Mechanochemical and Conformational Study of N-heterocyclic Carbonyl-Oxime Transformations

Ines Primožič,* Tomica Hrenar,* Krešimir Baumann, Lucija Krišto, Ivana Križić, and Srdanka Tomić

Department of Chemistry, Faculty of Science, University of Zagreb, Horvatovac 102a, HR-10000 Zagreb, Croatia

RECEIVED MAY 2, 2014; REVISED MAY 27, 2014; ACCEPTED MAY 28, 2014

Abstract. New mechanochemical pathways for the transformation of six N-heterocyclic carbonyl compounds into oximes using hydroxylamine hydrochloride were explored. Reactions were performed first without any base since the heterocyclic moieties (imidazole, benzimidazole, pyridine and quinuclidine) have an intrinsic basic nitrogen atom. This green, solvent free method was suitable for all compounds (up to quantitative yields) except for N-benzyl substituted imidazole and benzimidazole-2-carbaldehyde. For the slower reacting aldehydes, reactions with liquid assisted grinding and addition of sodium hydroxide were performed as well. Conformational analysis and quantum-chemical calculations revealed steric and electronic reasons for the lower reactivity of N-benzyl substituted derivatives.

Keywords: nitrogen heterocycles; oximes; mechanochemical synthesis; conformational analysis; DFT

INTRODUCTION

Oximes are used as building blocks for the synthesis of diverse biologically active compounds (e.g. some antimicrobial agents,1 insecticides,2 and vasodilators3). Among the most important oxime drugs are compounds that are used as antidotes of nerve agents.4 Nerve agent inactivates acetylcholinesterase molecules by phosphorylation of amino acid serine in the active site. Oximes can reactivate inhibited acetylcholinesterase by a nucleophilic attack of the phosphorus atom thus forming an oxime-phosphonate and the free enzyme. The most effective antidotes for nerve-agent poisoning are N-heterocyclic oximes based on a pyridine skeleton such as pralidoxime (2-PAM), obidoxime, HI-6, and TMB-4, Fig. 1. Since oxime functionalities share common electrostatic properties as the ester linkage, oximes have been used successfully as bioisosteres for the ester group in the muscarinic ligands.5,6 The oxime moiety is stable and represents a suitable base for a variety of structural modifications as well.

Beside their use as intermediates in organic synthesis,8–12 oximes are also of interest for protection, purification and characterization of carbonyl compounds.13 They can be efficient ligands in coordination chemistry for complexation of various metals,14 and in supramolecular chemistry since they are strong hydrogen-bond donors.15 As a result of their broad utilisation, there is a growing interest in finding convenient synthetic approaches (e.g. solvent free reactions, higher yields, shorter reaction times, etc.).

The classical method for the preparation of oximes includes the reaction of a carbonyl compound with hydroxylamine hydrochloride in the presence of an equimolar amount of base using solution-based methods (refluxing alcoholic/aqueous solutions).16 Characteristics for these reactions are usually long reaction time, elevated temperatures, and abundant use of organic solvents, which makes them expensive and environmentally harmful. Some advances have been made in the greener synthesis of aldoximes from carbonyl compounds, for example, by use of calcium oxide with elevated temperature and solvent free conditions,17 microwave irradiated synthesis in presence of basic Al2O3 and pestle & mortar synthesis with molecular sives,18 grinding of some aliphatic and aromatic ketones and aldehydes with the addition of sodium hydroxide without solvent19 and liquid assisted grinding,20 grinding in conjunction with Bi2O3.21 Up to now, only one study of solvent free mechanochemical transformation of N-heterocyclic aldehydes (pyridine-2-, 3- and 4-carbaldehydes) was conducted20 but no investigations concerning the use of the N-heterocyclic carbonyl compound as a substrate and base at the same time has been reported so far.

* Authors to whom correspondence should be addressed. (E-mails: ines@chem.pmf.hr, hrenar@chem.pmf.hr)
In the course of our work in the design and synthesis of new imidazolium, pyridinium and quinuclidinium oximes as antidotes in organophosphorus poisoning, we decided to explore possible mechanochemical paths in the search of a more efficient preparation of desired compounds. In this work, six different \(N\)-heterocyclic oximes were synthesized from their corresponding carbonyl derivatives, Fig. 2, in the presence of hydroxylamine hydrochloride under solvent free conditions using ball milling. The aim was to find the synthetic route that is green, thus a solvent-free method giving products in good yields, at ambient conditions and without any other additives. Transformations were monitored by IR spectroscopy in order to obtain the information about the reaction mixture composition depending upon time without the interference of added solvents necessary for other frequently used methods such as NMR. Molecular modelling was used to rationalize the observed differences in reactivity of different compounds by conformational analysis of reactants and products.

**EXPERIMENTAL**

Reagents and solvents used for the synthesis were purchased from Sigma-Aldrich, Co., and used without further purification. Appropriate imidazole and benzimidazole 2-carbaldehydes 1a–4a were prepared from \(N\)-alkyl imidazoles and benzimidazoles in the reaction with \(n\)-butyl lithium (\(n\)-BuLi) and DMF.\(^{27}\) Pyridine-3-carbaldehyde (5a) was commercially available (Sigma-Aldrich, Co.), while the only ketone used, quinuclidin-3-one (6a),\(^{28}\) was prepared from the commercially available quinuclidin-3-one hydrochloride (Fluka). The classical syntheses of \(N\)-heterocyclic oximes 1b–5b\(^{29,30}\) and 6b\(^{31}\) were carried out according to the published procedures. \(^1\)H and \(^{13}\)C NMR spectra were recorded on a Bruker AV-300 or 600 Spectrometer at 300 or 600 MHz, respectively. All NMR spectra were measured in DMSO-\(d_6\) using tetramethylsilane as a reference. IR spectra were recorded as thin films on NaCl plates or KBr pellets on a Perkin-Elmer Spectrum Two FT-IR and the signals reported in cm\(^{-1}\). All compounds were identified by comparison of their physical and spectral data with those reported in the literature.

**Mechanochemical Synthesis of Oximes**

A Retsch MM200 grinder mill operating at 25 Hz frequency was used for the mechanochemical synthesis. Ball mill experiments were performed in stainless steel jar of 10 mL volume using one ball. Reactions were monitored by TLC (DC-Alufolien Aluminiumoxide 60 F254 plates (Merck) with 9:1 chloroform-methanol as the eluent) and no side products have been detected. FT-IR spectra were recorded over the 4000–400 cm\(^{-1}\) range without baseline corrections up to 30 minutes except for compound 4a where the reaction was monitored during 90 minutes. In all reactions 100 mg of FTIR-grade KBr was added. **Procedure A**: in a jar, 100 mg of the carbonyl compound and equimolar amount of hydroxylamine hydrochloride were grounded together in a mill. **Procedure B**: (liquid assisted grinding, LAG): 100 mg of carbonyl compound, equimolar amount of hydroxylamine hydrochloride in the presence of 30 \(\mu\)L of methanol were grounded together in a mill. **Procedure C**: 100 mg of carbonyl compound, equimolar amount of hydroxylamine hydrochloride in the presence of 30 \(\mu\)L of methanol were grounded together in a mill.

**Quantum-Chemical Calculation**

Conformational search was performed by calculation of relaxed potential energy surfaces (PES). PES for 1a and 3a were spanned by two torsional coordinates \(\phi_1\) and \(\phi_2\).
in the relative range of 0–360° from the initial structure (Fig. 4) whereas PES for \(2a\) and \(4a\) were spanned by three torsional coordinates \(\varphi_1\), \(\varphi_2\) and \(\varphi_3\) (Fig. 5). PES scans were obtained by varying the torsional coordinates using the automatic conformational generator implemented in program \(qcc\) and optimizing geometries in all geometrical parameters apart from the frozen torsional angles. Data from relaxed scans were arranged in a two-way or a three-way array. Parallelized combinatorial optimization algorithm for the arbitrary number of ways (dimensions) implemented in program \(moonee\) was used to determine all local minimums on the investigated PES. All local minimums were reoptimized at the B3LYP/6-311++G(d,p) level. To insure that the obtained geometries were local minima, harmonic frequency calculations were performed. Natural bond orbital analysis (NBO) was carried out on the optimized structures of conformers. All electronic structure calculations were performed using the Gaussian 09 program. Spectra figures and PES were plotted using the Gnuplot graphing utility. Potential energy on 4-dimensional plots is represented with the colour gradient ranging from blue to white to red and simultaneously with the different sizes of spheres.

RESULTS AND DISCUSSION

It is known that mechanochemistry is a useful tool for the efficient synthesis of a variety of imines: the reactions can be performed in a single step with quantitative yields by gas-solid, liquid-solid or solid-solid reactions. Regarding the oxime synthesis, one study of solvent free mechanochemical transformations of pyridine-2-, 3- and 4-carbaldehydes into oximes was recently conducted were solvent-drop grinding in the presence of hydroxylamine hydrochloride and sodium hydroxide were done with a pestle and mortar. Reactions were performed for 9 minutes at room temperature and incomplete reaction was obtained only for 2-carbaldehyde (65%). However, no investigations concerning the use of \(N\)-heterocyclic carbonyl compounds as bases that can deprotonate hydroxylamine hydrochloride have been reported up to now. Therefore, we synthesized four \(N\)-heterocyclic aldehydes: \(N\)-methyl (1a) and \(N\)-benzylimidazole-2-carbaldehyde (2a); \(N\)-methyl (3a) and \(N\)-benzylbenimidazole-2-carbaldehyde (4a). Pyridine-3-carbaldehyde (5a) was commercially available, as well as quinuclidin-3-one hydrochloride from which quinuclidin-3-one (6a) was prepared. Those carbonyl compounds were selected because they all possess a heterocyclic moiety with a basic nitrogen atom ranging from the most basic quinuclidine ring (\(pK_a = 11.3\)) to imidazole (\(pK_a = 6.95\)), benzimidazole (\(pK_a = 5.53\)) and pyridine ring (\(pK_a = 5.25\)).

Table 1. Mechanochemical synthesis: time required to quantitatively convert carbonyl compounds into oximes.

<table>
<thead>
<tr>
<th>Carbonyl compound</th>
<th>Procedure A (t) / min</th>
<th>Procedure B (t) / min</th>
<th>Procedure C (t) / min</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>1</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>2a</td>
<td>(&gt;30^{(a)})</td>
<td>6</td>
<td>20</td>
</tr>
<tr>
<td>3a</td>
<td>5</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>4a</td>
<td>(&gt;90^{(b)})</td>
<td>(&gt;90^{(b)})</td>
<td>(&gt;90^{(b)})</td>
</tr>
<tr>
<td>5a</td>
<td>30</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>6a</td>
<td>(&gt;30^{(a)})</td>
<td>10</td>
<td>-</td>
</tr>
</tbody>
</table>

\((a)\) incomplete reaction
\((b)\) no product was detected
N-benzyl and carbonyl substituents at the heterocyclic moiety are lowering the pKₐ values (e.g. N-benzylimidazole has pKₐ=6.70, pyridine-3-carbaldehyde pKₐ=3.80 (Ref. 41) and quinuclidin-3-one, pKₐ=7.20), a series of carbonyl compounds with a significant differences in basicity were tested. From all six carbonyl derivatives respective oximes 1b–6b were synthesized using classical solution-based methods with hydroxylamine as described in literature in order to prepare standards for the analysis. Next, mechanochemical synthesis using grinder mill was studied, Scheme 1.

The ball milling of carbonyl compounds 1a–6a was done at room temperature in the stoichiometric ratio of 1:1 with hydroxylamine hydrochloride without addition of any solvent or a base and the reactions were monitored up to 30 minutes (90 minutes for the reaction of 4a), Table 1, Procedure A. Oximes 1b, 3b and 5b were obtained in quantitative yields, while in the reaction of ketone 6a, a small amount of protonated reactant remained, Fig. 3f. The fastest reaction was that of N-methylimidazole aldehyde 1a (1 minute) and the slowest was that of N-benzylimidazole derivative 2a, while no trace of product was detectable in the reaction.
of N-benzylbenzimidazole 4a. Since a very small amount (a drop) of liquid can significantly accelerate mechanochemical reactions (‘liquid assisted grinding’, LAG)\cite{43,44}, in a set of new experiments 30 μL of methanol was added in all reaction mixtures, Table 1, Procedure B. As expected, addition of a small quantity of solvent to the reaction mixture significantly enhanced the rate of the mechanochemical synthesis and quantitatively provided oximes 2b, 5b and 6b in less than 10 minutes. However, the mechanochemical synthesis of oxime 4b was not successful using LAG method even when sodium hydroxide was added as a base in equimolar amounts, Table 1, Procedure C. That indicated that the low basicity of the heterocyclic nitrogen atom of 4a was not the reason for the poor reactivity of that compound as compared to the structurally similar aldehydes 2a (bulky N-benzyl substituent) and 3a (benzimidazole moiety).

In order to obtain information about the reaction mixture composition, reactions were monitored by FT-IR spectroscopy, Fig. 3. By the use of this method, the mixture was taken directly from the reaction jar, KBr pellet made and spectrum recorded without the use of solvent. Prior to the measurements, the IR spectra of the reaction components and the product were studied. The IR spectra were predicted by B3LYP/6-311++G(d,p) quantum chemical calculations as well. Vibrational frequencies calculated were scaled and compared with
the experimental values. It was shown that the range of 1800–600 cm\(^{-1}\) was the most suitable for monitoring the oximation because all spectra had wide signals between 2700 and 3500 cm\(^{-1}\). That signals originate from N–H and O–H stretching vibrations in oximes and deliberated water molecules suggesting that N–H and O–H groups are in strong intramolecular and intermolecular hydrogen bonding. IR spectra of all six compounds are in strong intramolecular and intermolecular hydrogen bonding. IR spectra of all six compounds showed week bands corresponding to the C=N stretching vibration which were in all cases overlapped with the vibrational frequencies of the carbonyl compounds, thus, the complete disappearance of the carbonyl C=O peak in spectrum was monitored. The spectra of all compounds 1a–6a showed strong wide bands corresponding to the carboxylic C=O stretching at 1685, 1688, 1696, 1689, 1706 and 1735 cm\(^{-1}\), respectively, Fig. 3.

To explain the differences in reactivity of N-methyl and N-benzyl substituted imidazole and benzimidazole derivatives, conformational and NBO analysis for aldehydes 1a–4a were done. Initial geometries for the conformational analysis were obtained by analysis of PES scans calculated at the semiempirical level (PM6). PES for N-methyl 1a and 3a, as well as for N-benzyl derivatives 2a and 4a were spanned in the space of two and three torsional coordinates, respectively (Figs. 4 and 5). Optimization procedure for finding local minimums was utilizing brute-force search in

| Table 2. Calculated standard Gibbs energies of formation for conformers of 1a and 3a (B3LYP/6 311++G(d,p), relative to conformer 1) |
|----------------|----------------|----------------|
| Conformer      | \(\Delta G^r(1a)\) / kJ mol\(^{-1}\) | \(\Delta G^r(3a)\) / kJ mol\(^{-1}\) |
| 1              | 0.0            | 0.0            |
| 2              | 28.0           | 26.0           |

Conformers found at the semiempirical level were subsequently optimized using DFT (B3LYP/6-311++G(d,p)) method. To ensure that optimized structures were local minimums, harmonic frequency calculations were performed. Conformational space of 1a and 3a is consisted of two conformers different in the orientation of the conjugated aldehyde group. Conformers of 1a and 3a where the aldehyde oxygen atom is not pointing to the N-methyl group were significantly higher in energy (Fig. 4 and Table 2). According to the Boltzmann distribution, probability of finding these conformers is very low and thus could be excluded from further analysis. In the conformers that represent global minimums on the PES (carbonyl oxygen pointing toward the N-methyl group) lower energy is derived from the presence of the hydrogen bond (=O···H 2.38 Å). Since the geometry of nucleophilic attack at the carbon atom in carbonyl group should be ideal Bürgi–Dunitz angle (≈107°),\(^{45}\) it is evident that there is no steric hindrance of the N-methyl group. Moreover, NBO analysis showed that natural atomic orbital occupancies for the carbon atoms of carbonyl groups in 1a and 3a are similar thus giving the similar reaction rates (Table 1).

Conformational space of N-benzyl derivatives is consisted of two conformers in the case of 2a and three conformers in the case of 4a (Fig. 5). The second conformer of 2a and the third conformer of 4a, where the aldehyde oxygen atom is at the same side as the non-substituted nitrogen atom of imidazole ring, were again significantly higher in the energy (Fig. 5 and Table 3). Furthermore, in this conformer one side of the carbonyl group is completely blocked with a bulky benzyl group and the reaction with nucleophile is not possible at all.

In each of the remaining conformers (conformer 1 for 2a and conformers 1 and 2 for 4a) carbonyl oxygen is pointing toward the N-methylene group (=O···H 2.32 Å in 2a; 2.24 and 2.44 Å in 4a) and has a contact with the ortho hydrogen atom of the phenyl ring (Fig. 6). Although, the flexible N-benzyl group have less nega-

Figure 6. Overlay of conformers for aldehydes 2a (conformer 1: red) and 4a (conformer 1: blue, conformer 2: yellow). (Distances are given in ångströms.)
tive steric effect than that in previously described conformers, the electrostatic effect at the nucleophilic attack should be taken into account as well (Fig. 6). In the case of 4a, the conformer 2 (only 3.1 kJ mol\(^{-1}\) higher in energy) which has no equivalent in 2a due to the steric interactions between methylene and aromatic protons of the benzene ring, has the benzyl group in a position which can interfere even more with the nucleophile, and as a consequence, the slowing of the reaction rate can be expected. NBO analysis revealed that the natural atomic orbital occupancies for the carbon atoms of carbonyl groups in 2a and 4a are higher than in 1a and 3a. Higher occupancies mean that the nucleophilic attack is deflected in comparison to reactions with \(N\)-methyl derivatives 1a and 3a. Joint effect of steric hindrance and electronic effects are in accordance with the observed reduced reaction rates (Table 1).

**CONCLUSION**

Mechanochemistry proved to be an excellent alternative for the transformations of \(N\)-heterocyclic carbonyl compounds into oximes using hydroxylamine hydrochloride. There is no need for use of an additional base and only catalytic amount of solvent is sufficient to enhance the reaction, which makes this route environmentally friendly, and a better and greener alternative to the existing methods for \(N\)-heterocyclic oxime synthesis. It was not possible to prepare aldoxime of \(N\)-benzyl substituted benzimidazole, derivative with the bulky, electron withdrawing group. With the addition of an external base, it was shown that the low basicity of heterocyclic nitrogen atom is not responsible for the lowest reactivity. Conformational analysis pointed at the steric repulsions as the one of the main causes for the low reactivity in the solid state synthesis. NBO occupancies provided additional electronic reasons for the lower reactivity of \(N\)-benzyl substituted derivatives.

**Acknowledgements.** This work was supported by the Ministry of Science, Education and Sports of the Republic of Croatia, Research Projects 119-1191344-3121 and 119-1191342-2959.

**REFERENCES**


