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Inhibitors Targeting STAT5 Signaling in Myeloid Leukemias: New Tetrahydroquinoline Derivatives with Improved Antileukemic Potential

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Abstract: Signal transducers and activators of transcription 5A and 5B (STAT5A and STAT5B) are two closely related STAT family members that are crucial downstream effectors of tyrosine kinase oncoproteins such as FLT3-ITD in acute myeloid leukemia (AML) and BCR-ABL in chronic myeloid leukemia (CML). We recently developed and reported the synthesis of a first molecule called **17f** that selectively inhibits STAT5 signaling in myeloid leukemia cells and overcomes their resistance to chemotherapeutic agents. To improve the antileukemic effect of **17f**, we synthesized 10 analogs of this molecule and analyzed their impact on cell growth, survival, chemoresistance and STAT5 signaling. Two compounds, **7a** and **7a'**, were identified as having similar or higher antileukemic effects in various AML and CML cell lines. Both molecules were found to be more effective than **17f** to inhibit STAT5 activity/expression and to suppress the chemoresistance of CML.

Introduction

Among the STAT family members, STAT5A and STAT5B proteins play a critical role in the development of hematologic cancers that affect lymphoid and myeloid lineages.[1] Persistent tyrosine phosphorylation of STAT5A/5B (P-Y-STAT5) has been found in hematopoietic cancers driven by tyrosine kinase oncogenes (TKO) such as BCR-ABL in chronic myeloid and acute lymphoid leukemias (CML and Ph^+ALL), FLT3-internal tandem duplication (FLT3-ITD) in acute myeloid leukemia (AML) and $JAK2^{V617F}$ in myeloproliferative neoplasms (MPN).[2–4] In addition, gain of function (GOF) mutations of STAT5B have been described in different hematopoietic malignancies affecting the lymphoid compartment. This is best illustrated by the presence of the recurrent N642H mutation of STAT5B in some T cell leukemias.[5]

The development of Imatinib Mesylate (IM, Glivec®) and related BCR-ABL kinase inhibitors such as Dasatinib, Nilotinib, Bosutinib and Ponatinib has made a major breakthrough in the treatment of CML.[6–10] IM leads to complete inhibition of BCR-ABL-dependent STAT5 activation which is an important part of this molecule effectiveness. However, Imatinib Mesylate is not totally curative and is unable to completely eradicate leukemic stem cells (LSC) which is responsible of relapse of CML patients. Moreover, mutations on BCR-ABL tyrosine kinase domain occurs, which prevents IM binding and promote IM resistance of CML cells.[11–15]

Mutations affecting the tyrosine kinase receptor FLT3 can be found in up to one third of AML patients. FLT3-ITD is the most frequent one and usually confers a poor prognosis.[16] Targeting the kinase activity of FLT3 mutants has been therefore adopted to inhibit aberrant signaling in AML. First- and second-generations FLT3 inhibitors were employed as a single agent or in combination with other therapeutic drugs such as cytarabine (Ara-C) and anthracyclines in AML treatment.[17–20] Clinical activity of some of these molecules was evidenced in patients with FLT3-mutated AML but was often transient and relapse eventually occurred. Like BCR-ABL, secondary mutations in FLT3 have been shown to be responsible for the loss of therapeutic response to FLT3 inhibitors.[21] Alternative approaches targeting critical effectors of BCR-ABL and FLT3-ITD are therefore needed to cure CML and AML.

In this regard, STAT5 satisfy all criteria to be a druggable target in CML and AML diseases.[22,23] STAT5 proteins have been shown to play a crucial role in the maintenance of CML stem cells and to promote the resistance of CML cells against TKI treatments.[24–27] Expression of P-Y-STAT5 has also been associated with an increased risk of relapse in AML after achieving complete remission.[28] Thus, targeting STAT5 would contribute to abrogate *de novo* and acquired resistance of CML and AML to chemotherapy.

Several approaches have been used to target STAT5 in leukemias.[23] Among them, chromones- and salicylic acid-based molecules have been employed to identify selective inhibitors that bind to the SH2 domain of STAT5.[29–31] One of them, AC-4-130 suppressed P-Y-STAT5 and the growth of CML and AML cells as well as tumor formation in xenograft models of AML.[32,33] Finally, another small STAT5-SH2-binding compound containing a nucleotidic scaffold showing a therapeutic potential for prostate cancer and CML has been identified in structure-based screening studies.[34] In a similar vein, cell-based screening assays using a library of clinically used drugs were also

employed to identify the antipsychotic drug, pimozide, as a potential STAT5 inhibitor. This compound was shown to inhibit P-Y-STAT5 and survival of CML and AML cells without affecting the kinase activity of BCR-ABL or FLT3-ITD.[35,36] Synthesis of pimozide derivatives and structure-activity relationship (SAR) studies allowed to identify compounds with enhanced inhibitory effects on STAT5 activity and CML cell growth.[37] As repositioning drugs, antidiabetics such as pioglitazone and rosiglitazone have also been shown to possess antileukemic activity. Both synthetic compounds belong to the thiazolidinedione (TZD) class of ligands that bind to the nuclear receptor peroxisome proliferator-activated receptor gamma (PPARγ). Activation of PPARγ by pioglitazone not only inhibits the growth of CML cells but also reduces the expression of *STAT5* genes.[38–40] Importantly, the drug combination using pioglitazone with IM triggers apoptosis of these leukemic cells suggesting that besides phosphorylation, inhibition of STAT5 expression is of prime importance for resistant CML stem cell eradication.[40]

We used a library of PPAR agonists that were previously synthesized in our lab, to identify a hit compound that blocks P-Y-STAT5 and leukemic cell growth. 18 derivatives of this "hit" sharing a diversely substituted 4,4-dimethyl-1,2,3,4-tetrahydroquinoline scaffold, linked to an heteroaryl ring by an ethoxy chain were synthesized and tested in preclinical studies using various leukemic cell lines. Results of the cell proliferation and viability assays allowed us to select one compound called **17f** with improved antileukemic activity. This compound is composed of a 3-pyridinyl substituted 4,4-dimethyl-1,2,3,4-tetrahydroquinoline (THQ) and indole rings, linked by an ethoxy chain attached by carbon C-5 of the indole and nitrogen N-1 of the THQ (Figure 1). We showed that **17f** suppressed P-Y-STAT5 and growth of CML and AML cells.[41] We also demonstrated that **17f** sensitizes leukemic cells that acquired resistance to IM or Ara-C.[42]

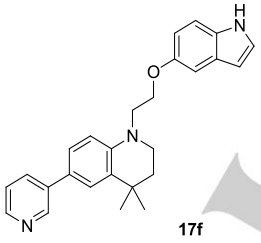
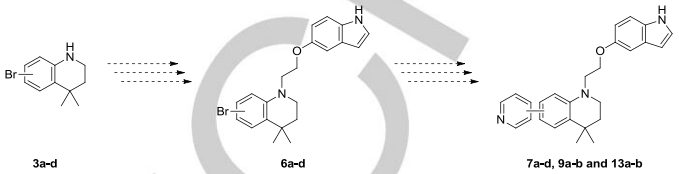


Figure 1. Compound **17f**

In order to improve the antileukemic potential of **17f**, we synthesized 10 new analogs with modifications around the tetrahydroquinoline (THQ) ring and analyzed their impacts on CML and AML cell growth. Among these analogs, two compounds, **7a** and **7a'**, containing respectively a 3- and 4-pyridinyl moiety in position 5 on the THQ ring, showed similar or higher growth inhibitory effects when compared to **17f**. We also demonstrated that **7a** and **7a'** were more effective than **17f** in blocking P-Y-STAT5 expression and activity. Lastly, both compounds showed a slightly better cytotoxic effect when combined with IM to kill CML cells that acquired resistance to this tyrosine kinase inhibitor.

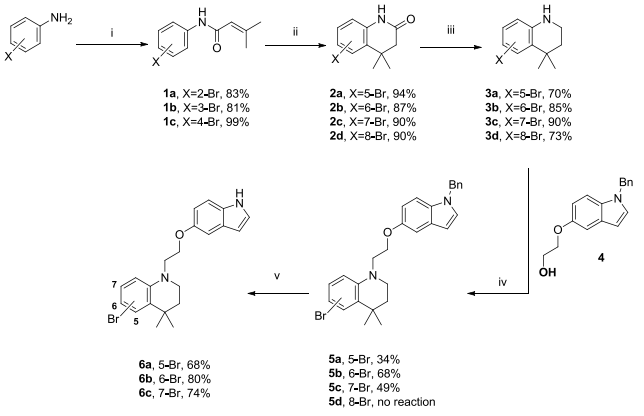
Chemistry

In order to study SAR around the tetrahydroquinoline (THQ) ring, we used a convergent pathway implemented and optimized from our previously described work. This synthetic route, setting good yields, has been extended to synthesize a series of new analogs using the brominated THQ and indole rings as starting materials with the linking strategy as described previously (Scheme 1).[41]



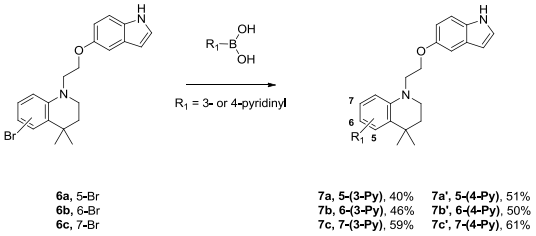
Scheme 1. Our work on new analogs

To obtain these analogs series, we needed to synthetize the brominated key intermediates 4,4-dimethyl-1,2,3,4-tetrahydroquinoline **6**, with bromine on positions 5, 7 and 8. From 2-bromoaniline, we performed the same method used in Juen *et al.* work with an acylation followed by a Friedel-Crafts alkylation to get lactam **2d**.[41] From 3-bromoaniline, we obtained as expected both lactam brominated on positions 5 and 7, and isolated each isomer by purification, respectively **2a** and **2c**. Each lactam was then reduced to the corresponding brominated THQ **3a**,**c**,**d**. Alcohol **4** was converted to aldehyde by 2-iodobenzoic acid (IBX) and then filtered on amines **3a** and **3c** in the presence of $NaBH(OAc)_3$, to afford by reductive amination **5a** and **5c**. From amine **3d**, compound **5d** was not observed, surely due to steric hindrance of the bromine in position 8 on the tetrahydroquinoline scaffold. Consequently, an alternative synthesis pathway from amine **3d** has been developed to obtain the desired 8-brominated THQ. Finally, deprotection of **5a** and **5c** using potassium *tert*-butoxide under air (1 atm) in DMSO led to key intermediates **6a** and **6c**, with 68% and 74% yields respectively (Scheme 2).



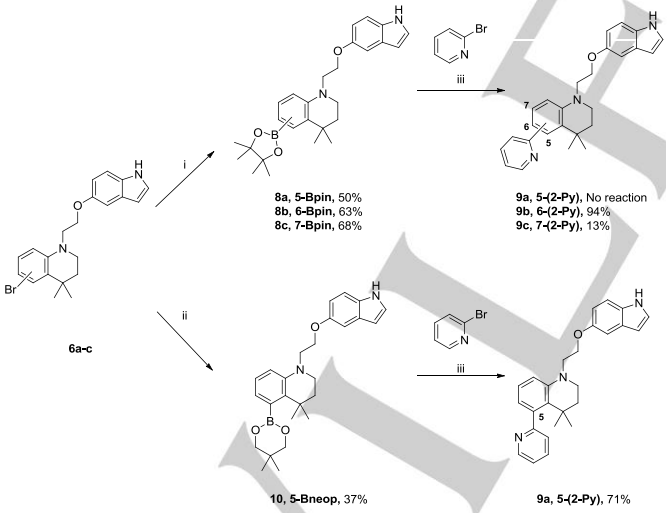
Scheme 2. Synthesis of key intermediates 6a-c. Reagents and conditions: (i) 3,3-dimethylacryloyl chloride, dry pyridine, 0 °C to rt, 5 h; (ii) $AlCl_3$, dry DCM, 0 °C to rt, 1 h; (iii) BH_3.THF, dry toluene, 0 °C to reflux, 5 h; (iv) 1) alcohol **4**, IBX, dry DCE, 80 °C, 2 h; 2) filtration; 3) amine **3**, $NaBH(OAc)_3$, rt, 3 h; (v) *t*-BuOK, DMSO, O_2, rt, 3 h.

Introduction of 3- and 4-pyridinyl groups on the THQ ring of both **6a-c** by Suzuki cross-coupling afforded respectively **7a-c** and **7a'-c'** analogs with satisfying yields (40-61%) (Scheme 3).



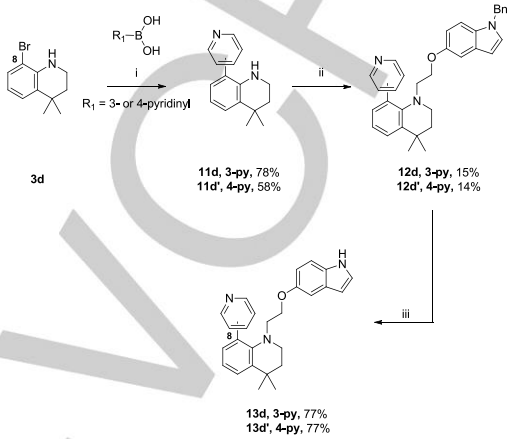
Scheme 3. Synthesis of analogs **7a-c** and **7a'-c'** by Suzuki coupling from compounds **6a-c**. Reagents and conditions: $R_1B(OH)_2$, $PdCl_2(PPh_3)_2$, K_2CO_3, DMF/water, 90 °C, 5 h.

However, as observed by Juen *et al.*, the reaction between **6a-c** and 2-pyridinylboronic acid did not occur and only led to degradation of the starting material. To bypass this issue, we converted aryl bromides **6a-c** into pinacol boronate esters **8a-c** with good yields (50-68%). Compounds **8b** and **8c** subsequently yielded **9b** and **9c** after Suzuki coupling using 2-bromopyridine.[43,44] Concerning compound **9b**, this optimized procedure allowed us to improve the yield from 24% to 60% in two steps.[41] Though, concerning pinacol boronate ester **8a,** coupling was not observed, surely due to steric hindrance between methyl groups of both pinacol boronate and THQ. Consequently, we synthesized the corresponding less hindered neopentyl boronate ester **10** from aryl bromide **6a** and used it in the same way in Suzuki coupling with 2-bromopyridine to yield **9a** with a satisfying 71% yield (Scheme 4).



Scheme 4. Synthesis of 2-pyridinyl analogs from compounds **6a-c**. Reagents and conditions: (i) bis(pinacolato)diboron, $PdCl_2(dppf)$, KOAc, dry dioxane, 100 °C, 12 h; (ii) bis(neopentylglycolato)diboron, $PdCl_2(dppf)$, KOAc, dry dioxane, 100 °C, 12 h; (iii) 2-bromopyridine, $PdCl_2(dppf)$, Na_2CO_3, dioxane/water, 100 °C, 5 h.

We then focused on analogs substituted in position 8 on the THQ ring. Indeed, as previously described, no reaction between amine **3d** and alcohol **4** was observed due to the bromine position. Therefore, Suzuki cross-coupling with 3- and 4-pyridinyl boronic acids were performed first on brominated THQ **3d** to afford **11d**,**d'**. Thereafter classic pathway was used to reach compounds **13d**,**d'** in two steps and unsurprisingly, the bridge formation first step gave low yields (Scheme 5).



Scheme 5. Synthesis of analogs substituted in position 8 on the tetrahydroquinoline ring. Reagents and conditions: (i) $R_1B(OH)_2$, $PdCl_2(PPh_3)_2$, K_2CO_3, DMF/water, 90 °C, 5 h; (ii) 1) alcohol **4**, IBX, dry DCE, 80 °C, 2 h; 2) filtration; 3) amine **12**, $NaBH(OAc)_3$, rt, 3 h; (iii) *t*-BuOK, DMSO, O_2, rt, 3 h.

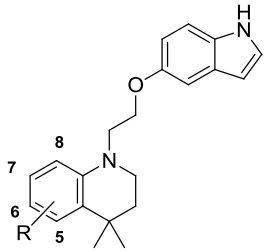
Introduction of 2-pyridinyl ring on position 8 was a double challenge, considering known difficulties with 2-pyridinyl coupling reactions and THQ position 8 hindrance, so several strategies were implemented. Previously described, formation of pinacol or neopentyl boronate ester were tested on aryl bromide **3d**, in order to perform Suzuki coupling with 2-bromopyridine. However, none of these reactions occurred certainly due to interactions between the free amine hydrogen and the bromine. To avoid this, we decided to protect the amine function by a benzyl group. Two strategies were employed starting from compound **3d**: reductive amination using benzaldehyde in the presence of $NaBH(OAc)_3$ and classical *N*-alkylation from benzylbromide using sodium hydride. Unfortunatly, none of the two reactions yielded the desired protected amine. Two different strategies of complete synthesis were then considered starting from 2-aminophenylboronic acid or 2-chloroaniline (Supplementary data). Synthetic route using 2-aminophenylboronic acid permitted to obtain the THQ substituted with a 2-pyridine group in position 8, however all attempts to yield the compound linked to indole by the ethyloxy chain failed. We explained this by the steric hindrance with the 2-pyridinyl group in this 8-position and thus by possible interaction between the hydrogen of amine function on tetrahydroquinoline scaffold and the 2-pyridine nitrogen. From 2-chloroaniline, we achieved synthesis of linked compound with chloride in position 8 on tetrahydroquinoline ring with a very low 5% yield. Then, deprotected compound was obtained and used to form the corresponding pinacol boronate or neopentyl boronate ester but all reactions failed and led to the starting chlorinated material.

Results and discussion

In order to improve the antileukemic activity of **17f,** SAR studies were conducted with focus on the pyridinyl heterocycle bound to the tetrahydroquinoline ring. In this work, we then addressed whether changes in the nitrogen position on the pyridinyl moiety as well as changes in the position of the latter on the THQ ring might directly impact the antileukemic potential of **17f**.

Initial screenings were carried out to determine IC_{50} values for newly synthesized compounds tested on CML (KU812 and K562) and AML (MV4-11) cell lines (Table 1).

Table 1. Structure of analogs **7a-c** and **7a'-c'**



IC_{50} (μM)					
Compound			CML		AML
	Substitution on THQ ring	R	KU812	K562	MV4-11
17f = 7b	6	3-py	5.38 ± 0.01	8.74 ± 1.54	3.55 ± 0.47
6a	5	Br	10.74 ± 3.07	17.17 ± 3.33	27.89 ± 2.91
6b	6	Br	7.59 ± 0.63	15.58 ± 1.67	7.14 ± 0.85
6c	7	Br	30.9 ± 8.27	55.13 ± 10.70	4.86 ± 1.25
7a	5	3-py	5.80 ± 0.80	3.12 ± 0.50	5.90 ± 0.70
7a'	5	4-py	2.50 ± 0.10	5.20 ± 0.70	2.80 ± 0.40
7b'	6	4-py	9.38 ± 1.18	22.65 ± 1,09	13.35 ± 2.83
7c	7	3-py	84.45 ± 19.60	103.70 ± 13.30	25.30 ± 11.32
7c'	7	4-py	93.21 ± 8.19	444 ± 6.02	945 ± 151.20
8b	6	Bpin	35.23 ± 11.78	21.46 ± 5.28	23.56 ± 4.28
8c	7	Bpin	3.15 ± 1.18	15.50 ± 1.14	14.88 ± 4.80
9a	5	2-py	16.00 ± 4.19	51.06 ± 10.77	14.88 ± 5.43
9b	6	2-py	>200	>900	15.25 ± 1.82
9c	7	2-py	89.50 ± 4.51	914.16 ± 195.30	88.15 ± 14.33
10	5	Bneop	41.23 ± 4.54	91.43 ± 2.07	6.57 ± 1.99
13d	8	3-py	21.40 ± 6.02	22.6 ± 7.35	4.25 ± 1.51
13d'	8	4-py	20.25 ± 6.04	15.5 ± 4.99	6.85 ± 0.34

[a]IC_{50} values of **17f** analogs and their precursors obtained for K562, KU812 and MV4-11 cell lines. Cells were treated with concentrations ranging from 100 nM to 100 μM for 48 h. Cytotoxicity was determined by MTT assays. IC_{50} values were calculated using GraphPad Prism 7 software (n = 3 in triplicate, mean ± SEM).

Compounds **7c**, **7c'** and **13d**, **13d'** substituted respectively in position 7 and 8 on the THQ ring were less effective in inhibiting leukemic cell growth than **17f**. In addition, results clearly indicated that substitution by the 2-pyridinyl group in each position on the THQ ring (analogs **9a-c**) dramatically reduced the effectiveness of **17f**. Precursor compounds **6a-c**, **8b**,**c** and **10** showed average IC_{50} values similar or higher than **17f** on both CML and AML cell lines. In sharp contrast, compounds **7a** and **7a'** substituted in position 5 on THQ ring respectively by the 3- and 4-pyridinyl moieties showed similar or enhanced antileukemic activity when compared to **17f**. Thereby, activity of compounds **7a**, **7a'** and **17f** seems to be promoted by their pyridinyl nitrogen similar position (i.e. possible formation of a hydrogen bond in a same area). This observation can explain also the lower activity of compound **7b'**, where pyridinyl nitrogen is slightly offset from this area. To confirm these results in other leukemic cells, IC_{50} values of **7a** and **7a'** were also determined in KCL-22 (CML), KG1a and MOLM-13 (AML) cell lines. Excepted for KG1a cells, IC_{50} values obtained for **7a** and **7a'** were nearly close to those obtained for **17f** (Table 2A). Besides, compounds **6c**, **10**, **13d** and **13d'**, which only showed a good activity on MV4-11 cells, were also evaluated on KG1a and MOLM-13 cell lines but did not exhibit relevant results (Supplementary data). Additionally,

cytotoxicity effects of **7a** and **7a'** were also determined on human bone marrow stromal cells (cell line: HS-27a and primary mesenchymal stem cells: MSC) which play a crucial role in the leukemic niche. Results showed that cytotoxic effects of **7a** and **7a'** on HS-27a and MSC were slightly higher than those observed for **17f** but IC_{50} values remained 5 to 10-fold higher than those required to inhibit leukemic cell growth (Table 2B).

Table 2. Cytotoxic effects of **7a**, **7a'** and **17f** in CML, AML and human bone marrow stromal cells

A

	IC_{50} (µM)		
Compound	**CML**	**AML**	
	KCL-22	**KG1a**	**MOLM-13**
17f	5.6 ± 0.8	3.4 ± 0.9	3.3 ± 1.0
7a	6.9 ± 3.1	7.8 ± 0.9	5.4 ± 0.5
7a'	8.7 ± 1.9	6.9 ± 0.8	4.7 ± 1.0

B

	Toxicity on Stromal Cells - IC_{50} (µM)	
Compound	**HS-27a**	**MSC**
17f	63.7 ± 0.1	33.6 ± 5.9
7a	27.0 ± 1.4	27.1 ± 1.4
7a'	23.2 ± 5.0	12.5 ± 0.5

[a](A) IC_{50} values of selected compounds obtained for KCL-22, KG1a and MOLM-13 cell lines. (B) IC_{50} values of selected compounds obtained for HS-27a and MSC stromal cells. (A-B) Cells were treated with concentrations ranging from 100 nM to 100 µM for 48 h and cytotoxicity was determined by MTT assays. IC_{50} values were calculated using GraphPad Prism 7 software (n = 3 in triplicate, mean ± SEM).

To analyze the growth inhibitory properties of **7a** and **7a'**, KU812 and MV4-11 cells were treated or not (DMSO as control) with 5µM (concentration that roughly corresponds to that of the **17f** IC_{50} values) of **7a**, **7a'** and **17f** during 72h. Cell viability and the number of living cells were daily determined by MTT and trypan blue dye exclusion assays respectively. Results showed that **7a** and **7a'**, when compared to **17f**, have a slightly higher or similar cytotoxic effect in KU812 and MV4-11 cells respectively (Figure 2A). Both compounds have also similar or enhanced growth inhibitory properties (Figure 2B) and were able to induce apoptosis in both leukemic cell lines (Figure 2C).

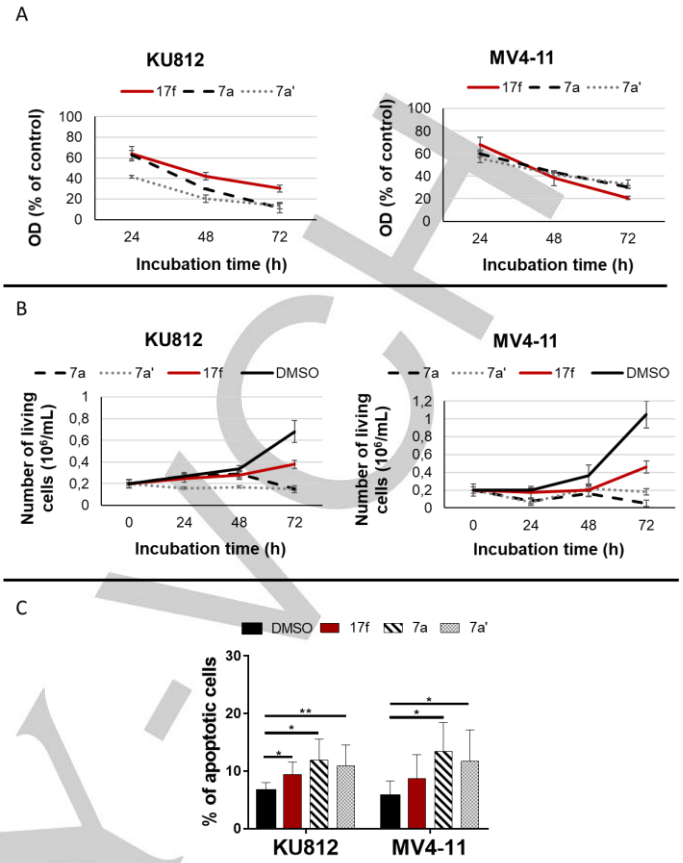


Figure 2. (A) Viability kinetics of KU812 and MV4-11 treated with compounds **17f**, **7a** and **7a'**. Cells were treated with 5 µM of inhibitors or with DMSO as control, for 24, 48 or 72 h. Cytotoxic effect was determined by MTT assays. Results are presented in percentage of control. Values were calculated using GraphPad Prism 7 software (n = 3 in triplicate, mean ± SEM). (B) Growth kinetics of KU812 (up) and MV4-11 (down) treated with 5 µM of compounds **17f**, **7a** and **7a'** or DMSO as control determined by Trypan blue exclusion assays (n = 3 in triplicate, mean ± SEM). (C) Representative flow cytometry histogram of KU812 and MV4-11 cells treated with 3 µM of **7a**, **7a'** and **17f** or DMSO. Cells were stained with FITC Annexin V and 7-AAD, and the percentage of apoptotic cells were then evaluated by flow cytometry (n = 3 in triplicate; mean ± SEM).

We previously demonstrated that **17f** inhibits STAT5 signaling in CML and AML cells, we then addressed whether **7a** and **7a'** also suppress STAT5 activity and expression in KU812 and MV4-11 cells. A concentration of 3 µM was used in the experiments to keep close to the IC_{50} values of both compounds. We first evaluated impact of **7a** and **7a'** on *STAT5A* and *STAT5B* gene expression in both leukemic cell lines by qRT-PCR experiments. Results showed that **7a** and **7a'** had no effect on STAT5A and STAT5B mRNA levels in leukemic cells treated for 15 h with these compounds (Figure 3A). We then analyzed the phosphorylation and expression of STAT5 in KU812 and MV4-11 cells exposed to **7a** and **7a'** (3µM) for 24 and 48 h. Results showed that both compounds inhibited the phosphorylation of STAT5 and reduced STAT5 protein expression at 48 h post-treatment suggesting that **7a** and **7a'** as **17f** might indirectly interfere with the stability/degradation of the proteins (Figure 3B).[42] Further

investigations are then required to determine impact of these molecules on the ubiquitin/proteasome system.

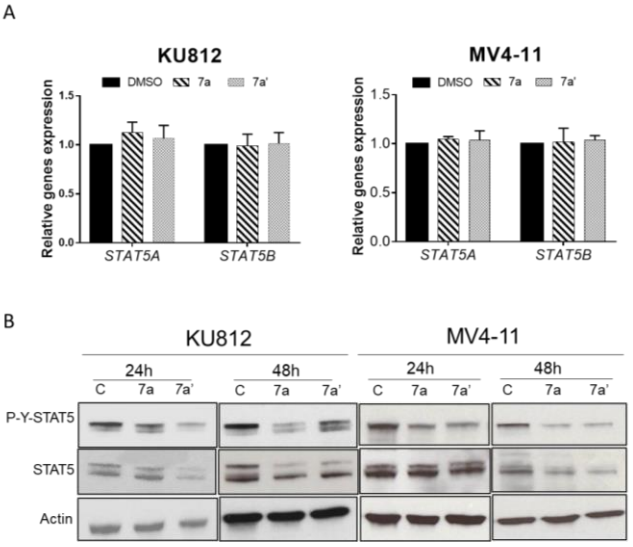


Figure 3. (A) qRT-PCR analysis of *STAT5A* and *STAT5B* transcripts in KU812 and MV4-11 cells treated or not with **7a**, **7a'** or DMSO as control for 24 h. Results are presented as the fold changes in gene expression in treated cells normalized to internal control genes (*GAPDH*, *ACTB*, *RPL13a*) and relative to control cells (normalized to 1) (n = 3 in triplicate; mean ± SEM). (B) Protein extracts from KU812, MV4-11 cells treated with **7a** and **7a'** (3 µM) or DMSO (–) for 48 h were analyzed by Western blotting to detect P-Y-STAT5 and STAT5 protein expression. Actin was used as a loading control (n = 3).

We then addressed whether **7a** and **7a'** inhibited STAT5 transcriptional activity. qRT-PCR experiments were conducted to determine effects of these compounds on STAT5-dependent expression of target genes such as *PIM1* and *CISH* in KU812 and MV4-11 cells. **17f** was used as a positive control in these experiments. We found that both molecules were as efficient as **17f** to downregulate *PIM1* and *CISH* gene expression (Figure 4).

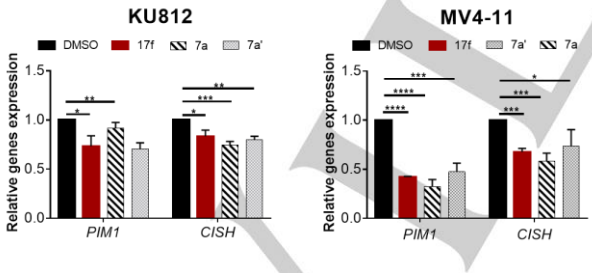


Figure 4. qRT-PCR analysis of STAT5 target genes, *PIM1* and *CISH* transcripts, in KU812 (left) and MV4-11 (right) cells treated or not with **7a**, **7a'**, **17f** (3 µM) or DMSO as the control for 24 h. Results are presented as the fold changes in gene expression in treated cells normalized to internal control genes (*GAPDH*, *ACTB*, *RPL13a*) and relative to control cells (normalized to 1) (n = 3 in triplicate; mean ± SEM).

We previously showed that **17f** overcomes the resistance of CML and AML cells to IM and Ara-C.[42] We then ask whether **7a** and **7a'** had a greater impact than **17f** to overcome the resistance of CML cells to IM. K562R cells that were previously used as an IM-resistant leukemic cell model, were treated with various concentrations of **17f**, **7a** and **7a'** (ranging from 0.1 to 10 µM) in combination with 1 µM IM. Results showed that compound **7a'** exhibited a slightly but significant better cytotoxic effect than **17f** and **7a** at 1 and 5 µM concentrations (figure 5A). This was also confirmed in kinetic experiments using 5 µM as the difference in the optimal concentration between each compound (figure 5B).

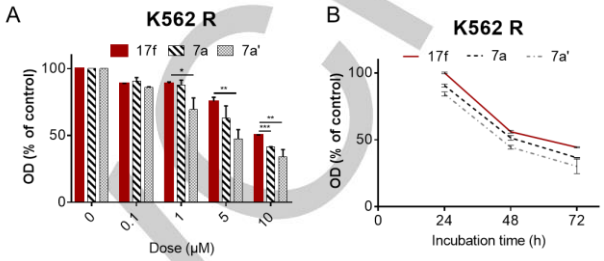


Figure 5. (A) Metabolic activity of IM-resistant cells K562 R treated with IM (1 µM) in combination with ranging doses from 100 nM to 10 µM of **7a**, **7a'** or **17f**, for 48 h. Cytotoxic effect was determined by MTT assays. Results are presented in percentage of control. Values were calculated using GraphPad Prism 7 software (n = 3 in triplicate, mean ± SEM). (B) Viability kinetics of IM-resistant cells K562 R treated with IM (1 µM) as control in combination with selected compound, **17f**, **7a** or **7a'** (5 µM) for 24, 48 or 72 h. Cytotoxic effect was determined by MTT assays. Results are presented in percentage of control. Values were calculated using GraphPad Prism 7 software (n = 3 in triplicate; mean ± SEM).

Conclusion

In summary, among the 10 new analogs of **17f**, compounds **7a** and **7a'** were found to be the most interesting inhibitors of STAT5 signaling and myeloid leukemia cell growth when compared to **17f**. Our SAR studies demonstrated that both positions of pyridinyl moiety on the THQ aromatic part and its nitrogen position were essential in the antileukemic properties of **7a** and **7a'**. Indeed, the C-5 tetrahydroquinoline 3- and 4- pyridinyl substitutions in **7a** and **7a',** gave better IC_{50} results than C-7 and C-8 THQ ring substitutions as well as all 2-pyridinyl substituted derivatives. This work also showed that, as **17f**, both **7a** and **7a'** block STAT5 transcriptional activity by affecting not only the phosphorylation but also expression of the proteins. Experiments are now conducted to determine whether both compounds can affect expression of STAT5A, STAT5B and most importantly the oncogenic $STAT5B^{N642H}$ mutant which is frequently expressed in different T cell leukemias/lymphomas and in T cell lymphoblastic leukemia.[45,46] SAR studies will be then continued on our compound central scaffold. In particular substitutions in positions C-5 and C-6 of the THQ ring by polynitrogen aromatic heterocycles like pyrimidine or pyrazine will be conducted to generate molecules with improved antileukemic potential while avoiding their cytotoxic effects on normal cells. This should help us to get a better understanding of the influence of nitrogen on the THQ part of our structure.

Experimental Section

CHEMISTRY

Materials and methods

Reagents and solvents were purchased from commercial sources (Acros, Aldrich and VWR international) and were used without further purification. For synthesis in anhydrous and inert conditions, the glassware was dried with a heat gun during several vacuum-dry argon cycles and maintained under argon. Reactions were monitored by using thin layer chromatography (TLC), pre-coated aluminum sheets silica gel 60 F254 marketed by Merck and visualized under UV fluorescence 254 and 365 nm. Purifications were carried out by chromatography on silica gel columns on an ISCO purification unit, Combi Flash RF 75 PSI, with Redisep flash silica gel columns (60 Å, 230-400 mesh, grade 9385). Melting point (mp) were determined on a Büchi melting point apparatus Model B-540 in open capillaries. NMR spectra were recorded at 300 MHz (^{1}H) or 75 MHz (^{13}C) on a Bruker AVANCE AV 300 instrument. All NMR experiments were realized in deuterated chloroform ($CDCl_3$) or dimethylsulfoxide (DMSO-d_6). Chemical shifts were reported in parts per million (ppm) (δ relative to residual solvent peak for ^{1}H and ^{13}C) and coupling constants (J) are reported in hertz (Hz). The notations used are: δ: chemical shift (ppm); s: singlet; d: doublet; dd: doublet of doublet; t: triplet; m: multiplet; br: broad signal; J: coupling constant (Hz). High-resolution mass spectra (HRMS) was performed by the mass spectrometry service on a Q-Exactive spectrometer from Thermo Scientific using the electrospray ionization (ESI) technique. If necessary, the purity was determined by high performance liquid chromatography (HPLC). Purity of all final compounds was 95% or higher. HPLC analyses were carried out with a LaChrom Elite system [Hitachi L-2130 (pump), L-2200 (autosampler) and L-2400 (UV-detector)] using 254 nm UV for detection. The column was a XBridge C-18 (250 × 10 mm, 4µm, 135Å); elution was performed with 0.1% (by volume) of TFA in water (solvent A), and 0.1% of TFA in acetonitrile (solvent B); gradient 20-100% of B over 35 minutes with a flow rate of 1 mL.min^{-1}; column temperature of 25 °C; injection of 10 µL in DMSO.

*Method A: Nucleophilic substitution (**1a, 1b**).*

To a solution of bromoaniline (1.0 equiv) in dry pyridine at 0 °C under argon, was added dropwise 3,3-dimethylacryloylchloride (1.2 equiv). The reaction mixture was stirred for 5 h at rt and then carefully poured into an Erlenmeyer flask containing 1 M HCl under magnetic stirring. The product was filtered, washed by water and heptane, then purified by flash chromatography on silica gel.

1a *N*-(2-Bromophenyl)-3-methylbut-2-enamide

Following **method A**, the reaction of 2-bromoaniline (4.60 mL, 40.70 mmol), 3,3-dimethylacryloyl chloride (5.50 mL, 48.84 mmol) in dry pyridine (17.70 mL) afforded, after purification by flash chromatography on silica gel (cyclohexane/ethyl acetate; 70/30), the title compound **1a** as a white solid (10.30 g, *quant.*); mp 100 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.28 (s, 1H), 7.68 – 7.58 (m, 2H), 7.40 – 7.30 (m, 1H), 7.15 – 7.07 (m, 1H), 6.05 – 5.96 (m, 1H), 2.13 (d, *J* = 1.1 Hz, 3H), 1.86 (d, *J* = 1.0 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 164.6, 152.1, 136.5, 132.6, 127.8, 127.1, 126.7, 118.6, 117.9, 27.0, 19.6. HRMS-ESI (*m/z*): found 254.01704, cacld for $C_{11}H_{13}{}^{79}BrNO^+$ $[M+H]^+$ 254.01750 and found 256.01461, cacld for $C_{11}H_{13}{}^{81}BrNO^+$ $[M+H]^+$ 256.01546.

1b *N*-(3-Bromophenyl)-3-methylbut-2-enamide

Following **method A**, the reaction of 3-bromoaniline (11.40 mL, 105.00 mmol), 3,3-dimethylacryloyl chloride (14.00 mL, 126.00 mmol) in dry pyridine (46.00 mL) afforded, after purification by flash chromatography on silica gel (cyclohexane/ethyl acetate; 70/30), the title compound **1b** as a white solid (26.70 g, *quant.*); mp 115 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.01 (s, 1H), 8.02 (dd, *J* = 1.8, 1.8 Hz, 1H), 7.48 (ddd, *J* = 7.8, 1.8, 1.8 Hz, 1H), 7.25 (dd, *J* = 7.8, 7.8 Hz, 1H), 7.19 (ddd, *J* = 7.8, 1.8, 1.8 Hz, 1H), 5.84 (dq, *J* = 1.1, 1.1 Hz, 1H), 2.15 (d, *J* = 1.1 Hz, 3H), 1.87 (d, *J* = 1.1 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 164.8, 152.4, 141.2, 130.7, 125.4, 121.5, 121.2, 118.8, 117.7, 27.1, 19.6. HRMS-ESI (*m/z*): found 254.01729, cacld for $C_{11}H_{13}{}^{79}BrNO^+$ $[M+H]^+$ 254.01750 and found 256.01488, cacld for $C_{11}H_{13}{}^{81}BrNO^+$ $[M+H]^+$ 256.01546.

*Method B: Cyclization (**2a, 2c, 2d**).*

To a solution of *N*-bromophenyl-3-methylbut-2-enamide **1** (1.0 equiv) in dry dichloromethane at 0 °C under argon was added portionwise aluminium chloride (4.0 equiv) over 30 min. The reaction mixture was stirred for 1 h at rt, then poured on ice and extracted with diethyl ether. After extraction, the combined organic extracts were washed with saturated aqueous $NaHCO_3$ solution, brine and then dried with $MgSO_4$. After evaporation under reduced pressure, the crude product was purified by flash chromatography on silica gel.

2a 5-Bromo-4,4-dimethyl-3,4-dihydroquinolin-2(*1H*)-one and **2c** 7-bromo-4,4-dimethyl-3,4-dihydroquinolin-2(*1H*)-one

Following **method B**, the reaction of *N*-(3-bromophenyl)-3-methylbut-2-enamide **1b** (20.00 g; 78.70 mmol) and aluminium chloride (42.00 g, 314.80 mmol) in dry dichloromethane (97 mL) afforded, after purification by flash chromatography on silica gel (cyclohexane/ethyl acetate; 70/30), the title compounds **2a** as a white solid (7.38 g, 43 %) and **2c** as a white solid (9.05 g, 47 %).

2a: mp 137 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.28 (brs, 1H), 7.20 (dd, J = 7.9, 1.3 Hz, 1H), 7.04 (d, J = 7.9, 7.9 Hz, 1H), 6.88 (dd, J = 7.9, 1.3 Hz, 1H), 2.43 (s, 2H), 1.44 (s, 6H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 168.4, 139.8, 130.0, 128.9, 128.6, 120.7, 115.8, 46.8, 36.5, 27.0 (2C). HRMS-ESI (*m/z*): found 254.01728, cacld for $C_{11}H_{13}{}^{79}BrNO^+$ $[M+H]^+$ 254.01750 and found 256.01501, cacld for $C_{11}H_{13}{}^{81}BrNO^+$ $[M+H]^+$ 256.01546.

2c: mp 175 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.25 (brs, 1H), 7.23 (d, *J* = 8.3 Hz, 1H), 7.13 (dd, *J* = 8.3, 2.0 Hz, 1H), 7.03 (d, *J* = 2.0 Hz, 1H), 2.36 (s, 2H), 1.20 (s, 6H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 169.4, 138.7, 131.5, 126.5, 124.8, 119.5, 117.6, 44.5, 33.4, 27.0 (2C). HRMS-ESI (*m/z*): found 254.01706, cacld for $C_{11}H_{13}{}^{79}BrNO^+$ $[M+H]^+$ 254.01750 and found 256.01466, cacld for $C_{11}H_{13}{}^{81}BrNO^+$ $[M+H]^+$ 256.01546.

2d 8-Bromo-4,4-dimethyl-3,4-dihydroquinolin-2(*1H*)-one

Following **method B**, the reaction of *N*-(2-bromophenyl)-3-methylbut-2-enamide **1a** (20.00 g; 78.70 mmol) and aluminium chloride (42.00 g, 314.80 mmol) in dry dichloromethane (97 mL) afforded, after purification by flash chromatography on silica gel (cyclohexane/ethyl acetate; 70/30), the title compound **2d** as a white solid (18.85 g, 94%); mp 108 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.17 (s, 1H), 7.46 (dd, *J* = 7.8, 1.2 Hz, 1H), 7.33 (dd, *J* = 7.8, 1.2 Hz, 1H), 6.96 (dd, *J* = 7.8, 7.8 Hz, 1H), 2.41 (s, 2H), 1.24 (s, 6H).^{13}C NMR (75 MHz, DMSO-d_6): δ 169.4, 135.5, 134.7, 130.9, 124.1, 123.9, 109.7, 44.6, 34.3, 26.9 (2C). HRMS-ESI (*m/z*): found 254.01710, cacld for $C_{11}H_{13}{}^{79}BrNO^+$ $[M+H]^+$ 254.01750 and found 256.01492, cacld for $C_{11}H_{13}{}^{81}BrNO^+$ $[M+H]^+$ 256.01546.

*Method C: Reduction (**3a, 3c, 3d**).*

To a solution of bromo-4,4-dimethyl-3,4-dihydroquinolin-2(*1H*)-one **2** (1.0 equiv) in dry toluene at 0 °C under argon was added dropwise a solution of BH_3.THF 1 M (2.5 equiv). The reaction mixture was refluxed for 4 h and then quenched at 0 °C by the addition of saturated aqueous $NaHCO_3$ solution. After extraction with ethyl acetate, the combined organic extracts were washed with brine and dried with $MgSO_4$. After evaporation under reduced pressure, the crude product was purified by flash chromatography on silica gel.

3a 5-Bromo-4,4-dimethyl-1,2,3,4-tetrahydroquinoline

Following **method C**, the reaction of 5-bromo-4,4-dimethyl-3,4-dihydroquinolin-2(*1H*)-one **2a** (3.00 g; 11.80 mmol) and BH_3.THF 1 M (30.00 mL, 29.51 mmol) in dry toluene (28 mL) afforded, after purification by flash chromatography on silica gel (cyclohexane/ethyl acetate; 80/20), the title compound **3a** as a light yellow oil (2.28 g, 81%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.91 (dd, *J* = 7.8, 1.4 Hz, 1H), 6.75 (dd, *J* = 8.0, 7.8 Hz, 1H), 6.45 (dd, *J* = 8.0, 1.4 Hz, 1H), 3.78 (brs, 1H), 3.24 – 3.16 (m, 2H), 1.85 – 1.78 (m, 2H), 1.57 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 146.7, 128.1, 127.6, 124.7, 124.1, 114.9, 41.9, 38.0, 33.9, 28.7 (2C). HRMS-ESI (*m/z*): found 240.03797, cacld for $C_{11}H_{15}{}^{79}BrN^+$ $[M+H]^+$ 240.03824 and found 242.03542, cacld for $C_{11}H_{15}{}^{81}BrN^+$ $[M+H]^+$ 242.03619.

3c 7-Bromo-4,4-dimethyl-1,2,3,4-tetrahydroquinoline

Following **method C**, the reaction of 7-bromo-4,4-dimethyl-3,4-dihydroquinolin-2(*1H*)-one **2c** (7.00 g; 27.50 mmol) and BH_3.THF 1 M (70.00 mL, 69.00 mmol) in dry toluene (65 mL) afforded, after purification by flash chromatography on silica gel (cyclohexane/ethyl acetate; 80/20), the title compound **3c** as a light yellow oil (5.95 g, 90%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.03 (d, *J* = 8.2 Hz, 1H), 6.74 (dd, *J* = 8.2, 2.1 Hz, 1H), 6.62 (d, *J* = 2.1 Hz, 1H), 3.87 (brs, 1H), 3.34 – 3.28 (m, 2H), 1.75 – 1.69 (m, 2H), 1.27 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 144.8, 129.3, 128.0, 120.0, 119.9, 116.6, 38.4, 36.8, 31.7, 30.8 (2C). HRMS-ESI (*m/z*): found 240.03807, cacld for $C_{11}H_{15}{}^{79}BrN^+$ $[M+H]^+$ 240.03824 and found 242.03541, cacld for $C_{11}H_{15}{}^{81}BrN^+$ $[M+H]^+$ 242.03619.

3d 8-Bromo-4,4-dimethyl-1,2,3,4-tetrahydroquinoline

Following **method C**, the reaction of 8-bromo-4,4-dimethyl-3,4-dihydroquinolin-2(*1H*)-one **2d** (10.00 g; 39.30 mmol) and BH_3.THF 1 M (100.00 mL, 98.20 mmol) in dry toluene (92 mL) afforded, after purification by flash chromatography on silica gel (cyclohexane/ethyl acetate; 80/20), the title compound **3d** as a light yellow oil (8.96 g, 76%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.26 (dd, J = 7.9, 1.4 Hz, 1H), 7.17 (dd, *J* = 7.7, 1.4 Hz, 1H), 6.53 (dd, *J* = 7.9, 7.7 Hz, 1H), 4.69 (brs, 1H), 3.49 – 3.36 (m, 2H), 1.83 – 1.71 (m, 2H), 1.32 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 140.5, 132.0, 130.1, 125.4, 117.2, 109.0, 38.5, 36.7, 32.4, 30.9 (2C). HRMS-ESI (*m/z*): found 240.03775, cacld for $C_{11}H_{15}{}^{79}BrN^+$ $[M+H]^+$ 240.03824 and found 242.03521, cacld for $C_{11}H_{15}{}^{81}BrN^+$ $[M+H]^+$ 242.03619.

*Method D: Reductive Amination (**5a**, **5c**, **12a**, **12b**).*

To a solution of 2-((1-benzyl-*1H*-indol-5-yl)oxy)ethanol **4** (1.0 equiv) in dry 1,2-DCE under argon, was added 2-iodoxybenzoic acid (IBX) (3.0 equiv). The resulting mixture was stirred 2 h at 80 °C and then cooled to rt. The reaction medium was filtered through a sintered glass funnel over a round bottom flask containing the corresponding amine (2.1 equiv) in dry 1,2-DCE. The resulting mixture was stirred for 15 minutes and then $NaBH(OAc)_3$ (2.0 equiv) was added. The reaction medium was stirred 2 h at rt and then hydrolyzed with a saturated aqueous $NaHCO_3$ solution. After extraction with ethyl acetate, the combined organic extracts were washed with brine and dried with $MgSO_4$. After evaporation under reduced pressure, the crude product was purified by flash chromatography on silica gel.

5a 1-(2-((1-Benzyl-*1H*-indol-5-yl)oxy)ethyl)-5-bromo-4,4-dimethyl-1,2,3,4-tetrahydroquinoline

Following **method D**, the reaction of 2-((1-benzyl-*1H*-indol-5-yl)oxy)ethanol **4** (0.35 g, 1.32 mmol), IBX (1.11 g, 3.96 mmol), 5-bromo-4,4-dimethyl-1,2,3,4-tetrahydroquinoline **3a** (0.66 g, 2.77 mmol), $NaBH(OAc)_3$ (0.56 g, 2.64 mmol) in dry 1,2-DCE (5 mL) afforded, after purification by flash chromatography on silica gel (cyclohexane/ethyl acetate; 80/20), a mixture of title compound **5a** with starting material **3a**. The mixture was dissolved in diethyl ether (200 mL), washed by HCl 3 M (3 × 100 mL) and brine (1 × 100 mL), dried with $MgSO_4$ and evaporated under reduced pressure to give **5a** as a light yellow oil (0.51 g, 78%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.44 (d, *J* = 3.1 Hz, 1H), 7.33 – 7.20 (m, 4H), 7.17 – 7.12 (m, 2H), 7.04 (d, *J* = 2.3 Hz, 1H), 6.88 – 6.69 (m, 4H), 6.35 (d, *J* = 3.1 Hz, 1H), 5.36 (s, 2H), 4.10 (t, *J* = 5.7 Hz, 2H), 3.68 (t, *J* = 5.7 Hz, 2H), 3.34 – 3.29 (m, 2H), 1.77 – 1.68 (m, 2H), 1.46 (s, 6H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 153.0, 147.5, 138.8, 131.6, 130.1, 129.2, 128.9 (2C), 128.3, 128.2, 127.7, 127.3 (2C), 123.6, 122.8, 112.0, 111.9, 111.3, 103.7, 101.0, 65.5, 51.9, 49.7, 46.0, 41.3, 34.1, 28.8 (2C). HRMS-ESI (*m/z*): found 489.15329, cacld for $C_{28}H_{30}{}^{79}BrN^+$ $[M+H]^+$ 489.15360 and found 491.15117, cacld for $C_{28}H_{30}{}^{81}BrN^+$ $[M+H]^+$ 491.15156.

5c 1-(2-((1-Benzyl-*1H*-indol-5-yl)oxy)ethyl)-7-bromo-4,4-dimethyl-1,2,3,4-tetrahydroquinoline

Following **method D**, the reaction of 2-((1-benzyl-*1H*-indol-5-yl)oxy)ethanol **4** (0.50 g, 1.87 mmol), IBX (1.57 g, 5.61 mmol), 7-bromo-4,4-dimethyl-1,2,3,4-tetrahydroquinoline **3c** (0.95 g, 3.92 mmol), $NaBH(OAc)_3$ (0.80 g, 3.74 mmol) in dry 1,2-DCE (7 mL) afforded, after purification by flash chromatography on silica gel (cyclohexane/ethyl acetate; 98/2), a mixture of title compound **5c** with starting material **3c**. The mixture was dissolved in diethyl ether (200 mL), washed by HCl 3 M (3 × 100 mL) and brine (1 × 100 mL), dried with $MgSO_4$ and evaporated under reduced pressure to give **5c** as a light yellow oil (0.45 g, 49%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.44 (d, *J* = 3.1 Hz, 1H), 7.34 – 7.21 (m, 4H), 7.17 – 7.12 (m, 2H), 7.06 (d, *J* = 2.4 Hz, 1H), 7.04 (d, *J* = 8.2 Hz, 1H), 6.80 (d, *J* = 1.9 Hz, 1H), 6.71 (dd, *J* = 8.9, 2.4 Hz, 1H), 6.62 (dd, *J* = 8.2, 1.9 Hz, 1H), 6.36 (d, *J* = 3.1 Hz, 1H), 5.36 (s, 2H), 4.12 (t, *J* = 5.4 Hz, 2H), 3.67 (t, *J* = 5.4 Hz, 2H), 3.45 – 3.39 (m, 2H), 1.66 – 1.60 (m, 2H), 1.17 (s, 6H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 152.5, 145.2, 138.4, 131.1, 129.8, 129.7, 128.7, 128.5 (2C), 127.4, 127.3, 126.9 (2C), 119.8, 117.4, 112.6, 111.5, 110.9, 103.2, 100.6, 65.1, 50.1, 49.2, 45.9, 35.9, 31.4, 30.0 (2C). HRMS-ESI (*m/z*): found 489.15317, cacld for $C_{28}H_{30}{}^{79}BrN^+$ $[M+H]^+$ 489.15360 and found 491.15109, cacld for $C_{28}H_{30}{}^{81}BrN^+$ $[M+H]^+$ 491.15156.

*Method E: Heterocyclic amine debenzylation (**6a**, **6c**, **13d-d'**).*

To a solution of the benzyl indole **5** or **12** (1.0 equiv) in DMSO at rt was added *t*-BuOK (14.0 equiv). Dry air was then bubbled into the solution and stirred 2 h at rt. The reaction mixture was quenched with saturated aqueous NH_4Cl solution, extracted with ethyl acetate, washed with brine and dried with $MgSO_4$. After evaporation under reduced pressure, the crude product was purified by flash chromatography on silica gel.

6a 1-(2-((*1H*-Indol-5-yl)oxy)ethyl)-5-bromo-4,4-dimethyl-1,2,3,4-tetrahydroquinoline

Following **method E**, the reaction of 1-(2-((1-benzyl-*1H*-indol-5-yl)oxy)ethyl)-5-bromo-4,4-dimethyl-1,2,3,4-tetrahydroquinoline **5a** (2.02 g; 4.13 mmol) and *t*-BuOK (6.50 g , 57.78 mmol) in DMSO

(122.50 mL) afforded, after purification by flash chromatography on silica gel (cyclohexane/ethyl acetate; 80/20), the title compound **6a** as a light yellow oil (1.61 g, 98%). HPLC purity: 98.8%, ^{t}R = 30.06 min. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.91 (brs, 1H), 7.28 – 7.23 (m, 2H), 7.02 (d, *J* = 2.4 Hz, 1H), 6.89 – 6.82 (m, 1H), 6.79 – 6.67 (m, 3H), 6.32 – 6.27 (m, 1H), 4.11 (t, *J* = 5.7 Hz, 2H), 3.69 (t, *J* = 5.7 Hz, 2H), 3.36 – 3.29 (m, 2H), 1.78 – 1.71 (m, 2H), 1.48 (s, 6H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 152.3, 147.1, 131.1, 128.0, 127.9, 127.7, 125.8, 123.2, 122.3, 112.0, 111.5, 111.4, 102.7, 100.8, 65.0, 51.5, 45.6, 40.8, 33.6, 28.4 (2C). HRMS-ESI (*m/z*): found 399.10623, cacld for $C_{21}H_{24}{}^{79}BrN_2O^+$ $[M+H]^+$ 399.10665 and found 401.10407, cacld for $C_{21}H_{24}{}^{81}BrN_2O^+$ $[M+H]^+$ 401.10461.

6c 1-(2-((*1H*-Indol-5-yl)oxy)ethyl)-7-bromo-4,4-dimethyl-1,2,3,4-tetrahydroquinoline

Following **method E**, the reaction of 1-(2-((1-benzyl-*1H*-indol-5-yl)oxy)ethyl)-5-bromo-4,4-dimethyl-1,2,3,4-tetrahydroquinoline **5c** (1.00 g; 2.04 mmol) and *t*-BuOK (3.21 g, 28.60 mmol) in DMSO (60 mL) afforded, after purification by flash chromatography on silica gel (cyclohexane/ethyl acetate; 80/20), the title compound **6c** as a yellow oil (0.60 g, 74%). HPLC purity: 99.4%, ^{t}R = 29.57 min. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.92 (brs, 1H), 7.31 – 7.23 (m, 2H), 7.09 – 7.01 (m, 2H), 6.82 (d, J = 2.0 Hz, 1H), 6.70 (dd, J = 8.7, 2.4 Hz, 1H), 6.63 (dd, J = 8.2, 2.0 Hz, 1H), 6.32 – 6.27 (m, 1H), 4.13 (t, J = 5.5 Hz, 2H), 3.68 (t, J = 5.5 Hz, 2H), 3.48 – 3.40 (m, 2H), 1.68 – 1.60 (m, 2H), 1.18 (s, 6H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 152.3, 145.2, 131.1, 129.8, 128.0, 127.4, 125.8, 119.9, 117.4, 112.7, 112.0, 111.4, 102.7, 100.8, 65.1, 50.1, 45.9, 35.9, 31.4, 30.0 (2C). HRMS-ESI (*m/z*): found 399.10635, cacld for $C_{21}H_{24}{}^{79}BrN_2O^+$ $[M+H]^+$ 399.10665 and found 401.10420, cacld for $C_{21}H_{24}{}^{81}BrN_2O^+$ $[M+H]^+$ 401.10461.

*Method F: Suzuki coupling (**7a-a'**, **7c-c'**, **11d-d'**)*

A mixture of the corresponding aryl bromide **6** (1.0 equiv), K_2CO_3 (1.5 equiv) and the corresponding boronic acid (3.0 equiv) in DMF and water was degassed with argon. $PdCl_2(PPh_3)_2$ (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 with $MgSO_4$. After evaporation under reduced pressure, the crude product was purified by flash chromatography on silica gel.

7a 1-(2-((*1H*-Indol-5-yl)oxy)ethyl)-4,4-dimethyl-5-(pyridin-3-yl)-1,2,3,4-tetrahydroquinoline

Following **method F**, the reaction of 1-(2-((*1H*-indol-5-yl)oxy)ethyl)-5-bromo-4,4-dimethyl-1,2,3,4-tetrahydroquinoline **6a** (75 mg, 0.19 mmol), pyridin-3-ylboronic acid (69 mg, 0.56 mmol), K_2CO_3 (78 mg, 0.56 mmol) and $PdCl_2(PPh_3)_2$ (13 mg, 0.02 mmol) in DMF (4.50 mL) and water (0.90 mL) afforded, after purification by flash chromatography on silica gel (cyclohexane/ethyl acetate; 70/30), the title compound **7a** as a yellow oil (30 mg, 40%). HPLC purity: 97.9%, ^{t}R = 16.57 min. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.93 (brs, 1H), 8.52 (dd, *J* = 4.8, 1.7 Hz, 1H), 8.42 (d, *J* = 1.7 Hz, 1H), 7.63 (ddd, *J* = 7.8, 1.7, 1.7 Hz, 1H), 7.38 (dd, *J* = 7.8, 4.8 Hz, 1H), 7.31 – 7.25 (m, 2H), 7.06 (d, *J* = 2.4 Hz, 1H), 6.99 (dd, *J* = 8.5, 7.3 Hz, 1H), 6.78 (dd, *J* = 8.5, 1.3 Hz, 1H), 6.74 (dd, *J* = 8.7, 2.4 Hz, 1H), 6.34 – 6.28 (m, 1H), 6.13 (dd, *J* = 7.3, 1.3 Hz, 1H), 4.18 (t, *J* = 5.6 Hz, 2H), 3.75 (t, *J* = 5.6 Hz, 2H), 3.47 (t, *J* = 6.0 Hz, 2H), 1.62 – 1.53 (m, 2H), 0.90 (s, 6H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 152.4, 149.3, 147.5, 144.5, 140.9, 138.4, 136.4, 131.1, 128.2, 128.0, 126.0, 125.8, 122.5, 119.5, 112.0, 111.5, 111.3, 102.7, 100.8, 65.0, 51.0, 45.7, 39.3, 32.9, 30.9, 30.7. HRMS-ESI (*m/z*): found 398.21964, cacld for $C_{26}H_{27}N_3O^+$ $[M+H]^+$ 398.22269.

7a' 1-(2-((*1H*-Indol-5-yl)oxy)ethyl)-4,4-dimethyl-5-(pyridin-4-yl)-1,2,3,4-tetrahydroquinoline

Following **method F**, the reaction of 1-(2-((*1H*-indol-5-yl)oxy)ethyl)-5-bromo-4,4-dimethyl-1,2,3,4-tetrahydroquinoline **6a** (80 mg, 0.20 mmol), pyridin-4-ylboronic acid (74 mg, 0.60 mmol), K_2CO_3 (83 mg, 0.60 mmol) and $PdCl_2(PPh_3)_2$ (14 mg, 0.02 mmol) in DMF (4.00 mL) and water (0.80 mL) afforded, after purification by flash chromatography on silica gel (cyclohexane/ethyl acetate; 70/30), the title compound **7a'** as a yellow solid (41 mg, 51%); mp 180 °C. HPLC purity: 99.8 %, ^{t}R = 16.65 min. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.92 (brs, 1H), 8.53 (d, *J* = 4.9 Hz, 2H), 7.31 – 7.24 (m, 4H), 7.05 (d, *J* = 2.4 Hz, 1H), 6.98 (dd, *J* = 8.4, 7.3 Hz, 1H), 6.80 – 6.76 (m, 1H), 6.73 (dd, *J* = 8.7, 2.4 Hz, 1H), 6.33 – 6.29 (m, 1H), 6.06 (dd, *J* = 7.3, 0.9 Hz, 1H), 4.18 (t, *J* = 5.6 Hz, 2H), 3.75 (t, *J* = 5.6 Hz, 2H), 3.51 – 3.43 (m, 2H), 1.62 – 1.54 (m, 2H), 0.95 (s, 6H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 152.4, 148.6 (2C), 144.4, 142.5, 139.5, 131.1, 128.0 (2C), 126.0, 125.8, 124.8 (2C), 118.4, 112.0, 111.5, 111.2, 102.7, 100.8, 65.0, 50.9, 45.7, 39.0, 32.9, 30.6 (2C). HRMS-ESI (*m/z*): found 398.21954, cacld for $C_{26}H_{28}N_3O^+$ $[M+H]^+$ 398.22269.

7c 1-(2-((*1H*-Indol-5-yl)oxy)ethyl)-4,4-dimethyl-7-(pyridin-3-yl)-1,2,3,4-tetrahydroquinoline

Following **method F**, the reaction of 1-(2-((*1H*-indol-5-yl)oxy)ethyl)-7-bromo-4,4-dimethyl-1,2,3,4-tetrahydroquinoline **6c** (100 mg, 0.25 mmol), pyridin-3-ylboronic acid (92 mg, 0.75 mmol), K_2CO_3 (104 mg, 0.75 mmol) and $PdCl_2(PPh_3)_2$ (17 mg, 0.025 mmol) in DMF (5.00 mL) and water (1.20 mL) afforded, after purification by flash chromatography on silica gel (cyclohexane/ethyl acetate; 60/40), the title compound **7c** as a yellow solid (60 mg, 61%); mp 209 °C. HPLC purity: 99.4 %, ^{t}R = 18.14 min. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.90 (brs, 1H), 8.84 (d, *J* = 1.9 Hz, 1H), 8.52 (dd, *J* = 4.8, 1.3 Hz, 1H), 7.99 – 7.94 (m, 1H), 7.42 (dd, *J* = 7.9, 4.8 Hz, 1H), 7.28 – 7.21 (m, 3H), 7.06 (d, *J* = 2.4 Hz, 1H), 6.91 (d, *J* = 1.5 Hz, 1H), 6.82 (dd, *J* = 7.8, 1.5 Hz, 1H), 6.70 (dd, *J* = 8.7, 2.4 Hz, 1H), 6.31 – 6.25 (m, 1H), 4.20 (t, *J* = 5.6 Hz, 2H), 3.80 (t, *J* = 5.6 Hz, 2H), 3.52 – 3.45 (m, 2H), 1.75 – 1.66 (m, 2H), 1.25 (s, 6H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 152.3, 147.6, 147.3, 144.1, 136.7, 135.4, 134.3, 131.1, 130.7, 128.0, 126.5, 125.8, 123.8, 113.9, 112.0, 111.5, 109.0, 102.7, 100.8, 65.2, 50.2, 46.0, 36.4, 31.5, 30.4 (2C). HRMS-ESI (*m/z*): found 398.21970, cacld for $C_{26}H_{27}N_3O^+$ $[M+H]^+$ 398.22269.

7c' 1-(2-((*1H*-Indol-5-yl)oxy)ethyl)-4,4-dimethyl-7-(pyridin-4-yl)-1,2,3,4-tetrahydroquinoline

Following **method F**, the reaction of 1-(2-((*1H*-indol-5-yl)oxy)ethyl)-7-bromo-4,4-dimethyl-1,2,3,4-tetrahydroquinoline **6c** (100 mg, 0.25 mmol), pyridin-4-ylboronic acid (92 mg, 0.75 mmol), K_2CO_3 (104 mg, 0.75 mmol) and $PdCl_2(PPh_3)_2$ (17 mg, 0.025 mmol) in DMF (5 mL) and water (1 mL) afforded, after purification by flash chromatography on silica gel (cyclohexane/ethyl acetate; 60/40), the title compound **7c'** as a white solid (58.70 mg, 59%); mp: 209 °C. HPLC purity: 99.9%, ^{t}R = 18.31 min. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.92 (brs, 1H), 8.53 – 8.48 (m, 2H), 7.56 – 7.51 (m, 2H), 7.30 – 7.22 (m, 3H), 7.07 (d, *J* = 2.3 Hz, 1H), 6.95 (d, *J* = 1.5 Hz, 1H), 6.89 (dd, *J* = 7.9, 1.5 Hz, 1H), 6.71 (dd, *J* = 8.7, 2.3 Hz, 1H), 6.31 – 6.26 (m, 1H), 4.21 (t, *J* = 5.5 Hz, 2H), 3.80 (t, *J* = 5.5 Hz, 2H), 3.52 – 3.44 (m, 2H), 1.74 – 1.68 (m, 1H), 1.25 (s, 6H).^{13}C NMR (75 MHz, DMSO-d_6): δ 152.3, 149.9 (2C), 148.0, 144.2, 135.6, 131.8, 131.1, 128.0, 126.5, 125.8, 121.1 (2C), 113.7, 112.0, 111.5, 108.7, 102.8, 100.8, 65.2, 50.1, 45.9, 36.3, 31.6, 30.3 (2C). HRMS-ESI (*m/z*): found 398.21947, cacld for $C_{26}H_{27}N_3O^+$ $[M+H]^+$ 398.22269.

*Method G: Boronate formation (**8a-c**, **10**)*

A mixture of the corresponding aryl bromide **6** (1.0 equiv), KOAc (5.0 equiv) and the corresponding bisdiboron (1.2 equiv) in dioxane was degassed with argon. Then $PdCl_2$(dppf) (0.07 equiv) was added and the resulting mixture was stirred at 100 °C for 12 h. The mixture was cooled to rt, filtered through a pad of dicalite, washed with water and

extracted with ethyl acetate. Combined organic extracts were washed with brine and dried with $MgSO_4$. After evaporation under reduced pressure, the crude product was purified by flash chromatography on silica gel.

8a 1-(2-((1*H*-Indol-5-yl)oxy)ethyl)-4,4-dimethyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1,2,3,4-tetrahydroquinoline

Following **method G**, the reaction of 1-(2-((*1H*-indol-5-yl)oxy)ethyl)-5-bromo-4,4-dimethyl-1,2,3,4-tetrahydroquinoline **6a** (335 mg, 0.84 mmol), bis(pinacolato)diboron (256 mg, 1.01 mmol), KOAc (412 mg, 4.20 mmol) and $PdCl_2$(dppf) (43 mg, 0.059 mmol) in dioxane (5 mL) afforded, after purification by flash chromatography on silica gel (cyclohexane/ethyl acetate; 80/20), the title compound **8a** as a light yellow solid (188 mg, 50%).

8b 1-(2-((1*H*-Indol-5-yl)oxy)ethyl)-4,4-dimethyl-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1,2,3,4-tetrahydroquinoline

Following **method G**, the reaction of 1-(2-((1*H*-indol-5-yl)oxy)ethyl)-6-bromo-4,4-dimethyl-1,2,3,4-tetrahydroquinoline **6b** (660 mg, 1.65 mmol), bis(pinacolato)diboron (504 mg, 1.98 mmol), KOAc (811 mg, 8.26 mmol) and $PdCl_2$(dppf) (84.7 mg, 0.116 mmol) in dioxane (8.3 mL) afforded, after purification by flash chromatography on silica gel (cyclohexane/ethyl acetate; 80/20), the title compound **8b** as a beige solid (468 mg, 63%); mp : 201 °C. HPLC purity: 95.2%, ^tR = 29.52 min. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.91 (s, 1H), 7.40 (d, *J* = 1.5 Hz, 1H), 7.32 – 7.22 (m, 3H), 7.02 (d, *J* = 2.3 Hz, 1H), 6.73 – 6.64 (m, 2H), 6.31 – 6.27 (m, 1H), 4.14 (t, *J* = 5.5 Hz, 2H), 3.72 (t, *J* = 5.5 Hz, 2H), 3.52 – 3.45 (m, 2H), 1.69 – 1.63 (m, 2H), 1.24 (s, 12H), 1.20 (s, 6H).^{13}C NMR (75 MHz, DMSO-d_6): δ 152.3, 146.1, 133.8, 131.6, 131.1, 129.5, 128.0, 125.8, 112.0, 111.4, 109.8, 102.7, 100.8, 82.8 (2C), 64.9, 49.9, 46.0, 36.2, 31.3, 30.2 (4C), 24.7 (2C). HRMS-ESI (*m/z*): found 447.28125, cacld for $C_{27}H_{36}BN_2O_3^+$ $[M+H]^+$ 447.28135.

8c 1-(2-((1*H*-Indol-5-yl)oxy)ethyl)-4,4-dimethyl-7-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1,2,3,4-tetrahydroquinoline

Following **method G**, the reaction of 1-(2-((*1H*-indol-5-yl)oxy)ethyl)-7-bromo-4,4-dimethyl-1,2,3,4-tetrahydroquinoline **6c** (260 mg, 0.65 mmol), bis(pinacolato)diboron (198 mg, 0.78 mmol), KOAc (319 mg, 3.25 mmol) and $PdCl_2$(dppf) (33.60 mg, 0.05 mmol) in dioxane (3.40 mL) afforded, after purification by flash chromatography on silica gel (cyclohexane/ethyl acetate; 80/20), the title compound **8c** as a white solid (198 mg, 68%); mp 194 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.91 (s, 1H), 7.28 – 7.22 (m, 2H), 7.14 (d, *J* = 7.6, 1H), 7.08 – 6.99 (m, 2H), 6.87 (d, *J* = 7.6 Hz, 1H), 6.74 (d, *J* = 8.6 Hz, 1H), 6.29 (br, 1H), 4.17 – 4.07 (m, 2H), 3.71 – 3.62 (m, 2H), 1.72 – 1.59 (m, 2H), 1.26 (s, 12H), 1.20 (s, 6H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 152.3, 143.2, 134.1, 131.1, 128.0, 125.8, 125.2, 121.9, 119.4, 116.5, 111.9, 111.4, 102.8, 100.8, 83.3 (2C), 65.1, 50.3, 45.7, 36.3, 31.8, 30.3 (2C), 24.7 (4C). HRMS-ESI (*m/z*): found 447.27817, cacld for $C_{27}H_{36}BN_2O_3^+$ $[M+H]^+$ 447.28135.

*Method H: Suzuki coupling of 2-bromopyridine (**9a-c**).*

A mixture of boronate ester **8** or **10** (1.3 equiv), 2-bromopyridine (1.0 equiv) and Na_2CO_3 (2.5 equiv) in dioxane and water was degassed with argon. $PdCl_2$(dppf) (0.07 equiv) was then added and the resulting mixture was stirred 5 h at 100 °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 with $MgSO_4$. After evaporation under reduced pressure, the crude product was purified by flash chromatography on silica gel.

9a 1-(2-((1*H*-Indol-5-yl)oxy)ethyl)-4,4-dimethyl-5-(pyridin-2-yl)-1,2,3,4-tetrahydroquinoline

Following **method H**, the reaction of 1-(2-((1*H*-indol-5-yl)oxy)ethyl)-5-(5,5-dimethyl-1,3,2-dioxaborinan-2-yl)-4,4-dimethyl-1,2,3,4-tetrahydroquinoline **10** (30 mg, 0.069 mmol), 2-bromopyridine (5.1 µL, 0.053 mmol), Na_2CO_3 (14 mg, 0.133 mmol) and $PdCl_2$(dppf) (3 mg, 0.004 mmol) in dioxane (0.34 mL) and water (0.09 mL) afforded, after purification by flash chromatography on silica gel (cyclohexane/ethyl acetate; 60/40), the title compound **9a** as a light yellow solid (15 mg, 71%); mp : 56 °C. HPLC purity: 98.2%, ^tR = 17.39 min. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.90 (s, 1H), 8.61 (d, *J* = 4.6 Hz, 1H), 7.83 – 7.74 (m, 2H), 7.41 – 7.35 (m, 1H), 7.32 – 7.17 (m, 5H), 7.06 (d, *J* = 1.9 Hz, 1H), 6.73 (dd, *J* = 8.7, 2.2 Hz, 1H), 6.32 – 6.25 (m, 1H), 4.21 (t, *J* = 5.8 Hz, 2H), 3.79 (t, *J* = 5.8 Hz, 2H), 3.52 – 3.44 (m, 2H), 1.75 – 1.68 (m, 2H), 1.25 (s, 6H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 156.9, 152.3, 149.2, 143.9, 137.1, 136.8, 131.7, 131.1, 128.0, 126.1, 125.8, 122.0, 120.0, 113.9, 112.0, 111.5, 108.6, 102.8, 100.8, 65.0, 50.2, 45.9, 36.4, 31.6, 30.4 (2C). HRMS-ESI (*m/z*): found 398.22185, cacld for $C_{26}H_{28}N_3O^+$ $[M+H]^+$ 398.22269.

9b 1-(2-((1*H*-Indol-5-yl)oxy)ethyl)-4,4-dimethyl-6-(pyridin-2-yl)-1,2,3,4-tetrahydroquinoline

Following **method H**, the reaction of 1-(2-((1*H*-indol-5-yl)oxy)ethyl)-4,4-dimethyl-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1,2,3,4-tetrahydroquinoline **8b** (70 mg, 0.157 mmol), 2-bromopyridine (12 µL, 0.12 mmol), Na_2CO_3 (32 mg, 0.30 mmol) and $PdCl_2$(dppf) (6.2 mg, 0.08 mmol) in dioxane (0.80 mL) and water (0.21 mL) afforded, after purification by flash chromatography on silica gel (cyclohexane/ethyl acetate; 75/25), the title compound **9b** as a yellow oil (45 mg, 94%). HPLC purity: 99.9%, ^tR = 17.15 min. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.92 (s, 1H), 8.54 (d, *J* = 4.6 Hz, 1H), 7.93 (d, *J* = 2.1 Hz, 1H), 7.81 – 7.67 (m, 3H), 7.26 (dd, *J* = 5.4, 2.7 Hz, 2H), 7.18 – 7.11 (m, 1H), 7.05 (d, *J* = 2.3 Hz, 1H), 6.77 (d, *J* = 8.8 Hz, 1H), 6.72 (dd, *J* = 8.8, 2.4 Hz, 1H), 6.32 – 6.27 (m, 1H), 4.17 (t, *J* = 5.6 Hz, 2H), 3.76 (t, *J* = 5.5 Hz, 2H), 3.54 – 3.44 (m, 2H), 1.76 – 1.64 (m, 2H), 1.28 (s, 6H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 156.7, 152.3, 149.0, 144.5, 136.8, 131.1, 130.5, 128.0, 125.8, 125.3, 125.1, 123.9, 120.5, 118.4, 112.0, 111.5, 110.6, 102.8, 100.8, 65.1, 50.1, 46.0, 36.3, 31.7, 30.3 (2C). HRMS-ESI (*m/z*): found 398.22189, calcd for $C_{26}H_{28}N_3O^+$ $[M+H]^+$ 398.22269.

9c 1-(2-((1*H*-Indol-5-yl)oxy)ethyl)-4,4-dimethyl-7-(pyridin-2-yl)-1,2,3,4-tetrahydroquinoline

Following **method H**, the reaction of 1-(2-((1*H*-indol-5-yl)oxy)ethyl)-4,4-dimethyl-7-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1,2,3,4-tetrahydroquinoline **8c** (50 mg, 0.11 mmol), 2-bromopyridine (8.20 µL, 0.086 mmol), Na_2CO_3 (23 mg, 0.22 mmol) and $PdCl_2$(dppf) (5 mg, 0.006 mmol) in dioxane (0.60 mL) and water (0.15 mL) afforded, after purification by flash chromatography on silica gel (cyclohexane/ethyl acetate; 75/25), the title compound **9c** as a yellow oil (8 mg, 18%). HPLC purity: 99.0 %, ^tR = 17.63 min. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.91 (br, 1H), 8.61 (d, *J* = 4.8 Hz, 1H), 7.81 – 7.73 (m, 2H), 7.38 (d, *J* = 1.2 Hz, 1H), 7.33 – 7.18 (m, 5H), 7.07 (d, *J* = 2.4 Hz, 1H), 6.73 (dd, *J* = 8.7, 2.4 Hz, 1H), 6.31 – 6.25 (m, 1H), 4.20 (t, *J* = 5.7 Hz, 2H), 3.79 (t, *J* = 5.7 Hz, 2H), 3.52 – 3.43 (m, 2H), 1.76 – 1.66 (m, 2H), 1.25 (s, 6H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 156.9, 152.3, 149.3, 143.9, 137.1, 136.9, 131.7, 131.1, 128.0, 126.1, 125.8, 122.0, 120.0, 113.9, 112.0, 111.5, 108.6, 102.8, 100.8, 65.0, 50.2, 45.9, 36.4, 31.6, 30.4 (2C). HRMS-ESI (*m/z*): found 398.21985, cacld for $C_{26}H_{28}N_3O^+$ $[M+H]^+$ 398.22269.

10 1-(2-((1*H*-Indol-5-yl)oxy)ethyl)-5-(5,5-dimethyl-1,3,2-dioxaborinan-2-yl)-4,4-dimethyl-1,2,3,4-tetrahydroquinoline

Following **method G**, the reaction of 1-(2-((1*H*-indol-5-yl)oxy)ethyl)-5-bromo-4,4-dimethyl-1,2,3,4-tetrahydroquinoline **6a** (400 mg, 1.00 mmol), bis(neopentyl glycolato)diboron (271 mg, 1.20 mmol), KOAc (491 mg, 5.00 mmol) and $PdCl_2$(dppf) (51 mg, 0.07 mmol) in dioxane (8.60 mL) afforded, after purification by flash chromatography on silica

gel (cyclohexane/ethyl acetate; 80/20), the title compound **10** as a light yellow solid (124 mg, 29%); mp 77 °C. HPLC purity: 96.6%, ^{t}R = 24.31 min. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.89 (br, 1H), 7.28 – 7.23 (m, 2H), 7.03 (d, *J* = 2.4 Hz, 1H), 6.90 (dd, *J* = 8.4, 7.1 Hz, 1H), 6.70 (dd, *J* = 8.7, 2.4 Hz, 1H), 6.65 (dd, *J* = 8.4, 1.0 Hz, 1H), 6.47 (dd, *J* = 7.1, 1.0 Hz, 1H), 6.31 – 6.27 (m, 1H), 4.12 (t, *J* = 5.7 Hz, 2H), 3.73 – 3.64 (m, 6H), 3.48 – 3.41 (m, 2H), 1.67 – 1.59 (m, 2H), 1.27 (s, 6H), 1.00 (s, 6H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 152.4, 143.1, 137.5, 132.5, 131.1, 128.0, 125.8, 125.7, 119.5, 111.9, 111.4, 106.0, 102.7, 100.8, 71.5 (2C), 65.0, 50.5, 45.7, 38.0, 32.8, 31.3, 29.8 (2C), 21.5 (2C). HRMS-ESI (*m/z*): found 433.26455, cacld for $C_{27}H_{36}BN_2O_3^+$ $[M+H]^+$ 433.26570.

11d 4,4-Dimethyl-8-(pyridin-3-yl)-1,2,3,4-tetrahydroquinoline

Following **method F**, the reaction of 8-bromo-4,4-dimethyl-1,2,3,4-tetrahydroquinoline **3d** (500 mg, 2.08 mmol), pyridin-3-ylboronic acid (768 mg, 6.25 mmol), K_2CO_3 (864 mg, 6.25 mmol) and $PdCl_2(PPh_3)_2$ (146 mg, 0.208 mmol) in DMF (30 mL) and water (6 mL) afforded, after purification by flash chromatography on silica gel (cyclohexane/ethyl acetate; 60/40), the title compound **11d** as an orange solid (389 mg, 78%); mp 88 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.55 – 8.50 (m, 2H), 7.78 (ddd, *J* = 7.8, 2.2, 1.8 Hz, 1H), 7.43 (ddd, *J* = 7.8, 4.8, 0.9 Hz, 1H), 7.19 (dd, *J* = 7.8, 1.4 Hz, 1H), 6.77 (dd, *J* = 7.6, 1.4 Hz, 1H), 6.58 (dd, *J* = 7.8, 7.6 Hz, 1H), 5.01 (brs, 1H), 3.17 – 3.09 (m, 2H), 1.66 – 1.58 (m, 2H), 1.26 (s, 6H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 149.8, 147.8, 141.3, 136.7, 135.6, 129.8, 128.0, 126.2, 123.7, 122.3, 115.6, 37.6, 36.4, 31.8, 31.1 (2C). HRMS-ESI (*m/z*): found 239.15213, cacld for $C_{16}H_{19}N_2^+$ $[M+H]^+$ 239.15428.

11d' 4,4-Dimethyl-8-(pyridin-4-yl)-1,2,3,4-tetrahydroquinoline

Following **method F**, the reaction of 8-bromo-4,4-dimethyl-1,2,3,4-tetrahydroquinoline **3d** (367 mg, 1.53 mmol), pyridin-4-ylboronic acid (563 mg, 4.58 mmol), K_2CO_3 (633 mg, 4.58 mmol) and $PdCl_2(PPh_3)_2$ (107 mg, 0.15 mmol) in DMF (22 mL) and water (4.50 mL) afforded, after purification by flash chromatography on silica gel (cyclohexane/ethyl acetate; 60/40), the title compound **11d'** as a yellow solid (320 mg, 88%); mp 174 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.60 – 8.56 (m, 2H), 7.42 – 7.38 (m, 2H), 7.20 (dd, *J* = 7.6, 1.6 Hz, 1H), 6.80 (dd, *J* = 7.6, 1.6 Hz, 1H), 6.58 (dd, *J* = 7.6, 7.6 Hz, 1H), 5.13 (s, 1H), 3.19 – 3.10 (m, 2H), 1.67 – 1.59 (m, 2H), 1.26 (s, 6H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 149.9 (2C), 147.6, 140.8, 130.0, 127.6, 126.6, 124.1 (2C), 123.0, 115.6, 37.6, 36.3, 31.8, 31.0 (2C). HRMS-ESI (*m/z*): found 239.15334, cacld for $C_{16}H_{19}N_2^+$ $[M+H]^+$ 239.15428.

12d 1-(2-((1-Benzyl-1*H*-indol-5-yl)oxy)ethyl)-4,4-dimethyl-8-(pyridin-3-yl)-1,2,3,4-tetrahydroquinoline

Following **method D**, the reaction of 2-((1-benzyl-*1H*-indol-5-yl)oxy)ethanol **4** (0.37 g, 1.37 mmol), IBX (1.15 g, 4.11 mmol), 4,4-dimethyl-8-(pyridin-3-yl)-1,2,3,4-tetrahydroquinoline **11d** (0.69 g, 2.89 mmol), $NaBH(OAc)_3$ (0.58 g, 2.74 mmol) in dry 1,2-DCE (6.00 mL) afforded, after purification by flash chromatography on silica gel (cyclohexane/ethyl acetate; 70/30), the title compound **12d** as a light yellow oil (100 mg, 15%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.68 (brs, 1H), 8.50 (brd, *J* = 4.0 Hz, 1H), 7.90 (ddd, *J* = 7.9, 1.9, 1.8 Hz, 1H), 7.46 – 7.42 (m, 2H), 7.36 (dd, *J* = 6.1, 3.3 Hz, 1H), 7.33 – 7.21 (m, 4H), 7.19 – 7.13 (m, 2H), 7.00 – 6.94 (m, 2H), 6.72 (d, *J* = 2.4 Hz, 1H), 6.49 (dd, *J* = 8.8, 2.4 Hz, 1H), 6.36 (d, *J* = 3.1 Hz, 1H), 5.34 (s, 2H), 3.46 (t, *J* = 6.3 Hz, 2H), 3.26 – 3.16 (m, 2H), 2.92 (t, *J* = 6.3 Hz, 2H), 1.73 – 1.63 (m, 2H), 1.30 (s, 6H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 152.2, 149.0, 145.0, 145.2, 138.6, 138.4, 137.7, 136.8, 131.0, 130.4, 129.6, 129.2, 128.6, 128.5 (2C), 127.3, 126.9 (2C), 126.8, 123.8, 121.4, 111.6, 110.7, 103.2, 100.6, 66.0, 54.0, 49.2, 44.4, 33.5, 32.5, 31.2 (2C). HRMS-ESI (*m/z*): found 488.26583, cacld for $C_{33}H_{34}N_3O^+$ $[M+H]^+$ 488.26964.

12d' 1-(2-((1-Benzyl-1*H*-indol-5-yl)oxy)ethyl)-4,4-dimethyl-8-(pyridin-4-yl)-1,2,3,4-tetrahydroquinoline

Following **method D**, the reaction of 2-((1-benzyl-1*H*-indol-5-yl)oxy)ethanol **4** (0.65 mg, 2.41 mmol), IBX (2.02 g, 2.28 mmol), 4,4-dimethyl-8-(pyridin-4-yl)-1,2,3,4-tetrahydroquinoline **11d'** (1.21 g, 5.07 mmol), $NaBH(OAc)_3$ (1.02 g, 4.82 mmol) in dry 1,2-DCE (10 mL) afforded, after purification by flash chromatography on silica gel (cyclohexane/ethyl acetate; 60/40), the title compound **12d'** as a yellow oil (164 mg, 14%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.58 – 8.54 (m, 2H), 7.47 – 7.44 (m, 2H), 7.43 (d, *J* = 3.0 Hz, 1H), 7.35 (dd, *J* = 4.7, 4.7 Hz, 1H), 7.33 – 7.12 (m, 6H), 6.97 – 6.92 (m, 2H), 6.73 (d, *J* = 2.4 Hz, 1H), 6.47 (dd, *J* = 8.9, 2.4 Hz, 1H), 6.35 (dd, *J* = 3.0, 0.7 Hz, 1H), 5.34 (s, 2H), 3.57 (t, *J* = 6.2 Hz, 2H), 3.29 – 3.19 (m, 2H), 2.95 (t, *J* = 6.2 Hz, 2H), 1.75 – 1.64 (m, 2H), 1.29 (s, 6H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 152.1, 149.8, 149.7 (2C), 144.7, 138.4 (2C), 131.1, 130.6, 129.6, 128.8, 128.6, 128.5 (2C), 127.3, 126.9 (2C), 126.9, 123.6 (2C), 120.8, 111.5, 110.7, 103.2, 100.6, 65.9, 54.3, 49.2, 44.6, 33.9, 32.5, 31.0 (2C). HRMS-ESI (*m/z*): found 488.26873, cacld for $C_{33}H_{34}N_3O^+$ $[M+H]^+$ 488.26964.

13d 1-(2-((1*H*-Indol-5-yl)oxy)ethyl)-4,4-dimethyl-8-(pyridin-3-yl)-1,2,3,4-tetrahydroquinoline

Following **method E**, the reaction of 1-(2-((1-benzyl-1*H*-indol-5-yl)oxy)ethyl)-4,4-dimethyl-8-(pyridin-3-yl)-1,2,3,4-tetrahydroquinoline **12d** (100 mg; 0.21 mmol) and *t*-BuOK (323 mg , 2.87 mmol) in DMSO (6.60 mL) afforded, after purification by flash chromatography on silica gel (cyclohexane/ethyl acetate; 70/30), the title compound **13d** as a white solid (64 mg, 77%); mp 158 °C. HPLC purity: 97.3%, ^{t}R = 18.54 min. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.90 – 10.87 (m, 1H), 8.65 (dd, *J* = 2.3, 0.7 Hz, 1H), 8.49 (dd, *J* = 4.8, 1.7 Hz, 1H), 7.87 (ddd, *J* = 7.8, 2.3, 1.7 Hz, 1H), 7.43 (ddd, *J* = 7.8, 4.8, 0.7 Hz, 1H), 7.37 (dd, *J* = 5.9, 3.6 Hz, 1H), 7.26 (dd, *J* = 2.8, 2.7 Hz, 1H), 7.19 (d, *J* = 8.7 Hz, 1H), 7.00 – 6.95 (m, 2H), 6.69 (d, *J* = 2.3 Hz, 1H), 6.47 (dd, *J* = 8.7, 2.3 Hz, 1H), 6.32 – 6.27 (m, 1H), 3.45 (t, *J* = 6.4 Hz, 2H), 3.25 – 3.19 (m, 2H), 2.93 (t, *J* = 6.4 Hz, 2H), 1.80 – 1.64 (m, 2H), 1.30 (s, 6H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 151.9, 149.5, 147.5, 145.3, 138.6, 137.5, 136.2, 131.1, 130.6, 129.1, 127.9, 126.7, 125.7, 123.5, 121.3, 111.8, 111.4, 102.6, 100.8, 66.0, 54.1, 44.5, 33.5, 32.5, 31.2 (2C). HRMS-ESI (*m/z*): found 398.21939, cacld for $C_{26}H_{28}N_3O^+$ $[M+H]^+$ 398.22269.

13d' 1-(2-((1*H*-Indol-5-yl)oxy)ethyl)-4,4-dimethyl-8-(pyridin-4-yl)-1,2,3,4-tetrahydroquinoline

Following **method E**, the reaction of 1-(2-((1-benzyl-1*H*-indol-5-yl)oxy)ethyl)-4,4-dimethyl-8-(pyridin-4-yl)-1,2,3,4-tetrahydroquinoline **12d'** (150 mg; 0.31 mmol) and *t*-BuOK (484 mg , 4.31 mmol) in DMSO (9.60 mL) afforded, after purification by flash chromatography on silica gel (cyclohexane/ethyl acetate; 70/30), the title compound **13d'** as a yellow oil (94 mg, 77%). HPLC purity: 99.6%, ^{t}R = 18.20 min. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.88 (brs, 1H), 8.60 – 8.56 (m, 2H), 7.50 – 7.46 (m, 2H), 7.36 (dd, *J* = 5.9, 3.5 Hz, 1H), 7.26 (dd, *J* = 2.8, 2.7 Hz, 1H), 7.19 (d, *J* = 8.8 Hz, 1H), 6.99 – 6.92 (m, 2H), 6.72 (d, *J* = 2.4 Hz, 1H), 6.47 (dd, *J* = 8.8, 2.4 Hz, 1H), 6.31 – 6.27 (m, 1H), 3.57 (t, *J* = 6.2 Hz, 2H), 3.30 – 3.21 (m, 2H), 2.96 (t, *J* = 6.2 Hz, 2H), 1.75 – 1.65 (m, 2H), 1.30 (s, 6H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 151.9, 149.8, 149.7 (2C), 144.8, 138.4, 131.1, 130.7, 128.9, 127.9, 126.9, 125.8, 123.7 (2C), 120.9, 111.8, 111.4, 102.7, 100.8, 65.9, 54.4, 44.6, 33.9, 32.5, 31.1 (2C). HRMS-ESI (*m/z*): found 398.22138, cacld for $C_{26}H_{28}N_3O^+$ $[M+H]^+$ 398.22269.

BIOLOGY

Cell cultures and reagents

K562, KU812, KCL-22, IM-sensitive (K562S), IM-resistant (K562R) BCR-ABL+ cells and MV4-11 cells were obtained from the Deutshe Sammlung von Mikroorganismens und Zellkulturen (DSMZ). MOLM-13 and HS-27a were obtained from the American Type Culture Collection (ATCC). All cell lines were obtained according to the supplier's recommendations. 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 µg/mL streptomycin (Gibco – Thermofisher, Waltham, MA, USA) at 37 °C, 5% CO2. Resistant 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.[47] Informed consent was obtained before BM samples were taken. MSC were cultured in αMEM (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 µg/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.

Cell proliferation assays

Cell viability and proliferation were studied using a MTT cell proliferation assay (Sigma-Aldrich, MO, USA). Briefly, $2x10^4$ 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 concentration-effect of the molecules, cells were treated with concentrations ranging from 100 nM to 100 µM for 48 h. To determine drugs toxicity on stromal cells, $1x10^4$ HS-27a or $3.5x10^3$ were cultured in 96 well plates, incubated for 24 h and then treated with concentrations ranging from 100 nM to 100 µM of drugs 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, Offenburg, Germany). Living cells were also enumerated using the trypan blue dye exclusion method.

Apoptosis analysis

KU812 and MV4-11 cells were treated for 48 h with 3 µM of each drugs and then washed with PBS, then stained (10^6 cells) in buffer containing FITC-annexin V and 7-amino-actinomycin D (7-AAD) (Beckmann Coulter, Fullerton, USA) for 15 min at 4 °C and analyzed by flow cytometry (Becton Dickinson Accuri™ *C6 flow cytometer)*. *The FlowJo® software was used to analyze data.*

Western blot

Cells were suspended in NP40 buffer (Bio-Rad, NY, USA), separated on SDS/PAGE and blotted onto nitrocellulose membrane. Blots were incubated with the following antibodies (Abs): P-Y694/699-STAT5, Actin (Cell signaling Technology, Danvers, USA), STAT5 (BD Transduction Laboratories, Franklin Lakes, USA), STAT5A and STAT5B (Zymed/ThermoFisher Scientific, Waltham, MA, USA). Membranes were developed with the ECL chemiluminescence detection system (GE Healthcare, Little Chalfont, UK) using specific peroxidase (HRP) conjugated to rabbit or mouse IgG antibodies (Cell signaling Technology, Danvers, USA).

qRT-PCR analysis

RNA samples were reverse-transcribed using SuperScript®VILO cDNA synthesis kit (Invitrogen, Carlsbad, USA) as recommended by the supplier. The resulting cDNAs were used for quantitative real-time PCR (qRT-PCR). PCR primers (PIM1: for 5'-TTTCGAGCATGACGAAGAGA-3', rev 5'-GGGCCAAGCACCATCTAAT-3'; CISH: for 5'-AGCCAAGACCTTCTCCTACCTT-3', rev 5'-TGGCATCTTCTGCAGGTGT-3'; STAT5A: for 5'-TCCCTATAACATGTACCCACA-3', rev 5'-ATGGTCTCATCCAGGTCGAA-3'; STAT5B: for 5'-TGAAGGCCACCATCATCAG-3', rev 5'-TGTTCAAGATCTCGCCACTG-3') were designed with the ProbeFinder software (Roche Applied Sciences, Basel, Switzerland) and used to amplify the RT-generated cDNAs. qRT-PCR analyses were performed on the Light Cycler 480 thermocycler II (Roche). GAPDH (glyceraldehyde-3-phosphate dehydrogenase), ACTB (actin beta) and RPL13A (ribosomal protein L13A) were used as reference genes for normalization of qRT-PCR experiments. Each reaction condition was performed in triplicate. Relative gene expression was analyzed using the 2-ΔΔCt method.[48]

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Supplementary Data

Detailed synthesis strategic pathways starting from 2-aminophenylboronic acid and 2-chloroaniline to reach 2-pyridinyl analog in position 8 on THQ ring, NMR spectra and biological evaluation of compounds **6c**, **10**, **13d** and **13d'** on other AML cell lines, KG1a and MOLM-13.

Abbreviations used

AKT, Protein Kinase B; AML, Acute Myeloid Leukemia; BCR-ABL, Breakpoint Cluster Region-Abelson; Bneop, neopentylglycolato borane; Bpin, pinacol borane CML, Chronic Myeloid Leukemia; DCE, Dichloroethane; DMF, Dimethylformamide; DMSO, Dimethyl sulfoxide; DNA, Deoxyribonucleic acid; FLT3-ITD, FMS like tyrosine kinase 3 - Internal Tandem Duplication; IBX, 2-iodoxybenzoic acid; IM, Imatinib Mesylate; JAK2, Janus Kinase 2; PIM1, Proviral Integration site for Moloney leukemia virus 1; PPARγ, Peroxisome Proliferator – Activated Receptor gamma; RNAi, interference ribonucleic acid; ROS, Reactive Oxygen Species; SAR, Structure-Activity Relationship; SMA-ZnPP, Styrene-Maleic Acid-copolymer conjugated ZnPP; STAT5, Signal transducer and activator of transcription 5; TFA, Trifluoroacetic Acid; THQ, Tetrahydroquinoline; TKI, Tyrosine Kinase Inhibitor.

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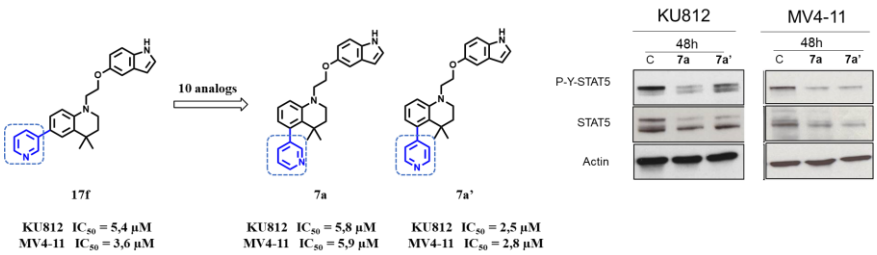

STAT5 inhibitors in myeloid leukemias: To improve the antileukemic activity of a first lead compound called **17f**, new derivatives were synthesized by pharmacomodulation. Two compounds, **7a** and **7a'**, showed similar or higher antileukemic effects in various acute and chronic myeloid leukemias cell lines. Both compounds were found to be more effective than **17f** to inhibit STAT5 activity/expression and to suppress the chemoresistance of CML cells.

Keywords: Inhibitors - Medicinal Chemistry - Myeloid Leukemias - STAT5 - Suzuki coupling