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Side chain modifications of (indol-3-yl)glyoxamides as antitumor agents

MAUD ANTOINE[1], PASCAL MARCHAND[1], GUILLAUME LE BAUT[1], MICHAEL CZECH[2], SILKE BAASNER[2], & ECKHARD GUNTHER[2]

[1]*Département de Pharmacochimie, Faculté de Pharmacie, Nantes Université, Nantes Atlantique Universités, IICiMed UPRES EA 1155, 1 rue Gaston Veil, Nantes F-44035, France, and* [2]*Æterna Zentaris GmbH, 50 Weismuellerstrasse, D-60314 Frankfurt/Main, Germany*

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Abstract

New series of analogues of *N*-(pyridin-4-yl)-2-[1-(4-chlorobenzyl)-indol-3-yl]glyoxamide D-24851 were synthesized, characterized and tested for their *in vitro* anticancer properties. In the first series, an amino acid spacer was introduced in the glyoxamide chain of D-24851. In the second series, the glyoxamide chain was moved to positions 4 and 5 of indole skeleton. These new compounds were tested on four cancer cell lines (KB, SK-OV-3, NCI-H460 and SF-268), with promising activity for the glycine derivative.

Keywords: *(Indol-3-yl)glyoxamides, microtubule destabilising agents*

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Introduction

Microtubules are dimers of tubulin, a cytoskeletal protein, that are playing an important role during the mitosis by constructing a bipolar spindle that enable the chromosome segregation [1,2]. By targeting microtubules, drugs inhibit cell proliferation by blocking the mitosis at the metaphase/anaphase transition and induce apoptosis; they are one of the most important classes of chemotherapeutics [3–5].

Anti-microtubule agents, also known as tubulin poisons or mitotic spindle poisons, have been isolated from a variety of natural and synthetic sources [6]. Among these agents, taxanes (paclitaxel and docetaxel) and vinca alkaloids (vinblastine and vincristine) are widely used clinically [7–11]. Because of toxicity and poor bioavailability of anti-microtubule agents, combined with multi-drug resistance, there is clearly a need to develop improved antimitotic drugs.

Several promising new compounds that interact with microtubules or tubulin have recently been characterized. Among them, *N*-(pyridin-4-yl)-2-[1-(4-chlorobenzyl)-indol-3-yl]glyoxamide D-24851 (Figure 1) destabilises microtubules and blocks cell cycle at G2-M transition [12–14]. D-24851 is highly cytotoxic *in vitro* and *in vivo*, with efficacy against tumor cell lines showing various resistance phenotypes. Moreover, this compound is orally available and has no neurotoxic effects; D-24851 is currently in phase 1 clinical trials. These encouraging results prompted us to investigate and design new analogues of D-24851.

In this work, we described the synthetic routes used for the modification of the glyoxamide chain of D-24851: the first series was based on the introduction of an amino acid spacer between the keto group of the amide and the nitrogen of the 4-aminopyridine while in the second series, we decided to move the glyoxamide chain in positions 4 and 5 of indole moiety.

Materials and methods

Chemistry

Instrumentation. Melting points were determined on an Electrothermal IA 9000 melting point apparatus in open capillary tubes and are uncorrected. ^{1}H NMR

Correspondence: P. Marchand, Département de Pharmacochimie, Faculté de Pharmacie, Nantes Atlantique Universités, IICiMed UPRES EA 1155, 1 rue Gaston Veil, Nantes F-44035, France. Tel: 33 0 240 412 874. E-mail: pascal.marchand@univ-nantes.fr

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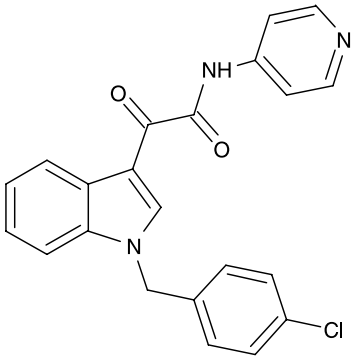

Figure 1. Structure of D-24851.

and ^{13}C spectra were recorded on a Brucker AC 250 or AVANCE 400 spectrometer. Chemical shifts (δ) are reported in part per million (ppm) relative to tetramethylsilane as internal standard (in NMR description, s = singulet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublet, dt = doublet of triplet, qd = quadruplet of doublet and br = broad). Coupling constants J are given in Hz. IR spectra were recorded on a Perkin-Elmer Paragon 1000 PC spectrometer; only the most significant absorption bands have been reported. Electrospray ionization (ESI) mass spectra were recorded on a ESQUIRE-LC Ion Trap System. Reactions were monitored by TLC analysis using Merck silica gel 60F-254 thin-layer plates. Column chromatography was carried out on silica gel Merck 60 (70-230 mesh ASTM). Chemicals and solvents used were commercially available.

For the numbering of hydrogens in the structures in the experimental section, see Figure 2.

*1-(4-Chlorobenzyl)-1H-indole (**2**)*. To a suspension of sodium hydride (1.5 g, 37.6 mmol) in 50 mL of DMSO under nitrogen was added slowly indole **1** (4.0 g, 34.1 mmol) in 15 mL of DMSO. The mixture was stirred at room temperature for 1 h. Then 4-chlorobenzyl chloride (6.1 g, 37.6 mmol) in 10 mL of DMSO was added and the resulting mixture was

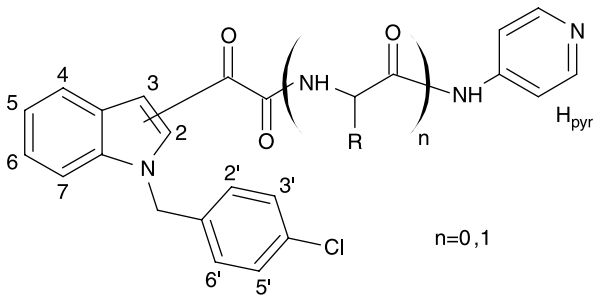

Figure 2. General structure of new synthesized indolylglyoxamides with our numbering of the hydrogens for the NMR spectra in the experimental section.

stirred at room temperature for 6 h. The organic layer was washed with water and the product was extracted into dichloromethane (3 × 200 mL). The organic layer was dried with Na_2SO_4, filtered, and the solvent was evaporated *in vacuo*. Yield 94% as an orange oil. ^1H NMR (DMSO-d_6): 5.46 (s, 2H, CH_2); 6.53 (dd, 1H, H_3, J = 3.1, 1.0); 7.05 (td, 1H, H_5, J = 7.0, 1.2); 7.14 (td, 1H, H_6, J = 7.0, 1.2); 7.23 (d, 2H, $H_{2'}$, $H_{6'}$, J = 8.6); 7.39 (d, 2H, $H_{3'}$, $H_{5'}$, J = 8.6); 7.43 (dd, 1H, H_7, J = 8.2, 1.0); 7.53 (d, 1H, H_2, J = 3.1); 7.60 (dd, 1H, H_4, J = 8.2, 1.0). IR: cm^{-1} 3100-3000, 1475, 1450. MS m/z: 242 (M + H).

*[1-(4-Chlorobenzyl)-1H-indol-3-yl](oxo)acetyl chloride (**3**)*. To a solution of oxalyl chloride (1.1 mL, 12.5 mmol) in 50 mL of Et_2O under nitrogen was added slowly at 0°C 1-(4-chlorobenzyl)-1*H*-indole **2** (3.0 g, 12.5 mmol) in 100 mL of Et_2O. The resulting solution was allowed to warm to room temperature and stirred for 1 h. The solvent was evaporated in vacuum. Recrystallization from diisopropyl ether gave the desired compound as a yellow powder. Yield 72%. Mp: 131-132°C. ^1H NMR (DMSO-d_6): 5.64 (s, 2H, CH_2); 7.30-7.45 (m, 6H, $H_{2'}$, $H_{3'}$, $H_{5'}$, $H_{6'}$, H_5, H_6); 7.61 (dd, 1H, H_7, J = 6.3, 1.0); 8.26 (dd, 1H, H_4, J = 6.3, 1.0); 8.76 (s, 1H, H_2). IR: cm^{-1} 1766, 1655. MS m/z: 333 (M + H).

*Benzyl 2-oxo-2-(pyridin-4-ylamino)ethylcarbamate (**7**)*. To a solution of benzyloxycarbonylglycine **4** (1.0 g, 4.8 mmol) in 30 mL of CH_2Cl_2 was added triethylamine (2.0 mL, 14.3 mmol) and 4-aminopyridine (0.45 g, 4.8 mmol). The resulting solution was cooled to 0°C and phenyl dichlorophosphate DCP (0.7 mL, 4.8 mmol) was added. The mixture was stirred at room temperature for 48 h. Organic layer was washed with saturated sodium hydrogenocarbonate solution and water. The organic layer was dried with Na_2SO_4, filtered and the solvent was evaporated in vacuo. The residue was purified by chromatography (CH_2Cl_2/EtOH: 99/1, 9/1). Yield 23%, yellow powder. Mp: 146-147°C. ^1H NMR (DMSO-d_6): 3.90 (d, 2H, CH_2, J = 6.1); 5.10 (s, 2H, CH_2); 7.26-7.40 (m, 5H, Ph); 7.61 (d, 2H, H_{pyr}, J = 5.8); 7.69 (t, 1H, NH, J = 6.1); 8.47 (d, 2H, H_{pyr}, J = 5.8); 10.47 (br, 1H, NH). IR: cm^{-1} 3450, 1655. MS m/z: 286 (M + H).

*Benzyl 1-methyl-2-oxo-2-(pyridin-4-ylamino)ethylcarbamate (**8**)*. **8** was obtained from benzyloxy carbonylalanine **5** using DCP as previously described for **7**. Yield 37% as a yellow oil. ^1H NMR (DMSO-d_6): 1.33 (d, 3H, CH_3, J = 7.0); 4.20-4.26 (m, 1H, CH); 5.06 (s, 2H, CH_2); 7.38-7.40 (s, 5H, Ph); 7.61 (d, 2H, H_{pyr}, J = 6.4); 7.74 (d, 1H, NH, J = 7.0); 8.46 (d,

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2H, H_{pyr}, J = 6.4); 10.44 (br, 1H, NH). IR: cm^{-1} 3287, 1702, 1682. MS m/z: 300 (M + H).

*Benzyl 2-methyl-1-[(pyridin-4-ylamino)carbonyl]butyl carbamate (**9**)*. To a solution of benzyloxycarbonylisoleucine **6** (1.0 g, 3.7 mmol) in 50 mL of CH_2Cl_2 was added 2-chloro-*N*-methylpyridinium iodide (0.96 g, 3.7 mmol), triethylamine (1.3 mL, 9.4 mmol) and 4-aminopyridine (0.35 g, 3.7 mmol). The resulting mixture was heated at reflux during 8 h. After cooling at room temperature, the organic layer was washed with brine. The organic layer was dried with Na_2SO_4, filtered and the solvent was evaporated in vacuo. The residue was purified by chromatography (CH_2Cl_2/EtOH: 99/1, 9/1). Yield 98%, yellow powder. Mp: 140-141°C. ^{1}H NMR (DMSO-d_6): 0.83-0.91 (m, 8H, CH_2, CH_3); 1.26 (m, 1H, CH); 4.07 (m, 1H, CH); 5.07 (s, 2H, CH_2); 7.39-7.46 (m, 5H, Ph); 7.62 (d, 2H, H_{pyr}, J = 6.4); 7.69 (d, 1H, NH, J = 8.2); 8.47 (d, 2H, H_{pyr}, J = 6.4); 10.52 (br, 1H, NH). IR: cm^{-1} 3279, 1698, 1674. MS m/z: 342 (M + H).

*2-Amino-N-(pyridin-4-yl)acetamide (**10**)*. A solution containing benzyl 2-oxo-2-(pyridin-4-ylamino)ethyl carbamate **7** and Pd/C 5% in catalytic quantity in 30 mL of methanol was hydrogenated at 1 bar for 1 h. The resulting solution was filtrated over celite and the solvent was evaporated under reduced pressure. Yield 84%, yellow oil. ^{1}H NMR (DMSO-d_6): 3.34 (s, 2H, CH_2); 3.37 (br, 2H, NH_2); 7.66 (d, 2H, H_{pyr}, J = 6.4); 8.45 (d, 2H, H_{pyr}, J = 6.4). IR: cm^{-1} 3290, 1642. MS m/z: 152 (M + H).

*2-Amino-N-(pyridin-4-yl)propanamide (**11**)*. **11** was obtained by hydrogenation of **8** as previously described for **10**. Yield 94%, yellow oil. ^{1}H NMR (DMSO-d_6): 1.22 (d, 3H, CH_3, J = 6.4); 3.48 (q, 1H, CH, J = 6.4); 7.63 (d, 2H, H_{pyr}, J = 6.3); 8.42 (d, 2H, H_{pyr}, J = 6.3). IR: cm^{-1} 3223, 1672. MS m/z: 166 (M + H).

*2-Amino-3-methyl-N-(pyridin-4-yl)pentanamide (**12**)*. **12** was obtained by hydrogenation of **9** as previously described for **10**. Yield 55%, yellow oil. ^{1}H NMR (DMSO-d_6): 0.83-0.94 (m, 6H, CH_3); 1.08-1.26 (m, 1H, CH); 1.40-1.76 (m, 2H, CH_2); 3.20 (d, 1H, CH, J = 5.8); 3.27 (br, 2H, NH_2); 7.67 (d, 2H, H_{pyr}, J = 6.1); 8.45 (d, 2H, H_{pyr}, J = 6.1). IR: cm^{-1} 3277, 1671. MS m/z: 208 (M + H).

*2-[1-(4-Chlorobenzyl)-1H-indol-3-yl]-2-oxo-N-{2-oxo-2-[(pyridin-4-yl)amino]ethyl} acetamide (**13**)*. To a solution of [1-(4-chlorobenzyl)-1*H*-indol-3-yl](oxo) acetyl chloride **3** (0.35 g, 1.1 mmol) in 5 mL of THF was added triethylamine (0.15 mL, 1.1 mmol). The resulting solution was cooled to 0°C and then 2-amino-*N*-(pyridin-4-yl)acetamide **10** (0.16 g, 1.1 mmol) in 2 mL of THF was added dropwise. The mixture was heated at reflux for 9 h. After cooling at room temperature, the resulting solution was filtered and the solvent was evaporated. The residue was dissolved in ethyl acetate and washed with water. The organic layer was dried with Na_2SO_4, filtered and the solvent was evaporated in vacuo. The residue was purified by chromatography (CH_2Cl_2/EtOH: 9/1). Yield 25%, orange powder. Mp: 121-122°C. ^{1}H NMR (DMSO-d_6): 4.12 (d, 2H, CH_2, J = 5.8); 5.65 (s, 2H, CH_2); 7.32-7.37 (m, 4H, H_{pyr}, H_5, H_6); 7.40 (dd, 2H, $H_{2'}$, $H_{6'}$, J = 8.2); 7.59-7.65 (m, 3H, $H_{3'}$, $H_{5'}$, H_7); 8.33 (dd, 1H, H_4, J = 9.1, 4.2); 8.48 (d, 2H, H_{pyr}, J = 6.2); 9.05 (t, 1H, NH, J = 5.8); 9.08 (s, 1H, H_2); 10.51 (br, 1H, NH). ^{13}C NMR (DMSO-d_6): 180.79 (C=O), 168.22 (C=O), 163.37 (C=O), 150.36 (2CH), 145.38 (C), 141.33 (CH), 136.09 (C), 135.66 (C), 132.42 (C), 129.23 (2CH), 128.71 (2CH), 126.93 (C), 123.70 (CH), 123.11 (CH), 121.63 (CH), 113.16 (2CH), 111.56 (C), 111.48 (CH), 49.02 (CH_2), 42.79 (CH_2). IR: cm^{-1} 3386, 1679, 1623, 1599, 1499. MS m/z: 447 (M + H).

*2-({2-[1-(4-Chlorobenzyl)-1H-indol-3-yl]-2-oxoacetyl}amino)-N-(pyridin-4-yl) Propanamide (**14**)*. **14** was obtained by reaction of **3** with **11** as previously described for **13**. Yield 44%, yellow powder. Mp: 107-108°C. ^{1}H NMR (DMSO-d_6): 1.49 (d, 3H, CH_3, J = 7.0); 4.57 (qd, 1H, CH, J = 7.0, 6.7); 5.64 (s, 2H, CH_2); 7.29-7.47 (m, 6H, $H_{2'}$, $H_{3'}$, $H_{5'}$, $H_{6'}$, H_5, H_6); 7.61-7.65 (m, 3H, H_{pyr}, H_7); 8.28-8.33 (m, 1H, H_4); 8.48 (d, 2H, H_{pyr}, J = 5.2); 8.97 (d, 1H, NH, J = 6.7); 9.02 (s, 1H, H_2); 10.52 (br, 1H, NH). ^{13}C NMR (DMSO-d_6): 181.06 (C=O), 171.80 (C=O), 163.07 (C=O), 150.36 (2CH), 145.50 (C), 141.22 (CH), 136.13 (C), 135.68 (C), 132.44 (C), 129.13 (2CH), 128.72 (2CH), 126.88 (C), 123.72 (CH), 123.13 (CH), 121.60 (CH), 113.34 (2CH), 111.59 (CH), 111.50 (C), 49.49 (CH), 49.04 (CH_2), 17.55 (CH_3). IR: cm^{-1} 3319, 1681, 1638, 1591, 1491. MS m/z: 462 (M + H).

*2-({2-[1-(4-Chlorobenzyl)-1H-indol-3-yl]-2-oxoacetyl}amino)-3-methyl-N-(pyridin-4-yl) Pentanamide (**15**)*. **15** was obtained by reaction of **3** with **12** as previously described for **13**. Yield 55%, yellow powder. Mp: 101-102°C. ^{1}H NMR (DMSO-d_6): 0.87-0.98 (m, 8H, CH_3, CH_2); 1.21-1.23 (m, 1H, CH); 4.37-4.48 (m, 1H, CH); 5.64 (s, 2H, CH_2); 7.31-7.46 (m, 6H, $H_{2'}$, $H_{6'}$, $H_{3'}$, $H_{5'}$, H_5, H_6); 7.60-7.66 (m, 3H, H_{pyr}, H_7); 8.30 (m, 1H, H_4); 8.49 (d, 2H, H_{pyr}, J = 6.1); 8.75 (d, 1H, NH, J = 8.5); 8.94 (s, 1H, H_2); 10.63 (br, 1H, NH). ^{13}C NMR (DMSO-d_6): 181.13 (C=O), 170.80 (C=O), 163.37 (C=O), 150.44 (2CH), 145.11 (C), 141.05 (CH), 136.18

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(C), 135.61 (C), 132.43 (C), 129.25 (2CH), 128.70 (2CH), 126.78 (C), 123.74 (CH), 123.15 (CH), 121.53 (CH), 113.32 (2CH), 111.56 (CH), 111.52 (C), 58.14 (CH), 49.02 (CH_2), 36.24 (CH), 24.56 (CH_2), 15.30 (CH_3), 10.67 (CH_3). IR: cm^{-1} 3255, 1698, 1634, 1599, 1499. MS m/z: 504 (M + H).

*Methyl 2-methyl-3-nitrobenzoate (**18**)*. A 0.5% chlorhydric acid methanolic solution was added to 2-methyl-3-nitrobenzoic acid **16** (10.0 g, 55.2 mmol). The resulting solution was heated at reflux for 48 h. The solvent was evaporated, and the residue was dissolved in CH_2Cl_2. The organic layer was washed with a saturated solution of sodium hydrogenocarbonate and then with brine. The organic layer was dried with Na_2SO_4, filtered and the solvent was evaporated. Yield 96%, yellow powder. Mp: 35-36°C. ^{1}H NMR (DMSO-d_6): 2.53 (s, 3H, CH_3); 3.94 (s, 3H, CH_3); 7.62 (t, 1H, H_5, J = 7.9); 8.06 (dd, 1H, H_6, J = 7.9, 1.2); 8.10 (dd, 1H, H_4, J = 7.9, 1.2). IR: cm^{-1} 1725, 1526, 1364. MS m/z: 196 (M + H).

*Methyl 3-methyl-4-nitrobenzoate (**19**)*. **19** was obtained by esterification of **17** as previously described for **18**. Yield 98%, white powder. Mp: 41-43°C. ^{1}H NMR (DMSO-d_6): 2.52 (s, 3H, CH_3); 3.94 (s, 3H, CH_3); 7.98-8.02 (m, 1H); 8.09-8.13 (m, 2H). IR: cm^{-1} 1742, 1530, 1354. MS m/z: 196 (M + H).

*Methyl 2-[(E)-2-(dimethylamino)vinyl]-3-nitrobenzoate (**20**)*. To a solution of methyl 2-methyl-3-nitrobenzoate **18** (5.0 g, 23.9 mmol) in 20 mL of DMF was added 7 mL of dimethylformamide dimethylacetal. The resulting solution was heated at 110°C for 8 h. After cooling at room temperature, Et_2O (200 mL) was added and the organic layer was washed twice with water. The organic layer was dried with Na_2SO_4, filtered and the solvent was evaporated. Yield 89% (crude), red oil. ^{1}H NMR (DMSO-d_6): 2.81 (s, 6H, CH_3); 3.83 (s, 3H, CH_3); 5.41 (d, 1H, $H_{1'}$, J = 13.4); 6.40 (d, 1H, $H_{2'}$, J = 13.4); 7.23 (t, 1H, H_5, J = 7.9); 7.75 (dd, 1H, H_6, J = 7.9, 0.8); 7.86 (dd, 1H, H_4, J = 7.9, 0.8). MS m/z: 251 (M + H).

*Methyl 3-[(E)-2-(dimethylamino)vinyl]-4-nitrobenzoate (**21**)*. **21** was obtained using the same procedure as **20**. Yield 93% (crude), red oil. ^{1}H NMR (DMSO-d_6): 2.95 (s, 6H, CH_3); 3.85 (s, 3H, CH_3); 5.55 (d, 1H, $H_{2'}$, J = 13.1); 7.47 (dd, 1H, H_6, J = 8.5, 1.5); 7.55 (d, 1H, $H_{1'}$, J = 13.1); 7.87 (d, 1H, H_5, J = 8.5); 8.19 (d, 1H, H_2, J = 1.5). MS m/z: 251 (M + H).

*Methyl 1H-indole-4-carboxylate (**22**)*. A solution containing methyl 2-[(*E*)-2-(dimethylamino)vinyl]-3-nitrobenzoate **20** and Pd/C 5% in catalytic quantity in 20 mL of benzene was hydrogenated at 1 bar for 6 h. The resulting solution was filtrated over celite and the solvent was evaporated in vacuo. The residue was purified by column chromatography (CH_2Cl_2). Yield 85%, yellow powder. Mp: 68-69°C. ^{1}H NMR (DMSO-d_6): 3.93 (s, 3H, CH_3); 6.98 (s, 1H, H_3); 7.23 (t, 1H, H_6, J = 7.8); 7.58 (d, 1H, H_2, J = 2.7); 7.75 (dd, 1H, H_5, J = 7.8, 0.9); 7.77 (dd, 1H, H_7, J = 7.8, 0.9); 11.50 (br, 1H, NH). IR: cm^{-1} 3338, 1696. MS m/z: 176 (M + H).

*Methyl 1H-indole-5-carboxylate (**23**)*. **23** was obtained using the same procedure as **22**. Yield 73%, beige powder. Mp: 62-64°C. ^{1}H NMR (DMSO-d_6): 3.88 (s, 3H, CH_3); 6.63-6.66 (m, 1H, H_2); 7.50-7.54 (m, 2H, H_6, H_3); 7.80 (dd, 1H, H_7, J = 8.5, 1.5); 8.31 (d, 1H, H_4, J = 0.9); 11.36 (s, 1H, NH). IR: cm^{-1} 3367, 1704. MS m/z: 176 (M + H).

*(1H-Indol-4-yl)methanol (**24**)*. To a solution of lithium aluminium hydride (0.43 g, 11.4 mmol) in 60 mL of THF under nitrogen at 0°C was added methyl 1*H*-indole-4-carboxylate **22** (2.0 g, 11.4 mmol). The reaction mixture was stirred at 0°C for 20 h and then hydrolyzed. The resulting mixture was filtered over celite. The filtrate was washed with water, the organic layer was dried with Na_2SO_4, filtered and the solvent was evaporated in vacuo. Yield 25% (crude), orange oil. ^{1}H NMR (DMSO-d_6): 4.80 (d, 2H, CH_2, J = 5.6); 5.12 (t, 1H, OH, J = 5.6); 6.53-6.55 (m, 1H, H_3); 7.05 (d, 1H, H_7, J = 7.2); 7.10 (t, 1H, H_6, J = 7.1); 7.33 (d, 1H, H_5, J = 7.3); 7.34-7.36 (m, 1H, H_2); 11.12 (br, 1H, NH). MS m/z: 148 (M + H).

*(1H-Indol-5-yl)methanol (**25**)*. **25** was obtained using the same procedure as **24**. Yield 76% (crude), orange oil. ^{1}H NMR (DMSO-d_6): 4.58 (d, 2H, CH_2, J = 5.7); 5.05 (t, 1H, OH, J = 5.7); 6.51 (d, 1H, H_3, J = 2.7); 7.11 (d, 1H, H_6, J = 8.4); 7.38-7.40 (m, 1H, H_7); 7.52 (d, 1H, H_2, J = 3.3); 7.54 (s, 1H, H_4); 11.12 (br, 1H, NH). MS m/z: 148 (M + H).

*H-Indole-4-carboxaldehyde (**26**)*. To a solution of (1H-indol-4-yl)methanol **24** (0.54 g, 3.7 mmol) in 20 mL of CH_2Cl_2, was added manganese dioxide (1.8 g, 21.1 mmol) and the reaction mixture was stirred for 20 h. The resulting solution was filtered over celite and the solvent was evaporated. Yield 49%, brown powder. Mp: 118-119°C. ^{1}H NMR (DMSO-d_6): 7.13 (d, 1H, H_3, J = 3.2); 7.36 (t, 1H, H_6, J = 7.6); 7.69 (d, 1H, H_2, J = 3.2); 7.70 (d, 1H, H_7, J = 7.2); 7.81 (d, 1H, H_5, J = 8.0); 10.23 (s, 1H, CHO); 11.65 (s, 1H, NH). IR: cm^{-1} 1666. MS m/z: 146 (M + H).

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*H-Indole-5-carboxaldehyde (**27**)*. **27** was obtained using the same procedure as **26**. Yield 23%, red oil. ^{1}H NMR (DMSO-d_6): 6.70 (br, 1H, H_3); 7.57 (d, 1H, H_7, J = 7.6); 7.60 (br, 1H, H_2); 7.67 (d, 1H, H_6, J = 7.9); 8.22 (s, 1H, H_4); 10.00 (s, 1H, CHO); 11.66 (br, 1H, NH). IR: cm^{-1} 1670. MS m/z: 146 (M + H).

*1-(4-Chlorobenzyl)-1H-indole-4-carbaldehyde (**28**)*. **Method A** (benzylation of **26**). To a solution of 1*H*-indole-4-carboxaldehyde **26** (2.0 g, 13.8 mmol) in 60 mL of CH_3CN was added cesium carbonate (9.0 g, 27.6 mmol). The resulting mixture was heated at reflux for 1 h and after cooling at room temperature 4-chlorobenzyl chloride (2.2 g, 13.8 mmol) was added. The reaction mixture was stirred for 2 h at reflux. The resulting solution was filtered over celite and the solvent was evaporated. Yield 90%, yellow powder.

Method B (oxidation of **31**). To a solution of [1-(4-chlorobenzyl)-1*H*-indol-4-yl]methanol **31** (0.38 g, 1.4 mmol) in 10 mL of CH_2Cl_2 was added portion-wise pyridinium dichromate (0.53 g, 1.4 mmol). The reaction mixture was stirred for 6 h and then the resulting solution was filtered over celite. The filtrate was washed with a solution of HCl 1 M. The organic layer was dried with Na_2SO_4, filtered and the solvent was evaporated. The residue was purified by chromatography (CH_2Cl_2). Yield 71%, yellow powder. Mp: 80-81°C. ^{1}H NMR (DMSO-d_6): 5.58 (s, 2H, CH_2); 7.19 (d, 1H, H_3, J = 3.1); 7.25 (d, 2H, $H_{2'}$, $H_{6'}$, J = 8.7); 7.38 (t, 1H, H_6, J = 7.9); 7.41 (d, 2H, $H_{3'}$, $H_{5'}$, J = 8.7); 7.73 (d, 1H, H_7, J = 7.9); 7.83 (d, 1H, H_2, J = 3.1); 7.90 (d, 1H, H_5, J = 7.9); 10.23 (s, 1H, CHO). IR: cm^{-1} 1671. MS m/z: 270 (M + H).

*1-(4-Chlorobenzyl)-1H-indole-5-carbaldehyde (**29**)*. To a solution of 1*H*-indole-5-carboxaldehyde **27** (2.0 g, 13.8 mmol) in 60 mL of DMSO under nitrogen was added sodium hydride (0.61 g, 15.2 mmol). The reaction mixture was stirred for 1 h at room temperature and then 4-chlorobenzyl chloride (2.2 g, 13.8 mmol) was added. The reaction mixture was stirred for 6 h. CH_2Cl_2 was added and the organic layer was washed twice with water. The organic layer was dried with Na_2SO_4, filtered and the solvent was evaporated.Yield 94%, beige powder. Mp: 86-87°C. ^{1}H NMR (DMSO-d_6): 5.55 (s, 2H, CH_2); 6.78 (d, 1H, H_3, J = 3.0); 7.26 (d, 2H, H_a, J = 8.4); 7.33 (d, 1H, H_7, J = 7.2); 7.42 (d, 2H, H_b, J = 8.4); 7.68 (m, 1H, H_2); 7.70 (m, 1H, H_6); 8.24 (s, 1H, H_4); 10.01 (s, 1H, CHO). IR: cm^{-1} 1676. MS m/z: 270 (M + H).

*Methyl 1-(4-chlorobenzyl)-1H-indole-4-carboxylate (**30**)*. **30** was obtained using the same procedure as **29**. Yield 90%, yellow powder. Mp: 65-66°C. ^{1}H NMR (DMSO-d_6): 3.93 (s, 3H, CH_3); 5.54 (s, 2H, CH_2); 7.05 (d, 1H, H_3, J = 3.1); 7.23 (d, 2H, $H_{2'}$, $H_{6'}$, J = 8.6); 7.25 (t, 1H, H_6, J = 8.0); 7.40 (d, 2H, $H_{3'}$, $H_{5'}$, J = 8.6); 7.75 (d, 1H, H_2, J = 3.1); 7.79-7.82 (m, 2H, H_5, H_7). IR: cm^{-1} 1702. MS m/z: 300 (M + H).

*1-(4-Chlorobenzyl)-1H-indol-4-yl]methanol (**31**)*. To a solution of methyl 1-(4-chlorobenzyl)-1*H*-indole-4-carboxylate **30** (0.5 g, 1.7 mmol) in 5 mL of THF was added lithium aluminium hydride (0.10 g, 2.5 mmol). The reaction mixture was stirred for 1 h. CH_2Cl_2 was added, and the organic layer was washed twice with water. The organic layer was dried with Na_2SO_4, filtered and the solvent was evaporated. Yield 92%, yellow powder. Mp: 62-63°C. ^{1}H NMR (DMSO-d_6): 4.78 (d, 2H, CH_2, J = 5.5); 5.14 (t, 1H, OH, J = 5.5); 5.46 (s, 2H, CH_2); 6.60 (d, 1H, H_3, J = 3.1); 7.05-7.13 (m, 2H, H_5, H_6); 7.21 (d, 2H, $H_{2'}$, $H_{6'}$, J = 8.4); 7.33 (dd, 1H, H_7, J = 6.1, 1.9); 7.40 (d, 2H, $H_{3'}$, $H_{5'}$, J = 8.4); 7.52 (d, 1H, H_2, J = 3.1). IR: cm^{-1} 3287. MS m/z: 272 (M + H).

*1-(Benzenesulfonyl)indoline (**33**)*. To a solution of indoline **32** (20.0 g, 168 mmol) in 200 mL of 1,2-dichloroethane was added dropwise triethylamine (26 mL, 168 mmol), and a solution of benzenesulfonyl chloride (29.6 g, 168 mmol) in 100 mL of 1,2-dichloroethane. The reaction mixture was stirred for 12 h and the solvent was evaporated. The residue was extracted with CH_2Cl_2 and the organic layer was washed twice with water. The organic layer was dried with Na_2SO_4, filtered and the solvent was evaporated. Yield 97%, white powder. Mp: 132-133°C. ^{1}H NMR (DMSO-d_6): 2.92 (t, 2H, H_3, J = 8.4); 3.95 (t, 2H, H_2, J = 8.4); 7.01 (t, 1H, H_5, J = 7.3); 7.19 (d, 1H, H_7, J = 8.3); 7.23 (t, 1H, H_6, J = 7.3); 7.52 (d, 1H, H_4, J = 8.3); 7.59-7.63 (m, 2H, $H_{3'}$, $H_{5'}$); 7.67-7.71 (m, 1H, $H_{4'}$); 7.82-7.86 (m, 2H, $H_{2'}$ $H_{6'}$). IR: cm^{-1} 1351, 1167. MS m/z: 260 (M + H).

*Ethyl 2-[1-(benzenesulfonyl)indolin-5-yl]-2-oxoacetate (**34**)*. To a solution of aluminium chloride (4.2 g, 31.5 mmol) in 100 mL of CH_2Cl_2 was added chloroxoacetyl chloride (3.5 mL, 31.5 mmol) and the reaction mixture was stirred for 1 h. 1-(Benzenesulfonyl)indoline **33** (5.1 g, 19.7 mmol) was added and the resulting solution was stirred for 5 h. The organic layer was washed with brine and then with aan aqueous saturated sodium hydrogen carbonate solution. The organic layer was dried with Na_2SO_4, filtered and the solvent was evaporated. Yield 97%, beige powder. Mp: 85-86°C. ^{1}H NMR (DMSO-d_6): 1.34 (t, 3H, CH_3, J = 7.1); 3.13 (t, 2H, H_3, J = 8.4); 4.05 (t, 2H, H_2, J = 8.4); 4.42 (q, 2H, CH_2, J = 7.1); 7.62-7.66 (m, 3H, $H_{3'}$, $H_{5'}$, $H_{4'}$); 7.76-7.78 (m, 2H, $H_{2'}$, $H_{6'}$); 7.87 (dd, 1H, H_6,

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J = 8.5, 1.8); 7.94-7.97 (m, 2H, H_7, H_4). IR: cm^{-1} 1740, 1671, 1363, 1178. MS m/z: 360 (M + H).

*Methyl 2-hydroxy-2-(indolin-5-yl)acetate (**35**)*. To a solution of ethyl 2-[1-(benzenesulfonyl)indolin-5-yl]-2-oxoacetate **34** (4.0 g, 11.3 mmol) in 100 mL of methanol was added magnesium (4.1 g, 0.2 mol). The reaction mixture was stirred for 2 h and then CH_2Cl_2 was added. The organic layer was washed with brine. The organic layer was dried with Na_2SO_4, filtered and the solvent was evaporated. Yield 90%, beige powder. Mp: 95-96°C. ^{1}H NMR (DMSO-d_6): 2.91 (t, 2H, H_3, J = 8.5); 3.43 (t, 2H, H_2, J = 8.5); 3.61 (s, 3H, CH_3); 4.96 (d, 1H, CH, J = 5.2); 5.55 (s, 1H, NH); 5.74 (d, 1H, OH, J = 5.2); 6.46 (d, 1H, H_7, J = 7.8); 6.94 (d, 1H, H_6, J = 7.8); 7.06 (s, 1H, H_4). IR: cm^{-1} 3328, 3148, 1742. MS m/z: 208 (M + H).

*Methyl 2-(indol-5-yl)-2-oxoacetate (**36**)*. To a solution of methyl 2-hydroxy-2-(indolin-5-yl)acetate **35** (1.2 g, 5.9 mmol) in 50 mL of toluene was added manganese dioxide (10.2 g, 0.1 mol) and the reaction mixture was stirred for 2 h. The solution is filtered over celite and the solvent was evaporated. Yield 88%, beige powder. Mp: 90-91°C. ^{1}H NMR (DMSO-d_6): 4.00 (s, 3H, CH_3); 5.74 (s, 1H, H_2); 6.72 (s, 1H, H_3); 7.61 (d, 1H, H_6, J = 8.7); 7.74-7.77 (m, 1H, H_7, H_2); 8.27 (s, 1H, H_4); 11.79 (br, 1H, NH). IR: cm^{-1} 3387, 1722, 1664. MS m/z: 204 (M + H).

*Methyl 2-[1-(4-chlorobenzyl)indol-5-yl]-2-oxoacetate (**37**)*. To a solution of methyl 2-(indol-5-yl)-2-oxoacetate **36** (0.17 g, 0.9 mmol) in 8 mL of DMSO was added slowly sodium hydride (0.04 g, 1 mmol). The resulting solution was stirred for 1 h and then a solution of 4-chlorobenzyl chloride (0.15 g, 0.9 mmol) in 4 mL of DMSO was added dropwise. The reaction mixture was stirred for 4 h. The residue was taken with CH_2Cl_2, the organic layer was washed twice with water. The organic layer was dried with Na_2SO_4, filtered and the solvent was evaporated. Yield 91%, yellow powder. Mp: 67-68°C. ^{1}H NMR (DMSO-d_6): 3.93 (s, 3H, CH_3); 5.51 (s, 2H, CH_2); 6.81 (d, 1H, H_3, J = 3.1); 7.26 (d, 2H, $H_{2'}$, $H_{6'}$, J = 8.3); 7.41 (d, 2H, $H_{3'}$, $H_{5'}$, J = 8.3); 7.71-7.78 (m, 3H, H_2, H_6, H_7); 8.30 (d, 1H, H_4, J = 1.5). IR: cm^{-1} 1743, 1666. MS m/z: 328 (M + H).

*[1-(4-Chlorobenzyl)-1H-indol-5-yl]oxoacetic acid (**38**)*. To a solution of methyl 2-[1-(4-chlorobenzyl)indol-5-yl]-2-oxoacetate **37** (1.5 g, 4.8 mmol) in 5 mL of EtOH was added a solution of sodium hydroxyde 1 M (0.6 mL, 6.0 mmol). The reaction mixture is heated at reflux for 3 h. After cooling at room temperature, a solution of hydrochlorid acid was added dropwise. The organic layer was extracted with CH_2Cl_2, dried with Na_2SO_4, filtered and the solvent was evaporated. Yield 82%, red powder. Mp: 120-121°C. ^{1}H NMR (DMSO-d_6): 5.55 (s, 2H, CH_2); 6.81 (d, 1H, H_3, J = 3.1); 7.26 (d, 2H, $H_{2'}$, $H_{6'}$, J = 8.6); 7.42 (d, 2H, $H_{3'}$, $H_{5'}$, J = 8.6); 7.70-7.75 (m, 3H, H_2, H_6, H_7); 8.25 (s,1H, H_4). IR: cm^{-1} 1697, 1666, 1605, 1487. MS m/z: 314 (M + H).

*2-[1-(4-Chlorobenzyl)-1H-indol-5-yl]-2-oxo-N-pyridin-4-ylacetamide (**39**)*. A solution containing [1-(4-chlorobenzyl)-1*H*-indol-5-yl]oxoacetic acid **38** (1.0 g, 3.2 mmol), 2-chloro-*N*-methylpyridinium iodide CNMPI (0.80 g, 3.2 mmol), triethylamine (1.1 mL, 8.0 mmol) and 4-aminopyridine (0.30 g, 3.2 mmol) in 80 mL of CH_2Cl_2 was heated at reflux for 4 days. The organic layer was washed with water, dried with Na_2SO_4 and the solvent was evaporated to give a yellow powder. The residue was purified by chromatography (CH_2Cl_2/EtOH: 95/5). Yield 42%, pale yellow powder. Mp: 167-168°C. ^{1}H NMR (DMSO-d_6): 5.56 (s, 2H, CH_2); 6.81 (d, 1H, H_3, J = 2.7); 7.24 (d, 2H, $H_{2'}$, $H_{6'}$, J = 8.5); 7.43 (d, 2H, $H_{3'}$, $H_{5'}$, J = 8.5); 7.69-7.75 (m, 2H, H_2, H_7); 7.76 (d, 2H, H_{pyr}, J = 6.4); 7.83 (dd, 1H, H_6, J = 7.0, 1.5); 8.40 (d, 1H, H_4, J = 1.5); 8.57 (d, 2H, H_{pyr}, J = 6.4); 11.33 (br, 1H, NH). ^{13}C NMR (DMSO-d_6): 188.67 (C=O), 165.33 (C=O), 150.49 (2CH), 144.67 (C), 138.99 (C), 136.63 (C), 132.15 (C), 131.61 (CH), 128.83 (2CH), 128.60 (2CH), 127.98 (C), 125.66 (CH), 123.99 (C), 122.32 (CH), 113.99 (2CH), 110.95 (CH), 103.90 (CH), 48.53 (CH_2). IR: cm^{-1} 3346, 1654, 1587, 1511. MS m/z: 390 (M + H).

Results and discussion

Chemistry

In the first series, with an amino acid spacer (Ala, Gly and Ile) between the keto group and the nitrogen of 4-aminopyridine of the glyoxamide chain, the key intermediate **3** was prepared in two steps consisting in a benzylation of indole and then reaction of 4-chlorobenzylindole **2** with oxalyl chloride (Scheme 1) [15]. Amines **10-12** were prepared from the corresponding *N*-CBz amino acids *via* amidification with 4-aminopyridine then using cleavage of the protecting group of the amine by hydrogenation with Pd on charcoal 5% in methanol. Reaction of glyoxyl chloride **3** with amines **10-12** in basic medium afforded **13-15** with moderate yields (25-55%).

In the second series, the glyoxamide chain of D-24851 was moved in positions 4 and 5 of the indole ring. The corresponding glyoxylic acids can be synthesized from aldehydes or esters, in our cases 4- and 5-formylindoles or methyl *1H*-indole-4- and 5-carboxylates. Leimgruber- Batcho method was used to build the indole ring [16]. Compounds **22-23** were synthesized in three steps from 2-methyl-3-nitrobenzoic

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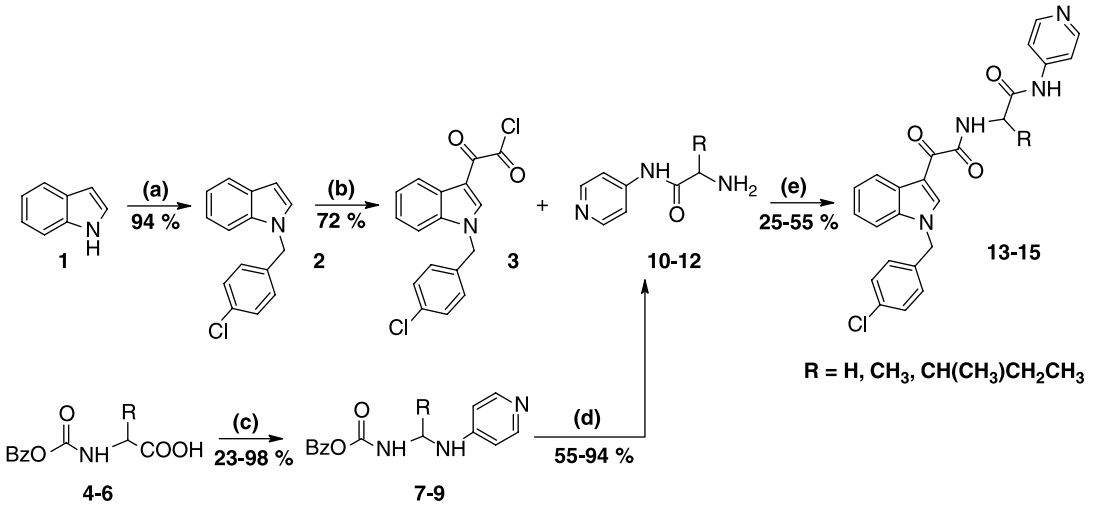
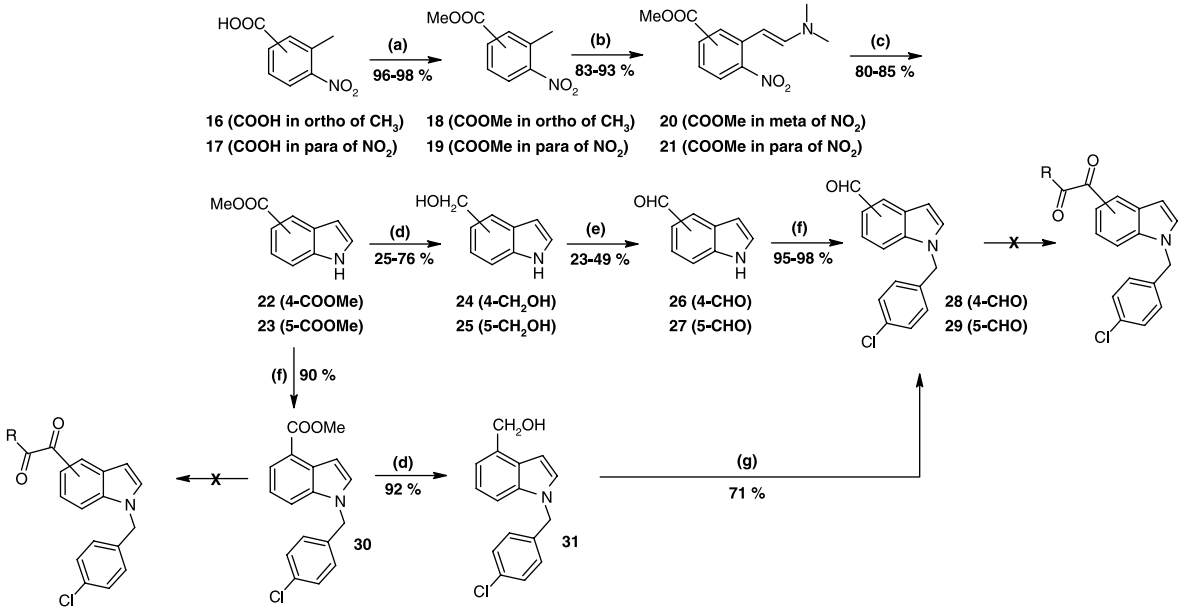


Scheme 1. Preparation of analogues of D-24851 with an amino spacer **13-15**. (a) i) NaH, DMSO, rt, 1 h ii) 4-chlorobenzyl chloride, DMSO, rt, 6 h (b) ClCOCOCl, Et_2O, rt, 1 h (c) DCP, Et_3N, CH_2Cl_2, rt, 2 days (R = H, CH_3) or CNMPI, Et_3N, CH_2Cl_2, Δ, 5 h (R = $CH(CH_3)CH_2CH_3$) (d) H_2, Pd/C 5%, MeOH, rt, 1 h (e) Et_3N, THF, Δ, 10 h.

acid **16** and 3-methyl-4-nitrobenzoic acid **17** with good yields (Scheme 2) [17]. Reduction of methyl esters **22-23** with lithium aluminium hydride in THF and subsequent oxidation of alcohols **24-25** with manganese dioxide afforded 4- and 5-formylindoles **26-27** with moderate yields (23-49%) [18]. Benzylation of **26** and **27** with 4-chlorobenzyl chloride using cesium carbonate in acetonitrile or sodium hydride in DMF gave **28** and **29** with excellent yields (95-98%). Compound **29** was also prepared from **22**, by benzylation of the indole in a first time leading to **30** (Scheme 2). Then the reduction of the ester function into alcohol using lithium aluminium hydride led to **31** with excellent yield and subsequent oxidation with



Scheme 2. Preparation of key intermediates for the synthesis of analogues of D-24851 with the glyoxamide chain in positions 4 and 5 of indole ring. (a) HCl, MeOH, Δ, 48 h (b) DMFDMA, DMF, 110°C, 8 h (c) H_2, Pd/C 5%, benzene, rt, 6 h (d) $LiAlH_4$, THF, 0°C or rt, 20 h (e) MnO_2, CH_2Cl_2, rt, 24 h (f) 4-chlorobenzyl chloride, Cs_2CO_3, CH_3CN, Δ, 3 h (g) i) NaH, DMSO, rt, 1 h ii) 4-chlorobenzyl chloride, DMSO, rt, 4 h (h) PDC, CH_2Cl_2, rt, 6 h.

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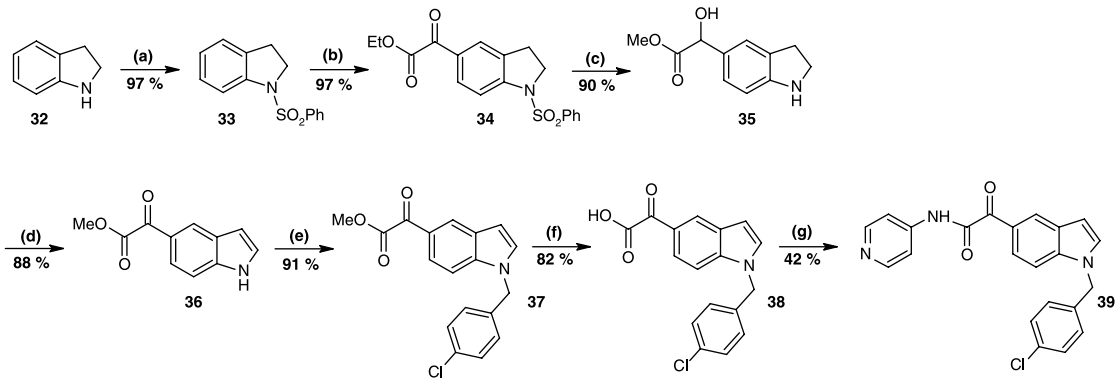

Scheme 3. Synthesis of analogue of D-24851 with the glyoxamide chain in position 5 of the indole ring. (a) $PhSO_2Cl$, Et_3N, $(CH_2)_2Cl_2$, rt, 12 h (b) $ClCOCOOEt$, $AlCl_3$, CH_2Cl_2, rt, 6 h (c) Mg, MeOH, rt, 2 h (d) MnO_2, toluene, Δ, 2 h (e) i) NaH, DMSO, rt, 1 h ii) 4-chlorobenzyl chloride, DMSO, rt, 4 h (f) NaOH 1 M, EtOH, Δ, 3 h (g) CNMPI, Et_3N, CH_2Cl_2, Δ, 4 days

PDC gave **29** with very good yield thus indicating that it was better to do first the benzylation of indole before the transformation of ester into aldehyde.

Various methods described in the literature [19,20] to obtain glyoxylic acids from aldehydes and esters were tried: 1) conversion of aldehydes and esters in α-ketonitriles, precursors of glyoxylic acids, *via* α-cyanhydrines [21] and acid chlorides [22] respectively, 2) oxidation of nitroaldols (obtained from aldehydes) with copper salts [23], 3) hydrolysis of α-iminonitriles (obtained from aldehydes) [24] or 4) hydrolysis of α-oxothioesters (obtained from esters) [25–27]. Using these methodologies, all the attempts to synthesize the corresponding and desired glyoxylic acids from ours aldehydes and esters were unsuccessful.

However, for compound bearing the glyoxamide chain in position 5 of the indole nucleus, another pathway was used, *via* the indoline **32**, which was first protected by a benzenesulfonyle in order to orient the Friedel-Crafts reaction in position 5 of the indoline moiety (Scheme 3) [28]. The reaction of 1-(benzenesulfonyl)indoline **33** with ethylchlorooxoacetate in the presence of aluminium trichloride in dichloromethane led to **34** with excellent yield. Cleavage of the protecting group was achevied using magnesium in methanol [29] with concomitant reduction of the keto group and transesterification to give **35**. Oxidation of indoline into indole with manganese dioxide in toluene led to **36**, with simultaneous re-oxidation of alcohol into ketone. Benzylation of indole as described above provided **37**. Then hydrolysis of **37** and amidification of glyoxylic acid **38** with 4-aminopyridine using CNMPI as coupling agent [30] gave 2-[1-(4-chlorobenzyl)-1*H*-indol-5-yl]-2-oxo-*N*-pyridin-4-ylacetamide **39**.

Pharmacology

The XTT assay quantifies cellular metabolic activity which correlates with cellular viability and/or cell number [31]. This cytotoxicity assessment has been conducted with four diverse tumor cell lines, namely KB/HeLa (ATCC CCL17, human cervix carcinoma), SK-OV-3 (ATCC HTB-77, human ovarian carcinoma), SF-268 (NCI 503138, CNS cancer glioma), and NCI-H460 (NCI 503473, large cell lung cancer).

Test compounds in 100% DMSO at 1 mg/mL are added to the tumor cell lines to final concentrations of 3.16 μg/mL compound and 0.3% DMSO. After 45 h of incubation at 37°C/5%CO_2 1 mg/mL XTT (Serva, cat. no 38450) and 76.6 μg/mL PMS (Sigma, cat. no P9625) are incubated with the cells for an additional 3 h. After 48 h of total compound incubation, cellular metabolic activity is quantified by single point measurement of absorbance at 490 nm. Non-treated cells and blank controls w/o cells are set as reference values of 0% and 100% inhibition, respectively. Compounds which show an inhibition of cellular viability of 50% in at least one of the four cell lines analysed are subjected subsequently to EC_{50} determination in the same panel of cell lines.

Compounds **13-15** and **39** were evaluated in XTT assay and the pharmacological results are reported in Table I. For the compounds with an amino acid spacer, alanine derivative **14** showed only poor activity ($1.49 < IC_{50} < 8.07$ μg/mL) compared with the lead compound D-24851 ($0.017 < IC_{50} < 0.077$ μg/mL). Isoleucine derivative **15** showed no activity on three cell lines (KB, SK-OV-3 and NCI-H460) but better activity on SF-268 (0.004 μg/mL) than D-24851 (0.077 μg/mL). Finally, glycine derivative **13** had better activity on SK-OV-3 (0.017 μg/mL) and SF-268 (0.015 μg/mL) than D-24851 (0.030 μg/mL on SK-OV-3 and 0.077 μg/mL on SF-268) but less activity on other cell lines (KB and NCI-H460). The introduction of an amino acid spacer did not improve the pharmacological profile of the glyoxamide series. Compound **39** with the glyoxamide chain in position-5 of indole was not active, indicating that the position 3 of the *N*-pyridin-4-ylglyoxamide on indole is essential for the interaction with the target.

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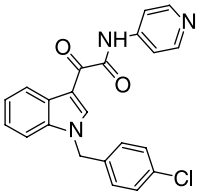
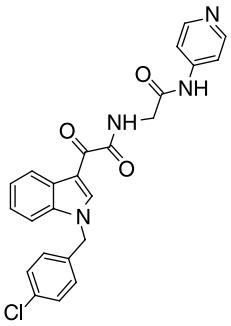
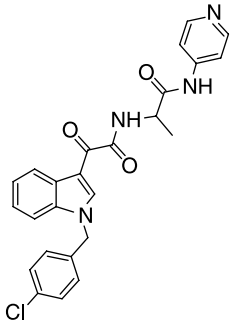
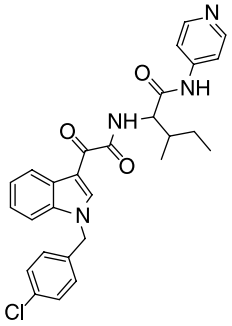
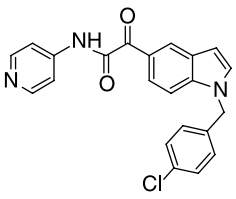
Table I. Anticancer activity of indolylgloxamides in cancer cell lines.

N°	Structure	Cell lines			
		KB IC_{50} (μg/mL)	**SK-OV-3** IC_{50} (μg/mL)	**NCI-H460** IC_{50} (μg/mL)	**SF-268** IC_{50} (μg/mL)
D-24851		0.017	0.030	0.064	0.077
13		0.216	0.017	0.136	0.015
14		6.44	8.07	1.49	2.15
15		270.8	0.224	1.11	0.004
39		ND	18.49	0.331	67.0

[a]All values are the mean of at least 2 determinations.

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Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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