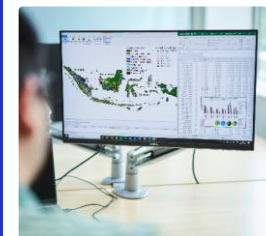
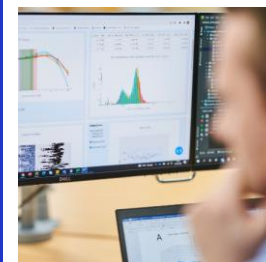


Updating reactivity profiling: reactivity of nucleophilic sensitisers with a model disulphide

**Suzanne Martin, Becky Lewis, Sandrine Spriggs, Richard Cubberley,
Nora Aptula, Maja Aleksic**

SERS

Safety, Environmental
& Regulatory Science



Nucleophilic sensitisers

**Chemical
Research in
Toxicology**

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Perspective

Updating Reaction Mechanistic Domains for Skin Sensitization: 1. Nucleophilic Skin Sensitizers

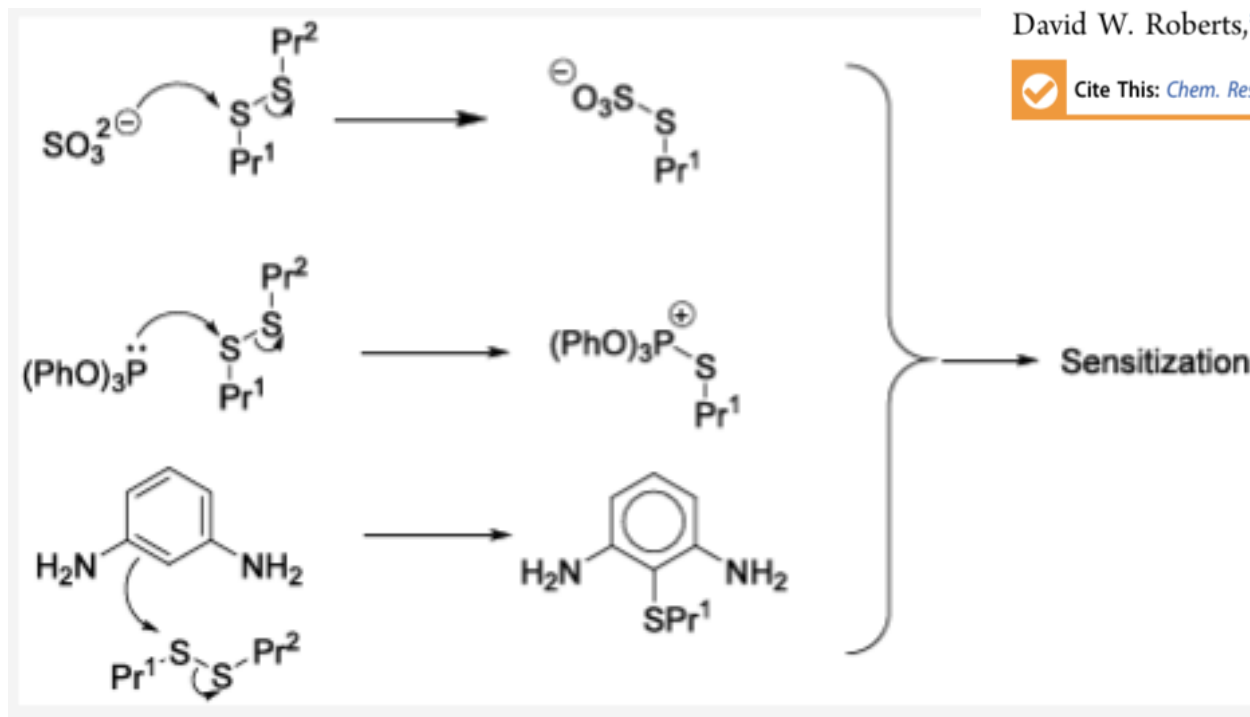
David W. Roberts,* Anne Marie Api, Aynur Aptula, Isabelle Lee, and Holger Moustakas



Cite This: *Chem. Res. Toxicol.* 2024, 37, 1757–1768



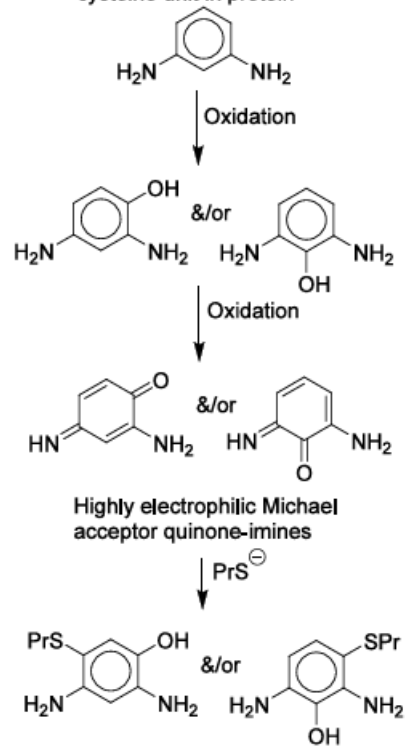
Read Online



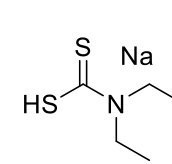
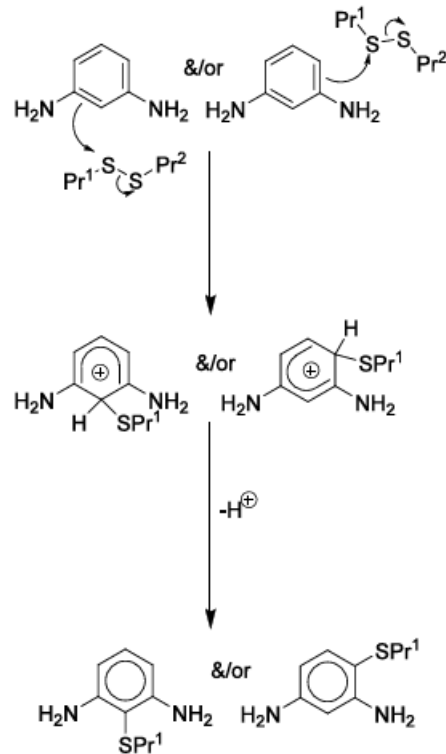
- Nucleophilic centers can be sulphur or phosphorus atom or aromatic carbon of a sensitiser
- Electrophile is the S-S bridge on proteins

Nucleophilic sensitisers (cont.)

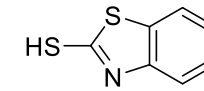
Prohaptten mechanism via oxidative hydroxylation, further oxidation and electrophilic attack on ionised SH group of cysteine unit in protein



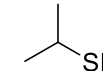
Direct hapttenation via nucleophilic attack on disulfide linkage in protein



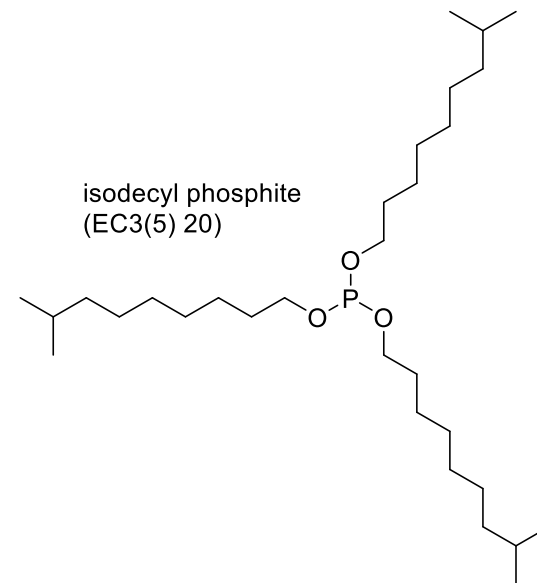
sodium diethyldithiocarbamate
(EC3(%) 1.66)



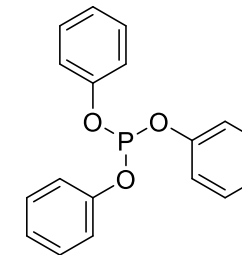
2-mercaptobenzothiazole
(EC3(%) 4.27)



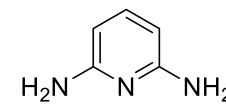
isopropyl mercaptan
(EC3(%) 75.5)



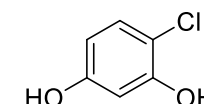
isodecyl phosphite
(EC3(5) 20)



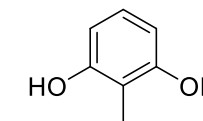
triphenyl phosphite
(EC3(%) 1.4)



2,6-diamino pyridine
(EC3(%) 0.25)



4-chlororesorcinol
(EC3(%) 5.8)



2-methyl-1,3-benzenediol
(EC3(%) 50)

Reactivity Profiling

Peptide design

AcFAA **C**AA
 AcFAA **K**AA
 AcFAA **H**AA
 AcFAA **R**AA
 AcFAA **Y**AA
NH₂FAAAAA
 AcFAAAAA (-ve control)
 AcFAGAGA (int std)

adduct formation/peptide depletion

0.5mM peptide (50µL of 2.5mM stock)
 +
 50mM chemical (100 µL of 125mM stock)
 +
 Buffer (90µL) + 0.1mM IS (10µL of 2.5mM stock)
 ↓
 24 hours incubation
 ↓
 LC/MS/MS

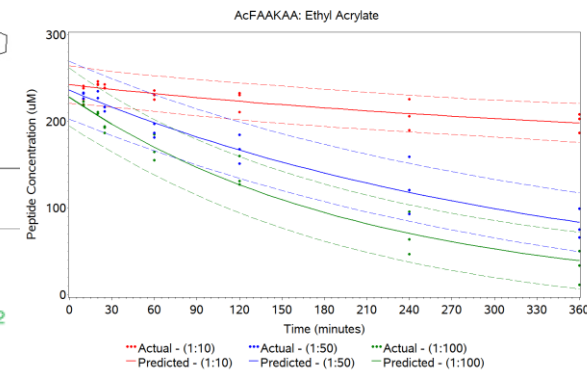
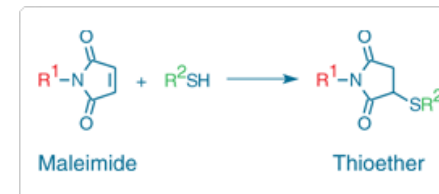
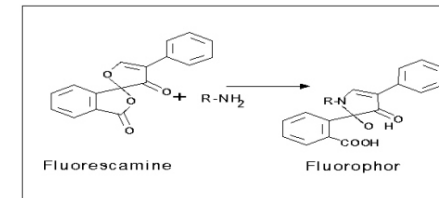
Output

- **Qualitative (adduct formation) – confirmation of reactivity; reaction mechanism(s)**
- **Quantitative (peptide depletion/reaction kinetics) – comparison with DPRA; reaction rate**

Experimental Procedure

reaction kinetics

Adapted a standard spectrophotometric protein quantification protocols for our needs using fluorescence readout (AcFAAKAA – fluorescamine; AcFAACAA – maleimide)



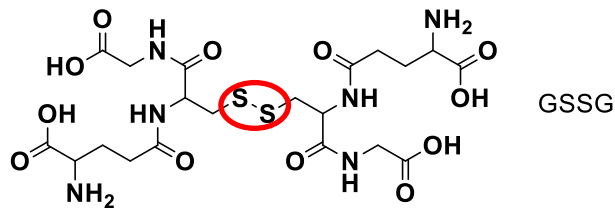
Updating reactivity profiling – method adaptation

Nucleophilic targets

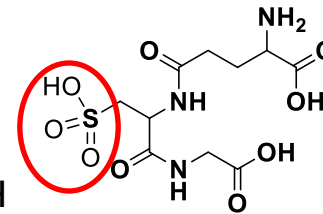


New signals observed with Cys peptide for m-phenylenediamine, 5-amino-2-methylphenol and 1-(2,4-Dihydroxyphenyl)-3-phenylprop-2-en-1-one) however not easily explained

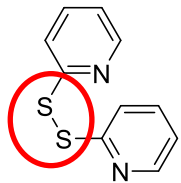
Electrophilic targets



No obvious adducts, noted m/z 356, which is tentatively heavily oxidised GSH



GSH sulphonate
m/z 356.08

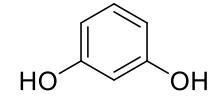


1,2-di(pyridin-2-yl)disulfane

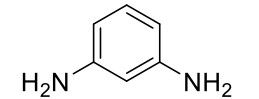


All chemicals showed new signals that could be explained by a nucleophilic sensitiser mechanism

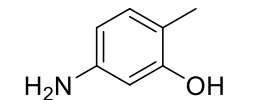
Test chemicals



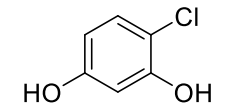
resorcinol
(EC3(%) 3.45)



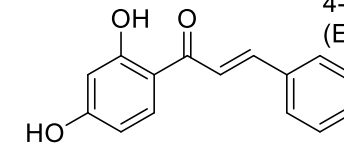
m-phenylenediamine



5-amino-2-methylphenol
EC3(%) 0.44



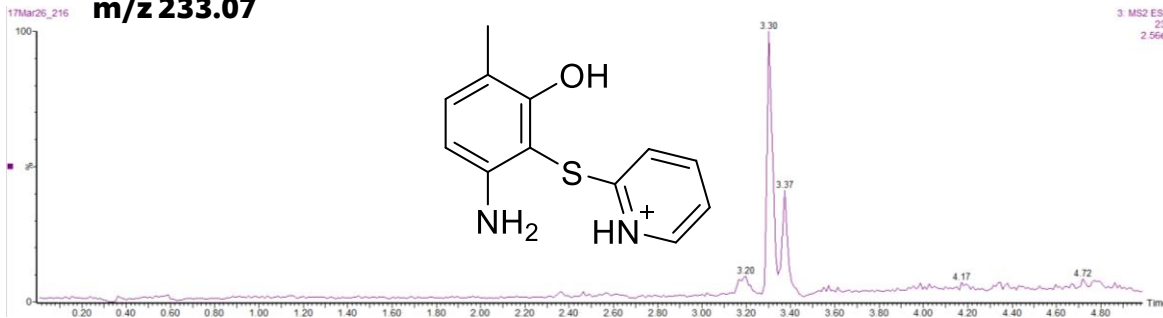
4-chlororesorcinol
(EC3(%) 5.8)



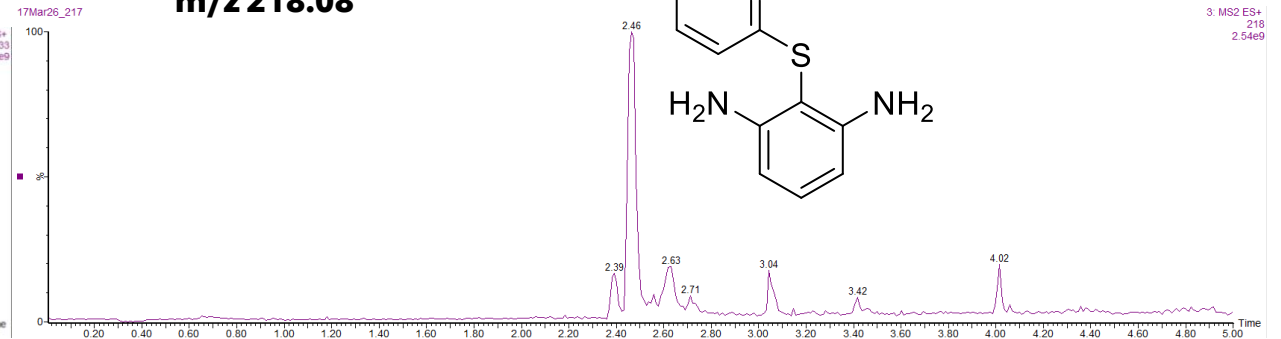
1-(2,4-Dihydroxyphenyl)-3-phenylprop-2-en-1-one

Plausible adduct structures observed

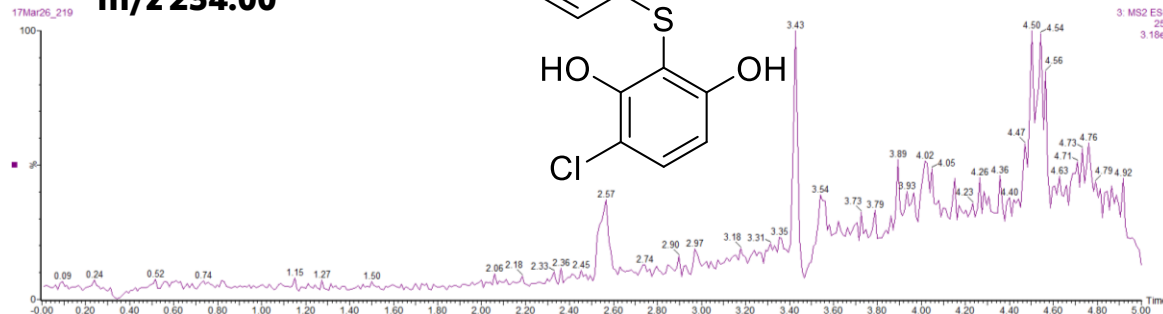
5-amino-2-methylphenol
m/z 233.07



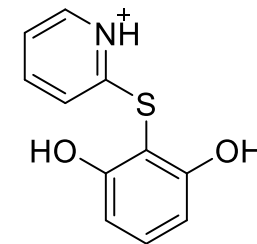
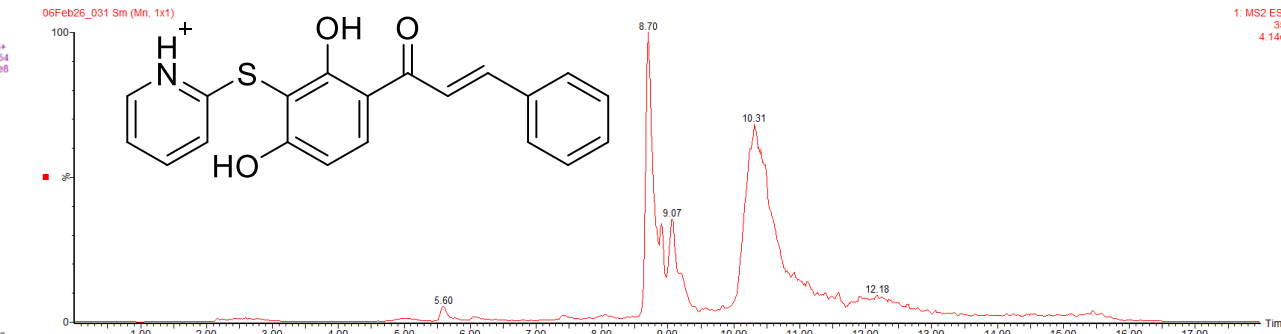
m-phenylenediamine
m/z 218.08



4-chlororesorcinol
m/z 254.00



1-(2,4-Dihydroxyphenyl)-3-phenylprop-2-en-1-one
m/z 350.09



resorcinol
m/z 220.04

Next steps

- **Expansion of the nucleophilic chemical domain**

Broaden chemical space across S-, N- and C-nucleophiles
Include sensitisers with known NAMs data

- **Quantification of nucleophilic reactivity**

Derive peptide depletion and reaction rate parameters
Establish concentration - response and kinetic descriptors

- **Integration with NAM performance and modelling**

Explore relationships between SERS' and published GARDskin Dose-Response nucleophilic reactivity
Support development of mechanistically informed predictive models

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Thank You



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