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RESEARCH ARTICLE

Synthesis and evaluation of the antiproliferative activity of novel pyrrolo[1,2-a]quinoxaline derivatives, potential inhibitors of Akt kinase. Part II

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Abstract

Attenuation of protein kinases by selective inhibitors is an extremely active field of activity in anticancer drug development. Therefore, Akt, a serine/threonine protein kinase, also known as protein kinase B (PKB), represents an attractive potential target for therapeutic intervention. Recent efforts in the development and biological evaluation of small molecule inhibitors of Akt have led to the identification of novel inhibitors with various heterocycle scaffolds. Based on previous results obtained on the antiproliferative activities of new pyrrolo[1,2-*a*]quinoxalines, a novel series was designed and synthesized from various substituted phenyl-1*H*-pyrrole-2-carboxylic acid alkyl esters via a 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. The first biological evaluation of our new substituted pyrrolo[1,2-*a*]quinoxalines showed antiproliferative activity against the tested cell lines. From a general SAR point of view, these preliminary biological results highlight the importance of substitution at the C-4 position of the pyrroloquinoxaline scaffold by a benzylpiperidinyl fluorobenzimidazole group, and also the need for a functionalization on the pyrrole ring.

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

Introduction

Cancer remains the leading cause of death in the world, and as a result there is a pressing need for novel and effective treatments. Cancer cells differ from their normal counterparts in a number of biochemical processes, particularly during the control of cell growth and division[1]. In the field of chemotherapeutic drugs, the search for new, more active, more selective, and less toxic compounds is still very intense, and new promising anticancer approaches are being tested[2,3]. Among these approaches, protein kinases (PKs) have been intensively investigated because of their role in the transduction of proliferative signals in mammalian cells. The dysregulation or inappropriate expression of these enzymes is associated with neoplasias. Consequently, attenuation of protein kinases by selective inhibitors is an extremely active field of activity in drug development. Recently, novel series of potent and selective Akt kinase inhibitors based on a 2,3-substituted quinoxaline or pyrazine skeleton (compounds **I–IV**) have been reported (Figure 1)[4–11]. Akt, a serine/threonine kinase belonging to the AGC superfamily of kinases, is a key regulator of apoptosis, cell cycle progression, cell proliferation, and growth[4–6,12–14]. Thus, Akt activation, which plays a critical role in tumorigenesis, is a critical downstream effector in the PI3K signal transduction pathway. Hence, it is frequently activated in tumors by growth factor overexpression and mutation in tumor suppressor PTEN (phosphatase and tensin homolog). Thus, inhibition of Akt kinase has been recognized as a potential target for cancer therapy. Therefore, it is challenging to develop isozyme-selective and Akt small molecule-specific inhibitors[5,6,12–14]. Structure–activity

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relationship (SAR) studies of these latter heterocyclic quinoxalines and pyrazinones **I–IV** indicated the importance of 2,3-diphenyl substituents and of the N-1 heteroatom. Alternative core heterocycles were also explored to identify more potent (and balanced dual) activity. Thus, quinoline, pyridine, naphthyridine, and pyridopyrimidine variations have been described[14–16]. Further optimization of these lead compounds on the 2-phenyl substituents was accomplished through library synthesis. This led to new bioactive compounds **V–VI** bearing a fluorobenzimidazole on the piperidine ring (Figure 1)[17,18].

In the course of our work devoted to discovering new compounds employed in anticancer chemotherapy as potential inhibitors of Akt kinase, we previously identified a series of substituted pyrrolo[1,2-*a*]quinoxaline derivatives designed as interesting bioactive isosteres of quinoxaline and pyrazine derivatives **I–IV**[19]. From these preliminary results, it appeared that the most promising pyrrolo[1,2-*a*]quinoxaline **JG454** (Figure 2) could initiate new, valuable anticancer chemistry scaffolding. Thus, taking into account our experience in the field of synthesis of new bioactive heterocyclic compounds based on our pyrrolo[1,2-*a*]quinoxaline heterocyclic core[19–23], we used the **JG454** pyrrolo[1,2-*a*]quinoxaline moiety as a template for the design of new derivatives in which the pyrrole nucleus is substituted in different positions by a phenyl in comparison with the reference compounds **I–VI**. We also decided to introduce a fluorobenzimidazole on the piperidine core in analogy to the new active reference compounds **V–VI**. Further pharmacomodulations were also considered, such as the introduction of an ester function on the pyrrolic ring. Hence, we report 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 proliferation of the

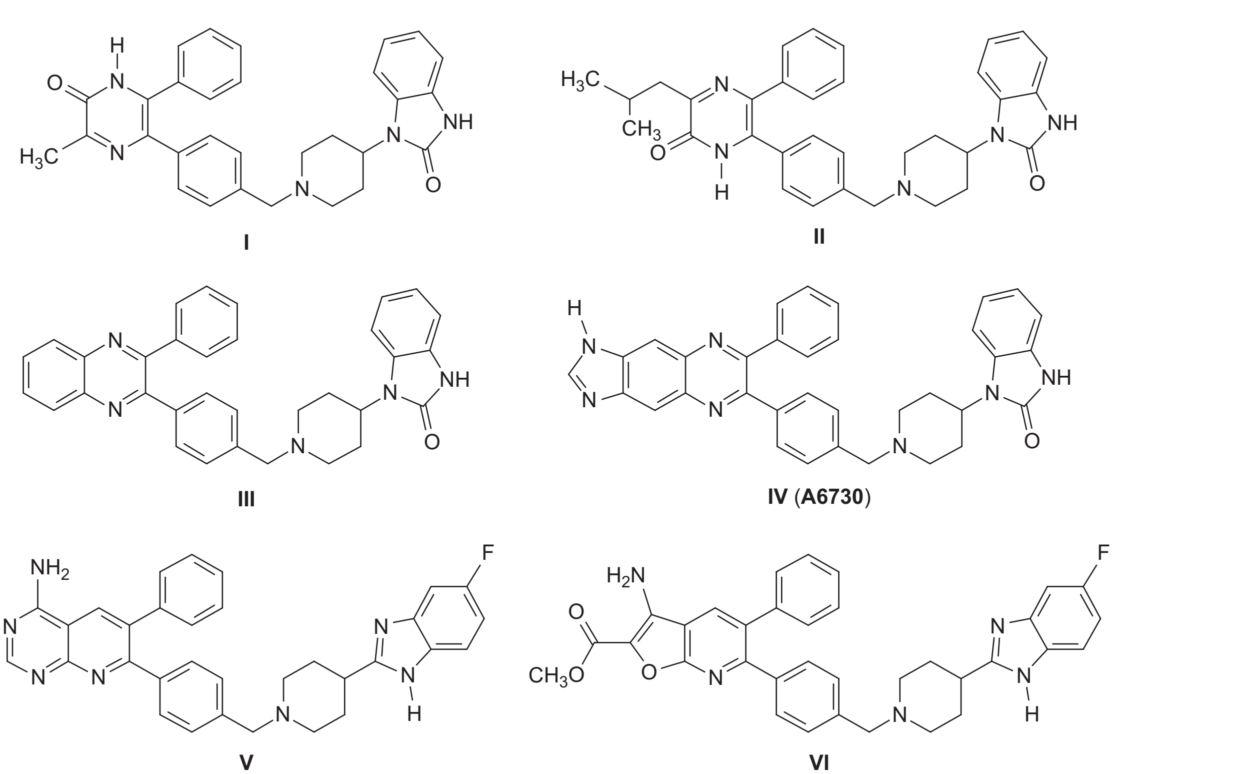


Figure 1. Structures of pyrazinones I–II, 2,3-diphenylquinoxalines III–**IV**, and fused pyridines **V–VI**, Akt kinase inhibitors.

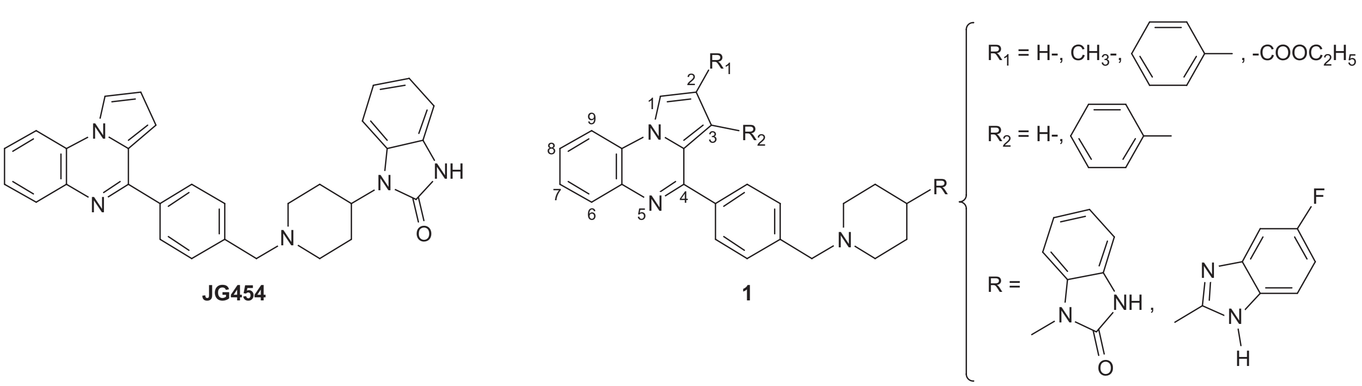


Figure 2. Structure of compound **JG454**, and general structure of new synthesized substituted pyrrolo[1,2-*a*]quinoxaline derivatives **1**.

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 are reported uncorrected. Nuclear magnetic resonance (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 (TLC) was carried out on 0.25 mm precoated silica gel plates (Polygram Sil G/UV_{254}) with visualization by irradiation with an ultraviolet (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.

*4-Bromo-1H-pyrrole-2-carboxylic acid methyl ester (**3**)* The 1*H*-pyrrole-2-carboxylic acid methyl ester (16 mmol) was dissolved in CCl_4 (50 mL) and cooled to–15°C. Then, a solution of Br_2 (16 mmol) in CCl_4 (100 mL) was added dropwise and the mixture was stirred for 1 h. After warming to room temperature, a 2 M aqueous solution of NaOH (110 mL) was added. After 10 min of stirring, the separated organic layer was dried over Na_2SO_4, filtered, and evaporated under reduced pressure to give **3**. Yield: 81%, white crystals, mp = 101°C[24]; IR ν_{max} (KBr)/cm^{-1} 3285 (NH), 1690 (CO); ^{1}H NMR δ (300 MHz, $CDCl_3$) 9.46 (bs, 1H, NH), 6.96 (dd, 1H, *J* 3.00 and 1.80 Hz, H-5), 6.90 (dd, 1H, *J* 2.55 and 1.80 Hz, H-3), 3.87 (s, 3H, CH_3).

*4-Bromopyrrole-1,2-dicarboxylic acid 1-tert-butyl ester 2-methyl ester (**4**)* To a solution of 4-bromopyrrole-1*H*-pyrrole-2-carboxylic acid methyl ester **3** (10 mmol) in anhydrous acetonitrile were successively added 4-(*N,N*-dimethylamino)pyridine (DMAP) (10 mmol) and di-*tert*-butyldicarbonate (13 mmol). The reaction was stirred at room temperature for 2 h. To the mixture were added 60 mL of diethyl ester and 30 mL of a 1 M aqueous solution of $KHSO_4$. The organic layer was separated and washed sequentially with a 1 M aqueous solution of $KHSO_4$, water, a saturated aqueous $NaHCO_3$ solution, and brine, and then dried with anhydrous sodium sulfate. The solvent was removed *in vacuo*. The resulting residue afforded a yellow oil[24]. Yield: 97%; IR ν_{max} (KBr)/cm^{-1} 1750 and 1735 (CO); ^{1}H NMR δ (300 MHz, $CDCl_3$) 7.32 (d, 1H, *J* 1.80 Hz, H-5), 6.0 (dd, 1H, *J* 1.80 Hz, H-3), 3.86 (s, 3H, CH_3), 1.59 (s, 9H, $C(CH_3)_3$).

*4-Phenyl-1H-pyrrole-2-carboxylic acid methyl ester (**2a**)* *Method A*: To a solution of 4-bromopyrrole-1, 2-dicarboxylic acid 1-*tert*-butyl ester 2-methyl ester **4** (10 mmol), phenylboronic acid (25 mmol) and tetrakis(triphenylphosphine)-palladium(0) (0.5 mmol) in 85 mL of dimethylformamide (DMF) was added 15 mL of 2 M aqueous Na_2CO_3. The reaction mixture was stirred at 110°C for 15 h. The reaction was quenched with 200 mL of water and extracted with ethyl acetate (3 × 100 mL). The combined organic layers were washed with water and brine and dried with anhydrous sodium sulfate. The solvent was removed under reduced pressure. The residue was triturated in methanol, then the resulting precipitate was filtered, washed with MeOH, and dried to give **2a** as white crystals. *Method B*: To a suspension of 4-bromopyrrole-1, 2-dicarboxylic acid 1-*tert*-butyl ester 2-methyl ester **4** (3.3 mmol) and $Pd(PPh_3)_4$ (0.164 mmol) in a mixture of toluene/EtOH (50/3 mL) under nitrogen were added K_2CO_3 (3.6 mmol) and phenylboronic acid (3.6 mmol). The reaction mixture was refluxed for 24 h, and the cooled suspension was extracted with CH_2Cl_2 (3 × 70 mL). The organic layer was washed with a saturated solution of NaCl (90 mL), and the combined organic extracts were dried over sodium sulfate, filtered, and evaporated under reduced pressure. The crude residue was solubilized in 20 mL of dichloromethane. To this reaction mixture was added 50 mL of a 10% trifluoroacetic acid solution in dichloromethane. The mixture was stirred at room temperature for 4 h, then neutralized with 75 mL of a saturated aqueous solution of potassium carbonate and extracted with 50 mL of dichloromethane. The organic layer was washed with water, then brine, and dried with anhydrous sodium sulfate. The solvent was removed under reduced pressure. The residue was triturated in methanol, then the resulting precipitate was filtered, washed with MeOH, and dried to give **2a** as white crystals. Yield: 39% (method A), 50% (method B), white crystals, mp = 175°C[24]; IR ν_{max} (KBr)/cm^{-1} 3310 (NH), 1700 (CO); ^{1}H NMR δ (300 MHz, $CDCl_3$) 9.26 (bs, 1H, NH), 7.55 (d, 2H, *J* 7.35 Hz, H-2' and H-6'), 7.38 (t, 2H, *J* 7.35 Hz, H-3' and H-5'), 7.27 (dd, 1H, *J* 3.05 and 1.70 Hz, H-5), 7.26 (t, 1H, *J* 7.35 Hz, H-4'), 7.22 (dd, 1H, *J* 2.60 and 1.70 Hz, H-3), 3.89 (s, 3H, CH_3).

Synthesis of 1-(2-nitrophenyl)-3- or -4-phenylpyrrole-2-carboxylic acid methyl ester (8a–b) and 1-(2-nitrophenyl)-4-methyl-3-phenylpyrrole-2-carboxylic acid ethyl ester (8c)

To the solution of methyl or ethyl 3- or 4-phenylpyrrole-2-carboxylate **2a–c** (3.4 mmol) in 12 mL of DMF was added cesium carbonate (4.06 mmol). 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 and was then diluted in AcOEt (60 mL), washed with water (2 × 50 mL), then brine (50 mL), and dried over sodium sulfate. The organic layer was concentrated under vacuum to give a brown oil. After triturating in Et_2O a solid was obtained and filtered off, washed with Et_2O, and dried to give the desired product **8**.

*1-(2-Nitrophenyl)-4-phenylpyrrole-2-carboxylic acid methyl ester (**8a**)* Yield: 83%, yellow crystals, mp = 134°C[24]; IR ν_{max} (KBr)/cm^{-1} 1710 (CO); ^{1}H NMR δ (300 MHz, $CDCl_3$) 8.16 (dd, 1H, *J* 7.85 and 1.30 Hz, H-3"), 7.74 (ddd, 1H, *J* 7.85, 7.20 and 1.30 Hz, H-4"), 7.65 (ddd, 1H, *J* 7.85, 7.20 and 1.30 Hz, H-5"), 7.57 (d, 2H, *J* 7.80 Hz, H-2' and H-6'), 7.50 (dd, 1H, *J* 7.85 and 1.30 Hz, H-6"), 7.43 (d, 1H, *J* 1.80 Hz, H-3), 7.40 (t, 2H, *J* 7.80 Hz, H-3' and H-5'), 7.27 (t, 1H, *J* 7.80 Hz, H-4'), 7.23 (d, 1H, *J* 1.80 Hz, H-3), 3.73 (s, 3H, CH_3).

*1-(2-Nitrophenyl)-3-phenylpyrrole-2-carboxylic acid methyl ester (**8b**)* Yield: 47%, yellow crystals, mp = 121°C; IR ν_{max} (KBr)/cm^{-1} 1705 (CO); ^{1}H NMR δ (300 MHz, $CDCl_3$) 8.15 (dd, 1H, *J* 7.90 and 1.40 Hz, H-3"), 7.75 (ddd, 1H, *J* 7.90, 7.30 and 1.40 Hz, H-4"), 7.64 (ddd, 1H, *J* 7.90, 7.30 and 1.40 Hz, H-5"), 7.52–7.47 (m, 3H, H-6", H-2' and H-6'), 7.43-7.33 (m, 3H, H-3', H-4' and H-5'), 6.93 (d, 1H, *J* 2.70 Hz, H-5), 6.44 (d, 1H, *J* 2.70 Hz, H-4), 3.50 (s, 3H, CH_3). Anal. Calcd. for $C_{18}H_{14}N_2O_4$: C, 67.07; H, 4.38; N, 8.69. Found: C, 66.82; H, 4.57; N, 8.86%.

*1-(2-Nitrophenyl)-4-methyl-3-phenylpyrrole-2-carboxylic acid ethyl ester (**8c**)* Yield: 92%, yellow oil; IR ν_{max} (KBr)/cm^{-1} 1710 (CO); ^{1}H NMR δ (300 MHz, $CDCl_3$) 8.09 (dd, 1H, *J* 8.10 and 1.50 Hz, H-3"), 7.58 (ddd, 1H, *J* 8.10, 7.40 and 1.50 Hz, H-4"), 7.48 (ddd, 1H, *J* 8.10, 7.40 and 1.50 Hz, H-5"), 7.38 (dd, 1H, *J* 8.10 and 1.50 Hz, H-6"), 7.36-7.28 (m, 5H, H-2', H-3', H-4', H-5' and H-6'), 6.76 (s, 1H, H-5), 3.87 (q, 2H, *J* 7.10 Hz, CH_2), 2.10 (s, 3H, CH_3), 0.82 (t, 3H, *J* 7.10 Hz, CH_3). Anal. Calcd. for $C_{20}H_{18}N_2O_4$: C, 68.56; H, 5.18; N, 7.99. Found: C, 68.74; H, 5.03; N, 8.26%.

Synthesis of 2- or 3-phenyl-5H-pyrrolo[1,2-a]quinoxalin-4-one (9a–b) and 2-methyl-3-phenyl-5H-pyrrolo[1,2-a]quinoxalin-4-one (9c)

A suspension of **8a–c** (12.3 mmol) and iron powder (49.1 mmol) in 55 mL of acetic acid was heated under reflux for 2 h. The reaction mixture was cooled, suspended in 150 mL of a 1 M aqueous solution of HCl, agitated, then filtered off, washed with HCl 1 M (80 mL), water, AcOEt, Et_2O, and dried to give a fluffy white solid.

*2-Phenyl-5H-pyrrolo[1,2-a]quinoxalin-4-one (**9a**)* Yield: 91%, white crystals, mp = 285°C[24]; IR ν_{max} (KBr)/cm^{-1} 1650 (CO); ^{1}H NMR δ (300 MHz, DMSO-d_6) 11.30 (s, 1H, NH), 8.71 (d, 1H, *J* 1.65 Hz, H-1), 8.11 (d, 1H, *J* 7.60 Hz, H-9), 7.82 (d, 2H, *J* 7.50 Hz, H-2' and H-6'), 7.46 (d, 1H, *J* 1.65 Hz, H-3), 7.43 (t, 2H, *J* 7.50 Hz, H-3' and H-5'), 7.32-7.24 (m, 4H, H-6, H-7, H-8 and H-4').

*3-Phenyl-5H-pyrrolo[1,2-a]quinoxalin-4-one (**9b**)* Yield: 92%, white crystals, mp = 240°C; IR ν_{max} (KBr)/cm^{-1} 1645 (CO); ^{1}H NMR δ (300 MHz, DMSO-d_6) 11.17 (s, 1H, NH), 8.28 (d, 1H, *J* 2.85 Hz, H-1), 8.08 (d, 1H, *J* 8.10 Hz, H-9), 7.73 (d, 2H, *J* 7.20 Hz, H-2' and H-6'), 7.39–7.17 (m, 6H, H-6, H-7, H-8, H-3', H-4', and H-5'), 6.86 (d, 1H, *J* 2.85 Hz, H-2). Anal. Calcd. for $C_{17}H_{12}N_2O$: C, 78.44; H, 4.65; N, 10.76. Found: C, 78.65; H, 4.50; N, 10.94%.

*2-Methyl-3-phenyl-5H-pyrrolo[1,2-a]quinoxalin-4-one (**9c**)* Yield: 72%, white crystals, mp = >260°C; IR ν_{max} (KBr)/cm^{-1} 1645 (CO); ^{1}H NMR δ (300 MHz, DMSO-d_6) 11.08 (s, 1H, NH), 8.08 (s, 1H, H-1), 7.98 (d, 1H, *J* 8.00 Hz, H-9), 7.40-7.26 (m, 8H, H-6, H-7, H-8, and H phenyl), 2.11 (s, 3H, CH_3). Anal. Calcd. for $C_{18}H_{14}N_2O$: C, 78.81; H, 5.14; N, 10.21. Found: C, 78.70; H, 4.97; N, 10.44%.

Synthesis of 4-chloro-2-phenylpyrrolo[1,2-a]quinoxaline (11a), 4-chloro-3-phenylpyrrolo[1,2-a]quinoxaline (11b), and 4-chloro-2-methyl-3-phenylpyrrolo[1,2-a]quinoxaline (11c)

A solution of 5*H*-pyrrolo[1,2-*a*]quinoxalin-4-one **9a–c** (10 mmol) in $POCl_3$ (35 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, dried, and recrystallized from ethyl acetate to give **11**.

*4-Chloro-2-phenylpyrrolo[1,2-a]quinoxaline (**11a**)* Yield: 83%, white crystals, mp = 131°C[24]; IR ν_{max} (KBr)/cm^{-1} 1605 (C=N); ^{1}H NMR δ (300 MHz, $CDCl_3$) 8.19 (d, 1H, *J* 1.55 Hz, H-1), 7.90 (dd, 1H, *J* 7.80 and 1.50 Hz, H-9), 7.85 (dd, 1H, *J* 7.80 and 1.50 Hz, H-6), 7.69 (d, 2H, *J* 7.90 Hz, H-2' and H-6'), 7.55 (ddd, 1H, *J* 7.80, 7.20 and 1.50 Hz, H-8), 7.48-7.42 (m, 3H, H-7, H-3' and H-5'), 7.33 (t, 1H, *J* 7.90 Hz, H-4'), 7.28 (d, 1H, *J* 1.55 Hz, H-3).

*4-Chloro-3-phenylpyrrolo[1,2-a]quinoxaline (**11b**)* Yield: 71%, white crystals, mp = 119°C; IR ν_{max} (KBr)/cm^{-1} 1610 (C=N); ^{1}H NMR δ (300 MHz, $CDCl_3$) 8.02 (d, 1H, *J* 2.75 Hz, H-1), 7.91 (dd, 1H, *J* 8.10 and 1.30 Hz, H-9), 7.87 (dd, 1H, *J* 8.10 and 1.30 Hz, H-6), 7.60–7.41 (m, 7H, H-7, H-8, H-2', H-3', H-4', H-5', and H-6'), 6.91 (d, 1H, *J* 2.75 Hz, H-2). Anal. Calcd. for $C_{17}H_{11}ClN_2$: C, 73.25; H, 3.98; N, 10.05. Found: C, 73.41; H, 4.13; N, 9.90%.

*4-Chloro-2-methyl-3-phenylpyrrolo[1,2-a]quinoxaline (**11c**)* Yield: 81%, white crystals, mp = 142°C; IR ν_{max} (KBr)/cm^{-1} 1605 (C=N); ^{1}H NMR δ (300 MHz, $CDCl_3$) 7.90 (dd, 1H, *J* 7.80 and 1.50 Hz, H-9), 7.88 (s, 1H, H-1), 7.83 (dd, 1H, *J* 7.80 and 1.50 Hz, H-6), 7.54 (ddd, 1H, *J* 7.80, 7.10 and 1.50 Hz, H-8), 7.48–7.41 (m, 4H, H-7, H-2', H-6', and H-4'), 7.38–7.36 (m, 2H, H-3' and H-5'), 2.20 (s, 3H, CH_3). Anal. Calcd. for $C_{18}H_{13}ClN_2$: C, 73.84; H, 4.47; N, 9.57. Found: C, 74.07; H, 4.72; N, 9.49%.

Synthesis of substituted 4-(pyrrolo[1,2-a]quinoxalin-4-yl)benzaldehydes (12a–d)

To a suspension of 4-chloropyrrolo[1,2-*a*]quinoxaline **11** (3.3 mmol) and $Pd(PPh_3)_4$ (0.164 mmol) in a mixture of toluene/EtOH (50/3 mL) under nitrogen were added K_2CO_3 (3.6 mmol) and phenylboronic acid (3.6 mmol). The reaction mixture was refluxed for 24 h, and the cooled suspension was extracted with CH_2Cl_2 (3 × 70 mL). The organic layer was washed with a saturated solution of NaCl (90 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 was filtered, washed with ethanol, and purified by column chromatography on silica gel using dichloromethane as eluent to give the pure product **12a–d**.

*4-(2-Phenylpyrrolo[1,2-a]quinoxalin-4-yl)benzaldehyde (**12a**)* Yield: 86%, yellow crystals, mp = 181°C; IR ν_{max} (KBr)/cm^{-1} 1700 (CHO); ^{1}H NMR δ (300 MHz, $CDCl_3$) 10.17 (s, 1H, CHO), 8.31 (d, 1H, *J* 1.35 Hz, H-1'), 8.22 (d, 2H, *J* 8.40 Hz, H-2 and H-6), 8.10 (d, 2H, *J* 8.40 Hz, H-3 and H-5), 8.09 (dd, 1H, *J* 8.10 and 1.50 Hz, H-9'), 7.96 (dd, 1H, *J* 8.10 and 1.50 Hz, H-6'), 7.70 (d, 2H, *J* 7.70 Hz, H-2" and H-6"), 7.60 (ddd, 1H, *J*

8.10, 7.60 and 1.50 Hz, H-8′), 7.51 (ddd, 1H, *J* 8.10, 7.60 and 1.50 Hz, H-7′), 7.44 (t, 2H, *J* 7.70 Hz, H-3″ and H-5″), 7.33 (t, 1H, *J* 7.70 Hz, H-4″), 7.24 (d, 1H, *J* 1.35 Hz, H-3′). Anal. Calcd. for $C_{24}H_{16}N_2O$: C, 82.74; H, 4.63; N, 8.04. Found: C, 82.98; H, 4.84; N, 8.23%.

*4-(3-Phenylpyrrolo[1,2-a]quinoxalin-4-yl)benzaldehyde (**12b**)* Yield: 43%, yellow crystals, mp = 145°C; IR ν_{max} (KBr)/cm^{-1} 1705 (CHO); ^{1}H NMR δ (300 MHz, $CDCl_3$) 9.94 (s, 1H, CHO), 8.12 (d, 1H, *J* 2.80 Hz, H-1′), 8.08 (dd, 1H, *J* 7.95and 1.25 Hz, H-9′), 7.96 (dd, 1H, *J* 7.95 and 1.25 Hz, H-6′), 7.63–7.49 (m, 6H, H-2, H-3, H-5, H-6, H-7′, and H-8′), 7.07–6.92 (m, 6H, H-2″, H-3″, H-4″, H-5″, H-6″, and H-2′). Anal. Calcd. for $C_{24}H_{16}N_2O$: C, 82.74; H, 4.63; N, 8.04. Found: C, 82.58; H, 4.71; N, 8.16%.

*4-(2-Methyl-3-phenylpyrrolo[1,2-a]quinoxalin-4-yl)benzaldehyde (**12c**)* Yield: 70%, yellow crystals, mp = 154°C; IR ν_{max} (KBr)/cm^{-1} 1705 (CHO); ^{1}H NMR δ (300 MHz, $CDCl_3$) 9.90 (s, 1H, CHO), 8.04 (d, 1H, *J* 8.00 Hz, H-9′), 7.96 (s, 1H, H-1′), 7.90 (d, 1H, *J* 8.00 Hz, H-6′), 7.56 (t, 1H, *J* 8.00 Hz, H-8′), 7.51 (d, 2H, *J* 8.25 Hz, H-2 and H-6), 7.46 (t, 1H, *J* 8.00 Hz, H-7′), 7.41 (d, 2H, *J* 8.25 Hz, H-3 and H-5), 7.03–6.95 (m, 3H, H-3″, H-4″, and H-5″), 6.86 (d, 2H, *J* 7.65 Hz, H-2″ and H-6″), 2.28 (s, 3H, CH_3). Anal. Calcd. for $C_{25}H_{18}N_2O$: C, 82.85; H, 5.00; N, 7.73. Found: C, 83.05; H, 4.93; N, 7.52%.

*Ethyl 4-(4-formylphenyl)pyrrolo[1,2-a]quinoxaline-2-carboxylate (**12d**)* Yield: 88%, yellow crystals, mp = 195°C; IR ν_{max} (KBr)/cm^{-1} 1705 (CHO); ^{1}H NMR δ (300 MHz, $CDCl_3$) 10.16 (s, 1H, CHO), 8.56 (d, 1H, *J* 1.40 Hz, H-1), 8.19 (d, 2H, *J* 8.10 Hz, H-2′ and H-6′), 8.12 (d, 1H, *J* 7.90 Hz, H-9), 8.04 (d, 2H, *J* 8.10 Hz, H-3′ and H-5′), 7.93 (d, 1H, *J* 7.90 Hz, H-6), 7.65–7.53 (m, 2H, H-7 and H-8), 7.40 (d, 1H, *J* 1.40 Hz, H-3), 4.39 (q, 2H, *J* 6.90 Hz, CH_2), 1.42 (t, 3H, *J* 6.90 Hz, CH_3). Anal. Calcd. for $C_{21}H_{16}N_2O_3$: C, 73.24; H, 4.68; N, 8.13. Found: C, 73.39; H, 4.47; N, 8.27%.

Synthesis of 5-fluoro-2-{1-[4-(pyrrolo[1,2-a]quinoxalin-1-yl)benzyl]piperidin-4-yl}-1H-benzimidazole (1a, 1c, 1f, 1h) and 1,3-dihydro-1-{1-[4-(pyrrolo[1,2-a]quinoxalin-4-yl)benzyl]piperidin-4-yl}-2H-benzimidazol-2-one (1b, 1d, 1e, 1g)

The pH of a solution of the aldehyde **12a–d** (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. Column chromatography of the residue on silica gel using methanol–chloroform (1/9) as eluent 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 **1**.

*5-Fluoro-2-{1-[4-(pyrrolo[1,2-a]quinoxalin-1-yl)benzyl]piperidin-4-yl}-1H-benzimidazole (**1a**)* Yield: 43%, pale-yellow crystals, mp = 138°C; IR ν_{max} (KBr)/cm^{-1} 3340 (NH), 1685 (C=O); ^{1}H NMR δ (300 MHz, DMSO-d_6) 12.30 (s, 1H, NH), 8.55 (dd, 1H, *J* 2.80 and 1.30 Hz, H-1″), 8.31 (d, 1H, *J* 8.10 Hz, H-9″), 7.96 (d, 2H, *J* 8.00 Hz, H-3′ and H-5′), 7.92 (d, 1H, *J* 8.10 Hz, H-6″), 7.56 (t, 1H, *J* 8.10 Hz, H-8″), 7.53–7.48 (m, 4H, H-2′, H-6′, H-7″, and H benzimid.), 7.40–7.20 (m, 2H, H benzimid.), 7.20 (dd, 1H, *J* 3.95 and 1.30 Hz, H-3″), 6.96 (dd, 1H, *J* 3.95 and 2.80 Hz, H-2″), 3.62 (s, 2H, CH_2N), 2.99–2.90 (m, 2H, CH_2 pip.), 2.87–2.83 (m, 1H, CH pip.), 2.20–2.13 (m, 2H, CH_2 pip.), 2.04–1.99 (m, 2H, CH_2 pip.), 1.94–1.86 (m, 2H, CH_2 pip.). Anal. Calcd. for $C_{30}H_{26}FN_5$: C, 75.76; H, 5.51; N, 14.73. Found: C, 75.52; H, 5.58; N, 14.94%.

*1,3-Dihydro-1-{1-[4-(2-phenylpyrrolo[1,2-a]quinoxalin-4-yl)benzyl]piperidin-4-yl}-2H-benzimidazol-2-one (**1b**)* Yield: 60%, pale-yellow crystals, mp = 270°C; IR ν_{max} (KBr)/cm^{-1} 3350 (NH), 1685 (C=O); ^{1}H NMR δ (300 MHz, $CDCl_3$) 9.46 (s, 1H, NH), 8.27 (d, 1H, *J* 1.35 Hz, H-1″), 8.06 (dd, 1H, *J* 8.10 and 1.40 Hz, H-9″), 8.02 (d, 2H, *J* 8.15 Hz, H-3′ and H-5′), 7.93 (dd, 1H, *J* 8.10 and 1.40 Hz, H-6″), 7.71 (d, 2H, *J* 7.65 Hz, H-2‴ and H-6‴), 7.59 (ddd, 1H, *J* 8.10, 7.55 and 1.40 Hz, H-8″), 7.52 (ddd, 1H, *J* 8.10, 7.55 and 1.40 Hz, H-7″), 7.47 (m, 1H, H-4‴), 7.41 (d, 2H, *J* 8.15 Hz, H-2′ and H-6′), 7.33–7.30 (m, 1H, H benzimid.), 7.28 (d, 1H, J 1.35 Hz, H-3″), 7.10–7.08 (m, 3H, H benzimid.), 4.46–4.44 (m, 1H, CH pip.), 3.72 (s, 2H, CH_2N), 3.15–3.11 (m, 2H, CH_2 pip.), 2.56–2.52 (m, 2H, CH_2 pip.), 2.30–2.27 (m, 2H, CH_2 pip.), 1.89–1.85 (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, 78.45; H, 5.85; N, 12.97%.

*5-Fluoro-2-{1-[4-(2-phenylpyrrolo[1,2-a]quinoxalin-1-yl)benzyl]piperidin-4-yl}-1H-benzimidazole (**1c**)* Yield: 64%, orange crystals, mp = 150°C; IR ν_{max} (KBr)/cm^{-1} 3350 (NH), 1680 (C=O); ^{1}H NMR δ (300 MHz, DMSO-d_6) 12.32 (s, 1H, NH), 9.09 (d, 1H, *J* 1.30 Hz, H-1″), 8.38 (d, 1H, *J* 8.25 Hz, H-9″), 8.05 (d, 2H, *J* 8.10 Hz, H-3′ and H-5′), 7.95 (d, 1H, *J* 8.25 Hz, H-6″), 7.91 (d, 2H, *J* 8.25 Hz, H-2‴ and H-6‴), 7.66 (t, 1H, *J* 8.25 Hz, H-8″), 7.64–7.27 (m, 9H, H-2′, H-6′, H-3″, H-7″, H-3‴, H-4‴, H-5‴, and 2H benzimid.), 6.99–6.96 (m, 1H, H benzimid.), 3.64 (s, 2H, CH_2N), 3.00–2.95 (m, 2H, CH_2 pip.), 2.88–2.85 (m, 1H, CH pip.), 2.19–2.16 (m, 2H, CH_2 pip.), 2.05–1.98 (m, 2H, CH_2 pip.), 1.94–1.87 (m, 2H, CH_2 pip.). Anal. Calcd. for $C_{36}H_{30}FN_5$: C, 78.38; H, 5.48; N, 12.69. Found: C, 78.46; H, 5.63; N, 12.48%.

*1,3-Dihydro-1-{1-[4-(3-phenylpyrrolo[1,2-a]quinoxalin-4-yl)benzyl]piperidin-4-yl}-2H-benzimidazol-2-one (**1d**)* Yield: 58%, pale-yellow crystals, mp = 275°C; IR ν_{max} (KBr)/cm^{-1} 3350 (NH), 1690 (C=O); ^{1}H NMR δ (300 MHz, $CDCl_3$) 9.46 (s, 1H, NH), 8.27 (d, 1H, *J* 2.85 Hz, H-1″), 8.06 (dd, 1H, *J* 8.10 and 1.40 Hz, H-9″), 7.94 (dd, 1H, *J* 8.10 and 1.40 Hz, H-6″), 7.56 (ddd, 1H, *J* 8.10, 7.50 and 1.40 Hz, H-8″), 7.47 (ddd, 1H, *J* 8.10, 7.50 and 1.40 Hz, H-7″), 7.35 (d, 2H, *J* 8.15 Hz, H-3′ and H-5′), 7.34–7.31 (m, 1H, H benzimid.), 7.20–6.96 (m, 10H, H-2′, H-6′, H-2‴, H-3‴, H-4‴, H-5‴, H-6‴, and 3 H benzimid.), 6.95 (d, 1H, *J* 2.85 Hz, H-2″), 4.49–4.43 (m, 1H, CH pip.), 3.48 (s, 2H, CH_2N), 3.01–2.97 (m, 2H, CH_2 pip.), 2.50–2.46 (m, 2H, CH_2 pip.), 2.17–2.10 (m, 2H, CH_2 pip.), 1.87–1.82 (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, 78.95; H, 5.63; N, 12.81%.

1,3-Dihydro-1-{1-[4-(2-methyl-3-phenylpyrrolo[1,2-a]quinoxalin-4-yl)benzyl]piperidin-4-yl}-2H-benzimidazol-2-one ***(1e)*** Yield: 40%, pale-yellow crystals, mp = 170°C; IR ν_{max} (KBr)/cm^{-1} 3345 (NH), 1680 (C=O); ^{1}H NMR δ (300 MHz, $CDCl_3$) 10.18 (s, 1H, NH), 8.03 (d, 1H, *J* 7.95 Hz, H-9"), 7.93 (s, 1H, H-1"), 7.89 (d, 1H, *J* 7.95 Hz, H-6"), 7.52 (t, 1H, *J* 7.95 Hz, H-8"), 7.44 (t, 1H, *J* 7.95 Hz, H-7"), 7.36–7.34 (m, 1H, H benzimid.), 7.27 (d, 2H, *J* 8.10 Hz, H-3', and H-5'), 7.11–6.99 (m, 8H, H-2', H-6', H-3''', H-4''', H-5''', and 3 H benzimid.), 6.93–6.91 (m, 2H, H-2''' and H-6'''), 4.44–4.42 (m, 1H, CH pip.), 3.49 (s, 2H, CH_2N), 3.02–2.98 (m, 2H, CH_2 pip.), 2.58–2.48 (m, 2H, CH_2 pip.), 2.26 (s, 3H, CH_3), 2.22–2.18 (m, 2H, CH_2 pip.), 1.90–1.84 (m, 2H, CH_2 pip.). Anal. Calcd. for $C_{37}H_{33}N_5O$: C, 78.83; H, 5.90; N, 12.42. Found: C, 78.61; H, 5.75; N, 12.69%.

5-Fluoro-2-{1-[4-(2-methyl-3-phenylpyrrolo[1,2-a]quinoxalin-1-yl)benzyl]piperidin-4-yl}-1H-benzimidazole ***(1f)*** Yield: 52%, pale-yellow crystals, mp = 201°C; IR ν_{max} (KBr)/cm^{-1} 3370 (NH), 1630 (C=N); ^{1}H NMR δ (300 MHz, DMSO-d_6) 12.31 (s, 1H, NH), 8.49 (s, 1H, H-1"), 8.27 (dd, 1H, *J* 8.10 and 1.20 Hz, H-9"), 7.86 (dd, 1H, *J* 8.10 and 1.20 Hz, H-6"), 7.60 (ddd, 1H, *J* 8.10, 7.60 and 1.20 Hz, H-8"), 7.52–7.19 (m, 3H, H-7" and 2H benzimid.), 7.16 (d, 2H, *J* 7.80 Hz, H-3' and H-5'), 7.03–6.86 (m, 8H, H-2''', H-3''', H-4''', H-5''', H-6''', H-2', H-6', and H benzimid.), 3.39–3.36 (m, 3H, CH pip. and CH_2 pip.), 3.32 (s, 2H, CH_2N), 2.88–2.82 (m, 2H, CH_2 pip.), 2.19 (s, 3H, CH_3), 2.07–2.02 (m, 2H, CH_2 pip.),1.87–1.82 (m, 2H, CH_2 pip.). Anal. Calcd. for $C_{37}H_{32}FN_5$: C, 78.56; H, 5.70; N, 12.38. Found: C, 78.77; H, 5.78; N, 12.46%.

Ethyl 4-{4-[(4-(2-oxo-2,3-dihydro-1H-benzimidazol-1-yl)piperidin-1-yl)benzyl]}pyrrolo[1,2-a]quinoxaline-2-carboxylate ***(1g)*** Yield: 76%, pale-yellow crystals, mp = 159°C; IR ν_{max} (KBr)/cm^{-1} 3360 (NH), 1710 and 1685 (C=O); ^{1}H NMR δ (300 MHz, $CDCl_3$) 9.28 (s, 1H, NH), 8.52 (d, 1H, *J* 1.45 Hz, H-1), 8.06 (dd, 1H, *J* 7.90 and 1.50 Hz, H-9), 7.99 (d, 2H, *J* 8.00 Hz, H-3' and H-5'), 7.93 (dd, 1H, *J* 7.95 and 1.40 Hz, H-6), 7.60 (d, 2H, *J* 8.00 Hz, H-2' and H-6'), 7.56–7.51 (m, 2H, H-7 and H-8), 7.41 (d, 1H, *J* 1.45 Hz, H-3), 7.37–7.34 (m, 1H, H benzimid.), 7.08–7.06 (m, 3H, 3H benzimid.), 4.43–4.36 (m, 3H, CH pip. and CH_2), 3.73 (s, 2H, CH_2N), 3.18–3.10 (m, 2H, CH_2 pip.), 2.58–2.55 (m, 2H, CH_2 pip.), 2.30–2.28 (m, 2H, CH_2 pip.), 1.87–1.83 (m, 2H, CH_2 pip.), 1.39 (t, 3H, *J* 6.95 Hz, CH_3). Anal. Calcd. for $C_{33}H_{31}N_5O_3$: C, 72.64; H, 5.73; N, 12.84. Found: C, 72.84; H, 5.70; N, 12.98%.

Ethyl 4-{4-[(4-(5-fluoro-1H-benzimidazol-2-yl)piperidin-1-yl)benzyl]}pyrrolo[1,2-a]quinoxaline-2-carboxylate ***(1h)*** Yield: 39%, pale-yellow crystals, mp = 147°C; IR ν_{max} (KBr)/cm^{-1} 3360 (NH), 1710 and 1685 (C=O); ^{1}H NMR δ (300 MHz, DMSO-d_6) 12.30 (s, 1H, NH), 9.04 (d, 1H, *J* 1.50 Hz, H-1), 8.46 (d, 1H, *J* 8.10 Hz, H-9), 7.95-7.91 (m, 3H, H-6, H-3' and H-5'), 7.62–7.43 (m, 5H, H-7, H-8, H-2', H-6', and H benzimid.), 7.25–7.23 (m, 1H, H benzimid.), 7.22 (d, 1H, *J* 1.50 Hz, H-3), 6.99–6.92 (m, 1H, H benzimid.), 4.31 (q, 2H, *J* 6.90 Hz, CH_2), 3.61 (s, 2H, CH_2N), 2.97–2.89 (m, 2H, CH_2 pip.), 2.88-2.85 (m, 1H, CH pip.), 2.28–2.13 (m, 2H, CH_2 pip.), 2.04-1.83 (m, 4H, 2CH_2 pip.), 1.33 (t, 3H, *J* 6.90 Hz, CH_3). Anal. Calcd. for $C_{33}H_{30}FN_5O_2$: C, 72.37; H, 5.52; N, 12.79. Found: C, 72.11; H, 5.78; N, 12.88%.

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 (FCS), antibiotics (100 U/mL penicillin, 100 µg/mL streptomycin), and L-glutamine, 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 the blood of healthy volunteers were obtained following centrifugation on a Ficoll gradient. Cells were then incubated in medium alone or induced to enter the cell cycle by the addition of PHA (5 µg/mL; Murex Biotech Ltd., Dartford, UK).

Cytotoxicity test

The 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 PBS (phosphate buffered 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 enzyme linked immunosorbent assay (ELISA) microplate reader (Thermo Electron Corp.) at 490 nm wavelength. The amount of color 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 day incubation period. The 50% inhibitory concentrations (IC_{50}) were determined by linear regression analysis, and are expressed in µM ± SD (Microsoft Excel).

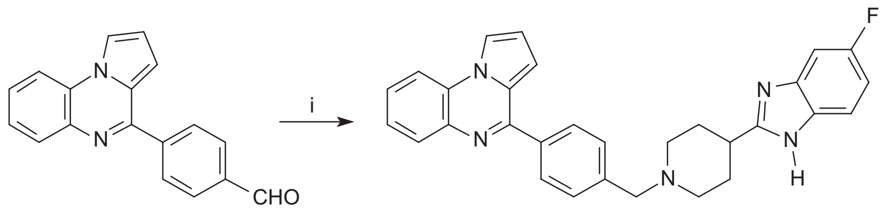
Results and discussion

Chemistry

The synthesis of the 5-fluoro-2-{1-[4-(pyrrolo[1,2-*a*]-quinoxalin-1-yl)benzyl]piperidin-4-yl}-1*H*-benzimidazole **1a** was accomplished from the previously described 4-(pyrrolo[1,2-*a*]quinoxalin-4-yl)benzaldehyde[19] coupled with 4-(5-fluorobenzimidazol-2-yl)piperidine[25] through a reductive amination using $NaBH_3CN$ (Scheme 1).

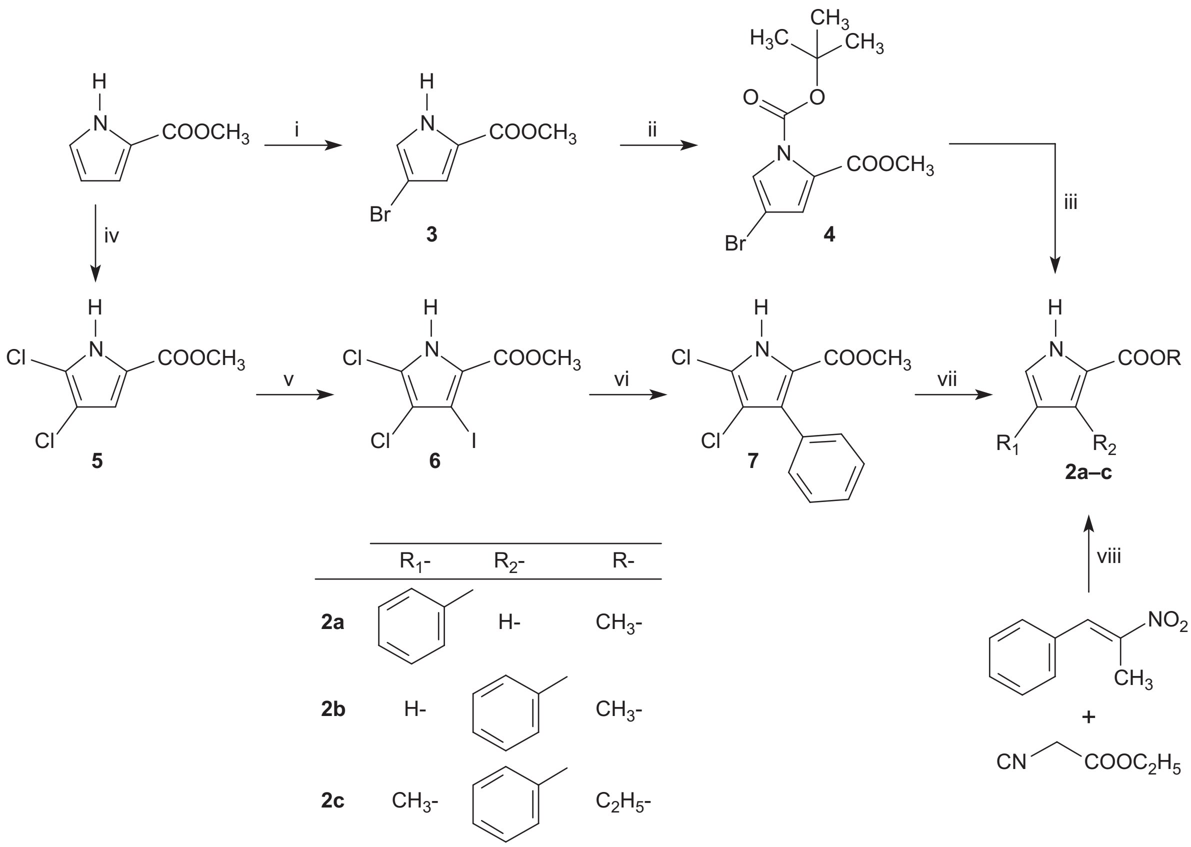
The other new pyrrolo[1,2-*a*]quinoxaline derivatives **1b–f** and **1g–h** were synthesized from various substituted phenyl-1*H*-pyrrole-2-carboxylic acid alkyl esters **2a–c** or from 3-methyl-2-quinoxalinol, respectively (Schemes 2 and 3). The synthesis of the 4-phenyl-1*H*-pyrrole-2-carboxylic acid methyl ester **2a** was accomplished in three steps starting from commercially available 1*H*-pyrrole-2-carboxylic acid methyl ester according to the sequence depicted in Scheme 2.

The 4-bromopyrrole carboxylate **3** was prepared by regioselective bromination of methyl pyrrole-2-carboxylate[26,27]. This bromopyrrole ester **3** was then protected



1a

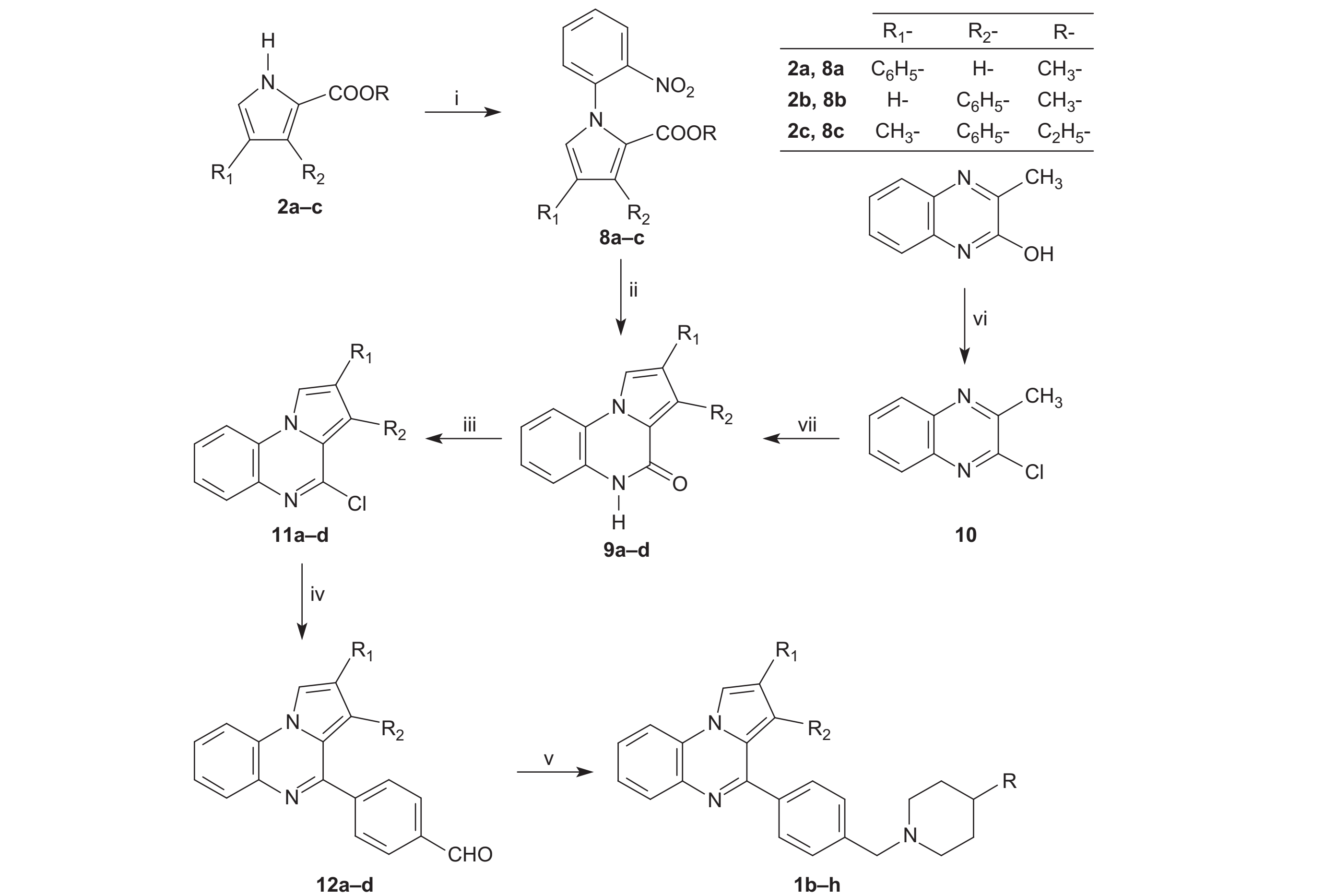
Scheme 1. Synthesis of 5-fluoro-2-{1-[4-(pyrrolo[1,2-*a*]quinoxalin-1-yl)benzyl]piperidin-4-yl}-1*H*-benzimidazole (**1a**). Reagents and conditions: (i) 4-(5-fluorobenzimidazolin-2-yl)piperidine, $NaBH_3CN$, MeOH, Δ.



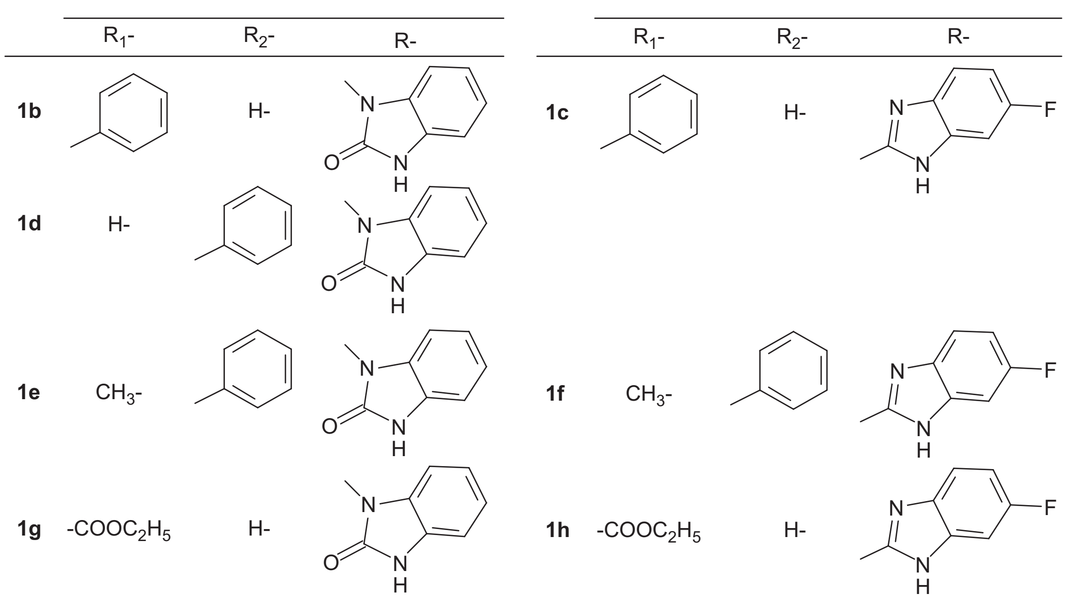
Scheme 2. Synthesis of phenyl-1*H*-pyrrole-2-carboxylic acid alkyl esters (**2a–c**). Reagents and conditions: (i) Br_2, CCl_4,–10°C; (ii) Boc_2O, DMAP, CH_3CN, RT; (iii) *Method A:* C_6H_5-$B(OH)_2$, $Pd[P(C_6H_5)_3]_4$, aq. Na_2CO_3, DMF, Δ; *Method B:* (1) C_6H_5-$B(OH)_2$, $Pd[P(C_6H_5)_3]_4$, toluene, K_2CO_3, EtOH, Δ; (2) CF_3COOH, CH_2Cl_2, RT; (iv) SO_2Cl_2, $CHCl_3$, RT; (v) I_2, CF_3COOAg, $CHCl_3$, RT; (vi) C_6H_5-$B(OH)_2$, $Pd(CH_3COO)_2$, acetone, aq. K_2CO_3, Δ; (vii) H_2, Pd/C, MeOH, 50 psi; (viii) DBU, THF/*t*-BuOH, 50°C.

as the corresponding *tert*-butyl carbamate to afford **4** in nearly quantitative yield[24,28,29]. The role of the Boc group is not to protect nitrogen but to reduce the electron density of the pyrrole to avoid extensive dehalogenation[30]. Coupling Boc-protected **4** with phenylboronic acid under Suzuki–Miyaura cross-coupling conditions[31] proceeded cleanly to afford the 4-phenyl-1*H*-pyrrole-2-carboxylic acid methyl ester **2a** with concomitant deprotection of the NH[28,29]. The 3-phenyl derivative **2b** was then targeted by first reacting the methyl pyrrole-2-carboxylate with two equivalents of sulfuryl chloride to give the C4, C5 dichloride **5** in 83% yield (Scheme 2). The iodination of this compound **5** gave the tetrasubstituted pyrrole derivative **6**. The Suzuki–Miyaura cross-coupling reaction of **6** with phenylboronic acid using palladium acetate in acetone led to the desired product **7**. Hydrogenation of **7** with palladium on carbon reduced the two remaining halogens and introduced the two hydrogens at C4 and C5 to afford the 3-phenylpyrrole **2b**[30]. The ethyl 4-methyl-3-phenylpyrrole-2-carboxylate **2c** was synthesized using a Barton–Zard reaction[32]; the (*E*)-1-phenyl-2-nitropropene reacted with one equivalent of ethyl isocyanide previously anionized with one equivalent of 1,8-diazabicyclo[5.4.0] undec-7-ene (DBU) in a mixture of tetrahydrofuran and *tert*-butyl alcohol leading to pyrrole **2c** (Scheme 2)[33,34].

The preparation of *N*-aryl pyrroles **8a–c** was achieved by nucleophilic substitution of the various pyrrole-2-carboxylates **2a–c** with 2-fluoro-nitrobenzene using cesium carbonate as the base in refluxing DMF solution (Scheme 3)[35,36].



	R_1-	R_2-
9,11,12a	C_6H_5-	H-
9,11,12b	H-	C_6H_5-
9,11,12c	CH_3-	C_6H_5-
9,11,12d	-$COOC_2H_5$	H-



Scheme 3. Synthesis of 1,3-dihydro-1-{1-[4-(pyrrolo[1,2-*a*]quinoxalin-4-yl)benzyl]piperidin-4-yl}-2*H*-benzimidazol-2-ones and 5-fluoro-2-{1-[4-(pyrrolo[1,2-*a*]quinoxalin-1-yl)benzyl]piperidin-4-yl}-1*H*-benzimidazoles **1a–h**. Reagents and conditions: (i) 2-fluoro-nitrobenzene, Cs_2CO_3, DMF, Δ; (ii) Fe, CH_3COOH, Δ; (iii) $POCl_3$, Δ; (iv) OHC-C_6H_4-$B(OH)_2$, $Pd[P(C_6H_5)_3]_4$, K_2CO_3, toluene, EtOH, Δ; (v) 4-(2-ketobenzimidazolin-1-yl)piperidine or 4-(5-fluorobenzimidazolin-2-yl)piperidine, $NaBH_3CN$, MeOH, Δ; (vi) $POCl_3$, Δ; (vii) $BrCH_2COCOOC_2H_5$, EtOH, Δ.

Reduction of the nitro moiety with iron in hot glacial acetic acid produced the spontaneous ring closure onto the ester to afford the desired tricyclic pyrrolo[1,2-*a*]-quinoxalines **9a–c** through a one-pot reduction–cyclization step[24,36]. The lactame **9d** was prepared in two steps by treatment of commercially available 3-methyl-2-quinoxalinol with phosphorus oxychloride leading to the chloro derivative **10** followed by condensation with ethyl bromopyruvate in dry ethanol[37]. The lactames **9a–d** were subsequently chlorodehydroxylated with phosphorus oxychloride, leading to the 4-chloroquinoxalines **11a–d**. 4-(Pyrrolo[1,2-*a*]quinoxalin-4-yl)benzaldehydes **12a–d** were easily prepared by a direct Suzuki–Miyaura cross-coupling reaction of 4-chloropyrroloquinoxalines **11a–d** with 4-formylphenylboronic acid performed in the presence of $Pd(PPh_3)_4$ as a catalyst, and in the presence of potassium carbonate used as the base[19]. The aldehydes **12a–d** 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 **1b–h**[19]. The 3D spatial determinations of **1b**, **1d**, and **1e** were established by X-ray crystallography[38], and confirmed the structures in the solid state as anticipated on the basis of infrared (IR) and ^{1}H nuclear magnetic resonance (NMR) data (Figure 3).

Biology

Cytotoxicity

All compounds **1a–h** were tested on activated human peripheral blood mononuclear cells (Table 1)[19,23].

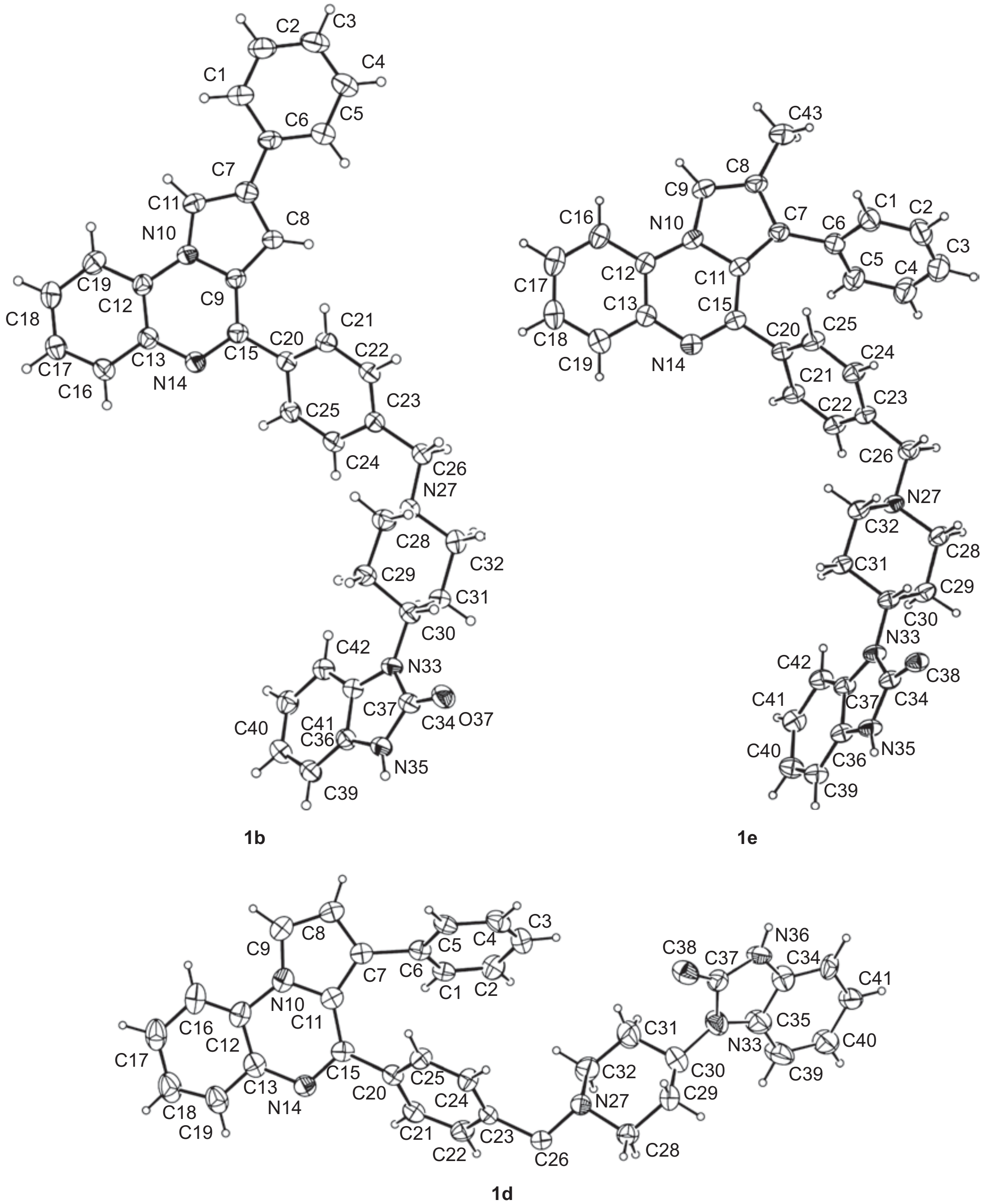


Figure 3. The ORTEP drawing of 1,3-dihydro-1-{1-[4-(pyrrolo[1,2-*a*]quinoxalin-4-yl)benzyl]piperidin-4-yl}-2*H*-benzimidazol-2-ones **1b**, **1d**, and **1e** with thermal ellipsoids at 30% level.

Table 1. *In vitro* activity of compounds **1a–h** on U937, K562, HL60, and MCF7 cells, and cytotoxicity on human peripheral blood mononuclear cells (PBMNC) + phytohemagglutinin (PHA).

Compound	IC_{50} value (μM)[a]				Cytotoxicity on activated human PBMNC (+ PHA)
	U937	K562	HL60	MCF7	
A6730	8 ± 0.2	8 ± 0.3	5.5 ± 0.2	>20	n.d.[b]
1a	11 ± 0.3	4 ± 0.2	7 ± 0.3	11 ± 0.5	7 ± 0.5
1b	>50	30 ± 1	50 ± 1	>50	>50
1c	4 ± 0.2	3 ± 0.3	14 ± 0.5	3 ± 0.4	4 ± 0.4
1d	12 ± 0.4	19 ± 0.5	2 ± 0.3	>50	>50
1e	17.5 ± 0.3	15 ± 0.4	5 ± 0.2	35 ± 1	6 ± 0.3
1f	3 ± 0.3	3 ± 0.4	50 ± 0.5	3 ± 0.4	4 ± 0.4
1g	>50	>50	>50	46 ± 1	>50
1h	6 ± 0.2	4 ± 0.4	11 ± 0.4	3.5 ± 0.2	>50

[a]The IC_{50} (μM) values correspond to the mean ± standard deviation from three independent experiments.
[b]n.d., not determined.

As expected, most of the pyrrolo[1,2-*a*]quinoxalines **1a–h** showed a 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.

Antiproliferative effect

Compounds **1a–h** 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 reference standard drug. The results are summarized in Table 1. The pyrrolo[1,2-*a*]quinoxalines **1c**, **1f**, and **1h** were found to be the most antiproliferative compounds on the growth of human myeloid U937 cell line with IC_{50} of 4, 3, and 6 μM, respectively. These three derivatives showed a better activity in comparison with the reference compound A6730 (IC_{50} = 8 μM). Interestingly, **1c**, **1f**, and **1h** were substituted by a benzylpiperidinyl fluorobenzimidazole moiety in position 4 of the pyrrolo[1,2-*a*]quinoxaline core, and were also substituted on the pyrrole ring. Moreover, the absence of substitution on this pyrrole structure (compound **1a**) induced a slight decrease in the antiproliferative activity on the U937 cell line (IC_{50} = 11 μM for **1a** compared with 3–6 μM for **1c**, **1f**, and **1h**).

All other compounds **1b**, **1d**, **1e**, and **1g** derived from incorporation of the benzylpiperidinyl benzimidazolone moiety, which was present in the reference compounds **I–IV**, into the 4-position of the heterocyclic pyrroloquinoxaline ring were found to be less active or inactive on the U937 cell line in comparison with their benzylpiperidinyl fluorobenzimidazole analogs **1c**, **1f**, and **1h**. Nevertheless, the two pyrrolo[1,2-*a*]quinoxalines, bearing a phenyl in position 3 of the tricyclic structure (compounds **1d** and **1e**), showed significant antiproliferative activities (IC_{50} = 12 and 17.5 μM, respectively). From a SAR point of view, these preliminary biological results on the U937 cell line highlight the importance of substitution at the C-4 position of the pyrroloquinoxaline scaffold by a benzylpiperidinyl fluorobenzimidazole group, and also the need for a functionalization on the pyrrole ring.

The antiproliferative potencies of these new derivatives **1a–h** were also examined toward the human myeloid leukemia cell lines K562 and HL60.

Among the eight compounds tested for antiproliferative activities on the K562 cell line, the four pyrrolo[1,2-*a*]-quinoxalines **1a**, **1c**, **1f**, and **1h**, always bearing a benzylpiperidinyl fluorobenzimidazole moiety in their 4-position, were found to be the most active compounds with an IC_{50} of 3–4 μM. The replacement of the benzylpiperidinyl fluorobenzimidazole substituent by a benzylpiperidinyl benzimidazolone group in position 4 of the pyrroloquinoxaline skeleton (compounds **1b**, **1d–e**, and **1g**) led to a decrease in the activity. However, as the substitution in position 2 by an ester function led to the inactive compound **1g** (IC_{50} > 50 μM), the substitution at position 2 or 3 by a phenyl (compounds **1b**, **1d**, and **1e**) only induced a slight decrease in the antiproliferative activity upon the K562 cell line, with IC_{50} from 15 to 30 μM. Moreover, it could be also noted that the phenyl substitution at position 3 (**1d** and **1e**) was less detrimental for the activity (IC_{50} of 19 and 15 μM, respectively) in comparison with their 2-phenyl analog **1b** (IC_{50} = 30 μM).

Against the HL60 human acute promyeloid leukemia cell line, most of the tested compounds showed antiproliferative activity with IC_{50} values from 2 to 50 μM, except **1g** that was found to be inactive (IC_{50} >50 μM). In a general way, pyrroloquinoxalines having a benzylpiperidinyl fluorobenzimidazole moiety at position 4 exhibited better activities than their benzylpiperidinyl benzimidazolone homologs (i.e. IC_{50} = 14 μM for **1c** vs. 50 μM for **1b**, 11 μM for **1h** vs. >50 μM for **1g**, and 7 μM for **1a** vs. 14 μM for **JG454**[19]). Surprisingly, this observation could not be applied to compounds **1e** and **1f**. Hence, the IC_{50} of **1e** (5 μM) was 10 times lower than that of compound **1f** (IC_{50} = 50 μM). Interestingly, the desmethyl structural analog **1d** of active pyrrolo[1,2-*a*]quinoxaline **1e** was found to be twice as active on this HL60 line than compound **1e** (IC_{50} = 2 μM for **1d**).

Against the MCF7 breast adenocarcinoma, the same pyrrolo[1,2-*a*]quinoxalines **1c**, **1f**, and **1h**, bearing a benzylpiperidinyl fluorobenzimidazole moiety in position 4 and substituted on the pyrrole ring, exhibited potent cytotoxicity (IC_{50} from 3 to 3.5 μM). Nevertheless, their unsubstituted pyrrole analog (compound **1a**) showed significant antiproliferative activity with an IC_{50} of 11 µM. However, the isosteric replacement of the benzylpiperidinyl fluorobenzimidazole group by a benzylpiperidinyl benzimidazolone was never found to be beneficial in terms of antiproliferative activity (i.e. **1b** compared to **1c**: IC_{50} >50 µM vs. 3 µM; **1e** to **1f**: IC_{50} = 35 µM vs. 3 µM; **1g** to **1h**: IC_{50} = 46 µM vs. 3.5 µM, and also **JG454**[19] vs. **1a**: IC_{50} =20 µM vs. 11 µM).

Conclusion

In the present report, we describe the synthesis of a new series of substituted pyrrolo[1,2-*a*]quinoxaline derivatives, and present 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 new substituted pyrrolo[1,2-*a*]quinoxalines showed antiproliferative activity against U937, K562, HL60, and MCF7 cell lines. From a general SAR point of view, these preliminary biological results highlight the importance of substitution at the C-4 position of the pyrroloquinoxaline scaffold by a benzylpiperidinyl fluorobenzimidazole group, and also the need of a functionalization on the pyrrole ring. However, compounds that demonstrated high selectivity (high index of selectivity) should offer a potentially safer therapy. Index of selectivity (IS) was defined as the ratio of the IC_{50} value on human mononuclear cells to the IC_{50} value on U937, K562, HL60, or MCF7 line. This led to the identification of compounds with IS >25 for compound **1d** on the human myeloid leukemic cell line HL60, and >14.2 for compound **1h** against the MCF7 breast adenocarcinoma. This potential inhibitor **1h** also showed interesting IS on U937 and K562 leukemic cell lines with values of >8.3 and >12.5, respectively. These two compounds could now constitute suitable candidates for further pharmacological studies.

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.

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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38. Supplementary X-ray crystallographic data of compounds 1b, 1d and 1e (CCDC-693149, CCDC-707577 and CCDC-693150): Cambridge Crystallographic Data Centre, University Chemical Laboratory, 12 Union Road, Cambridge, CB2 1EZ, UK.E-mail:deposit@ccdc.cam.ac.uk.