TLC plates as well as the detection of the compounds was performed as described in the preceding papers. Detection was realized by means of UV extinction at 254 and 366 nm, as well as by colorization with staining reagents. Changes in Rf-values indicate an interaction between ligand and DNA and are expressed by the ratio Rf/Rf, in which Rf represents the Rf-value without, and Rf represents the Rf-value with DNA.

**Cultivation Media**

Medium A: Soybean meal (degreased) 2% and mannitol 2% in deionized water, pH=7.5 prior to sterilization.

Medium B: Oat flakes 2% in deionized water and addition of 5 ml trace element concentrate per liter, pH=7.8 prior to sterilization.

Medium C: Glycerol 3%, casein peptone 0.2%, K₂HPO₄ 0.1%, NaCl 0.1%, MgSO₄ 0.05% in tap water and addition of 5 ml trace element concentrate per liter, pH=7.0 prior to sterilization.

Medium D: malt extract 2%, yeast extract 2%, glucose H₂O 1% and (NH₄)₂HPO₄ 0.05%, pH=6.0 prior to sterilization.

Medium E: Casein peptone 0.5%, meat peptone 0.5%, glucose H₂O 1% and maltose H₂O 1%, pH=5.4 prior to sterilization.

Medium F: Soluble starch 1%, maize starch 0.5%, glucose H₂O 1%, yeast extract 0.5%, corn steep 0.5% and CaCO₃ 0.2%; pH=7.0 prior to sterilization. Trace element concentrate contained CaCl₂·2H₂O 3 g, Fe-III-citrate 1 g, MnSO₄ 0.1 g, ZnCl₂ 0.1 g, CuSO₄·5H₂O 0.025 g, Na₂B₄O₇·10H₂O 0.05 g, H₂O 1000 ml.
0.02 g, CoCl₂ 0.004 g and NaMoO₄·2H₂O 0.01 g in 1 liter of deionized water.

Fermentation and Sample Preparation for TLC Screening
The strains were cultivated in 300 ml Erlenmeyer flasks each containing 100 ml of the production media on a rotary shaker at 180 rpm (Streptomyces strains at 28°C for 4 days in media A to C, Fungi imperfecti strains at 21°C for 11 days in media D to F). The culture broth was separated from the cells by centrifugation (4000 rpm, 10 minutes) and with 50 ml of the supernatant were loaded onto an Amberlite XAD-16 column (2.5X5 cm), washed with water (100 ml) and eluted with 50 ml of methanol-water (4:1). The eluate obtained was concentrated to dryness and dissolved in 1 ml of methanol-water (1:1).

Fermentation and Isolation of 5 and 6
Strain GT 46045 (Fungi imperfecti) was cultivated in a 200 liter stirred tank fermentor using the production medium F. The fermentor was inoculated with 5% (v/v) of shaking cultures grown for 48 hours in 300 ml Erlenmeyer flasks containing 100 ml of medium F at 180 rpm at 22°C. The fermentation was carried out at 22°C for 264 hours with an aeration rate of 0.5 v/v/minute and an agitation rate of 130 rpm. The culture filtrate was passed through an Amberlite XAD-16 column (12.5% resin volume relating to culture filtrate volume). The resin was washed with 90 liters of water and eluted with 90 liters of MeOH-water (4:1). After removing the organic solvent by evaporation under vacuum, the aqueous residue was extracted with 5 liters of ethyl acetate and the organic layer was evaporated to dryness. The obtained brown oil (7.8 g) was chromatographed on Sephadex LH-20 (6X100 cm) using MeOH as eluent to yield 1.8 g of a crude product which was further purified by preparative reversed-phase HPLC column using a stainless steel column (250X25 mm) filled with Licrosorb RP-18. Elution was performed with water-MeOH, starting with 5% MeOH over 5 minutes, followed by a linear gradient from 5% MeOH to 95% MeOH over 10 minutes and a 10 minutes hold at 95% MeOH at a flow rate of 20 ml/minute to yield 167 mg of 5 and 245 mg of 6.

(8S)-3-(2-Hydroxypropyl)-cyclohexanone (5)

$$\alpha^D_{20} = 36.0 \text{ (c 0.8, CHCl}_3)$$; m.p. 121-122°C; UV $\lambda_{max}^{MeOH}$ nm (c) 204 (2,100), 222 (2,000), 268 (500); IR (KBr) min⁻¹ 3520, 2975, 1706, 1445, 1394, 1350, 1206, 1062, 948; 1H NMR: (300 MHz, CD₃OD) $\delta$ 1.10 (m, 1H, 5-H), 1.32 (d, J=6.2 Hz, 3H, 3'-CH₃), 1.32 (m, 1H, 6-H), 1.53 (m, 1H, 4-H), 1.64 (m, 1H, 5-H), 1.75 (m, 1H, 6-H), 1.78 (m, 2H, 7-H₂), 2.10 (m, 1H, 4-H), 2.22 (m, 1H, 4a-H), 2.93 (dd, J=5.3, 5.3 Hz, 1H, 8a-H), 3.81 (m, 1H, 8-H), 4.51 (m, 1H, 3-H); 13C NMR (90.5 MHz, CD₂OD) $\delta$ 21.3 (q, 3'-CH₃), 22.2 (t, C-6), 32.2 (t, C-7), 32.8 (d, C-4a), 33.0 (t, C-5), 36.9 (t, C-4), 45.7 (d, C-8a), 70.6 (d, C-8), 76.2 (d, C-3), 176.6 (s, C-1); ESI-MS: $m/z = 185.1 (M+H)$; EI-MS: $m/z = 184.2113 (\Delta = +0.4 \text{ mmu}) = C_{10}H_{16}O_3 (M^+)$.}

Streptomyces strain GT 41006 was cultivated in a 150 liter fermentor at 300 rpm and 28°C in medium A for 4 days at an aeration rate of 0.5 v/v/minute. The culture filtrate was adsorbed on 25 liters of XAD-16 resin, which was then washed with 50 liters of water and eluted with 50 liters of MeOH-water (4:1). The eluate was concentrated to dryness and was further purified on a silica gel column (10X50 cm) by elution with ethyl acetate-MeOH (3:1) and Sephadex LH-20 (6X100 cm) with MeOH as eluent (twice) to yield 8.8 g of pure 7: $\alpha^D_{20} = 10.8 \text{ (c 1.1, 10.7, 11H, 2-H), 2.30 (m, 1H, 6-H), 2.35 (m, 1H, 2-H), 3.82 (m, 1H, 2'-H); }^{13}C \text{ NMR (90.5 MHz, CD}_2\text{OD)}: \delta 24.2 (q, C-3'), 26.4 (t, C-5), 31.8 (t, C-4), 37.3 (d, C-3), 42.2 (t, C-6), 46.9 (t, C-1'), 49.4 (t, C-2), 65.9 (d, C-2'), 214.0 (s, C-1); ESI-MS: $m/z = 157.0 (M+H)$; EI-MS: $m/z = 156.1145 (\Delta = +0.5 \text{ mmu}) = C_4H_7O_2 (M^+)$, 138.1045 (A=0.5 mmu)=$C_6H_5O (M^--CH_2O)$, 97.0658 (A=0.5 mmu)=$C_6H_4O (M^--C_3H_2O)$.

Esterification of 5 with (S)-(R)-α-Phenylbutyric Acid
12.2 mg (78.2 µmol) of 5 was dissolved in 10 ml of CH₂Cl₂ and 170 µl of (S)-α-phenylbutyric acid, 25 mg of dicyclohexylcarbodiimide and 30 mg of 4-dimethylaminopyridine was added. The mixture was stirred for 2 hours at room temperature and 10 ml of methanol was added. The solvent was evaporated and the remaining crude material was chromatographed on silica gel (column 30X2 cm, in ethyl acetate) to yield 8.1 mg (34%) of the (S)-phenylbutyric acid derivative of 5. In analogy, 12.0 mg of 5 was treated with (R)-α-phenylbutyric acid to yield 7.5 (32 %) of the R-derivative.}
Acid (9) (zl= -2.3 mmu)=C10H18O3 (M+).

4'), 82.9 (d, C-5), 180.1 (s, C-2); EI-MS: m/z=186.1279

purification was done by preparative RP-18 HPLC using
twice on Sephadex LH-20 (column 6X100cm, in MeOH; to yield 10.8 g of a brown oil which was chromatographed twice on Sephadex LH-20 (column 6×100 cm, in MeOH and in acetone) to yield 220 mg of pure colorless amorphous 9: [α]D 20 +15.4 (c 0.1, MeOH); m.p. 138–139°C; UV λ max nm (ε) 201 (1,800), 226 (4,600), 279 (1,800); UV λ max nm (ε) 209 (3,600), 225 (2,400), 279 (800); UV λ max nm (ε) 226 (4,000), 277 (1,400); IR (KBr) cm⁻¹ 3450, 2955, 1700, 1549, 1379, 1182, 1044; CD λ max nm (θ) 299 (11,270), 254 (+1,610), 216 (9,660); ¹H NMR: (500 MHz, CD₃OD) δ 0.85 (s, 3H, 30-H), 0.97 (s, 3H, 28-H), 1.01 (s, 3H, 29-H), 1.04 (s, 3H, 26-H), 1.11 (s, 3H, 25-H), 1.14 (m, 1H, 16-H), 1.17 (m, 1H, 15-H), 1.27 (s, 3H, 27-H), 1.29 (s, 3H, 23-H), 1.38 (m, 1H, 7-H), 1.38 (m, 1H, 9-H), 1.52 (m, 1H, 5-H), 1.52 (m, 1H, 7-H), 1.53 (m, 1H, 6-H), 1.61 (m, 1H, 6-H), 1.67 (m, 1H, 1-H), 1.77 (dd, J=14.2, 14.2, 4.3 Hz, 1H, 15-H), 1.91 (m, 1H, 9-H), 1.95 (m, 2H, 11-H), 1.98 (m, 1H, 21-H), 2.15 (m, 1H, 16-H), 2.20 (m, 1H, 1-H), 2.20 (m, 1H, 2-H), 2.20 (t, J=13.7 Hz, 1H, 19-H), 2.37 (dd, J=13.7, 3.5 Hz, 1H, 18-H), 2.45 (m, 1H, 2-H), 2.57 (d, J=24.2 Hz, 1H, 21-H), 3.48 (d, J=10.8 Hz, 1H, 24-H), 3.64 (d, J=10.8 Hz, 1H, 24-H), 5.35 (s br, 1H, 12-H); ¹³C NMR (90.5 MHz, CD₃OD): δ 17.5 (q, C-26), 20.7 (q, C-25), 21.1 (q, C-28), 21.8 (t, C-6), 24.5 (t, C-11), 25.5 (q, C-27), 25.6 (q, C-30), 25.9 (t, C-15), 26.2 (q, C-23), 28.4 (t, C-16), 31.6 (t, C-2), 32.2 (q, C-29), 33.2 (t, C-7), 35.1 (s, C-20), 36.3 (t, C-1), 40.2 (d, C-9), 40.7 (s, C-8), 41.9 (s, C-10), 43.8 (s, C-14), 47.5 (t, C-19), 49.2 (d, C-18), 49.1 (s, C-17), 51.0 (d, C-5), 51.7 (t, C-21), 69.0 (t, C-24), 77.9 (s, C-4), 125.0 (s, C-12), 142.0 (s, C-13), 177.1 (only by HMBC-Korrelation, C-3), 219.0 (s, C-22); ESI-MS: m/z=489.0 (M+H); EI-MS: m/z=470.4 (M−H₂O); FAB-MS: m/z=489.3612

Determination of Biological Activities

Antibiotic Activity

The antibiotic activity was tested by means of a standardized agar diffusion plate assay. The compounds were tested according to published procedures for their antibiotic activity against the following microorganisms: Bacillus subtilis ATCC 6633 (IMET 10880) NA, Staphylococcus aureus (IMET 10760) SG 511,
Escherichia coli SG 458, Pseudomonas aeruginosa SG 137 (IMET 10480), Pseudomonas aeruginosa K 799/61, 
Sporobolomyces salmonicolor SBUG 549, Candida albicans BMSY 212, Penicillium notatum JP 36, 
Mycobacterium phlei SG 346.

Inhibition of Xanthin Oxidase

The compounds were tested in a lucigenin-coupled cheniluminescence assay for their inhibitory activity on 
xanthine oxidase (SIGMA CHEMICAL Co.) with allopurinol used as standard39).

Cytotoxic Porperties

The testing for cytotoxic and antiproliferative activities 
against L-929 (mouse fibroblasts), K562 (human leukemia) 
and HeLa (human cervix carcinoma) was performed 
according to standard protocols38).

Antiviral Activity

The antiviral activity against coxsackie virus B3 (strain 
Nancy), influenza virus A and herpes simplex virus type I 
was tested by inhibition of virus-induced cytopathic effects 
(zpE) on HeLa, MDCK and GMK cells, respectively40.

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