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Short communication

Synthesis and evaluation of the cytotoxic activity of novel ethyl 4-[4-(4-substitutedpiperidin-1-yl)]benzyl-phenylpyrrolo[1,2-*a*]quinoxaline-carboxylate derivatives in myeloid and lymphoid leukemia cell lines

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ABSTRACT

Leukemia is the most common blood cancer, and its development starts at diverse points, leading to distinct subtypes that respond differently to therapy. This heterogeneity is rarely taken into account in therapies, so it is still essential to look for new specific drugs for leukemia subtypes or even for therapy-resistant cases. Among heterocyclic compounds that attracted a lot of attention because of its wide spread biological activities, the pyrrolo[1,2-*a*]quinoxaline heterocyclic framework has been identified as interesting scaffolds for antiproliferative activity against various human cancer cell lines. In the present study, novel ethyl 4-[4-(4-substitutedpiperidin-1-yl)]benzyl-phenylpyrrolo[1,2-*a*]quinoxaline-carboxylate derivatives **1a-l** have been designed and synthesized. Their cytotoxicities were evaluated against five different leukemia cell lines, including Jurkat and U266 (lymphoid cell lines), and K562, U937, HL60 (myeloid cell lines), as well as normal human peripheral blood mononuclear cells (PBMNCs). Then, apoptosis study was performed with the more interesting compounds. The new pyrrolo[1,2-*a*]quinoxaline series showed promising cytotoxic potential against all leukemia cell lines tested, and some compounds showed better results than the reference compound A6730. Some compounds, such as **1a**, **1e**, **1g** and **1h** are promising because of their high activity against leukemia and their low activity against normal hematopoietic cells. Structure-activity relationships of these new synthetic compounds **1a-l** are here also discussed.

1. Introduction

Acute leukemia is one of the most aggressive hematopoietic malignancies and is characterized by the abnormal proliferation of the immature cells and a premature block in lymphoid or myeloid differentiation. Adult acute leukemia have a poor prognosis due to a

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large number of relapses. Thus, identifying and understanding the treatment-related resistance mechanisms is of major interest to improve the therapeutic strategy [1]. Therefore, there is an urgent need to find new therapeutics, which could led to the development of novel treatment strategies with less or minimal side effects.

Heterocyclic compounds attracted a lot of attention because of its wide spread biological activities. Among them, the pyrrolo[1,2-*a*]quinoxaline heterocyclic framework constitutes the basis of an important class of compounds possessing interesting biological activities. These compounds have been reported to serve as key intermediates for the assembly of several heterocycles including antipsychotic agents [2], anti-HIV agents [3], adenosine A_3 receptor modulators [4], antiparasitic agents [5–10], and antitumor agents [11–13]. In this last field, the discovery and development of novel therapeutic agents are one of the most important goals in medicinal chemistry. In this context, we have recently published three series (Series **A-C**) of new interesting substituted pyrrolo[1,2-*a*]quinoxalines (Fig. 1) endowed with good activity towards the human leukemia cells [14–16]. These antiproliferative pyrrolo[1,2-*a*]quinoxaline derivatives have been previously designed as novel structural analogues of compound A6730, a well-described Akt inhibitor that presents antiproliferative activity against different human leukemia cell lines [14–17]. Continuing our efforts in this field and considering the pharmacological activities of pyrroloquinoxalines on human leukemic cells, a new series (Series **D**) was designed and synthesized. Thus, by taking into accounts the best results obtained in series **B** (Fig. 1), we decided to use the JG576 and JG572 pyrrolo[1,2-*a*]quinoxaline moieties as a template for the design of new derivatives **1a-l** in which the pyrrole nucleus is substituted in different positions by a phenyl and an ester function (Series **D**, Fig. 1). In relation to our previous works, further pharmacomodulations on the piperidine core have been considered, such as the introduction of new substituted heterocyclic systems [14–16]. The antiproliferative profile of the obtained derivatives **1a-l** was then evaluated *in vitro* against a panel of myeloid (U937, HL60, K562) or lymphoid (Jurkat, U266) leukemic cell lines. Moreover, to determine their respective cytotoxicity, the new ethyl 4-[4-(4-substitutedpiperidin-1-yl)]benzyl-phenylpyrrolo[1,2-*a*]quinoxaline-carboxylate derivatives **1a-l** were tested on activated human peripheral blood mononuclear cells, and assessment of apoptosis was performed with the more interesting compounds. Structure-activity relationships of these new synthetic compounds **1a-l** are here discussed. Finally, we used simple computational programs to predict the drug-like characteristics through the calculated

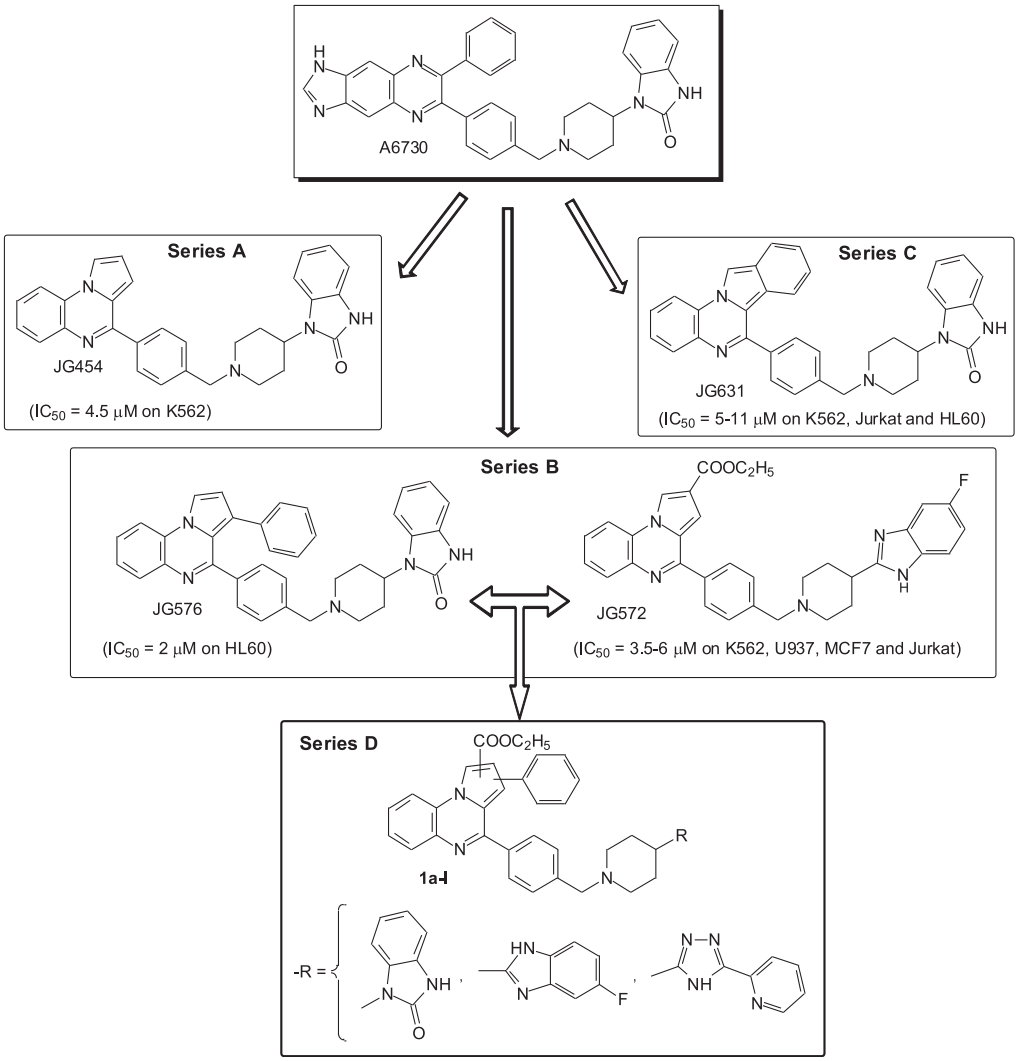
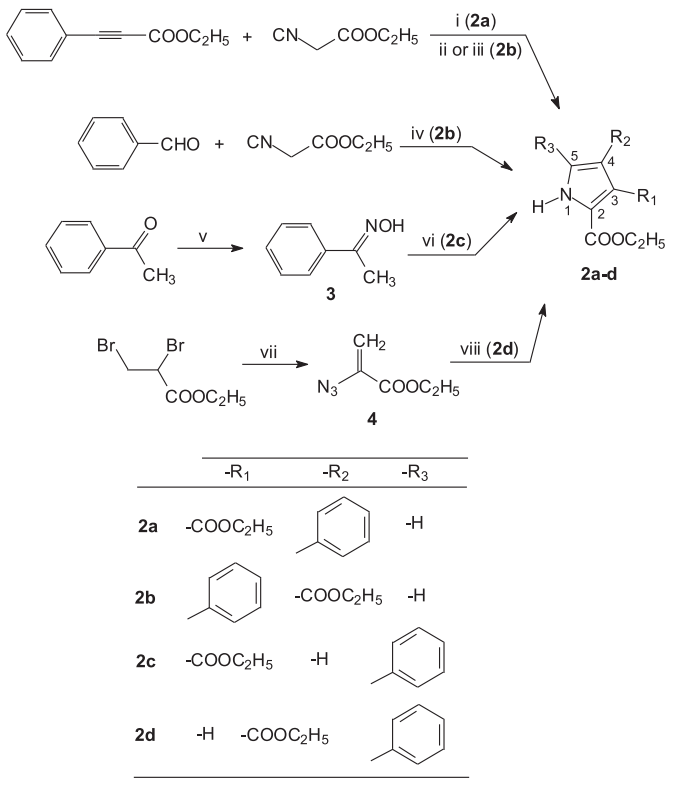


Fig. 1. Structure of bioactive compounds of previously described series A-C, and general structure of new synthesized substituted pyrrolo[1,2-*a*]quinoxaline derivatives **1a-l** (series D).

physicochemical and toxicological properties of these new ethyl 4-[4-(4-substitutedpiperidin-1-yl)]benzyl-phenylpyrrolo[1,2-*a*]quinoxaline-carboxylate derivatives to determine their potential anti-leukemia activity.

2. Chemistry

All reported pyrrolo[1,2-*a*]quinoxaline derivatives **1a-l** were synthesized from various substituted phenyl-1*H*-pyrrole-dicarboxylic acid ethyl ester **2a-d** (Schemes 1 and 2). Different strategies using classical or microwaves heating were considered for the synthesis of the phenyl-1*H*-pyrrole-diesters **2a-d** in order to introduce the phenyl and ester functions on the pyrrole ring (Table 1). The synthesis of the diethyl 4-phenyl-1*H*-pyrrole-2,3-dicarboxylate **2a** has been accomplished by treatment of ethyl isocyanide on ethyl phenylpropiolate under 1,3-bis(diphenylphosphino)propane (dppp) catalysis via a formal [3 + 2] cyclo-addition (Scheme 1, Table 1) [18,19]. Various attempts were investigated for the preparation of the diethyl 3-phenyl-1*H*-pyrrole-2,4-dicarboxylate **2b**. At first, this pyrrole **2b** was prepared by silver-catalyzed cycloaddition of commercially available ethyl phenylpropiolate with ethyl isocyanoacetate in 1,4-dioxane or DMF at 80 °C (Scheme 1, Methods A-C, Table 1) [20,21]. The very low yield obtained (12%, Table 1) using microwaves heating led us to investigate other methodologies. The copper-catalyzed reaction of ethyl isocyanide with the electron-deficient alkyne, ethyl phenylpropiolate, gave the diethyl 3-phenyl-1*H*-pyrrole-2,4-dicarboxylate **2b** with 62% in dioxane at 100 °C using microwave heating (Table 1)



Scheme 1. Synthesis of phenyl-1*H*-pyrrole-diesters **2a-d**; Reagents and conditions: (i) dppp, dioxane, 100 °C; (ii) Method A: Ag_2CO_3, dioxane, 80 °C; Method B: 1) Ag_2CO_3, dioxane, 25 °C; 2) 80 °C; Method C: 1) Ag_2CO_3, NMP, 25 °C; 2) 80 °C; (iii) Method D: Cu_2O, phénanthroline, dioxane, 100 °C; (iv) Method E: DBU, THF, 50 °C; (v) H_2NOH, HCl, Pyridine, EtOH, reflux; (vi) 1) $H_5C_2OOC{-}C{\equiv}C{-}COOC_2H_5$, DABCO, toluene, 80 °C; 2) 170 °C, P; (vii) NaN_3, DMF, 65 °C; (viii) $C_6H_5{-}CO{-}CH_2{-}COOC_2H_5$, $Mn(OAc)_3$&·$2H_2O$, 40 °C.

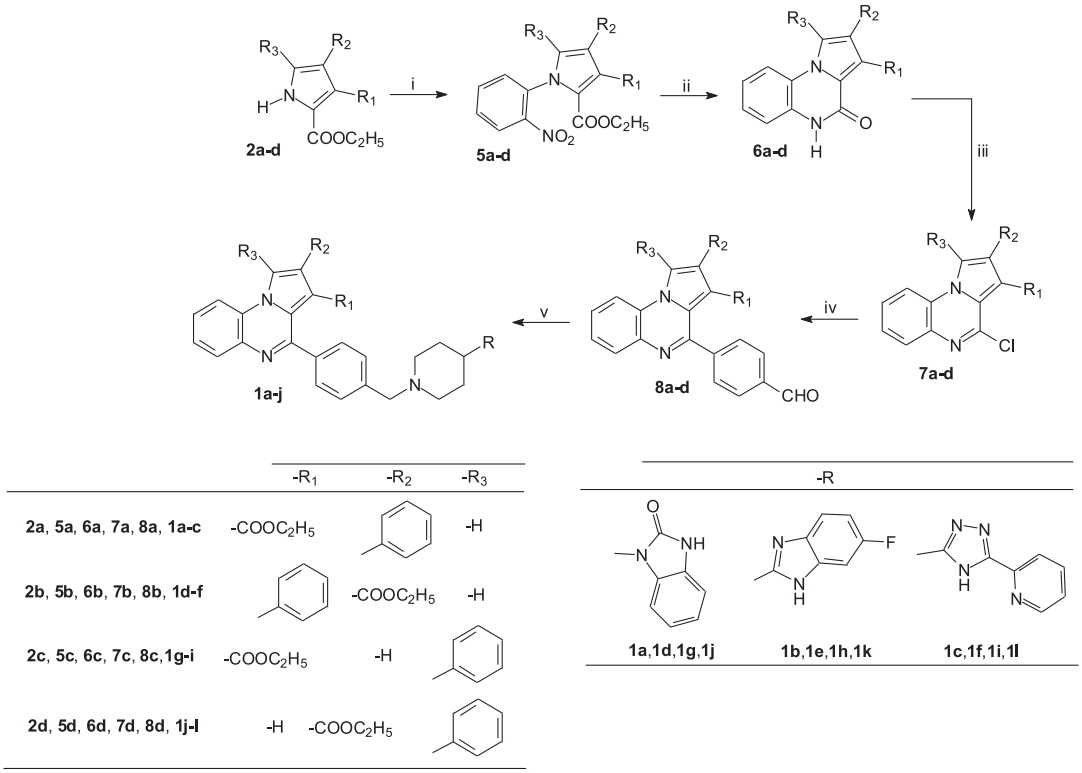
[1,2]. Pyrrole **2b** could be also synthesized by reaction of ethyl isocyanoacetate with benzaldehyde in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in THF [22,23]. The synthesis of the diethyl 5-phenyl-1*H*-pyrrole-2,3-dicarboxylate **2c** has been accomplished in two steps starting from commercially available acetophenone via its oxime **3** (Scheme 1). The acetophenone oxime **3**, synthesized by the reaction of hydroxylamine on acetophenone [7], was then reacted with diethyl acetylene in the presence of 1,4-diazabicyclo[2.2.2]octane (DABCO) to form pyrrole **2c** via a thermal rearrangement by Trofimov reaction [24,25]. Diethyl 5-phenyl-1*H*-pyrrole-2,4-dicarboxylate **2d** was prepared by manganese(III)-catalyzed formal [3 + 2] annulation of ethyl 2-azidoacrylate **4** and ethyl 3-oxo-3-phenylpropanoate [26]. The 2-azidoacrylate **4** was previously synthesized by treatment of ethyl 2,3-dibromopropanoate with three equivalents of sodium azide (NaN_3) in aprotic polar solvent such as dimethylformamide (DMF) [10,11]. A X-ray single crystal analysis was also performed on phenyl-1*H*-pyrrole-diesters **2b-d** in order to confirm the structures (Fig. 2). The preparation of *N*-aryl pyrroles **5a-d** were obtained by nucleophilic substitution of the various pyrrole-2-carboxylates **2a-d** with 2-fluoro-nitrobenzene using cesium carbonate as the base in refluxing DMF solution (Scheme 2) [15,16]. The preparation of **5a-d** was also performed under microwave irradiation. Reduction of the nitro moiety with iron in hot glacial acetic acid produced the spontaneous ring closure onto the ester in position 2 of the pyrrole moiety to afford the desired tricyclic pyrrolo[1,2-*a*]quinoxalines **6a-d** through a one-pot reduction-cyclization step [15,16]. The lactames **6a-d** were subsequently chlorodehydroxylated with phosphorous oxychloride, leading to the 4-chloroquinoxalines **7a-d**. 4-(Pyrrolo[1,2-*a*]quinoxalin-4-yl)benzaldehydes **8a-d** were easily prepared by a direct Suzuki-Miyaura cross-coupling reaction of 4-chloropyrroloquinoxalines **7a-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 [14–16]. The aldehydes **8a-d** were then engaged in a reductive amination with $NaBH_3CN$ and 4-(2-ketobenzimidazolin-1-yl)piperidine or 4-(5-fluorobenzimidazolin-2-yl)piperidine or 2-(3-piperidin-4-yl-1*H*-1,2,4-triazol-5-yl)pyridine to give the pyrrolo[1,2-*a*]quinoxalines **1a-l** [14–16]. The 3D spatial determinations of **1a**, **1d**, **1g** and **1j** were established by X-ray crystallography (Fig. 3), and confirmed the structures in the solid state as anticipated on the basis of IR and ^1H and ^{13}C NMR data.

3. Biological activity

3.1. Cytotoxicity in leukemia cell lines

The twelve new compounds **1a-l** were tested in MTS assay for their *in vitro* antiproliferative activity against five human leukemic cell lines (U937, K562, Jurkat, U266 and HL60). Compound A6730 (Fig. 1) was used in these tests as the reference standard drug. The results are summarized in Table 2. In addition, compound LY-294002, which showed antiproliferative activity against the HL60, U937 and K562 cell lines [27–30], was also applied as a referential cytotoxic agent.

The pyrrolo[1,2-*a*]quinoxalines **1c**, **1f**, **1g**, **1h, 1i, 1k** and **1l** were found the most antiproliferative compounds on the growth of human myeloid U937 cell line with IC_{50} of 3–11 μM. The two derivatives **1h** and **1k** with IC_{50} of 4 and 3 μM respectively, showed a better activity in comparison with the reference compound A6730 ($IC_{50} = 8$ μM). These two compounds **1h** and **1k** were substituted in position 4 by a benzylpiperidinyl fluorobenzimidazole group and in position 1 by a phenyl. In general, the substitution by a phenyl on position 1 of the pyrrole moiety led to active derivatives (**1g-l** excepted **1j**). Interestingly, **1c**, **1f, 1i** and **1l** were substituted by a



Scheme 2. Synthesis of pyrrolo[1,2-*a*]quinoxalines **1a-l**; 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 or 2-(3-piperidin-4-yl-1*H*-1,2,4-triazol-5-yl]pyridine, $NaBH_3CN$, MeOH, Δ.

Table 1
Synthesis of phenyl-1*H*-pyrrole-diester **2a-d** under standard reaction conditions.

	Reagents and conditions	Time	Yield (%)
2a	(i) dppp, dioxane, 100 °C	2 h	87[a]
2b	(ii) *Method A*: Ag_2CO_3, dioxane, 80 °C	1 h	9[a]
	(ii) *Method B*: 1) Ag_2CO_3, dioxane, 25 °C; 2) 80 °C	1) 5 min 2) 30 min	10[a]
	(ii) *Method C:* 1) Ag_2CO_3, NMP, 25 °C; 2) 80 °C	1) 5 min 2) 30 min	12[b]
	(iii) *Method D:* Cu_2O, phenanthroline, dioxane, 100 °C	40 min	62[b]
	(iv) *Method E:* DBU, THF, 50 °C	1 h	35[b]
2c	(vi) 1) $H_5C_2OOC–C\equiv C–COOC_2H_5$, DABCO, toluene, 80 °C; 2) 170 °C, P	1) 6 min 2) 45 min	28[b]
2d	(viii) $C_6H_5–CO–CH_2–COOC_2H_5$, $Mn(OAc)_3\&\cdot 2H_2O$, 40 °C	2 h	42[a]

[a] Conventional heating.
[b] Microwaves heating (200 W).

benzylpiperidinyl triazolylpyridine moiety in position 4 of the pyrrolo[1,2-*a*]quinoxaline core, All other compounds derived from the incorporation of the benzylpiperidinyl benzimidazolone moiety (compounds **1a, 1d** and **1j**), which was present in the reference compound A6730, into the 4-position of the heterocyclic pyrroloquinoxaline ring were found inactive on the U937 cell line in comparison with their benzylpiperidinyl fluorobenzimidazole or benzylpiperidinyl triazolylpyridine analogues, excepted **1g** that was found active (IC_{50} = 9 μM). From a SAR point of view, these preliminary biological results on U937 cell line enlightened the importance of the substitution at C-4 position of the pyrroloquinoxaline scaffold by a benzylpiperidinyl fluorobenzimidazole group, and also the need of a phenyl functionalisation in position 1 of the pyrrole ring (compounds **1h** and **1k**).

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

Among the twelve compounds tested for antiproliferative activities on K562 cell line, the five pyrrolo[1,2-*a*]quinoxalines **1a**, **1b**, **1e**, **1k** and **1l** were found the most active compounds with an IC_{50} of 3–4 μM. All the other pyrroloquinoxalines **1** also showed significant antiproliferative activity with IC_{50} ranging from 7 to 14 μM, better than the one found for the reference compound A6730 (IC_{50} = 17 μM). Moreover, in terms of structure-activity relationships discussion, it could be also noticed that the four quinoxalines **1b**, **1e**, **1h** and **1k**, bearing the benzylpiperidinyl fluorobenzimidazole moiety in their 4-position, were always found the most active compounds with IC_{50} of 3–7 μM in each subseries diversely substituted by a phenyl and an ester on the pyrrole ring.

Against the HL60 human acute promyeloid leukemia cell line, most of the tested compounds showed antiproliferative activity with IC_{50} values from 3 to 24 μM, excepted **1a** and **1j** that were

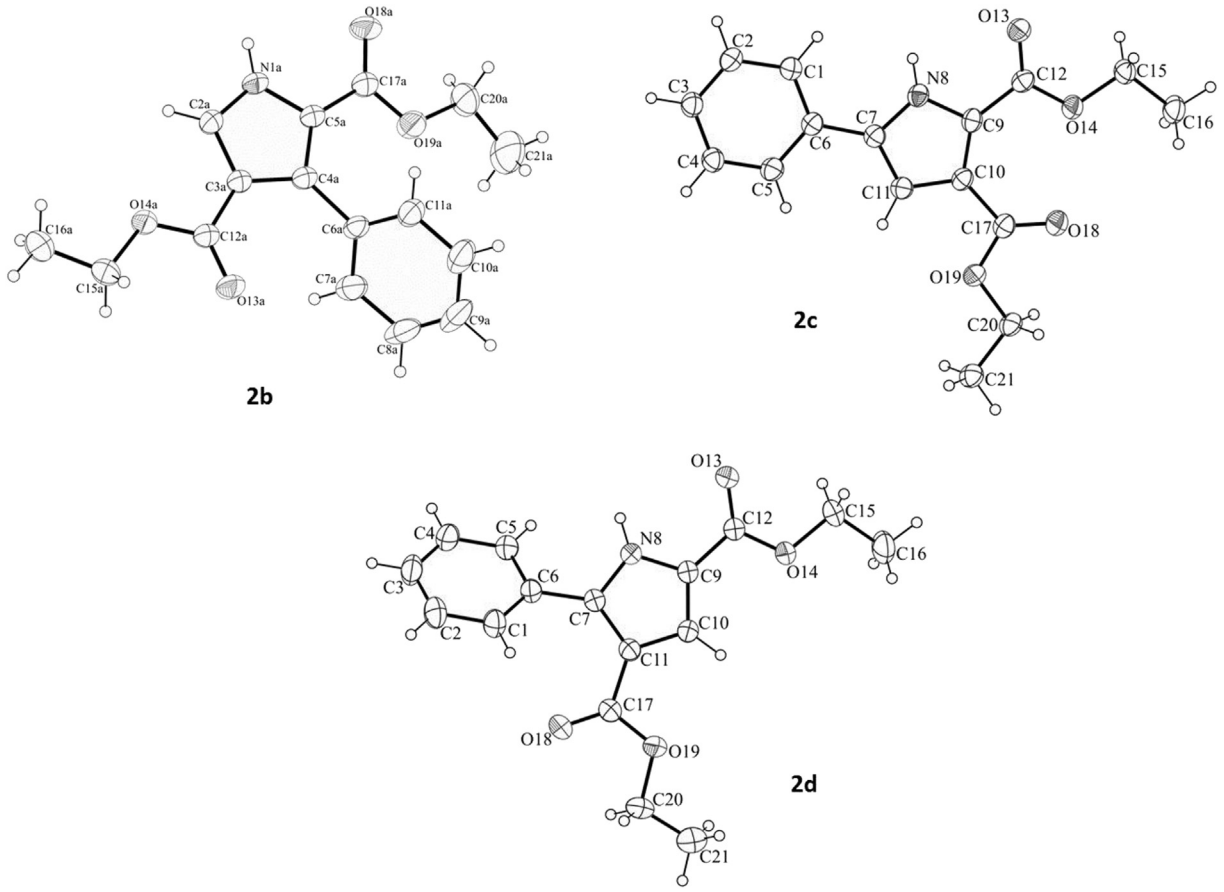


Fig. 2. The ORTEP drawing of phenyl-1*H*-pyrrole-diesters **2b-d** with thermal ellipsoids at 30% level.

found inactive (IC_{50} > 50 μM). The two pyrroloquinoxalines **1d** and **1f** having an ester function and a phenyl respectively in position 2 and 3 exhibited better activities than their other homologues. Compounds **1d** and **1k** showed a better activity that the one noticed for the reference compound A6730: i.e. IC_{50} = 3.5 and 3 μM for **1d** and **1k**, respectively in comparison with 5.5 μM for A6730.

The antiproliferative activities of compounds **1a-l** against the T-acute lymphoblastic leukemia Jurkat cell line were also investigated, and the results exhibited potent cytotoxicity for pyrroloquinoxalines **1b-l** (IC_{50} from 2 to 6.5 μM) as potent as the one observed for A6730 (IC_{50} = 3.5 μM). Nevertheless, the pyrrolo[1,2-*a*]quinoxaline **1a** showed low antiproliferative activity (IC_{50} = 41 μM). Hence in the subseries functionalized by a phenyl and an ester at C2 and C3 respectively, the IC_{50} of **1b** and **1c** (5 μM) was 8.2 times lower than those of compound **1a** (IC_{50} = 41 μM).

Against the human myeloma cell line U266, the same pyrrolo [1,2-*a*]quinoxalines **1b**, **1f**, **1h**, **1k** and **1l**, bearing a benzylpiperidinyl fluorobenzimidazole or a benzylpiperidinyl triazolylpyridine moiety in position 4 and substituted on the pyrrole ring, exhibited potent cytotoxicity (IC_{50} from 3 to 5 μM). All the other tested pyrroloquinoxalines **1** (compounds **1d-e**, **1g** and **1i**) were also found to be active on U266 cell line with IC_{50} ranging from 8 to 18 μM, with the exception of **1a**, **1c** and **1j** that presented an IC_{50} superior to 50 μM.

Against each human cancer cell lines, the antiproliferative activities of compounds **1a-l** were generally found superior to those of the other reference drug LY-294002.

3.2. Cytotoxicity activity in activated normal peripheral blood mononuclear cells

The compounds **1a-l** were tested on activated (PBMNC + PHA) human peripheral blood mononuclear cells to evaluate their respective cytotoxicity on normal cells (Table 2). As expected, most of the pyrrolo[1,2-*a*]quinoxalines **1a-l** showed significant level of cytotoxicity against lymphocytes with IC_{50} ranging from 8 to over 50 μM. These preliminary results were used to determine their respective range of toxic concentration.

Indexes of selectivity (IS) were defined as the ratio of the IC_{50} value on the human mononuclear cells to the IC_{50} value on the K562, U937, HL60, Jurkat and U266 lines. Compounds that demonstrated high selectivity (high index of selectivity) should offer a potential of safer therapy. This led to identify compounds with index of selectivity >16.7 and >12.5 for compounds **1e** and **1a**, respectively, on the human myeloid leukemic cell lines K562; and >12.5 for compound **1g** against the human leukemic cell lines Jurkat. We could notice that the more interesting new pyrroloquinoxaline structure (compound **1e**) could be considered as a direct combination of our previously bioactive described derivatives JG572 and JG576. Moreover, we could notice that the compound **1a** showed interesting selectivity towards K562 CML cell lines. The potential inhibitor **1h** also showed interesting IS on U937 and U266 leukemic cell lines with value of 12.5. These four compounds could now constitute suitable candidates for further pharmacological studies. The reference compound A6730 showed interesting selectivity with index of selectivity value noticed at 14.3 on the Jurkat cell line.

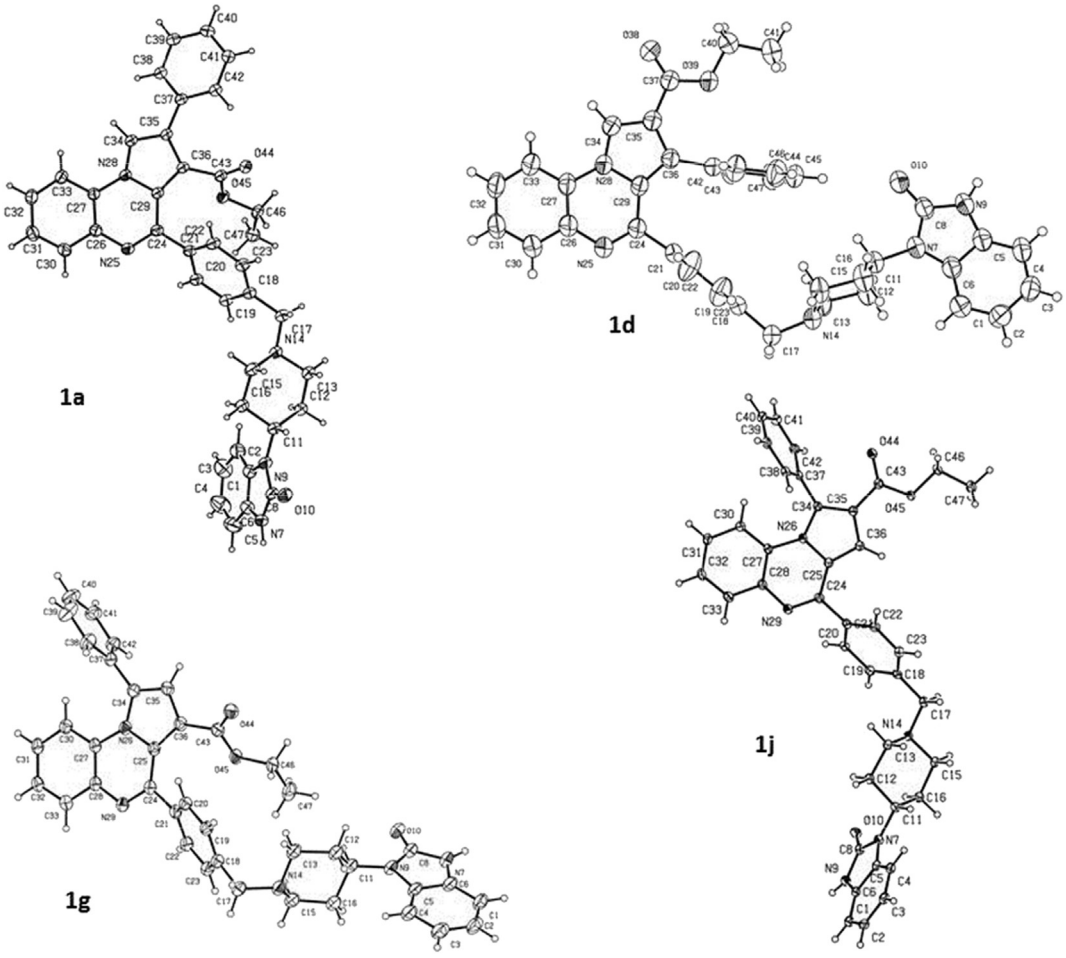


Fig. 3. The ORTEP drawing of pyrrolo[1,2-*a*]quinoxalines **1a, 1d, 1g** and **1j** with thermal ellipsoids at 30% level.

Table 2
In vitro activity of compounds **1a-l** on U937, K562, HL60, Jurkat and U266 cells, and cytotoxicity on human peripheral blood mononuclear cells PBMNC + PHA.

IC_{50} values (μM)[a]						
Compound	K562	U937	HL60	Jurkat	U266	Cytotoxicity on activated human peripheral blood mononuclear cells (PBMNC) PBMNC + PHA
A6730	17 ± 0.3	8 ± 0.2	5.5 ± 0.2	3.5 ± 0.2	n.d.[b]	>50
LY-294002	38 ± 1	14 ± 0.3	14 ± 0.3	22 ± 1	46 ± 2	>50
1a	4 ± 0.1	>50	>50	41 ± 1.2	>50	>50
1b	3 ± 0.1	21 ± 2.2	19 ± 2.8	5 ± 0.2	3.5 ± 0.1	14 ± 1
1c	14 ± 0.3	7 ± 0.3	12 ± 2.3	5 ± 0.1	>50	16 ± 2
1d	9 ± 0.3	>50	3.5 ± 0.1	4 ± 0.1	10 ± 0.8	41.3 ± 5
1e	3 ± 0.1	n.d.	10 ± 0.9	5 ± 0.2	18 ± 1.1	>50
1f	7 ± 0.2	7 ± 0.1	5 ± 0.1	2 ± 0.1	5 ± 0.1	8 ± 0.5
1g	8 ± 0.2	9 ± 0.4	6 ± 0.1	4 ± 0.1	9 ± 0.8	>50
1h	7 ± 0.3	4 ± 0.1	8 ± 0.2	6 ± 0.15	4 ± 0.1	50 ± 4
1i	12 ± 0.4	11 ± 0.9	24 ± 3	5 ± 0.1	8 ± 0.5	50 ± 6
1j	8.5 ± 0.3	>50	>50	6.5 ± 0.1	>50	>50
1k	3 ± 0.1	3 ± 0.1	3 ± 0.1	3.5 ± 0.1	3 ± 0.1	13 ± 1
1l	3.5 ± 0.1	8 ± 0.2	7 ± 0.3	3 ± 0.1	3 ± 0.1	12 ± 1

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

3.3. Determination of the action mode

The reference compound A6730, which showed interesting selectivity on the Jurkat cell line, is additionally described as an Akt inhibitor (Akt1 IC_{50} = 58 nM, Akt2 IC_{50} = 10 nM and IC_{50} Akt3 = 2.2 μM) [31]. To determine the possible mechanism of action of our compounds, we first evaluated their potency on isolated enzymes such as Akt 1, 2 and 3 as well as on mTOR. These tests have been performed by DiscoverX at 1 and 10 μM [32]. Nevertheless, only low effects have been detected. Akt2 activity was reduced of 38% and 35% with derivatives **1e** and **1j** at 10 μM, respectively. Such of enzymatic interaction could not explain the cell effects. Next assessment of apoptosis in K562 cell line was examined. Apoptosis induces loss of membrane asymmetry resulting in phosphatidyl

serine (PS) exposure and alterations in mitochondrial membrane potential. Cells were incubated with or without increasing doses **1e** for 3 days. Compound **1e**, which showed the most interesting index of selectivity on the human myeloid leukemic cell lines K562, induces a significantly increase in Annexin V positive cells from the second and third days (92% ± 2.8) (Fig. 4). These results could explain the inhibition of cell proliferation observed with this compound **1e**.

3.4. Predicted toxicity and other drug relevant properties

To speculate on any toxicity risks and drug-like characteristics of these novel synthesized compounds, we computed a set of drug relevant properties from their 2D chemical structures (Table 3). The calculated properties indicated that our molecules **1** present the same toxicological profile as the anticancer reference compound A6730. These parameters were calculated by the molinspiration web services [33]. All compounds **1a-l** were found lipophilic with Clog *P* values between 6.44 and 7.70. The Clog *P* of the reference compound A6730 was noticed at 5.55 slightly lower than those of our compounds. To predict intestinal absorption, we estimated the molecular polar surface areas (PSA) of these new pyrroloquinoxaline compounds **1a-l** from the calculated TPSA. PSA has been extensively used in medicinal chemistry for modeling absorption phenomena and to optimize a drug's ability to permeate cells. The PSA of a molecule is defined as the surface sum over all polar atoms, which primarily consist of oxygen and nitrogen as well as their attached hydrogens. Molecules with a polar surface area greater than 140 $Å^2$ tend to be poor at permeating cell membranes, while a PSA of less than 60 $Å^2$ is usually needed for molecules to penetrate the blood-brain barrier and thus act on the brain and other central nervous system tissues [34,35]. The PSA of our compounds could suggest an intestinal absorption (PSA < 140 $Å^2$). On the other hand, these pyrroloquinoxalines seem to be unlikely to cross the blood-brain barrier (PSA > 60 $Å^2$). Thus, based on these predicted data, the calculated PSA values for these derivatives **1** could indicate a possible better oral bioavailability with a smaller chance of CNS toxicity.

4. Conclusion

In the present work, we synthesized a series of twelve new ethyl 4-[4-(4-substitutedpiperidin-1-yl)]benzyl-phenylpyrrolo[1,2-*a*] quinoxaline-carboxylate derivatives **1a-l** and investigated their antileukemic activity on the human leukemic cell lines U937, K562, Jurkat, U266 and HL60. These results have been discussed in a preliminary SAR study. The first biological evaluation of our new substituted pyrrolo[1,2-*a*]quinoxalines showed cytotoxic activity in these myeloid and lymphoid leukemia cell lines. Consequently, compounds **1e**, **1a**, **1g** and **1h** are promising due to their high cytotoxic activity against some leukemia cells (IC_{50} ranging from 3 to 9 μM) and their lower toxicity against normal hematopoietic cells (estimated IC_{50} > 50 μM). These compounds showing interesting index of selectivity may constitute suitable candidates for further pharmacological studies. Moreover, it would be also interesting to enlarge the biological evaluation of these new bioactive pyrrolo[1,2-*a*]quinoxaline derivatives in order to precise now their mechanism of action.

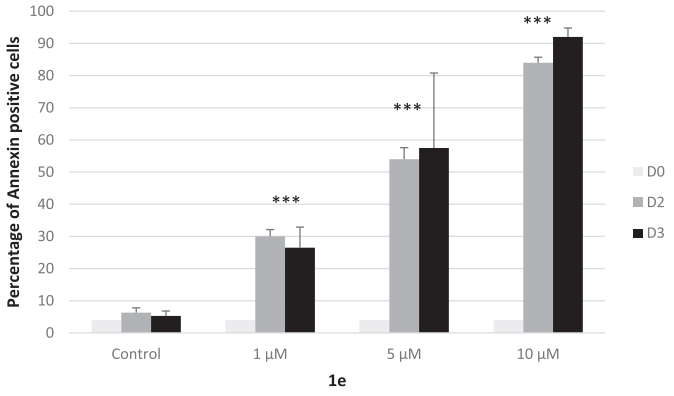


Fig. 4. Effect of **1e** on apoptosis of K562 cells. Cells were cultured with or without increasing doses of **1e** for 3 days in culture medium then stained with APC-Annexin V, and analyzed by flow cytometry. Results are expressed as Mean ± Standard Error (SEM) of three independent experiments. ***p < 0.001 compared with control (t-test).

Table 3
Predicted drug-relevant properties of compounds **1a-l**.

Compound	Clog *P*	TPSA	nON	nOH/NH	N violations[a]
A6730	5.55	95.50	8	2	2
1a	7.19	84.65	8	1	2
1b	7.70	75.53	7	1	2
1c	6.49	101.32	9	1	2
1d	7.19	84.65	8	1	2
1e	7.70	75.53	7	1	2
1f	6.49	101.32	9	1	2
1g	7.13	84.65	8	1	2
1h	7.65	75.53	7	1	2
1i	6.44	101.32	9	1	2
1j	7.13	84.65	8	1	2
1k	7.65	75.53	7	1	2
1l	6.44	101.32	9	1	2

[a] Number of violations to the Lipinski's "rule of five": log $P \leq 5$, molecular weight ≤500, number of hydrogen bond acceptors ≤10, and number of hydrogen bond donors ≤5.

5. Experimental

5.1. Chemistry

Commercially reagents were used as received without additional purification. Melting points were determined with an SMLUX-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 designated 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 precoated silica gel plates (POLYGRAM SIL G/UV_{254}) and 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 within ±0.4% of the theoretical values.

5.1.1. Ethyl 2-azidoacrylate (**4**)

To a solution of sodium azide (3.75 g, 57.68 mmol) in DMF (120 mL) at 65 °C was added ethyl 2,3-dibromopropionate (5.00 g, 19.26 mmol). After 10 min, the reaction mixture was cooled and poured into water (300 mL) and extracted with ether (3 × 120 mL). The combined organic extracts were washed with water (3 × 120 mL), dried over $MgSO_4$, filtered and evaporated *in vacuo* to afford ethyl 2-azidoacrylate as a yellow oil (79%). R_f = 0.38 (cyclohexane/Et_2O-98/2) [36,37].

*5.1.2. Diethyl 4-phenyl-1H-pyrrole-2,3-dicarboxylate (**2a**)*

To a 1,4-dioxane solution (10 mL) of 1,3-bis(diphenylphosphino) propane (dppp) (0.32 g, 0.78 mmol) were added ethyl isocyanoacetate (0.70 g, 6.19 mmol) and ethyl phenylpropiolate (0.90 g, 5.17 mmol). The solution was stirred at 100 °C for 2 h. After the consumption of ethyl isocyanoacetate, the reaction mixture was cooled to room temperature, filtered and evaporated *in vacuo*. The crude was purified on silica gel (eluent: cyclohexane/AcOEt-90/10 then cyclohexane/AcOEt-70/30, $R_f = 0.33$) to afford **2a** as a colorless oil (87%) [18,19].

*5.1.3. Diethyl 3-phenyl-1H-pyrrole-2,4-dicarboxylate (**2b**)*

Method A: To a mixture of ethyl phenylpropiolate (2.5 g, 14.3 mmol) and Ag_2CO_3 (0.264 g, 0.96 mmol) in 1,4-dioxane (40 mL) at 80 °C, ethyl isocyanoacetate (1.08 g, 9.55 mmol) was slowly added. The solution was stirred at 100 °C for 30 min. After the consumption of ethyl isocyanoacetate, the reaction mixture was cooled to room temperature, filtered and evaporated *in vacuo*. The crude was purified on silica gel (eluent: cyclohexane/AcOEt-90/10 then cyclohexane/AcOEt-70/30, $R_f = 0.33$) to afford **2b** as white crystals (9%). **Method B**: A mixture of ethyl phenylpropiolate (2.5 g, 14.3 mmol) and Ag_2CO_3 (0.264 g, 0.96 mmol) in 1,4-dioxane (40 mL) was heated at 80 °C for 5 min, then mixture was cooled with an ice bath. To this resulting solution cooled at room temperature, ethyl isocyanoacetate (1.08 g, 9.55 mmol) was added dropwise. The reaction mixture was stirred for 5 min at 25 °C, then heated for 30 min at 80 °C. The resulting slurry was concentrated under reduced pressure and taken up with dichloromethane. The organic layer was washed with brine, dried with Na_2SO_4, filtered and evaporated under *vacuum*. The crude was purified on silica gel as above leading to **2b** (10%). **Method C**: To a *N*-methyl-2-pyrrolidone (NMP) solution (40 mL) of Ag_2CO_3 (0.264 g, 0.96 mmol) was added ethyl phenylpropiolate (2.5 g, 14.3 mmol). After a prestirring of 30 s, the solution was irradiated during 1 min. The irradiation was programmed to maintain a constant temperature (80 °C) with a power of 200 W. To the resulting solution cooled at room temperature, ethyl isocyanoacetate (1.08 g, 9.55 mmol) was added dropwise. After a pre-stirring of 30 s, the solution was irradiated during 6 min with a first step of irradiation of 1 min at 25 °C followed by a second one of 5 min at 80 °C. The power was set at 200 W in both steps. The resulting slurry was concentrated under reduced pressure and taken up with dichloromethane. The organic layer was washed with brine, dried with Na_2SO_4, filtered and evaporated under *vacuum*. The crude was purified on silica gel as above to afford **2b** (12%). **Method D**: To a 1,4-dioxane solution (25 mL) of Cu_2O (0.085 g, 0.59 mmol) and 1,10-phenanthroline (0.215 g, 1.19 mmol) were added ethyl isocyanoacetate (1.35 g, 11.9 mmol) and ethyl phenylpropiolate (2.5 g, 14.3 mmol). After a prestirring of 30 s, the solution was irradiated during 40 min. The irradiation was programmed to maintain a constant temperature (100 °C) with a power of 200 W. The reaction mixture was cooled to room temperature, filtered and concentrated *in vacuo*. The crude was purified on silica gel as above to give **2b** (62%). **Method E**: To a THF solution (30 mL) of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (3.00 g, 19.7 mmol) was added ethyl isocyanoacetate (1.98 g, 17.5 mmol). After a prestirring of 30 s, the solution was irradiated during 2 min. The irradiation was programmed to maintain a constant temperature (50 °C) with a power of 200 W. To the resulting solution at the same temperature, ethyl isocyanoacetate (1.08 g, 9.55 mmol) was added dropwise. After a prestirring of 30 s, the solution was irradiated during 1 h. The irradiation was programmed to maintain a constant temperature (50 °C) with a power of 200 W. The reaction mixture was neutralized with acetic acid and then the solvent was removed under reduced pressure. The resulting residue was extracted with ethyl acetate and the extract was washed with hydrochloric acid and water, dried with Na_2SO_4, and then evaporated *in vacuo*. The crude was purified on silica gel as above to give **2b** (35%) [20–22].

*5.1.4. Diethyl 5-phenyl-1H-pyrrole-2,3-dicarboxylate (**2c**)*

To 1,4-diazabicyclo[2.2.2]octane (0.083 g, 0.74 mmol) and oxime **3** (1.00 g, 7.40 mmol) in dry toluene (20 mL) was added diethyl acetylenedicarboxylate (1.26 g, 7.40 mmol), and the resultant mixture was subjected to the two-stage microwave irradiation sequence (stage 1: 80 °C, 6 min; stage 2: 170 °C, 45 min). The power was set at 200 W in both steps. The reaction mixture was cooled to room temperature, filtered and concentrated *in vacuo*. The crude was purified on silica gel as above to give **2c** (28%) [24,25].

*5.1.5. Diethyl 5-phenyl-1H-pyrrole-2,4-dicarboxylate (**2d**)*

To a solution of ethyl 2-azidoacrylate **4** (1.70 g, 12.1 mmol) and ethyl phenylacetoacetate (3.47 g, 18.0 mmol) in MeOH (30 mL) was added AcOH (1.45 g, 24.1 mmol) and manganese(III) acetate dihydrate (1.29 g, 4.81 mmol), and the solution was stirred at 40 °C for 2 h. The reaction mixture was quenched with pH 9 ammonium buffer ($AcONH_4 + NH_4OH$), and then extracted twice with AcOEt. The combined organic extracts were washed with brine, dried with Na_2SO_4, filtered and concentrated *in vacuo*. The resulting residue was cooled, triturated in Et_2O and filtered. The crystals formed were filtered, and dried under reduced pressure to give **2d** as white crystals (42%) [26].

*5.1.6. General procedure for diethyl 1-(2-nitrophényl)-phenyl-pyrrole-dicarboxylate (**5a-d**)*

Conventional heating: To a solution of diethyl phenyl-1*H*-pyrrole-dicarboxylate **2a-d** (2.75 mmol) in 11 mL of DMF was added cesium carbonate (3.3 mmol). The mixture was stirred at room temperature for 10 min, then 2-fluoro-nitrobenzene (4.12 mmol) was added. The reaction mixture was refluxed for 1 h 30 (15 h for **5d**), then was diluted in AcOEt (35 mL). The organic layer was washed with water (2 × 30 mL), then brine (30 mL) and dried over sodium sulfate. The organic layer was concentrated under vacuo 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 **5**. *Microwave heating:* A suspension of diethyl phenyl-1*H*-pyrrole-dicarboxylate **2a-d** (3.4 mmol), 1-fluoro-2-nitrobenzene (5.1 mmol) and cesium carbonate (4.06 mmol) in 12 mL of DMF was irradiated during 10 min. The irradiation was programmed to maintain a constant temperature (150 °C) with a maximal power output of 200 W. The reaction mixture was then diluted in AcOEt (60 mL), washed with water (2 × 50 mL), then brine (50 mL) and dried over sodium sulphate. The organic layer was concentrated under vacuo to give products **5a-d** as an oil.

*5.1.6.1. Ethyl 1-(2-nitrophenyl)-4-phenyl-pyrrole-2,3-dicarboxylate (**5a**).* Orange oil (85%/89%). ^{1}H NMR ($CDCl_3$) δ: 8.13 (dd, 1H, $J = 7.90$ and 1.35 Hz, H-3′), 7.74 (ddd, 1H, $J = 7.90$, 7.90 and 1.35 Hz, H-4′), 7.63 (ddd, 1H, $J = 7.90$, 7.90 and 1.35 Hz, H-5′), 7.55–7.43 (m, 3H, H-6′, H-2 phenyl and H-6 phenyl), 7.40–7.28 (m, 3H, H-3 phenyl, H-4 phenyl and H-5 phenyl), 7.02 (s, 1H, H-5), 4.39–4.30 (m, 2H, OCH_2), 4.12 (q, 2H, $J = 7.20$ Hz, OCH_2), 1.31 (t, 3H, $J = 7.20$ Hz, CH_3), 1.15 (t, 3H, $J = 7.20$ Hz, CH_3). HRMS-ESI *m/z* $[M + Na]^+$ Calcd for $C_{22}H_{20}N_2O_6Na$: 431.1219, Found: 431.1224.

*5.1.6.2. Ethyl 1-(2-nitrophenyl)-3-phenyl-pyrrole-2,4-dicarboxylate (**5b**).* Yellow oil (89%/95%). ^{1}H NMR ($CDCl_3$) δ: 8.19 (dd, 1H, $J = 7.80$ and 1.50 Hz, H-3′), 7.77 (ddd, 1H, $J = 7.80$, 7.80 and 1.50 Hz, H-4′), 7.67 (ddd, 1H, $J = 7.80$, 7.80 and 1.50 Hz, H-5′), 7.59 (s, 1H, H-5), 7.51 (dd, 1H, $J = 7.80$ and 1.50 Hz, H-6′), 7.39–7.35 (m, 5H, 5H phenyl), 4.14 (q, 2H, $J = 7.20$ Hz, OCH_2), 3.89–3.80 (m, 2H, OCH_2),

1.13 (t, 3H, $J = 7.20$ Hz, CH_3), 0.77 (t, 3H, $J = 7.20$ Hz, CH_3). HRMS-ESI m/z $[M + Na]^+$ Calcd for $C_{22}H_{20}N_2O_6Na$: 431.1219, Found: 431.1236.

*5.1.6.3. Ethyl 1-(2-nitrophenyl)-5-phenyl-pyrrole-2,3-dicarboxylate (**5c**).* Yellow oil (65%/76%). ^1H NMR ($CDCl_3$) δ: 8.05–8.01 (m, 2H, H-3′ and H-6′), 7.62–7.56 (m, 2H, H-2 phenyl and H-6 phenyl), 7.31 (t, 1H, $J = 7.50$ Hz, H-4′), 7.25–7.04 (m, 4H, H-5′, H-3 phenyl, H-4 phenyl and H-5 phenyl), 6.77 (s, 1H, H-4), 4.34 (q, 2H, $J = 6.90$ Hz, OCH_2), 4.12 (q, 2H, $J = 6.90$ Hz, OCH_2), 1.38–1.14 (m, 6H, $2CH_3$). HRMS-ESI m/z $[M + Na]^+$ Calcd for $C_{22}H_{20}N_2O_6Na$: 431.1219, Found: 431.1248.

*5.1.6.4. Ethyl 1-(2-nitrophenyl)-5-phenyl-pyrrole-2,4-dicarboxylate (**5d**).* Yellow oil (28%/). ^1H NMR ($CDCl_3$) δ: 8.04 (dd, 1H, $J = 7.50$ and 1.80 Hz, H-3′), 7.66 (s, 1H, H-3), 7.52 (ddd, 1H, $J = 7.50$, 7.50 and 1.80 Hz, H-4′), 7.46 (ddd, 1H, $J = 7.50$, 7.50 and 1.80 Hz, H-5′), 7.26–7.16 (m, 6H, H-6′ and 5H phenyl), 4.17 (q, 2H, $J = 6.90$ Hz, OCH_2), 4.15 (q, 2H, $J = 6.90$ Hz, OCH_2), 1.27 (t, 3H, $J = 6.90$ Hz, CH_3), 1.18 (t, 3H, $J = 6.90$ Hz, CH_3). HRMS-ESI m/z $[M+Na]^+$ Calcd for $C_{22}H_{20}N_2O_6Na$: 431.1219, Found: 431.1240.

*5.1.7. General procedure for ethyl 4,5-dihydro-4-oxo-5H-phénylpyrrolo[1,2-a]quinoxaline-carboxylate (**6a-d**)*

A suspension of **5** (2.6 mmol) and iron powder (9.5 mmol) in 11 mL of acetic acid was heated under reflux for 2 h. The reaction mixture was cooled, suspended in 20 mL of a 1 M aqueous solution of HCl, agitated, then filtered off, washed with HCl 1 M (8 mL), water, Et_2O and dried to give **6** as a fluffy white solid.

*5.1.7.1. Ethyl 4,5-dihydro-4-oxo-5H-2-phenylpyrrolo[1,2-a]quinoxaline-3-carboxylate (**6a**).* Beige crystals (69%), mp 239–241 °C. IR (KBr) 3200, 2750 (NH), 1720 (COO), 1660 (CON). ^1H NMR (DMSO-d_6) δ: 11.49 (s, 1H, NH), 8.58 (s, 1H, H-1), 8.17 (d, 1H, $J = 8.10$ Hz, H-9), 7.55 (d, 2H, $J = 7.50$ Hz, H-2 phenyl and H-6 phenyl), 7.45 (t, 2H, $J = 7.50$ Hz, H-3 phenyl and H-5 phenyl), 7.34–7.27 (m, 4H, H-4 phenyl, H-6, H-7 and H-8), 4.30 (q, 2H, $J = 7.20$ Hz, OCH_2), 1.25 (t, 3H, $J = 7.20$ Hz, CH_3). ^{13}C NMR (DMSO-d_6) δ: 157.0 (C-4), 155.2 (C-7), 129.7 (C-9a), 122.6 (C-5a), 117.7 (C-1), 116.8 (C-3a), 116.0 (C-6), 112.3 (C-3), 111.0 (C-2), 108.9 (C-9), 100.8 (C-8), 55.4 (CH_3O). Anal. Calcd. for $C_{12}H_{10}N_2O_2$: C, 67.28; H, 4.71; N, 13.08. Found: C, 67.40; H, 4.81; N, 12.94.

*5.1.7.2. Ethyl 4,5-dihydro-4-oxo-5H-3-phenylpyrrolo[1,2-a]quinoxaline-2-carboxylate (**6b**).* Beige crystals (88%), mp > 310 °C. IR (KBr) 3200, 2800 (NH), 1720 (COO), 1665 (CON). ^1H NMR (DMSO-d_6) δ: 11.25 (s, 1H, NH), 8.80 (s, 1H, H-1), 8.28 (d, 1H, $J = 7.80$ Hz, H-9), 7.34–7.27 (m, 7H, H-6, H-8 and 5H phenyl), 7.21 (t, 1H, $J = 7.80$ Hz, H-7), 4.09 (q, 2H, $J = 6.90$ Hz, OCH_2), 1.09 (t, 3H, $J = 6.90$ Hz, CH_3). ^{13}C NMR (DMSO-d_6) δ: 157.0 (C-4), 155.2 (C-7), 129.7 (C-9a), 122.6 (C-5a), 117.7 (C-1), 116.8 (C-3a), 116.0 (C-6), 112.3 (C-3), 111.0 (C-2), 108.9 (C-9), 100.8 (C-8), 55.4 (CH_3O). Anal. Calcd. for $C_{12}H_{10}N_2O_2$: C, 67.35; H, 4.78; N, 13.15. Found: C, 67.47; H, 4.92; N, 13.30.

*5.1.7.3. Ethyl 4,5-dihydro-4-oxo-5H-1-phenylpyrrolo[1,2-a]quinoxaline-3-carboxylate (**6c**).* White crystals (58%), mp = 218–220 °C. IR (KBr) 3200, 2700 (NH), 1700 (COO), 1670 (CON). ^1H NMR (DMSO-d_6) δ: 11.50 (s, 1H, NH), 7.56–7.53 (m, 5H, 5H phenyl), 7.32 (d, 1H, $J = 7.70$ Hz, H-9), 7.24 (t, 1H, $J = 7.70$ Hz, H-8), 6.97 (d, 1H, $J = 7.70$ Hz, H-6), 6.84 (t, 1H, $J = 7.70$ Hz, H-7), 6.81 (s, 1H, H-2), 4.27 (q, 2H, $J = 6.90$ Hz, OCH_2), 1.30 (t, 3H, $J = 6.90$ Hz, CH_3). Anal. Calcd. for $C_{12}H_{10}N_2O_2$: C, 67.21; H, 4.64; N, 13.01. Found: C, 67.33; H, 4.74; N, 12.76.

*5.1.7.4. Ethyl 4,5-dihydro-4-oxo-5H-1-phenylpyrrolo[1,2-a]quinoxaline-2-carboxylate (**6d**).* White crystals (84%), mp = 229–231 °C. IR (KBr) 3200, 2700 (NH), 1695 (COO), 1665 (CON). ^1H NMR (DMSO-d_6) δ: 11.53 (s, 1H, NH), 7.61–7.53 (m, 3H, 3H phenyl), 7.50–7.47 (m, 2H, 2H phenyl), 7.44 (s, 1H, H-3), 7.29 (d, 1H, $J = 7.90$ Hz, H-6), 7.20 (t, 1H, $J = 7.90$ Hz, H-8), 6.75 (t, 1H, $J = 7.90$ Hz, H-7), 6.64 (d, 1H, $J = 7.90$ Hz, H-9), 4.02 (q, 2H, $J = 6.90$ Hz, OCH_2), 1.30 (t, 3H, $J = 6.90$ Hz, CH_3). Anal. Calcd. for $C_{12}H_{10}N_2O_2$: C, 67.31; H, 4.74; N, 13.11. Found: C, 67.43; H, 4.95; N, 12.86.

*5.1.8. General procedure for ethyl 4-chloro-phenylpyrrolo[1,2-a] quinoxaline-carboxylate (**7a-d**)*

A solution of 5*H*-pyrrolo[1,2-*a*]quinoxalin-4-one **6** (4 mmol) in $POCl_3$ (8 mL) was refluxed for 2 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 sodium carbonate. The precipitate was filtered, dried and recrystallized from ethyl acetate to give **7**.

*5.1.8.1. Ethyl 4-chloro-2-phenylpyrrolo[1,2-a]quinoxaline-3-carboxylate (**7a**).* White crystals (77%), mp = 109–111 °C. IR (KBr) 1715 (COO). ^1H NMR ($CDCl_3$) δ: 7.97 (s, 1H, H-1), 7.87 (dd, 1H, $J = 8.10$ and 1.20 Hz, H-9), 7.80 (dd, 1H, $J = 8.10$ and 1.20 Hz, H-6), 7.58–7.32 (m, 7H, H-7, H-8 and 5H phenyl), 4.41 (q, 2H, $J = 7.20$ Hz, OCH_2), 1.34 (t, 3H, $J = 7.20$ Hz, CH_3). HRMS-ESI m/z $[M+H]^+$ Calcd for $C_{20}H_{15}N_2O_2ClNa$: 351.0900, Found: 351.0918.

*5.1.8.2. Ethyl 4-chloro-3-phenylpyrrolo[1,2-a]quinoxaline-2-carboxylate (**7b**).* Beige crystals (96%), mp = 144–146 °C. IR (KBr) 1715 (COO). ^1H NMR ($CDCl_3$) δ: 8.56 (s, 1H, H-1), 7.93 (dd, 1H, $J = 8.10$ and 1.50 Hz, H-9), 7.88 (dd, 1H, $J = 8.10$ and 1.50 Hz, H-6), 7.59 (ddd, 1H, $J = 8.10$, 7.90 and 1.50 Hz, H-8), 7.52 (ddd, 1H, $J = 8.10$, 7.90 and 1.50 Hz, H-7), 7.44–7.37 (m, 5H, 5H phenyl), 4.19 (q, 2H, $J = 7.20$ Hz, OCH_2), 1.14 (t, 3H, $J = 7.20$ Hz, CH_3). HRMS-ESI m/z $[M+H]^+$ Calcd for $C_{20}H_{15}N_2O_2ClNa$: 351.0900, Found: 351.0933.

*5.1.8.3. Ethyl 4-chloro-1-phenylpyrrolo[1,2-a]quinoxaline-3-carboxylate (**7c**).* Beige crystals (94%), mp = 108–110 °C. IR (KBr) 1720 (COO). ^1H NMR ($CDCl_3$) δ: 7.91 (d, 1H, $J = 8.10$ Hz, H-9), 7.55–7.48 (m, 5H, 5H phenyl), 7.41 (t, 1H, $J = 8.10$ Hz, H-8), 7.33 (d, 1H, $J = 8.10$ Hz, H-6), 7.17 (t, 1H, $J = 8.10$ Hz, H-7), 7.09 (s, 1H, H-2), 4.44 (q, 2H, $J = 7.20$ Hz, OCH_2), 1.44 (t, 3H, $J = 7.20$ Hz, CH_3). HRMS-ESI m/z $[M+H]^+$ Calcd for $C_{20}H_{15}N_2O_2ClNa$: 351.0900, Found: 351.0903.

*5.1.8.4. Ethyl 4-chloro-1-phenylpyrrolo[1,2-a]quinoxaline-2-carboxylate (**7d**).* Orange crystals (90%), mp = 178 °C. IR (KBr) 1710 (COO). ^1H NMR ($CDCl_3$) δ: 7.86 (dd, 1H, $J = 8.10$ and 1.30 Hz, H-6), 7.63–7.55 (m, 4H, 3H phenyl and H-3), 7.50–7.46 (m, 2H, 2H phenyl), 7.37 (ddd, 1H, $J = 8.10$, 7.85 and 1.30 Hz, H-8), 7.10 (ddd, 1H, $J = 8.10$, 7.85 and 1.30 Hz, H-7), 7.01 (dd, 1H, $J = 8.10$ and 1.30 Hz, H-9), 4.19 (q, 2H, $J = 7.20$ Hz, OCH_2), 1.18 (t, 3H, $J = 7.20$ Hz, CH_3). HRMS-ESI m/z $[M + H]^+$ Calcd for $C_{20}H_{15}N_2O_2ClNa$: 351.0900, Found: 351.0925.

*5.1.9. General procedure for ethyl 4-(4-formylphenyl)-phenylpyrrolo[1,2-a]quinoxaline-carboxylate (**8a-d**)*

To suspension of compound **7a-d** (4.64 mmol), and $Pd(PPh_3)_4$ (0.232 mmol) in a mixture of toluene/EtOH (75/4.1 mL) under nitrogen were added K_2CO_3 (5.1 mmol) and 4-formylphenylboronic acid (5.1 mmol). The reaction mixture was refluxed for 24 h, and the cooled suspension was extracted with CH_2Cl_2 (3 × 80 mL). The organic layer was washed with a saturated solution of NaCl (95 mL), and the combined organic extracts were dried over sodium sulfate, filtered, and evaporated under reduced pressure. The crude residue was triturated in ethanol. The resulting precipitate was filtered, washed with ethanol, and purified by column chromatography on

silica gel using dichloromethane as eluent gave the pure product **8**.

*5.1.9.1. Ethyl 4-(4-formylphenyl)-2-phenylpyrrolo[1,2-a]quinoxaline-3-carboxylate (**8a**).* Yellow crystals (83%), mp = 76–78 °C. IR (KBr) 1720 (COO), 1700 (CHO). ^{1}H NMR ($CDCl_3$) δ: 10.13 (s, 1H, CHO), 8.12 (dd, 1H, *J* = 8.10 and 1.30 Hz, H-9), 8.10 (s, 1H, H-1), 8.04 (d, 2H, *J* = 7.80 Hz, H-3′ and H-5′), 8.00 (dd, 1H, *J* = 8.10 and 1.30 Hz, H-6), 7.96 (d, 2H, *J* = 7.80 Hz, H-2′ and H-6′), 7.67 (ddd, 1H, *J* = 8.10, 7.85 and 1.30 Hz, H-8), 7.64–7.52 (m, 3H, H-7 and 2H phenyl), 7.48–7.39 (m, 3H, 3H phenyl), 3.63 (q, 2H, *J* = 6.90 Hz, OCH_2), 0.89 (t, 3H, *J* = 6.90 Hz, CH_3). HRMS-ESI *m/z* $[M + H]^+$ Calcd for $C_{27}H_{20}N_2O_3Na$: 421.1552, Found: 421.1552.

*5.1.9.2. Ethyl 4-(4-formylphenyl)-3-phenylpyrrolo[1,2-a]quinoxaline-2-carboxylate (**8b**).* Yellow crystals (60%), mp = 160–162 °C. IR (KBr) 1715 (COO), 1700 (CHO). ^{1}H NMR ($CDCl_3$) δ: 9.93 (s, 1H, CHO), 8.69 (s, 1H, H-1), 8.09 (d, 1H, *J* = 7.80 Hz, H-9), 8.05 (d, 1H, *J* = 7.80 Hz, H-6), 7.66 (t, 1H, *J* = 7.80 Hz, H-8), 7.58 (t, 1H, *J* = 7.80 Hz, H-7), 7.53 (d, 2H, *J* = 7.20 Hz, H-3′ and H-5′), 7.38 (d, 2H, *J* = 7.20 Hz, H-2′ and H-6′), 7.03–6.95 (m, 5H, 5H phenyl), 4.23 (q, 2H, *J* = 6.90 Hz, OCH_2), 1.20 (t, 3H, *J* = 6.90 Hz, CH_3). HRMS-ESI *m/z* $[M+H]^+$ Calcd for $C_{27}H_{20}N_2O_3Na$: 421.1552, Found: 421.1532.

*5.1.9.3. Ethyl 4-(4-formylphenyl)-1-phenylpyrrolo[1,2-a]quinoxaline-3-carboxylate (**8c**).* Pale yellow crystals (79%), mp = 178–180 °C. IR (KBr) 1720 (COO), 1695 (CHO). ^{1}H NMR ($CDCl_3$) δ: 10.14 (s, 1H, CHO), 8.09–8.06 (m, 1H, H-9), 8.04 (d, 2H, *J* = 8.10 Hz, H-3′ and H-5′), 7.97 (d, 2H, *J* = 8.10 Hz, H-2′ and H-6′), 7.58–7.56 (m, 5H, 5H phenyl), 7.48–7.44 (m, 2H, H-6 and H-8), 7.25–7.20 (m, 1H, H-7), 7.21 (s, 1H, H-2), 3.80 (q, 2H, *J* = 7.20 Hz, OCH_2), 0.99 (t, 3H, *J* = 7.20 Hz, CH_3). HRMS-ESI *m/z* $[M + H]^+$ Calcd for $C_{27}H_{20}N_2O_3Na$: 421.1552, Found: 421.1573.

*5.1.9.4. Ethyl 4-(4-formylphenyl)-1-phenylpyrrolo[1,2-a]quinoxaline-2-carboxylate (**8d**).* Yellow crystals (89%), mp = 182–184 °C. IR (KBr) 1700 (COO and CHO). ^{1}H NMR ($CDCl_3$) δ: 10.18 (s, 1H, CHO), 8.20 (d, 2H, *J* = 7.95 Hz, H-3′ and H-5′), 8.12 (d, 2H, *J* = 7.95 Hz, H-2′ and H-6′), 8.02 (d, 1H, *J* = 7.80 Hz, H-6), 7.62–7.48 (m, 6H, 5H phenyl and H-3), 7.42 (t, 1H, *J* = 7.80 Hz, H-8), 7.13–7.09 (m, 2H, H-7 and H-9), 4.16 (q, 2H, *J* = 7.20 Hz, OCH_2), 1.13 (t, 3H, *J* = 7.20 Hz, CH_3). HRMS-ESI *m/z* $[M + H]^+$ Calcd for $C_{27}H_{20}N_2O_3Na$: 421.1552, Found: 421.1566.

*5.1.10. General procedure for ethyl 4-{4-[(4-(2-oxo-2,3-dihydro-1H-benzimidazol-1-yl)piperidin-1-yl)benzyl]}-phenylpyrrolo[1,2-a]quinoxaline-carboxylate, ethyl 4-{4-[(4-(5-fluoro-1H-benzimidazol-2-yl)piperidin-1-yl)benzyl]}-phenylpyrrolo[1,2-a]quinoxaline-carboxylate and ethyl 4-{4-[4-(3-(pyridin-2-yl)-1,2,4-triazol-5-yl)piperidin-1-yl)benzyl]}-phenylpyrrolo[1,2-a]quinoxaline-carboxylate (**1a-l**)*

The pH of a solution of the aldehyde **8a-d** (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 methanol was adjusted to 6 by the dropwise addition of acetic acid. Powered sodium cyanoborohydride (2.15 mmol) was then added, and the resultant mixture was refluxed for 5 h. After removal of the methanol by rotary evaporation, the residue was triturated in water and extracted with dichloromethane. The organic layer was washed with water, dried over magnesium sulfate and evaporated to dryness. Column chromatography of the residue on silica gel using ethyl acetate - cyclohexane (1/1) then methanol-chloroform (1/9) as eluents gave the crude product. This solid was then triturated with diethyl ether, filtered, washed with diethyl ether and dried under reduced pressure to give the compounds **1a-l**.

*5.1.10.1. Ethyl 4-{4-[(4-(2-oxo-2,3-dihydro-1H-benzimidazol-1-yl)piperidin-1-yl)benzyl]}-2-phenylpyrrolo[1,2-a]quinoxaline-3-carboxylate (**1a**).* White crystals (53%), mp 185–187 °C. ^{1}H NMR ($CDCl_3$) δ: 9.68 (s, 1H, NH), 8.10 (d, J = 7.8 Hz, 1H, H-9), 8.08 (s, 1H, H-1), 7.96 (d, J = 7.5 Hz, 1H, H-6), 7.78 (d, J = 7.8 Hz, 2H, H-2′ and H-6′), 7.64–7.53 (m, 6H, H-7, H-8, H-3′, H-5′, H-4 benzimid. and H-7 benzimid.), 7.45–7.30 (m, 4H, H-2 phenyl, H-3 phenyl, H-5 phenyl and H-6 phenyl), 7.10–7.07 (m, 3H, H-4 phenyl, H-5 benzimid. and H-6 benzimid.), 4.46–4.37 (m, 1H, CH pip.), 3.67 (q, J = 6.9 Hz, 2H, CH_2), 3.66 (s, 2H, CH_2N), 3.13–3.08 (m, 2H, NCH_2 pip.), 2.58–2.44 (m, 2H, NCH_2 pip.), 2.28–2.20 (m, 2H, CH_2 pip.), 1.88–1.84 (m, 2H, CH_2 pip.), 0.92 (t, J = 6.9 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$) δ: 166.94 (C═O), 156.52 (C-4), 155.69 (C═O benzimid.), 141.51 (C-3a and C-5a), 139.74 (C-4′ and C-1 phenyl), 137.42 (C-7a benzimid.), 134.64 (C-1′), 131.82 (C-6), 130.59 (C-3 and C-5 phenyl), 130.37 (C-3′ and C-5′), 129.86 (C-2′ and C-6′), 129.71 (C-4 phenyl), 129.33 (C-2 and C-6 phenyl), 129.04 (C-7), 127.57 (C-8), 127.19 (C-2 and C-3a benzimid.), 125.08 (C-9a), 122.49 (C-5 benzimid. and C-6 benzimid.), 115.18 (C-9), 114.61 (C-3), 114.37 (C-1), 111.15 (C-4 benzimid. and C-7 benzimid.), 64.05 (NCH_2), 62.47 (CH_2), 54.56 (NCH_2 pip.), 52.12 (CH pip.), 30.65 (CH_2 pip.), 15.08 (CH_3). HRMS-ESI *m/z* $[M+Na]^+$ Calcd for $C_{39}H_{35}N_5O_3Na$: 644.2637, Found: 644.2634.

*5.1.10.2. Ethyl 4-{4-[(4-(5-fluoro-1H-benzimidazol-2-yl)piperidin-1-yl)benzyl]}-2-phenylpyrrolo[1,2-a]quinoxaline-3-carboxylate (**1b**).* Cream crystals (61%), mp 162–164 °C. ^{1}H NMR ($CDCl_3$) δ: 8.08 (s, 1H, H-1), 8.04 (d, J = 7.8 Hz, 1H, H-9), 7.96 (d, J = 7.8 Hz, 1H, H-6), 7.70 (d, J = 7.2 Hz, 2H, H-2′ and H-6′), 7.59 (t, J = 7.2 Hz, 1H, H-7), 7.55–7.47 (m, 3H, H-3′, H-5′ and H-8), 7.44–7.33 (m, 7H, H-2 phenyl, H-3 phenyl, H-5 phenyl, H-6 phenyl, H-4 benzimid., H-6 benzimid. and H-7 benzimid.), 6.93 (t, J = 7.2 Hz, 1H, H-4 phenyl), 3.61 (q, J = 7.0 Hz, 2H, CH_2), 3.54 (s, 2H, CH_2N), 2.91 (d, 2H, J = 10.2 Hz, NCH_2 pip.), 2.84 (t, J = 11.4 Hz, 1H, CH pip.), 2.05 (t, J = 10.8 Hz, 2H, CH_2 pip.), 1.99 (d, J = 11.4 Hz, 2H, NCH_2 pip.), 1.94–1.85 (m, 2H, CH_2 pip.), 0.87 (t, J = 7.0 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$) δ: 165.68 (C═O), 160.05 (d, J = 234 Hz, C-5 benzimid.), 158.46 (C-4), 139.78 (C-4′), 138.14 (C-1 phenyl, C-1′, C-2 benzimid., C-5a and C-3a benzimid.), 135.91 (C-3a and C-5a), 133.21 (C-6 and C-7a benzimid.), 130.55 (C-2′ and C-6′), 129.99 (C-3′ and C-5′), 129.23 (C-7), 128.91 (C-8), 128.51 (C-4 phenyl), 127.95 (C-2 phenyl, C-3 phenyl, C-5 phenyl and C-6 phenyl), 127.73 (C-9a), 125.85 (C-2), 123.62 (C-3), 113.95 (C-9), 113.48 (C-1 and C-7 benzimid.), 113.24 (C-4 benzimid. and C-6 benzimid.), 62.71 (NCH_2), 61.16 (CH_2), 53.17 (NCH_2 pip.), 36.50 (CH pip.), 30.74 (CH_2 pip.), 13.63 (CH_3). HRMS-ESI *m/z* $[M+H]^+$ Calcd for $C_{39}H_{35}N_5O_2F$: 624.2775, Found: 624.2751.

*5.1.10.3. Ethyl 4-{4-[4-(3-(pyridin-2-yl)-1,2,4-triazol-5-yl)piperidin-1-yl)benzyl]}-2-phenylpyrrolo[1,2-a]quinoxaline-3-carboxylate (**1c**).* White crystals (56%), mp 142–144 °C. ^{1}H NMR ($CDCl_3$) δ: 8.73 (d, J = 3.0 Hz, 1H, H-6 pyr.), 8.22 (d, J = 7.8 Hz, 1H, H-5 pyr.), 8.08 (d, J = 8.4 Hz, 1H, H-6), 8.06 (s, 1H, H-1), 7.94 (d, J = 8.4 Hz, 1H, H-9), 7.84 (t, J = 7.8 Hz, 1H, H-4 pyr.), 7.74 (d, J = 7.8 Hz, 2H, H-2′ and H-6′), 7.58 (t, J = 7.8 Hz, 1H, H-7), 7.56–7.49 (m, 4H, H-3′, H-5′, H-2 phenyl and H-6 phenyl), 7.44–7.40 (m, 2H, H-3 phenyl and H-5 phenyl), 7.38–7.34 (m, 2H, H-4 phenyl and H-3 pyr.), 7.28 (m, 1H, H-8), 3.65 (s, 2H, CH_2N), 3.64 (q, J = 7.2 Hz, 2H, CH_2), 3.06 (d, 2H, J = 10.8 Hz, NCH_2 pip.), 2.97–2.89 (m, 1H, CH pip.), 2.23 (t, J = 10.2 Hz, 2H, CH_2 pip.), 2.12 (d, J = 12.0 Hz, 2H, NCH_2 pip.), 2.10–2.00 (m, 2H, CH_2 pip.), 0.91 (t, J = 7.2 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$) δ: 165.56 (C═O), 154.36 (C-4, C-3 Triazole and C-2 pyr.), 149.50 (C-6 pyr.), 147.15 (C-5 Triazole), 138.36 (C-4′ and C-4 pyr.), 137.48 (C-1 phenyl, C-1′ and C-5a), 136.04 (C-3a), 133.31 (C-6), 130.40 (C-2′ and C-6′), 129.41 (C-3′, C-5′ and C-7), 128.97 (C-8), 128.47 (C-4 phenyl), 128.31 (C-2 phenyl and C-6 phenyl), 127.95 (C-3 phenyl and C-5 phenyl), 127.62 (C-9a), 125.83 (C-2), 124.60 (C-3

pyr.), 123.69 (C-3), 121.79 (C-5 pyr.), 113.78 (C-9), 113.32 (C-1), 63.02 (CH_2N), 61.19 (CH_2), 53.39 (CH pip. and NCH_2), 30.74 (CH_2 pip.), 13.68 (CH_3). HRMS-ESI *m/z* $[M+Na]^+$ Calcd for $C_{39}H_{35}N_7O_2Na$: 656.2750, Found: 656.2768.

*5.1.10.4. Ethyl 4-{4-[(4-(2-oxo-2,3-dihydro-1H-benzimidazol-1-yl) piperidin-1-yl)benzyl]}-3-phenylpyrrolo[1,2-a]quinoxaline-2-carboxylate (**1d**).* White crystals (60%), mp 259–262 °C. ^1H NMR ($CDCl_3$) δ: 9.95 (s, 1H, NH), 8.67 (s, 1H, H-1), 8.07 (d, J = 8.0 Hz, 1H, H-6), 8.03 (d, J = 8.0 Hz, 1H, H-9), 7.61 (t, J = 8.0 Hz, 1H, H-7), 7.55 (t, J = 8.0 Hz, 1H, H-8), 7.34 (d, J = 7.2 Hz, 1H, H-4 benzimid.), 7.23 (d, J = 7.8 Hz, 2H, H-2′ and H-6′), 7.15–7.07 (m, 3H, H-5 benzimid., H-6 benzimid., H-7 benzimid.), 7.06–6.96 (m, 7H, H-3′, C-5′, H-2 phenyl, H-3 phenyl, H-4 phenyl, H-5 phenyl and H-6 phenyl), 4.45 (t, 1H, CH pip.), 4.23 (q, 2H, CH_2), 3.46 (s, 2H, CH_2N), 3.00 (d, J = 10.2 Hz, 2H, NCH_2 pip.), 2.52 (q, J = 7.2 Hz, 2H, CH_2 pip.), 2.15 (t, J = 11.4 Hz, 2H, NCH_2 pip.), 1.88 (d, J = 10.8 Hz, 2H, CH_2 pip.), 1.19 (t, J = 7.2 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$) δ: 165.54 (C═O), 158.05 (C-4), 156.58 (C═O benzimid.), 140.33 (C-4′), 137.93 (C-1′), 137.56 (C-3a and C-5a), 135.22 (C-1 phenyl), 132.62 (C-3a benzimid., C-3 phenyl, C-4 phenyl and C-5 phenyl), 131.80 (C-6), 130.70 (C-7a benzimid.), 130.06 (2′ and C-6′), 129.54 (C-7, C-3′ and C-5′), 128.20 (C-2 phenyl and C-6 phenyl), 127.80 (C-8), 126.81 (C-9a), 124.41 (C-2), 122.61 (C-5 benzimid.), 122.52 (C-6 benzimid.), 120.66 (C-3), 119.65 (C-1), 115.21 (C-9) 111.25 (C-7 benzimid.), 111.17 (C-4 benzimid.), 64.03 (CH_2N), 61.79 (CH_2), 54.50 (NCH_2 pip.), 52.37 (CH pip.), 30.81 (CH_2 pip.), 15.53 (CH_3). HRMS-ESI *m/z* $[M+H]^+$ Calcd for $C_{39}H_{36}N_5O_3$: 622.2818, Found: 622.2795.

*5.1.10.5. Ethyl 4-{4-[(4-(5-fluoro-1H-benzimidazol-2-yl)piperidin-1-yl)benzyl]}-3-phenylpyrrolo[1,2-a]quinoxaline-2-carboxylate (**1e**).* White crystals (78%), mp 244–247 °C. ^1H NMR ($CDCl_3$) δ: 9.87 (s, 1H, NH), 8.67 (s, 1H, H-1), 8.04 (d, J = 7.0 Hz, 1H, H-9), 8.03 (t, J = 7.0 Hz, 1H, H-6), 7.61 (t, J = 7.0 Hz, 1H, H-7), 7.54 (t, J = 7.0 Hz, 1H, H-8), 7.18 (d, J = 8.0 Hz, 2H, H-2′ and H-6′), 7.06–6.96 (m, 7H, H-3′, H-5′, H-2 phenyl, H-3 phenyl, H-4 phenyl, H-5 phenyl, H-6 phenyl, H-4 benzimid., H-6 benzimid. and H-7 benzimid.), 4.23 (q, J = 7.2 Hz, 2H, CH_2), 3.41 (s, 2H, CH_2N), 2.97–2.86 (m, 3H, NCH_2 pip. and CH pip.), 2.11 (d, J = 11.6 Hz, 2H, CH_2 pip.), 2.08 (t, J = 11.6 Hz, 2H, NCH_2 pip.), 1.93 (m, 2H, CH_2 pip.), 1.20 (t, J = 7.2 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$) δ: 165.56 (C═O), 161.10 (d, J = 236 Hz, C-5 benzimid.), 158.21 (C-4), 140.16 (C-4′), 138.00 (C-1 phenyl, C-1′, C-2 benzimid. and C-3a benzimid.), 137.57 (C-3a and C-5a), 135.19 (C-7a benzimid.), 131.62 (C-6), 130.11 (C-2′ and C-6′), 129.64 (3′ and C-5′), 129.54 (C-7), 128.19 (C-8), 128.04 (C-2 phenyl, C-3 phenyl, C-4 phenyl, C-5 phenyl and C-6 phenyl), 127.77 (9a), 124.44 (C-2), 120.69 (C-3), 119.69 (C-1), 115.31 (C-9 and C-7 benzimid.), 111.90 (C-4 benzimid. and C-6 benzimid.), 64.24 (CH_2N), 61.92 (CH_2), 54.66 (NCH_2 pip.), 38.34 (CH pip.), 32.34 (CH_2 pip.), 15.60 (CH_3). HRMS-ESI *m/z* $[M + H]^+$ Calcd for $C_{39}H_{35}N_5O_2F$: 624.2775, Found: 624.2783.

*5.1.10.6. Ethyl 4-{4-[4-(3-(pyridin-2-yl)-1,2,4-triazol-5-yl)piperidin-1-yl)benzyl]}-3-phenylpyrrolo[1,2-a]quinoxaline-2-carboxylate (**1f**).* Pale yellow crystals (53%), mp 138–140 °C. ^1H NMR ($CDCl_3$) δ: 8.73 (d, J = 3.0 Hz, 1H, H-6 pyr.), 8.64 (s, 1H, H-1), 8.23 (d, J = 7.8 Hz, 1H, H-5 pyr.), 8.02 (d, J = 8.4 Hz, 1H, H-9), 7.99 (d, J = 8.4 Hz, 1H, H-6), 7.84 (t, J = 7.8 Hz, 1H, H-4 pyr.), 7.56 (t, J = 7.8 Hz, 1H, H-7), 7.50 (t, J = 7.2 Hz, 1H, H-8), 7.36 (t, J = 6.0 Hz, 1H, H-3 pyr.), 7.17 (d, J = 7.2 Hz, 2H, H-2′ and H-6′), 7.06–7.02 (m, 1H, H-4 phenyl),7.01–6.94 (m, 6H, H-3′, H-5′, H-2 phenyl, H-3 phenyl, H-5 phenyl, and H-6 phenyl), 4.20 (q, J = 7.2 Hz, 2H, CH_2), 3.49 (s, 2H, CH_2N), 3.01–2.93 (m, 3H, NCH_2 pip. and CH pip.), 2.23–2.13 (m, 4H, NCH_2 pip. and CH_2 pip.), 2.12–2.03 (m, 2H, CH_2 pip.), 1.17 (t, J = 7.2 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$) δ: 164.07 (C═O), 156.45 (C-4, C-3 Triazole and C-2 pyr.), 149.47 (C-6 pyr.), 147.36 (C-5 Triazole), 138.47 (C-4′ and C-4 pyr.), 136.63 (C-1 phenyl and C-1′), 135.97 (C-5a), 133.63 (C-3a), 130.21 (C-6), 128.64 (C-2′ and C-6′), 128.48 (C-3′ and C-5′), 128.09 (C-7), 126.77 (C-8), 126.49 (C-3 phenyl, C-4 phenyl and C-5 phenyl), 126.42 (C-2 phenyl and C-6 phenyl), 126.31 (9a), 125.41 (C-3 pyr.), 122.87 (C-2), 121.87 (C-5 pyr.), 119.12 (C-3), 118.26 (C-9), 113.76 (C-1), 62.64 (CH_2N), 60.34 (CH_2), 53.03 (NCH_2 pip.), 35.14 (CH pip.), 30.41 (CH_2 pip.), 14.07 (CH_3). HRMS-ESI *m/z* $[M + H]^+$ Calcd for $C_{39}H_{36}N_7O_2$: 634.2930, Found: 634.2952.

*5.1.10.7. Ethyl 4-{4-[(4-(2-oxo-2,3-dihydro-1H-benzimidazol-1-yl) piperidin-1-yl)benzyl]}-1-phenylpyrrolo[1,2-a]quinoxaline-3-carboxylate (**1g**).* Pale yellow crystals (66%), mp 167–169 °C. ^1H NMR ($CDCl_3$) δ: 10.52 (s, 1H, NH), 8.10 (d, J = 8.0 Hz, 1H, H-6), 7.82 (d, J = 7.6 Hz, 2H, H-2′ and H-6′), 7.62–7.53 (m, 8H, H-4 benzimid., H-2 phenyl, H-3 phenyl, H-4 phenyl, H-5 phenyl, H-6 phenyl, H-3′ and H-5′), 7.44 (d, J = 8.0 Hz, 1H, H-9), 7.43 (t, J = 8.0 Hz, 1H, H-7), 7.33 (d, J = 8.0 Hz, 1H, H-5 benzimid.), 7.21–7.02 (m, 4H, H-2, H-8, H-6 benzimid. and C-7 benzimid.), 4.52–4.36 (m, 1H, CH pip.), 3.79 (q, J = 7.2 Hz, 2H, CH_2), 3.69 (s, 2H, CH_2N), 3.13 (d, J = 10.8 Hz, 2H, NCH_2 pip.), 2.62–2.48 (m, 2H, CH_2 pip.), 2.27 (t, J = 10.8 Hz, 2H, NCH_2 pip.), 1.86 (d, J = 14.8 Hz, 2H, CH_2 pip.), 0.98 (t, J = 7.2 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$) δ: 166.54 (C═O), 156.74 (C═O benzimid.), 155.97 (C-4), 141.21 (C-3a and C-4′), 140.77 (C-5a), 134.78 (C-1′), 133.44 (C-1 phenyl and C-3a benzimid.), 131.68 (C-6), 131.12 (C-3′ and C-5′), 130.56 (C-7a benzimid.), 130.37 (C-2 phenyl, C-3 phenyl, C-4 phenyl, C-5 phenyl and C-6 phenyl), 129.64 (C-2′ and C-6′), 129.46 (C-7), 128.66 (C-8), 127.22 (C-9a), 122.56 (C-1, C-3 and C-5 benzimid.), 120.22 (C-2 and C-6 benzimid.), 118.10 (C-9), 111.22 (C-7 benzimid.), 111.19 (C-4 benzimid.), 64.09 (CH_2N), 62.27 (CH_2), 54.60 (NCH_2 pip.), 52.18 (CH pip.), 30.69 (CH_2 pip.), 15.27 (CH_3). HRMS-ESI *m/z* $[M + H]^+$ Calcd for $C_{39}H_{36}N_5O_3$: 622.2818, Found: 622.2802.

*5.1.10.8. Ethyl 4-{4-[(4-(5-fluoro-1H-benzimidazol-2-yl)piperidin-1-yl)benzyl]}-1-phenylpyrrolo[1,2-a]quinoxaline-3-carboxylate (**1h**).* Pale yellow crystals (60%), mp 156–158 °C. ^1H NMR ($CDCl_3$) δ: 11.40 (s, 1H, NH), 8.02 (d, J = 7.6 Hz, 1H, H-6), 7.74 (d, J = 8.0 Hz, 2H, H-2′ and H-6′), 7.61–7.52 (m, 5H, H-2 phenyl, H-3 phenyl, H-4 phenyl, H-5 phenyl and H-6 phenyl), 7.46 (d, J = 8.0 Hz, 1H, H-9), 7.44–7.37 (m, 3H, H-7, H-3′ and H-5′), 7.21–7.15 (m, 4H, H-2, H-8, H-4 benzimid. and H-7 benzimid.), 6.96 (td, J = 9.2 Hz and 1.6 Hz, 1H, H-6 benzimid.), 3.72 (q, J = 7.2, 2H, CH_2), 3.55 (s, 2H, CH_2N), 2.98–2.84 (m, 3H, NCH_2 pip. and CH pip.), 2.13–1.98 (m, 4H, NCH_2 pip. and CH_2 pip.), 1.96–1.81 (m, 2H, CH_2 pip.), 0.92 (t, J = 7.2 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$) δ: 166.61 (C═O), 160.7 (d, J = 235 Hz, C-5 benzimid.), 156.16 (C-4), 141.49 (C-4′), 140.40 (C-3a and C-5a), 138.66 (C-2 benzimid. and C-3a benzimid.), 134.65 (C-1 phenyl), 133.67 (C-7a benzimid.), 131.22 (C-3′ and C-5′), 131.11 (C-3 phenyl, C-4 phenyl and C-5 phenyl), 130.63 (C-6, C-2 phenyl and C-6 phenyl), 130.39 (C-7), 129.35 (C-2′ and C-6′), 128.72 (C-8), 128.54 (C-9a), 127.38 (C-1, C-3 and C-7 benzimid.), 120.33 (C-6 benzimid.), 118.21 (C-9), 111.50 (C-4 benzimid.), 64.19 (CH_2N), 62.32 (CH_2), 54.70 (NCH_2 pip.), 38.34 (CH pip.), 32.34 (CH_2 pip.), 15.21 (CH_3). HRMS-ESI *m/z* $[M+H]^+$ Calcd for $C_{39}H_{35}N_5O_2F$: 624.2775, Found: 624.2800.

*5.1.10.9. Ethyl 4-{4-[4-(3-(pyridin-2-yl)-1,2,4-triazol-5-yl)piperidin-1-yl)benzyl]}-1-phenylpyrrolo[1,2-a]quinoxaline-3-carboxylate (**1i**).* Pale yellow crystals (69%), mp 145–147 °C. ^1H NMR ($CDCl_3$) δ: 9.12 (s, 1H, NH), 8.68 (d, J = 4.0 Hz, 1H, H-6 pyr.), 8.20 (d, J = 8.0 Hz, 1H, H-5 pyr.), 8.06 (dd, J = 8.2 and 1.6 Hz, 1H, H-6), 7.86 (td, J = 7.6 and 1.6 Hz, 1H, H-4 pyr.), 7.78 (d, J = 7.4 Hz, 2H, H-2′ and H-6′), 7.57–7.54 (m, 5H, H-2 phenyl, H-3 phenyl, H-4 phenyl, H-5 phenyl and H-6 phenyl), 7.52 (d, J = 7.4 Hz, 2H, H-3′ and H-5′), 7.43 (d, J = 8.2 Hz, 1H, H-9), 7.41 (t, J = 7.2 Hz, 1H, H-7), 7.38 (dd, J = 6.4 and 1.2 Hz, 1H, H-9), 7.37 (d, J = 7.2 Hz, 1H, H-3 pyr.), 7.18 (td, J = 7.2 Hz and 1.6 Hz, 1H, H-8), 7.17 (s, 1H, H-2), 3.78 (q, J = 7.2, 2H, CH_2), 3.73

(s, 2H, CH_2N), 3.14–3.12 (m, 2H, NCH_2 pip.), 3.00–2.88 (m, 1H, CH pip.), 2.37–2.30 (m, 2H, CH_2 pip.), 2.19–2.05 (m, 4H, 2 CH_2 pip.), 0.97 (t, J = 7.2 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$) δ: 166.40 (C=O), 157.58 (C-2 pyr.), 155.85 (C-4), 148.75 (C-3 triazole), 150.77 (C-6 pyr.), 140.93 (C-5 triazole), 139.77 (C-3a), 138.84 (C-4 pyr.), 138.70 (C-5a), 134.70 (C-4′), 133.41 (C-1′ and C-1 phenyl), 131.59 (C-7), 131.06 (C-3′ and C-5′), 130.91 (C-3 phenyl and C-5 phenyl), 130.49 (C-8), 130.32 (C-2′ and C-6′), 129.46 (C-2 phenyl and C-6 phenyl), 128.61 (C-9a), 128.37 (C-6), 127.16 (C-9), 125.92 (C-4 phenyl), 125.77 (C-3), 123.23 (C-3 pyr.), 120.22 (C-5 pyr.), 118.04 (C-2), 115.92 (C-1), 64.02 (NCH_2), 62.24 (CH_2), 54.44 (NCH_2 pip.), 36.29 (CH pip.), 31.67 (CH_2 pip.), 15.22 (CH_3). HRMS-ESI *m/z* $[M + H]^+$ Calcd for $C_{39}H_{36}N_7O_2$: 634.2930, Found: 634.2959.

*5.1.10.10. Ethyl 4-{4-[(4-(2-oxo-2,3-dihydro-1H-benzimidazol-1-yl) piperidin-1-yl)benzyl]}-1-phenylpyrrolo[1,2-a]quinoxaline-2-carboxylate (**1j**).* White crystals (70%), mp 165–167 °C. ^{1}H NMR ($CDCl_3$) δ: 10.19 (s, 1H, NH), 8.03 (d, J = 7.8 Hz, 1H, H-6), 8.02 (d, J = 7.8 Hz, 2H, H-2′ and H-6′), 7.67–7.57 (m, 6H, H-7, H-3 phenyl, H-4 phenyl, H-5 phenyl, H-3′ and H-5′), 7.55 (s, 1H, H-3), 7.54–7.51 (m, 2H, H-2 phenyl and H-6 phenyl), 7.41–7.33 (m, 2H, H-9 and H-4 benzimid.), 7.14–7.05 (m, 4H, H-8, H-5 benzimid., H-6 benzimid., H-7 benzimid.), 4.47 (t, J = 12.0 Hz, 1H, CH pip.), 4.17 (q, J = 7.2 Hz, 2H, CH_2), 3.73 (s, 2H, CH_2N), 3.23–3.08 (m, 2H, NCH_2 pip.), 2.65–2.50 (m, 2H, CH_2 pip.), 2.37–2.23 (m, 2H, NCH_2 pip.), 1.89 (d, J = 10.2 Hz, 2H, CH_2 pip.), 1.13 (t, J = 7.2 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$) δ: 165.59 (C=O), 156.75 (C-4), 156.60 (C=O benzimid.), 139.29 (C-5a and C-3a benzimid.), 138.22 (C-1′), 136.20 (C-3a and C-1 phenyl), 131.88 (C-6), 131.85 (C-2 phenyl and C-6 phenyl), 130.85 (C-7a benzimid.), 130.80 (C-4 phenyl), 130.51 (C-3′ and C-5′), 130.24 (C-7), 130.12 (C-3 phenyl and C-5 phenyl), 129.67 (C-2′ and C-6′), 128.19 (C-8), 127.34 (C-9), 126.61 (C-2), 120.81 (C-1), 118.12 (C-5 benzimid. and C-6 benzimid.), 111.94 (C-3 and C-4 benzimid.), 111.20 (C-7 benzimid.), 64.01 (CH_2N), 61.61 (CH_2), 54.61 (NCH_2 pip.), 52.15 (CH pip.), 30.67 (CH_2 pip.), 15.42 (CH_3). HRMS-ESI *m/z* $[M+H]^+$ Calcd for $C_{39}H_{36}N_5O_3$: 622.2818, Found: 622.2846.

*5.1.10.11. Ethyl 4-{4-[(4-(5-fluoro-1H-benzimidazol-2-yl)piperidin-1-yl)benzyl]}-1-phenylpyrrolo[1,2-a]quinoxaline-2-carboxylate (**1k**).* White crystals (46%), mp 195–197 °C. ^{1}H NMR ($CDCl_3$) δ: 7.97 (d, J = 7.8 Hz, 1H, H-6), 7.93 (d, J = 7.2 Hz, 2H, H-2′ and H-6′), 7.64–7.56 (m, 3H, H-7, H-9 and H-4 phenyl), 7.55–7.47 (m, 6H, H-3, H-3 phenyl, H-5 phenyl, H-3′, H-5′ and H-4 benzimid.), 7.36 (t, J = 6.6 Hz, 2H, H-2 phenyl and H-6 phenyl), 7.09 (d, J = 7.8 Hz, 1H, H-8), 7.08 (d, J = 7.8 Hz, 1H, H-7 benzimid.), 6.98 (t, J = 7.8 Hz, 1H, H-6 benzimid.), 4.16 (q, J = 7.2 Hz, 2H, CH_2), 3.62 (s, 2H, CH_2N), 2.99 (d, J = 11.4 Hz, 2H, NCH_2 pip.), 2.94 (t, J = 12 Hz, 1H, CH pip.), 2.15 (t, J = 11.4 Hz, 2H, NCH_2 pip.), 2.06 (d, J = 11.4 Hz, 2H, CH_2 pip.), 1.98–1.89 (m, 2H, CH_2 pip.), 1.11 (t, J = 7.2 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$) δ: 165.75 (C=O), 160.57 (d, J = 235 Hz, C-5 benzimid.), 156.90 (C-4), 142.10 (C-4′), 139.21 (C-5a), 137.97 (C-1′), 136.42 (C-2 benzimid. and C-3a benzimid.), 134.60 (C-1 phenyl), 132.51(C-9a), 131.90 (C-3′ and C-5′), 131.52 (C-3a), 130.91 (C-6), 130.84 (C-3 phenyl and C-5 phenyl), 130.32 (C-7a benzimid.), 130.05 (C-7, C-9 and C-4 phenyl.), 129.58 (C-2′ and C-6′), 128.37 (C-8), 127.52 (C-2 phenyl and C-6 phenyl), 120.93 (C-1 and C-2), 118.28 (C-7 benzimid.), 111.82 (C-3 and C-6 benzimid.), 111.64 (C-4 benzimid.), 64.24 (CH_2N), 61.79 (CH_2), 54.74 (NCH_2 pip.), 38.31 (CH pip.), 32.35 (CH_2 pip.), 15.41 (CH_3). HRMS-ESI *m/z* $[M+H]^+$ Calcd for $C_{39}H_{35}N_5O_2F$: 624.2775, Found: 624.2747.

*5.1.10.12. Ethyl 4-{4-[4-(3-(pyridin-2-yl)-1,2,4-triazol-5-yl)piperidin-1-yl)benzyl]}-1-phenylpyrrolo[1,2-a]quinoxaline-2-carboxylate (**1l**).* White crystals (42%), mp 144–146 °C. ^{1}H NMR ($CDCl_3$) δ: 8.69 (d, J = 4.6 Hz, 1H, H-6 pyr.), 8.19 (d, J = 7.8 Hz, 1H, H-5 pyr.), 7.99 (d, J = 7.0 Hz, 1H, H-6), 7.94 (d, J = 7.0 Hz, 2H, H-2′ and H-6′), 7.83 (td, J = 7.6 and 1.6 Hz, 1H, H-4 pyr.), 7.62–7.45 (m, 8H, H-3, H-7, H-9, H-3 phenyl, H-4 phenyl, H-5 phenyl, H-3′ and H-5′), 7.40–7.29 (m, 3H, H-2 phenyl, H-6 phenyl and H-3 pyr.), 7.04 (d, J = 7.0 Hz, 1H, H-8), 4.14 (q, J = 7.1 Hz, 2H, CH_2), 3.67 (s, 2H, CH_2N), 3.07 (d, J = 11.0 Hz, 2H, NCH_2 pip.), 2.99–2.79 (m, 1H, CH pip.), 2.34–1.90 (m, 6H, NCH_2 pip. and 2 × CH_2 pip.), 1.10 (t, J = 7.1 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$) δ: 165.65 (C=O), 156.79 (C-4 and C-2 pyr.), 150.99 (C-3 Triazole and C-6 pyr.), 148.41 (C-5 Triazole), 139.39 (C-4′), 138.86 (C-5a and C-4 pyr.), 138.18 (C-1′), 134.72 (C-1 phenyl), 131.94 (C-3a, C-3′ and C-5′), 130.98 (C-6), 130.86 (C-3 phenyl and C-5 phenyl), 130.11 (C-7, C-9 and C-4 phenyl), 129.56 (C-2′ and C-6′), 128.22 (C-8), 127.37 (C-3 pyr., C-2 phenyl and C-6 phenyl), 123.11 (C-2, C-9a and C-5 pyr.), 120.84 (C-1), 112.01 (C-3), 64.51 (CH_2N), 61.68 (CH_2), 55.03 (NCH_2 pip.), 32.32 (CH_2 pip. and CH pip.), 15.49 (CH_3). HRMS-ESI *m/z* $[M + H]^+$ Calcd for $C_{39}H_{36}N_7O_2$: 634.2930, Found: 634.2962.

5.2. X-ray data

The structure of compounds **2b-d**, **1a**, **1d**, **1g**, **1j** and **1k** has been established by X-ray crystallography (Figs. 3 and 4). Colorless single crystal of **2b** was obtained by slow evaporation from chloroform: monoclinic, space group $P2_1/c$, a = 11.9390(11) Å, b = 13.8893(9) Å, c = 18.5001(19) Å, α = 90°, β = 100.959(7)°, γ = 90°, V = 3011.8(5) Å^3, Z = 8, δ(calcd) = 1.267 Mg m^{-3}, FW = 287.31 for $C_{16}H_{17}NO_4$, F(000) = 1216. Colorless single crystal of **2c** was obtained by slow evaporation from methanol/dichloromethane (20/80) solution: monoclinic, space group $P2_1/n$, a = 14.653(3) Å, b = 5.1458(12) Å, c = 18.842(4) Å, α = 90°, β = 94.447(14)°, γ = 90°, V = 1416.4(5) Å^3, Z = 4, δ(calcd) = 1.347 Mg m^{-3}, FW = 287.31 for $C_{16}H_{17}NO_4$, F(000) = 608. Colorless single crystal of **2d** was obtained by slow evaporation from methanol/dichloromethane (20/80) solution: triclinic, space group $P\text{-}_1$, a = 8.628(9) Å, b = 9.798(2) Å, c = 10.095(3) Å, α = 73.02(2)°, β = 71.00(3)°, γ = 73.95(5)°, V = 756.1(8) Å^3, Z = 1, δ(calcd) = 1.262 Mg m^{-3}, FW = 574.61 for $C_{32}H_{34}N_2O_8$, F(000) = 304. Colorless single crystal of **1a** was obtained by slow evaporation from methanol/dichloromethane (30/70) solution: triclinic, space group $P\text{-}_1$, a = 10.0140(10) Å, b = 10.877(2) Å, c = 17.580(2) Å, α = 79.642(9)°, β = 86.041(8)°, γ = 65.016(8)°, V = 1707.3(4) Å^3, Z = 2, δ(calcd) = 1.311 Mg m^{-3}, FW = 673.96 for $C_{39}H_{35}N_5O_3$,0.48($CH_2Cl_2O_2$),0.19(O), F(000) = 710. Colorless single crystal of **1d** was obtained by slow evaporation from methanol/dichloromethane (30/70) solution: triclinic, space group $P\text{-}_1$, a = 8.3759(10) Å, b = 12.6418(13) Å, c = 16.6718(18) Å, α = 109.502(7)°, β = 96.482(8)°, γ = 103.928(7)°, V = 1578.7(3) Å^3, Z = 2, δ(calcd) = 1.308 Mg m^{-3}, FW = 621.72 for $C_{39}H_{35}N_5O_3$, F(000) = 656. Colorless single crystal of **1g** was obtained by slow evaporation from methanol/dichloromethane (20/80) solution: triclinic, space group $P\text{-}_1$, a = 9.6882(14) Å, b = 11.2689(13) Å, c = 19.639(2) Å, α = 77.504(8)°, β = 83.067(11)°, γ = 72.264(11)°, V = 1990.4(4) Å^3, Z = 2, δ(calcd) = 1.347 Mg m^{-3}, FW = 807.16 for $C_{39}H_{35}N_5O_3$,1.56($CHCl_3$), F(000) = 836. Colorless single crystal of **1j** was obtained by slow evaporation from methanol/dichloromethane (30/70) solution: triclinic, space group $P\text{-}_1$, a = 9.7518(8) Å, b = 11.1004(8) Å, c = 17.9247(12) Å, α = 86.622(5)°, β = 82.713(5)°, γ = 66.141(5)°, V = 1760.1(2) Å^3, Z = 2, δ(calcd) = 1.398 Mg m^{-3}, FW = 741.09 for $C_{39}H_{35}N_5O_3$,$CHCl_3$, F(000) = 772. Pale-yellow single crystal of **1k** was obtained by slow evaporation from methanol/dichloromethane (30/70) solution: monoclinic, space group C2/c, a = 18.0617(14) Å, b = 9.4210(8) Å, c = 43.021(3) Å, α = 90°, β = 93.345(5)°, γ = 90°, V = 7307.9(10) Å^3, Z = 8, δ(calcd) = 1.296 Mg m^{-3}, FW = 712.78 for $C_{39}H_{34}FN_5O_2$,5(H_2O) F(000) = 3016. Full crystallographic results have been deposited at the Cambridge Crystallographic Data Centre (CCDC-1014944, CCDC-891817, CCDC-891816, CCDC-891812, CCDC-

891811, CCDC-891813, CCDC-891814, CCDC-891815, respectively), UK, as Supplementary Material [38]. The data were corrected for Lorentz and polarization effects and for empirical absorption correction [39]. The structure was solved by direct methods Shelx 2013 [40] and refined using Shelx 2013 [40] suite of programs.

5.3. Biology

5.3.1. Cell culture

The human leukemic cell lines U937, K562, HL60, U266 and Jurkat were grown in RPMI 1640 medium (Life Technology, France) supplemented with 10% fetal calf serum (FCS), antibiotics (100 U/mL penicillin, 100 μg/mL streptomycin) and L-glutamin, (Eurobio, France) 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 (T lymphoproliferative agent) (PHA)-induced cells. PBMNC from blood of healthy volunteers were obtained following centrifugation on Ficoll gradient. Cells were then incubated in medium alone or induced to enter cell cycle by the addition of PHA (5 μg/mL, Murex Biotech Limited, Dartford, UK).

5.3.2. 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 Buffer Saline) and plated in quadruplicate into microtiter-plate wells in 100 μL culture media with or without our various compounds at increasing concentrations (0, 1, 5, 10, 20 and 50 μM) during 1, 2 and 3 days. After 3 h of incubation at 37 °C with 20 μL MTS/well, the plates were read by using an ELISA microplate reader (Thermo, Electrocorporation) at 490 nm wavelength. The amount of colour produced was directly proportional to the number of viable cells. The results are expressed as the concentrations inhibiting cell growth by 50% after a 3 days incubation period. The 50% cytotoxic concentrations (CC_{50}) were determined by linear regression analysis, expressed in μM ± SD (Microsoft Excel).

5.3.3. Annexin V staining by flow cytometry

Cells (2×10^5) were incubated for 3 days with increasing doses of **1e** (0, 1, 5, and 10). Experiments were performed with APC-Annexin V (Biolegend, CA) according to the manufacturer's instructions. Briefly, cells (2×10^4) were incubated with 5 mL of APC-Annexin V resuspended in 295 mL of 1X binding buffer for 10 min at room temperature in the dark. Then, cells were analyzed by flow cytometry. The APC-Annexin V-positive cells were considered as apoptotic. Flow cytometry analysis was performed with a BD FACS Canto II flow cytometer (Becton-Dickinson, France) and experiments were analyzed using the Diva software.

5.3.4. Computational prediction of toxicity and drug relevant properties

Calculations of Clog *P* and calculations of Topological Polar Surface Area (TPSA), number of hydrogen bond acceptor (nON) and donor (nNH/OH) atoms, and any violations to the Lipinski's "rule of five" (logP $\leq$ 5, molecular weight $\leq$500, number of hydrogen bond acceptors $\leq$10, and number of hydrogen bond donors $\leq$5) [34]; were performed using the MIPC server at http://www.molinspiration.com/cgi-bin/properties [33].

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2016.02.047.

References

[1] M.A. Lichtman, Battling the hematological malignancies: the 200 years' war, The Oncologist 13 (2008) 126–138.
[2] G. Campiani, S. Butini, C. Fattorusso, F. Trotta, S. Franceschina, M. De Angelis, K.S. Nielsen, Novel Aryl Piperazine Derivatives with Medical Utility, 2006. WO2006072608.
[3] G. Campiani, F. Aiello, M. Fabbrini, E. Morelli, A. Ramunno, S. Armaroli, V. Nacci, A. Garofalo, G. Greco, E. Novellino, G. Maga, S. Spadari, A. Bergamini, L. Ventura, B. Bongiovanni, M. Capozzi, F. Bolacchi, S. Marini, M. Coletta, G. Guiso, S. Caccia, Quinoxalinylethylpyridylthioureas (QXPTs) as potent non-nucleoside HIV-1 reverse transcriptase (RT) inhibitors. Further SAR studies and identification of a novel orally bioavailable hydrazine-based antiviral agent, J. Med. Chem. 44 (2001) 305–315.
[4] S. Schann, S. Mayer, S. Gardan, Pyrrolo[1,2-*a*]quinoxaline Derivatives as Adenosine A3 Receptor Modulators and Uses Thereof, 2007. EP1798233.
[5] J. Guillon, P. Grellier, M. Labaied, P. Sonnet, J.-M. Léger, R. Déprez-Poulain, I. Forfar-Bares, P. Dallemagne, N. Lemaître, F. Péhourcq, J. Rochette, C. Sergheraert, C. Jarry, Synthesis, antimalarial activity and molecular modeling of new pyrrolo[1,2-*a*]quinoxalines, bispyrrolo[1,2-*a*]quinoxalines, bispyrido[3,2-*e*]pyrrolo[1,2-*a*]pyrazines and bispyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazines, J. Med. Chem. 47 (2004) 1997–2009.
[6] J. Guillon, I. Forfar, M. Mamani-Matsuda, V. Desplat, M. Saliège, D. Thiolat, S. Massip, A. Tabourier, J.-M. Léger, B. Dufaure, G. Haumont, C. Jarry, D. Mossalayi, Synthesis, analytical behaviour and biological evaluation of new 4-substituted pyrrolo[1,2-*a*]quinoxalines as antileishmanial agents, Bioorg. Med. Chem. 15 (2007) 194–210.
[7] J. Guillon, I. Forfar, V. Desplat, S. Belisle-Fabre, D. Thiolat, S. Massip, H. Carrie, D. Mossalayi, C. Jarry, Synthesis of new 4-(*E*)-alkenylpyrrolo[1,2-*a*]quinoxalines as antileishmanial agents by suzuki-miyaura cross-coupling reactions, J. Enzyme Inhib. Med. Chem. 22 (2007) 541–549.
[8] J. Guillon, S. Moreau, E. Mouray, V. Sinou, I. Forfar, S. Belisle-Fabre, V. Desplat, P. Millet, D. Parzy, C. Jarry, P. Grellier, New ferrocenic pyrrolo[1,2-*a*]quinoxaline derivatives : synthesis, and in vitro antimalarial activity, Bioorg. Med. Chem. 16 (2008) 9133–9144.
[9] J. Guillon, E. Mouray, S. Moreau, C. Mullié, I. Forfar, V. Desplat, S. Belisle-Fabre, F. Ravanello, A. Le-Naour, N. Pinaud, J.-M. Léger, G. Gosmann, C. Jarry, G. Déléris, P. Sonnet, P. Grellier, New ferrocenic pyrrolo[1,2-*a*]quinoxaline derivatives: Synthesis, and *in vitro* antimalarial activity – part II, Eur. J. Med. Chem. 46 (2011) 2310–2326.
[10] L. Ronga, M. Del Favero, A. Cohen, C. Soum, P. Le Pape, S. Savrimoutou, N. Pinaud, C. Mullié, S. Daulouede, P. Vincendeau, N. Farvacques, P. Agnamey, F. Pagniez, S. Hutter, N. Azas, P. Sonnet, J. Guillon, Design, synthesis and biological evaluation of novel 4-Alkapolyenylpyrrolo[1,2-a]quinoxalines as antileishmanial agents – part III, Eur. J. Med. Chem. 81 (2014) 378–393.
[11] J. Milne, K.D. Normington, M. Milburn, Tetrahydroquinoxalinone Sirtuin Modulators, 2006. WO2006094210.
[12] F. Grande, F. Aiello, O. De Grazia, A. Brizzi, A. Garofalo, N. Meamati, Synthesis and antitumor activities of a series of novel quinoxalinhydrazides, Bioorg. Med. Chem. 15 (2007) 288–294.
[13] J. Guillon, M. Le Borgne, C. Rimbault, S. Moreau, S. Savrimoutou, N. Pinaud, S. Baratin, M. Marchivie, S. Roche, A. Bollacke, A. Pecci, L. Alvarez, V. Desplat, J. Jose, Synthesis and biological evaluation of novel substituted pyrrolo[1,2-*a*]quinoxaline derivatives as inhibitors of the human protein kinase CK2, Eur. J. Med. Chem. 65 (2013) 205–222.
[14] V. Desplat, A. Geneste, M.-A. Begorre, S. Belisle Fabre, S. Brajot, S. Massip, D. Thiolat, D. Mossalayi, C. Jarry, J. Guillon, Synthesis of new pyrrolo[1,2-*a*]quinoxaline derivatives as potential inhibitors of Akt kinase, J. Enzyme Inhib. Med. Chem. 23 (2008) 648–658.
[15] V. Desplat, S. Moreau, A. Gay, S. Belisle-Fabre, D. Thiolat, S. Massip, D. Mossalayi, C. Jarry, J. Guillon, Synthesis and evaluation of the anti-proliferative activity of novel pyrrolo[1,2-*a*]quinoxaline derivatives, potential inhibitors of akt kinase. Part II, J. Enzyme Inhib. Med. Chem. 25 (2010) 204–215.
[16] V. Desplat, S. Moreau, S. Belisle-Fabre, D. Thiolat, J. Uranga, R. Lucas, S. Massip, L. de Moor, C. Jarry, D. Mossalayi, P. Sonnet, G. Déléris, J. Guillon, Synthesis and evaluation of the antiproliferative activity of novel isoindolo[2,1-*a*]quinoxaline and Indolo[1,2-*a*]quinoxaline derivatives, J. Enzyme Inhib. Med. Chem. 26 (2011) 657–667.
[17] H.A. Jensen, R.P. Bunaciu, J.D. Varner, A. Yen, GW5074 and PP2 kinase inhibitors implicate nontraditional c-Raf and Lyn function as drivers of retinoic acid-induced maturation, Cell. Signal 27 (2015) 1666–1675.
[18] S. Kamijo, C. Kanazawa, Y. Yamamoto, Copper- or phosphino-catalyzed reaction of alkynes with isocyanides. Regioselective synthesis of substituted

pyrroles controlled by the catalyst, J. Am. Chem. Soc. 127 (2005) 9260–9266.
[19] A.V. Gulevich, A.G. Zhdanko, R.V.A. Orru, V.G. Nenajdenko, Isocyanoacetate derivatives: synthesis, reactivity, and application, Chem. Rev. 110 (2010) 5235–5331.
[20] J. Liu, Z. Fang, Q. Zhang, Q. Liu, X. Bi, Silver-catalyzed isocyanide-alkyne cycloaddition: a general and practical method to oligosubstituted pyrroles, Angew. Chem. Int. Ed. 52 (2013) 6953–6957.
[21] M. Gao, C. He, H. Chen, R. Bai, B. Cheng, A. Lei, Synthesis of pyrroles by click reaction: silver-catalyzed cycloaddition of terminal alkynes with isocyanides, Angew. Chem. Int. Ed. 52 (2013) 6958–6961.
[22] M. Suzuki, M. Miyoshi, K. Matsumoto, Synthesis of amino acids and related compounds. 7. A convenient synthesis of 3-substituted pyrrole-2,4-dicarboxylic acid esters, J. Org. Chem. 39 (1974) 1980.
[23] J. Guillon, H. Dumoulin, P. Dallemagne, R. Reynolds, S. Rault, Synthesis and antituberculosis activity of new phenylpyrrolo[1,2-*a*]quinoxalinylpyrrole carboxylic acid derivatives, Pharm. Pharmacol. Commun. 4 (1998) 33–38.
[24] S. Ngwerume, J.E. Camp, Synthesis of highly substituted pyrroles via nucleophilic catalysis, J. Org. Chem. 75 (2010) 6271–6274.
[25] B.A. Trofimov, Preparation of pyrroles from ketoximes and acetylenes, Adv. Heterocycl. Chem. 51 (1999) 177–301.
[26] Y.-F. Wang, K.K. Toh, S. Chiba, K. Narasaka, Mn(III)-catalyzed synthesis of pyrroles from vinyl azides and 1,3-dicarbonyl compounds, Org. Lett. 10 (2008) 5019–5022.
[27] P. Liu, B. Xu, J. Li, H. Lu, LY294002 inhibits leukemia cell invasion and migration through early growth response gene 1 induction independent of phosphatidylinositol 3-kinase-Akt pathway, Biochem. Biophys. Res. Commun. 377 (2008) 187–190.
[28] S. Matsuda, A. Nakanishi, Y. Wada, Y. Kitagishi, Roles of PI3K/AKT/PTEN pathway as a target for pharmaceutical therapy, Open Med. Chem. J. 7 (2013) 23–29.
[29] H.G. Jørgensen, E.K. Allan, S.M. Graham, J.L. Godden, L. Richmond, M.A. Elliott, J.C. Mountford, C.J. Eaves, T.L. Holyoake, Lonafarnib reduces the resistance of primitive quiescent CML cells to imatinib mesylate *in vitro*, Leukemia 19 (2005) 1184–1191.
[30] S. Zhao, M. Konopleva, M. Cabreira-Hansen, Z. Xie, W. Hu, M. Milella, Z. Estrov, G.B. Mills, M. Andreeff, Inhibition of phosphatidylinositol 3-kinase dephosphorylates BAD and promotes apoptosis in myeloid leukemias, Leukemia 18 (2004) 267–275.
[31] C.W. Lindsley, The Akt/PKB family of protein kinases: a review of small molecule inhibitors and progress towards target validation: a 2009 update, Curr. Top. Med. Chem. 10 (2010) 458–477.
[32] R. Boulahjar, A. Ouach, M. Chiurato, S. Bourg, M. Ravache, R. Le Guével, S. Marionneau-Lambot, T. Oullier, O. Lozach, L. Meijer, C. Guguen-Guillouzo, S. Lazar, M. Akssira, Y. Troin, G. Guillaumet, S. Routier, Novel tetrahydropyrido[1,2-*a*]isoindolone derivatives (Valmerins): potent cyclin dependant kinase/glycogen synthase kinase 3 inhibitors with antiproliferative activities and antitumor effects in human tumor xenografts, J. Med. Chem. 55 (2012) 9589–9606.
[33] http://www.molinspiration.com/cgi-bin/properties. Accession date: July 20, 2015.
[34] D.E. Clark, S.D. Pickett, Computational methods for the prediction of drug likeness, Drug Discov. Today 5 (2000) 49–58.
[35] M.F.C. Cardoso, P.C. Rodrigues, M.E.I.M. Oliveira, I.L. Gama, I.M.C.B. da Silva, I.O. Santos, D.R. Rocha, R.T. Pinho, V.F. Ferreira, M.C.B.V. de Souza, F. de C. da Silva, F. Paes Silva Jr., Synthesis and evaluation of the cytotoxic activity of 1,2-furanonaphthoquinones tethered to 1,2,3-1H-triazoles in myeloid and lymphoid leukemia cell lines, Eur. J. Med. Chem. 84 (2014) 708–717.
[36] J.F. Bickley, T.L. Gilchrist, R. Mendonça, Hydroxylation of 1-azabicyclo[4.1.0]hept-3-enes formed by Diels-Alder reactions of benzyl 2*H*-azirine-3-carboxylate, Arkivoc vi (2002) 192–204.
[37] T.L. Gilchrist, R. Mendonça, Addition of pyrimidine and purine bases to benzyl 2*H*-azirine-3-carboxylate, Synlett 12 (2000) 1843–1845.
[38] Supplementary X-ray Crystallographic Data: Cambridge Crystallographic Data Centre, University Chemical Lab, Lensfield Road, Cambridge, CB2 1EW, UK; E-mail: deposit@chemcrys.cam.ac.uk.
[39] G.M. Sheldrick, SADABS, University of Göttingen, Germany, 1996.
[40] G.M. Sheldrick, Acta Crystallogr. Sect. A 64 (2008) 112–122.