Contents

List of Contributors XVII Preface XXI A Personal Foreword XXIII

Part One Introduction 1

1 Metabolism in Drug Development 3

Bernard Testa

- 1.1 What? An Introduction 3
- 1.2 Why? Metabolism in Drug Development 4
- 1.2.1 The Pharmacological Context 4
- 1.2.2 Consequences of Drug Metabolism on Activity 6
- 1.2.3 Adverse Consequences of Drug Metabolism 7
- 1.2.4 Impact of Metabolism on Absorption, Distribution, and Excretion 10
- 1.3 How? From Experimental Results to Databases to Expert Software Packages 11
- 1.3.1 The Many Factors Influencing Drug Metabolism 11
- 1.3.2 Acquiring and Interpreting Experimental Results 13
- 1.3.3 Expert Software Tools and Their Domains of Applicability 14
- 1.3.4 Roads to Progress 16
- 1.4 Who? Human Intelligence as a Conclusion 17 References 19

Part Two Software, Web Servers and Data Resources to Study Metabolism 27

- 2 Software for Metabolism Prediction 29
- Lu Tan and Johannes Kirchmair
- 2.1 Introduction 29
- 2.2 Ligand-Based and Structure-Based Methods for Predicting Metabolism 30
- 2.3 Software for Predicting Sites of Metabolism 38



۷

VI Contents

- Knowledge-Based Systems 38 2.3.1
- 2.3.2 Molecular Interaction Fields 39
- 2.3.3 Docking 39
- 2.3.4 Reactivity Models 40
- 2.3.5 Data Mining and Machine Learning Approaches 41
- 2.3.6 Shape-Focused Approaches 42
- 2.4 Software for Predicting Metabolites 43
- 2.4.1 Knowledge-Based Systems 44
- 2.4.2 Data Mining and Machine Learning Approaches 46
- 2.4.3 Molecular Interaction Fields 46
- 2.5 Software for Predicting Interactions of Small Molecules with Metabolizing Enzymes 46
- 2.6 Conclusions 48 References 49
- 3 Online Databases and Web Servers for Drug Metabolism Research 53 David S. Wishart
- 3.1 Introduction 53
- 3.2 Online Drug Metabolism Databases 54
- 3.2.1 DrugBank 57
- 3.2.2 HMDB 59
- 3.2.3 PharmGKB 59
- 3.2.4 Wikipedia 60
- 3.2.5 PubChem 61
- 3.2.6 Synoptic Databases: ChEBI, ChEMBL, KEGG, and BindingDB 61
- 3.2.7 Specialized Databases: UM-BBD, SuperCYP, PKKB, and PK/DB 63
- 3.2.8 Online Database Summary 64
- 3.3 Online Drug Metabolism Prediction Servers 65
- Metabolite Predictors 66 3.3.1
- 3.3.2 SoM Predictors 66
- 3.3.3 Specialized Predictors 68
- 3.3.4 ADMET Predictors 70
- 3.3.5 Web Server Summary 71 References 71

Part Three Computational Approaches to Study Cytochrome P450 Enzymes 75

- Structure and Dynamics of Human Drug-Metabolizing Cytochrome 4 P450 Enzymes 77 Ghulam Mustafa, Xiaofeng Yu, and Rebecca C. Wade
- 4.1 Introduction 77
- 4.2 Three-Dimensional Structures of Human CYPs 78
- 4.3 Structural Features of CYPs 78

- 4.3.1 CYP-Electron Transfer Protein Interactions 81
- 4.3.2 Substrate Recognition Sites 82
- 4.3.3 Structural Variability and Substrate Specificity Profiles 83
- 4.3.3.1 CYP1A2 83
- 4.3.3.2 CYP2A6 85
- 4.3.3.3 CYP2C9 85
- 4.3.3.4 CYP2D6 86
- 4.3.3.5 CYP2E1 87
- 4.3.3.6 CYP3A4 87
- 4.4 Dynamics of CYPs 88
- 4.4.1 Active Site Flexibility 88
- 4.4.2 Active Site Solvation 93
- 4.4.3 Active Site Access and Egress Pathways 93
- 4.4.4 MD Simulations of CYPs in Lipid Bilayers 96
- 4.5 Conclusions 96 References 97

5 Cytochrome P450 Substrate Recognition and Binding 103 Andrew G. Leach and Nathan J. Kidley

- 5.1 Introduction 103
- 5.2 Substrate Recognition in the Catalytic Cycle of CYPs 103
- 5.3 Substrate Identity in Various Species 104
- 5.4 Structural Insight into Substrate Recognition by CYPs 107
- 5.4.1 CYP1A1, CYP1A2, and CYP1B1 108
- 5.4.2 CYP2A6 108
- 5.4.3 CYP2A13 109
- 5.4.4 CYP2C8 110
- 5.4.5 CYP2C9 112
- 5.4.6 CYP2D6 112
- 5.4.7 CYP2E1 113
- 5.4.8 CYP2R1 113
- 5.4.9 CYP3A4 115
- 5.4.10 CYP8A1 115
- 5.4.11 CYP11A1 116
- 5.4.12 CYP11B2 118
- 5.4.13 CYP19A1 118
- 5.4.14 CYP46A1 119
- 5.4.15 General Insights from Protein-Ligand Crystal Structures 119
- 5.5 The Challenges of Using Docking for Predicting Kinetic Parameters 120
- 5.6 Substrate Properties for Various Human Isoforms 120
- 5.6.1 Kinetic Parameters $K_{\rm m}$ and $k_{\rm cat}$ and Their Relationship with Substrate and Protein Structure 124
- 5.7 Conclusions 128
 - References 128

6	QM/MM Studies of Structure and Reactivity of Cytochrome P450
	Enzymes: Methodology and Selected Applications 133
	Sason Shaik, Hui Chen, Dandamudi Usharani, and Walter Thiel
6.1	Introduction 133
6.2	QM/MM Methods 135
6.2.1	Methodological Issues in QM/MM Studies 136
6.2.1.1	QM/MM Partitioning 136
6.2.1.2	QM Methods 137
6.2.1.3	MM Methods 138
6.2.1.4	Subtractive versus Additive QM/MM Schemes 139
6.2.1.5	Electrostatic QM/MM Interactions 139
6.2.1.6	QM/MM Boundary Treatments 139
6.2.1.7	QM/MM Geometry Optimization 140
6.2.1.8	QM/MM Molecular Dynamics and Free Energy Calculations 140
6.2.1.9	QM/MM Energy versus Free Energy Calculations 141
6.2.2	Practical Issues in QM/MM Studies 141
6.2.2.1	QM/MM Software 141
6.2.2.2	QM/MM Setup 142
6.2.2.3	Accuracy of QM/MM Results 143
6.2.2.4	QM/MM Geometry Optimization 143
6.2.2.5	Extracting Insights from QM/MM Calculations 144
6.3	Selected QM/MM Applications to Cytochrome P450 Enzymes 144
6.3.1	Formation of Cpd I from Cpd 0 146
6.3.1.1	Conversion of Cpd 0 into Cpd I in the T252X Mutants 148
6.3.2	Properties of Cpd I 151
6.3.2.1	Cpd I Species of Different Cytochrome P450s 154
6.3.3	The Mechanism of Cytochrome P450 StaP 155
6.3.4	The Mechanism of Dopamine Formation 160
6.3.4.1	The Electrostatic Effect is Not Due to Simple Bulk Polarity 163
6.4	An Overview of Cytochrome P450 Function Requires Reliable MD
	Calculations 163
6.5	Conclusions 164
	References 165
7	Computational Free Energy Methods for Ascertaining Ligand
	Interaction with Metabolizing Enzymes 179
	Mark J. Williamson
7.1	Introduction 179
7.2	Linking Experiment and Simulation: Statistical Mechanics 180
7.2.1	A Note on Chemical Transformations 182
7.3	Taxonomy of Free Energy Methods 183
7.3.1	Pathway Methods 183
7.3.1.1	Pathway Planning: Using the State Nature of the Free Energy
7010	Cycle 184
7.3.1.2	Free Energy Perturbation 185

-

- 7.3.1.3 Bennett Acceptance Ratio 185
- 7.3.1.4 Thermodynamic Integration 186
- 7.3.2 Endpoint Methods 186
- 7.3.2.1 Molecular Mechanics-Generalized Born Surface Area (MM-GBSA) 186
- 7.3.2.2 Linear Interaction Energy 187
- 7.3.2.3 QM Endpoint Methods 187
- 7.3.3 Summary of Free Energy Methods 187
- 7.4 Ligand Parameterization 188
- 7.5 Specific Examples 189
- 7.5.1 Cytochrome P450 (CYP) 189
- 7.5.2 Chorismate Mutase 192
- 7.6 Conclusions 192
 - References 193
- 8 Experimental Approaches to Analysis of Reactions of Cytochrome P450 Enzymes 199
 - Frederick Peter Guengerich
- 8.1 Introduction 199
- 8.2 Structural Data and Substrate Binding 199
- 8.3 Systems for Production of Reaction Products and Analysis of Systems 200
- 8.3.1 In Vivo Systems 201
- 8.3.2 Tissue Microsomal Systems 201
- 8.3.3 Purified CYPs in Reconstituted Systems 201
- 8.3.4 Membranes from Heterologous Expression Systems 202
- 8.3.4.1 Mammalian Cells 202
- 8.3.4.2 Insect Cell Systems (Using Baculovirus Infection for Expression) 202
- 8.3.4.3 Microbial Membrane Systems 202
- 8.4 Methods for Analysis of Products of Drugs 203
- 8.4.1 Separation Methods 203
- 8.4.1.1 High-Performance Liquid Chromatography 203
- 8.4.1.2 Other Separation Methods 204
- 8.4.2 Analysis Methods 204
- 8.4.2.1 HPLC-UV 204
- 8.4.2.2 LC-MS 205
- 8.4.2.3 LC-MS/MS 205
- 8.4.2.4 LC-HRMS 205
- 8.4.2.5 NMR 205
- 8.4.2.6 Other Spectroscopy of Metabolites 206
- 8.5 Untargeted Searches for CYP Reactions 208
- 8.6 Complex CYP Products 208
- 8.7 Structure-Activity Relationships Based on Products 210
- 8.7.1 SARs Based on Chemical Bond Energy 211
- 8.7.2 SARs Based on Docking 211

X Contents

8.7.3 8.8 8.9 8.10	Knowledge-Based SAR 212 SAR of Reaction Rates 213 Other Issues in Predictions 213 Conclusions 214 References 214	
Part Four Computational Approaches to Study Sites and Products of Metabolism 221		
9	Molecular Interaction Fields for Predicting the Sites and Products of Metabolism 223 Fabio Broccatelli and Nathan Brown	
9.1	Introduction 223	
9.2	CYP from a GRID Perspective 224	
9.3	From Lead Optimization to Preclinical Phases: the Challenge of SoM Prediction 226	
9.3.1	MetaSite: Accessibility Function 227	
9.3.2	MetaSite: Reactivity Function 229	
9.3.3	MetaSite: Site of Metabolism Prediction 230	
9.3.4	MetaSite: Validation and Case Studies 231	
9.3.5	MetaSite: Prediction of CYP Inhibition 234	
9.3.6	MassMetaSite: Automated Metabolite Identification 236	
9.4	Conclusions 239	
	References 241	
10	Structure-Based Methods for Predicting the Sites	
	and Products of Metabolism 243	
	Chris Oostenbrink	
10.1	Introduction 243	
10.2	6 Å Rule 243	
10.3	Methodological Approaches 245	
10.4	Prediction of Binding Poses 247	
10.5	Protein Flexibility 249	
10.6	Role of Water Molecules 254	
10.7	Effect of Mutations 256	
10.8	Conclusions 258	
10.0	References 259	
11	Reactivity-Based Approaches and Machine Learning Methods for Predicting the Sites of Cytochrome P450-Mediated Metabolism 265 Patrik Rydberg	
11.1	Introduction 265	
11.1	Reactivity Models for CYP Reactions 268	
11.4	Accession intraction of a contraction of the contra	

- 11.2.1 Hydroxylation of Aliphatic Carbon Atoms 268
- 11.2.2 Hydroxylation and Epoxidation of Aromatic and Double Bonded Carbon Atoms 271
- 11.2.3 Combined Carbon Atom Models 273
- 11.2.4 Comprehensive Models 273
- 11.3 Reactivity-Based Methods Applied to CYP-Mediated Site of Metabolism Prediction 274
- 11.3.1 Methods Only Applicable to Carbon Atoms 274
- 11.3.2 Comprehensive Methods 276
- 11.4 Machine Learning Methods Applied to CYP-Mediated Site of Metabolism Prediction 278
- 11.4.1 Atomic Descriptors 278
- 11.4.2 Machine Learning Methods and Optimization Criteria 279
- 11.5 Applications to SoM Prediction 280
- 11.5.1 Isoform-Specific Models 281
- 11.5.2 Isoform-Unspecific Models 283
- 11.6 Combinations of Structure-Based Models and Reactivity 284
- 11.7 Conclusions 285 References 286
- 12 Knowledge-Based Approaches for Predicting the Sites and Products of Metabolism 293 Philip Neville Judson
- 12.1 Introduction 293
- 12.2 Building and Maintaining a Knowledge Base 295
- 12.3 Encoding Rules in a Knowledge Base 299
- 12.4 Ways of Working with Rules 301
- 12.5 Using the Logic of Argumentation 303
- 12.6 Combining Absolute and Relative Reasoning 307
- 12.7 Combining Predictions from Multiple Sources 310
- 12.8 Validation and Assessment of Performance 312
- 12.9 Conclusions 314
 - References 314
- Part Five Computational Approaches to Study Enzyme Inhibition and Induction 319
- 13 Quantitative Structure–Activity Relationship (QSAR) Methods for the Prediction of Substrates, Inhibitors, and Inducers of Metabolic Enzymes 321 Oraphan Phuangsawai, Supa Hannongbua, and Mathew Paul Gleeson
- 13.1 Introduction 321
- 13.2 In Silico QSAR Methods 322
- 13.2.1 Experimental Variability 323

XII Contents

- 13.2.2 Data Curation and Manipulation 324
- 13.2.3 Molecular Descriptors 324
- 13.2.4 Training SAR, QSAR, and Machine Learning Models 325
- 13.2.5 Local versus Global OSAR Models 325
- 13.2.6 SAR and Classical QSAR Methods 326
- 13.2.7 Machine Learning OSAR Methods 327
- 13.2.8 Model Assessment and Validation 327
- 13.2.8.1 Assessing the Predictive Ability of QSAR Models 327
- 13.2.8.2 Applicability Domains of QSAR Models 328
- 13.3 QSAR Models for Cytochrome P450 328
- 13.3.1 Inhibition QSAR 328
- 13.3.1.1 SAR 328
- 13.3.1.2 Classical OSAR Models 329
- 13.3.1.3 Machine Learning OSAR Models 333
- 13.3.1.4 Classification Models 334
- 13.3.1.5 3D OSAR Models 335
- Enzyme Induction QSAR 336 13.3.2
- 13.4 Conjugative Metabolizing Enzymes 337
- Uridine Diphosphate Glucosyltransferase (UGT) QSAR 338 13.4.1
- 13.4.2 Sulfotransferases QSAR 338
- 13.5 In Vitro Clearance QSAR 339
- 13.6 Conclusions 340 References 341
- Pharmacophore-Based Methods for Predicting the Inhibition 14 and Induction of Metabolic Enzymes 351
 - Teresa Kaserer, Veronika Temml, and Daniela Schuster
- 14.1 Introduction 351
- 14.2 Substrate and Inhibitor Pharmacophore Models 354
- 14.2.1 Cytochrome P450 enzymes 354
- 14.2.1.1 CYP1A2 354
- 14.2.1.2 CYP2B6 355
- 14.2.1.3 CYP2C9 356
- 14.2.1.4 CYP2C19 357
- 14.2.1.5 CYP2D6 358
- 14.2.1.6 CYP3A4 359
- 14.2.1.7 CYP3A5 and CYP3A7 360
- 14.2.2 UDP-Glucuronosyltransferases (UGTs) 361
- 14.2.2.1 UGT1A1 361
- 14.2.2.2 UGT1A4 361
- 14.2.2.3 UGT1A9 361
- 14.2.2.4 UGT2B7 362
- 14.2.3 Interference with Recently Identified Phase I Metabolic Enzymes 362
- 14.3 Inducer Models 363
- 14.3.1 Hetero- and Autoactivation 363

- 14.3.1.1 CYP2C9 363
- 14.3.1.2 CYP3A4 364
- 14.3.2 Nuclear Receptors 364
- 14.3.2.1 Pregnane X Receptor 364
- 14.3.2.2 CAR 366
- 14.4 Conclusions 366 References 368
- 15 Prediction of Phosphoglycoprotein (*P-gp*)-Mediated Disposition in Early Drug Discovery 373 Simon Thomas and Richard J. Dimelow
- 15.1 Introduction 373
- 15.2 QSAR Modeling of Compounds Interacting with Transporters 376
- 15.2.1 Experimental Data and Assays 376
- 15.2.2 Descriptors Used in P-gp Substrate Identification 378
- 15.2.3 QSAR Methods Used in P-gp Substrate Identification 380
- 15.3 Influence of Compound Structure on P-gp Substrate Identity 380
- 15.4 QSAR Models for P-gp Substrates 385
- 15.5 Application to Drug Discovery 388
- 15.6 Conclusions 391 References 392
- 16 Predicting Toxic Effects of Metabolites 397 Andreas Bender
- 16.1 Introduction 397
- 16.2 Methods for Predicting Toxic Effects 401
- 16.2.1 Predicting Metabolites 401
- 16.2.2 Predicting Relative and Absolute Metabolism Likelihoods and Rates 401
- 16.2.3 Utilizing Pharmacogenetic Data to Anticipate Dose, Rate, and Time Information in an Individual Patient 402
- 16.2.4 Predicting the Effect of the Resulting Metabolites 402
- 16.2.4.1 Bioactivity-Based Mechanistic Models 403
- 16.2.4.2 Incorporating Pathway Information into Toxicity Models *404*
- 16.2.4.3 Toxicogenetic and Pharmacogenomic Approaches 406
- 16.2.4.4 Knowledge-Based Systems 407
- 16.2.4.5 Reactive Metabolites 407
- 16.2.5 Current Scientific and Political Developments Regarding Metabolism and Toxicity Prediction 408
- 16.3 Conclusions 408 References 409

XIV Contents

Part Six	Experimental Approaches to Study Metabolism 413
17	In Vitro Models for Metabolism: Applicability for
	Research on Food Bioactives 415
	Natalie D. Glube and Guus Duchateau
17.1	Introduction 415
17.1.1	Bioavailability 416
17.1.2	Intestinal Absorption 416
17.1.3	First-Pass Metabolism 418
17.2	Classification of In Vitro Models for Metabolism 418
17.3	Modifications via Gut (Colon) Microflora 419
17.3.1	Background Information 419
17.3.2	In Vitro Models 420
17.3.2.1	Fecal Slurry 421
17.3.2.2	Isolated Pure Bacterial Cultures 421
17.3.2.3	Complex Intestinal Models (TIM-2) 421
17.4	Intestinal (Gut Wall) Metabolism 421
17.4.1	Background Information 421
17.4.2	In Vitro Models 422
17. 4 .2.1	Tissue Intact Models 423
17.4.2.2	Subcellular and Cellular Models 423
17.5	Hepatic Metabolism 423
17.5.1	Background Information 423
17.5.2	In Vitro Models 424
17.5.2.1	Supersomes: Recombinant Phase I and
	Phase II Enzymes 424
17.5.2.2	•
17.5.2.3	S9 Fractions 426
17.5.2.4	Hepatocyte Cell Lines 426
17.5.2.5	
17.5.2.6	
17.5.2.7	Hepatocytes in Culture 429
17.6	Pharmacokinetic Data Obtainable from In Vitro Metabolism
	Models 431
17.6.1	Pharmacokinetic Analysis 431
17.6.1.1	•
	Formation 432
17.6.1.2	Mathematical Models for Metabolism: Well-Stirred,
	Parallel Tube, and Dispersion Models 432
17.7	Assay Validation 433
17.7.1	Selection and Preparation of Reference Compounds 433
17.7.2	Analytics 434
17.7.3	Theoretical Steps to Establish an In Vitro Model 434
17.8	Conclusions 435
17.8.1	What Can We Summarize from the Literature? 435

- 17.8.2 What Questions We Wish to Have Answered Will Determine Which Model We Select 436 References 438
- 18 In Vitro Approaches to Study Drug–Drug Interactions 441 Stephen S. Ferguson and Jessica A. Bonzo
- 18.1 Introduction 441
- 18.1.1 Additional Factors Influencing Drug Metabolism 442
- 18.2 Inhibition of Drug Metabolism 444
- 18.2.1 In Vitro Models for Predicting Inhibition of Drug Metabolism 444
- 18.2.1.1 Human Liver Microsomes 445
- 18.2.1.2 S9 and Cytosol 456
- 18.2.1.3 Recombinant Enzymes 457
- 18.2.1.4 Primary Hepatocytes 458
- 18.3 Transcriptional Regulation of Metabolism 460
- 18.3.1 Gene Induction Pathways 460
- 18.3.2 Gene Repression/Suppression 462
- 18.3.3 In Vitro Models for Predicting Induction of Drug Metabolism Enzymes 463
- 18.3.3.1 Ligand Binding Assays 463
- 18.3.3.2 Gene Reporter Assays 465
- 18.3.3.3 Cellular Models for Induction Studies 466
- 18.3.3.4 Induction Assays in Cellular Models 468
- 18.3.3.5 Treatment with Control and Test Compounds 470
- 18.3.3.6 Gene Expression in Cellular Models for Induction 471
- 18.3.3.7 Enzymatic Activity in Metabolically Competent Cellular Models of Induction 474
- 18.4 Next-Generation Models and Concluding Remarks 474 References 477
- 19 Metabolite Detection and Profiling 485
 - lan D. Wilson
- 19.1Introduction485
- 19.2 Chromatography 486
- 19.3 Mass Spectrometry 487
- 19.4 Sample Preparation for LC-MS-Based Metabolite Profiling 490
- 19.5 Metabolic Profiling by LC-MS 491
- 19.5.1 Metabolic Stability and Cytochrome P450 Inhibition Assays 491
- 19.5.2 Metabolite Profiling, Detection, and Identification from *In Vivo* and *In Vitro* Studies 492
- 19.5.3 Reactive Metabolite Detection 496
- 19.6 Conclusions 496 References 497

Index 499