Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

Short communication

Synthesis, kinase inhibitory potencies and in vitro antiproliferative activity of isoindigo and 7′-azaisoindigo derivatives substituted by Sonogashira cross-coupling

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ARTICLE INFO

ABSTRACT

In the course of structure–activity relationship studies we were interested in the synthesis of isoindigo and 7′-azaisoindigo derivatives substituted at the N-1 position by a 1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl), at the 5′-position by various chains introduced by Sonogashira cross-coupling and substituted or not at the 5-position by a bromine atom. To get an insight into the substitution pattern required for the best biological potencies, their kinase inhibitory potencies and their in vitro antiproliferative activities were evaluated. The derivatives were tested toward four protein kinases (CDK5/p25, GSK3, CK1, Dyrk1A) and their in vitro antiproliferative activity was tested against two human myeloid leukaemia cell lines (K562 and HL60).

1. Introduction

In the course of studies on the preparation of potential biologically active compounds, we were interested in the synthesis of indigoid derivatives. We have reported, in previous papers, the synthesis and biological activities 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 the aromatic rings at the 5-, 6- and/or 5′-positions [1–4]. 7′-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 [4].

The results obtained in these previous structure–activity relationship studies have shown that the pharmaceutical profile of this series could be optimized by substitutions of the upper oxindole moiety, bromination in the 5-position and the presence of acetyl groups on the sugar residue. Moreover, the 7′-azaisoindigo derivatives were more cytotoxic than their non-aza analogues, particularly toward two myeloid leukaemia cell lines (K562, HL60).

In the isoindigo series, the most active compound was the one bearing a 4-oxobutanoic acid side chain at the 5′-position [3]. Therefore, to get an insight into the substitution pattern required for the best biological potencies, we decided to introduce various functionalized alkynyl side chains at the C-5′ position of the upper indolin-2-one or 7-azaindolin-2-one moiety by Sonogashira

cross-coupling. Moreover, to our knowledge, the functionalization of the isoindigo or 7-azaisoindigo framework by palladium-catalyzed cross-coupling reactions has never been described.

The biological profile of these compounds was examined by in vitro antiproliferative potencies' testing toward two myeloid leukaemia cell lines (K562, HL60) using the classic colorimetric MTS test. Moreover, to identify the cellular target(s) implicated in the in vitro antiproliferative activity of these derivatives, their inhibitory potencies toward four different kinases (CDK5/p25, GSK3, CK1, DyrK1A) were examined.

2. Chemistry

In our previous work, we have described the synthesis of 5′-iodoglycosyl-isoindigo **3** by coupling in acidic conditions of the glycosylated isatin **1** with 5-iodoindolin-2-one [4]. The brominated analogue **4** was prepared in 68% yield, from 5-bromo glycosylated isatine **2**, using the same procedure (Scheme 1).

The alkynyl side chains were introduced in the 5′-position of the isoindigo or 7′-azaisoindigo framework by Sonogashira coupling using Pd(PPh$_3$)$_4$ in acetonitrile in the presence of CuI and Et$_3$N. In these conditions, compounds **5** and **6**, brominated or not and bearing a Cbz-protected aminopropyl side chain at the 5′-position were obtained in respectively 24% and 33% yields. Compounds **7** and **8** were obtained from **3** or **4** in 14 and 41% yield respectively, using the same catalytic conditions in the presence of but-3-yn-1-ol.

To optimize the chemical yields of these reactions, the use of Pd(PPh$_3$)$_2$Cl$_2$, CuI, Et$_3$N in acetonitrile as described previously for

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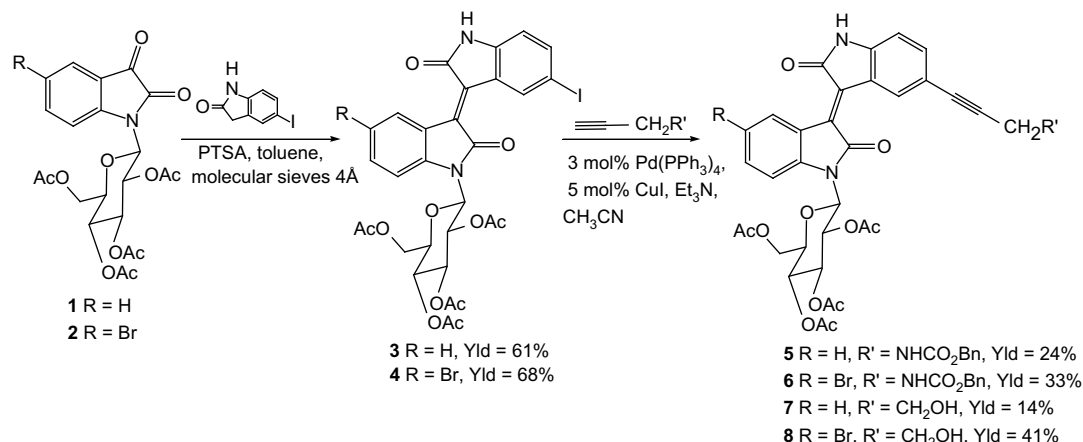
Scheme 1.

5-bromoindole derivatives under thermic conditions [5] or for 5-iodoindolin-2-one derivatives under microwaves irradiation [6] was also tested. In these latter catalytic conditions, none of the expected coupling products was observed.

As we had already described the preparation of the 7-aza-5-bromoindolin-2-one **9** [4], in the aza series, the Sonogashira cross-coupling reactions were first attempted with brominated intermediates. Compound **11** was prepared in 51% yield by coupling glycosylated isatin **2** and 7-aza-5-bromoindolin-2-one **9** in acidic conditions as already described for the synthesis of the mono-brominated analogue **10** [4] (Scheme 2).

From 7′-aza-5′-bromoisoindigo derivatives **10** and **11**, no coupling product was obtained when the Sonogashira reaction was carried out in the same catalytic conditions than for compounds **3** and **4**. So we tried to introduce the alkynyl side chain before the formation of the 7′-azaisoindigo framework by treating the 7-aza-5-bromoindolin-2-one **9** in Sonogashira conditions. Unfortunately, no coupling product was obtained (Scheme 2).

Then, we decided to activate the 5-position of the 7-aza-5-bromoindolin-2-one **9** by preparing the iodinated analogue either in the presence of KI and CuI in dimethylformamide as described for 3,5-dibromopyridine [7] or with BuLi and iodine in THF as described by Lavilla for 5-bromonicotinic acid [8]. In both conditions, no halogen exchange was observed.

The halogen exchange was only observed when the reaction was performed in the presence of CuI, NaI and a 1,2-diamine ligand (*N*,*N*′-dimethyl-1,2-cyclohexanediamine) in dioxane [9,10] (Scheme 3). In these conditions, compound **12** was obtained in 62% yield. The halogen exchange performed in the same conditions with the azaisoindigo derivative **10** led only to the recovery of starting material.

Compound **12** was reacted in acidic conditions with an isatine derivative to give the corresponding glycosylazaisoindigos **13** and **14** in 61 and 74% yield respectively.

Finally, the coupling reaction performed between the iodo derivatives **13**, **14** and the Cbz-protected propargylamine with Pd(PPh₃)₄ in acetonitrile in the presence of CuI, Et₃N led to the attempted products **15** and **16** in respectively 39 and 45% yields (Scheme 3).
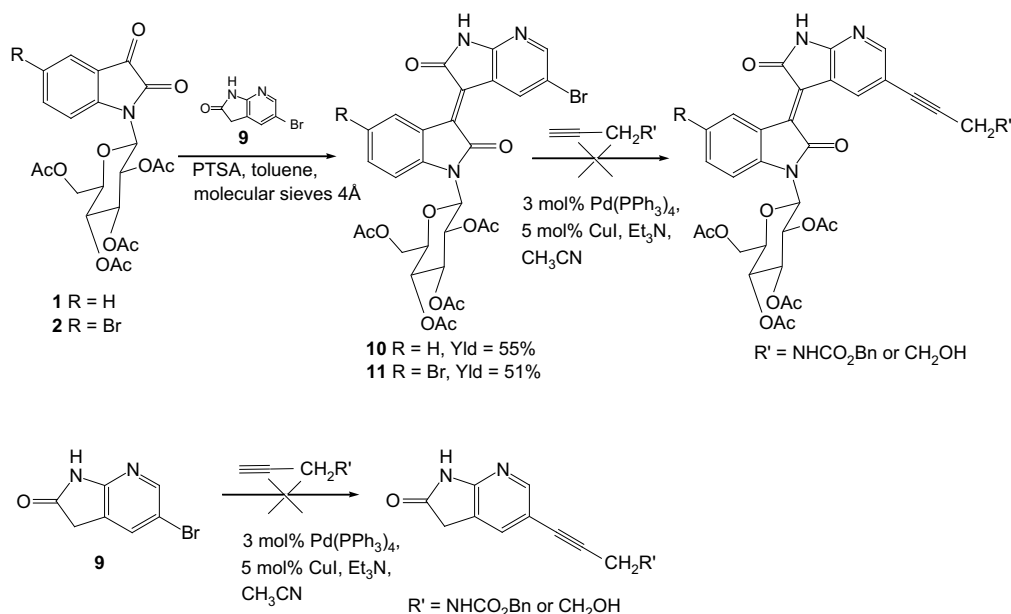
Scheme 2.
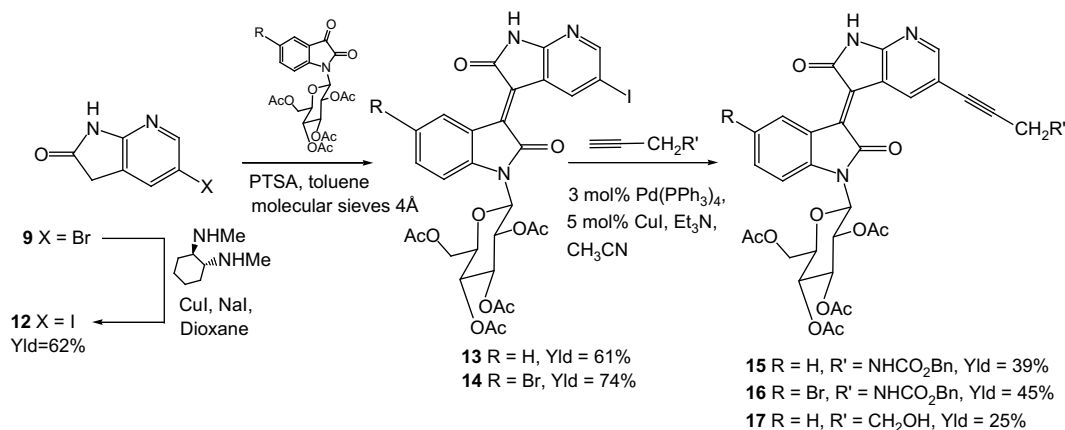
Scheme 3.

Compound **17** was obtained from **13** and but-3-yn-1-ol in 25% yield by using the same reaction conditions. When this reaction was applied to the brominated analogue **14**, the formation of the corresponding coupling product was observed but we never succeed in an efficient purification of this compound.

No further chemical transformations of the reported compounds **5–8** and **15–17** were carried out regarding the modest overall yields of these synthetic approaches.

3. Results and discussion

In vitro antiproliferative activities of compounds **3–8**, **10**, **11**, **13–17** were evaluted in triplicate toward human myeloid leukaemia cell lines (K562, HL60) using the classic colorimetric MTS test. Percentage of cell proliferation inhibition was defined as absorbance in experimental wells compared with absorbance in control wells, after subtraction of the blank values. None of the tested compounds have shown any cytotoxicity toward the cell lines tested when the final concentration of the tested compounds used was 10^{-6} M (Table 1). Only five compounds (two isoindigo derivatives (**3**, **8**) and three 7'-azaisoindigo (**10**, **13**, **15**) analogues) have shown modest antiproliferative activities when tested at 10^{-5} M. In the iso-indigo series, the most active compound was compound **3**, bearing a sugar moiety and an iodine atom at the 5'-position, which inhibited the cell proliferation for both cell lines nearby 50%. Moreover, compound **8**, bearing a sugar moiety and a hydroxybut-3-ynyl side chains in the 5'-position, suppressed the cell proliferation of K562 cells by 51%. In the 7'-azai-soindigo series, the most active compound was compound **10** bearing a sugar moiety and a bromine atom in the 5'-position which inhibited the cell proliferation in a 60–75% range for both cell lines. Furthermore, compounds **13** and **15** suppressed the cell proliferation of K562 cells in a 50–60% range.

In vitro kinase inhibitory potencies of compounds **3–7**, **10**, **11**, **13–15** were evaluated toward CDK5/p25, GSK3, CK1 and DyrK1A as already described in the literature [11] by Laurent Meijer's group (Station Biologique CNRS, Roscoff, France). Regarding the residual kinase activity (more than 65%) when the compounds were tested at 10 μM, none of them was particularly active toward these kinases.

In conclusion, we have synthesized various 1-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)isoindigos and 7'-azaisoindigos, substi-tuted by Sonogashira reaction. The results obtained in this structure–activity relationship study have shown that in both series, the introduction of a functionalized side chain in the 5'-position did not improve the cytotoxic activity of these series.

4. Experimental

4.1. Chemistry

IR spectra were recorded on a Perkin–Elmer Paragon 500 spec-trometer ($\bar{\nu}$ in cm^{-1}). NMR spectra were performed on a Bruker AVANCE 400 (^{1}H: 400 MHz, ^{13}C: 100 MHz) or AVANCE 500 (^{1}H: 500 MHz, ^{13}C: 125 MHz), chemical shifts δ in ppm, the following abbreviations are used: singlet (s), doublet (d), triplet (t), doubled doublet (dd), doublet of triplets (dt), multiplet (m), broad signal (br s), broad doublet (br d). 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-Iodo-1H-pyrrolo[2,3-b]pyridin-2(3H)-one 12

A mixture of 5-bromo-7-azaoxindole **9** (330 mg, 1.55 mmol), copper iodide (15 mg, 0.079 mmol, 5 mol%), sodium iodide (465 mg, 3.10 mmol) and trans-N,N'-dimethyl-1,2-cyclohexanediamine (22 mg, 0.155 mmol, 10 mol%) in anhydrous dioxane (15.8 mL) under argon atmosphere was heated at 110 °C in a sealed tube for 24 h in the darkness. After cooling, the mixture was diluted with a 28–30% NH₄OH solution (10 mL), water was added and the mixture was extracted with dichloromethane. After drying over MgSO₄, the solvent was removed and the crude product was purified by flash chromatography (cyclohexane/EtOAc 7:3 then 5:5) to give compound **12** (250 mg, 0.96 mmol, 62%) as a white solid (mp 70 °C).

IR (KBr) $\nu_{C=O}$ 1718 cm^{-1}; 1206 cm^{-1}.

HRMS (ES+) calcd for $C_7H_6IN_2O$ [M + H]$^+$ 260.9525, found 260.9522.

Table 1
Antiproliferative activity of compounds **3–17** (percentage of inhibition of prolifer-ation at $10^{-5}/10^{-6}$ M). Taxotere was used as a positive control at a drug concen-tration of 2.5×10^{-10} M with 46% of HL60 cell growth inhibition and 32% of K562 cell growth inhibition.

Compounds	3	4	5	6	7	8[a]	10	11	13	14	15	16	17
HL60	45/0	0/0	1/0	0/0	8/0	28/0	73/3	0/0	1/0	0/0	11/0	0/0	0/0
K562	49/16	3/0	6/0	2/1	33/2	51/7	64/19	4/3	47/0	3/0	59/4	5/0	10/5

[a] Compound **8** was tested as a mixture with oct-3,5-diyne-1,8-diol in a 94.3:5.7 w/w ratio.

^{1}H NMR (400 MHz, DMSO-d_6): 3.55 (2H, s), 7.85 (1H, m), 8.26 (1H, m), 11.10 (1H, s, NH).
^{13}C NMR (100 MHz, DMSO-d_6): 35.2 (CH$_2$), 139.2, 151.3 (CH$_{arom}$), 83.8, 123.3, 157.5 (C$_{arom}$), 175.2 (C=O).

4.1.2. General procedure for the preparation of compounds 4, 11, 13 and 14

To an anhydrous solution of PTSA (30 mol%) in toluene were added indolin-2-one derivatives (1.1 eq; 1.8 eq for **9**), acetylated glycosylisatine (18 mM; 21 mM for **1**), and 4 Å molecular sieves. The mixture was refluxed for 24 h (48 h for **11**) in the darkness (except for **11**). After cooling, EtOAc was added, the organic phase was washed with water. After drying over MgSO$_4$, the solvent was removed and the crude product was purified in different conditions depending on the compounds:

- Compounds **4**, **13** and **14**: flash chromatography (cyclohexane/ EtOAc 1:1) to give a residue which was precipitated in a mixture of AcOH/toluene 1:1 with a few drops of water. Filtration gave the attempted compound.
- Compound **11**: flash chromatography (cyclohexane/EtOAc 6:4 then 5:5) to give the attempted compound.

4.1.3. 5-Bromo-5′-Iodo-1-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranos-1-yl)isoindigo 4

Compound **2** (250 mg, 0.45 mmol); compound **4** (243 mg, 0.305 mmol, 68%) as a red solid (mp 270–273 °C).
IR (KBr): ν_{NH} 3385 cm^{-1}; $\nu_{C=O}$ 1775–1695 cm^{-1}.
HRMS (ES+) calcd for C$_{30}$H$_{26}$^{79}BrIN$_2$NaO$_{11}$ [M + Na]$^+$ 818.9662, found 818.9670.
^{1}H NMR (400 MHz, DMSO-d_6): 1.78 (3H, s, CH$_3$), 1.96 (3H, s, CH$_3$), 2.03 (3H, s, CH$_3$), 2.05 (3H, s, CH$_3$), 4.09–4.18 (2H, m), 4.32–4.38 (1H, m), 5.30–5.40 (1H, m), 5.52–5.64 (2H, m), 6.13–6.22 (1H, m), 6.72 (1H, d, J = 8.0 Hz), 7.55 (1H, br d, J = 7.5 Hz), 7.63 (1H, br d, J = 7.5 Hz), 7.71 (1H, dd, J$_1$ = 8.0 Hz, J$_2$ = 1.5 Hz), 9.34 (1H, s), 9.37 (1H, d, J = 1.5 Hz), 11.15 (1H, s, NH).
^{13}C NMR (125 MHz, DMSO-d_6): 20.0, 20.3, 20.4, 20.5 (CH$_3$), 61.9 (CH$_2$), 67.2, 67.8, 72.3, 73.2, 78.2 (CH), 112.3, 114.1, 131.1, 134.9, 137.4, 141.5 (CH$_{arom}$), 84.2, 114.5, 122.6, 123.5, 130.4, 134.8, 140.0, 144.3 (C$_{arom}$), 166.6, 168.1, 168.9, 169.4, 169.5, 170.1 (C=O).

4.1.4. 7′-Aza-5,5′-dibromo-1-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranos-1-yl)isoindigo 11

Compound **2** (50 mg, 0.090 mmol); compound **11** (34.6 mg, 0.046 mmol, 51%) as a red solid (mp 280 °C).
IR (KBr): ν_{NH} 3390 cm^{-1}; $\nu_{C=O}$ 1790–1715 cm^{-1}; 1600, 1458, 1368, 1232 cm^{-1}.
HRMS (ES+) calcd for C$_{29}$H$_{26}$^{79}Br$_2$N$_3$O$_{11}$ [M + H]$^+$ 749.9934, found 749.9951.
^{1}H NMR (400 MHz, DMSO-d_6): 1.79 (3H, s, CH$_3$), 1.96 (3H, s, CH$_3$), 2.03 (3H, s, CH$_3$), 2.04 (3H, s, CH$_3$), 4.09–4.17 (2H, m), 4.31–4.38 (1H, m), 5.29–5.41 (1H, m), 5.52–5.65 (2H, m), 6.11–6.19 (1H, m), 7.58 (1H, br d, J = 7.5 Hz), 7.68 (1H, d, J = 8.0 Hz), 8.37 (1H, d, J = 2.0 Hz), 9.40 (2H, m), 11.86 (1H, s, NH).
^{13}C NMR (100 MHz, DMSO-d_6): 20.0, 20.3, 20.4, 20.5 (CH$_3$), 61.9 (CH$_2$), 67.1, 67.8, 72.3, 73.2, 78.2 (CH), 114.1, 131.4, 135.5, 138.5, 150.7 (CH$_{arom}$), 112.0, 114.7, 117.7, 122.3, 131.9, 132.8, 140.5, 156.9 (C$_{arom}$), 166.6, 168.0, 169.0, 169.4, 169.5, 170.1 (C=O).

4.1.5. 7′-Aza-5′-iodo-1-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranos-1-yl)isoindigo 13

Compound **1** (100 mg, 0.209 mmol); compound **13** (91 mg, 0.127 mmol, 61%) as a red solid (mp 220–222 °C).
IR (KBr): ν_{NH} 3450 cm^{-1}; $\nu_{C=O}$ 1770–1700 cm^{-1}; 1227 cm^{-1}.

HRMS (ES+) calcd for C$_{29}$H$_{27}$IN$_3$O$_{11}$ [M + H]$^+$ 720.0690, found 720.0687.
^{1}H NMR (400 MHz, DMSO-d_6): 1.78 (3H, s, CH$_3$), 1.96 (3H, s, CH$_3$), 2.03 (3H, s, CH$_3$), 2.05 (3H, s, CH$_3$), 4.10–4.19 (2H, m), 4.32–4.38 (1H, m), 5.29–5.39 (1H, m), 5.58 (1H, t, J = 9.5 Hz), 5.55–5.71 (1H, br s), 6.11–6.20 (1H, m), 7.14 (1H, t, J = 8.0 Hz), 7.50 (1H, t, J = 7.5 Hz), 7.52–7.58 (1H, br s), 8.42 (1H, d, J = 2.0 Hz), 9.15 (1H, d, J = 8.0 Hz), 9.53 (1H, s), 11.73 (1H, s, NH).
^{13}C NMR (100 MHz, DMSO-d_6): 20.0, 20.3, 20.4, 20.5 (CH$_3$), 61.9 (CH$_2$), 67.2, 67.8, 72.5, 73.2, 78.2 (CH), 112.1, 122.7, 129.4, 133.7, 143.4, 155.0 (CH$_{arom}$), 83.9, 118.4, 120.5, 131.2, 133.2, 141.7, 156.7 (C$_{arom}$), 167.1, 167.7, 168.9, 169.4, 169.5, 170.0 (C=O).

4.1.6. 7′-Aza-5-bromo-5′-iodo-1-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranos-1-yl)isoindigo 14

Compound **2** (150 mg, 0.270 mmol); compound **14** (159 mg, 0.199 mmol, 74%) as a red solid (mp > 260 °C).
IR (KBr) ν_{NH} 3460 cm^{-1}; $\nu_{C=O}$ 1780–1710 cm^{-1}; 1601, 1458, 1365, 1234 cm^{-1}.
HRMS (ES+) calcd for C$_{29}$H$_{26}$^{79}BrIN$_3$O$_{11}$ [M + H]$^+$ 797.9795, found 797.9814.
^{1}H NMR (400 MHz, DMSO-d_6): 1.79 (3H, s, CH$_3$), 1.96 (3H, s, CH$_3$), 2.03 (3H, s, CH$_3$), 2.05 (3H, s, CH$_3$), 4.07–4.18 (2H, m), 4.31–4.37 (1H, m), 5.30–5.41 (1H, m), 5.52–5.64 (2H, m), 6.12–6.20 (1H, m), 7.58 (1H, br d, J = 8.0 Hz), 7.67 (1H, d, J = 8.0 Hz), 8.45 (1H, d, J = 2.0 Hz), 9.39 (1H, d, J = 2.0 Hz), 9.54 (1H, s), 11.81 (1H, s, NH).
^{13}C NMR (100 MHz, DMSO-d_6): 20.0, 20.3, 20.4, 20.5 (CH$_3$), 61.9 (CH$_2$), 67.1, 67.8, 72.3, 73.2, 78.2 (CH), 114.1, 131.3, 135.4, 143.8, 155.6 (CH$_{arom}$), 84.0, 114.7, 118.2, 122.3, 131.6, 132.8, 140.6, 157.0 (C$_{arom}$), 166.6, 167.8, 169.0, 169.4, 169.5, 170.1 (C=O).

4.1.7. General procedure for the preparation of compounds 5–8, 15–17

A mixture of iodinated isoindigo or 7′azaisoindigo and alkyne in acetonitrile in the presence of triethylamine (quantities indicated below), CuI (5 mol%) and Pd(PPh$_3$)$_4$ (3 mol%) was refluxed (see reaction time below). After cooling, water was added and the mixture was extracted with EtOAc. After drying over MgSO$_4$, the solvent was removed and the crude product was purified as indicated below to give the attempted compound.

4.1.8. 5′-(N-Benzyloxycarbonyl-3-aminoprop-1-ynyl)-1-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranos-1-yl)isoindigo 5

Compound **3** (200 mg, 0.278 mmol); Cbz-propargylamine (105 mg, 0.555 mmol); acetonitrile (0.64 mL); NEt$_3$ (42 μL, 0.301 mmol); 24 h. Purification by flash chromatography (cyclohexane/EtOAc 6:4 then 4:6) to give a residue which was dissolved in toluene and then precipitated by addition of cyclohexane. Filtration gave **5** (52 mg, 0.067 mmol, 24%) as a red solid (mp 200 °C).
IR (KBr) ν_{NH} 3386 cm^{-1}; $\nu_{C=O}$ 1790–1710 cm^{-1}; $\nu_{C=C}$ 1600 cm^{-1}.
HRMS (ES+) calcd for C$_{41}$H$_{37}$N$_3$NaO$_{13}$ [M + Na]$^+$ 802.2224, found 802.2202.
^{1}H NMR (500 MHz, DMSO-d_6): 1.77 (3H, s, CH$_3$), 1.96 (3H, s, CH$_3$), 2.03 (3H, s, CH$_3$), 2.05 (3H, s, CH$_3$), 4.08 (2H, d, J = 5.5 Hz), 4.10–4.18 (2H, m), 4.32–4.38 (1H, m), 5.07 (2H, s), 5.28–5.41 (1H, br s), 5.57 (1H, t, J = 8.5 Hz), 5.56–5.67 (1H, br s), 6.10–6.23 (1H, br s), 6.85 (1H, d, J = 8.0 Hz), 7.11 (1H, t, J = 8.0 Hz), 7.30–7.40 (5H, m), 7.42 (1H, d, J = 8.0 Hz), 7.46 (1H, t, J = 7.5 Hz), 7.50–7.58 (1H, br s), 7.85 (1H, t, J = 5.5 Hz, NH), 9.04 (1H, s), 9.11 (1H, d, J = 8.0 Hz), 11.17 (1H, s, NH).
^{13}C NMR (100 MHz, DMSO-d_6): 20.0, 20.3, 20.4, 20.5 (CH$_3$), 30.6, 61.9, 65.6 (CH$_2$), 67.3, 67.8, 72.5, 73.2, 78.3 (CH), 81.9, 85.3 (C$_{alkyne}$), 110.1, 111.8, 122.5, 127.9 (3C), 128.4 (2C), 129.0, 132.2, 133.0, 136.1 (CH$_{arom}$), 114.9, 120.7, 121.5, 131.8, 133.7, 136.9, 141.1, 144.4 (C$_{arom}$), 156.0, 166.9, 168.5, 168.8, 169.4, 169.5, 170.0 (C=O).

4.1.9. 5′-(N-Benzyloxycarbonyl-3-aminoprop-1-ynyl)-5-bromo-1-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranos-1-yl)isoindigo **6**

Compound **4** (50 mg, 0.063 mmol); Cbz-propargylamine (16.5 mg, 0.087 mmol); acetonitrile (1 mL); NEt₃ (20 μL, 0,143 mmol); 6 h. Purification by flash chromatography (cyclohexane/EtOAc 6:4 then 4:6) to give **6** (17.9 mg, 0.021 mmol, 33%) as a red solid (mp 130–132 °C).

IR (KBr) ν_{NH} 3420 cm^{-1}; $\nu_{C=O}$ 1770–1700 cm^{-1}; $\nu_{C\equiv C}$ 1610 cm^{-1}.
HRMS (ES+) calcd for $C_{41}H_{36}{}^{79}BrN_3NaO_{13}$ [M + Na]$^+$ 880.1329, found 880.1333.

^{1}H NMR (400 MHz, DMSO-d_6): 1.77 (3H, s, CH₃), 1.96 (3H, s, CH₃), 2.03 (3H, s, CH₃), 2.05 (3H, s, CH₃), 4.08 (2H, d, J = 5.5 Hz), 4.10–4.18 (2H, m), 4.32–4.37 (1H, m), 5.07 (2H, s), 5.30–5.40 (1H, m), 5.53–5.64 (2H, m), 6.11–6.21 (1H, br s), 6.86 (1H, d, J = 8.0 Hz), 7.29–7.40 (5H, m), 7.44 (1H, d, J = 8.5 Hz), 7.52–7.58 (1H, m), 7.63 (1H, br d, J = 8.0 Hz), 7.83 (1H, t, J = 5.5 Hz, NH), 9.05 (1H, s), 9.37 (1H, d, J = 2.0 Hz), 11.20 (1H, s, NH).

^{13}C NMR (100 MHz, DMSO-d_6): 20.0, 20.3, 20.4, 20.5 (CH₃), 30.6, 61.9, 65.6 (CH₂), 67.2, 67.8, 72.4, 73.2, 78.3 (CH), 81.7, 85.4 (C$_{alkyne}$), 110.3, 113.9, 127.9 (3C), 128.4 (2C), 131.1, 132.6, 134.8, 136.6 (CH$_{arom}$), 114.5, 115.1, 121.4, 122.6, 130.2, 135.3, 136.9, 140.1, 144.8 (C$_{arom}$), 156.0, 166.5, 168.6, 168.9, 169.4, 169.5, 170.1 (C=O).

4.1.10. 5′-(4-Hydroxybut-1-ynyl)-1-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranos-1-yl)isoindigo **7**

Compound **3** (100 mg, 0.139 mmol); but-3-yn-1-ol (16 μL, 0.210 mmol); acetonitrile (2 mL); NEt₃ (40 μL, 0.287 mmol); 14 h. Purification by flash chromatography (cyclohexane/EtOAc 6:4 then 5:5) to give a residue which was dissolved in toluene and then precipitated by addition of cyclohexane. Filtration gave **7** (12.5 mg, 0.0189 mmol, 14%) as a red solid (mp 304 °C).

IR (KBr) $\nu_{NH,OH}$ 3530–3160 cm^{-1}; $\nu_{C=O}$ 1775–1685 cm^{-1}; $\nu_{C\equiv C}$ 1610 cm^{-1}.
HRMS (ES+) calcd for $C_{34}H_{33}N_2O_{12}$ [M + H]$^+$ 661.2034, found 661.2043.

^{1}H NMR (500 MHz, DMSO-d_6): 1.77 (3H, s, CH₃), 1.96 (3H, s, CH₃), 2.03 (3H, s, CH₃), 2.05 (3H, s, CH₃), 2.56 (2H, t, J = 7.0 Hz), 3.59 (2H, dt, J_1 = 7.0 Hz, J_2 = 5.5 Hz), 4.11–4.18 (2H, m), 4.33–4.38 (1H, m), 4.90 (1H, t, J = 5.5 Hz), 5.29–5.38 (1H, br s), 5.57 (1H, t, J = 9.0 Hz), 5.56–5.68 (1H, br s), 6.10–6.20 (1H, br s), 6.82 (1H, d, J = 8.0 Hz), 7.10 (1H, t, J = 8.0 Hz), 7.39 (1H, d, J = 8.0 Hz), 7.46 (1H, t, J = 7.5 Hz), 7.49–7.56 (1H, br s), 9.01 (1H, s), 9.11 (1H, d, J = 8.0 Hz), 11.10 (1H, s, NH).

^{13}C NMR (100 MHz, DMSO-d_6): 20.0, 20.3, 20.4, 20.5 (CH₃), 23.2, 59.9, 61.9 (CH₂), 67.3, 67.8, 72.5, 73.1, 78.3 (CH), 81.1, 86.5 (C$_{alkyne}$), 110.0, 111.8, 122.4, 129.0, 132.1, 132.9, 136.0 (CH$_{arom}$), 116.0, 120.8, 121.5, 131.6, 133.9, 141.3, 143.9 (C$_{arom}$), 166.9, 168.5, 168.8, 169.4, 169.5, 170.1 (C=O).

4.1.11. 5-Bromo-5′-(4-hydroxybut-1-ynyl)-1-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranos-1-yl)isoindigo **8**

Compound **4** (50 mg, 0.063 mmol); but-3-yn-1-ol (8 μL, 0.105 mmol); acetonitrile (1 mL); NEt₃ (20 μL, 0.143 mmol); 3 h. Purification by flash chromatography (cyclohexane/EtOAc 6:4 then 5:5) to give **8** in a 94.3:5.7 w/w mixture with oct-3,5-diyne-1,8-diol (20.4 mg, 0,026 mmol, 41% – the ratio **8**/oct-3,5-diyne-1,8-diol was estimated from the ^{1}H NMR spectra).

IR (KBr) ν_{NH} 3630–3160 cm^{-1}, $\nu_{C=O}$ 1770–1700 cm^{-1}, $\nu_{C\equiv C}$ 1610 cm^{-1}.
HRMS (ES+) calcd for $C_{34}H_{32}{}^{79}BrN_2O_{12}$ [M + H]$^+$ 739.1139, found 739.1147.

^{1}H NMR (400 MHz, DMSO-d_6): 1.78 (3H, s, CH₃), 1.96 (3H, s, CH₃), 2.03 (3H, s, CH₃), 2.05 (3H, s, CH₃), 2.56 (2H, t, J = 7.0 Hz), 3.58 (2H, dt, J_1 = 7.0 Hz, J_2 = 5.5 Hz), 4.09–4.18 (2H, m), 4.32–4.38 (1H, m), 4.91 (1H, t, J = 5.5 Hz), 5.30–5.40 (1H, m), 5.51–5.65 (2H, m),

4.1.12. 7′-Aza-5′-(N-benzyloxycarbonyl-3-aminoprop-1-ynyl)-1-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranos-1-yl)isoindigo **15**

Compound **13** (50.0 mg, 0.069 mmol); Cbz-propargylamine (20.0 mg, 0.106 mmol); acetonitrile (1 mL); NEt₃ (20 μL, 0.143 mmol); 16 h. Purification by flash chromatography (cyclohexane/EtOAc 6:4 then 5:5) to give **15** (21.2 mg, 0.027 mmol, 39%) as a red solid (mp 248–250 °C).

IR (KBr) ν_{NH} 3430 cm^{-1}; $\nu_{C=O}$ 1780–1690 cm^{-1}; $\nu_{C\equiv C}$ 1610 cm^{-1}.
HRMS (ES+) calcd for $C_{40}H_{37}N_4O_{13}$ [M + H]$^+$ 781.2357, found 781.2358.

^{1}H NMR (400 MHz, DMSO-d_6): 1.77 (3H, s, CH₃), 1.96 (3H, s, CH₃), 2.02 (3H, s, CH₃), 2.05 (3H, s, CH₃), 4.11 (2H, d, J = 5.5 Hz), 4.12–4.18 (2H, m), 4.31–4.38 (1H, m), 5.07 (2H, s), 5.28–5.41 (1H, br s), 5.57 (1H, t, J = 9.0 Hz), 5.54–5.69 (1H, br s), 6.09–6.19 (1H, br s), 7.14 (1H, t, J = 8.0 Hz), 7.30–7.40 (5H, m), 7.49 (1H, t, J = 7.5 Hz), 7.53–7.60 (1H, m), 7.88 (1H, t, J = 5.5 Hz, NH), 8.28 (1H, d, J = 1.5 Hz), 9.15 (1H, d, J = 8.0 Hz), 9.25 (1H, s), 11.83 (1H, s, NH).

^{13}C NMR (100 MHz, DMSO-d_6): 20.0, 20.3, 20.4, 20.5 (CH₃), 30.6, 61.9, 65.7 (CH₂), 67.2, 67.8, 72.5, 73.2, 78.3 (CH), 78.9, 88.2 (C$_{alkyne}$), 112.1, 122.7, 127.9 (3C), 128.4 (2C), 129.3, 133.6, 138.7, 152.6 (CH$_{arom}$), 112.7, 115.9, 120.5, 131.5, 133.1, 136.9, 141.6, 157.1 (C$_{arom}$), 156.0, 167.0, 168.2, 168.9, 169.4, 169.5, 170.1 (C=O).

4.1.13. 7′-Aza-5′-(N-benzyloxycarbonyl-3-aminoprop-1-ynyl)-5-bromo-1-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)isoindigo **16**

Compound **14** (53.0 mg, 0.066 mmol); Cbz-propargylamine (13.0 mg, 0.069 mmol); acetonitrile (1 mL); NEt₃ (20 μL, 0,143 mmol); 6 h. Purification by flash chromatography (cyclohexane/EtOAc 6:4 then 4:6) to give **16** (25.5 mg, 0.030 mmol, 45%) as a red solid (mp 210–212 °C).

IR (KBr) ν_{NH} 3414 cm^{-1}; $\nu_{C=O}$ 1770–1700 cm^{-1}; $\nu_{C\equiv C}$ 1618 cm^{-1}.
HRMS (ES+) calcd for $C_{40}H_{36}{}^{79}BrN_4O_{13}$ [M + H]$^+$ 859.1462, found 859.1464.

^{1}H NMR (500 MHz, DMSO-d_6): 1.78 (3H, s, CH₃), 1.96 (3H, s, CH₃), 2.03 (3H, s, CH₃), 2.05 (3H, s, CH₃), 4.11 (2H, d, J = 5.5 Hz), 4.09–4.17 (2H, m), 4.31–4.37 (1H, m), 5.07 (2H, s), 5.30–5.42 (1H, br s), 5.52–5.64 (2H, m), 6.10–6.20 (1H, br s), 7.30–7.40 (5H, m), 7.55–7.62 (1H, br s), 7.67 (1H, br d, J = 7.5 Hz), 7.88 (1H, t, J = 5.5 Hz, NH), 8.30 (1H, s), 9.25 (1H, s), 9.39 (1H, d, J = 1.5 Hz), 11.90 (1H, s, NH).

^{13}C NMR (100 MHz, DMSO-d_6): 20.0, 20.2, 20.4, 20.5 (CH₃), 30.6, 61.9, 65.7 (CH₂), 67.1, 67.8, 72.4, 73.3, 78.3 (CH), 78.8, 88.3 (C$_{alkyne}$), 114.1, 127.9 (3C), 128.4 (2C), 131.3, 135.4, 139.0, 153.1 (CH$_{arom}$), 112.9, 114.7, 115.7, 122.3, 131.5, 133.1, 136.9, 140.4, 157.4 (C$_{arom}$), 156.0, 166.6, 168.3, 169.0, 169.4, 169.5, 170.0 (C=O).

4.1.14. 7′-Aza-5′-(4-hydroxybut-1-ynyl)-1-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranos-1-yl)isoindigo **17**

Compound **13** (90 mg, 0.125 mmol); but-3-yn-1-ol (14 μL, 0.184 mmol); acetonitrile (2 mL); NEt₃ (38 μL, 0.273 mmol); 14 h. Purification by flash chromatography (cyclohexane/EtOAc 6:4 then 4:6) to give **17** (20.6 mg, 0.031 mmol, 25%) as a red solid (mp 160 °C).

IR (KBr) $\nu_{NH,OH}$ 3580–3150 cm^{-1}; $\nu_{C=O}$ 1770–1695 cm^{-1}; $\nu_{C\equiv C}$ 1609 cm^{-1}.
HRMS (ES+) calcd for $C_{33}H_{32}N_3O_{12}$ [M + H]$^+$ 662.1986, found 662.1983.

^{1}H NMR (400 MHz, DMSO-d_6): 1.78 (3H, s, CH_3), 1.96 (3H, s, CH_3), 2.03 (3H, s, CH_3), 2.05 (3H, s, CH_3), 2.60 (2H, t, $J = 6.5$ Hz), 3.61 (2H, dt, $J_1 = 6.5$ Hz, $J_2 = 5.5$ Hz), 4.10–4.19 (2H, m), 4.32–4.38 (1H, m), 4.93 (1H, t, $J = 5.5$ Hz), 5.28–5.40 (1H, m), 5.57 (1H, t, $J = 9.5$ Hz), 5.54–5.73 (1H, br s), 6.09–6.18 (1H, m), 7.14 (1H, t, $J = 8.0$ Hz), 7.50 (1H, t, $J = 7.5$ Hz), 7.52–7.59 (1H, m), 8.25 (1H, d, $J = 1.5$ Hz), 9.15 (1H, d, $J = 8.0$ Hz), 9.23 (1H, s), 11.76 (1H, s, NH).

^{13}C NMR (100 MHz, DMSO-d_6): 20.0, 20.2, 20.4, 20.5 (CH_3), 23.3, 59.7, 61.9 (CH_2), 67.2, 67.8, 72.5, 73.2, 78.3 (CH), 78.1, 89.6 (C_{alkyne}), 112.2, 122.7, 129.3, 133.5, 138.6, 152.5 (CH_{arom}) 113.7, 115.8, 120.5, 131.6, 133.0, 141.4, 156.6 (C_{arom}), 167.0, 168.2, 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^2 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_2. 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 (10^{-5} M final concentration). Cell viability was assayed after 72 h continous 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 Cell-Titer 96 Aqueous One Solution Cell Proliferation Assay kit (Promega Corp) according to the manufacture's instructions. Absorbance was measured at 490 nm with a microplate reader.

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