

**CHEMISTRY AND BIOLOGY OF 3,4-DIHYDROPYRIMIDIN-2(1H)-ONE (OR THIONE)  
DERIVATIVES OBTAINED BY THE BIGINELLI MULTICOMPONENT REACTION**

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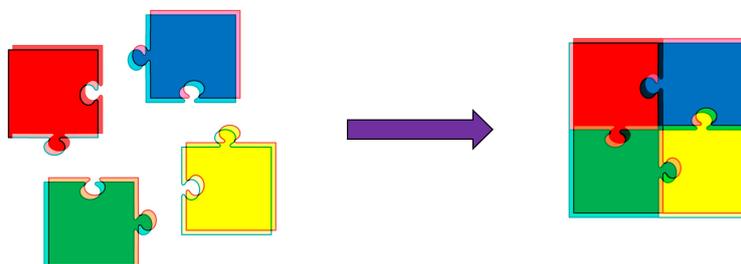
**Abstract.** *In this book chapter we evaluate the synthetic chemistry and biology associated with 3,4-dihydropyrimidin-2(1H)-ones (or thiones), known as DHPMs. These derivatives belong to a class of heterocyclic compounds typically obtained in a straightforward fashion via the Biginelli multicomponent reaction. The mechanisms associated with this multicomponent reaction (MCR) and the biology behind DHPMs have been the subject of heated debates. Herein, we describe the evolution of the reaction, biological application of these derivatives and perspectives associated with this important MCR*

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**1. Introduction**

Multicomponent reactions (MCRs) are a class of organic transformation which simultaneously engages three or more starting reagents brought together (preferentially, all at once), in a one-pot version, and affords a final product in which most (if not all) of the atoms from the starting materials are incorporated into the final framework<sup>1</sup> (see Scheme 1).



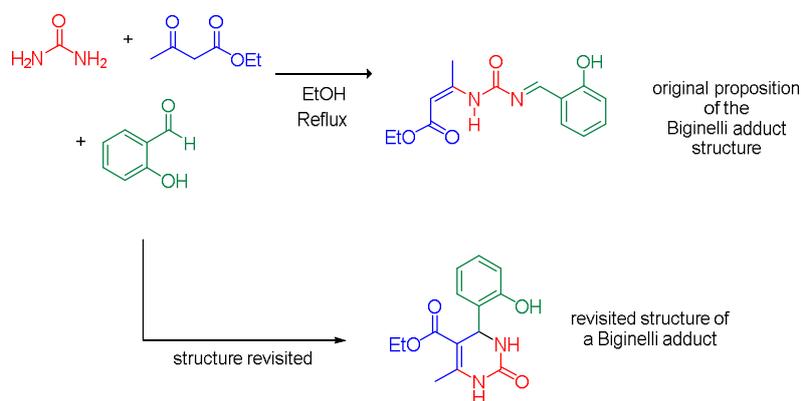
**Scheme 1.** Pictorial view of a four-component multicomponent reaction.

The opportunity of merging diversity and complexity in a single step with atom economy is a huge differential of MCRs.<sup>2-8</sup> In addition, several bioactive compounds may be obtained in a single and elegant step, thus allowing the creation of libraries of compounds with different biological activities and

responses.<sup>9-13</sup> Every year, new MCR approaches are described and several known and new heterocyclic derivatives are synthesized using this efficient synthetic methodology. Another possibility when MCRs are performed is the stereochemical control (diastereo- and enantioselective control) by using several available methodologies. There are dozens of compounds with different biological responses for the tested diastereo- and enantio- isomers. This is why the development of more efficient asymmetric methodologies to obtain enantiomerically pure MCR adducts is currently a challenge.<sup>14-16</sup>

Among the known MCRs, the Biginelli three-component reaction is an eponymous reaction named after Pietro Biginelli. The reaction was disclosed in 1891 by Biginelli himself.<sup>17-20</sup> Two years later, he published the full accounts of the reaction.<sup>21,22</sup> The history and the man behind this interesting three-component reaction have been thoroughly reviewed elsewhere.<sup>23</sup> The original proposition of the Biginelli adduct was initially wrongly described by P. Biginelli, and he himself had to revisit it (Scheme 2).<sup>23</sup>

As depicted in Scheme 2, the reaction was controversial from its inception, since the Biginelli adduct structure had to be revisited.<sup>23</sup> Despite the initial mistake, this MCR is among the most useful synthetic methodologies and, for some, this is still the most important MCR.<sup>24</sup> The Biginelli adducts, that is, 3,4-dihydropyrimidin-2(1*H*)-one (or thione) derivatives, are also known as DHPMs. The possibility of obtaining libraries of different DHPMs and evaluating their biological activities fosters interest in the development of more efficient catalytic methodologies to synthesize these structures using the Biginelli MCR.<sup>25-30</sup>



**Scheme 2.** The Biginelli synthesis as originally proposed and the revisited adduct.

Because of its importance, the Biginelli reaction is among the most studied MCRs. Using “Biginelli” as the keyword, one may evoke this importance by viewing the large number of studies indexed at ISI-Web of Science (Figure 1).

In this book chapter, we intend to show the readers the mechanistic issues related to this MCR, the fundamental role of catalysis for DHPMs syntheses, the development of enantioselective versions of the reaction and some features related to their biological activities.

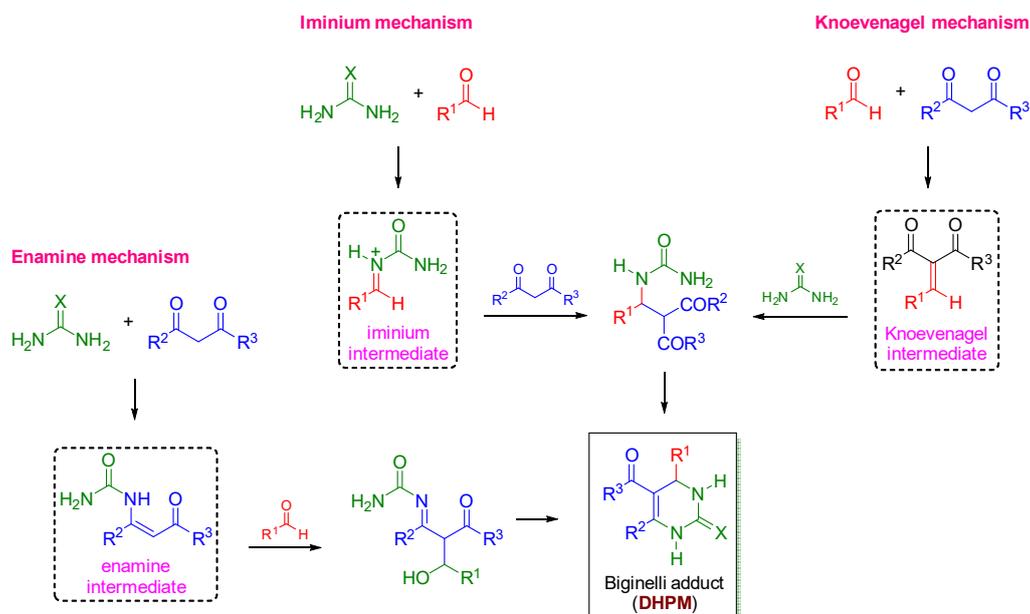
## 2. The Biginelli multicomponent reaction mechanisms

Today, three possibilities for the Biginelli reaction mechanism are accepted; that is, the iminium, the enamine- and the Knoevenagel mechanisms (Scheme 3). These three possible reaction pathways always lead to the same final Biginelli adduct (*i.e.* the DHPM). These three reaction pathways may be taking place simultaneously; hence, without the proper conditions to be carried out, it is not possible to warrant a preferred reaction pathway for the transformation. The evolution and an in-depth analysis of the Biginelli MCR mechanism has been reviewed by some of us elsewhere.<sup>31</sup> Typically, the formation of the first reaction intermediate of the initial bimolecular reaction determines the mechanism, as seen in Scheme 3. It is

important to remember, however, that these reactions may be in equilibria, and the formation of an intermediate may be just a dead end, as showed in the seminal work of Eberlin and co-workers.<sup>32</sup>



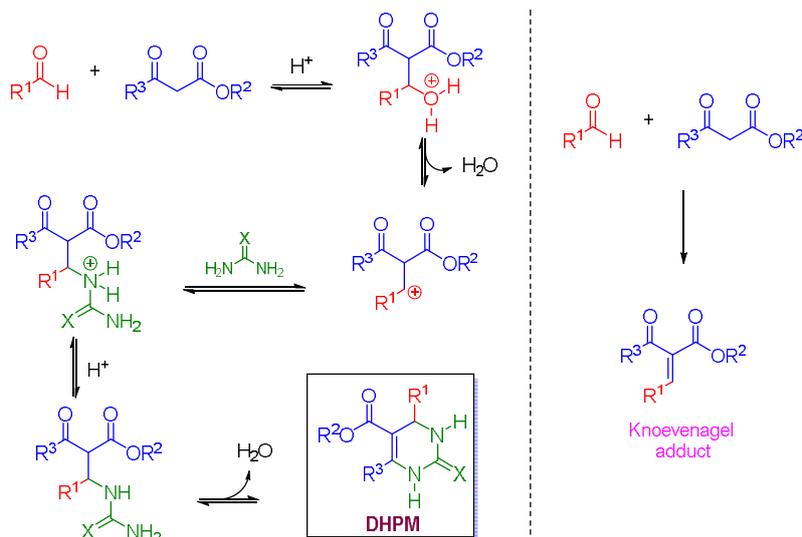
Figure 1. Results of searching "Biginelli" as the keyword at ISI-Web of Science (17<sup>th</sup> October 2018).



Scheme 3. Simplified versions of three possible reaction mechanisms for the Biginelli multicomponent reaction, as currently accepted.

The basis for these three propositions dates back to 1933, in the seminal works of Folkers and Johnson.<sup>33,34</sup> The possibility of these initial bimolecular reactions was investigated under different conditions. The two works are indeed the first rationales towards a more accurate mechanistic proposition for the Biginelli MCR. Many advances were reached by their work, and the enamine mechanism (Scheme 3) seemed to be favoured under the studied conditions.

Later, Sweet and Fissekis described an outstanding mechanistic proposition of what is today known as the Knoevenagel mechanism (Scheme 4). The proposition was based on a series of reactions and conditions tested by the authors. Many of the earlier reported experiments aiming at elucidating the reaction mechanism were repeated by the authors as an attempt to reach a better comprehension of the chemical transformation. According to their results, the “anomalous” behaviour of the Biginelli condensation could be explained by whether the reaction proceeds through the Knoevenagel intermediate. Several bimolecular reactions and their behaviour under acidic conditions were equally evaluated in the study, and the formation of their products and by-products were rationalized.

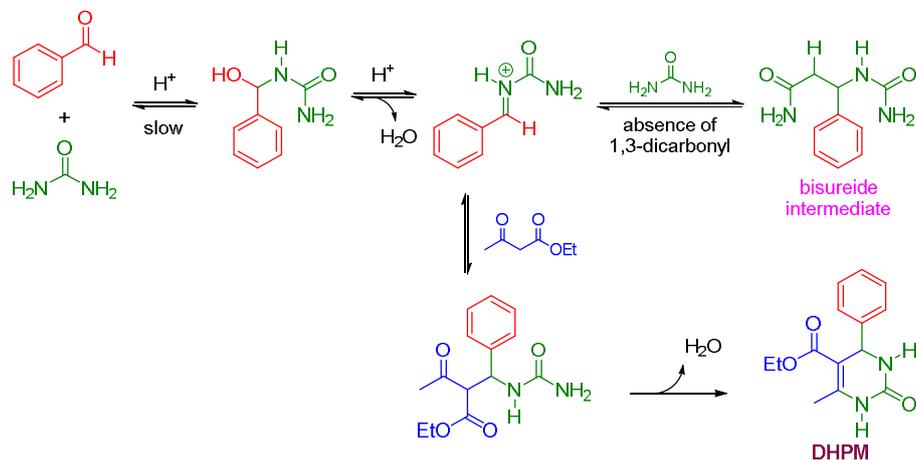


**Scheme 4.** Knoevenagel mechanism for the Biginelli multicomponent reaction (left) and the Knoevenagel condensation between the aldehyde and the 1,3-dicarbonyl compound (right).

Although the proposition of Sweet and Fissekis<sup>35</sup> was very accurate under the studied conditions, only a few catalytic systems that were actually investigated,<sup>36</sup> had a preference for the Knoevenagel pathway for the Biginelli MCR.

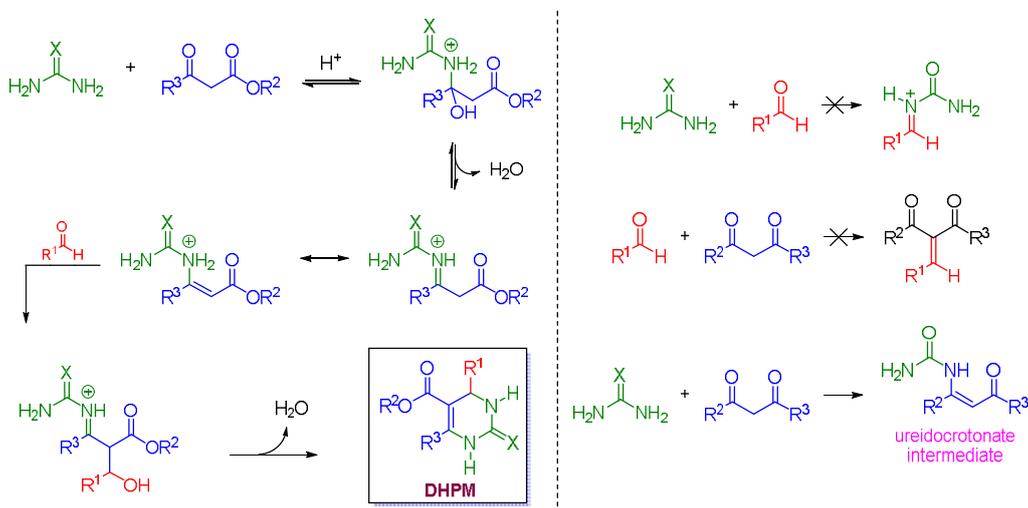
The iminium mechanism is currently the most accepted and it operates in most of the described catalytic systems. This mechanism has been re-examined by Kappe<sup>37</sup> and it showed some possibilities such as the double urea addition to the aldehyde (Scheme 5), affording the bisureide intermediate.

The reaction may proceed starting from the bisureide derivative, and the DHPM will be formed at the end of the reaction. With a likely basis that the addition to the aldehyde is what affords the iminium intermediate, urea excess is typically noted for the catalysed Biginelli reactions where this mechanism is assumed. Although this seems a good idea at first glance, we have demonstrated that this is indeed a misguided idea,<sup>38</sup> and that the kinetics for the second urea addition to form the bisureide intermediate (see Scheme 5) is highly favoured. We also demonstrated<sup>39</sup> that when the iminium mechanism is operating, the excess of aldehyde makes the reaction proceed faster and with higher yields, whereas the urea excess slows the reaction and lowers the yields of the Biginelli adduct.



**Scheme 5.** Re-examination of the iminium mechanism of the Biginelli multicomponent reaction.

In 2007, the report of Capanec and co-workers<sup>40</sup> showed that the Biginelli reaction proceeds through the enamine reaction pathway (Scheme 6) when promoted by  $\text{SbCl}_3$ . The promoter was tested from 10 mol% to equimolar quantities, depending on the reaction. In the bimolecular tests aiming at depicting the preferential mechanism of the developed conditions, only the ureidocrotonate could be isolated in the reaction between urea and ethyl acetoacetate (Scheme 6). The other two possible bimolecular reactions were equally tested but no product was observed under the conditions used to carry out the transformation.

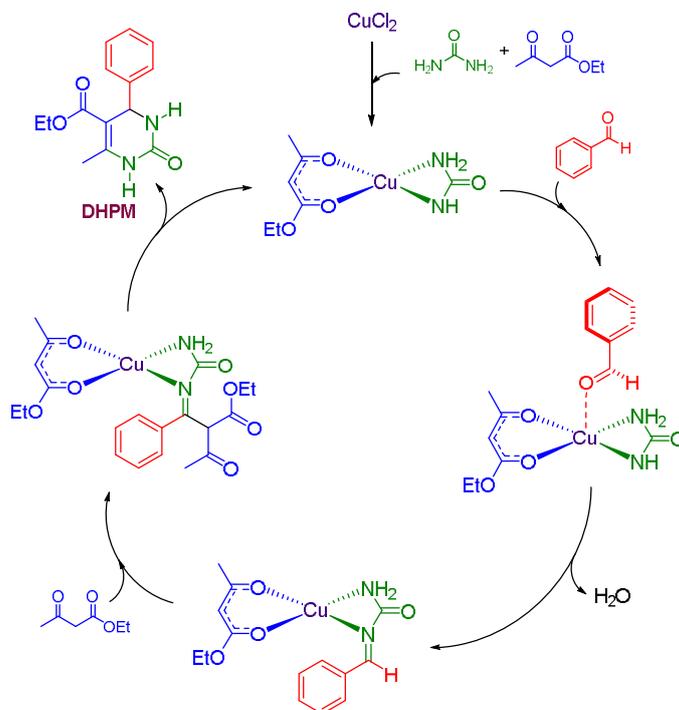


**Scheme 6.** The enamine reaction pathway of the Biginelli reaction (left) and the bimolecular reactions promoted by  $\text{SbCl}_3$  (right). Only the ureidocrotonate could be isolated and the formation of the other two intermediates failed.

The aforementioned authors showed strong evidence regarding the preference for the enamine mechanism. One important conclusion of the work<sup>40</sup> is that most of the available reports just assumed reaction mechanisms similar to those proposed before, without any real proof. A few years later,<sup>41</sup> the same

group demonstrated that the enamine mechanism was also the preferred pathway when  $[\text{Al}(\text{H}_2\text{O})_6](\text{BF}_4)_3$  was used as the catalyst to promote the Biginelli condensation. Indeed, there only a few reports (see the cited reviews) where the mechanism of the transformation is actually investigated rather than assumed. This is a problem, as will be discussed in due course, especially if we consider an enantioselective version of the reaction.

The mechanism of the reaction, when catalysed by a Lewis acid, was investigated by us (Scheme 7) using NMR, DFT calculations and ESI-MS(/MS).<sup>42</sup> It was demonstrated that the reagents were not always innocent when Lewis acids were tested and that, although the reaction proceed through an iminium-like mechanism, the catalytic cycle was far more complex when compared to those of Bronsted-catalysed reactions. Some formed complexes *in situ* and their equilibria could also be analysed in the manuscript, indicating that, depending on the Lewis acid, the mechanism of the reaction may proceed through unexpected pathways and not necessarily similar to those for a Bronsted catalysed reaction.

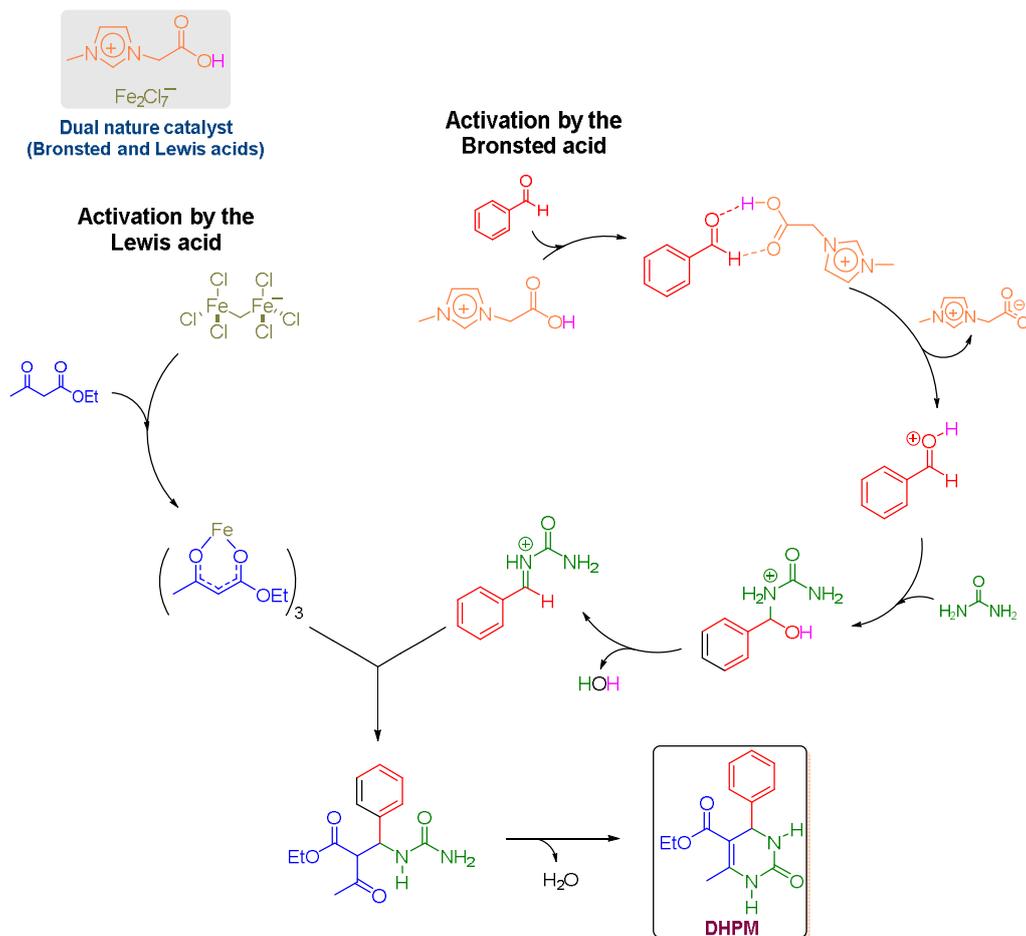


**Scheme 7.** Catalytic cycle proposed for a copper-catalysed Biginelli condensation. Several equilibria are involved in the transformation and they are omitted in this Scheme for clarity.

When catalysts of a dual nature (Lewis and Bronsted acids in the same structure) are used, there is a combined role of both moieties, as showed by us<sup>39</sup> in the only mechanism investigated and described so far (Scheme 8) for such a case. In the catalysis, an iminium-like mechanism was favoured and the Bronsted acid activates the electrophilic species, whereas the Lewis acid is responsible for improving the nucleophilicity of the other species, as seen in Scheme 8.

ESI-MS(/MS), NMR, DFT calculations and kinetics data supported the proposition. Note that as we described before,<sup>42</sup> the reagents could form a complex with the Lewis acid *in situ* and improve its nucleophilicity. Theoretical calculations pointed in this direction<sup>39</sup> and showed improved values of Fukui functions for both the electrophilic and nucleophilic species. The kinetic data presented in the work<sup>39</sup> was the first one available in the literature to support a mechanism of the Biginelli reaction. Interestingly, the data

showed that urea excess does not favour the reaction outcome, considering the iminium mechanism was operating under the developed conditions. Indeed, the reaction was slower when compared with those without urea excess. The use of aldehyde excess, on the other hand, had a dramatic and beneficial effect on the reaction yields. The use of an excess of ethyl acetoacetate did not return any significant effect on the reaction outcome.



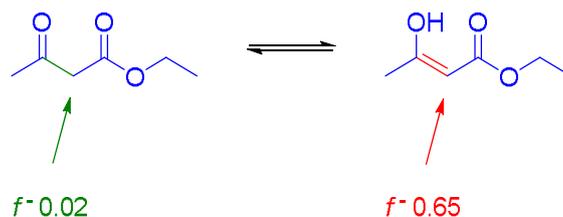
**Scheme 8.** Catalysed Biginelli reaction using a dual nature catalyst. Note the Bronsted and Lewis acids have different roles.

For base-catalysed versions, only a few reports are available. It was demonstrated that, depending on the reagent (urea or thiourea), when  $t\text{BuOK}$  was used as the promoter, the mechanism may proceed differently.<sup>43</sup> When thiourea was the reagent, the reaction proceeded in a Knoevenagel-like fashion, whereas it went through a bisureide-like intermediate when urea was the reagent. Another interesting report<sup>44</sup> investigated the reaction under basic conditions and concluded the mechanism operating for the Biginelli MCR was the enamine reaction pathway. As noted by these two reports, the three plausible mechanisms may happen, and there is still much room to be explored, so further investigations are needed to clarify the base-catalysed versions.

In general, it is still not possible to say what the crucial factors affecting the mechanism selection of the Biginelli MCR are. Indeed, any new catalytic system has to be investigated before proposing a mechanism for the catalysed reaction. In many cases, as described herein, the three most accepted mechanisms may be operating simultaneously.

### 3. Catalysis' roles and solvent effects

The number of available reports describing the effects of solvents on the Biginelli reaction is very limited. Clark and co-workers demonstrated<sup>45</sup> that there is a combined role of catalysis and solvent effects to improve the yields of the Biginelli reaction. The work also pointed out that the iminium mechanism is favoured under their studied conditions. A main point of the study was to show that 1,3-dicarbonyl reacts preferentially through the enol tautomer under Bronsted acid catalysis. Later, we demonstrated<sup>38</sup> the solvent effect in the absence of any catalyst and the vital role of the solvent in restoring the enol isomer to further the Biginelli reaction. Fukui functions ( $f^-$ ) were calculated to quantify the nucleophilicity trends of the isomers (Scheme 9). In general, both works<sup>38,45</sup> (under catalysed and non-catalysed conditions) returned similar results and pointed to the crucial role of solvent to the Biginelli reaction.



**Scheme 9.** Keto-enol equilibrium of ethyl acetoacetate and calculated Fukui functions ( $f^-$ ) of the indicated carbon atom.

It is known there are many works describing solvent-free versions of the Biginelli reaction, as reviewed elsewhere.<sup>46</sup> The idea of solventless reactions is attractive from the viewpoint of green chemistry.<sup>47-49</sup> It is not, however, a crucial issue regarding the Biginelli reaction, especially because, as discussed herein, there are many effects and important roles associated with the solvent for this MCR. If solvent is required to improve yields and selectivities, it is important to prioritize the use of solvents with green appeal, such as ionic liquids, ethanol, water, PEG and *p*-cymene.

The catalyst used to be another polemical issue and, in this sense, we considered this point requires some elucidations. A few reports wrongly described the so-called “catalyst-free” Biginelli reaction. All drawbacks associated with these works and the mistaken conclusions were addressed in our previous report.<sup>38</sup> We consider it a waste of time to discuss these mistakes herein, and hence we will focus on the discussion of the catalyst's roles for an appropriate Biginelli condensation. We wish, however, to make it very clear for readers that the Biginelli condensation may be processed without any catalyst (yields of  $\approx 50\%$ ), but yields and selectivities are deeply impaired. Catalysis is without a doubt a fundamental tool for efficiently performed chemical transformations; hence, the Biginelli reaction is also benefited when carried out under catalytic conditions.

Several reports described the Biginelli MCR in temperatures above 100 °C, even under catalytic conditions. One benefit of using catalysis is to try to reduce the temperature of the transformation. Not all substrates are stable at high temperatures, such as those used in the model Biginelli reaction *i.e.* a mixture of benzaldehyde, urea and ethyl acetoacetate.

The use of a catalytic system allows the catalyst to be recovered and reused with yields far above  $\approx 50\%$ , which are the average for the uncatalyzed Biginelli condensation reaction. There are several catalytic systems for the Biginelli MCR operating under homogeneous and heterogeneous conditions, and both have advantages and disadvantages, as we have demonstrated elsewhere.<sup>50</sup>

For the great majority of the Biginelli articles already published, at least one of the reagents is used in excess. For some reported conditions, two of the three reagents are used in excess. If one considers that the

reaction is not so simple to be carried out, again catalysis plays a vital role to further the reaction. The need for more efficient catalytic systems and conditions to perform the reaction using equimolar quantities may be only reached whether catalysis is involved and it points to the importance of a catalyst for the reaction.

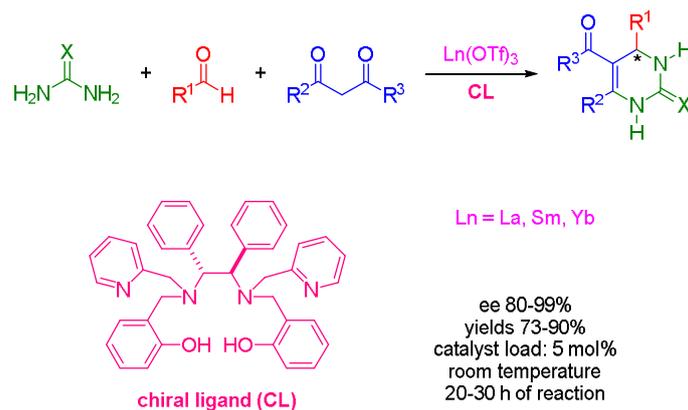
The reaction time for the Biginelli reaction under uncatalyzed conditions is typically 24 h. Catalysed versions of the reaction reduce this time to only a few hours. As is clear from the viewpoint of time, catalysis has a direct influence on the reaction outcome by shortening the time significantly.

Although there are several catalytic systems developed for the Biginelli reaction, it is not rare to find a case in which the catalyst is used above 10 mol%. This high catalyst load should be assiduously avoided. If there are many articles describing such conditions, one may fairly conclude there is still a need for more efficient conditions for this MCR and, in this sense, more efficient catalysts, as catalysis is the future to further the reaction, especially considering the green features which are expected by using MCRs.

The mechanism reaction pathway is only tuned when catalysed versions are performed. We demonstrated and proposed a kinetic model for the uncatalyzed Biginelli condensation.<sup>38</sup> It is shown that the reaction goes through the three possible reaction pathways; therefore, the presence of a catalyst also has a vital role for the mechanism selection. Even so, sometimes the presence of a catalyst is not a guarantee to select one reaction pathway exclusively, and the transformation may still be operating through different mechanisms. This is a major problem especially when aiming at efficient enantioselective versions of the Biginelli MCR. The enantioselective versions of the reaction will be analysed as a separate topic due to their importance.

#### 4. Enantioselective versions of the Biginelli multicomponent reaction

The development of enantioselective versions of the Biginelli reaction was a huge challenge for decades. But in 2005, Zhu and co-workers broke this paradigm and reported the first successful version of this important MCR. The achievement was possible using a chiral ligand in the presence of lanthanide triflates ( $\text{Ln}(\text{OTf})_3$ ), as seen in Scheme 10. Better ee values were obtained by using the Yb complex, and a series of DHPMs were synthesized using the methodology. The authors tested two different chiral ligands, but the ligand shown in Scheme 10 returned the best results. A series of enantiomerically enriched DHPMs was obtained using the developed methodology with relative success during the chiral induction step. The authors suggested that the reaction proceeded through an iminium-like mechanism, and the key step for chiral induction was the 1,3-dicarbonyl addition to the iminium ion.

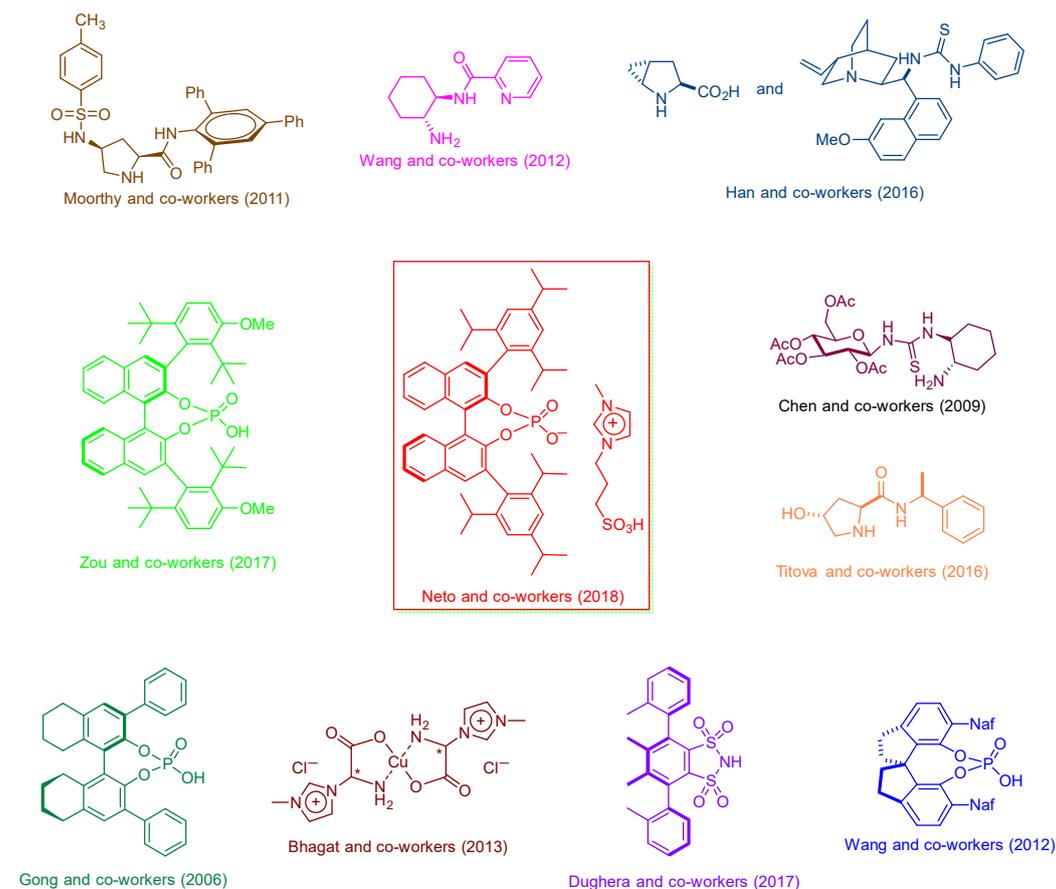


**Scheme 10.** The first successful example of an enantioselective version of the Biginelli multicomponent reaction.

Likely motivated by this breakthrough work,<sup>51</sup> new chiral catalysts and conditions were developed for the enantioselective version of the Biginelli reaction, as reviewed elsewhere.<sup>52-54</sup> Figure 2 shows a few examples of chiral catalysts (or ligands) used to promote the enantioselective version of the Biginelli MCR

with good to excellent ee values.<sup>55-64</sup> All these works brought important contributions to the development of the enantioselective Biginelli MCR, although there is still much room for improvements, considering the problems and conditions yet required to perform the reaction.

Despite the great progress already experienced for the enantioselective version, many drawbacks are still noted, thus requiring urgent improvements to further the enantioselective version of the reaction. The reaction time is too long, and sometimes more than one week is required to complete the reaction. This is mostly because the reaction must be performed at room temperature or at temperatures close to it to warrant good ee values. As a consequence, sometimes yields are lower than those achieved at a higher temperature. Another problem noted is the excessive use of reagents. It is not rare to use two (of three) reagents in excess and, therefore, the waste from the reaction is high. This feature is in opposition to what is expected from an ideal MCR, that is, to minimize waste and to maximize atom economy. Most of the reports available for the enantioselective version of the Biginelli MCR typically require the use of Brønsted acid as a co-catalyst. It means that beyond the presence of a chiral inductor, the reaction also requires the addition of another component, if it is to be carried out. Although cheap acids may be used, it is not a good idea from the viewpoint of green chemistry. As noted in the experimental section of such reports (see the cited reviews for examples), the amount of acid is characteristically 10-20 mol%.



**Figure 2.** Examples of chiral catalysts successfully applied in the enantioselective version of the Biginelli multicomponent reaction.

The beneficial effect of organic salts as additives has been demonstrated, aiming at improved yields and, mostly, for improved ee values.<sup>65</sup> This work described the use of several additives and concluded that a combination of <sup>t</sup>BuNH<sub>2</sub>·TFA returned the best results for the developed catalyst. The work also affirmed that excess of urea and 1,3-dicarbonyl reagent returned improved yields.

The mechanism of the enantioselective version of the Biginelli reaction was so far just assumed to be the iminium mechanism. The commonest procedure available to proceed with the reaction is described as a two-step procedure, although one-pot. Typically, the chiral inductor and additives (Bronsted acid and organic salt) are added alongside the aldehyde and the urea (or thiourea). After a few hours of reaction, the third component is added. This procedure aims to ensure the iminium ion formation and to avoid the other two discussed mechanisms. However, it is no guarantee that the reaction will indeed follow the iminium pathway, especially because its formation is reversible and in equilibrium.

A new generation of chiral catalysts was recently reported by our group<sup>55</sup> to overcome most of these drawbacks (see the central catalyst in Figure 2). In the work, we combined a chiral inductor (as the anion) and a Bronsted acid (as the cation) of a new class of task-specific ionic liquids.<sup>66-69</sup> The concepts of ACDC (asymmetric counteranion directed catalysis)<sup>70-72</sup> and ionic liquid effect<sup>73-75</sup> were merged and major problems associated with the reaction overcome. The catalyst had a Bronsted acid in its structure and therefore no additional H source was required. In addition, the catalyst, as a task-specific ionic liquid, is an organic salt in nature. The unique catalyst combined the chiral inductor, the Bronsted acid and organic salt in a single structure. Another important contribution of the article was an actual investigation of the reaction mechanism, which pointed firmly to the iminium mechanism as the single preferred reaction pathway under the described reaction conditions. The reagents and catalyst could also be added at once, at the beginning of the reaction, as is wished for MCRs. Based on the results, a plausible transition state could be proposed to explain the chiral induction during the 1,3-dicarbonyl addition to the formed iminium ion.

## 5. Biological activity of DHPMs

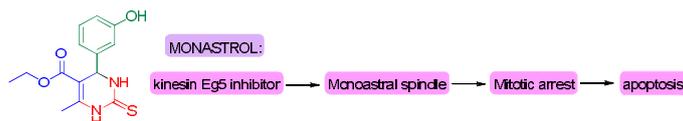
Due to the versatility of the Biginelli reaction, an infinity of products can be obtained in a very simple and direct way using this MCR with the proper reagents' selection.<sup>76</sup> Some good reviews on this topic were recently published,<sup>77-79</sup> in which it is possible to see a broad scope of products with several biological actions. In this part of the chapter, we are going to discuss some selected works with a focus on the main activities reported for DHPMs, that is, their anticancer, their antimicrobial, their anti-HIV activities and as calcium channel blockers.

### 5.1. Anticancer activity

Cancer is a set of diseases characterized by disorderly cell growth, often with the ability to invade health tissues and organs. It is still one of the leading causes of death worldwide and a serious public health problem.<sup>80</sup>

The most important treatment of cancer consists of chemotherapy. Despite the extensive development of substances in this area, efficacy is limited. Among the limitations, we can mention the lack of sensitivity, the notable side effects and the development of drug resistance, which justify the search for new active compounds.<sup>81</sup>

Monastrol, discovered in 1999,<sup>82</sup> is a small cell-permeable and central DHPM derivative, which is useful as a prototype for the development of anticancer drugs. Its action is on kinesin Eg5 (a motor protein) of mitotic cells, inducing monoastrol spindle instead of the bipolar spindle during cell division (Scheme 11).

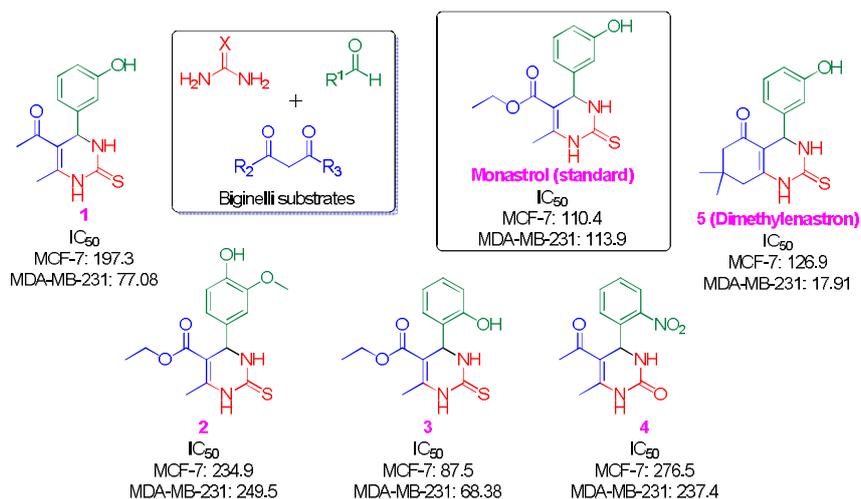


**Scheme 11.** Monastrol structure: a motor protein Eg5 inhibitor.

The net result of Monastrol use is mitotic arrest and, finally, induced apoptosis. An advantage of this mechanism of action is the reduction of neurotoxicity, justified by the absence of effects on the microtubules.

We have already studied the action of various DHPMs on breast cancer,<sup>83</sup> one of the main causes of death among women. After a screening of the active compounds in MCF-7 and MDA-MB-231 cells, more specific *in silico*, *in vitro* and *in vivo* assays were accomplished with the more promising DHPM derivatives (Figure 3).

Some synthesized compounds showed inhibitory activity on Eg5 and impaired the mitosis of tumor cells. They also decreased the cancer stem cells (CSC) in MDA-MB-231 cells, restraining tumor initiation and maintenance. Compounds **1**, **2**, **4** and **5** (see Figure 3) were secure for fibroblasts, being selective for cancer cells. The final result was the desired apoptosis for the treated cells. The *in vivo* assay using chorioallantoic membrane (CAM) of fertilized chicken eggs showed all derivatives subtly inhibit new blood vessel formation even at low doses.<sup>83</sup>



**Figure 3.** DHPM derivatives acting on Eg5 motor protein against breast cancer.

Another interesting work in anticancer research involving DHPM derivatives was described by the Rashid group.<sup>84</sup> In the paper, the inhibition of thymidine phosphorylase (TP), an angiogenic enzyme with a significant role in tumor growth and metastasis, was investigated. Among the eighteen synthesized compounds with structural change at the C-6 position, the authors found five structures with better TP-inhibition than the standard 7-deazaxanthine and showed compound **7** was the most potent one, acting in both *in vitro* and CAM *in vivo* assays (Figure 4).

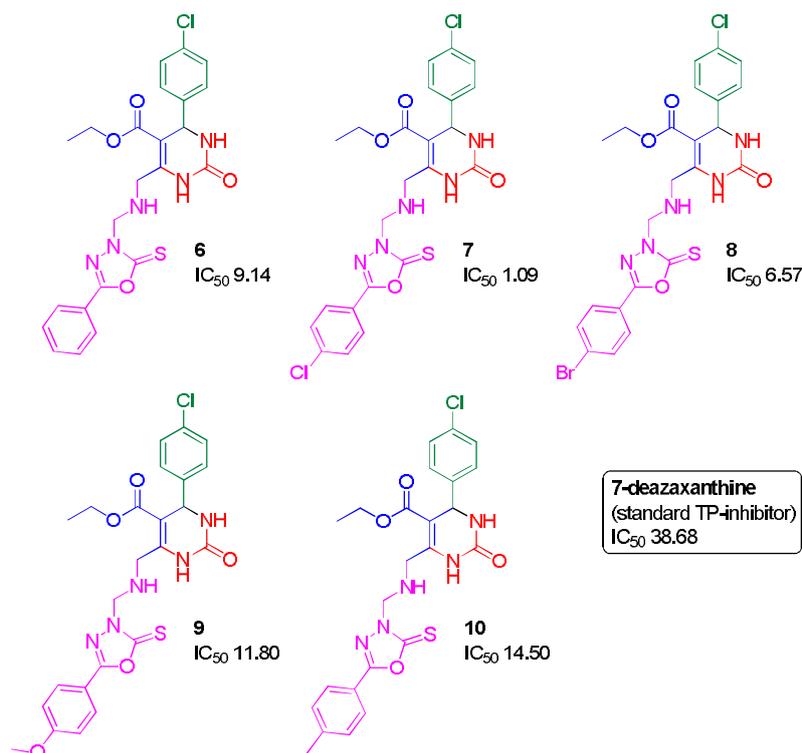
## 5.2. Antimicrobial activity

Making antimicrobial compounds is still a challenge, because resistance is a very common drawback and infections that were previously easily treated are now difficult to solve. The increasing prevalence of this resistance, together with a low number of new drugs coming on to the market, causes an important public health problem.<sup>85</sup>

Tuberculosis is one of the main infectious diseases causing mortality in the world. Beside the deaths by the disease, patients with HIV run a high risk of contracting mycobacterial infections, which increases the risk of death.<sup>86</sup>

Elumalai and co-workers<sup>87</sup> studied some DHPM derivatives with antimycobacterial activity. In the work, researchers used two pharmacophoric hybridized groups (2,4,5,6-pyrimidine and DHPM) to synthesize a set of new molecules. Two strains, *Mycobacterium fortuitum* CA10 and *Mycobacterium*

*tuberculosis* B814, were used for a preliminary assay. Active substances were tested against *M. tuberculosis* CIP 103471 and *M. tuberculosis* H37Rv ATCC 27294. Compound **14**, with 4-fluoro phenyl substituent at 4-position, was found to have the best action against both strains, with minimum inhibitory concentration (MIC) close to  $1 \mu\text{g mL}^{-1}$  (Figure 5).



**Figure 4.** DHPM derivatives with strong thymidine phosphorylase inhibition activity.

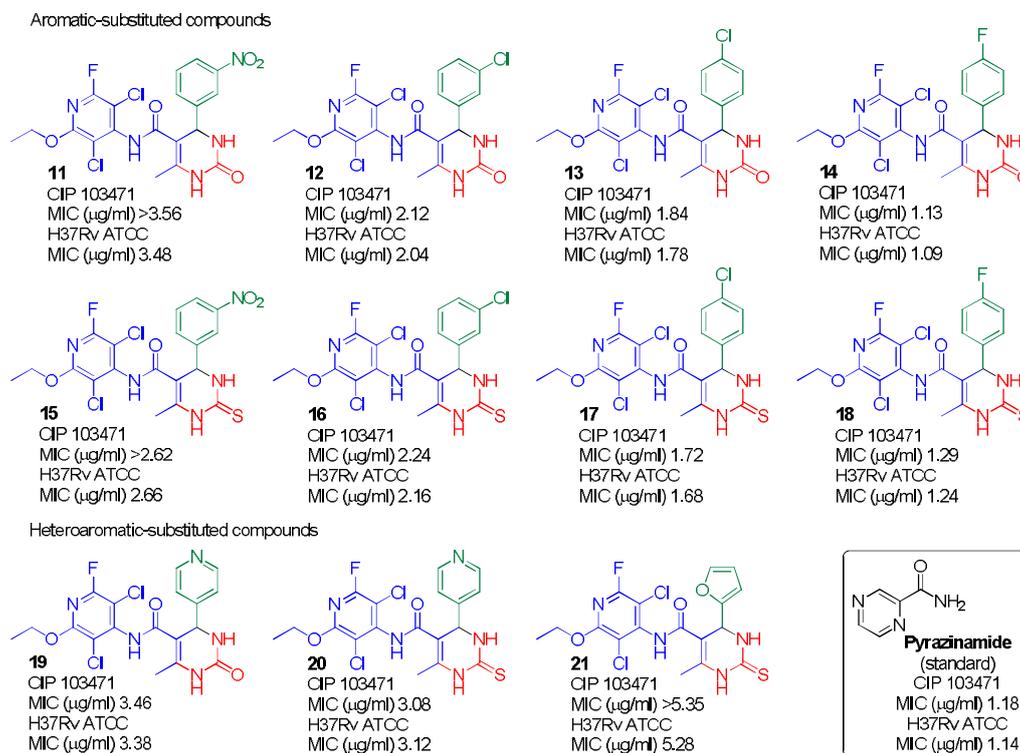
### 5.3. Anti-HIV activity

Acquired immunodeficiency syndrome (AIDS) is still one of the most important world health problems even with the success of highly active antiretroviral therapy (HAART). AIDS and HIV infection represent global health hazards and a complex scientific puzzle.<sup>88,89</sup> The toxicity of current available drugs and the inevitable emergence of multi-drug resistant strains make the problem even worse,<sup>90-92</sup> so, despite the great number of existing drugs, research in this area are still very important. According to the literature, DHPM analogues have potential against HIV.<sup>93-96</sup>

To illustrate the potential of DHPM compounds as anti-HIV in more detail, we will discuss Kim and co-workers' report<sup>93</sup> of activity and preliminary structure-activity relationship (SAR) of DHPMs as inhibitors of HIV-1 replication. The synthesis of compounds was performed by the Biginelli reaction. To evaluate the biological activity, a sequential screening of structural variation in distinct regions of a hit structure obtained in the work were performed (Figure 6), where the hit scaffolds were inspired by an inhibitor of HIV-gp120-human CD4 binding, an alkaloid isolated from a marine sponge.<sup>97</sup> The first result was that the thio- analogues and methylated analogues on N1 or N3 resulted in complete loss of activity.

The second round of analogues were prepared with structural variation only in the carbonyl R<sup>1</sup> moiety of the hit structures (Figure 6). Compound **26** was the only active one. Other esters or polar carboxylic acid (**22**) resulted in the loss of cellular activities. The conclusion of this evaluation is that a non-polar bulky R<sup>1</sup>

group is necessary to anti-HIV activity and the CH<sub>2</sub> spacer is a critical factor, provided that compound **27** without a methylene group had EC<sub>50</sub> > 10 μM. The following experiments evaluated the impact of substitution at R<sup>2</sup> of the hit structure (Figure 6); that is, the synthesized analogues incorporated the most active R<sup>1</sup> substituent. In this experiment it was observed that an increase in the size of R<sup>2</sup> from methyl to ethyl and propyl showed improved cellular activity from 0.529 μM from methyl (**26**) to 0.087 from ethyl (**28**) and 0.286 μM to propyl (**29**). The other analogs exhibited reduced or complete loss of activities.

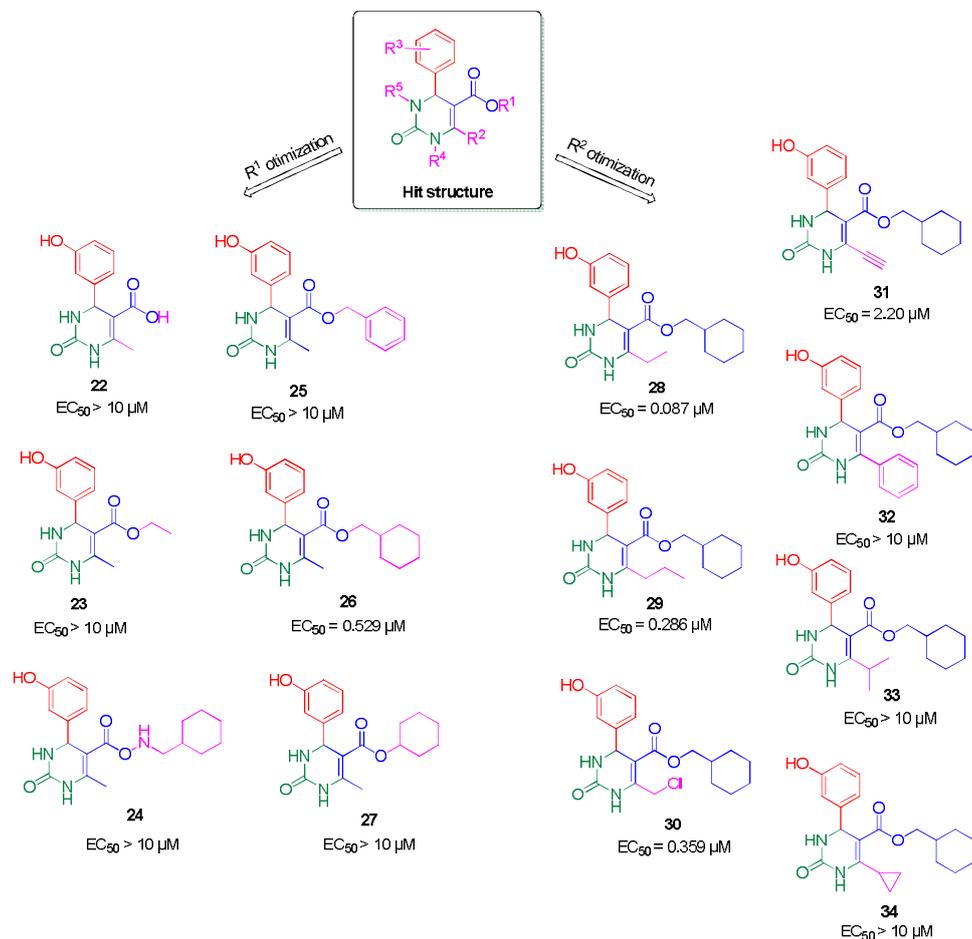


**Figure 5.** DHPM derivatives with antimycobacterial activity.

Another optimization procedure was performed by the authors, after examining the R<sup>1</sup> and R<sup>2</sup> moieties, *i.e.*, the evaluation of the substituent effect on the phenyl ring R<sup>3</sup> (Figure 7).

The most active R<sup>1</sup> and R<sup>2</sup> substituents were incorporated into the synthesized analogues. The *o*-OH analogue (**44**) was inactive, so no other *o*-analogues were prepared; from *m*- and *p*-substituted analogues the only inactive compound was the carbocyclic acid analog (**45**). In general, *p*-substituted compounds were more active than *m*-ones. Regarding the electronic characteristics of the substituents, there was no clear evidence about the effects of donor or withdrawal groups. Examples of this are compounds **14** and **19**; the former, with withdrawal group (*p*-NO<sub>2</sub>), was less active than the latter, with a donor group (*p*-OH). Another comparison, between compounds **17** and **20**, showed that the former, with withdrawal group (*p*-F) was more active than the latter, with donor group (*p*-NH<sub>2</sub>).

The last experiment was designed to evaluate if the configuration on C-4 could impart biological activity. Chiral resolution of compounds **5** and **7** generated their enantiomers, compounds **44-49**, with *ee* values up to 94%. The enantiomers were tested separately; for both compounds (*S*), enantiomers were at least twice as active as their racemic forms (compounds **5** and **7**) and (*R*) enantiomers were totally inactive up to 10 μM (Figure 8).



**Figure 6.** R<sup>1</sup> and R<sup>2</sup> substitution pattern and EC<sub>50</sub> values of DHPM analogues with anti-HIV activity.

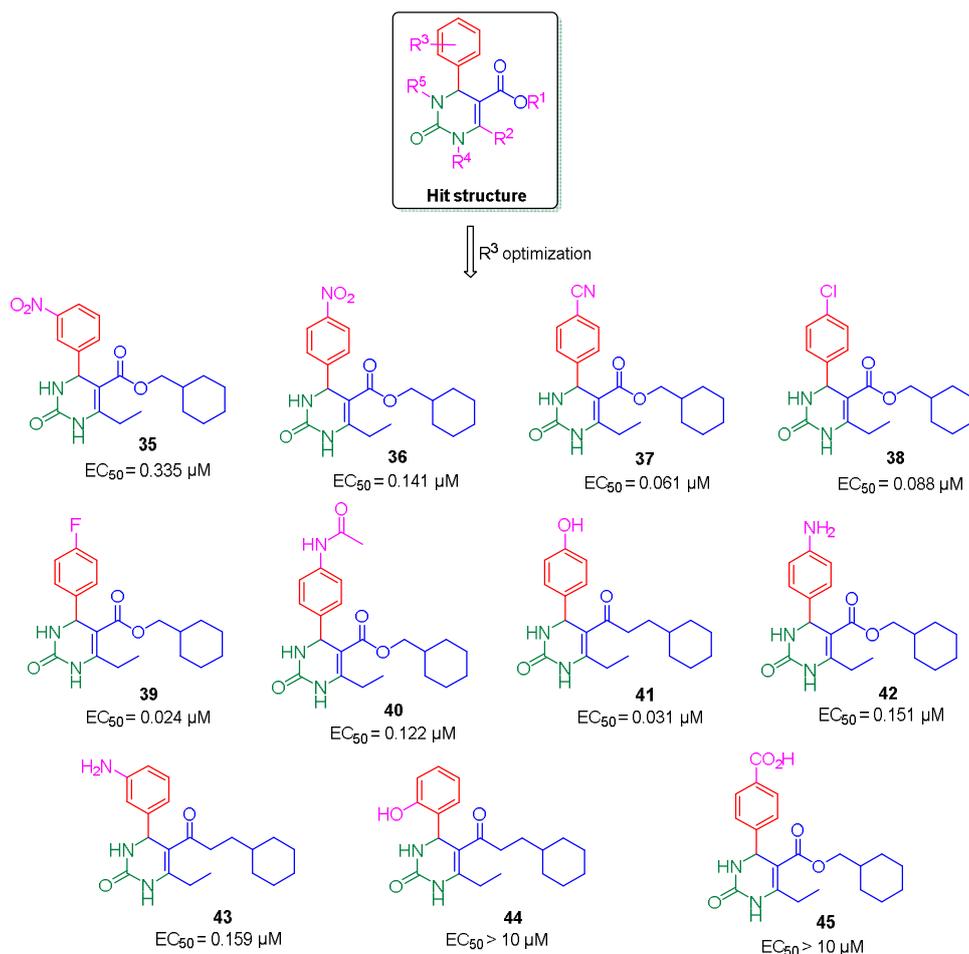
Several analogues presented activities superior to that of the positive control (Nevirapine, 0.150 μM), and only compounds **30** and **31** presented measurable cytotoxic activity against uninfected cells, while all the other compounds did not show cytotoxic activities.

#### 5.4. Calcium channel blockers (CCBs)

In 1969, Nifedipine, a dihydropyridine derivative, was developed by Bayer company. In 1972, it was confirmed that this compound reduces high blood pressure, angina and cardiac arrhythmia by calcium entry inhibition.<sup>79,98</sup> Experimental work in this area started in the 1960s, where the screening of a small organic molecule, like Nifedipine, with coronary dilator activity, led to the discovery of the mechanism of calcium entry blockade by drugs, which were later named calcium channel blockers (CCBs).<sup>79</sup> Today, this class of drugs is used to treat hypertension and stable angina.<sup>98</sup> Based on the structure of the pharmacophore of CCBs, there are three main classes: (i) arylalkylamine derivatives, (ii) benzothiazepine structures and (iii) 1,4-dihydropyridine derivatives (Figure 9).

Based on this finding, several other compounds were prepared using the molecular structure of Nifedipine as an inspiration, among them, DHPM analogues. Several structure-activity relationship studies with the DHPM class of compounds, performed by Squibb researchers, were reported in the 90s with respect

to their ability to target calcium channels<sup>99-104</sup> The most important conclusions of these works are summarized in Figure 10.

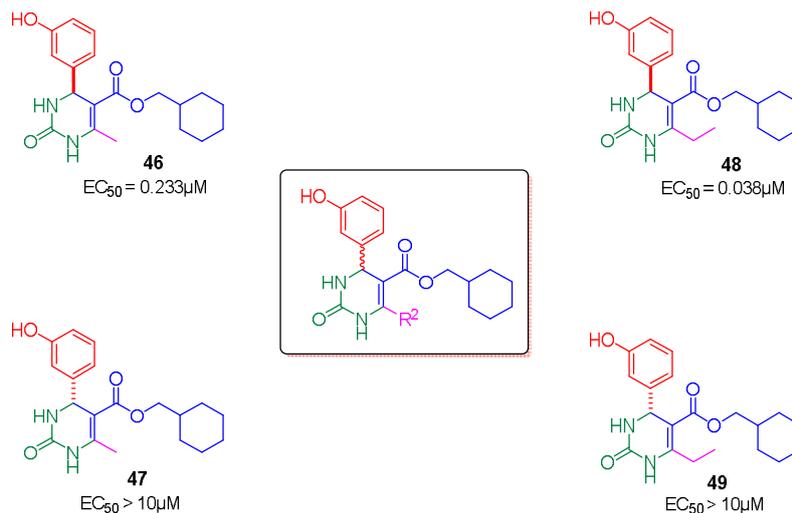


**Figure 7.** Phenyl substitution pattern and EC<sub>50</sub> values of DHPM analogues.

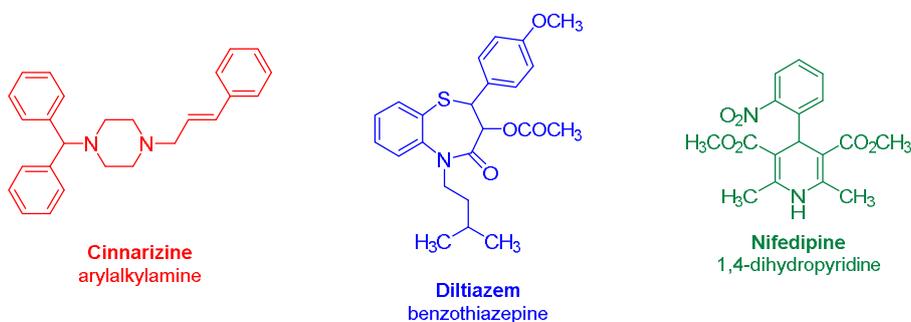
Although the first findings of these studies indicated thio-analogues as the most potent compounds when compared with oxo- and aza-analogues, they found oxo-analogues were more stable *in vivo*.<sup>100</sup> Similar findings were obtained with compounds bearing substituents at N3 (R<sup>3</sup>). Despite being very potent CCBs *in vitro*, their anti-hypertensive properties were negligible in *in vivo* experiments (in rats) as a result of metabolization.<sup>99</sup> Since then, several studies have aimed to produce analogues that could keep or increase the potency and improve *in vivo* stability by variation of N3 substituents.<sup>105-107</sup> The type of ester group at C5 (R<sup>2</sup>) had great effect. Isopropyl esters presented better potency than ethyl and methyl esters. Curiously, these results were very similar to the work presented previously on DHPM with anti-HIV activity.<sup>97</sup>

Another important finding, related to phenyl group substitution (R<sup>1</sup>), was that the adduct bearing a nitro group at *o*-position was more effective as an antagonist of the calcium channel than that containing CF<sub>3</sub> or Cl as a substituent.<sup>99</sup> In a recent work, Chakraborty and co-workers<sup>108</sup> reported CCB activity of DHPMs

with or without a N1-alkyl substitution. This study revealed that substitution in this position abolishes calcium channel inhibition, and perhaps the hydrogen bonding at this position is essential to the activity.



**Figure 8.** Two examples of enantiomerically pure DHPMs and  $EC_{50}$  values. (*S*) enantiomers are active while (*R*) enantiomers are not.



**Figure 9.** Examples of calcium channel blockers (CCBs) and structures of the basic three classes of CCBs.

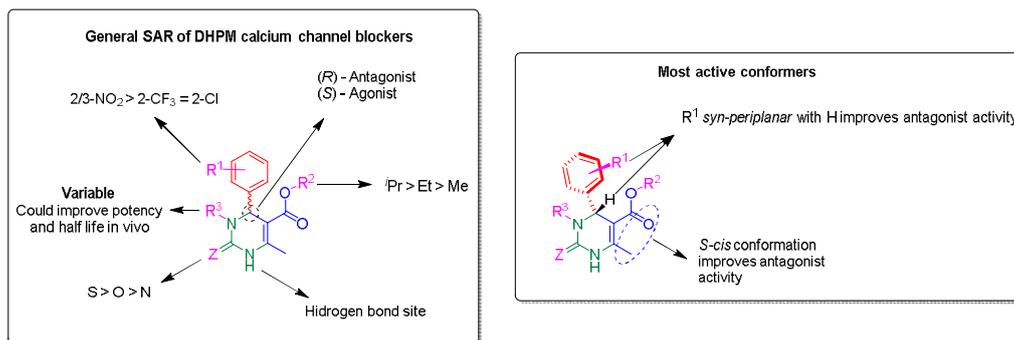
As presented before in the example of DHPMs with anti-HIV activity, the stereocenter at C4 also plays a key role in the activity toward the calcium channel. Contrary to the work of Kim and co-workers,<sup>93</sup> the *R* enantiomer was found to be 750-fold more potent as a calcium channel antagonist than the corresponding *S* enantiomer.

Finally, the preferential conformation of phenyl and ester moieties was investigated by the analogue's synthesis and X-ray crystallography. It was found that the most active conformers were those with substituent of phenyl group *syn-periplanar* with C-4 hydrogen and ester in the *S-cis* conformation for maximum receptor affinity.<sup>101</sup>

## 6. Concluding remarks

The potential of the Biginelli reaction was explored in this book chapter. The synthesis of DHPMs, although known for more than a century, is still challenging many groups. The role of catalysis is vital to the

synthesis of DHPMs, not only to improve yields and to shorten times, but also to select a proper reaction pathway. Enantioselective versions are now emerging, but there is still plenty of room for improvement in the reaction conditions, yields, ee values and mechanistic studies.



**Figure 10.** General structure-activity relationship of DHPM calcium channel blockers and most active conformations.

The potential of DHPMs as bioactive compounds is only now starting to emerge in several applications. The facility to generate libraries of derivatives and to test them as antitumoral agents, anti-HIV and CCBs are only a few examples we explored.

We hope that readers feel challenged to face the new horizons of this most important MCR and to contribute to the elucidation of the areas that are still foggy regarding the Biginelli reaction. Several avenues of opportunities are open in front of us and we just have to follow them.

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