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ENVIRONMENT DIRECTORATE  
JOINT MEETING OF THE CHEMICALS COMMITTEE AND  
THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY

**REPORT OF THE EXPERT CONSULTATION ON SCIENTIFIC AND REGULATORY EVALUATION  
OF ORGANIC CHEMISTRY MECHANISM-BASED STRUCTURAL ALERTS FOR THE  
IDENTIFICATION OF PROTEIN-BINDING CHEMICALS**

Series on Testing and Assessment  
No. 139

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**INTER-ORGANIZATION PROGRAMME FOR THE SOUND MANAGEMENT OF CHEMICALS**

A cooperative agreement among **FAO, ILO, UNEP, UNIDO, UNITAR, WHO, World Bank and OECD**

**Environment Directorate  
ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT  
Paris 2011**

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The Inter-Organisation Programme for the Sound Management of Chemicals (IOMC) was established in 1995 following recommendations made by the 1992 UN Conference on Environment and Development to strengthen co-operation and increase international co-ordination in the field of chemical safety. The Participating Organisations are FAO, ILO, UNEP, UNIDO, UNITAR, WHO, World Bank and OECD. UNDP is an observer. The purpose of the IOMC is to promote co-ordination of the policies and activities pursued by the Participating Organisations, jointly or separately, to achieve the sound management of chemicals in relation to human health and the environment.

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## **FOREWORD**

This document is a report of the expert consultation held on 20 October 2010 with the aim to evaluate a set of structural alerts for estimating covalent binding of chemicals with proteins. This consultation was held based on a key recommendation from the OECD Workshop on Structural Alerts for the OECD (Q)SAR Application Toolbox held in May 2008 [see ENV/JM/MONO(2009)4]. The resulting set of alerts will be implemented in version 2.0 of the OECD (Q)SAR Toolbox.

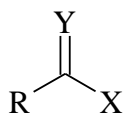
This document is published under the responsibility of the Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology of the OECD.

# **Mechanistic Domain: Acylation**

## Mechanistic Alert: Direct Acylation Involving a Leaving Group

A number of structural alerts have been suggested to be capable of directly acylating proteins resulting in adduct formation. The structural alerts covered by this mechanistic alert are as follows:

### Structural alert: Acyl halides (including benzyl and carbamoyl derivatives)



R = any carbon, nitrogen

X (leaving group) = halogen, azide

Y = oxygen, sulphur

### *Mechanism*

An acylation mechanism involving nucleophilic attack at the carbonyl (or sulfinyl) has been suggested as being responsible for the activity of these chemicals (Enoch et al 2009, Gerner et al 2004, Hulzebos et al 2005, Roberts et al 2007).

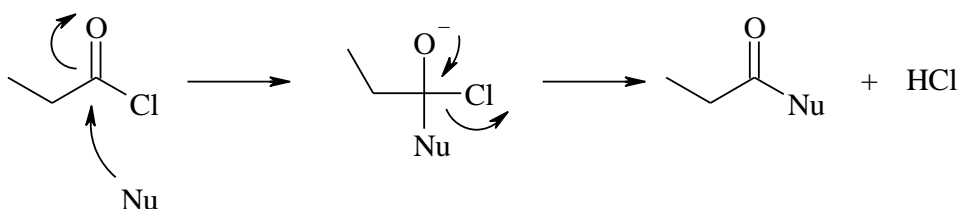
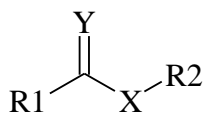


Figure 1: Acylation mechanism for acyl halides (Nu = biological nucleophile e.g. cysteine or lysine)

### Structural alert: Acetates



R1 = any carbon or hydrogen

R2 = aromatic, heteroaromatic, heterocyclic, alkene, alkyne

R1 and R2 can be part of a ring e.g. dihydro-coumarin.

Y = oxygen or sulphur

X = oxygen (acetates), sulphur (thioacetates), nitrogen (acetanilides)

### Mechanism

An acylation mechanism has been suggested for chemicals of this type (Payne et al 1994, Roberts et al 2007).

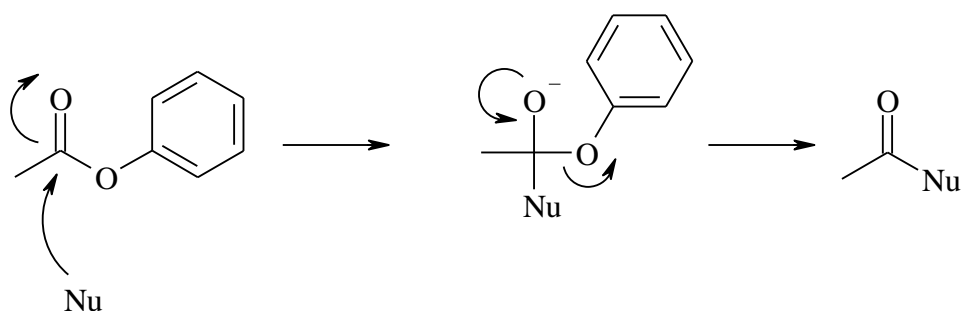
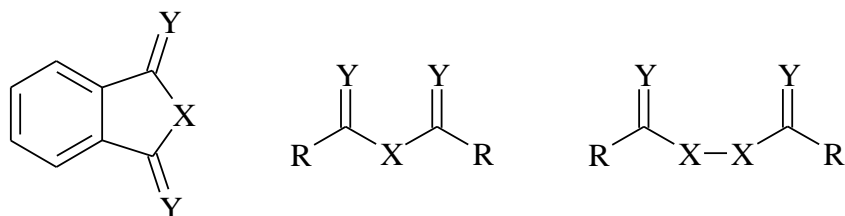


Figure 1: Acylation mechanism for acetate and related chemicals (Nu = biological nucleophile e.g. cysteine or lysine)

### Structural alert: Anhydrides



X = oxygen, sulphur

Y = oxygen (carbonyl), sulphur (sulfinyl)

R = any carbon or hydrogen

### Mechanism

An acylation mechanism has been suggested as being responsible for the protein binding ability of these chemicals (Enoch et al 2009, Gerner et al 2004, Roberts et al 2007).

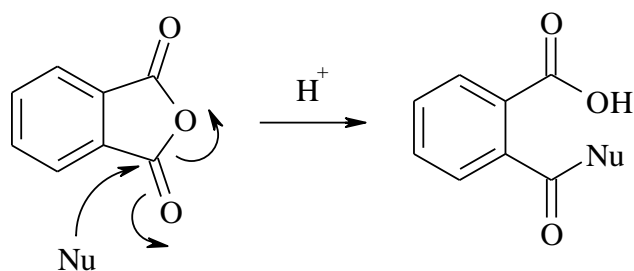
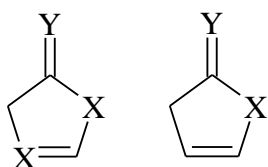


Figure 1: Acylation mechanism for anhydrides (Nu = biological nucleophile e.g. cysteine or lysine)

### Structural alert: Azlactones



Y = oxygen (carbonyl), sulphur (sulfinyl)

X = oxygen, sulphur, nitrogen

### *Mechanism*

An acylation mechanism involving ring opening has been suggested to be responsible for protein binding (Roberts et al 2007). Importantly, these chemicals are only active due to the ability of the unsaturated moiety to stabilise the leaving group anion (the equivalent  $\gamma$ -lactone-type structures are not protein reactive).

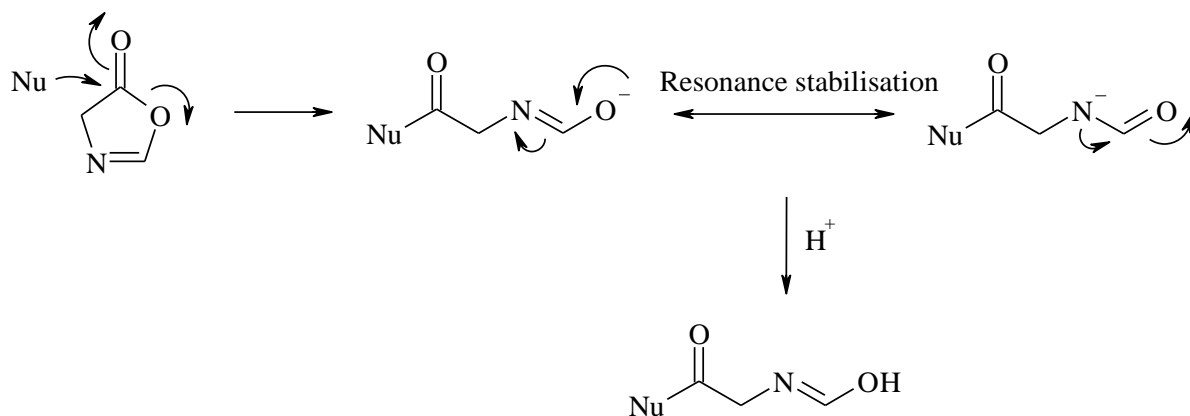
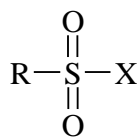


Figure 1: Ring opening acylation reaction for azlactones-type chemicals (Nu = biological nucleophile e.g. cysteine or lysine)

### Structural alert: Sulphonyl halides



R = any carbon or hydrogen

X = halogen, azide

### *Mechanism*

An acylation mechanism involving attack at the sulphur has been suggested for the protein binding potential of this class of chemicals (Ashby et al 1995, Payne et al 1994).

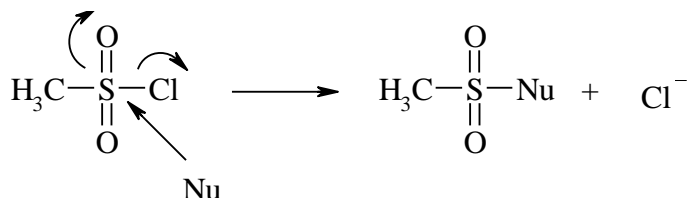
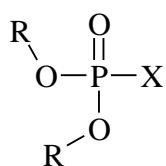


Figure 1: Acylation mechanism for sulphonyl halides and related chemicals (Nu = biological nucleophile e.g. cysteine or lysine)

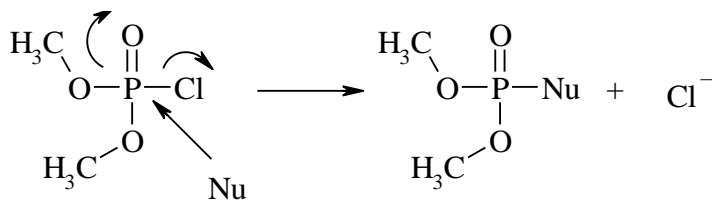
### Structural alert: Phosphonic acid halides



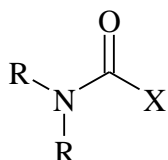
R = any carbon or hydrogen

### *Mechanism*

An acylation mechanism has been suggested as being responsible for the protein binding ability of this class of chemicals (Ashby et al 1995, Payne et al 1994)



### Structural alert: Dialkyl carbamoylhalides



X = halogen

R = any carbon or hydrogen

### *Mechanism*

An acylation mechanism has been suggested to be responsible for the protein binding ability of this class of chemicals (Hermens 1990).

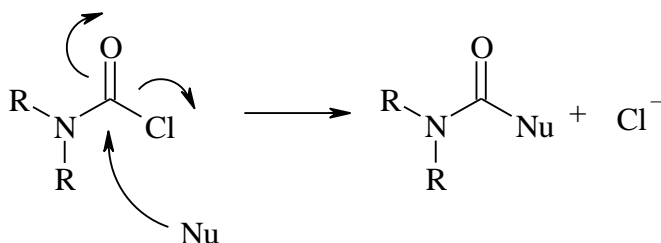


Figure 1: Acylation mechanism for dialkyl carbamoylchloride chemicals (Nu = biological nucleophile e.g. cysteine or lysine)

### Category mitigating factors

- No mitigating factors have been reported for any of the Mechanistic classes covered by this category

### References

Ashby J et al (1995) Toxicology, 103, p177

Enoch SJ et al (2009) Chemical Research in Toxicology, 22, p1447

Gerner I et al (2004) ATLA, 32, p487

Hermens JLM (1990) Environmental Health Perspectives, 87, p219

Hulzebos E et al (2005) QSAR and Combinatorial Science, 24, p332-342

Payne MP et al (1994) Journal of Chemical Information and Computer Science, 334, p154

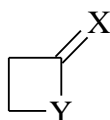
Roberts DW et al (2007) Chemical Research in Toxicology, 20, p44

Roberts DW et al (2007) Chemical Research in Toxicology, 20, p1019

## Mechanistic Alert: Ring Opening Acylation

Several structural alerts have been suggested to be capable of binding covalently to proteins via a ring opening acylation reaction. The structural alerts covered by this mechanistic alert are as follows:

### Structural alert: $\beta$ -Lactones



X = oxygen (carbonyl), sulphur (sulfinyl)

Y = oxygen, sulphur, nitrogen

### *Mechanism*

An acylation mechanism involving a ring opening reaction has been suggested to be responsible for the protein binding ability of these chemicals (Gerner et al 2004, Roberts et al 2007). Note: Only the four membered ring system is sufficiently reactive to be capable of protein binding. This is due to the additional energy gained upon release of the strain in the four membered ring. The equivalent five and six membered ring systems are not strained and are thus not capable of protein binding.

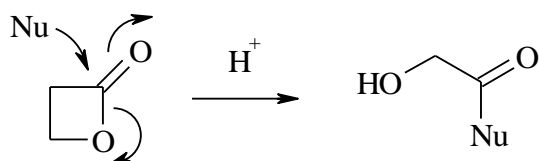
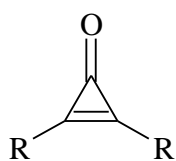


Figure 1: Ring opening acylation mechanism for  $\beta$ -lactone derivatives (Nu = biological nucleophile e.g. cysteine or lysine)

### Structural alert: Cyclopropenones



R = any carbon or hydrogen

### *Mechanism*

An acylation-type ring opening reaction has been suggested to be responsible for the protein binding ability of this class of chemicals (Turro et al 1966).

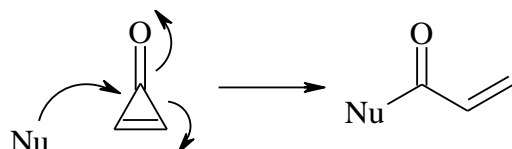


Figure 1: Ring opening acylation reaction (Nu = biological nucleophile e.g. cysteine or lysine)

### Category mitigating factors

- No mitigating factors have been reported for any of the Mechanistic classes covered by this category

### References

Gerner I et al (2004) ATLA, 32, p487

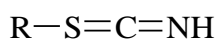
Roberts DW et al (2007) Chemical Research in Toxicology, 20, p44

Turro NJ et al (1966) Journal of the American Chemical Society, 88, p3672

## Mechanistic Alert: Isocyanates and Related Chemicals

Isocyanates and related chemicals have been shown to bind covalently to proteins via an acylation mechanism resulting in adduct formation. The structural alerts covered by this mechanistic alert are as follows:

### Structural alert: Thiocyanates



R = any carbon or hydrogen

### *Mechanism*

An acylation mechanism has been suggested to be responsible for the protein reactivity of these chemicals (Enoch et al 2009, Gerner et al 2004, Roberts et al 2007, Zinke et al 2002).

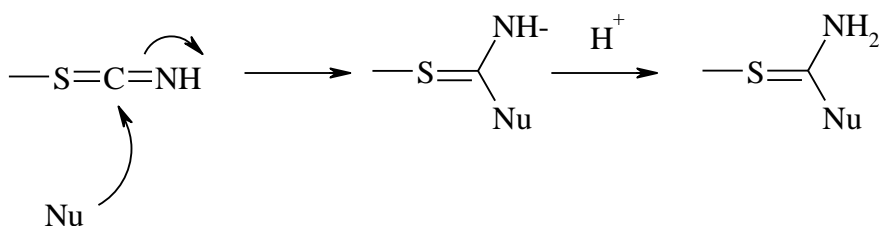
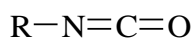


Figure 1: Acylation mechanism for thiocyanate chemicals (Nu = biological nucleophile e.g. cysteine or lysine)

### Structural alert: Isocyanates



R = any carbon or hydrogen

### *Mechanism*

An acylation mechanism has been suggested to be responsible for the protein reactivity of these chemicals (Enoch et al 2009, Gerner et al 2004, Roberts et al 2007, Zinke et al 2002).

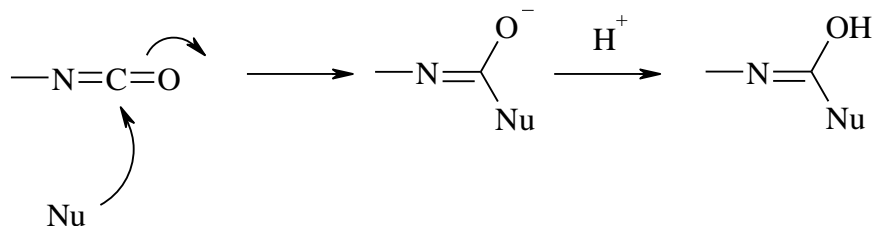
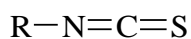


Figure 1: Acylation mechanism for isocyanate chemicals (Nu = biological nucleophile e.g. cysteine or lysine)

### Structural alert: Isothiocyanates



R = any carbon or hydrogen

### *Mechanism*

An acylation mechanism has been suggested to be responsible for the protein reactivity of these chemicals (Enoch et al 2009, Gerner et al 2004, Roberts et al 2007, Zinke et al 2002).

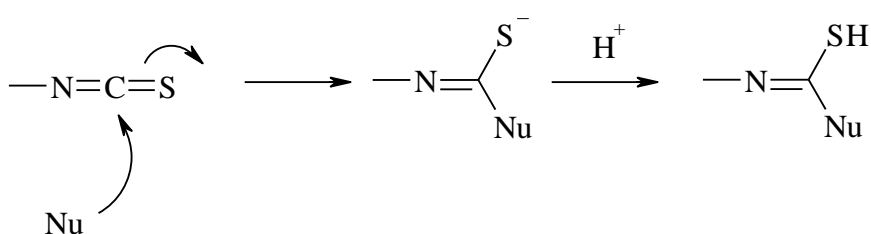
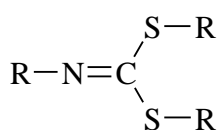


Figure 1: Acylation mechanism for isothiocyanate chemicals (Nu = biological nucleophile e.g. cysteine or lysine)

### Structural alert: Dithiocarbonimidic acid esters



R = any carbon or hydrogen

### *Mechanism*

An acylation mechanism has been suggested to be responsible for the protein reactivity of these chemicals (Enoch et al 2009, Gerner et al 2004, Roberts et al 2007, Zinke et al 2002).

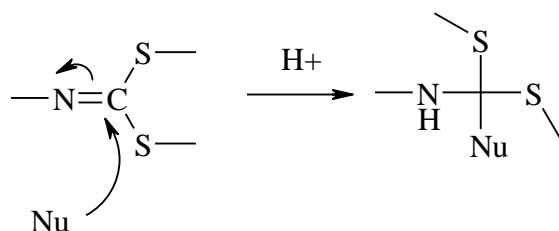
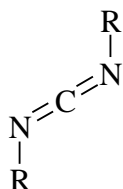


Figure 1: Acylation mechanism for dithiocarbonamidic acid ester chemicals (Nu = biological nucleophile e.g. cysteine or lysine)

### Structural alert: Carbodiimides



R = any carbon

### *Mechanism*

An acylation type mechanism has been proposed for this class of chemicals (Ashby et al 1995).

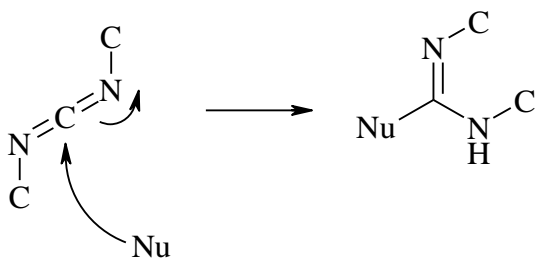
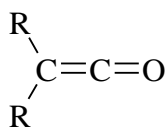


Figure 1: Acylation mechanism for carbodiimides (Nu = biological nucleophile e.g. cysteine or lysine)

### Structural alert: Ketenes



R = any carbon or hydrogen

### *Mechanism*

An acylation mechanism has been suggested for this class of chemicals (Hermens 1990).

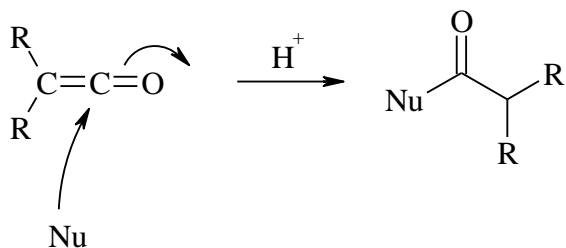


Figure 1: Acylation mechanism for ketenes (Nu = biological nucleophile e.g. cysteine or lysine)

### Category mitigating factors

- No mitigating factors have been reported for any of the Mechanistic classes covered by this category

### References

Ashby J et al (1995) Toxicology, 103, p177

Enoch SJ et al (2009) *Chemical Research in Toxicology*, 22, p1447

Gerner I et al (2004) *ATLA*, 32, p487

Hermens JLM (1990) *Environmental Health Perspectives*, 87, p219

Roberts DW et al (2007) *Chemical Research in Toxicology*, 20, p1019

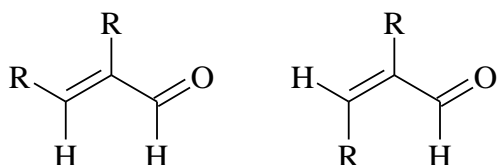
Zinke S et al (2002) *ATLA*, 30, p285

# **Mechanistic Domain: Michael Addition**

## Mechanistic Alert: Polarised Alkenes and Related Chemicals

A wide range of alkenes polarised by an electronegative group (or groups) have been shown to be capable of covalently binding to proteins via Michael addition. The structural alerts covered by this mechanistic alert are as follows:

### Structural alert: Polarised alkene - aldehydes



R = any carbon or hydrogen

### *Mechanism*

The mechanism has been suggested to involve attack by a nucleophile at the  $\beta$ -carbon atom (Aptula et al 2006, Hermens 1990, Roberts et al 2007, Schultz et al 2007, 2005, 2004, van der Ohe et al 2005, Verhaar et al 1992).

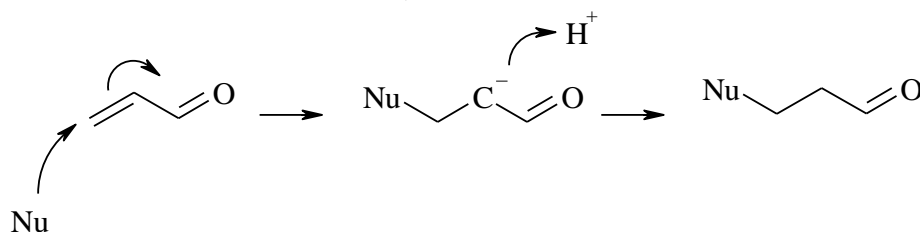
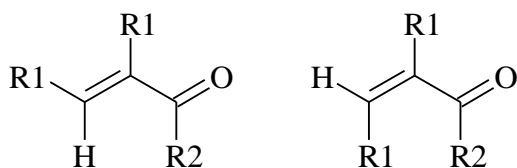


Figure 1: Michael addition for polarised alkene - aldehydes (Nu = biological nucleophile e.g. cysteine or lysine)

covered by this category are as follows:

### Structural alert: Polarised alkene - ketones



R1 = any carbon or hydrogen

R2 = any carbon

### Mechanism

The mechanism has been suggested to involve attack by a nucleophile at the  $\beta$ -carbon atom (Aptula et al 2006, Hermens 1990, Roberts et al 2007, Schultz et al 2007, 2005, 2004, van der Ohe et al 2005, Verhaar et al 1992).

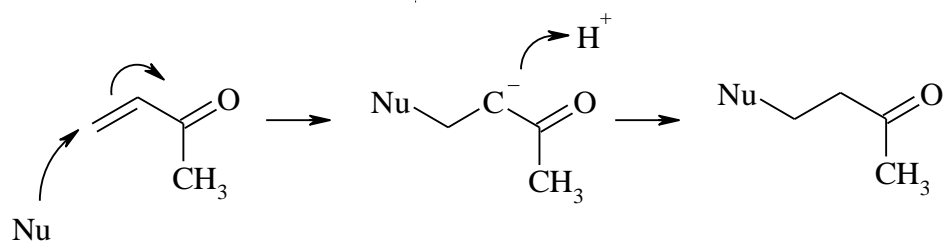
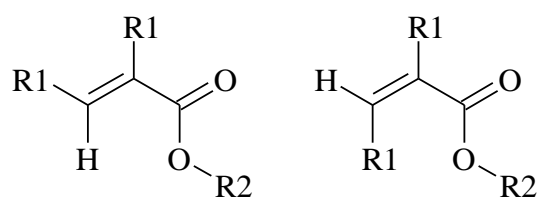


Figure 1: Michael addition for polarised alkene - ketones (Nu = biological nucleophile e.g. cysteine or lysine)

### Structural alert: Polarised alkene - esters



R1 = any carbon or hydrogen

R2 = any carbon

### Mechanism

The mechanism has been suggested to involve attack by a nucleophile at the  $\beta$ -carbon atom (Aptula et al 2006, Hermens 1990, Roberts et al 2007, Schultz et al 2007, 2005, 2004, van der Ohe et al 2005, Verhaar et al 1992).

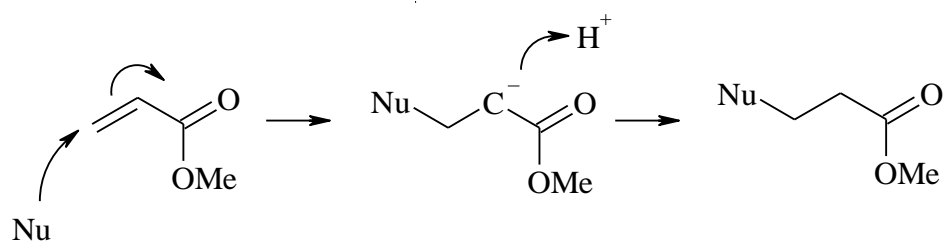
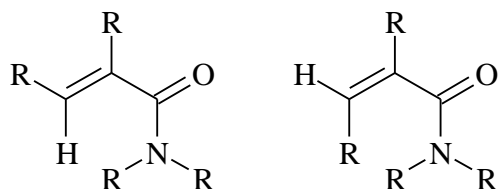


Figure 1: Michael addition for polarised alkene - esters (Nu = biological nucleophile e.g. cysteine or lysine)

Structural alert: Polarised alkene - amides



R = any carbon or hydrogen

*Mechanism*

The mechanism has been suggested to involve attack by a nucleophile at the  $\beta$ -carbon atom (Aptula et al 2006, Hermens 1990, Roberts et al 2007, Schultz et al 2007, 2005, 2004, van der Ohe et al 2005, Verhaar et al 1992).

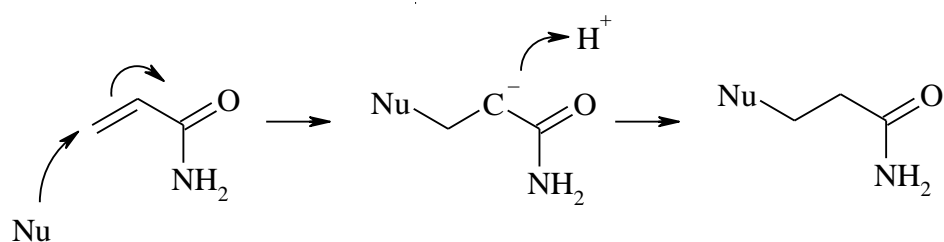
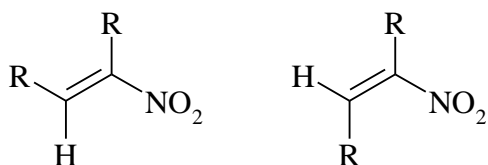


Figure 1: Michael addition for polarised alkene - amides (Nu = biological nucleophile e.g. cysteine or lysine)

Structural alert: Polarised alkene - nitros



R = any carbon or hydrogen

*Mechanism*

The mechanism has been suggested to involve attack by a nucleophile at the  $\beta$ -carbon atom (Aptula et al 2006, Hermens 1990, Roberts et al 2007, Schultz et al 2007, 2005, 2004, van der Ohe et al 2005, Verhaar et al 1992).

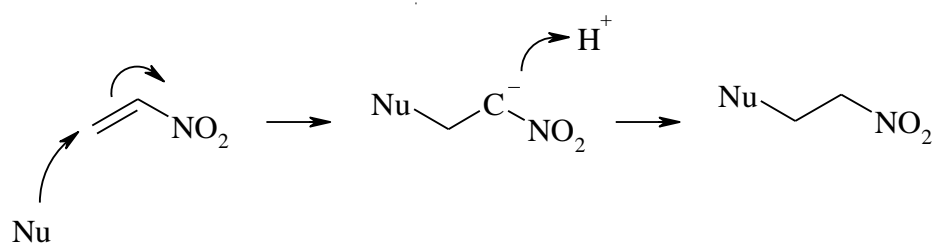
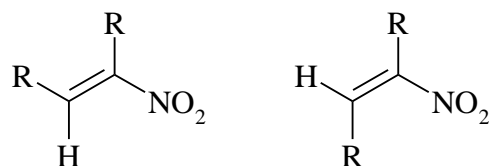


Figure 1: Michael addition for polarised alkene - nitro (Nu = biological nucleophile e.g. cysteine or lysine)

#### Structural alert: Polarised alkene - cyano



R = any carbon or hydrogen

#### *Mechanism*

The mechanism has been suggested to involve attack by a nucleophile at the  $\beta$ -carbon atom (Aptula et al 2006, Hermens 1990, Roberts et al 2007, Schultz et al 2007, 2005, 2004, van der Ohe et al 2005, Verhaar et al 1992).

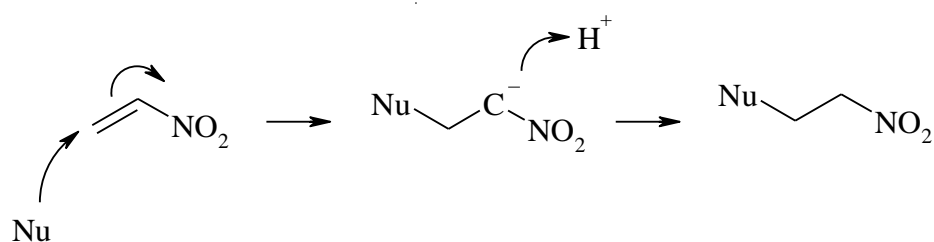
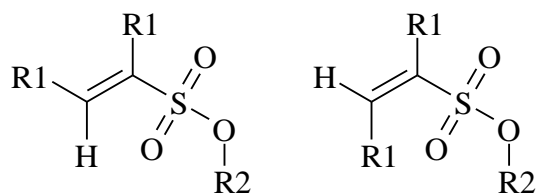


Figure 1: Michael addition for polarised alkene - cyano (Nu = biological nucleophile e.g. cysteine or lysine)

### Structural alert: Polarised alkene - sulfonate



R1 = any carbon or hydrogen

R2 = any carbon

### *Mechanism*

The mechanism has been suggested to involve attack by a nucleophile at the  $\beta$ -carbon atom (Aptula et al 2006, Hermens 1990, Roberts et al 2007, Schultz et al 2007, 2005, 2004, van der Ohe et al 2005, Verhaar et al 1992).

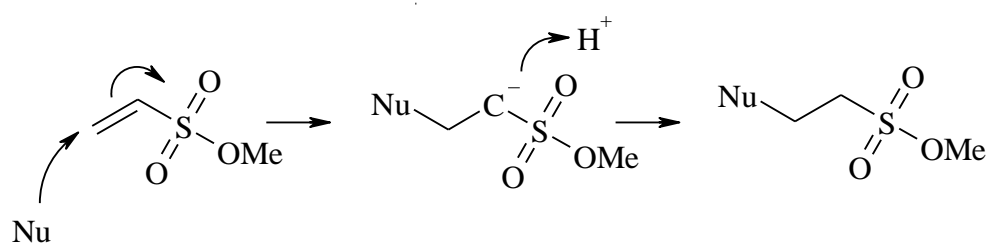
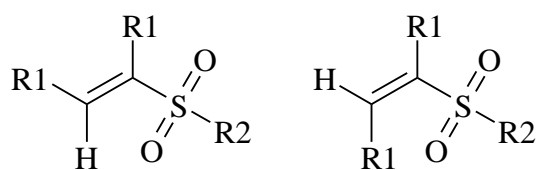


Figure 1: Michael addition for polarised alkene - sulfonate (Nu = biological nucleophile e.g. cysteine or lysine)

### Structural alert: Polarised alkene - sulfone



R1 = any carbon or hydrogen

R2 = any carbon

### *Mechanism*

The mechanism has been suggested to involve attack by a nucleophile at the  $\beta$ -carbon atom (Aptula et al 2006, Hermens 1990, Roberts et al 2007, Schultz et al 2007, 2005, 2004, van der Ohe et al 2005, Verhaar et al 1992).

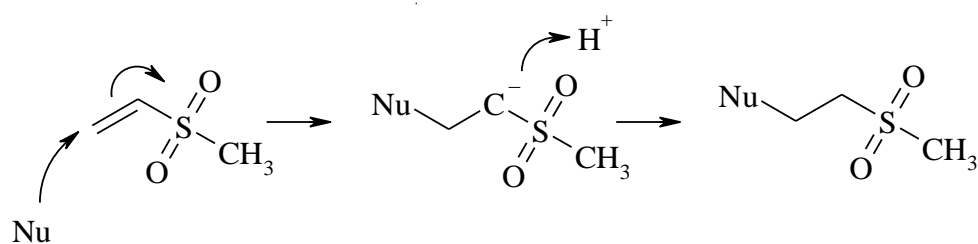
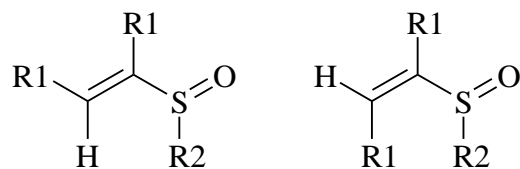


Figure 1: Michael addition for polarised alkene - sulfone (Nu = biological nucleophile e.g. cysteine or lysine)

#### Structural alert: Polarised alkene - sulfinyl



R1 = any carbon or hydrogen

R2 = any carbon

#### *Mechanism*

The mechanism has been suggested to involve attack by a nucleophile at the  $\beta$ -carbon atom (Aptula et al 2006, Hermens 1990, Roberts et al 2007, Schultz et al 2007, 2005, 2004, van der Ohe et al 2005, Verhaar et al 1992).

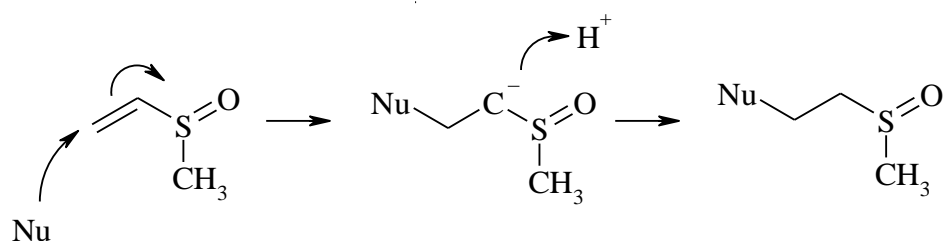
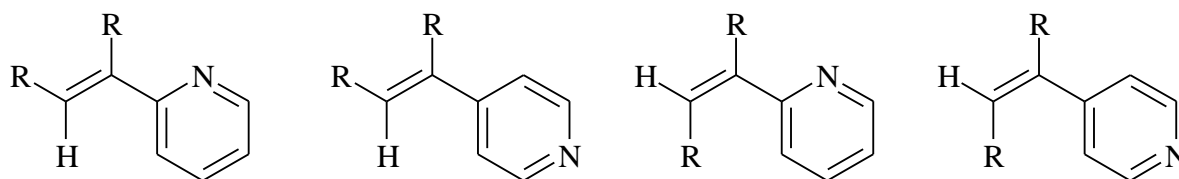


Figure 1: Michael addition for polarised alkene - sulfinyl (Nu = biological nucleophile e.g. cysteine or lysine)

### Structural alert: Polarised alkene - pyridines



R = any carbon or hydrogen

### *Mechanism*

The mechanism has been suggested to involve attack by a nucleophile at the  $\beta$ -carbon atom (Aptula et al 2006, Hermens 1990, Roberts et al 2007, Schultz et al 2007, 2005, 2004, van der Ohe et al 2005, Verhaar et al 1992).

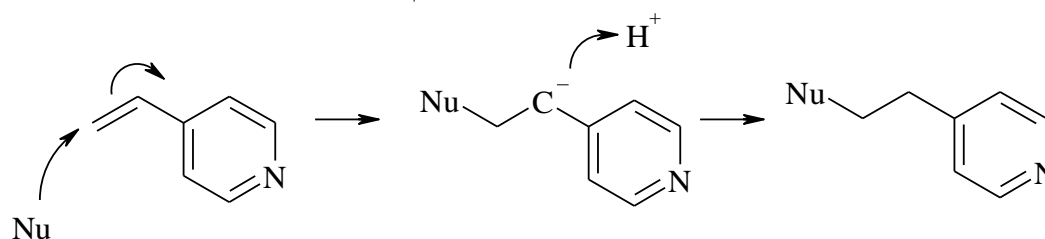
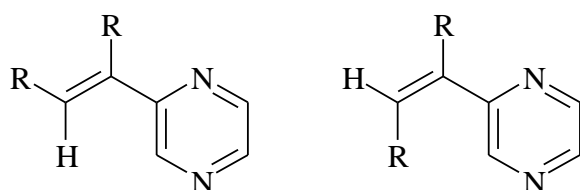


Figure 1: Michael addition for polarised alkene - pyridines (Nu = biological nucleophile e.g. cysteine or lysine)

### Structural alert: Polarised alkene - pyrazines



R = any carbon or hydrogen

### *Mechanism*

The mechanism has been suggested to involve attack by a nucleophile at the  $\beta$ -carbon atom (Aptula et al 2006, Hermens 1990, Roberts et al 2007, Schultz et al 2007, 2005, 2004, van der Ohe et al 2005, Verhaar et al 1992).

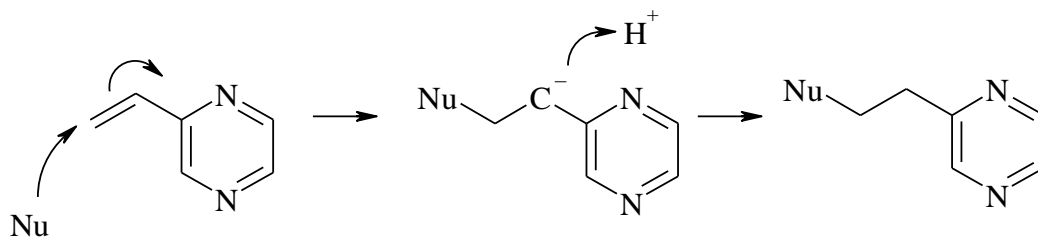
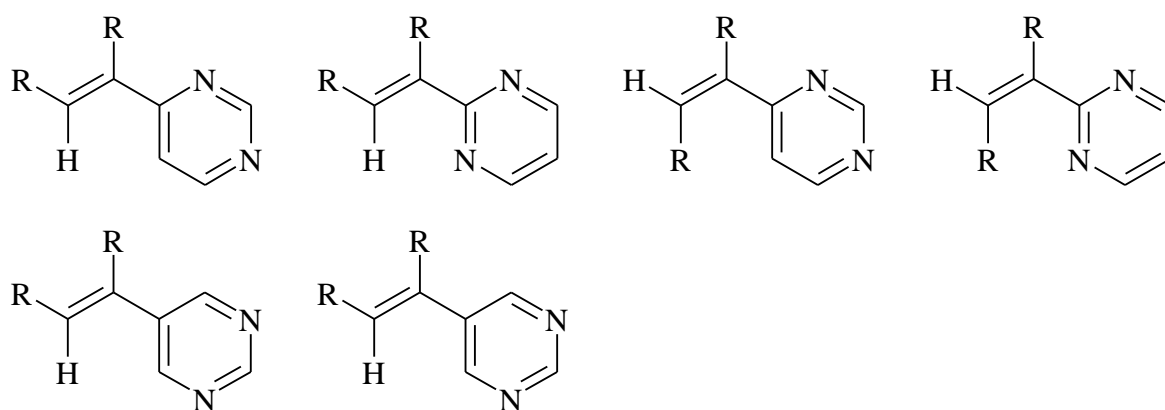


Figure 1: Michael addition for polarised alkene - pyrazines (Nu = biological nucleophile e.g. cysteine or lysine)

Structural alert: Polarised alkene - pyrimidines



R = any carbon or hydrogen

*Mechanism*

The mechanism has been suggested to involve attack by a nucleophile at the  $\beta$ -carbon atom (Aptula et al 2006, Hermens 1990, Roberts et al 2007, Schultz et al 2007, 2005, 2004, van der Ohe et al 2005, Verhaar et al 1992).

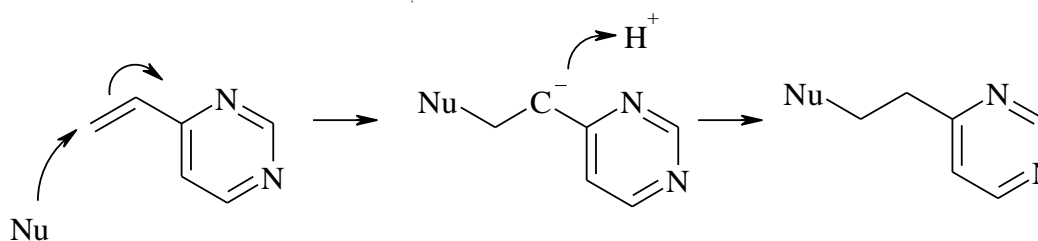
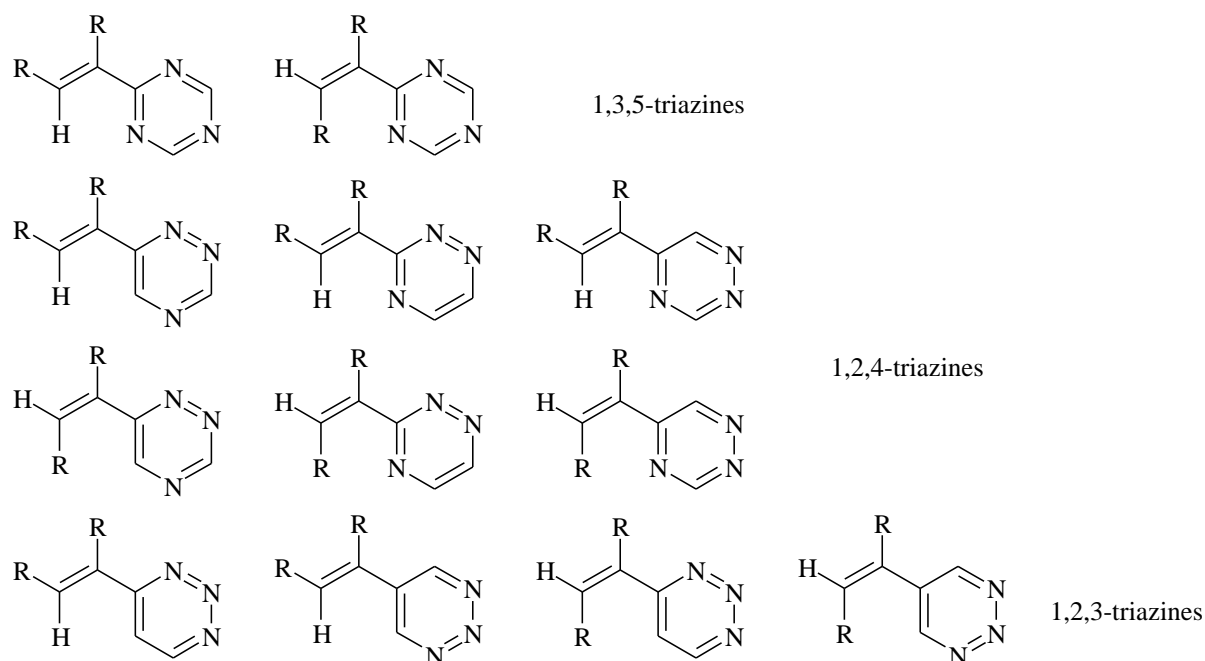


Figure 1: Michael addition for polarised alkene - pyrimidines (Nu = biological nucleophile e.g. cysteine or lysine)

## Structural alert: Polarised alkene - triazines



R = any carbon or hydrogen

### Mechanism

The mechanism has been suggested to involve attack by a nucleophile at the  $\beta$ -carbon atom (Aptula et al 2006, Hermens 1990, Roberts et al 2007, Schultz et al 2007, 2005, 2004, van der Ohe et al 2005, Verhaar et al 1992).

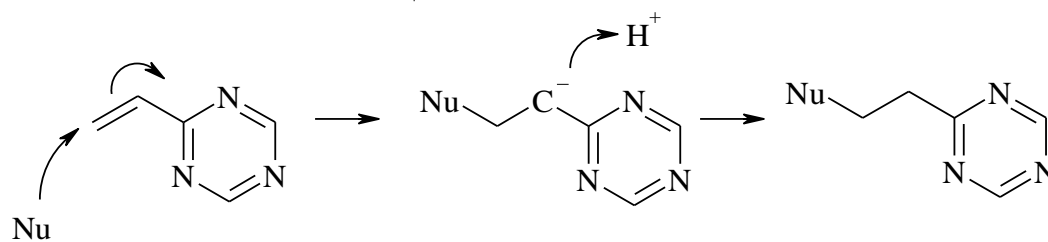
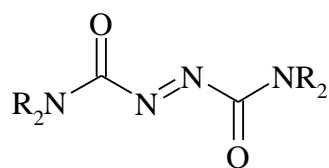


Figure 1: Michael addition for polarised alkene - triazines (Nu = biological nucleophile e.g. cysteine or lysine)

### Structural alert: Azocarbonamides



R = any carbon or hydrogen

#### *Mechanism*

A Michael addition mechanism has been reported as being responsible for the protein reactivity of this class of chemicals (Hill et al 1999).

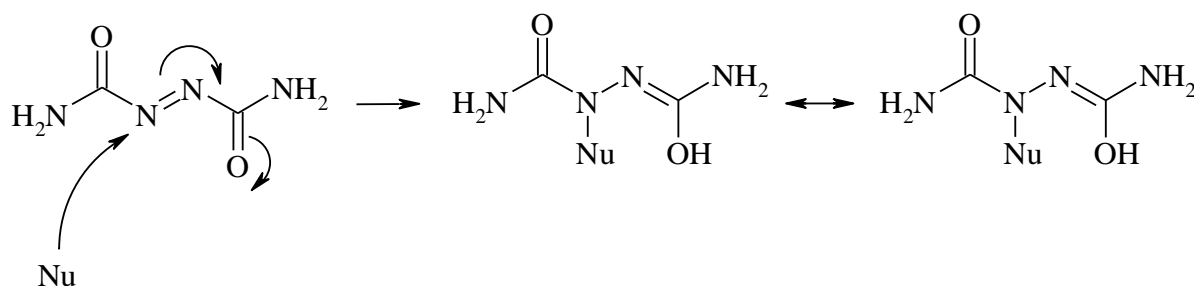


Figure 1: Michael addition mechanism for azocarbonamides (Nu = biological nucleophile e.g. lysine or cysteine).

#### Category mitigating factors

- Polarised alkenes: Di-substitution at the  $\beta$ -carbon removes Michael addition activity resulting in Schiff base formation dominating (Patlewicz et al 2003, Roberts et al 2006). However, if R is a further alkene that itself is not di-substituted at the  $\gamma$ -carbon then activity is restored due to the Michael reaction occurring at the  $\gamma$ -carbon rather than the  $\beta$ -carbon.

#### References

Aptula AO et al (2006) Chemical Research in Toxicology, 19, p1097

Hermens JLM (1990) Environment Health Perspectives, 87, p219

Hill RD et al (1999) Journal of Organic Chemistry, 64, p9538

Patlewicz G (2003) QSAR and Combinatorial Science, 22, p196

Roberts DW et al (2007) *Chemical Research in Toxicology*, 20, p1019

Roberts DW et al (2006) *Chemical Research in Toxicology*, 19, p1228

Schultz TW et al (2007) *Chemical Research in Toxicology*, 20, p1359

Schultz TW et al (2005) *SAR and QSAR in Environmental Research*, 16, p313

Schultz TW et al (2004) *SAR and QSAR in Environmental Research*, 15, p139

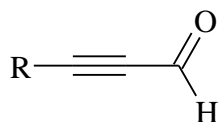
van der Ohe et al (2005) *Chemical Research in Toxicology*, 18, p536

Verhaar HJM et al (1992) *Chemosphere*, 25, p471

### Mechanistic Alert: Polarised Alkynes

A wide range of alkynes polarised by an electronegative group have been shown to be capable of covalently binding to proteins via Michael addition. The structural alerts covered by this mechanistic alert are as follows:

#### Structural alert: Polarised alkyne - aldehydes



R = any carbon or hydrogen

#### *Mechanism*

The mechanism has been suggested to involve attack by a nucleophile at the  $\beta$ -carbon atom (Aptula et al 2006, Roberts et al 2007, Schultz et al 2007, Verhaar et al 1992).

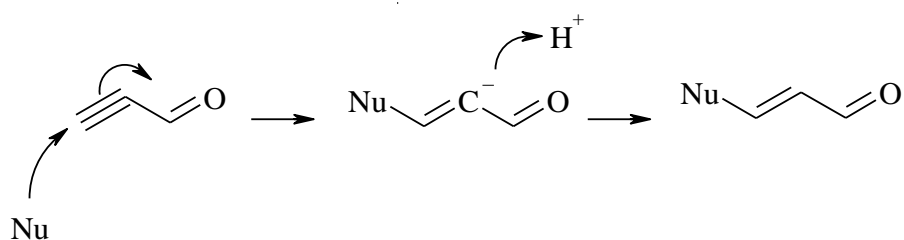
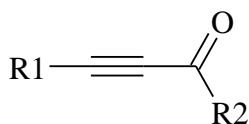


Figure 1: Michael addition for polarised alkyne – aldehydes (Nu = biological nucleophile e.g. cysteine or lysine)

#### Structural alert: Polarised alkyne - ketones



R1 = any carbon or hydrogen

R2 = any carbon

#### *Mechanism*

The mechanism has been suggested to involve attack by a nucleophile at the  $\beta$ -carbon atom (Aptula et al 2006, Roberts et al 2007, Schultz et al 2007, Verhaar et al 1992).

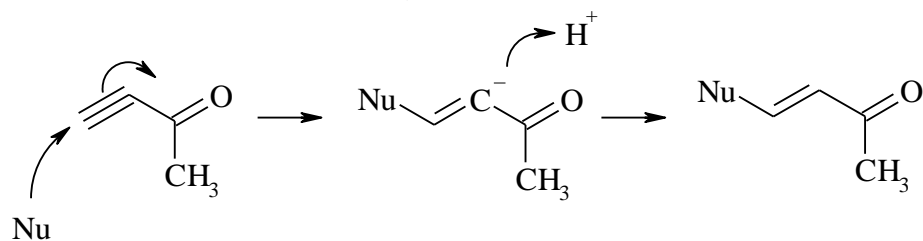
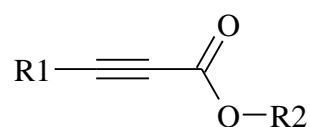


Figure 1: Michael addition for polarised alkyne – ketones (Nu = biological nucleophile e.g. cysteine or lysine)

Structural alert: Polarised alkyne - esters



R1 = any carbon or hydrogen

R2 = any carbon

*Mechanism*

The mechanism has been suggested to involve attack by a nucleophile at the  $\beta$ -carbon atom (Aptula et al 2006, Roberts et al 2007, Schultz et al 2007, Verhaar et al 1992).

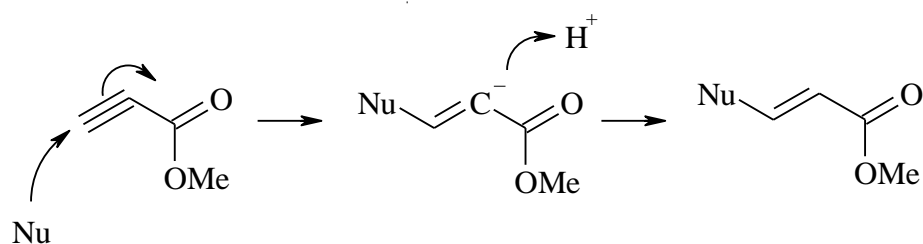
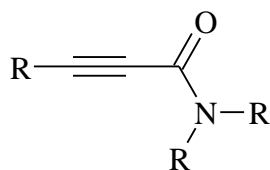


Figure 1: Michael addition for polarised alkyne – esters (Nu = biological nucleophile e.g. cysteine or lysine)

Structural alert: Polarised alkyne - amides



R = any carbon or hydrogen

*Mechanism*

The mechanism has been suggested to involve attack by a nucleophile at the  $\beta$ -carbon atom (Aptula et al 2006, Roberts et al 2007, Schultz et al 2007, Verhaar et al 1992).

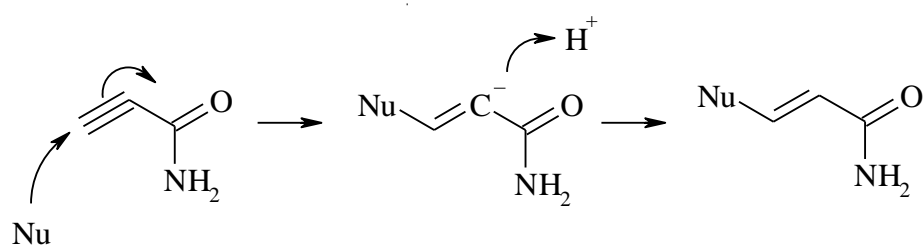
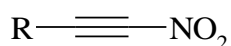


Figure 1: Michael addition for polarised alkyne – amides (Nu = biological nucleophile e.g. cysteine or lysine)

Structural alert: Polarised alkyne - nitros



R = any carbon or hydrogen

*Mechanism*

The mechanism has been suggested to involve attack by a nucleophile at the  $\beta$ -carbon atom (Aptula et al 2006, Roberts et al 2007, Schultz et al 2007, Verhaar et al 1992).

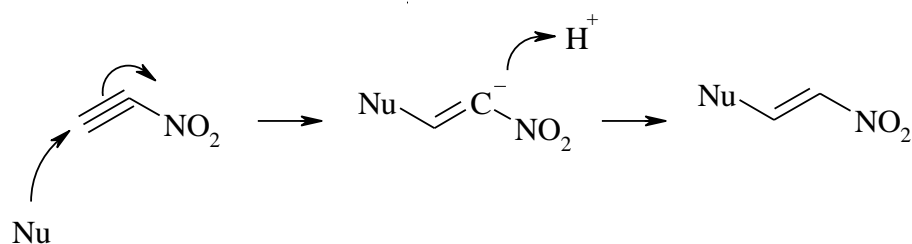
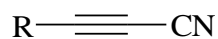


Figure 1: Michael addition for polarised alkyne – nitros (Nu = biological nucleophile e.g. cysteine or lysine)

Structural alert: Polarised alkyne - cyano



R = any carbon or hydrogen

*Mechanism*

The mechanism has been suggested to involve attack by a nucleophile at the  $\beta$ -carbon atom (Aptula et al 2006, Roberts et al 2007, Schultz et al 2007, Verhaar et al 1992).

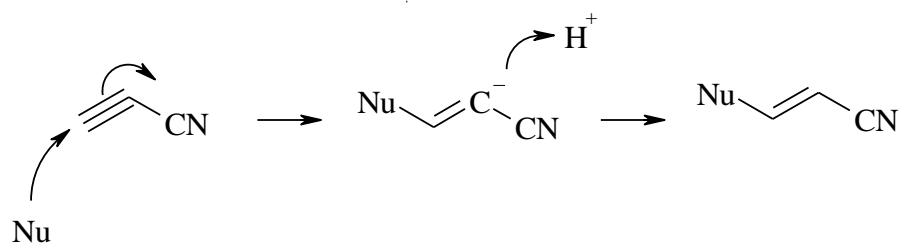
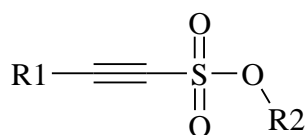


Figure 1: Michael addition for polarised alkyne – cyano (Nu = biological nucleophile e.g. cysteine or lysine)

Structural alert: Polarised alkyne - sulfonate



R1 = any carbon or hydrogen

R2 = any carbon

*Mechanism*

The mechanism has been suggested to involve attack by a nucleophile at the  $\beta$ -carbon atom (Aptula et al 2006, Roberts et al 2007, Schultz et al 2007, Verhaar et al 1992).

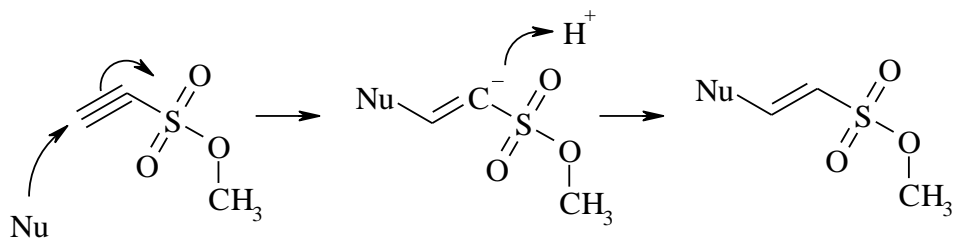
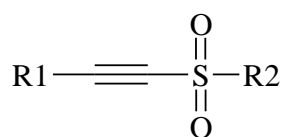


Figure 1: Michael addition for polarised alkyne – sulfonate (Nu = biological nucleophile e.g. cysteine or lysine)

Structural alert: Polarised alkyne - sulfone



R1 = any carbon or hydrogen

R2 = any carbon

*Mechanism*

The mechanism has been suggested to involve attack by a nucleophile at the  $\beta$ -carbon atom (Aptula et al 2006, Roberts et al 2007, Schultz et al 2007, Verhaar et al 1992).

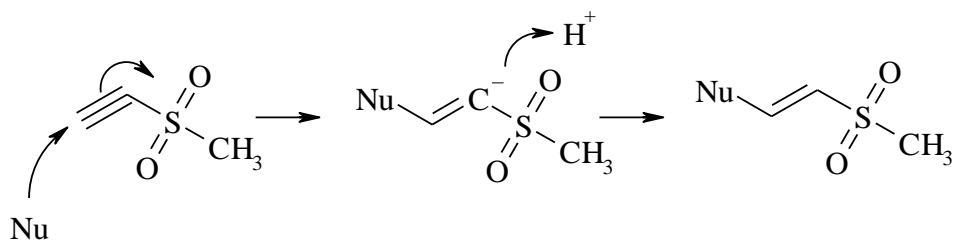
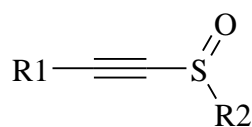


Figure 1: Michael addition for polarised alkyne – sulfone (Nu = biological nucleophile e.g. cysteine or lysine)

### Structural alert: Polarised alkyne - sulfinyl



R1 = any carbon or hydrogen

R2 = any carbon

### *Mechanism*

The mechanism has been suggested to involve attack by a nucleophile at the  $\beta$ -carbon atom (Aptula et al 2006, Roberts et al 2007, Schultz et al 2007, Verhaar et al 1992).

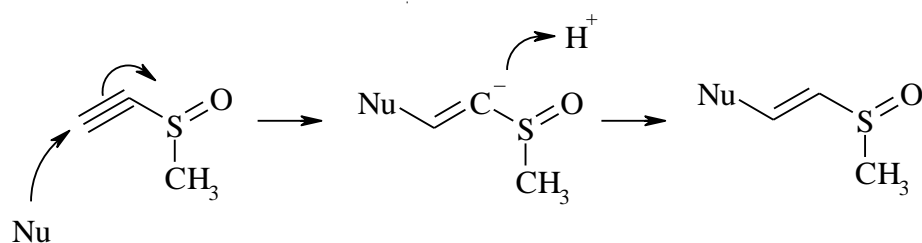
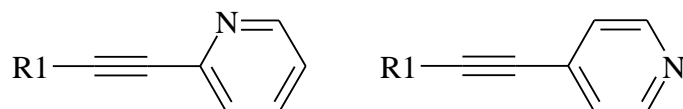


Figure 1: Michael addition for polarised alkyne – sulfinyl (Nu = biological nucleophile e.g. cysteine or lysine)

### Structural alert: Polarised alkyne - pyridine



R = any carbon or hydrogen

### *Mechanism*

The mechanism has been suggested to involve attack by a nucleophile at the  $\beta$ -carbon atom (Aptula et al 2006, Roberts et al 2007, Schultz et al 2007, Verhaar et al 1992).

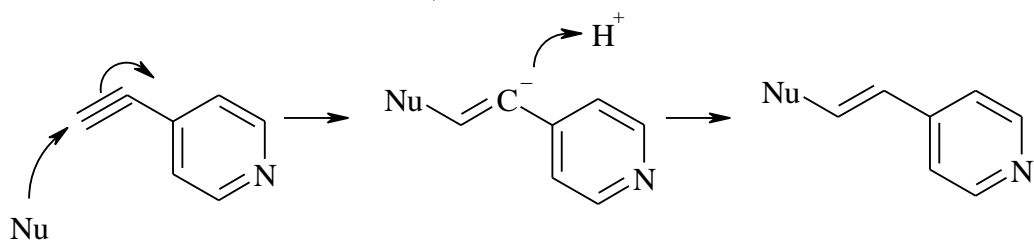
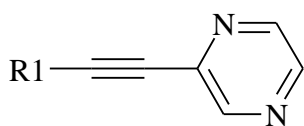


Figure 1: Michael addition for polarised alkyne – pyridine (Nu = biological nucleophile e.g. cysteine or lysine)

Structural alert: Polarised alkyne - pyrazine



R = any carbon or hydrogen

*Mechanism*

The mechanism has been suggested to involve attack by a nucleophile at the  $\beta$ -carbon atom (Aptula et al 2006, Roberts et al 2007, Schultz et al 2007, Verhaar et al 1992).

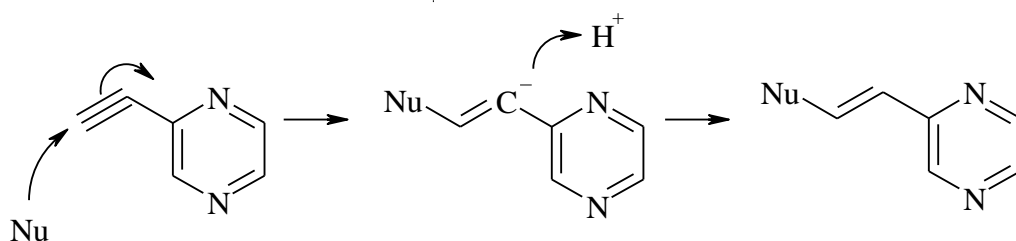
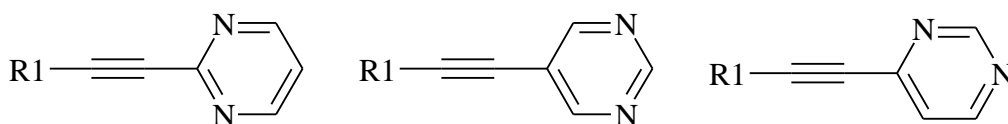


Figure 1: Michael addition for polarised alkyne – pyrazine (Nu = biological nucleophile e.g. cysteine or lysine)

Structural alert: Polarised alkyne - pyrimidine



R = any carbon or hydrogen

### Mechanism

The mechanism has been suggested to involve attack by a nucleophile at the  $\beta$ -carbon atom (Aptula et al 2006, Roberts et al 2007, Schultz et al 2007, Verhaar et al 1992).

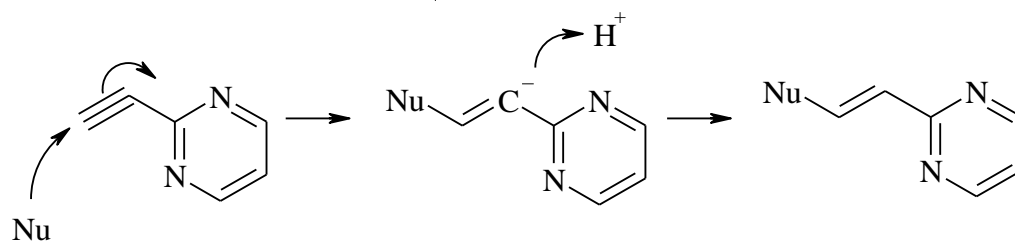
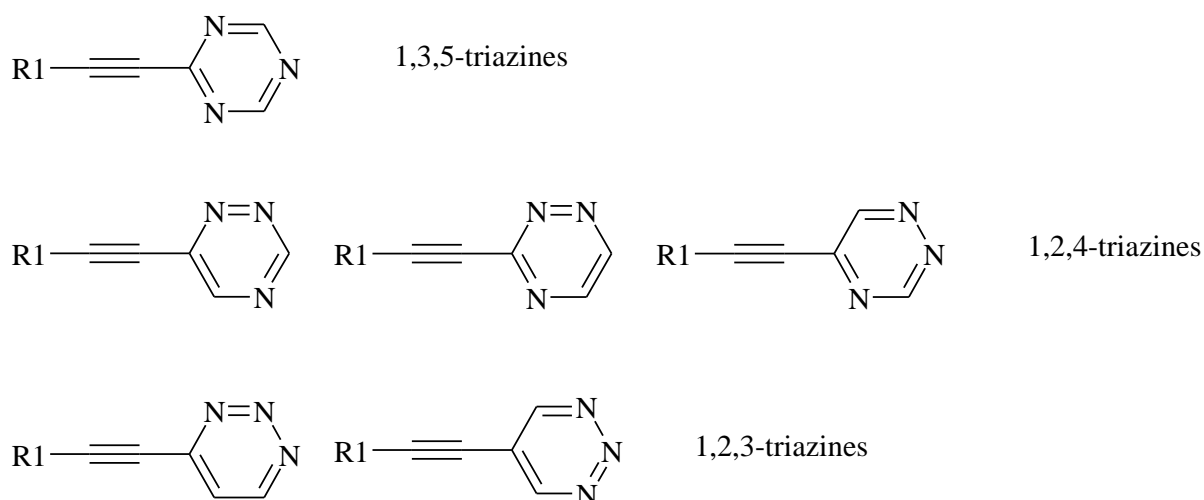


Figure 1: Michael addition for polarised alkyne – pyrimidine (Nu = biological nucleophile e.g. cysteine or lysine)

### Structural alert: Polarised alkyne - triazine



R = any carbon or hydrogen

### Mechanism

The mechanism has been suggested to involve attack by a nucleophile at the  $\beta$ -carbon atom (Aptula et al 2006, Roberts et al 2007, Schultz et al 2007, Verhaar et al 1992).

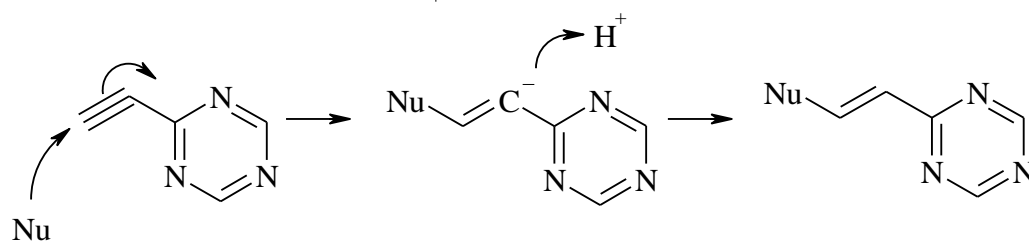


Figure 1: Michael addition for polarised alkyne – triazine (Nu = biological nucleophile e.g. cysteine or lysine)

Category mitigating factors

- No mitigating factors have been reported

References

Aptula AO et al (2006) Chemical Research in Toxicology, 19, p1097

Roberts DW et al (2007) Chemical Research in Toxicology, 20, p1019

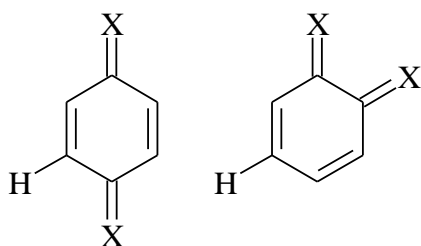
Schultz TW et al (2007) Chemical Research in Toxicology, 20, p1359

Verhaar HJM et al (1992) Chemosphere, 25, p471

## Mechanistic Alert: Quinones and Quinone-type Chemicals

Quinones and a number of closely chemical classes have been shown to be capable of reacting covalent with proteins via Michael addition. The structural alerts covered by this mechanistic alert are as follows:

### Structural alert: benzoquinones



X = O, NH<sub>2</sub>, NH

### *Mechanism*

The mechanism has been suggested to involve attack by a nucleophile at the  $\beta$ -carbon atom (Aptula et al 2006, Enoch et al 2009, Roberts et al 2007, Schultz et al 2007, Verhaar et al 1992).

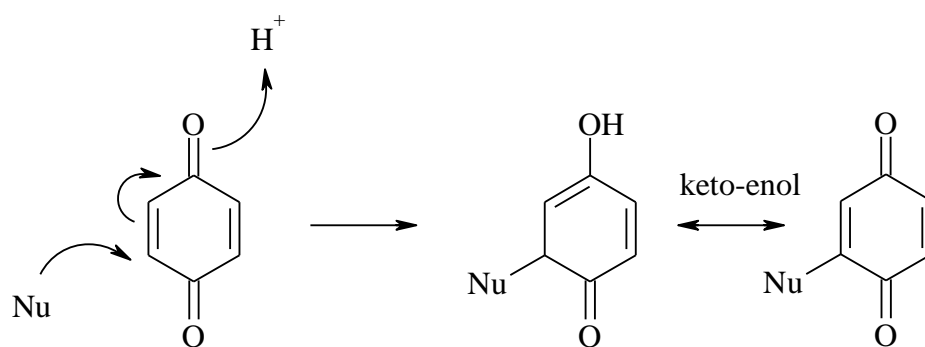
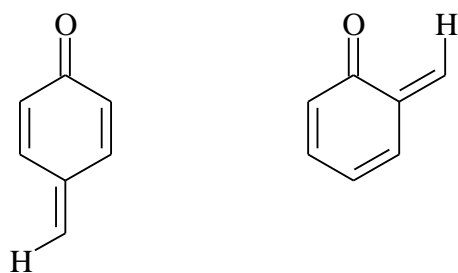


Figure 1: Michael addition for 1,4-quinone (Nu = biological nucleophile e.g. cysteine or lysine)

Structural alert: Quinone-methides



*Mechanism*

A Michael addition mechanism has been suggested for these chemicals (Aptula et al 2006, Enoch et al 2008, Roberts et al 2007).

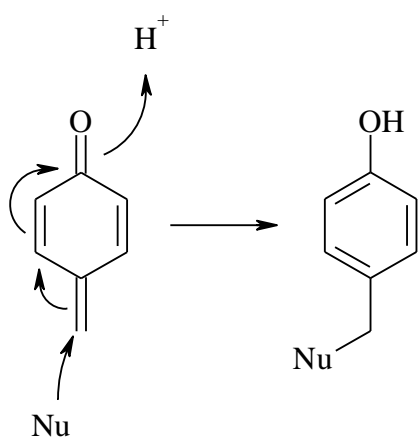
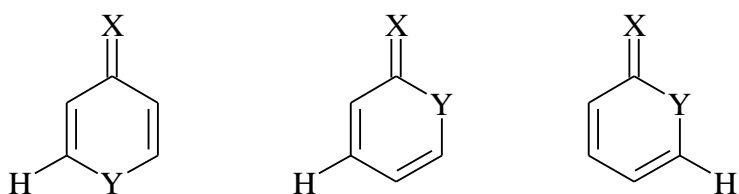


Figure 1: Michael addition mechanism for quinone-methides (Nu = biological nucleophile e.g. cysteine or lysine)

Structural alert: Pyranones (and related nitrogen chemicals)



X = O, NH

Y = O, N

*Mechanism*

A Michael addition mechanism has been suggested as being responsible for the protein binding ability of this class of chemicals (Mekenyan et al 2007).

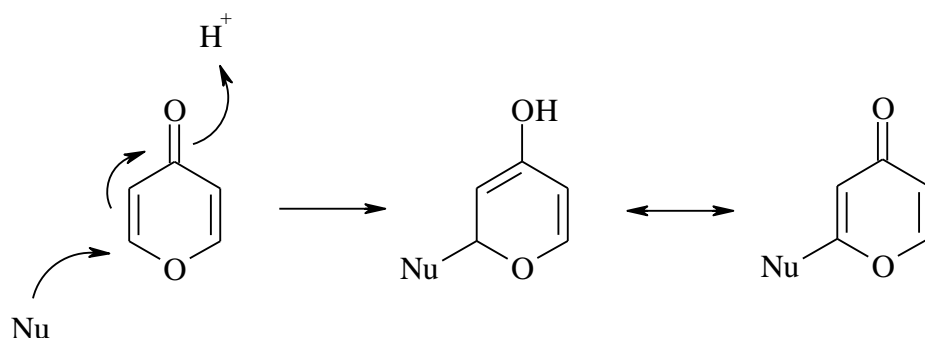


Figure 1: Michael addition mechanism for pyranones

#### Category mitigating factors

- No mitigating factors have been reported for the Mechanistic classes within this category

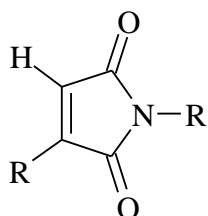
#### References

- Aptula AO et al (2006) *Chemical Research in Toxicology*, 19, p1097
- Enoch SJ et al (2009) *Chemical Research in Toxicology*, 22, p1447
- Enoch SJ et al (2008) *SAR and QSAR in Environmental Research*, 19, p555
- Mekenyan O et al (2007) *Chemical Research in Toxicology*, 20, p1927
- Roberts DW et al (2007) *Chemical Research in Toxicology*, 20, p44
- Roberts DW et al (2007) *Chemical Research in Toxicology*, 20, p1019
- Schultz TW et al (2007) *Chemical Research in Toxicology*, 20, p1359
- Verhaar HJM et al (1992) *Chemosphere*, 25, p471

## Mechanistic Alert: Acid Imides

Acid imides have been suggested to form covalent protein adducts via Michael addition.

### Structural alert: Acid imides



R = any carbon or hydrogen

### *Mechanism*

A Michael addition mechanism has been suggested for this class of chemicals (Gerner et al 2004).

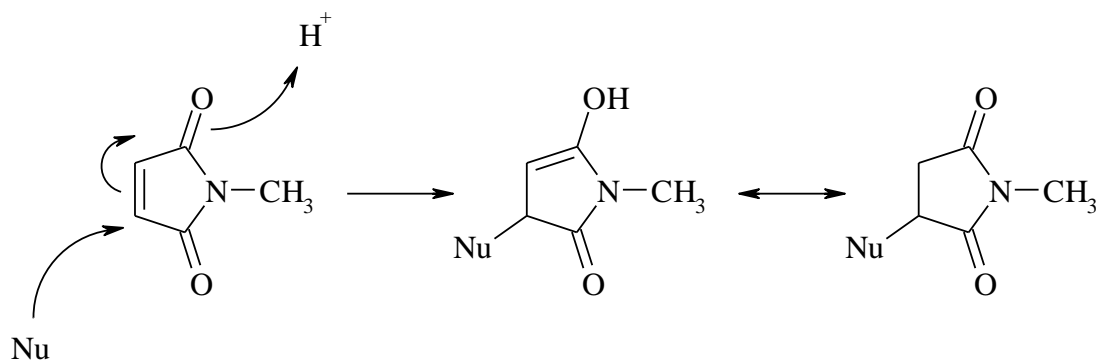


Figure 1: Michael addition mechanism for acid imides (Nu = biological nucleophile e.g. cysteine or lysine)

### Category mitigating factors

- None have been reported for this category

### References

Gerner I et al (2004), ATLA, 32, p487

## Profiling scheme:" Michael acceptors-potency categorization"

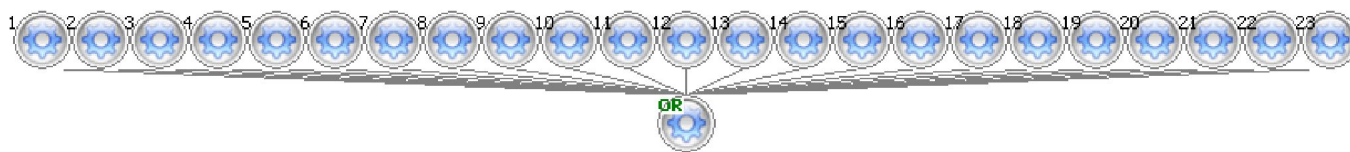
export date: Report created: 28.04.10 - 16:56

### Available Categories

- |    |                                  |    |                                |
|----|----------------------------------|----|--------------------------------|
| 1. | Extremely reactive-Category A    | 2. | Highly reactive-Category B     |
| 3. | Moderately Reactive - Category C | 4. | Slightly Reactive - Category D |
| 5. | Suspect - Category F             |    |                                |

### Categories Explanations

1. Extremely reactive-Category A

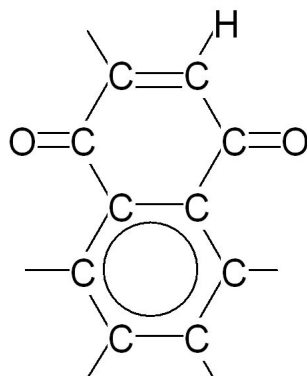


**Query index:** 1 of category: Extremely reactive-Category A

**type:** *Structural boundary:*

The target chemical should have the fragment C1(=O)c2c(C(=O)C{H}=C1)cccc2 in its structure.

fragment

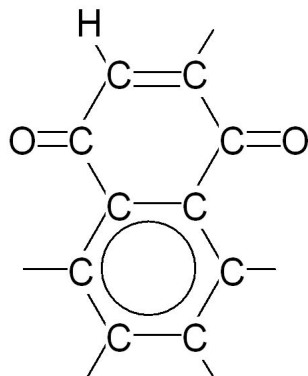


**Query index:** 2 of category: Extremely reactive-Category A

**type:** *Structural boundary:*

The target chemical should have the fragment C1(=O)c2c(C(=O)C=C{H}1)cccc2 in its structure.

fragment

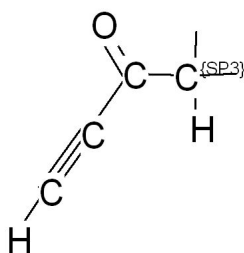


**Query index:** 3 of category: Extremely reactive-Category A

**type: Structural boundary:**

The target chemical should have the fragment C(C{sp3}{H})(=O)C#C{H} in its structure.

fragment

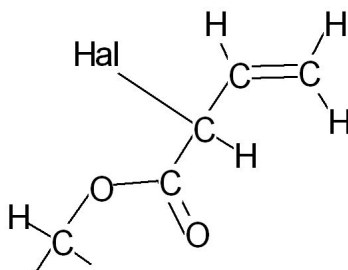


**Query index:** 4 of category: Extremely reactive-Category A

**type: Structural boundary:**

The target chemical should have the fragment C{H2}=C{H}C{H}(C(=O)OC{H})[V1]Hal in its structure.

fragment



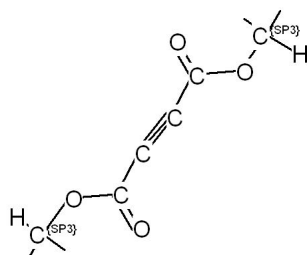
Definition	Options	Face 1	Face 2	Face 3	Face 4
1	Hal V <sub>1</sub>	F-X <sub>1</sub>	Cl-X <sub>1</sub>	Br-X <sub>1</sub>	I-X <sub>1</sub>

**Query index:** 5 of category: Extremely reactive-Category A

**type: Structural boundary:**

The target chemical should have the fragment C(=O)(C#CC(=O)OC{sp3}{H})OC{sp3}{H} in its structure.

fragment

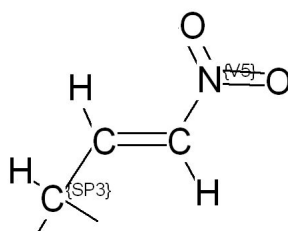


**Query index:** 6 of category: Extremely reactive-Category A

**type: Structural boundary:**

The target chemical should have the fragment C{H}(=C{H}C{sp3}{H})N{V5}(=O)=O in its structure.

fragment

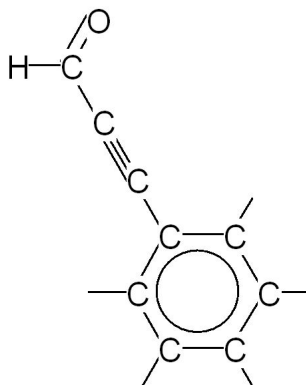


**Query index:** 7 of category: Extremely reactive-Category A

**type: Structural boundary:**

The target chemical should have the fragment c1(C#CC{H}=O)cccc1 in its structure.

fragment

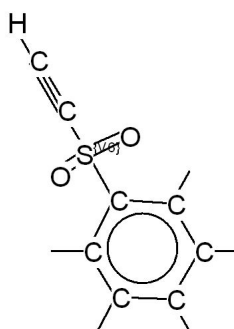


**Query index:** 8 of category: Extremely reactive-Category A

**type: Structural boundary:**

The target chemical should have the fragment c1(S{V6}(=O)(=O)C#C{H})ccccc1 in its structure.

fragment

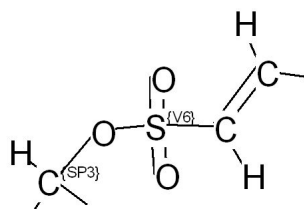


**Query index:** 9 of category: Extremely reactive-Category A

**type: Structural boundary:**

The target chemical should have the fragment C{H}(=C{H})S{V6}(=O)(=O)OC{sp3}{H} in its structure.

fragment

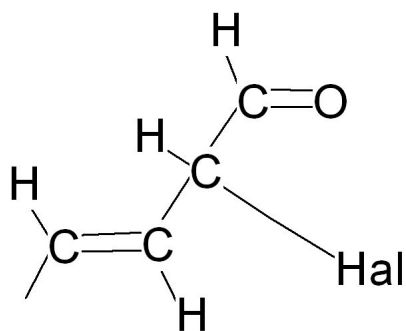


**Query index:** 10 of category: Extremely reactive-Category A

**type: Structural boundary:**

The target chemical should have the fragment C{H}(=O)C{H}(C{H}=C{H})[V1]Hal in its structure.

fragment



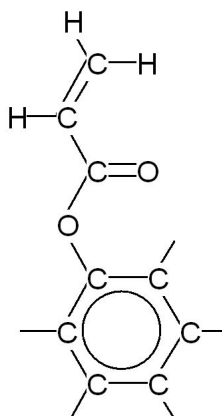
Definition	Options	Face 1	Face 2	Face 3	Face 4
1 Hal V <sub>1</sub>		F X <sub>1</sub>	Cl X <sub>1</sub>	Br X <sub>1</sub>	I X <sub>1</sub>

**Query index:** 11 of category: Extremely reactive-Category A

**type:** *Structural boundary:*

The target chemical should have the fragment C(=O)(C{H}=C{H2})Oc1ccccc1 in its structure.

fragment

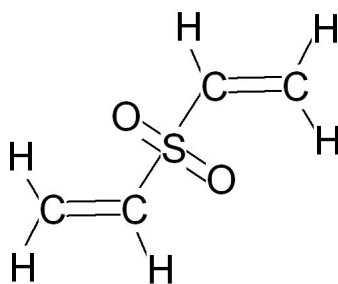


**Query index:** 12 of category: Extremely reactive-Category A

**type:** *Structural boundary:*

The target chemical should have the fragment C{H}(=C{H2})S(=O)(=O)C{H}=C{H2} in its structure.

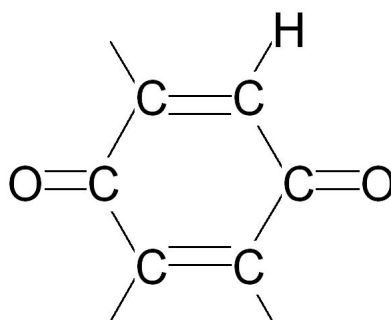
fragment



**Query index:** 13 of category: Extremely reactive-Category A

**type:** *Structural boundary:*

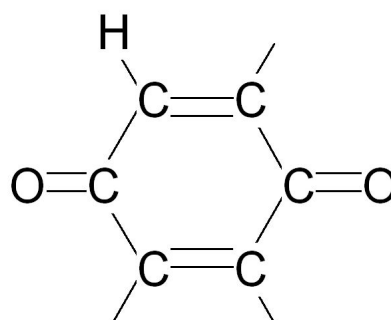
The target chemical should have the fragment C1=C{H}C(=O)C=CC1=O in its structure.  
fragment



**Query index:** 14 of category: Extremely reactive-Category A

**type:** *Structural boundary:*

The target chemical should have the fragment C{H}1=CC(=O)C=CC1=O in its structure.  
fragment

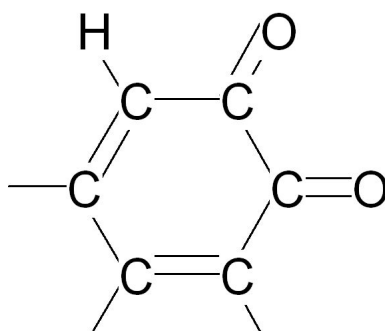


**Query index:** 15 of category: Extremely reactive-Category A

**type:** *Structural boundary:*

The target chemical should have the fragment C{H}1C(=O)C(=O)C=CC1=O in its structure.

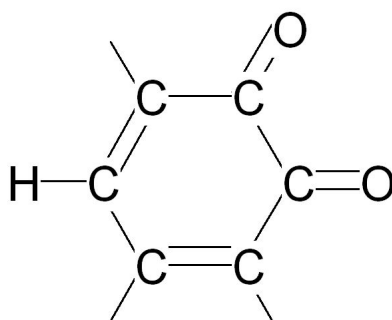
fragment



**Query index:** 16 of category: Extremely reactive-Category A

**type:** *Structural boundary:*

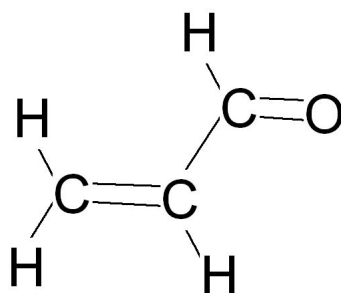
The target chemical should have the fragment C1C(=O)C(=O)C=CC{H}=1 in its structure.  
fragment



**Query index:** 17 of category: Extremely reactive-Category A

**type:** *Structural boundary:*

The target chemical should have the fragment C{H}(=O)C{H}=C{H2} in its structure.  
fragment

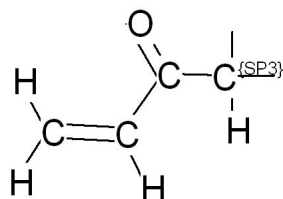


**Query index:** 18 of category: Extremely reactive-Category A

**type:** *Structural boundary:*

The target chemical should have the fragment C(C{sp3}{H})(=O)C{H}=C{H2} in its structure.

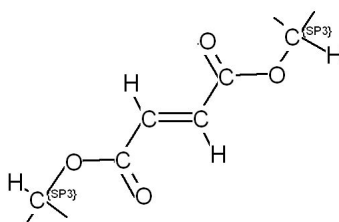
fragment



**Query index:** 19 of category: Extremely reactive-Category A

**type: Structural boundary:**

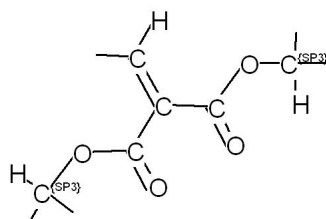
The target chemical should have the fragment  
C(=O)(C{H}=C{H})C(=O)OC{sp3}{H}OC{sp3}{H} in its structure.  
fragment



**Query index:** 20 of category: Extremely reactive-Category A

**type: Structural boundary:**

The target chemical should have the fragment  
C(=O)(C(=C{H})C(=O)OC{sp3}{H})OC{sp3}{H} in its structure.  
fragment

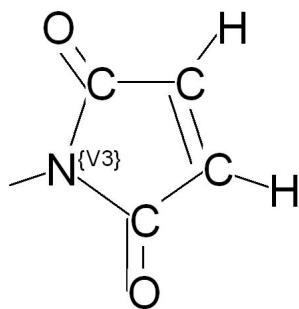


**Query index:** 21 of category: Extremely reactive-Category A

**type: Structural boundary:**

The target chemical should have the fragment C1(=O)C{H}=C{H}C(=O)N{V3}1 in its structure.

fragment

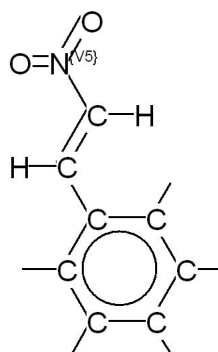


**Query index:** 22 of category: Extremely reactive-Category A

**type: Structural boundary:**

The target chemical should have the fragment c1(C{H}=C{H}N{V5}(=O)=O)cccc1 in its structure.

fragment

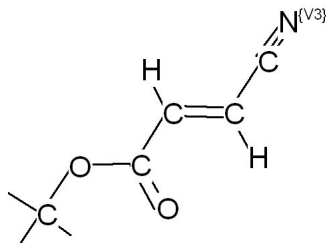


**Query index:** 23 of category: Extremely reactive-Category A

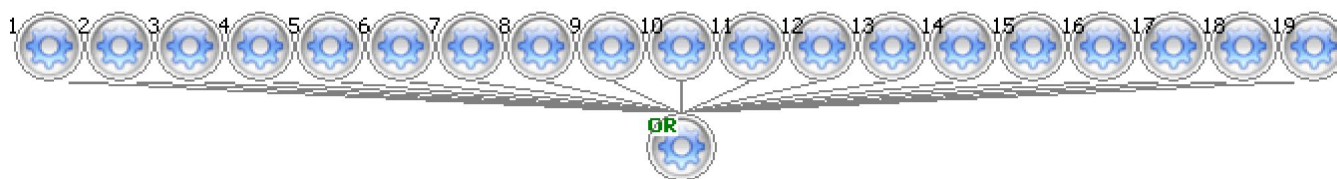
**type: Structural boundary:**

The target chemical should have the fragment C(=O)(C{H}=C{H}C#N{V3})OC in its structure.

fragment



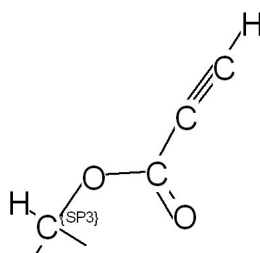
## 2. Highly reactive-Category B



**Query index:** 1 of category: Highly reactive-Category B

**type: Structural boundary:**

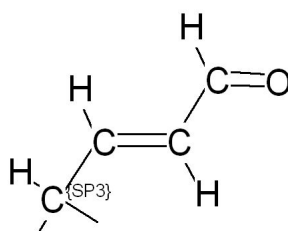
The target chemical should have the fragment C(=O)(C#C{H})OC{sp3}{H} in its structure.  
fragment



**Query index:** 2 of category: Highly reactive-Category B

**type: Structural boundary:**

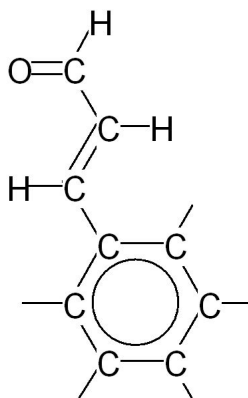
The target chemical should have the fragment C{H}(=O)C{H}=C{H}C{sp3}{H} in its structure.  
fragment



**Query index:** 3 of category: Highly reactive-Category B

**type: Structural boundary:**

The target chemical should have the fragment c1(C{H}=C{H}C{H}=O)ccccc1 in its structure.  
fragment

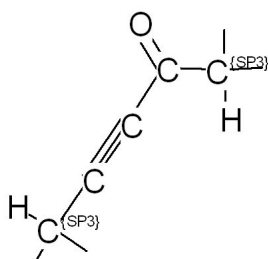


**Query index:** 4 of category: Highly reactive-Category B

**type: Structural boundary:**

The target chemical should have the fragment C(C{sp3}{H})(=O)C#CC{sp3}{H} in its structure.

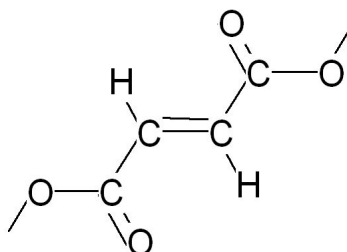
fragment



**Query index:** 5 of category: Highly reactive-Category B

**type: Structural boundary:**

The target chemical should have the fragment C(=O)(O)C{H}={t}C{H}C(=O)O in its structure.  
fragment



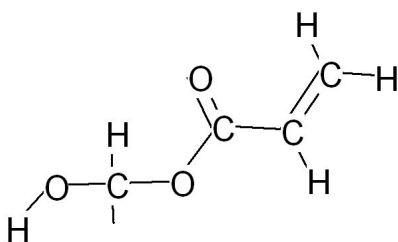
**Query index:** 6 of category: Highly reactive-Category B

**type: Structural boundary:**

The target chemical should have the fragment C(=O)(C{H}=C{H2})OC{H}O{H} in its

structure.

fragment

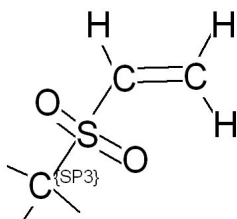


**Query index:** 7 of category: Highly reactive-Category B

**type: Structural boundary:**

The target chemical should have the fragment C{H}(=C{H2})S(C{sp3})(=O)=O in its structure.

fragment

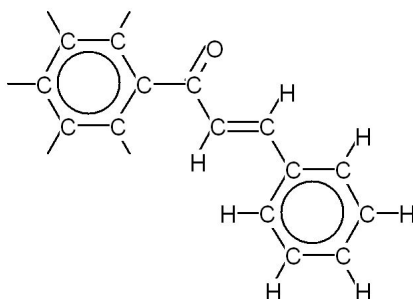


**Query index:** 8 of category: Highly reactive-Category B

**type: Structural boundary:**

The target chemical should have the fragment c1(C(=O)C{H}=C{H}c2c{H}c{H}c{H}c{H}c{H}2)cccc1 in its structure.

fragment

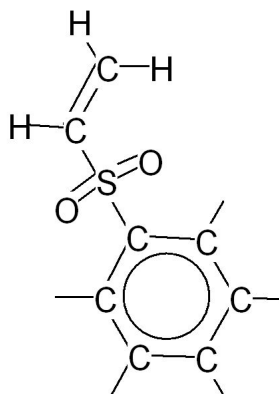


**Query index:** 9 of category: Highly reactive-Category B

**type: Structural boundary:**

The target chemical should have the fragment c1(S(=O)(=O)C{H}=C{H2})cccc1 in its structure.

fragment

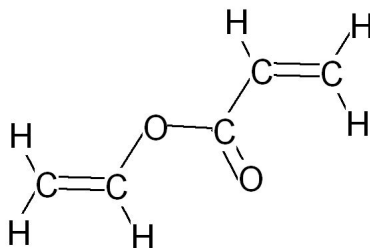


**Query index:** 10 of category: Highly reactive-Category B

**type:** *Structural boundary:*

The target chemical should have the fragment C(=O)(C{H}=C{H2})OC{H}=C{H2} in its structure.

fragment

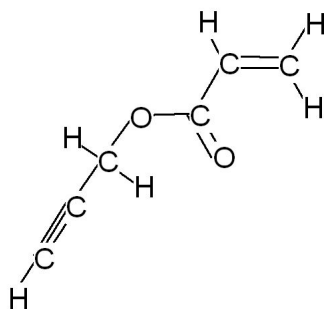


**Query index:** 11 of category: Highly reactive-Category B

**type:** *Structural boundary:*

The target chemical should have the fragment C(=O)(C{H}=C{H2})OC{H2}C#C{H} in its structure.

fragment

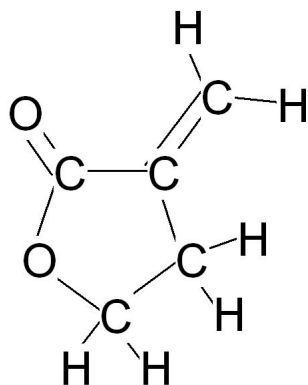


**Query index:** 12 of category: Highly reactive-Category B

**type:** *Structural boundary:*

The target chemical should have the fragment C1(=O)C(=C{H2})C{H2}C{H2}O1 in its structure.

fragment



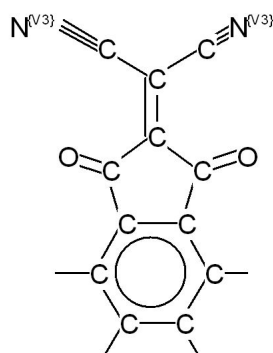
**Query index:** 13 of category: Highly reactive-Category B

**type: Structural boundary:**

The target chemical should have the fragment

C1(=O)C(=C(C#N{V3})C#N{V3})C(=O)c2c1cccc2 in its structure.

fragment

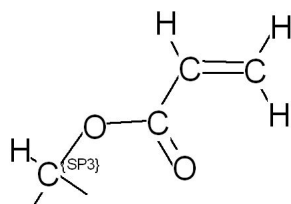


**Query index:** 14 of category: Highly reactive-Category B

**type: Structural boundary:**

The target chemical should have the fragment C(=O)(C{H}=C{H2})OC{sp3}{H} in its structure.

fragment

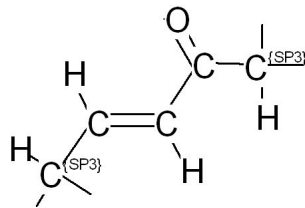


**Query index:** 15 of category: Highly reactive-Category B

**type: Structural boundary:**

The target chemical should have the fragment  $C(C\{sp3\}\{H\})(=O)C\{H\}=C\{H\}C\{sp3\}\{H\}$  in its structure.

fragment

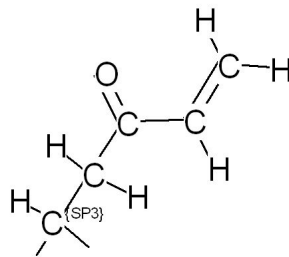


**Query index:** 16 of category: Highly reactive-Category B

**type: Structural boundary:**

The target chemical should have the fragment  $C(=O)(C\{H\}=C\{H_2\})C\{H_2\}C\{sp3\}\{H\}$  in its structure.

fragment

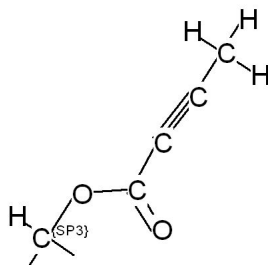


**Query index:** 17 of category: Highly reactive-Category B

**type: Structural boundary:**

The target chemical should have the fragment  $C(=O)(C\#CC\{H_3\})OC\{sp3\}\{H\}$  in its structure.

fragment

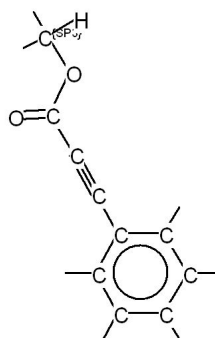


**Query index:** 18 of category: Highly reactive-Category B

**type: Structural boundary:**

The target chemical should have the fragment C(=O)(C#Cc1ccccc1)OC{sp3}{H} in its structure.

fragment

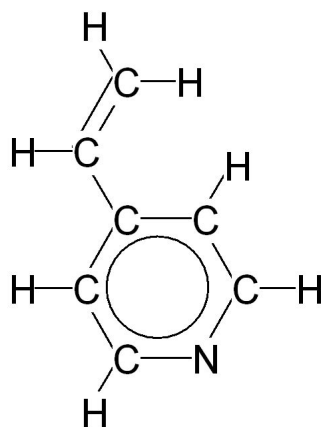


**Query index:** 19 of category: Highly reactive-Category B

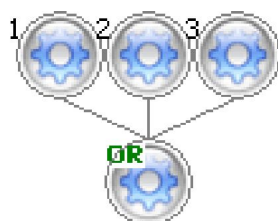
**type:** *Structural boundary:*

The target chemical should have the fragment c1(C{H}=C{H2})c{H}c{H}nc{H}c{H}1 in its structure.

fragment



## 3. Moderately Reactive - Category C

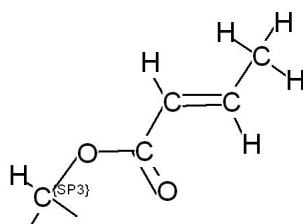


**Query index:** 1 of category: Moderately Reactive - Category C

**type: Structural boundary:**

The target chemical should have the fragment C(=O)(C{H}=C{H}C{H3})OC{sp3}{H} in its structure.

fragment

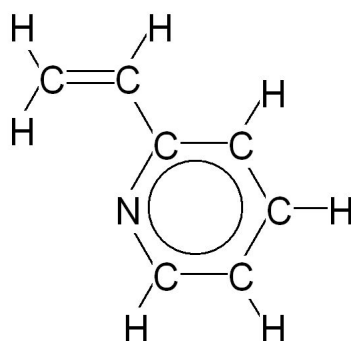


**Query index:** 2 of category: Moderately Reactive - Category C

**type: Structural boundary:**

The target chemical should have the fragment c1(C{H}=C{H2})c{H}c{H}c{H}c{H}n1 in its structure.

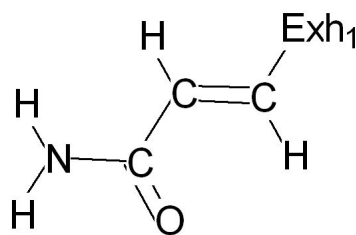
fragment



**Query index:** 3 of category: Moderately Reactive - Category C

**type: Structural boundary:**

The target chemical should have the fragment  
 $C\{H\}(*Exh_1;C\{sp3\}\{in[1-10]\},H)=C\{H\}C(=O)N\{H_2\}$  in its structure.  
fragment



	Definition	Options	Face 1	Face 2
1	Exh <sub>1</sub>	C{sp3}{in[1-10]}		

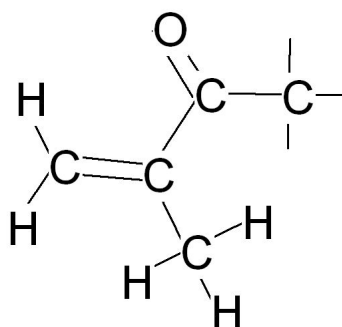
## 4. Slightly Reactive - Category D



**Query index:** 1 of category: Slightly Reactive - Category D

**type: Structural boundary:**

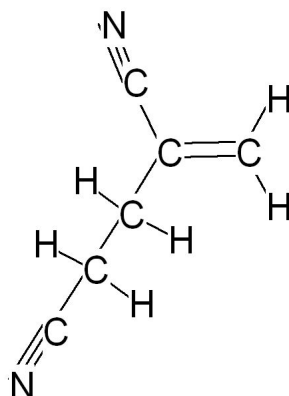
The target chemical should have the fragment C(C)(=O)C(=C{H2})C{H3} in its structure.  
fragment



**Query index:** 2 of category: Slightly Reactive - Category D

**type: Structural boundary:**

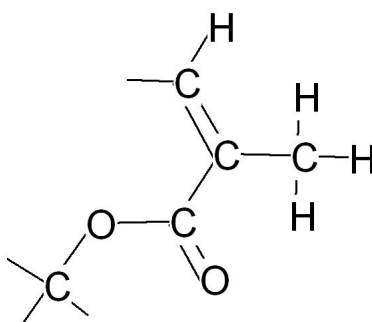
The target chemical should have the fragment C(#N)C(=C{H2})C{H2}C{H2}C#N in its structure.  
fragment



**Query index:** 3 of category: Slightly Reactive - Category D

**type: Structural boundary:**

The target chemical should have the fragment C(=O)(C(C{H3})=C{H})OC in its structure.  
fragment

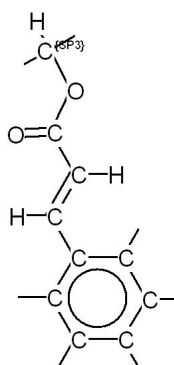


**Query index:** 4 of category: Slightly Reactive - Category D

**type: Structural boundary:**

The target chemical should have the fragment C(=O)(C{H}=C{H}c1ccccc1)OC{sp3}{H} in its structure.

fragment



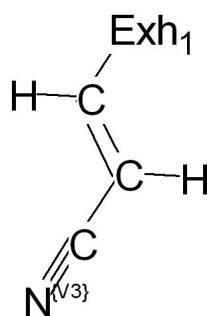
## 5. Suspect - Category F



Query index: 1 of category: Suspect - Category F

type: **Structural boundary:**

The target chemical should have the fragment  
 $C\{H\}(*Exh_1;C\{sp^3\}\{in[1-10]\},H)=C\{H\}C\#N\{v3\}$  in its structure.  
 fragment



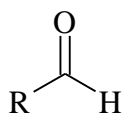
	Definition	Options	Face 1	Face 2
1	Exh <sub>1</sub>	C{sp3}{in[1-10]}		

# **Mechanistic Domain: Schiff Base Formers**

## Mechanistic Alert: Direct Acting Schiff Base Formers

Several classes of chemical have been shown to be capable of covalently binding to proteins via Schiff base formation. The structural alerts covered by this mechanistic alert are as follows:

### Structural alert: Mono-carbonyls



R = hydrogen, any carbon (R groups cannot be aromatic, heteroaromatic or heterocyclic, unless they are either mono- or di- ortho-substituted)

### *Mechanism*

A Schiff base formation mechanism has been suggested to be responsible for the protein binding ability of these types of chemicals (Gerner et al 2004, Roberts et al 2007, Verhaar et al 1992)

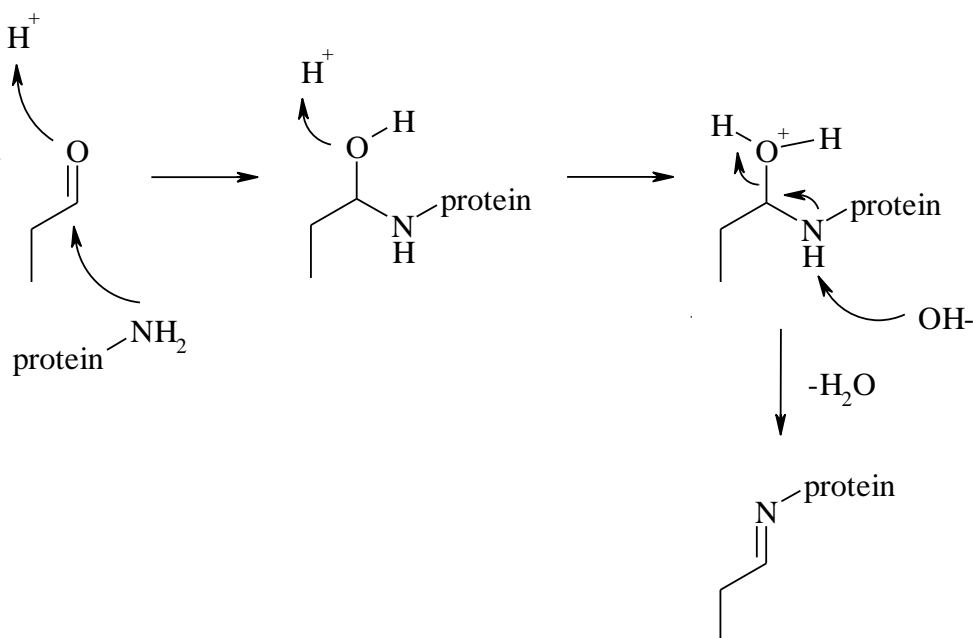
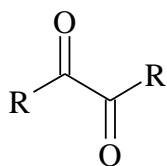


Figure 1: Schiff base formation mechanism for mono-carbonyls

### Structural alert: 1-2-Dicarbonyls



R = hydrogen, any carbon (both R groups cannot be aromatic, heteroaromatic or heterocyclic, unless they are either mono- or di- ortho-substituted)

### *Mechanism*

A Schiff base formation mechanism has been suggested to be responsible for the protein binding ability of these types of chemicals (Enoch et al 2009, Roberts et al 2007, Verhaar et al 1992). 1,2-Dicarbonyl chemicals have been shown to be able to undergo a second Schiff base reaction and thus cross-link protein chains (Marqui 2001).

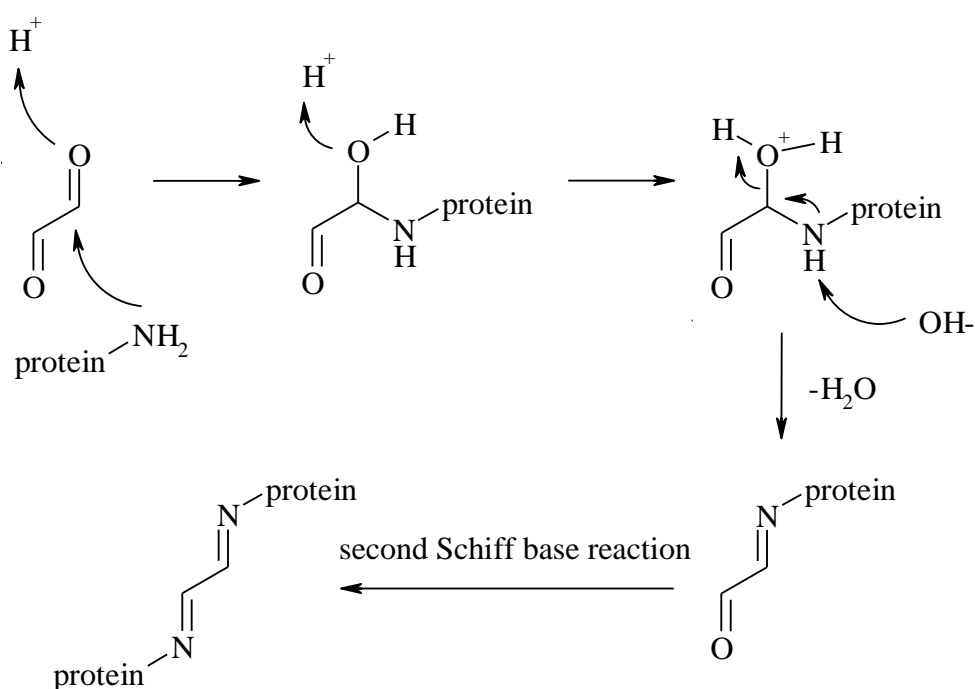
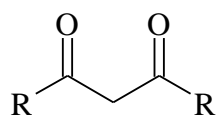


Figure 1: Schiff base formation mechanism for 1,2-dicarbonyls

### Structural alert: 1-3-Dicarbonyls



R = hydrogen, any carbon (both R groups cannot be aromatic, heteroaromatic or heterocyclic, unless they are either mono- or di- ortho-substituted)

### *Mechanism*

A Schiff base formation mechanism has been suggested to be responsible for the protein binding ability of these types of chemicals (Aptula et al 2006, Roberts et al 2006, Verhaar et al 1992). The potential for a second Schiff base reaction, resulting in possible protein cross linking also exists (depending on the substitution at the second carbonyl centre).

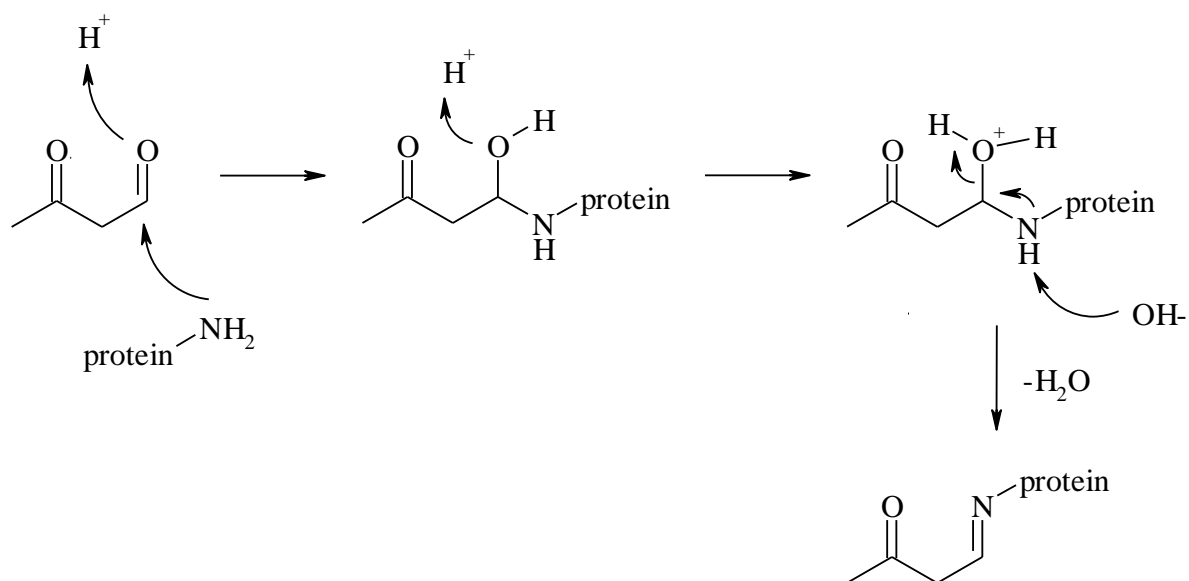


Figure 1: Schiff base formation mechanism for 1,3-dicarbonyls

### Category mitigating factors

- Mono-carbonyls: R cannot be aromatic, heteroaromatic or heterocyclic directly attached to the reactive carbonyl centre (unless they are ortho-substituted)
- Mono-carbonyls: R cannot be a second directly attached carbonyl moiety (such chemicals are a Mechanistic class)

- Mono-carbonyls: R cannot be a group such that the alerting group becomes a 1,3-dicarbonyl (such chemicals are a Mechanistic class)
- 1,2-Dicarbonyls: Both R groups cannot be aromatic, heteroaromatic or heterocyclic directly attached to the reactive carbonyl centre
- 1-3-Dicarbonyls: Both R groups cannot be aromatic, heteroaromatic or heterocyclic directly attached to the reactive carbonyl centre.
- 1,2 and 1-3-Dicarbonyls: The exception to this is if one of the aromatic groups is ortho-substituted. This substitution causes the delocalised  $\pi$ -system between the carbonyl group and aromatic ring system to be broken. This result in activity at the carbonyl group (see examples of active Schiff base reactive chemicals in Gerberick et al 2005).

### References

Aptula AO et al (2006) Chemical Research in Toxicology, 19, p1097

Enoch SJ et al (2009) Chemical Research in Toxicology, 22, p1447

Gerberick GF et al (2005) Dermatitis, 16, p157

Gerner I et al (2004) ATLA, 32, p487

Marqui C (2001) Journal of Agricultural and Food Chemistry, 49, p4676

Roberts DW et al (2007) Chemical Research in Toxicology, 20, p1019

Roberts DW et al (2006) Chemical Research in Toxicology, 19, p1228

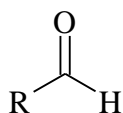
Verhaar HJM et al (1992) Chemosphere, 25, p471

# **Mechanistic Domain: Schiff Base Formers**

## Mechanistic Alert: Direct Acting Schiff Base Formers

Several classes of chemical have been shown to be capable of covalently binding to proteins via Schiff base formation. The structural alerts covered by this mechanistic alert are as follows:

### Structural alert: Mono-carbonyls



R = hydrogen, any carbon (R groups cannot be aromatic, heteroaromatic or heterocyclic, unless they are either mono- or di- ortho-substituted)

### *Mechanism*

A Schiff base formation mechanism has been suggested to be responsible for the protein binding ability of these types of chemicals (Gerner et al 2004, Roberts et al 2007, Verhaar et al 1992)

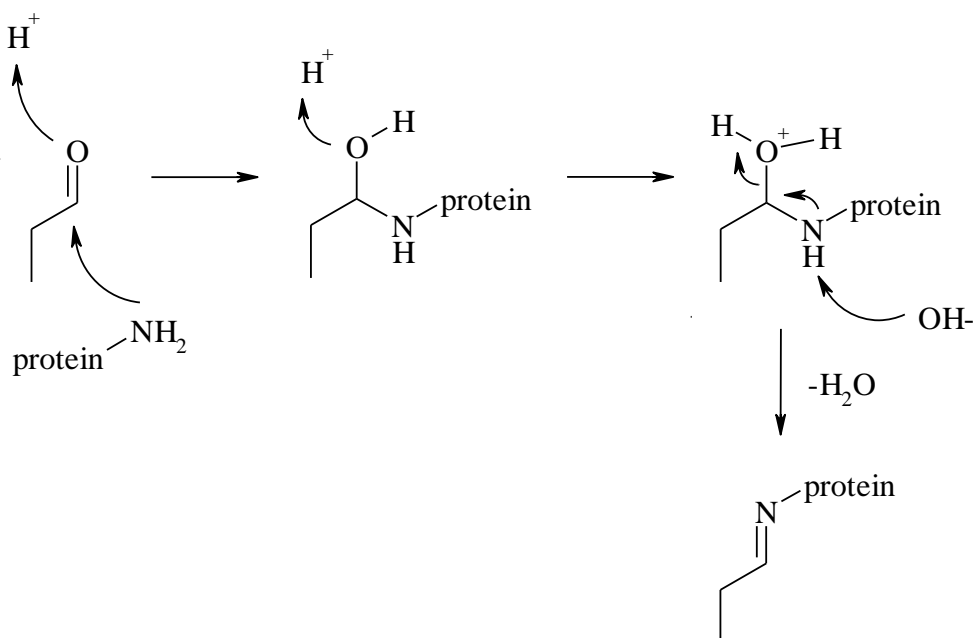
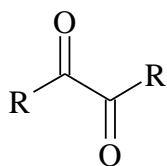


Figure 1: Schiff base formation mechanism for mono-carbonyls

### Structural alert: 1-2-Dicarbonyls



R = hydrogen, any carbon (both R groups cannot be aromatic, heteroaromatic or heterocyclic, unless they are either mono- or di- ortho-substituted)

### *Mechanism*

A Schiff base formation mechanism has been suggested to be responsible for the protein binding ability of these types of chemicals (Enoch et al 2009, Roberts et al 2007, Verhaar et al 1992). 1,2-Dicarbonyl chemicals have been shown to be able to undergo a second Schiff base reaction and thus cross-link protein chains (Marqui 2001).

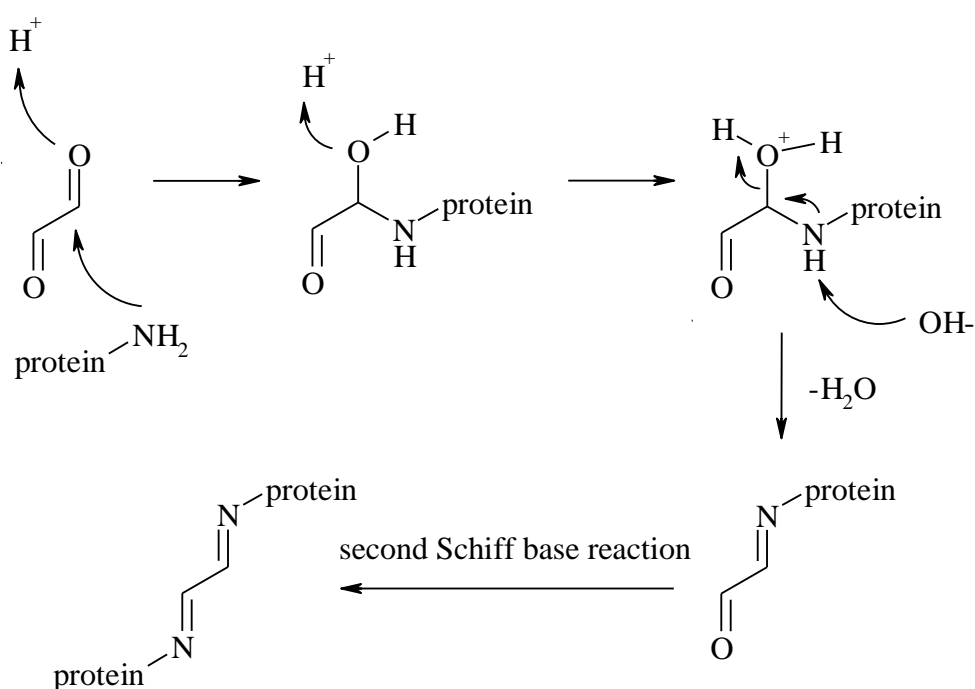
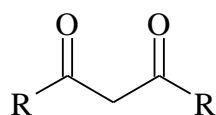


Figure 1: Schiff base formation mechanism for 1,2-dicarbonyls

### Structural alert: 1-3-Dicarbonyls



R = hydrogen, any carbon (both R groups cannot be aromatic, heteroaromatic or heterocyclic, unless they are either mono- or di- ortho-substituted)

### *Mechanism*

A Schiff base formation mechanism has been suggested to be responsible for the protein binding ability of these types of chemicals (Aptula et al 2006, Roberts et al 2006, Verhaar et al 1992). The potential for a second Schiff base reaction, resulting in possible protein cross linking also exists (depending on the substitution at the second carbonyl centre).

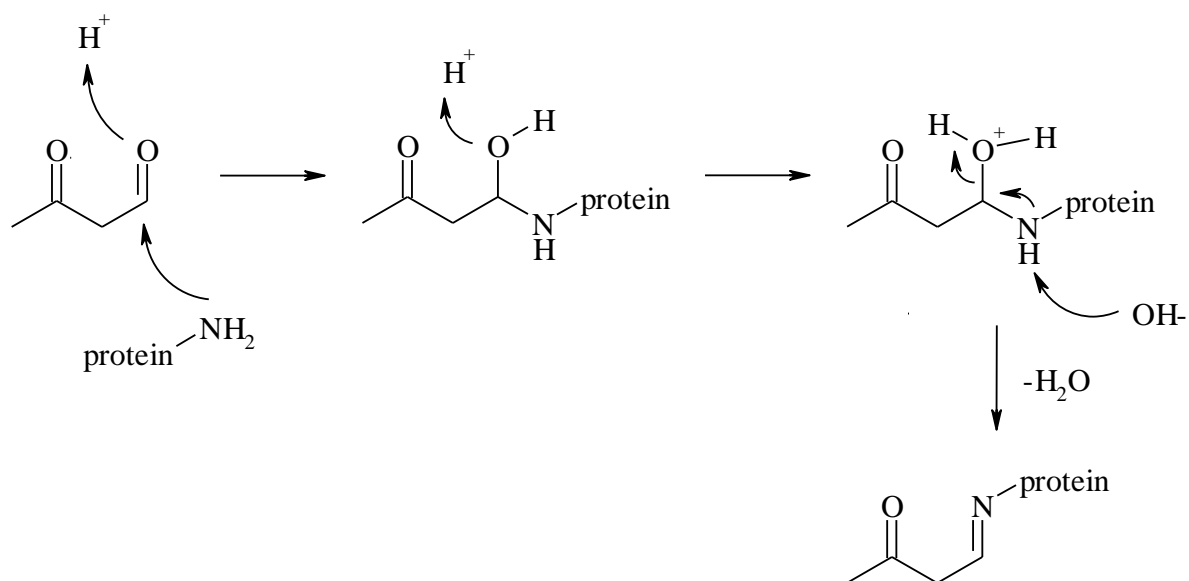


Figure 1: Schiff base formation mechanism for 1,3-dicarbonyls

### Category mitigating factors

- Mono-carbonyls: R cannot be aromatic, heteroaromatic or heterocyclic directly attached to the reactive carbonyl centre (unless they are ortho-substituted)
- Mono-carbonyls: R cannot be a second directly attached carbonyl moiety (such chemicals are a Mechanistic class)

- Mono-carbonyls: R cannot be a group such that the alerting group becomes a 1,3-dicarbonyl (such chemicals are a Mechanistic class)
- 1,2-Dicarbonyls: Both R groups cannot be aromatic, heteroaromatic or heterocyclic directly attached to the reactive carbonyl centre
- 1-3-Dicarbonyls: Both R groups cannot be aromatic, heteroaromatic or heterocyclic directly attached to the reactive carbonyl centre.
- 1,2 and 1-3-Dicarbonyls: The exception to this is if one of the aromatic groups is ortho-substituted. This substitution causes the delocalised  $\pi$ -system between the carbonyl group and aromatic ring system to be broken. This result in activity at the carbonyl group (see examples of active Schiff base reactive chemicals in Gerberick et al 2005).

### References

Aptula AO et al (2006) Chemical Research in Toxicology, 19, p1097

Enoch SJ et al (2009) Chemical Research in Toxicology, 22, p1447

Gerberick GF et al (2005) Dermatitis, 16, p157

Gerner I et al (2004) ATLA, 32, p487

Marqui C (2001) Journal of Agricultural and Food Chemistry, 49, p4676

Roberts DW et al (2007) Chemical Research in Toxicology, 20, p1019

Roberts DW et al (2006) Chemical Research in Toxicology, 19, p1228

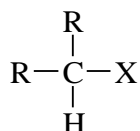
Verhaar HJM et al (1992) Chemosphere, 25, p471

## **Mechanistic Domain: S<sub>N</sub>2**

## Mechanistic Alert: S<sub>N</sub>2 Reaction at a sp<sup>3</sup> Carbon Atom

A number of chemical classes have been suggested to be capable of reacting covalently with proteins via an S<sub>N</sub>2 reaction at a sp<sup>3</sup> hybridised carbon atom. The structural alerts covered by this mechanistic alert are as follows:

### Structural alert: Alkyl halides



R = hydrogen, any carbon except the following:

R ≠ carbonyl (these chemicals fall under the α-halocarbonyl alert), -CS, -CN (these chemicals fall under the mustards alert)

X = halogen

### *Mechanism*

An S<sub>N</sub>2 mechanism has been suggested to be responsible for the protein reactivity of this class of chemicals (Aptula et al 2006, Gerner I et al 2004, Roberts et al 2007, 2010, Schultz et al 2007).

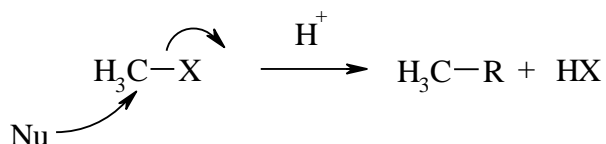
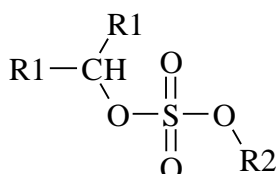


Figure 1: S<sub>N</sub>2 mechanism for primary and secondary alkyl halides (Nu = biological nucleophile e.g. cysteine or lysine)

### Structural alert: Sulfates



R1 = any carbon, hydrogen

R2 = any carbon

Note: R1 and R2 can be part of an aliphatic ring system

### *Mechanism*

An S<sub>N</sub>2 mechanism has been suggested to be responsible for protein reactivity (Aptula et al 2006, Roberts et al 2007).

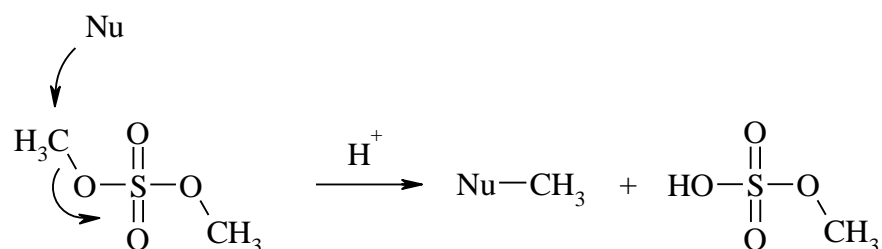
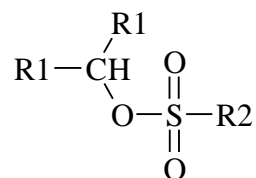


Figure 1: S<sub>N</sub>2 mechanism for sulfates (Nu = biological nucleophile e.g. cysteine or lysine)

### Structural alert: Sulfonates



R1 = any carbon, hydrogen

R2 = any carbon (R1 cannot be alkene or alkyne as these chemicals are Michael acceptors)

Note: R1 and R2 can be part of an aliphatic ring system e.g. sulfones

### *Mechanism*

An S<sub>N</sub>2 mechanism has been suggested to be responsible for protein reactivity (Aptula et al 2006, Roberts et al 2007).

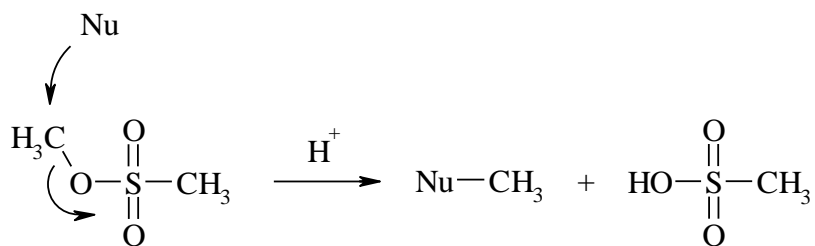
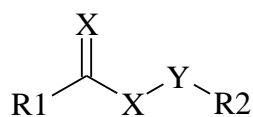


Figure 1:  $\text{S}_{\text{N}}2$  mechanism for sulfonates (Nu = biological nucleophile e.g. cysteine or lysine)

### Structural alert: Allyl acetates and related chemicals



X = oxygen, sulphur

Y =  $\text{CH}_2$ , CH

R1 = any carbon atom

R2 = carbon atom part of an alkene, alkyne, aromatic ring, heteroaromatic ring or heterocyclic ring

### *Mechanism*

An  $\text{S}_{\text{N}}2$  mechanism occurring at the activated carbon (atom Y in the alert) has been suggested to be responsible for the protein reactivity of these chemicals (Roberts et al 2007a, b).

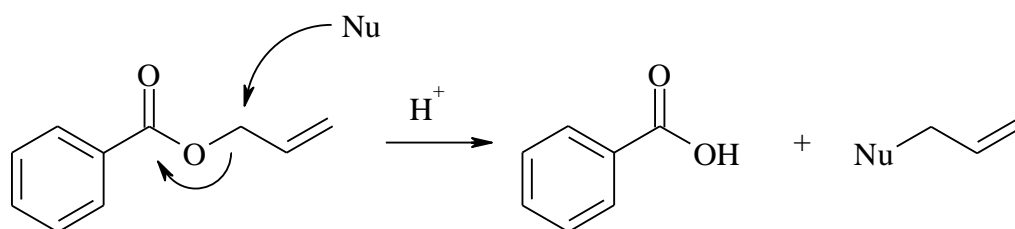
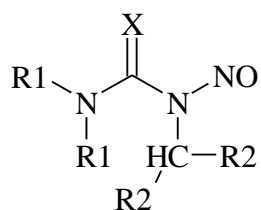


Figure 1:  $\text{S}_{\text{N}}2$  mechanism for allyl acetate and derivatives (Nu = biological nucleophile e.g. cysteine or lysine)

Structural alert: Nitrosoureas (carbon)



X = oxygen (nitrosourea derivatives), nitrogen (nitrosoguanidine derivatives)

R1 = any carbon, hydrogen

R2 = any carbon, hydrogen

*Mechanism*

An S<sub>N</sub>2 mechanism involving direct alkylation (Figure 1) has been proposed (Roberts et al 2007).

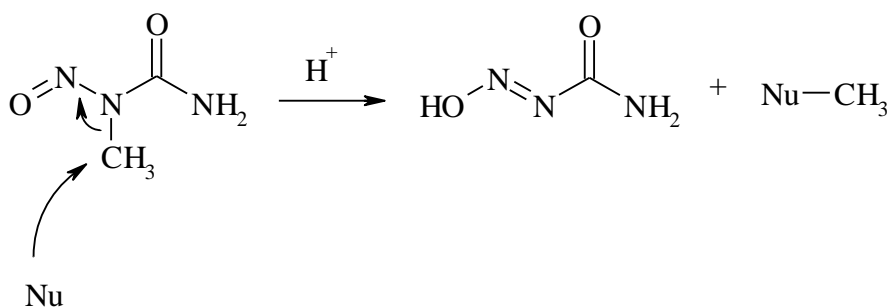
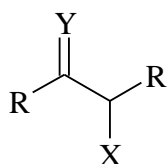


Figure 1: S<sub>N</sub>2 alkylation mechanism (Nu = biological nucleophile e.g. cysteine or lysine)

Structural alert:  $\alpha$ -Halocarbonyls



Y = oxygen, sulphur

X = halogen

R = any carbon, hydrogen

### Mechanism

An S<sub>N</sub>2 mechanism has been suggested to be responsible for the protein reactivity of this class of chemical (Gerner et al 2004, Hulzebos et al 2005, Schultz et al 2007).

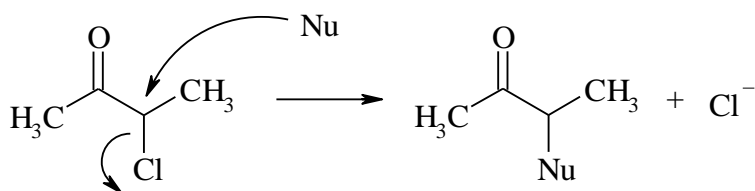
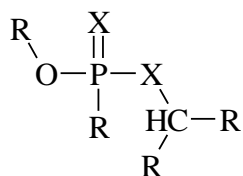


Figure 1: S<sub>N</sub>2 reaction for α-halocarbonyls (Nu = biological nucleophile e.g. cysteine or lysine)

### Structural alert: Phosphonates



R = any carbon, hydrogen

X = oxygen, sulphur

### Mechanism

An S<sub>N</sub>2 mechanism has been suggested as being responsible for the protein binding ability of these chemicals (Gerner et al 2004, von der Ohe et al 2005).

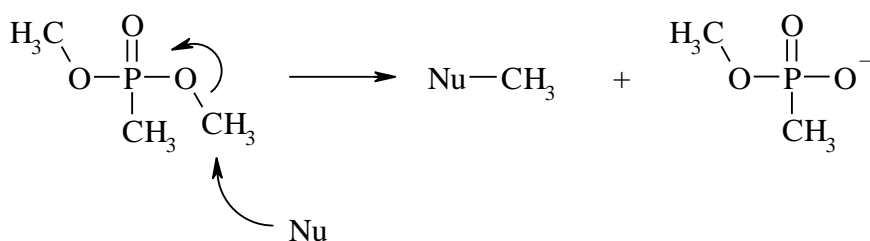


Figure 1: S<sub>N</sub>2 mechanisms for phosphonates (Nu = biological nucleophile e.g. cysteine or lysine)



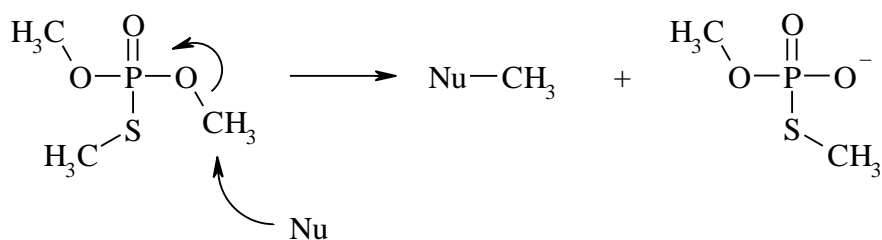
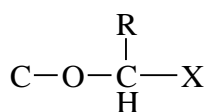


Figure 1:  $S_N2$  mechanisms for thiophosphates (Nu = biological nucleophile e.g. cysteine or lysine)

Structural alert:  $\alpha$ -Halo ethers



X = halogen

R = any carbon atom, hydrogen

*Mechanism*

An  $S_N2$  mechanism has been suggested to be responsible for the protein reactivity of this class of chemicals (Hermens 1990)

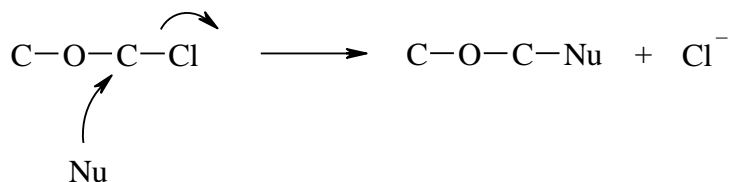
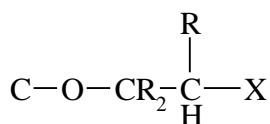


Figure 1:  $S_N2$  mechanism for  $\alpha$ -halo ethers (Nu = biological nucleophile e.g. cysteine or lysine)

Structural alert:  $\beta$ -Halo ethers



X = halogen

R = any carbon atom, hydrogen

### Mechanism

An S<sub>N</sub>2 mechanism has been suggested to be responsible for the protein reactivity of this class of chemicals (Hermens 1990)

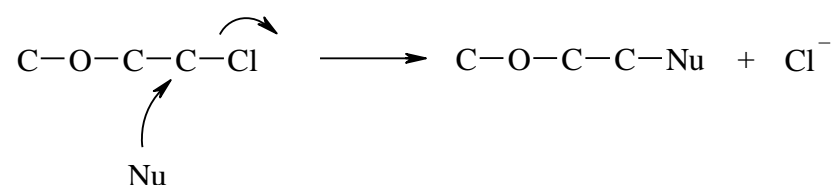
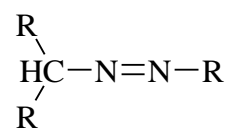


Figure 1: S<sub>N</sub>2 mechanism for β-halo ethers (Nu = biological nucleophile e.g. cysteine or lysine)

### Structural alert: Alkyl diazo



R = any carbon, hydrogen

### Mechanism

An S<sub>N</sub>2 mechanism is the most plausible route to protein binding for this class of chemicals (Hermens 1990).

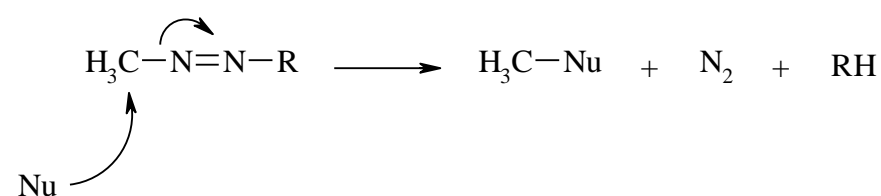
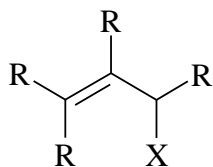


Figure 1: S<sub>N</sub>2 mechanism for alkyl diazo chemicals (Nu = biological nucleophile e.g. cysteine or lysine)

Structural alert:  $\alpha$ -Haloalkenes (and related cyano, sulfate and sulphonate substituted chemicals)



X = halogen, cyano, sulfate, sulphonate

R = any carbon, hydrogen

*Mechanism*

An  $\text{S}_{\text{N}}2$  mechanism is the most likely route to protein binding for this class of chemicals (Hulzebos et al 2005, Verhaar et al 1992).

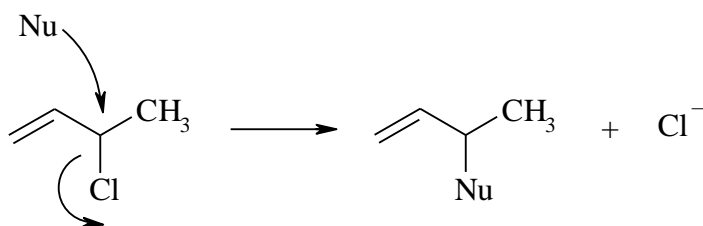
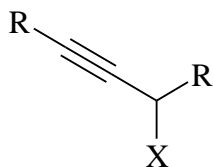


Figure 1:  $\text{S}_{\text{N}}2$  mechanism for  $\alpha$ -haloalkenes (Nu = biological nucleophile e.g. cysteine or lysine)

Structural alert:  $\alpha$ -Haloalkynes (and related cyano, sulfate and sulphonate substituted chemicals)



X = halogen, cyano, sulfate, sulphonate

R = any carbon, hydrogen

*Mechanism*

An S<sub>N</sub>2 mechanism is the most likely route to protein binding for this class of chemicals (Verhaar et al 1992).

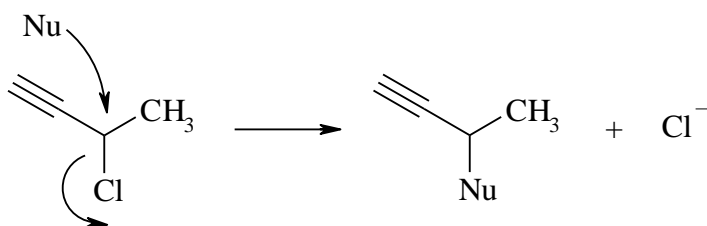
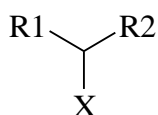


Figure 1: S<sub>N</sub>2 mechanism for alpha-haloalkynes (Nu = biological nucleophile e.g. cysteine or lysine)

Structural alert:  $\alpha$ -Halobenzyls (and related cyano, sulfate and sulphonate substituted chemicals)



X = halogen, cyano, sulfate, sulphonate

R1 = aromatic carbon

R2 = any carbon, hydrogen

### *Mechanism*

An S<sub>N</sub>2 mechanism is the most likely route to protein binding for this class of chemicals (Verhaar et al 1992).

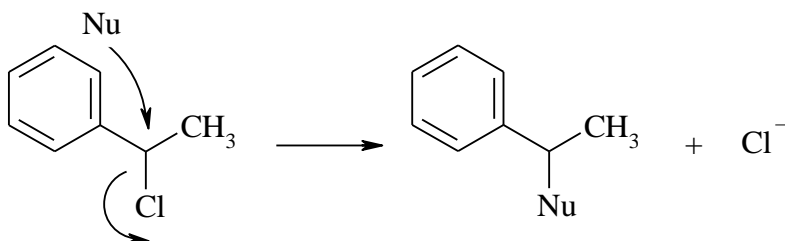


Figure 1: S<sub>N</sub>2 mechanism for alpha-halobenzyls (Nu = biological nucleophile e.g. cysteine or lysine)

### Category mitigating factors

- All Mechanistic classes within this category: Tertiary alkyl carbons do not undergo the S<sub>N</sub>2 reaction due to steric hindrance
- Alkyl halides: Tertiary alkyl halides are considered to be sterically hindered at the site of nucleophilic attack to be protein reactive
- Sulfonic esters: Site of nucleophilic attack (the carbon directly attached to the oxygen atom in the R1 group) cannot be tertiary due to increased steric hindrance.
- Sulfonic esters: R2 cannot be an alkene or alkyne if the chemical is a sulfonate (left hand alert) as these chemicals are Michael acceptors.
- Allyl acetates: R1 cannot be an alkene or alkyne directly attached to the carbonyl (or sulfinyl) as these chemicals would be Michael acceptors
- Allyl acetates: Y cannot be tertiary as steric hindrance prevents the S<sub>N</sub>2 reaction
- α-Halocarbonyls: Tertiary halides are not reactive due to increased steric hindrance at the site of electrophilic attack

### References

Aptula AO et al (2006) Chemical Research in Toxicology, 19, p1097

Gerner I et al (2004) ATLA, 32, p487

Hermens JLM (1990) Environmental Health Perspectives, 87, p219

Hulzebos E et al (2005) QSAR and Combinatorial Science, 24, p332

Robert DW et al (2007) Chemical Research in Toxicology, 20, p44

Roberts DW et al (2007a) Chemical Research in Toxicology, 20, p1019

Roberts DW et al (2007b) Chemical Research in Toxicology, 20, p1321

Roberts DW et al (2010) Chemical Research in Toxicology, 23, p228

Schultz TW et al (2007) SAR and QSAR in Environmental Research, 18, p21

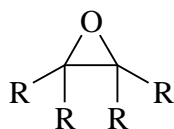
Verhaar HJM et al (1992) Chemosphere, 25, p471

von der Ohe et al (2006) Chemical Research in Toxicology, 18, p536

## Mechanistic Alert: Epoxides and Related Chemicals

Several chemical classes have been suggested to react with proteins via a three membered ring opening  $S_N2$  reaction. The structural alerts covered by this mechanistic alert are as follows:

### Structural alert: Epoxides



R = any carbon, hydrogen

### *Mechanism*

A ring opening  $S_N2$  mechanism has been suggested to be responsible for protein reactivity (Aptula et al 2006, Roberts et al 2007, Verhaar et al 1992).

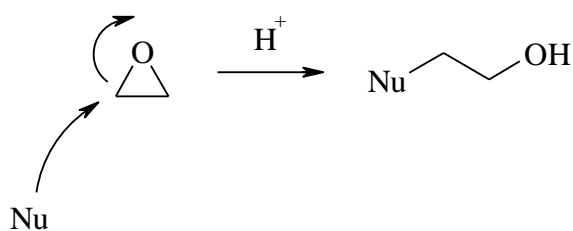
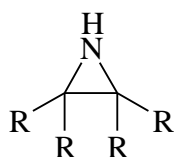


Figure 1: Ring opening  $S_N2$  reaction (Nu = biological nucleophile e.g. cysteine or lysine)

### Structural alert: Aziridines



R = any carbon, hydrogen

### *Mechanism*

A ring opening  $S_N2$  mechanism has been suggested to be responsible for protein reactivity (Aptula et al 2006, Roberts et al 2007, Verhaar et al 1992).

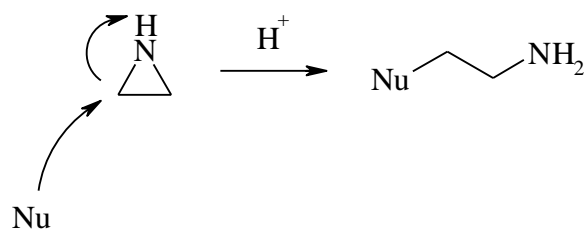
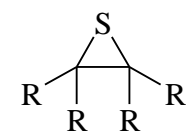


Figure 1: Ring opening  $S_N2$  reaction (Nu = biological nucleophile e.g. cysteine or lysine)

### Structural alert: Sulfuranes



R = any carbon, hydrogen

### *Mechanism*

A ring opening  $S_N2$  mechanism has been suggested to be responsible for protein reactivity (Aptula et al 2006, Roberts et al 2007, Verhaar et al 1992).

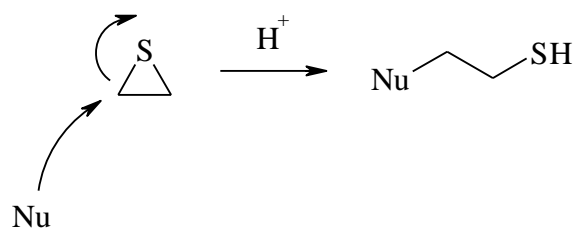


Figure 1: Ring opening  $S_N2$  reaction (Nu = biological nucleophile e.g. cysteine or lysine)

### Category mitigating factors

- No mitigating factors have been reported for any of the Mechanistic classes covered by this category

### References

Aptula AO et al (2006) *Chemical Research in Toxicology*, 19, p1097

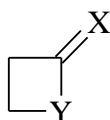
Roberts DW et al (2007) *Chemical Research in Toxicology*, 20, p1019

Verhaar HJM et al (1992) *Chemosphere*, 25, p471

## Mechanistic Alert: Ring Opening S<sub>N</sub>2 Reaction

Several chemical classes have been suggested to react with proteins via four membered ring opening S<sub>N</sub>2 reaction. The structural alerts covered by this mechanistic alert are as follows:

### Structural alert: β-Lactones



X = oxygen (carbonyl), sulphur (sulfinyl)

Y = oxygen, sulphur, nitrogen

### *Mechanism*

An S<sub>N</sub>2 mechanism involving a ring opening reaction has been suggested as being responsible for the protein binding ability of these chemicals (Enoch et al 2008, Roberts et al 2007). Note: Only the four membered ring system is sufficiently reactive to be capable of protein binding. This is due to the additional energy gained upon release of the strain in the four membered ring. The equivalent five and six membered ring systems are not strained and are thus not capable of protein binding.

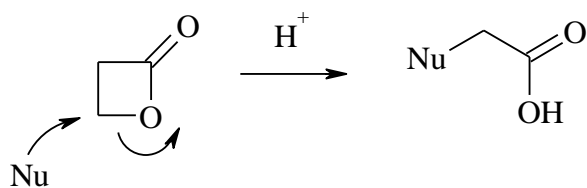


Figure 1: Ring opening S<sub>N</sub>2 mechanism for β-lactone derivatives (Nu = biological nucleophile e.g. cysteine or lysine)

### Category mitigating factors

- No mitigating factors have been reported for any of the Mechanistic classes covered by this category

## References

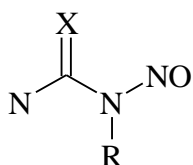
Enoch SJ et al (2008) SAR and QSAR in Environmental Research, 19, p555

Roberts DW et al (2007) Chemical Research in Toxicology, 20, p1019

### Mechanistic Alert: S<sub>N</sub>2 Reaction at a Nitrogen Atom

A number of chemical classes have been suggested to be capable of reacting covalently with proteins via an S<sub>N</sub>2 reaction at a nitrogen atom. The structural alerts covered by this mechanistic alert are as follows:

#### Structural alert: Nitrosoureas (nitrogen)



X = oxygen (nitrosourea derivatives), nitrogen (nitrosoguanidine derivatives)

R = any carbon, hydrogen

#### *Mechanism*

An S<sub>N</sub>2 nitrosation mechanism has been suggested to lead to the formation of protein adducts (Roberts et al 2007).

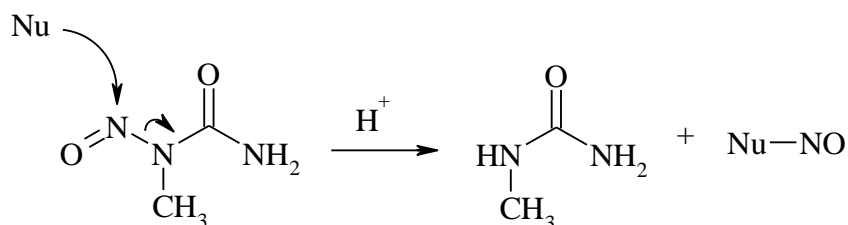
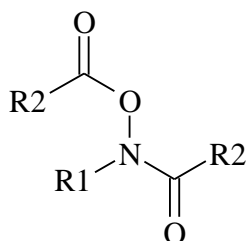


Figure 1: S<sub>N</sub>2 nitrosation mechanism (Nu = biological nucleophile e.g. cysteine or lysine)

#### Structural alert: N-Acetoxy-N-acetyl-phenyl



R1 = aromatic, heteroaromatic, heterocyclic ring system

R2 = any carbon, hydrogen

### Mechanism

A nitrogen centred  $S_N2$  mechanism has been suggested to be responsible for the protein reactivity of this class of chemicals (Roberts et al 2007).

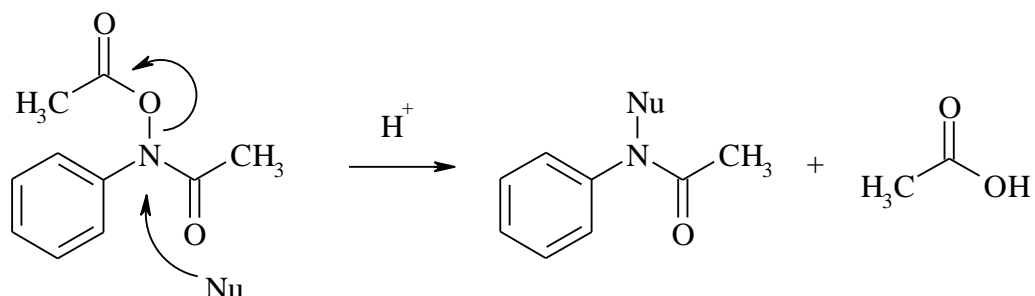
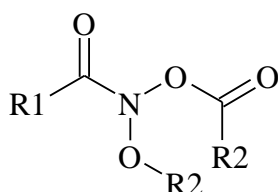


Figure 1: Nitrogen centred  $S_N2$  mechanism (Nu = biological nucleophile e.g. cysteine or lysine)

### Structural alert: N-Acyloxy-N-alkoxyamides



R1 = aromatic, heteroaromatic, heterocyclic ring system

R2 = any carbon, hydrogen

### Mechanism

An  $S_N2$  mechanism has been suggested to be responsible for the alkylation of biological macromolecules including proteins (Banks et al 2003).

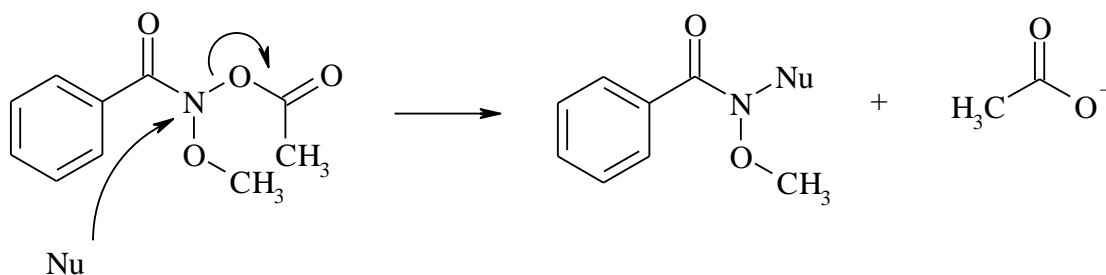


Figure 1: S<sub>N</sub>2 mechanism for N-acyloxy-N-alkoxyamides (Nu = biological nucleophile e.g. cysteine or lysine)

#### Category mitigating factors

- No mitigating factors have been reported for any of the Mechanistic classes within this category

#### References

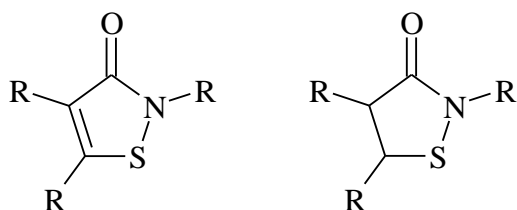
Banks et al (2003) Organic Biomolecular Chemistry, 1, p2238-2246

Roberts DW et al (2007) Chemical Research in Toxicology, 20, p44

### Mechanistic Alert: S<sub>N</sub>2 Reaction at a Sulphur Atom

A number of chemical classes have been suggested to be capable of reacting covalently with proteins via an S<sub>N</sub>2 reaction at a sulphur atom. The structural alerts covered by this mechanistic alert are as follows:

#### Structural alert: Isothiazol-3-ones (sulphur)



R = any carbon, hydrogen

#### *Mechanism*

A sulphur centred S<sub>N</sub>2 mechanism has been suggested to be responsible for the reactivity of this class of chemicals (Figure 1). This mechanism has been shown to exist for sulphur nucleophiles (e.g. cysteine) (Roberts et al 1997, Alvarez-Sanchez et al 2003).

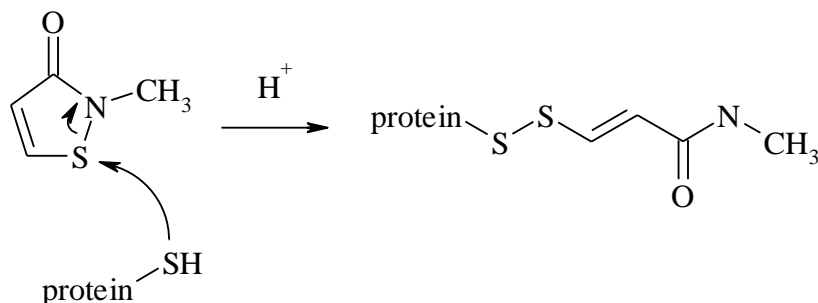
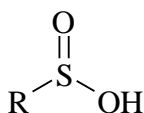


Figure 1: Sulphur centred S<sub>N</sub>2 mechanism involving thiol based nucleophile (e.g. cysteine)

#### Structural alert: Aromatic sulphonic acids



R = aromatic, heteroaromatic, heterocyclic ring system

#### *Mechanism*

An S<sub>N</sub>2 reaction involving disulphide exchange has been suggested to be responsible for the protein binding ability of these chemicals (Gerner et al 2004).

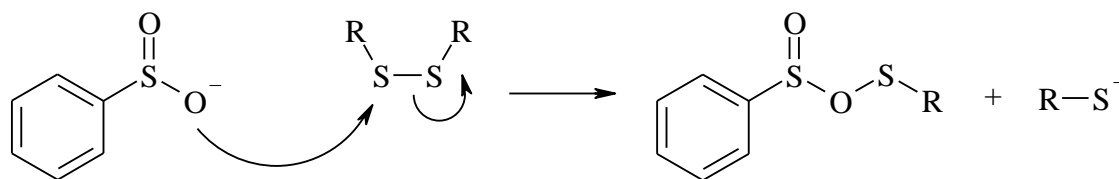
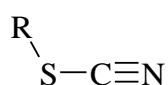


Figure 1: S<sub>N</sub>2 disulphide exchange mechanism for aromatic sulphonate acids

### Structural alert: Thiocyanates



R = any carbon

### *Mechanism*

An S<sub>N</sub>2 mechanism involving the cyano group acting as a leaving group has been suggested to be responsible for the protein reactivity of this class of chemicals (Hermens 1990).

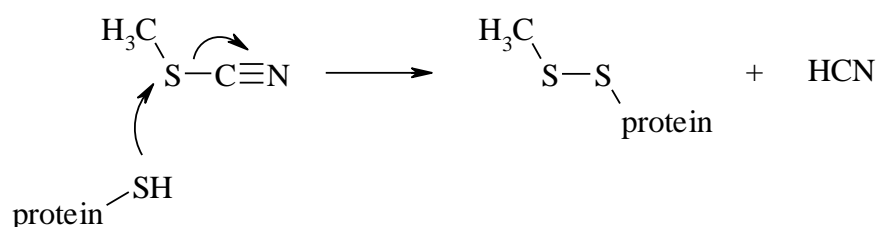
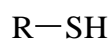


Figure 1: S<sub>N</sub>2 mechanism for thiocyanates

### Structural alert: Thiols



R = any carbon

### *Mechanism*

An S<sub>N</sub>2 type mechanism with thiol groups in biological macromolecules resulting in the formation of disulfide bridges has been suggested (Chipinda et al 2007, Hermens 1990).

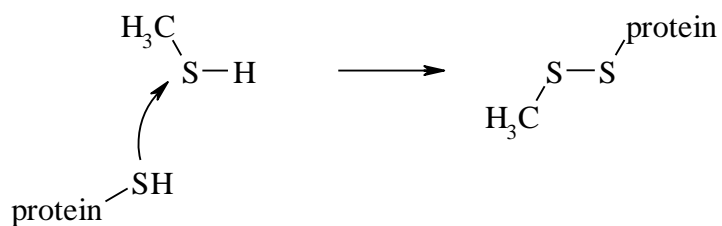
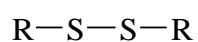


Figure 1: Disulfide bridge formation

Structural alert: Disulfides



R = any carbon

*Mechanism*

An S<sub>N</sub>2 type mechanism with thiol groups in biological macromolecules resulting in the formation of disulfide bridges has been suggested (Chipinda et al 2007, Hermens 1990).

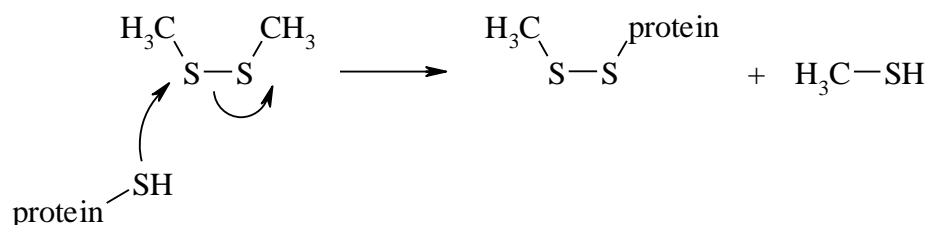
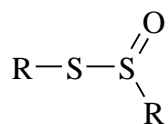


Figure 1: Disulfide bridge formation

Structural alert: Thiosulfonates



R = any carbon

*Mechanism*

An S<sub>N</sub>2 type mechanism with thiol groups in biological macromolecules resulting in the formation of disulfide bridges has been suggested (Chipinda et al 2007, Hermens 1990).

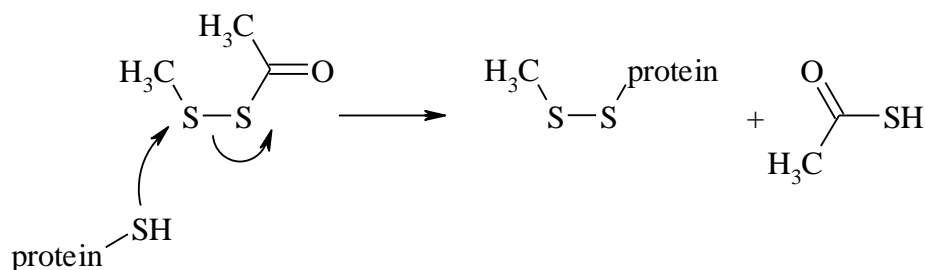
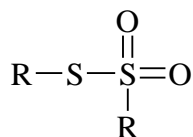


Figure 1: Disulfide bridge formation

Structural alert: Sulfoxides of disulfides



R = any carbon

*Mechanism*

An S<sub>N</sub>2 type mechanism with thiol groups in biological macromolecules resulting in the formation of disulfide bridges has been suggested (Chipinda et al 2007, Hermens 1990).

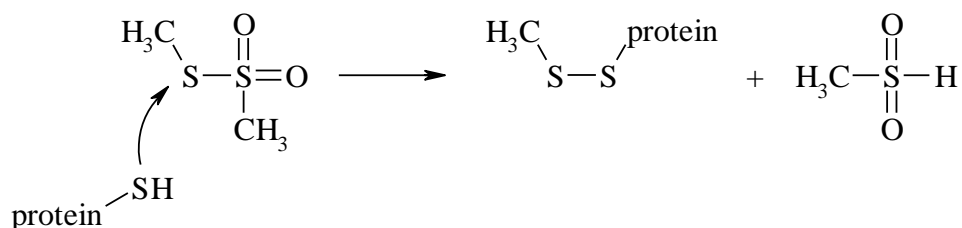
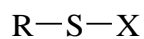


Figure 1: Disulfide bridge formation

Structural alert: Sulfenyl halides



X = halide

R = any carbon, hydrogen

### *Mechanism*

An S<sub>N</sub>2 mechanism has been suggested as being responsible for the protein binding ability of this class of chemicals (Hermens 1990).

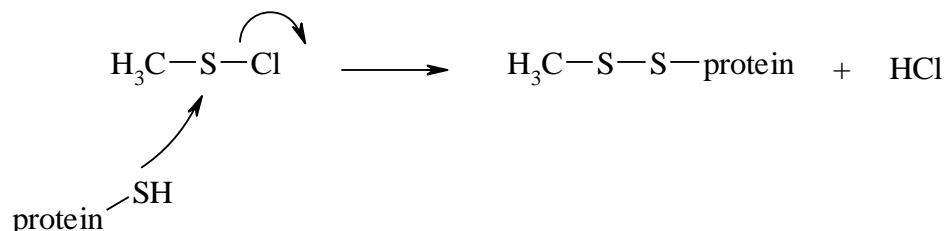


Figure 1: S<sub>N</sub>2 mechanism for sulfenyl halides

### Category mitigating factors

- The biological nucleophile must be sulphur based for of the mechanistic classes within this category

### References

Alvarez-Sanchez R et al (2003) Chemical Research in Toxicology, 16, p627

Chipinda I et al (2007) Chemical Research in Toxicology, 20, p1084

Gerner I et al (2004) ATLA, 32, p487

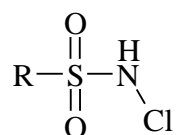
Hermens JLM (1990) Environment Health Perspectives, 87, p219

Roberts DW et al (2007) Chemical Research in Toxicology, 20, p44

### Mechanistic Alert: S<sub>N</sub>2 Reaction at a Halo Atom

A number of chemical classes have been suggested to be capable of reacting covalently with proteins via an S<sub>N</sub>2 reaction at a halo atom. The structural alerts covered by this mechanistic alert are as follows:

#### Structural alert: N-Chloro-sulphonamides



R = any carbon, hydrogen

#### *Mechanism*

An S<sub>N</sub>2 mechanism involving the chlorination of an amine moiety within a biological macromolecule has been suggested to be responsible for the toxicity of these chemicals (Grisham et al 1984, Peskin et al 2001, Piga et al 2005).

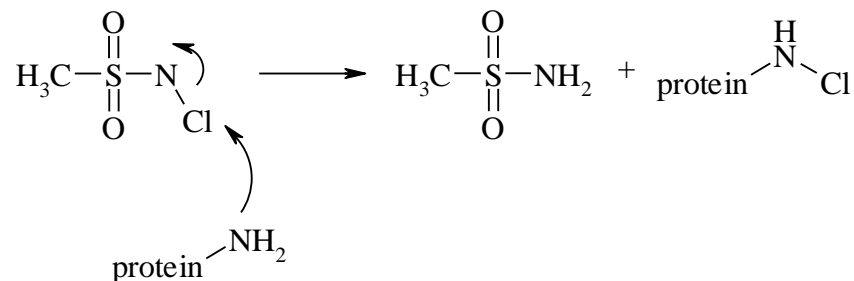
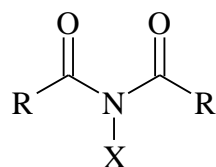


Figure 1: S<sub>N</sub>2 mechanism involving the chlorination of an amine unit within a biological macromolecule

#### Structural alert: N-Haloimides



R = any carbon, hydrogen

X = F, Cl, Br, I

### Mechanism

This alert has been linked with protein binding in skin sensitisation (Zinke et al 2002). However, no clear mechanism has been established in the literature. One can hypothesise an S<sub>N</sub>2 mechanism in which a nitrogen protein nucleophile (e.g. lysine) extracts the chlorine. This mechanism is analogous to that shown to exist in N-chloro sulphonamide derivatives (see alert SN2-14 and associated references) (Figure 1).

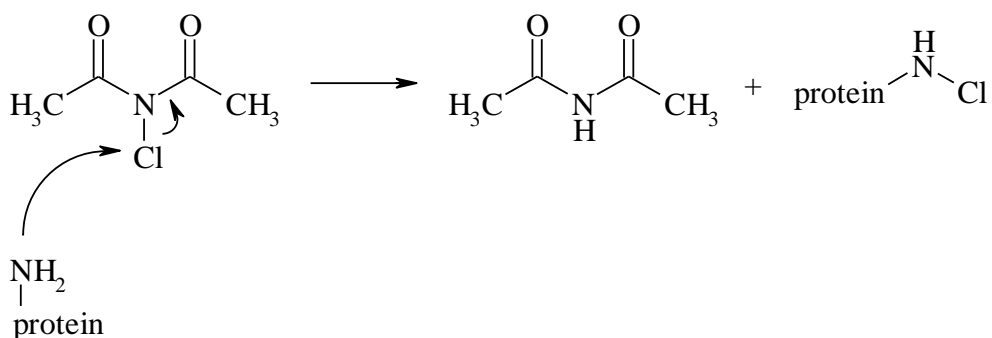


Figure 1: Possible S<sub>N</sub>2 mechanism responsible for protein binding for this class of chemicals

### Category mitigating factors

- The biological nucleophile must be nitrogen based for of the mechanistic classes within this category

### References

Grisham MB et al (1984) Journal of Biological Chemistry, 259, p10404

Peskin AV et al (2001) Free Radical Biology and Medicine, 30, p572

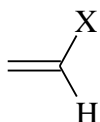
Piga R et al (2005) Archives of Biochemistry and Biophysics, 436, p101

Zinke I et al (2002) ATLA, 30, p285

### Mechanistic Alert: S<sub>N</sub>2 Reaction at a sp<sup>2</sup> carbon Atom

A number of chemical classes have been suggested to be capable of reacting covalently with proteins via an S<sub>N</sub>2 reaction at a sp<sup>2</sup> carbon atom. The structural alerts covered by this mechanistic alert are as follows:

#### Structural alert: Polarised alkenes with a halogen leaving group



X = F, Cl, Br, I

#### *Mechanism*

An S<sub>N</sub>2 type mechanism (commonly referred to an S<sub>N</sub>Vinyl mechanism) has been suggested to be responsible for the ability of this class of chemicals to react with proteins (Lei et al 2009).

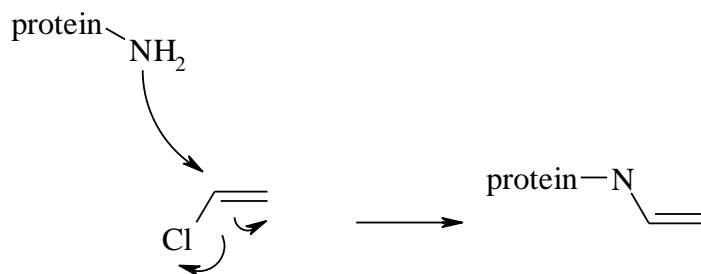
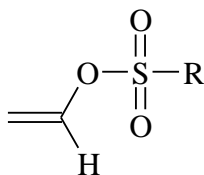


Figure 1: S<sub>N</sub>2 (S<sub>N</sub>Vinyl) mechanism

#### Structural alert: Polarised alkenes with a sulfonate leaving group



### Mechanism

An S<sub>N</sub>2 type mechanism (commonly referred to an S<sub>N</sub>Vinyl mechanism) has been suggested to be responsible for the ability of this class of chemicals to react with proteins (Lei et al 2009).

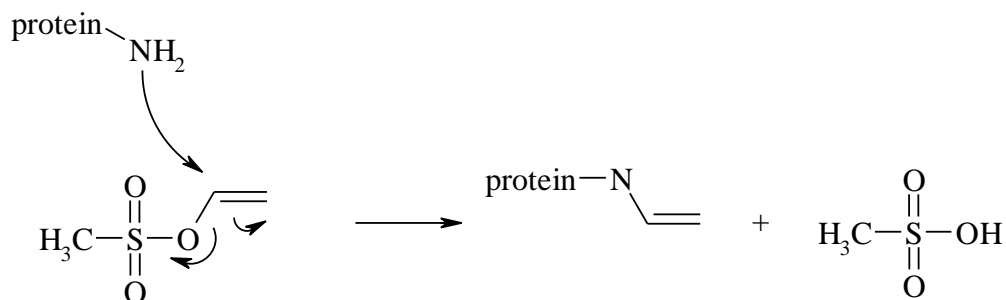
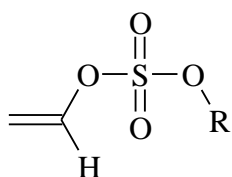


Figure 1: S<sub>N</sub>2 (S<sub>N</sub>Vinyl) mechanism

### Structural alert: Polarised alkenes with a sulfate leaving group



### Mechanism

An S<sub>N</sub>2 type mechanism (commonly referred to an S<sub>N</sub>Vinyl mechanism) has been suggested to be responsible for the ability of this class of chemicals to react with proteins (Lei et al 2009).

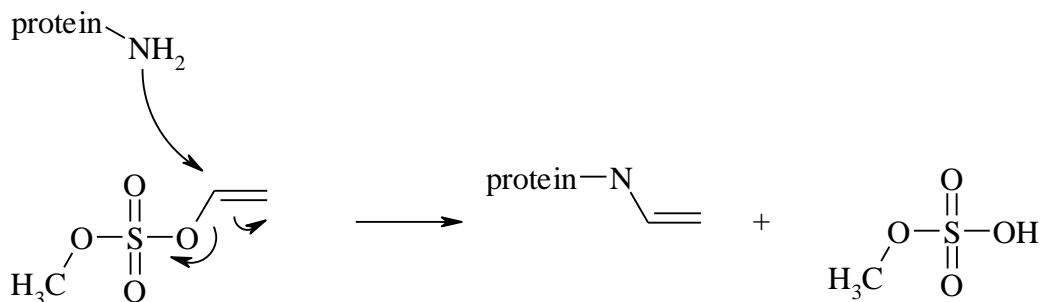
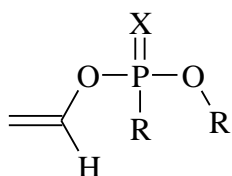


Figure 1: S<sub>N</sub>2 (S<sub>N</sub>Vinyl) mechanism

Structural alert: Polarised alkenes with a phosphonate leaving group



X = O (phosphonate), S (thiophosphonate)

*Mechanism*

An S<sub>N</sub>2 type mechanism (commonly referred to an S<sub>N</sub>Vinyl mechanism) has been suggested to be responsible for the ability of this class of chemicals to react with proteins (Lei et al 2009).

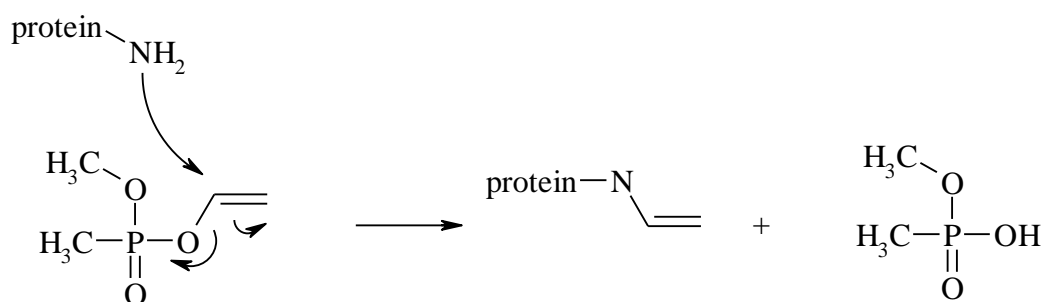
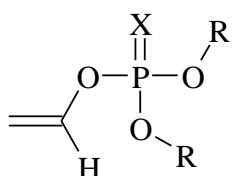


Figure 1: S<sub>N</sub>2 (S<sub>N</sub>Vinyl) mechanism

Structural alert: Polarised alkenes with a phosphate leaving group



X = O (phosphate), S (thiophosphate)

*Mechanism*

An S<sub>N</sub>2 type mechanism (commonly referred to an S<sub>N</sub>Vinyl mechanism) has been suggested to be responsible for the ability of this class of chemicals to react with proteins (Lei et al 2009).

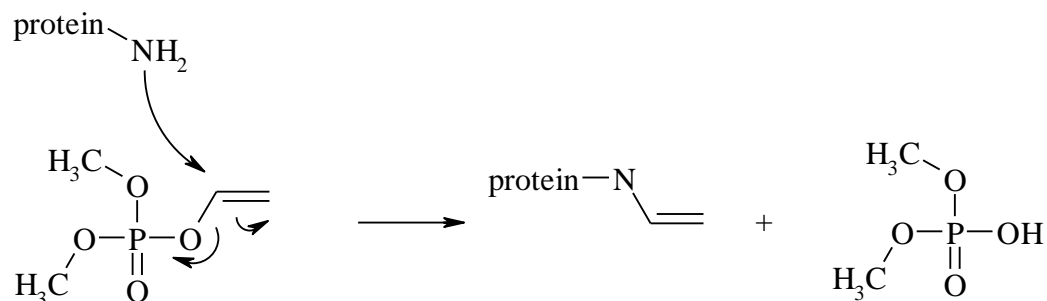


Figure 1: S<sub>N</sub>2 (S<sub>N</sub>Vinyl) mechanism

#### Category mitigating factors

- No mitigating factors have been reported for any of the mechanistic classes covered by this category

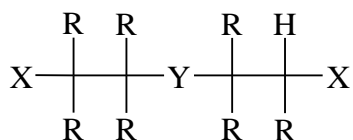
#### References

Lei M-Y et al (2009) Tetrahedron, 65, p6888

## Mechanistic Alert: Episulfonium Ion Formation

Several chemical classes have been shown to form reactive episulfonium ions resulting in reactive species capable of undergoing  $S_N2$  reaction resulting in protein adduct formation. The structural alerts covered by this mechanistic alert are as follows:

### Structural alert: Mustards



Y = nitrogen, sulphur (any oxidation state of sulphur is allowed as long as a lone pair remains free for the cyclisation reaction)

X = Cl, Br, I

R = any carbon, hydrogen

### *Mechanism*

Mustards have been suggested to undergo an intra-molecular cyclisation to form an electrophilic reactive episulfonium ion. The episulfonium ion is then susceptible to  $S_N2$  attack by biological nucleophiles (Noll et al 2006, Smith et al 1995, Hermens 1990).

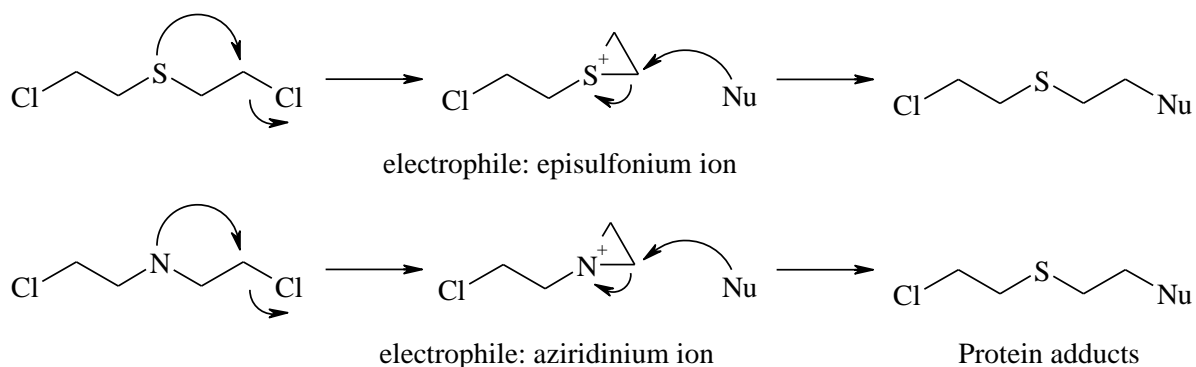
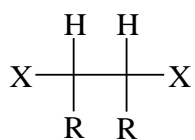


Figure 1: Cyclisation and subsequent  $S_N2$  mechanism for mustards (Nu = biological nucleophile).

### Structural alert: 1,2-Dihaloalkane

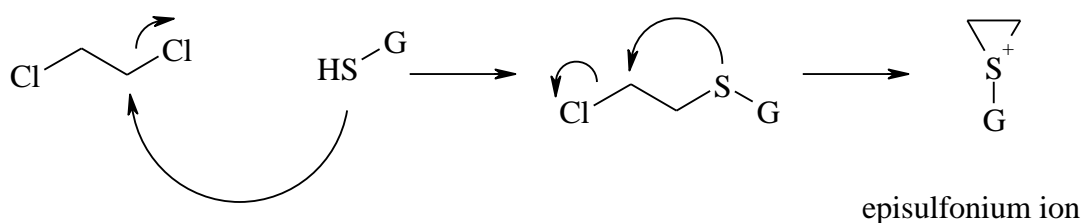


X = Cl, Br, I

R = hydrogen, any carbon

### *Mechanism*

It has been suggested that 1,2-dihaloalkanes undergo an initial attack by glutathione followed by internal cyclisation resulting in the formation of a reactive episulfonium ion. This ion can then undergo an S<sub>N</sub>2 type ring opening reaction (Granville et al 2005).



### Category mitigating factors

- Fluorine is excluded for both mechanistic classes within this category due to the strength of the C-F bond

### References

Granville CA et al (2005) *Mutation Research*, 572, 98-112

Hermens JLM et al (1990) *Environmental Health Perspectives*, 878, p219

Noll DM et al (2006) *Chemical Reviews*, 106, 277-301

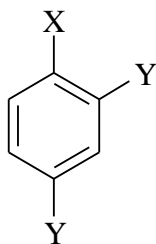
Smith KJ et al (1995) *Journal of the American Academy of Dermatology*, 32, 765-776

# Mechanistic Domain: $S_NAr$

## Mechanistic Alert: S<sub>N</sub>Ar

A number of activated aromatic chemicals have been shown to be capable of covalently binding to proteins via an S<sub>N</sub>Ar mechanism. The structural alerts covered by this mechanistic alert are as follows:

### Structural alert: Activated halo-benzenes



X (leaving group) = F, Cl, Br, I, CN

Y (activating group) = aldehyde, nitro, cyano, halogen, sulfinyl, sulfone, sulfonate, trifluoromethyl

### *Mechanism*

A nucleophilic aromatic substitution mechanism (S<sub>N</sub>Ar) has been proposed for chemicals of this type (Aptula et al 2006, Enoch et al 2008, Gerner et al 2004, Roberts et al 2007).

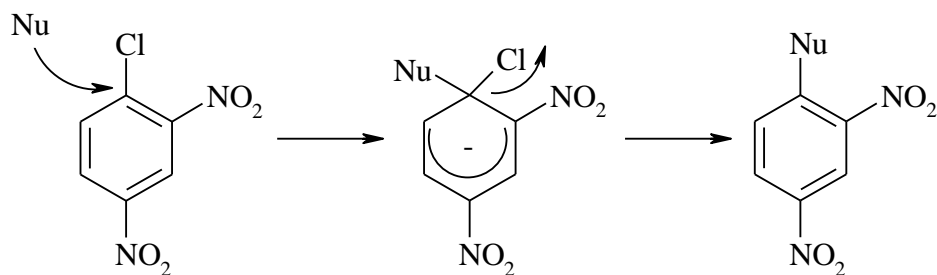
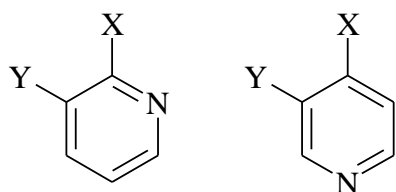


Figure 1: S<sub>N</sub>Ar mechanism for activated halo-benzenes (Nu = biological nucleophile e.g. cysteine or lysine)

### Structural alert: Activated halo-pyridines



X (leaving group) = F, Cl, Br, I, CN

Y (activating group) = aldehyde, nitro, cyano, halogen, sulfinyl, sulfone, sulfonate, trifluoromethyl

### *Mechanism*

A nucleophilic aromatic substitution mechanism ( $S_NAr$ ) has been proposed for chemicals of this type (Aptula et al 2006, Gerner et al 2004, Hulzebos et al 2005).

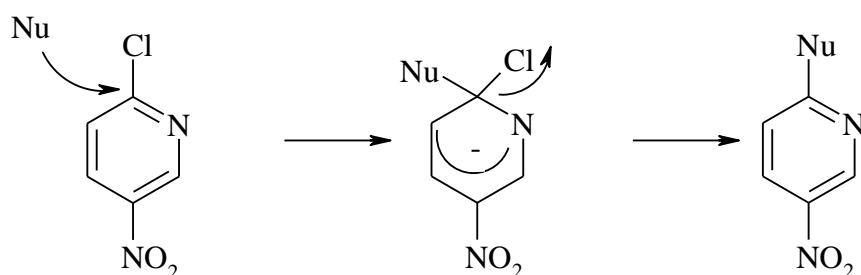
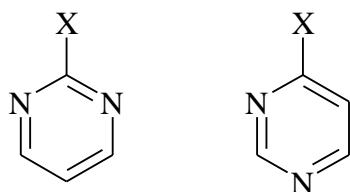


Figure 1:  $S_NAr$  mechanism for activated halo-pyridine derivatives (Nu = biological nucleophile e.g. cysteine or lysine)

### Structural alert: Halo-pyrimidines



X (leaving group) = F, Cl, Br, I, CN

### *Mechanism*

A nucleophilic aromatic substitution mechanism ( $S_NAr$ ) has been proposed for chemicals of this type (Aptula et al 2006, Gerner et al 2004, Hulzebos et al 2005).

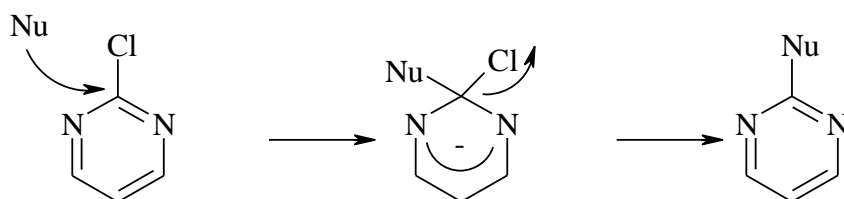
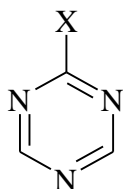


Figure 1:  $S_NAr$  mechanism for halo-pyrimidine derivatives (Nu = biological nucleophile e.g. cysteine or lysine)

### Structural alert: Halo-triazines



X (leaving group) = F, Cl, Br, I, CN

### *Mechanism*

A nucleophilic aromatic substitution mechanism ( $S_NAr$ ) has been proposed for chemicals of this type (Aptula et al 2006, Gerner et al 2004, Hulzebos et al 2005, Roberts et al 2007).

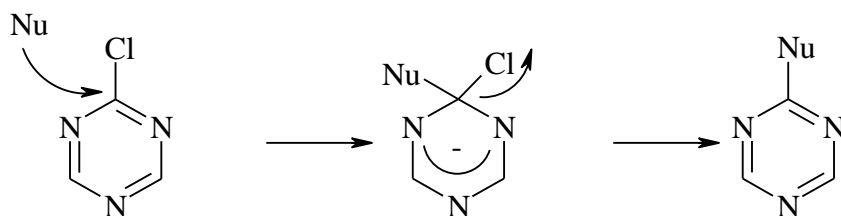


Figure 1:  $S_NAr$  mechanism for halo-triazine derivatives (Nu = biological nucleophile e.g. cysteine or lysine)

### Category mitigating factors

- No mitigating factors have been identified for any of the mechanistic classes within this category

### References

Aptula AO et al (2006) Chemical Research in Toxicology, 19, p1097

Enoch SJ et al (2009) Chemical Research in Toxicology, 22, p1447

Gerner I et al (2004) ATLA, 32, p487

Hulzebos et al (2005) QSAR and Combinatorial Science, 24, p332

Robert DW et al (2007) Chemical Research in Toxicology, 20, p1019