The Anti-inflammatory Sesquiterpene Lactone Helenalin Inhibits the Transcription Factor NF-κB by Directly Targeting p65*

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The sesquiterpene lactone helenalin is a potent anti-inflammatory drug whose molecular mechanism of action remains unclear despite numerous investigations. We have previously shown that helenalin and other sesquiterpene lactones selectively inhibit activation of the transcription factor NF-κB, a central mediator of the human immune response. These drugs must target a central step in NF-κB pathway, since they inhibit NF-κB induction by four different stimuli. It has previously been reported that sesquiterpene lactones exert their effect by inhibiting degradation of IκB, the inhibitory subunit of NF-κB. These data contradicted our report that IκB is not detectable in helenalin-treated, ocaida acid-stimulated cells. Here we use confocal laser scanning microscopy to demonstrate the presence of IκB-released, nuclear NF-κB in helenalin-treated, tumor necrosis factor-α stimulated cells. These data show that neither IκB degradation nor NF-κB nuclear translocation is inhibited by helenalin. Rather, we provide evidence that helenalin selectively alkylates the p65 subunit of NF-κB. This sesquiterpene lactone is the first anti-inflammatory agent shown to exert its effect by directly modifying NF-κB.

In traditional medicine, alcoholic preparations from flowers of Arnica montana and Arnica chamissonis ssp. foliosa are applied externally to treat hematomas, contusions, sprains, rheumatic diseases, and superficial inflammations of the skin. The secondary metabolites that mediate the anti-inflammatory effects are sesquiterpene lactones of the 10α-methylsudoguananolid type like helenalin, 11α,13-dihydrohelenalin, chamissonolid, and their ester derivatives (1–3). Several studies have investigated how these natural compounds exert their anti-inflammatory effect. Sesquiterpene lactones have been shown to modulate many processes that influence inflammatory reactions, for example, oxidative phosphorylation, platelet aggregation, histamine and serotonin release (3, 4). These activities are mediated chemically by α,β-unsaturated carbonyl structures, such as an α-methylene-γ-lactone or an α,β-unsubstituted cyclopentenone. These structure elements react with nucleophiles, especially cysteine sulfhydryl groups, by a Michael-type addition (5, 6). Therefore, exposed thiol groups, such as cysteine residues in proteins, appear to be the primary targets of sesquiterpene lactones. Some sesquiterpene lactones, for example, helenalin, possess two alkylant structure elements which may be responsible for their remarkably high activity (5–8). However, other factors, such as lipophilicity, molecular geometry, and the chemical environment of the target sulfhydryl may also influence the activity of sesquiterpene lactones (6).

Despite many studies which prove the anti-inflammatory activity of sesquiterpene lactones, the molecular mechanism by which they exert their anti-inflammatory effect has not been sufficiently explained. Recently, we were able to show that helenalin, 11α,13-dihydrohelenalin, and chamissonolid potently inhibit induction of the transcription factor NF-κB (9). This protein is a central mediator of the human immune response (10). In almost all cell types, NF-κB, composed of a p50 and p65 subunit, is retained in an inactive cytoplasmic complex by binding to a third, inhibitory subunit, IκB. A large variety of inflammatory conditions, such as bacterial and viral infection as well as inflammatory cytokines, rapidly induce NF-κB activity. Active NF-κB is released from the cytoplasmic complex by phosphorylation and proteolytic degradation of the IκB subunit. The activated factor then translocates to the nucleus, where it stimulates the transcription of its target genes. NF-κB regulates the transcription of various inflammatory cytokines, such as interleukin-1, -2, -6, and -8 and TNF-α,1 as well as genes encoding cyclooxygenase-II, nitric oxide synthase, immunoreceptors, cell adhesion molecules, hematopoetic growth factors, and growth factor receptors (see Refs. 10 and 11). Pharmacological inhibition of NF-κB in vivo may thus substantially attenuate inflammatory processes.

We have recently shown that the sesquiterpene lactones helenalin, 11α,13-dihydrohelenalin, and chamissonoid inhibit DNA binding activity of NF-κB (9). However, we were not able to identify the molecular target through which these natural compounds exert their effect. Since NF-κB activation by many different inducers is inhibited by helenalin, the sesquiterpene lactone must target a common step in these signal transduction pathways. In addition to the physiological induction of NF-κB by inflammatory mediators, the transcription factor can be activated in vitro by treatment of cell extracts with the detergent deoxycholate. This dissociates the inhibitor IκB from the p50 and p65 subunits, releasing an active NF-κB heterodimer. We were able to show that in cell extracts generated from helenalin-treated cells, deoxycholate treatment no longer activates NF-κB-DNA binding activity (9). These data suggest

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1 The abbreviations used are: TNF, tumor necrosis factor; DTT, dithiothreitol; EMSA, electromophoretic mobility shift assay; GSH, glutathione; MG132, carbobenzoxy-leucyl-leucyl-leucinal-H; PBS, phosphate-buffered saline; poly(dl-dc), polyoxyinosinic deoxyctydilic acid, double-stranded alternating copolymer.
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that helenalin either modifies the NF-κB-IκB complex in such a way that deoxycholate cannot dissociate the complex, or that it attacks the deoxycholate-released NF-κB so that DNA binding is impaired. We were subsequently able to demonstrate that the homodimer of subunit p50, which contains a redox sensitive cysteine residue in its DNA-binding domain (12–14), is not affected by helenalin. Helenalin-treated stimulated cells, which show no NF-κB binding activity in the EMSA, nonetheless show a decrease in the amount of IκB-α protein in Western blot analysis. These data allow two interpretations. Either, helenalin may modify the IκB-α protein so that it is no longer recognized by the anti-IκB-α antibody. Alternatively, IκB is degraded in helenalin-treated cells, releasing NF-κB, but the transcription factor is modified, so that it no longer binds DNA.

Hehner and colleagues (15) have recently presented evidence that the sesquiterpene lactone parthenolide inhibits degradation of IκB-α and IκB-β. From their data, these authors conclude that sesquiterpene lactones inhibit NF-κB activation by preventing the degradation of IκBs. These results contradict our previous report that IκB-α immunoreactivity decreases significantly in helenalin-treated, ocdacic acid-stimulated cells (9). In this study we show that in helenalin-treated cells, degradation of IκB, and NF-κB translocation to the nucleus are not inhibited. Using a confocal laser scanning microscope, we demonstrate the presence of IκB-released NF-κB in the nucleus of helenalin-treated, TNF-α-stimulated cells. Moreover, we show that helenalin directly interferes with NF-κB DNA binding due to its alkylating activity. Helenalin selectively modifies the p65 subunit of the transcription factor, thereby inhibiting its DNA binding. Most importantly we show that, unlike antioxidants such as acetylsalicylic acid, helenalin can inactivate the active NF-κB complex. This property is crucial for the treatment of inflammation, where previously activated NF-κB shows that helenalin directly interferes with NF-κB.

RESULTS

Helenalin Directly Modifies the Active NF-κB Heterodimer—We have previously shown that pretreatment of cells with helenalin (structure see Fig. 1) inhibits NF-κB activation by various inducers (1). A simple explanation for the inhibitory effect of helenalin would be a direct modification of the NF-κB DNA binding activity. Sesquiterpene lactones can react by a Michael-type addition to biological nucleophiles, especially with sulphydryl groups of cysteine residues (5, 6). The NF-κB heterodimers p50 and p65 contain cysteine residues in their DNA-binding domains (12, 13, 22). It has recently been reported that cell extracts from TNF-α-stimulated cells incubated with sesquiterpene lactones in vitro show no diminished NF-κB activity (9, 15). These observations suggested that sesquiterpene lactones do not directly modify the active NF-κB proteins. However, to be able to draw this conclusion, it is important that the in vitro conditions are comparable with those of the in vivo
experiments. This is not the case. There are differences in reaction time, temperature, and chemical conditions. Attempts to adapt the in vivo conditions to the in vitro parameters were not successful due to proteolysis of the cell extracts at 37 °C.

Therefore, we modified the experimental conditions in the in vivo system. Cells were pretreated with TNF-α for 20 min (Fig. 2A, lane 2). Subsequently, helenalin was added at a 10 μM concentration (Fig. 2A, lanes 4–9). Cell extracts were prepared after the indicated times and analyzed for NF-κB DNA binding by EMSA. Addition of helenalin caused complete inhibition of NF-κB DNA binding after 80 min (lane 7). This distinguishes helenalin from other NF-κB inhibitors, which only prevent transcription factor activation, but are unable to inhibit the activated protein (23). In a Western blot using an anti-p65 polyclonal antibody, we show that this inhibitory effect is not due to helenalin-induced degradation of NF-κB (Fig. 2B).

To prove that NF-κB inhibition is due to irreversible alkylation of free sulfhydryls on cysteine residues the experiment shown in Fig. 2A was modified. An excess of the thiol DTT (5 mM) was added 20 min after TNF-α stimulation and 10 min prior to helenalin addition (Fig. 3A, lanes 4, 6, and 8). If sesquiterpene lactones modify NF-κB by alkylation, an excess of DTT should prevent this reaction, since the sesquiterpene lactones would react with the large quantities of free sulfhydryls in the DTT instead of reacting with NF-κB. Indeed, addition of DTT completely suppressed the inhibitory effect of the sesquiterpene lactone on NF-κB DNA binding activity (Fig. 3A, lanes 6 and 8). In contrast, addition of DTT 80 min after helenalin treatment had no influence on the ability of helenalin to inhibit NF-κB DNA binding (Fig. 3B, lanes 6 and 8). These data are consistent with the hypothesis that helenalin irreversibly alkylates NF-κB, thereby inhibiting its DNA binding.

To support this hypothesis the following experiment was carried out. Jurkat cells were left untreated or incubated with 10 μM helenalin for 1 h. The cell extracts were treated either with the detergent deoxycholate alone or with deoxycholate/DTT. In cell extracts from untreated cells, deoxycholate is able to dissociate the IκB-NF-κB complex, releasing the active, DNA-binding form of NF-κB (Fig. 4, lane 2). DTT does not affect this reaction (Fig. 4, lane 3). In contrast, in cell extracts from cells treated for 1 h with helenalin, deoxycholate no longer able to generate active NF-κB. The addition of DTT to cell extracts has no effect on this reaction, suggesting that the effect of helenalin is irreversible, even after addition of excess DTT (Fig. 4, lane 6).

The question arises how helenalin can specifically modify sulfhydryl groups on NF-κB in the presence of a high (mM) concentration of glutathione (GSH) in the cytoplasm. Since GSH contains free sulfhydryl groups, helenalin could conceivably react with GSH, thereby becoming unavailable to react with NF-κB. We therefore investigated whether helenalin-2β-mono-glutathionyl adduct, the reaction product between helenalin and glutathione, is able to inhibit NF-κB activation. This GSH adduct, like native helenalin, completely inhibited NF-κB activation by TNF-α. The concentration required for complete inhibition (20 μM, data not shown) was only slightly higher than that of native helenalin. Thus, unlike DTT, glutathione does not inhibit helenalin reactivity. We propose that the difference between the reaction of helenalin with GSH or with DTT lies in the reversibility of the former but not the latter.

We investigated whether 11α,13-dihydrohelenalin and chamissonolid, which inhibited NF-κB binding activity to a much lesser degree, also modify the active NF-κB heterodimer. These two compounds differ from helenalin mainly because...
they are only monofunctional alkylants. The exocyclic methylene group present in helenalin is saturated in 11α,13-dihydro-helenalin. In contrast, chamissonolid still possesses the exocyclic methylene group, but lacks the cyclopentenone structure.

While helenalin exists in a rapidly exchanging equilibrium of

![FIG. 3. The effects of helenalin on NF-κB DNA binding in the presence of DTT. A, lane 1 shows unstimulated cells. In lane 2 cells were treated with 200 units/ml TNF-α alone for 20 min, in lane 3 for 110 min. In lanes 4–8, cells were stimulated with TNF-α. After 20 min DTT was added to a final concentration of 5 mM (lanes 4, 6, and 8). 10 min after DTT addition, thus 30 min after TNF-α stimulation, helenalin was added at the concentrations indicated (lanes 5–8). After a total reaction time of 20 (lane 2) or 110 min (lanes 1 and 3–8) cell extracts were prepared and analyzed for NF-κB activity by EMSA. B, lane 1 shows unstimulated cells. In lane 2 cells were treated with 200 units/ml TNF-α alone for 20 min, in lane 3 for 180 min. In lanes 4–8 cells were stimulated with TNF-α. After 20 min helenalin was added at the concentrations indicated (lanes 5–8). 80 min after helenalin addition, thus 100 min after TNF-α stimulation, DTT was added to a final concentration of 5 mM (lanes 4, 6, and 8). After a total reaction time of 20 (lane 2) or 180 min (lanes 1 and 3–8) cell extracts were prepared and analyzed for NF-κB activity by EMSA. A filled arrowhead indicates the position of the NF-κB DNA complex. The p65 homodimer DNA complex migrates slightly slower in the gel. The open circle denotes a nonspecific activity binding to the probe and the open arrowhead shows unbound oligonucleotide.]

![FIG. 4. The effect of helenalin and DTT on NF-κB DNA binding following desoxycholate (DOC) activation. Jurkat T-cells were left untreated (lanes 1–3) or treated for 1 h with 10 μM helenalin. In lanes 2, 3, 5, and 6 the detergent desoxycholate (0.4%) was added to the cell extracts. In lanes 3 and 6, 5 mM DTT was added simultaneously. After 10 min the extracts were analyzed by EMSA. A filled arrowhead indicates the position of the NF-κB DNA complex. The open circle denotes a nonspecific activity binding to the probe. The open arrowhead shows unbound oligonucleotide.]

![FIG. 5. The effect of helenalin on DNA binding by p65. 293 cells were transiently transfected with 6 μg of a p65 expression vector (lanes 2–6). Twenty-four hours after transfection, cells were treated with various concentrations of helenalin, as indicated, for 1 h. Lanes 8–12 show 293 cells treated for 1 h with the indicated amounts of helenalin and subsequently stimulated for 1 h with 200 units/ml TNF-α. Lanes 1 and 7 show untreated control cells. Total cell extracts were prepared and analyzed for NF-κB DNA binding by EMSA. A filled arrowhead indicates the position of NF-κB p50/p65 heterodimer DNA complex in TNF-α-treated cells. The p65 homodimer DNA complex migrates slightly slower in the gel. The open circle denotes a nonspecific activity binding to the probe and the open arrowhead shows unbound oligonucleotide.]

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two different twist-chair conformations (TC 7 and TC 10). 11α,13-dihydrohelenalin preferably adopts only one of these (TC 7); in contrast, chamissonolid adopts a twist-boat conformation (24, 25). The latter two compounds also directly interfere with NF-κB DNA binding, but only at 200 μM concentrations (data not shown). At this concentration both sesquiterpene lactones also prevent the release of active NF-κB by desoxycholate treatment (data not shown).

Helenalin Impairs NF-κB p65 DNA Binding in Vivo—We have previously shown that the p50 DNA-binding subunit of NF-κB is not modified by helenalin (9). Therefore, we investigated helenalin’s effect on the subunit p65. 293 cells were

![Image]

**Fig. 6. IκB degradation and NF-κB nuclear translocation are not inhibited by helenalin.** Confocal scanning microscopy images of untreated Jurkat T cells (A) or cells that were stimulated with 200 units/ml TNF-α for 1 h (B). Cells were pretreated with either 10 μM helenalin (C) or with 50 μM of the proteasome inhibitor MG132 (D) for 1 h before stimulation with TNF-α. Cells shown in E were pretreated with both helenalin and MG132 for 1 h and subsequently stimulated with TNF-α. Fixed samples were stained for filamentous actin with fluorescein isothiocyanate labeled phalloidin (blue label), for cell nuclei with TOTO-3 (red label) and for activated p65-NF-κB molecules using a mouse monoclonal anti-p65 NF-κB antibody, biotin-conjugated goat anti-mouse IgG antibody and Cy3-labeled ExtrAvidin (green label, appears yellow when colocalizing with red cell nuclei).
transfected with an expression vector for the p65 subunit of NF-κB. p65 constitutively binds DNA as a homodimer (26). Transfected cells expressing p65 NF-κB were treated with increasing concentrations of helenalin for 1 h after which cell extracts were analyzed by EMSA (Fig. 5, lanes 3–6). In addition, untransfected 293 cells were preincubated with the same concentrations of helenalin and subsequently stimulated with 200 units/ml TNF-α for 1 h (Fig. 5, lanes 9–12). 20 μM helenalin completely impaired both p65 DNA binding as well as TNF-α stimulated NF-κB activity.

Helenalin Does Not Prevent Translocation of Activated NF-κB into the Nucleus—We have previously shown that extracts of cells treated with helenalin exhibit no NF-κB binding activity in the EMSA. Nonetheless, IκB appears to be degraded in these cells, as measured by a loss of IκB-α immunoreactivity in the Western blot. Interestingly, in helenalin-treated cells this decrease occurred with a delay compared to untreated cells (9). At that time we suggested that this effect might be explained by two models: either, helenalin modifies the IκB-α protein so that it is no longer recognized by the anti-IκB-α antibody or, alternatively, IκB-α is degraded in helenalin-treated cells without concurrent NF-κB activation. In contrast
to our observation Hehner and colleagues (15) reported that the germacraneolid parthenolide inhibits NF-κB activation by preventing IκB degradation. Therefore we used immunocytochemistry to investigate whether NF-κB translocates to the nucleus in helenalin-treated cells.

Jurkat cells were stimulated with 200 units/ml TNF-α, in addition, two samples were pretreated with either 10 μM helenalin or 50 μM of the proteasome inhibitor MG132 for 1 h before stimulation with TNF. A fourth sample was pretreated with both helenalin and MG132 and subsequently stimulated with TNF. Cells were air-dried onto slides and stained with both helenalin and MG132 and subsequently stimulated before stimulation with TNF. A fourth sample was pretreated with MG132 prevents IκB degradation. Therefore we used immunocytochemistry to investigate whether NF-κB inhibition, helenalin isobutyrate was studied. This sesquiterpene lactone, which is more lipophilic than helenalin, inhibited NF-κB DNA binding at a 20 μM concentration (see Fig. 7A, lane 14), a similar concentration to that required of helenalin (9). To gain further insights about the role which the exocyclic methylene group and the configuration of the γ-lactone may play, Mexicanin I and 2,3-dihydroaromaticin were studied (structures see Fig. 1). In contrast to helenalin and helenalin isobutyrate, both possess a trans-configurated lactone ring and adopt a conformation approximate to a TC 6 twist-chair (TC 6) (27).2 20 μM Mexicanin I also completely inhibited NF-κB DNA binding (see Fig. 7A, lane 6). Therefore the influence of the conformation may be neglected. 2,3-Dihydroaromaticin, with the α-methylene-γ-lactone as the only reactive center, prevented NF-κB binding at a 50 μM concentration (see Fig. 7B, lane 3). This concentration is higher than that required of either helenalin or its ester, again suggesting the importance of two reactive centers for a strong inhibitory activity. Current studies are under way to determine whether additional structural features influence the inhibitory activity of sesquiterpene lactones.

**DISCUSSION**

Sesquiterpene lactones represent an active principle of many drugs used in traditional medicine as anti-inflammatory remedies. Flowerheads from *A. montana* and *A. chamissonis* ssp. *folliosa* are prominent examples (1). Their extracts are used to treat hematomas, contusions, sprains, rheumatic diseases, and superficial inflammations of the skin (see for Ref. 1). The anti-inflammatory effect of this remedy is mediated by esters of helenalin, 11α,13-dihydrohelenalin and chamissonidol. In several studies it was shown that these compounds interfere with

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2 T. J. Schmidt, unpublished results.
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Fig. 7. The effect of Mexicanin I, 2,3-dihydroaromaticin, and helenalinisobutyrate on NF-κB DNA binding. Jurkat cells were treated for 1 h with one of the sesquiterpene lactones at the concentrations indicated and subsequently stimulated with 200 units/ml TNF-α for 1 h. Lane 1 shows unstimulated control cells and in lane 2, cells were treated with TNF-α alone. Cell extracts were analyzed for NF-κB DNA binding activity by EMSA. A filled arrowhead indicates the position of NF-κB DNA complexes. The open circle denotes a nonspecific activity binding to the probe and the open arrowhead shows unbound oligonucleotide.

cellular processes including oxidative phosphorylation, platelet aggregation, histamine and serotonin release (3, 4). However, the molecular mechanism by which sesquiterpene lactones exert their anti-inflammatory effect could not be sufficiently explained. Recently we have shown that helenalin selectively inhibits DNA binding activity of the transcription factor NF-κB, and prevents the induction of κB-dependent gene expression (9). Since various inflammatory processes are directly dependent on NF-κB activity, we suggested that this is the molecular mechanism by which helenalin exerts its anti-inflammatory effect. However, at that time we were not able to explain in detail the mechanism by which helenalin inhibits NF-κB activity. Two experiments pointed to possible models. First, in helenalin-treated cells, the anti-IκB-α immunoreactivity decreases after ocdacic acid stimulation, even though no active NF-κB can be detected. Second, the detergent deoxycholate, which can release active NF-κB from IκB in untreated cell extracts, is no longer able to do so in helenalin-treated cells. These observations lead us to formulate two hypotheses. Either, helenalin may modify the NF-κB-IκB-α complex so that it cannot be dissociated by deoxycholate and IκB is no longer recognized by the anti-IκB-α antibody. Alternatively, IκB is degraded in helenalin-treated cells releasing NF-κB, but the transcription factor is modified so that it no longer binds DNA.

Here we show that the helenalin-mediated inhibition of NF-κB is due to alkylation of the active transcription factor. Interestingly, our data indicate that helenalin targets the cysteine sulfhydryl groups in the p65 subunit. Another alkylation agent shown to directly modify NF-κB, N-ethylmaleimide, reacts with cysteine 62 in the p50 subunit (28). ακB alkylation prevents DNA binding activity of NF-κB. This model is supported by the recent elucidation of the x-ray structure of the p65 homodimer, which shows that cysteine 38 is located within the DNA-binding domain and another cysteine, cysteine 120, is found in a proximal loop (22). It is possible that helenalin modifies either one or both of these residues. In addition, a single helenalin molecule may cross-link these two cysteines. In a computer based study, we are currently investigating possible alkylation sites in the p65 subunit.

We substantiate the observation that helenalin does not prevent IκB degradation. Using immunocytochemistry, we show that in helenalin-treated cells NF-κB is translocated to the nucleus, which can only occur after IκB-α degradation. Therefore, the recent report that the sesquiterpene lactone parthenolide prevents the induced degradation of IκB-α and IκB-β should be reinvestigated (15). Parthenolide possesses a germacranolide skeleton with two potentially reactive centers in the form of an exocyclic methylene group and an epoxide ring. Since we have shown here that sesquiterpene lactones with two reactive centers interfere directly with NF-κB DNA binding, the statement that parthenolide does not do so may also require revision. These discrepancies between the results of Hehner et al. (15) and our observations may be due to the different experimental conditions. We had also previously reported that helenalin does not interfere with NF-κB DNA binding (9). However, we realized that the conditions used in this in vitro experiment are not comparable to the conditions under which helenalin reacts in intact cells. Specifically, the reaction time allowed in vitro was shorter (20 min versus 1 h in vivo) and the temperature lower (room temperature versus 37 °C). Our attempts to adapt the in vitro conditions to those present in vivo were not successful. The higher temperature and longer incubation time led to degradation of the protein extracts. Therefore, we designed new experiments (Figs. 3 and 4) to investigate whether sesquiterpene lactones directly modify NF-κB. Using this approach, we were able to show that the direct inhibitory effect on NF-κB binding is not restricted to sesquitertene lactones of the pseudoguaianolide type but is observed also with the germacranolides diversifolin, tirotundin, and diversifolin methyl ether (29).

We have investigated the effects of lipophilicity and molecular geometry on NF-κB inhibition. Our results suggest that neither property influences sesquiterpene lactone activity. Helenalin isobutyrate is more lipophilic than helenalin, but both compounds have nearly the same inhibitory activity. In contrast to helenalin and helenalin isobutyrate, Mexicanin I possesses a trans-configurated lactone ring. We could show that Mexicanin I has the same inhibitory activity as the two cis-configurated lactones. Thus, lipophilicity and molecular geometry appear to be of marginal importance among these bifunctional alkylants. In contrast, the monofunctional 2,3-
Dihydroaromaticin is significantly less effective at inhibiting NF-κB (50 μM versus 20 μM in Mexicanin I). Therefore, the most important structural element is the occurrence of two reactive centers, one as an exocyclic methylene group and the other as an α,β-unsaturated cyclopentenone ring. However, the small number of compounds used do not allow us to draw final conclusions. We are currently investigating additional sesquiterpene lactones in order to define the structural requirements for NF-κB inhibition in more detail.

Taken together, we have shown that sesquiterpene lactones inhibit the NF-κB signaling cascade by directly targeting the active heterodimer. By selectively modifying the p65 subunit they use a unique mechanism of NF-κB inactivation, which is quite different from that of other anti-inflammatory agents (30). Based on our results, sesquiterpene lactones could serve as lead compounds for the development of novel, potent anti-inflammatory drugs for the treatment of inflammatory disorders such as rheumatoid arthritis or inflammatory bowel diseases. These drugs could also be important in the treatment or prevention of adult respiratory distress syndrome or systemic immune response syndrome.

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REFERENCES
1. Willuhn, G. (1981) Pharm. Unserer Zeit. 10, 1–7
2. Hall, I. H., Lee, K. H., Starnes, C. O., Sumida, Y., Wu, R. Y., Waddell, T. G., Cochran, J. W., and Gerhart, K. G. (1979) J. Pharmacol. Sci. 68, 537–541
3. Hall, I. H., Starnes, C. O., Lee, K.-H., and Waddell, T. G. (1980) J. Pharmacol. Sci. 69, 537–543
4. Schreider, H., Lüscher, W., Stroback, H., Leven, W., Willuhn, G., Till, U., and Schroe, K. (1990) Thromb. Res. 57, 839–845
5. Picman, A. K., Rodriguez, E., and Towers, C. H. N. (1979) Chem. Biol. Interact. 28, 83–89
6. Schmidt, T. J. (1997) Bioorg. Med. Chem. 5, 645–653
7. Kupchan, S. M., Eakin, M. A., and Thomas, A. M. (1971) J. Med. Chem. 14, 1147–1152
8. Lee, K. H., Meek, R., and Piantadosi, C. (1973) J. Med. Chem. 16, 299–301
9. Lyží, G., Schmidt, T. J., Merfort, I., and Pahl, H. L. (1997) Biol. Chem. 378, 951–961
10. Baeuerle, P., and Henkel, T. (1994) Annu. Rev. Immunol. 12, 141–179
11. Baeuerle, P., and Baltimore, D. (1996) Cell 87, 13–20
12. Kumar, S., Rabson, A. B., and Gilman, C. (1992) Mol. Cell. Biol. 12, 3094–3106
13. Matthews, J. R. Waksasugi, N., Virelizer, J.-L., Yodoi, J., and Hay, R. T. (1992) Nucleic Acids Res. 20, 3821–3830
14. Toldano, M. B., and Leonhard, W. J. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 4328–4332
15. Henehr, S. P., Heinrich, M., Bork, P. M., Vogt, M., Ratter, F., Lehmann, V., Schulze-Osthoff, K., Drige, W., and Schmitz, M. L. (1998) J. Biol. Chem. 273, 1288–1297
16. Graham, F. L., and Van Der Eb, A. J. (1973) Virology 52, 456–467
17. Ruben, S. M., Dillon, P. J., Schreck, R., Henkel, T., Chen, C.-H., Maher, M., Baeuerle, P. A., and Rosen, C. A. (1991) Science 251, 1490–1493
18. Schmitt, M. L., and Baeuerle, P. A. (1991) EMBO J. 10, 3805–3817
19. Willuhn, G., Kreßken, J., and Leven, W. (1990) Planta Med. 56, 111–114
20. Leven, W. (1988) Thesis, Heinrich-Heine-Universität, Düsseldorf
21. Merfort, I. and Wendisch, D. (1993) Phytochemistry 34, 1436–1437
22. Chen, Y.-Q., Ghosh, S., and Ghosh, G. (1998) Nat. Struct. Biol. 5, 67–73
23. Schreck, R., Meier, B., Mannel, D. N., Drige, W., and Baeuerle, P. A. (1992) J. Exp. Med. 175, 1181–1194
24. Wiebecke, M., Kreßken, J., Mootz, D., and Willuhn, G. (1982) Tetrahedron 38, 2709–2714
25. Schmidt, T. J. (1996) J. Mol. Struct. 385, 99–112
26. Ganchi, P. A., Sun, S. S., Green, W. C., and Ballard, D. W. (1993) Mol. Cell. Biol. 13, 7826–7835
27. Appendino, G., Calleri, M., Chiari, G., Garboldi, P., and Menichini, F. (1986) Gazz. Chim. Ital. 116, 637–641
28. Toldano, M. R., Ghosh, D., Trinh, P., and Leonard, W. J. (1993) Mol. Cell. Biol. 13, 852–860
29. Rüngeler, P., Lyží, G., Castro, V., Mura, G., Pahl, H. L., and Merfort, I. (1998) Planta Med. 64, 588–593
30. Baeuerle, P. A., and Baichwal, V. R. (1997) Adv. Immunol. 65, 111–137