Synthesis of 6*H*-indolo[2,3-*b*]quinoxaline-*N*-glycosides and their cytotoxic activity against human ceratinocytes (HaCaT)†

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N-Glycosides of 6*H*-indolo[2,3-*b*]quinoxalines were prepared and structurally characterized. The synthesis relies on the cyclocondensation of isatine-*N*-glycosides with 1,2-diaminobenzenes. Some products exhibit weak cytotoxic activity against human ceratinocytes (HaCaT).

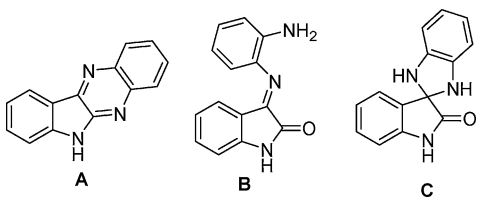
Introduction

N-Glycosides of indole and of related compounds are of remarkable pharmacological relevance, *e.g.* as anti-cancer agents. Well-known cancerostatic natural products include, for example, staurosporine, K-252d, rebeccamycin and the tjipanazoles.[1,2] Recently, the isolation of indigo-*N*-glycosides, the akashines A–C, has been reported.[3] Whereas the parent indigo is pharmacologically inactive, the akashines exhibit a considerable cancerostatic activity against various human cancer cell lines. Recently, we have reported the first synthesis of indigo-*N*-glycosides (blue sugars).[4] Indirubin, the red isomer of indigo, is the active ingredient of a traditional Chinese medicinal recipe which has been used for the treatment of myelocytic leukaemia.[5] Indirubin derivatives are potent inhibitors of several kinases such as GSK-3β and cyclin dependent kinases (CDK's).[6,7] We have recently reported the first synthesis of indirubin-*N*′-glycosides (red sugars) and their anti-proliferative activity against various human cancer cell lines.[8] The cancerostatic activities of the glycosides are higher than those of the aglycons. Isoindigo-*N*-glycosides also show a considerable anti-proliferative and kinase inhibitory activity.[9] It is worth mentioning that both deprotected and protected *N*-glycosides are of pharmacological interest. For example, the biological activity of so-called 'Natura', *i.e.* acetyl-protected β-D-xylopyranosyl-*N*-isoindigo, has been reported to be higher than the activity of its deprotected analogue.[10,11]

6*H*-Indolo-[2,3-*b*]quinoxalines combine the structural features of indoles and quinoxalines and the first derivative was prepared in 1895 by condensation of 1,2-diaminobenzene with isatine.[12] In recent years, 6*H*-indolo[2,3-*b*]quinoxalines have received much attention, due to their considerable pharmacological relevance.[13] For example, they show some DNA duplex stabilization.[14] It has been mentioned above that the pharmacological activity of *N*-glycosylated heterocycles is often higher than that of the aglycons. Therefore, we have studied the synthesis and structural characterization of novel 6*H*-indolo[2,3-*b*]quinoxaline-*N*-glycosides and the results of our efforts are reported herein.[15]

Results and discussion

The product distribution of the reaction of isatines with 1,2-diaminobenzenes has been reported to strongly depend on the solvent and the conditions (Scheme 1).[12,16,17] 6*H*-Indolo-[2,3-*b*]quinoxaline **A** is exclusively formed when the reaction is carried out in glacial acetic acid (AcOH). The employment of benzene or MeOH results in the formation of a mixture of **A** and of 3-imino-isatine **B**. Product **B** can be transformed into **A** by treatment with AcOH. The reaction of the starting materials at elevated temperatures in a polar-aprotic solvent, such as *N*,*N*-dimethylacetamide (DMA), has been reported to give spirobenzimidazole **C**.[16]



Scheme 1 Possible products of the reaction of 1,2-diaminobenzene with isatine.

It is well known that direct *N*-glycosylations of indole and related unsaturated *N*-heterocycles proceed with extremely low yields or are not possible at all.[18] Therefore, the direct *N*-glycosylation of 6*H*-indolo[2,3-*b*]quinoxalines is not a promising approach to 6*H*-indolo[2,3-*b*]quinoxaline-*N*-glycosides. Our strategy for the synthesis of these compounds relies on the reaction of isatine-*N*-glycosides with 1,2-diaminobenzenes. The starting materials, isatine-*N*-glycosides **2**, were prepared as follows:[8] The reaction of the free sugars with aniline and subsequent acetylation gave acetyl-protected *N*-glycosylated anilines[19] which were transformed into isatine-*N*-glycosides **2** by $AlCl_3$-mediated cyclization[20] with oxalyl chloride. Isatine-*N*-rhamnoside **β-2a**, isatine-*N*-mannoside **β-2b**, and isatine-*N*-glucoside **β-2c** were isolated in the form of the pure β-anomers.

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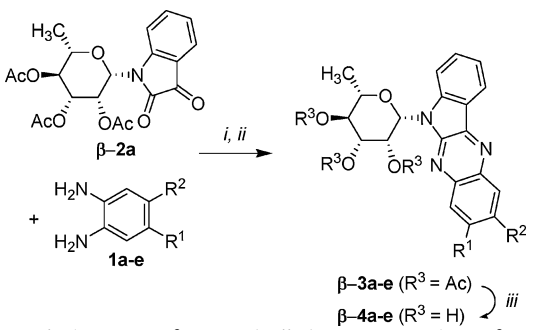
† Electronic supplementary information (ESI) available: Experimental procedures and compound characterization and biological studies. CCDC reference numbers 695574 (**β-3c**), 695575 (**β-3g**) and 695576 (**β-3a**). For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/b812308j

The reaction of a AcOH–1,4-dioxane solution of isatine-*N*-rhamnoside **β-2a** and 1,2-diaminobenzene (**1a**) at 80 °C afforded slightly impure 6*H*-indolo[2,3-*b*]quinoxaline-*N*-rhamnoside **β-3a** in 40% yield.[15] Due to the formation of many side-products of similar polarity, the chromatographic separation proved to be very difficult. Therefore, the conditions were optimized. The best yields of **β-3a** (up to 72%) were obtained when the starting materials (1 : 1.1 stoichiometry) were stirred in pure glacial acetic acid at 80 °C until no **β-2a** could be detected any more (TLC control, 3 h) (Table 1). The TLC control was necessary as some deglycosylation was observed when the reaction time was too long. Subsequently, the solvent was removed *in vacuo*, benzene and *p*-toluenesulfonic acid (PTSA) were added, and the solution was stirred at 80 °C for 45 min (TLC control). This step was important to complete the cyclization and dehydration. The deacetylated product **β-4a** could be isolated in 98% yield by reaction of **β-3a** with NaOMe–MeOH (*Zemplén* conditions).[21]

The reaction of isatine-*N*-rhamnoside **β-2a** with 1,2-diaminobenzene (**1a**), 1,2-diamino-4,5-dimethylbenzene (**1b**), 2,3-diaminonaphthalene (**1c**), 1,2-diamino-4,5-dichlorobenzene (**1d**), and 1,2-diamino-4-nitrobenzene (**1e**) afforded the novel indolo[2,3-*b*]quinoxaline-*N*-rhamnosides **β-3a**, **β-3b**, **β-3c**, **β-3d**, and **β-3e**, respectively (Table 1). Products **β-3a**, **β-3b**, and **β-3c** were isolated in good yields. In contrast, the yields of **β-3d** and **β-3e** were low. This can be explained by the low nucleophilicity of acceptor-substituted 1,2-diaminobenzenes **1d** and **1e**. The reaction of **β-2a** with (unsymmetrical) 1,2-diamino-4-nitrobenzene (**1e**) can, in principle, result in the formation of two regioisomers. Only one isomer (**β-3e**) could be isolated in pure form. However, it was not possible to unambiguously clarify whether the nitro group is located at carbon atom C-2 or C-3. The deacetylation under *Zemplén*-conditions afforded the novel deprotected 6*H*-indolo[2,3-*b*]quinoxaline-*N*-glycosides **β-4a**, **β-4b**, and **β-4c** in very good yields.

Table 1 Synthesis of β-L-(rhamnopyranosyl)indolo[2,3-*b*]quinoxalines **β-4a–e**

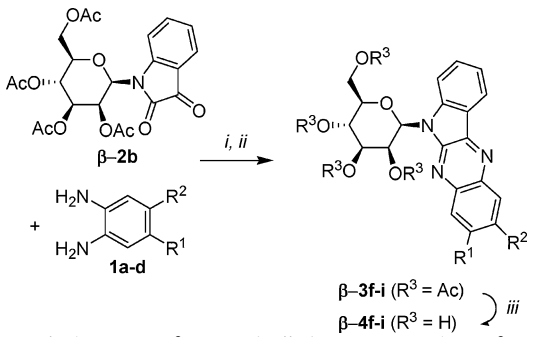


Conditions: i: AcOH, 80 °C, 1–3 h; ii: benzene, PTSA, 80 °C, 1–3 h; iii: NaOMe, MeOH, 7 h, 20 °C

Entry	R^1	R^2	% (**β-3**)[a]	% (**β-4**)[a]
a	H	H	72	98
b	Me	Me	63	85
c	–$(CH)_4$–		61	79
d	Cl	Cl	10	—[c]
e	NO_2[b]	H[b]	9	—[c]

[a] Yields of isolated products. [b] Assignment arbitrary. [c] Experiment was not carried out.

Table 2 Synthesis of β-D-(mannopyranosyl)indolo[2,3-*b*]quinoxalines **β-4f–i**

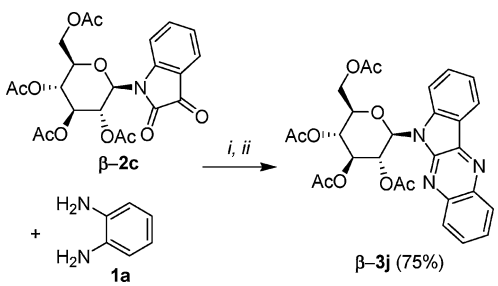


Conditions: i: AcOH, 80 °C, 1–3 h; ii: benzene, PTSA, 80 °C, 1–3 h; iii: NaOMe, MeOH, 7 h, 20 °C

Entry	R^1	R^2	% (**β-3**)[a]	% (**β-4**)[a]
f	H	H	69	72
g	Me	Me	72	94
h	–$(CH)_4$–		65	97
i	Cl	Cl	44	75

[a] Yields of isolated products.

The reaction of isatine-*N*-mannoside **β-2b** with **1a**, **1b**, **1c**, and **1d** afforded the 6*H*-indolo[2,3-*b*]quinoxaline-*N*-mannosides **β-3f**, **β-3g**, **β-3h**, and **β-3i**, respectively (Table 2). Interestingly, mannoside **β-3i**, derived from 1,2-diamino-4,5-dichlorobenzene (**1d**), was isolated in much better yield than the corresponding rhamnoside **β-3d**. This result suggests that the sugar moiety also has a significant influence on the yield of the cyclocondensation. The yields of mannosides **β-3f**, **β-3g**, **β-3h** were similar to those of **β-3a**, **β-3b**, and **β-3c**. The deacetylation afforded the novel deprotected 6*H*-indolo[2,3-*b*]quinoxaline-*N*-mannosides **β-4f**, **β-4g**, **β-4h**, and **β-4i** in very good yields. The reaction of **1a** with isatine-*N*-glucoside **β-2c** gave the 6*H*-indolo[2,3-*b*]quinoxaline-*N*-glucoside **β-3j** in good yield (Scheme 2).

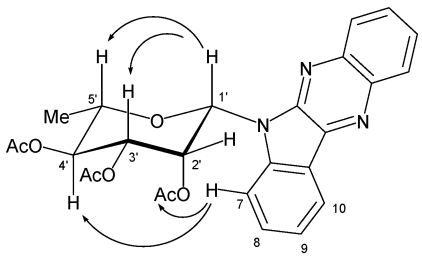


Scheme 2 Synthesis of β-D-(glucopyranosyl)indolo[2,3-*b*]quinoxaline **β-3j**. *Conditions*: *i*: AcOH, 80 °C, 1–3 h; *ii*: benzene, PTSA, 80 °C, 1–3 h.

All attempts to induce a cyclization of isatine-*N*-glycosides with 1,2-diaminoethane proved to be unsuccessful. This can be explained by the assumption that the rearomatization is an important driving force of the cyclocondensation.

The configuration and conformation of all products were studied in detail by NMR spectroscopy. The assignment of the signals was established by DEPT and 2D NMR techniques (^{1}H,^{1}H COSY, HETCOR, HSQC, HMBC, and ^{1}H,^{1}H NOESY). For example, in the ^{1}H,^{1}H NOESY spectrum of **β-3a**, NOE cross peaks were observed for proton H-7 with H-4′ and the *O*-acetyl

group attached to carbon atom C-2′ as well as proton H-1′ with H-3′ and H-5′. These findings confirm the assignments given for proton H-7, and furthermore, the stereochemistry of the pyranosyl ring ($^1C_4$ chair conformation and β configuration) (Scheme 3). ^{1}H,^{1}H NOESY spectra have been recorded and analyzed for compounds **β-3a–e**, **β-3g**, and **β-3h**. These experiments showed that β-$^1C_4$ and β-$^4C_1$ chair conformations were present for L-rhamno and D-manno configured sugar residues, respectively. The stereochemistry of the D-gluco derivative **β-3j** was found to be β-$^4C_1$ based on the coupling constants $^3J_{1',2'}$, $^3J_{2',3'}$, $^3J_{3',4'}$, and $^3J_{4',5'}$ in the range of 9.5 to 10.0 Hz, indicating all-axial positions of these protons. The structures of **β-3a**, **β-3c** and **β-3g** were independently confirmed by X-ray structure analyses (Fig. 1, 2 and 3).†



β–3a (β-L-*rhamno* $^1C_4$)

Scheme 3 Relevant NOE-correlations of **β-3a**.

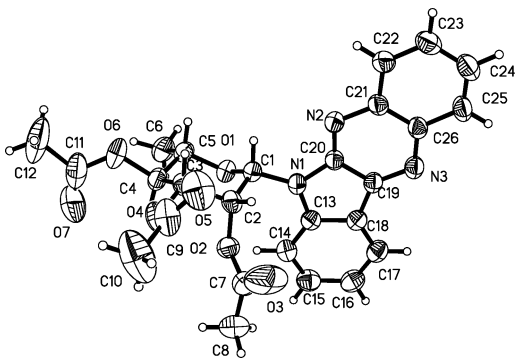


Fig. 1 ORTEP-plot of **β-3a** (50% probability level).

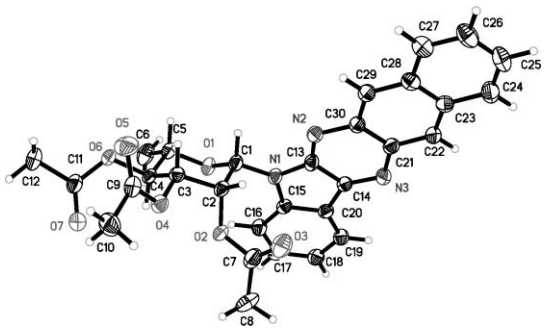


Fig. 2 ORTEP-plot of **β-3c** (50% probability level).

To evaluate the biological properties of the 6*H*-indolo[2,3-*b*]quinoxaline-*N*-glycosides a selection of the synthesiszed derivatives was tested towards their cytotoxic activity. The immortalized human keratinocytes (HaCaT) cell line was chosen for the evaluation of antiproliferative effects. Besides the unprotected compounds **β-4a**, **β-4b**, **β-4c** and **β-4g**, some acetylated quinoxaline-*N*-glycosides were also tested. The results of the biological studies are summarised in Table 3.

Table 3 Results of the antiproliferative screening[a]

Compound	IC_{50}/μmol L^{-1}
β-**3a**	>100
β-**3b**	49.8
β-**3c**	65.5
β-**3f**	>100
β-**3g**	71.3
β-**4a**	>100
β-**4b**	>100
β-**4c**	>100
β-**4g**	>100
Etoposide	0.8

[a] Inhibition studies were performed in 2 separate experiments including 4 parallel dilutions. The cell viability was detected using the "Neutral Red" assay.[22]

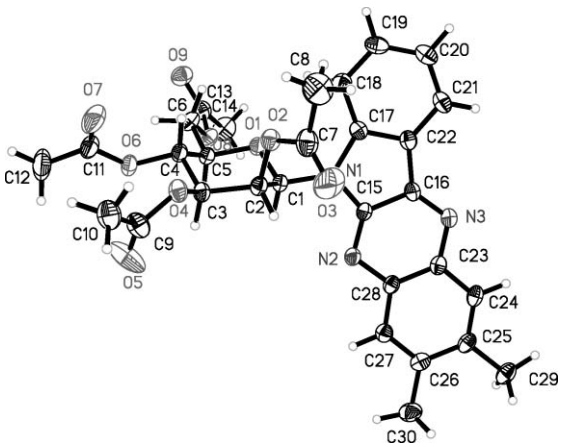


Fig. 3 ORTEP-plot of **β-3g** (50% probability level).

It becomes obvious that the compounds tested in this study show only moderate antiproliferative activities towards the HaCaT cell line. Compared to etoposide only a weak cytotoxic effect of the quinoxaline-*N*-glycosides can be assumed. In comparison to the recently described[8] high antiproliferative activities of indirubine-*N*-glycosides, the higher lipophilicity of the quinoxaline-*N*-glycosides is likely to be the main reason for the decreased effects towards eukaryotic cells. The cytotoxic effects observed in this study could arise from unspecific binding effects at the cell membrane. The much stronger inhibition of proliferation by indirubine-*N*-glycosides is likely induced by inhibition of CDK enzymes which can probably not be inhibited by the tested indoloquinoxaline-*N*-glycosides.

In conclusion, *N*-glycosides of 6*H*-indolo[2,3-*b*]quinoxalines were prepared by cyclocondensation of isatine-*N*-glycosides with 1,2-diaminobenzenes. Some products exhibit weak cytotoxic activity against human ceratinocytes (HaCaT).

Experimental section

General comments

All solvents were dried by standard methods. ^{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 DMSO-d_6 as solvents. The calibration of spectra was carried out on solvent signals ($CDCl_3$: δ (^{1}H) = 7.25, δ (^{13}C) = 77.0; DMSO-d_6: δ (^{1}H) = 2.50, δ (^{13}C) = 39.7). Mass spectrometric data (MS) were obtained by electron ionization (EI, 70 eV), chemical ionization (CI, isobutane) or electrospray ionization (ESI). Melting points are uncorrected. Analytical thin layer chromatography was performed on 0.20 mm 60A silica gel plates. Column chromatography was performed on 60A silica gel (60–200 mesh).

General procedure for the synthesis of 6*H*-indolo[2,3-*b*]quinoxaline-*N*-glycosides 3a–j

A solution of isatine-*N*-glycoside **β-2a** (1.0 equiv.) and of diamine **1** (1.1 equiv.) in glacial acetic acid (10 mL) was stirred at 80 °C for 1–3 h until no starting materials could be detected by TLC (heptanes–EtOAc = 3:1). The solution was allowed to cool to 20 °C and the solvent was removed *in vacuo*. To the residue was added dry benzene (10 mL) and a catalytic amount of toluenesulfonic acid. The solution was stirred at 80 °C for 1–3 h until the reaction was complete (TLC-control). The solution was allowed to cool to 20 °C and NEt_3 (for neutralization) and toluene (4 mL) were added. The solvent was concentrated *in vacuo* and the residue was purified by chromatography (heptane–EtOAc = 9 : 1 → 6 : 1 → 2 : 1).

General procedure for the deacetylation

Product **3** was suspended in dry MeOH under argon atmosphere. To the suspension was added a MeOH solution of NaOMe (0.1 M, prepared from 23 mg of sodium and 10 mL of dry MeOH). The solution was stirred at 20 °C until the reaction is complete (TLC-control, $CHCl_3$–EtOH = 5 : 1). The precipitate was filtered off washed several times with *n*-pentane and dried *in vacuo*.

6-(β-L-Rhamnopyranosyl)indolo[2,3-*b*]quinoxaline (β-4a)

Stirring of **β-2a** (300 mg, 0.71 mmol) and **1a** (85 mg, 0.79 mmol) for 3 h at 80 °C in HOAc and for 45 min at 80 °C in benzene (in the presence of PTSA) afforded **β-3a** (253 mg, 72%) as a slightly yellow solid. Starting with **β-3a** (200 mg, 0.41 mmol), 40 mL of MeOH and 0.5 mL of a 0.1 M MeOH solution of NaOMe, **β-4a** was isolated (146 mg, 98%) after stirring for 7 h as a slightly yellow solid. Mp. 339–341 °C (heptane–EtOAc); $[\alpha]_D$ = +9.63 (c = 0.53; T = 21.5 °C; DMSO); R_f = 0.40 ($CHCl_3$–EtOH = 5 : 1). ^{1}H-NMR (300 MHz, DMSO-d$_6$): δ = 8.32 (ddd, $^5J_{7,10}$ = 0.6 Hz, $^4J_{8,10}$ = 1.3 Hz, $^3J_{9,10}$ = 7.8 Hz, 1H, H-10); 8.29, 8.13 (2 ddd, $^5J_{1,4}$ = 0.6 Hz, $^4J_{1,3}$ = $^4J_{2,4}$ = 1.7 Hz, $^3J_{1,2}$ = $^3J_{3,4}$ = 8.1 Hz, 2H, H-1, H-4); 8.19 (d't', $^3J_{7,8}$ = 8.5 Hz, 1H, H-7); 7.84, 7.76 (2 ddd, $^4J_{1,3}$ = $^4J_{2,4}$ = 1.7 Hz, $^2J_{2,3}$ = 6.8 Hz, $^3J_{1,2}$ = $^3J_{3,4}$ = 8.1 Hz, 2H, H-2, H-3); 7.67 (ddd, $^4J_{8,10}$ = 1.3 Hz, $^3J_{8,9}$ = 7.3 Hz, $^3J_{7,8}$ = 8.5 Hz, 1H, H-8); 7.37 (d't', $^4J_{7,9}$ = 0.8 Hz, $^3J_{8,9}$ = 7.3 Hz, $^3J_{9,10}$ = 7.8 Hz, 1H, H-9); 6.38 (d, $^3J_{1',2'}$ = 1.0 Hz, 1H, H-1'); 5.25 (d, $^3J_{2',OH}$ = 4.9 Hz, 1H, $OH_{(2')}$); 5.06 (d, $^3J_{4',OH}$ = 5.1 Hz, 1H, $OH_{(4')}$); 4.93 (d, $^3J_{3',OH}$ = 5.8 Hz, 1H, $OH_{(3')}$); 4.10–4.06 (m, 1H, H-2'); 3.72–3.66 (m, 1H, H-3'); 3.63–3.45 (m, 2H, H-4', H-5'); 1.34 (d, $^3J_{5',6'}$ = 5.9 Hz, 3H, H-6'). ^{13}C-NMR (75.5 MHz, DMSO-d$_6$): δ = 144.7, 144.1, 139.9, 139.6, 139.0 (5 C_q); 130.8 (C-8); 129.3, 127.6 (C-1, C-4); 129.2, 126.6 (C-2, C-3); 121.3 (C-10); 121.2 (C-9); 119.0 (C-10a); 117.0 (C-7); 83.6 (C-1'); 75.7 (C-5'); 73.4 (C-3'); 72.1 (C-2'); 71.6 (C-4'); 18.4 (C-6'). MS (EI, 70 eV): m/z (%) = 365 (5) [M$^+$]; 248 (23); 219 (100) [aglycone + H]; 153 (2) [M$^+$ − aglycone − 2HOAc]. HRMS (EI): calcd. for $C_{20}H_{19}N_3O_4$ ([M$^+$]) 365.136463. Found: 365.13701.

2,3-Dimethyl-6-(β-L-rhamnopyranosyl)indolo[2,3-*b*]quinoxaline (β-4b)

Stirring of **β-2a** (300 mg, 0.71 mmol) and of **1b** (107 mg, 0.79 mmol) for 1.5 h at 80 °C in HOAc and for 2 h at 80 °C in benzene (in the presence of PTSA) afforded **β-3b** (231 mg; 63%) as a slightly yellow solid. Starting with **β-3b** (160 mg, 0.31 mmol), 20 mL of MeOH and 0.5 mL of a 0.1 M MeOH solution of NaOMe, **β-4b** was isolated (103 mg, 85%) after stirring for 24 h as a slightly yellow solid. Mp. 347–350 °C (heptane–EtOAc); $[\alpha]_D$ = +3.33 (c = 0.50; T = 21.6 °C; DMSO); R_f = 0.45 ($CHCl_3$–EtOH = 5 : 1). ^{1}H-NMR (250 MHz, DMSO-d$_6$): δ = 8.26 (ddd, $^5J_{7,10}$ = 0.6 Hz, $^4J_{8,10}$ = 1.3 Hz, $^3J_{9,10}$ = 7.7 Hz, 1H, H-10); 8.15 (d't', $^3J_{7,8}$ = 8.5 Hz, 1H, H-7); 8.03, 7.91 (2 br s, 2H, H-1, H-4); 7.63 (ddd, $^4J_{8,10}$ = 1.3 Hz, $^3J_{8,9}$ = 7.3 Hz, $^3J_{7,8}$ = 8.5 Hz, 1H, H-8); 7.33 (ddd, $^4J_{7,9}$ = 0.9 Hz, $^3J_{8,9}$ = 7.3 Hz, $^3J_{9,10}$ = 7.7 Hz, 1H, H-9); 6.33 (d, $^3J_{1',2'}$ = 1.0 Hz, 1H, H-1'); 5.31 (d, $^3J_{2',OH}$ = 4.6 Hz, 1H, $OH_{(2')}$); 5.07 (d, $^3J_{4',OH}$ = 5.0 Hz, 1H, $OH_{(4')}$); 4.94 (d, $^3J_{3',OH}$ = 5.7 Hz, 1H, $OH_{(3')}$); 4.06 (m, 1H, H-2'); 3.71–3.63 (m, 1H, H-3'); 3.61–3.46 (m, 2H, H-4', H-5'); 2.49 (2 s, 6H, 2 CH_3); 1.34 (d, $^3J_{5',6'}$ = 5.5 Hz, 3H, H-6'). ^{13}C-NMR (75.5 MHz, DMSO-d$_6$): δ = 144.1, 143.8, 139.5, 138.8, 138.3, 137.9, 136.4 (7 C_q); 130.2 (C-8); 128.2, 126.8 (C-1, C-4); 121.0 (C-10); 120.9 (C-9); 119.2 (C-10a); 116.7 (C-7); 83.6 (C-1'); 75.7 (C-5'); 73.4 (C-3'); 72.1 (C-2'); 71.6 (C-4'); 20.1, 19.8 (2 CH_3); 18.3 (C-6'). MS (EI, 70 eV): m/z (%) = 393 (7) [M$^+$]; 276 (33); 247 (100) [aglycone + H]; 246 (36); 232 (58). HRMS (EI): calcd. for $C_{22}H_{23}N_3O_4$ ([M$^+$]) 393.168130. Found: 393.16831.

13-(β-L-Rhamnopyranosyl)-5,12,13-triaza-indeno[1,2-*b*]anthracene (β-4c)

Stirring of **β-2a** (300 mg, 0.71 mmol) and **1c** (124 mg, 0.79 mmol) for 1.5 h at 80 °C in HOAc and for 1.5 h at 80 °C in benzene (in the presence of PTSA) afforded **β-3c** (197 mg, 61%) as a yellow solid. Starting with **β-3c** (100 mg, 0.19 mmol) in 15 mL of MeOH and 0.3 mL of a 0.1 M MeOH solution of NaOMe, **β-4c** was isolated (61 mg; 79%) after stirring for 24 h as a yellow to orange solid. Mp 359–360 °C (heptane–EtOAc); $[\alpha]_D$ = −20.85 (c = 047; T = 22.3 °C; DMSO); R_f = 0.52 ($CHCl_3$–EtOH = 5 : 1). ^{1}H-NMR (250 MHz, DMSO-d$_6$): δ = 8.93, 8.72 (2 s, 2H, H-6, H-11); 8.32 (br d, $^3J_{3,4}$ = 7.6 Hz, 1H, H-4); 8.27–8.19 (m, 2H, H-7, H-10); 8.14 (d, $^3J_{1,2}$ = 8.4 Hz, 1H, H-1); 7.71–7.56 (m, 3H, H-2, H-8, H-9); 7.36 ('t', $^3J_{2,3}$ = $^3J_{3,4}$ = 7.6 Hz, 1H, H-3); 6.35 (br s, 1H, H-1'); 5.32 (d, $^3J_{2',OH}$ = 5.0 Hz, 1H, $OH_{(2')}$); 5.09 (d, $^3J_{4',OH}$ = 5.2 Hz, 1H, $OH_{(4')}$); 4.96 (d, $^3J_{3',OH}$ = 5.9 Hz, 1H, $OH_{(3')}$); 4.14 (br 't', 1H, H-2'); 3.74–3.66 (m, 1H, H-3'); 3.63–3.47 (m, 2H, H-4', H-5'); 1.36 (d, $^3J_{5',6'}$ = 5.7 Hz, 3H, H-6'). ^{13}C-NMR (75.5 MHz, DMSO-d$_6$): δ = 145.9, 144.8, 142.6, 137.0, 136.9, 133.1, 131.7 (7 C_q); 131.3 (C-2); 128.4, 127.8 (C-7, C-10); 127.4, 124.6 (C-6, C-11); 126.6, 125.6 (C-8, C-9); 121.8 (C-4); 121.4 (C-3); 119.2 (C-4a); 116.8 (C-1); 83.7 (C-1'); 75.7, 73.4, 71.6 (3 s, C-3', C-4', C-5'); 71.9 (C-2'); 18.3 (C-6'). MS (EI, 70 eV): m/z (%) = 415 (7) [M$^+$]; 298 (10); 270 (48); 269 (100) [aglycone + H]; 140 (10). HRMS (EI): calcd. for $C_{24}H_{21}N_3O_4$ ([M$^+$]) 415.151893. Found: 415.15266.

6-(β-D-Mannopyranosyl)indolo[2,3-*b*]quinoxaline (β-4f)

Stirring of **β-2b** (300 mg, 0.63 mmol) and **1a** (75 mg, 0.69 mmol) for 2 h at 80 °C in HOAc and for 2 h at 80 °C in benzene (in the presence of PTSA) afforded **β-3f** (230 mg, 69%) as a slightly yellow solid. Starting with **β-3f** (50 mg, 0.10 mmol), 10 mL of MeOH and 0.5 mL of a 0.1 M solution of NaOMe, **β-4f** was isolated (25 mg, 72%) after stirring for 24 h as a slightly yellow solid. Mp. 325–327 °C (heptane–EtOAc); $[\alpha]_D = -20.52$ ($c = 0.47$; $T = 22.3$ °C; DMSO); $R_f = 0.29$ ($CHCl_3$–EtOH = 5 : 1). ^{1}H-NMR (250 MHz, DMSO-d_6): $\delta = 8.34$–8.23 (m, 3H), 8.14 (m, 1H) (H-1, H-4, H-7, H-10); 7.84, 7.76 (2 ddd, $^5J_{1,4} = 0.6$ Hz, $^4J_{1,3} = {}^4J_{2,4} = 1.7$ Hz; $^3J_{2,3} = 6.8$ Hz, $^3J_{1,2} = J_{3,4} = 8.1$ Hz, 2H, H-2, H-3); 7.65 (ddd, $^4J_{8,10} = 1.4$ Hz, $^3J_{8,9} = 7.5$ Hz, $^3J_{7,8} = 8.3$ Hz, 1H, H-8); 7.37 (d't', $^4J_{7,9} = 0.9$ Hz, $^3J_{8,9} = 7.5$ Hz, $^3J_{9,10} = 7.8$ Hz, 1H, H-9); 6.38 (d, $^3J_{1',2'} = 0.8$ Hz, 1H, H-1′); 5.26 (br s, 1H, $OH_{(2')}$); 5.02 (br s, 1H, $OH_{(4')}$); 4.97 (br s, 1H, $OH_{(3')}$); 4.63 (br t, 1H, $OH_{(6')}$); 4.07 (br s, 1H, H-2′); 3.88–3.47 (m, 5H, H-3′, H-4′; H-5′, H-6′a, H-6′b). ^{13}C-NMR (75.5 MHz, DMSO-d_6): $\delta = 144.8$, 144.1, 139.9, 139.6, 139.0 (5 C_q); 130.8 (C-8); 129.3, 127.6 (C-1, C-4); 129.2, 126.6 (C-2, C-3); 121.2, 121.2 (C-9, C-10); 119.0 (C-10a); 117.4 (C-7); 83.6 (C-1′); 81.1 (C-5′); 73.7 (C-3′); 71.9 (C-2′); 66.6 (C-4′); 61.3 (C-6′). HRMS (ESI): calcd. for $C_{20}H_{19}N_3O_5$ ($[M + H]^+$) 382.13975. Found: 382.12953.

2,3-Dimethyl-6-(β-D-mannopyranosyl)indolo[2,3-*b*]quinoxaline (β-4g)

Stirring of **β-2b** (300 mg, 0.63 mmol) and **1b** (94 mg, 0.69 mmol) for 1 h at 80 °C in HOAc and for 1.5 h at 80 °C in benzene (in the presence of PTSA) and recrystallization from EtOAc and heptane afforded **β-3g** (260 mg, 72%) as yellow to orange needles. Starting with **β-3g** (260 mg, 0.45 mmol), 25 mL of MeOH and 0.5 mL of a 0.8 M MeOH solution of NaOMe, **β-4g** was isolated (173 mg, 94%) after stirring for 24 h as a slightly yellow solid. Mp. 352–354 °C (heptane–EtOAc); $[\alpha]_D = -10.09$ ($c = 0.49$; $T = 21.7$ °C; DMSO); $R_f = 0.24$ ($CHCl_3$–EtOH = 5 : 1). ^{1}H-NMR (300 MHz, DMSO-d_6): $\delta = 8.27$ (br d, $^3J_{9,10} = 7.6$ Hz, 1H, H-10); 8.20 (br d, $^3J_{7,8} = 8.5$ Hz, 1H, H-7); 8.03, 7.91 (2 br s, 2H, H-1, H-4); 7.61 (ddd, $^4J_{8,10} = 1.0$ Hz, $^3J_{8,9} = 7.4$ Hz, $^3J_{7,8} = 8.5$ Hz, 1H, H-8); 7.34 (d't', $^4J_{7,9} = 0.9$ Hz, $^3J_{8,9} = 7.4$ Hz, $^3J_{9,10} = 7.6$ Hz, 1H, H-9); 6.33 (d, $^3J_{1',2'} = 1.0$ Hz, 1H, H-1′); 5.30 (br s, 1H, $OH_{(2')}$); 5.00 (br s, 1H, $OH_{(4')}$); 4.95 (br s, 1H, $OH_{(3')}$); 4.61 (br t, 1H, $OH_{(6')}$); 4.06 (br s, 1H, H-2′); 3.87–3.47 (m, 5H, H-3′, H-4′, H-5′, H-6′a, H-6′b); 2.50 (2 s, 6H, 2 CH_3). ^{13}C-NMR (75.5 MHz, DMSO-d_6): $\delta = 144.2$, 143.8, 139.5, 138.8, 138.3, 137.9, 136.4 (7 C_q); 130.2 (C-8); 128.2, 126.7 (C-1, C-4); 121.0; 120.9 (C-9; C-10); 119.2 (C-10a); 117.1 (C-7); 83.6 (C-1′); 81.2 (C-5′); 73.7 (C-3′); 71.9 (C-2′); 66.6 (C-4′); 61.5 (C-6′); 20.1, 19.8 (2 CH_3). MS (EI, 70 eV): m/z (%) = 409 (3) [M^+]; 276 (13); 248 (21); 247 (100) [aglycone + H]; 246 (21); 232 (32). HRMS (EI): calcd. for $C_{22}H_{23}N_3O_5$ ([M^+]) 409.163493. Found: 409.16322.

13-(β-D-Mannopyranosyl)-5,12,13-triaza-indeno[1,2-*b*]anthracene (β-4h)

Stirring of **β-2b** (300 mg, 0.63 mmol) and **1c** (110 mg, 0.69 mmol) for 1 h at 80 °C in HOAc and for 1 h at 80 °C in benzene (in the presence of PTSA) and recrystallization from EtOAc and heptane afforded **β-3h** (245 mg; 65%) as a yellow to orange solid. Starting with **β-3h** (78 mg, 0.13 mmol), 10 mL of MeOH and 0.5 mL of a 0.5 M MeOH solution of NaOMe, **β-4h** was isolated (54 mg; 97%) after stirring for 24 h as a yellow to orange solid. Mp. 320–322 °C (heptane–EtOAc); $R_f = 0.25$ ($CHCl_3$–EtOH = 5 : 1). ^{1}H-NMR (250 MHz, DMSO-d_6): $\delta = 8.95$, 8.74 (2 s, 2H, H-6, H-11); 8.35–8.10 (m, 4H, H-1, H-4, H-7, H-10); 7.71–7.57 (m, 3H, H-2, H-8, H-9); 7.37 ('t', $^3J_{2,3} = {}^3J_{3,4} = 7.5$ Hz, 1H, H-3); 6.35 (br s, 1H, H-1′); 5.31 (d, $^3J_{2',OH} = 4.8$ Hz, 1H, $OH_{(2')}$); 5.04 (d, $^3J_{4',OH} = 4.5$ Hz, 1H, $OH_{(4')}$); 4.97 (d, $^3J_{3',OH} = 5.0$ Hz, 1H, $OH_{(3')}$); 4.65 (t, $^3J_{6',OH} = 5.5$ Hz, 1H, $OH_{(6')}$); 4.12 (br s, 1H, H-2′); 3.90–3.47 (m, 5H, H-3′, H-4′, H-5′, H-6′a, H-6′b). ^{13}C-NMR (75.5 MHz, DMSO-d_6): $\delta = 145.9$, 144.8, 142.6, 137.0, 136.9, 133.0, 131.7 (7 C_q); 131.2 (C-2); 128.4, 127.8 (C-7, C-10); 127.4, 124.5 (C-6, C-11); 126.6, 125.6 (C-8, C-9); 121.6 (C-4); 121.4 (C-3); 119.1 (C-4a); 117.2 (C-1); 83.7 (C-1′); 81.1 (C-5′); 73.6 (C-3′); 71.8 (C-2′); 66.5 (C-4′); 61.4 (C-6′). HRMS (ESI): calcd. for $C_{24}H_{21}N_3O_4$ ($[M + H]^+$) 432.15540. Found: 432.15519.

2,3-Dichloro-6-(β-D-mannopyranosyl)indolo[2,3-*b*]quinoxaline (β-4i)

Stirring of **β-2b** (300 mg, 0.63 mmol) and **1d** (123 mg, 0.69 mmol) for 1 h at 80 °C in HOAc and for 1 h at 80 °C in benzene (in the presence of PTSA) and recrystallization from EtOAc and heptane afforded **β-3i** (170 mg; 44%) as yellow to orange crystals. Starting with **β-3i** (100 mg, 0.18 mmol), 15 mL of MeOH and 0.5 mL of a 0.5 M MeOH solution of NaOMe, **β-4i** was isolated (60 mg, 75%) after stirring for 24 h as a slightly yellow solid. Mp. 323–324 °C (heptane–EtOAc); $[\alpha]_D = -3.04$ ($c = 0.63$; $T = 22.6$ °C; DMSO); $R_f = 0.41$ ($CHCl_3$–EtOH = 5 : 1). ^{1}H-NMR (250 MHz, DMSO-d_6): $\delta = 8.52$, 8.38 (2 s, 2H, H-1, H-4); 8.30–8.23 (m, 2H, H-7, H-10); 7.68 (ddd, $^4J_{8,10} = 1.1$ Hz, $^3J_{8,9} = 7.3$ Hz, $^3J_{7,8} = 8.4$ Hz, 1H, H-8); 7.38 (ddd, $^4J_{7,9} = 0.9$ Hz, $^3J_{8,9} = 7.3$ Hz, $^3J_{9,10} = 7.7$ Hz, 1H, H-9); 6.34 (d, $^3J_{1',2'} = 0.8$ Hz, 1H, H-1′); 5.18 (br s, 1H, $OH_{(2')}$); 5.03 (br s, 1H, $OH_{(4')}$); 5.02 (br s, 1H, $OH_{(3')}$); 4.66 (t, 1H, $OH_{(6')}$); 4.05 (br s, 1H, H-2′); 3.90–3.45 (m, 5H, H-3′, H-4′, H-5′, H-6′a, H-6′b). ^{13}C-NMR (75.5 MHz, DMSO-d_6): $\delta = 145.2$, 144.4, 141.1, 138.7, 137.8, 131.5 (6 C_q); 131.5 (C-8); 129.8, 128.4 (C-1, C-4); 128.8 (C_q); 121.6 (C-9); 121.6 (C-10); 118.6 (C-10a); 117.7 (C-7); 83.7 (C-1′); 81.2 (C-5′); 73.6 (C-3′); 71.9 (C-2′); 66.4 (C-4′); 61.4 (C-6′). HRMS (ESI): calcd. for $C_{20}H_{17}Cl_2N_3O_5$ ($[M + H]^+$) 450.06180. Found: 450.06229.

Biological studies

The screening toward antiproliferative properties was performed in accordance to the NIH protocols. The cell viability was investigated using the Neutral Red assay with subsequent measurement of the absorption at 630 nm.[22] Stock solutions of the test compounds were prepared using DMSO. All experiments were performed in 2 independent experiments with 4 parallel dilutions over a period of 3 days. Etoposide was used as the positive control experiment and DMSO as the negative control experiment.

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