The analysis of photochemical anti-syn isomerization process across the –N–N= bond in heterocyclic imines

Michal Hricovíni¹,* , James Asher², and Miloš Hricovíni¹

¹ Institute of Chemistry, Slovak Academy of Sciences, Dúbravská cesta 9, 845 38 Bratislava, Slovak Republic;
² Institute of Inorganic Chemistry, Slovak Academy of Sciences, Dúbravská cesta 9, 845 36 Bratislava, Slovak Republic.

* Corresponding author: chemmike@savba.sk
The analysis of photochemical anti-syn isomerization process across the –N–N= bond in heterocyclic imines
Abstract: Many biologically active molecules experience photoisomerization processes after light-induced excitation (e.g. rhodopsin). Important photochemical properties showed particularly compounds possessing aromatic systems in the presence of conjugated heteroatoms. Such systems often constitute a part of natural biomolecules and play a crucial role in essential biochemical and biological processes. Due to photochemical nature of these aromatic compounds, such as Schiff bases, they are often studied for pharmacological applications and used in biochemistry and medicine. For this reason, we focused our study on photochemical processes of quinazolinone-based Schiff bases. The isomerization from the energetically more favourable anti-isomer to the syn-isomer by UV/vis excitation has been found namely in the systems possessing a double bond systems. Presented analysis deals with NMR spectroscopy and theoretical DFT analysis of photochemical processes of the Schiff base possessing a quinazolinone moiety with a series of model compounds to investigate the photochemical behaviour of the –N–N= linkage. The NMR experiments in solution showed that irradiation at 365 nm leads to photochemically-induced isomerization from the anti- to the higher-energy syn-form around the –N–N= linkage.

Keywords: NMR; Photoactive biomolecules; Photoisomerization; Schiff-bases
Introduction

- some chemical substances are photoactive when subjected to UV radiation
- can form many reactive species
- play a crucial role in essential biochemical processes
- exhibit structural changes and biological activities upon radiation
- act as the first step in a number of light-induced biological processes
- photochemical nature makes them applicable in many fields

Pepino, A. J. et al. *Phys. Chem. Chem. Phys.*, 2015, 17, 12927.
Yin, T.-T. et al. *Org. Electron.*, 2018, 52, 61.
Rashid, Md Al M. et al. *Nanomaterials*, 2019, 9, 119.
Compounds

- Schiff-base possessing a quinazolinone moiety (S1)
- Model compounds (M1-M4) possessing N–N=CH moiety
- Significance of Schiff bases can be attributed to the presence of the N=C or N=N double bond
- The –N–N=CH– array of atoms has different photochemical properties compared to the N=N double bond

Hricovíni, M.; Asher, J.; Hricovíni, M. RSC Adv. 2020, 10, 5540.
Outline

- study of photochemical behaviour
  - generation of ROS via photosystem I/II mechanism
  - structural changes upon irradiation

- spectroscopic characterisation
  - EPR (X-band)
  - NMR (1D and 2D)

- DFT calculations
  - geometry optimisation
  - excited states study

\[ \begin{align*}
0_P & \xrightarrow{h\nu} 1_P \xrightarrow{\text{ISC}} 3_P^* + 3_O_2 \\
3_P^* + S & \rightarrow P^- + S'^+ \\
3_P^* + 3_O_2 & \rightarrow P'^+ + O_2^- \\
3_P^* + 3_O_2 & \rightarrow 1_P + 1_O_2 \\
\end{align*} \]

P = photosensitizer, S = substrate, 0 = ground state, 1 = singlet, 3 = triplet, * = activated state, ISC = intersystem crossing
Results and discussion

- DMSO solvent, RT, continuous irradiation at 365 nm
- spin-trapping technique, spin traps used

- detected oxygen centred adducts, namely \( \cdot \text{DMPO-O}_2^- \) (a-c) and carbon centred adduct \( \cdot \text{ND-CH}_3 \) (d)

- reactive species are formed via consecutive reactions in DMSO under atmospheric conditions

\[
\begin{align*}
(\text{CH}_3)_2\text{SO} + \cdot \text{OOH/O}_2^- & \rightarrow \cdot \text{CH}_3 + \text{CH}_3\text{S(O)OO)/(CH}_3\text{S(O)OO}^- \\
\cdot \text{CH}_3 + \cdot \text{O}_2 & \rightarrow \text{CH}_3\text{OO}^- \\
2 \cdot \text{CH}_3\text{OO}^- & \rightarrow \text{CH}_3\text{O}^- + \cdot \text{O}_2
\end{align*}
\]

Hricovíni, M.; Asher, J.; Hricovíni, M. RSC Adv. 2020, 10, 5540.
Results and discussion

- 600 MHz, RT, DMSO, before irradiation (a)

- new resonances originating from the syn-isomer after 10 min irradiation at 365 nm (b)

- intensities had been increasing with irradiation

- isomerization varied in a manner correlated to the structure

- stabilisation of syn-isomer via H-bonds

High-resolution 600 MHz $^1$H NMR spectrum of S1 in DMSO at 25°C before irradiation (a) and after 10 minutes of UV irradiation ($\lambda_{max} = 365$ nm) (b). Smaller signals (marked with subscript s) belong to the syn-isomer, which formed upon irradiation; assignments without indices belong to the main form (anti-isomer).

Hricovín, M.; Asher, J.; Hricovíni, M. RSC Adv. 2020, 10, 5540.
Results and discussion

- temperature coefficients of the –N₃–N=C(H) protons and OH protons (Table 1)

- coefficients for the OH protons for 1 – 7 varied between -5.36 and -6.11 ppb/K

- OH groups are involved in intramolecular H-bonds with different strengths depending upon the structure and conformation

- the same behaviour also for the NH protons

- stability of syn-isomers due to the formation of intramolecular hydrogen bonds

| S1   | anti-                     | (20°C) | (25°C) | (35°C) | (45°C) | (55°C) | (65°C) | ppb/K |
|------|---------------------------|--------|--------|--------|--------|--------|--------|-------|
|      | –OH’                      | 10.233 | 10.203 | 10.142 | 10.080 | 10.020 | 9.958  | -6.11 |
|      | –OH’’                     | 11.434 | 11.409 | 11.353 | 11.299 | 11.246 | 11.193 | -5.36 |
|      | N₃=N=CH                   | 8.441  | 8.454  | 8.477  | 8.499  | 8.522  | 8.542  | -2.24 |
|      | –NH                       | 7.484  | 7.460  | 7.408  | 7.357  | 7.305  | 7.254  | -5.11 |

Hricovín, M.; Asher, J.; Hricovíni, M. RSC Adv. 2020, 10, 5540.
Results and discussion

- The relative energies between the anti- and syn-forms for M1-M4 and S1 are listed in Table 2 and the selected geometry parameters are in Table 3 (next slide).

- The N₃–N₉ bond has partial double-bond character (around 1.37 Å, see Table 3, row 3).

- Incorporation of the heterocyclic moiety (M2) to the structure of M1 caused the bond elongation of the bond (1.380 vs. 1.356 Å).

- A significant effect is also seen after addition of the aromatic ring C to the carbon atom C₁₀; there is a visible zigzag double-bond formation in the C₄–N₃–N₉–C₁₀ linkage, where the N₃–N₉ bond was shortened and the N₉–C₁₀ bond was prolonged (Table 3, rows 3 and 4).

**Table 2.** DFT-computed energy differences (kJ/mol) between the anti and syn-forms for all compounds using ωB97XD functional, 6-311++G(2d,2p) basis set and SMD solvent model (DMSO)

| Compound | M1 | M2 | M3 | M4 | S1 |
|----------|----|----|----|----|----|
| ΔE_{syn}−ΔE_{anti} | 7.88 | 6.09 | 4.99 | 2.39 | 7.56 |

Hricovín, M.; Asher, J.; Hricovín, M. *RSC Adv.* 2020, 10, 5540.
## Results and discussion

Table 3. Selected optimised bond lengths (Å), bond angles (degrees) and torsion angles (degrees) for M1–M4 and S1 obtained by DFT calculations at the ωB97XD/6-311++G(2d,2p) using SMD solvent model (DMSO).

| Bond length | M1 | M2 | M3 | M4 | S1 |
|-------------|----------|----------|----------|----------|----------|
| | anti- | syn- | anti- | syn- | anti- | syn- | anti- | syn- | anti- | syn- |
| C1–C6 | – | – | – | – | 1.410 | 1.409 | – | – | 1.410 | 1.410 |
| C10–C1 | – | – | – | – | 1.458 | 1.453 | – | – | 1.457 | 1.453 |
| C3–N3 | 1.365 | 1.371 | 1.365 | 1.375 | 1.357 | 1.387 | 1.374 | 1.402 | 1.360 | 1.386 |
| C10–N9 | 1.267 | 1.272 | 1.270 | 1.272 | 1.278 | 1.280 | 1.265 | 1.268 | 1.278 | 1.279 |
| C2–N3 | 1.356 | 1.356 | 1.380 | 1.368 | 1.385 | 1.385 | 1.358 | 1.349 | 1.388 | 1.384 |
| C2–N3 | – | – | 1.463 | 1.471 | 1.459 | 1.462 | 1.449 | 1.447 | 1.458 | 1.459 |

| Bond angle | M1 | M2 | M3 | M4 | S1 |
|-------------|----------|----------|----------|----------|----------|
| | anti- | syn- | anti- | syn- | anti- | syn- | anti- | syn- |
| N3–N9–C10 | 120.7 | 116.5 | 120.1 | 123.1 | 122.7 | 117.5 | 118.6 | 113.4 | 122.5 | 117.9 |
| N9–C10–C1 | – | – | – | – | 119.4 | 120.7 | – | – | 119.4 | 120.3 |
| C2–N3–C4 | – | – | 123.8 | 122.3 | 121.3 | 118.2 | 121.2 | 122.5 | 122.5 | 120.8 |
| C8a–N1–C2 | – | – | 110.4 | 109.8 | 116.7 | 115.2 | – | – | 116.8 | 116.3 |
| N1–C2–N3 | – | – | 110.4 | 110.2 | 109.0 | 108.0 | – | – | 108.2 | 107.4 |
| C4–N3–N9 | 121.6 | 130.8 | 115.8 | 126.5 | 114.9 | 119.8 | 117.1 | 120.8 | 115.0 | 120.6 |
| C2–C3–N3 | – | – | – | – | – | – | – | – | 112.4 | 113.4 |
| N3–N9–C10 | – | – | – | – | 180 | 0 | –170.9 | 14.8 | –165.7 | 58.9 | –180 | –67.1 | –167.3 | 57.4 |
| C2–N3–C10 | – | – | 3.4 | –177.7 | –1.5 | –156.8 | 0 | 131.1 | 1.3 | –151.9 |
| N9–C10–C1 | – | – | – | – | –179.5 | –178.0 | – | – | 179.9 | –178.1 |
| N9–C10–C1 | – | – | – | – | –2.2 | 1.0 | – | – | –2.3 | 2.0 |
| N3–C2–C4 | – | – | – | – | – | – | – | – | –9.3 | –11.7 |
| N1–C2–N3 | – | – | –21.8 | –31.1 | –41.8 | –53.6 | – | – | –38.7 | –48.1 |

Hricovíni, M.; Asher, J.; Hricovíni, M. RSC Adv. 2020, 10, 5540.
Results and discussion

- For $M_1$, both the molecule and its energy profile with respect to $D(C\text{–}N\text{–}N\text{–}C)$ are symmetric.

- Energy profiles of $S_1$ and $M_1$, $M_2$ and $M_3$ are compared.

- The $C_4N_2$ ring present in $M_2$ introduces a major part of the asymmetry - steric repulsion between the Schiff’s base $CH_2$ group and the ring $CH_2$ group.

- The difference between $S_1$ and $M_3$ is the aromatic Ring B, which does not participate in the large $\pi$-system.

Ground state energy curves (kJ/mol) against $C_4N_3N_9C_{10}$ dihedral angle for $S_1$ and model systems. Top: energy profiles for $S_1$ and $M_1$, $M_2$, and $M_3$ (zeroed to lowest energy point on curve). Calculated at the $\omega B97XD/6-311++G(2d,2p)$ level. Bottom: $S_1$ and $M_3$ (zeroed to syn minimum).

Hricovíni, M.; Asher, J.; Hricovíni, M. RSC Adv. 2020, 10, 5540.
Results and discussion

- small shoulder around 0° in S1, M2, and M3 is visible as a peak in the case of M4, less pronounced in case of M2

- steric repulsion between the C=O group and the electron pairs (two lone pairs, one N–C bond) on sp³-hybridised N⁻

- This suggests that the peak (or shoulder) at 0° arises from resonance structures with negative charge at the Schiff’s base nitrogen

- nearby electron-donating OH group shows such effects most strongly

Top: ground-state energy curves (in kJ/mol) for S1, M3, M4, and M3pcm against D(C–N–N–C). Bottom: resonance structure with negative charge on Schiff’s-base nitrogen.

Hricovíní, M.; Asher, J.; Hricovíní, M. RSC Adv. 2020, 10, 5540.
Results and discussion

- potential energy curves for ground and excited states against the dihedral angle \( D(C\text{--N--N--C}) \); studied for all model compounds and S1

- first excited state has its highest energy at 180° and its lowest energy at 0°

- The first state arises from an \( n-\pi^* \) excitation

- the dominant excitation is HOMO \( \rightarrow \) LUMO

Top: Energy curves for the ground state (GS) and first three excited states (ES1-ES3) of M1. Relative energies (kJ/mol) of the states have been heavily adjusted for clarity.
Bottom: Ground state (GS) and first two excited states (ES1, ES2) energy curves for M2. Relative energies (kJ/mol) of the curves have been adjusted for clarity.

Hricovíni, M.; Asher, J.; Hricovíni, M. RSC Adv. 2020, 10, 5540.
Results and discussion

- M3 and S1 – the situation is a little different
- the first two have similar energy profiles to the ground state; third is inverted
- the conical intersection between 2\textsuperscript{nd} and 3\textsuperscript{rd} excited state
- deexcitation mechanism is similar for both systems

Energy curves for ground state (GS) and excited states (ES\textsubscript{1}-ES\textsubscript{4}) of M3 (left) and S1 (right) with proposed mechanisms of excitation and deexcitation processes (E – excitation, 1 – structural relaxation, 2 – internal conversion, 3 – emission). Relative energies (kJ/mol) of the curves have been adjusted for clarity.

Hricovíni, M.; Asher, J.; Hricovíni, M. RSC Adv. 2020, 10, 5540.
Conclusions

- EPR measurements evidenced the generation of ROS using spin-trapping technique
  - the presence of oxygen- and carbon-centred adducts

- NMR spectra proved the photoisomerization process around the N–N bond within the –N–N=CH– array
  - two isomers, syn-isomer formed, 25% of conversion
  - presence of hydrogen bonds

- DFT calculations
  - the anti-form to be lower in energy than the syn-form, in accordance with experiment
  - excited state energy curves; photoisomerization involves n–π* excitations or proceeds purely via π–π* states; the mechanism is determined by the π-system size
  - **M3** and **S1**, the 3rd singlet-singlet excitation (π–π*) results in an excited state with an appropriate energy profile for isomerisation; lower excitations are unlikely to be involved
Acknowledgments

Institute of Chemistry
Slovak Academy of Sciences

Grant agencies:
VEGA 1/0041/15
VEGA 2/0100/14
APVV-15-0053
SP Grant 2003SP200280203

Computing Centre of the SAS using the supercomputing infrastructure acquired in projects ITMS 26230120002 and 26210120002

Department of Physical Chemistry and Chemical Physics
Faculty of Chemical and Food Technology, Slovak University of Technology