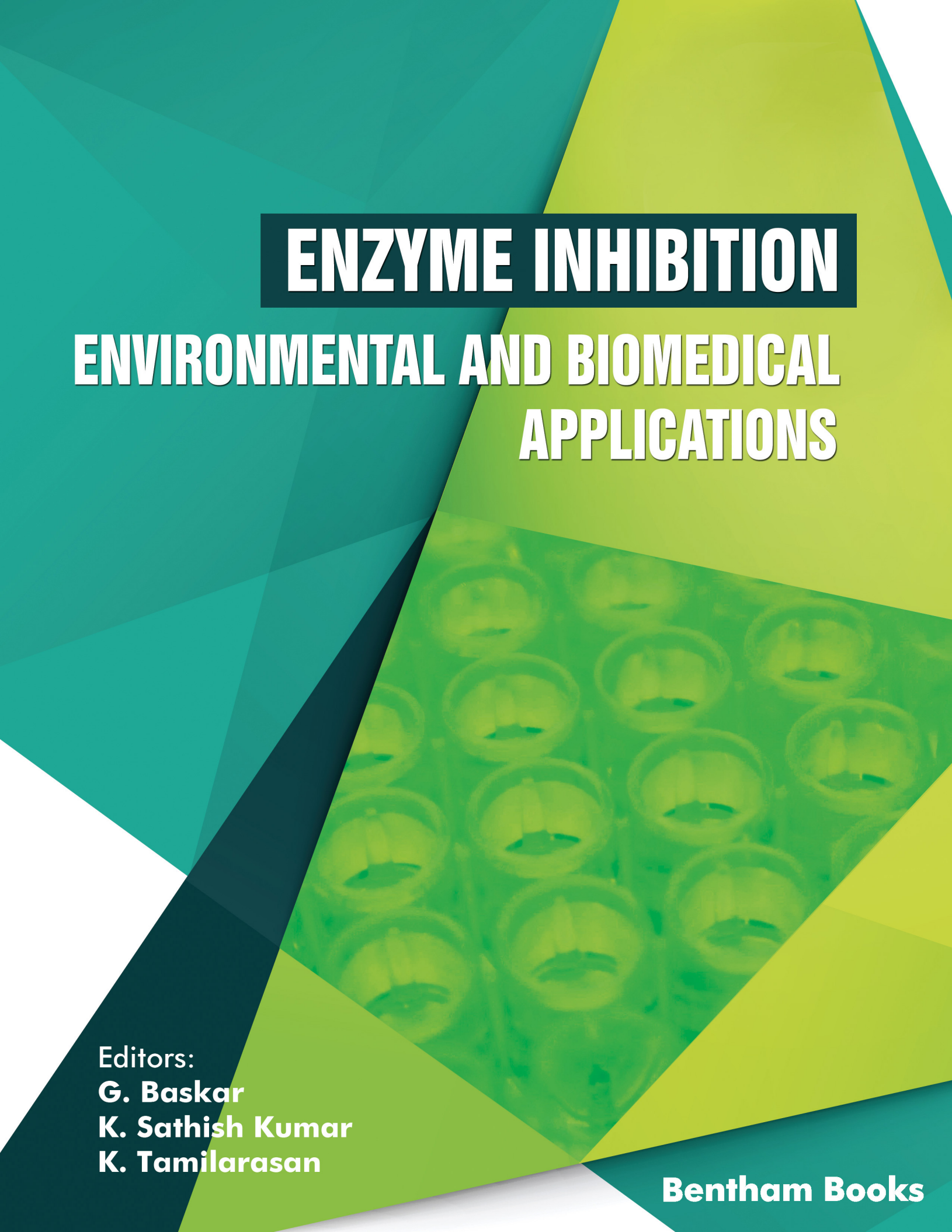
ENZYME INHIBITION

ENVIRONMENTAL AND BIOMEDICAL APPLICATIONS



Editors:
G. Baskar
K. Sathish Kumar
K. Tamilarasan

Bentham Books

Frontiers in Enzyme Inhibition

(Volume 1)

Enzyme Inhibition - Environmental and Biomedical Applications

Edited by

G. Baskar
Department of Biotechnology,
St. Joseph's College of Engineering,
Chennai,
India

K. Sathish Kumar
Department of Chemical Engineering,
SSN College of Engineering,
Chennai – 603110,
India

&

K. Tamilarasan
Department of Chemical Engineering,
SRM University,
Chennai – 603203,
India

Frontiers in Enzyme Inhibition

Volume # 1

Enzyme Inhibition - Environmental and Biomedical Applications

Editors: G. Baskar, K. Sathish Kumar and K. Tamilarasan

ISBN (Online): 978-981-14-6082-1

ISBN (Print): 978-981-14-6080-7

ISBN (Paperback): 978-981-14-6081-4

need for a court order if at any point you breach any terms of this License Agreement. In no event will any delay or failure by Bentham Science Publishers in enforcing your compliance with this License Agreement constitute a waiver of any of its rights.

3. You acknowledge that you have read this License Agreement, and agree to be bound by its terms and conditions. To the extent that any other terms and conditions presented on any website of Bentham Science Publishers conflict with, or are inconsistent with, the terms and conditions set out in this License Agreement, you acknowledge that the terms and conditions set out in this License Agreement shall prevail.

Bentham Science Publishers Pte. Ltd.
80 Robinson Road #02-00
Singapore 068898
Singapore
Email: subscriptions@benthamscience.net

CONTENTS

PREFACE i

LIST OF CONTRIBUTORS iv

CHAPTER 1 INTRODUCTION TO ENZYME INHIBITION – ENVIRONMENTAL AND BIOMEDICAL APPLICATIONS 1

G. Baskar, R. Aiswarya, K. Sathish Kumar and *K. Tamilarasan*

CONCLUSION 2

CONSENT FOR PUBLICATION 3

CONFLICT OF INTEREST 3

ACKNOWLEDGEMENTS 3

REFERENCES 3

CHAPTER 2 ENZYME INHIBITION IN THERAPEUTIC APPLICATIONS 5

A. Saravanan, R. Jayasree1, T.R. Sundaraman, R.V. Hemavathy, S. Jeevanantham P. Senthil Kumar and *P. R. Yaashikaa*

INTRODUCTION 5

ENZYME INHIBITION 6

TYPES OF ENZYME INHIBITION 8

Reversible Enzyme Inhibition 8

Competitive Reversible Inhibition 9

Non-competitive Reversible Inhibition 9

Uncompetitive Reversible Inhibition 10

Irreversible Inhibition 10

ENZYME INHIBITION: MECHANISM AND SCOPE 11

Inhibition of Asparaginase 11

Inhibition of Glycosidases 11

SOME THERAPEUTIC APPLICATIONS 12

Serine Protease Inhibitors 12

Serine Protease Inhibitors for Cancer Treatment 12

Serine Protease Inhibitors as Antiviral Specialists 12

Phosphodiesterases 12

Matrix Metalloproteinases 13

Histone Deacetylases 13

CONCLUSION 14

CONSENT FOR PUBLICATION 14

CONFLICT OF INTEREST 14

ACKNOWLEDGEMENTS 15

REFERENCES 15

CHAPTER 3 ANALYTICAL ASPECTS OF BIOSENSOR BASED ON ENZYME INHIBITION 17

A. Saravanan, S. Jeevanantham, P. Senthil Kumar and *P. R. Yaashikaa*

INTRODUCTION 17

Most Sensors Comprise of Three Chief Segments 18

WORKING OF BIOSENSORS 19

GENERAL FEATURES OF BIOSENSORS 20

BIOCOMPONENT 22

PRINCIPLE OF A BIOSENSOR 22

Transducer Selection for Biosensors 23

BIO SENSING METHOD 23

Sorts of Biosensors 23

Calorimetric Biosensors 24
Amperometric Biosensors 24
Optical Biosensors 24
USES OF BIOSENSORS IN VARIOUS FIELDS 25
The Biosensor in Light of Enzyme Inhibition 25
Strategies in View of Enzyme Inhibition Biosensors 26
Strategies in Light of Enzyme Inhibition Biosensing Frameworks 26
Nonstop Strategies in View of Enzyme Inhibition 26
IMMOBILIZATION EFFECT ON BIOSENSOR RESPONSE 27
PARAMETERS INFLUENCING THE EXECUTION OF ENZYMATIC BIOSENSORS ... 27
Impact of pH 27
Impact of Substrate Concentration 28
ENZYME INHIBITION BASED BIOSENSOR 28
Glucose Oxidation Inhibition 28
Tyrosinase Inhibition 28
Urease Inhibition 30
Cholinesterase Inhibition 31
ASSURANCE OF PESTICIDES 32
CONCLUSION 33
CONSENT FOR PUBLICATION 33
CONFLICT OF INTEREST 33
ACKNOWLEDGEMENTS 33
REFERENCES 33

CHAPTER 4 RNA SILENCING IN ENZYME INHIBITION AND ITS ROLE IN CROP IMPROVEMENT 36
S. Justin Packia Jacob
INTRODUCTION 36
Antisense System in Nature 37
Ribozymes 38
Antisense RNA 38
Antisense Mechanism 39
Delayed Fruit Ripening in Tomato by Silencing Polygalacturonase (PG) Enzyme using Antisense RNA 42
RNA Interference (RNAi) 43
Lysine and Threonine Increase in Maize by Silencing LKR/SDH Enzyme 43
High Amylose Sweetpotato using Silencing of 1,4-α-D-glucan-6-α- [1,4-glucan--transferase 44
Low Gossypol Cotton Seed by Silencing 45
Low PhytateRice by Silencing Inositol Phosphate Kinases 46
Enhancing β-Carotene Content in Potato by RNAi-mediated Silencing of the β-Carotene Hydroxylase 47
Lachrymatory Synthase Enzyme Suppression in Tearless Onion 49
CONCLUSION 50
CONSENT FOR PUBLICATION 50
CONFLICT OF INTEREST 51
ACKNOWLEDGEMENTS 51
REFERENCES 51

CHAPTER 5 ENZYME BIOSENSORS BASED ON ENZYME INHIBITION FOR PESTICIDE AND HEAVY METAL DETECTION 59
Elsa Cherian and *G Baskar*

INTRODUCTION 59
HISTORY OF BIOSENSOR 60
TYPES OF BIOSENSOR 61
Antigen-antibody Interaction-based Biosensors 61
Biosensors Based on Nucleic Acid 61
Biosensors Based on Microbial Cells 61
Enzyme Based Biosensors 62
INFLUENCE OF ENZYME INHIBITION ON THE WORKING OF BIOSENSOR 62
APPLICATION OF ENZYME INHIBITION BASED BIOSENSOR IN ENVIRONMENTAL FIELD 63
Pesticide Detection 63
Cholinesterase-Based Biosensors 64
Alkaline Phosphatase Based Biosensor 65
Peroxidase-Based Biosensors 66
Tyrosinase-Based Biosensors 66
Heavy Metal Detection 67
CONCLUSION 68
CONSENT FOR PUBLICATION 68
CONFLICT OF INTEREST 68
ACKNOWLEDGEMENTS 68
REFERENCES 68

CHAPTER 6 RECENT INSIGHTS OF MATRIX METALLOPROTEINASE INHIBITORS IN THERAPEUTIC APPLICATIONS 73
Ravichandran Rathna, Bethu Madhumitha, Ravichandran Viveka and *Ekambaram Nakkeeran*

INTRODUCTION 73
MATRIX METALLOPROTEINASE 74
MATRIX METALLOPROTEINASE INHIBITORS 76
Endogenous Inhibitors 80
Exogenous Inhibitors 81
THERAPEUTIC ACCOMPLISHMENTS 82
Arthritis 82
Autoimmune Disease 83
Inflammations 83
Cancer 84
Cardiovascular Diseases 85
CLINICAL IMPLICATIONS AND OBSTACLES 85
CONCLUDING REMARKS AND FUTURE DIRECTIONS 87
CONSENT FOR PUBLICATION 88
CONFLICT OF INTEREST 88
ACKNOWLEDGEMENTS 88
REFERENCES 88

CHAPTER 7 CURRENT POTENTIALITIES AND PERSPECTIVES OF ENZYME INHIBITION BASED BIOSENSOR 97
Senthil Nagappan, Rajvikram Madurai Elavarasan, Nilavunesan Dhandapani and *Ekambaram Nakkeeran*

INTRODUCTION 97
FUNDAMENTALS OF ENZYME INHIBITION BASED BIOSENSOR 98
Enzyme Inhibitors 98
Enzyme Inhibitors Kinetics 99

Components of Biosensor 100
Transducer 100
Substrate 101
Enzymes 102
Immobilization 102
ENZYME INHIBITION BASED BIOSENSOR IN ENVIRONMENTAL POTENTIALITIES 103
Heavy Metals 103
Pesticides 104
Toxins 105
ENZYME INHIBITION BASED BIOSENSOR IN PHARMACEUTICALS POTENTIALITIES 106
ENZYME INHIBITION BASED BIOSENSOR IN FOOD INDUSTRY 108
APPLICATIONS IN OTHER MAJOR FIELDS 109
FUTURE PERSPECTIVES 111
CONCLUDING REMARKS 112
CONSENT FOR PUBLICATION 112
CONFLICT OF INTEREST 112
ACKNOWLEDGEMENTS 112
REFERENCES 112

CHAPTER 8 PRODUCT INHIBITION IN BIOETHANOL FERMENTATION: AN OVERVIEW 122
E. Raja Sathendra, G. Baskar and *R. Praveen Kumar*
INTRODUCTION 122
LIGNOCELLULOSIC PRETREATMENT 123
Pretreatment Strategy 123
Detoxication of Lignocellulosic Hydrolysate 126
MICROORGANISM FOR ETHANOL FERMENTATION 127
Saccharification and Fermentation 131
Distillation 131
Dehydration 131
INHIBITION OF ETHANOLIC FERMENTATION 132
GROWTH KINETICS 132
PRODUCT INHIBITION VALIDATION 136
CONCLUSION 141
LIST OF ABBREVIATIONS 141
CONSENT FOR PUBLICATION 141
CONFLICT OF INTEREST 141
ACKNOWLEDGEMENTS 141
REFERENCES 142

CHAPTER 9 FERMENTATION STRATEGIES TO MINIMIZE PRODUCT INHIBITION IN BIOETHANOL PRODUCTION 148
Luciana Porto de Souza Vandenberghe, Nelson Libardi Junior, Cristine Rodrigues, Joyce Gueiros Wanderley Siqueira and *Carlos Ricardo Soccol*
INTRODUCTION 148
BIOETHANOL MICROBIAL STRAINS 149
BIOETHANOL PRODUCTION FROM DIFFERENT FEEDSTOCKS 151
Sucrose Sources 151
Starch Sources 152
Lignocellulose-based Feedstock 152

FEEDSTOCK PRETREATMENT AND ETHANOL PRODUCTION INHIBITION 154
Thermochemical Pretreatments 155
Acid Pretreatments 155
Steam Explosion 156
Ammonia Fiber Expansion (AFEX) 157
Alkaline Pretreatment 157
Hot Pressured Water 157
Organosolv 158
Oxidative Methods 158
ILs 158
Biological Pretreatment 159
Enzymatic Hydrolysis 159
FACTORS THAT AFFECT BIOETHANOL PRODUCTION 160
High Temperature 160
High Bioethanol Concentration 161
Substrate Composition 161
Other Factors 162
IMMOBILIZATION OF CELLS AS A SOLUTION TO REDUCE BIOETHANOL PRODUCTION INHIBITION 163
GENETIC MODIFICATION OF BIOETHANOL-PRODUCING STRAINS 164
CONCLUSION 167
CONSENT FOR PUBLICATION 168
CONFLICT OF INTEREST 168
ACKNOWLEDGEMENTS 168
REFERENCES 168

CHAPTER 10 TOXICITY AND STRUCTURAL ACTIVITY RELATIONSHIP OF PERSISTENT ORGANIC POLLUTANTS 174
Ankur Khare, Pradip Jadhao, Sonam Paliya and *Kanchan Kumari*

INTRODUCTION 175
DIFFERENT POPS (PCBS, PBDES, AND PFOS) KNOWN AND ALTERATION IN ENZYMATIC ACTIVITIES 177
Polychlorinated Biphenyls (PCBs) 177
Polybrominated Diphenyl Ether (PBDEs) 178
Perfluorooctane Sulfonate (PFOS) and Perfluorooctanoic Acid (PFOA) 179
NEUROTOXIC EFFECTS OF POPS 181
Possible Mode of Action(s) 182
Effects on Neurotransmitters System 182
Dose-Response Effects on Intracellular Signalling 182
Effect on Different Cellular Pathways and Molecules Involved 183
Effect on Aryl Hydrocarbon Receptor Pathway 184
Effect on the Immune System 186
Pathway of Genetic Toxicity 186
Effect on Thyroid Hormone System 187
Effect of PCB on Thyroid Hormone System 187
Effect of Polybrominated Diphenyl Ethers on Thyroid Hormone System 187
Effect of Perfluoroalkyl Substances (PFASs) on the Thyroid hormone system 188
MAJOR PATHWAY OF POPS METABOLISM AND ENZYMATIC ALTERATIONS 189
Effect on Cytochrome P (CYP) Enzyme 189
Effect on EROD Activity 190
Effect of PCBs on Xenobiotic Biotransformation Enzymes 191

STRUCTURE ACTIVITY RELATIONSHIP (SAR) 191
Why SAR? 191
Elements of SAR and SAR Models 192
SAR and Associated Toxicity Mechanism 194
CONCLUSION 196
CONSENT FOR PUBLICATION 197
CONFLICT OF INTEREST 197
ACKNOWLEDGEMENTS 197
REFERENCES 197

CHAPTER 11 ENZYME INHIBITORS FOR BREAST CANCER THERAPY 204
,Hariharan Jayaraman, Praveen Kumar Posa Krishnamoorthy, Lakshmi Suresh Mahalakshmi Varadan, Aparna Madan and *Balu Ranganathan*
INTRODUCTION 204
Mammary Gland Anatomical Construction 206
Breast Carcinogenesis 206
Cancer in the Duct 206
Cancer in the Lobules 207
Cancer in the Nipple 208
Enzymes Predominantly in the Breast 209
Aromatase 209
Cyclooxygenases 210
Location 210
Mechanism 210
COX-2 Inhibitors 211
LYSYL OXIDASE 212
Mechanism of Action 212
Genetics 212
Tumorigenesis 212
Mechanism of Action 213
HYDROXYSTEROID DEHYDROGENASES 214
Mechanism of Action 214
17β- Hydroxysteroid Dehydrogenase 214
3β- Hydroxysteroid Dehydrogenase 216
Tousled-like Kinases 217
Drugs Used to Inhibit Enzymes Involved in Breast Cancer 218
Anastrazole 218
Side-effects 219
Exemestane 219
Side-effects 219
Celecoxib 219
Side-effects 220
Drug Inhibitors of HSD17B 220
Drug Inhibitors of HSD3B 221
Potential Drugs as TLK2 Inhibitors 221
CONCLUSION 222
AUTHOR'S CONTRIBUTION 222
CONSENT FOR PUBLICATION 222
CONFLICT OF INTEREST 222
ACKNOWLEDGMENT 222
REFERENCES 222

CHAPTER 12 ENZYME INHIBITION APPLICATIONS IN TREATMENT OF HUMAN VIRAL DISEASES 228
Subasree Sekar, Arthi Udhayachandran and *P.K. Praveen Kumar*
INTRODUCTION 228
MECHANISM OF ANTIVIRAL DRUGS 229
Attachment 230
Entry 230
Uncoating 230
Transcription and Translation 230
Replication 230
Assembly & Maturation 231
Release 231
HUMAN VIRAL DISEASES 231
Chikungunya Virus (CHIKV) 231
Berberine 233
Harringtonine 234
Silymarin 235
Curcumin 236
Daphnane Diterpenoids 237
Dengue Virus 237
Houttuynia Cordata 239
Fucoidan 240
Boesenbergia Rotunda 240
Quercetin 241
Palmatine 242
Geneticin 242
Mycophenolic Acid (MPA) 243
Iminosugars 244
Dasatinib 246
Nipah Virus (NiV) 246
Favipiravir 247
Remdesivir 248
Galectin-1 248
HIV (Human Immunodeficiency Virus) 248
Coumarin 250
Rosmarinic Acid 251
Calanolides 252
Tenofovir 253
Abacavir 253
INFLUENZA 254
Isoquercetin 255
Oseltamivir 256
CONCLUSION 257
CONSENT FOR PUBLICATION 257
CONFLICT OF INTEREST 257
ACKNOWLEDGEMENTS 258
REFERENCES 258

CHAPTER 13 ENZYME INHIBITION APPLICATIONS IN TREATMENT OF NEUROLOGICAL DISORDERS 263
D. Lokapriya and *P.K. Praveen Kumar*

INTRODUCTION 263
NEURODEGENERATIVE DISEASES 264
Alzheimer's Disease (AD) 264
Inhibition of Cholinesterases 265
Drugs 266
Parkinson's Disease (PD) 266
Inhibition of Monoamine Oxidase (MAO) 266
Drugs 268
Stroke 269
Inhibition of Cyclooxygenase (COX) 270
Brain Tumor 271
Inhibition of Matrix Metalloproteinases (MMPs) 271
Inhibition of Phosphoinositide 3-Kinase (PI3k) 272
Inhibition of Ras: Farnesyl Transferase (FTase) 272
Inhibition of Proteasome 273
Inhibition of COX-2 273
Glioblastoma 274
Inhibition of Protein Kinase C (PKC) 274
Inhibition of Histone Acetylation 274
Multiple Sclerosis (MS) 275
Inhibition of Histone Deacetylases (HDAC) 275
ENZYME INHIBITORS FROM PLANTS 276
OTHER ENZYMES 278
Beta-site Amyloid Precursor Protein Cleaving Enzyme-1 [BACE-1] 278
γ-secretase 278
Sirtuin 2 (SIRT2) Deacetylase 278
Phospholipases A2 (PLA2) 278
Glutathione S Transferases (GST) 279
Dipeptidyl Peptidase IV [DPP-IV] 280
Carbonic Anhydrase [CA] 280
CLUSTERED REGULATORY INTERSPACED SHORT PALINDROMIC REPEAT-ASSOCIATED 9 (CRISPR/CAS9) FOR TREATMENT OF NEUROLOGICAL DISORDERS 280
CONCLUSION 283
CONSENT FOR PUBLICATION 283
CONFLICT OF INTEREST 283
ACKNOWLEDGEMENT 283
REFERENCES 283

SUBJECT INDEX 288

PREFACE

Enzyme, a protein molecule exhibits specific activity and binding towards the substrate molecule for the completion of biocatalytic reaction. Enzyme inhibitors are molecules which prevents the functioning and interaction of enzyme by specific substrate or analogue. The enzyme inhibitors alters and slow down the catalytic action of enzyme in various modes. The activity is mainly inhibited by reversible, irreversible inhibition, covalent, non-covalent binding. The inhibitors also act on specific and non-specific site including bacteria, virus, plants and animals. The inhibitors plays a vital role in pharmaceutical and other bio-chemical industries. The enzyme inhibitors are widely used as herbicide and pesticide for the destruction of pathogens. The action of inhibitors has paved way for the development in drug discovery and pharmaceutical industries. The enzyme inhibitors acts as drug molecules (Eg.antimicrobial drug) in the treatment of various disease. These drug deactivates the enzyme needed for the survival of pathogens. Enzyme inhibitors also finds its application in metabolic process by inhibiting the action of enzyme against animal predators. The new insight of enzyme inhibitors in environmental monitoring has gained wide attention among researchers for the development of biosensor. The main objective of this book on "Frontiers in Enzyme Inhibition – Environmental and Biomedical Application" is to provide basic information on recent development in the field of enzymology. This book also explain about the applied inhibition method in drug discovery. This book will mainly focus on enzyme inhibition and its application in pharmaceutical and environment. The book will be highly valuable for students and researchers for enhancing their knowledge on basic concept of applied inhibition in the field drug discovery related projects. This book is compiled based on the basic concept of specific inhibitors in environmental and pharmaceutical applications. The main theme of this book includes

1] Enzyme inhibition in development and assessment of biosensors

2) Product inhibition during fermentation process

3) Inhibition in crop management

4) Inhibition in the development and formulation of drug

This book will give wide knowledge and understanding in the development of drug and biosensors. This book gives a collective information on various mechanism and alterations that are noted during the developmental stage. This book consists of 13 chapters and the summary of each chapters is given below.

Chapter 1 gives a general background and significance of enzyme inhibition. The basic mechanism and scope of inhibitions is listed out in this chapter. This chapter outlines the outstanding application of Serine protease inhibitors in anticancer treatment and regulating blood coagulation factor. The importance of other remedial inhibitors is also summarized in this chapter.

Chapter 2 gives information about the enzyme based biosensors and its typical component. The importance of coupling electrochemical sensors with metabolites and antibodies is summarized. This chapter gives an idea about the intendment parameters influencing during the design of biosensors. The role of biosensor in clinical and management of pesticides is explained in detailed fashion. This chapter also highlight the strategies of enzyme inhibition in bio-sensing frameworks. The purport of biosensors in light of catalyst and its resistant is

explained in this chapter.

Chapter 3 discuss the role of enzyme inhibition in the design and assessment of biosensors. The recent advances and challenges faced during the fabrication of biosensors is explained in this chapter. This chapter explains about the different application of inhibition based biosensor focusing on clinical and environmental management.

Chapter 4 provides applications RNA Silencing in Enzyme Inhibition and its Role in Crop Improvement. Expression of *antisense* genes and the related gene silencing technique has been exploited as an applied *technique in plant* biotechnology for creating "metabolic engineered *plants*" in which the endogenous target gene, which is responsible for the unpleasant character is specifically suppressed. In the present chapter, the down regulations of the enzyme using the gene-silencing technique used in various crop varieties are discussed in detail.

Chapter 5 provides an overview about the history and development of biosensor based on different generation of biochemical signal including antigen –antibody interactions, nucleic acid and microbial cell. The role of enzyme inhibition and development of enzyme based biosensors is explained in this chapter. The importance of enzyme inhibition in the detection of pesticides and heavy metal is discussed in this chapter.

Chapter 6 deals with interesting insights of therapeutic accomplishments of matrix metalloproteinase inhibitors in arthritis, autoimmune disease, inflammations, cancer and cardiovascular disease. The functional role and inhibitors of metalloproteinase is explained in this chapter. This chapter also deals with the clinical implication of matrix metalloproteinase and its effect.

Chapter 7 reviews the potential biosensors based on enzyme inhibition and its application in various field. The kinetic parameter in the design of biosensor is explained in this chapter. The detection level of toxins, heavy metal and pesticides is detailed in this chapter. This chapter also highlights the significance of enzyme inhibition in assessing the drug and its development during various phases. The assessment of safety level of food by enzyme based biosensors is explained in this chapter.

Chapter 8 presents the detailed information on product inhibition during fermentation of ethanol. The cell growth and ethanol inhibitions and related kinetics is explained in this chapter. This chapter also explains about the validation of product inhibition.

Chapter 9 highlights the significance strategies to reduce the inhibition of bioethanol during fermentation process. The production of bioethanol from different strains and source is explained in this chapter. This chapter summarizes the different pre-treatment conditions and factors affecting the inhibition condition is explained in this chapter.

Chapter 10 explains on various Persistent Organic Pollutants (POPs) and its action with respect to the neurotoxic effects emphasising on dose-response and structure-activity relationships (SAR) are discussed in this chapter. The potential modes of actions and alteration in neurotransmitter systems and the mechanisms is discussed in detailed in this chapter.

Chapter 11 gives general introduction about the anatomy of breast carcinogenesis and the enzyme produced predominately produces in breast. The mechanism of different enzymes and the inhibitors used as drug in the treatment of breast cancer is given in this chapter.

Chapter 12 reviews about the various enzyme inhibitor molecule involved in the formulation and development of antiviral drug for HIV Chikungunya, Dengue, Ebola, Influenza, and Nipah viral diseases. The mode of action, inhibition entry and replication of host cells is discussed in this chapter. This chapter also explains about the emerging technologies like CRISPR used for the diagnosis, treatment and alleviation of viral disease progression.

Chapter 13 explains about the various neurological disorders and the enzyme inhibitors used as drug is explained in this chapter. This chapter summarizes the action and mechanism of various enzyme inhibition in the therapy of neurological disease.

The editors would like to express sincere thanks to all authors for their phenomenal contributions to this book. The editor thank all reviewers and well-wishers for the completion of this book.

Dr. G. Baskar
Department of Biotechnology
St. Joseph's College of Engineering
Chennai, India

Dr. K. Sathish Kumar
Department of Chemical Engineering
SSN College of Engineering
Chennai – 603110, India

K. Tamilarasan
Department of Chemical Engineering
SRM University
Chennai – 603203, India

List of Contributors

A. Saravanan	Department of Biotechnology, Rajalakshmi Engineering College, Chennai 600048, India
Ankur Khare	Environmental Impact and Sustainability Division, CSIR-NEERI, Nagpur, India Academy of Scientific and Innovative Research (AcSIR), Ghaziabad, India
Aparna Madan	Department of Biotechnology, Sri Venkateswara College of Engineering, Sriperumbudur Tk. - 602 117, Kancheepuram District, Tamil Nadu, India
Arthi Udhayachandran	Department of Biotechnology, Sri Venkateswara College of Engineering (Autonomous), Sriperumbudur Tk, Tamilnadu, India
Balu Ranganathan	Palms Connect Sdn Bhd, Shah Alam 40460, Selangor Darul Ehsan, Malaysia Palms Connect LLC, Showcase Lane, Sandy, UT, USA
Bethu Madhumitha	Department of Biotechnology, Sri Venkateswara College of Engineering (Autonomous), Sriperumbudur Tk - 602 117, Tamil Nadu, India
Carlos Ricardo Soccol	Biotechnology and Bioprocess Engineering Department, Federal University of Parana, Curitiba-PR, Brazil
Cristine Rodrigues	Biotechnology and Bioprocess Engineering Department, Federal University of Parana, Curitiba-PR, Brazil
D. Lokapriya	Department of Biotechnology, Sri Venkateswara College of Engineering (Autonomous), Sriperumbudur Tk, Tamilnadu, India
E. Raja Sathendra	Department of Biotechnology, Arunai Engineering College, Tiruvannamalai-606603, India
Ekambaram Nakkeeran	Department of Biotechnology, Sri Venkateswara College of Engineering (Autonomous), Sriperumbudur Tk - 602 117, Tamil Nadu, India
Elsa Cherian	Department of Food Technology, SAINTGITS College of Engineering, Kottayam, Pathamuttom P. O, Kerala-686532, India
G. Baskar	Department of Biotechnology, St. Joseph's College of Engineering, Chennai – 600119, India
Hariharan Jayaraman	Department of Biotechnology, Sri Venkateswara College of Engineering, Sriperumbudur Tk. - 602 117, Kancheepuram District, Tamil Nadu, India
Joyce Gueiros Wanderley Siqueira	Biotechnology and Bioprocess Engineering Department, Federal University of Parana, Curitiba-PR, Brazil
K. Sathish Kumar	Department of Chemical Engineering, SSN College of Engineering, Chennai – 603110, India
K. Tamilarasan	Department of Chemical Engineering, SRM Institute of Science and Technology, Chennai – 603203, India
Kanchan Kumari	Environmental Impact and Sustainability Division, CSIR-NEERI, Nagpur, India Academy of Scientific and Innovative Research (AcSIR), Ghaziabad, India

Lakshmi Suresh	Department of Biotechnology, Sri Venkateswara College of Engineering, Sriperumbudur Tk. - 602 117, Kancheepuram District, Tamil Nadu, India
Luciana Porto de Souza Vandenberghe	Biotechnology and Bioprocess Engineering Department, Federal University of Parana, Curitiba-PR, Brazil
Mahalakshmi Varadan	Department of Biotechnology, Sri Venkateswara College of Engineering, Sriperumbudur Tk. - 602 117, Kancheepuram District, Tamil Nadu, India
Nelson Libardi Junior	Biotechnology and Bioprocess Engineering Department, Federal University of Parana, Curitiba-PR, Brazil
Nilavunesan Dhandapani	Department of Biotechnology, Sri Venkateswara College of Engineering (Autonomous), Sriperumbudur Tk - 602 117, Tamil Nadu, India
P.K. Praveen Kumar	Department of Biotechnology, Sri Venkateswara College of Engineering (Autonomous), Sriperumbudur Tk, Tamilnadu, India
P.R. Yaashikaa	Department of Chemical Engineering, SSN College of Engineering, Chennai 603110, India
P. Senthil Kumar	Department of Chemical Engineering, SSN College of Engineering, Chennai 603110, India
Pradip Jadhao	Environmental Impact and Sustainability Division, CSIR-NEERI, Nagpur, India Academy of Scientific and Innovative Research (AcSIR), Ghaziabad, India
Praveen Kumar Posa Krishnamoorthy	Department of Biotechnology, Sri Venkateswara College of Engineering, Sriperumbudur Tk. - 602 117, Kancheepuram District, Tamil Nadu, India
R. Aiswarya	Department of Biotechnology, St. Joseph's College of Engineering, Chennai – 600119, India
R. Jayasree	Department of Biotechnology, Rajalakshmi Engineering College, Chennai 600048, India
R. Praveen Kumar	Department of Biotechnology, Arunai Engineering College, Tiruvannamalai-606603, India
R.V. Hemavathy	Department of Biotechnology, Rajalakshmi Engineering College, Chennai 600048, India
Rajvikram Madurai Elavarasan	Department of Electrical and Electronics Engineering, Sri Venkateswara College of Engineering (Autonomous), Sriperumbudur Tk - 602 117, Tamil Nadu, India
Ravichandran Rathna	Department of Biotechnology, Sri Venkateswara College of Engineering (Autonomous), Sriperumbudur Tk - 602 117, Tamil Nadu, India
Ravichandran Viveka	Department of Biotechnology, Sri Venkateswara College of Engineering (Autonomous), Sriperumbudur Tk - 602 117, Tamil Nadu, India
S. Jeevanantham	Department of Biotechnology, Rajalakshmi Engineering College, Chennai 600048, India
S. Justin Packia Jacob	Department of Biotechnology, St. Joseph's College of Engineering, Chennai – 600119, India
Senthil Nagappan	Department of Biotechnology, Sri Venkateswara College of Engineering (Autonomous), Sriperumbudur Tk - 602 117, Tamil Nadu, India

Sonam Paliya	Environmental Impact and Sustainability Division, CSIR-NEERI, Nagpur, India Academy of Scientific and Innovative Research (AcSIR), Ghaziabad, India
Subasree Sekar	Department of Biotechnology, Sri Venkateswara College of Engineering (Autonomous), Sriperumbudur Tk – 602117, Tamilnadu, India
T.R. Sundaraman	Department of Biotechnology, Rajalakshmi Engineering College, Chennai 600048, India

CHAPTER 1

Introduction to Enzyme Inhibition – Environmental and Biomedical Applications

G. Baskar[1,*], R. Aiswarya[1], K. Sathish Kumar[2] and **K. Tamilarasan[3]**

[1] *Department of Biotechnology, St. Joseph's College of Engineering, Chennai – 600119. India*

[2] *Department of Chemical Engineering, SSN College of Engineering, Chennai – 603110, India*

[3] *Department of Chemical Engineering, SRM Institute of Science and Technology, Chennai-603203, India*

Abstract: Enzyme inhibitors alter the activity of an enzyme molecule when an enzyme binds to it. The enzyme inhibition finds its applications in drug discovery and assessment of various environmental pollutants owing to its high specificity and potency. The study of enzyme inhibition mechanism is highly recommended as it mainly depends upon the structural requirement and site of enzyme action. The enzyme inhibition also plays a vital role in the design of biosensors for the detection and assessment of an analyte molecule.

Keywords: Biosensor, Drug discovery, Enzyme inhibitors, Environment assessments, Specificity.

The enzyme, a protein molecule acts as a catalyst in the various enzymatic reactions. The enzyme inhibitors inhibit the catalytic activity by modifying the amino acid. The design of the drug analogue is accomplished by a complete understanding of kinetics and structure-function relationship [1]. The combination of chemistry and high throughput screening technology against the catalytic site helps in the discovery of the drug [2]. The state of the art of enzyme inhibition plays a major quest in various fields. The development of enzyme-based inhibitions follows mainly 1) Slow-tight inhibition 2) Substrate and product inhibition. Antimetabolites, anti-enzyme, antibodies, and biosensors also follow enzyme inhibitions for the detection and assessment of a particular analyte molecule. The drug discovery is based on enzyme inhibitions represented by monolithic immobilized enzyme reactors (MIERs). The high throughput screening helps in the identification of target protein during drug development [3]. The

* **Corresponding author G. Baskar:** Department of Biotechnology, St. Joseph's College of Engineering, Chennai – 600119, India; E-mail: basg2004@gmail.com

major challenging factor in enzyme inhibition is the kinetic calculation using various software. The major impact on enzyme inhibition is based on the unpredictable synergistic mechanism. The enzyme inhibitor has created a new space for the therapeutics market and has potential therapeutic applications for various diseases like Chronic Obstructive Pulmonary Disease (COPD), gastrointestinal disorders, cardiovascular, and other inflammatory-related diseases. The conceptual models of the enzyme inhibition play a major role in the interaction of substrate-inhibitor. The use of immobilized enzyme technology has paved the way for the development of tools in drug discovery and the design of biosensors. The cytochrome (P450) (CYP) actively helps in drug interaction by enzyme induction [4]. The CYP1 enzyme inhibits the action of dimethylhydrazine and acts as chemo protectants [5]. The CYP1 and its regulation of aryl hydrocarbon receptors have been extensively studied for drug resistance of the carcinogenesis [6]. Matrix metalloproteinases (MMP) play a vital role in embryogenesis, wound healing, and stem cell mobilization. They cleave the intra and extracellular matrix molecules at the pericellular environment [7, 8]. MMP cleaves and regulates the enzyme involved during signal transduction at a particular site. The MMP actively helps in the regulation of tumor suppression and autoimmune disorders [9 - 12]. MMP as gelatinases helps in the progression of aneurysms by proteolytic activity of neutrophils [13]. The neutrophil extracellular traps HLE along with MMP-9 has therapeutic value, where oleoyl moiety is replaced by ß-Lactam [14]. The natural metabolites extracted from plant sources were reported to have the lead component of enzyme inhibitors known as acetylcholinesterase (AChE), glutathione S-transferase (GST), and α-glucosidase. The natural compounds exhibited their potential role in the treatment of Alzheimer's disease as evident from the structure-activity relationship [15].

The basic principle of biosensor lies in the interaction of specific chemical and biological agents in the form of inhibitors. The inhibition in biosensors helps in analysing the kinetic characterization of the process at a heterogeneous surface. The concentration of inhibitor helps in analysing the percentage of inhibition of the biocatalyst over immobilized biosensor. The inhibition biosensor plays a significant role in environmental assessment. Enzymes like alkaline phosphatase combined with electrochemical sensors help in the detection of heavy metals [16]. The enzymatic alterations of various Persistent Organic Pollutants (POPs) and the assessment of pollutant levels with respect to their cell signaling are formulated by inhibition mechanism [17]. Thus, the mechanism of enzyme inhibition plays a major in the discovery of drug and monitoring of environmental pollutants.

CONCLUSION

Enzyme inhibitors are acts as a significant tool to distinguish the enzyme reaction

and its parameters in biological industries. The measurement and accuracy of enzyme detections have entered a new era by using pico technology. The immobilized enzyme technology was widely used in drug discovery and sensor development industry due to the availability of various new enzyme inhibitors. The use of a single MMP or combination of MMP with Quantum Dots (QD) helps in the treatment of cancer. Various *in-vitro* and *in-silico* models help in the analysis of the drug development process to minimize the uneventful interaction of drugs. The enzyme inhibition plays a major role in improving the target of drugs to a particular site during the chemoprevention process. The enzyme inhibitor at the heterogeneous surface helps in the analysis of persistent organic pollutants and also helps in other environmental assessments.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The author(s) confirms that there is no conflict of interest.

ACKNOWLEDGEMENTS

Declared none.

REFERENCES

[1] Sami AJ, Shakoori AR. Cellulase activity inhibition and growth retardation of associated bacterial strains of Aulacophora foviecollis by two glycosylated flavonoids isolated from Mangifera indica leaves. J Med Plants Res 2011; 5(2): 184-90.

[2] El-Metwally TH, El-Senosi Y. Enzyme Inhibition Medical Enzymology: Simplified Approach. NY: Nova Publishers 2010; pp. 57-77.

[3] Bartolini M, Greig NH, Yu QS, Andrisano V. Immobilized butyrylcholinesterase in the characterization of new inhibitors that could ease Alzheimer's disease. J Chromatogr A 2009; 1216(13): 2730-8.
[http://dx.doi.org/10.1016/j.chroma.2008.09.100] [PMID: 18950780]

[4] Delgoda R, Westlake AC. Herbal interactions involving cytochrome p450 enzymes: a mini review. Toxicol Rev 2004; 23(4): 239-49.
[http://dx.doi.org/10.2165/00139709-200423040-00004] [PMID: 15898829]

[5] Chang TK, Chen J, Benetton SA. *In vitro* effect of standardized ginseng extracts and individual ginsenosides on the catalytic activity of human CYP1A1, CYP1A2, and CYP1B1. Drug Metab Dispos 2002; 30(4): 378-84.
[http://dx.doi.org/10.1124/dmd.30.4.378] [PMID: 11901090]

[6] King HWS, Osbourne MR, Beland FA, Harvey RG, Brookes P. (+)-7α,8β- Dihydroxy-9β,10β-epo-y-7,8,9,10-tetrahydrobenzo[a]-pyrene is an intermediate in the metabolism and binding to DNA of benzo[a]pyrene. Proc Natl Acad Sci 1976; 73: 2679-81.

[7] Cauwe B, Opdenakker G. Intracellular substrate cleavage: a novel dimension in the biochemistry, biology and pathology of matrix metalloproteinases. Crit Rev Biochem Mol Biol 2010; 45(5): 351-

CHAPTER 2

Enzyme Inhibition in Therapeutic Applications

A. Saravanan[1,*], R. Jayasree[1], T.R. Sundaraman[1], R.V. Hemavathy[1], S. Jeevanantham[1], P. Senthil Kumar[2] and **P. R. Yaashikaa[2]**

[1] *Department of Biotechnology, Rajalakshmi Engineering College, Chennai 600048, India*

[2] *Department of Chemical Engineering, SSN College of Engineering, Chennai 603110, India*

Abstract: Enzyme is a protein fragment that catalyzes the biological reactions by reducing the activation energy required for the reactions to occur. Enzyme inhibition is a vital method for regulating movement in living cells. Enzyme inhibition occurs by the substrate called inhibitors that can bind to an enzyme and reduce its activity, endogenous mixes and xenobiotics are compound. There are three fundamental kinds of enzyme inhibition: competitive, non-competitive, and uncompetitive. Among the measuring time frame, enzyme repressing drugs are anticipated to be presented for new signs including asthma and interminable obstructive pneumonic ailment, aspiratory blood vessel hypertension, hepatitis C and discontinuous claudication. This chapter offers an expansive point by point diagram of compound inhibitors at present available and those in late-organize medical trials. The data and examination exhibited in this report are vital resources in basic leadership for chiefs engaged with business advancement, advertising, statistical surveying, item improvement, mergers, and acquisitions, authorizing, business administration, speculation managing an account and arrangement creation, and to specialists to the pharmacological and biotechnology industry. The investigation gives a complete examination of the present markets for compound hindering medications and, specifically, the market capability of promising medications and innovations a work in progress.

Keywords: Biosynthetic, Biochemical, Covalent, Chronic obstructive pulmonary disease, Enzyme, Hepatitis C, Inhibition, Inorganic metal, Medication, Non-competitive, Pharmaceutical, Therapeutic, Uncompetitive, Xenobiotics.

INTRODUCTION

Enzymes have great potency towards the application of the pharmaceutical industry particularly for the treatment of cardiovascular diseases, treatment of cancer, wound debridement, bleeding disorders and digestive aids [1]. The catalytic property of therapeutic enzymes plays an important role in converting various target molecules into other desired products [2]. Most of the therapeutic

[*] **Corresponding author Saravanan Anbalagan:** Department of Biotechnology, Rajalakshmi Engineering College, Sriperumbudur Tk - 602 105, Tamil Nadu, India; Tel: +91-9003838356; E-mail: saravanan.a@rajalakshmi.edu.in

G. Baskar, K. Sathish Kumar & K. Tamilarasan (Eds.)

enzymes are required in less amount when compared to industrially imperative enzymes.

Animal tissues, plants, and microorganisms are the major sources of the enzymes. Among the various sources, microbial enzymes are chosen as suitable due to their consistency, optimization and economic production. Few fungal, bacteria and yeast strains contribute to the production of enzymes with the therapeutic application. Enzymes for therapeutic application should have a high degree of purity and specificity. During the protein treatment, the transport of mixes inside the host considerable cells is basic as a result of the tremendous nuclear size. In addition, the immune response generated by the host cells after receiving foreign enzyme is the major concern contributing to life-threatening conditions with severe allergic reactions. The short half-life of enzymes is another problem associated with therapeutic application and microencapsulation and counterfeit liposomal capture are a portion of the strategies used to increase the strength and half-existence of chemical medications [3]. As an end, headways in medication improvement and conveyance in recent decades have upset another path for chemical treatment. Therapeutic enzymes are marketed as preparations of pure lyophilized substances with the addition of buffering salts [4]. Table **1** summarizes the list of enzymes with their therapeutic applications.

Table 1. List of some enzymes with therapeutic applications.

Enzyme	Application
Alteplase	Treatment of cardiovascular diseases
Urokinase	Treatment of cardiovascular diseases
Anistreplase	Treatment of cardiovascular diseases
Carboxypeptidase G2	Treatment of cancer
β–glucuronidase	Treatment of cancer
Alkaline Phosphatase	Treatment of cancer
β Galactosidase	Digestive Aids
Glutenases	Digestive Aids
Proteases	Wound Debridement

ENZYME INHIBITION

Enzyme inhibitors are organic or inorganic compounds that can control the substance reactant development either rescindable or permanent. The inhibitor can transform one amino destructive, or a couple of side-chain required in compound synergist activity. Safely, the engineered change should be conceivable to test

inhibitor for any medicine regard.

Enzymes catalyse a reaction by reducing the activation energy needed for the reaction to occur. However, enzymes need to be tightly regulated to ensure that levels of the product do not rise to undesired levels. This is accomplished by enzyme inhibition. Some noticeable incredible delineations are medicine and toxic substance action and cure diagram for remedial utilizations *e.g.*, iodoacetamide deactivates Cys amino destructive in impetus side chain; methotrexate in threat chemotherapy through semi-explicitly control DNA mix of perilous cells; ibuprofen quells the amalgamation of the pro-inflammatory prostaglandins; drugs stifle the folic destructive amalgamation essential for the improvement of pathogenic tiny life forms in this way various distinctive prescriptions.

Protease inhibitors can work in many different ways to inhibit the action of proteases. These inhibitors can be classified by the type of proteases they inhibit and the mechanism by which they inhibit those enzymes. While commercial protease inhibitors are typically sold based on the class of protease they inhibit, understanding the various mechanisms by which inhibitors function is essential for a comprehensive understanding of inhibition and for developing protease inhibitors as a therapeutic strategy.

Regulation of enzyme activity can be attained through enzyme inhibition and usually resulted in reduced enzyme activity [5]. Enzyme inhibition can be reversible or irreversible. Reversible inhibition of enzyme can be distinct types of inhibition due to the possible non-covalent bond formed between the inhibitor molecule and the enzyme and thereby reduction in enzyme activity occurs completely inhibited or partially inhibited. Irreversible inhibition can cause chemical modification of the particular enzyme. There are diverse sorts of conceivable reversible restraints that may happen as focused kind, non-aggressive sort and uncompetitive, in spite of the fact that a blended kind now and then emerges. Degree of inhibition (i) of reversible inhibitor is calculated by using the following equation,

$$i = (V_o - V)/V_o \quad \textbf{(1)}$$

where V_o and V are the rates of uninhibited and inhibited reactions, respectively.

CHAPTER 3

Analytical Aspects of Biosensor Based on Enzyme Inhibition

A. Saravanan[1,*], S. Jeevanantham[1], P. Senthil Kumar[2] and **P. R. Yaashikaa[2]**

[1] *Department of Biotechnology, Rajalakshmi Engineering College, Chennai 602105, India*

[2] *Department of Chemical Engineering, SSN College of Engineering, Chennai 603110, India*

Abstract: An enzyme biosensor is an investigative device that joins an enzyme with a transducer to create a signal corresponding to target analyte fixation. An ideal enzyme activity is a basis for the maintenance of physiological homeostasis of biosensor. Both non-hereditary and hereditary disturbances can too much initiate or quiet characteristic enzyme activities. Due to its virtuous sensitivity, easy to operate, high precision and low instrumentation cost it can be used for recognition of various analytes in different fields than the traditional analytical methods. The enzyme based biosensor is most commonly used dominant tool for the determination of various biological importance such as antigens, antibodies, therapeutic drugs and metabolites. In regular enzyme-based biosensors, signal amplification is not satisfactory for the ultrasensitive identification of biomolecules and it was enhanced by consolidating enzymatic responses with redox cycling of multi-enzyme tags for every discovery test. This chapter explains the working principles, features, types, sensing methods and applications of enzyme based biosensor in various fields.

Keywords: Antigen, Antibody, Biomolecules, Chemical activators, Electrochemical, Enzyme biosensor, Homeostasis, Hereditary, Inhibitor, Metabolite, Nanomaterials, Transducer, Therapeutic drugs.

INTRODUCTION

The biosensor can be characterized as a minimal explanatory gadget fusing a natural or organically inferred detecting component either coordinated inside or personally connected with a physicochemical transducer. The biosensors have three essential components including transducer, signal processor and biologically recognition element (enzyme) for its effective performances in various fields. During the detection, enzyme in the biosensors can interact with the target molecule produces the biological signals. The produces signals reaches were converted into measurable form by the transducer [1, 2]. An enzyme biosensor is

* **Corresponding author A. Saravanan:** Department of Biotechnology, Rajalakshmi Engineering College, Chennai 602105, India; E-mail: saravanan.a@rajalakshmi.edu.in

G. Baskar, K. Sathish Kumar & K. Tamilarasan (Eds.)

a systematic device that combines a chemical with a transducer to create a standard corresponding to target analyte focus. The transducer changes over this flag into a quantifiable reaction, for example, present, potential, temperature change, or retention of light through electrochemical, warm, or optical means. This flag can be additionally increased, handled, or put away for later examination.

Most Sensors Comprise of Three Chief Segments

1. A receptor fit for perceiving the types of enthusiasm with a high level of selectivity. This is normally simultaneous with a coupling occasion between the receptor and an analyte;
2. A transducer, where the coupling occasion is converted into a quantifiable physical change. Cases could incorporate the age of electrons, protons, a change in conductivity or the age of an electrochemically dynamic concoction species, for example, hydrogen peroxide;
3. A strategy for estimating the change identified at the transducer and changing over this into valuable data.

Currently enzyme based biosensors gained many researchers' interest from various fields due to the utilization of enzyme as the recognition element in the sensor. Enzyme used in the biosensor shows remarkable selectivity towards specific analyte. For example, enzyme glucose oxidase will interact or bound only with glucose molecule among various sugars and produces the biological signals [3].

Numerous catalysts additionally show quick turnover rates and this is regularly fundamental to (a) maintain a strategic distance from immersion and (b) permit sufficient age of the dynamic species keeping in mind the end goal to be distinguishable. Sadly, there are likewise a few disservices. Though the catalyst gives the results within a short period of time, they may reduce the precision of the result and affects the enzyme activity. In some cases the amount spent for the catalyst will be higher. Immobilization of enzyme can increase or maintain the enzyme activity for longer periods [4, 5]. Inorganic examples, blood or salivation, there can likewise be solutes that are electrochemically dynamic and meddle with conclusions of the objective species. Then again, species might be available that predicament to the surface, causing fouling and loss of sensor reaction.

Enzyme - based sensors are more specific than cell-based sensors. Due to their shorter distribution behaviors, they react quickly. Glucose biosensor is the most commonly used enzyme based biosensor. Similarly chemical biosensor is a descriptive device which combines the chemical compound with a transducer and delivers the results like enzyme based biosensor. But they are too expensive than

enzyme based biosensor [6]. Biosensors allow the investigation in complex natural media. The discovery of an enormous number of mixes is of colossal pertinence for logical research and for process control in the nourishment and compound industry [7].

Compound biosensors utilize the fondness and selectivity of chemically dynamic proteins, towards their objective atoms. Commonly, enzyme immobilization with the transducer gains more attention and shows good results for longer period which reduces the harmful effects caused by the various substrates during the detection process. Inhibitors, co-substrate and co-factors in the analyte mixture are the different substrates which affect or reduce the effectiveness of the biosensor. Contingent upon the measure write, two key classes of protein sensors can be recognized. To begin with, the protein identifies the nearness of a substrate or co-substrate/co-factor. This is at that point, by method for a transducer, used to screen the expansion of enzymatic action. A run of the mill illustration is a glucose biosensor [8]. The most widely recognized case of this approach is the recognition of organophosphate mixes utilized as pesticides or fighting nerve specialists. The method of flag transduction can be electrochemical, optical, full (acoustic), warm and so on.

There have been critical enhancements in the field of enzymatic biosensors; the utilization of new, hereditarily built compounds has taken into consideration enhanced execution attributes of current biosensors for the recognition of setting up analytes (glucose, pesticides and so forth). Headway has been the usage of hereditarily altered catalysts to distinguish novel markers. An extra gathering of enhancements is the use of "non-conventional" transducer materials, *e.g.* carbon nanotubes (CNT), or diverse conductive polymers. Wonderful basic and electrical property headways have empowered new alternatives for the most part in the territory of electrochemical detecting advancements.

The innovation of enzymatic biosensors offers a strong blend of execution and explanatory highlights not accessible in some other bioanalytical framework. The posting of only a couple of alternatives in this review can energize future advancement, which could yield new ages of enzymatic biosensors for an extensive variety of utilizations in clinical, natural or mechanical diagnostics [9].

WORKING OF BIOSENSORS

The favored organic material like chemical is ideal for ordinary techniques like physical or membrane based methods. The favored organic material is in contact with the transducer. When the analyte contact with the biological material or enzyme in the biosensor will produce biochemical signals can be estimated once they converted into measurable signals by the transducer. Working principle of

CHAPTER 4

RNA Silencing in Enzyme Inhibition and its Role in Crop Improvement

S. Justin Packia Jacob*

Department of Biotechnology, St. Joseph's College of Engineering, Chennai, India

Abstract: Crop improvement represents the genetic alteration of plants to gratify human needs. Crop improvement, the art of engineering plants for the benefit of humankind, is as old as agriculture itself. Even though crop improvement programs focus on the development of novel crop varieties with enhanced quality and tolerance to ecological stresses (both biotic and abiotic) and making crops able to give more yield exhibiting good quality. Still, we cannot rely on the crops due to various reasons, like the irritating nature of onion, low lysine and threonine content in Maize, the presence of toxic Gossypol in cotton, *etc.* These irritating qualities of the crop must be reduced or removed by genetically modifying the crop plants, and then only it will be accepted for human consumption. Expression of *antisense* genes and the related gene-silencing technique has been exploited as an applied *technique in plant* biotechnology for creating "metabolic engineered *plants*" in which the endogenous target gene, which is responsible for the unpleasant character is specifically suppressed. The *antisense and its related technology* are used for various purposes such as silencing or ablating undesired genes. In the present chapter, the down-regulation of the enzyme using the gene-silencing technique used in various crop varieties are discussed in detail.

Keywords: Antisense, Crop, Down-regulation, Enzyme, Flavr-Savr, Gossypol, Improvement, Inhibition, Polygalacturonase, RNA-silencing, Tearless-onion.

INTRODUCTION

The global population was estimated to have reached 7.6 billion people as of May 2018 and it will be increased by more than 2 billion, till 2025. Hence to meet the worldwide demand for nourishment, the production of improved crops is required, particularly cereals, as they serve as the chief source of food for most of the human populace [1]. Antisense RNA is a recent technology and getting hold of popularity in the agricultural field. Genetic improvement of crops can be provided by RNAi (RNA interference) technology as it has established as a powerful appr-

* **Correspondence S. Justin Packia Jacob:** Professor, Department of Biotechnology, St. Joseph's College of Engineering, Chennai, Tamil Nadu, India; Email - drjpjacob@gmail.com

G. Baskar, K. Sathish Kumar & K. Tamilarasan (Eds.)

oach to improve the traits in crops by downregulating (silencing) genes. Antisense RNA, RNAi, and other related pathways retort to exogenous and endogenous genetic makeup (nucleic acids) along with basic cellular processes. Antisense RNA technology is an instrument for regulating the expression of the gene in the higher organisms and using this tool one can selectively inhibit the enzymes involved in a particular pathway. Thus, there is an enormous potential of Antisense RNA technology towards crop improvement and to encounter the agrarian-based demand of the growing population of our nation.

Antisense System in Nature

Naturally, arising antisense RNA was involved in the regulation of gene and this was confirmed during the study of E. coli ColE1 plasmid replication. E.coli based plasmid ColE1 replication includes the formation of an RNA primer, which is processed by RNase-H while bound to the DNA template. Inhibition of the processing of RNA primer and replication of the plasmid occurs by the binding of Antisense RNA with the primer; hence the plasmid copy number possibly will be regulated [2]. Replication of *Staphylococcus aureus* plasmid (pT 181) and copy number also appeared to be controlled by antisense RNA [3]. Antisense mRNA also inhibits E. coli Tn10 transposase mRNA translation.

Antisense control called an additional mechanism of translational control that occurs in bacterial cells. This form of regulation will be facilitated via antisense RNA since it comprises the sequence complementary to the sequence of a sense strand (mRNA). The initiation of translation is prevented by the pairing of the antisense RNA with the mRNA, which prevents the recognition of initiation codon and thereby 30S ribosomal subunit binding with the Shine Dalgarno sequence is prevented.

Transposase expression is encoded by the bacterial insertion sequence IS 10 is regulated by the antisense translation-control mechanism. The transposition of this mobile DNA element is catalyzed by Transposase. If the expression of transposase occurs considerably, as a result, abundant mutations may result from IS 10 transposition due to that the host cell may not be able to survive.

In general, this does not happen due to the occurrence of the antisense control, in which the IS 10 comprises two promoters: one called $P_{IN,}$ which directs transcription of the strand coding for transposase; the other called P_{OUT} lies within the transposase gene and directs transcription of the noncoding strand, producing an antisense RNA complementary to the 5′ end of transposase mRNA. Since P_{OUT} is promoter a stronger than a P_{IN}, therefore that antisense mRNA is produced in greater abundance than transposase mRNA.Antisense RNA hybridization to most

of the much rarer transposase mRNA prevents translation, thereby assuring that the rate of synthesis of transposase and, in turn, the frequency of transposition, is compatible with the survival of the host cells.

Antisense RNAs available naturally in plants are found to have a regulatory effect on gene expression. These include antisense RNA transcripts for the K-amylase mRNA of barley [4], antisense mRNA complementary to niv gene codes for the enzyme chalcone synthase (CHS) of the flavonoid pathway [5]. Two antisense transcripts were identified in barley [4] both were incorrectly complementary to the K-amylase gene whereas, in the case of niv gene, antisense transcripts occur due to an inverted duplication of un-translated leader sequences.

Therefore, a tentative mechanism has been proposed for the generation of antisense transcripts. Antisense RNA emerges when transcription of a gene proceeds in the sequence opposite to template in the absence of a strong termination site for transcription in the short intergenic region. In Brassica, self-incompatibility was controlled by antisense transcripts for the S locus receptor kinase gene [6]. There are different types of gene silencing mechanisms such as Ribozymes, RNAi technology, Antisense RNA technology, *etc.* let us discuss the role of these gene silencing mechanisms in the crop improvement program.

Ribozymes

A ribozyme is a catalytic RNA (RNA enzymes) which was first explained in *Tetrahymena thermophilic* in the early 1980s [7, 8]. The RNA processing competences of these ribozymes were subjugated as a possible antisense agent. Uhlenbeck [9] and Haseloff and Gerlach [10] first isolated this ribozyme from a viroid RNA. An excellent discussion about the ribozymes of hammerhead was given by Kurreck [11] and the mechanism of action of different ribozymes is elucidated by Doudna and Cech [12].

Antisense RNA

Antisense RNA (asRNA) [13] or oligonucleotide [14] is a single RNA strand that is complementary to the mRNA (codes for a protein) onto which the asRNA pairs and form the duplex structure, as a result, prevent its translation. Naturally occurring as RNAs were being identified in prokaryotic and eukaryotic organisms [13] antisense RNA can be classified into two types like shorter ones (less than 200 nucleotides) and longer ones (greater than 200 nucleotides) noncoding RNAs [15]. The prime function of asRNA is regulating the expression of a gene and found to have widespread usage as a research tool for gene down-regulation

CHAPTER 5

Enzyme Biosensors Based on Enzyme Inhibition for Pesticide and Heavy Metal Detection

Elsa Cherian[1,*] and **G Baskar**[2]

[1] *Department of Food Technology, SAINTGITS College of Engineering, Kottayam, Pathamuttom P. O, Kerala-686532, India*

[2] *Department of Biotechnology, St. Joseph's College of Engineering, Chennai – 600119, India*

Abstract: A biosensor is an analytical apparatus, which connects a biologically sensitive and selective component and a physiochemical transducer. Biologically sensitive elements include organisms, tissues, cells, *etc.* and the transducer includes electrochemical, optical, thermal or mechanical signals which are received and converted into a measurable signal. There are different categories of biosensors based on the principle of working. Some of them are electrochemical, amperometric, thermometric, optical, microbial and immunosensors. Biosensors are broadly used in different areas like the food industry, fermentation industry, pharmaceutical industry, *etc.* The present chapter explains the use of different types of biosensors which are based upon enzyme inhibition, as an investigative and diagnostic tool. Some of the enzyme inhibitors such as pollutants are strongly associated with human as well as environmental health, so these have to be monitored with strong significance. Thus enzyme inhibition based enzyme biosensors will be a precious tool for very fast performing and accurate for the above applications.

Keywords: Biosensor, Enzyme inhibition, Environmental applications, Heavy metals, Inhibitors, Pollutants.

INTRODUCTION

A biosensor is an investigative and diagnostic device with an immobilized biological material. Some of the examples for immobilized biological elements include enzymes, hormones, organelle or whole cell. This biological element particularly reacts with an analyte and produces physical, chemical or electrical signals as output that can be measured. The unknown compound for which concentration has to be measured is the analyte. Also a third element present in the biosensor acts as the reference signal or the control element. So the difference

[*] **Corresponding author Elsa Cherian:** Department of Food Technology, SAINTGITS College of Engineering, Kottayam, Pathamuttom P. O, Kerala-686532. India; E-mail: elsa.c@saintgits.org

between the control signal and the analyte will be proportional to the concentration of the material that has to be measured. Thus a characteristic biosensor connects two elements that are, the biological sensing element and a transducer which can detect analyte concentration. Thus biosensor is advantageous. Some of the advantages of biosensors include fast and continuous measurement, high specificity, easy calibration, fast response time, *etc.* This chapter explains the importance of biosensor with its history, types, and applications and also focuses on the influence of enzyme inhibition on the working of the biosensor.

HISTORY OF BIOSENSOR

The biosensor was first invented by Professor Leland C Clark Jnr, who is recognized as the father of the biosensor theory. Leland C Clark Jnr put forward a biosensor with an oxygen electrode in the year 1956. In the biosensor, Glucose oxidase was immobilized on the Clark oxygen electrode using the dialysis membrane [1]. The glucose concentration was calculated by the reduction in the dissolved oxygen concentration. The first potentiometric biosensor was made into reality by immobilizing urease enzyme on the ammonia electrode to confirm urea, in the year 1969 by Guilbault and Montalvo. A brief note on the history of the biosensor is given in Table **1**.

Table 1. History of biosensor.

Sl.No	Event	Year	Reference
1.	The first description on the immobilisation of proteins (invertase on activated charcoal)	1916	[2]
2.	Glass electrode	1922	[3]
3.	First glass electrode for analysis of blood samples	1925	[4]
4.	Carbon dioxide electrode	1954	[5]
5.	The invention of the oxygen electrode	1956	[6]
6.	The invention of a glucose electrode	1962	[7]
7.	The initial usage of the potentiometric biosensor using ammonia electrode immobilized with urease which was intended to confirm the presence of urea	1969	[8]
8.	The innovation of the ion-selective field-effect transistor (ISFET)	1970	[9]
9.	Microbe-based immunosensor	1975	[10]
10.	First biosensor for glucose-based upon fiber optics	1982	[11]
11.	Surface plasmon resonance (SPR) immunosensor	1983	[12]
12.	First mediated amperometric biosensor where ferrocene was used with glucose oxidase for the finding glucose	1984	[13]

(Table 1) cont.....

Sl.No	Event	Year	Reference
13.	Starting off the Pharmacia BIACore SPR-based biosensor system	1990	[14]

TYPES OF BIOSENSOR

Biosensors could be classified based on the mechanism used for biological signaling or the type of signal transduction they employ. Different biological signals that are generated for measurement include antigen-antibody interaction, enzymes, nucleic acid and microbial cells.

Antigen-antibody Interaction-based Biosensors

Biosensors working on the principle of antigen-antibody interaction are known as immunosensors where antigen-antibody complexes are confirmed and transformed using a transducer into an electrical signal, which is then processed, recorded and displayed. There are many types of transducers based on the signal that is generated for the complex formation. Immunosensors are mainly based on specific antigen-antibody interactions and detected, either directly or indirectly by the immunochemical reactions. For example, speedy, non-expensive and numerous assays can be carried out with immunosensors and this could play a major role during epidemics to make a correct judgment and follow the epidemic spreading.

Biosensors Based on Nucleic Acid

Another breakthrough in the biosensor field is the nucleic acid-based biosensor. Here DNA, RNA or nucleic acid analogue is used as the probe, interaction of which results in the generation of the signal [15]. The major trouble in this type of biosensor is the immobilization of the probe on the biosensor which affects analysis performance. Nucleic acid biosensors are mainly used in various fields, such as genotyping and gene expression studies [16], disease diagnosis [17], drug discovery [18], *etc.* The increased development of nucleic acid analogues results in its widespread application.

Biosensors Based on Microbial Cells

The microbial biosensors are based on the interaction between microbial cells and transducers. The microbial cells are highly biosensitive to the external environment, so they are considered to be efficient bioreceptors and also tend to attach to some surfaces. The major transducers that can be used include amperometric, potentiometric, calorimetric, conductometric, colorimetric, luminescence and fluorescence [19 - 21]. In this technique, choice and strategy of microbial immobilization play a major role since it greatly influences the

CHAPTER 6

Recent Insights of Matrix Metalloproteinase Inhibitors in Therapeutic Applications

Ravichandran Rathna, Bethu Madhumitha, Ravichandran Viveka and **Ekambaram Nakkeeran***

Research Laboratory, Department of Biotechnology, Sri Venkateswara College of Engineering (Autonomous), Sriperumbudur Tk - 602 117, Tamil Nadu, India

Abstract: Matrix metalloproteinases are proteolytic zinc-dependent enzymes that play a pivotal function in cell migration, proliferation, differentiation, programmed cell death, and other physiological processes. Recent studies demonstrated that the imbalance activation and inhibition of these enzymes resulted in unexpected physiological and pathological processes. Thus, it fueled the interest in matrix metalloproteinase and its inhibitors in medicinal and pharmaceutical chemists. This chapter discusses the therapeutic accomplishments of matrix metalloproteinase inhibitors in arthritis, autoimmune disease, inflammations, cancer, and cardiovascular disease. Further, the chapter discusses clinical trial implications, obstacles, and future research.

Keywords: Arthritis, Autoimmune disease, Classifications, Cancer, Cardiovascular disease, Clinical implications, Endogenous tissue inhibitors, Exogenous inhibitors, Functional roles, Inflammations, Matrix metalloproteinase, Matrix metalloproteinase inhibitors, Mechanism, Obstacles, Therapeutic accomplishments.

INTRODUCTION

In 1962, Gross and Lapiere investigated a compelling collagenolytic activity in small tissue fragments during metamorphosis in tadpoles [1]. The pioneer of interstitial collagenase from the amphibian, *Rana catesbeiana,* is a cornerstone for potent biological macromolecules, matrix metalloproteinases (MMPs). MMPs are also known as matrixins that belong to a metzincin superfamily, zinc-endopeptidases. It is a calcium-dependent zinc-containing endoproteases which are traditionally categorized into six types [2, 3] based on homology, substrate sp-

* **Corresponding author Ekambaram Nakkeeran:** Research Laboratory, Department of Biotechnology, Sri Venkateswara College of Engineering (Autonomous), Sriperumbudur Tk - 602 117, Tamil Nadu, India; E-mail: nakkeeran@svce.ac.in

G. Baskar, K. Sathish Kumar & K. Tamilarasan (Eds.)

ecificity and cellular localization (partly) as collagenases (MMP-1, -8, -13, -18), gelatinases (MMP-2, -9), stromelysins (MMP-3, -10, -11, -17), matrilysins (MMP-7, -26), membrane-type MMPs (MT-MMP-14, -15, -16, -17, -24, -25) and other MMPs (MMP-12, -19, -20, -21, -22, -23, -27, -28, -29). Recently, MMPs are classified into four types based on their domain structure as archetypal MMPs, matrilysins, gelatinases and furin-activatable MMPs [4]. All the MMPs consist of prodomain, a catalytic domain with the zinc metal active site, fibronectin-like domain, linker, and hemopexin domain that assist in the degradation of various extracellular matrix (ECM) and non-ECM [5, 6]. Further, they are released from fibroblasts [7], monocytes [8], macrophages [8], lymphocytes [9], epithelial cells [10], endothelial cells [11], polymorphonuclear leukocytes [12], and osteoblasts [13]. Catalytic domains of MMPs assist in cleaving the non-collagen substrates while N-terminal domains cleave the native fibrillar collagen molecules. In general, MMPs play a vital role in physiological or pathological processes and tissue remodeling *via* ECM protein turnover and degradation. This chapter discusses the therapeutic accomplishments of MMPI in arthritis, autoimmune disease, inflammations, cancer, and cardiovascular disease. Further, the chapter highlights the clinical trial implications, obstacles, and future researches.

MATRIX METALLOPROTEINASE

Archetypal MMPs consist of an inactive protein precursor (propeptide) with a critical cysteine residue and a zinc catalytic domain residing on the hemopexin domain. Interestingly, archetypal MMPs is sub-categorized into collagenases (MMP-1, -8, -13, -18), stromelysins (MMP-3, -10) and other archetypal MMPs (-12, -19, -20, -27). Collagenase was the first MMP discovered that is capable of cleaving triple helices collagen into fragments and essential for various biological functions such as for regulating proinflammatory factors [14], functions as an agonist of the non-ECM protein like protease-activated receptor-1 [15] and proteolysis insulin-like growth factor binding proteins [16]. Stromelysins are structurally the same as collagenase and have the capacity to degrade structural proteins of non-collagenous ECM like proteoglycans, glycoproteins, elastin, entactin, fibronectin and laminin and participate in proMMP activation. Jin *et al*. [17], reported the matriptase-dependent activation of MMP-3 increased the ECM degradation in the tumor cell microenvironment that was eventually promoting tumor development and angiogenesis. MMP-3 overexpression implicated in Osteoarthritis [18] while pulmonary hypertension associated with systemic sclerosis [19], esophageal cancer [20], and tumor progression [21] correlates to overexpression of MMP-10. MMP-12, -19, -20, and -27 are the four matrixins that are classified as other archetypal MMPs and have the same structure as archetypal MMPs but differ in their sequence and substrate specificity. MMP-12 is a macrophage metalloelastase capable of degrading elastin and also considered

as a prime therapeutic target for chronic obstructive pulmonary complications [22] and cutaneous melanoma [23]. MMP-19 is also known as matrix metalloproteinase RASI-1 which exhibits potent basement membrane degradation [24] and overexpressed in gastrointestinal diseases [25] and stimulates proliferation and cell migration of tumors [26]. MMP-20 is commonly known as enamelysin, or enamel metalloproteinase plays a vital role in tooth enamel formation. In pancreatic ductal adenocarcinoma, overexpression of MMP-19 and MMP-20 results in the progression and prognosis of carcinoma tissues [27]. MMP-27 is unique and consists of intracellular retention motif and exhibits expression on macrophages during ovarian and peritoneal endometriotic lesions [28].

Matrilysins are the smallest MMPs among other MMPs since it lacks the hemopexin domain. MMP-7 actively degrades the ECM components like collagen IV, laminin, glycoprotein, and mucoproteins to form soluble ectodomains from the cell surface. However, MMP-7 is the sole MMP released by epithelial tumor cells [29] and MMP-26 expressed in multiple human cancer tissues and smooth muscle cells [30]. Gelatinase is a type IV collagenase which includes gelatinase A (MMP-2) and B (MMP-9). It contains three single repeats of fibronectin in its structure to facilitate the binding of gelatin and collagen. MMP-2 prevents the autolytic inactivation while binding to the intact collagen. However, MMP-2 is resistant to the native interstitial collagens. Besides, MMP-2 and -9 play a vital function in tissue homeostasis [31] and tumor-associated tissue remodeling [32].

Furin-activated MMPs are sub-divided as secreted MMPs (MMP-11, -21, -28); type-I transmembrane MMPs (MMP-14, -15, -16, -24), type-II transmembrane MMPs (MMP-23), and glycosylphosphatidylinositol (GPI)-anchored MMPs (MMP-17, -25). Formerly, MMP-11 is categorized under stromelysins based on the structural similarity, but intracellular activation is owing to the occurrence of furin-cleavage sites between propeptide and a catalytic domain. Hsin *et al*. [33], reported the expression of MMP-11 in the development and aggression of tumors through the focal adhesion kinase/Src kinase (FAK/Src) pathway. MMP-21 is implicated in embryogenesis and tumor progression [34] and expressed in macrophages and fibroblasts [35]. Lohi *et al*. [36], identified MMP-28 (Epilysin) expressed in normal and intact tissues like testis, intestine, lung, skin, and play an important role in tissue homeostasis, cutaneous wound repair, and tumor progression. MMP-14 is a multifunctional Membrane Type-1 MMP (MT1-MMP) associated with metastatic progression for its ability to cleave ECM. Further, low-level MT1-MMP mediated ECM degradation enhances cell migration and tumorigenesis, but high-level MT1-MMP expression does not augment tumor invasion and vascularization [37]. MMP-15 (MT2-MMP) assists inducing proteolysis and function as a capable mediator of epithelial-mesenchymal

CHAPTER 7

Current Potentialities and Perspectives of Enzyme Inhibition Based Biosensor

Senthil Nagappan[1], Rajvikram Madurai Elavarasan[2], Nilavunesan Dhandapani[1] and **Ekambaram Nakkeeran[1,*]**

[1] *Department of Biotechnology, Sri Venkateswara College of Engineering (Autonomous), Sriperumbudur Tk - 602 117, Tamil Nadu, India*

[2] *Department of Electrical and Electronics Engineering, Sri Venkateswara College of Engineering (Autonomous), Sriperumbudur Tk - 602 117, Tamil Nadu, India*

Abstract: A biosensor is an electrical device encompassing biological components, with the intent to detect and measure the concentration of the analyte in a sample. The applications of the biosensor are numerous including, but not limited to food, healthcare and environmental sectors. In particular, biosensors based on enzymatic inhibition are highly sensitive in monitoring the inhibitory analytes affecting the catalytic activity of enzymes. A careful selection of transducer, a biosensor component for transforming the biochemical signal to an electrical signal along with the choice of enzymes is crucial in determining the commercial viability of enzymatic inhibitory biosensor. This chapter highlights the recent studies as well as products available in the market related to biosensors based on enzymatic inhibition. Besides, the economic analysis of existing and futuristic biosensors is also discussed.

Keywords: Analyte, Analytes, Allergens, Biosensor, Biochemicals, Detection, Enzymes, Environmental, Enzyme inhibition, Enzyme inhibition, Food industry, Healthcare, Heavy metal, Inhibition-based biosensors, Nerve agents, Pharmaceuticals, Potentiometric, Pesticide, Screening, Toxins, Transducer.

INTRODUCTION

Biosensors are finding applications in a wide range of fields such as healthcare, environment, food industry, *etc*. for the detection of the desired analyte. The popularity of biosensor can be mainly attributed to its characteristics of simplicity, affordability and rapid detection. Enzymatic inhibition based biosensor is a class of biosensor developed first in the 1960s by Guilbault for the detection of nerve agents [1]. The principle of enzymatic inhibition based biosensor is the correlation

* **Corresponding author Ekambaram Nakkeeran:** Research Laboratory, Department of Biotechnology, Sri Venkateswara College of Engineering (Autonomous), Sriperumbudur Tk - 602 117, Tamil Nadu, India; E-mail: nakkeeran@svce.ac.in

G. Baskar, K. Sathish Kumar & K. Tamilarasan (Eds.)

of inhibiting analyte concentration with the degree of enzyme inhibition. The parts of a typical biosensor are substrate, bioreceptor, transducer, and signal measuring device. The additional component in an enzyme inhibition biosensor is the inhibitor. Bioreceptors can be an enzyme, antibody, nucleic acid or the whole cell itself. The signal transducer senses chemical and physical cues arising from enzyme inhibition activity such as electroactive material, pH change, heat, light and mass change with the help of either electrode or optical sensing. Finally, an electrical signal is read from the transducer through signal conducting circuits like amplifiers, filters, multiplexers, analog to digital converters, linearizers and compressors.

The major advantages of enzymatic inhibition based biosensor are as follows:

- Shorter response time
- A wider range of detection
- Sensitivity
- Robustness

Furthermore, the enzyme immobilization is reported to improve response time, reusability and stability of inhibition biosensor [2]. Due to the above-said features, this class of biosensor is used for the detection of a variety of compounds such as pesticides, insecticides, surfactants, heavy metals, toxins, nerve agents, glycoalkaloids, pharmaceutical drugs, nicotine, fluoride, benzoic acid, *etc*. For detection by inhibitory effect, different enzymes are reported. This includes acetylcholinesterase, butyrylcholinesterase, acid phosphatase, alkaline phosphatase, ascorbic oxidase, catalase, chymotrypsin, urease, tyrosinase, protease, peroxidase, lipoxygenase, elastase, laccase, glucose oxidase, invertase, *etc*. The above-mentioned enzymes, although display effective inhibition at a lower concentration of analyte they suffer from the problem of selectivity. To overcome the problem of selectivity, pretreatment of samples to remove the inhibitory compounds of non-interest and protein engineering techniques have been employed. This chapter discusses the recent studies on biosensor based on inhibition of the enzyme.

FUNDAMENTALS OF ENZYME INHIBITION BASED BIOSENSOR

Enzyme Inhibitors

Enzyme inhibitors are of two types - reversible and irreversible inhibitors. Irreversible inhibitors form a stable binding with enzyme resulting in chemical modification of enzyme's active site. In the above case, modification of the enzyme’s active site is irreversible in nature and thus subsequent substrate or

inhibitor binding is impossible unless the active site is reactivated. In contrast, reversible inhibitors bind to the enzyme through weak interaction such as hydrogen bond, hydrophobic interaction, ionic bonds and van der Waals bond. The weak interaction allows the enzyme's active site to be reused for subsequent substrate or inhibitor binding. As a result, biosensors based on reversible inhibitors will have an advantage over one based on irreversible inhibitors in terms of reusability [2].

The mode of inhibition can be predicted Reversible inhibitors can be classified into four types - competitive, uncompetitive, non-competitive and mixed reversible inhibitors. Competitive inhibitors compete with the substrate for binding to the enzyme's active site. This class of inhibitor has similar structural confirmation to that of the substrate. Competitive inhibition happens only when the concentration of inhibitors is higher than the substrate [3]. Unlike competitive inhibitors, uncompetitive inhibitors bind to the enzyme-substrate complex rather than the active site of the enzyme. In such a case, there is no competition between substrate and inhibitor and thus the required inhibitor concentration for uncompetitive inhibition is lower than competitive inhibition. Non-competitive inhibitors bind both to enzyme and enzyme-substrate complex with the same affinity. They are not substrate analogues and bind to the enzyme on a site other than the active site. Similar to uncompetitive inhibitors, the required inhibitor concentration for non-competitive inhibition is lower than competitive inhibition. Mixed inhibitors bind to both enzyme and enzyme-substrate complex but with different affinity. Also, as in both cases of uncompetitive and non-competitive inhibitors, the site of binding in the enzyme is not the active site. A biosensor based on uncompetitive, non-competitive or mixed inhibition has the advantage of lower detection limits in comparison to a competitive inhibition biosensor [2]. Therefore understanding inhibition mechanism through enzyme inhibition kinetics is essential for constructing a biosensor.

Enzyme Inhibitors Kinetics

The mode of inhibition can be predicted by methods such as double reciprocal plots [4], Dixon plots [5], Cornish-Bowden plots [6]. Fig. (**1**) shows the double reciprocal plot for (a) competitive, (b) non-competitive (c) uncompetitive inhibition, and Dixon plot for (d) mixed inhibition. Fig. (**1A**) shows the characteristic pattern of competitive inhibition plot in which the lines representing enzymatic activity in absence of inhibitor (I=0) and enzymatic activity in the presence of inhibitor (I>0) intercept at a single point on y-axis, above the x-axis. Furthermore, dissociation constants of enzyme-substrate (Km) and enzyme-inhibitor (Km, app) can be estimated from the x-intercepts of their respective lines. Similarly, characteristic patterns are shown in Fig. (**1B**), (**1C**) and (**1D**)

CHAPTER 8

Product Inhibition in Bioethanol Fermentation: An Overview

E. Raja Sathendra[1,*], **G. Baskar**[2] and **R. Praveen Kumar**[1]

[1] *Department of Biotechnology, Arunai Engineering College, Tiruvannamalai-606603, India*

[2] *Department of Biotechnology, St. Joseph's College of Engineering, Chennai-600119, India*

Abstract: Many organisms are used for alcohol production in an industry that includes *Saccharomyces cerevisiae, Zymomonas mobilis*, and *Clostridium sp*are better microbes with respect to ethanol production and ethanol tolerance, Zymomonas mobilis can use glucose, sucrose, and fructose through Entner-Deodoroff pathway. In bioprocessing product inhibition is undesirable that limits the product's final titer and volumetric productivity more precisely known as product toxicity those utilizing the whole cell as biocatalyst. During the ethanol fermentation the yield of cell mass decreases gradually as the ethanol concentration increases progressively indicating product inhibition. The decrease in cell mass concentration is the accumulation of ethanol in the fermentation broth beyond the limits. This is because the increase in alcohol concentration during fermentation destroys the microorganism lipid bilayer membrane and denatures the enzymatic protein thereby creating instability conditions. The product inhibition is very well studied only in the batch reactor. Conventionally maximum ethanol concentration of 7-8% (v/v) is achieved in the time frame of 50-70 hr with the operating temperature of the 32-34ºC and stirring rate of 180rpm during fermentation. To overcome this problem the continuous product removal solves the product inhibition through maintaining the ethanol concentration below the inhibitory limit.

Keywords: Bioethanol, Cell mass, Ethanol fermentation, Ethanol concentration, *Escherichia coli*, Growth kinetics, Hinshelwood model, Lignocelluloses, *Lactis aerogenes*, Monod's model, Product inhibition, *Saccharomyces cerevisiae*, Substrate, Specific growth rate, Toxin concentration, *Zymomonas mobilis*.

INTRODUCTION

Bioethanol produced through biological processes is mostly used in pharmaceutical, biofuel, fuel additive and food industries. Plants are the main sources for bioethanol production (*e.g.* Cellulose and starch). These polysacc-

* **Corresponding author E. Raja Sathendra:** Department of Biotechnology, Arunai Engineering College, Tiruvannamalai-606603, India; E-mail: e.rajasathendra@gmail.com

G. Baskar, K. Sathish Kumar & K. Tamilarasan (Eds.)

harides can be hydrolyzed to release sugar for bioethanol fermentation. Lignocellulose is the most abundant and cheap source of fermentable sugars. A large volume of agricultural crop residues and non-edible plant biomass mainly contains 10–40% hemicellulose, 40–75% cellulose and 15–35% lignin, depending on the source. Hemicellulose and cellulose fraction of plant biomass can be used for bioethanol production. Lignocellulosic materials are highly recalcitrant nature and they need extensive pre-treatment to release sugars [1 - 3]. Tubers, grains, and roots are the main source of starch materials for bioethanol production [4]. Grains contain 62–89% fermentable starch typically.

LIGNOCELLULOSIC PRETREATMENT

Pretreatment changes the structural and physicochemical property of original lignocellulose by opening the biomass recalcitrance structure and then renders the direct microbial or enzymatic hydrolysis of cellulose to produce monomers [5]. Hemicellulose can be easily hydrolyzed using acids, alkalis and enzymes to release sugars for fermentation. The hydrolytic process mainly releases pentose sugars ($C_6H_{10}O_5$) *e.g*. rhamnose, arabinose and xylose and some hexose sugars ($C_6H_{10}O_6$) *e.g*. galactose and mannose. Hemicelluloses can release major parts of chemical constituents like monomers on thermochemical degradation *i.e*. xylose, mannose, acetic acid, galactose, and glucose, in conjunction with several inhibitors that are toxic to the fermenting microorganism. The important inhibitors generated on hydrolysis that include furans phenolics, (furfurals and 5-Hydroxy methyl furfural (5-HMF)), weak acids (acetic acid). Among inhibitors [6 - 9] Hibbert's ketones have also been noticed in the acid hydrolysate of pine and spruce [10, 11]. Fungi like yeast cells are highly exposed to growth inhibition during fermentation through such toxic compounds or at higher concentration of ethanol formed and it is the major drawback of using lignocellulosic material in converting to ethanol. During the bioethanol fermentation, microorganisms capable of tolerating the lignocellulose ethanol production by maintaining the high metabolic activity are desirable.

Pretreatment Strategy

Pre-treatment strategy plays an important part in the lignocellulose processing for bioethanol production. Extensive researches are made to improve the breaking down of lignocellulose material. Pre-treatment changes the structural and physicochemical property of original lignocellulose by opening the biomass recalcitrance structure and then renders the direct enzymatic hydrolysis of cellulose to produce monomers [12]. In the native form of C5 and C6 sugar-based hemicelluloses the major part of the component is the lignin in an intertwined and

complicated form [13] shown in Fig. (**1**).

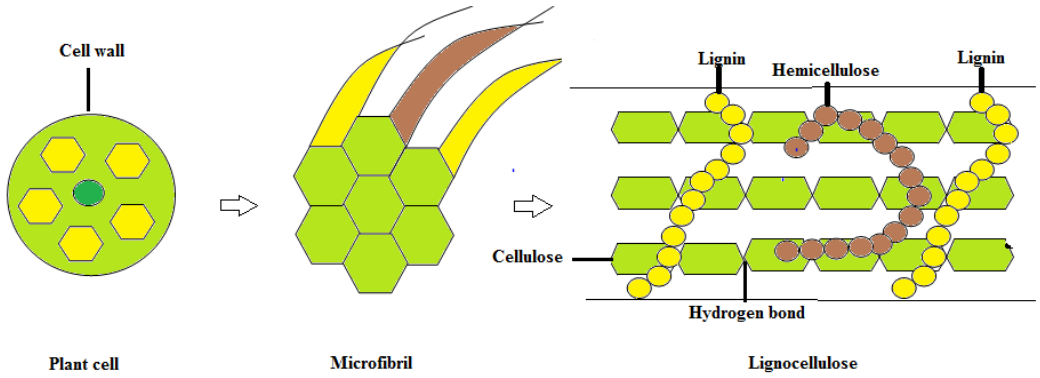


Fig. (1). showing the various internal components in the plant cell wall.

Lignocellulosic materials mostly contain a mixture of carbohydrate polymers (cellulose and hemicellulose), lignin, and ashes. The "holocellulose" is a term used to describe the total carbohydrate contained within a plant. Lignocellulosic materials are therefore comprised of cellulose and hemicellulose together referred to as Holocellulose. Cellulose, an unbranched linear polymer and hemicelluloses are a group of heterogeneous polysaccharides. The dry weight of the lignocellulosic material contains between 11% and 37% of hemicelluloses, can be easily hydrolyzed to their components of monomer consisting of xylose, mannose, glucose, galactose, arabinose, and small amounts of rhamnose, glucuronic acid, methyl glucuronic acid, and galacturonic acid by acids [14, 15]. Furthermore, among the various substrates lignocellulosic materials hold a good promise for biofuel production.

The non-fossil fluid fuel at present has an impact on a global scale is biofuels, including biodiesel and bioethanol. Utilization of bioethanol as a transportation fuel and as a gasoline supplement has been proved to be more environmentally friendly. The vehicle and industries are one of the major potential contributors to global warming by the release of carbon dioxide (CO_2) gas. The development of alternative energy sources such as biofuels becomes important to reduce these problems. The production of bioethanol from renewable sources such as plant biomass can reduce urban air pollution and the accumulation of carbon dioxide (CO_2), the so-called effect of greenhouse gases (GHG). Bioethanol is a complete and clean-burning of high octane fuel that can readily alter gasoline and its combustion results in significant reductions of toxic gas emissions such as 1-3 butadiene, benzene, and formaldehyde, while mixing ethanol with gasoline can

CHAPTER 9

Fermentation Strategies to Minimize Product Inhibition in Bioethanol Production

Luciana Porto de Souza Vandenberghe[*], **Nelson Libardi Junior, Cristine Rodrigues, Joyce Gueiros Wanderley Siqueira** and **Carlos Ricardo Soccol**

Biotechnology and Bioprocess Engineering Department, Federal University of Parana, Curitiba-PR, Brazil

Abstract: Bioethanol is the most used biofuel worldwide. Its use contributes to the reduction of fossil fuel consumption and environmental pollution. It is mainly produced from sucrose, which is available in alternative media. Yeast, mainly *Saccharomyces cerevisiae,* are the most employed microorganisms for ethanol production. These strains usually present high productivity, high ethanol tolerance, and the ability to ferment different sugars that are included in the composition of the highly utilized feedstock. Nevertheless, there are some barriers to yeast fermentation to overcome. They are linked to inhibitors of ethanol production, including high temperature, high ethanol concentration, and the ability to ferment pentose sugars. The efficiency and productivity of ethanol can be enhanced by the use of genetically modified yeast strains, including hybrid and recombinant. Other possibilities of limiting bioethanol processing inhibition are metabolic engineering of the medium and yeast cell immobilization. This chapter highlights some aspects that involve fermentation strategies to minimize bioethanol inhibition during its production.

Keywords: Biofuel, Bioethanol, Ethanol, Fermentation, Genetic modified, Hybrid, Inhibition, Metabolic engineering, Pentoses, Recombinant strains, *Saccharomyces cerevisiae*, Sucrose, Yeast.

INTRODUCTION

According to Rastogi and Shrivastava [1], bioethanol has an annual market of US$58 billion. Approximately half of the global sugar produced is used for ethanol production, with the USA and Brazil as the global leaders. These countries primarily use sugar-based crops like corn and sugarcane, respectively. In Brazil, biofuel accounts for 27.5% of the fuel market. Biofuels like ethanol have a strong global insertion. In terms of cellulosic biofuel production, approximately

[*] **Corresponding author Luciana Porto de Souza Vandenberghe:** Biotechnology and Bioprocess Engineering Department, Federal University of Parana, Curitiba-PR, Brazil; E-mail: lvandenberghe@ufpr.br

10% of the global residues would satisfy 50% of the biofuel demand by 2030. Nevertheless, there are some barriers in yeast fermentation to overcome that are linked to the inhibition of ethanol production: medium composition and processing conditions, including high temperature and product (bioethanol) concentrations (Fig. **1**). Another important point is the ability of strains to ferment pentose sugars, which are common components of lignocellulosic biomass hydrolysates.

Some actions for high ethanol efficiency and productivity can be achieved by the use of genetically modified yeast strains, including hybrid and recombinant microorganisms. Yeast cell immobilization is another important tool for better biofuel production. This chapter highlights recent information about strategies to minimize bioethanol inhibition during its production.

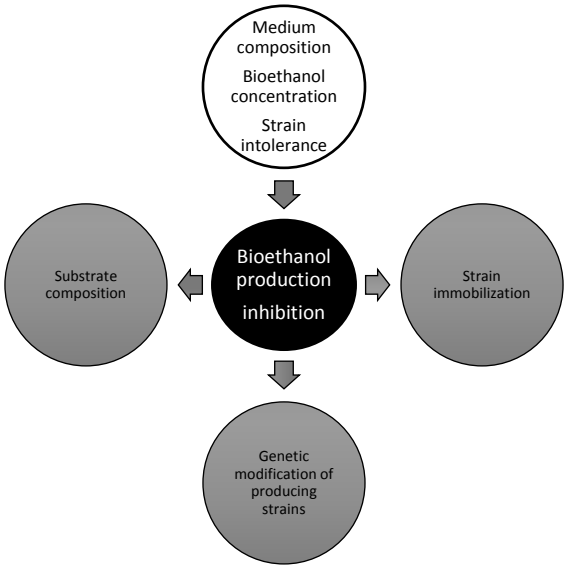


Fig. (1). The main causes of bioethanol production inhibition.

BIOETHANOL MICROBIAL STRAINS

Most microorganisms that produce ethanol by fermentation are mesophilic, with optimum metabolism between 30 and 37ºC. Yeast has been used for centuries for alcoholic beverages (*e.g.*, beer and wine) production. Currently, *Saccharomyces* strains are widely used for biofuel production. Approximately 2,500 yeast species have been discovered, and this number is expected to reach 150,000, namely because these microorganisms can live in diverse habitats, including terrestrial, aerial and aquatic environments [2].

Saccharomyces cerevisiae and *Zymomonas mobilis* are the most well-known ethanol producers. These microorganisms can convert glucose, sucrose, and fructose to ethanol, but they are incapable of converting pentose sugars such as xylose. *S. cerevisiae* uses glycolysis to convert sugars, mainly glucose, into ethanol. Under anaerobic conditions, 1 mole of glucose generates 2 moles of ethanol, 2 moles of carbon dioxide, and 2 two adenosine triphosphate (ATP) molecules. *Z. mobilis*, a gram-negative bacterium, has been used for ethanol fermentation because it has a higher ethanol tolerance and glucose uptake and enhanced ethanol yield and productivity when compared to *S. cerevisiae*. *Z. mobilis* metabolizes glucose by the Entner-Doudoroff pathway to yield 1 mole of ATP [3].

Some species of the genera *Candida*, *Pichia*, *Pachysolen*, and *Schizosaccharomyces* can convert pentose sugars to ethanol. *Pichia stipitis* (NRRL-Y-7124), *S. cerevisiae* (RL-11), and *Kluyveromyces fragilis* (Kf1) are reportedly very good ethanol producers (from different types of sugars) [2].

The natural characteristics of each microorganism are beneficial for ethanol production. Thermotolerant yeast like *Kluyveromyces marxianus* can co-ferment hexoses and pentoses as well as tolerate temperatures from 42 to 45ºC. The ability to ferment hexose sugars reduces the costs associated with the previous hydrolysis of feedstocks.

In general, yeast can sustain the main bottlenecks of the majority of ethanol fermentation processes, and thus they are the most suitable microorganisms for this purpose. The microorganism must have the simultaneous ability to produce high ethanol yields (> 90%) and tolerate concentrations up to 40 g/L, levels that allow productivities of 1g/L/h [2]. Yeast can grow in the simple and inexpensive culture medium, present resistance to inhibitors and contaminants, and tolerate a wide range of pH, mainly acidic conditions; all of these factors make them less susceptible to contamination [4]. Additionally, the property of floc formation is an advantage for the recovery and reuse of the biomass at the end of each fermentation batch. *Saccharomyces* strains are generally recognized as safe (GRAS), and thus they are still the most common microorganisms used for alcoholic beverages and other fermented food production as well as for biofuel production because they present some essential characteristics [3].

The co-culture of different strains has been also proposed as a method to overcome the problem of consumption of five and six-carbon sugars. For example, *S. cerevisiae* and *P. stipitis*, when cultivated together, consume glucose and xylose simultaneously, with a considerable augmentation in ethanol productivity. However, other problems, like the different ethanol tolerance of each

CHAPTER 10

Toxicity and Structural Activity Relationship of Persistent Organic Pollutants

Ankur Khare[1,2], Pradip Jadhao[1,2], Sonam Paliya[1,2] and **Kanchan Kumari[1,2,*]**

Environmental Impact and Sustainability Division, CSIR-NEERI, Nagpur, India

Academy of Scientific and Innovative Research (AcSIR), Ghaziabad-201002, India

Abstract: Persistent Organic Pollutants (POPs) are organic compounds of mainly anthropogenic origin posing a huge threat to human health and the ecosystem. Though the production and intended use of major POPs are banned by Stockholm Convention, still some POPs are being used in most developing countries around the globe. Although, environmental levels of some POPs, such as Polychlorinated biphenyls (PCBs) have declined, newly added POPs in the list of conventions, such as Polybrominated Diphenyl Ethers (PBDEs), Perfluorooctanesulfonate (PFOS) have emerged as new challenges. Exposure to POPs has been associated with a wide spectrum of health effects, including developmental, carcinogenic immunologic, reproductive, and neurotoxic effects. It is of major concern that the neurotoxic effects of some POPs have been observed in humans at low environmental concentrations. This chapter focuses on various POPs like PCBs, PBDEs, and PFOS as a representative chemical class of POPs and discusses the possible modes (s) of action for the neurotoxic effects with an emphasis on comparing dose-response and structure-activity relationships (SAR) with other structurally related chemicals. There are sufficient epidemiological and experimental studies carried out in different parts around the globe showing that PBDEs and PFOS exposure is associated with motor and cognitive deficits in humans and animal models. Several potential mechanisms were presumed for PBDEs and PFOs induced neurotoxic effects and alteration in neurotransmitter systems. Among them, the intracellular signaling processes and hormonal imbalance impacting the activity of thyroid hormone were reported as predominant. All these potential mechanisms are discussed in detail in the chapter. In addition to this, SAR will be highlighted for examining the toxicity of other relevant and structurally similar POPs to assess if they have a common mode(s) of action. Potency factors for several other POPs will also be described focusing on their effects on intracellular signaling processes and enzymatic activity and cell signaling pathways. This chapter is a comprehensive review, describes the alteration of enzymatic pathways and their associated toxicity at the biochemical level in different models for environmentally relevant POPs.

* **Corresponding author Kanchan Kumari:** Environmental Impact and Sustainability Division, CSIR-NEERI, Nagpur, Academy of Scientific and Innovative Research (AcSIR), Ghaziabad-201002, India; E-mail: onlinekanchan1@gmail.com

G. Baskar, K. Sathish Kumar & K. Tamilarasan (Eds.)

Keywords: Neurotoxicity, Persistent Organic Pollutants, Polychlorinated Biphenyls (PCBs), Polybrominated Diphenyl Ethers (PBDEs), Perfluorooctane Sulfonate(PFOS), Structure-Activity Relationships (SAR), Thyroid hormones.

INTRODUCTION

Persistent Organic Pollutants (POPs) are a group of persistent and extremely toxic chemicals in the environment even in trace amounts. They degrade very slowly. They are lipophilic (fat-loving), they have a tendency of bioaccumulation in adipose tissue of organisms and can travel from lower to higher trophic levels in the food chain. They have the ability of long-range transport and can be detected in the environment and biota of regions far away from its source of production. Therefore, they are a subject of regional, national and global concern. The United Nations Environment Programme (UNEP) Stockholm Convention (SC) on POPs was adopted in May 2001 and entered into force on17 May 2004. POPs are commonly known as Dirty Dozen which comprises of Polychlorinated biphenyls (PCBs), aldrin, dieldrin, endrin, polychlorinated dibenzofurans (PCDF), polychlorinated dibenzo-p-dioxins (PCDD), dichlorodiphenyltrichloroethane (DDT), hexachlorobenzene, mirex, toxaphene, chlordane, and heptachlor (Table **1**). Apart from the dirty dozen, some more chemicals were further added in the list of POPs called as new POPs. To protect human health and environment from POPs, article 16 of the SC has recommended the conference of the Parties, to check the effectiveness of the Convention. Apart from that, a Global Monitoring Plan (GMP) was also established by the SC to provide a framework for the necessary data collection on POPs from all regions. The main objective of this GMP was to identify the concentration of POPs over a time period. Level of POPs like PCBs, PBDEs have been accounted to be highest in the species at the top of the trophic level (polar bear, killer whales, eagles, and human beings). Apart from that, the concentration of POPs in every individual is accounted to be more than that of their ancestors [1 - 4]. Degradation of POPs occurs very slowly in the environment. They remain in the environment for a longer duration even if the provenance of POPs is instantly abolished. Scientific studies have revealed that a majority of the human population carries significant amounts of POPs in body fat, causing various health effects like endocrine and immune system disruption, cancer, reproductive and developmental problems, abnormal behavior and neurological disorders [5, 6]. POPs, upon their release in the environment, get transported by different transport media like air and water currents and reach places far from their origin point. This complete journey is a complex process consisting of numbers of “hops”; each hop is a sequence of three-stage which includes evaporation, atmospheric transportation followed by condensation at lower temperatures. Scientifically this complete phenomenon is termed as “grasshopper effect” (Fig. **1**). Similar to this process, POPs get transported to long

distances and get widely distributed in a short period. Vast water reservoirs like oceans, glaciers, icebergs, and huge mountains are known to be the ultimate fate of such chemicals.

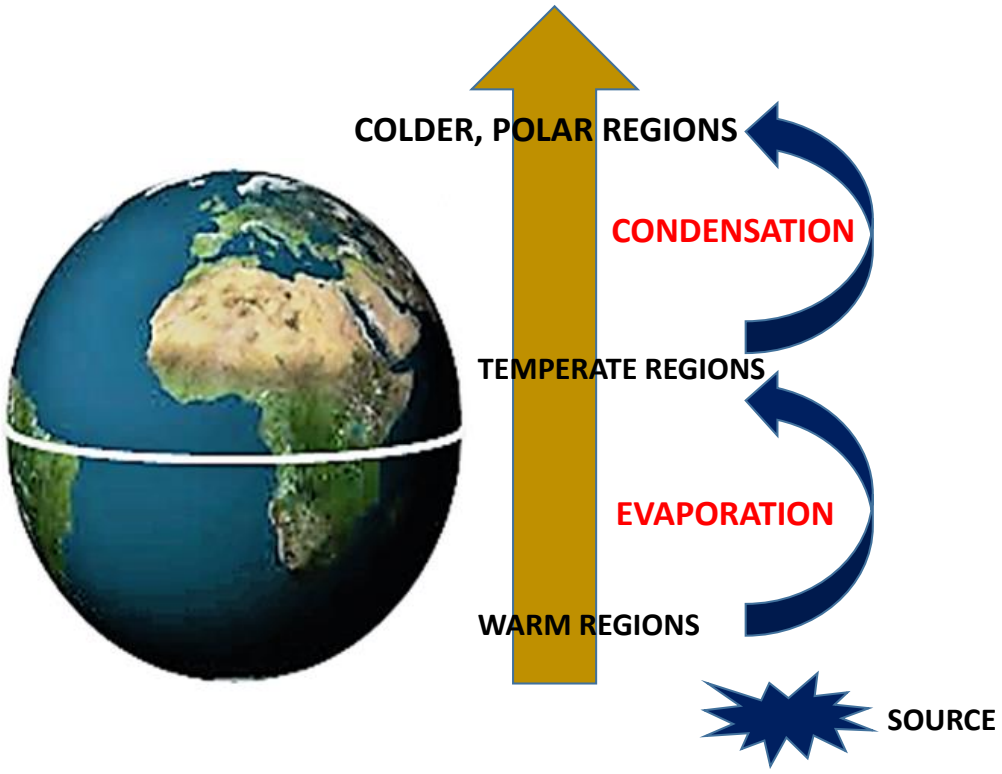


Fig. (1). Grasshopper Effect.

Table 1. Status of Twelve POPs in Stockholm Convention.

S.No.	Name	Listed Under	Category
1.	Aldrin	Annex A	Pesticide
2.	Chlordane	Annex A	Pesticide
3.	DDT	Annex B	Pesticide
4.	Dieldrin	Annex A	Pesticide
5.	Endrin	Annex A	Pesticide
6.	Heptachlor	Annex A	Pesticide
7.	Hexachlorobenzene	Annex A and Annex C	Pesticide/ Industrial Chemical
8.	Mirex	Annex A	Pesticide
9.	Toxaphene	Annex A	Pesticide

CHAPTER 11

Enzyme Inhibitors for Breast Cancer Therapy

Hariharan Jayaraman[1], Praveen Kumar Posa Krishnamoorthy[1], Lakshmi Suresh[1], Mahalakshmi Varadan[1], Aparna Madan[1] and **Balu Ranganathan[2,3,*]**

[1] *Department of Biotechnology, Sri Venkateswara College of Engineering, Sriperumbudur Tk. 602 117, Kancheepuram District, Tamil Nadu, India*

[2] *Palms Connect Sdn Bhd, Shah Alam 40460, Selangor Darul Ehsan, Malaysia*

[3] *Palms Connect LLC, Showcase Lane, Sandy, UT 84094, USA*

Abstract: Globally millions of women die of cancer, the most common cancer occurrence sites for women being breast, cervical and ovarian. Engineered enzyme inhibitors are a component of the drug regimen that has taken a drive into pharmaceutical international corporations' product portfolios which is by targeted delivery, moderating disease-free survival and leading to procrastination of death. Since 2002 the enzyme inhibitor anastrozole (Arimidex) is used as the first drug of choice for breast cancer which is available in the commercial market. Currently, there are several other FDA approved enzyme inhibitors like sulfonanilide analogs available in the pharmaceutical shelf decreasing aromatase expression and regulating enzyme activity for the treatment and cure of breast cancer.

Keywords: Anastrozole, Breast cancer, Celecoxib, Enzyme inhibitors, HSD17B, TLK.

INTRODUCTION

Breast cancer is one of the deadliest diseases occurring in women globally. Next to lung cancer, breast cancer ranks second accounting for 11.6% death. Whereas it ranks No.1 among the females with a high mortality rate subsequently followed by lung cancer and colorectal cancer [1]. Cancer is an uncontrolled proliferation of cells which is a result of changes in the genes such as oncogenes and tumor suppressor genes. Cancer is still a mystery due to the complex changes that occur in the cellular environment due to the genetic and non-genetic influences. The primary causative agents of cancer are much less understood until the 1980s. However, knowledge on the role of certain factors contributing to the etiology of breast cancer received attention in the 1980s. Risk factors associated with breast

* **Corresponding author Balu Ranganathan:** Palms Connect LLC, Showcase Lane, Sandy, UT 84094, USA; E-mail: ranga@palmsconnect.com

G. Baskar, K. Sathish Kumar & K. Tamilarasan (Eds.)

cancer include age, exposure to radiation, diet, body weight, family history and consumption of exogenous hormones as oral contraceptives [2]. Recent pieces of evidence suggest that single target based drug therapy is a limitation to efficiently treat cancer. Hence the pharmaceutical companies are currently exploring multiple target based drug therapy for their efficacy in controlling the disease as a more potent drug regimen. Currently, pharmaceutical companies are producing drugs for chemotherapy (small molecules), hormonal therapy (therapeutic hormones) and targeted therapy (therapeutic proteins) of breast cancer. Top 10 pharmaceutical companies which are involved in the production of oncology products in the year 2018 includes Roche, Novartis, Celgene, Johnson and Johnson, Bristol-Myers Squibb, Pfizer, Merck, AstraZeneca, Lilly and Abbvie [3]. Some of the breast cancer drugs manufactured by these companies are mentioned in the below table (Table. **1**) with their trade names and the type of therapy in which they are used.

The existing treatment strategies for breast cancer include unique or combination of chemotherapy, hormone therapy and targeted therapy. While chemotherapy is nonselective and hormonal therapy is partially specific to the estrogen producing and responding to cell types, targeted therapy specifically targets cells that overexpress HER2. Earlier the drugs which are used to treat cancer-targeted only single target, whereas now new drugs such as sorafenib and sunitinib are proven

Table 1. Breast cancer drugs.

S.No	Company	Brand Name	Generic Name	Therapy Type	Mechanism of Action
1.	Roche	Avastin	Bevacizumab	Targeted therapy	Blocks VEGF
2.	Roche	Herceptin	Transtuzumab	Targeted therapy	Block HER-2
3.	Pfizer	Ibrance	Palbociclib	Chemotherapy	CDK 4/6 Inhibitor
4.	Roche	Perjeta	Pertuzumab	Targeted therapy	Block HER-2
5.	Novartis	Afinitor	Everolimus	Chemotherapy	mTOR inhibitor
6.	Astra Zeneca	Nolvadex	Tamoxifen	Hormone therapy	Blocks Estrogen Receptors (SERM)
7.	Astra Zeneca	Faslodex	Fulvestrant	Hormone therapy	Blocks Estrogen Receptors (SERD)
8.	Arimidex	Arimidex	Anastrozole	Hormone therapy	Stops Estrogen synthesis (AIs)
9.	Novartis	Femara	Letrozole	Hormone therapy	Stops Estrogen synthesis (AIs)
10.	Pfizer	Aromasin	Exemestane	Hormone therapy	Stops Estrogen synthesis (AIs)

to have efficacy towards multiple targets such as PDGFR and VEGFR [4] making a milestone in the cancer treatment progression. A treatment regimen recommended to the breast cancer patient upon identification of the breast cancer subtype and the grade of the tumor. Because it is observed that breast cancer drugs respond differently to different molecular subtypes of cancer. For example, the drugs paclitaxel and doxorubicin showed a different gene expression profile in basal-like and erbB2+ subtypes of breast cancer than luminal and normal-like cancers [5].

Mammary Gland Anatomical Construction

The female breast is an extremely interlocking and tortuous organ involving lactation, emotional excitation and social blossom. It undergoes interchanged modifications in the life span of a female than any other part of the human body – from birth, upon puberty, during the periodical menstrual ovarian cycle, pregnancy leading to lactation and till menopause. The various parts of breast being:

- Lobule (tiny bulb-like structure) produces milk
- Lobe (15-20 sections of lobules)
- Duct, the tube through which milk travels through from lobes
- Nipple, milk is secreted through from the duct
- Areola

There is a complete absence of muscles and bones in the breast. Fat, connective tissue and ligaments act as the filler in between lobes and ducts which provides the shape, structure and aesthetics to the breasts. Basically carcinoma of the breast is seen in the lobules, ducts and rarely in the nipple.

Breast Carcinogenesis

Cancer in the Duct

Ductal carcinoma in situ (DCIS) is the earliest form of breast cancer where cancer is at the cellular level in the duct. Cancer cells are only present and not cancerous tissue. Cancerous cells are observed in the duct. It is being localized, contained to a local region and which has not spread across to the normal breast tissues (Fig. **1**).

CHAPTER 12

Enzyme Inhibition Applications in Treatment of Human Viral Diseases

Subasree Sekar, P.K. Praveen Kumar* and **Arthi Udhayachandran**

Department of Biotechnology, Sri Venkateswara College of Engineering (Autonomous), Sriperumbudur Tk – 602117, Tamilnadu, India

Abstract: Enzyme inhibitor molecules are used for the development of antiviral drugs. Understanding the mechanisms of enzyme inhibitors are needed for the treatment of HIV, Chikungunya, Dengue, Ebola, Influenza, and Nipah viral diseases. Inhibition of viral entry and its replication in the host cell was the most prominent mode of action against these viruses. In this chapter, the detailed list of plant compounds to be used as drugs for the treatment of above viral diseases through targeting of enzymes, reverse transcriptase, and RNA-dependent RNA polymerase is explained. Recent advancements such as emerging technologies, Next Generation Sequencing, and CRISPR used as an effective approach for the diagnosis, treatment, and alleviation of viral disease progression, are explained.

Keywords: Antiviral drugs, CRISPR, Enzyme inhibitors, Next Generation Sequencing, Reverse transcriptase, RNA-dependent RNA polymerase.

INTRODUCTION

Enzymes are biocatalyst that accelerates the chemical reactions. Enzyme inhibitors are chemical compounds with a low molecular weight that may scale back or completely inhibit the enzyme catalytic activity reversibly or irreversibly (permanently). An enzyme-inhibitor complex is formed once the enzyme is bound to the inhibitor. However, the complex is not formed if the enzyme is not bound. The presence of naturally occurring enzyme inhibitors, like antitrypsin, anti-thrombin, and anti-pepsin, controls the activity of an enzyme in the human body and under physiological circumstances ensures their extracellular and intracellular action [1].

Most commonly, competitive enzyme inhibitors are utilized as pharmaceutical drugs or agents. Competitive inhibition is an analog to biochemical substrates that

* **Corresponding author Praveen Kumar P.K:** Department of Biotechnology, Sri Venkateswara College of Engineering (Autonomous), Sriperumbudur Tk – 602117, Tamilnadu, India; Tel: +919444495008; Fax: +914427162462; Email: praveenpk@svce.ac.in

G. Baskar, K. Sathish Kumar & K. Tamilarasan (Eds.)

compete with the natural substrate for an enzyme's active site and prevent unwanted metabolic products [2]. Furthermore, additional inhibitors target enzymes that use bi - substrate only after a transition to the active site has occurred due to the binding either of the two reaction substrates. Such uncompetitive inhibitors bound to the substrate and hinder the enzyme catalysis [3]. For example, mycophenolic acid, which inhibits the inosine 5′-monophosphate dehydrogenase (IMDH) enzyme, is used in treating cancer and viral diseases [4].

Antiviral medicines are used in particular to treat viral infections. Most antivirals required activation by viral and cellular enzymes before antiviral use [5]. Moreover, some viruses have protease enzymes, which involve in cleavage of viral protein chains. Substantial research was carried out to identify HIV protease inhibitors as drugs for the treatment of HIV attacks in humans [6]. Protease inhibitors were found efficient in the 1990s but later it developed side effects [7]. Protease inhibitors development from natural sources is focused in the present era. For example, Shiitake mushroom (*Lentinus edodes*) possess protease inhibitors that have shown antiviral activity in *in vitro* [8, 9].

MECHANISM OF ANTIVIRAL DRUGS

In the following section, the drugs used for viral diseases are discussed and are shown in Fig. (**1**).

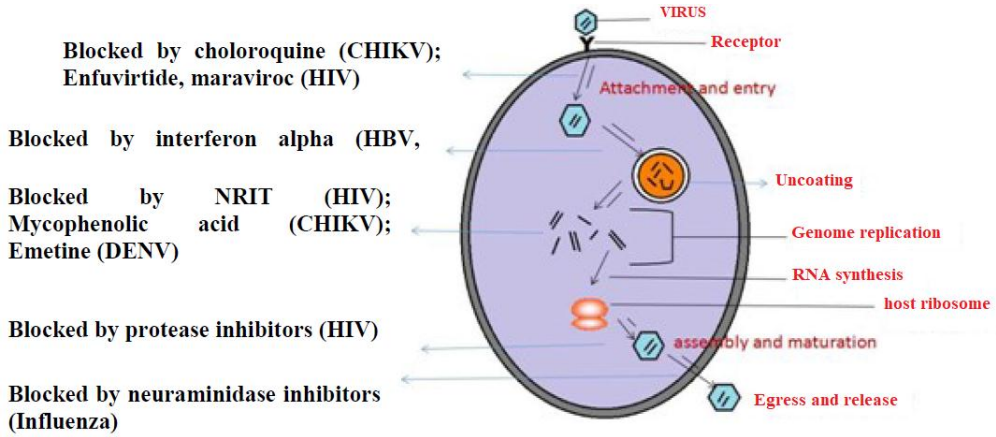


Fig. (1). Mechanism of action of antiviral drugs.

Attachment

Virus infections are initiated when virus capsid or envelope-related viral proteins bind to the specific host cell membrane receptors on host cells. The HIV envelope glycoproteins allow binding of the virus to bind to CD4+ T lymphocytes expressing the chemokine receptor 5 (CCR5) and/or the C-X-C motif chemokine receptor 4 (CXCR4) [10].

Entry

Viruses enter across host cell membranes into the cytoplasm. For example, the host cell (CD_4+ T lymphocyte) membranes are promoted by the HIV envelope protein (gp41) [11]. A virus-mediated fusion of the HIV envelope with a plasma membrane of the host CD4+ T lymphocytes is currently being offered as a virus - entry blocker.

Uncoating

Uncoating of viral entry involves the removal/degradation of nucleocapsids. Structural modification of nucleocapsids causes the viral genome to be released into the host cell cytoplasm and transported to the host cell nuclear nuclei. Currently, there are viral uncoating inhibitors that block influenza A virus M2 proton channel and prevent virus matrix protein dissociation dependent on pH [12].

Transcription and Translation

After the uncoating step, the gene expression of viral nucleic acid was determined, *i.e.*, the transcription of viral RNA or DNA into mRNA and mRNA translation into proteins, and viral polyproteins proteolytic cleavage into individual protein units, become available. Existing viral gene expression inhibitors presently disrupt HCV – related functional NS3/4A protease expression [13].

Replication

The ribonucleoside triphosphates or deoxyribonucleoside triphosphates generation is required for viral genome replication. In the host cell cytoplasm, most RNA viruses replicate their genomes. In the host cell nucleus, however, DNA viruses are replicating their genomes. Nucleoside analogue inhibitor phosphorylates viral

CHAPTER 13

Enzyme Inhibition Applications in Treatment of Neurological Disorders

D. Lokapriya and **P.K. Praveen Kumar***

Department of Biotechnology, Sri Venkateswara College of Engineering (Autonomous), Sriperumbudur Tk, 602117, Tamilnadu, India

Abstract: Enzyme inhibitors are widely prescribed drugs for many diseases including neurological disorders and today most of the drugs are enzyme inhibitors and are in the clinical/pre-clinical phase of the drug discovery. For many of the neurological disorders, especially neurodegenerative disorders, only symptomatic therapy is available rather than the therapy based on an understanding of the mechanism of these diseases. In this case, enzyme inhibitors become the solution as they inhibit the action of enzymes whose abnormal activity may be one of the causes of the disease. They also halt the progression of the disease and alleviate the symptoms. This chapter focuses on some of the enzyme inhibitors that have been prescribed as drugs for neurological disorders, their mechanism of action and discuss some inhibitors that are still in their research level of development.

Keywords: Drug discovery, Enzyme Inhibitors, Neurological disorders, Symptomatic therapy.

INTRODUCTION

Enzymes are protein molecules that act as catalysts in various biochemical reactions. Substances that inhibit the catalytic activity of enzymes are called enzyme inhibitors. Being the low molecular weight chemical compounds, enzyme inhibitors reduce the enzyme activity either reversibly or irreversibly [1]. Nowadays, chemo drugs are based on reducing the activity of overactive enzymes which in turn declines the progression of the disease and alleviates symptoms [2].

Generally, competitive enzyme inhibition is the mechanism used for employing enzyme inhibitors as pharmaceutical agents in which inhibitors structurally similar to normal biochemical substrates are used to compete with the natural substrate for the active site of the enzyme and it results in blocking the formation

* **Corresponding author Praveen Kumar P.K:** Department of Biotechnology, Sri Venkateswara College of Engineering (Autonomous), Sriperumbudur Tk, 602117, Tamilnadu, India; Tel: +919444495008; Fax: +914427162462; Email: praveenpk@svce.ac.in

G. Baskar, K. Sathish Kumar & K. Tamilarasan (Eds.)

of undesirable metabolic products [2]. Hence, enzyme inhibitors constitute a significant portion of the clinical usage of oral therapeutic drugs. Currently, enzymes are attractive targets for drug discovery and efforts are being made on identifying and optimizing drug candidates that specifically inhibit enzyme targets in the field of drug discovery and drug development [3].

Diseases that affect the brain, spine and nerves are called neurological disorders. Nearly, 600 common diseases of the nervous system are occurring in humans mainly: Stroke, Alzheimer's and, Parkinson, *etc.* The current population is aging. Hence, these disorders are very prevalent nowadays as aging is one of the significant risk factors of neurodegenerative disorders along with inflammation, apoptosis, and excitotoxicity [4]. Most of the drugs that are enzyme inhibitors for neurological disorders have been approved by the FDA and some enzyme inhibitors are still in the clinical and pre-clinical phase of the drug development. This chapter gives an overview of some enzyme inhibitors that have been used as drugs for neurological disorders along with their mechanisms.

NEURODEGENERATIVE DISEASES

Alzheimer's Disease (AD)

AD is one of the generally occurring neurodegenerative diseases. Symptomatic therapy is chosen for the treatment of AD. Dementia along with impaired learning and cognition is the primarily noticed clinical symptom but irritability, confusion, and behavioral changes occur later as the disease progresses. Peptides like β-amyloid (Aβ) plaques and neurofibrillary tangles are the pathological hallmarks of AD. They accumulate in the brains of patients of AD. Due to the mutations in amyloid precursor protein (APP), β-secretase (APP cleaving enzyme) does increase the proteolysis and it leads to the increased formation of Aβ plaques by γ-secretases. The accumulated Aβ plaques have destructive effects on neurons especially neuronal loss as it generates free radicals in the brain. Hence, neurotoxicity in the brain due to AD is directly proportional to aggregates formed by Aβ plaques [4].

There are five major types of drugs used for treating AD: cholinergic treatment, anti-glutamatergic treatment, nonsteroidal anti-inflammatory drugs (NSAIDs), vitamins and antioxidants, and pharmacological management of neuropsychiatric symptoms. Among these, acetylcholinesterase inhibitors (AChEIs) are widely used for treating AD [5].

Inhibition of Cholinesterases

Acetylcholine (Ach) is a neurotransmitter responsible for the conduction of electrical impulses among neurons. When the acetylcholinesterase (AChE) enzyme (EC 3.1.1.7) hydrolyses Ach, it's level decreases [6]. ACh is generated in neurons by the action of choline acetyltransferase concentrated in vesicles, and released from the presynaptic cell, which is shown in Fig. (**1**).

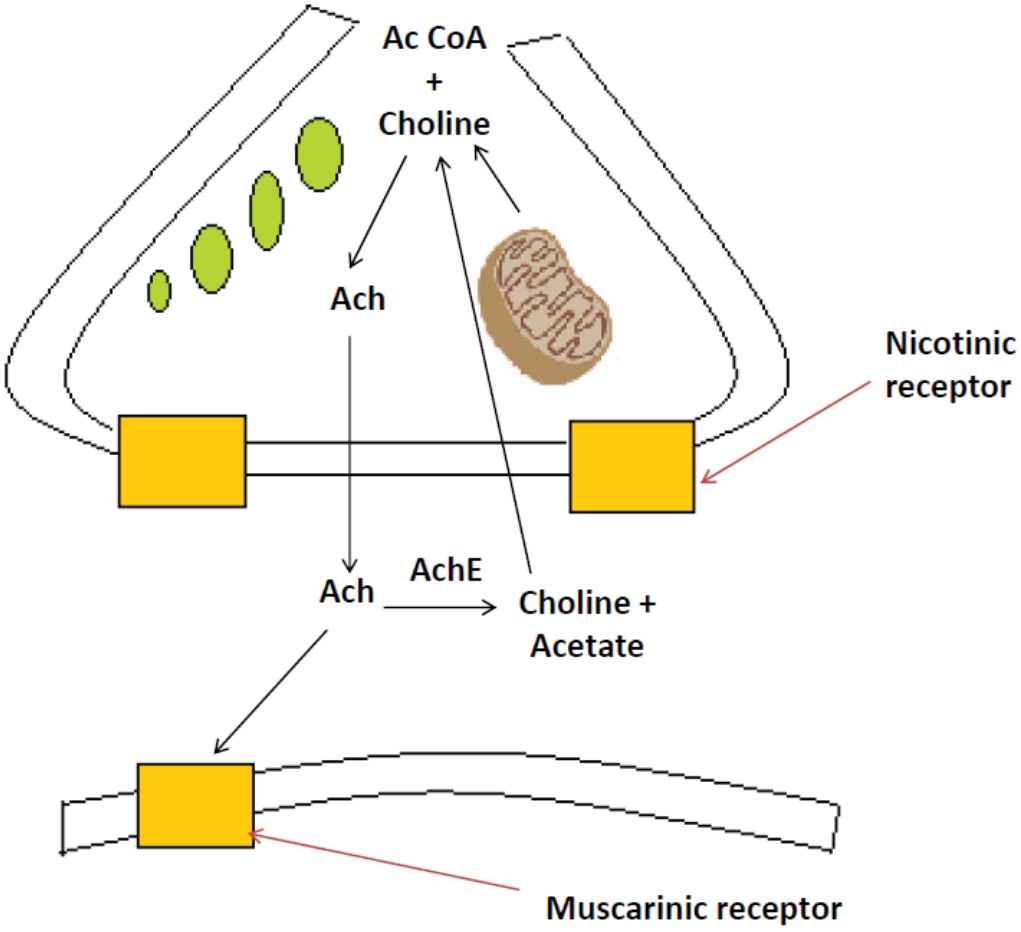


Fig. (1). Acetylcholine biosynthesis, and synaptic transmission.

ACh is released *via* nicotinic and muscarinic cholinergic receptors on post- and presynaptic cells. Once released, it interacts with the muscarinic receptor on postsynaptic cells. The interaction of Ach with muscarinic receptors leads to the hydrolysis of Ach by AChE. When AChE is inhibited, neurotransmission can be

SUBJECT INDEX

A

Acetic acid 64, 123, 127, 155, 156, 157, 158, 167
toxicity 156
Acetylation 13, 274, 275
histone 13, 274, 275
Acetylcholine 32, 64, 67, 101, 182, 265
biosynthesis 265
Acetylcholinesterase 2, 32, 64, 98, 101, 102, 104, 105, 106, 108, 109, 264
and choline oxidase 106
immobilized 102
inhibitors 264
Acids 49, 64, 82, 83, 98, 107, 108, 109, 111, 123, 124, 130, 132, 151, 154, 155, 156, 158, 162, 179, 189, 250, 252, 257, 275, 276, 277
aliphatic 155, 275
apetalic 250, 252
benzoic 98, 109, 156
betulinic 82
butyric 64
cinnamic 156
concentrated phosphoric 128
fatty 82
ferulic 156
formic 154, 155, 158
furoic 158
galacturonic 124
gallic 156
glucaric 158
glutamic 111
hydroxamic 82, 83
inorganic 156
isopetalic 252
levulinic 156
mercapturic 189
oleanolic 277
organic 132, 155, 162, 179
ortho-toluic 156
release uronic 155
sialic 257
suberoylanilide hydroxamic 275, 276
sulfenic 49
sulfuric 129, 155, 158
sulphuric 130
syringic 154, 156
uric 107, 108
ursolic 277
valproic 275, 276
Activated protein kinase (AMPK) 233, 234
Activation 41, 73, 74, 75, 77, 80, 81, 84, 105, 186, 211, 272
imbalance 73
-induced cell death (AICD) 186
intracellular 75
matriptase-dependent 74
thiol ester bonds 81
AIDS 248, 251, 252, 254
developing 254
inhibitors 252
Alkaline phosphatase 2, 6, 65, 67, 98, 102, 103, 104, 105
based biosensor 65
fabricating 65
inhibition of 103, 104
Alkaloids 239, 233, 242, 255
coptidis 242
isoquinoline 233
Allergic reaction 6, 87
Ammonia fiber expansion (AFEX) 157
Amperometric 100, 103
measurement of inhibition of catalase 103
transducer measures change 100
Amperometric biosensors 21, 24, 28, 65, 66
inhibition-based 65
screen-printed based 65
tyrosinase-based 66
Amylase 130
Amyloid precursor protein 264, 278
Amyotrophic lateral sclerosis 277, 283
Analgesic drug acetaminophen 107
Antisense 36, 37, 39, 43
effect 39
genes 36
mRNA 37
Antisense RNA 36, 37, 38, 39, 41, 42, 44, 50

G. Baskar, K. Sathish Kumar & K. Tamilarasan (Eds.)

complementary 37
effect 39
and RNAi 44
strand 39
technology 37, 38
transcripts 38, 41
Antitrypsin 228
Antiviral activity 229, 240, 242, 243, 244, 252
detected 240
Apoptosis 13, 14, 80, 185, 186, 211, 217, 218, 221, 264, 273, 274, 275
cellular 217
hepatic 186
increased 221
inducing 273
Arachidonic acid 210, 211, 278
metabolizing 210
Aromatase 205, 209, 210, 216, 218, 219, 220
active site of 209, 219
activity 209
enzyme 209
inhibition 219
inhibitors (AIs) 205, 209
protein 209
Arthritis 76, 81, 82, 83, 86
rheumatoid 76, 81, 83, 86
disease 82
Ascorbic oxidase 98
Aspartate kinases (AK) 43
Aspiratory blood vessel hypertension 5
Atherosclerosis 83, 85, 269
plaque 85
Atmospheric transportation 175
ATPases 182, 183
major calcium-stimulated 183
Autohydrolysis 166

B

Binding transcription factors 210
Biocatalytic action 29, 209
Bioethanol 131, 148, 149, 163, 167
fermentation process 163
fuel 131
inhibition 148, 149
synthesis 167
Bioethanol production 122, 123, 124, 125, 128, 148, 149, 151, 153, 155, 156, 160, 161, 163, 164, 166, 167, 168
factors influence 160
full-scale 164
inhibition 149, 163
Biofuel production 124, 148, 149, 150
cellulosic 148
Biomass 125, 129, 130, 137, 138, 139, 151, 150, 154, 155, 156, 157, 158, 159, 160
cellulosic 153
lignocellulosic 130, 151, 154, 158, 159, 160
sugar-based 151
Biosensors 1, 2, 17, 19, 20, 21, 22, 23, 24, 25, 27, 29, 30, 32, 33, 59, 60, 61, 62, 65, 66, 67, 97, 101, 102, 104, 107, 108, 112
chemical cluster 30
design of 1, 2
distinctive 25
effective 20, 112
electrochemical 66, 101
enzymatic 19, 27
enzymatic inhibitory 97
fluorescence quenching 104
fluorescent ALP-based 65
hindrance-based 25
hydrazine 29
immobilized 2
sensitive 104, 107
Biosynthesis 11, 42, 46, 47, 209, 214, 216, 243, 244
guanine nucleotide 243
intracellular asparagine 11
steroid 214
terpenoid 46
Blocks estrogen receptors 205
Blue-chitosan nanocomposites 106
Bovine serum albumin 65, 109
Brain tumors 76, 86, 271, 274, 276
advanced 86
malignant 274
Breast cancer 204, 205, 206, 207, 208, 209, 211, 214, 215, 216, 217, 218, 219, 220, 221, 222
advanced-stage 219
hormone-dependent 214, 216, 221
pre-menopausal 221
relapsed 221
treatment of 216, 219
Breast cancer cells 209, 215, 221
growth 209, 215
proliferation 215

C

Cancer 5, 6, 48, 73, 74, 76, 83, 84, 85, 86, 87, 204, 205, 206, 207, 208, 272
advanced solid 86
colorectal 87, 204
esophageal 74
gastric 76
ovarian 76, 87
prostate 48
Cancer cells 80, 83, 84, 206, 207, 273, 274
cervical 80
Cancer 84, 216, 217, 272, 273
drugs 273
genome atlas 217
metabolism 272
recurrence 216
therapy 84, 272
Cancer treatment 3, 5, 6, 12, 82, 84, 85, 206
progression 206
Carboxylic acids 83, 154, 157, 158
Carcinogenesis 2, 80, 179, 272, 273
Carcinomas 76, 180, 206
Cardiovascular diseases 73, 74, 83, 85, 87
Catalyses 10, 28, 155, 156, 158, 165, 214, 216, 280
ammonia-based 155
chemical 155
sulfite 158
Cell morphogenesis 217
Cellobiose 166, 167
Cell proliferation 77, 184, 187, 221, 244
eukaryotic 244
progenitor 187
Cellulose 122, 123, 124, 125, 127, 129, 130, 131, 152, 153, 154, 155, 156, 157, 158, 159, 160, 163
break 160
digestibility 125, 159
digestion 155
fibrils 153
structures 154
Central nervous system (CNS) 87, 181, 182, 282, 283
Chikungunya virus (CHIKV) 231, 232, 233, 235, 236, 237
Chronic obstructive pulmonary disease (COPD) 2, 5, 76
Collagen 74, 75, 271
cleaving triple helices 74
Collagenases 73, 77, 78
interstitial 73, 77
neutrophil 78
Collagen catabolic process 77, 78
Collagenolytic activity 73
Competitive reversible inhibition 9
Compound annual growth rate (CAGR) 152
Consumption 133, 159
microbial biomass 159
nutrient 133
Cyclodextrin 104
Cyclohexenyl chalcone derivatives 240
Cyclooxygenase 210, 211, 270
Cytokines 77, 83, 84, 186, 210, 248
inflammatory 83, 210
proinflammatory 77, 248
Cytosolic 11, 194
aryl hydrocarbon 194
chemotherapy medicate 11

D

Dendritic cells 248
Diarrhea 266
Diseases 46, 47, 48, 50, 73, 74, 75, 81, 82, 83, 85, 86, 87, 179, 204, 236, 254, 263, 264, 275, 276, 277
arthritic 82
atherosclerotic 85
autoimmune 73, 74, 81, 83
deadliest 204
gastrointestinal 75
immune 275
infectious 236, 254
neurodegenerative 264
Disorders 2, 5, 85, 178, 196, 264, 275, 277, 280
autoimmune 2, 275
bleeding 5
cardiovascular 85
gastrointestinal 2
human health-related 196
reproductive 178
DNA 22, 37, 39, 40, 41, 46, 61, 84, 165, 217, 230, 231, 244, 251, 257, 273, 275, 280, 281
activities, dependent 251
and histone proteins 275
and RNA virus replication 244
binding elements 165

damage response 217
duplexes 40, 41
helical structure 40
polymerases 231
synthesis 84
template 37
transcription 273
viruses 230, 280, 281
DNS method 131

E

ECM 74, 82, 85
processing 82
proteins 85
protein turnover and degradation 74
Elastin 74, 83, 212
degrading 74
Electrical 22, 100, 178, 187, 265
data stream 22
impulses 265
output 100
wires 178, 187
Electroactive 24, 98, 101
material 98
Electrocatalytic moieties 29
Electrochemical 17, 100, 105, 106, 107, 108, 109, 110
transducers 100
Electrode 60, 65, 103, 104, 107, 108, 109, 110
carbon nanopowder paste 65
disposable 108
exfoliated transition metal dichalcogenides 104
glass 60, 103
gold 107, 109
graphite 107, 109
multiwalled carbon nanotube 107, 108, 110
Embryogenesis 2, 75
Endocytosis 76
Environmental protection agency (EPA) 177
Enzymatic 123, 125, 130, 151, 152, 159, 161, 167, 214
biocatalytic activity 214
hydrolysis 123, 125, 130, 151, 152, 159, 161, 167
Enzyme 2, 3, 18, 19, 49, 65, 98, 102, 131, 228, 229, 275
catalysis 229
catalytic activity 228
cellulase 131
cleaves 49
detections 3
glucose oxidase 18
histone acetyltransferase 275
immobilization 18, 19, 65, 98, 102
induction 2
Enzyme Inhibition 8, 26, 28, 63, 97, 98, 103, 106, 108, 263
activity 98, 231
assay 111
biosensing frameworks 26
competitive 263
inhibition based biosensor 28, 63, 97, 98, 103, 106, 108
reversible 8
Enzyme inhibitors 1, 2, 3, 6, 11, 14, 98, 99211, 222, 228, 233, 263, 264, 266, 276
cancer-related 222
competitive 228
for breast cancer therapy and tumor necrosis factor 211
kinetics 99
Enzymes 60, 84, 155, 158, 160, 184, 189, 212, 214, 220, 229, 242, 243, 250, 274, 278
cellular 229
cellulolytic 155, 158, 160
collagenase 84
copper-dependent amine oxidase 212
histone deacetylases 274
immobilizing urease 60
inhibitors target 229
microsomal 184
monooxygenase 189
prominent oxidoreductase 214
protease 229, 242, 243, 278
steroidogenic 220
virus integrase 250
Ethanologenic yeast 160
Ethanol-producing yeasts 167
Ethanol product 137
Ethanol production 123, 126, 127, 131, 132, 148, 149, 150, 151, 152, 153, 154, 159, 161, 162, 165, 166
feedstocks 153
formation 136
global 152
lignocellulose 123
lignocellulosic 159

inhibition 149, 154, 165
processes 151
Exoglucosidase 11
Expression of antisense genes 36
Extracellular signal-regulated kinase 234
Extraction 14, 127, 158, 159, 161
liquid-liquid 161

F

Fas-dependent activation 186
Fed-batch 162, 163
Melle-Boinot process 163
processes 162
Fermentation 122, 123, 127, 131, 132, 136, 137, 138, 148, 149, 153, 154, 155, 157, 161, 162, 166
fed-batch 161
high cell density 166
inhibitors 127
products 157
reaction 127
Fermentative yeasts 158
Fermenting 139, 161, 166
broth 166
Ferment pentose 148, 149, 165
sugars 148, 149, 165
Fibrinolysis 270
Fibroblasts 74, 75, 271
Fibronectin 74, 75, 76, 271
turnover 76
Flavin adenine dinucleotide (FAD) 28
Food 46, 108
quality 108
resources 46
safety 108
Food production 104, 125, 150
boosted 104
fermented 150
Fucose containing sulfated polysaccharide (FCSP) 240

G

Gelatinases 2, 74, 75, 77, 78, 83, 271
Genes 37, 38, 39, 41, 42, 43, 47, 49, 165, 166, 184, 186, 204, 210, 273, 281, 282, 283
housekeeping 210
locus receptor kinase 38, 41
multiple 281
pentakisphosphate 2-kinase 47
protein-coding 166
recombinant microbial phytase 47
resistant 273
tumor suppressor 204
upregulate stress tolerance 165
Genes asRNA 42
Genes codes 43
Gene sequences 41, 275
Gene silencing 38, 49, 50, 281, 282
mediated 50
mechanisms 38
techniques 50
technologies 49
Genetic 166, 186
effects 186
engineering efforts 166
Genomes 42, 230, 244, 280
defective 244
Gliomas 86, 272, 274
abnormal 274
recurrent high-grade 86
Global monitoring plan (GMP) 175
Glucose 19, 24, 28, 62, 123, 124, 127, 132, 150, 151, 152, 153, 155, 159, 165, 166, 167
ferments 167
intracellular 165
measuring blood 62
fermentation 155, 159, 165
oxidation inhibition 28
Glucosidase 11, 245
Glucuronic acid 124, 240
methyl 124
Glutathione *S*-transferase (GST) 2, 279, 280
Glycolysis 150, 165
Glycoprotein(s) 74, 75, 231, 232, 240, 244, 245, 256, 257
envelope 232, 240
hemaggelutinin 256
oligosaccharides 245
precursors 244
surface envelope 231
viral 245
Growth 74, 81, 84, 85, 87, 125, 133, 134, 135, 136, 137, 141, 156, 161, 165, 211, 212, 274
carbon-dioxide 125

dysregulated 212
factor binding proteins 74
tumor 81, 84, 87

H

Heavy metal deposition 67
Hemicellulose 126, 161
fraction 126
hydrolysate composition 161
Hepatitis C virus (HCV) 244
Histone deacetylases (HDACs) 13, 274, 275, 276
HIV envelope 229, 230, 231
glycoproteins 230
protein 230
inhibitors 229, 231
HIV transcriptase effects 254
Holocellulose 124
Homeostasis 17, 187, 194
disrupt hormone 187
physiological 17
Homoharringtonine 233, 234
alkaloids 233
Hormones 59, 174, 175, 179, 183, 187, 188, 191, 194, 196, 205
steroid 191
therapeutic 205
thyroid 174, 175, 179, 187, 188, 194, 196
Hormone therapy 205
Host cell 230, 231
cytoplasm 230
detaches 231
Human 190, 248, 249, 250, 252, 253, 254, 257, 281, 282
immunodeficiency virus (HIV) 248, 249, 250, 252, 253, 254, 257, 281, 282
MCF breast cancer cells 190
Huntington's disease (HD) 278, 282
Hydrolysates 123, 127, 131, 155, 156, 160, 162, 164
acid 123, 127
Hydrolysing 32
Hydrolysis 11, 64, 123, 127, 128, 151, 153, 155, 156, 157, 159, 160
alkaline 157
thermochemical 155
Hydrolytic liquid fraction 156
Hydrolyze lignin structure 159
Hydrolyzes membrane phospholipids 278
Hydroxamate compounds trichostatin 275
Hypothalamus-pituitary-thyroid (HPT) 187
Hypoxia response element (HRE) 212

I

Inactivation 75, 216
autolytic 75
Infections 46, 87, 229, 234, 236, 240, 244, 245, 247, 256, 257, 280, 281, 282
henipavirus 247
opportunistic 282
treating neurological 281
viral 229, 236, 256, 257
Inflammations 73, 74, 76, 81, 82, 83, 87, 185, 219, 220, 264
active 83
atopy-associated 76
chronic airways 76
Inflammatory 2, 76, 86, 239, 278
bowel disease 76
disorders 86
reactions 239, 278
-related diseases 2
Influence 25, 162
bioethanol production 162
medicate adequacy 25
Inhibiting 85, 98, 240, 248
analyte concentration 98
MMP activity 85
viral infections 248
viral replication 240
Inhibition 2, 7, 10, 11, 36, 63, 66, 97, 98, 99, 102, 103, 104, 112, 168, 220, 221, 235, 245, 268, 272, 274, 278
avoiding cell 168
-based biosensors 97
effective 98
enzymatic 97, 98
horseradish peroxidase 103
host cell translation 235
irreversible 7, 10, 63
irreversible noncompetitive 268
Inhibition biosensor 2, 98, 99
competitive 99
Inhibition of 103, 110, 228, 271, 274
catalase 103, 110
matrix metalloproteinases 271
protein kinase 274
viral entry 228

Inhibitory 159, 250
activity on reverse transcriptase 250
effect on reverse transcriptase 250
molecules 159
Inhibitory activities 29, 81, 103, 233, 239, 240, 250, 255
competitive 240
elastase 81
Intellectual disability (ID) 218
Intracellular 75, 174, 182, 183, 196
retention motif 75
signaling 182, 183
signaling processes 174, 196
signalling 182
Invasive ductal carcinoma (IDC) 207
Ion-selective field-effect transistor (ISFET) 30, 60

J

Janus Kinase (JNK) 234

K

Kinases 43, 217, 218, 231, 233, 234, 272
activating 234
aspartate 43
cellular 231
encoded protein 217
lipid 272
mitogen-activated protein 233, 234
nuclear serine/threonine 217
Kinetic calculation 2

L

Lachrymatory factor synthase (LFS) 50
Lactose 128, 248
Lateral sclerosis 87
Leukemia 11, 249
acute lymphoblastic 11
Leukocytes traffic 83
Leukotrienes 278, 279
Ligand binding assay 214
Lignin 123, 124, 125, 127, 152, 158, 159, 153, 154, 156, 157, 158, 159
degrading 159
linkages 158
peroxidase 127, 159
Lignocellulose 122, 123, 130, 151, 152, 158, 159
-based Feedstock 152
degradation 159
material 123
processing 123
Lignocellulosic 123, 124, 125, 149, 151, 152, 153, 154, 155
biomass hydrolysates 149
biomass pretreatment processes 155
-derived by-products 154
ethanol production process 153
Hydrolysate 126
materials 123, 124, 125, 151, 152
Lipoxygenase 98
LKR and saccharopine dehydrogenase 43
Locked nucleic acids (LNAs) 40
Luciferases 24, 104, 106, 111
compound 24
Lysine-ketoglutarate reductase (LKR) 43, 44

M

Malignant 87, 88, 216, 272
cancer cell phenotypes 216
glioma Liver 87
pathologies 88
transformation 272
MAPK 234
kinase 234
signaling pathway 234
Marine ball sponge 248
Metabolic 11, 36, 49, 82, 136, 148, 184, 187, 229, 264
activity 82
engineered plants 36
engineering 49, 148
enzyme cytochrome P450 187
irregularity 11
path activity 136
pathways atrophy 184
products 229, 264
Metabolism 11, 47, 132, 136, 149, 162, 164, 165, 179, 187, 189, 214, 267, 269, 279
aerobic 269
cellular 11
complete 179
fermentative 165
optimum 149
oxidative 267, 279

steroid 214
Metabolites 2, 17, 179, 184, 187, 188, 189, 190, 191
hydroxylated 179, 189
natural 2
phenolic 189
water-soluble 191
Metamorphosis 73
Microbial 47, 59, 127, 132, 155, 156, 159, 160, 161
action 156
degradation 159
growth 132, 156, 161
phytase 47
strains 160
Mitogen-activated protein kinase (MAPK) 233, 234
Molasses 129, 151, 152, 153
Molecules 42, 74, 84, 85, 150, 153, 157, 182, 183, 186, 188, 189, 194, 205, 211, 220, 221, 222, 228, 274
antiapoptotic 211
biological 194
bondable 182
enzyme inhibitor 228
estrogen-mimicking 220
less-damaging 157
linking hemicellulose 153
lipid-soluble 274
native fibrillar collagen 74
thyroid hormone 188
Monod's model 122
Monolithic immobilized enzyme reactors (MIERs) 1
Monomers 28, 123, 124, 152
beta-glucose 152
Multiple sclerosis (MS) 76, 82, 83, 84, 275, 276, 277
Mutagenesis 111, 157, 248
cause DNA 157
involving site-directed 111
viral 248

N

Native 75, 125
interstitial collagens 75
production 125
Natural 2, 28, 30, 31, 32
compounds 2
contamination investigation 31
materials 32
operators frame 30
oxidation-diminishment 28
Neoplasia 13, 14, 184
Neuraminidase 231, 254, 256, 257
enzyme 257
influenza virus 256
Neurological pathology disorder 281
Neurons 182, 264, 265, 266, 278, 280, 283
dopaminergic 266
Neuroprotective activity 278
Neuroscientists 280
Neurospora crassa 43
Neurotransmitter systems 174, 181, 182, 196
Next-generation sequencers (NGS) 257
Nipah Virus (NiV) 246, 247, 248, 257
NIV gene 38, 41
Non-competitive reversible inhibition 9
Non-conventional transducer materials 19
Non-steroidal anti-inflammatory drugs (NSAIDs) 212, 219, 232, 264, 273
Nucleic acids 37, 39, 61, 98, 230, 242
viral 230, 242
Nucleoside 230, 237, 243, 248, 254
analog 243
carbocyclic 254
analogues target 248
monophosphate 248
triphosphatase 237
Nucleotide reverse transcriptase inhibitors (NRTIs) 253

O

Okadaic acid 102, 105
marine toxin 105
Open reading frames (ORFs) 231
Optical detection by fluorescence 111
Osteoporosis 76, 219
Oxidoreductase 104
Oxygen 23, 28, 29, 65, 132, 165, 189, 212, 269
atomic 28
depletion 212
molecular 189
sub-atomic 28
Oxygenation of PCBs 189

P

Paget's disease 208
Pancreatitis 12
Parkinson's disease (PD) 266, 267, 268, 276, 277, 278, 280
  pathogenesis 268
Pathogens, global human 237
Pathways 12, 24, 25, 42, 43, 47, 48, 49, 75, 76, 77, 78, 157, 165, 174, 179, 180, 183, 185, 213, 216, 232, 273, 283
  adrenal 216
  altering aryl hydrocarbon receptor 185
  amyloid 283
  carotenoid 49
  cyclooxygenase 273
  cytokine-mediated signaling 77, 78
  enzymatic 174, 213
  isoprenoid 42, 48
  metabolic 25, 47, 157, 179
  metastatic 76
  oxidative respiration 165
  saccharopine 43
  segment 12
  stress response 232
Pattern recognition receptor (PRR) 232
Pentose sugars 123, 150, 167
  converting 150
Perfluoroalkyl substances (PFASs) 188
Perfluorooctanoic Acid (PFOA) 179, 180, 181, 188
Phenolic compounds 66, 153, 154, 155, 157, 158, 162
  low molecular weight 157
  tested 66
Photovoltaics 111
Plasmid-mediated transformation 42
Polybrominated diphenyl ethers (PBDEs) 174, 175, 177, 178, 179, 180, 181, 184, 186, 187, 188, 194, 196
Polychlorinated biphenyls (PCBs) 174, 175, 177, 178, 179, 180, 184, 185, 186, 187, 189, 190, 191, 194, 196
Polymers 19, 107, 109, 110, 153, 179, 222
  conductive 19
  conjugated 109
  high molecular weight 179
  innovative 222
  polyhydroxyalkanoate 107
Polymorphonuclear leukocytes 74
Progesterone receptor (PR) 209, 249
Progressive multifocal leukoencephalopathy (PML) 282
Protease 81, 239
  cleavage 81
  enzyme activity 239
Protease inhibitors 7, 12, 229, 253
  aspartate 12
  commercial 7
  cysteine 12
Protein(s) 19, 24, 28, 43, 80, 122, 160, 189, 205, 221, 233, 235, 237, 239, 243, 274, 276, 283
  action 28
  activity 24
  enzymatic 122
  glucose-regulated 221
  heat-shock 160
  immobilized 24
  kinase C (PKC) 274, 276
  membrane 83
  membrane-associated 189
  membrane-bound 80
  plant 43
  secreted 80
  therapeutic 205
  toxic 283
  sensors 19
  synthesis 233, 235, 237, 239, 243
Proteinases 81, 82, 83, 212
Proteolysis 13, 74, 77, 78, 181, 264
Proteolytic 73, 231
  cleavage 231
  viral polyproteins 230

Q

Quantum dots (QD) 3

R

Reactions 1, 5, 7, 12, 18, 20, 22, 50, 61, 62, 65, 126, 155, 157, 160, 165, 194, 214, 217, 228, 263
  alkaline 157
  antiviral 12
  biochemical 263
  biological 5, 194
  catalyzed 65

chemical 228
electronic 22
enzymatic 1, 62
immunochemical 61
inhibited 7
oxidative 155
quantifiable 18
reverse 165
Reactive 156, 219
electrophile 219
radicals 156
Receptors 18, 22, 183, 187, 194, 209, 215, 230, 265, 267, 275
host cell membrane 230
muscarinic 265, 266
muscarinic cholinergic 265
nuclear hormone 275
particular 183
progesterone 209
protein-coupled membrane 215
thyroid 187
Release 123, 124, 155, 158, 159, 175, 231, 240, 244, 248, 254
extracellular surface 231
Reserve 45, 269
nutrient-enriched 45
respiratory 269
Respiratory 85, 86, 87
disease 85, 86
problems 87
system irritation 87
Response 17, 20, 21, 22, 24, 25, 26, 28, 31, 44, 46, 62, 78, 184, 194
biochemical 26
chemical catalyzed 25
enzymatic 17, 31
enzymatic receptor-mediated 194
immune surveillance 83
lesser postprandial glucose 44
toxic 184
Reverse integrase 249
Reverse transcriptase 228, 248, 250, 252, 253, 254
enzyme 253, 254
Reversible 8, 280
harmony 8
hydration 280
Reversible inhibitors 7, 8, 99, 102
mixed 99
predicted 99
RNA 38, 39, 40, 41, 43, 46, 50, 61, 231, 235, 239, 243, 248, 257
and protein synthesis 239
catalytic 38
genomic 231
noncoding 38
protein-encoding messenger 50
targeted 43
RNA-dependent RNA polymerase 228, 233, 238
proteins 233
RNAi-mediated 42, 47
method 42
Silencing 47
RNA interference (RNAi) 36, 37, 41, 42, 43, 44, 46, 47, 49, 50
and antisense RNA 50
pathway 41
RNAi technology 38, 50
RNA polymerase 239, 244, 248, 251
dependent 244
viral 246
RNA synthesis 233, 239, 240, 243, 248
decreased viral 243
decreasing intracellular 240
suppressing intracellular 240
viral 239
Rosmarinic acid 250, 251, 252

S

Saccharification 131, 151
Saponification 157
Sensor 18, 22, 23, 29, 30, 62, 101
cell-based 18
electrochemical 30
organic 23
valuable hydrazine 29
Sequence 37, 38, 39, 40, 50, 74, 175, 212, 244, 280
bacterial insertion 37
nucleotide 40
promoter 212
Signals 17, 19, 23, 24, 59, 61, 97, 98, 102, 182, 183, 234, 246
electrical 59, 61, 97, 98
electronic 24
extracellular 182
luciferase 234, 246
measurable 19, 59

mechanical 59
Skin 75, 86, 87, 184, 207, 208, 216, 219, 252
lesions 184, 219
rash 86
scarification 252
thickening 208
Soaking aqueous ammonia (SAA) 157
Species 18, 27, 29, 48, 49, 101, 110, 136, 150, 157, 159, 175, 180, 181, 184
cultivated crop 49
dynamic 18
dynamic concoction 18
freed quinone 29
ionic 101
reactive oxygen 110, 157, 159
Starches 44, 45, 122, 123, 129, 151, 152
amylose 44
cereal 44
fermentable 123
plant-based 44
Starchy 44, 151
crops for bioethanol production 151
materials 44, 151
Steroidogenesis 189, 214
Stirred-tank reactors 163
Stomach ulceration 220
Stroke 84, 86, 264, 269, 270, 277
ischemic 270, 277
mechanism of 269, 270
Structure 9, 10, 74, 75, 84, 85, 157, 158, 194, 206, 209, 210, 233, 234, 235
-activity relationships (SAR) 2, 174, 175, 177, 188, 191, 192, 194, 195, 196, 241, 234, 235, 236, 239, 240, 241, 242, 251, 253
of coumarin 250
of fucoidan and derivatives 241
of harringtonine 234, 235
of hyperoside 239
of isoquercetin 255
of quercetin 241, 242
of rosmarinic acid 251
of silymarin 235, 236
of tenofovir 253
Sugars 18, 25, 123, 125, 126, 127, 129, 131, 148, 150, 151, 153, 159, 161, 162, 165, 167
catabolize 165
complex 127
extractable 151
fermentable 123, 131, 151, 159
fermentable monomeric 126
five-carbon 153
global 148
hexose 123, 165
six-carbon 150, 153
Surface plasmon resonance (SPR) 60, 101, 111
Synthase 38, 41, 47, 210
enzyme chalcone 38, 41
prostaglandin-endoperoxide 210
Synthesis 38, 39, 40, 42, 50, 183, 189, 216, 235, 242, 244, 251, 266, 273
corticosteroid 216
phytoene 42
prostaglandin 273
Synthetic 11, 82, 250
coumarins 250
inhibitors 82
substances 11
System 10, 65, 66, 179, 187, 188, 281
cardiovascular 187
circulatory 179
endocrine 187, 188
enzymatic 188
gene editing 281
monosegmented sequential injection 66
neurological 187
nitrogenase 65
ordinary protein response 10

T

Technology 1, 280
facile gene manipulation 280
high throughput screening 1
Terpenoids 45, 46, 48
associated 46
defensive 45
protective 46
Therapies 84, 205, 221, 238, 249, 253, 256, 263, 264, 266, 274
anti-influenza 256
antiretroviral 249, 253
effectual 221
neurological disorder treatment 274
symptomatic 263, 264, 266
Thermotolerant 150, 162
microorganisms 162
yeast 150

Thyroid hormone 181, 187, 188
receptor 188
system 181, 187, 188
transport proteins 187
Thyroid receptor (TR) 187
Tissues 11, 13, 22, 25, 43, 48, 59, 64, 75, 83, 84, 128, 175, 178, 182, 189, 206, 209, 210, 212, 213, 214, 266
adipose 175, 209
cancerous 206
carcinoma 75
corn stem ground 128
endosperm 43
fat 178
healthy 84
multiple human cancer 75
nerve 182
neuronal 266
peripheral 214
photosynthetic 48
Toxicity 46, 65, 104, 109, 132, 162, 174, 180, 182, 184, 191, 193, 194, 233, 249, 251, 279
acute 104, 109, 233
associated 174
biological 193, 194
cottonseed 46
increasing cellular 251
metabolites cause 191
Toxicity 184, 192
equivalent factors (TEFs) 184, 192
equivalents (TEQ) 192
Transaminase 84
Transcriptase 249
Transcription 37, 38, 39, 40, 41, 42, 185, 210, 230, 233, 248, 274, 281
arresting 40
directs 37
reverse 248
Transcription 39, 230, 233, 234, 247, 275
and translation 230, 233
factors 234, 275
stage 39
studies 247
Transducer 17, 18, 19, 20, 21, 22, 23, 33, 59, 60, 61, 62, 97, 98, 100, 100, 101, 103
amperometric 101
chronoamperometric 103
conductometric 100
physicochemical 17
physiochemical 59
selection for biosensors 23
Translation 37, 38, 41, 50, 220, 230, 233, 243
bedside 220
viral 243
Transposase 37, 38
expression 37
gene 37
mRNA 37
Treating 244, 246, 267, 275, 277, 278
depression diseases 267
epilepsy 275
hepatitis 244
human illness 246
infections 244
neurological disorders 277, 278
Treatment 6, 12, 14, 127, 130, 264, 270, 280, 283
activated charcoal 127
anti-glutamatergic 264
antiviral 12
chemical 6
disease 14
effective 280, 283
enzymatic 130
preferred pharmacologic 270
radiation 14
Triplex-forming oligonucleotides (TFO) 40
TR receptor signaling 187
Tumor cells 12, 14, 74, 75, 76, 211, 213, 215, 271, 274
behavior 76
dispersals 12
epithelial 75
metastatic 271
microenvironment 74
Tumorigenesis 13, 75, 212
Tumor necrosis factor (TNF) 211
Tumors 2, 12, 13, 14, 75, 76, 84, 206, 209, 212, 213, 214, 215, 216, 218, 272, 273, 274
aggressive 218
breast cancer patient 215
counting CNS 13
malignant 84
metastatic 84
silencer qualities 14
silencer quality hushing 14
solid 212
suppression 2

tissues 12, 214
Tyrosinase 28, 29, 30, 66, 67, 68, 98, 102, 103, 107, 108, 109
activity 66, 67
and glucose oxidase 103
-Based Biosensors 66
biosensors 67
compound 29
immobilized 28
inhibitory activity 109
movement 29
Tyrosine 101, 107, 212, 246, 277
kinases 246, 277

U

Ulcer 219, 252
mucosal 219
Urease 30, 60, 62, 67, 98, 102, 103, 106
inhibition 30
Urokinase 6, 13
-type plasminogen activator 13
Utilization 7, 14, 18, 19, 25, 26, 27, 32, 44, 47, 156
remedial 7
single 25

V

Vaccines 232, 238, 246, 257
effective antiviral 238
licensed antiviral 232
veterinary 246
Viral 12, 242, 247
polymerases 242, 247
polyproteins 12
Viral proteins 230, 242, 243
envelope-related 230
Viral RNA 243, 244
accumulation 243
templates 244
Virus 42, 230, 231, 236, 237, 238, 239, 242, 244, 248, 249, 252, 257
cauliflower mosaic 42
cell leukemia 249
dengue 237, 238, 239, 244
-host interactions 257
human immunodeficiency 248, 249, 252
infections 230, 239
matrix protein dissociation 230
positive-sense RNA 231
proteases 231, 242
replicase proteins 236
Vomiting 86, 87, 266, 268

W

Waste 125, 129
glycerol 129
Water 46, 47, 129, 155, 157, 158, 161, 176, 179, 193, 189, 236
body pollution 46
distilled 129
high pressured 157
hot liquid 157
hot pressured 155, 157
partition coefficient 193
reservoirs 176
Women 214
premenopausal 214
post-menopause 214
World health organization 48

X

Xanthine oxidase 102
Xenobiotic 179, 189, 191
biotransformation enzymes 191
compounds, metabolism of 179, 189
Xylitol dehydrogenase genes 165

Y

Yeast 128, 148, 149, 166, 167
extract 128, 167
fermentation 148, 149
genome 166
Yeast cell(s) 123, 136, 148, 149, 163, 164, 166, 168
immobilization 148, 149, 163, 164, 168
immobilized 164
Yeast growth 161, 162, 164, 165
rate and metabolism 165

Z

Zinc 13, 74, 82

-binding groups (ZBG) 82
catalytic domain 74
-containing compounds 13

> The volume is a wonderful compilation of information about enzyme inhibition applications in healthcare and environmental science. The editors have identified key challenges and opportunities. This book is highly useful to drug development professionals, industrialists and academic researchers
>
> *S. Renganathan*
> *Centre for Biotechnology, Anna University, India*

G. BASKAR

Dr. G. Baskar completed his Ph.D from Anna University, Chennai, India. He is currently working as a Professor of Biotechnology in St. Joseph's College of Engineering, Chennai. He has published more than 150 research and review articles in National and International Journals, published 15 chapters in edited books and edited 3 books. His current research areas are food toxicology, biofuels and bioenergy, nanocatalysis, therapeutic proteins, microbial enzymes, nanomedicine and bioremediation. He is an active life member of various national and international professional bodies. He is the editorial board member of several journals, recipient of Outstanding Reviewer Awards from various Elsevier Journals. He received Outstanding Researcher on Renewable Energy Award from Indian Society for Technical Education, New Delhi in 2016 and Young Scientist Award 2015 from International Bioprocessing Association, France in 2017. He visited Swiss Federal Institute of Technology (EFPL), Switzerland as a visiting researcher in November and December 2018. Received 'Prof S B Chincholkar Memorial Award 2019' of the Biotech Research Society, India for his outstanding work in the area of Biofuels and Food Biotechnology in 2019.

K. SATHISH KUMAR

Dr. K. Sathish Kumar is an Associate Professor in the Department of Chemical Engineering, SSN College of Engineering, Chennai, India. He completed his PhD from Anna University Chennai. He has published over 40 research publications in refereed international journals and in proceedings of international conferences. He is a recognized supervisor of Anna University of Technology Chennai. His current research work includes synthesis of bimetallic particle, development of new nanocapsules for drug delivery, use of metallic nanoparticles as an adsorbent and its applications in water treatment. He is a life member of Indian Society for Technical Education (ISTE) and Biotech Research Society, India (BRSI).

K. TAMILARASAN

Dr. K. Tamilarasan is currently working as an Associate Professor in the Department of Chemical Engineering at SRM Institute of Science and Technology, Kattankulathur, Tamilnadu, India. He completed his Ph.D. in Biochemical Engineering (2012), Anna University, Chennai, India. His major research areas are bioenergy, platform chemical synthesis and photochemical degradation. He has published more than 30 publications in the International and National Journals and few book chapters. He had collaborative research work in the field of carbohydrate chemistry at University of Minnesota, USA. He is associated with several society including Indian Institute of Chemical Engineers (IIChE) and Biotech Research Society, India (BRSI).