Synthesis and antiproliferative activities of diversely substituted glycosyl-isoindigo derivatives.

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Abstract

In the course of structure–activity relationship studies, diversely substituted 1-(β-D-glucopyranosyl)-isoindigo derivatives were prepared from commercially available indolines. Their antiproliferative activities were evaluated toward a panel of human solid cancer cell lines (PC 3, DLD-1, MCF-7, M4Beu, A549, PA 1), a murine cell line (L929) and a human fibroblast primary culture to get an insight into the substitution pattern required for the best biological potencies.

Keywords: Indigoids; Isoindigos; Antiproliferative activities

1. Introduction

In the course of studies on the preparation of potential biologically active compounds, we were interested in the synthesis of indigoid derivatives. These heterocycles (indigo, indirubin, isoindigo) containing a bis-indole framework are derived from various natural sources (Scheme 1).

The bis-indole indirubin is the active ingredient of Danggui Longhui Wan, a mixture of plants used in traditional Chinese medicine to treat chronic diseases. Indirubins are potent inhibitors of several kinases such as GSK-3β and cyclin dependent kinases (CDKs) [1]. Indirubin is known to interact with the ATP-binding site of CDK-2, CDK-5 and GSK-3 [2]. Moreover, structure–activity relationship studies have shown that 5-nitro and 5-bromoindirubin are more potent kinase inhibitors (GSK-3β, CDK-1, CDK-5) than their parent indirubin [1]. Indirubin and indigo are also potent aryl hydrocarbon receptor ligands [3,4].

Indolin-2-one derivatives are usually known as ATP competitive inhibitors of receptor tyrosine kinase such as VEGFR, FGFR and PDGFR [5,6] (e.g. SU6668, SU11248). Moreover, some imidazo[2,1-*b*]thiazolylmethylene-2-indolinones such as compound A or indolylmethylene-2-indolinones such as compound B have been described as CDK1/cyclin B inhibitors [7]. Recently, SU9516, possessing an indolin-2-one framework was described as an ATP competitive inhibitor of CDKs (Scheme 2) [8].

We have described, in a previous paper, a method for the preparation of glycosyl-isoindigo derivatives substituted in the 5 position of one of the aromatic rings by either electron donor or acceptor substituents [9]. In this paper, the synthesis of isoindigo derivatives (indirubin isomers possessing two indolin-2-one moieties) bearing a sugar residue attached to one of the aromatic nitrogens and diversely substituted on both aromatic rings in the 5 or 5′ positions is described (Scheme 3).

It was reported in the literature [10,11] that the biological activities of the isoindigo derivative called Natura (1-(β-D-tri-*O*-acetylxylopyranosyl)-isoindigo) (Scheme 3) were better than those of the non-protected analogue, probably because of an enhanced cellular penetration. Accordingly, we have prepared glycosyl-isoindigo derivatives protected on the sugar residue by either benzyl or acetyl groups (Scheme 3). To get an insight into the effect of the substitution pattern on the biological ac-

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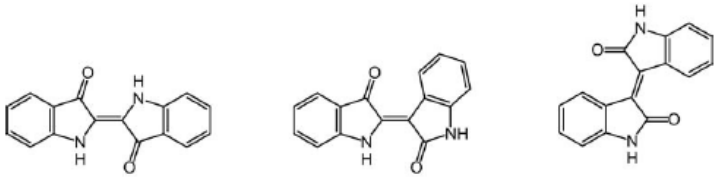
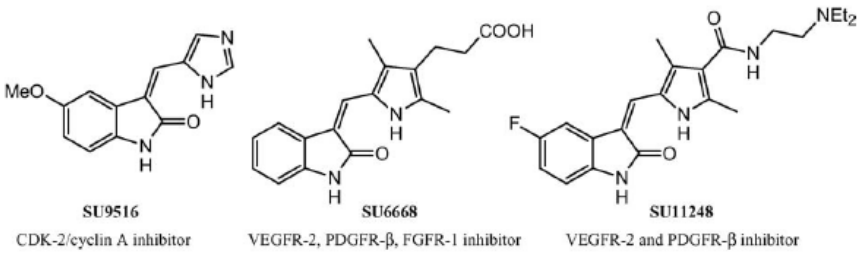
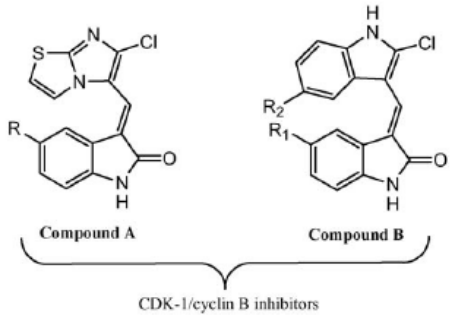
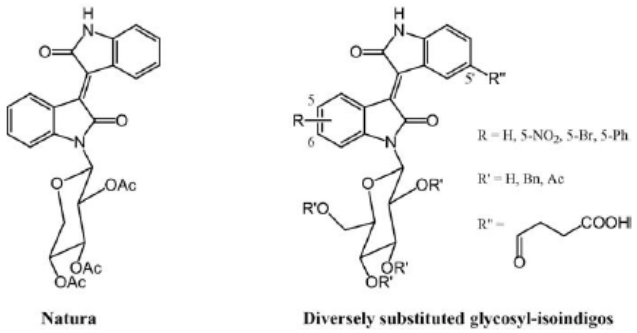


Scheme 1.





Scheme 2.



Scheme 3.

tivities of these compounds, their antiproliferative potencies were examined in vitro by two fluorometric assays: resazurin reduction test (RRT) and Hoechst 33342 dye DNA assay in a panel of human solid cancer cell lines (PC 3: prostatic adenocarcinoma, DLD-1: colon carcinoma, MCF-7: breast adenocarcinoma, M4Beu: melanoma teratocarcinoma, A549: lung carcinoma, PA 1: ovarian carcinoma), a murine cell line (L929) and a human fibroblast primary culture.

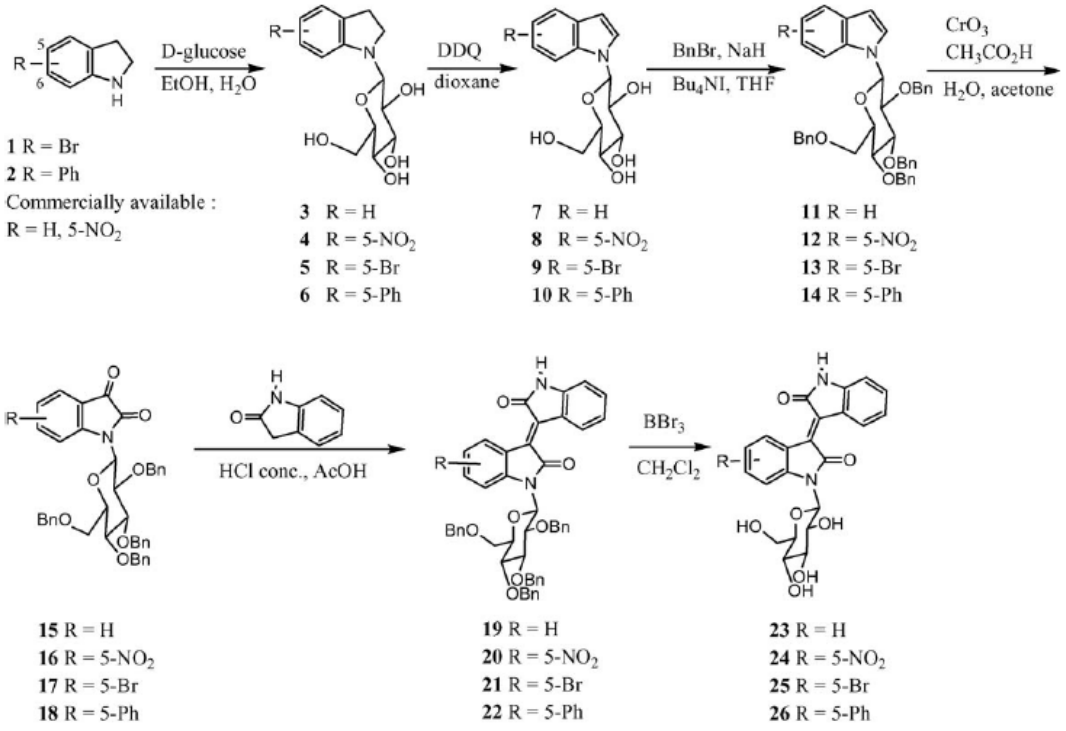
2. Chemistry

Recently, we described the synthesis and spectral data of compounds **23**–**25** [9]. The key intermediates in this approach are the protected glycosyl-isatines **15**–**17** which were prepared in four steps from the corresponding indolines. The preparation of compound **15** has been previously detailed [12]. The derivative substituted in the 5 position by a phenyl group was pre-

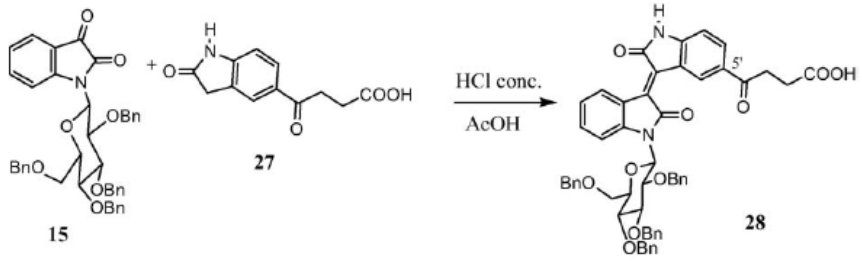
pared using the same synthetic pathway. Indoline **1** was prepared in 81% yield from the corresponding *N*-acetyl analogue by basic hydrolysis and phenyl analogue **2** was obtained from **1** in 45% yield by a Suzuki coupling reaction using phenyl boronic acid, palladium tetrakis(triphenylphosphine) and sodium carbonate as described by Sun et al. [13] for bromobenzene derivatives. Glycosyl-indolines **3**–**6** were prepared by glycosylation of the corresponding indolines [14]. After aromatization with 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ), the hydroxy groups of the intermediates **7**–**10** were first protected before oxidation with chromium oxide to give the glycosyl-isatines **15**–**18**. As mentioned in our previous paper [9], compared with reactions carried out with electron acceptor substituents, the first two steps were easier in the presence of electron donor substituents. Indeed, the glycosylation step was achieved in 24 h for compounds **3**, **5**, **6** and required 6 days for nitro analogue **4**. The aromatization step was carried out at room temperature during 12–18 h for compounds **7**, **9**, **10** and at 50 °C during 48 h for nitro compound **8**. Isatine derivatives **15**–**18** were prepared by oxidation of the corresponding indoles using chromium oxide [12]. To obtain the corresponding glycosyl-isoindigo derivatives, compounds **15**–**18** were treated in an acidic medium [15] in the presence of oxindole. Deprotection of the hydroxy groups of the glycosyl moiety was performed by reaction of derivatives **19**–**22** with boron tribromide [16] to give compounds **23**–**26** (Scheme 4).

Glycosyl-isoindigo derivative **28**, substituted in the 5′ position, was obtained by acid-catalyzed coupling of isatine **15** and substituted oxindole **27** which was prepared in 88% yield by acylation of the oxindole in the presence of succinic anhydride and aluminum chloride according to the procedure described by Kakushima et al. [17] for pyrrole derivatives. Unfortunately, the deprotection step performed in the same conditions as described above did not allow the obtention of the corresponding deprotected glycosyl-isoindigo (Scheme 5).

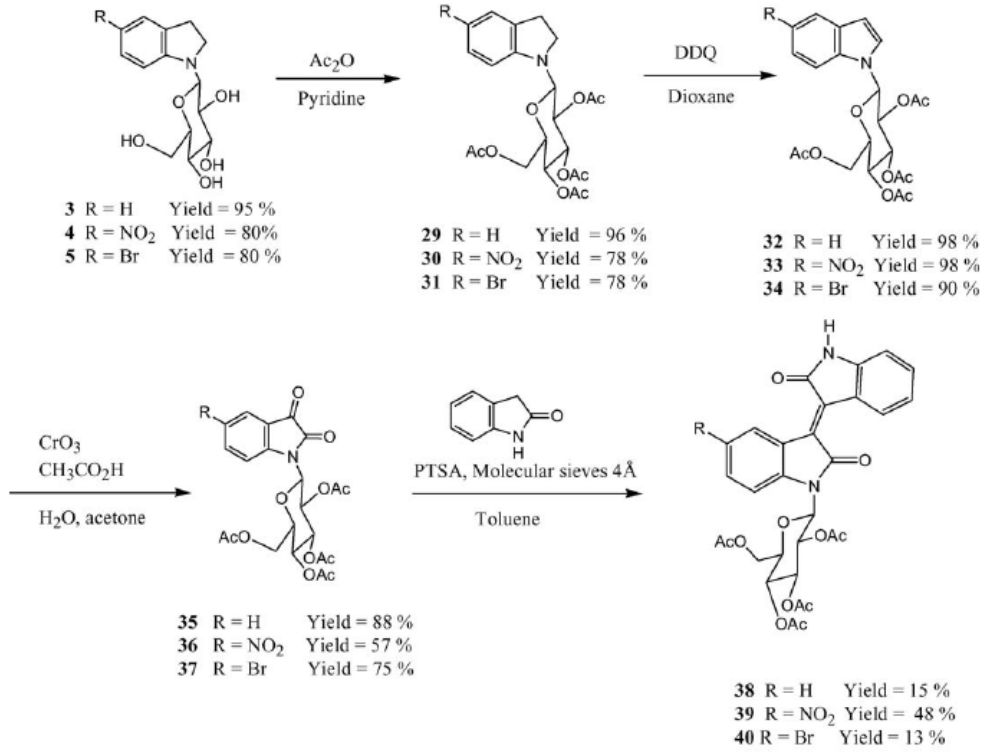
The synthesis of acetyl analogues **38**–**40** was performed by a similar synthetic pathway (Scheme 6). The key intermediates, acetylated glycosyl-isatines **35**–**37**, were prepared in four steps



Scheme 4.



Scheme 5.



Scheme 6.

from the corresponding indolines. The glycosyl-indolines **3**–**5** were acetylated in the presence of pyridine and acetic anhydride before aromatization with DDQ and oxidation with chromium oxide which were performed in the same conditions as described before for the benzylated series. Acetylated glycosyl-isatines **35**–**37** were then coupled with the oxindole moiety in an acidic medium [11] to give the acetylated glycosyl-isoindigos (Scheme 6). In this case, the coupling was achieved in the presence of *p*-toluenesulfonic acid (PTSA) instead of a mixture of acetic acid and HCl to avoid deacetylation.

The β configuration of the *N*-glycosyl derivatives **3**–**18** and **29**–**37** was determined by the measurement of the coupling constant between the $H_{1'}$ and $H_{2'}$ of the sugar residue. In all cases, the resulting signal of the $H_{1'}$ was a doublet with a coupling constant around 9 Hz.

Surprisingly, for several glycosyl-isoindigo derivatives **19**, **21**–**26**, **28**, **38**, **39**, the signal of the $H_{1'}$ of the sugar moiety was not a clear doublet. Therefore these signals were assigned as broad signals. NMR experiments were then run at higher temperatures (70 °C) or in other solvents ($CDCl_3$ or DMSO) to investigate if solvatation effects or the presence of different conformers could explain the shape of these signals. No changes were observed on the $H_{1'}$ signal in these conditions. The unusual appearance of these signals could be due to isomerization to the α configuration but this was never observed before in our previous works concerning glycosylated indole or azaindole derivatives. Moreover, the $H_{1'}$ signal of derivative **20**, benzylated glycosyl-isoindigo substituted by a nitro group, was a clear doublet with the usual 9 Hz coupling constant. The measurement of the coupling constant for the $H_{1'}$ signal clearly indicate a β configuration. In contrast, the $H_{1'}$ signal of the deprotected analogue **24** was a broad signal. On the basis that the presence or not of benzyl groups should not affect the β configuration of the *N*-glycosidic bond, the hypothesis of an isomerization was excluded and we assumed that the configuration of the *N*-glycosydic bond was β for all these compounds.

3. Results and discussion

3.1. In vitro antiproliferative activities

In vitro antiproliferative activities of compounds **19**–**26**, **28** and **38**–**40** were evaluated toward a panel of human solid cancer cell lines (PC 3, DLD-1, MCF-7, M4Beu, A549, PA 1), murine cell line (L929) and human fibroblast primary culture. The IC_{50}, defined as the drug concentration required to inhibit cell proliferation by 50%, was calculated from the curve of concentration-dependent survival percentage, defined as fluorescence in experimental wells compared with fluorescence in control wells, after subtraction of the blank values. The antiproliferative effect of the tested drug was assessed by both the RRT [18] and the determination of DNA cellular content after cell lysis by the Hoechst dye 33342 assay carried out according to the method described by Rago et al. [19] with minor mod-

Table 1
Antiproliferative activities of compounds **34**, **44** and **46** (IC_{50} in μM)

Compounds	Fibroblast	L929	A-549	DLD-1	M4Beu	MCF-7	PA 1	PC3
28	4.7	6.5	12.4	6.0	7.0	8.0	2.5	4.7
	(5.1)	(6.1)	(12.4)	(5.3)	(7.1)	(5.1)	(2.7)	(5.0)
38	Inactive	8.7	Inactive	17.3	Inactive	25.1	6.2	25
		(8.3)		(11.7)		(17.9)	(5.5)	(21.8)
40	Inactive	8.1	Inactive	17.0	Inactive	24.0	6.1	3.7
		(7.3)		(13.5)		(12.0)	(5.5)	(3.1)

Inactive means an IC_{50} value > 30 μM (Rezazurin test results are given first and Hoechst test results are given in brackets).

ifications. Under the conditions used, only three of the tested compounds have shown significant cytotoxic activities (Table 1).

The most potent compound was compound **28** which was cytotoxic toward all the cell lines tested. This cytotoxity is probably due to the presence of the carboxylic chain on the upper oxindole moiety because compound **19**, lacking this substitution, was completely inactive against the cell lines tested.

Compounds **38** and **40** had similar inhibition profiles except on PC3 cells on which compound **40** was slightly more active than its non brominated analogue **38**, they were both active on PA 1, L929, DLD-1 and MCF-7 cells. In contrast to compound **28**, they show selectivity between normal and tumor cell lines, they are both inactive against healthy human fibroblast. As previously described for Natura® [10,11], in our series, the acetylated derivatives **38** and **40**, were more cytotoxic than their benzylated analogues **19**, **21** or parent compounds **23**, **25**. In contrast, the nitro derivative **39** was inactive toward the cell lines tested indicating that the presence of a nitro group in position 5 is detrimental to the cytotoxicity.

In conclusion, we have synthesized diversely substituted 1-(β-D-glucopyranosyl)-isoindigos. The method described here allows the substitution of both aromatic rings. The oxindole moiety bearing the sugar residue was substituted by either electron donor or acceptor substituents in the 5 position. The results obtained in this structure–activity relationship studies have shown that the pharmaceutical profile of this series could be optimized by substitutions of the upper oxindole moiety and the presence of acetyl groups on the sugar residue.

4. Experimental

4.1. Chemistry

IR spectra were recorded on a Perkin–Elmer 881 spectrometer (ν in cm^{-1}). NMR spectra were performed on a Bruker AVANCE 400 (^1H: 400 MHz, ^{13}C: 100 MHz) (chemical shifts δ in ppm, the following abbreviations are used: singlet (s), doublet (d), triplet (t), doubled triplet (dt), doubled doublet (dd), doubled doubled doublet (ddd), multiplet (m), broad signal (br s). When necessary to identify all carbon atoms, complementary NMR experiments (HSQC, HMBC) were performed on a Bruker Avance 500. Mass spectra (ES) were determined on a high resolution Waters Micro Q-toff apparatus. Chromatographic purifications were performed by flash silica gel Geduran SI 60 (Merck) 0.040–0.063 mm column chromatography. For purity tests, TLC were performed on fluorescent silica gel plates (60 F254 from Merck).

4.1.1. 5-Bromoindoline **1**

A 40% aqueous NaOH solution (40 ml) was added to a solution of *N*-acetyl-5-bromoindoline (2 g, 8 mmol) in methanol (40 ml). The reaction mixture was refluxed for 18 h before hydrolysis. After extraction with dichloromethane, the organic phases were dried over $MgSO_4$. The residue obtained after concentration under vacuum, was purified by flash chromatography (eluent cyclohexane/EtOAc 70:30) to give **2** (1.28 g, 6.5 mmol) as a brown oil in 81% yield. C_8H_8BrN, IR (KBr) ν_{NH} 3380 cm^{-1}, $\nu_{C=C}$ 1600 cm^{-1}. ^1H (400 MHz, DMSO-d_6): 2.91 (t, $J = 8.5$ Hz, 2H, CH_2), 3.42 (t, $J = 8.5$ Hz, 2H, CH_2), 5.65 (s, 1H, NH), 6.42 (d, $J = 8.0$ Hz, 1H), 7.02 (dd, $J_1 = 8.0$ Hz, $J_2 = 1.5$ Hz, 1H), 7.14 (d, $J = 1.0$ Hz, 1H). ^{13}C (100 MHz, DMSO-d_6): 29.0, 46.6 (CH_2), 109.6, 126.8, 129.3 (CH), 107.1, 131.7, 151.9 (C).

4.1.2. 5-Phenylindoline **2**

Phenylboronic acid (332 mg, 2.72 mmol), 2 M aqueous sodium carbonate (2.5 ml) and palladium tetrakis (84.9 mg, 0.08 mmol) were added to a solution of 5-bromoindoline **1** (490 mg, 2.47 mmol) in a mixture of toluene (6 ml) and ethanol (3 ml). The reaction mixture was refluxed for 22 h. The solid residue obtained after concentration under vacuum and EtOAc addition was removed by filtration. The filtrate was washed with water, dried over $MgSO_4$, and concentrated under vacuum to give a residue, which was purified by flash chromatography (eluent cyclohexane/EtOAc from 90:10 to 60:40) to give the corresponding compound **2** (216 mg, 1.1 mmol) as a brown oil in 45% yield. $C_{14}H_{13}N$, IR (KBr) ν_{NH} 3417 cm^{-1}, $\nu_{C=C}$ 1599 cm^{-1}. ^1H (400 MHz, DMSO-d_6): 3.00 (t, $J = 8.5$ Hz, 2H, CH_2), 3.49 (td, $J_1 = 8.5$ Hz, $J_2 = 2.0$ Hz, 2H, CH_2), 5.63 (br s, 1H, NH), 6.59 (d, $J = 8.0$ Hz, 1H), 7.23–7.27 (m, 2H), 7.38–7.42 (m, 3H), 7.55–7.58 (m, 2H). ^{13}C (100 MHz, DMSO-d_6): 29.1, 46.5 (CH_2), 108.3, 122.6, 125.5 (2C), 125.6 (2C), 128.6 (2C) (CH), 128.9, 129.6, 141.1, 152.2 (C).

4.1.3. Typical procedure for the glycosylation reaction

D-Glucose (3.05 mmol) was added to a solution of indoline (6.1 mmol) in a mixture of ethanol (46.7 ml) and water (3.05 ml). The mixture was heated to 90 °C during 24 h for com-

pounds **3**, **5**, **6** and 6 days for compound **4**. Water (0.6 ml) was added after 7 and 14 h reflux. After concentration under vacuum, the resulting mixture was purified by flash chromatography (eluent EtOAc/MeOH from 98:2 to 90:10) to give the corresponding glycosyl-indoline.

1-(β-D-Glucopyranosyl)-indoline **3**: Obtained in 95% yield as a white solid (m.p. = 113 °C), IR (KBr) υ_{CO} 1270 cm^{-1}, $\upsilon_{C=C}$ 1610 cm^{-1}, υ_{OH} 3100–3700 cm^{-1}. MS (EI) M^+ Calcd. for $C_{14}H_{19}NO_5$, 281; Found: 281. ^1H NMR (400 MHz, DMSO-d_6): 2.91 (m, 2H), 3.12 (m, 1H), 3.21 (m, 1H), 3.30 (m, 2H), 3.38–3.54 (m, 2H), 3.62 (m, 2H), 4.35 (t, J = 5.0 Hz, 1H, OH), 4.64 (d, J = 8.0 Hz, 1H, $H_{1'}$), 4.91 (d, J = 5.0 Hz, 1H, OH), 5.02 (br s, 2H, OH), 6.59 (t, J = 8.0 Hz, 2H), 6.95 (d, J = 7.0 Hz, 1H), 7.00 (d, J = 8.0 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): 28.3 (CH_2), 45.7 (CH_2), 61.5 ($CH_{2\ sugar}$), 70.7, 71.4, 78.4, 78.8, 85.7 (CH_{sugar}), 108.1, 118.3, 125.0, 127.6 (CH), 130.2, 151.3 (C).

1-(β-D-Glucopyranosyl)-5-nitroindoline **4**: $C_{14}H_{18}N_2O_7$ obtained in 80% yield as an orange powder (m.p. = 70–75 °C), IR (KBr): ν_{OH} 3680–3040 cm^{-1}; $\nu_{C=C}$ 1610 cm^{-1}. ^1H (400 MHz, DMSO-d_6): 3.00–3.15 (m, 3H), 3.28–3.36 (m, 3H), 3.36–3.48 (m, 1H), 3.60–3.76 (m, 2H), 3.80–3.91 (m, 1H), 4.49 (t, J = 6.0 Hz, 1H, OH), 4.83 (d, J = 8.5 Hz, 1H, $H_{1'}$), 5.04 (d, J = 5.5 Hz, 1H, OH), 5.15 (d, J = 4.5 Hz, 1H, OH), 5.28 (d, J = 5.0 Hz, 1H, OH), 6.74 (d, J = 9.0 Hz, 1H), 7.91 (d, J = 2.5 Hz, 1H), 8.02 (dd, J_1 = 9.0 Hz, J_2 = 2.5 Hz, 1H). ^{13}C (100 MHz, DMSO-d_6): 26.3, 46.0 (CH_2), 60.9 ($CH_{2\ sugar}$), 69.9, 70.4, 77.3, 78.6, 84.2 (CH_{sugar}), 105.6, 120.5, 125.8 (CH), 130.6, 137.8, 157.0 (C).

1-(β-D-Glucopyranosyl)-5-bromoindoline **5**: $C_{14}H_{18}BrNO_5$ obtained in 80% yield as a brown gum, IR (NaCl): ν_{OH} 3402 cm^{-1}; $\nu_{C=C}$ 1606 cm^{-1}. ^1H (400 MHz, DMSO-d_6): 2.89–3.04 (m, 2H), 3.09–3.16 (m, 1H), 3.21–3.27 (m, 1H), 3.28–3.36 (m, 2H), 3.41–3.56 (m, 2H), 3.63 (ddd, J_1 = 12.0 Hz, J_2 = 5.5 Hz, J_3 = 2.0 Hz, 1H), 3.66–3.72 (m, 1H), 4.43 (t, J = 6.5 Hz, 1H, OH), 4.68 (d, J = 8.5 Hz, 1H, $H_{1'}$), 4.99 (d, J = 5.0 Hz, 1H, OH), 5.09 (d, J = 4.5 Hz, 1H, OH), 5.11 (d, J = 4.5 Hz, 1H, OH), 6.59 (d, J = 8.5 Hz, 1H), 7.17 (dd, J_1 = 8.5 Hz, J_2 = 2.0 Hz, 1H), 7.22 (d, J = 2.0 Hz, 1H). ^{13}C (100 MHz, DMSO-d_6): 27.3, 45.3 (CH_2), 60.8 ($CH_{2\ sugar}$), 69.9, 70.6, 77.5, 78.1, 84.8 (CH_{sugar}), 109.0, 126.9, 129.3 (CH), 108.1, 132.5, 150.1 (C).

1-(β-D-Glucopyranosyl)-5-phenylindoline **6**: $C_{20}H_{23}NO_5$ obtained in 85% yield as a brown gum, IR (NaCl): ν_{OH} 3400 cm^{-1}; $\nu_{C=C}$ 1620 cm^{-1}. ^1H (400 MHz, DMSO-d_6): 2.96–3.09 (m, 2H), 3.13–3.21 (m, 1H), 3.26–3.31 (m, 1H), 3.32–3.42 (m, 2H), 3.44–3.51 (m, 1H), 3.58 (dd, J_1 = 19.0 Hz, J_2 = 10.5 Hz, 1H), 3.66 (ddd, J_1 = 12.0 Hz, J_2 = 5.0 Hz, J_3 = 2.0 Hz, 1H), 3.70–3.77 (m, 1H), 4.42 (t, J = 6.5 Hz, 1H, OH), 4.73 (d, J = 9.0 Hz, 1H, $H_{1'}$), 4.97 (d, J = 5.0 Hz, 1H, OH), 5.08 (d, J = 4.5 Hz, 1H, OH), 5.09 (d, J = 5.0 Hz, 1H, OH), 6.69 (d, J = 8.0 Hz, 1H), 7.27 (t, J = 7.0 Hz, 1H), 7.34 (dd, J_1 = 8.5 Hz, J_2 = 1.5 Hz, 1H), 7.37–7.44 (m, 3H), 7.59 (dd, J_1 = 8.5 Hz, J_2 = 1.0 Hz, 2H). ^{13}C (100 MHz, DMSO-d_6): 27.6, 45.3 (CH_2), 60.9 ($CH_{2\ sugar}$), 70.0, 70.7, 77.7, 78.2, 85.1 (CH_{sugar}), 107.6, 122.7, 125.6, 125.7 (2C), 125.9, 128.7 (2C) (CH), 129.9, 130.5, 140.9, 150.4 (C).

4.1.4. Aromatization with DDQ

4.1.4.1. For compounds 7–9. DDQ (1.1 or 0.4 mmol for compound **9**) was added to a solution of glycosyl-indoline (0.36 mmol) in 1,4-dioxane (16 ml). Before hydrolysis with saturated aqueous $NaHCO_3$, the mixture was stirred at room temperature for 12 h for compound **7** or 18 h for compounds **9** and **10**. The reaction mixture was heated at 50 °C for 48 h for compound **8**. The solid residue ($DDQH_2$ and $NaHCO_3$) obtained after concentration under vacuum and EtOAc addition was removed by filtration. The filtrate was dried over $MgSO_4$, and concentrated under vacuum to give a residue, which was purified by flash chromatography (eluent EtOAc/MeOH from 100:0 to 90:10) to give the corresponding glycosyl-indole.

*4.1.4.2. For compound **10***. DDQ (0.4 mmol) was added to a solution of glycosyl-indoline (0.36 mmol) in 1,4-dioxane (16 ml). The mixture was stirred at room temperature for 18 h before hydrolysis with saturated aqueous $NaHCO_3$. After extraction with EtOAc, the organic phases were dried over $MgSO_4$, and concentrated under vacuum to give a residue, which was purified by flash chromatography (eluent EtOAc/MeOH 98:2) to give the corresponding glycosyl-indole.

1-(β-D-Glucopyranosyl)-indole **7**: $C_{14}H_{17}NO_5$ obtained in 74% yield as a salmon colored solid (m.p. = 90–95 °C), IR (KBr) $\upsilon_{C=C}$ 1610 cm^{-1}, υ_{OH} 3080–3700 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): 3.33 (m, 1H), 3.45 (m, 1H), 3.51 (m, 2H), 3.75 (m, 1H), 3.81 (m, 1H), 4.59 (br s, 1H, OH), 5.15 (d, J = 5.0 Hz, 1H, OH), 5.25 (d, J = 5.5 Hz, 2H, OH), 5.46 (d, J = 9.5 Hz, 1H, $H_{1'}$), 6.51 (d, J = 3.5 Hz, 1H), 7.09 (t, J = 7.0 Hz, 1H), 7.18 (dt, J_1 = 7.9 Hz, J_2 = 1.5 Hz, 1H), 7.56 (d, J = 3.5 Hz, 1H), 7.59 (dd, J_1 = 8.0 Hz, J_2 = 1.0 Hz, 1H), 7.61 (d, J = 8.0 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): 61.0 ($CH_{2\ sugar}$), 69.9, 71.7, 77.6, 79.4, 84.8 (CH_{sugar}), 101.6, 110.9, 119.7, 120.3, 121.2, 126.3 (CH), 128.5, 136.2 (C).

1-(β-D-Glucopyranosyl)-5-nitroindole **8**: $C_{14}H_{16}N_2O_7$ obtained in 65% yield as a yellow gum, IR (NaCl): ν_{OH} 3426 cm^{-1}; $\nu_{C=C}$ 1615 cm^{-1}. ^1H (400 MHz, DMSO-d_6): 3.30–3.37 (m, 1H), 3.43–3.58 (m, 3H), 3.71–3.78 (m, 2H), 4.61 (t, J = 6.0 Hz, 1H, OH), 5.19 (d, J = 5.5 Hz, 1H, OH), 5.28 (d, J = 5.0 Hz, 1H, OH), 5.34 (d, J = 6.0 Hz, 1H, OH), 5.59 (d, J = 9.0 Hz, 1H, $H_{1'}$), 6.83 (d, J = 3.5 Hz, 1H), 7.78 (d, J = 3.5 Hz, 1H), 7.84 (d, J = 9.0 Hz, 1H), 8.09 (dd, J_1 = 9.0 Hz, J_2 = 2.0 Hz, 1H), 8.61 (d, J = 2.0 Hz, 1H). ^{13}C (100 MHz, DMSO-d_6): 60.9 ($CH_{2\ sugar}$), 69.7, 72.0, 77.2, 79.6, 85.0 (CH_{sugar}), 104.2, 111.5, 116.6, 117.3, 130.1 (CH), 127.8, 139.2, 141.1 (C).

1-(β-D-Glucopyranosyl)-5-bromoindole **9**: $C_{14}H_{16}BrNO_5$ obtained in 88% yield as a brown gum, IR (NaCl): ν_{OH} 3395 cm^{-1}; $\nu_{C=C}$ 1606 cm^{-1}. ^1H (400 MHz, DMSO-d_6): 3.27–3.34 (m, 1H), 3.41–3.55 (m, 3H), 3.69–3.78 (m, 2H),

4.58 (t, $J = 5.5$ Hz, 1H, OH), 5.15 (d, $J = 5.5$ Hz, 1H, OH), 5.24 (d, $J = 5.0$ Hz, 1H, OH), 5.26 (d, $J = 6.0$ Hz, 1H, OH), 5.45 (d, $J = 9.0$ Hz, 1H, $H_{1'}$), 6.50 (d, $J = 3.5$ Hz, 1H), 7.29 (dd, $J_1 = 9.0$ Hz, $J_2 = 2.0$ Hz, 1H), 7.56 (d, $J = 3.5$ Hz, 1H), 7.59 (d, $J = 9.0$ Hz, 1H), 7.78 (d, $J = 2.0$ Hz, 1H). ^{13}C (100 MHz, DMSO-d_6): 60.9 ($CH_{2\ sugar}$), 69.8, 71.8, 77.4, 79.4, 84.8 (CH_{sugar}), 101.2, 113.0, 122.5, 123.6, 127.8 (CH), 112.1, 130.3, 134.9 (C).

1-(β-D-Glucopyranosyl)-5-phenylindole **10**: $C_{20}H_{21}NO_5$ obtained in 90% yield as a beige gum, IR (NaCl): ν_{OH} 3395 cm^{-1}; $\nu_{C=C}$ 1600 cm^{-1}. ^{1}H (400 MHz, DMSO-d_6): 3.27–3.35 (m, 1H), 3.43–3.56 (m, 3H), 3.71–3.84 (m, 2H), 4.59 (t, $J = 5.5$ Hz, 1H, OH), 5.15 (d, $J = 5.5$ Hz, 1H, OH), 5.24 (d, $J = 5.0$ Hz, 1H, OH), 5.26 (d, $J = 6.0$ Hz, 1H, OH), 5.48 (d, $J = 9.0$ Hz, 1H, $H_{1'}$), 6.57 (d, $J = 3.0$ Hz, 1H), 7.34 (t, $J = 7.5$ Hz, 1H), 7.46–7.51 (m, 3H), 7.54 (d, $J = 3.5$ Hz, 1H), 7.68 (d, $J = 8.5$ Hz, 1H), 7.71 (d, $J = 7.0$ Hz, 2H), 7.85 (d, $J = 1.5$ Hz, 1H). ^{13}C (100 MHz, DMSO-d_6): 61.0 ($CH_{2\ sugar}$), 69.9, 71.8, 77.5, 79.4, 84.9 (CH_{sugar}), 102.0, 111.4, 118.3, 120.5, 126.3, 126.7 (2C), 127.1, 128.8 (2C) (CH), 129.1, 132.2, 135.8, 141.6 (C).

4.1.5. Typical procedure for the benzylation step

NaH (60% dispersion in mineral oil, 8.2 mmol) was added at 0 °C to a solution of glycosyl-indole (0.86 mmol) in THF (12 ml) before addition of nBu$_4$NI (0.07 mmol) and benzyl bromide (6.7 mmol). The mixture was then stirred at 0 °C for 20 min before refluxing for 17 hours. After addition of florisil (60/100 mesh) to the reaction mixture, the solvent was removed under vacuum and the residue purified by flash chromatography (eluent: cyclohexane/EtOAc from 98:2 to 80:20) to give the protected glycosyl-indole.

1-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-indole **11**: $C_{42}H_{41}NO_5$ obtained in 78% yield as a yellow solid (m.p. = 90 °C), IR (KBr) $\upsilon_{C=C}$ 1610 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6): 3.65 (d, $J = 9.5$ Hz, 1H), 3.75 (m, 2H), 3.81 (d, $J = 9.5$ Hz, 1H), 3.95 (m, 2H), 4.15 (t, $J = 9.5$ Hz, 1H), 4.25 (d, $J = 11.0$ Hz, 1H), 4.50 (d, $J = 12.5$ Hz, 1H), 4.55 (d, $J = 12.0$ Hz, 1H), 4.65 (d, $J = 11.0$ Hz, 1H), 4.85 (d, $J = 10.5$ Hz, 1H), 4.90 (br s, 2H), 5.82 (d, $J = 9.0$ Hz, 1H, $H_{1'}$), 6.65 (d, $J = 3.0$ Hz, 1H), 6.74 (d, $J = 7.0$ Hz, 2H), 7.09–7.39 (m, 20H), 7.65 (d, $J = 8.0$ Hz, 1H), 7.70 (d, $J = 3.5$ Hz, 1H), 7.74 (d, $J = 8.0$ Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): 68.7, 72.3, 73.3, 74.1, 74.6 ($C_{sugar\ +\ benzyl}$), 76.3, 77.6, 80.2, 83.9, 84.6 (CH_{sugar}), 102.5, 111.1, 120.0, 120.6, 121.6, 126.5, 127.5–128.2 (CH), 128.5, 135.9, 137.5, 138.1 (2C), 138.5 (C).

1-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-5-nitroindole **12**: $C_{42}H_{40}N_2O_7$ obtained in 64% yield as a yellow gum, IR (NaCl): $\nu_{C=C}$ 1612 cm^{-1}. ^{1}H (400 MHz, DMSO-d_6): 3.71–3.77 (m, 3H), 3.81 (t, $J = 9.5$ Hz, 1H), 3.92–4.00 (m, 2H), 4.17 (t, $J = 9.0$ Hz, 1H), 4.38 (d, $J = 11.0$ Hz, 1H), 4.50 (d, $J = 12.0$ Hz, 1H), 4.56 (d, $J = 12.0$ Hz, 1H), 4.66 (d, $J = 11.0$ Hz, 1H), 4.84 (d, $J = 11.0$ Hz, 1H), 4.89 (s, 2H), 5.96 (d, $J = 9.0$ Hz, 1H, $H_{1'}$), 6.70 (dd, $J_1 = 7.5$ Hz, $J_2 = 1.0$ Hz, 2H), 6.94 (d, $J = 3.0$ Hz, 1H), 7.06–7.11 (m, 2H), 7.12–7.18 (m, 1H), 7.26 (dd, $J_1 = 7.5$ Hz, $J_2 = 1.5$ Hz, 2H), 7.30–7.41 (m, 13H), 7.92 (d, $J = 9.0$ Hz, 1H), 7.98 (d, $J = 3.0$ Hz, 1H), 8.09 (dd, $J_1 = 9.0$ Hz, $J_2 = 2.0$ Hz, 1H), 8.67 (d, $J = 2.0$ Hz, 1H). ^{13}C (100 MHz, DMSO-d_6): 68.5, 72.3, 73.6, 74.1, 74.6 ($CH_{2\ sugar\ +\ benzyl}$), 76.5, 77.5, 80.3, 83.8, 84.6 (CH_{sugar}), 105.1, 111.5, 116.9, 117.6, 127.4–128.2, 130.0 (CH), 127.8, 137.2, 138.0, 138.1, 138.4, 138.9, 141.4 (C).

1-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-5-bromoindole **13**: $C_{42}H_{40}BrNO_5$ obtained in 82% yield as a beige gum, IR (NaCl): $\nu_{C=C}$ 1604 cm^{-1}. ^{1}H (400 MHz, DMSO-d_6): 3.65 (d, $J = 10.5$ Hz, 1H), 3.71–3.75 (m, 2H), 3.78 (t, $J = 9.5$ Hz, 1H), 3.88–3.97 (m, 2H), 4.12 (t, $J = 9.0$ Hz, 1H), 4.29 (d, $J = 10.5$ Hz, 1H), 4.50 (d, $J = 12.0$ Hz, 1H), 4.55 (d, $J = 12.5$ Hz, 1H), 4.64 (d, $J = 11.0$ Hz, 1H), 4.83 (d, $J = 11.0$ Hz, 1H), 4.87 (s, 2H), 5.82 (d, $J = 9.0$ Hz, 1H, $H_{1'}$), 6.63 (d, $J = 3.5$ Hz, 1H), 6.73 (d, $J = 7.0$ Hz, 2H), 7.09–7.15 (m, 2H), 7.16–7.22 (m, 1H), 7.23–7.28 (m, 2H), 7.29–7.41 (m, 14H), 7.70 (d, $J = 8.5$ Hz, 1H), 7.77 (d, $J = 3.5$ Hz, 1H), 7.85 (d, $J = 2.0$ Hz, 1H). ^{13}C (100 MHz, DMSO-d_6): 68.6, 72.3, 73.4, 74.1, 74.5 ($CH_{2\ sugar\ +\ benzyl}$); 76.3, 77.5, 80.2, 83.9, 84.6 (CH_{sugar}), 102.2, 113.1, 122.9, 124.1, 127.5–128.3 (CH), 112.5, 130.3, 134.6, 137.4, 138.0, 138.1, 138.4 (C).

1-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-5-phenylindole **14**: $C_{48}H_{45}NO_5$ obtained in 68% yield as a beige gum, IR (NaCl): $\nu_{C=C}$ 1450, 1490, 1500 cm^{-1}. ^{1}H (400 MHz, DMSO-d_6): 3.71 (d, $J = 10.5$ Hz, 1H), 3.73–3.77 (m, 2H), 3.79 (t, $J = 9.5$ Hz, 1H), 3.91–3.98 (m, 2H), 4.16 (t, $J = 9.0$ Hz, 1H), 4.30 (d, $J = 10.5$ Hz, 1H), 4.51 (d, $J = 12.0$ Hz, 1H), 4.57 (d, $J = 12.5$ Hz, 1H), 4.65 (d, $J = 11.0$ Hz, 1H), 4.84 (d, $J = 11.0$ Hz, 1H), 4.86 (d, $J = 11.5$ Hz, 1H), 4.90 (d, $J = 11.5$ Hz, 1H), 5.84 (d, $J = 9.0$ Hz, 1H, $H_{1'}$), 6.70 (d, $J = 3.5$ Hz, 1H), 6.74-6.78 (m, 2H), 7.08–7.13 (m, 2H), 7.14–7.19 (m, 1H), 7.24–7.27 (m, 2H), 7.31–7.40 (m, 14H), 7.48–7.54 (m, 3H), 7.72–7.77 (m, 3H), 7.80 (d, $J = 8.5$ Hz, 1H), 7.93 (d, $J = 2.0$ Hz, 1H). ^{13}C (100 MHz, DMSO-d_6): 68.7, 72.3, 73.4, 74.1, 74.6 ($CH_{2\ sugar\ +\ benzyl}$), 76.3, 77.6, 80.2, 84.0, 84.6 (CH_{sugar}), 103.1, 111.6, 118.7, 121.0, 125.8–128.8 (CH), 129.1, 132.5, 135.5, 137.5, 138.1 (2C), 138.5, 141.4 (C).

4.1.6. Oxidation of the protected glycosyl-indole to the corresponding isatine derivatives

Chromium oxide (5.89 mmol) was added slowly to a solution of indole derivative (0.45 mmol) in acetone (1 ml), acetic acid (5 ml) and water (1.55 ml). The reaction mixture was stirred at room temperature for 2 hours before hydrolysis and extraction with CH_2Cl_2. The organic phases were washed with water and saturated aqueous NaCl until neutral pH. After drying over $MgSO_4$, the solvent was removed and the residue purified by flash chromatography (eluent cyclohexane/EtOAc 80:20) to give the corresponding isatines.

1-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-indolin-2,3-dione **15**: Obtained in 62% yield as a yellow gum, IR (NaCl) $\upsilon_{C=C}$ 1610 cm^{-1}, $\upsilon_{C=O}$ 1740 cm^{-1}, HRMS (ES) $[M + Na]^+$ Calcd. for $C_{42}H_{39}NNaO_7$ 692.2618; Found: 692.2633; ^{1}H NMR (400 MHz, DMSO-d_6): 3.71 (d, $J = 3.0$ Hz, 2H), 3.80 (m, 1H), 3.89 (dt, $J_1 = 9.5$ Hz, $J_2 = 3.5$ Hz, 1H), 3.96 (t,

J = 8.5 Hz, 1H), 4.11 (br s, 1H), 4.36 (d, J = 12.0 Hz, 1H), 4.50 (d, J = 3.0 Hz, 2H), 4.64 (d, J = 11.0 Hz, 1H), 4.69 (d, J = 11.5 Hz, 1H), 4.81 (d, J = 11.0 Hz, 1H), 4.90 (s, 2H), 5.54 (d, J = 9.0 Hz, 1H, $H_{1'}$), 6.95 (d, J = 7.0 Hz, 2H), 7.04–7.13 (m, 3H), 7.20 (t, J = 8.0 Hz, 1H), 7.25 (dd, J_1 = 7.5 Hz, J_2 = 2.5 Hz, 1H), 7.29–7.40 (m, 15H), 7.55 (d, J = 7.5 Hz, 1H), 7.60 (t, J = 8.0 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): 68.4, 72.2, 73.9, 74.2, 74.7 ($CH_{2\ sugar\ +\ benzyl}$), 76.3, 76.5, 77.3, 80.0, 84.9 (CH_{sugar}), 123.7, 124.8, 127.4-128.3, 138.2 (CH), 117.5, 137.6, 138.0, 138.1 (2C), 138.4 (C), 157.4, 182.0 (C=O).

1-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-5-nitroindolin-2,3-dione **16**: $C_{42}H_{38}N_2O_9$ obtained in 66% yield as a yellow gum, IR (NaCl): $\nu_{C=O}$ 1753 cm^{-1}; $\nu_{C=C}$ 1615 cm^{-1}. ^{1}H (400 MHz, DMSO-d_6): 3.68–3.78 (m, 2H); 3.88 (t; J = 9.0 Hz, 1H), 3.93–3.99 (m, 1H), 4.00–4.14 (m, 2H), 4.41 (d, J = 12.0 Hz, 1H), 4.49 (d, J = 12.5 Hz, 1H), 4.53 (d, J = 12.5 Hz, 1H), 4.66 (d, J = 11.0 Hz, 1H), 4.72 (d, J = 12.0 Hz, 1H), 4.86 (d, J = 11.0 Hz, 1H), 4.94 (s, 2H), 5.63 (d, J = 9.0 Hz, 1H, $H_{1'}$), 6.94–7.07 (m, 5H), 7.27–7.45 (m, 15H), 7.53 (d, J = 9.0 Hz, 1H), 8.16 (d, J = 2.5 Hz, 1H), 8.32 (dd, J_1 = 9.0 Hz, J_2 = 1.5 Hz, 1H). ^{13}C (100 MHz, $CDCl_3$): 68.0, 73.3, 74.5, 75.2, 76.0 ($CH_{2\ sugar\ +\ benzyl}$), 75.6, 76.8, 77.2, 80.8, 86.0 (CH_{sugar}), 113.9, 120.4, 127.4–128.6, 132.3 (CH), 117.2, 137.3, 137.7, 137.8 (2C), 143.7, 152.1 (C), 156.8, 180.4 (C=O).

1-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-5-bromoindolin-2,3-dione **17**: $C_{42}H_{38}BrNO_7$ obtained in 49% yield as yellow gum, IR (NaCl): $\nu_{C=O}$ 1747 cm^{-1}; $\nu_{C=C}$ 1606 cm^{-1}. ^{1}H (400 MHz, DMSO-d_6): 3.66–3.74 (m, 2H), 3.81 (t, J = 9.0 Hz, 1H), 3.87–3.93 (m, 1H), 3.98 (t, J = 9.0 Hz, 1H), 4.02–4.10 (m, 1H), 4.39 (d, J = 12.0 Hz, 1H), 4.48 (d, J = 12.5 Hz, 1H), 4.52 (d, J = 13.0 Hz, 1H), 4.63 (d, J = 11.0 Hz, 1H), 4.69 (d, J = 12.5 Hz, 1H), 4.83 (d, J = 10.5 Hz, 1H), 4.91 (s, 2H), 5.53 (d, J = 9.5 Hz, 1H, $H_{1'}$), 6.97 (d, J = 7.0 Hz, 2H), 7.05 (t, J = 7.0 Hz, 2H), 7.09–7.14 (m, 1H), 7.23–7.43 (m, 16H), 7.65–7.70 (m, 2H). ^{13}C (100 MHz, DMSO-d_6): 68.4, 72.2, 73.8, 74.2, 74.8 ($CH_{2\ sugar\ +\ benzyl}$), 76.1, 76.2, 77.3, 79.9, 85.0 (CH_{sugar}), 127.0, 127.4–128.3, 139.5 (CH), 115.6, 119.2, 137.6, 138.0, 138.1, 138.3, 146.9 (C), 156.9, 180.7 (C=O).

1-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-5-phenylindolin-2,3-dione **18**: $C_{48}H_{43}NO_7$ obtained in 22% yield as a yellow gum, IR (NaCl): $\nu_{C=O}$ 1741, 1694 cm^{-1}; $\nu_{C=C}$ 1610 cm^{-1}. ^{1}H (400 MHz, DMSO-d_6): 3.68–3.76 (m, 2H), 3.84 (t, J = 9.5 Hz, 1H), 3.93 (dt, J_1 = 9.5 Hz, J_2 = 3.0 Hz, 1H), 4.00 (t, J = 9.0 Hz, 1H), 4.07–4.18 (br s, 1H), 4.42 (d, J = 11.5 Hz, 1H), 4.51 (d, J = 12.5 Hz, 1H), 4.54 (d, J = 12.5 Hz, 1H), 4.65 (d, J = 11.0 Hz, 1H), 4.71 (d, J = 12.0 Hz, 1H), 4.84 (d, J = 11.0 Hz, 1H), 4.92 (s, 2H), 5.58 (d, J = 9.0 Hz, 1H, $H_{1'}$), 6.98 (d, J = 7.0 Hz, 2H), 7.04 (t, J = 7.5 Hz, 2H), 7.07–7.13 (m, 1H), 7.25–7.29 (m, 2H), 7.31–7.47 (m, 15H), 7.54 (t, J = 7.5 Hz, 2H), 7.72 (d, J = 7.5 Hz, 2H), 7.77 (d, J = 1.5 Hz, 1H), 7.83 (d, J = 7.5 Hz, 1H). ^{13}C (100 MHz, $CDCl_3$): 68.3, 73.5, 75.0, 75.4, 76.2 ($CH_{2\ sugar\ +\ benzyl}$), 76.4, 77.4, 77.5, 81.0, 86.4 (CH_{sugar}), 114.1, 123.8, 126.6, 127.6–128.7, 129.2, 136.3 (CH), 118.2, 137.2, 137.5, 138.0, 138.2, 138.3, 139.2, 147.5 (C), 157.7, 182.7 (C=O).

4.1.7. Typical procedure for the acid-catalyzed coupling reaction

A solution of glycosyl-isatine (0.19 mmol) and oxindole (0.19 mmol) in a mixture of acetic acid (0.4 ml) and concentrated HCl (2.6 μl) was refluxed for 24 hours. After cooling, EtOAc was added to the mixture, the organic phases were washed twice with H_2O, dried over $MgSO_4$ and concentrated under vacuum to give a residue which was purified by flash chromatography (eluent cyclohexane/EtOAc 80:20) to give the corresponding protected glycosyl-isoindigo derivatives.

1-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-isoindigo **19**: Obtained in 40% yield as a red solid (m.p. = 205 °C), IR (KBr): ν_{NH} 3410 cm^{-1}, $\nu_{C=O}$ 1700 cm^{-1}, $\nu_{C=C}$ 1600 cm^{-1}. HRMS (ES) Calcd. for $C_{50}H_{45}N_2O_7$ $[M + H]^+$: 785.3227; Found: 785.3224. ^{1}H (400 MHz, DMSO-d_6): 3.75 (d, J = 2.5 Hz, 2H), 3.80–3.87 (m, 1H), 3.88–3.94 (m, 1H), 3.96 (t, J = 9.0 Hz, 1H), 4.10–4.28 (m, 2H), 4.54 (d, J = 12.0 Hz, 1H), 4.57 (d, J = 12.5 Hz, 1H), 4.57–4.63 (m, 1H), 4.65 (d, J = 11.0 Hz, 1H), 4.84 (d, J = 11.0 Hz, 1H), 4.89 (s, 2H), 5.73 (br s, 1H, $H_{1'}$), 6.90–6.95 (m, 3H), 6.98 (t, J = 8.0 Hz, 2H), 7.01–7.10 (m, 2H), 7.13–7.18 (m, 1H), 7.27–7.47 (m, 18H), 8.97 (d, J = 8.0 Hz, 1H), 9.15 (d, J = 8.0 Hz, 1H), 11.0 (s, 1H, NH). ^{13}C (100 MHz, DMSO-d_6): 68.5, 72.2, 73.9, 74.2, 74.6 ($CH_{2\ sugar\ +\ benzyl}$), 76.5, 76.8, 77.5, 80.1, 84.8 (CH_{sugar}), 109.7, 121.2, 122.2, 127.4–128.2, 129.0, 129.3, 132.3, 133.2 (CH), 120.9, 121.6, 131.3, 134.6, 137.4, 138.0 (2C), 138.2, 138.5, 144.5 (C), 168.6 (2 C=O).

1-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-5-nitroisoindigo **20**: Obtained in 25% yield as a red gum, IR (NaCl): ν_{NH} 3409 cm^{-1}, $\nu_{C=O}$ 1702 cm^{-1}, $\nu_{C=C}$ 1615 cm^{-1}. HRMS (ES) Calcd. for $C_{50}H_{43}N_3NaO_9$ $(M + Na)^+$: 852.2897; Found: 852.2924. ^{1}H (400 MHz, DMSO-d_6): 3.72–3.76 (m, 2H), 3.84–3.96 (m, 2H), 4.00 (t, J = 9.5 Hz, 1H), 4.22–4.29 (m, 1H), 4.50 (d, J = 12.0 Hz, 1H), 4.55 (d, J = 12.0 Hz, 1H), 4.66 (d, J = 10.5 Hz, 1H), 4.62–4.69 (m, 1H), 4.85 (d, J = 10.5 Hz, 1H), 4.92 (s, 2H), 5.76 (d, J = 9.0 Hz, 1H, $H_{1'}$), 6.84–6.90 (m, 4H), 6.94–6.98 (m, 2H), 7.04–7.10 (m, 1H), 7.26–7.45 (m, 15H), 7.47–7.53 (m, 2H), 8.15–8.23 (m, 1H), 9.00 (d, J = 8.0 Hz, 1H), 10.11 (d, J = 2.5 Hz, 1H), 11.10 (s, 1H, NH). The signal of one proton of the sugar moiety is missing. ^{13}C (100 MHz, DMSO-d_6): 68.4, 72.2, 73.8, 74.1, 74.6 ($CH_{2\ sugar\ +\ benzyl}$), 76.5, 76.6, 77.3, 80.1, 85.0 (CH_{sugar}), 120.9, 121.2, 128.6, 137.2, 137.3, 138.0, 138.2, 138.3, 142.3, 145.2, 145.9 (C), 110.0, 121.5, 124.3, 127.2–128.3, 129.8, 134.3 (CH), 166.8, 168.6 (C=O).

1-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-5-bromoisoindigo **21**: Obtained in 43% yield as a red gum, IR (NaCl): ν_{NH} 3214 cm^{-1}; $\nu_{C=O}$ 1696, 1661 cm^{-1}; $\nu_{C=C}$ 1606 cm^{-1}. HRMS (ES) Calcd. for $C_{50}H_{43}BrN_2NaO_7$ $(M + Na)^+$: 885.2151/887.2131; Found: 885.2130/887.2128. ^{1}H (400 MHz, DMSO-d_6): 3.70–3.76 (m, 2H), 3.79–3.86 (m, 1H), 3.87–3.93 (m, 1H), 3.96 (t, J = 8.5 Hz, 1H), 4.03–4.26 (m, 2H), 4.51 (d, J = 12.0 Hz, 1H), 4.55 (d, J = 12.0 Hz,

1H), 4.59–4.67 (m, 2H), 4.84 (d, $J = 11.0$ Hz, 1H), 4.90 (s, 2H), 5.70 (br s, 1H, $H_{1'}$), 6.88–6.99 (m, 5H), 7.00–7.07 (m, 2H), 7.25–7.56 (m, 18H), 8.98 (d, $J = 8.5$ Hz, 1H), 9.36 (d, $J = 2.0$ Hz, 1H), 11.04 (s, 1H, NH). ^{13}C (100 MHz, DMSO-d_6): 68.4, 72.2, 73.9, 74.2, 74.6 ($CH_{2\ sugar\ +\ benzyl}$), 76.5 (2C), 77.4, 80.0, 84.9 (CH_{sugar}), 109.9, 113.3, 121.4, 127.4–128.3, 129.6, 131.1, 133.8, 134.1 (CH), 114.1, 121.3; 122.7, 129.8, 136.1, 137.4, 138.0, 138.2, 138.4, 140.5, 144.8 (C), 166.4, 168.7 (C=O).

1-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-5-phenylisoindigo **22**: Obtained in 60% yield as a red gum, IR (NaCl): ν_{NH} 3410 cm^{-1}; $\nu_{C=O}$ 1780 cm^{-1}; $\nu_{C=C}$ 1610 cm^{-1}. HRMS (ES) Calcd. for $C_{56}H_{48}N_2NaO_7$ $[M + Na]^+$: 883.3359; Found: 883.3333. ^{1}H (400 MHz, DMSO-d_6): 3.72–3.79 (m, 2H), 3.82–3.90 (m, 1H), 3.91–3.96 (m, 1H), 3.99 (t, $J = 8.5$ Hz, 1H), 4.12–4.30 (m, 2H), 4.54 (d, $J = 12.0$ Hz, 1H), 4.58 (d, $J = 12.0$ Hz, 1H), 4.61–4.66 (m, 1H), 4.67 (d, $J = 11.0$ Hz, 1H), 4.85 (d, $J = 10.5$ Hz, 1H), 4.91 (s, 2H), 5.75 (br s, 1H, $H_{1'}$), 6.90–6.99 (m, 5H), 7.01–7.07 (m, 2H), 7.27–7.48 (m, 18H), 7.55 (t, $J = 8.0$ Hz, 2H), 7.62–7.69 (m, 1H), 7.69 (d, $J = 8.0$ Hz, 2H), 9.00 (d, $J = 7.5$ Hz, 1H), 9.51 (d, $J = 2.0$ Hz, 1H), 10.95 (s, 1H, NH). ^{13}C (100 MHz, DMSO-d_6): 68.5, 72.3, 73.8, 74.2, 74.6 ($CH_{2\ sugar\ +\ benzyl}$), 76.6, 76.7, 77.5, 80.1, 84.9 (CH_{sugar}), 109.8, 112.4, 121.2, 126.2, 127.0, 127.3–128.2, 129.0, 129.4, 130.4, 133.3 (CH), 121.5, 121.6, 131.3, 134.1, 135.0, 137.4, 138.0, 138.2, 138.4, 140.1, 140.7, 144.5 (C), 167.0, 168.7 (C=O).

4.1.8. Typical procedure for the deprotection step

BBr_3 (1 M solution in CH_2Cl_2, 1.5 mmol) was added to a cooled (–80 °C) solution of protected glycosyl-isoindigo (0.10 mmol) in CH_2Cl_2 (9.5 ml). The mixture was stirred during 2 hours (compounds **24**–**26**) or 12 hours (compound **23**) before hydrolysis at –80 °C and warming up to room temperature. After decantation and extraction with EtOAc, the organic phases were dried over $MgSO_4$ and concentrated under vacuum to give a residue which was purified by flash chromatography (eluent EtOAc/MeOH from 95:5 to 85:15) to give the corresponding non-protected glycosyl-isoindigo.

1-(β-D-Glucopyranosyl)-isoindigo **23**: Obtained in 60% yield as a red solid (m.p. > 300 °C), IR (KBr): $\nu_{NH,\ OH}$ 3377 cm^{-1}; $\nu_{C=O}$ 1696; 1680 cm^{-1}; $\nu_{C=C}$ 1604 cm^{-1}. HRMS (FAB +) Calcd. for $C_{22}H_{21}N_2O_7$ $[M + H]^+$: 425.1349; Found: 425.1357. ^{1}H (400 MHz, DMSO-d_6): 3.29–3.46 (m, 3H), 3.52–3.59 (m, 1H), 3.78–3.84 (m, 1H), 3.86–4.02 (br s, 1H), 4.68 (t, $J = 5.5$ Hz, 1H, OH), 5.18 (d, $J = 5.0$ Hz, 1H, OH), 5.22 (d, $J = 5.0$ Hz, 1H, OH), 5.40 (d, $J = 5.0$ Hz, 1H, OH), 5.40 (br s, 1H, $H_{1'}$), 6.92 (d, $J = 7.5$ Hz, 1H), 7.04 (t, $J = 8.0$ Hz, 1H), 7.13 (t, $J = 7.5$ Hz, 1H), 7.28 (d, $J = 8.0$ Hz, 1H), 7.42 (t, $J = 7.5$ Hz, 1H), 7.48 (t, $J = 7.5$ Hz, 1H), 9.05 (d, $J = 8.0$ Hz, 1H), 9.19 (d, $J = 7.5$ Hz, 1H), 11.0 (s, 1H, NH). ^{13}C (100 MHz, DMSO-d_6): 61.0 (CH_2), 68.4, 69.8, 77.3, 80.1, 81.9 (CH_{sugar}), 109.7, 111.5, 121.2, 121.7, 128.8, 129.4, 132.3, 132.9 (CH), 121.1, 121.6, 132.1, 134.0, 142.3, 144.3 (C), 167.0, 168.7 (C=O).

1-(β-D-Glucopyranosyl)-5-nitroisoindigo **24**: Obtained in 18% yield as a red solid (m.p. > 300 °C), IR (KBr): $\nu_{NH,\ OH}$ 3424 cm^{-1}; $\nu_{C=O}$ 1720, 1703 cm^{-1}; $\nu_{C=C}$ 1618 cm^{-1}. HRMS (ES) Calcd. for $C_{22}H_{19}N_3NaO_9$ $[M + Na]^+$: 492.1019; Found: 492.1006. ^{1}H (400 MHz, DMSO-d_6): 3.30–3.45 (m, 3H), 3.47–3.58 (m, 1H), 3.80 (dd, 1H, $J_1 = 10.5$ Hz, $J_2 = 5.5$ Hz), 3.76–3.95 (m, 1H), 4.70 (t, $J = 5.5$ Hz, 1H, OH), 5.21 (d, $J = 5.0$ Hz, 1H, OH), 5.23 (d, $J = 4.5$ Hz, 1H, OH), 5.45 (d, $J = 4.5$ Hz, 1H, OH), 5.42 (br s, 1H, $H_{1'}$), 6.94 (d, $J = 7.5$ Hz, 1H), 7.09 (td, $J_1 = 8.0$ Hz, $J_2 = 1.0$ Hz, 1H), 7.47 (td, $J_1 = 7.5$ Hz, $J_2 = 1.0$ Hz, 1H), 7.51 (d, $J = 9.0$ Hz, 1H), 8.38 (dd, $J_1 = 9.0$ Hz, $J_2 = 2.5$ Hz, 1H), 9.05 (d, $J = 8.0$ Hz, 1H), 10.21 (d, $J = 2.5$ Hz, 1H), 11.10 (s, 1H, NH). ^{13}C (100 MHz, DMSO-d_6): 61.0 (CH_2), 68.7, 69.7, 77.0, 80.2, 82.2 (CH_{sugar}), 110.1, 111.7, 121.5, 124.2, 127.6, 130.0, 134.2 (CH), 121.1, 121.2, 129.2, 136.9, 142.1, 145.2, 147.0 (C), 167.1, 168.7 (C=O).

1-(β-D-Glucopyranosyl)-5-bromoisoindigo **25**: Obtained in 59% yield as a red solid (m.p. > 300 °C), IR (KBr): $\nu_{NH,\ OH}$ 3540, 3500–3200 cm^{-1}; $\nu_{C=O}$ 1700, 1680 cm^{-1} ; $\nu_{C=C}$ 1620, 1600 cm^{-1}. HRMS (ES) Calcd. for $C_{22}H_{19}BrN_2NaO_7$ $(M + Na)^+$: 525.0273/527.0253; Found: 525.0261/527.0282. ^{1}H (400 MHz, DMSO-d_6): 3.27–3.45 (m, 3H), 3.49–3.58 (m, 1H), 3.78 (dd, $J_1 = 11.0$ Hz, $J_2 = 5.0$ Hz, 1H), 3.79–3.96 (m, 1H), 4.66 (t, $J = 5.5$ Hz, 1H, OH), 5.17 (d, $J = 5.0$ Hz, 1H, OH), 5.21 (d, $J = 4.0$ Hz, 1H, OH), 5.37 (br s, 1H, $H_{1'}$), 5.40 (d, $J = 5.0$ Hz, 1H, OH), 6.91 (d, $J = 8.0$ Hz, 1H), 7.03 (td, $J_1 = 8.0$ Hz, $J_2 = 1.0$ Hz, 1H), 7.23 (d, $J = 8.5$ Hz, 1H), 7.43 (td, $J_1 = 7.5$ Hz, $J_2 = 1.0$ Hz, 1H), 7.64 (dd, $J_1 = 8.5$ Hz, $J_2 = 2.0$ Hz, 1H), 9.04 (d, $J = 8.0$ Hz, 1H), 9.43 (d, $J = 2.0$ Hz, 1H), 11.04 (s, 1H, NH). ^{13}C (100 MHz, DMSO-d_6): 61.0 (CH_2), 68.4, 69.7, 77.2, 80.1, 82.0 (CH_{sugar}), 109.9, 113.3, 121.3, 129.8, 130.9, 133.6, 134.2 (CH), 113.7, 121.4, 122.9, 130.4, 135.6, 141.2, 144.7 (C), 166.6, 168.7 (C=O).

1-(β-D-Glucopyranosyl)-5-phenylisoindigo **26**: Obtained in 66% yield as a red powder (m.p. > 300 °C), IR (KBr): $\nu_{NH,\ OH}$ 3400 cm^{-1}; $\nu_{C=O}$ 1706, 1683 cm^{-1}; $\nu_{C=C}$ 1616 cm^{-1}. HRMS (ES) Calcd. for $C_{28}H_{24}N_2NaO_7$ $(M + Na)^+$: 523.1481; Found: 523.1497. ^{1}H (400 MHz, DMSO-d_6): 3.30–3.46 (m, 3H), 3.52–3.60 (m, 1H), 3.77–3.84 (m, 1H), 3.88–4.03 (br s, 1H), 4.66 (t, $J = 5.5$ Hz, 1H, OH), 5.15 (d, $J = 5.0$ Hz, 1H, OH), 5.20 (d, $J = 5.0$ Hz, 1H, OH), 5.40 (br s, 1H, $H_{1'}$), 5.40 (d, $J = 5.0$ Hz, 1H, OH), 6.92 (d, $J = 7.5$ Hz, 1H), 7.03 (dt, $J_1 = 8.0$ Hz, $J_2 = 1.0$ Hz, 1H), 7.35 (d, $J = 8.5$ Hz, 1H), 7.38–7.44 (m, 2H), 7.51–7.56 (m, 2H), 7.67–7.71 (m, 2H), 7.77 (dd, $J_1 = 8.5$ Hz, $J_2 = 2.0$ Hz, 1H), 9.05 (d, $J = 7.5$ Hz, 1H), 9.55 (d, $J = 2.0$ Hz, 1H), 10.94 (s, 1H, NH). ^{13}C (100 MHz, DMSO-d_6) : 61.1 ($CH_{2\ sugar}$), 68.6, 69.9, 77.3, 80.1, 82.0 (CH_{sugar}), 109.8, 111.7, 121.2, 126.2 (2C), 127.0, 127.3, 129.0 (2C), 129.5, 130.5, 133.1 (CH), 121.6 (2C), 132.0, 133.9, 134.5, 140.3, 141.3, 144.4 (C), 167.1, 168.8 (C=O).

4-[5-(2-Oxo)-indolyl]-4-oxobutanoic acid **27**: Succinic anhydride (225 mg, 2.25 mmol) was added to a solution of aluminum chloride (600 mg, 4.5 mmol) in CH_2Cl_2 (3.1 ml). The mixture was stirred at room temperature for 15 min before addition of a solution of oxindole (99.9 mg, 0.75 mmol) in

CH_2Cl_2 (2 ml). The mixture was stirred at room temperature for 24 hours before hydrolysis to give a solid which was isolated by filtration and washed with water and CH_2Cl_2. The corresponding oxindole **27** was obtained in 88% yield as a gray solid (m.p. = 252–255 °C; Litt = 246–250 °C [20]), IR (KBr): $\nu_{OH,\ NH}$ 3560; 3300 cm^{-1}; $\nu_{C=O}$ 1730, 1710, 1680 cm^{-1}; $\nu_{C=C}$ 1610 cm^{-1}. HRMS (ES) Calcd. for $C_{12}H_{11}NNaO_4$ $(M + Na)^+$: 256.0586; Found: 256.0594. ^1H (400 MHz, DMSO-d_6): 2.59 (t, J = 6.0 Hz, 2H, CH_2), 3.21 (t, J = 6.0 Hz, 2H, CH_2), 3.59 (s, 2H, CH_2), 6.95 (d, J = 8.0 Hz, 1H), 7.86 (s, 1H), 7.92 (d, J = 8.0 Hz, 1H), 10.80 (s, 1H, NH), 12.15 (s, 1H, OH). ^{13}C (100 MHz, DMSO-d_6): 28.0, 32.6, 35.5 (CH_2), 108.7, 124.1, 129.0 (CH), 126.1, 130.1, 148.3 (C), 173.9, 176.8, 197.0 (C=O).

4-[5′-(1-(2,3,4,6-tetra-*O*-benzyl)-β-D-glucopyranosyl)-isoindigoyl)-4-oxobutanoic acid **28**: Obtained in 88% yield as a red solid (m.p. = 105–106 °C) by reaction of glycosyl-isatine **15** with oxindole **27** using the same conditions as described above. The only difference is that compound **28** precipitated when the reaction mixture was hydrolyzed. This precipitate was isolated by filtration and washed with water. IR (KBr): $\nu_{NH,\ OH}$ 3380, 3260 cm^{-1}; $\nu_{C=O}$ 1740, 1710, 1680 cm^{-1}; $\nu_{C=C}$ 1610 cm^{-1}. HRMS (ES) Calcd. for $C_{54}H_{49}N_2O_{10}$ $(M + H)^+$: 885.3387; Found: 885.3412. ^1H (400 MHz, DMSO-d_6): 2.61 (t, J = 6.0 Hz, 2H, CH_2), 3.18–3.25 (m, 2H, CH_2), 3.72 (d, J = 2.0 Hz, 2H, CH_2), 3.78–3.86 (m, 1H), 3.90–3.95 (m, 1H), 3.99 (t, J = 8.5 Hz, 1H), 4.08–4.24 (m, 2H), 4.47–4.55 (m, 3H), 4.62 (d, J = 11.0 Hz, 1H), 4.80 (d, J = 11.0 Hz, 1H), 4.86 (s, 2H), 5.79 (br s, 1H, $H_{1'}$), 6.86–6.95 (m, 4H), 6.96–7.03 (m, 2H), 7.12 (dt, J_1 = 8.0 Hz, J_2 = 1.5 Hz, 1H), 7.19–7.43 (m, 17H), 8.06 (dd, J_1 = 8.5 Hz, J_2 = 1.5 Hz, 1H), 9.11 (d, J = 8.0 Hz, 1H), 9.71 (s, 1H), 11.43 (s, 1H, NH). ^{13}C (100 MHz, DMSO-d_6): 27.9, 32.7 (CH_2), 68.5, 72.2, 73.9, 74.1, 74.6 ($CH_{2\ sugar\ +\ benzyl}$), 76.4, 76.9, 77.4, 80.0, 84.7 (CH_{sugar}), 109.6, 122.3, 123.7, 124.8, 127.4–128.2, 129.3, 129.8, 132.8, 133.2 (CH), 120.8, 121.3, 130.1, 132.6, 133.4, 137.6, 138.1 (2C), 138.2, 138.5, 148.1 (C), 167.0, 168.9, 173.9, 196.7 (C=O).

4.1.9. Typical procedure for the acetylation of the sugar residue

A solution of indoline (3.55 mmol) in pyridine (10.7 ml) was cooled to 0 °C before addition of acetic anhydride (7.9 ml). The reaction mixture was stirred at room temperature for 24 hours. After addition of water and extraction with EtOAc, the organic phases were washed with water and saturated aqueous $NaHCO_3$ until neutral pH. After drying over $MgSO_4$, the solvent was removed and the residue purified by flash chromatography (eluent cyclohexane/EtOAc from 80:20 to 40:60) to give the corresponding protected compounds.

1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-indoline **29**: $C_{22}H_{27}NO_9$ obtained in 96% yield as a white solid (m.p. = 100 °C), IR (KBr): $\upsilon_{C=O}$ 1780–1730 cm^{-1}; $\nu_{C=C}$ 1610 cm^{-1}. ^1H (400 MHz, DMSO-d_6): 1.95, 1.99, 2.00, 2.03 (4s, 12H, CH_3 acetate), 2.80–2.90 (m, 1H), 2.93–3.04 (m, 1H), 3.42–3.55 (m, 2H), 3.95–4.04 (m, 1H), 4.14 (m, J = 9.0 Hz, 2H), 4.97 (t, J = 9.0 Hz, 1H), 5.19 (t, J = 9.0 Hz, 1H), 5.46 (t, J = 9.0 Hz, 1H), 5.54 (d, J = 9.0 Hz, 1H, $H_{1'}$), 6.70 (t, J = 7.0 Hz, 1H), 6.80 (d, J = 7.0 Hz, 1H), 7.05–7.12 (m, 2H). ^{13}C (100 MHz, DMSO-d_6): 20.3, 20.4 (2C), 20.7 (CH_3 acetate), 27.5, 45.1 (CH_2 indoline), 61.9 (CH_2 sugar), 68.4, 68.6, 71.8, 73.1, 82.4 (CH_{sugar}), 108.1, 119.0, 124.6, 127.0 (CH), 129.9, 149.7 (C), 169.3, 169.4, 169.6, 170.0 (C=$O_{acetate}$).

1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-5-nitroindoline **30**: $C_{22}H_{26}N_2O_{11}$ obtained in 78% yield as a yellow solid (m.p. = 158 °C), IR (KBr): $\nu_{C=O}$ 1750 cm^{-1}; $\nu_{C=C}$ 1606 cm^{-1}. ^1H (400 MHz, DMSO-d_6): 1.94, 2.00, 2.04 (3 s, 12H, CH_3 acetate), 2.95-3.06 (m, 1H), 3.08–3.18 (m, 1H), 3.63–3.73 (m, 2H), 4.03–4.20 (m, 3H), 5.03 (t, J = 9.5 Hz, 1H), 5.25 (t, J = 9.5 Hz, 1H), 5.46 (t, J = 9.5 Hz, 1H), 5.67 (d, J = 9.5 Hz, 1H, $H_{1'}$), 6.89 (d, J = 9.0 Hz, 1H), 7.95 (d, J = 2.5 Hz, 1H), 8.09 (dd, J_1 = 9.0 Hz, J_2 = 2.5 Hz, 1H). ^{13}C (100 MHz, DMSO-d_6): 20.4 (2C), 20.5, 20.6 (CH_3 acetate), 26.5, 46.2 (CH_2 indoline), 61.9 (CH_2 sugar), 68.1, 68.4, 72.4, 72.8, 81.6 (CH_{sugar}), 106.4, 120.8, 125.6 (CH), 131.1, 139.1, 155.8, (C), 169.5–170.2 (C=$O_{acetate}$).

1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-5-bromoindoline **31**: $C_{22}H_{26}BrNO_9$ obtained in 78% yield as a yellow solid (m.p. = 142 °C), IR (KBr): $\nu_{C=O}$ 1741 cm^{-1}; $\nu_{C=C}$ 1597 cm^{-1}. ^1H (400 MHz, DMSO-d_6): 1.98, 1.99, 2.00, 2.04 (4s, 12H, CH_3 acetate), 2.83–2.94 (m, 1H), 2.96–3.05 (m, 1H), 3.45–3.56 (m, 2H), 3.97–4.04 (m, 1H), 4.09–4.16 (m, 2H), 4.98 (t, J = 9.5 Hz, 1H), 5.18 (t, J = 9.5 Hz, 1H), 5.44 (t, J = 9.5 Hz, 1H), 5.49 (d, J = 9.5 Hz, 1H, $H_{1'}$), 6.74 (d, J = 8.5 Hz, 1H), 7.23 (dd, J_1 = 8.5 Hz, J_2 = 2.0 Hz, 1H), 7.25 (d, J = 2.0 Hz, 1H). ^{13}C (100 MHz, DMSO-d_6): 20.2, 20.3 (2C), 20.4 (CH_3 acetate), 27.2, 45.4 (CH_2 indoline), 61.8 (CH_2 sugar), 68.2, 68.4, 71.8, 72.9, 82.1 (CH_{sugar}), 109.7, 127.4, 129.4 (CH), 109.7, 132.8, 149.1, (C), 169.2, 169.3, 169.5, 169.9 (C=$O_{acetate}$).

*4.1.10. Aromatization of compounds **29–31***

The aromatization of compounds **29–31** was performed using the same procedure as described before for the preparation of compounds **7–10**. The reaction mixture was stirred at room temperature during 12 hours for compound **32** and 24 hours for compound **34**. The aromatization was carried out at 100 °C during 3 days for compound **33**.

1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-indole **32**: $C_{22}H_{25}NO_9$ obtained in 98% yield as a white solid (m.p. = 140 °C), IR (KBr): $\upsilon_{C=O}$ 1790–1720 cm^{-1}; $\nu_{C=C}$ 1650, 1610 cm^{-1}. ^1H (400 MHz, DMSO-d_6): 1.67, 2.00, 2.02, 2.08 (4s, 12H, CH_3 acetate), 4.10–4.20 (m, 2H), 4.32–4.38 (m, 1H), 5.27 (t, J = 10.0 Hz, 1H), 5.58 (t, J = 9.5 Hz, 1H), 5.65 (t, J = 9.5 Hz, 1H), 6.26 (d, J = 9.0 Hz, 1H, $H_{1'}$), 6.55 (d, J = 3.5 Hz, 1H), 7.12 (dt, J_1 = 8.0 Hz, J_2 = 1.0 Hz, 1H), 7.24 (dt, J_1 = 8.0 Hz, J_2 = 1.0 Hz, 1H), 7.50 (d, J = 3.5 Hz, 1H), 7.58 (d, J = 8.0 Hz, 1H), 7.68 (d, J = 8.5 Hz, 1H). ^{13}C (100 MHz, DMSO-d_6): 19.8, 20.2, 20.4, 20.5 (CH_3 acetate), 62.1 (CH_2 sugar), 68.1, 69.9, 72.7, 73.0, 81.3 (CH_{sugar}), 103.2, 110.4, 120.2, 120.6, 121.8, 125.7 (CH), 128.4, 136.0 (C), 168.4, 169.4, 169.6, 170.1 (C=$O_{acetate}$).

1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-5-nitroindole **33**: $C_{22}H_{24}N_2O_{11}$ obtained in 98% yield as a yellow solid (m. p. = 163 °C), IR (KBr): $\nu_{C=O}$ 1749 cm^{-1}; $\nu_{C=C}$ 1616 cm^{-1}. ^1H (400 MHz, DMSO-d_6): 1.67, 2.01, 2.04, 2.09 (4s, 12H, $CH_{3\ acetate}$), 4.13–4.23 (m, 2H), 4.33–4.39 (m, 1H), 5.34 (t, J = 9.5 Hz, 1H), 5.58 (t, J = 9.5 Hz, 1H), 5.66 (t, J = 9.5 Hz, 1H), 6.36 (d, J = 9.0 Hz, 1H, $H_{1'}$), 6.86 (d, J = 3.5 Hz, 1H), 7.81 (d, J = 3.5 Hz, 1H), 7.92 (d, J = 9.5 Hz, 1H), 8.16 (dd, J_1 = 9.0 Hz, J_2 = 2.5 Hz, 1H), 8.61 (d, J = 2.5 Hz, 1H). ^{13}C (100 MHz, DMSO-d_6): 19.7, 20.2, 20.4, 20.5 ($CH_{3\ acetate}$), 62.0 ($CH_{2\ sugar}$), 67.9, 70.0, 72.4, 73.3, 81.8 (CH_{sugar}), 105.5, 111.1, 117.2, 117.6, 129.6 (CH), 127.9, 138.7, 141.6 (C), 168.4, 169.4, 169.6, 170.0 ($C{=}O_{acetate}$).

1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-5-bromoindole **34**: $C_{22}H_{24}BrNO_9$ obtained in 90% yield as a white solid (m.p. = 168 °C), IR (KBr): $\upsilon_{C=O}$ 1740 cm^{-1}. (400 MHz, DMSO-d_6): 1.68, 2.00, 2.03, 2.08 (4s, 12H, $CH_{3\ acetate}$); 4.10–4.21 (m, 2H), 4.30–4.35 (m, 1H), 5.29 (t, J = 10.0 Hz, 1H), 5.55 (t, J = 9.5 Hz, 1H), 5.63 (t, J = 9.0 Hz, 1H), 6.23 (d, J = 9.0 Hz, 1H, $H_{1'}$), 6.54 (d, J = 3.5 Hz, 1H), 7.37 (dd, J_1 = 9.0 Hz, J_2 = 2.0 Hz, 1H), 7.57 (d, J = 3.5 Hz, 1H), 7.69 (d, J = 9.0 Hz, 1H), 7.79 (d, J = 2.0 Hz, 1H). ^{13}C (100 MHz, DMSO-d_6): 19.8, 20.2, 20.4, 20.5 ($CH_{3\ acetate}$), 62.0 ($CH_{2\ sugar}$), 67.9, 69.8, 72.5, 73.1, 81.6 (CH_{sugar}), 102.7, 112.6, 122.9, 124.4, 127.5 (CH), 112.7, 130.3, 134.6 (C), 168.4, 169.4, 169.6, 170.0 ($C{=}O_{acetate}$).

*4.1.11. Oxidation of compounds **32–34***

Oxidation of compounds **32–34** using chromium oxide was performed according to the procedure described before.

1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-indoline-2,3-dione **35**: Obtained in 88% yield as a yellow gum, IR (NaCl): $\upsilon_{C=O}$ 1750 cm^{-1}; $\upsilon_{C=C}$ 1612 cm^{-1}. MS (IE): Calcd. for $C_{22}H_{24}NO_{11}$ ($M + H^+$): 478; Found: 478. HRMS (ES) $[M + Na]^+$ Calcd. for $C_{22}H_{23}NNaO_{11}$, 500.1163; Found: 500.1170, ^1H (400 MHz, $CDCl_3$): 1.92, 2.03, 2.09, 2.11 (4s, 12H, $CH_{3\ acetate}$), 3.96 (ddd, , J_1 = 10.0 Hz, J_2 = 4.5 Hz, J_3 = 2.0 Hz, 1H), 4.20 (dd, J_1 = 12.5 Hz, J_2 = 2.0 Hz, 1H), 4.28 (dd, J_1 = 12.5 Hz, J_2 = 4.5 Hz, 1H), 5.26 (t, J = 10.0 Hz, 1H), 5.41 (t, J = 9.5 Hz, 1H), 5.57 (t, J = 9.5 Hz, 1H), 5.70 (d, J = 9.5 Hz, 1H, $H_{1'}$), 7.22 (t, J = 8.0 Hz, 1H), 7.30 (d, J = 8.0 Hz, 1H), 7.63–7.68 (m, 2H). ^{13}C (100 MHz, $CDCl_3$): 20.3, 20.6, 20.7, 20.8 (CH_3 acetate), 61.7 ($CH_{2\ sugar}$), 67.7, 67.8, 73.0, 74.9, 79.9 (CH_{sugar}), 113.6, 124.7, 125.8, 138.7 (CH), 117.9, 148.0 (C), 157.6, 181.7 ($C{=}O_{isatine}$), 169.5, 169.6, 169.8, 170.5 ($C{=}O_{acetate}$).

1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-5-nitroindoline-2,3-dione **36**: $C_{22}H_{22}N_2O_{13}$ obtained in 57% yield as a yellow solid (m.p. = 60 °C), IR (KBr): $\nu_{C=O}$ 1754 cm^{-1}; $\nu_{C=C}$ 1616 cm^{-1}. ^1H (400 MHz, $CDCl_3$): 1.93, 2.04, 2.10, 2.12 (4s, 12H, $CH_{3\ acetate}$), 3.99 (ddd, J_1 = 10.0 Hz, J_2 = 4.5 Hz, J_3 = 2.0 Hz,1H), 4.20 (dd, J_1 = 12.5 Hz, J_2 = 2.0 Hz, 1H), 4.32 (dd, J_1 = 12.5 Hz, J_2 = 4.5 Hz, 1H), 5.28 (t, J = 10.0 Hz, 1H), 5.42-5.52 (m, 2H), 5.73 (d, J = 9.0 Hz, 1H, $H_{1'}$), 7.50 (d, J = 9.0 Hz, 1H), 8.53 (d, J = 2.5 Hz, 1H), 8.58 (dd, J_1 = 9.0 Hz, J_2 = 2.5 Hz, 1H). ^{13}C (100 MHz, $CDCl_3$): 20.3, 20.6 (2C), 20.8 ($CH_{3\ acetate}$), 61.5 ($CH_{2\ sugar}$), 67.6, 68.0, 72.5, 75.2, 80.2 (CH_{sugar}), 114.2, 121.3, 133.4 (CH), 117.7, 144.7, 152.1 (C), 156.9, 179.8 ($C{=}O_{isatine}$), 169.6, 169.7, 169.9, 170.3 ($C{=}O_{acetate}$).

1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-5-bromoindoline-2,3-dione **37**: $C_{22}H_{22}BrNO_{11}$ obtained in 75% yield as a yellow gum, IR (NaCl): $\nu_{C=O}$ 1752 cm^{-1}; $\nu_{C=C}$ 1607 cm^{-1}. ^1H (400 MHz, $CDCl_3$): 1.91, 2.01, 2.07, 2.09 (4 s, 12H, $CH_{3\ acetate}$), 3.94 (ddd, , J_1 = 10.0 Hz, J_2 = 4.5 Hz, J_3 = 2.0 Hz,1H), 4.17 (dd, J_1 = 12.5 Hz, J_2 = 2.5 Hz, 1H), 4.27 (dd, J_1 = 12.5 Hz, J_2 = 4.5 Hz, 1H), 5.23 (t, J = 9.5 Hz, 1H), 5.40 (t, J = 9.5 Hz, 1H), 5.48 (t, J = 9.0 Hz, 1H), 5.67 (d, J = 9.5 Hz, 1H, $H_{1'}$), 7.20 (dd, J_1 = 8.0 Hz, J_2 = 1.0 Hz, 1H), 7.74 (dd, J_1 = 8.0 Hz, J_2 = 2.5 Hz, 1H), 7.75 (d, J = 2.0 Hz, 1H). ^{13}C (100 MHz, $CDCl_3$): 20.3, 20.6 (2C), 20.7 ($CH_{3\ acetate}$), 61.6 ($CH_{2\ sugar}$), 67.7 (2C), 72.8, 74.9, 79.9 (CH_{sugar}), 115.4, 128.5, 140.9 (CH), 117.8, 119.1, 146.8 (C), 156.8, 180.5 ($C{=}O_{isatine}$), 169.6, 169.7, 169.8, 170.4 ($C{=}O_{acetate}$).

*4.1.12. General procedure for the preparation of the acetylated glycosyl-isoindigos **38–40***

To a solution of acetylated glycosyl-isatine (0.17 mmol) in toluene (9 ml) were added successively, 4 Å molecular sieves, oxindole (0.17 mmol) and PTSA (0.03 mmol). The mixture was refluxed for 24 hours. After cooling, EtOAc was added, the organic phase was washed with saturated aqueous $NaHCO_3$. After drying over $MgSO_4$, the solvent was removed and the residue purified by flash chromatography (eluent cyclohexane/EtOAc 50: 50) to give a red solid which was crystallized in a mixture of acetic acid, toluene and water. The acetylated glycosyl-isoindigos were isolated by filtration. The chemical yields for this coupling reaction were not optimized.

1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-isoindigo **38**: Obtained in 15% yield as a red solid (m.p. = 277 °C), IR (KBr): υ_{NH} 3446 cm^{-1}; $\upsilon_{C=O}$ 1742, 1699 cm^{-1}; $\upsilon_{C=C}$ 1618 cm^{-1}. HRMS (ES) Calcd. for $C_{30}H_{28}N_2NaO_{11}$ ($M + Na)^+$: 615.1591; Found: 615.1616. ^1H (400 MHz, $CDCl_3$): 1.85, 2.02, 2.09, 2.10 (4s, 12H, $CH_{3\ acetate}$), 3.92–3.99 (m, 1H), 4.19–4.31 (m, 2H), 5.30 (t, J = 10.0 Hz, 1H), 5.42 (t, J = 9.5 Hz, 1H), 5.70-5.78 (m, 1H), 5.86 (br s, 1H, $H_{1'}$), 6.81 (d, J = 7.5 Hz, 1H), 7.05 (dt, J_1 = 8.0 Hz, J_2 = 1.0 Hz, 1H), 7.12 (t, J = 8.0 Hz, 1H), 7.19 (d, J = 7.5 Hz, 1H), 7.34 (dt, J_1 = 7.5 Hz, J_2 = 1.0 Hz, 1H), 7.40 (t, J = 7.5 Hz, 1H), 7.57 (s, 1H, NH), 9.03 (d, J = 8.0 Hz, 1H), 9.14 (d, J = 8.0 Hz, 1H). ^{13}C (100 MHz, $CDCl_3$): 20.4, 20.7 (2C), 20.8 ($CH_{3\ acetate}$), 61.9 ($CH_{2\ sugar}$), 67.7, 68.1, 73.7, 74.9, 79.7 (CH_{sugar}), 121.7, 122.5, 132.3, 134.2, 141.3, 142.7 (C), 109.5, 111.0, 122.5, 123.2, 129.8, 130.1, 132.7, 133.0 (CH), 167.7, 169.0, 169.2, 169.6, 170.1, 170.6 (C=O).

1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-5-nitroisoindigo **39**: Obtained in 48% yield as a red solid (m.p. >300 °C), IR (KBr): ν_{NH} 3463 cm^{-1}; $\nu_{C=O}$ 1749 cm^{-1}; $\nu_{C=C}$ 1614 cm^{-1}. HRMS (ES) Calcd. for $C_{30}H_{27}N_3NaO_{13}$ ($M + Na)^+$: 660.1442; Found: 660.1467. ^1H (400 MHz, DMSO-d_6): 1.82, 2.01, 2.08, 2.09 (4s, 12H, $CH_{3\ acetate}$), 4.14–4.24 (m, 2H), 4.39–4.49 (m,

1H), 5.39–5.49 (m, 1H), 5.55–5.72 (m, 2H), 6.22 (br s, 1H, $H_{1'}$), 6.93 (d, $J = 7.5$ Hz, 1H), 7.06 (t, $J = 7.5$ Hz, 1H), 7.47 (t, $J = 7.5$ Hz, 1H), 7.83–7.90 (m, 1H), 8.33–8.40 (m, 1H), 8.98 (d, $J = 8.0$ Hz, 1H), 10.19 (d, $J = 2.0$ Hz, 1H), 11.12 (s, 1H, NH). ^{13}C (100 MHz, DMSO-d_6): 19.9, 20.2, 20.4, 20.5 ($CH_{3\ acetate}$), 61.8 ($CH_{2\ sugar}$), 67.4, 67.7, 72.2, 73.3, 78.3 (CH_{sugar}), 121.1, 121.2, 128.0, 137.7, 142.6, 145.4, 145.6 (C), 110.2, 112.3, 121.6, 124.1, 127.5, 129.8, 134.5 (CH), 166.8, 168.6, 169.0, 169.3, 169.5, 170.1 (C=O).

1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-5-bromoisoindigo **40**: Obtained in 13% yield as a red solid (m.p. = 285 °C), IR (KBr): ν_{NH} 3425 cm^{-1}; $\nu_{C=O}$ 1747, 1699 cm^{-1}; $\upsilon_{C=C}$ 1618 cm^{-1}. HRMS (ES) Calcd. for $C_{30}H_{27}BrN_2NaO_{11}$ $(M + Na)^+$: 693.0696/695.0675; Found: 693.0714/695.0713. ^1H (400 MHz, $CDCl_3$): 1.86, 2.02, 2.09, 2.10 (4s, 12H, $CH_{3\ acetate}$), 3.93–3.99 (m, 1H), 4.21 (dd, $J_1 = 12.5$ Hz, $J_2 = 2.5$ Hz, 1H), 4.27 (dd, $J_1 = 12.5$ Hz, $J_2 = 4.0$ Hz, 1H), 5.28 (t, $J = 9.5$ Hz, 1H), 5.42 (t, $J = 9.5$ Hz, 1H), 5.63–5.70 (m, 1H), 5.84 (br s, 1H, $H_{1'}$), 6.83 (d, $J = 7.5$ Hz, 1H), 7.03–7.11 (m, 2H), 7.36 (t, $J = 7.5$ Hz, 1H), 7.52 (dd, $J_1 = 8.5$ Hz, $J_2 = 2.0$ Hz, 1H), 7.64 (s, 1H, NH), 9.03 (d, $J = 8.5$ Hz, 1H), 9.39 (d, $J = 2.0$ Hz, 1H). ^{13}C (100 MHz, $CDCl_3$): 20.4, 20.7 (2C), 20.8 ($CH_{3\ acetate}$), 61.8 ($CH_{2\ sugar}$), 67.7, 68.0, 73.4, 74.9, 79.7 (CH_{sugar}), 116.2, 122.2, 123.3, 130.7, 135.6, 140.1, 143.2 (C), 109.8, 112.6, 122.7, 130.5, 132.4, 133.7, 135.0 (CH), 167.2, 169.0, 169.2, 169.6, 170.0, 170.6 (C=O).

4.2. Antiproliferative activities

4.2.1. Cell cultures

Stock cell cultures were maintained as monolayers in 75-cm^2 culture flasks in Glutamax Eagle's minimum essential medium (MEM) with Earle's salts supplemented with 10% fetal calf serum, 5 ml 100 mM sodium pyruvate, 5 ml of 100× non-essential amino acids and 2 mg gentamicin base. Cells were grown at 37 °C in a humidified incubator under an atmosphere containing 5% CO_2.

4.2.2. Survival assays

Cells were plated at a density of 5×10^3 cells in 190 µl culture medium in each well of 96-well microplates and were allowed to adhere for 16 h before treatment with tested drug. A stock solution 20 mM of each tested drug was prepared in DMSO and kept at –20 °C until use. Then 50 µl of each tested solution were added to the cultures. A 48 h continuous drug exposure protocol was used. The antiproliferative effect of the tested drug was assessed by both the RRT and determination of DNA cellular content after cell lysis.

4.2.3. RRT

Plates were rinsed with 200 µl PBS at 37 °C and emptied by overturning on absorbent toweling. Then 150 µl of a 25 µg ml^{-1} solution of resazurin in MEM without phenol red was added to each well. Plates were incubated for 1 h at 37 °C in a humidified atmosphere containing 5% CO_2. Fluorescence was then measured on an automated 96-well plate reader (Fluoroscan Ascent FL, Labsystem) using an excitation wavelength of 530 nm and an emission wavelength of 590 nm. Under the conditions used, fluorescence was proportional to the number of living cells in the well. The IC_{50}, defined as the drug concentration required to inhibit cell proliferation by 50%, was calculated from the curve of concentration-dependent survival percentage, defined as fluorescence in experimental wells compared with fluorescence in control wells, after subtraction of the blank values.

After reading, cells were prepared for cellular DNA quantitation with Hoechst dye 3342. They were rinsed with PBS, resazurin solution was then eliminated and plates were stored at –80 °C.

4.2.4. Hoechst dye 3342 test

On the day of assay, plates were thawed at room temperature for 10 min. Hundred microliters of a 0.01% (m/v) SDS solution in distilled water was then distributed into each well, the plates were incubated for 1 h at room temperature and frozen again at –80 °C for 1 h. After thawing, 100 µl of Hoechst dye 33342 solution at 30 µg ml^{-1} in a hypersaline buffer (10 mM Tris–HCl, pH 7.4, 1 mM EDTA and 2 M NaCl) were added to each well. The plates protected from light were incubated in this solution at room temperature for 1 h on a plate shaker. Fluorescence was then measured at 360/460 nm on a microplate fluorescence reader.

Under the conditions used, fluorescence was proportional to the amount of biomass and the IC_{50} was calculated as above.

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