Journal of Medicinal Chemistry

Article

A New Inhibitor Targeting Signal Transducer and Activator of Transcription 5 (STAT5) Signaling in Myeloid Leukemias

Ludovic Juen, Marie Brachet-Botineau, Cécile Parmenon, Jérôme Bourgeais, Olivier Hérault, Fabrice Gouilleux, Marie-Claude Viaud-Massuard, and Gildas Prié

J. Med. Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.jmedchem.7b00369 • Publication Date (Web): 27 Jun 2017

ACS Publications

Journal of Medicinal Chemistry is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

A New Inhibitor Targeting Signal Transducer and Activator of Transcription 5 (STAT5) Signaling in Myeloid Leukemias

Ludovic Juen,[†,#] Marie Brachet-Botineau,[§,‡,#] Cécile Parmenon,[†] Jérôme Bourgeais,[§,‡] Olivier Hérault,[§,‡] Fabrice Gouilleux,[*,‡] Marie-Claude Viaud-Massuard[†] and Gildas Prié[*,†]

[†]Equipe IMT "Innovation Moléculaire et Thérapeutique" - GICC UMR 7292 CNRS - Université de Tours - Labex SYNORG - Faculté de Pharmacie - 31 avenue Monge - 37200 Tours - France.

[‡]Equipe LNOx "Niche leucémique & métabolisme oxydatif" - GICC UMR 7292 CNRS - Université de Tours - Faculté de Médecine - Bâtiment Dutrochet - 10bis boulevard Tonnellé - 37032 Tours - France.

[§]CHRU de Tours, Service d'Hématologie Biologique, 2 boulevard Tonnellé - 37044 Tours - France.

KEYWORDS: STAT5, signal transduction, myeloid leukemias, pharmacological inhibitors, 4,4-dimethyl-1,2,3,4-tetrahydroquinoline.

ABSTRACT:

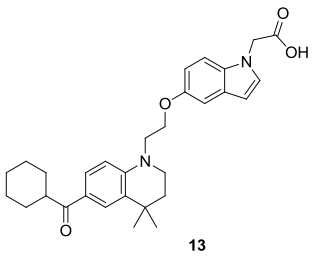
Signal Transducer and Activator of Transcription 5 (STAT5) are crucial effectors of tyrosine kinase oncogenes in myeloid leukemias. Inhibition of STAT5 would contribute to reducing the survival of leukemic cells and also tackling their chemoresistance. In a first screening experiment, we identified hit **13** as able to inhibit STAT5 phosphorylation and leukemic cell growth. The synthesis of 18 analogs of **13** allowed us to identify one compound, **17f,** as having the most potent antileukemic effect. **17f** inhibited the growth of acute and chronic myeloid leukemia cells and phosphorylation and transcriptional activity of STAT5. Importantly, **17f** had minimal effects on bone marrow stromal cells that play vital functions in the microenvironment of hematopoietic and leukemic cells. We also demonstrated that **17f** inhibits STAT5 but not STAT3, AKT or Erk1/2 phosphorylation. These results suggest that **17f** might be a new lead molecule targeting STAT5 signaling in myeloid leukemias.

INTRODUCTION

The Signal Transducer and Activator of Transcription factors 5A and B are two closely related STAT family members that play a major role in normal and leukemic haematopoiesis.[1] Persistent activation of these transcription factors is frequently found in leukemias as a consequence of deregulated tyrosine kinase activity. Phosphorylation of STAT5 is triggered by tyrosine kinase oncogenes (TKO) such as Fms-like receptor tyrosine kinase 3 with internal tandem duplications (Flt3-ITD), Kit^{D816V}, Bcr-Abl and $JAK2^{V617F}$ which have been characterized in various myeloid malignancies.[2–6] STAT5 is a crucial effector of Bcr-Abl, the major transforming agent in chronic myeloid leukemia (CML).[7,8] The development of Bcr-Abl tyrosine kinase inhibitors (TKI) such as Imatinib Mesylate (IM) has revolutionized the treatment of CML. Despite this success story, IM is not curative due to its inability to completely eradicate leukemic stem cells (LSC) that are responsible for initiation and relapse of CML. Moreover, the occurrence of Bcr-Abl mutations during progression of the disease promotes the resistance of CML cells to IM treatment.[9] Therefore, there is an urgent need for alternative therapeutic strategies to cure CML. In this regard, STAT5 fulfils all criteria of a major drug target in CML.[10] High STAT5 expression levels were shown not only to enhance IM resistance in CML cells but also to trigger Bcr-Abl mutations by inducing the production of reactive oxygen species (ROS) responsible for DNA damage.[8,11,12] Moreover, STAT5 was shown to play a key role in the maintenance of CML stem cells that are resistant to chemotherapy.[13] Several approaches have been used to target STAT5 in leukemias. Among them, cell-based screening with small molecules libraries of already approved drugs allowed the identification of pimozide as a potential STAT5 inhibitor in CML cells.[14] Although pimozide inhibits the transcriptional activity of STAT5, the use of high concentrations

suggests that effects of this molecule might be indirect. More recently, salicylic acid-based molecules targeting the SH2 domain required for dimerization and transcriptional activity of STAT5 were shown to inhibit the growth of CML cells.[15] One of these inhibitors binds STAT5 protein in a nanomolar range; furthermore it induces apoptosis and inhibits tyrosine phosphorylation of STAT5 in a micromolar range. A last approach is to target STAT5 activity through the activation of peroxisome proliferator-activated receptor gamma (PPARγ). Indeed the existence of a cross talk between protein PPAR and STAT5 has been discussed.[16–18] For instance, antidiabetic drugs such as glitazones that are PPARγ agonists were shown to have antileukemic activity. Activation of PPARγ by glitazones decreases expression and/or phosphorylation of STAT5 in CML cells.[18,19] Importantly, Prost et al. showed that combining pioglitazone with IM triggers apoptosis of CML stem cells.[11] The mechanism by which these leukemic stem cells are killed in response to this drug combination is not yet clear. Moreover, several reports indicated that antileukemic effects of glitazones might be dependent on "off-target" mechanisms.[20,21]

We previously described the synthesis and pharmacological evaluation of 24 PPARα/γ ligands targeting Type 2 diabetes.[22,23] All molecules synthesized during the project shared a diversely substituted 4,4-dimethyl-1,2,3,4-tetrahydroquinoline scaffold, linked to an aryl or an heteroaryl ring by an ethoxy chain. This series was comprised of PPARα/γ agonist, antagonist and inactive compounds. Inhibitory effects of some representative molecules of the series were evaluated on the growth of a Bcr-Abl expressing cell line (KU812 cells) and STAT5 activity.



13

Figure 1. Hit compound **13**

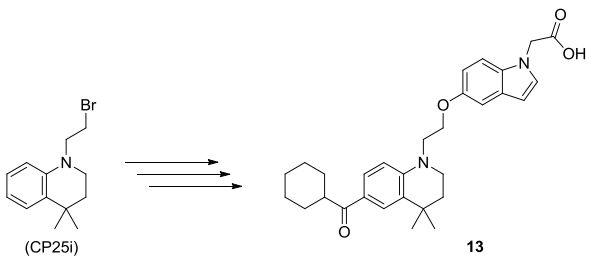
All molecules showed none to moderate activity on KU812 cell proliferation with a maximum inhibition of 55% at 10 μM for compound **13** (Figure 1). Interestingly the latter was the only compound to significantly inhibit STAT5 phosphorylation (supp. info). We also showed that **13** was inactive on PPARα/γ suggesting that inhibition of cell growth and STAT5 phosphorylation by this molecule occurs through a PPARγ independent pathway. Therefore, pharmacological exploitation of this "off-target" effect is of interest for the development of new antileukemic molecules that are devoid of side effects associated with PPARγ activation.[24]

A collection of 18 derivatives of hit compound **13** was synthesized and a structure/activity analysis was performed to determine their growth inhibitory effects on various myeloid leukemia cells. Experiments were conducted in CML cell lines (KU812 and K562) and acute myelogenous leukemia (AML) cell lines in which STAT5 is constitutively active (KG1a and MV-4-11). Among these molecules, **17f** was found to be the most active in blocking STAT5 phosphorylation and leukemic cell growth. We also demonstrated that **17f** selectively inhibits STAT5 phosphorylation but not STAT3, Erk1/2 and Akt signaling which also play a critical role in myeloid leukemia cell proliferation and survival. Interestingly, **17f** was almost inactive in inhibiting the growth of bone marrow stromal cells that are major components of the hematopoietic and leukemic microenvironment.

CHEMISTRY

Hit **13** scaffold was composed of indole and 4,4-dimethyl-1,2,3,4-tetrahydroquinoline rings, linked by an ethoxy chain attached by carbon C-5 of the indole and nitrogen N-1 of the tetrahydroquinoline (Scheme 1). This scaffold was substituted by an acetic acid group on the nitrogen N-1 of the indole and by a cyclohexylcarbonyl group on the carbon C-6 of the tetrahydroquinoline.

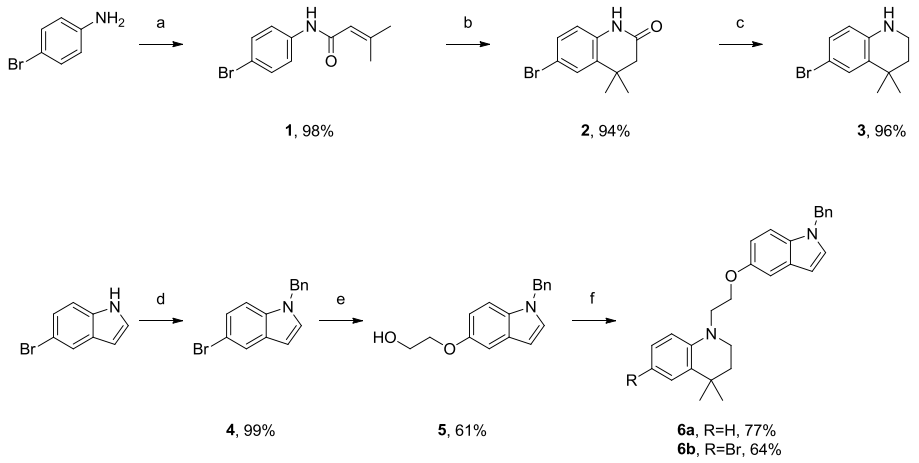
Scheme 1. Previous synthetic route of hit compound **13**



First convergent synthesis of hit **13** followed the synthetic route developed in our previous PPAR project.[22] Acylation of intermediate CP25i with cyclohexyl carbonyl chloride in the presence of graphite[25] followed by Williamson alkylation with ethyl 2-(5-hydroxy-*1H*-indol-1-yl)acetate and further saponification led to **13**. This method had some drawbacks including poor reproducibility of the acylation step and low yield of the alkylation step (<15%). Moreover, this procedure set the central scaffold substituents from the start, making new pharmacomodulations on indole and tetrahydroquinoline rings inconvenient. In order to study SAR in our current STAT5 project, we needed to readily synthetize a series of hit **13** analogs. To do this, we designed an alternative divergent synthesis. Starting from key intermediates **6a** and **6b** obtained

in satisfying yields, the central scaffold was further modified to produce the desired series (Scheme 2).

Scheme 2. Synthesis of key intermediates **6a** and **6b**[a]

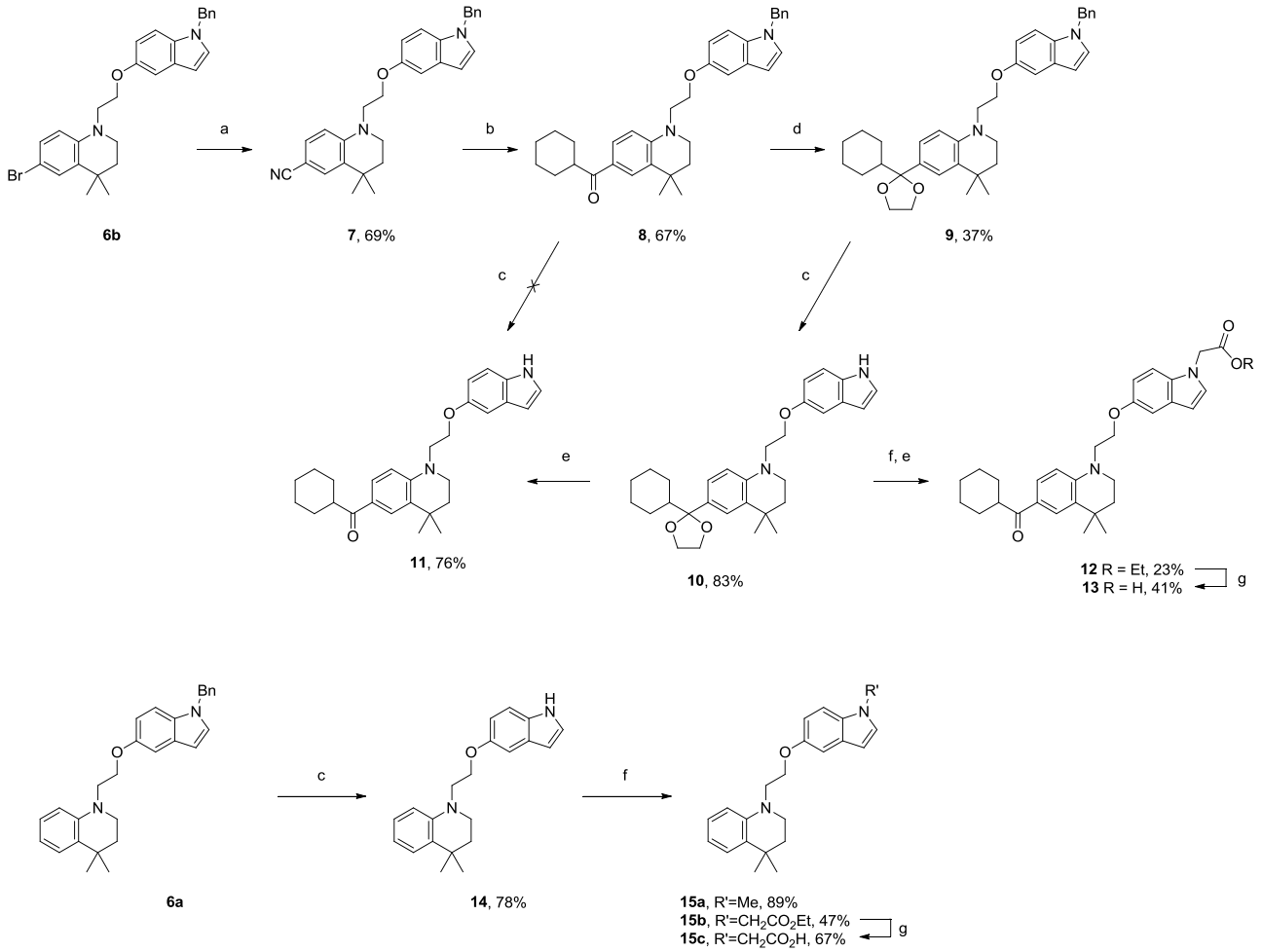


[a]Reagent and conditions: (a) 3,3-dimethyl-acryloyl chloride, dry pyridine, 0 °C to rt, 5 h; (b) $AlCl_3$, dry dichloromethane, 0 °C to rt, 1 h; (c) BH_3.THF, dry toluene, 0 °C to reflux, 12 h; (d) NaH, BnBr, dry DMF, 0 °C to rt, 2 h; (e) CuI, 3,4,7,8-tetramethyl-1,10-phenanthroline, Cs_2CO_3, dry ethylene glycol, 130 °C, 72 h; (f) 1) IBX, DCE, 80 °C, 2 h; 2) Filtration; 3) 4,4-dimethyl-1,2,3,4-tetrahydroquinoline or amine **3**, $NaBH(AcO)_3$, rt, 3 h.

Synthesis of the key intermediates **6a** and **6b** started with protection of the N-1 position of 5-bromoindole by a benzyl group. The protected indole **4** was then etherified by Ullmann-type coupling. To achieve this, several ligands were tested including 8-hydroxyquinoline[26] and 1,10-phenanthroline, however best results were obtained with electron rich 3,4,7,8-tetramethyl-1,10-phenanthroline[27] to afford **5** with a 61% yield. In order to carry out reductive amination, various reagents to oxidize alcohol **5** were assessed, including PCC, oxalyl chloride (according to Swern oxidation), Dess-Martin periodinane and 2-iodoxybenzoic acid (IBX).[28] As the aldehyde intermediate was unstable, IBX oxidation was chosen as it allowed an easy purification by simple filtration (indeed, excess of IBX and reduced by-product 2-iodosobenzoic acid (IBA) are insoluble at rt in DCE). Moreover, the filtrate was used in the next step without further treatment,

since DCE is one of the best reaction solvent for reductive aminations.[29] 4,4-Dimethyl-1,2,3,4-tetrahydroquinoline or 6-bromo-4,4-dimethyl-1,2,3,4-tetrahydroquinoline **3**, obtained from aniline or 4-bromoaniline,[22] were then introduced in the presence of $NaBH(AcO)_3$ to form **6a** and **6b** respectively with 77% and 64% yields over two steps (Scheme 2).

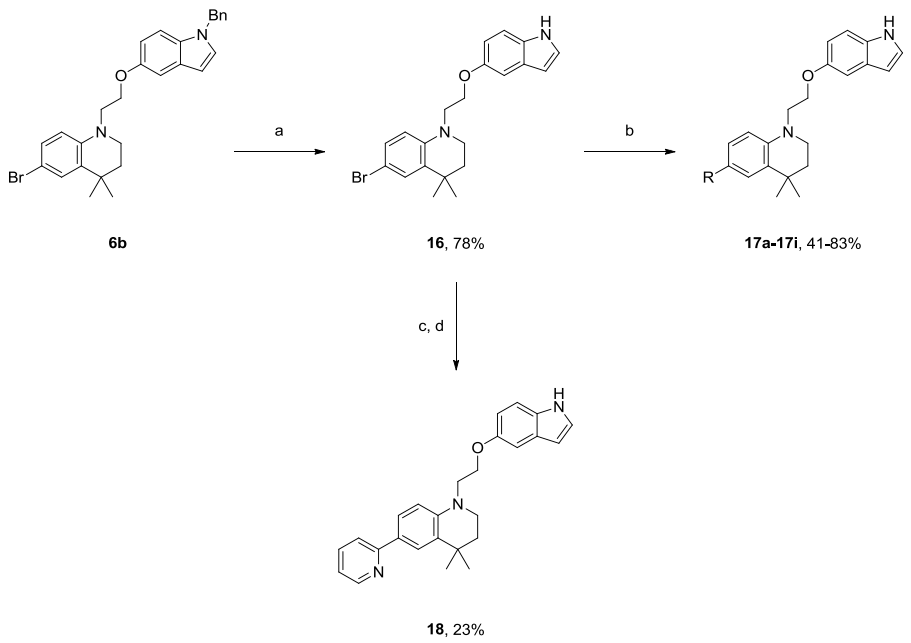
Scheme 3. Synthesis of hit compound 13 and its analogs[a]



[a]Reagent and conditions: (a) CuCN, DMF, reflux, 6 h; (b) 1) cyclohexylmagnesium chloride, CuBr, dry THF, reflux, 2 h; 2) H_2O, H_2SO_4 15%; (c) *t*-BuOK, DMSO, O_2, rt, 3 h; (d) ethylene glycol, *p*-toluenesulfonic acid monohydrate, toluene, reflux, 6 h; (e) HCl 0.1 M, THF, rt, 5 h; (f) NaH, ethyl bromoacetate or methyl iodide, dry DMF, 0 °C to rt, 2 h; (g) LiOH, THF/H_2O, 0 °C to rt, 2 h.

Conversion of aryl bromide **6b** to the corresponding nitrile **7**,[30] followed by nucleophilic addition of cyclohexylmagnesium chloride catalyzed by CuBr and subsequent hydrolysis led to the ketone **8** (Scheme 3).[31] Attempts of benzyl deprotection of **8** in the presence of potassium *tert*-butoxide under air (1 atm) led to hydroxylation at the α-carbon of the ketone.[32,33] To avoid this, several procedures were assessed to protect ketone **8** as the ketal **9**,[34,35] among which, the classical method using ethylene glycol and catalytic *p*-toluenesulfonic acid monohydrate with a Dean Stark trap gave the best result, nevertheless with a low 37% yield. The long reaction time required at high temperature led to partial decomposition of the starting ketone. However after 6 h, besides the product obtained, 34% of the starting material **8** was retrieved. The ketal **9** did undergo benzyl deprotection to afford **10** with 83% yield. Deprotection of the latter afforded ketone **11**, whereas *N*-alkylation with ethyl bromoacetate followed by saponification led to **13**. Deprotection of **6a**, conducted with potassium *tert*-butoxide under air (1 atm) in DMSO afforded **14** with 78% yield. N-alkylation of **14** by treatment with sodium hydride and methyl iodide led to analogs **15a**. Same reaction using ethyl bromoacetate, followed by saponification led to acid **15c** with 31% yield over two steps.

Scheme 4. Synthesis of analogs **16**, **17a-i** and **18**[a]

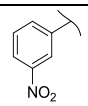
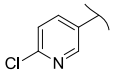
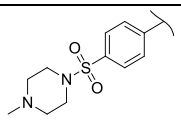


[a]Reagent and conditions: (a) *t*-BuOK, DMSO, O_2, rt, 3 h; (b) corresponding boronic acid, K_2CO_3, $PdCl_2(PPh_3)_2$, DMF/H_2O, 90 °C, 1-14 h; (c) bis(pinacolato)diboron, KOAc, $PdCl_2$(dppf), dry dioxane, 80 °C, 21 h; (d) 2-chloropyridine, K_3PO_4, $PdCl_2$(dppf), dioxane/H_2O, 100 °C, 4 h.

Various aryl and heteroaryl groups were introduced on the tetrahydroquinoline ring of **16** using Suzuki cross-coupling, affording **17a-i** analogs with moderate to good yields (41-83%) (Scheme 4, Table 1). Boronic acids used for the Suzuki coupling were commercially available except 1-methyl-4-[[4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]sulfonyl]piperazine which was synthesized in 2 steps from 4-bromobenzene-1-sulfonyl chloride.[36]

Table 1. Structure of analogs **17a-i**

Entry	Compound	R	Time (h)	Yield (%)
1	**17a**		1	50
2	**17b**		2	79

3	**17c**		2	68
4	**17d**		14	50
5	**17e**		1	83
6	**17f**		2.5	46
7	**17g**		2.5	50
8	**17h**		1	46
9	**17i**		1.5	41

Challenging introduction of the 2-pyridinyl group directly from aryl bromide **16** using MIDA boronic ester[37,38] led to only traces of product and degradation of the starting material. As a result, we decided to convert aryl bromide **16** to the corresponding boronic acid pinacol ester, which was directly engaged in a Suzuki coupling with 2-chloropyridine to yield compound **18**.

RESULT AND DISCUSSION

Hit compound **13**, an inactive analog of PPAR agonists that have been developed in our lab, was found to inhibit STAT5 phosphorylation in KU812 cells 72 h after treatment at 10 μM. However, proliferation and viability assays showed only a moderate cytotoxic efficiency on KU812 and K562 cells, with EC_{50} of 0.2 mM and 8 mM respectively.

As acid and ketone substituents of hit **13** were chosen in accordance with PPAR activity, we studied their relevance toward the inhibition of STAT5 activity and cytotoxic efficiency on CML cell lines, while keeping hit **13** central scaffold. In this purpose and in order to study structure activity relationships, pharmacomodulations on indole and tetrahydroquinoline rings were conducted.

Initial screenings were carried out to determine the growth inhibitory property of all newly synthesized compounds. KU812 and K562 cells were treated with 10 μM of each compound or DMSO as control and the kinetics of cell growth were determined by MTT and trypan blue dye exclusion assays (data for compounds with antiproliferative activity lower than **13** are shown in supp. info).

On one hand, deletion of the cyclohexyl carbonyl group on the tetrahydroquinoline led to compound **15c**, which lost antiproliferative efficiency after 48 h on K562 cells and had no effect on KU812 cells. On the other hand, removal of the acetic acid group led to compound **11**, which showed the same activity than **13** on K562 cells but lost it at 72 h. On KU812 cells, antiproliferative potency of **11** could be suspected at 24 h but was lost after 48 h. Finally, we removed both terminal substituents to yield **14**, which showed a better improvement of cytotoxic efficiency on K562 and KU812 cell lines. The activity was better than **13** and interestingly, growth inhibition was observed only after 24 h treatment (Figure 2).

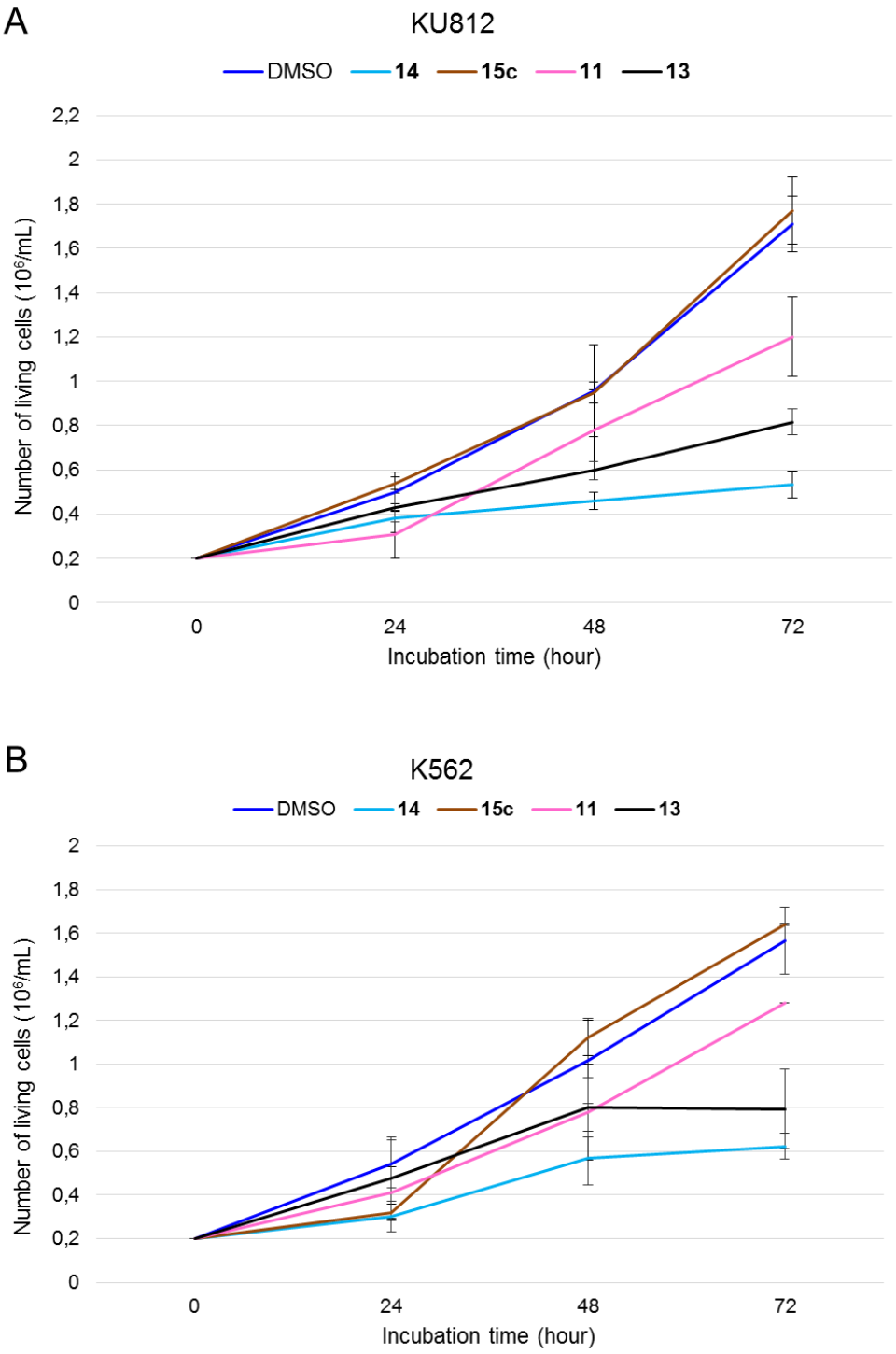
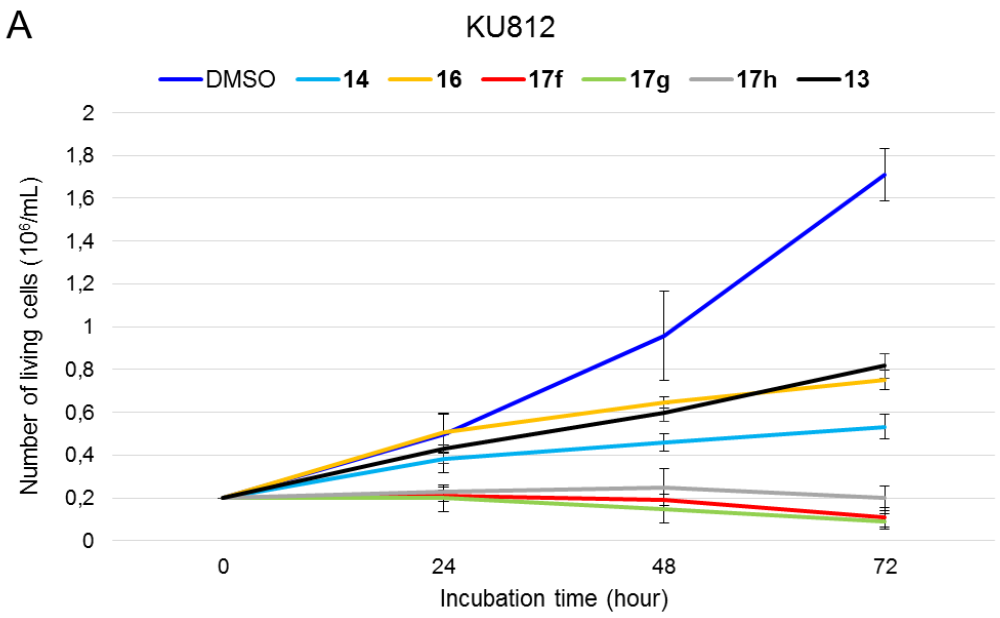


Figure 2. (A) Growth kinetics of KU812 cells treated with 10 μM of each compound as indicated or DMSO as control; (B) Growth kinetics of K562 cells treated with each compound or DMSO as control as in (A) (n = 3 in triplicates, data are mean ± SEM).

To investigate further the importance of the free indole, intermediates **6a** and **15b**, respectively N-alkylated with a benzyl group and an acetate group, were both evaluated and found to be inactive. To rule out steric hindrance effects and to validate the importance of the free indole as hydrogen bond donor, **14** was N-alkylated with the minimally hindering methyl group to yield compound **15a**. As the latter showed no activity, the free nitrogen was identified as essential for the efficiency.

From this point, the free nitrogen was retained on the indole while the 6-position of the tetrahydroquinoline was investigated further. To obtain a convergent and facile route for pharmacomodulations, introduction of a bromine atom on this position yielded **16**, found to have a similar activity than **13** (Figure 3).



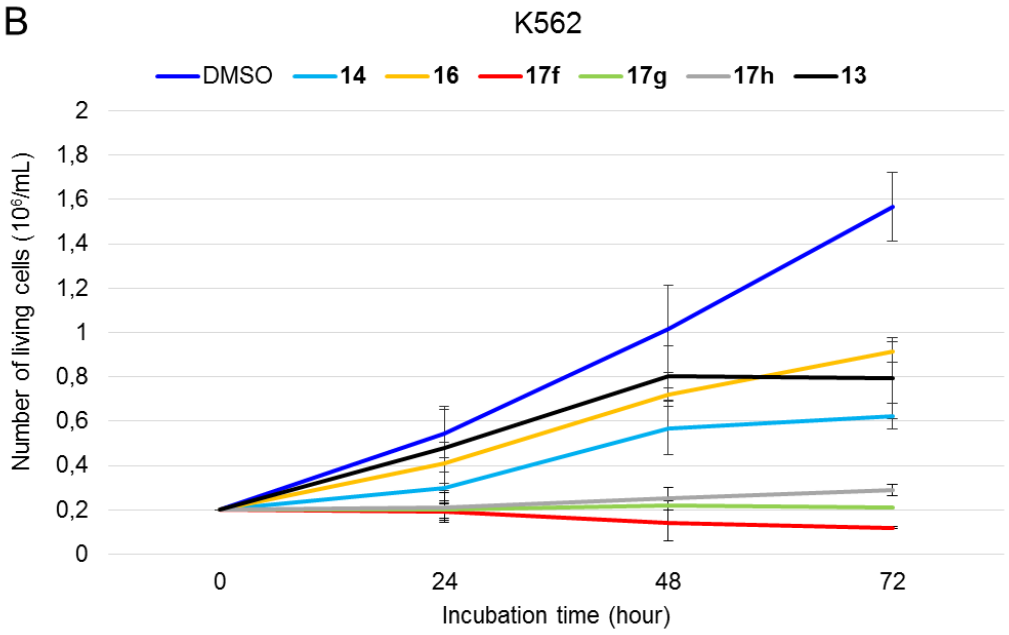


Figure 3. (A) Growth kinetics of KU812 cells treated with 10 µM of each compound as indicated or DMSO as control; (B) Growth kinetics of K562 cells treated with each compound or DMSO as control as in (A) (n = 3 in triplicates, data are mean ± SEM).

Starting from **16**, a small collection of aryl and heteroaryl substituents were introduced i.e. phenyl, 4-fluorophenyl, 3-nitrophenyl, 3-thiophenyl, 2-furanyl, 3-pyridinyl, 6-chloropyridin-3-yl, 4-pyrininyl, and 4-((4-methylpiperazin-1-yl)sulfonyl)phenyl which led to compounds **17a-i** (Figure 3).

17f was identified as the most antiproliferative molecule in KU812 and K562 cells while **17g** was as efficient as **17f** in the growth inhibition of KU812 cells. Compared to hit **13**, their activities were clearly increased. **17c** with a 3-nitrophenyl group possessed the same electronic distribution than **17f**, however was inactive. Moreover, **17a** with the phenyl group retained the same volume than **17f** but was inactive. We assumed the hydrogen bond acceptor character of the nitrogen of the pyridine of **17f** was required for the activity. By changing the position of the nitrogen of the pyridine, from 3 to 4, the antiproliferative efficiency of **17h**, still potent, was

slightly reduced compared to **17f** and **17g**. Changing the position of the nitrogen of the pyridine from 3 to 2, leading to **18**, resulted in a decrease of antiproliferative activity.

Five compounds (**14**, **16**, **17f-h**) with similar or better antiproliferative activities than hit **13**, were selected from this initial screening and tested for their capacity to induce apoptosis in KU812 cells. We observed that **17f**, **17g** and **17h** significantly increased the number of apoptotic cells as measured by AnnexinV/7AAD staining (Figure 4).

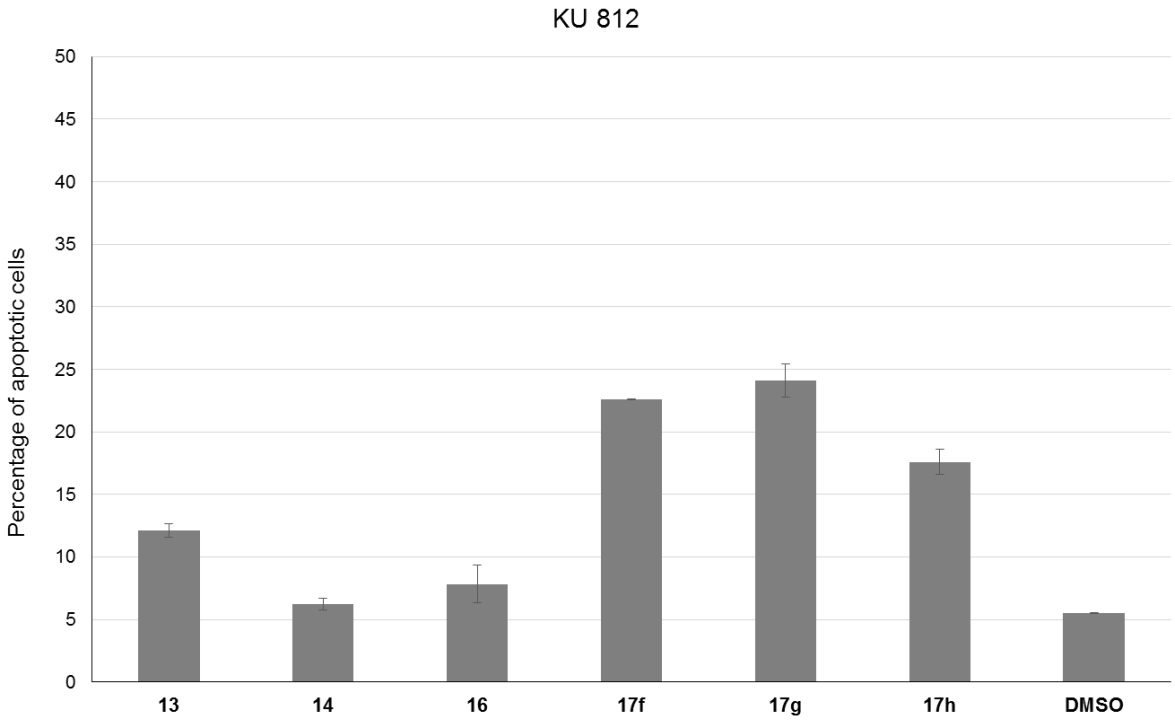


Figure 4. Representative flow cytometry histogram of KU812 cells cultured for 48 h with indicated compounds (10 μM) or control DMSO. Cells were stained with FITC-Annexin V and 7-AAD and the percentages of apoptotic cells were then evaluated by flow cytometry (n = 3 in triplicates, data are mean ± SEM).

EC_{50} values for these molecules were also calculated and clearly indicated that **17f** was the most active inhibitor of K562 and KU812 cell growth and viability (Table 2). Apoptosis experiments with **17f** in K562 cells were also realized by AnnexinV/7AAD staining (supp. info).

Table 2. EC_{50} values of selected compounds for K562 and KU812 cells[a]

	EC_{50} (μM)	
Compound	K562	KU812
13	8658.96 ± 59.52	196.57 ± 16.11
14	78.13 ± 2.73	14.63 ± 2.04
16	15.58 ± 1.67	7.59 ± 0.63
17f	8.74 ± 1.54	5.38 ± 0.01
17g	13.04 ± 3.21	5.41 ± 1.39
17h	22.65 ± 1.09	9.38 ± 1.18

[a]Cells were treated with concentrations ranging from 100 nM to 50 μM for 48 h. Cell viability was then determined by MTT assays and EC_{50} values were calculated using Origin® software (n = 3 in triplicates, data are mean ± SEM) (supp. info).

Interestingly, this inhibitory effect was not blocked by GW9662, an antagonist of PPARγ indicating that PPARγ activation was not involved in **17f**-mediated inhibition of cell growth (supp. info).[20] We then addressed whether the biological effect of this molecule correlated with the suppression of STAT5 activity in KU812 and K562 cells. We first evaluated impact of **17f** on *STAT5A* and *STAT5B* gene expression in KU812 cells by qRT-PCR experiments. Results showed that **17f** had no effect on *STAT5A* and *STAT5B* mRNA levels in KU812 cells treated for 15 h with this molecule (Figure 5A). We then analyzed the phosphorylation and expression of STAT5 in KU812 and K562 cells exposed to **17f** (10 μM) for 12 h. We observed that **17f** inhibited STAT5 phosphorylation in both leukemia cell lines (Figure 5B).

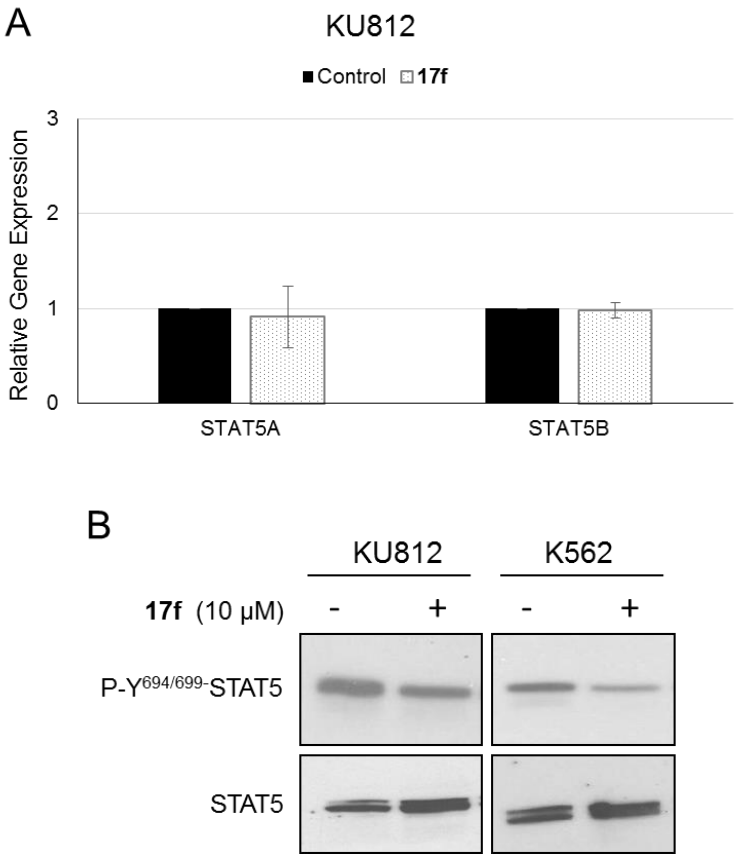


Figure 5. (A) qRT-PCR analysis of *STAT5A and STAT5B* transcripts in KU812 cells treated or not with **17f** (10 µM) or DMSO as control for 15 h. Results are presented as the fold changes in gene expression in **17f**-treated cells normalized to internal control genes (*GAPDH* and *ACTB*) and relative to control cells (normalized to 1) (n = 3 in triplicates, data are mean ± SEM). (B) Protein extracts from KU812 and K562 cells treated with **17f** (+) or DMSO (-) for 12 h were analyzed by Western blotting to detect P-Y-STAT5 and STAT5 protein expression (n = 2).

We then addressed whether **17f** inhibited STAT5 transcriptional activity in KU812 cells. qRT-PCR experiments were conducted to determine effects of **17f** on STAT5-dependent expression of target genes such as *PIM1, CYCLIN D1, C-MYC and MCL-1*. We found that **17f** was able to downregulate *PIM1* and *C-MYC* gene expression but had weak effects on *CYCLIN D1* and *MCL-1* gene expression (Figure 6A). We also compared expression of Pim1, Cyclin D1 and Mcl-1 proteins by Western blot analysis and found that expression of Pim1 was strongly inhibited by **17f** in KU812 cells while Cyclin D1 and Mcl-1 protein levels were less affected by this compound (figure 6B). Cyclin D1 and Mcl-1 expressions are also known to be regulated by distinct signaling pathways in Bcr-Abl-expressing cells through transcriptional and post-transcriptional mechanisms.[39,40] We then decided to determine whether **17f** might directly interfere with the transcriptional activation of a reporter gene driven by a STAT5-specific promoter. KU812 cells were transfected with a construct containing six tandem copies of the STAT5 response element in front of the minimal TK promoter fused to the luciferase reporter gene (6x(STAT5)-TK-luc). As control, cells were also transfected with a TK-luciferase vector without STAT5 response elements (TK-luc). Luciferase activity was then determined 48 hours post-transfection in cells treated or not with **17f**. As expected, constitutive STAT5 activity induced by Bcr-Abl increased luciferase activity in cells transfected with the STAT5-dependent promoter construct compared to cells transfected with the control TK-luc vector (figure 6C). This enhanced luciferase activity was selectively reduced after **17f** treatment. Collectively, these data strongly suggested that **17f** inhibits DNA binding and transcriptional activity of STAT5 in CML cells.

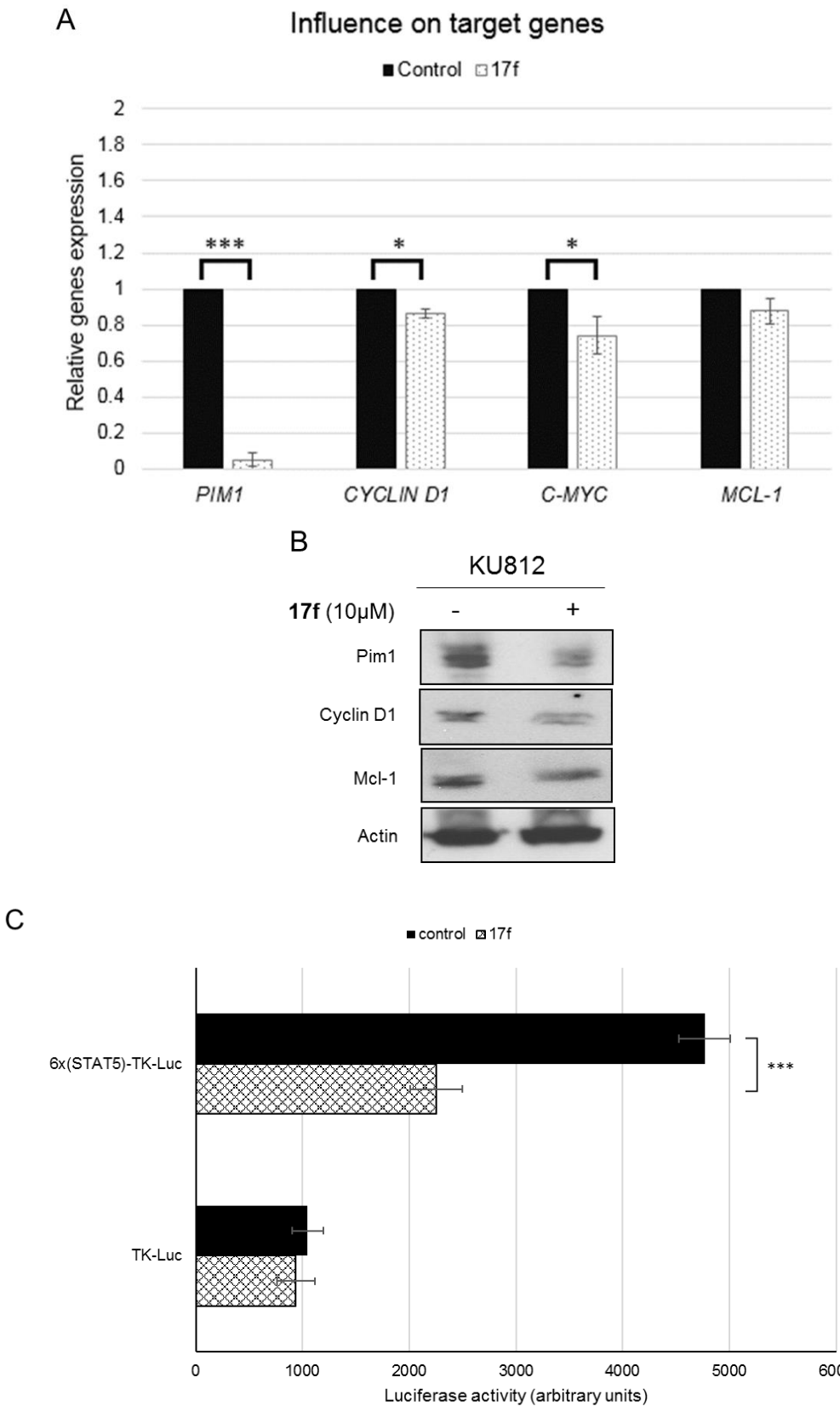
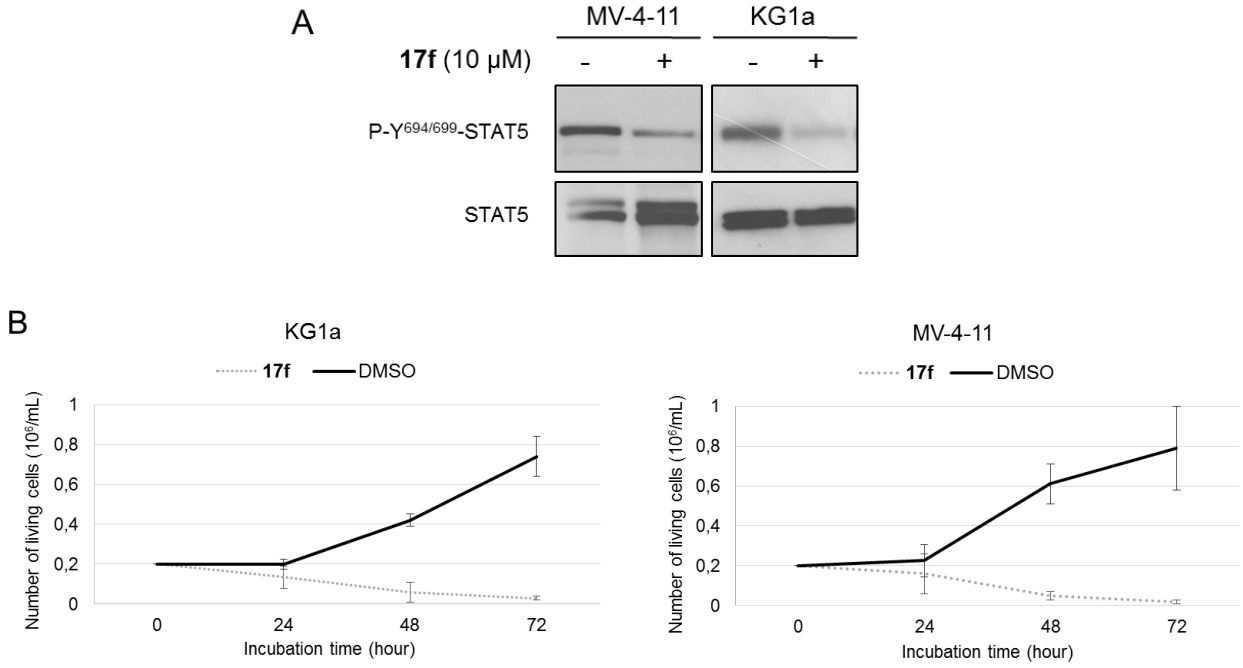

A
Influence on target genes
Control
17f
Relative genes expression
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
***
*
*
PIM1
CYCLIN D1
C-MYC
MCL-1
B
KU812
17f (10μM)
-
+
Pim1
Cyclin D1
Mcl-1
Actin
C
control
17f
6x(STAT5)-TK-Luc
TK-Luc
***
0
1000
2000
3000
4000
5000
6000
Luciferase activity (arbitrary units)

Figure 6. (A) qRT-PCR analysis of *PIM1, CYCLIN D1, C-MYC* and *MCL-1* expression in KU812 cells treated with **17f** (10 μM) or DMSO (control) for 15 h. Results are presented as the fold changes in *PIM1, CYCLIN D1, C-MYC* and *MCL-1* gene expression in **17f** cells normalized to internal control genes (*GAPDH* and *ACTB*) and relative to untreated cells (normalized to 1) (n = 3 in triplicates, data are mean ± SEM, *p < 0.05, ***p < 0.001). (B) Expression of Pim1, Cyclin D1 and Mcl-1 proteins in KU812 cells treated with 10 μM **17f** (+) or DMSO (-) for 24 hours. (C) KU812 cells transfected with a 6x(STAT5)-TK-luciferase reporter construct or a control TK-luciferase vector were treated with **17f** (10 μM) or DMSO (control) for 48 hours. Luciferase activities were next determined as described in material and methods. Luciferase activity (arbitrary units) in the histogram represents the relative luminescence units (rlu) values/mg of proteins (n = 3 in triplicates, data are mean ± SEM, ***p < 0.001).

STAT5 is also constitutively phosphorylated in AML cells and particularly in myeloid leukemia cells expressing the FLT3-ITD oncogene.[2] To exclude the possibility that **17f**-mediated inhibition of STAT5 and cell growth is a peculiarity of cells expressing Bcr-Abl, we evaluated effects of **17f** on STAT5 phosphorylation in two AML cell lines. MV-4-11 cells expressing FLT3-ITD and KG1a, an immature AML cell line, were exposed to **17f** for 12 h and phosphorylation of STAT5 was then analyzed by Western blot. Results showed that **17f** strongly reduced STAT5 phosphorylation in both cell lines (Figure 7A). We then explored whether **17f** also inhibited the growth of these leukemic cells. MV-4-11 and KG1a cells were treated with **17f** (10 μM) and growth kinetics were determined by MTT and trypan blue dye exclusion assays. We found that **17f** suppressed the growth and viability of these AML cells (Figure 7B). Importantly, EC_{50} values (KG1a: 2.64 μM; MV-4-11: 3.55 μM) showed that **17f** was even more

active in AML cells than in CML cells (Table 3). Interestingly enough, the EC_{50} value obtained for **17f** in MV-4-11 cells (3.55 µM) is close to the IC_{50} value reported for the compound 13a (3.5 µM), a STAT5-SH2 inhibitor that selectively binds to STAT5 in a nanomolar range.[15] In addition, we also found that **17f** induces apoptosis of KG1a and MV-4-11 cells indicating that **17f** inhibits STAT5-dependent cell survival (figure 7C).



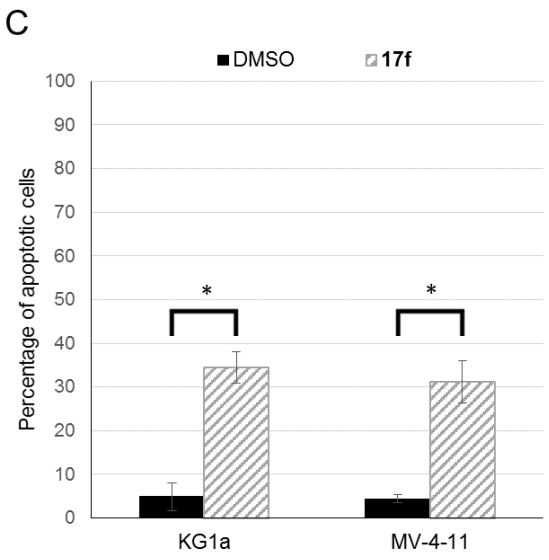


Figure 7. (A) Protein extracts from MV-4-11 and KG1a cells treated with **17f** (+) or DMSO (-) for 12 h were analyzed by Western blotting to detect P-$Y^{694/699}$-STAT5 and STAT5 protein expression. (B) Growth kinetics of KG1a (left) and MV-4-11 (right) cells cultured in absence (DMSO) or presence of **17f** (10 µM) were determined by MTT assays (n = 3 in triplicates, data are mean ± SEM). (C) Representative flow cytometry histogram of KG1a and MV-4-11 cells cultured for 48 h with **17f** (10µM) or DMSO (control). Cells were stained with FITC-Annexin V and 7-AAD and the percentages of apoptotic cells were then evaluated by flow cytometry (n = 3 in triplicates, data are mean ± SEM, *$p < 0.05$).

Table 3. EC_{50} values of **17f** for KG1a and MV-4-11 cells[a]

	17f
Cell Lines	EC_{50} (µM)
KG1a	2,638 ± 0,51
MV-4-11	3,549 ± 0,47

[a]Cells were treated with concentrations ranging from 100 nM to 50 µM for 48 h. Cell viability was then determined by MTT assays and EC_{50} values were calculated using Origin® software (n = 3 in triplicates, data are mean ± SEM) (supp. info).

To determine the selectivity of **17f** on STAT5 phosphorylation, we analyzed effects of this molecule on STAT3, Akt and Erk1/2 signaling. KG1a, MV-4-11 and KU812 cells were treated with **17f** (10 μM) for 24 h and phosphorylation levels of STAT3, Akt and Erk1/2 proteins were analyzed by Western blot analysis (Figure 8). Phosphorylation levels were also determined by band intensity quantification (supp. info). While phosphorylation of STAT5 was clearly inhibited by **17f** in all cell lines, effects on STAT3, Akt and Erk1/2 phosphorylation after 24 h treatment were almost negligible. Collectively, our findings suggest that the growth-suppressive effect of **17f** was as a result of blocking STAT5 phosphorylation in AML and CML cells.

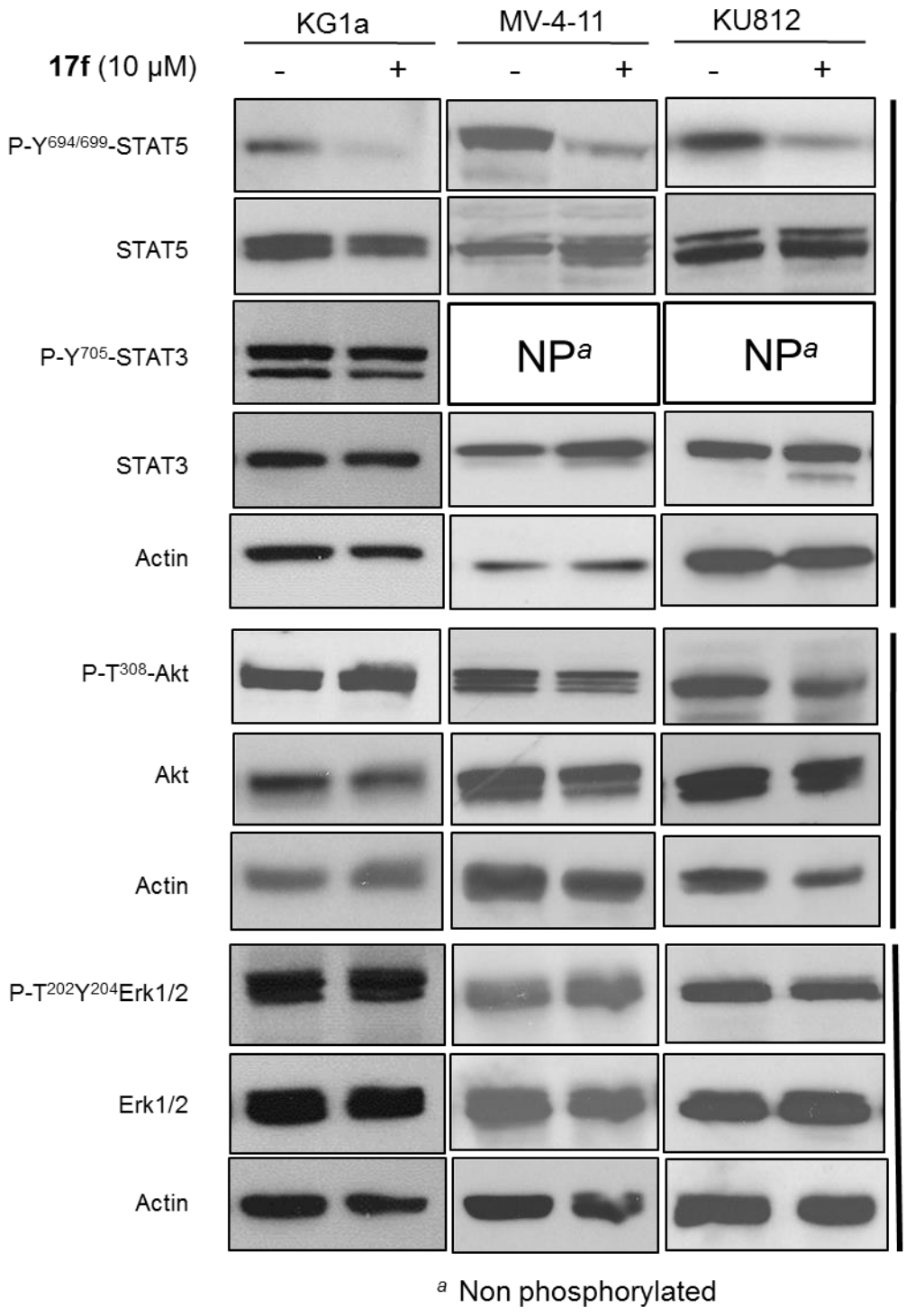
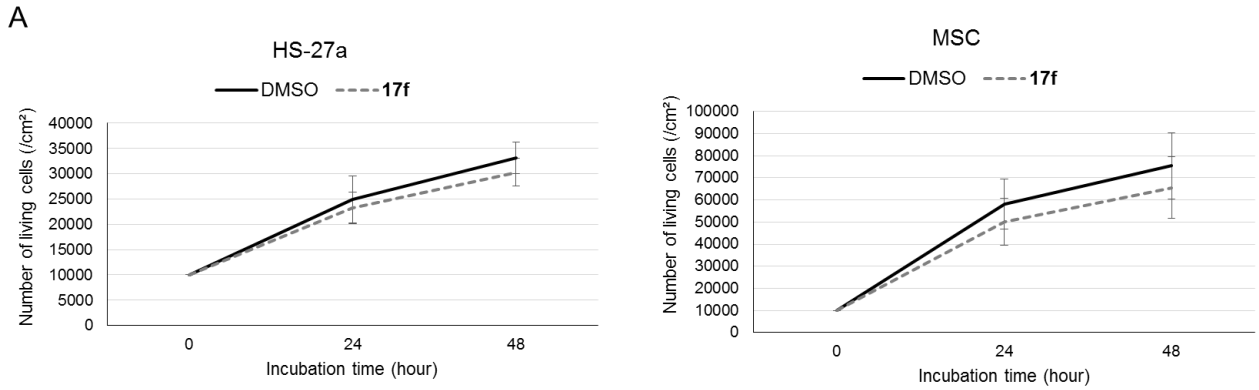


Figure 8. Protein extracts from KG1a, MV-4-11 and KU812 cells treated with **17f** (+) or DMSO (-) for 24 h were analyzed by Western blotting to detect the phosphorylated forms of STAT5, STAT3, Akt and Erk1/2 and the total forms of these proteins (n = 2). Actin expression

served as a loading control. Quantification of Western blot data is shown in supporting information.

Bone marrow stromal and mesenchymal stem cells (MSC) are major cellular components of the leukemic and hematopoietic microenvironment and play a vital role in the survival and chemoresistance of leukemic cells. We then analyzed effects of **17f** on freshly isolated MSC from human bone marrow. We also included in these experiments HS-27a cells, a human bone marrow stromal cell line that shares similar biological properties with MSC. HS-27a cells constitutively express phosphorylated STAT3 but not phosphorylated STAT5 proteins while phosphorylation of STAT5 and STAT3 are not detectable in primary MSC (data not shown). Results showed that **17f** does not significantly or weakly inhibits HS-27a and MSC cell growth with EC_{50} values 10 to 20 fold higher than those required to inhibit leukemic cell growth (Figure 9A, Table 4). Interestingly, we also found that **17f** does not affect the phosphorylation of STAT3 in HS-27a cells (Figure 9B). These findings indicated that **17f** selectively inhibits myeloid leukemia cell growth while sparing normal bone marrow stromal cells of the leukemic niche.



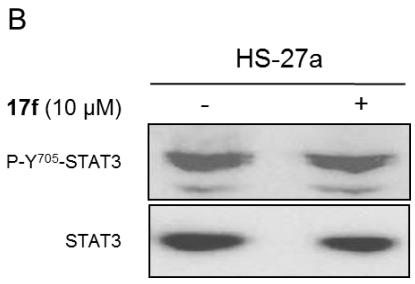


Figure 9. (A) Growth kinetics of HS-27a cells (left) and MSC (right) cultured in absence (DMSO) or presence of **17f** (10 µM) were determined by MTT assays (n = 3 in triplicates, data are mean ± SEM). (B) Extracts from HS-27a cells treated with **17f** (+) or DMSO (-) were analyzed by Western blotting to detect the phosphorylation and expression of STAT3.

Table 4. EC_{50} values of **17f** for HS-27a and MSC cells (3 donors)[a]

	17f
Cells	EC_{50} (µM)
HS27A	63.67 ± 0.05
MSC	33.65 ± 5.87

[a]Cells were treated with concentrations ranging from 1 µM to 100 µM for 48 h. Cell viability was then determined by MTT assays and EC_{50} values were calculated using Origin® software (n = 3 in triplicates, data are mean ± SEM) (supp. info).

CONCLUSION

In summary, among the 18 analogs of hit **13** that have been synthesized, compound **17f** was found to be the most potent inhibitor of AML and CML cell growth with encouraging EC values. Interestingly, the antileukemic effect of **17f** was even more pronounced in AML cells than in CML cells. Key structural features for **17f**-mediated antiproliferative effect are the free nitrogen of the indole ring along with the C-6 tetrahydroquinoline 3-pyridinyl substitution.

We also demonstrated that **17f** inhibits STAT5 phosphorylation in AML and CML and STAT5-dependent transcriptional activity. Importantly, we found that **17f** has no or weak impact on major cellular components of the leukemic niche and does not affect STAT3, Akt or Erk1/2 signaling in leukemia cells suggesting that **17f** selectively targets myeloid leukemia cells addicted to STAT5 signaling. These data also indicate that **17f** or derivatives might be of potential interest to analyze their impact on leukemia cells in contact with their stromal microenvironment.

In the aim of improving cell growth and STAT5 phosphorylation inhibition, in AML and CML cells, alternative modifications on the central scaffold of **17f** are being investigated. For instance, modification of the linker and substitution of indole by its isosteres indazole and 7-azaindole are currently undertaken.

EXPERIMENTAL SECTION

CHEMISTRY

Material and methods

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 pre-coated aluminium sheets silica gel (60 Å, F_{254}) 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 Redisep flash silica gel columns (60 Å, 230-400 mesh, grade 9385). NMR spectra were measured on a Bruker Ultrashield 300 spectrometer, 300 MHz (^{1}H), 282 MHz (^{19}F) and 75 MHz (^{13}C) in deuterated chloroform ($CDCl_3$) or dimethylsulfoxide (DMSO-d_6). Chemical shifts are given in parts per million (ppm) (δ relative to residual solvent peak for ^{1}H and ^{13}C). 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). The HRMS was performed by the mass spectrometry service on a Q-Exactive spectrometer from Thermo Scientific using the electrospray ionization (ESI) technique. Melting points (mp) were taken in open capillaries on a Büchi melting point apparatus Model B-540. 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) and L-2400 (UV-detector)] using 254 nm UV for detection. The column was a Phenomenex Synergi™ C-12, 4 µm particle size (100 mm × 4.6 mm), supported by Phenomenex Security Guard cartridge kit C12 (4.0 mm × 3.0 mm); elution was performed with 0.2% (by volume) of TFA in water (solvent A), and acetonitrile (solvent B);

gradient 35-100% of B with a flow rate of 1 mL.min^{-1}; column temperature of 25 °C; injection of 10 µL in DMSO.

1 *N*-(4-Bromophenyl)-3-methylbut-2-enamide

To a stirred solution of 4-bromoaniline (17.20 g, 100.00 mmol) in dry pyridine (43 mL) at 0 °C under argon, was added dropwise 3,3-dimethylacryloyl chloride (13.35 mL, 120.00 mmol). The reaction mixture was stirred 5 h at rt and then carefully poured into an erlenmeyer flask containing 1 M HCl (750 mL) under magnetic stirring. The product was filtered, washed by water (2 × 100 mL) and heptane (2 × 100 mL) then purified by flash chromatography on silica gel using cyclohexane/ethyl acetate (90/10) as eluent to give the title compound **1** as a white solid (24.9 g, 98%); mp 122 °C. ^{1}H NMR (DMSO, 300 MHz): δ 9.96 (s, 1H), 7.70 – 7.51 (m, 2H), 7.51 – 7.37 (m, 2H), 5.93 – 5.75 (m, 1H), 2.14 (d, *J* = 1.1 Hz, 3H), 1.86 (d, *J* = 1.0 Hz, 3H).^{13}C NMR (DMSO, 75 MHz): δ 164.7, 152.0, 138.9, 131.4 (2C), 120.9 (2C), 118.9, 114.4, 27.1, 19.5. HRMS-ESI (*m/z*): found 254.01618, calcd for $C_{11}H_{13}BrNO$ $[M+H]^+$ 254.01750.

2 6-Bromo-4,4-dimethyl-3,4-dihydroquinolin-2(*1H*)-one

To a stirred solution of *N*-(4-bromophenyl)-3-methylbut-2-enamide **1** (24.90 g, 97.98 mmol) in dry dichloromethane (120 mL) at 0 °C under argon, was added portionwise aluminum chloride (52.30 g, 391.92 mmol) over 30 min. The resulting mixture was stirred 1 h at rt, then poured on ice and extracted with diethyl ether (5 × 100 mL). The combined organic extracts were washed with saturated aqueous $NaHCO_3$ solution (1 × 200 mL), brine (1 × 100 mL) and then dried using $MgSO_4$. After evaporation under reduced pressure, the crude product was purified by recrystallization in ethanol to give the title compound **2** as a white solid (23.3 g, 94%); mp 176 °C. ^{1}H NMR (DMSO, 300 MHz): δ 10.26 (s, 1H), 7.40 (d, *J* = 2.2 Hz, 1H), 7.33 (dd, *J* = 8.4, 2.2 Hz, 1H), 6.82 (d, *J* = 8.4 Hz, 1H), 2.35 (s, 2H), 1.21 (s, 6H). ^{13}C NMR (DMSO, 75 MHz): δ

169.2, 136.4, 134.7, 129.9, 127.1, 117.4, 114.1, 44.4, 33.8, 27.0 (2C). HRMS-ESI (*m/z*): found 254.01628, calcd for $C_{11}H_{13}BrNO$ $[M+H]^+$ 254.01750.

3 6-Bromo-4,4-dimethyl-1,2,3,4-tetrahydroquinoline

To a stirred solution of 6-bromo-4,4-dimethyl-3,4-dihydroquinolin-2(*1H*)-one **2** (8.60 g, 33.84 mmol) in dry toluene (65 mL) at 0 °C under argon, was added dropwise a solution of BH_3.THF 1 M (85 mL, 85.00 mmol). The resulting mixture was refluxed 4 h and then quenched at 0 °C by the addition of saturated aqueous $NaHCO_3$ solution. The reaction medium was extracted with ethyl acetate (2 × 200 mL) then the combined organic extracts were washed with brine (1 × 100 mL) and dried with $MgSO_4$. After evaporation under reduced pressure, the crude product was purified by flash chromatography on silica gel using cyclohexane/ethyl acetate (90/10) as eluent to give the title compound **3** as a colorless oil (7.80 g, 96%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.25 (d, *J* = 2.3 Hz, 1H), 7.02 (dd, *J* = 8.5, 2.3 Hz, 1H), 6.35 (d, *J* = 8.5 Hz, 1H), 3.97 (s, 1H), 3.32 – 3.25 (m, 2H), 1.74 – 1.67 (m, 2H), 1.27 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 142.6, 132.3, 129.3, 129.2, 115.8, 108.5, 38.4, 36.8, 32.0, 30.8 (2C). HRMS-ESI (*m/z*): found 240.03699, calcd for $C_{11}H_{15}BrN$ $[M+H]^+$ 240.03824.

*Method A: Heterocyclic amine alkylation (**5**; **13a-13b**).*

To a stirred solution of the corresponding heterocyclic amine (1.0 equiv) in dry DMF at 0 °C was added portionwise NaH (1.2 equiv). The resulting mixture was stirred 30 minutes and then the corresponding alkyl halide (1.2 equiv) was added dropwise. The reaction mixture was stirred at rt for 2 h, carefully hydrolyzed by water and extracted with ethyl acetate. The combined organic extracts were washed four times with brine to remove DMF and dried using $MgSO_4$. After evaporation under reduced pressure, the crude product was purified by flash chromatography on silica gel.

4 1-Benzyl-5-bromo-*1H*-indole

The title compound was synthesized according to method A using 5-bromoindole (18.00 g, 91.82 mmol), NaH (4.42 g at 60%, 110.20 mmol), benzyl bromide (13.11 mL, 110.20 mmol) in dry DMF (117 mL). The crude product was purified by flash chromatography on silica gel using cyclohexane/ethyl acetate (98/2) as eluent to give the title compound **4** as a white solid (26.10 g, 99%); mp 91 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.82 – 7.70 (m, 1H), 7.35 – 7.21 (m, 4H), 7.16 – 7.04 (m, 4H), 6.49 (dd, *J* = 3.2, 0.8 Hz, 1H), 5.30 (s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 137.1, 135.1, 130.5, 129.6, 129.0 (2C), 127.9, 126.8 (2C), 124.6, 123.6, 113.0, 111.3, 101.4, 50.4. HRMS-ESI (*m/z*): found 286.02133, calcd for $C_{15}H_{13}BrN$ $[M+H]^+$ 286.02259.

5 2-((1-Benzyl-*1H*-indol-5-yl)oxy)ethanol

In a dry 50 mL round bottom Schlenk flask under argon was added 1-benzyl-5-bromo-*1H*-indole **4** (3.00 g, 10.48 mmol), Cs_2CO_3 (5.12 g, 15.72 mmol), copper(I) iodide (0.20 g, 1.05 mmol) and 3,4,7,8-tetramethyl-1,10-phenanthroline (0.50 g, 2.10 mmol). The flask was put under vacuum and refilled with argon three times then dry ethylene glycol (15 mL) was added as solvent. The resulting mixture was vigorously stirred at 130 °C for 72 h. The reaction mixture was 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 using cyclohexane/ethyl acetate (7/3) as eluent to give the title compound **5** as a white solid (1.71 g, 61%); mp 95°C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34 – 7.21 (m, 3H), 7.16 (d, *J* = 8.9 Hz, 1H), 7.14 – 7.06 (m, 4H), 6.85 (dd, *J* = 8.8, 2.4 Hz, 1H), 6.47 (dd, *J* = 3.1, 0.7 Hz, 1H), 5.30 (s, 2H), 4.15 – 4.10 (m, 2H), 4.00 – 3.94 (m, 2H), 2.11 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.1, 137.7, 132.1, 129.2,

129.2, 128.9 (2C), 127.7, 126.8 (2C), 112.6, 110.7, 104.2, 101.4, 70.2, 61.9, 50.4. HRMS-ESI (*m/z*): found 268.13294, calcd for $C_{17}H_{18}NO_2$ $[M+H]^+$ 268.13321.

*Method B: Reductive amination (**6a-b**).*

To a stirred solution of 2-((1-benzyl-*1H*-indol-5-yl)oxy)ethanol **5** (1.0 equiv) in dry 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 (2.0 equiv) in dry DCE (10 mL). The resulting mixture was stirred for 15 minutes and then $NaBH(AcO)_3$ (1.4 equiv) was added. The reaction medium was stirred 2 h at rt and then hydrolyzed with 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.

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

The title compound was synthesized according to method B using 2-((1-benzyl-*1H*-indol-5-yl)oxy)ethanol **5** (1.70 g, 6.36 mmol), IBX (5.34 g, 19.08 mmol), 4,4-dimethyl-1,2,3,4-tetrahydroquinoline (2.05 g, 12.72 mmol), $NaBH(AcO)_3$ (1.89 g, 8.90 mmol) in dry DCE (20 mL). In this case, the purification of the crude product by flash chromatography on silica gel using cyclohexane/ethyl acetate (98/2) gave a mixture of the title compound **6a** with 4,4-dimethyl-1,2,3,4-tetrahydroquinoline. The mixture was dissolved in diethyl ether (100 mL), washed by HCl 1 M (1 × 100 mL) and brine (1 × 100 mL), dried with $MgSO_4$ and evaporated under reduced pressure to give **6a** as a colorless oil (2.00 g, 77%); HPLC purity: 98.3%, ^tR = 24.94 min. ^1H NMR (300 MHz, $CDCl_3$) δ 7.33 – 7.23 (m, 3H), 7.20 (dd, *J* = 7.6, 1.6 Hz, 1H), 7.14 (d, *J* = 8.9 Hz, 1H), 7.12 – 7.00 (m, 5H), 6.82 (dd, *J* = 8.8, 2.4 Hz, 1H), 6.69 – 6.59 (m, 2H),

6.44 (dd, *J* = 3.1, 0.8 Hz, 1H), 5.29 (s, 2H), 4.20 (t, *J* = 6.3 Hz, 2H), 3.74 (t, *J* = 6.3 Hz, 2H), 3.51 – 3.42 (m, 2H), 1.79 – 1.71 (m, 2H), 1.28 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.4, 143.8, 137.7, 131.9, 131.2, 129.2, 129.0, 128.9 (2C), 127.7, 127.0, 126.8 (2C), 126.2, 115.8, 112.5, 110.7, 110.6, 103.9, 101.3, 65.5, 51.0, 50.4, 46.8, 37.2, 32.1, 30.8 (2C). HRMS-ESI (*m/z*): found 411.24285, calcd for $C_{28}H_{31}N_2O$ $[M+H]^+$ 411.24309.

6b 1-(2-((1-Benzyl-*1H*-indol-5-yl)oxy)ethyl)-6-bromo-4,4-dimethyl-1,2,3,4-tetrahydroquinoline

In this case 1.5 equivalent of amine **3** and 2.0 equivalents of $NaBH(AcO)_3$ were used. The title compound was synthesized according to method B, using 2-((1-benzyl-*1H*-indol-5-yl)oxy)ethanol **5** (3.40 g, 12.72 mmol), IBX (10.69 g, 38.16 mmol), 6-bromo-4,4-dimethyl-1,2,3,4-tetrahydroquinoline **3** (4.58 g, 19.08 mmol), $NaBH(AcO)_3$ (5.39 g, 25.44 mmol) in dry DCE (10 mL). In this case, the purification of the crude product by flash chromatography on silica gel using cyclohexane/ethyl acetate (98/2) gave a mixture of the title compound **6b** with **3**. 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 **6b** as a white solid (3.98 g, 64%); mp 89 °C. HPLC purity: 99.6%, ^{t}R = 28.82 min. ^{1}H NMR (300 MHz, DMSO) δ 7.43 (d, *J* = 3.0 Hz, 1H), 7.32 – 7.17 (m, 5H), 7.17 – 7.10 (m, 2H), 7.10 – 7.02 (m, 2H), 6.71 (dd, *J* = 8.9, 2.4 Hz, 1H), 6.62 (d, *J* = 8.9 Hz, 1H), 6.36 (d, *J* = 2.6 Hz, 1H), 5.35 (s, 2H), 4.11 (t, *J* = 5.6 Hz, 2H), 3.66 (t, *J* = 5.5 Hz, 2H), 3.43 – 3.38 (m, 2H), 1.67 – 1.59 (m, 2H), 1.18 (s, 6H). ^{13}C NMR (75 MHz, DMSO) δ 152.6, 142.7, 138.4, 133.1, 131.1, 129.7, 129.1, 128.7, 128.5 (2C), 128.0, 127.3, 126.9 (2C), 112.7, 111.6, 110.9, 106.2, 103.3, 100.6, 64.9, 50.1, 49.2, 45.8, 36.0, 31.8, 30.1 (2C). HRMS-ESI (*m/z*): found 489.15330, calcd for $C_{28}H_{30}BrN_2O$ $[M+H]^+$ 489.15360.

7 1-(2-((1-Benzyl-*1H*-indol-5-yl)oxy)ethyl)-4,4-dimethyl-1,2,3,4-tetrahydroquinoline-6-carbonitrile

A mixture of 1-(2-((1-benzyl-*1H*-indol-5-yl)oxy)ethyl)-6-bromo-4,4-dimethyl-1,2,3,4-tetrahydroquinoline **6b** (1.96 g, 4.01 mmol), copper(I) cyanide (3.59 g, 40.11 mmol) and DMF (17 mL) was refluxed for 6 h. After cooling to rt, the mixture was diluted with 30% aqueous NaCN solution (80 mL) and the resulting solution was extracted with ethyl acetate (3 × 100 mL). The combined organic extracts were washed with brine (4 × 100 mL) and dried with $MgSO_4$. After evaporation under reduced pressure, the crude product was purified by flash chromatography on silica gel using cyclohexane/ethyl acetate (96/4) as eluent to give the title compound **7** as a greenish oil (1.20 g, 69%). ^{1}H NMR (300 MHz, DMSO) δ 7.45 (d, *J* = 2.0 Hz, 1H), 7.43 (d, *J* = 3.0 Hz, 1H), 7.36 – 7.09 (m, 7H), 7.05 (d, *J* = 2.3 Hz, 1H), 6.78 (d, *J* = 8.8 Hz, 1H), 6.70 (dd, *J* = 8.9, 2.3 Hz, 1H), 6.36 (d, *J* = 2.9 Hz, 1H), 5.35 (s, 2H), 4.14 (t, *J* = 5.3 Hz, 2H), 3.76 (t, *J* = 5.3 Hz, 2H), 3.54 – 3.46 (m, 2H), 1.67 – 1.59 (m, 2H), 1.18 (s, 6H). ^{13}C NMR (75 MHz, DMSO) δ 152.5, 147.0, 138.4, 131.2, 131.1 (2C), 129.7, 129.2, 128.7, 128.5 (2C), 127.3, 126.9 (2C), 120.9, 111.5, 110.9, 110.8, 103.3, 100.6, 95.3, 65.0, 49.9, 49.2, 46.1, 35.2, 31.5, 29.5 (2C). HRMS-ESI (*m/z*): found 436.23811, calcd for $C_{29}H_{30}N_3O$ $[M+H]^+$436.23834.

8 (1-(2-((1-Benzyl-*1H*-indol-5-yl)oxy)ethyl)-4,4-dimethyl-1,2,3,4-tetrahydroquinolin-6-yl)(cyclohexyl)methanone

To a stirred solution of 1-(2-((1-benzyl-*1H*-indol-5-yl)oxy)ethyl)-4,4-dimethyl-1,2,3,4-tetrahydroquinoline-6-carbonitrile **7** (2.14 g, 4.91 mmol) in dry THF (30 mL) under argon was added cyclohexylmagnesium chloride (2 M solution in diethyl ether, 3.7 mL, 7.37 mmol) and copper(I) bromide (14 mg, 0.098 mmol). The resulting mixture was refluxed for 45 minutes. After cooling to rt, water (2.5 mL) was carefully added, followed by an aqueous solution of

H_2SO_4 (20 mL, 15%). After stirring for 20 h, the reaction mixture was extracted with dichloromethane (3 × 100 mL), the combined organic extracts were washed with saturated aqueous $NaHCO_3$ solution (1 × 100 mL), brine (1 × 100 mL) and dried with $MgSO_4$. After evaporation under reduced pressure, the crude product was purified by flash chromatography on silica gel using petroleum ether/ethyl acetate (90/10) as eluent to give the title compound **8** as a yellowish solid (1.71 g, 67%); mp 69 °C. HPLC purity: 98.9%, ^tR = 28.90 min. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.91 (d, *J* = 2.1 Hz, 1H), 7.69 (dd, *J* = 8.8, 2.1 Hz, 1H), 7.35 – 7.19 (m, 3H), 7.14 (d, *J* = 8.9 Hz, 1H), 7.12 – 6.96 (m, 4H), 6.80 (dd, *J* = 8.9, 2.4 Hz, 1H), 6.63 (d, *J* = 8.8 Hz, 1H), 6.45 (d, *J* = 3.0 Hz, 1H), 5.29 (s, 2H), 4.23 (t, *J* = 5.9 Hz, 2H), 3.81 (t, *J* = 5.8 Hz, 2H), 3.61 – 3.50 (m, 2H), 3.26 – 3.13 (m, 1H), 1.90 – 1.68 (m, 7H), 1.60 – 1.33 (m, 5H), 1.31 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 202.1, 153.1, 147.6, 137.7, 131.9, 130.8, 129.2, 129.2, 128.9 (2C), 128.6, 127.7, 126.8 (3C), 124.1, 112.4, 110.6, 109.4, 104.0, 101.3, 65.5, 51.0, 50.4, 47.1, 44.9, 36.3, 32.0, 30.2 (2C), 29.9 (2C), 26.2, 26.2 (2C). HRMS-ESI (*m/z*): found 521.31702, calcd for $C_{35}H_{41}N_2O_2$ $[M+H]^+$ 521.31625.

9 1-(2-((1-Benzyl-*1H*-indol-5-yl)oxy)ethyl)-6-(2-cyclohexyl-1,3-dioxolan-2-yl)-4,4-dimethyl-1,2,3,4-tetrahydroquinoline

To a stirred solution of (1-(2-((1-benzyl-*1H*-indol-5-yl)oxy)ethyl)-4,4-dimethyl-1,2,3,4-tetrahydroquinolin-6-yl)(cyclohexyl)methanone **8** (0.50 g, 0.96 mmol) in dry toluene (11 mL) under argon was added dry ethylene glycol (2.15 mL, 38.40 mmol) and *p*-toluenesulfonic acid monohydrate (6 mg, 0.03 mmol). The biphasic mixture was vigorously stirred at reflux with a Dean-Stark trap for 6 h. After cooling to rt, the mixture was washed with saturated aqueous $NaHCO_3$ solution (2 × 50 mL) and brine (2 × 50 mL), dried with $MgSO_4$. After evaporation under reduced pressure, the crude product was purified by flash chromatography on silica gel

using cyclohexane/ethyl acetate (96/4) as eluent to give the title compound **9** as a pale yellow solid (0.20 g, 37%); mp 66 °C. ^{1}H NMR (300 MHz, DMSO) δ 7.43 (d, *J* = 3.0 Hz, 1H), 7.32 – 7.17 (m, 4H), 7.17 – 7.11 (m, 2H), 7.06 (d, *J* = 1.7 Hz, 2H), 6.90 (dd, *J* = 8.5, 1.9 Hz, 1H), 6.72 (dd, *J* = 8.9, 2.3 Hz, 1H), 6.59 (d, *J* = 8.6 Hz, 1H), 6.34 (d, *J* = 3.0 Hz, 1H), 5.35 (s, 2H), 4.12 (t, *J* = 5.6 Hz, 2H), 3.84 (t, *J* = 6.8 Hz, 2H), 3.65 (t, *J* = 5.6 Hz, 2H), 3.57 (t, *J* = 6.7 Hz, 2H), 3.44 – 3.38 (m, 2H), 1.75 – 1.47 (m, 8H), 1.18 (s, 6H), 1.14 – 0.82 (m, 5H). ^{13}C NMR (75 MHz, DMSO) δ 152.7, 142.9, 138.4, 131.1, 129.6, 129.4, 128.7, 128.5 (2C), 127.4, 127.3, 126.9 (2C), 124.7, 123.7, 111.6, 111.3, 110.8, 109.7, 103.3, 100.6, 65.1, 63.8 (2C), 50.2, 49.2, 46.6, 45.9, 36.5, 31.5, 30.7 (2C), 26.8 (2C), 26.0, 25.7 (2C). HRMS-ESI (*m/z*): found 565.34142, calcd for $C_{37}H_{45}N_2O_3$ $[M+H]^+$ 565.34247.

*Method C: Heterocyclic amine debenzylation (**10**, **14**, **16**).*

To a stirred solution of the benzyl indole (1.0 equiv) in DMSO at rt was added *t*-BuOK (7.0 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.

10 1-(2-((*1H*-Indol-5-yl)oxy)ethyl)-6-(2-cyclohexyl-1,3-dioxolan-2-yl)-4,4-dimethyl-1,2,3,4-tetrahydroquinoline

In this case 14.0 equivalents of *t*-BuOK were used. The title compound was synthesized according to method C using 1-(2-((1-benzyl-*1H*-indol-5-yl)oxy)ethyl)-6-(2-cyclohexyl-1,3-dioxolan-2-yl)-4,4-dimethyl-1,2,3,4-tetrahydroquinoline **9** (180 mg, 0.32 mmol), *t*-BuOK (502 mg, 4.47 mmol) in DMSO (30 mL). The crude product was purified by flash chromatography on silica gel using cyclohexane/ethyl acetate (90/10) as eluent to give **10** as a white solid (126 mg,

83%); mp 71 °C. ^{1}H NMR (300 MHz, DMSO) δ 10.91 (s, 1H), 7.35 – 7.20 (m, 2H), 7.07 (d, *J* = 1.9 Hz, 1H), 7.04 (d, *J* = 2.1 Hz, 1H), 6.91 (dd, *J* = 8.5, 1.8 Hz, 1H), 6.72 (dd, *J* = 8.7, 2.3 Hz, 1H), 6.60 (d, *J* = 8.6 Hz, 1H), 6.32 – 6.26 (m, 1H), 4.13 (t, *J* = 5.5 Hz, 2H), 3.84 (t, *J* = 6.8 Hz, 2H), 3.66 (t, *J* = 5.5 Hz, 2H), 3.58 (t, *J* = 6.7 Hz, 2H), 3.46 – 3.38 (m, 2H), 1.75 – 1.50 (m, 8H), 1.19 (s, 6H), 1.14 – 0.87 (m, 5H). ^{13}C NMR (75 MHz, DMSO) δ 152.4, 142.9, 131.1, 129.4, 128.0, 127.4, 125.8, 124.7, 123.6, 112.0, 111.5, 111.4, 109.7, 102.7, 100.8, 65.1, 63.8 (2C), 50.3, 46.6, 45.9, 36.5, 31.5, 30.7 (2C), 26.8 (2C), 26.0, 25.7 (2C). HRMS-ESI (*m/z*): found 475.29434, calcd for $C_{30}H_{38}N_2O_3$ $[M+H]^+$ 475.29552.

11 (1-(2-((*1H*-Indol-5-yl)oxy)ethyl)-4,4-dimethyl-1,2,3,4-tetrahydroquinolin-6-yl)(cyclohexyl)methanone

To a stirred solution of 1-(2-((*1H*-indol-5-yl)oxy)ethyl)-6-(2-cyclohexyl-1,3-dioxolan-2-yl)-4,4-dimethyl-1,2,3,4-tetrahydroquinoline **10** (60 mg, 0.126 mmol) in THF (10 mL) under argon was added HCl (1 M solution in water, 2.5 mL, 2.50 mmol). The resulting mixture was stirred at rt for 2 h. After evaporation of the solvent under reduced pressure, the residue was extracted with ethyl acetate. The combined organic extracts were washed with saturated aqueous $NaHCO_3$ solution (1 × 50 mL), brine (1 × 50 mL) and dried with $MgSO_4$. After evaporation under reduced pressure, the crude product was purified by flash chromatography on silica gel using cyclohexane/ethyl acetate (85/15) as eluent to give the title compound **11** as a white solid (41 mg, 76%); mp 77 °C. HPLC purity: 99.5%, ^{t}R = 24.00 min. ^{1}H NMR (300 MHz, DMSO) δ 10.91 (s, 1H), 7.72 (d, *J* = 2.0 Hz, 1H), 7.64 (dd, *J* = 8.8, 1.9 Hz, 1H), 7.29 – 7.22 (m, 2H), 7.03 (d, *J* = 2.2 Hz, 1H), 6.76 – 6.67 (m, 2H), 6.31 – 6.27 (m, 1H), 4.17 (t, *J* = 5.4 Hz, 2H), 3.79 (t, *J* = 5.3 Hz, 2H), 3.57 – 3.50 (m, 2H), 3.29 – 3.19 (m, 1H), 1.79 – 1.59 (m, 7H), 1.35 (m, 5H), 1.22 (s, 6H). ^{13}C NMR (75 MHz, DMSO) δ 200.3, 152.3, 147.5, 131.2, 130.0, 128.3, 128.0, 125.9,

125.7, 122.7, 112.0, 111.4, 109.6, 102.8, 100.8, 65.0, 50.1, 46.2, 43.4, 35.6, 31.5, 29.8 (2C), 29.5 (2C), 25.7, 25.3 (2C). HRMS-ESI (*m/z*): found 431.27000, calcd for $C_{28}H_{35}N_2O_2$ $[M+H]^+$ 431.26930.

12 Ethyl 2-(5-(2-(6-(cyclohexanecarbonyl)-4,4-dimethyl-3,4-dihydroquinolin-1(*2H*)-yl)ethoxy)-*1H*-indol-1-yl)acetate

The title compound was synthesized according to method A using 1-(2-((*1H*-indol-5-yl)oxy)ethyl)-6-(2-cyclohexyl-1,3-dioxolan-2-yl)-4,4-dimethyl-1,2,3,4-tetrahydroquinoline **10** (85 mg, 0.179 mmol), NaH (9 mg at 60%, 0.215 mmol), ethyl bromoacetate (24 µL, 0.215 mmol) in dry DMF (2 mL). After treatment, the crude product was treated by HCl (1 M solution in water, 0.9 mL, 0.90 mmol) in THF (5 mL) under argon and stirred at rt for 3 h. After evaporation of the solvent under reduced pressure, the residue was extracted with ethyl acetate. The combined organic extracts were washed with saturated aqueous $NaHCO_3$ solution (1 × 50 mL), brine (1 × 50 mL) and dried with $MgSO_4$. After evaporation under reduced pressure, the crude product was purified by flash chromatography on silica gel using cyclohexane/acetone (94/6) as eluent to give the title compound **12** as a colorless oil (21 mg, 23%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.72 (d, *J* = 1.5 Hz, 1H), 7.64 (d, *J* = 8.8 Hz, 1H), 7.30 – 7.21 (m, 2H), 7.05 (d, *J* = 1.8 Hz, 1H), 6.80 – 6.68 (m, 2H), 6.33 (d, *J* = 2.3 Hz, 1H), 5.05 (s, 2H), 4.23 – 4.05 (m, 4H), 3.85 – 3.75 (m, 2H), 3.58 – 3.48 (m, 2H), 3.24 (s, 1H), 1.79 – 1.59 (m, 7H), 1.46 – 1.24 (m, 5H), 1.25 – 1.12 (m, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 200.3, 169.1, 152.7, 147.5, 131.8, 130.2, 130.0, 128.5, 128.3, 125.7, 122.7, 111.5, 110.5, 109.6, 103.3, 100.9, 65.1, 60.8, 50.0, 47.1, 46.2, 43.5, 35.6, 31.5, 29.8 (2C), 29.5 (2C), 25.7, 25.3 (2C), 14.1. HRMS-ESI (*m/z*): found 517.30665, calcd for $C_{32}H_{41}N_2O_4$ $[M+H]^+$ 517.30608.

*Method D: Ester saponification (**13** and **15c**).*

To a stirred solution of the ester (1.0 equiv) in THF and water (1/1) at rt was added LiOH (4.0 equiv). The reaction medium was stirred 2 h at rt. After THF was removed by evaporation under reduced pressure, HCl 1 M (20 mL) was added and the aqueous phase was extracted with dichloromethane (3 × 20 mL). The combined organic extracts were washed with brine (1 × 100 mL) and dried with $MgSO_4$. After evaporation under reduced pressure, the crude product was purified by flash chromatography on silica gel.

13 2-(5-(2-(6-(Cyclohexanecarbonyl)-4,4-dimethyl-3,4-dihydroquinolin-1(*2H*)-yl)ethoxy)-*1H*-indol-1-yl)acetic acid

The title compound was synthesized according to method D using ethyl 2-(5-(2-(6-(cyclohexanecarbonyl)-4,4-dimethyl-3,4-dihydroquinolin-1(*2H*)-yl)ethoxy)-*1H*-indol-1-yl)acetate **12** (18 mg, 0.035 mmol), LiOH (6 mg, 0.14 mmol), THF (1 mL) and water (1 mL). After treatment, the crude product was purified by flash chromatography on silica gel using dichloromethane/methanol (80/20) as eluent to give the title compound **13** as a white solid (7 mg, 41%); mp 104 °C. HPLC purity: 95.9%, ^{t}R = 21.25 min. ^{1}H NMR (300 MHz, DMSO) δ 7.73 (d, *J* = 1.6 Hz, 1H), 7.69 – 7.59 (m, 1H), 7.31 – 7.20 (m, 2H), 7.05 (d, *J* = 2.0 Hz, 1H), 6.80 – 6.68 (m, 2H), 6.31 (d, *J* = 2.9 Hz, 1H), 4.93 (s, 2H), 4.18 (t, *J* = 5.1 Hz, 2H), 3.80 (t, *J* = 4.9 Hz, 2H), 3.59 – 3.49 (m, 2H), 3.26 – 3.18 (m, 1H), 1.69 (m, 7H), 1.49 – 1.27 (m, 5H), 1.23 (s, 6H). ^{13}C NMR (75 MHz, DMSO) δ 200.3, 170.6, 152.6, 147.5, 131.8, 130.3, 130.0, 128.4, 128.3, 125.7, 122.7, 111.4, 110.5, 109.5, 103.2, 100.5, 65.1, 50.0, 47.3, 46.2, 43.4, 35.6, 31.5, 29.8 (2C), 29.5 (2C), 25.7, 25.3 (2C). HRMS-ESI (*m/z*): found 489.27545, calcd for $C_{30}H_{37}N_2O_4$ $[M+H]^+$ 489.27478.

14 1-(2-((*1H*-Indol-5-yl)oxy)ethyl)-4,4-dimethyl-1,2,3,4-tetrahydroquinoline

The title compound was synthesized according to method C using 1-(2-((1-benzyl-*1H*-indol-5-yl)oxy)ethyl)-4,4-dimethyl-1,2,3,4-tetrahydroquinoline **6a** (186 mg, 0.45 mmol), *t*-BuOK (356 mg, 3.17 mmol) in DMSO (5 mL). The crude product was purified by flash chromatography on silica gel using cyclohexane/ethyl acetate (90/10) as eluent to give **14** as a white solid (30 mg, 78%); mp 140 °C. HPLC purity: 99.7%, ^{t}R = 17.77 min. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.04 (s, 1H), 7.32 – 7.15 (m, 3H), 7.13 – 7.01 (m, 2H), 6.91 – 6.81 (m, 1H), 6.75 – 6.57 (m, 2H), 6.50 – 6.41 (m, 1H), 4.22 (t, *J* = 6.0 Hz, 2H), 3.75 (t, *J* = 6.2 Hz, 2H), 3.54 – 3.42 (m, 2H), 1.83 – 1.70 (m, 2H), 1.29 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.5, 143.8, 131.3, 131.2, 128.4, 127.0, 126.2, 125.0, 115.9, 112.9, 111.8, 110.7, 103.7, 102.5, 65.6, 51.1, 46.8, 37.2, 32.1, 30.8 (2C). HRMS-ESI (*m/z*): found 321.19556, calcd for $C_{21}H_{25}N_2O$ $[M+H]^+$ 321.19614.

15a 4,4-Dimethyl-1-(2-((1-methyl-*1H*-indol-5-yl)oxy)ethyl)-1,2,3,4-tetrahydroquinoline

The title compound was synthesized according to method A using 1-(2-((*1H*-indol-5-yl)oxy)ethyl)-4,4-dimethyl-1,2,3,4-tetrahydroquinoline **14** (50 mg, 0.16 mmol), NaH (8 mg at 60%, 0.19 mmol), methyl iodide (12 µL, 0.19 mmol) in dry DMF (0.5 mL). The crude product was purified by flash chromatography on silica gel using cyclohexane/ethyl acetate (95/5) as eluent to give the title compound **15a** as a white solid (47 mg, 89%); mp 77 °C. HPLC purity: 98.7%, ^{t}R = 20.92 min. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.24 – 7.16 (m, 2H), 7.12 – 6.97 (m, 3H), 6.88 (dd, *J* = 8.8, 2.4 Hz, 1H), 6.81 – 6.59 (m, 2H), 6.37 (d, *J* = 3.0 Hz, 1H), 4.30 – 4.17 (m, 2H), 3.76 (s, 3H), 3.76 – 3.70 (m, 2H), 3.53 – 3.44 (m, 2H), 1.84 – 1.71 (m, 2H), 1.29 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.2, 143.7, 132.4, 131.4, 129.5, 128.9, 127.1, 126.3, 116.1, 112.4, 111.0, 110.0, 103.9, 100.5, 65.6, 51.3, 46.7, 37.0, 33.1, 32.1, 30.9 (2C). HRMS-ESI (*m/z*): found 335.21194, calcd for $C_{22}H_{27}N_2O$ $[M+H]^+$ 335.21179.

15b Ethyl 2-(5-(2-(4,4-dimethyl-3,4-dihydroquinolin-1(*2H*)-yl)ethoxy)-*1H*-indol-1-yl)acetate

The title compound was synthesized according to method A using 1-(2-((*1H*-indol-5-yl)oxy)ethyl)-4,4-dimethyl-1,2,3,4-tetrahydroquinoline **14** (0.77 g, 2.41 mmol), NaH (0.12 g at 60%, 2.89 mmol), ethyl bromoacetate (0.32 mL, 2.89 mmol) in dry DMF (20 mL). The crude product was purified by flash chromatography on silica gel using cyclohexane/acetone (90/10) as eluent to give the title compound **15b** as a colorless oil (0.46 g, 47%); HPLC purity: 99.5%, ^{t}R = 20.69 min. ^{1}H NMR (300 MHz, DMSO) δ 7.31 – 7.23 (m, 2H), 7.13 (dd, *J* = 7.6, 1.5 Hz, 1H), 7.07 (d, *J* = 2.3 Hz, 1H), 7.01 – 6.92 (m, 1H), 6.77 (dd, *J* = 8.8, 2.3 Hz, 1H), 6.66 (d, *J* = 7.9 Hz, 1H), 6.56 – 6.48 (m, 1H), 6.35 (d, *J* = 3.0 Hz, 1H), 5.06 (s, 2H), 4.22 – 4.06 (m, 4H), 3.68 (t, *J* = 5.7 Hz, 2H), 3.46 – 3.39 (m, 2H), 1.71 – 1.63 (m, 2H), 1.21 (s, 6H), 1.20 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (75 MHz, DMSO) δ 169.1, 152.8, 143.5, 131.7, 130.6, 130.2, 128.5, 126.6, 125.7, 115.3, 111.6, 110.6, 110.5, 103.2, 100.9, 65.1, 60.8, 50.2, 47.1, 46.0, 36.6, 31.5, 30.6 (2C), 14.1. HRMS-ESI (*m/z*): found 407.23188, calcd for $C_{25}H_{31}N_2O_3$ $[M+H]^+$ 407.23292.

15c 2-(5-(2-(6-Bromo-4,4-dimethyl-3,4-dihydroquinolin-1(*2H*)-yl)ethoxy)-*1H*-indol-1-yl)acetic acid

The title compound was synthesized following the method D using ethyl 2-(5-(2-(4,4-dimethyl-3,4-dihydroquinolin-1(*2H*)-yl)ethoxy)-1H-indol-1-yl)acetate **15b** (50 mg, 0.12 mmol), LiOH (21 mg, 0.49 mmol), THF (2.5 mL) and water (2.5 mL). The crude product was purified by flash chromatography on silica gel using dichloromethane/methanol (80/20) as eluent to give the title compound **15c** as a white solid (31 mg, 67%); mp 158 °C. HPLC purity: 99.5%, ^{t}R = 14.71 min. ^{1}H NMR (300 MHz, DMSO) δ 12.89 (s, 1H), 7.25 (d, *J* = 8.9 Hz, 2H), 7.12 (d, *J* = 7.5 Hz, 1H), 7.08 – 7.02 (m, 1H), 6.99 – 6.90 (m, 1H), 6.75 (d, *J* = 8.7 Hz, 1H), 6.65 (d, *J* = 8.2 Hz, 1H), 6.56 – 6.46 (m, 1H), 6.31 (d, *J* = 2.6 Hz, 1H), 4.95 (s, 2H), 4.14 (t, *J* = 5.4 Hz, 2H), 3.68 (t, *J* = 5.2 Hz, 2H), 3.47 – 3.39 (m, 2H), 1.70 – 1.63 (m, 2H), 1.21 (s, 6H). ^{13}C NMR (75

MHz, DMSO) δ 170.5, 152.7, 143.5, 131.7, 130.6, 130.2, 128.5, 126.6, 125.7, 115.3, 111.5, 110.6, 110.5, 103.2, 100.6, 65.1, 50.1, 47.2, 46.0, 36.6, 31.5, 30.6 (2C). HRMS-ESI (*m/z*): found 379.19927, calcd for $C_{23}H_{27}N_2O_3$ $[M+H]^+$ 379.20162.

16 1-(2-((*1H*-Indol-5-yl)oxy)ethyl)-6-bromo-4,4-dimethyl-1,2,3,4-tetrahydroquinoline

The title compound was synthesized according to method C using 1-(2-((1-benzyl-*1H*-indol-5-yl)oxy)ethyl)-6-bromo-4,4-dimethyl-1,2,3,4-tetrahydroquinoline **6b** (1.83 g, 3.74 mmol), *t*-BuOK (2.94 g, 26.18 mmol) in DMSO (10 mL). The crude product was purified by flash chromatography on silica gel using cyclohexane/ethyl acetate (90/10) as eluent to give the title compound **16** as a white solid (1.16 g, 78%); mp 121 °C. HPLC purity: 98.7%, ^tR = 23.68 min. ^1H NMR (300 MHz, $CDCl_3$) δ 8.04 (s, 1H), 7.32 – 7.20 (m, *J* = 8.7 Hz, 2H), 7.20 – 7.16 (m, 1H), 7.16 – 7.05 (m, 2H), 6.85 (dd, *J* = 8.8, 2.2 Hz, 1H), 6.55 (d, *J* = 8.8 Hz, 1H), 6.49 – 6.44 (m, 1H), 4.20 (t, *J* = 6.0 Hz, 2H), 3.72 (t, *J* = 6.0 Hz, 2H), 3.56 – 3.34 (m, 2H), 1.84 – 1.57 (m, 2H), 1.27 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.3, 142.9, 133.5, 131.2, 129.5, 128.8, 128.4, 125.1, 112.8, 112.4, 111.8, 107.6, 103.7, 102.5, 65.4, 51.0, 46.7, 36.7, 32.3, 30.6 (2C). HRMS-ESI (*m/z*): found 399.10624, calcd for $C_{21}H_{24}BrN_2O$ $[M+H]^+$ 399.10665.

*Method E: Suzuki coupling **(17a-i)***

A mixture of 1-(2-((*1H*-indol-5-yl)oxy)ethyl)-6-bromo-4,4-dimethyl-1,2,3,4-tetrahydroquinoline **16** (1.0 equiv), K_2CO_3 (1.5 equiv), the corresponding boronic acid (3.0 equiv), DMF (10 mL) and water (2 mL) was degassed with argon. Then $PdCl_2(PPh_3)_2$ (0.1 equiv) was added and the resulting mixture was stirred at 90 °C. Upon completion of the reaction (monitored by TLC), the mixture was cooled to rt, filtered through a pad of dicalite and extracted with ethyl acetate (3 × 50 mL). Combined organic extracts were washed with brine (4 × 50 mL)

and dried with $MgSO_4$. After evaporation under reduced pressure, the crude product was purified by flash chromatography on silica gel.

17a 1-(2-((*1H*-Indol-5-yl)oxy)ethyl)-4,4-dimethyl-6-phenyl-1,2,3,4-tetrahydroquinoline

The title compound was synthesized following the method E using 1-(2-((*1H*-indol-5-yl)oxy)ethyl)-6-bromo-4,4-dimethyl-1,2,3,4-tetrahydroquinoline **16** (200 mg, 0.50 mmol), phenylboronic acid (183 mg, 1.50 mmol), K_2CO_3 (207 mg, 1.50 mmol), $PdCl_2(PPh_3)_2$ (35 mg, 0.05 mmol), DMF (10 mL) and water (2 mL). In this case the reaction time was 1 h. The crude product was purified by flash chromatography on silica gel using petroleum ether/ethyl acetate (95/15) as eluent to give the title compound **17a** as a brownish solid (100 mg, 50%); mp 121 °C. HPLC purity: 97.7%, ^{t}R = 24.17 min. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.05 (s, 1H), 7.59 – 7.52 (m, 2H), 7.48 (d, *J* = 2.2 Hz, 1H), 7.45 – 7.36 (m, *J* = 7.6 Hz, 2H), 7.33 (dd, *J* = 8.5, 2.2 Hz, 1H), 7.30 – 7.22 (m, 2H), 7.20 – 7.16 (m, 1H), 7.12 (d, *J* = 2.2 Hz, 1H), 6.88 (dd, *J* = 8.8, 2.4 Hz, 1H), 6.78 (d, *J* = 8.3 Hz, 1H), 6.49 – 6.45 (m, 1H), 4.26 (t, *J* = 6.1 Hz, 2H), 3.80 (t, *J* = 6.2 Hz, 2H), 3.57 – 3.49 (m, 2H), 1.86 – 1.78 (m, 2H), 1.36 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.4, 143.1, 141.8, 131.8, 131.3, 128.7 (3C), 128.4, 126.4 (2C), 126.0, 125.7, 125.1 (2C), 112.8, 111.8, 111.3, 103.7, 102.5, 65.5, 51.3, 46.8, 37.0, 32.2, 30.8 (2C). HRMS-ESI (*m/z*): found 397.22532, calcd for $C_{27}H_{29}N_2O$ $[M+H]^+$ 397.22744.

17b 1-(2-((*1H*-Indol-5-yl)oxy)ethyl)-6-(4-fluorophenyl)-4,4-dimethyl-1,2,3,4-tetrahydroquinoline

The title compound was synthesized following the method E using 1-(2-((*1H*-indol-5-yl)oxy)ethyl)-6-bromo-4,4-dimethyl-1,2,3,4-tetrahydroquinoline **16** (200 mg, 0.50 mmol), (4-fluorophenyl)boronic acid (209 mg, 1.50 mmol), K_2CO_3 (207 mg, 1.50 mmol), $PdCl_2(PPh_3)_2$ (35 mg, 0.05 mmol), DMF (10 mL) and water (2 mL). In this case the reaction time was 2 h. The

crude product was purified by flash chromatography on silica gel using petroleum ether/dichloromethane (60/40) as eluent to give the title compound **17b** as a brownish solid (165 mg, 80%); mp 123 °C. HPLC purity: 99.2%, ^{t}R = 24.53 min. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.04 (s, 1H), 7.55 – 7.43 (m, 2H), 7.40 (d, *J* = 2.2 Hz, 1H), 7.32 – 7.20 (m, 2H), 7.20 – 7.13 (m, 1H), 7.14 – 7.02 (m, 3H), 6.87 (dd, *J* = 8.8, 2.4 Hz, 1H), 6.75 (d, *J* = 8.5 Hz, 1H), 6.52 – 6.40 (m, 1H), 4.24 (t, *J* = 6.1 Hz, 2H), 3.79 (t, *J* = 6.1 Hz, 2H), 3.58 – 3.43 (m, 2H), 1.87 – 1.70 (m, 2H), 1.34 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.4, 160.1, 153.4, 143.2, 138.0, 131.7, 131.3, 128.5, 127.8, 127.7, 125.6, 125.1, 124.8, 115.6, 115.3, 112.8, 111.8, 111.2, 103.7, 102.6, 65.6, 51.1, 46.8, 37.1, 32.2, 30.8 (2C). ^{19}F NMR (282 MHz, $CDCl_3$) δ -118.04. HRMS-ESI (*m/z*): found 415.21734, calcd for $C_{27}H_{28}FN_2O$ $[M+H]^+$ 415.21802.

17c 1-(2-((*1H*-Indol-5-yl)oxy)ethyl)-4,4-dimethyl-6-(3-nitrophenyl)-1,2,3,4-tetrahydroquinoline

The title compound was synthesized following the method E using 1-(2-((*1H*-indol-5-yl)oxy)ethyl)-6-bromo-4,4-dimethyl-1,2,3,4-tetrahydroquinoline **16** (100 mg, 0.25 mmol), (3-nitrophenyl)boronic acid (125 mg, 0.75 mmol), K_2CO_3 (104 mg, 0.75 mmol), $PdCl_2(PPh_3)_2$ (21 mg, 0.03 mmol), DMF (10 mL) and water (2 mL). In this case the reaction time was 2 h. The crude product was purified by flash chromatography on silica gel using petroleum ether/ethyl acetate (90/10) as eluent to give the title compound **17c** as an orange solid (75 mg, 68%); mp 63 °C. HPLC purity: 97.6%, ^{t}R = 24.50 min. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.42 – 8.33 (m, 1H), 8.16 – 7.99 (m, 2H), 7.90 – 7.81 (m, 1H), 7.56 – 7.48 (m, 1H), 7.47 (d, *J* = 2.3 Hz, 1H), 7.35 (dd, *J* = 8.6, 2.3 Hz, 1H), 7.29 (d, *J* = 8.8 Hz, 1H), 7.21 – 7.17 (m, 1H), 7.11 (d, *J* = 2.4 Hz, 1H), 6.87 (dd, *J* = 8.8, 2.4 Hz, 1H), 6.77 (d, *J* = 8.6 Hz, 1H), 6.48 – 6.44 (m, 1H), 4.25 (t, *J* = 6.1 Hz, 2H), 3.81 (t, *J* = 6.0 Hz, 2H), 3.58 – 3.51 (m, 2H), 1.84 – 1.76 (m, 2H), 1.36 (s, 6H). ^{13}C NMR (75

MHz, $CDCl_3$) δ 153.4, 148.9, 144.3, 143.5, 132.0, 131.9, 131.3, 129.5, 128.4, 125.8, 125.7, 125.1, 124.7, 120.8, 120.4, 112.9, 111.9, 111.2, 103.7, 102.6, 65.6, 51.0, 46.8, 36.9, 32.3, 30.6 (2C). HRMS-ESI (*m/z*): found 442.21305, calcd for $C_{27}H_{28}N_3O_3$ $[M+H]^+$ 442.21252.

17d 1-(2-((*1H*-Indol-5-yl)oxy)ethyl)-4,4-dimethyl-6-(thiophen-3-yl)-1,2,3,4-tetrahydroquinoline

The title compound was synthesized following the method E using 1-(2-((*1H*-indol-5-yl)oxy)ethyl)-6-bromo-4,4-dimethyl-1,2,3,4-tetrahydroquinoline **16** (200 mg, 0.50 mmol), thiophen-3-ylboronic acid (192 mg, 1.50 mmol), K_2CO_3 (207 mg, 1.50 mmol), $PdCl_2(PPh_3)_2$ (35 mg, 0.05 mmol), DMF (10 mL) and water (2 mL). In this case the reaction time was 14 h. The crude product was purified by flash chromatography on silica gel using petroleum ether/ethyl acetate (90/10) as eluent to give the title compound **17d** as a beige solid (100 mg, 50%); mp 135 °C. HPLC purity: 99.1%, ^tR = 22.84 min. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.05 (s, 1H), 7.45 (d, *J* = 2.2 Hz, 1H), 7.36 – 7.32 (m, 2H), 7.32 – 7.24 (m, 3H), 7.21 – 7.14 (m, 1H), 7.11 (d, *J* = 2.4 Hz, 1H), 6.87 (dd, *J* = 8.8, 2.4 Hz, 1H), 6.72 (d, *J* = 8.5 Hz, 1H), 6.48 – 6.43 (m, 1H), 4.24 (t, *J* = 6.2 Hz, 2H), 3.78 (t, *J* = 6.2 Hz, 2H), 3.55 – 3.47 (m, 2H), 1.84 – 1.75 (m, 2H), 1.34 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.4, 143.2, 143.1, 131.5, 131.2, 128.4, 126.4, 125.7, 125.3, 125.1, 124.5, 124.0, 117.5, 112.9, 111.8, 111.0, 103.7, 102.6, 65.6, 51.1, 46.8, 37.1, 32.2, 30.8 (2C). HRMS-ESI (*m/z*): found 403.18422, calcd for $C_{25}H_{27}N_2OS$ $[M+H]^+$ 403.18386.

17e 1-(2-((*1H*-Indol-5-yl)oxy)ethyl)-6-(furan-2-yl)-4,4-dimethyl-1,2,3,4-tetrahydroquinoline

The title compound was synthesized following the method E using 1-(2-((*1H*-indol-5-yl)oxy)ethyl)-6-bromo-4,4-dimethyl-1,2,3,4-tetrahydroquinoline **16** (100 mg, 0.25 mmol), furan-2-ylboronic acid (84 mg, 0.75 mmol), K_2CO_3 (104 mg, 0.75 mmol), $PdCl_2(PPh_3)_2$ (21 mg, 0.03 mmol), DMF (10 mL) and water (2 mL). In this case the reaction time was 1 h. The crude

product was purified by flash chromatography on silica gel using cyclohexane/ethyl acetate (95/5) as eluent to give the title compound **17e** as a brown solid (80 mg, 83%); mp 58 °C. HPLC purity: 98.9%, ^{t}R = 22.74 min. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.04 (s, 1H), 7.52 (d, *J* = 2.1 Hz, 1H), 7.41 – 7.37 (m, 1H), 7.35 (dd, *J* = 8.6, 2.2 Hz, 1H), 7.29 (s, 1H), 7.20 – 7.16 (m, 1H), 7.10 (d, *J* = 2.4 Hz, 1H), 6.86 (dd, *J* = 8.8, 2.4 Hz, 1H), 6.68 (d, *J* = 8.6 Hz, 1H), 6.49 – 6.43 (m, 1H), 6.43 – 6.35 (m, 2H), 4.23 (t, *J* = 6.2 Hz, 2H), 3.77 (t, *J* = 6.2 Hz, 2H), 3.54 – 3.45 (m, 2H), 1.82 – 1.72 (m, 2H), 1.32 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 155.3, 153.3, 143.2, 140.6, 131.5, 131.3, 128.4, 125.1, 123.1, 122.1, 119.5, 112.8, 111.8, 111.5, 111.0, 103.7, 102.5, 101.8, 65.6, 51.2, 46.8, 36.9, 32.1, 30.7 (2C). HRMS-ESI (*m/z*): found 387.20697, calcd for $C_{25}H_{27}N_2O_2$ $[M+H]^+$ 387.20670.

17f 1-(2-((*1H*-Indol-5-yl)oxy)ethyl)-4,4-dimethyl-6-(pyridin-3-yl)-1,2,3,4-tetrahydroquinoline

The title compound was synthesized following the method E using 1-(2-((*1H*-indol-5-yl)oxy)ethyl)-6-bromo-4,4-dimethyl-1,2,3,4-tetrahydroquinoline **16** (180 mg, 0.45 mmol), pyridin-3-ylboronic acid (166 mg, 1.35 mmol), K_2CO_3 (187 mg, 1.35 mmol), $PdCl_2(PPh_3)_2$ (31 mg, 0.045 mmol), DMF (10 mL) and water (2 mL). In this case the reaction time was 2.5 h. The crude product was purified by flash chromatography on silica gel using cyclohexane/ethyl acetate (60/40) as eluent to give the title compound **17f** as a yellow solid (84 mg, 47%); mp 133 °C. HPLC purity: 99.2%, ^{t}R = 11.70 min. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.80 (d, *J* = 2.3 Hz, 1H), 8.51 – 8.41 (m, 1H), 8.11 (s, 1H), 7.90 – 7.78 (m, 1H), 7.43 (d, *J* = 2.3 Hz, 1H), 7.36 – 7.27 (m, 3H), 7.21 – 7.16 (m, 1H), 7.11 (d, *J* = 2.2 Hz, 1H), 6.86 (dd, *J* = 8.8, 2.4 Hz, 1H), 6.77 (d, *J* = 8.6 Hz, 1H), 6.50 – 6.41 (m, 1H), 4.25 (t, *J* = 6.0 Hz, 2H), 3.80 (t, *J* = 6.0 Hz, 2H), 3.59 – 3.48 (m, 2H), 1.85 – 1.74 (m, 2H), 1.34 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.4, 147.1, 146.4, 144.1, 137.5, 133.8, 131.9, 131.3, 128.5, 125.7, 125.1, 124.8, 124.5, 123.7, 112.9, 111.8, 111.2,

103.7, 102.6, 65.6, 51.2, 46.8, 36.9, 32.3, 30.7 (2C). HRMS-ESI (*m/z*): found 398.22215, calcd for $C_{26}H_{28}N_3O$ $[M+H]^+$ 398.22269.

17g 1-(2-((*1H*-Indol-5-yl)oxy)ethyl)-6-(6-chloropyridin-3-yl)-4,4-dimethyl-1,2,3,4-tetrahydroquinoline

The title compound was synthesized following the method E using 1-(2-((*1H*-indol-5-yl)oxy)ethyl)-6-bromo-4,4-dimethyl-1,2,3,4-tetrahydroquinoline **16** (100 mg, 0.25 mmol), (6-chloropyridin-3-yl)boronic acid (118 mg, 0.75 mmol), K_2CO_3 (104 mg, 0.75 mmol), $PdCl_2(PPh_3)_2$ (21 mg, 0.03 mmol), DMF (10 mL) and water (2 mL). In this case the reaction time was 1.5 h. The crude product was purified by flash chromatography on silica gel using petroleum ether/ethyl acetate (85/15) as eluent to give the title compound **17g** as a yellow solid (46 mg, 43%); mp 75 °C. HPLC purity: 99.3%, ^tR = 23.14 min. ^{1}H NMR (300 MHz, DMSO) δ 10.91 (s, 1H), 8.63 (d, *J* = 2.3 Hz, 1H), 8.04 (dd, *J* = 8.4, 2.7 Hz, 1H), 7.50 (d, *J* = 2.3 Hz, 1H), 7.47 (d, *J* = 8.6 Hz, 1H), 7.36 (dd, *J* = 8.6, 2.2 Hz, 1H), 7.29 – 7.23 (m, 2H), 7.04 (d, *J* = 2.3 Hz, 1H), 6.79 (d, *J* = 8.7 Hz, 1H), 6.72 (dd, *J* = 8.7, 2.4 Hz, 1H), 6.33 – 6.27 (m, 1H), 4.16 (t, *J* = 5.5 Hz, 2H), 3.75 (t, *J* = 5.5 Hz, 2H), 3.53 – 3.46 (m, 2H), 1.74 – 1.67 (m, 2H), 1.28 (s, 6H). ^{13}C NMR (75 MHz, DMSO) δ 152.8, 147.6, 147.0, 144.4, 136.7, 136.0, 131.8, 131.6, 128.4, 126.3, 125.6, 124.5, 124.5, 122.3, 112.4, 111.9, 111.7, 103.2, 101.3, 65.5, 50.5, 46.5, 36.8, 32.2, 30.7 (2C). HRMS-ESI (*m/z*): found 432.18430, calcd for $C_{26}H_{27}ClN_3O$ $[M+H]^+$ 432.18372.

17h 1-(2-((*1H*-Indol-5-yl)oxy)ethyl)-4,4-dimethyl-6-(pyridin-4-yl)-1,2,3,4-tetrahydroquinoline

The title compound was synthesized following the method E using 1-(2-((*1H*-indol-5-yl)oxy)ethyl)-6-bromo-4,4-dimethyl-1,2,3,4-tetrahydroquinoline **16** (100 mg, 0.25 mmol), pyridin-4-ylboronic acid (92 mg, 0.75 mmol), K_2CO_3 (104 mg, 0.75 mmol), $PdCl_2(PPh_3)_2$ (17 mg, 0.025 mmol), DMF (5 mL) and water (1 mL). In this case the reaction time was 1 h. The

crude product was purified by flash chromatography on silica gel using dichloromethane/methanol (98/2) as eluent to give the title compound **17h** as a yellow solid (46 mg, 46%); mp 142 °C. HPLC purity: 98.7%, ^{t}R = 11.72 min. ^{1}H NMR (300 MHz, DMSO) δ 10.91 (s, 1H), 8.57 – 8.40 (m, 2H), 7.63 – 7.55 (m, 3H), 7.47 (dd, *J* = 8.7, 2.2 Hz, 1H), 7.30 – 7.21 (m, 2H), 7.04 (d, *J* = 2.3 Hz, 1H), 6.80 (d, *J* = 8.7 Hz, 1H), 6.71 (dd, *J* = 8.8, 2.4 Hz, 1H), 6.33 – 6.26 (m, 1H), 4.17 (t, *J* = 5.5 Hz, 2H), 3.77 (t, *J* = 5.4 Hz, 2H), 3.55 – 3.45 (m, 2H), 1.76 – 1.64 (m, 2H), 1.29 (s, 6H). ^{13}C NMR (75 MHz, DMSO) δ 152.3, 149.9 (2C), 147.3, 144.7, 131.1, 131.1, 128.0, 125.8, 125.2, 123.9, 122.9, 119.6 (2C), 112.0, 111.5, 111.1, 102.8, 100.8, 65.1, 50.1, 46.0, 36.2, 31.7, 30.2 (2C). HRMS-ESI (*m/z*): found 398.22231, calcd for $C_{26}H_{28}N_3O$ $[M+H]^+$ 398.22269.

17i 1-(2-((*1H*-Indol-5-yl)oxy)ethyl)-4,4-dimethyl-6-(4-((4-methylpiperazin-1-yl)sulfonyl)phenyl)-1,2,3,4-tetrahydroquinoline

The title compound was synthesized following the method E using 1-(2-((*1H*-indol-5-yl)oxy)ethyl)-6-bromo-4,4-dimethyl-1,2,3,4-tetrahydroquinoline **16** (200 mg, 0.50 mmol), 1-methyl-4-((4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)sulfonyl)piperazine (550 mg, 1.50 mmol), K_2CO_3 (207 mg, 1.50 mmol), $PdCl_2(PPh_3)_2$ (35 mg, 0.05 mmol), DMF (10 mL) and water (2 mL). In this case the reaction time was 1.5 h. The crude product was purified by flash chromatography on silica gel using dichloromethane/methanol (99/1) as eluent to give the title compound **17i** as a white solid (117 mg, 42%); mp 205 °C. HPLC purity: 99.8%, ^{t}R = 14.45 min. ^{1}H NMR (300 MHz, DMSO) δ 10.91 (s, 1H), 7.82 (d, *J* = 8.4 Hz, 2H), 7.68 (d, *J* = 8.4 Hz, 2H), 7.54 (d, *J* = 1.7 Hz, 1H), 7.41 (dd, *J* = 8.6, 1.5 Hz, 1H), 7.26 (dd, *J* = 5.6, 2.9 Hz, 2H), 7.05 (d, *J* = 1.8 Hz, 1H), 6.80 (d, *J* = 8.8 Hz, 1H), 6.72 (dd, *J* = 8.7, 2.1 Hz, 1H), 6.35 – 6.24 (m, 1H), 4.17 (t, *J* = 5.2 Hz, 2H), 3.84 – 3.68 (m, 2H), 3.57 – 3.45 (m, 2H), 3.00 – 2.76 (m, 4H), 2.43 – 2.27

(m, 4H), 2.12 (s, 3H), 1.79 – 1.63 (m, 2H), 1.28 (s, 6H). ^{13}C NMR (75 MHz, DMSO) δ 152.4, 145.3, 144.2, 131.1 (2C), 131.1, 128.2 (2C), 128.0, 125.8, 125.7 (2C), 125.5, 124.4, 124.3, 112.0, 111.5, 111.2, 102.8, 100.8, 65.1, 53.5 (2C), 50.1, 46.0, 45.8 (2C), 45.3, 36.3, 31.7, 30.2 (2C). HRMS-ESI (*m/z*): found 559.27319, calcd for $C_{32}H_{39}N_4O_3S$ $[M+H]^+$ 559.27374.

18 1-(2-((1*H*-indol-5-yl)oxy)ethyl)-4,4-dimethyl-6-(pyridin-2-yl)-1,2,3,4-tetrahydroquinoline

A mixture of 1-(2-((*1H*-indol-5-yl)oxy)ethyl)-6-bromo-4,4-dimethyl-1,2,3,4-tetrahydroquinoline **16** (200 mg, 0.50 mmol), KOAc (245 mg, 2.50 mmol), bis(pinacolato)diboron (254 mg, 1.00 mmol) and dioxane (2.5 mL) was degassed with argon. Then $PdCl_2$(dppf) (4 mg, 0.005 mmol) was added and the resulting mixture was stirred at 80 °C for 21.5 h. The mixture was cooled to rt, filtered through a pad of dicalite, washed with water and extracted with ethyl acetate (3 × 50 mL). Combined organic extracts were washed with brine (50 mL) and dried with $MgSO_4$. After evaporation under reduced pressure, the crude product was purified by flash chromatography on silica gel using petroleum ether/ethyl acetate (85/15) as eluent to give intermediate 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 as a brownish solid (110 mg). A mixture of the latter (103 mg, 0.23 mmol), K_3PO_4 (98 mg, 0.46 mmol), 2-chloropyridine (24 µL, 1.10 mmol), dioxane (1.0 mL) and water (0.25 mL) was degassed with argon. Then $PdCl_2$(dppf) (4 mg, 0.005 mmol) was added and the resulting mixture was stirred at 100 °C for 4.5 h. The mixture was cooled to rt, filtered through a pad of dicalite, washed with water and extracted with ethyl acetate (3 × 50 mL). Combined organic extracts were washed with brine (50 mL) and dried with $MgSO_4$. After evaporation under reduced pressure, the crude product was purified by flash chromatography on silica gel using dichloromethane/ethyl acetate (95/5) as eluent to give title

compound **18** as a yellow solid (42 mg, 21%); mp 65 °C. HPLC purity: 98.2%, ^{t}R = 10.88 min. ^{1}H NMR (300 MHz, DMSO) 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) 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.

BIOLOGY

Cell cultures and reagents

KU812, K562, MV-4-11, KG1a and HS-27a cell lines were obtained from the American Type Culture Collection (ATCC) and maintained according to the supplier's recommendations. All cell lines were cultured in RPMI, with 10% Fetal Bovine Serum, 1% Glutamine and 1% Penicillin/Streptomycin at 37°C, 5% CO_2. Bone marrow (BM) MSC from donors undergoing orthopedic surgery were isolated and cultured as described.[41] Informed consent was obtained before BM samples were taken. GW 9662 and rosiglitazone were purchased from Sigma-Aldrich (Lyon, France).

Cell proliferation assays

Cell viability and proliferation were studied using an MTT cell proliferation assay. Briefly, $0.2x10^5$ cells were incubated in 100 µL of X-Vivo red phenol free medium (Lonza, Basel, Switzerland) in 96 well plates. In initial screening assays, cells were incubated with 10 µM of each compound for 24, 48 and 72 h. To determine the concentration-effect of the molecules, cells

were treated with concentrations ranging from 100 nM to 50 µM for 24 or 48 h. Cells were incubated with 10 µL of MTT working solution (5 g/L of methylthiazolyldiphenyl-tetrazolium bromide) during 4 h. Cells were then lysed overnight at 37 °C with 100 µL of SDS 10%, HCl 0.003%. Optical density (OD) at 570 nm was measured using a spectrophotometer (Dynex, Chantilly, United States). Living cells were also counted with the trypan blue dye exclusion method.

Apoptosis analysis

For apoptosis studies, cells were stained (10^6 cells) in buffer containing FITC-Annexin V and 7-amino-actinomycin D (7-AAD) (Beckmann Coulter, Fullerton, United States) for 15 min at 4 °C and then analyzed by flow cytometry (Becton Dickinson Accuri™ C6 flow cytometer).

Western Blot

Cells were suspended in Laemmli's 2x buffer, separated on SDS/PAGE and blotted onto nitrocellulose membrane (Bio-Rad). Blots were incubated with the following antibodies (Abs): P-$Y^{694/699}$-STAT5, P-Y^{701}-STAT3, P-T^{308}-Akt, Akt, P-$T^{202}Y^{204}$-Erk1/2, Erk1/2, Pim1, Cyclin D1 , Mcl-1 and Actin (Cell signaling Technology, Danvers, USA), STAT3 and STAT5 (BD Transduction Laboratories, Franklin Lakes, 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).

Plasmids, transfection and measurement of luciferase activity

The 6x(STAT5)-TK-luc containing six tandem copies of the STAT5 binding site linked to the minimal TK-luciferase reporter gene and control TK-luc plasmids were previously described.[42] For transient transfection assays, cells were electroporated (270V, 960µF) with the different constructs (25 µg). Transfected cells were expanded for 48h hours in medium and cell extracts

were next prepared in luciferase buffer according to the manufacturer's protocol (One Glo luciferase assay kit, Promega). Luciferase activities were measured in a luminometer (Mithras LB940, Berthold).

qRT-PCR analysis

RNA samples were reverse-transcribed using SuperScript®VILO cDNA synthesis kit (Invitrogen, Carlsbad, United States) as recommended by the supplier. The resulting cDNAs were used for quantitative real-time PCR (qRT-PCR). PCR primers (*STAT5A*: *for* 5'-TCCCTATAACATGTACCCACA-3', *rev* 5'-ATGGTCTCATCCAGGTCGAA-3'; *STAT5B*: *for* 5'-TGAAGGCCACCATCATCAG-3', *rev* 5'-TGTTCAAGATCTCGCCACTG-3'; *PIM1*: *for 5'*-TTTCGAGCATGACGAAGAGA-3', *rev* 5'-GGGCCAAGCACCATCTAAT-3'; *c-MYC*: *for* 5'-GCTGCTTAGACGCTGGATTT-3', *rev* 5'-TAACGTTGAGGGGCATCG-3'; *MCL-1*: *for* 5'-AAGCCAATGGGCAGGTCT-3', *rev* 5'-TGTCCAGTTTCCGAAGCAT-3' and *Cyclin D1*: *for* 5'-CCATCCAGTGGAGGTTTGTC-3', *rev* 5'-GTGGGACAGGTGGCCTTT-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). Both *GAPDH* (glyceraldehyde-3-phosphate dehydrogenase) and *ACTB* (actin beta) 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 $2^{-}\Delta\Delta Ct$ method.[43]

ASSOCIATED CONTENT

Supporting Information

Additional figures illustrating inhibition of STAT5 phosphorylation of hit **13**, cell growth inhibition data, apoptosis experiments, dose-response curve for EC_{50} calculation, quantification of Western Blot data, NMR spectra and Molecular Formula Strings. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Authors

*E-mail: gildas.prie@univ-tours.fr. Phone: +33(0)247367228. ORCID: 0000-0001-6369-7712.

*E-mail: fabrice.gouilleux@univ-tours.fr. Phone: +33(0)247366291.

Author Contributions

[#]L. J. and M. B.-B. contributed equally

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENT

This work was supported by the Region Centre, the European funds for the economical and regional development (FEDER), the Laboratory of excellence Labex SYNORG, ANR-11-LABX-0029 and La Ligue Contre le Cancer. We thank Emmanuel Pecnard for excellent technical assistance. We thank the Plateforme Scientifique et Technique Analyse des Systèmes Biologiques (PST-ASB) for analytical data.

ABBREVIATIONS USED:

7-AAD, 7-Aminoactinomycin D; ACTB, Actin Beta; Akt, Protein Kinase B; AML, Acute Myeloid Leukemia; BCR-ABL, Break point cluster-Abelson; CML, Chronic Myeloid Leukemia; FACS, fluorescence-activated cell sorting; FLT3-ITD, FMS like tyrosine kinase 3 - Internal Tandem Duplication; GAPDH, Glyceraldehyde-3-phosphate dehydrogenase; IBA, 2-iodosobenzoic acid; IBX, 2-iodoxybenzoic acid; IM, Imatinib Mesylate; qRT-PCR, quantitative reverse transcription-polymerase chain reaction; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PIM1, Provirus Integration site for Moloney leukemia virus; PPAR, peroxisome proliferator-activated receptor; RNA, ribonucleic acid; STAT5, Signal transducer and activator of transcription 5; TKI, Tyrosine Kinase Inhibitor.

REFERENCES

(1) Bunting, K. D. STAT5 Signaling in Normal and Pathologic Hematopoiesis. *Front. Biosci.* **2007**, *12*, 2807–2820.

(2) Mizuki, M.; Fenski, R.; Halfter, H.; Matsumura, I.; Schmidt, R.; Müller, C.; Grüning, W.; Kratz-Albers, K.; Serve, S.; Steur, C.; Büchner, T.; Kienast, J.; Kanakura, Y.; Berdel, W. E.; Serve, H. Flt3 Mutations from Patients with Acute Myeloid Leukemia Induce Transformation of 32D Cells Mediated by the Ras and STAT5 Pathways. *Blood* **2000**, *96*, 3907–3914.

(3) Harir, N.; Boudot, C.; Friedbichler, K.; Sonneck, K.; Kondo, R.; Martin-Lanneree, S.; Kenner, L.; Kerenyi, M.; Yahiaoui, S.; Gouilleux-Gruart, V.; Gondry, J.; Benit, L.; Dusanter-Fourt, I.; Lassoued, K.; Valent, P.; Moriggl, R.; Gouilleux, F. Oncogenic Kit Controls Neoplastic Mast Cell Growth through a Stat5/PI3-Kinase Signaling Cascade. *Blood* **2008**, *112*, 2463–2473.

(4) Shuai, K.; Halpern, J.; ten Hoeve, J.; Rao, X.; Sawyers, C. L. Constitutive Activation of STAT5 by the BCR-ABL Oncogene in Chronic Myelogenous Leukemia. *Oncogene* **1996**, *13*, 247–254.

(5) James, C.; Ugo, V.; Le Couédic, J.-P.; Staerk, J.; Delhommeau, F.; Lacout, C.; Garçon, L.; Raslova, H.; Berger, R.; Bennaceur-Griscelli, A.; Villeval, J. L.; Constantinescu, S. N.; Casadevall, N.; Vainchenker, W. A Unique Clonal JAK2 Mutation Leading to Constitutive Signalling Causes Polycythaemia Vera. *Nature* **2005**, *434*, 1144–1148.

(6) Levine, R. L.; Wadleigh, M.; Cools, J.; Ebert, B. L.; Wernig, G.; Huntly, B. J. P.; Boggon, T. J.; Wlodarska, I.; Clark, J. J.; Moore, S.; Adelsperger, J.; Koo, S.; Lee, J. C.; Gabriel, S.; Mercher, T.; D'Andrea, A.; Fröhling, S.; Döhner, K.; Marynen, P.; Vandenberghe, P.; Mesa, R. A.; Tefferi, A.; Griffin, J. D.; Eck, M. J.; Sellers, W. R.; Meyerson, M.; Golub, T. R.; Lee, S. J.; Gilliland, D. G. Activating Mutation in the Tyrosine Kinase JAK2 in Polycythemia Vera, Essential Thrombocythemia, and Myeloid Metaplasia with Myelofibrosis. *Cancer Cell* **2005**, *7*, 387–397.

(7) Ye, D. STAT5 Signaling Is Required for the Efficient Induction and Maintenance of CML in Mice. *Blood* **2006**, *107*, 4917–4925.

(8) Hoelbl, A.; Schuster, C.; Kovacic, B.; Zhu, B.; Wickre, M.; Hoelzl, M. A.; Fajmann, S.; Grebien, F.; Warsch, W.; Stengl, G.; Hennighausen, L.; Poli, V.; Beug, H.; Moriggl, R.; Sexl, V. Stat5 Is Indispensable for the Maintenance of Bcr/abl-Positive Leukaemia. *EMBO Mol. Med.* **2010**, *2*, 98–110.

(9) Jilani, I.; Kantarjian, H.; Gorre, M.; Cortes, J.; Ottmann, O.; Bhalla, K.; Giles, F. J.; Albitar, M. Phosphorylation Levels of BCR-ABL, CrkL, AKT and STAT5 in Imatinib-

Resistant Chronic Myeloid Leukemia Cells Implicate Alternative Pathway Usage as a Survival Strategy. *Leuk. Res.* **2008**, *32*, 643–649.

(10) Cumaraswamy, A. a.; Todic, A.; Resetca, D.; Minden, M. D.; Gunning, P. T. Inhibitors of Stat5 Protein Signalling. *Med. Chem. Commun.* **2012**, *3*, 22–27.

(11) Prost, S.; Relouzat, F.; Spentchian, M.; Ouzegdouh, Y.; Saliba, J.; Massonnet, G.; Beressi, J.; Verhoeyen, E.; Raggueneau, V.; Maneglier, B.; Castaigne, S.; Chomienne, C.; Chrétien, S.; Rousselot, P.; Leboulch, P. Erosion of the Chronic Myeloid Leukaemia Stem Cell Pool by PPARγ Agonists. *Nature* **2015**, *525*, 380–383.

(12) Warsch, W.; Grundschober, E.; Berger, A.; Gille, L.; Cerny-Reiterer, S.; Tigan, A.-S.; Hoelbl-Kovacic, A.; Valent, P.; Moriggl, R.; Sexl, V. STAT5 Triggers BCR-ABL1 Mutation by Mediating ROS Production in Chronic Myeloid Leukaemia. *Oncotarget* **2012**, *3*, 1669–1687.

(13) Berger, A.; Sexl, V.; Valent, P.; Moriggl, R. Inhibition of STAT5: A Therapeutic Option in BCR-ABL1-Driven Leukemia. *Oncotarget* **2014**, *5*, 9564–9576.

(14) Nelson, E. A.; Walker, S. R.; Weisberg, E.; Bar-Natan, M.; Barrett, R.; Gashin, L. B.; Terrell, S.; Klitgaard, J. L.; Santo, L.; Addorio, M. R.; Ebert, B. L.; Griffin, J. D.; Frank, D. A. The STAT5 Inhibitor Pimozide Decreases Survival of Chronic Myelogenous Leukemia Cells Resistant to Kinase Inhibitors. *Blood* **2011**, *117*, 3421–3429.

(15) Cumaraswamy, A. A.; Lewis, A. M.; Geletu, M.; Todic, A.; Diaz, D. B.; Cheng, X. R.; Brown, C. E.; Laister, R. C.; Muench, D.; Kerman, K.; Grimes, H. L.; Minden, M. D.; Gunning, P. T. Nanomolar-Potency Small Molecule Inhibitor of STAT5 Protein. *ACS*

Med. Chem. Lett. **2014**, *5*, 1202–1206.

(16) Liu, H.; Zang, C.; Fenner, M. H.; Liu, D.; Possinger, K.; Koeffler, H. P.; Elstner, E. Growth Inhibition and Apoptosis in Human Philadelphia Chromosome-Positive Lymphoblastic Leukemia Cell Lines by Treatment with the Dual PPARα/γ Ligand TZD18. *Blood* **2006**, *107*, 3683–3692.

(17) Saiki, M.; Hatta, Y.; Yamazaki, T.; Itoh, T.; Enomoto, Y.; Takeuchi, J.; Sawada, U.; Aizawa, S.; Horie, T. Pioglitazone Inhibits the Growth of Human Leukemia Cell Lines and Primary Leukemia Cells While Sparing Normal Hematopoietic Stem Cells. *Int. J. Oncol.* **2006**, *29*, 437–443.

(18) Bertz, J.; Zang, C.; Liu, H.; Wächter, M.; Possinger, K.; Phillip Koeffler, H.; Elstner, E. Compound 48, a Novel Dual PPAR α/γ Ligand, Inhibits the Growth of Human CML Cell Lines and Enhances the Anticancer-Effects of Imatinib. *Leuk. Res.* **2009**, *33*, 686–692.

(19) Prost, S.; Le Dantec, M.; Augé, S.; Le Grand, R.; Derdouch, S.; Auregan, G.; Déglon, N.; Relouzat, F.; Aubertin, A.; Maillere, B.; Dusanter-Fourt, I.; Kirszenbaum, M. Human and Simian Immunodeficiency Viruses Deregulate Early Hematopoiesis through a Nef/PPARγ/STAT5 Signaling Pathway in Macaques. *J. Clin. Invest.* **2008**, *118*, 1765–1775.

(20) Seargent, J. M.; Yates, E. A.; Gill, J. H. GW9662, a Potent Antagonist of PPAR γ , Inhibits Growth of Breast Tumour Cells and Promotes the Anticancer Effects of the PPAR γ Agonist Rosiglitazone, Independently of PPAR γ Activation. *Br. J. Pharmacol.* **2004**, *143*, 933–937.

(21) Wei, S.; Yang, J.; Lee, S.; Kulp, S. K.; Chen, C. PPARγ-Independent Antitumor Effects of Thiazolidinediones. *Cancer Lett.* **2009**, *276*, 119–124.

(22) Parmenon, C.; Guillard, J.; Caignard, D.-H.; Hennuyer, N.; Staels, B.; Audinot-Bouchez, V.; Boutin, J.-A.; Dacquet, C.; Ktorza, A.; Viaud-Massuard, M.-C. 4,4-Dimethyl-1,2,3,4-Tetrahydroquinoline-Based PPARα/γ Agonists. Part I: Synthesis and Pharmacological Evaluation. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 1617–1622.

(23) Parmenon, C.; Guillard, J.; Caignard, D.-H.; Hennuyer, N.; Staels, B.; Audinot-Bouchez, V.; Boutin, J.-A.; Dacquet, C.; Ktorza, A.; Viaud-Massuard, M.-C. 4,4-Dimethyl-1,2,3,4-Tetrahydroquinoline-Based PPARα/γ Agonists. Part. II: Synthesis and Pharmacological Evaluation of Oxime and Acidic Head Group Structural Variations. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2683–2687.

(24) Garcia-Bates, T. M.; Lehmann, G. M.; Simpson-Haidaris, P. J.; Bernstein, S. H.; Sime, P. J.; Phipps, R. P. Role of Peroxisome Proliferator-Activated Receptor Gamma and Its Ligands in the Treatment of Hematological Malignancies. *PPAR Res.* **2008**, *2008*, 1–18.

(25) Kodomari, M.; Suzuki, Y.; Yoshida, K. Graphite as an Effective Catalyst for Friedel–Crafts Acylation. *Chem. Commun.* **1997**, No. 16, 1567–1568.

(26) Niu, J.; Guo, P.; Kang, J.; Li, Z.; Xu, J.; Hu, S. Copper(I)-Catalyzed Aryl Bromides To Form Intermolecular and Intramolecular Carbon−Oxygen Bonds. *J. Org. Chem.* **2009**, *74*, 5075–5078.

(27) Altman, R. A.; Shafir, A.; Choi, A.; Lichtor, P. A.; Buchwald, S. L. An Improved Cu-Based Catalyst System for the Reactions of Alcohols with Aryl Halides. *J. Org. Chem.*

2008, *73*, 284–286.

(28) More, J. D.; Finney, N. S. A Simple and Advantageous Protocol for the Oxidation of Alcohols with O-Iodoxybenzoic Acid (IBX). *Org. Lett.* **2002**, *4*, 3001–3003.

(29) Abdel-Magid, A. F.; Carson, K. G.; Harris, B. D.; Maryanoff, C. A.; Shah, R. D. Reductive Amination of Aldehydes and Ketones with Sodium Triacetoxyborohydride. Studies on Direct and Indirect Reductive Amination Procedures. *J. Org. Chem.* **1996**, *61*, 3849–3862.

(30) Dhar, A.; Liu, S.; Klucik, J.; Berlin, K. D.; Madler, M. M.; Lu, S.; Ivey, R. T.; Zacheis, D.; Brown, C. W.; Nelson, E. C.; Birckbichler, P. J.; Benbrook, D. M. Synthesis, Structure−Activity Relationships, and RARγ−Ligand Interactions of Nitrogen Heteroarotinoids. *J. Med. Chem.* **1999**, *42*, 3602–3614.

(31) Weiberth, F. J.; Hall, S. S. Copper(I)-Activated Addition of Grignard Reagents to Nitriles. Synthesis of Ketimines, Ketones, and Amines. *J. Org. Chem.* **1987**, *52*, 3901–3904.

(32) Haddach, A. A.; Deaton-rewolinski, M. V. An Efficient Method for the N-Debenzylation of Aromatic Heterocycles. *Tetrahedron Lett.* **2002**, *43*, 399–402.

(33) Liang, Y. F.; Jiao, N. Highly Efficient C-H Hydroxylation of Carbonyl Compounds with Oxygen under Mild Conditions. *Angew. Chem. Int. Ed. Engl.* **2014**, *53*, 548–552.

(34) Lucas, P.; Mehdi, N. El; Ho, H. A.; Bélanger, D.; Breau, L. Expedient Synthesis of Symmetric Aryl Ketones and of Ambient-Temperature Molten Salts of Imidazole. *Synthesis (Stuttg).* **2000**, *2000*, 1253–1258.

(35) Ciceri, P.; Demnitz, F. W. J. An Efficient, Rapid and Highly Selective Preparation of the

Wieland-Miescher Ketone-9-Ethylene Ketal. *Tetrahedron Lett.* **1997**, *38*, 389–390.

(36) Devine, W.; Woodring, J. L.; Swaminathan, U.; Amata, E.; Patel, G.; Erath, J.; Roncal, N. E.; Lee, P. J.; Leed, S. E.; Rodriguez, A.; Mensa-Wilmot, K.; Sciotti, R. J.; Pollastri, M. P. Protozoan Parasite Growth Inhibitors Discovered by Cross-Screening Yield Potent Scaffolds for Lead Discovery. *J. Med. Chem.* **2015**, *58*, 5522–5537.

(37) Knapp, D. M.; Gillis, E. P.; Burke, M. D. A General Solution for Unstable Boronic Acids: Slow-Release Cross-Coupling from Air-Stable MIDA Boronates. *J. Am. Chem. Soc.* **2009**, *131*, 6961–6963.

(38) Dick, G. R.; Woerly, E. M.; Burke, M. D. A General Solution for the 2-Pyridyl Problem. *Angew. Chem. Int. Ed. Engl.* **2012**, *51*, 2667–2672.

(39) Sonoyama, J.; Matsumura, I.; Ezoe, S.; Satoh, Y.; Zhang, X.; Kataoka, Y.; Takai, E.; Mizuki, M.; Machii, T.; Wakao, H.; Kanakura, Y. Functional Cooperation among Ras, STAT5, and Phosphatidylinositol 3-Kinase Is Required for Full Oncogenic Activities of BCR/ABL in K562 Cells. *J. Biol. Chem.* **2002**, *277*, 8076–8082.

(40) Aichberger, K. J.; Mayerhofer, M.; Krauth, M.-T.; Skvara, H.; Florian, S.; Sonneck, K.; Akgul, C.; Derdak, S.; Pickl, W. F.; Wacheck, V.; Selzer, E.; Monia, B. P.; Moriggl, R.; Valent, P.; Sillaber, C. Identification of Mcl-1 as a BCR/ABL-Dependent Target in Chronic Myeloid Leukemia (CML): Evidence for Cooperative Antileukemic Effects of Imatinib and Mcl-1 Antisense Oligonucleotides. *Blood* **2005**, *105*, 3303–3311.

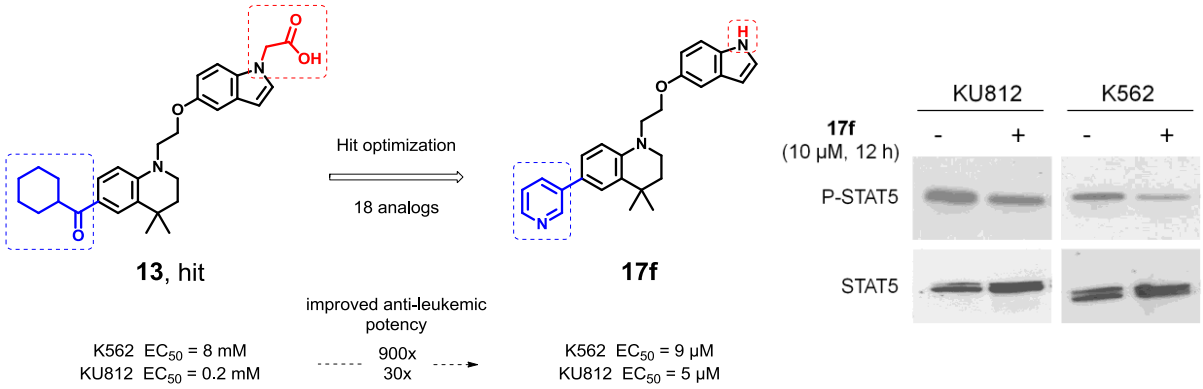
(41) Ponte, A. L.; Ribeiro-Fleury, T.; Chabot, V.; Gouilleux, F.; Langonné, A.; Hérault, O.; Charbord, P.; Domenech, J. Granulocyte-Colony-Stimulating Factor Stimulation of Bone

Marrow Mesenchymal Stromal Cells Promotes CD34+ Cell Migration Via a Matrix Metalloproteinase-2-Dependent Mechanism. *Stem Cells Dev.* **2012**, *21*, 3162–3172.

(42) Moriggl, R.; Gouilleux-Gruart, V.; Jähne, R.; Berchtold, S.; Gartmann, C.; Liu, X.; Hennighausen, L.; Sotiropoulos, A.; Groner, B.; Gouilleux, F. Deletion of the Carboxyl-Terminal Transactivation Domain of MGF-Stat5 Results in Sustained DNA Binding and a Dominant Negative Phenotype. *Mol. Cell. Biol.* **1996**, *16*, 5691–5700.

(43) Livak, K. J.; Schmittgen, T. D. Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2−ΔΔCT Method. *Methods* **2001**, *25*, 402–408.

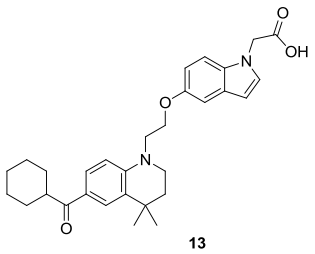
TABLE OF CONTENTS GRAPHIC



A New Inhibitor Targeting Signal Transducer and Activator of Transcription 5 (STAT5) Signaling in Myeloid Leukemias

Ludovic Juen,[†,#] Marie Brachet-Botineau,[§,‡,#] Cécile Parmenon,[†] Jérôme Bourgeais,[§,‡] Olivier Hérault,[§,‡] Fabrice Gouilleux,[*,‡] Marie-Claude Viaud-Massuard[†] and Gildas Prié[*,†]

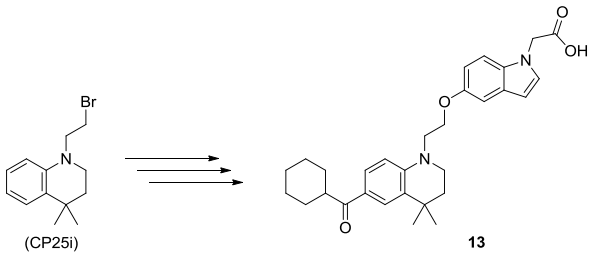
GRAPHIC



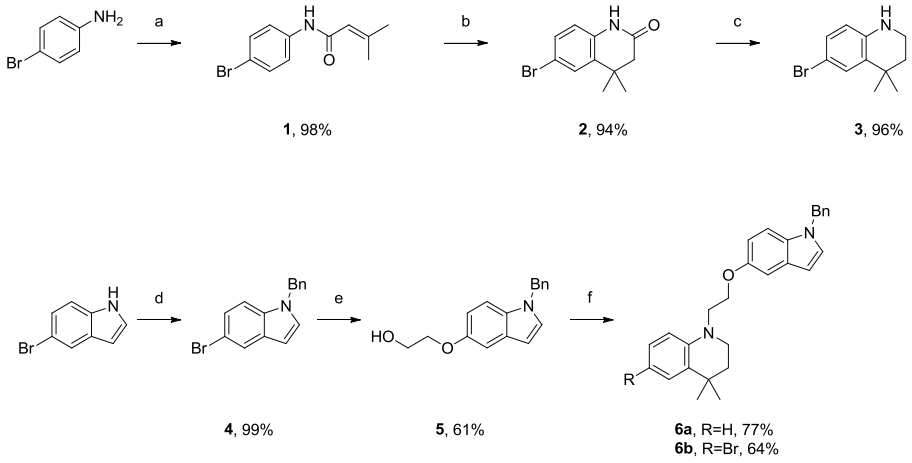
13

Figure 1. Hit compound **13**

Scheme 1. Previous synthetic route of hit compound **13**

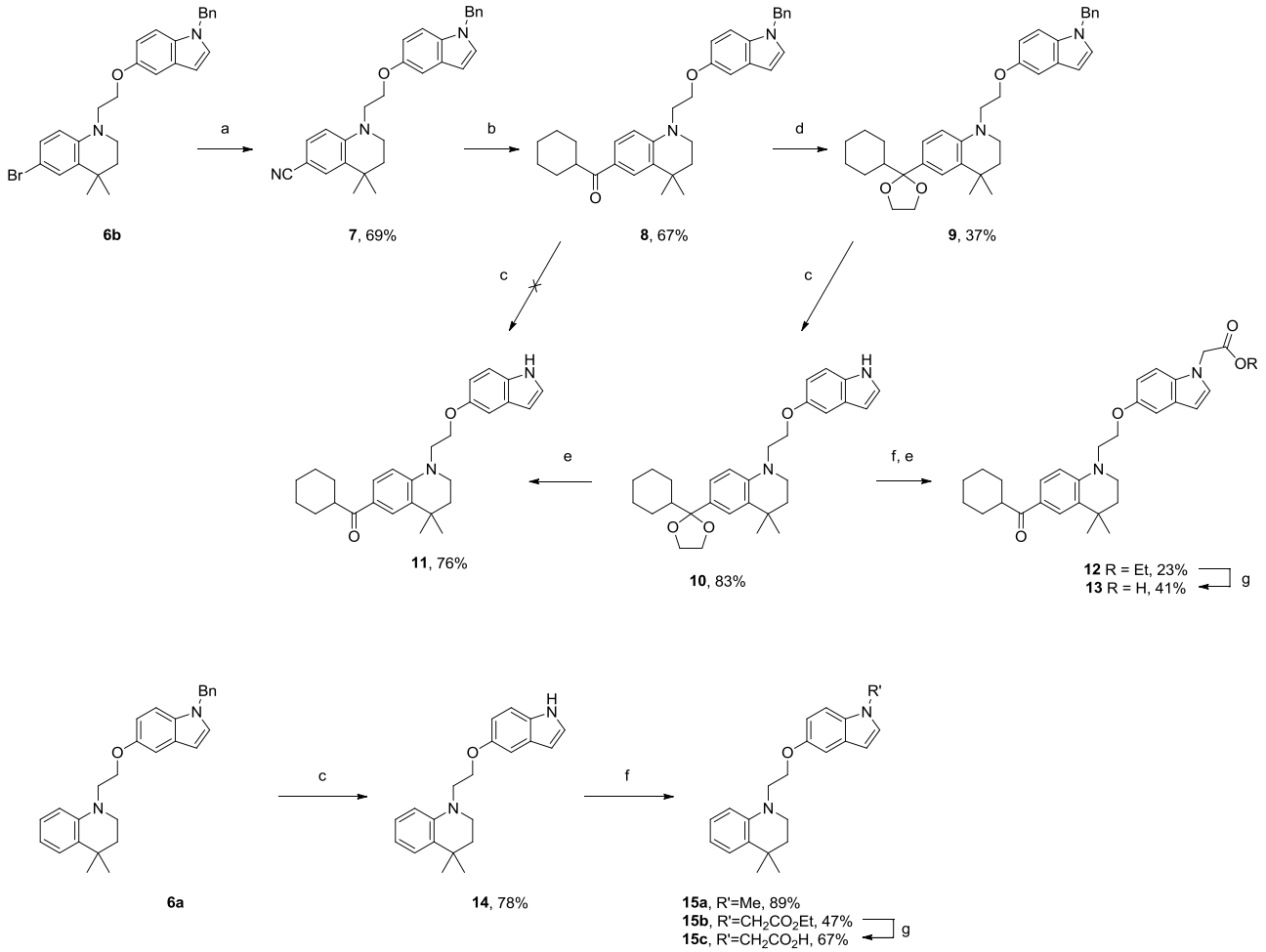


Scheme 2. Synthesis of key intermediates **6a** and **6b**[a]



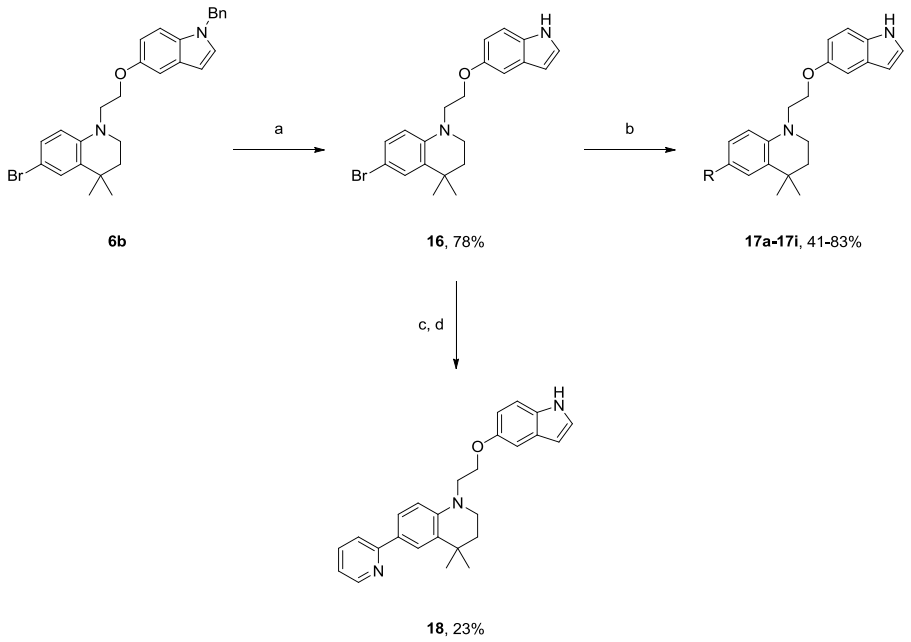
[a]Reagent and conditions: (a) 3,3-dimethyl-acryloyl chloride, dry pyridine, 0 °C to rt, 5 h; (b) $AlCl_3$, dry dichloromethane, 0 °C to rt, 1 h; (c) BH_3.THF, dry toluene, 0 °C to reflux, 12 h; (d) NaH, BnBr, dry DMF, 0 °C to rt, 2 h; (e) CuI, 3,4,7,8-tetramethyl-1,10-phenanthroline, Cs_2CO_3, dry ethylene glycol, 130 °C, 72 h; (f) 1) IBX, DCE, 80 °C, 2 h; 2) Filtration; 3) 4,4-dimethyl-1,2,3,4-tetrahydroquinoline or amine **3**, $NaBH(AcO)_3$, rt, 3 h.

Scheme 3. Synthesis of hit compound **13** and its analogs[a]



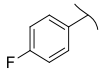
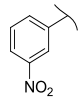
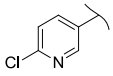
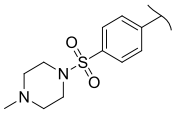
[a]Reagent and conditions: (a) CuCN, DMF, reflux, 6 h; (b) 1) cyclohexylmagnesium chloride, CuBr, dry THF, reflux, 2 h; 2) H_2O, H_2SO_4 15%; (c) *t*-BuOK, DMSO, O_2, rt, 3 h; (d) ethylene glycol, *p*-toluenesulfonic acid monohydrate, toluene, reflux, 6 h; (e) HCl 0.1 M, THF, rt, 5 h; (f) NaH, ethyl bromoacetate or methyl iodide, dry DMF, 0 °C to rt, 2 h; (g) LiOH, THF/H_2O, 0 °C to rt, 2 h.

Scheme 4. Synthesis of analogs **16**, **17a-i** and **18**[a]



[a]Reagent and conditions: (a) *t*-BuOK, DMSO, O_2, rt, 3 h; (b) corresponding boronic acid, K_2CO_3, $PdCl_2(PPh_3)_2$, DMF/H_2O, 90 °C, 1-14 h; (c) bis(pinacolato)diboron, KOAc, $PdCl_2$(dppf), dry dioxane, 80 °C, 21 h; (d) 2-chloropyridine, K_3PO_4, $PdCl_2$(dppf), dioxane/H_2O, 100 °C, 4 h.

Table 1. Structure of analogs **17a-i**

Entry	Compound	R	Time (h)	Yield (%)
1	**17a**		1	50
2	**17b**		2	79
3	**17c**		2	68
4	**17d**		14	50
5	**17e**		1	83
6	**17f**		2.5	46
7	**17g**		2.5	50
8	**17h**		1	46
9	**17i**		1.5	41

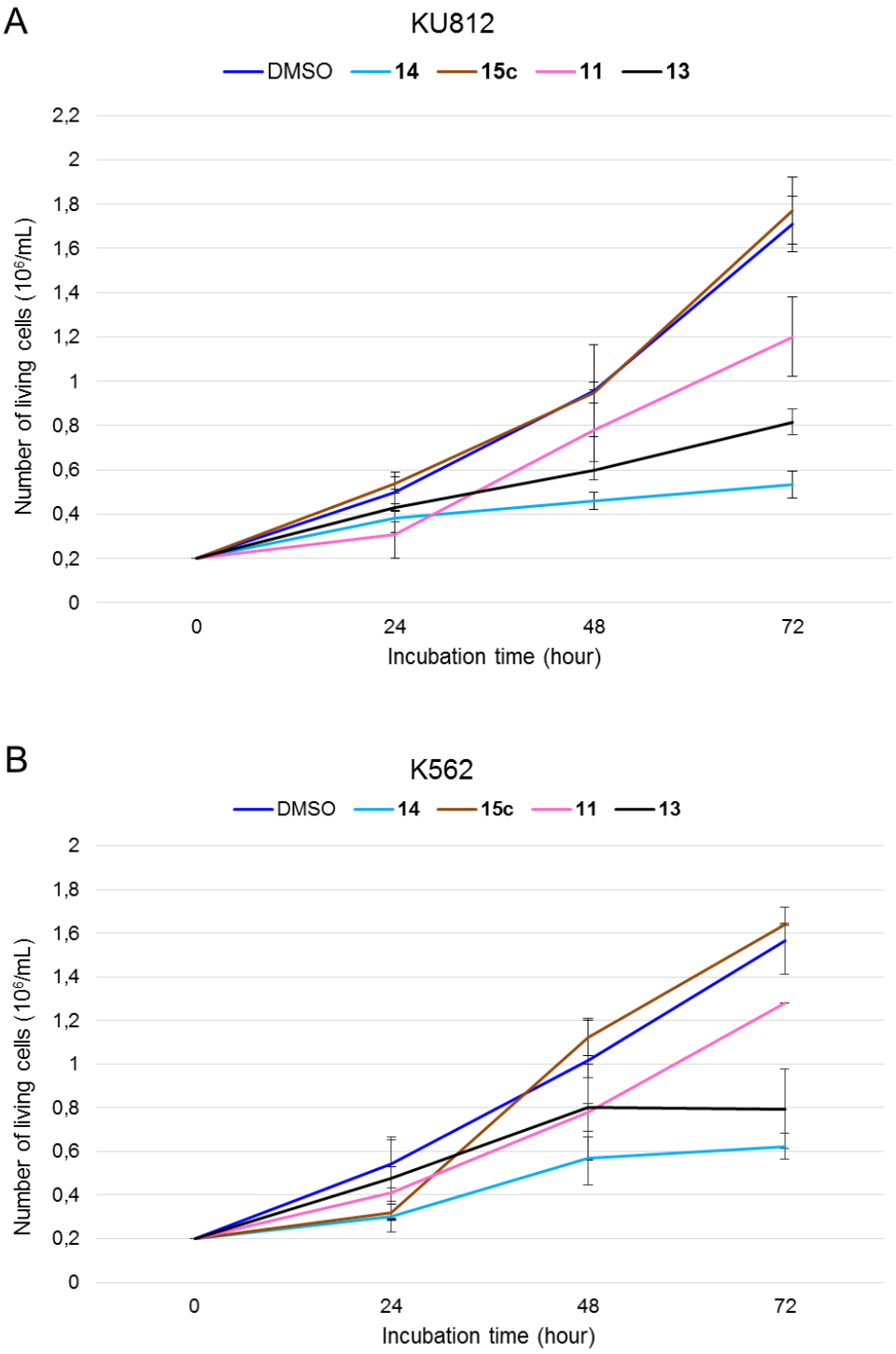


Figure 2. (A) Growth kinetics of KU812 cells treated with 10 μM of each compound as indicated or DMSO as control; (B) Growth kinetics of K562 cells treated with each compound or DMSO as control as in (A) (n = 3 in triplicates, data are mean ± SEM).

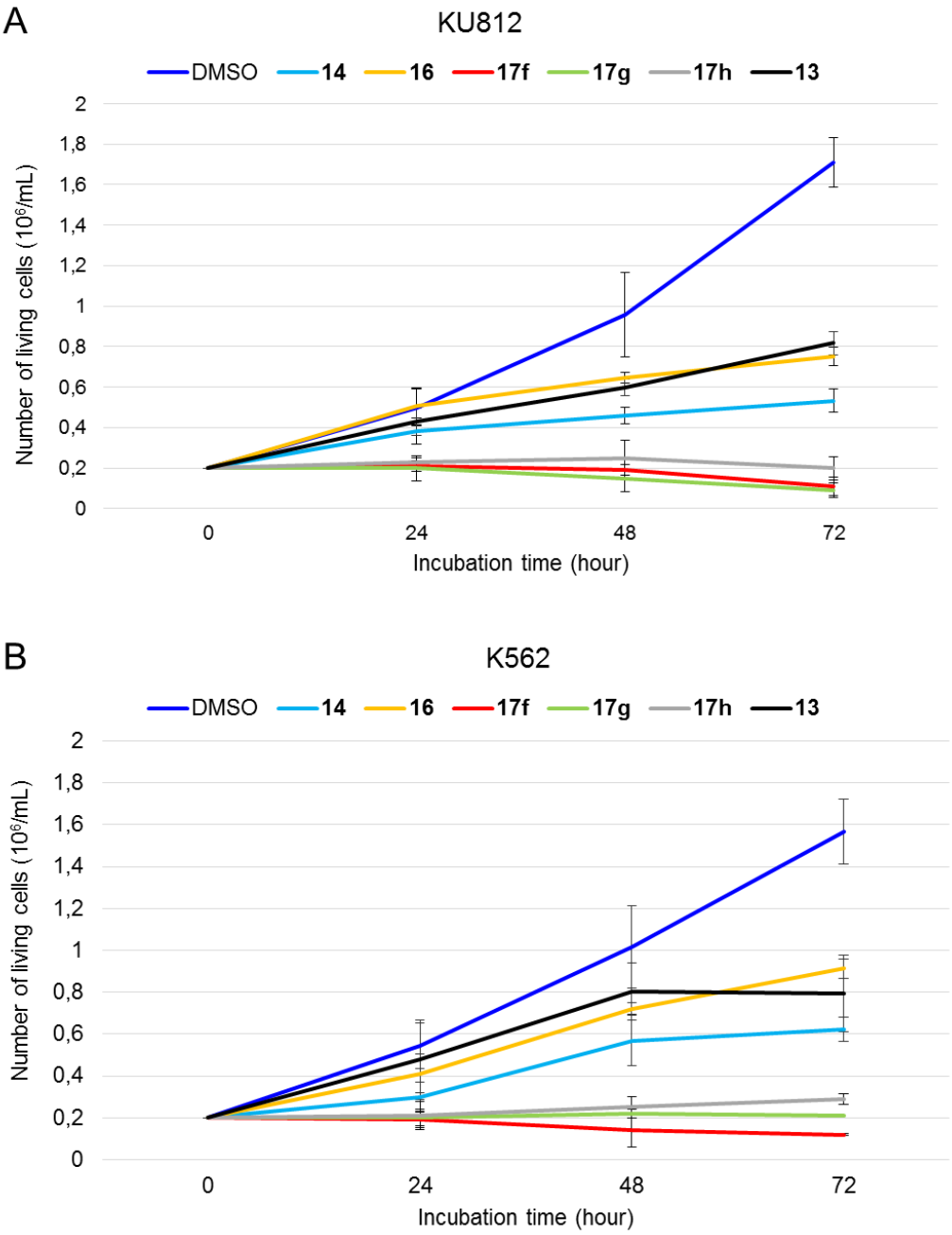


Figure 3. (A) Growth kinetics of KU812 cells treated with 10 μM of each compound as indicated or DMSO as control; (B) Growth kinetics of K562 cells treated with each compound or DMSO as control as in (A) (n = 3 in triplicates, data are mean ± SEM).

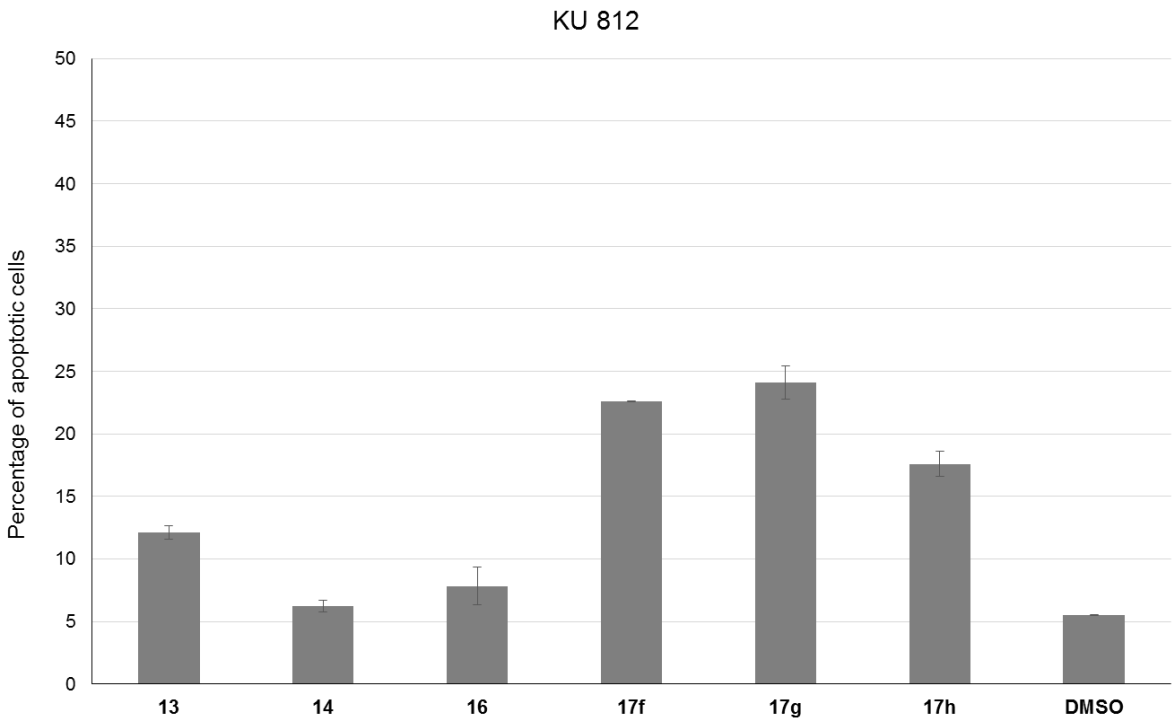


Figure 4. Representative flow cytometry histogram of KU812 cells cultured for 48 h with indicated compounds (10 μM) or control DMSO. Cells were stained with FITC-Annexin V and 7-AAD and the percentages of apoptotic cells were then evaluated by flow cytometry (n = 3 in triplicates, data are mean ± SEM).

Table 2. EC_{50} values of selected compounds for K562 and KU812 cells[a]

	EC_{50} (μM)	
Compound	K562	KU812
13	8658.96 ± 59.52	196.57 ± 16.11
14	78.13 ± 2.73	14.63 ± 2.04
16	15.58 ± 1.67	7.59 ± 0.63
17f	8.74 ± 1.54	5.38 ± 0.01
17g	13.04 ± 3.21	5.41 ± 1.39
17h	22.65 ± 1.09	9.38 ± 1.18

[a]Cells were treated with concentrations ranging from 100 nM to 50 μM for 48 h. Cell viability was then determined by MTT assays and EC_{50} values were calculated using Origin® software (n = 3 in triplicates, data are mean ± SEM) (supp. info).

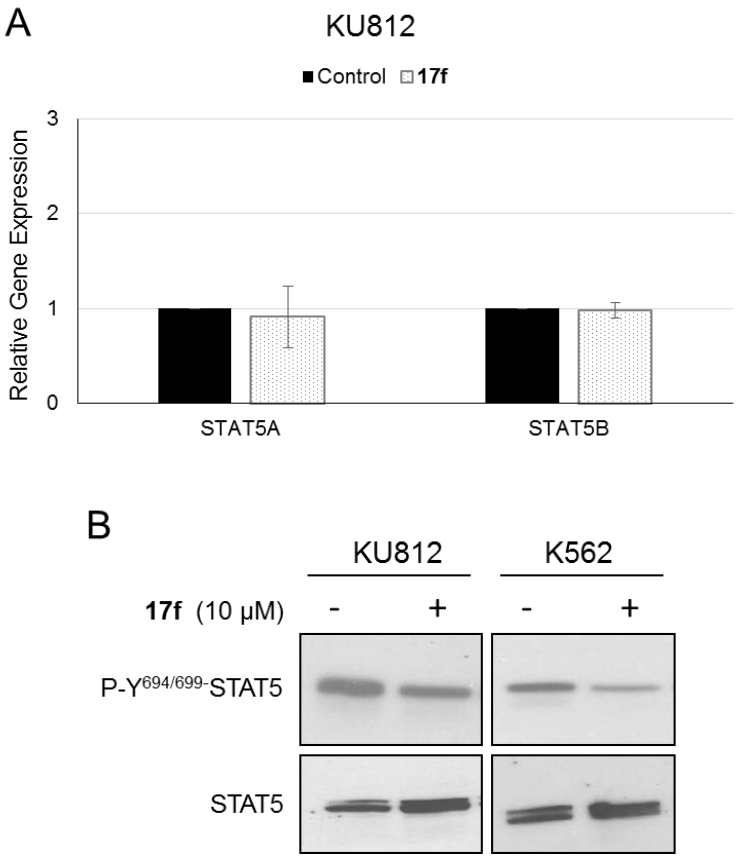


Figure 5. (A) qRT-PCR analysis of *STAT5A and STAT5B* transcripts in KU812 cells treated or not with **17f** (10 μM) or DMSO as control for 15 h. Results are presented as the fold changes in gene expression in **17f**-treated cells normalized to internal control genes (*GAPDH* and *ACTB*) and relative to control cells (normalized to 1) (n = 3 in triplicates, data are mean ± SEM). (B) Protein extracts from KU812 and K562 cells treated with **17f** (+) or DMSO (-) for 12 h were analyzed by Western blotting to detect P-Y-STAT5 and STAT5 protein expression (n = 2).

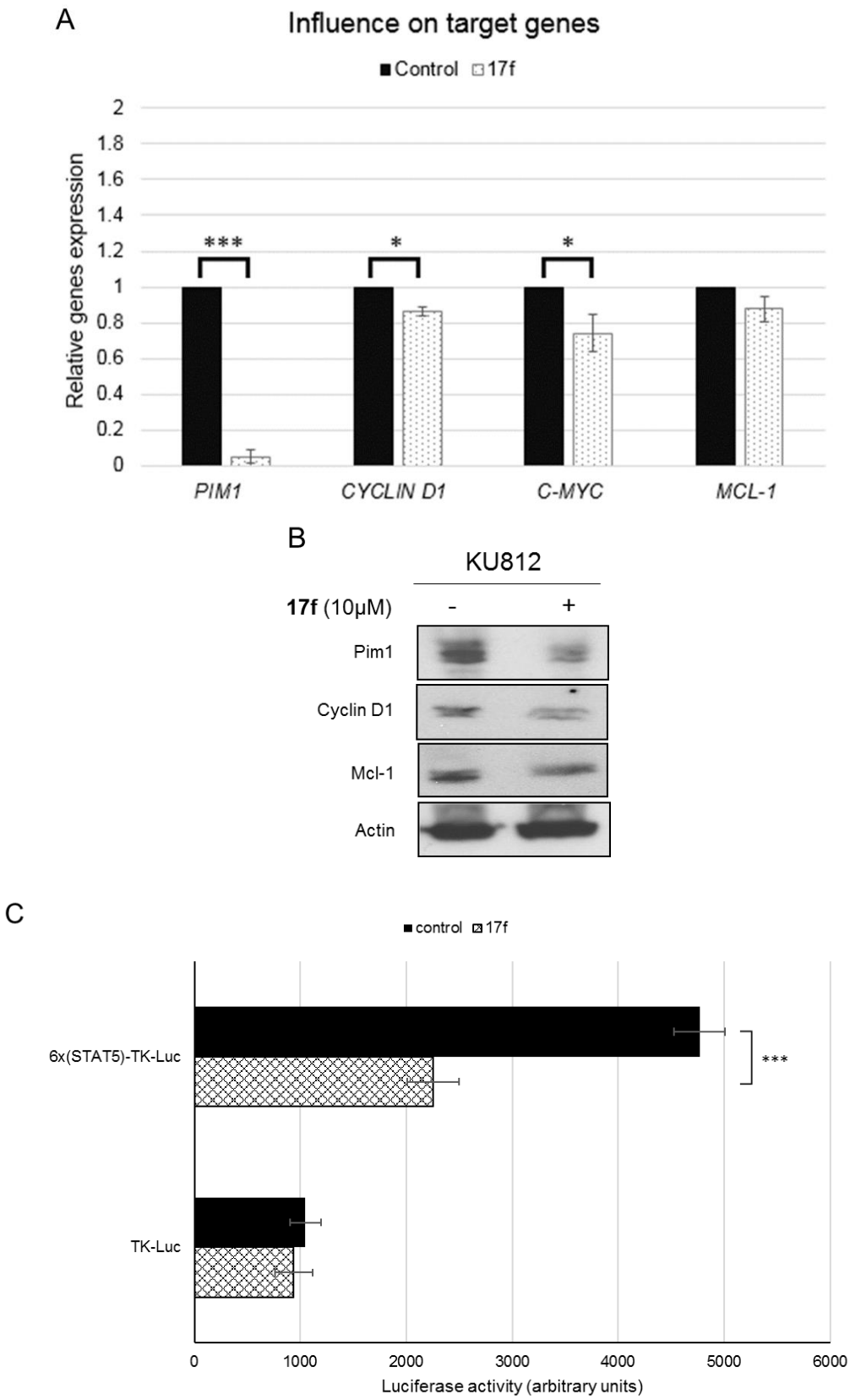

A
Influence on target genes
Control
17f
Relative genes expression
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
***
*
*
PIM1
CYCLIN D1
C-MYC
MCL-1
B
KU812
17f (10μM)
-
+
Pim1
Cyclin D1
Mcl-1
Actin
C
control
17f
6x(STAT5)-TK-Luc
TK-Luc
***
0
1000
2000
3000
4000
5000
6000
Luciferase activity (arbitrary units)

Figure 6. (A) qRT-PCR analysis of *PIM1, CYCLIN D1, C-MYC* and *MCL-1* expression in KU812 cells treated with **17f** (10 μM) or DMSO (control) for 15 h. Results are presented as the fold changes in *PIM1, CYCLIN D1, C-MYC* and *MCL-1* gene expression in **17f** cells normalized to internal control genes (*GAPDH* and *ACTB*) and relative to untreated cells (normalized to 1) (n = 3 in triplicates, data are mean ± SEM, *p < 0.05, ***p < 0.001). (B) Expression of Pim1, Cyclin D1 and Mcl-1 proteins in KU812 cells treated with 10 μM **17f** (+) or DMSO (-) for 24 hours. (C) KU812 cells transfected with a 6x(STAT5)-TK-luciferase reporter construct or a control TK-luciferase vector were treated with **17f** (10 μM) or DMSO (control) for 48 hours. Luciferase activities were next determined as described in material and methods. Luciferase activity (arbitrary units) in the histogram represents the relative luminescence units (rlu) values/mg of proteins (n = 3 in triplicates, data are mean ± SEM, ***p < 0.001).

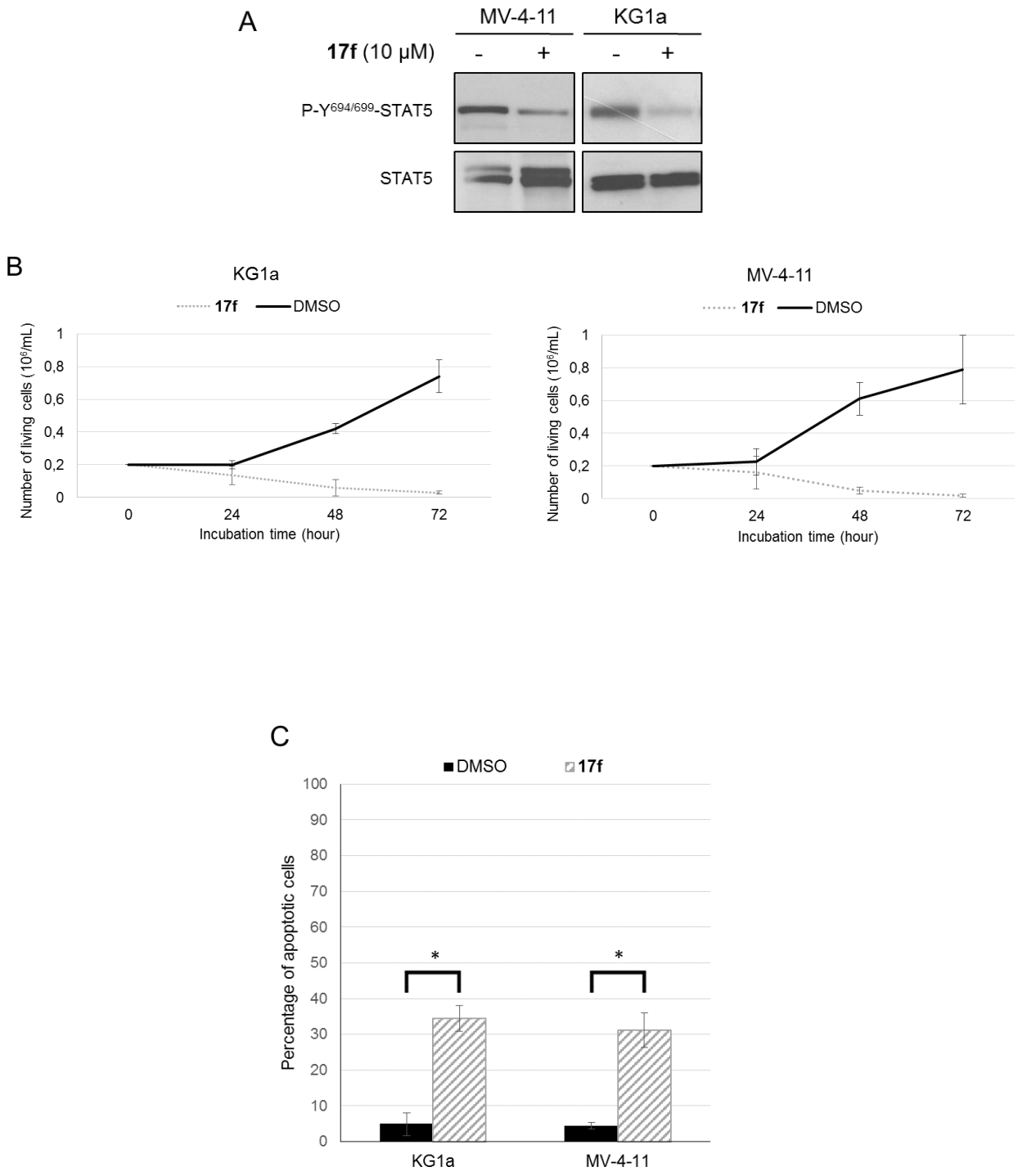


Figure 7. (A) Protein extracts from MV-4-11 and KG1a cells treated with **17f** (+) or DMSO (-) for 12 h were analyzed by Western blotting to detect P-Y$^{694/699}$-STAT5 and STAT5 protein expression. (B) Growth kinetics of KG1a (left) and MV-4-11 (right) cells cultured in absence

(DMSO) or presence of **17f** (10 μM) were determined by MTT assays (n = 3 in triplicates, data are mean ± SEM). (C) Representative flow cytometry histogram of KG1a and MV-4-11 cells cultured for 48 h with **17f** (10μM) or DMSO (control). Cells were stained with FITC-Annexin V and 7-AAD and the percentages of apoptotic cells were then evaluated by flow cytometry (n = 3 in triplicates, data are mean ± SEM, *$p < 0.05$).

Table 3. EC_{50} values of **17f** for KG1a and MV-4-11 cells[a]

	17f
Cell Lines	EC_{50} (μM)
KG1a	2,638 ± 0,51
MV-4-11	3,549 ± 0,47

[a]Cells were treated with concentrations ranging from 100 nM to 50 μM for 48 h. Cell viability was then determined by MTT assays and EC_{50} values were calculated using Origin® software (n = 3 in triplicates, data are mean ± SEM) (supp. info).

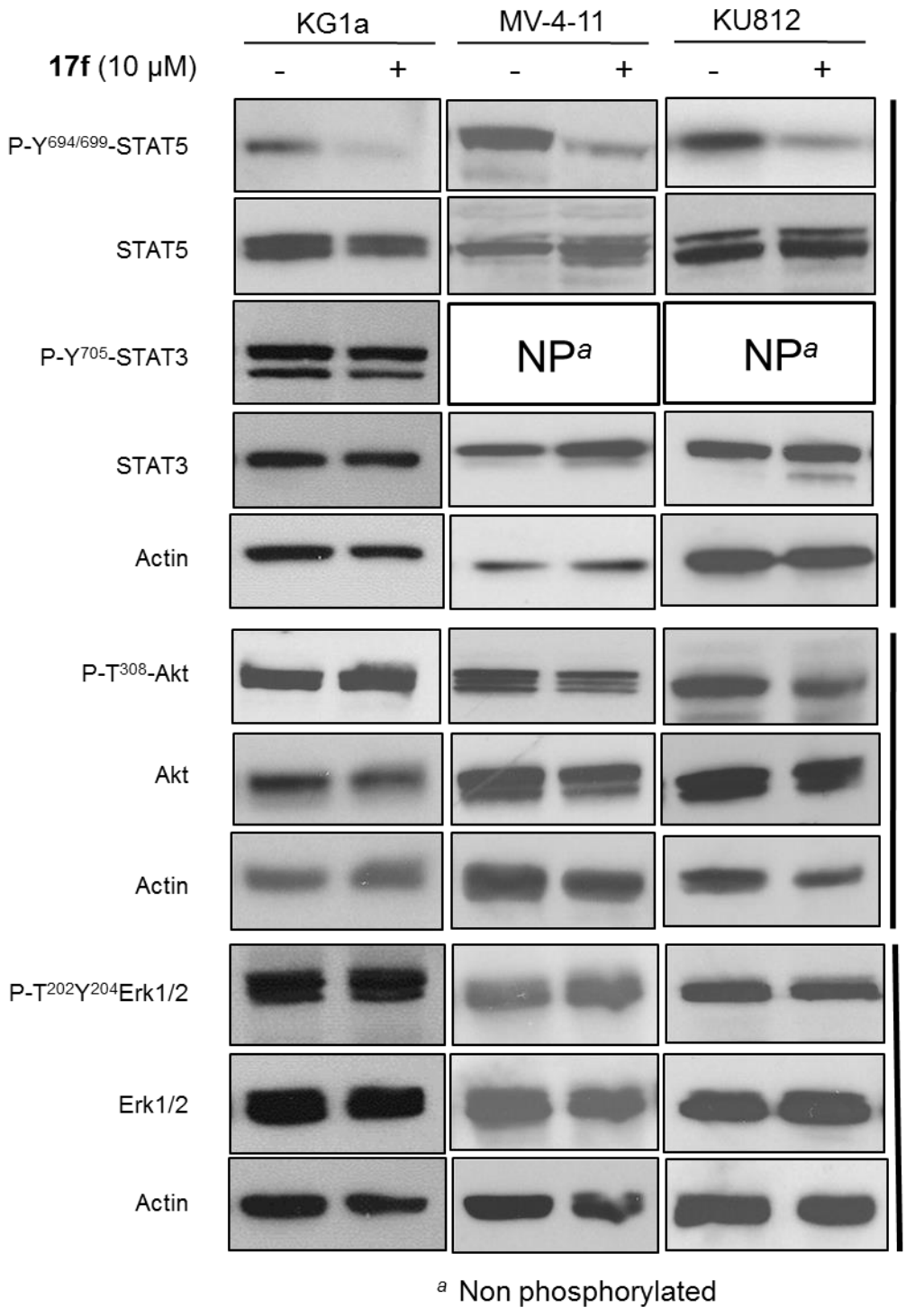


Figure 8. Protein extracts from KG1a, MV-4-11 and KU812 cells treated with **17f** (+) or DMSO (-) for 24 h were analyzed by Western blotting to detect the phosphorylated forms of STAT5, STAT3, Akt and Erk1/2 and the total forms of these proteins (n = 2). Actin expression

served as a loading control. Quantification of Western blot data is shown in supporting information.

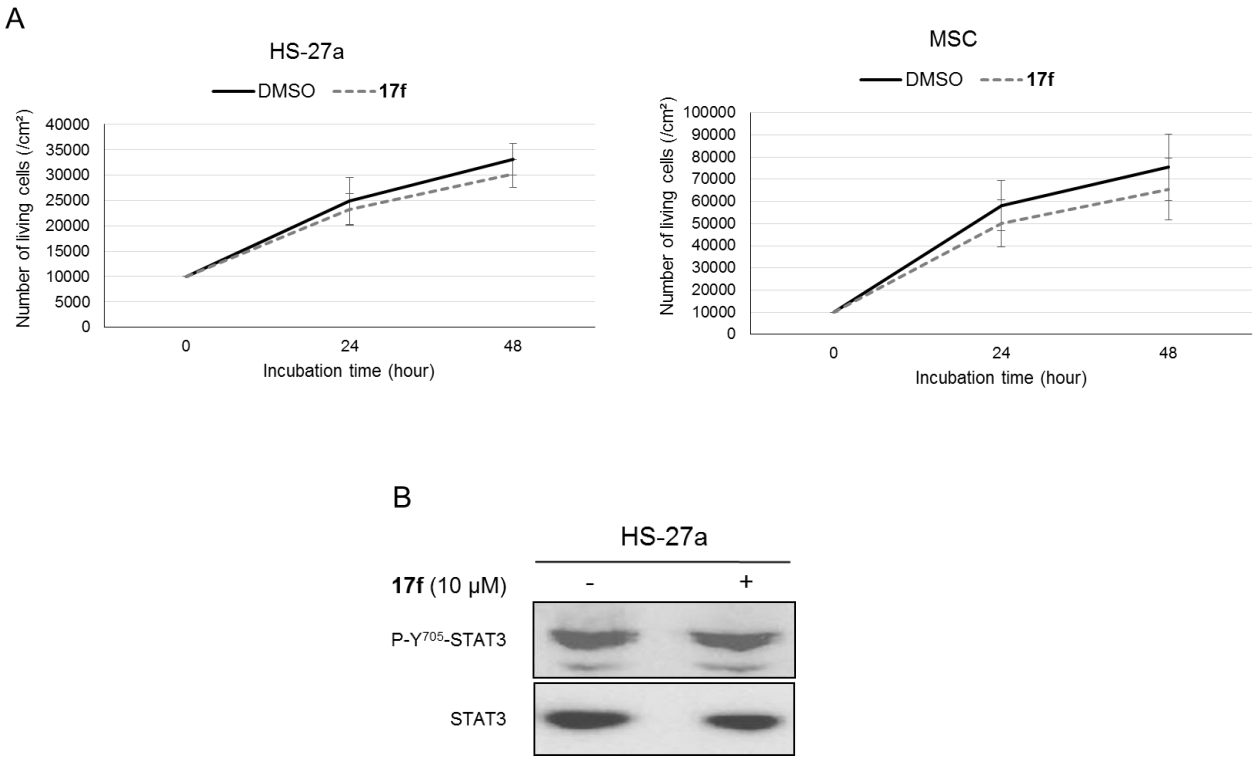


Figure 9. (A) Growth kinetics of HS-27a cells (left) and MSC (right) cultured in absence (DMSO) or presence of **17f** (10 μM) were determined by MTT assays (n = 3 in triplicates, data are mean ± SEM). (B) Extracts from HS-27a cells treated with **17f** (+) or DMSO (-) were analyzed by Western blotting to detect the phosphorylation and expression of STAT3.

Table 4. EC_{50} values of **17f** for HS-27a and MSC cells (3 donors)[a]

	17f
Cells	EC_{50} (μM)
HS27A	63.67 ± 0.05
MSC	33.65 ± 5.87

[a]Cells were treated with concentrations ranging from 1 μM to 100 μM for 48 h. Cell viability was then determined by MTT assays and EC_{50} values were calculated using Origin® software (n = 3 in triplicates, data are mean ± SEM) (supp. info).

TABLE OF CONTENTS GRAPHIC