Molecules **2006**, *11*, 1–33

molecules

ISSN 1420-3049
http://www.mdpi.org

Review

Reactivity and Biological Activity of the Marine Sesquiterpene Hydroquinone Avarol and Related Compounds from Sponges of the Order Dictyoceratida

Dušan Sladić [1,*] and Miroslav J. Gašić [2,*]

[1] Faculty of Chemistry, University of Belgrade, Studentski trg 12-16, POB 158, 11001 Belgrade, Serbia and Montenegro, Tel. +381-64-2795778; Fax. +381-11-636061
[2] IHTM Department of Chemistry, University of Belgrade, Studentski trg 12-16, POB 473, 11001 Belgrade, Serbia and Montenegro

[*] Authors to whom correspondence should be addressed; e-mails: dsladic@chem.bg.ac.yu; mjgasic@chem.bg.ac.yu

Received: 17 August 2005; in revised form: 22 September 2005 / Accepted: 22 September 2005 / Published: 31 January 2006

Abstract: A review of results of bioactivity and reactivity examinations of marine sesquiterpene (hydro)quinones is presented. The article is focused mostly on friedo-rearranged drimane structural types, isolated from sponges of the order Dictyoceratida. Examples of structural correlations are outlined. Available results on the mechanism of redox processes and examinations of chemo- and regioselectivity in addition reactions are presented and, where possible, analyzed in relation to established bioactivities. Most of the bioactivity examinations are concerned with antitumor activities and the mechanism thereof, such as DNA damage, arylation of nucleophiles, tubulin assembly inhibition, protein kinase inhibition, inhibition of the arachidonic cascade, etc. Perspectives on marine drug development are discussed with respect to biotechnological methods and synthesis. Examples of the recognition of validated core structures and synthesis of structurally simplified compounds retaining modes of activity are analyzed.

Keywords: Sponges**,** sesquiterpene, quinone, hydroquinone, bioactivity.

Contents

1. Introduction

The wealth of the oceans, the origin, number, versatility and coexistence of species and their survival mechanisms are fundamental questions that have occupied the imagination of scientists for ages. However, practical problems, mostly notably the restricted accessibility of organisms, have delayed a systematic and concerted approach to the study of marine life. In recent times, the improvements in the engineering of diving equipment and collection tools have enabled access to numerous species from various environments in sufficient amounts to allow reliable taxonomic classifications and detailed examinations of chemical constituents. As the result of the concerted efforts of investigators from various life science specialities over the last few decades, marine organisms are constantly yielding inspiring ideas related to the variety of unusual chemical structures of their metabolites, biosynthesis and the wide spectrum of biological functions and properties. The current progress in understanding the evolutionary history of marine organisms and of the effects of environmental conditions on adaptability is providing more rational guidelines for their selection as potential sources of bioactive secondary metabolites.

Among the species within the marine animal kingdom, sponges are the most primitive multicellular organisms. As typical filter-feeders, they capture and digest organic particles to produce metabolic energy. Being sessile organisms, devoid of any physical capability for defense and found in environmentally different areas, sponges needed to develop adaptive responses and specific means for self-protection [1,2]. Consequently, their chemical constituents, primarily their secondary metabolites and their origin, structure and function, have attracted the most attention regarding several aspects of marine chemistry.

2. Quinones with a rearranged drimane skeleton from sponges of the order Dictyoceratida

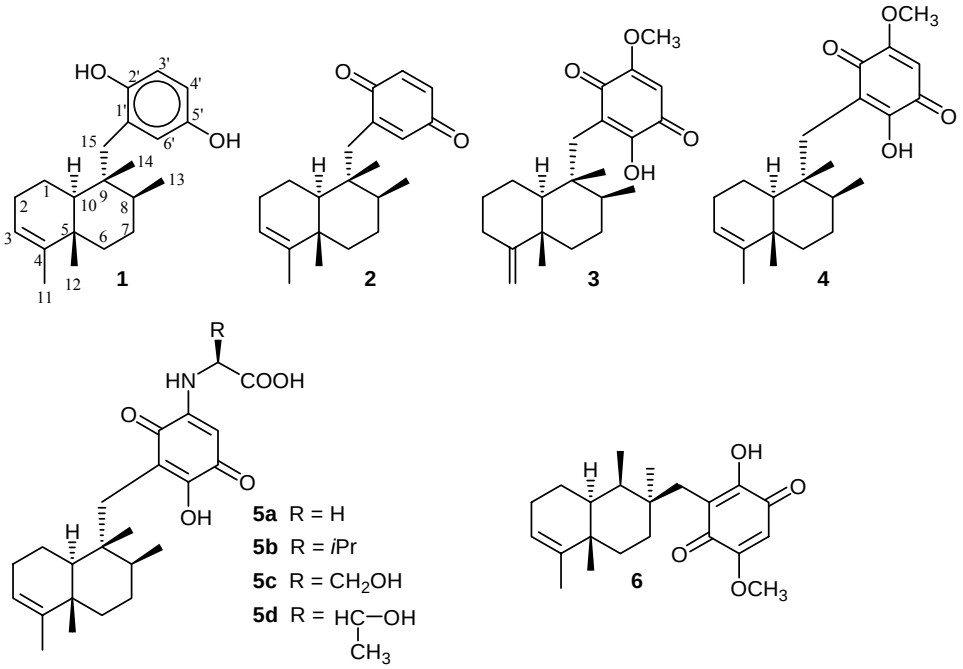
The identification of secondary metabolites, isolated from different species, and correlation of the obtained data at the level of a particular class or order are of special importance in taxonomic determinations. Structural types of isolated compounds are usually classified according to standard principles of organic chemistry.

One of the most widespread groups of constituents found in sessile organisms including sponges, are terpenoid quinones and hydroquinones, continuously reviewed and updated [3-5]. Since their great structural diversity, differences in origin, biosynthetic pathways and biological activities require further divisions, in this report primarily one subgroup is considered, namely the quinoid compounds

formed by a mixed shikimate-mevalonate biogenesis, resulting in formation of a 4,9-friedodrimane rearranged bicyclic sesquiterpene skeleton attached to a (hydro)quinone moiety. A representative group of compounds: avarol (**1**) [6] and avarone (**2**) [6] from *Dysidea avara*, ilimaquinone (**3**) [7,8] from *Petrosaspongia metachromia* (previously named *Hippospongia metachromia*), isospongiaquinone (**4**) [9,10] from *Stelospongia conulata*, the nakijiquinones **5a-d** [11] from *Spongia* sp., bolinaquinone (**6**) [12] from *Dysidea* sp., is presented in Figure 1 and their chemistry and biological effects analyzed in the sections that follow.

From the taxonomic point of view, it is important to mention that these structurally related compounds **1-6** were isolated from sponges belonging to the order Dictyoceratida, collected in different areas. They possess a common structural feature comprising a *trans*-decalin system in the rearranged drimane skeleton. Structural variations are found in the double bond position and in the substitution pattern in the quinone ring. Although similar in structure and having identical configuration of all asymmetric centers, (except for C-8 in bolinaquinone) these compounds present a wide variety of biological effects.

Figure 1. Hydroquinones and quinones with friedo-rearranged drimane skeleton.

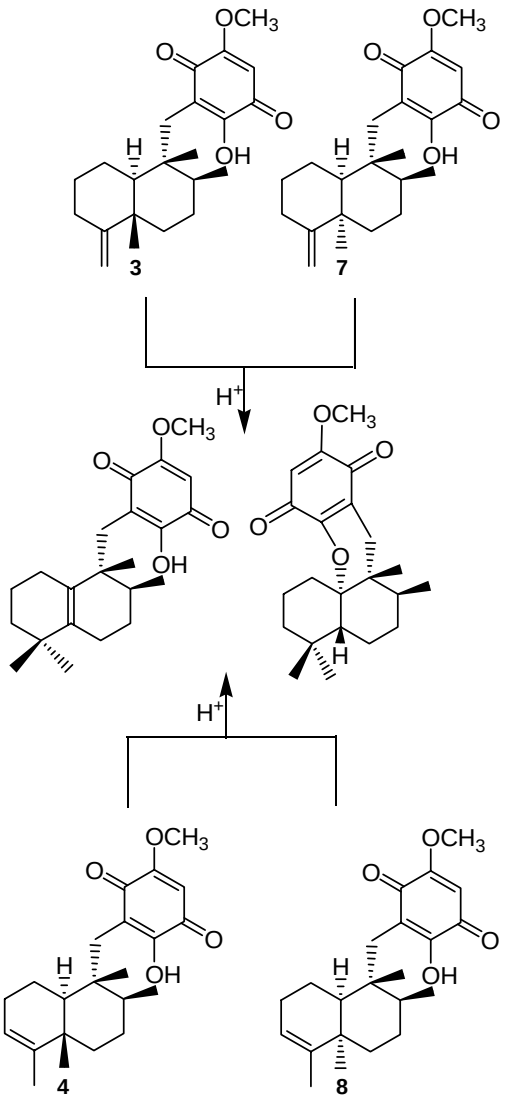


3. Structure

The general structure of the first rearranged drimane containing sesquiterpenes, namely avarol (**1**), avarone (**2**) and their derivatives, was established by standard analytical methods, chemical degradation [13-15] and later by stereocontrolled synthesis [16]. However, in some other cases, the assignment of absolute stereochemistry of asymmetric carbon atoms was not simple and was shown to be unreliable if based on correlations of circular dichroism (CD) measurements of low magnitude. For instance, the configuration of the carbon atoms at positions C-5,8,9 and 10 in ilimaquinone (**3**) was initially assigned as enantiomeric to those in avarol (**1**) [7]. The error was later corrected [8] by chemical degradation and correlation of the CD measurement data of the obtained products with those

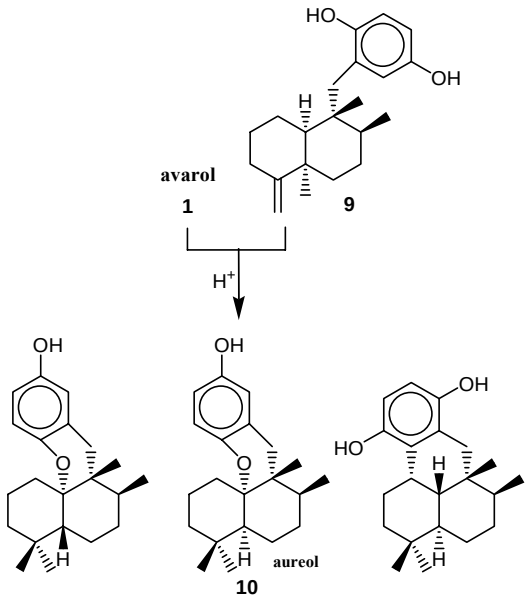
of compounds with firmly established stereochemistry to show that sesquiterpenoids **1, 2** and **3** have the same absolute stereochemistry, *i.e.* 5*S*,8*S*,9*R*,10*S*. This finding was of great importance for the determination of the absolute stereochemistry of other structurally related quinones which often occur as co-metabolites. Along with the efforts to correlate the stereochemistry of drimane- and 4,9-friedo-drimane sesquiterpene quinones by instrumental methods, it was also shown that through adequate reasoning and the use of simple chemical reactions, relevant conclusions can be reached concerning chirality, (*i.e.* absolute stereochemistry) at particular carbon atoms. In this respect, by an acid catalyzed rearrangement of ilimaquinone (**3**) and isospongiaquinone (**4**), and of 5-*epi*-ilimaquinone (**7**) and 5-*epi*-isospongiaquinone (**8**) (and *vice versa*), it was confirmed that both pairs yield the same mixture of products, unambiguously connected to ilimaquinone (Scheme 1) [17].

Scheme 1

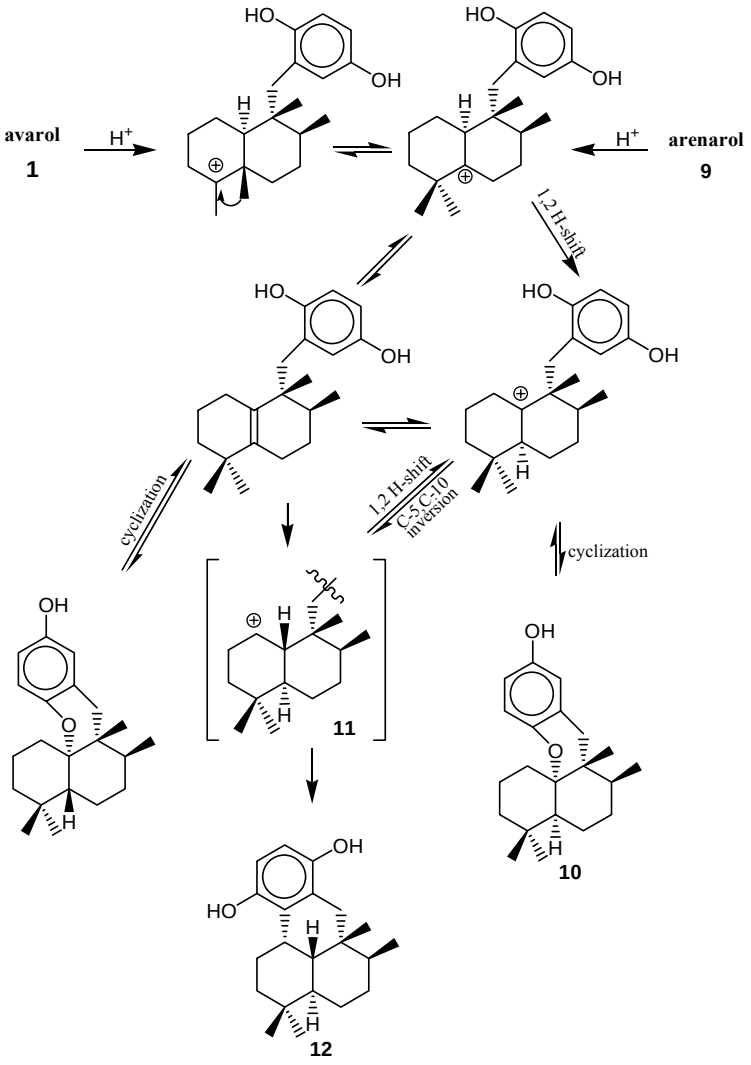


By the same approach, the correlation between avarol (**1**) and arenarol (**9**) was also established, so that it was possible to stereochemically define an assembly of related sponge metabolites [17], including some with an unrearranged drimane skeleton [18] (Scheme 2).

Scheme 2



Scheme 3

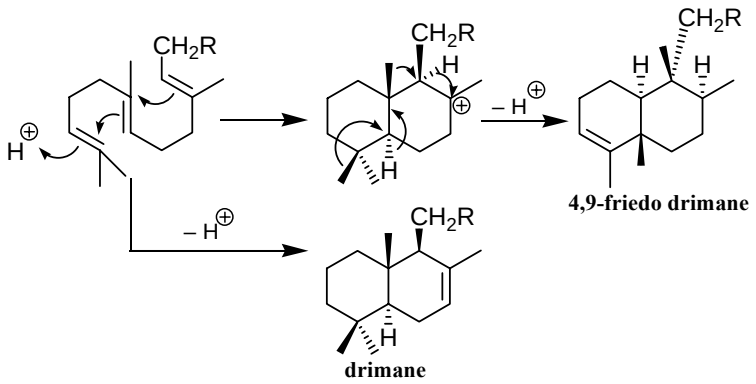


It is interesting to note that in acid catalyzed reactions of both ilimaquinone and avarol, a reversal of a friedo-like rearrangement takes place; formation of the tricyclic product **12** by avarol alkylation at C-1 (Scheme 3) was suggested to occur by a 1,2-*H* shift [17], involving an *a priori* less stable carbocation intermediate **11**.

4. Biosynthesis

The pathway of formation of 4,9-friedo-rearranged marine sesquiterpenoids, was first proposed for avarol (**1**) [6]. As can be seen in Scheme 4, the cyclization of farnesyl pyrophosphate (FPP) takes place by an initial electrophillic attack at the *head* position of FPP giving rise to a concerted process leading to a bicyclic carbocationic intermediate from which the final products, *i.e.* drimane or 4,9-friedo-drimane structural types are formed. Structurally, bolinaquinone (**6**) is an exception with respect to the position of the quinone moiety [12]; at present, no biosynthetic pathway has been proposed for this compound, although a 1,2 alkyl (instead of a hydride) shift to C-8 seems plausible. Recently, a few more C-8 substituted compounds were isolated from the *Dysidea sp.* sponges [19].

Scheme 4



5. Reactivity

The chemistry of simple quinones and hydroquinones has been extensively studied and is well documented [20-22]. However, in specific and more complex systems, steric requirements and electronic distribution frequently affect reactivity in an unexpected fashion.

Quinone reactivity studies generally rest on redox reactions and Michael addition/elimination reactions; in biological systems, both reactions require further subtle differentiations (Diels-Alder reactions not considered). From the physiological point of view, it is important to notice that the two processes are competitive and that both might be therapeutically effective but at the same time could be the cause of pronounced and non-selective cell toxicity.

i) *Redox reactivity*

Regarding the mechanism of the quinone/hydroquinone redox processes which lead to non-bonding transformations of the substrate, the proposals of a one-electron transfer reaction (I) *vs.* the hydride

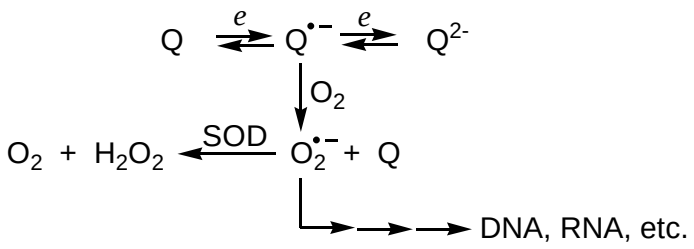
transfer process (II) may be one of the issues determining toxicity and/or the type of biological activity.

$$Q \rightleftharpoons [Q, NADH] \rightleftharpoons [Q^{\cdot-}, NADH^{\cdot+}] \rightleftharpoons [QH^{\cdot}, NAD^{\cdot}] \rightleftharpoons [QH^{-}, NAD^{+}] \rightleftharpoons QH^{-} + NAD^{+} \quad \text{I}$$

$$Q + NADH \rightleftharpoons QH^{-} + NAD^{+} \quad \text{II}$$

However, the interpretation of the pathway of quinone redox cycling processes has been associated with a long-lasting controversy regarding the above mechanisms (reaction I *vs.* II). Various simple model systems, but also enzyme (or enzyme-like) models, under differently designed experimental conditions, offered mostly indirect evidence regarding the reaction pathway [23]. The frequently proposed successive one-electron transfer reaction (*e-p-e*) based on the well known redox potential depending ability of the quinone/hydroquinone (Q/QH_2) couple to reversibly form radical intermediates by chemical activation processes led to a quite conceivable interpretation that these intermediates can affect cellular components either directly, or indirectly, by producing toxic oxygen radicals responsible for cell damage [24-26] (Scheme 5).

Scheme 5



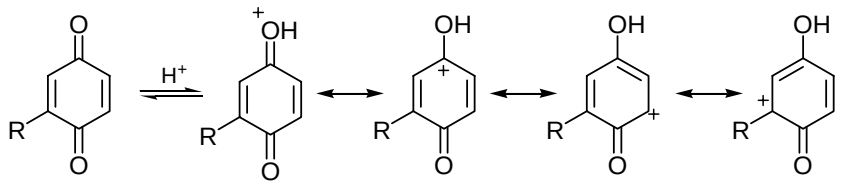
On the other hand, the proposal that the one-step two-electron transfer by the reduced form of nicotinamide adenine dinucleotide (NADH) or its derivatives, equivalent to a hydride shift (reaction II), takes place in an important biological model reaction, such as the reduction of carbonyl compounds, including quinones, implies an essentially different mechanism, affecting also biological effects. References to both opposing views are presented in several stimulating publications but differences in interpretation still persist [27-35].

ii) *Addition reactions*

Quinone addition-elimination processes greatly depend on the charge distribution within this conjugated system, influenced by reaction conditions, substituent effects, steric requirements and non-bonding interactions between the quinone, nucleophile and the medium. For instance, in hydroxy-substituted quinones, polarization can lead even to zwitterionic structures, *i.e.* to synthetically useful ylide formation [36]. Reaction conditions such as solvent changes, temperature etc., were examined in a study of nucleophilic substitutions of methoxy-alkyl-*p*-benzoquinones related to a regioselective quinone-enamine coupling process for the formation of the *nat*-mitomycin backbone: a solvent induced reversal of regioselectivity was established by changing polar protic to polar aprotic solvents [37]. The regioselectivity of addition reactions of thiols, amines, methanol and hydrogen chloride to avarone (**2**) was investigated and it was shown to be influenced by the electrophilicity of position 4' in

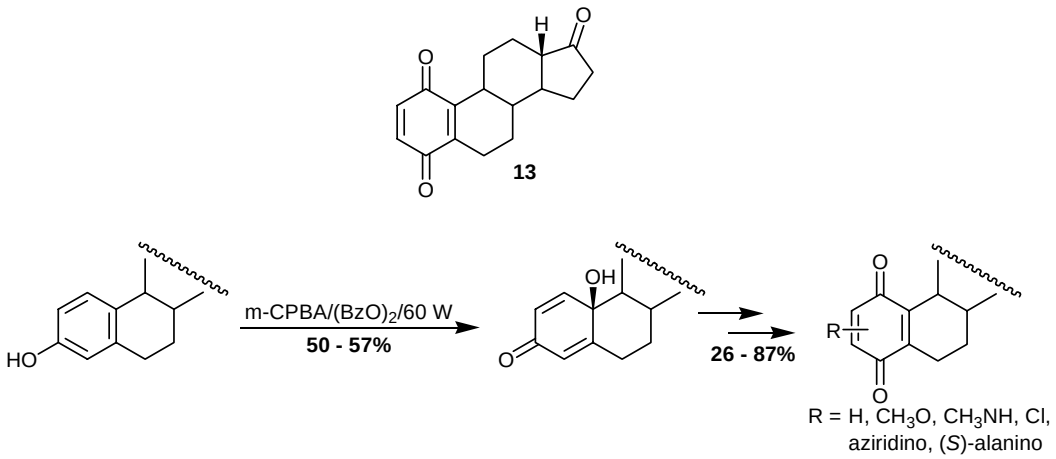
unprotonated avarone, the increased electrophilicity of position 3' in acidic medium (Figure 2), and by the acidity of the intermediate hydroquinones [38]. Substitution in position 6' was never observed.

Figure 2. Activation of position 3' in avarone by protonation.



Our further efforts to synthesize on a larger scale quinones with a different skeleton and to examine their reactivity[39-41], resulted in preparation of steroidal ring A *p*-quinone **13** and derivatives in good yield (Scheme 6).

Scheme 6

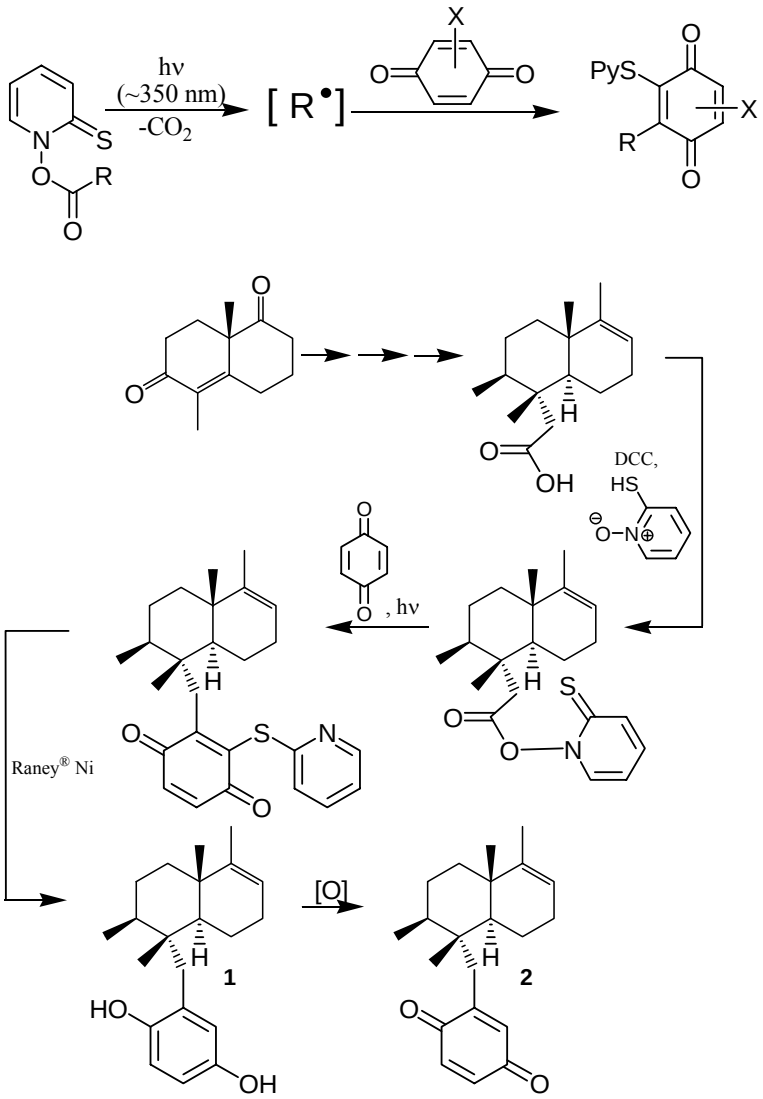


The process of regiospecific acid catalyzed methanol addition to **13** was also examined by conformational analysis (PM3, AM1, MNDO) of the parent quinone and of the possible protonated intermediates [42]. It was suggested that the geometry of the unsubstituted ring A *p*-quinone is characterized by deviations from planarity (Figure 3), while protonation flattens the ring, requiring twice the energy compared to *p*-benzoquinone [43]. The high regiospecificity of addition to C-2 was ascribed to differences in angle deformations in transition states.

Figure 3. The PM3 optimized geometry of **13**.

The effect of substituents on chemo- and regioselectivity in addition reactions was also clearly demonstrated in radical reactions. To secure efficiency in an enantioselective synthesis of **1**, **2** and **3** [44], a series of differently substituted quinones and their respective reactivity in photochemical radical decarboxylation reactions using thiopyridone derivatives [45] and quinone addition reactions on symmetrical and unsymmetrical quinones was examined. It was concluded that chemoselective addition takes place only on unsubstituted, conjugated double bonds, while regioselectivity of addition in quinones acting as radical traps is greatly influenced by the resonance effect of heteroatoms attached to the quinone ring; in some cases it reached a ratio of 15:1 or higher. A part of this unified approach to the synthesis of sesquiterpene quinones **1** and **2** is presented in Scheme 7.

Scheme 7

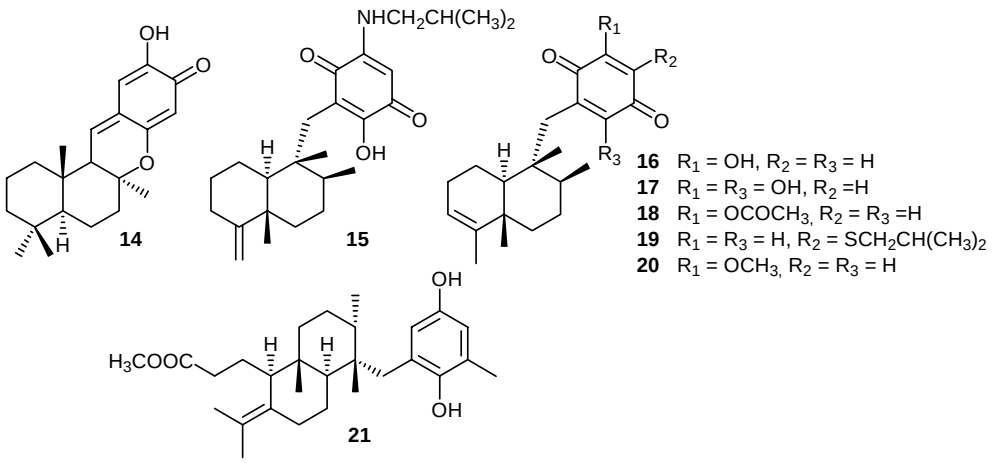


6. Biological effects

i) Antitumor activity

Many marine sesquiterpenoid quinones and hydroquinones are of considerable interest with regards to their versatile biological activities; also, a particular (hydro)quinone may display multiple activities. Compounds of structural types combining a decalin-type terpene unit and a quinoid moiety isolated from marine organisms are often characterized by pronounced cytotoxic activities. Such compounds may have *in vitro* IC_{50} values towards cell lines in the micromolar range. Most of them are sesquiterpenoids, (usually with the drimane skeleton, *e.g.* puupehenone (**14**) from the sponge *Hyrtios* sp. [46,47], which also shows a potent antituberculosis activity [48])**.** Among those are also the ones with a 4,9-*friedo*-rearranged skeleton, such as ilimaquinone (**3**) [49], and a related amino derivative **15** from the sponge *Dactylospongia elegans* [50], the nakijiquinones **5** [11] and especially avarone (**2**) and avarol (**1**), with strong ED_{50} activities of 0.62 μM and 0.93 μM, respectively, for mouse lymphoma cells [51,52]. Also, strong activity was evidenced for several hydroxy and acetoxy derivatives **16-18** of avarol and avarone, isolated from the marine sponge *Dysidea cinerea* [53] (Figure 4). It is interesting that arenarol (**9**), structurally similar to avarol but with a *cis*-decalin system, has approximately 20 times lower activity towards P388 lymphotic leukemia cells than avarol [54,55]. On the other hand, in this system, 3,4-dihydroavarol has only a slightly weaker activity than avarol [52]. The *in vitro* specificity of avarol, avarone and their derivatives toward leukemia cell lines, higher for an order of magnitude than against any other tumor cell lines (*e.g.* 4'-isobutylthioavarone (**19**) has GI_{50} against CCRF-CEM leukemia cells 1.3 μM, compared to an average GI_{50} of 11 μM against all cell lines in the NCI panel) makes them good lead compounds in search for new therapeutic agents. The avarol/avarone redox couple also showed antileukemic activity *in vivo*, with a therapeutic index of 11.7 for avarone and 4.5 for avarol [52]. Of several avarone derivatives synthesized by nucleophilic addition of O-, N-, and S-nucleophiles, only a few had a higher antileukemic activity to certain cell lines *in vitro* than the parent compound, e.g. 3'-methoxyavarone (**20**) [15, 42] and 3'-alkylamino avarone derivatives [56]. Avarol and avarone have manifold other biological activities: antiviral, antiinflammatory (see below), moderate antibacterial against Gram-positive strains and antifungal [57].

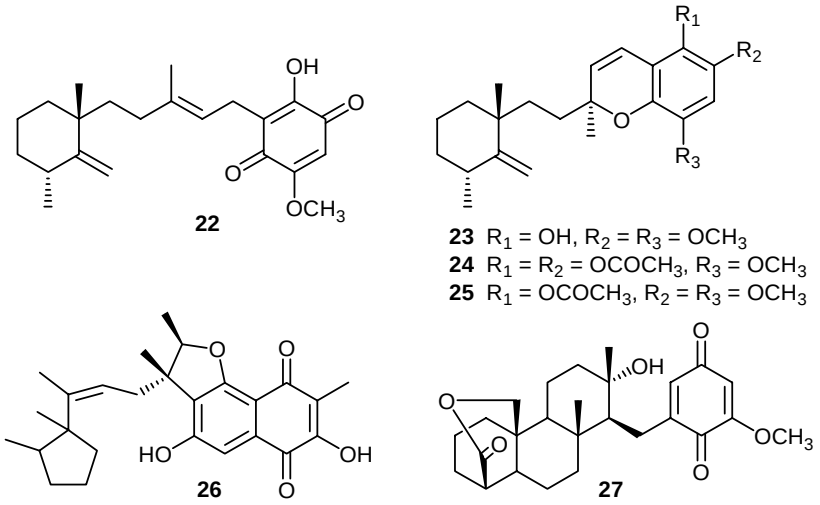
Figure 4



Among similar compounds some are diterpenoids, such as the ester **21**, related to atomaric acid, isolated from the brown alga *Stypopodium zonale* [58], with IC_{50} values against human lung and colon carcinoma cell lines of 2.5 μM or less.

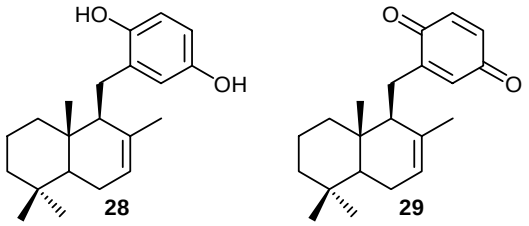
The presence of a bicyclic system is not a prerequisite for a significant cytotoxic activity. Metachromins A (**22**), and B (**23**), hypochromin A diacetate (**24**) and metachromin B monoacetate (**25**) (Figure 5) exhibit potent cytotoxicities against human colon and nasopharyngeal tumor cells with IC_{50} values of less than 1 μM [59]. Another example is the moderately cytotoxic naphthoquinone sesquiterpene neomarinone (**26**) produced by a marine actinomycete [60]. A tricyclic meroterpenoid quinine, strongylophorine (**27**), from the marine sponge *Petrosia corticata*, shows a significant activity in an anti-invasion assay [61]. Since tissue invasion *via* cell migration is a common process in angiogenesis and metastasis, the structure of this substance might also be helpful in the development of new anticancer drugs.

Figure 5



Several structure activity relationships (SAR) studies were performed with the aim of improving the activity of compounds based on natural product structural models. One possible approach is the synthesis of unnatural enantiomers to improve the understanding of SAR requirements. Some of these analogues, such as enantiomers **28** and **29** of isozonarol and isozonarone, respectively, showed antitumor activity (IC_{50}) in a micromolar range or less [62], i.e. higher than the corresponding natural products (Figure 6).

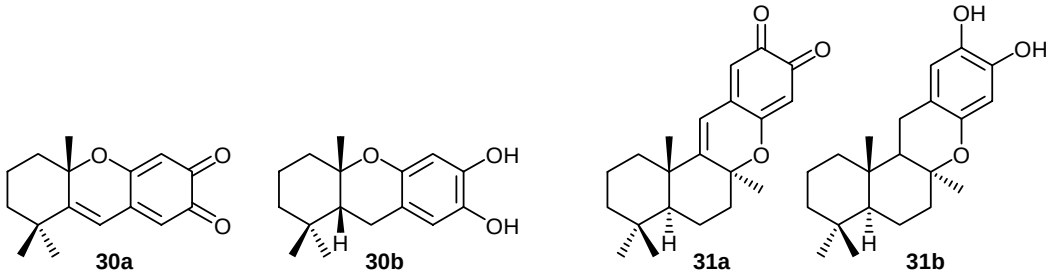
Figure 6



An alternative approach was made by changing the isoprenoid moiety. In one study, monoterpene models **30a** and **30b** of puupehedione (**31a**) and puupehediol (**31b**), respectively, were synthesized

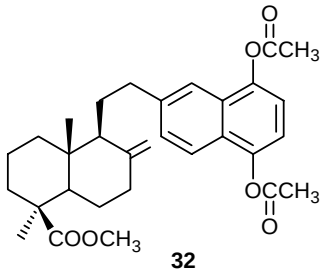
(Figure 7). Both compounds were active against tumor cells, the catechol compound having similar activity to the natural product, and the *o*-quinone having 4 times higher activity than the corresponding natural product (IC_{50} less than 1 μM) [63].

Figure 7



In another biomimetic study, a new series of diterpenyl quinones and hydroquinones were prepared by Diels-Alder reactions between three labdanic diterpenoids and *p*-benzoquinone or 1,4-naphthoquinone. A representative example is compound **32** (Figure 8). Several of the obtained compounds showed IC_{50} values under the micromolar level, and an important selectivity toward renal cancer lines, identifying these compounds as a very promising group of antineoplastics. The structure of the compound **32** could be partially superimposed on the crystal structure of avarol, which allows speculation that these molecules might interact at the same site and in a similar molecular orientation [55].

Figure 8



Some of the earlier mentioned steroidal ring A *p*-quinones [39-42] and related structural types show an expected anti-ovarian tumor activity since they are derived from estrogens (to be published).

ii) Mechanisms of cytotoxic activity

As mentioned, some type of biological activity was recorded for practically all of the sesquiterepene quinones with a rearranged drimane skeleton. In advanced approaches, additional evidence shedding more light on the likely mechanism of action was obtained. However, it is obvious that a rational design of drugs requires thorough understanding of the reactivity of functional groups, as parts of an envisaged pharmacophore, in chemically different environments.

Cytotoxicity of friedo-rearranged sesquiterpenoid quinones is well documented but differences in experimental techniques, types of cells and targeted components etc. impede correlations leading to conclusions. Also, chemical reactivity examinations, especially those allowing extrapolation of

obtained data to the mechanism of action of particular compounds in biological systems, are usually lacking.

General mechanisms

In most relevant publications, quinone/hydroquinone cytotoxicity is usually attributed to two processes [26,64]:

1) redox cycling of quinones, resulting in generation of reactive oxygen species which can damage biomolecules, and inhibition of the mitochondrial electron transport and/or of oxidative phosphorylation;
2) electrophilic arylation of critical cellular nucleophiles, either by quinones or quinone methides formed after bioreductive activation of quinones [65].

To identify the principal mechanism is difficult for quinones showing various chemical reactivities. Both redox cycling and arylation of nucleophiles can result in oxidative stress and cell killing. One-electron reduction of quinones to the corresponding semiquinone radicals is catalyzed by a number of NAD(P)H-oxidoreductases, including those associated to the mitochondrial electron-transport chain and the cytochrome P_{450} system of the endoplasmic reticulum [64], while two-electron reduction to hydroquinones is catalyzed by DT-diaphorase [66].

The above reactions were examined in other model systems in relatively close connection to quinone reactivity in biological systems. With simple naphthoquinones, it was found that NADPH-cytochrome P-450 reductase catalyzes the reduction by initial one-electron transfer to form semiquinone anion-radicals which react with molecular oxygen to yield superoxide anion-radicals [65]. With DT-diaphorase, however, naphthoquinones are reduced to hydroquinones which can subsequently be autooxidized to semiquinones. By investigating the mechanism of acid catalyzed reduction of quinones by acridine (as a model for NADH), it was found that a hydride shift is clearly implicated [35]. Contrary to this finding, in a recent work [67], it was shown by electron paramagnetic resonance (EPR) that 9,10-phenanthrenequinone reduction takes place by a one-electron transfer process catalyzed by an NADPH-dependent quinone oxidoreductase. The same conclusion was reached by following the redox process of some naturally occurring quinones, also by EPR [68]. It must be mentioned that in most of the attempts to clarify the mechanism, experiments were carried out under conditions which could hardly be adopted as a proof for the course of events in a biological system.

An appropriate general opinion, encompassing the proposed alternative mechanisms, was epitomized in a summary stating that the well known mitochondrial disfunction resulting in cytotoxicity may take place by any of the proposed mechanisms, including addition/elimination, depending primarily on the chemical structure and reactivity of the individual quinine [64].

When considering biochemical behaviour of quinone/hydroquinone couples, it has to be taken into account that hydroquinones are planar while quinones can adopt, with low energy requirements, various degrees of non-planarity [43], depending on the structure of quinone and the stereochemistry of enzyme-substrate interactions.

DNA damage

The chemical reactivity in relation to the mechanism of bioactivity of avarol and avarone has been examined earlier and more systematically than that of other rearranged drimane-containing (hydro)quinones [69]. It was recognized that this quinone-hydroquinone couple undergoes redox interconversion so that in any biological assay the (time and pH dependant) presence of both compounds in some ratio should be taken into account.

DNA damage was found to be one of principal causes of avarol/avarone cytotoxicity [70]. It was shown that avarol produces a significant DNA damage, primarily single-strand breaks, most probably indirectly by generating oxygen radicals. The effect is partly reversible, since the repair mechanisms are not affected. Participation of reactive hydroxyl radicals in DNA damage caused by avarol was established by experiments showing that tryptophan, a potent scavenger for hydroxyl radicals, inhibits the formation of strand breaks by cytotoxic concentrations of avarol in Friend erythroleukemia cells (FLC) [71], and by the study of effects of iron(II), and/or corresponding complexing agents on the amount of DNA damage induced by avarol in FLC [72].

The importance of effects of oxygen radicals in the antileukemic activity of avarol and its specificity to leukemia cells is confirmed by a good correlation between the activities of the protective enzyme superoxide dismutase (SOD) in various cell lines and their sensitivities to avarol. Tumor tissues with the lowest levels of SOD were found to be the most susceptible to avarol [70]. There is also evidence that avarol and avarone inhibit the cytoplasmatic Cu/Zn-SOD, as well as the SOD in intact L5178y mouse lymphoma cell system [73,74], potentiating effects of oxygen radicals. Covalent binding of avarol to DNA was not observed [70].

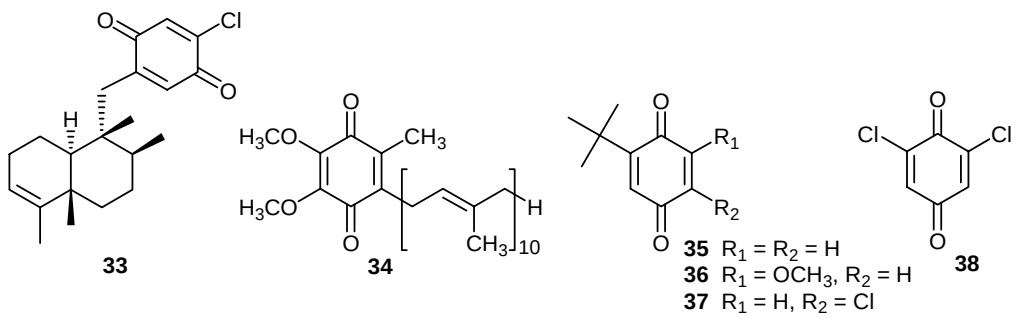
The formation of avarol semiquinone anion radicals was confirmed by electrochemical methods [75,76] and by EPR spectroscopy of products of air-oxidation of avarol and methanol reduction of avarone [77]. Still, we believed that evidence of semiquinone radical formation in biochemical reactions was insufficient. It seemed that the investigation of a relevant redox process such as the reaction of naturally occuring quinones with NAD(P)H models under conditions aimed to mimic those in biological systems, might be an adequate reaction although earlier examinations led to contradictiory conclusions (see above, [27-35]).

Therefore, in our initial investigations, in analogy with earlier published studies, relative rates of oxidation of NADH model compound, 1-benzyl-1,4-dihydronicotinamide (BNAH) by quinones **2, 20, 33-38** in anhydrous acetonitrile, in presence of various concentrations of magnesium perchlorate, and the respective cyclic voltammetric cathodic half-peak potentials ($E_{p/2}$) *vs.* saturated calomel electrode (SCE) [78] were correlated (Table 1). The relative rates follow the order of the respective half-peak potentials, with this tendency becoming more apparent in presence of Mg^{2+} ions. The interpretation of this trend rests on positive shifts of redox potentials E_0 (Q/Q^{-}) as the consequence of the formation of the semiquinone-magnesium complex. The same trend in relative rates was observed in ethanol-water solutions, with Mg^{2+} effects being less pronounced because of the lower stability of the intermediate complex in presence of protons.

Table 1. Relative rate constants (k_{rel}) of BNAH oxidation by quinones **2, 20, 33-38** in anhydrous acetonitrile in the presence of various concentrations of magnesium perchlorate, [Mg^{2+}], and the respective cyclic voltammetric cathodic half-peak potentials ($E_{p/2}$) *vs.* SCE.

Compound	k_{rel} [Mg^{2+}]/mol dm^{-3}			$E_{p/2}$, V [Mg^{2+}]/mol dm^{-3}	
	0	1.0×10^{-2}	1.0×10^{-1}	0	1.0×10^{-2}
2	1.4	5.1	5.4	-0.56	-0.23
20	1.6	5.0	5.3	-0.62	-0.34
33	4.2	8.9	9.0	-0.43	-0.20
34	5.9	11.8	14.3	-0.56	-0.36
35	2.4	5.6	6.4	-0.58	-0.22
36	2.3	5.9	6.5	-0.57	-0.34
37	3.9	4.6	5.0	-0.38	-0.20
38	7.2	7.8	7.8	-0.18	–

Figure 9



In order to mimic biological conditions and to improve the understanding of the nature of intermediates, the same reaction was examined in presence of different concentrations of cationic, anionic and neutral surfactants (Table 2). The results clearly show that in presence of cationic micelles (cetyltrimethylammonium bromide, CTAB), depending on concentrations up to critical micellar concentration (CMC, 1.5×10^{-3}M), a rate increase for compounds **2, 20, 33, 34** (Figure 9) was recorded, while in presence of anionic micelles (sodium dodecyl sulfate, SDS) a slight rate decrease takes place; in neutral micelles (Tween), essentially no change in rate, relative to those in EtOH/water, was noticed. Based on these results and supported by the established stability of the quinone radical anion/CTA^+ complex [79] with a stability constant of $1.4\text{x}10^{-2}$ at pH 8.1, the following stepwise electron transfer reaction pathway, involving quinone anion radical formation, is proposed:

$$Q + BNAH \rightleftharpoons [Q^{\bullet-}, BNAH^{\bullet+}] \overset{CTA^+}{\rightleftharpoons} [Q^{\bullet-}, CTA^+] + BNAH^{\bullet+}$$

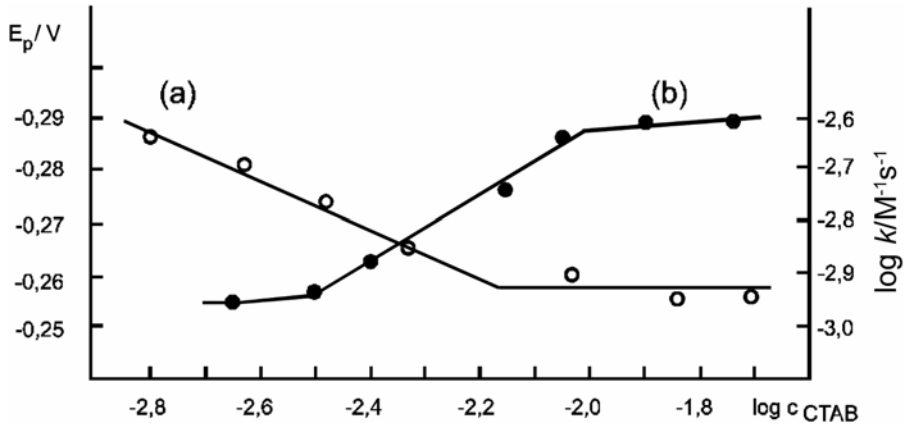
Table 2. Relative rate constants (k_{rel}) of BNAH oxidation by quinones **2**, **21**, **33-38** in ethanol/water (3:2), pH 7, in the presence of various surfactants (1.5×10^{-3} mol dm^{-3}) and magnesium perchlorate.

Surfactant	$[Mg^{2+}]$	k_{rel}							
	(mol dm^{-3})	**2**[a]	**20**	**33**	**34**	**35**	**36**	**37**	**38**
CTAB	0	3.1[*]	3.1	6.0	9.2	1.9	2.8	4.1	7.9
CTAB	1.0×10^{-2}	5.2[§]	5.0	7.1	13.8	2.7	3.5	4.4	7.9
CTAB	1.0×10^{-1}	5.2	5.2	7.0	14.0	3.0	3.5	4.6	8.1
SDS	0	1.0[†b]	1.4	2.5	4.8	1.9	2.0	2.9	6.6
SDS	1.0×10^{-2}	1.1[‡]	1.6	2.6	5.0	2.1	2.1	3.1	6.6
SDS	1.0×10^{-1}	1.1	1.7	2.7	5.1	2.1	2.2	3.1	6.7
TWEEN 80	0	1.3	2.4	4.1	4.9	2.1	2.7	4.2	6.1
TWEEN 80	1.0×10^{-2}	1.9	3.4	4.6	5.1	2.4	2.8	4.4	6.4
TWEEN 80	1.0×10^{-1}	2.1	4.0	4.6	5.2	2.8	3.4	4.5	6.7

([a]) Cyclic voltammetric cathodic half-peak potentials (in V *vs.* SCE) were as follows: -0.13([*]), -0.04([§]), -0.14([†]), -0.08([‡]); ([b]) Reference rate.

The lower reactivity of quinones **35-38** can be explained by the less lipophilic character of these compounds (less solubilized in the micellar pseudophase). In another study the *e-p-e* mechanism was supported by the fact that long-chain *N*-alkyl-1,4-dihydronicotinamides, compared to less lipophilic short-chain analogues, show a significant increase in the reaction rates of avarone reduction in protic solvents. This effect is a consequence of the stabilization of the intermediate radical-ion pair by automicellization [80]. A more precise description of the nature of the intermediate in avarone reduction was obtained by cyclic and rotating disc electrode voltammetry in micellar systems [79]. In presence of the cationic micellar agent CTAB, two well-defined peaks in avarone reduction can be observed in cyclic voltammograms, confirming one-electron reduction, whereby the intermediate avarone radical-anion was stabilized by the cationic micellar medium. The relevance of electrochemical data to understanding of biological avarone reductions could be seen from similar dependence of one-electron reduction potential and the rate constant for the reaction between avarone and BNAH on concentration of CTAB (Figure 10).

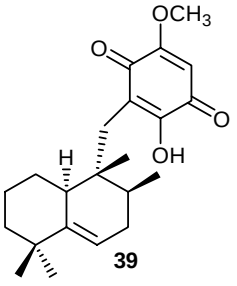
Figure 10. (a) Plot of E_p *vs.* log c_{CTAB} for 1 mM avarone at pH 9,1 (o); (b) Plot of log *k* *vs.* log c_{CTAB} at pH 9.1 for the reaction of BNAH and avarone (•).



In accordance with this mechanism, derivatives with more negative redox potential generally tend to have a higher cytotoxicity. It is interesting that DNA damage effected by oxygen radicals was shown for synthetic phenolic antioxidant *tert*-butylhydroquinone [81], which can be considered as a crude model of avarol.

DNA damage was suggested to be one mode of cytotoxicity for mamanuthaquinone (**39**, Figure 11), a metabolite of the sponge *Fasciospongia* sp. showing activity against human colon tumor cells [82].

Figure 11

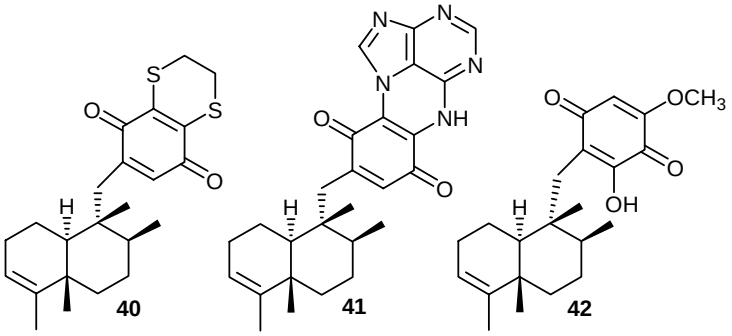


Arylation of nucleophiles

Avarone (**2**) was found to modify sulfhydryl groups both in glutathione and in bovine serum albumin, causing depletion of soluble and protein thiols both *in vitro* and *in vivo* [83,84]. Modification of sulfhydryls can be related to oxidative stress and/or enzyme inhibition.

In our studies [85,86] it was found by SDS polyacrylamide gel electrophoresis and isoelectrofocusing that β-lactoglobulin is modified *via* lysine amino groups, both by avarone and the synthetic steroidal quinone **13** (see Scheme 6 above) and their derivatives. The hydrolytic enzyme lysozyme and the hydrolytic enzyme hexokinase are also modified and inactivated by forementioned compounds (to be published). The derivative 3'4'-ethylenedithioavarone (**40**) was shown not to modify the proteins, since only the least reactive 6'-position of the quinone nucleus is available for addition. The derivative **40** has a much lower antitumor activity than avarone and its monosubstituted derivatives, so it becomes obvious that the nucleophilic addition is also a relevant mechanism of cytotoxicity of avarone and its derivatives (to be published). It must be pointed out that the derivative **40** has the redox potential similar to those of protein-modifying monoalkylthio derivatives, implying that lower activity is not always a consequence of the difference in redox properties.

Figure 12



Other disubstituted avarone derivatives, such as **41** [14], and **42** [53] are also much less active than the similar monosubstituted compounds (Figure 12). The notable exception are the nakijiquinones, natural derivatives of avarone, which act as tyrosine kinase inhibitors (see below).

A compound whose structure differs from typical natural sesquiterpene quinones in that it has a quinone-methide system is puupehenone (**14**), and it was suggested that it interacts with DNA through addition to the quinone methide [87].

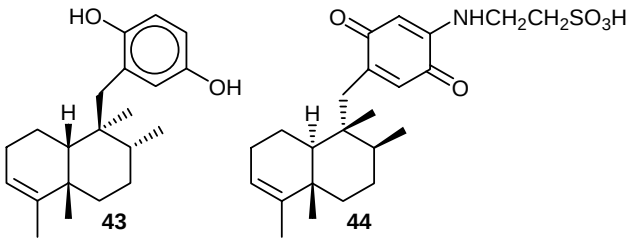
Tubulin assembly inhibition

Avarol (**1**) is an antimitotic agent, arresting cells during mitosis, and preventing karyokinesis without changing the appearance of the metaphase chromosomes [88]. Tubulin is crucial to cell division by depolymerization/repolymerization of microtubules to form a mitotic spindle. Prevention of this cycle is important in cancer chemotherapy. Avarol strongly inhibits polymerization of brain microtubule protein *in vitro*, as shown by viscosimetric analyses and electron microscopic studies [88]. Its effects are similar to those of colchicine and podophyllotoxin, although there is a significant difference [89] in that avarol inhibits protofilament elongation, rather than lateral association of tubulin during protofilament formation. At the same time, this mechanism differs from that of taxol, which stimulates the formation of microtubules by preventing the depolymerization process [90].

Protein kinase inhibition

Protein kinases, and in particular receptor tyrosine kinases (RTKs) have been identified as relevant molecular targets for treating cancer [91,92]. Several antitumor hydroquinones and quinones with a 4,9-friedodrimane skeleton showed potent, and often selective, protein *in vitro* kinase inhibitory activity. For instance, ilimaquinone (**3**) inhibits tyrosine kinase by 87 % at a concentration 1 μg/mL [93], and isoarenarol (**43**, Figure 13) and arenarol **9** from *Dysidea arenaria* inhibit several medically relevant protein kinases, with IC_{50} as low as 4 μM [94]. Interestingly, avarol (**1**) and several derivatives of avarone (**2**) were inactive against $pp60^{v\text{-}arc}$ protein tyrosine kinase, with the only exception of the taurino derivative melemeleone B (**44**, Figure 13), which was moderately active (IC_{50} = 28 μM) [95].

Figure 13

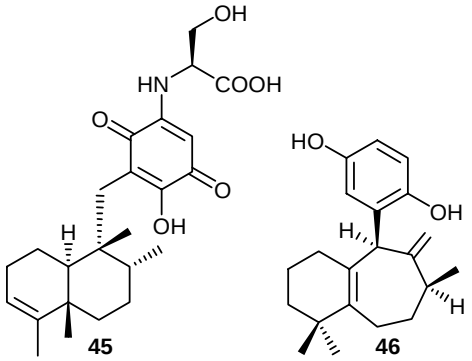


A comprehensive study was carried out with nakijiquinones (**5**) [96], after the discovery that nakijiquinones have a selective inhibitory activity against Her-2/Neu (also called erb B-2) receptor tyrosine kinase [11]. The nakijiquinones are the only naturally occurring inhibitors of this important oncogene product, vastly overexpressed in about 30% of primary breast, ovary and gastric carcinomas. Fourteen nakijiquinones and some related compounds, such as isospongiaquinone (**4**) and

ilimaquinone were also investigated as possible inhibitors of several different receptor tyrosine kinases [96], but almost all tested compounds were found to be only poor inhibitors of the investigated kinases. However, the synthetic 2-*epi*-nakijiquinone (**45**, Figure 14) is a good and selective inhibitor of VEGFR2 (KDR) with an IC_{50} value of 21 μM at adenosine triphosphate (ATP) concentration of 25 μM. Obviously, even minor stereochemical differences between agents might be an important determinant of the tyrosine kinase inhibiting potency. By molecular modelling studies probable interactions of the ligand with the ATP-binding domain of the kinase were identified. Inhibitors of the KDR receptor are very promising agents for cancer chemotherapy, since they prevent angiogenesis. Based on the structure of nakijiquinone C, a library of analogues was synthesized and investigated for inhibition of kinases with highly similar ATP-binding domains (see below). Using molecular modeling, studies in constructing homology models of some of the RTKs were also undertaken, with the aim to reveal sites in the kinase structure essential for the development of tailor-made new structural types for a more selective and efficient inhibition of different RTKs, chosen as crucial targets.

Frondosin A (**46**, Figure 14), a hydroquinone with a novel skeleton, and several related compounds from the sponge *Dysidea frondosa* showed a strong inhibitory activity against protein kinase C-α, with IC_{50} as low as 1.8 μM [97].

Figure 14



The presented results do provide insights allowing reasonable speculations with respect to some of the mechanisms by which quinones exert antitumor activity. It is to be expected that advanced examinations aimed at specific targets, will provide new ideas and lead compounds for cancer therapy.

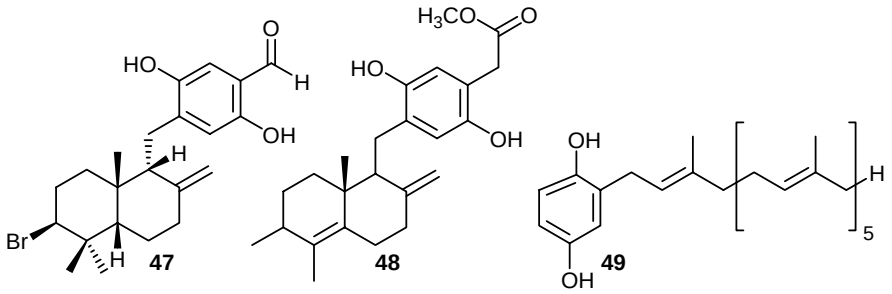
iii) Antiviral activity

Several naturally occurring sesquiterpene hydroquinones and quinones were found to inhibit various activities of HIV (human immunodeficiency virus) reverse transcriptases. Avarol (**1**) and avarone (**2**) were the subject of extensive studies, due to their ability to effectively inhibit HIV replication *in vitro* [98]. Unfortunately, clinical usefulness of the compounds could not be confirmed in AIDS patients. In a study, a series of avarone derivatives was tested in culture systems as antiviral agents, but none of them showed anti-HIV activity superior to that of the parent compounds [56]. However, most of them behaved as potent and selective inhibitors of polio virus (also a RNA virus) multiplication. Avarol and avarone and some other related natural products were the subject of

extensive studies. From the compounds tested, it appears that one of the necessary prerequisites for inhibition of HIV-1 reverse transcriptase activities is the presence of hydroxyl group at the hydroquinone or quinone ring, as shown by the structure of the most active compound **42** (Figure 12) [99]. The compounds inhibit indiscriminately the RNA-dependent and DNA-dependent DNA polymerase as well as the ribonuclease H activity of HIV-1 reverse transcriptase. Ilimaquinone (**3**) was also active, but it was found to inhibit only the ribonuclease H and not DNA polymerase activities [100]. It should be mentioned that ilimaquinone was found to inhibit the lyase activity of eukariotic DNA polymerase β [101]. This enzyme can repair damage after exposure to DNA-damaging agents, and inhibitors of this enzyme can potentiate cytotoxic activity and can be used as chemopotentiating agents in cancer treatment.

Avarol was suggested to exert its anti-HIV activity through a mechanism not involving inhibition of reverse transcriptase activity, but affecting its biosynthesis [102]. A hydroxyl group bound to hydroquinone or quinone ring is not always necessary for inhibitory activity against reverse transcriptase. For instance, peyssonols A (**47**) and B (**48**) (Figure 15) from the Red Sea red alga *Peyssonelia* sp. are potent inhibitors of RNA-directed DNA synthesis activity of the reverse transcriptases of HIV-1 and HIV-2 [103], the DNA-dependent activity being less inhibited and RNAse H activity unaffected. Also, 2-hexaprenylhydroquinone (**49**, Figure 15) from the sponge *Ircinia* sp. was shown to be an inhibitor of retroviral reverse transcriptases from HIV-1, HIV-2 and murine leukemia virus, as well as of cellular DNA polymerases [104]. Its inhibition was attributed to the effect on nucleotidyl-transfer catalytic reaction. Marine natural products with anti-HIV activity have been recently extensively reviewed [105].

Figure 15



iv) *Antiinflammatory activity*

Avarol (**1**) and some related compounds show antiinflammatory activity. Avarol was found to be a moderate inhibitor of human recombinant synovial phospholipase A_2 and cyclooxygenase and a good inhibitor of lipoxygenase [106,107]. Both avarol and, especially, avarone (**2**) inhibited the platelet aggregatory process [84]. Antiinflammatory effects of avarol were also ascribed to its behaviour as chain-breaking antioxidant of the arachidonate cascade, and/or as a scavenger of radicals. Of particular interest is the anti-psoriasis activity of avarol and some of its and avarone derivatives [108].

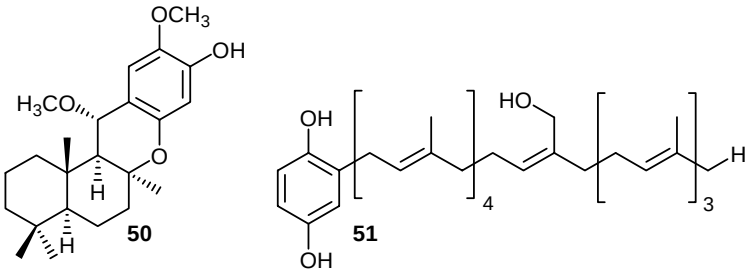
The secretory phospholipase A_2 (PLA_2) is strongly inhibited by bolinaquinone (**6**), with a potency on the human synovial enzyme (group II) higher than that of manoalide [109]. Bolinaquinone also inhibits 5-lipoxygenase activity. These activities contribute to modulatory effect of this metabolite on

acute and chronic inflammatory processes. It was recognized that PLA_2 is involved in a wide spectrum of pathophysiological conditions, including cancer [110].

Puupehenone (**14**) showed a better activity to 12-human, 15-human and 15-soybean lipoxygenases than the well known redox inhibitor nordihydroguaiaretic acid [111]. It behaves as a redox inhibitor, reducing the iron site. Good inhibitory potency and notable selectivity were exhibited by the non-reducing inhibitor dimethoxypuupehenol (**50**, Figure 16)

Prenyl hydroquinones of the type **51** (Figure 16) isolated from the sponge *Ircinia spinosula* showed an antiinflammatory activity which was at least in part a consequence of phospholipase A_2 inhibition [112].

Figure 16

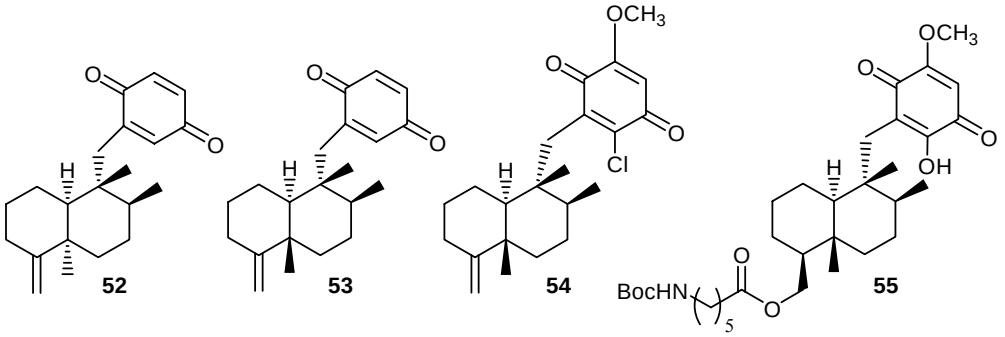


v) *Golgi-disturbing activity*

Contrary to usual approach of modern pharmacology which deals with defined molecular targets, interfering with Golgi function is more complex and is recognized by blocking secretion, altering morphology by dispersing Golgi structural elements, loss of cisternal stacks or inhibition of vesicular transport, the molecular targets often being unknown [113].

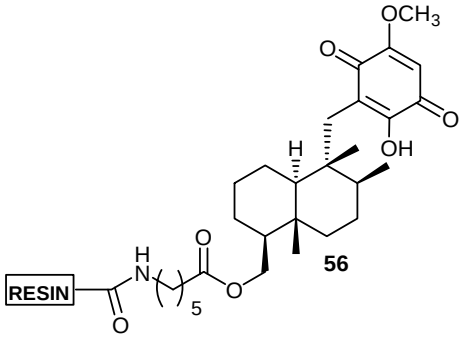
Ilimaquinone (**3**) has the ability to disrupt the Golgi apparatus [114] into small vesicles blocking cellular secretion in a reversible manner. In a detailed study of several ilimaquinone analogs and derivatives (Figure 17), it was found that rearranged drimane quinones without substituents in the quinone moiety have a lower activity than ilimaquinone in the order ilimaquinone > arenarone (**52**) > isoavarone (**53**) > avarone (**2**). On the other hand, ilimaquinone derivatives **54** and **55** are more active. The effect was not directly connected to the antitumor activity, but it was suggested that it could have implications on antitrypanosomal activity of ilimaquinone [115].

Figure 17



The active derivative **55** with a tether binding ilimaquinone structure to a protected amino group was used for preparing affinity resins **56** (Figure 18) with the aim of identifying potential target for antisecretory activity [116]. Affinity chromatography experiments showed that ilimaquinone interacts with enzymes of the activated methyl cycle: *S*-adenosylmethionin synthetase, *S*-adenosylhomocysteinase and methyl transferases. These results offer new opportunities for therapies of viral infections and atherothrombosis therapies considering the involvement of *S*-adenosylhomocysteinase in these diseases.

Figure 18



7. Marine pharmacology: perspectives

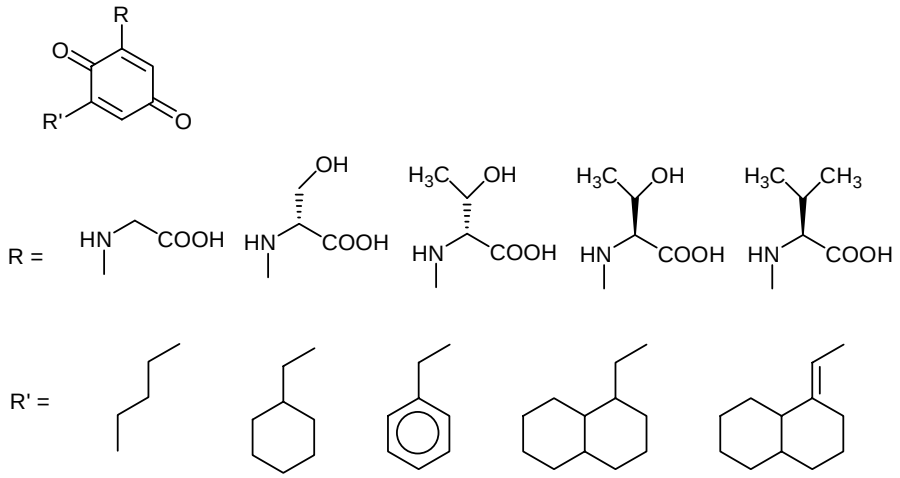
A great number of compounds with "promising biological activities" were isolated from marine sources, but not many were the subject of advanced biochemical investigation and only a few reached clinical evaluation. The reasons for a modest interest for marine organisms research by the pharmaceutical industry essentially rest on availability and regular, economically acceptable, supply of these "rich sources of new compounds". Nevertheless, this discouraging attitude has not affected the search for new chemical components as general indicators of marine life processes contributing to the ecological and environmental awareness as the *conditio sine qua non* for the development of civilisation. Therefore, the use of some marine natural products to be used for practical purposes truely depends on surmounting concomitant limitations to secure sufficient amounts of organisms without disturbing the envionmental balance. Among the ways to solve this problem are biotechnological methods. Substantial progress was already made in biomass production of sponges based on the regenerative power of their undifferentiated cells [117,118]. Also, in spite of the difficulties of sponge cultivation in natural habitat, several small-scale experiments with different sponge species were relatively fruitful [119], while in a closed system, a high growth rate of *Pseudosuberites andrews,* using microalgae as food source, was achieved. The attempts to produce secondary metabolites by sponge cell cultures are still mostly unsuccessful. However, achievement in production of cell aggregates from single cell cultures of a sponge species *Suberites domuncula*, and preserving them in culture for 5 months without a food source, is very encouraging [120,121]. Another line of investigation, with a great metabolite production potential, are marine bacteria and fungi. To some extent, this approach is complicated by the facts that in many cases it is not clear whether an isolated metabolite originates from the host organism or the symbiotic bacteria and also the question whether the symbiont and/or the host, if separated, will retain the same metabolic production. As suggested in recent publications, there is an apparent need for better understanding of the host – microbe

interactions in an ecologically relevant context [122]. In the search for new actinomycete**s** from deep ocean muds (beyond 1500 m), it was established that even organisms living in extreme conditions, can be cultivated to produce medicinally valuable products as such or as lead compounds [123,124]. Obviously, current advances in bioprocessing methodology and (bio)technology, also relevant for the aforementioned efforts to improve supply, seem to be a feasible element in the strategy to ensure economically justified production of secondary metabolites.

Organic synthesis, although a mainstay of the pharmaceutical industry, is not largely engaged in the production of marine natural products, even of those with an apparent pharmacological potential. The usual structural complexity, *i.e.* multistep procedures resulting in low overall yields, do not comply with the cost-benefit principle. Therefore, new reactions discovered and brilliant strategic principles developed in the total synthesis of natural products over the last years [125] are frequently applied in competitive production processes of other products, compatible with economic rules. Nevertheless, since great progress is being achieved in recognising the pharmacophore in a given bioactive natural product, new ideas based on core structures for the preparation of structurally simple drug candidates, are developing with increasing success.

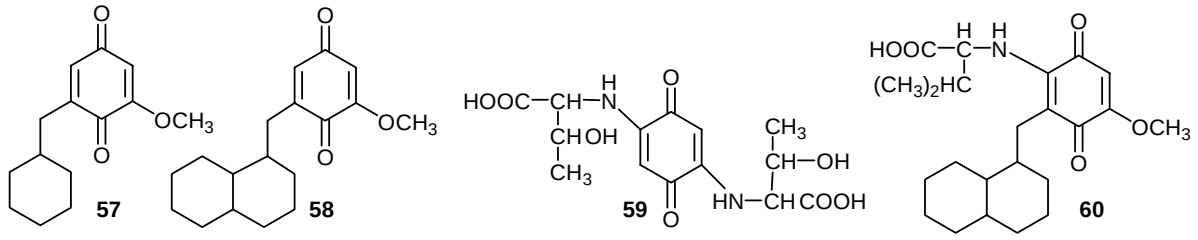
A very good example confirming the usefulness of this concept is an elaborated approach to the targeted design of a library of antitumor agents based on a "biologically validated" core structure of nakijiquinones [96,126,127] **5a-d** (see above). Among the newly synthesized compounds, some were found to be pronounced inhibitors of receptor tyrosine kinases (RTKs), critically involved in angiogenesis. In order to establish main structural features of compounds affecting the activity of these enzymes, the same group extended the library to more than 70 nakijiquinone analogues which could be biologically evaluated. The first set of analogues was structurally closely related to the selected nakijiquinones, while the second contained compounds modified both in the terpene hydrophobic unit and the quinone ring. The working hypothesis was that the terpene part will occupy the hydrophobic pocket close to the binding site of ATP in the receptor, while the quinone ring was expected to interact with the hinge region of the RTKs. The method of choice was the solution-phase synthesis, guided by the modular composition of the natural product. Some of the components of the modular composition of the nakijiquinone library are outlined in Figure 19. In analogy with parent nakijiquinones, a series of different RTKs were chosen as crucial targets in the search for anticancer drug development.

Figure 19. Examples of nakijiquinone library structural types.



The results obtained have shown that nearly 10% of the simple, easily available compounds in this library are inhibitors of RTKs, and four of them (**57-60**, Figure 20), of the Tie-2 receptor kinase. In parallel, molecular modeling studies in construction of homology models of different RTKs were also undertaken with the aim to reveal sites in the kinase structure essential for the development of more selective and efficient inhibitors of RTKs. As presented, even a change on a single chiral center results in pronounced activity differences of RTKs inhibitors.

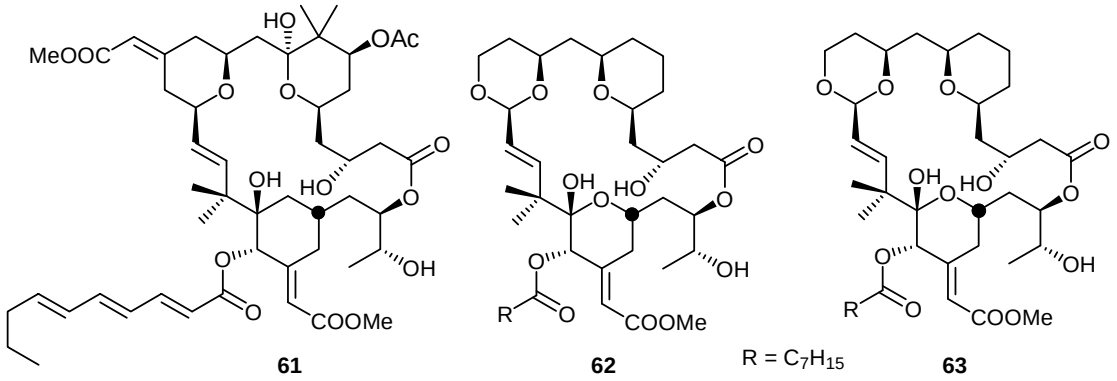
Figure 20



Using such and similar approaches, in conjunction with molecular modeling of the receptor binding site, tailor made compounds with a more selective and powerful effect of inhibition of RTKs can be developed.

Another illustrative general example of successful modification of biologically active compounds, structurally unrelated to sesqiterpene quinones**,** is the attempt to simplify structures of bryostatin-1 (**61**, Figure 21), a powerful antineoplastic macrolide from a marine bryozoan (128,129) and to establish SAR requirements [128,129] for preserving its exceptional pharmacological properties.

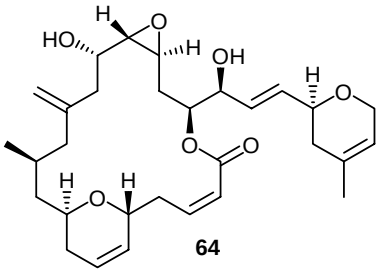
Figure 21



In a series of experiments, important discoveries and goals were achieved: *a)* the experimental data validate the design of the simplified pharmacological model structure; *b)* the newly developed analogues **62** and **63** (Figure 21) have *in vitro* and *in vivo* biological activities similar or higher than bryostatin and *c)* by practical total synthesis, the analogues are made available in sufficient amounts for clinical trials. In a similar approach [130] a series of designed analogues of laulimalide (**64**, Figure 22) a powerful microtubule stabilizing agent, isolated from a marine sponge *Cacospongia mycofijensis* [131] were prepared. This 20-membered macrolyde is relatively unstable, under mildly acidic conditions being transformed to a substantially less active isomer. Some of the analogues of simplified structures, however, are stable and retain the antiproliferative activity against tumor cells. Subtle

differences in activities between analogues provide information for the design of the new and more effective therapeutic agents.

Figure 22



The few of the results presented in this short "exercise in belief" and numerous other efforts over the last years confirm that pharmacophore recognition and organic synthesis and reaction mechanisms, in conjunction with structure-(re)activity relationship investigations, computer aided modeling, and organic synthesis including combinatorial methodologies, as well as biomass production, represent reliable assets in the process of discovery and production of important drugs.

Acknowledgements

The authors are grateful to Mr. Mario Zlatović and Mr. Srđan Tufegdžić for technical assistance in the preparation of the paper. The work carried out at the University of Belgrade which is included in the review was partly supported by the Ministry of Science and Environmental Protection of the Republic of Serbia (Project no. 1586).

References and Notes

1. Berquist, P.R. *Sponges*; Hutchinson: London, **1978**.
2. Simpson, T.L. *The cell biology of sponges*; Springer: New York, **1984**.
3. Faulkner, D.J. Marine natural products. *Nat. Prod. Rep.* **2002**, *19*, 1-48, and earlier reviews in this series.
4. Blunt, J.W.; Copp, B.R.; Munro, M.H.G.; Northcote, P.T.; Prinsep, M.R. Marine natural products. *Nat. Prod. Rep.* **2005**, *22*, 15-61, and earlier reviews in this series.
5. Faulkner, D.J. Marine Pharmacology. *A. van Leeuwenhoek* **2000**, *77*, 135-145.
6. Minale, L.; Riccio, R.; Sodano, G. Avarol, a novel sesquiterpenoid hydroquinone with a rearranged drimane skeleton from the sponge *Disidea avara*. *Tetrahedron Lett.* **1974**, 3401-3404.
7. Luibrand, R.T.; Erdman, T.R.; Vollmer, J.J.; Scheur, P.J.; Finer, J.; Clardy, J.C. Ilimaquinone, a sesquiterpenoid quinone from marine sponge. *Tetrahedron* **1979**, *35*, 609-612.
8. Capon, R.J.; McLeod, J.K. A Revision of the Absolute Stereochemistry of Ilimaquinone. *J. Org. Chem.* **1987**, *52*, 5059-5060.
9. Kazlauskas, R.; Murphy, R.T.; Warren, R.G.; Wells, R.J.; Blount, J.F. New quinones from a dictyoceratid sponge. *Aust. J. Chem.* **1978**, *31*, 2685-2697.
10. Capon, R.J. The acid-catalyzed rearrangement and absolute stereochemistry of isospongiaquinone. *J. Nat. Prod.* **1990**, *53*, 753-756.

11. Kobayashi, J.; Madono, T.; Shigemori, H. Nakijiquinone C and D, New Sesquiterpenoid Quinones with a Hydroxy Amino Acid Residue from a Marine Sponge Inhibiting c-erbB-2 Kinase. *Tetrahedron* **1995**, *51*, 10867-10874.
12. de Guzman, F.; Cop, B.R.; Mayne, C.L.; Concepcion, G.P.; Mangalindan, G.C.; Barrows, L.R.; Ireland, C.M. Bolinaquinone: A Novel Cytotoxic Sesquiterpene Hydroxyquinone from a Philippine *Dysidea* Sponge *J. Org. Chem.* **1998**, *63*, 8042-8044.
13. De Rosa, S.; Minale, L.; Riccio, R.; Sodano, G. The absolute configuration of avarol, a rearranged sesquiterpenoid hydroquinone from a marine sponge. *J. Chem. Soc. Perkin Trans.1* **1976**, 1408-1414.
14. Cozzolino, R.; De Giulio, A.; De Rosa, S.; Strazzullo, G.; Gašić, M.J.; Sladić, D.; Zlatović, M. Biological activities of avarol derivatives, 1. amino derivatives. *J. Nat. Prod.* **1990**, *53*, 699-702.
15. Trifunović, I.; Sladić, D.; Dogović, N.; Gašić, M.J. Carbon-13 chemical shift assignment in substituted 1,4-cyclohexadienones. *J. Serb. Chem. Soc.* **1987**, *52*, 559-563.
16. Sarma, A.S.; Chattopadhyay, P.; Synthetic Studies of *trans*-Clerodane Diterpenoids and Congeners: Stereocontrolled Total Synthesis of (±)-Avarol. *J. Org. Chem.* **1982**, *47*, 1727-1731
17. Capon, R.J. In *Studies in Natural Products Chemistry*; Atta-ur-Rahman, (Ed.); Elsevier Science: Amsterdam, **1995**; Vol. 15, pp. 289-326.
18. N.K. Utkina, V.A. Denisenko, O.V. Scholokova, A.E. Makarchenko. Determination of the Absolute Stereochemistry of Cyclosmenospongine E. *J. Nat. Prod.* **2003**, *66*, 1263-1265.
19. Giannini, C.; Debitus, C.; Posadaas, I.; Paya, M.; D Auria, M.V. Dysidotronic acid, a new and selective human phospholipase A_2 inhibitor from the sponge *Dysidea* sp. *Tetrahedron Lett.* **2000**, *41*, 3257-3260.
20. Patai, S.; Rapaport, Z., (Eds.); *The Chemistry of the Quinoid Compounds*; Wiley-Interscience: New York, **1988**.
21. Eberson, L. *Electron Transfer Reactions in Organic Chemistry*; Springer Verlag: Berlin, **1987**.
22. Kutyrev, A. A. Nucleophilic reactions of quinones. *Tetrahedron* **1991**, *47*, 8043-8065.
23. Verhoeven, J.W.; van Gerresheim, W.; F. M. Martens, F. M.; van der Kerk, S. M. Mechanism and transition-state structure of hydride-transfer reactions mediated by NAD(P)H-models. *Tetrahedron* **1986**, *42*, 975-992
24. Fridovich, I. Superoxide Dismutases. *Ann. Rev. Biochem.* **1975**, *44*, 147-159.
25. Fridovich, I. The biology of oxygen radicals. *Science* **1978**, *201*, 875-880 and references therein.
26. O'Brien, P.J. Molecular mechanisms of quinone cytotoxicity. *Chem.-Biol. Interact.* **1991**, *80*, 1-41.
27. Fukuzumi, S.; Wong, C. L.; Kochi, J. K. Unified view of Marcus electron transfer and Mulliken charge transfer theories in organometallic chemistry. Steric effects in alkylmetals as quantitative probes for outer-sphere and inner-sphere mechanisms. *J. Am. Chem. Soc.* **1980**, *102*, 2928-2939.
28. Fukuzumi, S.; Koumitsu, S.; Hironaka, K.; Tanaka, T. Energetic Comparison between Photoinduced Electron-Transfer Reactions from NADH Model Compounds to Organic and Inorganic Oxidants and Hydride-Transfer Reactions from NADH Model Compounds to *p*-Benzoquinone Derivatives. *J. Am. Chem. Soc.* **1987**, *109*, 305-316.
29. Fukuzumi, S.; Fujii, Y.; Suenobu, T.; Metal Ion-Catalyzed Cycloaddition vs Hydride Transfer Reactions of NADH Analogues with *p*-Benzoquinones. *J. Am. Chem. Soc.* **2001**, *123*, 10191-10199

30. Fukuzumi, S.; Ishikawa, M.; Tanaka, T. Acid-catalyzed electron-transfer processes in hydride-transfer reactions from 10-methylacridan to *p*-benzoquinone derivatives. *Chem. Lett.* **1989**, 1227-1230.
31. Ishikawa, M.; Fukuzumi, S. Primary Kinetic Isotope Effects on Acid-catalysed Reduction of *p*-Benzoquinone Derivatives by an Acid-stable NADH Analogue. *J. Chem. Soc. Faraday Trans.* **1990**, *86*, 3531-3536.
32. Abeles, R.H.; Hutton, R.F.; Westheimer, F.H. The Reduction of Thioketones by a Model for a Coenzyme. *J. Am. Chem. Soc.* **1957**, *79*, 712-716.
33. Carlson, B.W.; Miller, L.L. Mechanism of the Oxidation of NADH by Quinones. Energetics of One-Electron and Hydride Routes. *J. Am. Chem. Soc.***1985**, *107*, 479-485.
34. Miller, L.; Valentine, J.R.; On the Electron-Proton-Electron Mechanism for 1-Benzyl-1,4-dihydronicotinamide Oxidations *J. Am. Chem. Soc.* **1988**, *110*, 3982-3989..
35. Coleman, C.J.; Rose, J.G.; Murray, C.J. General acid catalysis of the reduction of *p*-benzoquinone by an NADH analog. Evidence for concerted hydride and hydron transfer. *J. Am. Chem. Soc.* **1992**, *114*, 9755-9762.
36. Spyroudis, S. Hydroxyquinones: Synthesis and Reactivity. *Molecules* **2000**, *5*, 1291-1330.
37. Cox, A.L.; Johnston, J.N. Use of the Vicinal Element Effect for Regiochemical Control of Quinone Substitutions and Its Implication for Convergent Mitomycin Construction. *Org. Lett.* **2001**, *3*, 3695-3697.
38. Božić, T.; Sladić, D.; Zlatović, M.; Novaković, I.; Trifunović, S.; Gašić, M.J. Regioselectivity of conjugate additions to monoalkyl-1,4-benzoquinones. *J. Serb. Chem. Soc.* **2002**, *67*, 547-551.
39. Šolaja, B.A.; Milić, D.R.; Gašić, M.J. A Novel *m*-CPBA Oxidation: *p*-Quinols and Epoxyquinols from Phenols. *Tetrahedron Lett.* **1996**, *37*, 3765-3768.
40. Milić, D.R.; Gašić, M.J.; Muster, W.; Csanádi, J.J.; Šolaja, B.A. The Synthesis and Biological Evaluation of A-Ring Substituted Steroidal p-Quinones. *Tetrahedron* **1997**, *53*, 14073-14084.
41. Milić, D.R.; Kapor, A.; Markov, B.; Ribar, B.; Strümpel, M.; Juranić, Z.; Gašić, M.J.; Šolaja, B.A. X-Ray Crystal Structure of 10β-Hydroxy-4β,5β-epoxyestr-1-en-3,17-dione and Antitumor Activity of its Congeners. *Molecules* **1999**, *4*, 338-352.
42. Milić, D.R.; Šolaja, B.A.; Došen-Mićović, Lj.; Ribar,B.; Kapor, A.; Sladić, D.; Gašić. M.J. Structure and reactivity of steroidal quinones. *J. Serb. Chem. Soc.* **1997**, *62*, 755-768.
43. Raber, D.J.; Rodriguez, W. Conformational Properties of Oxidation-Reduction Cofactors. *J. Am. Chem. Soc.* **1985**, *107*, 4146-4147.
44. Ling, T.; Poupon, E.; Rueden, E.J.; Kim, S.H.; Theodorakis, E.A. Unified Synthesis of Quinone Sesquiterpenes Based on a Radical Decarboxylation and Quinone Addition Reaction. *J. Am. Chem. Soc.* **2002**, *124*, 12261-12267.
45. Barton, D.H.R. The Invention of Chemical-Reactions of Relevance to the Chemistry of Natural Products. *Pure Appl. Chem.* **1994**, *66*, 1943-1954.
46. Ravi, B.N.; P. Perzanowski, P. H.; Ross, R.; Erdman, T.R.; Scheuer, P.J. Recent research in marine natural products: the puupehenones. *Pure Appl. Chem.* **1979**, *51*, 1893-1900.
47. Bourguet-Kondracki, M-L.; Lacombe, F.; Guyot, M. Methanol Adduct of Puupehenone, a Biologically Active Derivative from the Marine Sponge *Hyrtios* sp. *J. Nat. Prod.* **1999**, *62*, 1304-1305.

48. El Sayed, K.A.; Bartyzel, P.; Shen, X.; Perry, T.L.; Zjawiony, J.K.; Hamann, M.T. Marine Natural Products as Antituberculosis Agents. *Tetrahedron* **2000**, *56*, 949-953.
49. Shen, Y-C.; Hsieh, P-W. New Sesquiterpene Hydroquinones from a Taiwanese Marine Sponge *Polyfibrospongia australis. J. Nat. Prod.* **1997**, *60*, 93-97.
50. Rodríguez, J.; Quiñoá, E.; Riguera, R.; Peters, B.M.; Abrell, L.M.; Crews, P. The structures and stereochemistry of cytotoxic sesquiterpene quinones from *Dactylospongia elegans*. *Tetrahedron* **1992**, *48*, 6667-6680.
51. Müller, W.E.G.; Zahn, R.K.; Gašić, M.J.; Dogović, N.; Maidhof, A.; Becker, C.; Diehl-Seifert, B.; Eich, E. Avarol, a cytostatically active compound from the marine sponge *Dysidea avara*. *Comp. Biochem. Physiol.* **1985**, *80C*, 47-52.
52. Müller, W.E.G.; Maidhof, A.; Zahn, R.K.; Schröder, H.C.; Gašić, M.J.; Heidemann, D.; Bernd, A.; Kurelec, B.; Eich, E.; Seibert, G. Potent Antileukemic Activity of the Novel Cytostatic Agent Avarone and Its Analogues *in vitro* and *in vivo*. *Cancer Res.* **1985**, *45*, 4822-4826.
53. Hirsch, S.; Rudi, A.; Kashman, Y.; Loya, Y. New avarone and avarol derivatives from the marine sponge *Dysidea cinerea*. *J. Nat. Prod.* **1991**, *54*, 92-97.
54. Schmitz, F.J.; Lakshmi, V.; Powell, D.R.; Van der Helm, D. Arenarol and arenarone: sesquiterpenoids with rearranged drimane skeletons from the marine sponge *Dysidea arenaria*. *J. Org. Chem.* **1984**, *49*, 241-244.
55. Del Corral, J.M.M.; Gordaliza, M.; Castro, M.A.; Mahiques, M.M.; Chamorro, P.; Molinari, A.; García-Grávalos, M.D.; Broughton, H.B.; San Feliciano, A. New Selective Cytotoxic Diterpenylquinones and Diterpenylhydroquinones. *J. Med. Chem.* **2001**, *44*, 1257-1267.
56. De Giulio, A.; De Rosa, S.; Strazzullo, G.; Diliberto, L.; Obino, P.; Marongiu, M.E.; Pani, A.; La Colla, P. Synthesis and evaluation of cytostatic and antiviral activities of 3' and 4'-avarone derivatives. *Antivir. Chem. Chemother.* **1991**, *2*, 223-227.
57. Seibert, G.; Raether, W.; Dogović, N.; Gašić, M.J.; Zahn, R.K.; Müller, W.E. G. Antibacterial and antifungal activity of avarone and avarol. *Zbl. Bakt. Hyg.* **1985**, *260A*, 379-386.
58. Dorta, E.; Cueto, M.; Brito, I.; Darias, J. New Terpenoids from the Brown Alga *Stypopodium zonale*. *J. Nat. Prod.* **2002**, *65*, 1727-1730.
59. Shen, Y-C.; Chen, C-Y.; Kuo, Y-H. New Sesquiterpene Hydroquinones from a Taiwanese Marine Sponge, *Hippospongia metachromia*. *J. Nat. Prod.* **2001**, *64*, 801-803.
60. Hardt, I.H.; Jensen, P.R.; Fenical, W. Neomarinone, and new cytotoxic marinone derivatives, produced by a marine filamentous bacterium (actinomycetales). *Tetrahedron Lett.* **2000**, *41*, 2073-2076.
61. Warabi, K.; McHardy, L.M.; Matainaho, L.; Van Soest, R.; Roskelley, C.D.; Roberge, M.; Andersen, R.J. Strongylophorine-26, a New Meroterpenoid Isolated from the Marine Sponge *Petrosia* (Strongylophora) *corticata* That Exhibits Anti-invasion Activity. *J. Nat. Prod.* **2004**, *67*, 1387-1389.
62. Barrero, A.F.; Alvarez-Manzaneda, E.J.; Herrador, M.M.; Chahboun, R.; Galera, P. Synthesis and Antitumoral Activities of Marine ent-Chromazonarol and Related Compounds. *Bioorg. Med. Chem.* **1999**, *9*, 2325-2328.
63. Barrero, A.F.; Alvarez-Manzaneda, E.J.; Herrador, M.M.; Valdivia, M.V.; Chahboun, R. Synthesis of Monoterpenic Analogues of Puupehenone and Puupehedione. *Tetrahedron Lett.* **1998**, *39*, 2425-2428.

64. Henry, R.T.; Wallace, B.K. Different Mechanisms of Cell Killing by Redox Cycling and Arylating Quinones. *Arch. Toxicol.* **1996**, *70*, 482-489.
65. Guilivi, C.; Cadenas, E. One- and two-electron reduction of 2-methyl-1,4-naphthoquinone bioreductive alkylating agents: kinetic studies, free-radical production, thiol oxidation and DNA-strand-break formation. *Biochem. J.* **1994**, *301*, 21-30.
66. Ernster, L. DT diaphorase: a historical review, *Chem. Scripta* **1987**, *27A*, 1-13.
67. Maruyama, A.; Kumagai, Y.; Morikawa, K.; Taguchi, K.; Hayashi, H.; Ohta, T. Oxidative-stress-inducible qorA encodes an NADPH-dependent quinone oxidoreductase catalysing a one-electron reduction in *Staphylococcus aureus*. *Microbiology* **2003**, *149*, 389-398.
68. Pedersen, J.A. On the application of electron paramagnetic resonance in the study of naturally occurring quinones and quinols. *Spectrochim. Acta A* **2002**, *58*, 1257-1270.
69. Gašić, M.J. Biologically active compounds from marine sponges: an approach to chemical and biochemical characterization of the avarol/avarone redox couple. *J. Serb. Chem. Soc.* **1988**, *53*, 229-249.
70. Müller, W.E.G.; Sladić, D.; Zahn, R.K.; Bässler, K-H.; Dogović, N.; Gerner, H. Gašić, M.J.; Schröder, Heinz C. Avarol-induced DNA strand breakage in vitro and in Friend erythroleukemia cells. *Cancer Res.* **1987**, *47*, 6565-6571.
71. Schröder, H.C.; Wenger, R.; Gerner, H.; Reuter, P.; Kuchino, Y.; Sladić, D.; Müller, W.E.G. Suppression of the Modulatory Effects of the Antileukemic an the Anti-Human Immunodeficiency Virus Compound Avarol on Gene Expression by Tryptophan. *Cancer Res.* **1989**, *49*, 2069-2076.
72. Sladić, D.; Gašić, M.J. Effects of iron(II) compounds on the amount of DNA damage in Friend erythroleukemia cells induced by avarol. Role of hydroxyl radicals. *J. Serb. Chem. Soc.* **1994**, *59*, 915-920.
73. Batke, E.; Schröder, H.C.; Prellwitz, W.; Maidhof, A.; Eich, E.; Zahn, R.K.; Gašić, M.J.; Müller W.E.G. Influence of the antileukemic agents avarone and avarol on superoxide dismutase in vitro as well as in vivo (L5178y mouse lymphoma cells). *Biochem. Arch.* **1987**, *3*, 275-284.
74. Batke, E.; Ogura, R.; Vaupel, P.; Hummel, K.; Kallinowski, F.; Gašić, M.J.; Schröder, H.C.; Müller, W.E.G. Action of the antileukemic and anti-HTLV-III (anti-HIV) agent avarol on the levels of superoxide dismutases and glutathione peroxidase activities in L5178y mouse lymphoma cells. *Cell. Biochem. Funct.* **1987**, *6*, 123-129.
75. Gašić, M.J.; Sladić, D.; Tabaković, I.; Davidović, A. The Avarol-Avarone Redox Behaviour in Acetonitrile. *Croat. Chem. Acta* **1985**, *58*, 531-536.
76. Tabaković, I.; Davidović, A.; Müller, W.E.G.; Zahn, R.K.; Sladić, D.; Dogović, N.; Gašić, M.J. Electrochemical reactivity of biologically active quinone/hydroquinone sesquiterpenoids on glassy carbon electrodes. *Bioelectrochem. Bioener.* **1987**, *17*, 567-577.
77. Müller, W.E.G.; Diehl-Seifert, B. Sobel, C.; Bechtold, A.; Kljajić,Z.; Dorn,A. Sponge secondary metabolites: biochemical and ultrastructural localization of the antimitotic agent avarol in *Dysidea avara*. *J. Histochem. Cytochem.* **1986**, *34*, 1687-1690.
78. Dogović, N.; Sladić, D.; Gašić, M.J.; Tabaković, I.; Davidović, A.; Gunić, E. Reactivity of avarone and related *p*-benzoquinones with NADH model compounds. *Gazz. Chim. Ital.* **1991**, *121*, 63-66.

79. Davidović, A.; Tabaković, I.; Sladić, D.; Dogović, N.; Gašić, M.J. Mechanism of oxidation of 1-benzyl-1,4-dihydronicotinamide by the biologically active *p*-benzoquinone derivative, avarone, in a cationic micellar medium. *Bioelectrochem. Bioener.* **1991**, *26*, 457-468.
80. Zlatović, M.; Sladić, D.; Gašić, M.J. The kinetics of the reduction of the lipophilic quinone avarone by *n*-alkyl-1,4-dihydronicotinamides of various lipophilicities. *J. Serb. Chem. Soc.* **1999**, *64*, 647-654.
81. Okubo, T.; Nagai, F.; Ushiyama, K.; Kano, I. Contribution of oxygen radicals to DNA cleavage by quinone compounds derived from phenolic antioxidants, *tert*-butylhydroquinone and 2,5-di-*tert*-butylhydroquinone. *Toxicol. Lett.* **1997**, *90*, 11-18.
82. Swersey, J.C. Barrows, L.R.; Ireland, C.M. Mamanuthaquinone: an antimicrobial and cytotoxic metabolite of *Fasciospongia* sp. *Tetrahedron Lett.* **1991**, *46*, 6687-6690.
83. Belisario, M.A.; Pecce, R.; Maturo, M.; De Rosa, S. Arylation of sulfhydryl groups *in vitro* by the naturally occurring sesquiterpenoid benzoquinone avarone. *Toxicology* **1994**, *86*, 89-108.
84. Belisario, M.A.; Maturo, M.; Avagnale, G.; De Rosa, S.; Scopacasa, F.; De Caterina, M. *In vitro* Effect of Avarone and Avarol, a Quinone/Hydroquinone Couple of Marine Origin, on Plattelet Aggregation. *Pharmacol. Toxicol.* **1996**, *79*, 300-304.
85. Novaković, I.; Vujčić, Z.; Božić, T.; Božić, N.; Milosavić, N.; Sladić, D. Chemical modification of β-lactoglobulin by quinones. *J. Serb.Chem.Soc.* **2003**, *68*, 243-248.
86. Sladić, D.; Novaković, I.; Vujčić, Z.; Božić, T.; Božić, N.; Milić, D.; Šolaja, B.; Gašić, M. J. Protein covalent modification by biologically active quinones. *J. Serb. Chem. Soc.* **2004**, *69*, 901-907.
87. Zjawiony, J.K.; Bartyzel, P.; Hamann, M.T.; Chemistry of Puupehenone: 1,6-Conjugate Addition to Its Quinone-Methide System, *J. Nat. Prod.* **1998**, *61*, 1502.
88. Müller W.E.G.; Dogović, N.; Zahn, R.K.; Maidhof, A.; Diehl-Seifert, B.; Becker. C.; Sachsse, W.; Gašić, M.J.; Schröder, H.C. Inhibition of mitosis by avarol, a natural product isolated from the sponge *Dysidea avara*. *Bas. Appl. Histochem.* **1985**, *29*, 321-330.
89. Sandoval, I.V.; Weber, K. Polymerization of tubulin in the presence of colchicine or podophyllotoxin. Formation of a ribbon structure induced by guanylyl-5'-methylene diphosphonate. *J. Mol. Biol.* **1979**, *134*, 159-172.
90. Rowinsky, E.K.; Tolcher, A.W. In *Cancer: Principles and Practice of Oncology*; V.T. DeVita, Jr., S. Hellman, S.A. Rosenberg, (Eds.): Lippincott Williams & Wilkins: Philadelphia, PA, **2001**; Vol. 1, 431-447.
91. Cohen, P. Protein kinases - the major drug targets of the twenty-first century? *Nat. Rev. Drug Discov.* **2002**, *1*, 309-315.
92. Bridges, A.J. Chemical Inhibitors of Protein Kinases. *Chem. Rev.* **2001**, *101*, 2541-2571.
93. Wessels, M.; König, G.M.; Wright, A.D. A New Tyrosine Kinase Inhibitor from the Marine Brown Alga *Stypopodium zonale*. *J. Nat. Prod.* **1999**, *62*, 927-930.
94. Yoo, H.-D.; Leung, D.; Sanghara, J.; Daley, D.; van Soest, R.; Andersen, R.J. Isoarenarol, A New Protein Kinase Inhibitor from the Marine Sponge *Dysidea arenaria*. *Pharm. Biol.* **2003**, *41*, 223-225.
95. Alvi, K.A.; Diaz, M.C.; Crews, P.; Slate, D.L., Lee, R.H.; Moretti, R. Evaluation of New Sesquiterpene Quinones from Two Dysidea Sponge Species as Inhibitors of Protein Tyrosine Kinase. *J. Org. Chem.* **1992**, *57*, 6604-6607.

96. Stahl, P.; Kissau, L.; Mazitschek, R.; Huwe, A.; Furet, P.; Giannis, A.; Waldmann, H. Total Synthesis and Biological Evaluation of the Nakijiquinones. *J. Am. Chem. Soc.* **2001**, *123*, 11586-11593.
97. Patil, A.D.; Freyer, A.J.; Killmer, L.; Offen, P.; Carte, B.; Jurewicz, A.J.; Johnson, R.K. Frondosins, Five New Sesquiterpene Hydroquinone Derivatives with Novel Skeletons from the Sponge *Dysidea frondosa*: Inhibitors of Interleukin-8 Receptors. *Tetrahedron* **1997**, *53*, 5047-5060.
98. Sarin, P.S.; Sun, D.; Thornton, A.; Müller, W.E.G. Inhibition of replication of the etiological agent of acquired immune deficiency syndrome (human T-lymphotropic retrovirus/lymphadenopathy-associated virus) by avarol and avarone. *J. Natl. Cancer Inst.* **1987**, *78*, 663-666.
99. Loya, S.; Hizi, A. The inhibition of human immunodeficiency virus type 1 reverse transcriptase by avarol and avarone derivatives. *FEBS Lett.* **1990**, *269*, 131-134.
100. Loya, S.; Tal. R.; Kashman, Y.; Hizi, A. Illimaquinone, a selective inhibitor of the RNase H activity of human immunodeficiency virus type 1 reverse transcriptase. *Antimicrob. Agents Chemother.* **1990**, *34*, 2009-2012.
101. Cao, S.; Gao, Z.; Thomas, S.J.; Hecht, S.M.; Lazo, J.S.; Kingston, D.G.I. Marine Sesquiterpenoids that Inhibit the Lyase Activity of DNA Polymerase β. *J. Nat. Prod.* **2004**, *67*, 1716-1718.
102. Kuchino, Y.; Nishimura, S.; Schröder, H.C.; Rottmann, M.; Müller, W.E.G. Selective inhibition of formation of suppressor glutamine tRNA in Moloney murine leukemia virus-infected NIH-3T3 cells by Avarol. *Virology* **1988**, *165*, 518-526.
103. Loya, S.; Bakhanashvili, M.; Kashman, Y.; Hizi, A. Peyssonols A and B, two novel inhibitors of the reverse transcriptases of human immunodeficiency virus types 1 and 2. *Arch. Biochem. Biophys.* **1995**, *316*, 789-796.
104. Loya, S.; Rudi, A.; Kashman, Y.; Hizi, A. 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.
105. Tziveleka, L.-A.; Vagias, C.; Roussis, V. Natural products with anti-HIV activity from marine organisms. *Curr. Top. Med. Chem.* **2003**, *3*, 1512-1535
106. Schroeder, H.C.; Begin, M.E.; Kloecking, R.; Matthes, E.; Sarma, S.S.; Gašić, M.J.; Mueller, W.E. G. Avarol restores the altered prostaglandin and leukotriene metabolism in monocytes infected with human immunodeficiency virus type 1. *Virus Research* **1991**, *21*, 213-23
107. Ferrándiz, M.L.; Sanz, M.J.; Bustos, G.; Payá, M.; Alcaraz, M.J.; De Rosa, S. Avarol and avarone, two new anti-inflammatory agents of marine origin. *Eur. J. Pharmacol.* **1994**, *253*, 75-82.
108. Amigó, M.; Terencio, M.C.; Mitova, M.; Iodice, C.; Payá, M.; De Rosa, S. Potential Antipsoriatic Avarol Derivatives as Antioxidants and Inhibitors of PGE2 Generation and Proliferation in the HaCaT Cell Line. *J. Nat. Prod*. **2004,** *67*, 1459-1463.
109. Lucas, R.; Giannini, C.; D'Auria, M.V.; Payá, M. Modulatory Effect of Bolinaquinone, a Marine Sesquiterpenoid, on Acute and Chronic Inflammatory Processes. *J. Pharmacol. Exp. Ther.* **2003**, *304*, 1172-1180.

110. Balsinde, J.; Balboa, M.A.; Insel, P.A.; Dennis, E.A. Regulation and inhibition of phospholipase A2. *Annu. Rev. Pharmacol. Toxicol.* **1999**, *39*, 175-189.
111. Amagata, T.; Whitman, S.; Johnson, T.A.; Stessman, C.C.; Loo, C.P.; Lobkovsky, E.; Clardy, J.; Crews, P.; Holman, T.R. Exploring Sponge-Derived Terpenoids for Their Potency and Selectivity against 12-Human, 15-Human, and 15-Soybean Lipoxygenases. *J. Nat. Prod.* **2003**, *66*, 230-235.
112. Gil, B.; Sanz, M.J.; Terencio, M.C.; De Giulio, A.; De Rosa, S.; Alcaraz, M.J.; Payá, M. Effects of marine 2-ployprenyl-1,4-hydroquinones on phospholipase A_2 activity and some inflammatory responses. *Eur. J. Pharmacol.* **1995**, *285*, 281-288.
113. Dinter, A.; Berger, E.G. Golgi-disturbing agents. *Histochem. Cell. Biol.* **1998**, *109*, 571-590.
114. Takizawa, P.A.; Yucel, J.K.; Veit, B.; Faulkner, D.J.; Deerinck, T.; Soto, G.; Ellisman, M.; Malhotra, V. Complete vesiculation of Golgi membranes and inhibition of protein transport by a novel sea sponge metabolite, ilimaquinone. *Cell* **1993**, *73*, 1079.
115. Rangel, H.R., Dagger, F.; Compagnone, R.S. Antiproliferative effect of illimaquinone on *Leishmania mexicana*. *Cell. Biol. Internat*. **1997**, *21*, 337-339.
116. Radeke, H.S.; Digits, C.A.; Casaubon, R.L.; Snapper, M.L. Interactions of (-)-ilimaquinone with methylation enzymes: implications for vesicular-mediated secretion. *Chem. Biol.* **1999**, *6*, 639-647.
117. Battershill, C.N.; Page, M.J. Sponge aquaculture for drug production. *Aquacult. Updates* **1996**, *16*, 5-6.
118. Adams, C.; Stevely, J.M.; Sweat, D.J. Economic feasibility of small-scale sponge farming in Pohnpei, Federated States of Micronesia. *J. World Aquacult.* **1995**, *26*, 132-142.
119. Barthel, D.; Theede, H. A new method for the culture of marine sponges and its application for experimental studies. *Ophelia* **1986**, *25*, 75-82.
120. De Rosa, S. In *Natural Products in the New Millennium: Prospects and Industrial Application*; Rauter, A.P.; Palma, F.B.; Araujo, M.A.; dos Santos; S.P. (Eds.); Kluwer Academic Publishers: Dordrecht, **2002**; pp. 441-461.
121. Müller, W.E.G.; Wiens, M.; Batel, R.; Steffen, R.; Borojevic, R.; Custodio, M.R. Established of a primary cell culture from a sponge: primmorphs from *Suberites domuncula*. *Mar. Ecol. Prog. Ser.* **1999**, *178*, 205-219.
122. Engel, S.; Jensen P.R.; Fenical,W. Chemical ecology of marine microbial defense. *J. Chem.Ecol.* **2002**, *28*, 1971-1985.
123. Fenical, W. *et al.* Microbes from deep ocean sediments, accessing a new antibiotic resource. *International Symposium "Chemistry & Biology of Marine Organisms"*; Crete, **2003;** Sept. 21-26, 2003, Kolympari, Crete, Greece.
124. Fenical, W.; Sethna, K.; Lloyd, G.K. *Pharma. News*. **2002**, *9*, 489-494.
125. Nicolaou, K.C.; Vourloumis, D.; Winssinger, N.; Baran, P.S. The Art and Science of Total Synthesis at the Dawn of the Twenty-First Century. *Angew. Chem. Int. Ed.* **2000,** *39*, 44 – 122 and ref. therein
126. Stahl, P.; Kissau, L.; Mazitschek, R.; Huwe, A.; Furet, P.; Giannis, A.; Waldmann, H. Total Synthesis and Biological Evaluation of the Nakijiquinones. *J. Am. Chem. Soc.* **2001**, *47*, 11586 – 11593.

127. Stahl, P.; Kissau, L.; Mazitschek, R.; Giannis A.; Waldmann, H. Natural Product Derived Receptor Tyrosine Kinase Inhibitors: Identification of IGF1R, Tie-2, and VEGFR-3 Inhibitors. *Angew. Chem. Int. Ed.* **2002**, *41*, 1174-1178.
128. Wender, P.A.; Hilinski, M.K.; Mayweg, A.V.W. Late-State Intermolecular CH Activation for Lead Diversification: A Highly Chemoselective Oxyfunctionalization of the C-9 Position of Potent Bryostatin Analogues. *Org. Lett.* **2005**, *7*, 79-82.
129. Wender, P.A.; Baryza, J.L.; Bennet C.E.; Bi, F.C.; Brenner, S.E.; Clarke, M.O.; Horan, J.C.; Kan, C.; Lacôte, E.; Lippa, B.; Nell, P.G.; Turner,T.M. The Practical Synthesis of a Novel and Highly Potent Analogue of Bryostatin *J. Am. Chem. Soc.* **2002**, *46*, 13648 – 13649 and references therein.
130. Mooberry, S.L.; Randall-Hlubek, D.A.; Leal, R.M.; Hegde, S.G.; Hubbard, R.D.; Zhang, L.; Wender, P.A. Microtubule-stabilizing agents based on designed laulimalide analogues. *Proc. Natl. Acad. Sci. USA*, **2004**, *101*, 8803-8808.
131. Mooberry, S.L.; Tien, G.; Hernandez, A.H.; Plubrukarn, A.; Davidson, B.S. Laulimalide and isolaulimalide, new paclitaxel-like microtubule-stabilizing agents. *Cancer Res.* **1999**, *59*, 653-660.

Sample availability: Not applicable