Synthesis and Antiproliferative Effect of Ethyl 4-[4-(4-Substituted Piperidin-1-yl)]benzylpyrrolo[1,2-a]quinoxalinecarboxylate Derivatives on Human Leukemia Cells

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Acute leukemia is a hematological malignancy with high incidence and recurrence rates and is characterized by an accumulation of blasts in bone marrow due to proliferation of immature lymphoid or myeloid cells associated with a blockade of differentiation. The heterogeneity of leukemia led us to look for new specific molecules for leukemia subtypes or for therapy-resistant cases. Among heterocyclic derivatives that attracted attention due to their wide range of biological activities, we focused our interest on the pyrrolo[1,2-a]quinoxaline heterocyclic framework that has been previously identified as an interesting scaffold for antiproliferative activities against various human cancer cell lines. In this work, new ethyl 4-[4-(4-substituted piperidin-1-yl)]benzylpyrrolo[1,2-a]quinoxalinecarboxylate derivatives (1a–o) were designed, synthesized, and evaluated against five different leukemia cell lines, including Jurkat and U266 (lymphoid cell lines) and K562, U937, and HL60 (myeloid cell lines), as well as on normal human peripheral blood mononuclear cells (PBMCs). This new pyrrolo[1,2-a]quinoxaline series showed interesting cytotoxic potential against all tested leukemia cell lines. In particular, pyrroloquinoxalines 1a and 1m,n seem to be interesting due to their high activity against leukemia and their low activity against normal hematopoietic cells, leading to a high index of selectivity.

Introduction

Acute leukemia is one of the most common lethal leukemias and is characterized by an accumulation of blasts in bone marrow due to the proliferation of immature lymphoid or myeloid cells associated with a blockade of differentiation. Adult acute leukemia presents a poor prognosis due to a high incidence of relapses. As treatment has not been modified for many years, development of new, more selective therapeutic approaches is a challenge and is necessary to improve overall survival in patients.[1]

A heterocyclic framework constitutes the basis of an important class of compounds possessing interesting biological activities. In the recent years, pyrrolo[1,2-a]quinoxalines have attracted persistent interest due to practically important properties of some their derivatives, specifically, pronounced biological activities. Thus, these heterocyclic derivatives have many

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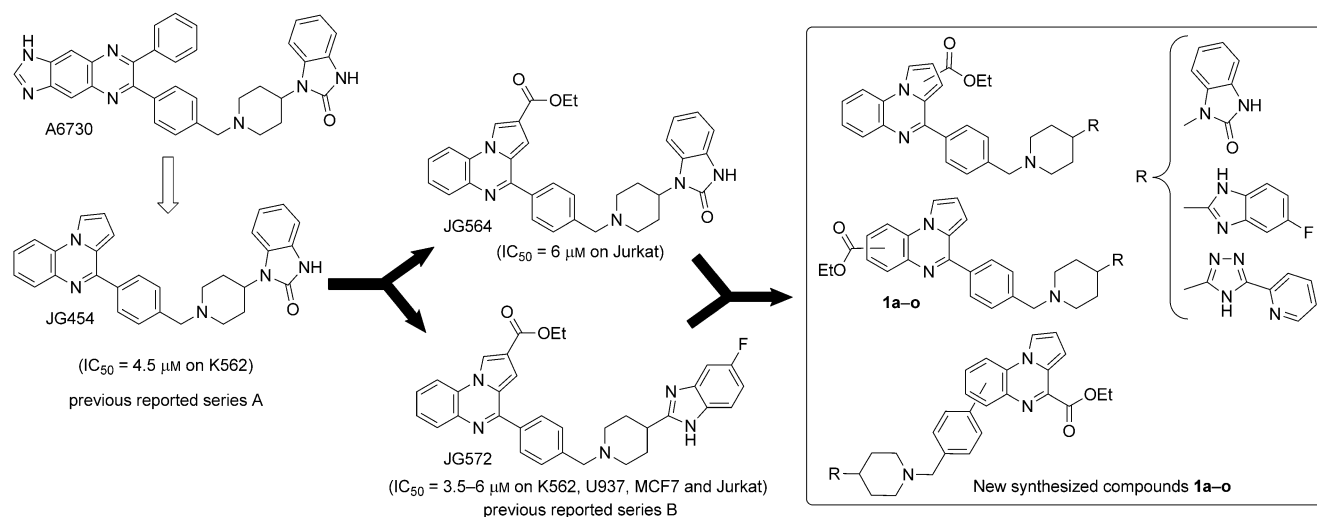http://onlinelibrary.wiley.com/doi/10.1002/cmdc.v12.12/issuetoc

Figure 1. Structure of A6730 and bioactive compounds of previously described series A–B, and general structure of new synthesized substituted pyrrolo[1,2-a]quinoxaline derivatives **1 a–o**.

biological activities and have been found in many pharmaceutical molecules, including antipsychotic agents,[2] antiviral agents,[3] adenosine receptor modulators,[4] antiparasitic agents,[5–11] and anticancer agents.[12–14] The discovery of new therapeutic agents against cancer is one of the most important goals in medicinal chemistry. Thus, we recently published four series of new pyrrolo[1,2-a]quinoxaline derivatives (Figure 1) endowed with interesting activity toward human leukemia cells.[15–18] These antileukemia pyrroloquinoxaline compounds have been previously reported as new structural analogues of derivative A6730, a reference Akt inhibitor that exhibits antiproliferative activity against various human leukemia cell lines.[15–20]

In this context, and by considering the biological activities of previous pyrrolo[1,2-a]quinoxalines on human leukemia cells, we undertook and investigated the synthesis of a new series of ethyl 4-[4-(4-substituted piperidin-1-yl)]benzylpyrrolo[1,2-a]quinoxalinecarboxylate derivatives. Taking into account the best results obtained in the previous series (A–B, Figure 1), we decided to refer to the JG564 and JG572 pyrrolo[1,2-a]quinoxaline pharmacophores[15–18] as a template for the design of new compounds **1 a–o**, in which the pyrrolo[1,2-a]quinoxaline ring is replaced in various positions by an ethyl ester functionality (Figure 1). In addition, further modulations on the piperidine core were also considered, such as the introduction of new substituted heterocyclic moieties (benzimidazolone, fluorobenzimidazole, and pyridinyltriazole substituents), in analogy to previously described antileukemia compounds.[15–20] The antiproliferative activity of the resulting derivatives (**1 a–o**) was then evaluated in vitro against various myeloid (U937, HL60, K562) or lymphoid (Jurkat, U266) leukemia cell lines. To determine their respective cytotoxicity, derivatives **1 a–o** were also tested on activated human peripheral blood mononuclear cells (PBMCs). Structure–activity relationships of these new derivatives (**1 a–o**) were also developed.

Results and Discussion

Chemistry

The reported pyrrolo[1,2-a]quinoxaline derivatives **1 a–k** were synthesized from different ethyl 4,5-dihydro-4-oxo-5H-pyrrolo[1,2-a]quinoxalinecarboxylates (**2 a–e**) (Schemes 1 and 2). Different strategies were considered for the synthesis of pyrrolo[1,2-a]quinoxaline esters **2 a–e** in order to introduce the ester functionality at various positions of the heterocyclic skeleton. The synthesis of ester **2 a** was accomplished in six steps according to the following pathway (Scheme 1): ethyl 3-hydroxymethylpyrrole-2-carboxylate (**3**) was prepared by silver-catalyzed cycloaddition of commercially available propargyl alcohol with ethyl isocyanoacetate in 1,4-dioxane at 80 °C.[21,22] The conversion of **3** to ethyl 3-formyl-1H-pyrrole-2-carboxylate (**4**) was achieved using manganese dioxide (MnO_2) in chloroform.[21] The oxidation of the aldehyde functionality of **4** into a carboxylic acid group was realized with $KMnO_4$, leading to pyrrole **5**, which was converted into diester derivative **6** by treatment with thionyl chloride in ethanol. Preparation of N-aryl pyrrole **7** was obtained by nucleophilic substitution of diethyl pyrrole-2,3-dicarboxylate (**6**) with 2-fluoro-nitrobenzene, using cesium carbonate as the base in DMF solution at reflux.[14,17]

Reduction of the nitro moiety of **7** with iron in hot glacial acetic acid produced spontaneous ring closure onto the ester to afford the desired tricyclic pyrrolo[1,2-a]quinoxaline (**2 a**) through a one-pot reduction–cyclization step.[14,17] The Clauson–Kaas reaction of **8 a,b** with 2,5-dimethoxytetrahydrofuran (DMTHF) in acetic acid gave pyrrolic derivatives **9 a,b**.[23] The resulting 1-(2-nitrophenyl)pyrrole ester intermediates **9 a,b** were subsequently reduced into the attempted 1-(2-aminophenyl)-pyrrole esters **10 a,b** using sodium borohydride–copper(II) sulfate in ethanol at 0 °C.[10,14,17] This $NaBH_4$–$CuSO_4$ system was found to be quite powerful in reducing the aromatic nitro
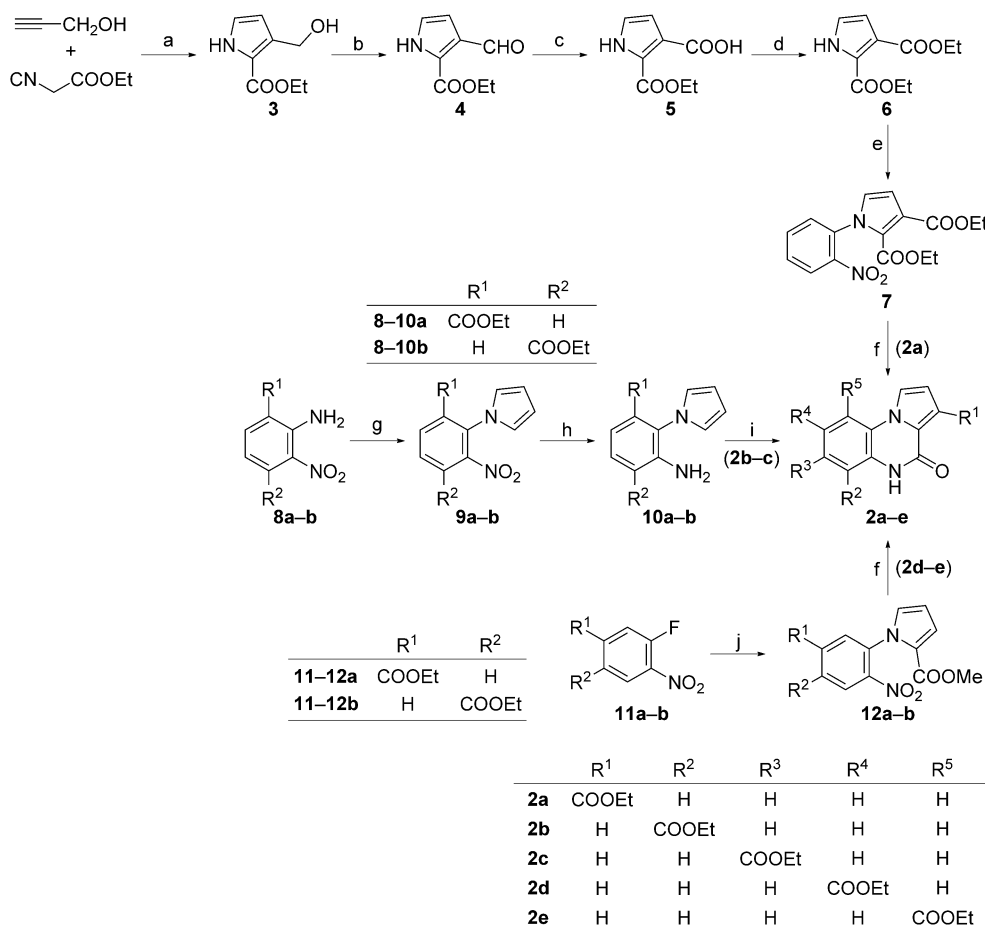
Scheme 1. Synthesis of ethyl 4,5-dihydro-4-oxo-5H-pyrrolo[1,2-a]quinoxalinecarboxylates **2a–e**. *Reagents and conditions*: a) Ag₂CO₃, dioxane, 80 °C, 2 h, 86 %; b) MnO₂, CHCl₃, reflux, 2.5 h, 70 %; c) KMnO4, acetone/H₂O, 40 °C, 1 h, then RT, 1 h, 31 %; d) EtOH, SOCl₂, reflux, 3 h, 87 %; e) 2-fluoronitrobenzene, Cs₂CO₃, DMF, reflux, 1.5 h, 92 %; f) Fe, CH₃COOH, reflux, 2 h, 28–92 %; g) DMTHF, AcOH, reflux, 1 h, 64–93 %; h) CuSO₄/NaBH₄, EtOH, 0 °C, 1 h, 88–90 %; i) (Cl₃CO)₂CO, toluene, reflux, 4 h, 48–81 %; j) methyl pyrrole-2-carboxylate; Cs₂CO₃, DMF, reflux, 2 h, 24–62 %.

groups. The reaction of **10a,b** with triphosgene in toluene gave lactams **2b,c**. Substitution of commercially methyl pyrrole-2-carboxylate with various ethyl fluoronitrobenzenecarboxylates (**11a,b**) led to the methyl 1-(4- or 5-ethoxycarbonyl-2-nitrophenyl)pyrrole-2-carboxylates **12a,b**. Mixing compounds **12a,b** in acetic acid at reflux with iron powder gave lactams **2d,e**.

Lactams **2a–e** were subsequently chlorodehydroxylated with phosphorous oxychloride, leading to 4-chloropyrrolo[1,2-a]quinoxalines **13a–e** (Scheme 2).[23] 4-(Pyrrolo[1,2-a]quinoxalin-4-yl)benzaldehydes **14a–e** were easily prepared by a direct Suzuki–Miyaura cross-coupling reaction of **13a–e** with 4-formylphenylboronic acid, performed in the presence of Pd(PPh₃)₄ as a catalyst, and in the presence of potassium carbonate as the base.[15–18] The 3D structural determination of **1c** was established by X-ray crystallography, and the structure was confirmed by solid-state NMR data (Figure 2).
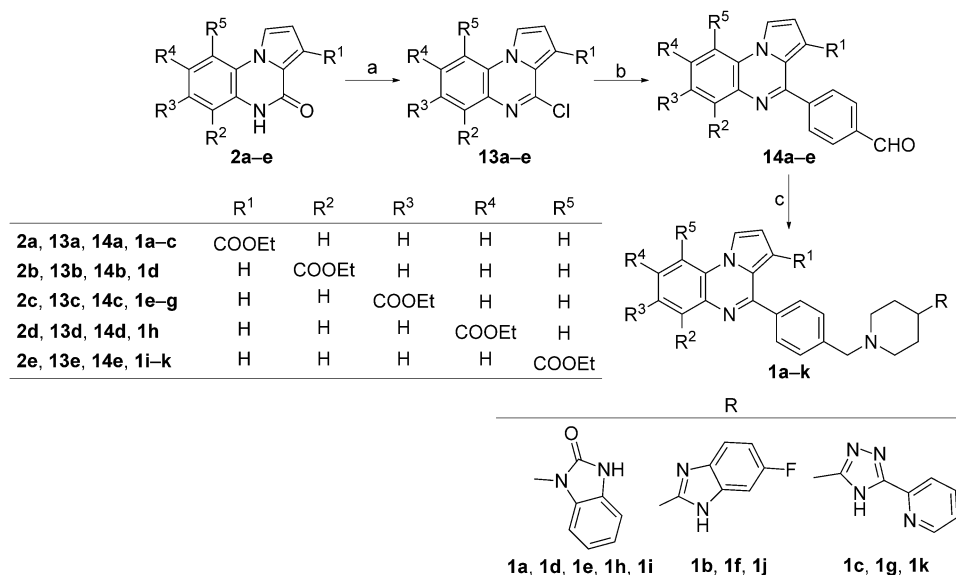
Pyrrolo[1,2-a]quinoxaline derivatives **1l–o** were synthesized in six steps from various halogenated 2-nitroaniline according to Scheme 3. Preparation of 1-(4-bromo-2-nitrophenyl)pyrroles **15a,b** was performed according to the Clauson–Kaas reaction, run under microwave irradiation, starting from halogeno-2-ni-

troaniline and 2,5-dimethoxytetrahedrofuran in acetic acid.[14] The resulting phenylpyrrole **15a,b** intermediates were subsequently reduced, using a NaBH₄–CuSO₄ treatment, into the attempted 1-(2-amino-4-bromophenyl)pyrroles **16a,b**.[10,14,17] Addition of **16a,b** to ethyl chlorooxoacetate in the presence of triethylamine provided esters **17a,b**.[14,24] Ethyl halogenopyrrolo[1,2-a]quinoxaline-4-carboxylates **18a,b** were then prepared by cyclization of amido esters **17a,b** in phosphorus oxychloride at reflux.[24] Coupling halogenated compounds **18a,b** with 4-formylphenylboronic acid under Suzuki–Miyaura cross-coupling conditions proceeded cleanly to afford pyrrolo[1,2-a]quinoxaline esters **19a,b**. Reaction of substituted piperidines with **19a,b** using sodium cyanoborohydride as the reducing agent in methanol gave amines **1l–o** (Scheme 3).

Biological activity

Cytotoxicity in leukemia cell lines

Fifteen new compounds (**1a–o**) were tested by MTS assay for their in vitro antiproliferative activity against five human leukemia cell lines (U937, K562, Jurkat, U266, and HL60). In addition,

	R¹	R²	R³	R⁴	R⁵
2a, 13a, 14a, 1a–c	COOEt	H	H	H	H
2b, 13b, 14b, 1d	H	COOEt	H	H	H
2c, 13c, 14c, 1e–g	H	H	COOEt	H	H
2d, 13d, 14d, 1h	H	H	H	COOEt	H
2e, 13e, 14e, 1i–k	H	H	H	H	COOEt

1a, 1d, 1e, 1h, 1i   **1b, 1f, 1j**   **1c, 1g, 1k**

Scheme 2. Synthesis of pyrrolo[1,2-*a*]quinoxalines **1a–k**. *Reagents and conditions*: a) POCl₃, reflux, 2 h, 68–89%; b) OHC-C₆H₄-B(OH)₂, Pd[P(C₆H₅)₃]₄, K₂CO₃, toluene, EtOH, reflux, 24 h, 65–86%; c) 4-(2-ketobenzimidazolin-1-yl)piperidine or 4-(5-fluorobenzimidazol-2-yl)piperidine or 2-(3-piperidin-4-yl-1*H*-1,2,4-triazol-5-yl]pyridine, NaBH₃CN, MeOH, reflux, 5 h, 33–77%.
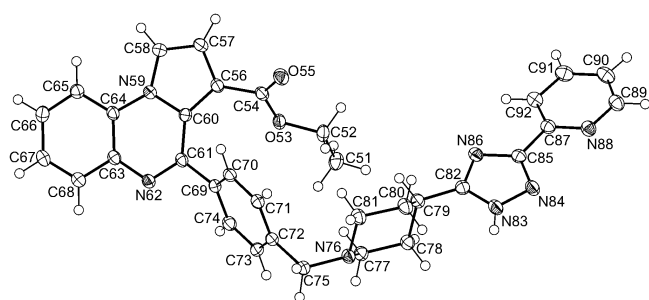

Figure 2. ORTEP image of pyrrolo[1,2-*a*]quinoxaline **1c** with thermal ellipsoids at 30% level.

compound A6730 (Figure 1) was used in these tests as the reference standard drug. The biological results are presented in Table 1. Moreover, compound LY-294002, which showed antiproliferative activity against the HL60, U937, and K562 cell lines,[25–28] was also applied as a reference cytotoxic agent.

Firstly, the antiproliferative potencies of these new derivatives (**1a–o**) were examined toward the human myeloid leukemia cell lines K562 and HL60. Among the fifteen compounds tested for antiproliferative activities in the K562 cell line, the four pyrrolo[1,2-*a*]quinoxalines **1a–b**, **1j**, and **1m** were found to be the most active, with IC₅₀ values of 0.5–4 μM. The other pyrroloquinoxalines (**1c,d**, **1i**, and **1k,l**) also showed significant antiproliferative activity, with IC₅₀ values ranging from 6 to 15 μM, making them more potent than reference compound A6730 (IC₅₀ = 17 μM). Pyrrolo[1,2-*a*]quinoxalines **1e–g**, bearing an ethyl ester functionality at position 7 of the heterocyclic skeleton, were found to have low antiproliferative activities (IC₅₀ = 34–37 μM) or were inactive (IC₅₀ > 50 μM). The same observation was found for compounds **1h**, with an ester group at position 8, and derivative **1o** (IC₅₀ > 50 μM). Surprisingly,

compound **1n**, a structural analogue of pyrroloquinoxalines **1l,m**, showed only moderate activity against the leukemia cell line K562, with an IC₅₀ of 35 μM. Moreover, in terms of structure–activity relationships (SAR), it was also noticed that 4-substituted benzylpiperidinylpyrroloquinoxalines **1a–c**, **1i–k**, and **1d**, bearing an ethyl ester moiety at positions 3, 9, and 6, respectively, were found to be the most active compounds, with IC₅₀ values ranging from 0.5 to 15 μM. This observation was also noticed for compounds **1l,m**, structural analogues of compound **1d** in which the benzylpiperidinyl moiety and the ester substitutions are simply reversed.

Against the HL60 human acute myeloblastic leukemia cell line, most of the tested compounds showed antiproliferative activity (IC₅₀ values ranging from 0.5 to 17 μM), with the exception of **1d**, **1h**, and **1n,o**, which were found to be less active or inactive (IC₅₀ > 50 μM for **1d**, **1h** and **1o**; IC₅₀ = 40 μM for **1n**). The three 4-substituted benzylpiperidinylpyrroloquinoxalines (**1a–c**), each with an ester functionality at position 3 (IC₅₀ = 1–4 μM), and pyrroloquinoxaline **1j**, with an ester substitution at C9 (IC₅₀ = 0.5 μM), exhibited better activities than their other homologues. These four derivatives showed better activity that that observed for reference compound A6730 (IC₅₀ = 5.5 μM). Moreover, it was noticed that the substitution of the ester at position 7 in pyrroloquinoxaline derivatives **1e–g** was more detrimental for activity (IC₅₀ = 7–17 μM). In addition to this subseries, replacement of the benzylpiperidinyl benzimidazolone or benzylpiperidinyl fluorobenzimidazole substituent (compounds **1e,f**) by a benzylpiperidinyl triazolylpyridine group at position 4 of the fused heterocyclic skeleton (compound **1g**) led to an increase in activity (IC₅₀ ≈ 7 μM). However, while substitution at position 7 by an ethyl ester functionality led to the moderately bioactive compounds **1e–g** (IC₅₀ = 7–17 μM), substitution at position 8 by this ester (compound **1h**) induced a decrease in antiproliferative activity in
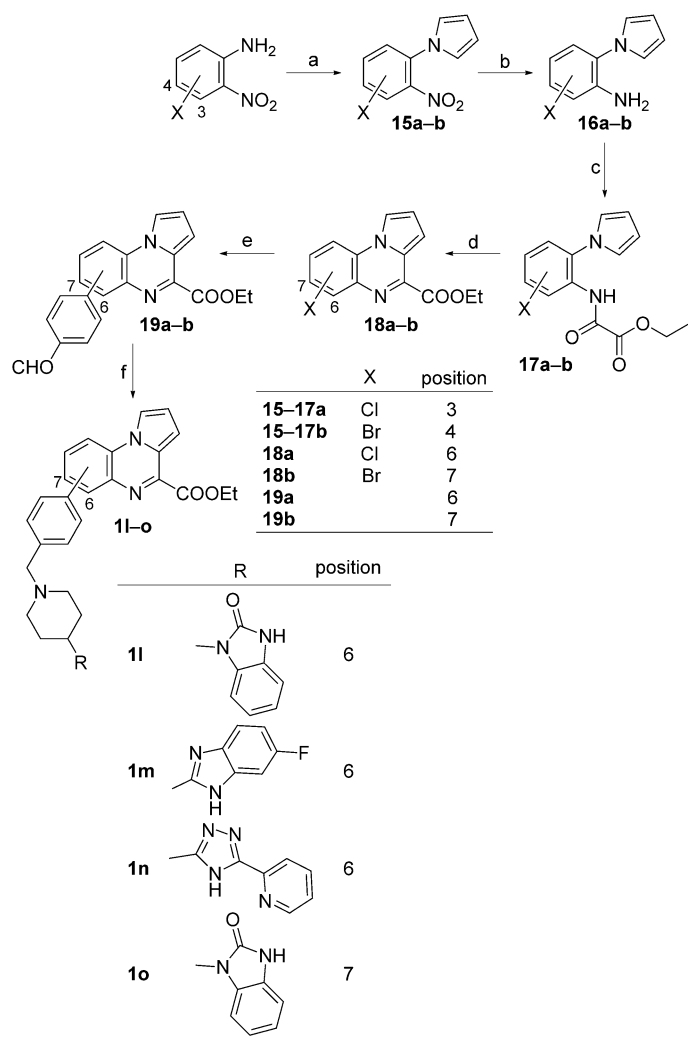
Scheme 3. Synthesis of pyrrolo[1,2-*a*]quinoxalines **1 l–o**. *Reagents and conditions*: a) DMTHF, AcOH, reflux, MW, 10 min, 82–86 %; b) CuSO$_4$/NaBH$_4$, EtOH, 0 °C to RT, 1 h, 74–80 %; c) H$_5$C$_2$-OOC-CO-Cl, TEA, THF, RT, 14 h, 85–94 %; d) POCl$_3$, reflux, 20 min, 57–64 %; e) OHC-C$_6$H$_4$-B(OH)$_2$, Pd[P(C$_6$H$_5$)$_3$]$_4$, K$_2$CO$_3$, toluene, EtOH, reflux, 24 h, 58–80 %; f) 4-(2-keto-benzimidazolin-1-yl)piperidine or 4-(5-fluorobenzimidazolin-2-yl)piperidine or 2-(3-piperi-din-4-yl-1*H*-1,2,4-triazol-5-yl)pyridine, NaBH$_3$CN, MeOH, reflux, 5 h, 48–70 %.

activity against the T-lymphocyte Jurkat cell line, with IC$_{50}$ values ranging from 12 to 20 μM. Surprisingly, substitution with this ester functionality in the C9 position of the pyrrolo[1,2-*a*]quinoxaline skeleton (compounds **1 i–k**) led to more active derivatives (IC$_{50}$ = 3–8 μM) than their other structural analogues **1 d–h**. We also noted that pyrroloquinoxalines **1 l–n**, the structural analogues of **1 d** in which substitution of the benzylpiperidinyl moiety and the ethyl ester groups are reversed between positions 4 and 6, exhibited potent cytotoxicity, with IC$_{50}$ values ranging from 3 to 4 μM, similar to that of reference compound A6730. When, the benzylpiperidinyl moiety was displaced at position 7 (compound **1 o**), the antiproliferative activity against Jurkat cells decreased (IC$_{50}$ = 30 μM).

Among compounds **1 a–o**, benzylpiperidinyl derivatives **1 a–c**, bearing an ester at position 3, also exhibited the best antiproliferative activity toward the growth of the human myeloid U937 cell line (IC$_{50}$ = 3–6 μM), in comparison with analogues **1 d–o**, which were found to be less active (IC$_{50}$ = 8 = 39 μM) or inactive (IC$_{50}$ > 50 μM). Moreover, pyrroloquinoxaline **1 j** also showed significant antiproliferative activity against the U937 cell line, similar to that found for reference compound A6730 (IC$_{50}$ = 8 μM).

Against the human myeloma cell line U266, pyrrolo[1,2-*a*]quinoxalines **1 a,b**, **1 k**, and **1 m**, exhibited the most potent cytotoxicity, with IC$_{50}$ ranging from 5 to 7 μM. All other tested pyrroloquinoxalines (**1**) exhibited moderate antiproliferative activity (IC$_{50}$ = 10–20 μM for compounds **1 c**, **1 g**, **1 i**, **1 l**, and **1 n**) or were inactive (IC$_{50}$ > 50 μM for derivatives **1 d–f**, **1 h**, **1 j**, and **1 o**).

From an SAR point of view, these preliminary biological results in leukemia cell lines highlighted the importance of substitution at the C4 position of the pyrroloquinoxaline scaffold by a benzylpiperidinyl group, as well as the need for an ethyl ester functionality at position 3 of the pyrrolo[1,2-*a*]quinoxaline ring (compounds **1 a–c**). With regard to these bioactive compounds, it was also noticed that compound **1 a**, derived from incorporation of the benzylpiperidinyl benzimidazolone moiety (present in reference compound A6730) into position 4 of the heterocyclic pyrroloquinoxaline ring, was found to be the most active candidate against all of the leukemia cell lines relative to their benzylpiperidinyl fluorobenzimidazole or benzylpiperidinyl triazolylpyridine analogues. Against each human cancer cell line, the antiproliferative activities of compounds **1** were generally found to be superior to those of the other reference drug, LY-294002.

the HL60 cell line (IC$_{50}$ > 50 μM. Among the 15 compounds tested for antiproliferative activities in the HL60 cell line, pyrrolo[1,2-*a*]quinoxalines **1 b**, **1 f**, **1 m**, and **1 j**, all bearing a benzylpiperidinyl fluorobenzimidazole moiety at position 4, were found to be the most active compounds in each subseries (IC$_{50}$ = 0.5–14 μM).

Against the T-acute lymphoblastic leukemia Jurkat cell line, the biological results of compounds **1 a–o** were quite similar to those observed against the HL60 cell line in terms of SAR. Pyrrolo[1,2-*a*]quinoxalines **1 a–c** (IC$_{50}$ from 1 to 2.1 μM) showed potent cytotoxicity—more potent than that observed for reference A6730 (IC$_{50}$ = 3.5 μM). In addition, compounds **1 d** and **1 h**, the structural analogues of pyrroloquinoxalines **1 a**, bearing an ester group at positions 6 and 8 of the heterocyclic system, were found to be inactive (IC$_{50}$ > 50 μM). Nevertheless, their homologues—pyrrolo[1,2-*a*]quinoxalines **1 e–g** with the ester at the C7 position—showed moderate antiproliferative

Cytotoxicity activity in activated normal PBMCs

Compounds **1 a–o** were tested in activated human peripheral blood mononuclear cells with phytohemagglutinin (PBMC + PHA) to evaluate their respective cytotoxicity in normal cells

Table 1. In vitro activity of compounds **1a–o** on U937, K562, HL60, Jurkat, and U266 cells, and cytotoxicity on human peripheral blood mononuclear cells (PBMC + PHA).
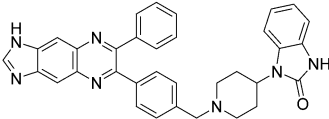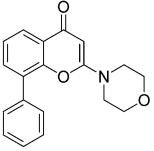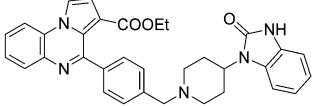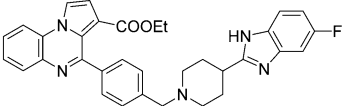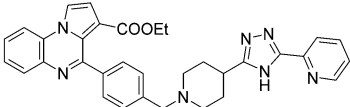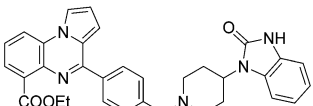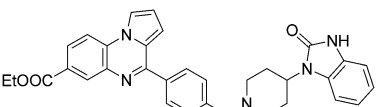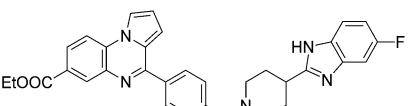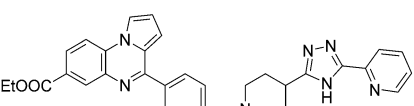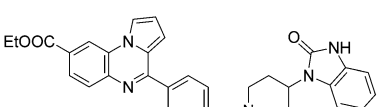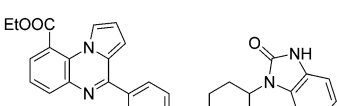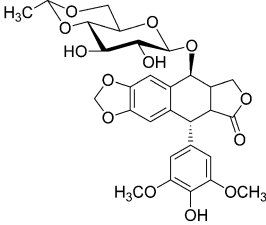
Compound	IC$_{50}$ [µM][a]					
	K562	U937	HL60	Jurkat	U266	PBMC + PHA
A6730	17 ± 0.3	8 ± 0.2	5.5 ± 0.2	3.5 ± 0.2	ND[b]	> 50
LY-294002	38 ± 1	14 ± 0.3	14 ± 0.3	22 ± 1	46 ± 2	> 50
1a	4 ± 0.1	3 ± 0.1	1 ± 0.1	1 ± 0.1	5 ± 0.2	> 50
1b	3 ± 0.2	4 ± 0.2	1.5 ± 0.1	1 ± 0.1	7 ± 0.2	9 ± 0.6
1c	9 ± 1	6 ± 0.2	4 ± 0.2	2.1 ± 0.1	17 ± 1.5	17 ± 1.2
1d	7 ± 0.1	> 50	> 50	> 50	> 50	> 50
1e	34 ± 2	> 50	17 ± 1.2	20 ± 1.3	> 50	> 50
1f	37 ± 1.2	33 ± 1.7	17 ± 1.6	14 ± 0.6	> 50	> 50
1g	> 50	20 ± 1.2	7 ± 0.3	12 ± 0.5	10 ± 1	> 50
1h	> 50	> 50	> 50	> 50	> 50	> 50
1i	15 ± 0.3	39 ± 2	16 ± 2	8 ± 0.4	10 ± 1.2	> 50

Table 1. (Continued)

Compound		IC$_{50}$ [μM][a]					
		K562	U937	HL60	Jurkat	U266	PBMC+PHA
1j		0.5±0.1	8±0.2	0.5±0.1	3±0.1	>50	6±0.3
1k		7±0.1	13±0.5	12±1.1	7±0.5	6±0.2	18±1.3
1l		6±0.2	23±1.3	15±2.2	4±0.2	20±2	>50
1m		3±0.2	12±0.5	7±0.3	3±0.1	5±0.2	>50
1n		35±2.1	36±2.1	40±2	3±0.2	16±1.5	>50
1o		>50	15±1.5	>50	30±1.6	>50	>50
cytarabine[31,32]		60.05	–	8.84	1.29±0.45	–	–
chlorambucil[31,33]		79.04	36.16	–	35.01±3.32	–	–

Table 1. (Continued)						
Compound	IC$_{50}$ [µM][a]					
	K562	U937	HL60	Jurkat	U266	PBMC + PHA
etoposide[31,34]	10.26 ± 0.20	14.10 ± 0.54	–	6.01 ± 1.66	–	–

[a] Values are the mean ± SD of three independent experiments. [b] Not determined.

(Table 1). In this biological evaluation, most of the pyrroloquinoxalines **1a–o** showed significant levels of cytotoxicity against lymphocytes, with IC$_{50}$ values ranging from 6 to >50 µM. These results were used to determine their respective range of toxic concentration. Indices of selectivity (IS) were defined as the ratio of the IC$_{50}$ value in human mononuclear cells to the IC$_{50}$ value in leukemia (K562, U937, HL60, Jurkat, and U266) cell lines. Compounds that demonstrate high selectivity (high selectivity index, SI) should offer the potential of safer therapy. This led us to identify compound **1a**, with SI > 50 for the HL60 and Jurkat cell lines and SI > 16.7 for the U937 cell line. Moreover, we noticed that compounds **1m,n** showed interesting selectivity toward the Jurkat cell line (SI > 16.7). These three compounds constitute potential new candidates for further pharmacomodulations and pharmacological studies.

Mode of action

The reference imidazoquinoxaline A6730, which showed interesting selectivity for the Jurkat cell line, is also described as an Akt inhibitor (Akt1 IC$_{50}$ = 58 nM, Akt2 IC$_{50}$ = 10 nM, and IC$_{50}$ Akt3 = 2.2 µM).[29] To determine the possible biological mechanism of action of these new derivatives, we first evaluated their potency toward isolated enzymes such as Akt 1, 2, and 3, as well as mTOR. These pharmacological tests were performed by DiscoverX at concentrations of 1 and 10 µM.[30] Nevertheless, only low effects were detected. Akt3 activity was decreased by 40 % with derivative **1b** at 10 µM. This enzymatic interaction could not explain alone the cellular effects.

Assessment of apoptosis in leukemia cell lines

Apoptosis induces loss of membrane asymmetry, resulting in phosphatidyl serine (PS) exposure detected by annexin V. The effect of compound **1a** on apoptosis, which showed the most interesting SI for human myeloid leukemia cell lines, was studied. Treatment of HL60 and K562 cells with **1a** induced a significant increase in Annexin V-positive cells after two and three days of incubation (Figure 3). These results could explain the inhibition of cell proliferation observed with this compound.
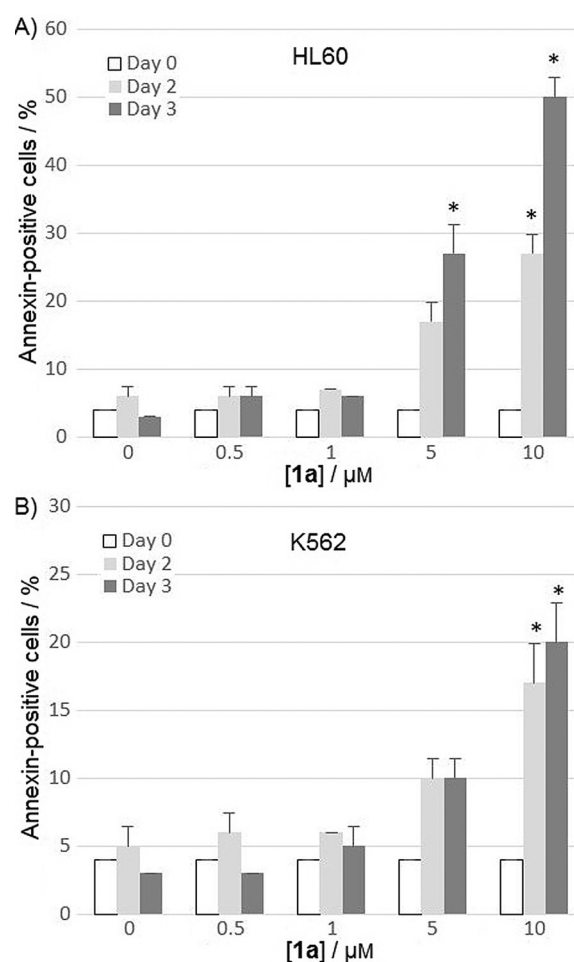
Figure 3. Effect of pyrrolo[1,2-a]quinoxaline **1a** on apoptosis of A) HL60 and B) K562 cells. Cells were cultured with or without increasing doses (0.5, 1, 5, and 10 µM) of compound **1a** for two and three days in culture medium, then stained with APC–Annexin V and analyzed by flow cytometry. Results are the mean ± SEM of two independent experiments; *$p < 0.05$ relative to controls (t-test).

Conclusions

In summary, by taking into account our previous works using the pyrrolo[1,2-a]quinoxaline template, we designed and syn-

thesized a series of 15 new ethyl 4-[4-(4-substituted piperidin-1-yl)]benzylpyrrolo[1,2-*a*]quinoxalinecarboxylate derivatives **1 a–o** and then evaluated their antileukemia activity against the human leukemia cell lines U937, K562, Jurkat, U266, and HL60. Using these biological results, a preliminary SAR study was discussed. The pharmacological evaluations of new compounds **1** showed cytotoxic activity in the different myeloid and lymphoid leukemia cell lines. Through these new pharmacomodulations on the pyrroloquinoxaline moiety, biological data demonstrated that derivatives **1 a** and **1 m,n** were interesting compounds, due to their high cytotoxic activity against some leukemia cells ($IC_{50} = 1–3$ μM) and their lower toxicity against normal hematopoietic cells (estimated $IC_{50} > 50$ μM). In comparison with the previously described pyrrolo[1,2-*a*]quinoxaline series,[15–18] these novel antileukemia heterocyclic compounds with high SI values could constitute more suitable candidates for further pharmacological modulations and investigations. Further studies are required to understand the mechanism of action of these new bioactive pyrrolo[1,2-*a*]quinoxaline derivatives. These studies are currently under progress.

Experimental Section

Chemistry

General: Commercially available reagents were used as received without additional purification. Melting points were determined with an SM-LUX-POL Leitz hot-stage microscope and are uncorrected. IR spectra were recorded on an NICOLET 380FT-IR spectrophotometer. NMR spectra were recorded with tetramethylsilane as an internal standard using a Bruker Avance 300 spectrometer. Splitting patterns have been reported as follows: s = singlet; bs = broad singlet; d = doublet; t = triplet; q = quartet; dd = double doublet; ddd = double double doublet; dt = double triplet; m = multiplet. Analytical TLC were carried out on 0.25 pre-coated silica gel plates (Polygram SIL G/UV$_{254}$) with visualization of compounds after UV light irradiation. Silica gel 60 (70–230 mesh) was used for column chromatography. Microwave experiments were carried out using a focused microwave reactor (CEM Discover). High-resolution mass spectra (electrospray in positive mode, ESI+) were recorded on a Waters Q-TOF Ultima apparatus. Elemental analyses were found to be within ±0.4% of the theoretical values.

Ethyl 3-hydroxymethylpyrrole-2-carboxylate (3): A mixture of propargyl alcohol (17.8 mmol) and Ag$_2$CO$_3$ (1.8 mmol) in 1,4-dioxane (60 mL) was heated at 80 °C for 5 min, then the mixture was cooled with an ice bath. This solution was cooled to 0 °C, then ethyl isocyanoacetate (27 mmol) was added dropwise. The reaction mixture was stirred for 10 min at room temperature, then heated 2 h at 80 °C. The resulting slurry was concentrated under reduced pressure and re-dissolved with dichloromethane. The organic layer was washed with brine, dried with Na$_2$SO$_4$, filtered, and evaporated under vacuum. The crude was purified on silica gel with EtOAc/petroleum ether as an eluent (5:5) to give **3** as pale-yellow crystals (86%, 2.59 g): mp: 91 °C.[21,22]

Ethyl 3-formyl-1*H*-pyrrole-2-carboxylate (4): Manganese dioxide (38 mmol) was added to a solution of ethyl 3-hydroxymethylpyrrole-2-carboxylate (**3**; 3.8 mmol) in chloroform (25 mL). The reaction mixture was then stirred at reflux for 2.5 h. The black solid was removed by filtration and washed with chloroform. The filtrate and washings were combined, dried with Na$_2$SO$_4$, filtered, and

evaporated to dryness under reduced pressure to give **4** as white crystals (70%, 440 mg): mp: 104 °C.[21]

2-Ethoxycarbonyl-1*H*-pyrrole-3-carboxylic acid (5): A solution of KMnO$_4$ (5.2 mmol) in acetone/water (1:1, *v/v*) was added dropwise to a solution of **4** (2.6 mmol) in acetone (6 mL). The resulting mixture was then heated at 40 °C for 1 h, then stirred at room temperature for an additional 1 h. An 85% aqueous solution of Na$_2$S$_2$O$_4$ (0.26 mmol) was added to the reaction mixture. After 10 min of stirring, the reaction solution was filtered over Celite. The Celite was washed with a solution of NaOH (1 M, 7 mL). The acetone of the filtrate was then evaporated under reduced pressure; the resulting filtrate was cooled and acidified with an aqueous solution of HCl (1 M) until a precipitate appeared. The precipitate was filtered, washed with water, and dried to give **5** as white crystals (31%, 148 mg): mp: 184 °C; ^{1}H NMR (CDCl$_3$): $\delta = 12.86$ (s, 1H, CO$_2$H), 12.46 (s, 1H, NH), 7.07 (t, 1H, $J = 5.90$ Hz, H-5), 6.60 (t, 1H, $J = 5.70$ Hz, H-4), 4.33 (q, 2H, $J = 7.20$ Hz, CH$_2$), 1.31 ppm (t, 3H, $J = 7.20$ Hz, CH$_3$); Anal. calcd for C$_8$H$_9$NO$_4$: C 52.46, H 4.95, N 7.65, found: C 52.63, H 5.12, N 7.92.

Diethyl 1*H*-pyrrole-2,3-dicarboxylate (6): Thionyl chloride (0.77 mmol) was added to a suspension of 2-ethoxycarbonyl-1*H*-pyrrole-3-carboxylic acid **5** (0.7 mmol) in EtOH (5 mL). The reaction mixture was heated at reflux for 3 h. EtOH was evaporated under reduced pressure. The residue was triturated with water and extracted with diethyl ether. The organic layer was then washed with a saturated aqueous solution of NaHCO$_3$, dried with Na$_2$SO$_4$, filtered, and evaporated to dryness to give **6** as a yellow oil (87%).[14]

General procedure for ethyl (4-formylphenyl)pyrrolo[1,2-*a*]quinoxalinecarboxylate (14 a–e, 19 a,b): K$_2$CO$_3$ (5.1 mmol) and 4-formylphenylboronic acid (5.1 mmol) were added to a suspension of compound **13 a–e** or **18 a,b** (4.64 mmol) and Pd(PPh$_3$)$_4$ (0.232 mmol) in a mixture of toluene/EtOH (75/4.1 mL) under nitrogen. The reaction mixture was stirred at reflux 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 EtOH, and the resulting precipitate was filtered, washed with EtOH, and purified by column chromatography on silica gel using dichloromethane as eluent to give pure product **14** or **19**.

Ethyl 4-(4-formylphenyl)pyrrolo[1,2-*a*]quinoxaline-3-carboxylate (14 a): Pale-yellow crystals (86%, 1.37 g): mp = 195 °C; ^{1}H NMR (CDCl$_3$): $\delta = 10.13$ (s, 1H, CHO), 8.12 (dd, 1H, $J = 8.00$ Hz and 1.40 Hz, H-6), 8.05 (d, 1H, $J = 3.00$ Hz, H-1), 8.03 (d, 2H, $J = 7.80$ Hz, H-3′ and H-5′), 7.98 (dd, 1H, $J = 8.20$ Hz and 1.40 Hz, H-9), 7.93 (d, 2H, $J = 7.50$ Hz, H-2′ and H-6′), 7.67 (td, 1H, $J = 8.00$ Hz and 1.40 Hz, H-7), 7.58 (td, 1H, $J = 8.00$ Hz and 1.40 Hz, H-8), 7.35 (d, 1H, $J = 3.00$ Hz, H-2), 3.83 (q, 2H, $J = 7.20$ Hz, OCH$_2$), 0.97 ppm (t, 3H, $J = 7.20$ Hz, CH$_3$); IR (KBr): $\tilde{\nu} = 1720$ (COO), 1705 cm^{-1} (CHO); HRMS-ESI *m/z* [M+Na]$^+$ calcd for C$_{21}$H$_{16}$N$_2$O$_3$Na: 367.1059, found: 367.1063.

Ethyl 4-(4-formylphenyl)pyrrolo[1,2-*a*]quinoxaline-6-carboxylate (14 b): Yellow crystals (65%, 1.04 g): mp = 172 °C; ^{1}H NMR (CDCl$_3$): $\delta = 10.15$ (s, 1H, CHO), 8.29 (d, 2H, $J = 8.40$ Hz, H-3′ and H-5′), 8.08 (dd, 1H, $J = 4.20$ Hz and 1.20 Hz, H-1), 8.07 (d, 2H, $J = 8.40$ Hz, H-2′ and H-6′), 8.05 (dd, 1H, $J = 7.20$ Hz and 1.20 Hz, H-7), 7.75 (dd, 1H, $J = 7.20$ Hz and 1.20 Hz, H-9), 7.60 (t, 1H, $J = 7.20$ Hz, H-8), 7.11 (dd, 1H, $J = 3.50$ Hz and 1.20 Hz, H-3), 6.99 (dd, 1H, $J = 4.20$ Hz and 3.50 Hz, H-2), 4.54 (q, 2H, $J = 7.20$ Hz, OCH$_2$), 1.48 ppm (t, 3H, $J = 7.20$ Hz, CH$_3$); IR (KBr): $\tilde{\nu} = 1715$ (COO), 1700 cm^{-1} (CHO); HRMS-ESI *m/z* [M+Na]$^+$ calcd for C$_{21}$H$_{16}$N$_2$O$_3$Na: 367.1059, found: 367.1054.

Ethyl 4-(4-formylphenyl)pyrrolo[1,2-*a*]quinoxaline-7-carboxylate (14 c): Yellow crystals (81 %, 1.29 g): mp = 216 °C; ^{1}H NMR (CDCl$_3$): δ = 10.17 (s, 1 H, CHO), 8.80 (dd, 1 H, *J* = 2.70 and 1.20 Hz, H-1), 8.26 (dd, 1 H, *J* = 8.70 Hz and 1.80 Hz, H-8), 8.21 (d, 2 H, *J* = 7.40 Hz, H-3′ and H-5′), 8.12 (d, *J* = 1.80 Hz, 1 H, H-6), 8.10 (d, 2 H, *J* = 7.40 Hz, H-2′ and H-6′), 7.97 (d, 1 H, *J* = 8.70 Hz, H-9), 7.07 (dd, 1 H, *J* = 4.20 Hz and 1.20 Hz, H-3), 7.02 (dd, 1 H, *J* = 4.20 Hz and 2.70 Hz, H-2), 4.46 (q, 2 H, *J* = 7.20 Hz, OCH$_2$), 1.47 ppm (t, 3 H, *J* = 7.20 Hz, CH$_3$); IR (KBr): $\tilde{v}$ = 1715 (COO), 1700 cm^{-1} (CHO); HRMS-ESI *m/z* [*M*+Na]$^+$ calcd for C$_{21}$H$_{16}$N$_2$O$_3$Na: 367.1059, found: 367.1054.

Ethyl 4-(4-formylphenyl)pyrrolo[1,2-*a*]quinoxaline-8-carboxylate (14 d): Yellow crystals (68 %, 1.09 g): mp = 215 °C; ^{1}H NMR (CDCl$_3$): δ = 10.17 (s, 1 H, CHO), 8.65 (dd, 1 H, *J* = 2.70 and 0.90 Hz, H-1), 8.28–8.19 (m, 3 H, H-9, H-3′ and H-5′), 8.16 (dd, 1 H, *J* = 8.40 Hz and 1.50 Hz, H-7), 8.12–8.04 (m, 3 H, H-6, H-2′ and H-6′), 7.07 (dd, 1 H, *J* = 4.20 Hz and 0.90 Hz, H-3), 7.00 (dd, 1 H, *J* = 4.20 Hz and 2.70 Hz, H-2), 4.50 (q, 2 H, *J* = 7.20 Hz, OCH$_2$), 1.50 ppm (t, 3 H, *J* = 7.20 Hz, CH$_3$); IR (KBr): $\tilde{v}$ = 1720 (COO), 1700 cm^{-1} (CHO); HRMS-ESI *m/z* [*M*+Na]$^+$ calcd for C$_{21}$H$_{16}$N$_2$O$_3$Na: 367.1059, found: 367.1062.

Ethyl 4-(4-formylphenyl)pyrrolo[1,2-*a*]quinoxaline-9-carboxylate (14 e): Pale-yellow crystals (79 %, 1.26 g): mp = 120 °C; ^{1}H NMR (CDCl$_3$): δ = 10.16 (s, 1 H, CHO), 8.21 (d, 2 H, *J* = 8.10 Hz, H-3′ and H-5′), 8.19–8.17 (m, 1 H, H-8), 8.09 (d, 2 H, *J* = 8.10 Hz, H-2′ and H-6′), 7.88 (dd, 1 H, *J* = 2.60 and 1.10 Hz, H-1), 7.74 (dd, 1 H, *J* = 7.90 Hz and 1.50 Hz, H-6), 7.52 (t, 1 H, *J* = 7.90 Hz, H-7), 7.03 (dd, 1 H, *J* = 3.90 Hz and 1.10 Hz, H-3), 6.93 (dd, 1 H, *J* = 3.90 and 2.60 Hz, H-2), 4.58 (q, 2 H, *J* = 7.20 Hz, OCH$_2$), 1.49 ppm (t, 3 H, *J* = 7.20 Hz, CH$_3$); IR (KBr): $\tilde{v}$ = 1720 (COO), 1715 cm^{-1} (CHO); HRMS-ESI *m/z* [*M*+Na]$^+$ calcd for C$_{21}$H$_{16}$N$_2$O$_3$Na: 367.1059, found: 367.1059.

Ethyl 6-(4-formylphenyl)pyrrolo[1,2-*a*]quinoxaline-4-carboxylate (19 a): Yellow crystals (80 %, 1.28 g): mp = 214 °C; ^{1}H NMR (CDCl$_3$): δ = 10.13 (s, 1 H, CHO), 8.11 (dd, 1 H, *J* = 2.70 Hz and 1.20 Hz, H-1), 8.05–8.02 (m, 4 H, H-2′, H-3′, H-5′ and H-6′), 7.99 (dd, 1 H, *J* = 8.40 Hz and 1.20 Hz, H-9), 7.73 (t, 1 H, *J* = 7.50 Hz, H-8), 7.63 (dd, 1 H, *J* = 7.80 Hz and 1.50 Hz, H-7), 7.56 (dd, 1 H, *J* = 3.90 Hz and 1.20 Hz, H-3), 7.04 (dd, 1 H, *J* = 3.90 Hz and 2.70 Hz, H-2), 4.48 (q, 2 H, *J* = 7.20 Hz, OCH$_2$), 1.47 ppm (t, 3 H, *J* = 7.20 Hz, CH$_3$); IR (KBr): $\tilde{v}$ = 1720 (COO), 1700 cm^{-1} (CHO); HRMS-ESI *m/z* [*M*+Na]$^+$ calcd for C$_{21}$H$_{16}$N$_2$O$_3$Na: 367.1059, found: 367.1056.

Ethyl 7-(4-formylphenyl)pyrrolo[1,2-*a*]quinoxaline-4-carboxylate (19 b): Yellow crystals (58 %, 926 mg): mp = 123 °C; ^{1}H NMR (CDCl$_3$): δ = 10.11 (s, 1 H, CHO), 8.49 (d, 1 H, *J* = 8.20 Hz, H-8), 8.10 (dd, 1 H, *J* = 2.70 Hz and 0.90 Hz, H-1), 8.03 (d, 2 H, *J* = 8.20 Hz, H-3′ and H-5′), 8.01 (s, 1 H, H-6), 7.97 (d, 1 H, *J* = 8.20 Hz, H-9), 7.90 (d, 2 H, *J* = 8.20 Hz, H-2′ and H-6′), 7.61 (dd, 1 H, *J* = 3.90 Hz and 0.90 Hz, H-3), 7.07 (dd, 1 H, *J* = 3.90 Hz and 2.70 Hz, H-2), 4.63 (q, 2 H, *J* = 7.20 Hz, OCH$_2$), 1.58 ppm (t, 3 H, *J* = 7.20 Hz, CH$_3$); IR (KBr): $\tilde{v}$ = 1720 (COO), 1700 cm^{-1} (CHO); HRMS-ESI *m/z* [*M*+Na]$^+$ calcd for C$_{21}$H$_{16}$N$_2$O$_3$Na: 367.1059, found: 367.1070.

General procedure for ethyl 4-{4-[(4-(2-oxo-2,3-dihydro-1*H*-benzimidazol-1-yl)piperidin-1-yl)benzyl]}phenylpyrrolo[1,2-*a*]quinoxalinecarboxylate, ethyl 4-{4-[(4-(5-fluoro-1*H*-benzimidazol-2-yl)piperidin-1-yl)benzyl]}phenylpyrrolo[1,2-*a*]quinoxalinecarboxylate, ethyl 4-{4-[4-(3-(pyridin-2-yl)-1,2,4-triazol-5-yl)piperidin-1-yl)benzyl]}phenylpyrrolo[1,2-*a*]quinoxalinecarboxylate (1 a–l), ethyl {4-[(4-(2-oxo-2,3-dihydro-1*H*-benzimidazol-1-yl)piperidin-1-yl)benzyl]}pyrrolo[1,2-*a*]quinoxaline-4-carboxylate (1 l, 1 o), ethyl 6-{4-[(4-(5-fluoro-1*H*-benzimidazol-2-yl)piperidin-1-yl)benzyl]}-pyrrolo[1,2-*a*]quinoxaline-4-carboxylate (1 m), and ethyl 6-{4-[4-(3-(pyridin-2-yl)-1,2,4-triazol-5-yl)piperidin-1-yl)benzyl]}pyrrolo[1,2-*a*]quinoxaline-4-carboxylate (1 n): The solution of the alde-hyde **14 a–e** or **19 a,b** (0.784 mmol) and 4-(2-ketobenzimidazolin-1-yl)piperidine or 4-(5-chloro-2-ketobenzimidazolin-1-yl)piperidine or 2-(3-piperidin-4-yl-1*H*-1,2,4-triazol-5-yl)pyridine (0.941 mmol) in 15 mL MeOH was adjusted to pH 6.0 by dropwise addition of acetic acid. Powered sodium cyanoborohydride (2.15 mmol) was then added, and the resulting mixture was stirred at reflux for 5 h. After removal of the MeOH 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 EtOAc/cyclohexane (1:1) and then MeOH/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 compounds **1 a–o**.

Ethyl 4-{4-[(4-(2-oxo-2,3-dihydro-1*H*-benzimidazol-1-yl)piperidin-1-yl)benzyl]}pyrrolo[1,2-*a*]quinoxaline-3-carboxylate (1 a): White crystals (77 %, 329 mg): mp: 124 °C; ^{1}H NMR (CDCl$_3$): δ = 9.87 (s, 1 H, NH), 8.12 (d, *J* = 7.80 Hz, 1 H, H-6), 8.02 (d, *J* = 2.70 Hz, 1 H, H-1), 7.96 (d, *J* = 8.10 Hz, 1 H, H-9), 7.77 (d, *J* = 7.80 Hz, 2 H, H-2′ and H-6′), 7.64–7.52 (m, 4 H, H-7, H-8, H-3′ and H-5′), 7.34–7.28 (m, 2 H, H-2 and H-4 benzimid.), 7.12–7.06 (m, 3 H, H-5 benzimid., H-6 benzimid. and H-7 benzimid.), 4.47–4.39 (m, 1 H, CH pip.), 3.79 (q, *J* = 7.20 Hz, 2 H, OCH$_2$), 3.68 (s, 2 H, CH$_2$N), 3.11 (dl, *J* = 10.80 Hz, 2 H, CH$_2$ pip.), 2.55–2.47 (m, 2 H, CH$_2$ pip.), 2.29–2.22 (m, 2 H, CH$_2$ pip.), 1.85 (dl, *J* = 11.10 Hz, 2 H, CH$_2$ pip.), 0.93 ppm (t, *J* = 7.20 Hz, 3 H, CH$_3$); ^{13}C NMR (CDCl$_3$): δ = 166.4 (C=O), 156.8 (C=O benzimid.), 155.7 (C4), 141.1 (C3a), 140.6 (C5a), 137.5 (C4′), 133.4 (C7), 131.6 (C8), 130.5 (C3′ and C5′), 130.4 (C7a benzimid.), 130.0 (C6), 129.7 (C1′), 129.4 (C2′ and C6′), 127.6 (C9), 127.4 (C3a benzimid.), 124.8 (C9a), 122.5 (C5 benzimid.), 122.4 (C6 benzimid.), 118.2 (C4 benzimid.), 116.0 (C3), 115.5 (C7 benzimid.), 115.3 (C1), 111.2 (C2), 64.0 (CH$_2$), 62.1 (CH$_2$), 54.5 (NCH$_2$ pip.), 52.1 (CH pip.), 30.6 (CH$_2$ pip.), 15.2 ppm (CH$_3$); HRMS-ESI *m/z* [*M*+Na]$^+$ calcd for C$_{33}$H$_{31}$N$_5$O$_3$Na: 568.2325, found: 568.2314.

Ethyl 4-{4-[(4-(5-fluoro-1*H*-benzimidazol-2-yl)piperidin-1-yl)benzyl]}pyrrolo[1,2-*a*]quinoxaline-3-carboxylate (1 b): Off-white crystals (50 %, 215 mg): mp: 111 °C; ^{1}H NMR ([D$_6$]DMSO): δ = 11.32 (s, 1 H, NH), 8.05 (d, *J* = 7.80 Hz, 1 H, H-6), 8.01 (d, *J* = 2.60 Hz, 1 H, H-1), 7.95 (d, *J* = 7.80 Hz, 1 H, H-9), 7.69 (d, *J* = 7.20 Hz, 2 H, H-2′ and H-6′), 7.60 (t, *J* = 7.20 Hz, 1 H, H-7), 7.52 (t, *J* = 7.20 Hz, 1 H, H-8), 7.44 (s, 1 H, H-4 benzimid.), 7.39 (d, *J* = 7.20 Hz, 2 H, H-3′ and H-5′), 7.30 (d, *J* = 2.60 Hz, 1 H, H-2), 7.20 (dc, 1 H, H-7 benzimid.), 6.94 (td, *J* = 9.00 Hz and 1.20 Hz, 1 H, H-6 benzimid.), 3.76 (q, *J* = 7.20 Hz, 2 H, OCH$_2$), 3.56 (s, 2 H, CH$_2$N), 2.95 (d, *J* = 11.80 Hz, 2 H, CH$_2$ pip.), 2.92–2.84 (m, 1 H, CH pip.), 2.09 (t, *J* = 10.80 Hz, 2 H, CH$_2$ pip.), 2.01 (d, *J* = 12.00 Hz, 2 H, CH$_2$ pip.), 1.97–1.88 (m, 2 H, CH$_2$ pip.), 0.90 ppm (t, *J* = 7.20 Hz, 3 H, CH$_3$); ^{13}C NMR ([D$_6$]DMSO): δ = 165.0 (C=O), 159.3 (d, *J* = 236 Hz, C5 benzimid.), 154.5 (C4), 139.2 (C4′), 139.1 (C1′, C2 benzimid. and C3a benzimid.), 136.0 (C3a, C5a and C7a benzimid.), 129.2 (C3′ and C5′), 128.6 (C2′ and C6′), 127.9 (C8), 126.4 (C9a), 123.4 (C3), 116.9 (C2), 114.8 (C9), 114.2 (C1), 114.0 (C7 benzimid.), 110.3 (C4 benzimid.), 110.1 (C6 benzimid.), 62.8 (CH$_2$), 60.8 (CH$_2$), 53.2 (NCH$_2$ pip.), 36.7 (CH pip.), 30.7 (CH$_2$ pip.), 13.8 ppm (CH$_3$); IR (KBr): $\tilde{v}$ = 3140 (NH), 1705 cm^{-1} (COO); HRMS-ESI *m/z* [*M*+H]$^+$ calcd for C$_{33}$H$_{30}$N$_5$O$_2$F: 548.2462, found: 548.2461.

Ethyl 4-{4-[4-(3-(pyridin-2-yl)-1,2,4-triazol-5-yl)piperidin-1-yl)benzyl]}pyrrolo[1,2-*a*]quinoxaline-3-carboxylate (1 c): White crystals (58 %, 253 mg): mp: 125 °C; ^{1}H NMR (CDCl$_3$): δ = 9.85 (s, 1 H, NH), 8.72 (d, *J* = 4.20 Hz, 1 H, H-6 pyr.), 8.19 (d, *J* = 7.80 Hz, 1 H, H-5 pyr.), 8.09–8.05 (m, 1 H, H-6), 7.96 (d, *J* = 3.00 Hz, 1 H, H-1), 7.93–7.86 (m, 1 H, H-9), 7.85–7.76 (m, 1 H, H-4 pyr.), 7.70 (d, *J* = 7.80 Hz, 2 H, H-2′ and H-6′), 7.61–7.42 (m, 4 H, H-7, H-8, H-3′ and H-5′), 7.40–7.29 (m,

1H, H-3 pyr.), 7.26 (d, J = 3.00 Hz, 1H, H-2), 3.74 (q, J = 7.00 Hz, 2H, OCH$_2$), 3.62 (s, 2H, CH$_2$N), 3.12–2.77 (m, 3H, CH$_2$ pip. and CH pip.), 2.30–1.88 (m, 6H, 3CH$_2$ pip.), 0.88 ppm (t, J = 7.00 Hz, 3H, CH$_3$); ^{13}C NMR (CDCl$_3$): δ = 165.0 (C=O), 155.6 (C2 pyr.), 154.5 (C4 and C3 triazole), 149.5 (C6 pyr.), 147.1 (C5 triazole), 139.9 (C4′), 139.2 (C1′), 137.4 (C4 pyr.), 136.2 (C3a and C5a), 130.4 (C6), 129.2 (C3′ and C5′), 128.3 (C7), 127.9 (C2′ and C6′), 126.2 (C8), 126.1 (C9a), 124.6 (C3 pyr.), 123.4 (C3), 121.8 (C5 pyr.), 116.7 (C2), 114.7 (C9), 114.0 (C1), 63.0 (CH$_2$), 60.7 (CH$_2$), 53.4 (NCH$_2$ pip.), 35.6 (CH pip.), 30.8 (CH$_2$ pip.), 13.8 ppm (CH$_3$); IR (KBr): $\tilde{\nu}$ = 3420 (NH), 1705 cm^{-1} (COO); HRMS-ESI m/z [M+Na]$^+$ calcd for C$_{33}$H$_{31}$N$_7$O$_2$Na: 580.2437, found: 580.2419.

Ethyl 4-{4-[(4-(2-oxo-2,3-dihydro-1H-benzimidazol-1-yl)piperidin-1-yl)benzyl]}pyrrolo[1,2-a]quinoxaline-6-carboxylate (1 d): Pale-yellow crystals (47%, 201 mg): mp: 125°C; ^{1}H NMR (CDCl$_3$): δ = 10.58 (s, 1H, NH), 8.13 (d, J = 7.80 Hz, 2H, H-2′ and H-6′), 8.04 (dd, J = 2.70 and 1.20 Hz, H-1), 8.01 (dd, J = 7.50 and 1.50 Hz, H-7), 7.73 (dd, J = 7.50 Hz and 1.50 Hz, 1H, H-9), 7.57 (d, J = 7.80 Hz, 2H, H-3′ and H-5′), 7.51 (t, J = 8.70 Hz, 1H, H-8), 7.36–7.31 (m, 1H, H benzimid.), 7.15 (dd, J = 3.90 Hz and 1.20 Hz, 1H, H-3), 7.14–7.04 (m, 3H, H benzimid.), 6.95 (dd, J = 3.90 Hz and 2.70 Hz, 1H, H-2), 4.54 (q, J = 7.20 Hz, 2H, OCH$_2$), 4.47–4.35 (m, 1H, CH pip.), 3.70 (s, 2H, CH$_2$N), 3.20–3.12 (m, 2H, CH$_2$ pip.), 2.61–2.58 (m, 2H, CH$_2$ pip.), 2.33–2.24 (m, 2H, CH$_2$ pip.), 1.91–1.83 (m, 2H, CH$_2$ pip.), 1.50 ppm (t, J = 7.20 Hz, 3H, CH$_3$); ^{13}C NMR (CDCl$_3$): δ = 168.1 (C=O), 155.1 (C=O benzimid.), 154.0 (C4), 134.2 (C4′), 133.1 (C1′ and C3a benzimid.), 129.2 (C3a and C5a), 129.1 (C6, C3′ and C5′), 128.0 (C7, C2′, C6′ and C7a benzimid.), 127.3 (C8), 126.5 (C9a), 125.2 (C6 benzimid.), 124.9 (C5 benzimid.), 116.1 (C9), 114.3 (C1), 109.8 (C2 and C3), 109.7 (C4 benzimid.), 109.0 (C7 benzimid.), 62.5 (CH$_2$), 61.5 (CH$_2$), 53.1 (NCH$_2$ pip.), 50.9 (CH pip.), 29.3 (CH$_2$ pip.), 14.4 ppm (CH$_3$); HRMS-ESI m/z [M+Na]$^+$ calcd for C$_{33}$H$_{31}$N$_5$O$_3$Na: 568.2325, found: 568.2321.

Ethyl 4-{4-[(4-(2-oxo-2,3-dihydro-1H-benzimidazol-1-yl)piperidin-1-yl)benzyl]}pyrrolo[1,2-a]quinoxaline-7-carboxylate (1 e): White crystals (63%, 269 mg): mp: 277°C; ^{1}H NMR (CDCl$_3$): δ = 11.90 (s, 1H,NH), 8.86–8.84 (m, 1H, H-1), 8.78 (d, J = 1.20 Hz, H-6), 8.22 (dd, J = 8.70 and 1.20 Hz, H-8), 8.07–8.03 (m, 4H, H-2′, H-3′, H-5′ and H-6′), 7.95 (d, 1H, H benzimid.), 8.45 (d, J = 8.70 Hz, 1H, H-9), 7.64–7.59 (m, 1H, H benzimid.), 7.18–7.05 (m, 4H, H-3 and H benzimid.), 6.97 (dd, J = 3.90 and 2.70 Hz, 1H, H-2), 4.44 (q, J = 7.20 Hz, 2H, OCH$_2$), 4.41–4.39 (m, 1H, CH pip.), 3.72 (s, 2H, CH$_2$N), 3.16–3.13 (m, 2H, CH$_2$ pip.), 2.56–2.28 (m, 2H, CH$_2$ pip.), 1.90–1.68 (m, 4H, 2CH$_2$ pip.), 1.45 ppm (t, J = 7.20 Hz, 3H, CH$_3$); ^{13}C NMR ([D$_6$]DMSO): δ = 165.2 (C=O), 154.2 (C=O benzimid.), 153.0 (C4), 135.1 (C4′), 134.1 (C3a benzimid.), 135.6 (C1′), 131.5 (C3a and C5a), 130.2 (C7), 129.9 (C7a benzimid.), 129.3 (3′ and C5′), 129.0 (C6), 128.9 (C2′ and C6′), 127.9 (C8), 127.8 (C9a), 124.4 (C5 benzimid. and C6 benzimid.), 117.8 (C9), 116.5 (C4 benzimid.), 115.1 (C7 benzimid.), 115.0 (C3), 110.2 (C2), 109.7 (C3), 61.9 (CH$_2$), 58.9 (CH$_2$), 51.5 (NCH$_2$ pip.), 47.2 (CH pip.), 25.9 (CH$_2$ pip.), 14.8 ppm (CH$_3$); IR (KBr): $\tilde{\nu}$ = 3180 (NH), 1700 (COO), 1680 cm^{-1} (NCON); HRMS-ESI m/z [M+Na]$^+$ calcd for C$_{33}$H$_{31}$N$_5$O$_3$Na: 568.2325, found: 568.2323.

Ethyl 4-{4-[(4-(5-fluoro-1H-benzimidazol-2-yl)piperidin-1-yl)benzyl]}pyrrolo[1,2-a]quinoxaline-7-carboxylate (1 f): White crystals (61%, 262 mg): mp: 132°C; ^{1}H NMR ([D$_6$]DMSO): δ = 11.26 (s, 1H,NH), 8.66 (d, J = 1.20 Hz, 1H, H-6), 8.48–8.44 (m, 2H, H-8 and H-1), 8.11 (dd, J = 8.20 and 1.20 Hz, H-8), 8.01 (d, J = 7.80 Hz, 2H, H-2′ and H-6′), 7.55 (d, J = 7.80 Hz, 2H, H-3′ and H-5′), 7.40–7.22 (m, 2H, 2H benzimid.), 7.15 (dd, J = 3.90 and 1.20 Hz, 1H, H-3), 7.06 (dd, J = 3.90 and 2.70 Hz, 1H, H-2), 6.98–6.92 (m, 1H, H benzimid.), 4.38 (q, J = 7.20 Hz, 2H, OCH$_2$), 3.63 (s, 2H, CH$_2$N), 3.00–2.96 (m, 2H, CH$_2$

pip.), 2.87–2.85 (m, 1H, CH pip.), 2.21–2.17 (m, 2H, CH$_2$ pip.), 2.04–2.00 (m, 2H, CH$_2$ pip.), 1.93–1.86 (m, 2H, CH$_2$ pip.), 1.38 ppm (t, J = 7.20 Hz, 3H, CH$_3$); ^{13}C NMR ([D$_6$]DMSO): δ = 166.0 (C=O), 159.4 (d, J = 236 Hz, C5 benzimid.), 154.5 (C4), 138.3 (C4′, C2 benzimid. and C3a benzimid.), 135.7 (C1′ and C7a benzimid.), 132.1 (C3a and C5a), 130.4 (C7), 130.2 (C3′ and C5′), 129.0 (C6), 128.6 (C2′ and C6′), 127.5 (C8 and C9a), 115.5 (C9), 115.0 (C1), 113.8 (C2 and C7 benzimid.), 110.7 (C3), 110.5 (C4 benzimid.), 109.6 (C6 benzimid.), 62.1 (CH$_2$), 61.3 (CH$_2$), 52.7 (NCH$_2$ pip.), 35.8 (CH pip.), 29.4 (CH$_2$ pip.), 14.4 ppm (CH$_3$); HRMS-ESI m/z [M+H]$^+$ calcd for C$_{33}$H$_{30}$N$_5$O$_2$F: 548.2462, found: 548.2455.

Ethyl 4-{4-[4-(3-(pyridin-2-yl)-1,2,4-triazol-5-yl)piperidin-1-yl)benzyl]}pyrrolo[1,2-a]quinoxaline-7-carboxylate (1 g): Pale-yellow crystals (62%, 271 mg): mp: 134°C; ^{1}H NMR (CDCl$_3$): δ = 9.85 (s, 1H, NH), 8.77–8.64 (m, 2H, H-6 and H-6 pyr.), 8.22–8.18 (m, 2H, H-1 and H-5 pyr.), 8.04–7.99 (m, 1H, H-8), 7.98 (d, J = 7.80 Hz, H-2′ and H-6′), 7.91 (d, J = 8.40 Hz, H-9), 7.86–7.83 (m, 1H, H-4 pyr.), 7.55 (d, 2H, J = 7.80 Hz, H-3′ and H-5′), 7.41–7.35 (m, 1H, H-3 pyr.), 7.08 (dd, J = 3.90 and 1.20 Hz, 1H, H-3), 6.96 (dd, J = 3.90 and 2.70 Hz, 1H, H-2), 4.46 (q, J = 7.20 Hz, 2H, OCH$_2$), 3.69 (s, 2H, CH$_2$N), 3.10–30.6 (m, 2H, 2H, CH$_2$ pip.), 2.94–2.89 (m, 1H, CH pip.), 2.29–2.01 (m, 6H, 3CH$_2$ pip.), 1.45 ppm (t, J = 7.20 Hz, 3H, CH$_3$); ^{13}C NMR (CDCl$_3$): δ = 166.0 (C=O), 156.0 (C2 pyr.), 154.9 (C4 and C3 triazole), 149.4 (C6 pyr.), 147.3 (C5 triazole), 140.2 (C4′), 137.4 (C4 pyr.), 135.7 (C1′), 132.0 (C3a and C5a), 130.1 (C7), 129.6 (C3′ and C5′), 128.5 (C6), 128.2 (C2′ and C6′), 127.3 (C8 and C9a), 124.5 (C3 pyr.), 121.8 (C5 pyr.), 115.3 (C9), 114.8 (C1), 113.7 (C2), 109.6 (C3), 62.9 (CH$_2$), 62.2 (CH$_2$), 53.4 (NCH$_2$ pip.), 35.4 (CH pip.), 30.7 (CH$_2$ pip.), 14.4 ppm (CH$_3$); HRMS-ESI m/z [M+Na]$^+$ calcd for C$_{33}$H$_{31}$N$_7$O$_2$Na: 580.2437, found: 580.2426.

Ethyl 4-{4-[(4-(2-oxo-2,3-dihydro-1H-benzimidazol-1-yl)piperidin-1-yl)benzyl]}pyrrolo[1,2-a]quinoxaline-8-carboxylate (1 h): Yellow crystals (48%, 205 mg): mp: 277°C; ^{1}H NMR (CDCl$_3$): δ = 13.40 (s, 1H, NH), 8.67–8.62 (m, 2H, H-7 and H-1), 8.20–8.00 (m, 5H, H-9, H-6, H-2′, H-6′ and H benzimid.), 7.61–7.56 (m, 2H, H-3′ and H-5′), 7.35–7.25 (m, 2H, H benzimid.), 7.18–6.95 (m, 2H, H-3 and H benzimid.), 6.96 (dd, J = 3.90 and 2.70 Hz, H-2), 4.49 (q, J = 7.20 Hz, 2H, OCH$_2$), 4.40–4.32 (m, 1H, CH pip.), 3.70 (s, 2H, CH$_2$N), 3.15–3.10 (m, 2H, CH$_2$ pip.), 2.51–2.46 (m, 2H, CH$_2$ pip.), 2.36–2.20 (m, 2H, CH$_2$ pip.), 1.95–1.81 (m, 2H, CH$_2$ pip.), 1.48 ppm (t, J = 7.20 Hz, 3H, CH$_3$); ^{13}C NMR ([D$_6$]DMSO + 2 drops of CF$_3$COOD): δ = 166.4 (C=O), 155.4 (C=O benzimid.), 154.2 (C4), 135.6 (C3a), 134.8 (C5a), 133.7 (C3′ and C5′), 131.8 (C2′ and C6′), 130.6 (C4′), 130.2 (C7a benzimid.), 130.1 (C1′), 128.8 (C9), 128.0 (C8 and C3a benzimid.), 126.5 (C6), 125.5 (C9a), 122.6 (C7), 122.0 (C5 and C6 benzimid.), 119.8 (C4 and C7 benzimid.), 119.1 (C1), 110.8 (C2), 110.2 (C3), 63.2 (CH$_2$), 60.4 (CH$_2$), 53.3 (NCH$_2$ pip.), and 48.6 (CH pip.), 27.4 ppm (CH$_2$ pip.), 15.6 (CH$_3$); HRMS-ESI m/z [M+Na]$^+$ calcd for C$_{33}$H$_{31}$N$_5$O$_3$Na: 568.2325, found: 568.2327.

Ethyl 4-{4-[(4-(2-oxo-2,3-dihydro-1H-benzimidazol-1-yl)piperidin-1-yl)benzyl]}pyrrolo[1,2-a]quinoxaline-9-carboxylate (1 i): White crystals (62%, 265 mg): mp: 151°C; ^{1}H NMR ([D$_6$]DMSO): δ = 10.89 (s, 1H, NH), 8.10 (dd, J = 7.80 and 1.50 Hz, 1H, H-8), 8.00 (d, J = 8.10 Hz, 2H, H-2′ and H-6′), 7.85–7.83 (m, 1H, H-1), 7.74 (dd, J = 8.20 and 1.20 Hz, 1H, H-6), 7.61–7.58 (m, 1H, H-8), 7.57 (d, J = 8.10 Hz, 2H, H-3′ and H-5′), 7.26–7.23 (m, 1H, H benzimid.), 7.11–7.09 (m, 1H, H-3), 7.03–6.95 (m, 4H, H-2 and 3H benzimid.), 4.51 (q, J = 6.90 Hz, 2H, OCH$_2$), 4.23–4.14 (m, 1H, CH pip.), 3.64 (s, 2H, CH$_2$N), 3.03–2.98 (m, 2H, CH$_2$ pip.), 2.42–2.34 (m, 2H, CH$_2$ pip.), 2.22–2.08 (m, 2H, CH$_2$ pip.), 1.69–1.65 (m, 2H, CH$_2$ pip.), 1.36 ppm (t, J = 6.90 Hz, 3H, CH$_3$); ^{13}C NMR (CDCl$_3$): δ = 169.0 (C=O), 155.4 (C=O benzimid.), 154.8 (C4), 140.6 (C4′), 137.5 (C1′), 136.9 (C3a ben-

zimid.), 133.1 (C3a and C5a), 129.4 (C7, C8, C9a and C7a benzimid.), 129.1 (C3′ and C5′), 128.7 (C6), 128.2 (C2′ and C6′), 126.4 (C5 benzimid.), 124.7 (C9), 121.7 (C6 benzimid.), 114.0 (C4 benzimid.), 109.8 (C7 benzimid. and C1), 109.8 (C2), 109.3 (C3), 62.7 (CH$_2$), 62.3 (CH$_2$), 53.2 (CH pip.), 50.8 (NCH$_2$ pip.), 29.3 (CH$_2$ pip.), 14.2 ppm (CH$_3$); HRMS-ESI *m/z* [M+Na]$^+$ calcd for C$_{33}$H$_{31}$N$_5$O$_3$Na: 568.2325, found: 568.2318.

Ethyl 4-{4-[(4-(5-fluoro-1*H*-benzimidazol-2-yl)piperidin-1-yl)benzyl]}pyrrolo[1,2-*a*]quinoxaline-9-carboxylate (1 j): Pale-yellow crystals (33 %, 142 mg): mp: 98 °C; ^{1}H NMR (CDCl$_3$): δ = 11.15 (s, 1 H, NH), 8.14 (dd, J = 7.80 Hz and 1.50 Hz, 1 H, H-8), 7.99 (d, J = 8.10 Hz, 2 H, H-2′ and H-6′), 7.84 (dd, J = 2.70 Hz and 1.20 Hz, 1 H, H-1), 7.69 (dd, J = 7.80 Hz and 1.50 Hz, 1 H, H-6), 7.55 (d, J = 8.10 Hz, 2 H, H-3′ and H-5′), 7.52–7.45 (m, 1 H, H benzimid.), 7.48 (t, J = 7.80 Hz, H-7), 7.28–7.25 (m, 1 H, H benzimid.), 7.05 (dd, J = 4.20 Hz and 1.20 Hz, 1 H, H-3), 7.00–6.96 (m, 1 H, H benzimid.), 6.90 (dd, J = 4.20 Hz and 2.70 Hz, 1 H, H-2), 4.58 (q, J = 7.00 Hz, 2 H, OCH$_2$), 3.71 (s, 2 H, CH$_2$N), 3.11–2.98 (m, 2 H, CH$_2$ pip.), 3.02–2.85 (m, 1 H, CH pip.), 2.25–1.94 (m, 6 H, 3 CH$_2$ pip.), 1.48 ppm (t, J = 7.00 Hz, 3 H, CH$_3$); ^{13}C NMR (CDCl$_3$): δ = 169.0 (C=O), 159.3 (d, J = 236 Hz, C5 benzimid.), 155.0 (C4), 140.3 (C4′), 137.3 (C2 benzimid. and C3a benzimid.), 138.0 (C1′), 136.4 (C2 benzimid. and C3a benzimid.), 136.7 (C1′), 132.6 (C8, C3a and C5a), 131.0 (C9a, C3′ and C5′), 129.4 (C6), 128.9 (C7), 128.5 (C2′ and C6′), 126.3 (C7a benzimid.), 124.7 (C9), 121.9 (C1), 120.0 (C4 benzimid.), 114.2 (H-2 and C7 benzimid.), 110.2 (C6 benzimid.), 109.6 (C3), 64.7 (CH$_2$), 62.4 (CH$_2$), 53.2 (NCH$_2$ pip.), 36.8 (CH pip.), 30.8 ppm (CH$_2$ pip.), 14.2 (CH$_3$); HRMS-ESI *m/z* [M+H]$^+$ calcd for C$_{33}$H$_{30}$N$_5$O$_2$F: 548.2462, found: 548.2469.

Ethyl 4-{4-[4-(3-(pyridin-2-yl)-1,2,4-triazol-5-yl)piperidin-1-yl)benzyl]}pyrrolo[1,2-*a*]quinoxaline-9-carboxylate (1 k): Pale-yellow crystals (41 %, 179 mg): mp: 87 °C; ^{1}H NMR (CDCl$_3$): δ = 9.75 (s, 1 H, NH), 8.71 (d, J = 4.50 Hz, 1 H, H-6 pyr.), 8.20 (d, J = 8.10 Hz, 1 H, H-8), 8.16 (dd, J = 8.10 Hz and 1.50 Hz, 1 H, H-5 pyr.), 7.99 (d, J = 8.10 Hz, 2 H, H-2′ and H-6′), 7.87 (dd, J = 7.80 Hz and 1.50 Hz, 1 H, H-4 pyr.), 7.82 (dd, J = 3.00 and 1.20 Hz, 1 H, H-1), 7.68 (dd, J = 7.50 Hz and 1.50 Hz, 1 H, H-6), 7.57 (d, J = 8.10 Hz, 2 H, H-3′ and H-5′), 7.48 (t, J = 7.80 Hz, 1 H, H-7), 7.39 (dd, J = 7.50 Hz and 5.10 Hz, 1 H, H-3 pyr.), 7.05 (dd, J = 3.90 Hz and 1.20 Hz, 1 H, H-3), 6.89 (dd, J = 3.90 Hz and 3.00 Hz, 1 H, H-2), 4.58 (q, J = 7.20 Hz, 2 H, OCH$_2$), 3.71 (s, 2 H, CH$_2$N), 3.11–3.07 (m, 2 H, CH$_2$ pip.), 2.98–2.90 (m, 1 H, CH pip.), 2.32–1.99 (m, 6 H, 3 CH$_2$ pip.), 1.49 ppm (t, J = 7.20 Hz, 3 H, CH$_3$); ^{13}C NMR (CDCl$_3$): δ = 169.0 (C=O), 156.0 (C2 pyr.), 155.9 (C4), 154.9 (C3 triazole), 149.5 (C6 pyr.), 147.3 (C5 triazole), 140.3 (C4′), 137.4 (C4 pyr.), 136.8 (C1′), 133.0 (C8, C3a and C5a), 129.5 (C6), 128.9 (C7), 128.6 (C2′ and C6′), 124.7 (C9), 124.5 (C3 pyr.), 121.8 (C5 pyr.), 121.7 (C1), 114.1 (C2), 109.3 (C3), 62.9 (CH$_2$), 62.3 (CH$_2$), 53.4 (NCH$_2$ pip.), 35.4 (CH pip.), 30.7 (CH$_2$ pip.), 14.2 ppm (CH$_3$); HRMS-ESI *m/z* [M+Na]$^+$ calcd for C$_{33}$H$_{31}$N$_7$O$_2$Na: 580.2437, found: 580.2421.

Ethyl 6-{4-[(4-(2-oxo-2,3-dihydro-1*H*-benzimidazol-1-yl)piperidin-1-yl)benzyl]}pyrrolo[1,2-*a*]quinoxaline-4-carboxylate (1 l): Yellow crystals (70 %, 299 mg): mp: 180 °C; ^{1}H NMR (CDCl$_3$): δ = 8.63 (s, 1 H, NH), 8.08 (dd, J = 2.70 and 1.20 Hz, 1 H, H-1), 7.93 (dd, J = 8.10 and 1.30 Hz, 1 H, H-9), 7.87 (d, J = 8.10 Hz, 2 H, H-2′ and H-6′), 7.71 (t, J = 8.10 Hz, 1 H, H-8), 7.65 (d, J = 7.20 Hz, 1 H, H benzimid.), 7.58–7.51 (m, 3 H, H-7, H-3′ and H-5′), 7.39–7.34 (m, 1 H, H benzimid.), 7.19–7.14 (m, 1 H, H benzimid.), 7.14–7.07 (m, 2 H, H-3 and H benzimid.), 7.04 (dd, J = 3.90 and 2.70 Hz, 1 H, H-2), 4.49 (q, J = 7.20 Hz, 2 H, OCH$_2$), 4.45–4.41 (m, 1 H, CH pip.), 3.68 (s, 2 H, CH$_2$N), 3.20–3.16 (m, 2 H, CH$_2$ pip.), 2.67–2.50 (m, 2 H, CH$_2$ pip.), 2.29 (m, 2 H, CH$_2$ pip.), 1.95–1.84 (m, 2 H, CH$_2$ pip.), 1.50 ppm (t, J = 7.20 Hz, 3 H, CH$_3$); ^{13}C NMR (CDCl$_3$): δ = 165.2 (C=O), 156.1 (C=O benzimid.), 143.4 (C4), 143.2 (C3a), 138.9 (C5a), 138.2 (C1′ and C4′), 133.2 (C6 and

C3a benzimid.), 132.3 (C2′ and C6′), 130.5 (C8), 130.2 (C9a), 129.5 (C7a benzimid.), 129.4 (C3′ and C5′), 127.6 (C7), 122.1 (C5 benzimid.), 122.0 (C6 benzimid.), 116.0 (C9), 113.9 (C4 benzimid.), 110.8 (C7 benzimid. and C1), 110.7 (C2), 110.1 (C3), 63.8 (CH$_2$), 62.8 (CH$_2$), 54.2 (NCH$_2$ pip.), 51.9 (CH pip.), 30.3 (CH$_2$ pip.), 15.1 ppm (CH$_3$); HRMS-ESI *m/z* [M+Na]$^+$ calcd for C$_{33}$H$_{31}$N$_5$O$_3$Na: 568.2325, found: 568.2308.

Ethyl 6-{4-[(4-(5-fluoro-1*H*-benzimidazol-2-yl)piperidin-1-yl)benzyl]}pyrrolo[1,2-*a*]quinoxaline-4-carboxylate (1 m): Yellow crystals (63 %, 270 mg): mp: 192 °C; ^{1}H NMR (CDCl$_3$): δ = 9.55 (s, 1 H, NH), 8.08 (dd, J = 2.70 Hz and 1.20 Hz, 1 H, H-1), 7.93 (dd, J = 8.40 Hz and 1.20 Hz, 1 H, H-9), 7.82 (d, J = 8.10 Hz, 2 H, H-2′ and H-6′), 7.69 (t, J = 8.40 Hz, 1 H, H-8), 7.61 (dd, J = 8.40 and 1.20 Hz, 1 H, H-7), 7.53 (dd, J = 4.20 and 1.20 Hz, H-3), 7.46 (d, 2 H, J = 8.10 Hz, H-3′ and H-5′), 7.50–7.47 (m, 1 H, H benzimid.), 7.28–7.24 (m, 1 H, H benzimid.), 7.02 (dd, J = 4.20 and 2.70 Hz, H-2), 7.00–6.93 (m, 1 H, H benzimid.), 4.49 (q, J = 7.20 Hz, 2 H, OCH$_2$), 3.68 (s, 2 H, CH$_2$N), 3.34–3.20 (m, 2 H, CH$_2$ pip.), 3.05–2.94 (m, 1 H, CH pip.), 2.31–2.22 (m, 2 H, CH$_2$ pip.), 2.19–1.99 (m, 4 H, 2 CH$_2$ pip.), 1.48 ppm (t, J = 7.20 Hz, 3 H, CH$_3$); ^{13}C NMR (CDCl$_3$): δ = 165.3 (C=O), 160.4 (d, J = 235 Hz, C5 benzimid.), 143.33 (C4), 143.31 (C3a), 138.4 (C5a, C2 benzimid. and C3a benzimid.), 138.11 (C4′), 138.07 (C1′), 134.3 (C7a benzimid.), 133.2 (C6), 132.3 (C2′ and C6′), 130.5 (C8, C3′ and C5′), 129.6 (C9a), 127.7 (C7), 116.01 (C1), 115.98 (C4 benzimid.), 113.9 (C9), 110.1 (C2, C3, C6 benzimid. and C7 benzimid.), 63.9 (CH$_2$), 62.8 (CH$_2$), 54.2 (NCH$_2$ pip.), 37.7 (CH pip.), 31.7 (CH$_2$ pip.), 15.2 ppm (CH$_3$); IR (KBr): $\tilde{v}$ = 3140 (NH), 1715 cm^{-1} (COO); HRMS-ESI *m/z* [M+H]$^+$ calcd for C$_{33}$H$_{31}$N$_5$O$_2$F: 548.2462, found: 548.2448.

Ethyl 6-{4-[4-(3-(pyridin-2-yl)-1,2,4-triazol-5-yl)piperidin-1-yl)benzyl]}pyrrolo[1,2-*a*]quinoxaline-4-carboxylate (1 n): Yellow crystals (70 %, 306 mg): mp: 187 °C; ^{1}H NMR ([D$_6$]DMSO): δ = 8.75–8.60 (m, 2 H, NH and H-6 pyr.), 8.37 (dd, J = 8.40 Hz and 1.20 Hz, 1 H, H-5 pyr.), 8.05 (d, J = 7.50 Hz, 1 H, H-9), 7.95–7.93 (m, 1 H, H-1), 7.79 (t, J = 7.80 Hz, 1 H, H-8), 7.73 (d, J = 7.80 Hz, 2 H, H-2′ and H-6′), 7.65–7.61 (m, 2 H, H-7 and H-4 pyr.), 7.45–7.42 (m, 3 H, H-3′, H-5′ and H-3 pyr.), 7.36 (dd, J = 4.00 Hz and 1.20 Hz, 1 H, H-3), 7.07 (dd, J = 4.00 and 2.70 Hz, 1 H, H-2), 4.38 (q, J = 6.90, 2 H, OCH$_2$), 3.59 (s, 2 H, CH$_2$N), 3.08–2.94 (m, 2 H, CH$_2$ pip.), 2.87–2.75 (m, 1 H, CH pip.), 2.25–2.15 (m, 2 H, CH$_2$ pip.), 2.06–1.95 (m, 2 H, CH$_2$ pip.), 1.90–1.71 (m, 2 H, CH$_2$ pip.), 1.35 ppm (t, J = 6.90 Hz, 3 H, CH$_3$); ^{13}C NMR ([D$_6$]DMSO): δ = 164.1 (C=O), 154.5 (C3 triazole and C2 pyr.), 150.0 (C6 pyr.), 147.2 (C5 triazole), 142.5 (C4), 141.8 (C3a), 138.1 (C5a, C4′ and C4 pyr.), 137.5 (C1′), 131.8 (C6), 131.3 (C2′ and C6′), 130.4 (C8, C9a, C3′ and C5′), 128.7 (C7), 123.9 (C3 pyr.), 121.8 (C5 pyr.), 115.6 (C1), 114.7 (C9), 109.1 (C2 and C3), 62.5 (CH$_2$), 62.0 (CH$_2$), 57.0 (NCH$_2$ pip.), 53.3 (CH pip.), 30.9 (CH$_2$ pip.), 14.4 ppm (CH$_3$); HRMS-ESI *m/z* [M+Na]$^+$ calcd for C$_{33}$H$_{31}$N$_7$O$_2$Na: 580.2437, found: 580.2413.

Ethyl 7-{4-[(4-(2-oxo-2,3-dihydro-1*H*-benzimidazol-1-yl)piperidin-1-yl)benzyl]}pyrrolo[1,2-*a*]quinoxaline-4-carboxylate (1 o): White crystals (48 %, 205 mg): mp: 138 °C; ^{1}H NMR ([D$_6$]DMSO): δ = 10.86 (s, 1 H, NH), 8.67 (dd, J = 2.70 and 1.20 Hz, 1 H, H-1), 8.48 (d, J = 8.70 Hz, 1 H, H-9), 8.31 (d, J = 1.80 Hz, 1 H, H-6), 8.02 (dd, J = 8.40 Hz and 2.10 Hz, 1 H, H-8), 7.86 (d, J = 8.10 Hz, 2 H, H-2′ and H-6′), 7.49 (d, J = 8.10 Hz, 2 H, H-3′ and H-5′), 7.41 (dd, J = 3.90 and 1.20 Hz, 1 H, H-3), 7.26–7.24 (m, 1 H, H benzimid.), 7.10–7.08 (dd, J = 3.90 and 2.70 Hz, 1 H, H-2), 7.04–6.95 (m, 3 H, 3 H benzimid.), 4.48 (q, J = 6.70 Hz, 2 H, OCH$_2$), 4.20–4.15 (m, 1 H, CH pip.), 3.61 (s, 2 H, CH$_2$N), 3.08–2.95 (m, 2 H, CH$_2$ pip.), 2.42–2.27 (m, 2 H, CH$_2$ pip.), 2.18–2.15 (m, 2 H, CH$_2$ pip.), 1.75–1.60 (m, 2 H, CH$_2$ pip.), 1.41 ppm (t, J = 6.70 Hz, 3 H, CH$_3$); ^{13}C NMR ([D$_6$]DMSO): δ = 163.2 (C=O), 153.3 (C=O benzimid.), 142.8 (C3a and C4), 137.0 (C5a), 136.9 (C1′ and C4′),

134.1 (C7 and C3a benzimid.), 129.2 (C2′, C6′ and C7a benzimid.), 128.7 (C6, C9a, C3′ and C5′), 128.1 (C8), 123.1 (C5 benzimid. and C6 benzimid.), 116.6 (C1), 114.7 (C9), 108.9 (C2 and C3), 108.5 (C7 benzimid.), 108.4 (C4 benzimid.), 61.4 (CH_2), 61.0 (CH_2), 52.2 (NCH_2 pip.), 49.6 (CH pip.), 28.1 (CH_2 pip.), 13.7 ppm (CH_3); HRMS-ESI m/z $[M+Na]^+$ calcd for $C_{33}H_{31}N_5O_3Na$: 568.2325, found: 568.2314.

1-(3-Chloro-2-nitrophenyl)pyrrole (15a): A mixture of commercial 3-chloro-2-nitroaniline (30 mmol) and 2,5-dimethoxytetrahydrofuran (33 mmol) in acetic acid (65 mL) was stirred at reflux for 10 min by microwave with vigorous stirring. The irradiation was programmed to maintain a constant temperature (120 °C) with a maximal output power of 200 W. After cooling, the reaction mixture was poured into water at 0 °C. The mixture was then extracted twice with diethyl ether. The organic layer was dried over Na_2SO_4 and filtered. The solvent was removed under reduced pressure, and the crude product was purified by flash chromatography (eluent: cyclohexane/EtOAc, 7:3) to give **15a** as off-white crystals (86%, 5.73 g): mp: 61 °C; ^{1}H NMR (CDCl$_3$): $\delta=7.53$ (d, 1H, $J=7.95$ Hz, H-4), 7.51 (d, 1H, $J=7.95$ Hz, H-6), 7.40 (t, 1H, $J=7.95$ Hz, H-5), 6.84 (dd, 2H, $J=1.95$ and 1.95 Hz, H-α), 6.37 ppm (dd, 2H, $J=1.95$ and 1.95 Hz, H-β). Anal. calcd for $C_{10}H_7ClN_2O_2$: C 53.95, H 3.17, N 12.58, found: C 54.06, H 3.32, N 12.68.

1-(2-Amino-3-chlorophenyl)pyrrole (16a): A 2 M aqueous solution of $CuSO_4$ (19 mL) was added to a solution of 1-(3-chloro-2-nitrophenyl)pyrrole (**15a**) (28 mmol) in EtOH (120 mL). Sodium borohydride (138 mmol) was added portionwise at 0 °C to the reaction mixture, which was then stirred at room temperature for 1 h. The reaction mixture was then diluted with EtOAc and filtered. The organic layer was separated, dried over Na_2SO_4, and evaporated to dryness under reduced pressure to give **16a** after purification by flash chromatography (eluent: CH_2Cl_2/MeOH, 9:1) as a yellow oil (74%, 3.98 g): mp: 79 °C; ^{1}H NMR (CDCl$_3$): $\delta=7.31$ (d, 1H, $J=7.80$ Hz, H-4), 7.11 (d, 1H, $J=7.80$ Hz, H-6), 6.86 (dd, 2H, $J=1.95$ and 1.95 Hz, H-α), 6.75 (t, 1H, $J=7.80$ Hz, H-5), 6.40 (dd, 2H, $J=1.95$ and 1.95 Hz, H-β), 4.16 ppm (s, 2H, NH_2); IR (KBr): $\tilde{\nu}=3455$, 3375 cm^{-1} (NH_2); Anal. calcd for $C_{10}H_9ClN_2$: C 62.35, H 4.71, N 14.54, found: C 62.23, H 5.02, 14.57.

Ethyl 2-[(6-chloro-2-pyrrolo-1-ylphenyl)amino]-2-oxo-acetate (17a): Et$_3$N (17 mmol) was added to a solution containing 1-(2-amino-4-bromophenyl)pyrrole (**16a**) (17 mmol) in 140 mL of THF at 0 °C, followed by dropwise addition of ethyl oxalyl chloride (17 mmol) over 15 min. The reaction mixture was warmed to room temperature and stirred for 14 h. The reaction mixture was filtered, and the filter cake was washed with THF, then with EtOAc. The organic phase was washed twice with 25 mL of a 1 M HCl aqueous solution, dried over sodium sulfate, filtered, and concentrated. The residue was cooled, triturated with diethyl ether, and decanted, and the crude product was purified by flash chromatography (eluent: cyclohexane/EtOAc, 8:2) to give **17a** as a yellow oil (94%, 4.67 g): ^{1}H NMR (CDCl$_3$): $\delta=8.45$ (s, 1H, NH), 7.53–7.27 (m, 2H, H-4 and H-6), 6.84–6.73 (m, 3H, H-5 and H-α), 6.31 (dd, 2H, $J=1.95$ and 1.95 Hz, H-β), 4.39–4.27 (m, 2H, CH_2), 1.43–1.30 ppm (m, 3H, CH_3); IR (KBr): $\tilde{\nu}=3280$ (NH), 1690 and 1680 cm^{-1} (CO); Anal. calcd for $C_{14}H_{13}ClN_2O_3$: C 57.45, H 4.48, N 9.57, found: C 57.25, H 4.66, N 9.74.

Ethyl 6-chloropyrrolo[1,2-a]quinoxaline-4-carboxylate (18a): A solution of **17a** (7 mmol) in POCl$_3$ (10 mL) was stirred at reflux for 20 min. After removing excess reactants under vacuum, the residue was carefully dissolved in water at 0 °C, and the resulting solution was basified with ammonium hydroxide. The precipitate was filtered, washed with diethyl ether, and dried to give **18a**, which was

purified by flash chromatography (eluent: CH_2Cl_2) as yellow crystals (57%, 1.09 g): mp: 110 °C; ^{1}H NMR (CDCl$_3$): $\delta=8.03$ (dd, 1H, $J=2.70$ and 1.20 Hz, H-1), 7.81 (d, 1H, $J=7.80$ Hz, H-9), 7.60–7.49 (m, 3H, H-3, H-7 and H-8), 7.02 (dd, 1H, $J=3.90$ and 2.70 Hz, H-2), 4.58 (q, 2H, $J=7.20$ Hz, CH_2), 1.54 ppm (t, 3H, $J=7.20$ Hz, CH_3); IR (KBr): $\tilde{\nu}=1735$ cm^{-1} (COO); Anal. calcd for $C_{14}H_{11}ClN_2O_2$: C 61.21, H 4.04, N 10.20, found: C 61.14, H 3.89, N 10.04.

X-ray crystallography data

The structure of compound **1c** was established by X-ray crystallography (Figure 2). A colorless single crystal of **1c** was obtained by slow evaporation from a MeOH/CHCl$_3$ solution (20:80, v/v): triclinic, space group $P1$, $a=10.5656(7)$ Å, $b=14.2740(10)$ Å, $c=22.6078(14)$ Å, $\alpha=83.453(4)°$, $\beta=89.521(4)°$, $\gamma=81.077(5)°$, $V=3346.2(4)$ Å^3, $Z=4$, δ(calcd)$=1.304$ Mg m^{-3}, FW$=656.74$ for $C_{33}H_{31}N_7O_2,5.5 H_2O$, $F(000)=1396$.[35] The data were corrected for Lorentz and polarization effects and for empirical absorption correction.[36] The structure was solved by direct methods using ShelX 2013[37] and refined using the ShelX 2013[37] suite of programs.

Biology

Cell culture: The human U937, K562, HL60, U266, and Jurkat leukemia cell lines were grown in RPMI 1640 medium (Life Technology, France), supplemented with 10% fetal calf serum (FCS), antibiotics (100 U mL$^-$ penicillin, 100 µg mL^{-1} streptomycin) and L-glutamine, (Eurobio, France) at 37 °C and 5% CO$_2$ in air. The toxicities of various molecules were also evaluated on non-activated, freshly isolated normal human PBMCs, as well as phytohemagglutinin (T lymphoproliferative agent; PHA)-induced cells. PBMCs from blood of healthy volunteers were obtained following centrifugation on Ficoll gradient. Cells were then incubated in medium alone or induced to enter the cell cycle by the addition of PHA (5 µg mL^{-1}, Murex Biotech Ltd., Dartford, UK).

Cytotoxicity evaluation: The MTS cell proliferation assay (Promega, France) is a colorimetric assay system that measures the reduction of a tetrazolium component (MTS) into formazan, produced by the mitochondria of viable cells. Cells were washed twice in phosphate-buffered saline (PBS) and plated in quadruplicate into microtiter plate wells in 100 µL culture media with or without various test compounds at increasing concentrations (0, 1, 5, 10, 20, and 50 µM) for 1, 2, and three days. After 3 h of incubation at 37 °C with 20 µL MTS per well, the plates were read using an ELISA microplate reader (Thermo Fisher Scientific) at λ 490 nm. 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 three-day incubation period. The 50% cytotoxic concentrations (CC$_{50}$) were determined by linear regression analysis, expressed as µM ± SD (Microsoft Excel).

Annexin V staining by flow cytometry: Cells (10^5) were incubated for three days with increasing doses of **1a** (0, 1, 5, and 10 µM diluted with DMSO). Experiments were performed with APC–Annexin V (Biolegend, CA), according to the manufacturer's instructions. Briefly, cells (2×10^4) were incubated with APC–Annexin V (5 µL), resuspended in 1× binding buffer (295 µL) for 10 min at room temperature in the dark. Cells were then analyzed by flow cytometry. The APC–Annexin V-positive cells were considered apoptotic. Flow cytometry analysis was performed with a BD Accuri (Becton-Dickinson, France), and experiments were analyzed using the BD Accuri C6 software.

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