JPP Journal of Pharmacy and Pharmacology

JPP 2009, 61: 919–924

Received June 03, 2008
Accepted March 30, 2009
DOI 10.1211/jpp/61.07.0011
ISSN 0022-3573

Research Paper

Hexa-, hepta- and nonaprenylhydroquinones isolated from marine sponges *Sarcotragus muscarum* and *Ircinia fasciculata* inhibit NF-κB signalling in H4IIE cells

Wim Wätjen[a], Annika Putz[b*], Yvonni Chovolou[a], Andreas Kampkötter[a], Frank Totzke[c], Michael H.G. Kubbutat[c], Peter Proksch[b] and Belma Konuklugil[d]

[a]Institute of Toxicology, [b]Institute of Pharmaceutical Biology and Biotechnology, Heinrich-Heine-Universität, Düsseldorf, Germany, [c]ProQinase GmbH, Freiburg, Germany and [d]Ankara University, Faculty of Pharmacy, Pharmacognosy, Ankara, Turkey

Abstract

Objectives Marine organisms have proven to be a rich source of potent pharmacologically active compounds. Three polyprenyl-1,4-hydroquinone derivates (hexaprenyl-1,4-hydroquinone, heptaprenyl-1,4-hydroquinone and nonaprenyl-1,4-hydroquinone) were isolated from the Zoobenthos-inhabiting sponges *Sarcotragus muscarum* and *Ircinia fasciculata* from the Eastern Mediterranean Sea (phylum: Porifera; class: Demospongiae).
Methods Hexa-, hepta- and nonaprenylhydroquinone were identified by ^{1}H-NMR, H,H-COSY, heteronuclear multiple bond correlation, FAB-MS and UV spectroscopy. The effects of the compounds on cell viability was determined using the MTT assay; antioxidative potential was measured using the Trolox equivalent antioxidative capacity assay. Inhibition of nuclear factor-κB activity was detected by secreted alkaline phosphatase assay. Activity against an array of protein kinases was determined in 96-well FlashPlates.
Key findings All compounds had prominent antioxidative activity, comparable to that of the synthetic vitamin E derivate Trolox. Hexaprenylhydroquinone showed the greatest cytotoxicity in H4IIE hepatoma cells (EC50 2.5 μM). All three compounds inhibited NF-κB signalling in this cell line, with heptaprenylhydroquinone being the most active. Screening of 23 kinases involved in signal transduction pathways (cell proliferation, survival, angiogenesis and metastasis) showed that hexaprenylhydroquinone and heptaprenylhydroquinone inhibited the activity of the epidermal growth factor receptor (IC50 1.6 and 1.4 μg/ml, respectively), and heptaprenylhydroquinone also inhibited the activity of other kinases (Src tyrosine kinase, vascular endothelial growth factor receptor 3 and insulin-like growth factor 1 receptor).
Conclusions The prenylated hydroquinones isolated from the marine sponges *S. muscarum* and *I. fasciculata* showed cytotoxic and antioxidative activities and inhibited NF-κB signalling in H4IIE hepatoma cells and protein kinases. These findings may result in the generation of new lead substances in cancer therapy.
Keywords cancer; cytotoxicity; marine natural products; nuclear factor-κB (NF-κB); oxidative stress; protein kinases

Introduction

With an increasing cancer rate worldwide, there is an urgent need for the improvement of existing and discovery of new anti-cancer drugs. Natural products are an important source of chemotherapeutic agents. About two-thirds of anti-cancer drugs approved today are either natural products or have been developed on the basis of knowledge gained from natural products.[1] Marine organisms have proven to be a rich source of potent pharmacologically active compounds.[2,3] The bioactivity of the diverse structures have made them attractive targets for both biomedical and synthetic purposes (e.g. as chemotherapeutic modalities). We analysed the cytotoxic activities of prenylated hydroquinones isolated from the Zoobenthos-inhabiting sponges *Sarcotragus muscarum* and *Ircinia fasciculata* (phylum Porifera; class Demospongiae) against a tumour cell line.

Correspondence: Wim Wätjen, Heinrich-Heine-Universität, Institute of Toxicology, PO Box 101007, 40001 Düsseldorf, Germany. E-mail: wim.waetjen@uni-duesseldorf.de

*This work forms part of Annika Putz's ongoing PhD thesis.

Sponges of the genus *Sarcotragus* are reported to contain various compounds such as variabilin, 8-hydroxy-variabilin, 14-furan-3-yl-3,7,11-trimethyltetradeca-7,11-dienoic acid octa- and nonaprenylhydroquinone sulfate and geranylfarnesylacetone. Gil and colleagues[4] reported cyclitol derivatives from this species. Ceramides (e.g. ircisulfamide) and glycosphingolipids (ircicerebroside) have been isolated from *I. fasciculata*. Fusetani and colleagues[5] isolated hexaprenylhydroquinone sulfate from a marine sponge *Dysidea* sp. and reported inhibitory activities on K-ATPase and phospholipase A2. Liu and colleagues analysed cytotoxic constituents of *Sarcotragus* sp., since the methanol extract of the sponge displayed cytotoxicity against five human tumour cell lines (A549, SK-OV-3, SK-MEL-2, XF498 and HCT15) and toxicity against brine shrimp larvae (LD50 93 μg/ml).[6] Guided by the brine shrimp assay, this extract was successively fractionated, yielding furanosesterterpene tetronic acids that had moderate-to-significant activity against five human tumour cell lines.[6] Furthermore, cytotoxic sesterterpenoids and norsesterterpenoids were isolated from two *Sarcotragus* sponges:[7] norsesterterpenoids, a sesterterpenoid, pyrroloterpenoids and a stereoisomer of kurospongin. These compounds were analysed for cytotoxicity in five human tumour cell lines; the sesterterpenoid and pyrroloterpenoids were not toxic (ED50 > 30 μg/ml); the other compounds (furanoterpenoids) showed marginal-to-moderate cytotoxic effects (highest toxicity was shown for sarcotin O in SK-MEL-2 cells: ED50 3 μg/μl). In addition, pyrrolosesterterpenes, furanosesterpene derivatives and furanoterpenoids (including two trinorsesterterpenes and two diterpenes) with cytotoxic effects were isolated (highest toxicity was shown for epi-sarcotrine A in SK-MEL-2 cells; ED50 3.4 μg/μl).[8]

We isolated hexaprenyl-1,4-hydroquinone, heptaprenyl-1,4-hydroquinone and nonaprenyl-1,4-hydroquinone from the Zoobenthos-inhabiting sponges *S. muscarum* and *I. fasciculata* from the Eastern Mediterranean Sea and studied their cytotoxic activity against different tumour cell lines as well as their effects on important protein kinases and the nuclear factor (NF)-κB signalling pathway.

Materials and Methods

All chemicals were of analytical grade and were purchased from Sigma (Deisenhofen, Germany) or Merck (Darmstadt, Germany). All tissue culture reagents were purchased from PAA (Coelbe, Germany); plastic materials for cell culture were obtained from Falcon (Heidelberg, Germany).

Sponge materials

S. muscarum material was collected in Mersin, Turkey in September 2005 (voucher specimen ZMA19034, retained at the Zoological Museum of the University of Amsterdam, the Netherlands); *I. fasciculata* was collected in Fethiye, Turkey in February 2006 (voucher specimen ZMAPOR19099). Tissues were preserved in 70% ethanol.

The sponge tissues (500 g wet weight *S. muscarum*, 950 g *I. fasciculata*) were exhaustively extracted in methanol overnight. Extraction was repeated twice for 1 h on the following day. The dried methanolic extract was reconstituted in aqueous methanol (90 : 10) and subjected to liquid/liquid partitioning against *n*-hexane. The aqueous phase was evaporated to dryness and subjected to liquid/liquid extraction against ethyl acetate.

Isolation of hexa- and nonaprenylhydroquinone

The *S. muscarum* ethyl acetate phase of 2.6 g was separated by gel filtration (Sephadex LH-20, 100% methanol) and the hydroquinone fraction (1.2 g) further fractionated by vacuum liquid chromatography (Silica gel; Kieselgel 60; Merck), yielding the hydroquinones in the fraction eluted with *n*-hexane/ethyl acetate (60 : 40, 855 mg). This fraction was then subjected to gel filtration over Sephadex LH-20 (methanol : dichloromethane 50 : 50). The fraction containing the hydroquinones (392 mg) was further purified using Licroprep diol (40–63 μm, Merck; *n*-hexane : acetone 90 : 10), yielding a mixture of hexa- and nonaprenylhydroquinone (143 mg), which could be separated by semipreparative HPLC (PrepStar (Varian, Darmstadt, Germany), Microsorb 60-8 C18 column, 250 × 21.4 mm (Dynamax, Houston, TX, USA), eluent 100% methanol), yielding 87 mg hexaprenylhydroquinone and 18 mg nonaprenylhydroquinone.

Isolation of heptaprenylhydroquinone

The 950 g (wet weight) *I. fasciculata* tissue extracted yielded 5 g ethyl acetate phase. The latter was subjected to gel filtration over Sephadex LH-20 (methanol). The fraction yielding heptaprenylhydroquinone (4.5 g) was further purified with Sephadex LH-20 (methanol : dichloromethane 50 : 50), yielding 2.7 g of a fraction containing heptaprenylhydroquinone. Of this fraction, 300 mg was further purified via LiCroprep DIOL (40–63 μm, Merck; *n*-hexane : acetone 70 : 30 and 80 : 20 and 100% dichloromethane, 25 mg). Final isolation was achieved by semipreparative HPLC (LaChrom L-7100, Merck/Hitachi, Eurospher 100-C18 250 × 8 mm column (Knauer, Berlin, Germany), eluent 93% methanol), when 8 mg heptaprenylhydroquinone was obtained.

Structural elucidation of compounds

All three compounds were identified by online UV spectrophotometry (P580A; Dionex, Idstein, Germany), fast atom bombardment MS (LCQ Deca, Thermo Finnigan, Dreieich, Germany) and NMR (DRX 500; Bruker, Rheinstetten, Germany) equipped with a triple broadband inverse gradient head for ^{1}H and heteronuclear multiple bond correlation (HMBC) spectra (500 MHz), and a quadro nuclear probe head for ^{13}C spectra (125 MHz). Structure elucidation was derived from the interpretation of ^{1}H NMR and two-dimensional techniques such as H,H-COSY and HMBC and comparison with literature data.[9–11]

Antioxidant activity

Trolox-equivalent antioxidative capacity (TEAC) was measured spectrophotometrically by analysing decoloration of the stable radical cation 2,2′-azino-bis(3-ethylbenz-thiazoline-6-sulfonic acid (ABTS) at 734 nm in comparison with the synthetic antioxidant Trolox.[12] Absorption was measured after 4 min of mixing the hydroquinones with the ABTS solution.

Cell culture

H4IIE rat hepatoma cells were grown in Dulbecco's modified essential medium (DMEM) containing 4.5 g/l glucose and 2 mmol/l L-glutamine, 10% fetal bovine serum, 100 units/ml penicillin and 100 μg/ml streptomycin. The medium was changed twice a week. The cells were maintained in a humidified atmosphere at 37°C with 5% CO_2.

Determination of cytotoxicity

The effect of isolated compounds on cell viability was determined using the MTT assay.[13] Cells were plated into 96-well plates (10 000 cells per well) and allowed to attach for 24 h. They were then treated with different concentrations of the prenylated hydroquinones for 24 h. After this treatment the medium was changed and the cells were incubated for 3 h under cell culture conditions with 700 μg/ml MTT, after which cells were lysed in 50% ethanol/49% water/1% acetic acid. The concentration of reduced MTT, measured photometrically at 560 nm, was taken as a marker of cell viability.

Inhibition of NF-κB

H4IIE cells were stably transfected with HiFect transfection reagent (Lonza, Cologne, Germany) according to the manufacturer's instructions. Briefly, H4IIE cells were seeded at a density of 1.5×10^5 cells per 35 mm Petri dish and incubated overnight. Cells were then transfected with 1.6 μg pNF-κB–SEAP and 0.4 μg pTK-hygromycin selection vector using 10 μl HiFect transfection reagent in 1 ml serum-free DMEM and 48 h later were transferred into 100 mm Petri dishes; stably transfected cell clones (H4IIE–SEAP) were selected with 400 μg/ml hygromycin.

For the reporter gene assay, H4IIE–SEAP cells were seeded in 12-well plates at a density of 2×10^5 cells per well and incubated for 48 h. Cells were preincubated with hexaprenylhydroquinone (0.5 and 1 μM), nonaprenylhydroquinone and heptaprenylhydroquinone (both 5 and 10 μM) for 1 h and then stimulated with 5 ng/ml tumour necrosis factor (TNF)α for 24 h. Activity of the reporter enzyme secreted alkaline phosphatase (SEAP) in the medium was measured using a chemiluminescence-based detection method. In brief, 30 μl conditioned cell culture medium was mixed with 30 μl 1 × dilution buffer (50 mM Tris, 150 mM NaCl, pH 7.4) and incubated for 30 min at 65°C to heat inactivate endogenous alkaline phosphatase activity. Samples were mixed with 30 μl assay buffer (2 M diethanolamine, 28 mM L-homoarginine) and 30 μl CSPD (chemiluminescent substrate: disodium 3-(4-methoxyspiro {1,2-dioxetane-3,2′-(5′-chloro)tricyclo[3.3. $1.1^{3,7}$]decan}-4-yl) phenyl phosphate)). After 15 min' incubation in the dark, SEAP activity was measured using a plate luminometer (Victor 1420, Wallac, PerkinElmer, Rodgau-Jügesheim, Germany). In each experiment we confirmed that inhibition of SEAP activity was not due to cytotoxic effects (MTT assay).

Protein kinase activity

Assays for the measurement of protein kinase activity were performed in 96-well FlashPlates (Perkin Elmer/NEN, Boston, MA, USA) in a 50 μl reaction volume. The reaction cocktail contained 20 μl assay buffer, 5 μl ATP solution (in water), 5 μl test compound (in 10% DMSO), 10 μl substrate and 10 μl purified recombinant protein kinase. The final concentration of ATP was 1 μM. The assay for all enzymes contained 60 mM HEPES-NaOH, pH 7.5, 3 mM $MgCl_2$, 3 mM $MnCl_2$, 3 μM Na-orthovanadate, 1.2 mM DTT, 50 μg/ml PEG20000 and 1 μM [γ-^{33}P]-ATP (approximately 5×10^5 cpm per well). The following substrates were used: glycogen synthase kinase 3 (GSK3) (14–27): Akt1 serine–threonine kinase; tetra(LRRWSLG): Aurora serine/threonine kinases A and B; MEK kinase 1: B-Raf kinase; Histone H1: cyclin-dependent kinase (CDK)2/cyclin A; Rb-CTF: CDK4/cyclin D1; Poly(Glu,Tyr)4:1: epidermal growth factor receptor (EGF-R), ephrin type B receptor 4 (EphB4), ErbB2 receptor protein tyrosine kinase, focal adhesion kinase (FAK), insulin-like growth factor 1 receptor (IGF1-R), Src tyrosine kinase, vascular endothelial growth factor receptor (VEGF-R)2, VEGF-R3, Tie2 cell-surface receptor; poly(Ala,Glu,Lys, Tyr): FMS-like tyrosine kinase 3 (FLT3), Met, platelet-derived growth factor(PDGF)-Rb; casein: Polo-like kinase (PLK)1, anti-casein kinase 2 alpha (CK2a1). Autophosphorylation was measured for ARK5 serine/threonine kinase, COT kinase and SAK kinase. In a primary screen, compounds were tested at a final concentration of 1 μg/ml (corresponding to 1.93 μM hexaprenylhydroquinone, 1.38 μM nonaprenylhydroquinone and 1.68 μM heptaprenylhydroquinone; $n = 1$) against the 23 protein kinases. The IC50 was then determined for compounds that inhibited the activity of at least one kinase by at least 45%. IC50 determinations for all 23 protein kinases were performed using ten concentrations ranging from 10 μg/ml to 30 ng/ml in semi-logarithmic dilution steps ($n = 1$ per concentration). This corresponds to calculated molar concentration ranges of 57.9 nM to 19.3 μM for hexaprenylhydroquinone, and 50.3 nM to 16.8 μM for heptaprenylquinone.

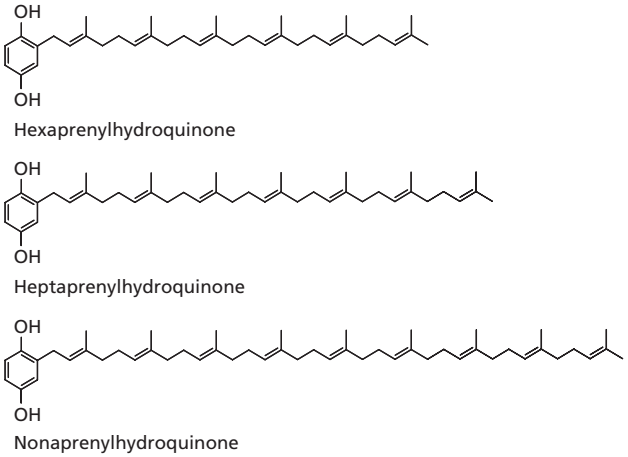
Statistical analysis

Values are given as means ± SEM of at least three independent experiments. The significance of changes in the test responses was assessed using the non-parametric Kruskal–Wallis test, followed by Dunn's multiple comparison test using GraphPad Prism 5 (La Jolla, CA, USA). Differences were considered to be significant at $P < 0.05$.

Results

Isolation of prenylated hydroquinones

Hexa-, hepta- and nonaprenylhydroquinones were isolated from the marine sponges *S. muscarum* and *I. fasciculata* (Figure 1). FAB-MS spectra showed a series of peaks characteristic for prenylated 1,4-benzoquinones at *m/z* M^+-69-(68)n arising from successive loss of isoprene units, as described by Cimino and colleagues.[9] ^{1}H-NMR (500 MHz, CD_3OD) data exhibited signals at δ_H 6.52 (1H, d, J = 2.8 Hz), 6.42 (1H, dd, J = 2.8, 8.5 Hz) and 6.56 (1H, d, J = 8.5), corresponding to the aromatic protons of a hydroquinone. The presence of a side chain of isoprenic units could be inferred by signals δ_H 3.23 (2H, d, J = 7.3 Hz), 5.31



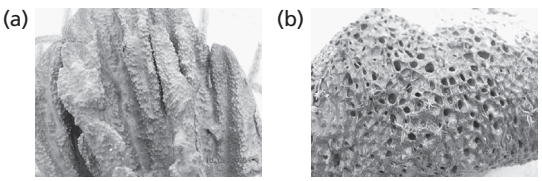


Figure 1 Structures of isolated compounds: hexaprenylhydroquinone, heptaprenylhydroquinone and nonaprenylhydroquinone, and the marine sponges (a) *Sarcotragus muscarum* and (b) *Ircinia variabilis.*

(1H, t, J = 7.3 Hz), 2.13 (2H, t, J = 6.8) and 1.69 (3H, s), representing the isoprene unit attached directly to the ring, and the slightly shifted signals of the remaining isoprene units. An all-*trans* polyprenylic side chain was identified by comparison with previously reported data.[9] Peak integrals allowed for the determination of the number of isoprene units attached to the hydroquinone. H,H-COSY and HMBC correlations confirmed these observations. Careful inspection of ^{1}H- NMR, H,H-COSY and HMBC data, UV spectra and MS spectra and comparison with literature data[9–11] identified the isolated compounds as hexa-, hepta- and nonaprenylhydroquinone.

Antioxidative activity of prenylated hydroquinones

The antioxidative activity of the prenylated hydroquinones measured using the TEAC assay showing a strong antioxidative potential similar to that of the synthetic vitamin E derivative Trolox (Figure 2). However, radical-scavenging effects were less than those of quercetin, a flavonoid widely used for its antioxidative properties (10 μM quercetin reduced the absorption to 0.102 ± 0.018 (data not shown).

Cytotoxicity of prenylated hydroquinones

The cytotoxicity of the isolated prenylhydroquinones was determined in H4IIE hepatoma cells using the MTT assay (Figure 3). The EC50 value for hexaprenylhydroquinone was in the low micromolar range (2.5 μM). EC50 values for hepta- and nonaprenylhydroquinone were about tenfold higher (approximately 25 μM).

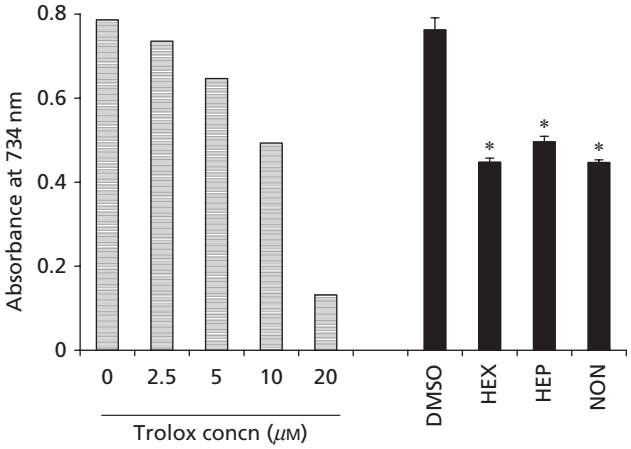


Figure 2 Antioxidative activity of prenylated hydroquinones. The antioxidative capacity of hexaprenylhydroquinone (HEX), heptaprenylhydroquinone (HEP) and nonaprenylhydroquinone (NON) is shown in comparison with the synthetic antioxidant Trolox. Values are means ± SEM ($n > 3$); *$P < 0.05$ vs control (DMSO).

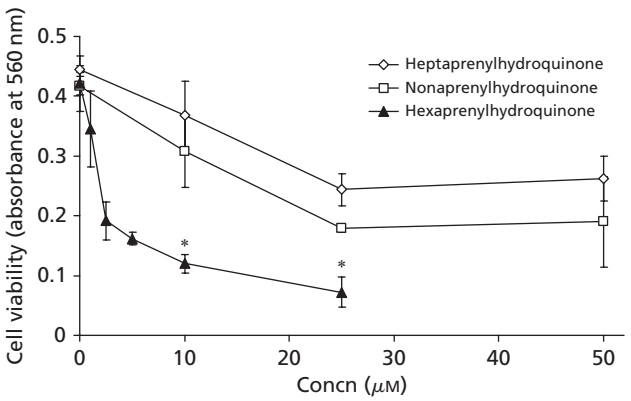


Figure 3 Cytotoxicity of isolated compounds in H4IIE cells. Cells were incubated with the prenylated hydroquinones for 24 h, then MTT reduction was measured as a marker of cell viability (absorbance at 560 nm). Values are means ± SEM (n = 2–5); *$P < 0.05$ vs corresponding control (DMSO).

Inhibition of NF-κB

The transcription factor NF-κB regulates a variety of physiological processes, including cell growth, oxidative stress response and apoptosis. We therefore analysed whether the prenylated hydroquinones interfered with the activation of NF-κB by the cytokine TNFα in H4IIE cells stably transfected with the pNF-κB–SEAP reporter plasmid. Incubation with TNFα (5 ng/ml, 24 h) increased SEAP activity about 3.3-fold. Preincubation with 1 μM (1 h) hexaprenylhydroquinone or 10 μM heptaprenylhydroquinone or nonaprenylhydroquinone significantly lowered NF-κB activity after stimulation by TNFα to 73 ± 7.7% and 72 ± 5.3% of the TNFα-stimulated value, respectively. On the other hand, basal NF-κB activity was not modulated by these substances (Figure 4). As a positive control, the known NF-κB inhibitor caffeic acid ethylphenylester (CAPE) was used: incubation of H4IIE cells with 40 μM CAPE (1 h)

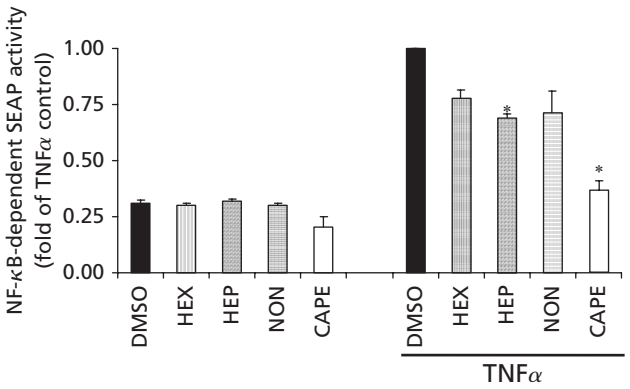


Figure 4 Effects of isolated compounds on activation of nuclear factor κB. H4IIE cells stably transfected with pNF-κB–SEAP reporter plasmid were preincubated for 1 h with isolated compounds (1 μM hexaprenylhydroquinone (HEX); 10 μM heptaprenylhydroquinone (HEP) and nonaprenylhydroquinone (NON)) or 40 μM caffeic acid ethylphenylester (CAPE; nuclear factor-κB (NF-κB) inhibitor) and then stimulated with 5 ng/ml tumour necrosis factor (TNF)α for 24 h. Cell culture supernatants were assayed for secreted alkaline phosphatase (SEAP) activity. Results are expressed as fold activity of the TNFα-stimulated cells (means ± SEM, $n = 3$). $P < 0.05$ vs corresponding control (TNFα + DMSO).

inhibited basal NF-κB-dependent transcriptional activity and completely blocked TNFα-induced SEAP activity to control levels.

Inhibition of protein kinases

We also analysed the effects of the prenylated hydroquinones on a range of protein kinases involved in signal transduction pathways, including protein kinases associated with cell proliferation (ARK5, Aurora A, Aurora B, B-Raf, CDK2, CDK4, COT, EGF-R, ErbB2, FLT3, PDGF-Rb, PLK1, SAK), survival (Akt1, CK2, IGF1-R), angiogenesis (EphB4, Tie2, VEGF-R2, VEGF-R3), and metastasis (FAK, Met, SRC). In a primary screen, nonaprenylhydroquinone did not inhibit any of these kinases at a single concentration of 1 μg/ml, whereas hexaprenylhydroquinone and heptaprenylhydroquinone showed some inhibitory activity. The IC50 values against all 23 protein kinases were therefore determined for these two compounds. Hexaprenylhydroquinone showed significant inhibition only of EGF-R (IC50 1.6 μg/ml (3.1 μM)), whereas heptaprenylhydroquinone inhibited EGF-R (IC50 1.4 μg/ml (2.3 μM)), SRC (IC50 3.7 μg/ml (6.2 μM)), VEGF-R3 (IC50 8.9 μg/ml (14.9 μM)) and IGF1-R (IC50 7.4 μg/ml (12.4 μM)) activity.

Discussion

Marine organisms have proven to be a source of potent pharmacologically active compounds. Since there is an urgent quest for new anti-cancer drugs, we analysed the pharmacological effects of hexa-, hepta- and nonaprenylhydroquinone isolated from the marine sponges *S. muscarum* and *I. fasciculata*.

Prenylated hydroquinones of the kind isolated in this study are widely distributed in different sponge species – they are typically found in sponges of the genera *Sarcotragus*, *Ircinia, Dysidea*, *Spongia* and *Hippospongia*; all of them are Demospongiae of the order Dictyoceratida.[14] (and references therein) The structural class of hydroquinones, especially polyprenylated hydroquinones, possess interesting pharmacological activities. 2-Hexaprenylhydroquinone, isolated from the Red Sea sponge *Ircinia* species, has been shown to be a general inhibitor of retroviral reverse transcriptases and cellular DNA polymerases. The pattern of inhibition was found to be similar for all DNA polymerases tested by Loya and colleagues.[15,16] Heptaprenylhydroquinone isolated from the Mediterranean sponge *l. spinosula* showed no effect against human recombinant synovial phospholipase A2, but octaprenylhydroquinone inhibited this enzyme in a concentration-dependent manner.[17] Nonaprenylhydroquinone isolated from Australian marine sponge *Sarcotragus* species inhibited α-1,3-fucosyltransferase (Fuc TVII), a key enzyme in the biosynthesis of selectin ligands, with an IC50 value of 2.4 μg/ml.[11]

We investigated whether the prenylated hydroquinones possess antioxidative capacity comparable to that of the synthetic vitamin E derivate Trolox. The antioxidative properties of prenylated hydroquinones have been investigated previously by Gil and colleagues,[17] who found that 2-polyprenyl-1,4-hydroquinones failed to scavenge the superoxide anion generated by the hypoxanthine/xanthine oxidase system. Treatment of human neutrophils with 2-polyprenyl-1,4-hydroquinones also did not block the release of superoxide anions. On the other hand, Terencio and colleagues reported that 2-prenyl-1,4-hydroquinone (H1), 2-diprenyl-1,4-hydroquinone (H2), 2-triprenyl-1,4-hydroquinone (H3) and 2-tetraprenyl-1,4-hydroquinone (H4) scavenged reactive oxygen species and inhibited 5-lipoxygenase activity in human neutrophils.[18] In line with these results, our study showed that the prenylated hydroquinones possess antioxidative potential, at least in a cell-free system. We also showed great differences in the toxicity of these compounds: hexaprenylhydroquinone was the most potent compound, while heptaprenyl- and nonaprenylhydroquinone were less toxic in H4IIE cells. EC50 values were in the low micromolar range for hexaprenylhydroquinone but 10-fold higher for the other compounds.

We also determined whether intracellular signal transduction pathways were modulated by the prenylated hydroquinones. Terencio and colleagues reported that prenylated hydroquinones suppressed the production of TNFα in J774 cells stimulated with lipopolysaccharide.[18] Here we report that the prenylated hydroquinones inhibited the activity of NF-κB, a transcription factor that is involved in many critical physiological responses. Unregulated NF-κB activity contributes to many human diseases and many cancers. The prenylated hydroquinones did not inhibit the basal activity of this transcription factor, but the amount of activation after stimulation with TNFα was decreased. Since NF-κB is commonly discussed as a transcription factor responsible for cell survival, this inhibition may contribute to the toxic effects of the prenylated hydroquinones.

In-vitro testing against 23 protein kinases involved in signal transduction pathways (cell proliferation, survival, angiogenesis and metastasis) showed that the hepta- and

hexaprenylhydroquinones had inhibitory activity, whereas nonaprenylhydroquinone had no such activity. Hexa- and heptaprenylhydroquinones inhibited EGF-R (IC50 values 1.6 μg/ml (3.1 μM) and 1.4 μg/ml (2.4 μM), respectively), and heptaprenylhydroquinone also inhibited SRC, VEGF-R and IGF1-R activity. The inhibition of EGF-R is important for potential use in tumour therapy. To date, two classes of EGF-R antagonists have been successfully tested in phase 3 trials and are now in clinical use: anti-EGF-R monoclonal antibodies such as cetuximab, and small-molecule EGF-R tyrosine kinase inhibitors such as gefitinib and erlotinib. EGF-R antagonists are currently available for the treatment of four metastatic epithelial cancers: non-small-cell lung cancer, squamous cell carcinoma of the head and neck, colorectal cancer and pancreatic cancer.[19]

Conclusions

We conclude that the prenylated hydroquinones, especially heptaprenylhydroquinone, may be useful for the development of new chemical compounds in cancer therapy as they are cytotoxic, inhibit NF-κB-signalling and disrupt the extracellular-signal regulated kinase signalling pathway by inhibition of EGF-R. Further analysis of these substances is necessary to analyse their potential significance in cancer therapy.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

Funding

We thank the Bundesministerium für Bildung und Forschung (BMBF (IB) and Tübitak (SBAG-Jülich-3 (104S09)) for financial support.

Acknowledgements

Help with identification of sponges by Dr Rob van Soest (Zoological Museum of the University of Amsterdam, the Netherlands) is gratefully acknowledged. We also thank Dr Dieter Hafner (Institute of Pharmacology, Heinrich-Heine-University, Düsseldorf) for statistical analysis.

References

1. Newman DJ *et al*. Natural products and derivatives as leads to cell cycle pathway targets in cancer chemotherapy. *Curr Cancer Drug Targets* 2002; 2: 279–308.
2. Scheuer PJ. Marine natural products. Diversity in molecular structure and bioactivity. *Adv Exp Med Biol* 1996; 391: 1–8.
3. Faulkner DJ. Marine natural products. *Nat Prod Rep* 2001; 18: 1–49.
4. Gil JH *et al*. Structural determination of cyclitol derivatives by fast-atom bombardment tandem mass spectrometry. *Rapid Commun Mass Spectrom* 2006; 20: 1253–1256.
5. Fusetani N *et al*. Isolation of a hexaprenylhydroquinone sulfate from the marine sponge Dysidea sp. as an H,K-ATPase inhibitor. *Experientia* 1987; 43: 1233–1234.
6. Liu Y *et al*. New cytotoxic sesterterpenes from the sponge Sarcotragus species. *J Nat Prod* 2001; 64: 1301–1304.
7. Liu Y *et al*. New cytotoxic sesterterpenoids and norsesterterpenoids from two sponges of the genus Sarcotragus. *J Nat Prod* 2003; 66: 1451–1456.
8. Liu Y *et al*. Cytotoxic pyrrolo- and furanoterpenoids from the sponge sarcotragus species. *J Nat Prod* 2002; 65: 1307–1314.
9. Cimino G *et al*. Polyprenyl derivatives from the sponge *Ircinia spinulosa*. *Tetrahedron* 1972; 28: 1315–1324.
10. Venkateswarlu Y, Reddy MVR. Three new heptaprenylhydroquinone derivatives from the sponge *Ircinia fasciculata*. *J Nat Prod* 1994; 57: 1286–1289.
11. Wakimoto T *et al*. Octa- and nonaprenylhydroquinone sulfates, inhibitors of alpha1,3-fucosyltransferase VII, from an Australian marine sponge Sarcotragus sp. Bioorg. *Med Chem Lett* 1999; 9: 727–730.
12. Re R *et al*. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic Biol Med* 1999; 26: 1231–1237.
13. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods* 1983; 65: 55–63.
14. Bifulco G *et al*. Bioactive prenylhydroquinone sulfates and a novel C_{31} furanoterpene alcohol sulfate from the marine sponge, *Ircinia* sp. *J Nat Prod* 1995; 58: 1444–1449.
15. Loya S *et al*. Hexaprenoid hydroquinones, novel inhibitors of the reverse transcriptase of human immunodeficiency virus type 1. *J Nat Prod* 1993; 56: 2120–2125.
16. Loya S *et al*. Mode of inhibition of HIV reverse transcriptase by 2-hexaprenylhydroquinone, a novel general inhibitor of RNA- and DNA-directed DNA polymerases. *Biochem J* 1997; 324: 721–727.
17. Gil B *et al*. Effects of marine 2-polyprenyl-1,4-hydroquinones on phospholipase A2 activity and some inflammatory responses. *Eur J Pharmacol* 1995; 285: 281–288.
18. Terencio MC *et al*. Suppression of leukotriene B4 and tumour necrosis factor a release in acute inflammatory responses by novel prenylated hydroquinone derivatives. *Naunyn Schmiedebergs Arch Pharmacol* 1998; 357: 565–572.
19. Ciardiello F, Tortora G. EGFR antagonists in cancer treatment. *N Engl J Med* 2008; 358: 1160–1174.