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# Sustainable Mechanochemistry of Biologically Active Molecules

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Synthetic mechanochemistry, the use of mechanical force produced by milling, grinding or extruding, as an activation technique, provides access to chemical entities of all kinds. This Review is focused on examples reported so far of known Active Pharmaceutical Ingredients (APIs), and effectively evaluated

bioactive molecules, all prepared by mechanochemistry. Three families of molecules are discussed: heterocyclic compounds, metal complexes and peptides. Advantages of the mechanochemistry approach are highlighted especially from a green chemistry and efficacy point of view.

## 1. Introduction

The International Union of Pure and Applied Chemistry (IUPAC) identified recently mechanochemistry as one of the 10 technologies of the future in chemistry, contributing positively to the sustainable development of the planet, and which will revolutionize the world.<sup>[1]</sup> The use of mechanical forces to generate chemical transformations has been known for a very long time.<sup>[2]</sup> Various fields of chemistry initially benefited from this technological approach (inorganic chemistry, chemistry of oxides and alloys, and so on).<sup>[3]</sup> It is more recently that synthetic organic chemistry,<sup>[4]</sup> capitalizing on the possibility of eliminating the use of solvents in a sustainable approach, has developed in the light of mechanochemistry. Mechanical forces are generated by various types of equipment, mostly mortar and pestle, ball mills or extruders. Pioneering work from the groups of Toda<sup>[5]</sup> and Kaupp<sup>[6]</sup> described the application of ball milling in organic synthesis. More recently, reactive extrusion was introduced as a continuous flow approach, making possible the handling of solids and concentrated mixtures, for the same purpose. Many families of organic molecules have now been mechanochemically synthesized and these on-going developments went along with the expansion of the mechanochemical toolbox. This approach complies with an important principle among the 12 principles of Green Chemistry,<sup>[7]</sup> namely avoiding waste. Most organic syntheses require a large amount of solvent, in the production or purification steps. By implicating mechanochemistry in a synthetic scheme, the volume of solvent is drastically reduced

in the reaction set up. Furthermore, thanks to mechanochemistry, the overall efficiency of a reaction is generally improved. Thus, in most cases, purification is facilitated because of the efficiency of the process, hence avoiding classical solvent-consuming column chromatography.<sup>[8]</sup>

A large number of recent reviews summarize these results, highlighting the potential contribution to sustainable chemistry as well as unique features belonging to the mechanochemical approach.<sup>[8–9]</sup> Concerning the topic of biologically active molecules prepared by mechanochemistry, many studies were reported in the field of pharmaceutical science concerning the preparation of co-crystals including Active Pharmaceutical Ingredients (APIs), and, to a lesser extent, for the preparation of APIs or chemical libraries for biological testing purposes (medicinal mechanochemistry).

Given the involvement for many years of our research team in this field, it seemed relevant to focus this review on the preparation *via* mechanochemistry of biologically active molecules, either APIs or effectively biologically evaluated compounds. The preparations of API-based co-crystals<sup>[9f,10]</sup> and coordination polymers such as BioMOFs (Metal–Organic Frameworks),<sup>[11]</sup> which were previously reviewed, were excluded from this report.

## 2. Equipment

The first mechanochemical reaction documented was realized in 315 B.C. by Theophrastus of Eresus using a copper mortar and pestle for the reduction of cinnabar into metallic mercury.<sup>[2]</sup> Since then, the milling technique has evolved. If the combination mortar/pestle is still the first equipment to use for testing the feasibility of a solid-state reaction, it lacks reproducibility and possibility to go for long reaction times. If suppliers are selling automated mortar and pestle, the most used equipment nowadays to perform mechanochemical reactions are vibratory and planetary ball-mills (vbm and pbm, respectively). In a vbm, the cylindrical or ovoid milling jar containing the reagents and usually one to three balls is agitated following an oscillating movement, horizontal or vertical, respectively. An example of

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<https://www.iufrance.fr/les-membres-de-liuf/membre/2233-xavier-bantreil.html>

vbm, operating with horizontal movements is shown in Figure 1a. In a pbm (Figure 1b, Figure 1d), the milling jar, which is rotating on itself, is placed on a horizontal wheel rotating in the opposite direction. In this case, the jars usually contain more milling balls (50–100). The differences in the type of agitation

and number of balls have therefore an impact on the forces applied by the balls to the chemical reagents during the milling. In the first case, the ball trajectory will induce more direct impact and compression and on a lower basis shearing and friction (mostly on the walls of the jar), while in the latter case,



From left to right: Nicolas Pétry, Frédéric Lamaty, Ophélie Bento, Tharwat Mohy El Dine, Xavier Bantreil and Florian Luttringer.

**Nicolas Pétry.** After achieving a Ph.D. in 2007 at ICSN (Gif-sur-Yvette) under the supervision of Prof. J.-M. Campagne, working on the total synthesis of dolastatin 14, he joined in 2010 the CNRS as engineer and carried out expertise and training for the Chemical Risk Prevention Unit in Gif-sur-Yvette. In 2012, he moved to Nancy (Université de Lorraine) in the group of Prof. Y. Fort and worked on the syntheses of biomolecules for medical imaging applications (PET scan). In June 2015, he arrived in Dr. F. Lamaty's group and is now specialized in mechanosynthesis of biomolecules.

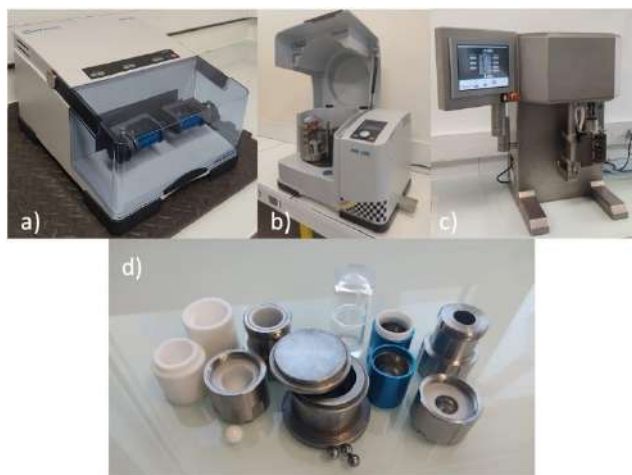
**Frédéric Lamaty.** Frédéric Lamaty received in 1992 a Ph.D. from Purdue University (West Lafayette, IN USA) under the supervision of Professor Ei-chi Negishi in the field of Pd-catalyzed cyclizations. He then joined the group of Professor Marc Julia at the Ecole Normale Supérieure in Paris to work on the synthesis of Vitamin A. In 1994, he obtained a permanent position at the Centre National de la Recherche Scientifique (CNRS) in Montpellier. He is currently Senior Scientist at the Max Mousseron Institute of Biomolecules (IBMM) and head of the Green Chemistry and Enabling Technologies team ([www.greenchem.cnrs.fr](http://www.greenchem.cnrs.fr)). Mechanochemistry is one of his major research topics.

**Ophélie Bento.** After performing in 2020 a 6-month internship at ICSN (Institut de Chimie des Substances Naturelles) in the team of Dr. F. Roussi where she worked on the synthesis of PROTACs derived from a natural product with anticancer properties, Ophélie obtained a Master in Drug Science-Pharmaceutical Chemistry at the Faculty of Pharmacy of Châtenay-Malabry (Paris XI). She then joined the teams of Dr. F. Lamaty (IBMM) and Dr. P. Marin (Institut de Génomique Fonctionnelle) in Montpellier for an interdisciplinary thesis. She is currently working on the sustainable synthesis of new ligands of the 5-HT<sub>6</sub> receptor for applications in autistic spectrum disorders.

**Tharwat Mohy El Dine.** In 2016, Tharwat Mohy El Dine obtained her Ph.D. in Organic chemistry from the University of Caen-Normandy (France) within the group of Pr. J. Rouden (LCMT) with a PhD thesis entitled "New Insights in Amide Bond Formation Mediated by Boron-Based Catalysts". Afterwards, she got a postdoctoral position for one year at the University of British Columbia (Vancouver, Canada) under the supervision of Pr. D. Perrin (The Perrin Laboratory). In 2017, she started her 2nd post-doctorate (Move-in Louvain fellowship) with Pr. S. Vincent at the University of Namur (Namur, Belgium). In 2019, she joined Dr. F. Lamaty's group in Montpellier (IBMM) for a two-year post-doctorate in collaboration with Polypeptide Group (Strasbourg) where she is working on the development of green peptide synthesis.

**Xavier Bantreil.** In 2008, Xavier Bantreil obtained a Ph.D. from Université Pierre et Marie Curie (UPMC – Paris VI) for his work on palladium-catalyzed allylic alkylations under the supervision of Prof. G. Poli. He then took a post-doctoral position in the group of Prof. S. P. Nolan and Dr. C. S. J. Cazin (ICIQ, Spain) and St Andrews University, UK), working on the synthesis of new catalysts for olefin metathesis. He then joined Dr. P. Belmont at Institut Curie (Paris) for 1-year, working on silver-catalyzed synthesis of quinoline derivatives. In September 2011, he joined Dr. F. Lamaty's group in Montpellier as Assistant Professor. In 2021, he was nominated as Junior Member of the Institut Universitaire de France (IUF) for his work on catalysis, mechanochemistry and flow chemistry in a view to develop sustainable chemistry.

**Florian Luttringer.** After obtaining a Master in Green Chemistry in 2019 from the University of Strasbourg during which he did a 6-month internship in Dr. F. Lamaty's group in Montpellier, he joined the group in 2020 as a PhD student. He is currently working on innovative mechanochemical procedures for the synthesis of new organometallic complexes.



**Figure 1.** Examples of mechanochemical equipment: a) vibratory ball-mill; b) planetary ball-mill; c) twin-screw extruder; d) milling jars for vbm and pbm. Copyright, Institut des Biomolécules Max Mousseron (IBMM). Reproduced with permission.

shearing/friction will be more prominent than impact/compression forces. Another difference between these devices is the internal volume of the milling jars. While the vbm will be more dedicated to small scale reactions, with milling jars having internal volumes  $V_i$  between 1.5 and 55 mL, the pbm allows to work on small scale, with  $V_i$  around 12 mL, as well as on larger scale with up to 500 mL  $V_i$  or more. However, the same apparatus may not be able to achieve the full range of reaction scales.

Nevertheless, if pbm allows to perform scale-up by using larger mills and milling jars, an alternative and productive approach for industrial scale-up is the use of reactive extrusion (Figure 1c). Indeed, such approach allows to work with solids and make them react along one or two screws. In theory, this counterbalances the limitations observed in classical solution flow chemistry, where clogging due to the formation or use of solids is dramatic. In an extruder, the reagents will be mixed and conveyed through compression and shearing forces very similar to the ones observed in milling jars. Another advantage of extrusion is the possibility to control the temperature inside the extruder barrel, which is an important parameter to modify the viscosity of the mixture and reduce reaction times. To date, the number of publications dealing with reactive extrusion in organic chemistry is limited and some examples, in connection with the preparation of APIs or bioactive molecules, will be outlined in this review.

When going from classical solution chemistry to mechanochemical milling, one of the first questions would be: what parameters can be changed to modulate the reactivity?

**Frequency:** The frequency of milling has a strong impact on the kinetics of the reaction. As shown by Ondruschka and co-workers for a Suzuki-Miyaura cross-coupling reaction, the kinetic energy is proportional to the square of the frequency.<sup>[12]</sup> Mack studied a series of Diels-Alder reactions with different activation energies.<sup>[13]</sup> The results obtained clearly showed a correlation

between the frequency and the pre-exponential factor of the Arrhenius equation. This seems logical as a higher frequency should provide an increased number of collisions between particles, as well as higher energy shocks. Such conclusion is consistent with the observations from Julien et al. in the formation of a quinoxaline derivative at different frequencies.<sup>[14]</sup>

**Material of the jar/balls:** Depending on the material chosen, the reaction outcome might be different. Indeed, as shown in some examples,<sup>[13]</sup> the energy resulting from the collisions between the ball and the walls of the jar will increase with the hardness of the material. Hence, PTFE might provide lower energy shocks than tungsten carbide.

**Number/size of balls:** The number of balls used can influence the reactivity, but this is difficult to predict as it depends in part on the physical aspect of the reaction mixture along the milling and on the reaction itself.<sup>[15]</sup> As an example for vbm, Kubota et al. showed that the best conversion was obtained in a palladium-catalyzed Buchwald-Hartwig cross-coupling reaction when three balls were present in order to allow for a better mixing of reagents, and especially the catalyst, than with one or two balls.<sup>[16]</sup> The size of the balls can also influence the reactivity. For example, in the mechanochemical alkylation of phenol with epichlorohydrin, the use of a 1.5 cm diameter ball instead of a 1.0 cm diameter ball, thus providing higher energy shocks, allowed to increase the conversion from 43 to 61%.<sup>[17]</sup>

**Solid additives:** A common factor that can be tuned in solution to avoid side-reactions is concentration. This parameter is not available at first glance in a ball-mill. However, it is possible to add inert solids (NaCl, MgSO<sub>4</sub>, SiO<sub>2</sub>, sand...) to dilute the reaction mixture. Such additive can also be helpful when the physico-chemical properties of the reaction mixture do not allow for an efficient mixing, for example if making a sticky paste. Adding a solid might help avoiding the formation of this kind of paste. The addition of solid can also have an influence on the selectivity of a reaction. In the development of the mechanochemical Strecker reaction, grinding without additive furnished a mixture of desired  $\alpha$ -aminonitrile and intermediate imine, while the addition of silica allowed to exclusively obtain the  $\alpha$ -aminonitrile.<sup>[18]</sup>

**Liquid additives:** Even though the first aim of using ball-mills is to avoid solvents, it was shown that the addition of a small quantity of liquid additive could have a tremendous effect on reaction kinetics or outcome.<sup>[19]</sup> The quantity of liquid added to the total mass of reagents introduced in the milling jar is defined as the  $\eta$  ratio ( $\mu\text{L}\cdot\text{mg}^{-1}$ ). In the case of a mechanochemical reaction, the  $\eta$  ratio is usually below  $1.0\ \mu\text{L}\cdot\text{mg}^{-1}$ , so that the reaction mixture looks like a humid paste. This Liquid-Assisted Grinding (LAG) approach allows for a better homogeneity of the mixture while maintaining an excellent mixing thanks to ball-milling. For example, our group reported the mechanochemical formation of NHC-copper complexes (NHC = N-heterocyclic carbene) directly from an imidazolium salt, copper powder and oxygen from air.<sup>[20]</sup> Without additive,



around 80% conversion was obtained after 8 h of milling. On the other hand, addition of a small quantity of water ( $\eta = 0.3 \mu\text{L}\cdot\text{mg}^{-1}$ ) drastically improved the kinetics of the reaction, and full conversion was attained in solely 3 h of grinding.

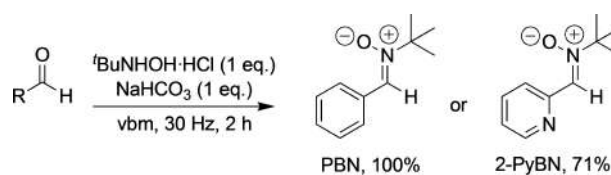
With the same idea in mind, it is possible to perform ILAG (Ion and Liquid Assisted Grinding),<sup>[21]</sup> POLAG (Polymer Assisted Grinding)<sup>[22]</sup> or IL-AG (Ionic Liquid Assisted Grinding)<sup>[23]</sup> to play on the properties of the reaction mixture.

**Monitoring:** While common techniques (IR, NMR, UV, powder X-Ray Diffraction (PXRD), solid-state NMR...) can be used for reaction monitoring as *ex situ* analysis, there might be a doubt concerning the analytical data as soon as a solvent is used for sample preparation. Additional experiments may be needed to ensure that the reaction is indeed occurring in the milling jar and not in solution. Also, even when no solvent is used, the fact of intimately mixing the reagents together for a few minutes can initiate the chemical reaction even if the mixture is left to stand. This technique was described as accelerated aging and proved useful for different transformations.<sup>[24]</sup> To undoubtedly demonstrate and understand what is happening in a milling jar, mechanochemists have developed new tools for in situ monitoring. It is indeed possible to perform in situ PXRD with a modified vbm<sup>[25]</sup> or specially-designed jars<sup>[26]</sup> inside a synchrotron, in situ solid-state HR-MAS NMR with a modified NMR probe,<sup>[27]</sup> as well as in situ Raman spectroscopy using transparent milling jars.<sup>[28]</sup> Hence, the number of in situ monitoring techniques is growing, but for the moment, if these techniques can complement each other and sometimes be used simultaneously, none of them can be universally applied to all mechanochemical systems.

### 3. Mechanochemical Synthesis of Biologically Active Heterocyclic Compounds

Most active ingredients currently on the market have a structure with at least one heterocycle. However, the synthesis of heterocyclic compounds often requires the use of solvent and requires purification at the end of the reaction. This represents a major problem for industrial synthesis, in particular for APIs, as the use of solvent is the first cause of pollution in the pharmaceutical industry. Because of the ecological and economic issues related to the cessation or reduction of the use of solvents in the pharmaceutical industry, many research teams have worked on the development of mechanochemical synthesis of APIs.

In 2008, our group reported for the first time the synthesis of active compound *C*-phenyl-*N*-*tert*-butylnitron (PBN) and one of its analogues *C*-2-pyridyl-*N*-*tert*-butylnitron (2-PyBN) by mechanochemistry (Scheme 1).<sup>[29]</sup> PBN, commonly used as a free radical spin trap, inhibits oxidation of lipoproteins, displays neuroprotective activity, exhibits potent antisenescent effects in neurodegenerative diseases such as Parkinson's, Alzheimer's disease, and Stroke syndrome, and is widely used as anti-aging agent.<sup>[29]</sup> We showed that it was possible to synthesize these



Scheme 1. Mechanochemical synthesis of PBN and 2-PyBN.

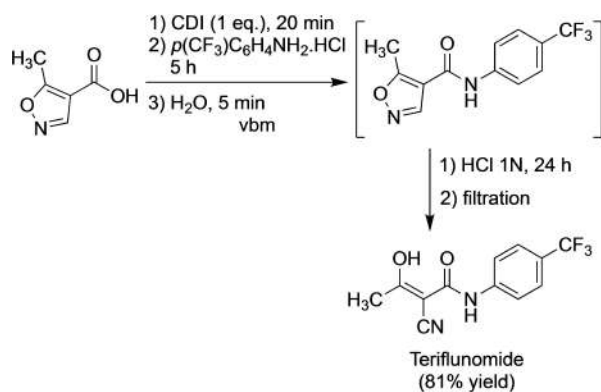
benzene- and heterocyclic-containing compounds using this new eco-compatible method. Many other groups have reported the mechanochemical synthesis of biologically active heterocyclic compounds, and this section will disclose the results obtained in this area.

#### 3.1. Amide-containing compounds

In 2014, Brown reported that more than 50% of the synthesis of therapeutic compounds contain at least one amide bond formation step.<sup>[30]</sup> Traditionally these couplings have been performed using toxic and dangerous reagents such as EDC, HATU or oxalyl chloride between a carboxylic acid and a primary or secondary amine. Moreover, one of the major sources of pollution is the use of solvents, and many alternatives are beginning to be developed to achieve solvent-free synthesis, but often requiring the use of solvents to recover or purify the products at the end of the reaction. Recently it has been shown that it is possible to carry out these reactions mechanochemically by using CDI or KO<sup>t</sup>Bu to avoid the use of hazardous and polluting substances.

One of the first examples of mechanochemical synthesis of APIs was the synthesis of active molecules via carboxylic acid/amine coupling. In 2012, our group described the first mechanochemical amide synthesis by activation of the carboxylic acid with CDI<sup>[31]</sup> in the total absence of solvent in a ball mill.<sup>[32]</sup> Indeed, no solvent was used during the milling and at the end of the reaction, only water was added, allowing to recover the desired product by simple filtration. This technique was applicable to various primary amines, used as their ammonium form. When enantiopure starting substrates were used, no epimerization was observed, and it allowed to considerably reduce the reaction time, from a few hours in solution to only a few minutes by mechanochemistry. Going further, this method was applied to the two-step synthesis of teriflunomide, an API used for the treatment of multiple sclerosis. 5-methyl-4-isoxazolecarboxylic acid and CDI were first milled for 20 min, then 4-(trifluoromethyl)aniline hydrochloride was added and the mixture was further milled for 5 hours. Upon treatment with aqueous HCl, the isoxazole ring opened giving the expected pure compound, in 81% yield (Scheme 2).

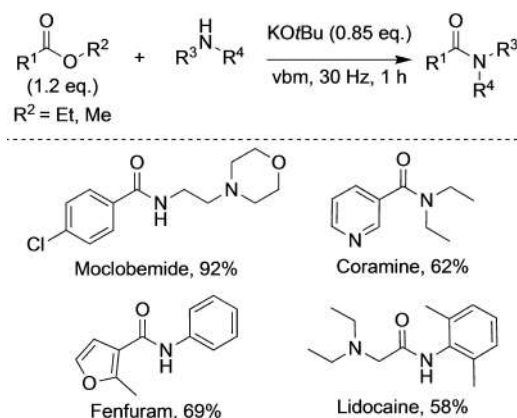
More recently, other teams also found an interest in the synthesis of amide bonds by mechanochemistry. In 2021, Browne's group showed that it is possible to synthesize commercially available APIs such as moclobemide, coramine, fenfuram, and lidocaine, by reacting the corresponding ethyl or



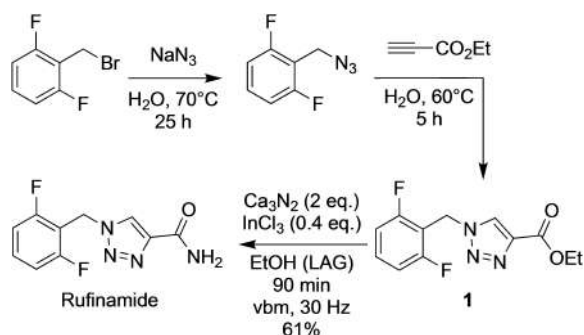
Scheme 2. Mechanochemical synthesis of teriflunomide.

methyl ester with the appropriate amine. The compounds were obtained in short reaction time (1 h) and yields ranging from 58 to 92% (Scheme 3). In this approach, only potassium *tert*-butoxide is necessary to promote the reaction, thus circumventing the use of classical toxic and dangerous coupling reagents at this stage.<sup>[33]</sup>

The same year, Gómez-Carpintero et al. reported the synthesis of primary amides, using calcium nitride as solid nitrogen source. This approach was used efficiently in the synthesis of



Scheme 3. Mechanochemical synthesis of amide-containing APIs.



Scheme 4. Mechanochemical synthesis of rufinamide.

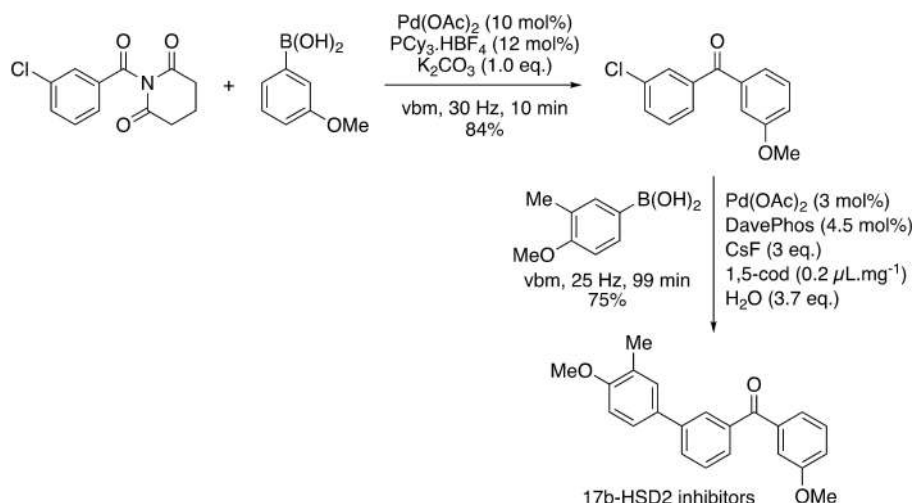
rufinamide, an anti-epileptic compound (Scheme 4).<sup>[34]</sup> Access to triazole-ester precursor **1** was performed according to literature procedures in solution but using water as solvent, through an azide formation following by a 3+2 Huisgen cycloaddition reaction. Finally, **1** was converted into rufinamide by grinding the ethyl ester with  $\text{Ca}_3\text{N}_2$  and a catalytic quantity of indium(III) chloride as Lewis acid. Of note, access to pure primary amide did not require any purification.

Amides can also be used as substrates for further transformations. In 2022, Zhang et al. reported the selective C–N bond cleavage of amides derived from glutarimide under Suzuki-Miyaura cross-coupling conditions for the late-stage modification of high value molecules.<sup>[35]</sup> They also applied their strategy to the two-step synthesis of a 17b-HSD2 inhibitor, used against osteoporosis (Scheme 5). After a palladium-catalyzed C–N cleavage, the resulting intermediate was submitted to previously developed mechanochemical Suzuki-Miyaura cross-coupling conditions<sup>[36]</sup> to yield the 17b-HSD2 inhibitor.

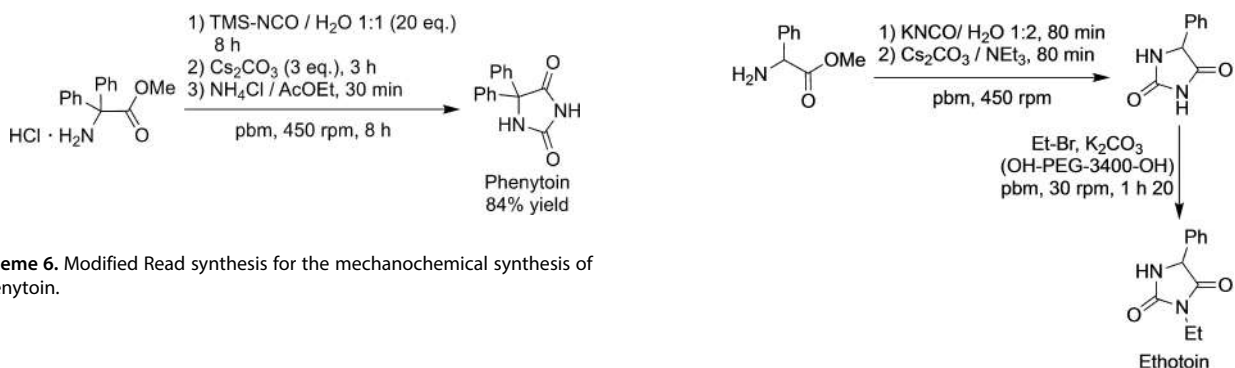
### 3.2. Hydantoins and derivatives

Hydantoins are a family of molecules presenting a 2,4-imidazolidinedione scaffold and showing many biological activities such as antiepileptic, anticonvulsant, antiandrogenic, antidiabetic or antifungal, few side effects and low toxicity. The structure of hydantoins, due to the presence of H-bond donors and H-bond acceptors, gives the molecule the ability to adjust its rigidity/flexibility in the active site, its hydrophobicity/hydrophilicity, its chirality or the ionic character of its scaffold. In the past, these molecules were synthesized in solution via multi-component reactions such as the Read, Bucherer-Bergs or Biltz reactions, but these approaches required cumbersome and expensive processes to obtain the pH and temperature variations required during the syntheses.<sup>[37]</sup> After having tried to synthesize these compounds by microwave, flow chemistry, sonication, photochemistry, since 2014, different research groups are now interested in the synthesis of these molecules by mechanochemistry. In this section, we will focus on the mechanochemical synthesis of some of them that present interesting pharmaceutical properties: Phenytoin, ethotoin, nitrofurantoin and dantrolene.<sup>[38]</sup>

Phenytoin was traditionally synthesized in solution through a Biltz reaction, requiring a condensation between benzil and an excess of urea, followed by a 1,2-shift rearrangement of the phenyl group.<sup>[38]</sup> Since then, different strategies were reported using innovative technologies (sonochemical, microwave, solventless, nano- $\text{Fe}_3\text{O}_4$  catalysts). More recently, this compound was obtained mechanochemically by our group.<sup>[39]</sup> A modified mechanochemical Read reaction, that is to date the only inexpensive and eco-friendly alternative to the Biltz reaction, was used to synthesize phenytoin. 2,2-Diphenylglycine methyl ester hydrochloride was grinded with TMS-NCO to form an intermediate urea, which in the presence of cesium carbonate will form phenytoin by in situ cyclisation (Scheme 6). Thus, phenytoin was obtained pure after an eco-friendly work-up, with a yield of 84%. However, in this synthesis, the first step



Scheme 5. Mechanochemical selective C–N bond cleavage.



Scheme 6. Modified Read synthesis for the mechanochemical synthesis of Phenytoin.

remains limiting as it requires a large excess of reagent (20 eq.) to obtain the desired intermediate.<sup>[39]</sup>

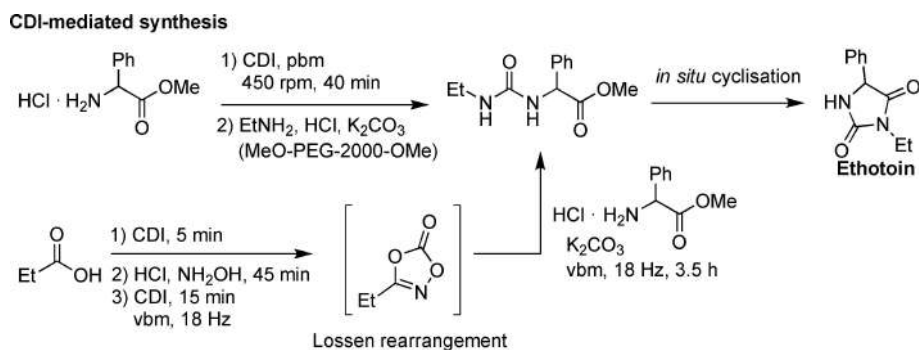
Next, our group studied the mechanochemical synthesis of the anticonvulsant ethotoin. This heterocycle can be obtained in solution by two closely related methods involving a cyclization reaction of 5-phenyl hydantoic acid or the rearrangement of non-commercial ethyl-*N*-Boc-phenylglycinamide, both giving a yield of about 65% after chromatography. Thanks to mechanochemistry, it is possible to synthesize this valuable compound while reducing the environmental impact, the reaction time and increasing the safety procedures. As for phenytoin, a first approach using a modified Read protocol involving ethyl isocyanate and potassium carbonate led directly to the desired compound. Alternatively, potassium isocyanate could be used to furnish 5-phenyl hydantoin as intermediate, which could react mechanochemically with ethyl bromide and deliver the anticonvulsant drug (Scheme 7).

Since isocyanate compound are often toxic, an alternative approach was envisioned using CDI<sup>[31]</sup> to activate either the aminoester or propanoic acid (Scheme 8). In the first case, further reaction with ethylamine furnished the ureido intermediate, which cyclized in situ to furnish ethotoin. In the latter case, reaction with hydroxylamine followed by a Lossen rearrangement in the presence of  $\alpha$ -phenylglycine methyl ester and potassium carbonate gave the same ureido intermediate, which further cyclized.<sup>[38,40]</sup> It should be noted that in all cases, it

Scheme 7. Mechanochemical approaches for Ethotoin synthesis.

was shown that the use of PEG (polyethylene glycol) as grinding assistant allowed to improve at least one of the synthetic steps,<sup>[41]</sup> also in the preparation of antimicrobial additives 3-allyl-5,5'-dimethyl hydantoin (ADMH) and 1-chloro-3-ethyl-5,5'-dimethyl hydantoin (CEDMH).<sup>[42]</sup>

Nitrofurantoin and dantrolene, compounds with antibacterial properties, are hydantoins that feature an *N*-acylhydrazone moiety, function which is present in medicinal chemistry as well as in marketed drugs. The synthesis of these compounds in solution requires excess reagent, pH adjustment, heating and cooling of the mixture. All these steps are energy consuming and polluting. Therefore, an alternative has been sought, mechanochemistry, capitalizing on previously reported methods<sup>[43]</sup> allowing to develop a greener synthesis of these drugs. To obtain these products, 1-amino hydantoin hydrochloride was ground with an aldehyde forming the desired *E* stereoisomers without post-reaction treatment and purification. The advantages of the mechanochemical approach are multiple: it allows i) to reduce the number of synthesis steps as well as the amount of reagent used (no excess), ii) to decrease the reaction time and to suppress the work-up step, thus decreasing the number of wastes generated, iii) to avoid



**Scheme 8.** CDI mediated for Ethotoin synthesis.

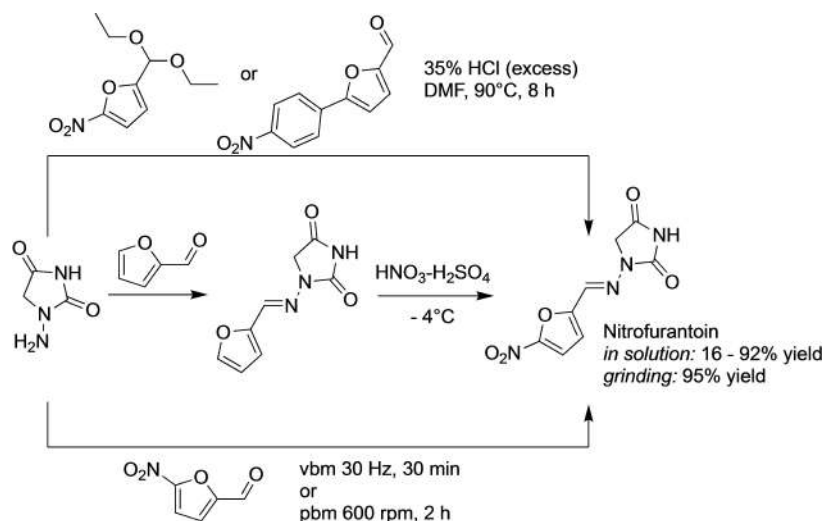
the use of solvent, and iv) work in safer reaction conditions. In addition, it reduces the overall energy cost of the reaction by eliminating the heating and cooling of the mixture (Scheme 9).<sup>[44]</sup> The scale-up of these syntheses has been studied by Crawford et al., demonstrating that it is possible to obtain these compounds by twin-screw extrusion in excellent yields, without the use of bases, solvents or end-of-reaction purification.<sup>[45]</sup> The mechanism of the reaction was studied by Sovic et al. using *in situ* Raman spectroscopy.<sup>[46]</sup>

### 3.3. Hydrazine-hydrazone derivatives: Isoniazid derivatives

As shown above with the synthesis of dantrolene and nitrofurantoin, hydrazine/hydrazone motifs are often present in therapeutic compounds. Their synthesis traditionally performed in solution requires the use of large amounts of solvent, refluxing conditions, and can lead to the formation of by-products, thus producing a lot of waste. Alternative technologies have been used, such as microwave irradiation, but again the use of solvent is necessary. In this context, Oliveira et al. reported the mechanochemical synthesis of isoniazid

derivatives.<sup>[47]</sup> They developed an eco-friendly synthesis of acylhydrazones derived from phenols, not requiring any solvent and resulting from the condensation of an hydrazine with an aldehyde. Among the compounds obtained, ftivazid, known for its antibiotic properties, was isolated in 90% after grinding isoniazid and vanillin for 4 hours. In solution, such condensation requires up to 48 hours to reach completion. Interestingly, the authors calculated the partial atomic charges using NBO (Natural Bond Orbital) analysis to determine the nucleophilicity of the different hydrazines used. It was shown that in solution, a correlation between the reactivity of hydrazine and the conversion rate and yield obtained existed. However, in the solid-state, it was not systematically the case. This observation tends to support the fact that not only electronics have an impact on the reactivity in the solid state, but also the properties of the solids involved (hydrogen bonding, density, mobility of the reactive site).

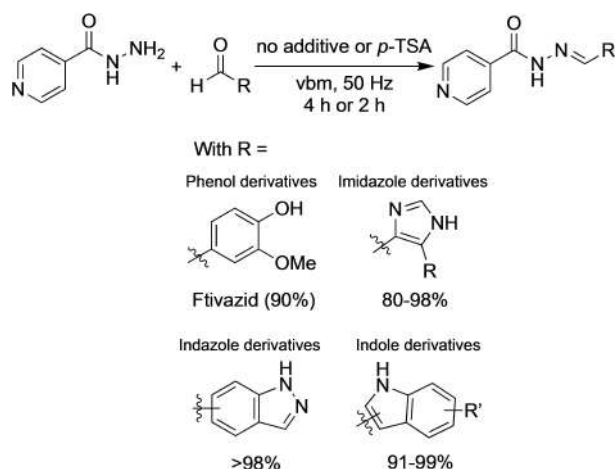
In 2017, the same team worked on the mechanochemical synthesis of several isoniazid derivatives, which were evaluated against *Mycobacterium tuberculosis* cell growth.<sup>[48]</sup> A similar protocol led to the efficient production of 14 compounds derived from phenols, imidazoles, indazole or indoles



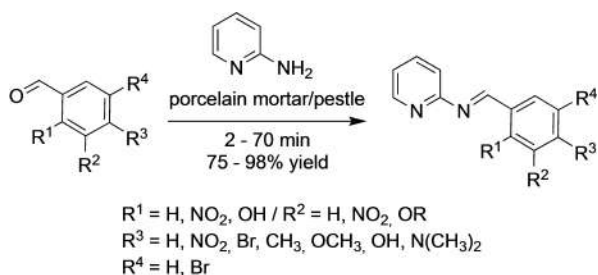
**Scheme 9.** In solution vs in mechanochemistry nitrofurantoin synthesis.



(Scheme 10). In the case of heterocyclic aldehydic partners, activation with *p*-TSA (*para*-toluene sulfonic acid) is necessary to obtain good yields. All isoniazid-derived compounds have been tested, some of them showing promising results for their use as antitubercular agents. Thus, it was shown that this synthesis was not only possible for phenol-derived substrates but could also be extended to heterocyclic substrates.



Scheme 10. Antitubercular agents.



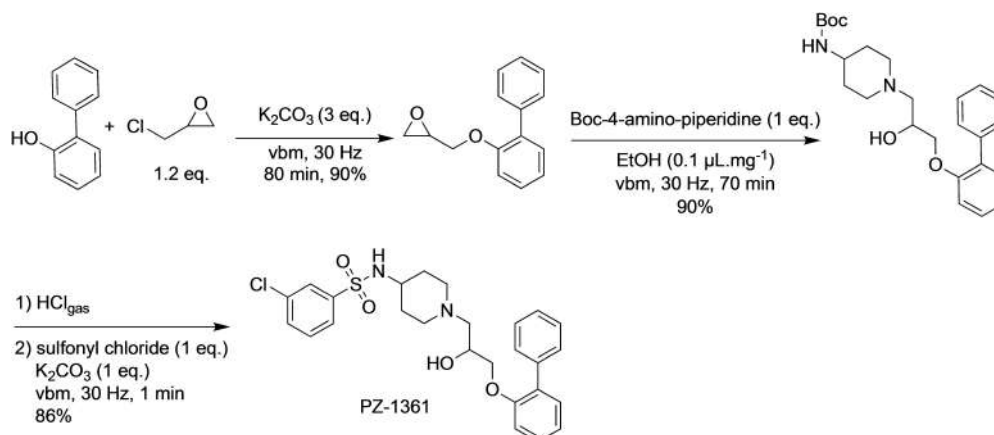
Scheme 11. Mechanochemically synthesized azomethine derived from 2-aminopyridine.

### 3.4. Azomethines derivatives, a pyridine scaffold for biologically active molecules

Azomethine is a pharmacophore often found in natural products and bioactive compounds, such as in ancistrocladine, a natural naphthylisoquinoline alkaloid. Das Sarma et al. have worked on the synthesis of azomethine derivatives from 2-aminopyridine and aromatic aldehyde, using mechanochemistry to make this reaction eco-friendlier by avoiding the need for heating and solvents.<sup>[49]</sup> The electronic nature of the substituents on the aldehyde partner had little influence on the reaction outcome. Interestingly, when a hydroxyl group was in ortho position (Scheme 11,  $R^1 = \text{OH}$ ) with respect to the aldehyde, reaction time was drastically decreased to minutes. The authors thus suggested an intramolecular assistance of this group through hydrogen bonding. Several analogues were efficiently synthesized and subsequently tested as antioxidant (DPPH (2,2'-diphenyl-1-picrylhydrazyl) radical scavenging activity) and antibiotic agents on gram+ and gram- bacteria. Among these compounds, the ones featuring a hydroxyl group had an antioxidant activity comparable to *L*-ascorbic acid. Some of these analogues have also shown interesting antibiotic activity compared to ampicillin.

### 3.5. 5-HT<sub>7</sub> receptor antagonists

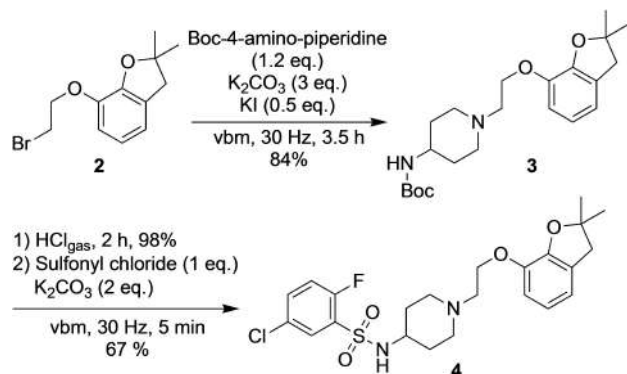
Our group together with Zajdel's team was also interested in the synthesis of biologically active molecules, targeting serotonergic receptors. In 2020, a sustainable synthesis of compound **PZ-1361**, an arylsulfonamide derivative of an alicyclic (aryloxy)alkyl amine, which was shown to be a 5-HT<sub>7</sub> receptor antagonist<sup>[50]</sup> with antidepressant and pro-cognitive properties *in vivo* was optimized (Scheme 12).<sup>[17]</sup> In batch, four steps are required to synthesize this compound, one of them being the alkylation of the 2-phenylphenol, a critical step requiring the addition of a large excess of alkylating agent (3 to 6 eq.) to limit the dimerization or opening of the epoxide. Overall, the synthesis in solution was found costly in terms of reagents, and



Scheme 12. Sustainable synthesis of a 5-HT<sub>7</sub> antagonist.

with a poor atom economy. Moreover, a purification by flash chromatography was necessary at each step to obtain the expected pure products. By mechanochemistry, the synthesis also required 4 steps, but after optimization, each step could be performed under almost stoichiometric conditions, thus allowing to perform solely liquid-liquid extractions to afford the pure intermediates and final compound. Other benefits are reduced reaction time, use of less toxic carbonate bases, higher yields. Through this work, we showed that it was possible to produce compound **PZ-1361** in an eco-compatible way, as proven by the E factor that was reduced by 2.7 (from 1932 in solution to 715 for the mechanochemistry).

In continuation of this work, our groups developed the mechanochemical synthesis of a new family of dual  $\alpha_2$ -adrenoreceptor/5-HT<sub>7</sub>-receptor antagonist with anti-depressant properties.<sup>[51]</sup> Compounds were studied *in vitro* and *in vivo*, allowing to highlight their pharmacological properties. These arylsulfonamide derivatives of (dihydrobenzofuranoxy)ethyl piperidine were fully synthesized by mechanochemistry, showing that it is indeed possible to merge medicinal chemistry and sustainability (medicinal mechanochemistry). Here, we will only focus on the synthesis of 5-chloro-2-fluoro-N-[1-[2-(2,2-dimethyl-2,3-dihydrobenzofuran-7-yloxy)ethyl]piperidin-4-yl]benzenesulfonamide **4**, which was the only compound from this library showing antidepressant properties without affecting the locomotion of the treated mice (Scheme 13). Alkylation of Boc-protected 4-aminopiperidine by 7-(2-bromoethoxy)-2,2-dimethyl-2,3-dihydrobenzofuran **2** was performed in a vibratory ball-mill at 30 Hz, in a PTFE jar. Then the obtained compound **3** was deprotected using gaseous HCl. Finally, reaction with 5-Cl, 2-F-phenylsulfonyl chloride in equimolar quantity and in the presence of non-toxic potassium carbonate allowed to obtain the desired product **4** in 5 min. *In fine*, this mechanochemical approach allowed obtaining a totally new series of compounds in less than 6 hours, without using any excess of costly reactive, any solvent and any time and solvent-consuming purification by flash chromatography.



**Scheme 13.** Sustainable synthesis of a dual  $\alpha_2$ -adrenoreceptor/5-HT<sub>7</sub>-receptor antagonist.

### 3.6. Pyrrole-derived compounds: Atorvastatin

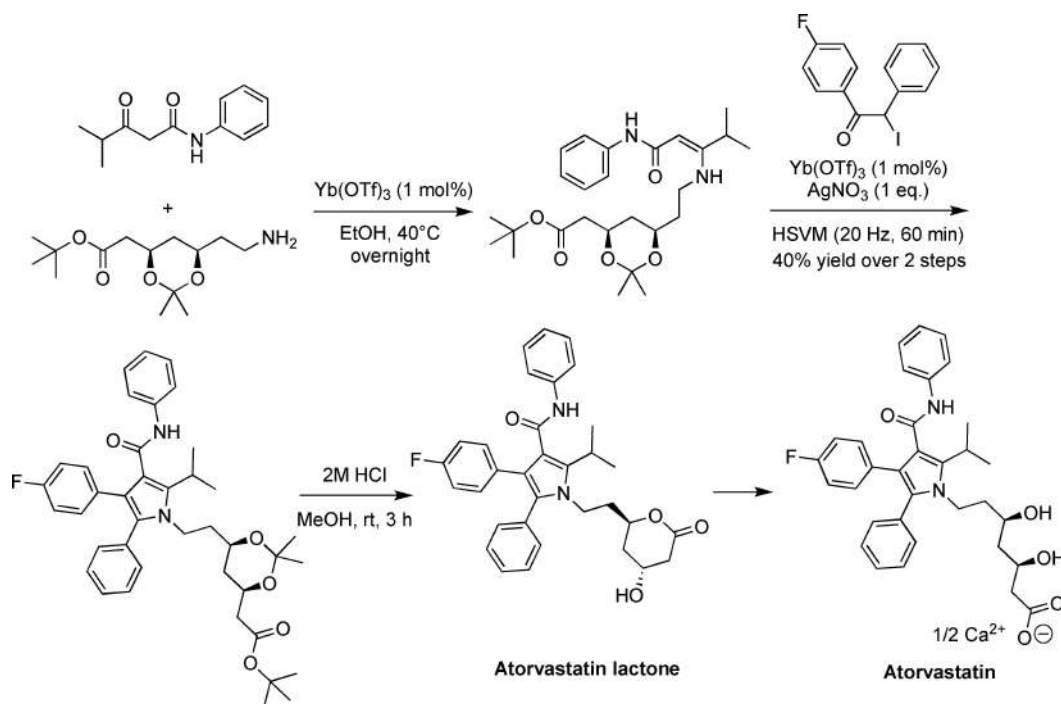
In recent years, multi-component reactions (MCR) have been of great interest to chemists as they allow the simultaneous formation of carbon-carbon and carbon-heteroatom bonds while reducing the number of steps, isolation, purification and the amount of solvent used. Atorvastatin, one of the best-known statins, used as a cholesterol-lowering drug, is synthesized in solution in a linear manner followed by cyclization via Paal-Knorr-type reactions or 1,3-dipolar cycloadditions. In 2014, Estévez et al. synthesized mechanochemically atorvastatin lactone, a precursor of atorvastatin, by high-speed vibrational grinding *via* a Hantzsch-type reaction in the presence of ytterbium triflate and silver nitrate (Scheme 14).<sup>[52]</sup> This work was the first example of a target-oriented synthesis with a mechanochemical multicomponent reaction step and shows that it is also possible to perform these MCR syntheses mechanochemically.

### 3.7. 2-Phenyl-imidazo(1,2- $\alpha$ )pyridine: Example of zolimidine

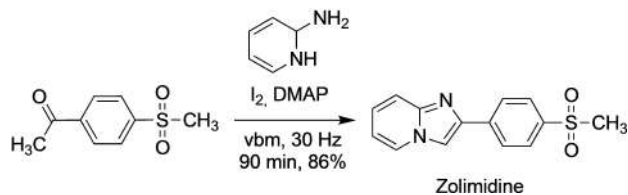
Imidazopyridine is a chemical motif present in many compounds with biological properties such as zolpidem, alpidem, olprinone, minodronic acid or zolimidine. Zolimidine, a gastro-protective compound of the imidazopyridine family, was synthesized mechanochemically in 2016 by Wang et al.<sup>[53]</sup> To construct the imidazopyridine core, several techniques have been described. However, these syntheses require condensation steps, multi-component reactions, oxidative coupling, tandem reactions, aminooxygenation or hydroamination reactions, which often require heating, use of toxic organic solvents, long reaction times, difficult treatments, or even metals. The work of Wang et al. showed that it is possible to obtain imidazopyridine through an I<sub>2</sub>/DMAP-mediated amination/cyclization, in the absence of metal, without solvent, without heating and in only 90 min, by mechanochemistry in a vbm (Scheme 15).

### 3.8. Pyrimidine and derivatives: Pyrimethamine

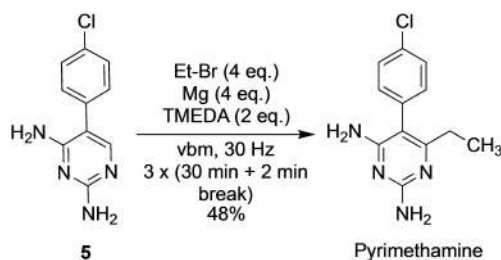
The pyrimidine unit is a chemical unit that is prevalent in many pharmaceutical compounds or natural products. This is for example the case of the anti-malarial drug pyrimethamine. Wu et al. have shown that it is possible to regioselectively alkylate pyrimidines by mechanochemistry using a Minisci-type reaction.<sup>[54]</sup> Traditionally this type of reaction requires the use of precious metals/ligands, high reaction temperature, and are limited since the addition of tertiary alkyl radicals was impossible. Photoredox catalysis was found to be the best alternative before the discovery of the possibility to perform this reaction by grinding, even when tertiary alkylhalides are used. In the case of the pyrimethamine synthesis, intermediate **5** was alkylated in the presence of an excess of bromoethane, magnesium and TMEDA. After 90 min of grinding, the desired pyrimethamine was isolated in 48% yield (Scheme 16).



Scheme 14. Mechanochemical multi-component reaction for Atorvastatin synthesis.



Scheme 15. Solvent-free zolimidine synthesis.



Scheme 16. Minisci-type reaction.

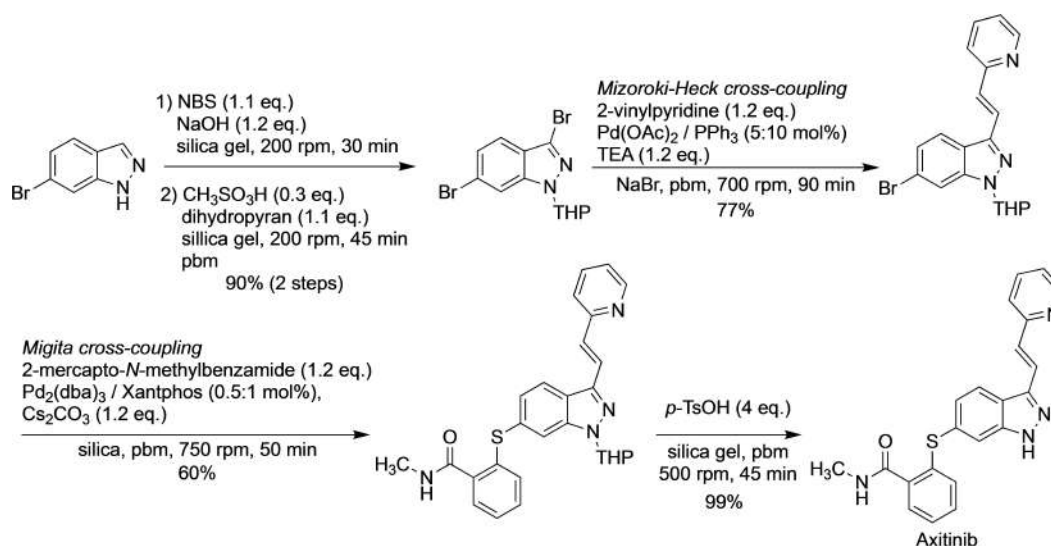
### 3.9. Indazole derivative: Axitinib

To achieve the synthesis of active molecules such as the indazole derivative axitinib, used in the treatment of kidney cancer, the use of palladium-catalyzed cross-coupling reactions is necessary. Yu et al. developed a bromide-assisted mechanochemical chemoselective Mizoroki-Heck reaction, using 3-bromoindazole as a substrate, and applied it for the synthesis of axitinib.<sup>[55]</sup> After extensive tuning of the reaction conditions,

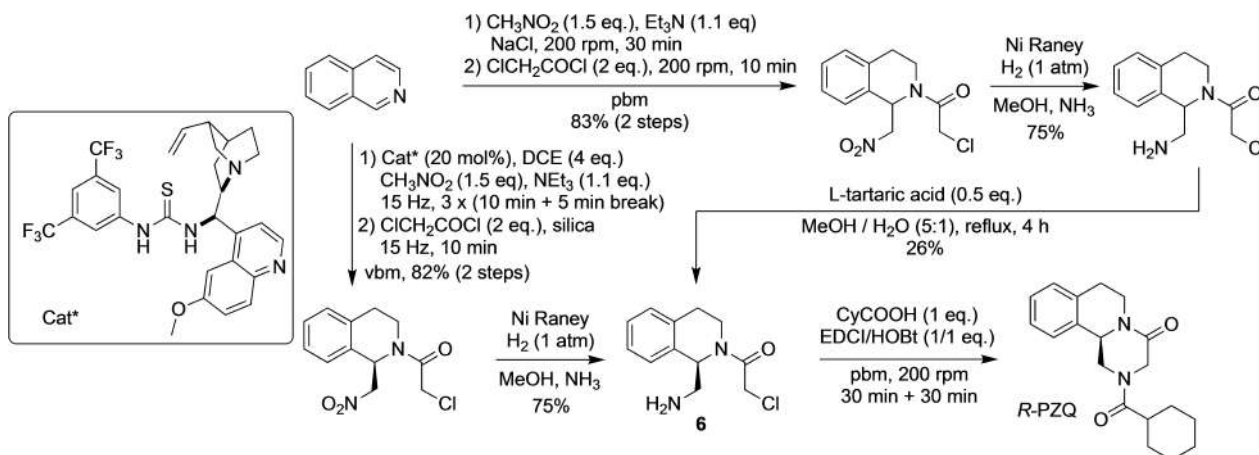
they showed that sodium bromide as grinding assistant played an important role as stabilizer for the catalytically active Pd(0) species and to prevent the competitive dehalogenation side-reaction. This mechanochemical Mizoroki-Heck reaction therefore allows to limit the solvents, the heating steps but also to decrease the quantities of reagents used, thus reducing the costs and the ecological impact of the reaction. This reaction was integrated into a 5-step fully mechanochemical synthesis of axitinib (Scheme 17), involving a bromination of 6-bromo-1*H*-indazole, protection of the primary amine, Mizoroki-Heck cross-coupling reaction, a Migita cross-coupling, and a deprotection. The final API was isolated in an overall yield of 44%. However, purifications were necessary at each synthetic step, which remains a point for improvement.

### 3.10. Isoquinoline derivative: Levo-praziquantel (*R*-PZQ)

Praziquantel (PZQ) is an antiparasitic drug traditionally administered in its racemic form although only the *R*-enantiomer is active. To overcome this, researchers have developed an enantioselective synthesis of intermediate **6**, which in one step would provide *R*-PZQ, by chemical or enzymatic resolution. The drawbacks of these methods are the need to use large amounts of solvent, protection/deprotection steps, toxic reagents, or difficult purifications to obtain the expected compound. Hence, Shou et al. have developed two synthetic routes, involving mechanochemical steps, for the synthesis of *R*-PZQ (Scheme 18).<sup>[56]</sup> The first route involves a racemic aza-Henry/acylation reaction, followed by a hydrogenation of the nitro group. Then, a chiral resolution using L-tartaric acid allowed to



Scheme 17. Mechanochemical axitinib synthesis.



Scheme 18. Two strategies for the sustainable synthesis of R-PZQ.

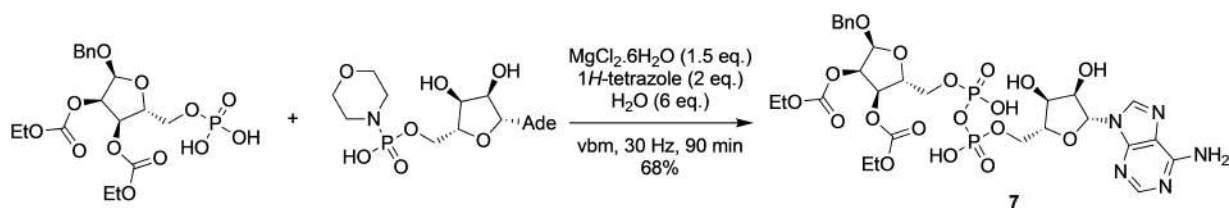
isolate the desired enantiomer, albeit with a poor yield. This enantiopure intermediate **6** could be obtained directly through a first enantioselective aza-Henry reaction in the presence of a thiourea-modified quinine alkaloid as catalyst. Acylation and hydrogenation allowed to isolate the enantiopure intermediate in 62% over the three steps. The last cyclization/acylation step was performed in the ball-mill using EDCI/HOBt as coupling agents. The desired *R*-PZQ was isolated in high yield (84%); however, this final step still requires the use of an explosive reagent, HOBt.

### 3.11. Dihydropyridine derivatives: Calcium antagonist felodipine, nitrendipine, nifedipine and nemadipine B

Jiang et al. developed the synthesis of 1,4-dihydropyridine calcium antagonists used in the treatment of hypertension by mechanochemistry using an enzyme: Lipozyme<sup>®</sup> RM. After

screening the reaction conditions, they showed that it was possible to synthesize felodipine, nifedipine, nitrendipine and nemadipine B in correct yields (Table 1). The synthesis of this compound is classically done via multi-component reactions in solution. The authors have shown here that it is possible to

Table 1. Enzyme-catalyzed mechanochemical synthesis of 1,4-DHP calcium antagonists.				
Compound	R <sup>1</sup>	R <sup>2</sup>	R <sup>3</sup>	Yield [%]
Felodipine	2,3-Cl <sub>2</sub> C <sub>6</sub> H <sub>3</sub>	Me	Et	69
Nifedipine	3-NO <sub>2</sub> C <sub>6</sub> H <sub>4</sub>	Me	Me	54
Nitrendipine	2-NO <sub>2</sub> C <sub>6</sub> H <sub>5</sub>	Me	Et	54
Nemadipine B	2,3-Cl <sub>2</sub> C <sub>6</sub> H <sub>3</sub>	Et	Et	56



Scheme 19. Mechanochemical synthesis of a sirtuin inhibitor.

carry out these same reactions without solvent, thus reducing the ecological impact of the synthesis of these compounds.<sup>[57]</sup>

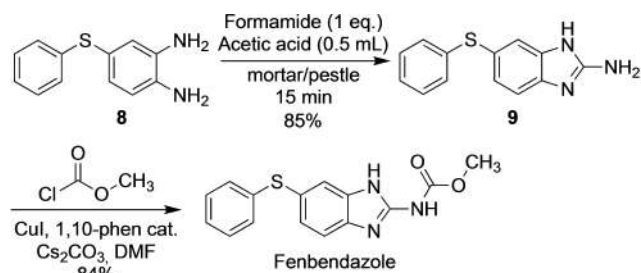
### 3.12. Nucleotides and nucleosides

Among the heterocyclic compounds, nucleotides and nucleosides have attracted attention in the mechanochemical community.<sup>[58]</sup> Inspired by the work of Ravalico et al.,<sup>[59]</sup> Dvorakova et al. reacted in a ball-mill an AMP (Adenosine monophosphate) in its activated morpholidate form with phosphorylated riboses in the presence of 1H-tetrazole and hexahydrate of magnesium chloride to furnish analogs of *O*-acetyl-ADP-ribose (ADP=Adenosine diphosphate) in significantly higher yields than in solution.

Some of these nucleosides, such as compound **7** (Scheme 19), were found to act as SIRT1 (human sirtuin homolog) inhibitors, with  $IC_{50}$  values down to 53  $\mu$ M, which is one order of magnitude higher than the reference compound Suramin ( $IC_{50}$  = 5.8  $\mu$ M).<sup>[60]</sup>

### 3.13. Benzimidazole derivatives

Recently, Zhang et al. have shown that it is possible to synthesize benzimidazole without using metal catalysts and under mild reaction conditions. This chemical motif is present in many therapeutic compounds such as Micardis, Vermox or Nexium. Providing a successful solvent-free and metal-free synthesis is therefore an interesting challenge for the multitude of possible pharmaceutical applications. In their work, the authors reported the synthesis of fenbendazole, a broad-spectrum, low-toxicity anthelmintic, via the condensation of a diamine **8** and formamide in the presence of a catalytic amount of acetic acid, which makes it possible to avoid metals at this stage in order to form the benzimidazole unit **9** in 85% yield (Scheme 20).<sup>[61]</sup> However, the second step of fenbendazole synthesis was performed in DMF using copper as catalyst. The solvent-free methodology was also applicable to benzothiazole and benzoxazole, a motif often found in biologically active compounds. This approach offers a promising alternative for the pharmaceutical industry by avoiding the use of metals, which are often difficult to eliminate at the end of the reaction.

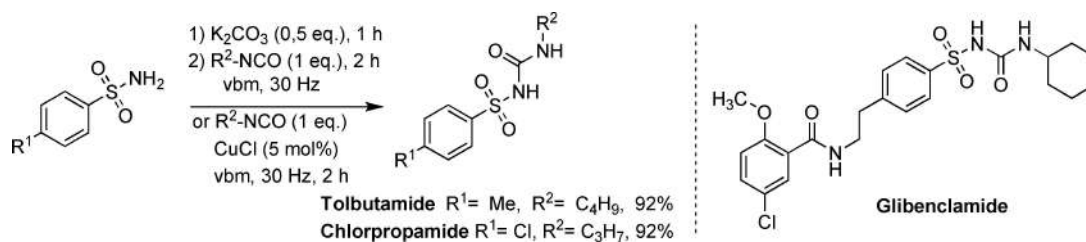


Scheme 20. Application of solvent-free benzimidazole formation for Fenbendazole synthesis.

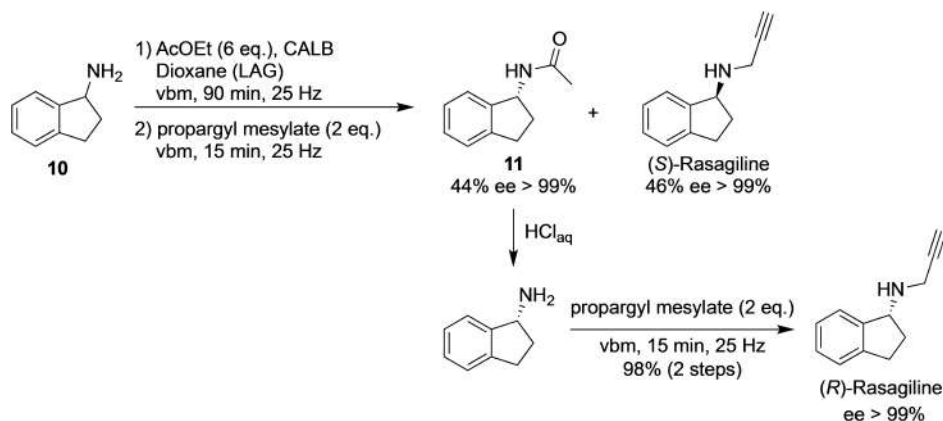
## 4. Arene-Containing Therapeutic Compounds

### 4.1. Synthesis of sulfonyl-(thio)Ureas: The case of the antidiabetic drugs tolbutamide, chlorpropamide, glibenclamide

Tan et al. have shown that it is possible to synthesize sulfonyl-(thio)urea by mechanochemistry. Different synthetic strategies have been explored but the one chosen is the coupling of sulfonamides with isocyanates. Thus, several anti-diabetic compounds have been obtained using this method, such as tolbutamide by reacting *p*-toluenesulfonamide with *n*-butyl isocyanate. During the reaction the sulfonamide is first activated by deprotonation in the presence of the weak base potassium carbonate, then the isocyanate is added. The desired product was obtained in an excellent yield of 92% (Scheme 21).<sup>[62]</sup> Alternatively, the sulfonyl-(thio)ureas could be formed directly by reaction of the sulfonamide and the isocyanate in the presence of copper(I) chloride as catalyst and without base. These conditions, originally developed in solution by Cervello and Sastre (1990) requires the use of DMF and up to 24 hours of reaction time. Thanks to ball-milling, the desired products, including 1<sup>st</sup> generation anti-diabetic chlorpropamide, were synthesized in solely 2 h in high yield. In addition, the scale-up to >1 gram of this copper-catalyzed protocol was demonstrated for tolbutamide and chlorpropamide. Glibenclamide, a 2<sup>nd</sup> generation antidiabetic drugs, was also synthesized in 60% over 2 steps involving the formation of an amide bond in the presence of EDC and pyridine and subsequent copper-catalyzed thio-urea formation.



Scheme 21. Mechanochemical synthesis of sulfonyl-(thio)urea.



Scheme 22. Mechanochemical enzymatic kinetic resolution of amine for the synthesis of Rasagiline.

#### 4.2. Chiral amine by mechanoenzymatic reaction: Rasagiline

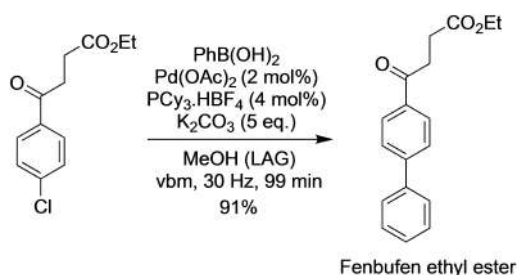
More and more, syntheses requiring the formation of a stereogenic center involve the use of enzymes. Enzymes are green catalysts that can replace organometallic catalysts traditionally used in chemistry and which are often toxic, polluting and difficult to remove from the reaction medium. This approach can also be applied to mechanochemistry as shown by the work of Pérez-Venegas et al. on the kinetic resolution of racemic amines.<sup>[63]</sup> Such an approach is important for the synthesis of compounds presenting a chiral amine such as cinacalcet (hyperparathyroidism), sertraline (depression) or sitagliptin (diabetes). The mechanochemical method was applied for the synthesis of both enantiomers of rasagiline (the *R* enantiomer is administered in Parkinson's disease). For this purpose, racemic amine **10** was milled in the presence of ethyl acetate, used here as acylating agent, and *Candida antartica* lipase B (CALB) in the presence of dioxane as liquid assistant, for 90 min at 25 Hz. After this first step, propargyl mesylate was added and the mixture was ground for a further 15 min to provide (*S*)-rasagiline and the enantiopure acylated amine **11**. After separation using column chromatography, the latter was deprotected with aqueous HCl and the chiral amine was ground with propargyl mesylate to afford (*R*)-rasagiline in 98% yield (Scheme 22). Coupled with mechanochemistry, without restrictive reaction conditions (inert atmosphere, high temperature), the use of enzymes becomes even more eco-responsible by allowing in addition to reduce drastically the use of solvent,

major pollutant in the pharmaceutical industry, to decrease drastically the time of reaction, passing from a few hours or days to only 90 min, but also to decrease the heating temperature.

#### 4.3. Synthesis of biphenyl derivatives: Fenbufen ethyl ester

The biphenyl motif is found in some APIs, such as in fenbufen, a non-steroidal anti-inflammatory drug. Jiang et al. described the synthesis of several biphenyl compounds including fenbufen ethyl ester via a Suzuki-Miyaura cross-coupling reaction involving aryl chlorides.<sup>[64]</sup> In their study, different liquid additives were evaluated in order to improve the reaction yields. It was shown that depending on the ligand used (Davephos or  $\text{PCy}_3$ ), the solvent used impacted the yield, with polar protic solvents giving the best results overall. Importantly, a tiny addition of solvent ( $\eta = 0.045 \mu\text{L}\cdot\text{mg}^{-1}$ ) allowed to drastically improve the yield, from 38% under solvent-free conditions to 97% with a liquid additive. To synthesize fenbufen ethyl ester, methanol was chosen as additive because it gave the best results when tricyclohexylphosphine was used (Scheme 23).

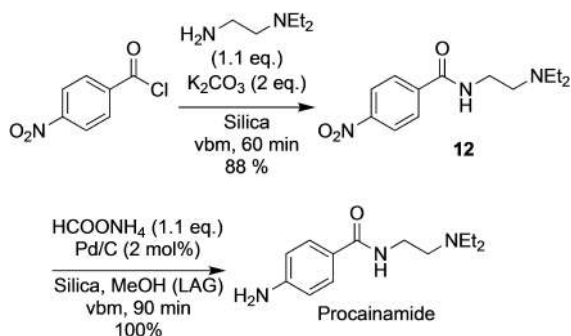




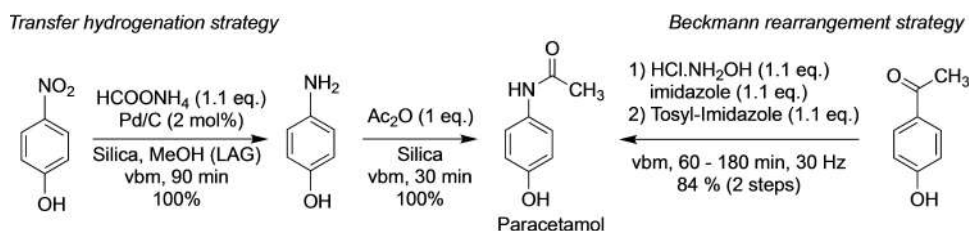
Scheme 23. Mechanochemical Suzuki-Miyaura cross-coupling reaction.

#### 4.4. Aromatic nitro derivatives: Procainamide and paracetamol

Amino-benzamide derivatives constitute a class of compounds with interesting biological activities such as procainamide, used in the treatment of cardiac arrhythmia, or paracetamol, one of the most widely used analgesics and antipyretics. In the case of these widely used drugs, the interest in developing a more eco-responsible synthesis is important because it would make it possible to considerably reduce the waste generated by the pharmaceutical industry for their production. Portada et al. have shown that it is possible to synthesize these products mechanochemically by catalytic transfer hydrogenation of aromatic nitro derivatives.<sup>[65]</sup> Previously, only one example of transfer hydrogenation of nitro derivatives was reported using AuNP (NP = nanoparticles). In this work, ammonium formate was used as hydrogen donor, in the presence of Pd/C, allowing the synthesis of procainamide and paracetamol in an easy and more sustainable way. Performed on a laboratory scale (a few



Scheme 24. Mechanochemical procainamide synthesis.



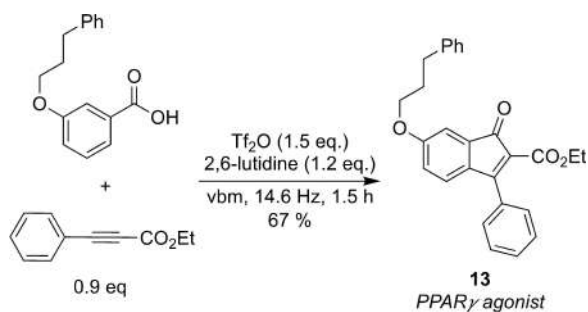
Scheme 25. Mechanochemical approaches towards paracetamol.

mg) and on a gram scale, the two-step synthesis of procainamide involved a first step of amide bond formation between 4-nitrobenzoyl chloride and 2-(diethylamino)-ethylamine. The intermediate **12** was then submitted to catalytic transfer hydrogenation conditions, in the presence of a liquid assistant, methanol, and silica to homogenize the reaction mixture, to give the procainamide (Scheme 24).

Using these conditions, paracetamol was synthesized by catalytic transfer hydrogenation of 4-nitrophenol followed by an acylation reaction (Scheme 25, left). Of note, for the synthesis of these APIs, Teflon reactors were used to limit the risk of corrosion of the stainless steel in the presence of acetic acid and to limit the stainless-steel residues in the reaction medium. The products are therefore obtained without purification at the end of the reaction, thus avoiding one of the most polluting stages in the industry. Mocci et al. were also interested in the synthesis of paracetamol by mechanochemistry, this time *via* a Beckmann rearrangement (Scheme 25, right). Paracetamol was thus obtained in two steps with a yield of 84%, although the corresponding carbonyl was required to carry out this reaction.<sup>[66]</sup>

#### 4.5. Synthesis of indenones: PPAR $\gamma$ agonist

Indenones represent a family of molecules that are very present in natural substances or compounds with biological properties. This is the case, for example, of a peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$ ) agonist, which is involved in transcript processes controlling cell proliferation, differentiation, and apoptosis, and which was efficiently synthesized by mechanochemistry. Classically, the synthesis of indenones requires the use of expensive transition metals, expensive starting materials, solvents, and harsh reaction conditions. Some alternatives have been developed in the past such as a metal-free synthesis, but requiring the use of highly toxic aryl nitrile and MeOTf, high temperature (150 °C) and inert atmosphere.<sup>[67]</sup> Inspired by the work of Khodaei et al.,<sup>[68]</sup> a mechanochemical method was developed to obtain these compounds through a direct annulation of an aromatic carboxylic acid with an alkyne and Tf<sub>2</sub>O as activating agent, while trying to avoid solvents, major pollutants in industrial solution synthesis (Scheme 26).<sup>[69]</sup> Using this method, the PPAR $\gamma$  agonist **13** was synthesized in less than 2 hours, resulting in a 67% yield after a purification by flash chromatography on silica gel. This compound was previously obtained through a four-

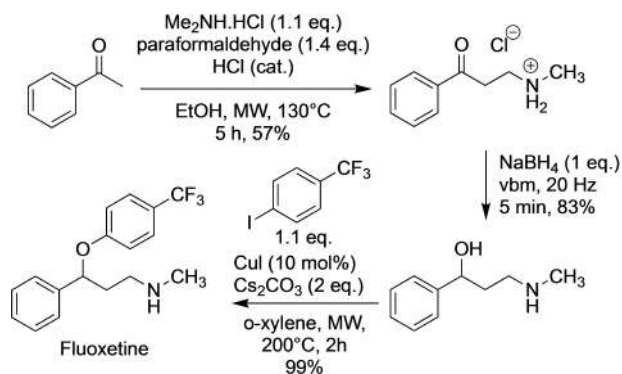


Scheme 26. Mechanochemical preparation of indenones.

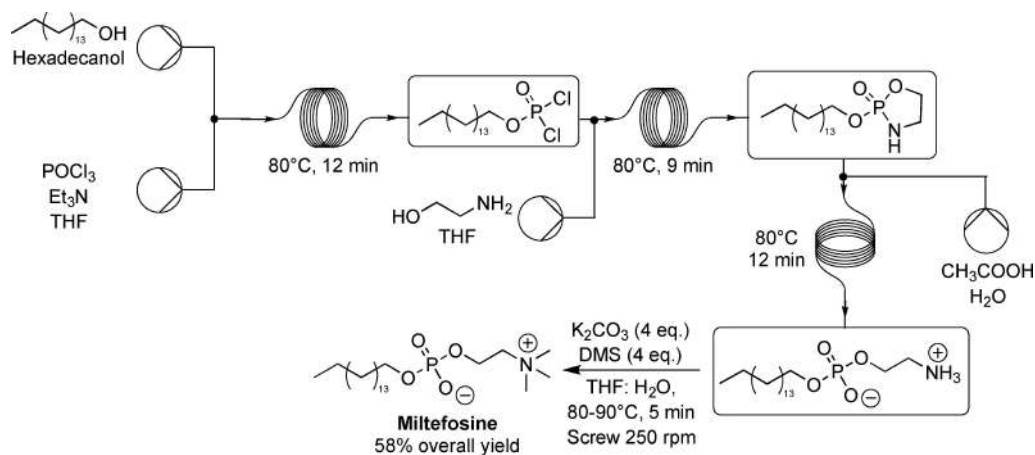
step synthesis and in 20% overall yield, thus demonstrating the benefits of the milling approach. It should be noted that the scale-up to 5 mmol of starting alkyne was found to be efficient on model substrates.

#### 4.6. Fluoxetine synthesis

Fluoxetine is a widely marketed antidepressant compound. Recently, Solà et al. developed a synthetic approach merging



Scheme 27. Merging microwave irradiation and ball-milling for API synthesis.



Scheme 28. Flow synthesis of miltefosine.

mechanochemistry and microwave irradiation to allow for a considerable reduction in reaction times and improved yields, while reducing the amount of solvent used and the energy consumed. Two very similar synthesis routes were developed, with only one depicted in Scheme 27.<sup>[70]</sup> The first step involves a Mannich reaction, followed by a *N*-demethylation step happening upon high temperature heating and prolonged microwave irradiation. Subsequent reduction with sodium borohydride in a ball-mill, and microwave-assisted *O*-arylation furnished fluoxetine in an overall yield of 47%.

## 5. Miscellaneous

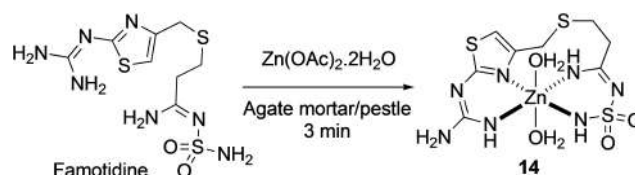
### 5.1. Miltefosine

Leishmaniasis is a tropical disease, caused by the bite of a parasite: phlebotomine sandflies. This disease, depending on the case, can cause severe muscle damage or even be fatal. To combat this, an antiparasitic with also antitumoral effect is available, miltefosine. However, its traditional synthesis requires long reaction times, difficult reaction and processing conditions, the use of expensive reagents, and very high temperature and pressure, to finally obtain the product with a low yield. Patil et al. have developed the first synthesis combining solution chemistry and mechanochemistry in a flow mode to obtain miltefosine with a 58% overall yield, a short reaction time (34 min) and without difficult processing and purification, allowing to produce this API at a rate of  $10\text{ g}\cdot\text{h}^{-1}$ .<sup>[71]</sup> While the first three steps were optimized using liquid flow chemistry, the final step required the use of a single screw extruder to circumvent reactor clogging (Scheme 28).

## 6. Mechanochemistry of Biologically Active Coordination Complexes

Since the discovery of the biological properties of cisplatin [Pt(NH<sub>2</sub>)<sub>2</sub>Cl<sub>2</sub>] in 1965, metallodrugs have gained interest.<sup>[72]</sup> Much effort has been done to discover analogs of cisplatin and metal complexes that are more potent than Pt(II)-based anticancer agents while being more biocompatible and more specific towards cancer cells.<sup>[73]</sup> Nevertheless, platinum-based drugs still remain the most widely used chemotherapeutics in clinical use. Knowledge of drugs containing frequently occurring biological scaffolds combined with modern calculation techniques such as DFT or modeling (e.g. docking assays) gave way to new opportunities for the scientific community and paved the way to the modern medicinal inorganic chemistry. The wide use of antibiotics over the last decades and bacteria developing resistance towards them raised a growing concern amongst the world population. In 2015, the World Health Organization (WHO) came up with a global action plan on antimicrobial resistance among which one of the 5 objectives was “to optimize the use of antimicrobial medicines in human”.<sup>[74]</sup> The first strategy that consists of complexing preexisting drugs is usually the direct answer addressed to this concern.<sup>[75]</sup> More recently, some researchers focused their attention on the use of metal compounds to treat other diseases, such as neglected tropical diseases<sup>[76]</sup> or the virus SARS-CoV-2 causing the COVID-19 pandemic.<sup>[77]</sup> Metallodrugs have also found use as anticancer, antimicrobial, anti-arthritis,<sup>[78]</sup> antifungal,<sup>[79]</sup> or antiviral<sup>[80]</sup> agents.

In the search for new biologically active complexes synthesized by mechanochemistry, two strategies are usually envisioned. The first one is in theory more straightforward and consists of complexing already described biologically active compounds such as a nonsteroidal anti-inflammatory drug (NSAID) or antibiotics with metal precursors to enhance their physicochemical and biological properties while the second one is based on the discovery and the development of new complexes and bioactive scaffolds.



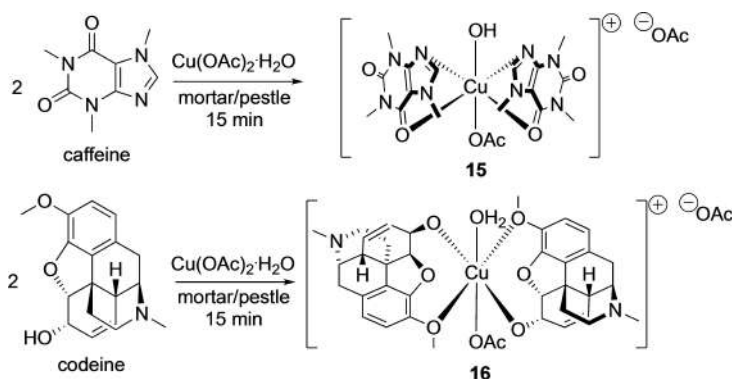
Scheme 29. Zn(famotidine).2H<sub>2</sub>O synthesis by mechanochemistry.

### 6.1. Complexation of already existing drugs

Bacteria are becoming more and more resistant to antibiotics, representing a growing concern in our modern society. Hence there is an urge to enhance drugs' activity against these bacteria. One way to achieve this is to use pre-existing drugs and to complex them with metal salts. In this regard, Amin et al. were pioneers in evaluating a bioactive complex synthesized by mechanochemistry and to compare its physicochemical properties with the same compound obtained by conventional way.<sup>[81]</sup> Practically, one equivalent of famotidine was ground manually with Zn(OAc)<sub>2</sub>.2H<sub>2</sub>O for 2–3 min (Scheme 29).

The complex **14** thus obtained was characterized by elemental analysis, <sup>1</sup>H NMR and FT-IR before and after MeOH washing as workup procedure. The analyses showed that no significant change was due to the work-up, thus proving that the complexation occurred in the solid-state. The activity of **14** was then tested against 34 clinical isolates and 2 strains of *Helicobacter pylori* as reference. The different strains were comprising antibiotic-susceptible and antibiotic-resistant ones and the results showed an enhanced activity against both kinds of strains compared to the free ligand. In addition, the complex was found to be less toxic than famotidine.

Another example of the enhanced activity of a biologically active compound complexed to a metal was made by Tella et al.<sup>[82]</sup> The authors compared the activity of the complex obtained by mechanochemical synthesis with that obtained by solution-based synthesis. They ground in a mortar and pestle two equivalents of caffeine or codeine with Cu(OAc)<sub>2</sub>.H<sub>2</sub>O (Scheme 30). The new complexes **15** and **16** obtained by both ways were characterized by elemental analysis, FT-IR, NMR, UV-

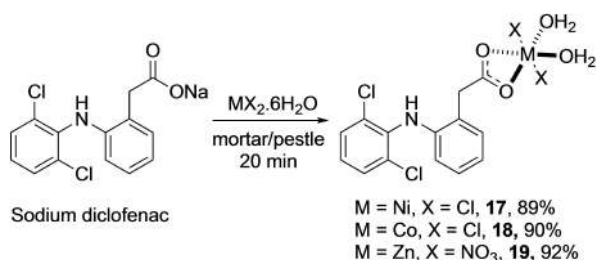


Scheme 30. Synthesis of [Cu(CAF)<sub>2</sub>(H<sub>2</sub>O)(OAc)]OAc and [Cu(COD)<sub>2</sub>(H<sub>2</sub>O)(OAc)]OAc complexes using manual grinding.

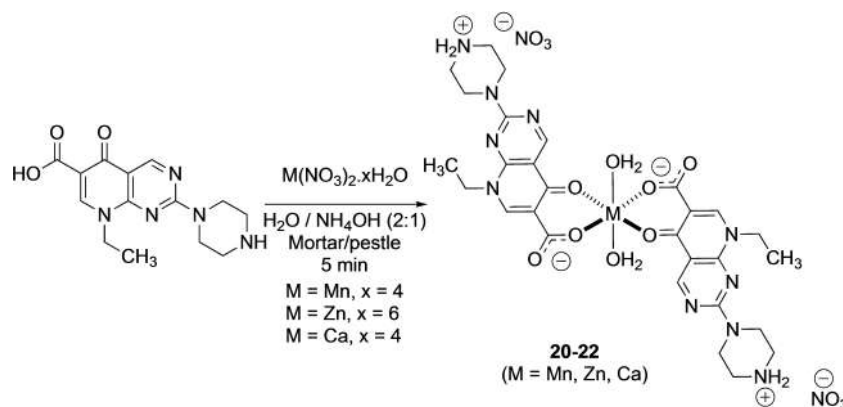
vis spectroscopy and PXRD. The complexes were then subjected to antibacterial and antifungal activity studies and the results showed similar activity between solution-based and mechanochemically synthesized complexes. Both showed higher activity than the free ligands.

Complexation of sodium diclofenac (NaD) with Ni(II), Co(II) and Zn(II) precursors was realized by Osunniran et al. by manual grinding in a mortar and pestle and by conventional way.<sup>[83]</sup> In both cases, equimolar amounts of hydrated metal precursors and NaD were used. Complexes **17–19** were obtained in yields ranging from 58 to 76% by refluxing starting materials for 1 h in MeOH. The yields were improved to 89–93% after 20 min of solvent-free grinding and a simple washing with methanol as workup (Scheme 31). Antioxidant activity was evaluated using the DPPH (1,1-diphenyl-2-picrylhydrazyl) assay and the three complexes showed better IC<sub>50</sub> than the reference ascorbic acid.

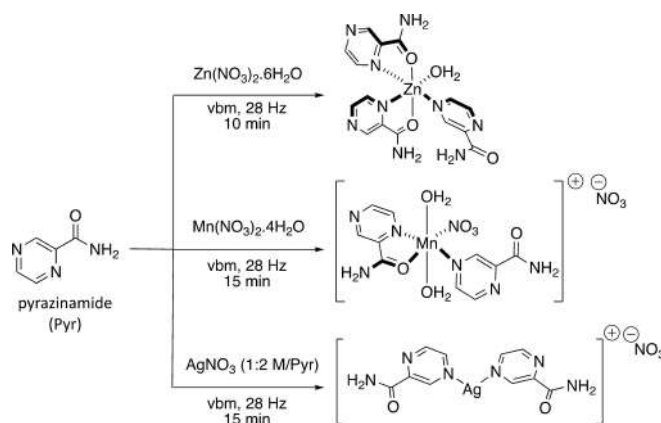
Another addition to the enhanced activity of antibiotics used as ligands was made by Alves et al. by grinding manually for 5 min pipemidic acid (PA), a quinolone derivative, with hydrates of Mn(II), Zn(II) and Ca(II) nitrate precursors in LAG conditions (Scheme 32).<sup>[84]</sup> The complexes **20–22** obtained were recrystallised in water and exhibited a 1:2 molar ratio (M:PA). Their antimicrobial activity against *S. aureus* (Gram positive) and *E. coli* (Gram negative) bacteria strains was assessed and the Mn(II) complex showed the most encouraging results. Although with a minimum inhibitory concentration (MIC) equal to that of its precursor for *S. aureus*, the activity was 2-fold better than the free ligand PA for both strains. Based on the same reaction conditions, the group more recently studied the formation of



Scheme 31. Synthesis of diclofenac-Metal complexes using manual grinding.



Scheme 32. Solid-state synthesis of complexes derived from pipemidic acid with antimicrobial activity.



Scheme 33. Metal complexes derived from pyrazinamide with antimicrobial activities.

the Cu(II) complex with pipemidic acid and Cu(NO<sub>3</sub>)<sub>2</sub> which was shown to have a 1D hydrogen bonded network.<sup>[85]</sup> Quaresma et al. also explored the formation of metal complexes based on pyrazinamide, an antimycobacterial agent, with zinc, manganese and silver nitrates exhibiting for some 1D coordination frameworks (Scheme 33).<sup>[86]</sup> Antimicrobial activity of complexes derived from manganese and silver was found to be higher than the one of pyrazinamide. Of note, the silver complex tested resulted from a stoichiometric reaction between silver(I) nitrate and pyrazinamide and its structure could not be properly identified.

Several groups are currently developing mechanochemical syntheses to obtain active pharmaceutical ingredients (API), compounds interesting from an industrial perspective. The crystal structure of metal complexes based on APIs obtained by solvent-less or solvent-free synthesis may differ from the one obtained by classical way. Ancillary ligands like organic solvents can coordinate to metals and the cost of purification of these compounds in the industry is usually high. It is the case for the metallodrug bismuth subsalicylate **23** obtained by André et al., synthesized from Bi<sub>2</sub>O<sub>3</sub> and salicylic acid. Interestingly, as shown by PXRD and FTIR analyses, different forms of bismuth salicylate

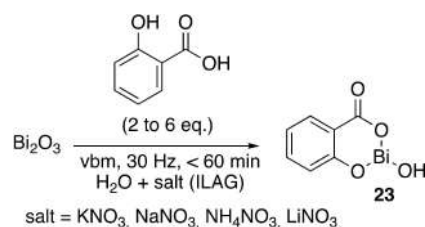
could be obtained depending on the stoichiometry used in the reaction. Additionally, it was found necessary to use water as liquid assistant with a catalytic amount of nitrate salt to obtain efficient and selective conditions (Scheme 34).<sup>[87]</sup>

Sević et al. performed the *in situ* monitoring of the mechanochemical formation of the metallodrug silver sulfadiazine **24**, an antibacterial API, by raman spectroscopy.<sup>[46]</sup> The reaction took place in presence of the ligand and silver nitrate with aqueous ammonia as liquid additive allowing the deprotonation of the ligand (Scheme 35).

Considering the possible use of mechanochemistry in the pharmaceutical industry for the production of APIs, a Cu(II)-NSAID (nonsteroidal anti-inflammatory drug) complex was synthesized both by ball-milling and in solution to compare their biological activity.<sup>[88]</sup> The synthesis based on meloxicam (H<sub>2</sub>Mel) and Cu(OAc)<sub>2</sub>·H<sub>2</sub>O in the presence of a mixture of EtOH and DMF gave [Cu(HMel)<sub>2</sub>(DMF)<sub>2</sub>] **25a** in 15 min at 15 Hz with a ball mill and a different complex [Cu(HMel)<sub>2</sub>(DMF)]<sub>2</sub>·0.25H<sub>2</sub>O by conventional synthesis (Scheme 36). As a green approach, DMF was then replaced with more innocuous solvents yielding [Cu(HMel)<sub>2</sub>(S)<sub>2</sub>] **25a-e** where S=THF, DMSO, CH<sub>3</sub>CN and H<sub>2</sub>O, respectively. The complexes obtained were then tested *in vitro* against ovarian cancer cell lines IGROV-1 and SKOV-3, showing an enhanced cytotoxicity for the mechanochemically obtained compounds compared to the free ligand and to the solution-based complex [Cu(HMel)<sub>2</sub>(DMF)]<sub>2</sub>·0.25H<sub>2</sub>O.

## 6.2. Discovery and development of original bioactive scaffolds

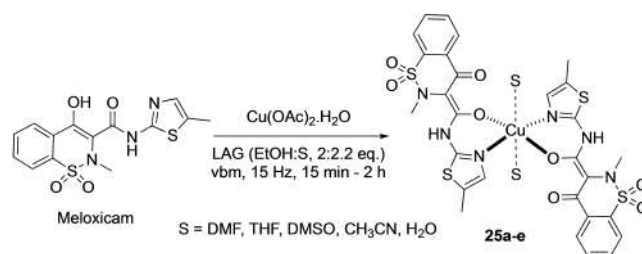
Alzahrani et al. synthesized a Cd(II) and a Zn(II) complex **28** and **29** respectively, bearing a *N'*-benzylidene-3-hydroxy-2-naphthohydrazide tridentate ligand **27** which was obtained by a condensation reaction between 3-hydroxy-2-naphthohydrazide **26** and benzaldehyde performed in refluxing glacial acetic acid (Scheme 37).<sup>[89]</sup> The two complexes were fully characterized by spectroscopic and analytical techniques and revealed a 1:2 (M:L) molar ratio in both cases. **28** and **29** were then tested for their biological activities, including antimicrobial, antioxidant, and antitumor assays. MIC were assessed by agar-streak dilution technique and the results showed almost no antimicrobial activity for the compounds. The antioxidant assay was realized with a referenced ABTS method by measuring the scavenged free radicals compared to ascorbic acid and showed promising results. Finally, the complexes were tested against HCT116 (human colorectal carcinoma) cell line and compared to 5-fluorouracil (5-FU). **28** had the best IC<sub>50</sub> value of 3.16 μg.mL<sup>-1</sup>. The same group further explored the biological activity of the 3-hydroxy-2-naphthohydrazide **26** moiety by condensation with cyclohexanone in solution and subsequent complexation with Ni(II), Co(II) and Cu(II) precursors by mechanochemistry.<sup>[85]</sup> The ligand and the related complexes were all evaluated for their antimicrobial, antioxidant and antitumor activities but the compounds did not show significant antimicrobial activity. The nickel complex was the only one exhibiting a notable antioxidant activity (83.4%) which is close to *L*-ascorbic acid



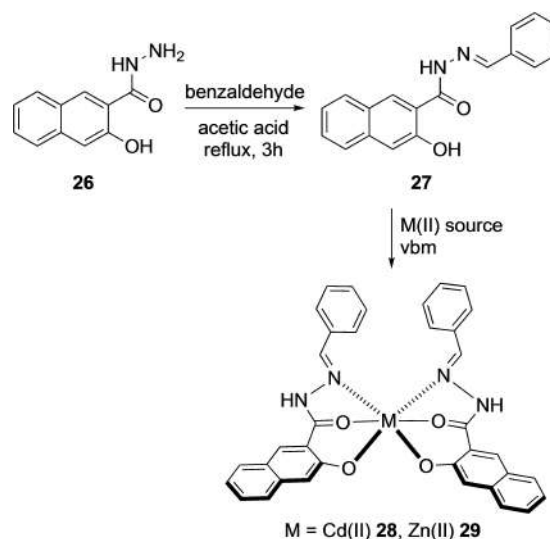
Scheme 34. Mechanochemical synthesis of bismuth salicylate complexes.



Scheme 35. Mechanochemical preparation of silver sulfadiazine.



Scheme 36. Mechanochemical synthesis of differently solvated copper(meloxicam) complexes as anticancer agents.

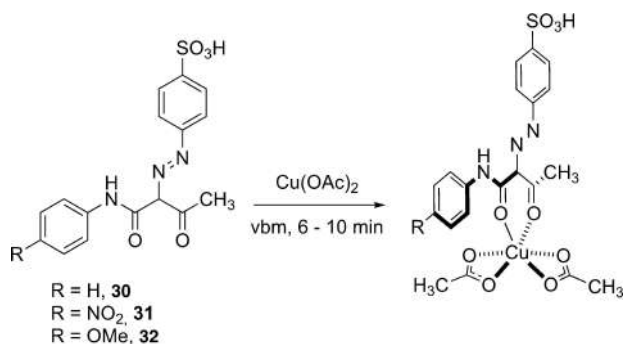


Scheme 37. Mechanochemical coordination of Cd(II) and Zn(II).

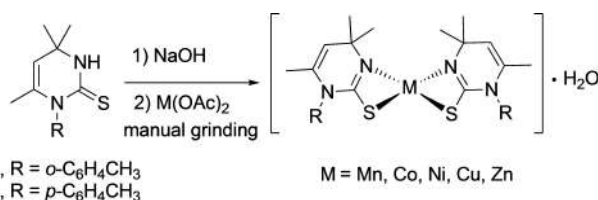
used as standard (85.95%). The cytotoxic activity was this time evaluated against hepatocellular carcinoma cell line (HepG2) and compared with 5-FU as a positive control. The best IC<sub>50</sub> value obtained corresponded to the cobalt complex (2.9 μg.mL<sup>-1</sup>) and is comparable to that of 5-FU (2.6 μg.mL<sup>-1</sup>).

Zaky and Fekri developed three series of Ni(II), Co(II) and Cu(II) complexes by grinding equimolar amounts of the corresponding metal acetate precursor to three substituted  $\beta$ -diketones **30–32** acting as bidentate ligands in the ball mill (Scheme 38).<sup>[90]</sup> All the complexes were formed in 6–10 minutes at 40 Hz and have yields ranging from 97–98%. They were tested as well as the free ligands using the well diffusion method. The biological activity of the compounds was evaluated by determining the minimum inhibitory concentration (MIC) against gram-positive bacteria, gram-negative bacteria and pathogenic fungi with ciprofloxacin and fluconazole as standard controls. Surprisingly, the free ligands exhibited a better antimicrobial and antifungal activity than all the complexes tested with the lowest MICs for each strain but remained less active than the control compounds. Results showed a higher activity in the case of electron-withdrawing substituents on the phenyl ring of the ligand than with electron-donating substituents.

Sethi et al. were able to develop a series of M(II) complexes (M = Mn, Co, Ni, Cu, Zn) with two pyrimidine-2-thione bidentate N,S-ligands **33** and **34** by manual grinding in a mortar and pestle (Scheme 39).<sup>[91]</sup> The *in vitro* antibacterial activity of the complexes was assessed using agar well diffusion method against *Bacillus subtilis*, *S. Aureus*, *P. syringae* and *P. aeruginosa*. and compared to oxacillin or penicillin. **34** and its metal complexes were found to be generally more active than their *ortho*-tolyl analogues against all the strains and the complexes were more potent than the free ligands. Of note, cobalt, nickel and copper complexes featuring ligand **34** had the most promising results. The authors suggested that the lower polarity of these metals that comes from partial sharing of their positive charge with donor groups and overlap of the ligand orbital



Scheme 38. Representative example of mechanochemical synthesis of dicarbonyl ligated complexes.

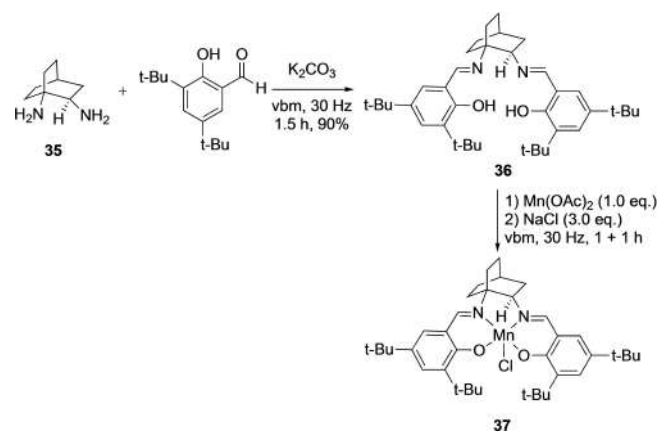


Scheme 39. Pyrimidine-2-thione derived complexes.

enables them to be more easily adsorbed on the surface of the cell wall of microorganisms. These ions can then stop the respiratory process of the cells and their proteins synthesis. DNA photocleavage study followed the same trend for the tested compounds.

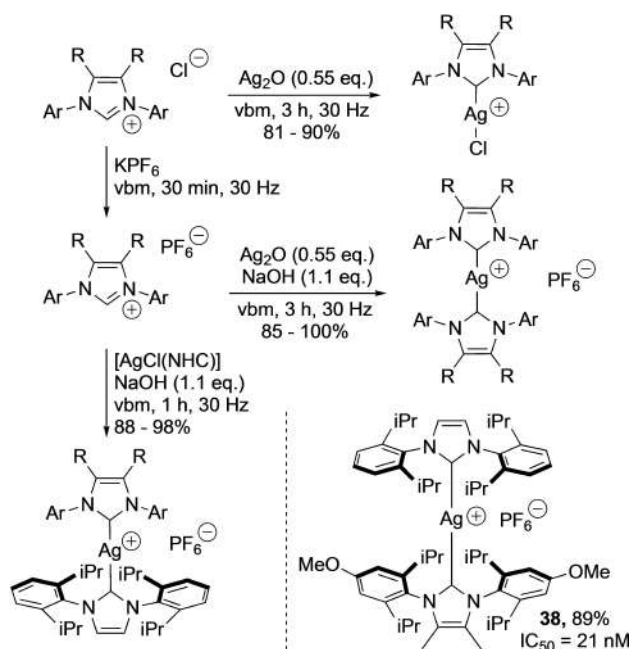
In 2021, our group reported the synthesis of five metal-salen complexes starting from bicyclic diamine **35** and 3,5-di-*tert*-butylsalicylaldehyde.<sup>[92]</sup> Condensation reaction in solution gave the salen compound **36** (91% yield), which was then reacted with different metal acetates (Pd, Cu, Co, Ni, Mn) to yield corresponding complexes with good to excellent yields. The ligand **36** and the manganese complex **37** were also obtained by ball milling in 90% and 83% yield respectively (Scheme 40). Of note, the Mn(II) complex obtained after chelation was treated with brine opened to air to replace an acetate anion and oxidize manganese to Mn(III) and furnish complex **37**. All the compounds were tested against HCT116 colorectal cancer cell line compared to doxorubicin and cisplatin as references. Manganese complex **37** was found to be the most promising compound exhibiting an  $IC_{50}$  of 336 nM, making it 16 times more cytotoxic than cisplatin and 2.4 times more than doxorubicin. The higher biological activity observed for this metal compared to the other complexes may be due to the tendency of Mn(III) salen complexes to act as mimetics of enzymes, thus suggesting an oxido-reduction reaction.

In 2020, our group also reported the synthesis of series of silver complexes bearing sterically encumbered and poorly soluble NHC ligands by mechanochemistry, giving rise to a library of homoleptic and heteroleptic cationic Ag(I) complexes as well as heteroleptic neutral Ag(I) complexes (Scheme 41).<sup>[93]</sup> The mechanochemical approach allowed to obtain complexes that could not be synthesized using classical solution techniques. Their cytotoxic activity was evaluated against colorectal HCT116 cancer cell lines and compared to 5-fluorouracil and cisplatin. The percentage inhibition of cell proliferation was measured at  $10^{-5}$  M and  $10^{-6}$  M. Compounds showing a high percentage of inhibition were then tested by recording half growth inhibition concentration ( $IC_{50}$ ). All the  $IC_{50}$  values obtained, except for one compound, showed a higher cytotoxic-



Scheme 40. Mechanochemical synthesis of biologically active Mn-salen complex.





Scheme 41. Synthesis of NHC–Ag(I) complexes showing antitumoral activity.

icity than the standard control, in the nanomolar range. Notably, the heteroleptic complex **38** was found to be the most active, with an  $IC_{50}$  of 21 nM, which makes it 256 times more potent than cisplatin.

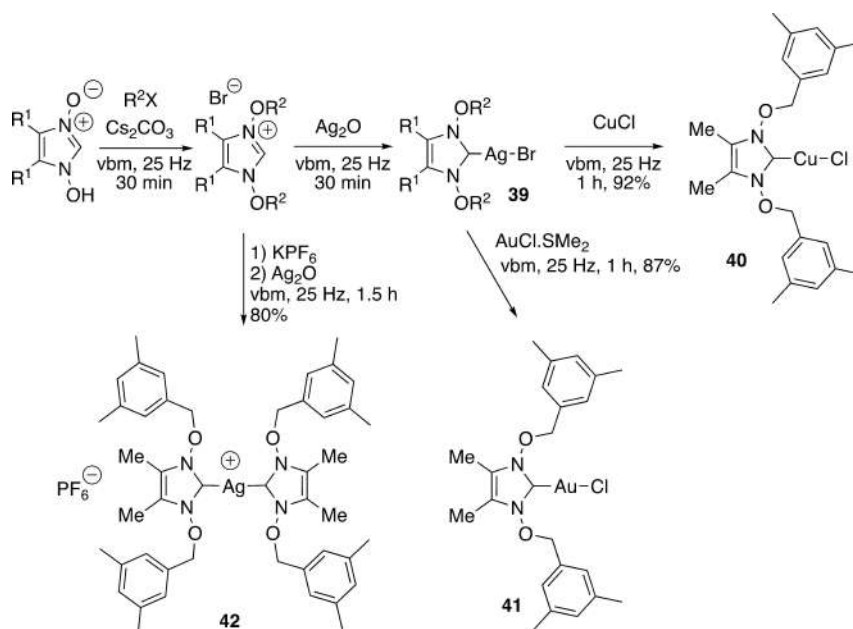
Having these good results in hand, our group developed, in collaboration with A. Wróblewska and G. Mlostoń, the mechanosynthesis of silver complexes bearing *N*-Oxy-Heterocyclic Carbenes (NOHC) ligands.<sup>[94]</sup> The imidazolium salts were synthe-

sized by alkylation of 1-hydroxyimidazole-3-oxides obtained from a diketone, hydroxylamine and formaldehyde. Metalation with silver(I) oxide furnished different 1,3-di(alkyloxy)imidazole-2-ylidenes metal complexes, including one example of a cationic bis-NOHC silver complex **42**. Transmetalation of silver complex **39** with copper(II) and gold(I) chloride allowed to obtain corresponding NOHC-copper and gold complexes **40** and **41** (Scheme 42). The compounds were then tested for their activity against HCT116 cell line compared to doxorubicin and the most promising results were obtained for the cationic silver complex with an  $IC_{50}$  of 0.75  $\mu$ M.

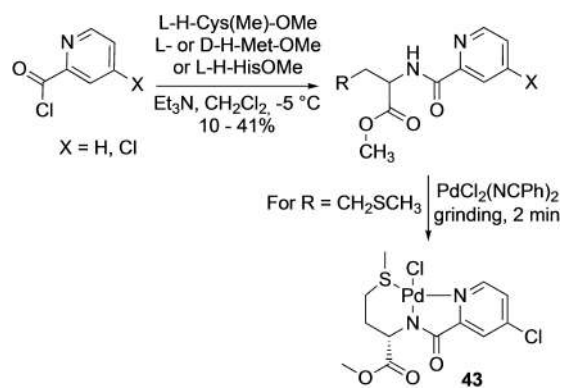
Churusova et al. developed a series of Pd(II) complexes based on tridentate picolinylamide ligands synthesized from picolinyl chloride and methyl esters of 3 different amino acids (Scheme 43).<sup>[95]</sup> The ligands were obtained in solution with poor to moderate yields (10–48%) and then ground in a mortar and pestle for 2 min with a Pd(II) precursor. The authors found the sulfide ancillary donor group obtained with *S*-methyl-*L*-cysteine and *L*- or *D*-methionine methyl esters to be crucial for the cytotoxic effect of the complexes when tested against HCT116, MCF7 and PC3 cancer cell lines. These compounds all exhibited better  $IC_{50}$  than the standard cisplatin, in the range of  $\mu$ M. Of note, palladium complex **43** showed the best results with an  $IC_{50}$  of 0.45  $\mu$ M against human colon cancer cell line HCT116.

## 7. Mechanosynthesis of Biologically Active Peptides

Peptides constitute an intriguing class of lead compounds that find many applications in the fields of material science, cosmetics, nutriment, and pharmaceuticals. Notably, such



Scheme 42. Mechanosynthesis of novel NOHC-metal complexes.



**Scheme 43.** Synthesis of palladium complexes bearing picolinylamide ligands.

molecules play central roles in medicinal chemistry, acting as diagnostic agents or therapeutics.<sup>[96]</sup> Industrial pharmaceutical companies are showing a renewed interest in therapeutic peptides translated by the increase in investment and research efforts in this field.<sup>[97]</sup> Indeed, these therapeutics are very interesting due to their remarkable potency, high therapeutic index, high selectivity, low toxicity and biodegradability.<sup>[98]</sup> Currently, there are nearly 80 peptide drugs on the global market, more than 150 peptides in the stage of clinical development and another 400–600 peptides subjected to preclinical studies.<sup>[98c,99]</sup> In the recent years, a considerable progress has been made through the development of new routes of administration and new strategies to improve their low oral bioavailability, *in vivo* plasma stability and short circulation time.<sup>[96b,100]</sup> With their tremendous potential as promising drug candidates, the market of synthetic therapeutic bulk peptides is expected to continue its momentum and further broaden over the coming years.

So far, the accessibility to many peptidic drugs using the classical ways of stepwise peptide synthesis in solution or on solid supports is still impeded by their expensive production, challenging purification and major environmental issues.<sup>[101]</sup> Hence, huge amounts of volatile, toxic and hazardous organic solvents (DMF, NMP, THF, DCM, 1,4-dioxane), coupling reagents and bases (Et<sub>3</sub>N, DIPEA, piperidine) are usually required during the synthesis and isolation steps.<sup>[102]</sup> Therefore, with regard to atom economy and environmental sustainability, the chemical production of peptides is among the most problematic synthetic procedures.<sup>[102–103]</sup>

Peptide synthesis can be made greener and more sustainable<sup>[104]</sup> by designing coupling reagents with improved safety profile<sup>[105]</sup> and replacing standard organic solvents with green substitutes.<sup>[106]</sup> One particularly active area is the use of aqueous media to perform the coupling of amino acids.<sup>[107]</sup> Complementary alternatives include the use of solvent-free conditions,<sup>[5c,108]</sup> the use of minimal volumes of solvents,<sup>[109]</sup> and the use of continuous-flow conditions.<sup>[110]</sup> In addition to these approaches, ball-milling mechanochemical<sup>[111]</sup> or mechanoenzymatic<sup>[112]</sup> solvent-free conditions were also applied to the synthesis of peptides. Homogeneity and reactivity of

solid-state reaction mixtures can be improved by LAG where small amounts of solvents are used as an additive.<sup>[111c,113]</sup>

To complement this review, we will only be discussing the synthesis of biologically active peptides by mechanochemistry.

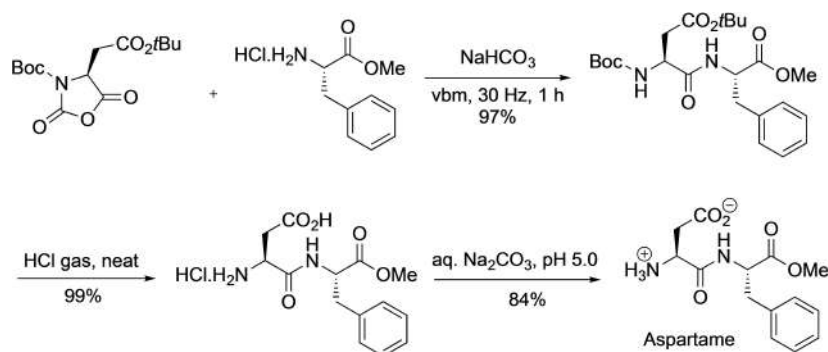
## 7.1. Peptides synthesis by mechanochemical ball-milling and extrusion

Ball milling has gained a remarkable interest in peptide synthesis in recent years owing to the possibility of avoiding the use of problematic organic solvents, reducing the reaction time and isolating elusive reaction intermediates.<sup>[4,114]</sup> Yet, the wide spreading of ball milling processes among the peptide production industry is still limited due to their discontinuous batch production and difficulty in further up-scaling which is not straightforward.<sup>[4]</sup> The scalability problem can be overcome using traditional solution-based continuous flow peptide production processes which proved beneficial but are seriously confined when considering highly concentrated reaction mixtures and the related appearance of solids.<sup>[115]</sup> Recently, reactive extrusion enabled mechanochemical peptide synthesis with very short reaction times and conditions free from CMR chemicals.<sup>[116]</sup>

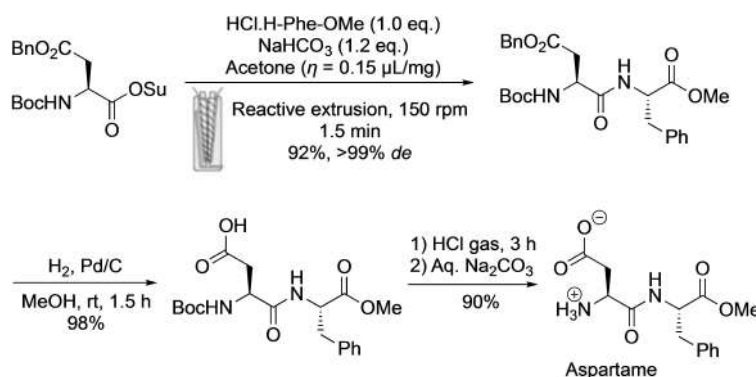
## 7.2. Aspartame

The field of mechanochemical peptide synthesis was pioneered by our group by reporting in 2009 a novel strategy for the synthesis of  $\alpha$ -peptides by the coupling of urethane-protected  $\alpha$ -amino acid *N*-carboxyanhydride (UNCA) derivatives and  $\alpha$ -amino esters under solvent-free conditions enabled by ball-milling activation.<sup>[111a]</sup> This methodology has been exemplified by the synthesis of the dipeptide aspartame, the world's most widely used artificial sweetener marketed as NutraSweet, Equal, Sugar Twin and AminoSweet. Ball-milling of Boc-Asp(OtBu)-NCA and HCl·H-Phe-OMe in the presence of NaHCO<sub>3</sub> for 1 h yielded the protected dipeptide as the only product beside inorganic salts (Scheme 44). Boc and *t*Bu protecting groups were then cleaved, without solvent, under acidic conditions in a gas–solid reaction for 3 h to yield the hydrochloride form of aspartame, along with volatile by-products. The latter form was then dissolved in water, neutralized with an aqueous solution of Na<sub>2</sub>CO<sub>3</sub> to the isoelectric point (pH 5.0), filtered and dried *in vacuo* to yield aspartame as white powder (84% yield).<sup>[111a]</sup>

Recently, this industrially relevant dipeptide aspartame was synthesized by the same group, however using the mechanical reactive extrusion.<sup>[116]</sup> Accordingly, a pre-mixed mixture of Boc-Asp(OBn)-OSu, HCl·H-Phe-OMe, NaHCO<sub>3</sub> and reagent grade acetone was slowly poured into the extruder's barrel that was heated at 40 °C, the speed of screws being set at 150 rpm and the output valve turned towards the recirculation pipe. After a residence time of 1 minute 30 seconds, crude Boc-Asp(Bn)-Phe-OMe was collected from the extruder's die as a white paste (Scheme 45). After purification of the crude by aqueous acid workup, removal of the benzyl group by hydrogenation of Boc-

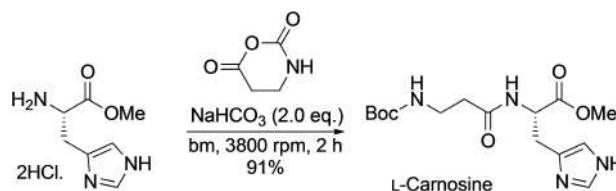


Scheme 44. Solvent-free synthesis of aspartame.



Scheme 45. Aspartame synthesis using twin-screw extrusion.

Asp(OBn)-Phe-OMe followed by deprotection of the Boc group under solvent-free gaseous HCl conditions and further precipitation at the isoelectric point (pH 5.0) furnished aspartame in 81 % overall yield over three steps. Calculating the space-time yields (STY) for the reactive extrusion process and comparing them to ball-milling or solution syntheses, the efficiency of the extrusion was clearly demonstrated.



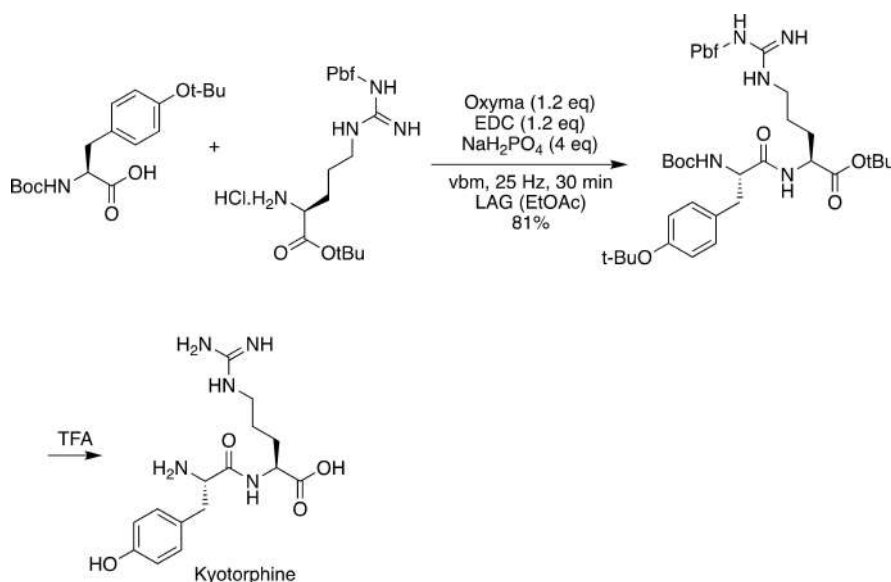
Scheme 46. Dipeptide synthesis involving a  $\beta$ -amino acid NCA.

### 7.3. L-Carnosine

L-Carnosine is a mammalian dipeptide composed of  $\beta$ -alanine and L-histidine amino acids and found in brain and muscle tissues. It was isolated from Liebig's meat extract and first synthesized by Baumann and Ingvaldsen<sup>[117]</sup> and Barger and Tutin.<sup>[118]</sup> It has proven to display hydroxylradical scavenger properties and act as an antiglycating agent, and antioxidant-based on *in vitro* tests.<sup>[119]</sup> Hernandez et al. applied the NCA strategy<sup>[111a]</sup> to the synthesis of  $\alpha,\beta$ - and  $\beta,\beta'$ -dipeptides, by starting from  $\beta$ -amino acid NCA (Scheme 46). Ball-milling of Boc- $\beta$ -Ala-NCA and 2HCl.H-His-OMe in the presence of NaHCO<sub>3</sub>, under solvent-free conditions, for 2 h yielded the protected  $\alpha,\beta$ -dipeptide in excellent yield (91 %).<sup>[111d]</sup>

### 7.4. Kyotorphine

Kyotorphine is a dipeptide (Tyr-Arg) which was first isolated from bovine brain by Takagi in 1979<sup>[120]</sup> and synthesized by the same group in 1980.<sup>[121]</sup> This short peptide presents potent analgesic properties, being four times more potent than endogenous opioids.<sup>[122]</sup> Making use of a procedure for peptide mechanochemistry previously developed in our group,<sup>[113b]</sup> we showed that a protected kyotorphine could easily be prepared by mechanochemistry.<sup>[123]</sup> Indeed, the direct coupling of the protected tyrosine Boc-Tyr(OtBu)-OH with the amino ester hydrochloride HCl.H-Arg(Pbf)-OtBu was achieved by reacting both reagents for 30 min in a vibrating ball mill in the presence of Oxyma/EDC as coupling agent and Na<sub>2</sub>HPO<sub>4</sub> as a base (Scheme 47). The expected protected dipeptide was obtained as a pure compound with a very good yield (81 %). Kyotorphine could then be obtained by a one-pot deprotection using TFA,



Scheme 47. Milling approach to kyotorphine.

confirming that short bioactive peptides can be prepared by mechanochemistry in a very efficient and sustainable way.

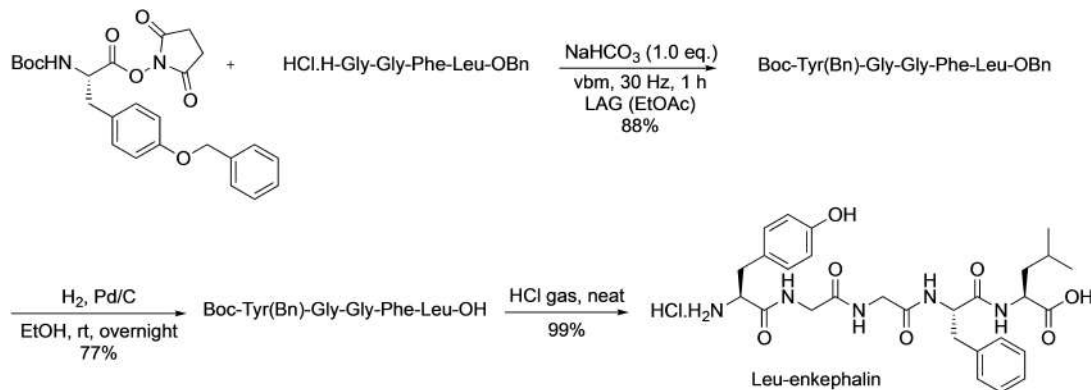
### 7.5. Leu-Enkephalin

Leu-enkephalin is a pentapeptide which was isolated from the porcine brain and synthesized for the first time by Hughes and coworkers in 1975.<sup>[124]</sup> This long peptide is an endogenous ligand which binds the opioid receptors and has a prominent role in processing sensory information in the brain.<sup>[125]</sup> Our group reported the first efficient and environmental benign syntheses of tetra- and pentapeptides by liquid-assisted ball milling of stoichiometric amounts of non-hazardous reactants, Boc-protected  $\alpha$ -amino acid *N*-carboxyanhydrides (Boc-AA-NCA) or Boc-protected  $\alpha$ -amino acid *N*-hydroxysuccinimide esters (Boc-AA-OSu) with  $\alpha$ -amino acid alkyl ester salts, in the presence of  $\text{NaHCO}_3$  and minimal amounts of EtOAc as a green

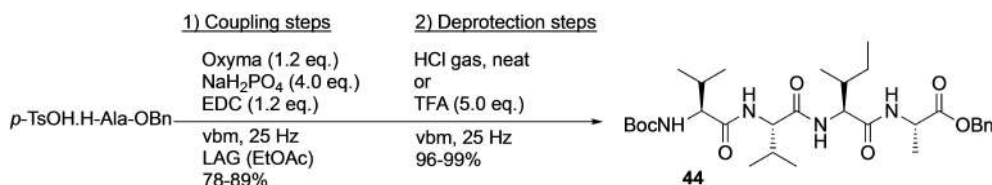
solvent. By iteration, this approach was successfully extended to the synthesis of Leu-enkephalin with 46% overall yield, over 9 steps (Scheme 48). In the last steps, treatment of the tetrapeptide  $\text{HCl}\cdot\text{H-Gly-Gly-Phe-LeuOBn}$  with Boc-Tyr(Bn)-OSu furnished the pentapeptide Boc-Tyr(Bn)-Gly-Gly-Phe-Leu-OBn in 88% yield. Hydrogenation of the benzyl moieties using Pd/C under a  $\text{H}_2$  atmosphere provided Boc-Tyr(Bn)-Gly-Gly-Phe-Leu-OH in 77% yield which upon further gaseous HCl-mediated deprotection of the Boc group afforded Leu-enkephalin in a quantitative yield.<sup>[113a]</sup>

### 7.6. VWIA tetrapeptide

One of the promising therapeutic strategies for Alzheimer's disease (AD) consists of targeting the early oligomerization of amyloid  $\beta$  protein ( $\text{A}\beta$ ).  $\text{A}\beta(39-42)$  tetrapeptide is a small therapeutic peptide that inhibits  $\text{A}\beta(42)$ -induced neurotoxicity by



Scheme 48. Leu-enkephalin synthesis.



Scheme 49. Mechanochemical approach to a small therapeutic peptide.

preventing A $\beta$  aggregation.<sup>[126]</sup> The Boc-VVIA-OBn tetrapeptide **44** was synthesized in our group using the previously developed ball-milling strategy with 56% overall yield (88% purity), over 5 steps (Scheme 49).<sup>[113b]</sup> Accordingly, the coupling steps were achieved by ball-milling from the amino ester salts (*p*-toluenesulfonate or hydrochloride) with Boc-AA-OH in the presence of Oxyma, NaH<sub>2</sub>PO<sub>4</sub>, EDC and small amounts of EtOAc as the liquid grinding assistant. The desired coupling products were isolated in good yields and pleasing purities (78-89%) following the conventional post-treatments based on aqueous acid/base workups (extractions and washings). The removal of the protecting groups was either performed by treating the Boc-protected peptides with gaseous HCl to provide the amino esters as hydrochlorides in high yield and purity or by treating them with TFA under mechanochemical conditions to furnish the corresponding esters as TFA salts in quantitative yield.<sup>[111e]</sup>

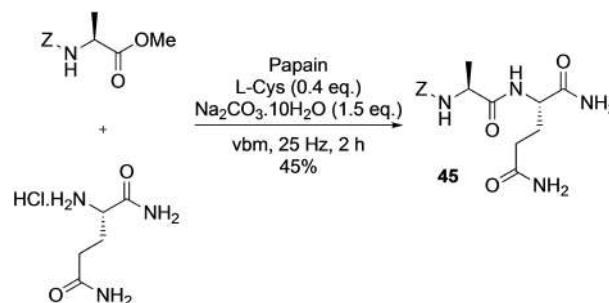
In this study, the same peptide was also synthesized in solution and in solid phase (SPPS). Efficiency and environmental impact were evaluated for each method. Comparison of the three different synthetic approaches revealed that in terms of yield and purity, ball-milling and SPPS were similar, both being superior to solution. Regarding the environmental impact, the mechanosynthesis presented the lowest generation of waste.

### 7.7. L-Alanyl-L-glutamine

The dipeptide L-alanyl-L-glutamine is widely used in cell culture and as an important nutrient supplement.<sup>[127]</sup> Hernandez et al. reported the synthesis of Z-L-Ala-L-Gln-NH<sub>2</sub> **45**, a derivative of the valuable dipeptide L-alanyl-L-glutamine, using papain enzyme as a catalyst and sodium carbonate decahydrate under ball-milling conditions. Accordingly, Z-Ala-OMe, HCl.H-Gln-NH<sub>2</sub>, papain, L-Cys, and Na<sub>2</sub>CO<sub>3</sub>·10H<sub>2</sub>O were ground in the ball mill for 2 h to furnish the desired dipeptide **45** in 45% yield (Scheme 50). The moderate yield could be associated to the lower degree of recognition of the glutamine amide by the enzyme.<sup>[112a]</sup>

### 7.8. Endomorphin-1

The bioactive tetrapeptide endomorphin-1 (EM1), H-Tyr-Pro-Trp-Phe-NH<sub>2</sub>, is an endogenous ligand of the  $\mu$ -opioid receptor that is currently regarded as a substantial lead for the design of painkillers lacking any harmful side effects.<sup>[128]</sup> Recently, Anselmi et al. reported the unprecedented use of nanocrystalline

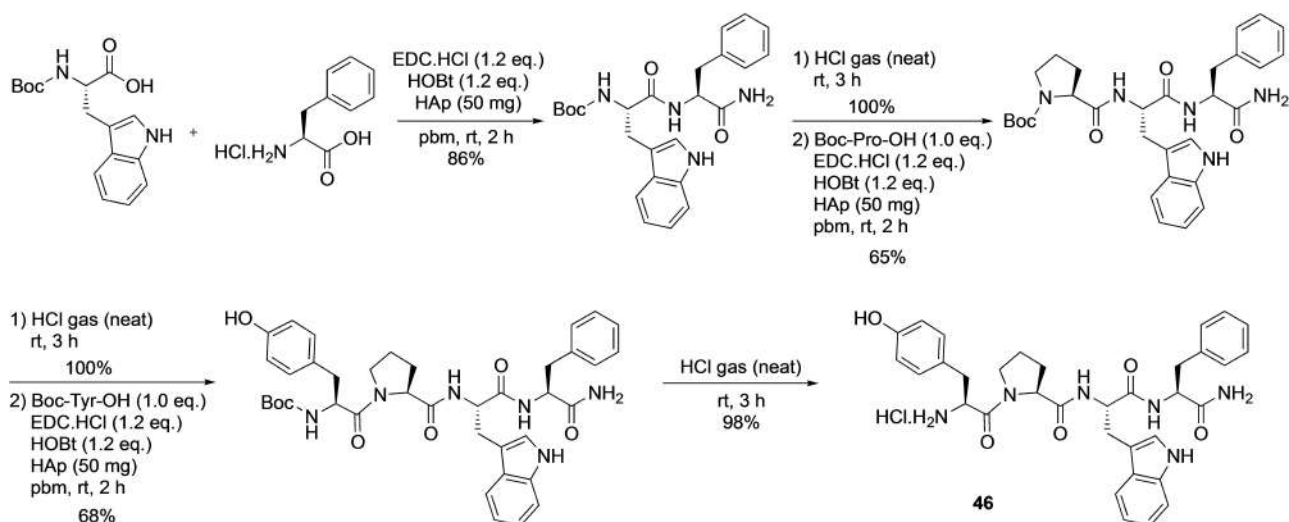


Scheme 50. Mechanoenzymatic peptide coupling.

hydroxyapatite (HAP) powder to promote the minimal solvent-assisted mechanochemical coupling between *N*-protected amino acids and amino·HCl partners, in the presence of the coupling reagents EDC·HCl/HOBt.<sup>[129]</sup> Accordingly, EM1·HCl salt **46** was synthesized in 6 steps by solvent-free planetary ball milling (Scheme 51). The mechanical milling of Boc-Trp-OH and HCl.H-Phe-NH<sub>2</sub> in the presence of EDC·HCl, HOBt and HAP for 2 h at rt gave the dipeptide Boc-Trp-Phe-NH<sub>2</sub> in 86% yield (94% purity). The coupling reaction is very efficient, even if the use of explosive and toxic HOBt should be avoided from a safety and sustainability point of view. The treatment of Boc-Trp-Phe-NH<sub>2</sub> with gaseous HCl allowed the removal of the *N*-Boc protecting group and provided H-Trp-Phe-NH<sub>2</sub>·HCl salt in quantitative yield. Further coupling of this salt with Boc-Pro-OH under the same previous ball milling conditions delivered the tripeptide Boc-Pro-Trp-Phe-NH<sub>2</sub> in 65% yield (77% purity). Deprotection of *N*-Boc group as before, and subsequent coupling with Boc-Tyr-OH afforded the *N*-Boc-protected EM1 in 68% yield (89% purity). Finally, Boc deprotection of the latter followed by its suspension in EtOAc, sonication and centrifugation furnished EM1·HCl **46** in almost quantitative yield and excellent purity (94%).

### 7.9. 13-Mer PTP7

13-mer PTP7 **47**, a truncated derivative of the antimicrobial peptide gaegurin6 isolated from the frog *Rana rugosa*, is a peptide of sequence H-Phe-Leu-Gly-Ala-Leu-Phe-Lys-Ala-Leu-Ser-Lys-Leu-Leu-OH. This peptide is of some interest for its substantial toxicity against miscellaneous cancer cell lines as prostate, human lung, breast and hepatocellular carcinomas.<sup>[130]</sup> Recently, the synthesis of this cytotoxic 13-mer PTP7 was described by Santino et al.<sup>[131]</sup> using the previously reported

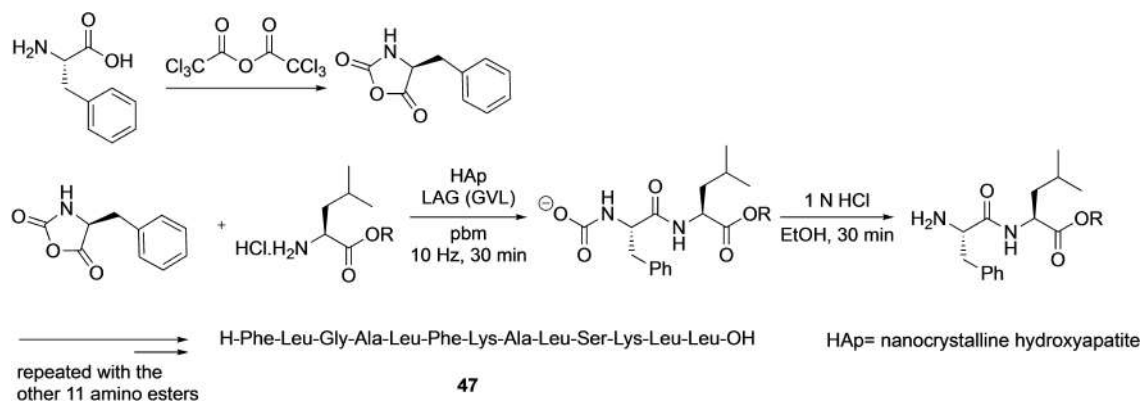


Scheme 51. Synthesis of endomorphin-1.

nanocrystalline HAp powder together with activated amino acid as *N*-carboxyanhydride (Scheme 52).<sup>[129]</sup> Ser was introduced as Ser(OtBu)NCA, and Lys as Lys(Boc)NCA. For each coupling, an equimolar mixture of NCA and the amino partner hydrochloride salt was mixed with HAp powder and  $\gamma$ -valerolactone (GVL). After milling into a pre-cooled agate jar equipped with three balls at a frequency of 10 Hz for 30 min, the crude mixture was diluted with ethanol, the suspension was separated by centrifugation to recover HAp crystals and the collected organic layers were treated with HCl (1 M) in ethanol for 30 min at RT. Following each coupling step and after solvent evaporation under reduced pressure, traces of insoluble materials were removed by filtration, and the intermediates were crystallized as HCl salts from water/ethanol. Next, the partially protected sequence was treated with HCl (0.5 N) in ethanol for 5 h at 0 °C and the residues were suspended in EtOAc and sonicated for 15 min. The peptide-HCl salt was collected by centrifugation (3000 rpm), and the procedure was repeated to furnish crude PTP7 **47** in 55% overall yield (76% purity).

## 8. Conclusion

In this review was summarized research work reporting the synthesis of bioactive molecules, Active Pharmaceutical Ingredients or compounds evaluated in biological tests, using mechanochemistry technique. On a small scale, the vibratory and planetary mills are widely used and allow quick and efficient access to the desired product, while limiting the consumption of organic solvents. In addition, the mechanochemical approach is not limited to research laboratory scale. High-capacity mills make the preparation of larger quantities of product possible. However, the preferred approach in this case is reactive extrusion which allows working with solids or concentrated mixtures and producing the desired compound continuously, in large quantities. The structural diversity of the molecules synthesized so far is significant and the mechanochemist's toolbox continues to expand. This suggests that in the future mechanochemistry could become central in the discovery of new bioactive molecules and drugs (medicinal mechanochemistry) and that the production of drugs, including



Scheme 52. Mechanochemical synthesis of cytotoxic 13-mer PTP7.



large-scale synthesis and formulation, could be carried out using extruders. Noteworthy, chemists in industry are showing an increased interest in mechanochemistry.<sup>[33]</sup> Once widely adopted, because of the drastic reduction of the amount of organic solvents used, this strategy will contribute decisively to the development of sustainable chemistry with a positive impact on the environment.

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## Conflict of Interest

The authors declare no conflict of interest.

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