Contents

Preface XIII A Personal Foreword XV

| 1 | Origin and Historical Perspective on Reactive Metabolites 1 |
|------|---|
| | Abbreviations 1 |
| 1.1 | Mutagenesis and Carcinogenesis 1 |
| 1.2 | Detection of Reactive Metabolites 3 |
| 1.3 | Induction and Inhibition: Early Probes for Reactive Metabolites and Hepatotoxicants 4 |
| 1.4 | Covalent Binding and Oxidative Stress: Possible Mechanisms of Reactive Metabolite Cytotoxicity 5 |
| 1.5 | Activation and Deactivation: Intoxication and Detoxification 6 |
| 1.6 | Genetic Influences on Reactive Metabolite Formation 6 |
| 1.7 | Halothane: the Role of Reactive Metabolites in Immune-Mediated |
| | Toxicity 7 |
| 1.8 | Formation of Reactive Metabolites, Amount Formed, and Removal of |
| | Liability 8 |
| 1.9 | Antibodies: Possible Clues but Inconclusive 8 |
| 1.10 | Parent Drug and Not Reactive Metabolites, Complications in |
| | Immune-Mediated Toxicity 9 |
| 1.11 | Reversible Pharmacology Should not be Ignored as a Primary |
| | Cause of Side Effects 10 |
| 1.12 | Conclusions: Key Points in the Introduction 10 |
| | References 11 |
| 2 | Role of Reactive Metabolites in Genotoxicity 13 |
| - | Abbreviations 13 |
| 2.1 | Introduction 13 |
| 2.2 | Carcinogenicity of Aromatic and Heteroaromatic Amines 13 |
| 2.3 | Carcinogenicity of Nitrosamines 17 |
| 2.4 | Carcinogenicity of Quinones and Related Compounds 19 |
| 2.5 | Carcinogenicity of Furan 23 |
| 2.6 | Carcinogenicity of Vinyl Halides 26 |

v

2.6 Carcinogenicity of Vinyl Halides 26

VI Contents

| 2.7 2.8 | Carcinogenicity of Ethyl Carbamate 26 Carcinogenicity of Dihaloalkanes 28 |
|------------|--|
| 2.9 | Assays to Detect Metabolism-Dependent Genotoxicity in Drug Discovery 28 |
| 2.10 | Case Studies in Eliminating Metabolism-Based Mutagenicity in Drug Discovery Programs 29 References 36 |
| 3 | Bioactivation and Inactivation of Cytochrome P450 and Other |
| | Drug-Metabolizing Enzymes 43 |
| | Abbreviations 43 |
| 3.1 | Introduction 43 |
| 3.2 | Pharmacokinetic and Enzyme Kinetic Principles Underlying |
| | Mechanism-Based Inactivation and Drug–Drug Interactions 44 |
| 3.2.1 | Enzyme Kinetic Principles of Mechanism-Based Inactivation 44 |
| 3.2.2 | Pharmacokinetic Principles Underlying DDIs Caused by |
| | Mechanism-Based Inactivation 46 |
| 3.3 | Mechanisms of Inactivation of Cytochrome P450 Enzymes 47 |
| 3.3.1 | Quasi-Irreversible Inactivation 47 |
| 3.3.2 | Heme Adducts 48 |
| 3.3.3 | Protein Adducts 49 |
| 3.4 | Examples of Drugs and Other Compounds that are Mechanism-Based Inactivators of Cytochrome P450 Enzymes 49 |
| 3.4.1 | Amines 49 |
| 3.4.2 | Methylenedioxyphenyl Compounds 51 |
| 3.4.3 | Quinones, Quinone Imines, and Quinone Methides 52 |
| 3.4.4 | Thiophenes 53 |
| 3.4.5 | Furans 55 |
| 3.4.6 | Alkynes 56 |
| 3.4.7 | 2-Alkylimidazoles 57 |
| 3.4.8 | Other Noteworthy Cytochrome P450 Inactivators 58 |
| 3.5 | Mechanism-Based Inactivation of Other Drug-Metabolizing |
| 3.5.1 | Enzymes 60 Aldehvde Oxidase 60 |
| 3.5.2 | Aldehyde Oxidase 60 Monoamine Oxidases 61 |
| 3.6 | Concluding Remarks 64 |
| 5.0 | References 65 |
| | Kitches 05 |
| 4 | Role of Reactive Metabolites in Drug-Induced Toxicity – The Tale ofAcetaminophen, Halothane, Hydralazine, and Tienilic Acid71Abbreviations71 |
| 4.1 | Introduction 71 |
| 4.2 | Acetaminophen 71 |
| 4.2.1 | Metabolism of Acetaminophen 72 |
| 4.2.2 | Metabolic Activation of Acetaminophen 73 |

4.3 Halothane 75 Metabolism of Halothane 76 4.3.1 Hepatotoxicity following Halothane Administration 78 4.3.2 Hydralazine 79 4.4 4.5 Tienilic Acid 82 References 84 5 Pathways of Reactive Metabolite Formation with **Toxicophores/-Structural Alerts** 93 Abbreviations 93 5.1 Introduction 93 5.2 Intrinsically Reactive Toxicophores 93 Electrophilic Functional Groups 94 5.2.1 Metal Complexing Functional Groups 96 5.2.2 5.3 Toxicophores that Require Bioactivation to Reactive Metabolites 98 5.3.1 Aromatic Amines (Anilines) 98 5.3.2 ortho- and para-Aminophenols 101 5.3.3 Nitroarenes 103 5.3.4 Hydrazines 105 5.3.5 Five-Membered Heteroaromatic Rings 107 5.3.5.1 Furans 107 5.3.5.2 Thiophenes 109 Thiazoles and 2-Aminothiazoles 109 5.3.5.3 3-Alkyl Pyrrole and 3-Alkylindole Derivatives 112 5.3.5.4 1,3-Benzdioxole (Methylenedioxyphenyl) Motif 115 5.3.5.5 5.3.6 Terminal Alkenes and Alkynes 117 5.4 Concluding Remarks 121 References 121 6 Intrinsically Electrophilic Compounds as a Liability in Drug Discovery 131 Abbreviations 131 6.1 Introduction 131 Intrinsic Electrophilicity of β-Lactam Antibiotics as a 6.2 Causative Factor in Toxicity 131 6.3 Intrinsically Electrophilic Compounds in Drug Discovery 133 6.3.1 Linking Innate Electrophilicity with Drug Toxicity 135 6.4 Serendipitous Identification of Intrinsically Electrophilic Compounds in Drug Discovery 136 References 141 7 Role of Reactive Metabolites in Pharmacological Action 145 Abbreviations 145 7.1 Introduction 145

VIII Contents

| 7.2 | Drugs Activated Nonenzymatically and by Oxidative |
|---|---|
| 7.2.1 | Metabolism 145 |
| | Proton Pump Inhibitors 145 Nitrosoureas 147 |
| 7.2.2 | |
| 7.2.3 | Imidazotriazenes 148 Thiomatalandaren 150 |
| 7.2.4 | Thienotetrahydropyridines 150 |
| 7.2.5 | Oxazaphosphorines 152 |
| 7.2.6 | N, N, N', N', N', N' Hexamethylmelamine 153 |
| 7.3 | Bioreductive Activation of Drugs 153 Bioreduction to Radical Intermediates 157 |
| 7.3.1 | |
| 7.3.1.1 | Tirapazamine 157 |
| 7.3.1.2 | Anthracyclines 157 |
| 7.3.1.3 | Enediynes 158 |
| 7.3.1.4 | Artemisinin Derivatives 166 |
| 7.3.2 | Bioreductive Activation to Electrophilic Intermediates 168 |
| 7.3.2.1 | Mitomycins 168 |
| 7.3.2.2 | Aziridinylbenzoquinones 170 Diamakatian Antoniana (Anthermalianata Allahatian Spaniana 172 |
| 7.3.2.3 | Bioreductive Activation of Anthracyclines to Alkylating Species 173 |
| 7.3.2.4 | Bioreductive Activation of Nitroaromatic Compounds 174 |
| 7.4 | Concluding Remarks 175 |
| | References 176 |
| 8 | Retrospective Analysis of Structure–Toxicity Relationships |
| • | |
| | of Drugs 185 |
| | of Drugs 185 Abbreviations 185 |
| 8.1 | Abbreviations 185 |
| 8.1 8.2 | Abbreviations 185 Introduction 185 |
| | Abbreviations 185 Introduction 185 Irreversible Secondary Pharmacology 189 |
| 8.2 | Abbreviations 185 Introduction 185 Irreversible Secondary Pharmacology 189 Common Structural Features: Carboxylic Acids 189 |
| 8.2 8.2.1 | Abbreviations 185 Introduction 185 Irreversible Secondary Pharmacology 189 Common Structural Features: Carboxylic Acids 189 Primary Pharmacology and Irreversible Secondary Pharmacology 191 |
| 8.2 8.2.1 8.3 | Abbreviations 185 Introduction 185 Irreversible Secondary Pharmacology 189 Common Structural Features: Carboxylic Acids 189 Primary Pharmacology and Irreversible Secondary Pharmacology 191 Primary or Secondary Pharmacology and Reactive Metabolites: the |
| 8.2 8.2.1 8.3 | Abbreviations 185 Introduction 185 Irreversible Secondary Pharmacology 189 Common Structural Features: Carboxylic Acids 189 Primary Pharmacology and Irreversible Secondary Pharmacology 191 Primary or Secondary Pharmacology and Reactive Metabolites: the Possibility for False Structure–Toxicity Relationships 192 |
| 8.28.2.18.38.48.5 | Abbreviations 185 Introduction 185 Irreversible Secondary Pharmacology 189 Common Structural Features: Carboxylic Acids 189 Primary Pharmacology and Irreversible Secondary Pharmacology 191 Primary or Secondary Pharmacology and Reactive Metabolites: the Possibility for False Structure–Toxicity Relationships 192 Multifactorial Mechanisms as Causes of Toxicity 196 |
| 8.2 8.2.1 8.3 8.4 | Abbreviations 185 Introduction 185 Irreversible Secondary Pharmacology 189 Common Structural Features: Carboxylic Acids 189 Primary Pharmacology and Irreversible Secondary Pharmacology 191 Primary or Secondary Pharmacology and Reactive Metabolites: the Possibility for False Structure–Toxicity Relationships 192 |
| 8.28.2.18.38.48.5 | Abbreviations 185 Introduction 185 Irreversible Secondary Pharmacology 189 Common Structural Features: Carboxylic Acids 189 Primary Pharmacology and Irreversible Secondary Pharmacology 191 Primary or Secondary Pharmacology and Reactive Metabolites: the Possibility for False Structure–Toxicity Relationships 192 Multifactorial Mechanisms as Causes of Toxicity 196 Clear Correlation between Protein Target and Reactive Metabolites 197 |
| 8.2 8.2.1 8.3 8.4 8.5 8.6 | Abbreviations 185 Introduction 185 Irreversible Secondary Pharmacology 189 Common Structural Features: Carboxylic Acids 189 Primary Pharmacology and Irreversible Secondary Pharmacology 191 Primary or Secondary Pharmacology and Reactive Metabolites: the Possibility for False Structure–Toxicity Relationships 192 Multifactorial Mechanisms as Causes of Toxicity 196 Clear Correlation between Protein Target and Reactive Metabolites 197 Conclusion – Validation of Reactive Metabolites as Causes of |
| 8.2 8.2.1 8.3 8.4 8.5 8.6 | Abbreviations 185 Introduction 185 Irreversible Secondary Pharmacology 189 Common Structural Features: Carboxylic Acids 189 Primary Pharmacology and Irreversible Secondary Pharmacology 191 Primary or Secondary Pharmacology and Reactive Metabolites: the Possibility for False Structure–Toxicity Relationships 192 Multifactorial Mechanisms as Causes of Toxicity 196 Clear Correlation between Protein Target and Reactive Metabolites 197 Conclusion – Validation of Reactive Metabolites as Causes of |
| 8.2 8.2.1 8.3 8.4 8.5 8.6 | Abbreviations 185 Introduction 185 Irreversible Secondary Pharmacology 189 Common Structural Features: Carboxylic Acids 189 Primary Pharmacology and Irreversible Secondary Pharmacology 191 Primary or Secondary Pharmacology and Reactive Metabolites: the Possibility for False Structure–Toxicity Relationships 192 Multifactorial Mechanisms as Causes of Toxicity 196 Clear Correlation between Protein Target and Reactive Metabolites 197 Conclusion – Validation of Reactive Metabolites as Causes of Toxicity 198 |
| 8.2 8.2.1 8.3 8.4 8.5 8.6 | Abbreviations 185 Introduction 185 Irreversible Secondary Pharmacology 189 Common Structural Features: Carboxylic Acids 189 Primary Pharmacology and Irreversible Secondary Pharmacology 191 Primary or Secondary Pharmacology and Reactive Metabolites: the Possibility for False Structure–Toxicity Relationships 192 Multifactorial Mechanisms as Causes of Toxicity 196 Clear Correlation between Protein Target and Reactive Metabolites 197 Conclusion – Validation of Reactive Metabolites as Causes of Toxicity 198 |
| 8.2 8.2.1 8.3 8.4 8.5 8.6 8.7 | Abbreviations 185 Introduction 185 Irreversible Secondary Pharmacology 189 Common Structural Features: Carboxylic Acids 189 Primary Pharmacology and Irreversible Secondary Pharmacology 191 Primary or Secondary Pharmacology and Reactive Metabolites: the Possibility for False Structure–Toxicity Relationships 192 Multifactorial Mechanisms as Causes of Toxicity 196 Clear Correlation between Protein Target and Reactive Metabolites 197 Conclusion – Validation of Reactive Metabolites as Causes of Toxicity 198 References 200 |
| 8.2 8.2.1 8.3 8.4 8.5 8.6 8.7 | Abbreviations 185 Introduction 185 Irreversible Secondary Pharmacology 189 Common Structural Features: Carboxylic Acids 189 Primary Pharmacology and Irreversible Secondary Pharmacology 191 Primary or Secondary Pharmacology and Reactive Metabolites: the Possibility for False Structure–Toxicity Relationships 192 Multifactorial Mechanisms as Causes of Toxicity 196 Clear Correlation between Protein Target and Reactive Metabolites 197 Conclusion – Validation of Reactive Metabolites as Causes of Toxicity 198 References 200 Bioactivation and Natural Products 203 |
| 8.2 8.2.1 8.3 8.4 8.5 8.6 8.7 9 | Abbreviations 185 Introduction 185 Irreversible Secondary Pharmacology 189 Common Structural Features: Carboxylic Acids 189 Primary Pharmacology and Irreversible Secondary Pharmacology 191 Primary or Secondary Pharmacology and Reactive Metabolites: the Possibility for False Structure–Toxicity Relationships 192 Multifactorial Mechanisms as Causes of Toxicity 196 Clear Correlation between Protein Target and Reactive Metabolites 197 Conclusion – Validation of Reactive Metabolites as Causes of Toxicity 198 References 200 Bioactivation and Natural Products 203 Abbreviations 203 |
| 8.2 8.2.1 8.3 8.4 8.5 8.6 8.7 9 9.1 | Abbreviations 185 Introduction 185 Irreversible Secondary Pharmacology 189 Common Structural Features: Carboxylic Acids 189 Primary Pharmacology and Irreversible Secondary Pharmacology 191 Primary or Secondary Pharmacology and Reactive Metabolites: the Possibility for False Structure–Toxicity Relationships 192 Multifactorial Mechanisms as Causes of Toxicity 196 Clear Correlation between Protein Target and Reactive Metabolites 197 Conclusion – Validation of Reactive Metabolites as Causes of Toxicity 198 References 200 Bioactivation and Natural Products 203 Abbreviations 203 Introduction 203 |
| 8.2 8.2.1 8.3 8.4 8.5 8.6 8.7 9 9.1 | Abbreviations185Introduction185Irreversible Secondary Pharmacology189Common Structural Features: Carboxylic Acids189Primary Pharmacology and Irreversible Secondary Pharmacology191Primary or Secondary Pharmacology and Reactive Metabolites: thePossibility for False Structure–Toxicity Relationships192Multifactorial Mechanisms as Causes of Toxicity196Clear Correlation between Protein Target and ReactiveMetabolites197Conclusion – Validation of Reactive Metabolites as Causes of Toxicity198References200203Bioactivation and Natural Products203Abbreviations203Introduction203Well-Known Examples of Bioactivation of Compounds Present in |
| 8.2 8.2.1 8.3 8.4 8.5 8.6 8.7 9 9.1 9.2 | Abbreviations 185 Introduction 185 Irreversible Secondary Pharmacology 189 Common Structural Features: Carboxylic Acids 189 Primary Pharmacology and Irreversible Secondary Pharmacology 191 Primary or Secondary Pharmacology and Reactive Metabolites: the Possibility for False Structure–Toxicity Relationships 192 Multifactorial Mechanisms as Causes of Toxicity 196 Clear Correlation between Protein Target and Reactive Metabolites 197 Conclusion – Validation of Reactive Metabolites as Causes of Toxicity 198 References 200 Bioactivation and Natural Products 203 Abbreviations 203 Introduction 203 Well-Known Examples of Bioactivation of Compounds Present in Herbal Remedies 205 |

- 9.2.3 Aristolochia and Aristolochic Acid 208
- 9.2.4 Comfrey, Coltsfoot, and Pyrrolizidine Alkaloids 210
- 9.3 Well-Known Examples of Bioactivation of Compounds Present in Foods 212
- 9.3.1 Cycasin 212
- 9.3.2 Aflatoxin 214
- 9.3.3 3-Methylindole 216
- 9.3.4 Polycyclic Azaheterocyclic Compounds in Cooked Meats 216
- 9.3.5 Nitrosamines 219
- 9.4 Summary 220 References 220

10 Experimental Approaches to Reactive Metabolite Detection 225

- Abbreviations 225
- 10.1 Introduction 225
- 10.2 Identification of Structural Alerts and Avoiding them in Drug Design 225
- 10.3 Assays for the Detection of Reactive Metabolites 227
- 10.3.1 Qualitative Electrophile Trapping Assays 227
- 10.3.2 Quantitative Electrophile Trapping Assays 230
- 10.3.3 Covalent Binding Assays 231
- 10.3.4 Detecting and Characterizing Bioactivation by Enzymes Other than Cytochrome P450 233
- 10.4 Other Studies that can Show the Existence of Reactive Metabolites 234
- 10.4.1 Metabolite Identification Studies 234
- 10.4.2 Radiolabeled Metabolism and Excretion In Vivo 235
- 10.4.3 Whole-Body Autoradiography and Tissue Binding 236
- 10.4.4 Inactivation of Cytochrome P450 Enzymes 237
- 10.5 Conclusion 237 References 238
- 11
 Case Studies on Eliminating/Reducing Reactive Metabolite

 Formation in Drug Discovery
 241

Abbreviations 241

- 11.1 Medicinal Chemistry Tactics to Eliminate Reactive Metabolite Formation 241
- 11.2 Eliminating Reactive Metabolite Formation on Heterocyclic Ring Systems 242
- 11.2.1 Mechanism(s) of Thiazole Ring Bioactivation and Rational Chemistry Approaches to Abolish Reactive Metabolite Formation 242
- 11.2.2Mechanism(s) of Isothiazole Ring Bioactivation and Rational
Chemistry Approaches to Abolish Reactive Metabolite Formation249
- 11.3 Medicinal Chemistry Strategies to Mitigate Bioactivation of Electron-Rich Aromatic Rings 251

X Contents

| 11.4 | Medicinal Chemistry Strategies to Mitigate Bioactivation on a Piperazine Ring System 256 |
|--------|--|
| 11.5 | 4-Fluorofelbamate as a Potentially Safer Alternative to Felbamate 258 |
| 11.5 | Concluding Remarks 263 |
| 11.0 | References 263 |
| | |
| 12 | Structural Alert and Reactive Metabolite Analysis for the Top 200 Drugs |
| | in the US Market by Prescription 269 |
| | Abbreviations 269 |
| 12.1 | Introduction 269 |
| 12.2 | Structural Alert and Reactive Metabolite Analyses for the Top 20 Most |
| | Prescribed Drugs in the United States for the Year 2009 270 |
| 12.2.1 | Daily Dose Trends 270 |
| 12.2.2 | Presence of Structural Alerts 270 |
| 12.2.3 | Evidence for Metabolic Activation to Reactive Metabolites 275 |
| 12.3 | Insights Into the Excellent Safety Records for Reactive |
| | Metabolite–Positive Blockbuster Drugs 280 |
| 12.4 | Structural Alert and Reactive Metabolite Analyses for the Remaining |
| | 180 Most Prescribed Drugs 282 |
| 12.4.1 | Structural Alert and/or Reactive Metabolite "False Positives" 289 |
| 12.5 | Structure Toxicity Trends 302 |
| 12.5.1 | Meloxicam versus Sudoxicam 304 |
| 12.5.2 | Zolpidem versus Alpidem 304 |
| 12.5.3 | Quetiapine versus Olanzapine versus Clozapine 304 |
| | References 306 |
| 13 | Mitigating Toxicity Risks with Affinity Labeling Drug Candidates 313 |
| | Abbreviations 313 |
| 13.1 | Introduction 313 |
| 13.2 | Designing Covalent Inhibitors 313 |
| 13.2.1 | Selection of Warheads 316 |
| 13.2.2 | Reversible Covalent Modification 322 |
| 13.3 | Optimization of Chemical Reactivity of the Warhead Moiety 326 |
| 13.3.1 | Experimental Approaches 326 |
| 13.3.2 | In Silico Approaches 328 |
| 13.3.3 | Additional Derisking Factors 329 |
| 13.4 | Concluding Remarks 329 |
| | References 330 |
| 14 | Dealing with Reactive Metabolite–Positive Compounds in Drug |
| | Discovery 335 |
| | Abbreviations 335 |
| 14.1 | Introduction 335 |
| 14.2 | Avoiding the Use of Structural Alerts in Drug Design 336 |
| 14.3 | Structural Alert and Reactive Metabolite Formation 338 |

- 14.4 Should Reactive Metabolite–Positive Compounds be Nominated as Drug Candidates? *340*
- 14.4.1 Impact of Competing, Detoxification Pathways 341
- 14.4.2 The Impact of Dose Size 342
- 14.4.3 Consideration of the Medical Need/Urgency 345
- 14.4.4 Consideration of the Duration of Treatment 345
- 14.4.5 Consideration of Novel Pharmacological Targets 346
- 14.5 The Multifactorial Nature of IADRs 348
- 14.6 Concluding Remarks 350
 - References 351
- 15 Managing IADRs a Risk–Benefit Analysis 357

Abbreviations 357

- 15.1 Risk–Benefit Analysis 357
- 15.2 How Common is Clinical Drug Toxicity? 359
- 15.3 Rules and Laws of Drug Toxicity 363
- 15.4 Difficulties in Defining Cause and Black Box Warnings 365
- 15.5 Labeling Changes, Contraindications, and Warnings: the Effectiveness of Side Effect Monitoring 367
- 15.6 Allele Association with Hypersensitivity Induced by Abacavir: Toward a Biomarker for Toxicity 369
- 15.7 More Questions than Answers: Benefit Risk for ADRs 373 References 374

Index 377