Drug Metabolism Tools to Assess Bioactivation Potential of Drug Candidates - minimizing metabolism-related DILI or genotoxicity

(评价候选药物的代谢物激活风险的药物代谢模型 --- 尽可能降低药物导致的肝损伤和基因毒性)

Zhoupeng Zhang
(张洲鹏)

Dept. of Pharmacokinetics, Pharmacodynamics and Drug Metabolism
Merck Sharp & Dohme Corp., West Point, PA19486, USA

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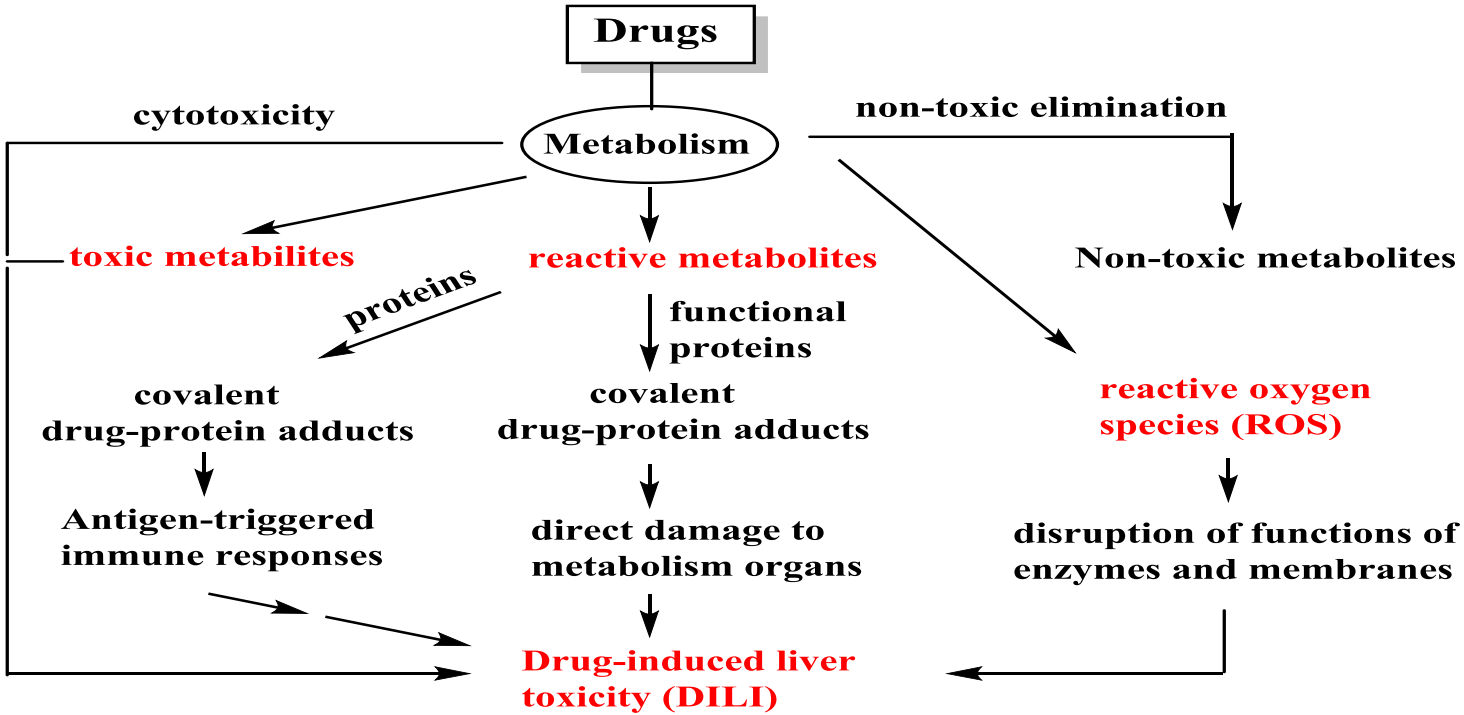
Outlines

- **Background for bioactivation/metabolic activation**
- **Available metabolism assays to assess potential mechanism of bioactivation**
- **Approaches to minimize the bioactivation potential of drug candidates (case studies)**
- **Summary**

Drug-induced liver injury (DILI)

- A rare but potentially serious idiosyncratic adverse drug reaction associated with treatment of certain drugs

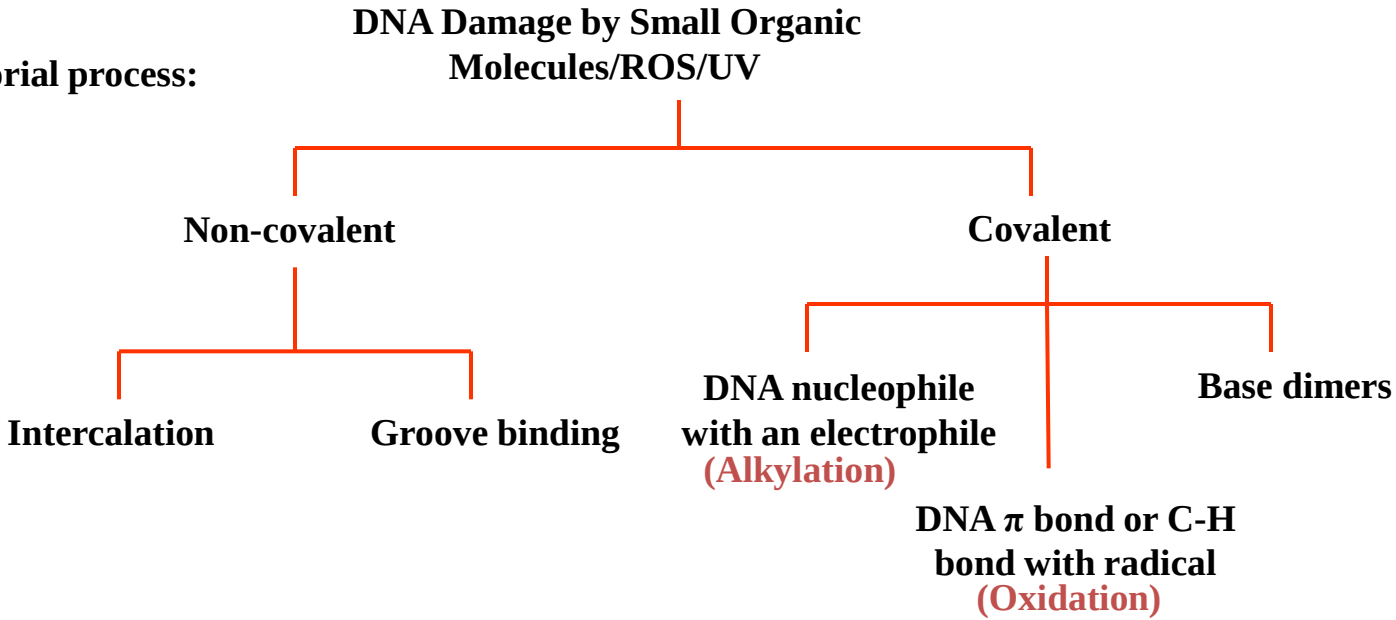


- Bioactivation is only ONE of the several possible causes for DILI

Genotoxicity

- **The property of chemical agents that damages the genetic information within a cell causing mutations, which may lead to cancer.**

A multifactorial process:



- **Metabolism is only ONE of the causes for genotoxicity.**
- **Drug-induced liver injury and genotoxcity may or may not share the same mechanism.**

Available assays for assessing bioactivation potential

- **Metabolite Identification studies**
  - **Potential reactive intermediate-related metabolites, toxic metabolites**
  - **Adducts of GSH, N-acetylcysteine, amino acids, drug-proteins, etc.**
  - **In vitro trapping studies (adducts of GSH, cyanide, SCB, DNA bases, DNAs, etc.)**
- **Covalent protein binding studies using**
  - **in vitro in liver microsomes or hepatocytes ([^{3}H] or [^{14}C])**
  - **in vivo in rats ([^{3}H] or [^{14}C])**
  - **SDS-PAGE analysis of drug-protein adducts ([^{3}H] or [^{14}C])**
  - **LC/MS analysis of drug-amino acid adducts**
- **Studies for potential oxidative stress:**
  - **Peroxide formation**
  - **Depletion of intracellular GSH in hepatocytes**
- **Others**
  - **Comet assay (DNA damages)**

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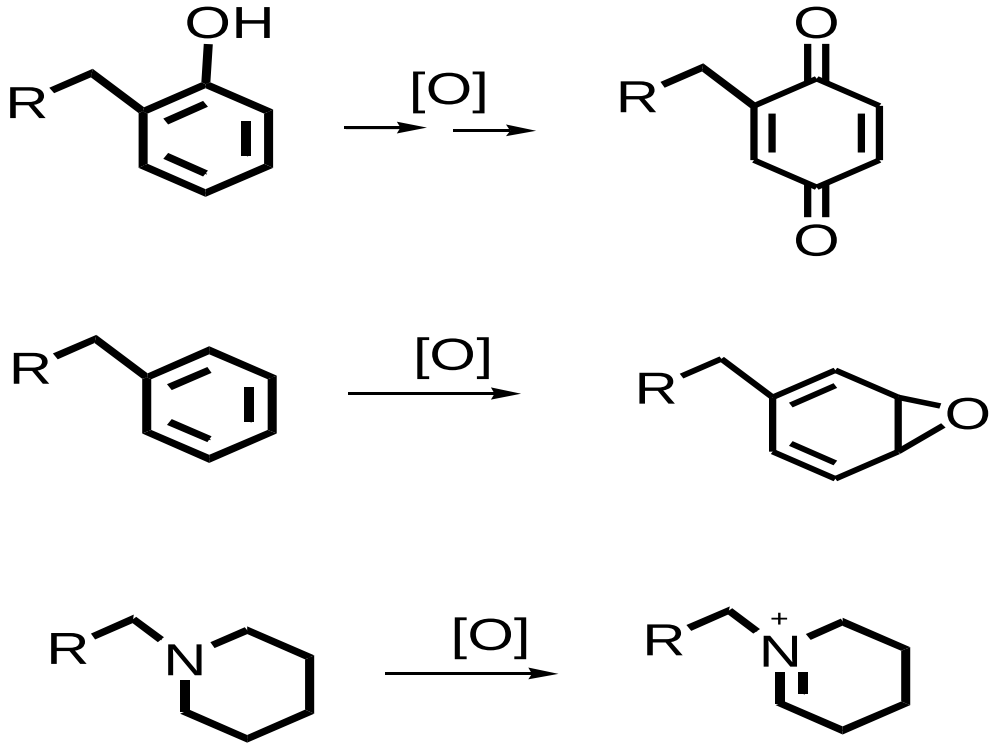
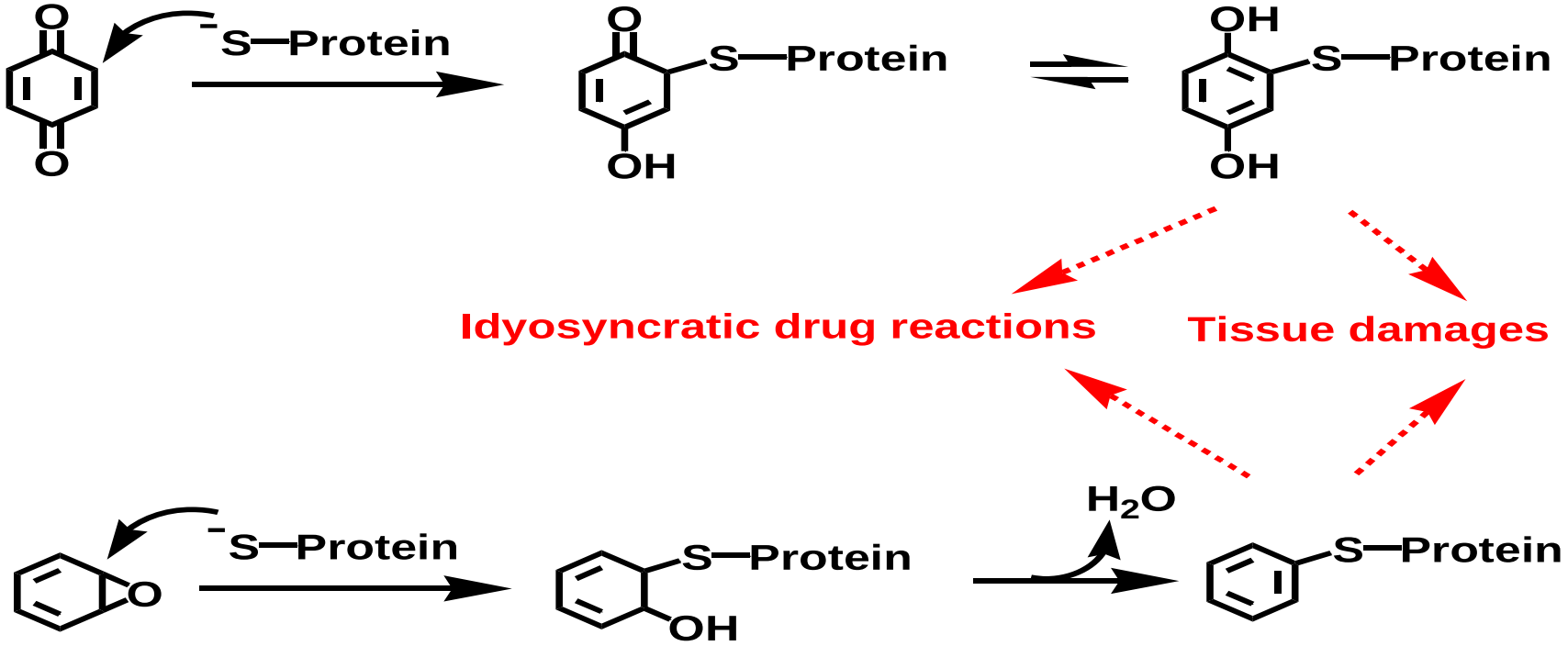
Metabolism of drugs to reactive metabolites

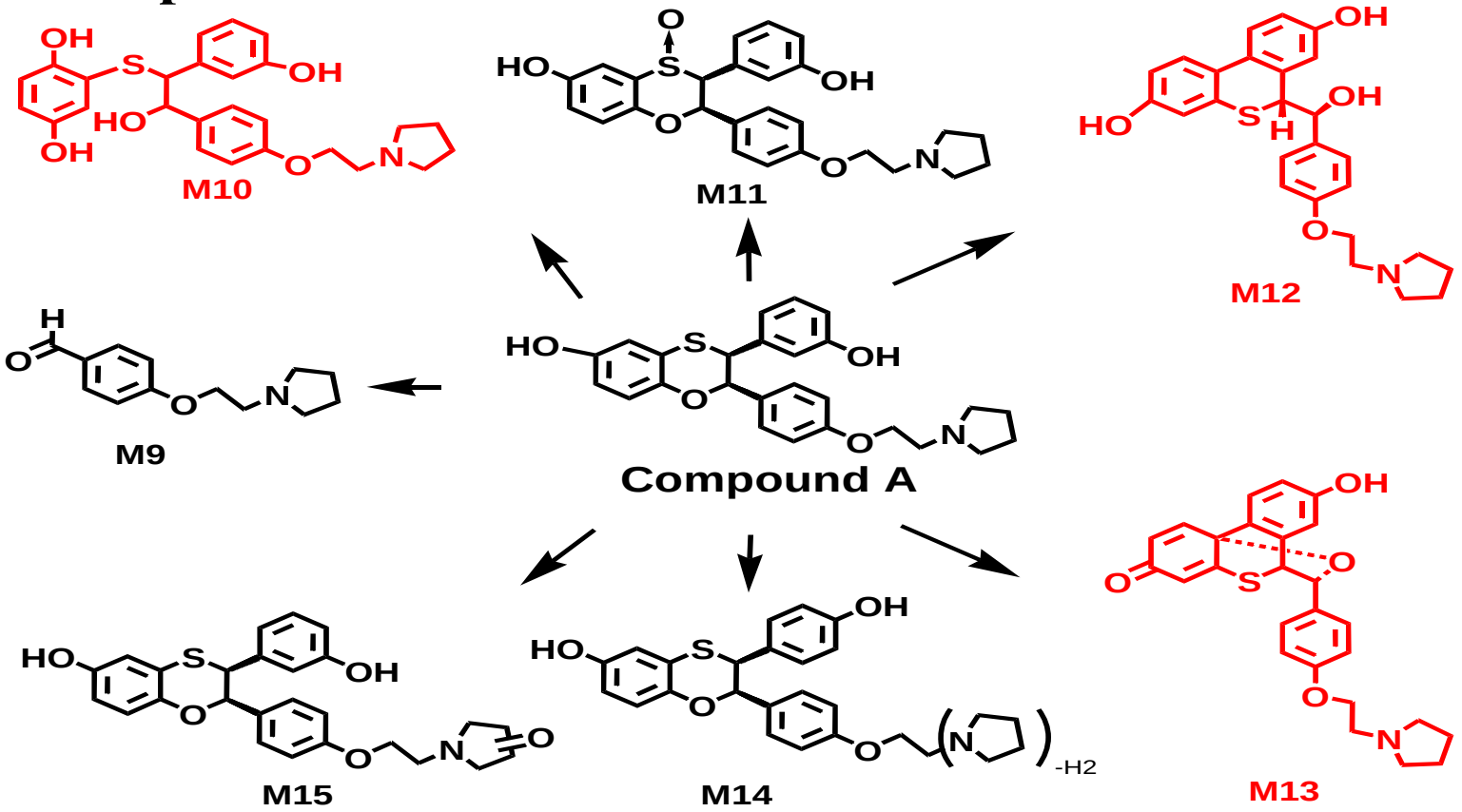


Formation of drug-protein adducts



- Direct correlation between bioactivation (protein adduct formation) and toxicity is not clear.
- Some toxicity findings were thought to be related to bioactivation of drug molecules.

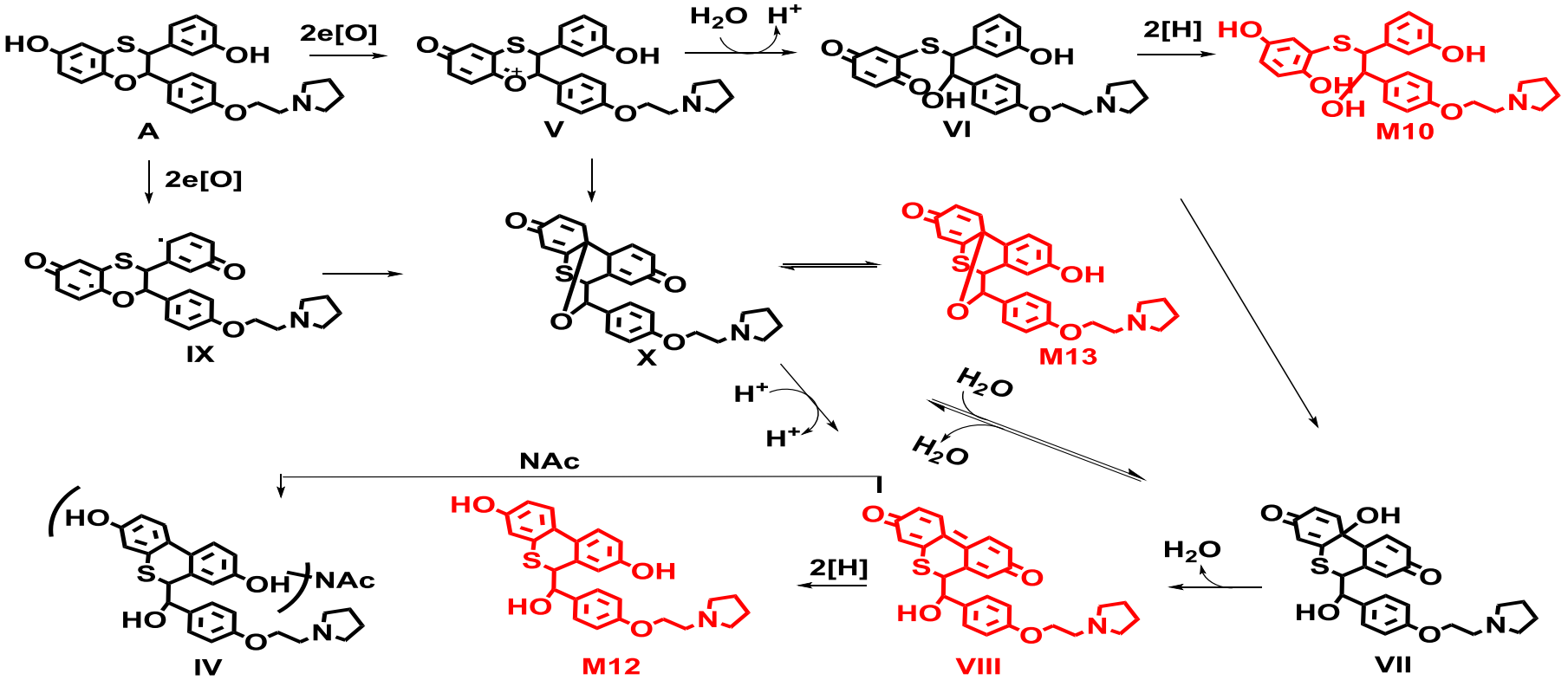
Formation of potential reactive metabolites in incubations of compound A with rat and human liver microsomes



- Three potential reactive metabolite-related species were detected.

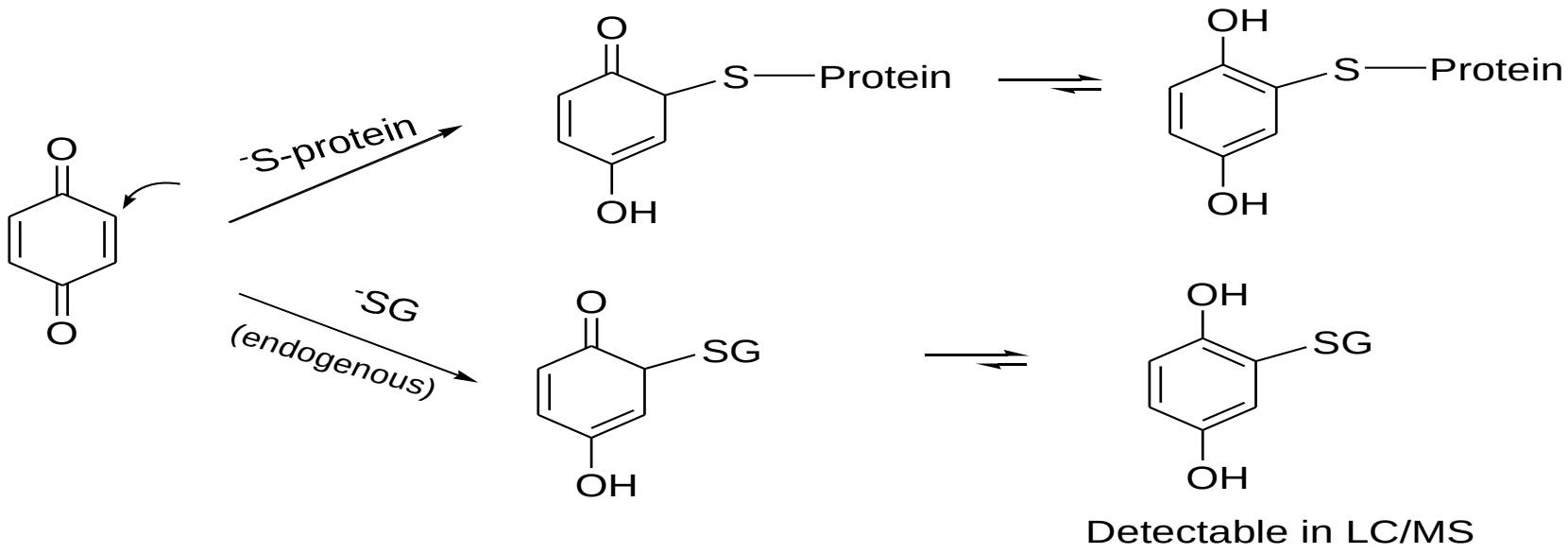
(Z. Zhang, *Chem. Res. Toxicol.* 2005, 18, 675)

CYP3A4-mediated biotransformation of compound A



(Z. Zhang, *Chem. Res. Toxicol.* 2005, 18, 675)

Formation of drug-protein and drug-GSH adducts

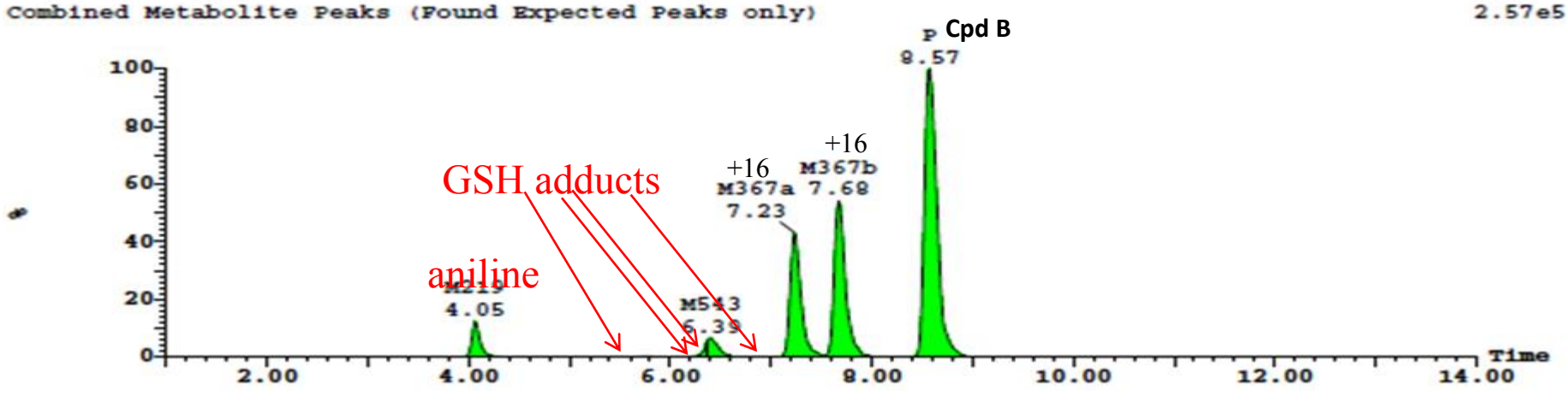


Detection of GSH adducts in liver extract of rats dosed with compound B

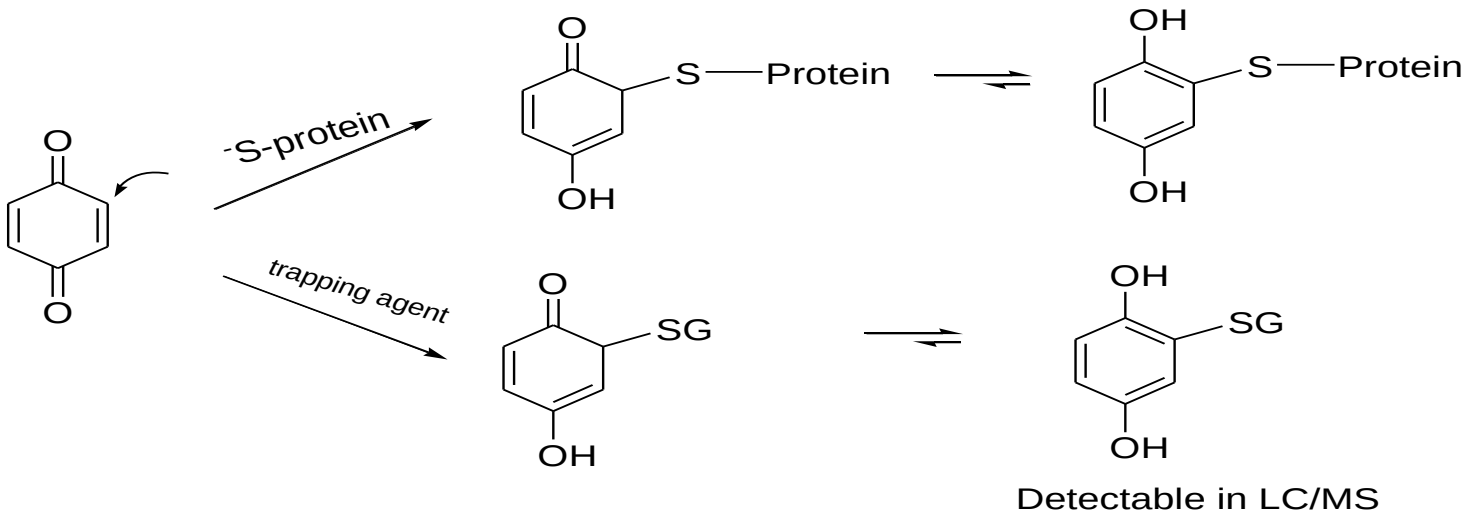
Expected Metabolites:

Label	Metabolite Name	Formula	Time	m/z Found	mDa	PPM	Area Abs		Area %
P	Parent	C16H13F4N5	8.57	352.1135	-5.0	-14.3	36404.90	49.48	(15.59)
M367b	+O	C16H13F4N5O	7.68	368.1105	-2.9	-8.0	18409.60	25.02	(7.88)
M367a	+O	C16H13F4N5O	7.23	368.1106	-2.8	-7.7	13323.50	18.11	(5.70)
M219	Parent-C6H4N4 (R_2)	C10H9F4N	4.05	220.0765	1.6	7.2	2862.70	3.89	(1.23)
M543	+GSH+O	C22H21F4N5O7	6.39	544.1458	0.3	0.5	2255.50	3.07	(0.97)
M658	+GSH-2H	C26H28F4N8O6S	6.36	657.1866	-0.1	-0.1	113.50	0.15	(0.05)
M527	+GSH+O-C6H5N5 (R_3)	C20H25F4N3O7S	5.76	528.1436	0.9	1.6	100.30	0.14	(0.04)
M525	+GSH-2H+O-C6H5N5 (R_3)	C20H23F4N3O7S	6.24	526.1241	-3.0	-5.7	86.30	0.12	(0.04)
M672	+GSH-2H+O	C26H28F4N8O7S	6.11	673.1770	-4.6	-6.8	23.40	0.03	(0.01)

Total metabolites: 9



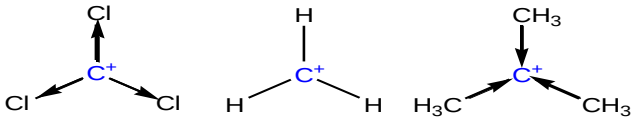
In vitro trapping studies



“Hard” vs. “soft” electrophiles/nucleophiles

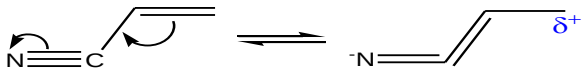
- Chemical “hardness” and “softness” is a function of polarization.

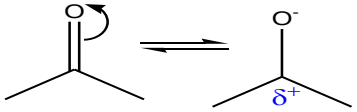
- Hard electrophiles have high positive charge density at the electrophilic center (the charge is localized, ex. carbocations)



Harder----------Softer
(EWG) (EDG)

- Soft electrophiles have low positive charge density at the electrophilic center (the charge is delocalized) – often as a result of diffuse electron density of π bond





- Hard nucleophiles have high negative charge density (not diffuse and localized charge)

- Soft nucleophiles have less negative charge density (more diffuse and delocalized charge)

“Hard” and “soft” electrophiles and nucleophiles

Electrophiles

Aldehydes, polarized double bonds

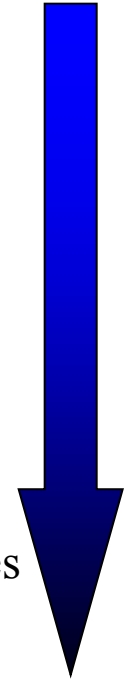
Epoxides, strained ring lactones, alkyl sulfates, alkyl halides

Arylcarbocations

Benzylic carbocations, nitrenium ions

Alkylcarbocations, iminium ions, imines

softest



hardest

Nucleophiles

Thiol groups in cysteinyl protein residues and glutathione

Sulfur atoms of methinoyl protein residues

Primary and secondary amino protein residues (arginine, lysine and histidine)

Amino groups of purine bases (RNA & DNA)

Phosphate oxygen of RNA and DNA

Cyanide

- Hard electrophiles react with hard nucleophiles
- Soft electrophiles react with soft nucleophiles

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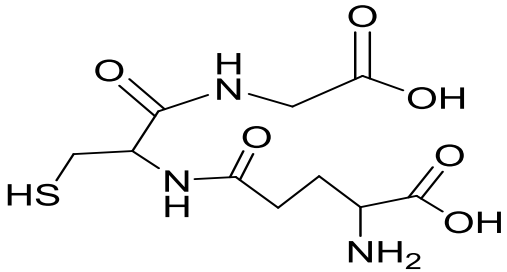
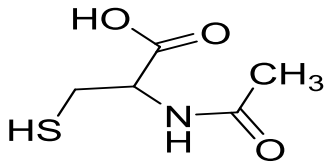
Common *in vitro* trapping agents

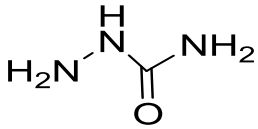


Glutathinone (GSH)

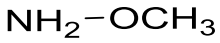


N-acetylcysteine (NAc)

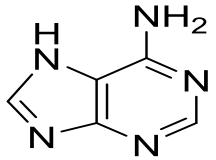
Cyanide



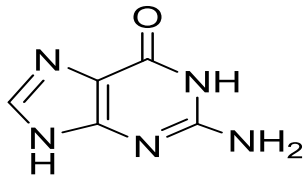
Semicarbazide



Hydroxyamine

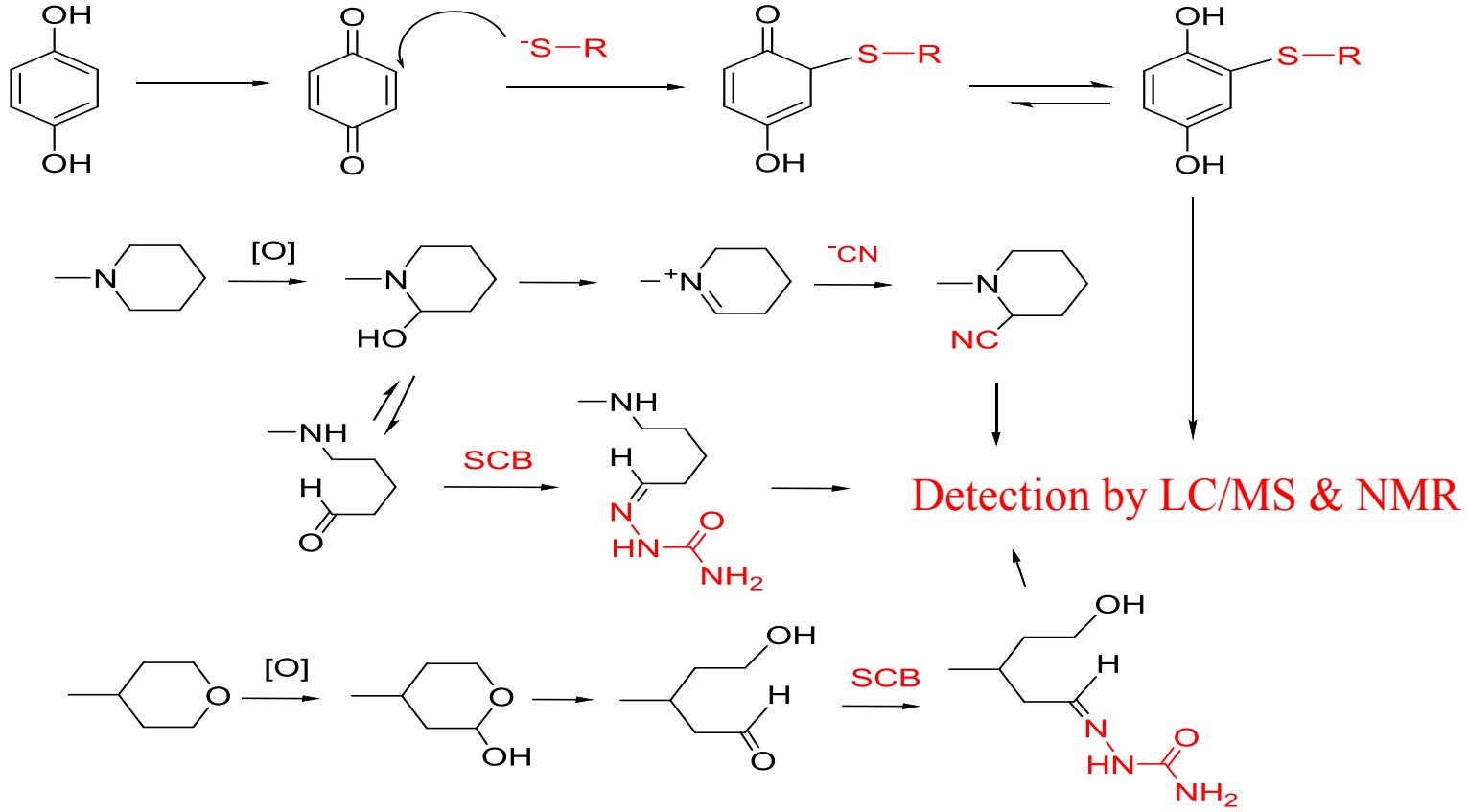


Adenine



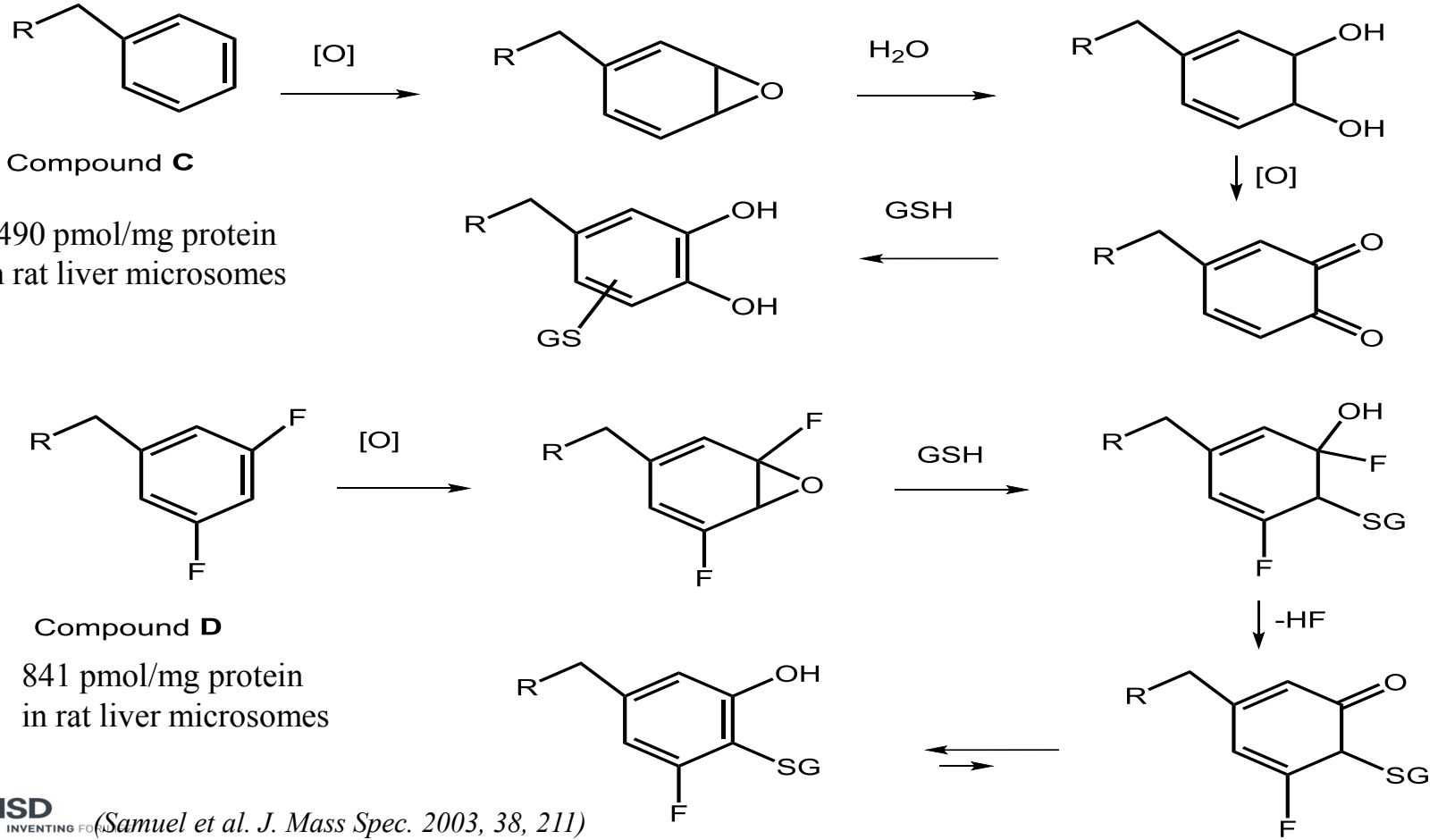
Guanine

In vitro trapping



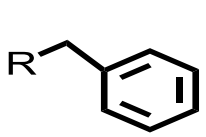
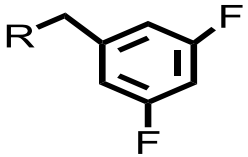
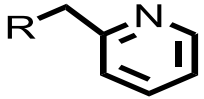
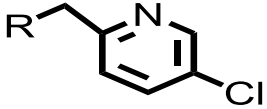
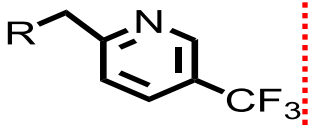
- **Select appropriate trapping agents based on structures of compounds and SAR**

In vitro trapping and in vitro covalent protein binding



(Samuel et al. J. Mass Spec. 2003, 38, 211)

Covalent Protein Binding of [^{3}H]Compounds in Liver Microsomes of Rats (RLM) and human (HLM) (pmol/mg protein)

	 Compound **C**	 Compound **D**	 Compound **E**	 Compound **F**	 Compound **G**
RLM	**1490**	**841**	**535**	**190**	**111**
HLM	**3870**	**1690**	**911**	**303**	**88**

(Samuel et al. J. Mass Spec. 2003, 38, 211)

In vivo covalent protein binding of [^{3}H]A in rats*

Tissues	Covalent binding (pmol/mg protein)			Drug concentration (μM)		
	2 h	6 h	24 h	2 h	6 h	24 h
Plasma	0.4 ± 0.6	0	0.6 ± 2.4	1.8	1.1	0.1
Liver	2.8 ± 1.7	6.7 ± 2.8	8.2 ± 0.7	7.0	3.4	0.1
Kidney	3.2 ± 2.5	5.1 ± 4.0	1.1 ± 1.0	10.5	6.6	0.3

*: Male Sprague-Dawley rats were dosed orally with [^{3}H]A at 10 mg/kg (N = 3 each time point).

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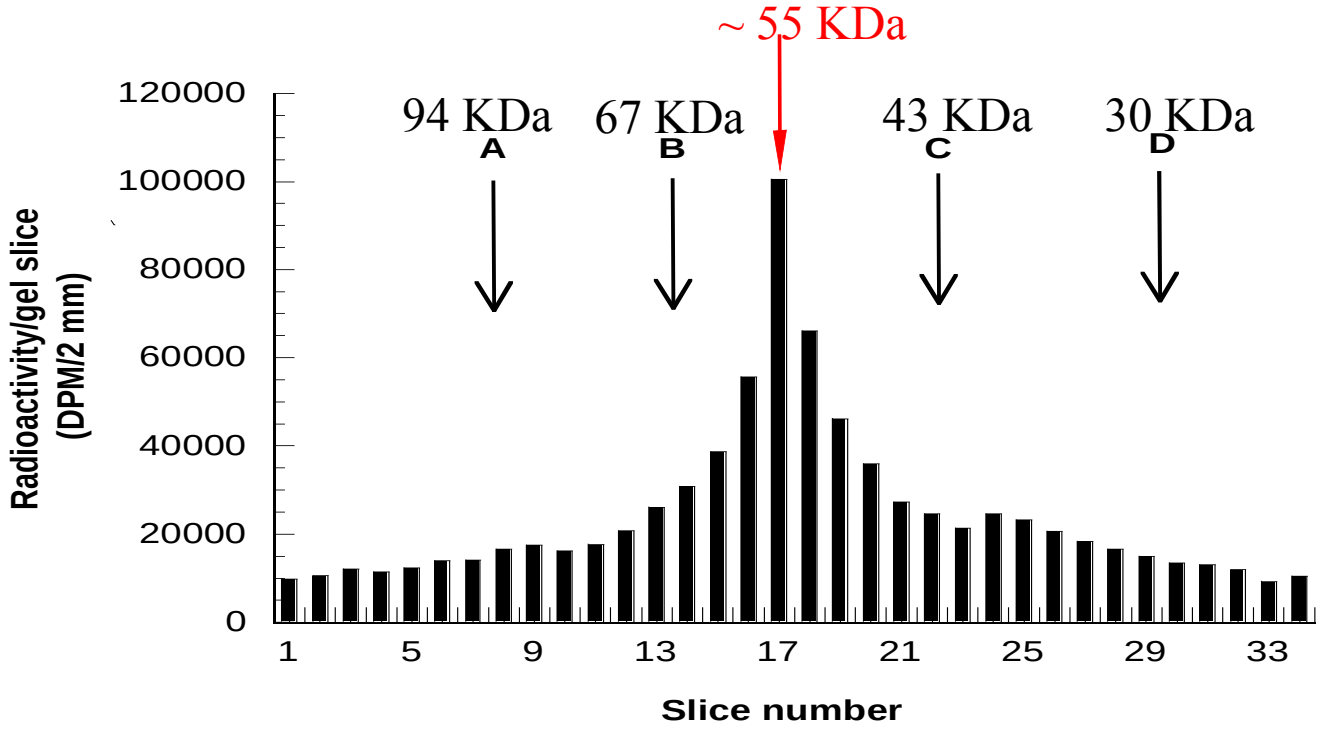
(Zhang, et al., Chem. Res. Toxicol. 2005, 18, 675)

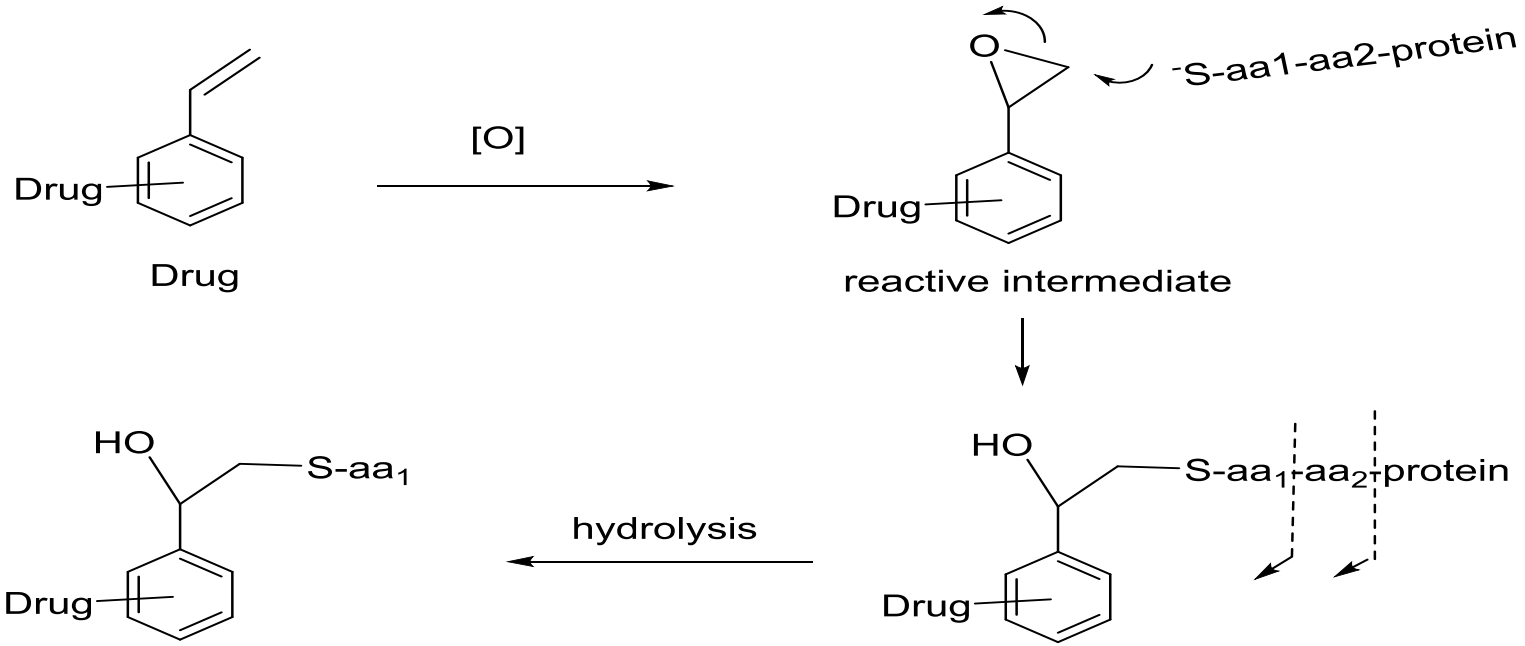
SDS-PAGE analysis of drug-protein adducts
- covalent binding of [^{3}H]A to proteins in human liver microsomes



- [^{3}H]A covalently bound to proteins with MW ~ 55KDa.

(Zhang, et al., Chem. Res. Toxicol. 2005, 18, 675)

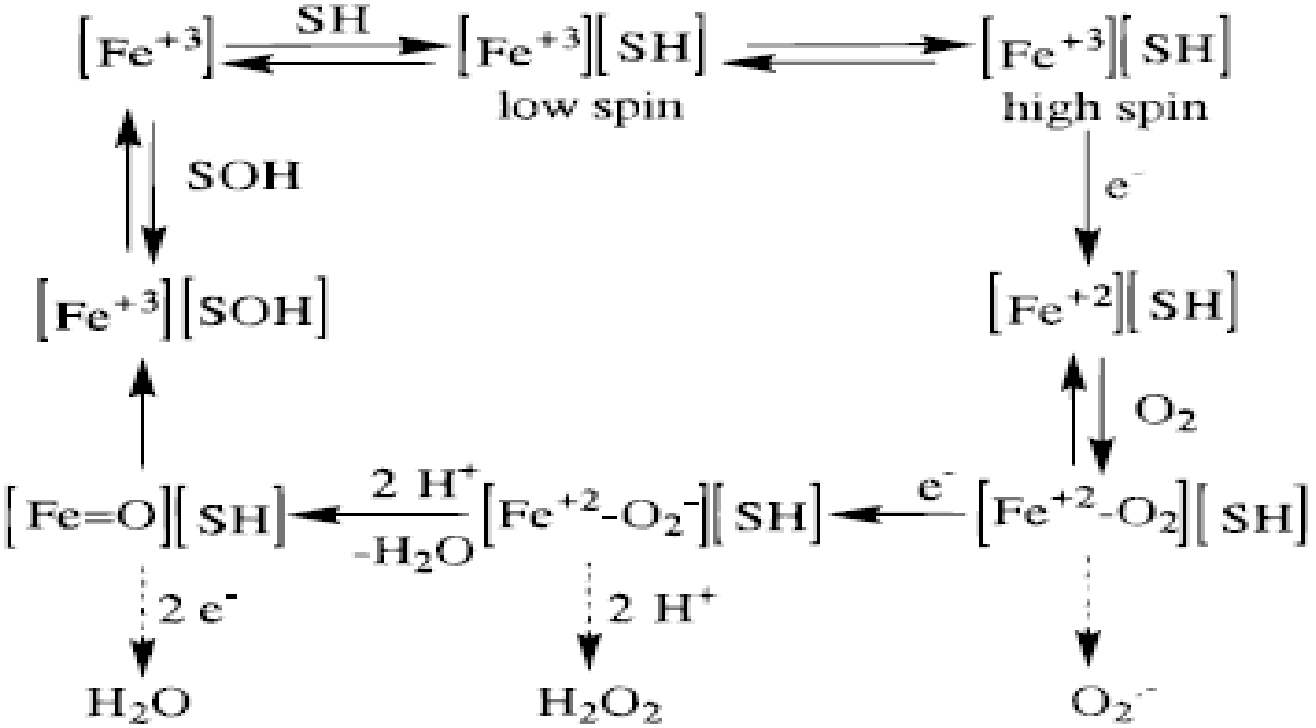
LC/MS analysis of drug-amino acid adducts



Drug-amino acid adducts
(detected by LC/MS)

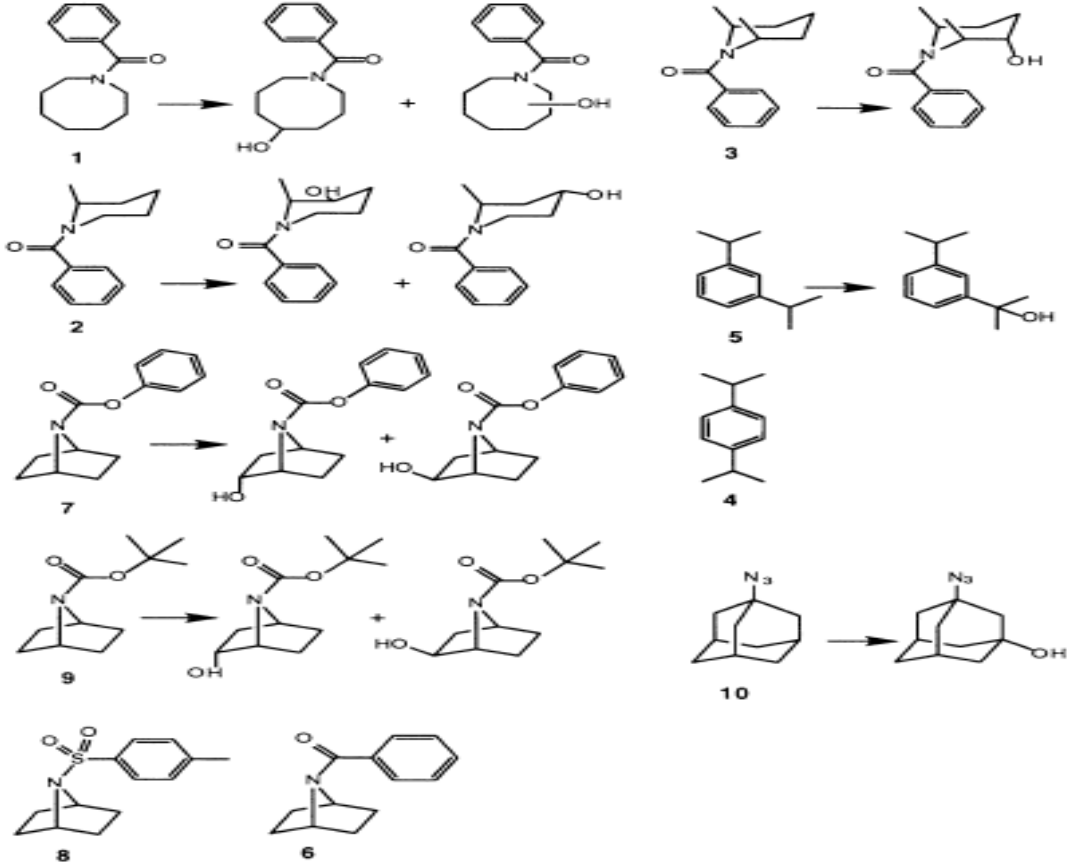
Drug-protein adducts

Studies for potential oxidative stress - formation of peroxides



- Highly uncoupled P450-medicated oxidations lead to formation of H_2O_2 and superoxide species.
- Some other enzymes, e.g, peroxidase can also cause oxidative damage in host tissues.

The substrate specificity of cytochrome P450cam



Z. Zhang et al./Bioorg. Med. Chem. 6 (1998) 1501–1508

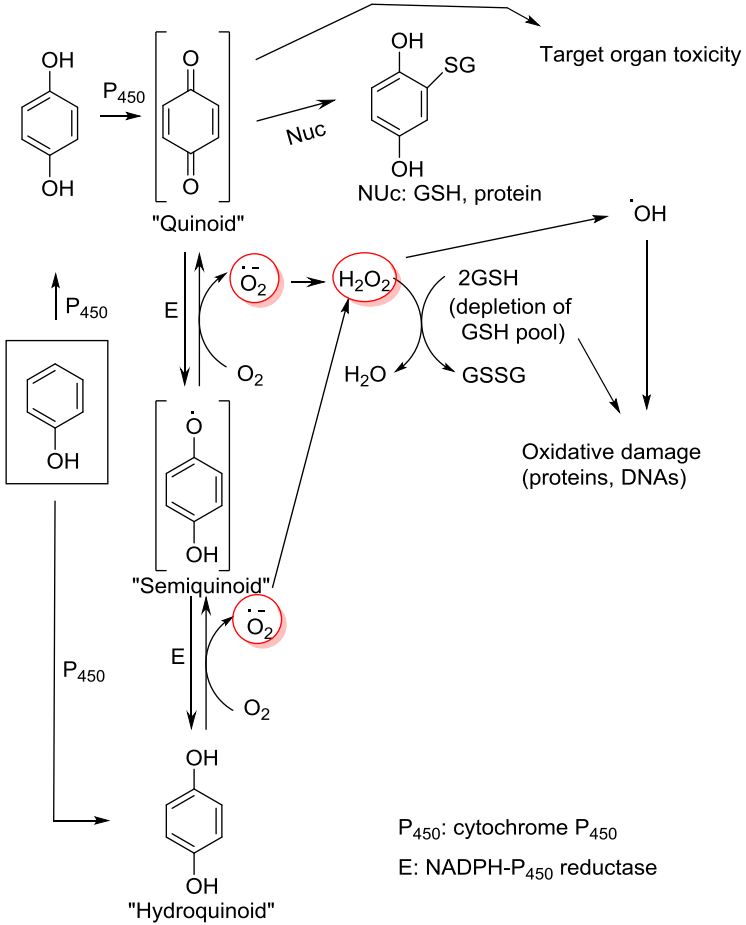
Table 1. Parameters for the interaction of DOCK-predicted substrates with wild-type $P450_{cam}$

Compd	K_s	Spin state change	NADH Used[a]	O_2 Used	H_2O_2 Formed	Organic products	Product formation[b]
	μM	%	nmol·min^{-1} nmol^{-1}	nmol·min^{-1} nmol^{-1}	%		%
Camphor	1.1	100	262	250	2	+	96
1	2.8	59	26	32	18	+	55
2	16.0	69	45	40	65	+	31
3	3.0	57	41	33	38	+	94
4	NA	<4	6	9	100	–	<1
5	1.4	32	8	8	100	+	1
6	0.4	59	32	28	58	+	37
7	2.1	36	37	26	39	+	4
8	0.9	24	ND[c]	ND	ND	ND	ND
9	4.5	33	14	8	72	+	1
10	0.6	78	49	31	25	+	20

[a]Background NADH consumption is 4–6 nmol · min^{-1} nmol^{-1}.
[b]The turnover is the ratio of the area of the organic product peak divided by the area of the starting material plus organic product after both have been normalized versus the internal standard peak times ×100.
[c]Compound **8** precipitated at the substrate concentration (1 mM) utilized to measure catalytic turnover. No product was detected when lower concentrations were used.

- **The H_2O_2 formed from decoupled turnover can oxidize (consume) GSH to GSSG !**

P450-mediated quinoid formation - toxicological implications



H_2O_2 and ROS:

- From decoupled P450 rxns
- From bioactivation

$$2O_2^{\bullet -} + 2H^+ \xrightarrow[\text{dismutase}]{\text{superoxide}} O_2 + H_2O_2$$

Metabolite analysis and characterization

A 1 mL solution containing 1 μM camphor-free $P450_{cam}$, 2 μM putidaredoxin reductase, 8 μM putidaredoxin, 1 μM catalase (to remove any H_2O_2 formed in the reaction), 1 mM test compound (added in 10 μL ethanol), and 5 mM NADH was incubated at 25 °C for 1.5 h.

Catalase catalyzes the following reaction:

$$2\ H_2O_2 \rightarrow 2\ H_2O + O_2$$

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Printed in U.S.A.

Radical Rebound Mechanism in Cytochrome P-450-catalyzed Hydroxylation of the Multifaceted Radical Clocks α- and β-Thujone*

Xiang He and Paul R. Ortiz de Montellano‡

From the Department of Pharmaceutical Chemistry, University of California, San Francisco, California 94143-2280

Enzyme Incubations—Unless otherwise indicated, the buffer was 100 mM KH_2PO_4, pH 7.4. In the case of $P\text{-}450_{cam}$, the incubations contained $P\text{-}450_{cam}$ (0.5 μM), Pd (5 μM), Pd reductase (5 μM), α-thujone (2000 μM), superoxide dismutase (2 μM), and catalase (100 μg/ml) in the reaction buffer. The reaction was initiated by the addition of NADH (2000 μM).

Catalase :

$$2\,H_2O_2 \rightarrow 2\,H_2O + O_2$$

$$2O_2^{\bullet -} + 2H^+ \xrightarrow[\text{dismutase}]{\text{superoxide}} O_2 + H_2O_2$$

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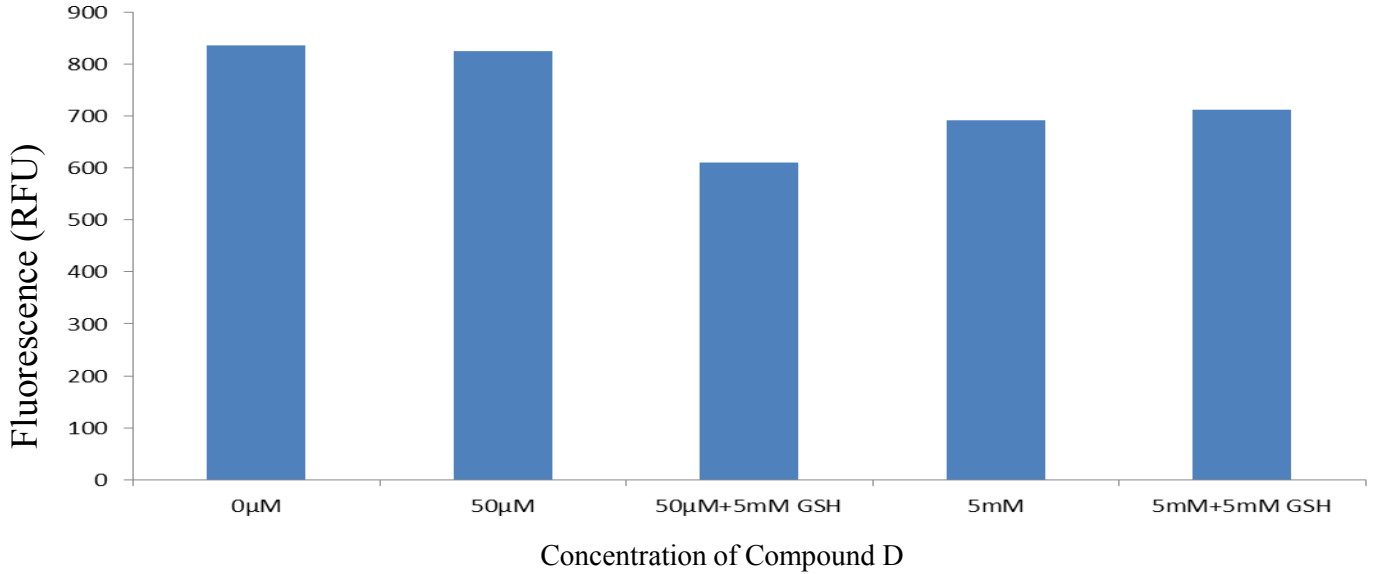
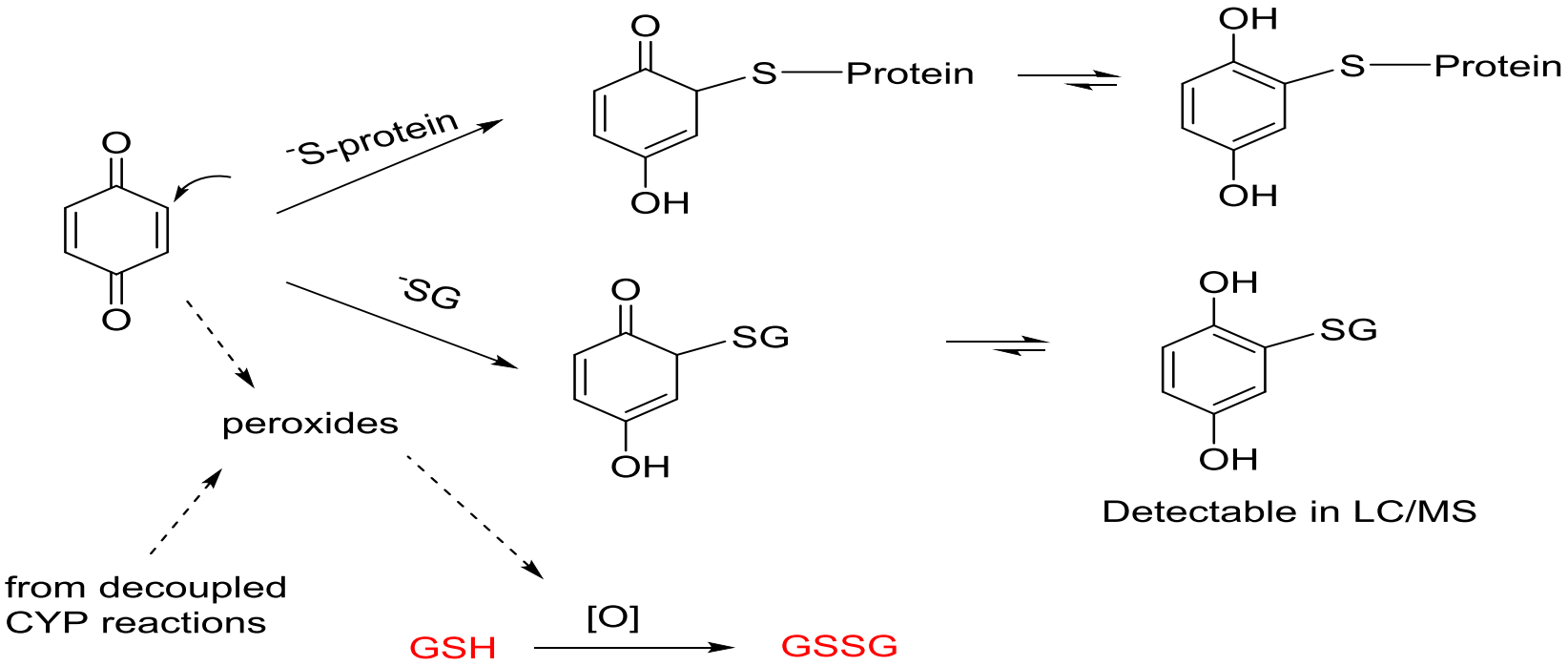
Comparison of formation of H_2O_2 in *Salmonella* in the presence or absence of compound H (Ames +) or GSH



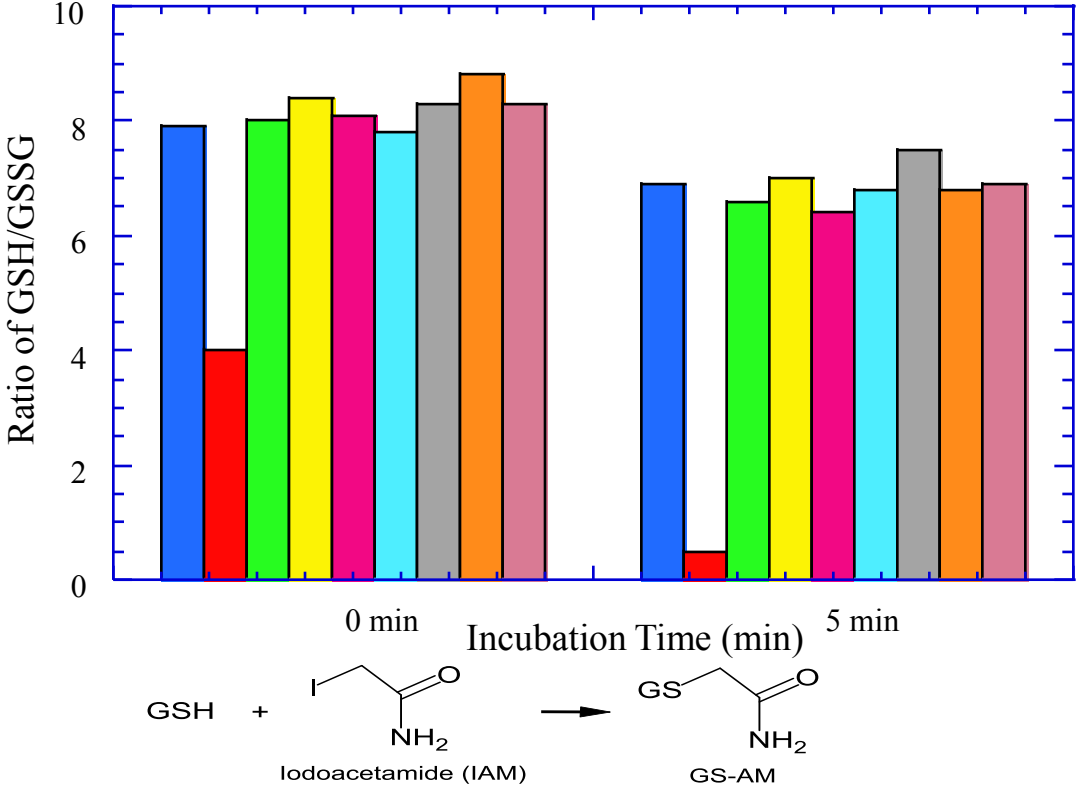
*: *Salmonella* was incubated in the presence or absence of compound H (50 μM or 5 mM) or GSH (5 mM) for 60 min prior to the H_2O_2 assay.

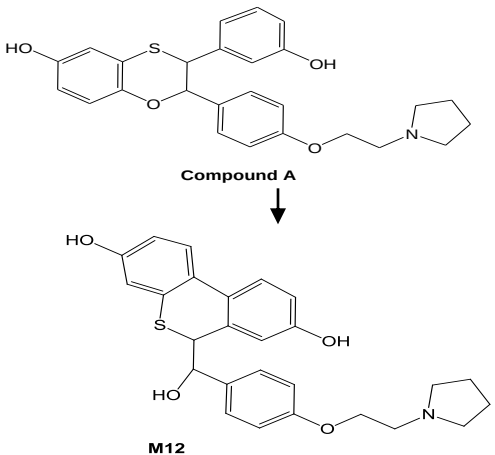
- **The presence of compound H in *Salmonella* did not increase the formation of H_2O_2 compared to the controls.**

Studies for potential oxidative stress – depletion of intracellular GSH in hepatocytes



Effect of compound A and its quinone-like metabolite M12 on the ratio of GSH/GSSG in rat hepatocytes

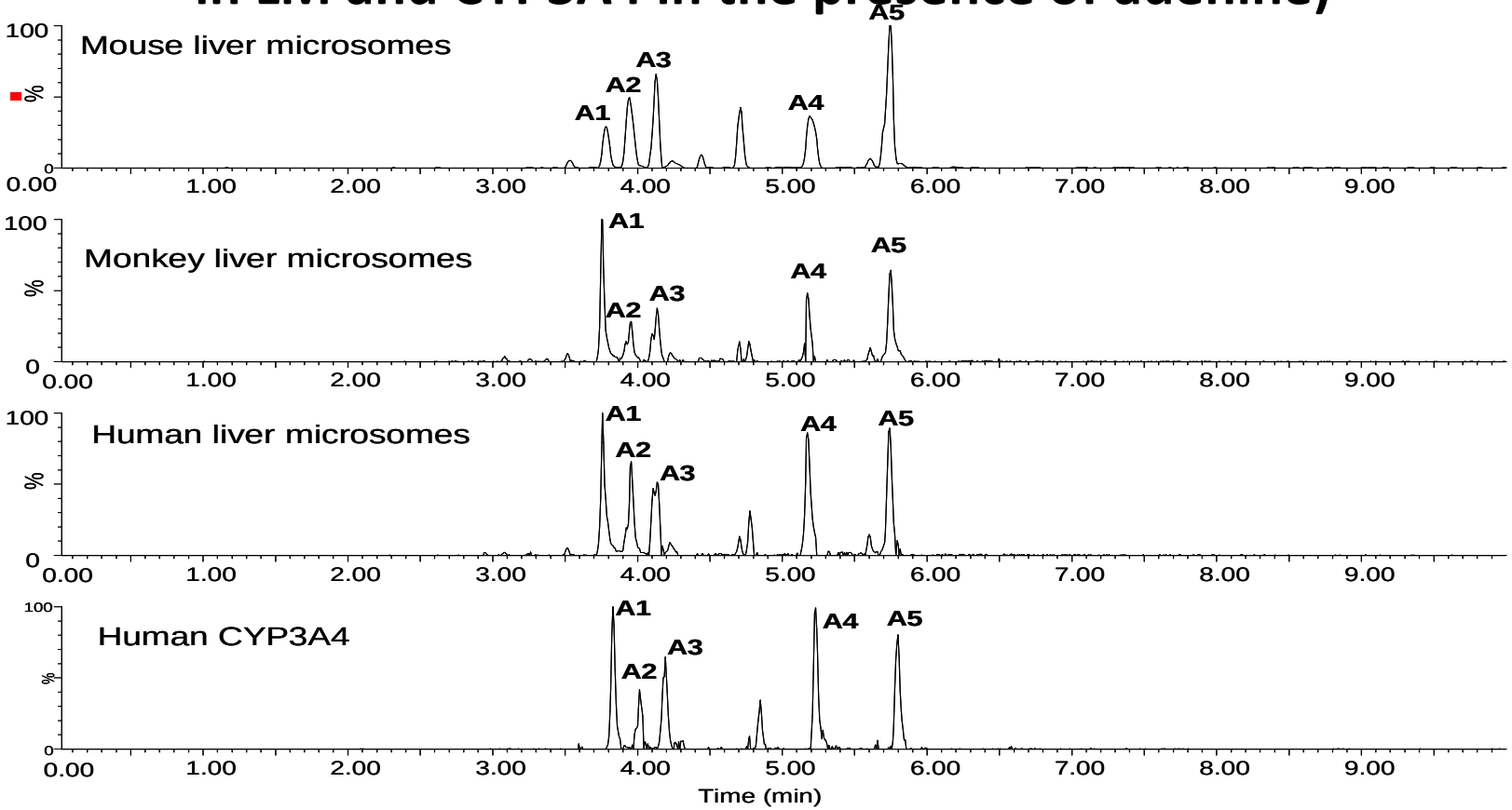




- **Cpd A and its hydroquinone metabolite M12 did not cause depletion of intracellular GSH level.**

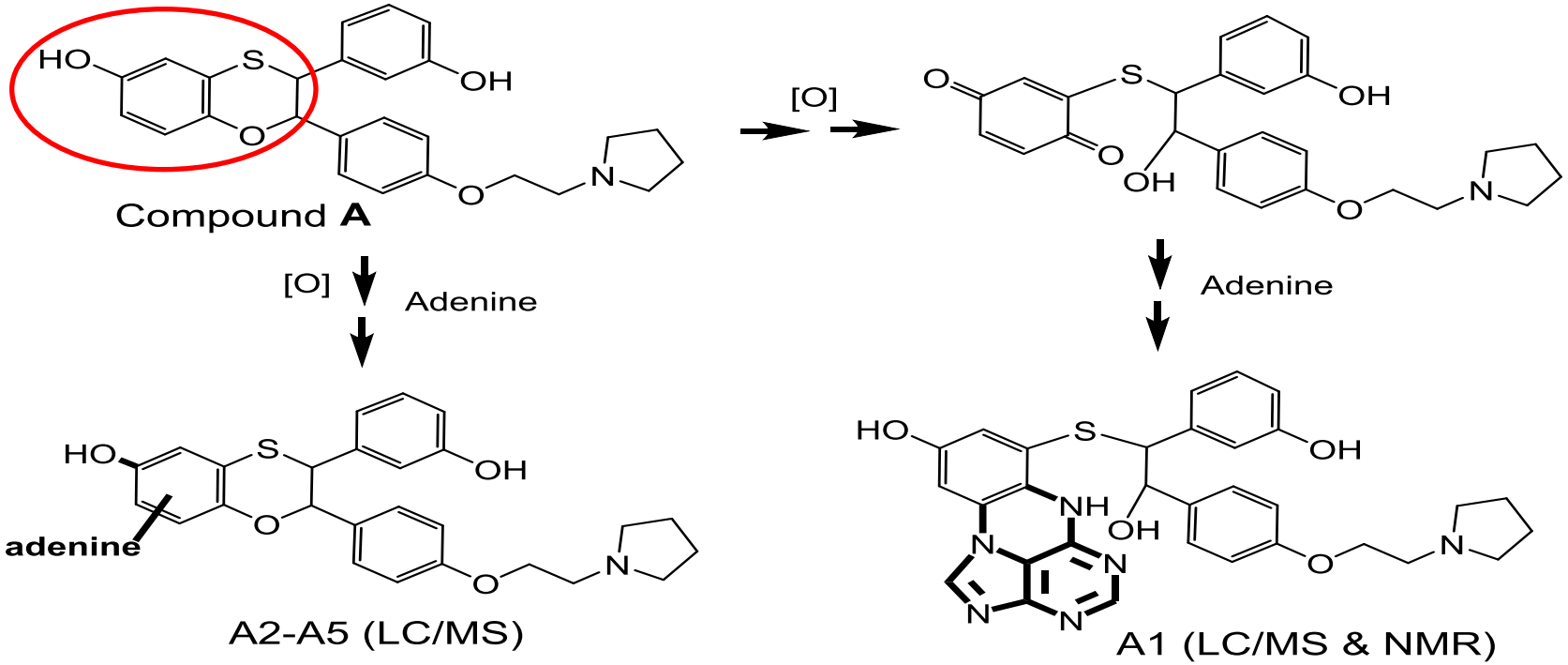
(Zhang, et al., Chem. Res. Toxicol. 2005, 18, 675)

Detection of adenine adducts A1-A5 of compound A in LM and CYP3A4 in the presence of adenine)



(Y. Li, *Chem. Res. Toxicol.* 2012; 25:2368)

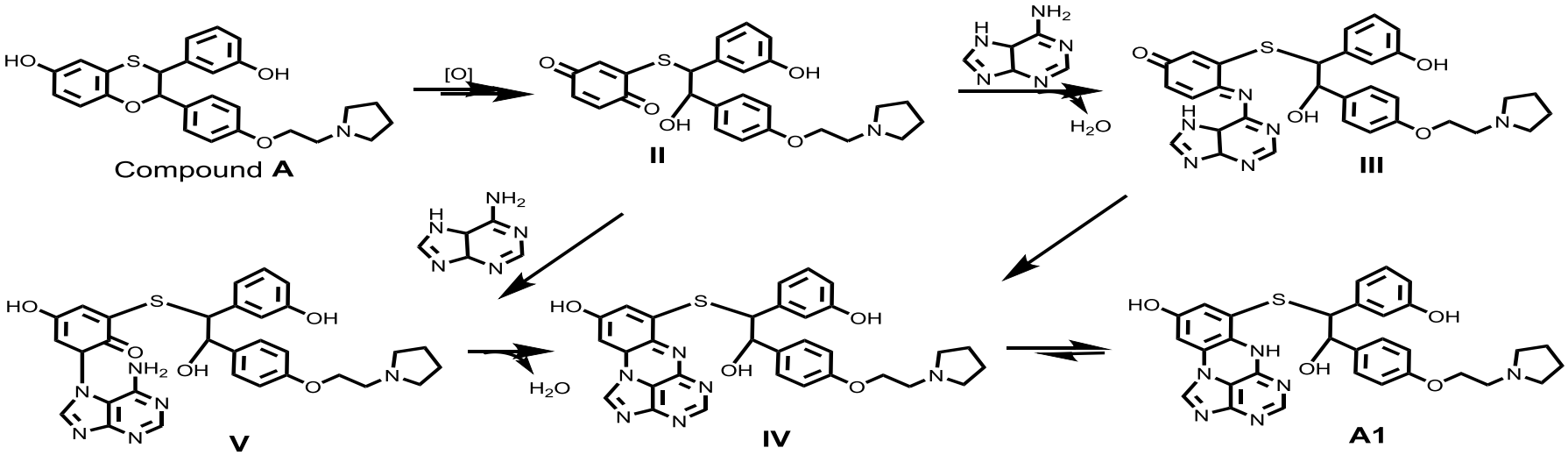
Structures of adenine adducts A1-A5 of compound A



- The 1,4-benzoxathiin-6-ol ring is subject to bioactivation

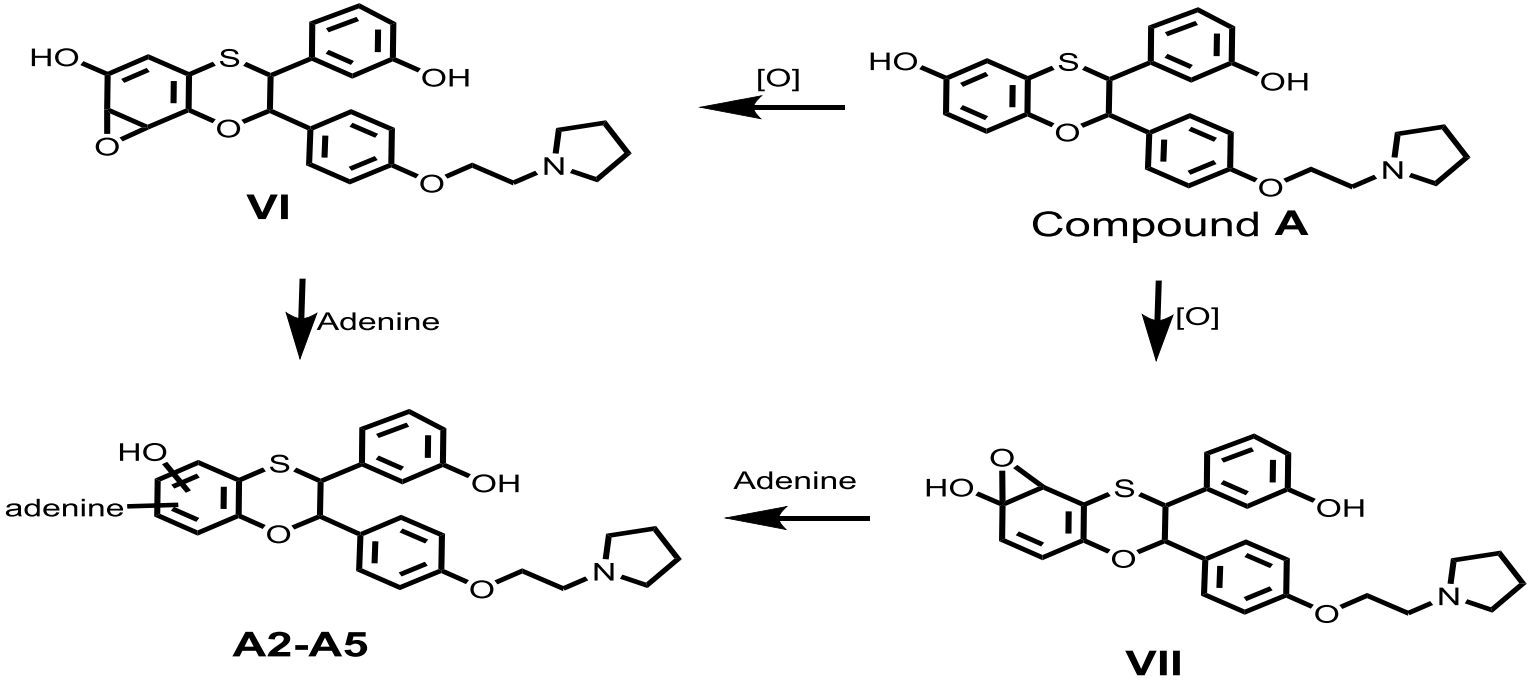
(Y. Li, *Chem. Res. Toxicol.* 2012; 25:2368)

Proposed mechanism for the formation of the adenine adduct A1



(Y. Li, *Chem. Res. Toxicol.* 2012; 25:2368)

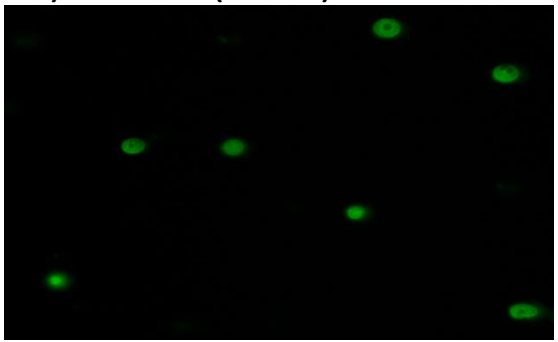
Proposed mechanism for the formation of the adenine adducts A2-A5



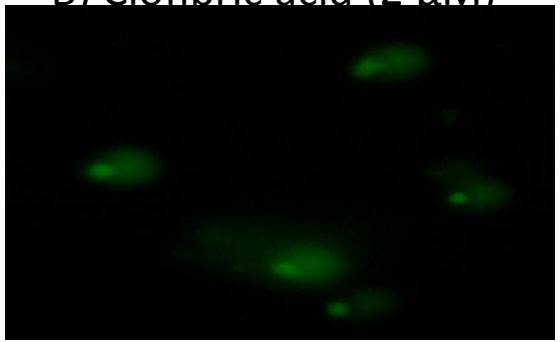
(Y. Li, *Chem. Res. Toxicol.* 2012; 25:2368)

Fluorescence microscopy photographs (Comet assay) of human hepatocytes treated with compounds for 90 min

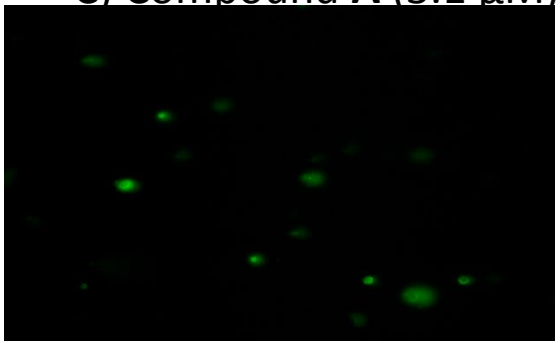
A) DMSO (0.1%)



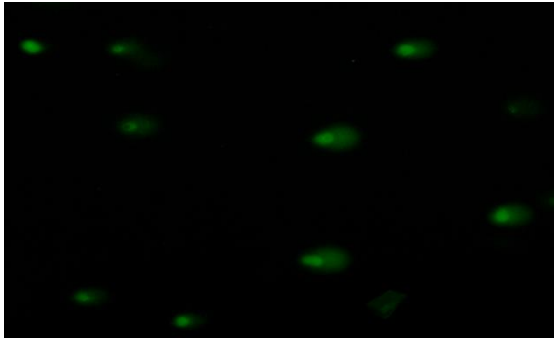
B) Clofibric acid (2 µM)



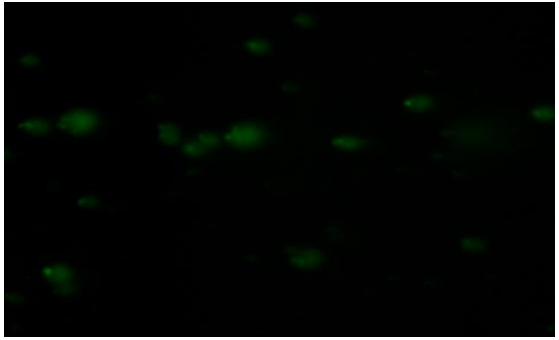
C) Compound **A** (3.1 µM)



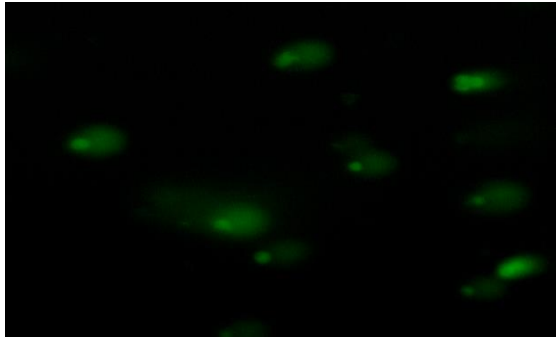
D) Compound **A** (6.2 µM)



E) Compound **A** (12.5 µM)



F) Compound **A** (25 µM)



- Compound **A** caused DNA damages in the concentration range of 3.1 and 25 µM)

(Y. Li, *Chem. Res. Toxicol.* 2012; 25:2368)

Available assays for assessing bioactivation potential

- **Metabolite Identification studies**
  - **Potential reactive intermediate-related metabolites, toxic metabolites**
  - **Adducts of GSH, N-acetylcysteine, amino acids, drug-proteins, etc.**
  - **In vitro trapping studies (adducts of GSH, cyanide, SCB, DNA bases, DNAs, etc.)**
- **Covalent protein binding studies using**
  - **in vitro in liver microsomes or hepatocytes ([^{3}H] or [^{14}C])**
  - **in vivo in rats ([^{3}H] or [^{14}C])**
  - **SDS-PAGE analysis of drug-protein adducts ([^{3}H] or [^{14}C])**
  - **LC/MS analysis of drug-amino acid adducts**
- **Studies for potential oxidative stress:**
  - **Peroxide formation**
  - **Depletion of intracellular GSH in hepatocytes**
- **Others**
  - **Comet assay (DNA damages)**

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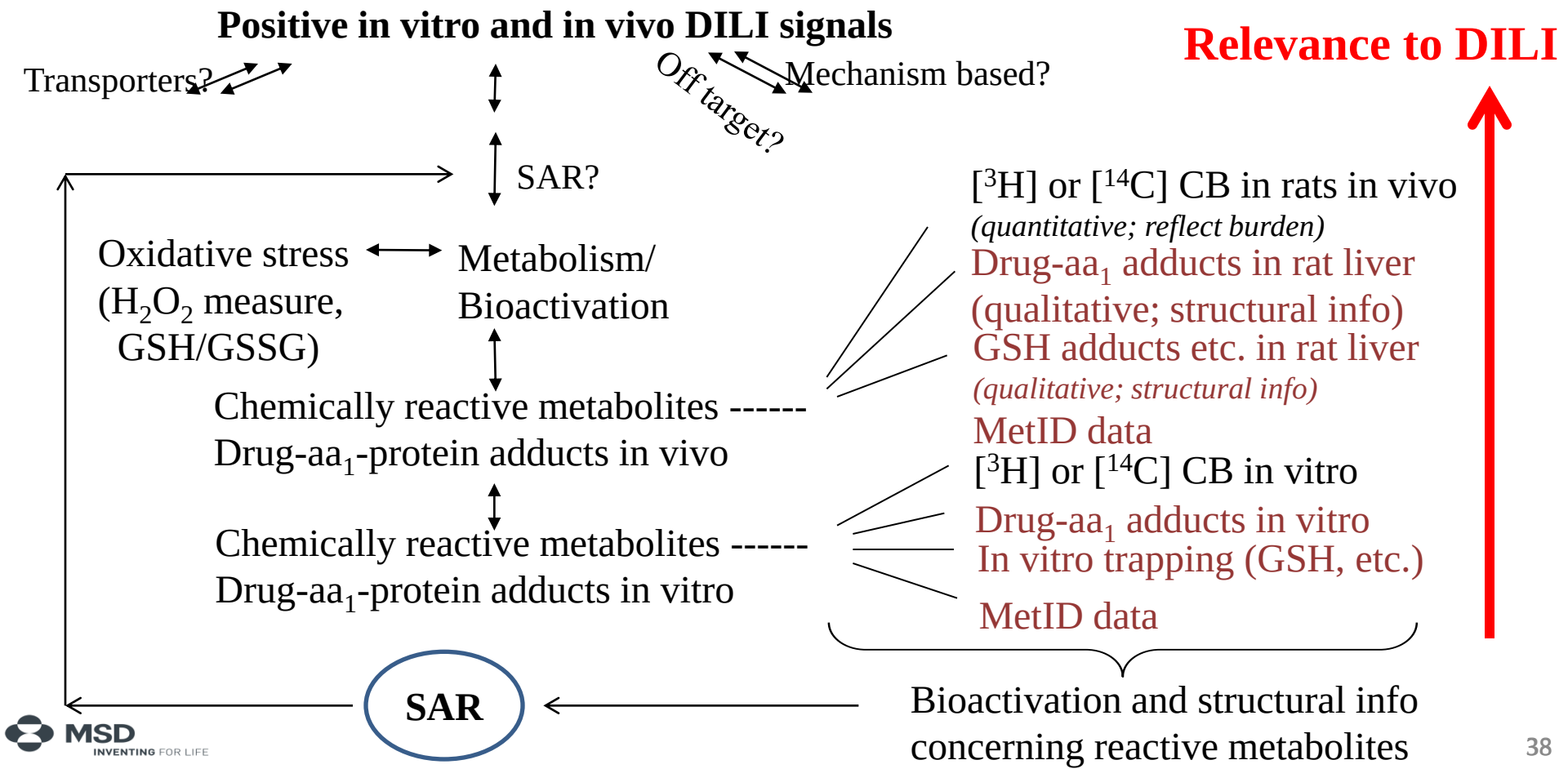
Approaches to select assays to minimize bioactivation



Closing remarks

- **Some drugs with DILI signals have generated appreciable amount of reactive metabolites.**
- **Some other drugs that form a lot of reactive metabolites do not have obvious DILI signals.**
- **Some drugs with DILI signals are not metabolized to reactive metabolites.**
- **Bioactivation of drug molecules (covalent binding to proteins) is only one of the possible causes for the DILI, and may or may not play a major role for the observed liver toxicity.**
- **The relevance between protein targets of reactive metabolites and the toxicity is not well understood. Structural modification based on metabolism data is a valuable approach to minimize bioactivation of drug candidates, but not predictive to toxicity.**
- **Several drug metabolism assays are available for assessing bioactivation potential of drug molecules. Study designs should be hypothesis-driven and should be based on the specific properties of compounds, including mechanism of action.**

Acknowledgements

Julie Li, Qing Chen, Jason Ngui, Wei Tang, Ralph Stearns, David Evans, Thomas Baillie, Ian Mcintosh, Dan Cui, Kaushik Mitra, Mark Cancilla

Selected references used in this presentation

Positioning paper:

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Li Y, Doss GA, Li Y, Chen Q, Tang W, Zhang Z. **In vitro bioactivation of a selective estrogen receptor modulator (2S,3R)-(+)-3-(3-hydroxyphenyl)-2-[4-(2-pyrrolidin-1-ylethoxy)phenyl]-2,3-dihydro-1,4-benzoxathiin-6-ol (I) in liver microsomes: formation of adenine adducts.** Chem Res Toxicol. 2012; 25(11):2368-77.