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Synthesis of New Pyrrolo[1,2-*a*]quinoxaline Derivatives as Potential Inhibitors of Akt Kinase

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Abstract

Akt kinases are attractive targets for small molecule drug discovery because of their key role in tumor cell survival/proliferation and their overexpression/activation in many human cancers. Recent efforts in the development and biological evaluation of small molecule inhibitors of Akt have led to the identification of novel Akt kinase inhibitors, based on a quinoxaline or pyrazinone scaffold. A series of new substituted pyrrolo[1,2-*a*]quinoxaline derivatives, structural analogues of these active quinoxaline or pyrazinone pharmacophores, was synthesized from various substituted 2-nitroanilines or 1,2-phenylenediamine *via* multistep heterocyclization process. These new compounds were tested for their *in vitro* ability to inhibit the proliferation of the human leukemic cell lines K562, U937 and HL60, and the breast cancer cell line MCF7. Three of these human cell lines (K562, U937 and MCF7) exhibited an active phosphorylated Akt form. The most promising active pyrroloquinoxalines were found to be **1a** that inhibited K562 cell line proliferation with an IC_{50} of 4.5 μM, and **1h** that inhibited U937 and MCF7 cell lines with IC_{50} of 5 and 8 μM, respectively. These two candidates exhibited more potent activities than the reference inhibitor A6730.

Keywords: *Pyrrolo[1,2-a]quinoxaline, Akt kinase, inhibitor, antiproliferative agents*

Introduction

Protein kinase B (PKB), also known as Akt, is a serine/threonine kinase that has recently garnered a great deal of attention as a promising molecular target for cancer therapy due to its critical role as a regulator of the cell's apoptotic machinery [1–5]. Akt is comprised of three mammalian isoforms, namely Akt1, Akt2, and Akt3. Akt as a downstream target of PI-3 kinase can induce a variety of biological responses. Overexpression of Akt can result from inactivation of tumor suppressor PTEN and has been correlated with an increasing number of human cancers. Akt is also responsible for promoting survival signals that downregulate apoptotic pathways and contribute to cancer progression. Thus, Akt has a wide range of downstream targets that regulate tumor-associated cell processes such as cell growth, cell cycle progression, survival, migration, epithelial-mesenchymal transition and angiogenesis. Correlation between resistance to chemotherapy and Akt activation has also been observed in prostate cancer cell lines and in human tumors tissue [3]. Inhibition of Akt alone or in combination with other standard cancer chemotherapeutics results in increased programmed death of cancer cells, leading to decreased tumor growth and tumor resistance to chemotherapy [3]. However, the development of Akt inhibitors as small molecule therapeutics for the treatment of cancer has been

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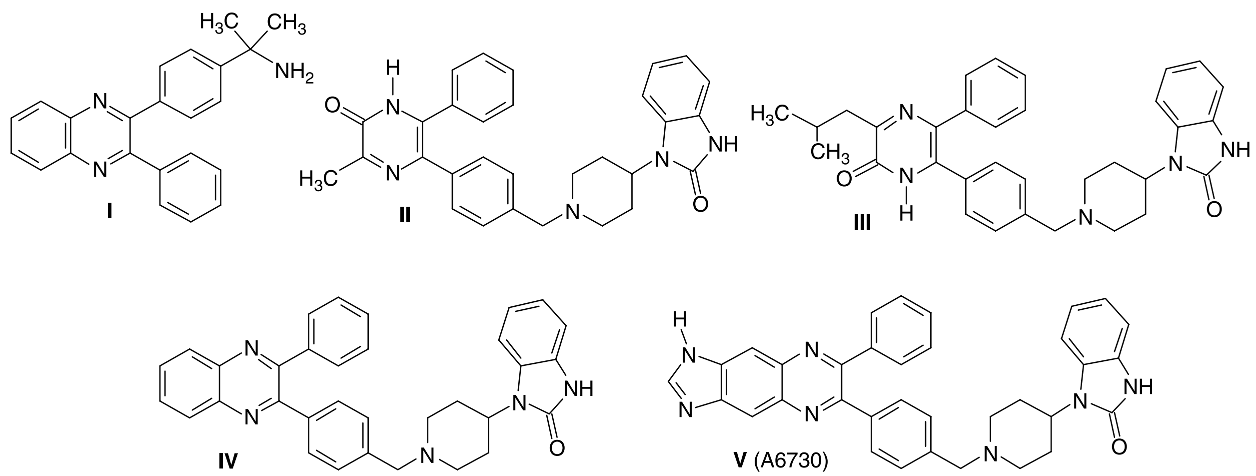


Figure 1. Structures of 2,3-diphenylquinoxaline **I**, pyrazinones **II-III**, quinoxaline **IV** and **V** (A6730), Akt kinase inhibitors.

hindered by a lack of Akt specific inhibitors (*versus* the AGC family of kinases) and isozyme selective (Akt1, Akt2, and Akt3) Akt inhibitors due to high sequence identity homology [1–5]. The 2,3-diphenylquinoxaline **I** was identified through a high throughput screening effort devoted to select compounds capable of inhibiting the three Akt isozymes (Figure 1) [6,7]. Further optimisation around this initial hit **I** resulted in the identification of more potent Akt1 (**II**)-, Akt2 (**III**)- and dual Akt1/2-selective (**IV** and **V**) kinase inhibitors [4–6,8–10].

We previously described a novel synthetic approach to pyrrolo[1,2-*a*]quinoxaline derivatives designed as interesting bioactive analogues of quinoline, quinoxaline or pyridine derivatives [11–14]. They could be developed as new isosteres of quinoxalines **I, IV-V** and pyrazinones **II-III**. Hence, we reported here the synthesis of a series of pyrrolo[1,2-*a*]quinoxaline derivatives **1** (Figure 2), and the preliminary results of their *in vitro* ability to inhibit the proliferation of the human leukemic cell lines U937, K562 and HL60, and the breast cancer cell line MCF7. Three of these human cell lines (K562, U937 and MCF7) exhibited an active phosphorylated Akt form.

Materials and methods

Chemistry

Instrumentation. Melting points were determined with an SM-LUX-POL Leitz hot-stage microscope and reported uncorrected. NMR spectra were recorded on

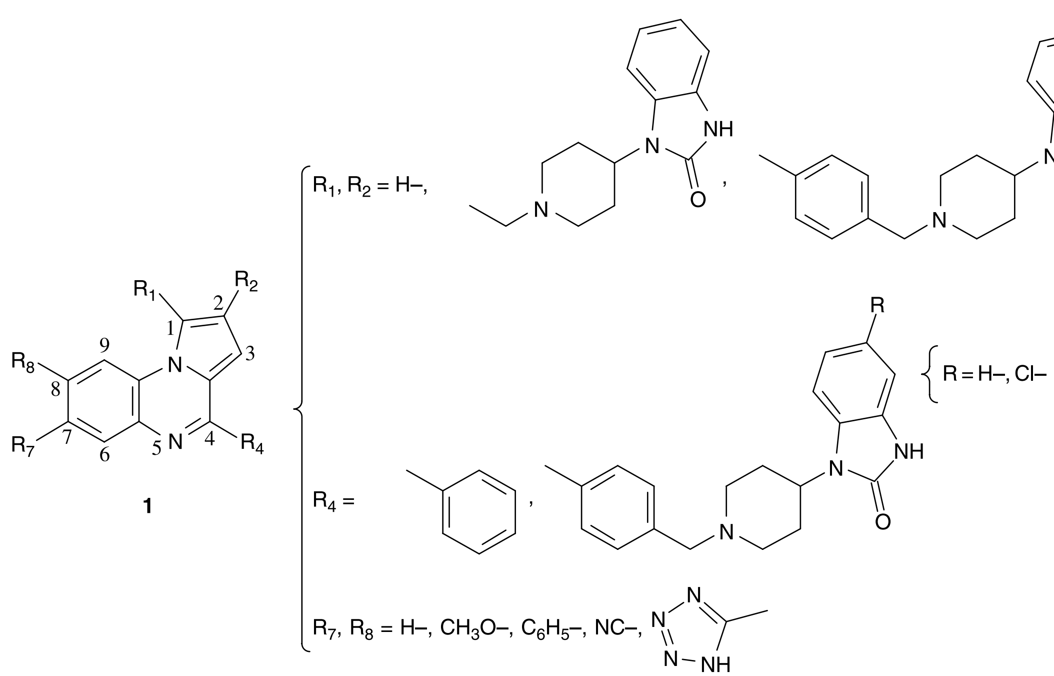


Figure 2. General structure of synthesized substituted pyrrolo[1,2-*a*]quinoxaline derivatives **1**.

a BRUKER AVANCE 300 spectrometer (300 MHz). Chemical shifts refer to tetramethylsilane which was used as an internal reference. Analytical TLC 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. Elemental analyses were conducted by CNRS, Vernaison, France. Compound A6730 was purchased from Sigma-Aldrich.

*Synthesis of 4-(pyrrolo[1,2-a]quinoxalin-4-yl) benzaldehydes **6a-e**. Method A:* A mixture of the 4-chloropyrrolo[1,2-*a*]quinoxaline **5a-e** [11,12,14] (5 mmol), the 4-formylphenylboronic acid (5.5 mmol) and $Pd(PPh_3)_4$ (0.15 mmol) in benzene (25 mL), ethanol (1.6 mL) and 2 M aqueous sodium carbonate solution (5.4 mL) was stirred and heated at reflux under nitrogen for 24 h. It was then cooled, transferred to a separating funnel, and the reaction flask washed out with water (3 × 50 mL) and dichloromethane (3 × 90 mL), the washings being added to the separating funnel. The organic layer was separated and the aqueous phase extracted with dichloromethane (2 × 100 mL). The combined organic extracts were then washed with water (3 × 130 mL), dried over Na_2SO_4, filtered and the filtrate evaporated under reduced pressure. The crude residue was triturated in a mixture of diethyl ether-petroleum ether (1/3). The resulting precipitate was filtered, washed with diethyl ether-petroleum ether (1/3), then with ethanol, dried and crystallized from ethanol to give the pure product **6a-e**. *Method B:* To a suspension of potassium 4-formylphenyltrifluoroborate (1.5 mmol), cesium carbonate (4.5 mmol), $PdCl_2(dppf) \cdot CH_2Cl_2$ (0.15 mmol), and 4-chloropyrrolo[1,2-*a*]quinoxaline **5a-b** (1.65 mmol), in THF (15 mL) was added water (1.5 mL) under a nitrogen atmosphere. The reaction mixture was stirred at reflux for 18 h, then cooled to room temperature, diluted with water (25 mL), and extracted with diethyl ether. The combined organic extracts were washed with brine and then dried over Na_2SO_4, filtered and the filtrate evaporated under reduced pressure. The crude residue was triturated in a mixture of diethyl ether-petroleum ether (1/3). The resulting precipitate was filtered, washed with diethyl ether-petroleum ether (1/3), then with ethanol, dried and crystallized from ethanol to give the pure product **6a-b**.

*4-(Pyrrolo[1,2-a]quinoxalin-4-yl) benzaldehyde (**6a**).* Yield: 88% (method A), 34% (method B), yellow crystals, mp = 114°C; IR ν_{max} (KBr)/cm^{-1} 1700 (CHO); ^{1}H NMR δ (300 MHz, $CDCl_3$) 10.16 (s, 1H, CHO), 8.21 (d, 2H, *J* 8.20 Hz, H-2 and H-6), 8.08 (d, 2H, *J* 8.20 Hz, H-3 and H-5), 8.08-8.04 (m, 2H, H-1′ and H-9′), 7.94 (dd, 1H, *J* 8.15 and 1.30 Hz, H-6′), 7.59 (ddd, 1H, *J* 8.15, 7.40 and 1.30 Hz, H-8′), 7.51 (ddd, 1H, *J* 8.15, 7.40 and 1.30 Hz, H-7′), 7.01 (dd, 1H, *J* 4.05 and 1.30 Hz, H-3′), 6.96 (dd, 1H, *J* 4.05 and 2.70 Hz, H-2′). Anal. Calcd. for $C_{18}H_{12}N_2O$: C, 79.39; H, 4.44; N, 10.29. Found: C, 79.55; H, 4.72; N, 10.08%.

*4-(7-Methoxypyrrolo[1,2-a]quinoxalin-4-yl) benzaldehyde (**6b**).* Yield: 74% (method A), 31% (method B), yellow crystals, mp = 168°C; IR ν_{max} (KBr)/cm^{-1} 1710 (CHO); ^{1}H NMR δ (300 MHz, $CDCl_3$) 10.15 (s, 1H, CHO), 8.21 (d, 2H, *J* 8.10 Hz, H-2 and H-6), 8.10 (d, 2H, *J* 8.10 Hz, H-3 and H-5), 8.06-8.03 (m, 1H, H-1′), 7.87 (d, 1H, *J* 9.10 Hz, H-9′), 7.62 (m, 1H, H-6′), 7.22 (dd, 1H, *J* 9.10 and 2.60 Hz, H-8′), 7.05-7.02 (m, 1H, H-3′), 6.96-6.92 (m, 1H, H-2′), 3.96 (s, 3H, CH_3O). Anal. Calcd. for $C_{19}H_{14}N_2O_2$: C, 75.48; H, 4.67; N, 9.27. Found: C, 75.32; H, 4.47; N, 9.44%.

*4-(8-Methoxypyrrolo[1,2-a]quinoxalin-4-yl) benzaldehyde (**6c**).* Yield: 90%, yellow crystals, mp = 196°C; IR ν_{max} (KBr)/cm^{-1} 1705 (CHO); ^{1}H NMR δ (300 MHz, $CDCl_3$) 10.13 (s, 1H, CHO), 8.18 (d, 2H, *J* 8.00 Hz, H-2 and H-6), 8.05 (d, 2H, *J* 8.00 Hz, H-3 and H-5), 7.99 (d, 1H, *J* 9.00 Hz, H-6′), 7.95-7.93 (m, 1H, H-1′), 7.30 (d, 1H, *J* 2.85 Hz, H-9′), 7.09 (dd, 1H, *J* 9.00 and 2.85 Hz, H-7′), 6.98-6.93 (m, 2H, H-3′ and H-2′), 3.99 (s, 3H, CH_3O). Anal. Calcd. for $C_{19}H_{14}N_2O_2$: C, 75.48; H, 4.67; N, 9.27. Found: C, 75.62; H, 4.80; N, 9.13%.

*4-(8-Phenylpyrrolo[1,2-a]quinoxalin-4-yl) benzaldehyde (**6d**).* Yield: 80%, yellow crystals, mp = 176°C; IR ν_{max} (KBr)/cm^{-1} 1700 (CHO); ^{1}H NMR δ (300 MHz, $CDCl_3$) 10.16 (s, 1H, CHO), 8.23 (d, 2H, *J* 7.93 Hz, H-2 and H-6), 8.17-8.08 (m, 3H, H-1′, H-6′ and H-9′), 7.78-7.75 (m, 3H, H-2″, H-6″ and H-7′), 7.55 (t, 2H, *J* 7.20 Hz, H-3″ and H-5″), 7.46 (t, 1H, *J* 7.20 Hz, H-4″), 7.06-7.03 (m, 1H, H-3′), 7.00-6.98 (m, 1H, H-2′). Anal. Calcd. for $C_{24}H_{16}N_2O$: C, 82.74; H, 4.63; N, 8.04. Found: C, 82.57; H, 4.69; N, 7.93%.

*4-(7-Cyanopyrrolo[1,2-a]quinoxalin-4-yl) benzaldehyde (**6e**).* Yield: 55%, yellow crystals, mp = 310°C; IR ν_{max} (KBr)/cm^{-1} 2240 (N≡C), 1705 (CHO); ^{1}H NMR δ (300 MHz, $CDCl_3$) 10.16 (s, 1H, CHO), 8.38 (dd, 1H, *J* 2.80 and 1.10 Hz, H-1′), 8.20 (d, 2H, *J* 8.30 Hz, H-2 and H-6), 8.11-8.09 (m, 3H, H-3, H-5 and H-6′), 8 (d, 1H, *J* 8.5 Hz, H-9′), 7.81 (dd, 1H, *J* 8.5 and 1.80 Hz, H-8′), 7.10 Hz (dd, 1H, *J* 4.05 and 1.10 Hz, H-3′), 7.05 (dd, 1H, *J* 4.05 and 2.80 Hz, H-2′). Anal. Calcd. for $C_{19}H_{11}N_3O$: C, 76.75; H, 3.73; N, 14.13. Found: C, 76.89; H, 3.64; N, 14.27%.

*Synthesis of 1,3-dihydro-1-{1-[4-(pyrrolo[1,2-a] quinoxalin-4-yl) benzyl]piperidin-4-yl}-2H-benzimidazol-2-one (**1a-f**), 1,3-dihydro-1-{1-[(4-phenylpyrrolo[1,2-a]quinoxalin-1-yl) methyl]piperidin-4-*

*yl}-2H-benzimidazol-2-one (**1h**), and 1,3-dihydro-1-{1-[4-(4-phenylpyrrolo[1,2-a]quinoxalin-1-yl)benzyl]piperidin-4-yl}-2H-benzimidazol-2-one (**1i**).* The pH of a solution of the aldehyde **6a-e**, **9**, or **11** (2.5 mmol) and secondary amine (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. Solids were crystallized from propan-2-ol, filtered, washed with diethyl ether and dried under reduced pressure to give the compounds **1a-f**, **1h-i**.

*1,3-Dihydro-1-{1-[4-(pyrrolo[1,2-a]quinoxalin-4-yl)benzyl]piperidin-4-yl}-2H-benzimidazol-2-one (**1a**).* Yield: 45%, white crystals, mp = 215°C; IR ν_{max} (KBr)/cm^{-1} 3360 (NH), 1685 (C=O); ^{1}H NMR δ (300 MHz, $CDCl_3$) 10.15 (s, 1H, NH), 8.08 (dd, 1H, *J* 7.95 and 1.45 Hz, H-9″), 8.03 (dd, 1H, *J* 2.75 and 1.40 Hz, H-1″), 8.01 (d, 2H, *J* 8.15 Hz, H-3′ and H-5′), 7.90 (dd, 1H, *J* 7.95 and 1.45 Hz, H-6″), 7.57 (d, 2H, *J* 8.15 Hz, H-2′ and H-6′), 7.51 (ddd, 1H, *J* 7.95, 7.30 and 1.45 Hz, H-8″), 7.48 (ddd, 1H, *J* 7.95, 7.30 and 1.45 Hz, H-7″), 7.35-7.32 (m, 1H, H benzimid.), 7.10-7.06 (m, 3H, H benzimid.), 7.03 (dd, 1H, *J* 4.05 and 1.40 Hz, H-3″), 6.93 (dd, 1H, *J* 4.05 and 2.75 Hz, H-2″), 4.44-4.41 (m, 1H, CH pip.), 3.69 (s, 2H, CH_2N), 3.13-3.09 (m, 2H, CH_2 pip.), 2.54-2.50 (m, 2H, CH_2 pip.), 2.27-2.23 (m, 2H, CH_2 pip.), 1.86-1.83 (m, 2H, CH_2 pip.). ^{13}C NMR δ (75 MHz, $CDCl_3$) 155.0 (CO), 154.2 (C4″), 140.4 (C5a″), 137.2 (C9a″), 136.2 (C1′), 130.1 (C6″), 129.2 (C3a), 129.0 (C3′ and C5′), 128.5 (C7a), 127.9 (C2′ and C6′), 127.4 (C8″), 127.1 (C3a″), 125.3 (C4′), 125.2 (C7″), 121.0 (C6), 120.9 (C5), 114.5 (C9″), 113.9 (C3″), 113.6 (C1″), 109.8 (C4), 109.6 (C7), 108.6 (C2″), 62.6 (CH_2N), 53.1 (CH_2 pip.), 53.0 (CH_2 pip.), 50.7 (CH pip.), 29.3 (CH_2 pip.), 29.2 (CH_2 pip.). Anal. Calcd. for $C_{30}H_{27}N_5O$: C, 76.08; H, 5.75; N, 14.79. Found: C, 75.87; H, 5.86; N, 14.94%.

*1,3-Dihydro-1-{1-[4-(7-methoxypyrrolo[1,2-a]quinoxalin-4-yl)benzyl]piperidin-4-yl}-2H-benzimidazol-2-one (**1b**).* Yield: 53%, pale-yellow crystals, mp = 110°C; IR ν_{max} (KBr)/cm^{-1} 3365 (NH), 1680 (C=O); ^{1}H NMR δ (300 MHz, $CDCl_3$) 8.84 (s, 1H, NH), 8.01 (d, 2H, *J* 7.95 Hz, H-3′ and H-5′), 7.96 (dd, 1H, *J* 2.70 and 1.20 Hz, H-1″), 7.82 (d, 1H, *J* 9.05 Hz, H-9″), 7.60 (d, 2H, *J* 7.95 Hz, H-2′ and H-6′), 7.55 (d, 1H, *J* 2.80 Hz, H-6″), 7.35-7.32 (m, 1H, H benzimid.), 7.13 (dd, 1H, *J* 9.05 and 2.80 Hz, H-8″), 7.11-7.08 (m, 3H, H benzimid.), 7.03 (dd, 1H, *J* 4.05 and 1.20 Hz, H-3″), 6.89 (dd, 1H, *J* 4.05 and 2.70 Hz, H-2″), 4.45-4.41 (m, 1H, CH pip.), 3.94 (s, 3H, CH_3O), 3.70 (s, 2H, CH_2N), 3.13-3.10 (m, 2H, CH_2 pip.), 2.54-2.51 (m, 2H, CH_2 pip.), 2.27-2.24 (m, 2H, CH_2 pip.), 1.89-1.85 (m, 2H, CH_2 pip.). Anal. Calcd. for $C_{31}H_{29}N_5O_2$: C, 73.93; H, 5.80; N, 13.91. Found: C, 74.05; H, 5.89; N, 13.72%.

*1,3-Dihydro-1-{1-[4-(8-methoxypyrrolo[1,2-a]quinoxalin-4-yl)benzyl]piperidin-4-yl}-2H-benzimidazol-2-one (**1c**).* Yield: 48%, pale-yellow crystals, mp = 156°C; IR ν_{max} (KBr)/cm^{-1} 3355 (NH), 1680 (C=O); ^{1}H NMR δ (300 MHz, $CDCl_3$) ^{1}H NMR δ (300 MHz, $CDCl_3$) 10.27 (s, 1H, NH), 8.01 (d, 2H, *J* 8.00 Hz, H-3′ and H-5′), 7.98 (d, 1H, *J* 8.65 Hz, H-6″), 7.92 (dd, 1H, *J* 2.75 and 1.30 Hz, H-1″), 7.57 (d, 2H, *J* 8.00 Hz, H-2′ and H-6′), 7.37-7.33 (m, 2H, 2H benzimid.), 7.30 (d, 1H, *J* 2.85 Hz, H-9″), 7.11-7.06 (m, 3H, H-7″ and 2H benzimid.), 7.01 (dd, 1H, *J* 4.00 and 1.30 Hz, H-3″), 6.92 (dd, 1H, *J* 4.00 and 2.75 Hz, H-2″), 4.46-4.42 (m, 1H, CH pip.), 3.98 (s, 3H, CH_3O), 3.71 (s, 2H, CH_2N), 3.15-3.11 (m, 2H, CH_2 pip.), 2.54-2.51 (m, 2H, CH_2 pip.), 2.27-2.23 (m, 2H, CH_2 pip.), 1.88-1.83 (m, 2H, CH_2 pip.). Anal. Calcd. for $C_{31}H_{29}N_5O_2$: C, 73.93; H, 5.80; N, 13.91. Found: C, 74.05; H, 5.89; N, 13.72%.

*1,3-Dihydro-1-{1-[4-(8-phenylpyrrolo[1,2-a]quinoxalin-4-yl)benzyl]piperidin-4-yl}-2H-benzimidazol-2-one (**1d**).* Yield: 61%, pale-yellow crystals, mp = 150°C; IR ν_{max} (KBr)/cm^{-1} 3365 (NH), 1680 (C=O); ^{1}H NMR δ (300 MHz, $CDCl_3$) 9.17 (s, 1H, NH), 8.12 (d, 2H, *J* 8.00 Hz, H-3′ and H-5′), 8.09 (dd, 1H, *J* 2.75 and 1.25 Hz, H-1″), 8.05 (m, 2H, H-6″ and H-9″), 7.76-7.72 (m, 3H, 3H phényl), 7.61-7.57 (m, 4H, 2H phenyl, H-2′ and H-6′), 7.44 (d, 1H, *J* 8.40 Hz, H-7″), 7.35-7.31 (m, 1H, H benzimid.), 7.08 (m, 3H, H benzimid.), 7.03 (dd, 1H, *J* 4.00 and 1.25 Hz, H-3″), 6.95 (dd, 1H, *J* 4.00 and 2.75 Hz, H-2″), 4.46-4.42 (m, 1H, CH pip.), 3.74 (s, 2H, CH_2N), 3.14-3.10 (m, 2H, CH_2 pip.), 2.56-2.52 (m, 2H, CH_2 pip.), 2.28-2.24 (m, 2H, CH_2 pip.), 1.87-1.83 (m, 2H, CH_2 pip.). Anal. Calcd. for $C_{36}H_{31}N_5O$: C, 78.66; H, 5.68; N, 12.74 Found: C, 78.86; H, 5.49; N, 12.57%.

*1,3-Dihydro-1-{1-[4-(7-cyanopyrrolo[1,2-a]quinoxalin-4-yl)benzyl]piperidin-4-yl}-2H-benzimidazol-2-one (**1e**).* Yield: 68%, pale-yellow crystals, mp = 152°C; IR ν_{max} (KBr)/cm^{-1} 3370 (NH), 2230 (N≡C), 1695 (C=O); ^{1}H NMR δ (300 MHz, $CDCl_3$) 9.57 (s, 1H, NH), 8.35 (d, 1H, *J* 1.75 Hz, H-6″), 8.06 (dd, 1H, *J* 2.90 and 1.05 Hz, H-1″), 8.02 (d, 2H, *J* 8.15 Hz, H-3′ and H-5′), 7.96 (d, 1H, *J* 8.60 Hz, H-9″), 7.76 (dd, 1H, *J* 8.60 and 1.75 Hz, H-8″), 7.57 (d, 2H, *J* 8.15 Hz, H-2′ and H-6′), 7.36-7.32 (m, 1H, H benzimid.), 7.14 (dd, 1H, *J* 4.00 and 1.05 Hz, H-3″), 7.12-7.09 (m, 3H, H benzimid.), 7.02 (dd, 1H, *J* 4.00 and 2.90 Hz, H-2″), 4.46-4.42 (m, 1H, CH pip.), 3.79 (s, 2H, CH_2N), 3.22-3.19 (m, 2H, CH_2 pip.), 2.58-2.56 (m, 2H, CH_2 pip.), 2.36-2.33 (m, 2H, CH_2 pip.), 1.89-1.86 (m, 2H, CH_2 pip.). Anal. Calcd. for

$C_{31}H_{26}N_6O$: C, 74.68; H, 5.25; N, 16.86. Found: C, 74.81; H, 5.10; N, 17.03%.

*1,3-Dihydro-1-{1-[4-(pyrrolo[1,2-a]quinoxalin-4-yl)benzyl]piperidin-4-yl}-5-chloro-2H-benzimidazol-2-one (**1f**)*. Yield: 21%, white crystals, mp = 245°C; IR ν_{max} (KBr)/cm^{-1} 3350 (NH), 1680 (C=O); ^{1}H NMR δ (300 MHz, $CDCl_3$) 10.22 (s, 1H, NH), 8.13 (d, 1H, *J* 8.10 Hz, H-9″), 8.11-8.04 (m, 3H, H-1″, H-3′ and H-5′), 7.91 (d, 1H, *J* 8.10 Hz, H-6″), 7.57-7.52 (m, 4H, H-2′, H-6′, H-7″ and H-8″), 7.17-7.15 (m, 1H, H benzimid.), 7.08-7.04 (m, 2H, H benzimid.), 6.96-6.93 (m, 2H, H-2″ and H-3″), 4.41-4.38 (m, 1H, CH pip.), 3.67 (s, 2H, CH_2N), 3.02-2.97 (m, 2H, CH_2 pip.), 2.42-2.33 (m, 2H, CH_2 pip.), 2.24-2.215 (m, 2H, CH_2 pip.), 1.81-1.71 (m, 2H, CH_2 pip.). Anal. Calcd. for $C_{30}H_{26}ClN_5O$: C, 70.93; H, 5.16; N, 13.79. Found: C, 71.13; H, 5.31; N, 14.01%.

*1,3-Dihydro-1-{1-[(4-phenylpyrrolo[1,2-a]quinoxalin-1-yl)methyl]piperidin-4-yl}-2H-benzimidazol-2-one (**1h**)*. Yield: 31%, white crystals, mp = 257°C; IR ν_{max} (KBr)/cm^{-1} 3370 (NH), 1685 (C=O); ^{1}H NMR δ (300 MHz, $CDCl_3$) 9.44 (s, 1H, NH), 8.75 (dd, 1H, *J* 8.05 and 1.40 Hz, H-9″), 8.11 (dd, 1H, *J* 8.05 and 1.40 Hz, H-6′), 7.99-7.96 (m, 2H, H-2″ and H-6″), 7.63-7.54 (m, 5H, H-7′, H-8′, H-3″, H-4″ and H-5″), 7.15-7.03 (m, 4H, 4H benzimid.), 6.95 (d, 1H, *J* 3.85 Hz, H-2″), 6.80 (d, 1H, *J* 3.85 Hz, H-3″), 4.52-4.49 (m, 1H, CH pip.), 4.07 (s, 2H, CH_2N), 3.29-3.26 (m, 2H, CH_2 pip.), 2.52-2.36 (m, 4H, $2CH_2$ pip.), 1.93-1.89 (m, 2H, CH_2 pip.). Anal. Calcd. for $C_{30}H_{27}N_5O$: C, 76.08; H, 5.75; N, 14.79. Found: C, 75.98; H, 5.55; N, 14.70%.

*1,3-Dihydro-1-{1-[4-(4-phenylpyrrolo[1,2-a]quinoxalin-1-yl)benzyl]piperidin-4-yl}-2H-benzimidazol-2-one (**1i**)*. Yield: 70%, pale-yellow crystals, mp = 165°C; IR ν_{max} (KBr)/cm^{-1} 3360 (NH), 1680 (C=O); ^{1}H NMR δ (300 MHz, $CDCl_3$) 9.20 (s, 1H, NH), 8.04 (d, 2H, *J* 8.15 Hz, H-3′ et H-5′), 7.99 (dd, 1H, *J* 7.95 and 1.30 Hz, H-9″), 7.56-7.47 (m, 8H, H-2′, H-6′, H-6″, H-7″, H-8″, H-2‴, H-4‴ and H-6‴), 7.37 (t, 2H, *J* 7.85 Hz, H-3‴ and H-5‴), 7.17-7.09 (m, 4H, 4H benzimid.), 7.06 (d, 1H, *J* 4.05 Hz, H-3″), 6.81 (d, 1H, *J* 4.05 Hz, H-2″), 4.48-4.45 (m, 1H, CH pip.), 3.74 (s, 2H, CH_2N), 3.20-3.16 (m, 2H, CH_2 pip.), 2.60-2.57 (m, 2H, CH_2 pip.), 2.34-2.31 (m, 2H, CH_2 pip.), 1.93-1.89 (m, 2H, CH_2 pip.). Anal. Calcd. for $C_{36}H_{31}N_5O$: C, 78.66; H, 5.68; N, 12.74. Found: C, 78.42; H, 5.45; N, 12.93%.

*1,3-Dihydro-1-{1-[4-(7-(1H-tetrazol-5-yl)pyrrolo[1,2-a]quinoxalin-4-yl)benzyl]piperidin-4-yl}-2H-benzimidazol-2-one (**1g**)*. A solution of **1e** (1 mmol), sodium azide (5 mmol), and ammonium chloride (5 mmol) in DMF (8 mL) was heated to 110°C for 8 h. After the solution cooled to room temperature, water was added and the resultant solid filtered, washed with ethanol then with diethyl ether, and dried to give **1g**. Yield: 73%, beige crystals, mp = 271°C; IR ν_{max} (KBr)/cm^{-1} 3380 (NH), 1690 (C=O); ^{1}H NMR δ (300 MHz, DMSO-d_6) 10.92 (s, 1H, NH), 8.60 (dd, 1H, *J* 2.95 and 1.10 Hz, H-1″), 8.55 (d, 1H, *J* 1.95 Hz, H-6″), 8.47 (d, 1H, *J* 8.75 Hz, H-9″), 8.24 (dd, 1H, *J* 8.75 and 1.95 Hz, H-8″), 8.07 (d, 2H, *J* 7.90 Hz, H-3′ and H-5′), 7.66 (d, 2H, *J* 7.90 Hz, H-2′ and H-6′), 7.28-7.25 (m, 1H, H benzimid.), 7.10 (dd, 1H, *J* 3.95 and 1.10 Hz, H-3″), 7.03-6.99 (m, 4H, H benzimid. and H-2″), 4.36-4.33 (m, 1H, CH pip.), 4.03 (s, 2H, CH_2N), 3.32-3.28 (m, 2H, CH_2 pip.), 2.70-2.55 (m, 4H, $2CH_2$ pip.), 1.82-1.77 (m, 2H, CH_2 pip.). Anal. Calcd. for $C_{31}H_{27}N_9O$: C, 68.74; H, 5.02; N, 23.28. Found: C, 68.90; H, 5.13; N, 23.07%.

*Synthesis of 1,3-dihydro-1-{1-[4-(4-phenylpyrrolo[1,2-a]quinoxalin-2-yl)methyl]piperidin-4-yl}-2H-benzimidazol-2-one (**1j**) and 1,3-dihydro-1-{1-[α-cyano-4-(4-phenylpyrrolo[1,2-a]quinoxalin-2-yl)methyl]piperidin-4-yl}-2H-benzimidazol-2-one (**1k**)*. The pH of a solution of the aldehyde **15** (2.5 mmol) and secondary amine (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 resulting precipitate was filtered, washed with methanol then with diethyl ether and dried under reduced pressure to give **1k**. After evaporation of the solvents, the second residue was triturated in water and extracted with dichloromethane. The organic layer was washed with water, dried over magnesium sulfate and evaporated to dryness. Solid was crystallized from propan-2-ol, filtered, washed with diethyl ether and dried under reduced pressure to give compound **1j**.

*1,3-Dihydro-1-{1-[4-(4-phenylpyrrolo[1,2-a]quinoxalin-2-yl)methyl]piperidin-4-yl}-2H-benzimidazol-2-one (**1j**)*. Yield: 43%, white crystals, mp = 148°C; IR ν_{max} (KBr)/cm^{-1} 3185 (NH), 1690 (C=O); ^{1}H NMR δ (300 MHz, $CDCl_3$) 10.41 (s, 1H, NH), 8.07 (dd, 1H, *J* 8.00 and 1.35 Hz, H-9′), 8.06-8.02 (m, 3H, H-1′, H-2″ and H-6″), 7.89 (dd, 1H, *J* 8.00 and 1.35 Hz, H-6′), 7.59-7.56 (m, 4H, H-3″, H-4″, H-5″ and H-8′), 7.48 (ddd, 1H, *J* 8.00, 7.45 and 1.35 Hz, H-7′), 7.33-7.30 (m, 1H, H benzimid.), 7.13-7.02 (m, 3H, 3H benzimid.), 7.00 (d, 1H, *J* 1.20 Hz, H-3′), 4.42-4.40 (m, 1H, CH pip.), 3.76 (s, 2H, CH_2N), 3.20-3.17 (m, 2H, CH_2 pip.), 2.55-2.52 (m, 2H, CH_2 pip.), 2.25-2.22 (m, 2H, CH_2 pip.), 1.86-1.83 (m, 2H, CH_2 pip.). Anal. Calcd. for $C_{30}H_{27}N_5O$: C, 76.08; H, 5.75; N, 14.79. Found: C, 76.28; H, 5.82; N, 14.83%.

*1,3-Dihydro-1-{1-[α-cyano-4-(4-phenylpyrrolo[1,2-a]quinoxalin-2-yl)methyl]piperidin-4-yl}-2H-benzimidazol-2-one (**1k**)*. Yield: 25%, white crystals, mp = 260°C; IR ν_{max} (KBr)/cm^{-1} 3190 (NH), 2230 (N≡C), 1695 (C=O); ^{1}H NMR δ (300 MHz, $CDCl_3$) 8.75 (s, 1H, NH), 8.19 (d, 1H, *J* 1.15 Hz, H-

1′), 8.08 (dd, 1H, *J* 8.05 and 1.35 Hz, H-9′), 8.05-8.02 (m, 2H, H-2″ and H-6″), 7.96 (dd, 1H, *J* 8.05 and 1.35 Hz, H-6′), 7.62-7.58 (m, 4H, H-3″, H-4″, H-5″ and H-8′), 7.50 (ddd, 1H, *J* 8.05, 7.40 and 1.30 Hz, H-7′), 7.22-7.18 (m, 1H, H benzimid.), 7.12 (d, 1H, *J* 1.15 Hz, H-3′), 7.10-7.07 (m, 3H, H benzimid.), 5.15 (s, 1H, CHCN), 4.38-4.36 (m, 1H, CH pip.), 3.22-3.19 (m, 1H, CH_2 pip.), 2.98-2.95 (m, 1H, CH_2 pip.), 2.72-2.70 (m, 2H, CH_2 pip.), 2.44-2.41 (m, 2H, CH_2 pip.), 2.01-1.98 (m, 1H, CH_2 pip.), 1.86-1.83 (m, 1H, CH_2 pip.). ^{13}C NMR δ (75 MHz, $CDCl_3$) 154.2 (CO), 153.4 (C-4′), 138.0 (C-5a′), 136.0 (C-9a′), 130.6 (C-1″), 130.1 (C-4″), 129.8 (C-6′), 129.1 (C-3″ and C-5″), 128.9 (C-2″ and C-6″), 128.8 (C-3a), 128.7 (C-7a), 126.8 (C-8′), 126.4 (C-3a′), 124.8 (C-7′), 123.0 (C-6), 121.0 (C-5), 120.8 (C-1′), 116.9 (N≡C), 115.9 (C-9′), 115.6 (C-3′), 109.2 (C-4), 109.0 (C-2′), 108.0 (C-7), 55.4 (CH), 52.0 (CH_2 pip.), 50.4 (CH_2 pip.), 47.4 (CH pip.), 29.0 (CH_2 pip.), 28.7 (CH_2 pip.). Anal. Calcd. for $C_{31}H_{26}N_6O$: C, 74.68; H, 5.26; N, 16.86. Found: C, 74.41; H, 5.46; N, 17.04%.

*Synthesis of 1-bromo-4-phenylpyrrolo[1,2-a]quinoxaline (**10**)*. To a solution of 4-phenylpyrrolo[1,2-*a*]quinoxaline **8** (15 mmol) in CH_2Cl_2 (65 mL) was added a solution of *N*-bromosuccinimide (15 mmol) CH_2Cl_2 (30 mL). After the mixture was stirred at room temperature for 30 min, a 2% aqueous solution of NaOH (65 mL) was added and the mixture extracted with CH_2Cl_2 (3 × 35 mL). The combined organic extracts were dried over sodium sulfate and concentrated under reduced pressure. The residue was triturated in ethanol, filtered, washed with ethanol then with petroleum ether and dried, yielding **10**. Yield: 82%, white crystals, mp = 159°C; ^{1}H NMR δ (300 MHz, $CDCl_3$) 9.35 (dd, 1H, *J* 7.95 and 1.75 Hz, H-9″), 8.07 (dd, 1H, *J* 7.95 and 1.75 Hz, H-6), 7.95-7.92 (m, 2H, H-2′ and H-6′), 7.57-7.54 (m, 5H, H-7, H-8, H-3′, H-4′ and H-5′), 6.97 (d, 1H, *J* 4.25 Hz, H-2), 6.92 (d, 1H, *J* 4.25 Hz, H-3). Anal. Calcd. for $C_{17}H_{11}BrN_2$: C, 63.18; H, 3.43; N, 8.67. Found: C, 63.09; H, 3.22; N, 8.95%.

*4-(4-Phenylpyrrolo[1,2-a]quinoxalin-1-yl) benzaldehyde (**11**)*. To suspension of 1-bromo-4-phenylpyrrolo[1,2-*a*]quinoxaline **10** (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_2CO_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_2Cl_2 (3 × 80 mL). The organic layer was washed with a saturated solution of NaCl (70 mL), and the combined organic extracts were dried over sodium sulfate, filtered, and evaporated under reduced pressure. The residue was triturated in ethanol, filtered, washed with ethanol then with petroleum ether and dried to give **11**. Yield: 84%, yellow crystals, mp = 215°C; IR ν_{max} (KBr)/cm^{-1} 1705 (C=O); ^{1}H NMR δ (300 MHz, $CDCl_3$) 10.16 (s, 1H, CHO), 8.06 (d, 2H, *J* 8.20 Hz, H-2 and H-6), 8.06-8.02 (m, 2H, H-6′ and H-9′), 7.78 (d, 2H, *J* 8.20 Hz, H-3 and H-5), 7.62-7.58 (m, 2H, H-2″ and H-6″), 7.47-7.44 (m, 3H, H-7′, H-8′ and H-4″), 7.26-7.14 (m, 3H, H-2′, H-3″ and H-5″), 6.92 (d, 1H, *J* 3.85 Hz, H-3′). Anal. Calcd. for $C_{24}H_{16}N_2O$: C, 82.74; H, 4.63; N, 8.04. Found: C, 82.97; H, 4.57; N, 8.11%.

Pharmacology

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 (FCS), 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), as well as phytohemagglutinin (lymphoproliferative agent) (PHA)-induced cells. PBMNC from 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 MTS cell proliferation assay 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 PBS (Phosphate Buffer Saline) and plated in quadruplicate into microtiter-plate wells in 100 μL culture media without or with our various compounds at increasing concentration (0, 1, 5, 10 and 20 μM). After 3 h of incubation 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.

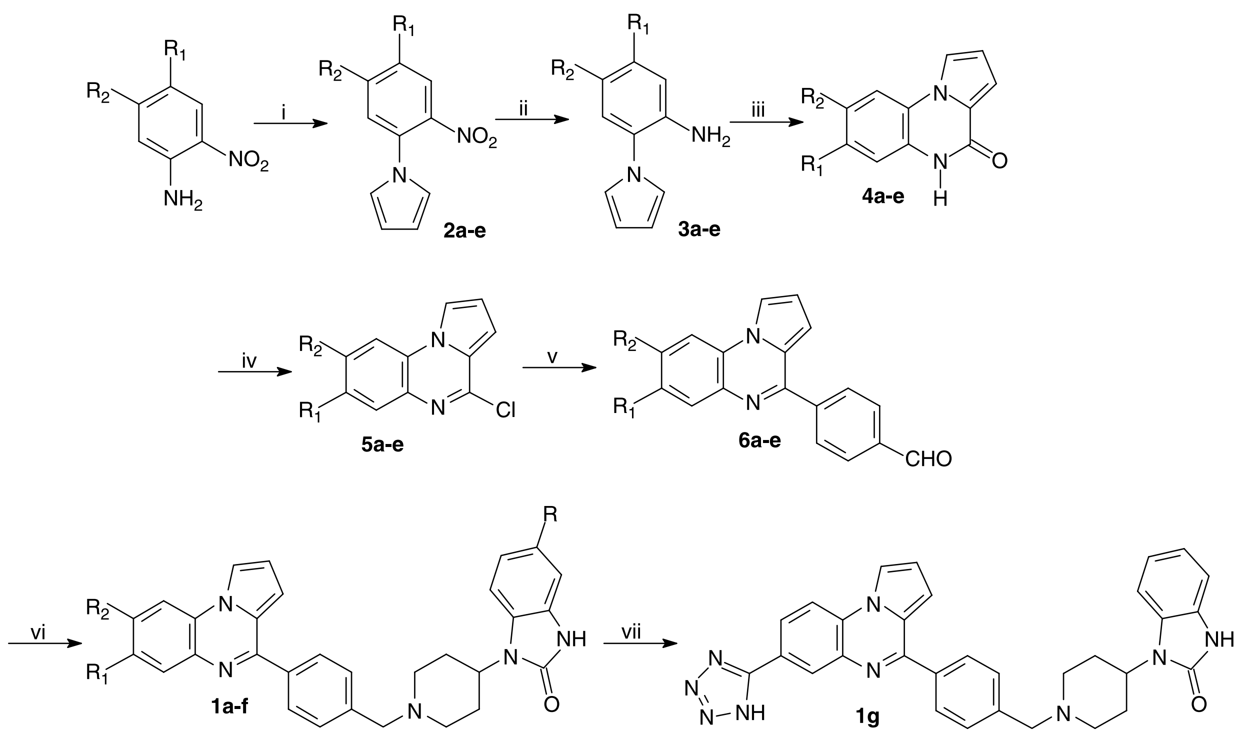
Results and discussion

Chemistry

The synthesis of the 1,3-dihydro-1-{1-[4-(pyrrolo[1,2-*a*]quinoxalin-4-yl)benzyl]piperidin-4-yl}-

2*H*-benzimidazol-2-ones **1a-f** has been accomplished in six or seven steps starting from 2-nitroaniline according to the sequence depicted in Schbeme 1. The Clauson-Kaas reaction of 2-nitroanilines with 2,5-dimethoxytetrahydrofuran (DMTHF) in acetic acid gave the pyrrolic derivatives **2a-e**, which were reduced using a $NaBH_4$-$CuSO_4$ system to provide the attempted 1-(2-aminophenyl)pyrroles **3a-d** [14] or using a $SnCl_2$, $2H_2O$ treatment to give **3e** [15]. The reaction of **3a-e** with triphosgene in toluene gave the lactams **4a-e**, which were subsequently chlorodehydroxylated with phosphorous oxychloride, leading to the 4-chloroquinoxalines **5a-e** [12,14]. 4-(Pyrrolo[1,2-*a*]quinoxalin-4-yl)benzaldehydes **6a-e** were easily prepared in quite good yields (55-90%) by a direct Suzuki-Miyaura cross-coupling reaction of 4-chloropyrroloquinoxalines **5a-e** with 4-formylphenylboronic acid performed in the presence of $Pd(PPh_3)_4$ as a catalyst, and in the presence of sodium carbonate used as the base (method A) [14,16,17]. The Suzuki-Miyaura-type reaction was then expanded to the use of potassium (*E*)-4-formylphenyltrifluoroborate and 4-chloropyrrolo[1,2-*a*]quinoxaline **5a-b** as coupling partner by using $PdCl_2(dppf){\cdot}CH_2Cl_2$ as the catalyst, cesium carbonate as the base, and THF-H_2O as the solvent system (method B) [14,18,19]. The aldehydes **6a-e** were then engaged in a reductive amination with $NaBH_3CN$ and 4-(2-ketobenzimidazolin-1-yl)piperidine or 4-(5-chloro-2-ketobenzimidazolin-1-yl)piperidine to give the pyrroloquinoxalines **1a-f** [20]. The tetrazole derivative **1g** was synthesized by reacting sodium azide with **1e** [21].

The 4-phenylpyrrolo[1,2-*a*]quinoxaline **8** was prepared by cyclisation of the amide **7** in refluxing phosphorus oxychloride. Under Vilsmeier-Haack reaction conditions, formylation of **8** occurs selectively using a $POCl_3$/DMF complex at position 1 to give the 4-phenylpyrrolo[1,2-*a*]quinoxaline-1-carbaldehyde **9** [22]. Reaction of **8** and one equivalent of *N*-bromosuccinimide (NBS) afforded the 1-bromo-4-phenylpyrrolo[1,2-*a*]quinoxaline **10** as the sole reac-



	R_1–	R_2–	R–
1-6a	H–	H–	H–
1-6b	CH_3O–	H–	H–
1-6c	H–	CH_3O–	H–
1-6d	H–	C_6H_5–	H– H–
1-6e	NC–	H–	Cl
1f	H–	H–	–

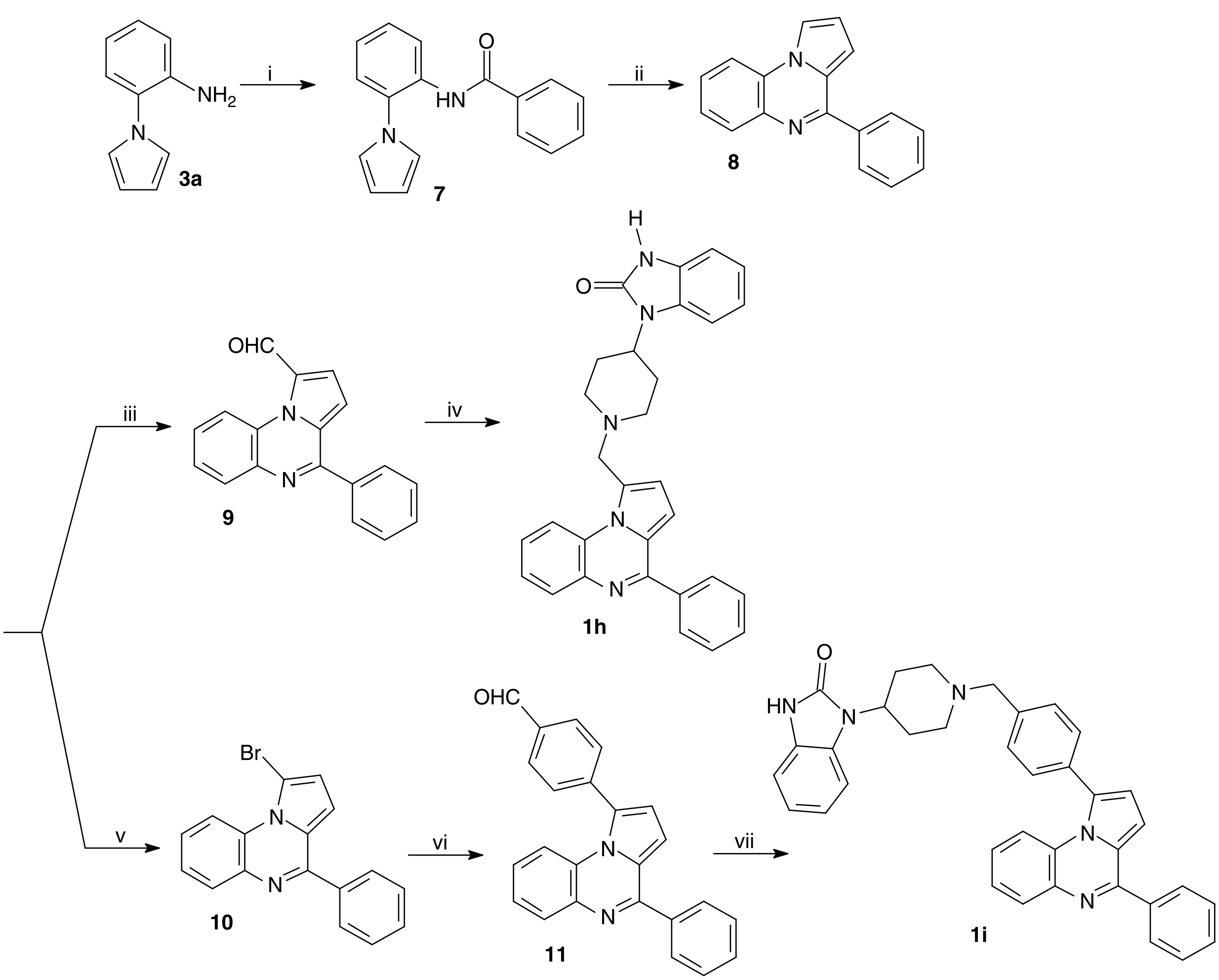
Scheme 1. Synthesis of the 1,3-dihydro-1-{1-[4-(pyrrolo[1,2-*a*]quinoxalin-4-yl)benzyl]piperidin-4-yl}-2*H*-benzimidazol-2-ones **1a-g**. Reagents and conditions: (i) DMTHF, AcOH, Δ; (ii) *Method A:* $CuSO_4$, $NaBH_4$, EtOH, RT for **3a-d**; *Method B:* $SnCl_2$, $2H_2O$, EtOH, Δ for **3e**; (iii) $CO(OCCl_3)_2$, toluene, Δ; (iv) $POCl_3$, Δ; (v) *Method A*: OHC-C_6H_4-$B(OH)_2$, $Pd[P(C_6H_5)_3]_4$, Na_2CO_3, C_6H_6, EtOH, Δ; *Method B*: OHC-C_6H_4-BF_3K, $PdCl_2(dppf){\cdot}CH_2Cl_2$, Cs_2CO_3, THF-H_2O, Δ; (vi) 4-(2-ketobenzimidazolin-1-yl)piperidine, $NaBH_3CN$, MeOH, Δ; (vii) **1e**, NaN_3, NH_4Cl, DMF, Δ.

tion product. It was then followed by the Suzuki-type cross-coupling of **10** with 4-formylphenylboronic acid in order to introduce the benzaldehyde moiety in position 1 of this 4-phenylpyrrolo[1,2-*a*]quinoxaline skeleton to give **11** [23,24]. Reaction of 4-(2-ketobenzimidazolin-1-yl)piperidine with **9** and **11** and using sodium cyanoborohydride as reductive agent in methanol gave the amines **1h** and **1i**, respectively (Scheme 2).

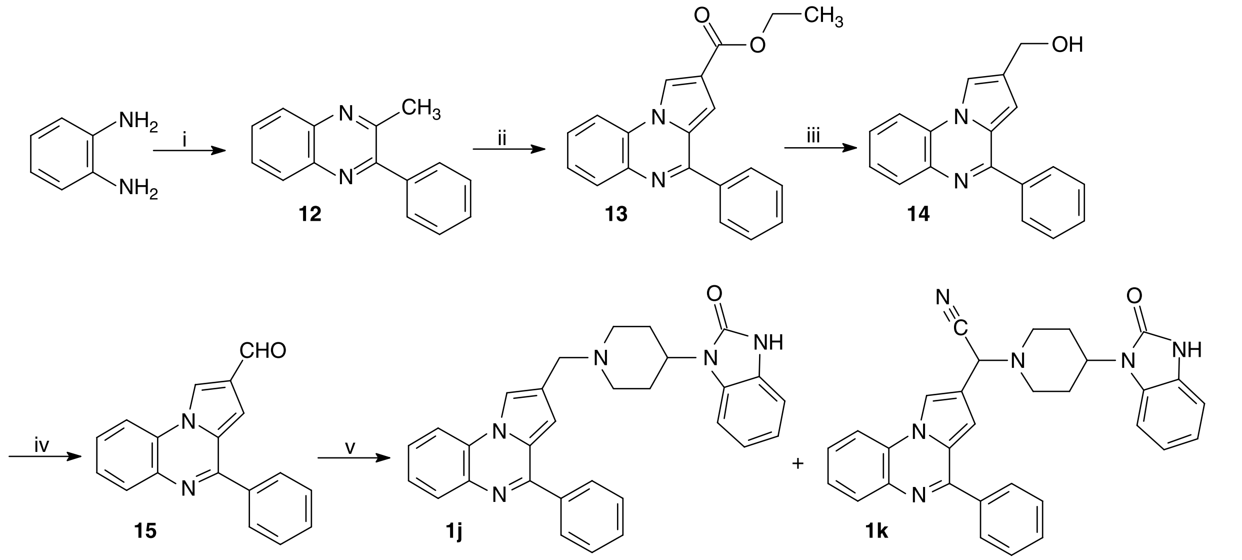
The 2-(aminomethyl)-4-phenylpyrrolo[1,2-*a*]quinoxalines **1j-k**, structural analogues of compound **1h** in position 2 of the pyrrolo[1,2-*a*]quinoxaline moiety, were prepared according to the sequence as shown in Scheme 3. Reaction of commercially available phenylenediamine with 1-phenylpropan-1,2-dione in acetic acid gave the methylphenylquinoxaline **12**. Treatment of compound **12** with ethyl bromopyruvate in refluxing ethanol led to ethyl 4-phenylpyrrolo[1,2-*a*]quinoxaline-2-carboxylate **13**. Reduction of the ester group of **13** with $LiAlH_4$ in anhydrous tetrahydrofuran gave the alcohol **14**, subsequently oxidized into the attempted aldehyde **15** using MnO_2 in refluxing chloroform [22]. The aldehyde **15** was then engaged in a reductive amination with $NaBH_3CN$ and 4-(2-ketobenzimidazolin-1-yl)piperidine to give in majority (43%) the pyrroloquinoxaline **1j** by applying the same experimental procedure as described in the synthesis of compounds **1a-f** and **1h-i**.

During this synthesis we also isolated a second amine **1k** (25%) presenting a nitrile group on the methylene function. The mechanism of formation of this new compound is probably due to the nucleophilic addition of a cyanide ion (CN^-), formed by hydrolysis of the cyanoborohydride in the reactional medium [25,26], on the iminium intermediate during the reductive amination.

The 3D spatial determinations of **1j** and **1k** were established by X-ray crystallography [27], and confirmed the structures in the solid state as anticipated on the basis of IR, ^1H and ^{13}C NMR data (Figure 3). Moreover, **1k** was found as a racemic (*R*)/(*S*) mixture as highlighted by the determined spatial group (C2/c).



Scheme 2. Synthesis of the 1,3-dihydro-1-{1-[4-(4-phenylpyrrolo[1,2-*a*]quinoxalin-1-yl)benzyl- or -methyl]piperidin-4-yl} -2*H*-benzimidazol-2-ones **1h-i**. Reagents and conditions: (i) C_6H_5COCl, toluene, pyridine, Δ; (ii) $POCl_3$, Δ; (iii) $POCl_3$/DMF, DMF, Δ; (iv) 4-(2-ketobenzimidazolin-1-yl)piperidine, $NaBH_3CN$, MeOH, Δ; (v) NBS, CH_2Cl_2, RT; (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, $NaBH_3CN$, MeOH, Δ.

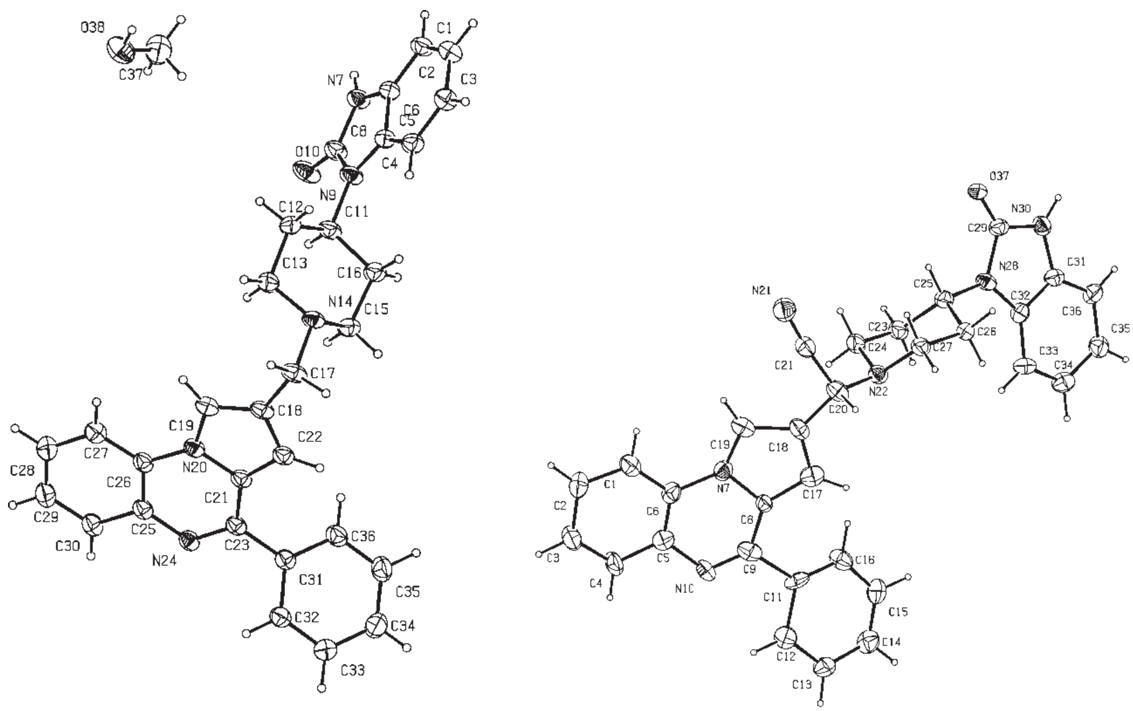


Scheme 3. Synthesis of the 1,3-dihydro-1-{1-[4-(4-phenylpyrrolo[1,2-*a*]quinoxalin-2-yl)methyl]piperidin-4-yl}-2*H*-benzimidazol-2-ones **1j-k**. Reagents and conditions: (i) $C_6H_5COCOCH_3$, AcOH, Δ; (ii) $BrCH_2COCOOC_2H_5$, EtOH, Δ; (iii) $LiAlH_4$, THF, Δ; (iv) MnO_2, $CHCl_3$, Δ; (v) 4-(2-ketobenzimidazolin-1-yl)piperidine, $NaBH_3CN$, MeOH, Δ.

Pharmacology

Cytotoxicity. All compounds **1a-k** were tested on activated (PBMNC + PHA) human peripheral blood mononuclear cells (Table I) [14]. As expected, most of the pyrrolo[1,2-*a*]quinoxalines **1a-k** showed significant level of cytotoxicity against lymphocytes with IC_{50} ranging from 5 to > 50 μM. These preliminary results were used to determine their respective range of toxic concentration.

Antiproliferative effect. Compounds **1a-k** were assessed for their ability to inhibit the *in vitro* proliferation of the human leukemic cell lines U937, K562 and HL60, and the breast carcinoma line MCF7. Compound A6730 (Figure 1) was used in these tests as the



1j **1k**

Figure 3. The ORTEP drawing of 1,3-dihydro-1-{1-[4-(4-phenylpyrrolo[1,2-*a*]quinoxalin-2-yl)methyl]piperidin-4-yl}-2*H*-benzimidazol-2-ones **1j** and **1k** with thermal ellipsoids at 30% level.

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

Compound	IC_{50} values (μM)[a] U937	K562	HL60	MCF7	Cytotoxicity on activated human peripheral blood mononuclear cells (PBMNC) PBMNC + PHA
A6730	8 ± 0.2	8 ± 0.3	5.5 ± 0.2	>20	n.d.[b]
1a	>20	4.5 ± 0.2	14 ± 0.4	20 ± 1	10 ± 0.5
1b	>20	>20	>20	>20	43 ± 2
1c	>20	10 ± 0.3	10 ± 0.3	>20	9 *** ± 0.4
1d	>20	16 ± 0.4	>20	>20	>50
1e	>20	>20	10 ± 0.4	>20	44 ± 2
1f	>20	9 ± 0.3	>20	>20	8 ± 0.4
1g	>20	>20	>20	>20	>50
1h	5 ± 0.1	>20	20 ± 1	8 ± 0.3	9 ± 0.4
1i	>20	>20	15.5 ± 0.4	>20	>50
1j	16 ± 0.3	8 ± 0.2	14 ± 0.3	17 ± 0.4	5 ± 0.1
1k	17 ± 0.3	17 ± 0.4	>20	>20	35 ± 3

[a] The IC_{50} (μM) values correspond to the mean + /− standard deviation from 3 independent experiments.; [b] n.d. = not determined.

reference standard drug. The results are summarized in Table I. The pyrrolo[1,2-*a*]quinoxalines **1h**, **1j** and **1k** were found the most antiproliferative compounds on the growth of human myeloid U937 cell line with IC_{50} from 5 to 17 μM. In particular, **1h** displayed strong cytotoxic properties on U937 cell line with an IC_{50} of 5 μM, and showed a better activity in comparison with the reference compound A6730 (IC_{50} = 8 μM). Interestingly, these three pyrroloquinoxalines **1h**, **1j** and **1k** were substituted by a methylpiperidinyl benzimidazolone moiety in position 1 or 2 and by a phenyl ring in position 4 of the pyrrolo[1,2-*a*]quinoxaline core. Moreover, the displacement of the methylpiperidinyl benzimi-dazolone substitutent from the position 1 (compound **1h**) to position 2 (compound **1j**) induced a slight decrease in the antiproliferative activity on U937 cell line (IC_{50} 16 μM for **1j** compared with 5 μM for **1h**). All other compounds **1a-f** derived from the incorporation of the benzylpiperidinyl benzimidazolone moiety, which was present in the reference compounds **II-V**, into the 4-position of the pyrroloquinoxaline ring were found inactive at 20 μM on the U937 cell line. It was also interesting to notice the difference of antiproliferative activities between the structural analogues **1i** and **1h** in which the replacement of the benzylpiperidinyl benzimidazolone moiety (compound **1i**) with a methylpiperidinyl benzimidazolone group (compound **1h**) was beneficial for inhibitory activity (IC_{50} = 5 μM for **1h** compared with > 20 μM for **1i**). From a SAR point of view, these preliminary biological results on U937 cell line enlightened the importance of the substitution at C-1 position of the pyrroloquinoxaline scaffold by a methylpiperidinyl benzimidazolone group.

The antiproliferative potencies of these new derivatives **1a-k** were also examined towards the human myeloid leukaemia cell lines K562 and HL60.

Among the eleven compounds tested for antiproliferative activities on K562 cell line, pyrroloquinoxaline **1a** was found the most active compound with an IC_{50} of 4.5 μM. In the same series, the substitution of the 7- or 8-position of the pyrrolo[1,2-*a*]quinoxaline moiety (compounds **1b-e** and **1g**) seems to slightly or totally decrease the activity in comparison with their unsubstituted derivative **1a** with IC_{50} ranging from 10 to > 20 μM. However, as the substitution in position 8 led to slightly active compounds **1c-d** (IC_{50} = 10 and 16 μM, respectively), the substitution at position 7 of the pyrroloquinoxaline heterocycle by one methoxy, one cyano or one tetrazole group (compounds **1b**, **1e** and **1g**) decreased once more the antiproliferative activity upon K562 cell line with IC_{50} superior to 20 μM. On the other hand, introduction of a chlorine atom on the 5 position of the benzimidazolone moiety reduced the activity up to two times (i.e.; IC_{50} = 9 μM for **1f** compared to 4.5 μM for **1a**). Introduction of the methyl- or benzylpiperidinyl benzimidazolone group in position 1 of the pyrroloquinoxaline skeleton (compounds **1h**, **1i**) led to a decrease in the activity, whereas introduction of this methylpiperidinyl benzimidazolone group in position 2 (compound **1j**) provided a better antiproliferative activity (IC_{50} = 8 μM). The presence of a cyano group on the methylene in position 2 (compound **1k**) also decreased the activity (IC_{50} = 17 μM).

Against the HL60 human acute promyeloid leukemia cell line, most of the tested compounds had only weak antiproliferative activity with IC_{50} values from 10 to 20 μM. The most active derivatives on this HL60 line were the 8-methoxy and 7-cyano pyrroloquinoxalines **1c** and **1e** which, with an IC_{50} value of 10 μM, were 2-fold less potent than A6730 (IC_{50} = 5.5 μM). The results against the HL60 cell line do not enable us to determine precisely the structure-activity relationship in this series of compounds.

Against the MCF7 breast adenocarcinoma, none of the pyrroloquinoxalines **1a-g**, **1i** and **1k** exhibited relevant cytotoxicity ($IC_{50} \geqq 20\,\mu M$). Nevertheless, the two compounds, bearing a methylpiperidinyl benzimidazolone group in position 1 and 2 of the tricyclic structure (compounds **1h** and **1j**), have shown significant antiproliferative activities ($IC_{50} = 8$ and $17\,\mu M$, respectively).

Conclusion. In the present report, we described the synthesis of new pyrrolo[1,2-a]quinoxaline derivatives bearing the piperidin-4-yl-2*H*-benzimidazol-2-one moiety *via* Suzuki cross-coupling and reductive amination reactions and presented their antiproliferative activities on the human leukemic cell lines U937, K562 and HL60, and the breast cancer cell line MCF7. These results have been discussed in a preliminary SAR study. The first biological evaluation of our compounds showed antiproliferative activity against U937, K562 and MCF7 cell lines. The most promising active pyrroloquinoxalines were found to be **1a** that inhibited K562 cell line proliferation with an IC_{50} of $4.5\,\mu M$, and **1h** that inhibited U937 and MCF7 cell lines with IC_{50} of 5 and $8\,\mu M$, respectively. These two candidates exhibited more potent activities than the reference inhibitor A6730. None of these new synthesized compounds **1a-k** had a significant effect on the HL60 cell line proliferation, which expressed an inactive Akt form, suggesting that these compounds exhibited a specificity for phosphorylated Akt form. Moreover, it would be now interesting to enlarge the biological evaluation of these two new pyrrolo[1,2-*a*]quinoxaline derivatives by studying the phosphorylation level of Akt by Western-Blot using (Ser473 or Thr308) phosphoAkt antibodies, as well as their isoenzyme selectivity. Finally, the new substituted pyrrolo[1,2-*a*]quinoxalines could open the way to new valuable medicinal chemistry scaffolding in the oncology domain.

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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