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Synthesis and Cytotoxic Activity of Steroid-Anthraquinone Hybrids

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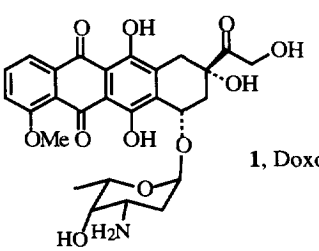
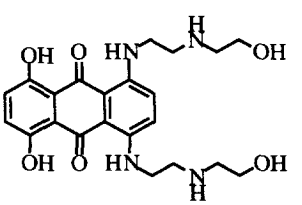
Abstract: Synthesis of cytotoxic steroidal derivatives containing a quinone moiety is described. The synthetic strategy is based on an unsual A + CD -> ABCD Diels-Alder approach which generates 9β-H cholestane analogs. The adducts formed are efficiently aromatized in basic media to give steroid-anthaquinones hybrids showing interesting cytotoxic activity on four tumor cell lines.

Novel potentially bioactive compounds can be designed combining in a single molecule different classes of natural products. This approach has been succesfully applied *e.g.* in the design of hybrid molecules containing a neotropsin-type minor groove binder linked to neocarzionostatin chromophore analogs,[1] or by combination of classic β-lactam antibiotics and gyrase inhibitors, which give rise to extremely potent antibiotics.[2] Other examples of potentially biologically active hybrid compounds synthetized are: taxamicins,[3] lactendiynes,[4] calichearubicins,[5] retinoid-anthraquinones hybrids,[6] β-lactam-nucleosides.[7] Attention has also been paid to the synthesis of drug-steroid hybrids, as for example estramycins,[8] steroidal polyamines,[9] a cholesterol-baccatin III hybrid[10] and opioid-steroid hybrids.[11]

In consideration of the powerful antineoplastic activity of the anthracycline antibiotics great efforts have been made in the design and synthesis of analogs of these compounds presenting low molecular complexity and similar mechanism of action. Starting from doxorubicin (Fig. 1, **1**), the most commonly used intercalating agent in the treatment of cancer,[12] new synthetic anthracenediones have been developed. Mitoxantrone (**2**), in particular, has shown clinical effectiveness against human malignancies.[13]



1, Doxorubicin



2, Mitoxantrone

Figure 1

Because of the importance of steroids as vectors for biological activities (see, for example, the potent antiviral colasane)[14] as a part of a development of new approaches to compounds related to steroids, we designed, synthesized and evaluated the cytotoxic activity of a novel class of steroid-anthraquinone hybrids of general formula **3** (Fig. 2). The new compounds were synthesized in two series differing for the side chains. The number and position of hydroxyl groups were also varied in order to gain information on the requirements for cytotoxic activity.

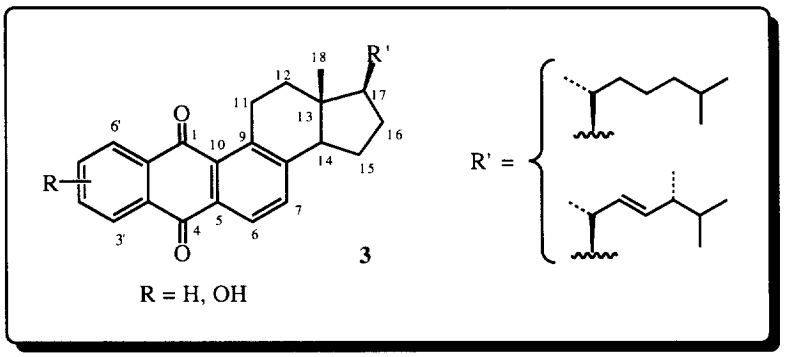


Figure 2

For the synthesis of the steroid-anthraquinone hybrids we took advantage of a recent application of the rare[15] A + CD -> ABCD Diels-Alder approach for the construction of cholestane analogs. Using this strategy we synthesized remarkable cholestane analogs, such as a C_2 symmetric *bis*-cholestane derivative and a fullerene adduct.[16] The CD ring fragments used in the Diels-Alder reaction were dienes **4a** and **4b**, the latter recently prepared for the synthesis of (17*R*)-17-methylincisterol,[17] which were obtained respectively from vitamin D_3 and vitamin D_2.

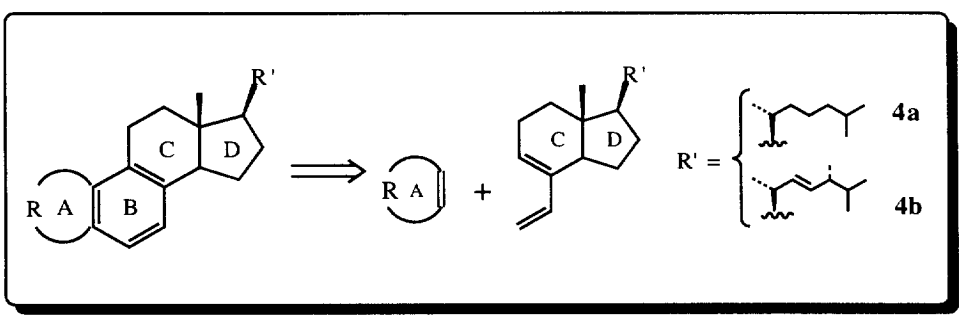
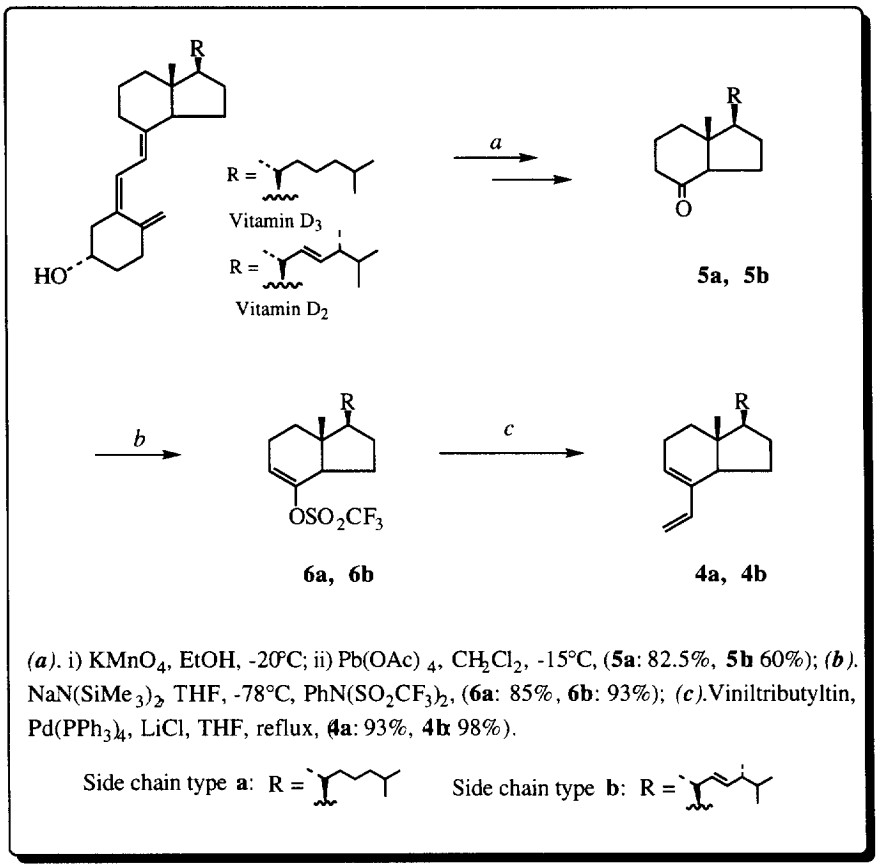


Figure 3

We report here full experimental details on the preparation of **4a** and **4b**, their reaction with a series of naphthoquinones and the aromatization of the resulting adducts to give antiproliferative steroid-anthraquinone hybrids.

Dienes **4a** and **4b** were obtained respectively from vitamin D_2 (65% overall yield) and vitamin D_3 (55% overall yield), through a general protocol reported in Scheme 1.

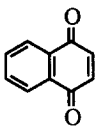
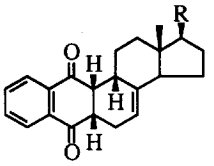
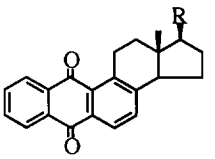
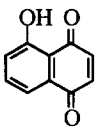
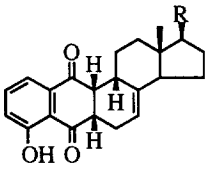
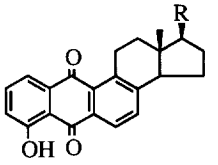
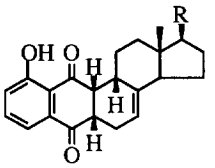
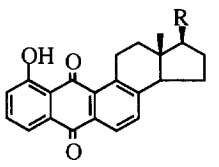
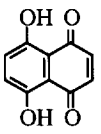
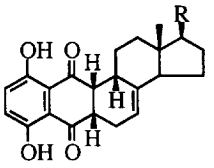
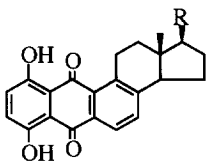
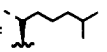
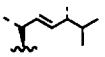


Scheme 1.

The ready kinetic enolization of Grundmann's ketones[18] **5a** and **5b** was achieved at -78°C using the bulky base sodium *bis*(trimethylsilyl)amide. The resulting enolates were quenched with N-phenyltrifluoromethanesulfonimide[19] to afford the triflates **6a** and **6b**. Stille reaction[20] between **6a** and **6b** and vinyltributyltin, catalyzed by tetrakis(triphenylphosphine)palladium(0) gave the dienes **4a** and **4b**.

The reaction of both **4a** and **4b** with different anthraquinones in refluxing toluene afforded stereochemically pure 9β–H unnatural cholestane analogues **7-10** (Table 1) as the sole detectable Diels-Alder adducts. The stereochemistry of the reaction was elucidated by comparison of the NMR data of **7b-10** with those of the adduct **7a**, whose structure was secured by X-ray diffraction analysis.[16]

Table 1

Dienophiles	Adducts		Yields	Steroid-Quinone Hybrids		Yields
		**7a** **7b**	(98%) (71%)		**11a** **11b**	(90%) (90%)
		**8a** **8b**	(73%) (64%)		**12a** **12b**	(75%) (95%)
		**9a** **9b**	(13%) (10%)		**13a** **13b**	(61%) (85%)
		**10a** **10b**	(81%) (66%)		**14a** **14b**	(90%) (90%)
Side chain type **a**: R = 				Side chain type **b**: R = 		

Aromatization of adducts **7-10** was easily accomplished by the action of air in presence of DBU (1,8-diazabicyclo[5.4.0]undec-7-ene)[21] to afford compounds **11-14**, hydrogen peroxyde being concevaibly the other product.

The reaction of **4a,b** with 5-hydroxynaphthoquinone afforded the two pairs **8a-9a** and **8b-9b**. The single components of each pair were separated by silica gel column chromatography and the structures were assigned by comparison of the ^{13}C chemical shift values of the carbonyl carbons in the compounds **12a,b** and **13a,b** with those of **14a,b**. In fact, in the ^{13}C NMR spectrum of **14a** the two carbonyl resonances at δ 190.1 and 187.1 were assigned to C-4 and C-1, respectively, since the latter suffers the γ-effect due to the C-11. In compound **12a** the same carbonyl carbons resonate at δ 191.8 and 182.9, while in **13a** the chemical shift values were 188.9 and 185.0, making clear that the variation of the chemical shift values at both carbons in the pair **12a-13a**, as compared to **14a**, was due to the presence or the absence of the *peri*-OH group.

Compounds **11-14** were tested *in vitro* on four tumor cell lines for the evaluation of their antiproliferative activity[22,23] in comparison with doxorubicin. The results are reported in Table 2 as IC_{50} (concentration of compound expressed in μg/ml required to inhibit the cellular growth by 50% after 24, 48 and 72 hours of drug exposure).

Table 2. *In vitro* cytotoxic activity (IC_{50}, μg/ml) of compounds **11-14** and doxorubicin on J774 (murine monocyte/macrophage), GM7373 (bovine aortic endothelial), IGR-1 (human melanoma) and P388 (murine leukemia) cell lines.[a]

Cell-line / *h*	**11a**	**11b**	**12a**	**12b**	**13a**	**13b**	**14a**	**14b**	***doxorub.***
J774									
24	*n. d.*	100.1±8.2	*n. d.*	**30.7±5.1**	117.6±9.6	*n. d.*	143.7±2.3	**19.7±4.1**	*86.3±9.3*
48	112.6±14.9	**25.3±5.4**	95.9±2.1	**10.1±1.1**	62.5±4.3	**43.4±3.4**	133.3±21.7	**10.9±1.3**	*54.2±7.1*
72	83.3±7.2	16.6±2.1	80.9±6.4	**0.35±0.01**	25.9±7.2	35.4±4.1	80.1±4.3	**7.7±1.1**	*12.1±3.9*
GM7373									
24	*n. d.*	*n. d.*	*n. d.*	*n. d.*	*n. d.*	*n. d.*	*n. d.*	*n. d.*	*69.7±9.4*
48	71.4±2.9	35.0±10.3	85.3±4.2	22.3±1.7	50.8±6.6	54.7±4.2	44.1±5.2	**16.3±2.1**	*17.6±3.5*
72	35.7±5.5	12.5±2.4	8.8±2.5	**0.55±0.08**	30.7±5.2	33.3±3.1	31.7±2.3	8.8±2.2	*4.6±2.9*
IGR-1									
24	*n. d.*	*n. d.*	*n. d.*	87.0±6.2	*n. d.*	150.9±19.3	*n. d.*	46.7±4.1	*22.9±6.4*
48	*n. d.*	44.1±5.3	111.1±11.3	30.7±5.2	19.7±3.3	87.0±14.1	231.3±22.6	**10.9±2.3**	*11.5±1.4*
72	83.3±10.3	31.7±4.3	81.3±6.4	9.5±1.9	12.5±2.4	50.6±3.4	66.6±12.3	8.2±1.3	*0.34±0.09*
P388									
24	*n. d.*	*n. d.*	*n. d.*	**43.4±3.4**	*n. d.*	*n. d.*	*n. d.*	**33.3±2.1**	*52.3±7.3*
48	274.0±12.3	181.1±9.2	164.0±14.1	34.5±4.1	75.0±10.3	125.0±15.8	174.0±5.7	23.2±1.6	*10.3±3.9*
72	132.0±7.1	111.1±5.3	77.1±6.3	18.5±1.3	50.0±8.1	133.3±21.6	153.8±4.1	12.5±0.9	*2.1±0.9*

n.d.: activity not detectable. Bolded data refer to IC_{50} lower than that found for doxorubicin

[a] The results are expressed as mean ± S.E.M. of three separate experiments in triplicate.

The results show that compounds with the ergosterol side chain (**11b-14b**) were more cytotoxic than those with the saturated side chain (**11a-14a**). In particular, the most active compounds were those possessing a C-3' hydroxyl group, *e.g.* **12b** and **14b**, which show cytotoxic activities comparable to those of doxorubicin.

In conclusion the *in vitro* data appears quite promising and warrant further investigation to fully assess the potentials of these hybrids.

Acknowledgements. This work has been supported by MURST (40%) and CNR (Rome). The mass spectra were obtained from the "Servizio di Spettrometria di Massa del CNR e dell'Università di Napoli"; the staff is gratefully acknowledged. We thank Dr. Gianluca Giorgi ("Centro Interdipartimentale di Analisi e Determinazioni Strutturali" of the University of Siena) for accurate high resolution mass spectra. We wish to thank Dr. Paolo Lombardi (Menarini Ricerche Sud, Pomezia, Roma), Prof. Manlio Palumbo (University of Padova) for helpful discussions and miss Rossella De Caro for valuable aid in the experimental work.

EXPERIMENTAL SECTION

General techniques

All reactions were carried out under a dry argon atmosphere using freshly distilled solvents unless otherwise noted. Tetrahydrofuran was distilled from sodium and benzophenone. Toluene and methylene chloride were distilled from calcium hydride. Glassware was flame dried (0.05 torr) before use. When necessary, compounds were dried by azeotropic removal of water with toluene under reduced pressure. Reactions were monitored by thin layer chromatography (TLC) on Merck silica gel plates (0.25 mm) and visualized using UV light, spraying with H_2SO_4-$Ce(SO_4)_2$ solution and drying. Reaction temperatures were measured externally. Flash chromatography was performed on Merck silica gel (60, particle size: 0.040-0.063 mm). Yields refer to chromatographically and spectroscopically (^{1}H-NMR) pure materials.

NMR spectra were recorded in $CDCl_3$ solutions on a Bruker AM-250 spectrometer at ambient temperature. Chemical shifts are reported relative to the residual solvent peak ($CHCl_3$: δ=7.26, $^{13}CDCl_3$: δ=77.0). Optical rotations were recorded in $CHCl_3$ solutions on a JASCO DIP-370 or JASCO DIP-1000 polarimeters. Mass spectra (E.I., 70 eV) were recorded on a VG TRIO 2000 mass spectrometer. High resolution mass spectra were recorded on a VG 70-250SE (70eV, source temp.: 200°C, resolution: 5000 m/Δm, 10% valley). IR were recorded on a Nicolet FT 5DXB spectrometer. UV-Vis spectra were recorded in dioxane on a Varian DMS 90 spectrometer. For the cytotoxic assays the optical density (OD) of the wells were measured with a microplate spectrophotometer (Titertek Multiscan MCC/340) equipped with a 620 nm filter. All the reagents for cell culture were from Cellbio; MTT and doxorubicin were purchased from Sigma.

Preparation and selected data of compounds.

Ketones 5a and 5b.[18]

5b. To a -20°C cooled solution of vitamin D_2 (5.00 g, 13.1 mmol) in absolute ethanol (500 ml) was slowly added a solution of $KMnO_4$ (4.20 g, 26.5 mmol) in distilled water (125 ml). The resulting mixture was stirred for additional 15 min, and then warmed at 40°C for 30 min. Filtration through a shorth path af celite and washing of the solid with absolute ethanol afforded a yellow solution. Removal of the solvent in vacuo afforded a residue which was flash chromatographed (silica gel, 2-3% methanol in choroform) to give the corresponding triol (3.25 g, 65%) as a white solid. $[\alpha]_D$= +63.4° (c=0.44, $CHCl_3$); ^{1}H NMR δ: 0.80 (3H, s, Me-18), 0.81 (3H, d, J= 6.6 Hz, Me-26), 0.82 (3H, d, J= 6.6 Hz, Me-27), 0.90 (3H, d, J= 6.6 Hz, Me-28), 0.97 (3H, d, J= 6.6 Hz, Me-21), 2.19 (1H, ddd, J= 13.5, 12.0, 1.6 Hz, H-4), 2.44 (1H, ddd, J= 13.0, 4.0, 3.9 Hz, H-1), 2.58 (1H, bdd, J=12.0, 3.5 Hz, H-4'), 3.78 (1H, m, H-3), 4.91 (1H, d, J= 9.7 Hz, H-7), 4.92 (1H, bs, H-19), 5.01 (1H, bs, H-19'), 5.16 (2H, m, H-22 and H-23), 5.56 (1H, dd, J=9.7, 1.6 Hz, H-6); ^{13}C NMR δ: 13.0, 17.1, 19.6, 20.0, 20.4, 20.6, 21.8, 27.7, 33.1, 33.2, 36.0, 37.6, 39.9, 40.0, 42.8, 44.0, 46.5, 57.3, 59.9, 70.5, 70.7, 74.9, 111.4, 125.2, 132.1, 135.4, 140.7, 145.3; I.R. (film) ν_{max}3282, 1045 cm^{-1}, MS, *m/z*: 412 (M^+-H_2O), 154, 136.

To a -15°C cooled solution of triol (0.20 g, 0.46 mmol) in dry methylene chloride (4 ml), $Pb(OAc)_4$ (0.24 g, 0.55 mmol) was added in portions. The resulting mixture was stirred for 20 min at the same temperature and then without the cooling bath for additional 20 min. Filtration through a short column of celite-silica gel (1:1) and

washing of the solid with methylene chloride afforded a solution which was concentrated in vacuo. The residue was flash chromatographed (silica gel; 5-30% ethyl acetate in petroleum ether) to give **5b** (0.107 g, 93% yield) as a colorless oil. $[\alpha]_D$= +11.6° (c=1.01, $CHCl_3$); ^{1}H NMR δ: 0.61 (3H, s, Me-18), 0.78 (3H, d, J= 6.6 Hz, Me-26), 0.79 (3H, d, J= 6.6 Hz, Me-27), 0.87 (3H, d, J= 6.6 Hz, Me-28), 1.00 (3H, d, J= 6.6 Hz, Me-21), 2.41 (1H, dd, J= 10.9, 7.6 Hz, H-14), 5.18 (2H, m, H-22 and H-23); ^{13}C NMR δ: 12.6, 17.5, 19.0, 19.6, 19.9, 20.9, 24.0, 27.7, 33.0, 38.8, 39.8, 40.9, 42.8, 49.7, 56.5, 62.0, 132.4, 134.8, 211.9; I.R. (film) ν_{max} 1717 cm^{-1}; MS, *m/z*: 276 (M^+), 261, 233, 205, 179, 151.

5a. To a -20°C cooled solution of vitamin D_3 (15.0 g, 39.0 mmol) in absolute ethanol (1350 ml) was slowly added a solution of $KMnO_4$ (12.4 g, 78.5 mmol) in distilled water (450 ml). The resulting mixture was stirred for additional 75 min, and then warmed at 40°C for 30 min. Filtration through a shorth path af celite and washing of the solid with absolute ethanol afforded a yellow solution. Removal of the solvent in vacuo afforded a residue which was dried by azeotropic removal of water with toluene under reduced pressure and dissolved in dry methylene chloride (400 ml). This solution was cooled to -15°C and $Pb(OAc)_4$ (24.4 g, 55.0 mmol) was added in four portions. The resulting mixture was stirred for 15 min at the same temperature and without the cooling bath for additional 60 min. Filtration through a short column of celite-silica gel (1:1) and washing of the solid with methylene chloride afforded a solution which was concentrated in vacuo. The residue was flash chromatographed (silica gel, 5-30% ethyl acetate in petroleum ether) to give **5a** (8.51 g, 82.5% yield) as a colorless oil. $[\alpha]_D$= +8.9° (c=1.59, $CHCl_3$); Lit.:[18b] $[\alpha]_D$= +7.9° (c=2.80, $CHCl_3$); ^{1}H NMR δ: 0.62 (3H, s, Me-18), 0.86 (6H, d, J= 6.6 Hz, Me-26 and -27), 0.94 (3H, d, J= 6.6 Hz, Me-21), 2.44 (1H, dd, J= 10.9, 7.5 Hz, H-14); MS, *m/z*: 264 (M^+), 221, 179, 151.

Vinyl triflates 6a and 6b.[19b]

6b. To a solution of sodium *bis*(trimethylsilyl)amide (0.75 ml, 1.0 M in THF, 0.75 mmol) at -78°C **5b** (85.0 mg, 0.30 mmol) dissolved in dry THF (1.0 ml) was added. After 1 h, N-phenyltrifluoromethanesulfonimide (260 mg, 0.72 mmol) was added to the resulting enolate. After 20 min the reaction mixture was slowly warmed at r.t. and stirred for 2 h. The reaction was quenched with water, concentrated in vacuo to remove the excess of THF, and extracted with petroleum ether. The organic phase was dried (Na_2SO_4), filtered and concentrated in vacuo. The residue was flash chromatographed (silica gel, petroleum ether) to give **6b** (115 mg, 93% yield) as a colorless oil. $[\alpha]_D$= +85.8° (c=2.31, $CHCl_3$); ^{1}H NMR δ: 0.80 (3H, s, Me-18), 0.84 (3H, d, J= 6.6 Hz, Me-26), 0.86 (3H, d, J= 6.6 Hz, Me-27), 0.94 (3H, d, J= 6.6 Hz, Me-28), 1.05 (3H, d, J= 6.6 Hz, Me-21), 2.31 (2H, m, H_2-11), 2.50 (1H, m, H-14), 5.18 (1H, dd, J= 15.2, 8.6 Hz, H-22 or H-23), 5.30 (1H, dd, J= 15.2, 7.0 Hz, H-23 or H-22), 5.58 (1H, d, J= 7.4, 3.7 Hz, H-9); ^{13}C NMR δ: 11.4, 17.5, 19.9, 20.8, 21.5, 21.7, 23.8, 33.1, 34.8, 40.3, 42.9, 45.1, 50.3, 54.2, 118.6 (q, J= 312 Hz), 132.9, 135.0, 149.9; MS, *m/z*: 410 (M^++2), 408 (M^+), 365, 327, 283, 259.

6a[19b] was synthesized from **5a** as described above for **6b** (85% yield, colorless oil). $[\alpha]_D$= +18.2° (c=1.02, $CHCl_3$); ^{1}H NMR δ: 0.78 (3H, s, Me-18), 0.88 (6H, d, J= 6.6 Hz, Me-26 and Me-27), 0.94 (3H, d, J= 6.6 Hz, Me-21), 2.40 (2H, m H-11), 2.48 (1H, m, H-14), 5.58 (1H, dd, J= 7.4, 3.7 Hz, H-9); MS, *m/z*: 398 (M^++2), 396 (M^+).

Dienes 4a and 4b

4b. To a suspention of $Pd(PPh_3)_4$ (85 mg, 0.07 mmol), LiCl (475 mg, 11.3 mmol) and vinyltributyltin (0.85 ml, 2.94 mmol), a solution of **6b** (1.0 g, 2.45 mmol) in dry THF (10 ml) was slowly added. The reaction

mixture was stirred at reflux for 2 h and then quenched with water (10 ml), concentrated in vacuo to remove the excess of THF and extracted with petroleum ether. The organic phase was washed with water, a 10% aqueous solution of NH_4OH and brine. The organic phase was dried (Na_2SO_4), filtered and concentrated in vacuo. The residue was flash chromatographed (silica gel, petroleum ether) to give **4b** (696 mg, 98% yield) as a colorless oil. $[\alpha]_D$= -11.5° (c=2.35, $CHCl_3$); ^1H NMR δ: 0.70 (3H, s, Me-18), 0.84 (3H, d, J= 6.6 Hz, Me-26), 0.85 (3H, d, J= 6.6 Hz, Me-27), 0.93 (3H, d, J= 6.6 Hz, Me-28), 1.06 (3H, d, J= 6.6 Hz, Me-21), 4.84 (1H, d, J= 11.2, H-6), 5.20 (1H, d, J= 17.2 Hz, H-6'), 5.21 (2H, m, H-23 and H-24), 5.67 (1H, m, H-9), 6.20 (1H, dd, J= 17.2, 11.2 Hz, H-7); ^{13}C NMR δ: 11.3, 17.4, 19.5, 19.8, 20.9, 23.7, 24.6, 28.7, 32.9, 35.6, 40.2, 42.5, 42.7, 49.7, 53.7, 111.3, 125.7, 131.8, 135.5, 138.0, 138.7; MS, *m/z*: 286 (M^+), 259, 161.

4a was synthesized from **6a** as described above for **4b** (93% yield, colorless oil). $[\alpha]_D$= -21.0° (c=0.86, $CHCl_3$); ^1H NMR δ: 0.78 (3H, s, Me-18), 0.89 (6H, d, J= 6.6 Hz, Me-26 and Me-27), 0.96 (3H, d, J= 6.6 Hz, Me-21), 4.83 (1H, d, J= 11.2, H-6), 5.20 (1H, d, J= 17.2 Hz, H-6'), 5.68 (1H, m, H-9), 6.20 (1H, dd, J= 17.2, 11.2 Hz, H-7); ^{13}C NMR δ: 11.2, 18.9, 22.5, 22.8, 23.9, 24.0, 24.8, 28.0, 28.7, 35.9, 36.1, 36.2, 39.5, 42.7, 49.8, 54.0, 111.4, 125.8, 138.2, 138.8; MS, *m/z*: 274 (M^+).

Diels-Alder adducts 7-10

General procedure: diene (1.0 mmol) and dienophile (1.0 mmol) were dissolved in anhydrous toluene (3.0 ml) and heated at 100°C until the reaction was complete (5-12 h, TLC analysis). Concentration under reduced pressure gave a residue which was flash chromatographed (silica gel) to give adducts **7-10.**

7a was eluted with petroleum ether - toluene 1:1 (98% yield) as a colorless solid and recrystallized from ethyl acetate; m. p.: 159 - 160°C. $[\alpha]_D$= +190.9° (c=0.49, $CHCl_3$); ^1H NMR δ: 0.71 (3H, s, Me-18), 0.86 (6H, d, J= 6.5, Me-26 and Me-27), 0.91 (3H, d, J= 5.8 Hz, Me-21), 3.40 (2H, m, H-5 and H-10), 5.06 (1H, m, H-7), 7.70 (2H, dd, J= 5.8, 3.3 Hz, H-3' and H-6'), 7.90 (1H, dd, J= 5.8, 3.3 Hz, H-4' or H-5'), 8.03 (1H, dd, J= 5.8, 3.3 Hz, H-5' or H-4'); ^{13}C NMR δ: 18.4 (C-18), 18.8 (C-21), 22.5, 22.8 (C-26, C-27), 22.8 (C-11), 23.9 (C-23), 24.5 (C-15), 27.1 (C-6), 28.0 (C-25), 28.7 (C-16), 35.9 (C-22), 36.1 (C-20), 36.7 (C-9), 37.7 (C-12), 39.4 (C-24), 41.6 (C-13), 49.2 (C-14), 50.7 (C-5), 50.8 (C-10), 57.1 (C-17), 114.3 (C-7), 126.2, 127.0 (C-3', C-6'), 132.5, 135.9 (C-2, C-3), 133.8, 134.2 (C-4', C-5'), 142.0 (C-8), 198.0 (C-1), 199.2 (C-4); UV: λ= 210-240 (ε= 9654-9221), λ_{max}= 257 (ε= 6311), λ= 295-307 (ε= 1412-1175); MS, *m/z*: 432 (M^+), 417, 319.

7b was eluted with petroleum ether - toluene 1:1 (71% yield) as a colorless solid. $[\alpha]_D$= +109.8° (c=0.21, $CHCl_3$); ^1H NMR δ: 0.72 (3H, s, Me-18), 0.82 (3H, d, J= 6.6 Me-26), 0.83 (3H, d, J= 6.6, Me-27), 0.92 (3H, d, J= 6.6 Hz, Me-28), 1.02 (3H, d, J= 6.6 Hz, Me-21), 3.40 (2H, m, H-5 and H-10), 5.08 (1H, m, H-7), 5.20 (2H, m, H-22 and H-23), 7.70 (2H, dd, J= 5.8, 3.3 Hz, H-3' and H-6'), 7.90 (1H, dd, J= 5.8, 3.3 Hz, H-4' or H-5'), 8.02 (1H, dd, J= 5.8, 3.3 Hz, H-5' or H-4'); MS, *m/z*: 444 (M^+).

8a, **9a** were eluted with petroleum ether - ethyl acetate - acetic acid 99:1:0.1 as yellow solids in 73% (**8a**) and 13% (**9a**) yield, respectively.

8a: $[\alpha]_D$= +220.0° (c=0.45, $CHCl_3$); ^1H NMR δ: 0.71 (3H, s, Me-18), 0.87 (6H, d, J= 6.6 Hz, Me-26 and Me-27), 0.92 (3H, d, J= 5.8 Hz, Me-21), 3.38 (2H, m, H-5 and H-10), 5.08 (1H, m, H-7), 7.22 (1H, dd, J= 7.5, 1.3 Hz, H-4'), 7.53 (1H, dd, J= 7.5, 1.3 Hz, H-6') 7.60 (1H, t, J= 7.5 Hz, H-5'), 11.90 (1H, s, O*H*); ^{13}C NMR δ: 18.4 (C-18), 18.8 (C-21), 22.5, 22.7 (C-26, C-27), 22.8 (C-11), 23.9 (C-23), 24.8 (C-15), 26.9 (C-6), 28.0 (C-25), 28.7 (C-16), 35.9 (C-22), 36.1 (C-20), 37.0 (C-9), 37.7 (C-12), 39.4 (C-24), 41.7 (C-13), 49.2 (C-14), 49.9 (C-5), 50.3 (C-10), 57.1 (C-17), 114.5 (C-7), 117.7 (C-3), 118.4, 124.0, 136.4

(C-4', C-5', C-6'), 132.7 (C-2), 142.3 (C-8), 160.8 (C-3'), 198.6 (C-1), 205.3 (C-4); UV: λ= 200-233 (ε= 8912-8710), λ_{max}= 260 (ε= 5754), λ= 323-348 (ε= 6166-6761); MS, *m/z*: 448 (M^+), 335.

9a: $[\alpha]_D$= +216.7° (c=0.24, $CHCl_3$); ^1H NMR δ: 0.70 (3H, s, Me-18), 0.87 (6H, d, J= 6.6 Hz, Me-26 and Me-27), 0.92 (3H, d, J= 5.8 Hz, Me-21), 3.36 (2H, m, H-5 and H-10), 5.08 (1H, m, H-7), 7.22 (1H, d, J= 8.4 Hz, H-5'), 7.39 (1H, d, J= 7.3 Hz, H-3'), 7.60 (1H, dd, J= 8.4, 7.3 Hz, H-4') 12.02 (1H, s, O*H*); ^{13}C NMR δ: 18.4 (C-18), 18.8 (C-21), 22.5, 22.8 (C-26, C-27), 22.8 (C-11), 23.9 (C-23), 24.4 (C-15), 28.0 (C-6), 28.0 (C-25), 28.7 (C-16), 35.9 (C-22), 36.1 (C-20), 36.4 (C-9), 37.6 (C-12), 39.5 (C-24), 41.7 (C-13), 49.2 (C-14), 50.1 (C-5), 50.7 (C-10), 57.2 (C-17), 114.1 (C-7), 115.1 (C-2), 117.5, 123.4, 136.9 (C-3', C-4', C-5'), 136.1 (C-3), 142.0 (C-8), 162.1 (C-6'), 197.0 (C-4), 206.4 (C-1); UV: λ= 200-230 (ε= 9332-8511), λ_{max}= 260 (ε= 5880), λ= 339-346 (ε= 5623-6309); MS, *m/z*: 448 (M^+), 335.

8b, **9b** were isolated as above as yellow solids in 64% and 10% yield, respectively.

8b: $[\alpha]_D$= +85.2° (c=0.64, $CHCl_3$); ^1H NMR δ: 0.72 (3H, s, Me-18), 0.82 (3H, d, J= 6.6, Me-26), 0.83 (3H, d, J= 6.6, Me-27), 0.91 (3H, d, J= 6.6 Hz, Me-28), 1.02 (3H, d, J= 6.6 Hz, Me-21), 3.37 (2H, m, H-5 and H-10), 5.08 (1H, m, H-7), 5.20 (2H, m, H-22 and H-23), 7.22 (1H, dd, J= 7.5, 1.3 Hz, H-4'), 7.53 (1H, dd, J= 7.5, 1.3 Hz, H-6') 7.60 (1H, t, J= 7.5 Hz, H-5'), 11,88 (1H, s, O*H*); ^{13}C NMR δ: 17.6, 18.9, 19.6, 19.9, 20.6, 22.7, 24.7, 26.7, 29.1, 33.0, 37.0, 37.4, 40.6, 41.5, 42.8, 49.3, 49.9, 50.3, 56.9, 114.6, 117.7, 118.5, 124.1, 132.2, 132.7, 135.3, 136.4, 142.2, 160.8, 198.6, 205.3; MS, *m/z*: 460 (M^+), 335, 317, 203.

9b: $[\alpha]_D$= +119.7° (c=0.15, $CHCl_3$); ^1H NMR δ: 0.73 (3H, s, Me-18), 0.82 (3H, d, J= 6.6, Me-26), 0.83 (3H, d, J= 6.6, Me-27), 0.92 (3H, d, J= 6.6 Hz, Me-28), 1.02 (3H, d, J= 6.6 Hz, Me-21), 3.37 (2H, m, H-5 and H-10), 5.08 (1H, m, H-7), 5.20 (2H, m, H-22 and H-23), 7.21 (1H, d, J= 8.4 Hz, H-5'), 7.38 (1H, d, J= 7.3 Hz, H-3'), 7.60 (1H, dd, J= 8.4, 7.3 Hz, H-4') 12.01 (1H, s, O*H*); ^{13}C NMR δ: 17.6, 18.9, 19.6, 19.9, 20.6, 22.8, 24.3, 27.9, 29.1, 33.0, 36.3, 37.4, 40.6, 41.5, 42.8, 49.3, 50.0, 50.6, 56.9, 114.6, 115.1, 117.5, 123.4, 132.1, 135.3, 136.1, 136.9, 141.9, 162.1, 197.0, 206.4; MS, *m/z*: 460 (M^+), 335, 317, 203.

10a was eluted as a yellow solid with petroleum ether - ethyl acetate - acetic acid 99:1:0.1 in 81% yield. $[\alpha]_D$= +280.0° (c=0.38, $CHCl_3$); ^1H NMR δ: 0.72 (3H, s, Me-18), 0.88 (6H, d, J=6.6 Hz, Me-26 and Me-27) 0.92 (3H, d, J= 5.8 Hz, Me-21), 3.33 (2H, m, H-5 and H-10), 5.12 (1H, m, H-7), 7.22 (2H, s, H-4' and H-5'), 11.69 (1H, s, O*H*), 11.88 (1H, s, O*H*); ^{13}C NMR δ: 18.4 (C-18), 18.8 (C-21), 22.7, 22.8 (C-26, C-27), 22.8 (C-11), 23.9 (C-23), 24.7 (C-15), 27.7 (C-6), 28.0 (C-25), 28.7 (C-16), 35.9 (C-22), 36.1 (C-20), 36.7 (C-9), 37.6 (C-12), 39.4 (C-24), 41.6 (C-13), 49.2 (C-14), 49.3 (C-5), 49.4 (C-10), 57.1 (C-17), 114.1 (C-7), 112.2, 114.7 (C-2, C-3), 127.8, 128.5 (C-4', C-5') 142.0 (C-8), 154.2, 155.7 (C-3', C-6'), 203.8 (C-1), 205.2 (C-4); UV: λ= 212-245 (ε= 15849-14125), λ_{max}= 260 (ε= 5888), λ= 394 (ε= 8318) λ(s)= 413 (ε= 6760); MS, *m/z*: 464 (M^+), 446, 351.

10b. Yellow solid isolated as above in 66% yield. ^1H NMR δ: 0.72 (3H, s, Me-18), 0.82 (3H, d, J= 6.6 Me-26), 0.83 (3H, d, J= 6.6, Me-27), 0.91 (3H, d, J= 6.6 Hz, Me-28), 1.01 (3H, d, J= 6.6 Hz, Me-21), 3.32 (2H, m, H-5 and H-10), 5.10 (1H, m, H-7), 5.20 (2H, m, H-22 and H-23), 7.20 (2H, s, H-4' and H-5'), 11.66 (1H, s, O*H*), 11.85 (1H, s, O*H*); MS, *m/z*: 476 (M^+).

Steroid-anthraquinone hybrids 11-14

General procedure: to a solution of Diels-Alder adducts **7-10** (1 mmol) in methylene chloride (15 ml), DBU (1,8-diazabicyclo[5.4.0]undec-7-ene) was added (1 mmol for **7**, 2.5 mmol for **8-10**). The resulting dark

solution was stirred for 3 h in an open flask. The reaction was quenched with HCl (0.5 N, 7 ml) and water (10 ml). The organic layer was washed with water and dried (Na_2SO_4), filtered and concentrated in vacuo. The residue was flash cromatographed (silica gel) to give hybrids **11-14**.

11a. Yellow solid. Eluted with petroleum ether - toluene 1:1; 90% yield. $[\alpha]_D$= +27.6° (c=0.42, $CHCl_3$); ^{1}H NMR δ: 0.57 (3H, s, Me-18), 0.88 (6H, d, J= 6.6 Hz, Me-26 and Me-27), 1.01 (3H, d, J= 5.8 Hz, Me-21), 1.74 (1H, dt, J= 14, 8.5 Hz, H-12), 2.13 (2H, m, H_2-15), 2.30 (1H, dt, J= 14.0, 5.7 Hz, H-12), 2.76 (1H, dd, J= 14.2, 6.8 Hz, H-14), 3.53 (2H, dd, J= 8.5, 5.7 Hz, H_2-11), 7,44 (1H, d, J= 7.8 Hz, H-7), 7.74 (2H, m, H-4' and H-5'), 8.19 (1H, d, J= 7.8 Hz, H-6), 8.23 (2H, m, H-3' and H-6'); ^{13}C NMR δ: 11.5 (C-18), 18.7 (C-21), 22.5, 22.8 (C-26, C-27), 23.8 (C-23), 24.3 (C-15), 27.4 (C-11), 28.0 (C-25), 28.5 (C-16), 36.0 (C-22), 36.1 (C-20), 37.0 (C-12), 39.4 (C-24), 41.4 (C-13), 52.5 (C-14), 55.6 (C-17), 125.6, 126.4, 127.2, 131.5, 133.2, 133.9 (C-3', C-4', C-5', C-6', C-6, C-7), 130.1, 132.7, 132.9, 135.2 (C-2, C-3, C-5, C-10), 141.2 (C-9), 149.1 (C-8), 183.7 (C-1), 185.7 (C-4); UV: λ= 200-218 (ε= 6607), λ_{max}= 266 (ε= 14454), λ= 320-328 (ε= 3311-3467); MS, *m/z*: 428 (M^+), 315; HRMS, *m/z*: 428.2704 (428.2715 calcd for $C_{30}H_{36}O_2$).

11b. Yellow solid. Eluted with petroleum ether - toluene 1:1; 90% yield. $[\alpha]_D$= +26.8° (c=1.73, $CHCl_3$); ^{1}H NMR δ: 0.59 (3H, s, Me-18), 0.85 (3H, d, J= 6.6 Hz, Me-26), 0.86 (3H, d, J= 6.6 Hz, Me-27), 0.95 (3H, d, J= 6.6 Hz, Me-28), 1.11 (3H, d, J= 6.6 Hz, Me-21), 2.74 (1H, dd, J= 14.2, 6.8 Hz, H-14), 3.53 (2H, dd, J= 8.5, 5.7 Hz, H_2-11), 5.25 (2H, m, H-22 and H-23), 7,42 (1H, d, J= 7.8 Hz, H-7), 7.74 (2H, m, H-4'and H-5'), 8.17 (1H, d, J= 7.8 Hz, H-6), 8.23 (2H, m, H-3' and H-6'); MS, *m/z*: 440 (M^+), 315; HRMS, *m/z*: 440.2710 (440.2715 calcd for $C_{31}H_{36}O_2$).

12a. Yellow solid. Eluted with petroleum ether - AcOEt - AcOH 95:5:0.1; 75% yield. $[\alpha]_D$= +48.5° (c=0.68, $CHCl_3$); ^{1}H NMR δ: 0.56 (3H, s, Me-18), 0.88 (6H, d, J= 6.6 Hz, Me-26 and Me-27), 1.01 (3H, d, J= 5.8 Hz, Me-21), 2.12 (2H, m, H_2-15), 2.30 (1H, dt, J= 14.2, 5.7 Hz, H-12), 2.70 (1H, dd, J= 14.2, 6.8 Hz, H-14), 3.50 (2H, dd, J= 8.5, 5.7 Hz, H_2-11), 7.24 (1H, d, J= 8.4 Hz, H-4'), 7.41 (1H, d, J= 7.9 Hz, H-7), 7.58 (1H, dd, J= 8.4, 7.4 Hz, H-5') 7.72 (1H, d, J= 7.4 Hz, H-6'), 8.13 (1H, d, J= 7.9 Hz, H-6), 12.92 (1H, s,O*H*); ^{13}C NMR δ: 11.4 (C-18), 18.7 (C-21), 22.5, 22.8 (C-26, C-27), 23.8 (C-23), 24.4 (C-15), 27.8 (C-11), 28.0 (C-25), 28.5 (C-16), 36.0 (C-22), 36.1 (C-20), 37.0 (C-12), 39.5 (C-24), 41.3 (C-13), 52.5 (C-14), 55.6 (C-17), 117.4 (C-3), 118.5 (C-4'), 124.3 (C-6'), 125.8 (C-6), 129.6 (C-5), 132.1 (C-7), 132.9 (C-2, C-10), 135.8 (C-5'), 141.8 (C-9), 149.1 (C-8), 162.3 (C-3'), 182.9 (C-1), 191.8 (C-4); UV: λ= 208-252 (ε= 13803-13182), λ_{max}= 263 (ε= 25119), λ(sh)= 274 (ε= 10000), λ= 360-404 (ε= 7244-7413) λ= 384 (ε= 7762); MS, *m/z*: 444 (M^+), 331, 289; HRMS, *m/z*: 444.2662 (444.2664 calcd for $C_{30}H_{36}O_3$).

12b. Yellow solid. Eluted with petroleum ether - AcOEt - AcOH 95:5:0.1, 85% yield. $[\alpha]_D$= +45.2° (c=0.50, $CHCl_3$); ^{1}H NMR δ: 0.59 (3H, s, Me-18), 0.84 (3H, d, J= 6.6 Hz, Me-26), 0.87 (3H, d, J= 6.6 Hz, Me-27), 0.94 (3H, d, J= 6.6 Hz, Me-28), 1.11 (3H, d, J= 6.6 Hz, Me-21), 2.75 (1H, dd, J= 14.2, 6.8 Hz, H-14), 3.54 (2H, m, H_2-11), 5.25 (2H, m, H-22 and H-23), 7.27 (1H, d, J= 8.4 Hz, H-4'), 7.44 (1H, d, J= 7.9 Hz, H-7), 7.61 (1H, dd, J= 8.4, 7.4 Hz, H-5') 7.75 (1H, d, J= 7.4 Hz, H-6'), 8.17 (1H, d, J= 7.9 Hz, H-6), 12.96 (1H, s,O*H*); MS, *m/z*: 456 (M^+), 332, 331, 329, 289; HRMS, *m/z*: 456.2669 (456.2664 calcd for $C_{31}H_{36}O_3$).

13a. Yellow solid. Eluted with petroleum ether - AcOEt - AcOH 95:5:0.1, 61% yield. $[\alpha]_D$= +47.4° (c=0.19, $CHCl_3$); ^{1}H NMR δ: 0.59 (3H, s, Me-18), 0.89 (6H, d, J= 6.6 Hz, Me-26 and Me-27), 1.00 (3H, d, J= 5.8 Hz, Me-21), 2.16 (2H, m, H_2-15), 2.30 (1H, m, H-12), 2.76 (1H, m, H-14), 3.52 (2H, m, H_2-11), 7.24 (1H, d, J= 8.4 Hz, H= 5'), 7.48 (1H, d, J= 7.9 Hz, H-7), 7.65 (1H, dd, J= 8.4, 7.4 Hz, H-4'), 7.76

(1H, d, J= 7.4 Hz, H-3'), 8.22 (1H, d, J= 7.9 Hz, H-6), 12.58 (1H, s, O*H*); ^{13}C NMR δ: 11.5 (C-18), 18.7 (C-21), 22.5, 22.8 (C-26, C-27), 23.8 (C-23), 24.4 (C-15), 27.4 (C-11), 28.0 (C-25), 28.5 (C-16), 36.0 (C-22), 36.1 (C-20), 36.9 (C-12), 39.5 (C-24), 41.5 (C-13), 52.6 (C-14), 55.6 (C-17), 115.7 (C-2), 119.3 (C-5'), 123.0 (C-3'), 125.3 (C-6), 130.2 (C-5), 131.5 (C-7), 132.5 (C-10), 135.3 (C-3), 136.5 (C-4') 141.7 (C-9) 150.0 (C-8), 161.8 (C-6'), 185.0 (C-4), 188.9 (C-1); UV: λ= 200-228 (ε= 13833-13148), λ_{max}= 258 (ε= 20892), λ(s)= 273 (ε= 10471), λ= 398-413 (ε= 7079-6918); MS, *m/z*: 444 (M^+), 331, 289; HRMS, *m/z*: 444.2661 (444.2664 calcd for $C_{30}H_{36}O_3$).

13b. Yellow solid. Eluted with petroleum ether - AcOEt - AcOH 95:5:0.1, 85% yield. $[\alpha]_D$= +28.6° (c=0.88, $CHCl_3$); ^{1}H NMR δ: 0.59 (3H, s, Me-18), 0.85 (3H, d, J= 6.6 Hz, Me-26), 0.88 (3H, d, J= 6.6 Hz, Me-27), 0.94 (3H, d, J= 6.6 Hz, Me-28), 1.11 (3H, d, J= 6.6 Hz, Me-21), 2.75 (1H, dd, J= 14.2, 6.8 Hz, H-14), 3.53 (2H, m, H_2-11), 5.25 (2H, m, H-22 and H-23), 7.26 (1H, d, J= 8.4 Hz, H= 5'), 7.47 (1H, d, J= 7.9 Hz, H-7), 7.65 (1H, dd, J= 8.4, 7.4 Hz, H-4'), 7.76 (1H, d, J= 7.4 Hz, H-3'), 8.22 (1H, d, J= 7.9 Hz, H-6), 12.61 (1H, s, O*H*); MS, *m/z*: 456 (M^+), 331, 332, 290, 289, 275; HRMS, *m/z*: 456.2667 (456.2664 calcd for $C_{31}H_{36}O_3$).

14a. Red solid. Eluted with petroleum ether - AcOEt - AcOH 90:10:0.1; 90% yield. $[\alpha]_D$= + 82.3° (c=0.53, $CHCl_3$); ^{1}H NMR δ: 0.54 (3H, s, Me-18), 0.87 (6H, d, J= 6.6 Hz, Me-26 and Me-27), 1.00 (3H, d, J= 5.8 Hz, Me-21), 2.12 (2H, m, H_2-15), 2.28 (1H, m, H-12), 2.80 (1H, m, H-14), 3.48 (2H, m, H_2-11), 7.20 (2H, s, H= 4' and H= 5'), 7.41 (1H, d, J= 7.9 Hz, H= 7), 8.13 (1H, d, J= 7.9 Hz, H-6), 12.87 (1H, s, O*H*), 13.12 (1H, s, O*H*); ^{13}C NMR δ: 11.4 (C-18), 18.7 (C-21), 22.5, 22.8 (C-26, C-27), 23.8 (C-23), 24.4 (C-15), 27.8 (C-11), 28.0 (C-25), 28.5 (C-16), 36.1 (C-20, C-22), 37.0 (C-12), 39.4 (C-24), 41.2 (C-13), 52.6 (C-14), 55.6 (C-17), 112.5, 113.9 (C-2, C-3), 125.3 (C-6), 127.9, 129.1 (C-4', C-5'), 129.7 (C-5), 132.0 (C-7), 132.6 (C-10), 142.2 (C-9) 149.8 (C-8), 156.8, 157.2 (C-3', C-6'), 187.1 (C-1), 190.1 (C-4); UV: λ= 200-234 (ε= 14125-131804), λ_{max}= 272 (ε= 13490), λ(s)= 283 (ε= 5370), λ_{max}= 356 (ε= 1995), λ= 467-494 (ε= 5754-5495); MS, *m/z*: 460 (M^+), 347; HRMS, *m/z*: 460.2615 (460.2614 calcd for $C_{30}H_{36}O_4$).

14b. Red solid. Eluted with petroleum ether - AcOEt - AcOH 90:10:0.1; 90% yield. $[\alpha]_D$= + 91.5° (c=0.60, $CHCl_3$); ^{1}H NMR δ: 0.59 (3H, s, Me-18), 0.84 (3H, d, J= 6.6 Hz, Me-26), 0.85 (3H, d, J= 6.6 Hz, Me-27), 0.94 (3H, d, J= 6.6 Hz, Me-28), 1.11 (3H, d, J= 6.6 Hz, Me-21), 2.79 (1H, dd, J= 14.2, 6.8 Hz, H-14), 3.57 (2H, m, H_2-11), 5.25 (2H, m, H-22 and H-23), 7.26 (2H, s, H= 4' and H= 5'), 7.48 (1H, d, J= 7.9 Hz, H= 7), 8.24 (1H, d, J= 7.9 Hz, H-6), 12.95 (1H, s, O*H*), 13.21 (1H, s, O*H*); MS, *m/z*: 472 (M^+), 347, 305, 291; HRMS, *m/z*: 472.2628 (472.2614 calcd for $C_{31}H_{36}O_4$).

Determination of the cytotoxic activity

Cells. J774 cells (murine monocyte/macrophage cells) were grown in suspension culture, in Techne stirrer bottles, spun at 25 rpm and incubated at 37°C in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% FBS (heat-inactivated foetal bovine serum), 25 mM HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid), glutamine (2mM), penicillin (100 U/ml) and streptomycin (100 μg/ml). GM7373 cells (bovine aortic endothelial cell line) were maintained in adhesion flask with MEM (Eagle's Minimum Essential Medium) with Earle's salts, supplemented with 10% FBS, 25 mM HEPES, glutamine (2mM), penicillin (100 U/ml), streptomycin (100 μg/ml) and enriched with 2% MEM non essential aminoacids and 2% MEM vitamin solution. IGR-1 cells (human melanoma cell lines) were grown in adhesion on

Petri dishes with MEM supplemented with 10% FBS, 25 mM HEPES, penicillin (100 U/ml) and streptomycin (100 µg/ml). P388 cells (murine leukemia cell lines) were grown in adhesion on Petri dishes with L-15 (Leibovitz) medium supplemented with 10% FBS, 25 mM HEPES, penicillin (100 U/ml) and streptomycin (100 µg/ml).

Cytotoxicity assay. J774, GM7373, IGR-1, P388, ($1x10^4$ cells) were plated on 96-well microtiter plates and allowed to adhere at 37°C in 5% CO_2/95% air for 2h. Thereafter the medium was replaced with 50µl of fresh medium and 75 µl aliquot of 1:2 v/v serial dilution of each test compound (dissolved in culture medium plus 10% dimethoxyethane) was added and then the cells incubated for 24, 48, 72 h. The cells viability was assessed through an [3-(4,5-dimethyltiazol-2yl)-2,5-phenyl-2H-tetrazolium bromide] (MTT) conversion assay.[22] Briefly, 25 µg of MTT (5 mg/ml) was added and the cells were incubated for additional 3 h. Following this time the cells were lysed and the dark blue crystals solubilized with 100 µl of a solution containing 50% (v:v) N,N-dimethylformamide, 20% (w:v) SDS (sodium dodecyl sulfate) with an adjusted pH of 4.5.[23] The optical density (OD) of each well was measured with a microplate spectrophotometer equipped with a 620 nm filter. The viability of each cell line in response to treatment with compounds **11-14** and doxorubicin was calculated as:

% dead cell = 100 - (OD treated/OD control) x 100; (OD: optical density).

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