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DIPLOMARBEIT

**CURCUMIN:
NOVEL THERAPEUTIC APPLICATIONS
OF AN OLD TRADITIONAL DRUG.
WITH FOCUS ON ALZHEIMER'S DISEASE**

zur Erlangung des akademischen Grades
Magistra Pharmaciae (Mag.pharm.)
an der Fakultät für Lebenswissenschaften
der Universität Wien

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Wien, Jänner 2007

UNIVERSITÄT WIEN

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Fakultät für Lebenswissenschaften



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Curcumin:
Novel Therapeutic Applications
of an Old Traditional Drug.
With Focus on Alzheimer's Disease.

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*I would like to thank Prof. Christian Noe,
Prof. Roberto Pellicciari and Dr. Hannelore Kopelent
for opening me the possibility for this great experience
and for their support during the work of my thesis.*

*I want to thank all of my friends, especially Gaby,
who were always there not only during my stay in Perugia.*

*Last but not least I would like to express
my deepest gratitude to my father,
who offered me all opportunities
and my grandma for always being there.*

Thanks for everything.

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PERSPECTIVES

In the academic year 2005/2006 I had the possibility to participate in the Erasmus exchange program. I was offered the opportunity to carry out a diploma thesis project in the research group of Professor Pellicciari at the University of Perugia.

Within one of the research topics of this group my thesis project should be dedicated to synthesis of new derivatives of Curcumin. Unfortunately, upon starting experimental work it was recognized that a strong allergic reaction affected my state of health in a way making it impossible to continue the experiments. Professor Pellicciari kindly proposed to change the topic of the thesis project from the synthesis of novel Curcumin derivatives to a theoretical survey of the to date important synthesized derivatives and the synthesis of Curcumin. Furthermore biological activities of the respective compounds and the connection so far proposed between the structure and its influence into the pathogenesis of Alzheimer's disease should be investigated.

Therefore in this work the most important facts about AD, the influence of Curcumin in the pathogenesis so far proposed, its origin and chemical structure and as mentioned the SAR of some selected derivatives are ascribed.

1. INTRODUCTION

In this part an overview is given about the most important facts in respect of Curcumin, its origin and use and the idea why it could be beneficial in AD.

Curcumin has been used in the form of turmeric, the so called powder of the rhizome, as an antioxidant food preservative, as an anti-inflammatory turmeric extract and has also been widely employed in traditional Indian medicine.¹ Curcumin, in particular, exhibits plenty of biological properties.^{2,3}

Seven major species of *Curcuma* (Zingiberaceae) including *Curcuma longa* Linn., *C. xanthorrhiza* Roxb., *C. wenyujin* Y.H. Chen and C. Ling, *C. sichuanensis*, *C. kwangsiensis*, *C. aeruginosa* Roxb., and *C. elata* Roxb., are cultivated in tropical regions of Asia, and Curcumin is the major yellow pigment isolated from the ground rhizome of *Curcuma longa* Linn.¹ Ethnologically *Curcuma longa* occupies an important position as nearly every food contains in India, and religious ceremonies always make use of the rhizome powder, called as mentioned turmeric, in any form.



The rhizome is normally used for colouring and flavouring food, but as a powder it is also used for medical purposes.^{1,3} In old Hindu texts it is ascribed for its aromatic, stimulant and carminative properties. A locally applied mixture of turmeric with slaked lime is known as a household remedy for the treatment of sprains and swellings caused by injury.¹ Current traditional Indian medicine claims the use of turmeric against biliary disorders, anorexia, coryza, cough, diabetic wounds, hepatic disorders, rheumatic diseases and sinusitis.⁴

Traditional medicine in China uses *Curcuma longa* for diseases which are associated with abdominal pains and icterus.^{3,5} It has been used for centuries in India and elsewhere as a dietary spice and as a herbal medicine for treatment of wounds, jaundice, and rheumatoid arthritis and has been shown as non-toxic.¹

It has been reported to be able to confer significant protection against neurotoxic and genotoxic agents, and therefore is thought to be able to decrease the oxidative damage and inflammation associated with Alzheimer's disease (AD). Among the neurodegenerative diseases AD represents the most common and devastating disorder of human brain and is due to a multiple cascade, including crucial events such as oxidative damage and chronic-inflammation caused by amyloid- β aggregation.⁶⁻¹⁰ In this connection, it has strongly been suggested that antioxidant, as well as anti-inflammatory or combined anti-oxidant/NSAIDs therapies may prove useful for AD prevention or treatment.¹¹ After extensive research in the last years NSAIDs in particular have entered the therapeutic practice^{6, 12, 13} while antioxidants are currently being evaluated in order to elucidate their beneficial properties in decreasing A β -aggregation.¹⁴ The yellow curry spice Curcumin reported to be endowed with both antioxidant and anti-inflammatory properties may substantiate particularly promising for AD treatment. It has also been observed that Curcumin is structurally related to Congo Red, a dye known to bind amyloid- β oligomers and fibrils in vitro.¹⁵ Unlike the negatively charged Congo Red which is both toxic and unable to cross the blood-brain barrier, Curcumin's nature might allow it to enter brain and is not as toxic.¹⁶ Recent reports, moreover, have shown statistics in which the percentage of AD in 80 years old population in India is only a quarter compared to that in the US.¹⁷

The idea was to compile literature relating to the most interesting derivatives of Curcumin, the synthesis of the compound and its derivatives and the influence in the pathogenesis of AD. Therefore a brief overview to the pathogenesis is given as well in chapter 2.

2. ALZHEIMER'S DISEASE

2.1. Pathogenesis of AD

2.1.1. Pathophysiological and Clinical Principles of AD

Alzheimer's disease (AD) is a neurodegenerative disorder presenting the most common form of dementia in the elderly population¹⁸ and can be divided into an early onset type (onset < 60 years) and in the more common late onset type (onset > 60 years).¹⁹ It is currently not possible to predict the onset of AD before the first signs of cognitive decline appear.⁹

The disease usually begins with gradual failure of recent memory, with preserved alertness and motorfunction and progresses slowly to involve many cognitive spheres and shortens life expectancy, with most patients ultimately dying of secondary respiratory complications.⁹ The aetiological events leading to AD pathogenesis are not clear and although age and the inheritance of predisposing genetic factors appear to play a major role, more recent evidence suggests that the development and progression of AD is subject to a wide variety of both environmental and genetic modifiers.

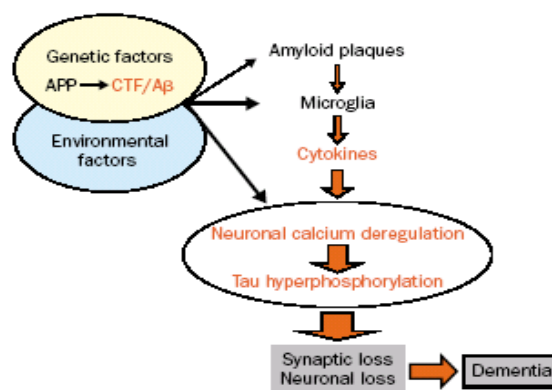


Fig. 1 Schematic representation of the events leading to dementia of the Alzheimer's disease.

There is no single gene that accounts for AD heritability, despite some clues that have been provided by genetic analysis of rare cases of early-onset familial Alzheimer's disease (FAD) which are caused by missense mutations in the amyloid precursor protein (APP) and presenilin-1 and -2 (PS1 and PS2) genes. The vast majority of late-onset AD cases are spontaneous (also called sporadic AD). Mutations and polymorphism in multiple genes are likely to contribute to sporadic AD pathogenesis together with non-genetic factors. There is plenty of evidence that the pathology of AD involves amyloid- β (A β) accumulation which leads to numerous extracellular neuritic (amyloid) plaques and intracellular neurofibrillary tangles (NFTs) in the hippocampus, amygdala and association neocortex, oxidative damage and neuroinflammation.^{9, 16, 20} The inflammatory response promoting and sustaining compounds found in AD brain includes besides the β -amyloid protein the pentraxins C-reactive protein and amyloid P complement proteins, the inflammatory cytokines interleukin-1 (IL-1), interleukin-6 (IL-6) and tumor necrosis factor- α , the protease inhibitors α -2-macroglobulin and α -1-antichymotrypsin and the prostaglandine generating cyclooxygenases COX-1 and COX-2.⁷

The presence of chronic neuroinflammation as in AD also appears in other neurodegenerative disorders including Parkinson's disease (PD) and Creutzfeldt-Jacob disease (CJD).¹² In the last years of intensive research it has been elucidated that the specific accumulation of neurotoxic amyloid- β (A β) derived from the proteolysis of APP in the central nervous system (CNS) appears to represent a major pathological step in the progression of AD.²¹ Since A β accumulation in the form of senile plaques has been recognized as one of the major potential causes of AD pathology,²² β -amyloid protein, its precursor and metabolism have become the subjects of intensive studies to find new targets for novel therapeutic methods and prevention of AD.

Currently the treatment of the dementing symptoms of AD is restricted to two types of drugs: acetylcholinesterase inhibitors (for example Donezipil) and the *N*-methyl-D-aspartate receptor complex antagonist Memantine.⁹ As these drugs only temporarily relieve some symptoms for a period of time and do not address the pathologic process or substantially slow clinical progression there is a need to develop new potential disease-modifying treatments.

2.1.2. The AAP and its Cleavage

As mentioned a major cause of AD is thought to be the A β -accumulation and the resulting formation of amyloid plaques and neurofibrillary tangles. Accordingly, in the last years research efforts have been directed to the elucidation of crucial aspects of the production and the deposition of the β -amyloid peptide. A β is derived from the larger amyloid precursor protein (APP)²³⁻²⁵ which is encoded by a single gene on chromosome 21.^{26, 27} APP presents an ubiquitously expressed type 1 membrane glycoprotein²⁷ with a single transmembrane domain, a large extracellular domain (ectodomain, N-terminal region) and a short cytoplasmic (C-terminal) tail (Fig. 2).^{25, 26}

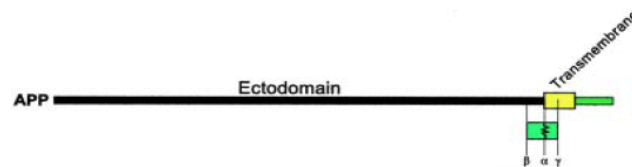


Fig. 2 The Amyloid precursor protein.

The function of APP is unknown, but roles in maintaining metal homeostasis, cell viability and blood hemostasis have been confirmed.²⁸ By alternative splicing of a 19-exon gene - which represents APP - multiple isoforms are obtained ranging from 563 to 770 amino acid residues.²⁶ A β is derived from the region of the protein encoded by parts of exons 16 and 17 and the predominant transcript isoform APP770 containing exon 7 domain which also contains a serine protease inhibitor called Kunitz proteinase inhibitor (KPI).²⁰ Cleavage of APP is complex and can occur via several different routes, which have been categorized as either amyloidogenic or nonamyloidogenic pathway. In particular it is processed by three proteases designated as α -, β - and γ -secretase.^{24, 25} Cleavage by either α - or β -secretases produces large soluble N-terminal fragments (sAPP α and sAPP β), C83 and C99 membrane-bound C-terminal fragments, respectively and further cleavage by γ -secretase releases non pathogenic p3 peptide, a truncated A β fragment²⁷ and A β (Fig. 3).²⁰

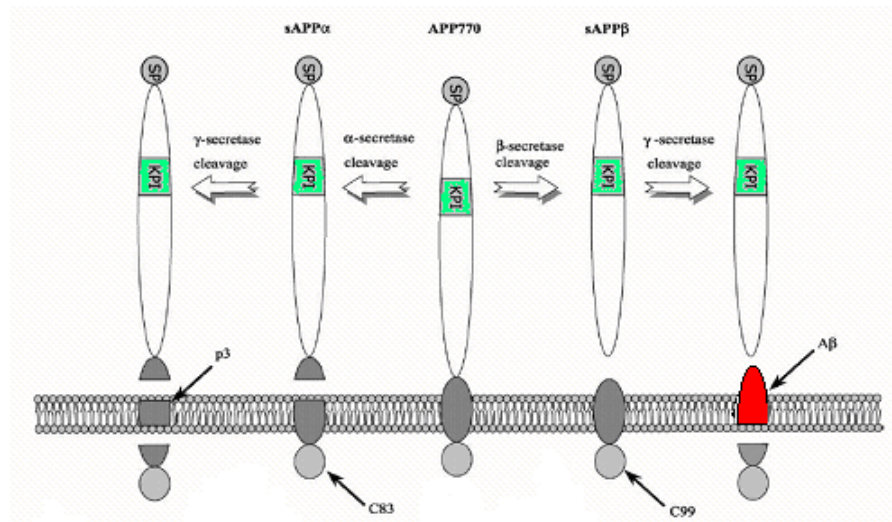


Fig. 3 Schematic diagrams of the amyloid precursor protein (APP) and its metabolic derivatives. The largest APP isoform is APP770 (shown centre) with a large extracellular domain and short intracellular segment. The drawing is not to scale; the transmembrane region has been magnified to permit clear visualisation of the cleavage events. SP represents the signal peptide and KPI the Kunitz proteinase inhibitor domain.

α -Secretase cleaves APP within the A β region²⁰ and is usually considered as non-amyloidogenic cleavage because it does not produce A β .²⁶ However, its product p3 is sometimes found in plaques. Some representatives of the family of disintegrin metalloproteases (or adamalysins) have been postulated as α -secretase candidates like TACE (also called tumor necrosis factor alpha (TNF- α)), ADAM-10 and MDC-9.^{20, 23, 26} All members display a common domain organisation and possess four potential functions: cell fusion, cell adhesion, intracellular signaling and proteolysis.²⁰

β -Secretase corresponds to the alternative amyloidogenic pathway and cleaves APP at the β -cleaving site at the APP ectodomain generating membrane bound C-terminal fragments^{20, 23} and A β peptide is left anchored in the membrane with a free N-terminus.¹¹⁶ Two aspartyl protease homologues BACE and BACE-2 were recently identified to cleave APP at the β -secretase sites.^{20, 29}

γ -Secretase cleavage represents the critical step because fragments resulting from α - and β -secretase cleavage remain anchored in the membrane and may become degraded or further processed by γ -secretase²⁶ to form p3 (non toxic) and A β (including A β 42, that is associated with AD pathogenesis).²⁰ The integrity of γ -secretase remains elucidative but it has been evaluated that the presenilins PS1

and PS2, which were initially discovered through genetic analysis of early-onset FAD, are two candidates for catalytic components of γ -secretase.^{20, 26, 30} Recent studies have shown that γ -secretase is a more complex entity than presenilin alone and that nicastrin interacts strongly with the presenilins, co-immunoprecipitates with APP C-terminal fragments in the context of presenilin FAD mutations and deletion of specific regions within the nicastrin molecule. This leads to significantly reduced A β -production.²⁰ The overall structure of α -, β -, γ -secretase-like proteins are depicted in Fig. 4.

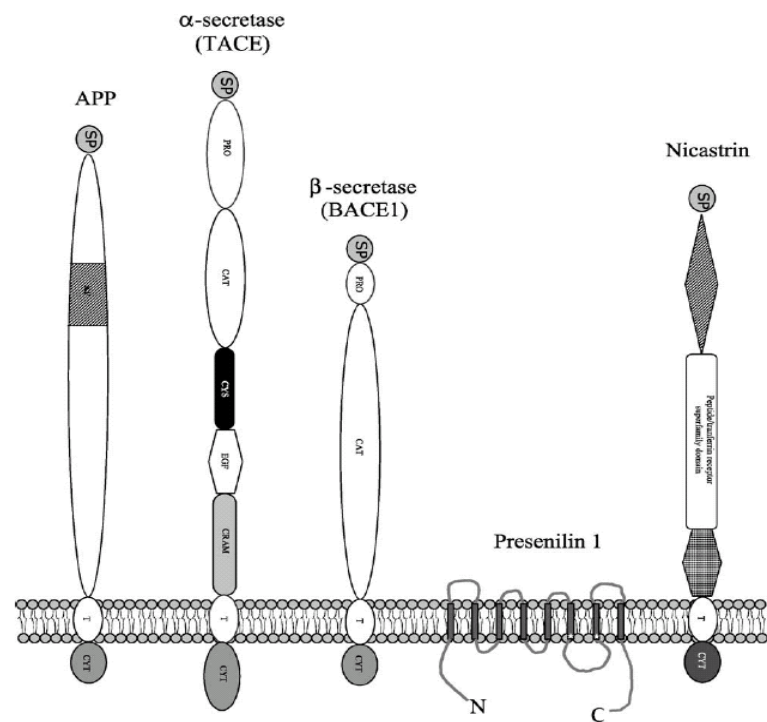


Fig. 4 Domain organisation of membrane bound APP, one of the α -secretases (TACE), β -secretase (BACE1), presenilin-1 and nicastrin. The two aspartic acid residues in transmembrane domains 6 and 7 of PS1 are shown as stars. Abbreviations: SP: signal peptide, KPI: Kunitz proteinase inhibitor domain, CAT: catalytic domain, CYS: cysteine rich domain, PRO: prodomain (also called inhibitor domain), CYT: cytoplasmic domain, CRAM: crambin-like domain, T: transmembrane region.²⁰

Despite the recent findings the physiological functions of APP are poorly understood.

2.1.3. The Biology of β -Amyloid

A β is a peptide of 39-43 amino acids,²³ that is heterogeneous at both its amino and its carboxyl termini,^{19, 31} attributed to differences in local tissue processing and A β is also found at low concentrations as a normal constituent of biological fluids.³¹ The C-terminus determines the rate of fibril formation and the N-terminus is necessary for polymerization.²⁵ The cleavage of APP gives rise to mainly two different types of A β : the soluble sA β (predominantly A β 1-40) and the A β (A β 1-42) which is found in amyloid deposits.¹⁹ The length of A β -peptide dramatically influences its physical properties and it has been evaluated that the longer forms which end at position 42 (A β 42) and 43 are more amyloidogenic²⁵ because of the presence of Leu41 and Val42 at the C-terminus,³² which also has been suggested to be particularly important for the initiation of A β deposits and cytotoxicity.¹⁹

The difference between sA β and A β is caused by many different factors including mutations. Soluble A β is predominantly a random coil and a α -helical folded peptide,²⁵ whereas the insoluble A β has a predominantly toxic β -sheet content, consisting of two β -sheet domains spanning residues 10-25 and 30-42.³² This β -sheet conformation is associated with its relative insolubility, its resistance to degradation^{19, 25} and its ability to form fibrillar or amorphous deposits which compromise neuritic and diffuse plaques, respectively.²⁵ Fibrillogenesis can be modulated by lipoproteins, glycosaminoglycans and metals. The apolipoprotein E molecule (apoE2, E3, E4), which bind to A β and ApoE4, is a another risk factor, which lowers age of onset by presumably altering A β aggregation.²⁸

A β is also able to stimulate inflammatory response from microglia, to inhibit neurite outgrowth, activate protein phosphorylation and is neurotoxic.²⁸ It was shown that low concentrations of A β are neurotrophic³³ leading to the suggestion that disruptions of A β generation, as occurs in AD, may be replacing a trophic activity with a toxic one.²⁵

2.1.4. The Role of A β and Other Associated Factors in AD

The pathogenesis of AD is complex and is driven by both environmental and genetic factors. Although most of the cases are sporadic with an obscure etiology, some of the disease are inherited and several genes were found to be clearly implicated in familial Alzheimer's disease (FAD).¹⁰

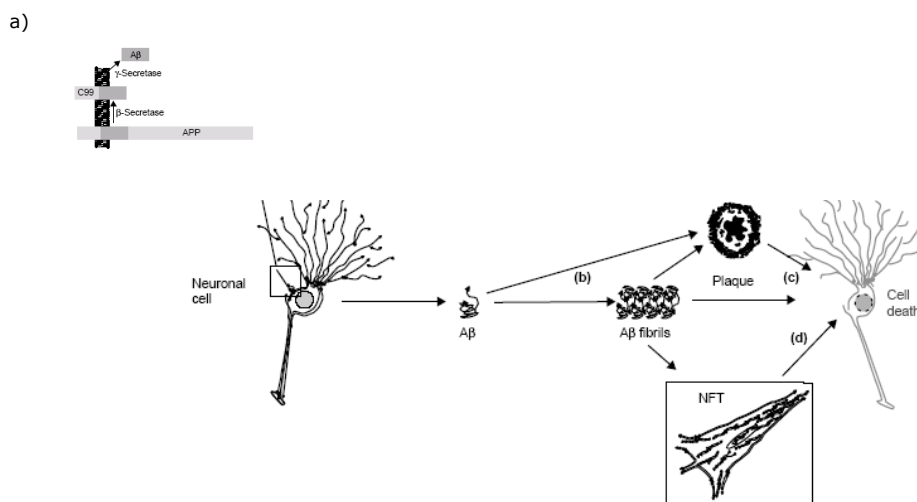
To the early onset inherited forms three genetic loci are linked and found in the genes encoding for the APP, presenilin-1 (PS1) and presenilin-2 (PS2) and a genetic risk factor for developing late-onset dementia represents the inheritance of the ϵ 4 allele of apolipoprotein E.³⁴

Chromosome	Gene Defect	Phenotype
21	<i>β-Amyloid-precursor-protein mutations</i>	<i>Increased production of all β-amyloid proteins or β-amyloid protein 42</i>
19	<i>ApolipoproteinE4 polymorphism</i>	<i>Increased density of β-amyloid plaques and vascular deposits</i>
14	<i>Presenilin 1 mutations</i>	<i>Increased production of β-amyloid protein 42</i>
1	<i>Presenilin 2 mutations</i>	<i>Increased production of β-amyloid protein 42</i>

Table 1 Genetic factors predisposing to AD: Relationship to the β -Amyloid phenotype.⁹

From the neuropathological perspective, the major hallmarks of AD is the presence of two characteristic lesions: the senile amyloid plaques (SP)³⁵ – extracellular insoluble congophilic protein aggregates composed of amyloid β ⁸ - and neurofibrillary tangles (NFTs)³⁵ –intracellular lesions consisting of paired helical filaments (PHFs) from hyperphosphorylated cytoskeletal protein tau.⁸ It has been a longstanding debate whether they are essential components in the pathogenetic process or only markers of nerve degeneration, but since it has been evaluated that autosomal dominant mutations in the gene encoding the APP were causal for

early-onset familial AD (FAD), it is strongly suggested that A β plays a primary role in the pathogenesis.⁸ As mentioned currently 4 genes in which mutations or polymorphisms cause Alzheimer's disease have been confirmed (shown in table 1) and additional candidate genes await confirmation. Thus because the apolipoproteinE4 allele is a normal genetic polymorphism that confers an increased risk (but not certainty) of AD, it is indicated that genetic factors predisposing to the disease do not need to occur in a dominant pattern and may thus be hard to recognize in genetic epidemiological studies.⁹ All identified mutations cause increased formation of total A β or, specifically, the more fibrillogenic A β 1-42³⁵ and lead to excessive accumulation of A β 42.¹⁰ Therefore, they support the amyloid cascade hypothesis, which is formed by the basis that APP participate in neuronal cell death in AD forms.³⁵ Besides all debates A β is proposed as central trigger of pathological changes as synapse loss, activation of inflammatory process, the induction of neurofibrillary changes leading to formation of PHF and ultimately neuronal death as shown in scheme 1.⁸



Scheme 1 Illustration of the amyloid cascade hypothesis. **(a)** After processing of APP by β - and γ -secretase, A β is secreted and **(b)** aggregates into fibrils that deposit in senile plaques. Fibrils and plaques can induce neurotoxicity.⁸

Other evidences for the amyloid hypothesis derive from numerous studies. It is proposed that additional factors may be as important as A β in the pathogenesis of AD and that the cascade hypothesis is an oversimplification.¹⁹ Nevertheless the amyloid theory remains still controversial because of the weak correlation between the amyloid load and several parameters of neurological dysfunction.¹⁰

However, it is widely believed that the progressive accumulation of A β -aggregates

is fundamental to the initial development of neurodegenerative pathology and triggers a cascade of events as neurotoxicity, oxidative damage and inflammation that contribute to the progression of AD.^{16, 34} Nevertheless it remains elucidative how the senile plaques and neurofibrillar tangles are built and the influence of the metabolites and mutations found so far in the development of the pathogenesis. Furthermore, which microglial products, alone or combinatorially, mediate neurotoxic response, to find targets for inhibition and novel therapeutic methods.

2.2. Therapeutical Approaches in AD

2.2.1. *Nonsteroidal Anti-inflammatory Drugs (NSAIDs) in AD*

Before discussing the potencies of Curcumin in AD the influence of NSAIDs in the pathogenesis should be avowed. On one hand because Curcumin is also thought to exhibit NSAIDs properties and on the other hand because NSAIDs are currently used as therapeutic methods in AD.

As we know, the major cause of neurotoxicity in AD pathology is represented by the deposition of β -amyloid into insoluble plaques, and fibrillar peptide formation and deposition caused by mutations in the gene encoding the APP, and that the presenilin genes are key events in the pathophysiology.^{9, 34, 36} Now we are focusing on the other hallmark of AD, the inflammatory event that occurs in the brain as a response to the extracellular deposition of A β -fibrils.³⁷ The primary immune effector cell responsible for this inflammatory part of the disease is the microglial cell.¹²⁸ The microglial respond to the deposited amyloid fibrils and plaques exhibits enhanced expression of a variety of cell surface proteins, and the consequence of microglial activation is a fulminating process resulting in the astrogliosis and neuronal cell death that also characterizes the pathology of the AD brain.³⁸⁻⁴⁰ The activated microglia secretes a diverse range of acute-phase proteins and complement components, notable is the secretion and synthesis of the proinflammatory cytokines interleukin-1 β (IL-1 β), IL-6, and tumor necrosis factor α (TNF α) and the chemokine macrophage chemotactic protein-1. This appearance of inflammatory products in the AD brain has been interpreted as evidence of a chronic inflammatory component in the

disease process, and has led to studies exploring the effect of anti-inflammatory drug treatment on the incidence of AD-related dementia.⁴¹ This concept is favoured by epidemiological studies of patients with arthritis, where it was shown that there is a reduced risk of AD when consuming NSAIDs for treatment³⁹ and numerous studies support the finding that chronic intake of nonsteroidal anti-inflammatory drugs (NSAIDs) can decrease risk for AD by more than 50%.^{13, 38}

2.2.1.1. *Nonsteroidal Anti-inflammatory Drugs*

Non-steroidal anti-inflammatory drugs (NSAIDs) are a group of pharmacologically related compounds with analgetic, antipyretic and anti-inflammatory properties. Their shared characteristics is inhibition of cyclooxygenase (COX) enzymes. In low to moderate doses NSAIDs act centrally to inhibit fever and pain. Higher doses inhibit local inflammation by blocking the induction of interleukin-1 (IL-1), interleukin-1 β (IL- β) and possibly IL-6 via pathways that rely on prostaglandin synthesis.⁷

2.2.1.2. *The Influence via Cyclooxygenases*

The anti-inflammatory actions of NSAIDs and their therapeutic benefit in treating AD have been attributed to the ability of these drugs to inhibit the cyclooxygenases which are the rate limiting enzymes responsible for the conversion of arachidonic acid into inflammatory mediators, including prostaglandin E₂ (PGE₂).³⁸ Two enzyme isoforms, COX-1 and COX-2, have been identified in the brain and periphery. These two forms are encoded by distinct genes on different chromosomes, but share approximately 80% sequence homology and have similar catalytic activity. COX-1 is constitutively expressed by many cell types, while COX-2 is induced at sites of local inflammation.⁷ Therapeutic benefits of NSAIDs are typically observed at doses much higher than those required to inhibit the cyclooxygenases, that suggested that there are other targets of NSAIDs' action,⁴² and also because it became apparent that only

a subset of NSAIDs lower A β 42 levels, whereas all NSAIDs by definition inhibit COX.¹³ Furthermore, it was demonstrated that the effects of non-selective NSAIDs differ from that of COX-selective compounds, and also COX-1 and COX-2-selective compounds differently affect A β 40- and A β 42 secretion. The elucidation of the acting mechanism of NSAIDs is of great interest also because prolonged treatment with existing NSAIDs results in severe side effects. Most prominently in the gastrointestinal tract, that occur primarily as a consequence of the inhibition of COX-1 activity³⁸ and actually the benefits of NSAIDs in AD are still a matter of debate. To date two strategies to overcome the gastrointestinal side-effects have been identified. One is the use of the R-enantiomer of flurbiprofen, which maintain the A β -lowering properties without causing gastric damage because of lacking COX-inhibition⁴³ and the other is based on using the NO-releasing derivatives of flurbiprofen which have been shown to reduce brain inflammation and A β -burden.⁴⁴ Nevertheless further biological and clinical investigations should be done to clarify the mechanism of NSAIDs in AD and whether the mentioned strategies will lead to innovative and effective treatment options.

2.2.1.3. Influence via PPAR γ

Another study provided evidence that the mechanism by which NSAIDs intervene in disease progression could be their capacity to act as agonist for PPAR γ and alter, at the transcriptional level, microglial production of proinflammatory products. The PPARs are a class of nuclear receptor superfamily members called termed peroxisome proliferator-activated receptors (PPAR). They are lipid-activated DNA-binding proteins structurally related to the steroid and retinoic acid receptor families and are associated with sequence-specific promoter elements and transcriptionally regulate gene expression after ligand binding.⁴⁵ There are three isoforms (PPAR α , γ and δ) which are differently expressed. The natural ligands for this receptor family are fatty acids and metabolites³⁸ and has well described roles in adipocytes and serves to regulate enzymes of lipid metabolism in these cells.³⁸ It was found that the PPAR γ isoform is expressed in monocytes and macrophages in which its principal action is to suppress the expression of proinflammatory cytokines IL1- β ,

TNF α , and IL-6 and other proinflammatory products. The natural ligand of PPAR γ is the J class prostaglandine PGJ2 (15d-PGJ2) and its immediate metabolites and its activation affects negatively the macrophage activation and cytokine expression by antagonizing the activity of the transcription factors NF κ B, AP-1, and STAT proteins.⁴⁵ The PPAR γ isoform can also be activated by indomethacin and other NSAIDs⁴² as the antidiabetic drugs of thiazolidinedione class, as well as the naturally occurring fatty acid docosahexaenoic acid (DHA).³⁸ It was reported that PPAR γ agonists act to inhibit the production of proinflammatory and neurotoxic products produced by β -amyloid (A β)-stimulated microglial cells and monocytes.³⁸ It was noted that therapeutically efficacious doses of NSAIDs are achieved at greater concentrations than those required to inhibit COX activity⁴² and that Aspirin, a potent COX inhibitor, does not reduce risk of AD. These findings led to the suggestion that NSAIDs also act via another mechanism, and particular in AD the potency of these drugs is attributed to their ability to act as PPAR γ agonist rather than to inhibit COX activity.¹²⁸ Because PPAR γ agonists are little or non toxic and exhibit a substantial bioavailability after oral administration these drugs would offer better agents in treating or maybe preventing AD than COX inhibition.³⁸

2.2.1.4. Via Direct Modulation of γ -Secretase Activity

More recently NSAIDs were suggested to represent a class of compounds which lower A β 42 production by direct modulation of γ -secretase activity or the enzyme substrate, *i.e.* APP.¹³ In the study of Weggen et al.¹³ it was shown that some NSAIDs which on the one hand do not show the same subtle switch in AB cleavage to produce shorter AB peptides than the molecules which were proposed to be γ -secretase inhibitors, on the other hand exhibit features that are in common with these previous evaluated γ -secretase inhibitors. First, NSAIDs lower A β 42 both in cell-based assays and in vitro, and notable regarded to this NSAIDs inhibited A β 42 but not A β 40 in an in vitro γ -secretase assay and second, their ability to reduce A β 42 is altered by PS and APP mutations. The primary evaluated γ -secretase inhibitors cleave also substrates such as Notch and ErbB-4 and it is unclear whether inhibition of γ -secretase through these molecules can be used clinically without causing

serious side effects.⁴⁶ Considering that NSAIDs which specially inhibit A β 42 production do not appear to affect Notch processing and as mentioned above only some NSAIDs inhibit A β -formation, complete elucidation of the exact molecular mechanism by which NSAIDs lower A β generation represents an interesting aspect for developing novel A β 42-specific γ -secretase inhibitors.¹³

However, also other proposed mechanisms by which NSAIDs are able to influence A β induced neurotoxicity were discussed, but further investigations are required to explore the effects of NSAIDs and their mechanisms in neuronal cells.

2.2.2. Dyes as Ligands for Attacking Amyloid ?

Various diseases including Alzheimer's disease are characterized by proteinaceous deposits in the affected organs which contain several common elements, among them amyloid fibrils.⁴⁷ Besides the specificity for each disease these amyloid fibrils have remarkably similar properties as they all tend to be long (>1 μ m), thin (10 – 20 nm), straight and unbranching. Their internal structure is largely a cross β -sheet and the strands which make up the β -sheet are arranged perpendicular to the long axis of the fibrils.⁴⁸

Various approaches in trying to inhibit the production and reduce the extent of accumulation of fibrillar A β in the brain are currently being evaluated as potential therapies for AD.⁴⁹⁻⁵⁴ It is therefore of great interest to develop ligands that specifically bind fibrillar A β aggregates. Since extracellular senile plaques (SPs) are accessible targets, these new ligands could be used as in vivo diagnostic tools and as probes to visualize the progressive deposition of A β in studies of AD amyloidogenesis in living patients, and therefore maybe potential targets for prevention and cure.⁵⁵ It is widely accepted that A β is only toxic when it is aggregated and like all amyloid fibrils, A β fibrils present two characteristic features. First, amyloid deposits observed in tissue sections appear orange under the light microscope. Second, amyloid deposits stained with Congo Red (CR) display a green birefringence when viewed between crossed polarizers in a light microscope. This birefringence is the result of the ordered structure of the amyloid fibrils, which have

multiple dye-binding sites with orientation related symmetry.⁵⁶ Consequently, it was tried to evaluate if some of these dyes which are used to visualize amyloid plaques have also the possibility to inhibit or decrease the fibrillation.

2.2.2.1. Congo Red

Congo Red (CR) is a hydrophilic dye that has an absorption spectrum that changes in the presence of proteins with a β -pleated sheet conformation.⁵⁶ It is widely used as a histological dye for staining amyloid¹⁶³ and in addition to this use as a diagnostic agent for amyloidosis CR has been demonstrated to inhibit pathological aggregation of the amyloid β -peptide.⁵⁸ Since this finding experiments were aimed at the elucidation of the molecular mechanism of the CR - amyloid interactions and other structurally similar dyes and derivatives were tested for their properties in binding to amyloid fibrils as well. This should lead to new diagnostic agents with higher amyloid specificity and anti-amyloidosis drugs that inhibit amyloid formation and/or solubilize already deposited amyloid fibrils.⁵⁷ The interaction of CR with A β became also of interest as it was shown that CR when added after A β , reverses the A β -induced effect. This effect could not be explained by the inhibition of A β fibril formation and therefore revealed a new aspect of the interaction with A β .⁵⁹ Several binding assays of CR to polypeptides with known secondary structures indicated that an extensive β -sheet structure was necessary for CR to bind to polypeptides.⁶⁰ The affinity of CR for amyloid was also proposed to depend on the structure of CR: the two aminosubstituted naphthalenesulfonic acid moieties connected by azo linkages to a central biphenyl group (Fig. 5) and modifications of the naphthalene ring largely effects the affinity for amyloid fibrils. Thus it was suggested that the conjugated rings as well as the sulfonic group are involved in the interaction with amyloid.⁵⁷

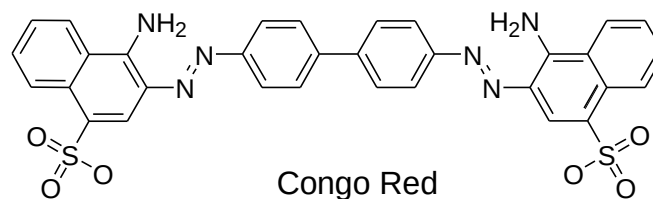


Fig. 5 The structure of Congo Red.⁵⁷

The first model of CR binding to fibrillar β -amyloid was developed by Klunk et al. in 1994 which tried to explain the relative affinities of various CR congeners for the β -amyloid fibril considering that CR does not bind to a single β -amyloid peptide but requires the formation of a β -sheet containing fibril and CR binds with its long axis parallel to the fibril axis.⁶¹ Thus, the fibrils were never defined precisely by x-ray diffraction for the reason of their noncrystalline-insoluble nature another model was evaluated. In that model CR was co-crystallized with a protein showing an internal β -sheet structure. The crystal coordinates of CR bound into the internal β -sheet in dimeric insulin were used,¹⁵ because of the high amino acid similarity of the porcine insulin CR binding site and the residues 15 – 23 (QKLVFFAED) of β -amyloid (Fig.6).

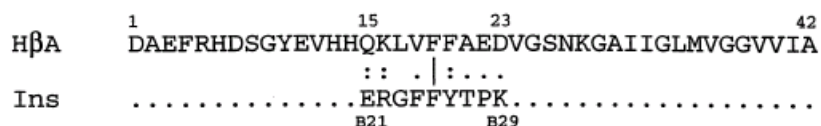


Fig. 6 Sequence alignment of human β -Amyloid peptide 1-42 with porcine insulin β -strand.¹⁵

The different conceptions of the alignment of CR to the peptide is demonstrated in Fig. 7. (a) is the perpendicular alignment proposed by Klunk et al.¹⁶⁷ and (b) shows the parallel alignment of CR to the peptide which was postulated by Carter and Chou.¹⁵

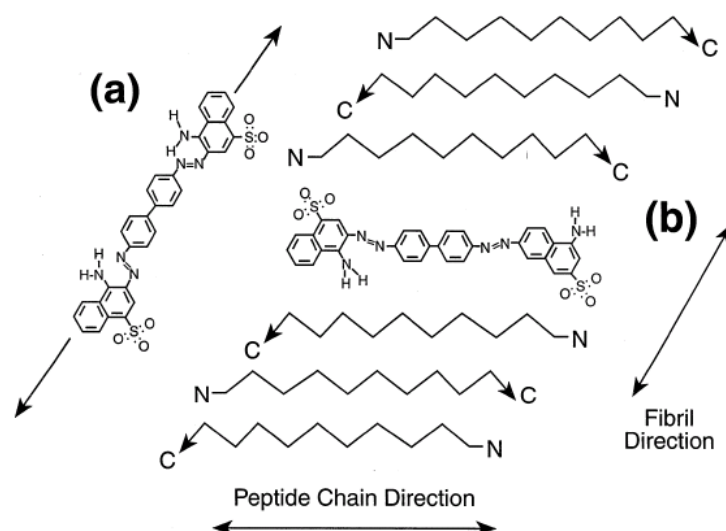


Fig. 7 Model of alternative CR binding orientation in Alzheimer's β -amyloid fibril.¹⁵

In Fig. 8a the orientation of the CR molecule in the dimeric insulin structure is shown and when the 9 amino acid peptide from insulin, which forms the pseudo β -sheet, is substituted with the similar 9 amino acids from human β -amyloid peptide (amino acids 15-23) and subjected to energy minimization, structure 8b is obtained and the similarity in these figures is evident.¹⁵

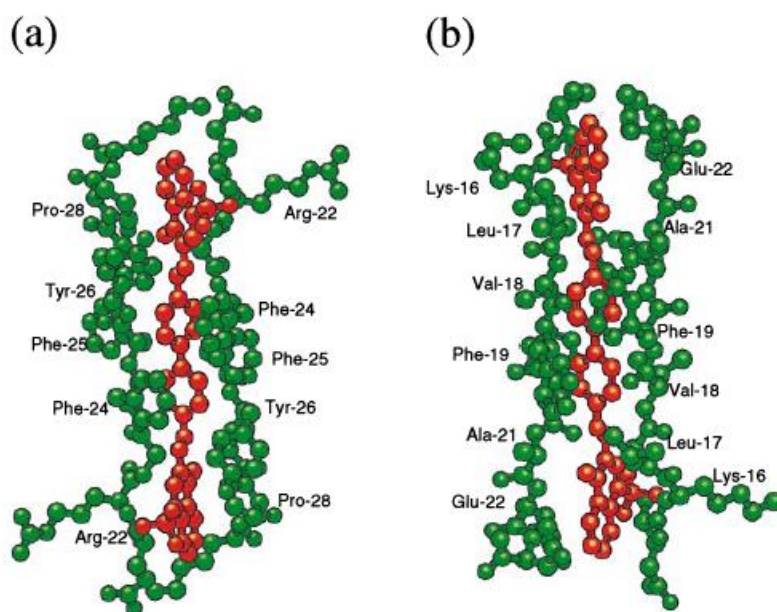


Fig. 8 Ball and Stick model of both, porcine insulin β -sheet CR binding site and the model of the proposed structure of CR binding to β -amyloid antiparallel β -sheet strands, res. 15 – 23.¹⁵

Phenylalanine-24 (Phe24) in the insuline peptide seems to interact with the CR ring the same way as Phe19 in the β -amyloid chain. In Fig. 9 demonstrating a line drawing format of energy minimized structure the common features are as well obvious.

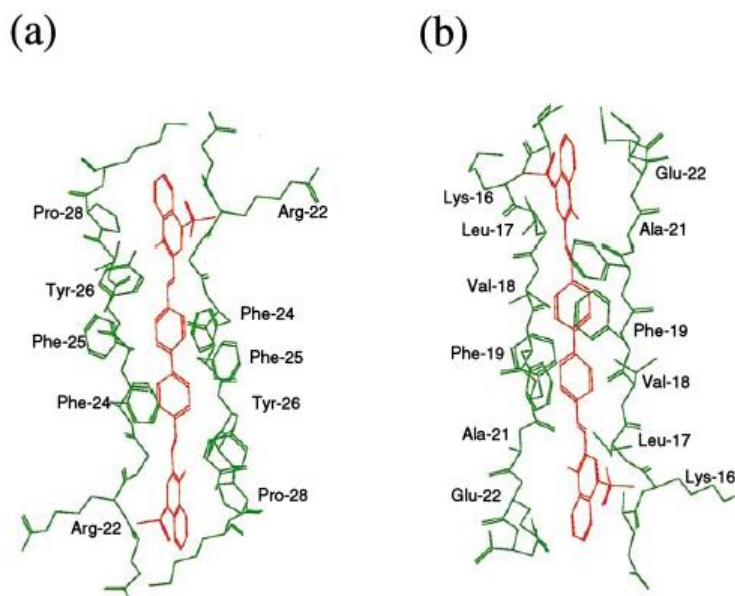


Fig. 9 Line drawing model of the insuline peptide.¹⁵

They replaced the 15 – 23 residue in antiparallel conformation without CR and observed significant changes in the structures. The suggested model presents a very possible model of CR binding to a simple dimeric form of β -amyloid that plausibly is a precursor to the fully formed fibril that may explain the effects of CR binding to β -amyloid fibrils.¹⁵ In that work it was as well proposed that Phe19, due to its similarity to Phe24 in porcine insulin and the observation that substitution of Phe19 through threonine (Thr) abolish plaque-forming competence is a key in determining the properties of the fibril.⁶² The interaction of CR with performed fibrils was also thought to take place with those antiparallel strands that participate in the formation of the surface and ends of the fibrils. Therefore, CR may disturb the interaction of the fibril with the plasma membrane, which would explain the decrease of the A β -fibril-CR complex and its effectiveness in both preventing and reversing the effects of A β .^{15, 59} It was also shown that CR did not decrease the cellular uptake of A β and did not increase the cellular A β secretion. Furthermore, it was indicated that CR could not account by itself for the striking rise in A β monomer and no evidence of a cell-associated A β -degrading protease inhibitable by CR was found. Regarding all mechanisms of the effects of CR, it was speculated that CR binds A β monomers and

prevents its interaction with other monomers – as competitive antagonist for A β ⁵⁹ – and/or works together with other factors released by the cells that are capable of promoting A β polymerization.⁶³ Through explorations where the binding mode of CR to Alzheimer's A β peptide by UV Raman spectroscopy was examined, it was observed that the intensity of the UV Raman bands of CR significantly increases upon binding to A β . This spectral change is in line with that observed when the average molecular planarity was increased by crystallization and opposite to that observed when the planar conformation of the biphenyl group is inhibited by methylation. Therefore it was suggested that in the CR-A β complex, the biphenyl conformation may be closer to planar than in the unbound CR, and the torsional mobility of the biphenyl group may be required in approaching the binding site through the mesh of β -sheet and this mobility should also be retained in new derivatives.⁵⁷ Another approach is based on highly conjugated chrysamine-G (CG) (Fig 10) but as both CG and CR are too large (molecular weight > 700), making them unlikely candidates for penetrating the intact blood-brain barrier, and therefore, they are not suitable as imaging agents for detecting fibrillar A β aggregates in patients with AD.⁵⁵ Potential ligands for detecting A β aggregates in the living brain must cross the intact blood-brain barrier. In order to follow this approach the two derivatives X-34 and BSB (Fig. 10) were designed and thought to exhibit strong potencies.³²

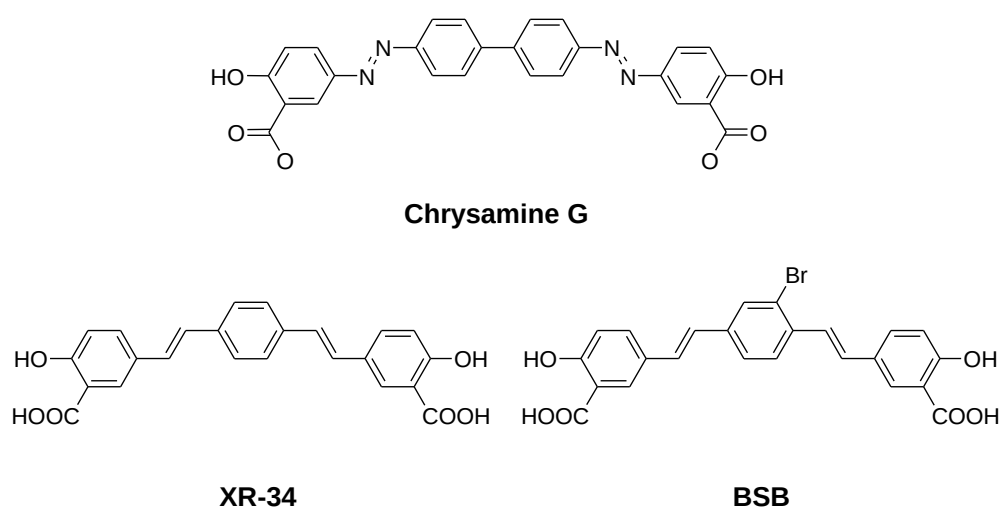


Fig. 10 Structures of CG, XR-34, BSB.³²

XR-34 contains instead of the diazo group a simple vinyl group and has several advantages over CR and CG because it is smaller, therefore may penetrate intact blood brain barrier more readily. The vinyl group improves the in vitro and in vivo stability. BSB was shown to bind specifically to A β in vitro and specifically to A β (1-42) aggregates,⁵⁵ labels A β plaques in the sections from AD brains, cross the blood brain barrier in Tg mouse models of amyloidosis and labels senile plaques with a sensitivity and specificity equal to that of CR.³²

2.2.2.2. Benzothiazoles Thioflavin S and T and Styrylbenzenes

Research also concentrated on other dyes in purpose to evaluate new small ligands that specifically bind fibrillar A β aggregates and to improve brain ligands with a smaller molecular size and increased lipophilicity than CR and CryG. To generate ligands with those properties it was concentrated on highly conjugated thioflavins S and T, which are commonly used as dyes for staining the A β aggregates in the AD brain.⁶⁴ These compounds are known to bind amyloid specifically. They are based on benzothiazole which is relatively small in molecular size. However, the thioflavins contain an ionic quaternary amine, which is permanently charged and unfavorable for brain uptake. Therefore it was reported that small molecule-based radioiodinated ligands, showing selective binding to A β aggregates, cross the intact blood-brain barrier by simple diffusion. Four novel ligands showing preferential labeling of amyloid aggregates of A β (1-40) and A β (1-42) peptides. Two ¹²⁵I-labeled styrylbenzenes, (*E,E*)-1-iodo-2,5-bis(3-hydroxycarbonyl-4-hydroxy)- styrylbenzene (ISB), and (*E,E*)-1-iodo-2,5-bis(3-hydroxycarbonyl-4-methoxy)styrylbenzene (IMSB), and two ¹²⁵I-labeled thioflavins, 2-[4'-(dimethylamino)phenyl]-6-iodobenzothiazole (TZDM), and 2-[4'-(4''-methylpiperazin-1-yl)phenyl]-6-iodobenzothiazole (TZPI), were prepared at a high specific activity (Fig. 11).⁶⁴ Using these four radioiodinated probes with high specific activity, studies of the binding of the ligands to aggregates of A β (1-40) and A β (1-42) peptides in solution were carried out. All of the ligands displayed a saturable binding in vitro and in human brain⁵⁵ and it was provided compelling evidence that there are distinctive and mutually exclusive binding sites on

A β (1-40) and A β (1-42) aggregates for these two series of compounds.

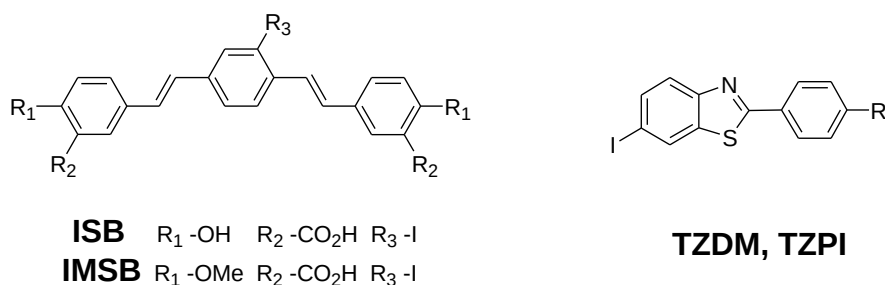


Fig. 11 Synthesized styrylbenzenes and thioflavins.⁶⁴

It is known that if A β (1-40) and A β (1-42) peptides are aggregated, they assemble into a fibrillary coil containing β -sheet structures. It is evident that under the conditions used in that work A β (1-40) and A β (1-42) peptides form aggregates containing two different binding pockets for styrylbenzenes and benzothiazoles (thioflavins). Clearly, there are significant structural differences between these two aggregates, and additionally at least two different binding pockets, possibly more, exist on these aggregates.^{65, 66} Neither detailed structural information was available on the potential binding sites, nor the full extent of functional linkages of these styrylbenzene or benzothiazole binding sites on A β aggregates was known. It has been suggested previously that binding of small molecules to the A β aggregates may prevent further aggregation and reduce their toxicity.^{65, 67} Further testing is warranted to investigate any functional or physiological roles of these binding sites in amyloid plaque (aggregates) formation and cellular toxicity. To test the permeability through the intact bloodbrain barrier, these new agents were injected into untreated mice. The results suggest that these two styrylbenzenes showed relatively low initial permeability on crossing the blood-brain barrier. The potential usefulness of these agents for in vivo imaging after a bolus i.v. injection appears to be limited. The compounds derived from benzothiazole were prepared to improve the brain uptake after an i.v. injection. These compounds are derivatives of thioflavins, but do not contain a quaternary ammonium ion; therefore, they are relatively small in size, neutral, and lipophilic. It was assumed that thioflavins may provide better candidates for further development of the in vivo imaging agents critically important for evaluation of Alzheimer's disease. Nevertheless all of them may be useful as

biomarkers for studies of A β (1-40) as well as A β (1-42) aggregates for detecting amyloid aggregates in the brain by in vivo and in vitro techniques.⁵⁵ Further derivatives of styrylbenzene-, and thioflavin as well as *m*-I-stilbene have been tested and modified to overcome the low brain penetration in a genetically altered transgenic mouse mode for AD.⁶⁸ In that approach it was clearly suggested the suitability of the detection of amyloid deposits in the living brain and if these derivatives can act as ligands in AD patients it may be possible to detect the A β plaques in the living human brains by in vivo imaging techniques. A very recent work proposes a general binding model for amyloid-specific dyes to amyloid fibrils through examinations based on thioflavin T.⁶⁹ Through observation of spherulites which are formed by peptides and proteins under amyloidogenic conditions by confocal microscopy using thioflavin-T as the dye and exciting the dye with polarised light a consistent pattern was noticed.⁷⁰ This phenomenon can only occur if thioflavin-t binds in a specific orientation with respect to the amyloid fibrils, thus the binding orientation can be deduced. Therefore the anisotropic fluorescence emission from amyloid fibril-containing spherulites in the presence of thioflavin-T indicates that the dye binds in a regular fashion to amyloid fibrils. By analyzing the emission intensity as a function of the angle between the plane of polarisation of the exciting laser beam and the orientation of dye molecules, it was shown that the long molecular axis of the dye needs to be parallel to the plane of polarization of the light to be excited. This implies that the dye binds to the fibrils with its long axis parallel to that of the fibrils. As channels are running in the same direction on the β -sheet of the amyloid fibrils they seem likely to act as the binding site of the dye. In Fig. 12 the thioflavin-T together with the diagram of the β -sheet with backbone atoms N, C, C α and the side chain R for one residue is shown.

Thioflavin-T

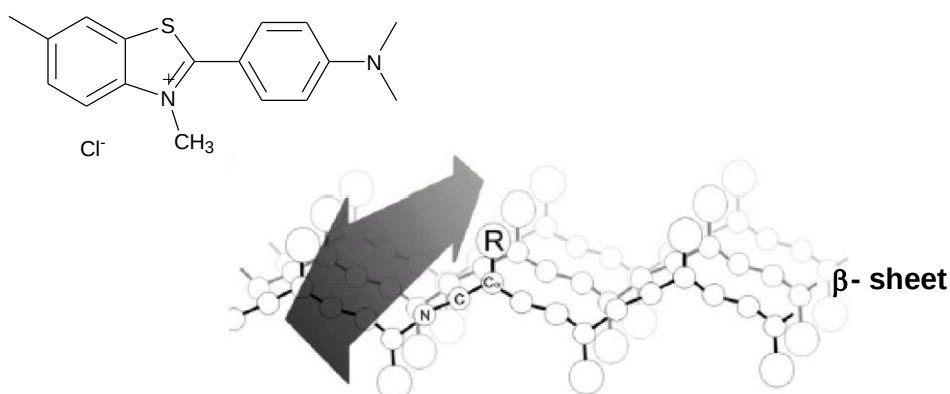


Fig. 12 Thioflavin-T and the diagram of a β -sheet.⁶⁹

This scheme of β -sheet is valid for both parallel and anti-parallel sheets. As part of a fibril the long axis of the fibrils would be perpendicular to the surface of the page and one of the binding channels is indicated with a double headed arrow. Thioflavin-T is therefore thought to bind with its long axis parallel to the long axis of the arrow.⁶⁹

Fig. 13 demonstrates a schematic representation of a protofilament with, arbitrarily, three β -sheets. Individual β -strands are shown by 'zig-zag' lines in black, dark and light grey. Sidechains accessible from the solvent are shown as black circles and point out of the plane of the paper. Note that sidechains on the third, light grey, β -sheet are also accessible from the solvent and point below the plane of the paper (not shown for clarity).

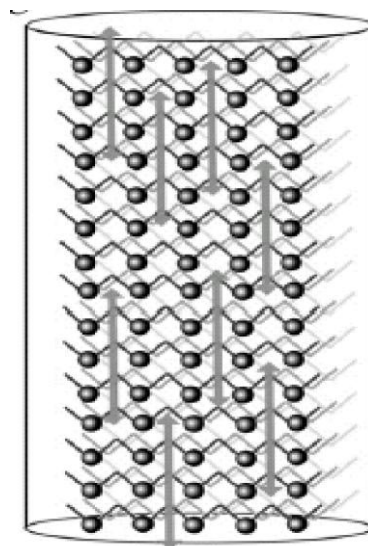


Fig. 13 Schematic representation of a protofilament with 3 β -sheets.⁷¹

Dye molecules are represented by double headed arrows. When several protofilaments form a fibril, some of these surfaces will be obscured, resulting in impeded dye binding in those areas. The steric constraints imposed by this environment are largely unique to amyloid fibrils, offering an explanation for the specificity of structural similar dyes.¹⁷⁵ The understanding will promote aid for developing new derivatives. The dye thioflavin-S has so far not been explored which may be due to the fact that thioflavin-S exists as a mixture and is no pure chemical entity.⁷¹

2.2.2.3. Cyanines

Further intense research in finding new ligands concentrated on Cyanines which have also been used as sensors to examine β -sheet structures in solutions and thin layers. The cationic, conjugated cyanine dye Pinacyanol chloride (PIN), which was proved to be an useful tool in studying the aggregation characteristics of ovalbumin in a β -sheet conformation presents an advantage in comparison to CR.¹⁷⁸ Its spectral changes make it possible to distinguish between aggregated and nonaggregated forms or between α and β conformations and it has to be shown two fold more sensitiv in A β -binding. The interaction between PIN and A β is mainly electrostatic. Inouye et al.⁷³ found that A β has an isoelectric point of 5.5, and at the pH used in the study (7.4) the peptide presents a net negative charge due to the carboxyl groups of aspartic acid and glutamic acid. Hence, the peptide interacted electrostatically with the positive PIN. Such an interaction would be independent of the peptide conformation, and it is the motif by which spectral changes produced by peptides in an α -helix conformation can be observed. However, there is a factor that is related to the β conformation, suggesting that PIN can be used as a probe for fibrillar A β .⁷²

2.2.3. Curcumin and its Properties in A β -Induced Cytotoxicity

Since this subject represents also a main part of the literature research it is more precisely presented in the chapter 4.3 at pages 59 ff.

3. METHODS & CRITERIA FOR THE LITERATURE RESEARCH

Following data bases were used for the literature research:

- SciFinder [<http://www.cas.org/SCIFINDER/>]
- PubMed [<http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?DB=pubmed>]
- Elsevier Science [http://www.elsevier.com/wps/find/journal_browse.cws_home]
- Emeroteca Virtuale [<http://www.caspur.it/sitispecialistici/emerotecavirtuale.html>]
- American Chemical Society [<http://pubs.acs.org/about.html>]
- The New England Journal of Medicine [<http://content.nejm.org/search.dtl>]

Relating to the subject following keywords were used:

- Curcumin
- Curcumin analogues and derivatives
- Biological Activities of Curcumin
- Dyes and Alzheimer's disease
- Synthesis of Curcumin
- Curcumin and Alzheimer's disease

According to the broad field of research on Curcumin a great number of papers have been published so far. A restriction were done with regard to the significance for the present study in the most interesting papers concerning the subject without temporally defined limitations towards the publication year.

The results of the selected papers and conclusions are presented in chapter 4. Results and Discussion.

4. RESULTS OF THE LITERATURE RESEARCH

4.1. Biological Activities of Curcumin

As current Indian medicine claims the use of turmeric in various medical cases, furthermore because of its extensive use in Asian regions for a very long time as household remedy, spice and flavouring, and because it has been shown to be very non-toxic,¹ research has concentrated on evaluating the biological properties of this medicinal used plant, in order to develop new drugs which show less toxicity to humans.³

For the last few decades extensive work has been done to establish the biological activities and pharmacological actions of turmeric and its extracts. Curcumin (diferuloylmethane), the main yellow component of turmeric, has been shown to have a wide spectrum of biological actions. These include its antioxidant, anti-inflammatory, anticarcinogenic, antimutagenic, anticoagulant, antifertility, antidiabetic, antibacterial, antifungal, antiprotozoal, antiviral, antifibrotic, antivenom, antiulcer, hypotensive and hypocholesteremic activities.⁷⁴⁻¹³⁷

4.1.1. Antioxidant Effect

The antioxidant activity of Curcumin was reported as early as 1975. The compound has shown to be a more potent antioxidant than α -tocopherol and acts as a scavenger of oxygen free radicals. Curcumin and its three derivatives (demethoxycurcumin, bisdemethoxycurcumin and diacetylcurcumin) provide a protection of haemoglobin against oxidation at a concentration as low as 0.08mM *in vitro*, whereby diacetylcurcumin possesses the lowest inhibitory activity.⁷⁴⁻⁷⁶ Curcumin can significantly inhibit the generation of reactive oxygen species (ROS) like superoxide anions, H_2O_2 and nitrite radical generation by activated macrophages, which play an important role in inflammation, and it also lowers the production of ROS *in vivo*.^{2,77} Its derivatives, demethoxycurcumin and bisdemethoxycurcumin also have antioxidant effect.⁷⁸ Curcumin exerts powerful inhibitory effect against

H₂O₂-induced damage in human keratinocytes and fibroblasts and in NG 108-15 cells.^{79, 80} It was shown to reduce oxidized proteins in amyloid pathology in Alzheimertransgenic mice⁸¹ and possesses the potency to inhibit lipid peroxidation, which plays a main role in the inflammation of heart diseases and in cancer, in rat liver microsomes, erythrocyte membranes and brain homogenates.⁸² This property is developed by maintaining the activities of antioxidant enzymes like superoxide dismutase, catalase and glutathione peroxidase, which play an important role in the regulation of lipid peroxidation at higher levels.⁸³ Since ROS have been implicated in the development of various pathological conditions, Curcumin has the potential to control these diseases through its potent antioxidant activity.⁸⁴

Contradictory to the above-mentioned antioxidant effect, Curcumin has pro-oxidant activity. Kelly et al.⁸⁵ reported that Curcumin not only failed to prevent single-strand DNA breaks by H₂O₂, but also caused DNA damage. As this damage was prevented by antioxidant α -tocopherol, the pro-oxidant role of Curcumin has been proved. The pro-oxidant activity appears to be mediated through generation of phenoxyl radical of Curcumin by peroxidase–H₂O₂ system, which co-oxidizes cellular glutathione or NADH, accompanied by O₂ uptake to form ROS.⁸⁶ However, the antioxidant mechanism of Curcumin is attributed to its unique conjugated structure, which includes two methoxylated phenols and an enol form of β -diketone.^{87, 88} The structure shows typical radical-trapping ability as a chain-breaking antioxidant, depending upon reaction conditions.⁸⁹

4.1.2. Anti-inflammatory Activity

A great number of papers exist relating the activity of compounds extracted from *C. longa* L. being potent inhibitors of inflammation. These substances can be classified as curcuminoids, analogues of diarylheptanoids. Anti-inflammatory activity can be studied in two different models. In the chronic models (cotton pellet and granuloma pouch) where the inflammation and granulomas are developing during a period of time, indicating the proliferative phase of inflammation, and the acute model, where acute effects of anti-inflammatory agents can be studied, testing their inhibitory action on the development of rat paw edema.³ Srimal and Dhawan have examined

pharmacological actions of Curcumin⁹⁰ and they reported that the compound was effective in acute as well as chronic models of inflammation. The potency was shown as approximately equal to phenylbutazone in the acute model, but it is only half active in the chronic experiments. Namely it was observed to be less toxic than the reference drug (no mortality up to a dose of 2g kg⁻¹). Curcumin was shown to be effective against carrageenin-induced rat paw edema and cotton pellet granuloma models of inflammation in rats.⁹¹ The natural analogues of Curcumin, [feruloyl-(4-hydroxycinnamoyl)-methane] (FHM) and [bis-(4-hydroxycinnamoyl)-methane] (BHM), are also potent anti-inflammatory agents^{1,92} as well as the volatile oil,⁹³ the petroleum ether, alcohol and water extracts of *C. longa* show antiinflammatory effects.⁹⁴ Recently it has been reported that Curcumin stimulates stress-induced expression of stress proteins and may act in a way similar to indomethacin and salicylate.⁹⁵ Curcumin offers anti-inflammatory effect through inhibition of NFκB activation⁹⁶ and has also been shown to reduce the TNF-α-induced expression of the tissue factor gene in bovine aortic-endothelial cells by repressing activation of both AP-1 and NFκB.⁹⁷ The antiinflammatory role of Curcumin is also mediated through downregulation of cyclooxygenase-2 and inducible nitric oxide synthetase through suppression of NFκB activation.⁹⁸ Curcumin enhances wound-healing in diabetic rats and mice⁹⁹ and in H₂O₂-induced damage in human keratinocytes and fibroblasts.⁷⁹ It was also demonstrated as an inhibitor of leucotriene formation in rat peritoneal polymorphonuclear neutrophils (PMNL), with an EC₅₀ of 27 x 10⁻⁷ M, in contrast, the hydrocortisone did not show any effect.¹⁰⁰

4.1.3. Anticarcinogenic Effect – Induction of Apoptosis

Curcumin acts as a potent anticarcinogenic compound. Among various mechanisms, induction of apoptosis plays an important role in its anticarcinogenic effect. Curcumin induces apoptosis and inhibits cell-cycle progression, both of which are instrumental in preventing cancerous cell growth in rat aortic smooth muscle cells.¹⁰¹ The antiproliferative effect is mediated partly through inhibition of protein tyrosine kinase and c-myc mRNA expression and the apoptotic effect may partly be mediated through inhibition of protein tyrosine kinase, protein kinase C, c-myc mRNA

expression and bcl-2 mRNA expression.¹⁰¹ Curcumin induces apoptotic cell death by DNA-damage in human cancer cell lines, TK-10, MCF-7 and UACC-62 by acting as topoisomerase II poison.¹⁰² It causes rapid decrease in mitochondrial membrane potential and release of cytochrome *c* to activate caspase 9 and caspase 3 for apoptotic cell death.¹⁰³ Recently, an interesting observation was made regarding curcumin-induced apoptosis in human colon cancer cell and role of heat shock proteins (hsp) thereon. In that study, SW480 cells were transfected with hsp 70 cDNA in either the sense or antisense orientation and stable clones were selected and tested for their sensitivity to Curcumin. Curcumin was found to be ineffective to cause apoptosis in cells having hsp 70, while cells harbouring antisense hsp 70 were highly sensitive to apoptosis by Curcumin as measured by nuclear condensation, mitochondrial transmembrane potential, release of cytochrome *c*, activation of caspase 3 and caspase 9 and other parameters for apoptosis.¹⁰⁴ Expression of glutathione S-transferase P1-1 (GSTP1-1) is correlated to carcinogenesis and Curcumin has been shown to induce apoptosis in K-562 leukaemia cells by inhibiting the expression of GSTP1-1 at transcription level.¹⁰⁵ The mechanism of Curcumin-induced apoptosis has also been studied in Caki cells, where Curcumin causes apoptosis through downregulation of Bcl-XL and IAP, release of cytochrome *c* and inhibition of Akt, which are markedly blocked by *N*-acetylcysteine, indicating a role of ROS in curcumin-induced cell death.¹⁰⁶ In LNCaP prostate cancer cells Curcumin induces apoptosis by enhancing tumour necrosis factor-related apoptosis-inducing ligand (TRAIL).¹⁰⁷ The combined treatment of the cell with Curcumin and TRAIL induces DNA fragmentation, cleavage of procaspase 3, 8 and 9, truncation of Bid and release of cytochrome *c* from mitochondria, indicating involvement of both external receptor-mediated and internal chemical-induced apoptosis in these cells.¹⁰⁷ In colorectal carcinoma cell line, Curcumin delays apoptosis along with the arrest of cell cycle at G1 phase. Curcumin also reduces *P53* gene expression, which is accompanied with the induction of HSP-70 gene through initial depletion of intracellular Ca^{2+} .¹⁰⁸ Curcumin also produces nonselective inhibition of proliferation in several leukaemia, nontransformed haematopoietic progenitor cells and fibroblast cell lines.¹⁰⁹ That Curcumin induces apoptosis and large-scale DNA fragmentation has also been observed in $\text{V}\gamma 9\text{V}\delta 2^+$ T cells through inhibition of isopentenyl pyrophosphate-induced NF κ B activation, proliferation and chemokine production.¹¹⁰ Curcumin induces apoptosis in human leukaemia HL-60 cells, which is blocked by

some antioxidants.¹¹¹ Curcumin suppresses human breast carcinoma through multiple pathways and downregulates matrix metalloproteinase (MMP)-2 and upregulates tissue inhibitor of metalloproteinase (TIMP)-1, two common effector molecules involved in cell invasion.¹¹² However, Curcumin affects different cell lines differently. Whereas leukaemia, breast, colon, hepatocellular and ovarian carcinoma cells undergo apoptosis in the presence of Curcumin, lung, prostate, kidney, cervix and CNS malignancies and melanoma cells show resistance to cytotoxic effect of Curcumin.¹¹³ Curcumin also suppresses tumor growth through various pathways. Nitric oxide (NO) and its derivatives play a major role in tumour promotion. Curcumin inhibits iNOS and COX-2 production by suppression of NFkB activation.^{85, 91} Curcumin also increases NO production in NK cells after prolonged treatment, culminating in a stronger tumouricidal effect.¹¹⁴ Curcumin also induces apoptosis in AK-5 tumour cells through upregulation of caspase-3.¹¹⁵ Recently, Curcumin has been shown to prevent glutathione depletion in Jurkat cells, thus protecting cells from caspase-3 activation and oligonucleosomal DNA fragmentation. Curcumin also inhibits proliferation of rat thymocytes. These strongly imply that cell growth and cell death share a common pathway at some point and that Curcumin affects a common step, presumably involving modulation of AP-1 transcription factor.¹¹⁶

4.1.4. Other Biological Activities

Pro/antimutagenic Activity

Curcumin exerts both pro- and antimutagenic effects. It was shown to reduce the number of aberrant cells in cyclophosphamide-induced chromosomal aberration in Wistar rats.¹¹⁷ Turmeric also prevents mutation in urethane (a powerful mutagen) models.¹¹⁸

Anticoagulant Activity

Curcumin shows anticoagulant activity by inhibiting collagen and adrenaline-induced platelet aggregation *in vitro* as well as *in vivo* in rat thoracic aorta.¹¹⁹

Antifertility Activity

Petroleum ether and aqueous extracts of turmeric rhizomes show 100% antifertility effect in rats when fed orally.¹²⁰ Implantation is completely inhibited by these extracts.¹²¹ Curcumin inhibits 5 α -reductase, which converts testosterone to 5 α -dihydrotestosterone, thereby inhibiting the growth of flank organs in hamster.¹²² Curcumin also inhibits human sperm motility and has the potential for the development of a novel intravaginal contraceptive.¹²³

Antidiabetic Effect

Curcumin prevents galactose-induced cataract formation at very low doses.¹²⁴ Both turmeric and Curcumin decrease blood sugar level in alloxan-induced diabetes in rat.¹²⁵ Curcumin also decreases advanced glycation and products-induced complications in diabetes mellitus.¹²⁶

Antibacterial Activity

Both Curcumin and the oil fraction suppress growth of several bacteria like *Streptococcus*, *Staphylococcus*, *Lactobacillus*, etc. The aqueous extract of turmeric rhizomes has antibacterial effects.¹²⁷ Curcumin also prevents growth of *Helicobacter pylori* CagA⁺ strains *in vitro*.¹²⁸

Antifungal Effect

Ether and chloroform extracts and oil of *C. longa* have antifungal effects.¹²⁹ Crude ethanol extract also possesses antifungal activity.¹³⁰ Turmeric oil is also active against *Aspergillus flavus*, *A. parasiticus*, *Fusarium moniliforme* and *Penicillium digitatum*.¹³¹

Antiprotozoan Activity

The ethanol extract of the rhizomes has anti-*Entamoeba histolytica* activity. Curcumin exhibits anti-*Leishmania* activity *in vitro* and several synthetic derivatives of Curcumin have anti-*L. amazonensis* effect.¹³² Anti-*Plasmodium falciparum* and anti-*L. major* effects of Curcumin have also been reported.¹³³

Antiviral Effect

Curcumin has been shown to have antiviral activity.³ It acts as an efficient inhibitor of Epstein-Barr virus (EBV) key activator Bam H fragment z left frame 1 (BZLF1) protein transcription in Raji DR-LUC cells. EBV inducers such as 12-O-tetradecanoylphorbol-13-acetate, sodium butyrate and transforming growth factor-beta increase the level of BZLF1 m-RNA in these cells, which is effectively blocked by Curcumin.¹³⁴ Most importantly, Curcumin also shows anti-HIV (human immunodeficiency virus) activity by inhibiting the HIV-1 integrase needed for viral replication.¹³⁵ It also inhibits UV lightinduced HIV gene expression.¹³⁶ Thus Curcumin and its analogues may have the potential for novel drug development against HIV.

Antifibrotic Effect

Curcumin suppresses bleomycin-induced pulmonary fibrosis in rats. Oral administration of Curcumin at 300 mg/kg dose inhibits bleomycin-induced increase in total cell counts and biomarkers of inflammatory responses. It also suppresses bleomycin-induced alveolar macrophage-production of TNF- α , superoxide and nitric oxide. Thus Curcumin acts as a potent antiinflammatory and antifibrotic agent.¹³⁷

Antivenom Effect

Ar-turmerone, isolated from *C. longa*, neutralizes both haemorrhagic activity of *Bothrops* venom and 70% lethal effect of *Crotalus* venom in mice.³ It acts as an enzymatic inhibitor of venom enzymes with proteolytic activities.

4.2. Chemistry of Curcumin

4.2.1. Chemical Structure

Curcumin, 5-hydroxy-1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-1,4,6-trien-3-one (diferuloylmethane) is a secondary metabolite and as mentioned the main yellow compound of *Curcuma longa* rhizomes and exhibits numerous biological activities.⁷⁴⁻¹³⁷ In the rhizome it is present together with 5-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-7-(4-hydroxyphenyl)hepta-1,4,6-trien-3-one (demethoxycurcumin), and 5-hydroxy-1,7-bis(4-hydroxyphenyl)hepta-1,4,6-trien-3-one (bisdemethoxycurcumin) (Fig.14).¹³⁸

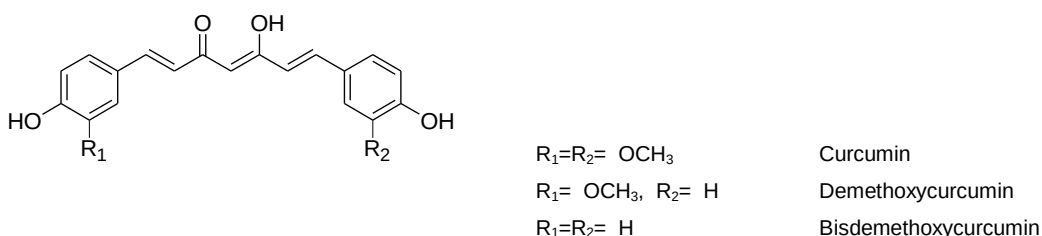


Fig. 14 Chemical structure of Curcumin, demethoxycurcumin and bisdemethoxycurcumin.

Curcumin was first isolated in 1870 and its chemical structure was determined in 1910, which was subsequently confirmed by synthesis.¹³⁹ Curcumin itself has a unique conjugated structure including two methoxylated phenols, exists in equilibrium between the diketo and keto-enol forms, whereby the keto-enol form is strongly favored by intramolecular H-bonding¹⁴⁰ (Fig. 15) and Curcumin shows potent antioxidant activity by its radical chainbreaking ability.¹⁴¹

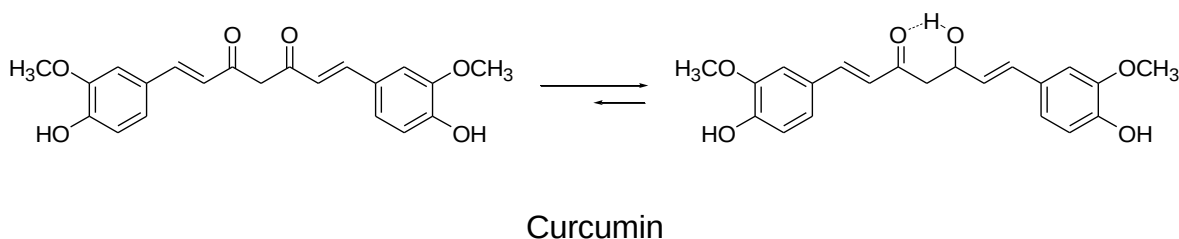
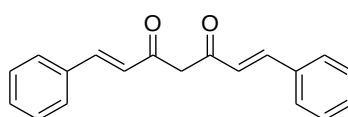


Fig. 15 Diketo and keto-enol form of Curcumin.¹⁴¹

The major constituent, Curcumin (diferuloylmethane), is in the most important fraction of *C. longa* L.. It melts at 176-177°C and forms red-brown salts with alkalis. Curcumin is insoluble in water and ether and soluble in ethanol, alkalis, ketone, acetic acid and chloroform. In the molecule of Curcumin the main chain is aliphatic, unsaturated and the aryl group can be substituted or not.³

4.2.2. Synthesis of Curcumin

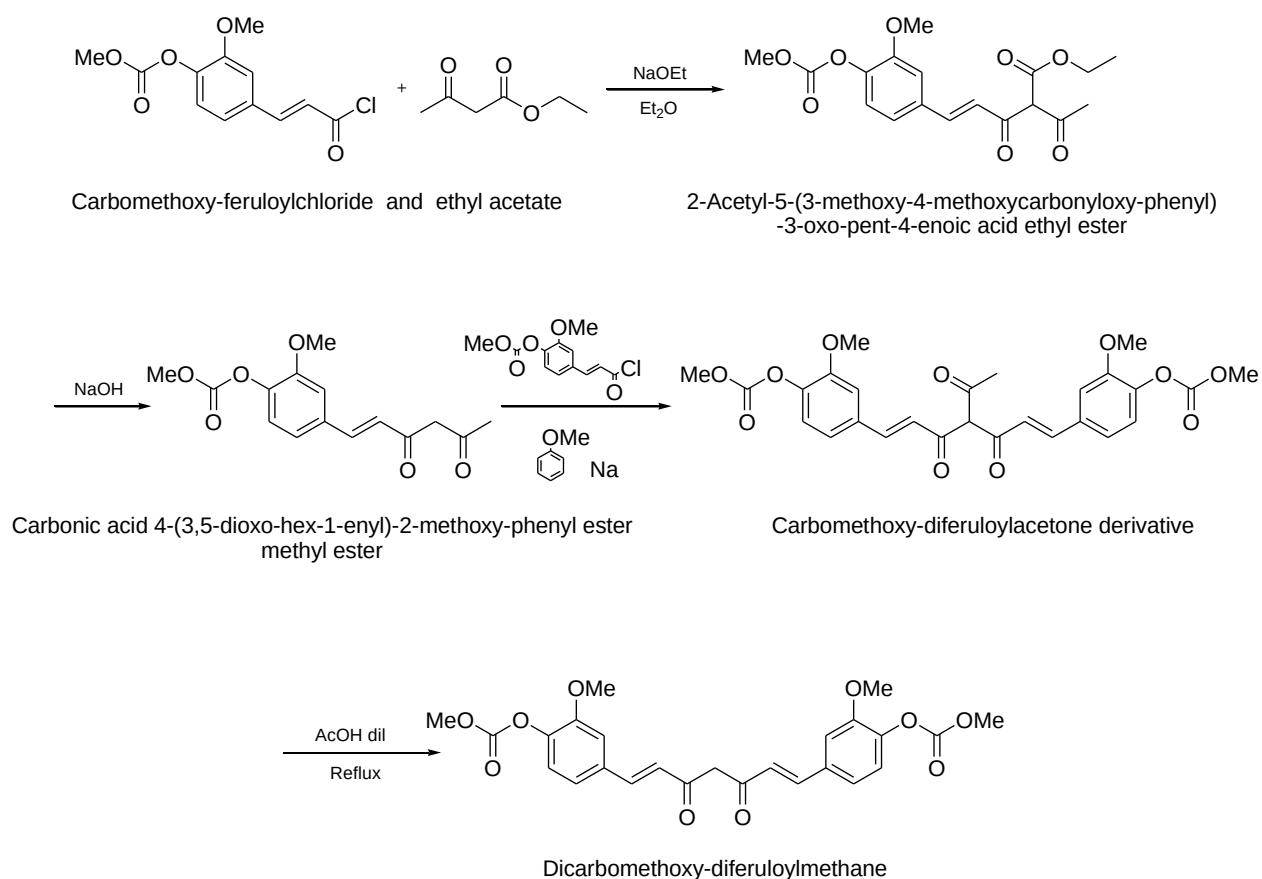
Vogel and Pelletier have been the first who tried to isolate Curcumin as a dye in 1815,¹⁴² but Curcumin was obtained in a crystalline condition not before 1870 by synthesis of two independent from each other working scientists named Daube and Iwanof-Gajewsky.¹⁴³ Their estimation differed in respect of the structural composition of Curcumin. Daube suggested that Curcumin has a structural formula of $C_{10}H_{10}O_3$ whereby Iwanof-Gajewsky claimed an easier formula of C_4H_4O for Curcumin. Discomposure arose with another suggested formula by Jackson and Mencke in 1884 who suggested for Curcumin a formula of $C_{14}H_{14}O_4$.¹⁴⁴ This formula was shown to be wrong by Ciamician and Silber¹⁴⁵ who hereupon proposed a formula of $C_{21}H_{20}O_6$, which was proved to be true by Milobedzka, Kostanecki and Lampe in 1910.¹⁴⁶ Through several evaluations they showed the ferulic acid to be a part of Curcumin and therefore they proposed for Curcumin the structural formula of a diferuloylmethane as shown in Fig. 15. To confirm the formula to be the right one Lampe and Milobedzka started to synthesize Curcumin in 1913 and first tried to build dicinnamoylmethane (Fig. 16) as they thought that its the precursor of Curcumin.¹⁴⁷



Dicinnamoylmethane

Fig. 16 Structure of dicinnamoylmethane.¹⁴⁷

The worked out synthetisizing method was shown to be correct, because following this mechanism it was also possible to synthetisize diferuloylmethane which corresponds in its physical and chemical properties exactly with the natural dye Curcumin. The pathway of Lampe et al. shown in scheme 2¹⁴⁸ includes 5 steps and starts with the reaction of the ethyl acetate with carbomethoxy-feruloylchloride in basic conditions to achieve the product of the condensation. Following saponification and decarboxylation gave the corresponding carboxymethoxy-diferuloylacetone derivative. Refluxing product with acetic acid dil. afforded dicarbomethoxy-diferuloylmethan. Milobedzka and coworkers compared this structure with the natural dicarbomethoxy-curcumin showing complete identity.

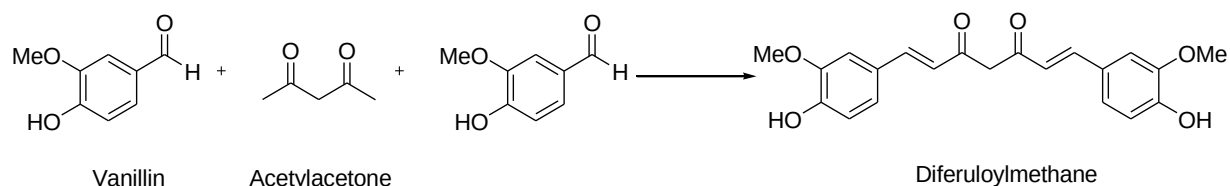


Scheme 2 First pathway of Curcumin synthesis.¹⁴⁸

Furthermore as the carbomethoxygroups were eliminated and Curcumin was obtained, the pathway was again shown to be the right one.¹⁴⁸

Another work was developed by Ghosh in 1919 in order to prove the structure of Curcumin through demonstrating the presence of the -CO-CH₂-CO- group.

Through several evaluations Gosh and coworkers additionally discovered that dicarboethoxycurcumin could be converted into dicarbethoxyisocurcumin very easily. The authors showed Curcumin and Isocurcumin to be two geometric isomers.¹⁴⁹ A new method to synthesize Curcumin was proposed by Pabon in 1964¹⁵⁰ in consideration of the work of Pavoli et al.,^{143, 144} who prepared Curcumin by heating vanillin, acetylacetone and boric anhydride (2:1:2) over a free flame for 30 min. and claimed a yield of 10% in this one step procedure as shown in scheme 2. Thus the method yielded in practice unsatisfying 1,5% of the product scientists proposed different variations of the reaction.



Scheme 3 Curcumin synthesis proposed by Pavolini et al.^{143, 144}

The low yield was not unexpected due to the high temperatures in this reaction, which on the other hand were necessary to get any reaction at all. Pabon and coworkers suggested that the low yield is caused by the fact that the vanillin condenses with the less reactive methyl groups of acetylacetone and in their opinion this behavior must be ascribed to the properties of the acetylacetone/boric anhydride complex which was not mentioned by Pavolini and probably has the suggested structure shown in Fig 17.^{151, 152}

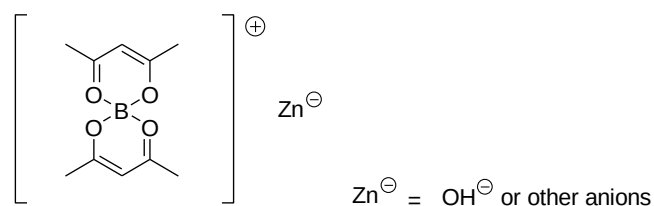


Fig. 17 Acetylacetone/boric anhydride complex.¹⁵¹

The structure occurring in the reaction was proposed to be as shown in Fig. 18 and this complex is decomposed by diluted acids and bases.¹⁵³

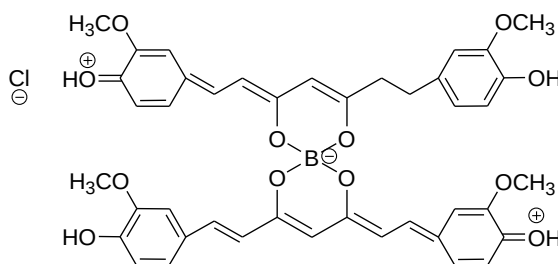


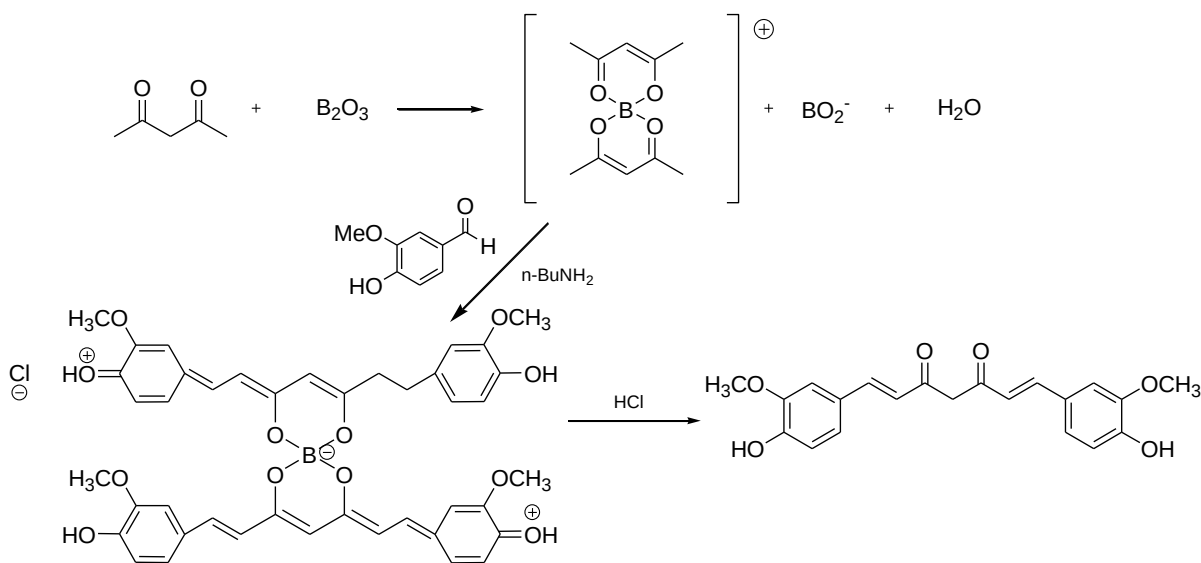
Fig. 18 Curcumin-acetylacetone/boric anhydride complex.¹⁵³

Hence the new idea of Pabon et al.¹⁵⁰ was to use diluted acids because Curcumin itself is unstable towards alkali. Keeping these proposals in consideration and because they observed that the condensation of vanillin and acetylacetone in the presence of boric anhydride took place at 150°C, if minor amounts of butanol and piperidine were also present, they thought that boric esters and bases like piperidine might be used with success for this type of condensation. Their experiment with tributyl borate, piperidine and the reaction product of acetylacetone and boric anhydride prompted them to study all factors involved systematically. They found that the best temperature range for this condensation was 80 -110°C and higher yields could be obtained by using a solvent. The results are shown in table 2.¹⁵⁰

<u>Trialkyl borate</u>	<u>Yield of Curcumin (%)</u>
Trimethyl borate	63
Triethyl borate	71
Tripropyl borate	72
Tri-isopropyl borate	80
Tri-n-butyl borate	73
Tri(2-butyl) borate	78
Tri-isobutyl borate	69
Tri-tert.butyl borate	58
Tripentyl borate	59
Tri(2-methylbutyl) borate	66
Trioctyl borate	57
Trioctadecyl borate	+/-5

Table 2 Influence of the nature of the trialkyl borate on condensation at room temperature of 0.4 mole of vanillin with the reaction product from 0.2 mole acetylacetone and 0.14 mole of boric anhydride in 200 ml of ethyl acetate, adding 0.8 mole trialkyl borate and 4 ml butylamine.¹⁵⁰

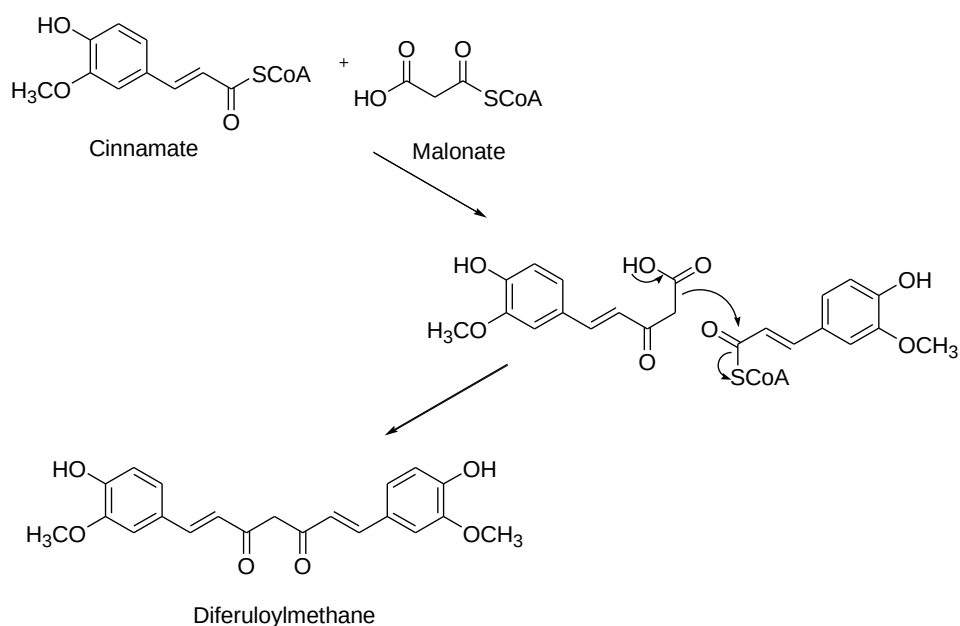
The improvement of this synthesis (shown in scheme 4) by using tributyl borate and piperidin as catalysts and the freshly prepared complex of acetylacetone and boric anhydride led to a significant increase of the yields. Some Curcumin derivatives, using vanilin (or benzyldehyde derivatives), tributyl borate, ethyl acetate, the complex of acetylacetone and boric anhydride have been synthesized using this method.¹³⁹



Scheme 4 Synthesis of Curcumin as proposed by Pabon.¹³⁹

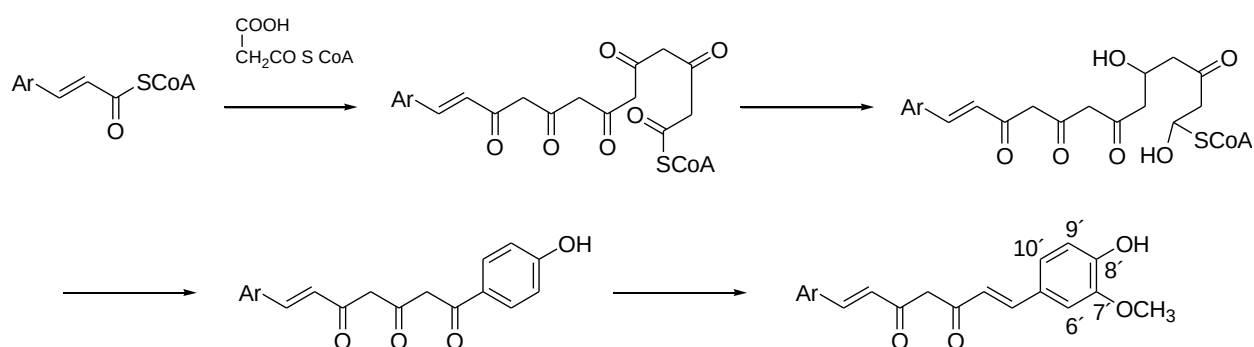
In a work of Roughly and Whiting¹⁵⁴ investigating the biosynthesis of natural diarylheptanoids with particular reference to Curcumin it was shown that the biosynthesis of Curcumin involves two cinnamate units which are coupled to a central carbon provided by malonate and therefore showed a new way of achieving Curcumin (scheme 5).

If biosynthesis does follow the steps shown in scheme 5 some activity might be expected in the cinnamate-derived units, incorporated either via eventual participation of acetate, through pyruvate, in phenyl-alanine metabolism, or through degradation to carbon dioxide. It would be surprising if such processes were more effective in acetate and malonate utilisation than the direct incorporation into the central methylene, unless some form of compartmentalisation happens.



Scheme 5 Curcumin biosynthesis involving two cinnamate units.¹⁵⁴

A possible alternative route for Curcumin formation was suggested as shown in scheme 6.¹⁵⁴



Scheme 6 Alternative pathway of curcumin biosynthesis.¹⁵⁴

The route involved a cinnamate starter, extended by five acetate (malonate) units, the cyclization of the chain gives the second aromatic ring (reduction before cyclization removes the 6'- and the 10'-hydroxy-function) and the biosynthesis would be completed by hydroxylation at C-7'. A lot of variations are possible because

of different unknown influences. It was proposed that ferulic and rosmarinic acid are acceptable precursors to Curcumin and to date the most used synthesis of Curcumin is the synthesis of Curcumin proposed by Pabon (scheme 4).¹³⁹

4.2.3. SAR of Synthesized Curcumin Analogues

The various properties of Curcumin have attracted much attention and in the years after its synthesis several analogues have been obtained through chemical modifications on the basic structure of Curcumin in attempt to increase its effectiveness and decrease the potential toxic effects. Some of these analogues have been reported to possess a higher anti-inflammatory activity than phenylbutazone.¹³²

On the basis of the biological activity and the easy synthetical approach, Curcumin was used as a lead compound to design Curcumin derivatives with the purpose of examining their anti-inflammatory activity.¹³⁹ Therefore, the anti-inflammatory activity of Curcumin, diacetylcurcumin (DAC), triethylcurcumin (TEC), tetrahydrocurcumin (THC) and phenylbutazone (PB) has been studied (Fig.19).

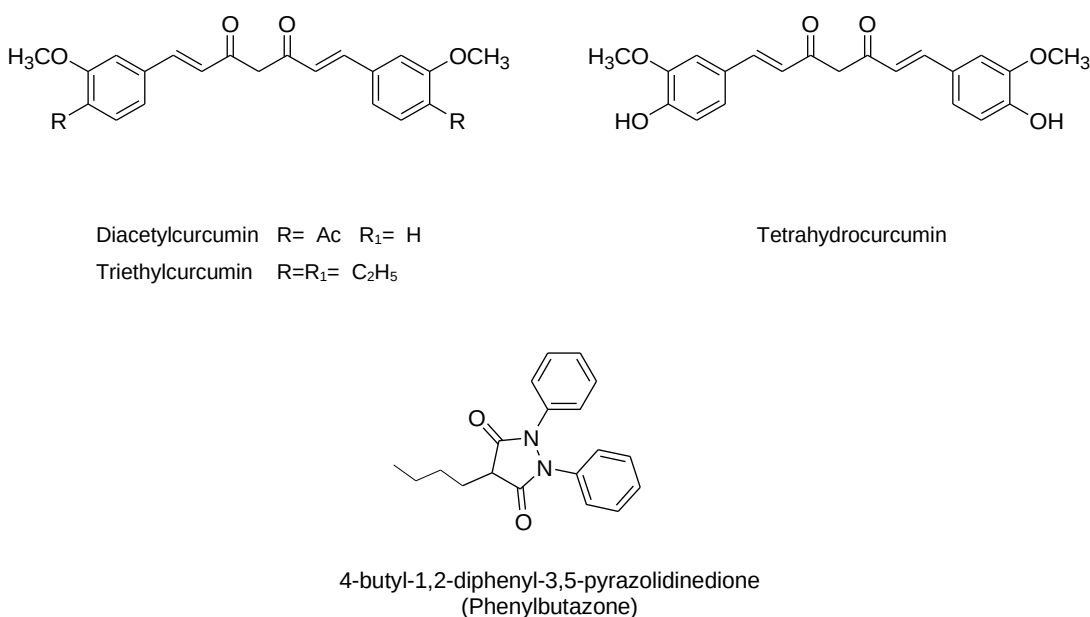


Fig.19 The substances tested on anti-inflammatory activity.¹³⁹

These analogues showed anti-inflammatory (protective) as well as inflammation-increasing (irritant) effects. The highest potency showed tetrahydrocurcumin. Altogether 16 Curcumin analogues were prepared and some physico-chemical properties could be summarized as follows.¹³⁹

The compound *without substituents* at the phenyl rings shows little inhibition in anti-inflammatory assays, whereas *substitution at the 4-position* of each phenyl ring by hydroxy, methoxy or methyl significantly increases the activity over the unsubstituted derivatives. Introduction of a *chloro* atom in the *4-position* does not increase the anti-inflammatory effect, which may suggest that an electron-donating substituent in the *para* position is favorable for activity. Among the *mono-substituted* compounds the hydroxy derivative showed the highest activity (ED_{50} = 73mg/kg). Moreover, addition of a *methoxy group* in the *3- or 2- position* instead of the *4-position* causes a significant decrease in activity, therefore mono-substitution at *ortho* and *meta* position is not sufficient for activity. Furthermore, the introduction of *methyl groups* in the *3- and 5-position* affords the most active compound (ED_{50} = 13mg/kg) in the series of methoxylated and alkylated 4-hydroxy derivatives. Compared to this they found the opposite effect by substituting the *3- or 5- position* of the 4-methoxy analogue, here the activity was increased with a methoxy group, and the introduction of benzoyloxy groups in the *3- and 4-position*, maybe because of sterical reasons, caused a complete loss of activity.¹³⁹

Altogether it was shown that the olefinic double bonds, the 4-hydroxyl groups and the presence of one 3-methoxy group are important for the anti-inflammatory activity, and besides this, the substituents at *meta* positions are important for activity as well. Thus the bigger substituents (tert-butyl) lead to inactivity, analogues without 4-hydroxygroups have low or no activity and the compound with 3-methyl groups shows the highest activity in the inflammatory tests based on the inhibition of the carageenin-induced swelling of rat paw.¹³⁹

In another approach Curcumin analogues were assayed in vitro against *L. amazonensis* promastigotes with using pentamidine isethionate as reference drug.¹³² Several modified analogues were tested. Comparing the results in table 3 it can be observed that the compounds 1,7-bis-(3,4-dimethoxyphenyl)-1,6-heptadien-3,5-dione and 1,7-bis-(4-propargyl-3-methoxyphenyl)-1,6-heptadiene-5,5-dione (Fig. 20) show

the highest activity against *L. amazonensis*, followed by the derivatives shown in table 3. It was observed that the substitution of the hydroxyl group in hydrazinocurcumin with a propargyl group brings an enhancement of nearly 10 times in the leishmanicidal activity whereas the absence of *para* propargyl group decreases the effect.¹³²

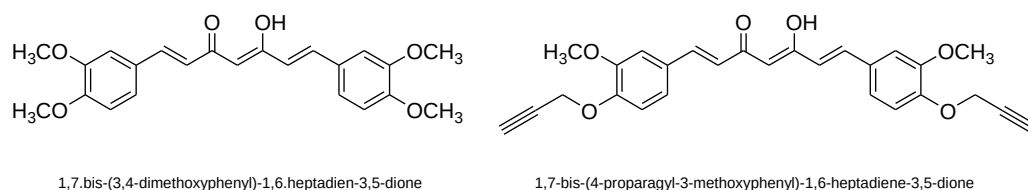


Fig. 20 Most potent derivatives in leishmanicidal activity.¹³²

<u>Compound</u>	<u>LD₅₀ (μg/ml)³</u>	
1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,4,6-heptatrien-3-one	9.00	±2
1,7-bis-(4-hydroxy-3-methoxyphenyl)-heptane-3,5-dione	23.10	±2
1,7-bis-(4-hydroxy-3-methoxyphenyl)-heptane-3,5-diol	51.23	±3
1,7-bis-(3,4-dimethoxyphenyl)-1,6-heptadiene-3,5-dione	2.00	±5
1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-diol	39.24	±4
1,7-bis-(4-acetoxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione	17.55	±3
1,7-bis-(4-acetoxy-3-methoxyphenyl)-heptane-3,5-dione	27.10	±4
1,7-bis-(4-propargyl-3-methoxyphenyl)-1,6-heptadiene-3,5-dione	1.24	±5
1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-acetonide	14.12	±2
1,7-bis-(3,4-dimethoxyphenyl)-1,6-heptadiene-3,5-diol	25.32	±5
Pentamidine	0.278	±0.001

Table 3 Compounds and results of the tested anti leishmanicidal activity.¹³²

Unfortunately the results did not allow to postulate any correlation between the leishmanicidal activity and the chemical structures. Although it was suggested that the phenol group is essential in Curcuminoids for good biological effects, and the non phenolic compounds 1,7-bis-(3,4-dimethoxyphenyl)-1,6-heptadien-3,5-dione and 1,7-bis-(4-propargyl-3-methoxyphenyl)-1,6-heptadiene-3,5-dione were much more effective against *L. amazonensis*. This leads to the suggestion that the leishmanicidal activity may be related to other structural features, such as the presence of the β-dicarbonyl system, the α,β-unsaturated system and the increasing electronic density in the aromatic ring.¹³²

Shim and coworkers designed several Curcumin analogues in expectation to enhance the biological, especially anti-angiogenic, activity. The evaluation of in vitro and in vivo assays of these derivatives shows that hydrazinocurcumin (Fig. 21) possesses a 30 times higher inhibitor-potency against bovine aortic endothelial cells (BAECs) than Curcumin.¹⁵⁵

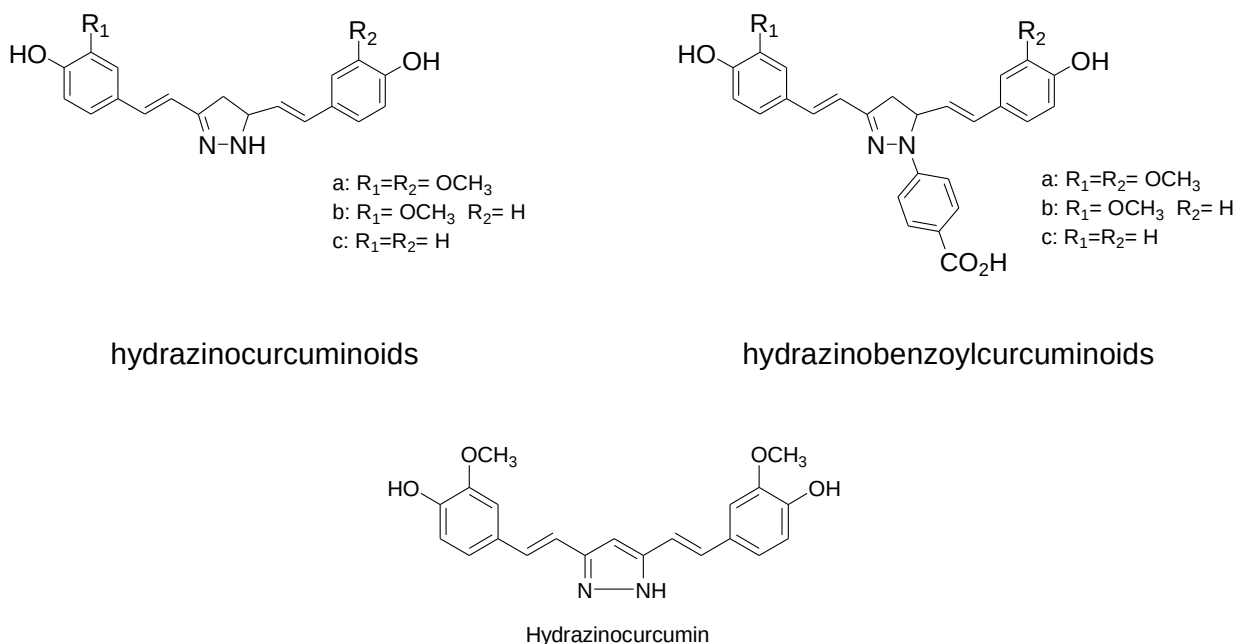


Fig. 21 Tested hydrazino- and hydrazinobenzoylcurcuminoids by Shim and coworkers.¹⁵⁵

Several studies on the SAR of Curcuminoids have revealed that the presence of the aromatic moieties is essential for the antioxidant activity. The diketone moiety of tetrahydrocurcumin also involves antioxidant mechanism and in addition, phenolic hydroxyl or methoxyl groups were modified to enhance the potency of other biological activities of Curcumin. Studies suggest that the diketone moiety of Curcumin may be essentially involved in its tremendous biological activities. In that work the diketone moieties of each Curcumin analogue were replaced with hydrazine derivatives in the presence of acetic acid and the highest activity showed hydrazinocurcumin with an IC₅₀ of 0.52 μ M. The compounds with bulky benzoic acid moiety also obtain enhanced anti-proliferative activity, but in a weaker potency than hydrazinocurcumin.¹⁵⁵

In the frame of another investigation devoted to the development of selective cytotoxic antitumor agents 32 Curcumin analogues (diarylheptanoides) were prepared and their cytotoxic effects were evaluated. In the same work also

26 diketo compounds structurally related to Curcumin were designed and a selected number of them were tested in a drug resistance reversal assay.¹⁵⁶ Among the 32 diarylheptanoids the hydrogenated (saturation of olefinic bond) as well as the cyclic diarylheptanoids (Fig. 22) were inactive, therefore the presence of the conjugated β -diketone in the cyclic carbon chain appears to play an important role for cytotoxicity in this class of compounds.

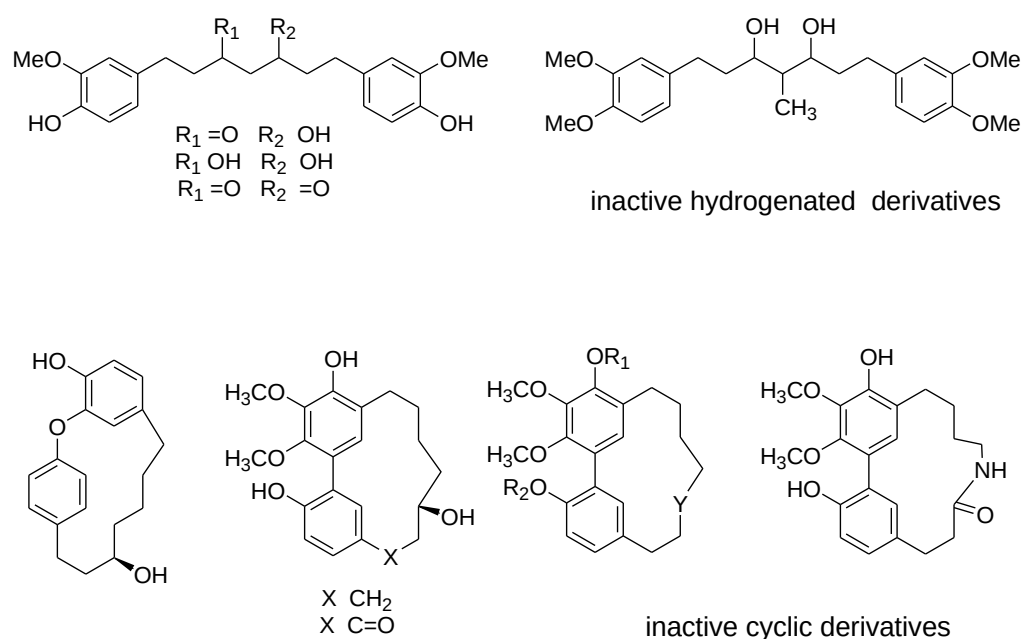


Fig. 22 Tested hydrogenated and cyclic diarylheptanoids.¹⁵⁶

The bis-substituted diarylheptanoid compound which is o-fluorinated on each benzene ring displays a broad cytotoxicity spectrum but borderline activity. The remaining fluorinated diarylheptanoids were inactive (Fig 23).

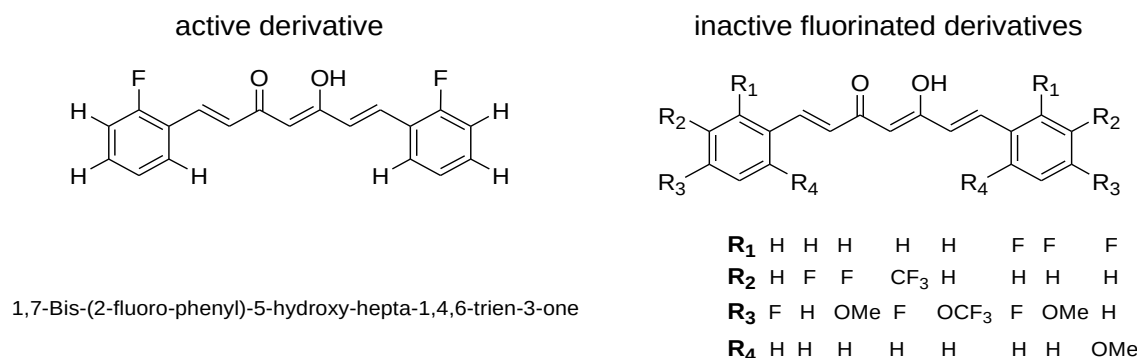


Fig. 23 Tested fluorinated compounds.¹⁵⁶

By comparing the cytotoxicity results of the remaining diarylderivatives following SARs were established.¹⁵⁶ Converting the keto-enol moiety to the corresponding pyrazole (Fig. 24) lead to increased cytotoxicity against various cell lines. Thus, the ring substituents affected the activity in the pyrazole derivatives.

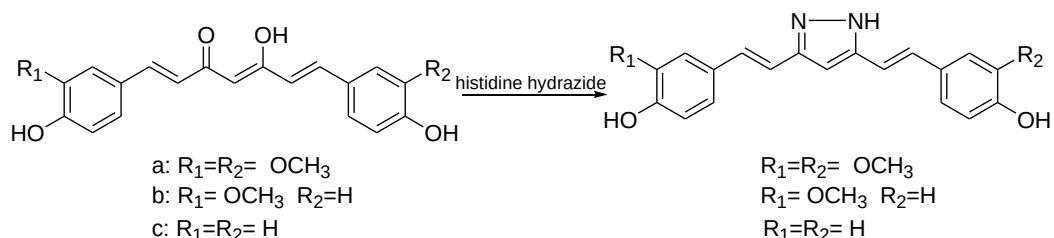


Fig. 24 The conversion of the keto-enol moiety to the pyrazole leads to increased cytotoxicity.¹⁵⁶

Demethylation or methylation of Curcumin to form the dihydroxy- and the trimethyl derivatives (Fig. 25), respectively, increased cytotoxicity against determined cells. Therefore, the presence of catechol or 3,4-dimethoxyphenyl substituents enhanced the cytotoxic properties.¹⁵⁶

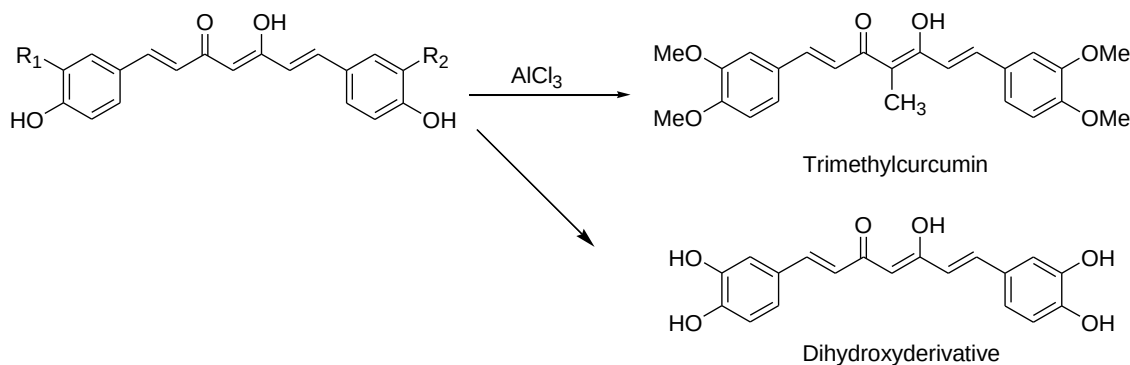
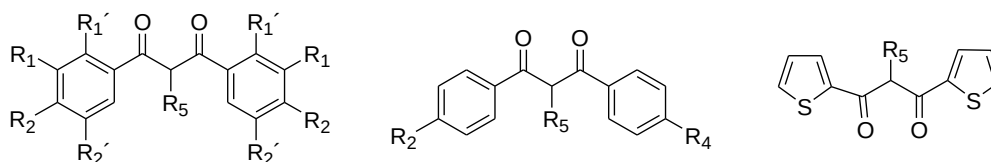


Fig. 25 Achieved compounds through methylation and demethylation of Curcumin.¹⁵⁶

It was concluded that the position and nature of the substituents on the benzene ring modulate antitumor activity.¹⁵⁶

Among the 26 1,3-diaryl-1,3-diketone-propane and related analogues (Fig. 26) following SARs can be summarized. The β -diketone moiety enhances the cytotoxic properties, introduction of *tert*-butyl group (an electron-donating substituent) on the phenyl ring and replacement of the hydrogen atom with fluorine (an electron-withdrawing substituent) at the *para* position, respectively, leads to increase

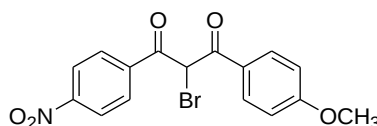
cytotoxicity against certain cells. Compared to unsubstituted the β -bromination between the keto groups led to enhanced activity but substitution by nitroso-, benzoyl-, methyl- and furan moieties at this position abolished activity.



1,3-diaryl-1,3-diketone-propane and analogues

Fig. 26 Delineation of synthesized propane analogues.¹⁵⁶

The compound which has an α -bromo substituent and 4-nitro and 4-methoxy groups on separate benzene rings (Fig. 27) demonstrated the strongest cytotoxic effects against HOS (bone cancer) and 1A9 (breast cancer) cells with ED₅₀ values of 0.97 and <0.63 μ g/mL, respectively.



2-bromo-1-(4-methoxy-phenyl)-3-(4-nitro-phenyl)-propane-1,3-dione

Fig. 27 Compound with the strongest cytotoxic effects against HOS and 1A9.¹⁵⁶

It was confirmed that asymmetrical substitution led to enhanced activity and different electronegative aryl substituents (4-nitro, 4-methoxy) to increased activity against 1A9 and HOS cells. In addition to that, replacing the phenyl groups with thiophenyl groups increased cytotoxicity against HOS and A19 cell lines.¹⁵⁶ The efforts towards the design of new Curcumin analogues in another work have focused on the truncation of the general structure to either a single enone or dienone system.¹⁵⁷ All new compounds were prepared according to the same general synthetic procedure, the utilization of classic Claisen-Schmidt reaction, or obtained commercially. A reasonable approach was to explore compounds with systematic

differences in the carbon chain connecting the two aromatic regions and small-molecule anti-angiogenic analogues of Curcumin are attractive targets. Examination of Curcumin suggested that the two aromatic regions might be critical for potential ligand-receptor binding, therefore a preliminary pharmacological model divided Curcumin in three regions (Fig. 28). Region A required an aromatic ring, region B was composed of a symmetrical dienedione linker, and region C also required an aromatic ring.¹⁵⁷

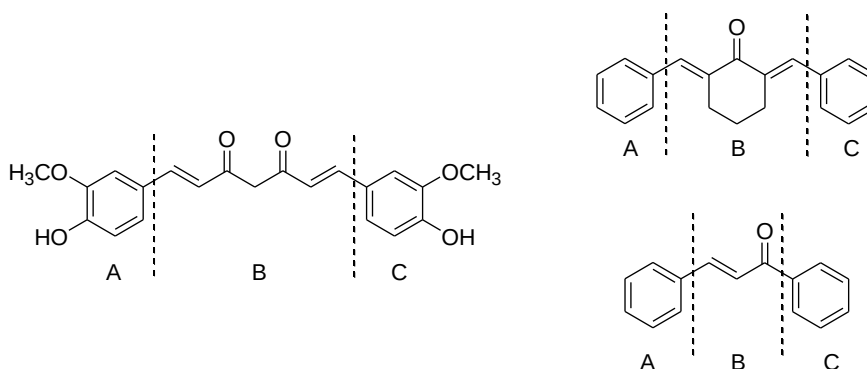


Fig. 28 Schematic demonstration of the differentiation into three regions.¹⁵⁷

Based on this simple model, various linkers in region B were prepared or obtained commercially and by modifying the linker to an enone or dienone, two new series possessing antiangiogenic properties were discovered (Fig. 29).¹⁵⁷

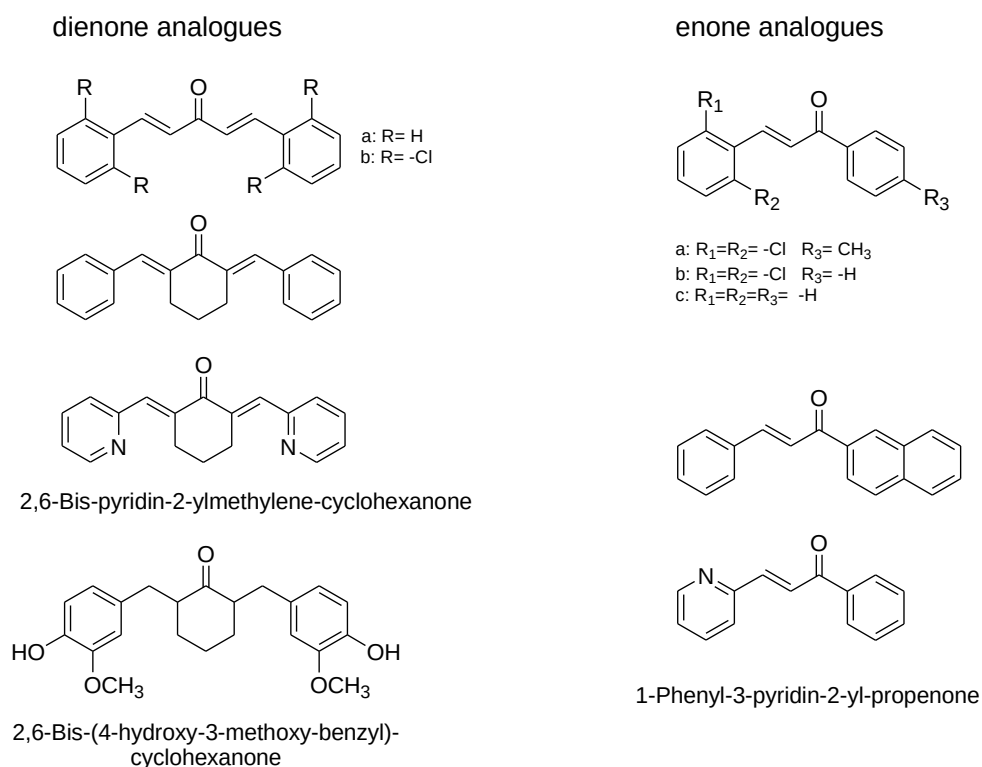


Fig. 29 Synthesized enone and dienone derivatives with antiangiogenic properties.¹⁵⁷

Among the enone compounds it was initially hypothesized, assuming potential nucleophilic addition with the enone, that substituents in position 2 and 6 of the aromatic ring in Region A might affect inhibition via stereoelectronic effects resulting from a conformational change. Adjusting the aromatic rings in Regions A and C and substitution of the phenyl ring in Region A with a pyridyl ring yielded the compound 1-Phenyl-3-pyridin-2-yl-propenone with excellent inhibition. The parent aromatic dienone compounds are cyclohexanone and acetone derivatives. As before in the enone series, the importance of stereoelectronic effects and the effect of conformational changes were explored by altering the aromatic substituents in Regions A and C, substitution of both phenyl rings, Region A and C, with pyridyl rings yielded 2,6-Bis-pyridin-2-ylmethylene-cyclohexanone which also possessed excellent inhibition. 2,6-Bis-(4-hydroxy-3-methoxybenzyl)-cyclohexanone was examined because of the similar functional group patterns to Curcumin (3-methoxy 4-hydroxy aromatic substitution patterns). The work led to the conclusion that aromatic enone and aromatic dienone analogues of Curcumin were excellent antiangiogenic compounds, having inhibition patterns equivalent or better than the parent natural product.¹⁵⁷

After several studies focusing mainly on changes in the β -diketone structure and aryl substitution, the intent of a novel work was to explore mono-carbonyl derivatives and diarylpentanoides to see whether they would possess increased anti-cancer and anti-angiogenic activity.¹⁵⁸ Through database searching two compounds were found with high similarity to Curcumin (Fig 30), the monoketones 1,5-bis(3,4-dimethoxyphenyl)-1,4-pentadiene-3-one (BDMPP) and 2,6-bis((3-methoxy-4-hydroxyphenyl)-methylene)-cyclohexanone (BMHPC).

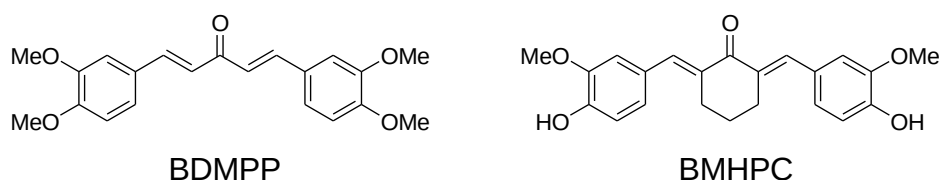
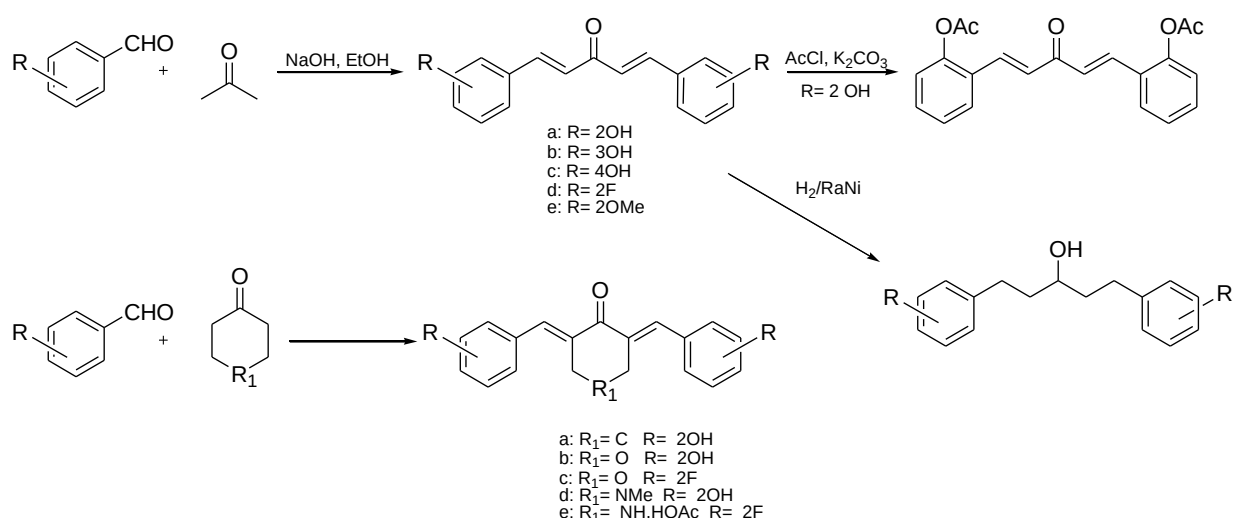


Fig. 30 The through database searching found compounds with high similarity to Curcumin.¹⁵⁸

These compounds were tested in a number of in vitro cell viability screens and it was evaluated that they possessed a higher activity compared to Curcumin. As a follow up, a number of novel mono-ketone lead compounds were synthesized and

biologically evaluated as potential anti-cancer and anti-angiogenesis agents. Similar studies on mono-ketones were evaluated before but an important distinction between the old studies and the new work is the location of the aryl substituents in the *ortho*-position. The main object of present investigations is the synthesis and testing of a number of symmetrical (scheme 7), but truncated Curcumin analogues in order to develop novel, active and selective anti-cancer and anti-angiogenesis agents.¹⁵⁸



Scheme 7 Synthesis of symmetrical, truncated Curcumin analogues.¹⁵⁸

α - β -Unsaturated ketones were produced to allow biological assessments by varying the substituents on the aromatic ring and the α -carbons of ketones, respectively. All three mono hydroxy groups were explored because of reported increase of activity. Further the replacement of the hydroxy groups with methoxy groups or fluorine were studied and the annulation of α -carbons allowed examinations of the preferred steric environment for this region as well as to study the bioeffects through modifying hydrophobicity.¹⁵⁸ The tested analogues showed a broad spectrum of activity as well as distinctive patterns of selectivity. Among all tested derivatives the *ortho* fluoro substituted piperidone analog (Fig 31) proved to be the most active compound, even more active than Curcumin and cisplatin, a DNA crosslinking agent that is commonly used in the treatment of a number of different cancers.

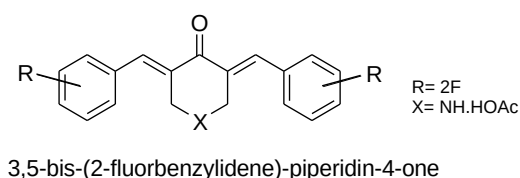


Fig. 31 The *ortho* fluoro substituted piperidone analogue with the highest activity.¹⁵⁸

3,5-bis-(2-fluorbenzylidene)-piperidin-4-one, acetic acid salt was also tested in vivo and seems to have potential as a chemotherapeutic agent since it demonstrated better activity but lower toxicity than a commonly used drug. Following general conclusions could be summarized:

As previously shown *ortho*-Substitution on the aromatic rings enhanced the activity of the symmetrical analogues while substitution in the *meta*- or *para*- position led to inactive compounds. The symmetrical α - β -unsaturated ketone structure found in the novel analogues showed increased in vitro anti-cancer and anti-angiogenesis activity compared to the diketone structure of Curcumin. Introduction of heteroatoms in the cyclic ketone portion yielded compounds with improved anti-cancer and anti-angiogenesis activity. Saturation of the olefinic bond of the α - β -unsaturated ketones produced an aryethyl alcohol that possessed little anti-cancer and direct anti-angiogenic activity.¹⁵⁸

In order to search new antitumor agents acting on mitochondrial various analogues of Curcumin were synthesized and compared to the well known uncoupler agent carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) and it was presented evidence that two of these agents (Fig 32) obtained by chemical substitution of Curcumin are able to combine the pro-oxidant PTP (permeability transmission pore) inducing properties of the parent drug Curcumin with potent mitochondrial uncoupling properties.¹⁵⁹

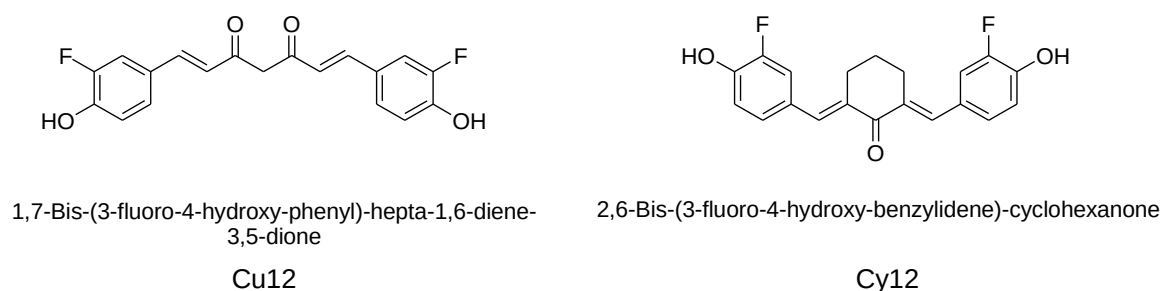


Fig. 32 Synthesized compounds with promising anti-tumor activities.¹⁵⁹

The replacement of methoxygroups by fluorine atoms induced three significant physico-chemical changes, namely an enhancement of the phenolic acidity, a large increase in partition coefficient in n-octanol/water biphasic system, and a large increase in $\Delta\log P^{\text{N}}_{\text{oct-npoe}}$ parameter, thus compared to the methoxy derivatives the fluorinated derivatives showed higher lipophilicity and higher acidity which suggests that the substitution of fluoride increases the concentration of anionic forms in organic media such as mitochondrial membranes. The measurement of lipophilicity of the anionic form was not possible, but the accumulation of anions in the organic phase could be seen qualitatively in partition experiments by changes in organic phase coloration. These effects are markedly important for fluorine derivatives at pH around pH 7.0 underlining that not only high lipophilicity but also high acidity and high complexation power may be responsible for the increase in uncoupler effects of Cu12 and Cy12. Therefore, these two compounds may represent interesting anti-tumor agents.¹⁵⁹

Based on previous results of Curcumin potencies it was hypothesized that the rigidity of symmetrical aromatic moieties plays an important role to enhance antiangiogenic activity. Therefore symmetrical bis-aromatic alkynyl pyridine and thiophene derivatives representing the rigid structure of Curcumin (Fig. 32) and bis-aromatic alkyl pyridine and thiophene derivatives were synthesized.¹⁶⁰

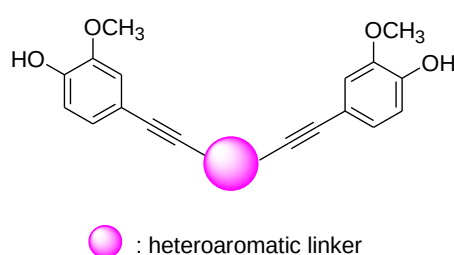
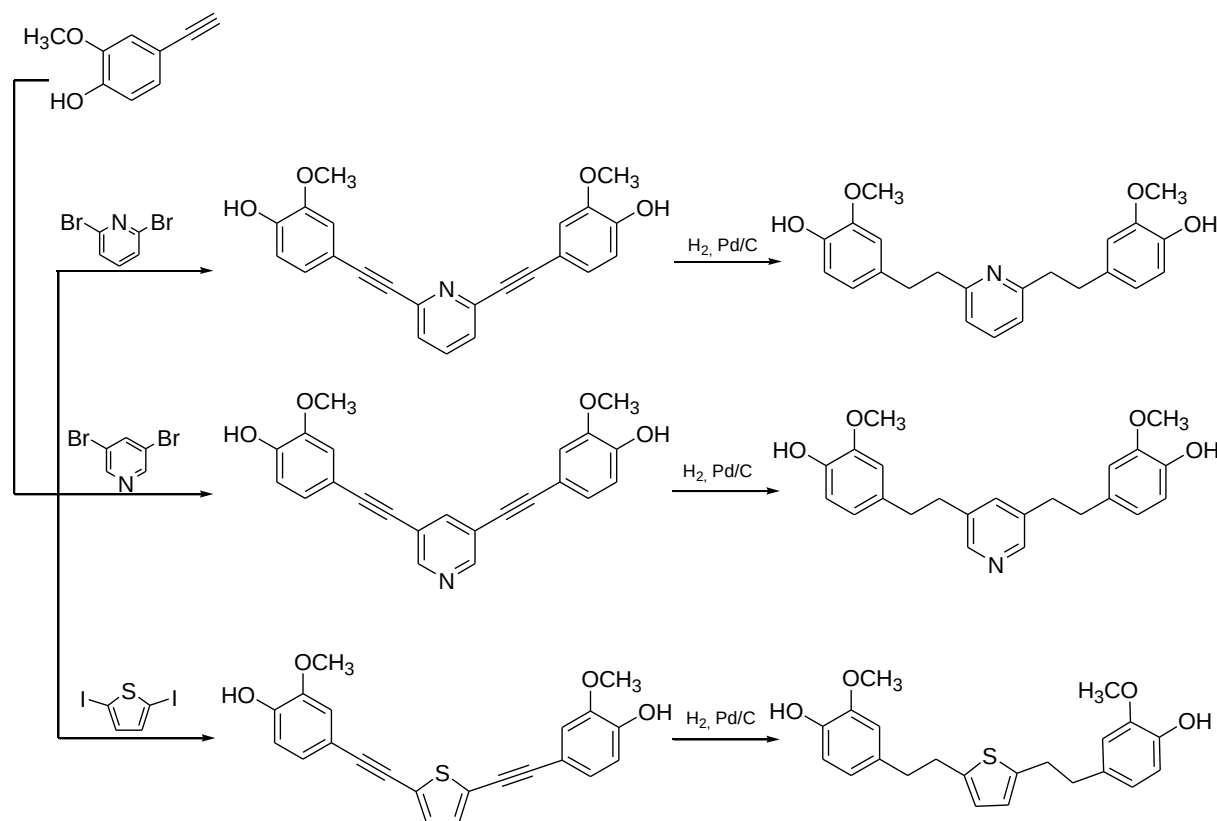


Fig. 32 Demonstration of the structural rigidity which is thought to be important for enhanced antiangiogenic activity.¹⁶⁰

For the ability to study the relationship between structural rigidity and activity the alkynyl compounds were hydrogenated with Pd/C at room temperature in the H₂ atmosphere, respectively (scheme 8). As expected the bis-alkynyl compounds, rigid mimetic structure of Curcumin, showed more potent growth inhibitory activity than Curcumin itself. The bis-alkynyl-pyridine derivatives possess stronger inhibitory

activity than the thiophene derivative.¹⁴ The watersoluble derivatives of these bis-alkynyl compounds, which were also obtained through hydrogenation and had more flexible alkyl chain, showed similar inhibition activity to Curcumin. The authors found that the rigid mimetic structure has a strong anti-angiogenic activity and improved the biological activity.¹⁶⁰



Scheme 8 Synthesized compounds which were achieved through hydrogenation.¹⁶⁰

A recent work described the covalent attachment of Curcumin with ligands that internalize with cellular environment of infected cells. They used the aminoacids glycine and D-alanine, which are essential compounds of bacterial cells, and glucose and acetic acid being food components and also recognized by cells. Since piperin has been proved to enhance bioavailability of drugs they also linked Curcumin with piperin to achieve more efficient bioconjugates. The aims were to ease the transmembran passage of Curcumin and a decrease of metabolism inside the cell, respectively. Furthermore to enhance hydrophobicity and to have a biodegradable linkage in the bioconjugate, which could be degraded by the cellular enzymes and Curcumin released at the drug target site (pro-drug).¹⁶¹ The conjugates shown in

Fig. 33 were synthesized and following results were concluded. Tested in vitro on a lot of multiresistant strains of bacteria and fungi the compounds proved to have higher hydrophobicity as Curcumin and the antibacterial and antifungal activity is significantly increased.¹⁵ Thus there is a need to develop novel nonantibiotic drugs to overcome antibiotic resistance these compounds are promising targets for further investigations.¹⁶¹

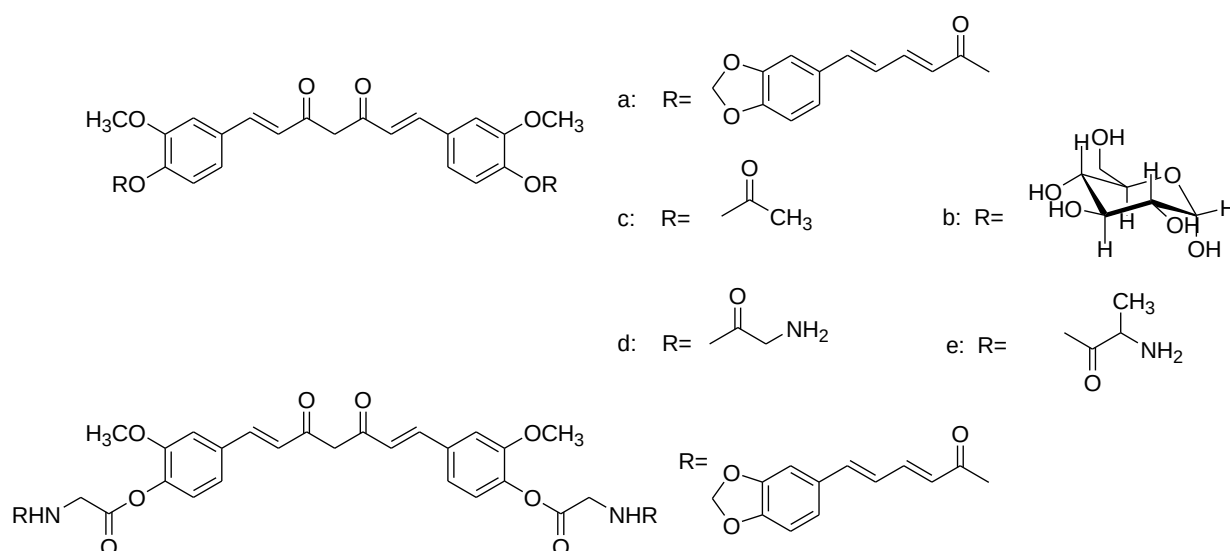
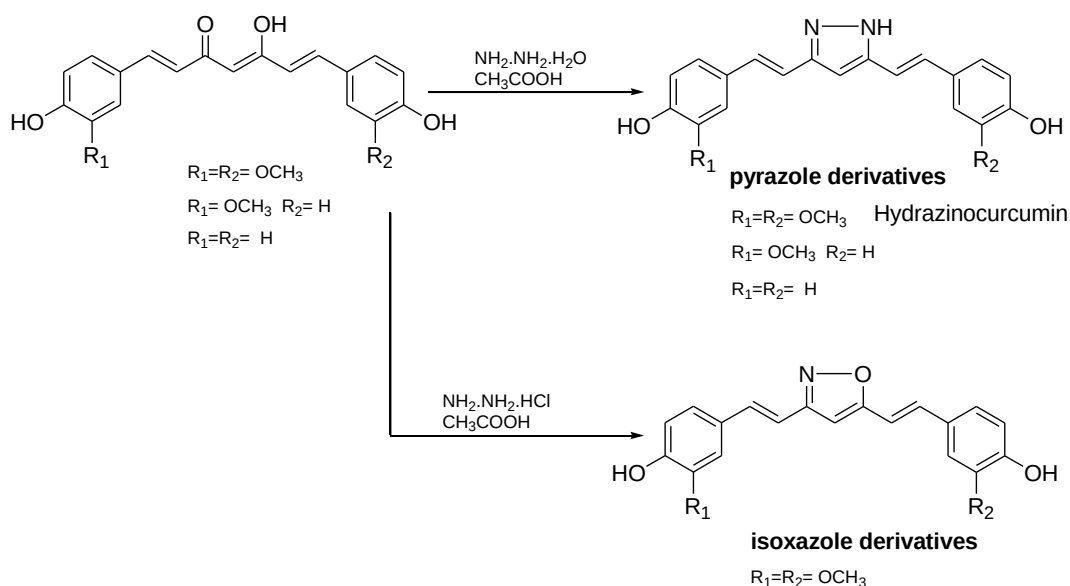


Fig. 33 Derivatives that were achieved through connection with glycine, D-alanine, glucose, acetic acid and piperin.¹⁶¹

In a novel study of Curcumin analogues as potential COX-1/COX-2 inhibitor pyrazol and isoxazol derivatives were designed and showed a higher antioxidant activity than trolox and a significantly enhanced COX-1/ COX-2 selectivity.¹⁶² In previous literature it was found that Curcumin was investigated for COX inhibitory activity. One pyrazol analog of Curcumin was investigated for lipoxygenase inhibitory and the three pyrazole derivatives of curcuminoids were synthesized and evaluated for endothelial cell proliferation and cytotoxic activity.^{155, 156} The curcuminoids Curcumin, demethoxycurcumin and bisdemethoxycurcumin were isolated and the pyrazole and isoxazole analogues were prepared by treating the CH_2Cl_2 extract as shown in scheme 9.¹⁶²



Scheme 9 Synthesized pyrazole and isoxazole derivatives with antioxidant and COX1/COX2 selectivity.¹⁶²

Concordantly to previous studies the o-methoxyphenolic moiety of the pyrazole and isoxazole derivatives showed to be essential for antioxidant property. Hydrazinocurcumin was found to be more potent than Curcumin¹⁵⁵ and the isoxazole derivative shown in scheme 8 was equipotent. Compared to trolox compounds Curcumin, hydrazinocurcumin, the isoxazole derivative showed higher antioxidant activity and among them hydrazinocurcumin showed the highest, most probably because of the presence of pyrazole NH.¹⁶² In COX catalyzed prostaglandin biosynthesis assay in vitro COX-1/COX-2 inhibitory activity was tested and hydrazinocurcumin and the isoxazole derivative demonstrated better COX-2 inhibitory activity in comparison to Curcumin. Since these analogues exhibited good COX inhibitory and antioxidant activities they were investigated for in vivo anti-inflammatory activity assay and hydrazinocurcumin showed the highest activity as well. Therefore molecular docking studies were performed and these further supported the strong inhibitory activity of hydrazinocurcumin compared to the others.¹⁶² SAR studies revealed that replacement of the β -diketo fragment of Curcumin by a pyrazole ring significantly enhances COX-1/COX-2 selectivity. The mixed antioxidant and COX inhibitory properties may provide superior anti-inflammatory activity. Thus, the investigation of the real antioxidant activity, the good radical scavenging and the better understanding for the ligands/enzyme interactions

in detail through molecular docking studies could lead to novel potent inhibitors in future.

Because of the possibility to prepare derivatives of Curcumin with feruloyl acid, a work has to be considered where 14 feruloyl-myo-inositol derivatives were prepared and the relationship between their stereostructure and inhibitory activity towards the 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced superoxide (O_2^-) generation and their suppressive effect on the TPA-induced Epstein-Barr Virus (EBV), respectively, was evaluated.¹⁶³ Out of the three structural groups, monoferuloyl-, bisferuloyl-, and trisferuloyl-myo-inositol three derivatives showed high biological activity (Fig. 34). It was substantiated that the derivative 3-(4-Acetoxy-3-methoxy-phenyl)-acrylic acid 2,3,4,5-tetraacetoxy-6-(3-phenyl-acryloyloxy)-phenylester in which two feroyl moieties are introduced to the hydroxylgroups bond to the 1,2-vicinal carbons in myo-inositol exhibit a high suppressive activity towards the O_2^- generation. This observation suggests that the inhibitory effect of bisferuloyl-myo-inositol derivatives depends on if the phenolic and inositol hydroxyl groups are protected by acetyl group or not, hydrophobicity of the molecules and stereostructure of the molecules.¹⁶³

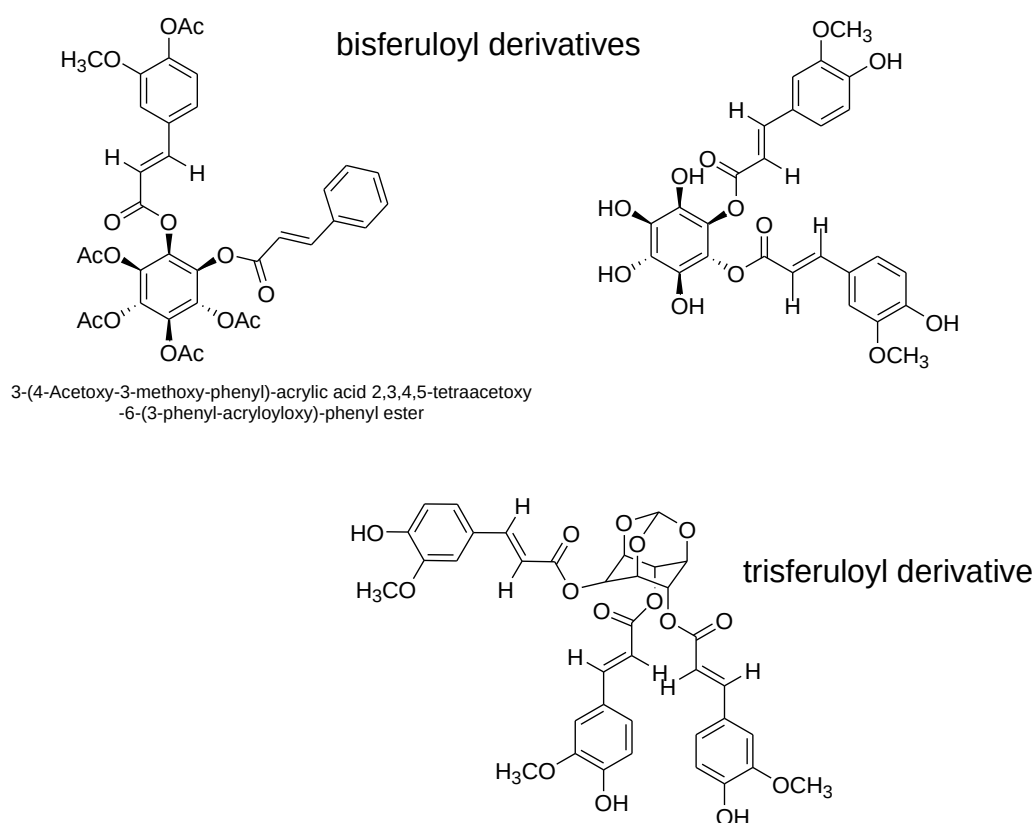


Fig. 34 Synthesized bisferuloyl and trisferuloyl derivatives.¹⁶³

Further they summarized that stereostructures in which two feruloyl moieties were introduced into equatorial hydroxyl groups bonded to the 3 and 6 carbons in myo-inositol demonstrated low activities and the structure which had two feroyl moieties introduced into the equatorial hydroxyl groups bonded to the vicinal 1 and 6 carbons in myo-inositol and the hydroxyl group of the system was not protected showed high suppressive activity on the O_2^- generation. The trisferuloyl derivative showed a distinct inhibitory activity toward the TPA- induced EBV activation. It was suggested that the suppressive activity of this compound may be attributed to its special molecular structure in which ferulic acid are facing each other by the use of a myo-inositol 1,3,5-orthoformate skeleton.¹⁶³

Thus the results showed that in the bisferuloyl group protection of the hydroxy group enhances activity, however in the group of trisferuloyl derivatives an increase of activity was achieved with protection of the hydroxy group. Concluding, it can be said, that the inhibitory potencies of these derivatives may depend on the stereostructure of the molecules rather than on the hydrophobicity of molecules.¹⁶³

4.3. Curcumin and its Properties in A β -Induced Cytotoxicity

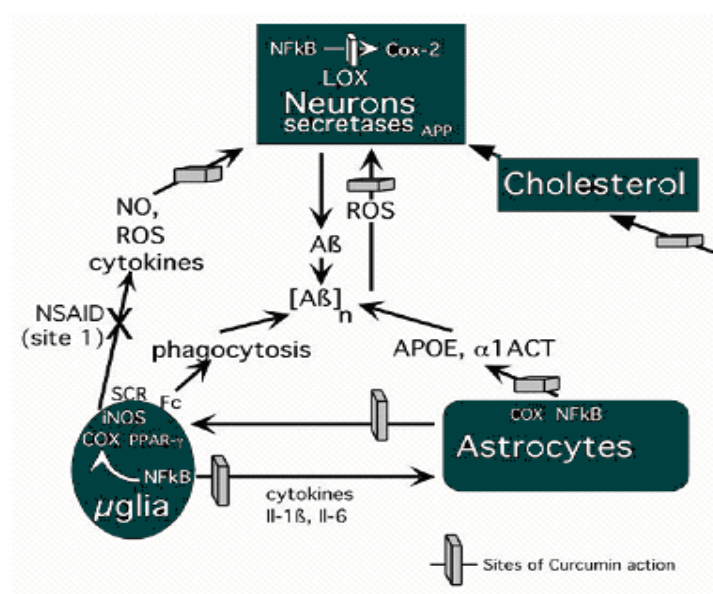
Since it is known that in neurodegenerative diseases including Alzheimer's disease besides other aspects also both oxidative damage and inflammation have been implicated,¹¹ and because of strong evidence that antioxidants can protect from in vitro β -amyloid toxicity and resulting oxidative damage,¹⁶⁴ a clinical trial testing the ability of high dose of the anti-oxidant α -tocopherol to slow AD progression was carried out.¹⁶⁵ The modest success of this trial in slowing the decline in activities of daily living has stimulated interest in antioxidant approaches. It remained unclear to what extent the tested vitamin E in form of α -tocopherol affected the oxidative damage, neuronal dysfunction, synapse loss and other pathogenic events, but it was shown, that one natural alternative antioxidant to vitamin E is the polyphenolic antioxidant Curcumin, found in turmeric.¹¹

Knowing that the yellow curry spice Curcumin shows antioxidant and anti-inflammatory activities,¹ and that age-adjusted Alzheimer's prevalence in India, where Curcumin is used almost every day, is roughly one quarter of the rates in the United States in those aged 80 years or older¹⁶⁶ research started to concentrate on the properties of Curcumin. Curcumin was reported to be several times more potent than vitamin E as a free radical scavenger⁸¹ and effective against nitric oxide based radicals.¹⁶⁷ Furthermore oral administration of Curcumin has been shown to be centrally neuroprotective.¹⁶⁸

Therefore, considering that Curcumin with its antioxidant and anti-inflammatory properties protects against neurotoxic and genotoxic agents, it was hypothesized that dietary Curcumin acting as combined antioxidant and NSAID agent might also have significant preventive activity against A β -induced neurotoxicity and cognitive deficits.¹¹ In an assay where 22 month Sprague-Dawley (SD) rats were used to compare the effects of the conventional NSAID, ibuprofen, and Curcumin for their ability to protect against amyloid β -protein (A β)-induced damage. It was shown that Curcumin besides other good influences on oxidative damages markedly reduced total A β plaque numbers compared to control diet fed animals and its potencies were shown to be greater than ibuprofen.¹¹

Furthermore Curcumin was tested for its ability to inhibit the combined inflammatory and oxidative damage that occurs as a response to amyloid in the transgenic mouse model APPs. This model carries a human familial AD gene (amyloid precursor protein with the “Swedish” double mutation)¹⁶⁹ and shows age-related neuritic plaque pathology, an inflammatory response, oxidative damage and age-related memory deficits linked to defective long-term potentiation (LTP),¹⁷⁰ representing the factors that are implicated in AD. It was established that Curcumin in a dose dependent manner is able to suppress the inflammatory cytokine IL-1 β and the astrocytic inflammatory marker GFAP, to reduce oxidative damage and it decreased overall insoluble amyloid, soluble amyloid and plaque burden.⁸¹ Recognizing that these A β -lowering effects were not mediated by reductions in APP expression, because there was found no decrease in APP production, the mechanisms underlying these Curcumin treatment effects are thought to be multifunctional as shown in scheme 10.⁸¹

It was suggested that Curcumin blocks AD pathogenesis at multiple sites. Curcumin can act as a scavenger of ROS, including NO and peroxynitrite generated by reactive glia and hydroxyl radicals generated by neurons as a result of direct A β toxicity. Ibuprofen (NSAID action at *site 1*) can inhibit microglial activation and cytokine production, but was not sufficient to reduce oxidative damage. Curcumin also limits damage by inhibiting NF κ B (nuclear factor κ B) induced iNOS (inducible nitric oxide synthase), cyclooxygenase 2, and inflammatory cytokine production by reactive glia.



Scheme 10 Multifunctional mechanism of Curcumin in AD.¹⁷¹

By blocking NF κ B and reducing interleukines IL-1 β , IL-6, and Apolipoprotein ApoE, Curcumin also should reduce proamyloidogenic factors (ApoE, α 1ACT (alpha-1-antichymotrypsin)). Furthermore Curcumin can lower plasma and tissue cholesterol, potentially lowering A β -production as well as Lipoxygenase (LOX), cyclooxygenase-2 (COX-2), scavenger receptors (SCR) and Fc Ig receptors (Fc).¹⁷¹⁻¹⁷⁴ Besides all these findings the direct effects of Curcumin on the formation and destabilization of A β -fibrillogenesis remained unclear.

Consequently other investigations concentrated on examining the effects of Curcumin and its analogue, rosmarinic acid (RA), on the formation and extension of A β (1-40)fibrils and A β (1-42)fibrils, as well as their activity to destabilize A β -fibrils at pH 7.5 at 37 °C in vitro. It was shown that Curcumin and rosmarinic acid dose-dependently inhibit β -amyloid fibrils (fA β) formation from fresh A β , and destabilize preformed fA β in vitro.¹² The two analogues were compared to nordihydroguaiaretic acid (NDGA) which possess the ability of inhibiting fA β formation from A β to fA β extension in vitro and showed a similar anti-amyloidogenic activity as Cur and RA because of structural similarity (Fig. 35).¹⁷⁵

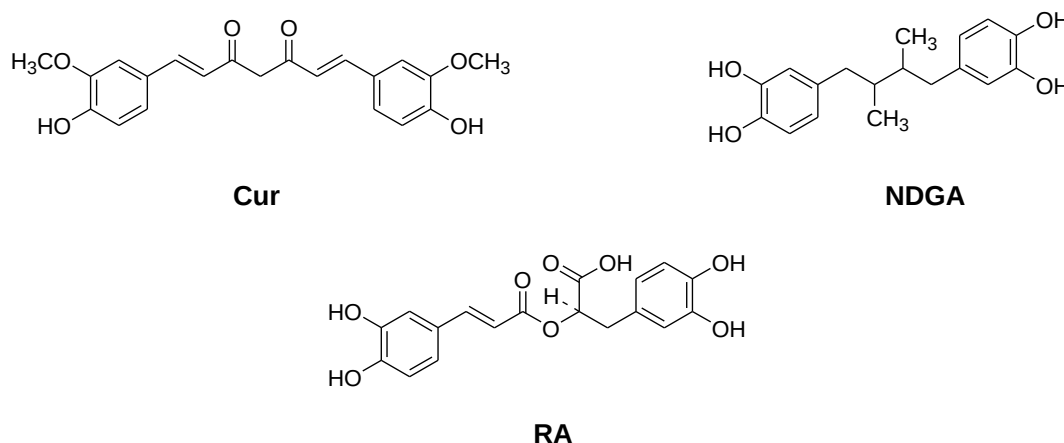


Fig. 35 Structure of Curcumin (Cur), nordihydroguaiaretic acid (NDGA) and rosmarinic acid (RA).¹⁷⁵

All of these molecules own two 3,4-dihydroxyphenyl rings (NDGA, RA) or 3,4-methoxyhydroxyphenyl rings (Cur) symmetrically bound by a short carbohydrate chain and this compact and symmetric structure might be suitable for specifically

binding to free A β and subsequently inhibiting polymerization of A β into fA β .¹⁴ Cur, RA and NDGA were proposed to have different mechanism compared to ApoE, which was suggested to inhibit through formation of complexes with A β s and thus eliminating free A β s from the reaction mixture.¹⁷⁵ Cur is thought to be able to bind to the end of extending fibril-A β (1-40) and increase the rate of depolymerization by destabilizing the conformation of A β (1-40) that just has been incorporated into the fibrils end.¹⁴ Further efforts to clarify the mechanisms by which Cur as well as RA inhibit formation in vitro are essential.

In another assay considering the interaction of soluble A β (1-40) or fibrillar A β (1-42) caused activation of nuclear transcript factor, early growth response-1 (Egr-1), which resulted in increased expression of cytokines (TNF- α and IL-1 β) and chemokines (MIP-1 β , MCP-1 and IL-8) in monocytes¹⁷⁶ and because Curcumin was shown to inhibit phorbol-ester (4 β -phorbol 12-myristate 13-acetate; PMA) – induced activation of Egr-1, AP-1 and NF- κ B in endothelial cells.^{177, 178} Egr-1 was explored as a molecular target for preventing inflammation utilizing a small organic molecule like Curcumin.²⁶ It was found that Curcumin inhibited A β -induced expression of Egr-1 protein and Egr-1 DNA-binding activity in THP-1 monocytic model cells that results concomitantly of the attenuation of the A β (1-40)- mediated gene expression of TNF- α , IL-1 β , IL-8 and MCP-1, indicating either a direct or causal effect. This study thus provided one mechanism, among several multifactorial effects, by which Curcumin abrogates amyloid peptide-induced inflammation. Furthermore it was shown that the chemotaxis of monocytes, which can occur in response to chemokines from activated microglia and astrocytes in the brain can be attenuated by Curcumin.²⁶

In a recent work¹⁶ an in vitro model of A β fibrillization was used to show that Curcumin can bind amyloid to inhibit A β aggregation as well as fibril and oligomer formation with dosing at achievable levels. It was demonstrated that Curcumin can label plaques in vitro and in vivo, block toxic oligomers in vitro and significantly reduce amyloid levels in aged Tg2576 mice (22 months) fed Curcumin diet beginning at 17 months after established amyloid deposition. Nevertheless it remained unclear from which Curcumin activity the amyloid suppressing function is achieved. It was discovered that Congo Red which inhibits A β 42 fibrillogenesis and oligomer formation through β -sheet breaking,¹⁷⁹ its derivative chrysamine G, and RS-0406 a novel

A β -aggregation inhibitor (Fig. 36) and Curcumin are similar in activity since all bind to plaques, prevent oligomer formation at similar low ID₅₀, and recognize secondary structure in fibrillar and oligomeric A β .

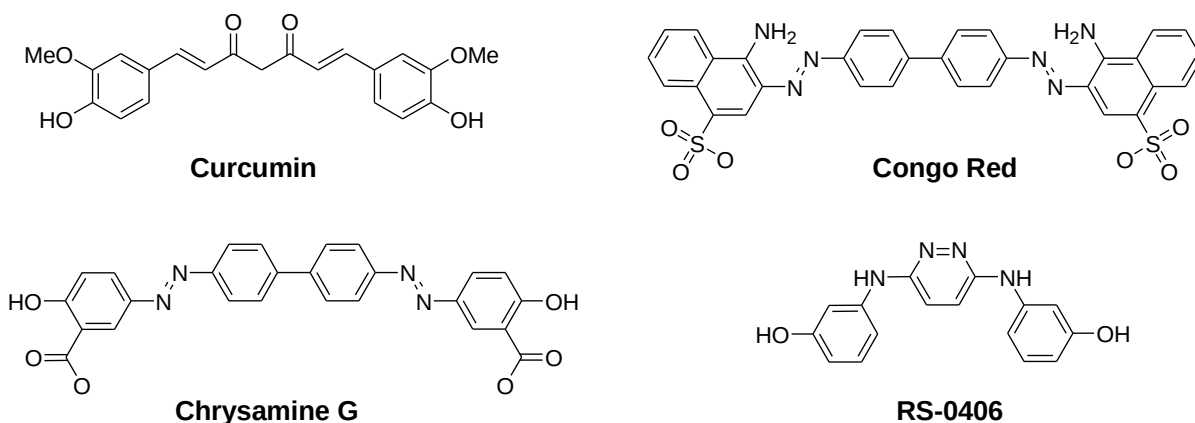


Fig. 36 Structures of Curcumin, Congo Red and related molecules.¹⁶

The advantage of Curcumin is to be more brain-permeable than these other molecules and therefore able to cross the blood-brain barrier easier to bind to plaque in vitro.¹⁶ The ELISA and aggregation studies showed that Curcumin was able to inhibit aggregation or promote A β -fibril disaggregation at low concentrations (IC₅₀= 0.81-1 μ M) and monomeric A β formed fewer aggregates in the presence of Curcumin. Higher doses of Curcumin promoted disassembly of performed A β aggregates. In that work it was also suggested that the effect of Curcumin did not depend on A β sequence, but on fibril-related conformation and they tested the Curcumin chelation of both iron and copper, which was proposed as one mechanism potentially contributing to amyloid reduction in animal models. It remained still unclear whether Curcumin's avidity for copper and potential concentration in the brain will be high enough to directly alter central nervous system A β metal binding. Besides the recent evaluations of Curcumin's potencies to decrease A β amyloid burden, and promoting disaggregation of A β -fibrils to reduce the ongoing causes for β -amyloid induced neurotoxicity, the real mechanism by which Curcumin is able to develop its properties remain unclear, and research in vivo for achieving more knowledge about this compound is necessary. Amyloid accumulation begins decades before diagnosis and

therefore anti-amyloid therapie should ideally start before clinical symptoms but also efficous approaches at advanced stages of amyloid accumulation are needed. In vivo test suggest that Curcumin could be beneficial even after the disease has developed.¹⁶

5. DISCUSSION & FUTURE PERSPECTIVES

A wealth of studies from laboratories all over the world support the amyloid hypothesis after it has become the focus of extensive AD research over 10 years ago.⁶ According to the amyloid hypothesis that accumulation of A β in the brain is the primary influence in driving the pathogenesis and the resulting disease process is derived through an imbalance between A β -production and A β -clearance,^{19, 26} resulting in chronic-inflammation,¹⁴⁰ oxidative damage and following neurotoxicity,³⁷ various therapeutic strategies have been proposed for treating and preventing the pathogenesis so far. Even if to date some treatment strategies which show good influence on AD progress are available,^{6, 180} there are a lot of problems to overcome. Currently used NSAIDs are still a matter of debate, not only because of their side-effects in producing gastrointestinal problems. Furthermore, tested dyes that demonstrated to exhibit strong potencies as anti-aggregates showed bad membrane permeability in vitro and therefore obtain bad bioavailability and other tested targets did not show the expected results or failed in in vitro testing.¹⁸¹

Nevertheless the development of anti-A β aggregation therapeutics remains a rational approach for treating AD and other amyloid influenced pathologies. What we need is a potent orally available inhibitor of A β -amyloidogenesis, that is small enough to cross the blood brain barrier, and big enough to interfere with the big β -sheet conformation of the fibrils.¹⁸¹ Considering the multifactoral process in AD it is obvious, that the therapeutic targets do not adress only one event. Thus, there is a need to develop compounds, small molecule mimetics, that adress multiple underlying disease mechanisms in parallel, and a multifaceted therapeutic strategy will be required for the successful treatment of AD.³² However, to develop a potent anti-aggregate target the describing and understanding of the exact kinetic mechanisms of amyloid fibril-formation and the binding of Curcumin, dyes and other potent molecules to the fibrils is primary important. Since this is still far from clear a lot of further elaboration in vitro and in vivo are necessary to develop a potential, safe and very non toxic anti-amyloidogenesis agent for treating AD and other A β -derived neurodegenerative disorders. In the old traditional drug Curcumin exhibiting a broad range of activity research hope to find a potential candidate.

6. SUMMARY

Alzheimer's disease represents the most common and devastating neurological disorder and research is concentrated in finding new potential candidates that not only temporarily relieve some symptoms but address the pathological process. Knowing that the crucial event of the pathogenesis is the amyloid aggregation research was concentrated on molecules that possess multifaceted activity, are small enough to cross the blood brain barrier easily, show good bioavailability and additionally desirable have no side effects and less toxicity. During the last years of research it became increasingly clear that dyes acting as markers for amyloid fibrils also exhibit the potency to interfere in the aggregation of β -amyloid. Curcumin promoted in great interest because of its use as a dye and for the treatment of several diseases associated with inflammation in Traditional Indian medicine. As it was noticed that in India the percentage of AD affected persons is only a quarter to that in the US Curcumin was started to be intensively examined whether it exhibits features to represent a candidate for a potent anti aggregative agent.

In this work the most important biological activities as well as the chemistry and synthesis of Curcumin and the SAR of selected derivatives are exemplified. Furthermore, the pathophysiological and clinical principles of AD and the assumption of its beginning and possible mechanisms which are thought to induce the pathogenesis of AD are briefly explained. Notably the so far supposed influence of NSAIDs, Curcumin and other dyes on the onset and progression is explicated. Summing up the findings of the literature research it could be seriously assumed that Curcumin with its broad range of activity and its derivatives opens an interesting perspective as possible AD treatment candidates in the future.

So far further investigations are necessary to clarify the exact physiological and pharmacokinetical role of Curcumin in $A\beta$ -aggregation and contemporary findings could be helpful in the search of new compounds for the treatment and prevention of AD and other $A\beta$ aggregation induced CNS disorders.

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