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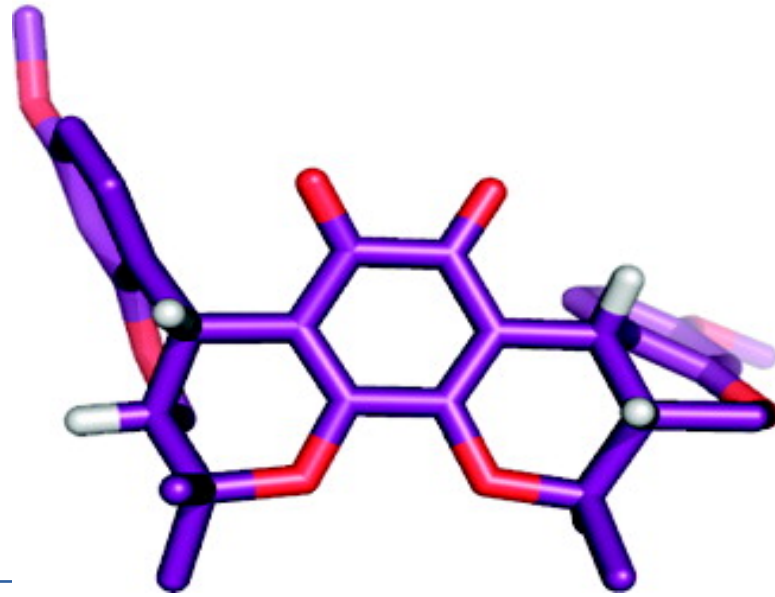

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Bis-pyranobenzoquinones as a New Family of Reversal Agents of the Multidrug Resistance Phenotype Mediated by P-Glycoprotein in Mammalian Cells and the Protozoan Parasite *Leishmania*

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We have synthesized a set of bis-pyranobenzoquinones through a direct and highly efficient approach based on a double intramolecular domino Knoevenagel hetero Diels−Alder reaction. These bis-pyranobenzoquinone derivatives are compounds whose skeletons have similarities to those of some anticancerous and leishmanicidal drugs. Considering that these drugs are substrates for some members of the ATP-binding cassette (ABC) family of proteins that confers a multidrug resistance (MDR) phenotype, we have carried out the biological evaluation of 20 bis-pyranobenzoquinones as modulators of the MDR phenotype in mammalian cell lines overexpressing P-glycoprotein, MRP1, or BCRP. Moreover, we also tested some of these compounds as potential MDR modulators in a *Leishmania tropica* line overexpressing a P-glycoprotein-like transporter. Compounds **9** and **10** are, in this work, the most promising reversal agents of MDR in human cancer cell lines, while compounds **4** and **20** showed potent reversal activity of MDR phenotype in the protozoan parasite *Leishmania*.

Introduction

Drug resistance has emerged in the past years as one of the major impediments for the successful treatment of cancer and diseases caused by protozoan parasites like *Leishmania* spp. One drug resistance mechanism is the overexpression of drug efflux pumps belonging to the ATP-binding cassette (ABC[a]) family of proteins.[1] This is one of the largest protein family known from archaeobacteria to higher eukaryotes.[2] These proteins use the energy of ATP hydrolysis to either import or export a wide spectrum of substrates ranging from small inorganic ions to large peptides, sugar, lipids, or drugs.[2] Among the ABC proteins, P-glycoprotein (Pgp, MDR1, or ABCB1), the multidrug resistance associated protein 1 (MRP1 or ABCC1), and the breast cancer resistance protein (BCRP or ABCG2) are able to transport different structurally and functionally unrelated drugs, conferring a multidrug resistance (MDR) phenotype. ABC transporters are involved in the resistance to anticancer drugs[3] and, at least in vitro, to many antiparasitic drugs.[4−7] Therefore, the development of inhibitors of these transporters is of high clinical relevance. The combined therapy, using drugs along with drug resistance modulators, is thought to be an effective solution. During the past years, a significant number of inhibitors of ABC proteins have been reported. For mammalian Pgp, the most active modulators are those known as third-generation modulators: LY335979 (zosuquidar), GF120918 (elacridar), and XR9576 (tariquidar), among others. These modulators are able to revert efficiently the activity of this transporter;[8] however, the expectations raised by the in vitro results have not correlated well with those obtained in clinical trials.[9] This is due to the fact that zosuquidar influences the pharmacokinetics and biodistribution of the coadministered drug,[10] elacridar is not specific for Pgp,[1] and tariquidar showed limited clinical activity to restore sensitivity to anthracycline- or taxane-based chemotherapy.[11] These results suggest that inhibition of Pgp function with currently available MDR inhibitors is generally ineffective for restoring the sensitivity to chemotherapy in the majority of patients, although a small minority of them may benefit.[11]

We have described natural dihydro-β-agarofuran sesquiterpenes as potent and specific modulators of human and *Leishmania* P-glycoproteins in vitro,[12−16] but we still lack evidence for their in vivo activities. In the meantime, our laboratory continues on the research of more active and less toxic compounds. For human MRP1, very few modulators have been described and most are nonspecific modulators; only PAK-104P[17] is considered efficient and specific for this protein. Among the human BCRP inhibitors, fumitremorgin C[18] was the first described specific modulator, although its neurotoxicity limited its use in clinical patients; afterward, the fumitremorgin C analogue Ko143 was described as a new specific and nontoxic BCRP inhibitor.[19] Future in vivo assays will provide more

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[a] Abbreviations: IKHDA, intramolecular Knoevenagel hetero Diels−Alder; MDR, multidrug resistance; Pgp, P-glycoprotein; ABC, ATP binding cassette; MRP1, multidrug resistance protein 1; BCRP, breast cancer resistance protein; SAR, structure−activity relationships; K_i, inhibition constant; B_{max}, maximum percentage of Pgp inhibition; RI, resistance index; EDDA, ethylenediammonium diacetate; DNR, daunorubicin; TMDs, transmembrane domains; NIH-3T3 MDR1 cells, NIH-3T3 cells transfected with human multidrug resistance 1 gene that encodes the wild-type (G185) P-glycoprotein multidrug efflux pump.

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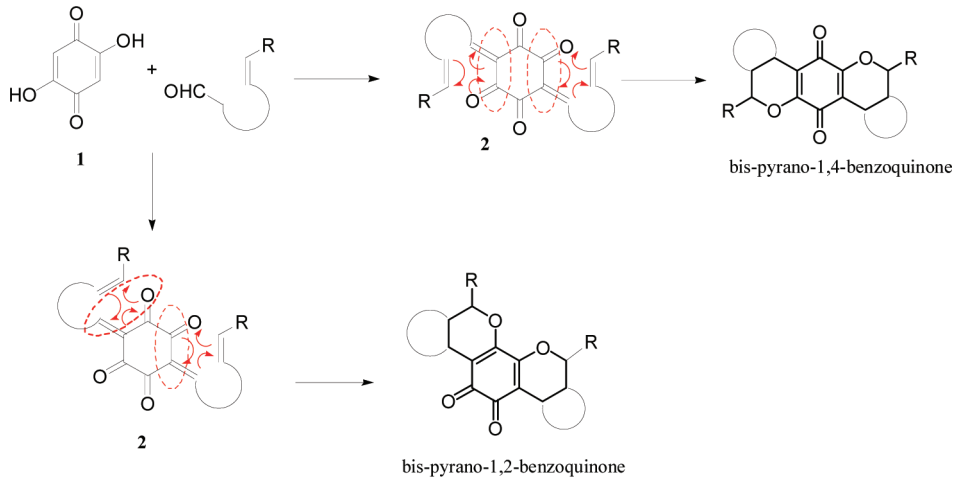
Scheme 1. Plausible Formation of Bis-pyranobenzoquinones



information about this compound. The most recent human BCRP modulators to be reported are some hemisynthetic flavonoids.[20]

The scarce number of known MDR modulators for protozoan parasites made it necessary to describe new specific molecules for those ABC transporters involved in the MDR phenotype of these infectious agents. But such new modulators should not interfere with the normal physiological functions ascribed to their human homologues.

The bis-pyranobenzoquinones present benzopyran and benzoquinone cores, both designated as privileged structures in medicinal chemistry. These bis-pyranobenzoquinone derivatives share similarities to some anticancerous and leishmanicidal drugs (e.g., anthracyclines and mitoxantrone and camptothecin). Considering this structural resemblance, we decided to research if they were able to inhibit the ABC drug transporters. As a part of our investigation into reversal agents of MDR phenotype, we evaluated the biological effect of 20 bis-pyranobenzoquinones as potential modulators of the MDR phenotype in mammalian cell lines overexpressing Pgp, MRP1, or BCRP. We also tested some of these compounds as potential MDR modulators in a *Leishmania tropica* line overexpressing a Pgp-like transporter. We employed two types of assays for evaluating these compounds: (a) monitoring of the accumulation of different fluorescent MDR substrates to determine if our compounds were interfering with the ABC transporter-mediated efflux of fluorescent drugs[14,20,21] and (b) the MTT-based assay[22] to know their intrinsic cytotoxicities and to determine the ability of the selected compounds to decrease cell survival, due to the inhibition of ABC proteins in the presence of increasing concentrations of cytotoxic MDR substrates.

We have found several bis-pyranobenzoquinones that specifically inhibit the activity of Pgp of mammalian cells and the protozoan parasite *L. tropica.* No active compounds were found against MRP1 and BCRP1.

Results and Discussion

Chemistry. Among the strategies that can lead to the discovery of new drugs, the identification and use of privileged structures,[23] molecular fragments that are able to interact with more than one target, have gained particular attention. The combination of the concept of privileged structures with efficient synthetic methodology, such as domino reactions,[24] can guide the fast identification of a new lead. Driven by our interest in antitumoral[25] and anti-MDR agents,[12−15,21,26] we decided to explore the possibility of preparing a new series of bis-pyranobenzoquinones utilizing domino reactions. Domino reactions continue to be of substantial interest because of their ability to maximize molecular complexity while minimizing waste.

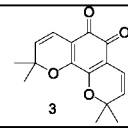
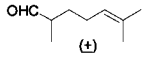
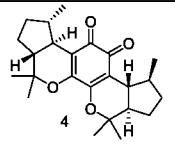
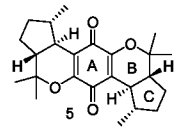
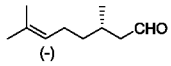
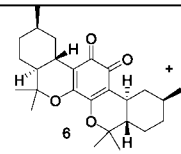
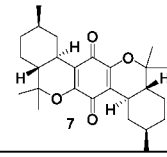
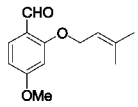
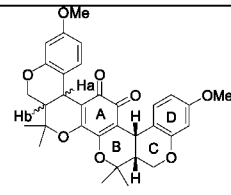
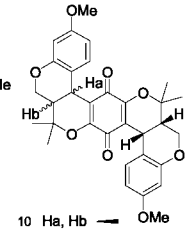
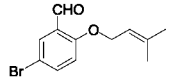
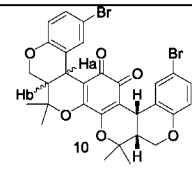
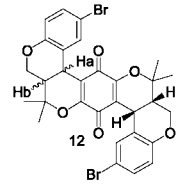
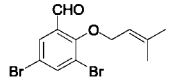
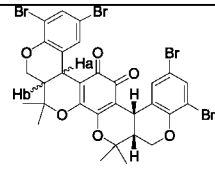
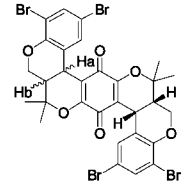
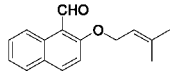
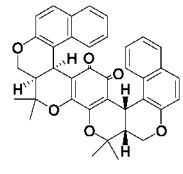
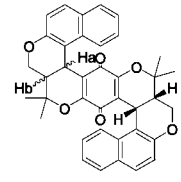
In our efforts to generate high molecular complexity in a one-pot reaction we encouraged the preparation of complex and structurally diverse bis-pyranobenzoquinones through a double intramolecular domino Knoevenagel hetero Diels−Alder reaction.

Our approach was based on the use of 2,5-dihydroxy-1,4-benzoquinone[27] as an adequate and symmetric synthetic equivalent to 1,3-dicarbonyl compound. It was reacted with an aldehyde containing an alkene in the presence of a catalytic amount of ethylenediammonium diacetate (EDDA). The Knoevenagel condensation of 2,5-dihydroxy-1,4-benzoquinone (**1**) and the corresponding aldehyde possessing a double bond in the side chain leads to a reactive intermediate (**2**) (Scheme 1), which undergoes a double intramolecular hetero Diels−Alder reaction with the dienophile moieties, yielding the corresponding bis-pyranobenzoquinones. The polyfunctional intermediate (**2**) has two heterodiene systems that can react following two possibilities. In one of them, the two heterodienes in contiguous disposition react to afford angular bis-pyrano-1,2-benzoquinones. In the other case the two heterodienes yield the linear bis-pyrano-1,4-benzoquinones. The formation of the two pyran rings may occur simultaneously starting from the intermediate (**2**), or alternatively one of the two pyran rings may form first to give the other intermediate, which can then undergo a second hetero Diels−Alder reaction. In terms of the structural diversity, the synthetic sequence is very attractive because in one pot we obtain the corresponding 1,2- and 1,4-benzoquinone adducts, allowing a direct comparison between the two in the SAR (structure−activity relationship) study.

This paper describes the first examples of a double intramolecular domino Knoevenagel hetero Diels−Alder reaction. We selected three aliphatic and four aromatic aldehydes for our purposes. Table 1 summarizes the results obtained in the formation of the corresponding bis-pyranobenzoquinones. The course of the reaction seemed quite substrate-dependent. The best yields were achieved using 2.5 equiv of aldehydes, a catalytic amount of EDDA (0.8 mmol %), and EtOH (0.018 mmol/mL) as solvent under reflux conditions.

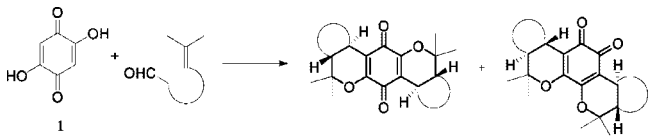
When the reaction was carried out using 3,3-dimethylacrolein (entry 1), the process was found to be regioselective, since only compound **3** was formed in high yield. With the longer chain aliphathic aldehydes, 2,6-dimethylhept-5-enal (entry 2) and (*s*)-(−)-citronellal (entry 3), both types of adducts were obtained

Table 1. Results Obtained in the Double Intramolecular DKHDA of **1** and Several Unsaturated Aldehydes

Entry	Dienophile	Adducts	Ratio of diastereomers	Yield (%)
1		 3	-----	70
2	 (±)	 4 +  5	1.0:1.0	81
3	 (-)	 6 +  7	1.6:1.0	79
4		 8 Ha, Hb 9 Ha, Hb  10 Ha, Hb 11 Ha, Hb	1.0:4.0:5.0:5.0	60
5		 10 12 Ha, Hb 13 Ha, Hb  12 14 Ha, Hb 15 Ha, Hb	5.0:1.0:2.0:1.8	80
6		 16 Ha, Hb 17 Ha, Hb  18 Ha, Hb 19 Ha, Hb	8.0:5.0:6.0:1.0	80
7		 20  21 Ha, Hb 22 Ha, Hb	3.6:1.4:1.0	57

(**4**, **5** and **6**, **7**), respectively, but only as their trans adducts in a ratio of 1:1 and 1.6:1. It would appear that the stereogenic center α or *β* to the carbonyl in these aldehydes was able to produce high asymmetric induction. The high diastereoselectivity found agrees with the results obtained by Tietze et al. in the preparation of several trans-annulated products using aliphatic aldehydes with a stereogenic center (Scheme 2).[28] In the formation of trans-annulated products (**4**−**7**) only the exo-*E*-anti transition state can be operative, since the endo-*Z*-anti form is not possible for geometrical reasons.[29] The structures

Scheme 2. Bis-pyranobenzoquinones from **1** and Unsaturated Aliphatic Aldehydes



of the new compounds were verified by NMR spectroscopic and X-ray analysis. Figure 1 shows the X-ray molecular structures of **4**, **6**, and **7**.

The desired aromatic aldehydes were prepared by the O-alkylation of the corresponding phenol derivatives with dimethylallyl bromide. The reaction of these aldehydes with **1** yielded cis-annulated cycloadducts (Scheme 3). In this case there are two possible orientations in the transition state (endo-*E*-syn and exo-*Z*-syn),[28] leading to a mixture of four cis-annulated cycloadducts. We obtained different ratios depending of the nature of the aromatic aldehyde employed. Thus, the reaction of 4-methoxy-2-(3-methylbut-2-enyloxy)benzaldehyde (entry 4) with **1** gave a mixture of two bis-pyrano-1,2-benzoquinones (**8** and **9**) and two bis-pyrano-1,4-benzoquinones (**10** and **11**) in 1:4:5:5 ratio, respectively. The adducts are formed from the different possibilities of the transition states in the cycloaddition process.[30] Identical orientations for the two heterodiene systems with respect to the side chain lead to cis fused rings in the same face of the molecule (adducts **9** and **10**), while different orientations produce cis fused rings in opposite faces (adducts **8** and **11**). These adducts present high symmetry. Adducts **9** and **10** are chirals (±) and present a C_2 symmetry, while adducts **8** and **11** are achirals because of the existence of a center of symmetry. With 5-bromo-2-(3-methylbut-2-enyloxy)benzaldehyde (entry 5), we also obtain two bis-pyrano-1,2-benzoquinones (**12**, **13**) and two bis-pyrano-1,4-benzoquinones (**14**, **15**) in a 5:1:2:1.8 ratio, respectively. The introduction of one additional bromine group in the aromatic ring of the aldehyde (entry 6) gave similar adducts (**16**−**19**) in 8:5:6:1 ratio. In the reaction of **1** with 2-(3-methylbut-2-enyloxy)naphthalene-1-carbaldehyde (entry 7) only one of the two possible orthoquinones was formed. For compounds **20** and **21** we obtained suitable crystals for X-ray diffraction, and the corresponding X-ray crystal structures are illustrated in Figure 2. X-ray molecular structures of compounds **20** and **21** highlight the significant differences in 3D structure that these molecules present.

The synthetic approach is highly efficient allowing the construction of complex polycyclic scaffolds with six new σ-bonds (four C−C σ-bonds and two O−C σ-bonds) and the formation of four rings in a one-pot reaction.

Biological Assays and SAR. For mammalian cells, the initial screening for anti-MDR activities of 19 different bis-pyranobenzoquinones (compound **3** was not assayed because of its insolubility in DMSO) was assessed by measuring the intracellular accumulation of the fluorescent substrates daunorubicin, calcein-AM, and mitoxantrone in cells expressing Pgp (MDR1), MRP1, and BCRP, respectively.[20,21,31] Accumulation assays using 10 μM daunorubicin as the fluorescent substrate showed that compounds **7**−**11** and **19** at 10 μM were the most potent modulators of Pgp in NIH-3T3 MDR1-G185 cells (MDR1 cells), compared with 10 μM verapamil as the control modulator (Figure 3), with K_i values ranging from 0.42 μM for compound **9** to 1.04 μM for compound **8** (Table 2). Compounds **8**−**11** were able to block up 100% of the Pgp drug transport activity, inducing a 5- to 6-fold increase in daunorubicin accumulation compared with the MDR1 cells without modulators. Neverthe-

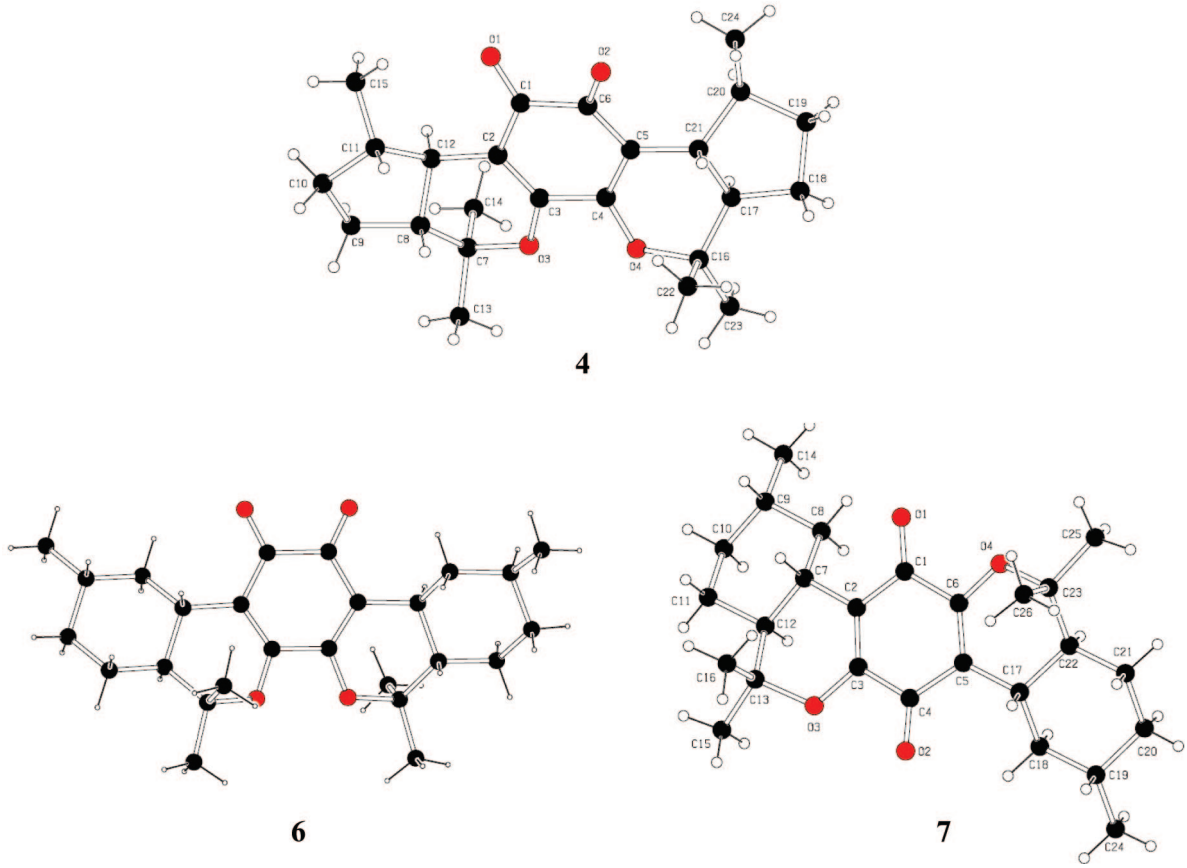
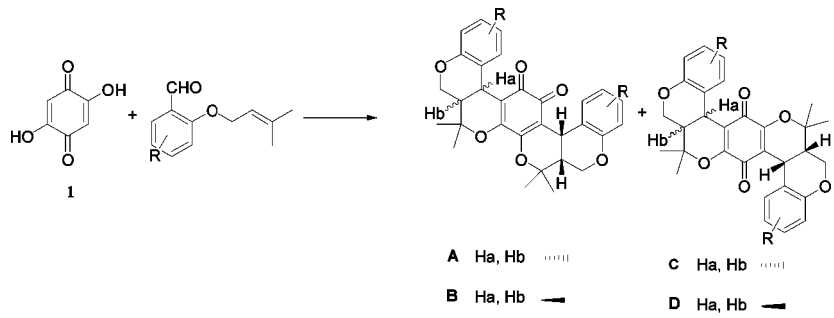


Figure 1. X-ray molecular structures of compounds **4**, **6**, and **7**.

Scheme 3. Bis-pyranobenzoquinones from **1** and Unsaturated Aromatic Aldehydes



less compounds **7** and **19** were unable to inhibit completely P-glycoprotein; compound **7** inhibited transport activity with values around 72−80%. Meanwhile compound **19** showed an 88−100% inhibition, depending on the employed methods (Table 2 and Figure 3). None of the tested compounds were active inhibitors of the drug transport mediated by MRP1 and BCRP in the same concentration range, as determined by the fluorescent-based accumulation assays using calcein-AM and mitoxantrone as respective substrates (data not shown). These results suggest that these compounds are specific inhibitors of the MDR phenotype mediated by Pgp.

The compounds that specifically inhibited each ABC protein were selected and their intrinsic cytotoxicities were measured using the MTT-based[22] assay in order to discard the most toxic compounds at a fixed concentration of 10 μM. The intrinsic toxicities of the above compounds (Table 3) indicated that

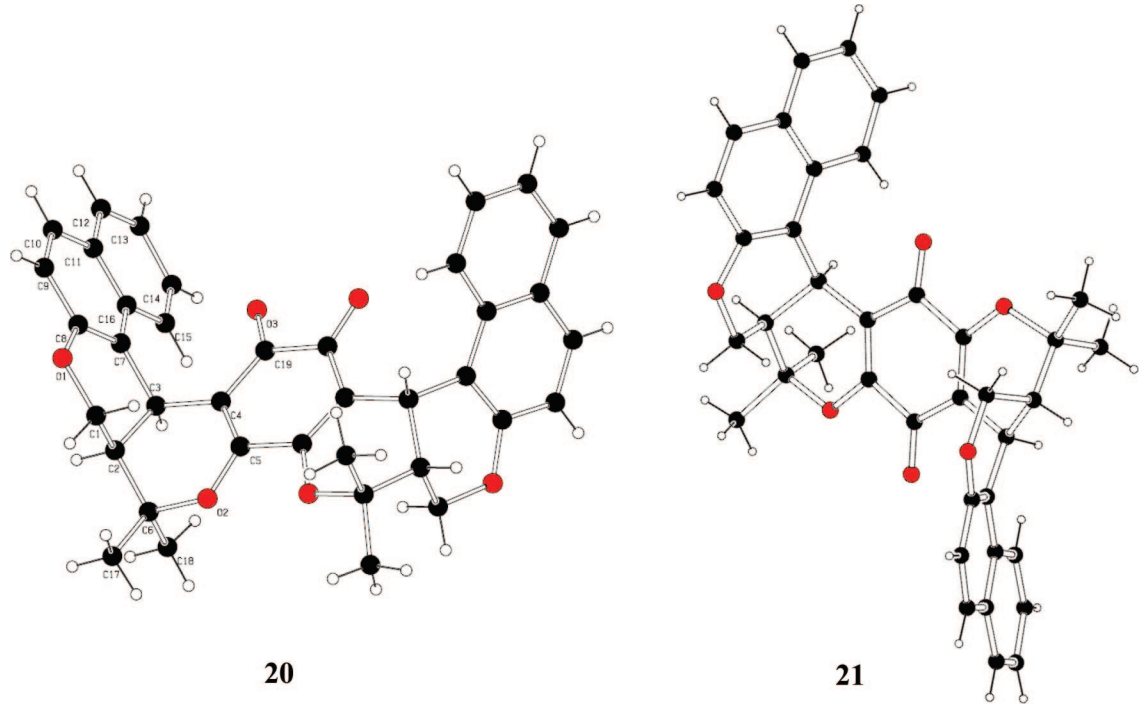


Figure 2. X-ray molecular structures of compounds **20** and **21**.

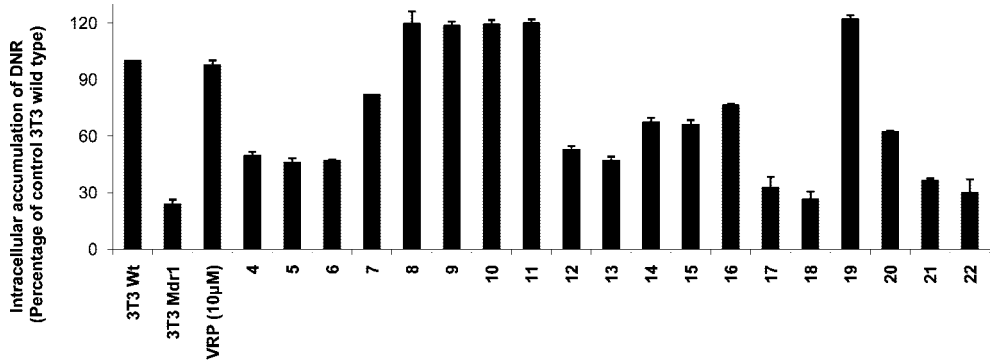


Figure 3. Modulation of daunorubicin accumulation by 10 μM bis-pyranobenzoquinone derivatives in NIH-3T3 MDR1 cells. Results are expressed as percentage of daunorubicin uptake in NIH-3T3 MDR1 cells in the presence or absence of compounds with respect to the accumulation in NIH-3T3 Wt cells (100%). 10 μM verapamil was used for comparison. The data are the average of three independent experiments ± SD.

Table 2. Inhibition of Pgp-Mediated Daunorubicin Transport by the Selected Bis-pyranobenzoquinones in NIH-3T3 MDR1 Cells[a]

compd	K_i (μM)	B_{max}
7	0.77 ± 0.50	72.0 ± 10.2
8	1.04 ± 0.36	112.5 ± 8.9
9	0.42 ± 0.13	105.8 ± 6.9
10	0.51 ± 0.19	104.3 ± 8.2
19	1.01 ± 0.75	88.9 ± 14.9

[a] The K_i was defined as the concentration of bis-pyranobenzoquinones that produced 50% inhibition of Pgp, and B_{max} was defined as the maximal inhibition. K_i and B_{max} values were determined using the equation described in Materials and Methods. Results are expressed as the mean ± SD ($P \leq 0.0001$) of three independent experiments performed in triplicate.

Table 3. Intrinsic Cytotoxicity of 10 μM Selected Bis-pyranobenzoquinones on NIH-3T3 Wild-Type (Wt) and NIH-3T3 MDR1 Cells[a]

	% inhibition of cell growth	
compd	NIH-3T3 MDR1	NIH-3T3 Wt
7	18.1 ± 3.2	0.0 ± 0.1
8	10.8 ± 1.4	24.7 ± 0.0
9	3.5 ± 1.5	9.7 ± 0.1
10	0.0 ± 7.0	6.5 ± 1.1
11	54.1 ± 3.2	53.3 ± 0.7
19	9.6 ± 1.1	12.9 ± 2.5
zosuquidar	3.1 ± 0.6	2.4 ± 4.9
verapamil	11.2 ± 1.1	19.6 ± 2.8

[a] Verapamil and zosuquidar were used at 10 and 3 μM, respectively. Results are expressed as the mean ± SD of three independent experiments performed in triplicate.

Table 4. Daunorubicin Resistance Reversal Ability of Selected Bis-pyranobenzoquinones in NIH-3T3 MDR1 Cells[a]

	10 μM[b]		3 μM[b]			1 μM[b]
compd	IC_{50} (μM)	RI[c]	IC_{50} (μM)	RI[c]	IC_{50} (μM)	RI[c]
7	0.16 ± 0.01	3.6	0.46 ± 0.15	10.4	0.87 ± 0.20	19.7
8	0.11 ± 0.08	2.5	0.12 ± 0.03	2.7	0.25 ± 0.06	5.7
9	0.09 ± 0.03	2.0	0.11 ± 0.00	2.4	0.17 ± 0.08	3.9
10	0.07 ± 0.01	1.6	0.11 ± 0.05	2.4	0.20 ± 0.11	4.5
19	0.10 ± 0.04	2.3	0.16 ± 0.16	3.5	0.35 ± 0.04	8.0
zosuquidar			0.04 ± 0.01	0.9	0.10 ± 0.04	2.3
verapamil	0.11 ± 0.01	2.5				

[a] IC_{50} and RI values for daunorubicin in MDR1 cells without compounds were 1.01 ± 0.31 μM and 23.0, respectively. The data shown are the average of three independent experiments ± SD. [b] Concentration of bis-pyranobenzoquinones. [c] RI: resistance index, the ratio of the IC_{50} of the MDR line to the IC_{50} of the Wt line (0.044 ± 0.030 μM).

compound **11** caused a cell growth inhibition of 50−60% in both drug-sensitive and MDR1 cells. Consequently, this compound was discarded as unsuitable for modulation of mammalian Pgp. Compounds **9**, **10**, and **19** were less toxic for the NIH-3T3 cells than the classical Pgp modulator verapamil at 10 μM; specifically, compound **10** showed a cytotoxic value of only 6.5% at 10 μM. The selected compounds present similar toxicity in comparison with the third-generation inhibitor zosuquidar (3 μM) (Table 3). Subsequently, we investigated their MDR-reversing potencies at 1, 3, or 10 μM in the presence of increasing concentrations of cytotoxic MDR substrates. We determined the IC_{50} (concentration of a drug that diminishes the cellular growth by 50%) and the RI (resistance index). The RI is the ratio of the IC_{50} of the MDR line (with and without modulators) to the IC_{50} of the wild-type (Wt) line. The RI parameter allows a quantitative comparison between the efficiencies of different compounds as MDR modulators.

We determined the IC_{50} values for daunorubicin in the presence of these compounds using the MTT-based assay in Wt and Pgp expressing NIH-3T3 cells (Table 4). Compounds **9** and **10** at 10 μM were the most active inhibitors of Pgp activity, being able to shift the IC_{50} value of MDR1 cells from 1.01 μM to 0.09 and 0.07 μM, respectively. The MDR1 cells were 2.5-fold more resistant than the wild-type cells in the presence of 10 μM verapamil, but when compound **10** was used at the same concentration, the MDR1 cells were 1.6-fold more resistant. Meanwhile for compound **9** the resistant index was 2.0. For comparison, the MDR1 cells without modulators were 23-fold more resistant to daunorubicin than the Wt cells. Compounds **8** and **19** at 10 μM reversed resistance to daunorubicin with efficiencies comparable to that of 10 μM verapamil (Table 4), but compound **19** showed lower cytotoxicity (Table 3). Additionally, compound **7** has a lower reversal activity than verapamil but with the advantage that this compound does not present any intrinsic cytotoxicity in Wt cells (Table 3). The selected compounds at 3 μM showed a lower reversal activity than 3 μM zosuquidar, which inhibited completely Pgp; only compounds **8**−**10** at 3 μM have a comparable reversal activity (Table 4). Additionally, the assays at 1 μM showed that compounds **9** and **10** have similar potency compared to zosuquidar. Consequently, the bis-pyranobenzoquinones represent hit compounds for future design of powerful and specific reversal agents.

After examination of the obtained results some conclusion about structure−activity relationships were made. Among the best modulators of Pgp in MDR1 cells, four out of six are bis-pyrano-1,4-benzoquinones. In the series of the trans adducts (**4**−**7**) synthesized from aliphatic unsaturated aldehydes, only compound **7** showed relevant inhibitory activity. In this sense, the size of the adducts plays an important role for the anti-MDR1 activity. If we compared the structure of compound **5** with that of **7**, attending to the size of the ring C, the five-member ring works worse than the six-member ring.

In the series of the cis adducts (**8**−**22**) obtained from aromatic unsaturated aldehydes, the position and stereoelectronic properties of the substituents on the aromatic ring are important for the activity. The best results were achieved with the adducts having a methoxy group (entry 4).

Compounds **8**, **9** and **10**, **11** show similar structural characteristics. These structures show cis-fused dihydropyran rings (B/C), but in compounds **9** and **10** all hydrogens implied in the joint of these heterocycles are located in the same face, while in the case of compounds **8** and **11** they are located in different face. Consequently, compounds **8**, **11** and **9**, **10** adopt different spatial conformations[32] (see Figure 4) that are responsible for the cytotoxic profile of these compounds. In fact, compound **8** has significantly more toxicity than **9** and compound **11** exhibits a high intrinsic toxicity compared with compound **10** (see Table 3).

The adducts **12**−**15**, which have a bromine group in the aromatic ring, showed lower reversal activity than adducts **8**−**11** and verapamil. The introduction of a second bromine group in the aromatic ring leads to three adducts (**16**−**18**) also less active than verapamil, and only compound **19** whose heterocycles are localized in the same face (with the same spatial arrangement that **10**) was successful at inhibiting the mammaliam Pgp with potency similar to that of verapamil but lower than that of zosuquidar (see Table 4). The adducts **20**−**22** with two fused aromatic rings also showed lower reversal activity than verapamil (see Figure 3; some data not shown).

Thus, according to the mentioned results, compounds **9** and **10** represent the most promising hits of this work that would need further derivatization and screening for possible optimization into leads to be tested in future in vivo assays with reversal activities comparable to that of 1 μM zosuquidar (Table 4).

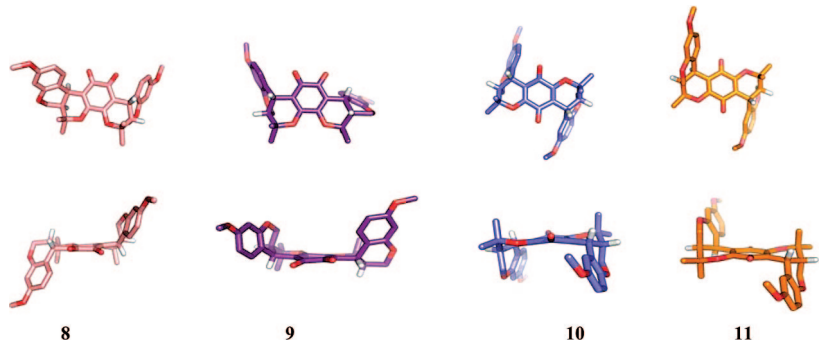


Figure 4. Minimum energy conformations for compounds **8**−**11**.

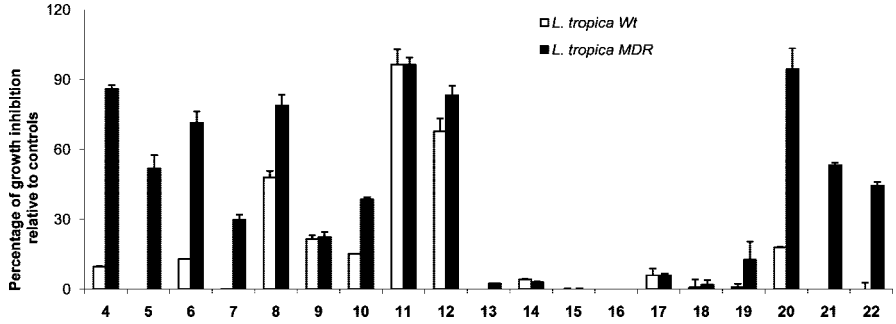


Figure 5. *L. tropica* Wt and MDR lines were exposed to 10 μM of selected bis-pyranobenzoquinones, in the absence (Wt line) or presence of 150 μM daunorubicin (MDR line) for 72 h. The results are expressed as percentage of growth inhibition relative to the control of each cell line without bis-pyranobenzoquinones. The data are the average of three independent experiments ± SD.

Considering **9** and **10** as hit compounds, several chemical modifications will be undertaken to obtain a set of representative compounds and to realize 3D-QSAR studies. We think that compound **19** is an interesting molecule for the future rational designs of more active and less toxic reversal Pgp modulators because it showed high resistance reversal activity with low cytotoxicity in both Wt and Pgp-expressing cells.

Parallel studies have been developed on the drug resistance reversal effect at 10 μM 19 bis-pyranobenzoquinones in a MDR *Leishmania tropica* line maintained in the presence of daunorubicin. Drug resistance in the MDR *L. tropica* line is related to a decreased intracellular drug accumulation, mainly due to the overexpression of a Pgp-like transporter.[21] The results, determined by MTT-based assay, indicated that compounds **4**, **6**, **8**, **11**, **12**, and **20** caused more than 75% cell growth inhibition of MDR parasites, specifically compounds **11** and **20**, with values around 100%. Nevertheless, **11** and **12** were shown to be significantly toxic by itself in the Wt line, producing around 75−95% growth inhibition of the Wt cells, while compounds **4**, **6**, and **20** showed low intrinsic cytotoxic effects in this Wt line (Figure 5). Interestingly the most effective molecules at reversing Pgp in *L. tropica* cell line were bis-pyrano-1,2-benzoquinones instead of bis-pyrano-1,4-benzoquinones. In regard to the adducts **4**−**7** synthesized from aliphatic unsaturated aldehydes (entries 2 and 3, Table 1), the 1,2-benzoquinones (**4** and **6**) show better capacity to inhibit the *Leishmania* Pgp protein. No compounds of entries 4, 5, and 6 (Table 1) showed ability to inhibit the growth of *Leishmania tropica* MDR line; only **8**, **11**, and **12** are interesting structures in accordance with their cytotoxicity properties per se. In the entry 7, the bis-pyrano-1,2-benzoquinone **20** exhibits significantly more capacity to inhibit the Pgp protein with respect to the bis-pyrano1,4-benzoquinones **21** and **22**.

In summary, we have reported an efficient synthesis of complex bis-pyranobenzoquinones based on a novel double intramolecular domino Knoevenagel hetero Diels−Alder reaction strategy. These bis-pyranobenzoquinones constitute a new family of compounds with potential to reverse MDR in human cancer cell lines and, therefore, to be used as hits for future derivatives to be screened for possible optimization into leads for future in vivo assays. They may also be reversal agents of MDR phentoype in the protozoan parasite *Leishmania*. For mammalian cells the bis-pyrano-1,4-benzoquinones with the heterocyclic fused rings in the same face are the best modulators. For the MDR *L. tropica* line the bis-pyrano-1,2-benzoquinones exhibited the higher cytotoxicities. Consequently, bis-pyranobenzoquinones behave quite differently depending on the type of P-glycoprotein considered. Our results represent a starting point for the preparation of new hits as more powerful and selective reversal agents of MDR.

Materials and Methods

Chemistry. General. All solvents and reagents were purified by standard techniques (as 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_{254} 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_{254} (Machery-Nagel) were used for preparative TLC purification. ^{1}H NMR spectra were recorded in $CDCl_3$ or C_6D_6 at 300 or 400 MHz, using a Bruker AMX300 or Bruker AMX400 instruments. For ^{1}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 ^{1}H−^{1}H COSY and ROESY where necessary. Data are reported in the following manner: chemical shift (multiplicity, coupling constant if appropriate, integration). Coupling constants (*J*) are given in hertz (Hz) to the nearest 0.5 Hz. ^{13}C NMR spectra were recorded at 75 and 100

MHz using a Bruker AMX300 or Bruker AMX400 instrument. Carbon spectra assignments are supported by DEPT-135 spectra and $^{13}C-^{1}H$ (HMQC) and $^{13}C-^{1}H$ (HMBC) correlations where necessary. Chemical shifts are quoted in ppm and are referenced to the appropriate residual solvent peak. MS and HRMS were recorded on a VG Micromass ZAB-2F. All compounds were named using ACD40 Name-Pro program, which is based on IUPAC rules. Melting points were recorded using a Buchi B540 capillary apparatus and are uncorrected. IR spectra were taken on a Bruker IFS28/55 spectrophotometer. The synthesis of 2-prenyloxybenzaldehydes used in entries 4−7 was based on the procedures described in ref 33.

General Procedure for the Synthesis of Bis-pyranobenzoquinones through Double IKHDA. Compound **1** (50 mg, 0.357 mmol) dissolved in anhydrous EtOH (5 mL) was treated with 6.0 equiv of the corresponding aldehyde and catalytic amounts of EDDA (ethylenediammonium diacetate) (4 mg, 0.022 mmol). The mixture was heated at reflux until all the starting quinone was consumed (generally <30 min). Then the solvent was removed under reduced pressure and the residue was purified by preparative TLC.

2,2,9,9-Tetramethyl-2,9-dihydropyrano[3,2-*h*]chromene-5,6-dione (3). Following the general procedure described above, **1** (30 mg, 0.21 mmol) was treated with of 3,3-dimethylacrolein (0.126 mL, 1.28 mmol, 6.0 equiv) and EDDA (2.4 mg, 0.013 mmol) in EtOH (5 mL). The mixture was heated at reflux for 18 min. The solvent was removed under reduced pressure and the residue was purified by preparative TLC (Hex/EtOAc, 4:1) to yield 40.6 mg (70%) of compound **3** as an amorphous orange solid. R_f (Hex/EtOAc, 4:1) = 0.36. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.50 (s, 12H), 5.54 (d, J = 10 Hz, 2H), 6.45 (d, J = 10 Hz, 2H) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz) δ 28.3 × 2 (q), 80.8 (s), 113.9 (s), 114.7 (d), 128.6 (d), 150.7 (s), 178.6 (s) ppm. EIMS m/z (%) 272 (M^+, 100), 257 ($M^+ - CH_3$, 50), 229 ($M^+ - CH_3 - CO$, 54), 201 (11). HR-EI-MS m/z 272.1043 [(M^+) calcd for $C_{16}H_{16}O_4$ 272.1049]. IR ($CHCl_3$) ν_{max} (cm^{-1}) 2974, 2361, 1634, 1577, 1466, 1343, 1303, 1187, 1136, 974, 873, 769, 740, 643.

Reaction of 1 with 2,6-Dimethylhept-5-enal To Obtain Adducts 4 and 5. Following the general procedure described above, **1** (50 mg, 0.357 mmol) was treated with 2,6-dimethylhept-5-enal (0.341 mL, 2.141 mmol) and EDDA (3.7 mg, 0.019 mmol) in EtOH (7 mL). The mixture was heated at reflux for 19 min. The solvent was removed under reduced pressure and the residue was chromatographed by preparative-TLC (Hex/EtOAc, 4:1) to yield compound **4** (53.5 mg, 39%) as a crystalline orange solid and compound **5** (57.5 mg, 41.9%) as an crystalline yellow solid.

Bis-pyrano-1,2-benzoquinone 4. Crystalline orange solid. R_f (Hex/EtOAc, 4:1) = 0.57. Mp 206 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.15 (s, 6H), 1.19 (m, 2H), 1.38 (d, J = 4.7 Hz, 6H), 1.43 (s, 6H), 1.57 (m, 2H), 1.68 (m, 2H), 2.00 (m, 6H) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz) δ 20.3 (q), 23.1 (q), 24.5 (t), 27.9 (q), 34.0 (t), 35.9 (d), 42.6 (d), 52.2 (d), 83.6 (s), 115.6 (s), 158.3 (s), 178.9 (s) ppm. EI-MS m/z (%) 384 (M^+, 100), 369 ($M^+ - CH_3$, 67), 356 ($M^+ - CO$, 29), 338 ($M^+ - 2 \times CH_3$, 8), 247 (12). HR-EI-MS 384.2300 [(M^+) calcd for $C_{24}H_{32}O_4$ 384.2301]. IR ($CHCl_3$) (cm^{-1}) ν_{max} 2949, 2870, 2360, 2090, 1657, 1569, 1463, 1355, 1319, 1282, 1240, 1168, 1122, 1010, 966, 900, 857, 755, 501.

Bis-pyrano-1,4-benzoquinone 5. Crystalline yellow solid. R_f (Hex/EtOAc, 4:1) = 0.65. Mp 219 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.16 (s, 6H), 1.19 (m, 2H), 1.38 (d, J = 4.7 Hz, 6H), 1.43 (s, 6H), 1.43 (m, 2H), 1.56 (m, 2H), 1.67 (m, 2H), 1.90 (m, 6H) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz) δ 20.1 (q), 23.2 (q), 24.6 (t), 27.9 (q), 34.2 (t), 35.3 (d), 42.8 (d), 52.7 (d), 82.6 (s), 117.8 (s), 158.2 (s), 180.9 (s) ppm. EI-MS m/z (%) 384 (M^+, 100), 369 ($M^+ - CH_3$, 71), 356 ($M^+ - CO$, 47), 341 (356 − CH_3, 22). HR-EI-MS m/z 384.2282 [(M^+) calcd for $C_{24}H_{32}O_4$ 384.2301]. IR ($CHCl_3$) (cm^{-1}) ν_{max} 2951, 2871, 2360, 1725, 1654, 1574, 1465, 1383, 1329, 1284, 1239, 1169, 1129, 1006, 922, 755, 668, 473.

Reaction of 1 with (*s*)-(−)-Citronellal To Obtain Adducts 6 and 7. Following the general procedure described above, **1** (40 mg, 0.285 mmol) was treated with (*s*)-(−)-citronellal (0.310 mL, 1.71 mmol) and EDDA (2.85 mg, 0.015 mmol) in EtOH (7 mL). The mixture was heated at reflux for 22 min. The solvent was removed under reduced pressure and the residue was chromatographed by preparative TLC (Hex/EtOAc, 4:1) to yield compound **6** (57.0 mg, 48.5%) as a crystalline orange solid and compound **7** (35.6 mg, 30.3%) as a crystalline yellow solid.

Bis-pyrano-1,2-benzoquinone 6. Crystalline orange solid. R_f (Hex/EtOAc, 4:1) = 0.46. $[\alpha]_D^{25}$ −33 (*c* 1.0, $CHCl_3$). Mp 195 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.52 (dd, J = 11.7, 12.4 Hz, 2H), 0.85 (d, J = 6.51 Hz, 6H), 1.02 (m, 2H), 1.10 (s, 6H), 1.39 (m, 2H), 1.46 (s, 6H), 1.56 (m, 2H), 1.77 (m, 6H), 2.24 (td, J = 11.0, 3.0 Hz, 2H), 2.74 (d, J = 10.9 Hz, 2H) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz) δ 19.6 (q), 22.0 (q), 26.7 (q), 27.0 (t), 31.2 (d), 33.2 (d), 35.0 (t), 37.5 (t), 47.2 (d), 82.1 (s), 115.3 (s), 157.6 (s), 179.8 (s) ppm. EI-MS m/z (%) 414 (M^+ + 2, 29.5), 412 (M^+, 4.7), 384 ($M^+ - CO$, 100). HR-EI-MS m/z 412.2536 [(M^+) calcd for $C_{26}H_{36}O_4$ 412.2614]. IR ($CHCl_3$) (cm^{-1}) ν_{max} 2982, 2866, 2102, 1648, 1586, 1454, 1389, 1316, 1278, 1208, 1138, 1107, 1014, 989, 932, 791, 752, 664, 557, 531, 482.

Bis-pyrano-1,4-benzoquinone 7. Crystalline yellow solid. R_f (Hex/EtOAc, 4:1) = 0.58. $[\alpha]_D^{25}$ +310 (*c* 0.1, $CHCl_3$). Mp 229 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.63 (dd, J = 11.6, 12.5 Hz, 2H), 0.90 (d, J = 6.5 Hz, 6H), 1.00 (m, 2H), 1.09 (s, 6H), 1.27 (m, 2H), 1.46 (s, 6H), 1.56 (m, 2H), 1.77 (m, 6H), 2.24 (td, J = 11.0, 3.0 Hz, 2H), 2.74 (d, J = 10.9 Hz, 2H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 19.2 (q), 22.0 (q), 26.8 (q), 27.2 (t), 32.3 (d), 33.4 (d), 35.1 (t), 38.3 (t), 47.9 (d), 81.4 (s), 117.6 (s), 152.2 (s), 181.0 (s) ppm. EI-MS m/z (%) 412 (M^+, 100), 397 ($M^+ - CH_3$, 20), 369 ($M^+ - CH_3 - CO$, 9). HR-EI-MS 412.2579 [(M^+) calcd for $C_{26}H_{36}O_4$ 412.2614]. IR ($CHCl_3$) (cm^{-1}) ν_{max} 2922, 2867, 1656, 1591, 1455, 1374, 1354, 1331, 1307, 1273, 1211, 1142, 1121, 1017, 996, 961, 936, 868, 754, 665.

Reaction of 1 with 4-Methoxy-2-(3-methylbut-2-enyloxy)benzaldehyde To Obtain Adducts 8−11. Following the general procedure described above, 30.0 mg (0.21 mmol) of **1** was treated with 282.4 mg (1.28 mmol) of 4-methoxy-2-(3-methylbut-2-enyloxy)benzaldehyde and 2.1 mg of EDDA (0.01 mmol) in EtOH (5 mL). The mixture was heated at reflux for 20 min. The solvent was removed under reduced pressure, and the residue was chromatographed by preparative TLC (Hex/EtOAc, 7:3) to yield 4.96 mg (4.3%) of **8**, 17.45 mg (15.3%) of **9**, 21.93 mg (19.2%) of **10**, and 24.65 mg (21.6%) of **11**.

Bis-pyrano-1,2-benzoquinone 8. Amorphous orange solid. R_f (Hex/EtOAc, 1:1) = 0.05. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.22 (s, 6H), 1.56 (s, 6H), 2.10 (dd, J = 9.4, 4.0 Hz, 2H), 3.72 (s, 6H), 4.27 (d, J = 6.0 Hz, 2H), 4.29 (m, 2H), 4.39 (dd, J = 11.9, 3.5 Hz, 2H), 6.25 (d, J = 2.3 Hz, 2H), 6.40 (dd, J = 8.4, 2.3 Hz, 2H), 7.14 (d, J = 8.6 Hz, 2H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.5 (q), 27.4 (d), 27.9 (q), 37.8 (d), 55.0 (q), 64.3 (t), 81.7 (s), 100.8 (d), 107.1 (d), 113.2 (s), 114.6 (s), 130.5 (d), 155.4 (s), 157.3 (s), 159.1 (s), 181.1 (s) ppm. EI-MS m/z (%) 544 (M^+,46), 516 ($M^+ - CO$, 100). HR-EI-MS 544.2116 [(M^+) calcdfor $C_{32}H_{32}O_8$ 544.2097]. IR ($CHCl_3$) ν_{max} (cm^{-1}) 2923, 1649, 1617, 1617, 1591, 1503, 1441, 1396, 1341, 1270, 1165, 1127, 1090, 1037, 1010, 849, 835, 757.

Bis-pyrano-1,2-benzoquinone 9. Amorphous orange solid. R_f (Hex/EtOAc, 1:1) = 0.2. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.28 (s, 6H), 1.55 (s, 6H), 2.10 (dd, J = 9.4, 4.0 Hz, 2H), 3.72 (s, 6H), 4.22 (d, J = 6.0 Hz, 2H), 4.27 (m, 2H), 4.39 (dd, J = 11.9, 3.5 Hz, 2H), 6.25 (d, J = 2.5 Hz, 2H), 6.42 (dd, J = 8.7, 2.6 Hz, 2H), 7.27 (d, J = 8.3 Hz, 2H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.8 (q), 27.1 (d), 28.1 (q), 37.8 (d), 55.0 (q), 64.7 (t), 81.6 (s), 100.7 (d), 107.0 (d), 113.0 (s), 114.0 (s), 131.2 (d), 154.2 (s), 157.0 (s), 159.2 (s), 178.3 (s) ppm. EI-MS m/z (%) 546 (M^+ + 2, 100), 544 (M^+, 23), 418 (M^+ − 126, 2). HR-EI-MS: 544.2108 [(M^+) calcd for $C_{32}H_{32}O_8$ 544.2097]. IR ($CHCl_3$) ν_{max} (cm^{-1}) 2932, 2358,1618, 1586, 1503, 1442, 1395, 1341, 1269, 1197, 1163, 1126, 1036, 1010, 946, 836, 755, 486.

Bis-pyrano-1,4-benzoquinone 10. Amorphous yellow solid. R_f (Hex/EtOAc, 1:1) = 0.67. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.53 (s, 6H), 1.38 (s, 6H), 2.10 (dd, J = 9.5, 5.8 Hz, 2H), 3.73 (s, 6H), 4.18 (d, J = 5.9 Hz, 2H), 4.23 (m, 2H), 4.42 (dd, J = 9.3, 5.3 Hz, 2H), 6.28 (d, J = 2.5 Hz, 2H), 6.44 (dd, J = 8.7, 2.6 Hz, 2H), 7.32

(d, $J = 8.7$ Hz, 2H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 24.2 (q), 26.6 (q), 28.6 (d), 38.0 (d), 55.5 (q), 63.8 (t), 80.4 (s), 100.6 (d), 107.1 (d), 112.2 (s), 116.2 (s), 132.2 (d), 151.9 (s), 154.5 (s), 159.4 (s), 181.2 (s) ppm. EI-MS *m/z* (%) 546 ($M^+ + 2$, 57), 544 (M^+, 100), 443 ($M^+ - C_5H_9O$, 3), 418 ($M^+ - 126$, 44). HR-EI-MS 544.2107 [(M^+) calcd for $C_{32}H_{32}O_8$ 544.2097]. IR ($CHCl_3$) (cm^{-1}) ν_{max} 2932, 1719, 1657, 1619, 1591, 1504, 1443, 1393, 1341, 1269, 1164, 1126, 1010, 946, 835, 755.

Bis-pyrano-1,4-benzoquinone 11. Amorphous yellow solid. R_f (Hex/EtOAc, 1:1) = 0.5. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.29 (s, 6H), 1.57 (s, 6H), 2.12 (dd, $J = 9.3$, 5.4 Hz, 2H), 3.74 (s, 6H), 4.20 (d, $J = 5.2$ Hz, 2H), 4.23 (m, 2H), 4.42 (dd, $J = 9.3$, 5.3 Hz, 2H), 6.29 (d, $J = 2.6$ Hz, 2H), 6.45 (dd, $J = 8.7$, 2.6 Hz, 2H), 7.29 (d, $J = 9.0$ Hz, 2H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 24.2 (q), 26.6 (q), 28.6 (d), 38.0 (d), 55.5 (q), 63.8 (t), 80.4 (s), 100.6 (d), 107.1 (d), 112.2 (s), 116.2 (s), 132.2 (d), 151.9 (s), 154.5 (s), 159.4 (s), 181.2 (s) ppm. EI-MS *m/z* (%) 544 (M^+, 100), 418 (50), 391 (25), 363 (10), 203 (30). HR-EI-MS 544.2070 [(M^+) calcd for $C_{32}H_{32}O_8$ 544.2097]. IR ($CHCl_3$) (cm^{-1}) ν_{max} 2932, 1717, 1655, 1617, 1591, 1443, 1393, 1341, 1269.

Reaction of 1 with 5-Bromo-2-(3-methylbut-2-enyloxy)benzaldehyde To Obtain Adducts 12−15. Following the general procedure described above, **1** (50 mg, 0.357 mmol) was treated with 5-bromo-2-(3-methylbut-2-enyloxy)benzaldehyde (574.05 mg, 2.14 mmol) and EDDA (4 mg, 0.02 mmol) in EtOH (5 mL). The mixture was heated at reflux for 25 min. The solvent was removed under reduced pressure and the residue was chromatographed by preparative TLC (Hex/EtOAc, 4:1) to yield compound **12** (87.9 mg, 38.4%) as an amorphous orange solid, 31.2 mg of compound **13** (13.6%) as an amorphous orange solid, 33.5 mg of compound **14** (14.6%) as an amorphous yellow solid, and 31.2 mg of compound **15** (13.6%) as an amorphous yellow solid.

Bis-pyrano-1,2-benzoquinone 12. Amorphous orange solid. R_f (Hex/AcOEt, 4:1) = 0.23. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.29 (s, 6H), 1.58 (s, 6H), 2.13 (t, $J = 5.1$ Hz, 2H), 4.29 (m, 4H), 4.40 (dd, $J = 12.1$, 3.6 Hz, 2H), 6.60 (d, $J = 8.6$ Hz, 2H), 7.18 (dd, $J = 8.5$, 1.8 Hz, 2H), 7.49 (s, 2H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.7 (q), 27.1 (q), 28.8 (d), 37.5 (d), 64.1 (t), 81.7 (s), 112.5 (s), 113.3 (s), 117.6 (d), 122.7 (s), 131.0 (d), 132.9 (d), 152.6 (s), 157.8 (s), 177.8 (s) ppm. EI-MS *m/z* (%): 644 ($M^+ + 2$, 100), 642 (M^+, 28), 614 ($M^+ - CO$, 16). HR-EI-MS: 642.0085 [(M^+) calcd for $C_{30}H_{26}O_6Br$ 642.0076]. IR ($CHCl_3$) ν_{max} (cm^{-1}) 2854, 1728, 1647, 1585, 1482, 1407, 1373, 1219, 1178, 1124, 1087, 817, 772, 671, 536, 494, 471.

Bis-pyrano-1,2-benzoquinone 13. Amorphous orange solid. R_f (Hex/EtOAc, 4:1) = 0.60. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.25 (s, 6H), 1.58 (s, 6H), 2.14 (m, 2H), 4.33 (d, $J = 5.7$ Hz, 2H), 4.37 (m, 2H), 4.44 (d, $J = 3.5$ Hz, 2H), 6.60 (d, $J = 8.6$ Hz, 2H), 7.20 (dd, $J = 8.4$, 2.1 Hz, 2H), 7.33 (d, $J = 9.0$ Hz, 2H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.5 (q), 27.5 (q), 28.4 (d), 37.3 (d), 64.3 (t), 82.0 (s), 112.9 (s), 113.6 (s), 117.6 (d), 123.0 (s), 130.9 (d), 132.1 (d), 152.6 (s), 157.6 (s), 179.6 (s) ppm. EI-MS *m/z* (%) 644 ($M^+ + 2$, 25), 642 (M^+, 18), 616 (644 − CO, 50), 614 ($M^+ - CO$, 100). HR-EI-MS 642.0067 [(M^+) calcd for $C_{30}H_{26}O_6Br$ 642.0076]. IR ($CHCl_3$) ν_{max} (cm^{-1}) 2923, 2104, 1644, 1482, 1406, 1239, 1181, 1125, 1014, 794, 751, 551.

Bis-pyrano-1,4-benzoquinone 14. Amorphous yellow solid. R_f (Hex/EtOAc, 4:1) = 0.37. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.38 (s, 6H), 1.57 (s, 6H), 2.11 (dd, $J = 9.4$, 5.5 Hz, 2H), 4.24 (m, 4H), 4.43 (dd, $J = 11.8$, 3.1 Hz, 2H), 6.63 (d, $J = 8.7$ Hz, 2H), 7.20 (d, $J = 8.7$ Hz, 2H), 7.52 (s, 2H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 24.0 (q), 26.7 (q), 29.2 (d), 37.6 (d), 63.9 (t), 80.6 (s), 112.5 (s), 115.3 (s), 117.7 (d), 122.2 (s), 131.2 (d), 133.6 (d), 152.4 (s), 152.8 (s), 180.5 (s) ppm. EI-MS *m/z* (%): 644 ($M^+ + 2$, 20), 642 (M^+, 100), 640 ($M^+ - 2$, 15), 624 ($M^+ - H_2O$, 4). HR-EI-MS 642.0065 [(M^+) calcd for $C_{30}H_{26}O_6Br$ (M^+) 642.0076]. IR ($CHCl_3$) ν_{max} (cm^{-1}) 2984, 2360, 1659, 1589, 1482, 1394, 1336, 1255, 1175, 1123, 1009, 815, 769, 608, 472.

Bis-pyrano-1,4-benzoquinone 15. Amorphous yellow solid. R_f (Hex/EtOAc, 4:1) = 0.72. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.29 (s, 6H), 1.61 (s, 6H), 2.14 (dd, $J = 9.2$, 4.5 Hz, 2H), 4.26 (m, 4H), 4.43 (dd, $J = 12.0$, 3.9 Hz, 2H), 6.62 (d, $J = 7.1$ Hz, 2H), 7.20 (d, $J = 8.7$ Hz, 2H), 7.41 (s, 2H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.7 (q), 27.2 (q), 28.9 (d), 37.6 (d), 64.2 (t), 80.9 (s), 112.6 (s), 115.0 (s), 117.7 (d), 122.7 (s), 131.1 (d), 132.9 (d), 152.8 (s), 153.3 (s), 181.0 (s) ppm. EI-MS *m/z* (%) 644 ($M^+ + 2$, 17), 642 (M^+, 100), 640 ($M^+ - 2$, 16), 624 ($M^+ - H_2O$, 4). HR-EI-MS 642.0072 [(M^+) calcd for $C_{30}H_{26}O_6Br$ 642.0076]. IR ($CHCl_3$) ν_{max} (cm^{-1}) 2924, 2356, 1729, 1655, 1588, 1481, 1395, 1335, 1253, 1231, 1174, 1122, 1009, 811, 757, 630, 552.

Reaction of 1 with 3,5-Dibromo-2-(3-methylbut-2-enyloxy)benzaldehyde To Obtain Adducts 16−19. Following the general procedure described above, **1** (30 mg, 0.21 mmol) was treated with 5-bromo-2-(3-methylbut-2-enyloxy)benzaldehyde (442.9 mg, 1.28 mmol) and EDDA (2.5 mg, 0.02 mmol) in EtOH (5 mL). The mixture was heated at reflux for 26 min. The solvent was removed under reduced pressure and the residue was chromatographed by preparative TLC (Hex/EtOAc, 7:3) to yield 52.8 mg of compound **16** (31.4%) as an amorphous orange solid, 35.4 mg of compound **17** (21.1%) as an amorphous orange solid, 39 mg of compound **18** (23.2%) as an amorphous yellow solid, and 6.6 mg of compound **19** (3.9%) as a yellow solid.

Bis-pyrano-1,2-benzoquinone 16. Amorphous orange solid. R_f (Hex/EtOAc, 7:3) = 0.20. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.27 (s, 6H), 1.58 (s, 6H), 2.16 (m, 2H), 4.28 (d, $J = 5.4$ Hz, 2H), 4.41 (dd, $J = 4.4$ Hz, 2H), 4.50 (dd, $J = 12.0$, 3.6 Hz, 4H), 7.45 (s, 2H), 7.50 (s, 2H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.7 (q), 27.0 (q), 29.1 (d), 37.2 (d), 64.9 (t), 81.7 (s), 110.6 (s), 112.6 (s), 112.8 (s), 123.7 (s), 132.3 (d), 134.0 (d), 149.2 (s), 157.3 (s), 177.6 (s) ppm. EI-MS *m/z* (%) 804 ($M^+ + 4$, 18), 802 ($M^+ + 2$, 29), 800 (M^+, 25), 772 ($M^+ - CO$, 100). HR-EI-MS 800.8196 [(M^+) calcd for $C_{30}H_{24}O_6Br_4$ 800.8167]. IR ($CHCl_3$) ν_{max} (cm^{-1}): 2927, 1726, 1652, 1585, 1471, 1447, 1373, 1271, 1241, 1127, 1019, 983, 926, 863, 813, 756, 668, 611, 581, 490.

Bis-pyrano-1,2-benzoquinone 17. Amorphous orange solid. R_f (Hex/EtOAc, 7:3) = 0.46. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.24 (s, 6H), 1.60 (s, 6H), 2.19 (m, 2H), 4.33 (d, $J = 5.73$ Hz, 2H), 4.33 (d, $J = 5.7$ Hz, 2H), 4.48 (dd, $J = 11.2$, 3.8 Hz, 4H), 7.32 (d, $J = 1.2$ Hz, 2H), 7.50 (d, $J = 1.8$ Hz, 2H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.5 (q), 27.3 (q), 28.9 (d), 37.3 (d), 65.1 (t), 81.8 (s), 110.7 (s), 112.7 (s), 113.3 (d), 124.1 (s), 131.7 (d), 133.9 (d), 149.2 (s), 157.5 (s), 179.3 (s) ppm. EI-MS *m/z* (%): 804 ($M^+ + 4$, 18), 802 ($M^+ + 2$, 29), 800 (M^+, 25), 772 ($M^+ - CO$, 100). HR-EI-MS 800.8198 [(M^+) calcd for $C_{30}H_{24}O_6Br_4$ 800.8167]. IR ($CHCl_3$) ν_{max} (cm^{-1}) 2925, 2855, 1727, 1659, 1591, 1447, 1394, 1335, 1266, 1172, 1126, 1010, 756.

Bis-pyrano-1,4-benzoquinone 18. Amorphous yellow solid. R_f (Hex/EtOAc, 7:3) = 0.31. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.36 (s, 6H), 1.55 (s, 6H), 2.13 (m, 2H), 4.22 (d, $J = 5.34$ Hz, 2H), 4.31 (m, 2H), 4.55 (dd, $J = 11.8$, 3.8 Hz, 2H), 7.51 (s, 4H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 24.0 (q), 26.5 (q), 29.5 (d), 31.3 (d), 64.6 (t), 80.5 (s), 110.8 (s), 112.2 (s), 114.8 (s), 123.2 (s), 133.2 (d), 134.2 (d), 149.4 (s), 152.4 (s), 180.2 (s) ppm. EI-MS *m/z* (%) 804 ($M^+ + 4$, 20), 802 ($M^+ + 2$, 22), 800 (M^+, 18), 772 ($M^+ - CO$, 100). HR-EI-MS 800.8198 [(M^+) calcd for $C_{30}H_{24}O_6Br_4$ 800.8167]. IR ($CHCl_3$) ν_{max} (cm^{-1}): 2926, 2356, 1661, 1588, 1471, 1446, 1394, 1335, 1270, 1173, 1127, 1013, 983, 946, 862, 755, 697, 612.

Bis-pyrano-1,4-benzoquinone 19. Amorphous yellow solid. R_f (Hex/EtOAc, 7:3) = 0.6. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.30 (s, 6H), 1.60 (s, 6H), 2.17 (m, 2H), 4.23 (d, $J = 5.2$ Hz, 2H), 4.37 (dd, $J = 12.0$, 5.3 Hz, 2H), 4.53 (dd, $J = 12.1$, 3.6 Hz, 2H), 7.42 (s, 2H), 7.52 (s, 2H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 24.0 (q), 27.1 (q), 29.6 (d), 37.8 (d), 65.0 (t), 80.8 (s), 110.9 (s), 112.7 (s), 114.7 (s), 124.0 (s), 132.0 (d), 134.5 (d), 149.7 (s), 153.4 (s), 180.8 (s) ppm. EI-MS *m/z* (%): 804 ($M^+ + 4$, 10), 802 ($M^+ + 2$, 18), 800 (M^+, 14), 772 ($M^+ - CO$, 100). HR-EI-MS 800.8193 [(M^+) calcd for $C_{30}H_{24}O_6Br_4$ 800.8167]. IR ($CHCl_3$) ν_{max} (cm^{-1}): 2924, 1727, 1659, 1590, 1471, 1446, 1335, 1266, 1272, 1126, 1012, 865, 813, 755, 667, 527, 475.

Reaction of 1 with 3-(3-Methyl-but-2-enyloxy)naphthalene-2-carbaldehyde To Obtain Adducts 20−22. Following the general procedure described above, **1** (30 mg, 0.21 mmol) was treated with 3-(3-methylbut-2-enyloxy)naphthalene-2-carbaldehyde (308.3 mg (1.28 mmol) and EDDA (2.1 mg, 0.012 mmol) in EtOH (5 mL). The mixture was heated at reflux for 25 min. The solvent was removed under reduced pressure and the residue was purified by TLC preparative (Hex/EtOAc, 7:3) to yield compound **20** (43.9 mg, 35.8%) as a yellow solid, 16.9 mg of compound **21** (13.8%) as a orange crystalline solid, and 12.0 mg of compound **22** (7.1%) as an amorphous orange solid.

Bis-pyrano-1,2-benzoquinone 20. Crystalline orange solid. R_f (Hex/EtOAc, 7:3) = 0.28. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.59 (s, 12H), 2.20 (m, 2H), 4.09 (t, J = 11.3 Hz, 2H), 4.44 (m, 2H), 4.60 (d, J = 3.4 Hz, 2H), 6.91 (d, J = 8.8 Hz, 2H), 7.39 (t, J = 8.3 Hz, 2H), 7.55 (t, J = 8.8 Hz, 2H), 7.56 (d, J = 8.8 Hz, 2H), 7.64 (d, J = 8.2 Hz, 2H), 7.93 (d, J = 8.6 Hz, 2H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ: 25.5 (d), 26.0 (q), 26.8 (q), 37.4 (d), 62.7 (t), 79.3 (s), 112.7 (s), 113.7 (s), 117.7 (d), 122.7 (d), 123.9 (d), 125.3 (d), 127.8 (d), 128.7 (s), 129.1 (d), 134.3 (s), 150.3 (s), 156.1 (s), 177.1 (s) ppm. EIMS m/z (%): 586 (M^+ + 2, 100), 584 (M^+, 20). HR-EI-MS: 584.2198 [calcd for $C_{38}H_{32}O_6$ (M^+) 584.2199]. IR ($CHCl_3$) ν_{max} (cm^{-1}): 2358, 1657, 1624, 1601, 1572, 1513, 1473, 1433, 1370, 1287, 1258, 1135, 1083, 1027, 955, 812, 750, 626, 479.

Bis-pyrano-1,4-benzoquinone 21. Orange crystalline solid. R_f (Hex/EtOAc, 7:3) = 0.57. Mp 206 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.40 (s, 6H), 1.65 (s, 6H), 2.14 (m, 2H), 4.00 (t, J = 11.2 Hz, 2H), 4.29 (m, 2H), 4.67 (d, J = 4.1 Hz, 2H), 7.00 (d, J = 3.7 Hz, 2H), 7.36 (t, J = 7.4 Hz, 2H), 7.53 (t, J = 3.3 Hz, 2H), 7.57 (d, J = 3.6 Hz, 2H), 7.88 (d, J = 3.7 Hz, 2H), 7.65 (d, J = 3.9 Hz, 2H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.3 (d), 25.7 (q), 26.2 (q), 38.2 (d), 62.9 (t), 78.7 (s), 114.4 (s), 114.5 (s), 118.1 (d), 122.8 (d), 123.4 (d), 125.7 (d), 128.0 (d), 128.8 (s), 129.1 (d), 134.0 (s), 151.4 (s), 152.2 (s), 179.0 (s) ppm. EI-MS m/z (%) 584 (M^+, 100), 569 (M^+ − Me, 10). HR-EI-MS 584.2192 [(M^+) calcd for $C_{38}H_{32}O_6$ 584.2199]. IR ($CHCl_3$) ν_{max} (cm^{-1}) 2360, 2084, 1642, 1369, 773, 684, 648, 590, 498, 472.

Bis-pyrano-1,4-benzoquinone 22. Amorphous orange solid. R_f (Hex/EtOAc, 7:3) = 0.1. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.43 (s, 12H), 2.15 (m, 2H), 4.04 (t, J = 11.1 Hz, 2H), 4.35 (m, 2H), 4.65 (d, J = 4.1 Hz, 2H), 7.00 (d, J = 8.9 Hz, 2H), 7.36 (t, J = 7.4 Hz, 2H), 7.55 (t, J = 7.4 Hz, 2H), 7.67 (d, J = 8.8 Hz, 2H), 7.75 (d, J = 7.9 Hz, 2H), 8.04 (d, J = 8.6 Hz, 2H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.3 (d), 25.8 (q), 26.5 (q), 38.2 (d), 63.2 (t), 78.8 (s), 114.2 (s), 114.9 (s), 118.0 (d), 122.9 (d), 123.3 (d), 125.8 (d), 128.9 (d), 128.8 (s), 129.1 (d), 134.0 (s), 151.2 (s), 152.4 (s), 179.0 (s) ppm. EI-MS m/z (%) 584 (M^+, 100), 569 (M^+ − CH_3, 10). HR-EI-MS 584.2192 [(M^+) calcd for $C_{38}H_{32}O_6$ 584.2199]. IR ($CHCl_3$) ν_{max} (cm^{-1}) 2356, 1665, 1623, 1581, 1454, 1226, 1088, 957, 814, 748, 476.

Crystal Structure Analysis. Intensity data were collected at room temperature on a Enraf-Nonius KappaCCD difractometer with Mo Ka radiation (λ = 0.717 Å). Cell refinement and data reduction were performed with COLLECT[34] and DENZO.[35] Structures were solved by direct methods.[36] All non-H-atoms were refined anisotropically,[37] and H atoms were placed in calculated position and refined with a riding model. Calculations and the computing of molecular graphics and publication material were mainly performed with the WinGX[38] set of programs.

Crystal Data for 4. $C_{24}H_{32}O_4$, M_w = 384,5, monoclinic, space group $P2_1/c$, a = 9.137(4) Å, b = 18.800(8) Å, c = 12.346(7) Å, β = 103.73(9)°, V = 2100.7(17) Å^3, Z = 4, μ (Mo Kα) = 0.081 mm^{-1}, ρ_{calc} = 1.22 g·cm^{-3}, S = 1.05. Final R indices: R_1 = 0.076 and R_w = 0.189 for 2668 observed reflections (θ_{max} = 27.5 and $I > 2\sigma(I)$ criterion) and 260 parameters; maximum and minimum residues are 0.37 and −0.26 e·Å^3, respectively.

Crystal Data for 6. $C_{26}H_{36}O_4$, M_w = 412.6, orthorhombic, space group $P2_12_12_1$, a = 12.120(6) Å, b = 12.354(4) Å, c = 15.557(8) Å, V = 2329.4(2) Å^3, Z = 4, μ (Mo Kα) = 0.078 mm^{-1}, ρ_{calc} = 1.18 g·cm^{-3}, S = 1.03. Final R indices: R_1 = 0.076 and R_w = 0.189 for 3064 observed reflections (θ_{max} = 28.6 and $I > 2\sigma(I)$ criterion) and 272 parameters; maximum and minimum residues are 0.19 and −0.14 e·Å^3, respectively.

Crystal Data for 7. $C_{26}H_{36}O_4$, M_w = 412.6, orthorhombic, space group $P2_12_12_1$, a = 13.405(11) Å, b = 18.714(9) Å, c = 19.223(9) Å, V = 4822(5) Å^3, Z = 8 (two independent molecules in the asymmetric unit), μ (Mo Kα) = 0.070 mm^{-1}, ρ_{calc} = 1.14 g.cm^{-3}, S = 1.11. Final R indices: R_1 = 0.072 and R_w = 0.170 for 4728 observed reflections (θ_{max}= 27.0 and $I > 2\sigma(I)$ criterion) and 542 parameters; maximum and minimum residues are 0.22 and −0.23 e·Å^3, respectively.

Crystal Data for 20. $C_{38}H_{32}O_6$, M_w = 584.7, cubic, space group $Ia\bar{3}d$, a = 34.588(3) Å, V = 41378(62) Å^3, Z = 48, μ (Mo Kα) = 0.076 mm^{-1}, ρ_{calc} = 1.13 g·cm^{-3}, S = 1.15. Final R indices: R_1 = 0.089 and R_w = 0.199 for 2226 observed reflections (θ_{max} = 26 and $I > 2\sigma(I)$ criterion) and 200 parameters; maximum and minimum residues are 0.34 and −0.17 and e·Å^3, respectively.

Crystal Data for 21. $C_{38}H_{32}O_6.2(CHCl_3)$, M_w = 823.4, trigonal, space group $P\bar{3}$, a = 21.492(6) Å, c = 7.483(3) Å, V = Å^3, Z = 3, μ (Mo Kα) = 0.47 mm^{-1}, ρ_{calc} = 1.37 g·cm^{-3}, S = 1.07. Final R indices: R_1 = 0.078 and R_w = 0.208 for 3700 observed reflections (θ_{max} = 27.5 and $I > 2\sigma(I)$ criterion) and 470 parameters; maximum and minimum residues are 0.76 and −0.23 e·Å^3, respectively.

Biological Assays. Chemicals. Verapamil, mitoxantrone, and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma-Aldrich (Madrid, Spain). Danorubicin was from Pfizer (Madrid, Spain). Calcein-acetoxymethyl ester (calcein-AM) was purchased from Molecular Probes Europe BV (Leiden, The Netherlands). Zosuquidar was from Lilly Laboratories (France). HPMI-glucose was composed of 10 mM HEPES, 120 mM NaCl, 5 mM Na_2HPO_4, 0.4 mM $MgCl_2$, 0.04 mM $CaCl_2$, 10 mM $NaHCO_3$, 10 mM glucose, 5 mM KCl, pH 7.4. PBS was composed of 1.2 mM KH_2PO_4, 8.1 mM Na_2HPO_4, 130 mM NaCl, 2.6 mM KCl, adjusted to pH 7.4. Dulbecco's modified Eagle medium (DMEM), RPMI medium, and RPMI-1640 modified medium were purchased from Gibco (Invitrogen SA, Spain)

Cell Cultures. The mammalian cell lines used in the present work were parental NIH-3T3 Wt, drug-sensitive and transfected with the human MDR1-G185 gene (MDR1 cells), provided by Dr. I. Pastan (National Cancer Institute, NIH, Bethesda, MD),[12,13] HEK293 (human embryonic kidney) cells stably transfected with the pcDNA empty vector and with the vector containing the cDNA that codes for Wt BCRP (HEK-293-BCRP R482 cells), obtained from Dr. Susan Bates.[21] Both cells lines were cultured at 37 °C in a humidified atmosphere with 5% or 10% CO_2, respectively, in DMEM supplemented with 10% heat-inactivated fetal bovine serum (HIFBS). The 2008 ovarian drug-sensitive carcinoma cells and 2008 cells transfected with the cDNA that codes for MRP1 (2008-MRP1 cells), provided by Dr. P. Borst (Division of Molecular Biology, The Netherlands Cancer Institute, Amsterdam, The Netherlands),[21] were cultured at 37 °C in a humidified atmosphere with 5% CO_2 in RPMI supplemented with 10% HIFBS.

The Wt *L. tropica* LRC strain was a clone obtained by agar plating.[38] A *L. tropica* line highly resistant to daunorubicin was maintained in the presence of 150 μM daunorubicin as previously described.[38] This resistant line possesses a MDR phenotype similar to that of tumor cells, which confers cross-resistance to several drugs because of the overexpression of a Pgp-like multidrug efflux transporter.[14,39] Promastigote forms were grown at 28 °C in RPMI 1640 modified medium (Gibco) and supplemented with 20% HIFBS.

Modulation of Drug Efflux in Pgp (MDR1), BCRP, and MRP1-Expressing Cells. The bis-pyranobenzoquinones studied in the present work were screened for their ability to block the Pgp-dependent daunorubicin efflux, the MRP1-dependent calcein efflux, or the BCRP-dependent mitoxantrone efflux in a microplate assay, as described.[16] Cells were incubated in the presence/absence of 10 μM bis-pyranobenzoquinone derivatives in DMEM + 10% HIFBS with 10 μM daunorubicin for MDR1 cells, 2 μM calcein-AM for 2008-MRP1 cells, or 5 μM mitoxantrone for HEK-BCRP cells for 2 h at 37 °C. After the incubation step with daunorubicin, calcein-

AM, or mitoxantrone, the fluorescent probes were removed and the monolayers were washed twice with cell culture medium + 10% HIFBS in order to remove any trace of extracellular fluorescent. Finally, an amount of 50 μL of lysis buffer (Tris-HCl, 20 mM, pH 7.4, SDS, 0.2%) was added to each well and incubated at 4 °C overnight in the dark. The accumulated intracellular fluorescence due to daunorubicin ($\lambda_{excitation}$ = 480 nm; $\lambda_{emission}$ = 590 nm) or calcein-AM ($\lambda_{excitation}$ = 490 nm; $\lambda_{emission}$ = 520 nm) or mitoxantrone ($\lambda_{excitation}$ = 635 nm; $\lambda_{emission}$ = 670 nm) was measured in a microplate spectrofluorometer "SpectraMax Gemini EM" (Molecular Devices, Sunnyvale CA) using the SoftmaxPro 4.3 software (also from Molecular Devices).

Flow Cytometry Analysis of Inhibition of Daunorubicin Accumulation in a NIH-3T3 Cell Line Overexpressing a Pgp (MDR1) Transporter. The accumulation of daunorubicin in Wt and MDR1 NIH-3T3 lines was estimated by flow cytometry using a Becton Dickinson FacScan in order to determine K_i and B_{max} values (see below). Briefly, cells were incubated with 2 μM daunorubicin for 0.5 h in DMEM + 10% HIFBS at 37 °C in a humidified atmosphere with 5% CO_2 in the presence or in the absence of different concentrations of bis-pyranobenzoquinone derivatives. Finally, cells were extensively washed with medium and PBS, tripsinized, resuspended in cold PBS, and immediately analyzed. Cells were gated on the basis of size and complexity to eliminate dead cells and debris from the analysis. Quantification of intracellular fluorescence was carried out by scanning the emission in FL2 with a Becton Dickinson FacScan (BD European HQ, Erembodegem-Aalst, Belgium). All of these values were converted to percentage inhibition of Pgp normalized for wild-type cells and fitted to the equation for one site saturation, using SigmaPlot 2000 software for Windows (SPSS Inc., Chicago, IL): $y = B_{max}x/(K_i + x)$, where y is the inhibition of daunorubicin efflux at a given bis-pyranobenzoquinone concentration, B_{max} is the maximum percentage of Pgp inhibition, K_i is the concentration of compound needed to achieve a half-maximal inhibition at equilibrium, and x is the concentration of bis-pyranobenzoquinone derivatives (μM).

Cytotoxicity Assays and Reversion of Daunorubicin Resistance Mediated by MDR1 Cells. Exponentially growing NIH-3T3 Wt and MDR1 cells were trypsinized and plated at a density of 3.5×10^3 cells per well (in 96-well plates), and they were allowed to attach for 24 h at 37 °C in a humidified atmosphere with 5% CO_2. Reversion of drug resistance was determined in MDR1 cells after 72 h of incubation at 37 °C in a humidified atmosphere with 5% CO_2 with increasing daunorubicin concentrations in the presence of 10, 3, or 1 μM of each compound. Verapamil (10 μM) and zosuquidar (3 and 1 μM) were used as classical and third-generation MDR1 reversal agents, respectively. Dose−response curves were generated as described previously[12] by nonlinear regression of the data points to four-parameter logistic curves using SigmaPlot 2000 for Windows (SPSS Inc., Chicago, IL). Bis-pyranobenzoquinones (10 μM), verapamil (10 μM), and zosuquidar (3 μM) cytotoxicities were evaluated, also by the MTT colorimetric assay in 96-well plates.[12,13,22]

Experiments of Chemosensitization to Daunorubicin in *L. tropica* MDR. The viability of parasites in the presence of bis-pyranobenzoquinone derivatives was analyzed by a MTT-based assay.[14] The screening was performed in 96-well plates maintained for 72 h at 28 °C. Promastigote forms in the logarithmic phase of growth were suspended in fresh medium to yield 6×10^6 cells/mL. Each well was filled with 50 μL of the parasite suspension (3×10^5 cells). To assess the chemosensitizing activity, the promastigotes of *L. tropica* MDR line were exposed to both 150 μM daunorubicin and 10 μM of each bis- pyranobenzoquinone derivative, and the Wt line was exposed only to bis-pyranobenzoquinone derivatives to study their intrinsic toxicity. The results were expressed as percentage of growth inhibition (GI).

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Supporting Information Available: ^{1}H NMR ($CDCl_3$), ^{13}C NMR ($CDCl_3$), and purity determinations for all synthesized bis-pyranobenzoquinones. This material is available free of charge via the Internet at http://pubs.acs.org.

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