



**HAL**  
open science

## Synthesis and antiproliferative activities of isoindigo and azaisoindigo derivatives

Fadoua Bouchikhi, Fabrice Anizon, Pascale Moreau

► **To cite this version:**

Fadoua Bouchikhi, Fabrice Anizon, Pascale Moreau. Synthesis and antiproliferative activities of isoindigo and azaisoindigo derivatives. *European Journal of Medicinal Chemistry*, 2008, 43, pp.755-762. hal-00270595

**HAL Id: hal-00270595**

**<https://hal.science/hal-00270595v1>**

Submitted on 7 Apr 2008

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

# Synthesis and antiproliferative activities of isoindigo and azaisoindigo derivatives

Fadoua Bouchikhi, Fabrice Anizon, Pascale Moreau\*

*Laboratoire SEESIB, Université Blaise Pascal, UMR 6504 du CNRS, 24 avenue des Landais, F-63177 Aubière Cedex, France*

## Abstract

In the course of structure–activity relationship studies, diversely substituted 1-( $\beta$ -D-acetylatedglucopyranosyl)isoindigo derivatives were prepared from indolines. New 7'-azaisoindigo analogues were also synthesized by coupling a glycosylated isatine and a 7-azaindolin-2-one derivative. Compounds containing a 7'-azaisoindigo framework have never been described before. To get an insight into the substitution pattern required for the best biological potencies, their antiproliferative activities were evaluated toward a human buccal carcinoma cell line (KB) and two human myeloid leukaemia cell lines (K562, HL60).

*Keywords:* Indigoids; Isoindigos; 7'-Azaisoindigo; In vitro antiproliferative activities

## 1. Introduction

As part of our ongoing studies concerning the preparation of potential biologically active compounds, we were interested in the synthesis of diversely substituted isoindigo derivatives.

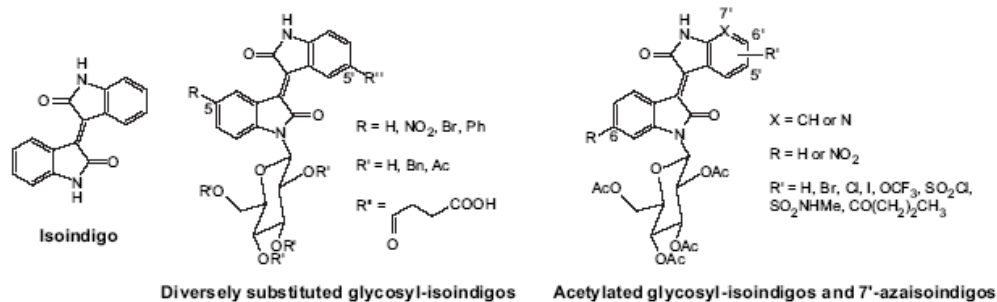
We have reported, in previous papers, the synthesis and antiproliferative activities of isoindigo derivatives bearing a sugar residue attached to one of the indole nitrogens and diversely substituted on the aromatic rings in the 5 or 5' position (Scheme 1). The sugar moieties were either protected with benzyl or acetyl groups or unprotected. The results obtained in these previous structure–activity relationship studies have shown that the pharmaceutical profile of this series could be optimized by substitution of the upper oxindole moiety and the presence of acetyl groups on the sugar residue [1,2]. The acetylated-glycosyl derivatives we prepared in the past were only substituted on the lower oxindole part in the 5 position. Therefore, in this paper, the synthesis of acetylated-glycosyl-isoindigo derivatives diversely substituted in the 6 and/or 5'

and/or 6' positions is described. Moreover, azaisoindigo analogues were also prepared to evaluate the influence of a 7'-azaindolin-2-one moiety instead of an indolin-2-one part on the biological activities of these compounds (Scheme 1). To our knowledge, this 7'-azaisoindigo framework, substituted or not, has never been described before. To get an insight into the effect of the substitution pattern on the biological activities of these compounds, their antiproliferative potencies were examined toward a human buccal carcinoma cell line (KB) and two human myeloid leukaemia cell lines (K562, HL60) using the classic colorimetric MTS test.

## 2. Chemistry

The key intermediates in this synthesis are the protected glycosyl-isatines **7** and **8** which were prepared in four steps from the corresponding glycosyl-indolines **1** and **2**, respectively. The preparation of compound **7** has been previously detailed [2]. Glycosyl-indolines **1** and **2** were prepared by glycosylation of the corresponding indolines. The hydroxy groups of the sugar moiety were first acetylated before aromatization with 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ)

\* Corresponding author. Tel.: +33 4 73 40 79 63; fax: +33 4 73 40 77 17.  
E-mail address: Pascale.MOREAU@univ-bpclermont.fr (P. Moreau).



Scheme 1.

and oxidation with chromium oxide to give the glycosyl-isatines **7** and **8** (Scheme 2).

As mentioned in our previous paper for the synthesis of 1-(2,3,4,6-tetra-*O*-acetyl-β-*D*-glucopyranosyl)-5-nitroindolin-2,3-dione [2], the synthesis was more difficult in the presence of electron acceptor substituents. Indeed, the glycosylation step was achieved in 24 h for compound **1** and required 6 days for nitro analogue **2**. The aromatization step was carried out at room temperature for 12 h for compound **5** and at 100 °C for 3 days for nitro compound **6**. At last, the oxidation with chromium oxide was performed and it resulted in 88% yield for compound **7** and only 46% yield for the 6-nitro analogue **8**.

The required diversely substituted indolin-2-one derivatives were either commercially available (**5** and 6-chlorooxindole) or prepared by standard methods (Scheme 3).

5-Iodoindolin-2-one **9** was prepared in 64% yield by treatment of 5-iodoindole with pyridinium bromide perbromide (PBPB) as previously described for indolin-2-one derivatives [3]. 7-Azaindolin-2-one **10** was prepared in 55% overall yield by treatment with pyridinium bromide perbromide followed by debromination [4,5]. Bromo derivative **11** was prepared in 95% yield by bromination of the corresponding indolin-2-one using *N*-bromosuccinimide [6]. 7-Aza-5-bromoindolin-2-one **12** was prepared in two steps from the corresponding 7-azaindole as previously described [7]. Trifluoromethoxy derivative **13** was obtained in 45% yield by reduction of the corresponding isatine in the presence of hydrazine hydrate as described in the literature for the bromo derivative [8].

5-(1-Oxobutyl)indolin-2-one **14** was prepared in 48% yield by acylation of the indolin-2-one in the presence of butyryl chloride and aluminium chloride according to the procedure described by Kakushima et al. for pyrrole derivatives [9]. 5-*N*-Methylaminosulfonylindolin-2-one **16** was obtained in 63% yield by amidation of 5-chlorosulfonylindolin-2-one **15** that was prepared by sulfonylation of indolin-2-one with chlorosulfonic acid [10,11].

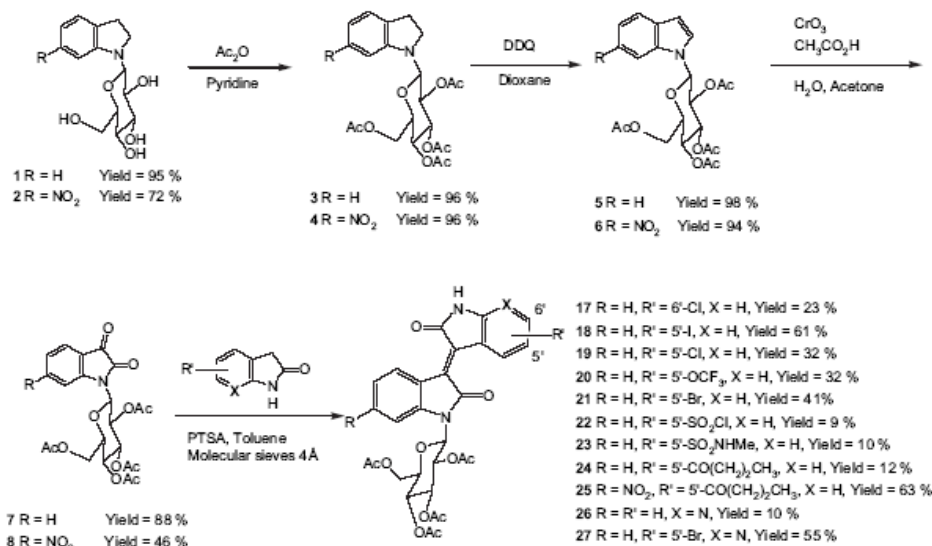
The final coupling step between isatines **7**, **8** and indolin-2-one or 7-azaindolin-2-one derivatives **9–16** was achieved in the presence of *p*-toluenesulfonic acid (PTSA) to give acetylated-glycosyl-isoidindigo or 7'-azaisoidindigo derivatives **17–27**[11] (Scheme 2, Table 1). In the isoidindigo series, the yields of the coupling step were usually lower when the

reactions were performed with indolin-2-one substituted in the 5 position with an electron acceptor substituent (compounds **22–24**), except when the isatine was activated with an electron acceptor substituent (compound **25**). For the 7'-azaisoidindigos, the reaction was also easier when the 7-azaindolin-2-one was substituted with an electron donor substituent.

### 3. Results and discussion

In vitro antiproliferative activities of isoidindigo (Scheme 1) and compounds **17–27** were evaluated in triplicate toward human buccal carcinoma cell line (KB) and human myeloid leukaemia cell lines (K562, HL60) using the classic colorimetric MTS test (Table 2). Taxotere was used as a positive control at a drug concentration of  $2.5 \times 10^{-10}$  M. Percentage of cell proliferation inhibition was defined as absorbance in experimental wells compared with absorbance in control wells, after subtraction of the blank values. The IC<sub>50</sub>, defined as the drug concentration required to inhibit cell proliferation by 50%, was calculated from the curve of concentration-dependent survival percentage. Under the conditions used ( $10^{-5}$  M final concentration of tested compounds), isoidindigo was active (IC<sub>50</sub> = 1.6 μM) toward KB cells whereas it was slightly cytotoxic toward HL60 and K562 cell lines. None of the glycosyl-isoidindigo derivatives (**17–25**) exhibited relevant cytotoxicity toward the cell lines tested. Nevertheless, the two compounds bearing a 7'-azaindolin-2-one moiety (**26**, **27**) have shown significant antiproliferative activities. Compound **26** inhibited the cell proliferation of all the cell lines tested in a 75–80% range (KB IC<sub>50</sub> = 1.6 μM). Compound **27** suppressed the cell proliferation of HL60 cells with a percentage of 73% whereas the cell proliferation of KB (IC<sub>50</sub> = 13.9 μM) and K562 cells was inhibited in a 60% range. Therefore, the presence of an extra nitrogen atom seems to be favourable for the cytotoxicity of these compounds.

In conclusion, we have synthesized diversely substituted 1-(2,3,4,6-tetra-*O*-acetyl-β-*D*-glucopyranosyl)isoidindigos. The method described here allows the substitution of both aromatic rings by either electron donor or acceptor substituents in the 6 and/or 5',6' positions. Moreover, this synthetic approach allowed also the preparation of 7'-azaindolin-2-one analogues. The results obtained in this structure–activity relationship study have shown that the antiproliferative activities of this series



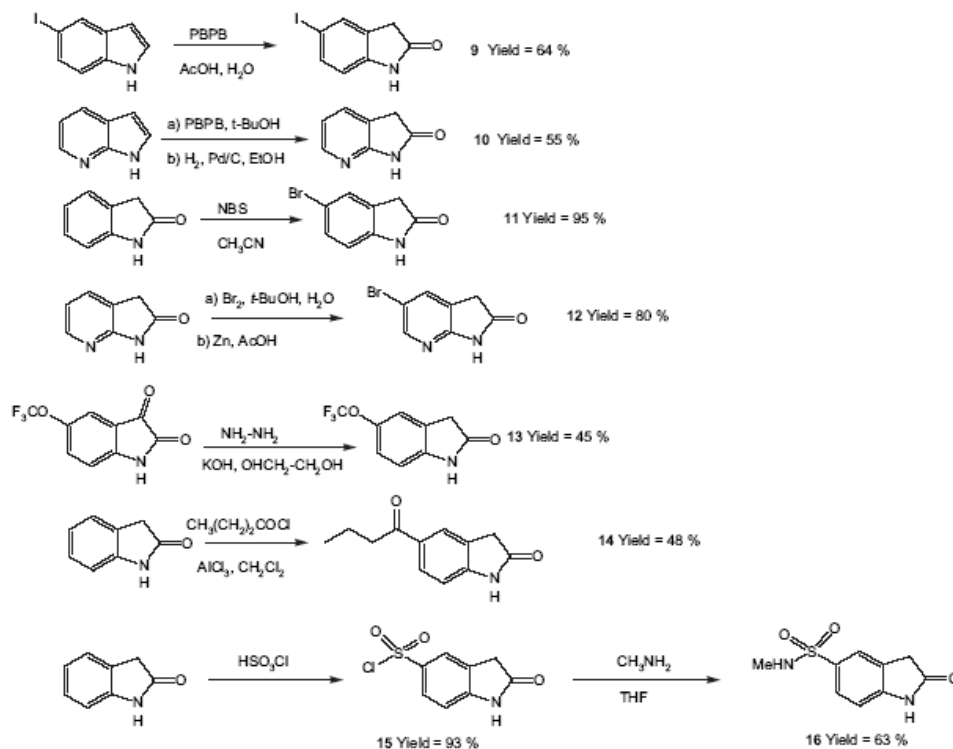
Scheme 2.

could be optimized by the presence of a 7-azaindolin-2-one moiety. In this case, the replacement of a methine group by a nitrogen atom could reinforce the interaction with the biological target(s) involved in the cytotoxicity of these compounds. The preparation of diversely substituted 7-azaindolin-2-one derivatives and the identification of the biological target(s) of these compounds are currently under investigation.

## 4. Experimental

### 4.1. Chemistry

IR spectra were recorded on a Perkin-Elmer Paragon 500 spectrometer ( $\nu$  in  $\text{cm}^{-1}$ ). NMR spectra were performed on a Bruker AVANCE 400 (<sup>1</sup>H: 400 MHz, <sup>13</sup>C: 100 MHz) or



Scheme 3.



Table 1

Compound	Isatine	Indolin-2-one (equiv)	Reaction time (h)	Purification method
17	7	6-Cl-oxindole (2)	48	A
18 <sup>a</sup>	7	9 (1.2)	30	B
19	7	5-Cl-oxindole (2)	48	A
20	7	13 (1.1)	36	C
21	7	11 (1.2)	24	C
22	7	15 (1.1)	48	C
23	7	16 (1.1)	24	C
24	7	14 (2)	48	D
25 <sup>b</sup>	8	14 (2)	48	A
26 <sup>b</sup>	7	10 (1.2)	48	C
27	7	12 (1.2)	48	E

<sup>a</sup> The reaction was performed in darkness.

<sup>b</sup> Isatine concentration was 15 mM for compound 25 and 45 mM for compound 26.

AVANCE 500 (<sup>1</sup>H: 500 MHz, <sup>13</sup>C: 125 MHz), chemical shifts  $\delta$  in ppm, the following abbreviations are used: singlet (s), doublet (d), triplet (t), quadruplet (q), doubled doublet (dd), sextuplet (sext), 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, TLCs were performed on fluorescent silica gel plates (60 F<sub>254</sub> from Merck).

#### 4.1.1. 1-( $\beta$ -D-Glucopyranosyl)-6-nitroindoline 2

D-Glucose (549 mg, 3.1 mmol) was added to a solution of 6-nitroindoline (1 g, 6.1 mmol) in a mixture of ethanol (46.7 mL) and water (3.1 mL). The mixture was refluxed for 6 days. Water (0.6 mL) was added after 7 h and 14 h. After concentration under vacuum, the resulting mixture was purified by flash chromatography (eluent: EtOAc/MeOH from 98:2 to 90:10) to give compound 2 in 72% yield as an orange

Table 2

Compound	KB	HL60	K562
Taxotere <sup>a</sup>	71	46	32
Isoindigo	92 (1.6)	53	64
17	42	22	30
18	49	45	49
19	38	47	56
20	0	4	3
21	34	49	58
22	35	0	1
23	19	0	13
24	13	29	33
25	41	58	50
26	76 (1.6)	70	75
27	59 (13.9)	73	64

Percentage of cell proliferation inhibition at a drug concentration of  $10^{-5}$  M; IC<sub>50</sub> values [ $\mu$ M] are reported in brackets.

<sup>a</sup> Taxotere was used as a positive control at a drug concentration of  $2.5 \times 10^{-10}$  M.

solid (mp = 195 °C). IR (KBr):  $\nu_{C=C}$  1610, 1615 cm<sup>-1</sup>;  $\nu_{OH}$  3400 cm<sup>-1</sup>. HRMS (ES) calcd for C<sub>14</sub>H<sub>18</sub>N<sub>2</sub>NaO<sub>7</sub> (M + Na)<sup>+</sup> 349.1012, found 349.0995. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): 3.00–3.13 (m, 3H), 2.28–3.36 (m, 3H), 3.38–3.44 (m, 1H), 3.58–3.65 (m, 2H), 3.72–3.80 (m, 1H), 4.40 (t, *J* = 6.5 Hz, 1H, OH), 4.80 (d, *J* = 9.0 Hz, 1H, H<sub>V</sub>), 4.95 (d, *J* = 5.0 Hz, 1H, OH), 5.07 (d, *J* = 4.5 Hz, 1H, OH), 5.10 (d, *J* = 5.0 Hz, 1H, OH), 7.24 (d, *J* = 8.5 Hz, 1H), 7.30 (d, *J* = 2.0 Hz, 1H), 7.48 (dd, *J*<sub>1</sub> = 8.5 Hz, *J*<sub>2</sub> = 2.0 Hz, 1H). <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>): 27.4, 45.7 (CH<sub>2</sub>), 60.9 (CH<sub>2</sub> sugar), 70.0, 70.5, 77.3, 78.2, 84.4 (CH<sub>sugar</sub>), 100.6, 113.3, 124.5 (CH), 138.3, 147.9, 152.0 (C).

#### 4.1.2. 6-Nitro-1-(2,3,4,6-tetra-O-acetyl- $\beta$ -D-glucopyranosyl)indoline 4

A solution of indoline 2 (960 mg, 2.94 mmol) in pyridine (10.3 mL) was cooled to 0 °C before addition of acetic anhydride (7.5 mL). The reaction mixture was stirred at room temperature for 24 h. After addition of water and extraction with EtOAc, the organic phases were washed with water, saturated aqueous NaHCO<sub>3</sub> and water until neutral pH. After drying over MgSO<sub>4</sub>, the solvent was removed and the residue was purified by flash chromatography (eluent: cyclohexane/EtOAc 80:20 then 50:50) to give compound 4 in 96% yield as a yellow solid (mp = 58 °C). IR (KBr):  $\nu_{C=O}$  1752 cm<sup>-1</sup>. HRMS (ES): calcd for C<sub>22</sub>H<sub>26</sub>N<sub>2</sub>NaO<sub>11</sub> (M + Na)<sup>+</sup> 517.1434, found 517.1453. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): 1.92 (s, 3H, CH<sub>3</sub>), 1.94 (s, 3H, CH<sub>3</sub>), 1.97 (s, 3H, CH<sub>3</sub>), 2.01 (s, 3H, CH<sub>3</sub>), 2.90–3.02 (m, 1H), 3.05–3.15 (m, 1H), 3.56–3.64 (m, 2H), 3.98–4.12 (m, 2H), 4.15–4.21 (m, 1H), 4.97 (t, *J* = 10.0 Hz, 1H), 5.18 (t, *J* = 9.5 Hz, 1H), 5.45 (t, *J* = 9.5 Hz, 1H), 5.72 (d, *J* = 9.5 Hz, 1H), 7.28 (d, *J* = 8.5 Hz, 1H), 7.55–7.58 (m, 2H). <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>): 20.3–20.4 (4C) (CH<sub>3</sub>), 27.4, 45.7, 61.9 (CH<sub>2</sub>), 68.2, 68.4, 72.0, 72.8, 81.6 (CH), 101.7, 114.7, 125.0 (CH), 138.5, 147.9, 151.0 (C), 169.3, 169.4, 169.6, 170.0 (C=O).

#### 4.1.3. 6-Nitro-1-(2,3,4,6-tetra-O-acetyl- $\beta$ -D-glucopyranosyl)indole 6

DDQ (210 mg, 0.93 mmol) was added in portions to a solution of indoline 4 (350 mg, 0.71 mmol) in dioxane (46 mL). The mixture was stirred at 100 °C for 3 days before addition of saturated aqueous NaHCO<sub>3</sub>. After extraction with EtOAc, the organic phases were washed with a brine solution, dried over MgSO<sub>4</sub> and evaporated. The residue was purified by flash chromatography (eluent: cyclohexane/EtOAc 50:50) to give 6 in 94% yield as a yellow solid (mp = 67 °C). IR (KBr):  $\nu_{C=O}$  1753 cm<sup>-1</sup>. HRMS (ES) calcd for C<sub>22</sub>H<sub>24</sub>N<sub>2</sub>NaO<sub>11</sub> (M + Na)<sup>+</sup> 515.1278, found 515.1257. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): 1.62 (s, 3H, CH<sub>3</sub>), 1.98 (s, 3H, CH<sub>3</sub>), 2.00 (s, 3H, CH<sub>3</sub>), 2.06 (s, 3H, CH<sub>3</sub>), 4.09–4.18 (m, 2H), 4.30–4.36 (m, 1H), 5.28 (t, *J* = 9.5 Hz, 1H), 5.56 (t, *J* = 9.5 Hz, 1H), 5.64 (t, *J* = 9.0 Hz, 1H), 6.48 (d, *J* = 9.0 Hz, 1H), 6.76 (d, *J* = 3.5 Hz, 1H), 7.77 (d, *J* = 9.0 Hz, 1H), 7.96 (d, *J* = 3.5 Hz, 1H), 7.98 (dd, *J*<sub>1</sub> = 9.0 Hz, *J*<sub>2</sub> = 2.0 Hz, 1H), 8.71 (d, *J* = 2.0 Hz, 1H). <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>): 19.7, 20.2, 20.4, 20.5 (CH<sub>3</sub>), 61.9 (CH<sub>2</sub>), 67.8, 70.2, 72.3,

73.2, 81.2 (CH), 104.3, 107.0, 115.4, 121.2, 132.2 (CH), 133.3, 134.7, 142.8 (C), 168.4, 169.4, 169.6, 170.0 (C=O).

#### 4.1.4. 6-Nitro-1-(2,3,4,6-tetra-O-acetyl- $\beta$ -D-glucopyranosyl)indoline-2,3-dione **8**

Chromium oxide (285 mg, 2.85 mmol) was added in portions to a suspension of indole derivative **6** (150 mg, 0.305 mmol) in a mixture of acetone (0.5 mL), acetic acid (2.3 mL) and water (0.8 mL). The reaction mixture was stirred at room temperature for 5 h before addition of cold water. The solid was isolated by filtration to give the corresponding isatine **8** in 46% yield as a yellow solid (mp = 110 °C). IR (KBr):  $\nu_{\text{C=O}}$  1715–1800  $\text{cm}^{-1}$ . HRMS (ES) calcd for  $\text{C}_{22}\text{H}_{22}\text{N}_2\text{NaO}_{13}$  ( $\text{M} + \text{Na}$ )<sup>+</sup> 545.1020, found 545.1003. <sup>1</sup>H NMR (400 MHz,  $\text{CDCl}_3$ ): 1.94 (s, 3H, CH<sub>3</sub>), 2.06 (s, 3H, CH<sub>3</sub>), 2.10 (s, 3H, CH<sub>3</sub>), 2.21 (s, 3H, CH<sub>3</sub>), 3.97–4.01 (m, 1H), 4.21 (dd,  $J_1 = 12.5$  Hz,  $J_2 = 2.0$  Hz, 1H), 4.38 (dd,  $J_1 = 13.0$  Hz,  $J_2 = 3.0$  Hz, 1H), 5.37 (t,  $J = 9.5$  Hz, 1H), 5.44 (t,  $J = 9.5$  Hz, 1H), 5.48 (t,  $J = 9.5$  Hz, 1H), 5.74 (d,  $J = 9.0$  Hz, 1H), 7.85 (d,  $J = 8.0$  Hz, 1H), 8.10 (dd,  $J_1 = 8.0$  Hz,  $J_2 = 2.0$  Hz, 1H), 8.17 (d,  $J = 1.5$  Hz, 1H). <sup>13</sup>C NMR (100 MHz,  $\text{CDCl}_3$ ): 20.3, 20.6 (3C) (CH<sub>3</sub>), 60.8 (CH<sub>2</sub>), 67.2, 68.1, 72.4, 75.0, 80.4 (CH), 109.2, 119.9, 126.5 (CH), 121.2, 148.6, 153.5 (C), 156.3, 169.4, 169.8, 169.9, 170.8, 180.6 (C=O).

#### 4.1.5. 5-Iodoindolin-2-one **9**

To a solution of 5-iodoindole (500 mg, 2.06 mmol) in a mixture of acetic acid and water (100:10 mL) was added a solution of pyridinium bromide perbromide (790 mg, 2.47 mmol) in acetic acid (100 mL). The reaction mixture was heated at 80 °C for 24 h before evaporation and addition of 10% aqueous  $\text{Na}_2\text{CO}_3$ . After extraction with EtOAc, the combined organic phases were dried over  $\text{MgSO}_4$ , evaporated and the residue was purified by flash chromatography (eluent: cyclohexane/EtOAc 80:20) to give compound **9** in 64% yield as a grey solid (mp = 170–172 °C). IR (KBr):  $\nu_{\text{C=C}}$  1609  $\text{cm}^{-1}$ ;  $\nu_{\text{C=O}}$  1704  $\text{cm}^{-1}$ ;  $\nu_{\text{NH}}$  3150  $\text{cm}^{-1}$ . HRMS (ES) calcd for  $\text{C}_8\text{H}_7\text{INO}$  ( $\text{M} + \text{H}$ )<sup>+</sup> 259.9572, found 259.9577. <sup>1</sup>H NMR (400 MHz,  $\text{DMSO}-d_6$ ): 3.48 (s, 2H), 6.65 (d,  $J = 8.0$  Hz, 1H), 7.48–7.52 (m, 2H), 10.47 (s, 1H, NH). <sup>13</sup>C NMR (100 MHz,  $\text{DMSO}-d_6$ ): 35.5 (CH<sub>2</sub>), 111.5, 132.7, 135.9 (CH), 83.9, 128.8, 143.5 (C), 175.8 (C=O).

#### 4.1.6. 5-Trifluoromethoxyindolin-2-one **13**

Potassium hydroxide (147 mg) and hydrazine hydrate (678  $\mu\text{L}$ , 12.0 mmol) were added to a solution of 5-trifluoromethoxyisatine (100 mg, 0.43 mmol) in ethylene glycol (0.6 mL). The mixture was stirred at 70 °C for 30 min. After addition of water, the mixture was neutralized with 10% aqueous HCl and extracted with EtOAc. The combined organic phases were dried over  $\text{MgSO}_4$ , evaporated and the residue was purified by flash chromatography (eluent: cyclohexane/EtOAc 70:30) to give compound **13** in 45% yield as a pink solid (mp = 140 °C). IR (KBr):  $\nu_{\text{C=C}}$  1669  $\text{cm}^{-1}$ ;  $\nu_{\text{C=O}}$  1705  $\text{cm}^{-1}$ ;  $\nu_{\text{NH}}$  3298  $\text{cm}^{-1}$ . HRMS (ES) calcd for  $\text{C}_9\text{H}_7\text{F}_3\text{NO}_2$  ( $\text{M} + \text{H}$ )<sup>+</sup> 218.0429, found 218.0431. <sup>1</sup>H NMR

(400 MHz,  $\text{DMSO}-d_6$ ): 3.54 (s, 2H), 6.86 (d,  $J = 8.5$  Hz, 1H), 7.16 (d,  $J = 8.5$  Hz, 1H), 7.24 (s, 1H), 10.47 (br s, 1H, NH). <sup>13</sup>C (100 MHz,  $\text{DMSO}-d_6$ ): 36.0 (CH<sub>2</sub>), 109.6, 118.2, 120.6 (CH), 120.2 (q,  $J_{\text{C,F}} = 255$  Hz,  $\text{OCF}_3$ ), 127.7, 142.8, 142.9 (C), 176.3 (C=O).

#### 4.1.7. 5-(1-Oxobutyl)indolin-2-one **14**

Butanoyl chloride (1.19 mL, 11.5 mmol) was added to a suspension of aluminium chloride (3.0 g, 22.5 mmol) in  $\text{CH}_2\text{Cl}_2$  (15.5 mL). The mixture was stirred at room temperature for 15 min before addition of a solution of oxindole (500 mg, 3.76 mmol) in  $\text{CH}_2\text{Cl}_2$  (10 mL). The mixture was stirred at room temperature for 48 h before hydrolysis and extraction with EtOAc. The combined organic phases were washed with saturated aqueous  $\text{NaHCO}_3$ , dried over  $\text{MgSO}_4$ , evaporated and the residue was purified by flash chromatography (eluent: cyclohexane/EtOAc from 90:10 to 60:40) to give compound **14** in 48% yield as a white solid (mp = 143–144 °C). IR (KBr):  $\nu_{\text{C=C}}$  1610  $\text{cm}^{-1}$ ;  $\nu_{\text{C=O}}$  1675, 1717  $\text{cm}^{-1}$ ;  $\nu_{\text{NH}}$  3000–3280  $\text{cm}^{-1}$ . HRMS (ES) calcd for  $\text{C}_{12}\text{H}_{14}\text{NO}_2$  ( $\text{M} + \text{H}$ )<sup>+</sup> 204.1025, found 204.1029. <sup>1</sup>H NMR (400 MHz,  $\text{DMSO}-d_6$ ): 0.91 (t,  $J = 7.5$  Hz, 3H, CH<sub>3</sub>), 1.61 (sext,  $J = 7.5$  Hz, 2H, CH<sub>2</sub>), 2.91 (t,  $J = 7.0$  Hz, 2H, CH<sub>2</sub>), 3.55 (s, 2H), 6.89 (d,  $J = 8.0$  Hz, 1H), 7.81 (s, 1H), 7.86 (dd,  $J_1 = 8.0$  Hz,  $J_2 = 1.5$  Hz, 1H), 10.75 (br s, 1H, NH). <sup>13</sup>C NMR (100 MHz,  $\text{DMSO}-d_6$ ): 13.7 (CH<sub>3</sub>), 17.6, 35.5, 39.4 (CH<sub>2</sub>), 108.7, 124.1, 129.0 (CH), 126.1, 130.4, 148.2 (C), 176.8, 198.5 (C=O).

#### 4.1.8. 5-Chlorosulfonylindolin-2-one **15**

Oxindole (5.0 g, 37.6 mmol) was added in portions in chlorosulfonic acid (10.2 mL) at 30 °C. The mixture was stirred at room temperature for 1.5 h and heated at 70 °C for 1 h. After addition of water, the solid was collected by filtration and washed with water to give compound **15** in 93% yield as a pink solid (mp = 185 °C). IR (KBr):  $\nu_{\text{C=C}}$  1610  $\text{cm}^{-1}$ ;  $\nu_{\text{C=O}}$  1715  $\text{cm}^{-1}$ ;  $\nu_{\text{NH}}$  3110  $\text{cm}^{-1}$ . HRMS (ES) calcd for  $\text{C}_8\text{H}_6\text{NO}_3\text{S}$  ( $\text{M} - \text{Cl}$ )<sup>+</sup> 196.0068, found 196.0078. <sup>1</sup>H NMR (400 MHz,  $\text{DMSO}-d_6$ ): 3.46 (s, 2H), 6.72 (d,  $J = 8.0$  Hz, 1H), 7.40–7.45 (m, 2H), 10.43 (br s, 1H, NH). <sup>13</sup>C NMR (100 MHz,  $\text{DMSO}-d_6$ ): 36.1 (CH<sub>2</sub>), 108.5, 122.3, 125.6 (CH), 125.7, 140.3, 144.9 (C), 177.0 (C=O).

#### 4.1.9. 5-N-Methylaminosulfonylindolin-2-one **16**

Methylamine 2 M/THF (1.16 mL, 2.32 mmol) was added dropwise to a solution of 5-chlorosulfonylindolin-2-one **15** (200 mg, 0.86 mmol) in THF cooled to 0 °C. The mixture was stirred at room temperature for 24 h before evaporation and purification of the residue by flash chromatography (eluent: cyclohexane/EtOAc 50:50 then 30:70) to give compound **16** in 63% yield as a pink solid (mp = 190 °C). IR (KBr):  $\nu_{\text{C=C}}$  1620  $\text{cm}^{-1}$ ;  $\nu_{\text{C=O}}$  1696  $\text{cm}^{-1}$ ;  $\nu_{\text{NH}}$  3050–3410  $\text{cm}^{-1}$ . HRMS (ES) calcd for  $\text{C}_9\text{H}_{10}\text{N}_2\text{NaO}_3\text{S}$  ( $\text{M} + \text{Na}$ )<sup>+</sup> 249.0310, found 249.0314. <sup>1</sup>H NMR (400 MHz,  $\text{DMSO}-d_6$ ): 2.38 (d,  $J = 4.5$  Hz, 3H), 3.59 (s, 2H), 6.97 (d,  $J = 8.0$  Hz, 1H), 7.22–7.27 (m, 1H,  $\text{NHCH}_3$ ), 7.58 (s, 1H), 7.61 (dd,  $J_1 = 8.0$  Hz,  $J_2 = 2.0$  Hz, 1H), 10.76 (br s, 1H, NH).

$^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ ): 28.7 (CH<sub>3</sub>), 35.6 (CH<sub>2</sub>), 108.9, 122.9, 127.4 (CH), 126.7, 131.6, 147.4 (C), 176.5 (C=O).

#### 4.1.10. Typical procedure for the acid-catalysed coupling reaction

To an anhydrous solution of PTSA (0.3 equiv) in toluene were added acetylated-glycosyl-isatine (21 mM), indolin-2-one derivative and 4 Å molecular sieves. The mixture was refluxed from 24 to 48 h. After cooling, EtOAc was added, the organic phase was washed with water. After drying over MgSO<sub>4</sub>, the solvent was removed and the residue purified in different conditions depending on the compounds (Table 1):

- Method A: flash chromatography (eluent: cyclohexane/EtOAc 40:60 then cyclohexane/EtOAc 40:60 (0.01% AcOH)) to give crude solid which was refluxed in cyclohexane before filtration to give the attempted compound.
- Method B: flash chromatography (eluent: cyclohexane/EtOAc 50:50 then cyclohexane/EtOAc 50:50 (0.01% AcOH)) to give a residue which was precipitated in a mixture of AcOH/toluene 50:50 with a few drops of water. After filtration, the solid was refluxed in cyclohexane before filtration to give the attempted compound.
- Method C: flash chromatography (eluent: cyclohexane/EtOAc 50:50 then cyclohexane/EtOAc 50:50 (0.01% AcOH)) to give a residue which was precipitated in a mixture of AcOH/toluene 50:50 with a few drops of water before filtration to give the attempted compound.
- Method D: flash chromatography (eluent: cyclohexane/EtOAc 40:60 then cyclohexane/EtOAc 40:60 (0.01% AcOH)) to give the attempted compound without further purification.
- Method E: flash chromatography (eluent: cyclohexane/EtOAc 50:50 then cyclohexane/EtOAc 50:50 (0.01% AcOH)) to give directly the attempted compound.

4.1.10.1. *6'-Chloro-1-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)isoindigo 17*. Yield: 23%. Red solid (mp > 280 °C). IR (KBr):  $\nu_{\text{C}=\text{C}}$  1615 cm<sup>-1</sup>;  $\nu_{\text{C}=\text{O}}$  1700–1800 cm<sup>-1</sup>;  $\nu_{\text{NH}}$  3215–3615 cm<sup>-1</sup>. HRMS (ES) calcd for C<sub>30</sub>H<sub>27</sub><sup>35</sup>ClN<sub>2</sub>NaO<sub>11</sub> (M + Na)<sup>+</sup> 649.1201, found 649.1196.  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ ): 1.77 (s, 3H, CH<sub>3</sub>), 1.96 (s, 3H, CH<sub>3</sub>), 2.02 (s, 3H, CH<sub>3</sub>), 2.05 (s, 3H, CH<sub>3</sub>), 4.09–4.21 (br s, 2H), 4.31–4.40 (m, 1H), 5.24–5.39 (m, 1H), 5.52–5.71 (m, 2H), 6.03–6.13 (m, 1H), 6.88 (s, 1H), 7.05 (d,  $J = 8.0$  Hz, 1H), 7.10 (t,  $J = 7.5$  Hz, 1H), 7.42–7.57 (m, 2H), 8.92 (d,  $J = 8.5$  Hz, 1H), 9.09 (d,  $J = 8.0$  Hz, 1H), 11.12 (s, 1H, NH).  $^{13}\text{C}$  NMR (125 MHz, DMSO- $d_6$ ): 20.0, 20.3, 20.4, 20.5 (CH<sub>3</sub>), 61.9 (CH<sub>2</sub>), 67.3, 67.8, 72.5, 73.1, 78.3 (CH), 109.7, 112.2, 121.0, 122.4, 128.9, 130.6, 132.9 (CH), 120.3, 120.8, 131.3, 133.3, 137.0, 141.2, 145.7 (C), 166.9, 168.6, 168.8, 169.4, 169.5, 170.0 (C=O).

4.1.10.2. *5'-Iodo-1-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)isoindigo 18*. Yield: 61%. Red solid (mp = 228–230 °C). IR (KBr):  $\nu_{\text{C}=\text{C}}$  1610 cm<sup>-1</sup>;  $\nu_{\text{C}=\text{O}}$  1675–1785 cm<sup>-1</sup>;  $\nu_{\text{NH}}$

3285–3615 cm<sup>-1</sup>. HRMS (ES) calcd for C<sub>30</sub>H<sub>27</sub>I<sub>N</sub><sub>2</sub>NaO<sub>11</sub> (M + Na)<sup>+</sup> 741.0557, found 741.0574.  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ ): 1.78 (s, 3H, CH<sub>3</sub>), 1.96 (s, 3H, CH<sub>3</sub>), 2.03 (s, 3H, CH<sub>3</sub>), 2.05 (s, 3H, CH<sub>3</sub>), 4.11–4.18 (m, 2H), 4.32–4.38 (m, 1H), 5.28–5.38 (m, 1H), 5.52–5.68 (m, 1H), 5.58 (t,  $J = 9.0$  Hz, 1H), 6.13–6.21 (m, 1H), 6.71 (d,  $J = 8.0$  Hz, 1H), 7.10 (t,  $J = 8.0$  Hz, 1H), 7.46 (t,  $J = 8.0$  Hz, 1H), 7.49–7.56 (m, 1H), 7.69 (dd,  $J_1 = 8.0$  Hz,  $J_2 = 1.5$  Hz, 1H), 9.11 (d,  $J = 8.0$  Hz, 1H), 9.33 (s, 1H), 11.09 (s, 1H, NH).  $^{13}\text{C}$  NMR (125 MHz, DMSO- $d_6$ ): 19.9, 20.3, 20.4, 20.5 (CH<sub>3</sub>), 61.9 (CH<sub>2</sub>), 67.3, 67.8, 72.5, 73.1, 78.3 (CH), 112.1 (2C), 122.5, 129.0, 133.1, 137.0, 140.9 (CH), 84.0, 120.7, 123.7, 132.0, 133.2, 141.2, 143.9 (C), 167.0, 168.0, 168.8, 169.4, 169.5, 170.0 (C=O).

4.1.10.3. *5'-Chloro-1-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)isoindigo 19*. Yield: 32%. Red solid (mp = 242 °C). IR (KBr):  $\nu_{\text{C}=\text{C}}$  1611 cm<sup>-1</sup>;  $\nu_{\text{C}=\text{O}}$  1695–1795 cm<sup>-1</sup>;  $\nu_{\text{NH}}$  3120–3570 cm<sup>-1</sup>. HRMS (ES) calcd for C<sub>30</sub>H<sub>27</sub><sup>35</sup>ClN<sub>2</sub>NaO<sub>11</sub> (M + Na)<sup>+</sup> 649.1201, found 649.1213.  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ ): 1.78 (s, 3H, CH<sub>3</sub>), 1.96 (s, 3H, CH<sub>3</sub>), 2.03 (s, 3H, CH<sub>3</sub>), 2.05 (s, 3H, CH<sub>3</sub>), 4.11–4.18 (m, 2H), 4.32–4.38 (m, 1H), 5.29–5.37 (m, 1H), 5.58 (t,  $J = 9.5$  Hz, 1H), 5.54–5.70 (m, 1H), 6.10–6.18 (m, 1H), 6.87 (d,  $J = 8.5$  Hz, 1H), 7.11 (t,  $J = 8.5$  Hz, 1H), 7.43 (dd,  $J_1 = 8.5$  Hz,  $J_2 = 2.0$  Hz, 1H), 7.47 (t,  $J = 7.5$  Hz, 1H), 7.50–7.56 (m, 1H), 9.03 (d,  $J = 1.5$  Hz, 1H), 9.13 (d,  $J = 8.0$  Hz, 1H), 11.11 (s, 1H, NH).  $^{13}\text{C}$  NMR (125 MHz, DMSO- $d_6$ ): 20.0, 20.3, 20.4, 20.5 (CH<sub>3</sub>), 61.9 (CH<sub>2</sub>), 67.3, 67.8, 72.5, 73.2, 78.3 (CH), 111.1, 112.0, 122.5, 128.6, 129.1, 132.4, 133.2 (CH), 120.7, 122.7, 125.1, 132.2, 133.4, 141.3, 143.2 (C), 167.0, 168.3, 168.8, 169.4, 169.5, 170.0 (C=O).

4.1.10.4. *1-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl)-5'-trifluoromethoxyisoindigo 20*. Yield: 32%. Red solid (mp = 270 °C). IR (KBr):  $\nu_{\text{C}=\text{O}}$  1690–1795 cm<sup>-1</sup>;  $\nu_{\text{NH}}$  3220–3605 cm<sup>-1</sup>. HRMS (ES) calcd for C<sub>31</sub>H<sub>27</sub>F<sub>3</sub>N<sub>2</sub>NaO<sub>12</sub> (M + Na)<sup>+</sup> 699.1414, found 699.1416.  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ ): 1.77 (s, 3H, CH<sub>3</sub>), 1.96 (s, 3H, CH<sub>3</sub>), 2.03 (s, 3H, CH<sub>3</sub>), 2.05 (s, 3H, CH<sub>3</sub>), 4.10–4.19 (m, 2H), 4.33–4.40 (m, 1H), 5.28–5.39 (m, 1H), 5.53–5.71 (m, 2H), 6.11–6.21 (m, 1H), 6.94 (d,  $J = 8.5$  Hz, 1H), 7.12 (t,  $J = 8.0$  Hz, 1H), 7.41 (dd,  $J_1 = 8.5$  Hz,  $J_2 = 1.5$  Hz, 1H), 7.48 (t,  $J = 7.5$  Hz, 1H), 7.51–7.57 (m, 1H), 9.01 (s, 1H), 9.15 (d,  $J = 8.0$  Hz, 1H), 11.16 (s, 1H, NH).  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ ): 19.9, 20.2, 20.4, 20.5 (CH<sub>3</sub>), 61.9 (CH<sub>2</sub>), 67.3, 67.8, 72.5, 73.1, 78.3 (CH), 110.5, 112.2, 122.3, 122.5, 126.1, 129.1, 133.3 (CH), 120.3 (q,  $J_{\text{C,F}} = 255$  Hz, OCF<sub>3</sub>), 120.7, 122.1, 132.4, 133.5, 141.3, 142.3, 143.4 (C), 167.0, 168.5, 168.8, 169.4, 169.5, 170.0 (C=O).

4.1.10.5. *5'-Bromo-1-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)isoindigo 21*. Yield: 41%. Red solid (mp > 290 °C). IR (KBr):  $\nu_{\text{C}=\text{C}}$  1610 cm<sup>-1</sup>;  $\nu_{\text{C}=\text{O}}$  1700–1790 cm<sup>-1</sup>;  $\nu_{\text{NH}}$  3310–3600 cm<sup>-1</sup>. HRMS (ES) calcd for C<sub>30</sub>H<sub>27</sub><sup>79</sup>BrN<sub>2</sub>NaO<sub>11</sub> (M + Na)<sup>+</sup> 693.0696, found 693.0717.  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ ): 1.78 (s, 3H, CH<sub>3</sub>), 1.96 (s, 3H, CH<sub>3</sub>), 2.03 (s,



3H, CH<sub>3</sub>), 2.05 (s, 3H, CH<sub>3</sub>), 4.10–4.19 (m, 2H), 4.32–4.39 (m, 1H), 5.28–5.39 (m, 1H), 5.58 (t,  $J = 8.5$  Hz, 1H), 5.54–5.70 (m, 1H), 6.11–6.20 (m, 1H), 6.82 (d,  $J = 8.5$  Hz, 1H), 7.11 (t,  $J = 7.5$  Hz, 1H), 7.47 (t,  $J = 7.5$  Hz, 1H), 7.49–7.58 (m, 2H), 9.13 (d,  $J = 8.0$  Hz, 1H), 9.17 (s, 1H), 11.11 (s, 1H, NH). <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>): 19.9, 20.3, 20.4, 20.5 (CH<sub>3</sub>), 61.9 (CH<sub>2</sub>), 67.3, 67.8, 72.5, 73.1, 78.3 (CH), 111.6, 112.1, 122.5, 129.1, 131.3, 133.2, 135.1 (CH), 112.8, 120.7, 123.2, 132.2, 133.3, 141.3, 143.5 (C), 167.0, 168.2, 168.8, 169.4, 169.5, 170.0 (C=O).

**4.1.10.6. 5'-Chlorosulfonyl-1-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)isoindigo 22.** Yield: 9%. Red solid (mp = 235 °C). IR (KBr):  $\nu_{\text{C}=\text{C}}$  1611 cm<sup>-1</sup>;  $\nu_{\text{C}=\text{O}}$  1690–1795 cm<sup>-1</sup>;  $\nu_{\text{NH}}$  3200–3600 cm<sup>-1</sup>. HRMS (ES) calcd for C<sub>30</sub>H<sub>27</sub><sup>35</sup>ClN<sub>2</sub>NaO<sub>13</sub>S (M + Na)<sup>+</sup> 713.0820, found 713.0841. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): 1.78 (s, 3H, CH<sub>3</sub>), 1.95 (s, 3H, CH<sub>3</sub>), 2.03 (s, 3H, CH<sub>3</sub>), 2.04 (s, 3H, CH<sub>3</sub>), 4.12–4.18 (m, 2H), 4.33–4.40 (m, 1H), 5.27–5.37 (m, 1H), 5.51–5.68 (m, 2H), 6.11–6.22 (m, 1H), 6.79 (d,  $J = 8.0$  Hz, 1H), 7.09 (t,  $J = 7.0$  Hz, 1H), 7.44 (t,  $J = 7.0$  Hz, 1H), 7.48–7.54 (m, 1H), 7.63 (d,  $J = 7.5$  Hz, 1H), 9.10 (d,  $J = 7.5$  Hz, 1H), 9.28 (s, 1H), 11.04 (s, 1H, NH). <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>): 20.0, 20.3, 20.4, 20.5 (CH<sub>3</sub>), 61.8 (CH<sub>2</sub>), 67.3, 67.8, 72.6, 73.1, 78.3 (CH), 108.6, 112.0, 122.3, 127.0, 128.7, 130.8, 132.6 (CH), 120.6, 120.9, 134.8, 141.1, 142.1 (C), 144.5 (C), 166.7, 168.8, 168.9, 169.4 (2C), 170.1 (C=O).

**4.1.10.7. 5'-N-Methylaminosulfonyl-1-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)isoindigo 23.** Yield: 10%. Red solid (mp = 231 °C). IR (KBr):  $\nu_{\text{C}=\text{C}}$  1617 cm<sup>-1</sup>;  $\nu_{\text{C}=\text{O}}$  1700–1780 cm<sup>-1</sup>;  $\nu_{\text{NH}}$  3230–3610 cm<sup>-1</sup>. HRMS (ES) calcd for C<sub>31</sub>H<sub>31</sub>N<sub>3</sub>NaO<sub>13</sub>S (M + Na)<sup>+</sup> 708.1475, found 708.1496. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): 1.78 (s, 3H, CH<sub>3</sub>), 1.96 (s, 3H, CH<sub>3</sub>), 2.03 (s, 3H, CH<sub>3</sub>), 2.05 (s, 3H, CH<sub>3</sub>), 2.44 (d,  $J = 4.5$  Hz, 3H), 4.10–4.19 (m, 2H), 4.35–4.42 (m, 1H), 5.29–5.39 (m, 1H), 5.53–5.68 (m, 2H), 6.13–6.22 (m, 1H), 7.05 (d,  $J = 8.0$  Hz, 1H), 7.12 (t,  $J = 7.5$  Hz, 1H), 7.29–7.35 (m, 1H, NH), 7.48 (t,  $J = 7.5$  Hz, 1H), 7.52–7.57 (m, 1H), 7.78 (d,  $J = 8.0$  Hz, 1H), 9.13 (d,  $J = 8.0$  Hz, 1H), 9.46 (s, 1H), 11.40 (s, 1H, NH). <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>): 19.9, 20.3, 20.4, 20.5, 28.7 (CH<sub>3</sub>), 61.9 (CH<sub>2</sub>), 67.4, 67.8, 72.5, 73.1, 78.3 (CH), 110.1, 112.3, 122.5, 128.1, 129.1, 131.6, 133.4 (CH), 120.7, 121.2, 132.0, 132.5, 133.1, 141.4, 147.3 (C), 166.9, 168.7, 168.9, 169.4, 169.5, 170.1 (C=O).

**4.1.10.8. 5'-(1-Oxobutyl)-1-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)isoindigo 24.** Yield: 12%. Red solid (mp = 210 °C). IR (KBr):  $\nu_{\text{C}=\text{C}}$  1612 cm<sup>-1</sup>;  $\nu_{\text{C}=\text{O}}$  1675–1785 cm<sup>-1</sup>;  $\nu_{\text{NH}}$  3175–3520 cm<sup>-1</sup>. HRMS (ES) calcd for C<sub>34</sub>H<sub>34</sub>N<sub>2</sub>NaO<sub>12</sub> (M + Na)<sup>+</sup> 685.2009, found 685.1990. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): 0.95 (t,  $J = 7.5$  Hz, 3H, CH<sub>3</sub>), 1.67 (sext,  $J = 7.5$  Hz, 2H, CH<sub>2</sub>), 1.78 (s, 3H, CH<sub>3</sub>), 1.96 (s, 3H, CH<sub>3</sub>), 2.03 (s, 3H, CH<sub>3</sub>), 2.05 (s, 3H, CH<sub>3</sub>), 2.95 (t,  $J = 7.0$  Hz, 2H, CH<sub>2</sub>), 4.12–4.19 (m, 2H), 4.36–4.43 (m, 1H), 5.28–5.41 (m, 1H), 5.55–5.70 (m, 2H), 6.13–6.24 (m, 1H), 6.95 (d,  $J = 8.0$  Hz, 1H), 7.12 (t,  $J = 7.0$  Hz, 1H), 7.47 (t,

$J = 7.5$  Hz, 1H), 7.49–7.57 (m, 1H), 8.02 (dd,  $J_1 = 8.5$  Hz,  $J_2 = 1.5$  Hz, 1H), 9.11 (d,  $J = 8.0$  Hz, 1H), 9.64 (s, 1H), 11.36 (s, 1H, NH). <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>): 13.7, 19.9, 20.3, 20.4, 20.5 (CH<sub>3</sub>), 17.5, 39.4, 61.9 (CH<sub>2</sub>), 67.4, 67.8, 72.5, 73.0, 78.3 (CH), 109.6, 112.1, 122.4, 129.0, 129.6, 133.1, 133.4 (CH), 120.8, 121.3, 130.5, 131.9, 133.7, 141.2, 148.0 (C), 166.9, 168.8, 168.9, 169.4, 169.5, 170.0, 198.4 (C=O).

**4.1.10.9. 6-Nitro-5'-(1-oxobutyl)-1-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)isoindigo 25.** Yield: 63%. Red solid (mp > 250 °C). IR (KBr):  $\nu_{\text{C}=\text{C}}$  1618 cm<sup>-1</sup>;  $\nu_{\text{C}=\text{O}}$  1690–1780 cm<sup>-1</sup>;  $\nu_{\text{NH}}$  3125–3670 cm<sup>-1</sup>. HRMS (ES) calcd for C<sub>34</sub>H<sub>33</sub>N<sub>3</sub>NaO<sub>14</sub> (M + Na)<sup>+</sup> 730.1860, found 730.1886. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): 0.95 (t,  $J = 7.5$  Hz, 3H, CH<sub>3</sub>), 1.68 (sext,  $J = 7.5$  Hz, 2H, CH<sub>2</sub>), 1.79 (s, 3H, CH<sub>3</sub>), 1.98 (s, 3H, CH<sub>3</sub>), 2.06 (s, 3H, CH<sub>3</sub>), 2.07 (s, 3H, CH<sub>3</sub>), 2.95 (t,  $J = 7.0$  Hz, 2H, CH<sub>2</sub>), 4.14 (dd,  $J_1 = 12.5$  Hz,  $J_2 = 2.0$  Hz, 1H), 4.24 (dd,  $J_1 = 12.5$  Hz,  $J_2 = 3.5$  Hz, 1H), 4.42–4.48 (m, 1H), 5.26–5.37 (m, 1H), 5.49–5.68 (m, 1H), 5.68 (t,  $J = 9.0$  Hz, 1H), 6.29 (d,  $J = 9.0$  Hz, 1H), 6.97 (d,  $J = 8.0$  Hz, 1H), 8.00 (dd,  $J_1 = 9.0$  Hz,  $J_2 = 2.0$  Hz, 1H), 8.07 (d,  $J = 8.0$  Hz, 1H), 8.15 (d,  $J = 1.5$  Hz, 1H), 9.32 (d,  $J = 9.0$  Hz, 1H), 9.62 (s, 1H), 11.47 (s, 1H, NH). <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>): 13.7, 19.9, 20.3 (2C), 20.4 (CH<sub>3</sub>), 17.4, 39.5, 61.1 (CH<sub>2</sub>), 67.2, 67.7, 72.0, 73.1, 78.8 (CH), 105.9, 110.0, 117.6, 129.5, 130.4, 134.8 (CH), 121.0, 126.4, 129.2, 130.7, 137.8, 141.5, 149.0, 149.1 (C), 166.5, 168.5, 169.1, 169.3, 169.5, 170.0, 198.2 (C=O).

**4.1.10.10. 7'-Aza-1-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)isoindigo 26.** Yield: 10%. Red solid (mp = 196 °C). IR (KBr):  $\nu_{\text{C}=\text{C}}$  1603 cm<sup>-1</sup>;  $\nu_{\text{C}=\text{O}}$  1690–1790 cm<sup>-1</sup>;  $\nu_{\text{NH}}$  3240–3625 cm<sup>-1</sup>. HRMS (ES): calcd for C<sub>29</sub>H<sub>28</sub>N<sub>3</sub>O<sub>11</sub> (M + H)<sup>+</sup> 594.1724, found 594.1712. <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>): 1.77 (s, 3H, CH<sub>3</sub>), 1.96 (s, 3H, CH<sub>3</sub>), 2.02 (s, 3H, CH<sub>3</sub>), 2.05 (s, 3H, CH<sub>3</sub>), 4.10–4.18 (m, 2H), 4.33–4.40 (m, 1H), 5.27–5.41 (m, 1H), 5.52–5.68 (m, 2H), 6.04–6.16 (m, 1H), 7.06 (dd,  $J_1 = 7.5$  Hz,  $J_2 = 5.5$  Hz, 1H), 7.13 (t,  $J = 7.5$  Hz, 1H), 7.48 (t,  $J = 7.5$  Hz, 1H), 7.51–7.59 (m, 1H), 8.20 (d,  $J = 5.0$  Hz, 1H), 9.14 (d,  $J = 8.0$  Hz, 1H), 9.17 (d,  $J = 8.0$  Hz, 1H), 11.59 (s, 1H, NH). <sup>13</sup>C NMR (125 MHz, DMSO-*d*<sub>6</sub>): 20.0, 20.3, 20.4, 20.5 (CH<sub>3</sub>), 61.9 (CH<sub>2</sub>), 67.3, 67.8, 72.5, 73.1, 78.3 (CH), 111.6, 117.7, 122.6, 129.1, 133.2, 136.4, 150.5 (CH), 116.1, 120.6, 132.1, 132.5, 141.3, 157.9 (C), 167.0, 168.2, 168.8, 169.4, 169.5, 170.0 (C=O).

**4.1.10.11. 7'-Aza-5'-bromo-1-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)isoindigo 27.** Yield: 55%. Red solid (mp = 228–230 °C). IR (KBr):  $\nu_{\text{C}=\text{C}}$  1617 cm<sup>-1</sup>;  $\nu_{\text{C}=\text{O}}$  1715–1800 cm<sup>-1</sup>;  $\nu_{\text{NH}}$  3300–3600 cm<sup>-1</sup>. HRMS (ES): calcd for C<sub>29</sub>H<sub>27</sub><sup>79</sup>BrN<sub>3</sub>O<sub>11</sub> (M + H)<sup>+</sup> 672.0829, found 672.0833. <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>): 1.78 (s, 3H, CH<sub>3</sub>), 1.96 (s, 3H, CH<sub>3</sub>), 2.03 (s, 3H, CH<sub>3</sub>), 2.05 (s, 3H, CH<sub>3</sub>), 4.10–4.18 (m, 2H), 4.32–4.38 (m, 1H), 5.29–5.40 (m, 1H), 5.52–5.68 (m, 1H), 5.58 (t,  $J = 9.0$  Hz, 1H), 6.10–6.20 (m, 1H), 7.15



(t,  $J = 8.0$  Hz, 1H), 7.50 (t,  $J = 7.5$  Hz, 1H), 7.53–7.60 (m, 1H), 8.34 (d,  $J = 2.0$  Hz, 1H), 9.15 (d,  $J = 8.0$  Hz, 1H), 9.40 (br s, 1H), 11.80 (br s, 1H, NH).  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ ): 20.0, 20.2, 20.4, 20.5 (CH<sub>3</sub>), 61.9 (CH<sub>2</sub>), 67.2, 67.8, 72.5, 73.2, 78.3 (CH), 111.8, 122.7, 129.4, 133.8, 138.2, 150.1 (CH), 111.9, 117.8, 120.5, 131.2, 133.5, 141.6, 156.5 (C), 167.1, 167.9, 168.9, 169.3, 169.5, 170.0 (C=O).

## 4.2. Antiproliferative activities

### 4.2.1. Cell cultures and proliferation assays

Stock cell cultures were maintained as monolayers in 75 cm<sup>2</sup> culture flasks in Glutamax RPMI medium with 10% fetal calf serum containing penicillin and streptomycin. Cells were grown at 37 °C in a humidified incubator under an atmosphere containing 5% CO<sub>2</sub>. Cells were plated at a density of 500–800 cells in 200 µL culture medium in each well of 96-well microplates and were allowed to adhere for 24 h before treatment with tested drugs in DMSO solution (final concentration of 10<sup>-5</sup> M). Cell viability was assayed after 72 h continuous drug exposure with a tetrazolium compound [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium salt, MTS, Promega Corp] by CellTiter 96 Aqueous One Solution Cell Proliferation Assay kit (Promega Corp) according to the manufacturer's instructions. Absorbance was measured at 490 nm with a microplate reader.

## Acknowledgements

The authors are grateful to Prof. Michelle Prudhomme for helpful discussions and to Bertrand Légeret for mass spectra analysis.

## References

- [1] M. Sassatelli, E. Saab, F. Anizon, M. Prudhomme, P. Moreau, *Tetrahedron Lett.* 45 (2004) 4827–4830.
- [2] M. Sassatelli, F. Bouchikhi, S. Messaoudi, F. Anizon, E. Debiton, C. Barthomeuf, M. Prudhomme, P. Moreau, *Eur. J. Med. Chem.* 41 (2006) 88–100.
- [3] P. Stjernlof, M.D. Ennis, L.O. Hansson, R.L. Hoffman, N.B. Ghazal, S. Sundell, M.W. Smith, K. Svensson, A. Carlsson, H. Wikstrom, *J. Med. Chem.* 38 (1995) 2202–2216.
- [4] A. Marfat, M.P. Carta, *Tetrahedron Lett.* 28 (1987) 4027–4030.
- [5] S. Okuda, M.M. Robison, *J. Am. Chem. Soc.* 81 (1959) 740–743.
- [6] L. Sun, N. Tran, F. Tang, H. App, P. Hirth, G. McMahon, C. Tang, *J. Med. Chem.* 41 (1998) 2588–2603.
- [7] D. Mazéas, G. Guillaumet, M.-C. Viaud, *Heterocycles* 50 (1999) 1065–1080.
- [8] M.S.C. Pedras, M. Jha, *J. Org. Chem.* 70 (2005) 1828–1834.
- [9] M. Kakushima, P. Hamel, R. Frenette, J. Rokach, *J. Org. Chem.* 48 (1983) 3214–3219.
- [10] L. Sun, N. Tran, C. Liang, F. Tang, A. Rice, R. Schreck, K. Waltz, L.K. Shawver, G. McMahon, C. Tang, *J. Med. Chem.* 42 (1999) 5120–5130.
- [11] H. Guan, A.D. Laird, R.A. Blake, C. Tang, C. Liang, *Bioorg. Med. Chem. Lett.* 14 (2004) 187–190.