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Research paper

Novel antileukemic compound with sub-micromolar potency against STAT5 addicted myeloid leukemia cells

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ABSTRACT

Signal Transducer and Activator of Transcription 5A and 5B (STAT5A/5B) are key effectors of tyrosine kinase oncogenes in myeloid leukemias. It is now clearly evidenced that inhibition of STAT5A/5B not only blocks the growth and survival of myeloid leukemia cells but also overcomes the resistance of leukemic cells to chemotherapy. Previous screening experiments allowed us to identify **17f** as a lead compound with promising anti-leukemic activity that blocks the phosphorylation and transcriptional activity of STAT5A/5B in myeloid leukemia cells addicted to these proteins. In light of these findings, we initiated further pharmacomodulations of **17f** to develop new derivatives with enhanced antileukemic activity. Our screening assays identified **14a**, an aminopyrimidine derivative of **17f**, as a new lead compound that: 1) blocks the growth and survival of myeloid leukemia cells at sub-micromolar concentrations, 2) targets the phosphorylation of STAT5 but also the expression of STAT5B and 3) relieves the resistance of Chronic and Acute Myeloid leukemia cells to conventional chemotherapy.

1. Introduction

Cancer is the second leading cause of death worldwide, accounting for nearly 10 million deaths in 2020 (<https://www.who.int/news-room/fact-sheets/detail/cancer>). According to the Global Cancer Observatory, leukemia ranked 13th in incidence and 10th in mortality in 2022 and is the most common cancer in children and adolescent (<https://gco.iarc.who.int/media/globocan/factsheets/cancers/36-leukaemia-fact-sheet.pdf>). Signal transducers and activators of transcription 5A (STAT5A) and 5B (STAT5B) belong to the JAK/STAT signaling pathway. These two transcription factors, which share 90 % homology, are critical downstream effectors of tyrosine kinase oncogenes (TKOs). The latter include BCR-ABL and Fms-like receptor tyrosine kinase 3 with internal tandem duplications (FLT3-ITD), which are critical transforming agents in CML and AML, respectively [1]. Both STAT5A and STAT5B are important drivers of CML development and AML cell proliferation and survival [2–6].

Moreover, as resistance and relapse occur in CML and AML cases,

alternative approaches targeting key effectors of BCR-ABL and FLT3-ITD are needed. To this end, STAT5 fulfills all the criteria of an important drug target in CML and AML [7,8]. Indeed, in the case of CML, STAT5 proteins play a key role in the maintenance of the leukemic stem cell pool and stimulate resistance to TKI (tyrosine kinase inhibitor) treatment [2,9,10]. In AML, the maintenance of P-STAT5 expression increases the risk of relapse [11]. Therefore, targeting STAT5 would help to overcome the resistance of CML and AML to chemotherapy.

Since the late 2000s, the development of inhibitors targeting STAT5 has been the subject of intense research. Indirect STAT5 inhibitors with antileukemic activity include repositioning drugs such as the antidiabetic PPAR ligands or the antipsychotic pimozide (Fig. 1). It was demonstrated that pioglitazone, one of these PPAR ligands, reduces STAT5 gene expression and resensitizes TKI-resistant CML stem cells when combined with Imatinib Mesylate (IM) [12–15]. Pimozide, for its part, decreases the survival of CML cell lines by inhibiting STAT5 phosphorylation. It also overcomes TKI resistance in BCR-ABL T315I mutant cells [16]. In AML cells expressing FLT3-ITD, pimozide

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inhibits P–Y-STAT5 and STAT5-dependent gene expression and synergizes with FLT3 inhibitors to induce apoptosis [17]. On the other hand, high-throughput assays based on fluorescence polarization, surface plasmon resonance (SPR) binding experiments combined with *in silico* studies allowed the identification of STAT5 inhibitors that block protein dimerization and consequently the transcriptional activity of STAT5 [18–20]. Among these is the salicylic acid-based AC-4-130 and the catechol biphosphate derivative Stafib2, two selective blockers of the STAT5B SH2 domain (Fig. 1) [20,21]. AC-4-130 and Stafib2 inhibit the phosphorylation of STAT5 and the growth and/or survival of CML and AML cell lines. In addition, using xenograft tumor models, AC-4-130 was shown to reduce the expansion of AML cells *in vivo* [22].

Over the past decade, our research group has focused on the design and synthesis of inhibitors of STAT5 signaling in myeloid leukemias. Following an initial cell-based screening of PPAR ligands selected from our chemolibrary [23,24] and subsequent pharmacomodulation studies, we identified 17f as a novel inhibitor of STAT5A/5B signaling with promising antileukemic activity in CML and AML cells addicted to these proteins [25]. Compound 17f pharmacophore is based on a tetrahydroquinoline (THQ) scaffold bearing a 3-pyridinyl group at position 6. We also showed that 17f was able to sensitize leukemic cells that had acquired resistance to IM and cytarabine (Ara-C), two conventional therapeutic agents used in the treatment of CML and AML, respectively. Indeed, 17f strongly inhibited the growth and survival of resistant CML and AML cells when combined with IM or Ara-C [26]. Based on these encouraging results, we recently published the synthesis of 10 new analogs of 17f. Among them, compounds 1 and 2 (Fig. 1), which contain a 3- and 4-pyridinyl moiety, respectively, at position 5 on the THQ ring, showed comparable or slightly elevated growth inhibitory effects in CML and AML cells, in addition to the inhibition of P–Y-STAT5 expression and activity [27]. Finally, compounds 1 and 2 slightly enhanced the sensitization of resistant CML cells when used in combination with IM.

In order to improve the antileukemic activity of 17f, 1 and 2 compounds, we prepared two series of derivatives focusing on the THQ ring positions 5, 6 and 7, which were found to be essential for the antileukemic activity of our molecules (Fig. 2). The first series contained 9 compounds designed by introduction of 2-, 3- and 4-pyridylmethyl moieties on the THQ cycle with the aim of including a degree of freedom between the pyridinyl and THQ rings. The second series of derivatives is based on the synthesis of 15 compounds bearing variously substituted polynitrogen heterocycles such as pyridazine, pyrazine and

pyrimidine on the THQ ring. All these molecules were biologically tested on CML (KU812) and AML (MV-4-11) cell lines, which are dependent on STAT5 signaling. Cell growth inhibition experiments showed that among these two series, compound 14a was the most potent inhibitor, with sub-micromolar EC₅₀ values in blocking leukemic cell growth. These results were confirmed in other CML and AML cell lines. Importantly, at sub-micromolar concentrations, 14a was found to be inactive in inhibiting the growth of primary bone marrow stromal cells, which are important components of the hematopoietic and leukemic micro-environment. Interestingly, we also found that 14a blocks not only STAT5A/5B phosphorylation but also STAT5B expression in these leukemic cells. Finally, 14a was shown to relieve the resistance of CML and AML cell lines to IM and Ara-C, respectively.

2. Results and discussion

2.1. Chemistry

Two series of new derivatives of compounds 17f, 1 and 2 were synthesized by pharmacomodulations on the tetrahydroquinoline (THQ) ring of the pharmacophore. The first series consists of 2-, 3- and 4-pyridylmethyl derivatives at positions 5, 6 and 7 of the THQ, and the second comprises analogs bearing diversely substituted polynitrogen heterocycles at positions 5 and 6 of the THQ cycle. All compounds were prepared using 1-(2-((1*H*-indol-5-yl)oxy)ethyl)-5-bromo-4,4-dimethyl-1,2,3,4-tetrahydroquinoline 3a, 1-(2-((1*H*-indol-5-yl)oxy)ethyl)-6-bromo-4,4-dimethyl-1,2,3,4-tetrahydroquinoline 3b and 1-(2-((1*H*-indol-5-yl)oxy)ethyl)-7-bromo-4,4-dimethyl-1,2,3,4-tetrahydroquinoline 3c as starting brominated reagents, previously reported in foregoing works [25,27].

In order to enhance the antileukemic effect and STAT5 inhibition, the first modification investigated aimed to increase the conformational freedom of the key pyridine-THQ backbone, strongly limited by the presence of the THQ gem-dimethyl group, especially when the pyridinyl group is located at positions 5 or 6 of the THQ. As foregoing results have shown that this dimethyl group was important for biological activities (unpublished work), we chose to study homologation between the THQ core and the pyridine, to compare activities with more restrained 17f, 1 and 2 compounds. Homologation strategy has been extensively used in medicinal chemistry and has proved to be successful in numerous lead optimization steps [28]. Introducing a methylene should permit to enhance spatial arrangements possibilities due the higher

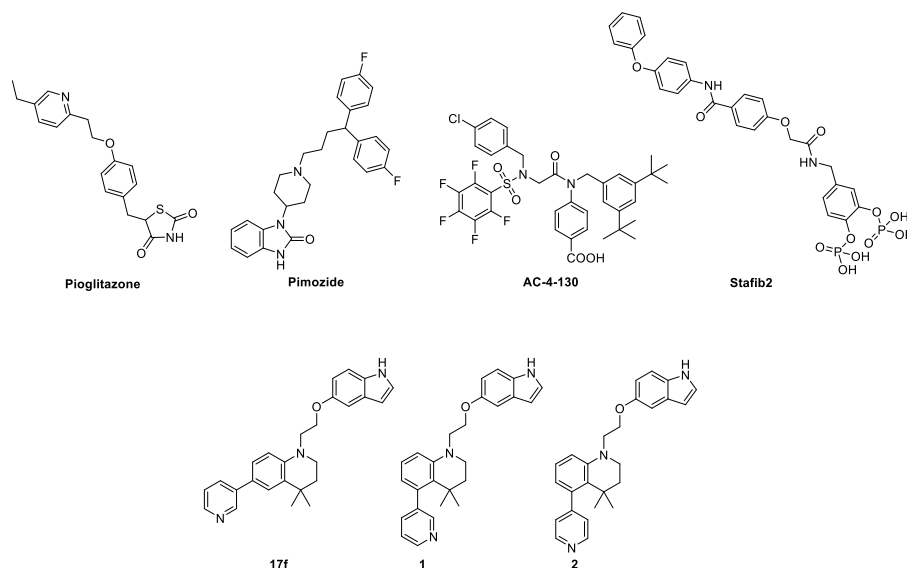


Fig. 1. STAT5 inhibitors.

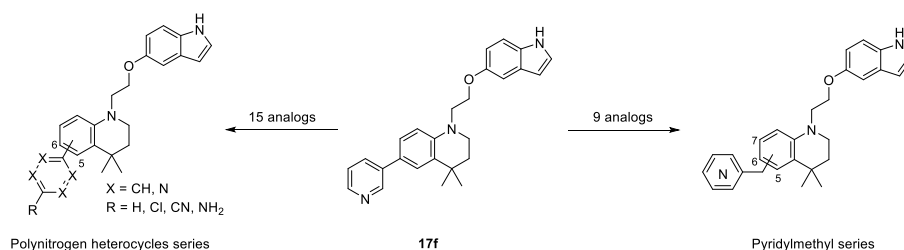


Fig. 2. Pharmacomodulations performed on compound **17f**.

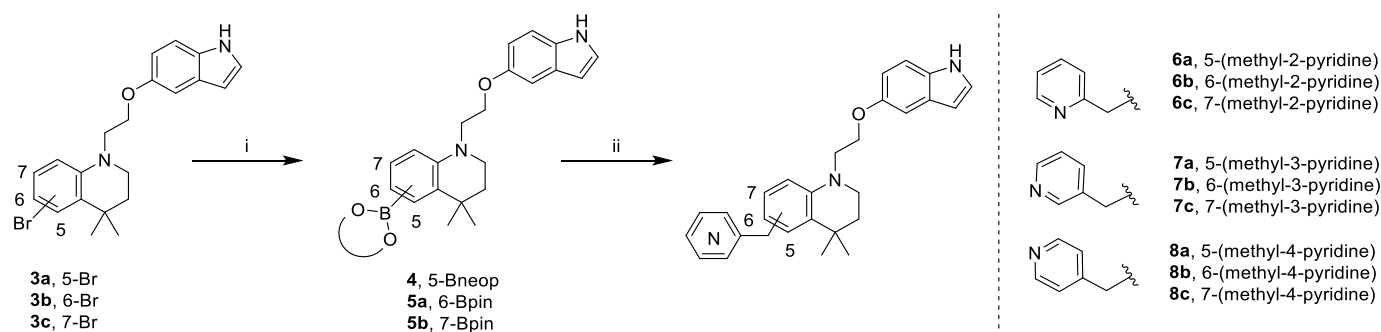
conformational freedom obtained, thus avoiding the steric hindrance between the dimethyl group and the substituent particularly at positions 5 and 6 of the THQ (Scheme 1). As 2-, 3- and 4-pyridylmethyl boronic derivatives were not commercially available, the chosen strategy was to convert starting brominated reagents **3a-c** into respective boronate esters. As previously reported, **3a-c** have been converted into neopentyl boronate ester **4** with moderate yields and pinacol boronate esters **5a,b** with good yields [27]. Suzuki coupling of **4**, **5a** and **5b** with various (bromomethyl)pyridine derivatives using conditions developed by Zhu et al., afforded compounds **6a-c**, **7a-c** and **8a-c** with low to excellent yields (4%-quantitative) [29]. The lower yields in C5 substitution (**6a**, **7a** and **8a**) could be explained by the sterically hindrance due to *gem*-dimethyl tetrahydroquinoline substitution and to the formation of deborylated by-product.

In a second phase, we investigated the introduction of polynitrogen heterocycles such as pyridazine, pyrazine and pyrimidine on the THQ ring. Indeed, we have previously shown that the addition of mononitrogen hydrogen-bonding acceptor substituents, i.e. pyridine, on the THQ ring, enhances STAT5 inhibition along with anti-leukemic activity [25]. Due to the previous modest biological results with analogs substituted at position 7 of the THQ compounds (**6c**, **7c**, **8c**), only positions 5 and 6 of the THQ were explored. Following the same strategy as in the pyridylmethyl series, we used boronate esters **4** and **5a** to introduce polynitrogen heterocycles at position 5 and 6 of the THQ by Suzuki cross-coupling reactions with corresponding commercial brominated heterocycles. However, the introduction of the 2-amino-5-pyrimidine substituent, for which the corresponding boronic acid (2-amino-pyrimidine-5-boronic acid) was commercially available, was carried out directly from starting brominated reagents **3a** and **3b** to yield **14a** and **14b** (Scheme 2). All these polynitrogen derivatives (**9a-16b**) were obtained with low to very good yields (28–93%). Though, compound **10a** was not isolated due to the poor reaction conversion rate combined with fruitless purification.

2.2. In vitro antileukemic activity of pyridylmethyl and polynitrogen derivatives

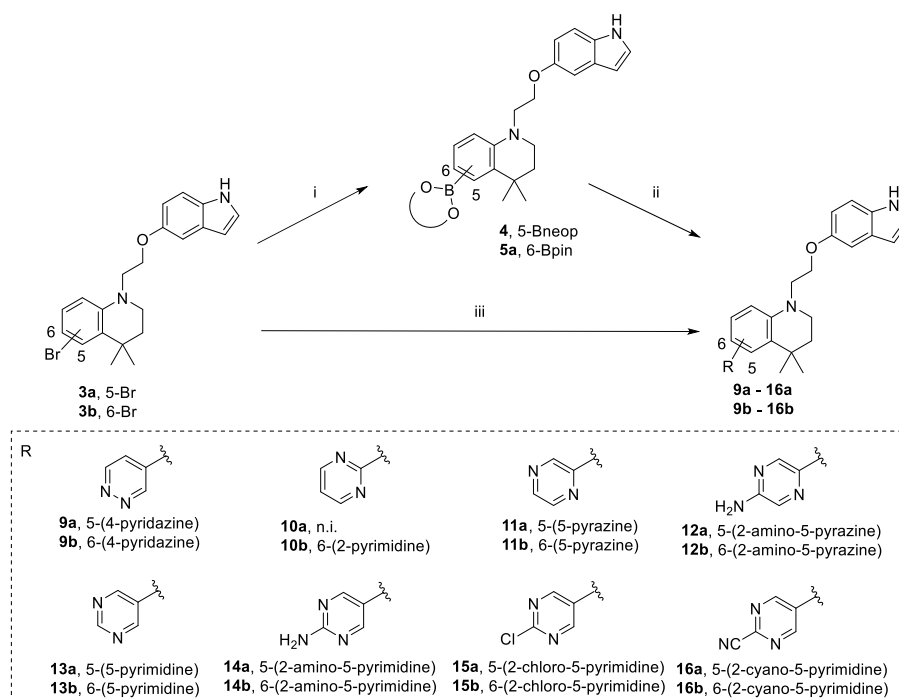
Initial screenings were carried out to determine the growth inhibitory property (EC₅₀ value) of pyridylmethyl and polynitrogen derivatives of **17f**, **1** and **2** that were first tested on two different leukemic cells: KU812 (CML) and MV-4-11 (AML) cell lines.

Firstly, in the pyridylmethyl series, as shown in Table 1, compounds **6c**, **7c** and **8c** substituted at position 7 on the THQ ring were less effective in inhibiting leukemic cell growth than **17f**, **1** and **2**. These results confirmed previous work hypothesis that pharmacomodulations at positions 7 or 8 of the THQ ring led to a significant loss of antileukemic activity [27]. It is worth mentioning that introduction of a methyl-2-pyridine group (**6a-c**) on the THQ part did not improve the activity. Overall in this first series, only compounds **7a** and **7b**, bearing a methyl-3-pyridine group in positions 5 and 6, and **8a**, carrying a methyl-4-pyridine group in position 5 of the THQ, showed similar antileukemic activity compared to **17f**, **1** and **2**. Secondly, in the polynitrogen series, 4-pyridazine (**9a,b**), 5-pyrazine (**11a,b** and **12a,b**) and non-substituted 2- or 5-pyrimidine (**10b** and **13a,b**) derivatives showed less growth inhibitory activity than **17f** (Table 2). However, the introduction of a 2-substituted 5-pyrimidine moiety on the THQ ring led to similar or higher inhibitory activity compared to **17f**. Indeed, compounds **15a,b** and **16a,b** bearing respectively a 2-chloro- or 2-cyano-5-pyrimidine showed a similar antileukemic activity as **17f**. More interestingly, analogs **14a** and **14b**, carrying a 2-amino-5-pyrimidine in positions 5 and 6, respectively, led to the highest anti-leukemic activity among all compounds, **14a** being the most potent inhibitor with sub-micromolar EC₅₀ values on both evaluated cell lines. These results were confirmed in K562 and KCL-22 (CML) as well as in MOLM-13 (AML) cell lines (Table 3) for analogs **14a** and **14b**. All other pyridylmethyl and polynitrogen derivatives molecules that were not retained in the first screening were definitively discarded after testing in other leukemic cell lines (Supporting information; Tables S1 and S2). Importantly, EC₅₀ values obtained for **14a** in all leukemic cell lines were below the micromolar range indicating that **14a** was much more efficient than **17f** in inhibiting leukemic cell viability.



Scheme 1. Synthesis of compounds **6a-8c**^a.

^aReagents and conditions: (i) bis(neopentylglycolato)diborane (for compound **4**) or bis(pinacolato)diborane (for compound **5a** and **5b**), PdCl₂(dppf), KOAc, dry dioxane, 100 °C, 12 h, 63–68%; (ii) (bromomethyl)pyridine, Pd(PPh₃)₄, Na₂CO₃, 1,2-DME/water, 95 °C, 2 h, 4%-quantitative.



Scheme 2. Synthesis of compounds **9a-16b**^a.

^aReagents and conditions: (i) bis(neopentylglycolato)diborane (for compound **4**) or bis(pinacolato)diborane (for compound **5a**), PdCl₂(dppf), KOAc, dry dioxane, 100 °C, 12 h, 63–65 %; (ii) RBr, PdCl₂(dppf), Na₂CO₃, dioxane/water, 100 °C, 5 h, 61–93 %; (iii) (2-Aminopyrimidin-5-yl)boronic acid, PdCl₂(PPh₃)₂, K₂CO₃, DMF/eau, 90 °C, 5 h, 28–93 %; n.i.: not isolated.

Table 1

EC₅₀ values of pyridylmethyl derivatives **6a-8c** for KU812 and MV-4-11 cell lines.

compound	EC ₅₀ (μM) ^a	
	KU812	MV-4-11
17f	5.4 ± 0.0	3.6 ± 0.5
1	5.8 ± 0.8	5.9 ± 0.7
2	2.5 ± 0.1	2.8 ± 0.4
6a	74.4 ± 11.1	12.4 ± 2.0
6b	11.5 ± 1.9	7.7 ± 1.7
6c	>100	34.2 ± 2.8
7a	9.3 ± 1.1	3.1 ± 0.3
7b	5.3 ± 0.9	4.9 ± 1.1
7c	25.1 ± 4.5	20.5 ± 4.1
8a	2.7 ± 0.4	1.3 ± 0.1
8b	21.3 ± 3.6	25.8 ± 2.0
8c	>100	14.3 ± 1.2

^a Cells were treated with concentrations ranging from 100 nM to 10 μM for 48 h. Cytotoxicity was determined by MTT assays, and EC₅₀ values were calculated using GraphPad Prism 8 software (n = 3 in triplicate, mean ± SEM).

2.3. **14a**, a novel inhibitor with enhanced antileukemic activity

To analyze the growth inhibitory properties of **14a** and **14b**, CML and AML cells were treated with 5 μM (concentration that approximately corresponds to that of the **17f** EC₅₀ values) of **14a**, **14b** and **17f** during 72 h and cell viability was daily determined (Fig. 3). Data clearly indicated that **14a** has the most important antiproliferative activity compared to **17f** and to a lesser extent **14b** in all tested leukemic cell lines.

We then determined the cytotoxic effect of **14a** and **14b** on human bone marrow mesenchymal stromal cells (BM MSC), HS27A and primary MSC. Results showed that cytotoxicity of **14a** and **14b** on HS27A and MSC cells was almost identical and approximately 4- to 5-fold higher (MSC) and less than 2-fold (HS27A) than cytotoxicity observed for **17f**

Table 2

EC₅₀ values of polynitrogen compounds **9a-16b** for KU812 and MV-4-11 cell lines.

compound	EC ₅₀ (μM) ^a	
	KU812	MV-4-11
17f	5.4 ± 0.0	3.6 ± 0.5
9a	23.5 ± 11.6	14.0 ± 6.6
9b	44.6 ± 10.7	16.0 ± 3.6
10b	29.5 ± 10.7	4.5 ± 2.0
11a	39.3 ± 16.7	15.3 ± 4.6
11b	48.1 ± 6.9	7.7 ± 2.3
12a	21.2 ± 4.8	14.6 ± 2.2
12b	29.2 ± 4.0	13.7 ± 5.0
13a	>100	51.9 ± 20.6
13b	8.3 ± 1.7	4.2 ± 0.8
14a	0.6 ± 0.1	0.3 ± 0.1
14b	2.3 ± 0.5	0.4 ± 0.1
15a	6.7 ± 3.2	9.0 ± 2.3
15b	1.3 ± 0.5	2.4 ± 0.6
16a	4.7 ± 2.0	5.1 ± 1.0
16b	2.4 ± 0.9	3.5 ± 0.5

^a Cells were treated with concentrations ranging from 100 nM to 10 μM for 48 h. Cytotoxicity was determined by MTT assays, and EC₅₀ values were calculated using GraphPad Prism 8 software (n = 3 in triplicate, mean ± SEM).

(Table 3). We then focus on compound **14a** to compare its efficacy with pimozide and AC-4-130, two previously identified STAT5 inhibitors with antileukemic properties [16,20]. EC₅₀ values were determined for both CML and AML cell lines (Table 4). Results clearly demonstrated that **14a** was 5–20 times more efficient in inhibiting leukemic cell growth than AC-4-130 and pimozide. The cytotoxicity of these three compounds against stromal cells was also compared to demonstrate minimal differences between them.

Table 3EC₅₀ values of **14a** and **14b** compared to **17f** for CML, AML and bone marrow mesenchymal stromal cells (BM MSC).

EC ₅₀ (μM) ^a	CML			AML		BM MSC	
	KU812	K562	KCL-22	MV-4-11	MOLM-13	HS27A	MSC
17f	5.4 ± 0.0	8.7 ± 1.5	5.6 ± 0.8	3.6 ± 0.5	3.3 ± 1	63.3 ± 9.7	57.6 ± 6.6
14a	0.6 ± 0.1	0.8 ± 0.2	0.5 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	44.1 ± 11.7	12.0 ± 4.4
14b	2.3 ± 0.5	2.9 ± 0.9	0.3 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	43.9 ± 8.5	14.5 ± 4.8

^a Cells were treated with concentrations ranging from 100 nM to 10 μM for 48 h. Cytotoxicity was determined by MTT assays, and EC₅₀ values were calculated using GraphPad Prism 8 software (n = 3 in triplicate, mean ± SEM).

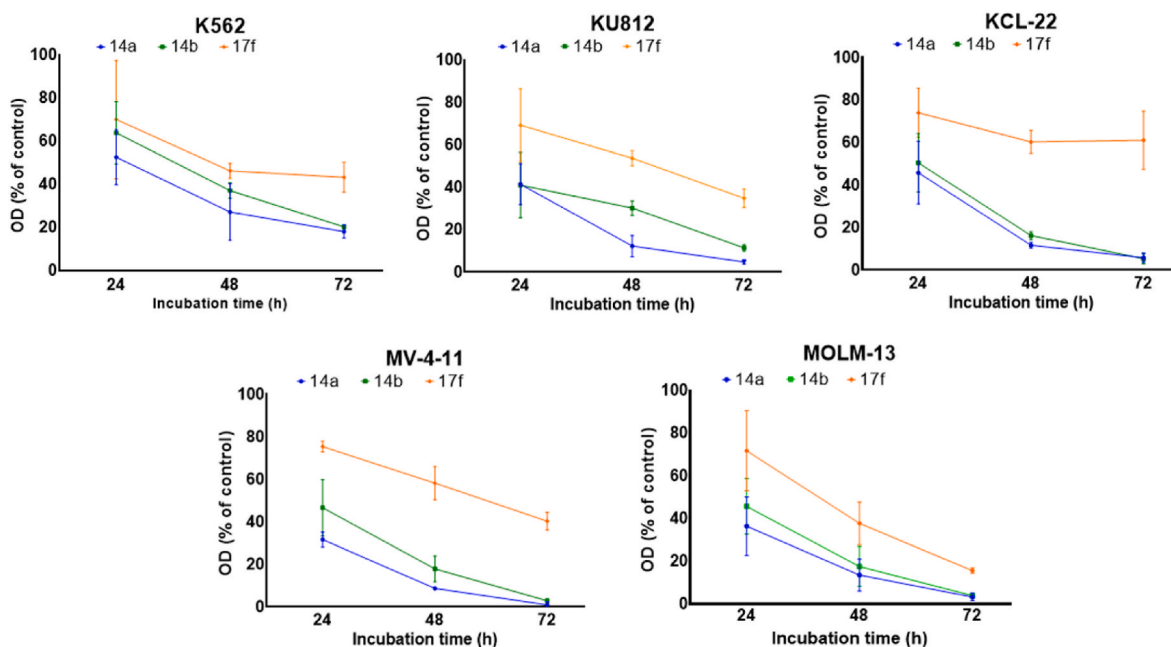


Fig. 3. **14a** induces the best growth inhibitory effect on CML and AML cells. Growth kinetics of K562, KU812, KCL-22, MV-4-11 and MOLM-13 cells treated with compounds **17f**, **14a** and **14b**. Cells were treated with 5 μM of inhibitors for 24, 48 or 72 h cytotoxic effect was determined by MTT assays. Results are presented as percentage of control. Values were calculated by using GraphPad Prism 8 software (n = 3 in triplicate, mean ± SEM).

Table 4EC₅₀ values of **14a**, **AC-4-130** and pimoziide for CML, AML and bone marrow mesenchymal stromal cells (BM MSC).

EC ₅₀ (μM) ^a	CML			AML		BM MSC	
	KU812	K562	KCL-22	MV-4-11	MOLM-13	HS27A	MSC
AC-4-130	4.3 ± 0.6	10.1 ± 0.1	15.3 ± 1.2	2.6 ± 0.9	2.8 ± 0.9	27.8 ± 4	18.1 ± 7.9
Pimoziide	2.9 ± 0.9	3.5 ± 0.5	9.3 ± 1	3.2 ± 0.6	5.9 ± 0.7	57.5 ± 6	18.2 ± 7.9
14a	0.6 ± 0.1	0.8 ± 0.2	0.5 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	44.1 ± 11.7	12.0 ± 4.4

^a Cells were treated with concentrations ranging from 100 nM to 10 μM for 48 h. Cytotoxicity was determined by MTT assays, and EC₅₀ values were calculated using GraphPad Prism 8 software (n = 3 in triplicate, mean ± SEM).

2.4. **14a** inhibits the phosphorylation/expression of STAT5A/5B

We then evaluated the impact of **14a** on the phosphorylation and expression of STAT5A/5B in STAT5 addicted KU812 and MV-4-11 cell lines (Fig. 4A). Concentrations corresponding to EC₅₀ values of **14a** obtained for KU812 (0.6 μM) and MV-4-11 (0.3 μM) cells were used to determine its effect on STAT5 activity and/or expression. Extracts from leukemic cells treated or not (DMSO) with **14a** for 24h were prepared and analyzed by immunoblotting. Membranes were simultaneously blotted with anti-P^{Y694/699} STAT5A/5B and STAT5A antibodies or with anti-P^{Y694/699} STAT5A/5B and STAT5B antibodies, then revealed with anti-rabbit or anti-mouse antibodies coupled to the corresponding fluorophores as mentioned in the experimental section. Results showed

that the phosphorylation of STAT5A/5B was clearly inhibited in KU812 and MV-4-11 cells treated with **14a**. Surprisingly, we also found that STAT5B but not STAT5A expression was markedly reduced indicating that **14a** might target STAT5B expression in these leukemic cells. A recurrent activating mutation of the *STAT5B* gene (*STAT5B*^{N642H}) has been described in some types of leukemia and lymphoma patients [30, 31]. Data from the literature also showed that *STAT5B*^{N642H} behave as an oncogene both *in vitro* and *in vivo* [32]. We then reasoned that **14a** might be able to inhibit expression of *STAT5B*^{N642H} in cells expressing this oncogene. The hematopoietic cell line Ba/F3 overexpressing a flag-tagged form of *STAT5B*^{N642H} was treated with increasing concentrations of **14a**. Results clearly showed that **14a** was able to reduce expression of *STAT5B*^{N642H} in a dose dependent fashion (Fig. 4B). These

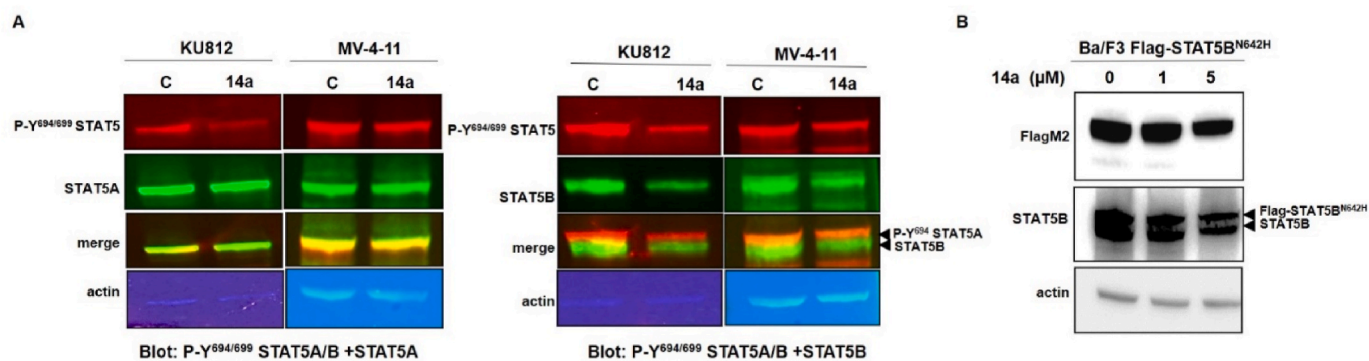


Fig. 4. 14a inhibits the phosphorylation of STAT5A/5B and expression of STAT5B or STAT5B^{N642H}. A) Protein extracts from KU812 or MV-4-11 cells treated with 14a (KU812: 0.6 μM; MV-4-11: 0.3 μM) or DMSO (C) for 24 h were analyzed by western blot. Membranes were incubated with both anti-P-Y^{694/699} STAT5A/5B and anti-STAT5A antibodies (left panel) or with anti-P-Y^{694/699} STAT5A/5B and anti-STAT5B (right panel). Actin served as loading control; B) Ba/F3 cells overexpressing a flag-tagged form of the STAT5B^{N642H} oncogene were treated with indicated concentrations of 14a for 24 h. Protein extracts were prepared and analyzed by western blot with an anti-Flag antibody (FlagM2). Membranes were reprobed with an anti-STAT5B antibody. Actin served as loading control.

data are in line with our previously published results indicating that 17f also interferes with expression of STAT5B^{N642H} [26]. Collectively, our data indicated that STAT5B and STAT5B^{N642H} expression might be indirectly targeted by 14a through mechanisms that probably involve the stability/degradation of the protein.

2.5. 14a overcomes the resistance of CML and AML cells to chemotherapy

In the next set of experiments, we asked whether 14a would break down the resistance of myeloid leukemia cells to the chemotherapeutic agents IM and Ara-C that are clinically used to treat CML and AML respectively. Different types of resistance have been described in the literature. First, resistance to imatinib (IM) and Ara-C can be acquired by

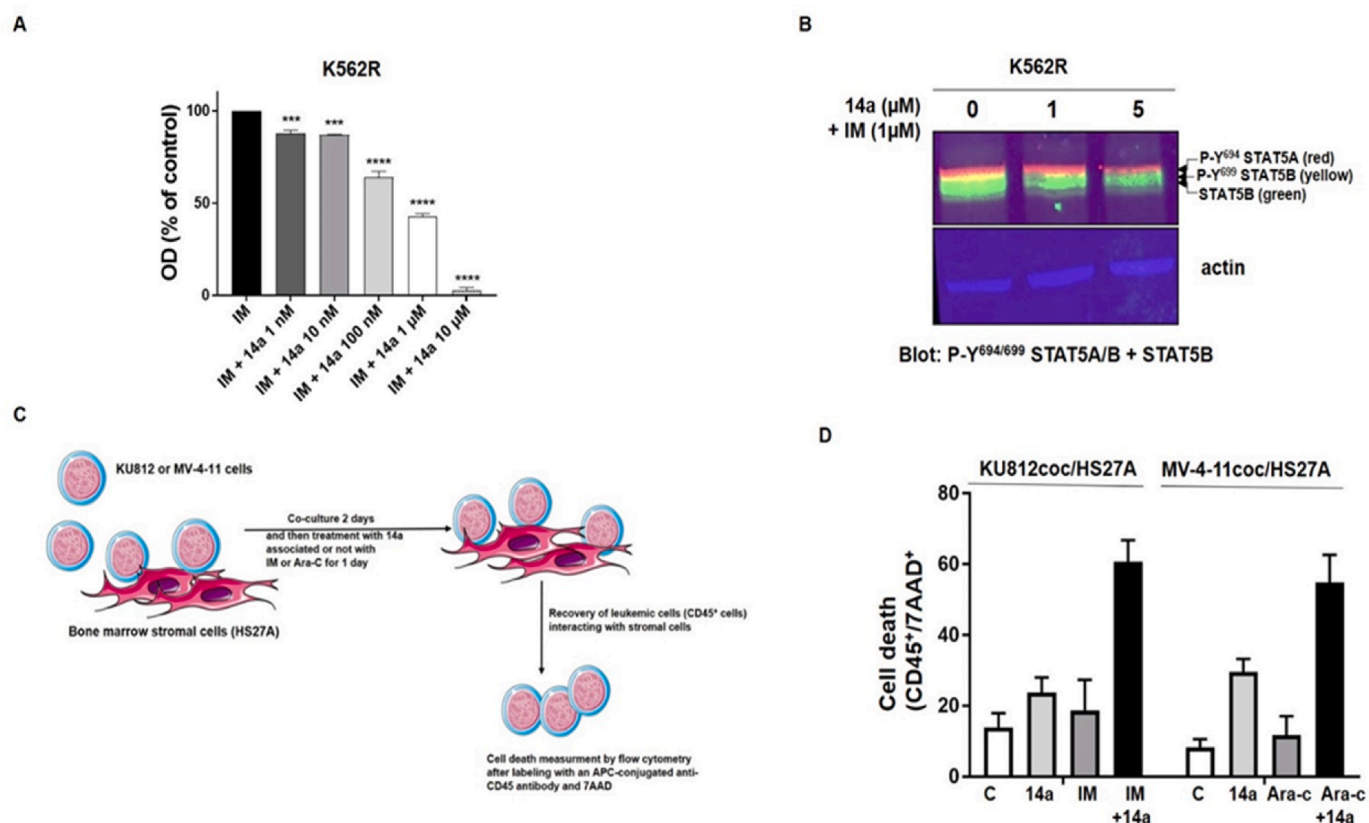


Fig. 5. 14a overcomes the resistance of myeloid leukemia cells to IM and Ara-C treatment. A) K562R cells were treated with IM (1 μM) and with increasing concentrations of 14a for 48 h. Cell viability was determined by MTT assays; B) Protein extracts from K562R cells treated with 14a or not (DMSO) with or without IM (1 μM) for 24 h were analyzed by western blotting to detect P-Y^{694/699}-STAT5 and STAT5B protein expression (n = 2). Actin served as loading control; C) Experimental protocol used to detect *de novo* resistance of KU812 and MV-4-11 cells to IM or Ara-C treatment (see experimental section for details); D) KU812 and MV-4-11 cells interacting with HS27A stromal cells were treated or not (C) with 14a (1 μM), IM (1 μM), Ara-C (1 μM) or a combination of these molecules. The percentage of leukemic cell death (CD45⁺/7AAD⁺ cells) was next evaluated by flow cytometry.

CML and AML, respectively, when leukemic cells are exposed to increasing concentrations of these therapeutic agents [33,34]. Second, *de novo* resistance is related to the bone marrow microenvironment. Bone marrow stromal cells interact directly with leukemic cells to promote their survival and escape from therapy [35,36]. We then addressed whether **14a** could directly interfere with *de novo* or acquired resistance of CML and AML cells. Results indicated that **14a** was able to inhibit the growth of IM-resistant K562 cells at concentrations as low as 100 nM (Fig. 5A). According to these data, we found that **14a** reduced the phosphorylation of STAT5A/5B as well as expression of STAT5B (Fig. 5B).

We then analyzed the impact of **14a** on the resistance of KU812 and MV-4-11 to IM and Ara-C when co-cultured with bone marrow stromal cells HS27A (Fig. 5C). Results showed that KU812 and MV-4-11 cells interacting with stromal cells over a three-day period are more resistant to treatment with IM or Ara-C, respectively. However, the use of **14a** in conjunction with IM or Ara-C resulted in a reversion to a more sensitive state (Fig. 5D). Collectively, these data suggest that **14a** by inhibiting STAT5 activity and expression directly impact the resistance of CML and AML cells to chemotherapy.

3. Conclusion

In summary, among the pyridylmethyl and polynitrogen derivatives of lead molecules **17f**, **1** and **2** that have been synthesized, compound **14a** was found to be the most potent inhibitor of myeloid leukemia cell growth along with STAT5 signaling. Our studies first demonstrated that increasing the conformational freedom by methylene homologation between the pyridine and the THQ cycle didn't improve antileukemic activity. Results obtained with pyridylmethyl series confirmed that pharmacomodulation at positions 5 and 6 of the THQ cycle led to a better antileukemic activity, compared to position 7, which had been previously hypothesized with pyridines analogs [27]. In a second time, introduction of pyridazine, pyrazine and pyrimidine cycles in position 5 and 6 of the THQ showed that only 2-substituted-5-pyrimidine led to interesting antileukemic property, with sub-micromolar activity for compounds **14a** and **14b**, bearing a 2-amino-5-pyrimidine moiety in positions 5 and 6 of the THQ, respectively. In particular, compound **14a** showed the most potent inhibitory effect with sub-micromolar EC₅₀ values in all cell lines analyzed. Importantly, these sub-micromolar concentrations have no cytotoxic effect on primary human bone marrow stromal cells suggesting that these crucial cellular components of the leukemic niche might not be impacted by **14a**. This is particularly important for our co-culture experiments in which we analyzed the impact of **14a** on the chemoresistance of leukemic cells mediated by stromal cells. We also demonstrated that **14a** inhibits STAT5 phosphorylation and STAT5B expression in STAT5 addicted AML and CML cells through mechanisms that remain to be elucidated. Interestingly, we also found that **14a** inhibits the expression of the STAT5B^{N642H} protein in hematopoietic cells transformed by this oncogene. This recurrent mutation of STAT5B has been described in patients with T cell leukemias and lymphomas suggesting that **14a** might also be used in the treatment of hematopoietic cancers associated with expression of this active STAT5B mutant. Finally, we also showed that **14a** when associated with IM or Ara-C overcomes the acquired or *de novo* resistance of CML and AML cells to these conventional therapeutic agents. In conclusion, compound **14a** represents a promising lead compound for further optimization towards a STAT5-targeted therapy for the treatment of myeloid leukemias. In particular, work on the indole cycle and the ethanolamine THQ-indole linker is ongoing in the lab.

4. Experimental section

4.1. Chemistry

4.1.1. General information

Compounds **3**, **4** and **5** were synthesized according to the previous synthesis strategy [25,27]. All commercial materials were used without further purification. For anhydrous and inert reactions, the glassware was heated with a heat gun, while several vacuum-dry argon cycles were performed. The thin layer chromatography (TLC) studies were performed using commercial precoated aluminum sheets silica gel (60 Å, F254) marketed by Merck and revealed under UV 254 and 365 nm lighting. The purifications by chromatography on silica gel columns were carried out on an ISCO purification unit, Combi Flash RF 75 PSI, with Rediseplash silica gel columns (60 Å, 230–400 mesh, grade 9385). NMR spectra were realized on a Bruker AVANCE AV 300 instrument or on a Bruker Avance Neo 400 spectrometer, HRMS was performed by the mass spectrometry service on a Q-Exactive spectrometer from Thermo Scientific using the electrospray ionization (ESI) technique. The purity was determined by high performance liquid chromatography (HPLC). HPLC analyses were carried out with a LaChrom Elite system [Hitachi L-2130 (pump) and L-2400 (UV-detector)] using UV 254 nm for detection. The column was a XBridge C-18 (250 × 10 mm, 4 µm, 135 Å); elution was performed with 0.1 % (by volume) of TFA in water (solvent A), and 0.1 % (by volume) of TFA in acetonitrile (solvent B); gradient 20–100 % of B over 35 min with a flow rate of 1 mL min⁻¹; column temperature of 25 °C; injection of 10 µL in DMSO. All compounds are >95 % pure by HPLC.

4.1.2. General procedure

4.1.2.1. General procedure A. A mixture of 1-(2-((1*H*-indol-5-yl)oxy)ethyl)-4,4-dimethyl(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1,2,3,4-tetrahydroquinoline **4** or **5a,b** (1.0 equiv), Na₂CO₃ (4.8 equiv) and pyridylmethyl bromide (1.5 equiv) in DME (6 mL) and water (2 mL) was degassed with argon. Pd(PPh₃)₄ (0.075 equiv) was then added and the resulting mixture was stirred 2 h at 95 °C. The mixture was cooled to rt, filtered through a pad of dicalite and extracted with ethyl acetate. Combined organic extracts were washed with brine and dried over MgSO₄. After evaporation under reduced pressure, the crude product was purified by flash chromatography on silica gel.

4.1.2.2. General procedure B. A mixture of boronate ester **4** or **5a** (1.0 equiv), the corresponding bromo derivatives (1.3 equiv) and Na₂CO₃ (2.5 equiv) in dioxane and water was degassed with argon. PdCl₂(dppf) (0.07 equiv) was then added and the resulting mixture was stirred 5 h at 100 °C. The mixture was cooled to room temperature, filtered through a pad of dicalite and extracted with ethyl acetate. Combined organic extracts were washed with brine and dried over MgSO₄. After evaporation under reduced pressure, the crude product was purified by flash chromatography on silica gel.

4.1.2.3. General procedure C. A mixture of the corresponding aryl bromide **3** (1.0 equiv), K₂CO₃ (1.5 equiv) and 2-amino-5-pyrimidinyl-5-boronic acid (3.0 equiv) in DMF and water was degassed with argon. PdCl₂(PPh₃)₂ (0.1 equiv) was then added and the resulting mixture was stirred 5 h at 90 °C. The mixture was cooled to rt, filtered through a pad of dicalite and extracted with ethyl acetate. Combined organic extracts were washed with brine and dried over MgSO₄. After evaporation under reduced pressure, the crude product was purified by flash chromatography on silica gel.

4.1.3. Spectral analysis of compounds **6a-16b**

1-(2-((1*H*-Indol-5-yl)oxy)ethyl)-4,4-dimethyl-5-(pyridin-2-ylmethyl)-1,2,3,4-tetrahydroquinoline (**6a**). This compound was

prepared according to the general procedure A. Light yellow oil; yield 4 %; ^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ 10.89 (s, 1H), 8.48 (d, $J = 4.9$ Hz, 1H), 7.63 (td, $J = 7.6, 2.0$ Hz, 1H), 7.28–7.22 (m, 2H), 7.16 (dd, $J = 7.6, 4.9$ Hz, 1H), 7.06–6.95 (m, 2H), 6.89 (dd, $J = 8.2, 7.4$ Hz, 1H), 6.72 (dd, $J = 8.7, 2.4$ Hz, 1H), 6.61 (d, $J = 8.2$ Hz, 1H), 6.32–6.28 (m, 1H), 6.21 (d, $J = 7.4$ Hz, 1H), 4.27 (s, 2H), 4.13 (t, $J = 5.5$ Hz, 2H), 3.68 (t, $J = 5.5$ Hz, 2H), 3.38–3.34 (m, 2H), 1.73–1.65 (m, 2H), 1.30 (s, 6H); ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$): 162.0, 152.3, 148.8, 145.3, 137.7, 136.3, 131.1, 129.1, 128.0, 126.3, 125.7, 122.7, 120.9, 120.5, 111.9, 111.4, 110.2, 102.8, 100.8, 65.2, 51.6, 45.7, 43.0, 41.2, 32.4, 30.2; HRMS m/z $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{27}\text{H}_{30}\text{N}_3\text{O}^+$ 412.2383, found 412.2369; purity (HPLC) 95.6 %.

1-(2-((1*H*-Indol-5-yl)oxy)ethyl)-4,4-dimethyl-6-(pyridin-2-ylmethyl)-1,2,3,4-tetrahydroquinoline (**6b**). This compound was prepared according to the general procedure A. Yellow oil; yield 11 %; ^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ 10.89 (s, 1H), 8.45 (ddd, $J = 4.9, 1.8, 1.0$ Hz, 1H), 7.65 (td, $J = 7.6, 1.8$ Hz, 1H), 7.27–7.22 (m, 2H), 7.21–7.13 (m, 2H), 7.06 (d, $J = 2.1$ Hz, 1H), 7.01 (d, $J = 2.4$ Hz, 1H), 6.83 (dd, $J = 8.4, 2.1$ Hz, 1H), 6.69 (dd, $J = 8.7, 2.4$ Hz, 1H), 6.58 (d, $J = 8.4$ Hz, 1H), 6.30–6.27 (m, 1H), 4.10 (t, $J = 5.8$ Hz, 2H), 3.89 (s, 2H), 3.63 (t, $J = 5.8$ Hz, 2H), 3.42–3.37 (m, 2H), 1.65 (t, $J = 5.9$ Hz, 2H), 1.18 (s, 6H); ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$): δ 161.7, 152.3, 148.9, 141.9, 136.50, 131.1, 130.6, 128.0, 127.0, 126.4, 126.3, 125.8, 122.7, 121.1, 112.0, 111.5, 110.8, 102.7, 100.8, 65.0, 50.3, 45.9, 43.2, 36.7, 31.5, 30.6. HRMS m/z $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{27}\text{H}_{30}\text{N}_3\text{O}^+$ 412.2383, found 412.2379; purity (HPLC) 97.4 %.

1-(2-((1*H*-Indol-5-yl)oxy)ethyl)-4,4-dimethyl-7-(pyridin-2-ylmethyl)-1,2,3,4-tetrahydroquinoline (**6c**). This compound was prepared according to the general procedure A. Yellow oil; yield 8 %; ^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ 10.89 (s, 1H), 8.42 (ddd, $J = 4.9, 1.9, 1.0$ Hz, 1H), 7.61 (td, $J = 7.6, 1.9$ Hz, 1H), 7.28–7.23 (m, 2H), 7.21 (dt, $J = 7.6, 1.0, 1\text{H}$), 7.14 (ddd, $J = 7.6, 4.9, 1.0$ Hz, 1H), 7.03–6.98 (m, 2H), 6.68 (dd, $J = 8.7, 2.4$ Hz, 1H), 6.60 (d, $J = 1.6$ Hz, 1H), 6.40 (dd, $J = 7.8, 1.6$ Hz, 1H), 6.32–6.30 (m, 1H), 4.09 (t, $J = 5.8$ Hz, 2H), 3.89 (s, 2H), 3.64 (t, $J = 5.8$ Hz, 2H), 3.44–3.39 (m, 2H), 1.67–1.60 (t, $J = 5.9$ Hz, 2H), 1.16 (s, 6H); ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$): δ 161.2, 152.4, 148.9, 143.5, 137.9, 136.5, 131.2, 128.6, 128.0, 125.9, 125.8, 123.0, 121.2, 116.0, 112.0, 111.5, 111.4, 102.8, 100.9, 64.9, 50.3, 46.0, 44.1, 36.7, 31.3, 30.6; HRMS m/z $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{27}\text{H}_{30}\text{N}_3\text{O}^+$ 412.2383, found 412.2384; purity (HPLC) 96.3 %.

1-(2-((1*H*-Indol-5-yl)oxy)ethyl)-4,4-dimethyl-5-(pyridin-3-ylmethyl)-1,2,3,4-tetrahydroquinoline (**7a**). This compound was prepared according to the general procedure A. Whitish solid; yield 13 %; m.p. 166–168 °C; ^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ 10.91 (s, 1H), 8.38–8.34 (m, 2H), 7.44–7.38 (m, 1H), 7.32–7.21 (m, 3H), 7.04 (d, $J = 2.4$ Hz, 1H), 6.90 (dd, $J = 8.4, 7.5$ Hz, 1H), 6.72 (dd, $J = 8.7, 2.4$ Hz, 1H), 6.62 (d, $J = 8.4$ Hz, 1H), 6.32–6.28 (m, 1H), 6.13 (dd, $J = 7.5, 1.1$ Hz, 1H), 4.17 (s, 2H), 4.14 (t, $J = 5.7$ Hz, 2H), 3.68 (t, $J = 5.7$ Hz, 2H), 3.43–3.38 (m, 2H), 1.71 (t, $J = 5.7$ Hz, 2H), 1.32 (s, 6H); ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$): δ 152.4, 149.9, 146.9, 145.3, 137.9, 137.8, 136.0, 131.1, 129.1, 128.0, 126.4, 125.8, 123.3, 120.3, 112.0, 111.5, 110.3, 102.8, 100.8, 65.2, 51.6, 45.7, 41.3, 37.1, 32.5, 30.3; HRMS m/z $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{27}\text{H}_{30}\text{N}_3\text{O}^+$ 412.2383, found 412.2384; purity (HPLC) 95.2 %.

1-(2-((1*H*-Indol-5-yl)oxy)ethyl)-4,4-dimethyl-6-(pyridin-3-ylmethyl)-1,2,3,4-tetrahydroquinoline (**7b**). This compound was prepared according to the general procedure A. Yellow oil; yield *quant.*; ^1H NMR (300 MHz, CDCl_3): δ 8.53 (s, 1H), 8.46 (d, $J = 4.6$ Hz, 1H), 8.32 (s, 1H), 7.54 (d, $J = 7.8$ Hz, 1H), 7.30–7.21 (m, 2H), 7.19 (t, $J = 2.8$ Hz, 1H), 7.10 (d, $J = 2.3$ Hz, 1H), 7.03 (d, $J = 2.1$ Hz, 1H), 6.85 (dd, $J = 8.7, 2.3$ Hz, 1H), 6.83 (dd, $J = 8.4, 2.1$ Hz, 1H), 6.62 (d, $J = 8.4$ Hz, 1H), 6.49–6.43 (m, 1H), 4.21 (t, $J = 6.2$ Hz, 2H), 3.88 (s, 2H), 3.74 (t, $J = 6.2$ Hz, 2H), 3.51–3.43 (m, 2H), 1.80–1.72 (m, 2H), 1.27 (d, $J = 1.4$ Hz, 6H); ^{13}C NMR (75 MHz, CDCl_3): δ 153.8, 150.0, 147.2, 142.9, 138.5, 137.4, 132.0, 131.7, 128.8, 127.7, 127.1, 126.9, 125.5, 124.1, 113.2, 112.2, 111.3, 104.0, 102.8, 65.9, 51.5, 47.1, 38.8, 37.6, 32.5, 31.3, 25.4; HRMS

m/z $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{27}\text{H}_{30}\text{N}_3\text{O}^+$ 412.2383, found 412.2374; purity (HPLC) 98.8 %.

1-(2-((1*H*-Indol-5-yl)oxy)ethyl)-4,4-dimethyl-7-(pyridin-3-ylmethyl)-1,2,3,4-tetrahydroquinoline (**7c**). This compound was prepared according to the general procedure A. Yellow oil; yield 48 %; ^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ 10.90 (s, 1H), 8.47 (d, $J = 2.1$ Hz, 1H), 8.36 (dd, $J = 4.8, 1.8$ Hz, 1H), 7.58 (ddd, $J = 7.8, 2.1, 1.8$ Hz, 1H), 7.29–7.20 (m, 3H), 7.02 (d, $J = 7.8$ Hz, 1H), 7.00 (d, $J = 2.3$ Hz, 1H), 6.67 (dd, $J = 8.8, 2.3$ Hz, 1H), 6.58 (d, $J = 1.7$ Hz, 1H), 6.36 (dd, $J = 7.8, 1.7$ Hz, 1H), 6.32–6.29 (m, 1H), 4.11 (t, $J = 5.7$ Hz, 2H), 3.78 (s, 2H), 3.65 (t, $J = 5.7$ Hz, 2H), 3.43–3.38 (m, 2H), 1.64 (t, $J = 5.8$ Hz, 2H), 1.17 (s, 6H); ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$): δ 152.3, 149.6, 147.1, 143.6, 138.6, 137.3, 135.9, 131.1, 128.5, 128.0, 125.9, 125.8, 123.4, 115.5, 111.9, 111.5, 111.0, 102.7, 100.8, 64.9, 50.2, 45.9, 38.3, 36.6, 31.3, 30.5; HRMS m/z $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{27}\text{H}_{30}\text{N}_3\text{O}^+$ 412.2383, found 412.2372; purity (HPLC) 99.9 %.

1-(2-((1*H*-Indol-5-yl)oxy)ethyl)-4,4-dimethyl-5-(pyridin-4-ylmethyl)-1,2,3,4-tetrahydroquinoline (**8a**). This compound was prepared according to the general procedure A. Light yellow oil; yield 14 %; ^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ 10.91 (s, 1H), 8.41 (d, $J = 5.2$ Hz, 2H), 7.29–7.24 (m, 2H), 7.09–7.06 (m, 2H), 7.04 (d, $J = 2.4$ Hz, 1H), 6.93 (dd, $J = 8.6, 7.5$ Hz, 1H), 6.72 (dd, $J = 8.7, 2.4$ Hz, 1H), 6.64 (dd, $J = 8.6, 1.3$ Hz, 1H), 6.31–6.29 (m, 1H), 6.17 (dd, $J = 7.5, 1.3$ Hz, 1H), 4.20–4.10 (m, 4H), 3.69 (t, $J = 5.7$ Hz, 2H), 3.45–3.40 (m, 2H), 1.72–1.66 (m, 2H), 1.27 (s, 6H); ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$): δ 152.4, 151.5, 149.3, 145.4, 137.0, 131.1, 129.3, 128.0, 126.5, 125.8, 124.0, 120.6, 112.0, 111.5, 110.5, 102.8, 100.8, 65.2, 51.6, 45.7, 41.2, 39.3, 32.5, 30.3; HRMS m/z $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{27}\text{H}_{30}\text{N}_3\text{O}^+$ 412.2383, found 412.2380; purity (HPLC) 96.6 %.

1-(2-((1*H*-Indol-5-yl)oxy)ethyl)-4,4-dimethyl-6-(pyridin-4-ylmethyl)-1,2,3,4-tetrahydroquinoline (**8b**). This compound was prepared according to the general procedure A. Greenish oil; yield 39 %; ^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ 10.89 (s, 1H), 8.46–8.38 (m, 2H), 7.29–7.15 (m, 4H), 7.04 (d, $J = 2.0$ Hz, 1H), 7.02 (d, $J = 2.5$ Hz, 1H), 6.80 (dd, $J = 8.4, 2.0$ Hz, 1H), 6.69 (dd, $J = 8.7, 2.5$ Hz, 1H), 6.59 (d, $J = 8.4$ Hz, 1H), 6.30–6.26 (m, 1H), 4.10 (t, $J = 5.8$ Hz, 2H), 3.78 (s, 2H), 3.64 (t, $J = 5.8$ Hz, 2H), 3.47–3.37 (m, 2H), 1.65 (t, $J = 5.9$ Hz, 2H), 1.19 (s, 6H); ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$): δ 152.4, 151.2, 149.5, 142.1, 131.1, 130.7, 128.0, 127.0, 126.4, 125.83, 125.79, 123.9, 112.0, 111.5, 110.9, 102.8, 100.8, 65.1, 50.3, 45.9, 39.6, 36.6, 31.6, 30.6; HRMS m/z $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{27}\text{H}_{30}\text{N}_3\text{O}^+$ 412.2383, found 412.2383; purity (HPLC) 96.3 %.

1-(2-((1*H*-Indol-5-yl)oxy)ethyl)-4,4-dimethyl-7-(pyridin-4-ylmethyl)-1,2,3,4-tetrahydroquinoline (**8c**). This compound was prepared according to the general procedure A. Yellow oil; yield 30 %; ^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ 10.90 (s, 1H), 8.39 (d, $J = 5.3$ Hz, 2H), 7.29–7.19 (m, 4H), 7.03 (d, $J = 7.8$ Hz, 1H), 7.00 (d, $J = 2.4$ Hz, 1H), 6.66 (dd, $J = 8.7, 2.4$ Hz, 1H), 6.58 (d, $J = 1.4$ Hz, 1H), 6.37 (dd, $J = 7.8, 1.4$ Hz, 1H), 6.32–6.30 (m, 1H), 4.11 (t, $J = 5.7$ Hz, 2H), 3.77 (s, 2H), 3.65 (t, $J = 5.7$ Hz, 2H), 3.42–3.38 (m, 2H), 1.68–1.61 (m, 2H), 1.17 (s, 6H); ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$): δ 152.3, 150.8, 149.3, 143.7, 137.5, 131.1, 128.7, 128.0, 125.9, 125.8, 124.0, 115.7, 111.9, 111.5, 111.2, 102.8, 100.8, 64.9, 50.2, 45.9, 40.5, 36.6, 31.3, 30.5; HRMS m/z $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{27}\text{H}_{30}\text{N}_3\text{O}^+$ 412.2383, found 412.2375; purity (HPLC) 97.3 %.

1-(2-((1*H*-Indol-5-yl)oxy)ethyl)-4,4-dimethyl-5-(pyridazin-4-yl)-1,2,3,4-tetrahydroquinoline (**9a**). This compound was prepared according to the general procedure B. Light yellow solid; yield 28 %; ^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ 10.90 (s, 1H), 9.20 (dd, $J = 5.2, 1.2$ Hz, 1H), 9.15 (dd, $J = 2.4, 1.2$ Hz, 1H), 7.61 (dd, $J = 5.3, 2.4$ Hz, 1H), 7.31–7.21 (m, 2H), 7.09–6.95 (m, 2H), 6.88–6.80 (m, 1H), 6.73 (dd, $J = 8.8, 2.4$ Hz, 1H), 6.33–6.28 (m, 1H), 6.13–6.04 (m, 1H), 4.19 (t, $J = 5.6$ Hz, 2H), 3.77 (d, $J = 5.6$ Hz, 2H), 3.48 (t, $J = 6.1$ Hz, 2H), 1.65–1.55 (m, 2H), 0.92 (s, 6H); ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$): δ 152.3, 151.9, 150.6, 144.5, 143.8, 135.7, 131.1, 128.0, 127.7, 126.5, 126.3, 125.8, 118.5, 111.9, 111.9, 111.4, 102.8, 100.8, 65.0, 50.8, 45.6, 39.4, 32.8, 30.8;

HRMS m/z $[M+H]^+$ caclcd for $C_{25}H_{27}N_4O^+$ 399.2179, found 399.2168; purity (HPLC) 98.2 %.

1-(2-((1*H*-Indol-5-yl)oxy)ethyl)-4,4-dimethyl-6-(pyridazin-4-yl)-1,2,3,4-tetrahydroquinoline (**9b**). This compound was prepared according to the general procedure B. Red oil; yield 58 %; 1H NMR (300 MHz, DMSO- d_6) δ 10.89 (s, 1H), 9.55 (d, $J = 2.6$ Hz, 1H), 9.04 (d, $J = 5.6$ Hz, 1H), 7.85 (dd, $J = 5.6, 2.6$ Hz, 1H), 7.71 (d, $J = 2.3$ Hz, 1H), 7.60 (dd, $J = 8.8, 2.3$ Hz, 1H), 7.29–7.21 (m, 2H), 7.04 (d, $J = 2.4$ Hz, 1H), 6.84 (d, $J = 8.8$ Hz, 1H), 6.71 (dd, $J = 8.8, 2.4$ Hz, 1H), 6.33–6.27 (m, 1H), 4.17 (t, $J = 5.5$ Hz, 2H), 3.78 (t, $J = 5.5$ Hz, 2H), 3.57–3.47 (m, 2H), 1.70 (t, $J = 6.0$ Hz, 2H), 1.29 (s, 6H); ^{13}C NMR (75 MHz, DMSO- d_6) 152.8, 151.6, 148.9, 145.9, 137.9, 131.9, 131.6, 128.4, 126.3, 126.1, 124.7, 121.1, 119.6, 112.5, 111.9, 111.7, 103.3, 101.3, 65.6, 50.5, 46.5, 36.5, 32.2, 30.5; HRMS m/z $[M+H]^+$ caclcd for $C_{25}H_{27}N_4O^+$ 399.2179, found 399.2184; purity (HPLC) 96.1 %.

1-(2-((1*H*-Indol-5-yl)oxy)ethyl)-4,4-dimethyl-6-(pyrimidin-2-yl)-1,2,3,4-tetrahydroquinoline (**10b**). This compound was prepared according to the general procedure B. White solid; yield 53 %; 1H NMR (300 MHz, DMSO- d_6) δ 10.90 (s, 1H), 8.73 (d, $J = 4.8$ Hz, 2H), 8.22 (d, $J = 2.1$ Hz, 1H), 8.04 (dd, $J = 8.8, 2.1$ Hz, 1H), 7.28–7.23 (m, 2H), 7.19 (t, $J = 4.8$ Hz, 1H), 7.05 (d, $J = 2.4$ Hz, 1H), 6.80 (d, $J = 8.8$ Hz, 1H), 6.72 (dd, $J = 8.8, 2.4$ Hz, 1H), 6.32–6.25 (m, 1H), 4.19 (t, $J = 5.6$ Hz, 2H), 3.79 (t, $J = 5.6$ Hz, 2H), 3.53 (t, $J = 6.0$ Hz, 2H, H_2), 1.72 (t, $J = 6.0$ Hz, 2H), 1.27 (s, 6H); ^{13}C NMR (75 MHz, DMSO- d_6) δ 164.0, 157.3, 152.3, 145.9, 131.1, 130.1, 128.0, 126.9, 125.8, 125.2, 123.7, 117.7, 112.0, 111.4, 110.4, 102.8, 100.8, 65.1, 50.1, 46.1, 36.0, 31.6, 30.2. HRMS m/z $[M+H]^+$ caclcd for $C_{25}H_{27}N_4O^+$ 399.2180, found 399.2176; purity (HPLC) 98.0 %.

1-(2-((1*H*-Indol-5-yl)oxy)ethyl)-4,4-dimethyl-5-(pyrazin-2-yl)-1,2,3,4-tetrahydroquinoline (**11a**). This compound was prepared according to the general procedure B. Yellow oil; yield 47 %; 1H NMR (300 MHz, DMSO- d_6) δ 10.90 (s, 1H), 8.65–8.58 (m, 3H), 7.29–7.25 (m, 2H), 7.08–7.01 (m, 2H), 6.85 (dd, $J = 8.5, 1.2$ Hz, 1H), 6.74 (dd, $J = 8.8, 2.4$ Hz, 1H), 6.33–6.29 (m, 1H), 6.25 (dd, $J = 7.3, 1.2$ Hz, 1H), 4.19 (t, $J = 5.7$ Hz, 2H), 3.76 (t, $J = 5.7$ Hz, 2H), 3.53–3.46 (m, 2H), 1.64–1.53 (m, 2H), 0.89 (s, 6H); ^{13}C NMR (75 MHz, DMSO- d_6) δ 158.9, 152.3, 144.9, 144.4, 142.7, 142.5, 137.7, 131.1, 128.4, 128.0, 126.1, 125.8, 118.6, 111.9, 111.9, 111.4, 102.8, 100.8, 65.1, 50.8, 45.6, 38.8, 32.8, 30.0; HRMS m/z $[M+H]^+$ caclcd for $C_{25}H_{27}N_4O^+$ 399.2179, found 399.2167; purity (HPLC) 97.3 %.

1-(2-((1*H*-Indol-5-yl)oxy)ethyl)-4,4-dimethyl-6-(pyrazin-2-yl)-1,2,3,4-tetrahydroquinoline (**11b**). This compound was prepared according to the general procedure B. Yellow oil; yield 75 %; 1H NMR (300 MHz, DMSO- d_6) δ 10.90 (s, 1H), 9.09 (brs, 1H), 8.57–8.51 (m, 1H), 8.37 (d, $J = 2.5$ Hz, 1H), 7.96 (d, $J = 2.1$ Hz, 1H), 7.83–7.72 (m, 1H), 7.30–7.20 (m, 2H), 7.04 (d, $J = 2.3$ Hz, 1H), 6.81 (d, $J = 8.7$ Hz, 1H), 6.71 (dd, $J = 8.9, 2.3$ Hz, 1H), 6.31–6.27 (m, 1H), 4.18 (t, $J = 5.6$ Hz, 2H), 3.78 (t, $J = 5.6$ Hz, 2H), 3.52 (t, $J = 6.0$ Hz, 2H), 1.71 (t, $J = 6.0$ Hz, 2H), 1.28 (s, 6H); ^{13}C NMR (75 MHz, DMSO- d_6) δ 152.3, 152.2, 145.2, 143.8, 140.9, 140.7, 131.1, 130.9, 128.0, 125.8, 125.4, 124.0, 122.2, 112.0, 111.4, 110.8, 102.8, 100.8, 65.2, 50.1, 46.1, 36.1, 31.7, 30.1; HRMS m/z $[M+H]^+$ caclcd for $C_{25}H_{27}N_4O^+$ 399.2179, found 399.2182; purity (HPLC) 98.9 %.

5-(1-(2-((1*H*-Indol-5-yl)oxy)ethyl)-4,4-dimethyl-1,2,3,4-tetrahydroquinolin-5-yl)pyrazin-2-amine (**12a**). This compound was prepared according to the general procedure B. Yellow oil; yield 28 %; 1H NMR (300 MHz, DMSO- d_6) δ 10.90 (s, 1H), 7.85 (d, $J = 1.5$ Hz, 1H), 7.78 (d, $J = 1.5$ Hz, 1H), 7.30–7.23 (m, 2H), 7.05 (d, $J = 2.4$ Hz, 1H), 6.98 (dd, $J = 7.8, 7.3$ Hz, 1H), 6.79–6.70 (m, 2H), 6.34–6.29 (m, 3H), 6.22 (d, $J = 7.3$ Hz, 1H), 4.17 (t, $J = 5.7$ Hz, 2H), 3.73 (t, $J = 5.7$ Hz, 2H), 3.50–3.43 (m, 2H), 1.62–1.53 (m, 2H), 0.96 (s, 6H); ^{13}C NMR (75 MHz, DMSO- d_6) δ 154.1, 152.4, 145.9, 144.4, 141.1, 138.7, 131.1, 129.3, 129.0, 128.0, 125.9, 125.7, 119.5, 111.9, 111.4, 111.3, 102.8, 100.8, 65.1, 50.9, 45.7, 39.1, 32.8, 30.0; HRMS m/z $[M+H]^+$ caclcd for $C_{25}H_{28}N_5O^+$ 414.2884, found 414.2276; purity (HPLC) 96.9 %.

5-(1-(2-((1*H*-Indol-5-yl)oxy)ethyl)-4,4-dimethyl-1,2,3,4-

tetrahydroquinolin-6-yl)pyrazin-2-amine (**12b**). This compound was prepared according to the general procedure B. Brown solid; yield 69 %; 1H NMR (300 MHz, DMSO- d_6) δ 10.89 (s, 1H), 8.34 (s, 1H), 7.88 (s, 1H), 7.74–7.63 (m, 1H), 7.51 (d, $J = 8.7$ Hz, 1H), 7.31–7.13 (m, 2H), 7.10–6.97 (m, 1H), 6.72 (d, $J = 8.8$ Hz, 2H), 6.32–6.21 (m, 3H), 4.15 (t, $J = 5.7$ Hz, 2H), 3.72 (t, $J = 5.7$ Hz, 2H), 3.51–3.42 (m, 2H), 1.70 (t, $J = 5.9$ Hz, 2H), 1.26 (s, 6H); ^{13}C NMR (75 MHz, DMSO- d_6) δ 153.8, 152.3, 143.2, 140.6, 137.2, 131.1, 130.9, 130.7, 128.0, 125.8, 124.2, 123.4, 122.3, 112.0, 111.5, 110.8, 102.8, 100.8, 65.1, 50.2, 46.0, 36.5, 31.7, 30.4; HRMS m/z $[M+H]^+$ caclcd for $C_{25}H_{28}N_5O^+$ 414.2288, found 414.2292; purity (HPLC) 97.3 %.

1-(2-((1*H*-Indol-5-yl)oxy)ethyl)-4,4-dimethyl-5-(pyrimidin-5-yl)-1,2,3,4-tetrahydroquinoline (**13a**). This compound was prepared according to the general procedure B. White solid; yield 79 %; 1H NMR (300 MHz, DMSO- d_6) δ 10.91 (s, 1H), 9.16 (s, 1H), 8.69 (s, 2H), 7.32–7.19 (m, 2H), 7.08–6.97 (m, 2H), 6.84 (dd, $J = 8.5, 1.1$ Hz, 1H), 6.73 (dd, $J = 8.7, 2.5$ Hz, 1H), 6.33–6.28 (m, 1H), 6.17 (dd, $J = 7.3, 1.1$ Hz, 1H), 4.19 (t, $J = 5.6$ Hz, 2H), 3.76 (t, $J = 5.6$ Hz, 2H), 3.48 (t, $J = 6.1$ Hz, 2H), 1.60 (t, $J = 6.1$ Hz, 2H), 0.90 (s, 6H); ^{13}C NMR (75 MHz, DMSO- d_6) δ 156.6, 156.0, 152.4, 144.6, 138.9, 134.6, 131.1, 128.6, 128.0, 126.3, 125.8, 119.5, 112.0, 111.9, 111.5, 102.8, 100.8, 65.1, 50.9, 45.7, 39.1, 32.9, 31.1; HRMS m/z $[M+H]^+$ caclcd for $C_{25}H_{27}N_4O^+$ 399.2180, found 399.2189; purity (HPLC) 96.8 %.

1-(2-((1*H*-Indol-5-yl)oxy)ethyl)-4,4-dimethyl-6-(pyrimidin-5-yl)-1,2,3,4-tetrahydroquinoline (**13b**). This compound was prepared according to the general procedure B. Yellow oil; yield 85 %; 1H NMR (300 MHz, DMSO- d_6) δ 10.90 (s, 1H), 9.03 (s, 2H), 9.01 (s, 1H), 7.57 (d, $J = 2.3$ Hz, 1H), 7.44 (dd, $J = 8.6, 2.3$ Hz, 1H), 7.29–7.22 (m, 2H), 7.04 (d, $J = 2.4$ Hz, 1H), 6.82 (d, $J = 8.6$ Hz, 1H), 6.71 (dd, $J = 8.7, 2.4$ Hz, 1H), 6.32–6.27 (m, 1H), 4.17 (t, $J = 5.4$ Hz, 2H), 3.77 (t, $J = 5.4$ Hz, 2H), 3.51 (t, $J = 6.0$ Hz, 2H), 1.71 (t, $J = 6.0$ Hz, 2H), 1.29 (s, 6H); ^{13}C NMR (75 MHz, DMSO- d_6) δ 155.5, 153.3, 152.3, 144.3, 133.7, 131.5, 131.1, 128.0, 125.8, 125.1, 124.0, 119.8, 112.0, 111.4, 111.3, 102.8, 100.8, 65.1, 50.1, 46.0, 36.3, 31.8, 30.2; HRMS m/z $[M+H]^+$ caclcd for $C_{25}H_{27}N_4O^+$ 399.2180, found 399.2168; purity (HPLC) 99.5 %.

5-(1-(2-((1*H*-Indol-5-yl)oxy)ethyl)-4,4-dimethyl-1,2,3,4-tetrahydroquinolin-5-yl)pyrimidin-2-amine (**14a**). This compound was prepared according to the general procedure C. Uncolored oil; yield 61 %; 1H NMR (300 MHz, DMSO- d_6) δ 10.89 (s, 1H), 8.03 (s, 2H), 7.31–7.21 (m, 2H), 7.05 (d, $J = 2.4$ Hz, 1H), 6.97 (dd, $J = 8.4, 7.3$ Hz, 1H), 6.78–6.70 (m, 2H), 6.57 (s, 2H), 6.32–6.28 (m, 1H), 6.15 (dd, $J = 7.3, 1.2$ Hz, 1H), 4.17 (t, $J = 5.6$ Hz, 2H), 3.73 (t, $J = 5.6$ Hz, 2H), 3.45 (t, $J = 6.0$ Hz, 2H), 1.60 (t, $J = 5.9$ Hz, 2H), 1.00 (s, 6H); ^{13}C NMR (75 MHz, DMSO- d_6) δ 162.1, 156.8, 152.3, 144.6, 136.1, 131.1, 129.3, 128.0, 127.4, 126.1, 125.8, 120.4, 111.9, 111.4, 111.3, 102.8, 100.8, 65.1, 51.0, 45.7, 39.4, 32.9, 31.1; HRMS m/z $[M+H]^+$ caclcd for $C_{25}H_{28}N_5O^+$ 414.2884, found 414.2288; purity (HPLC) 98.4 %.

5-(1-(2-((1*H*-Indol-5-yl)oxy)ethyl)-4,4-dimethyl-1,2,3,4-tetrahydroquinolin-6-yl)pyrimidin-2-amine (**14b**). This compound was prepared according to the general procedure C. Off-white oil; yield 93 %; 1H NMR (300 MHz, DMSO- d_6) δ 10.90 (s, 1H), 8.44 (s, 2H), 7.34 (d, $J = 2.2$ Hz, 1H), 7.28–7.22 (m, 2H), 7.19 (dd, $J = 8.5, 2.2$ Hz, 1H), 7.03 (d, $J = 2.4$ Hz, 1H), 6.77–6.67 (m, 2H), 6.53 (s, 2H), 6.31–6.27 (m, 1H), 4.15 (t, $J = 5.6$ Hz, 2H), 3.71 (t, $J = 5.6$ Hz, 2H), 3.45 (t, $J = 5.7$ Hz, 2H), 1.69 (t, $J = 5.7$ Hz, 2H), 1.26 (s, 6H); ^{13}C NMR (75 MHz, DMSO- d_6) δ 162.1, 154.8, 152.4, 142.8, 131.3, 131.1, 128.0, 125.8, 123.8, 123.1, 122.8, 122.1, 111.9, 111.4, 111.3, 102.8, 100.8, 65.1, 50.2, 46.0, 36.6, 31.7, 30.4; HRMS m/z $[M+H]^+$ caclcd for $C_{25}H_{28}N_5O^+$ 414.2888, found 414.2292; purity (HPLC) 97.3 %.

1-(2-((1*H*-Indol-5-yl)oxy)ethyl)-5-(2-chloropyrimidin-5-yl)-4,4-dimethyl-1,2,3,4-tetrahydroquinoline (**15a**). This compound was prepared according to the general procedure B. Off-white solid; yields 35 %; 1H NMR (400 MHz, DMSO- d_6) δ 10.90 (s, 1H), 8.69 (s, 2H), 7.29–7.23 (m, 2H), 7.07–7.00 (m, $J = 9.2, 6.5$ Hz, 2H), 6.85 (d, $J = 8.4$ Hz, 1H), 6.73 (dd, $J = 8.7, 2.2$ Hz, 1H), 6.31 (s, 1H), 6.19 (d, $J = 7.3$ Hz, 1H), 4.19 (t, $J = 5.5$ Hz, 2H), 3.76 (t, $J = 5.5$ Hz, 2H), 3.48 (t, $J = 5.5$ Hz, 2H), 1.61

(t, $J = 5.5$ Hz, 2H), 0.93 (s, 6H); ^{13}C NMR (101 MHz, DMSO- d_6) δ 159.2, 158.3, 152.4, 144.6, 137.9, 133.2, 131.2, 128.7, 128.0, 126.4, 125.9, 119.5, 112.2, 112.03, 111.5, 102.8, 100.8, 65.0, 50.9, 45.8, 38.9 (under DMSO), 32.9, 31.3; HRMS m/z $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{25}\text{H}_{26}\text{ClN}_4\text{O}^+$ 433.1790, found 433.1787; purity (HPLC) 98.6 %.

1-(2-((1*H*-Indol-5-yl)oxy)ethyl)-6-(2-chloropyrimidin-5-yl)-4,4-dimethyl-1,2,3,4-tetrahydroquinoline (**15b**). This compound was prepared according to the general procedure B. Yellow solid; yield 78 %; ^1H NMR (400 MHz, DMSO- d_6) δ 10.89 (s, 1H), 9.01 (s, 2H), 7.58 (d, $J = 2.3$ Hz, 1H), 7.44 (dd, $J = 8.7$, 2.3 Hz, 1H), 7.28–7.23 (m, $J = 6.0$, 3.2 Hz, 2H), 7.03 (d, $J = 2.4$ Hz, 1H), 6.82 (d, $J = 8.7$ Hz, 1H), 6.71 (dd, $J = 8.7$, 2.4 Hz, 1H), 6.29 (m, $J = 2.1$ Hz, 1H), 4.17 (t, $J = 5.6$ Hz, 2H), 3.77 (t, $J = 5.6$ Hz, 2H), 3.50 (t, 2H), 1.70 (t, 2H), 1.28 (s, 6H); ^{13}C NMR (101 MHz, DMSO- d_6) δ 156.82, 156.39, 152.35, 144.54, 132.78, 131.52, 131.13, 127.97, 125.84, 125.25, 124.12, 118.31, 111.98, 111.44, 111.35, 102.77, 100.79, 65.08, 50.05, 46.03, 36.23, 31.77, 30.14; ^{13}C NMR (101 MHz, DMSO- d_6) δ 156.8, 156.4, 152.4, 144.5, 132.8, 131.5, 131.1, 128.0, 125.8, 125.3, 124.1, 118.3, 112.0, 111.4, 111.4, 102.8, 100.8, 65.1, 50.1, 46.0, 36.2, 31.8, 30.1; HRMS m/z $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{25}\text{H}_{26}\text{ClN}_4\text{O}^+$ 433.1790, found 433.1785; purity (HPLC) 98.6 %.

5-(1-(2-((1*H*-Indol-5-yl)oxy)ethyl)-4,4-dimethyl-1,2,3,4-tetrahydroquinolin-5-yl)pyrimidine-2-carbonitrile (**16a**). This compound was prepared according to the general procedure B. White solid; yield 38 %; ^1H NMR (400 MHz, DMSO- d_6) δ 10.90 (s, 1H), 8.95 (s, 2H), 7.32–7.23 (m, 2H), 7.12–6.99 (m, 2H), 6.88 (d, $J = 8.5$ Hz, 1H), 6.73 (d, $J = 8.7$ Hz, 1H), 6.31 (s, 1H), 6.18 (d, $J = 7.4$ Hz, 1H), 4.19 (t, $J = 5.3$ Hz, 2H), 3.77 (t, $J = 5.2$ Hz, 2H), 3.49 (t, $J = 5.3$ Hz, 2H), 1.61 (t, $J = 5.2$ Hz, 2H), 0.91 (s, 6H); ^{13}C NMR (101 MHz, DMSO- d_6) δ 158.0, 152.8, 145.1, 142.6, 142.2, 133.7, 131.6, 128.9, 128.4, 126.8, 126.3, 119.6, 116.7, 112.8, 112.5, 111.9, 103.2, 101.3, 65.4, 51.3, 46.2, 39.3, 33.3, 31.7; HRMS m/z $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{26}\text{H}_{26}\text{N}_5\text{O}^+$ 424.2132, found 424.2128; purity (HPLC) 99.8 %.

5-(1-(2-((1*H*-Indol-5-yl)oxy)ethyl)-4,4-dimethyl-1,2,3,4-tetrahydroquinolin-6-yl)pyrimidine-2-carbonitrile (**16b**). This compound was prepared according to the general procedure B. Yellow solid; yield 80 %; ^1H NMR (400 MHz, DMSO) δ 10.89 (s, 1H), 9.27 (s, 2H), 7.71 (d, $J = 2.3$ Hz, 1H), 7.59 (dd, $J = 8.9$, 2.3 Hz, 1H), 7.29–7.22 (m, 2H), 7.04 (d, $J = 2.4$ Hz, 1H), 6.86 (d, $J = 8.8$ Hz, 1H), 6.71 (dd, $J = 8.8$, 2.4 Hz, 1H), 6.29 (s, 1H), 4.18 (t, $J = 5.6$ Hz, 2H), 3.80 (t, $J = 5.6$ Hz, 2H), 3.53 (t, $J = 5.8$ Hz, 2H), 1.71 (t, $J = 5.8$ Hz, 2H), 1.29 (s, 6H). ^{13}C NMR (101 MHz, DMSO) δ 153.9, 152.3, 145.5, 139.9, 135.8, 131.7, 131.1, 128.0, 125.9, 125.9, 124.5, 117.6, 116.7, 112.0, 111.5, 111.4, 102.8, 100.8, 65.1, 50.0, 46.1, 36.0, 31.8, 30.0; HRMS m/z $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{26}\text{H}_{26}\text{N}_5\text{O}^+$ 424.2132, found 424.2127; purity (HPLC) 98 %.

4.2. Biology and biochemistry

4.2.1. Cell cultures and reagents

K562, KU812, KCL-22, IM-resistant (K562R) BCR-ABL⁺ cells and MV-4-11 cells were obtained from the Deutsche Sammlung für Mikroorganismen und Zellkulturen (DSMZ). MOLM-13 and HS27A were obtained from the American Type Culture Collection (ATCC). The Ba/F3 cell line expressing STAT5B^{N642H} was previously described⁸. Cells were cultured in RPMI 1640 (Gibco-ThermoFisher, Waltham, MA, USA), with 10 % fetal bovine serum (Thermoscientific-Sigma-Aldrich St Louis, MO, USA), 2 mM glutamine (Gibco-ThermoFisher, Waltham, MA, USA), 100 U/mL penicillin and 100 $\mu\text{g}/\text{mL}$ streptomycin (Gibco-ThermoFisher, Waltham, MA, USA) at 37 °C, 5 % CO₂. Resistant K562 cells were cultured with 1 μM IM. IM was purchased from Selleckchem (Houston, USA). Bone marrow (BM) MSC obtained from donors undergoing orthopedic surgery were isolated and cultured as described. Informed consent was obtained before BM samples were taken. MSC were cultured in α MEM (Gibco - ThermoFisher, Waltham, MA, USA), with 10 % fetal bovine serum (Thermoscientific - Sigma-Aldrich St Louis, MO, USA), 2 mM glutamine (Gibco-ThermoFisher, Waltham, MA, USA), 100 U/mL penicillin, 100 $\mu\text{g}/\text{mL}$ streptomycin (Gibco-ThermoFisher, Waltham,

MA, USA), 0.01 % fungizone (Gibco-ThermoFisher, Waltham, MA, USA) and 0.005 % fibroblast growth factor 2 (Gibco-ThermoFisher, Waltham, MA, USA) at 37 °C, 5 % CO₂.

4.2.2. Co-cultures and magnetic cell separation

HS27A stromal cells were grown up to confluence in 150 cm² flask. Medium was changed one day before starting the co-culture. 2.5x10⁶ KU812 or MV-4-11 cells were next added on stromal cell monolayers for 48 h and then treated with **14a** alone or with IM or Ara-C for 24 h. To discriminate between stromal cells and leukemic cells non-adherent and adherent, leukemic cells were first labelled with an APC-conjugated anti-CD45 antibody (Becton-Dickinson) and then stained with 7-amino-actinomycin D (7-AAD) (Sigma-Aldrich, Lyon, France) for 30 min at room temperature. The percentage of leukemic cell death was next determined by flow cytometry (Becton Dickinson Accuri™ C6 flow cytometer). The FlowJo® software was used to analyze data.

4.2.3. Cell proliferation assays

Cell viability and proliferation were studied using a MTT cell proliferation assay (Sigma-Aldrich, MO, USA). Briefly, 2x10⁴ leukemic cells were cultured in 100 μL of RPMI medium in 96 well plates and treated with 10 μM of each drug for 24, 48 and 72 h. To determine the EC₅₀ values, cells were treated with concentrations ranging from 100 nM to 100 μM for 48 h. Drug cytotoxicities were tested on BM stromal cells. For that purpose, 1x10⁴ HS27A or 3.5x10³ MSC cells were cultured in 96 well plates, incubated for 24 h and then treated with concentrations of drugs ranging from 100 nM to 100 μM for 48 h. Cells were then incubated with 10 μL of MTT working solution (5 g/L of methylthiazolyldiphenyl-tetrazolium bromide) during 4 h. Cells were lysed overnight at 37 °C with 100 μL of SDS 10 %, HCl 0.003 %. Optical density (OD) at 570 nm was then measured using a spectrophotometer CLARIOstar® (BMG Labtech, Offenbach, Germany). Living cells were also enumerated using the trypan blue dye exclusion method.

4.2.4. Western blot

Cells were suspended in NP40 buffer (Bio-Rad, NY, USA), separated on SDS/PAGE and blotted onto nitrocellulose membrane. Blots were simultaneously incubated with a polyclonal rabbit anti-P-Y^{694/699}-STAT5A/B antibody and a specific mouse monoclonal anti-STAT5A or anti-STAT5B antibody (Zymed/ThermoFisher Scientific, Waltham, MA, USA). Membranes were revealed with an anti-mouse conjugated to the Dylight800 fluorophore (STAT5A and STAT5B) (Biorad) and with an anti-rabbit conjugated to the StarbrightB700 fluorophore (P-Y^{694/699}STAT5) (Biorad). Actin was used as a loading control and was detected with an anti-mouse conjugated to the StarbrightB520 fluorophore as a secondary antibody (Biorad). The ChemiDoc MP Imaging System (Biorad) was used to address multiplex fluorescent western blotting. Monoclonal anti-Flag M2 (Stratagene, Santa Clara, United States), anti-STAT5B and anti-actin antibodies were used to detect expression of a Flag-tagged STAT5B^{N642H} and the actin control. In this context, membranes were developed with the ECL chemiluminescence detection system (GE Healthcare, Little Chalfont, UK) using specific peroxidase (HRP) conjugated to mouse IgG antibodies (Cell signaling Technology, Danvers, USA).

CRedit authorship contribution statement

Marion Polomski: Methodology, Investigation, Data curation. **Marie Brachet-Botineau:** Methodology, Investigation, Data curation. **Benjamin Victoir:** Methodology, Investigation, Data curation. **Cécile Croix:** Writing – review & editing, Methodology, Investigation, Data curation, Conceptualization. **Fabrice Gouilleux:** Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Gildas Prié:** Writing – review & editing, Writing – original draft, Validation, Supervision, Project

administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Abbreviations

7-AAD, 7-Aminoactinomycin D; AKT, Protein Kinase B; AML, Acute Myeloid Leukemia; Ara-C, 1 β -arabino-furanosylcytosine or Cytarabine; BCR-ABL, Breakpoint Cluster Region-Abelson; BM, Bone Marrow; BM MSC, Bone Marrow Mesenchymal Stromal Cells; CML, Chronic Myeloid Leukemia; DMF, Dimethylformamide; DMSO, Dimethyl sulfoxide; ERK1/2, Extracellular Signal-Regulated Kinases 1/2; FLT3-ITD, FMS like tyrosine kinase 3 - Internal Tandem Duplication; IM, Imatinib Mesylate; JAK, Janus Kinase; MSC, Primary Mesenchymal Stromal Cells; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PPAR, Peroxisome Proliferator – Activated Receptor; (SPR) surface plasmon resonance; SH2 domain, Src Homology-2 domain; STAT5, Signal transducer and activator of transcription 5; TFA, Trifluoroacetic Acid; THQ, Tetrahydroquinoline; TKI, Tyrosine Kinase Inhibitor; TKO, tyrosine kinase oncogene; TLC, Thin Layer Chromatography.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ejmech.2024.117211>.

Data availability

Data will be made available on request.

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