FLAVONOIDS AND PHENOLICS

Editor:
Simone Carradori

Bentham Books

Medicinal Chemistry Lessons From Nature

(Volume 1)

Flavonoids and Phenolics

Edited By

Simone Carradori
Department of Pharmacy, G. d'Annunzio
University of Chieti-Pescara
Italy

Medicinal Chemistry Lessons From Nature

Volume # 1

Flavonoids and Phenolics

Editor: Simone Carradori

ISSN (Online): 2972-340x

ISSN (Print): 2972-3396

ISBN (Online): 978-981-5079-09-8

ISBN (Print): 978-981-5079-10-4

ISBN (Paperback): 978-981-5079-11-1

First published in 2022.

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

FOREWORD

The intention of this volume is to give an overview of the latest discoveries in the research on natural products-derived compounds, through a medicinal chemistry approach of the most exciting topics on flavonoids and (poly)phenolics derivatives. It is structured with innovative book setting outlines and a clear distinction between experimental and clinical results, in order to aid the reader to know at which step of the pipeline each compound is. Due to the scarcity of information of the other competing books, the Guest Editor wants to fulfil the gap of the acquired knowledge in the last few years with the aim to provide a guide for academic and professional researchers and clinicians.

The exploration of the chemical space ranges from flavonoids to phenolic compounds, covering all the aspects relevant for medicinal chemistry (drug design, structure-activity relationships, permeability data, cytotoxicity, appropriate statistical procedures, molecular modelling studies and technological formulations). Each chapter reviews on agents with common chemical features, considering them as scaffolds to obtain various derivatives aiming at the biological activity. The chemical modifications of these agents could increase their intrinsic properties, overcome limitations as drug candidates and introduce new properties. Thus, the volume is intended to be useful to researchers for more concrete applications in the natural product field. As far as I know, this is the first time these data are organized focusing on the synthetic methods and their strategies comprehending the last years.

KEY FEATURES

1. Updated information on synthetic/natural compounds;
2. In-depth analysis of novel findings and promising translational applications;
3. Use of organic reactions as a powerful tool in drug discovery to improve the biological activity or give new chemical and biological properties to the parent molecules;
4. Molecular mechanisms with innovative approaches for the readers to improve their own research investigations.

Rosa Amoroso
Department of Pharmacy
G. d'Annunzio University of Chieti-Pescara
Via dei Vestini 31, 66100 Chieti (Italy)

PREFACE

Natural products are often used in drug development due to their ability to provide unique and chemically diverse structures unmatched by any synthetic chemical collection. Medicinal Chemists have always been inspired by nature because natural products are often perceived as safer and for their capability to interact with biological targets. Indeed, in recent years, there has been emerging research on traditional herbal medicines based on their efficacy in the treatment of diseases for which they have been traditionally applied.

Conversely, natural compounds suffer from several issues such as scarce availability and seasonality, high differences in the production/extraction/isolation, low purity in commercial products from worldwide suppliers, and side effects. Moreover, due to their chemical complexity and the optional presence of different chiral centers, the total synthesis of a natural compound can be also challenging and expensive.

This book series would propose the latest discoveries in the field of compounds inspired by nature and obtained by chemical/enzymatic modification of a natural compound in the search for biologically active molecules for the treatment of human/animal ailments and permit the disposal of a wider arsenal for clinicians. The natural compounds are grouped into three clusters. The chapters are built in the following format: • General background on the (phyto)chemistry of the scaffold; • General background on the pharmacological profile of the scaffold; • Description of the proposed derivatives and their potentialities with respect to the parent compounds (with a particular emphasis on the synthetic approaches and structure-activity relationships); • *In silico* analysis of the crucial interactions with the biological target, when available; • Clinical studies and patent surveys (if available) on the new and proposed structures.

The readership of this book is represented primarily by Academies, Researchers, Specialists in the pharmaceutical field, Industry sector, Contract Research Organizations and hospitals dealing with clinical research.

Simone Carradori
Department of Pharmacy
G. d'Annunzio University of Chieti-Pescara
Italy

List of Contributors

Adriana Trifan	Department of Pharmacognosy, Faculty of Pharmacy, "Grigore T. Popa" University of Medicine and Pharmacy Iasi, 16 Universitatii Street, 700115 Iasi, Romania
Alessandra Ammazzalorso	Department of Pharmacy, Medicinal Chemistry Unit, "G. d'Annunzio" University of Chieti-Pescara, *Via* dei Vestini, 66100 Chieti, Italy
Alessandro Bonardi	Department of NEUROFARBA, Section of Pharmaceutical and Nutraceutical Sciences, Pharmaceutical and Nutraceutical Section, University of Florence, *Via* U, Schiff 6, 50019 Sesto Fiorentino, Firenze, Italy
Alessio Nocentini	Department of NEUROFARBA, Section of Pharmaceutical and Nutraceutical Sciences, Pharmaceutical and Nutraceutical Section, University of Florence, *Via* U, Schiff 6, 50019 Sesto Fiorentino, Firenze, Italy
Andrea Angeli	Department of NEUROFARBA, Section of Pharmaceutical and Nutraceutical Sciences, Pharmaceutical and Nutraceutical Section, University of Florence, *Via* U., Schiff 6, 50019 Sesto Fiorentino, Firenze, Italy
Annabella Vitalone	Department of Physiology and Pharmacology "V. Ersparmer", Sapienza University of Rome, P.le Aldo Moro 5, 00185 Rome, Italy
Antonella Capperucci	Department of Chemistry "Ugo Schiff", University of Florence, *Via* della Lastruccia 13, I-50019 Sesto Fiorentino, Italy
Antonella Di Sotto	Department of Physiology and Pharmacology "V. Ersparmer", Sapienza University of Rome, P.le Aldo Moro 5, 00185 Rome, Italy
Arafa Musa	Department of Pharmacognosy, College of Pharmacy, Jouf University, Sakaka, Al Jouf, 2014, Saudi Arabia
Arafa Musa	Department of Pharmacognosy, College of Pharmacy, Jouf University, Sakaka, Al Jouf, 2014, Saudi Arabia
Atilla Akdemir	Computer-aided Drug Discovery Laboratory, Department of Pharmacology, Faculty of Pharmacy, Bezmialem Vakif University, Fatih, Istanbul 34093, Turkey
Barbara De Filippis	Department of Pharmacy, "G. d'Annunzio" University of Chieti-Pescara, *Via* dei Vestini 31, 66100 Chieti, Italy
Bijo Mathew	Department of Pharmaceutical Chemistry, Amrita School of Pharmacy, Amrita Vishwa Vidyapeetham, Amrita Health Science Campus, Kochi-682 041, India
Celeste De Monte	Department of Drug Chemistry and Technology, Sapienza, University of Rome, 00185 Rome, Italy
Claudio Ferrante	Department of Pharmacy, Medicinal Plant Unit (MPU), Botanic Garden Giardino dei Semplici, G. d'Annunzio University of Chieti-Pescara, *Via* dei Vestini, 66100 Chieti, Italy
Claudiu T. Supuran	Department of NEUROFARBA, Section of Pharmaceutical and Nutraceutical Sciences, Pharmaceutical and Nutraceutical Section, University of Florence, *Via* U. Schiff 6, 50019 Sesto Fiorentino, Firenze, Italy

Giovanna Poce	Department of Drug Chemistry and Technology, Sapienza, University of Rome, 00185 Rome, Italy
Heiko Lange	Department of Environmental and Earth Sciences, University of Milano-Bicocca, Milan, Italy
Leena K Pappachen	Department of Pharmaceutical Chemistry, Amrita School of Pharmacy, Amrita Vishwa Vidyapeetham, Amrita Health Science Campus, Kochi-682 041, India
Luc Zongo	Department of Pharmacy, University of Saint Dominic of West Africa (USDAO), Doulougou, Burkina Faso
Mohamed A. Abdelgawad	Department of Pharmaceutical Chemistry, College of Pharmacy, Jouf University, Sakaka, Al Jouf, 2014, Saudi Arabia
Manoj Kumar Sachidanandan	Department of Oral and maxillofacial surgery and diagnostics, College of Dentistry, Hail University, Hail Province, 2440, Saudi Arabia
Maria Antonietta Casadei	Department of Drug Chemistry and Technologies, Sapienza University of Rome, P.le A. Moro 5, 00185 Rome, Italy
Melissa D'Ascenzio	D'Arcy Thompson Unit, Biological and Biomedical Sciences Education, School of Life Sciences, University of Dundee, DD1 4HN, Dundee, UK
Mohammed A. Abdelgawad	Department of Pharmaceutical Chemistry, College of Pharmacy, Jouf University, Sakaka, AlJouf, 72341, Saudi Arabia
Mutairah Shaker Alshammari	Department of Pharmaceutical Analytical Chemistry, Faculty of science, Jouf University, Sakaka, Al Jouf, 2014, Saudi Arabia
Paolo Guglielmi	Department of Drug Chemistry and Technologies, Sapienza University of Rome, P.le A. Moro 5, 00185 Rome, Italy
Rania Bakr	Department of Pharmaceutical Chemistry, College of Pharmacy, Jouf University, Sakaka, Al Jouf, 2014, Saudi Arabia
Sara Consalvi	Department of Drug Chemistry and Technology, Sapienza, University of Rome, 00185 Rome, Italy
Stefania Cesa	Department of Drug Chemistry and Technology, Sapienza, University of Rome, 00185 Rome, Italy
Stefania Garzoli	Department of Drug Chemistry and Technologies, Sapienza University of Rome, P.le A. Moro 5, 00185 Rome, Italy
Stefania Petralito	Department of Drug Chemistry and Technologies, Sapienza University of Rome, P.le A. Moro 5, 00185 Rome, Italy
Trond Vidar Hansen	Department of Pharmacy, Section for Pharmaceutical Chemistry, University of Oslo, PO Box 1068 Blindern, N-0316 Oslo, Norway
Virginia Pontecorvi	Department of Drug Chemistry and Technologies, Sapienza University of Rome, P.le A. Moro 5, 00185 Rome, Italy
Vaishnav Bhaskar	Department of Pharmaceutical Chemistry, Amrita School of Pharmacy, Amrita Vishwa Vidyapeetham, Amrita Health Science Campus, Kochi-682 041, India

Leena K Pappachen	Department of Pharmaceutical Chemistry, Amrita School of Pharmacy, Amrita Vishwa Vidyapeetham, Amrita Health Science Campus, Kochi-682 041, India
Luc Zongo	Department of Pharmacy, University of Saint Dominic of West Africa (USDAO), Doulougou, Burkina Faso
Luigi Menghini	Department of Pharmacy, Medicinal Plant Unit (MPU), Botanic Garden "Giardino dei Semplici", "G. d'Annunzio" University of Chieti-Pescara, *Via* dei Vestini, 66100 Chieti, Italy
Mohamed A. Abdelgawad	Department of Pharmaceutical Chemistry, College of Pharmacy, Jouf University, Sakaka, Al Jouf, 2014, Saudi Arabia
Manoj Kumar Sachidanandan	Department of Oral and maxillofacial surgery and diagnostics, College of Dentistry, Hail University, Hail Province, 2440, Saudi Arabia
Marco Gullì	Department of Physiology and Pharmacology "V. Ersparmer", Sapienza University of Rome, P.le Aldo Moro 5, 00185 Rome, Italy
Maria Antonietta Casadei	Department of Drug Chemistry and Technologies, Sapienza University of Rome, P.le A. Moro 5, 00185 Rome, Italy
Marialuigia Fantacuzzi	Department of Pharmacy, "G. d'Annunzio" University of Chieti-Pescara, *Via* dei Vestini 31, 66100 Chieti, Italy
Mariangela Biava	Department of Drug Chemistry and Technology, Sapienza, University of Rome, P.le A. Moro 5, 00185 Rome, Italy
Mario R. Meneghetti	, Institute of Chemistry and Biotechnology, Federal University of Alagoas, Maceió, Brazil
Mattia Spano	Department of Drug Chemistry and Technologies, Sapienza University of Rome, P.le A. Moro 5, 00185 Rome, Italy
Melissa D'Ascenzio	D'Arcy Thompson Unit, Biological and Biomedical Sciences Education, School of Life Sciences, University of Dundee, DD1 4HN, Dundee, UK
Mutairah Shaker Alshammari	Department of Pharmaceutical Analytical Chemistry, Faculty of science, Jouf University, Sakaka, Al Jouf, 2014, Saudi Arabia
Nathalie Saraiva Rosa	Department of Chemistry, Royal College of Surgeons in Ireland, Dublin, Ireland
Niccolò Chiaramonte	Department of Chemistry, Royal College of Surgeons in Ireland, Dublin, Ireland
Özlen Güzel-Akdemir	Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Istanbul University, Beyazit, 34116 Istanbul, Turkey
Paolo Guglielmi	Department of Drug Chemistry and Technologies, Sapienza University of Rome, P.le A. Moro 5, 00185 Rome, Italy
Patrizia Paolicelli	Department of Drug Chemistry and Technologies, Sapienza University of Rome, P.le A. Moro 5, 00185 Rome, Italy
Paulo F. Silva Santos-Júnior	Institute of Chemistry and Biotechnology, Federal University of Alagoas, Maceió, Brazil
Rafael J. M. Omena	Group of Catalysis and Chemical Reactivity, Chemistry and Biotechnology Institute, Federal University of Alagoas, Maceió, Brazil

Rania Bakr	Department of Pharmaceutical Chemistry, College of Pharmacy, Jouf University, Sakaka, Al Jouf, 2014, Saudi Arabia
Sara Consalvi	Department of Drug Chemistry and Technology, Sapienza, University of Rome, 00185 Rome, Italy
SilVia Di Giacomo	Department of Physiology and Pharmacology "V. Ersparmer", Sapienza University of Rome, P.le Aldo Moro 5, 00185 Rome, Italy
Simone Carradori	Department of Pharmacy, "G. d'Annunzio" University of Chieti-Pescara, *Via* dei Vestini 31, 66100 Chieti, Italy
Stefania Cesa	Department of Drug Chemistry and Technology, Sapienza, University of Rome, 00185 Rome, Italy
Stefania Garzoli	Department of Drug Chemistry and Technologies, Sapienza University of Rome, P.le A. Moro 5, 00185 Rome, Italy
Stefania Petralito	Department of Drug Chemistry and Technologies, Sapienza University of Rome, P.le A. Moro 5, 00185 Rome, Italy
Thiago M. Aquino	Institute of Chemistry and Biotechnology, Federal University of Alagoas, Maceió, Brazil
Trond Vidar Hansen	Department of Pharmacy, Section for Pharmaceutical Chemistry, University of Oslo, PO Box 1068 Blindern, N-0316 Oslo, Norway
Virginia Pontecorvi	Department of Drug Chemistry and Technologies, Sapienza University of Rome, P.le A. Moro 5, 00185 Rome, Italy
Vaishnav Bhaskar	Department of Pharmaceutical Chemistry, Amrita School of Pharmacy, Amrita Vishwa Vidyapeetham, Amrita Health Science Campus, Kochi-682 041, India

CHAPTER 1

Polyphenols and Flavonoids: Chemical, Pharmacological and Therapeutic Aspects

Stefania Cesa[1,*], **Francesco Cairone**[1] and **Celeste De Monte**[1]

[1] *Department of Drug Chemistry and Technology, Sapienza, University of Rome, 00185 Rome, Italy*

Abstract: Polyphenols and flavonoids represent a group of compounds characterized by a large assortment of phenolic structures, which can be naturally found in vegetables, roots, stems, flowers, grains, and fruits. Thanks to their biological activities, molecules belonging to these classes of compounds, besides their nutritional role, have found applications in several fields such as pharmaceutical, cosmetic, and nutraceutical. In fact, like many natural derivatives from plants, they possess several therapeutic properties, including antitumor, anti-oxidative, anti-neurodegenerative, antimicrobial and anti-inflammatory effects. Nowadays, the growing interest in polyphenolics and flavonoids translates into constant research to better define their pharmacological mechanism of action. Extraction studies in order to obtain pure compounds with a more defined biological activity, as well as pharmacokinetic studies to understand the bioavailability, the involved metabolic pathways and the related active metabolites, are carried out. Molecular docking studies are also continuously in progress to expand the field of application. Moreover, toxicity experiments to clarify their safety and studies about the interaction with other compounds to understand their selectivity of action are continuously forwarded and deepened. Consequently, many recent studies are aimed at introducing polyphenols, more specifically flavonoids, and their semi-synthetic derivatives, in the prevention, management and treatment of several diseases.

Keywords: Bioavailability, Biological properties, Chemical structures, Disease prevention, Flavonoids, Metabolism, Polyphenols, Semisynthetic derivatives.

INTRODUCTION

Polyphenols are the plant's secondary metabolites, contained in specialized cells in small quantities and not necessary for cell viability that vegetal organisms produce to perform different functions. They include several classes of chemical molecules characterized by the presence of aromatic rings bearing more than a hydroxylic function, up to complex polycyclic and polymeric compounds.

[*] **Corresponding author Stefania Cesa:** Department of Drug Chemistry and Technology, Sapienza, University of Rome, 00185 Rome, Italy; E-mail: stefania.cesa@uniroma1.it

Simone Carradori (Ed.)

All molecules presenting a simple phenolic group are theoretically able to act as anti-radical species since they could react with endogen radicals to undergo new and more stable radical residues, which tend to react by neutralizing rather than attacking macromolecules such as DNA or proteins causing damages up to mutagenesis or unfolding. Moreover, the presence of *ortho*-diphenol groups also allows the metals chelation, improving the antioxidant properties. Polyphenols are frequently classified in relation to their chemical structure into four principal molecules, represented by phenolic acids, flavonoids, stilbenes and lignans. Natural products have always been the subject of great interest thanks to their biological activities and pharmacological properties. The attention towards bioactive compounds is growing more and more over the years because they found applications in pharmaceutical, nutraceutical, cosmetic and medical fields [1]. By virtue of appropriate pharmacodynamic, pharmacokinetic, bioavailability and toxicity studies, they can potentially be included among dietary supplements and therapeutic tools for the prevention and treatment of many human diseases with important applications in phytotherapy and herbal medicine [2]. A recent review [3] reports the protection exerted by a polyphenol-rich diet, highlighting the potential ability of pure polyphenols and of phytocomplex to reverse oxidative stress-relative diseases and a "promising chemopreventive efficacy" through modulation of apoptosis and cellular growth, inhibition of DNA synthesis and modulation of signal transduction. Among the bioactive natural products, a preeminent position is occupied by flavonoids, plants and fungi secondary metabolites, of supreme interest both for their ubiquitous distribution in nature and for the wide structural diversification, to which is often correlated a specific bioactivity. They can be found in many parts of plants, including leaves, flowers (where flavonoids constitute the colored pigments of petals), roots, fruits, stems, seeds, rhizome, bark, gum and shell [4]. The reason for their ubiquitous location in many plant organs may be due to the flavonoids' important role in protecting them against oxidative stress and ultraviolet radiation, as well as in attracting pollinating animals [5].

Flavonoids have gained a special prominence among natural compounds of pharmaceutical and therapeutic interest, thanks to the wide range of chemical subclasses and their wide variety of pharmacological properties, such as the modulation of enzymatic activities by inhibiting lipid peroxidation and cyclo-oxygenase and lipoxygenase activity, anti-inflammatory, anti-mutagenic, antioxidative and antitumor effects [6].

In a recent review [7], authors underline the high interest, not only for polyphenols contained in several by-products of agroindustry processes but also for bound polyphenols, which could need hydrolytic treatment of the containing matrices, to make efficient their extraction yields. Analogously, Jablonsky *et al*.

[8] studied the bioactivity potential of phenolics extracted by softwood bark, emphasizing the extract complexity and the wide applications in the pharmacologic field as cytotoxic, antioxidant, fungicidal and antibacterial substances. Moreover, Cotas *et al*. [9] evaluated the potential applications of polyphenols, tannins and many others extracted from seaweed (Chlorophyta, Rhodophyta and Phaeophyceae). As polyphenols are one of the most represented classes of seaweed phytochemicals and seaweeds are one of the most available organic matrices in nature, they could be more exploited for large-scale production of polyphenolic compounds.

Healthy food containing polyphenols found also application in the prevention of skin aging and skin cancer, as functional foods or as sources of nutraceuticals to be used both in food supplements, cosmetic products or a topical formulation for dermatologic applications. This application field was also recently reviewed, but authors concluded that ingredients used with this aim, are often poorly characterised or represent part of complex mixtures by which it is difficult to establish the relationship between a single molecule and its biological effect [10].

The antioxidant and free radical scavenging activities were shown for many compounds of this class, as well as the cardioprotective, antidiabetic and antiviral potential. Most researchers are actually involved in the deepening of the mechanisms underlying the anti-cancer activity and the apoptosis induction, focusing the attention on the key enzyme involved in cellular proliferation, angiogenesis progression, and metastatic processes [11]. Anyway, most parts of these results were obtained by experiments performed *in vitro* or *ex vivo*, but the real potential of a molecule or of a class of compounds needs to be evaluated on the basis of its ability to be absorbed and metabolized while maintaining its biological effect, and finally its capability to reach the active site. As flavonoids generally display low water solubility and consequently low bioavailability, a topic of greatest interest for the scientific community is the application of several strategies aiming to solve this problem. So, a valent strategy for the polyphenolic fraction valorization is represented by the possibility of enhancing their efficacy, bioavailability and release to the action site, mediated by the exploitation of nanotechnologies capable of solving many and different problems [12] related to the specific structures, which could undergo low intestinal absorption, rapid metabolism and excretion, low plasmatic contents. This research field found application in the formulation based on many different systems, differently organized, such as liposomes, solid lipid nanoparticles, nanostructured lipid carriers, nanosuspensions, and nanoemulsions. These could be able to enhance the solubility of single nutraceuticals rather than solving problems inherent with the more or less complex nature of organic extracts obtained by food, non-edible

CHAPTER 2

Recent Development of Hybrids and Derivatives of Resveratrol in Neurodegenerative Diseases

Barbara De Filippis[1,*] and **Marialuigia Fantacuzzi**[1]

[1] *Department of Pharmacy, G. d'Annunzio University of Chieti-Pescara, via dei Vestini 31, 66100 Chieti, Italy*

Abstract: Neurodegenerative diseases (NDs) are characterized by the progressive loss of neurons in different regions of the nervous system, being Alzheimer's disease (AD) and Parkinson's disease (PD) the most common NDs. Despite their high incidence, the pharmacological treatments are mainly symptomatic. For this reason, in recent years, the research has been focused on the discovery of new molecules able to target neuropathological pathways involved in NDs. In the last decades, several researchers investigated the neuroprotective actions of naturally occurring polyphenols, such as resveratrol, that has attracted special interest since its ability to interact simultaneously with the multiple targets implicated in NDs. Thanks to the structural simplicity of the stilbene core, the broad spectrum of possible modifications, and the improved synthetic strategies, resveratrol is an attractive chemical starting point for the searching of new entities with extended therapeutic uses in NDs. In this review, a systematic update of the stilbene-based hybrids and derivatives, and SAR analysis were provided for the development of new drugs potentially useful as NDs multitarget directed ligands.

Keywords: Antioxidant, Molecular Hybrids, Neurodegeneration, Polyphenols, Resveratrol, Stilbene Derivatives.

INTRODUCTION

Neurodegenerative diseases (NDs) are characterized by progressive disorders and devastating damages of the structure and function of neurons. NDs etiology is still not clear, despite the increased current knowledge of their neurobiology, but different contributing factors, such as aging, lifestyle and genetic factors are involved. Despite the differences in clinical signs, among others, the pathological processes appear similar, suggesting common neurodegenerative pathways. The pathogenesis of several NDs, including Alzheimer's (AD), Parkinson's (PD),

* **Corresponding Author Barbara De Filippis:** Department of Pharmacy, G. d'Annunzio University of Chieti-Pescara, *via* dei Vestini 31, 66100 Chieti, Italy; E-mail: barbara.defilippis@unich.it

Simone Carradori (Ed.)

Huntington's (HD) and amyotrophic lateral sclerosis diseases are associated with multiple factor risks [1]. Neuroinflammation and oxidative stress represent the main causes of induction of NDs, but also excitotoxicity, mitochondrial dysfunction, and apoptosis [2]. The role of oxidative stress in neurodegeneration is well documented and is correlated to the progression of AD and PD [3]. Microglia cells are resident cells in the central nervous system (CNS), with immune function under normal conditions. The activation of brain microglia, and the subsequent extra production of inflammatory mediators, such as nitric oxide (NO), may result in uncontrolled neuroinflammation in NDs [4]. Lipoxygenase (LOX) and cyclooxygenase (COX) cascades are upregulated in chronic and age-related brain pathologies [5].

AD is the most common form of dementia and the most studied ND. It is characterized by two neuropathological hallmarks: deposition of extracellular β-amyloid (Aβ) plaques and intracellular neurofibrillary tangles, even if other factors as neuroinflammation play an important role in progression of AD [6]. Due to the complexity of the involved pathways, it is difficult to control the progression of this pathology. Current treatment for AD is only symptomatic; thus, the development of drugs with the potential to change the progression of the disease is a priority. One of the major pharmacological approaches of AD regards the inhibition of acetyl and butyryl cholinesterase (AChE and BuChE). They are key enzymes that play important roles in cholinergic transmission by hydrolyzing the acetylcholine (ACh) [7]. In AD, the AChE level in the brain decreases progressively, but BuChE activity remains or increases compared to the basal level. Some research studies reported that the peripheral anionic site (PAS) of AChE can locate with Aβ protein and enhances the formation of amyloid fibrils in the senile plaques indicating that inhibiting the AChE activity is a promising approach to prevent Aβ aggregation [8]. However, AChE inhibition may cause the classical cholinergic toxicity [9]. $A\beta_{1\text{-}42}$ is responsible for the initial self-aggregation of Aβ, and the resulting β-amyloid oligomers and fibrils are toxic to neurons [10]. Moreover, high concentrations of copper, zinc and iron ions accelerate amyloid deposits in AD patients [11, 12]. Although the mechanisms that underlie NDs pathophysiology are not completely clarified, a series of studies described the critical role of inflammation and oxidative stress in the degeneration of neurons [13, 14], promoting the Aβ aggregation [3].

Monoamine oxidases (MAOs) have been received increasing attention in recent years due to their roles in the treatment of AD and PD. MAOs are FAD-containing enzymes that bind tightly to the outer mitochondrial membrane in brain, liver, intestinal mucosa, and other organs and catalyze the oxidative deamination of biogenic and xenobiotic amines. There are two types of isoenzymes, MAO-A and MAO-B, which can be distinguished by their

differential primary DNA sequences, tissue distribution, substrates, and inhibitor selectivity. MAO-A is situated predominantly in catecholaminergic neurons and especially oxidizes serotonin, adrenaline and noradrenaline, while MAO-B is placed in serotonergic neurons and glia where deaminates dopamine and 2-phenylethylamine (2-PEA). Therefore, the study of MAO inhibitors has attracted increasing interest in recent years for their therapeutic effect on NDs. Selective MAO-B inhibitors, such as rasagiline and selegiline, are used as adjuvant therapy in the treatment of PD and AD [15]. However, the high levels of MAO-B in neuronal tissue could lead to an increase in the levels of H_2O_2 and oxidative free radicals, which ultimately contribute to the etiology of NDs. Thus, selective inhibition of MAO-B becomes another valuable approach for the treatment of AD [16].

PD is the second most common neurodegenerative disorder caused by progressive loss of dopaminergic nigrostriatal neurons. The neuropathological hallmark of PD involves the disruption to mitochondria, the oxidative stress, alterations to the presynaptic protein α-synuclein, resulting in the accumulation of intracellular protein aggregates, Lewy bodies, and Lewy neurites, and neuroinflammatory processes [17]. Unfortunately, current drugs are mainly focused on symptomatic controls, and a long-term application leads to the loss of drug efficacy and important adverse effects. Since conventional therapeutics are not sufficient for the treatment of PD, the development of new agents is crucial [18].

Currently available drugs only provide symptomatic treatment, and has modest benefits, rather than preventing or curing neurodegeneration [19], and effective therapeutic agents are still far to success. In this contest, it is essential to develop novel therapeutic approaches to fight the NDs. Since the complexity of the involved pathways, a single biological target often proves ineffective in the treatment of diseases with a complex path mechanism. This fact inspired the research to design and develop a single drug containing structural features able to act on multiple biological targets [20]. In this way, the design of poly-pharmacophores represents a further modern approach that provides effective pharmacological responses for diverse receptors or enzymatic systems, and responds to the ADMET limits, showing antioxidant, neuroprotective, and brain permeable properties [21]. These multitarget-directed ligands (MTDLs) could contain a variety of scaffolds. During the past decades, many studies reported the positive effect of natural compounds against different diseases such as cardiovascular, diabetes, and cancer. On the other hand, natural products have emerged as potential neuroprotective agents for the treatment of NDs [22]. In this context, natural products have been used as structural models in the drug design of ligands against AD, as evidenced by the high number of published studies [23].

CHAPTER 3

Biological Activities of Synthetic Derivatives of Xanthones: An Update (2016-2020)

Cristina Scarpecci[1] and **Sara Consalvi**[1,*]

[1] *Department of Chemistry and Technologies of Drug, Sapienza University of Rome, P.le A. Moro 5, 00185 Rome, Italy.*

Abstract: Natural xanthones are a class of secondary metabolites widely distributed in nature and with a broad spectrum of biological activities. Their scaffold is amenable to several modifications and has emerged as a "privileged structure" for drug development, representing a very attractive point for medicinal chemistry optimization. A combination of innovative synthetic methodologies and medicinal chemistry studies have provided several xanthone synthetic derivatives for different therapeutic purposes, including cancer, inflammation, Alzheimer's disease (AD), cardiovascular and infectious diseases. The aim of this chapter is to give an update on the significance of synthetic xanthones in medicinal chemistry over the last five years (2016-2020), with a focus on their biological activities and structure-activity relationship (SAR).

Keywords: anticancer, drug discovery, natural xanthones, synthetic xanthones, SAR analysis.

INTRODUCTION

Xanthones are a class of *O*-heterocycles symmetrical compounds characterized by a dibenzo-γ-pyrone scaffold (Fig. **1**) They can be extracted from different sources [1, 2] (fungi, lichens [3], higher plants [4] and marine organisms [5]) and are widely distributed in nature.

Natural xanthones have been a rich source for the discovery of novel therapeutic agents for many decades [4, 6]. One of the most representative examples is the polyprenylated xanthone gambogic acid (GA), isolated from *Garcinia hanburyi* (Clusiaceae), which entered clinical trials for the treatment of patients with advanced malignant tumors, including non-small cell lung cancer [7, 8]. Another well-known xanthone is mangosteen, isolated from *Garcinia mangostana* and sold worldwide as a nutritional supplement with anti- oxidant, anti- inflammatory

* **Corresponding author Sara Consalvi:** Department of Chemistry and Technologies of Drug, Sapienza University of Rome, P.le A. Moro 5, 00185 Rome, Italy; E-mail: sara.consalvi@uniroma1.it

Simone Carradori (Ed.)

and immunostimulating properties [9, 10]. Depending on their structures and position of substituents, natural xanthones have an incredibly broad spectrum of biological activities [11], such as cytotoxic [12], antiinfective [13], antioxidant [14], cardioprotective [15, 16], anti-inflammatory [17], and antihypertensive [18]. The xanthone scaffold has then emerged as a "privileged structure" for drug development and represents a very attractive point for medicinal chemistry optimization. The xanthone core is amenable to several modifications. However, even though natural sources offer a wide variety of differently substituted xanthones, the enzymes involved in their biosynthetic pathways could limit their structural diversity. Furthermore, some of them are difficult to obtain through conventional extraction methods and are present only at low concentrations. On the other hand, total synthesis can be a *via*ble strategy to easily obtain xanthones and explore the chemical space and the structure-activity relationship (SAR) around their scaffold [1, 12]. Therefore, combining novel synthetic methodologies and medicinal chemistry optimization is essential to allow the generation of libraries of xanthone synthetic derivatives with enhanced activities, improved safety profiles and acceptable drug-like properties. The importance of xanthone synthetic derivatives has been extensively reviewed [13, 19, 20]. The aim of this chapter is to give an update on the significance of synthetic xanthones in medicinal chemistry over the last five years (2016-2020), with a focus on their biological activities and SAR.

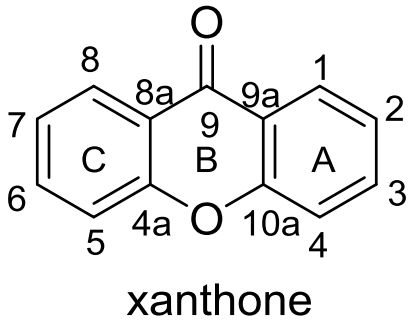


Fig. (1). General scaffold of xanthones.

XANTHONE SYNTHETIC DERIVATIVES FOR CANCER THERAPY

The anticancer properties of xanthones, such as gambogic acid (GA) [21], α-mangostin and 5,6-dimethylxanthenone-4-acetic acid (DMXAA), have been amply studied, and some of them underwent clinical trials [12]. Xanthones can inhibit tumor growth both *in vitro* and *in vivo*. As shown in Fig. **(2).,** they have several putative mechanisms of action, including apoptosis induction, cell cycle arrest, anti-angiogenesis and anti-metastatic effects, and antioxidant or reactive oxygen species (ROS)-stimulating activity. However, their exact molecular

mechanism is yet to be clarified. The planar scaffold of xanthones, involving a three-ring system, might intercalate DNA and establish non-covalent DNA interactions [22]. Consequently, these promising activities prompted a continuous search for novel xanthone-based anticancer candidates.

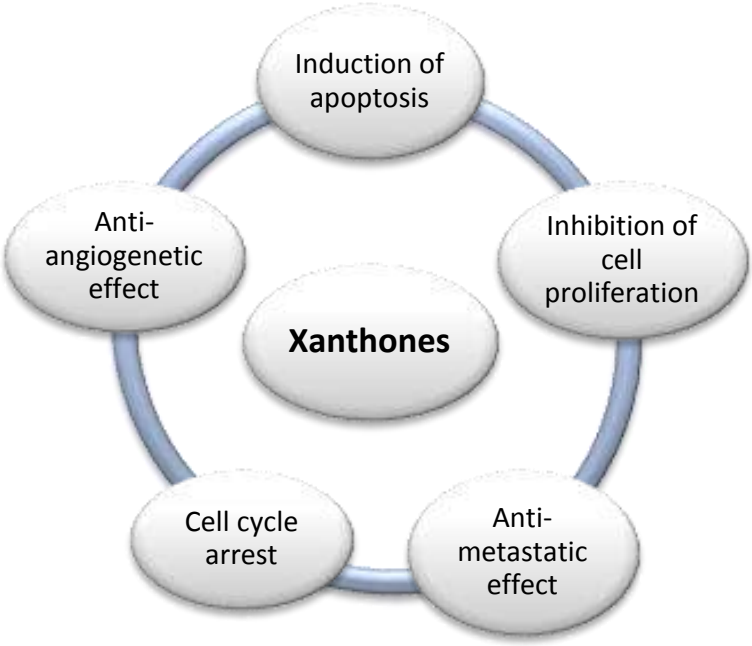


Fig. (2). Proposed Anticancer mechanisms for xanthones.

Caged Xanthones (CXs)

Gambogic acid (GA) represents the main bioactive natural product isolated from the gambogin resin secreted by the tropical trees of *Garcinia hanburyi.* GA has a unique prenylated caged xanthone structure and exhibits a wide range of biological activities, including anticancer. Notably, GA inhibits the growth of a broad panel of cancer cell lines *in vitro* and *in vivo* and entered clinical trials in China for the treatment of non-small cell lung, colon, and renal cancers. Several biological targets of GA are described in the literature, including transferrin receptor, nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) pathway, inhibitory kappa B kinase-β (IKKβ), heat shock protein 90 (Hsp90) ATPase, p53-Mouse double minute 2 homolog (MDM2) interaction, B-cell lymphoma 2 (bcl-2) pathway [7]. However, as common setbacks of natural products, pharmacokinetic drawbacks such as poor aqueous solubility and a short half-life limited its use as an anticancer drug. Thus, structural modifications were made to improve its drug-like profile and obtain analogues as potential anticancer agents [12]. Recently, Zhang's research group identified SARs around the unique

CHAPTER 4

Combretastatin Derivatives as Tubulin Inhibitors: A Fascinating Journey from Nature to Drug Discovery Strategies

Alessandra Ammazzalorso[1,*] and **Trond Vidar Hansen**[2]

[1] *Department of Pharmacy, Medicinal Chemistry Unit, G. d'Annunzio University of Chieti-Pescara, via dei Vestini, 66100 Chieti, Italy*

[2] *Department of Pharmacy, Section for Pharmaceutical Chemistry, University of Oslo, PO Box 1068 Blindern, N-0316 Oslo, Norway*

Abstract: The combretastatins are a family of stilbene phenolic natural products isolated from the bark of the South African bush willow tree *Combretum caffrum*. Since their isolation and structural elucidation, these molecules have attracted a lot of interest due to their potent cytotoxic activity against several human cancer cell lines. Combretastatin A-4, a *cis*-stilbene, is the most potent member of these natural products, has the ability to strongly inhibit tubulin polymerization, resulting in high cytotoxic activity. Indeed, it also displays an additional activity as a potent vascular disrupting agent. This interesting double bioactive profile accounts for the potent antiproliferative and antivascular action in tumors. However, combretastatin A-4, due to the sensitive *cis*-stilbene moiety, is prone to isomerization giving the less bioactive *trans*-isomer and exhibits diminished water solubility. Hence, a wide panel of synthetic derivatives were therefore developed with the aim of overcoming these limitations. The development of prodrugs such as fosbretabulin, ombrabulin and Oxi4503 isrepresentative of successful attempts to overcome pharmacokinetic disadvantages, whereas the most recent approaches aim to develop combretastatin prodrugs able to selectively target tumor site, possessing also theranostic properties. Herein, miscellaneous and the most potent synthetic analogues are presented. In addition, a general outlook on combretastatin derivatives and drug delivery approaches based on innovative nanoformulations is also presented.

Keywords: Anticancer, Combretastatin, Cytotoxic, Colchicine Binding Site, Drug Delivery System, Heterocyclic Derivatives, Natural Compounds, Nanoformulation, Prodrugs, Photoresponsive Hybrid, Stilbene, Structure-activity Relationships, Tubulin Polymerization, Vascular Disrupting Agent.

[*] **Corresponding author Alessandra Ammazzalorso:** Department of Pharmacy, G. d'Annunzio University, *via* dei Vestini 31, 66100 Chieti, Italy; E-mail: alessandra.ammazzalorso@unich.it

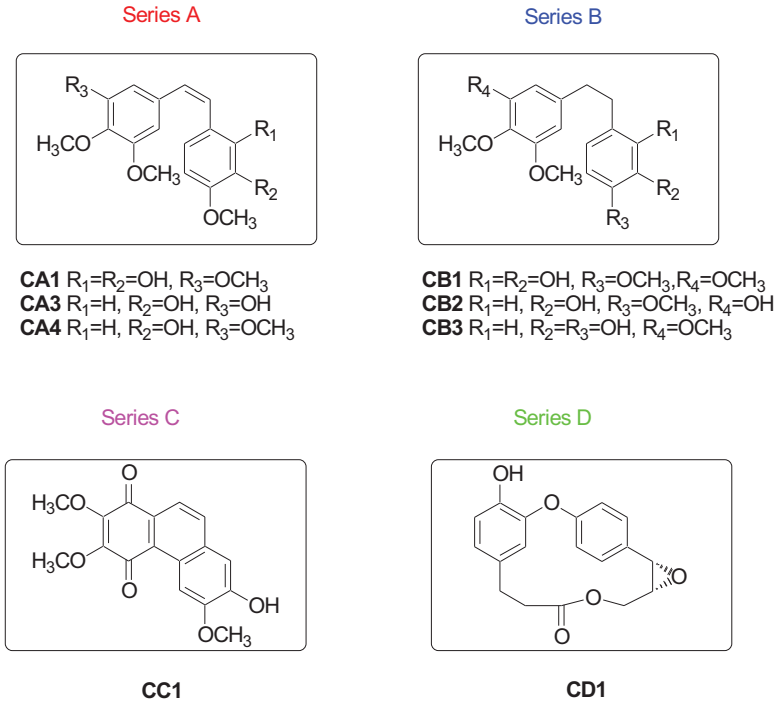
Simone Carradori (Ed.)

INTRODUCTION

Natural combretastatins were firstly isolated by Pettit in the 1980s from the bark of *Combretum caffrum* (Eckl. & Zeyh.) Kuntze, an African willow tree [1 - 3]. In the same years, their antimitotic activity was determined. Extracts from *Combretum caffrum* were traditionally used as a folk medicine for the treatment of scorpion stings, cardiovascular disorders and worm related diseases by the Xhosa tribe in South Africa. From a structural point of view, combretastatins are closely related to stilbenes, representing their *cis*-isomers. These bioactive compounds were classified into four families, namely combretastatin A (CA1 to CA6), combretastatin B (CB1 to CB4), combretastatin C-1 and combretastatin D (D1 to D4). The family A comprises stilbene-based compounds, whereas the family B dihydrostilbenes, phenanthrenes for C and macrocyclic lactones for D. In Fig. **1** the chemical structures of representative members for each family are presented. Overall, their general features are two phenyl rings (one trimethoxy substituted), linked by a *cis*-configure.d double bond. The mode of action of the combretastatins is closely related to colchicine, sharing with it the ability to bind tubulin and act as polymerization inhibitors. In fact, it was demonstrated that combretastatins bind at the colchicine binding site (CBS) on the β-tubulin in a similar orientation as colchicine. The most prominent representative of this group of compounds is combretastatin A-4 (CA4, 3'- hydroxy-3,4,4 ',5- tetramethoxy-*cis* -stilbene), whose principal structural features include a 3,4,5-trimethox--substituted phenyl ring (A), a B ring containing C3'-OH and C4'-OCH_3 substituents, and an ethylene bridge providing proper rigidity and spatial orientation of aromatic rings. These structural features were found essential to produce a potent interaction in CBS of tubulin and provide high levels of cytotoxicity [4]. CA4 displays potent antiproliferative effects as an inhibitor of tubulin polymerization, but it induces also marked anti-vascular and anti-angiogenic effects by acting as a vascular disrupting agent (VDA) [5]. The selective disruption of tumor microvessels determines the loss of nutrients, oxygen deprivation and irreversible vascular damage, leading to haemorrhagic necrosis and cell death.

It is interesting to underline that combretastatin A-1 (CA1) and its prodrugs can undergo activation to a cytotoxic *ortho*-quinone intermediate, which interacts with structural elements in proteins and nucleic acids producing oxidative stress through superoxide/hydrogen peroxide production. This chemical behaviour explains the superior antitumor effect played by CA1 compared to CA4, pointing out how little structure differences could affect different pathways into the organisms. Despite the potent cytotoxic, rather simple chemical structure and anti-angiogenic activity exerted by CA4 and CA1, these compounds suffer from some drawbacks, such as the low water solubility and the instability of the *cis*

configuration. Many CA4 and CA1 derivatives were synthesized to overcome the solubility problems, including phosphate prodrugs: CA4 phosphate (fosbretabulin, Zybrestat®) represents a successful derivative, widely studied in many clinical studies alone or in combination with traditional chemotherapeutic agents or with radiotherapy. The isomerization of the *cis* configuration, occurring after *in vivo* administration or in the presence of light, heat, or acidic media, leads to the *trans*-stilbene isomer, significantly less potent at inhibiting tubulin polymerization and diminishing cancer cell growth [6].



CA1 R_1=R_2=OH, R_3=OCH_3
CA3 R_1=H, R_2=OH, R_3=OH
CA4 R_1=H, R_2=OH, R_3=OCH_3

CB1 R_1=R_2=OH, R_3=OCH_3,R_4=OCH_3
CB2 R_1=H, R_2=OH, R_3=OCH_3, R_4=OH
CB3 R_1=H, R_2=R_3=OH, R_4=OCH_3

Fig. (1). Chemical structures of representative members of four families of combretastatins.

The potent anticancer profile of combretastatins attracted a lot of attention in medicinal chemistry research, stimulating the efforts of researchers to obtain derivatives with improved pharmacokinetic properties and tumor targeting selectivity [7, 8].

INSIGHTS ON MECHANISM OF ACTION OF COMBRETASTATINS

Combretastatins belong to the family of microtubule-binding agents, usually categorized in microtubule-stabilizing (taxanes, epothilones) and microtubule-destabilizing agents (colchicine, vinca alkaloids, combretastatins) when used in

CHAPTER 5

Natural Flavonoid and Chalcone Scaffolds as Leads for Synthetic Antitubercular Agents

Federico Appetecchia[1], Mariangela Biava[1] and **Giovanna Poce[1,*]**

[1] *Department of Chemistry and Technologies of Drug, Sapienza University of Rome, piazzale A. Moro 5, 00185 Rome, Italy*

Abstract: Tuberculosis is a leading cause of mortality and morbidity worldwide, claiming 1.2 million deaths (including 208 000 people with HIV) and 10 million new cases in 2019. Current treatment suffers from significant shortcomings such as length, dosage regimen, toxicity, and resistance development to currently used medicines. The emergence of multidrug-resistant tuberculosis and extensively drug-resistant tuberculosis is a major concern in controlling the disease. Therefore, there is an urgent need for new antitubercular drugs that are active against resistant strains, less toxic, and that act upon a different mechanism than the current drugs. Natural products can be a great source for the development of new anti-tubercular agents because of their rich chemical diversity with privileged antimicrobial activity. In this chapter, we focus our attention on flavonoids and chalcone scaffolds as leads for the development of new antitubercular agents.

Keywords: Antimicrobials, Antimycobacterials, Catechins, Chalcones, Coumarin, Epigallocatechin gallate, Flavanones, Flavonoids, Formononetin, Isoflavones, Liquiritigenin, Multi-drug resistant tuberculosis, *Mycobacterium tuberculosis*, Natural products, Quinolines, Secondary metabolites, Tuberculosis.

INTRODUCTION

Tuberculosis (TB) is a leading cause of mortality and morbidity worldwide, claiming 1.2 million deaths (including 208 000 people with HIV) and 10 million new cases in 2019. The annual number of TB deaths is falling globally (between 2015 and 2019 was 14%) less than halfway towards the 2020 milestone of a 35% reduction between 2015 and 2020 [1]. *Mycobacterium tuberculosis* (*Mtb*), the causative agent of TB, can survive within the host, switching between active and latent disease states and evading the immune system defenses. The current frontline TB therapy consists of a co-administration for 2 months of isoniazid

* **Corresponding Author Giovanna Poce:** Department of Chemistry and Technologies of Drug, Sapienza University of Rome, piazzale A. Moro 5, 00185 Rome, Italy; Tel.: +39 06 49913593; fax: +39 06 499133333; E-mail: giovanna.poce@uniroma1.it

Simone Carradori (Ed.)

(INH), rifampicin (RIF), ethambutol (EMB), and pyrazinamide (PZA) (Fig. **1**) followed by RIF and INH administration for 4 months. This treatment suffers from significant shortcomings such as length, dosage regimen, toxicity, and resistance development to currently used medicines. The emergence of multidrug-resistant TB (MDR-TB), defined as TB that is resistant to INH and RIF, and extensively drug-resistant TB (XDR-TB), classified as being resistant to INH and RIF in addition to any fluoroquinolone and injectable second-line drugs, is a major concern. The World Health Organization (WHO) estimates that in 2018 there were 484 000 new cases with resistance to rifampicin (RIF) (RR-TB), of which 78% had MDR-TB, and 8.5% of MDR-TB cases had XDR-TB [1].

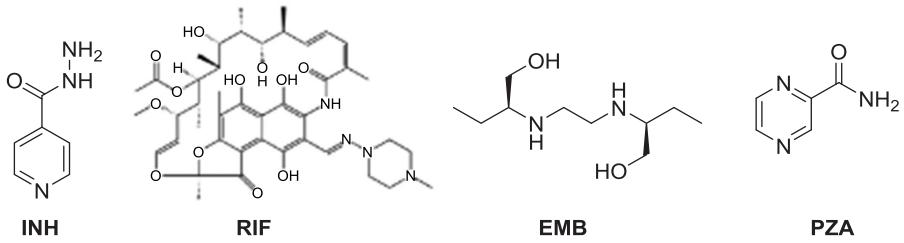


Fig. (1). Chemical structures of the frontline medicines isoniazid (INH), rifampicin (RIF), ethambutol (EMB), and pyrazinamide (PZA).

Therefore, there is an urgent need for new anti-TB drugs active against MDR and XDR strains, less toxic, and that act upon a different mechanism than the current drugs. Except for bedaquiline [2] and pretomanid [3] (Fig. **2**) approved by the Food and Drug Administration (FDA) in 2012 and 2019, respectively, and delamanid [4] (Fig. **2**)., approved by the European Medicines Agency (EMA) in 2014, very few molecules make it through the stringent bottlenecks of TB drug discovery.

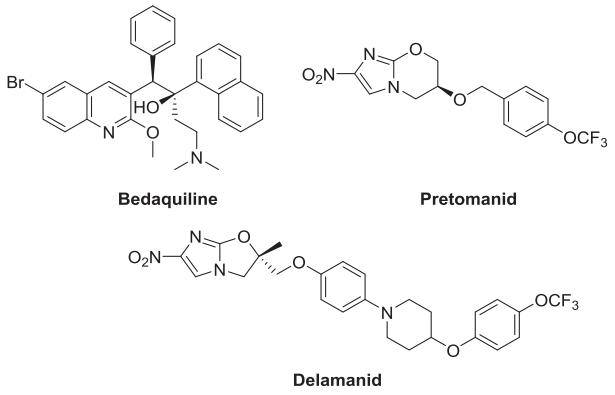


Fig. (2). Chemical structures of bedaquiline, pretomanid and delamanid.

Unfortunately, TB drug discovery was completely neglected for a long period of time, and only in the last two decades research all around the globe led to the discovery of new molecules with anti-TB potential [5, 6]. The majority of drugs that are currently being evaluated in clinical trials were identified using phenotypic screening. Indeed, most of the compounds identified by high-throughput screening campaigns and target-based drug design approaches failed to demonstrate activity against *Mtb* in a whole-cell screening assay. Natural products can be a great source for the development of new anti-tubercular agents because of their rich chemical diversity with privileged antimicrobial activity. In this chapter, we will focus our attention on flavonoids and chalcone scaffolds as leads for the development of new antitubercular agents.

FLAVONOIDS

Flavonoids are polyphenolic natural products commonly found in plants and fungi. A significant amount of literature is available that reports the antimycobacterial potential of naturally occurring flavonoids [7].

Epigallocatechin gallate (EGCG, (Fig. **3**) the main polyphenol found in green tea, showed several biological activities such as reducing inflammation and oxidative-stress [8] as well as anti-carcinogenic [9] and antimicrobial activity [10 - 12]. Unfortunately, EGCG showed low bioavailability and poor pharmacokinetics because of its rapid biotransformation and degradation after oral or parenteral administration in animal studies and the good *in vitro* results could not be translated into *in vivo* tests [13 - 15]. Banerjee *et al.* prepared triazolyl-flavonoids hybrids by combining the catechin/epicatechin fragment to a 1,4-triazole compounds **1**-**4**, (Fig. **3**) moiety as inhibitors of the FabG4 enzyme of *Mtb*. The role of the different fragments on the activity was evaluated building up structure-activity relationship (SAR) studies by substituting fragments linked to the 1- and 4-positions of the triazole ring with less interacting moieties.

Compounds **1** and **2** showed an inhibition constant (K*i*) of 3.97 and 0.88 μM, respectively, against FabG4 and a minimum inhibitory concentration (MIC) of 20 and 5 μg/ml against *M. smegmatis*, proving that both the catechin/epicatechin and the galloyl fragments play a crucial role in the inhibition potency [16].

Gaur *et al.* prepared a series of semi-synthetic derivatives of liquiritigenin LTG, (Fig. **4**) a flavanone found in a variety of plants, including *Glycyrrhiza glabra* that showed a MIC of 25 μg/ml against *Mtb* [17]. Plant extracted LTG was derivatized to four analogues: LTG-oxime (**5**), LTG-7,4'-diacetate (**6**), LTG-4'-acetate (**7**) and LTG-7,4'-dibenzoate (**8**) (Fig. **4**) Only the oxime **5** and the mono-acetate **7** showed some activity against *Mtb* with a MIC of 25 μg/ml, comparable to that of LTG, proving that the hydroxyl group at position 7 is essential for the activity.

CHAPTER 6

In Silico Approaches to Naturally Existing Chalcones and Flavonoids on Mao Inhibitory Action: A Boon to CNS Drug Discovery

Arafa Musa[1,2], Della Grace Thomas Parambi[3], Mutairah Shaker Alshammari[4], Rania Bakr[3], Mohammed A. Abdelgawad[3,5], Dibya Sundar Panda[6], Manoj Kumar Sachidanandan[7], Vaishnav Bhaskar[8], Leena K. Pappachen[8] and **Bijo Mathew[8,*]**

[1] *Department of Pharmacognosy, College of Pharmacy, Jouf University, Sakaka, Al Jouf, 72341, Saudi Arabia*

[2] *Department of Pharmacognosy, Faculty of Pharmacy, Al-Azhar University, Cairo-11371, Egypt*

[3] *Department of Pharmaceutical Chemistry, College of Pharmacy, Jouf University, Sakaka, Al Jouf, 72341, Saudi Arabia*

[4] *Department of Pharmaceutical Analytical Chemistry, Jouf University, Sakaka, Al Jouf, 72341, Saudi Arabia*

[5] *Department of Pharmaceutical Organic Chemistry, Beni-Suef University, Beni-Suef, 62514Egypt*

[6] *Department of Pharmaceutics, College of Pharmacy, Jouf University, Sakaka, Al Jouf, 72341, Saudi Arabia*

[7] *Department of Oral and maxillofacial surgery and diagnostics, College of Dentistry, Hail University, Hail Province,2440, Saudi Arabia*

[8] *Department of Pharmaceutical Chemistry, Amrita School of Pharmacy, Amrita Vishwa Vidyapeetham, Amrita Health Science Campus, Kochi-682 041, India*

Abstract: *In silico* studies or computer-aided drug design (CADD) have led to advancement in drug discovery and development of neurodegenerative disorders (NDDs) and neuropsychiatric disorders. CADD is being increasingly used by universities and industries and provides a clear understanding of molecular interactions. Predicting molecular interactions provides relevant information to extract the potential of bioactive compounds. At present, more interest is on natural entities as therapeutic agents with different heterocyclic categories. Various heterocyclic structures are suggested to show MAO (monoamine oxidase) inhibitory activity by CADD and preclinical studies. Among these, chalcones and flavonoids play a major role in MAO inhibitory action because of the phenolic ring. In this chapter, we discuss *in silico* studies of natural chalcones and flavonoids with MAO inhibitory by considering the

[*] **Corresponding author Bijo Mathew, Associate Professor:** Department of Pharmaceutical Chemistry, Amrita School of Pharmacy, Amrita Vishwa Vidyapeetham, Amrita Health Science Campus, Kochi-682 041, India; E-mails: bijomathew@aims.amrita.edu, bijovilaventgu@gmail.com

Simone Carradori (Ed.)

complexity of the brain and the multifactorial nature of neurodegenerative disorders. These *in silico* studies prove that phytoconstituents from herbal medicine with therapeutic properties can serve as lead drug molecules for the treatment of NDDs.

Keywords: Apigenin, Chalcone, Flavonoids, *In silico*, Isoflavonoids, Kaempferol, Luteolin, MAO-inhibitor, Neurodegenerative Disorders, Quercetin, Xanthones.

INTRODUCTION

Neurodegenerative disorders are remarked as disorders of the central nervous system often resulting in selective loss of neurons. The research illustrated that deposition of amyloid proteins causes neuronal degeneration. As information pile up to date, no treatments are available for NDDs and are increasing the mortality and morbidity rates in developed countries and the same in developing countries. The reports say that abnormalities in MAOs (monoamine oxidases) lead to various neurological disorders and they have a major role in CNS and peripheral organs. Recent reports also indicate that abnormalities in mitochondria and the enzymes present in the mitochondria, like monoamine oxidases (MAOs), catechol-*O*-methyl transferases, and dopa decarboxylase [1 - 3], lead to NDDs. At this moment, the focus is on the flavin-containing enzyme called monoamine oxidase B (MAO-B), which is present in the outer membrane of mitochondria. MAO-B acts by catalyzing the oxidative deamination of neurotransmitters (dopamine, noradrenaline, and serotonin) and exogenous amines, which are targets for the diseases like Alzheimer's disease (AD), Parkinson's disease (PD), cardiovascular diseases, and also depression [4, 5]. MAO-B is directly involved in damaging neuronal cells by creating reactive oxygen species (ROS), which is the key reason for cell oxidative injury [5]. Identifying the MAO enzymes was a breakthrough in the history of drug discovery.

It is a long-standing tradition to use herbal drugs to treat and cure many diseases, mainly as medicines or sources of unique lead molecules for drug discovery and development of novel medicines [6 - 8]. Reports indicate that half of the top best-selling medicines are natural products, and their sales account for US 16 billion dollars, indicating that herbal medicines can be pre-optimized to be potentially bioactive compounds. Several chemoinformatics analyses also reveal that natural products can act as" drug-like" or "lead-like" and their physicochemical and structural properties can make them leads or drugs. Numerous reports suggest that natural products can act as therapeutic and lead compounds in neurodegenerative diseases [9]. The rational treatment of CNS disorders by natural products is still in its infancy due to the multifactorial nature and complexity of CNS and the complex chemistry and pharmacology of natural compounds.

Several human MAO inhibitors are now used as antianxiety anti-depression agents, whereas human MAO-B inhibitors can be used alone or in combination therapy for Alzheimer's and Parkinson's disease. Phytochemicals have the capacity to alternate the line of treatment in neurodegenerative disease. Herbal compounds have antioxidant properties and the capability to interact with several targets especially signaling pathways, neuroinflammation, and protein folding [10 - 13]. For example, several reports depict that natural compounds containing chalcones and flavonoids exert antioxidant properties directly interacting in Nrf2 pathways by scavenging reactive oxygen species (ROS). It is reported that many antioxidant properties of echinatin, licochalcone, and chalcones are due to proton transfer and electron transfer mechanisms [14], and natural and synthetic analogues like morpholine, naphthoquinone, amphetamine, coumarins, piperine, β-carboline, and caffeine have shown to exhibit appreciable MAO and neuroprotection [15 - 17]. Several synthetic compounds were developed incorporating the basic structure of chalcone and flavonoids as the basic moiety [18 - 23]. This book chapter mainly emphasizes the chalcones and flavonoids containing natural products related to their MAO-B action.

CHALCONES

The name" chalcone" was coined by the scientists Stanislaw Kostanneki and Josef Tambor. Chalcones are open-chain and chemically a three α, β-carbon unsaturated carbonyl system joined to two aromatic rings [24, 25] (Fig. **1**) They are also known to be the major precursor in the biosynthesis of some heterocyclic compounds like pyrazolines, benzothiazepine, flavones, and 1,4-diketones.

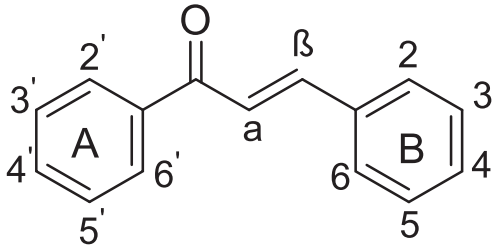


Fig. (1). Chemical structure of chalcone.

Chalcones are known as a biogenetic precursor of most flavonoids found in fruits (apple, citrus, and tomato), vegetables (potatoes, bean sprouts, and shallots), edible plants like licorice, but do not accumulate much in plants and are abundantly found in Leguminosae, Moraceae, and Asteraceae families. Chalcones are studied for their broad-spectrum activities like antimicrobial, antimicrobials,

CHAPTER 7

Lignins and Lignans – Recent Developments and Trends Regarding their Pharmaceutical Properties

Luc Zongo[1] and **Heiko Lange**[2,*]

[1] *Department of Pharmacy, University of Saint Dominic of West Africa (USDAO), Doulougou, Burkina Faso*

[2] *Department of Environmental and Earth Sciences, University of Milano-Bicocca, Milan, Italy*

Abstract: Lignins and lignans as natural polyphenols exhibit a rather broad variety of common physico-chemical features that can be of interest with respect to their use in the pharmaceutical sector. While polyphenol types have antioxidant, antiinflammatory, antibacterial and eventually antiviral activities in common, structural features beyond the polyphenol aspect differ enormously: isolated lignins are oligomers and/or polymers of monolignol C9-building blocks, while lignans are based on dimers thereof. The structural differences caused lignin to be exploited in the pharmaceutical sector mainly as material for the generation of matrices and carrier for drug delivery, while lignans are tested for the suitability as APIs. The chapter gives an overview of this situation, including the biological backgrounds of the two interesting natural polyphenols, isolation and methods for their characterisation.

Keywords: Antioxidant, Anti-Inflammatory, Antibacterial, Antivirus, Antitumor, Carrier, Delivery, Film, Lignin, Lignan, Microcapsules, Nanoparticles, Nanocapsules, Polyphenols, Renewable Resources.

INTRODUCTION

From all the renewable components present in land-based and aqueous biomass, natural polyphenols, namely lignin, lignans and tannins, are respectively important structural materials in the support tissues of vascular plants and valuable products of secondary plant metabolism that have a fundamental role in different stages of plant life, participating mostly in ecological mechanisms of interaction with other organisms and with the surrounding environment, and allowing plants to cope with the adversities. The natural polyphenols offer a wide range of heterogeneous intrinsic reactivities and activities that render them ideal starting oligomeric and polymeric materials for the preparation of functional

* **Corresponding author Heiko Lange:** Department of Environmental and Earth Sciences, University of Milano-Bicocca, Piazza della Scienza 1, 20126 Milan, Italy; E-mail: heiko.lange@unimib.it

Simone Carradori (Ed.)

macromolecules and the development of highly value-added materials. Nevertheless, due to the complexity and heterogeneity of their structures, especially in case of lignin and its isolated forms, and a type of historical lack in suitable, *i.e.*, useful detailed structural characterisation and the slow adaption of new structural insights by the wider community, their potential remains somewhat underexploited. Benefitting, however, more than ever simply from sheer necessity to actually find *via*ble substitutes for fossil-based materials, valorisation of natural polyphenols in the form of lignin, lignans and tannins is a booming research area, as the development of publications related to the field is indicating.

LIGNIN

Lignin interacts chemically and physically with the other two major components of the plant biomass, *i.e.*, cellulose and hemicelluloses [1]. The tight interplay between these three major plant biopolymers renders the plant cell wall impermeable, confers mechanical strength and rigidity, and overall serves to provide stability to the plants and confers resistance to microbial attacks.

The different functions of lignin in the plant cause its distribution to vary significantly within the different parts of the plant, *i.e.*, among stem, branching points, branches and leaves, and between the different walls of the plant cells themselves [2, 3]. The concentrations of lignin in the middle lamella and the primary cell wall are higher than the lignin concentration in the secondary cell wall. Nonetheless, the majority of the total amount of lignin present in the plant, 75–85%, is located in the secondary wall, due to its considerably large volume. Lignin abundancy is different for every plant species, ranging from ca. 20% in hardwoods, ca. 28% in softwoods and herbaceous angiosperms, to ca. 15% in monocots, accounting overall for 15–35% in average in dry wood [2, 4 - 6]. Unlike the structurally very regular and well-understood cellulose and hemicelluloses, lignin seems to exhibit only random sequences of various interunit bonding motifs [7].

Biosynthesis and Structural Features of Lignins

Lignin formation in cells has been proposed to be a *post-mortem* process in plant cells [8, 9]. The three monolignol building blocks for lignin, *i.e.*, *p*-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol (Fig. **1**) are produced presumably from *l*-tyrosine [10 - 13] or phenylalanine [14, 15] in adjacent living cells and transported into dead cells for lignification of the dead cell in a radical polymerisation process. While being induced by enzymatic activation of the monomers, the polymerisation proceeds most probably without any influence of the dirigent protein.

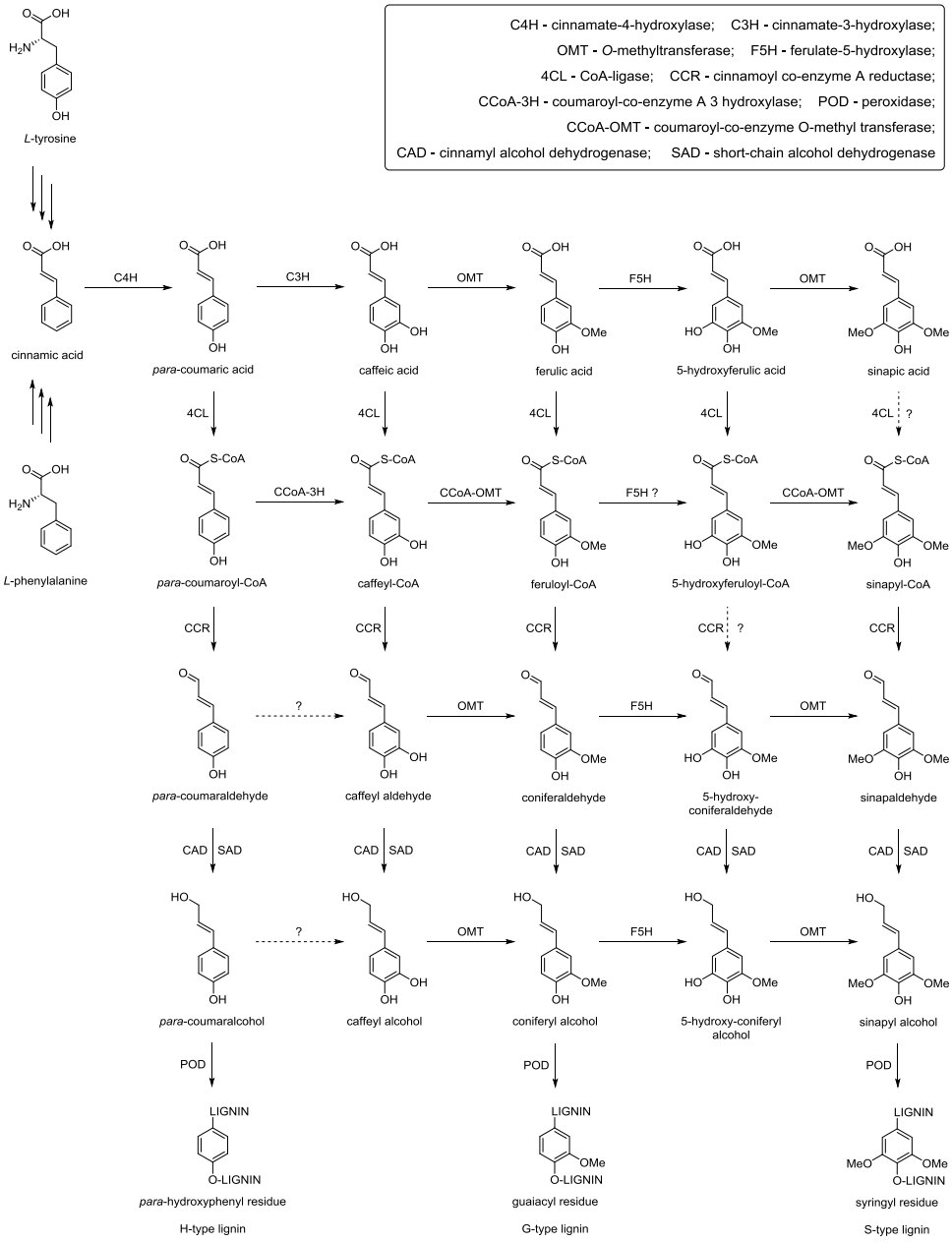


Fig. (1). Elements of the biosynthesis of lignins. For references, refer to the main text.

CHAPTER 8

Semisynthetic Resveratrol-derived Systems: A Synergism between Nature and Organic Synthesis

Antonella Capperucci[1] and **Damiano Tanini**[1,*]

[1] *Department of Chemistry Ugo Schiff, University of Florence, Via della Lastruccia 13, I-50019 Sesto Fiorentino, Italy*

Abstract: Structural modifications of the resveratrol scaffold are valuable tools in order to develop new derivatives with potential biomedical and pharmacological applications. The investigation of the biological properties of resveratrol-derived semisynthetic systems and the study of their structure-activity relationships are attracting growing interest from medicinal chemists and biologists. In this context, the synthesis of novel resveratrol-derived systems characterisaed by elevated molecular complexity is highly sought after. Over the past years, a wide variety of resveratrol derivatives have been prepared and studied for their biological properties. Therefore, a number of stilbenoid-related potential anticancer, antioxidant, antiviral, analgesic, and anti-neurodegenerative systems have been investigated. This chapter focuses on recent studies related to the preparation and the study of semisynthetic resveratrol-derived systems.

Keywords: Antioxidants, Anticancer Agents, Functionalization, Organic Synthesis, Prodrugs, Resveratrol, Stilbenoids, Structural Modifications.

INTRODUCTION

Resveratrol is a polyphenol-based natural product which can be isolated from a broad range of plants, where it has been demonstrated to act as phytoalexin [1]. Resveratrol is widely known because of its occurrence in red wine and its implication in the "French paradox" [2]. Owing to its antioxidant activity, resveratrol prevents the oxidation of LDL *in vitro* and reduces the markers of oxidative stress *in vivoi* [3]. A number of activities, including antioxidant, anti-inflammatory, anticancer, estrogenic, neuroprotective, anti-atherosclerosis, cardioprotective, anti-diabetic, anti-osteoporosis, anti-obesity, and anti-aging properties, have been described for resveratrol. The abundance and the diversity of resveratrol molecular targets reasonably account for the multiplicity of phar-

* **Corresponding author Damiano Tanini:** Department of Chemistry Ugo Schiff, University of Florence, *Via* della Lastruccia 13, I-50019 Sesto Fiorentino, Italy; E-mail: damiano.tanini@unifi.it

Simone Carradori (Ed.)

macological effects of this stilbenoid. For these reasons, resveratrol and related stilbenoids have been widely studied over the past years. However, the clinical benefits of resveratrol are hampered by unfavourable pharmacokinetic and pharmacodynamics, including poor bioavailability, low aqueous solubility, and chemical instability. In this context, considerable efforts have been devoted to evaluate whether suitable structural modifications could improve or modulate the biological properties of the parent stilbenoid [4, 5]. On the other hand, structural modifications of resveratrol can also be performed in order to improve its metabolic stability and to access new hybrids through the conjugation of two biologically active molecules. Indeed, a number of differently functionalised resveratrol derivatives and resveratrol-based hybrids have been proposed as potential pharmacological tools, exhibiting anticancer [6], antiviral [7], antibacterial [8], analgesic [9] and antioxidant properties [10]. Additionally, resveratrol derivatives have also been studied for their potential role in preventing or treating cardiovascular [11], Alzheimer's, and Parkinson's diseases [12].

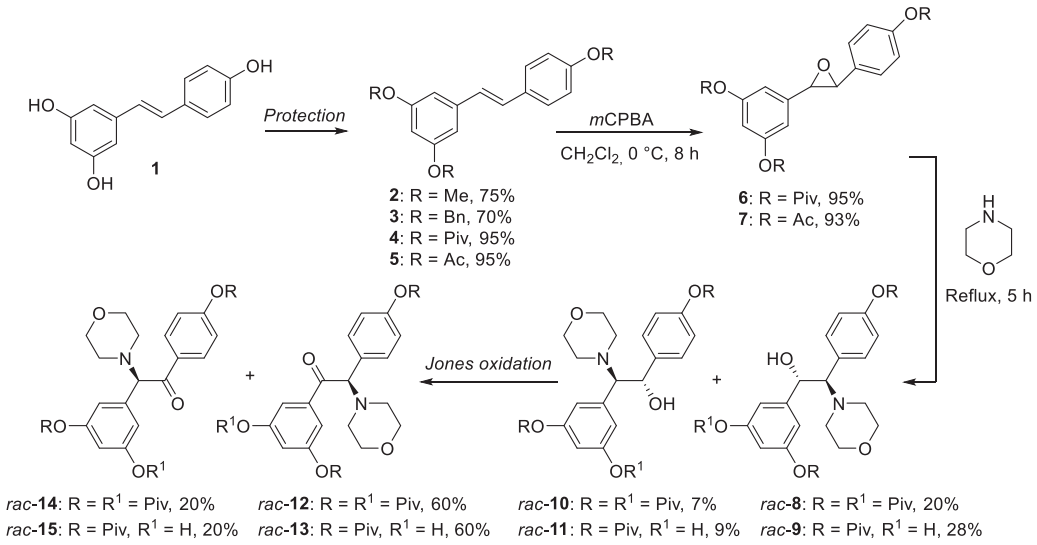
This chapter focuses on recent studies on the preparation and the biological properties of semisynthetic resveratrol-derived systems. Only synthetic procedures employing resveratrol as the starting material will be discussed herein. However, several approaches for the preparation of resveratrol-based hybrids rely on the construction of the functionalised resveratrol skeleton through the coupling of suitably decorated and substituted building blocks.

RESVERATROL ETHERS AND RELATED DERIVATIVES

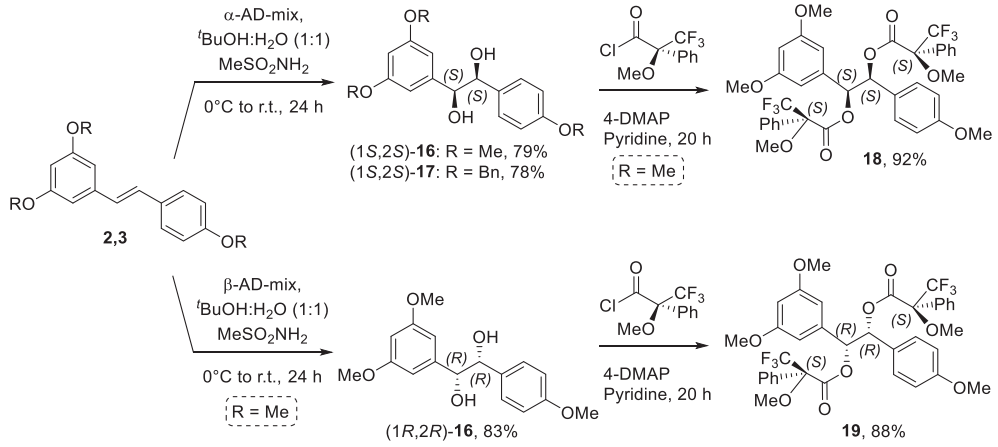
The anticancer activity of a series of resveratrol derivatives, prepared through functionalization of the stilbene double bond and/or protection of phenolic moieties, was reported by Orsini *et al*. [13]. Phenolic groups of resveratrol **1** could be protected as methyl ethers or benzyl ethers to afford compounds **2** and **3** in good yields. Tri-pivaloyl ester and tri-acetyl esters of resveratrol **4** and **5** were, respectively, obtained upon the reaction of resveratrol with pivaloyl chloride or acetyl chloride. Epoxides **6** and **7** were efficiently prepared through the epoxidation of the stilbene double bond of **4** and **5** with *m*-chloroperbenzoic acid (*m*CPBA). Epoxides **6** and **7** could be employed as precursors for a variety of resveratrol derivatives. Ring-opening reaction of **6** with morpholine enabled the formation of regioisomeric amino alcohols **8**,**9** and **10**,**11**. Oxidation of these regioisomeric alcohols under Jones conditions was exploited for the synthesis of functionalised ketones **12**,**13** and **14**,**15** Scheme **(1).**

Resveratrol tri-*O*-methyl ether **2** and tri-*O*-benzyl ether **3** were also employed as substrates for the synthesis of enantio-enriched diols (1*S*,2*S*)-**16** and (1*S*,2*S*)-**17** through asymmetric Sharpless dihydroxylation using AD-mix-α. Similarly,

resveratrol-derived enantio-enriched diol (1*R*,2*R*)-**16** was efficiently prepared from **2** by using AD-mix-β. The enantiomeric purity of both enantiomers of **16** was determined by the application of the Mosher method Scheme **(2).** Therefore, ^{1}H and ^{19}F NMR spectra of esters **18** and **19**, were prepared upon the reaction of the aforementioned enantio-enriched diols with (*R*)-α-methoxy, α-trifluoromethyl phenylacetyl chloride, were studied and the optical purity was assessed to be greater than 99%.



Scheme (1). Synthesis of resveratrol derivatives through the reactivity of resveratrol epoxides.



Scheme (2). Synthesis of enantio-enriched resveratrol-derived diols and preparation of corresponding Mosher esters.

CHAPTER 9

Aurone Scaffold and Structural Analogues for the Development of Monoamine Oxidase (MAO) Inhibitors

Paolo Guglielmi[1], Virginia Pontecorvi[1,*] and **Atilla Akdemir[2]**

[1] *Department of Drug Chemistry and Technologies, Sapienza University of Rome, P.le A. Moro 5, 00185Rome, Italy*

[2] *Computer-aided Drug Discovery Laboratory, Department of Pharmacology, Bezmialem Vakif University, Fatih, Istanbul 34093, Turkey*

Abstract: Continuous efforts in the development of monoamine oxidase inhibitors prompted the search for effective strategies for the design of novel drugs candidate. Thankfully, nature often provides scaffolds useful for the promotion of novel exploitable chemical entities. In this regard, aurones (a class of uncommon flavonoids) and their structural related analogues may play an important role in the development of monoamine oxidase inhibitors. The target prediction of the simplest aurone (2-benzylidenebenzofuran-3(2*H*)-one) clearly suggests that this compound probably affects MAO (monoamine oxidase) enzymes, which is in accordance with the recently reported literature. The current chapter reports the recent discoveries involving aurones and their structurally related analogues as MAO inhibitors, describing detailed structure-activity relationships (SARs) for each subgroup of compounds.

Keywords: Aurones, Homoisoflavonoids, Indanones, MAO inhibitors, Monoamine oxidase, Neurodegenerative diseases, Tetralones.

INTRODUCTION

Human monoamine oxidases (hMAOs) are flavoenzymes that catalyze the oxidative deamination of dietary amines, monoamine neurotransmitters and hormones [1, 2]. The common substrates for MAO include important monoamine neurotransmitters such as dopamine, noradrenaline, adrenaline and serotonin [3]; this leads MAOs to play an important role in behavioral, cognitive, and endocrine regulation [4]. The specificity of MAO for its substrate depends on the concentration, affinity, and turnover rate of the substrate as well as the conc-

* **Corresponding author Paolo Guglielmi Ph.D:** Department of Drug Chemistry and Technologies, Sapienza University of Rome, P.le A. Moro 5, 00185 Rome, Italy; E-mail: paolo.guglielmi@uniroma1.it

Simone Carradori (Ed.)

entration of the enzyme [5]. Two human isoforms have been proposed and discovered, i.e., hMAO-A and hMAO-B. They are characterized by structural homology, active site differences and catalytic efficiency, tissue localization, substrate and inhibitor selectivity [6]. The two isoforms are quite similar, sharing ~70% overall sequence identity [7].

These enzymes are bound to the mitochondrial intermembrane *via* their transmembrane (TM) domains and their catalytic domain is located in the cytosol (Fig. **1**). They have subunit molecular weights of 59,700 (hMAO-A) and 58,000 (hMAO-B), consisting of 527 and 520 amino acids, respectively [3]. The (dimeric) holoenzyme has a covalently bound flavin adenine dinucleotide (FAD) cofactor for each subunit, which interacts with Cys406 of hMAO-A or Cys397 of hMAO-B, and an 'aromatic cage' which is the site where the amine group of the substrate binds and undergoes oxidation [3, 4, 7].

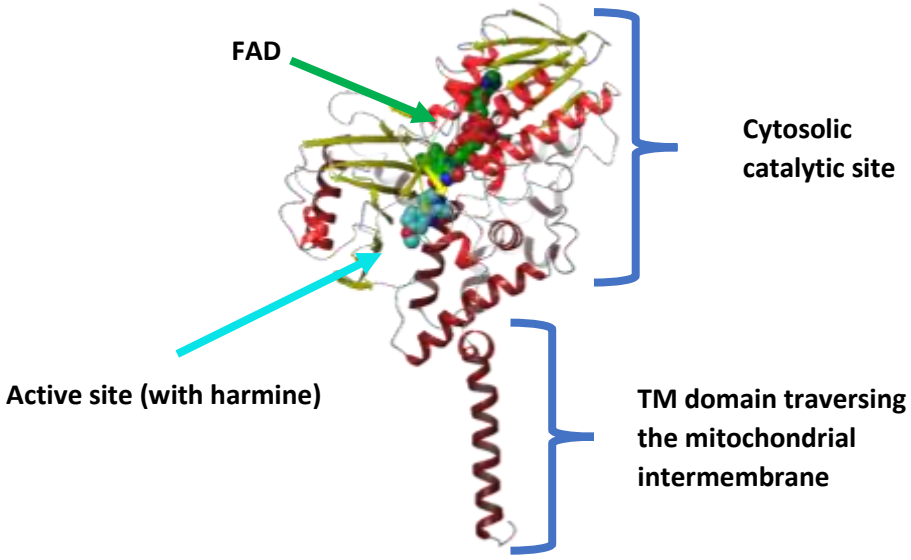


Fig. (1). The overall 3D structure of human MAO-A in complex with FAD (green CPK) and harmine (turquoise CPK). The cytosolic catalytic domain and the transmembrane (TM) domain that traverses the mitochondrial intermembrane are indicated.

The covalent binding of FAD seems to act as a structural core for the active conformation instead of being required for catalytic activity [5]. Both the isoforms share a substrate binding cavity of ~400 $Å^3$. hMAO-B is also endowed with a hydrophobic cavity of 290 $Å^3$, the "entrance cavity", which works as a "gate" for the "closed" or "open" conformations [7]. It has been discovered that aromatic and aliphatic residues seem to contribute to substrate selectivity of hMAO-A and hMAO-B [5].

The substrate binding site is located adjacent to the FAD binding site and consists of the conserved residues Tyr69, Tyr197, Phe208, Phe352, Tyr407, Trp441 and Tyr444 (Fig. **2**). Also conserved in both isozymes are Phe112, Trp128, Leu176 and Phe177, however, these residues have a significantly different conformation in hMAO-A and hMAO-B (Fig. **2**). The amino acids Ala111, Phe173, Ile180, Asn181 and Ile335 in hMAO-A are not conserved and their hMAO-B counterparts are Pro102, Leu164, Leu171, Cys172 and Tyr326. This changes the shape and binding characteristics of the substrate binding sites.

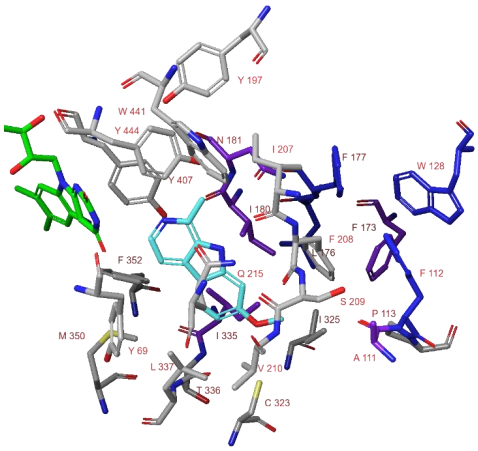


Fig. (2). The active site of hMAO-A in complex with harmine (pdb: 2z5y). The conserved amino acids are indicated in light grey. The amino acids that are different in hMAO-B are indicated in purple (A111, F173, I180, N181 and I335). The amino acids that are conserved but have different conformations are indicated in dark blue (F112, W128, L176 and F177). FAD is indicated in green and only partly shown for clarity reasons. The ligand harmine is indicated in turquoise.

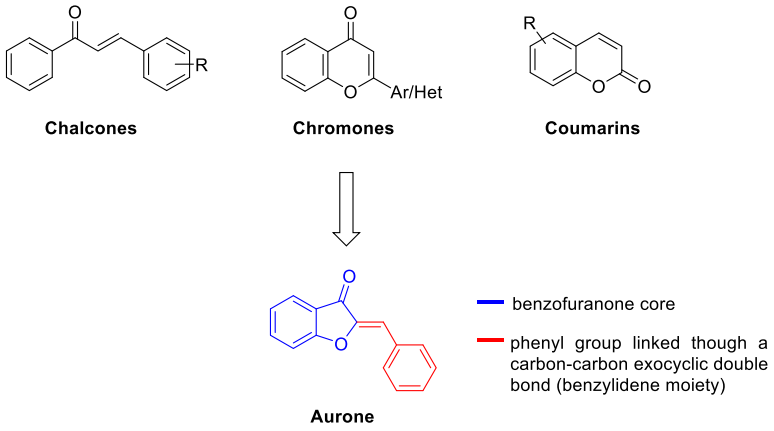


Fig. (3). Structure of chalcones, chromones and coumarins. Structural correlation between flavonoids and aurones.

CHAPTER 10

Coumarins as Carbonic Anhydrase Inhibitors

Claudiu T. Supuran[1,*]

[1] *Department of NEUROFARBA, Section of Pharmaceutical and Nutraceutical Sciences, Pharmaceutical and Nutraceutical Section, University of Florence, via U. Schiff 6, 50019 Sesto Fiorentino, Firenze, Italy*

Abstract: Carbonic anhydrases (CAs, EC 4.2.1.1) are metalloenzymes and relevant drug targets with many medicinal chemistry applications. Their classes of inhibitors are in clinical use as diuretics, or drugs for the management of glaucoma, epilepsy, obesity, tumors and infectious diseases. Among the inhibitors discovered so far, coumarins constitute an interesting class. They undergo CA-catalyzed hydrolysis and act as "prodrug inhibitors", forming 2-hydroxy-cinnamic acids, which bind at the entrance of the enzyme active site, which has a relevant variability of amino acid residues among the different CA isoforms present in mammals, humans included. Coumarins act as isoform-selective CA inhibitors against pharmacologically relevant enzymes, such as the tumor-associated CA IX and XII. Coumarins present as metabolites in many species of bacteria, fungi, plants and ascidians showed relevant CA inhibitory properties and were used as leads for obtaining synthetic derivatives with enhanced enzyme inhibitory action belonging to a variety of classes, such as polysubstituted coumarins on both rings, thiocoumarins, thioxocoumarins, sulfocoumarins, *etc.*

Keywords: Carbonic Anhydrase, Coumarin, Prodrug Inhibitor, Sulfocoumarins, Thioxocoumarins, Tumors.

CARBONIC ANHYDRASE INHIBITORS AND ACTIVATORS

Carbonic anhydrases (CAs, EC 4.2.1.1) are metalloenzymes present in organisms throughout the life kingdoms (Prokaryotes and Eukaryotes), being encoded by eight genetically diverse families [1 - 10]. They catalyze the carbon dioxide hydration to bicarbonate and H^+ ions. This is a simple reaction but crucial for a variety of processes, both physiologic and pathologic, due to the fact that CO_2 and water, two neutral molecules, generate a weak base, bicarbonate, and a strong acid, and as a consequence, this enzyme plays a key role in acid-base equilibria, pH regulation and metabolism [1, 11 - 15].

[*] **Corresponding author Claudiu T. Supuran:** Department of NEUROFARBA, Section of Pharmaceutical and Nutraceutical Sciences, Pharmaceutical and Nutraceutical Section, University of Florence, *via* U. Schiff 6, 50019 Sesto Fiorentino, Firenze, Italy; Tel: +39-055-4573729; Fax: +39-055-4573385; E-mail: claudiu.supuran@unifi.it

Simone Carradori (Ed.)

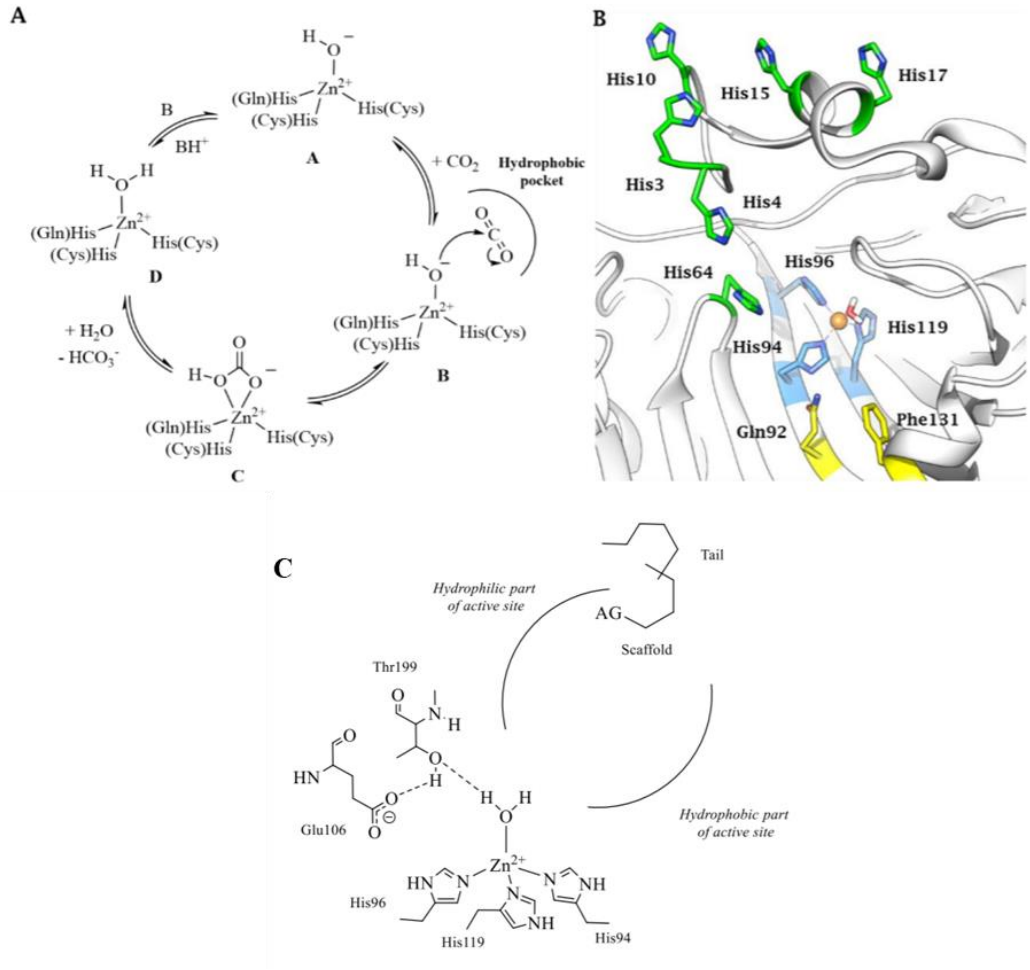


Fig. (1). A. CA catalytic cycle for the CO_2 hydration/bicarbonate dehydration reaction. **B**. α-CA (hCA II) active site, with the zinc ion and its ligands (His94, 96, and 119) as well as His residues involved in proton shuttling. **C**. α-CA inhibition mechanism by occlusion of the active site entrance [16].

The metal ion is usually zinc(II) coordinated by three amino acid residues and a water molecule/hydroxide ion, acting s a nucleophile in the catalytic cycle – (Fig. **1A**) [1 - 9, 16]. The species with zinc hydroxide within the active site is the nucleophilic, catalytically active one, which converts CO_2 bound within the enzyme active site cleft to bicarbonate, originally coordinated to the metal and then released in solution, with the formation of the acidic species, with water coordinated to the zinc. Fig. (**1A**). This form must lose a proton, a process which is achieved by the so-called proton shuttling residues, usually, His residues, shown in Fig. (**1B**) [1 - 9, 16]. This is also the rate-limiting step of the catalytic

cycle, but CAs are among the fastest and most effective enzymes known to date [16].

This reaction is crucial for a host of physiologic functions connected to pH regulation processes in all types of organisms and cells [17 - 21]. For this reason, modulating CA activity by using inhibitors and activators, leads to pharmacological applications for the treatment of multiple human diseases. The CA inhibitors (CAIs) are used as diuretics since the '50s [22 - 24], as anti-glaucoma agents [25, 26], antiepileptic drugs [27, 28], antiobesity agents [29, 30], and for the management of metastatic, hypoxic tumors [31 - 36]. The CA activators (CAAs) [37 - 39] started to be used for pharmacological applications more recently, initially for memory therapy [40], or for the modulation of emotional memory, but they may show other applications in areas such as generalised anxiety, post-traumatic stress, phobias, as well as obsessive-compulsive disorders [41, 42].

Among the four CA inhibition mechanisms reported to date for α-CAs [16, 43 - 45], the compounds which occlude the entrance to the active site Fig. (**1C**). will be discussed. These CAIs bind far away from the catalytic metal ion (> 10 Å away from it), in a region that is rather variable among the many mammalian isoforms, which also favoured the discovery of isoform-selective CAIs [16, 43 - 45]. Coumarins were the first compounds for which this inhibition mechanism has been disclosed by Quinn's group [46], both from the inhibition mechanism and drug design viewpoints. In fact, the discovery of coumarins as CAIs and their particular inhibition mechanism [46, 47] allowed for a rational drug design campaign of a large number of isoform-selective inhibitor classes for many catalytically active human (h) CA isoforms (of the 15 hCA isoforms present in humans, 12 possess catalytic activity, with isoforms VIII, X and XI being devoid of it [1]).

COUMARINS WITH CA INHIBITORY ACTION

Natural Product Coumarins

Among the many natural products that are widespread in organisms all over the phylogenetic tree [48], coumarins play a relevant role in medicinal chemistry due to their multiple pharmacologic applications [49 - 55]. They show a variety of biological activities, such as the anticoagulant effects [55], inhibitors of monoamine oxidase (MAO) [52, 53], antibacterial, antifungal and antiviral actions [51, 54], antioxidant activity [50, 54], antitumor effects [51, 54], as well as anti-inflammatory action [50, 51, 54]. The precise mechanisms of action of coumarins against so many different targets are poorly understood, except for the MAO inhibition and anticoagulant activities [53 - 55]. Coumarins incorporate two

CHAPTER 11

Phenols and Polyphenols as Carbonic Anhydrase Inhibitors

Alessandro Bonardi[1], **Claudiu T. Supuran**[1] and **Alessio Nocentini**[1,*]

[1] *Department of NEUROFARBA, Section of Pharmaceutical and Nutraceutical Sciences, Pharmaceutical and Nutraceutical Section, University of Florence, Via U. Schiff 6, 50019 Sesto Fiorentino, Firenze, Italy*

Abstract: Thousands of phenolic derivatives have been identified in the plant kingdom, which exert crucial roles in plant physiology. Many such derivatives were shown to produce pharmacological effects in humans which address their use in medicine as antiaging, anti-inflammatory, antioxidant, antidiabetic, and antiproliferative agents among others. Numerous such pharmacological activities are likely to derive from the inhibition of human carbonic anhydrase (CAs, EC 4.2.1.1) isoforms. Phenols, in fact, are able to anchor to the zinc-bound nucleophile present in the enzyme active site, blocking the catalytic action of CAs in humans and/or encoded in various microorganisms. This chapter discusses natural, semisynthetic and synthetic phenol derivatives that exhibited a CA inhibitory action. The discussion over the CA inhibition profiles is categorized as the inhibition of human CAs and inhibition of CAs from microorganisms. Multiple types of inhibition mechanisms by phenolic derivatives are discussed according to X-ray crystallographic resolutions and *in silico* studies.

Keywords: Antitumor, Antibiotic, Anti-infective, Bacteria, Carbonic anhydrase, Enzymology, Fungi, Inhibitor, *In silico*, Phenol, Polyphenol, Protozoa.

PHENOLS AND POLYPHENOLS

Since ancient times, *Homo sapiens* have been using plants and herbal extracts as natural medicines to treat health issues. In modern medicine, the research for natural derivatives as precursors for the development of drugs has spread considerably [1].

More than five thousand phenol and polyphenol derivatives have been identified in plants. They play key roles as structural polymers (*i.e.* lignin), antioxidants, UV screens (*i.e.* flavonoids), signal compounds (*i.e.* flavonoids and salicylic acid),

* **Corresponding author Alessio Nocentini:** Department of NEUROFARBA, Section of Pharmaceutical and Nutraceutical Sciences, Pharmaceutical and Nutraceutical Section, University of Florence, *via* U. Schiff 6, 50019 Sesto Fiorentino, Firenze, Italy; E-mail: alessio.nocentini@unifi.it

Simone Carradori (Ed.)

attractants to accelerate pollination (*i.e.* flavonoids and carotenoids), and defense response chemicals (*i.e.* tannins and phytoalexins), such as coloring for camouflage and defense against herbivores, as well as antibacterials and antifungals [2 - 4].

Numerous phenol derivatives are actively used nowadays in medicine according to demonstrated anti-aging [5], anti-inflammatory [6], antioxidant [7], anti-diabetic (*i.e.* stilbenoids) [8, 9], and antiproliferative activities (*i.e.* flavonoids, phenolic alcohols, lignans, and secoiroides) [10, 11]. Other polyphenol derivatives, such as resveratrol, have been demonstrated to prevent cardiovascular diseases by improving the function of the endothelial tissue, inhibiting platelet aggregation and reducing blood pressure and flogosis [12 - 17].

Carbonic Anhydrases

The superfamily of metalloenzymes carbonic anhydrases (CAs, EC 4.2.1.1) is widely expressed among all life kingdoms. Eight distinct classes are encoded by eight evolutionarily unrelated gene families: α-, β-, γ-, δ-, ζ-, η-, θ- and ι-CAs [18 - 29]. The CAs chiefly catalyze the reversible hydration of carbon dioxide (CO_2) to bicarbonate (HCO_3^-) and proton (H^+). This reaction is physiologically crucial for all living beings as it is involved in respiration, pH and CO_2 homeostasis, transport of CO_2/HCO_3^- and a multitude of biosynthetic reactions [18 - 21]. Fifteen α-class CA isoforms are encoded in humans and are implicated in numerous physiological processes such as electrolytes secretion, metabolic reactions (*i.e.* gluconeogenesis, lipogenesis, ureagenesis), bone resorption, and calcification [29 - 39]. An anomalous expression and/or activity of specific human CA isoforms was observed in a multitude of human pathological processes. Hence, many such diseases can be pharmacologically treated with CA inhibitors (CAIs) or activators (CAAs) [18].

Also human pathogens, among which bacteria, protozoa, and fungi, encode for α-, β-, γ- and η-CAs. In fact, CAs are crucial for the parasite virulence, growth or acclimatization in the hosts. The inhibition of these CAs produces growth impairment and defects in the pathogen, being a promising strategy for anti-infective intervention [39, 40].

CA Inhibition Mechanism of Phenol Derivatives

The mechanisms through which CAs are inhibited or activated have been studied for decades and are well-understood processes [18, 19, 41]. To date, four inhibition mechanisms have been discovered and characterized:

1. The zinc-binders, which include inorganic anions, sulfonamides and their bioisosteres (sulfamates, sulfamides), dithiocarbamates (and bioisosteres), hydroxamates, carboxylic acids, phosphates, benzoxaboroles, selenols, phosphonamidates [42 - 50].
2. Compounds that anchor to the zinc-bound water molecule/hydroxide ion, such as phenols, polyamines, sulfocoumarins, and thioxocoumarins [51 - 55].
3. Compounds that occlude the entrance of the active site, that are coumarins and their bioisosteres [56 - 58].
4. Compounds binding out of the active site, that is 2-(benzylsulfonyl)-benzoic acid [42, 59].

The CA inhibition mechanism of phenolic derivatives was described for the first time in 1994 by Nair *et al*. [51], which solved the crystal structure of hCA II in adduct with phenol **1** (Fig. **1A-B**). Successively, Lomelino *et al*. reported an analogous binding mode to hCA II for resorcinol **3** (pdb 4E49) and hydroquinone **4** (pdb 4E3H) (Fig. **1C-D**) [60]. The phenol OH group anchors to the zinc-bound water molecule/hydroxide through an H-bond as a donator, while it receives a second H-bond by the NH backbone of Thr199, a conserved amino acid in the active site of all α-CAs. The benzene ring accommodates within the active site forming hydrophobic interactions with Val121, Val143, Leu198, and Trp209 (Fig. **1**). The *m*-OH group of resorcinol **3** formed a water-bridged H-bond with Gln92. In contrast, two coexisting binding orientations were found for hydroxyquinone **4** within hCA II active site which differ from the orientation of the *p*-OH group. In only one of these, the *p*-OH formed an H-bond with Gln92 side chain NH_2.

Lately, in 2020 a new inhibition mechanism was reported crystallographically by D'Ambrosio *et al*. for catechols [61], which are 1,2-dihydroxybenzene derivatives. Multiple crystallization experiments were conducted on hCA II and chlorogenic acid **49** as CAI produced analogues, *i.e.* the shape of the ligand electron density within the active site was not compatible with **49**, but well matched with its hydrolysis product caffeic acid **42** (Fig. **2**). The authors demonstrated that **49** is hydrolyzed to **42** only in the simultaneous presence of hCA II and the crystallization buffer. Thus, under physiological conditions, this molecule does not act as a suicide inhibitor as coumarins or sulfocoumarins did instead. In detail, **42** is anchored to the enzyme by means of the two OH groups of the catechol which are H-bonded to both the zinc-bound nucleophile and to another water molecule characteristic of α-CAs active site, that is named "deep water" (W_d in Fig. **(2**). One of these OH groups is also at H-bond distances from the Thr200 side chain OH and Thr199 amide NH. The organic scaffold of the inhibitor establishes several hydrophobic interactions with residues Val121, Phe131 and Leu198, whereas the carboxylate functionality points towards the protein surface and does not interact with any protein residue.

CHAPTER 12

The Role of Flavonoids and other Selected (Poly) Phenols in Cancer Prevention and Therapy: A Focus on Epigenetics

Melissa D'Ascenzio[1,*]

[1] *D'Arcy Thompson Unit, Biological and Biomedical Sciences Education, School of Life Sciences, University of Dundee, DD1 4HN, Dundee, UK*

Abstract: The importance of diet in determining the incidence of chronic illnesses such as diabetes, cardiovascular disorders, neurodegenerative diseases, and cancer has inspired extensive research on the role of individual dietary components in chemoprevention. Flavonoids and (poly)phenols have often been identified as the ideal candidates for these types of studies, as they represent large classes of natural products that are widely available in fruit and vegetables. In this chapter, we will discuss the antiproliferative properties of flavonols, flavanols, flavones, isoflavones, anthocyanins, curcuminoids and resveratrol derivatives, with a particular focus on their ability to interfere with epigenetic processes and modulate gene expression. We will look at the challenges encountered during the optimisation of the pharmacokinetic and pharmacodynamic properties of these natural products and, where possible, we will define structure-activity relationships.

Keywords: Anthocyanins, Anticancer agents, Apigenin, Curcumin, Chemoprevention, Epigallocatechin-3-gallate, Epigenetics, Flavonoids, Flavonols, Flavones, Genistein, Isoflavones, Kaempferol, Polyphenols, Quercetin, Resveratrol.

INTRODUCTION

Chemoprevention and the Epigenetic Mechanisms Associated with Chronic Diseases

According to the latest World Population Prospect published in 2019 by the United Nations, the world's population is predicted to reach 9.7 billion by the year 2050 [1]. Thanks to the progress made by medicine and the implementation of

[*] **Corresponding author Melissa D'Ascenzio:** D'Arcy Thompson Unit, Biological and Biomedical Sciences Education, School of Life Sciences, University of Dundee, DD1 4HN, Dundee, UK; Tel.: +44 (0) 1382 384682; E-mail: m.dascenzio@dundee.ac.uk

Simone Carradori (Ed.)

public health measures aimed at improving the quality of care and raising awareness around healthy lifestyles, life expectancy is also expected to increase significantly over the next thirty years. As of today, the fastest-growing age group in terms of percentage of the overall population, is the one represented by persons aged 65 or over. Almost a fifth of the European and Northern American population is constituted by over 65s and this number is predicted to increase until it will reach 25% by 2050 [1]. Unfortunately, an extension in life expectancy does not necessarily translate into an increase in a healthy lifespan. In fact, ageing is one of the major contributing factors to the incidence of chronic diseases such as neurodegenerative disorders, cardiovascular diseases, chronic respiratory diseases, diabetes, obesity, and cancer [2]. In many instances, the decreased mortality associated with these pathologies leads to the development of comorbidities and the overall complication of the patient's clinical picture [3]. In 2002, the growing body of evidence linking dietary and lifestyle habits with the incidence of chronic diseases led the WHO and FAO to conduct a joint expert consultation aimed at producing evidence-based recommendations for the cost-effective prevention and control of chronic diseases [4]. In 2013, these recommendations were incorporated into a Global Action Plan for the prevention of non-communicable diseases (NCDs) and have since been translated into regional and national policies worldwide [5]. While WHO guidelines tend to focus mostly on limiting tobacco and alcohol consumption, reducing salt, sugar, and calorie intake, as well as promoting physical activity, scientific evidence demonstrates that the connection between diet and chronic diseases goes further than that. Recent studies report that the Mediterranean diet and the DASH (Dietary Approaches to Stop Hypertension) diet can slow down cognitive decline [6, 7], while high compliance to a hybrid Mediterranean-DASH diet reduces the incidence of Alzheimer's disease by more than 50% in patients aged 58-98 [8]. In a similar way, the regular consumption of fruits and vegetables has been associated with a significant reduction in the risk of developing several types of cancer, especially those involving the gastrointestinal tract and the respiratory system, but also including breast, bladder, prostate, kidney, cervix, ovary and endometrium cancer [9, 10]. These findings constitute the foundation upon which several dietary components, including micronutrients and phytochemicals, have been studied and adopted in clinical settings as adjuvants in cancer therapy and prevention [11]. This approach takes the name of chemoprevention.

Although there seems to be a general consensus around the fact that most dietary components show neuroprotective and anticancer behaviour thanks to their antioxidant properties, *i.e.* their ability to reduce the oxidative stress caused by the production of reactive oxygen and nitrogen species (ROS & RNS), there is a consolidated body of evidence suggesting that common phytochemicals found in vegetables and fruit can activate stress-response pathways that involve a large

variety of effectors, including transcription factors, protein kinases, and epigenetic modifiers [12, 13].

The dramatic effect that dietary components can have on gene expression through the modulation of epigenetic mechanisms is beautifully captured in the honeybee (*Apis mellifera*) model, where the development of larvae with identical genetic material into queen or worker bees is determined by the food they receive [14]. While the queen bee is fed exclusively with royal jelly, worker bees receive royal jelly only for three days and then move onto a diet made of worker jelly, pollen, and beebread [15]. These dissimilar diets have been proven responsible for the physical and behavioural differences observed in the two honeybee phenotypes. At the cellular level, this phenomenon is caused by changes in DNA methylation levels, particularly at alternative splicing sites [14]. DNA methylation is an archaic mechanism that traces its origin back to bacteria and unicellular organisms and involves transferring a methyl group to the C-5 position of a cytosine base [16]. In humans, methylation usually happens at cytosine-rich regions known as CpG islands, which constitute over two-thirds of mammalian promoters [17]. Although humans do not exhibit the same developmental flexibility as honeybees, DNA methylation of gene promoters has a powerful silencing effect on downstream genes and it plays a fundamental role in embryonic development [18]. Changes in DNA methylation levels at this stage of development can dramatically increase the risk of chronic diseases later in life, as shown by studies conducted on individuals who were prenatally exposed to famine during the Dutch Hunger Winter in 1944-45 [19, 20]. Although these results would be difficult to confirm in humans due to ethical implications, it has been demonstrated in several animal studies that in the uterus and early life exposure to phytochemicals that are known for their ability to inhibit DNA methylation can reduce the incidence of cardiovascular diseases, obesity, and cancer [21 - 24]. In fact, maintaining a balanced DNA methylation status is particularly important to prevent cell transformation, as genome-wide demethylation associated with expression of oncogenes and hypermethylation of CpG islands promoters of onco-suppressor genes constitute a clear hallmark of cancer [17]. However, DNA methylation is not the only epigenetic process that has been found to be dysregulated in cancer development and progression. Epigenetic mechanisms associated with the onset of cancerous phenotypes include post-translational modification (PTM) of histone and non-histone proteins through acetylation, methylation, phosphorylation, ubiquitinylation, citrullination, ribosylation, and sumoylation, as well as non-coding RNA (ncRNA) mediated gene silencing [25]. In this chapter, we will discuss the antiproliferative activity of flavonoids and other selected (poly)phenols of plant origin, with particular reference to their ability to interfere with specific epigenetic mechanisms, such as DNA methylation and the methylation/acetylation of both histone and non-histone proteins.

SUBJECT INDEX

A

B

C

D

E

F

G

H

I

J

K

L

M

N

O

P

Q

R

S

T

U

V

W

X

Simone Carradori

Prof. Simone Carradori has carried out his scientific work in Medicinal Chemistry collaborating with several foreign departments. Currently, he is an associate professor at the Department of Pharmacy of "G. d'Annunzio" University of Chieti-Pescara (Italy). The scientific activity is mainly focused on the characterization and synthesis of natural/ synthetic derivatives with potential biological activity and is documented from over 225 papers in international peer-reviewed journals, 6 book chapters, 1 European patent and participations in numerous conferences as invited lecturer. Furthermore, he is "Visiting Professor" at "Faculty of Pharmacy of Bezmialem Vakif University" (Istanbul, Turkey) and member of the PhD committee at "G. d'Annunzio" University of Chieti-Pescara.

He is Editor-in-chief of "Anti-Cancer Agents in Medicinal Chemistry" (Bentham Science Publishers), co-editor of "Current Medicinal Chemistry" (Bentham Science Publisher) and "Anti-Inflammatory and Anti-Allergy Agents in Medicinal Chemistry" (Bentham Science Publishers).