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ORIGINAL ARTICLE

Synthesis and evaluation of the antiproliferative activity of novel isoindolo[2,1-*a*]quinoxaline and indolo[1,2-*a*]quinoxaline derivatives

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Abstract
A novel series of isoindolo[2,1-*a*]quinoxaline and indolo[1,2-*a*]quinoxaline derivatives was synthesized and evaluated *in vitro* against various human cancer cell lines for antiproliferative activity. These new compounds displayed activity against leukemia and breast cancer cell lines in the 3- to 18-µM concentration range.

Keywords: Isoindolo[2,1-a]quinoxaline, indolo[1,2-a]quinoxaline, antiproliferative agents

Introduction

The pyrrolo[1,2-*a*]quinoxaline heterocyclic framework constitutes the basis of an important class of compounds possessing interesting biological activities. These compounds have been reported to serve as key intermediates for the assembly of several heterocycles including antipsychotic agent[1], anti-HIV agent[2], adenosine A_3 receptor modulator[3], antiparasitic agents[4–7], and antitumor agents[8,9]. In this last field, the discovery and development of novel therapeutic agents are one of the most important goals in medicinal chemistry. Recently, we have designed and developed a series of new interesting antiproliferative-substituted pyrrolo[1,2-*a*]quinoxalines **I**–**II**[10,11] (Figure 1).

As an extension of our work on the development of anticancer drugs of the pyrroloquinoxaline type[10,11], we synthesized new conformationally restricted analogues of our previously synthesized compounds **II** such as isoindolo[2,1-*a*]quinoxaline and indolo[1,2-*a*]quinoxaline

structures **1**, which could be defined as new bioisosteres of the pyrrolo[1,2-*a*]quinoxaline skeleton (Figure 1). Cyclizing open structures or creating an additional ring system in a given structure represents one of the useful methods in the search for biologically active conformations. The end result is a more constrained molecule, with an imposed conformation[12]. Moreover, these new isoindolo[2,1-*a*]quinoxaline and indolo[1,2-*a*]quinoxaline moieties **1** were substituted by a benzylpiperidinyl benzimidazolone substituent or benzylpiperidinyl fluorobenzimidazole group in analogy to our previously bioactive reference compounds **I**–**II**[10,11]. The antiproliferative profile of the obtained derivatives **1** was then evaluated *in vitro* against a panel of five leukemic cell lines: U937, K562, Jurkat, U266, and HL60, and against one breast cancer cell line: MCF7. Moreover, to determine their respective cytotoxicity, the new isoindolo[2,1-*a*]quinoxaline and indolo[1,2-*a*]quinoxaline derivatives were tested on activated human peripheral blood mononuclear cells.
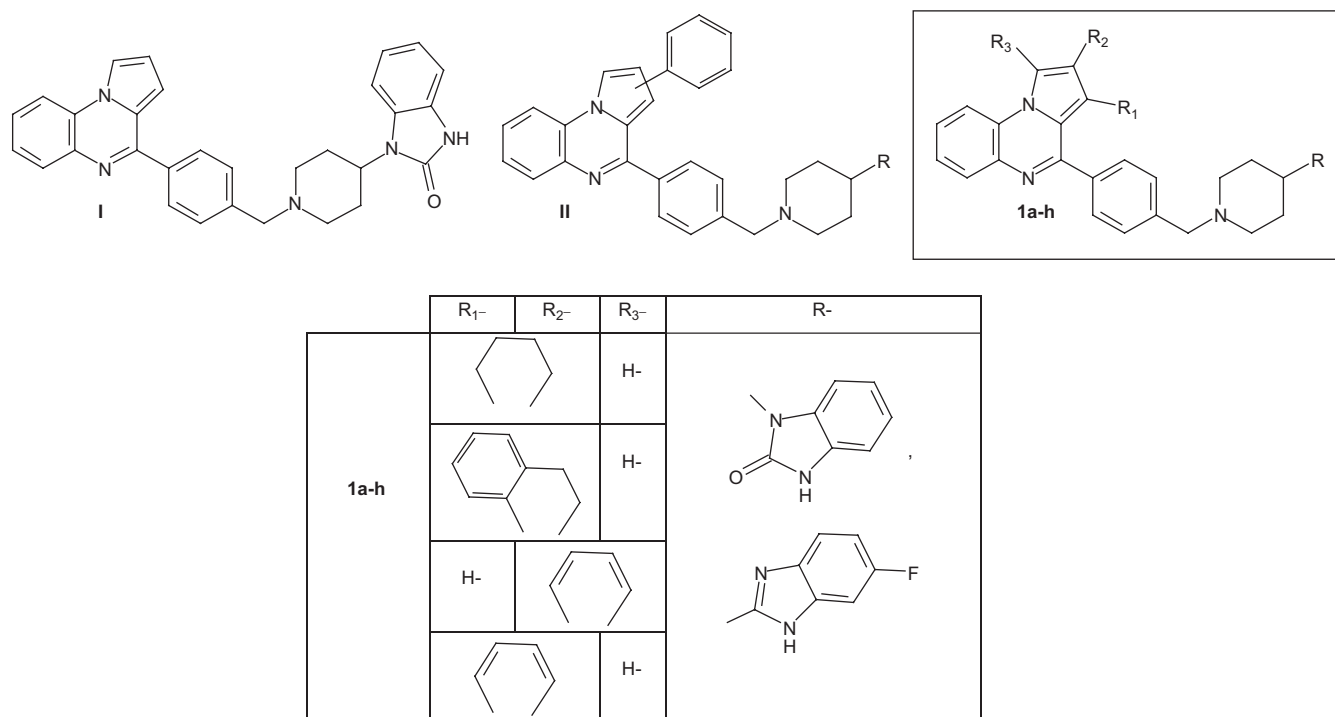
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Figure 1. Structure of compounds I, II and new synthesized substituted isoindolo- or indoloquinoxaline derivatives **1a-h**.

Materials and methods

Chemistry

Instrumentation

Melting points were determined with an SM-LUX-POL Leitz hot-stage microscope and reported uncorrected. IR spectra were recorded on a BRUKER IFS-25 spectrophotometer. NMR spectra were recorded on a BRUKER AVANCE 300 spectrometer (300 MHz). Chemical shifts refer to tetramethylsilane, which was used as an internal reference. Analytical thin layer chromatography was carried out on 0.25 precoated silica gel plates (POLYGRAM SIL G/UV$_{254}$) with visualisation by irradiation with a UV lamp. Silica gel 60 (70–230 mesh) was used for column chromatography. Mass spectra were recorded on a Micromass-Waters Q-TOF Ultima spectrometer. Elemental analyses were conducted by CNRS, Vernaison, France. Compound LY-294002 was purchased from Sigma-Aldrich. Microwave experiments were carried out at atmospheric pressure using a microwave reactor (MILESTONE *Microwave Laboratory Systems* Start S). The instrument consists of a continuous focused microwave power output from 0 to 850 W.

Synthesis of ethyl 2-(2-nitrophenyl)-4,5,6,7-tetrahydroisoindole-1-carboxylate, ethyl 2-(2-nitrophenyl)-4,5-dihydrobenzo[e]isoindole-1-carboxylate and ethyl 1-(2-nitrophenyl)indole-2-carboxylate (3a-c). Method A: To the solution of methyl or ethyl substituted pyrrole-2-carboxylate **2a**–**c** or (3.4 mmol) in 12 mL of dimethylformamide (DMF), cesium carbonate (4.06 mmol) was added. The mixture was stirred at room temperature for 10 min, then 1-fluoro-2-nitrobenzene (5.1 mmol) was added. The reaction mixture was refluxed for 1 h 30 min, then diluted in AcOEt (60 mL) after cooling. The reaction mixture was washed with water (2 × 50 mL), then brine (50 mL) and dried over sodium sulfate. The organic layer was concentrated under vacuum to give products **3a**–**c**. Method B: A suspension of methyl- or ethyl-substituted pyrrole-2-carboxylate **2a**–**b** (3.4 mmol), 1-fluoro-2-nitrobenzene (5.1 mmol), and cesium carbonate (4.06 mmol) in 12 mL of DMF was irradiated for 10 min. The irradiation was programmed to maintain a constant temperature (200°C) with a maximal output power of 850 W. The reaction mixture was then diluted in AcOEt (60 mL), washed with water (2 × 50 mL) and brine (50 mL) and then dried over sodium sulfate. The organic layer was concentrated under vacuum to give products **3a**–**b** as oil.

Ethyl 2-(2-nitrophenyl)-4,5,6,7-tetrahydroisoindole-1-carboxylate (3a). Yield: 95% (method A), 88% (method B), yellow oil; IR v_{max} (KBr)/cm^{-1} 1705 (CO); ^{1}H NMR (300 MHz, CDCl$_3$) δ 8.04 (dd, 1H, J = 7.95 and 1.55 Hz, H-3′), 7.66 (ddd, 1H, J = 7.95, 7.80 and 1.55 Hz, H-4′), 7.54 (ddd, 1H, J = 7.95, 7.80 and 1.55 Hz, H-5′), 7.38 (dd, 1H, J = 7.95 and 1.55 Hz, H-6′), 6.63 (s, 1H, H-3), 4.10 (q, 2H, J = 7.00 Hz, CH$_2$), 2.91–2.86 (m, 2H, CH$_2$), 2.61–2.58 (m, 2H, CH$_2$), 1.83–1.78 (m, 4H, 2 CH$_2$), 1.16 (t, 3H, J = 7.00 Hz, CH$_3$). Anal. Calcd. for C$_{17}$H$_{18}$N$_2$O$_4$: C, 64.96; H, 5.77; N, 8.91. Found: C, 65.12; H, 5.72; N, 8.79.

Ethyl 2-(2-nitrophenyl)-4,5-dihydrobenzo[e]isoindole-1-carboxylate (3b). Yield: 97% (method A), 86% (method B), orange oil; IR v_{max} (KBr)/cm^{-1} 1710 (CO); ^{1}H NMR (300 MHz, CDCl$_3$) δ 8.19 (dd, 1H, J = 8.00 and 2.00 Hz, H-9), 8.06 (dd, 1H, J = 8.10 and 1.50 Hz, H-3′), 7.69 (ddd, 1H, J = 8.10, 7.50 and 1.50 Hz, H-4′), 7.58 (ddd, 1H, J = 8.10, 7.50 and 1.50 Hz, H-5′), 7.44 (dd, 1H, J = 8.10 and 1.50 Hz, H-6′), 7.31–7.18 (m, 3H, H-6, H-7 and H-8), 4.11 (q, 2H, J = 7.15 Hz, CH$_2$), 2.92–2.88 (m, 2H, CH$_2$), 2.72-2.66 (m,

2H, CH$_2$), 1.05 (t, 3H, $J = 7.15$ Hz, CH$_3$). Anal. Calcd. for C$_{21}$H$_{18}$N$_2$O$_4$: C, 69.60; H, 5.01; N, 7.73. Found: C, 69.85; H, 4.92; N, 7.96.

Ethyl 1-(2-nitrophenyl)indole-2-carboxylate (*3c*). Yield: 75% (method A), yellow crystals, mp = 88°C[13].

Synthesis of 7,8,9,10-tetrahydro-5H-isoindolo[2,-1-a]quinoxalin-6-one, 11,12-dihydro-5H-benzo[e]isoindolo[2,1-a]quinoxalin-6-one and 5H-indolo[1,2-a]quinoxalin-6-one (4a–c). Method A: A suspension of 3a–c (2.5 mmol) and iron powder (10 mmol) in 12 mL of acetic acid was heated under reflux for 2 h. The reaction mixture was cooled, suspended in 35 mL of a 1-M aqueous solution of HCl, agitated, then filtered off, washed with 1 M (25 mL) HCl, followed by water, AcOEt, Et$_2$O and then dried to give 4a–c as a white solid. Method B: A mixture of pyrrole-2-carboxylate ester 2a (0.25 mmol), *o*-iodotrifluoroacetanilide (0.375 mmol), CuI (0.025 mmol), *l*-proline (0.05 mmol), and K$_2$CO$_3$ (0.75 mmol) in 5 mL of DMSO was stirred at 80°C for 24 h. To the cooled solution, 1.5 mL of water was added. The mixture was then heated at 60°C for 15 h. The mixture was cooled to room temperature and diluted with 150 mL of ethyl acetate, washed with water, dried over Na$_2$SO$_4$, and concentrated in vacuum. The residue was purified by column chromatography on silica gel (eluting with 1:1 petroleum ether/ethyl acetate) to provide the desired product 4a.

7,8,9,10-Tetrahydro-5H-isoindolo[2,1-a]quinoxalin-6-one (4a). Yield: 81% (method A), 46% (method B), beige crystals, mp = 273°C; IR ν_{max} (KBr)/cm^{-1} 1645 (CO); ^{1}H NMR (300 MHz, DMSO-d$_6$) δ 10.96 (s, 1H, NH), 7.87 (dd, 1H, $J = 7.90$ and 1.45 Hz, H-1), 7.83 (s, 1H, H-11), 7.21–7.10 (m, 3H, H-2, H-3, and H-4), 2.95–2.91 (m, 2H, CH$_2$), 2.66–2.63 (m, 2H, CH$_2$), 1.74–1.71 (m, 4H, 2 CH$_2$). Anal. Calcd. for C$_{15}$H$_{14}$N$_2$O: C, 75.61; H, 5.92; N, 11.76. Found: C, 75.47; H, 6.04; N, 11.71.

11,12-Dihydro-5H-benzo[e]isoindolo[2,1-a]quinoxalin-6-one (4b). Yield: 79% (method A), beige crystals, mp = 297°C; IR ν_{max} (KBr)/cm^{-1} 1650 (CO); ^{1}H NMR (300 MHz, DMSO-d$_6$) δ 11.26 (s, 1H, NH), 8.85 (dd, 1H, $J = 8.10$ and 1.90 Hz, H-7), 8.12 (s, 1H, H-13), 7.98 (dd, 1H, $J = 7.95$ and 1.50 Hz, H-1), 7.27–7.17 (m, 6H, H-2, H-3, H-4, H-8, H-9 and H-10), 2.85–2.82 (m, 2H, CH$_2$), 2.76–2.73 (m, 2H, CH$_2$). Anal. Calcd. for C$_{19}$H$_{14}$N$_2$O: C, 79.70; H, 4.93; N, 9.78. Found: C, 79.93; H, 4.99; N, 9.67.

5H-Indolo[1,2-a]quinoxalin-6-one (4c). Yield: 94% (method A), beige crystals, mp > 300°C[13].

Synthesis of 6-chloro-isoindolo[2,1-a]quinoxalines and 6-chloro-indolo[1,2-a]quinoxaline (5a–d). A solution of 4a–d (30 mmol) in POCl$_3$ (60 mL) was refluxed for 4 h. After removing excess of reactive under *vacuum*, the residue was carefully dissolved in water at 0°C and the resulting solution was made basic with 32% aqueous ammonium hydroxide solution. The precipitate was filtered, washed with water, dried and extracted with dichloromethane. The organic layer was washed with water, dried over magnesium sulfate, and evaporated to dryness to give 5.

6-*Chloro-7,8,9,10-tetrahydroisoindolo[2,1-a]quinoxaline (5a).* Yield: 84%, beige crystals, mp = 127°C; ^{1}H NMR

(300 MHz, CDCl$_3$) δ 7.79 (dd, 1H, $J = 8.0$ and 1.40 Hz, H-1), 7.66 (dd, 1H, $J = 8.0$ and 1.40 Hz, H-4), 7.63 (s, 1H, H-11), 7.45 (ddd, 1H, $J = 8.0$, 7.75 and 1.40 Hz, H-2), 7.36 (ddd, 1H, $J = 8.0$, 7.75 and 1.40 Hz, H-3), 3.17 (t, 2H, $J = 5.80$ Hz, CH$_2$), 2.78 (t, 2H, $J = 5.80$ Hz, CH$_2$), 1.90–1.80 (m, 4H, 2 CH$_2$). Anal. Calcd. for C$_{15}$H$_{13}$ClN$_2$: C, 70.18; H, 5.10; N, 10.91. Found: C, 69.96; H, 5.38; N, 11.05.

6-Chloro-11,12-dihydro-benzo[e]isoindolo[2,1-a]quinoxaline (5b). Yield: 83%, beige crystals, mp = 173°C; ^{1}H NMR (300 MHz, CDCl$_3$) δ 8.07 (dd, 1H, $J = 8.20$ and 1.40 Hz, H-7), 7.88 (dd, 1H, $J = 7.80$ and 1.20 Hz, H-1), 7.86 (s, 1H, H-13), 7.77 (dd, 1H, $J = 7.80$ and 1.20 Hz, H-4), 7.52 (ddd, 1H, $J = 7.80$, 7.20 and 1.20 Hz, H-2), 7.42 (ddd, 1H, $J = 7.80$, 7.20 and 1.20 Hz, H-3), 7.40–7.32 (m, 2H, H-8 and H-10), 7.25 (ddd, 1H, $J = 8.20$, 7.55 and 1.40 Hz, H-9), 2.94–2.89 (m, 2H, CH$_2$), 2.86–2.81 (m, 2H, CH$_2$). Anal. Calcd. for C$_{19}$H$_{13}$ClN$_2$: C, 74.88; H, 4.30; N, 9.19. Found: C, 74.74; H, 4.19; N, 9.30.

6-Chloro-indolo[1,2-a]quinoxaline (5c). Yield: 84%, pale-yellow crystals, mp = 129°C; ^{1}H NMR (300 MHz, CDCl$_3$) δ 8.46 (dd, 1H, $J = 8.20$ and 1.30 Hz, H-11), 8.41 (dd, 1H, $J = 8.40$ and 1.10 Hz, H-1), 7.98 (dd, 1H, $J = 8.40$ and 1.10 Hz, H-4), 7.95 (dd, 1H, $J = 8.20$ and 1.30 Hz, H-8), 7.68–7.57 (m, 2H, H-2 and H-3), 7.51–7.43 (m, 2H, H-9 and H-10), 7.37 (s, 1H, H-7). Anal. Calcd. for C$_{15}$H$_9$ClN$_2$: C, 71.30; H, 3.59; N, 11.09. Found: C, 71.25; H, 3.82; N, 10.87.

6-Chloro-isoindolo[2,1-a]quinoxaline (5d). Yield: 47%, pale-yellow crystals, mp = 183°C; (Found M$^+$: 253.0522, C$_{15}$H$_9$ClN$_2$ requires 253.0533); ^{1}H NMR (300 MHz, CDCl$_3$) δ 8.65 (m, 1H, H-1), 8.54 (s, 1H, H-11), 8.12 (m, 1H, H-10), 8.03 (m, 1H, H-7), 7.92 (m, 1H, H-4), 7.65–7.61 (m, 2H, H-2 and H-3), 7.51–7.46 (m, 2H, H-8 and H-9). Anal. Calcd. for C$_{15}$H$_9$ClN$_2$: C, 71.30; H, 3.59; N, 11.09. Found: C, 71.56; H, 3.54; N, 10.93.

Synthesis of 4-(isoindolo[2,1-a]quinoxalin-6-yl)benzaldehydes and 4-(indolo[1,2-a]quinoxalin-6-yl)benzaldehyde (6a–d). To suspension of compound 5a–d (4.64 mmol) and Pd(PPh$_3$)$_4$ (0.232 mmol) in a mixture of toluene/EtOH (75/4.1 mL) under nitrogen were added K$_2$CO$_3$ (5.1 mmol) and 4-formylphenylboronic acid (5.1 mmol). The reaction mixture was refluxed for 24 h, and the cooled suspension was extracted with CH$_2$Cl$_2$ (3 × 80 mL). The organic layer was washed with a saturated solution of NaCl (95 mL), and the combined organic extracts were dried over sodium sulfate, filtered, and evaporated under reduced pressure. The crude residue was triturated in ethanol. The resulting precipitate when was filtered, washed with ethanol, and purified by column chromatography on silica gel using dichloromethane as eluent gave the pure product 6a–d.

4-(7,8,9,10-Tetrahydroisoindolo[2,1-a]quinoxalin-6-yl)benzaldehyde (**6a**). Yield: 89%, yellow crystals, mp = 172°C; IR ν_{max} (KBr)/cm^{-1} 1705 (CO); ^{1}H NMR (300 MHz, CDCl$_3$) δ 10.14 (s, 1H, CHO), 8.05 (d, 2H, $J = 8.10$ Hz, H-2 and H-6), 8.03 (dd, 1H, $J = 8.05$ and 1.20 Hz, H1′), 7.87 (s, 1H, H-11′), 7.82 (dd, 1H, $J = 8.05$ and 1.20 Hz, H-4′), 7.79 (d, 2H, $J = 8.10$, H-3 and H-5), 7.54 (ddd,

1H, $J = 8.05$, 7.70 and 1.20 Hz, H-2′), 7.44 (ddd, 1H, $J = 8.05$, 7.70 and 1.20 Hz, H-3′), 2.84 (t, 2H, $J = 6.20$ Hz, CH$_2$), 2.18 (t, 2H, $J = 6.20$ Hz, CH$_2$), 1.85–1.75 (m, 2H, CH$_2$), 1.69–1.60 (m, 2H, CH$_2$). Anal. Calcd. for C$_{22}$H$_{18}$N$_2$O: C, 80.96; H, 5.56; N, 8.58. Found: C, 81.05; H, 5.70; N, 8.55.

4-(11,12-Dihydrobenzo[e]isoindolo[2,1-a]quinoxalin-6-yl)benzaldehyde (6b). Yield: 86%, yellow crystals, mp = 174°C; IR ν_{max} (KBr)/cm^{-1} 1710 (CO); ^{1}H NMR (300 MHz, CDCl$_3$) δ 10.07 (s, 1H, CHO), 8.09 (dd, 1H, $J = 8.10$ and 1.35 Hz, H-7′), 7.98 (s, 1H, H-13′), 7.88 (dd, 1H, $J = 8.10$ and 1.20 Hz, H-1′), 7.86 (d, 2H, $J = 8.10$ Hz, H-2 and H-6), 7.83 (d, 2H, $J = 8.10$ Hz, H-3 and H-5), 7.58 (ddd, 1H, $J = 8.00$, 7.65 and 1.20 Hz, H-2′), 7.55 (ddd, 1H, $J = 8.00$, 7.65 and 1.20 Hz, H-3′), 7.26 (dd, 1H, $J = 8.00$ and 1.20 Hz, H-4′), 6.98 (ddd, 1H, $J = 8.10$, 7.60, and 1.35 Hz, H-8′), 6.58 (ddd, 1H, $J = 8.10$, 7.60, and 1.35 Hz, H-9′), 6.15 (dd, 1H, $J = 8.10$ and 1.35 Hz, H-10′), 3.00–2.95 (m, 2H, CH$_2$), 2.92–2.87 (m, 2H, CH$_2$). Anal. Calcd. for C$_{26}$H$_{18}$N$_2$O: C, 83.40; H, 4.85; N, 7.48. Found: C, 83.58; H, 4.97; N, 7.62.

4-(Indolo[1,2-a]quinoxalin-6-yl)benzaldehyde (6c). Yield: 83%, yellow crystals, mp = 196°C; IR ν_{max} (KBr)/cm^{-1} 1690 (CO); ^{1}H NMR (300 MHz, CDCl$_3$) δ 10.18 (s, 1H, CHO), 8.57 (d, 1H, $J = 8.10$ Hz, H-11′), 8.53 (d, 1H, $J = 8.30$ Hz, H-1′), 8.24 (d, 2H, $J = 8.10$ Hz, H-2 and H-6), 8.14–8.11 (m, 3H, H-3, H-5 and H-4′), 7.97 (d, 1H, $J = 8.10$, H-8′), 7.69 (t, 1H, $J = 8.30$ Hz, H-2′), 7.62 (t, 1H, $J = 8.30$ Hz, H-3′), 7.53–7.47 (m, 2H, H-9′ and H-10′), 7.29 (s, 1H, H-7′). Anal. Calcd. for C$_{22}$H$_{14}$N$_2$O: C, 81.97; H, 4.38; N, 8.69. Found: C, 82.17; H, 4.42; N, 8.89.

4-(Isoindolo[2,1-a]quinoxalin-6-yl)benzaldehyde (6d). Yield: 79%, yellow crystals, mp = 213°C; IR ν_{max} (KBr)/cm^{-1} 1700 (CO); ^{1}H NMR (300 MHz, CDCl$_3$) δ 10.23 (s, 1H, CHO), 8.62 (s, 1H, H-11′), 8.24–8.20 (m, 1H, H-1′), 8.19–8.16 (m, 1H, H-10′), 8.15 (d, 2H, $J = 8.40$ Hz, H-2 and H-6), 8.01 (d, 2H, $J = 8.40$ Hz, H-3 and H-5), 7.93 (d, 1H, $J = 8.70$, H-4′), 7.69–7.65 (m, 2H, H-2′ and H-3′), 7.41–7.35 (m, 1H, H-9′), 7.31–7.28 (m, 1H, H-7′), 7.20–7.14 (m, 1H, H-8′). Anal. Calcd. for C$_{22}$H$_{14}$N$_2$O: C, 81.97; H, 4.38; N, 8.69. Found: C, 82.09; H, 4.34; N, 8.77.

Synthesis of 1,3-dihydro-1-{1-[4-(isoindolo[2,1-a]quinoxalin-6-yl)benzyl]piperidin-4-yl}-2-benzimidazol-2-ones and 1,3-dihydro-1-{1-[4-(indolo[1,2-a]quinoxalin-6-yl)benzyl]piperidin-4-yl}-2-benzimidazol-2-one (1a–d). The pH of a solution of the aldehyde 6a–d (2.5 mmol) and 4-(2-ketobenzimidazol-1-yl)piperidine (3.0 mmol) in 40 mL methanol was adjusted to 6 by the dropwise addition of acetic acid. Powered sodium cyanoborohydride (6.9 mmol) was then added, and the resultant mixture was refluxed for 5 h. The hot precipitate formed was filtered, washed with methanol then with diethyl ether and dried under reduced pressure to give the compounds 1a–d.

1,3-Dihydro-1-{1-[4-(7,8,9,10-tetrahydroisoindolo[2,1-a]quinoxalin-6-yl)benzyl]piperidin-4-yl}-2H-benzimidazol-2-one (1a). Yield: 43%, pale-yellow crystals, mp = 289°C; IR ν_{max} (KBr)/cm^{-1} 3350 (NH), 1685 (C=O); ^{1}H NMR (300 MHz, CDCl$_3$) δ 10.86 (s, 1H, NH), 8.29 (s, 1H, H-11″), 8.18 (dd, 1H, $J = 8.00$ and 1.25 Hz, H-1″), 7.80 (dd, 1H, $J = 8.00$ and 1.25

Hz, H-4″), 7.56–7.38 (m, 6H, H-2′, H-3′, H-5′, H-6′, H-2″ and H-3″), 7.26–7.23 (m, 1H, H benzimid.), 6.98–6.96 (m, 3H, H benzimid.), 4.18–4.17 (m, 1H, CH pip.), 3.65 (s, 2H, CH$_2$N), 3.02–2.99 (m, 2H, CH$_2$ pip.), 2.75 (t, 2H, $J = 5.90$ Hz, CH$_2$), 2.43–2.38 (m, 2H, CH$_2$ pip.), 2.18–2.09 (m, 4H, CH$_2$ pip. and CH$_2$), 1.70–1.65 (m, 4H, CH$_2$ and CH$_2$ pip.), 1.57–1.54 (m, 2H, CH$_2$). Anal. Calcd. for C$_{34}$H$_{33}$N$_5$O: C, 77.39; H, 6.30; N, 13.27. Found: C, 77.33; H, 6.21; N, 13.45.

1,3-Dihydro-1-{1-[4-(11,12-dihydrobenzo[e]isoindolo[2,1-a]quinoxalin-6-yl)benzyl]piperidin-4-yl}-2H-benzimidazol-2-one . 2 HCl (1b). Yield: 76%, orange crystals, mp = 298°C; IR ν_{max} (KBr)/cm^{-1} 3400 (NH), 2650–2350 (NH$^+$), 1695 (C=O); ^{1}H NMR (300 MHz, DMSO-d$_6$) δ 11.93 (s, 1H, NH$^+$), 11.04 (s, 1H, NH), 9.03 (s, 1H, H-13″), 8.49 (d, 1H, J = 7.50 Hz, H-7″), 8.18 (d, 1H, $J = 7.30$ Hz, H-1″), 7.86–7.75 (m, 6H, H-2′, H-3′, H-5′, H-6′, H-2″ and H benzimid.), 7.62 (t, 1H, J = 7.30 Hz, H-3″), 7.27 (d, 1H, $J = 7.30$ Hz, H-4″), 7.03–6.98 (m, 4H, H-8″ and 3H benzimid.), 6.69 (t, 1H, J = 7.50 Hz, H-9″), 6.11 (d, 1H, $J = 7.50$ Hz, H-10″), 4.61–4.59 (m, 1H, CH pip.), 4.45 (s, 2H, CH$_2$N), 4.40 (bs, 1H, NH$^+$), 3.47–3.42 (m, 2H, CH$_2$ pip.), 3.21–3.13 (m, 2H, CH$_2$ pip.), 3.08–2.88 (m, 6H, CH$_2$ pip. and 2 CH$_2$), 1.95–1.91 (m, 2H, CH$_2$ pip.). Anal. Calcd. for C$_{38}$H$_{35}$Cl$_2$N$_5$O: C, 70.37; H, 5.44; N, 10.80. Found: C, 70.46; H, 5.57; N, 11.04.

1,3-Dihydro-1-{1-[4-(indolo[1,2-a]quinoxalin-6-yl)benzyl]piperidin-4-yl}-2H-benzimidazol-2-one(1c). Yield: 69%, yellow crystals, mp = 236°C; IR ν_{max} (KBr)/cm^{-1} 3350 (NH), 1685 (C=O); ^{1}H NMR (300 MHz, DMSO-d$_6$) δ 10.87 (s, 1H, NH), 8.77–8.73 (m, 2H, H-11″ and H-1″), 8.05–8.00 (m, 4H, H-3′, H-5′, H-4″ and H-8″), 7.71 (t, 1H, $J = 8.10$ Hz, H-2″), 7.61 (t, 1H, $J = 8.10$ Hz, H-3″), 7.59 (d, 2H, $J = 8.10$ Hz, H-2′ and H-6′), 7.54-7.48 (m, 2H, H-9″ and H-10″), 7.39 (s, 1H, H-7″), 7.28–7.25 (m, 1H, H benzimid.), 7.01–6.96 (m, 3H, H benzimid.), 4.23–4.14 (m, 1H, CH pip.), 3.66 (s, 2H, CH$_2$N), 3.05–3.01 (m, 2H, CH$_2$ pip.), 2.45–2.40 (m, 2H, CH$_2$ pip.), 2.21–2.13 (m, 2H, CH$_2$ pip.), 1.70–1.64 (m, 2H, CH$_2$ pip.). Anal. Calcd. for C$_{34}$H$_{29}$N$_5$O: C, 77.99; H, 5.58; N, 13.37. Found: C, 78.20; H, 5.62; N, 13.45.

1,3-Dihydro-1-{1-[4-(isoindolo[2,1-a]quinoxalin-6-yl)benzyl]piperidin-4-yl}-2H-benzimidazol-2-one (1d). Yield: 72%, yellow crystals, mp = 255°C; IR ν_{max} (KBr)/cm^{-1} 3345 (NH), 1690 (C=O); ^{1}H NMR (300 MHz, DMSO-d$_6$) δ 10.83 (s, 1H, NH), 9.24 (s, 1H, H-11″), 8.64 (dd, 1H, $J = 8.50$ and 1.80 Hz, H-10″), 8.02–7.99 (m, 1H, H-1″), 7.95 (d, 1H, $J = 8.50$ Hz, H-4″), 7.75 (d, 2H, $J = 7.95$ Hz, H-3′ and H-5′), 7.73–7.67 (m, 2H, H-2″ and H-3″), 7.62 (d, 2H, $J = 7.95$ Hz, H-2′ and H-6′), 7.36–7.27 (m, 1H, H-9″), 7.26–7.22 (m, 2H, H-7″ and H benzimid.), 7.17–7.11 (m, 1H, H-8″), 7.01–6.96 (m, 3H, H benzimid.), 4.26–4.16 (m, 1H, CH pip.), 3.73 (s, 2H, CH$_2$N), 3.09–3.05 (m, 2H, CH$_2$ pip.), 2.50–2.37 (m, 2H, CH$_2$ pip.), 2.25–2.17 (m, 2H, CH$_2$ pip.), 1.73–1.68 (m, 2H, CH$_2$ pip.). Anal. Calcd. for C$_{34}$H$_{29}$N$_5$O: C, 77.99; H, 5.58; N, 13.37. Found: C, 78.17; H, 5.43; N, 13.30.

Synthesis of 5-fluoro-2-{1-[4-(isoindolo[2,1-a]quinoxalin-6-yl)benzyl]piperidin-4-yl}-1H-benzimidazoles and 5-fluoro-2-{1-[4-(indolo[1,2-a]quinoxalin-6-yl)benzyl]piperidin-4-yl}-1H-benzimidazole (1e–h). The

pH of a solution of the aldehyde **6a**–**d** (2.5 mmol) and 4-(5-fluorobenzimidazol-2-yl)piperidine (3.0 mmol) in 40 mL methanol was adjusted to 6 by the dropwise addition of acetic acid. Powered sodium cyanoborohydride (6.9 mmol) was then added, and the resultant mixture was refluxed for 5 h. After removal of the methanol by rotary evaporation, the residue was triturated in water and extracted with dichloromethane. The organic layer was washed with water, dried over magnesium sulfate and evaporated to dryness. Column chromatography of the residue on silica gel using ethyl acetate–cyclohexane (1/1) then methanol–chloroform (1/9) as eluents gave the crude product. This solid was then triturated with diethyl ether, filtered, washed with diethyl ether and dried under reduced pressure to give the compounds **1e**–**h**.

5-Fluoro-2-{1-[4-(7,8,9,10-tetrahydroisoindolo[2,-1-a]quinoxalin-6-yl)benzyl]piperidin-4-yl}-1H-benzimidazole (1e). Yield: 63%, pale-yellow crystals, mp = 152°C; IR ν_{max} (KBr)/cm^{-1} 3340 (NH), 1685 (C=O); ^{1}H NMR (300 MHz, DMSO-d$_6$) δ 12.41 (s, 1H, NH), 8.30 (s, 1H, H-11″), 8.19 (d, 1H, J=8.10 Hz, H-1″), 7.80 (d, 1H, J=8.10 Hz, H-4″), 7.57–7.31 (m, 7H, H-2′, H-3′, H-5′, H-6′, H-2″, H-3″ and H benzimid.), 7.23–7.20 (m, 1H, H benzimid.), 6.99–6.96 (m, 1H, H benzimid.), 3.63 (s, 2H, CH$_2$N), 2.98–2.93 (m, 2H, CH$_2$ pip.), 2.87–2.85 (m, 1H, CH pip.), 2.76 (t, 2H, J=5.85 Hz, CH$_2$), 2.17–2.12 (m, 4H, CH$_2$ and CH$_2$ pip.), 2.00–1.98 (m, 2H, CH$_2$ pip.), 1.90–1.84 (m, 2H, CH$_2$ pip.), 1.71–1.68 (m, 2H, CH$_2$), 1.56–1.54 (m, 2H, CH$_2$). Anal. Calcd. for C$_{34}$H$_{32}$FN$_5$: C, 77.10; H, 6.09; N, 13.22. Found: C, 77.24; H, 6.32; N, 13.15.

5-Fluoro-2-{1-[4-(11,12-dihydrobenzo[e]isoindolo[2,1-a]quinoxalin-6-yl)benzyl]piperidin-4-yl}-1H-benzimidazole (1f). Yield: 40%, yellow crystals, mp = 179°C; IR ν_{max} (KBr)/cm^{-1} 3340 (NH), 1690 (C=O); ^{1}H NMR (300 MHz, DMSO-d$_6$) δ 12.33 (s, 1H, NH), 8.54 (s, 1H, H-13″), 8.29 (d, 1H, J=7.80 Hz, H-7″), 7.91 (dd, 1H, J=8.10 and 1.20 Hz, H-1″), 7.61 (ddd, 1H, J=8.10, 7.90 and 1.20 Hz, H-2″), 7.51 (d, 2H, J=8.10 Hz, H-3′ and H-5′), 7.50–7.48 (m, 2H, H-3″ and H benzimid.), 7.23 (d, 2H, J=8.10 Hz, H-2′ and H-6′), 7.22–7.20 (m, 2H, H-4″ and H benzimid.), 7.02–6.91 (m, 2H, H-8″ and H benzimid.), 6.52 (t, 1H, J=7.80 Hz, H-9″), 6.11 (d, 1H, J=7.80 Hz, H-10″), 3.57 (s, 2H, CH$_2$N), 2.97–2.81 (m, 7H, CH$_2$ pip., CH pip. and 2 CH$_2$), 2.15–2.04 (m, 4H, 2 CH$_2$ pip.), 1.91–1.83 (m, 2H, CH$_2$ pip.). Calcd. for C$_{38}$H$_{32}$FN$_5$: C, 79.01; H, 5.58; N, 12.12. Found: C, 78.95; H, 5.57; N, 12.04.

5-Fluoro-2-{1-[4-(indolo[1,2-a]quinoxalin-6-yl)-benzyl]piperidin-4-yl}-1H-benzimidazole (1g). Yield: 86%, yellow crystals, mp = 139°C; IR ν_{max} (KBr)/cm^{-1} 3345 (NH), 1690 (C=O); ^{1}H NMR (300 MHz, DMSO-d$_6$) δ 12.27 (s, 1H, NH), 8.76-8.73 (m, 2H, H-11″ and H-1″), 8.04–7.98 (m, 4H, H-3′, H-5′, H-4″ and H-8″), 7.70 (ddd, 1H, J=8.40, 7.20 and 1.20 Hz, H-2″), 7.62–7.54 (m, 3H, H-2′, H-6′ and H-3″), 7.51–7.45 (m, 2H, H-9″ and H-10″), 7.39 (s, 1H, H-7″), 7.36-7.31 (m, 1H, H benzimid.), 7.22–7.18 (m, 1H, H benzimid.), 7.02–6.94 (m, 1H, H benzimid.), 3.64 (s, 2H, CH$_2$N), 2.99–2.90 (m, 2H, CH$_2$ pip.), 2.87–2.83 (m, 1H, CH pip.), 2.21–2.13 (m, 2H, CH$_2$ pip.), 2.04–2.00 (m, 2H, CH$_2$

pip.), 1.93–1.82 (m, 2H, CH$_2$ pip.). Calcd. for C$_{34}$H$_{28}$FN$_5$: C, 77.69; H, 5.37; N, 13.32. Found: C, 77.56; H, 5.48; N, 13.11.

5-Fluoro-2-{1-[4-(isoindolo[2,1-a]quinoxalin-6-yl)benzyl]piperidin-4-yl}-1H-benzimidazole (1h). Yield: 66%, yellow crystals, mp = 196°C; IR ν_{max} (KBr)/cm^{-1} 3350 (NH), 1690 (C=O); ^{1}H NMR (300 MHz, DMSO-d$_6$) δ 12.48 (bs, 1H, NH), 9.24 (s, 1H, H-11″), 8.64 (dd, 1H, J=8.10 and 2.10 Hz, H-10″), 8.03–7.99 (m, 1H, H-1″), 7.95 (d, 1H, J=8.40 Hz, H-4″), 7.72 (d, 2H, J=7.85 Hz, H-3′ and H-5′), 7.70–7.66 (m, 2H, H-2″ and H-3″), 7.58 (d, 2H, J=7.85 Hz, H-2′ and H-6′), 7.48–7.44 (m, 1H, H benzimid.), 7.35–7.21 (m, 3H, H-7″, H-9″ and H benzimid.), 7.16–7.10 (m, 1H, H-8″), 6.99–6.92 (m, 1H, H benzimid.), 3.68 (s, 2H, CH$_2$N), 3.02–2.97 (m, 2H, CH$_2$ pip.), 2.92–2.83 (m, 1H, CH pip.), 2.23-2.15 (m, 2H, CH$_2$ pip.), 2.08–2.01 (m, 2H, CH$_2$ pip.), 1.92–1.78 (m, 2H, CH$_2$ pip.). Anal. Calcd. for C$_{34}$H$_{28}$FN$_5$: C, 77.69; H, 5.37; N, 13.32. Found: C, 77.42; H, 5.21; N, 13.57.

2-(2-Aminophenyl)-2H-isoindole-1-carboxylic acid (8). A solution of 1-cyano-2-(2-aminophenyl)isoindole 7 (3 mmol) in acetic acid (10 mL) was refluxed for 30 min. After cooling, the reaction mixture was poured into ice water (150 mL) and stirred for 15 min. The resulting solution was evaporated under reduced pressure. The residue was cooled and triturated in diethyl ether. The precipitate was collected by filtration and dried to give 8. Yield: 98%, orange crystals, mp > 300°C; IR ν_{max} (KBr)/cm^{-1} 3360–2500 (NH$_2$ and OH), 1660 (C=O); ^{1}H NMR (300 MHz, DMSO-d$_6$) δ 12.03 (bs, 1H, COOH), 8.92 (s, 1H, H-3), 8.44 (dd, 1H, J=8.10 and 0.90 Hz, H-7), 8.37 (dd, 1H, J=8.10 and 0.90 Hz, H-4), 7.89–7.86 (m, 1H, H-6′), 7.57 (dd, 1H, J=8.10 and 1.20 Hz, H-3′), 7.46 (ddd, 1H, J=8.10, 7.50 and 1.20 Hz, H-4′), 7.34–7.28 (m, 3H, H-5, H-6 and H-5′), 6.91 (s, 2H, NH$_2$). Anal. Calcd. for C$_{15}$H$_{12}$N$_2$O$_2$: C, 71.42; H, 4.79; N, 11.10. Found: C, 71.37; H, 4.96; N, 11.39.

2-(2-Fluorophenyl)-2H-isoindole-1-carbonitrile (9). To a solution of sodium hydrogen sulfite (0.015 mol) in water (38 mL) was added phthalaldehyde (0.015 mol). The mixture was stirred until the solid was dissolved, and 2-fluoroaniline (0.015 mol) was added. The reaction was heated at 70°C for 30 min, cooled, KCN (0.052 mol) in water (8.0 mL) was added, and the mixture was heated at 70°C for an additional 90 min. The solid formed upon cooling was filtered, washed with water and extracted with dichloromethane. The organic layer was dried over magnesium sulfate and evaporated to dryness. Column chromatography of the residue on silica gel using dichloromethane as eluent gave 9. Yield: 32%, pale-yellow crystals, mp = 120°C; IR ν_{max} (KBr)/cm^{-1} 2200 (C≡N); ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.73 (dd, 1H, J=8.40 and 0.90 Hz, H-4), 7.69 (dd, 1H, J=8.40 and 0.90 Hz, H-7), 7.59–7.48 (m, 3H, H-4′, H-5′ and H-6′), 7.38–7.28 (m, 3H, H-3′, H-3 and H-5), 7.17 (ddd, 1H, J=8.40, 7.80 and 0.90 Hz, H-6). Anal. Calcd. for C$_{15}$H$_9$FN$_2$: C, 76.26; H, 3.84; N, 11.86. Found: C, 76.36; H, 3.77; N, 11.65.

5H-Isoindolo[2,1-a]quinoxalin-6-one (4d). A suspension of 2-(2-fluorophenyl)-2H-isoindole-1-carbonitrile

9 (3 mmol) and 85% potassium hydroxide (12 mmol) in *tert*-butanol (21 mL) was heated at 80°C for 4 h, then cooled, poured into crushed ice, and stirred for 10 min. The resulting precipitate was filtered, washed with water then with diethyl ether, and dried under reduced pressure to give 4d. Yield: 80%, white crystals, mp > 260°C; (Found MNa+: 257.0699, $C_{15}H_{10}N_2O^{23}Na$ requires 257.0691); IR ν_{max} (KBr)/cm^{-1} 1645 (CO); ^{1}H NMR (300 MHz, DMSO-d$_6$) δ 11.40 (s, 1H, NH), 8.78 (s, 1H, H-11), 8.35 (dd, 1H, J = 8.10 and 1.00 Hz, H-1), 8.31 (dd, 1H, J = 7.90 and 0.90 Hz, H-10), 7.83 (dd, 1H, J = 7.90 and 0.90 Hz, H-7), 7.45–7.39 (m, 2H, H-2 and H-4), 7.33–7.22 (m, 3H, H-3, H-8 and H-9). Anal. Calcd. for $C_{15}H_{10}N_2O$: C, 76.91; H, 4.30; N, 11.96. Found: C, 77.09; H, 4.10; N, 12.07.

Biology

Cell culture. The human leukemic cell lines U937, K562, and HL60, and the breast cancer cell-line MCF7 were grown in RPMI 1640 medium (Life Technology, France) supplemented with 10% fetal calf serum, antibiotics (100 U/mL penicillin, 100 μg/mL streptomycin), and L-glutamin, at 37°C, 5% CO_2 in air. The toxicity of various molecules was also evaluated on non-activated, freshly isolated normal human peripheral blood mononuclear cells (PBMNC) and phytohemagglutinin (lymphoproliferative agent) (PHA)-induced cells. PBMNC from blood of healthy volunteers were obtained following centrifugation on Ficoll gradient. Cells were then incubated in medium alone or induced to enter cell cycle by the addition of PHA (5 μg/mL, Murex Biotech Limited, Dartford, UK).

Cytotoxicity Test. The 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethonyphenol)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) cell proliferation assay (Promega, France) is a colorimetric assay system, which measures the reduction of a tetrazolium component (MTS) into formazan produced by the mitochondria of viable cells. Cells were washed twice in phosphate buffer saline and plated in quadruplicate into microtiter-plate wells in 100-μL culture media without or with our various compounds at increasing concentrations (0, 1, 5, 10, 20, and 50 μM). After 3 h of incubation at 37°C with 20 μL MTS/well, the plates were read using an ELISA microplate reader (Thermo, Electrocorporation) at 490 nm wavelength. The amount of colour produced was directly proportional to the number of viable cells. The results are expressed as the concentrations inhibiting cell growth by 50% after a 3 days incubation period. The 50% inhibitory concentrations (IC$_{50}$) were determined by linear regression analysis, expressed in μM ± SD (Microsoft Excel).
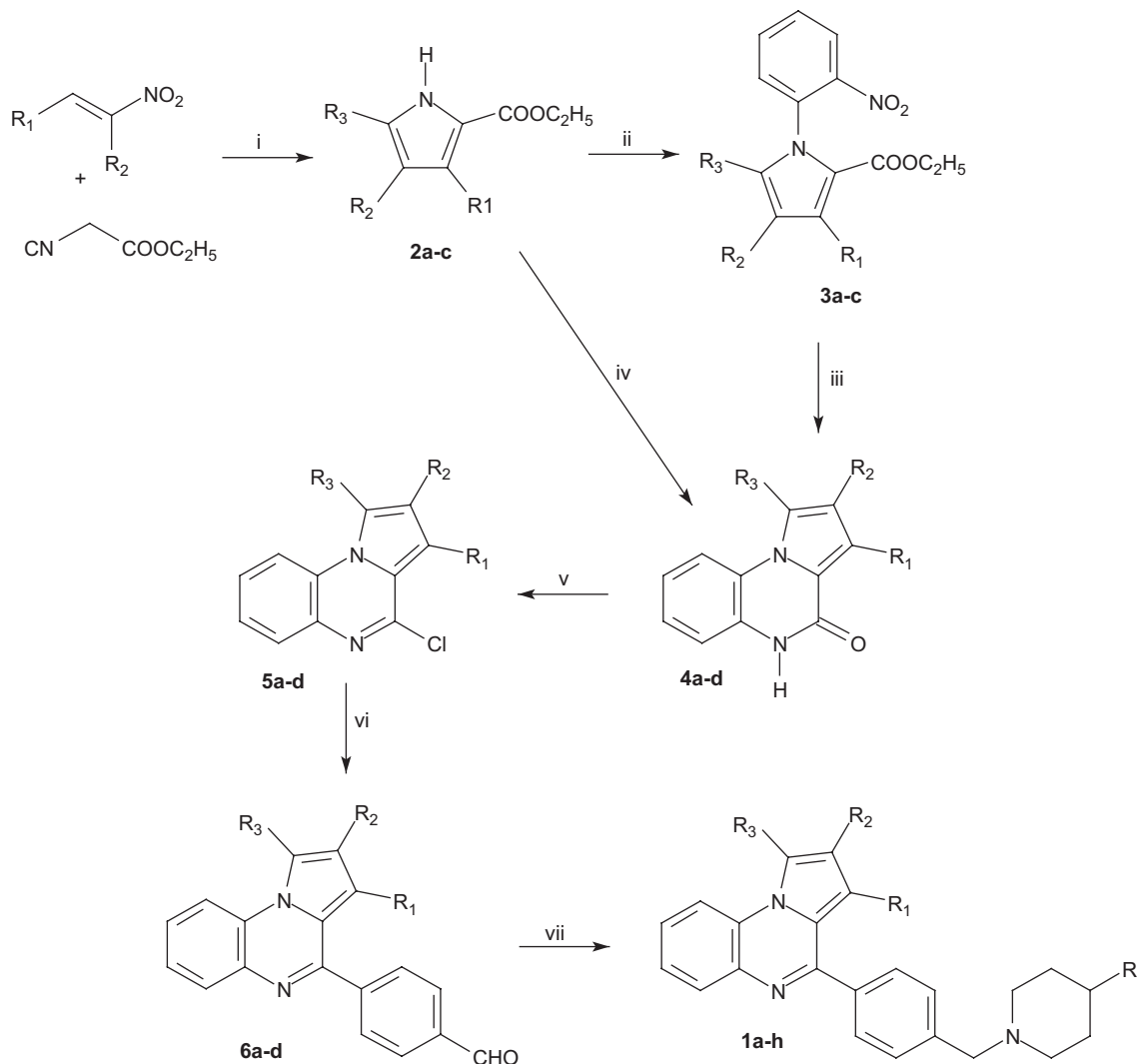
Results and discussion

Chemistry

The synthesis of the new isoindolo- or indoloquinoxaline derivatives **1a**–**h** has been accomplished according to the sequence depicted in Scheme 1. Condensation of nitrocyclohexenes with ethyl isocyanoacetate in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) afforded the corresponding fused pyrroles **2a**–**b** using a Barton-Zard reaction[14-16]. The not commercially available, starting material 2-nitro-3,4-dihydronaphthalene was readily prepared by the nitration of the corresponding alkene[17]. The preparation of *N*-aryl pyrroles **3a**–**c** was then obtained by nucleophilic substitution of the various alkyl pyrrole-2-carboxylates **2a**–**c** with 1-fluoro-2-nitrobenzene using cesium carbonate as the base in refluxing DMF solution[3,10]. The preparation of **3a** and **3b** was also performed under microwave irradiation.

Reduction of the nitro moiety of **3a**–**c** with iron in hot glacial acetic acid produced the spontaneous ring closure onto the ester to afford the desired isoindolo- or indoloquinoxalinones **4a**–**c** through a one-pot reduction–cyclization step[3,13]. CuI/*L*-proline-catalyzed coupling of 2-iodotrifluoroacetanilide with pyrrole-2-carboxylate ester **2a** in DMSO at 80–90°C followed by *in situ* hydrolysis at 60°C also afforded the isoindolo[2,1-*a*]quinoxalin-6-one **4a**[18]. The lactams **4a**–**d** were subsequently chlorodehydroxylated with phosphorous oxychloride, leading to the 6-chloroquinoxalines **5a**–**d**. Coupling chloro derivatives **5a**–**d** with 4-formylphenylboronic acid in the presence of Pd(PPh$_3$)$_4$ as a catalyst under Suzuki-Miyaura cross-coupling conditions proceeded to afford the substituted benzaldehydes **6a**–**d**[19,10]. Reductive amination of benzaldehydes **6a**–**d** with 4-(2-ketobenzimidazolin-1-yl) piperidine or 4-(5-fluorobenzimidazol-2-yl)piperidine using NaBH$_3$CN provided the final products **1a**–**h**. The 3D spatial determinations of **1a** and **1c** were established by X-ray crystallography[20] and confirmed the structures in the solid state as anticipated on the basis of IR and ^{1}H NMR data (Figure 2).

The synthesis of the isoindoloquinoxalinone derivative **4d** was first supported by the work of the Diana et al.[21,22] using 2-(2′-aminophenyl)-1-cyano-isoindole **7** as a key intermediate (Scheme 2). The isolation of derivative **7** was possible by a Strecker-type synthesis between substituted 1,2-phenylendiamine and phthalaldehyde in water and in the presence of potassium cyanide and sodium hydrogensulfite[21,22]. Refluxing compound **7** in acetic acid followed by an *in situ* hydrolysis failed in the synthesis of lactam **4d**, and only furnished the 2-(2-aminophenyl)-2H-isoindole-1-carboxylic acid **8**. The structural identification of compound **8** was unambiguously established on the basis of IR and ^{1}H NMR data. The zwitterionic structure of **8** has been confirmed by IR spectrometry. The IR spectrum indicated the presence of a carboxylate COO$^-$ band and a sharp ammonium NH$_3^+$ band, respectively, observed at 1660 and 1910 cm^{-1}. Moreover, the ^{1}H NMR spectrum showed a broad singlet at 12.03 ppm (1H), highly characteristic for a carboxylic acid proton, and a singlet at 6.91 ppm (2H), attributed to the amine group.

The preparation of **4d** was achieved by using the following strategy via the 2-(2-fluorophenyl)-2H-isoindole-1-carbonitrile **9** (Scheme 2). This cyano-substituted isoindole **9** was also preliminary prepared by a

	R₁−	R₂−	R₃−	R-
1a	(cyclohexyl)		H -	(1-methyl-2-ketobenzimidazolyl)
1b	(2-methylphenylethyl)		H -	(1-methyl-2-ketobenzimidazolyl)
1c	H -	(cyclohexenyl)		(1-methyl-2-ketobenzimidazolyl)
1d	(cyclohexenyl)		H -	(1-methyl-2-ketobenzimidazolyl)

	R₁−	R₂−	R₃−	R-
1e	(cyclohexyl)		H -	(2-methyl-5-fluorobenzimidazolyl)
1f	(2-methylphenylethyl)		H -	(2-methyl-5-fluorobenzimidazolyl)
1g	H -	(cyclohexenyl)		(2-methyl-5-fluorobenzimidazolyl)
1h	(cyclohexenyl)		H -	(2-methyl-5-fluorobenzimidazolyl)

Scheme 1. Reagents and conditions: (i) DBU, THF/t-BuOH, 50°C; (ii) 1-fluoro-2-nitrobenzene, Cs_2CO_3, DMF, Δ; (iii) Fe, CH_3COOH, Δ; (iv) 1) o-iodotrifluoroacetanilide, K_2CO_3, CuI, L-proline, DMSO, 80°C; 2) H_2O, 60°C (v) $POCl_3$, Δ; (vi) $OHC-C_6H_4-B(OH)_2$, $Pd[P(C_6H_5)_3]_4$, K_2CO_3, toluene, EtOH, Δ; (vii) 4-(2-ketobenzimidazolin-1-yl)piperidine or 4-(5-fluorobenzimidazolin-2-yl)piperidine, $NaBH_3CN$, MeOH, Δ.
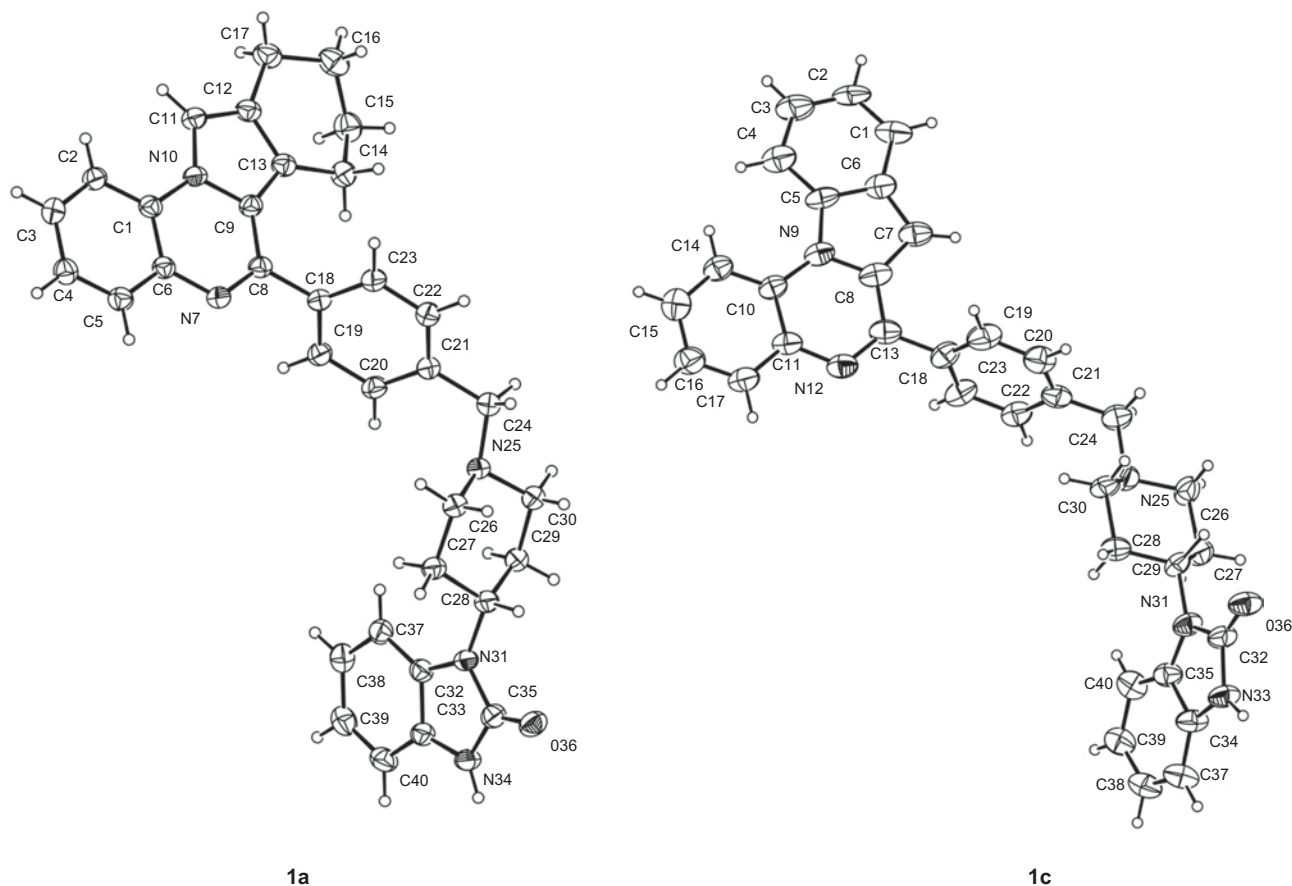
Figure 2. The ORTEP drawing of 1,3-dihydro-1-{1-[4-(pyrrolo[1,2-*a*]quinoxalin-4-yl)benzyl]piperidin-4-yl}-2*H*-benzimidazol-2-ones **1a** and **1c** with thermal ellipsoids at 30% level.

Strecker-type synthesis between 2-fluoroaniline and *o*-phthalodicarboxaldehyde in water and in the presence of potassium cyanide and sodium hydrogensulfite[23]. Treatment of **9** with potassium hydroxide in *tert*-butanol at 80°C led directly to the tetracyclic lactam **4d** involving selective hydrolysis to amide, which was able to carry out intramolecular displacement of aromatic fluorine through an anionic ring closure[24–27]. Structure elucidation of **4d** was performed using high-resolution mass spectrometry analysis (experimental mass 257.0699, theoretical mass for $C_{15}H_{10}N_2O^{23}Na$ 257.0691). Structural confirmation of compound **4d** was also achieved by IR and ¹H NMR spectroscopy on the basis of previous results for 5*H*-pyrrolo[1,2-*a*]quinoxalin-4-one series published by our group[4–7,10,11]. In the ¹H NMR spectrum, we observed a D_2O exchangeable singlet at 11.40 ppm characteristic of the NH lactam function. Moreover, the data also showed a singlet at 8.78 ppm attributed to the proton in position 11 of the isoindolo[2,1-*a*]quinoxaline skeleton. In the IR spectrum, lactam **4d** showed a strong stretching carbonyl band at 1645 cm⁻¹.

Biology

Antiproliferative Effect. The eight new compounds **1a**–**h** were tested in MTS assay for their *in vitro* antiproliferative activity against five human leukemic cell lines (U937, K562, Jurkat, U266, and HL60), and one breast cancer

cell line (MCF7). Compound LY-294002 (Figure 3), which showed antiproliferative activity against the HL60, U937 and K562 cell lines[28–31], was applied as a referential cytotoxic agent. The results of the antiproliferative activity studies are summarized in Table 1.

Among compounds **1a**–**h**, the benzylpiperidinyl fluorobenzimidazole derivatives **1e**–**g** exhibited the best antiproliferative activity on the growth of human myeloid U937 cell line (IC₅₀ from 3.5 to 4 μM) in comparison with their benzylpiperidinyl benzimidazolone analogues **1a**–**c** that were found inactive (IC₅₀ > 50 μM). Surprisingly, the isoindoloquinoxaline **1h**, the structural aromatic analogue of the tetrahydroisoindoloquinoxaline **1e**, showed moderate antiproliferative activity with an IC₅₀ value of 42 μM. Moreover, in terms of structure–activity relationships discussion, it could be also noticed that the IC₅₀ of compound **1g** bearing an indole moiety (IC₅₀ = 4 μM) was found 10 times lower than those of its isoindolo analogue **1h** (IC₅₀ = 42 μM). Introduction of a fused benzene nucleus (compound **1f**) onto the tetrahydroisoindole ring (compound **1e**) did not lead to significant modification in the antiproliferative activity (IC₅₀ = 4 μM for **1f** *versus* 3.5 μM for **1e**).

Similar profile of antiproliferative activity was observed against the human myeloma cell line U266. Hence, the three quinoxalines **1e**–**g**, bearing the benzylpiperidinyl fluorobenzimidazole moiety in their 4-position, were
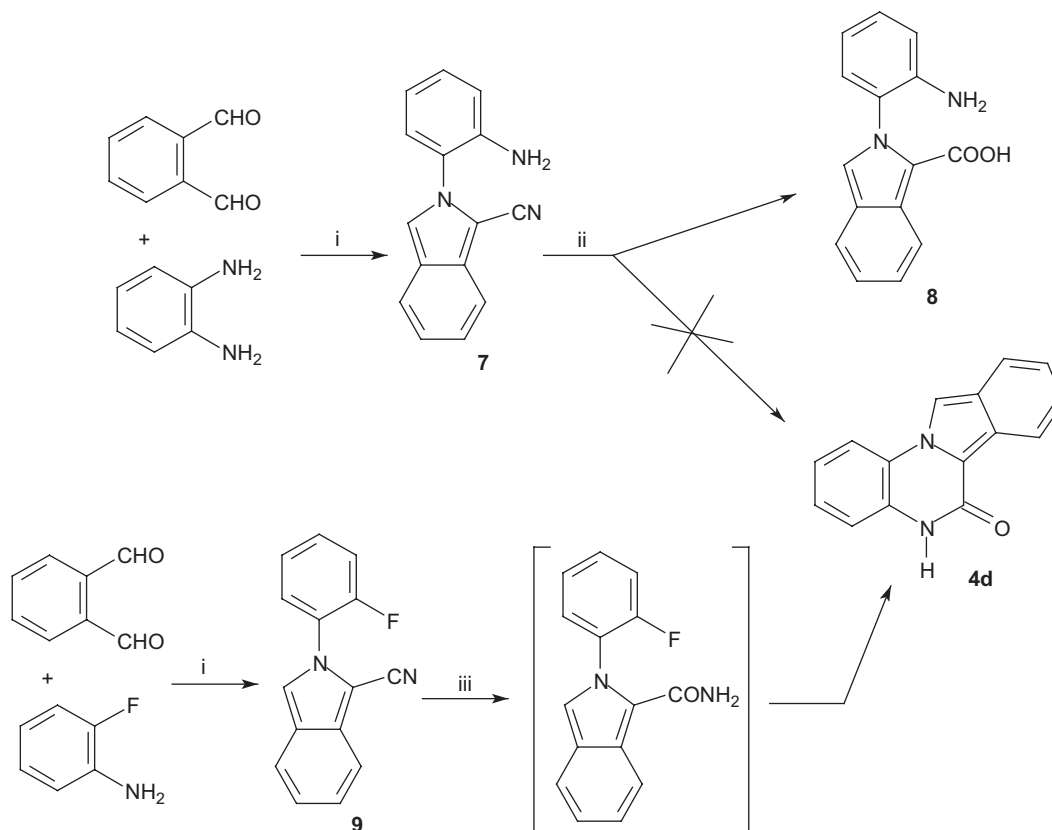
Scheme 2. Reagents and conditions: (i) NaHSO$_3$, KCN, H$_2$O, 70°C; (ii) CH$_3$COOH, Δ; (iii) KOH, *tert*-BuOH, 80°C.
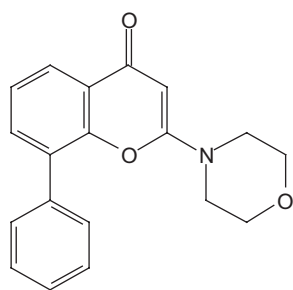
Figure 3. Structure of compound LY-294002.

always found the most active compounds with an IC$_{50}$ of 3–4 μM. The fourth fluorobenzimidazole derivative **1h** also showed significant antiproliferative activity (IC$_{50}$ = 14 μM). Concerning the fused substitutions on the quinoxaline ring, we noticed a similar biological profile as those observed against the U937 cell line.

The antiproliferative potencies of these new derivatives **1a**–**h** were also examined toward the human myeloid leukaemia cell lines K562 and HL60. On K562 cell line, **1e**–**g** were also found as the most active compounds (IC$_{50}$ = 3–4 μM). The replacement of the benzylpiperidinyl fluorobenzimidazole substituent by a benzylpiperidinyl benzimidazolone group in position 4 of the fused heterocyclic skeleton (compounds **1a**–**c**) led to a decrease in the activity (IC$_{50}$ ~ 46 μM). However, it could be noticed that the

nature of the substitution at position 4 in the isoindoloquinoxaline derivatives **1d** and **1h** was less detrimental for the activity (IC$_{50}$ of 11 and 13 μM, respectively). The benzylpiperidinyl fluorobenzimidazole compounds possessing a fused tetrahydroisoindole, dihydrobenzo[*e*]isoindole, or indole nucleus (compounds **1e**–**g**) exhibited better antiproliferative activities than their isoindolo analogue **1h**. In a general way, this kind of structure–activity profile was also observed against the HL60 and Jurkat cell lines.

Against the HL60 human acute promyeloid leukemia cell line, most of the tested compounds showed antiproliferative activity with IC$_{50}$ values from 3 to 18 μM, except **1b** and **1c** that were found inactive (IC$_{50}$ > 50 μM). As in a general way, isoindolo- and indoloquinoxalines having a benzylpiperidinyl fluorobenzimidazole moiety at position 4 exhibited better activities than their benzylpiperidinyl benzimidazolone homologues (i.e., IC$_{50}$ = 4 μM for **1e** *versus* 13.5 μM for **1a**, 3 μM for **1f** compared >50 μM for **1b**, and 4 μM for **1g** *versus* >50 μM for **1c**). Surprisingly, this kind of observation could not be applied to compounds **1d** and **1h**. Hence, the IC$_{50}$ of **1d** (9 μM) was twice lower than those of compound **1h** (IC$_{50}$ = 18 μM).

The antiproliferative activities of compounds **1a**–**h** against the T-lymphocyte Jurkat cell line were similar to those observed against the HL60 cell line. The same observation concerning antiproliferative activity of benzimidazolone **1d** *versus* fluorobenzimidazole **1h** on Jurkat

Table 1. In vitro activity of compounds **1a-h** on U937, K562, HL60, Jurkat, U266, and MCF7 cells, and cytotoxicity on human peripheral blood mononuclear cells PBMNC + PHA.

IC_{50} values $(\mu M)^a$

Compound	U937	K562	HL60	Jurkat	U266	MCF7	Cytotoxicity on activated human peripheral blood mononuclear cells (PBMNC) PBMNC + PHA
LY-294002	14 ± 0.3	38 ± 1	14 ± 0.3	22 ± 1	46 ± 2	7 ± 0.5	>50
1a	>50	46.5 ± 1	13.5 ± 0.3	44 ± 1	>50	11 ± 0.5	>50
1b	>50	45.5 ± 1	>50	>50	>50	9 ± 0.5	>50
1c	>50	45.5 ± 1	>50	>50	>50	>50	41 ± 1
1d	>50	11 ± 0.5	9 ± 0.4	5 ± 0.2	> 50	> 50	>50
1e	3.5 ± 0.3	3 ± 0.2	4 ± 0.2	3.5 ± 0.2	3.5 ± 0.3	4 ± 0.3	6 ± 0.3
1f	4 ± 0.3	4 ± 0.3	3 ± 0.5	3 ± 0.2	4 ± 0.3	3 ± 0.4	7 ± 0.5
1g	4 ± 0.3	3.5 ± 0.2	4 ± 0.3	3.5 ± 0.3	3 ± 0.2	4 ± 0.4	4 ± 0.3
1h	42 ± 1	13 ± 0.5	18 ± 0.5	12 ± 0.5	14 ± 0.5	33 ± 1	>50

^{a}The IC_{50} (μM) values correspond to the mean ± standard deviation from three independent experiments.

cell line could also be noticed ($IC_{50} = 5$ μM for **1d** *versus* 12 μM for **1h**).

In MCF7 cell line, the pyrrolo[1,2-*a*]quinoxaline derivatives **1e**–**g**, bearing a benzylpiperidinyl fluorobenzimidazole moiety in position 4 exhibited potent cytotoxicity with IC_{50} ranging from 3 to 4 μM. However, the isoindolo derivative **1h** showed low activity ($IC_{50} = 33$ μM) against this breast cancer line. Moreover, two of their benzylpiperidinyl benzimidazolone analogues bearing an hydrogenated isoindole ring (compounds **1a** and **1b**) have shown significant antiproliferative activity with an IC_{50} of 11 and 9 μM, respectively. Aromatization of the tetrahydroisoindole nucleus of **1a** (compound **1d**) led to a decrease in the antiproliferative activity; i.e., $IC_{50} = 11$ μM for **1a** and >50 μM for **1d**.

Against each human cancer cell lines, the antiproliferative activities of compounds **1e**–**g** were always found superior to those of the reference drug LY-294002.

Cytotoxicity. All compounds **1a**–**h** were tested on activated human peripheral blood mononuclear cells (Table 1). As expected, most of the pyrrolo[1,2-*a*]quinoxalines **1a**–**h** showed significant level of cytotoxicity against lymphocytes with IC_{50} ranging from 4 to >50 μM. These preliminary results were used to determine their respective range of toxic concentration.

In a general way, the less cytotoxic molecules were the less active on the various tumor cell lines except compounds **1d** and **1h**. Moreover, the benzylpiperidinyl benzimidazolone derivatives **1a**–**d** were found less cytotoxic than their benzylpiperidinyl fluorobenzimidazole analogues **1e**–**g** in the exception of compound **1h** that presented an IC_{50} superior to 50 μM against lymphocytes. Indexes of selectivity were defined as the ratio of the IC_{50} value on the human mononuclear cells to the IC_{50} value on the U937, K562, HL60, Jurkat, U266, or MCF7 lines. Compounds that demonstrated high selectivity (high index of selectivity) should offer a potential of safer therapy. This led to identify compounds with index of selectivity >10 for compound **1d** on the human leukemic cell

lines Jurkat, and >5.5 for compound **1b** against the MCF7 breast adenocarcinoma.

Conclusion

In the present report, we described the synthesis of a new series of substituted isoindolo- or indoloquinoxaline derivatives and presented their antiproliferative activities on the human leukemic cell lines U937, K562, Jurkat, U266, and HL60, and the breast cancer cell line MCF7. From the biological activity data, some structure–activity relationships can be inferred. The presence at C-4 position of the benzylpiperidinyl fluorobenzimidazole moiety on the isoindolo- and indoloquinoxaline skeletons resulted in active compounds. Nevertheless, the nature of this substitution at position 4 in the isoindolo[2,1-*a*] quinoxalines **1d** and **1h** seems less detrimental for the antiproliferative activity. In addition, the two antiproliferative compounds **1b** and **1d** showing interesting index of selectivity may constitute suitable candidates for further pharmacological studies. Moreover, it would be also interesting to enlarge the biological evaluation of these two new pyrrolo[1,2-*a*]quinoxaline derivatives **1b** and **1d** in order to precise now their mechanism of action.

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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