RSC Advances

PAPER

View Article Online
View Journal | View Issue

Published on 02 May 2014. Downloaded by University of Waikato on 15/07/2014 10:07:17.

Synthesis and antiproliferative activity of *N*-glycosyl-3,3-diaryloxindoles†

Cite this: *RSC Adv.*, 2014, **4**, 22828

Dennis Kleeblatt,[a] Christoph A. Cordes,[a] Philipp Lebrenz,[a] Martin Hein,[a] Holger Feist,[a] Abdul Matin,[b] Rabia Raza,[c] Jamshed Iqbal,[c] Omar Munshi,[ad] Qamar Rahman,[d] Alexander Villinger[a] and Peter Langer*[ae]

Received 25th March 2014
Accepted 2nd May 2014

DOI: 10.1039/c4ra02627f

www.rsc.org/advances

N-Glycosylated 3,3-diaryloxindoles were prepared by Lewis acid catalyzed reaction of acetylated *N*-glycosylisatins with various benzene derivatives and subsequent deprotection. Some of the products showed antiproliferative activity against malignant cutaneous melanoma cells HT-144 (ATCC HTB-63) and lung carcinoma (H157) cell lines (ATCC CRL-5802).

Introduction

The 3,3-diaryloxindole core structure is of considerable pharmacological relevance. For example, oxyphenisatin and its derivative acetyl-oxyphenisatin were used until the 1970s as nonprescription laxatives. Later a relationship between oxyphenisatin derivatives and chronic liver-diseases was observed and the molecule was taken from the market.[1,2] However, it is known today that these liver-injuries were not caused by a general toxicity of oxyphenisatin, but instead by a hypersensitivity of liver cells. Several recent studies showed that oxyphenisatin and other 3,3-diaryoxindoles show considerable antiproliferative activity against various human cancer-cell-lines.[3–6] 3,3-Diaryloxindole derivatives can be prepared by reaction of isatin with arenes in the presence of acid.[7,8] 3,3-Diaryloxindoles containing two different aryl groups were prepared by Grignard reaction of isatin with arylmagnesium halides followed by acid-catalyzed condensation reaction with benzene derivatives (Fig. 1).[9]

In recent years, we and others studied the synthesis and antiproliferative activity of various *N*-glycosylated indole and bisindole derivatives. In this context, it was observed that the pharmacological activity of the *N*-glycosides is often considerably higher than that of the non-glycosylated heterocycles. For example, the akashines, indigo-*N*-glycosides isolated from *Streptomyces* sp. GW48/1497, proved to be active against various human cancer cell lines.[10] Indirubin derivatives are potent selective inhibitors of cyclin-dependent kinases (CDKs), which play an important role in proliferation, and show promising anti-cancer activities.[11–13] *N*-Glycosides of thia- and selenaindirubins show a remarkable activity against melanoma and lung carcinoma cells, respectively.[14] Moreau and Sassatelli and their coworkers reported the synthesis and anti-cancer activity of various isoindigo-*N*-glycosides.[15] In this context, oxindole-*N*-glycosides were prepared as synthetic building blocks, (1-(2,3,4-tri-*O*-acetyl-β-D-xylopyranosyl)isoindigo), so-called NATURA, represents a pharmacologically active natural *O*-glycosylated isoindigo.[16] Recently, we reported the synthesis of a number of *N*,*N*-diglycosylated indirubin derivatives.[17]

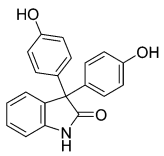


Fig. 1 Structure of oxyphenisatine.

Due to the considerable pharmacological relevance of non-glycosylated 3,3-diaryloxindoles and due to our ongoing interest in the synthesis and pharmacological evaluation of new *N*-glycosylated indole and bisindole derivatives, we decided to study the synthesis of hitherto unknown *N*-glycosides of these molecules. Herein, we report an efficient synthesis of 3,3-diaryloxindole-*N*-glycosides and their anti-proliferative activity against various cancer cell lines.

Results and discussion

Acetyl-protected isatin-*N*-glycosides **4a–d** were prepared as previously reported.[13,18–21] For example, *N*-(β-D-mannopyranosyl)-

[a]*Institut für Chemie, Universität Rostock, Albert-Einstein-Str. 3a, 18059 Rostock, Germany. E-mail: peter.langer@uni-rostock.de; Fax: +49 (381)4986412; Tel: +49 (381)4986410*

[b]*Department of Medical Lab Technology, University of Haripur, Hattar Road, Haripur, Khyber Pakhtunkhwa, 22620-Pakistan*

[c]*Department of Pharmaceutical Sciences, COMSATS Institute of Information Technology, Abbottabad, Postal Code 22060, Pakistan*

[d]*Amity University, Lucknow Campus, Viraj Khand-5, Gomti Nagar, Lucknow – 226010, India*

[e]*Leibniz-Institut für Katalyse e. V. an der Universität Rostock, Albert-Einstein-Str. 29a, 18059 Rostock, Germany*

† Electronic supplementary information (ESI) available. CCDC 960490. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c4ra02627f

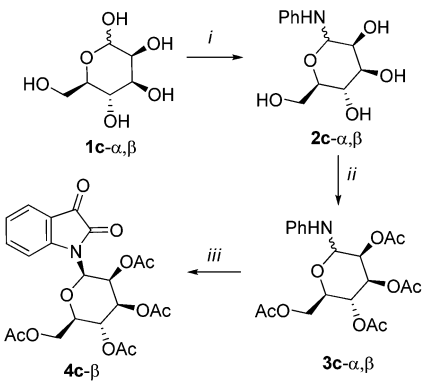
This journal is © The Royal Society of Chemistry 2014

Published on 02 May 2014. Downloaded by University of Waikato on 15/07/2014 10:07:17.

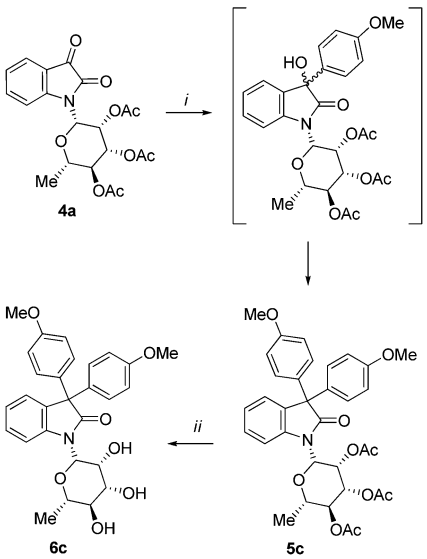
isatin **4c** was prepared by reaction of D-mannose (**1b**) with aniline, followed by acetylation of the sugar moiety and cyclization with oxalyl chloride (Scheme 1). The isatin-*N*-glycosides of L-rhamnose **4a**, D-glucose **4b** and D-galactose **4d** were synthesized under similar conditions.[13,18–21]

The reaction of isatin-*N*-glycosides **4** with benzene derivatives was next studied. In the literature, several Lewis and Brönstedt acids ($TiCl_4$, $BF_3 \cdot OEt_2$, $AlCl_3$, $AlEtCl_2$, H_2SO_4, H_2SO_4–AcOH, $MeSO_3H$) were used as catalysts for the synthesis of non-glycosylated 3,3-diaryloxindoles.[22–24] To find the best conditions for the reaction of *N*-(2,3,4-tri-*O*-acetyl-β-L-rhamnopyranosyl) isatin (**4a**) with anisole, we systematically varied the catalyst and the conditions. It proved to be advantageous to use anisole in a high excess as a solvent. The use of $TiCl_4$ or $BF_3 \cdot OEt_2$ resulted in decomposition of the starting material and complex mixtures (temperature: −78 °C). After much experimentation, only the employment of $AlCl_3$ as catalyst was successful and gave the expected 3,3-diaryloxindole-*N*-glycoside **5c** in excellent yield (Scheme 2).

With optimized reaction conditions in hand, we studied next the scope of the reaction. The $AlCl_3$ catalyzed reaction of *N*-glycosylated isatines **4a–d** with different benzene derivatives afforded the desired 3,3-diaryloxindole-*N*-glycosides **5a–l** (Table 1). The best yields were obtained for products **5c–e,h,i,l** derived from electron-rich arenes (like anisole and *N*,*N*-dimethylaniline). In fact, the yields were excellent for all sugar moieties, due to the high nucleophilicity of the arenes. These transformations required relatively short reaction times and reactions had to be performed at low temperatures. The high yields can be explained by the high nucleolphilic character of electron-rich arenes. Furthermore, $AlCl_3$ proved to be better soluble in anisole and *N*,*N*-dimethylaniline as compared to benzene or toluene. The yields of the reaction of benzene with isatine-*N*-glycosides **4a–d** were dependent on the carbohydrate moiety. While an excellent yield was obtained for rhamnoside **5a**, only moderate yields were obtained for mannoside **5f** and galactoside **5j**. The use of electron-poor arenes, such as bromo- or chlorobenzene, was unsuccessful (no reaction and various conditions and decomposition of the starting material).



Scheme 1 Synthesis of acetyl protected *N*-(β-D-mannopyranosyl) isatin **4b**. Reagents and conditions: (i) EtOH (abs.), $PhNH_2$, 78 °C, 12 h; (ii) pyridine, Ac_2O, 0 °C, 24 h; (iii) oxalyl chloride, $AlCl_3$, 55 °C, 1.5 h.



Scheme 2 Synthesis of *N*-glycosylated 3,3-diaryl-oxindole **5c**. Reagents and conditions: (i) anisole, $AlCl_3$, 20 °C, 10 min; (ii) MeOH, NaOMe, 12 h, 20 °C.

The deprotection of **5a–l** was successfully performed under Zemplén conditions (Scheme 2 and Table 1). Products **5a–l** were dissolved in dry methanol and treated with catalytic amounts of sodium methoxide at 20 °C. After stirring for 12 h, the reactions were complete. After standard work-up procedure, the pure products were isolated as white solids in 75–88% yields (Table 1).

For the confirmation of the structure of compounds **5** and **6**, several NMR experiments, like ^1H and ^{13}C NMR, $^1H,^1H$ COSY, $^1H,^1H$ NOESY and HMBC, were performed. The $^1H,^1H$ NOESY-spectra show correlations between H-1, H-3 and H-5 for each series of the *N*-glycosides. These correlations indicate a $^1C_4$-chair conformation with β-configuration for rhamno-configurated products. In contrast, a $^4C_1$-chair conformation with β-configuration was observed for gluco-, manno- and galacto-configurated derivatives. As a typical example, the diagnostic ^1H, ^1H NOESY correlations of **5e** and **5h** are shown in Fig. 2.

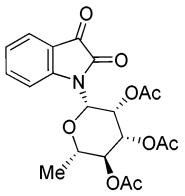
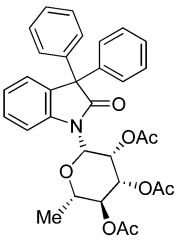
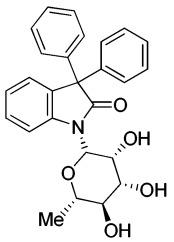
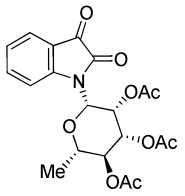
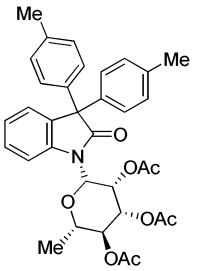
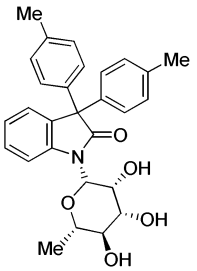
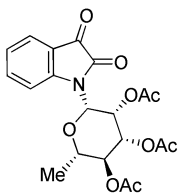
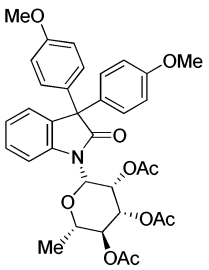
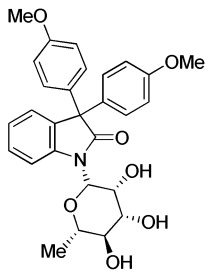
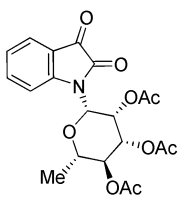
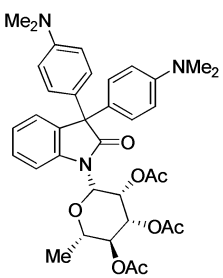
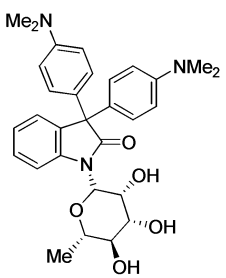
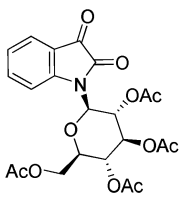
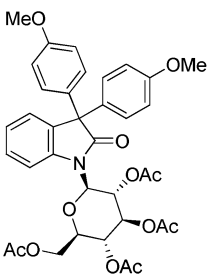
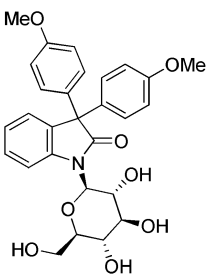
The structure of **5a** was independently confirmed by X-ray crystal structure analysis (Fig. 3). Compound **5a** crystallized in a orthorhombic crystal lattice with the space-group $P2_12_12_1$. The $^1C_4$-chair conformation and the β-configuration of the sugar-moiety are clearly visible. Bond-lengths and angles do not show any significant divergences to theoretically expected values.

Antiproliferative activity

3,3-Diaryloxindoles have been reported for their anti-proliferative activity. Natarajan *et al.* found that this group of compounds depletes intra-cellular Ca^{2+} stores leading to inhibition of translation initiation.[25] Uddin *et al.*[26] evaluated oxyphenisatin as potent antiproliferative agent, particularly against the human breast carcinoma cell line. Keeping in view this property, we evaluated our synthesized *N*-glycosylated 3,3-diaryloxindoles for their antiproliferative activities against two

Published on 02 May 2014. Downloaded by University of Waikato on 15/07/2014 10:07:17.

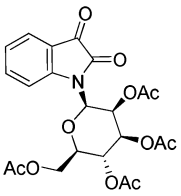
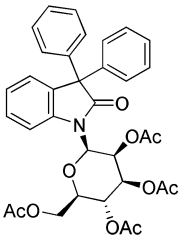
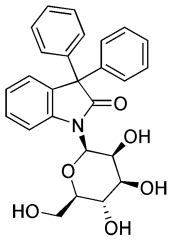
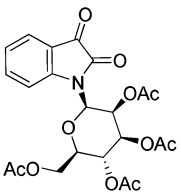
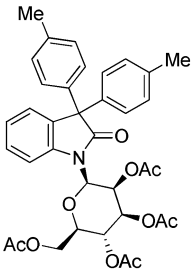
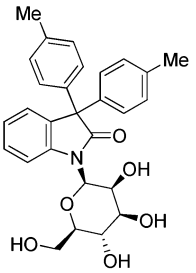
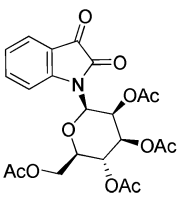
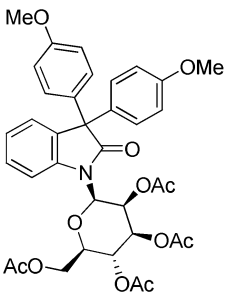
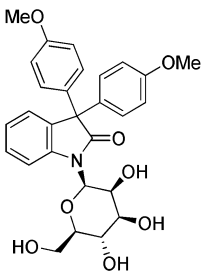
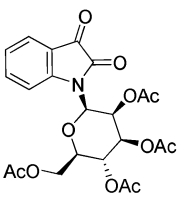
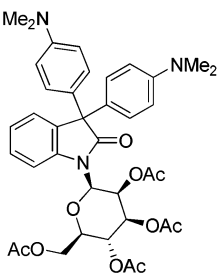
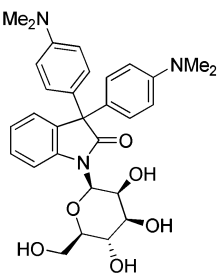
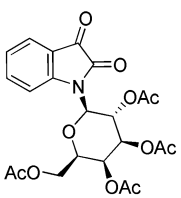
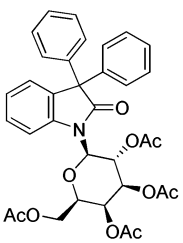
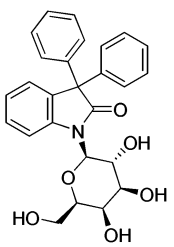
Table 1 Syntheses of *N*-glycosylated 3,3-diaryloxindoles **5a–l** and **6a–l**

4	Time [min]	Temp. [°C]	% (5)[a]	% (6)[a]
 **4a**	120	80	 **5a**, 94%	 **6a**, 88%
 **4a**	120	80	 **5b**, 56%	 **6b**, 77%
 **4a**	10	20	 **5c**, 98%	 **6c**, 81%
 **4a**	10	20	 **5d**, 90%	 **6d**, 86%
 **4b**	10	20	 **5e**,85%	 **6e**, 82%

This journal is © The Royal Society of Chemistry 2014

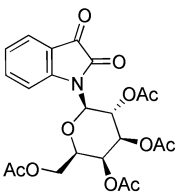
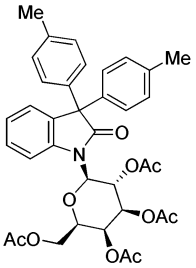
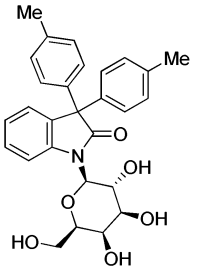
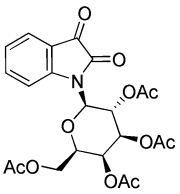
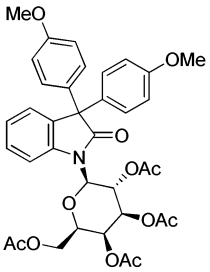
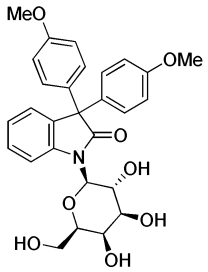
Published on 02 May 2014. Downloaded by University of Waikato on 15/07/2014 10:07:17.

Table 1 (*Contd.*)

4	Time [min]	Temp. [°C]	% (5)[a]	% (6)[a]
 **4c**	120	80	 **5f**, 59%	 **6f**, 76%
 **4c**	120	80	 **5g**, 55%	 **6g**, 78%
 **4c**	10	20	 **5h**, 95%	 **6h**, 84%
 **4c**	10	20	 **5i**, 80%	 **6i**,80%
 **4d**	120	80	 **5j**, 66%	 **6j**, 75%

Published on 02 May 2014. Downloaded by University of Waikato on 15/07/2014 10:07:17.

Table 1 (*Contd.*)

4	Time [min]	Temp. [°C]	% (5)[a]	% (6)[a]
 **4d**	120	80	 **5k**, 62%	 **6k**, 78%
 **4d**	10	20	 **5l**, 92%	 **6l**, 79%

[a] Yields are given for isolated products.

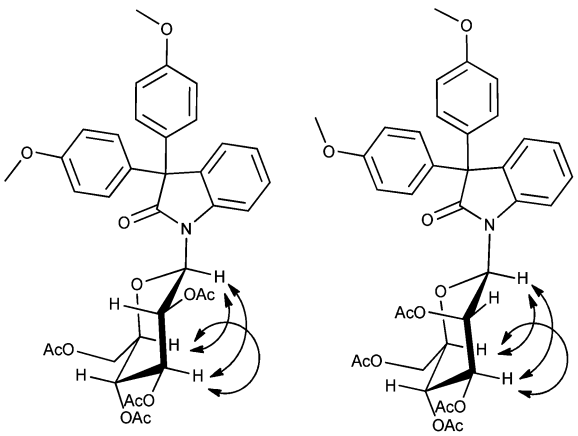


Fig. 2 Diagnostic $^{1}H,^{1}H$ NOESY correlations of compound **5e** and **5h**.

cancer cell lines, *i.e.* H157 cancer cell line derived from human oral squamous cell carcinoma and HT-144 cell line derived from malignant cutaneous melanoma and a normal cell line HCEC derived from human corneal epithelial cells. All the experiments were performed by exposing cells to 1 μM, 25 μM, 50 μM, and 100 μM end concentration of compounds. The results obtained revealed some of the compounds as antiproliferative agents while rest of the compounds were almost inactive against both cancer cell lines. The active compounds inhibited the growth of cancer cell lines at 100 μM concentration while

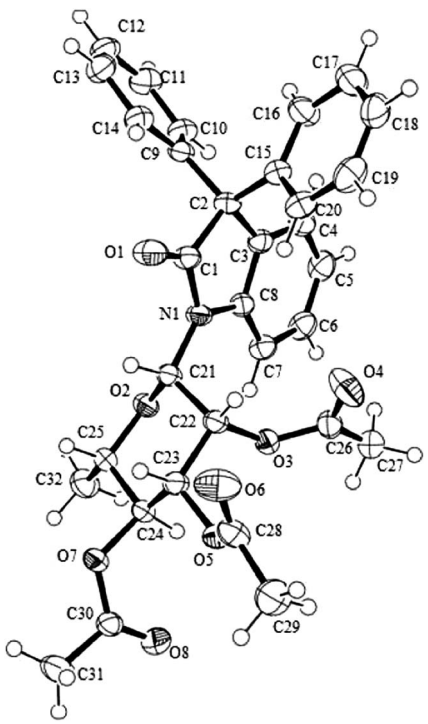


Fig. 3 ORTEP plot of compound **5a**.

 This journal is © The Royal Society of Chemistry 2014

Published on 02 May 2014. Downloaded by University of Waikato on 15/07/2014 10:07:17.

below this concentration no considerable inhibition was observed. The results given in Table 2 show the percentage antiproliferative activity of the compounds against H157 cells, HT-144 cells and HCEC cells along with standard deviation (Fig. 4).

The results obtained after exposing the cancer to a range of concentration of test compounds revealed that some of the compounds were active in inhibiting the growth of cancer cells at 100 μM end concentration, with 60% inhibition as the best value (compound **6i**) while the standard dug methotrexate showed only up to 21% growth inhibition while vincristine exhibited 66% growth inhibition at the same concentration. A very positive aspect of the results was that the compounds did not show any inhibition activity towards normal cell line HCEC. It was also observed that there is a relation between growth inhibition potency of compounds with a little difference in activity against both cell lines. Among all the tested *N*-glycosyl-3,3-diaryloxindoles, **6i** was most active with 60.21 ± 5.22 growth inhibition against H157 cells and 53.1 ± 4.81% growth inhibition against HT-144 cells while the second highest growth inhibition was exhibited by compound **5g** with 55.6 ± 5.23% growth inhibition against H157 cells and 51.55 ± 6.34% activity against HT-144 cells. By observing the chemical structures of active compounds, it can be inferred that simultaneous presence of hydroxyl and dimethyl amino groups imparts good growth inhibition characteristics to the compound as can be seen in compound **6i**. Similar substitution of functional groups was present in compounds **6d** but the replacement of one hydroxyl group with methyl group in **6d** resulted in a big change in activity of compound thus making it much lesser active than **6i**. In case of series **5**, the compound **5g** exhibited maximum growth inhibition which can be result of simultaneous substitution of *O*-acetyl and methyl groups on tested compound. Moreover, it was also observed that stereochemistry of the compounds also play a vital role in biological activity of the compounds. This can be observed in the activity difference of **5g** and **5k** which contain the same heterocyclic moiety, but a different stereochemistry. The same type of difference can be observed for the activities of **6e** and **6h**. In conclusion, some of the compounds tested in this study provide a direction towards the discovery of potent and antiproliferative compounds. Further work on such compounds with varying substitution patterns may lead to the discovery of some novel and value able antiproliferative compounds. Additionally, inactivity of these compounds towards normal cell lines renders them an interesting class of compounds for cancer studies.

Table 2 Antiproliferative activity of compounds against H157, HT-144 and HCEC cells

	H157 GI ± STD[a] [%]	HT-144 GI ± STD[b] [%]	HCEC GI ± STD[c] [%]
5a	15.93 ± 4.53	18.76 ± 5.84	4.43 ± 1.27
5b	38.97 ± 4.35	36.72 ± 7.33	6.94 ± 2.44
5c	23.54 ± 2.87	21.38 ± 4.57	6.25 ± 2.19
5d	18.52 ± 1.09	19.27 ± 6.05	6.82 ± 3.93
5e	28.94 ± 6.12	29.76 ± 3.12	4.92 ± 1.84
5f	17.21 ± 7.91	16.43 ± 7.17	4.4 ± 1.27
5g	55.6 ± 5.23	51.55 ± 6.34	8.12 ± 4.82
5h	14.52 ± 1.11	10.28 ± 4.88	7.4 ± 3.81
5i	20.91 ± 5.44	16.18 ± 3.78	7.25 ± 4.65
5j	16.52 ± 1.14	20.15 ± 5.13	5.4 ± 0.14
5k	18.98 ± 5.78	21.77 ± 6.55	7.56 ± 4.83
5l	13.39 ± 8.45	16.59 ± 4.98	4.8 ± 1.91
6a	28.53 ± 4.81	27.7 ± 8.77	6.94 ± 2.44
6b	20.14 ± 7.33	29.87 ± 5.76	6.25 ± 2.19
6c	32.94 ± 2.81	30.81 ± 6.31	4.92 ± 1.84
6d	14.65 ± 9.41	17.6 ± 3.16	6.4 ± 2.17
6e	52.79 ± 4.65	44.58 ± 6.95	3.4 ± 1.44
6f	17.91 ± 4.88	19.35 ± 5.37	4.4 ± 1.27
6g	18.88 ± 6.94	21.31 ± 7.13	7.25 ± 4.65
6h	12.59 ± 7.09	9.17 ± 3.62	5.4 ± 0.14
6i	60.21 ± 5.22	53.1 ± 4.81	6.9 ± 3.32
6j	16.28 ± 3.45	27.34 ± 7.56	7.56 ± 4.83
6k	21.07 ± 9.83	16.35 ± 5.33	5.4 ± 0.14
6l	9.33 ± 5.22	14.89 ± 4.54	5.4 ± 0.14
Methotrexate	21.03 ± 1.1	20.71 ± 0.9	3.1 ± 0.13
Vincristine	66.07 ± 9.1	68.01 ± 3.2	6.2 ± 0.76

[a] Growth Inhibition (GI) (%) of compounds along with standard deviation (STD) when tested against H157 cells at 100 μM end concentration. [b] Growth inhibition (GI) (%) of compounds along with standard deviation (STD) when tested against HT-144 cells at 100 μM end concentration. [c] Growth inhibition (GI) (%) of compounds along with standard deviation (STD) when tested against HCEC cells at 100 μM end concentration.

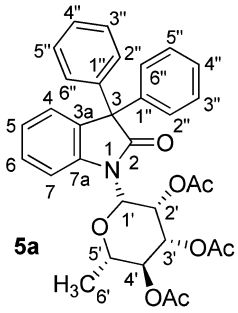


Fig. 4 Numbering of compound **5a** and related structures (**5**, **6**).

Conclusion

In conclusion, *N*-glycosylated 3,3-diaryloxindoles were prepared by Lewis acid catalyzed reaction of acetylated *N*-glycosylisatins with various benzene derivatives and subsequent deprotection. Some of the products showed good antiproliferative activity against malignant cutaneous melanoma cells HT-144 (ATCC HTB-63) and Lung carcinoma (H157) cell lines (ATCC CRL-5802) and no effect towards normal body cells.

Experimental

All solvents were anhydrous and commercially available and all reactions were performed under argon atmosphere. Yields refer to isolated products. ^{1}H NMR spectra (250.13 MHz, 300.13 MHz and 500.13 MHz) and ^{13}C NMR spectra (62.9 MHz, 75.5 MHz and 125.8 MHz) were recorded on Bruker spectrometers AV 250, AV 300 and AV 500 in $CDCl_3$ and acetone-d_6 as solvents. The

Published on 02 May 2014. Downloaded by University of Waikato on 15/07/2014 10:07:17.

calibration of spectra was carried out on solvent signals ($CDCl_3$: δ (^{1}H) = 7.25, δ (^{13}C) = 77.0; acetone-d_6: δ (^{1}H) = 2.05, δ (^{13}C) = 30.8). Mass spectrometric data (MS) were obtained by electron ionization (EI, 70 eV) or electrospray ionization (ESI). The mass spectra and high-resolution mass spectra (HRMS) were recorded on the following MS instruments: Time-of-Flight LC/MS 6210 (Agilent Technologies) for ESI and Finnigan MAT 95 XP (Thermo Electron Corporation) for EI. Elemental analyses were performed on a C,H,N,S analyser (Thermo Quest Flash EA 1112). The melting points were measured with a polarizing microscope (Leitz Laborlux 12 Pol-S) equipped with a hot stage (Mettler FP 90). Thin layer chromatography was performed on silica-gel foil (Merck DC-foil, silica gel 60, F_{254}) and silica gel plates (Merck HPTLC precoated plates, silica gel 60, F_{254}) pursues. Detection was carried out *via* UV absorbance at 254 nm or 366 nm and/or development with 10% methanolic sulfuric acid and subsequent heat treatment. Column chromatography was performed on Macherey-Nagel silica gel 60 (particle size 63–200 nm, 70–230 mesh).

General procedure A: preparation of the *N*-glycosylated 3,3-diarylindolin-2-ones 5a–l

The isatin-*N*-glycoside **4** was dissolved in the corresponding aromatic compound and $AlCl_3$ was added. After heating for 3 h at 80 °C the reaction was complete (TLC-control). Water was given to the reaction mixture and the organic layer was separated. The aqueous layer was extracted three times with ethyl acetate. The combined organic layer was washed three times with 1 M $NaHCO_3$-solution, one time with water and dried over sodium sulfate. After evaporation the residue was purified by column chromatography (heptane–ethyl acetate = 3 : 1).

1-(2,3,4-Tri-*O*-acetyl-β-L-rhamnopyranosyl)-3,3-diphenylindolin-2-one (5a). Starting with **4a** (200 mg, 0.48 mmol), benzene (20 mL) and $AlCl_3$ (200 mg, 1.50 mmol) **5a** was isolated as a colorless solid (250 mg, 94%), mp = 265–267 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.78–7.76 (m, 1H, Ar), 7.37–7.19 (m, 10H, Ar), 7.13–7.06 (m, 3H, Ar), 6.04 (d, $^3J_{1',2'}$ = 1.5 Hz, 1H, H-1′), 5.65 (dd, $^3J_{2',1'}$ = 1.5 Hz, $^3J_{2',3'}$ = 3.4 Hz, 1H, H-2′), 5.38 (dd, $^3J_{3',2'}$ = 3.4 Hz, $^3J_{3',4'}$ = 10.4 Hz, 1H, H-′), 5.24 ("t", J = 9.8 Hz, 1H, H-4), 4.09–4.00 (m, 1H, H-5′), 2.08, 1.94, 1.78 (3 s, 3 × OCOC*H*$_3$), 1.33 (d, $^3J_{6',5'}$ = 6.0 Hz, 3H, H-6′). ^{13}C NMR (63 MHz, $CDCl_3$): δ = 178.0 (C-2), 171.4, 171.2, 171.1 (3 × *C*OOCH$_3$), 144.5, 142.9, 134.3, 134.3 (4 × C_{Qu}), 130.4 (C–H), 130.2 (C–H), 130.1 (C–H), 130.0 (C–H), 129.7 (C–H), 129.3 (C–H), 128.9 (C–H), 127.5 (C–H), 124.5 (C–H), 116.3 (C–H), 82.5 (C-1′), 75.3 (C-5′), 72.4 (C-4′), 71.9 (C-3′), 71.8 (C-2′), 63.8 (C-3), 21.7, 21.7, 21.4 (3 × OCO*C*H$_3$), 18.8 (C-6′). MS (EI, 70 eV): m/z (%) = 557 (M^+, 57.47), 314 (10), 285 (40), 269 (25), 273 (61), 256 (22), 213 (19), 171 (25), 153 (100), 111 (73). HRMS (EI): calcd. for $C_{32}H_{31}N_2O_8$ $[M]^+$ 557.20442. Found 557.204724. Anal. calc. for $C_{32}H_{31}NO_8$ (557.59): C, 68.93; H, 5.60; N, 2.51. Found: C, 68.94; H, 5.77; N, 2.46.

1-(2,3,4-Tri-*O*-acetyl-β-L-rhamnopyranosyl)-3,3-bis(4-methylphenyl)indolin-2-one (5b). Starting with **4a** (200 mg, 0.48 mmol), toluene (20 mL) and $AlCl_3$ (200 mg, 1.50 mmol) **5b** was isolated as a colorless solid (156 mg, 56%), mp = 114–116 °C. ^{1}H NMR (300 MHz, acetone-d_6): δ = 7.78–7.75 (m, 1H, Ar), 7.36–7.31 (m, 1H, Ar), 7.20–7.06 (m, 8H, Ar), 7.01–6.98 (m, 2H, Ar), 6.02 (d, $^3J_{1',2'}$ = 1.5 Hz, 1H, H-1′), 5.63 (dd, $^3J_{2',1'}$ = 1.5 Hz, $^3J_{2',3'}$ = 3.4 Hz, 1H, H-2′), 5.38 (dd, $^3J_{3',2'}$ = 3.4 Hz, $^3J_{3',4'}$ = 10.2 Hz, 1H, H-3′), 5.24 ("t", J = 9.6 Hz, 1H, H-4′), 4.10–4.00 (m, 1H, H-5′), 2.29, 2.27 (2 s, 2 × Me), 2.09, 1.94, 1.78 (3 s, 3 × OCOC*H*$_3$), 1.34 (d, $^3J_{6',5'}$ = 6.2 Hz, 3H, H-6′). ^{13}C NMR (63 MHz, acetone-d_6): δ = 178.3 (C-2), 171.4, 171.2, 171.1 (3 × *OCO*CH$_3$), 142.4, 141.6, 140.0, 138.9, 138.4, 134.7 (4 × C_{Qu}), 130.8 (2 × C–H), 130.7 (2 × C–H), 130.3 (2 × C–H), 129.9 (2 × C–H), 129.5 (C–H), 127.4 (C–H), 124.4 (C–H), 116.2 (C–H), 82.4 (C-1′), 75.3 (C-5′), 72.4 (C-4′), 71.9 (C-3′), 71.8 (C-2′), 63.1 (C-3), 21.9 (2 × Me), 21.7, 21.7, 21.4 (3 × OCO*C*H$_3$), 18.8 (C-6′). MS (EI, 70 eV): m/z (%) = 585 (M^+, 30), 312 (43), 284 (14), 273 (34), 222 (13), 213 (16), 171 (19), 153 (100), 111 (53). HRMS (ESI): calcd for $C_{34}H_{36}NO_8$ $[M + H]^+$ 586.24354 and for $C_{34}H_{35}NaNO_8$ $[M + Na]^+$ 608.22549. Found 586.24393 and 608.2261.

1-(2,3,4-Tri-*O*-acetyl-β-L-rhamnopyranosyl)-3,3-bis(4-methoxyphenyl)indolin-2-one (5c). Starting with **4a** (300 mg, 0.72 mmol), anisole (20 mL) and $AlCl_3$ (200 mg, 1.50 mmol) **5c** was isolated as a colorless solid (433 mg, 98%), mp = 114–116 °C. ^{1}H NMR (300 MHz, acetone-d_6): δ = 7.62–7.60 (m, 1H, Ar), 7.21–7.15 (m, 1H, Ar), 7.05–6.99 (m, 3H, Ar), 6.97–6.94 (m, 1H, Ar), 6.92–6.86 (m, 2H, Ar), 6.75–6.67 (m, 4H, Ar), 5.87 (d, $^3J_{1',2'}$ = 1.5 Hz, 1H, H-1′), 5.49 (dd, $^3J_{2',1'}$ = 1.5 Hz, $^3J_{2',3'}$ = 3.6 Hz, 1H, H-2′), 5.24 (dd, $^3J_{3',2'}$ = 3.5 Hz, $^3J_{3',4'}$ = 10.3 Hz, 1H, H-3′), 5.10 ("t", J = 9.8 Hz, 1H, H-4′), 3.95–3.85 (m, 1H, H-5′), 3.63, 3.62 (2 s, 2 × OMe), 1.95, 1.80, 1.65 (3 s, 3 × OCOC*H*$_3$), 1.19 (d, $^3J_{6',5'}$ = 6.2 Hz, 3H, H-6′). ^{13}C NMR (63 MHz, acetone-d_6): δ = 178.6 (C-2), 171.4, 171.2, 171.1 (3 × *OCO*CH$_3$), 161.0, 160.7, 142.3, 136.6, 135.1, 134.9 (6 × C_{Qu}), 131.5 (2 × C–H), 131.1 (2 × C–H), 129.4 (C–H), 127.4 (C–H), 124.4 (C–H), 116.2 (C–H),115.4 (2 × C–H), 82.4 (C-1′), 75.3 (C-5′), 72.4 (C-4′), 71.9 (C-3′), 71.8 (C-2′), 62.4 (C-3), 56.5, 56.4 (2 × OMe), 21.7, 21.7, 21.4 (3 × OCO*C*H$_3$), 18.9 (C-6′). MS (EI, 70 eV): m/z (%) = 617 (M^+, 20), 344 (97), 316 (16), 273 (21), 238 (12), 207 (28), 171 (12), 153 (54), 111 (35). HRMS (EI): calcd for $C_{34}H_{35}NO_{10}$ $[M]^+$ 617.22555. Found 617.22491. Anal. calc. for $C_{36}H_{37}NO_{12}$ (617.64): C, 66.12; H, 5.71; N, 2.27. Found: C, 66.33; H, 5.53; N, 2.36.

1-(2,3,4-Tri-*O*-acetyl-β-L-rhamnopyranosyl)-3,3-bis[4-(dimethylamino)phenyl]indolin-2-one (5d). Starting with **4a** (1.0 g, 2.38 mmol), *N*,*N*-dimethylaniline (20 mL) and $AlCl_3$ (600 mg, 4.50 mmol) **5d** was isolated as a colorless solid (1.380 g, 90%), mp = 130–132 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.58–7.55 (m, 1H, Ar), 7.22–7.10 (m, 4H, Ar), 7.03–6.96 (m, 3H, Ar), 6.65–6.62 (m, 4H, Ar), 5.87 (d, $^3J_{1',2'}$ = 1.5 Hz, 1H, H-1′), 5.66 (dd, $^3J_{2',1'}$ = 1.5 Hz, $^3J_{2',3'}$ = 2.8 Hz, 1H, H-2′), 5.28–5.18 (m, 2H, H-3′, H-4′), 3.73–3.64 (m, 1H, H-5′), 2.91, 2.90 (2 s, 2 × NMe_2), 2.08, 1.97, 1.81 (3 s, 3 × OCOC*H*$_3$), 1.34 (d, $^3J_{6',5'}$ = 6.2 Hz, 3H, H-6′). ^{13}C NMR (63 MHz, $CDCl_3$): δ = 177.7 (C-2), 170.0, 169.5, 169.5 (3 × *OCO*CH$_3$), 149.5, 149.2, 140.1, 133.9 (4 × C_{Qu}), 129.4 (C–H), 128.6 (C–H), 126.9 (C–H), 125.5 (C–H), 122.6 (C–H), 113.7 (C–H), 112.4 (C–H), 112.1 (C–H), 80.5 (C-1′), 76.4 (C-5′), 73.8 (C-4′), 70.6 (C-3′), 70.4 (C-2′), 60.6 (C-3), 20.7, 20.7, 20.4 (3 × OCO*C*H$_3$), 17.6 (C-6′). MS (EI, 70 eV): m/z (%) = 643 (M^+, 24), 370 (18), 157 (33), 135 (9), 115 (25). HRMS (EI): calcd. for $C_{36}H_{41}N_3O_8$ $[M]^+$ 643.28882. Found 643.28907. Anal. calc. for $C_{36}H_{41}N_3O_8$ (643.73): C, 67.17; H, 6.42; N, 6.53. Found: C, 67.13; H, 6.50; N, 5.97.

 This journal is © The Royal Society of Chemistry 2014

Published on 02 May 2014. Downloaded by University of Waikato on 15/07/2014 10:07:17.

1-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-3,3-bis(4-methoxyphenyl)indolin-2-one (5e). Starting with **4b** (200 mg, 0.42 mmol), anisole (10 mL) and $AlCl_3$ (220 mg, 1.65 mmol) **5e** was isolated as a colorless solid (241 mg, 85%), mp = 108–110 °C. ^{1}H NMR (250 MHz, acetone-d_6): δ = 7.63–7.60 (m, 1H, Ar), 7.38–7.32 (m, 1H, Ar), 7.25–7.22 (m, 1H, Ar), 7.16–7.04 (m, 5H, Ar), 6.90–6.84 (m, 4H, Ar), 5.94 (d, $^3J_{2',1'}$ = 9.5 Hz, 1H, H-1′), 5.73 ("t", ^3J = 9.4 Hz, 1H, H-2′), 5.49 ("t", ^3J = 9.4 Hz, 1H, H-3′), 5.37 ("t", ^3J = 9.4 Hz, 1H, H-4′), 4.32–4.20 (m, 3H, H-5′, H-6′), 3.76, 3.75 (2 s, 6H, 2 × OMe_2), 2.04, 2.03, 1.92, 1.41 (4 s, 4 × OCOC*H*$_3$). ^{13}C NMR (63 MHz, acetone-d_6): δ = 179.2 (C-2), 171.6, 171.1, 171.0, 170.2 (4 × *OCO*CH_3) 160.9, 160.8, 141.1, 136.2, 135.2, 134.6 (6 × C_{Qu}), 131.5 (C–H), 130.0 (C–H), 127.8 (C–H), 124.8 (C–H), 113.7 (C–H), 115.5 (C–H), 115.4 (C–H), 114.6 (C–H), 81.1 (C-1′), 76.1 (C-5′), 75.0 (C-3′), 69.8 (C-4′), 69.0 (C-2′), 63.5 (C-6′), 62.6 (C-3), 56.5 (2 × NMe_2), 21.6, 21.6, 21.4, 20.9 (4 × OCO*C*H$_3$). MS (EI, 70 eV): *m*/*z* (%) = 675 (M^+, 30.42), 344 (99), 331 (29), 169 (100), 127 (11), 109 (34). HRMS (EI): calcd. for $C_{36}H_{37}NO_{12}$ $[M]^+$ 675.23103, found 675.23139. Anal. calc. for $C_{36}H_{37}NO_{12}$ (675.68): C, 63.99; H, 5.52; N, 2.07. Found: C, 64.17; H, 5.33; N, 1.87.

1-(2,3,4,6-Tetra-*O*-acetyl-β-D-mannopyranosyl)-3,3-diphenylindolin-2-one (5f). Starting with **4c** (200 mg, 0.42 mmol), benzene (20 mL) and $AlCl_3$ (220 mg, 1.65 mmol) **5f** was isolated as a colorless solid (152 mg, 59%), mp = 95–97 °C. ^{1}H NMR (250 MHz, acetone-d_6): δ = 7.81–7.77, (m, 1H, Ar), 7.38–7.20 (m, 10H, Ar), 7.13–7.07 (m, 3H, Ar), 6.14 (d, $^3J_{1',2'}$ = 1.5 Hz, 1H, H-1′), 5.67 (dd, $^3J_{2',1'}$ = 1.7 Hz, $^3J_{2',3'}$ = 3.0 Hz, 1H, H-2′), 5.56–5.45 (m, 2H, H-3′, H-4′), 4.35–4.23 (m, 2H, H-5′, H-6′), 2.08, 2.08, 1.95, 1.78 (4 s, 4 × OCOC*H*$_3$). ^{13}C NMR (63 MHz, acetone-d_6): δ = 178.0 (C-2), 171.6, 171.2, 171.2, 171.1 (4 × *OCO*CH_3), 144.5, 142.8, 142.4, 134.3 (4 × C_{Qu}), 130.4 (2 × C–H), 130.2 (2 × C–H), 130.1 (2 × C–H), 130.0 (2 × C–H), 129.7 (C–H), 129.4 (C–H), 129.0 (C–H), 127.6 (C–H), 124.6 (C–H), 116.4 (C–H), 82.5 (C-1′), 76.7 (C-5′), 72.4 (C-3′), 71.6 (C-4′), 66.9 (C-2′), 63.8 (C-3), 63.7 (C-6′), 21.6, 21.6, 21.6, 21.4 (4 × $OCOCH_3$). MS (EI, 70 eV): *m*/*z* (%) = 615 (M^+, 11.19), 331 (23), 285 (24), 256 (14), 169 (97), 109 (68). HRMS (EI): calcd. for $C_{34}H_{33}NO_{10}$ $[M]^+$ 615.20990. Found 615.210250. Anal. calc. for $C_{34}H_{33}NO_{10}$ (615.63): C, 66.33; H, 5.40; N, 2.28. Found: C, 66.86; H, 5.74; N, 2.28.

1-(2,3,4,6-Tetra-*O*-acetyl-β-D-mannopyranosyl)-3,3-bis(4-methylphenyl)indolin-2-one (5g). Starting with **4c** (200 mg, 0.42 mmol), toluene (20 mL) and $AlCl_3$ (230 mg, 1.72 mmol) **5g** was isolated as a colorless solid (148 mg, 55%), mp = 107–109 °C. ^{1}H NMR (300 MHz, acetone-d_6): δ = 7.79–7.76, (m, 1H, Ar), 7.35–7.30 (m, 8H, Ar), 7.19–7.05 (m, 1H, Ar), 7.00–6.96 (m, 2H, Ar), 6.11 (d, $^3J_{1',2'}$ = 1.5 Hz, 1H,H-1′), 5.65 (dd, $^3J_{2',1'}$ = 1.5 Hz, $^3J_{2',3'}$ = 3.0 Hz, 1H, H-2′), 5.55–5.44 (m, 2H, H-3′, H-4′), 4.35–4.22 (m, 2H, H-5′, H-6′), 2.29, 2.27 (2 s, 2 × Me), 2.08, 2.07, 1.95, 1.78 (4 s, 4 × OCOC*H*$_3$). ^{13}C NMR (63 MHz, acetone-d_6): δ = 178.3 (C-2), 171.6, 171.2, 171.2, 171.1 (4 × *OCO*CH_3), 142.3, 141.6, 140.0, 138.9, 138.5, 134.7 (6 × C_{Qu}), 130.8 (2 × C–H), 130.7 (2 × C–H), 130.3 (2 × C–H), 129.9 (C–H), 129.5 (C–H), 127.4 (C–H), 124.5 (C–H), 116.3 (C–H), 82.4 (C-1′), 76.7 (C-5′), 72.4 (C-3′), 71.6 (C-4′), 66.9 (C-2′), 63.8 (C-6′), 63.2 (C-3), 21.9, 21.6, 21.6, 21.4 (4 × $OCOCH_3$). MS (EI, 70 eV): *m*/*z* (%) = 643 (M^+, 31), 331 (22), 312 (57), 284 (12), 222 (10), 169 (100), 109 (33). HRMS (EI): calcd. for $C_{36}H_{37}NO_{10}$ $[M]^+$ 643.24120. Found 643.24124.

1-(2,3,4,6-Tetra-*O*-acetyl-β-D-mannopyranosyl)-3,3-bis(4-methoxyphenyl)indolin-2-one (5h). Starting with **4c** (300 mg, 0.63 mmol), anisole (10 mL) and $AlCl_3$ (300 mg, 2.25 mmol) **5h** was isolated as a colorless solid (403 mg, 95%), mp = 119–121 °C. ^{1}H NMR (300 MHz, acetone-d_6): δ = 7.78–7.75, (m, 1H, Ar), 7.35–7.29 (m, 1H, Ar), 7.18–6.98 (m, 6H, Ar), 6.88–6.80 (m, 4H, Ar), 6.10 (d, $^3J_{1',2'}$ = 1.7 Hz, 1H,H-1′), 5.60 (dd, $^3J_{2',1'}$ = 1.7 Hz, $^3J_{2',3'}$ = 3.0 Hz, 1H, H-2′), 5.55–5.44 (m, 2H, H-3′, H-4′), 4.35–4.20 (m, 2H, H-5′, H-6′), 3.76, 3.75 (2 s, 2 × OMe), 2.07, 2.07, 1.94, 1.77 (4 s, 4 × OCOC*H*$_3$). ^{13}C NMR (63 MHz, acetone-d_6): δ = 178.6 (C-2), 171.6, 171.2, 171.2, 171.1 (4 × *OCO*CH_3), 161.0, 160.7, 142.3, 136.5, 135.0, 134.8 (6 × C_{Qu}), 131.5 (2 × C–H), 131.1 (2 × C–H), 129.4 (C–H), 127.4 (C–H), 124.5 (C–H), 116.4 (C–H), 115.4 (2 × C–H), 82.4 (C-1′), 76.7 (C-5′), 72.4 (C-3′), 71.6 (C-4′), 66.9 (C-2′), 63.8 (C-3), 63.8 (C-6′), 62.4 (C-3), 56.4 (2 × OMe), 21.6, 21.6, 21.6, 21.4 (4 × $OCOCH_3$). MS (EI, 70 eV): *m*/*z* (%) = 675 (M^+, 28), 344 (100), 331 (12), 169 (47), 109 (15). HRMS (EI): calcd. for $C_{36}H_{37}NO_{12}$ $[M]^+$ 675.23103. Found 675.23126. Anal. calc. for $C_{36}H_{37}NO_{12}$ (675.68): C, 63.99, H; 5.52; N, 2.07. Found: C, 64.02; H, 5.44; N, 2.13.

1-(2,3,4,6-Tetra-*O*-acetyl-β-D-mannopyranosyl)-3,3-bis[4-(dimethylamino)phenyl]-indolin-2-one (5i). Starting with **4c** (200 mg, 0.42 mmol), *N*,*N*-dimethylaniline (10 mL) and $AlCl_3$ (220 mg, 1.65 mmol) **5i** was isolated as a colorless solid (235 mg, 80%), mp = 102–104 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.56–7.53 (m, 1H, Ar), 7.20–7.10 (m, 3H, Ar), 7.03–6.95 (m, 4H, Ar), 6.64–6.61 (m, 4H, Ar), 5.90 (d, $^3J_{2',1'}$ = 1.5 Hz, 1H, H-1′), 5.68 (dd, $^3J_{1',2'}$ = 1.5 Hz, $^3J_{3',2'}$ = 3.4 Hz, 1H, H-2′), 5.43 (t, $^3J_{3',4'}$ = 10.0 Hz 1H, H-4′), 5.30 (dd, $^3J_{4',3'}$ = 10.0 Hz, $^3J_{2',3'}$ = 3.4 Hz, 1H, H-3′), 4.33–4.18 (m, 2H, H-6′), 3.85–3.79 (m, 1H, H-5′), 2.90, 2.89 (2 s, 12H, 2 × NMe_2), 2.12, 2.07, 1.97, 1.81 (4 s, 4 × OCOC*H*$_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 177.7 (C-2), 170.5, 169.7, 169.4, 169.4 (4 × *OCO*CH_3) 149.6, 149.3, 139.9, 133.9 (4 × C_{Qu}), 129.4 (C–H), 128.5 (C–H), 128.4 (C–H), 126.8 (C–H), 125.5 (C–H), 122.7 (C–H), 113.7 (C–H), 112.3 (C–H), 112.0 (C–H) 80.7 (C-1′), 75.3 (C-2′), 70.5 (C-3′), 70.0 (C-4′), 65.4 (C-5′), 62.2 (C-6′), 60.6 (C-3), 40.4 (4 × NMe_2), 20.7, 20.7, 20.6, 20.4 (4 × OCO*C*H$_3$). MS (EI, 70 eV): *m*/*z* (%) = 701 (M^+, 31.94), 370 (14), 281 (10), 231 (12), 181 (21), 169 (23), 131 (28), 119 (29). HRMS (ESI): calcd for $C_{23}H_{44}N_3O_{10}$ $[M + H]^+$ 702.30212 and for $C_{23}H_{43}N_3NaO_{10}$ $[M + Na]^+$ 724.28407. Found 702.30336 and 724.28526. Anal. calc. for $C_{38}H_{43}N_3O_{10}$ (701.76): C, 65.04; H, 6.18; N, 5.99. Found: C, 64.91; H, 6.12; N, 5.39.

1-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-3,3-diphenylindolin-2-one (5j). Starting with **4d** (200 mg, 0.42 mmol), benzene (20 mL) and $AlCl_3$ (400 mg, 3.00 mmol) **5j** was isolated as a colorless solid (170 mg, 66%), mp = 110–112 °C. ^{1}H NMR (300 MHz, acetone-d_6): δ = 7.64–7.61 (m, 1H, Ar), 7.44–7.28 (m, 8H, Ar), 7.23–7.14 (m, 5H, Ar), 5.95–5.86 (m, 2H, H-1, H-2), 5.58 (dd, $^3J_{3',4'}$ = 3.4 Hz, $^3J_{5',4'}$ = 0.9 Hz, 1H, H-4′), 5.41–5.37 (m, 1H, H-3′), 4.56 (dt, $^3J_{6',5'}$ = 6.4 Hz, $^3J_{4',5'}$ = 0.8 Hz, 1H, H-5′), 4.31–4.11 (m, 2H, H-6′), 2.30, 1.98, 1.92, 1.36 (4 s, 4 × OCOC*H*$_3$). ^{13}C NMR (63 MHz, acetone-d_6): δ = 178.5 (C-2), 171.5, 171.5, 171.1, 170.4 (4 × *OCO*CH_3), 144.4, 143.0, 141.5, 134.0 (4 × C_{Qu}), 130.6 (C–H), 130.3 (C–H), 130.2 (C–H), 130.0 (C–H), 130.0 (C–H), 129.3 (C–H), 129.1 (C–H), 128.0 (C–H), 124.9 (C–H), 114.3 (C–H),

Published on 02 May 2014. Downloaded by University of Waikato on 15/07/2014 10:07:17.

81.4 (C-1′), 74.7 (C-2′), 73.1 (C-3′), 69.5 (C-4′), 66.7 (C-5′), 64.0 (C-6′), 63.3 (C-3), 21.7, 21.5, 21.4, 20.8 (4 × OCO*C*H$_3$). MS (EI, 70 eV): *m*/*z* (%) = 615 (M$^+$, 28.01), 331 (61), 285 (25), 169 (100), 127 (14), 109 (32). HRMS (EI): calcd for $C_{34}H_{33}NO_{10}$ [M]$^+$ 615.20990. Found 615.21111. Anal. calc. for $C_{34}H_{33}NO_{10}$ (615.63): C, 66.33; H, 5.40; N, 2.28. Found: C, 66.33; H, 5.05; N, 1.94.

1-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-3,3-bis(4-methylphenyl)indolin-2-one (5k). Starting with **4d** (200 mg, 0.42 mmol), toluene (10 mL) and $AlCl_3$ (220 mg, 1.65 mmol) **5k** was isolated as a colorless solid (157 mg, 62%), mp = 111–113 °C. ^{1}H NMR (300 MHz, acetone-d_6): δ = 7.62–7.59 (m, 1H, Ar), 7.42–7.36 (m, 1H, Ar), 7.27–7.24 (m, 1H, Ar), 7.16–7.05 (m, 9H, Ar), 5.94–5.86 (m, 2H, H-1, H-2), 5.58 (dd, $^3J_{3',4'}$ = 3.4 Hz, $^3J_{5',4'}$ = 0.9 Hz, 1H, H-4′), 5.40–5.36 (m, 1H, H-3′), 4.54 (dt, $^3J_{6',5'}$ = 6.6 Hz, $^3J_{4',5'}$ = 0.9 Hz, 1H, H-5′), 4.30–4.10 (m, 2H, H-6′), 2.29, 2.28 (2 s, 2 × Me), 2.30, 1.98, 1.92, 1.36 (4 s, 4 × OCOC*H*$_3$). ^{13}C NMR (75 MHz, acetone-d_6): δ = 178.8 (C-2), 171.5, 171.5, 171.1, 170.3 (4 × *OCO*CH$_3$), 141.4, 140.3, 138.8, 138.6, 134.4 (5 × C_{Qu}), 130.8 (C–H), 130.6 (C–H), 130.0 (C–H), 130.0 (C–H), 129.9 (C–H), 127.9 (C–H), 124.8 (C–H), 114.1 (C–H), 81.3 (C-1′), 74.7 (C-2′), 73.2 (C-3′), 69.5 (C-4′), 66.7 (C-5′), 63.4 (C-6′), 63.3 (C-3), 21.9 (2 × Me) 21.7, 21.5, 21.4, 20.8 (4 × OCO*C*H$_3$). MS (EI, 70 eV): *m*/*z* (%) = 643 (M$^+$, 52.00), 331 (77), 312 (52), 284 (10), 169 (100), 127 (8), 109 (20). HRMS (EI): calcd for $C_{36}H_{37}NO_{10}$ [M]$^+$ 643.24120. Found 643.24204. Anal. calc. for $C_{36}H_{37}NO_{10}$ (643.68): C, 67.17; H, 5.79; N, 2.18. Found: C, 67.49; H, 5.75; N, 1.80.

1-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-3,3-bis(4-methoxyphenyl)indolin-2-one (5l). Starting with **4d** (200 mg, 0.42 mmol), anisole (10 mL) and $AlCl_3$ (220 mg, 1.65 mmol) **5l** was isolated as a colorless solid (260 mg, 92%), mp = 104–106 °C. ^{1}H NMR (250 MHz, $CDCl_3$): δ = 7.44–7.41 (m, 1H, Ar), 7.33–7.25 (m, 1H, Ar), 7.18–7.06 (m, 6H, Ar), 6.83–6.77 (m, 4H, Ar), 5.84 ("t", $^3J_{1',2'}$ = 9.5 Hz, $^3J_{3',2'}$ = 9.8 Hz, 1H, H-2′), 5.73 (d, $^3J_{2',1'}$ = 9.5 Hz, 1H, H-1′), 5.55 (d, $^3J_{3',4'}$ = 3.2 Hz, 1H, H-4′), 5.16 (dd, $^3J_{2',3'}$ = 9.8 Hz, $^3J_{4',3'}$ = 3.2 Hz, 1H, H-3′), 4.25–4.09 (m, 3H, H-5′, H-6′), 3.76 (s, 2 × OMe), 2.29, 2.06, 1.97, 1.33 (4 s, 4 × OCOC*H*$_3$). ^{13}C NMR (63 MHz, $CDCl_3$): δ = 178.1, 170.3, 169.9, 169.8 (4 × *OCO*CH$_3$), 168.9 (C-2), 158.9, 158.6 (2 × C-4″), 139.0 (C-7a), 134.5 (C-1″), 132.8 (C-3a), 132.7 (C-5), 129.9, 129.2 (2 × C-2″), 128.1 (C-4), 126.0 (C-6), 123.3 (C-5), 113.8, 113.6 (2 × C-3″), 112.0 (C-7), 79.7 (C-1′), 73.1 (C-2′), 71.5 (C-3′), 67.3 (C-4′), 64.6 (C-5′), 61.3 (C-6′), 61.0 (C-3), 55.2, 55.2 (2 × OMe), 20.7, 20.6, 20.5, 19.6 (4 × OCOC*H*$_3$). MS (EI, 70 eV): *m*/*z* (%) = 675 (M$^+$, 27), 344 (85), 331 (74), 169 (75), 127 (12), 109 (28). HRMS (EI): calcd for $C_{36}H_{37}NO_{12}$ [M]$^+$ 675.23103. Found 675.23040. Anal. calc. for $C_{36}H_{37}NO_{12}$ (675.68): C, 63.99; H, 5.52; N, 2.07. Found: C, 64.03; H, 5.59; N, 2.06.

General procedure B: synthesis of the deprotected *N*-glycosylated 3,3-diarylindolin-2-ones 6a–l

One equivalent of the acetyl-protected *N*-glycosylated 3,3-diarylindolin-2-one **5** was solved in dry MeOH. To the stirred solution 5 mol% NaOMe (0.1% solution) was added and the mixture stirred for 12 h. When the reaction was complete (TLC-control) the reaction mixture was neutralized by addition of acetic acid. The solvent was removed under reduced pressure, the remaining residue solved in ethyl acetate and the solution washed three times with water. After separation of the organic layer and evaporation of the solvent the solid product **6** was obtained.

3,3-Diphenyl-1-(β-L-rhamnopyranosyl)indolin-2-one (6a). Starting with **5a** (100 mg, 0.18 mmol), dray methanol (4 mL) and 0.1% solution of NaOMe (0.49 mL, 5 mol%) **6a** was isolated as a colorless solid (62 mg, 80%), mp = 123–125 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.75–7.72 (m, 1H, Ar), 7.34–7.20 (m, 12H, Ar), 7.07–7.02 (m, 1H, Ar), 5.63 (d, $^3J_{1',2'}$ = 1.1 Hz, 1H, H-1′), 4.53 (brs, 1H, OH), 4.22–4.02 (m, 3H, H-2′, 2 × OH), 3.70–3.47 (m, 3H, H-3′, H-4′, H-5′), 1.35 (d, $^3J_{6',5'}$ = 5.8 Hz, 3H, H-6′). ^{13}C NMR (63 MHz, $CDCl_3$): δ = 178.4 (C-2), 144.5, 144.0, 143.7, 134.0 (4 × C_{Qu}), 130.4 (2 × C–H), 130.2 (2 × C–H), 130.1 (2 × C–H), 130.0 (2 × C–H), 129.3 (C–H), 129.1 (C–H), 128.9 (C–H), 127.0 (C–H), 124.1 (C–H), 117.2 (C–H), 113.8 (C–H), 84.6 (C-1′), 77.3 (C-2′), 75.8 (C-3′), 74.2 (C-4′), 73.8 (C-5′), 63.9 (C-3), 19.3 (C-6′). MS (EI, 70 eV): *m*/*z* (%) = 431 (M$^+$, 25), 285 (100), 256 (32), 208 (21), 165 (10). HRMS (EI): calcd for $C_{26}H_{25}NO_5$ [M]$^+$ 431.17272. Found 431.17278.

3,3-Bis(4-methylphenyl)-1-(β-L-rhamnopyranosyl)indolin-2-one (6b). Starting with **5b** (100 mg, 0.17 mmol), dray methanol (4 mL) and 0.1% solution of NaOMe (0.46 mL, 5 mol%) **6b** was isolated as a colorless solid (60 mg, 77%), mp = 128–129 °C. ^{1}H NMR (300 MHz, acetone-d_6): δ = 7.71–7.68 (m, 1H, Ar), 7.24–7.00 (m, 13H, Ar), 5.60 (d, $^3J_{1',2'}$ = 1.3 Hz, 1H, H-1′), 4.53 (brs, 1H, OH), 4.25–4.01 (m, 3H, H-2′, 2 × OH), 3.70–3.46 (m, 3H, H-3′, H-4′, H-5′), 2.29, 2.28 (2 s, 2 × OMe), 1.35 (d, $^3J_{6',5'}$ = 5.6 Hz, 3H, H-6′). ^{13}C NMR (63 MHz, acetone-d_6): δ = 178.8 (C-2), 143.9, 141.6, 140.9, 138.6, 138.4, 134.5 (6 × C_{Qu}), 130.7 (2 × CH), 130.6 (2 × CH), 130.3 (2 × CH), 129.2 (2 × CH), 126.8 (2 × CH), 124.0 (CH), 117.0 (CH), 84.6 (C-1′), 77.3 (C-5′), 75.8 (C-4′), 74.3 (C-3′), 73.8 (C-2′), 63.3 (C-3), 21.9 (2 × Me), 19.3 (C-6′). MS (EI, 70 eV): *m*/*z* (%) = 459 (M$^+$, 49), 313 (100), 298 (20), 284 (31), 270 (29), 222 (43), 194 (13). HRMS (EI): calcd for $C_{28}H_{29}NO_5$ [M]$^+$ 459.20402. Found 459.20427.

3,3-Bis(4-methoxyphenyl)-1-(β-L-rhamnopyranosyl)indolin-2-one (6c). Starting with **5c** (200 mg, 0.32 mmol), dray methanol (4 mL) and 0.1% solution of NaOMe (0.87 mL, 5 mol%) **6c** was isolated as a colorless solid (129 mg, 81%), mp = 125–127 °C.^{1}H NMR (300 MHz, acetone-d_6): δ = 7.71–7.68 (m, 1H, Ar), 7.24–7.12 (m, 6H, Ar), 7.06–7.01 (m, 1H, Ar), 6.88–6.82 (m, 4H, Ar), 5.60 (d, $^3J_{1',2'}$ = 1.1 Hz, 1H, H-1′), 4.54 (brs, 1H, OH), 4.15–3.99 (m, 3H, H-2′, 2 × OH), 3.79–3.46 (m, 9H, H-3′, H-4′, H-5′, 2 × OMe), 1.35 (d, $^3J_{6',5'}$ = 5.6 Hz, 3H, H-6′). ^{13}C NMR (63 MHz, acetone-d_6): δ = 178.8 (C-2), 160.6, 160.4, 143.6, 136.2, 135.5, 134.5 (6 × C_{Qu}), 131.2 (2 × CH), 131.0 (2 × CH), 128.8 (CH), 126.6 (CH), 123.7 (CH), 116.7 (CH), 115.1 (2 × CH), 115.1 (2 × CH), 84.3 (C-1′), 77.1 (C-5′), 75.6 (C-4′), 74.0 (C-3′), 73.5 (C-2′), 62.3 (C-3), 56.2 (2 × NMe_2), 19.1 (C-6′). MS (EI, 70 eV): *m*/*z* (%) = 491 (M$^+$, 19), 344 (100), 298 (12), 316 (22), 302 (11), 238 (18). HRMS (EI): calcd for $C_{28}H_{29}NO_7$ [M]$^+$ 491.19385. Found 491.19386. Anal. calc. for $C_{28}H_{29}NO_7$ (491.53): C, 68.42; H, 5.95; N, 2.85. Found: C, 68.07; H, 6.16; N, 2.29.

3,3-Bis[4-(dimethylamino)phenyl]-1-(β-L-rhamnopyranosyl) indolin-2-one (6d). Starting with **5d** (200 mg, 0.31 mmol), dray methanol (4 mL) and 0.1% solution of NaOMe (0.84 mL,

 This journal is © The Royal Society of Chemistry 2014

Published on 02 May 2014. Downloaded by University of Waikato on 15/07/2014 10:07:17.

5 mol%) **6d** was isolated as a colorless solid (138 mg, 86%), mp = 156–158 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.65–7.62 (m, 1H, Ar), 7.21–6.98 (m, 7H, Ar), 6.67–6.63 (m, 4H, Ar), 5.56 (d, $^3J_{1',2'}$ = 1.1 Hz, 1H, H-1′), 4.64 (brs, 1H, OH), 4.15–3.94 (m, 3H, H-2′, 2 × OH), 3.69–3.45 (m, 3H, H-3′, H-4′, H-5′), 2.89, 2.88 (2 × NMe_2), 1.34 (d, $^3J_{6',5'}$ = 5.8 Hz, 3H, H-6′). ^{13}C NMR (63 MHz, $CDCl_3$): δ = 179.8 (C-2), 151.6, 151.5, 143.9, 135.7, 132.2, 131.5 (6 × C_{Qu}), 130.9 (2 × CH), 130.7 (2 × CH), 128.7 (CH), 126.8 (CH), 123.7 (CH), 116.5 (CH), 113.8 (C–H), 84.6 (C-1′), 77.3 (C-5′), 75.9 (C-4′), 75.9 (C-3′), 74.4 (C-2′), 62.4 (C-3), 41.5 (2 × NMe_2), 19.3 (C-6′). MS (EI, 70 eV): *m*/*z* (%) = 517 (M^+, 73), 370 (100), 342 (50), 262 (9), 223 (9), 183 (8). HRMS (EI): calcd for $C_{30}H_{35}N_3O_5$ $[M]^+$ 517.25712. Found 517.25699. Anal. calc. for $C_{30}H_{35}N_3O_5$ (517.62): C, 69.61; H, 6.82. Found: C, 69.47; H, 6.96%.

1-(β-D-Glucopyranosyl)-3,3-bis(4-methoxyphenyl)indolin-2-one (6e). Starting with **5e** (100 mg, 0.15 mmol), dray methanol (4 mL) and 0.1% solution of NaOMe (0.40 mL, 5 mol%) **6e** was isolated as a colorless solid (61 mg, 82%), mp = 145–147 °C. ^{1}H NMR (300 MHz, acetone-d_6): δ 7.36–7.33 (m, 1H, Ar), 7.28–7.16 (m, 6H, Ar), 7.10–7.05 (m, 1H, Ar), 6.89–6.78 (m, 4H, Ar), 5.49 (d, $^3J_{1',2'}$ = 9.4 Hz, 1H, H-1′), 4.18–3.49 (m, 16H, H-2′, H-3′, H-4′, H-5′, H-6′, 4 × OH, 2 × OMe). ^{13}C NMR (75 MHz, acetone-d_6): δ = 179.2 (C-2), 160.9, 160.6, 142.4, 137.1, 135.4, 135.2 (6 × C_{Qu}), 131.6 (2 × C–H), 131.4 (2 × C–H), 129.6 (C–H), 127.7 (C–H), 124.2 (C–H), 115.3 (2 × C–H), 114.2 (C–H), 84.3 (C-1′), 81.7 (C-5′), 80.3 (C-3′), 72.3 (C-2′), 70.9 (C-4′), 63.5 (C-6′), 62.7 (C-3), 56.5, 56.4 (2 × OMe). MS (EI, 70 eV): *m*/*z* (%) = 507 (M^+, 30), 344 (100), 330 (11), 316 (35), 302 (16), 238 (26), 210 (9). HRMS (EI): calcd for $C_{28}H_{29}NO_8$ $[M]^+$ 507.18877. Found 507.18861. Anal. calc. for $C_{28}H_{29}NO_8$ (507.53): C, 66.26; H, 5.76. Found: C, 65.76; H, 5.82.

1-(β-D-Mannopyranosyl)-3,3-diphenylindolin-2-one (6f). Starting with **5f** (100 mg, 0.16 mmol), dray methanol (8 mL)/dray THF (2 mL) and 0.1% solution of NaOMe (0.43 mL, 5 mol%) **6f** was isolated as a colorless solid (55 mg, 76%), mp = 133–134 °C. ^{1}H NMR (300 MHz, acetone-d_6): δ = 7.76–7.73 (m, 1H, Ar), 7.35–7.18 (m, 7H, Ar), 7.07–7.01 (m, 1H, Ar), 5.67 (d, $^3J_{1',2'}$ = 1.1 Hz, 1H, H-1′), 4.54 (brs, 1H, OH), 4.23–3.47 (m, 9H, H-2′, H-3′, H-4′, H-5′, H-6′, 3 × OH). ^{13}C NMR (63 MHz, acetone-d_6): δ = 178.5 (C-2), 144.4, 144.0, 143.7, 134.0 (4 × C_{Qu}), 130.4 (2 × C–H), 130.2 (2 × C–H), 130.1 (2 × C–H), 130.0 (2 × C–H), 129.4 (C–H), 128.9 (C–H), 126.9 (C–H), 124.0 (C–H), 117.5 (C–H), 84.8 (C-1′), 82.1 (C-5′), 76.0 (C-3′), 73.7 (C-4′), 69.3 (C-2′), 63.9 (C-3), 63.7 (C-6′). MS (EI, 70 eV): *m*/*z* (%) = 447 (M^+, 23), 285 (100), 270 (10), 256 (47), 208 (22), 180 (12), 165 (13). HRMS (EI): calcd for $C_{26}H_{25}NO_6$ $[M]^+$ 447.16764. Found 447.16804.

1-(β-D-M annopyranosyl)-3,3-bis(4-methylphenyl)indolin-2-one (6g). Starting with **5g** (100 mg, 0.16 mmol), dray methanol (4 mL) and 0.1% solution of NaOMe (0.43 mL, 5 mol%) **6g** was isolated as a colorless solid (58 mg, 78%), mp = 139–140 °C. ^{1}H NMR (300 MHz, acetone-d_6): δ = 7.73–7.70 (m, 1H, Ar), 7.22–7.00 (m, 11H, Ar), 5.64 (d, $^3J_{1',2'}$ = 1.3 Hz, 1H, H-1′), 4.55 (brs, 1H, OH), 4.23–3.72 (m, 9H, H-2′, H-3′, H-4′, H-5′, H-6′, 3 × OH), 3.52–3.46 (m, 1H, H-5′), 2.29, 2.28 (2 s, 2 × Me). ^{13}C NMR (63 MHz, acetone-d_6): δ = 178.8 (C-2), 143.9, 141.5, 140.9, 138.6, 138.4, 134.4 (6 × C_{Qu}), 130.7 (2 × C–H), 130.6 (2 × C–H), 130.3 (2 × C–H), 130.1 (2 × C–H), 129.2 (C–H), 126.8 (C–H), 123.9 (C–H), 117.3 (C–H), 84.8 (C-1′), 82.1 (C-5′), 76.0 (C-3′), 73.6 (C-4′), 69.3 (C-2′), 63.7 (C-6′), 63.3 (C-3), 21.9 (2 × Me). MS (EI, 70 eV): *m*/*z* (%) = 475 (M^+, 23), 313 (100), 298 (17), 284 (21), 270 (21), 222 (20). HRMS (EI): calcd for $C_{28}H_{29}NO_6$ $[M]^+$ 475.19894. Found 475.199822.

1-(β-D-Mannopyranosyl)-3,3-bis(4-methoxyphenyl)indolin-2-one (6h). Starting with **5h** (200 mg, 0.30 mmol), dray methanol (8 mL)/dray THF (2 mL) and 0.1% solution of NaOMe (0.80 mL, 5 mol%) **6h** was isolated as a colorless solid (126 mg, 84%), mp = 135–137 °C. ^{1}H NMR (300 MHz, acetone-d_6): δ = 7.72–7.69 (m, 1H, Ar), 7.22–7.00 (m, 7H, Ar), 6.87–6.83 (m, 4H, Ar), 5.63 (d, $^3J_{1',2'}$ = 1.5 Hz, 1H, H-1′), 4.54–4.53 (m, 1H, OH), 4.18–3.72 (m, 7H, H-2′, H-3′, H-4′, H-6′, 2 × OMe, 2 × OH), 3.52–3.46 (m, 1H, H-5′).^{13}C NMR (63 MHz, acetone-d_6): δ = 179.1 (C-2), 160.8, 160.6 (2 × C-4′′), 143.9, 136.4, 135.7, 134.7 (4 × C_{Qu}), 131.4 (2 × C–H), 131.3 (2 × C–H), 129.2 (C–H), 126.8 (C–H), 123.9 (C–H), 117.3 (C–H), 115.4 (2 × C–H), 115.3 (2 × C–H), 84.8 (C-1′), 82.2 (C-5′), 76.0 (C-3′), 73.6 (C-4′), 69.4 (C-2′), 63.8 (C-6′), 62.6 (C-3), 56.4 (2 × OMe). MS (EI, 70 eV): *m*/*z* (%) = 507 (M^+, 30), 344 (100), 330 (13), 316 (36), 302 (17), 238 (26), 210 (9). HRMS (EI): calcd for $C_{28}H_{29}NO_8$ $[M]^+$ 507.18877. Found 507.18870.

1-(β-D-Mannopyranosyl)-3,3-bis[4-(dimethylamino)phenyl]-indolin-2-one (6i). Starting with **5i** (200 mg, 0.28 mmol), dray methanol (6 mL) and 0.1% solution of NaOMe (0.76 mL, 5 mol%) **6i** was isolated as a colorless solid (122 mg, 80%), mp = 149–151 °C. ^{1}H NMR (300 MHz, acetone-d_6): δ = 7.65–7.62 (m, 1H, Ar), 7.19–6.98 (m, 7H, Ar), 6.67–6.63 (m, 4H, Ar), 5.59 (d, $^3J_{1',2'}$ = 1.5 Hz, 1H, H-1′), 4.61–4.14 (m, 3H, H-2′, 2 × OH), 3.95–3.40 (m, 7H, H-3′, H-4′, H-5′, H-6′, 2 × OH), 2.89 (s, 2 × NMe_2). ^{13}C NMR (63 MHz, acetone-d_6): δ = 179.8 (C-2), 151.6, 151.5, 143.9, 135.6, 132.1, 131.5, (6 × C_{Qu}), 130.9 (2 × C–H), 130.8 (2 × C–H), 128.8 (C–H), 126.8 (C–H), 123.7 (C–H), 116.3 (C–H), 113.9 (2 × C–H), 113.8 (2 × C–H), 84.8 (C-1′), 82.1 (C-5′), 76.1 (C-3′), 73.5 (C-4′), 69.5 (C-2′), 63.8 (C-6′), 62.4 (C-3), 41.5 (2 × OMe). MS (EI, 70 eV): *m*/*z* (%) = 277 (M^+, 53), 413 (19), 370 (100), 342 (65), 326 (17), 281 (12), 251 (13), 223 (17), 207 (16). HRMS (EI): calcd for $C_{30}H_{35}N_3O_6$ $[M]^+$ 533.25204. Found 533.25108. Anal. calc. for $C_{30}H_{35}NO_6$ (533.62): C, 67.52; H, 6.61; N, 7.87. Found: C, 67.46; H, 6.40; N, 7.10.

1-(β-D-Galaktopyranosyl)-3,3-diphenylindolin-2-one (6j). Starting with **5j** (100 mg, 0.16 mmol), dray methanol (4 mL) and 0.1% solution of NaOMe (0.43 mL, 5 mol%) **6j** was isolated as a colorless solid (54 mg, 75%), mp = 149–151 °C. ^{1}H NMR (300 MHz, acetone-d_6): δ = 7.58–7.56 (m, 1H, Ar), 7.35–7.22 (m, 12H, Ar), 7.11–7.06 (m, 4H, Ar), 5.49 (d, $^3J_{1',2'}$ = 1.5 Hz, 1H, H-1′), 4.48–3.74 (m, 10H, H-2′, H-3′, H-4′, H-5′, H-6′, 4 × OH). ^{13}C NMR (75 MHz, acetone-d_6): δ = 178.4 (C-2), 145.2, 143.4, 142.5, 134.4 (4 × C_{Qu}), 130.6 (2 × C–H), 130.3 (2 × C–H), 130.0 (2 × C–H), 129.9 (2 × C–H), 129.7 (C–H), 129.1 (C–H), 128.7 (C–H), 127.7 (C–H), 124.2 (C–H), 114.6 (C–H), 84.3 (C-1′), 79.6 (C-2′), 76.7 (C-3′), 71.2 (C-4′), 68.5 (C-5′), 64.2 (C-3), 63.5 (C-6′). MS (EI, 70 eV): *m*/*z* (%) = 447 (M^+, 38), 285 (100), 256 (61), 208 (26), 180 (13), 165 (13). HRMS (EI): calcd. for $C_{26}H_{25}NO_6$ $[M]^+$ 447.16764. Found 447.16766.

1-(β-D-Galactopyranosyl)-3,3-bis(4-methylphenyl)indolin-2-one (6k). Starting with **5k** (100 mg, 0.16 mmol), dray methanol (4 mL) and 0.1% solution of NaOMe (0.43 mL, 5 mol%) **6k** was isolated as a colorless solid (58 mg, 78%), mp = 153–155 °C.

Published on 02 May 2014. Downloaded by University of Waikato on 15/07/2014 10:07:17.

^{1}H NMR (300 MHz, acetone-d_6): δ = 7.56–7.53 (m, 1H, Ar), 7.28–7.04 (m, 11H, Ar), 5.47 (d, $^3J_{1',2'}$ = 9.2 Hz, 1H, H-1′), 4.48–3.72 (m, 10H, H-2′, H-3′, H-4′, H-5′, H-6′, 4 × OH), 2.29, 2.27 (2 × Me). ^{13}C NMR (75 MHz, acetone-d_6): δ = 178.7 (C-2), 142.5, 142.3, 140.6, 138.6, 138.1, 134.8 (6 × C_{Qu}), 130.6 (2 × C–H), 130.5 (2 × C–H), 130.5 (2 × C–H), 130.2 (2 × C–H), 129.5 (C–H), 127.6 (C–H), 124.1 (C–H), 114.5 (C–H), 84.3 (C-1′), 79.6 (C-2′), 76.7 (C-3′), 71.2 (C-4′), 68.5 (C-5′), 63.5 (C-3), 63.5 (C-6′), 21.9 (2 × Me). MS (EI, 70 eV): m/z (%) = 475 (M^+, 15), 313 (100), 298 (12), 284 (17), 270 (18), 222 (16). HRMS (EI): calcd for $C_{28}H_{29}NO_6$ $[M]^+$ 475.19894. Found 475.19925.

3,3-Bis(4-methoxylphenyl)-1-(β-D-galactopyranosyl)indolin-2-on (6l). Starting with **5l** (100 mg, 0.15 mmol), dray methanol (4 mL) and 0.1% solution of NaOMe (0.41 mL, 5 mol%) **6l** was isolated as a colorless solid (59 mg, 79%), mp = 147–149 °C. ^{1}H NMR (300 MHz, acetone-d_6): δ = 7.56–7.53 (m, 1H, Ar), 7.27–7.15 (m, 11H, Ar), 7.09–7.04 (m, 11H, Ar), 6.88–6.78 (m, 11H, Ar), 5.47 (d, $^3J_{1',2'}$ = 9.2 Hz, 1H, H-1′), 4.48–4.40 (m, 1H, H-2′), 4.33–4.31 (m, 1H, H-3′), 4.22–4.19 (m, 1H, H-4′), 4.10–4.08 (m, 1H, H-5′), 3.93–3.72 (m, 12H, H-6′, 3 × OH, 2 × OMe). ^{13}C NMR (75 MHz, acetone-d_6): δ = 179.0 (C-2), 160.8, 160.5 (2 × C-4′′), 142.4, 137.3, 135.4, 135.1 (4 × C_{Qu}), 131.6 (2 × C–H), 131.3 (2 × C–H), 129.5 (C–H), 127.6 (C–H), 124.1 (C–H), 114.5 (C–H), 84.3 (C-1′), 79.6 (C-2′), 76.7 (C-3′), 71.2 (C-4′), 68.5 (C-5′), 63.5 (C-3), 62.8 (C-6′), 56.5, 56.4 (2 × OMe). MS (EI, 70 eV): m/z (%) = 507 (M^+, 20), 344 (100), 316 (27), 302 (13), 238 (20), 210 (7). HRMS (EI): calcd for $C_{28}H_{29}NO_8$ $[M]^+$ 507.18877. Found 507.18846.

Antiproliferative activity

Cell lines and cell cultures

For the purpose of Antiproliferative activity, malignant cutaneous melanoma cells HT-144 (ATCC HTB-63), Lung carcinoma (H157) cell lines (ATCC CRL-5802) and human corneal epithelial cells (HCEC) obtained from RIKEN Bio Resource Center, Japan were used. These H157 were maintained in RPMI-1640 medium as described previously.[27] The medium was supplemented with heat-inactivated fetal bovine serum (10%), L-glutamine (2 mM), pyruvate (1 mM), penicillin (100 U mL^{-1}) and streptomycin (100 μg mL^{-1}). The cultured was proceeded at 37 °C in a 5% CO_2 incubator, horizontally in T75 cm^2 sterile tissue culture flasks. HT-144 (ATCC HTB-63) cells were cultured in minimal essential medium (MEM) with 10% fetal calf serum, with the addition of essential amino acids. HCEC were grown in DMEM medium [containing 5% heat-inactivated fetal bovine serum, insulin (5 μg mL^{-1}), epidermal growth factor human (10 ng mL^{-1}) and DMSO (0.5%)]. Under these conditions, HCEC exhibited corneal epithelial cell-specific properties. For experiments, both cancerous cell lines (H157 and HT-144) and normal cell line (HCEC) were grown in 96-well plates by inoculating at a cell density of 10^4 and 5 × 10^4 cells per 100 μL per well respectively and these well plates were incubated at 37 °C in a 5% CO_2 incubator in controlled humidity. Confluent monolayers of all the three cell lines were formed within 24 h, which were subsequently used for further experiments.

Antiproliferative activity by sulforhodamine B (SRB) assay

Antiproliferative activity was studied using the method reported by Skehan *et al.*[28] The cell monolayers were washed with Hank's Balanced Salt Solution (HBSS) to remove non adherent cells. All the three cell lines were incubated with a range of concentrations of test compounds (1, 25, 50 and100 μM concentration) for up to 24 h at 37 °C in a 5% CO_2 incubator. On completion of incubation period, cells were fixed with 50% ice cold TCA buffer and placed for 1 h at 4 °C. The excess TCA was removed by washing plates at least 5 times with HBSS and then air dried. Fixed cells were further treated with sulforhodamine B solution (0.4%) and allowed to stain for 20–30 min. After staining cells were rinsed with 1% acetic acid and plates were allowed to dry. Plates were treated with 10 mM Tris for 5–10 min at room temperature and their absorbance was recorded at 565 nm. Blank background optical density was measured in wells incubated with growth medium and without cells. Negative control values were obtained from H157 incubated in RPMI medium, HT-144 in MEM medium and HCEC in DMEM medium without inhibitors. Vincristine and methotrexate was used as a positive control. % growth inhibition of the compounds was calculated using the formula: % growth inhibition = 100 − [(OD_{sample}/$OD_{control}$) × 100].

Acknowledgements

Financial support by the BMBF "REMEDIS"-project "Höhere Lebensqualität durch neuartige Mikroimplantate" (Grant no. 03IS2081A) and by the DAAD (Deutsch-Pakistanische Hochschulzusammenarbeit) is gratefully acknowledged.

References

1 T. B. Reynolds and A. G. Redeker, *JAMA, J. Am. Med. Assoc.*, 1970, **213**, 2273.
2 T. B. Reynolds, R. L. Peters and S. Yamada, *N. Engl. J. Med.*, 1971, **285**, 813–820.
3 M. K. Uddin, S. G. Reignier, T. Coulter, C. Montalbetti, C. Grånäs, S. Butcher, C. Krog-Jensen and J. Felding, *Bioorg. Med. Chem. Lett.*, 2007, **17**, 2854–2857.
4 A. Natarajan, Y. H. Fan, H. Chen, Y. Guo, J. Iyasere, F. Harbinski, W. J. Christ, H. Aktas and J. A. Halperin, *J. Med. Chem.*, 2004, **47**, 1882–1885.
5 M. K. Christensen, K. D. Erichsen, C. Trojel-Hansen, J. Tjørnelund, S. J. Nielsen, K. Frydenvang, T. N. Johansen, B. Nielsen, M. Sehested, P. B. Jensen, M. Ikaunieks, A. Zaichenko, E. Loza, I. Kalvinsh and F. Björkling, *J. Med. Chem.*, 2010, **53**, 7140–7145.
6 S. Takada, N. Ishizuka, T. Sasatani, Y. Makisumi and H. Jyoyama, *Chem. Pharm. Bull.*, 1984, **32**, 877–886.
7 A. Baeyer and M. J. Lazarus, *Ber. Dtsch. Chem. Ges.*, 1885, **18**, 2637–2643.
8 N. Danaila, *Chem. Zentralbl.*, 1910, **81**, 1148–1849.
9 K. C. Nicolaou, D. Y.-K. Chen, X. Huang, T. Ling, M. Bella and S. A. Snyder, *J. Am. Chem. Soc.*, 2004, **126**, 12888–12896.

 This journal is © The Royal Society of Chemistry 2014

Published on 02 May 2014. Downloaded by University of Waikato on 15/07/2014 10:07:17.

10 R. P. Maskey, I. Grün-Wollny, H. H. Fiebig and H. Laatsch, *Angew. Chem.*, 2002, **114**, 623–625; *Angew. Chem., Int. Ed.*, 2002, **41**, 597–599.

11 (*a*) W. F. De Azevedo, S. Leclerc, L. Meijer, L. Havlicek, M. Strnad and S. H. Kim, *Eur. J. Biochem.*, 1997, **243**, 518–526; (*b*) R. Hoessel, S. Leclerc, J. Endicott, M. Noble, A. Lawrie, P. Tunnah, M. Leost, E. Damiens, D. Marie, D. Marko, E. Niederberger, W. Tang, G. Eisenbrand and L. Meijer, *Nat. Cell Biol.*, 1999, **1**, 60–67.

12 (*a*) A. Beauchard, Y. Ferandin, S. Frère, O. Lozach, M. Blairvacq, L. Meijer, V. Thiéry and T. Besson, *Bioorg. Med. Chem.*, 2006, **14**, 6434–6443; (*b*) Y. Ferandin, K. Bettayeb, M. Kritsanida, O. Lozach, P. Polychronopoulos, P. Magiatis, A. L. Skaltsounis and L. Meijer, *J. Med. Chem.*, 2006, **49**, 4638–4649; (*c*) J. Ribas, K. Bettayeb, Y. Ferandin, X. Garrofé-Ochoa, M. Knockaert, F. Totzke, C. Schächtele, J. Mester, P. Polychronopoulos, P. Magiatis, A. L. Skaltsounis, J. Boix and L. Meijer, *Oncogene*, 2006, **25**, 6304–6318; (*d*) M. Mapelli, L. Massimiliano, C. Crovace, M. Seeliger, L. H. Tsai, L. Meijer and A. Musacchio, *J. Med. Chem.*, 2005, **48**, 671–679; (*e*) Z. L. Wu, P. Aryal, O. Lozach, L. Meijer and F. P. Guengerich, *Chem. Biodiversity*, 2005, **2**, 51–65; (*f*) S. Duensing, A. Duensing, D. C. Lee, K. M. Edwards, S. O. Piboonniyom, E. Manuel, L. Skaltsounis, L. Meijer and K. Münger, *Oncogene*, 2004, **23**, 8206–8215; (*g*) F. P. Guengerich, J. L. Sorrells, S. Schmitt, J. A. Krauser, P. Aryal and L. Meijer, *J. Med. Chem.*, 2004, **47**, 3236–3241; (*h*) E. Droucheau, A. Primot, V. Thomas, D. Mattei, M. Knockaert, C. Richardson, P. Sallicandro, P. Alano, A. Jafarshad, B. Baratte, C. Kunick, D. Parzy, L. Pearl, C. Doerig and L. Meijer, *Biochim. Biophys. Acta, Proteins Proteomics*, 2004, **1697**, 181–196; (*i*) N. Sato, L. Meijer, L. Skaltsounis, P. Greengard and A. Brivanlou, *Nat. Med.*, 2004, **10**, 55; (*j*) L. Meijer, A. L. Skaltsounis, P. Magiatis, P. Polychronopoulos, M. Knockaert, M. Leost, X. P. Ryan, C. D. Vonica, A. Brivanlou, R. Dajani, A. Tarricone, A. Musacchio, A. M. Roe, L. Pearl and P. Greengard, *Chem. Biol.*, 2003, **10**, 1255–1266; (*k*) E. Damiens, B. Baratte, D. Marie, G. Eisenbrand and L. Meijer, *Oncogene*, 2001, **20**, 3786–3796; (*l*) T. G. Davies, P. Tunnah, L. Meijer, D. Marko, G. Eisenbrand, J. A. Endicott and M. E. M. Noble, *Structure*, 2001, **9**, 389–397.

13 S. Libnow, K. Methling, M. Hein, D. Michalik, M. Harms, K. Wende, A. Flemming, M. Koeckerling, H. Reinke, P. J. Bednarski, M. Lalk and P. Langer, *Bioorg. Med. Chem.*, 2008, **16**, 5570–5583.

14 (*a*) M. Kunz, I. Hohensee, K. M. Driller, M. Hein, S. Libnow, P. Langer, R. Ramer, B. Hinz, A. Berger and J. Eberle, *ChemMedChem*, 2010, **5**, 534–539; (*b*) F. Erben, D. Kleeblatt, M. Sonneck, M. Hein, H. Feist, T. Fahrenwaldt, C. Fischer, A. Matin, J. Iqbal, M. Plötz, J. Eberle and P. Langer, *Org. Biomol. Chem.*, 2013, **11**, 3963–3978.

15 (*a*) M. Sassatelli, E. Saab, F. Anizon, M. Prudhomme and P. Moreau, *Tetrahedron Lett.*, 2004, **45**, 4827–4830; (*b*) M. Sassatelli, F. Bouchikhi, S. Messaoudi, F. Anizon, E. Debiton, C. Barthomeuf, M. Prudhomme and P. Moreau, *Eur. J. Med. Chem.*, 2006, **41**, 88–100; (*c*) M. Sassatelli, F. Bouchikhi, B. Aboab, F. Anizon, F. Doriano, M. Prudhomme and P. Moreau, *Anti-Cancer Drugs*, 2007, **18**, 1069–1074; (*d*) F. Bouchikhi, F. Anizon and P. Moreau, *Eur. J. Med. Chem.*, 2008, **43**, 755–762; (*e*) F. Bouchikhi, F. Anizon and P. Moreau, *Eur. J. Med. Chem.*, 2009, **44**, 2705–2710.

16 (*a*) L. Wang, X. Liu, R. Chen, *US Pat.* 6566341, 2003, Chem. Abstr., 138, 379213; (*b*) L. Wang, X. Liu, R. Chen, *WO Patent* 03051900, 2003, Chem. Abstr., 139, 47135.

17 D. Kleeblatt, B. Siyo, M. Hein, V. Iaroshenko, J. Iqbal, A. Villinger and P. Langer, *Org. Biomol. Chem.*, 2013, **11**, 886–895.

18 R. Stollé, *Chem. Ber.*, 1913, **46**, 3915–3916.

19 J. F. M. da Silva, S. J. Garden and A. C. Pinto, *J. Braz. Chem. Soc.*, 2001, **12**, 273–324.

20 (*a*) C. Chavis, C. De Gourcy, F. Dumont and J.-L. Imbach, *Carbohydr. Res.*, 1983, **113**, 1–20; (*b*) J. G. Douglas and J. Honeyman, *J. Chem. Soc.*, 1955, 3674–3678.

21 M. N. Preobrazhenskaya, I. V. Yartseva and L. V. Ektova, *Dokl. Chem.*, 1974, **215**, 219–222.

22 J. Bergman and N. Eklund, *Tetrahedron*, 1980, **36**, 1445–1450.

23 A. S. Ijaz, J. Parrick and A. Yahya, *J. Chem. Res., Miniprint*, 1990, 833–848.

24 D. A. Klumpp, K. Y. Yeung, K. S. Prakash and G. A. Olah, *J. Org. Chem.*, 1998, **63**, 4481–4484.

25 A. Natarajan, Y.-h. Fan, H. Chen, Y. Guo, J. Iyasere, F. Harbinski, W. J. Christ, H. Aktas and J. A. Halperin, *J. Med. Chem.*, 2004, **47**, 1882–1885.

26 M. K. Uddin, S. G. Reignier, T. Coulter, C. Montalbetti, C. Granas, S. Butcher, K. C. Jensen and J. Fielding, *Bioorg. Med. Chem. Lett.*, 2007, **17**, 2854–2857.

27 R. Raza, A. Matin, S. Sarwar, M. Barsukova-Stuckart, M. Ibrahim, U. Kortz and J. Iqbal, *Dalton Trans.*, 2012, **41**, 14329–14336.

28 P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. McMahon, D. Vistica, J. T. Warren, H. Bokesch, S. Kenney and M. R. Boyd, *J. Natl. Cancer Inst.*, 1990, **82**, 1107–1112.