Bioorganic & Medicinal Chemistry Letters 19 (2009) 1858–1860

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Inhibition of NF-*k*B and metalloproteinase-9 expression and secretion by parthenolide derivatives

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ARTICLE INFO

Article history:
Received 13 January 2009
Revised 18 February 2009
Accepted 20 February 2009
Available online 25 February 2009

Keywords:
Metalloproteinase-9
Gene expression
Parthenolide
Sesquiterpenes
Parthenolide derivatives
NF-*k*B driven transcription

ABSTRACT

Semisynthetic derivatives of parthenolide (**1**) were tested on NF-*k*B driven transcription and metalloproteinase-9 (MMP-9) expression and secretion. The four membered ring compounds **5** and **6,** obtained by acidic treatment of **1**, exhibited a higher activity with respect to **1** in all the biological assays. Then an increased ability of the **5** and **6** to inhibit NF-*k*B driven transcription may lead to a down-regulation of MMP-9 expression and secretion. This work provides new details about the structural requisites for NF-*k*B inhibition.

Parthenolide is the major active principle of *Tanacetum parthenium* (L.) Schultz Bip. (Asteraceae) used for the treatment of migraine and other pathologic situations related to inflammatory processes.[1] The anti-inflammatory activity of parthenolide and other sesquiterpene lactones (SLs) is exerted through the inhibition of the nuclear transcription factor-*k*B (NF-*k*B).[2–5] This transcription factor, when activated, induces the expression of a variety of genes that are known to be involved in atherosclerosis, cancer, and inflammatory diseases.[6] Matrix metalloproteinase-9 (MMP-9) is a member of a family of structurally related zinc-containing enzymes,[7] whose expression is partly controlled by NF-*k*B.[8] MMP-9 is overexpressed in many pathological conditions like cancer invasion and metastasis,[9] cartilage destruction in arthritis, atherosclerotic plaque rupture,[10] and gastric ulcer.[11]

Previous studies investigated the influence of chemical functionalities and molecular conformations of SLs in order to find a relationship between chemical structure and biological activity.[12–18] Despite this intensive research, the question of defining the pharmacophore for designing better pharmaceutical compounds remains open. In a previous study we prepared and tested semisynthetic derivatives of parthenolide on human leukocyte chemotaxis.[19] The results lead to the new hypothesis that the pharmacophore structure should be searched in the bicyclic core of the sesquiterpene compound. Since NF-*k*B represents the central mediator of the immune system and the target for parthenolide antinflammatory activity, in the present study we investigated the effect of the parthenolide semisynthetic derivatives previously described[19] on the NF-*k*B driven transcription and MMP-9 secretion and expression, with the aim to obtain more information about the structural requirements for NF-*k*B inhibition. The present study helps to highlight that stereochemistry and the hydroxyl functional groups of the carbocyclic skeleton modulate the parthenolide bioactivity.

NF-*k*B driven transcription was evaluated by transient transfection in Human Embryonic Kidney cells (HEK293) challenged with phorbol myristate acetate (PMA) 100 nM, as pro-inflammatory agent.[20] MMP-9 transcription was performed in HEK293 cells,[20] and MMP-9 secretion[21] was evaluated in THP-1 macrophages treated with 10 nM PMA.[22] Cytotoxicity of the compounds towards THP-1 and HEK293 cells was investigated by the MTT assay.[23] Structures of the compounds under study are reported in Figure 1. Electrophilic transannular cyclization reaction on **1** performed in methanol at acidic pH lead to the synthesis of compounds **3**–**5**.[19] Taking into account that the physiological environment is aqueous, the cyclization was carried out in acetone/water mixture, thus obtaining **6**,[24] which revealed to be spectroscopically identical to michampanolide previously isolated from *Michelia champaca* L. (Magnoliaceae).[25] We also performed this reaction in water and strong proton exchanger beads as catalyst thus affording identical products.

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doi:10.1016/j.bmcl.2009.02.080

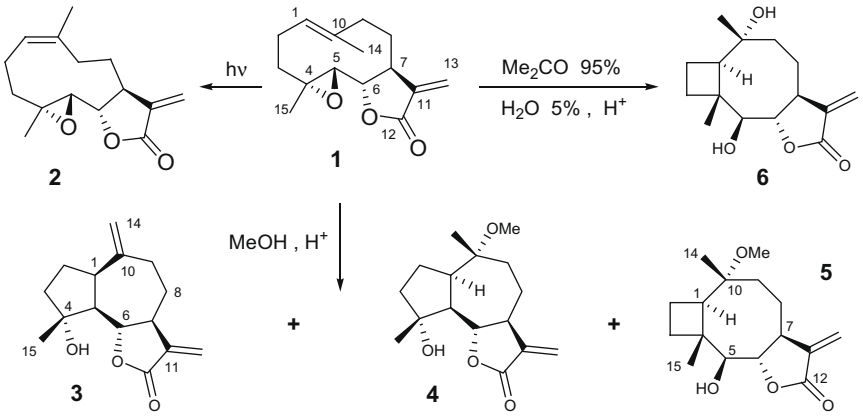

Figure 1. Chemical modifications of parthenolide (**1**).

Table 1
Effect of parthenolide derivatives on NF-*k*B driven transcription

Compound	IC_{50} ± SD (μM)
1	9.76 ± 0.83
2	7.4 ± 0.85
3	8.8 ± 0.42
4	6.0 ± 1.31[a]
5	2.04 ± 0.36[a]
6	2.8 ± 0.12[a]

[a] IC_{50} of **4** ($p < 0.05$), **5**, and **6** ($p < 0.01$) are statistically different with respect to **1** (parthenolide).

Table 2
Effects of parthenolide derivatives on MMP-9 gene transcription

Compound	IC_{50} ± SD (μM)
1	6.6 ± 1.2
2	8.5 ± 1.1
3	6.6 ± 1.7
4	8.6 ± 1.6
5	3.2 ± 0.5[a]
6	3.8 ± 0.3[a]

[a] IC_{50} of **5** ($p < 0.01$) and **6** ($p < 0.05$) are statistically different with respect to **1** (parthenolide).

The results of the bioassays are shown in Tables 1 and 2 and Figure 2. Statistical analysis was performed by one-way Anova analysis of variance with post-hoc Bonferroni test, using Graph Pad Prism 4. Log*P* values were calculated by a software ACD/ChemSketch.11.

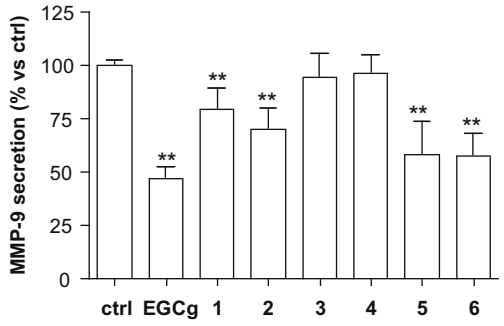

Figure 2. Effects of parthenolide derivatives on MMP-9 secretion in THP-1 macrophages. Compounds were tested at 20 μM; this concentration was not cytotoxic, as assessed by the MTT test. Results are expressed as OD units normalized for the intracellular protein content, and are calculated as % versus ctrl treated only with vehicle. Results are the mean ± SD of three experiments in triplicate. Epigallocatechin-3-gallate (EGCg) 20 μM was used as reference compound. **5** and **6** are statistically different with respect to parthenolide ($p < 0.05$). $^{**}p < 0.01$ versus ctrl.

Regarding the effect on NF-*k*B driven transcription, IC_{50}s of compound **2**, and **3** were not statistically different when compared with **1**, while compounds **5** and **6** showed IC_{50}s 3–5 fold lower than that of **1** (Table 1). The inhibitory effect on MMP-9 transcription and secretion exerted by **2**–**4** did not significantly differ from that of **1**. Conversely, the higher inhibitory potency against NF-*k*B was reflected in a higher effect of **5** and **6** on MMP-9 transcription (Table 2) and secretion (Fig. 2). At 20 μM, the highest concentration used in the experiments, the derivatives were not cytotoxic.

In previous studies,[12,17,18] the biological activity of **1** and other SLs has been related to the alkylating ability of the conjugated exomethylene lactone. This moiety is kept unaltered in the derivatives presently tested, therefore the alkylating potential is not sufficient to explain the differences highlighted in our experiments and other chemical and structural properties must be taken into consideration.

Compounds **3**–**6** are more polar than **1** because of their hydroxyl group at C4 (**3** and **4**, 3° alcohol oriented below the main molecular plane) or C5 (**5**, 2° alcohol oriented above the main molecular plane) or C5 and C10 (**6**, 2° and 3° alcohols oriented above and below, respectively). Such a different polarity is mirrored by the shift of log*P* values, from 2.42 ± 0.42 of **1** to 0.86 ± 0.42 (mean ± SD) of **6**. A linear correlation was found between the log*P* values and the IC_{50}s calculated for NF-*k*B inhibition ($r = 0.83$). The direct correlation between partition coefficient and inhibitory activity may suggest two different possibilities: either compounds **5** and **6** are more accessible to the site of action because they diffuse better, or the higher is the hydrophilia the better is the conformation in aqueous solution thus facilitating the interaction with the target protein.

From a structural point of view, compounds **3** and **4** or **5** and **6** belong to the [5.3.0] or [6.2.0] decane ring systems so that their docking features may be different and also modulated by the different accessibility of 3° versus 2° alcohol. Moreover, the hydroxyl group at C10 in **6** gives additional proton donor character in the hydrogen bond formation.

At this stage, we may conclude that the bioactivity of parthenolide derivatives depends on the carbocyclic skeleton as well as on its stereochemistry and functionalization.

One more clue from this study is the instability of **1** in aqueous medium at acidic pH (Fig. 1), from which we may assume that in the gastric environment **1** is transformed into more active metabolites. The hypothesis that **1** may behave as a pro-drug was previously formulated[19] and receives further support from this study. Data on metabolic transformation of **1** in vivo are not reported, but patients treated daily with an oral dose of a feverfew preparation containing

up to 4 mg of parthenolide had undetectable plasma concentrations of parthenolide.[26] If further ADME studies confirm our hypothesis, new perspectives will open for drug optimization.

Acknowledgements

This work was supported by private grants to Centro Studi e Ricerche sulla Caratterizzazione e Sicurezza d'Uso di Sostanze Naturali-Giovanni Galli. We are grateful to Dr. Carla Vender and the CRA (Consiglio per la Ricerca e la Sperimentazione in Agricoltura, Villazzano—Trento) for providing the aerial part of *Tanacetum parthenium* L.

References and notes

1. Knight, D. W. *Nat. Prod. Rep.* **1995**, *12*, 271.
2. Hehner, S. P.; Heinrich, M.; Bork, P. M.; Vogt, M.; Ratter, F.; Lehmann, V.; Schulze-Osthoff, K.; Droge, W.; Schmitz, M. L. *J. Biol. Chem.* **1998**, *273*, 1288.
3. Lyss, G.; Knorre, A.; Schmidt, T. J.; Pahl, H. L.; Merfort, I. *J. Biol. Chem.* **1998**, *273*, 33508.
4. Garcia-Pineres, A. J.; Castro, V.; Mora, G.; Schmidt, T. J.; Strunck, E.; Pahl, H. L.; Merfort, I. *J. Biol. Chem.* **2001**, *276*, 39713.
5. Garcia-Pineres, A. J.; Lindenmeyer, M. T.; Merfort, I. *Life Sci.* **2004**, *75*, 841.
6. Aggarwal, B. B.; Shishodia, S. *Ann. N.Y. Acad. Sci.* **2004**, *1030*, 434.
7. Massova, I.; Kotra, L. P.; Fridman, R.; Mobashery, S. *FASEB J.* **1998**, *12*, 1075.
8. Moon, S. K.; Cha, B. Y.; Kim, C. H. *J. Cell. Physiol.* **2004**, *198*, 417.
9. Johansson, N.; Ahonen, M.; Kahari, V. M. *Cell. Mol. Life Sci.* **2000**, *57*, 5.
10. Beaudeux, J. L.; Giral, P.; Bruckert, E.; Foglietti, M. J.; Chapman, M. J. *Clin. Chem. Lab. Med.* **2004**, *42*, 121.
11. Mori, N.; Sato, H.; Hayashibara, T.; Senba, M.; Geleziunas, R.; Wada, A.; Hirayama, T.; Yamamoto, N. *Gastroenterology* **2003**, *124*, 983.
12. Rodriguez, E.; Towers, G. H. N.; Mitchell, J. C. *Phytochemistry* **1976**, *15*, 1573.
13. Fischer, N. H.; Weidenhamer, J. D.; Riopel, J. L.; Quijano, L.; Menelaou, M. A. *Phytochemistry* **1990**, *29*, 2479.
14. Macias, F. A.; Galindo, J. C.; Massanet, G. M. *Phytochemistry* **1992**, *31*, 1969.
15. Fischer, N. H.; Lu, T.; Cantrell, C. L.; Castaneda-Acosta, J.; Quijano, L.; Franzblau, S. G. *Phytochemistry* **1998**, *49*, 559.
16. Rungeler, P.; Castro, V.; Mora, G.; Goren, N.; Vichnewski, W.; Pahl, H. L.; Merfort, I.; Schmidt, T. J. *Bioorg. Med. Chem.* **1999**, *7*, 2343.
17. Siedle, B.; Garcia-Pineres, A. J.; Murillo, R.; Schulte-Monting, J.; Castro, V.; Rungeler, P.; Klaas, C. A.; Da Costa, F. B.; Kisiel, W.; Merfort, I. *J. Med. Chem.* **2004**, *47*, 6042.
18. Wagner, S.; Hofmann, A.; Siedle, B.; Terfloth, L.; Merfort, I.; Gasteiger, J. *J. Med. Chem.* **2006**, *49*, 2241.
19. Neukirch, H.; Kaneider, N. C.; Wiedermann, C. J.; Guerriero, A.; D'Ambrosio, M. *Bioorg. Med. Chem.* **2003**, *11*, 1503.
20. Human Embryonic Kidney cells (HEK293) were plated in 24-well plates (70,000 cell/well) two days before transfection in DMEM/F-12 nutrient mixture, supplemented with 10% fetal calf serum, in the presence of compounds under study or vehicle. Transient transfection was performed by calcium phosphate co-precipitation technique. Briefly, a unique co-precipitate containing each reporter plasmid/luciferase (NF-kB luc or 2.2 luc) was prepared and aliquoted in each well to ensure that all samples were transfected with the same amount of plasmid DNA (1.2 μg of luciferase plasmid/well). After 16 h at 37 °C, cells were washed with PBS and incubated for 24 h in medium containing compounds (0.5–20 μM) to be tested or the vehicle (DMSO, 0.1%) in the presence of PMA 100 nM.
21. Dell'Agli, M.; Bellosta, S.; Rizzi, L.; Galli, G. V.; Canavesi, M.; Rota, F.; Parente, R.; Bosisio, E.; Romeo, S. *Cell. Mol. Life Sci.* **2005**, *62*, 2896.
22. THP-1 cells were plated in 24-well plated (1.2×10^6 cells/well) in the presence of 10 nM PMA to induce differentiation. 48 h after, THP-1 macrophages were treated with compounds under study (20 μM).
23. Denizot, F.; Lang, R. *J. Immunol. Methods* **1986**, *89*, 271.
24. *Experimental procedure for the preparation of* **6**: (−)(3a*S*,6*R*,6a*R*,8a*R*,9*S*,9a*S*)-Decahydro-6,9-dihydroxy-6,8a-dimethyl-3-methylenecyclobuta[6,7]cycloocta[1,2-*b*]furan-2(3*H*)-one. To a solution of parthenolide **1** (40.0 mg, 0.161 mmol) in 10 mL of acetone/water 95:5 was added amberlyst® 15 (0.5 g) at room temperature under stirring. After 3 days, the reaction mixture was subject to flash chromatography on silica (Merck LiChroprep Si60, 15–25 μm), and eluted with gradient of diethylether in hexane to afford 15 fractions. The final fraction, eluted with ethyl acetate, was evaporated and the residues were submitted to HPLC purification (Reinin Dynamax 60A SiO_2 (8 μm, 25 × 1 cm) column, solvent flux 3 mL/min, $\lambda = 225$ nm, hexane/isopropanol 7:3, $R_t = 11.8$ min), thus furnishing **6** (4.3 mg). NMR: Bruker AV400 (^{1}H and 2D spectra at 400 MHz, δ in ppm using residual solvent signals as internal standard ($CDCl_3 = 77.0$, $CHCl_3 = 7.26$), ^{13}C frequencies were obtained from edited HSQC and HMBC maps. NOESY data are reported as correlation map(s) between protons ^{1}H ↔ ^{1}H; HMBC data are reported as ^{13}C → correlated to ^{1}H. Mass spectra: *Kratos MS80* with home-built acquisition system.
^{1}H NMR: 6.27 (d, *J* = 3.6 Hz, 1H, H-13a), 5.59 (d, *J* = 3.2 Hz, 1H, H-13b), 4.19 (dd, *J* = 9.8, 7.5 Hz, 1H, H-6), 3.60 (d, *J* = 9.8 Hz, 1H, H-5), 3.13 (m, 1H, H-7), 2.53 (dd, *J* = 11.0, 8.3 Hz, 1H, H-1), 2.41 (dddd, *J* = 14.5, 12.9, 6.1, 3.9 Hz, 1H, H-8α), 2.08 (dddd, *J* = 11.0, 11.0, 10.8, 9.1 Hz, 1H, H-2β), 1.95 (dddd, *J* = 11.0, 8.3, 8.3, 1.6 Hz, 1H, H-2α), 1.85 (ddd, *J* = 15.2, 12.9, 3.8 Hz, 1H, H-9β), 1.82 (ddd, *J* = 10.9, 10.8, 8.3 Hz, 1H, H-3α), 1.71 (ddd, *J* = 10.9, 9.1, 1.6 Hz, 1H, H-3β), 1.66 (ddd, *J* = 15.2, 4.6, 3.9 Hz, 1H, H-9α), 1.49 (dddd, *J* = 14.5, 11.0, 4.6, 3.8 Hz, 1H, H-8β), 1.32 (s, 3H, H-14), 1.27 (s, 3H, H-15). ^{13}C NMR ($CDCl_3$): δ 168.9 (s, C-12), 140.5 (s, C-11), 122.0 (t, C-13), 84.4 (d, C-5), 82.1 (d, C-6), 73.2 (s, C-10), 48.1 (d, C-1), 43.1 (s, C-4), 41.1 (d, C-7), 37.3 (t, C-9), 33.4 (t, C-3), 29.3 (q, C-14), 26.7 (t, C-8), 19.9 (t, C-2), 14.1 (q, C-15). NOESY: H-7 ↔ H-1, H-5 ↔ H-1 and H-7 and H-3α, 3H-15 ↔ H-6 and H-9β, 3H-14 ↔ H-2β, H-13b ↔ H-8α. HMBC: C-12 → 2H-13, C-11 → H-13a, C-10 → H-1 and 3H-14, C-4 → H-1 and H-5 and 3H-15.
EIMS (70 eV) *m/z* (rel. int.): 267 $[M+H]^+$ (1), 251 $[M-Me]^+$ (1), 248 $[M-H_2O]^+$ (2), 233 $[M-Me-H_2O]^+$ (3), 230 $[M-H_2O-H_2O]^+$ (3), 220 $[M\text{-}46]^+$ (7), 205 $[M\text{-}46\text{-}15]^+$ (9), 43 (100). HREIMS *m/z* 251.1282 ± 0.005 $[C_{14}H_{19}O_4]^+$, calcd 251.1283; 248.1401 ± 0.005 $[C_{15}H_{20}O_3]^+$, calcd 248.1412; 233.1179 ± 0.005 $[C_{14}H_{17}O_3]^+$, calcd 233.1178; 230.1296 ± 0.005 $[C_{15}H_{18}O_2]^+$, calcd 230.1307.
25. Jacobsson, U.; Kumar, V.; Saminathan, S. *Phytochemistry* **1995**, *39*, 839.
26. Curry, E. A., 3rd; Murry, D. J.; Yoder, C.; Fife, K.; Armstrong, V.; Nakshatri, H.; O'Connell, M.; Sweeney, C. J. *Invest. New Drug* **2004**, *22*, 299.