Inhibition of TNF-α Promoter Activity and Synthesis by A11-99-1, a New Cyclopentenone from the Ascomycete *Mollisia melaleuca*

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In a search for inhibitors of the inducible tumor necrosis factor-α (TNF-α) promoter activity and synthesis, a new chlorinated cyclopentenone was isolated from fermentations of the ascomycete *Mollisia melaleuca*. The structure was determined by a combination of spectroscopic techniques. The compound blocked the inducible human TNF-α promoter activity and synthesis with IC_{50}-values of 2.5–5 μg/ml (8.1–16.1 μM). Studies on the mode of action of the compound revealed that the inhibition of TNF-α promoter activity is caused by an inhibition of the phosphorylation of the I$\varkappa$B protein which prevents the activation of the transcription factor NF-$\varkappa$B. No cytotoxic, antibacterial and antifungal activities could be observed up to 100 μg/ml (323 μM) of the compound.

Key words: Mollisia melaleuca, Cyclopentenone, TNF-α, NF-$\varkappa$B

Introduction

Tumor necrosis factor-α (TNF-α) is a potent pro-inflammatory cytokine, which is released by monocytes, macrophages and T-cells in response to diverse extracellular stimuli including lipopolysaccharide (LPS), antigens, viruses, cytokines or exposure to calcium ionophore/12-*O*-tetradecanoylphorbol-13-acetate (TPA) (Aggarwal, 2003). TNF-α is among the earliest activated cytokines in inflammation and it contributes to the pathogenesis of inflammatory and auto-immune diseases by induction of further cytokines especially IL-1β and IL-6 (Feldmann and Maini, 2001). Studies of the promoter region of TNF-α have shown that interactions of the transcription factors NF-$\varkappa$B, NF-AT, Ets-1, Elk-1 and the AP-1 family members ATF2/Jun contribute to the transcriptional regulation of the TNF-α gene in various cell lines (Tsai *et al.*, 1996a, b). Thus low molecular weight compounds which interfere with the regulation of TNF-α expression may open novel therapeutic strategies to manipulate TNF-α levels in immunopathological processes in which TNF-α plays a destructive role such as superantigen induced septic shock, rheumatoid arthritis and Crohn's disease.

In a search for compounds inhibiting the inducible TNF-α promoter activity in T-cells, a new chlorinated cyclopentenone, designated A11-99-1, was isolated from fermentations of the ascomycete *Mollisia melaleuca*. In the current study the fermentation, isolation, structure elucidation and some biological activities of A11-99-1 are described. Species of the genus *Mollisia* are common and widespread saprotrophs, producing large numbers of minute apothecia on decaying leaves and decorticated twigs. *Mollisia melaleuca* (Fr.) Sacc. (Dermateaceae, Helotiales) is distinguished from the most common species *M. cinerea* by its whitish apothecial disk delimited by a dark brown margin, and the size of its ascospores (Dennis, 1981; Breitenbach and Kränzlin, 1984).

Materials and Methods

General experimental procedures

For analytical HPLC a Hewlett Packard 1090 series II instrument and for preparative HPLC a Jasco model PU-980 instrument was used. The content of A11-99-1 in samples taken during fermentation and in fractions during purification was determined by analytical HPLC [Merck LiChrosphere RP-18; 5 μm, column 125 × 4 mm; H_3PO_4 (0.1%)-acetonitrile gradient (% acetonitrile); flow 1 ml/min; 0–10 min 0–100%, 10–15 min 100%; retention time: A11-99-1, 6.6 min].

Spectral data were obtained with the following instruments: ^{1}H NMR (500 MHz) and ^{13}C NMR

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(125 MHz) spectra at room temperature with a Bruker DRX500 spectrometer with an inverse multinuclear 5 mm probe head equipped with a shielded gradient coil. The spectra were recorded in $CDCl_3$, and the solvent signals (7.26 and 77.0 ppm, respectively) were used as reference. The chemical shifts (δ) are given in ppm, and the coupling constants (J) in Hz. COSY, HMQC and HMBC experiments were recorded with gradient enhancements using sine shaped gradient pulses. For the 2D heteronuclear correlation spectroscopy the refocusing delays were optimized for $^1J_{CH}$ = 145 Hz and $^nJ_{CH}$ = 10 Hz. The raw data were transformed and the spectra were evaluated with the standard Bruker XWIN-NMR software (rev. 010101). The UV and the IR spectra were recorded with a Perkin Elmer λ 16 and a Bruker IFS 48 spectrometer. EI (70 eV), CI (NH_3 and CH_4) and FAB mass spectra were recorded with a Jeol SX102 spectrometer, APCI spectra with a HP Series 1100 LCD-MSD HPLC-MS set up (Hewlett-Packard, Waldbronn, Germany), and ESI spectra with a Micromass QTOF micro instrument. The optical rotation was measured with a Perkin-Elmer 141 polarimeter at 22 °C.

Producing organism, fermentation and isolation of A11-99-1

The ascomycete strain A11–99 was collected on decaying wood and identified as *Mollisia melaleuca* (Fr.) Sacc. according to Breitenbach and Kränzlin, 1984. The strain is deposited in the culture collection of the Department of Biotechnology, University of Kaiserslautern, Germany.

For maintenance on agar slants the strain was kept on YMG medium (10 g/l malt extract; 10 g/l glucose; 4 g/l yeast extract and 1.5% agar for solid media; pH 5.5). For submerged cultivation, strain A11-99 was grown in YMG medium (pH 5.5). A well-grown seed culture of *M. melaleuca* A11-99 (200 ml YMG medium) was used to inoculate a Biolafitte C-6 fermenter containing 20 l of YMG medium with aeration (3 l air/min) and agitation (120 rpm) at 22 °C. The production of A11-99-1 was followed by the inhibitory effect of various concentrations of a crude extract of the culture fluid on the TNF-α promoter activity as described below. After 50 h, the culture fluid was separated by filtration and extracted with EtOAc (15 l). The solvent was evaporated and the crude product (1 g) was separated by chromatography on Sephadex LH20 with MeOH as eluent resulting in 130 mg of an enriched product, which was further purified by preparative HPLC (LiChrosorb RP18, column 2.5 × 25 cm) with H_2O/acetonitrile (75:25). A11-99-1 (30 mg) was purified from the enriched product (62 mg) by gel permeation chromatography (SP 300/25 Nucleogel GPC 50-10, Macherey and Nagel, Düren, Germany) with 2-propanol as eluent. Yield: 30 mg of A11-99-1.

Physicochemical properties of A11-99-1

A11-99-1 (3-chloro-4-dichloromethyl-5-dichloromethylene-2,4-dihydroxy-cyclopent-2-enone) was obtained as brownish oil. $[\alpha]_D$ – 11° (c 5.0 in $CHCl_3$). – UV (MeOH): λ_{max} (log ε) = 307 nm (3.0), 226 nm (3.2). – IR (KBr): ν = 3415, 2925, 1710, 1670, 1585, 1380, 1285, 1175, 1140, 1050, 985, 900, 810, 790, 735, 705, and 645 cm^{-1}. – ^1H NMR ($CDCl_3$): δ (mult.) = 6.29, s, 7-H. – ^{13}C NMR ($CDCl_3$): δ = 180.2 (C-1), 152.9 (C-2), 132.7 (C-6), 130.6 (C-3), 129.4 (C-5), 81.1 (C-4), 73.1 (C-6). – HRFABMS $[M+H]^+$: m/z = 310.8593 (required for $C_7H_4O_3Cl_5$, 310.8603).

Biological assays

Jurkat cells (ATCC TIB 152), U-937 cells (ATCC CRL-1593), HL-60 cells (ATCC CCL 240), and RAW 264.7 cells (ATCC TIB-71) were grown in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS) und 65 μg/ml penicillin G and 100 μg/ml streptomycin sulfate. HepG2 cells (ATCC HB 8065), COLO 320 cells (DSMZ ACC 144) and HeLa S3 (ATCC CCL 2.2) were maintained in DMEM medium supplemented with 10% fetal calf serum. The assays for antimicrobial activity were carried out as described previously (Becker *et al.*, 1997). Cytotoxicity was measured using the cell Titer96^{TM} Aqueous One Cell Proliferation Assay (Promega, Mannheim, Germany) according to the manufacturer's instructions.

The influence of A11-99-1 on the syntheses of macromolecules in Jurkat cells was analyzed as follows. Jurkat cells were seeded in 24-well plates at 1×10^6 cells/ml in OPTIMEM medium containing 10% FCS. 3700 Bq of the radioactive precursors (2-^{14}C)-thymidine, (2-^{14}C)-uridine or (1-^{14}C)-leucine were added and the cells were incubated for an additional 24 h with or without test compound. The cells were lysed by addition of 0.5 ml 15% trichloroacetic acid (TCA) per well. The precipitate was collected by centrifugation at 21,000 ×

g for 10 min at 4 °C, washed with 1 ml TCA (10%) and the radioactivity in the acid insoluble material was measured with a liquid scintillation counter.

Reporter gene assays. The 1.2 kb human TNF-α promoter was amplified by PCR from genomic DNA extracted from HeLa S3 cells as described by Takashiba *et al.* (1993). The PCR product was cloned into the XhoI-HindIII site of the pGL3-basic vector (Promega, Mannheim, Germany) to generate the TNF-α promotor driven luciferase reporter plasmid pJR-TNF-pro. The plasmid pRL-CMV for normalizing transfection efficiency was obtained from Promega (Dual-Luciferase-Reporter-Assay). Jurkat cells were electroporated essentially as described and seeded in 96-well plates (6×10^5 cells/ml in OPTIMEM containing 10% FCS) with and without test compounds (Weidler *et al.*, 2000a). Reporter gene expression was induced as indicated and the reporter gene activity was measured 24 h after transfection using the luciferase assay system (Promega) according to the manufacturer's instructions with a luminometer.

ELISA. TNF-α production in U-937 and Jurkat cells pretreated for 1 h with or without test compounds and stimulated with 50 ng/ml TPA (U-937) or 32 nM TPA and 2 μM ionomycin (Jurkat) for additional 16 h was determined by ELISA (QuantiGlo™ human TNF-α ELISA, R&D Systems, Wiesbaden, Germany) according to the manufacturer's instructions.

For western blotting, Jurkat cells were starved for 24 h in RPMI 1640 medium containing 0.5% FCS, treated for 1 h with test compounds and induced with 25 ng/ml TPA and 2.5 μM ionomycin for 30 min. Total cell extracts were prepared using RIPA detergent buffer (137 mM NaCl, 2.7 mM KCl, 8.1 mM $Na_2HPO_4 \times 2\ H_2O$, 1.5 mM KH_2PO_4, pH 7.4). 1% NP-40, 0.5% Na-deoxycholate, 0.1% SDS, 1 μM Na_3VO_4, complete protease inhibitor cocktail 1:50 (Roche Diagnostics, Mannheim, Germany) and cell extracts (50–100 μg protein) were subjected to 10% SDS-PAGE, transferred onto a nitrocellulose membrane, probed either with antibodies specific for the phosphorylated form of I$\varkappa$B (New England Biolabs, Beverly, USA) and then with the appropriate secondary antibody conjugated to horseradish peroxidase. Immunoreactive proteins were visualized by the enhanced chemoluminiscent detection system (ECL system, Amersham International, Freiburg, Germany). After stripping of the corresponding membrane, the blots were reprobed with an anti-I$\varkappa$B antibody and developed as described above.

Results and Discussion

To identify novel inhibitors of TNF-α expression from fermentations of fungi, we used a human TNF-α (hTNF-α) transcriptional reporter in transiently transfected Jurkat T-cells, which reflects the activation of the transcription factors binding to the regulatory sites of the TNF-α gene. Screening of 1,700 culture fluid and mycelial extracts of basidiomycetes, ascomycetes and imperfect fungi for compounds inhibiting the inducible expression of the hTNF-α promoter driven luciferase reporter gene resulted in the identification of the new fungal metabolite A11-99-1 (Fig. 1) from fermentations of *Mollisia melaleuca.*

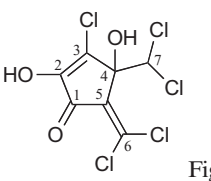

Fig. 1. Structure of A11-99-1.

Structure elucidation

A11-99-1 was isolated from the crude culture filtrate extract by bioactivity guided fractionation as described in Materials and Methods, and the structure was determined from the spectroscopic data. It proved to be difficult to obtain a mass spectrum of the compound, and although the initial APCI mass spectrum obtained from the isolation procedure suggested a molecular weight of 292 g/mol and (from the isotope pattern) the presence of 5 chlorines, the fragmentation observed was difficult to explain. EI, CI or ESI ionisation did not give any useful spectra at all. However, the indication that the compound is highly chlorinated was confirmed by the FAB mass spectrum recorded with a mixture of glycerol and polyethylene glycol as matrix. It was then revealed that the molecular weight is 310 g/mol, and isotope pattern as well as high resolution data definitely establish the degree of chlorination. The exact mass of the compound corresponds to $C_7H_3O_3Cl_5$, and with the presence of 7 signals in the ^{13}C NMR spectrum this should be the elemental composition. This corresponds to an unsaturation index of 4, and as the ^{13}C NMR spectrum suggests the presence of

one carbonyl group and two carbon, carbon double bonds the compound should have one ring. The ^{1}H NMR spectrum contained only one signal, a singlet at 6.29 ppm. This correlated with the carbon signal at 73.1 ppm, and the chemical shifts as well as the direct ^{1}H-^{13}C coupling constant (181 Hz) indicated that this is a -$CHCl_2$ proton (Nakanishi *et al.*, 1989). In the HMBC spectrum the proton signal correlates with the second saturated carbon atom (C-4) and with the two unsaturated carbon atoms C-3 and C-5, which consequently have to be part of the ring. The ^{13}C shifts for C-5 and C-6 are in agreement with an exocyclic dichlorinated double bond (Nakanishi *et al.*, 1989), determining the positions for C-1 and C-2. The remaining substituents on C-2 and C-3 are a hydroxyl group and a chlorine atom, and the positioning of the hydroxyl group on C-2 is suggested by its chemical shift. An alternative structure with a six-membered ring and the dichlorinated double bond between C-5 and C-6 endocyclic is less plausible as this structure would be expected to be quite unstable due to the possibility to become aromatic. In addition, the carbon shifts for such a double bond would be expected to be more differentiated than observed. In our hands, A11-99-1 is chemically stable and consists of only one isomer, and the spectroscopic data we have been able to collect all point towards the structure presented in Fig. 1. Unfortunately the hydroxyl protons did not give sharp signals in the ^{1}H NMR spectrum, and did not provide additional information.

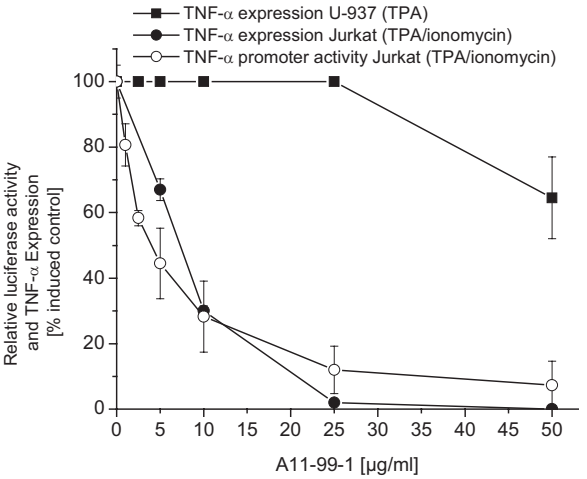

Fig. 2. Effect of A11-99-1 on TNF-α promoter activity and synthesis.
Jurkat cells were transfected with a hTNF-α promoter-dependent luciferase reporter gene plasmid and stimulated with 32 nM TPA/2 μM ionomycin for 24 h with or without test compounds. Control (100%): stimulation only. The expression of the reporter gene luciferase was determined as described in Materials and Methods. The data represent the mean ± SEM of three independent experiments.
U-937 and Jurkat cells were pretreated for 1 h with or without A11-99-1 and stimulated with 50 ng/ml TPA (U-937) or 32 nM TPA/2 μM ionomycin (Jurkat) for additional 16 h. TNF-α concentrations from cell supernatants were determined by ELISA. Induced control (100%): 800–905 pg/ml TNF-α. The data represent the mean ± SEM of three independent evaluations.

Biological activities

Effect of A11-99-1 on TNF-α promoter activity and synthesis

Transfection of Jurkat T-cells with the hTNF-α promoter driven luciferase reporter gene plasmid and stimulation with 32 nM 12-*O*-tetradecanoylphorbol-13-acetate (TPA) and 2 μM ionomycin resulted in a 17- to 20-fold activation over the basal level of luciferase expression. As shown in Fig. 2, A11-99-1 inhibited the inducible hTNF-α promoter activity in a dose-dependent manner with IC_{50}-values of 2.5–5 μg/ml (8.1–16.1 μM).

We next analyzed the influence of A11-99-1 on the TNF-α production in Jurkat and U-937 cells, which release significant amounts of TNF-α following TPA/ionomycin or TPA treatment. Pretreatment of Jurkat T-cells with various concentrations of A11-99-1 and stimulation with 10 ng/ml TPA/2.5 μM ionomycin resulted in a dose-dependent inhibition of TNF-α synthesis, which was comparable to the results obtained in the hTNF-α promoter reporter gene assays. As shown in Fig. 2, A11-99-1 blocked the TNF-α synthesis in Jurkat cells with IC_{50}-values of 5–10 μg/ml (16.1–32.3 μM). Pretreatment of the human promonocytic cell line U-937 with A11-99-1 and stimulation with 50 ng/ml TPA resulted in an incomplete inhibition of TNF-α synthesis with a remaining activity of 60% of the induced control even at the highest concentration tested (161.3 μM). These results indicate a preferential target within the signaling pathways leading to TNF-α expression in T-cells.

Cellular DNA-, RNA-, and protein syntheses were examined in Jurkat cells by determining the incorporation of (2-^{14}C)-thymidine, (2-^{14}C)-uridine and (1-^{14}C)-leucine in TCA-insoluble fractions. Up to a concentration of 50 μg/ml of A11-99-1 no effect on DNA- and RNA-syntheses could

Table I. Effect of A11-99-1 on SEAP or luciferase reporter gene expression in HeLa S3 and Jurkat cells.

Reporter gene assay	Cell line	Stimulus	A11-99-1 IC_{50} [μg/ml (μM)]
pTK-AP-1	HeLaS3	TPA (25 ng/ml)	25–50 (80.1–161.3)
pGE3-NF-AT/IL-2	Jurkat	TPA (10 ng/ml)/ionomycin (2.5 μM)	5–10 (16.1–32.3)
pGL3-NF-κB	Jurkat	TPA (10 ng/ml)/ionomycin (2.5 μM)	5–10 (16.1–32.3)
pGL3-COX-2	Jurkat	TPA (10 ng/ml)/ionomycin (2.5 μM)	50 (161.3)

The cells lines were transiently transfected with the indicated reporter gene constructs and the expression of the reporter gene was induced as described in Materials and Methods section.

be observed, whereas protein synthesis was inhibited to 25% during a 24 h incubation period, suggesting that the compound does not interfere with replication, transcription and translation in a general manner (data not shown). Cytotoxic properties of A11-99-1 were evaluated against various neoplastic cell lines by measuring the reduction of the tetrazolium compound 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium (MTS) into a colored formazan product which is directly proportional to the living cells in culture. No cytotoxic activities against Jurkat, U-937, HL-60, RAW 264.7, HepG2, COLO 320 and HeLa S3 cells could be observed up to 100 μg/ml of the compound during a 24 h incubation period (data not shown). In the plate diffusion assay A11-99-1 exhibited no antibacterial or antifungal activities up to 100 μg/paper disc.

Effect of A11-99-1 on NF-κB, NF-AT and AP-1-dependent reporter gene expression

The transcriptional activation of the human TNF-α promoter is dependent on binding sites for NF-κB, NF-AT and AP-1 families of transcription factors (Tsytsykova and Goldfield, 2002). We therefore investigated the influence of A11-99-1 on NF-κB, NF-AT and AP-1-dependent transcriptional reporters. As shown in Table I, A11-99-1 inhibited the TPA induced AP-1 and TPA/ionomycin induced NF-AT:AP-1 mediated SEAP expression in transiently transfected HeLa S3 or Jurkat cells with IC_{50}-values of 25–50 μg/ml (80–161 μM) and 5–10 μg/ml (16–32 μM), respectively. The NF-κB-dependent reporter gene activity was inhibited with IC_{50}-values of 5–10 μg/ml (16–32 μM). Beside TNF-α, the inducible isoform of the cyclooxygenase COX-2 is upregulated in many cell types by pro-inflammatory stimuli and it has been shown that the signaling mechanisms governing COX-2 expression also contribute to the inducible TNF-α expression (Íñiguez et al., 2000; Mestre *et al.*, 2001). We therefore investigated the influence of A11-99-1 on a human COX-2 transcriptional reporter (Table I). The TPA/ionomycin stimulated COX-2 promoter activity was inhibited by A11-99-1 far less efficient than the TNF-α promoter activity with an IC_{50}-value of 50 μg/ml (161 μM).

Effect of A11-99-1 on NF-κB activation

To further characterize the influence of A11-99-1 on NF-κB activation, Jurkat cells were pretreated with A11-99-1 and stimulated with TPA/ionomycin. Total cell extracts were analyzed by western blotting using a polyclonal IκB antibody. The immunoblotting demonstrated that activation of Jurkat cells with TPA/ionomycin resulted in a loss of the IκB protein (Fig. 3A). The degradation of

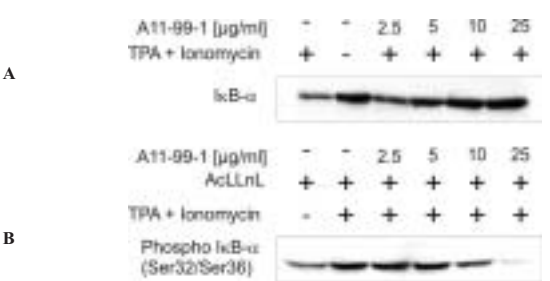

Fig. 3. Effect of A11-99-1 on NF-κB activation.
Jurkat cells were treated with A11-99-1 for 1 h in the presence or absence of 100 μM of the proteasome inhibitor *N*-Acetyl-Leu-Leu-norleucinal (AcLLnL), which stabilizes the phosphorylated form of IκB and stimulated for 30 min with 25 ng/ml TPA and 2.5 μM ionomycin. Subsequently total cell extracts were prepared and equal amounts of protein (50–80 μg) analyzed by western blotting using antibodies against IκBα (A) and phospho-IκBα (Ser32/36) (B). Results are a representative experiment repeated three times with essentially similar findings.

I$\varkappa$B was diminished in the presence of A11-99-1 at 5–10 μg/ml (16–32 μM). These results suggest that A11-99-1 interrupts the NF-$\varkappa$B signaling pathway by inhibiting the phosphorylation or degradation of I$\varkappa$B. To characterize whether A11-99-1 prevents NF-$\varkappa$B activation by inhibiting the phosphorylation of the I$\varkappa$B protein, Jurkat cells were pretreated with A11-99-1 and stimulated with TPA/ionomycin in the presence of the proteasome inhibitor *N*-Acetyl-Leu-Leu-norleucinal (AcLLnL), which stabilizes the phosphorylated form of I$\varkappa$B-α. As shown in Fig. 3B, treatment of the cells with 25 μg/ml A11-99-1 reduced the phosphorylation of the I$\varkappa$B protein to the basal level. These results indicate that A11-99-1 inhibits NF-$\varkappa$B activation by preventing the phosphorylation of I$\varkappa$B and therefore sequestering the NF-$\varkappa$B-I$\varkappa$B complex in an inactive form.

Similar highly chlorinated cyclopentenones have been isolated from fermentations of *Mollisia ventosa* as inhibitors of the calcium/calmodulin-dependent cyclic nucleotide phosphodiesterase and it has been proposed that these compounds interact with calmodulin (CaM) to inhibit Ca^{2+}/CaM-dependent enzymes (Nakanishi *et al.*, 1989). The chlorinated cyclopentenone CPDHC has been reported to inhibit the IL-6 and IFN-γ-dependent activation of the STAT1 and STAT3 transcription factors by preventing their phosphorylation (Weidler *et al.*, 2000b). Structurally related chlorinated cyclopentenons have also recently been isolated from fermentations of a *Dasyscyphus* species (Mierau *et al.*, 2004). These compounds showed strong cytotoxic activities, which were due to an interference with the cellular macromolecule syntheses. The biological activities of the naturally occurring cyclopentenone prostaglandins (cyPGs) have been attributed to the α,β-unsaturated carbonyl group in the cyclopentenone ring which reacts with sulfhydryl groups of cysteine residues of proteins by Michael addition. Most inhibitory effects of cyPGs like 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2) on gene transcription have been ascribed to the formation of adducts with components of the NF-$\varkappa$B and AP-1 pathways (Castrillo *et al.*, 2000; Straus *et al.*, 2000; Cernuda-Morrollón *et al.*, 2001; Pérez-Sala *et al.*, 2003). In addition inhibitory effects on the activation of c-Jun NH_2-terminal kinase and STAT1 phosphorylation have been described (Sawano *et al.*, 2002; Weber *et al.*, 2003). The importance of the α,β-unsaturated carbonyl group for the biological activity has been demonstrated by incubation with glutathione or dithiothreitol (DTT) which eliminates the activity of the cyPGs. But unlike the cyPGs, the α,β-unsaturated carbonyl group of A11-99-1 seems not to be solely responsible for the biological activities of the compound since preincubation with cysteine or DTT neither resulted in an adduct formation nor abolished the inhibition of the TNF-α promoter activity (data not shown).

In summary we have identified a novel fungal cyclopentenone, A11-99-1, which inhibits the inducible expression of TNF-α in Jurkat T-cells. The mechanism by which A11-99-1 inhibits the expression of pro-inflammatory genes seems to be the interference with signaling pathways leading to the activation of the transcription factors NF-$\varkappa$B and NF-AT. This compound may serve as lead structure for the development of transcription-based inhibitors of pro-inflammatory genes.

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