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Noduliprevenone: A Novel Heterodimeric Chromanone with Cancer Chemopreventive Potential

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Chemoprevention is considered a new strategy in fighting diverse types of cancer. To influence cancer development and progression, signaling pathways,[1] for example, enzymes like cyclooxygenase-2,[2] transcription factors like NF-κB,[3] and several metabolic processes leading to activation or deactivation of carcinogenic agents, are envisaged as promising approaches.[4,5]

Cytochrome P450 (CYP) enzymes activate xenobiotics and thus contribute to the generation of potent carcinogens. Accordingly, inhibition of cytochrome P450 isoforms, such as CYP1A enzymes, which play an important role in polycyclic aromatic hydrocarbon (PAH) induced cancer forms, is advantageous for chemoprevention.[6] In contrast, the induction of phase II enzymes inactivates carcinogens and accelerates their renal or biliar elimination, forming conjugates with polar ligands, such as glutathione, glucuronic acid, and acetic or sulfuric acid.[7] NAD(P)H:quinone reductase (QR) contributes in detoxification by catalysing the two-electron-accepted reduction of quinones to their corresponding, low-mutagenic hydroquinones and is therefore often used as biomarker.[8] Accordingly, the aim of cancer chemopreventive strategy can include the inhibition of phase I enzymes accompanied with an increased phase II metabolism.

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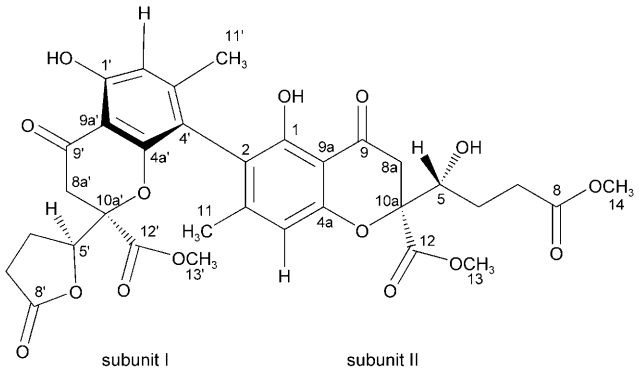
The fungus *Nodulisporium* sp. is an endophyte of a Mediterranean alga, and produces the novel polyketide noduliprevenone (**1**), an unprecedented structure consisting of two



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uniquely modified xanthone-derived units. The absolute configuration of compound **1**, including four chiral centres and a chiral axis, was assigned using CD-spectroscopy, Mosher's method and selective NOE gradient measurements. Compound **1** was identified as a competitive inhibitor of cytochrome P450 1A activity with an IC_{50} value 6.5 ± 1.6 μM and induces at the same time twofold NAD(P)H:quinone reductase (QR) activity in Hepa 1c1c7 mouse culture cells with a concentration of 5.3 ± 1.1 μM.

The molecular formula of compound **1** was deduced by accurate mass measurement (HRESIMS) to be $C_{33}H_{34}O_{15}$, implying 17 degrees of unsaturation. UV absorption maxima at 279 and 353 nm gave proof for an extended aromatic system. The ^{13}C NMR spectrum depicted 33 resonances, 32 of which could be recognised as pairs with close to identical chemical shifts, indicating a heterodimeric structure with almost the same substitution pattern of the subunits (Table S1 in the Supporting Information). Each subunit includes three carbonyl groups and six sp^2 carbons, the latter forming the aromatic nucleus. Two of the aromatic carbon

resonances were characterised by a downfield shift due to substitution with oxygen (δ_C=161.7, C-1′/159.5 ppm, C-1; δ_C=157.8, C-4a′/159.8 ppm, C-4a). The ^{1}H NMR spectrum contained two typical signals for chelated hydroxyl groups (δ_H=11.47 ppm, OH-1′; δ_H=11.94 ppm, OH-1) characteristic for the presence of phenolic moieties adjacent to carbonyl groups (δ_C=195.4 ppm, C-9′; δ_C=197.7 ppm, C-9). Since only one uncoupled aromatic proton resonance (δ_H= 6.49 ppm, CH-2′; δ_H=6.43 ppm, CH-4) was present on each aromatic system, the phenyl rings had to be fivefold substituted. From the data of ^{1}H-^{13}C HMBC experiments a heterodimeric chromanone structure was deduced (Figure S1 in the Supporting Information). The architecture of subunit I was clarified by HMBC correlations of H_2-8a′ to C-9a′, H-2′ to C-9′ and OH-1′ to C-9′. The substitution pattern of the aromatic moiety was deduced on the basis of HMBC couplings and supported by the NOESY cross-peaks from both OH-1′ and CH_3-11′ to CH-2′ proving their *meta* position to each other. The chromanone heterocycle of subunit II was assigned by HMBC correlations of H_2-8a to C-9a, H-4 to C-9, CH_3-11 to C-4a and OH-1 to C-9. In contrast to subunit I, here NOESY cross-peaks were only observed between the vicinal residues CH_3-11 and CH-4, proving that both benzene moieties are not identically substituted. Taking all information of spectroscopic analyses into account, the subunits were linked asymmetrically through the remaining quaternary carbons C-4′ of subunit I and C-2 of subunit II. According to the degree of unsaturation one more ring had to be present in **1**. The ^{1}H-^{1}H COSY experiment revealed two aliphatic spin systems due to couplings between H-5′, H_2-6′ and H_2-7′, as well as from OH-5 through to H_2-7. Long-range HMBC correlations between H_2-6′, H_2-7′ and the carbonyl group C-8′ (δ_C=176.0 ppm), but also between H-5′ and C-8′ indicated a γ-lactone ring in subunit I. HMBC correlations of H_2-6 and H_2-7 to the carbonyl group C-8 (δ_C=173.8 ppm) in addition to the correlation of the methoxyl group CH_3-14 (δ_H=3.63 ppm) to C-8 suggested a 4-hydroxy-butyric acid methyl ester moiety for subunit II. The connection of the lactone ring to C-10a′ followed from the HMBC cross-peaks between H-5′ and C-4a′, C-8a′ as well as C-10a′, while the ester chain was found to be bonded to C-10a because of cross-peaks between H-5 to both C-8a and C-10a. Further HMBC correlations between the methoxyl groups CH_3-13′ (δ_H=3.70 ppm), CH_3-13 (δ_H=3.77 ppm) and the corresponding carbonyl groups C-12′ (δ_C=169.8 ppm), C-12 (δ_C=170.9 ppm) evidenced two carboxymethyl functionalities. Correlations of H_2-8a′ to C-12′ and H_2-8a to C-12 positioned these ester groups at the quaternary carbons 10a′ and 10a, respectively.

Compound **1** has a chiral axis between C-4′and C-2 as well as four chiral centres. The absolute configuration of the chiral axis was determined by using CD spectroscopy. The CD spectrum of **1** was compared with that of the reference molecule (*M*)-orsellinic acid camphanate, the absolute configuration of which was established by X-ray crystallographic studies (Figure S2 in the Supporting Information).[9] Sector rule lines depicted for the ^1L_b band show that the substitution pattern for both molecules is comparable.[10,11] The nearly congruent CD curves with the same sign for the ^1L_a and ^1L_b band CD suggested the *M* absolute configuration for compound **1** (Figure S2 in the Supporting Information).

NOE enhancements observed for the ^{1}H NMR resonance of H-8aα′ upon irradiation of the H-5′ and CH_3-13′ resonances indicated that the methylene proton H-8aα′, the carboxymethyl group and H-5′ were located on the same side of the molecule with proton 8aα′ and the carboxymethyl group in pseudo-axial positions (Figure 1, and Figure S3 in the Supporting Information). Accordingly, as expected for bulkier moieties the lactone ring must have pseudo-equatorial orientation. This was confirmed by a further NOE correlation between the lactone methylene group H_2-6′ and H-8aβ′. For subunit II irradiation of the resonance for H-5 caused enhancements of H-8aα and CH_3-13, suggesting the same orientation of the carboxymethyl group, H-5 and H-8aα, with the ester chain in a pseudo-equatorial position (Figure S3 in the Supporting Information). Consequently, the relative configuration of the chiral centres was deduced as 5′*R**, 10a′*S** and 5*R**, 10a*S**, respectively. Furthermore, two important NOESY correlations between CH_3-13′ and CH_3-11, as well as between H-7′α and 1-OH allowed us to deduce, in conjunction with the *M*-configuration of the chiral axis, the absolute configuration of both chiral carbons of subunit I as 5′*S* and 10a′*R* (Figure 2 and Figure S4 in the Supporting Information). By using a modified Mosher's method, the absolute configuration of the secondary alcohol group at the chiral centre C-5 was assigned as 5*R* (Figure S5 in the Supporting Information).[12] Taking into account the relative configuration deduced from NOE measurements the absolute configuration of subunit II was assigned as 5*R* and 10a*S*. We propose the trivial name noduliprevenone for compound **1**.

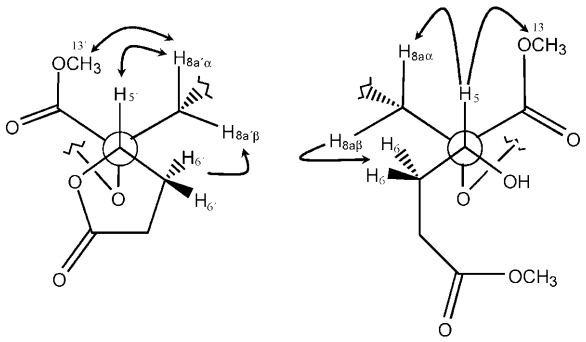

Figure 1. Selective gradient NOEs (black arrows) of both monomeric subunits depicted as Newman projections (along the 5′-10a′/5-10a axis, respectively).

Biosynthetically, noduliprevenone (**1**) is an octaketide presumably derived from anthraquinone and xanthone precursor molecules.[13] The chromanone basic structures of **1** are supposedly formed through a ring cleavage of the xan-

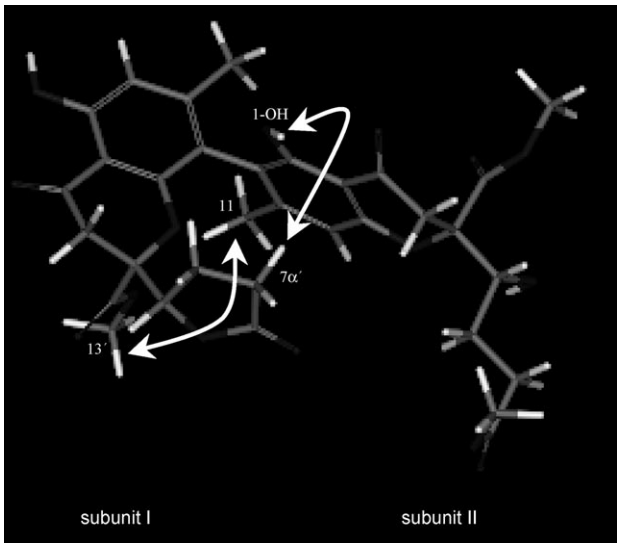

Figure 2. Selective gradient NOEs (white arrows) between the two subunits of **1**. The preferred conformation was calculated using Cerius2 4.0 (MSI) molecular modeling software package.

thone nucleus, finally creating the lactone and acyclic methyl ester chain of subunits I and II as shown in Figure S6 in the Supporting Information.[14] Chromanone-type structures substituted with a butanolide ring (called ergochrome F unit) are extremely rare and have only been observed in a few fungal derived compounds, that is, xanthoquinodin A3 and B3,[14] ergoxanthin,[15] chaetomanone,[16] and the lachnones 3, 4 and 5[17] (Figure S7 in the Supporting Information). Substitution with an acyclic hydroxybutanoic methyl ester is unprecedented. Also, noduliprevenone is unique in being the first heterodimeric compound that incorporates two such unusually modified chromanone units. The connection of the subunits probably resulted from oxidative phenol coupling, involving two different activated sites of the aromatic rings. Moreover, the linkage of the subunits must be stereoselectively controlled, thus leading to the *M* conformer of **1**.

The chemopreventive potential of compound **1** was investigated applying two phase I and II metabolic enzymes. First, the influence on the initiation of cancer development was analysed by performing a CYP1A inhibition assay. Noduliprevenone (**1**) was identified as an inhibitor of CYP1A activity in vitro, with an IC_{50} value of 6.5 ± 1.6 μM ($n=3$; Figure 3A). The compound demonstrated competitive inhibition with respect to the substrate 3-cyano-7-ethoxycoumarin, determined by Dixon– and Cornish–Bowden plots (Figure 3B). Competitive inhibition was also reflected by a decrease in IC_{50} values obtained with decreasing substrate concentrations (data not shown). Otherwise, after a 48 h treatment period, noduliprevenone (**1**) was found to induce the twofold specific activity of QR in a dose-dependent manner with a concentration of 5.3 ± 1.1 μM ($n=3$) (Figure 3C) without evident cytotoxicity at concentrations below 25 μM.

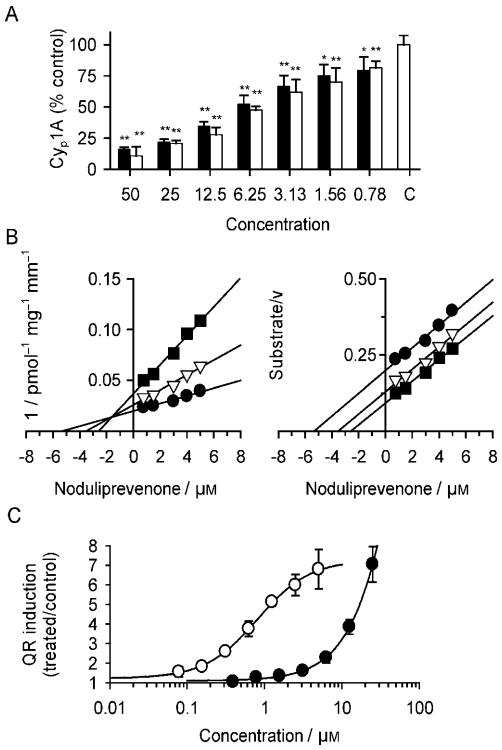

Figure 3. A) Dose-dependent inhibition of CYP1A enzymatic activity by **1** (black bars, μM scale) in comparison with α-naphthoflavone (white bars, nM scale), respectively. Means significantly different from control (C) (*$P<0.01$, **$P<0.001$). B) Competitive inhibition of CYP1A activity by **1**. Dixon (left) and Cornish Bowden plot (right) of the results of kinetic experiments using 2.5 μM (■), 5 μM (▽), and 10 μM (●) CEC, respectively, as a substrate. C) Induction of QR activity by **1** (●) in comparison with sulforaphane used as a positive control (○).

Experimental Section

The algal sample originates from Corfu (Greece). The sample was processed immediately after collection. The isolation of the fungus was carried out using an indirect isolation method. Algal samples were rinsed three times with sterile water. After surface sterilisation with 70% EtOH for 15 s the alga was rinsed in sterile artificial sea water (ASW). Subsequently the alga was aseptically cut into small pieces and placed on agar plates containing isolation medium: agar (15 g/L), ASW (800 mL L^{-1}), glucose (1 g L^{-1}), peptone from soymeal (0.5 g L^{-1}), yeast extract (0.1 g L^{-1}), benzyl penicillin (250 mg L^{-1}) and streptomycin sulfate (250 mg L^{-1}). Fungi growing out of the algal tissue were separated on biomalt medium (biomalt 20 g L^{-1}, agar 10 g L^{-1}, ASW 800 mL L^{-1}) until the culture was pure. The strain 707/GrKo3 was assigned as *Nodulisporium* sp. and was identified by Dr. R. A. Samson, Centralbureau voor Schimmel Cultures, Utrecht (The Netherlands).

The fungal strain was cultivated for ten weeks on 10 L solid Czapek-Dox medium (Becton Dickinson, France) with agar (15 g L^{-1}) at room temperature in Fernbach flasks. Fungal biomass and media were homogenised by using an Ultra-Turrax apparatus and extracted with ethyl acetate. The ethyl acetate extract (16.35 g) of the *Nodulisporium* fermentation was separated by liquid–liquid extraction (hexane–MeOH). The MeOH extract was fractionated by using column chromatography over silica gel (Merck, 63–200 μm, 7×23 cm) with a CH_2Cl_2/acetone/MeOH gradient yielding 12 fractions. Fraction 4 (1.53 g) was further fractionated by RP column chromatography (Macherey–Nagel Polygoprep 60–50 C_{18}, 4×23 cm) with a MeOH/H_2O gradient. Subfraction 4 was then purified by RP HPLC (Knauer C_{18} Eurosphere 100 250×8 mm, 5 μm) with a mobile phase (2 mL min^{-1}) consisting of 55/45 MeOH/H_2O resulting in 18 frac-

tions. Fraction 17 (18.2 mg) was finally separated (same RP18 HPLC column) with mixture MeOH/H_2O 55/45 (2.5 mL min^{-1}) affording the pure compound **1** (12.0 mg).

Compound **1** was tested noneffectively in agar diffusion assays[18] against the bacteria *Bacillus megaterium*, *Escherichia coli*, the fungi *Microbotryum violaceum*, *Eurotium rubrum* and *Mycotypha microspora*, and the green micro-alga *Chlorella fus*ca. Cytotoxicity of the compound was investigated by using 36 cancer cell lines.[19] The pure compound showed no activity in the assay at concentrations of 1 μg mL^{-1} and 10 μg mL^{-1}, respectively.

Data for 1: White solid; $[\alpha]_D^{24} = +89.44$ ($c = 0.15$ in $CHCl_3$); 1D and 2D NMR data: see Table S1 in the Supporting Information; IR (ATR): $\tilde{\nu} =$ 2922, 2359, 1732, 1633, 1365, 1155 cm^{-1}; UV/Vis (MeOH): λ_{max} ($\log\varepsilon$) = 279 (4.13), 353 nm (3.60 mol^{-1} dm^3 cm^{-1}); HR ESIMS: *m/z* calcd for $[C_{33}H_{34}O_{15}Na]^+$: 693.1795; found: 693.1807.

Keywords: cancer chemoprevention · heterodimeric chromanone · medicinal chemistry · natural products · structure elucidation

[1] A. Albini, M. B. Sporn, *Nat. Rev. Cancer* **2007**, *7*, 139–147.
[2] M.-T. Wang , K. V. Honn, D. Nie, *Cancer Metastasis Rev.* **2007**, *26*, 525–534.
[3] M. Karin, *Nature* **2006**, *441*, 431–436.
[4] A. Krick, S. Kehraus, C. Gerhäuser, K. Klimo, M. Nieger, A. Maier, H.-H. Fiebig, I. Atodiresei, G. Raabe, J. Fleischhauer, G. M. König, *J. Nat. Prod.* **2007**, *70*, 353–360.
[5] R. Patel, R. Garg, S. Erande, G. B. Maru, *J. Clin. Biochem. Nutr.* **2007**, *40*, 82–91.
[6] P. A. Tsuji, T. Walle, *Chem.-Biol. Interact.* **2007**, *169*, 25–31.
[7] Y. Deng, Y.-W. Chin, H. Chai, W. J. Keller, A. D. Kinghorn, *J. Nat. Prod.* **2007**, *70*, 2049–2052.
[8] C. Gerhäuser, K. Klimo, E. Heiss, I. Neumann, A. Gamal-Eldeen, J. Knauft, G.-Y. Liu, S. Sitthimonchai, N. Frank, *Mutat. Res.* **2003**, *523/524*, 163–172.
[9] D. Drochner, W. Hüttel, S. E. Bode, M. Müller, U. Karl, M. Nieger, W. Steglich, *Eur. J. Org. Chem.* **2007**, *11*, 1749–1758.
[10] G. Snatzke, P. C. Ho, *Tetrahedron* **1971**, *27*, 3645–3653.
[11] G. Snatzke, M. Kajtár, F. Werner- Zamojska, *Tetrahedron* **1972**, *28*, 281–288.
[12] J. M. Seco, E. Quiñoá, R. Riguera, *Chem. Rev.* **2004**, *104*, 17–117.
[13] G. Bringmann, T. F. Noll, T. A. M. Gulder, M. Grüne, M. Dreyer, C. Wilde, F. Pankewitz, M. Hilker, G. D. Payne, A. L. Jones, M. Goodfellow, H.-P. Fiedler, *Nat. Chem. Biol.* **2006**, *2*, 429–433.
[14] N. Tabata, H. Tomoda, Y. Iwai, S. Ōmura, *J. Antibiot.* **1996**, *49*, 267–271.
[15] B. Franck, H. Flasch, *Fortschr. Chem. Org. Naturst.* **1973**, *30*, 151–206.
[16] S. Kanokmedhakul, K. Kanokmedhakul, N. Phonkerd, K. Soytong, P. Kongsaeree, A. Suksamrarn, *Planta Med.* **2002**, *68*, 834–836.
[17] V. Rukachaisirikul, S. Chantaruk, W. Pongcharoen, M. Isaka, S. Lapanun, *J. Nat. Prod.* **2006**, *69*, 980–982.
[18] B. Schulz, J. Sucker, H.-J. Aust, K. Krohn, K. Ludewig, P. G. Jones, D. Döring, *Mycol. Res.* **1995**, *99*, 1007–1015.
[19] H.-H. Fiebig, A. Maier, A. M. Burger, *Eur. J. Cancer* **2004**, *40*, 802–820.

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