RESEARCH ARTICLE

Benign synthesis of therapeutic agents: domino synthesis of unsymmetrical 1,4-diaryl-1,4-dihydropyridines in the ball-mill

Cristina Blazquez-Barbadillo, Juan Francisco González, Andrea Porcheddu, David Virieux, José Carlos Menéndez and Evelina Colacino

ABSTRACT

The solvent-free preparation of unsymmetrical N-aryl-5,6-unsubstituted-1,4-dihydropyridines (DHPs) by ball-milling was investigated. Three different mechanochemical domino reactions (one-pot/one step or stepwise) were studied, the process parameters underlying any mechanochemical process were modulated and the differences with solution-based mechanistic pathways were disclosed. The selection of the most suitable method was driven by the physical state of both the reactants and the intermediates, while the physical state of the final 1,4-DHP directed the choice of the catalyst (Lewis vs Brønsted) and the type of work-up to recover the final products (by column chromatography vs precipitation in water). The results herein described are unprecedented in the arena of synthetic methods to access diversely substituted 1,4-DHPs, an N-heterocyclic scaffold relevant to both synthetic and medicinal purposes.

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Introduction

Nowadays, the combination of enabling technologies with synthetic strategies and processes 'benign by design' and characterized by minimal waste generation represents a powerful approach to address the quest for a sustainable organic chemistry. In this context, synthetic strategies combining tandem, domino or cascade (1, 2), with multicomponent reactions (3) (MCR) have attracted increasing attention, being usually characterised by step and intrinsically high atom, step and pot economy while generating structural complexity and molecular diversity with operational simplicity and waste minimization. The efficiency and the low ecological footprint of these powerful synthetic tools can be additionally improved by conducting the reactions using mechanochemistry, an environmentally friendly technology closely intertwined with the 12 principles of Green Chemistry (4,5). The low environmental impact displayed by mechanochemical processes has been recently demonstrated for the preparation of Active Pharmaceutical Ingredients (APIs) by life cycle assessment studies in the continuous flow mechanochemical manufacturing of nitrofurantoin (6), and its quantitative assessment against the 12 principles of green chemistry by using the DOZ N2.0 tool (7). Therefore, the possibility to access complex biologically relevant structures and value-added compounds from simple precursors by mechanochemical domino or multicomponent reactions is very appealing (8). Mechanochemically-activated synthetic approaches to heterocyclic systems were previously reported for both: (i) domino or cascade reactions to prepare dihydropyrroles and pyrroles (via oxidative cyclocondensation reactions) (9), pyrazolones and isoxazolones (by asymmetric organocatalytic Mannich reaction-fluorination) (10), indolylquinones (by Michael addition-oxidation sequence) (11), and (ii) multicomponent reactions to access pyrroles (by Hantzsch pyrrole synthesis) (12, 13), thiophenes (by Gewald reaction) (14), coumarine thiazolidinones (15), 4H-chromene derivatives.
(16), dihydropyrimidones (17) and 2,3-dihydro-1,2,6-thia-
diazine 1-oxides (18) (by Biginelli-type reaction), benzoa-
zines (by condensation/Mannich base ring-closure
reactions) (19), α-amino phosphonate derivatives (by
Kabachnik-Fields reaction) (20), or peptidic-like adducts
(by Ugi 4-CR) and α-acyloxy amides (by Passerini-3CR) (21).

In our ongoing work in the field of medicinal mechan-
chemistry (22–25), applied to the sustainable prepara-
ion of pharmacologically relevant scaffolds (26, 27),
Active Pharmaceutical Ingredients (APIs) (28–31) and
World Health Organisation (WHO) essential medicines
(28, 32–35), we pointed our attention pointed towards
the development of mechanochemical methods to
access 1,4-dihydropyridines (1,4-DHPs), a
N-heterocyclic scaffold relevant to both synthetic and medicinal
purposes. The great versatility of the plethora of solution-
based synthetic methods already available (36–39), and
the careful choice of the appropriate precursors, make
1,4-DHPs privileged scaffolds with a biological activity
that can be tuned, in potency and selectivity, to the
desired extent to target specific pharmacological

effects in diverse therapeutic areas (40–43). Several
1,4-DHPs are therapeutic agents used in medical prac-
tice worldwide as calcium-channel-blockers (e.g. nifedi-
pine) for treating cardiovascular diseases (44).

Hantzsch’s method, a 4-CR involving an aromatic alde-
hyde, a β-ketoester and ammonia, is the most common
synthetic strategy to access N-unsubstituted symmetrical
1,4-DHPs (Scheme 1(a)) (45). However, this method is
unsuitable for preparing some relevant derivatives such as
unsymmetrical N-aryl-1,4-dihydropyridines and N-aryl-
5,6-unsubstituted-1,4-DHPs, which are still scarcely inves-
tigated for their biological activity, due to the lack of
efficient synthetic methods. Moreover, due to the pres-
ence of a C₉-C₆-unsubstituted positions, they show
enantioselective reactivity enabling the preparation of
complex heterocyclic frameworks. To overcome these
limitations, one of us (46) previously reported a straight-
forward solution-based domino 3-CR of 1,4-DHPs from
aromatic amines, αβ-unsaturated aldehydes and ethyl
acetoacetate catalysed by cerium(IV) ammonium nitrate
(CAN) at room temperature during one hour (Scheme 1
(b)) (46). The synthetic strategy relies on the use of αβ-
unsaturated aldehydes, very often used as inexpensive
bifunctional reagents employed as C-building blocks in
the preparation of pyridines, 1,2-DHPs and 1,4-DHPs,
due to the presence of two electrophilic centers (37).

To the best of our knowledge, there are very few
reports dealing with the use of ball-milling processes
to prepare 1,4-DHPs, including some Hantzsch reaction
of aromatic aldehydes, alkyl acetoacetates and alkyl
3-aminocrotonates promoted by Lipozyme® RM IM (tria-
cylglycerol acyldrolase) (48), and one report on the
synthesis of unsymmetrical N-aryl-1,4-dihydropyridines
by manual grinding (Scheme 1(c)) (47). In the latter
reaction, the domino MCR involved the stoichiometric
coupling of an aromatic aldehyde with an active methylene
compound (malononitrile, ethyl cyanocetate or cy-
noacetamide) and an aza-Michael reaction of an aniline
on diethyl acetylenedicarboxylate (DEAD). The
rearrangement of the intermediates generated the cor-
responding N-aryl-1,4-dihydropyridines (Scheme 1(c))
(47, 49).

By virtue of the importance of 1,4-DHP scaffold in syn-
thetic and medicinal chemistry the use of mechano-
chemical methods to access them becomes particularly
appealing. Based on our previous findings in solution
(Scheme 1(b)) (46), we report herein the solvent-free
preparation of N-aryl-5,6-unsubstituted-1,4-DHPs by
ball-milling. The purpose of this endeavor is to: (i)

improve the ecological footprint of the solution-based
methods, (ii) avoid the use of any critical raw materials
(50) (e.g. the rare light earth elements such as cerium)
or heterogeneous catalysts (38), and (iii) investigate the
reactivity and selectivity of the reactive system by
three different mechanochemical methods, one-pot/

one step or stepwise, to highlight the differences with
solution-based mechanistic pathways (51). Process
parameters were investigated in neat or liquid-assisted
grinding (52) (LAG) conditions, including the influence
of the milling speed, milling stress and milling media,
the size and number of the milling balls, with or

without additives.

Results and discussion
Optimization of Reaction Conditions. Based on the
C-anchored synthetic strategy previously reported by us
in solution (Scheme 1(b)) (46), aniline, cinnamaldehyde
and ethyl acetoacetate were selected as benchmark sub-
strates to both optimize the reaction conditions to
prepare 1,4-DHP 3 and to investigate the influence of
process parameters in the ball mill. The study was con-
ducted according to three different synthetic pathways:
(i) the one-pot/one-step domino 3-CR (Method A,

Scheme 2), based on the previously reported method in
solution (46), (ii) the one-pot /two-step 3-CR involving
the formation of an imine 1 (step 1), followed by the
1,4-Michael conjugate addition/ cyclocondensation
sequence (step 2) (Method B1, Scheme 2), and (iii) the
one-pot /two-step 3-CR involving the formation of a
β-enamino ester 2 (step 1), telescoped with the cinna-
maldehyde through a 1,4-Michael addition/cyclization
and dehydration sequence (step 2) (Method B2,

Scheme 2). In all cases, water was generated as the

only by-product of the reaction.
After some unsuccessful experiments with a vibratory ball mill (see Supporting Information), Method A was investigated in a planetary ball mill. To this end, equimolar amounts of the reactants and the Lewis catalyst CAN (46, 53) (5 mol %) were ball milled for 4 h at 450 rpm, in a ZrO2 jar containing 25 balls of 5 mm diameter of the same material. Even if the analysis of the crude by GC-MS was promising, showing that 1,4-DHP was the major product (60%), a residual amount of aniline (13%) was still present, together with a by-product (27%) that was tentatively identified, based on its GC-MS fragmentation, as the corresponding compound generated by the competitive 1,2-addition reaction of ethyl acetoacetate to E-cinnamaldehyde, not previously reported in solution nor detected under vibrating ball milling. Furthermore, liquid-assisted

**Scheme 1.** MCRs for the preparation of diverse 1,4-DHP scaffolds: (a) and (b) in solution (45, 46) or (c) solvent-free by manual grinding (47) (EWG = electron-withdrawing group).

**Scheme 2.** Screened pathways to 1,4-DHP 3 by ball-milling.
grinding (52) (LAG) conditions with EtOH (55.1 μL, η = 0.1 μL/mg) were detrimental, leading to an additional unidentified by-product, as indicated by the GC-MS analysis of the crude mixture. In all cases, GC-MS analyses of the crude showed that the rapid (and undesired) oxidative aromatization of 1,4-DHPs to the corresponding pyridines, previously reported with excellent yields in the presence of CAN (1.5 equiv) by manual grinding over 15 min (54, 55), did not occur at all. Replacement of the Lewis acidic catalyst CAN by p-toluene sulfonic acid (56, 57) (p-TsOH, 5 mol %) led to identical results, while no reaction occurred when using the organocatalyst L-proline (58) (10 mol %). In this case, the 1,4-DHP 3 was not formed at all and the 1,2-addition reaction was predominant. Whatever the reaction and process conditions, the formation of the 1,2-addition by-product could not be avoided, strongly suggesting that the one-pot/one-step 3-CR procedure commonly successful in solution was unsuitable for ball-milling. To overcome the above-mentioned limitations and to circumvent the formation of the 1,2-addition by-product, the reaction was also studied in stepwise manners (Methods B1 and B2, Scheme 2).

To optimise the reaction conditions and the mechanoochemical parameters, the synthesis of imine 1 from E-cinnamaldehyde and aniline was investigated (step 1, Method B1, Scheme 2) and a selection of data is reported in Table 1. In addition, process parameters such as type of mechanical stress (in a planetary or vibrating ball-mill), milling speed, milling time, type of grinding media (stainless steel or zirconium oxide), number and size of the milling balls, in neat conditions or using liquid-assisted grinding (LAG) procedures, with or without additive, were investigated.

| Entry | t (h) | rpm | ZrO2 ball diameter | H^+C = O (%)b | Conversion (%)b |
|-------|------|-----|--------------------|---------------|----------------|
| 1<sup>c</sup> | 2<sup>c</sup> | / | / | / | 12.7<sup>e</sup> / 25 | / | / | 26<sup>c</sup> | 74 |
| 2<sup>c</sup> | 2<sup>c</sup> | 450<sup>c</sup> | / | / | / | 10.3<sup>c</sup> / 25 | / | 10<sup>e</sup> (21)<sup>d</sup> | 87<sup>c</sup> (77)<sup>d</sup> |
| 3<sup>c</sup> | 2 | 450<sup>c</sup> | / | / | / | 10.3 / 25 | / | 12<sup>e</sup> | 86 |
| 4 | 2 | 450<sup>c</sup> | / | / | / | / | 10.3/25 | / | 8.8/3 | 13 | 85 |
| 5 | 2 | / | / | / | / | / | / | / | / | 12 | 84 |
| 6 | 2 | / | / | / | / | / | / | / | / | 8.8/3 | 28 | 78 |
| 7 | 2 | 450<sup>c</sup> | / | / | / | / | / | / | / | 10.3 | / | 9 | 88 |
| 8 | 2 | 450<sup>c</sup> | / | / | / | / | / | / | / | 20.6 | / | 13 | 87 |
| 9<sup>e</sup> | 2 | 450<sup>c</sup> | / | / | / | / | / | / | / | 10.3 | / | 11<sup>e</sup> | 89 |
| 10 | 2 | 450<sup>c</sup> | / | / | / | / | / | / | / | 10.3 | / | 11 | 89 |
| 11 | 2 | 450<sup>c</sup> | / | / | / | / | / | / | / | 8.0 | / | 14 | 86 |
| 12<sup>e</sup> | 2<sup>e</sup> | 450<sup>c</sup> | / | 5.5 | 10.3 | / | / | / | 6 | 94 |
| 13<sup>c</sup> | 2<sup>c</sup> | 450<sup>c</sup> | / | 5.5 | 10.3 | / | / | / | 25<sup>e</sup> (20)<sup>d</sup> | 75<sup>c</sup> (80)<sup>d</sup> |
| 14 | 2 | 450<sup>c</sup> | / | 10.3 | 10.3 | / | / | / | / | / | / | 19 | 75<sup>c</sup> |
| 15 | 2<sup>c</sup> | 450<sup>c</sup> | / | / | / | / | / | / | / | / | / | / | 19 | 75<sup>c</sup> |

<sup>a</sup>Reaction scale: 1.5 mmol (R = H, NO2) using ZrO2 or stainless steel (SS) as grinding media, in 12 mL jar (for planetary ball-mill) or in 10 mL jar (for vertical mixer-mill);<sup>b</sup> Residual amount of aldehyde (R = H) and conversion were determined by 1H NMR in CDCl3 by comparing the area of H^+C = O proton of E-cinnamaldehyde (at 9.75 ppm) to the area of proton H^+C = N (at 8.34 ppm) for 1. Data for R = NO2 are given in entry 14;<sup>c</sup> The grinding media was SS;<sup>d</sup> The reaction time was 3 h;<sup>e</sup> MgSO4 (100 mg) was added as dehydrating agent;<sup>f</sup> Liquid assisted grinding conditions (LAG) using EtOH (682 μL, η = 2 μL/mg), with η value defined<sup>22</sup> as the volume of the solvent (in μL) / the sample weight (in mg);<sup>g</sup> o-nitrocinammaldehyde was used as substrate (H^+C = O proton at 9.82 ppm, H^+C = N proton at 8.43 ppm for 4 in CDCl3).
surface-mediated phenomena influencing the outcome of the reaction.

No substantial improvements were observed when milling the reactants in the presence of MgSO₄, as solid support and dehydrating agent (entry 4) or reducing the milling stress frequency (59) (using 3 ball of 10 mm diameter, total weight \( m_{\text{tot}} = 8.8 \text{ g} \), entry 5), leading to a similar amount of residual aldehyde (12-13%). The reaction was repeated by extending the milling time to 3 h (entry 2) but the conversion was decreased, indeed, the amount of water formed during ball-mill could be responsible for aldehyde hydration, which hamper the formation of the E-imine 1. When changing the type of mechanical stress and the milling stress frequency (59) (using 3 balls of 10 mm diameter at 50 Hz, total weight \( m_{\text{tot}} = 8.8 \text{ g} \), entry 6), conversion was also lower, and the amount of residual aldehyde increased (21% and 28% respectively).

It was also speculated that the outcome of the reaction was driven by the rheology of the mixture: at the beginning of the synthesis, both reactants are liquids, so that no mechanical effects can be evoked in this stage. However, as soon as the E-imine 1 is formed, the mixture turned to a solid, desirable in view of the subsequent mechanochemical step (Scheme 2, Method B1, step 2). The visual inspection of the crude mixture containing the newly formed solid E-imine 1 showed a non-homogenous aspect, with zones characterized by a finely dispersed pale-yellow powder and others appearing as wet clumps. Postulating that better conversions could be achieved by avoiding the formation of the clumps, a set of experiments was planned to increase the milling stress frequency. The modulation of this process parameter was achieved by reducing the size of the balls, while keeping their weighted amount close to that one used in the most promising experiment (entry 2). Therefore, the 25 balls in ZrO₂ (5 mm, \( m_{\text{tot}} = 10.3 \text{ g} \)) were replaced by 1 mm or 2 mm ZrO₂ balls (\( m_{\text{tot}} = 10.3 \text{ g} \), Table 1, entries 7 and 10). The results were comparable to those already obtained so far, and were not improved by adding MgSO₄ (entry 9) or by doubling the weighted amount of 2 mm balls (entry 8), (i.e. 9-13% of residual aldehyde still detectable by GC-MS in the crude mixture).

A possible strategy to reduce the clumps, to keep both increased stress frequency and the individual impacts more energetic, was to mix balls of different sizes (Table 1, entries 11–15). Their relative weighted amount was also investigated: in two cases (entries 12 and 15), better results were obtained (e.g. the residual amount of cinnamaldehyde was 6–7%), while the addition of EtOH as LAG solvent was detrimental, also when a solid reactant such as o-nitrocinnamaldehyde (entry 13) was used, or by increasing the amount of 1 mm ZrO₂ balls (entry 14). Despite all the attempts, it was impossible to achieve complete conversion of the starting materials, even when an excess of aniline (1.5 equiv) was added.

Having disclosed the suitable process conditions to prepare E-imine 1 (Table 1, entry 12) in the ball mill, the optimization of the cascade reaction, i.e. 1,4-conjugate addition / cyclisation / dehydration occurring in the second step (Scheme 2, Method B1, step 2) was directly carried out on the crude obtained from step 1.

Therefore, aiming to disclose the most suitable catalyst between CAN or p-TsOH, two experiments were run in parallel. To the jar already containing E-imine 1 and the mixture of zirconium oxide balls having 1 mm (\( m_{\text{tot}} = 5.5 \text{ g} \)) and 2 mm (\( m_{\text{tot}} = 10.3 \text{ g} \)) diameter, a stoichiometric amount of ethyl acetoacetate was added, together with the catalyst (15 mol%) and EtOH (1.27 mL, \( \eta = 2 \mu \text{L/mg} \) (60). The mixture was ball-milled at 450 rpm for two hours, then the crudes were analysed by GC-MS. It was found that, for both experiments, the E-imine was fully converted, but the 1,4-DHP 3 was formed together with the by-product 5, arising from the competing 1,2-nucleophilic addition to the aldehyde function. This result was also confirmed by the \(^1H\) NMR analyses of the crude mixture. Aiming to avoid the formation of by-product 5, the new set of experiments was conducted in the same milling conditions as before (for both steps 1 and 2), except for the milling speed, now reduced to 250 rpm (Scheme 3).

For both catalysts, the GC-MS analyses showed similar reactivity and selectivity profiles: 1,4-DHP 3 was the major product of the reaction together with traces (1%) of by-product 5. \(^1H\) NMR analyses of both crudes in CDCl₃ revealed that the 1,4-DHP 3 obtained by the CAN-catalysed reaction underwent to a fast degradation during the analyses, as also confirmed later by an aging experiment during 12 h in the NMR tube, complemented by GC-MS analyses run in parallel. This is not surprising, considering the strong oxidizing character of CAN catalyst, particularly effective for the oxidative aromatisation of 1,4-DHP to pyridines (54). However, this side reaction did not occur during ball-milling, as demonstrated by GC-MS analyses of the crude mixture immediately after the jar was opened. No characteristic signals of the corresponding pyridine by-product could be detected immediately after milling, the degradation occurring in solution upon exposure of the sample to the air.

Any attempt to recover the final product 3 by precipitation from water or any other (green) solvents was unsuccessful for both crude mixtures issued by CAN or p-TsOH catalysed reaction. This was due to the oily
physical state of the final product, that could be recovered later on only by a chromatographic purification, impacting negatively the sustainability of the entire process, and being the cause of low yield. Moreover, if the CAN-catalysed reaction lead 1,4-DHP 3 in a disappointing 11% yield after column, the chromatographic purification of the crude mixture issued from p-TsOH catalysed reaction was not successful, due to the concomitant formation of an unidentified by-product that was co-eluted with the desired 1,4-DHP 3.

To verify the hypotheses above and to potentially reconsider p-TsOH as a valuable alternative catalyst to CAN, solid 1,4-DHPs needed to be prepared, in order to allow an easier recovery by a straightforward work-up based on precipitation in water, followed by *in vacuo* filtration.

Therefore, solid reactants such as 1-naphthylamine and o-nitrocinnamaldehyde were selected as starting reagents for step 1. Two reactions were run in parallel for two hours at 450 rpm in the same milling conditions disclosed so far (Scheme 3), leading to the corresponding *E*-imine 4 (Table 1). In the second step, *p*-TsOH (15 mol%) was added, and different alkyl acetoacetates (Alkyl = Et, *t*-Bu) in each experiment. The mixtures were ball-milled at 250 rpm for two hours according to Method B1 (Scheme 2), and the solid final product was recovered by precipitation from water followed by *in vacuo* filtration.

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To confirm that the recovery of the product by a standard ‘organic’ solvent-based work-up negatively impacted the final yields, the experiments were repeated and the products recovered by a work-up involving a liquid–liquid extraction followed by a purification by column chromatography. As a result, the yields dropped drastically, to 52% and 50% respectively for compounds 6 and 7, confirming the initial assumption.

Being the reactivity and the selectivity of both catalysts the same in the milling conditions disclosed for Method B1, the choice of the most appropriate catalyst strictly depended on the physical state of the final 1,4-DHP, driving the choice of the work-up to be used for its recovery. Generally speaking, CAN is the suitable catalyst if the final target is an oil requiring a purification by column chromatography. At the same time, for solid 1,4-DHPs that can be recovered by precipitation in water, *p*-TsOH is a valuable alternative, not only because as Brønsted acid enhances the electrophilicity of the imine, favoring the 1,4-conjugate addition of the β-ketoester (62), but also to avoid the risks associated to the CAN-promoted aerobic over-oxidation to pyridines.

To study the mechanism of the reaction in the ball mill, the preparation of 1,4-DHP 3 was also explored.
by Method B2 (Scheme 1), involving the formation of a \( \beta \)-enaminoester intermediate, combining the nucleophilicity of the enamine and the electrophilicity of enones. For Method B2, the same process conditions disclosed in Method B1 (Scheme 3) were used. Aniline and ethyl acetoacetate were milled together in the first step for two hours at 450 rpm, leading to the corresponding \( \beta \)-enaminocarboxylic intermediate. The 1,4-Michael addition to cinnamaldehyde/cyclisation/dehydration occurred in the second step in the presence of Lewis catalysts CAN (15 mol%), milling the mixture for two additional hours. Even if the reactivity, selectivity and yield of the system were comparable to those obtained starting from the \( E \)-imine derivative (Method B1), the reactivity corresponding to the formation of the \( \beta \)-enaminocarboxylic derivative corresponded to an oily texture, ruling out the possibility to evoke any mechanochemical activation for both the first and the second step of the reaction. Thus, it can be concluded that the preparation of 1,4-DHPs was equally possible by both stepwise addition pathways (Methods B1 and B2) or by the simultaneous addition of the three reaction components. However, Method B1 remains the most promising, mainly due to: (i) its better selectivity in comparison with Method A, and (ii) its better rheological profile, leading to a solid \( E \)-imine intermediate (step 1).

The reaction scope towards the preparation of unsymmetrical \( N \)-aryl-5,6-unsubstituted-1,4-diaryl DHPs was investigated using the optimized ball-milling Method B1, with structural variations introduced simultaneously at the \( N \)-1 (\( Ar^1 \) substituent), at \( C \)-4 (\( Ar^2 \) substituent) and at the ester functional group (\( R^2 \) substituent) (Scheme 4). Beyond the physical state of the final target (solid or liquid), key to choose the catalyst to be used, the reactivity of the system seemed to be driven by the nature of substitution: (i) on the enals (e.g. for compounds 3 vs 10, 8 vs 13, and 9 vs 14), with slightly better results obtained when \( \omega \)-nitrocinnamaldehyde was used, (ii) on the aniline component, with the better results obtained with electron-donating groups (EDG) (e.g. EDG = OMe, Me vs Ph, Cl, for compounds 11 and 16 vs 10, 11 vs 13 and 12 vs 14), enhancing the nucleophilicity of the amino group, while (iii) the reactivity was independent on the \( \beta \)-ketoester alkyl substitution (e.g. with alkyl = Et vs \( \text{Bu} \) for compounds 8 vs 9, 11 vs 12, 13 vs 14 and 16 vs 17).

Unfortunately, the yields of 1,4-DHPs prepared by Method B1 were only moderate in many cases, especially in comparison with CAN-promoted synthesis in solution (46), and in general, with respect to other solution-based methods with different catalysts (e.g. \( I_2 \), (63) \( \text{Mg(ClO}_4\text{)}_2 \) (64) or \( \text{HClO}_4\text{SiO}_2 \), silica-supported sulfonic acid,56 and metal oxides such as nano CuO (65), nano \( \text{Fe}_3\text{O}_4 \) (66) or \( \text{SO}_4^{2-}/\text{Ce}_x\text{Zr}_{1-x}\text{O}_2 \) (67) (Scheme 4), despite that the crude of the reactions were generally clean, as assessed by GC/MS and \( ^1\text{H} \) NMR analyses. The isolation of compounds 8–18 presented the same difficulties already encountered for the purification of 1,4-DHP 3, due to the lability of these compounds, which quickly oxidized on \( \text{SiO}_2 \) in the presence of an oxygen atmosphere (64). The limited number of synthetic methods to prepare \( N \)-aryl-5,6-unsubstituted-1,4-DHPs and their unstable character in some conditions have limited the number of studies finalized to investigate their biological activity. However, 1,4-DHP 3 (\( R^2 = \text{Et} \)) displayed antidysslipidemic activity, lowering cholesterol or triglyceride levels in plasma, while compound 14 (\( R^2 = \text{Bu} \)) acted as a potent antioxidant, the activity \textit{in vitro} being modulated by the nature of the \( R^2 \) substituent at the ester functional group (Scheme 4) (63).

The first quantitatively assessment of the greenness of mechanochemical processes against the 12 Principles of Green Chemistry was applied to the preparation of the World Health Organisation (WHO) Essential Medicine nitrofurantoin (7), using the free, web-based scoring matrix DOZN\textsuperscript{TM} 2.0 tool (68, 69). It provides a unified set of metrics, organizing the 12 Principles of Green Chemistry into three major groups: improved resource use (G1, Group 1: principles # 1, 2, 7, 8, 9 and 11), increased energy efficiency (G2, Group 2: principle # 6), and reduced human and environmental hazards (G3, Group 3: principles # 3, 4, 5, 10 and 12), providing a score for the individual 12 principles, and aggregating the greenness scores (\textit{aggregated score}) of processes on a scale of 0–100, with 0 being the most desirable one (68).

Therefore, DOZN 2.0 greenness scores were generated for the synthesis of compound 1,4-DHP 3 by ball milling process (Method B1) in comparison with the solvent-based procedure (46) (Table 2).

Both processes display a DOZN 2.0 aggregated score of 1, however, the scores are lower for each group of principles in the ball mill (Method B1) indicating a greener synthesis compared to solution-based process. The scores were calculated by neglecting the quantities and nature of raw materials and solvents used for the work-up.

Although a purification by chromatographic column is necessary for both solution-based and by ball-mill methods, fewer numbers of raw materials (and solvents) were used in the work-up for method B1 (3) by ball milling than for the solution-based process (6), also requiring several washings by liquid–liquid extraction (71). This makes the process by ball milling greener and economic (# 2, # 4, and # 7) and also less hazardous and inherently safer (# 3 and # 12).
Both processes use of the same reactants to make the same product (1,4-DHP 3), the differences in scores arises from the amount of EtOH used in the synthesis.

For Group 1 (improved resource use), Method B1 received low scores because the raw material EtOH is used as grinding additive and not as solvent. This adds to the overall resource use without adding to the final product yield, generating a higher score for solvent-based process in Group 1 and for Group 3 (reduced human and environmental hazards), that quantifies the amount of waste produced, the severity of the waste, and the hazard level of the product being produced. In the case of Group 2 (energy efficiency) both processes received a zero score because there is no deviation from ambient conditions with regard to temperature and pressure.

Table 2. Comparative scores for the preparation of 1,4-DHP 3 by ball milling vs. solvent-based procedure (70).

|                      | By ball milling | In solution$^1$ |
|----------------------|----------------|-----------------|
| Improved resource use (G1) | 5.50           | 9.14            |
| Increased energy efficiency (G2) | 0              | 0               |
| Reduced human and environmental hazards (G3) | 11.76          | 32.5            |

$^1$The scores were calculated by neglecting the raw materials, solvents and process conditions used for the work-up.

Scheme 4. Reaction scope using Method B1. Legend: comparative yields are given in black: for ball-milling Method B1, in blue: for solution-based synthesis via one-pot/one-step domino reaction using CAN as catalyst, in purple: for other solution-based methods involving catalysts other than CAN and p-TsOH.
Conclusions
The preparation of N-aryl-5,6-unsubstituted-1,4-DHPs was disclosed in the ball mill and the reactivity of the system was investigated according to three different domino reactions (one-pot/one-step or stepwise), and the process parameters underlying any mechanochemical process where modulated. As a result, the selection of the most suitable method was driven by the physical state of both the reactants and the intermediates, while the physical state of the final 1,4-DHP directed the choice of the catalyst (Lewis vs Brønsted) and the type of work-up to recover the final products (by column chromatography vs precipitation in water). The quantitatively assessment of the greenness of Method B1 by ball milling against the 12 Principles of Green Chemistry was done in comparison with the solution-based procedure, showing the advantages of ball milling approach.

Despite the challenges to be faced in the preparation of N-aryl-5,6-unsubstituted-1,4-DHPs, in solution or by ball-milling, the results herein described are unprecedented in the arena of synthetic methods to access diversely substituted 1,4-DHPs. Hopefully they will pave the way towards the systematic use of mechanochemical procedures to prepare other pharmacologically relevant compounds, containing 1,4-DHP scaffolds.

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Supporting information
Experimental procedures, 1H and 13C spectral data and full characterization for all of the synthesized compounds are available in the supporting information file.

Data availability
Data are available as part of the electronic supplementary material.

Authors’ contribution
C.B.B. experimental data production, analyses and manuscript preparation; E.C. conceptualization, supervision, data curation, manuscript preparation and funding; J.C.M.: supervision, conceptualization, manuscript editing and funding; J.F.G. manuscript editing; D.V.: manuscript editing and funding; A.P. manuscript editing and data curation.

Research ethics
No requirement for completing an ethical assessment prior to conducting this research.

ORCID
Evelina Colacino http://orcid.org/0000-0002-1179-4913

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