

## Design and Synthesis of a Novel Series of Pyranonaphthoquinones as Topoisomerase II Catalytic Inhibitors

Sandra Jiménez-Alonso,<sup>†,‡</sup> Haydee Chávez Orellana,<sup>†,||</sup> Ana Estévez-Braun,<sup>\*,†,‡</sup> Angel G. Ravelo,<sup>†,‡</sup> Elisa Pérez-Sacau,<sup>†,‡</sup> and Felix Machín<sup>\*,§</sup>

*Instituto Universitario de Bio-Organica "Antonio González", Universidad de La Laguna, Avda. Astrofísico Fco, Sánchez 2, 38206 La Laguna, Tenerife, Spain, Instituto Canario de Investigaciones del Cáncer (ICIC) (<http://www.icic.es>), Spain, Facultad de Farmacia Bioquímica, Universidad San Luis Gonzaga de Ica, Peru, and Unidad de Investigación, Hospital Universitario Nuestra Señora de Candelaria, Carretera del Rosario, 145, 38010, Santa Cruz de Tenerife, Spain*

Received April 30, 2008

On the basis of previous pharmacophore modeling studies of naphthoquinones derivatives, we have designed and synthesized a new set of pyranonaphthoquinones. These compounds were obtained through a direct and highly efficient approach based on an intramolecular domino Knoevenagel hetero Diels–Alder reaction from lawsone (2-hydroxynaphthoquinone) and a variety of aldehydes containing an alkene. The synthesized pyranonaphthoquinones were evaluated against the  $\alpha$  isoform of human topoisomerase II (hTopoII $\alpha$ ). Among the 11 derivatives studied, we found that six of them act as catalytic inhibitors of the enzyme in vitro. These six derivatives strongly preclude the enzyme from decatenating or relaxing suitable substrates. Finally, we correlate their active/inactive status with docking studies of these novel compounds into the ATPase domain of hTopoII $\alpha$ .

### Introduction

Mass screening programs of natural products by the National Cancer Institute have identified the quinone moiety as an important pharmacophoric element for cytotoxic activity.<sup>1</sup> A representative group of quinonoid compounds are 1,2- and 1,4-naphthoquinones, which are widely distributed in nature and play important physiological roles in animals and plants.<sup>2</sup> Some illustrative examples of antitumoral naphthoquinones are plumbagin,<sup>3</sup> juglone,<sup>4</sup>  $\beta$ -lapachone,<sup>5</sup> and rhinacanthone.<sup>6</sup> These 1,2- and 1,4-naphthoquinones are considered privileged structures in medicinal chemistry on the basis of their numerous biological activities and structural properties.<sup>7</sup> In most cases, the biological activity is related to the ability of quinones to accept one or two electrons to form highly reactive radical anion intermediates, which are responsible for the oxidative stress observed in the cells.<sup>8</sup> But there are several genotoxic properties<sup>9</sup> attributed to quinonoid compounds such as DNA intercalation or alkylation of DNA that might also contribute to their cytotoxicity. Especially relevant is the fact that quinones may have topoisomerase II as a target.<sup>10,11</sup>

Topoisomerases are important targets in antitumoral chemotherapy. In fact, after more than 30 years of clinical use, about 50% of current treatment protocols still employ at least one drug directed against topoisomerases.<sup>12</sup>

From a physiological point of view, these enzymes catalyze changes in the topology of the DNA molecule and facilitate events such as transcription, replication, and physical separation of sister chromatids in mitosis.<sup>13</sup>

Two types of topoisomerases can be differentiated on the basis of their molecular reactions. Type I topoisomerases catalyze

the relaxation of positive supercoiling by making single strand breaks in one of the DNA strands while allowing the other one to rotate around it. Type II topoisomerases make transient double strand breaks and allow other DNA molecule to pass through it. Type II topoisomerases, like TopoI, can also relax supercoiled DNA, but they are the only enzymes capable of unknotting the replicated sister chromatids in order to allow a faithful segregation of the genetic material to the daughter cells during cell division.<sup>14–17</sup>

The key to understanding why topoisomerases are good antitumoral targets comes from different angles. First of all they are highly active in cells that are proliferating and therefore need to replicate and segregate the DNA.<sup>13,17–19</sup> Moreover, in humans, one of the type II topoisomerases, TopoII $\alpha$ , is up-regulated in proliferating cells.<sup>18</sup> On the other hand, as they can generate strand breaks, they are potential cytotoxic molecular scissors. In fact, this seems to be the cytotoxic mechanism for a number of antitumoral drugs that target topoisomerases.<sup>20–26</sup> These drugs, known as topoisomerase “poisons”, stabilized the topoisomerase-induced breaks and therefore generate DNA damage, the ultimate cause of induced cell death for most cancer cells.<sup>22,27,28</sup> Examples of such poisons can be found for both topoisomerases. Camptothecin and its derivatives are specific TopoI poisons,<sup>29</sup> whereas epipodophyllotoxins (etoposide), aminoacridines (amsacrine), and antracyclines (doxorubicin, daunorubicin, etc.) are potent TopoII poisons.<sup>30–34</sup> Aside from the poisons, other groups of drugs with potent antitumoral activity have been shown to act as pure catalytic inhibitors of the enzymes. This is the case of the anti-TopoII agents merbarone and the family of bisdioxopiperazines.<sup>35–38</sup> The actual mechanism by which these later group exerts their cytotoxic properties remains controversial, but it is likely that cell death occurs through mitotic collapse as cells fail to segregate chromosomes because of reduced TopoII activity.<sup>39–43</sup>

Here, we present the synthesis of novel pyranonaphthoquinones based on previous pharmacophore modeling studies.<sup>44</sup> We have also evaluated their potential as drugs that target human topoisomerase II $\alpha$  and find that six of them do act as TopoII

\* To whom correspondence should be addressed. For A.E.-B.: phone, +34 922 318576; fax, +34 922 318571; e-mail, aestebra@ull.es. For F.M.: phone, +34 922 602951; fax, +34 922 600562; e-mail, fmacconw@canarias.org.

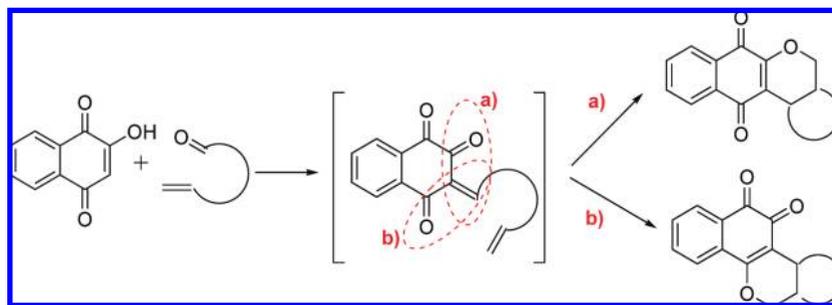
<sup>†</sup> Universidad de La Laguna.

<sup>‡</sup> Instituto Canario de Investigaciones del Cáncer.

<sup>||</sup> Universidad San Luis Gonzaga de Ica.

<sup>§</sup> Hospital Universitario Nuestra Señora de Candelaria.

## Scheme 1



catalytic inhibitors. Finally, we correlate their active/inactive status with docking studies of these novel compounds into the ATPase domain of hTopoII $\alpha$ .

## Chemistry

Recently we have published the synthesis and pharmacophore modeling of naphthoquinone derivatives with cytotoxic activity in human HL-60 cell line.<sup>44</sup> In the CoMSIA model the steric map of prenylpyranonaphthoquinones contains a favorable area located in the pyran ring, which indicates that bulky substituents or an extension of the structure in this part of the molecule produces high cytotoxicity. Continuing with our interest in synthesizing bioactive naphthoquinone derivatives,<sup>44,45</sup> we aim at achieving complex prenylpyranonaphthoquinones by extension of the structure through the pyran ring.

One of the most efficient synthetic methodology creating complexity and diversity are the domino reactions.<sup>46</sup> In this sense, the domino Knoevenagel hetero Diels–Alder reaction in its intramolecular version has emerged as a powerful process that not only allows the efficient synthesis of complex compounds such as natural products<sup>47</sup> starting from simple substrates but also permits the preparation of highly diverse molecules. Representative examples of this methodology using  $\alpha,\beta$ -unsaturated carbonyl compounds are the preparation of tetra-substituted dihydropyrans,<sup>48</sup> tetracyclic pyrazoles,<sup>49</sup> substituted 2*H*,5*H*-pyrano[4,3-*b*]pyran-5-ones,<sup>50</sup> and a variety of  $\alpha$ -laponone derivatives.<sup>51</sup>

As reported by Ferreira et al.,<sup>51b</sup> the reaction of 2-hydroxy-1,4-naphthoquinone with aliphatic aldehydes bearing a double bond at an appropriate distance generates two new fused rings next to the naphthoquinone core. Thus, the Knoevenagel condensation of 2-hydroxy-1,4-naphthoquinone with an unsaturated aldehyde produces the intermediate shown in Scheme 1. This species presents two heterodiene systems that will provide the formation of *o*- or *p*-naphthoquinones.

We selected 2,6-dimethyl-5-heptenal **1** to search for the best reactions conditions, and then we use different ratios of reagents, different solvents (EtOH, MeOH, C<sub>7</sub>H<sub>8</sub>, DCE, dioxane, acetonitrile), and different bases (ethylene diammonium diacetate, triethylamine, *N*-acetylpiperidine). The best yield was obtained using 3 equiv of aldehyde, 6% mol of EDDA, and EtOH as solvent under reflux. In these conditions, the corresponding adducts were obtained in quantitative yield in 30 min (Table 1, entry 1). Then we applied these conditions to several aliphatic and aromatic aldehydes. With the aliphatic aldehydes 2,6-dimethyl-5-heptenal (entry 1) and citronellal (entry 2), we obtained high diastereoselectivity since trans-adducts were obtained. The products were obtained as a mixture of *o*- and *p*-naphthoquinones, easy to separate by chromatography. The stereogenic center in  $\alpha$ -position or  $\beta$ -position to the carbonyl in the aldehydes **1** and **2** renders a high asymmetric induction,

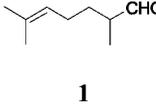
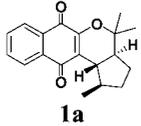
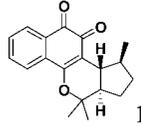
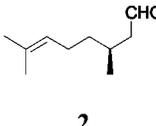
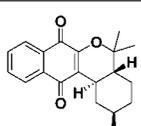
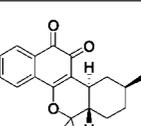
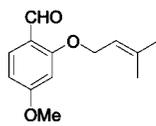
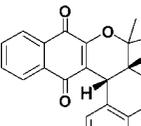
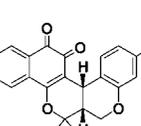
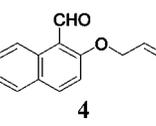
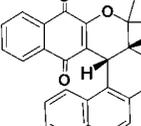
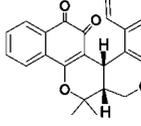
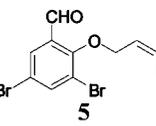
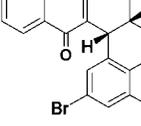
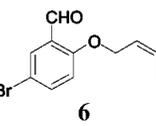
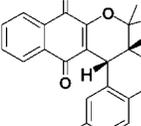
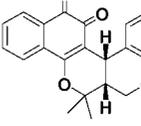
and the adducts **1a**, **1b**, **2a**,<sup>51b</sup> and **2b**<sup>51b</sup> were produced with excellent selectivity and high yield. Attending to the structural diversity, the synthetic sequence is very attractive because 1,2- and 1,4-naphthoquinone adducts are generated in a one-pot reaction, allowing after biological evaluation the direct comparison between 1,2- and 1,4-naphthoquinones in the SAR (structure–activity relationship) study. The trans stereochemistry of the adducts **1a**, **1b**, **2a**, and **2b** was established on the basis of coupling constants and ROESY experiments. This stereochemistry agrees with the results by Tietze and co-workers<sup>47</sup> for similar pyrano fused systems described. In the formation of trans-annulated products only the *exo*-(*E*)-*anti* transition state can be operative, since the *exo*-(*E*)-*anti* form is not possible for geometrical reasons.<sup>47a</sup>

The aromatic aldehydes **3–6** were prepared from the *O*-alkylation of the corresponding phenol derivatives, using 1-bromo-3-methylbutene and K<sub>2</sub>CO<sub>3</sub> in dry acetone under reflux. Only *cis* adducts were obtained from these aromatic aldehydes, with overall yields slightly lower than those of **1** and **2**. An *endo*-(*E*)-*syn* orientation can be assumed as transition structure because an *exo*-(*Z*)-*syn* transition seems less likely owing to steric interferences.<sup>47a,d</sup> The *cis* nature of the bridging hydrogens of the adducts was strongly supported by their coupling constant ( $\sim 4.4$  Hz) in the <sup>1</sup>H NMR spectrum and by the NOE effects detected in the ROESY spectrum. With the aldehyde **5** (entry 5) only one compound **5a** was detected. The no formation of the corresponding orthoquinone is probably due to steric hindrance of the bromine group in the transition state. The energetic difference HOMO<sub>dienophile</sub> – LUMO<sub>heterodiene</sub> of the two possible 1-oxa-1,3-butadiene must be similar for all but entry 5 in Table 1, a conclusion following from the ratio of resulting adducts. Aiming at improving the degree of regioselectivity, we repeated the reaction under microwave irradiation<sup>52</sup> following different conditions; however, microwaving only improves the reaction time (from 20 to 3 min; see Table 1) with no significant change of the yields corresponding to reflux conditions.

## Biological Results and Discussion

To evaluate the potential biological activities of the new compounds, we decided to check their abilities as anti-topoisomerase II agents. This first and direct approach was chosen because (i) quinones have already been shown to act on TopoII without the need to take into account possible, and more complex, intracellular conversions to other more reactive species,<sup>13</sup> (ii) topoisomerase II is possibly the most important molecular target of many antitumoral agents in use,<sup>12,13</sup> and (iii) regardless of the cytotoxic potency and mechanism of quinones in vivo, the knowledge of their anti-topoisomerase II activities has important implications to take into consideration if they want to be developed for clinical use.<sup>13</sup>

**Table 1.** Reaction of 2-Hydroxy-1,4-naphthoquinone with Several Unsaturated Aldehydes

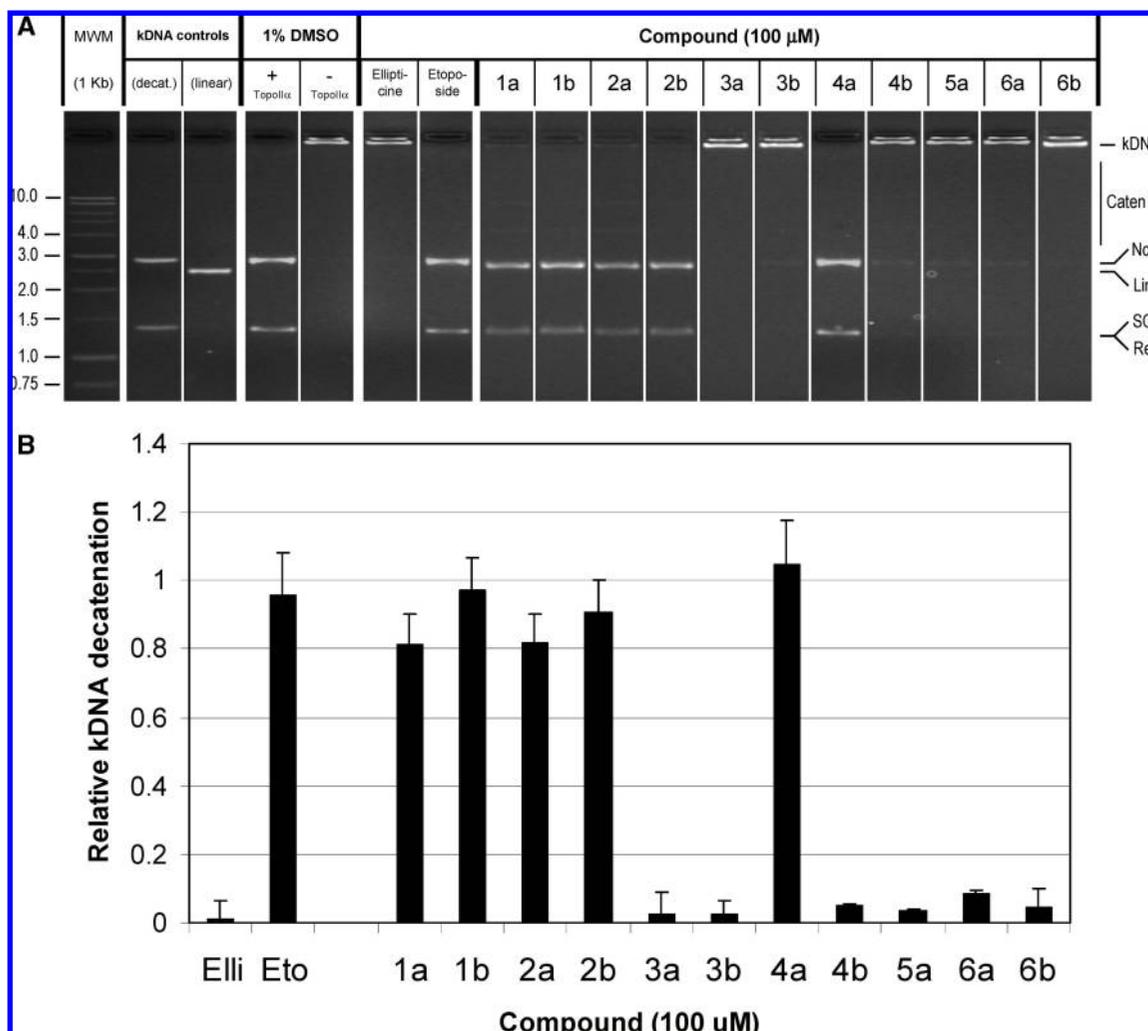
Entry	Aldehyde	<i>Para</i> -adduct	<i>Ortho</i> -adduct	Ratio <sup>a</sup> (Yield)	Ratio <sup>b</sup> (Yield)
1				1:1.5 (100)	1:1 (98)
2				1:1 (94)	1:1 (85)
3				1:1.6 (70)	1:1 (85)
4				1:1.6 (70)	1:1 (66)
5			----	---- (62)	---- (59)
6				1:1 (72)	1.4:1 (68)

<sup>a</sup> Heating under reflux using EtOH as solvent. <sup>b</sup> Heating using a CEM-Discover microwave and EtOH as solvent.

To test whether compounds were active against human topoisomerase II $\alpha$ , we employed the purified enzyme and a set of small molecules of circular DNA as substrates in a series of different in vitro experiments. The use of these small DNA molecules greatly facilitates the visualization of the topoisomerase reactions by simple electrophoretic means in the three assays described below: “decatenation”, “relaxation”, and “stabilization of the cleavage complex”.

For the decatenation assay, the kinetoplast DNA (kDNA) was employed as substrate. Kinetoplast DNA, obtained from *Crithid-*

*ia fasciculata*, is a network consisting of thousands of interlocked closed circular DNA molecules called “minicircles”. Because of its overall size, kDNA cannot enter a typical agarose gel during electrophoresis unless minicircles are previously released. A transient double strand break is the only way to take a minicircle out of the network and preserve its circular nature. This can be accomplished through the decatenation activity of topoisomerase II. In fact, this assay is considered to be the most specific one for topoisomerase II activity.<sup>53</sup> Minicircles, once released, enter the agarose gel and appear as



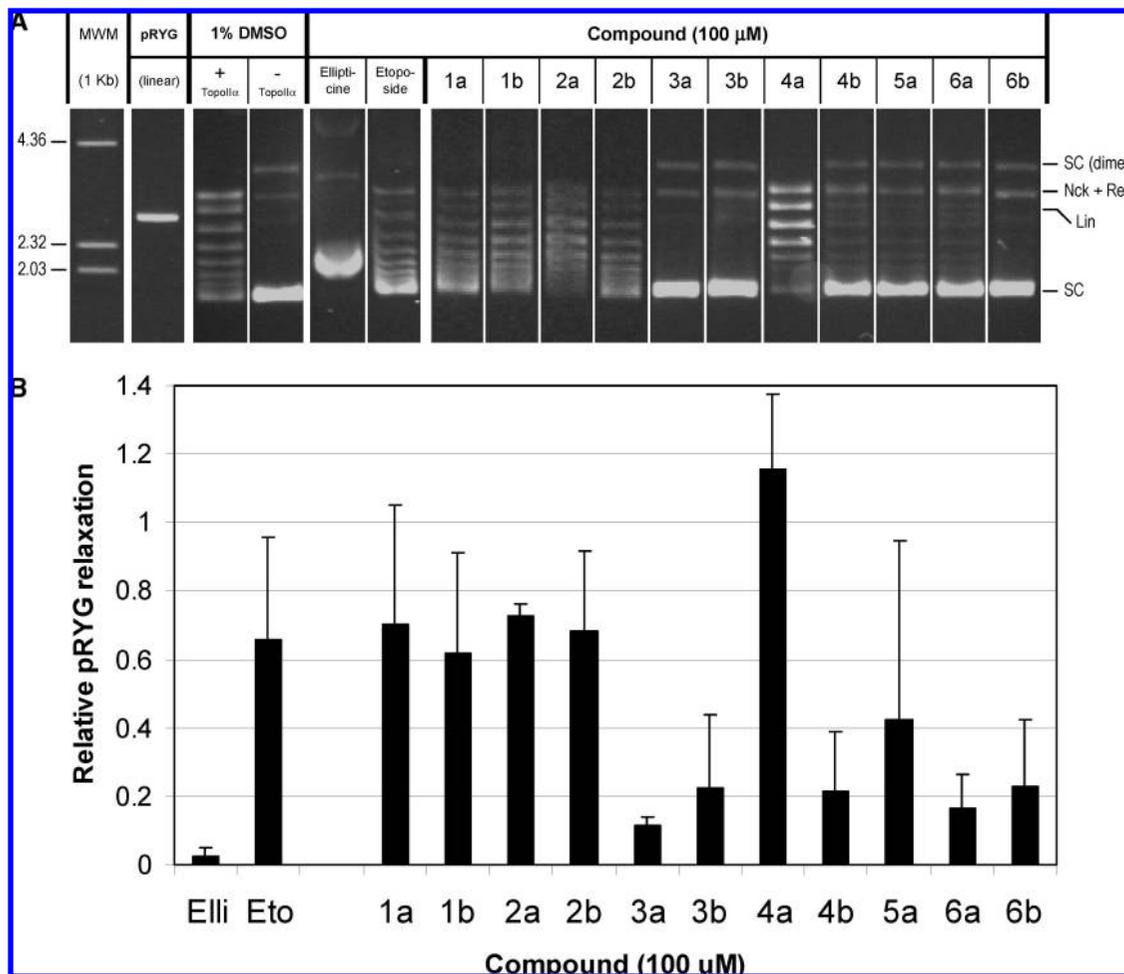
**Figure 1.** Inhibition of hTopoII $\alpha$ -mediated kDNA decatenation by pyranonaphthoquinones **3a**, **3b**, **4a**, **5a**, **6a**, **6b**. Catenanes minicircles from kinetoplast DNA were treated with hTopoII $\alpha$  in the presence of either just 1% DMSO or 100  $\mu$ M ellipticine, etoposide, or a novel set of pyranonaphthoquinones in 1% DMSO: (A) agarose gel electrophoresis to separate substrates (kDNA) and products of the hTopoII $\alpha$  reaction (Nck + Rel + SC); (B) quantitation of product formation of three independent experiments relative to 1% DMSO. Note how the presence of pyranonaphthoquinones **3a**, **3b**, **4b**, **5a**, **6a**, and **6b** completely inhibit hTopoII $\alpha$  (compared to controls without enzyme or with ellipticine): kDNA (intact kinetoplast DNA), Caten (catenanes subproducts), Nck (nicked minicircles), Lin (linearized minicircles), SC (supercoiled minicircles), Rel (relaxed minicircles).

a mixture of covalently closed topoisomers and nicked products ("Nck" band in Figure 1A). To simplify the overall number of visible products, the electrophoresis is carried out in the presence of an excess of an intercalative agent (ethidium bromide in these assays) to convert all covalently closed topoisomers to a unique band of maximum supercoiled unique topoisomer ("SC" band in Figure 1A).

Thus, we incubated the purified enzyme with the synthesized pyranonaphthoquinones and immediately added the kDNA (Figure 1). Several controls were included to confirm the correct performance of the assays. First samples with just the final concentration of the solvent (1% DMSO) were used with or without the enzyme to set up our 100% or 0% of activity, respectively. These samples were also used to titrate the amount of enzyme, kDNA, and incubation time to just complete the reaction under the presence of the enzyme ("end-point" reaction, not shown). Two well-known anti-TopoII agents were also included. Etoposide is a topoisomerase II highly specific nonintercalative poison that stabilizes the cleavage complex. As the cleavage step is indeed reversible,<sup>26</sup> etoposide does not inhibit greatly the overall reaction (Figure 1). On the other hand, ellipticine, while it modestly promotes the formation of more

cleavage intermediates, can strongly inhibit topoisomerase II decatenation at the same time that it intercalates into the DNA molecule<sup>54</sup> (Figure 1). So far, complete decatenation was seen in the control without any drugs and the sample with etoposide, whereas ellipticine inhibited the minicircles release to a great extent as seen by comparison with the sample that lacked the enzyme (Figure 1). The same complete absence of decatenation activity was observed when compounds **3a**, **3b**, **4b**, **5a**, **6a**, and **6b** were present, whereas no inhibition was apparent for the pyranonaphthoquinones **1a**, **1b**, **2a**, **2b**, and **4a**.

To further check that decatenation failed by an inhibition of topoisomerase II $\alpha$  in the presence of the pyranonaphthoquinones, we performed another assay for TopoII activity. In this assay we measured the TopoII-dependent relaxation of a plasmid (pRYG) that contained a hot spot sequence for TopoII.<sup>55</sup> Plasmids, as they are in bacteria, are negatively supercoiled in its natural form ("SC" band in Figure 2A), and this basic form is preserved during DNA extraction. However, two more species are marginally obtained as well: a "relaxed" covalently closed form ("Rel" band in Figure 2A) and a "nicked" single-strand break form (termed "Nck" in Figure 2A). All these topological species can be resolved in an agarose gel electrophoresis under



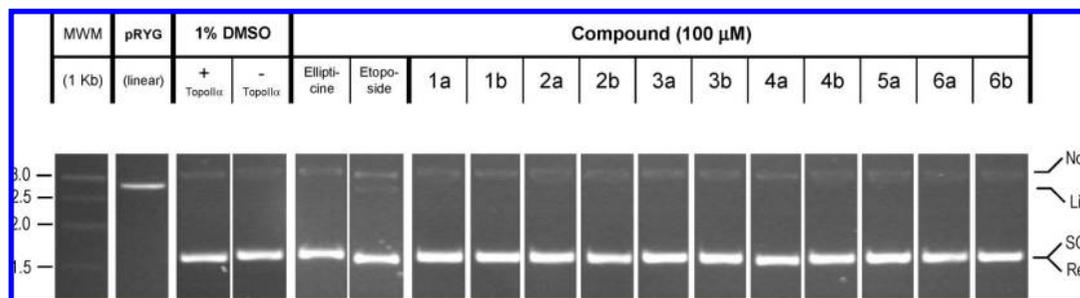
**Figure 2.** Inhibition of hTopoII $\alpha$ -mediated supercoiled DNA relaxation by pyranonaphthoquinones **3a**, **3b**, **4a**, **5a**, **6a**, **6b**. Covalently closed negative supercoiled pRYG plasmid (SC form) was treated with hTopoII $\alpha$  in the presence of either just 1% DMSO or 100  $\mu$ M ellipticine, etoposide, or a novel set of pyranonaphthoquinones in 1%DMSO: (A) agarose gel electrophoresis (without ethidium bromide) to separate substrates (SC form) and products (relaxed topoisomers) of the htopo II $\alpha$  reaction; (B) quantitation of product formation of three independent experiments relative to 1% DMSO. Note how the presence of pyranonaphthoquinones **3a**, **3b**, **4b**, **5a**, **6a**, and **6b** greatly inhibit hTopoII $\alpha$  (compared to controls without enzyme or with ellipticine): Nck (nicked pRYG), Rel (relaxed pRYG), Lin (linearized pRYG), SC (supercoiled pRYG).

specific conditions, where the order of migration is, from fastest to slowest, SC, Nck, Rel. Either topoisomerase I or II can relax supercoiling in vitro to an equilibrium of topoisomers. As it is shown in Figure 2A and quantified in Figure 2B, the SC form of pRYG is reduced and appeared as a set of variably relaxed topoisomers that migrate more slowly than the SC form when the enzyme is present without any drugs. This pattern of topoisomers is also obtained for etoposide and some of the compounds studied (**1a**, **1b**, **2a**, **2b**, and **4a**) (Figure 2). By contrast, ellipticine and the other novel pyranonaphthoquinones tested (**3a**, **3b**, **4b**, **5a**, **6a**, and **6b**) showed a pattern that resembled that of the control without the enzyme. Interestingly, this pattern of inhibition of relaxation fully coincides with the pattern observed in the decatenation assay (Figure 1). Thus, this double-check of inhibition strongly points out compounds **3a**, **3b**, **4b**, **5a**, **6a**, and **6b** as active drugs against human topoisomerase II $\alpha$ .

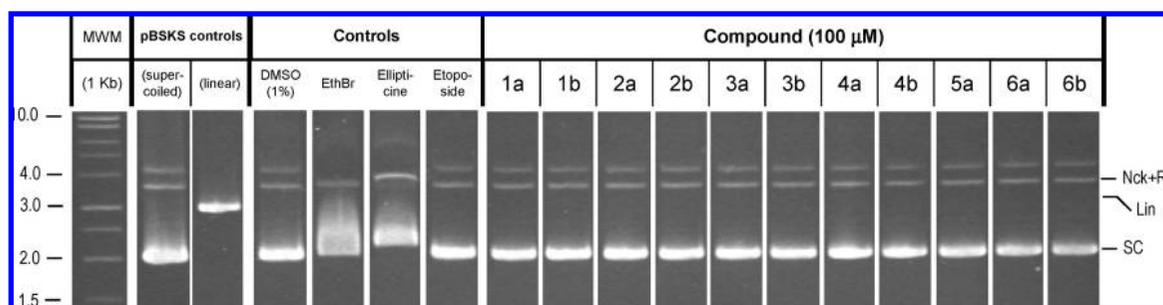
Although some of the compounds analyzed did not show any inhibition of TopoII $\alpha$  in these assays, this does not preclude that those derivatives do not act against TopoII $\alpha$ . In fact, as shown here in the previous assays, etoposide has little effect on the overall TopoII activity in spite of being a strong topoisomerase poison (Figures 1 and 2). Therefore, we tested our compounds to see whether they could poison the TopoII reaction by increasing the steady state of the cleavage complex,

as etoposide does. To do so, we repeated the assay with pRYG as substrate. In this case, the tested drug was added once the relaxation reaction had already started and the electrophoresis was run under the presence of an excess of ethidium bromide to fully positively supercoil all the topoisomers (make them run as a sole band during the electrophoresis) and thus clearly unmask the linear form ("Lin" band in Figure 3) of the DNA. This form is the result of the cut by the enzyme, and although it is normally rather transient and difficult to detect because the enzyme quickly reseals it, it can be seen with potent inhibitors of the ligation step of the catalytic cycle of TopoII, as is the case of etoposide.<sup>26</sup> In fact, in our hands etoposide did show this linear form of the pRYG plasmid (Figure 3). However, this form was not visible for the other controls, including ellipticine<sup>54</sup> and the novel pyranonaphthoquinones tested. Therefore, we can conclude that none of the compounds behaved as the classical poisons of TopoII, at least at the concentration tested.

Finally, two types of catalytic inhibitors of TopoII can be differentiated with regard to their action on the DNA molecule itself: those that can intercalate between the bases of the DNA and those that cannot. Normally, although not necessarily, intercalating agents can inhibit topoisomerase II in an indirect fashion as they overwound DNA, a situation that might change the efficiency of the topoisomerase II reaction. On the other



**Figure 3.** Pyranonaphthoquinones do not enhance the cleavage complex. Covalently closed negative supercoiled pRYG plasmid was treated with hTopoII $\alpha$  and then immediately incubated with just 1% DMSO, 100  $\mu$ M ellipticine, 100  $\mu$ M etoposide, or 100  $\mu$ M of a novel set of pyranonaphthoquinones, all in 1% (v/v) DMSO. An agarose gel electrophoresis (with ethidium bromide) separates substrates and products of the hTopoII $\alpha$  reaction from the linear intermediate (Lin band). Note how none of the pyranonaphthoquinones lead to the presence of the Lin form in comparison to etoposide.



**Figure 4.** Pyranonaphthoquinones do not intercalate within the DNA. Covalently closed negative supercoiled pBSKS plasmid was incubated with just 1% DMSO, 1  $\mu$ g/mL ethidium bromide, 100  $\mu$ M ellipticine, 100  $\mu$ M etoposide, or 100  $\mu$ M of a novel set of pyranonaphthoquinones, all in 1% (v/v) DMSO. An agarose gel electrophoresis (without ethidium bromide) allows us to see a retardation for the DNA treated with intercalators such as ethidium bromide and ellipticine, whereas no such phenomenon is observed for the synthesized pyranonaphthoquinones.

hand, those drugs that do not intercalate in the DNA may indeed be more specific for the enzyme. To discern between these two possibilities, we employed a simple but reliable test of intercalation.<sup>56</sup> We incubated a negatively supercoiled small circular DNA (in this case the plasmid pBSKS) with the compounds used in this paper, plus the intercalative agent ethidium bromide as an additional control, followed by an electrophoresis without EthBr in low-field conditions. If any compound intercalates into the DNA, it would (i) change the degree of supercoiling toward a positive (overwound) molecule and/or (ii) change the apparent mass of the DNA molecule. For a small circular DNA molecule negatively supercoiled the result of any of these changes would be a noticeable retardation in the migration during electrophoresis. In fact, this can be clearly seen under the presence of the intercalatives ethidium bromide and ellipticine, whereas etoposide does not change the mobility (Figure 4). None of the pyranonaphthoquinones studied led to DNA retardation (Figure 4). Thus, we conclude that they are not inhibiting the TopoII reaction by previously intercalating within the DNA bases.

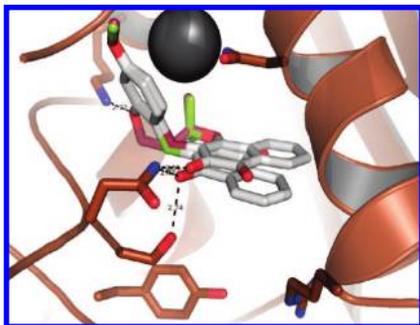
Eukaryotic topoisomerase II are multisubunit proteins that modulate topology by passing an intact helix through a transient double-stranded break they create in the DNA backbone.<sup>13,57</sup> Six steps in the catalytic cycle of the enzyme can be envisioned. First, TopoII binds the DNA. Second, it cleaves one of the DNA molecules under the presence of a divalent cation. Third, ATP binds the enzyme and allows a conformational change that directs the strand passage of the second DNA molecule. Fourth, the enzyme relegates the first DNA. Fifth, ATP hydrolysis opens the enzyme to liberate the DNA molecule that has just passed through, and finally, the other DNA is released from the enzyme.

ATP is an important cofactor for the overall catalytic activity, in spite of its role in the catalytic cycle being restricted to the latter stages. A number of antitumoral agents have been shown

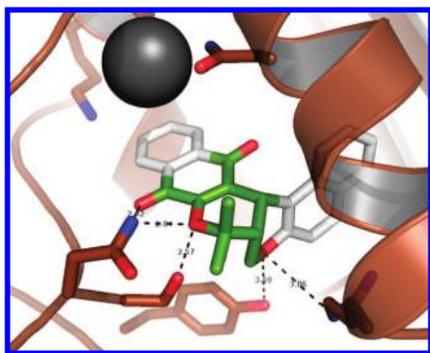
to act on how the ATP binds to or is hydrolyzed by the enzyme. Most of them have been shown to be competitive inhibitors of ATP. Remarkably, they do not enhance dramatically the cleavage complex even though ATP binding is needed to continue the catalytic cycle toward the religation step. In fact, they seem to stop the reaction at the step of ATP hydrolysis, trapping the enzyme in a closed clamp with the DNA molecules.<sup>36</sup> Therefore, it seems likely that these agents simulate the ATP binding step, in relation to the conformational changes that allow strand passage and DNA strand breaks resealing, but they are not easily “excluded” out of the pocket when needed to finish up the reaction cycle.<sup>36</sup> All these would explain why they strongly inhibit decatenation and relaxation activities while keeping the DNA molecules free of double strand breaks. In fact, our molecular docking studies support this hypothesis.

### Docking

We first tried docking our pyranonaphthoquinones into various possible pockets of TopoII. We obtain the best results into the ATP pocket, so we used an accurate model for this target site. This fact is consistent with the biological results that point out that active pyranonaphthoquinones act as catalytic inhibitors of TopoII. The program AutoDock 3.0.5<sup>58</sup> was used to dock separately *o*- and *p*-pyranonaphthoquinones. The model shows the compounds acting as hTopoII inhibitors by binding to the ATP pocket in the ATPase domain. We have found that all active compounds (**3a**, **3b**, **4b**, **5a**, **6a**, and **6b**) were superimposed in the binding site and they showed an effective  $\pi$  interaction with the magnesium (gray ball) (Figure 5). This type of interaction seems essential, since the inactive compounds **1a**, **1b**, **2a**, **2b** do not have aromatic rings. These docking results also predicted favorable interactions between the compounds and key residues at the ATPase domain such as K140, N122,



**Figure 5.** Docking model of compounds **3a** and **3b** binding into the ATP pocket of hTopo II. Both compounds showed a  $\pi$  interaction with the magnesium (gray ball). The Lys140 is predicted to bind the oxygen of the pyrane ring. Meanwhile, the residues Asn122 and Ser121 bind the carbonyl at position 2 for the ortho derivatives or the carbonyl at position 1 for the *p*-pyranonaphthoquinones.



**Figure 6.** Docking model of compound **4a** binding into the ATP pocket of hTopoII. The relative disposition between the magnesium (gray ball) and the aromatic ring of the naphthoquinone produces a poor  $\pi$  interaction. In this case the oxygen atoms of both pyrane rings and the carbonyl in position 2 were situated at an adequate distance from key residues such as Asn122, Ser121, Tyr379, and Arg70.

and S121. The model also explains that 1,2- and 1,4-pyranonaphthoquinones show similar inhibitory activity. In Figure 5 we can observe how the carbonyl at C-4 of **3a** superimposes with the carbonyl at C-2 of **3b**, and both carbonyls bind the residues Asn122 and Ser 121, respectively. However, compounds **4a**, with an additional but nonactivated aromatic ring, docked into the binding site, adopting different poses compared with the activated compounds. In this case the naphthoquinone motif was situated near the magnesium cation but their interaction is less effective compared with those in the active compounds (see Figure 6). The additional interactions with the key residues were fitted only partially. All these results are consistent with the obtained experimental data, and the generated model explains the TopoII inhibitory activity of the pyranonaphthoquinones synthesized. Interestingly, salvicine, an ATP-competing hTopoII $\alpha$  inhibitor with a naphthoquinone ring gives a same pattern of in vitro inhibition of the enzyme.<sup>59</sup> Remarkably, other *o*- and *p*-pyranonaphthoquinones such as  $\beta$ -lapachone have been shown to be catalytic inhibitors of the hTopoII $\alpha$ , in spite of not having the second aromatic ring.<sup>60</sup> However, they inhibited the topoisomerase activity more weakly and required higher concentrations of the drug. Since no docking models were available for these naphthoquinones, we included them in our studies and compared them to our active and inactive compounds (see Supporting Information Figures S-1, S-2, S-3, and S-4). Again, in this case the best docking pose into the ATP binding pocket reveal a  $\pi$  stacking interaction between the aromatic ring A of the naphthoquinone moiety and the magnesium cation (see

Supporting Information). This interaction is less effective than the one with the additional aromatic ring observed in the active compounds. In addition these docking results do not predict favorable interactions between the compounds and key residues at the ATPase domain such as K140, N122, and S121, which were predicted for the active compounds.

## Conclusion

In summary, on the basis of previous pharmacophore modeling studies of naphthoquinones derivatives, we have designed and synthesized a novel set of pyranonaphthoquinones. A simple and rapid protocol of the intramolecular domino Knoevenagel hetero Diels–Alder reaction was developed for the preparation of these pyranonaphthoquinones in a single preparative step. The reactions are very efficient, since two new fused rings are formed through three new  $\sigma$ -bonds (two C–C  $\sigma$  bonds, and one O–C  $\sigma$ -bond). The biological activities of the synthesized naphthoquinones, focused on the action against human topoisomerase II $\alpha$ , revealed that compounds **3a**, **3b**, **4b**, **5a**, **6a**, and **6b** act as topoisomerase II catalytic inhibitors. In support of the proposed action of the active pyranonaphthoquinones, we show that (i) no linear DNA molecules (result for cleavage complex enhancement) are seen (Figure 3) and (ii) potent decatenation and relaxation inhibition are present with the compounds that better fit within the ATP pocket (Figures 1, 2, 5, and 6). We have further checked that the inhibition was not due to any action of the compound on the DNA itself, which would inhibit the very first two steps of the catalytic cycle, by showing that none were DNA intercalators (Figure 4).

## Experimental Section

**Chemistry. General Methods.** All solvents and reagents were purified by standard techniques reported in Perrin, D. D.; Amarego, W. L. F. *Purification of Laboratory Chemicals*, 3rd ed.; Pergamon Press: Oxford, U.K., 1988 or used as supplied from commercial sources as appropriate. Reactions were monitored by TLC (on silica gel POLYGRAM SIL G/UV<sub>254</sub> foils). Purification by column flash chromatography used Merk Kiesel 60-H (0.063–0.2 mm) as adsorbent and different mixtures of hexanes–ethyl acetate as eluent. Precoated TLC plates SIL G-100 UV<sub>254</sub> (Machery-Nagel) were used for preparative TLC purification. <sup>1</sup>H NMR spectra were recorded in CDCl<sub>3</sub> or C<sub>6</sub>D<sub>6</sub> at 300 and 400 MHz, using Bruker AMX300 and Bruker AMX400 instruments. For <sup>1</sup>H spectra, chemical shifts are given in parts per million (ppm) and are referenced to the residual solvent peak. The following abbreviations are used: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. Proton assignments and stereochemistry are supported by <sup>1</sup>H–<sup>1</sup>H COSY and ROESY where necessary. Data are reported in the following manner: chemical shift (integration, multiplicity, coupling constant if appropriate). Coupling constants (*J*) are given in hertz (Hz) to the nearest 0.5 Hz. <sup>13</sup>C NMR spectra were recorded at 75 and 100 MHz using Bruker AMX300 and Bruker AMX400 instruments. Carbon spectra assignments are supported by DEPT-135 spectra, <sup>13</sup>C–<sup>1</sup>H (HMQC), and <sup>13</sup>C–<sup>1</sup>H (HMBC) correlations where necessary. Chemical shifts are quoted in ppm and are referenced to the appropriate residual solvent peak. MS and HRMS were recorded at VG Micromass ZAB-2F. IR spectra were taken on a Bruker IFS28/55 spectrophotometer.

**Microwave Irradiation Experiments.** All microwave irradiation experiments were carried out in a Discover-CEM monomode microwave apparatus operating at a frequency of 2.45 GHz with continuous irradiation power from 0 to 300 W with utilization of the standard absorbance level of 300 W maximum power. The reactions were carried out in 10 mL glass tubes sealed with aluminum/Teflon crimp tops, which can be exposed up to 250 °C and 20 bar of internal pressure. Temperature was measured with an IR sensor on the outer surface of the process vial. After the

irradiation period, the reaction vessel was cooled rapidly to ambient temperature by air jet cooling.

**General Procedure for the Reaction of 2-Hydroxynaphthoquinone with the Unsaturated Aldehydes by Heating (Procedure A).** An amount of 100 mg (0.547 mmol) of 2-hydroxy-1,4-naphthoquinone in 10 mL of EtOH was treated with 3 equiv of aldehyde and catalytic amounts of EDDA (ethylenediamino diacetate, 6% mmol). The reaction mixture was heated under reflux and checked by TLC until disappearance of the starting naphthoquinone. Then the reaction mixture was cooled and the EtOH was removed under reduced pressure. The crude was purified by silica gel column chromatography with hexanes/EtOAc as solvent.

**General Procedure by Microwave (MW) Irradiation (Procedure B).** Twenty-five mg of 2-hydroxy-1,4-naphthoquinone (0.143 mmol), 3 equiv of aldehyde and catalytic amount of EDDA (5.5 mg, 0.03 mmol) were suspended in 2 mL of EtOH in a 10 mL reaction glass containing a stirring magnet. The vial was sealed tightly with an aluminum-Teflon crimp top. After the irradiation period, the reaction vessel was cooled rapidly to ambient temperature by air jet cooling. The solvent was removed under reduced pressure and the residue was purified by TLC-preparative chromatography.

**Reaction of 2-Hydroxy-1,4-naphthoquinone with 2,6-Dimethyl-5-heptenal.** Following the general procedure A described above, an amount of 174.96 mg (1.0 mmol) of 2-hydroxy-1,4-naphthoquinone in 10 mL of EtOH was treated with 0.47 mL (3.0 mmol) of 2,6-dimethyl-5-heptenal and 10 mg of EDDA (0.06 mmol). The reaction mixture was heated under reflux for 30 min. The solvent was removed under vacuum, and the crude product was purified by flash chromatography with 10% hexanes/EtOAc to yield 136.9 mg (46.3%) of adduct **1a** and 159.3 mg (53.8%) of adduct **1b**. Following the procedure B described above, the reaction mixture was irradiated for 2 min at a preselected temperature of 130 °C, with an irradiation power of 150 W. The crude was purified by preparative TLC using hexanes/EtOAc (7:3) to provide 21.4 mg of **1a** (50.6%) and 20.1 mg (47.4%) of **1b**.

**Data for 1a.** It was isolated as an amorphous yellow solid.  $R_f$  (Hex/AcOEt, 4:1) = 0.58.  $^1\text{H NMR}$  (300 MHz,  $\text{CDCl}_3$ )  $\delta$  1.26 (s, 3H), 1.30 (m, 1H), 1.50 (s, 3H), 1.50 (d,  $^3J_{\text{H,H}} = 5.7$  Hz, 3H), 1.55 (m, 1H), 1.66 (m, 1H), 1.83 (m, 1H), 2.07 (m, 1H), 2.20 (m, 2H), 7.63 (m, 2H), 8.00 (m, 2H) ppm.  $^{13}\text{C NMR}$  (75 MHz,  $\text{CDCl}_3$ )  $\delta$  20.3 (CH<sub>3</sub>), 23.5 (CH<sub>3</sub>), 24.6 (CH<sub>2</sub>), 28.0 (CH<sub>3</sub>), 34.2 (CH<sub>2</sub>), 35.3 (CH), 43.5 (CH), 52.6 (CH), 82.5 (C), 124.1 (C), 125.7 (CH  $\times$  2), 130.5 (C), 132.2 (C), 132.3 (CH), 133.5 (CH), 154.7 (C), 179.9 (C), 183.8 (C) ppm. EI-MS  $m/z$  (%) 296 ( $\text{M}^+$ , 100), 281 ( $\text{M}^+ - \text{CH}_3$ , 97), 253 ( $\text{M}^+ - \text{CO}$ , 32), 225 ( $\text{M}^+ - 2 \times \text{CO}$ , 13). EI-HRMS  $m/z$  296.1402 [ $\text{M}^+$ ] calcd for  $\text{C}_{19}\text{H}_{20}\text{O}_3$  296.1412]. IR ( $\text{CHCl}_3$ )  $\nu_{\text{max}}$  2948, 2870, 1678, 1649, 1594, 1561, 1460, 1362, 1334, 1303, 1272, 1244, 1207, 1132, 1039, 980, 838, 723  $\text{cm}^{-1}$ .

**Data for 1b.** It was isolated as an amorphous orange solid.  $R_f$  (Hex/AcOEt, 4:1) = 0.45.  $^1\text{H NMR}$  (300 MHz,  $\text{CDCl}_3$ )  $\delta$  1.31 (s, 3H), 1.31 (m, 1H), 1.48 (d,  $^3J_{\text{H,H}} = 5.3$  Hz, 3H), 1.55 (s, 3H), 1.68 (m, 2H), 1.85 (m, 1H), 2.07 (m, 3H), 7.46 (t,  $^3J_{\text{H,H}} = 7.5$  Hz, 1H), 7.61 (t, 1H,  $^3J_{\text{H,H}} = 7.5$  Hz, H-17), 7.82 (d, 1H, d,  $J = 7.8$  Hz, H-19), 8.01 (d, 1H,  $^3J_{\text{H,H}} = 7.5$  Hz, H-16) ppm.  $^{13}\text{C NMR}$  (75 MHz,  $\text{CDCl}_3$ )  $\delta$  20.5 (CH<sub>3</sub>), 23.2 (CH<sub>3</sub>), 24.8 (CH<sub>2</sub>), 28.2 (CH<sub>3</sub>), 32.6 (CH<sub>2</sub>), 34.0 (CH), 39.4 (CH), 52.6 (CH), 83.7 (C), 117.2 (C), 124.6 (CH), 127.9 (CH), 130.0 (C), 130.2 (CH), 132.9 (C), 134.4 (CH), 161.9 (C), 178.3 (C), 180.2 (C) ppm. EI-MS  $m/z$  (%) 296 ( $\text{M}^+$ , 100), 281 ( $\text{M}^+ - \text{CH}_3$ , 91), 253 ( $\text{M}^+ - \text{CO}$ , 48), 225 ( $\text{M}^+ - 2\text{CO}$ , 33). EI-HRMS 296.1412 [ $\text{M}^+$ ], calcd for  $\text{C}_{19}\text{H}_{20}\text{O}_3$  296.1412]. IR ( $\text{CHCl}_3$ )  $\nu_{\text{max}}$  2947, 2869, 2359, 1797, 1644, 1590, 1451, 1375, 1291, 1231, 1129, 1085, 927, 862, 776, 718, 691, 661, 469  $\text{cm}^{-1}$ .

**Reaction of 2-Hydroxy-1,4-naphthoquinone with (s)-(-)-Citronellal.** Following the general procedure described above, an amount of 100 mg (0.57 mmol) of 2-hydroxy-1,4-naphthoquinone in 10 mL of EtOH was treated with 0.31 mL (1.71 mmol) of (s)-(-)-citronellal and 5.5 mg of EDDA (0.03 mol). The reaction mixture was heated under reflux for 30 min, cooled to room temperature, and freed from the solvent in vacuo. The residue was purified by flash chromatography using hexanes/AcOEt (9:1) to

yield 80.7 mg of adduct **2a** (45.7%) and 85.9 mg (48.6%) of adduct **2b**. Following the procedure B described above, the reaction mixture was irradiated for 2.5 min at a preselected temperature of 130 °C, with an irradiation power of 136 W. The crude was purified by preparative TLC using hexanes/EtOAc (7:3) to provide 18.1 mg of **2a** (40.8%) and 19.5 mg (44.0%) of **2b**.

**Data for 2a.**<sup>51b</sup> It was isolated as an amorphous yellow solid.  $R_f$  (Hex/AcOEt, 4:1) = 0.58.  $[\alpha]_{\text{D}}^{25} + 183$  (c 0.57,  $\text{CHCl}_3$ ).  $^1\text{H NMR}$  (300 MHz,  $\text{CDCl}_3$ )  $\delta$  0.68 (dd,  $^3J_{\text{H,H}} = 11.7, 12.0$  Hz, 1H), 0.92 (d, 3H,  $^3J_{\text{H,H}} = 6.5$  Hz), 1.10 (m, 1H, H-13a), 1.12 (s, 3H, CH<sub>3</sub>), 1.32 (m, 1H), 1.50 (s, 3H, CH<sub>3</sub>), 1.65 (m, 1H), 1.83 (m, 3H), 2.41 (td,  $^3J_{\text{H,H}} = 11.0, 2.7$  Hz, 1H), 2.83 (d,  $^3J_{\text{H,H}} = 12.5$  Hz, 1H), 7.64 (m, 2H), 8.00 (m, 2H) ppm.  $^{13}\text{C NMR}$  (75 MHz,  $\text{CDCl}_3$ )  $\delta$  19.1 (CH<sub>3</sub>), 22.0 (CH<sub>3</sub>), 26.7 (CH<sub>3</sub>), 27.3 (CH<sub>2</sub>), 32.5 (CH), 34.3 (CH), 35.0 (CH<sub>2</sub>), 38.6 (CH<sub>2</sub>), 47.9 (CH), 82.2 (C), 123.6 (C), 125.6 (CH), 125.8 (CH), 130.7 (C), 132.4 (CH), 132.6 (C), 133.6 (CH), 154.0 (C), 179.9 (C), 184.1 (C) ppm. EI-MS  $m/z$  (%) 310 ( $\text{M}^+$ , 100), 295 (31), 267 (28), 240 (9). EI-HRMS 310.1566 [ $\text{M}^+$ ] calcd for  $\text{C}_{20}\text{H}_{22}\text{O}_3$  310.1569]. IR ( $\text{CHCl}_3$ )  $\nu_{\text{max}}$  2923, 2867, 1679, 1600, 1574, 1456, 1372, 1334, 1303, 1270, 1208, 1136, 1089, 1041, 1014, 980, 963, 933, 794, 755, 725, 667, 528  $\text{cm}^{-1}$ .

**Data for 2b.**<sup>51b</sup> It was isolated as an amorphous orange solid.  $[\alpha]_{\text{D}}^{25} + 18$  (c 0.39,  $\text{CHCl}_3$ ).  $R_f$  (Hex/AcOEt, 4:1) = 0.45.  $^1\text{H NMR}$  (300 MHz,  $\text{CDCl}_3$ )  $\delta$  0.59 (dd,  $^3J_{\text{H,H}} = 11.5, 12.0$  Hz, 1H), 0.92 (d,  $^3J_{\text{H,H}} = 6.5$  Hz, 3H), 1.05 (m, 1H), 1.18 (s, 3H, CH<sub>3</sub>), 1.34 (m, 1H), 1.51 (s, 3H, CH<sub>3</sub>), 1.60 (m, 1H), 1.81 (m, 3H), 2.36 (td,  $^3J_{\text{H,H}} = 11.0, 2.7$  Hz, 1H), 2.87 (d,  $^3J_{\text{H,H}} = 12.6$  Hz, 1H), 7.45 (t,  $^3J_{\text{H,H}} = 7.5$  Hz, 1H), 7.61 (t,  $^3J_{\text{H,H}} = 7.0$  Hz, 1H), 7.78 (d,  $^3J_{\text{H,H}} = 7.7$  Hz, 1H), 8.00 (d,  $^3J_{\text{H,H}} = 7.6$  Hz, 1H) ppm.  $^{13}\text{C NMR}$  (75 MHz,  $\text{CDCl}_3$ )  $\delta$  19.18 (CH<sub>3</sub>), 22.0 (CH<sub>3</sub>), 26.9 (CH<sub>3</sub>), 27.1 (CH<sub>2</sub>), 32.1 (CH), 33.6 (CH), 35.0 (CH<sub>2</sub>), 37.7 (CH<sub>2</sub>), 47.98 (CH), 82.3 (C), 116.5 (C), 124.1 (CH), 127.9 (CH), 130.1 (C), 130.3 (CH), 132.6 (C), 134.5 (CH), 161.3 (C), 178.7 (C), 180.1 (C) ppm. EI-MS  $m/z$  (%) 310 ( $\text{M}^+$ , 100), 282 ( $\text{M}^+ - \text{CO}$ , 14). EI-HRMS 310.1565 [ $\text{M}^+$ ] calcd for  $\text{C}_{20}\text{H}_{22}\text{O}_3$  310.1569]. IR ( $\text{CHCl}_3$ )  $\nu_{\text{max}}$  2925, 2867, 2360, 1679, 1641, 1594, 1563, 1453, 1378, 1286, 1209, 1136, 1090, 972, 963, 930, 861, 754, 692, 662  $\text{cm}^{-1}$ .

**Synthesis of 4-Methoxy-2-(3-methylbut-2-enyloxy)benzaldehyde.** An amount of 1 mol (152.15 mg) of 2-hydroxy-4-methoxybenzaldehyde was added to a solution of 1-bromo-3-methylbut-2-ene (2 mol, 0.115 mL) and  $\text{K}_2\text{CO}_3$  (1.2 mol, 168.85 mg) in dry acetone (30 mL). The mixture was heated under reflux for 18 h under nitrogen atmosphere. Then the mixture was filtered and the solvent removed under vacuum. The residue was treated with an aqueous solution of 5% NaOH and extracted three times with  $\text{Et}_2\text{O}$ . The organic layers were dried over  $\text{MgSO}_4$ . After removal of solvent the crude was purified by flash chromatography (silica gel, 9:5:0.5, hexanes/EtOAc) to provide 187.6 mg (74%) of 4-methoxy-2-(3-methylbut-2-enyloxy)benzaldehyde.  $^1\text{H NMR}$  ( $\text{CDCl}_3$ , 300 MHz)  $\delta$  1.69 (s, 3H, CH<sub>3</sub>), 1.73 (s, 3H, CH<sub>3</sub>), 3.78 (s, 3H, CH<sub>3</sub>), 4.53 (d,  $^3J_{\text{H,H}} = 6.6$  Hz, 2H), 5.42 (t,  $^3J_{\text{H,H}} = 6.6$  Hz, 1H), 6.37 (d,  $^4J_{\text{H,H}} = 2.2$  Hz, 1H), 6.45 (dd,  $^3J_{\text{H,H}} = 8.7$ ,  $^4J_{\text{H,H}} = 2.0$ , 1H), 7.71 (d,  $^3J_{\text{H,H}} = 8.7$  Hz, 1H), 10.24 (s, 1H).  $^{13}\text{C NMR}$  (75 MHz,  $\text{CDCl}_3$ )  $\delta$  17.7 (CH<sub>3</sub>), 25.2 (CH<sub>3</sub>), 55.0 (CH<sub>3</sub>), 64.8 (CH<sub>2</sub>), 98.3 (CH), 105.4 (CH), 118.3 (CH), 118.6 (C), 129.6 (CH), 138.0 (C), 162.5 (C), 165.5 (C), 187.7 (CH). EIMS  $m/z$  (%) 220 ( $\text{M}^+$ , 5), 152 ( $\text{M}^+ - \text{C}_5\text{H}_8$ , 100), 151 ( $\text{M}^+ - \text{C}_5\text{H}_9$ , 78), 69 ( $\text{C}_4\text{H}_5\text{O}$ , 24). EIHRMS 220.1092 [ $\text{M}^+$ ] calcd para  $\text{C}_{13}\text{H}_{16}\text{O}_3$  220.1099]. IR ( $\text{CHCl}_3$ )  $\nu_{\text{max}}$  2856, 1678, 1601, 1501, 1443, 1384, 1260, 1202, 1167, 1113, 1035, 998, 815, 643  $\text{cm}^{-1}$ .

**Reaction of 2-Hydroxy-1,4-naphthoquinone with 4-Methoxy-2-(3-methylbut-2-enyloxy)benzaldehyde.** Following the general procedure described above, an amount of 100 mg (0.57 mmol) of 2-hydroxy-1,4-naphthoquinone in 10 mL of EtOH was treated with 378.8 mg (1.71 mmol) of 4-methoxy-2-(3-methylbut-2-enyloxy)benzaldehyde and 5.5 mg of EDDA. The reaction mixture was refluxed for 30 min, cooled to room temperature, and freed from the solvent in vacuum. The residue was purified by preparative TLC using hexanes/EtOAc (9:1) to yield 74.1 mg (34.6%) of adduct **3a** and 77.0 mg (35.9%) of **3b**. Following the procedure B described above, the reaction mixture was irradiated for 3 min at a preselected

temperature of 130 °C, with an irradiation power of 150 W. The crude was purified by preparative TLC using hexanes/EtOAc (7:3) to provide 23.6 mg of **3a** (43.9%) and 22.0 mg (40.9%) of **3b**.

**Data for 3a.** It was isolated as an amorphous yellow solid.  $R_f$  (Hex/EtOAc, 7:3) = 0.37.  $^1\text{H NMR}$  (300 MHz,  $\text{CDCl}_3$ )  $\delta$  1.30 (s, 3H,  $\text{CH}_3$ ), 1.60 (s, 3H,  $\text{CH}_3$ ), 2.15 (dd,  $^3J_{\text{H,H}} = 9.4, 4.4$  Hz, 1H), 3.71 (s, 3H,  $\text{OCH}_3$ ), 4.30 (dd,  $^3J_{\text{H,H}} = 11.9, 4.4$  Hz, 1H), 4.46 (dd,  $^3J_{\text{H,H}} = 12.0, 6.6$  Hz, 2H), 6.27 (d,  $^3J_{\text{H,H}} = 2.5$  Hz, 1H), 6.42 (dd,  $^3J_{\text{H,H}} = 8.6, 2.5$  Hz, 1H), 7.18 (d,  $^3J_{\text{H,H}} = 8.6$  Hz, 1H), 7.67 (m, 2H), 8.08 (m, 2H) ppm.  $^{13}\text{C NMR}$  (75 MHz,  $\text{CDCl}_3$ )  $\delta$  23.8 ( $\text{CH}_3$ ), 27.2 ( $\text{CH}_3$ ), 28.7 (CH), 37.9 (CH), 54.9 ( $\text{OCH}_3$ ), 64.3 ( $\text{CH}_2$ ), 80.4 (C), 100.7 ( $\text{CH}_{\text{arom}}$ ), 107.1 ( $\text{CH}_{\text{arom}}$ ), 112.9 ( $\text{C}_{\text{arom}}$ ), 121.3 ( $\text{C}_{\text{arom}}$ ), 126.0 ( $\text{CH}_{\text{arom}}$ ), 126.1 ( $\text{CH}_{\text{arom}}$ ), 130.5 ( $\text{C}_{\text{arom}}$ ), 130.8 ( $\text{CH}_{\text{arom}}$ ), 132.1 ( $\text{C}_{\text{arom}}$ ), 132.8 ( $\text{CH}_{\text{arom}}$ ), 133.9 ( $\text{CH}_{\text{arom}}$ ), 153.6 ( $\text{C}_{\text{arom}}$ ), 154.5 ( $\text{C}_{\text{arom}}$ ), 159.2 ( $\text{C}_{\text{arom}}$ ), 179.6 (C, C=O), 184.9 (C, C=O) ppm. EI-MS  $m/z$  (%) 376 ( $\text{M}^+$ , 100), 361 ( $\text{M}^+ - \text{CH}_3$ , 19). EI-HRMS 376.1324 [ $\text{M}^+$ ] calcd for  $\text{C}_{23}\text{H}_{20}\text{O}_5$  376.1311]. IR ( $\text{CHCl}_3$ )  $\nu_{\text{max}}$  = 2982, 1680, 1646, 1578, 1503, 1463, 1364, 1336, 1269, 1240, 1202, 1164, 1129, 1079, 1036, 987, 954, 833, 797, 756, 725, 665, 562, 478  $\text{cm}^{-1}$ .

**Data for 3b.** It was isolated as an amorphous orange solid.  $R_f$  (Hex/EtOAc, 7:3) = 0.45.  $^1\text{H NMR}$  (300 MHz,  $\text{CDCl}_3$ )  $\delta$  1.34 (s, 3H,  $\text{CH}_3$ ), 1.67 (s, 3H,  $\text{CH}_3$ ), 2.21 (dd,  $^3J_{\text{H,H}} = 6.7, 4.1$  Hz, 1H), 3.72 (s, 3H,  $\text{OCH}_3$ ), 4.40 (m, 3H), 6.27 (d,  $^4J_{\text{H,H}} = 2.6$  Hz, 1H), 6.40 (dd,  $^3J_{\text{H,H}} = 8.6$  Hz,  $^4J_{\text{H,H}} = 2.5$  Hz, 1H), 7.16 (d,  $^3J_{\text{H,H}} = 8.6$  Hz, 1H), 7.54 (t,  $^3J_{\text{H,H}} = 7.5$  Hz, 1H), 7.65 (t,  $^3J_{\text{H,H}} = 7.5$  Hz, 1H), 7.85 (d,  $^3J_{\text{H,H}} = 7.8$  Hz, 1H), 8.10 (d,  $^3J_{\text{H,H}} = 7.6$  Hz, 1H) ppm.  $^{13}\text{C NMR}$  (75 MHz,  $\text{CDCl}_3$ )  $\delta$  24.0 ( $\text{CH}_3$ ), 27.7 ( $\text{CH}_3$ ), 28.1 (CH), 38.1 (CH), 54.9 ( $\text{OCH}_3$ ), 64.4 ( $\text{CH}_2$ ), 81.6 (C), 100.8 (CH), 107.1 ( $\text{CH}_{\text{arom}}$ ), 113.5 ( $\text{C}_{\text{arom}}$ ), 115.1 ( $\text{C}_{\text{arom}}$ ), 124.6 ( $\text{CH}_{\text{arom}}$ ), 128.3 ( $\text{CH}_{\text{arom}}$ ), 130.2 ( $\text{C}_{\text{arom}}$ ), 130.5 ( $\text{CH}_{\text{arom}}$ ), 130.9 ( $\text{CH}_{\text{arom}}$ ), 132.5 ( $\text{C}_{\text{arom}}$ ), 134.7 ( $\text{CH}_{\text{arom}}$ ), 154.2 ( $\text{C}_{\text{arom}}$ ), 159.1 ( $\text{C}_{\text{arom}}$ ), 161.5 ( $\text{C}_{\text{arom}}$ ), 179.5 (C, C=O), 179.8 (C, C=O) ppm. EI-MS  $m/z$  (%) 376 ( $\text{M}^+$ , 100), 361 ( $\text{M}^+ - \text{CH}_3$ , 50). EI-HRMS 376.1304 [ $\text{M}^+$ ] calcd for  $\text{C}_{23}\text{H}_{20}\text{O}_5$  376.1311]. IR ( $\text{CHCl}_3$ )  $\nu_{\text{max}}$  = 2982, 1680, 1646, 1578, 1503, 1463, 1364, 1336, 1269, 1240, 1202, 1164, 1129, 1079, 1036, 987, 954, 833, 797, 756, 725, 665, 562, 478  $\text{cm}^{-1}$ .

**Reaction of 2-Hydroxynaphthoquinone with 4-Methoxy-2-[(3-methyl-2-butenyl)oxy]-1-naphthaldehyde.** Following the general procedure described above, an amount of 100 mg (0.57 mmol) of 2-hydroxy-1,4-naphthoquinone in 10 mL of EtOH was treated with 413.48 mg (1.722 mmol) of 2-[(3-methyl-2-butenyl)oxy]-1-naphthaldehyde and 5.5 mg of EDDA. The reaction mixture was refluxed for 25 min, cooled to room temperature, and freed from solvent in vacuum. The residue was purified by preparative TLC using hexanes/EtOAc (9:1) to afford 60.1 mg (26.6%) of **4a** and 100 mg (44.3%) of **4b**. Following the procedure B described above, the reaction mixture was irradiated for 4 min at a preselected temperature of 130 °C, with an irradiation power of 108 W. The crude was purified by preparative TLC using hexanes/EtOAc (7:3) to provide 18.7 mg of **4a** (33%) and 18.7 mg (33%) of **4b**.

**Data for 4a.** It was isolated as an amorphous yellow solid.  $R_f$  (Hex/AcOEt 7:3) = 0.53.  $^1\text{H NMR}$  (300 MHz,  $\text{CDCl}_3$ )  $\delta$  1.61 (s, 6H), 2.30 (m, 1H), 4.0 (t,  $^3J_{\text{H,H}} = 11.0$  Hz, 1H), 4.41 (m, 1H), 4.87 (d,  $^3J_{\text{H,H}} = 4.6$  Hz, 1H), 7.01 (d,  $^3J_{\text{H,H}} = 8.8$  Hz, 1H), 7.42 (t,  $^3J_{\text{H,H}} = 7.1$  Hz, 1H), 7.59 (m, 3H), 7.73 (d,  $^3J_{\text{H,H}} = 6.2$  Hz, 2H), 7.80 (t,  $^3J_{\text{H,H}} = 8.5$  Hz, 2H), 8.05 (d,  $^3J_{\text{H,H}} = 7.1$  Hz, 1H), 8.19 (d,  $^3J_{\text{H,H}} = 8.6$  Hz, 1H).  $^{13}\text{C NMR}$  (75 MHz,  $\text{CDCl}_3$ )  $\delta$  25.5 ( $\text{CH}_3$ ), 26.4 (CH), 26.6 ( $\text{CH}_3$ ), 38.9 (CH), 63.7 ( $\text{CH}_2$ ), 78.5 (C), 116.2 ( $\text{C}_{\text{arom}}$ ), 118.2 ( $\text{CH}_{\text{arom}}$ ), 120.1 ( $\text{C}_{\text{arom}}$ ), 123.0 ( $\text{CH}_{\text{arom}}$ ), 123.5 ( $\text{CH}_{\text{arom}}$ ), 126.2 ( $\text{CH}_{\text{arom}}$ ), 128.2 ( $\text{CH}_{\text{arom}}$ ), 128.9 ( $\text{C}_{\text{arom}}$ ), 129.0 ( $\text{CH}_{\text{arom}}$ ), 130.3 ( $\text{C}_{\text{arom}}$ ), 132.1 ( $\text{C}_{\text{arom}}$ ), 133.8 ( $\text{CH}_{\text{arom}}$ ), 133.9 ( $\text{C}_{\text{arom}}$ ), 134.2 ( $\text{CH}_{\text{arom}}$ ), 151.5 ( $\text{C}_{\text{arom}}$ ), 153.8 ( $\text{C}_{\text{arom}}$ ), 179.5 (C, C=O), 182.8 (C, C=O) ppm. EI-MS  $m/z$  (%) 396 ( $\text{M}^+$ , 100), 381 ( $\text{M}^+ - \text{Me}$ , 14). EI-HRMS 396.1354 [ $\text{M}^+$ ], calcd for  $\text{C}_{26}\text{H}_{20}\text{O}_4$  396.1362]. IR ( $\text{CHCl}_3$ )  $\nu_{\text{max}}$  = 2922, 1730, 1622, 1594, 1568, 1512, 1474, 1374, 1349, 1328, 1300, 1262, 1203, 1131, 1008, 943, 805, 746, 720, 672, 470  $\text{cm}^{-1}$ .

**Data for 4b.** It was isolated as an amorphous orange solid.  $R_f$  (hexanes/EtOAc, 7:3) = 0.45.  $^1\text{H NMR}$  (300 MHz,  $\text{CDCl}_3$ )  $\delta$  1.62 (s, 3H), 1.65 (s, 3H), 2.30 (m, 1H), 4.15 (t,  $^3J_{\text{H,H}} = 11.1$  Hz, 1H), 4.43 (m, 1H), 4.79 (d,  $^3J_{\text{H,H}} = 4.0$  Hz, 1H), 6.97 (d,  $^3J_{\text{H,H}} = 8.8$

Hz, 1H), 7.34 (t,  $^3J_{\text{H,H}} = 7.4$  Hz, 1H), 7.50 (m, 2H), 7.65 (m, 2H), 7.74 (d,  $^3J_{\text{H,H}} = 8.0$  Hz, 1H), 7.91 (d,  $^3J_{\text{H,H}} = 7.8$  Hz, 1H), 7.99 (d,  $^3J_{\text{H,H}} = 7.4$  Hz, 1H), 8.13 (d,  $^3J_{\text{H,H}} = 8.5$  Hz, 1H) ppm.  $^{13}\text{C NMR}$  (75 MHz,  $\text{CDCl}_3$ )  $\delta$  25.8 (C), 26.1 (CH), 26.8 (C), 38.2 (CH), 63.1 ( $\text{CH}_2$ ), 78.7 (C), 113.2 ( $\text{C}_{\text{arom}}$ ), 115.2 ( $\text{C}_{\text{arom}}$ ), 117.8 ( $\text{CH}_{\text{arom}}$ ), 122.8 ( $\text{CH}_{\text{arom}}$ ), 123.4 ( $\text{CH}_{\text{arom}}$ ), 124.6 ( $\text{CH}_{\text{arom}}$ ), 125.5 ( $\text{CH}_{\text{arom}}$ ), 127.9 ( $\text{CH}_{\text{arom}}$ ), 128.2 ( $\text{CH}_{\text{arom}}$ ), 128.8 ( $\text{C}_{\text{arom}}$ ), 129.0 ( $\text{CH}_{\text{arom}}$ ), 130.0 ( $\text{C}_{\text{arom}}$ ), 132.2 ( $\text{C}_{\text{arom}}$ ), 134.2 ( $\text{C}_{\text{arom}}$ ), 134.6 ( $\text{CH}_{\text{arom}}$ ), 151.0 ( $\text{C}_{\text{arom}}$ ), 160.5 ( $\text{C}_{\text{arom}}$ ), 177.6 (C, C=O), 178.8 (C, C=O) ppm. EI-MS  $m/z$  (%) 396 ( $\text{M}^+$ , 58), 379 (100). EI-HRMS 396.1360 [ $\text{M}^+$ ] calcd for  $\text{C}_{26}\text{H}_{20}\text{O}_4$  396.1362]. IR ( $\text{CHCl}_3$ )  $\nu_{\text{max}}$  = 2926, 2357, 1698, 1657, 1573, 1513, 1452, 1359, 1290, 1258, 1231, 1135, 1087, 923, 812, 750, 705, 660, 533  $\text{cm}^{-1}$ .

**Synthesis of 3,5-Dibromo-2-(3-methylbut-2-enyloxy)benzaldehyde (5).** An amount of 419.89 mg (1.5 mmol) of 3,5-dibromo-2-hydroxybenzaldehyde was added to a solution of 1-bromo-3-methylbut-2-ene (3 mmol, 0.345 mL) and  $\text{Cs}_2\text{CO}_3$  (1.8 mmol, 586.7 mg) in dry THF (30 mL). The mixture was heated under reflux for 18 h under nitrogen atmosphere. Then the mixture was filtered and the solvent removed under vacuum. The residue was treated with an aqueous solution of 5% NaOH and extracted three times with  $\text{Et}_2\text{O}$ . The organic layers were dried over  $\text{MgSO}_4$ . After removal of solvent the crude was purified by flash chromatography (silica gel, 9.5:0.5, hexanes/EtOAc) to provide 441.0 mg (85%) of 3,5-dibromo-2-(3-methylbut-2-enyloxy)benzaldehyde.  $^1\text{H NMR}$  ( $\text{CDCl}_3$ , 300 MHz)  $\delta$  1.46 (s, 3H), 1.64 (s, 3H), 4.51 (d,  $^3J_{\text{H,H}} = 6.7$  Hz, 2H), 6.82 (t,  $^3J_{\text{H,H}} = 6.4$  Hz, 1H), 7.21 (s, 1H), 7.80 (d,  $^3J_{\text{H,H}} = 2.5$  Hz, 1H), 10.15 (s, 1H) ppm.  $^{13}\text{C NMR}$  ( $\text{CDCl}_3$ , 75 MHz)  $\delta$  17.7 ( $\text{CH}_3$ ), 25.2 ( $\text{CH}_3$ ), 71.8 ( $\text{CH}_2$ ), 117.6 ( $\text{CH}_{\text{arom}}$ ), 119.5 ( $\text{C}_{\text{arom}}$ ), 129.7 ( $\text{CH}_{\text{arom}}$ ), 132.2 ( $\text{C}_{\text{arom}}$ ), 140.7 ( $\text{CH}_{\text{arom}}$ ), 141.9 (C), 157.3 (C), 187.4 (C) ppm.

**Reaction with 3,5-Dibromo-2-(3-methylbut-2-enyloxy)benzaldehyde.** Following the general procedure described above, an amount of 100 mg (0.57 mmol) of 2-hydroxy-1,4-naphthoquinone in 10 mL of EtOH was treated with 0.310 mL (1.72 mmol) of 3,5-dibromo-2-(3-methylbut-2-enyloxy)benzaldehyde and 5 mg of EDDA (5.5 mg, 0.03 mmol). The reaction mixture was refluxed for 25 min and cooled to room temperature and the solvent removed under vacuum. The residue was purified by flash chromatography using hexanes/EtOAc, 9:1, to yield 180.2 mg of the adduct **5a** (62%). Following the procedure B described above, the reaction mixture was irradiated for 3 min at a preselected temperature of 130 °C, with an irradiation power of 100 W. The crude was purified by preparative TLC using hexanes/EtOAc (7:3) to provide 42.3 mg (58.6%) of **5a**.

**Data for 5a.** It was isolated as an amorphous yellow solid.  $R_f$  (Hex/EtOAc 7:3) = 0.5.  $^1\text{H NMR}$  (300 MHz,  $\text{CDCl}_3$ )  $\delta$  1.20 (s, 3H,  $\text{CH}_3$ ), 1.62 (s, 3H,  $\text{CH}_3$ ), 2.21 (dd,  $^3J_{\text{H,H}} = 4.2$  Hz,  $^3J_{\text{H,H}} = 9.5$  Hz, 1H), 4.48 (m, 3H), 4.56 (dd,  $^2J_{\text{H,H}} = 12$  Hz,  $^3J_{\text{H,H}} = 3.4$  Hz, 1H), 7.32 (s, 1H), 7.46 (s, 1H), 7.68 (m, 2H), 8.08 (m, 2H) ppm.  $^{13}\text{C NMR}$  (75 MHz,  $\text{CDCl}_3$ )  $\delta$  23.6 ( $\text{CH}_3$ ), 27.3 ( $\text{CH}_3$ ), 29.3 (CH), 37.5 ( $\text{CH}_2$ ), 65.2 ( $\text{CH}_2$ ), 80.4 (C), 110.8 ( $\text{C}_{\text{arom}}$ ), 112.4 ( $\text{C}_{\text{arom}}$ ), 119.5 ( $\text{C}_{\text{arom}}$ ), 124.2 ( $\text{C}_{\text{arom}}$ ), 126.1 ( $\text{CH}_{\text{arom}}$ ), 126.3 ( $\text{CH}_{\text{arom}}$ ), 130.5 ( $\text{C}_{\text{arom}}$ ), 131.9 ( $\text{CH}_{\text{arom}} + \text{C}_{\text{arom}}$ ), 133.0 ( $\text{CH}_{\text{arom}}$ ), 133.8 ( $\text{CH}_{\text{arom}}$ ), 134.1 ( $\text{CH}_{\text{arom}}$ ), 149.4 ( $\text{C}_{\text{arom}}$ ), 154.0 ( $\text{C}_{\text{arom}}$ ), 179.2 (C, C=O), 184.5 (C, C=O) ppm. EI-MS  $m/z$  (%) 505 ( $\text{M}^+$ , 52), 503 (100), 488 ( $\text{M}^+ - \text{CH}_3$ , 15). EI-HRMS 505.9381 [ $\text{M}^+$ ] calcd para  $\text{C}_{22}\text{H}_{16}\text{O}_4\text{Br}_2$  505.9374]. IR ( $\text{CHCl}_3$ )  $\nu_{\text{max}}$  2984, 1681, 1646, 1608, 1575, 1446, 1394, 1360, 1337, 1306, 1267, 1212, 1181, 1129, 988, 862, 755, 723, 668, 584  $\text{cm}^{-1}$ .

**Synthesis of 5-Bromo-2-(3-methylbut-2-enyloxy)benzaldehyde (6).** An amount of 301.53 mg (1.5 mmol) of 2-hydroxy-5-bromobenzaldehyde was added to a solution of 1-bromo-3-methylbut-2-ene (3 mmol, 0.345 mL) and  $\text{Cs}_2\text{CO}_3$  (1.8 mmol, 586.7 mg) in dry THF (30 mL). The mixture was heated under reflux for 18 h under nitrogen atmosphere. Then the mixture was filtered and the solvent removed under vacuum. The residue was treated with an aqueous solution of 5% NaOH and extracted three times with  $\text{Et}_2\text{O}$ . The organic layers were dried over  $\text{MgSO}_4$ . After removal of solvent the crude was purified by flash chromatography (silica gel, 9.5:0.5, hexanes/EtOAc) to provide 281.4 mg (70%) of

5-bromo-2-(3-methylbut-2-enyloxy)benzaldehyde.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz)  $\delta$  1.69 (s, 3H,  $\text{CH}_3$ ), 1.73 (s, 3H,  $\text{CH}_3$ ), 4.53 (d,  $^3J_{\text{H,H}} = 7.7$  Hz, 2H), 6.82 (d,  $^3J_{\text{H,H}} = 8.9$  Hz, 1H), 7.49 (dd,  $^3J_{\text{H,H}} = 8.8$ ,  $^4J_{\text{H,H}} = 2.6$  Hz, 1H), 7.7 (d,  $^3J_{\text{H,H}} = 2.5$  Hz, 1H), 10.3 (s, 1H) ppm.  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 75 MHz)  $\delta$  14.6 ( $\text{CH}_3$ ), 18.7 ( $\text{CH}_3$ ), 66.2 ( $\text{CH}_2$ ), 113.6 (C), 115.4 (CH), 118.9 (CH), 126.6 (C), 131.0 (CH), 138.4 (CH), 139.5 (C), 160.5 (C), 187.5 (CH, CHO).

**Reaction of 2-Hydroxy-1,4-naphthoquinone with 5-Bromo-2-(3-methylbut-2-enyloxy)benzaldehyde.** Following the general procedure described above, an amount of 50 mg (0.26 mmol) of 2-hydroxy-1,4-naphthoquinone in 10 mL of EtOH was treated with 212.2 mg (0.79 mmol) of 5-bromo-2-(3-methylbut-2-enyloxy)benzaldehyde and 3.0 mg of EDDA. The reaction mixture was refluxed for 28 min and cooled to room temperature and the solvent removed under vacuum. The residue was purified by flash chromatography using hexanes/EtOAc (4:1) to yield 39.5 mg (35.7%) of **6a** and 40.5 mg (36.6%) of **6b**. Following the procedure B described above, the reaction mixture was irradiated for 3 min at a preselected temperature of 130 °C, with an irradiation power of 110 W. The crude was purified by preparative TLC using hexanes/EtOAc (7:3) to provide 23.9 mg (39.2%) of **6a** and 17.3 mg (28.4%) of **6b**.

**Data for 6a.** It was isolated as an amorphous yellow solid.  $R_f$  (Hex/EtOAc 7:3) = 0.52.  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  1.27 (s, 3H,  $\text{CH}_3$ ), 1.63 (s, 3H,  $\text{CH}_3$ ), 2.18 (m, 1H), 4.36 (dd,  $^2J_{\text{H,H}} = 12.1$ ,  $^3J_{\text{H,H}} = 3.8$  Hz, 1H), 4.47 (m, 2H), 6.63 (d,  $^3J_{\text{H,H}} = 8.6$  Hz, 1H), 7.19 (dd,  $^3J_{\text{H,H}} = 8.5$ ,  $^4J_{\text{H,H}} = 1.6$  Hz, 1H), 7.35 (s, 1H), 7.71 (m, 2H), 8.13 (m, 2H). ppm.  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  23.6 ( $\text{CH}_3$ ), 27.3 ( $\text{CH}_3$ ), 29.0 (CH), 37.6 (CH), 64.4 ( $\text{CH}_2$ ), 88.5 (C), 112.7 ( $\text{C}_{\text{arom}}$ ), 117.7 ( $\text{CH}_{\text{arom}}$ ), 120.2 ( $\text{C}_{\text{arom}}$ ), 123.0 ( $\text{C}_{\text{arom}}$ ), 126.1 ( $\text{CH}_{\text{arom}}$ ), 126.3 ( $\text{CH}_{\text{arom}}$ ), 130.6 ( $\text{C}_{\text{arom}}$ ), 130.9 ( $\text{CH}_{\text{arom}}$ ), 132.0 ( $\text{C}_{\text{arom}}$ ), 132.3 ( $\text{CH}_{\text{arom}}$ ), 132.9 ( $\text{CH}_{\text{arom}}$ ), 134.0 ( $\text{CH}_{\text{arom}}$ ), 152.8 ( $\text{C}_{\text{arom}}$ ), 154.0 ( $\text{C}_{\text{arom}}$ ), 179.4 (C, C=O), 184.7 (C, C=O) ppm. EI-MS  $m/z$  (%) 426 ( $\text{M}^+$ , 100), 408 ( $\text{M}^+ - \text{H}_2\text{O}$ , 18). EI-HRMS 426.0266 (calcd for  $\text{C}_{22}\text{H}_{17}\text{O}_4\text{Br}$  ( $\text{M}^+$ ) 426.0290). IR ( $\text{CHCl}_3$ )  $\nu_{\text{max}}$  2922, 2359, 1680, 1645, 1608, 1575, 1480, 1361, 1337, 1255, 1211, 1186, 1227, 1082, 988, 954, 815, 769, 723, 469  $\text{cm}^{-1}$ .

**Data for 6b.** It was isolated as an amorphous orange solid.  $R_f$  (Hex/EtOAc, 7:3) = 0.32.  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  1.27 (s, 3H,  $\text{CH}_3$ ), 1.65 (s, 3H,  $\text{CH}_3$ ), 2.21 (m, 1H), 4.34–4.45 (m, 3H), 6.60 (d,  $^3J_{\text{H,H}} = 8.6$  Hz, 1H), 7.11 (dd,  $^3J_{\text{H,H}} = 8.8$ ,  $^4J_{\text{H,H}} = 1.6$  Hz, 1H), 7.25 (s, 1H), 7.51 (t,  $^3J_{\text{H,H}} = 7.5$  Hz, 1H), 7.66 (t,  $^3J_{\text{H,H}} = 5.4$  Hz, 1H), 7.82 (d,  $^3J_{\text{H,H}} = 7.41$  Hz, 1H), 8.03 (d,  $^3J_{\text{H,H}} = 6.8$  Hz, 1H).  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  23.8 ( $\text{CH}_3$ ), 27.8 ( $\text{CH}_3$ ), 28.5 (CH), 37.5 (CH), 64.5 ( $\text{CH}_2$ ), 81.8 (C), 112.8 ( $\text{C}_{\text{arom}}$ ), 113.9 ( $\text{C}_{\text{arom}}$ ), 117.6 ( $\text{CH}_{\text{arom}}$ ), 123.6 ( $\text{C}_{\text{arom}}$ ), 124.7 ( $\text{CH}_{\text{arom}}$ ), 128.3 ( $\text{CH}_{\text{arom}}$ ), 130.1 ( $\text{C}_{\text{arom}}$ ), 130.7 ( $\text{CH}_{\text{arom}}$ ), 131.1 ( $\text{CH}_{\text{arom}}$ ), 131.8 ( $\text{C}_{\text{arom}}$ ), 131.9 ( $\text{CH}_{\text{arom}}$ ), 134.8 ( $\text{CH}_{\text{arom}}$ ), 152.6 ( $\text{C}_{\text{arom}}$ ), 162.1 ( $\text{C}_{\text{arom}}$ ), 179.1 (C, C=O), 179.2 (C, C=O). EI MS  $m/z$  (%) 426 ( $\text{M}^+$ , 100), 409 ( $\text{M}^+ - \text{H}_2\text{O}$ , 27), 354 (21), 327 (19). EI HRMS 426.0288 [ $\text{M}^+$ ] calcd for  $\text{C}_{22}\text{H}_{17}\text{O}_4\text{Br}$  426.0290]. IR ( $\text{CHCl}_3$ )  $\nu_{\text{max}}$  2924, 1643, 1595, 1564, 1481, 1369, 1253, 1126, 1090, 816, 755, 543  $\text{cm}^{-1}$ .

**Biology. Biological Assay.** The solvent for all the stocks of the chemical agents employed was dimethyl sulfoxide (DMSO), Molecular Biology grade (DNase and RNase-free), from Sigma-Aldrich. Ellipticine and etoposide were purchased from Sigma-Aldrich and stored at 10 mM in DMSO stock at  $-20$  °C. Synthesized pyranonaphthoquinones were also stored in DMSO as a 10 mM stock at  $-20$  °C until their use.

**TopoII-Mediated DNA Decatenation Assay.** The TopoII assay kit from TopoGEN (Columbus, OH) was used. Purified hTopoII $\alpha$  was also purchased from the same vendor. DNA decatenation assays were performed according to the manufacturer's instructions and earlier procedures<sup>61</sup> with some minor modifications. The assay was performed in a total reaction volume of 20  $\mu\text{L}$  containing 50 mM Tris-HCl (pH 8.0), 120 mM KCl, 10 mM  $\text{MgCl}_2$ , 0.5 mM dithiothreitol, 0.5 mM ATP, 30  $\mu\text{g}/\text{mL}$  bovine serum albumin, and 150 ng of kDNA. All chemical compounds were then added to a final concentration of 100  $\mu\text{M}$  in 1% DMSO. Reactions were initiated by addition of 2–4 units of hTopoII $\alpha$ , and mixtures were incubated for 30 min at 37 °C. The reaction was terminated by the

addition of 5  $\mu\text{L}$  of stock solution (5% SDS, 25% ficoll, and 0.05% bromophenol blue) followed by treatment with 0.25 mg/mL proteinase K (Roche) at 55 °C for 30 min to eliminate the protein. Samples were resolved by electrophoresis on a 1% (w/v) agarose gel containing 0.5  $\mu\text{g}/\text{mL}$  ethidium bromide in TAE buffer (100 mM Tris-acetate and 2 mM  $\text{Na}_2\text{EDTA}$ , pH 8.3). DNA bands were visualized by UV and photographed and documented with QuantityOne (BioRad).

For quantitation of the substrate (kDNA) and products of the TopoII reaction (Nck, SC, and catenanes), the numerical values of intensity profiles of the different lanes were obtained after background subtraction and exported to Microsoft Excel. For each lane, the overall intensity was used to obtain the fraction of intensity for the different bands (kDNA, Nck, and SC). Here, we measured hTopoII $\alpha$  activity based on the final products (nicked plus supercoiled minicircles). Positive control (1% DMSO, +hTopoII $\alpha$ ) and negative control (1% DMSO, -hTopoII $\alpha$ ) were used to set up the limit values (1 and 0, respectively).

**TopoII-Mediated DNA Relaxation Assay.** The TopoII drug kit from TopoGEN (Columbus, OH) was used. The 20  $\mu\text{L}$  reaction mixture contained 250 ng of pRYG plasmid DNA and 0.5 mM ATP in the assay buffer [10 mM Tris-HCl, 50 mM KCl, 50 mM NaCl, 0.1 mM EDTA, 5 mM  $\text{MgCl}_2$ , and 2.5% (v/v) glycerol, pH 8.0]. Chemical compounds were at 100  $\mu\text{M}$  in 1% DMSO. The reaction components were added as follows: assay buffer, DNA, chemical compound from DMSO stock or just DMSO, and finally 1–2 units of hTopoII $\alpha$ . The reaction mixture was incubated at 37 °C for 30 min, and the reaction was quenched with 1% (w/v) SDS and 25 mM  $\text{Na}_2\text{EDTA}$ . The mixture was treated with 0.25 mg/mL proteinase K (Roche) at 55 °C for 30 min to digest the protein. pRYG DNA topoisomers were resolved in 1% (w/v) agarose gel electrophoresis in 1 $\times$  TBE buffer (89 mM Tris-borate and 2 mM  $\text{Na}_2\text{EDTA}$ , pH 8) without ethidium bromide. The photograph was taken as above after staining with ethidium bromide.

Quantitation of the products was done as above only that the SC form was considered as substrate of the reaction and the sum of all topoisomers (and nicked DNA) as products.

**Stabilization of the Cleavage Complex.** The same TopoII drug kit was used as above, but this time an amount of 4–8 units of hTopoII $\alpha$  was added 1–3 min before the compound addition. Electrophoresis was run in TAE buffer with 0.5  $\mu\text{g}/\text{mL}$  ethidium bromide.

**DNA Intercalation Assay.** Negatively supercoiled pBSKS plasmid was prepared using a plasmid mega kit (Qiagen) as described by the manufacturer. An amount of 250 ng of pBSKS was then incubated for 10 min at 37 °C with 100  $\mu\text{M}$  of the analyzed drugs in DMSO 1% (v/v) and loaded in a 1% (w/v) agarose gel. Electrophoresis was carried out in 1 $\times$  TBE buffer without ethidium bromide (0.5 V/cm).

**Molecular Docking.** The crystal structure of hTopoII $\alpha$  was retrieved from the Protein Data Bank (PDB entry 1ZXM).<sup>62</sup> The hydrogen atoms of the protein were added using the software package ADT Tools. Protein atom types and potentials were assigned according to the Amber 4.0 force field with Kollman united-atom charges,<sup>63</sup> and the magnesium ion was assigned a charge of +0.9. The initial structures of naphthoquinones were optimized using Gaussian 03 at level HF/3-21G with RESP charges. The minimized geometry was used to prepare the ligands for their use in docking program AutoDock 3.0.5 (<http://www.scripps.edu/mb/olson/doc/autodock/>) following the standard procedure. The three-dimensional grid with 60  $\times$  60  $\times$  60 points and a spacing of 0.375 Å was created by the AutoGrid algorithm to evaluate the binding energies between the ligands and the proteins. Default docking parameters were used except number of generations, population size, and docking runs, which are fixed at 27 000, 100, and 25, respectively. The Lamarckian genetic algorithm<sup>63</sup> was applied to analyze protein–ligand interactions. The docked structures of the ligands were generated after a reasonable number of evaluations. Figures for best scoring dockings were generated by PyMOL.<sup>64</sup>

**Acknowledgment.** This work has been partly funded by the Spanish MEC (Project SAF 2006-06720) and ICIC (Instituto Canario de Investigación del Cáncer) to A.E.-B. and by FIS (Project PI06/1211) to F.M. Ramon y Cajal program (Spanish Ministry of Science & Technology) contributes to the financial support of F.M. S.J.-A. thanks the MEC for a predoctoral fellowship. The authors also thank to B. Delgado and V. González for granting access to the HLC apparatus.

**Note Added after ASAP Publication.** This manuscript was released ASAP on September 25, 2008, with two references missing from ref 51. The updated version was posted on October 17, 2008.

**Supporting Information Available:**  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectra, purity determinations for all pyranonaphthoquinones synthesized, and figures showing docking models of lapachones and **2a**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

## References

- (1) (a) Driscoll, J. S.; Hazard, G. F.; Wood, H. B. Quinone structure-antitumor activity relationships. *Cancer Chemother. Rep., Part 2* **1974**, *4*, 1-362. (b) Liu, K. C.; Li, J.; Sakya, S. Synthetic approaches to the 2003 new drugs. *Mini-Rev. Med. Chem.* **2004**, *4*, 1105-1125.
- (2) Thomson, R. H. *Naturally Occurring Quinones IV. Recent Advances*; Blackie A&P: London, 1997.
- (3) (a) Ding, Y.; Chen, Z. J.; Liu, S.; Che, D.; Vetter, M.; Chang, C. H. Inhibition of Nox-4 activity by plumbagin, a plant-derived bioactive naphthoquinone. *J. Pharm. Pharmacol.* **2005**, *7* (1), 111-116. (b) Tiwari, S. B.; Pai, R. M.; Udupa, N. Temperature sensitive liposomes of plumbagin: characterization and in vivo evaluation in mice bearing melanoma B16F1. *J. Drug Targeting* **2002**, *10* (8), 585-591. (c) Oommen, E.; Shenoy, B.; Dinesh, U.; Kamath, N.; Ravindra, D.; Uma, P. Antitumor efficacy of cyclodextrin-complexed and niosome-encapsulated plumbagin in mice bearing melanoma B16F1. *Pharm. Pharmacol. Commun.* **1999**, *5* (4), 281-285. (d) Fujii, N.; Yamashita, Y.; Arima, Y.; Yasushi, N.; Nagashima, M.; Minoru, N.; Nakano, H. Induction of topoisomerase II-mediated DNA cleavage by the plant naphthoquinones plumbagin and shikonin. *Antimicrob. Agents Chemother.* **1992**, *36* (12), 2589-2594.
- (4) (a) Polonik, S. G.; Prokof'eva, N. G.; Agafonova, I. G.; Uvarova, N. I. Antitumor and immunostimulating activity of 5-hydroxy-1,4-naphthoquinone (Juglone) *O*- and *S*-acetylglucosides. *Pharm. Chem. J.* **2003**, *37* (8), 397-398. (b) Kapadia, G. J.; Balasubramanian, V.; Tokuda, H.; Konoshima, T.; Takasaki, M.; Koyama, J.; Tagahaya, K.; Nishino, H. Anti-tumor promoting effects of naphthoquinone derivatives on short term Epstein-Barr early antigen activation assay and in mouse skin carcinogenesis. *Cancer Lett.* **1997**, *113*, 47-53.
- (5) (a) Li, C. J.; Li, Y. Z.; Pinto, A. V.; Pardee, A. B. Potent inhibition of tumor survival in vivo by  $\beta$ -lapachone plus Taxol: combining drugs imposes different artificial checkpoints. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 13369-13374. (b) Pink, J. J.; Planchon, S. M.; Tagliarino, C.; Varnes, M. E.; Siegel, D.; Boothman, D. A. *J. Biol. Chem.* **2000**, *275*, 5416-5424. (c) Pardee, A. B.; Li, Y. Z.; Li, C. J. NAD(P)H:quinone oxidoreductase activity is the principal determinant of  $\beta$ -lapachone cytotoxicity. *Curr. Cancer Drug Targets* **2002**, *2*, 227-242. (d) Li, Y.; Sun, X.; LaMont, J. T.; Pardee, A. B.; Li, C. J. Selective killing of cancer cells by  $\beta$ -lapachone: direct checkpoint activation as a strategy against cancer. *Proc. Nat. Acad. Sci. U.S.A.* **2003**, *100* (5), 2674-2678. (e) Boorstein, R. J.; Pardee, A. B. Coordinate inhibition of DNA synthesis and thymidylate synthase activity following DNA damage and repair. *Biochem. Biophys. Res. Commun.* **1983**, *117* (1), 30-36.
- (6) Thirumurugan, R. S.; Kavimani, S.; Srivastava, R. S. Antitumor activity of rhinacanthone against Dalton's ascitic lymphoma. *Biol. Pharm. Bull.* **2000**, *23* (12), 1438-1440.
- (7) (a) Costantino, L.; Barlocco, D. Privileged structures as leads in medicinal chemistry. *Curr. Med. Chem.* **2006**, *13*, 65-85. (b) Li, J.; Liu, K.; Sakya, S. Synthetic approaches to the 2004 new drugs. *Mini-Rev. Med. Chem.* **2005**, *5*, 1133. (c) DeSimone, R. W.; Currie, K. S.; Mitchell, S. A.; Darrow, J. W.; Pippin, D. A. Privileged structures: applications in drug discovery. *Comb. Chem. High Throughput Screening* **2004**, *7*, 473. (d) Li, J.; Liu, K. Synthetic approaches to the 2002 new drugs. *Mini-Rev. Med. Chem.* **2004**, *4*, 207.
- (8) O'Brien, P. J. Molecular mechanisms of quinone cytotoxicity. *Chem. Biol. Interact.* **1991**, *80*, 1-41.
- (9) (a) Plyta, Z. F.; Li, T.; Papageorgiou, V. P.; Mellidis, A. S.; Assimopoulou, A. N.; Pitsinos, E. N.; Couladouros, E. A. Inhibition of topoisomerase I by naphthoquinone derivatives. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3385-3390. (b) Miller, M. G.; Rodgers, A.; Cohen, G. M. Mechanisms of toxicity of naphthoquinones to isolated hepatocytes. *Biochem. Pharmacol.* **1986**, *35*, 1177-1184. (c) Salmon-Chemin, L.; Buissine, E.; Yardley, V.; Kohler, S.; Debreu, M. A.; Landry, V.; Sergheraert, C.; Croft, S. L.; Krauth-Siegel, R. L.; Davioud-Charvet, E. 2- and 3-Substituted 1,4-naphthoquinone derivatives as subversive substrates of trypanothione reductase and lipamide dehydrogenase from *Trypanosoma cruzi*: synthesis and correlation between redox cycling activities and in vitro cytotoxicity. *J. Med. Chem.* **2001**, *44*, 548-565.
- (10) Lindsey, R. H. J.; Bromberg, K. D.; Felix, C. A.; Osheroff, N. 1,4-Benzoquinone is a topoisomerase II poison. *Biochemistry* **2004**, *43*, 7563-7574.
- (11) Lindsey, R. H. J.; Bender, R. P.; Osheroff, N. Effects of benzene metabolites on DNA cleavage mediated by human topoisomerase II  $\alpha$ : 1,4-hydroquinone is a topoisomerase II poison. *Chem. Res. Toxicol.* **2005**, *18*, 761-770.
- (12) Wilstermann, A. M.; Osheroff, N. Stabilization of eukaryotic topoisomerase II-DNA cleavage complexes. *Curr. Top. Med. Chem.* **2003**, *3*, 321-338.
- (13) McClendon, A. K.; Osheroff, N. DNA topoisomerase II, genotoxicity, and cancer. *Mutat. Res.* **2007**, *623*, 83-97.
- (14) Osheroff, N. DNA topoisomerases. *Biochim. Biophys. Acta* **1998**, *1400*, 1-2.
- (15) Wang, J. C. Cellular roles of DNA topoisomerases: a molecular perspective. *Nat. Rev. Mol. Cell Biol.* **2002**, *3*, 430-440.
- (16) Champoux, J. J. DNA topoisomerases: structure, function, and mechanism. *Annu. Rev. Biochem.* **2001**, *70*, 369-413.
- (17) Holm, C.; Goto, T.; Wang, J. C.; Botstein, D. DNA topoisomerase II is required at the time of mitosis in yeast. *Cell* **1985**, *41*, 553-563.
- (18) Heck, M. M.; Hittelman, W. N.; Earnshaw, W. C. Differential expression of DNA topoisomerases I and II during the eukaryotic cell cycle. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 1086-1090.
- (19) Heck, M. M.; Earnshaw, W. C. Topoisomerase II: a specific marker for cell proliferation. *J. Cell Biol.* **1986**, *103*, 2569-2581.
- (20) Osheroff, N.; Corbett, A. H.; Robinson, M. J. Mechanism of action of topoisomerase II-targeted antineoplastic drugs. *Adv. Pharmacol.* **1994**, *29B*, 105-126.
- (21) Robinson, M. J.; Corbett, A. H.; Osheroff, N. Effects of topoisomerase II-targeted drugs on enzyme-mediated DNA cleavage and ATP hydrolysis: evidence for distinct drug interaction domains on topoisomerase II. *Biochemistry* **1993**, *32*, 3638-3643.
- (22) Corbett, A. H.; Osheroff, N. When good enzymes go bad: conversion of topoisomerase II to a cellular toxin by antineoplastic drugs. *Chem. Res. Toxicol.* **1993**, *6*, 585-597.
- (23) Capranico, G.; Zunino, F. DNA topoisomerase-trapping antitumor drugs. *Eur. J. Cancer* **1992**, *28A*, 2055-2060.
- (24) Robinson, M. J.; Osheroff, N. Stabilization of the topoisomerase II-DNA cleavage complex by antineoplastic drugs: inhibition of enzyme-mediated DNA religation by 4'-(9-acridinylamino)methanesulfonamide. *Biochemistry* **1990**, *29*, 2511-2515.
- (25) Gale, K. C.; Osheroff, N. Uncoupling the DNA cleavage and religation activities of topoisomerase II with a single-stranded nucleic acid substrate: evidence for an active enzyme-cleaved DNA intermediate. *Biochemistry* **1990**, *29*, 9538-9545.
- (26) Osheroff, N. Effect of antineoplastic agents on the DNA cleavage/religation reaction of eukaryotic topoisomerase II: inhibition of DNA religation by etoposide. *Biochemistry* **1989**, *28*, 6157-6160.
- (27) Sabourin, M.; Nitiss, J. L.; Nitiss, K. C.; Tatebayashi, K.; Ikeda, H.; Osheroff, N. Yeast recombination pathways triggered by topoisomerase II-mediated dna breaks. *Nucleic Acids Res.* **2003**, *31*, 4373-4384.
- (28) Howard, M. T.; Neece, S. H.; Matson, S. W.; Kreuzer, K. N. Disruption of a topoisomerase-DNA cleavage complex by a DNA helicase. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 12031-12035.
- (29) Bjornsti, M. A.; Benedetti, P.; Viglianti, G. A.; Wang, J. C. Expression of human DNA topoisomerase I in yeast cells lacking yeast DNA topoisomerase I: restoration of sensitivity of the cells to the antitumor drug camptothecin. *Cancer Res.* **1989**, *49*, 6318-6323.
- (30) Wasserman, R. A.; Wang, J. C. Mechanistic studies of amsacrine-resistant derivatives of DNA topoisomerase II. Implications in resistance to multiple antitumor drugs targeting the enzyme. *J. Biol. Chem.* **1994**, *269*, 20943-20951.
- (31) Nitiss, J. L.; Liu, Y. X.; Hsiung, Y. A temperature sensitive topoisomerase II allele confers temperature dependent drug resistance on amsacrine and etoposide: a genetic system for determining the targets of topoisomerase II inhibitors. *Cancer Res.* **1993**, *53*, 89-93.

- (32) Nitiss, J. L.; Liu, Y. X.; Harbury, P.; Jannatipour, M.; Wasserman, R.; Wang, J. C. Amsacrine and etoposide hypersensitivity of yeast cells overexpressing DNA topoisomerase II. *Cancer Res.* **1992**, *52*, 4467–4472.
- (33) Guano, F.; Pourquier, P.; Tinelli, S.; Binaschi, M.; Bigioni, M.; Animati, F.; Manzini, S.; Zunino, F.; Kohlhagen, G.; Pommier, Y.; Capranico, G. Topoisomerase poisoning activity of novel disaccharide anthracyclines. *Mol. Pharmacol.* **1999**, *56*, 77–84.
- (34) Zunino, F.; Capranico, G. DNA topoisomerase II as the primary target of anti-tumor anthracyclines. *Anti-Cancer Drug Des.* **1990**, *5*, 307–317.
- (35) Fortune, J. M.; Osheroff, N. Merbarone inhibits the catalytic activity of human topoisomerase II $\alpha$  by blocking DNA cleavage. *J. Biol. Chem.* **1998**, *273*, 17643–17650.
- (36) Roca, J.; Ishida, R.; Berger, J.M.; Andoh, T.; Wang, J. C. Antitumor bisdioxopiperazines inhibit yeast DNA topoisomerase II by trapping the enzyme in the form of a closed protein clamp. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 1781–1785.
- (37) Andoh, T.; Ishida, R. Catalytic inhibitors of dna topoisomerase II. *Biochim. Biophys. Acta* **1998**, *1400*, 155–171.
- (38) Tanabe, K.; Ikegami, Y.; Ishida, R.; Andoh, T. Inhibition of topoisomerase II by antitumor agents bis(2,6-dioxopiperazine) derivatives. *Cancer Res.* **1991**, *51*, 4903–4908.
- (39) Andoh, T.; Sato, M.; Narita, T.; Ishida, R. Role of DNA topoisomerase II in chromosome dynamics in mammalian cells. *Biotechnol. Appl. Biochem.* **1993**, *18* (2), 165–174.
- (40) Skladanowski, A.; Côme, M.; Sabisz, M.; Escargueil, A. E.; Larsen, A. K. Down-regulation of DNA topoisomerase II $\alpha$  leads to prolonged cell cycle transit in g2 and early m phases and increased survival to microtubule-interacting agents. *Mol. Pharmacol.* **2005**, *68*, 625–634.
- (41) Cortés, F.; Pastor, N. Induction of endoreduplication by topoisomerase II catalytic inhibitors. *Mutagenesis* **2003**, *18*, 105–112.
- (42) Cortés, F.; Pastor, N.; Mateos, S.; Domínguez, I. Roles of DNA topoisomerases in chromosome segregation and mitosis. *Mutat. Res.* **2003**, *543*, 59–66.
- (43) Jensen, L. H.; Dejligbjerg, M.; Hansen, L. T.; Grauslund, M.; Jensen, P. B.; Sehested, M. Characterisation of cytotoxicity and DNA damage induced by the topoisomerase II-directed bisdioxopiperazine anticancer agent icrf-187 (dexrazoxane) in yeast and mammalian cells. *BMC Pharmacol.* **2004**, *4*, 31.
- (44) Pérez-Sacau, E.; Díaz-Peñate, R.; Estévez-Braun, A.; Ravelo, A. G.; García-Castellano, J. M.; Campillo, M.; Pardo, L. Synthesis and pharmacophore modeling of naphthoquinone derivatives with cytotoxic activity in human promyelocytic leukemia HL60 cell line. *J. Med. Chem.* **2007**, *50*, 696–706.
- (45) (a) Pérez-Sacau, E.; Estévez-Braun, A.; Ravelo, A. G.; Ferro, E.; Tozuda, H.; Mukainaka, H.; Nishino, H. Inhibitory effects of lapachol derivatives on Epstein–Barr virus activation. *Bioorg. Med. Chem.* **2003**, *11*, 483. (b) Ravelo, A. G.; Estévez-Braun, A.; Chávez-Orellana, H.; Pérez-Sacau, E.; Mesa-Siverio, D. Recent studies on natural products as anticancer agents. *Curr. Top. Med. Chem.* **2004**, *4*, 241. (c) Pérez-Sacau, E.; Soto-Delgado, J.; Estévez-Braun, A.; Ravelo, A. G. Synthesis of 9 and 10-membered macrolactones by selective ozonolysis of 1,4-diaza-phenanthrene derivatives. *Tetrahedron* **2005**, *61*, 437. (d) Ravelo, A. G.; Estévez-Braun, A.; Pérez-Sacau, E. The Chemistry and Biology of Lapachol and Related Natural Products  $\alpha$  and  $\beta$  Lapachones. In *Studies in Natural Products Chemistry*; Atta-Ur-Rahman, Ed.; Elsevier Science Publishers: Amsterdam, 2003; Vol. 29, Part J, pp 719–60, and references cited therein. (e) Pérez-Sacau, E.; Estévez-Braun, A.; Ravelo, A. G.; Gutiérrez, D.; Giménez, A. Antiplasmodial activity of naphthoquinones related to lapachol and  $\beta$ -lapachone. *Chem. Biodiversity* **2005**, *2*, 264–274. (f) Jiménez-Alonso, S.; Estévez-Braun, A.; Ravelo, A. G.; Zárate, R.; López, M. Double domino Knoevenagel hetero Diels–Alder strategy towards bispyrano-1,4-benzoquinones. *Tetrahedron* **2007**, *63*, 3066–3074.
- (46) (a) Tietze, L. F. Domino reactions in organic synthesis. *Chem. Rev.* **1996**, *96*, 115, and references cited therein. (b) Tietze, L. F.; Modi, A. Multicomponent domino reactions for the synthesis of biologically active natural products and drugs. *Med. Res. Rev.* **2000**, *20*, 304. (c) Tietze, L. F.; Rackelmann, N. Domino reactions in the synthesis of heterocyclic natural products and analogs. *Pure Appl. Chem.* **2004**, *76*, 1967. (d) Bhar, S.; Ramana, M. M. *J. Org. Chem.* **2004**, *69*, 8935. (e) Bunte, J. O.; Rinne, S.; Mattay, J. *Synthesis* **2004**, *4*, 619. (f) Tietze, L. F.; Beifuss, U. Sequential transformation in organic chemistry: a synthesis strategy with a future. *Angew. Chem., Int. Ed.* **1993**, *32*, 131. (g) Ho, T. L. *Tandem Organic Reactions*; Wiley: New York, 1992.
- (47) (a) Tietze, L. F.; Brumby, T.; Pretor, M.; Remberg, G. Intra- and intermolecular hetero-Diels–Alder reactions. 17. Intramolecular hetero-Diels–Alder reaction of alkylidene- and benzylidenepyrazolones and benzylideneisoxazolones. Investigations toward the conformation of the transition state. *J. Org. Chem.* **1988**, *53*, 810. (b) Tietze, L. F.; Siegbert, B.; Brumby, T.; Pretor, M.; Remberg, G. Effect of substituents in the chain between diene and dienophile moieties on the intramolecular hetero-Diels–Alder reaction of oxadienes. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 665. (c) Tietze, L. F.; Bratz, M.; Machinek, R.; Kiedrowski, G. V. Intra- and intermolecular hetero-Diels–Alder reactions. 16. Stereospecificity in intramolecular hetero-Diels–Alder reactions of 2-benzylidene-1,3-dicarbonyl compounds. *J. Org. Chem.* **1987**, *52*, 1638–1640. (d) Zhu, J.; Bienaymé, H., Eds. *Multicomponent Reactions*; Wiley-VCH: Weinheim, Germany, 2005; Chapter 5, pp 121–168.
- (48) Xing, C.; Li, X.; Zhu, S.; Zhao, J.; Zhu, S. Synthesis and hetero-Diels–Alder reactions of (*E*)- $\alpha$ -perfluoroalkanesulfonyl- $\alpha$ , $\beta$ -unsaturated ketones. *Tetrahedron Lett.* **2006**, *47*, 4951–4955.
- (49) Ceulemans, E.; Voets, M.; Emmers, S.; Uytterhoeven, K.; Meervelt, L. V.; Dehaen, W. Diastereoselective intramolecular hetero Diels–Alder approach towards polycyclic heterocycles. *Tetrahedron* **2002**, *58*, 531–544.
- (50) Leutbecher, H.; Conrad, J.; Klaiber, I.; Beifuss, U. Microwave assisted domino Knoevenagel condensation/ $6\pi$ -electron electrocyclicization reactions for the rapid and efficient synthesis of substituted 2*H*,5*H*-pyrano[4,3-*b*]pyran-5-ones and related heterocycles. *QSAR Comb. Sci* **2004**, *23*, 895–898.
- (51) (a) Lee, Y. R.; Choi, J. H.; Trinh, D. T. L.; Kim, N. W. A concise route for the synthesis of pyranonaphthoquinone derivatives. *Synthesis* **2005**, *18*, 3026–3034. (b) Ferreira, V. F.; Coutada, L. C.; Pinto, M. C. F. R.; Pinto, A. V. A novel synthesis of  $\alpha$  and  $\beta$ -pyran-naphthoquinone tetracyclic. *Synth Commun.* **1982**, *12*, 195–199. (c) Pinto, A. V.; Ferreira, V. F.; Coutada, L. C. M. Síntese de Análogos de Xiloidonas e Lapachonas. *An. Acad. Bras. Cienc.* **1980**, *52* (3), 477–479.
- (52) (a) Kappe, C. O.; Stadler, A. *Methods and Principles in Medicinal Chemistry: Microwaves in Organic and Medicinal Chemistry*; Wiley-VCH: Weinheim, Germany, 2005; Vol. 25. (b) Kappe, C. O. Synthetic methods. Controlled microwave heating in modern organic synthesis. *Angew. Chem., Int. Ed.* **2004**, *43*, 6250. (c) Hoz, A. D.; Diaz-Ortiz, A.; Moreno, A. Selectivity in organic synthesis under microwave irradiation. *Curr. Org. Chem.* **2004**, *8*, 903. (d) Loupy, A. In *Microwaves in Organic Synthesis*; Wiley-VCH: Weinheim, Germany, 2002.
- (53) Muller, M. T.; Helal, K.; Soisson, S.; Spitzner, J. R. A rapid and quantitative microtiter assay for eukaryotic topoisomerase II. *Nucleic Acids Res.* **1989**, *17*, 9499.
- (54) Cline, S. D.; Macdonald, T. L.; Osheroff, N. Azatoxin is a mechanistic hybrid of the topoisomerase II-targeted anticancer drugs etoposide and ellipticine. *Biochemistry* **1997**, *36*, 13095–13101.
- (55) Spitzner, J. R.; Chung, I. K.; Muller, M. T. Eukaryotic topoisomerase II preferentially cleaves alternating purine–pyrimidine repeats. *Nucleic Acids Res.* **1990**, *18*, 1–11.
- (56) Bures, N. S.; Sazesh, S.; Gunawardana, G. P.; Clement, J. J. Antitumor activity and nucleic acid binding properties of dercitin, a new acridine alkaloid isolated from a marine dercitus species sponge. *Cancer Res.* **1989**, *49*, 5267–5274.
- (57) Froelich-Ammon, S. J.; Osheroff, N. Topoisomerase poisons: harnessing the dark side of enzyme mechanism. *J. Biol. Chem.* **1995**, *270*, 21429–21432.
- (58) Morris, G. M.; Goodsell, D. S.; Huey, R.; Hart, W. E.; Halliday, S.; Belew, R.; Olson, A. J. *AutoDock: Automated Docking of Flexible Ligands to Receptors*, version 3.0.3; Molecular Graphics Laboratory, Department of Molecular Biology, Mail Drop MB-5, The Scripps Research Institute: La Jolla, CA, 1999; pp 1–82.
- (59) Hu, C. X.; Zuo, Z. L.; Xiong, B.; Ma, J. G.; Geng, M. Y.; Lin, L. P.; Jiang, H. L.; Ding, J. Salvicine functions as novel topoisomerase II poison by binding to ATP pocket. *Mol. Pharmacol.* **2006**, *70*, 1593–1601.
- (60) Krishnan, P.; Bastow, K. F. Novel mechanisms of DNA topoisomerase II inhibition by pyranonaphthoquinone derivatives—eleutherin, alpha lapachone, and beta lapachone. *Biochem. Pharmacol.* **2000**, *60*, 1367–1379.
- (61) Sahai, B. M.; Kaplan, J. G. A quantitative decatenation assay for type II topoisomerases. *Anal. Biochem.* **1986**, *156*, 364–379.
- (62) Wei, H.; Ruthenburg, A. J.; Bechis, S. K.; Verdine, G. L. Nucleotide-dependent domain movement in the ATPase domain of a human type IIa DNA topoisomerase. *J. Biol. Chem.* **2005**, *280*, 37041–37047.
- (63) Morris, G. M.; Goodsell, D. S.; Hallyday, R. S.; Huey, R.; Hart, W. E.; Belew, R. K.; Olson, A. J. Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function. *J. Comput. Chem.* **1998**, *19*, 1639–1662.
- (64) DeLano, W. *PyMOL*, version 0.99; DeLano Scientific LLC: South San Francisco, CA; <http://pymol.sourceforge.net/>.