Modeling of Degradation Chemistry at AstraZeneca

Anders Broo
Science of Stability – 2015 12-14 October 2015, Connecticut, USA
Outline

• Why is it of importance to understand degradation chemistry?

• Assessing risk for autooxidation

• Recent development in modeling of oxidation of hetroatoms
  - A predictive method to access risk for amine oxidation
  - Pitfalls and side reactions

• Summary and outlook
Why is it of importance to understand degradation chemistry?

- Regulatory requirement to control potential genotoxic impurities
- Accessing risk for late stage surprises
- Get mechanistic insight to link forced degradation studies to accelerated stability studies
  - Poor link between forced degradation results and observed impurities in formulated products

Eli Lilly experience

Modeling of chemical stability

Autoxidation

Degradation pathways

• Oxidation
  - Peroxides
  - Autoxidation
• Hydrolysis
• Thermal
• Photolysis

Autoxidation

• A major pathway
• Hard to predict
• Difficult to verify experimentally
• Easiest to study with calculations

Initiation

\[ \text{InH} \rightarrow \text{In}^\cdot \]

\[ \text{RH} + \text{In}^\cdot \rightarrow \text{R}^\cdot + \text{InH} \]

\[ \text{R}^\cdot + \text{O}_2 \rightarrow \text{R-O-O}^\cdot \]

\[ \text{R-O-O}^\cdot + \text{RH} \rightarrow \text{R}^\cdot + \text{R-O-OH} \]

\[ \text{R-O-O}^\cdot + \text{R-O-O}^\cdot \rightarrow \text{R-O-O-O-R} \]

\[ \text{R-O-O-O-R} \rightarrow \text{Nonradical products} \]

Propagation

Termination

Overall:

\[ \text{R} \xrightarrow{\text{In}_2, \text{In}^\cdot} \text{ROO}^\cdot \xrightarrow{\text{RH}, \text{R}^\cdot} \text{ROOH} \xrightarrow{\text{O}_2} \text{Products} \]
Modeling of chemical stability

Autoxidation

• Assumption
  - The risk for autoxidation can be estimated from bond dissociation energy (BDE) of the hydrogen that is easiest extracted
• BDE < 78-80 kcal/mol → risk for autoxidation

\[ \text{BDE} = \text{E(mol)} - \text{E(rad)} - \text{E(H}_{\text{rad}}) + \Delta\text{ZPE} \]

![Chemical structures and bond dissociation energy calculations]

**Table 1. The Validation of the Method**

<table>
<thead>
<tr>
<th>Compound</th>
<th>BDE + DZPE</th>
<th>DZPE</th>
<th>BDE (Calculated)</th>
<th>BDE (Experimental)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetic acid</td>
<td>104.7</td>
<td>8.5</td>
<td>96.1</td>
<td>94.6 ± 3.0</td>
</tr>
<tr>
<td>Propylene</td>
<td>94.5</td>
<td>8.3</td>
<td>86.1</td>
<td>88.8 ± 0.4</td>
</tr>
<tr>
<td>Trimethylamine</td>
<td>87.7</td>
<td>8.4</td>
<td>89.4</td>
<td>86.5 ± 2.2</td>
</tr>
<tr>
<td>BNAH</td>
<td>78.6</td>
<td>8.5</td>
<td>70.1</td>
<td>67.9 ± 1</td>
</tr>
<tr>
<td>Formaldehyde</td>
<td>95.5</td>
<td>8.5</td>
<td>87.0</td>
<td>88.144 ± 0.008</td>
</tr>
<tr>
<td>Ethanolamine</td>
<td>88.6</td>
<td>8.9</td>
<td>89.8</td>
<td>90.7 ± 2</td>
</tr>
<tr>
<td>Toluene</td>
<td>96.7</td>
<td>8.3</td>
<td>88.4</td>
<td>89.8 ± 0.6</td>
</tr>
<tr>
<td>Tetrahydronaphthalene</td>
<td>92.0</td>
<td>8.6</td>
<td>83.4</td>
<td>82.9 ± 1.2</td>
</tr>
<tr>
<td>Methanol</td>
<td>102.8</td>
<td>8.6</td>
<td>94.2</td>
<td>96.1 ± 0.3</td>
</tr>
</tbody>
</table>

The calculated values of the BDE are compared with experimental values, all results in kcal/mol.

**Figure 5.** Tetrazepam with the calculated BDE values.

---

Autooxidation risk assessment tool

- Automated process
  - Pipline Pilot Web Port
  - Corina, Szybki, Gaussian09
  - Scripts

Autoxidation
When BDE might fail to identify risk

- The BDE values are very good at predicting the regioselectivity of the autoxidation reaction
- Some cases where BDE ~ 92 kcal/mol like ethers result in reactive oxidation products on storage under air
- Some cases where low BDE ~79 kcal/mol don’t lead to degradation amino acids in peptides

- Abundant product will only be found when the chain reaction propagates
  - “Chain breakers” or anti-oxidants will scavenge the API radical
  - Eg Phenol (Ph-O3) not very stable

Per-Ola Norrby, Carina Bäcktorp, Thomas Andersson, Emma Evertsson, David Benstead, Helen Williams
Autoxidation

• Our tool is used to access risk in most drug development projects
  - If a risk is flagged an expert on chemical reactivity is consulted

• Guideline
  - BDE > 80 – 87 kcal/mol low risk
  - BDE between 72 – 80 kcal/mol intermediate risk
  - BDE < 72 kcal/mol high risk – standard antioxidants migth not be powerfull enough
Modeling of chemical stability

Heteroatom oxidation

- Small amounts of peroxide from excipients may oxidize heteroatoms in drug molecules

- Could this process be modeled using a robust modeling protocol?
  - Calculated reaction barriers $\Delta G^\ddagger$ was correlated to reaction energies $\Delta G_r$ and relative pKa of aliphatic and aromatic amines
  - DFT calculations M06/6-31G**

The relative pK$_a$ is estimated by calculating the Gibbs free energy of reaction $\Delta G_r$ and
\[ pK_a = pK_{a,\text{ref}} + \frac{\Delta G_r}{RT\ln10}, \]
where pK$_{a,\text{ref}}$ = 5.2 in case of pyridine
Modeling of chemical stability

Heteroatom oxidation

\[ \Delta G^{\ddagger}_{\text{calc pKa}} > 40 \text{ low risk} \]
Insight to stress test pitfalls

Oxidation of pyridine

- Hydrogen peroxide as oxidant
  - Slow reaction at room temperature
  - In many cases catalysis is needed to get good conversion
  - $\text{H}_2\text{O}_2$ reacts with unprotonated amine (nucleophilic)
- Apparent reaction barrier $\Delta G^{\ddagger}_{\text{app}}$ of 27.4 kcal/mol
- Calculated barrier is much higher ~43 kcal/mol

- Water might “activate” $\text{H}_2\text{O}_2$
- Influence of $\text{CO}_2$ from air
- Influence of pH
- Influence of co-solvent, acetonitrile

A 13 mM pyridine solution was reacted with 1.2 M hydrogen peroxide and the reaction was monitored for 3 days. After 3 days about 1.2% pyridine N-oxide was observed in the reaction solution.

Experimental and quantum chemical evaluations of pyridine oxidation under drug development stress test conditions; C. Bäcktorp, E. Örnskov, E. J. Ottosson, Evertsson, J. Remmelgas, A. Broo Accepted J. Pharm. Sci. 2015
Water might “activate” H$_2$O$_2$

Pre-complex of amine with water and H$_2$O$_2$

$$\Delta G^\ddagger_{\text{app}} \text{ of 27.4 kcal/mol}$$

<table>
<thead>
<tr>
<th>Oxidant</th>
<th>$\Delta G^\ddagger_{\text{intr}}$</th>
<th>$\Delta G^\ddagger_{\text{corr}}$</th>
<th>$\Delta G^\ddagger_{\text{preact}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>H$_2$O$_2$ (n=0)</td>
<td>45.3</td>
<td>45.2</td>
<td>44.4</td>
</tr>
<tr>
<td>H$_2$O$_2$ +1H$_2$O (n=1)</td>
<td>39.9</td>
<td>37.5</td>
<td>35.7</td>
</tr>
<tr>
<td>H$_2$O$_2$ +2H$_2$O (n=2)</td>
<td>37.8</td>
<td>33.1</td>
<td>32.1</td>
</tr>
<tr>
<td>H$_2$O$_2$ +3H$_2$O (n=3)</td>
<td>39.5</td>
<td>32.4</td>
<td>32.3</td>
</tr>
<tr>
<td>H$_2$O$_2$ +4H$_2$O (n=4)</td>
<td>41.7</td>
<td>32.3</td>
<td>30.2</td>
</tr>
</tbody>
</table>

$\Delta G^\ddagger_{\text{intr}} = \text{from QM calculations}$

$\Delta G^\ddagger_{\text{corr}} = \Delta G^\ddagger_{\text{intr}} - RT \ln[\text{Ox}]$

$\Delta G^\ddagger_{\text{preact}} = \Delta G^\ddagger_{\text{corr}} + \Delta E_{\text{bind}}(\text{H}_2\text{O})$

$\Delta G^\ddagger_{\text{app}} = \text{Apparent activation barrier}$

a) pre-activated reaction complex

b) transition state

Reaction faster than expected from calculated activation barriers
Influence of CO₂ on reaction rate

When reaction solution was gassed with CO₂ the reaction rate increased and more N-oxide was generated
Air: 1.2 % after 3 days ΔG°ₐₚₚ = 27.4 kcal/mol
CO₂ gassed: 4.1 % after 19.25 h ΔG°ₐₚₚ = 26.0 kcal/mol

Carbon dioxide is soluble in water where it can form carbonic acid H₂CO₃ and hydrogen carbonate HCO₃⁻

Calculations suggest that the carbonic acid system contribute to lowering the apparent activation barrier
Influence of pH

Carbonic acid pKₐ

6.35 (HCO₃⁻)  10.33 (H₂CO₃)

ΔG_{intr}^{=} = 35  ΔG_{intr}^{=} = 17

Influence of the carbonic acid system might explain that bell shape
Influence of co-solvent acetonitrile

Pyridine was oxidized by hydrogen peroxide in water solution with different levels of acetonitrile. Temperature was kept at T=22° C. Product is given as area percent of main peak at 254 nm after 20 h reaction time.

<table>
<thead>
<tr>
<th>ACN:H₂O (%)</th>
<th>Oxidant (M)</th>
<th>ACN (M)</th>
<th>Prod. (%)</th>
<th>∆G‡_{app}</th>
<th>∆G‡_{intr}</th>
<th>∆G‡_{corr}</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 : 100</td>
<td>1.2</td>
<td>0</td>
<td>0.8</td>
<td>27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 : 99</td>
<td>1.2</td>
<td>0.2</td>
<td>27.0</td>
<td>25</td>
<td>25.0</td>
<td>26.0</td>
</tr>
<tr>
<td>20 : 80</td>
<td>1.2</td>
<td>3.8</td>
<td>78.6</td>
<td>24</td>
<td>25.0</td>
<td>24.9</td>
</tr>
<tr>
<td>50 : 50</td>
<td>1.2</td>
<td>9.6</td>
<td>62.5</td>
<td>24</td>
<td>25.0</td>
<td>24.9</td>
</tr>
</tbody>
</table>

Small amount of co-solvent lead to more N-oxide

Experimental and quantum chemical evaluations of pyridine oxidation under drug development stress test conditions; C. Bäcktorp, E. Örnskov, E. J. Ottosson, Evertsson, J. Remmelgas, A. Broo Accepted J. Pharm. Sci. 2015
Summary

- BDE calculations can be used to highlight risk for autoxidation
  - Low BDE will not always lead to propagation of chain reaction

- A model for predicting risk for peroxide induced oxidation of amines

- Some sources for unexpected degradation pathways
  - Control pH
  - Co-solvent can react with H₂O₂ to form a better oxidant

- Outlook
  - Further work is needed to understand the effect of CO₂
  - How to translate solution reactivity to solid state
Acknowledgment

- Carina Bäcktorp – former post-doc: most modeling work
- Per-Ola Norrby – professor: autoxidation tool and insight
- Emma Evertsson, Thomas Andersson, Eivor Örnskov, Johan Remmelgas, Jenny Ottosson, David Benstead, Helen Williams, Johan Westin

Confidentiality Notice

This file is private and may contain confidential and proprietary information. If you have received this file in error, please notify us and remove it from your system and note that you must not copy, distribute or take any action in reliance on it. Any unauthorized use or disclosure of the contents of this file is not permitted and may be unlawful. AstraZeneca PLC, 2 Kingdom Street, London, W2 6BD, UK, T: +44(0)20 7604 8000, F: +44 (0)20 7604 8151, www.astrazeneca.com