

Contents

Dedication	<i>V</i>
List of Contributors	<i>XXI</i>
Preface	<i>XXVII</i>
A Personal Foreword	<i>XXXI</i>

Volume 68a

Part I Introduction to Lead Generation *1*

1	Introduction: Learnings from the Past – Characteristics of Successful Leads	3
	<i>Mike Hann</i>	
	Acknowledgments	<i>10</i>
	References	<i>10</i>
2	Modern Lead Generation Strategies	13
	<i>Jörg Holenz and Dean G. Brown</i>	
2.1	Lead Generation Greatly Influences Clinical Candidate Quality	<i>14</i>
2.2	Screening of Compound Libraries has Undergone a Major Paradigm Change	<i>15</i>
2.3	New Chemical Modalities are Available to Tackle Difficult Targets	<i>15</i>
2.4	As Demands have Increased, New Lead Generation Methods Emerged	<i>16</i>
2.5	How do Lead Generation Chemists Meet These Challenges and Subsequently Provide Their Lead Optimization Colleagues with High-Quality Lead Series?	<i>17</i>
2.5.1	Learnings can be Drawn from LG Project Failures	<i>17</i>
2.5.2	How Many Compounds to Screen to Generate High-Quality Leads?	<i>18</i>
2.5.3	Which Compounds to Screen to Generate High-Quality Leads?	<i>19</i>
2.5.4	Developing Project-Customized, Concerted, and Comprehensive Lead Generation Strategies will Increase LG Success Rates: the <i>CREATION</i> of Leads	<i>20</i>
2.5.5	Selecting the Target Defines LG Success Rates	<i>21</i>

- 2.5.6 Lead Generation should be Complemented by Auxiliary Technologies to Characterize Hits 21
- 2.5.7 Phenotypic Screens are Often Complemented by a Chemical Biology Arm 22
- 2.5.8 The Lead Generation Strategy is Defined by the Budget Allocated 22
- 2.5.9 Cost-Efficient but Information-Rich Lead Generation Strategies 23
- 2.5.10 The Revival of Potency as the Most Important Lead Criterion? 24
- 2.5.11 When has a LG Campaign Delivered Successfully? 27
- References 31

Part II The Importance of Target Identification for Generating Successful Leads 35

- 3 "Ligandability" of Drug Targets: Assessment of Chemical Tractability via Experimental and *In Silico* Approaches 37
Udo Bauer and Alexander L. Breeze
 - 3.1 Introduction 37
 - 3.2 The Concept of Ligandability 39
 - 3.2.1 General Characteristics of Ligandable Targets 39
 - 3.3 The Intersection of Ligandability and Human Disease Target Space 40
 - 3.3.1 Experimental Techniques for Assessing Target Ligandability 42
 - 3.3.1.1 High-Throughput Screening and Subset/"Validation Set" Screening 43
 - 3.3.1.2 Fragment Screening 44
 - 3.4 Practical Examples of the Use of Fragment Screening for Ligandability Assessment 50
 - 3.4.1 Chemical Tractability Assessment by *in silico* Approaches 54
 - 3.4.1.1 Pocket-Finding Algorithms 54
 - 3.4.1.2 Discrimination Functions and Validation Sets 55
 - 3.4.1.3 Simulation-Based Methods for Identifying Interaction Potentials 56
 - 3.5 Conclusions and Outlook 56
 - References 58
- 4 Chemistry-Driven Target Identification 63
Iván Cornella-Taracido, Ryan Hicks, Ola Engkvist, Adam Hendricks, Ronald Tomlinson, and M. Paola Castaldi
 - 4.1 Introduction 63
 - 4.2 Chemistry-Driven Target Discovery: Enabling Biology 65
 - 4.2.1 Biological Samples 65
 - 4.2.2 Cells Cultured in 2D 66
 - 4.2.3 Cells Cultured in 3D, Organoids, and Tissues 67
 - 4.2.4 Nonhuman Cells and Whole-Organism Screening 68
 - 4.2.5 Functional Assays and Readouts 68
 - 4.3 Chemistry for Target Discovery 71
 - 4.3.1 Screening Deck Selection 71

4.3.2	Triaging and Prioritization of Chemical Matter	72
4.3.3	SAR Expansion and Probe Synthesis for Target Deconvolution	73
4.4	Small-Molecule Target Identification Techniques	75
4.4.1	<i>In Silico</i> Target Deconvolution	75
4.4.2	Biochemical Profiling	77
4.4.3	Target Deconvolution Correlational Tools	78
4.4.4	Subcellular Localization	79
4.4.5	Chemical Genetics	79
4.4.6	Affinity Chemical Proteomics	81
4.4.7	Target Corroboration	84
4.5	Conclusions	86
	References	89

Part III Hit Generation Methods 93

5	Lead Generation Based on Compound Collection Screening	95
	<i>Dirk Weigelt and Ismet Dorange</i>	
5.1	Introduction	95
5.2	Screening of Existing Collections: the General Workflow	96
5.2.1	High-Throughput Screening	96
5.2.2	Medium-Throughput Screening: Selection Methods	98
5.3	Generation of New Screening Compounds	99
5.3.1	Collection Enhancement Programs	102
5.3.2	Library Design and Compound Selection	102
5.3.2.1	Number of Dimensions	103
5.3.2.2	Enumeration and Filtering	104
5.3.2.3	Layout	106
5.3.3	Focus on Synthetic Feasibility	107
5.3.3.1	Multicomponent Reactions	107
5.3.3.2	Click Chemistry	108
5.3.3.3	Diversity-oriented Synthesis	108
5.3.4	Structure-driven Approaches	109
5.3.4.1	Privileged Structures	110
5.3.4.2	Structure-driven Approaches Toward Uncharted Territory	112
5.3.5	Target Focus	114
5.3.5.1	Kinases	114
5.3.5.2	G-Protein-Coupled Receptors	115
5.3.5.3	Ion Channels	116
5.3.5.4	Protein-Protein Interactions	117
5.4	Other Concepts	117
5.4.1	Natural Products	118
5.4.2	DNA-Encoded Libraries	119
5.4.3	Spatially Addressed Libraries	120
5.4.4	On-bead Screening	120
5.4.5	Dynamic Combinatorial Chemistry	121

5.4.6	Cocktails and Mixtures	121
5.5	Summary and Outlook	122
	References	123
6	Fragment-Based Lead Generation	133
	<i>Ivan V. Efremov and Daniel A. Erlanson</i>	
6.1	Introduction	133
6.2	Screening Methods	135
6.3	Hit Validation	137
6.4	Ligand Efficiency and Other Metrics	138
6.5	Hit Optimization	139
6.6	Fragment Growing	140
6.7	Fragment Linking	144
6.8	Protein–Protein Interactions	147
6.9	GPCRs	151
6.10	Computational Approaches	152
6.11	Conclusions	153
	References	154
7	Rational Hit Generation	159
	<i>Bernd Wellenzohn and Alexander Weber</i>	
7.1	Introduction	159
7.2	Lead Generation: Transition State and Substrate Analogs	161
7.3	Hit Generation by Rational Library Design	165
7.4	Hit Generation by Virtual Screening	167
7.4.1	Structure-based VS in Enumerated Molecules	170
7.4.2	Ligand-based VS in Nonenumerated Virtual Chemical Spaces	171
7.5	Hit Generation by Scaffold Replacement Technologies	173
7.6	Hit Generation by Chemogenomics Approaches	174
7.7	Summary	178
	References	178
8	Competitive Intelligence–based Lead Generation and Fast Follower Approaches	183
	<i>Yu Jiang, Ziping Liu, Jörg Holenz, and Hua Yang</i>	
8.1	Introduction	183
8.2	Competitive Intelligence-based Approach	185
8.2.1	Example A: A Case Study for the Hybrid Strategy	190
8.2.2	Example C: A Case Study for the Fused Strategy	192
8.2.3	Example C: A Case Study for the Fused Strategy	193
8.2.4	Example D: A Case Study for the Fused Strategy	196
8.2.5	Example E: A Case Study for the Chimera Strategy	197
8.3	Fast Follower Approach	201
8.3.1	Salfanilamide-based Fast Follower Approaches	202
8.3.2	Omeprazole-based Fast Follower Approaches	203
8.3.3	Rimonabant-based Fast Follower Approach	210
	References	214

9 Selective Optimization of Side Activities: An Alternative and Promising Strategy for Lead Generation 221

Norbert Handler, Andrea Wolkerstorfer, and Helmut Buschmann

- 9.1 Introduction 221
- 9.1.1 Drug Selectivity and Unwanted or Desired Side Effects 222
- 9.2 Definition, Rational, and Concept of the SOSA Approach 223
- 9.2.1 Multiple Ligands and Polypharmacology 224
- 9.2.2 Safety and Bioavailability 225
- 9.3 Drugs in Other Drugs: Drug as Fragments 225
- 9.4 Drug Repositioning and Drug Repurposing 226
- 9.4.1 Old Drugs 226
- 9.5 The SOSA Approach and Analog Design 227
- 9.6 Patentability and Interference Risk of the SOSA Approach 230
- 9.6.1 Analogization, Optimization, and Isosterism 230
- 9.7 Case Studies and Examples 231
- 9.7.1 Sulfonamides 231
- 9.7.2 Morphine Analogs 232
- 9.7.3 Warfarin 232
- 9.7.4 Sildenafil (Viagra) 232
- 9.7.5 Thalidomide Analogs 233
- 9.7.6 Bupropion 234
- 9.7.7 Chlorpromazine 235
- 9.7.8 Chlorothiazide 235
- 9.7.9 Propranolol 235
- 9.7.10 Minaprine Analogs 236
- 9.7.11 Viloxazine Analogs 237
- 9.7.12 Methylation in the SOSA Strategy of Drug Design 237
- 9.7.13 Discovery of New Antiplasmodial Compounds 239
- 9.7.14 Drugs Acting on Central Nervous System Targets as Leads for Non-CNS Targets 241
- 9.7.15 Mexiletine Derivatives as Orally Bioavailable Inhibitors of Urokinase-Type Plasminogen Activator 242
- 9.7.16 Amiloride Analogs as Inhibitors of the Urokinase-type Plasminogen Activator 245
- 9.7.17 Flavonoids with an Oligopolysulfated Moiety: A New Class of Anticoagulant Agents 246
- 9.7.18 Clioquinol 249
- 9.8 Conclusions 251
- References 252

10 Lead Generation for Challenging Targets 259

Jinqiao Wan, Dengfeng Dou, Hongmei Song, Xian-Hui Wu, Xuemin Cheng, and Jin Li

- 10.1 Introduction 259
- 10.2 DNA-Encoded Library Technology in Lead Generation 260

10.2.1	Background	260
10.2.2	DNA-Recorded Synthesis-Assisted Libraries	262
10.2.3	DNA-Templated Synthesis-Assisted Libraries	264
10.2.4	Encoded Self-Assembling Chemical Libraries	266
10.2.5	Summary and Perspective	267
10.3	Stapled Peptide	276
10.3.1	Background	276
10.3.2	Structure, Design, and Synthesis of Stapled Peptide	278
10.3.2.1	Stapled Peptide Structure	278
10.3.2.2	Stapled Peptide Design	280
10.3.2.3	Stapled Peptide Synthesis	282
10.3.3	Stapled Peptide Solution α -Helix Conversion Measurement	283
10.3.4	Stapled Peptide Affinity Evaluation and α -Helix Content Correlation	284
10.3.4.1	Surface Plasmon Resonance Binding Assays	284
10.3.4.2	Fluorescence Polarization Assay	284
10.3.4.3	Stapled Peptide Affinity and α -Helix Content Correlation	285
10.3.5	Stapled Peptide Permeability	286
10.3.6	Peptide Stability Assay	288
10.3.7	Outlook	288
10.4	Phenotypic Screening	289
10.4.1	Introduction	289
10.4.2	Basics for Establishing a Phenotypic Screen	291
10.4.2.1	Identify a “Druggable” Phenotype and the Type of Readout	291
10.4.2.2	Assay Design	291
10.4.2.3	Hit Selection and Secondary Assay	291
10.4.3	Typical Phenotypic Assays	292
10.4.3.1	Cell-Viability Assay	292
10.4.3.2	Fluorescent Imaging Plate Reader Technology	293
10.4.3.3	High-Content Screening	293
10.4.4	<i>In Vitro</i> Phenotypic Screening	293
10.4.4.1	Classic Phenotypic Screening	293
10.4.4.2	Patient-Derived Stem Cell in Drug Discovery	294
10.4.4.3	Phenotypic Screening on iPSC-Derived Disease Models	295
10.4.4.4	High-Content Cytotoxicity Screening by iPSC-Derived Hepatocytes	296
10.5	Summary	297
	References	298
11	Collaborative Approaches to Lead Generation	307
	<i>Fabrizio Giordanetto, Anna Karawajczyk, and Graham Showell</i>	
11.1	Introduction	307
11.2	Creativity	308
11.3	Speed	308
11.4	Risk Sharing	308
11.5	Intellectual Property	309
11.6	Costs	309

11.7	Management	310
11.8	Lilly's Open Innovation Drug Discovery	310
11.9	Molecular Library Program	312
11.10	EU Openscreen	314
11.11	European Lead Factory	315
11.12	Medicines for Malaria Venture	317
11.13	Open Source Malaria Project	320
11.14	Drugs for Neglected Diseases Initiative	320
11.15	Open Lab Foundation	321
11.16	Scientists Against Malaria	322
11.17	Open Source Drug Discovery	323
11.18	TB Alliance	323
11.19	Summary	324
	References	325

Volume 68b

Dedication V

List of Contributors XXI

Part IV Converting Hits to Successful Leads 329

12	A Medicinal Chemistry Perspective on the Hit-to-Lead Phase in the Current Era of Drug Discovery	331
	<i>Dean G. Brown</i>	
12.1	Introduction	331
12.2	Active to Hit Processes	333
12.3	Target Potency: Energetics of Binding	336
12.4	Addressing Vast Chemical Space: HtL Strategies	345
12.5	Matched Pair Analysis	348
12.6	The Role of Hydrophobicity and HtL	351
12.7	Probing H-Bond Donors and Acceptors	353
12.8	Structure Based DD in HtL	356
12.9	Statistical Molecular Design	358
12.10	Hit to Lead is not Lead Optimization	359
12.11	Summary	362
	References	363
13	Molecular Recognition and Its Importance for Fragment-Based Lead Generation and Hit-to-Lead	367
	<i>Thorsten Nowak</i>	
13.1	Introduction	367
13.2	Brief Summary of the Main Factors that Govern Molecular Interactions	368

13.3	Thermodynamics of Molecular Interactions and Impact on Hit Finding and Optimization	369
13.4	Enthalpy as a Key Decision Tool in Medicinal Chemistry	371
13.5	Importance of Enthalpic Interactions: Drivers of Selectivity and Specificity?	373
13.6	Fragment Screening Hit Optimization: Fragment Linking	374
13.7	Interstitial Waters and Their Usefulness: Case Studies on HSP-90	381
13.8	Fragments to Find Hot Spots in Binding Pockets	385
13.9	Nonclassical Hydrogen Bonds – Interactions of Halogen Atoms with Π -Systems and Carbonyl Groups: Factor Xa and Cathepsin L	386
13.10	Binding Mode Dependency of the Experimental Conditions and Chemical Framework of Ligand	390
13.11	Cooperativity in Binding: DAO or DAAO D-Amino Acid Oxidase	391
	References	394
14	Affinity-Based Screening Methodologies and Their Application in the Hit-to-Lead Phase	401
	<i>Stefan Geschwindner</i>	
14.1	Introduction	401
14.2	Nuclear Magnetic Resonance Spectroscopy	402
14.3	Optical Biosensors: Surface Plasmon Resonance and Optical Waveguide Grating	404
14.4	Isothermal Titration Calorimetry	407
14.5	Thermal Shift Assay	411
14.6	Mass Spectrometry Approaches	412
14.7	Encoded Library Technologies	414
14.8	Emerging Technologies: Microscale Thermophoresis and Backscattering Interferometry	417
	References	418
15	Predictive Methods in Lead Generation	425
	<i>Matthew D. Segall and Peter Hunt</i>	
15.1	Introduction	425
15.2	Compound Property Prediction	427
15.3	Multiparameter Optimization: Identifying High-Quality Compounds	430
15.3.1	Drug-like Properties	430
15.3.2	Filters	431
15.3.3	Desirability Functions and Probabilistic Scoring	432
15.3.4	Pareto Optimization	435
15.3.5	Example	436
15.4	<i>De Novo</i> Design: Guiding the Exploration of Novel Chemistry	439
15.4.1	Example Application	442
15.5	Selection: Balancing Quality with Diversity	443
15.6	Conclusions	445
	References	447

16	Lead Quality	451
	<i>J. Willem M. Nissink, Sebastien Degorce, and Ken Page</i>	
16.1	Introduction	451
16.2	Properties in Drug Design	452
16.2.1	Primary Activity Assays	453
16.2.2	Physicochemical Properties	453
16.2.3	DMPK	454
16.2.4	Safety	454
16.2.5	Overall Profiles	456
16.3	Optimizing Properties: Useful Rules, Guides, and Simple Metrics for Early-Stage Projects	457
16.3.1	Rules for Potency: Ligand Efficiency Measures	457
16.3.2	Rules for Safety	462
16.3.3	Rules for DMPK and Mode of Administration: Early-Stage Structure-Based Profiling	464
16.3.3.1	Simple Design Rules for Good DMPK	464
16.3.3.2	Other DMPK Design Rules	465
16.3.4	Multiobjective Optimization	466
16.4	Predicted Dose to Man as a Measure of Early- and Late-Stage Lead Quality	467
16.4.1	Introduction	467
16.4.2	Description of Models and Data	469
16.4.3	Data Supporting Technique	471
16.4.3.1	Matching eD2M Doses with Normalized Observed Clinical Doses	472
16.4.3.2	Matching C_{\max} Values from eD2M and Clinical Studies	472
16.4.4	Flagging Potential Candidate Drugs Using eD2M	473
16.4.5	Determining Properties that Drive eD2M Predictions for a Series	474
16.5	Summary	480
	References	481

Part V Hypothesis-driven Lead Optimization 487

17	The Strategies and Politics of Successful Design, Make, Test, and Analyze (DMTA) Cycles in Lead Generation	489
	<i>Steven S. Wesolowski and Dean G. Brown</i>	
17.1	DMTA Cycles: Perspectives from History	490
17.2	Test: What Assays, in What Order, and Why?	494
17.3	Additional Advice for “Test” Component of DMTA	496
17.4	Design: What to Make and Why?	496
17.5	Additional Advice for “Design” Component of DMTA	500
17.6	Make: Challenges and Strategies for Synthesis	501
17.7	Additional Advice for the “Make” Component of DMTA	502

- 17.8 Analyze: Making Sense of What's Been Done and Formulating Sensible Plans for the Next Designs 502
- 17.9 Additional Advice for "Analyze" Component of DMTA 508
- 17.10 Results: Do Lead Optimization Teams Get What They Need? 508
- References 509

Part VI Recent Lead Generation Success Stories 513

- 18 **Lead Generation Paved the Way for the Discovery of a Novel H₃ Inverse Agonist Clinical Candidate 515**
Christophe Genicot and Laurent Provins
 - 18.1 Introduction 515
 - 18.2 Hit Identification 517
 - 18.3 Lead Generation 521
 - 18.3.1 Exploration of Oxazoline Substitution 523
 - 18.3.2 Rigidification of Propoxy Linker 531
 - 18.3.3 Oxazoline/Oxazole Surrogates: Lactams 533
 - 18.3.4 Conclusions 536
 - 18.4 Lead Optimization and Candidate Selection 537
 - 18.5 Conclusions 543
 - Acknowledgments 544
 - References 544
- 19 **Vorapaxar: From Lead Identification to FDA Approval 547**
Samuel Chackalamannil and Mariappan Chelliah
 - 19.1 Introduction 547
 - 19.2 Background Information on Antiplatelet Agents 549
 - 19.3 Thrombin Receptor (Protease-activated Receptor-1) Antagonists as a Novel Class of Antiplatelet Agents 550
 - 19.4 Mechanism of Thrombin Receptor Activation 550
 - 19.5 Preclinical Data Supporting the Antiplatelet Effect of Thrombin Receptor Antagonists 551
 - 19.6 Himbacine-derived Thrombin Receptor Antagonists 552
 - 19.6.1 Lead Identification 552
 - 19.6.2 Lead Generation of Himbacine-derived Thrombin Receptor Antagonist Hit 553
 - 19.6.2.1 Structure–Activity Relationship Studies 555
 - 19.6.2.2 First-Generation Thrombin Receptor Antagonists 556
 - 19.6.2.3 *In vivo* Metabolism of Himbacine Derivatives 558
 - 19.6.2.4 Generation of Aryl Himbacine Leads 561
 - 19.6.2.5 Second-Generation Leads that Incorporate Heteroatoms in the C-ring 562
 - 19.6.2.6 Identification of nor-seco Himbacine Lead 564

19.6.3	Discovery of Vorapaxar (SCH 530348)	565
19.6.3.1	Clinical Studies of Vorapaxar	567
19.7	Conclusions	569
	Abbreviations	570
	Acknowledgments	570
	References	571
20	Lead Generation Approaches Delivering Inhaled β_2-Adrenoreceptor Agonist Drug Candidates	575
	<i>Michael Stocks and Lilian Alcaraz</i>	
20.1	Introduction	575
20.2	Lead Generation Exercises to Discover β_2 AR Agonist Clinical Candidates	577
20.3	AstraZeneca Lead Generation Exercises to Discover β_2 AR Agonist Clinical Candidates	587
20.4	Summary	593
	References	593
21	GPR81 HTS Case Study	597
	<i>Eric Wellner and Ola Fjellström</i>	
21.1	General Remarks	597
21.2	The Target	598
21.3	Screening Cascade	599
21.4	Compound Selection (10 K Validation Set)	602
21.5	HTS	606
21.5.1	CSE	608
21.5.2	Single-Concentration Counterscreen	614
21.5.3	Clustering	615
21.5.4	Cluster Expansion and Nearest Neighbours	618
21.6	Hit Evaluation	618
21.6.1	Potency, Efficacy, and Curves	618
21.6.2	Binding Kinetics	621
21.6.3	Concentration–Response Counterscreen	622
21.6.4	Hit Assessment	622
21.6.4.1	Size and Lipophilicity Efficiency Assessment	622
21.6.4.2	Secondary Pharmacology Assessment	626
21.6.5	Secondary Screening Cascade and Hit Expansion	630
21.6.6	Biological Effect Assay	634
21.7	Alternative Lead Generation Strategies	638
21.7.1	Pepducins and Other Modified Peptides	641
21.8	Conclusions	645
	References	646
22	Development of Influenza Virus Sialidase Inhibitors	651
	<i>Mauro Pascolutti, Robin J. Thomson, and Mark von Itzstein</i>	
22.1	Introduction	651

22.2	Targets for Anti-influenza Drug Development: Receptor Binding and Receptor Cleavage	652
22.2.1	Targeting Receptor Binding by Haemagglutinin	654
22.2.2	Targeting Receptor Destruction by Sialidase	655
22.2.3	Influenza Virus Sialidase: Structure and Mechanism	656
22.3	Development of Influenza Virus Sialidase Inhibitors	658
22.3.1	The Development of Zanamivir: Proof of Concept and First-in-Class Sialidase Inhibitor Drug	659
22.3.1.1	Template Selection	659
22.3.1.2	Structure-based Inhibitor Design	662
22.3.1.3	X-Ray Crystallographic Confirmation of Inhibitor Binding Mode	665
22.3.1.4	Selectivity for Influenza Virus Sialidase over Human Sialidases	666
22.3.1.5	Efficacy against Virus Replication	667
22.3.1.6	Mode of Administration of the Highly Polar Drug	667
22.3.1.7	Modifying the Presentation of Zanamivir: Prodrugs and Multivalency	668
22.3.2	Sialidase Inhibitor Development on Noncarbohydrate Scaffolds	671
22.3.2.1	A Sialidase Inhibitor Based on a Cyclohexene Scaffold: The Development of Oseltamivir	671
22.3.2.2	A Sialidase Inhibitor Based on a Cyclopentane Scaffold: The Development of Peramivir	673
22.3.3	Monitoring Resistance to Influenza Virus Sialidase Inhibitors	675
22.4	Summary and Future Directions	676
	References	676
23	The Discovery of Cathepsin A Inhibitors: A Project-Adapted Fragment Approach Based on HTS Results	687
	<i>Sven Ruf, Christian Buning, Herman Schreuder, Wolfgang Linz, Dominik Linz, Hartmut Rütten, Georg Horstick, Markus Kohlmann, Katja Kroll, Klaus Wirth, and Thorsten Sadowski</i>	
23.1	General Background	687
23.2	Cathepsin A enzyme	687
23.2.1	Structural Biology and Catalytic Mechanism	687
23.2.2	Structural and Catalytic Functions of CatA	689
23.2.3	Tissue Distribution and Substrates	689
23.2.4	Natural Products and Synthetic Peptides as Inhibitors of CatA	690
23.3	CatA and the Link to Cardiovascular Disease	691
23.4	Lead Discovery	692
23.4.1	High-Throughput Screening and Data Analysis	692
23.4.2	Evaluation of Hit Series	693
23.4.2.1	Covalent Inhibitor Series	693
23.4.2.2	Malonamide Series	697
23.4.2.3	Pyrazolone Hit Series	698
23.4.3	Explorative Chemistry Delivers a Novel Lead Structure	699
23.4.3.1	Crystal Structure of 9b Bound to CatA	705

23.5	Lead Optimization	705
23.6	Toward an <i>in vivo</i> Proof of Concept	711
23.7	Summary and Conclusions	713
	References	714
24	Lead Structure Discovery for Neglected Diseases: Product Development Partnerships Driving Drug Discovery	717
	<i>Jeremy N. Burrows and Takushi Kaneko</i>	
24.1	Introduction	717
24.2	Malaria and Medicines for Malaria Venture	719
24.3	Malaria Lead Generation Strategy	719
24.4	Hit Identification Strategies	722
24.5	Optimization of a Marketed Antimalarial Chemotype	723
24.6	Target-Based Approaches	723
24.7	Asexual Blood-Stage Phenotypic Screening	724
24.8	Whole-Cell Screening: Results	725
24.9	Repositioning of Clinical Candidates Developed for Other Indications	726
24.10	Case Studies	727
24.10.1	Dihydroorotate Dehydrogenase (DHODH)	727
24.10.2	Whole-Cell Screening	728
24.11	Screening for Malaria Eradication	729
24.12	Tuberculosis and the Global Alliance for Tuberculosis Drug Development (TB Alliance)	729
24.13	Target Product Profiles	730
24.14	TB Alliance's Mission	730
24.15	Hit Generation Strategies for TB	732
24.16	Examples of Phenotypic Screens	733
24.17	Conclusions	741
	References	741
25	A Fragmentation Enumeration Approach to Generating Novel Drug Leads	747
	<i>Pravin S. Iyer and Manoranjan Panda</i>	
25.1	Introduction	747
25.2	Principle	748
25.3	Research Methodology	748
25.3.1	Fragmentation	749
25.3.1.1	Origin of Parent Molecules	749
25.3.1.2	Cores and Daughters	749
25.3.1.3	Nonflat Cores	751
25.3.2	Intelligent Recombination and Enumeration	754
25.4	Evaluation	754
25.4.1	Preliminary Experimental Evaluation	755
25.4.2	<i>In Silico</i> Evaluation	755

25.4.3 Virtual Screening Using Enzyme–Ligand Docking 756

25.5 Summary 758

References 759

Index 761