Novel glycoconjugates of diospyrin, a quinonoid plant product: synthesis and evaluation of cytotoxicity against human malignant melanoma (A375) and laryngeal carcinoma (Hep2)

Madhushree Das Sarma,[a] Rina Ghosh,*[b] Amarendra Patra,[c] Rajdeep Chowdhury,[d] Keya Chaudhuri[d] and Banasri Hazra*[a]

Received 24th May 2007, Accepted 6th August 2007
First published as an Advance Article on the web 22nd August 2007
DOI: 10.1039/b707851j

Glycoside derivatives of diospyrin (**1**) were synthesized for the first time, and the cytotoxicity of the novel compounds *vis-à-vis* their precursors were evaluated against two human cancer cell lines, *viz.* malignant melanoma (A375) and laryngeal carcinoma (Hep2). The IC_{50} values were in the low micromolar range for all the compounds tested, and A375 cells showed comparatively greater sensitivity than Hep2. Most of the compounds exhibited enhanced activity as compared to the plant-derived quinonoid precursor of the series (**1**), while the aminophenyl mannosyl (**6**) was found to be the most effective derivative. In A375 cells, **6** ($IC_{50} = 0.02$ μM) showed the maximum increase in cytotoxicity (~35-fold) over that of **1** ($IC_{50} = 0.82$ μM). Again, when the glycosides were evaluated at a given concentration (0.1 μM) for their relative capacity to generate ROS from A375 cells, the compound **6** could produce the highest amount of ROS. Incidentally, this derivative also showed a comparatively lower toxicity ($IC_{50} \sim 41$ μM) when tested against normal human peripheral blood mononuclear cells, indicating a fair prospect of its development as a novel chemotherapeutic agent for the treatment of malignant melanoma.

Introduction

Natural products, many of which are glycosylated secondary metabolites, have inspired the development of a large number of modern pharmaceuticals, particularly against microbial and cancerous diseases.[1,2] The sugar moiety imparts unique chemical diversity to these metabolites, and also contributes towards modulation of their biological activity and pharmacokinetic properties at the tissue, cellular and molecular levels.[3] Furthermore, the carbohydrate has recently been shown to play critical roles in the recognition of DNA (calicheamicin),[4] RNA (streptomycin),[5] and membrane (amphotericin),[6] inhibition of translation (erythromycin),[7] as well as targeting of specific proteins (staurosporine)[8] and protein complexes (cardiac glycosides, *e.g.*, digitoxin).[9] Natural product-derived glycoconjugates like vancomycin, etoposide, anthracycline and aminoglycoside antibiotics have been designed to target microbial and viral infections as well as cancer.[10–15] Recently, assorted strategies such as total or semisynthesis and genetic engineering have been adopted to alter the glycosylation state of natural products, leading to combinatorial libraries of structurally diverse analogues.[16,17]

[a]*Department of Pharmaceutical Technology, Jadavpur University, Calcutta 700 032, India. E-mail: hazra1@vsnl.com, banasrihazra@yahoo.co.in; Fax: +91 33 2337 0752; Tel: +91 33 2337 0843*
[b]*Department of Chemistry, Jadavpur University, Calcutta 700 032, India. E-mail: ghoshrina@yahoo.com*
[c]*Department of Chemistry, University College of Science, Calcutta 700 009, India*
[d]*Molecular & Human Genetics Division, Indian Institute of Chemical Biology, Jadavpur, Calcutta 700 032, India*

Anthracycline antibiotics, which are highly functionalised glycosides containing the quinonoid chromophore, have been extensively used for more than four decades, and currently constitute the second largest class of anticancer drugs in clinical application.[18,19] Naturally occurring as well as semisynthetic anthracyclines have played an ever-increasing role, either alone or in combination with other clinical agents against cancer.[20] For example, daunorubicin and its hydroxylated analogue doxorubicin, isolated from various *Streptomyces* strains, are the prototypes of this family of antibiotics widely used for the treatment of various types of malignancy.[21] Daunorubicin is particularly useful against acute myelocytic and lymphocytic leukemia, whereas doxorubicin is the most effective single agent against soft-tissue sarcomas in adults, and also exhibits a broader spectrum of action in many solid tumors, such as osteosarcoma, non-Hodgkin's lymphoma, and carcinomas of breast, ovary, thyroid and lung.[22] However, the potential clinical utility of anthracyclines, as for the majority of antitumor drugs, is often limited by dose-limiting side effects like bone marrow suppression and cardiotoxicity, and also by the appearance of multi-drug resistance in tumor cells.[23] Hence, continuous efforts are in progress to design less toxic anthracycline analogues. Also, quinonoid prodrugs were created for antibody-directed enzyme-linked therapy to achieve a targeted concentration of the drug at the tumor site.[24]

Thus, designing of quinone-sugar conjugates would be an attractive proposition in the pursuit of novel anticancer chemotherapeutics with minimal side effects.[25] Presumably, the sugar moiety would play a dual role in the recognition of the receptor at the malignant site, and influence the pharmacokinetic drug distribution,[26] while the quinone part would contribute to the lipophilicity and redox cycling property of the compound.[27]

This journal is © The Royal Society of Chemistry 2007

Incidentally, several plant-derived anthraquinones (such as aloe-emodin, chrysophanol, quinizarin, *etc.*) and naphthoquinones (*e.g.* lapachol, lawsone, naphthazarin, 7-methyl juglone, *etc.*) have been successfully employed as synthons for preparing quinone–sugar hybrids with excellent regio and stereo-specificity.[28–31] It may be mentioned that several derivatives of diospyrin, a naturally occurring bisnaphthoquinone, were found to be active against murine and human cancer cells.[32–36] In this context, we envisaged that appropriately glycosylated adducts of diospyrin may have antitumor activity. Consequently, coupling of diospyrin and its derivatives with several carbohydrate moieties was achieved, which is presented here for the first time. Thereafter, cytotoxicity of the novel glycosides was evaluated in two human cancer cell lines, *viz.* A375 (malignant skin melanoma) and Hep2 (epidermoid laryngeal carcinoma), *in vitro*, followed by the estimation of reactive oxygen species (ROS) generation in A375 cells. Further, the toxicity of the test compounds on human peripheral blood mononuclear cells (PBMC) was estimated for a preliminary assessment of the therapeutic prospect of the glycosylated quinones.

Results and discussion

1. Synthesis

Diospyrin (**1**; crude; ~1 g) was isolated from the stem bark (1 kg) of *Diospyros montana* Roxb. (family: Ebenaceae), and purified following methods developed in our laboratory.[37] The structure has finally been confirmed to be 2,6′-bis (5-hydroxy-7-methyl-1,4-naphthoquinone) through total synthesis,[38] and recently, by crystallographic analysis.[39] In order to synthesise a glycoside derivative of **1**, Koenigs–Knorr glycosidation[40] of **1** with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide[41] was attempted, using freshly prepared silver oxide.[31] However, most of the starting material **1** (~80%) remained 'unreacted', even continuing the reaction for 3 days, while the desired product was obtained as an anomeric mixture (**2**; Fig. 1) with very poor yield (~15%).[42] Change of solvent or promoter in this reaction did not lead to any notable improvement in the overall yield of **2**. Incidentally, in a similar work carried out on another plant-derived hydroxynaphthoquinone (naphthazarin), Caygill *et al.* had reported the recovery of ~35% of the starting material, and the glycosylated product was obtained at a yield of ~29% only.[31] However, a moderate yield (~40%) was reported for a similar reaction carried out on 2-hydroxy-1,4-naphthoquinone (lawsone).[28] Probably, the peri-hydroxy groups in diospyrin and naphthazarin would not allow the optimum participation of these quinonoids in this glycosidation reaction.

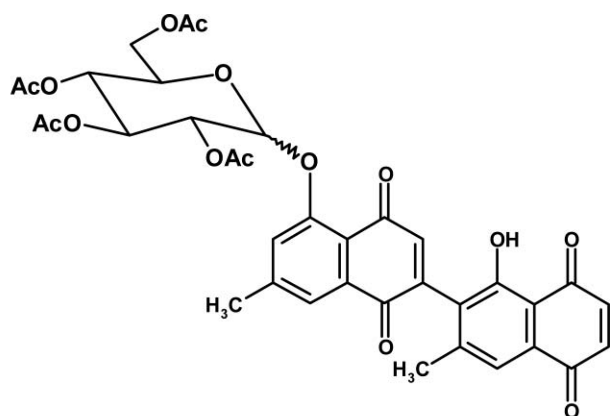


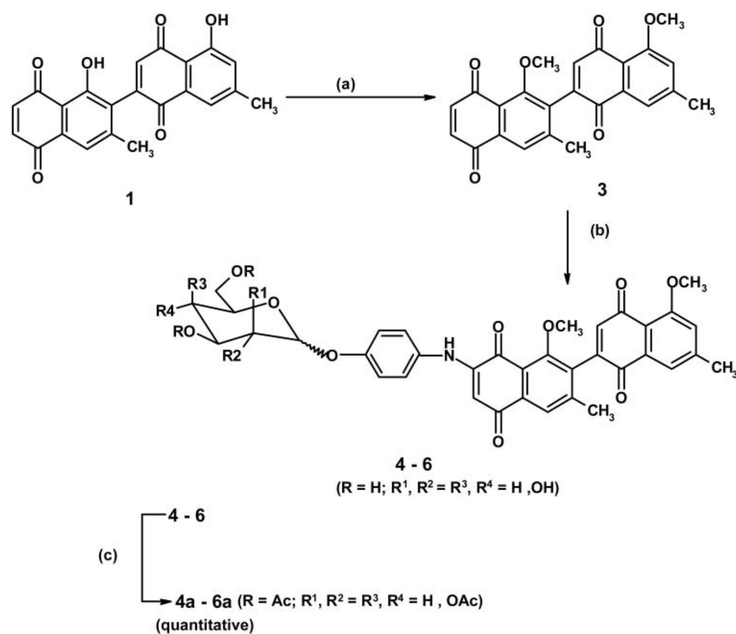
Fig. 1 Structure of 5-(2,3,4,6-tetra-*O*-acetyl-D-glucopyranosyloxy)diospyrin **2**.

Hence, in order to obtain a series of novel glycosyl analogues in substantial amounts for carrying out the desired biological studies, it was decided to utilise **3**, the dimethyl ether derivative of **1** as a synthon. Actually, the use of **3** had another advantage, because preparation of highly pure **1** through repeated crystallization of the 'crude' sample would have caused substantial loss of the precious natural product. On the other hand, 'crude' **1** could be converted almost quantitatively to **3** by treatment with methyl iodide and silver oxide, followed by purification over a neutral alumina column.[37] In fact, for carrying out the synthesis of various other derivatives in this hydroxy-bisnaphthoquinonoid series, **3** had always been preferred as a substrate in view of the apparent reluctance of **1** to undergo most of the chemical transformations involving the quinonoid moiety. It may be worth mentioning here that a series of amino-derivatives with promising antiproliferative activity were also obtained from **3**.[36,43] Hence, *p*-aminophenyl-D-glycosides were chosen to carry out 1,4-Michael addition on **3**, whereupon the expected adducts (**4–6**) were obtained in fairly good yields (80–92%; Scheme 1). It may be noted that although the aforesaid glycoside reagent has been widely documented in formulation of glycosylated liposomes for encapsulation and targeted delivery of chemotherapeutic agents,[44,45] to the best of our knowledge, the same has not been commonly encountered in the literature for the synthesis of anchored natural product–sugar conjugates. Further modification of **4–6** was carried out by using pyridine–acetic anhydride reagent to produce the corresponding acetyl derivatives (**4a–6a**). Incidentally, under the reaction condition given in Scheme 1, only the –OH groups were preferentially acetylated leaving the –NH–group unreacted, as confirmed by the NMR, MS and elemental analyses.

The position of the side-chain substitutions with the *p*-aminophenyl-D-glycoside moiety in compounds **4**, **4a**, **5**, **5a**, **6** and **6a**, was established unequivocally from one-dimensional NMR [^{1}H, ^{13}C (NDC and DEPT-135)] spectral studies, and two-dimensional ^{13}C-^{1}H NMR correlations optimized for $^1J_{C-H} \approx 160$ Hz and $^3J_{C-H} \approx 7$ Hz. Taking **4** as a representative structure (Fig. 2), the most downfield proton resonance observed at δ 7.75 (s) was assignable to H-8′, and in the long-range HETCORR spectrum, this proton showed correlation to the carbonyl carbon (C-1′) resonating at δ 184.6. Incidentally, the NH proton resonating at δ 7.49 had correlation with a different carbonyl carbon resonance (displayed at δ 180.6 in **4**) and was assignable to C-4′. The amino-sugar substituents in **4** could be either at C-2′ or C-3′. The location at C-3′ was conclusively settled from the long-range correlation observed between the C-4′ signal and H-2′ signal (δ 5.94) in consonance with the structures of 3′-substituted analogues of diospyrin dimethyl ether.

In Scheme 2, we describe the synthesis of a couple of modified sugar derivatives, based on an ethanolamine derived from diospyrin dimethyl ether (**7**),[36] in which the aromatic (phenyl) spacer has been replaced by an aliphatic one (**8** and **9**). Thus, **7** was treated with a tri-*O*-acetyl-D-glycal[46] in the presence of anhydrous

 This journal is © The Royal Society of Chemistry 2007



Sugar substrate (**A**)	Glycoside adduct (anomer) [Acetyl adduct]	R	R^1	R^2	R^3	R^4	Time	% Yield
p-Aminophenyl-*β*-D-glucopyranoside	**4** (*β*)	H	H	OH	H	OH	25 h	80
	[**4a** (*β*)]	Ac	H	OAc	H	OAc	12 h	quantitative
p-Aminophenyl-*β*-D-galactopyranoside	**5** (*β*)	H	H	OH	OH	H	41 h	92
	[**5a** (*β*)]	Ac	H	OAc	OAc	H	12 h	quantitative
p-Aminophenyl-*α*-D-mannopyranoside	**6** (*α*)	H	OH	H	H	OH	34 h	86
	[**6a** (*α*)]	Ac	OAc	H	H	OAc	12 h	quantitative

Scheme 1 Synthesis of glycosylated adduct of diospyrin dimethyl ether. *Reagents and conditions:* (a) CH_3I, Ag_2O, $CHCl_3$, stir, rt; (b) *p*-aminophenyl α or β-D-glycopyranoside (A), $CHCl_3$, EtOH, reflux, N_2 atm; (c) Ac_2O, pyridine, rt, 12 h.

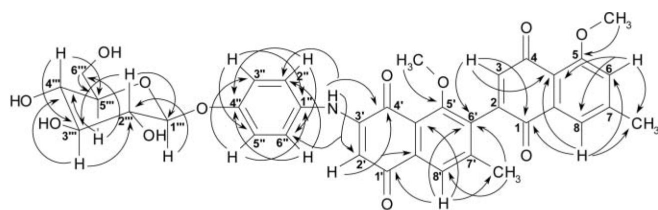


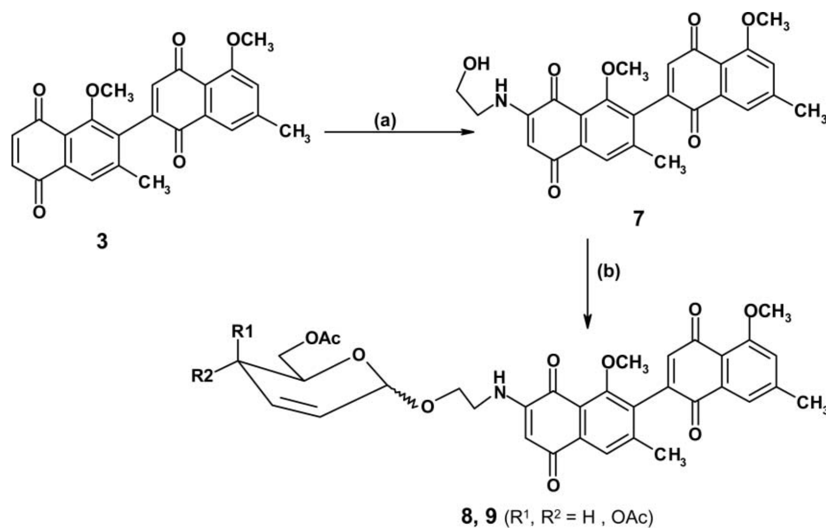
Fig. 2 Long range ^{13}C-^{1}H correlations of **4**.

indium chloride, when the corresponding 2,3-unsaturated glycosides were obtained through Ferrier rearrangement in ~72–80% yield, with the α-anomer as the major product.[47] The anomeric mixtures were separated by semi-preparative RP-HPLC. In the case of the galactal derivative (**9**), both the anomers were isolated, whereas only the major α-isomer of the glucal derived product could be collected for further assessment. Incidentally, in this case, anhydrous $InCl_3$[48–50] was found to be the most effective activator, whereas other Lewis acids, like $In(OTf)_3$[51] and $Yb(OTf)_3$,[52,53] failed to give the desired result.

2. Antitumor activity

The aforesaid glycoconjugates, their precursors and two standard clinical agents (doxorubicin and camptothecin) were evaluated for antiproliferative activity against two human cancer cell lines, *viz.* malignant melanoma (A375) and laryngeal carcinoma (Hep2) by MTT assay, based on colorimetric estimation of the blue formazan compound produced by reduction of the tetrazolium ring in MTT.[54] Subsequently, the effect of these compounds on PBMC were also assessed similarly. Marked inhibition was observed in the growth of tumor cells incubated with each of the compounds for 24 h. It was obvious from the respective IC_{50} values (Table 1) that the cytotoxicity profile of the natural product could be substantially enhanced, in most of the cases, through its conversion to glycosides. Incidentally, as compared to Hep2, the A375 tumor cells were found to be more sensitive to treatment with these drugs, as had been observed previously with a few other derivatives of diospyrin.[36]

The derivative **2** obtained through the direct glycosylation of diospyrin (Fig. 1) did not show notable improvement in its cytotoxicity profile. Hence, apropos of the earlier discussion on its poor yield, it was not worthwhile to expand the study on this



Sugar substrate (**B**)	Glycoside	R^1	R^2	% Yield	(α: β) [a]
Tri-*O*-acetyl–D-glucal	**8**	H	OAc	72	(10: 1) [b]
Tri-*O*-acetyl–D-galactal	**9**	OAc	H	80	(3: 2) [c]

[a] (α: β) diastereomeric ratio obtained from semipreparative RP-HPLC, eluted with isocratic mobile phase CH_3CN: H_2O at a flow rate of 0.6 mL/min at ambient temperature.

[b] CH_3CN: H_2O = 80: 20, v/v.

[c] CH_3CN: H_2O = 85: 15, v/v.

Scheme 2 Synthesis of glycosylated adduct of ethanolamine derivative of diospyrin dimethyl ether. *Reagents and conditions:* (a) $HOCH_2CH_2NH_2$, $CHCl_3$, EtOH 0–10 °C, 3 h; (b) tri-*O*-acetyl-D-glycal (B), anhydrous $InCl_3$, DCM, rt, 2 days.

Table 1 Evaluation of cytotoxicity towards tumor cells and PBMC by diospyrin and its derivatives

Compound	$IC_{50}/\mu M$ [a]		
	A375	Hep2	PBMC
1	0.82 ± 0.03	3.58 ± 0.56	78.32 ± 3.41
2 [b]	1.03 ± 0.05	4.24 ± 1.04	72.65 ± 2.53
3	0.20 ± 0.02	0.39 ± 0.03	53.26 ± 3.80
4	0.52 ± 0.02	1.80 ± 0.08	>100.0
4a	0.67 ± 0.02	0.48 ± 0.04	32.71 ± 4.64
5	0.13 ± 0.01	4.54 ± 1.28	41.91 ± 4.12
5a	0.17 ± 0.07	0.85 ± 0.08	>100.0
6	0.02 ± 0.01	0.26 ± 0.05	41.27 ± 2.82
6a	0.19 ± 0.03	2.95 ± 0.26	24.63 ± 2.13
7	3.18 ± 0.09	1.41 ± 0.16	21.82 ± 1.40
8α	0.19 ± 0.04	5.68 ± 1.38	59.34 ± 3.62
9α	0.06 ± 0.01	0.66 ± 0.07	74.98 ± 1.84
9β	0.06 ± 0.01	1.01 ± 0.10	79.15 ± 6.81

[a] Inhibitory concentration to reduce 50% cell growth evaluated by MTT assay. Data represent mean values (±SE) for three independent determinations. [b] With anomeric mixture (α–β 1 : 4) $IC_{50}/\mu M$ of doxorubicin, clinically used anticancer drug with a quinonoid structure, in A375: 0.007 ± 0.001; Hep2: 0.42 ± 0.04; PBMC: 15.51 ± 1.74. $IC_{50}/\mu M$ of camptothecin, anticancer drug as 'standard clinical agent', in A375: 0.003 ± 0.001; Hep2: 20.13 ± 2.42; PBMC: 32.28 ± 2.04.

series of analogues any further. On the other hand, among the next series of six aminophenyl glycopyranosides (Scheme 1), four showed enhanced cytotoxicity in A375 cells, and a marked change was observed in the mannosyl adduct (**6**), which was found to be approximately 10-fold more active than its synthon, **3**, with IC_{50} values of 0.02 and 0.20 μM, respectively. However, these conjugates did not exhibit such activity against Hep2 cells, although the mannosyl adduct (**6**) was the sole exception, showing an IC_{50} value of 0.26 μM, which was comparatively less than that of **3** (0.39 μM). Further, the structural modifications of **3** led to significant reduction in cytotoxicity of two of the derivatives (**4** and **5a**; IC_{50} > 100 μM) towards 'normal' PBMC. Again, it was interesting to compare the IC_{50} values of the three glycopyranosides (**4**, **5** and **6**) with respect to the corresponding *O*-acetylated products (**4a**, **5a** and **6a**). In the case of the mannosyl conjugate (**6a**), acetylation did not lead to any improvement, considering its effect on the tumors as well as on the 'normal' PBMC. However, for both glucosyl (**4**) and galactosyl (**5**) adducts, the acetylated sugars produced 4- to 5-fold enhanced cytotoxicity towards Hep2 cells.

In Scheme 2, the compound **7** (IC_{50} = 3.18 μM), when converted to its glucal derivative, **8**α, produced ~17-fold enhanced activity in A375 cells, while a more dramatic improvement (>50-fold) was observed for its galactal analogues (**9**α and **9**β). In addition, the glycals, **8**α, **9**α and **9**β, were relatively less toxic to the normal lymphocytes as compared to **7**.

Taken together, the ten glycosides presented above generally displayed a greater specificity towards the human melanoma cell line, rather than Hep2. The compounds **6**, **9**α and **9**β were the most cytotoxic in A375, with IC_{50} values at a low micromolar level, while the rest were also more effective than the natural diospyrin (**1**),

only **2** being an exception. However, when tested against Hep2, no substantial advantage of glycosylation could be observed; in fact, compounds **2**, **5** and **8α** showed IC_{50} values higher than the respective precursors.

In view of above, diospyrin glycoconjugates could be considered as prospective 'leads' for designing novel therapeutic agents against malignant melanoma, which is the most aggressive form of cutaneous cancer.[55] Reportedly growing at an annual rate of 5% in USA, the incidence of melanoma is becoming rather alarming, particularly among Caucasians in many countries.[56] For the treatment of disseminated melanoma, chemotherapy would be the standard option, despite the recent advances in immunotherapy and vaccines against cancer. Several plant-derived chemotherapeutic agents, such as vinorelbine tartrate and paclitaxel, have been clinically applied to patients with advanced malignant melanoma.[57,58] Unfortunately, metastatic melanoma cells tend to escape the induction of apoptotic death, and thereby acquire resistance to conventional chemotherapeutic agents. Thus, multidrug resistance has been frequently observed in such cases, hence, new drugs with novel modes of action might help to increase the sensitivity of melanoma to chemotherapeutic treatments.[59] Since diospyrin analogues are known to induce apoptosis in several human cancer cell lines, it would be relevant to explore the mechanism of action of its glycosides in this respect.[34] Further, involvement of a ROS-mediated pathway was established,[35] revealing dramatic changes in mitochondrial transmembrane potential and other associated events signaling apoptotic cell death, in MCF-7 cells, caused by diospyrin diethyl ether.[60,61] This prompted us to evaluate glycoside analogues for the ability to generate ROS in melanoma cells using fluorimetric assessment.[35,62] It was found that all quinonoids generated ROS in a dose-dependent manner (data not shown). However, for a comparative analysis, the R.F.I. values for all of them was determined at a concentration of 0.1 μM, and substantial generation of ROS was recorded by all the quinonoids as presented in Fig. 3. It was interesting to find that among all the glycosides, the highest ROS-generators, *viz.* **6**, **9α** and **9β** (~5.5 to 7-fold with respect to the untreated control cells) were the most active in terms of the IC_{50} values as well (Table 1).

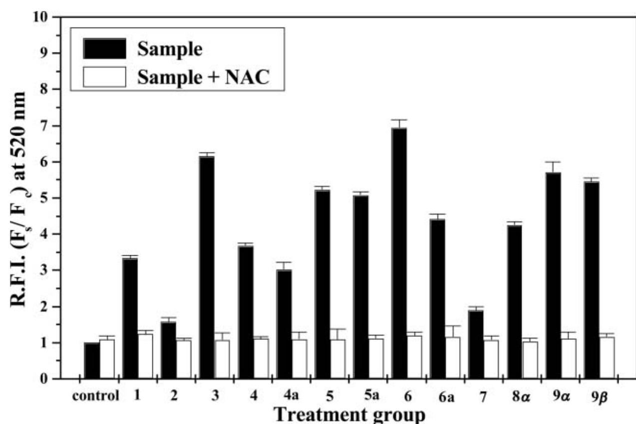


Fig. 3 ROS generation, *in vitro*, in A375 cells (2×10^5 per mL) treated with quinonoids (0.1 μM) in the presence or absence of pre-incubation with NAC (100 μM), followed by incubation with DCFH-DA (10 μM) for 20 min at 37 °C. R.F.I. = relative fluorescence intensity of sample (Fs) with respect to control (Fc). Error bars represent the standard error in each group ($n = 3$).

Table 2 Retention time of diospyrin derivatives

Compound	R_t/min [a]
3	12.3
6	3.8
7	5.0
8α	15.2

[a] Retention time for pure diospyrin analogues obtained from analytical RPHPLC using isocratic eluent, acetonitrile–water = 50 : 50 (v/v) at a flow rate of 1.0 mL min^{-1} at ambient temperature followed by UV detection at 255 nm.

Again, a parallel experiment with addition of *N*-acetyl cysteine (NAC), a specific scavenger for H_2O_2, showed dramatic reduction in fluorescence intensity, almost to the level of 'untreated control'. This observation would confirm the formation of H_2O_2 as the predominant ROS generated by **1** and its derivatives.[62]

In view of the above observation on generation of intracellular ROS through cellular uptake of the compounds, it was decided to determine the fate of the glycosides following their incubation with the A375 tumor cells. Thus, **6** and **8α** were chosen for a stability study involving HPLC analysis of the sample containing the cellular metabolites, as given in Table 2 and Fig. 4. HPLC chromatograms, with appropriate spiking, confirmed the presence of both glycosides in the sample, even after 24 h of incubation, with formation of one major and a few minor metabolites, none of which corresponded with their respective quinonoid precursors (Fig. 4). When estimated after 3 and 24 h, the amount of the 'unchanged' glycoside derivative, **6**, in the sample was found to be ~62 and 42%, respectively, while the major metabolite accounted for ~13 and 30%, respectively. A similar trend was observed for the derivative **8α**, as given in Fig. 4. The results indicate that the glycoside derivatives (**6** and **8α**) are responsible for enhanced antiproliferative activity in A375 cells and not their precursors, **3** and **7**, respectively.

The A375 cells treated for 24 h with compounds **1** and **6** were fixed and stained with Giemsa, and suitably mounted for photomicroscopic observation (Fig. 5). Rounding of cells with condensed nuclei was observed at 2 μM concentration of **1**. Additionally, irregular plasma membrane shapes with blebbing were also found, which was not observed at 1μM concentration of **1** (data not shown). With compound **6**, at 0.1 μM, the cells were found to be in condensed form with visible intracellular granules. In contrast, the control cells maintained a regular plasma membrane without blebbing, with few intracellular granules and little condensation of cytoplasm.

Experimental section

1. Chemistry

p-Aminophenyl-β-D-glucopyranoside, *p*-aminophenyl-β-D-galactopyranoside and *p*-aminophenyl-α-D-mannopyranoside were purchased from Sigma Chemical Company, USA; anhydrous indium trichloride was procured from CDH, India. All other reagents and solvents used were obtained from Sisco Research Laboratory, India. Column chromatography was performed on silica gel (60–120 mesh) and preparative TLC on 20 cm × 20 cm glass plates coated with a 2 mm layer of silica gel G from Merck,

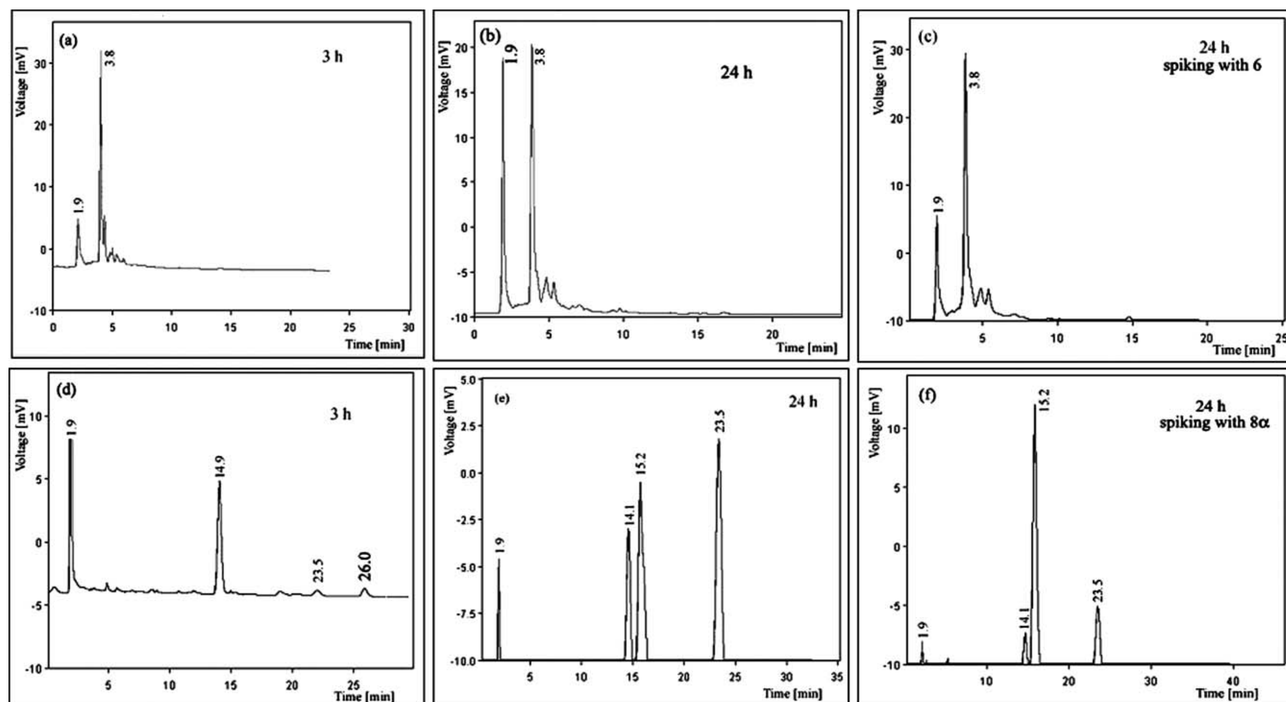


Fig. 4 *In vitro* stability of glycoside derivatives of diospyrin at 37 °C in A375 tumor cells by RPHPLC analysis. The chromatograms indicate analyses after (a) 3 h incubation of compound **6**; (b) 24 h incubation of compound **6**; (c) spiking with pure **6** after 24 h incubation; (d) 3 h incubation of compound **8**α; (e) 24 h incubation of compound **8**α; (f) spiking with pure **8**α after 24 h incubation.

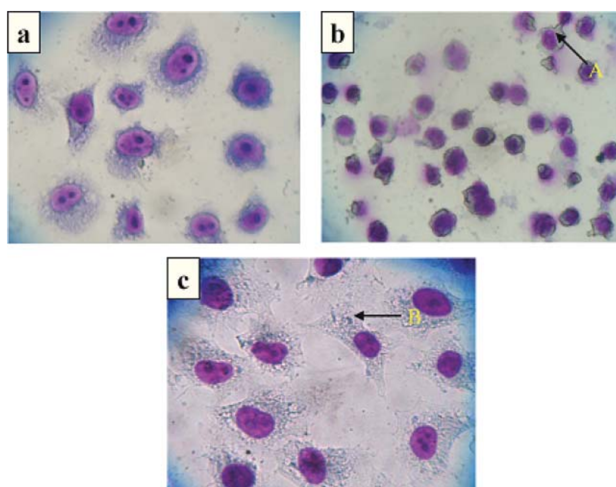


Fig. 5 Morphological observation of the cultured A375 cells under a light microscope [magnification 1000 ×]. (a) Untreated control cells showing regular plasma membrane without blebbing. (b) Cells treated with **1** (2 μM) for 24 h showing condensed nuclei with plasma membrane blebbing (A). (c) Cells treated with **6** (0.1 μM) for 24 h showing intracellular granules (B).

India. Petroleum ether was used in the boiling range of 60–80 °C. All organic solvents were distilled prior to use. Melting points were determined on Toshniwal melting point apparatus (cat no: CL-0301) and are uncorrected. Optical rotations were determined with an Atago, POLAX 2 L polarimeter, Japan. UV–Vis absorption spectra were recorded with a Shimadzu UV 1601 spectrophotometer. IR spectra were obtained on a Perkin-Elmer RXI FT-IR spectrophotometer system in KBr pellets. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AM 300 L Supercon NMR spectrometer operating at 300.13 and 75.47 MHz, respectively. Chemical shifts were expressed in ppm (δ) downfield relative to the internal reference Me_4Si and J values were reported in Hertz (Hz). The splitting pattern abbreviations in the ^{1}H spectra are as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, bs = broad singlet. FAB MS was run on a JEOL JMS600 and ESI MS were run on a WATERS Micromass Q-Tof microinstrument. Elemental analyses were carried out on a Perkin-Elmer instrument 2400 Series II CHN analyzer. Results obtained were within ±0.3% of the theoretical value. Semipreparative HPLC was performed on a Shimadzu LC-10 ATVP model instrument using a reverse phase C_{18} phenomenex column (250 mm × 10 mm i.d.; particle size 5 μm), with a UV–Vis variable wavelength detector set at 255 nm. Elution was carried out with isocratic mobile phase acetonitrile–water at a flow rate of 0.6 mL min^{-1} at ambient temperature.

General procedure for the preparation of 3′-[4-(D-glycopyranosyloxy)anilinyl]diospyrin dimethyl ether 4, 5 and 6

A mixture of diospyrin dimethyl ether (80 mg, 0.2 mmol) and *p*-aminophenyl-D-glycopyranoside (27 mg, 0.1 mmol) in chloroform (4 mL), ethanol (2 mL) and distilled water (4 drops) was refluxed at 80 °C under a nitrogen atmosphere. The mixture was then evaporated to dryness under reduced pressure. The residue thus obtained was subjected to preparative TLC on silica gel G using the solvent mixture $CHCl_3$–EtOAc–MeOH = 3 : 3 : 2 (v/v/v) to furnish the desired glycosyl derivative.

This journal is © The Royal Society of Chemistry 2007

Acetylation of 4, 5 and 6

The prepared glycosides *viz.* **4**, **5** and **6** were subjected to acetylation by stirring overnight with acetic anhydride and pyridine to furnish, after usual work-up, the corresponding acetylated products **4a**, **5a** and **6a** respectively in almost quantitative yield.

3′-[4-(β-D-Glucopyranosyloxy)anilinyl]diospyrin dimethyl ether 4

53.5 mg, yield 80%, dark red powder, mp 130–138 °C (from chloroform–petroleum ether). TLC R_f 0.45 (chloroform–ethyl acetate–methanol = 3 : 3 : 1, v/v/v). $[\alpha]_D^{29}$ −11.6° (*c* 1.18 in CH_3OH). Found: C, 64.51; H, 4.97; N, 2.16. Calc. for $C_{36}H_{33}NO_{12}$: C, 64.38; H, 4.95; N, 2.09%. UV–Vis λ_{max} (CH_3OH)/nm 224, 266 and 406 (ε/dm^3 mol^{-1} cm^{-1} 15 488, 15 849 and 3630). IR ν_{max}(KBr)/cm^{-1} 3428, 2925, 1658, 1606, 1515, 1460, 1354, 1257 and 1071. δ_H (300 MHz; CD_3OD; Me_4Si) 2.27 (3H, s, 7′-CH_3), 2.43 (3H, s, 7-CH_3), 3.34–3.43 (4H, m, H-2‴, H-3‴, H-4‴, H-5‴), 3.62 (3H, s, 5′-OCH_3), 3.67 (1H, dd, J_1 = 12.0 Hz, J_2 = 5.1 Hz, H_a-6‴), 3.86 (1H, dd, J_1 = 12.0 Hz, J_2 = 1.7 Hz, H_b-6‴), 3.92 (3H, s, 5-OCH_3), 4.87 (1H, d, J = 7.3 Hz, H-1‴), 5.94 (1H, s, H-2′), 6.79 (1H, s, H-3), 7.12 (2H, d, J = 9.0 Hz, H-2″ and H-6″), 7.18 (1H, s, H-6), 7.21 (2H, d, J = 9.0 Hz, H-3″ and H-5″), 7.49 (1H, s, -NH), 7.68 (1H, s, H-8), 7.75 (1H, s, H-8′). δ_C (75 MHz; CD_3OD; Me_4Si) 21.0 (7′-CH_3), 22.3 (7-CH_3), 56.9 (5-OCH_3), 62.6 (C-6‴), 62.8 (5′-OCH_3), 71.4 (C-3‴), 74.9 (C-4‴), 77.9 (C-5‴), 78.2 (C-2‴), 101.2 (C-1‴), 102.5 (C-2′), 118.5 (C-4a), 118.8 (C-2″ and C-6″), 120.1 (C-6), 121.4 (C-8), 121.5 (C-4′a), 124.7 (C-8′), 126.4 (C-3″ and C-5″), 133.3 (C-1″), 135.2 (C-8′a), 135.3 (C-6′), 136.6 (C-3′), 141.3 (C-3), 145.1 (C-8a), 147.2 (C-7′), 148.8 (C-7), 149.5 (C-2), 157.2 (C-4″), 159.8 (C-5′), 161.4 (C-5), 180.6 (C-4′), 184.6 (C-1′), 184.9 (C-4), 185.4 (C-1). ESI-MS: 672 (M + H), 694 (M + Na).

3′-[4-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyloxy)anilinyl]diospyrin dimethyl ether 4a

Yield almost quantitative, orange powder, mp 174 °C (from dichloromethane–diethyl ether). TLC R_f 0.59 (chloroform–ethyl acetate = 3 : 2, v/v). $[\alpha]_D^{28}$ −13.8° (*c* 1.0 in $CHCl_3$). Found: C, 62.88; H, 4.86; N, 1.61. Calc. for $C_{44}H_{41}NO_{16}$: C, 62.93; H, 4.92; N, 1.67%. UV–Vis λ_{max} ($CHCl_3$)/nm 262, and 406 (ε/dm^3 mol^{-1} cm^{-1} 25 119 and 5754). IR ν_{max}(KBr)/cm^{-1} 3335, 2930, 1753, 1658, 1598, 1503, 1351, 1252, 1065. δ_H (300 MHz; $CDCl_3$; Me_4Si) 2.04–2.09 (12H, 4 × s, 4 × $COCH_3$), 2.29 (3H, s, 7′-CH_3), 2.51 (3H, s, 7-CH_3), 3.72 (3H, s, 5′-OCH_3), 3.86–3.90 (1H, m, H-5‴), 4.04 (3H, s, 5-OCH_3), 4.19 (1H, dd, J_1 = 12.4 Hz, J_2 = 1.4 Hz, H_a-6‴), 4.31 (1H, dd, J_1 = 12.3 Hz, J_2 = 5.1 Hz, H_b-6‴), 5.08 (1H, d, J = 7.2 Hz, H-1‴), 5.18 (1H, t, J = 9.3 Hz, H-4‴), 5.25–5.35 (2H, m, H-2‴ and H-3‴), 6.22 (1H, s, H-2′), 6.80 (1H, s, H-3), 7.05 (2H, d, J = 8.7 Hz, H-2″ and H-6″), 7.17 (1H, s, H-6), 7.21 (2H, d, J = 8.9 Hz, H-3″ and H-5″), 7.58 (1H, s, -NH), 7.62 (1H, s, H-8), 7.88 (1H, s, H-8′). δ_C (75 MHz; $CDCl_3$; Me_4Si) 20.4, 20.5, 20.6, 20.7 (4 × $COCH_3$), 20.9 (7′-CH_3), 22.3 (7-CH_3), 56.5 (5-OCH_3), 61.9 (5′-OCH_3), 62.3 (C-6‴), 68.2 (C-4‴), 71.2 (C-2‴), 72.2 (C-5‴), 72.7 (C-3‴), 99.3 (C-1‴), 101.8 (C-2′), 117.7 (C-4a), 118.3 (C-2″ and C-6″), 118.5 (C-6), 120.7 (C-4′a), 120.7 (C-8), 124.2 (C-8′), 124.7 (C-3″ and C-5″), 132.4 (C-1″), 132.8 (C-8′a), 134.0 (C-6′), 135.1 (C-3′), 140.0 (C-3), 143.4 (C-8a), 146.1 (C-7), 146.2 (C-7′), 146.7 (C-2), 154.6 (C-4″), 158.6 (C-5′), 159.9 (C-5), 169.3, 169.4, 170.2, 170.5 (4 × $OCOCH_3$), 179.7 (C-4′), 183.0 (C-1′), 183.3 (C-4), 184.1 (C-1). ESI-MS: 862 (M + Na).

3′-[4-(β-D-Galactopyranosyloxy)anilinyl]diospyrin dimethyl ether 5

Yield 61.5 mg, 92%, dark red powder, mp 142–146 °C (from chloroform–petroleum ether). TLC R_f 0.45 (chloroform–ethyl acetate–methanol = 3 : 3 : 1, v/v/v). $[\alpha]_D^{29}$ −23.8° (*c* 0.58 in CH_3OH). Found: C, 64.29; H, 4.89; N, 2.03. Calc. for $C_{36}H_{33}NO_{12}$: C, 64.38; H, 4.95; N, 2.09%. UV–Vis λ_{max} (CH_3OH)/nm 219, 267 and 405 (ε/dm^3 mol^{-1} cm^{-1} 28 840, 25 119 and 6166). IR ν_{max}(KBr)/cm^{-1} 3398, 2927, 1657, 1606, 1514, 1459, 1353, 1256, 1069. δ_H (300 MHz; CD_3OD; Me_4Si) 2.26 (3H, s, 7′-CH_3), 2.44 (3H, s, 7-CH_3), 3.37–3.42 (4H, m, H-2‴, H-3‴, H-4‴ and H-5‴), 3.57 (1H, dd, J_1 = 11.9 Hz, J_2 = 4.7 Hz, H_a-6‴), 3.64 (3H, s, 5′-OCH_3), 3.85 (1H, br d, J = 11.9 Hz, H_b-6‴), 3.94 (3H, s, 5-OCH_3), 4.85–4.89 (1H, m, H-1‴), 5.96 (1H, s, H-2′), 6.80 (1H, s, H-3), 7.11 (2H, dd, J_1 = 9.2 Hz, J_2 = 2.6 Hz, H-2″ and H-6″), 7.14 (1H, s, H-6), 7.22 (2H, dd, J_1 = 9.1 Hz, J_2 = 2.6 Hz, H-3″ and H-5″), 7.33 (1H, s, -NH), 7.51 (1H, s, H-8), 7.75 (1H, s, H-8′). δ_C (75 MHz; $CDCl_3$; Me_4Si) 20.9 (7′-CH_3), 22.2 (7-CH_3), 56.9 (5-OCH_3), 62.6 (C-6‴), 62.8 (5′-OCH_3), 71.4 (C-3‴), 74.9 (C-4‴), 78.0 (C-5‴), 78.2 (C-2‴), 101.2 (C-1‴), 102.5 (C-2′), 118.5 (C-4a), 118.8 (C-2″ and C-6″), 120.2 (C-6), 121.4 (C-8), 121.6 (C-4′a), 124.7 (C-8′), 126.5 (C-3″ and C-5″), 133.3 (C-1″), 135.2 (C-8′a), 135.4 (C-6′), 136.6 (C-3′), 144.4 (C-3), 145.2 (C-8a), 147.2 (C-7′), 148.8 (C-7), 149.6 (C-2), 157.2 (C-4″), 159.8 (C-5′), 161.4 (C-5), 180.7 (C-4′), 184.7 (C-1′), 184.9 (C-4), 185.4 (C-1). ESI-MS: 672 (M + H), 694 (M + Na).

3′-[4-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyloxy)anilinyl]diospyrin dimethyl ether 5a

Yield almost quantitative, orange powder, mp 168 °C (from dichloromethane–diethyl ether). TLC R_f 0.59 (chloroform–ethyl acetate = 3 : 2, v/v). $[\alpha]_D^{28}$ +11.6° (*c* 1.5 in $CHCl_3$). Found: C, 62.84; H, 4.90; N, 1.72. Calc. for $C_{44}H_{41}NO_{16}$: C, 62.93; H, 4.92; N, 1.67%. UV–Vis λ_{max} ($CHCl_3$)/nm 264, and 404 (ε/dm^3 mol^{-1} cm^{-1} 26 303 and 6026). IR ν_{max}(KBr)/cm^{-1} 3325, 2928, 1752, 1661, 1606, 1515, 1460, 1363, 1224, 1070. δ_H (300 MHz; $CDCl_3$; Me_4Si) 2.06–2.08 (12H, 4 × s, 4 × $COCH_3$), 2.30 (3H, s, 7′-CH_3), 2.52 (3H, s, 7-CH_3), 3.73 (3H, s, 5′-OCH_3), 4.05 (3H, s, 5-OCH_3), 4.09–4.13 (2H, m, H-5‴ and H_a-6‴), 4.20–4.35 (1H, m, H_b-6‴), 5.39 (1H, t, J = 9.9 Hz, H-2‴), 5.48 (1H, bs, H-4‴), 5.54 (1H, bs, H-1‴), 5.56 (1H, dd, J_1 = 10.2 Hz, J_2 = 3.4 Hz, H-3‴), 6.23 (1H, s, H-2′), 6.81 (1H, s, H-3), 7.15 (2H, d, J = 8.8 Hz, H-2″ and H-6″), 7.18 (1H, s, H-6), 7.27 (2H, d, J = 9.7 Hz, H-3″ and H-5″), 7.61 (1H, bs, -NH), 7.63 (1H, bs, H-8), 7.89 (1H, s, H-8′). δ_C (75 MHz; $CDCl_3$; Me_4Si) 20.6, 20.7, 20.8 (4 × $COCH_3$), 20.9 (7′-CH_3), 22.3 (7-CH_3), 56.5 (5-OCH_3), 62.1 (C-6‴), 62.3 (5′-OCH_3), 65.9 (C-4‴), 68.8 (C-2‴), 69.3 (C-5‴), 69.4 (C-3‴), 96.1 (C-1‴), 101.8 (C-2′), 117.7 (C-2″ and C-6″), 118.2 (C-4a), 118.5 (C-6), 120.2 (C-4′a), 120.7 (C-8), 124.2 (C-8′), 124.7 (C-3″ and C-5″), 132.4 (C-1″), 134.0 (C-8′a and C-6′), 135.2 (C-3′), 140.0 (C-3), 143.4 (C-8a), 146.1 (C-7 and C-7′), 146.7 (C-2), 153.4 (C-4″), 158.6 (C-5′), 159.9 (C-5), 169.7, 170.0, 170.1, 170.5 (4 × $OCOCH_3$), 179.7 (C-4′), 183.0 (C-1′), 183.4 (C-4), 184.1 (C-1). ESI-MS: 862 (M + Na).

3′-[4-(α-D-Mannopyranosyloxy)anilinyl]diospyrin dimethyl ether 6

Yield 57.5 mg, 86%, dark red powder, mp 160–165 °C (from chloroform–petroleum ether). TLC R_f 0.66 (chloroform–ethyl

acetate–methanol = 3 : 3 : 2, v/v/v). $[\alpha]_D^{29}$ +31.0° (c 0.76 in CH_3OH). Found: C, 64.32; H, 4.90; N, 2.01. Calc. for $C_{36}H_{33}NO_{12}$: C, 64.38; H, 4.95; N, 2.09%. UV λ_{max} (CH_3OH)/nm 218, 251 and 358 (ε/dm^3 mol^{-1} cm^{-1} 25 119, 17 378 and 38 906). IR ν_{max}(KBr)/cm^{-1} 3399, 2923, 1658, 1604, 1512, 1460, 1353, 1258, 1031. δ_H (300 MHz; CD_3OD; Me_4Si) 2.23 (3H, s, 7′-CH_3), 2.45 (3H, s, 7-CH_3), 3.58 (1H, m, H-4‴), 3.65 (3H, s, 5′-OCH_3), 3.69 (1H, m, H-3‴), 3.73 (2H, m, H_2-6‴), 3.86 (1H, m, H-5‴), 3.91 (1H, m, H-2‴), 3.95 (3H, s, 5-OCH_3), 5.97 (1H, d, J = 2.7 Hz, H-1‴), 6.55 (1H, s, H-2′), 6.80 (1H, s, H-3), 7.15 (2H, dd, J_1 = 12.6 Hz, J_2 = 8.9 Hz, H-2″ and H-6″), 7.17 (1H, s, H-6), 7.23 (2H, dd, J_1 = 12.6 Hz, J_2 = 8.9 Hz, H-3″ and H-5″), 7.35 (1H, s, -NH), 7.53 (1H, s, H-8), 7.73 (1H, s, H-8′). δ_C (75 MHz; $CDCl_3$; Me_4Si) 20.9 (7′-CH_3), 22.2 (7-CH_3), 56.9 (5-OCH_3), 62.4 (C-6‴), 62.8 (5′-OCH_3), 71.3 (C-3‴), 74.8 (C-4‴), 77.8 (C-5‴), 78.4 (C-2‴), 101.1 (C-1‴), 102.3 (C-2′), 118.3 (C-4a), 118.7 (C-2″ and C-6″), 120.2 (C-6), 121.4 (C-4′a), 124.7 (C-8), 126.0 (C-8′), 126.5 (C-3″ and C-5″), 133.5 (C-1″), 135.1 (C-8′a), 136.5 (C-6′), 138.1 (C-3′), 141.3 (C-3), 145.1 (C-8a), 147.3 (C-7′), 148.9 (C-7), 149.4 (C-2), 157.0 (C-4″), 159.6 (C-5′), 161.3 (C-5), 182.5 (C-4′), 184.7 (C-1′), 185.0 (C-4), 185.4 (C-1). FAB-MS: 672 (M + H), 694 (M + Na).

3′-[4-(2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyloxy)anilinyl]diospyrin dimethyl ether 6a

Yield almost quantitative, orange powder, mp 90 °C (from dichloromethane–diethyl ether). TLC R_f 0.59 (chloroform–ethyl acetate = 3 : 2, v/v). $[\alpha]_D^{28}$ +14.0° (c 1.12 in $CHCl_3$). Found: C, 62.84; H, 4.89; N, 1.65. Calc. for $C_{44}H_{41}NO_{16}$: C, 62.93; H, 4.92; N, 1.67%. UV λ_{max} ($CHCl_3$)/nm 255 and 380 (ε/dm^3 mol^{-1} cm^{-1} 17 378 and 3715). IR ν_{max}(KBr)/cm^{-1} 3324, 2932, 1751, 1662, 1608, 1514, 1459, 1361, 1222, 1036. δ_H (300 MHz; $CDCl_3$; Me_4Si) 2.02–2.06 (12H, 4 × s, 4 × $COCH_3$), 2.21 (3H, s, 7′-CH_3), 2.51 (3H, s, 7-CH_3), 3.71 (3H, s, 5′-OCH_3), 3.86–3.92 (1H, m, H-5‴), 4.04 (3H, s, 5-OCH_3), 4.19 (1H, dd, J_1 = 12.3 Hz, J_2 = 2.0 Hz, H_a-6‴), 4.31 (1H, dd, J_1 = 12.3 Hz, J_2 = 2.0 Hz, H_b-6‴), 5.10 (1H, d, J = 7.4 Hz, H-1‴), 5.13–5.25 (1H, m, H-3‴), 5.28–5.35 (2H, m, H-2‴ and H-4‴), 6.21 (1H, s, H-2′), 6.80 (1H, s, H-3), 7.05 (2H, dd, J_1 = 8.9 Hz, J_2 = 2.7 Hz, H-2″ and H-6″), 7.17 (1H, s, H-6), 7.22 (2H, d, J = 8.9 Hz, H-3″ and H-5″), 7.60 (2H, bs, H-8 and -NH), 7.87 (1H, s, H-8′). δ_C (75 MHz; $CDCl_3$; Me_4Si) 20.4, 20.5, 20.57, 20.64 (4 × CO*C*H_3), 20.9 (7′-CH_3), 22.3 (7-CH_3), 56.4 (5-OCH_3), 61.9 (C-6‴), 62.2 (5′-OCH_3), 68.2 (C-3‴), 71.1 (C-4‴), 72.1 (C-5‴), 72.7 (C-2‴), 99.1 (C-1‴), 101.8 (C-2′), 117.7 (C-4a), 118.2 (C-2″ and C-6″), 118.5 (C-6), 120.1 (C-4′a), 120.6 (C-8), 124.2 (C-8′), 124.7 (C-3″ and C-5″), 132.7 (C-1″), 133.9 (C-8′a and C-6′), 135.1 (C-3′), 139.9 (C-3), 143.4 (C-8a), 146.1 (C-7′), 146.2 (C-7), 146.7 (C-2), 154.5 (C-4″), 158.5 (C-5′), 159.9 (C-5), 169.2, 169.3, 170.1, 170.5 (4 × O*C*OCH_3), 179.6 (C-4′), 182.9 (C-1′), 183.3 (C-4), 184.1 (C-1). ESI-MS: 862 (M + Na).

General procedure for the preparation of 3′-[2-(4,6-di-*O*-acetyl-2,3-dideoxy-D-hex-2-enopyranosyloxy)ethyl]amino diospyrin dimethyl ether 8 and 9

To a mixture of ethanolamine derivative of diospyrin dimethyl ether (**6**, 40 mg, 0.087 mmol) and tri-*O*-acetyl-D-glycal (50 mg, 0.184 mmol) in dry dichloromethane (3 mL), anhydrous $InCl_3$ (42.2 mg, 0.191 mmol) and activated molecular sieves (4 Å, 90 mg) were added at ambient temperature. The mixture was stirred at room temperature for 2 days. Then the mixture was filtered through a celite bed, washed well with dichloromethane (10 mL), and the combined filtrate and washings (20 mL) was finally washed with saturated $NaHCO_3$ solution (2 × 10 mL) followed by water (2 × 10 mL). The organic layer was dried over anhydrous Na_2SO_4 and evaporated to dryness to get a crude product. It was then chromatographed over silica gel using a mixture of $CHCl_3$–EtOAc–petroleum ether (3 : 2 : 1, v/v/v) as eluent to furnish an orange residue. The α and β diastereomers were separated by preparative reverse phase HPLC. Elution was carried out with an isocratic mobile phase acetonitrile–water at a flow rate of 0.6 mL min^{-1} at ambient temperature.

3′-[2-(4,6-Di-*O*-acetyl-2,3-dideoxy-D-*erythro*-hex-2-enopyranosyloxy)ethyl] amino diospyrin dimethyl ether 8

Combined column chromatographic yield 72%, (α–β = 10 : 1 by HPLC). Anomers were separated by semipreparative RPHPLC; diastereomeric mixture (15 mg) eluted with an isocratic mobile phase CH_3CN–H_2O = 80 : 20, v/v, at a flow rate of 0.6 mL min^{-1} at ambient temperature, furnished 12 mg (57.5%) of pure α-anomer. β-Anomer could not be separated in analytically pure form.

3′-[2-(4,6-Di-*O*-acetyl-2,3-dideoxy-α-D-*erythro*-hex-2-enopyranosyloxy)ethyl] amino diospyrin dimethyl ether 8α

Orange powder, mp 72 °C (from dichloromethane–diethyl ether). TLC R_f 0.57 (chloroform–ethyl acetate = 3 : 2, v/v). $[\alpha]_D^{28}$ +17.5° (c 1.1 in $CHCl_3$). Found: C, 64.26; H, 5.33; N, 2.12. Calc. for $C_{36}H_{35}NO_{12}$: C, 64.18; H, 5.24; N, 2.08%. UV λ_{max} ($CHCl_3$)/nm 244, 275 and 358 (ε/dm^3 mol^{-1} cm^{-1} 21 878, 13 378 and 6166). IR ν_{max}(KBr)/cm^{-1} 3379, 2927, 1742, 1658, 1614, 1511, 1459, 1359, 1246, 1051. δ_H (300 MHz; $CDCl_3$; Me_4Si) 2.07 (3H, s, $COCH_3$), 2.08 (3H, s, $COCH_3$), 2.27 (3H, s, 7′-CH_3), 2.49 (3H, s, 7-CH_3), 3.42 (2H, q, J = 5.4 Hz, -NHCH_2CH_2), 3.67 (3H, s, 5′-OCH_3), 3.80 (1H, t, J = 5.2 Hz, -NHCH_2C$H_a$$H_b$), 4.02 (1H, t, J = 5.0 Hz, -NHCH_2C$H_a$$H_b$), 4.03 (3H, s, 5-$OCH_3$), 4.06–4.16 (1H, m, H-5″), 4.19–4.30 (2H, m, H-6″ and H-6‴), 5.08 (1H, bs, H-1″), 5.31 (1H, dd, J_1 = 9.6 Hz, J_2 = 1.4 Hz, H-4″), 5.73 (1H, s, H-2′), 5.84 (1H, dt, J_1 = 10.3 Hz, J_2 = 2.0 Hz, H-3″), 5.92 (1H, d, J = 11.1 Hz, H-2″), 6.30 (1H, t, J = 5.5 Hz, -NH), 6.77 (1H, s, H-3), 7.15 (1H, s, H-6), 7.60 (1H, s, H-8), 7.87 (1H, s, H-8′). δ_C (75 MHz; $CDCl_3$; Me_4Si) 20.8, 20.90 (2 × CO*C*H_3), 20.93 (7′-CH_3), 22.3 (7-CH_3), 42.4 (-NH*C*$H_2$$CH_2$O), 56.5 (5-$OCH_3$), 62.2 (5′-$OCH_3$), 62.9 (C-6″), 65.1 (C-4″), 65.9 (-NHCH_2*C*H_2O), 67.3 (C-5″), 94.8 (C-1″), 100.0 (C-2′), 117.7 (C-4a), 118.5 (C-6), 120.3 (C-4′a), 120.7 (C-8), 124.3 (C-8′), 127.1 (C-3″), 129.7 (C-2″), 133.7 (C-8′a), 133.9 (C-6′), 135.5 (C-3′), 140.0 (C-3), 143.5 (C-8a), 145.9 (C-7′), 146.7 (C-7), 148.7 (C-2), 158.4 (C-5′), 159.9 (C-5), 170.2, 170.7 (2 × O*C*OCH_3),179.5 (C-4′), 182.1 (C-1′), 183.4 (C-4), 184.1 (C-1). ESI-MS: 696 (M + Na).

3′-[2-(4,6-Di-*O*-acetyl-2,3-dideoxy-D-*threo*-hex-2-enopyranosyloxy)ethyl] amino diospyrin dimethyl ether 9

Combined column chromatographic yield 80%, (α–β = 3 : 2 by HPLC). Anomers were separated by semipreparative RPHPLC; diastereomeric mixture (15 mg) eluted with an isocratic mobile

This journal is © The Royal Society of Chemistry 2007

phase $CH_3CN–H_2O$ = 85 : 15, v/v, at a flow rate of 0.6 mL min^{-1} at ambient temperature, furnished 7.3 mg (39%) of pure α-anomer, and 5.2 mg (28%) of pure β-anomer.

3′-[2-(4,6-Di-*O*-acetyl-2,3-dideoxy-α-D-*threo*-hex-2-enopyranosyloxy)ethyl] amino diospyrin dimethylether 9α

Orange powder, mp 68 °C (from dichloromethane–diethyl ether). TLC R_f 0.57 (chloroform–ethyl acetate = 3 : 2, v/v). $[\alpha]_D^{28}$ −54.1° (*c* 1.4 in $CHCl_3$). Found: C, 64.03; H, 5.15; N, 2.10. Calc. for $C_{36}H_{35}NO_{12}$: C, 64.18; H, 5.24; N, 2.08%. UV λ_{max} ($CHCl_3$)/nm 243, 277 and 357 (ε/dm^3 mol^{-1} cm^{-1} 18 621, 21 380 and 7943). IR ν_{max}(KBr)/cm^{-1} 3375, 2924, 1741, 1691, 1606, 1511, 1460, 1342, 1239, 1027. δ_H (300 MHz; $CDCl_3$; Me_4Si) 2.04 (3H, s, $COCH_3$), 2.08 (3H, s, $COCH_3$), 2.46 (3H, s, 7′-CH_3), 2.48 (3H, s, 7-CH_3), 3.42 (2H, dt, J_1 = 5.3 Hz, J_2 = 5.3 Hz, -NHCH_2CH$_2$), 3.72 (3H, s, 5′-OCH_3), 3.88 (1H, m, -NHCH$_2$CH_aH$_b$), 3.96 (1H, m, -NHCH$_2$CH$_a$$H_b$), 3.98 (3H, s, 5-$OCH_3$), 4.22 (2H, t, J = 6.2 Hz, H$_2$-6″), 4.32–4.36 (1H, m, H-5″), 5.03 (1H, dd, J_1 = 5.3 Hz, J_2 = 2.3 Hz, H-4″), 5.11 (1H, d, J = 2.5 Hz, H-1″), 5.29 (1H, s, H-2′), 5.73 (1H, s, H-3), 6.04 (1H, dd, J_1 = 10.0 Hz, J_2 = 3.0 Hz, H-2″), 6.15 (1H, dd, J_1 = 10.0 Hz, J_2 = 5.3 Hz, H-3″), 6.30 (1H, m, -NH), 7.13 (1H, s, H-6), 7.44 (1H, s, H-8), 7.84 (1H, s, H-8′). δ_C (75 MHz; $CDCl_3$; Me_4Si) 20.3, 20.7 (2 × COCH_3), 20.8 (7′-CH_3), 22.3 (7-CH_3), 42.4 (-NHCH_2CH$_2$O), 56.5 (5-OCH_3), 62.1 (5′-OCH_3), 62.6 (C-5″), 62.8 (C-6″), 65.6 (-NHCH$_2$$CH_2$O), 67.2 (C-4″), 94.3 (C-1″), 100.0 (C-2′), 117.9 (C-4a), 118.7 (C-6), 119.9 (C-4′a), 120.6 (C-8), 124.7 (C-8′), 125.7 (C-3″), 129.9 (C-2″), 130.0 (C-8′a), 133.8 (C-6′), 135.9 (C-3′), 140.2 (C-3), 146.9 (C-8a), 148.6 (C-7, C-7′), 148.8 (C-2), 159.4 (C-5′), 159.7 (C-5), 170.3, 170.5 (2 × O$COCH_3$), 179.4 (C-4′), 181.9 (C-1′), 189.7 (C-4), 191.6 (C-1). ESI-MS: 674 (M + H), 696 (M + Na).

3′-[2-(4,6-Di-*O*-acetyl-2,3-dideoxy-β-D-*threo*-hex-2-enopyranosyloxy)ethyl] amino diospyrin dimethylether 9β

Orange powder, mp 76 °C (from dichloromethane–diethyl ether). TLC R_f 0.57 (chloroform–ethyl acetate = 3 : 2, v/v). $[\alpha]_D^{28}$ −114.3° (*c* 0.9 in $CHCl_3$). Found: C, 64.11; H, 5.21; N, 2.05. Calc. for $C_{36}H_{35}NO_{12}$: C, 64.18; H, 5.24; N, 2.08%. UV λ_{max} ($CHCl_3$)/nm 244, 275 and 357 (ε/dm^3 mol^{-1} cm^{-1} 21 417, 15 488 and 5888). IR ν_{max}(KBr)/cm^{-1} 3374, 2922, 1740, 1692, 1608, 1513, 1462, 1349, 1246, 1052. δ_H (300 MHz; $CDCl_3$; Me_4Si) 2.04 (3H, s, $COCH_3$), 2.08 (3H, s, $COCH_3$), 2.27 (3H, s, 7′-CH_3), 2.50 (3H, s, 7-CH_3), 3.43 (2H, q, J = 5.3 Hz, -NHCH_2CH$_2$), 3.67 (3H, s, 5′-OCH_3), 3.80–3.88 (1H, m, -NHCH$_2$CH_aH$_b$), 3.96–3.99 (1H, m, -NHCH$_2$CH$_a$$H_b$), 4.03 (3H, s, 5-$OCH_3$), 4.21–4.24 (2H, m, H$_2$-6″), 4.32–4.35 (1H, m, H-5″), 5.03 (1H, dd, J_1 = 5.4 Hz, J_2 = 2.4 Hz, H-4″), 5.11 (1H, d, J = 2.6 Hz, H-1″), 5.73 (1H, s, H-2′), 6.04 (1H, dd, J_1 = 10.1 Hz, J_2 = 2.8 Hz, H-2″), 6.16 (1H, dd, J_1 = 9.8 Hz, J_2 = 5.4 Hz, H-3″), 6.29 (1H, t, J = 5.3 Hz, -NH), 6.77 (1H, s, H-3), 7.15 (1H, s, H-6), 7.60 (1H, s, H-8), 7.87 (1H, s, H-8′). δ_C (75 MHz; $CDCl_3$; Me_4Si) 20.3, 20.6, 20.7 (2 × COCH_3), 22.2 (7′-CH_3), 22.3 (7-CH_3), 42.3 (-NHCH_2CH$_2$O), 56.4 (5-OCH_3), 62.2 (5′-OCH_3), 62.5 (C-5″), 62.8 (C-6″), 65.6 (-NHCH$_2$$CH_2$O), 67.1 (C-4″), 94.2 (C-1″), 99.8 (C-2′), 118.4 (C-4a), 118.6 (C-6), 120.5 (C-4′a), 120.6 (C-8), 124.2 (C-8′), 125.6 (C-2″), 129.8 (C-3″), 133.7 (C-6′), 134.0 (C-8′a), 135.3 (C-3′), 139.9 (C-3), 143.5 (C-8a), 145.8 (C-7′), 146.7 (C-7), 148.6 (C-2), 159.2 (C-5′), 159.8 (C-5), 170.2, 170.5 (2 × O$COCH_3$), 179.4 (C-4′), 182.1 (C-1′), 183.3 (C-4), 184.1 (C-1). ESI-MS: 674 (M + H), 696 (M + Na).

2. Biological studies

Cell culture. Two human cancer cell lines, *viz.* A375 (malignant skin melanoma) and Hep2 (epidermoid laryngeal carcinoma) were obtained from the National Centre for Cell Science, Pune, India. The cells were grown in DMEM (GIBCO-BRL, Gaithersburg, MD, USA) supplemented with 10% heat-inactivated FCS (GIBCO-BRL, Gaithersburg, MD, USA) containing a 5% mixture of penicillin (100 U mL^{-1}), streptomycin (100 μg mL^{-1}) and gentamicin (3 μg mL^{-1}) in the presence of 5% CO_2 in humidified air at 37 °C, and routinely subcultured using a 0.25% trypsin–0.02% EDTA solution.

Fresh heparinized whole blood was collected from a normal human volunteer with informed consent. PBMC were isolated by Ficoll-Paque density gradient centrifugation.[63] The blood (5 mL) was layered carefully over the Hypaque (3 mL, Sigma Diagnostics, USA) and centrifuged at room temperature at 1000 rpm for 45 min. The buffy coat layer containing PBMC at the interface was carefully taken out, washed twice with PBS and centrifuged at 1500–2000 rpm for 10 min. The cells were suspended in RPMI 1640 with phenol red (GIBCO-BRL, Gaithersburg, MD, USA), supplemented with 20% FCS and antibiotics (as above), and incubated in the presence of 5% CO_2 in air at 37 °C.

Assessment of cytotoxicity *in vitro*

The *in vitro* growth inhibition effect of the test-compounds on A375, Hep2 and normal human PBMC was assessed by colorimetric determination of the conversion of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT; Sigma Chemicals, USA) into 'formazan blue' by the living cells.[54] Briefly, cells (2 × 10^5 per mL) were seeded in 96-well flat-bottomed microplates (Nunc, Roskilide, Denmark), and treated with different concentrations, in triplicate, of the test compounds appropriately diluted with DMSO. After 24 h incubation at 37 °C in a 5% CO_2 atmosphere, the medium was replaced with MTT solution (100 μL, 1 mg mL^{-1} in sterile PBS) for a further 24 h incubation. The supernatant was aspirated carefully, the precipitated crystals of 'formazan blue' were solubilized by adding DMSO (200 μL) to each well, and the optical density was measured with a microplate reader (Emax precision microplate reader, Molecular Devices, USA) at a wavelength of 570 nm. Doxorubicin and camptothecin (Sigma Chemicals, USA) were used as the positive controls in this experiment. The result represents the mean of three independent experiments and are expressed as IC_{50}, the concentration at which the optical density of the treated cells were reduced by 50% with respect to the untreated control.

Evaluation of ROS generation in tumor cells

The production of intracellular ROS in A375 cells was assessed with the oxidation sensitive, lipid permeable fluorescence probe 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA; Sigma Chemicals, USA),[62] which readily diffuses into the cells and hydrolysed by intracellular esterase to form 2′,7′- dichlorodihydrofluorescein (DCFH). This is trapped within the cells and

oxidised by cellular hydrogen peroxide or other oxidizing ROS to produce highly fluorescent compound 2′,7′-dichlorofluorescein DCF. A375 cells (2×10^5 cells per mL) were first loaded with DCFH-DA (10 μM) at 37 °C for 20 min followed by treatment with diospyrin and its derivatives (0.1 μM) for 1 h. The increase in fluorescence intensity of DCF, as a measure of ROS, was determined by a spectrofluorimeter (Perkin-Elmer LS 55) at $\lambda_{ex}/\lambda_{em} = 490/520$ nm (slit width, 5 nm). The same determination was repeated in the presence of *N*-acetyl-L-cysteine (NAC; Sigma Chemicals, USA), an antioxidant, by preincubating the cells with NAC (100 μM) for 2 h, followed by treatment with DCFH-DA and incubating with the sample as done before.[62] A minimum of three separate determinations was carried out for each compound.

Stability of glycosides in A375 cell culture

A375 tumor cells (2×10^5 per mL; 2 mL) were seeded in two sets of 6-well tissue culture plates, and grown in the same culture conditions as described before. After 24 h, when the cells reached 95% confluency, the two sets of culture plates were treated with the compounds **6** and **8**α (1 μM each), respectively. At selected time points (3 and 24 h), the supernatant was discarded from the respective culture plate, and the adherent tumor cells were washed with phosphate buffered saline (PBS; 3 × 1 mL), followed by trypsinisation and centrifugation in PBS at 2500 rpm for 5 min. The pellet was centrifuged at 12 000 rpm for 10 min with 1 mM sodium chloride solution (500 μL), to disrupt the cells by hypotonic shock. A mixture of methanol and chloroform (1 : 4, v/v; 500 μL) was added to this sample, centrifuged at 5000 rpm for 10 min, and the clear organic layer containing the compound and its metabolites was collected. After evaporation of the solvent, the residue was dried, dissolved in acetonitrile (100 μL), and filtered (Millipore; 0.45 μm). HPLC analysis was performed on a reverse phase C_{18} phenomenex column (250 mm × 4.6 mm i.d.; particle size 5 μm), under isocratic conditions (acetonitrile–water = 50 : 50, v/v), at a flow rate of 1.0 mL min^{-1} at ambient temperature, followed by UV detection at 255 nm.[64] The compounds, *viz.* **6** and **8**α, and their respective precursors, *viz.* **3** and **7**, were analysed separately for necessary standardization of their retention times under the same HPLC conditions.

Microscopic study

A375 cells (2×10^5 per mL; 200 μL) were seeded in coverslips and grown in DMEM till 95% confluency as described above and treated with the compounds **1** and **6**. After 24 h of incubation at 37 °C in a 5% CO_2 atmosphere, the supernatant was removed, and the adhering cells were fixed with methanol after washing with normal saline. The samples on the coverslips were left to dry in open air, followed by staining with Giemsa solution (Sigma Chemicals, USA) for 15 min, and then washed thrice with PBS for microscopic observation (Olympus, Japan).

Statistical analysis

The IC_{50} values were calculated by using linear regression analysis (MINITAB Release 13.31, USA).

Conclusion

Starting with diospyrin (**1**), a plant-derived quinonoid, a variety of glycoconjugates were synthesized for the first time in fairly good yields. In general, the glycoconjugates exhibited significant enhancement of the antiproliferative activity against A375 and Hep2 cells at low micromolar concentrations. However, the aminophenyl mannosyl derivative (**6**) was found to be the most potent, particularly against the malignant melanoma cell line (IC_{50} ~23nm). Obviously, this would necessitate synthesis and evaluation of a wider variety of glycosidic quinonoids. This is currently precluded by the scarcity of diospyrin, which might be overcome by carrying out the total synthesis of this plant-derived starting material in future.

Acknowledgements

This work was supported by BRNS, Department of Atomic Energy, Mumbai (Grant No. 2002/37/19/BRNS), to B.H. and R.G and by CSIR, New Delhi (Grant No. CMM0016) for K.C. Authors acknowledge CSIR, New Delhi for Senior Research Fellowships to M.D.S. [Grant No. 9/96(377)/2002-EMR-I] and R.C. [Grant No. 31/2(555)/2004-EMR-I]. Thanks are accorded to the instrumentation facility of the CAS on Natural Products, Department of Chemistry, Calcutta University for spectroscopic measurements. Facility for fluorimetry was kindly provided by Dr Chittaranjan Sinha, Department of Chemistry, Jadavpur University, and Dr Anup Bhattacharya, Medicinal Chemistry Division, IICB, Calcutta, is acknowledged for the help to carry out HPLC separation. Authentic stem-bark of *D. montana* Roxb. was kindly provided by Dr R.B. Mishra, Nagarjuna Education Society, Bolangir, Orissa.

References

1 A. C. Weymouth-Wilson, *Nat. Prod. Rep.*, 1997, **14**, 99.
2 J. S. Thorson and T. Vogt, in *Carbohydrate-Based Drug Discovery*, ed. C.-H. Wong, Wiley-VCH, Weinheim, 2003, vol. 2, ch. 25, pp. 685–711.
3 D. Kahne, D. Silva and S. Walker, in *Bioorganic Chemistry-Carbohydrates*, ed. S. M. Hecht, Oxford University Press, New York, 1999, ch. 5, pp. 174–197.
4 A. Kalben, S. Pal, A. H. Andreotti, S. Walker, D. Gange, K. Biswas and D. Kahne, *J. Am. Chem. Soc.*, 2000, **122**, 8403.
5 S. T. Wallace and R. Schroeder, *RNA*, 1998, **4**, 112.
6 M. Baginski, K. Sternal, J. Czub and E. Borowski, *Acta Biochim. Pol.*, 2005, **52**, 655.
7 W. S. Champney and R. Burdine, *Antimicrob. Agents Chemother.*, 1996, **40**, 1301.
8 S. R. Seo, Y. J. Kim, S. J. Moon, H. Sugiya, D. M. Shin, S. Lee and J. T. Seo, *Arch. Oral Biol.*, 2003, **48**, 553.
9 J. M. Langenhan, N. R. Peters, I. A. Guzei, M. F. Hoffmann and J. S. Thorson, *Proc. Natl. Acad. Sci. U. S. A.*, 2005, **102**, 12305.
10 M. Ge, Z. Chen, H. R. Onishi, J. Kohler, L. L. Silver, R. Kerns, S. Fukuzawa, C. Thompson and D. Kahne, *Science*, 1999, **284**, 507.
11 M. Gordaliza, M. A. del Corral, J. M. Castro and A. S. Feliciano, *Curr. Pharm. Des.*, 2000, **18**, 1811.
12 B. S. Kim, S. S. Moon and B. K. Hwang, *J. Agric. Food Chem.*, 1988, **48**, 1875.
13 O. Kutsch, D. N. Levy, P. J. Bates, J. Decker, B. R. Kosloff, G. M. Shaw, W. Priebe and E. N. Benveniste, *Antimicrob. Agents Chemother.*, 2004, **48**, 1652.
14 S. Erbeck, X. Liang, D. Hunkler, R. Krieger and H. Prinzbach, *Eur. J. Org. Chem.*, 1998, 1935.
15 F. Arcamone, *Doxorubicin, Anticancer Antibiotics; Medicinal Chemistry Series*, Academic Press, New York, 1981, vol. 17, pp. 1–369.

This journal is © The Royal Society of Chemistry 2007

16 C. Sanchez, L. Zhu, A. F. Brana, A. P. Salas, J. Rohr, C. Mendez and J. A. Salas, *Proc. Natl. Acad. Sci. U. S. A.*, 2005, **102**, 461.
17 S. N. Lopez, I. A. Ramallo, M. G. Sierra, S. A. Zacchino and R. L. E. Furlan, *Proc. Natl. Acad. Sci. U. S. A.*, 2007, **104**, 441.
18 P. J. O'Brien, *Chem.-Biol. Interact.*, 1991, **80**, 1.
19 C. Asche, *Mini–Rev. Med. Chem.*, 2005, **5**, 449.
20 F. Arcamone, F. Animati, G. Capranico, P. Lombardi, G. Pratesi, S. Manzini, R. Supino and F. Zunino, *Pharmacol. Ther.*, 1997, **76**, 117.
21 P. H. Wiernik and J. P. Dutcher, *Leukemia*, 1992, **6**(Suppl 1), 67.
22 G. N. Hortobagyi, *Drugs*, 1997, **54**(Suppl 4), 1.
23 R. H. Adamson, *Cancer Chemother. Rep.*, 1974, **58**, 293.
24 P. D. Senter, M. G. Saulnier, G. J. Schreiber, D. L. Hirschberg, J. P. Brown, I. Hellstrom and K. E. Hellstrom, *Proc. Natl. Acad. Sci. U. S. A.*, 1988, **85**, 4842.
25 J. B. Chaires, F. Leng, T. Przewloka, I. Fokt, Y.-H. Ling, R. Perez-Soler and W. Priebe, *J. Med. Chem.*, 1997, **40**, 261.
26 N. Sharon and H. Lis, *Sci. Am.*, 1993, **268**, 82.
27 G. Powis, *Free Radical Biol. Med.*, 1989, **6**, 63.
28 P. N. Cote and L. Goodman, *Carbohydr. Res.*, 1973, **26**, 247.
29 M. C. F. Linardi, M. M. de Oliveira and M. R. P. Sampaio, *J. Med. Chem.*, 1975, **18**, 1159.
30 K. Krohn, *Tetrahedron*, 1990, **46**, 291.
31 G. B. Caygill, D. S. Larsen and S. Brooker, *J. Org. Chem.*, 2001, **66**, 7427.
32 B. Hazra, P. Sur, D. K. Roy and A. Banerjee, *Planta Med.*, 1984, **51**, 295.
33 S. Pal, B. Hazra, A. Banerjee, R. Ray and D. K. Bhattacharya, *Phytother. Res.*, 1996, **10**, 393.
34 S. Chakrabarty, M. Roy, B. Hazra and R. K. Bhattacharya, *Cancer Lett.*, 2002, **188**, 85.
35 B. Hazra, M. Das Sarma, B. Kumar, S. Basu, K. Das, B. N. Pandey and K. P. Mishra, *Chemotherapy*, 2007, **53**, 173.
36 M. Das Sarma, R. Ghosh, A. Patra and B. Hazra, *Bioorg. Med. Chem.*, 2007, **15**, 3672.
37 U. Sanyal, S. Bhattacharyya, A. Patra and B. Hazra, *J. Chromatogr., A*, 2003, **1017**, 252.
38 M. Yoshida and K. Mori, *Eur. J. Org. Chem.*, 2000, 1313.
39 W. T. A. Harrison and O. C. Musgrave, *Acta Crystallogr.*, 2004, **c60**, 399.
40 W. Koenigs and E. Knorr, *Ber.*, 1901, **34**, 957.
41 P. G. Scheurer and F. Smith, *J. Am. Chem. Soc.*, 1954, **76**, 3224.
42 A cooled mixture of diospyrin (50 mg, 0.13 mmol), 2,3,4,6-tetra-*O*-acetyl-α-D-glucosyl bromide (70 mg, 0.17 mmol), freshly prepared silver oxide (31 mg, 0.13 mmol) and activated molecular sieves (4 Å, 90 mg) in acetonitrile (4 mL) was stirred at −10 °C under a nitrogen atmosphere. After 3 days, the mixture was filtered through a celite bed, washed well with dichloromethane (12 mL) and the combined filtrate and washings (20 mL) were finally washed with water (2 × 15 mL). The pooled organic layer was dried over anhydrous Na_2SO_4, concentrated and the crude residue was chromatographed on a silica-gel column. Elution with chloroform–benzene–ethyl acetate = 4 : 3 : 1 (v/v/v), furnished **2** (14 mg, 15%, α–β 1 : 4; Fig. 1) as indicated by ^1H NMR spectra and HRMS (ESI, positive ion): calculated for $C_{36}H_{32}O_{15}Na$, *m*/*z* 727.1672, found *m*/*z* 727.1672.
43 M. Das Sarma, R. Ghosh, A. Patra and B. Hazra, unpublished work.
44 P. Ghosh and B. K. Bachhawat, *Biochim. Biophys. Acta*, 1980, **632**, 562.
45 S. Dumont, C. D. Muller, F. Schube and J. Bartholeyns, *Anticancer Res.*, 1990, **10**, 155.
46 B. K. Shull, Z. Wu and M. A. Koreeda, *J. Carbohydr. Chem.*, 1996, **15**, 955.
47 B. S. Babu and K. K. Balasubramanian, *Tetrahedron Lett.*, 2000, **41**, 1271.
48 B. C. Ranu, *Eur. J. Org. Chem.*, 2000, 2347.
49 K. K. Chauhan and C. G. Frost, *J. Chem. Soc., Perkin Trans. 1*, 2000, 3015.
50 R. Ghosh, *Indian J. Chem.*, 2001, **40B**, 550.
51 R. Ghosh and S. Maiti, *J. Mol. Catal. A: Chem.*, 2007, **264**, 1.
52 M. Adinolfi, A. Iadonisi, A. Ravida and M. Schiattarella, *Tetrahedron Lett.*, 2004, **45**, 4485.
53 K. N. Jayaprakash and B. Fraser-Reid, *Synlett*, 2004, 301.
54 T. Mosmann, *J. Immunol. Methods*, 1983, **65**, 55.
55 A. Jemal, T. Murray, E. Ward, A. Samuels, R. C. Tiwari, A. Ghafoor, E. J. Feuer and M. J. Thun, *CA Cancer J. Clin.*, 2005, **55**, 10.
56 A. G. Glass and R. N. Hoover, *J. Am. Med. Assoc.*, 1989, **262**, 2097.
57 L. G. Ferun, N. Savaraj, J. Hurley, A. Marini and S. Lai, *Cancer*, 2000, **88**, 584.
58 F. E. Nathan, D. Berd, T. Sato and M. J. Mastrangelo, *Cancer*, 2000, **88**, 79.
59 R. M. Mackie, *Arch. Dermatol.*, 2000, **136**, 71.
60 B. Kumar, Ph.D. Thesis, Jadavpur University, 2006.
61 B. Kumar, J. Joshi, A. Kumar, B. N. Pandey, B. Hazra and K. P. Mishra, *Mol. Cell. Biochem.*, DOI: 10.1007/s11010-007-9511-9.
62 F. Gao, J. Yi, J. Q. Yuan, G. Y. Shi and X. M. Tang, *Cell Res.*, 2004, **14**, 81.
63 X. Chen, M. Lowe, T. Herliczek, M. J. Hall, C. Danes, D. A. Lawrence and K. Keyomarsi, *J. Natl. Cancer Inst.*, 2000, **92**, 1999.
64 A. Trouet, A. Passioukov, K. Van derpoorten, A.-M. Fernandez, J. Abarca-Quinones, R. Baurain, T. J. Lobl, C. Oliyali, D. Shochat and V. Dubois, *Cancer Res.*, 2001, **61**, 2843.

This journal is © The Royal Society of Chemistry 2007