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Solving the Challenging Synthesis of Highly Cytotoxic Silver Complexes bearing Sterically Hindered NHC Ligands with Mechanochemistry

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Dedicated to Professor Ei-ichi Negishi on the occasion of his 85th birthday

The use of ball-mills enabled the straightforward synthesis of a variety of silver(I) complexes featuring challenging NHC ligands. Sterically hindered including electron-poor or with very low solubility imidazolium salts were grinded with silver(I) oxide to furnish heteroleptic or homoleptic complexes in high yield and short reaction times. The synthesis of heteroleptic bis-NHC silver(I) complexes was also performed for the first time in a ball-mill. The efficiency and rapidity of the mechanochemical approach enabled the generation of a library of unprecedented NHC silver complexes, which cytotoxicity on HCT116 colorectal cancer cell line was evaluated providing a rare example of medicinal mechanochemistry. The cationic silver complexes were found more potent than the neutral analogues, with IC50 values down to 21 nM, 256 times more potent than cisplatin.

Introduction

N-heterocyclic carbenes (NHC) are widely used for the stabilization of transition metals, especially compared to phosphines in catalytic systems.1 Additionally, NHC-based complexes have found interest due to their biological properties, for example for their antibacterial, antifungal or anticancer properties.2 Besides, many groups have shown that NHC-gold(I) and NHC-gold(III) complexes could be valid alternatives to cisplatin due to their different mode of action.3 Silver(I) complexes have also been studied because they might behave similarly to gold complexes.2a–e 4 However, NHC-silver(I) complexes have mostly been studied for their antibacterial and antimicrobial activity,5 and only some of them were found to be active as anticancer agents.6 However, most of these complexes were either neutral [AgCl(NHC)] complexes or cationic homoleptic [Ag(NHC)2] complexes featuring N,N-dialkyl ligands. Cationic homoleptic [Ag(NHC)2] complexes bearing N,N-diaryl NHC ligands have been less studied because their synthesis is known to be more challenging. Yet, we recently developed a mechanochemical7 solvent-free method that enables a rapid and highly efficient access to such complexes.8 Additionally, ball-milling permits to easily synthesize neutral [AgCl(NHC)] complexes, either with alkyl or aryl substituents.9 In this manuscript, we initially focused our attention on the mechanochemistry of less conventional silver complexes bearing sterically hindered NHC ligands including one with electron withdrawing groups, to assess if the mechanochemical approach could solve synthetic problems encountered in solution.7e Then, we evaluated the cytotoxicity of the corresponding complexes, as well as heteroleptic bis-NHC complexes, as anticancer agents on colorectal HCT116 cancer cell line. Of note, this represents one of the rare examples of mechanochemical synthesis of molecules for biological testing, a branch of medicinal mechanochemistry.10

Results and discussion

Mechanosynthesis of heteroleptic neutral silver(I) complexes

First, the synthesis of neutral heteroleptic complexes was realized by milling highly encumbered imidazolium salts 1a–e and silver(I) oxide in slight excess in a stainless steel jar containing a 1 cm diameter stainless steel ball, using a vibratory MM400 ball-mill (Scheme 1). Gratifyingly, after 3 h at 30 Hz, full conversion was obtained in all cases. Complexes 2a and 2b, featuring ligands IPr3Cl (1,3-bis(4-methoxy-2,6-disopropylphenyl)imidazol-2-ylidene) and tIPr3Cl (1,3-bis(4-methoxy-2,6-disopropylphenyl)-4,5-dimethylimidazol-2-ylidene) could be isolated in 81% and 86% yield, respectively. In solution, the use of electron poor tIPr-HCl 1c (1,3-bis(2,6-disopropylphenyl)-4,5-dichloroimidazolium chloride) revealed problematic. Indeed, even when full conversion was obtained, isolated yield of corresponding complex 2c did not exceed

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31%. Of note, the same trend was observed in the synthesis of [CuCl(IPr)], with 40% being the highest reported isolated yield. Using the ball-mill, full conversion of 1c was also observed, but, in sharp contrast to the solution, upon treatment, complex 2c was isolated in an excellent 90% yield. Increased steric hindrance was not a limit to the method as complexes 2d and 2e, featuring IPr*OMe ([1,3-bis(4-methoxy-2,6-diphenylmethyl)phenyl]imidazol-2-ylidene)13 and IPr* ([1,3-bis(4-methyl-2,6-diphenylmethyl)phenyl]imidazol-2-ylidene)14 ligands, were isolated in 89% and 81%, respectively. While the exact reason for such positive results is not exactly known at the moment, this could be reasonably explained by a kinetic effect, in the absence of solvent, together with the efficient mixing of highly concentrated mixtures. It is important to highlight that the solvent-free method revealed highly practical in these cases since the imidazolium salts 1d and 1e are poorly soluble in organic solvents and water.

Mechanosynthesis of homoleptic cationic silver(I) complexes

We then turned our attention to unprecedented homoleptic [Ag(NHC)]PF$_6$ complexes. Imidazolium salts 1a-i were first converted into their PF$_6$ counterpart 3a-i using either the classical method, which consists in solubilizing the chloride salt in water in the presence of KPF$_6$ and recover the hexafluorophosphate salt that precipitates, or our previously reported solvent-free milling approach. This latter technique enabled the reduction of the quantity of KPF$_6$ and water used, and was found to be particularly efficient for salt 1e that reacts poorly in water due to a reduced solubility. In the ball-mill, anion metathesis occurred in 30 min under solvent-free conditions. We first focused on the synthesis of complex 4c, featuring electron poor ClPr ligand (Table 1). As discussed above, isolation of complexes featuring this ligand is difficult when the reaction is performed in solution. Reaction of 3c with...
silver(I) oxide in refluxing chloroform gave only 31% NMR conversion after 24h, and 90% after 48h (Table 1, entry 1). In dichloromethane at room temperature, with the addition of sodium hydroxide, it was possible to obtain full conversion of \(\text{Cl}_{2}\text{Ag-IPr}^{13}\text{PF}_6\) in 24 h (Table 1, entry 2). However, after filtration on Celite® and evaporation, complex 4c was obtained in only 45% yield. When imidazolium salt 3c was ball-milled with NaOH and Ag₂O under solvent-free conditions at 30 Hz for 3h, 100% conversion was also observed. More importantly, after the same treatment as when the reaction was performed in solution, homoleptic complex \([\text{Ag}(\text{IPr}^{13})]_{2}\text{PF}_6\) 4c was isolated in 85% yield (Table 1, entry 3). The desired complex 4c may thus easily decompose in solution while the solvent-free approach gives a much faster access to the complex and in a two-fold higher isolated yield.

The mechanochemical approach was then applied to salts 3a-e in the presence of silver oxide and sodium hydroxide (Scheme 2). As for complexes 2a-e, full conversion was observed in all cases in 3 h of milling. Homoleptic complexes 4a and 4b were isolated in excellent yields. The milling method was then applied successfully to poorly soluble \(\text{IPr}^*\text{OMe}_2\) (compared to IPr*), probably because of the worse σ-donation of the nitrogen atoms, was found to be slightly slower than with IPr*. More importantly, after 4h at 450 rpm, full conversion was obtained, yielding complex 4e in 80% yield. With a similar ligand bearing a benzyl instead of the picolyl group on the aromatic ring with a 2,6-diisopropylphenyl group resulted in slightly lower yields of 70% and 88% for 4h and 4i, respectively.

Mechanosynthesis of heteroleptic cationic silver(I) complexes

Finally, heteroleptic bis-NHC silver(I) complexes were synthesized using the vibratory ball-mill, starting from \([\text{AgCl}(\text{IPr}))\) 2f (IPr = 1,3-bis(2,6-diisopropylphenyl)-imidazol-2-ylidene), which was prepared via mechanochemistry. To the best of our knowledge, the synthesis of heteroleptic silver(I) complexes was never attempted in a ball-mill. Reaction was thus first realized using classical IMes-HPFe (1,3-bis(2,4,6-trimethylphenyl)imidazolium) and SiMes-HPFe (1,3-bis(2,4,6-trimethylphenyl)imidazolium) salts in the presence of sodium hydroxide (Scheme 4).

After 1 h of milling at 30 Hz in a vibratory ball-mill, full conversions were obtained and heteroleptic complexes 5a and 5b were isolated in 90% and 91% yield, respectively. Reaction with \(\text{Cl}_{2}\text{Ag-IPr}^{13}\text{PF}_6\) 3c and \(\text{Me}_{2}\text{Ag-IPr}^{13}\text{PF}_6\) 3b yielded corresponding complexes bearing N-alkyl, N-aryl ligands.
complexes 5c and 5d in 88% and 89%, respectively. To widen the scope of attainable heteroleptic complexes using this methodology, \(N,N\)-dibenzylimidazolium hexafluorophosphate and 1,3,4-triphenyl-1,2,4-triazolium hexafluorophosphate were reacted efficiently to furnish 5e and 5f in excellent yields. Of note, as shown by \(^1\)H NMR and HRMS analyses, only traces of ligand scrambling, resulting in the formation of undesired homoleptic silver complexes, were observed.\(^{16}\) X-ray quality crystals could be grown by slow diffusion of diethyl ether into a dichloromethane solution of complexes 2a, 2b, 4a and 4b (Figure 2). XRD analysis allowed to evaluate the steric properties of the ligands by calculating the %V\textsubscript{bur} (percent buried volume) of each complex using the SambVca web application (Table 2).\(^{17}\) As already witnessed with other metals, the introduction of methyl groups on the NHC backbone (2a vs 2b and 4a vs 4b), which push the aromatic moieties towards the metal center, induces an increase in %V\textsubscript{bur} in each case. The increase of the steric hindrance also

![Scheme 4. Mechanosynthesis of cationic heteroleptic silver complexes](image)

**Table 2. %V\textsubscript{bur} for the different complexes**

<table>
<thead>
<tr>
<th>Entry</th>
<th>Complex</th>
<th>(d_{C-Ag}) (Å)</th>
<th>%V\textsubscript{bur}(^a)</th>
<th>%V\textsubscript{bur}(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2a</td>
<td>2.080</td>
<td>40.2</td>
<td>41.3</td>
</tr>
<tr>
<td>2</td>
<td>2b</td>
<td>2.087</td>
<td>42.8</td>
<td>42.5</td>
</tr>
<tr>
<td>3</td>
<td>2e(^{14})</td>
<td>2.078</td>
<td>52.9</td>
<td>52.9</td>
</tr>
<tr>
<td>4</td>
<td>4a</td>
<td>2.099</td>
<td>38.7</td>
<td>38.7</td>
</tr>
<tr>
<td>5</td>
<td>4b</td>
<td>2.122</td>
<td>40.2</td>
<td>40.7</td>
</tr>
</tbody>
</table>

\(^a\) %V\textsubscript{bur} calculated with real C-Ag distance, sphere radius of 3.5 Å. \(^b\) Distance C-Ag normalized at 2.1 Å, sphere radius 3.5 Å.

![Figure 2. ORTEPs (at 50% probability level) of compounds (a) 2b, (b) 4a. Hydrogen atoms and PF\(_6\) anion are omitted for clarity.](image)
results in a longer NHC-metal distance. In comparison, ligand 
IPr* was found to be extremely hindered as %Vis calculated 
for 2e reaches a value of 52.9 while the %Vis of the isopropyl 
analogues do not exceed the value of 42.8.

**Cytotoxic activity of NHC silver(I) complexes**

Since homolectic silver(I) complexes featuring benzimidazole or 
imidazopyridine-based NHC ligand have already shown 
promising activity against colorectal HCT116 cancer cell lines 
compared to 5-fluorouracil or cisplatin, respectively, the 
unique capacity of mechanochemistry to generate rapidly a 
library of NHC silver complexes (2a-5f) prompted us to 
evaluate their cytotoxicity against this cell line. The 
percentage inhibition of cell proliferation was firstly measured 
using 10⁻⁵ and 10⁻⁶ M solutions of the complexes (Figure 3). 
Half growth inhibition concentration (IC₅₀) was then measured 
only for compounds that showed high percentage of inhibition 
at 10⁻⁶ M (Figure 4). As a comparison, doxorubicin and 
cisplatin, which are commonly used to treat cancer, were 
evaluated on the same cancer cell line and possess IC₅₀ of 810 
and 5.37 μM, respectively. In addition, [AgClIPr] 2f and 
[Ag(IPr)₂]PF₆ 4j complexes were also tested to evaluate the 
influence of substitutions of the ligand on cytotoxicity. Among 
the neutral heteroleptic complexes 2a-f, only 2d and 2e were 
found almost inactive. Such behavior could be assigned to the 
poor solubility of the complexes in DMSO. Complex 2a showed 
an IC₅₀ of 259 nM. As a comparison, 2f, which contains the 
classical IPr ligand, exhibited an IC₅₀ of 390 nM, thus showing 
the positive influence of the methoxy groups on the NHC. The 
introduction of methyl group on the backbone of the NHC 
resulted in another positive effect as 2b was found to be active 
at 96.8 nM. On the other hand, 2c, featuring chlorine atoms on 
the backbone of the NHC, displayed an IC₅₀ of 616 nM. Cationic 
homelectic complexes having N,N-diaryl NHC ligands 4a-c 
displayed a higher cytotoxicity, with IC₅₀ values down to 24.9 
M for 4b. Once again, the positive effect of the methoxy 
group was demonstrated as [Ag(IPr)₂]PF₆ complex displayed an 
IC₅₀ of 140 nM (vs 35.4 nM for 4a). The addition of methyl 
groups on the NHC backbone results in even lower IC₅₀ value 
(35.4 nM for 4a vs 24.9 nM for 4b). Homoleptic complexes 4f-i, 
containing N-aryl, N-alkyl NHC ligands, were found to be less 
active. In this family, 4h was the most active, with an IC₅₀ at 
293 nM. Cationic bis-NHC silver complexes 5a-f also showed 
promising activity. 5a, featuring an IMes ligand, was more 
cytotoxic than 5b that contains the analogous saturated ligand. 
While very similar in structure, 5d, having one IPr ligand in 
place of MeIPrOMe, was found to be slightly more active than 
homeletic 4b, with the best IC₅₀ of 21 nM. 5d is thus 256 and 
38 times more active than cisplatin and doxorubicin, 
respectively. Interestingly, 5f, containing a TPT ligand, showed 
an IC₅₀ of 163 nM, thus demonstrating a possible 
diversification of the structure of the active complexes. On the 
other hand, 5c and 5e, featuring a ClIPr and a N,N-dibenzyl NHC 
ligand, respectively, were not as active, with a % inhibition of 
cell proliferation below the values obtained for the other 
complexes. Thus, this preliminary study shows that introducing 
chlorine atoms on the backbone of the NHC, or using N-alkyl, 
N-aryl NHC ligands is detrimental to the biological activity. In 
the case of complexes featuring chlorine-substituted NHC 
ligands, the lower activity could be due to instability issues, as 
observed during their synthesis in solution. On the other hand, 
the MeIPrOMe ligand, either in neutral heteroleptic or cationic 
complexes, seems to present the best positive effect on the 
cytotoxicity of the silver complexes. Of note, apart from 4g, all 
the silver complexes tested displayed a higher cytotoxicity 
than doxorubicin and cisplatin.
Conclusions

In conclusion, mechanochemistry overcame solution-based chemistry, permitting to access rapidly and efficiently novel families of neutral heteroleptic and cationic homo- and heteroleptic complexes featuring NHC ligands bearing sterically hindering groups, and also electron-donating or - withdrawing substituents. Importantly, the use of ball-mills enabled the isolation of complexes difficult if not impossible to prepare and isolate using solution-based strategy. As most of the compounds obtained displayed a novel structure, their biological activity was evaluated. As preliminary results, the silver complexes showed high cytotoxic activity against colorectal HCT116 cancer cell line, with IC_{50} down to 21 nM, which is 256 times better than cisplatin.

Conflicts of interest

There are no conflicts to declare.

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Notes and references


16. See Supporting Information for details.