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# Development of prohibitin ligands against osteoporosis

Redouane Tabti,<sup>1,2</sup> François Lamoureux,<sup>3</sup> Céline Charrier,<sup>3</sup> Benjamin Ory,<sup>3</sup> Dominique Heymann,<sup>4,5,6</sup> Embarek Bentouhami,<sup>2</sup> and Laurent Désaubry<sup>1,7</sup>

<sup>1</sup> Laboratory of Regenerative Nanomedicine (RNM), INSERM U 1260, CRBS, Rue Eugène Boeckel, 67000, Strasbourg, France.

<sup>2</sup> LCIMN Laboratory, Faculty of Technology, University Ferhat Abbas, Sétif, Algeria

<sup>3</sup> INSERM, UMR 1238, Nantes, France; Université de Nantes, Nantes Atlantique Universités, Laboratoire « Sarcome osseux et remodelage des tissus osseux calcifiés », Faculté de Médecine, Nantes, France.

<sup>4</sup> Institut de Cancérologie de l'Ouest, site René Gauducheau, Boulevard Professeur Jacques Monod, Saint-Herblain, France.

<sup>5</sup> Université de Nantes, Faculty of Medicine, Nantes, France.

<sup>6</sup> University of Sheffield, Dept of Oncology and Metabolism, Sheffield, UK.

<sup>7</sup> Sino-French Joint Lab of Food Nutrition/Safety and Medicinal Chemistry, College of Biotechnology, Tianjin University of Science and Technology, Tianjin, China

**Correspondence to:** Laurent Désaubry, **email:** Désaubry@unistra.fr

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**ABSTRACT:** Current therapeutic approaches to osteoporosis display some potential adverse effects and a limited efficacy on non-vertebral fracture reduction. Some sulfonylamidines targeting the scaffold proteins prohibitins-1 and 2 (PHB1/2) have been showed to inhibit the formation of osteoclasts in charge of bone resorption. Herein, we report the development of a second generation of anti-osteoclastic PHB ligands. The most potent compound, **IN45**, showed 88 % inhibition at the low concentration of 5  $\mu$ M, indicates that it might serve as a basis for the development of new antiosteoporotic drugs.

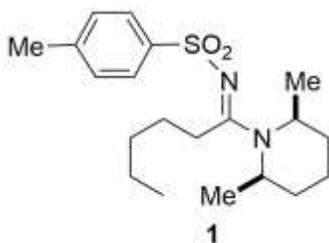
## 1. Introduction

Aging accelerates bone loss and skeletal fragility, leading to morbidity and mortality in both men and women [1]. A continual dynamic self-regeneration process in bone called remodeling is a coordinated cycle of resorption of old bone by osteoclasts followed by the deposition of new bone by osteoblasts in response to mechanical loadings and micro damages. With aging this balance shifts in a negative direction, favoring greater bone resorption and less bone formation, affecting the musculoskeletal system and ultimately resulting in osteoporosis, thus increasing fracture risk. In Europe, fractures caused by osteoporosis (usually hip or spinal) affect 1 in 2 women and 1 in 5 men aged more than 50 years old [2]. While numerous anti-catabolic (e.g. bisphosphonates, anti-RANKL) and pro-anabolic (e.g. PTH fragments) drugs have been developed in the recent past, most of them reduce bone resorption and increase bone mineral density by artificially suppressing the physiological bone remodeling process, leading to a new formed bone prone to breakage [3]. In addition current treatments of osteoporosis display some potential adverse effects and a limited efficacy on non-vertebral fracture reduction. Due to these limitations and the ageing of the population, the discovery of new anti-osteoporotic agents is a matter of public health.

Osteoclasts are generated by the fusion of mononuclear progenitors of bone marrow monocyte / macrophage lineage that resorb bone in the presence of the receptor activator of NF- $\kappa$ B ligand (RANKL) [4]. Indeed, binding of RANKL to its cell-surface receptor, RANK, activates several signaling pathways leading notably to the activation of the mitogen-activated protein (MAP) kinases p38 and ERK, the nuclear factor  $\kappa$ B (NF- $\kappa$ B), and the nuclear factor of activated T cells c1 (NFATc1), which are critical to regulate osteoclastogenesis and maintain the survival of mature osteoclasts. NFATc1 regulates in particular the expression of the tartrate-resistant acid phosphatase (TRAP) and cathepsin K, which are involved in the cell fusion of osteoclast progenitors and in the bone resorptive activity of osteoclasts.

In addition to RANKL, several humoral factors, such as interleukin-6 (IL-6), can also promote osteoclastogenesis through a regulation of the transcription factor STAT3 [5]. Indeed, a basal level of STAT3 phosphorylated at serine-727 associated with an absence of phosphorylation on tyrosine-705 is required for osteoclast differentiation and STAT3 inhibitors abolish RANKL-induced osteoclastogenesis. Importantly, STAT3 has been shown to be regulated by PHBs in several cell types [6-8].

To identify a novel class of antiosteoporotic agents, Chang *et al.* screened a library of sulfonylamidines for their *in vitro* ability to block pre-osteoclast differentiation into osteoclasts, which mediate the removal of bone cells [9]. The sulfonylamidine **1** (Figure 1) was found to be a particularly potent inhibitor of osteoclast differentiation with an IC<sub>50</sub> of 3.3 μM. Using affinity chromatography, prohibitin-1 (PHB1) was identified as the molecular target of these compounds [10]. PHB1 and its homolog PHB2 are ubiquitous scaffold proteins that coordinate many signaling pathways to regulate all aspects of cell functions, including metabolism, apoptosis, cell migration, division, and differentiation [11]. As such, PHBs are involved in the etiology of cancers, in inflammatory, cardiovascular, and neurodegenerative diseases, diabetes, obesity, and osteoporosis. The various functions and intracellular localization of PHBs are regulated by a wide array of post-translational modifications.



**Figure 1.** Structure of anti-osteoclastic sulfonylamidine **1** [10].

Since the identification of PHB1 as the molecular target of anti-osteoporotic sulfonylamidines, PHB1 has been demonstrated to inhibit the formation of mature osteoclasts [12], but the engagement of PHB1 in this activity of **1** has not been established yet, even though **1** has shown to block the PHB-dependent entry of Chikungunya virus into microglial cells [13]. Yet, some key events in the mechanism of action of these compounds have been clarified [14]. Osteoclast differentiation is initiated by the binding of RANK ligand (RANKL) to its receptor RANK, which activates TRAF6 and consecutively, IKK and ERK, leading to the activation of the transcription factors NF-κB, c-Fos and NFATc1. Sulfonylamidines were shown to dose-dependently inhibit the RANKL-induced activation of ERK, and both the translocation to the nucleus and the transcriptional activity of NF-κB. In addition, they repressed mRNA expression levels of c-Fos and NFATc1 induced by RANKL, indicating that sulfonylamidines mitigate RANKL-induced signaling and transcription factors required for osteoclast differentiation. Sulfonylamidines also repressed the expression of the matrix metalloproteinase 9 (MMP-9) and the kinase c-Src that both promote osteoclastic bone resorption.

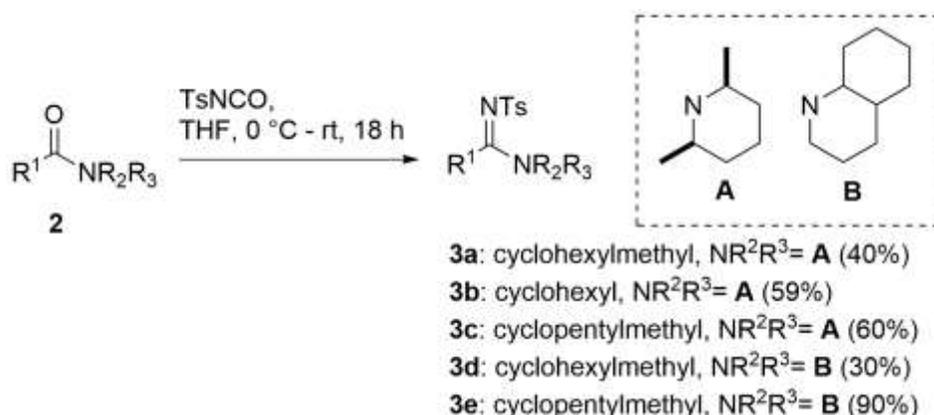
Hitherto, no further study on sulfonylamidines as PHB ligands has been reported, possibly

because the moderate activity of these compounds. Thus we aimed to develop more active analogues to explore the therapeutic potential of these PHB ligands in models of osteoporosis.

## 2. Results

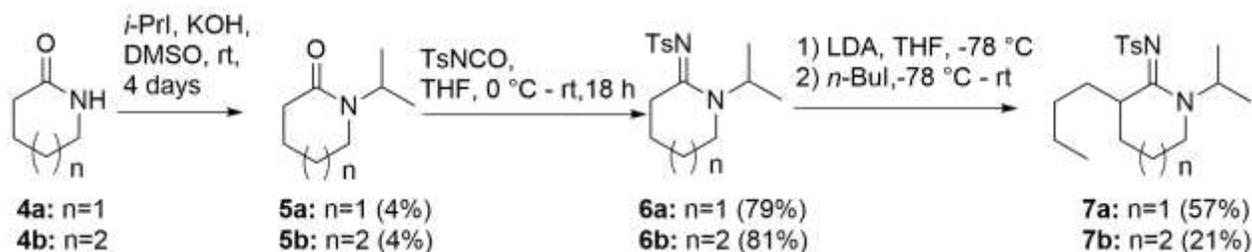
### 2.1. Chemical synthesis

A first approach to prepare sulfonylamidines relies on the condensation of amides **2** with tosylisocyanate following King's method (Scheme 1) [15].



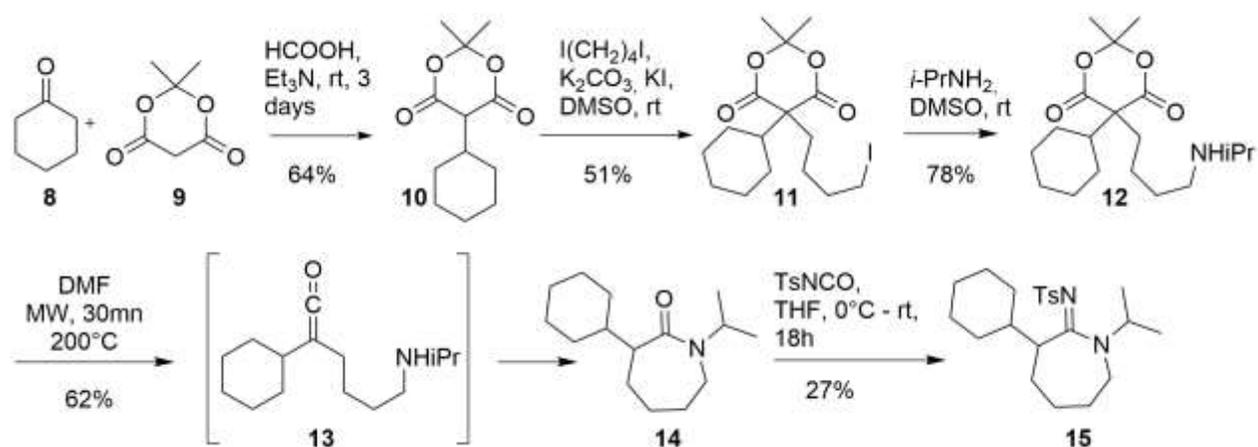
**Scheme 1.** Synthesis of sulfonylamidines **3a-e** by condensation of amides with tosylisocyanate.

To restrain the conformational flexibility of these PHB ligands, we synthesized the cyclic sulfonylamidines from valerolactam (**4a**) and caprolactam (**4b**) (Scheme 2). *N*-alkylation of these compounds with isopropyl iodide occurred in low yield. Condensation with tosylisocyanate conveniently furnished **6a/b** [15]. Deprotonation with LDA and reaction with *n*-iodobutane following Magnus' procedure [16] afforded the monoadducts **7a/b**. Unfortunately, this reaction did not proceed with cyclohexyl bromide or iodide. To overcome this limitation we explored several routes and finally developed an original synthesis of substituted caprolactams (Scheme 3).



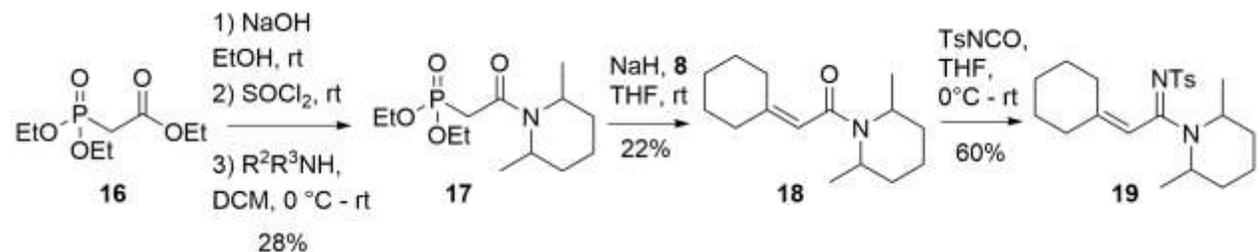
**Scheme 2.** Synthesis of sulfonylamidines **6a/b** and **7a/b**.

Our synthesis commenced with the reductive condensation of aldehyde **8** with Meldrum acid **9** in the presence of formic acid and trimethylamine to furnish the monoadduct **10** (Scheme 3). A second alkylation with 1,4-diodobutane delivered the adduct **11** in 51% yield. A condensation of the latter with isopropylamine gave the secondary amine **12** in 78% yield. With this advanced intermediate in hand, we proceeded to its cyclization via the formation of ketene intermediate **13**. Original attempts to perform this reaction at reflux in DMF afforded the expected caprolactam **14** in only 10% yield. Using microwave conditions improved the yield up to 62%. As far as we know, it is the first time that a caprolactam is synthesized by this type of strategy. Completion of the synthesis was accomplished by a condensation with tosylisocyanate in a 27% yield.



**Scheme 3.** Synthesis of sulfonylamidine **15**.

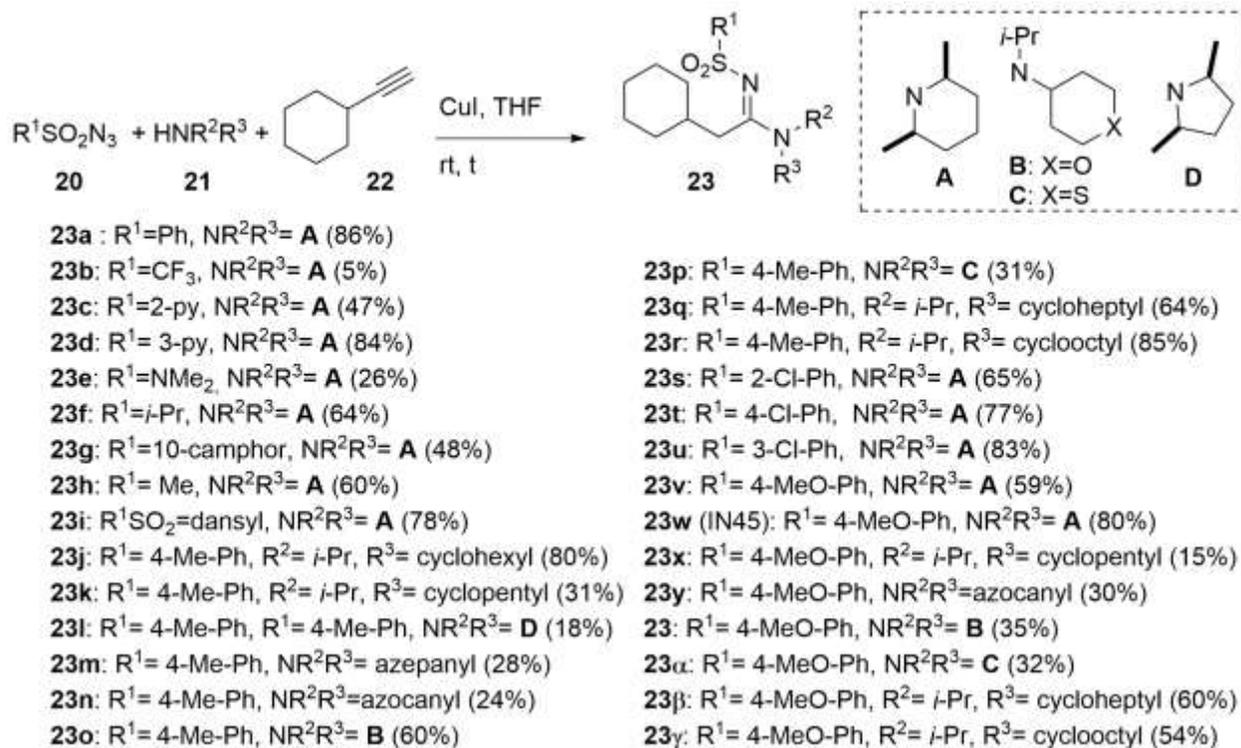
To restrict the conformational freedom of **3a**, we synthesized the alkene **19** using a Wadsworth–Emmons reaction and a condensation of the obtained amide with tosylisocyanate (Scheme 4).



**Scheme 4.** Synthesis of alkene **19**.

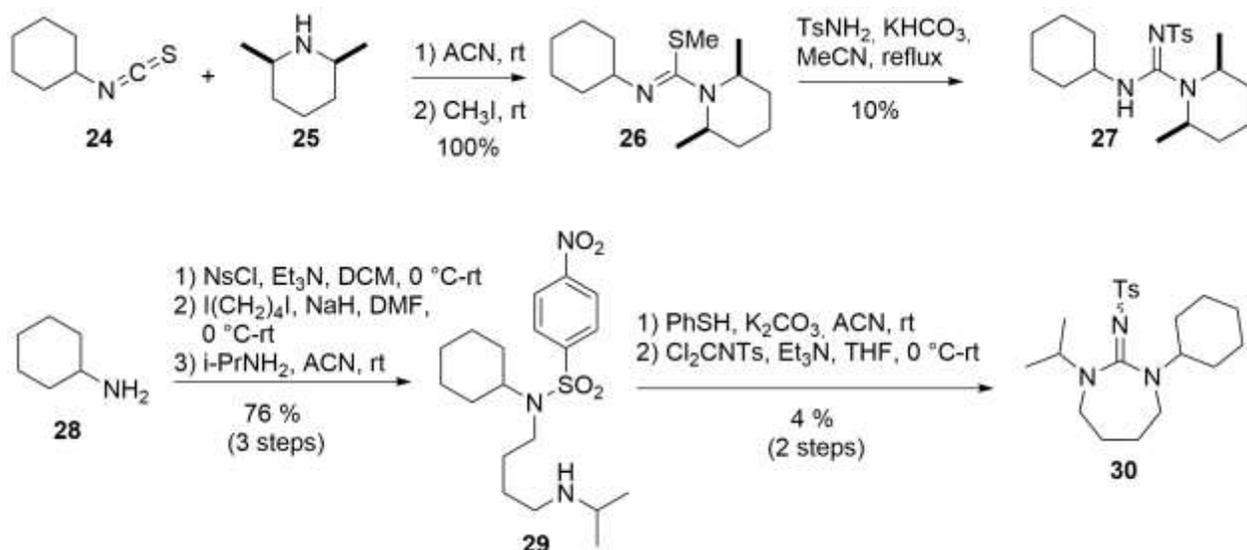
Sulfonylamidines **23a-y** were synthesized in a straightforward manner using a Cu-

catalyzed three-component coupling of sulfonyl azides **20**, alkynes **21**, and amines **22** (Scheme 5) [17].



**Scheme 5.** Synthesis of sulfonylamidines **23a-y**.

We examined the isosteric replacement of the tosylamide moiety synthesizing the tosylguanidines **27** and **30** (Scheme 6). Aza-isostere **27** was prepared in 3 steps starting from the addition of amine **25** to cyclohexylisothiocyanate **24**, followed by *S*-methylation and condensation with tosylamide [18]. The synthesis of the cyclic tosylguanidine **30** started by the protection of cyclohexylamine **28** as a nosylamide, which was alkylated by 1,4-diiodobutane, condensed with isopropylamine and deprotected. The obtained diamine **29** reacted with *N*-tosyldichloromethanimine [19] to afford the cyclic tosylguanidine **30**.



**Scheme 6.** Synthesis of the tosylguanidines **27** and **30**.

## 2.2. Inhibition of osteoclastogenesis

We functionally examined whether our new sulfonylamidines inhibit osteoclastogenesis. Osteoclast differentiation was induced from human CD14<sup>+</sup> precursors cultured in the presence of M-CSF and RANKL and the formation of multinucleated cells was followed by TRAP staining (Table 1) [20]. We confirmed that **1** indeed blocked osteoclast differentiation (54 % inhibition at 10  $\mu$ M). Importantly, replacement of the *n*-pentyl by a cyclohexylmethyl **3a** greatly inhibited osteoclast differentiation.

The replacement by a cyclohexyl (**3b**) was unfavorable. Restraining the conformational flexibility through the inclusion of the alkyl chain in a 6-membered ring was slightly detrimental (**7a**), but switching to a 7 membered ring (**7b**) restored a good inhibition of osteoclast differentiation. Deleting the *n*-butyl substituents on these two latter compounds (**6a** and **6b**) abolished the activity. The replacement of the cyclohexylmethyl of **3a** by a cyclopentylmethyl (**3c**) did not modify the activity. Exchanging the 2,6-dimethylpiperidine moiety by a decahydroquinoline (**3d**) was somewhat unfavorable. Introduction of an unsaturation between the cyclohexyl and the sulfoamidine moiety led to an inactive compound (**19**).

**Table 1.** Inhibition of osteoclastogenesis by the 1<sup>st</sup> series of sulfonylamidines.

Cpd	Structure	Inhibition %		Cpd	Structure	Inhibition %	
		1 $\mu$ M	10 $\mu$ M			1 $\mu$ M	10 $\mu$ M
<b>1</b>		20.9 $\pm$ 12.1	99.8 $\pm$ 1.0	<b>6a</b>		27.9 $\pm$ 4.06	6.1 $\pm$ 4.1
<b>3a</b>		35.0 $\pm$ 13.3	97.9 $\pm$ 2.4	<b>6b</b>		27.1 $\pm$ 9.5	39.3 $\pm$ 6.1
<b>3b</b>		22.8 $\pm$ 4.3	24.1 $\pm$ 9.8	<b>3c</b>		32.7 $\pm$ 11.4	91.5 $\pm$ 6.1
<b>7a</b>		31.9 $\pm$ 4.1	80.6 $\pm$ 8.1	<b>3d</b>		33.1 $\pm$ 0.5	78.9 $\pm$ 7.8
<b>7b</b>		12.1 $\pm$ 5.4	90.1 $\pm$ 2.7	<b>19</b>		25.5 $\pm$ 19.2	23.3 $\pm$ 10.8

Bone marrow-derived CD14<sup>+</sup> preosteoclasts were treated by sulfonylamidines with 100 ng/mL RANKL and 25 ng/mL M-CSF for 10 days. TRAP staining was then performed. Nuclei greater than 3 were counted as osteoclasts. The percentage of inhibition is an average corresponding to the number of osteoclasts in each treated condition compared to corresponding positive control, which represents the 100 % value.

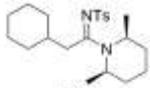
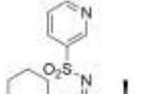
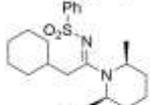
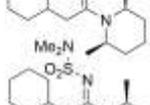
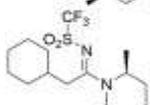
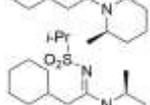
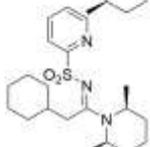
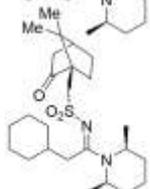
The following compounds have been tested with different lots of human monocytes which may have various sensitivity to the RANK ligand (RANKL). To compare all of these results, the compound **3a** was used as the reference compound.

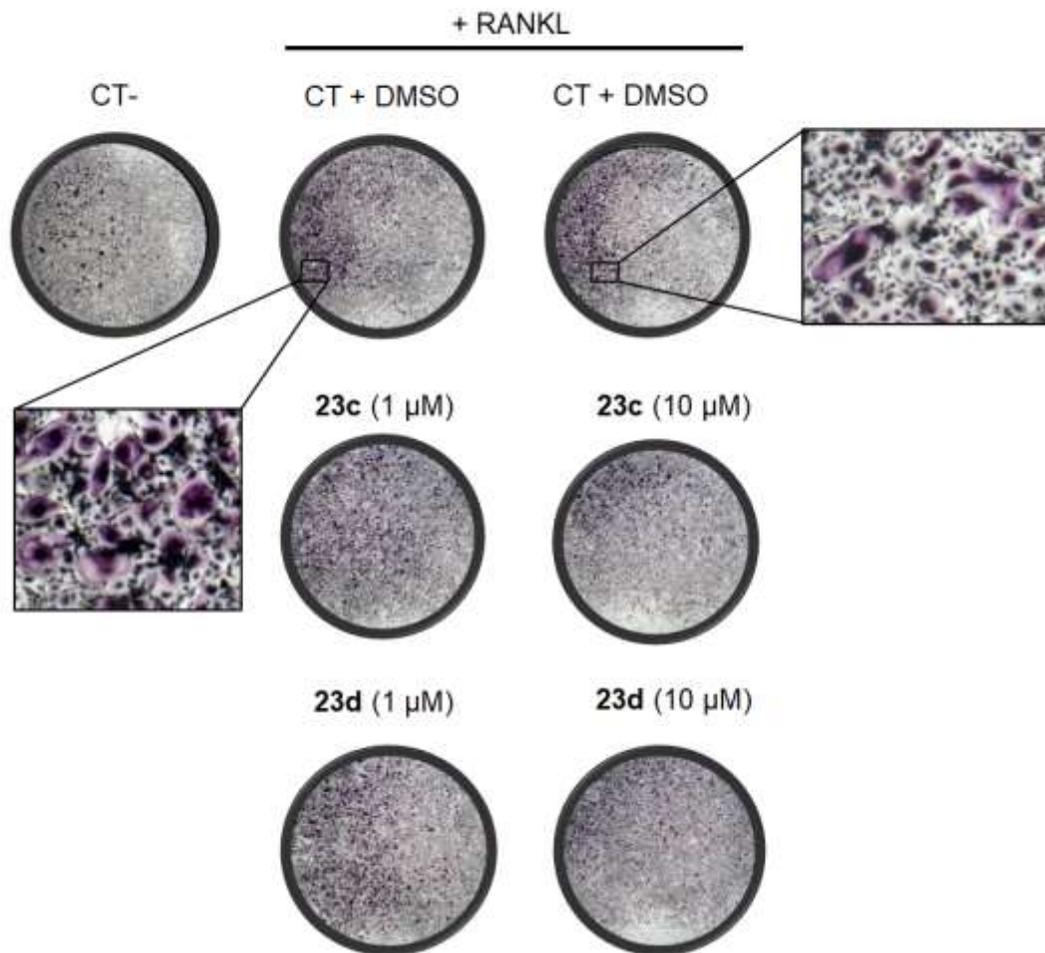
In accordance with **7b** activity, the rigidification of **3a** through a 7-membered ring (**15**) was beneficial (Supplementary table S1). The isosteric replacement of the tosylamidino moiety by a tosylguanidine (**27** and **30**) suppressed the activity. In contrast to what we observed with **3d**, the introduction of a decahydroquinoline (**3e**) was highly detrimental, indicating that the substituent effects of the alkyl and tosylamidino moieties are highly interdependent

Next, we pursued our SAR investigation by changing the nature of the arylsulfonyl group (Table 2 and supplementary table S2). Replacement of the 4-tolyl by a phenyl (**23a**), a trifluoromethyl (**23b**) or a 5-(dimethylamino)naphthalenyl (**23i**) was well tolerated, but replacement by a 2-pyridyl, 3-pyridyl, dimethylamino or camphorsulfonyl was unfavorable at the dose of 10  $\mu$ M (**23c**, **23d**, **23e**, **23g**, Figure 2). This deleterious effect was more pronounced with an isopropyl or a methyl or (**23f** and **23h**). **23c** and **23d** displayed a biphasic effect: at 1  $\mu$ M they promoted osteoclastogenesis, but at 10  $\mu$ M they

displayed the opposite effect. This increase in the number of osteoclasts can be explained by a transient release of pro-osteoclastogenic agents in the culture medium.

**Table 2.** Inhibition of osteoclastogenesis by the 3<sup>rd</sup> series of sulfonylamidines.

Cpd	Structure	Inhibition %		Cpd	Structure	Inhibition %	
		1 $\mu$ M	10 $\mu$ M			1 $\mu$ M	10 $\mu$ M
<b>3a</b>		14.4 $\pm$ 13.9	97.6 $\pm$ 2.1	<b>23d</b>		-23.6 $\pm$ 33.9	56.1 $\pm$ 39.7
<b>23a</b>		3.3 $\pm$ 26.41	97.9 $\pm$ 0.5	<b>23e</b>		11.8 $\pm$ 16.5	68.4 $\pm$ 5.5
<b>23b</b>		-1.1 $\pm$ 22.9	96.1 $\pm$ 3.9	<b>23f</b>		1.6 $\pm$ 8.96	12.6 $\pm$ 28.1
<b>23c</b>		-20.2 $\pm$ 35.6	44.9 $\pm$ 49.1	<b>23g</b>		-1.5 $\pm$ 10.4	57.5 $\pm$ 29.8



**Figure 2.** Representative pictures of CD14<sup>+</sup> monocytes treated (positive control) or not with RANKL (negative control), and +/- treated with **23c** or **23d** at indicated doses. Cells were then stained with TRAP staining kit to reveal multinucleated cells (osteoclasts).

The replacement of the tosyl by a mesyl (**23h**) or dansyl (**23i**) were respectively very and slightly detrimental (Supplementary table S2). Gratefully, the replacement of 2,6-dimethylpiperidine by an *N*-isopropylcyclohexylamine (**23j**) maintained the anti-osteoclastogenic efficacy. At the doses of 1  $\mu$ M and 10  $\mu$ M, **23j** suppressed osteoclastogenesis by 10% and 99% respectively.

In the next set of experiments, **3a** was found to promote osteoclastogenesis at 1  $\mu$ M, confirming that the observed effect is highly dependent of the monocyte donors. Gratefully, the introduction of a *N*-isopropylcyclopentylamine (**23k**) significantly enhanced the anti-osteoclastogenic efficacy (Table S3). Curiously the transition to a 2,6-dimethylpyrrolidine **23l** rendered the compound

pro-osteoclastogenic at 1  $\mu\text{M}$  and cytotoxic at 10  $\mu\text{M}$ . Switching to an azepane **23m** had a deleterious effect, but an azocane **23n** was well tolerated. The tetrahydropyran derivative **23o** was less active than its larger, less polar, sulfur isostere **23p**. Cycloheptanic derivatives **23q** had an activity at least equal to that of **3a**.

The cyclooctanic derivative **23r** was less active than **3a** at 1  $\mu\text{M}$ , but it was more active at 10  $\mu\text{M}$  (Supplementary table S4). Interestingly, this compound was found to display some cytotoxicity. The introduction of chlorine on the arylsulfonyl group improved the activity, especially in position ortho (**23s**). The introduction of an isopropyl in position para (**23w**) also increased the anti-osteoclastogenic activity.

Next, we replaced the 2,6-dimethylpiperidino moiety in **23v** by various hydrophobic amines, and we observed that the *N*-isopropylcyclooctanamine (**23y**) increased slightly the anti-osteoclastogenic activity (Table 3). Interestingly this compound, called **IN45**, was found to be cytotoxic at 10  $\mu\text{M}$ .

**Table 3.** Inhibition of osteoclastogenesis by the 7<sup>th</sup> series of sulfonylamidines.

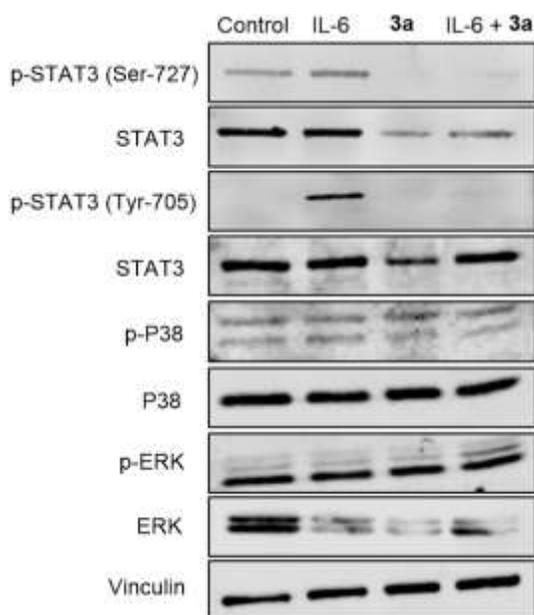
		Inhibition %					Inhibition %		
		1 $\mu\text{M}$	5 $\mu\text{M}$	10 $\mu\text{M}$			1 $\mu\text{M}$	5 $\mu\text{M}$	10 $\mu\text{M}$
<b>3a</b>		-9 $\pm$ 31	72 $\pm$ 13	99 $\pm$ 2	<b>23<math>\alpha</math></b>		-10 $\pm$ 25	53 $\pm$ 10	93 $\pm$ 4
<b>23v</b>		0 $\pm$ 32	62 $\pm$ 9	98 $\pm$ 2	<b>23<math>\beta</math></b>		-6 $\pm$ 18	74 $\pm$ 4	99 $\pm$ 2
<b>23x</b>		-86 $\pm$ 26	26 $\pm$ 12	99 $\pm$ 1	<b>23y</b>		5 $\pm$ 16	88 $\pm$ 8	100 $\pm$ 0
<b>23z</b>		-30 $\pm$ 20	49 $\pm$ 4	88 $\pm$ 7					
		-14 $\pm$ 5	28 $\pm$ 20	39 $\pm$ 21					

### 2.3. Effects of sulfonylamidine **3a** on cell signaling

Having probes with inhibition of osteoclastogenesis in hand; we next examined the molecular

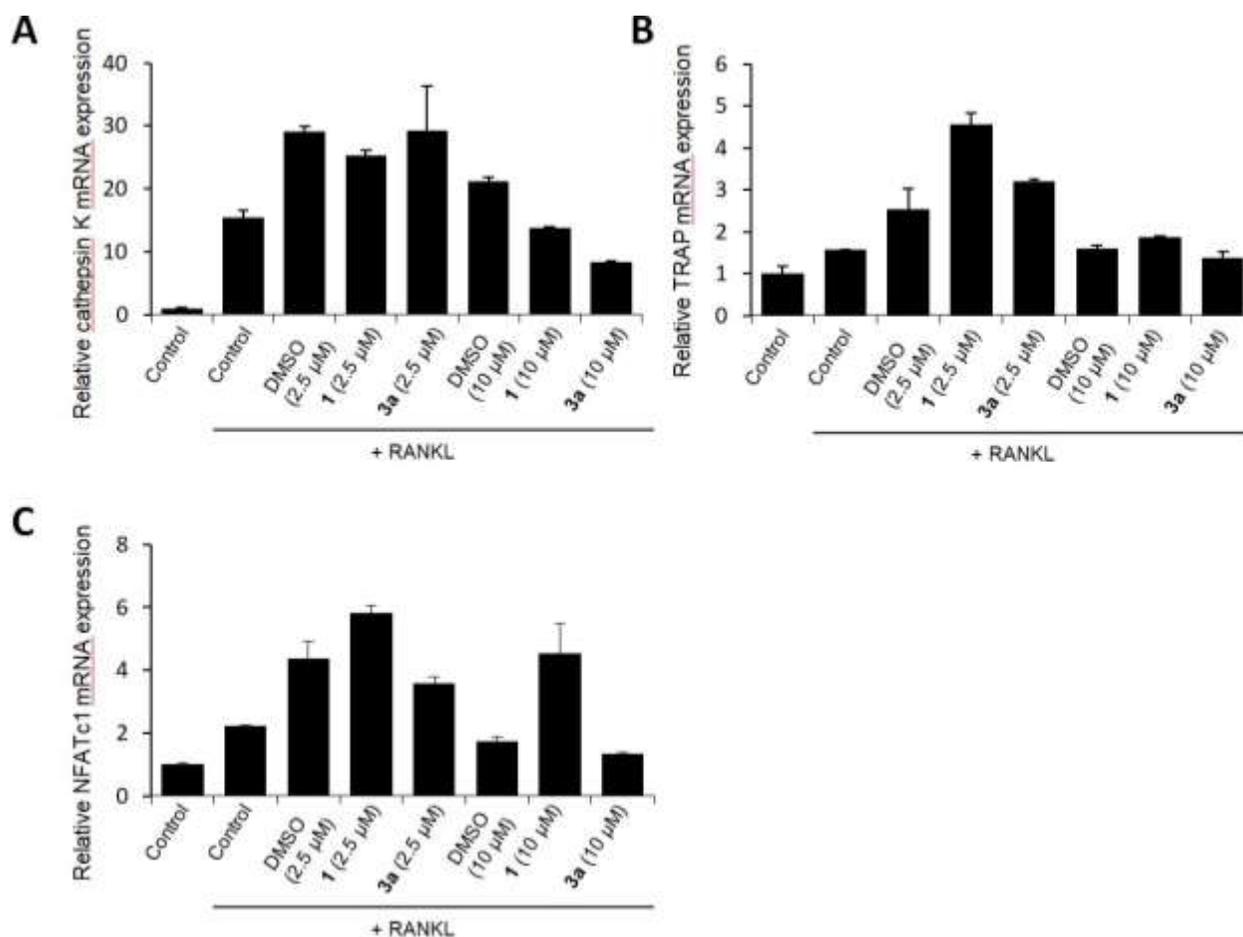
mechanisms of **3a** on key signaling pathways that regulate the differentiation of CD14+ monocytes in osteoclasts.

p38 and ERK are two MAP kinases involved in osteoclastic differentiation that have been shown to be activated by other PHB ligands in several cell types [21-26]. We observed that **3a** did not modulate the expression or phosphorylation of these 2 kinases at protein level after IL-6 stimulation. However, **3a** inhibited IL6-induced STAT3 phosphorylation at serine-727 (Figure 3), which is involved in osteoclast differentiation by regulating the transcription of NFATc1 [5, 27].



**Figure 3.** Effects of compound **3a** on the activation of STAT3, P38 and ERK in CD14+ monocytes. Human CD14+ monocytes were treated with 25 ng/ml for 3 days. At day 3, human CD14+ monocytes were treated with vehicle or 10  $\mu$ M **3a** for 4h without FBS and M-CSF, followed by IL-6 stimulation (25 ng/mL) for 15 min. Western blot analysis was performed on cell lysate to determine the phosphorylation level of STAT3 (at Ser-727 and Tyr-705), P38 and ERK.

Sulfonylamidines **1** and **3a** were found to decrease the mRNA expression of the matrix-degrading enzyme cathepsin K, the tartrate-resistant acid phosphatase (TRAP) and NFATc1, indicating that these compounds inhibit osteoclastogenesis through inhibition of RANKL-induced NFATc1 expression (Figure 4). This inhibition was more pronounced with **3a** than with reference compound **1**.



**Figure 4.** Effects of compounds **1** and **3a** on mRNA expression of cathepsin K, TRAP and NFATc1. At day 3, Human CD14<sup>+</sup> monocytes were treated with RANKL with or without **1** or **3a** (1 or 10 μM) for 4 days. Total RNA was isolated and analyzed by RT-qPCR. Each assay was performed in triplicate. **A.** Cathepsin K mRNA expression. **B.** TRAP mRNA expression. **C.** NFATc1 mRNA expression.

#### 4. Conclusion

With the aging of the population, the need to improve the treatment of osteoporosis will become more and more preminent. Therefore, new drugs targeting novel therapeutic agents are in great need. Although PHBs are involved in the regulation of osteoclastogenesis, these signaling proteins are not yet established therapeutic targets against osteoporosis. Since the discovery of anticlastogenic sulfonamidines targeting PHBs in 2010, no medicinal chemistry project aimed at improving these compounds has been reported.

Here, a series of 50 new analogues was synthesized, and evaluated for their *in vitro* osteoclastogenesis inhibitory activities. The most potent compound, **IN45**, showed 88 % inhibition at a concentration as low as 5  $\mu$ M. We have also contributed to better define the mechanism of action of these compounds. Kim *et al.* had shown that these compounds bind to PHB1 [10] and block ERK activation and NFATc1 expression [14]. Herein, we confirmed that these compounds indeed inhibit the expression of NFATc1 and found that they also inhibit the phosphorylation of the transcription factor STAT3 that is also necessary to osteoclast differentiation [27]. Considering that PHB1 overexpression inhibits NFATc1 and blocks osteoclast differentiation [12], and also that PHB1 regulate STAT3 signaling in several cell types, these observations support an action of sulfonylamidines on PHB1 to inhibit osteoclastogenesis, through a cascade of events that we are currently exploring. In addition, since sulfonylamidine **1** has been shown to block the entry of Chikungunya virus into microglial cells [13], sulfonylamidines might also block also the PHB-dependent entry of other viruses, such as chronic hepatitis C virus, dengue virus or enterovirus 71 (responsible of hand, foot and mouth disease [28]). Considering that PHBs are expressed in every tissue, these compounds might also be of interest to treat other types of diseases where modulation of PHB signaling is beneficial [26].

Overall, our current study sets up a basis for the development of new antiosteoporotic drugs with an original mechanism of action and also consolidate PHB1 as a novel therapeutic target against osteoporosis.

## **5. Material and methods**

### **5.1. Synthetic Procedures**

Detailed synthetic methods can be found in SI Materials and Methods.

### **5.2. Selection of CD14+ cells**

Blood sample from different human donors are first diluted with phosphate buffer to obtain final 100ml and diluted samples are layered onto Ficoll solution in a centrifuge tube. Human peripheral blood mononuclear cells (PBMCs) were then isolated by centrifugation over Ficoll gradient for 25 minutes at 500g. Next, enrichment and purification of osteoclast precursors (CD14+) allow the differentiation of high number of osteoclasts. CD14<sup>+</sup> cells are magnetically labeled with CD14 Microbeads and positively selected by MACS technology.

### 5.3. Osteoclast differentiation

To induce osteoclast formation, CD14<sup>+</sup> cells are seeded at 45 x 10<sup>4</sup> cells/well in 96-well plates in  $\alpha$ -MEM (Invitrogen Life Technologies) supplemented with 10%FCS and 25 ng/ml hM-CSF (hM-CSF; R&D Systems). From day 3 of the culture, medium is changed twice a week with medium containing 10%FCS, 25 ng/ml hM-CSF and 100 ng/ml hRANKL +/- chemical compounds. The formation of osteoclasts occurs after around 11 days and is confirmed by Tartrate-Resistant Acid Phosphatase (TRAP) staining (Sigma, France).

### 5.4. Quantitative reverse transcription PCR

CD14<sup>+</sup> cells were seeded in 96 wells plates. Three days after seeding, CD14<sup>+</sup> cells were treated with 25 ng/ml hM-CSF and 100ng/ml hRANKL +/-chemical compounds at the indicated doses for 4 days. Total RNA was extracted using the Macherey-Nagel Nucleospin RNA. Following a reverse transcription on 1  $\mu$ g of total RNA, cDNA was diluted to 10 ng/ $\mu$ L. A Real-time monitoring of PCR amplification of cDNA was performed using DNA primers on CFX96 Detector System (Bio-Rad) with SYBR PCR Master Mix (Bio-Rad). Target gene expression was normalized to GAPDH level in respective samples as an internal standard, and the comparative cycle threshold (Ct) method was used to calculate relative quantification of target mRNAs. Each assay was performed in triplicate.

### 5.5. Western blot

Samples containing equal amounts of protein from lysates of cultured osteosarcoma cells underwent electrophoresis on SDS-polyacrylamide gel and were transferred to PVDF filters blocked in odyssey blocking buffer (LI-COR). Blots were probed overnight at 4°C with primary antibodies. After incubation, the filters were washed in PBS containing 0.05% Tween. Filters were then probed with a fluorescent antibody (Li-COR IRDye) diluted in the odyssey blocking buffer. Antibody binding was visualized using the LI-COR odyssey Fc.

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### References

- [1] J.E. Compston, M.R. McClung, W.D. Leslie. Osteoporosis. *Lancet* (2019) **393**: 364-376.
- [2] K.E. Poole, J.E. Compston. Osteoporosis and its management. *BMJ* (2006) **333**: 1251-1256.

- [3] T. Sozen, L. Ozisik, N.C. Basaran. An overview and management of osteoporosis. *Eur. J. Rheumatol.* (2017) **4**: 46-56.
- [4] J.H. Park, N.K. Lee, S.Y. Lee. Current Understanding of RANK Signaling in Osteoclast Differentiation and Maturation. *Mol Cells.* (2017) **40**: 706-713.
- [5] L. Duplomb, M. Baud'huin, C. Charrier, M. Berreur, V. Trichet, F. Blanchard, D. Heymann. Interleukin-6 inhibits receptor activator of nuclear factor kappa B ligand-induced osteoclastogenesis by diverting cells into the macrophage lineage: Key role of Serine(727) phosphorylation of signal transducer and activator of transcription 3. *Endocrinology* (2008) **149**: 3688-3697.
- [6] J. Han, C. Yu, R.F. Souza, A.L. Theiss. Prohibitin 1 modulates mitochondrial function of Stat3. *Cell Signal.* (2014) **26**: 2086-2095.
- [7] A.S. Kathiria, W.L. Neumann, J. Rhees, E. Hotchkiss, Y.L. Cheng, R.M. Genta, S.J. Meltzer, R.F. Souza, A.L. Theiss. Prohibitin attenuates colitis-associated tumorigenesis in mice by modulating p53 and STAT3 apoptotic responses. *Cancer Res.* (2012) **72**: 5778-5789.
- [8] R. Qureshi, O. Yildirim, A. Gasser, C. Basmadjian, Q. Zhao, J.P. Wilmet, L. Desaubry, C.G. Nebigil. FL3, a Synthetic Flavagline and Ligand of Prohibitins, Protects Cardiomyocytes via STAT3 from Doxorubicin Toxicity. *Plos One* (2015) **10**.
- [9] M.Y. Lee, M.H. Kim, J. Kim, S.H. Kim, B.T. Kim, I.H. Jeong, S. Chang, S.H. Kim, S.Y. Chang. Synthesis and SAR of sulfonyl- and phosphoryl amidine compounds as anti-resorptive agents. *Bioorg. Med. Chem. Lett.* (2010) **20**: 541-545.
- [10] S.-Y. Chang, S.J. Bae, M.Y. Lee, S.-h. Baek, S. Chang, S.H. Kim. Chemical affinity matrix-based identification of prohibitin as a binding protein to anti-resorptive sulfonyl amidine compounds. *Bioorg. Med. Chem. Lett.* (2011) **21**: 727-729.
- [11] C. Basmadjian, F. Thuaud, N. Ribeiro, L. Desaubry. Flavaglines: potent anticancer drugs that target prohibitins and the helicase eIF4A. *Future Med. Chem.* (2013) **5**: 2185-2197.
- [12] C.H. Lee, S.W. Choi, J.Y. Kim, S.H. Kim, K.H. Yoon, J. Oh, M.S. Lee. Overexpression of prohibitin-1 inhibits RANKL-induced activation of p38-Elk-1-SRE signaling axis blocking MKK6 activity. *Biochem. Biophys. Res. Commun.* (2015) **463**: 1028-1033.
- [13] P. Wintachai, F. Thuaud, C. Basmadjian, S. Roytrakul, S. Ubol, L. Desaubry, D.R. Smith. Assessment of flavaglines as potential chikungunya virus entry inhibitors. *Microbiol Immunol* (2015) **59**: 129-141.
- [14] M.H. Kim, M. Park, J.S. Song, S.J. Park, S.H. Kim. Anti-resorptive activity and pharmacokinetic study of N(1),N(1)-diisopropyl-N(2)-(diphenylphosphoryl)-2-(4-nitrophenyl)acetamide. *Bioorg. Med. Chem. Lett.* (2011) **21**: 4263-4266.
- [15] C. King. Some Reactions of p-Toluenesulfonyl Isocyanate. *J. Org. Chem.* (1960) **25**: 352-356.
- [16] P. Magnus, J. Moursounidis. Reactions and diastereoselectivity of N2-arylsulfonyl amidine anions. *J. Org. Chem.* (1991) **56**: 1529-1534.
- [17] I. Bae, H. Han, S. Chang. Highly Efficient One-Pot Synthesis of N-Sulfonylamidines by Cu-Catalyzed Three-Component Coupling of Sulfonyl Azide, Alkyne, and Amine. *J. Am. Chem. Soc.* (2005) **127**: 2038-2039.
- [18] J.H.M. Lange, S. Verhoog, H.J. Sanders, A. van Loevezijn, C.G. Kruse. A novel atom-efficient, one-pot synthesis of sulfonylguanidines and sulfamoylguanidines. *Tetrahedron Lett.* (2011) **52**: 3198-3200.
- [19] C. Fest, H.-J. Riebel, H.-J. Santel, R.R. Schmidt, R.H. Strang. Phenylsulphonylisourea herbicides. in, EP 264020, 1989.
- [20] M. Baud'huin, F. Lamoureux, C. Jacques, L.R. Calleja, T. Quillard, C. Charrier, J. Amiaud, M. Berreur, B. Brounais-LeRoyer, R. Owen, G.C. Reilly, J.E. Bradner, D. Heymann, B. Ory. Inhibition of BET proteins and epigenetic signaling as a potential treatment for osteoporosis. *Bone* (2017) **94**: 10-21.
- [21] F. Emhemmed, S. Ali Azouaou, F. Thuaud, V. Schini-Kerth, L. Désaubry, C.D. Muller, G. Fuhrmann. Selective anticancer effects of a synthetic flavagline on human Oct4-expressing cancer stem-like cells via a p38 MAPK-dependent caspase-3-dependent pathway. *Biochem. Pharmacol.* (2014) **89**: 185-196.
- [22] J. Han, Q. Zhao, C. Basmadjian, L. Desaubry, A.L. Theiss. Flavaglines Ameliorate Experimental Colitis and Protect Against Intestinal Epithelial Cell Apoptosis and Mitochondrial Dysfunction. *Inflamm. Bowel Dis.* (2016) **22**: 55-67.
- [23] B. Hausott, H. Greger, B. Marian. Flavaglines: A group of efficient growth inhibitors block cell cycle progression and induce apoptosis in colorectal cancer cells. *Int. J. Cancer* (2004) **109**: 933-940.
- [24] P. Proksch, M. Giiasi, M.K. Treiber, K. Palfi, A. Merling, H. Spring, P.H. Krammer, M. Li-Weber. Rocaglamide derivatives are immunosuppressive phytochemicals that target nf-at activity in T cells. *J. Immunol.* (2005) **174**: 7075-7084.

- [25] J.Y. Zhu, I.N. Lavrik, U. Mahiknecht, M. Giaisi, P. Proksch, P.H. Krammer, L.-W. Min. The traditional Chinese herbal compound rocaglamide preferentially induces apoptosis in leukemia cells by modulation of mitogen-activated protein kinase activities. *Int. J. Cancer* (2007) **121**: 1839-1846.
- [26] A. Djehal, M. Krayem, A. Najem, H. Hammoud, T. Cresteil, C.G. Nebigil, D. Wang, P. Yu, E. Bentouhami, G.E. Ghanem, L. Desaubry. Targeting prohibitin with small molecules to promote melanogenesis and apoptosis in melanoma cells. *Eur. J. Med. Chem.* (2018) **155**: 880-888.
- [27] Y. Yang, M.R. Chung, S. Zhou, X. Gong, H. Xu, Y. Hong, A. Jin, X. Huang, W. Zou, Q. Dai, L. Jiang. STAT3 controls osteoclast differentiation and bone homeostasis by regulating NFATc1 transcription. *J. Biol. Chem.* (2019) **294**: 15395-15407.
- [28] C.G. Nebigil, C. Moog, S. Vagner, N. Benkirane-Jessel, D.R. Smith, L. Désaubry. Flavaglines as natural products targeting eIF4A and prohibitins: From traditional Chinese medicine to antiviral activity against coronaviruses. *Eur. J. Med. Chem.* (2020) **203**: 112653.