Activities of Prenylphenol Derivatives from Fruitbodies of Albatrellus spp. on the Human and Rat Vanilloid Receptor 1 (VR1...
Activities of Prenylphenol Derivatives from Fruitbodies of *Albatrellus* spp. on the Human and Rat Vanilloid Receptor 1 (VR1) and Characterisation of the Novel Natural Product, Confluentin

Several prenylphenols from basidiocarps of European and Chinese *Albatrellus* spp., namely grifolin (1), neogrigolin (2), confluentin (3), scutigeral (4), and albaconol (5) were investigated concerning their activities in test models for vanilloid receptor modulation. The isolation of these compounds from *A. confluens* and structure elucidation of the novel natural product confluentin (3) are described. The effects of scutigeral and neogrigolin on vanilloid receptors were studied by means of electrophysiological methodology on rat dorsal root ganglion neurons as well as on recombinant cell lines expressing the rat VR1 receptor. Concurrently, the effects of compounds 1–5 on a reporter cell line expressing the human vanilloid receptor VR1 were measured. In contrast to previous studies reported in the literature, the results of these investigations suggest that fungal prenylphenols act as weak antagonists (activity in the µM range), rather than exhibiting agonistic activities.

**Keywords:** Basidiomycetes; Pain; Scutigeral; Neogrigolin; Grifolin; Albaconol

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Introduction

The fruitbodies of Basidiomycetes of the mycorrhizal cosmopolitan genus *Albatrellus* S.F. Gray 1821 appear during late summer and autumn, preferably in mountainous conifer forests of temperate and boreal climates. Five species of *Albatrellus* are listed (as *Scutiger* spp.) in the most recent mycofloristic review of Western Germany [1]. The nomenclature and systematic position of these fungi is still being disputed. Stalpers [2] placed the genus into a family named Scutigeraceae within the Cantharellales. According to the current edition of the *Dictionary of the Fungi* and concurrent information on the Internet, it is included within the Albatrellaceae (Polyporales) [3, 4]. As the identity of *Scutiger* is considered dubious by Ryvarden [5], the generic name *Albatrellus* is preferred in the present paper.

A recent study on CNS activities of extracts from Basidiomycetes revealed that extracts from *A. ovinus* showed strong inhibition of the dopamine D1 receptor, and several prenylphenols were isolated as active principles [6]. Many of these compounds had already been previously identified as characteristic components of *Albatrellus* spp. [7–9]. Later, the main metabolic obtained in the course of the aforementioned studies, scutigeral (4), was subjected to a broad characterisation of CNS receptor activities, and interesting effects on the vanilloid receptor were reported [10]. In the course of the same study, minor components of the extract such as grifolin (1) and neogrigolin (2) had been isolated and tested. During preliminary experiments, these congeners exhibited activities similar to those observed with scutigeral (4), but they were not available in sufficient quantities for intensified characterisation. This was accomplished in the present study [11]. Moreover, we included albaconol (5), a novel structurally and biogenetically interesting cyclic derivative of these compounds, which was recently obtained from a Chinese *Albatrellus* sp. referred to as *A. confluens* [12].

In the following, we wish to report the isolation and structure elucidation of confluentin (3), a novel natural prenylphenol from specimens of *A. confluens* collected in Germany and the results of our studies on the biological effects of compounds 1–5 in electrophysiological and reporter cell test models.

Results and discussion

Isolation and identification of fungal metabolites

**Grifolin and neogrigolin**

Two main components 1 and 2 were isolated pure from the crude extract of *A. confluens*, (see Experimental).

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Figure 1. Chemical structures of prenylphenols 1–5 from Albatrellus sp.

Table 1. $^1$H and $^{13}$C NMR data [400/100 MHz] of grifolin (1), neogrin (2), and confluentin (3) in [D$_6$]DMSO.

<table>
<thead>
<tr>
<th>C-atom</th>
<th>$\delta$$_C$ [ppm]$^a$</th>
<th>$\delta$$_C$ [ppm]$^b$</th>
<th>$\delta$$_C$ [ppm]$^a$</th>
<th>H-atom</th>
<th>$\delta$$_H$ [ppm]</th>
<th>$J_{HH}$ [Hz]</th>
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<tr>
<td>C-1</td>
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<td>152.9</td>
<td>1-OH</td>
<td>9.38</td>
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<td>116.6</td>
<td>106.2</td>
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<tr>
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<td>155.6</td>
<td>153.6</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>C-4</td>
<td>106.7</td>
<td>100.0</td>
<td>107.6</td>
<td>4-H</td>
<td>6.03</td>
<td>s</td>
</tr>
<tr>
<td>C-5</td>
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<td>155.5</td>
<td>138.8</td>
<td></td>
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<tr>
<td>C-6</td>
<td>106.7</td>
<td>107.9</td>
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<td>6-H</td>
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<tr>
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<td>117.3</td>
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<td>124.2</td>
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<td>5.03</td>
<td>m</td>
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<tr>
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<td>15.3</td>
<td>14'-CH$_3$</td>
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<td>15'-CH$_3$</td>
<td>1.27</td>
<td></td>
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</table>

$^a$ The $^{13}$C NMR shifts are deduced from the HSQC and HMBC correlation spectra.

$^b$ Signals may be interchanged.
Comparing the NMR spectroscopic properties (Table 1) of grifolin (1) and neogri- 
folin (2), and are included here for comparison with those of the novel con- 
gen, confluentin (3).

Structure elucidation of confluentin
A minor component 3 co-eluted during preparative HPLC with metabolites 1 and 2. The ESI-LC-MS of 3 revealed a [M + H]+ ion peak at m/z 329 corresponding to the molecular formula C22H32O2 as deduced from HR-EI-MS. Accordingly, compound 3 is a formal dehydro derivative of grifolin (1) and neogri- 
folin (2).

Comparing the NMR spectroscopic properties (Table 1) of 3 with those of grifolin (1) and neogri- 
folin (2), the main features of 3 are the disappearance of one of the phenolic OH protons as well as of one of the vinyl protons and one of the vinyl methyl signals in the farnesyl side chain. Instead of these signals two additional doublets for olefinic protons with JHH = 9.9 Hz according to a cis double bond and an additional signal accounting for a terti- 
arly methyl group in the high field region of the 1H NMR were observed. Therefore, the structural formula 3 with a 2H-chromenol system and a geranyl side chain [14] was deduced for the new compound, which we named con- 
flu entin. The most important HMBC correlation signals supporting the constitution of 3 are given in Figure 2. The NOESY experiment was in agreement with the given substitution pattern in the aromatic nucleus (see Figure 3).

Conflu entin (3) was detected in the native crude extract of A. confluens, prepared from specimens which had been freeze-dried immediately after collection. Therefore it should not be an isolation artefact, but may be derived biogenetically. However, conflu entin (3) showed no optical activity, possibly indicating a non-enzymatic formation reaction or an equilibrium between the two epimers [15].

Formally, conflu entin (3) constitutes the dehydration product of two farnesylphenols with 2H-chromenol core, which were described in literature as constituents of Polyporus (syn: Albatrellus) dispansus [16]. Therefore, preliminary investigations on the occurrence of these and other compounds were carried out by analytical HPLC methodology in an attempt to detect further con- 
geners that might be interesting to include in the biologi- 

c evaluation. Neither these compounds nor confluentin (3) were detected in extracts of A. ovinus and A. pescaprae collected in Germany, both of which contained grifolin (1), neogri- 
folin (2), and scutigeral (4) as main metab- 

toles, according to HPLC analyses of crude extracts. Moreover, neither albacanol (5) nor any other main metab- 

toles with the expected molecular weights of 344 (C22H30O2), corresponding to the metabolites described from Japanese Albatrellus spp. [17, 18] were detected by the employed HPLC-MS and HPLC-UV methodology in the investigated fungi.

The references cited in the introductory section and an- 
other recent report of further novel metabolites of this structural type from A. ovinus collected in Japan [19] indicate that there is a great variety of prenylphenols to be encountered in this fungal genus. The production of grif- 
olin (1), scutigeral (4), and related metabolites appears to be a common feature of Albatrellus spp. All previous reports on these compounds from other fungal genera such as “Polyporus” and “Grifola” were based on outdated taxonomic literature. They most probably dealt with fungi that belong to Albatrellus, according to the currently valid taxonomy. Remarkably, Agerer et al. [9] had detected thelephoric acid or a metabolite with a similar UV spectrum during HPLC-based studies besides the typi- 
cal farnesylphenols in the course of their investigations of Albatrellus species and discussed its possible affini- 

ties to the Thelephorales. A chemotaxonomical study re- 
lying on a significant number of specimens appears promising to verify whether there are species-specific differences in the metabolite profiles within the genus Al- 
batrellus in Asia, Europe and other localities. However, this was beyond the scope of the present study.

Biological characterisation of prenylphenols

Electrophysiology

Scutigeral (4) had been reported to induce a dose-de- 

dependent 45Ca uptake in adult rat DRG neurons (IC50 =
0.16 ± 0.3 μM) which was blockable by the competitive vanilloid receptor VR1 antagonist capsazepine [10]. However, in the current study, no scutigeral-induced inward currents were observed under voltage-clamp conditions in these cells. Instead, 5 μM scutigeral (4) significantly decreased the proportion of DRG neurons that responded to capsaicin. In those neurons that maintained the responsiveness, it delayed the maximal current amplitude by 72 s. It was therefore discussed by Szallasi et al. [10] that scutigeral (4) gates the channel with prolonged kinetics as compared to capsaicin, thereby inducing a long lasting desensitisation of VR channels.

The goal of our studies was to compare the effects of scutigeral (4) and neogrifolin (2), the latter of which exhibited five-fold stronger activities in binding assays, on cloned rat VR1 receptors as well as on native vanilloid receptors expressed in rat primary sensory neurons. In the voltage clamp configuration, inward currents induced by repetitive capsaicin stimulation were recorded.

Figure 4. Effects of scutigeral (4; 0.5–5 μM, A–D) and neogrifolin (2; 0.5 μM, E, F) on capsaicin-induced currents from DRG-neurons (A, B) and VR1 cells (C–F).
by measuring vanilloid receptor activity (blockable by the competitive antagonist capsazepine, data not shown). Overall, 20 whole cell patch clamp recordings were performed on recombinant rat VR1 cells and rat DRG neurons. Experimental conditions were varied with respect to concentration (scutigeral (4): 0.5–5 µM, neogrolin (2): 0.1–1 µM) and application time (10–120 s). In accordance with the literature, neither scutigeral (4, n = 10) nor neogrolin (2, n = 10) elicited a detectable inward current on either cell type. Furthermore, neither compound delayed the responses of either cell type to subsequent capsaicin administration. Instead, in 70% of the recordings a remarkable effect on tachyphylaxis to capsaicin was observed. This phenomenon (repeated application of capsaicin leads to constantly decreasing current amplitudes) requires external calcium and is therefore reduced or even prevented under Ca-free conditions (normal calcium levels), the observed acceleration in tachyphylaxis to capsaicin was observed. This phenomenon (repeated application of capsaicin leads to constantly decreasing current amplitudes) requires external calcium and is therefore reduced or even prevented under Ca-free conditions. However, after application of scutigeral (4) or neogrolin (2) in concentrations ≥0.5 µM, tachyphylaxis started again even in the absence of external calcium (examples are shown in Figure 4).

If this effect were to be seen even under physiological conditions (normal calcium levels), the observed acceleration in tachyphylaxis might have the same net effect as the reduction of the neurons’ responsiveness to further capsaicin stimulation, as described by Szallasi et al. [10]. In either case the challenge with the fungal vanilloids reduces the excitability of the VR receptor expressing neuron.

**Biological activity in cell based bioluminescent assay**

To clarify the biological activity of the fungal compounds, parental CHO cells which stably express apoaequorin were used to establish a cell line which stably coexpresses hVR1. Stimulation of these cells with the VR1 agonists capsaicin, olvanil, and anandamide led to an activation of the receptor, resulting in a fast influx of calcium within 60 s after compound application, inducing aequorin luminescence peaking after 20 s. Agonist-induced responses were dose-dependent with EC50 values of 90 ± 45 nM for capsaicin (n = 6), 127 ± 44 nM for olvanil (n = 3), and 18.560 ± 4.760 nM for anandamide.

Capsazepine antagonised the response to a 100 nM capsaicin stimulus in a dose-dependent manner with an IC50 of 271 ± 126 nM (n = 3). No responses to agonists or antagonists were detected in the non-transfected parental CHO cells. Under identical experimental conditions none of the fungal metabolites (1–5) elicited any calcium influx into the recombinant cells in concentrations up to 50 µM within the first 60 s after application.

To check whether the lack of agonist-like activity might be the result of delayed kinetics of activation, exposure time was prolonged to 9 min. Even in this timeframe, no increase in intracellular calcium concentration was detected in this highly sensitive assay, suggesting that none of the compounds exhibited agonistic activity on the human VR1 receptor.

In accordance with the present study, the lack of activities of scutigeral (4) on hVR1 has been recently reported [20]. Since the inhibitory effects of the fungal vanilloids could be either the result of VR1 desensitisation or might in contrast be the result of antagonistic influence, the compounds were tested for antagonist-like properties in the VR1 assay. Indeed, grifolin (1), neogrolin (2), and albaconol (5) were able to antagonise the capsaicin-induced calcium response (100 nM) in a dose-dependent manner, with IC50 = 30.0 ± 3.5 µM ([1], n = 2), 7.1 ± 0.5 µM ([2], n = 2) and 17.0 ± 2.0 µM ([5], n = 2). Results for compounds 1 and 2 are shown in Figure 5. Neither confluentin (3) nor scutigeral (4) displayed any antagonist-like activity up to 50 µM (Table 2). Albaconol (5) showed no agonistic effects on human and rat VR1 and its antagonistic effects on both receptor orthologs were rather weak (IC50 = 17 µM in the human VR1 and 5.5 µM in the rat VR1), i.e. in about the same range as determined for compounds 1 and 2.

**Table 2. Biological activities of reference compounds and fungal metabolites 1–5 determined in a cell based bioluminescent assay.**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Agonistic activity EC50/nM</th>
<th>Antagonistic activity IC50/nM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capsaicin</td>
<td>90 + 45 (n = 6)</td>
<td>_b</td>
</tr>
<tr>
<td>Olvanil</td>
<td>127 + 44 (n = 3)</td>
<td>_</td>
</tr>
<tr>
<td>Anandamide</td>
<td>18.560 + 4.760 (n = 2)</td>
<td>_</td>
</tr>
<tr>
<td>Capsazepine</td>
<td>_</td>
<td>271 ± 126 (n = 3)</td>
</tr>
<tr>
<td>Grifolin (1)</td>
<td>_</td>
<td>25.975 ± 3455 (n = 2)</td>
</tr>
<tr>
<td>Neogrolin (2)</td>
<td>_</td>
<td>7052 + 507 (n = 2)</td>
</tr>
<tr>
<td>Confluentin (3)</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>Scutigeral (4)</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>Albaconol (5)</td>
<td>_</td>
<td>16.800 + 1800 (n = 2)</td>
</tr>
</tbody>
</table>

_b inactive up to >50 µM
Figure 5. Influence of grifolin (1) and neogrifolin (2) on the capsaicin-induced calcium response in CHO cells transfected with the VR1 receptor.

Taken together, the results obtained by electrophysiological recordings and the VR1 luminescence assay clearly demonstrated that the tested fungal vanilloids displayed no agonist-like activity. Furthermore, the antagonistic activity of grifolin (1) and neogrifolin (2) pointed toward a direct inhibitory effect of these compounds rather than a desensitising mode of action.

Besides the CNS activities (modulation of vanilloid and dopamine D1 receptors) mentioned in the introductory section, grifolin (1), scutigeral (4), and further chemically related metabolites also showed various other activities in biological systems. For instance, they were previously published as phytotoxic agents [17], as tyrosinase inhibitors [18], and as antioxidants [19].

Acknowledgements

We gratefully acknowledge the co-operation of Prof. Dr. W. Steglich (Ludwig-Maximilians-Universität Munich, Germany), who kindly provided an authentic sample of scutigeral. We wish to thank Mrs. I. Höschler, Mr. O. Mertens, Mrs. S. Reddig, Mrs. H. Wieland, and Mrs. C. Wotsch for expert technical assistance. Furthermore, the work of Dr. P. Schmitt, Mr. K. Schmidt, and Dipl. Ing. H. Musche, who recorded NMR and MS spectra, is gratefully acknowledged.

Experimental

General

If not indicated otherwise, all chemicals and solvents were obtained from Sigma-Aldrich, Deisenhofen, Germany.

Biological assays

Electrophysiology

Preparation of rat dorsal root ganglion cells: Dorsal root ganglia of male Wistar rats (120–180 g) were dissected aseptically, incubated in 4 mL collagenase, 1.25 mg/mL DMEM for 90 min and afterwards in 4 mL 0.25 % trypsin-EDTA solution for 30 min. After centrifugation (2000 rpm, 10 min, addition of horse serum) the pellet was resuspended in Nutrient Mixture HAM’s F12 with 10 % horse serum, 10 mM L-glutamine, 40 mM glucose, triturated through fire-polished Pasteur pipettes and plated onto glass cover slips previously coated with polyornithine and laminin. 2 h later 200 ng/mL NGF was added. Cells were kept and used for recordings for 5 days.

Recordings: All voltage clamp experiments were performed at room temperature using an EPC 9 amplifier controlled via TIDA software (HEKA Electronik, Lambrecht, Germany). Whole cell recordings were performed either from CHO cells stably transfected with rat VR1 receptor or from cultivated rat DRG neurons, respectively. In order to prevent tachyphylaxis of currents upon repetitive capsaicin stimulation, external calcium was omitted. After recording of 3–4 consecutive capsaicin induced currents with no or minor desensitisation, either scutigeral [4, (0.5–5 µM, in 0.01 % DMSO)] or neogrifolin [2, (0.1–1 µM, in 0.01 % DMSO)] were applied via the U-tube for at least 10 s and maximal 120 s. Thereafter, repetitive capsaicin stimuli were continued. Effects of scutigeral (4) and neogrifolin (2) per se and on following capsaicin induced currents were analysed.

Cell based bioluminescent assay

A recombinant CHO cell line stably expressing apoaequorin and the human VR1 was kindly provided by Bayer Milan (Milano, Italy). Cells were seeded into 96 well plates (10,000 cells/well) and cultivated for 24 h at 37 °C/5 % CO2 in MEM alpha medium + Glutamax (Gibco, Karlsruhe, Germany) containing 10 % inactivated FCS, 0.5 mg/mL genetin. After 24 h, medium was replaced by 50 µL tyrode (130 mM NaCl, 0 mM MgCl2, 5 mM NaHCO3, 20 mM HEPES, 1 mM CaCl2, pH 7.4) containing 1/5.000 coelenteracin (50 µM in 100 % DMSO). After 4 h, 50 µL compound (diluted in tyrode) was added and the luminescence detected immediately in a luminescence camera (Bayer). 10 min after compound application, cells were stimulated by adding 50 µL 100 nM capsaicin in the camera and luminescence recorded immediately. Data analysis was performed by means of GraphPad Prism software (Innotech, Schönaich, Germany).

Biological materials, isolation and physicochemical characterisation

Specimens and pure natural compounds investigated

Basidiocarps of Albatrellus confluens (Abt. & Schw. ex Fr.) Kottl. & Pouz. were collected near Pinus sylvestris in the vicinity of Trippstadt, Rheinland-Pfalz, Germany, in September of 1997, while those of A. pes-caprae (Pers.) Pouzar were found in a mixed forest near Picea sp. in the vicinity of Kaiserslautern.
Mölschbach, Rheinland-Pfalz, Germany, in October of 1995. The specimens of A. ovinus (Schaeff. ex Fr.) Koll. & Pouz. were encountered in October 2000 near Johanniskreuz, Rheinland-Pfalz, Germany, in a mixed softwood forest of Pseudotsuga sp. and Picea abies. All fruitbodies were frozen to −20 °C immediately after collection, brought to the laboratory in frozen state and freeze-dried. Voucher specimens, which corresponded to the description of the genus and species according to [21], are kept in the personal herbarium of M.S. A sample of scutigeral (4), isolated previously from A. ovinus, was kindly provided by Prof. W. Steglich (LMU Munich, Germany). Albaconol (5) was obtained as reported recently from a Chinese Albatrellus sp. referred to as A. confluentin [10]. The specimen had been collected in July 1999 in the vicinity of unidentified conifers in Wudin County, Yunnan Province, PR. China [12]. For the physicochemical data of compounds 1, 2 (see also Experimental and Table 1), 4, and 5, the reader is referred to the aforementioned publications.

Analytical/Chromatography
HPLC-UV/Vis analyses were performed using a HP 1100 with automated injection and diode array detection (System 1): Column: Merck (Darmstadt, Germany) LiChroSpher C18; 125 × 4 mm; 5 μm; mobile phase 0.01 % phosphoric acid/ acetonitrile; flow: 1 mL/min; linear gradient from 0–100 % acetonitrile in 9 min, followed by isocratic elution at 90 % acetonitrile; temperature: 50 °C. MS parameters: EI-MS and HR-MS were performed with a MAT 900S mass spectrometer (Finnigan, Bremen, Germany), 70 eV, CI: CHCl3. NMR: δ(H) and δ(C) spectra were recorded at 300 K with a Bruker DRX 400 spectrometer. Optical rotation was determined with a Perkin-Elmer 341 (Rodgau-Jügesheim, Germany) polarimeter using a sodium lamp and a cell with a length of 1 dm. The temperature and concentration in g/100 mL of the solvent are given.

Extraction and isolation of compounds 1–3
One gram of the freeze-dried fruitbody materials of Albatrellus confluentin was extracted twice with 300 mL methanol in an ultrasonic bath for 30 min each. The extracts were pooled, and the solvent was evaporated in vacuo to yield 214 mg of an oily crude product. This crude product was dissolved in 3 mL of methanol and filtered through a Baker (Deventer, The Netherlands) C18 Bond Elut 3 mL solid phase extraction column immediately before preparative HPLC. Compounds 1–3 were isolated by preparative HPLC (Hardware Gilson Abimed (Langenfeld, Germany) HPLC UV detector at 210 nm, binary pump system; Software Gilson Unipoint 1.71; Flow 7 mL/min; Mobile phase 0.1 % trifluoroacetic acid:acetonitrile; Stationary phase Merck LiChroSorb RP-18 (7 μm, 250 × 25 mm); Gradient: starting with t = 0 min (60 % acetonitrile); linear to t = 30 min (80 % acetonitrile); isocratic to t = 60 min (80 % acetonitrile); followed by linear gradient to t = 80 (100 % acetonitrile). Rf were determined as follows: (1): 47–52 min, (2): 54–59 min; (3): 76–79 min. This isolation procedure yielded approximately 55 mg of grifolin (1), 33 mg of neogrinolin (2), and 4 mg of confluentin (3).

Physico-chemical data
Grifolin (1)
Colourless solid. – HPLC-UV: Rf = 10.07 min (System 1); – 1H NMR: see [7, 13]. – 13C NMR (100 MHz, [D6]DMSO): see Table 1. – LC-MS: Rt = 5.5 min (System 2); ESI pos.: m/z (%) = 330 (17) [M + 2H]+, 329 (69) [M + H]+, 273 (6), 261 (4), 247 (2), 233 (3), 219 (2), 205 (19), 191 (7), 179 (19), 178 (80), 177 (15), 165 (2), 151 (15).

Neogrinolin (2)
Colourless solid. – HPLC-UV: Rf = 10.37 min (System 1); – 1H NMR: see [7]. – 13C NMR (100 MHz, [D6]DMSO): see Table 1. – LC-MS: Rf = 5.7 min (System 2); ESI pos.: m/z (%) = 330 (25) [M + 2H]+, 329 (100) [M + H]+, 273 (6), 205 (10), 179 (12), 178 (100), 177 (8), 151 (3).

Confluentin (3)
Colourless solid. – HPLC: Rf = 10.89 min (System 1). [α]D20 = +0.1 (c = 0.7, CHCl3). – 1H NMR (400 MHz, [D6]DMSO): see Table 1. – 13C NMR (100 MHz, [D6]DMSO): see Table 1. – LC-MS: Rf = 6.1 min (system 2); ESI pos.: m/z (%) = 328 (24) [M + 2H]+, 327 (100) [M + H]+, 271 (10), 245 (18), 231 (14), 217 (10), 203 (3), 191 (14), 178 (24), 177 (84), 175 (19). – EI-MS: m/z (%) = 326 (10) [M]+, 176 (17), 175 (100). – C19H19O2: calcld. 326.2246, found 326.2234.

References
[11] The sample of scutigeral was freshly reisolated for the studies and kindly provided by N.Feling (Prof. Steglich, Univ. Munich), and obtained in the course of her Ph. D. Thesis, University Munich, 2000.


